

Absolute cell number determination

Flow cytometry provides a rapid method to quantify cell characteristics, however most flow cytometers cannot directly provide the cell concentration or absolute count of cells in a sample.

In order to obtain absolute counts, a suspension of beads with known concentration is added to a specific volume of sample, so that the ratio of sample volume to microsphere volume is known. The volume of sample analyzed can be calculated from the number of microsphere events, and can be used with cell event counts to determine cell concentration. In general, at least 1,000 bead events should be acquired to assure a statistically significant determination of sample volume.

Reagent

CountBright™ Absolute Counting Beads *for flow cytometry*
Molecular probes. Catalog number: C36950.

Protocol

1. Take out the beads from the fridge 30 minutes before use. Allow beads to come to room temperature
2. Vortex during 15 seconds
3. Add 50µl of bead suspension to the sample. Sample volume should be at least 300µl.
4. Acquire the samples: Vortex just before acquisition. Set up FSC threshold low enough to include the beads on the FSC-SSC dot plot.
5. Beads will appear in the upper right region of all fluorescent dot plots. Gate the beads and make sure to acquire at least 1000 beads.
6. Make a gate on your cells and display the number of cells and beads acquired.
7. Calculate cell concentration

$$[\text{cells}/\mu\text{l}] = \frac{N \text{ of cell event}}{N \text{ of beads events}} \times \frac{\text{Beads in } 50\mu\text{l}}{\text{Vol. of the sample}} \times \text{Sample dilution}$$

Values determined by the cytometer.

Example:

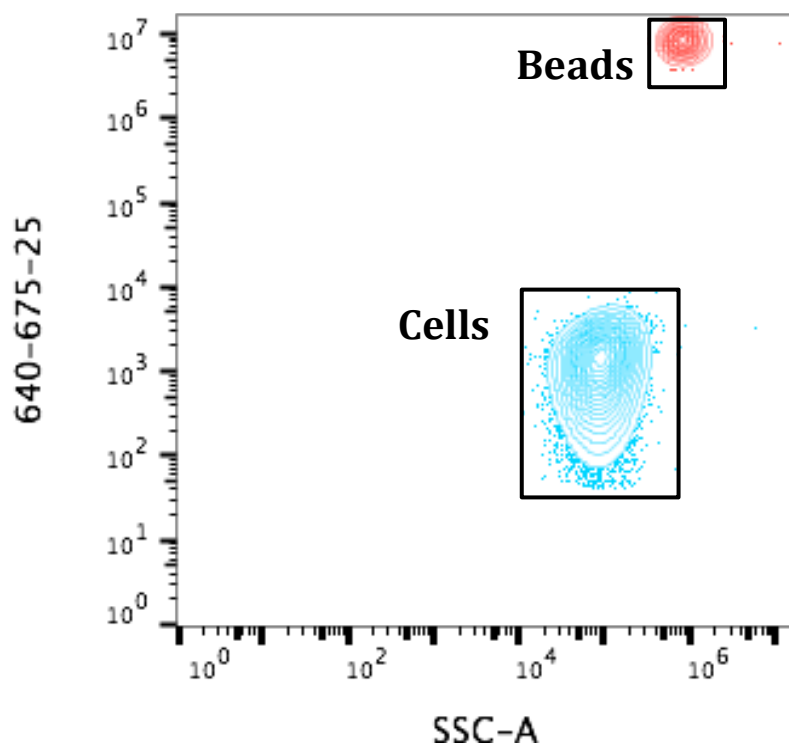
50 μl of sample + 50 μl of beads + 200 μl of PBS1X ----- Sample dilution (1/6).

Number of beads in 50 μl : 49500 beads (Depending on the reagent lot)

N of cells: 1700

N of beads: 1080

$$[\text{cells}/\mu\text{l}] = \frac{1700}{1080} \times \frac{49500}{300\mu\text{l}} \times 6 = 1481 \text{ cells}/\mu\text{l}$$



Sample from: Natalia Gabrielli-Patil Group, acquired in the BD Accuri C6.