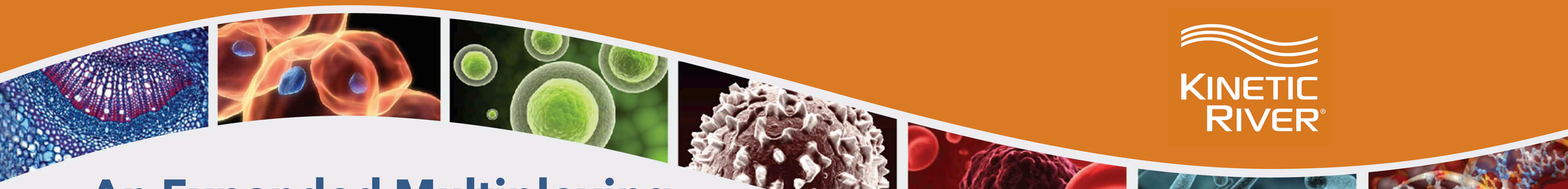

An Expanded Multiplexing Flow Cytometry Platform

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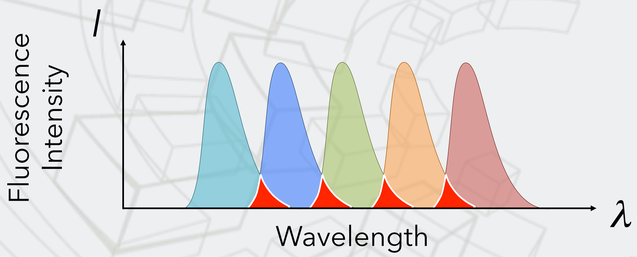
BACKGROUND

Spectral spillover of fluorescence channels, or spectral crosstalk, is a longstanding issue in flow cytometry. The limited window of wavelengths in the visible spectrum (about 350 nm), combined with the typical bandwidth of fluorophore emission spectra (30 to 80+ nm) and their long tails, forces a zero-sum trade-off: Either reduce the number of fluorescence detection channels, or tolerate a significant amount of cross-channel spillover, with the attendant compensation headaches. For those applications requiring a high degree of multiplexing, such as, *e.g.*, immunophenotyping, this trade-off is a significant constraint.



METHODS

Time-resolved cytometry offers the opportunity to expand multiplexing capabilities by exploiting the additional parameter of time, separately from wavelength. By resolving individual light-cell interactions on a sufficiently short time scale, the decay of fluorescence can be measured, creating an entirely new family of multiplexing options. We have designed and built a proof-of-concept prototype flow cytometer platform based on this principle.

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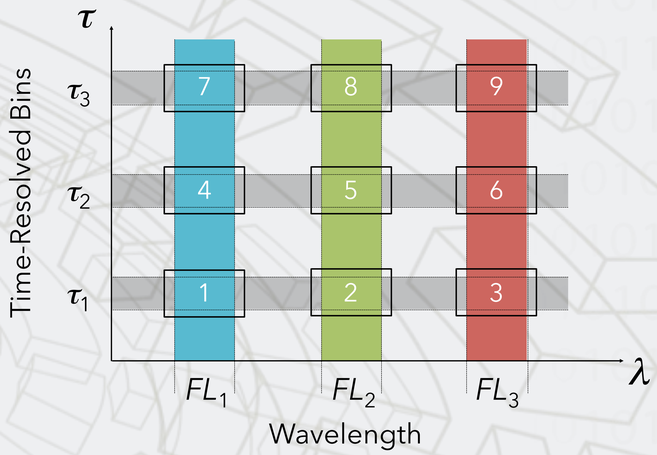
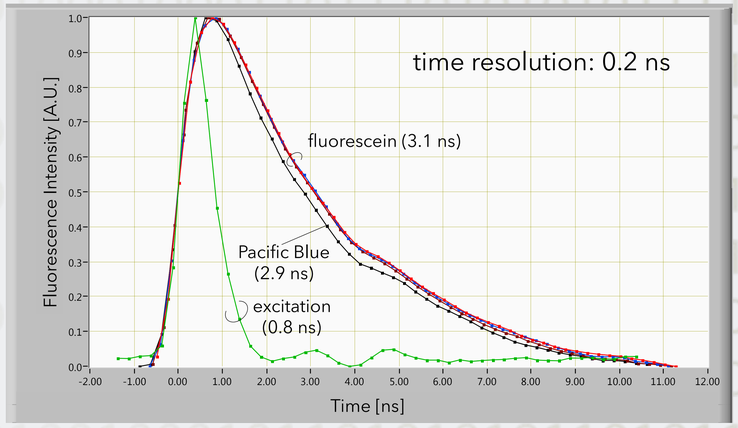
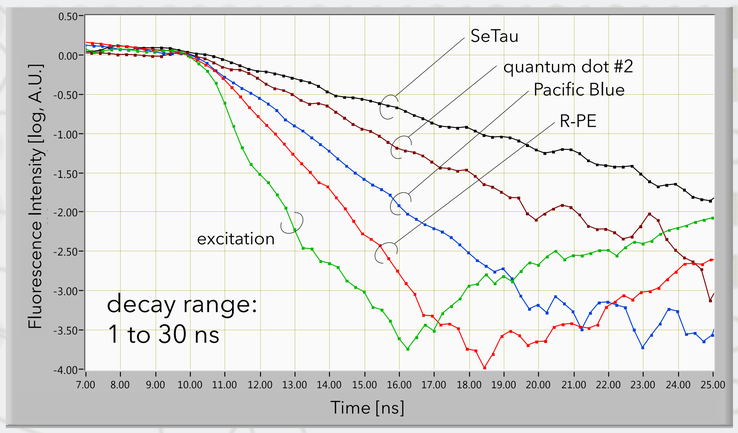
By exciting particles in the sample with sufficiently short pulses of 405-nm light (sub-picosecond, average power 30 mW; and 14 ns, average power 94 mW), and measuring the decay of fluorescence with adequate detection speed (detector/amplifier bandwidths >1 GHz and digitization rates up to 4 GS/s), we measured the fluorescence decays of different fluorophores using the same optical detection channel.



RESULTS

The system was characterized by measuring time-resolved fluorescence decays of 10 different fluorophores and SRPs under stationary and flow conditions. The impulse response function of our detection system under picosecond excitation was approx. 800 ps; under nanosecond excitation, the lower limit of detection for decay (1/e value) was 0.6 ns. We achieved a fluorescence decay reproducibility of +/- 0.1 ns and a decay resolution of 0.2 ns. Titration series of two fluorophores with different decays were measured on a single detection channel, resulting in the simultaneous resolution of emission from two spectrally overlapping fluorophores solely based on time-resolved analysis.





CONCLUSION

Our approach to time-resolved cytometry can expand multiplexing beyond current limits. By using time-resolved analysis, one can double or triple the number of simultaneous fluorescent labels that can be detected on a single cell. This approach preserves accepted workflows, protocols, and infrastructures; and is completely compatible with sorting.