DATA VARIATION ON DIFFERENT SPEED OF FLOWCYTOMETRY:

Flow cytometers in general are not made to provide precise volumetric counting (rather relative percentages between particles in the same sample). For "absolute counts", you have to add counting beads like Flow-Count beads(10 µm from Beckman-Coulter), Tru-Count beads provided in tubes (~4.5 µm from Becton-Dickinson), Cyto-Count (~6µm beads from DAKO), and Perfect-Count Microspheres (~6µm beads from Cytognos, Spain). All those are made for counting cells in suspension. If you increase the flow rate you increase the introduction of particles through the laser. Your counts should theoretically be proportional to the speed at each level (LOW - MED - HIGH). Explained below:

A **lower flow** rate decreases the width of the sample core and restricts the position of the cells to a smaller area. The majority of cells passes through the centre of the laser beam; thus the light illuminating the cells and emitted from the cells is more uniform. A lower rate is generally used in applications where greater resolution is critical, such as DNA analysis.

A **higher flow** rate is generally used for qualitative measurements such as immunophenotyping. The data are less resolved as its diameter increases, the fluids are less laminar (more turbulences between sample fluid/core-stream and sheath fluid) and the small particles go everywhere but are acquired more quickly.

This is how speed may variate the flow of cells but question is how does flow rate effect data resolution and specificity of the acquired data.

The experiment done by acquiring fix number of event on both speed at Gallios. At high speed as shown below the resolution in dot plot and histogram is not that good as both populations are merging. The % of negative population is 40.9, dim positive population is 50.5 and positive population is 3.1. The Median of the population are 0.357, 7.61 and 35.1 respectively.

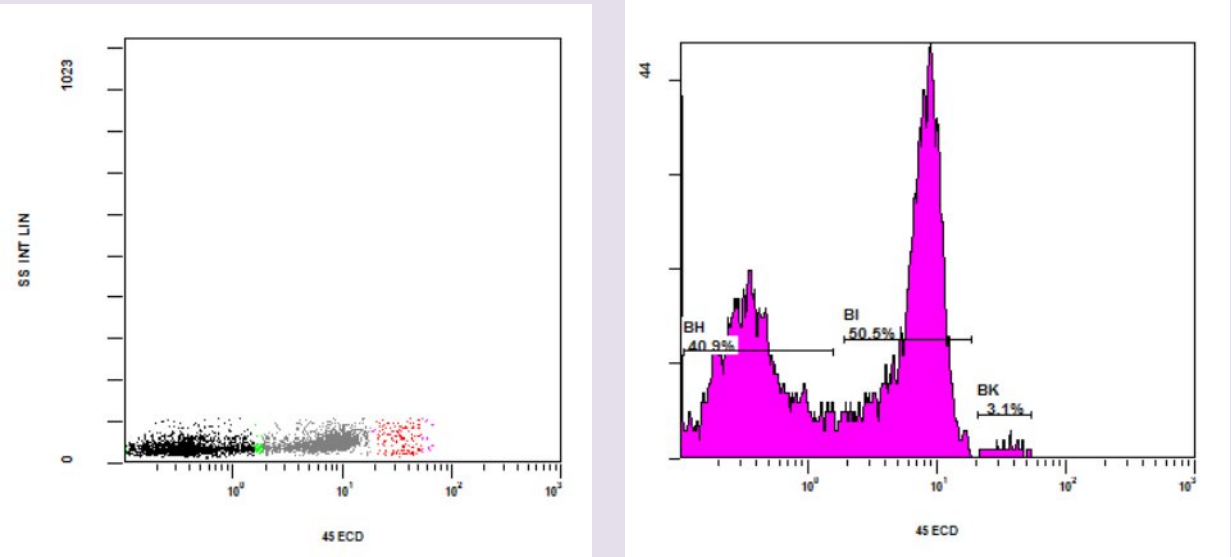

Figure 1

At low speed as shown below the resolution in dot plot and histogram is good as both populations are separated widely. The % of negative population is 16.1, dim positive population is 73.6 and positive population is 6.7. The median of the population are 0.259, 7.08 and 28.8 respectively.

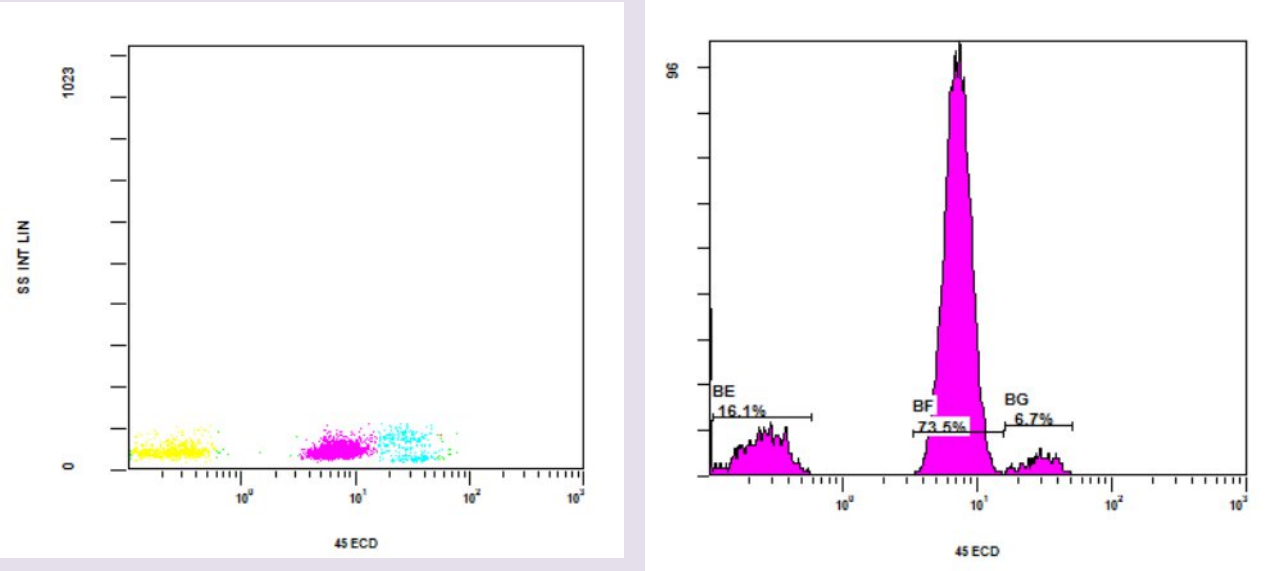

Figure 2

The population percentage is high for the positive population because the negative population is percentage is low in low speed data as compare to the high speed data as we have already discussed above why this high number of negative population is coming. Also the median of the population complied that the population is more spread and less compact which mean more non-specific population are present to vary the percentage. So overall result come that for qualitative data acquisition we need to run on low speed otherwise result variates and you may be facing some artefacts.