Instrument Start-up

Power up the computer workstation.

Depress the **Power** button on the BD Accuri C6 instrument face panel for 1 second. The instrument will begin a 5 minute auto start cycle. If the BD Accuri software displays the message *Extra Start-up time needed due to cleaning or improper shut down* it will take the instrument several more minutes for the instrument to get to the green-light ready state. Dripping from the SIP at the beginning of the Auto-Start cycle is normal.

Open the Accuri C6 software or a workspace template.

When the instrument is ready, the traffic light within the workspace will turn from yellow to green. It is recommended that you install a tube of DI water and run in Fast mode for several minutes to verify fluid uptake. See "Clogs/Obstructions" on Pg 4 if no fluid is taken up.
Important
Handle the SIP and Sample Support Arm Gently! The sample arm slides backwards and forwards unlike sideways on the Calibur. Your tube sits loosely on the SIP because the BD Accuri works via sample aspiration, not tube pressurization.

Workspace Set-Up

Setting Fluidics Rate
The system can accommodate an upper limit of 10,000 events per second, but it is recommended to acquire samples at a rate of 2,500 events per second or less to ensure the best data resolution.
To set the fluidics rate:

- Select from the Slow(14ul/min), Medium(35ul/min), or Fast(66 ul/min) radio button in the Fluidics section of the Collect tab.

ADVANCED: Use the Custom button to select a different rate between 10 and 100 ul/min

Threshold
Use thresholds to eliminate light scatter and/or fluorescence signals caused by debris in samples and electronic noise inherent in the system. By default, BD Accuri C6 Software is set to a primary threshold of channel 80,000 on FSC-H. Use the THRESHOLD button on the workspace.

- Threshold settings can be changed before, during, or after data acquisition.
- All thresholds are set on the Height signal for any given parameter.

- Width parameter is based on Threshold parameter setting (must select the appropriate parameter for cell cycle application).
- FSC Threshold should be set from 200K-500K for most mammalian cells with the exception of very large cells 0.5M-1.0M or platelets (10K-30K). Begin at a value low in the range and raise as needed.

Setting a Run Limit

The run limit defines when data acquisition will stop. *Time*, *Volume*, or *Number of Events* in a specified gate are used to define the run limit. If multiple run limits are set, data collection stops on whichever limit is reached first. If the *Run Unlimited* check box is checked the sample will not stop running until 1 million events(the maximum allowed per sample) are collected.

Creating and Editing Plots

Create a plot(s) using the buttons located in each designated plot area in the Accuri workspace. Plots cannot be resized.
Use the Plot Spec Tool found in each plot to manipulate the data display in a plot, including axis parameter selection, channel range specification, and selection of linear or logarithmic axis scale. Set up or modify plot specifications at any time before or after collecting data.

To modify the event number to be displayed, Select **Display>Events Display Settings** from the top menu. Choose from All, custom number or custom percentage.

To re-name plot axis labels, click on a plot axis and Choose **Rename Parameters** from the dropdown menu. Enter a new name in the **Rename to** field.

Statistics

When a plot is selected, statistics for that plot and any gates drawn in that plot will show up at the top of the plot analysis area. You can select for Median Statistics to be revealed or hidden at anytime by clicking the Display tab > Show Median Statistics.

Gating

There are a number of gating types available. These include quadrant, rectilinear, and polygonal for 2-parameter plots and vertical and horizontal markers for histograms. BD Accuri C6 Software automatically displays the percentage of cells within the region.

- Gates cannot only be deleted from the workspace by deleting the plot on which it was drawn. Gate names are assigned sequentially by type(R1, R2 R3, P1, P2, P3, M1, M2 etc). as created.
- Custom gate names can be entered by double-clicking on the label and typing a new gate name in the dialog box.

Compensation

Compensation is the process used to correct for the spillover of fluorescence into the wrong detector(s). On the Accuri C6, all compensation is performed manually. Post-acquisition compensation can be performed using Accuri C6 software or third-party applications.
To perform compensation,

1. Open the Compensation Template from the desktop
2. Collect at least 500 positive events for each single-stain control
3. Adjust compensation manually using the Set Color Compensation dialog box. In the row associated with the detector to correct, click on the FL button of the fluorochrome channel that is creating the spillover. Adjust the value while monitoring the quadrant statistical means for the plot containing both FL readouts. Repeat this with all fluorochrome-detector combinations in the experiment.
4. Apply the compensation values to all sample tubes.

Statistical readouts should ALWAYS be used when adjusting compensation. The UFCR recommends the use of quadrant gating when adjusting compensation values.

Acquiring Sample Data

The BD Accuri C6 aspirates your sample when it is in Run. It is important that there is a sample or water on the SIP whenever the instrument is in Run to avoid loading air into the fluidics.

- The rate of aspiration can be modified in the Fluidics area of the Workspace. *Slow, Medium* or *Fast* modes may be chosen for set flow rates of 14ul/min, 35ul/min or 66ul/min, respectively. The rate can also be set manually within a range of 10-100ul/min through the use of the Custom radio button and slider bar.
- Advance to the next well position on the plate to record your next sample *or* append data to the same sample.
- Delete saved information in a well by selecting the well and clicking the *Delete Events* button.
- It is recommended that a *backflush cycle(1 min)* is run between samples if doing rare event detection, or working with sticky samples. Wiping the SIP and back flushing between samples will prevent carryover between samples. Use the BACKFLUSH button on the workspace.

Clogs/ Obstructions

The BD Accuri C6 has a 1-minute UNCLOG cycle which may be run any time an obstruction is suspected. Place an empty tube on the sample port and select the UNCLOG button in the workspace. Fluid should accumulate in the tube. The cycle may be repeated until fluid is observed in the tube.

Exporting Data

Data can be exported from sample wells as individual FCS data files. Files will automatically be saved to a folder named EXPORT on the desktop. Transfer a copy of the files to your UFCR server space and move them to your lab folder in the My Documents folder. Choose File > Export FCS File. Delete any old data from your folder at each visit.

Cleaning and Shutdown

Close your workspace and launch the Accuri Cleaning Template. Run 10% bleach and DI water for 5 minutes each in well A01. Delete the well data after completion.

Shut Down
The last customer of the day is responsible for shutting down the cytometer. The BD Accuri C6 automatically runs a fluidics system shutdown. Depress the **Power** button on the Accuri instrument face panel for 1 second and the instrument will auto shutdown. Once the shutdown cycle has begun(Flashing blue light on front instrument panel) the computer can be powered off.

Specifications

Optics
20 mW 488 nm and 15 mW 640 nm lasers with a beam profile of 10 uM high x 75 uM wide
Detectors: 4 Fluorescent, 2 scatter

Detector	Filter	Fluorochrome
FL1	530/30	FITC, GFP, CFSE
FL2	585/40	PE, PI, PETX Red
FL3	670LP	PERCP, PERCPCY5.5, PE-CY7
FL4	675/25	APC, AF647

Fluidics
System based on ***sample aspiration*** instead of ***tube pressurization***
200 um ID Quartz Capillary Flow Cell
Minimum Detectable Particle Size: 5 um
Minimum Sample Volume: 50 ul
Sample Flow Rates: 10-100 ul/min
Core Diameter: 5-40um
Maximum Events Per Sample: 1 million events/well
Fluid Bottle Capacity: 2 L sheath fluid, 2 L waste, 250 mL cleaning solution, 250 mL decontamination solution
Tubes: Must be 12mm x 75mm or smaller. IF IT FITS, IT SIPS.

Performance
24 Bit Data (16,777,215 channels of resolution)
Detector sensitivity is factory pre-set. No adjustments are available.
Digital Acquisition Board
Sensitivity <150 MESF FITC; <100 MESF PE
Measures Area(A) and Height(H) of all parameters. Width(W) of one parameter.
Data Acquisition Rate 10,000 Events Per Second Max 2500 EPS Suggested
Max sample Conc: 5 million cells per ml

Computer
Dell Optiplex 7010 w/ 4GB RAM and a 500GB Hard drive
OS: Windows 7