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CORRELATIVE FLUORESCENCE IN MICROSCOPY AND FLOW CYTOMETRY: ADVANCES IN INSTRUMENTATION AND COMPENSATION

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As we develop a better understanding of correlative and comparative studies in flow cytometry and laser microscopies, new changes in instrumentation and advances in software change our technical environment. There is a divergence in how fluorescence instrumentation is treated in the different situations, however. Flow cytometry continues to add multiple detectors and separation of signal parameters [1]. Confocal microscopy continues to focus on visual separation with less attention to quantitative documentation. This divergence makes correlative observations more difficult, and requires our attention to details of precision. A review of filter options in flow cytometry shows how signal precision can be achieved [2].

The scientific and educational traditions in microscopy, fluorescence and flow cytometry differ by company, country and instrumentation. The development of flow cytometry as a quantitative analytical method initially placed great emphasis on spectral separation and compensation in order to gain a better understanding of individual fluorescence points [2]. Scanning laser microscopies paid attention to constructive obstacles to color images with much less attention to issues of spectral overlap and compensation as long as the visual construct was sufficient to provide an imaging separation. The methods sections of papers and reports often omit both instrument standardization and filter settings, making any comparison between projects difficult at best [3]. Figure 1 shows a typical dichroic filter set in multiparameter flow cytometry [4]. Similarly Figure 2 shows typical filter separations in multiparameter flow cytometry [4] as a demonstration of possible combinations.

The emerging technology of Evanescent Field Microscopy has developed around the traditional optical bench, but changes in software are expected to emerge soon. These trends have continued. The emergence of multiparameter flow cytometry makes a reexamination of spectral separation an important issue in microscopy [3]. The filter sets necessary for these separations should be reviewed more often and included in the methods of published papers and reports.

In recent years, in flow cytometry, 9, 10 and 11 color measurements are much more feasible, making 11, 12 and 13 parameter measurements possible [3]. It is not only reasonable but also essential to include multiparameter evaluations in order to separate the several sub-sets of lymphocytes and other cells. Multiparameter cytometry is required to delineate these sets.

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TABLE 1 DICHROIC FILTERS IN MULTIPARAMETER FLOW CYTOMETRY: TABLE OF TYPICAL DICHROIC SEPARATIONS IN FLOW CYTOMETRY

DICHROIC PASS FILTERS	SOME EXAMPLES OF 3-LASER SEPARATIONS POSSIBLE USES OF DICROIC SEPARATION
485 LP	Separation of Cascade Blue from Cascade Yellow.
560 LP	Separation of FITC from PE. A long-time standard.
600 LP	Separation of PE-Texas Red from FITC and PE.
640 SP	Separation of Pe-Cy5 from FITC, PE and PE-TEXAS RED.
685 LP	Separation of APC from APC-Cy5.5 and APC-Cy7.
685 SP	Separation of PE-Cy5.5 from PE-Cy5.5.
740 LP	Separation of PE-Cy5.5 from PE-Cy7, and APC-Cy5.5 from APC-Cy7.

TABLE 2 TYPICAL FILTERS IN MULTIPARAMETER FLOW CYTOMETRY: TABLE OF POSSIBLE FILTER SETS AND THEIR USE

FILTER	FILTER USE FOR TYPICAL FLUOROCHROME SETS IN FLOW CYTOMETRY IN A THREE LASER SYSTEM WITH A VIOLET OPTION
440/10	Cascade Blue, Pacific Blue. Narrow range with Violet lasers.
440/40	Hoechst 33258 and Hoechst 33342, Cascade Blue, Pacific Blue.
463/50	Alternate for Cascade Blue, Pacific Blue with a 488 Notch Filter.
488/No	Several reports recommend a Notch Filter to exclude 488 laser light.
530/30	FITC, Alexa Fluor 488 A long-time standard.
545/80	Cascade Yellow, ELF-97, YFP (Yellow Fluorescent Protein).
575/42	PE, PI (A narrow range standard).
610/20	PE-Texas Red, DsRed.
660/20	APC, Alexa Fluor 633.
667/30	PE-Cy5, Cy5, Alexa Fluor 647.
710/50	PE-Cy5.5, APC-Cy5.5, PE-Alexa Fluor 680, APC-Alexa Fluor 680, APC-Alexa Fluor 700.
780/60	PE-Cy7, APC-Cy7, PE-Alexa Fluor 750, APC-Alexa Fluor 750.

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