Duke University School of Medicine

IQA
Immunology Quality Assessment Program
NIH, NIAID, DAIDS

Manual Gating of Lymphocytes using BD MultiSET Software

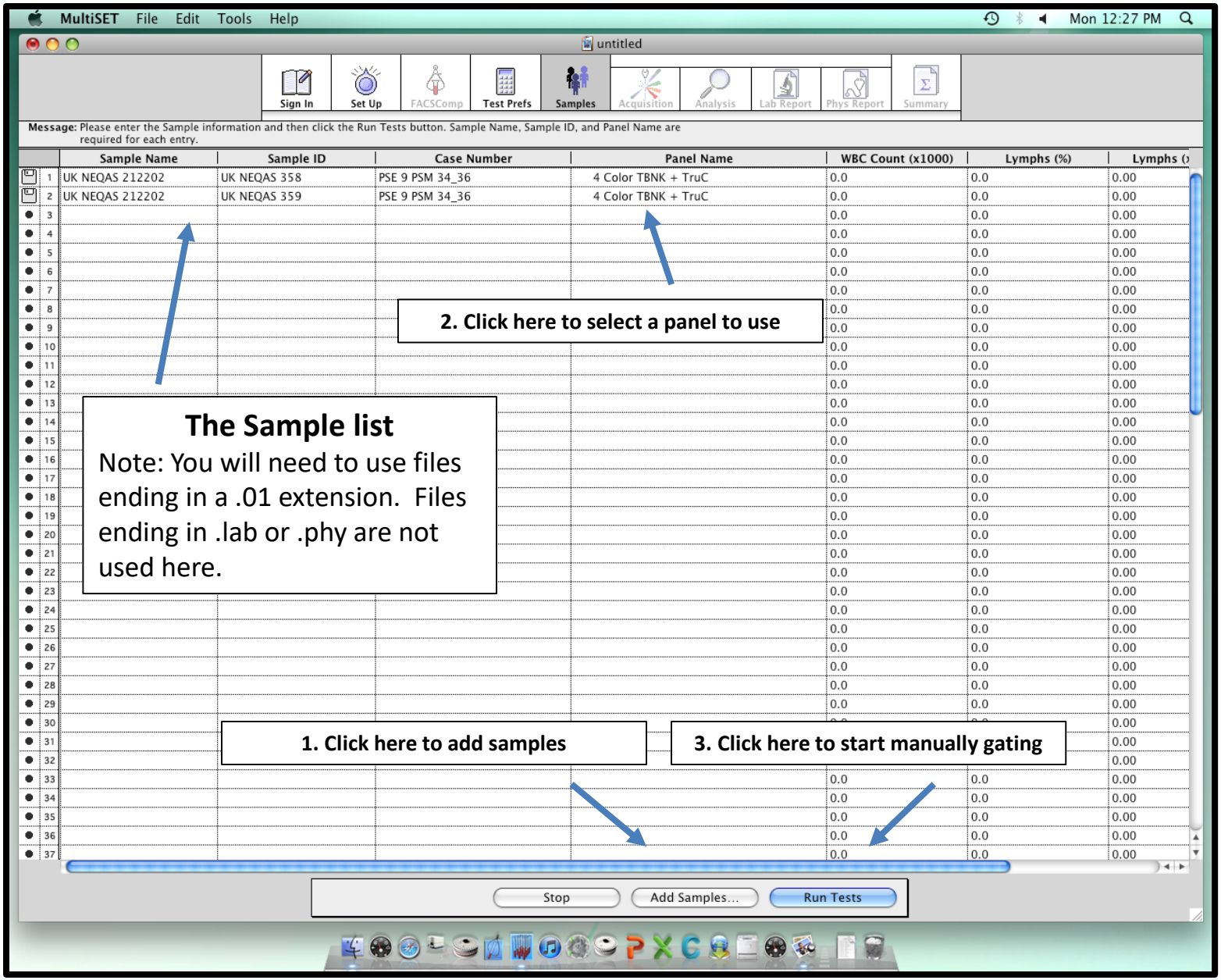
Prepared by Sylvester Hood for Immunology Quality Assessment

IQA Program Manager: Raul Louzao

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.


MultiSET File Edit Tools Help
Mon 12:27 PM
untitled
Sign In
Set Up
FACSComp
Test Prefs
Samples
Acquisition
Analysis
Lab Report
Phys Report
Summary
Message: Please enter the Sample information and then click the Run Tests button. Sample Name, Sample ID, and Panel Name are required for each entry.
Sample Name
Sample ID
Case Number
Panel Name
WBC Count (x1000)
Lymphs (%)
Lymphs (
1 UK NEQAS 212202 UK NEQAS 358 PSE 9 PSM 34_36 4 Color TBNK + TruC 0.0 0.0 0.00
2 UK NEQAS 212202 UK NEQAS 359 PSE 9 PSM 34_36 4 Color TBNK + TruC 0.0 0.0 0.00
2. Click here to select a panel to use
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
3. Click here to start manually gating
Stop
Add Samples...
Run Tests

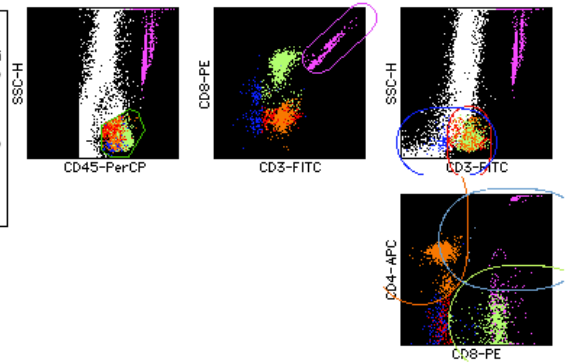
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: UK NEQAS 35806.01

Reagent Lot ID: 78222 Events Acquired: 15000 Abs Cnt Bd Lot ID: 20071 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 6B849D7F-52A6-476F-A1C7-AD01208F7719 Beads Per Pellet: 47100

Lymph Events	3000	
Bead Events	2841	
CD3+ %Lymph	85.97	Hi
CD3+ Abs Cnt	855	Lo
CD3+CD8+ %Lymph	35.00	
CD3+CD8+ Abs Cnt	348	
CD3+CD4+ %Lymph	32.87	
CD3+CD4+ Abs Cnt	327	Lo
CD3+CD4+CD8+ %Lymph	0.27	
CD3+CD4+CD8+ Abs Cnt	3	
CD45+ Abs Cnt	995	
T H/S Ratio	0.94	



QC Messages:

Code 3: Sample integrity questionable. Visually inspect.

Code 4: The CD3+ %Lymph value lies outside the normal reference range.

Code 4: The CD3+ Abs Cnt value lies outside the normal reference range.

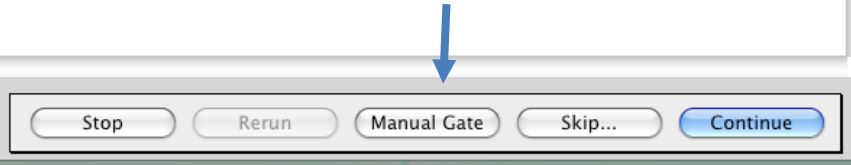
Code 4: The CD3+CD4+ Abs Cnt value lies outside the normal reference range.

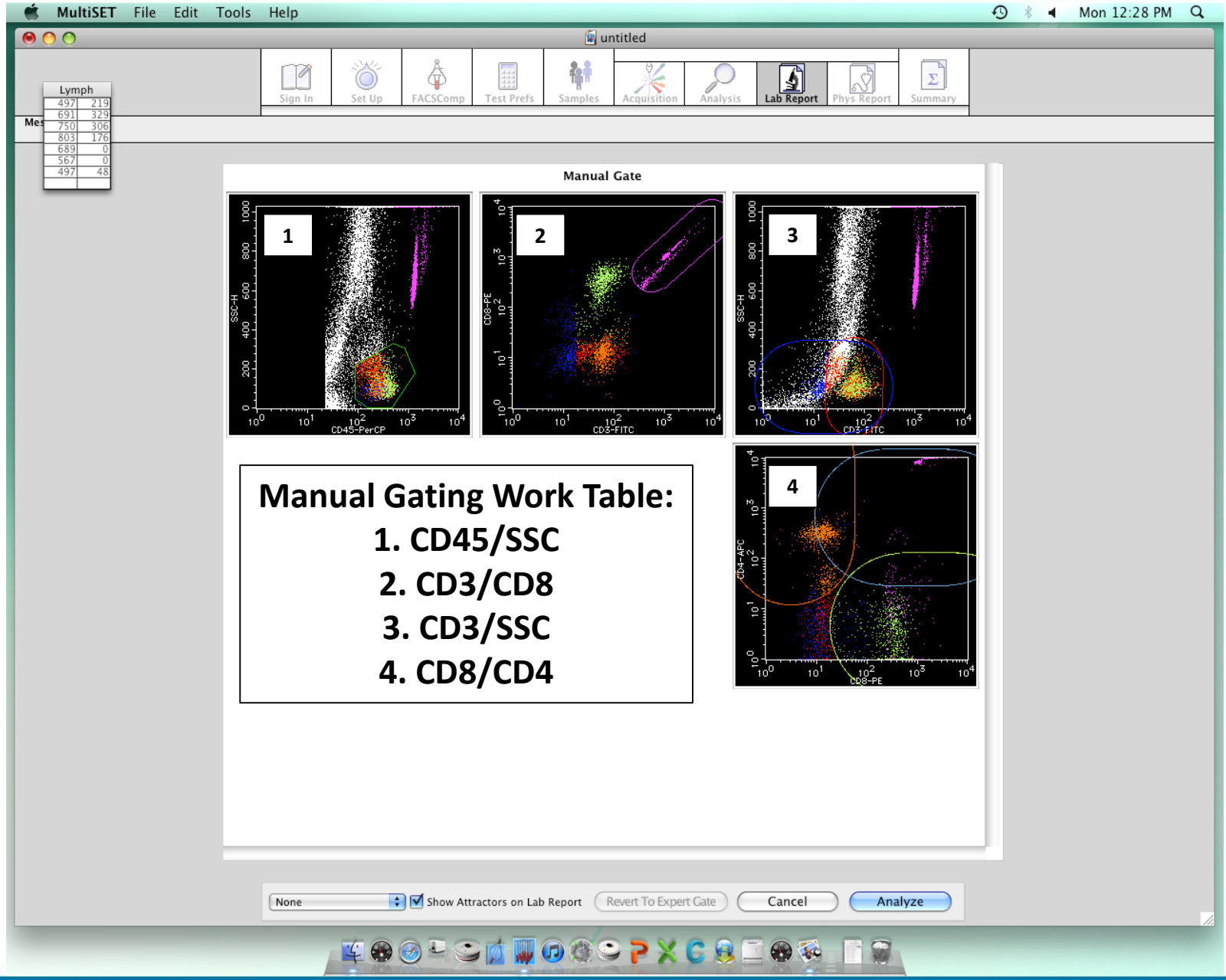
Tube Name: CD3/CD16+56/CD45/CD19 TruC

Status: Not yet run

File Name:

Select “Manual Gate”




MultiSET File Edit Tools Help
Mon 12:28 PM
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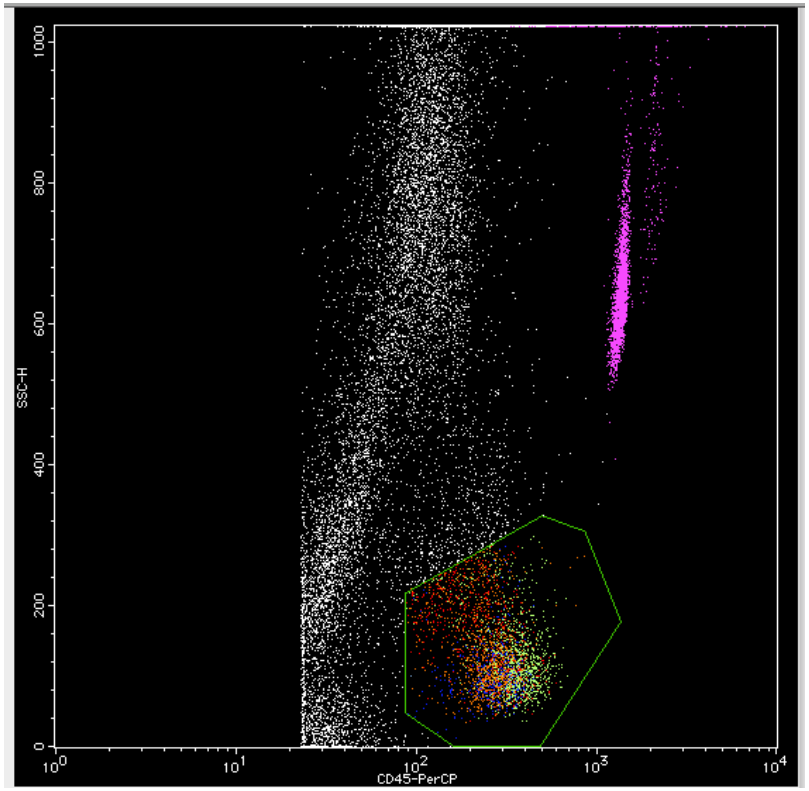
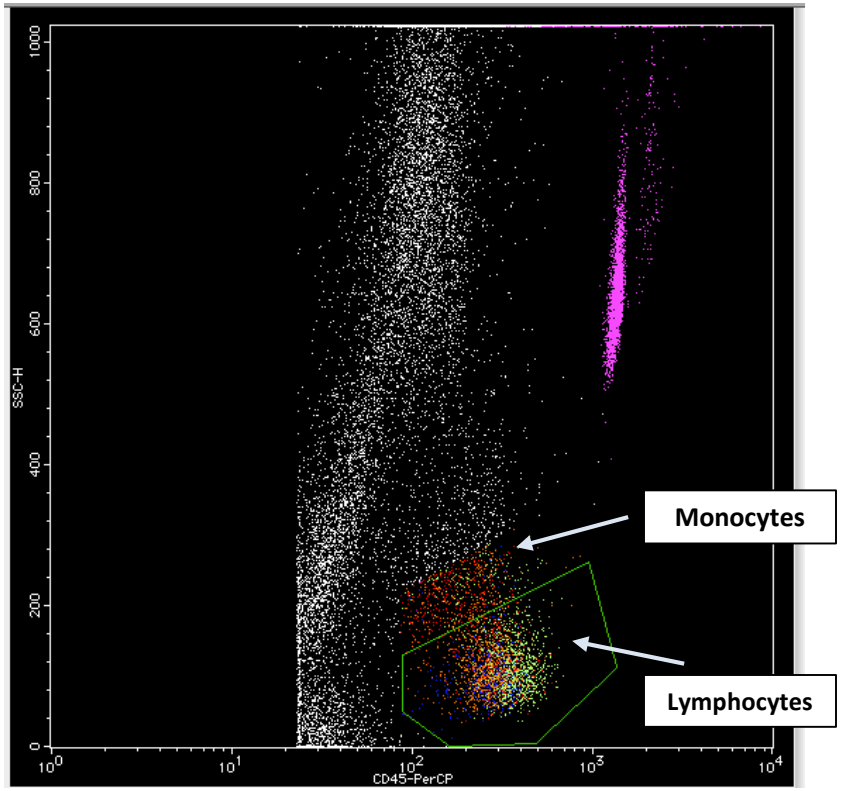
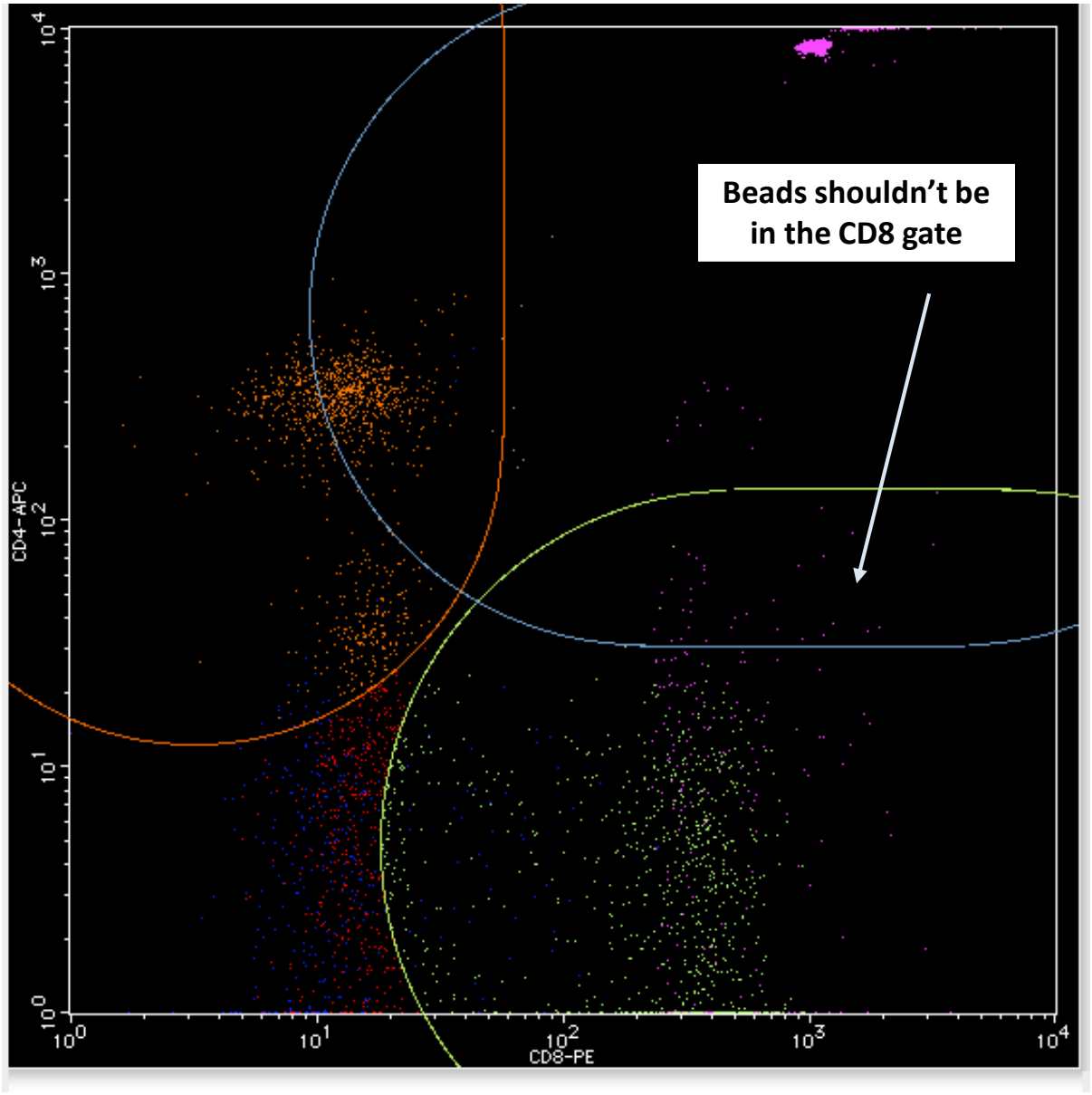
Sign In
Set Up
FACSComp
Test Prefs
Samples
Acquisition
Analysis
Lab Report
Phys Report
Summary
Lymph
497 219
691 329
750 306
803 176
689 0
567 0
497 48
Manual Gate
1
2
3
4
SSC-H
CD45-PerCP
CD8-PE
CD3-FITC
CD4-APC
Manual Gating Work Table:
1. CD45/SSC
2. CD3/CD8
3. CD3/SSC
4. CD8/CD4
None
Show Attractors on Lab Report
Revert To Expert Gate
Cancel
Analyze

- 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.

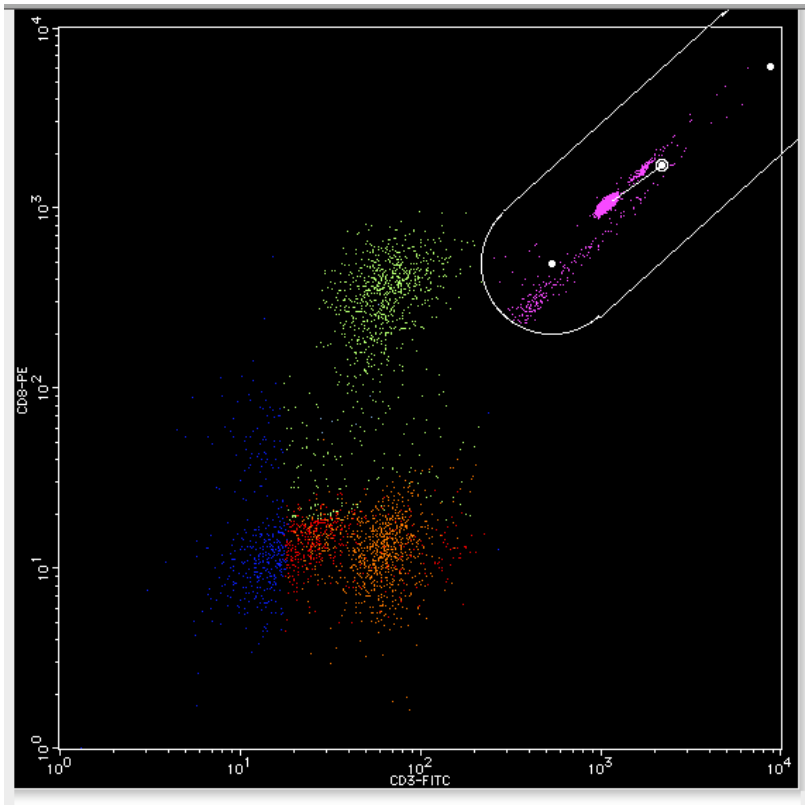




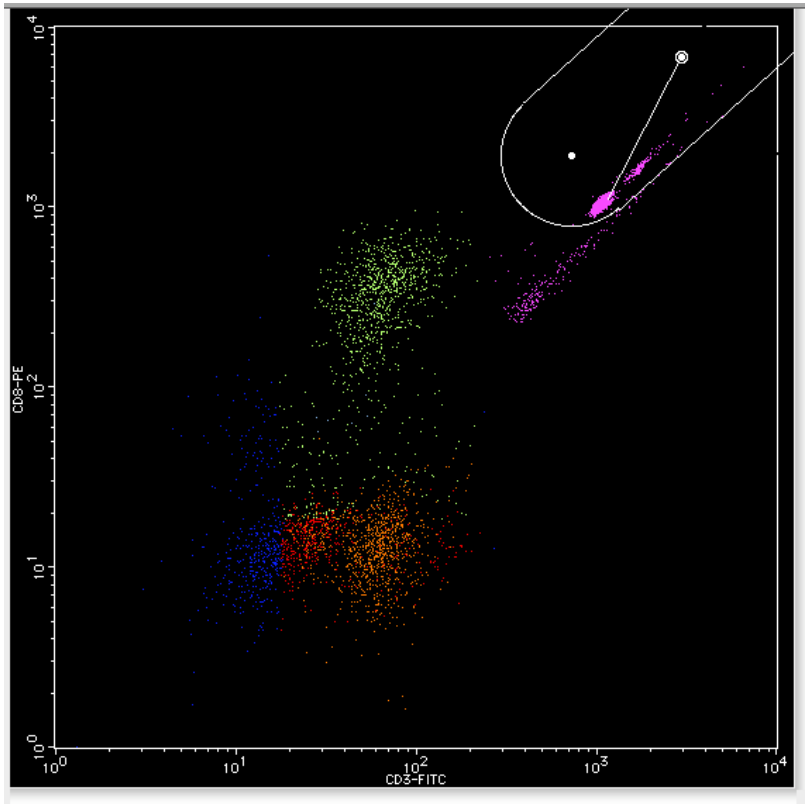


- **You may observe events from the bead gate in the CD8 gate. This is an indication that you need to tighten the bead gate.**

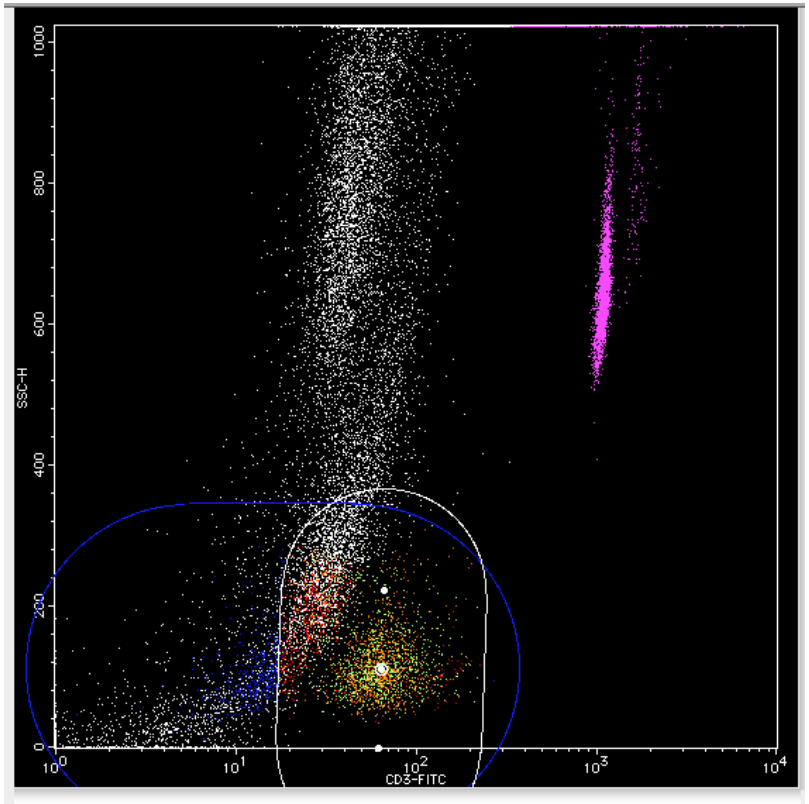
Before

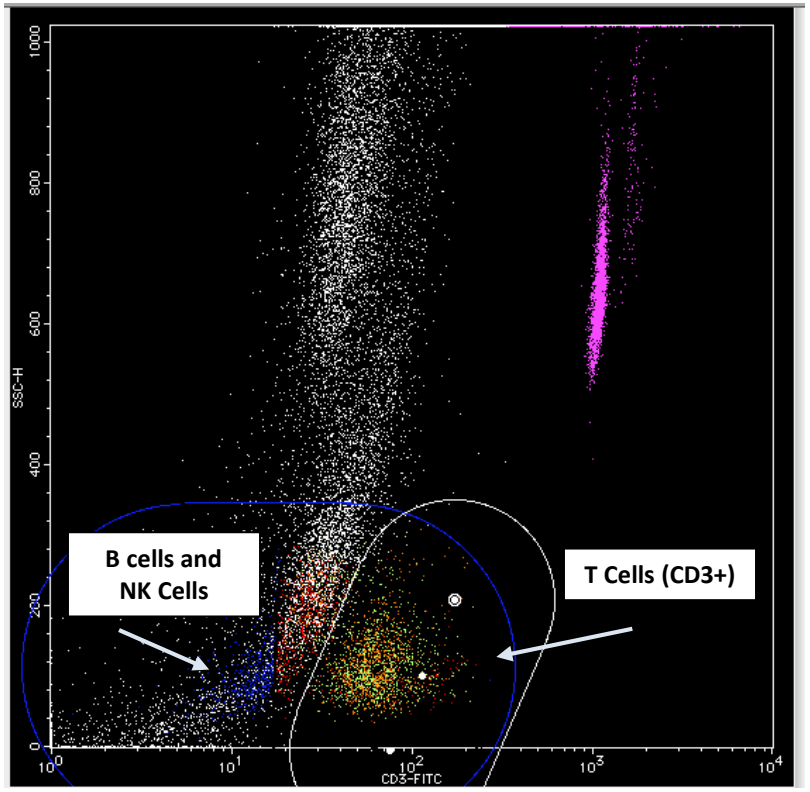


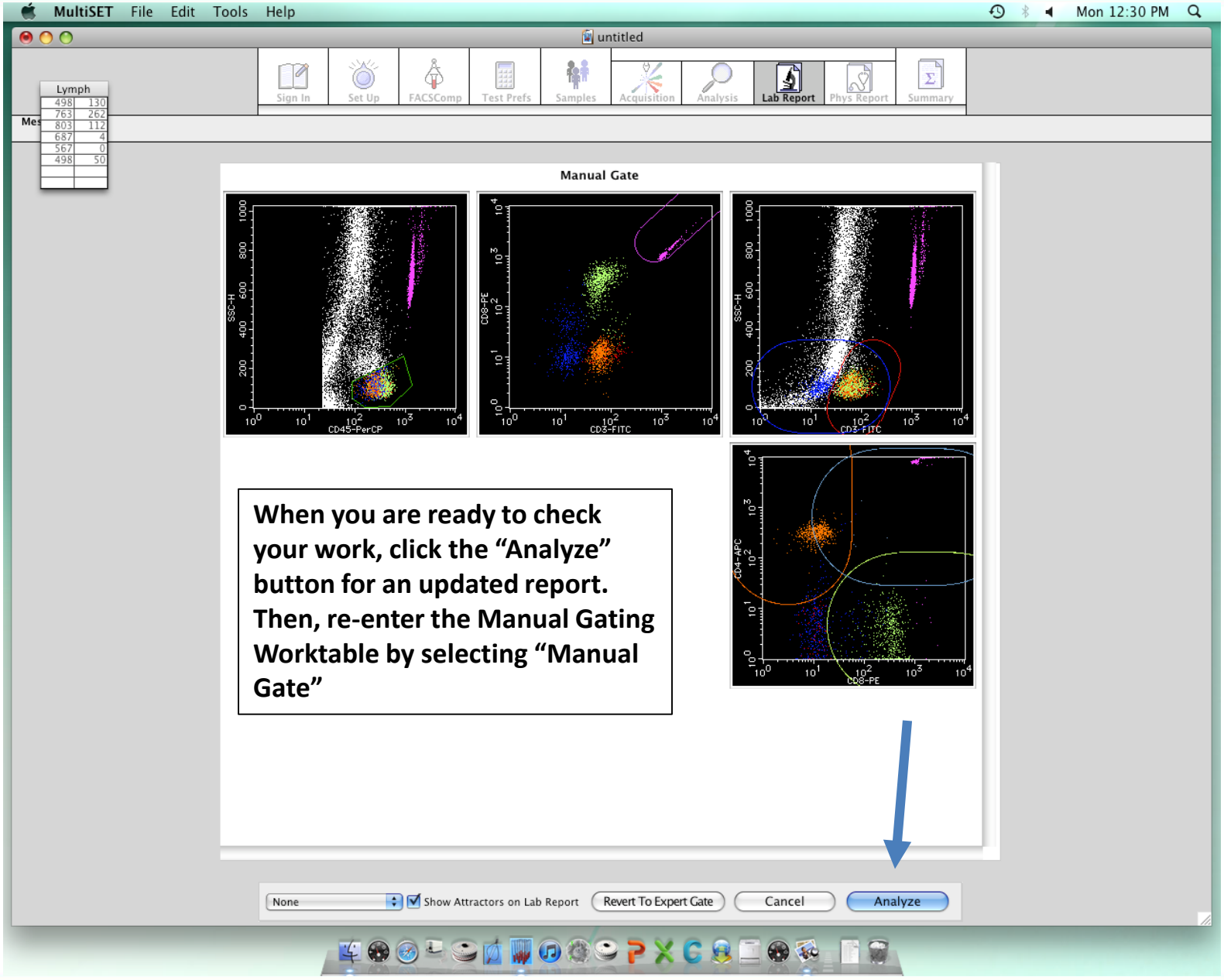
After



- 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.






MultiSET File Edit Tools Help
Mon 12:30 PM
untitled
Sign In
Set Up
FACSComp
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Samples
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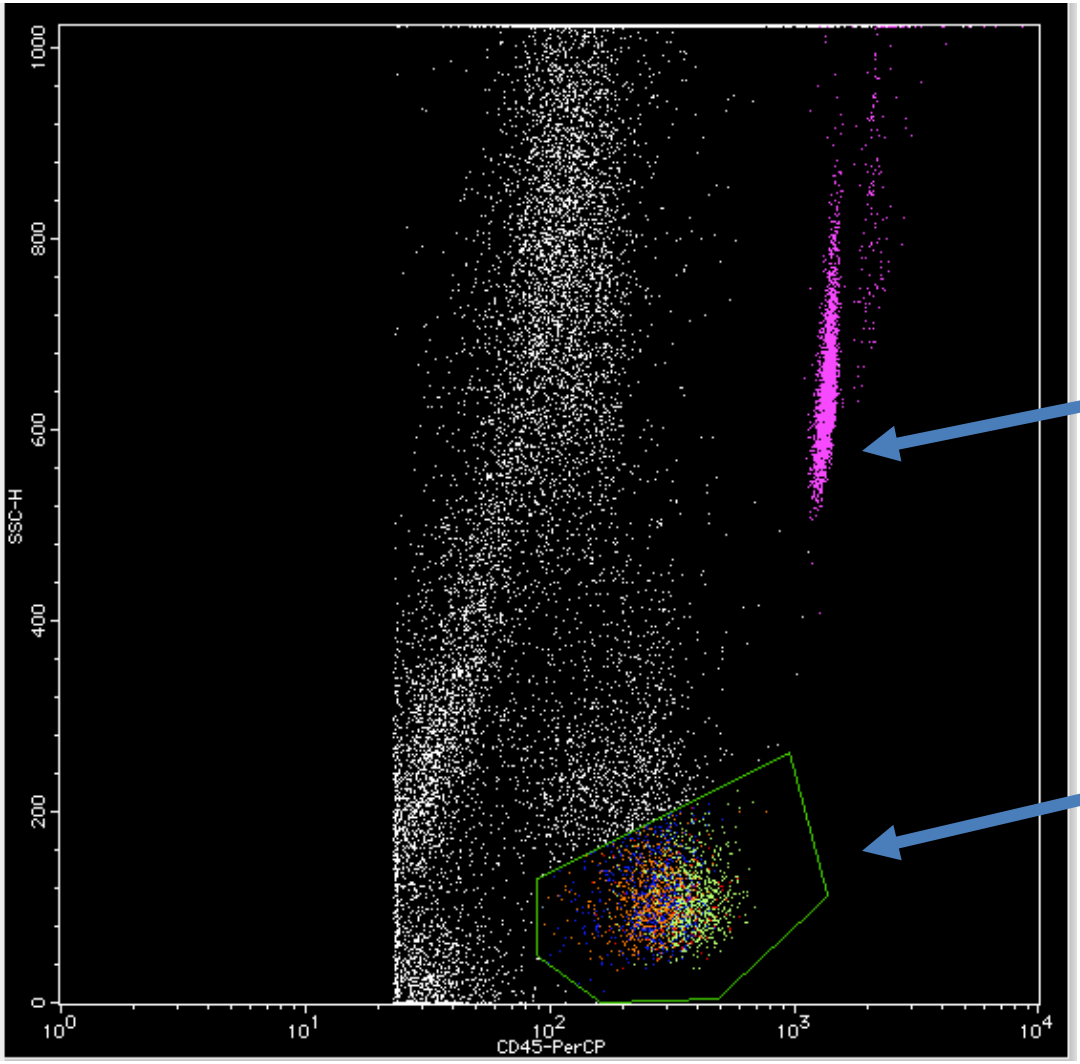
Lab Report
Phys Report
Summary
Lymph
498 130
763 262
803 112
687 4
567 0
498 50
Manual Gate
SSC-H
CD45-PerCP
CD8-PE
CD3-FITC
SSC-H
CD3-FITC
CD4-APC
CD8-PE
When you are ready to check your work, click the "Analyze" button for an updated report. Then, re-enter the Manual Gating Worktable by selecting "Manual Gate"
None
Show Attractors on Lab Report
Revert To Expert Gate
Cancel
Analyze



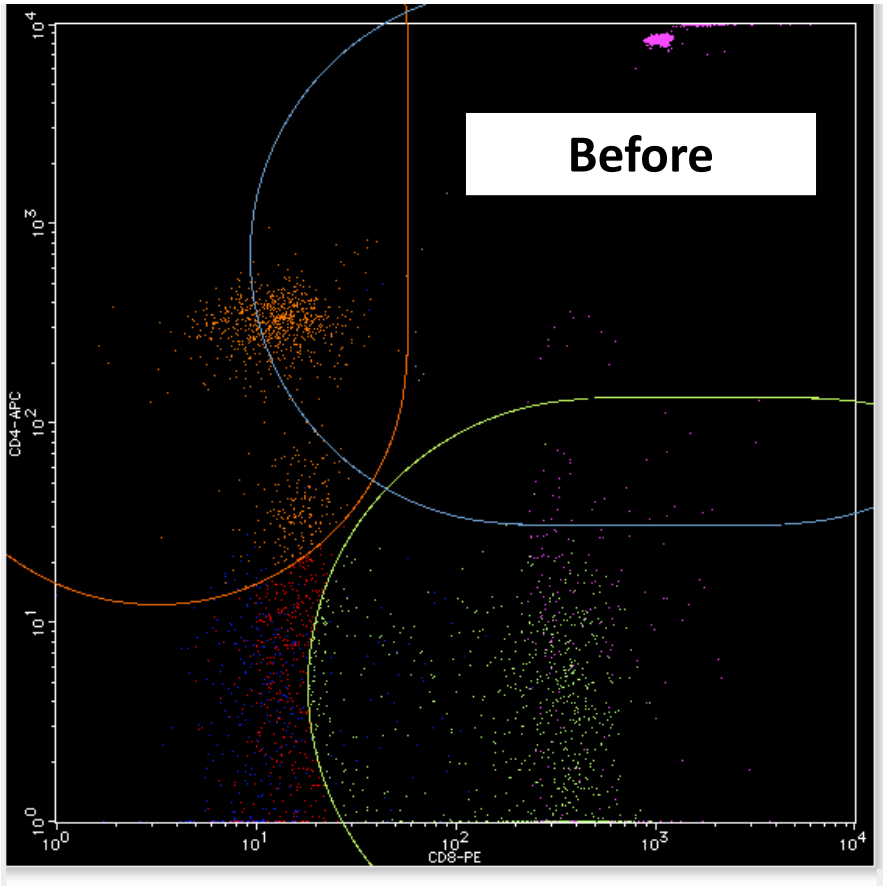
Checking the CD45/SSC Plot

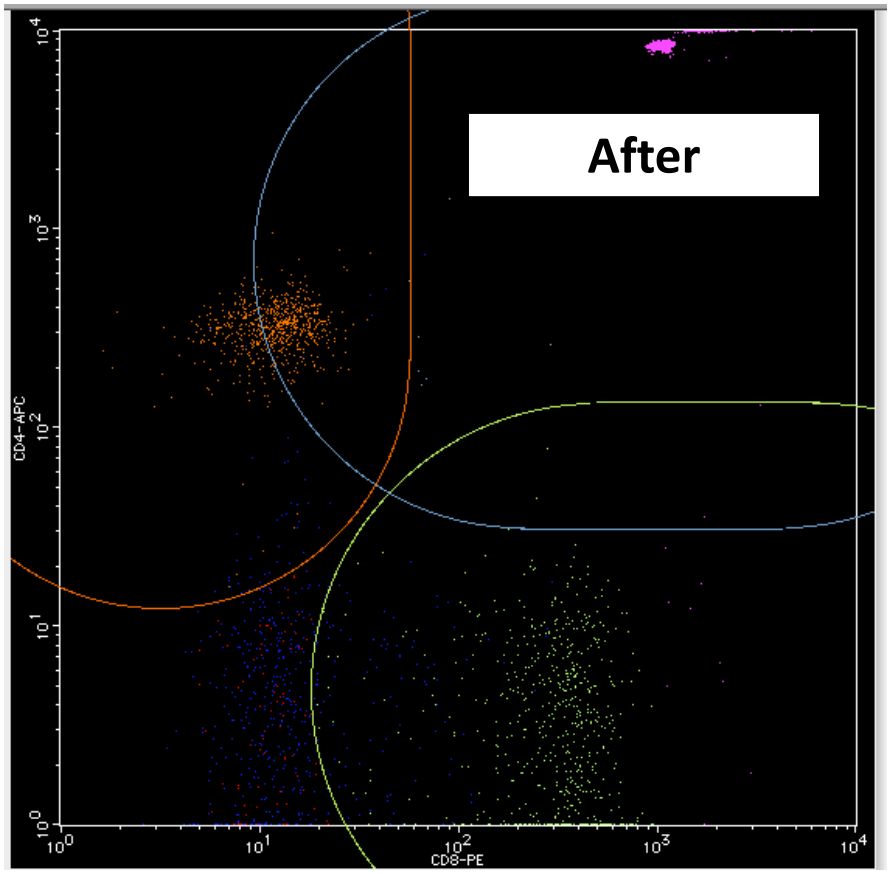
The bead population is completely purple. If there is any white, the bead gate on the CD3/CD8 plot is pulled too high.

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

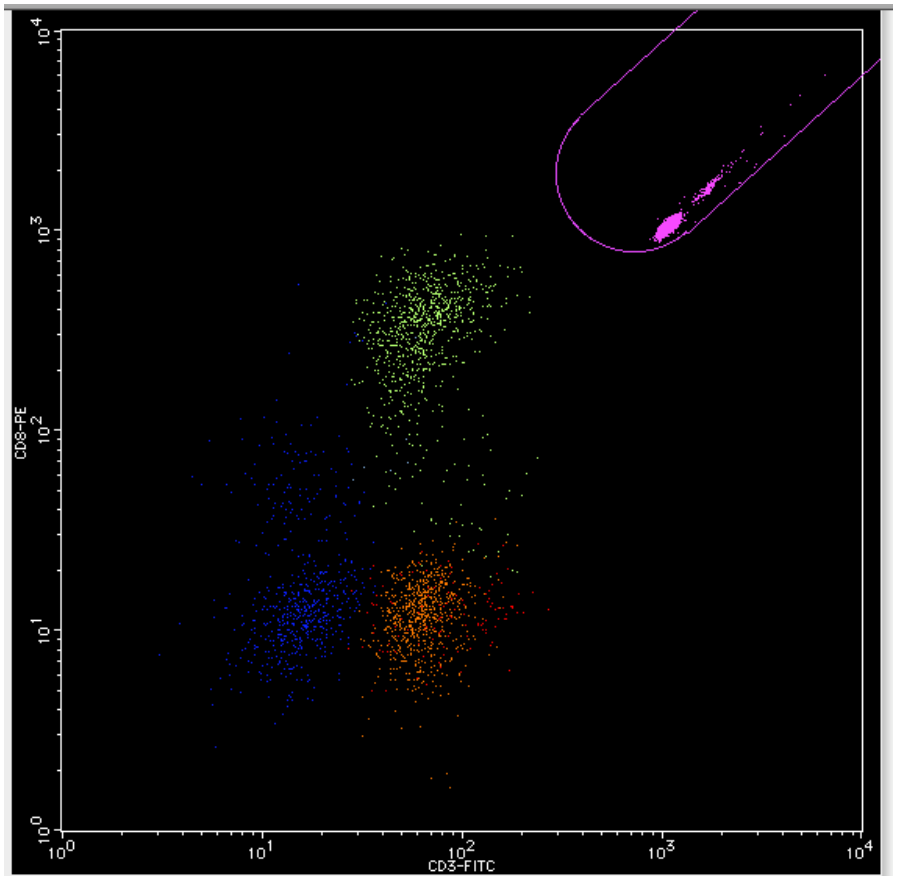
- There are few or no bead events in the CD8 gate.
- The CD4+ and CD8+ events are in tight separate clusters.

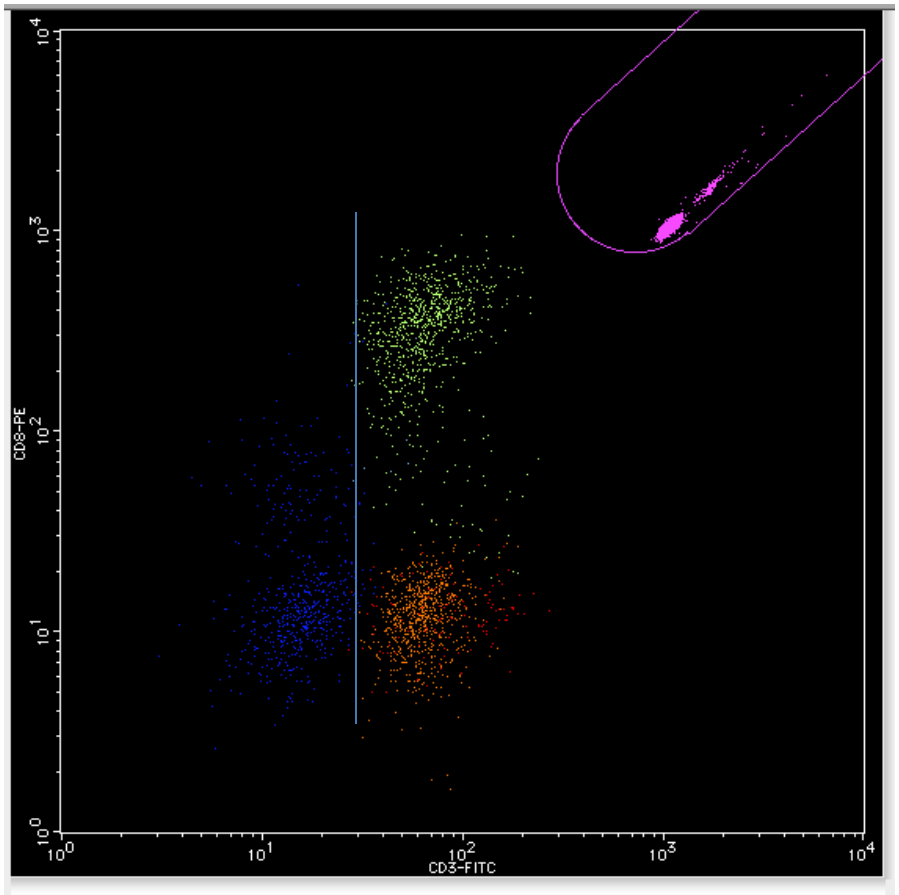




Checking the CD3 Gating using the CD3/CD8 Plot

- **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

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