THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

AMNIS IMAGESTREAM X INFORMATION AND GUIDELINES

INSTRUMENT INFORMATION

- The ImageStream (Amnis) produces multiple high-resolution images of every cell directly in flow, including bright field and dark field (SSC).
- This instrument has 12 Imaging Channels and 2 CCD camera, which are capable of taking twelve images per cell. It also has a filter wheel, inline filters, and spectral decomposition assembly which support in taking good quality image with corrected compensation.
- There are 4 Lasers, Violet-405, Blue-488, Yellow/Green-561 and Red-640nm. 10 fluorescent channels to choose from and 2 channels are used for bright field. For more detail on choosing a colors, please see Table 1 below.
- The Amnis has 3 objective, *20X, 40X and 60X and* 3 different speeds of acquisition *Low, Medium and High.* It can analyze up to 5,000 events/sec (High Speed).
- The cell/particle size should be less than 45 microns in diameter and your samples should be filtered through a 70 micron mesh filter before acquisition.
- In general, any established labeling protocol used for flow cytometry will work with the ImageStream. Stain cells on ice in the presence of azide when possible to reduce nonspecific capping of antibody. The major difference is the much higher sample concentration recommended.
- Use polypropylene or siliconized tubes to process samples to avoid cell loss.
- EDF™ technology dramatically extends the depth of field, enabling new applications and improving the performance of existing applications – especially spot counting applications such as FISH, autophagy, and nuclear translocation.

EXPERIMENT PLANNING GUIDELINES

- Data quality is significantly better when the reagents excited with the same laser are titrated such that the brightness levels of all probes are balanced to within a log of each other.
- **Controls:** It is important to have unlabeled cells (unstained has to be cells, not beads). Positive and negative biological controls.
- **Compensation Controls:** Cells or comp beads labeled with a single-color positive control for each fluorochrome used (i.e. FITC only, PE only).
- **Number of Samples:** • Positive biologic control • Negative biologic control • Experimental samples • Single color and unlabeled controls in separate tubes

Note: It takes approximately 4 minutes to process a single sample and longer if the sample is below the recommended concentration or the target cell population is less.

- **Final Sample Concentration and Volume**: At least 1 million cells in 50µL ($2x10^7$ cells/ml) in PBS/2%FBS in a 1.5mL siliconized micro-centrifuge tube. Final volume should be in between 25uL to 50uL. Lower concentrations increase data collection time a great deal.

- **Sample Acquisition:** Preferably micro-centrifuge tubes or 96 well plates. If you plan to bring 96 well plate, bring all single color and comp controls in tubes.

- **Cell Aggregation:** We strongly advise removing clumps by straining the sample through a 70 micron nylon mesh strainer. If sample aggregation is a problem, we suggest using an anti-clumping buffer such as 0.5M EDTA in the final suspension buffer.

- **Fixation:** If fixation is desired, fix cells using 1% PFA on ice for 20 minutes.

- **Application:** The ImageStreamX Mark II is designed to be a general-purpose platform for cellular studies. The ImageStreamX Mark II utilizes the same dyes and markers employed in microscopy and flow cytometry and can perform virtually any standard flow cytometry assay with the added value of visual confirmation.

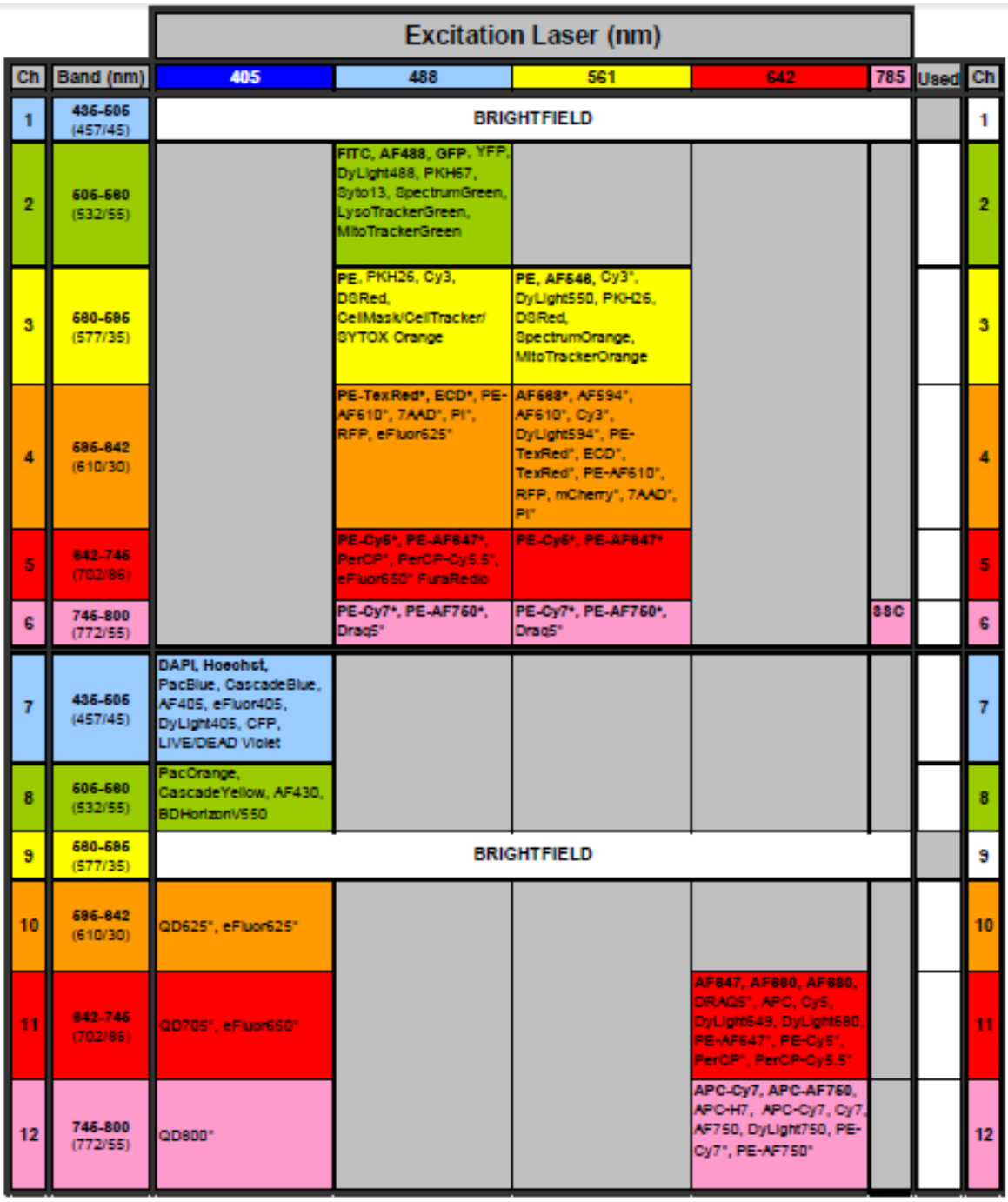
  Novel Applications include: Translocation, Co-localization, Cell classification, Cell cycle & Apoptosis.

- **Important Note:** We allocate at least a half day for experiment setup and data acquisition, especially for the first time, Therefore, conforming to the above sample guidelines is very crucial. Please consult with the Flow Core staff while planning an experiment and before purchasing reagents. Plan to do at least one pilot study to confirm reagent concentrations and brightness of probes.

Choice of fluorochrome & Brightness of Stain and Stain Balancing:

- Choose traditional cytometry/imaging fluorochromes.
- Try to achieve at least a full log shift in fluorescence, as measured by FACS (titration of antibodies).
- 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.



Ch	Band (nm)	Excitation Laser (nm) 405	488	561	642	785	Used	Ch
1	**436-505** (457/45)	**BRIGHTFIELD**						1
2	**505-550** (532/55)		**FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, SpectrumGreen, LysoTrackerGreen, MitoTrackerGreen**					2
3	**550-585** (577/35)		**PE, PKH26, Cy3, DSRed, CellMask/CellTracker/ SYTOX Orange**	**PE, AF546, Cy3*, DyLight550, PKH26, DSRed, SpectrumOrange, MitoTrackerOrange**				3
4	**585-642** (610/30)		**PE-TexRed*, ECD*, PE-AF610*, 7AAD*, PI*, RFP, eFluor625***	**AF568*, AF594*, AF610*, Cy3*, DyLight594*, PE-TexRed*, ECD*, TexRed*, PE-AF610*, RFP, mCherry*, 7AAD*, PI***				4
5	**642-746** (702/86)		**PE-Cy5*, PE-AF647*, PerCP*, PerCP-Cy5.5*, eFluor650* FuraRedlo**	**PE-Cy5*, PE-AF647***				5
6	**746-800** (772/55)		**PE-Cy7*, PE-AF750*, Draq5***	**PE-Cy7*, PE-AF750*, Draq5***		**SSC**		6
7	**436-505** (457/45)	**DAPI, Hoechst, PacBlue, CascadeBlue, AF405, eFluor405, DyLight405, CFP, LIVE/DEAD Violet**						7
8	**505-550** (532/55)	**PacOrange, CascadeYellow, AF430, BDHorizonV550**						8
9	**550-585** (577/35)	**BRIGHTFIELD**						9
10	**585-642** (610/30)	**QD625*, eFluor625***						10
11	**642-746** (702/86)	**QD705*, eFluor650***			**AF647, AF680, AF680, DRAQ5*, APC, Cy5, DyLight649, DyLight680, PE-AF647*, PE-Cy5*, PerCP*, PerCP-Cy5.5***			11
12	**746-800** (772/55)	**QD800***			**APC-Cy7, APC-AF750, APC-H7, APC-Cy7, Cy7, AF750, DyLight750, PE-Cy7*, PE-AF750***			12

Recommended dyes (based on optimal excitation and detection channels) are in boldface.
1 laser (488) system: Ideal dyes are AF488, PE, PE-TxRed, PE-Cy5, SSC-Ch6,
2 laser (488,642) system: Ideal dyes are AF488, PE, PE-TxRed, SSC-Ch6, and AF647, APC Cy7
3 laser (405,488,642) system: Ideal dyes are AF488, PE, PE-TxRed, SSC-Ch6, and DAPI, AF647, APC Cy7
Band-pass filter values assume 3laser configuration.

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RELATED PROCEDURES

This handout is related to ACSF SOP IN009: Amnis ImageStream^X Use and Maintenance. Please see the full SOP for further information.