Flow Post-its
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Why are Single Cell Suspensions Important?

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Flow cytometry allows for high throughput multiparametric analysis of **single cells**. Ensuring samples are in a single cell suspension is a critical step for a successful experiment, since the presence of **cell aggregates** means **fewer cells available** for **analysis** or **sorting** and can potentially lead to **clogs.** Therefore, it is important to **know the source of aggregation** and address it accordingly.

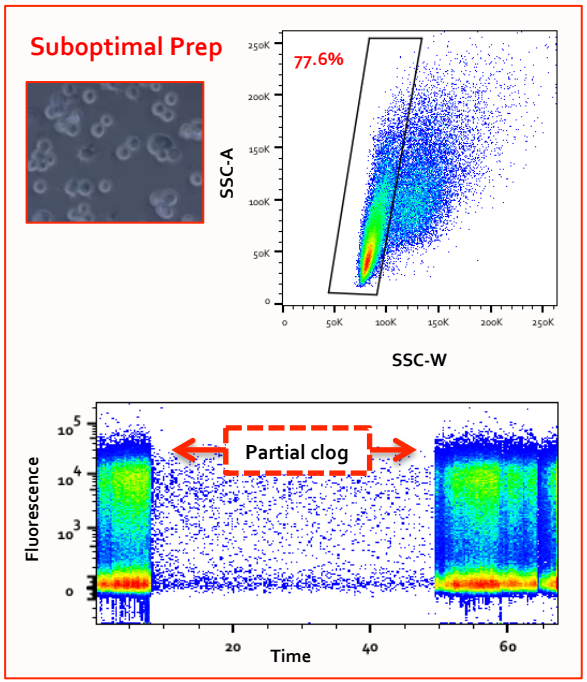
Sources and Solutions For Cell Aggregation

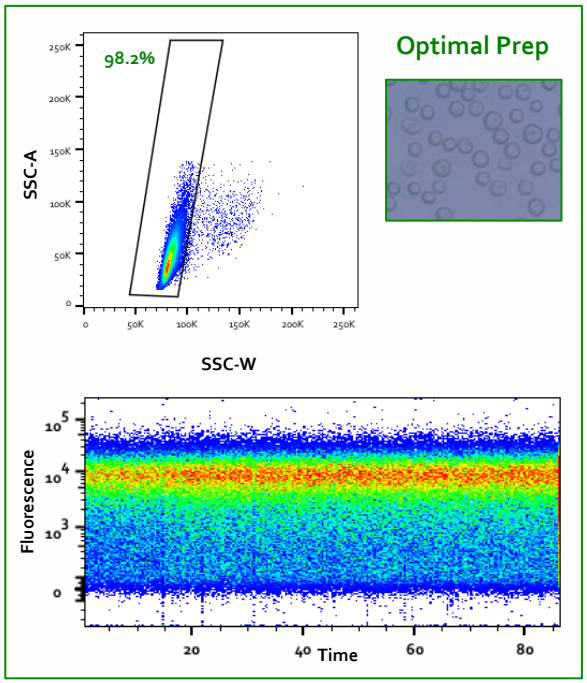
Adherent Cells

- ✓ Use Soybean Trypsin to quench Trypsin instead of media/serum, when harvesting cells
  Note: There are different options for enzymatic digestion. Optimization of cell preparation is key.
- ✓ Use calcium- and magnesium-free buffers
- ✓ Use BSA (0.1 – 0.5%) instead of FBS as a protein component
- ✓ If cells do not tolerate BSA, use dialyzed FBS
- ✓ Add EDTA (2-5 mM) to your samples
- ✓ Optimize cell concentration to prevent reaggregation

Cell Death – DNA released from lysed cells:

- ✓ Treat cells with 100 µg/mL DNAse I with 5 mM $MgCl_2$ in HBSS at room temperature for 15-30 minutes.
  Note: EDTA inhibits DNAse and should not be used.
- ✓ Wash cells once in HBSS with 5 mM $MgCl_2$.
- ✓ Resuspend cells in HBSS containing 1-5 mM $MgCl_2$ and 25-50 µg/mL DNAse I.





Important Notes

- Cell aggregates can cause instability in fluidics and eventually clog the flow cell
- Positive and negative cells stuck together will result in false positive signal
- Buildup of aggregates can cause fluctuating event rates
- Samples should be in a single cell suspension before acquisition at the instrument
- All samples should be checked under the microscope
- Filtration only removes large aggregates, it does not ensure single cell suspension