ImageStream sample preparation guide

V04

1. **Choice of cell type/magnification:** The particle type should be less than 120µm using 20x magnification, 60µm using 40x, and 40µm using 60x.
2. **Final concentration and volume:** 1-5 x 10^6 cells/ml in 50µL in PBS/2%FBS in a 1.5 mL siliconized eppendorf tube.
3. **Number of samples:** We recommend that you limit the number of samples.
    a. positive and negative biological controls
    b. compensation controls
    c. experiment samples
4. **Protocols:** In general, any established labelling protocol used for flow cytometry will work with the ImageStream (see *Current Protocols in Cytometry* for general labelling techniques). Remember to titrate your antibodies. Stain cells on ice in the presence of azide when possible to reduce non-specific capping of antibody. Capping may affect the ability to analyse your data.
5. **Choice of fluorochromes:** Chose fluorochromes that are excited by the lasers either the 405nm, 488nm, 561nm, or 642nm laser. Be aware that the 488nm and 561nm lasers are co-linear. Likewise, for 405nm and 642nm lasers. Use the chart on FACScore.au.dk or use a spectra viewer that will help you plan which dyes will work the best.
6. **Compensation:** Have a sample of cells (or beads) each labelled with a single colour for each fluorochrome used in your experimental samples (i.e. FITC only cells, PE only cells, etc.)
7. **Cell aggregation:** Minimize aggregation problems by straining the sample through a 70µm nylon mesh strainer, or by using an anti-clumping buffer such as EDTA prior to fixation.
8. **Fixation:** If fixation is desired, thoroughly fix cells with 1%PFA or 0.9% formalin on ice for 20 min.
9. **Titration:** Titrate your antibody preferably to a 2 log difference between pos. and neg. population and a Raw Max Pixel value preferable between 200 and 4095.