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Multispectral Flow Cytometry: Next Generation Tools For Automated Classification

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Flow cytometry has moved from a relatively simple technology 30 years ago, to a very sophisticated and high-speed detection technology today. However, the number of simultaneous fluorescence dyes that can be separated is limited by the difficulty in overlapping spectra and the complexity of resolving this spectral overlap problem. High-speed multianode PMTs may change this situation. The system we propose utilizes such a technology to allow full spectral analysis of cells and particles as they flow past the light source. Making these measurements is very complex and the necessity for advanced spectral overlap calculations creates a number of difficult problems to solve in a very short period of time. Next-generation instruments can either increase the number of detectors or modify the principles of collection. If the detector system were simplified, the overall cost and complexity of single-cell analytical systems might be reduced. This requires changes in both hardware and software that allow for the analysis of 30 or more spectral signals. Analysis of complex data sets requires some completely new analytical approaches, particularly in the area of multispectral analysis. This presentation discusses a next-generation instrument, which can collect simultaneously 32 bands of fluorescence from a particle in less than 5 microseconds. This opens new opportunities for analysis of bioparticles in a very fast and high content fashion.

New developments in flow cytometry technology and a reawakening of interest in automated classification may rekindle flow cytometry technology as a killer application in the next decade. No other technology can analyze 3 million cells a minute with every single cell getting identical attention and carefully standardized measurements. Flow cytometry is a mature technology that is used in many laboratories. 2005 is the 40th anniversary of the invention of the cell sorter by Mack Fulwyler in 1965. For 40 years flow cytometry has had a track record of being the most accurate and well-defined technology for measuring properties of single cells. Nonetheless, it is poised to accept the drug discovery challenge –a challenge currently being driven by the new field of Cytomics, which demands both high-content screening and high-throughput capabilities. With new lasers and detectors in the marketplace, there are clearly many opportunities for taking flow cytometry to the next level.

Early flow cytometers could measure only light scatter and Coulter volume. Shortly after there was a minimal ability to measure fluorescence. Although the most desirable direction in the early days of cytometry was advanced image analysis, the lack of any substantial computational power from the 1950s to 1980s made the analytical task very difficult indeed. The technology was initially driven from the perspective of image analysis because of the interest in discovering the differences between normal and cancerous cells. Because of the initial difficulty in designing functional image-based systems, Kamentsky and others focused on flow-based systems that were functional predecessors of current-day instruments [1,2]. As noted earlier, others had also been going in this direction, including Mack Fulwyler, who had observed the developments of Richard Sweet in 1963-1965 in his design of the high-speed inkjet printer [3]. It was a direct interaction with Richard Sweet that drove Fulwyler to design his first electrostatic-based cell sorter[4] in 1965. [4]

Today, however, the technology of flow cytometry is quite standard and the instrument is an essential workhorse in the fields of immunology and cell biology. The real problem facing us is how do we advance to a really essential change in technology when the current operational models are so ingrained in research and clinical laboratories? The answer is that we need to make some fundamental changes in photon-collection systems and simultaneously advance the state of multivariate data analysis to provide serious classification systems.

Flow cytometry is a technique whereby every single particle or cell in a suspension can be individually analyzed for its optical characteristics very rapidly. As the particles or cells pass through a liquid-handling system, they are organized into a single file via hydrodynamic forces within the flow chamber. Once this hydrodynamic focusing has been accomplished, the particles/cells usually pass through a very narrow beam of intense laser light, during which time a large number of variables such as light scatter and spectral signatures are collected (*see Figure 1*).

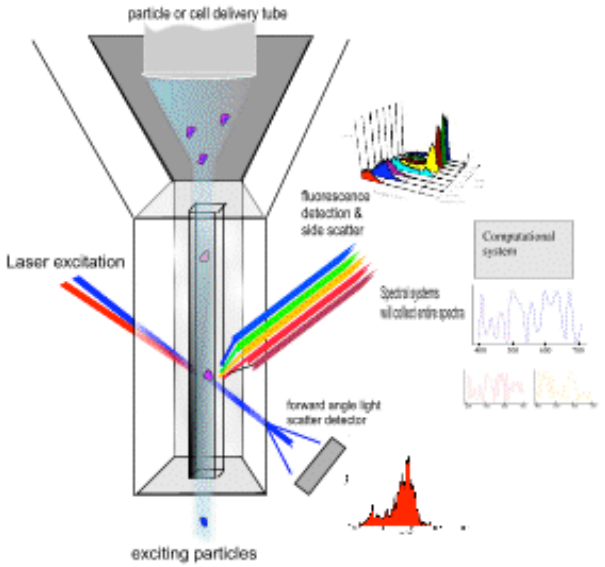

Figure 1: The flow chamber, through which suspended cells are driven through a laser beam, during which time the fluorochromes on the cells or particles are excited and emit fluorescence. Next generation instruments will collect entire spectral bands.

A fluorescence spectral signature can be collected from each cell. This signature can be multiple bands of a subset of wavelengths, depending on the excitation sources and the type and number of fluorochromes used. Application of advanced multivariate software allows the identification of population information from these cells. This also allows the possibility that complex mixtures of cells can be electronically separated into distinct populations with ease. Further, if required, more advanced flow cytometers called cell sorters can physically separate individual cells either by depositing a cell of interest into a single well, or by collecting entire purified populations if desired. This powerful capability makes flow cytometry a unique and indispensable technology in the world of advanced cell biology, with a significant change in instrument capabilities as we approach automated classification and better alignment. This presentation will outline the innovations that will be seen in the next decade of technology advances.

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