# FACScan Users' Guide

## Cytometer
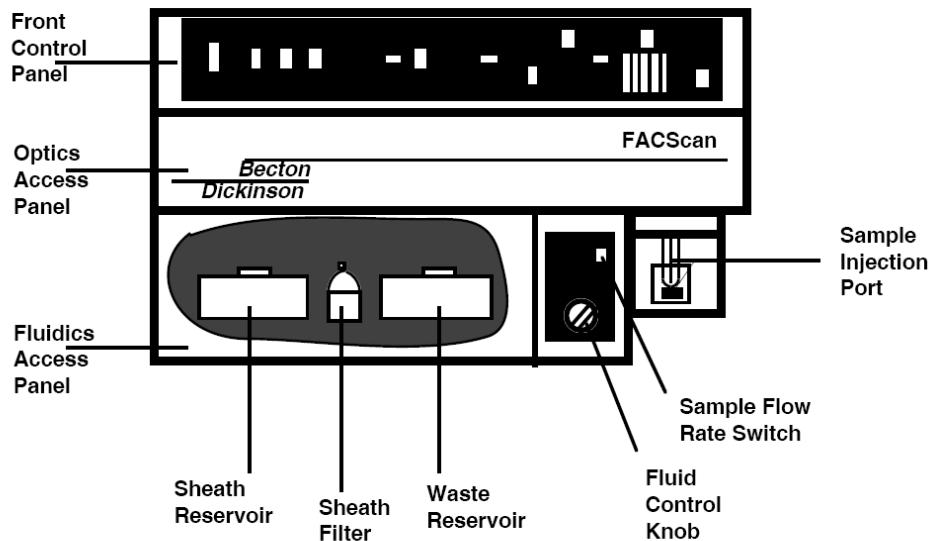


**Figure 2-1** BD FACScan Sensor Module

# FACScan Startup Procedures

## If the Instrument is OFF

1. **Check the sheath and waste tanks.**
   Open the fluidics door (front of instrument) and check the levels of sheath fluid and waste in reservoirs. Sheath fluid should be filled to 3/4 capacity (marked line). Waste should be empty except for ~10ml of bleach.
2. **Pressurize the sheath tank.**
   Flip the switch between the two fluidic reservoirs upward (close VENT VALVE).
3. **Turn on cytometer.**
   The FACScan must be turned ON **before** the computer. If you have a problem connecting to the cytometer, then the cytometer was not turned on in time to be recognized by the computer.
4. **Wait for STANDBY light**
   Make sure the tube of water is on the SIP and the dial is set to STANDBY. Once the STANDBY light (upper right hand side of instrument) is on the cytometer is ready.

## If the Instrument is ON already

1. **Make sure the Cytometer is in STANDBY**
   Make sure the Fluidic Control on the front of the instrument is turned to STANDBY, and a tube of water is on the SIP.

2. **Check the sheath and waste tanks.**
   Open the fluidics door (front of instrument) and check the levels of sheath fluid and waste in reservoirs. Sheath fluid should be filled to 3/4 capacity (marked line). Waste should be empty except for ~10ml of bleach.  If this needs to be changed:

3. **Depressurize the Sheath tank**
   Flip the switch between the two fluidic reservoirs downward to vent the tank

4. **Fill and Empty Sheath and Waste**

5. **Repressurize the Sheath tank**

6. **Wait for STANDBY light**

# CELLQuest Software Start Up & Sample Acquisition

File  Edit  Cytometer  Plots  Gates  Stats  Batch  Acquire  Windows

1. **Turn on computer, log in and open CELLQuest**
***The FACScan must be turned on before the computer. If you have problems connecting to the cytometer, then the cytometer was not turned on in time to be recognized by the computer.**
Click on the apple icon in the upper left hand corner of the screen.  A list should appear, and CellQuest is about 1/3 of the way down the list.  Choose CellQuest.

2. **Open your Acquisition Template**. Choose **File>Open...**  If you have a template saved, look in the CIC-FACScan drive, under:

   - Cellquest Data
   - Lab folder
   - Template folder

If you are making a newTemplate, use the toolbar on the left side of the screen to draw histograms and dot plots on the blank template (See New Template Protocol).  Be sure to SAVE the Template in your folder!!!

| File | |
|---|---|
| New | ⌘N |
| Open... | ⌘O |
| Close | ⌘W |
| Save | ⌘S |
| Save As... | |
| Save FCS File... | |
| Export Statistics... | |
| Append Statistics... | ⌘' |
| Document Size... | |
| Page Setup... | |
| Print... | ⌘P |
| Print One | |
| Quit | ⌘Q |

3. **Connect to the Cytometer**. Choose **Acquire>Connect to Cytometer**. The browser window will automatically open (but can also be found under **Acquire>Parameter Description** in the top menu bar).



4. **Set up your Acquisition and Storage** (the number of events to collect etc). Go to **Acquire>Acquisition and Storage**.

   a. Make sure your **Acquisition Gate** is set to "Accept" "All" events.
   b. Make sure your **Collection Criteria** is set properly. For example, if you would like to save 10,000 events in your 'live gate', you want to set collection criteria to "event count" and acquisition will stop when "10,000" of "G1=R1" (your primary population gate) events are counted.
   c. Make sure your **Storage Gate** data file is set to contain "All" events and the file **Resolution** is 1024.
   d. Click on OK to exit Acquisition and Storage.
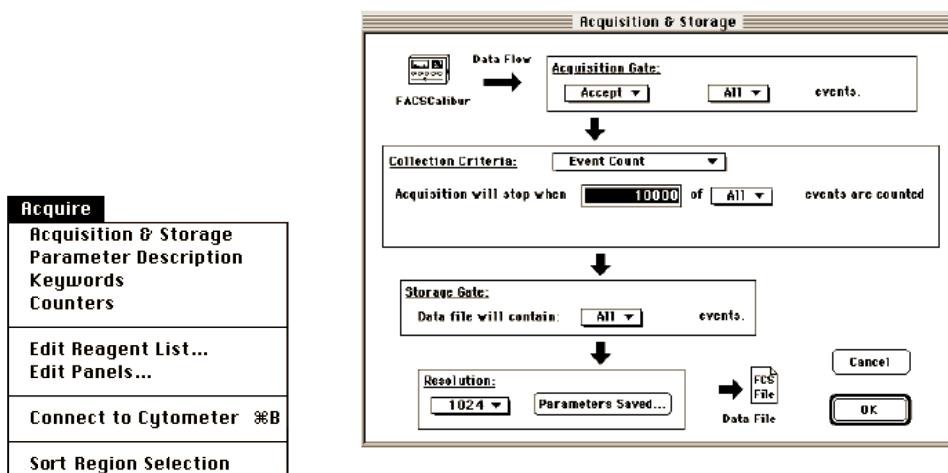


5. **Create a folder for your data**.
   a. Open the Parameter Description Window (**Acquire>Parameter Description**)

b. In the Parameter Description window click on the '**Folder…**' button next to the Directory and choose a location in which to save your data files.
c. Create a new folder for each day by clicking on '**Documents**' in the left of the window and then '**Create New Folder**'.
d. Name the folder
e. Click on "Select [your chosen folder name]" to select that folder and exit Folder Information.
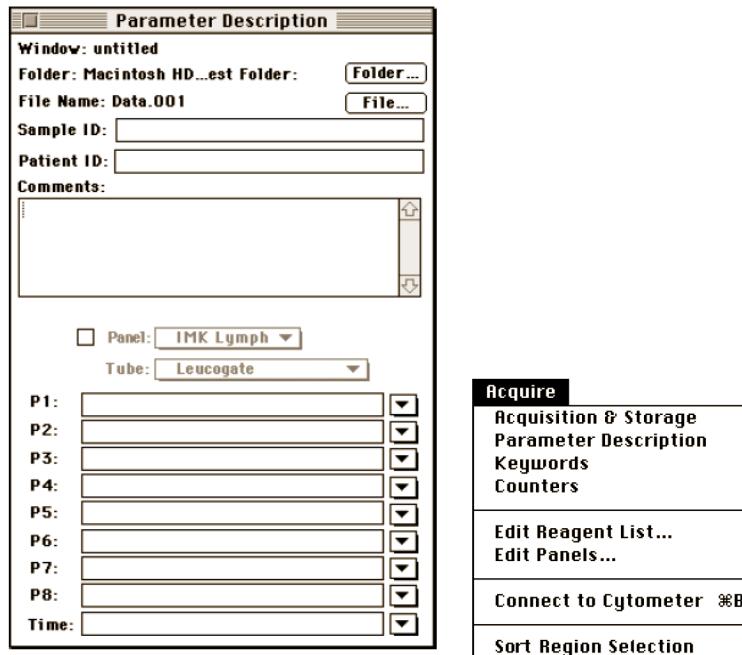


6. **Change the filename**.
    a. In the Parameter Description window click on the '**File…**' button next to the File and choose a name for your data files
    b. Set the file name prefix to "Custom Prefix".
    c. Set the file name suffix to "File Count" and start file count from 001.
    d. Click on OK to exit File Information.
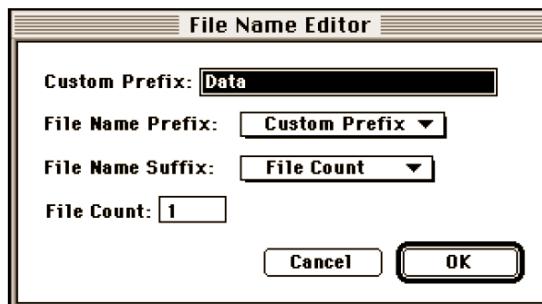


7. **Enter the names of your parameters** (e.g. Antibody conjugates, fluorescent dyes).
    Type the name directly into the box for each parameter.
    • P1: FSC (Forward Scatter)
    • P2: SSC (Side Scatter)

- P3: FL1 (FITC, GFP, Alexa 488)
- P4: FL2 (PE, Cy3)
- P5: FL3 (PI, PE-Cy5, PE-Cy7, 7AAD)

```
┌──────────── Parameter Description ────────────┐
Window: untitled
Folder: Macintosh HD...est Folder:    [Folder...]
File Name: Data.OO1                    [File...]
Sample ID: [                              ]
Patient ID: [                             ]
Comments:
          [                              ]

         ☐ Panel: [ IMK Lymph ▼ ]
            Tube: [ Leucogate      ▼ ]
P1: [                        ] [▼]
P2: [                        ] [▼]
P3: [                        ] [▼]
P4: [                        ] [▼]
P5: [                        ] [▼]
P6: [                        ] [▼]
P7: [                        ] [▼]
P8: [                        ] [▼]
Time: [                      ] [▼]
```

**Acquire**
Acquisition & Storage
Parameter Description
Keywords
Counters

Edit Reagent List...
Edit Panels...

Connect to Cytometer ⌘B

Sort Region Selection
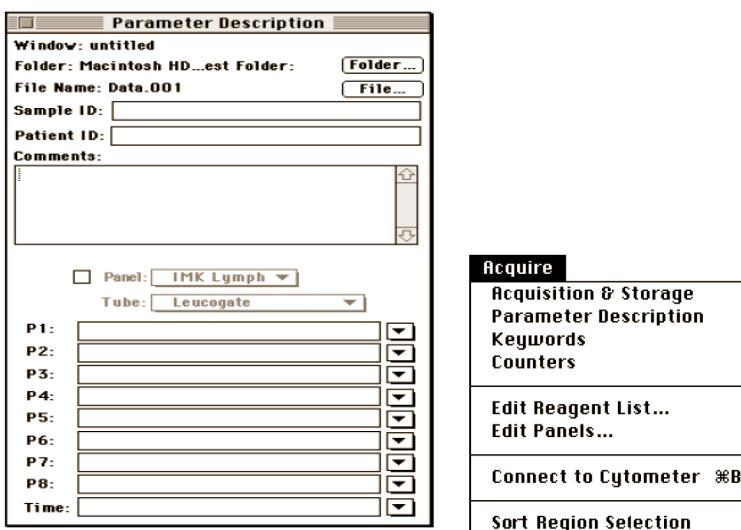
8. **Open the Counters window**. Go to **Acquire>Counters**.

**Acquire**
Acquisition & Storage
Parameter Description
Keywords
Counters

Edit Reagent List...
Edit Panels...

Connect to Cytometer ⌘B

Sort Region Selection

```
┌───────── Counters ─────────┐
Total Events:  0
Events/Second: 0      [ Reset ]
Elapsed Time:  0: 0: 0
```
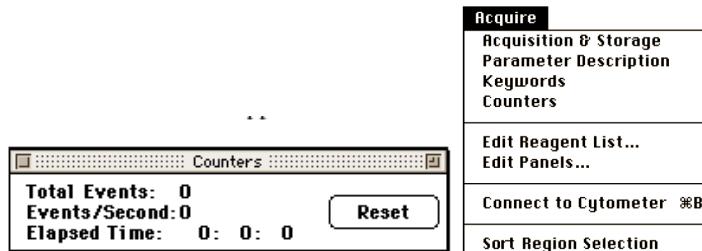
9. **Load Instrument Settings**. Go to **Cytometer>Instrument settings**.

10.    **Open AND SET your Instrument Settings**
       Click the **OPEN** button.  You can choose a specific Instrument Settings file *or* a previously recorded data file from your folder (choosing to use a previously recorded data file will copy the instrument settings from when that file was collected). Remember that when you **SET** the instrument settings the color wheel will spin, and it might take a few seconds to set! Click on **DONE** to exit Instrument Settings.

```
┌──────────── Instrument Settings ────────────┐
Cytometer Type:  FACSCalibur

Detectors/Amps:
Param  Detector  Voltage  AmpGain   Mode
P1     FSC       E00      1.00      Log
P2     SSC       150      1.00      Log
P3     FL1       150      1.00      Log
P4     FL2       150      1.00      Log
P5     FL3       150      1.00      Log
P6     FL1-A              1.00      Lin
P7     FL1-W              1.00      Lin

Displaying: Current Status              [Revert]
[Print...] [Save...] [Open...]  [Set]  [Done]
```

**Cytometer**
Detectors/Amps    ⌘1
Threshold         ⌘2
Compensation      ⌘3
Status            ⌘4

Instrument Settings...

Sort Counters

**11.** **Check sample settings before recording a file.** Tick the '**setup**' box in the Acquisition Control window. Press 'Acquire' to start viewing sample data. *__**you cannot record a data file in setup mode**__*

**12.** **Check Detectors/Amps settings**. Go to **Cytometer>Detectors/Amps** the window below left will appear. Adjust each parameter as required.

| Detectors/Amps | | | | |
|---|---|---|---|---|
| **Param** | **Detector** | **Voltage** | **Amp Gain** | **Mode** |
| P1 | FSC | E00 ▼ | 1.00 | Log ▼ |
| P2 | SSC | 150 | 1.00 | Log ▼ |
| P3 | FL1 | 150 | 1.00 | Log ▼ |
| P4 | FL2 | 150 | 1.00 | Log ▼ |
| P5 | FL3 | 150 | 1.00 | Log ▼ |

| Cytometer | |
|---|---|
| Detectors/Amps | ⌘1 |
| Threshold | ⌘2 |
| Compensation | ⌘3 |
| Status | ⌘4 |
| Instrument Settings... | |
| Sort Counters | |
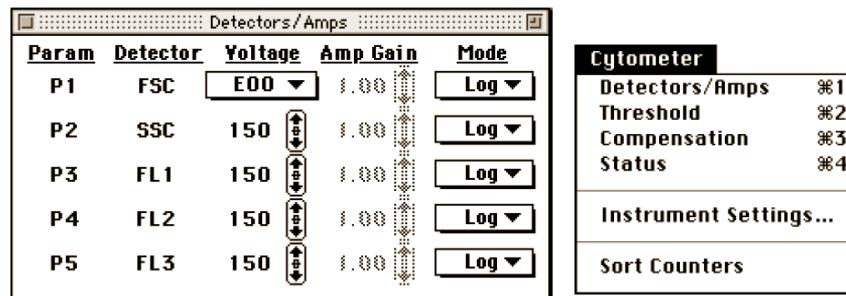
**13.** **Check Compensation settings**. Go to **Cytometer>Compensation** and the window above right will appear. (see Compensation tutorial for FACScan)

| Compensation | |
|---|---|
| FL1 – 0.0 % FL2 | |
| FL2 – 0.0 % FL1 | |
| FL2 – 0.0 % FL3 | |
| FL3 – 0.0 % FL2 | |

| Cytometer | |
|---|---|
| Detectors/Amps | ⌘1 |
| Threshold | ⌘2 |
| Compensation | ⌘3 |
| Status | ⌘4 |
| Instrument Settings... | |
| Sort Counters | |
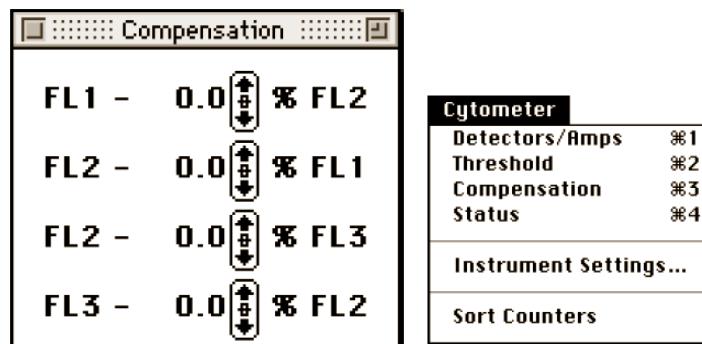
**14.** **Record a data file**.
a. Enter your '**Sample ID**' in the Parameter Description window.
b. Uncheck the **Setup** box on the Acquisition Control window. Your first file (name.001) is ready for recording.
c. Vortex your sample (or flick tube with your fingers), double check that there are no aggregates in your sample, and place on SIP.
d. **Run** cytometer on **Low** and click '**Acquire**' on the Acquisition Control window.
e. The number of events per second (Counter panel) should not exceed 2000. If your cells/events are running faster then remove from the SIP and dilute. If they are running slower then increase you sample speed to High (toggle switch above the RUN/STANDBY dial).
f. If you do not reach the required number of events before the sample runs out click '**Pause**' and then '**Save**'.
g. Repeat steps A-F for each sample.

**15.** **Backup your data at the end of each run.**
Either Bring a USB drive, or see the '**Data Transfer Guide**' for transferring your data to another computer

The CIC facility is not equipped for data storage.  Data is erased from all CIC computers (including workstations) on a monthly basis.

16.     **Please see the Data Analysis Protocols for analysis options.**

# FACScan Shut Down Procedure

**1. Disconnect the software from the cytometer.**
- After running the last sample, set the cytometer to STANDBY, make all necessary saves in CELLQuest and choose **Disconnect from Cytometer** in the Acquire menu.

**2. Clean flow cell with 10% Contrad for 3 minutes.**
- Place a tube with 3 ml of 10% Contrad on the SIP with the arm in the open position and aspirate for 30 seconds. Then close the arm and RUN the tube on HIGH for 3 minutes.

**3. Rinse flow cell with water for 3 minutes.**
- Place a tube with 3 ml of water on the SIP and aspirate for 30 seconds with the arm in the open position. Then close the arm and RUN on HIGH for another 3 minutes.

**Please Note:**

- *If someone **is not** on the instrument within 2 hours after your appointment*, shut down the instrument
- *If someone **is** on the instrument within 2 hours after your appointment*, keep the instrument turned on *(and in STANDBY mode)*
- *You can check the reservation schedule by double clicking on the reservation schedule shortcut on your desktop or going to* *http://biotech.missouri.edu/cgi-bin/ureserve.pl*