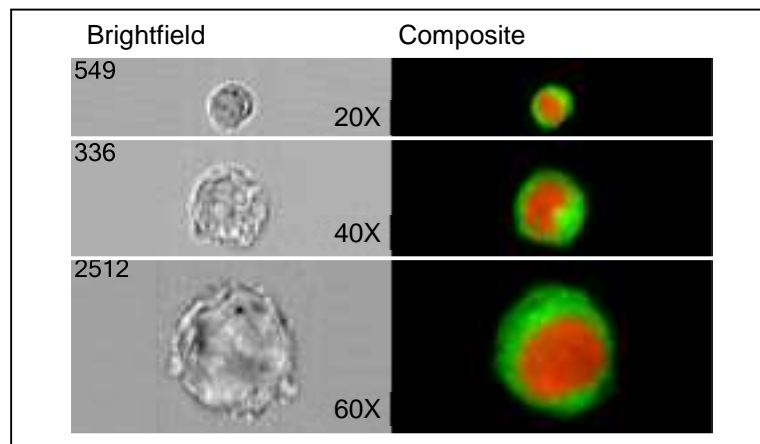


Sample Preparation Guide

Experimental Design: The ImageStream system quantifies the intensity, location, and distribution of fluorescent signals within tens of thousands of cells per sample. It can perform most flow cytometric assays, but the best applications take advantage of the technology's imaging capabilities to discriminate subtle morphologic or signal distribution changes within individual cells and cell populations.

- 1. Choice of Cell Type:** The particle size should be less than 120um using 20x magnification, 60um using 40x, and 40um using 60x. Images below are THP1 cells (~15um diameter) labeled with FITC NFKB and DraQ5 displayed at each magnification.



- 2. Final Sample Concentration and Volume:** At least 1 million cells in 50 uL (2×10^7 cells/ml) in PBS/2%FBS in a 1.5mL siliconized microcentrifuge tube. Will run ~400 cells per second on low speed.
- 3. Protocols:** In general, any established labeling protocol used for flow cytometry will work with the ImageStream (see *Current Protocols in Cytometry* for general labeling techniques). Staining on ice in the presence of 0.1% Na-azide when possible will reduce non-specific capping of antibody. Use siliconized polypropylene tubes when possible.
- 4. Choice of Fluorochromes:** Choose fluorochromes that are excited by the lasers in your ImageStream (405,488,642nm are most common). Use the chart on p.3 or look online for a spectra viewer that will help you plan which dyes will work the best.
- 5. Compensation:** Have a sample of cells each labeled with a single-color for each fluorochrome used (i.e. FITC only cells, PE only cells, etc.).
- 6. Cell Aggregation:** Minimize aggregation problems by straining the sample through a 70um nylon mesh strainer, or by using an anti-clumping buffer such as EDTA or Accumax prior to fixation.
- 7. Fixation:** If fixation is desired, fix cells with 1% PFA on ice for 20 min.
- 8. Number of samples:** No more than 30 total for feasibility experiments. Please limit the samples to the following; Positive and Negative **biologic controls**, **compensation controls**, and **experiment samples**.

Sample Preparation Guide

9. Brightness of Stain and Stain Balancing: Quantifying the location and distribution of signals in an image is a demanding task that requires optimized labeling. Below are a few suggestions to help design the experiment:

- Try to achieve at least a full log shift in fluorescence, as measured by FACS.
- Use the brightest dye for the antigen with the smallest copy number.
- The brightness of probes can be independently controlled by changing the laser power. However, data quality is enhanced when the brightness levels of all probes excited off a single laser are balanced to within a log of each other. Probe balancing avoids the saturation of bright stains when they are combined with dim stains in the same sample.

10. Shipping recommendations:

- **Include the data acquisition form and data that verifies the experiment worked.**
- Samples must be fixed and non-pathogenic.
- Wrap sample tubes in Parafilm.
- Insulate by placing in 15ml conical rack with Styrofoam lid. Pack in Styrofoam lined shipping box with refrigerant packs and paper. For winter delivery, use room temperature packs to prevent freezing of sample. For summer delivery use frozen packs.
- Label outside of box 'Do Not Freeze'.
- Email the package tracking number to your Amnis contact person.

11. International Shipments

To ship non-viable (fixed) material internationally to Amnis, a written statement for US Customs and Border Protection, Department of Homeland Security **must** accompany the shipment. ***This written statement must:***

- Be addressed to; US Customs and Border Protection, Department of Homeland Security.
- Be an original copy on institutional letterhead signed by the laboratory worker responsible for preparing the samples.
- Identify the material and name of the species from which the material was derived.
- state that the animals from which the material was derived:
 - a. have not been exposed to, or inoculated with, any livestock or poultry disease agents exotic to the United States, and
 - b. did not originate from a facility where work with exotic disease agents affecting livestock or avian species is conducted
- State that the material is non-viable
- Be placed in an envelope addressed to 'US Customs and Border Protection, Department of Homeland Security' and attached to the **outside** of the shipping box.

Shipping Address:

MilliporeSigma

Amnis Corporation

645 Elliott Avenue West, Suite 100

Seattle WA 98119

Pn: (855) 240-8045

Fx: (206) 576-6895

Email : applicationsupport@amnis.com

TU www.emdmillipore.com/amnisUT



Sample Preparation Guide

		Excitation Laser (nm)											
Ch	Band (nm)	375***	405	488	561	592	642	730	785	Used	Ch		
1	435-480 (457/45)	375 & 405*** Ch1/Ch9 BF or *DAPI, BV421, AF350, Hoechst, PB	BRIGHTFIELD									1	
2	480-560 (528/65)	*BV510, PacOrange, QD525,		FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, SpectrumGreen, LysoTrackerGreen, MitoTrackerGreen,							2		
3	560-595 (577/35)	*QD565, QD585, eFluor565		PE, PKH26, Cy3, DSRed, CellMask/CellTracker/SY TOX Orange	PE, AF546, DyLight550, PKH26, DSRed, Cy3, SpctmOrange						3		
4	595-642 (610/30)	Ch4/Ch10 BF or *QD625, eFluor625, BV605		Ch4/Ch10 BF or PE- TexRed*, ECD*, PE- AF610*, RFP, QD625*, eFluor625*	Ch4/Ch10 BF or AF568*, DyLight594*, PE-TxRed*, ECD*, PE- AF610*, RFP, mCherry*						4		
5	642-745 (702/85)	*QD705, eFluor700, BV711		PE-Cy5*, PE-AF647*, 7AAD*, PI* PerCP*, PerCP-Cy5.5*, eFluor650*, FuraRedlo, Draq5*, LDS751*,	PE-Cy5*, PE- AF647*, 7AAD*, Draq5*, LDS751*,						5		
6	745-785 (765/40)	*QD800, BV786		PE-Cy7*, PE-AF750*,	PE-Cy7*, PE- AF750*,				SSC		6		
7	435-505 (457/45)	375 No 405*** *DAPI, BV421, AF405, Hoechst, PacBlue, CascadeBlue, eFluor450, DyLight405, CFP, LIVE/DEAD Violet AF350									7		
8	505-570 (537/65)	*BV510, PacOrange, CascadeYellow, AF430,	*BV510, PacOrange, CascadeYellow, AF430, eFluor525, QD525								8		
9	570-595 (582/25)	Ch1/Ch9 BF or *QD565, QD585	Ch1/Ch9 BF or *QD565, QD585, eFluor565	BRIGHTFIELD									9
10	595-642 (610/30)	Ch4/Ch10 BF or *QD625, eFluor625, BV605	Ch4/Ch10 BF or *QD625, eFluor625, BV605		Ch4/Ch10 BF or TexRed*, AF594*, DyLight594*, mCherry*						10		
11	642-745 (702/85)	*QD705, eFluor700, BV711	*QD705, eFluor700, BV711		APC, AF647, AF660, Cy5, DyLight649, DRAQ5*	AF647, AF660, AF680, APC, Cy5, DyLight649, DyLight680, Draq5* PE- AF647*, PE-Cy5*, PerCP*, PerCP-Cy5.5*					11		
12	745-785 (765/40)	*QD800, BV786	*QD800, BV786		APC-Cy7, APC-AF750, APC-H7, APC- eFluor750	APC-Cy7, APC-AF750, APC-H7, APC- eFluor750, Cy7, AF750, DyLight750, PE-Cy7*, PE-AF750*	AF750, Cy7, DyLight750	SSC			12		

Recommended dyes (based on optimal excitation and detection channels) are in boldface.

*Many dyes will excite by more than one laser, and this can increase cross camera compensation.

**Channel bandpass may change depending on which lasers are on. Values listed are assuming 405,488, and 642 excitation.

***375 laser is aligned to Ch1 if the system also has a 405 laser, if not its aligned to Ch7. In cases where Ch1 is used for 375 excited dyes brightfield should be placed in Ch4 and Ch10.

1 laser (488): ideal dyes are AF488, PE, PE-TxRed, PE-Cy5, PE Cy7, SSC-Ch12,

2 laser (488,642): ideal dyes are AF488, PE, PE-TxRed, SSC-Ch6, and AF647, APC Cy7

3 laser (405,488,642): ideal dyes are AF488, PE, PE-TxRed, SSC-Ch6, and DAPI (or BV421), AF647, APC Cy7