CYTO 2023
May 20–24, 2023
Montreal, Québec, Canada

Poster #175

www.KineticRiver.com
info@KineticRiver.com
+1 (650) 439-7413

KINETIC RIVER®
Where Light Meets Life®

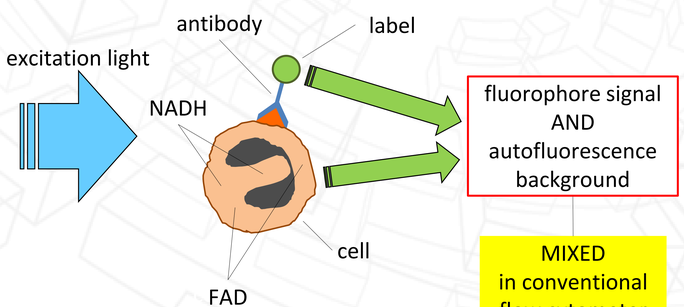
Progress Towards Real-Time Automated Autofluorescence Removal in a 16-Color Flow Cytometer

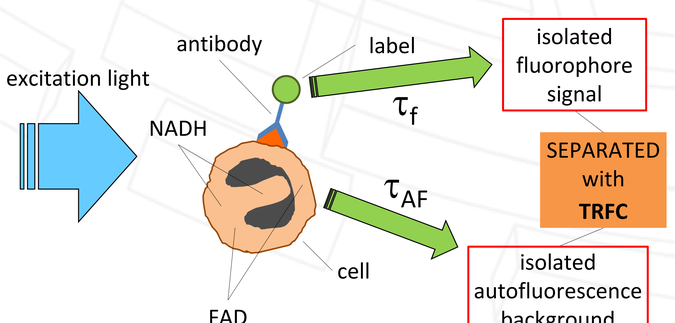
G. Vacca[1], A. H. Chin[1], K. LaneLutter[1], R. Hanson[1], P. Chattopadhyay[2], R. McKay[1,3] , H. Sillin[4]

[1]Kinetic River Corp., Mountain View, CA, United States; [2]Talon Biomarkers, Mendham, NJ, United States; [3]Full Spectrum Scientific LLC, East Windsor, NJ, United States; [4]JKI, Walnut Creek, CA, United States;

BACKGROUND

Most assays in flow cytometry rely on detection of signals from exogenously added fluorescent labels. However, cellular autofluorescence (AF) often contributes an unwanted background to such signals. This background, primarily from the metabolic cofactors nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), is high for commonly used UV and violet laser wavelengths and is present in most detection channels. Kinetic River is leveraging our **proprietary time-resolved flow cytometry (TRFC) technology** to allow for discrimination between this unwanted autofluorescence and the desired signal from the fluorescent label. By measuring two different parameters of fluorescent emission–the fluorescence intensity and the fluorescence lifetime decay–we can distinguish between the two sources and automatically eliminate the contribution of cellular autofluorescence, leaving only the desired signal from the fluorescent label.



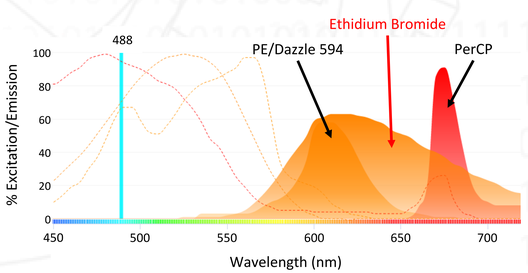


MATERIALS & METHODS

The *Colorado* time-resolved multiparametric analyzer system under development is designed to have pulsed laser excitation at 355 nm, 405 nm, and 488 nm along with six TRFC detection channels in the blue/green that have significant overlap with the emission spectra of NADH and/or FAD. The system will also have separate channels for FSC and SSC, as well as continuous-wave (CW) laser excitation at 636 nm and ten CW fluorescence channels (2 each for 355 nm, 405 nm, and 488 nm excitation, along with 4 for 636 nm excitation) in the red/NIR that do not overlap with AF. Ultrastable sheath flow will be established with our *Shasta* fluidic control system. Data acquisition will be based on a National Instrument PXI platform and custom-written LabVIEW code combined with signal processing performed on a computing platform running custom algorithms to **distinguish fluorophores of the same color but different lifetime *during* data acquisition**. In the development phase, parts of the system were tested using reconstituted lyophilized leukocyte cells stained with a viability dye (as a proxy for AF) combined with exogenous fluorescent labels emitting in the spectral range of the viability stain. Note that distinguishing between exogenous fluorescent labels and viability stains is another possible application of TRFC.

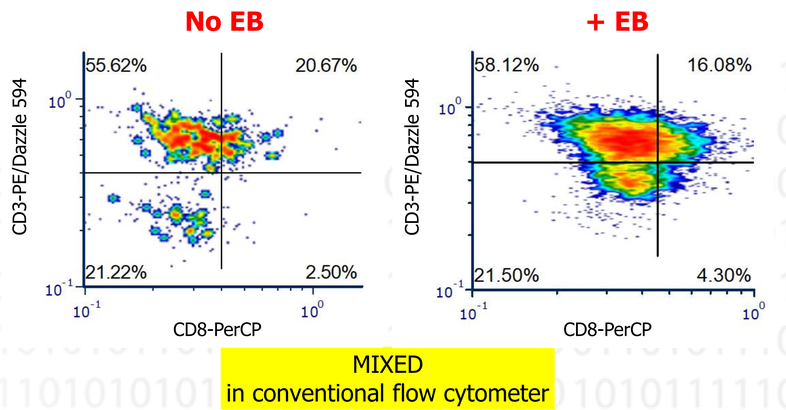
RESULTS

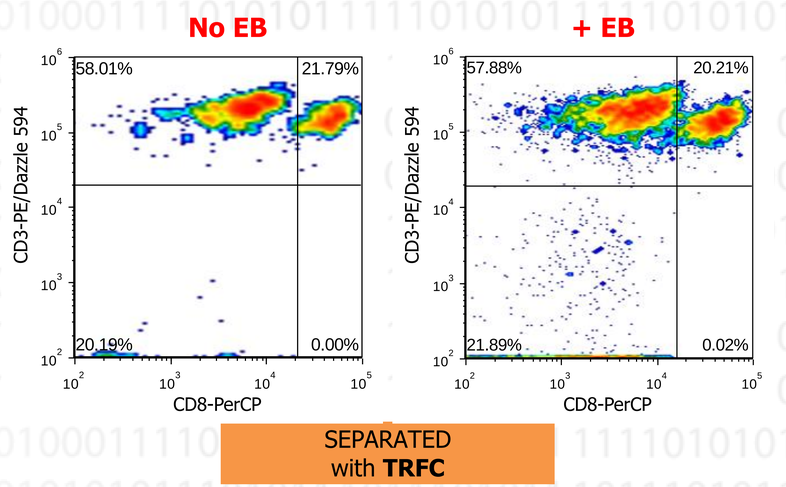
The *Colorado* successfully resolved exogenous fluorescence in the presence of a viability stain. In the figure shown in the next column, cells were stained with both ethidium bromide (EB) and exogenous fluorophore markers of Peridinin-Chlorophyll-Protein (PerCP) conjugated to a CD8 antibody marker (CD8-PerCP) and PE/Dazzle 594 conjugated to a CD3 antibody marker (CD3-PEDazzle594). Note that EB has high spectral overlap with both PerCP and PE/Dazzle 594; thus, a conventional cytometer cannot clearly identify positive or negative populations of CD8 or CD3 because of the strong EB emission. Using TRFC, we successfully extracted both the CD8-PerCP signal and the CD3-PEDazzle594 signal from the high EB background signal. The case of no EB staining is also shown for the respective mixed and separated with TRFC cases. A different compensation matrix was used for the mixed case compared to the TRFC case.



CONCLUSION

We demonstrated that the *Colorado* system can distinguish true signal from a high background signal without alterations in the user's experimental workflow or analysis methods. A complete immunophenotyping study of Acute Myeloid Leukemia patients will be performed using the completed 16-color *Colorado* system.





Without TRFC, the ethidium bromide signal is dominant and affects cluster separation (top). In the Colorado, *with the EB signal automatically rejected, CD3 and CD8 positive and negative populations can be easily identified (bottom).*

This work was made possible in part by U.S. government support under award number 2R44GM131619-02A1 from the NIH.