**Troubleshooting/ Precautions while Acquiring and Sorting of samples:**

- Need single cell suspension- pass through 40 µm cell strainer.
- Minimum 1 x $10^6$ cells/ml required while acquiring. For sorting of cells number depends upon users requirement.
- Fix the cells with Paraformaldehyde (0.25-4% PFA) or Ethanol for acquiring.
- After staining keep the samples in Dark.
- Control samples should be prepared the same as the sort sample from the same type of cells as experiment samples.
- Each control sample should contain a minimum of ~ 200,000 cells.
- Negative control (Unstained cells) essential to minimize the background signal due to autofluorescence.
- Single fluorochrome stained tubes for compensation.
- **Proper selection of the fluorochromes.**
    - ✓ Know your instrument's optical configuration and fluorophore spectra.
    - ✓ Divide fluorophores across multiple lasers and distant emission ranges.
    - ✓ Put bright fluorophores on rare cells/low expression markers, dim fluorophores on abundant cells/highly expressed markers
    - ✓ Put high spillover fluorophores on mutually exclusive markers
    - ✓ Use a single dump channel to exclude undesired populations
- For sorting student should provide Cleaning solutions like Sterile distilled water, 70% ethanol.
- Collection tubes or plate filled with appropriate quantity of media (Do not fill sample tubes over 2/3 full).