Multicolor Flow Cytometry

Overview

Antigen Density and Fluorochrome Selection

Fluorochromes vary with respect to the signal intensity they provide for each binding site. In general, use the brightest fluorochrome with the most sparsely-expressed target antigen. This typically means using Phycoerythrin for the least abundant target in an assay. Target antigens whose expression is discontinuous, such as activation markers and cytokines, also fit into this category. In these cases, a dim fluorochrome may readily detect the antigen on cells that express it at a high level; but cells with low levels of expression may be indistinguishable from the negative population. Another approach to amplify staining for dimly-expressed antigens is the use of indirect (2-3 step) staining protocols which utilize purified and/or biotinylated antibodies in conjunction with Avidin/Streptavidin-fluorochrome conjugated detection system. However, for most multi-color analysis, it is generally most convenient to use directly fluorochrome conjugated antibodies.

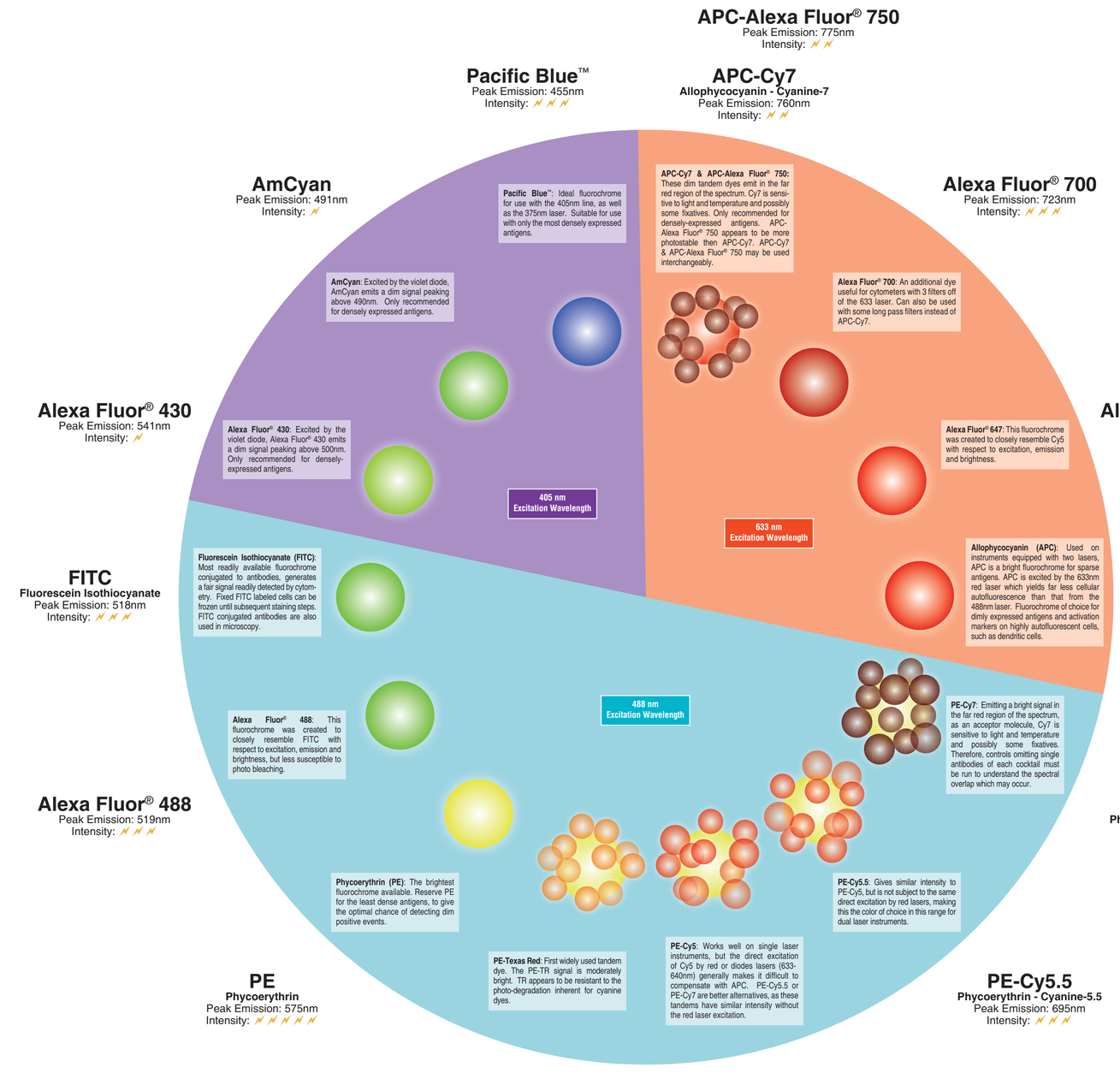
Tandem Dyes

The need for additional fluorochromes without adding extra lasers to flow cytometers led to the creation of tandem dyes. With tandem dyes, two fluorochromes are coupled together, with one fluorochrome being excited by the light source, and the second one excited by the emission of the first and eventually emitting at longer wavelengths. While this offers the user a wider selection of probes, several caveats arise with specific combinations. First, as is recommended for any other multi-color flow cytometry experiments, running single-color stained compensation controls with each individual conjugate should provide useful information on the intensity of each probe. Second, those tandem dyes which use cyanine dyes as acceptors are photo-labile and must be used with this in mind. Most tandem dyes will emit some signal from the donor molecule, and upon exposure to light the photo-labile cyanine molecules will become photo-bleached, leaving only the donor fluorochrome to fluoresce. This means compensation can vary from assay to assay if proper storage and handling conditions for these tandem dyes are not followed. Since there are usually differences between flow cytometers, it is also important to ensure that the cytometers used are equipped with proper filter sets.

Fluorochromes

eBioscience Prefix/Cat. No.	Fluorochrome	Emission Max (nm)
11-	FITC	518
12-	PE	575
Please Inquire	PE-Texas Red®	615
15-	PE-Cy5	670
35-	PE-Cy5.5	690
25-	PE-Cy7	760
	PerCP	675
	Propidium Iodide	617
00-6993-50	7-AAD	647
Please Inquire	Texas Red®	615
17-	APC	660
19-	Cy5	670
30-	APC-Cy5.5	690
10-	APC-Cy7	760
27-	APC-Alexa Fluor® 750	775
	Rhodamine Green™	527
	Rhodamine Red™	590
	TO-PRO 3	660
Please Inquire	Alexa Fluor® 350	442
52-	Alexa Fluor® 405	421
Please Inquire	Alexa Fluor® 430	541
53-	Alexa Fluor® 488	519
51-	Alexa Fluor® 647	668
56-	Alexa Fluor® 700	723
Please Inquire	Cascade Blue®	420
Please Inquire	Cascade Yellow™	545
	Marina Blue®	460
57-	Pacific Blue™	455
	Pacific Orange®	551
	AmCyan	491

Updated July 2006
Designed by Steven Lee

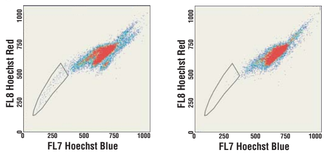


Best Selling Cell Sorter & Analyzer in Japan... Now Available Worldwide!

Easy and Reliable Sorting:
- Automatic droplet control
- Droplet monitoring for optimal droplet formation adjustment
- Automatic protection of sample
- Sorting speed of up to 10,000 cells per second

Flexible Configurations for Your Research Applications:
- 375nm (UV), 405nm (Violet), 488nm (Blue), 638nm (Red) lasers available
- Fixed and pre-optimized laser alignment
- 6-8 colors capability, easily upgraded
- Compact size (950mm x 660mm x 620mm)
- Easy access and cleaning of flow cell

SP Sorting and Analysis on the JSAN™:



Characterization of Hoechst 33342 fluorescence on whole mouse bone marrow and inhibition of mdr activity with Reserpine. The boxed region is the stem cell (SP) population.
***Left**: The stem cell (SP) can be observed in the box.*
***Right**: The stem cell (SP) population was specifically eliminated in the presence of Reserpine.*

Laser, Filter and Fluorochrome Configuration Example:

488nm Excitation (DPSS laser); Output Power: 20mW

Detector	Filter Type	Fluorochromes
FL1	535DF45	FITC, GFP, Alexa Fluor® 488, Bodipy, Fluo-3
FL2	580DF30	R-Phycoerythrin (PE), Propidium Iodide (PI)
FL3	670DF40	PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5, PI, PE-Texas Red®, 7-AAD
FL4	748ALP	PE-Cy7, PE-Alexa Fluor® 750

638nm Excitation (Diode Laser); Output Power: 12mW

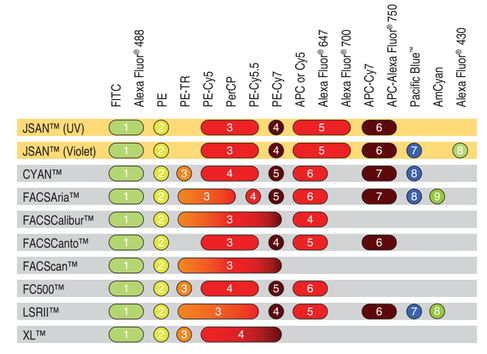
Detector	Filter Type	Fluorochromes
FL5	695AF55	Allophycocyanin (APC), Cy5, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 700
FL6	748ALP	APC-Cy7, APC-Alexa Fluor® 750

375nm Excitation (Diode Laser); Output Power: 8mW

Detector	Filter Type	Fluorochromes
FL7	424DF44	Hoechst33342-Blue, DAPI
FL8	590DF35 or 695AF55	Hoechst33342-Red

405nm Excitation (Diode Laser); Output Power: 50mW

Detector	Filter Type	Fluorochromes
FL7	450DF50	Pacific Blue™, Cascade Blue®, Alexa Fluor® 405
FL8	550DF30	Pacific Orange®



Instruments and Fluorochrome Compatiblity

For each instrument, the corresponding channel which detects each specific fluorochrome is indicated. Where more than one fluorochrome is marked, any of these may be used in the indicated detector. Verify which lasers and detectors are on your instrument, as not all are standard hardware.