Manual Gating of Lymphocytes using BD MultiSET Software

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In this presentation, you will learn how to adjust:

- CD45 gating
- Bead gating
- CD3/SSC gating

We will be using BD MultiSET software, but the same principles may be applied to other flow cytometry gating programs and platforms.
The Sample list
Note: You will need to use files ending in a .01 extension. Files ending in .lab or .phy are not used here.

1. Click here to add samples
2. Click here to select a panel to use
3. Click here to start manually gating
Select “Manual Gate”
Manual Gating Work Table:
1. CD45/SSC
2. CD3/CD8
3. CD3/SSC
4. CD8/CD4
• As samples degrade, monocytes may present lower than usual on the CD45/SSC plot. The automated gating may not always detect this anomaly.
• Double click on the CD45/SSC plot to enlarge it. Redraw the lymphocyte gate if necessary.
Beads shouldn’t be in the CD8 gate.

- You may observe events from the bead gate in the CD8 gate. This is an indication that you need to tighten the bead gate.
• As samples degrade, the CD3+ population will appear dimmer. It may even start to overlap with the CD3- population. The automated software may not always detect this anomaly.
• Double click on the CD3/SSC plot to enlarge it. Adjust the CD3 gate if necessary.
When you are ready to check your work, click the “Analyze” button for an updated report. Then, re-enter the Manual Gating Worktable by selecting “Manual Gate”
The bead population is completely purple. If there is any white, the bead gate on the CD3/CD8 plot is pulled too high.

The entire lymphocyte population is included. If you see a section that is 90% “blue” events at the top of the gate, you may need a tighter gate. The gate pictured here is well-adjusted.
Checking the CD8/CD4 Plot

- There are few or no bead events in the CD8 gate.
- The CD4+ and CD8+ events are in tight separate clusters.
Checking the CD3 Gating using the CD3/CD8 Plot

- The CD4 and CD8 clusters should be tight and separate. CD3- “Blue” events should not be present in the CD3+ space, and CD4 and CD8 events should not be in the CD3- space. A line break is included below to help visualize.
The staff at the IQA Center is available for technical consultation concerning laboratory techniques for these procedures. You may contact Raul Louzao at 919-684-5861 raul.louzao@duke.edu or Sylvester Hood at 919-613-4469 sfh7@duke.edu