Carboxyfluorescein Diacetate

Carboxyfluorescein Diacetate (CFSE) can be used to track asynchronous cell division. Cell division results in sequential halving of the initial fluorescence, producing a cellular fluorescence histogram. CFSE has a green emission that is collected in the FL1 detector of a FACSCalibur.

Cy3 and Cy5

Cy3 and Cy5 are excited by the 488-nanometer line of an argon laser and the 633-nanometer line of a helium-neon diode or laser, respectively. These conjugates can be used in flow cytometry but typically do not give the fluorescence intensity comparable to that of PE or APC. Applications where a smaller dye is required are more appropriate for these dyes. These fluorochromes are well suited for fluorescent microscopy.

Fluorescein Isothiocyanate

Fluorescein isothiocyanate (FITC) is currently the most commonly used fluorescent dye for flow cytometry analysis. When excited at 488 nanometers, FITC has a green emission that is usually collected at 530 nanometers, the FL1 detector of a FACSCalibur. FITC has a high quantum yield (efficiency of energy transfer from absorption to emission fluorescence) and approximately half of the absorbed photons are emitted as fluorescent light. FITC is seldom used for fluorescent microscopy applications as it photobleaches rather quickly although in flow cytometry applications, its photobleaching effects are not observed due to a very brief interaction at the laser intercept. FITC is highly sensitive to pH extremes.

Fluorescein Proteins

Fluorescein Proteins (see eGFP and eYFP listed in this section, as well as the references listed in the **Protocols & Useful Links** section).

Green Fluorescent Protein

Green Fluorescent Protein (eGFP) can be excited at 488 nanometers with a peak emission at 509 nanometers and is detected in the FL1 detector on the FACSCalibur. The FACSCanto and LSRII and all of the resource's sorter flow cytometers are able to distinguish between concurrently expressing eGFP and eYFP cells when the proper optical filters and experimental controls exist. More detailed discussion of this molecule can be found in the references listed in the **Protocols & Useful Links** section.

Peridinin Chlorophyll Protein

Peridinin Chlorophyll Protein (PerCP) has a 677-nanometer maximum emission, red, when excited at 488 nanometers and is detected on the FL3 detector of a FACSCalibur. A PerCP tandem dye is also available (PerCP-Cy5.5, also written Cy5.5-PerCP). PerCP is not suited for the high-powered laser (>150mW) applications, such as on a jet-in-air sorter like a MoFlo, due to its photobleaching characteristics.

Phycoerythrin

Phycoerythrin (PE or R-PE) has a huge absorption coefficient and almost perfect quantum efficiency. *In vivo*, it functions to transfer light energy to chlorophyll during photosynthesis. It is one of the brightest dyes used today and emits in the yellow/orange at about 570 nanometers. Those accustomed to fluorescent microscopy may not be familiar with this fluorochrome as it photobleaches rather quickly under a microscope.

Phycoerythrin-Cy5

Phycoerythrin-Cy5 (PE-Cy5, also written Cy5-PE) is a tandem conjugate where PE is coupled to the cyan dye, Cy5. When excited by 488-nanometer light, the excited fluorochrome (PE) is able to transfer its fluorescent energy to the cyanine molecule, which then fluoresces at a longer wavelength in the red range at 670 nanometers. This tandem dye is known by a confusing myriad of names that including Beckman Coulter's PC5. Other PE conjugates exist, e.g., PE-Cy5.5 and PE-Cy7, that

will not be discussed in this introductory fluorophore section. It is recommended that special precautions be taken with this conjugate, and cells stained with them, to protect the fluorochrome from long-term exposure to visible light.

Phycoerythrin-Texas Red

Phycoerythrin-Texas Red (PE-Texas Red, also written Texas Red-PE) is a tandem conjugate where PE is coupled to Texas Red dye. Similar to other tandem conjugates, when excited by 488-nanometer light, the excited fluorochrome (PE) is able to transfer its fluorescent energy to the Texas Red molecule, which then fluoresces at a longer wavelength with a peak in the orange range at 612 nanometers. This tandem is also known by other names such as ECD (Electron Coupled Dye). PE-Texas Red conjugates run on a FACSCalibur will result in dull expression due to the extant optical filters. This is not observed on the other cytometers in the facility shared resource when they are equipped with the appropriate optical filters for this conjugate. There is considerable overlap of emission when running PE and PE-Texas Red specimens.

Propidium Iodide

Propidium Iodide (PI) is a membrane-impermeant dye that stains by nondiscriminately intercalating into every 4th or 5th nucleic acid base pair, binding both DNA and RNA. Once bound, PI undergoes a conformational change and becomes ~40 times brighter. PI has a broad emission spectrum with a peak in the orange range at 620 nanometers. A number of assays employ, alcohol-fixed, RNAse-treated, PI stained cells or nuclei with altered DNA content to determine cell cycle compartment percentages. Propidium iodide has also been employed for many years as a marker for viability as the disrupted membranes of dead cells allow the dye to pass freely to the nucleic acids. However, this dye is very sticky; it will stick to sample tubes and, given sufficient time exposure to living cells, living cells will appear to be propidium iodide positive. Given this dye's broad emission spectrum and its sticky properties, contemporary flow cytometry labs have replaced propidium iodide with

other nucleic acid dyes, e.g., 7-AAD (listed below), TO-PRO-3 iodide, or DAPI, among many others, for viability measurements, although propidium iodide remains the most commonly used dye for DNA content analysis.

Texas Red

Texas Red has an excitation maximum in the yellow-orange range of the color spectrum. It can best be detected using a cytometer in the facility that is equipped with a yellow laser.

Yellow Fluorescent Protein

Yellow Fluorescent Protein (eYFP), a yellow-shifted variant of the eGFP molecule, is also excited at 488 nanometers with a peak emission at 535 nanometers and is also detected in the FL1 detector on a FACSCalibur, However, the FACSCanto, the LSRII and the FACSAria IIu are able to distinguish between concurrently expressing eGFP and eYFP cells if the appropriate optical filters and experimental controls exist. More detailed discussion of this eyfp can be found in the references listed in the **Protocols & Useful Links** section.