

FAQs for Image Cytometry (ImageStream)

What Concentration should be my sample be?

Cells should be resuspended as a single cell suspension at 2×10^7 - 5×10^7 to 10^8 cells/ml. Samples at lower concentrations result in increased acquisition time.

What Buffer should my sample be resuspended in?

Cells should ideally be resuspended in PBS 1X with either no or minimal protein (e.g. 0.5% BSA). Excess protein results in foaming of sample buffer that can introduce air bubbles into the flow cell that will affect image focus.

What volume should my sample be resuspended in?

A minimum of 50-60ul in a 1.5ml Eppendorf Tube.

How many fluorophores can be used?

A maximum of up to 12 fluorophores. Most users generally run panels of 4-6 fluorophores or less.

What magnifications can be acquired on the Imagestream?

20X, 40X and 60X. Choose an appropriate magnification based on the size of cells/objects in your sample. 20X can be used for cells ranging between 60um-120um, 40X can be used for cells between 40um-60um and 60X can be used for objects less than 40um.

What is the brightness of fluorophores as compared to a standard cytometer?

The Imagestream has very low background compared to a traditional flow cytometer and therefore is well suited for detecting small particles such as bacteria, liposomes, EVs, sometimes up to 10x more sensitive. However, due to spatial distribution of markers of interest, re-titration when moving panels from a standard cytometer to the Imagestream often requires re-titration for optimal staining of samples for image cytometry.

What controls do I need?

Single stained compensation controls are necessary to allow for subtraction of spectral overlap between channels. These can be cells or beads. An unstained control is not necessary unless you wish to acquire

that for your own reference. Positive and negative experimental controls are highly recommended as with any assay to help ascertain biological changes between samples/groups.

Are there any special consideration for sample preparation?

In general, standard flow cytometry protocols should transfer well to sample preparation for the Imagestream with the exception of having to potentially re-titrate antibodies.