Compensation-Free Flow Cytometry Based on Fluorescence Lifetime

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Background. The emergence of technologies such as mass cytometry and spectral flow cytometry, as well as the recent development of bright polymer-based tandem dyes with large Stokes shifts, is evidence for the continued need for greater multiparameter capabilities in flow cytometry (FC). However, mass cytometry destroys the cells during analysis and therefore cannot be used for sorting; and spectral unmixing requires complex and expensive optics. Both have lower sensitivity than traditional FC. Even with new dyes, current FC approaches to multiplexing suffer from compensation issues, causing complex workflows and lack of reproducibility.

Methods. We have developed multiparametric instrumentation that addresses the issues of spectral spillover and compensation to achieve increased multiplexing in FC. Based on high-speed, direct time-domain capture of fluorescence decays following pulsed excitation, our *Arno* platform is capable of distinguishing spectrally overlapping fluorophore emissions based solely on fluorescence lifetime, an intrinsic property of fluorophores. By partitioning fluorophores into "bins" with broad lifetime characteristics (e.g.: $\leq$ 10 ns, 15–30 ns, etc.), our approach can rapidly determine the contribution of each fluorophore to the observed emission decays. Each spectral detection channel, therefore, can be used for multiple fluorescent parameters, multiplying the number of labels available for simultaneous cell tagging.

Results. The *Arno* platform recently performed a common 4-color benchmark assay for HIV status monitoring (CD3 / CD4 / CD8 / CD45) using a single laser (405 nm) and only two fluorescence detectors (collecting light in bands of 420–520 nm and $\geq$ 560 nm), successfully reproducing results obtained on a traditional 4-color FC. The data gathered on our system needed no spectral compensation whatsoever, due to patented innovation in optical architecture, signal processing, and assay design. Our system delivered pulsed excitation to the samples (4-ns pulses at 12.5 MHz), digitized fluorescence decays at high speed (2GS/s/ch), and processed the resulting data to automatically extract contributions from two lifetime bins in each detection channel.

Conclusion. Our proof-of-concept platform demonstrates the validity of our lifetime-based approach to multiplexing. We are currently expanding our platform capabilities to build a compensation-free 14-parameter instrument ("No-Comp") that uses only 2 lasers and 6 fluorescence detectors (plus FSC and SSC). Planned platform extensions include a High-Multiplexing compensated version ("Hi-Mux") capable of collecting 27 parameters using only 2 lasers and 15 detectors.