P. Keller 10/2008

Flow Cytometry on the FACS Calibur: General Instructions

NOTE: The software has been updated since these instructions were written, while they are still good general instructions on using the Caliber, there are some differences in operation of the software so use with caution!

Sign up for time with the calendar in the cytometer room, 8th floor Jaharis http://cytometry.med.tufts.edu (note you need a password to do this, to get one, contact Allen or Steve)

Allen.Parmelee@tufts.edu or Stephen.Kwok@tufts.edu (for questions)

Use of the cytometer

- Check that the sheath tank has enough fluid and that it is pressurized
  - To add fluid, vent the pressure with the switch on the right side, add dH20, recap tightly and then repressurize

- Log in to the computer
  - Login: Kuperwasser
  - Password: 75Kneel@nd

- Click on the CellQuest Icon
  - From the Acquire menu, select 'connect to cytometer'
  - From the Cytometer menu, select Detector/Amps and Compensation
  - From the Plots menu, select dot plots or histogram (or both)
    - The plot source should be acquisition unless you want to gate live cells at the cytometer, then, use acquisition to analysis
    - Make a FSC vs. SSC dot plot, histograms for each channel (FL1-FL4) and dot plots for compensation if needed (FL1 vs. FL2, FL2 vs. FL3, FL3 vs. FL4 etc.)
  - From the Acquire menu, select parameter description
    - Make a new folder on the desktop to save your files in
    - Name the file (Set Sample ID as identifier when you hit the file button), change w/ each new sample
    - Label the axes (EpCAM etc), check the detector amps for correlation between P number and channel

- Hit run-low on the cytometer to run the unstained sample
  - Check the setup box in the acquire box
  - Make sure P1 is thes to E-1 in Detector/Amps for large cells
  - Set FSC and SSC scales to linear, log for everything else (except PI if you don't expect many dead cells)

  - Adjust all of the compensation sliders to 0% if they are not already there
  - Adjust the voltage for FSC to bring the cells to the middle of the dot plot, do the same for SSC
  - Check the histograms for the different channels, adjust the voltage for that channel so the peak is between 10^0 and 10^1 and not too much of the peak is smooshed against the axis
  - Periodically pause, abort and then resume the run so the counts are cleared off the plots so you can see where the peaks fall

- If you do not need to compensate samples then you are ready to collect data
  - Vortex sample
  - Uncheck 'setup' and click acquire
  - Count to 20,000 to 100,000 events (Acquire menu, counter window to change, if you gated live cells change it to count to your number within the gate but makes sure it saves all counts in the data file)
  - Change the file name with each sample

- Compensation…note this is not a complete description and the jury is still out on whether we want to do this by hand or with software
  - Use single stained samples to adjust the compensation
  - Compensation is a way to account for bleeding of the signals between channels
  - With a dot plot of FL1 vs. FL2 and a single stain of an antibody fluorescing in FL1, adjust the compensation so the cells positive for FL1 do not exceed a value of 10^1 for FL2. Use the minimum amount of compensation (FL2 = FL2-%FL1) to achieve this.
  - Repeat with additional single stained controls (FL2 vs FL1, adjust FL1 = FL1-%FL2; FL2 vs FL3, adjust FL3 = FL3-%FL2; FL3 vs FL2, adjust FL2 = FL2-%FL3 etc.)

- Every FACS run should include:
  - Unstained sample
  - Isotype controls for each antibody
  - Single stained samples for setting compensation manually or on the software
  - Stain of interest (double, triple etc.)
  - If doing PI staining, use PI at 0.1 μg/ml and add near time of running the sample (i.e. at the Calibur), run these samples last in your run if possible
    - You may need to run a PI-labeled sample earlier for compensation…if so, run bleach for 30 sec then water for 1' to get rid of PI before proceeding with non-PI samples

- When done run bleach for 5 minutes (very important to prevent clogs if PI was used) and then run dH20 for 5'
- Place the Cytometer on standby when done using it
- Quit CellQuest, transfer data files to a USB drive, trash the data on the computer (do not store on the computer)
- Check that the sheath fluid levels, if needed, depressurize, fill the tank and then repressurize for the next user

Channels corresponding to various color fluorophores:

FL1 = FITC, GFP, YFP, Hoechst
FL2 = PE, PI for DNA content
FL3 = PerCP, PE-Cy5, PerCP-Cy5.5, PI for viability
FL4 = APC

Compensation for commonly used ones (limited to Patty's working knowledge):

FITC and GFP will bleed in FL2
PE bleeds slightly in FL1
PE-Cy5 bleeds a lot in FL4
PerCP-Cy5.5 bleeds minimally in F2 and FL4
APC has minimal bleed in FL3
PI bleeds into both FL2 (a lot) and FL4 (a little)