CYTO 2022
June 3–7, 2022
Philadelphia

Poster #43

KINETIC RIVER®
Where Light Meets Life™
www.KineticRiver.com
info@KineticRiver.com
+1 (650) 439-7413

Automated Autofluorescence Removal in Flow Cytometry

Giacomo Vacca[1], Kshitija Pratip Shevgaonkar[1,2], Alan H. Chin[1], Elijah S. Kashi[1,3], Richard R. McKay[1,4]

[1]R&D, Kinetic River Corp., Mountain View, CA, United States; [2]currently at FastDetect Inc., Irvine, CA, United States; [3]currently at Cana Technology Inc., San Francisco, CA, United States; [4]Full Spectrum Scientific, East Windsor, NJ, United States

BACKGROUND

Most assays in flow cytometry rely on detection of signals from exogenously added fluorescent labels. However, cellular autofluorescence often contributes an unwanted background to such signals (**Fig. 1**, top). This background, primarily from nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), is high for commonly used ultraviolet and violet laser wavelengths and is present in most detection channels. **Kinetic River is leveraging our proprietary Time-Resolved Flow Cytometry (TRFC) technology in our *Colorado* system to allow for discrimination between this unwanted autofluorescence and the desired signal from the fluorescent label.**

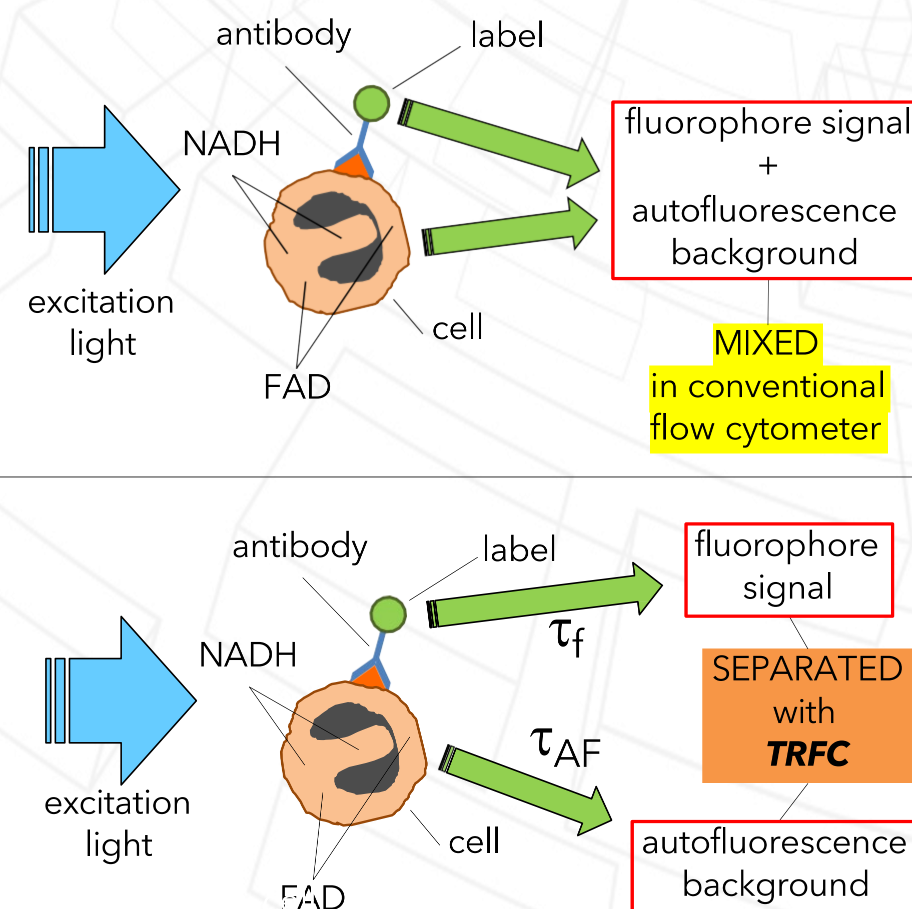


Fig. 1. Time-Resolved Flow Cytometry (TRFC) allows the discrimination between autofluorescence and signals from exogenous fluors–even when they are spectrally indistinguishable.

Using TRFC, the fluorescence lifetime decay of the fluorophore (τ_f) and the cellular autofluorescence (τ_{AF}) are used for automatic elimination of the contribution of cellular autofluorescence, leaving only the desired signal from the fluorescent label (**Fig. 1**, bottom).

MATERIALS & METHODS

The *Colorado* time-resolved multiparametric analyzer system uses pulsed laser excitation at 375 nm (Quixx, Omicron Laserage). Signals are collected by three fluorescence detection channels: 460/36, 510/42, 593LP (the first two of which have significant overlap with the emission spectra of NADH and/or FAD), plus forward- and side-scatter. Ultrastable sheath flow is established with our previously described *Shasta* fluidic control system (CYTO 2021) and monitored using our always-on *Cavour* flowcell monitoring. module. Data acquisition is based on a National Instruments PXI platform and custom-written LabVIEW code combined with signal processing performed on a computing platform running custom algorithms. The analyzer is operated using our *Panama* flow cytometry software for instrument control and data visualization (**Fig. 2**). The system was tested using cells stained with exogenous fluorescent labels emitting in the blue FL1 channel (strong NADH autofluorescence) and green FL2 channel (strong FAD autofluorescence).

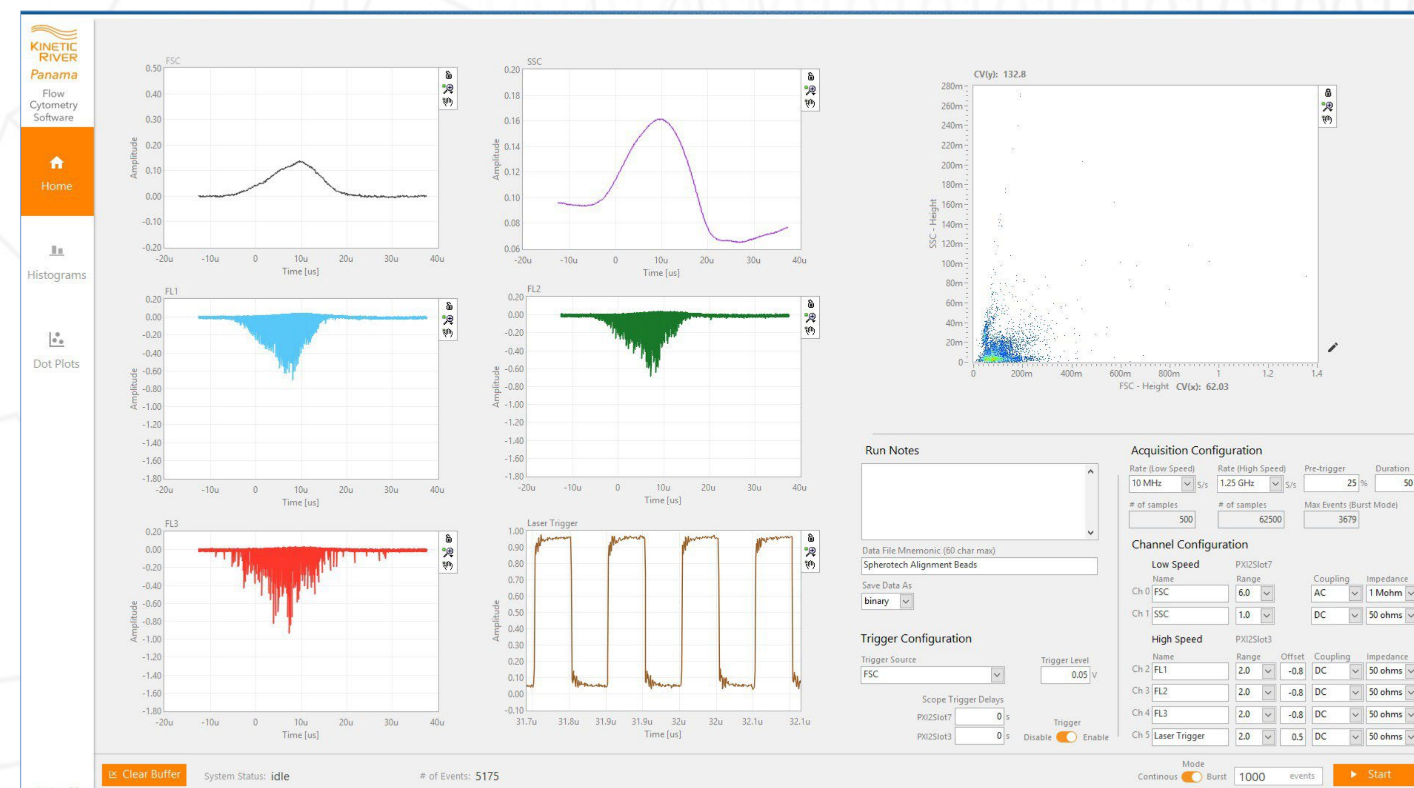
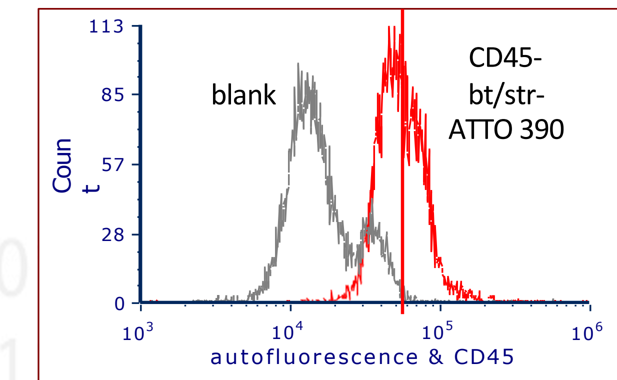


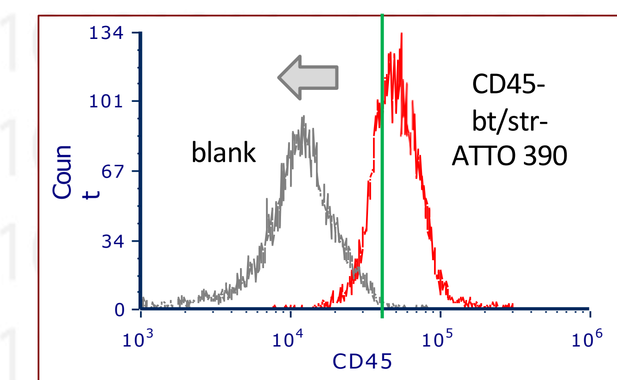
Fig. 2. The Panama instrument control and data visualization software.

RESULTS

The *Colorado*'s ability to resolve exogenous fluorescence from cellular autofluorescence based on fluorescence lifetime, regardless of the amount of spectral overlap of the signals, was demonstrated in cells stained with common fluorophores emitting in the blue and the green channels, with emphasis on the blue channel (autofluorescence from NADH). To best highlight the effectiveness of autofluorescence removal, we consider a case where the autofluorescence signal is comparable to the fluorophore signal. Shown in **Fig. 3** are results from the blue channel of eosinophils stained with CD45-biotin streptavidin-ATTO390 (gray is without staining, red is with staining) for the cases of no separation (top) and separation using TRFC (bottom). Note the removal of the high autofluorescence peak in both the signal and the blank with TRFC. Also note that the effect of autofluorescence would be much more severe if the marker in use was one with a high negative population instead of CD45; in this case, the autofluorescence signal would appear as an incorrectly labeled positive population without the use of TRFC.



MIXED (conventional analyzer)



SEPARATED with ***TRFC***

Fig. 3. (Top) High autofluorescence background contaminates more than half of the CD45 population. (Bottom) With TRFC, autofluorescence can be singled out, increasing separation between the CD45 signal and background.

CONCLUSION

We demonstrated that the *Colorado* system can distinguish true signal from high autofluorescence background. Current follow-on work is aimed at expanding the system's multiplexing capabilities by increasing the number of lasers and detection channels to create a cell analyzer platform that can automatically eliminate unwanted interference from cellular autofluorescence without alterations in the user's experimental workflow or analysis methods.

This work was made possible in part by U.S. government support under one or more grants awarded by the NIH.

The Colorado, Shasta, Cavour, and Panama, or use thereof, may be covered in whole or in part by patents in the U.S. and other jurisdictions. A current list of applicable patents can be found at https://www.kineticriver.com/kinetic-river-corp-patents.