Useful Tips to Improve Sample Quality for the Flow Cytometric Analysis and Cell Sorting (Live Cells)

Low cellular viability, auto-fluorescence, and cell aggregates will result in poor flow cytometric analysis and sorts. Improved cell preparation will result in better sort purity, yield, and cell viability.

- Make all cell preparations strictly on ice, unless otherwise stated in the procedure
- Buffer suggestions
    - Use Ca/Mg++ free buffers (for example: DPBS, 1X without calcium & magnesium, Sterile)
    - Use BSA (0.1 - 1%) or dialyzed FBS (1-5%) (any source will be good) for the protein support of the buffer. Addition of non-dialyzed serum to the buffers will replace the Ca/Mg++. Use lowest sufficient concentration of BSA to improve data quality.
    - Use EDTA at up to 5mM concentration (for example: ready to use 0.02% in DPBS (0.5 mM), Cat# E8008, Sigma-Aldrich Co.; or ~0.5 M in H_2O, Cat#03690, Sigma-Aldrich Co.). It may help to prevent cation-dependent cell-cell adhesion
    - Add 25-50ug/ml of DNAse I (Cat# D4513, Sigma-Aldrich Co.) and 5mM $MgCl_2$ (Magnesium chloride hexahydrate, Cat# M2670, Sigma-Aldrich Co.) to the buffer. It may help if cells are clumping due to cell death.
- Single cell suspension
    - At any stage syringe the sample through the 18 gauge needle by 3-5 slow passages to dissociate cell clusters
    - Filter samples just prior to analysis or cell sorting to remove remaining clumps. The 12 x 75-mm tube with the cell-strainer cap ("blue-cap tubes"; #161900, RU Stockroom; or Cat#352235, BD Falcon) offers the convenient way to filter laboratory samples. *Note: It's important to prevent clumping (using tips above) before you filter your samples through a nylon mesh, otherwise you will lose too many cells*
- Keep cells at reasonable concentration of 1-10 x 10^6cells/ml (dependent on the cell type). Avoid keeping cells at unnecessary high concentration for the prolonged periods of time
- Use Dead Cell Exclusion (DCE)/discrimination dyes to "eliminate" dead cells from analysis and sort. Choice of DCE dye depends on the color combination of fluorochromes within the sample
    - For non-fixed cells use conventional DCE dyes, for example - DAPI, PI, 7AAD, TO-PRO-3, SYTOX Blue, Green, or Red; etc. *Note: DO NOT USE conventional DCE dyes (listed above) on the fixed cells*
    - For fixed cells - use fixable DCE dyes, for example LIVE/DEAD Fixable Amino Dyes from Invitrogen, BioLegend, BD Bioscience, Tonbo, eBioscience, etc.
- For sample acquisition on the BD LSR Fortessa:
    - Use only regular FACS Tubes (Cat#352008, BD Falcon)
    - In case of limited cell numbers, use the Cluster Tubes 4408 (1.1 ml, Cat#29442-604, VWR) and load them on the analyzers as inserts into the regular FACS Tubes (see above)

Avoid:

- Vigorous vortexing. It could damage fragile cells
- Excessive and unnecessary centrifuge spin. Use minimal necessary forces (rpm) to sediment cells
- Aspirating the entire buffer, leaving dry pellet of cells. Cells will die in the dry pellet.
- Bubbles: Cells tend to die in bubbles due to the surface tension forces.