Counting Cells with the LSR II Flow Cytometer

Introduction: Cell counting is normally performed manually with a hemocytometer or with a dedicated, cell counting instrument such as an Invitrogen Countess, or a Beckman Coulter Vicell. Flow cytometers also count cells and because flow cytometers measure many parameters simultaneously, much more can be learned about the counted cells. For example, cell types can be determined by size and immunophenotyping, and functional dyes and stains can assess the state of the cell. Counting cells with a flow cytometer is fast and easy. LSR II cell counting requires counting beads because we cannot know with precision the exact sample volume the instrument is aspirating.

Example Experiment: Human peripheral blood mononuclear cells (PBMC) were obtained from ficolled whole blood. The PBMC were counted with a hemocytometer and re-suspended to 1×10^6 c/ml in PBS supplemented with 1% BSA. The live/dead dye Propidium iodide (PI) was used to determine the percentage of dead cells.

1. 350 µl of PBMC was aliquoted into two tubes. 0.2 µl of Propidium iodide (Life Technologies, cat #P3566) was added to one of the tubes generating a final concentration of 0.6 µg/ml. It is essential to know the exact volume of the buffer the cells are suspended in.
2. 50 µl of Invitrogen CountBright beads (catalog # C36950) was added to the sample. Vortex the beads vigorously before adding them to the tube. Counting beads are available from multiple vendors (eBioscience, Spherotech, Bang Labs, etc). The CountBright beads are excited by all lasers emitting light from UV to 635 nm and fluoresce brightly in all channels between 385 nm to 800 nm. **Note:** Most bead preparations contain a detergent. Detergent prevents the beads from aggregating but a side effect may be cell damage. This is why it's a good idea to dilute cells to a volume > 300 µl and add only 50 µl of the bead preparation.
3. Acquire your samples on the LSR II. Gate on the live PBMC as shown in **Figure #1**.

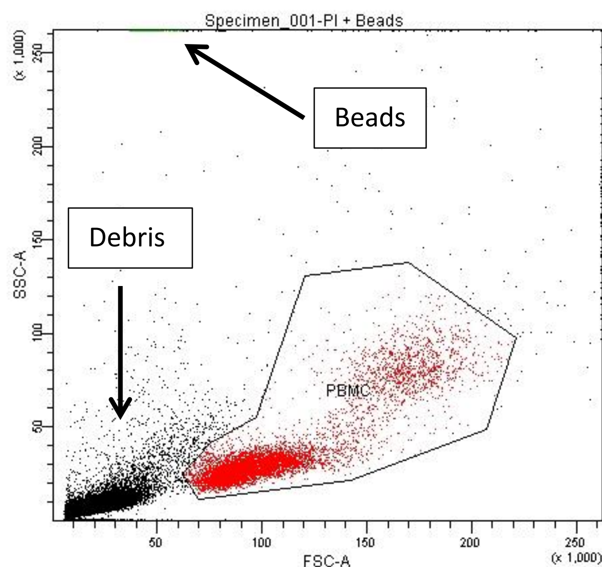


Figure #1 Gated on the PBMC, excluding debris and the beads.

4. Gate on the counting beads as shown in **Figure #2**, they are very bright and fluoresce in every channel.

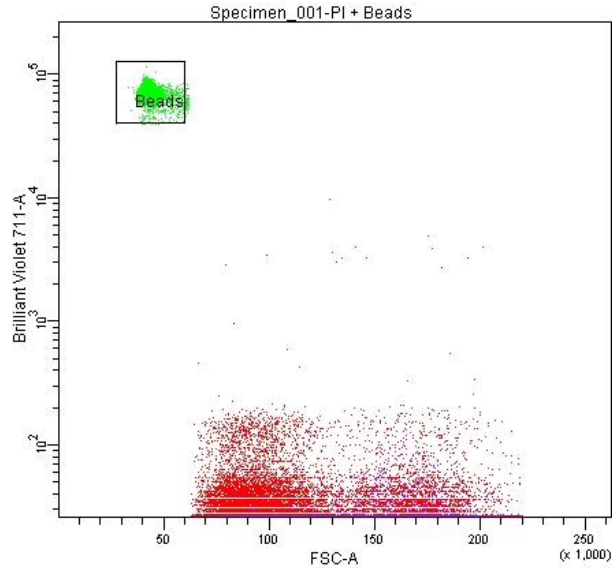
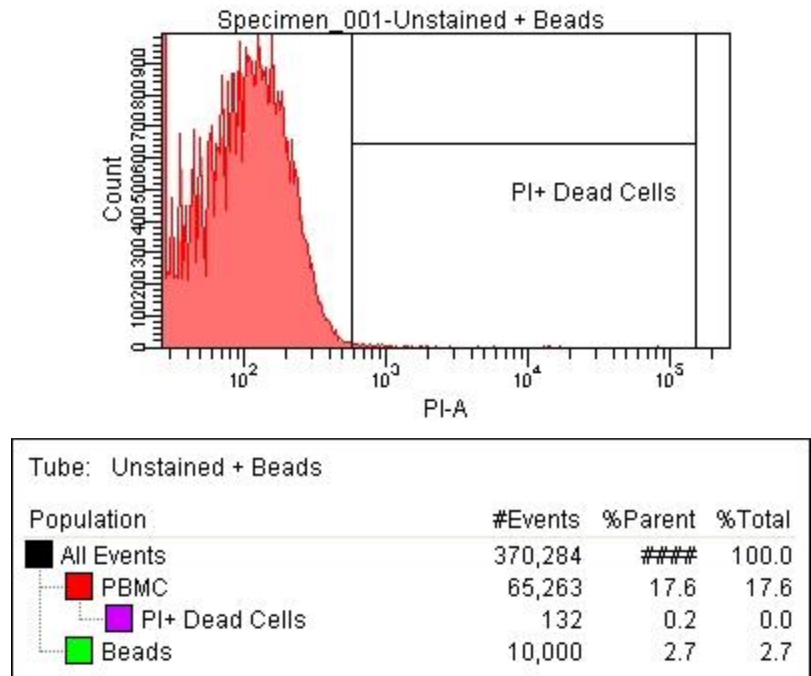


Figure #2 Gated on the beads in the Brilliant Violet 711 channel.

5. In the acquisition dashboard, choose the bead gate as the acquisition gate and instruct the instrument to stop recording once it reaches 10,000 events in the bead gate.
6. Gate the unstained histogram for PI as shown in **Figure #3.**

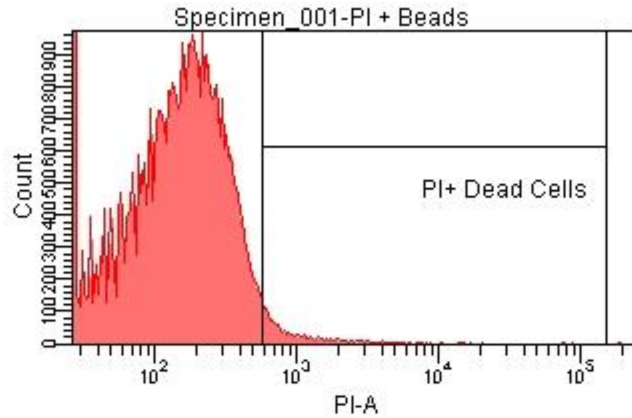


Tube: Unstained + Beads

Population	#Events	%Parent	%Total
All Events	370,284	####	100.0
PBMC	65,263	17.6	17.6
PI+ Dead Cells	132	0.2	0.0
Beads	10,000	2.7	2.7

Figure #3 Gated off the PI- Cells.

7. Determine the percentage of PI+ dead cells from the PI stained cells as shown in **Figure #4.**



Tube: PI + Beads

Population	#Events	%Parent	%Total
All Events	347,881	####	100.0
PBMC	64,615	18.6	18.6
PI+ Dead Cells	1,405	2.2	0.4
NOT(PI+ Dead Cells)	63,210	97.8	18.2
Beads	10,000	2.9	2.9

Figure #4 Gated off the PI+ Cells.

8. To calculate the concentration of cells the following formula is used:

A/B*C/D = concentration of sample as cells/ul

A = # live PBMC

B = fixed number of beads we made the instrument count (10,000 beads)

C = 54,000 number of counting beads in sample (50 ul)

D = final volume of sample (350 ul)

9. Variable C is determined by the specific bead lot number from Life Technologies. In this case, lot #1414119 contains 0.54 x 10^5 beads/50 µl.
10. Calculate the concentration/ml in Excel as shown in **Figure #5.**

Calculation A/B*C/D = concentration of sample as cells/ul

	Unstained	**Live Cells, PI-**
A = # live PBMC	65263	63210
B = fixed number of beads we made the instrument count (10000 beads)	10000	10000
C = 54,000 number of counting beads in sample (50 ul)	54000	54000
D = final volume of sample (350 ul)	350	350
A/B*C/D = concentration of sample as cells/ul =	**1006.914857**	**975.24**
Multiply by 1000 to get to cells/ml:	**1.01E+06**	**9.75E+05**

Results: The LSR II counted 1.01 (total cells) and 0.975 (live cells) x 10^6 c/ml as compared to 1 x 10^6 c/ml determined by the hemocytometer count.