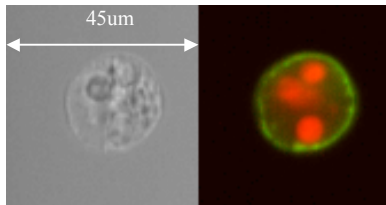


ImageStream[®] Sample Preparation Guide

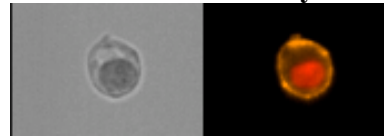
Experimental Design: The ImageStream[™] provides high information content data for individual cells and cell populations within heterogeneous samples. Using morphology and intensity based features the ImageStream provides high content morphological analysis on tens of thousands of cells per sample.

- Choice of Cell Type:** The cell or particle size should be less than 45 microns in diameter (see below for examples).

Apoptotic AnnexinV+ Jurkat



HuPB CD14+ Monocyte



- Choice of Fluorochromes:** Choose 488 nm-excitable fluorochromes. Brightfield imagery may be placed into any unused channel.

Channel 1	Channel 2	Channel 3	Channel 4	Channel 5	Channel 6
470-500nm	400-470nm	500-560nm	560-595nm	595-660nm	660-735nm
488 Scatter	Brightfield	Brightfield	Brightfield	Brightfield	Brightfield
		Fluorescein	PE	7-AAD	PE-Cy5
		AlexaFluor 488	Cy-3	Alexa610/PE	PE-Cy5.5
		GFP	AlexaFluor 546	Propidium Iodide	Alexa680/PE
		Syto Green	AlexaFluor 555	PE -TexasRed	Alexa647/PE
		Spectrum Green	DSRed	ECD	PerCP
		YFP			PerCP-Cy5.5
		Sybr Green			Draq-5

- Controls:** For spectral compensation it is important to have unlabeled cells, and cells labeled with a single color positive control for each fluorochrome used (i.e. FITC only cells, PE only cells, etc.), collected with brightfield off.
- Cell Aggregation:** De-aggregate clumps in the final step by straining the sample through a 70 micron nylon mesh, or by using an anti-clumping buffer.
- Final Sample Concentration and Volume:** Ideally 5×10^7 cells/ml in at least 50 μ l in a 500 μ l siliconized microcentrifuge tube.