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Triple-UV/Violet Excitation Analyzer for Label-Free Flow Cytometry

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BACKGROUND

Standard off-the-shelf flow cytometers have limited flexibility, leaving little choice to the user interested in more exotic excitation sources, or requiring users to buy a "bundle" of lasers, simply to get the single laser line desired. There is unmet demand for flow cytometry systems that are customized and modular; and in particular, for analyzers tailored to operate in the violet, UV, and deep UV, from customers interested in label-free analysis of cells, bacteria, and algae.

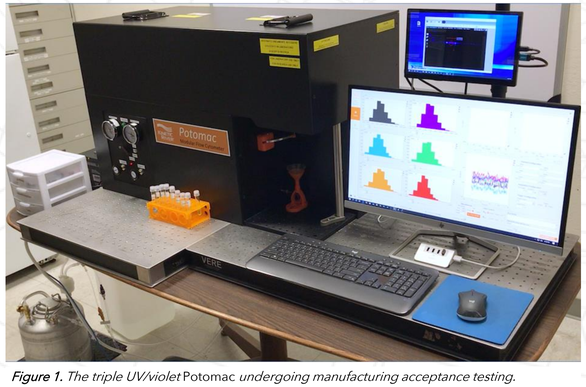

Figure 1. *The triple UV/violet* Potomac *undergoing manufacturing acceptance testing.*

METHODS

We have previously described our *Potomac* modular flow cytometry platform (CYTO 2017), designed to flexibly accommodate up to 7 lasers and 20 detectors. Using this flexible architecture, we have designed and built a *Potomac* analyzer customized for label-free analysis of cell samples (**Figure 1**) for the Italian National Research Council (IREA-CNR, Naples, Italy). It uses three interchangeable excitation sources: two built-in (405 and 375 nm, both from Pavilion Integration Corporation), and one external add-on (266 nm, from CryLas). It has six detectors (all PMTs for increased nanoparticle sensitivity): FSC, SSC, and four fluorescence channels (Hamamatsu Corp.), with three extra channels built-in to allow for future expansion. Detection channels (UV to IR) use high performance removable Semrock dichroics and filters (IDEX Health & Science). Ultrastable sheath flow is established with our newly introduced *Shasta* fluidic control system (see Poster #261 – "A Highly Customizable Fluidics Control Module for Flow Cytometry"). It incorporates our always-on flowcell monitoring system, the *Cavour* (see Poster #262 – "An Always-On Remote Flowcell Monitoring Module") for simple optimization and troubleshooting. Data is collected with proprietary electronics and a PXI National Instruments data acquisition system. Our new, proprietary *Panama* software operates the system, performs a full suite of sample processing and data visualization, and is intuitive and user-friendly. The system has built-in capability to expand the number of lasers and/or detection channels.

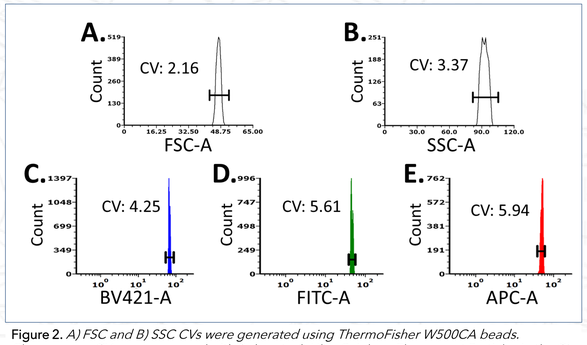

Figure 2. *A) FSC and B) SSC CVs were generated using ThermoFisher W500CA beads. Fluorescence CVs were assessed with Spherotech Ultra Rainbow Fluorescent Particles in the C) BV421, D) FITC, and E) APC channels (excitation at 405 nm).*

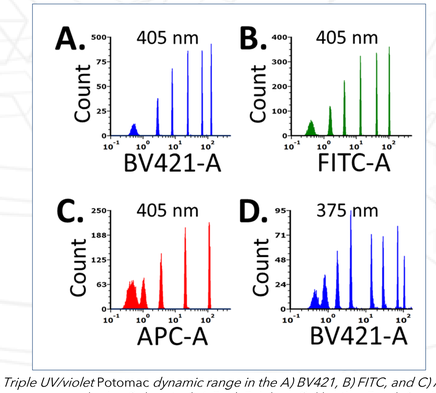

Figure 3. *Triple UV/violet* Potomac *dynamic range in the A) BV421, B) FITC, and C) APC channels was measured using Spherotech 6-Peak Rainbow Calibration Beads (excitation at 405 nm). Panel D shows 375-nm excitation of Spherotech 8-Peak Beads in the BV421 channel.*

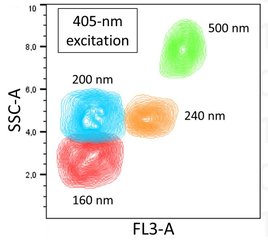

Figure 4. *The* Potomac *demonstrates excellent nanoparticle size discrimination using BioCytex Megamix-Plus SSC beads, despite the low excitation efficiency at 405 nm.*

RESULTS

The system was tested after shipping and installation by our European sales and service partner, Cytoflowservice (Milan, Italy) for performance using a variety of dyes, microspheres, and cell-based assays. Coefficients of variation (CVs) for the scatter channels (**Figure 2A and B**) and each fluorescence channel (**Figure 2C-E**) were measured using non-fluorescent and fluorescent microparticles respectively. Dynamic range was assessed using fluorescent 6-peak and 8-peak beads (**Figure 3**) and yielded excellent agreement with manufacturer's data (not shown). Megamix-Plus SSC beads (BioCytex) were used to assess the cytometer's performance in nanoparticle analysis (**Figure 4**). The *Potomac* demonstrated excellent discrimination down to 160 nm diameter (smallest size tested) despite sub-optimal excitation efficiency of these beads at 405 nm. Cell-based assays included cell cycle (**Figure 5**) and cell viability (**Figure 6**), both using DAPI. In both assays, cell populations (G1 vs. G2 and live vs. dead) are easily distinguished.

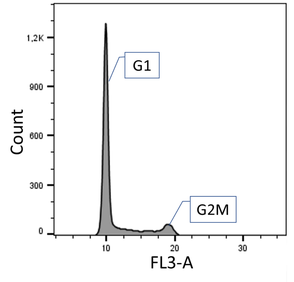

Figure 5. *A cell cycle assay on the triple UV/violet* Potomac *using DAPI-stained SH-SY5Y cells (375-nm excitation). The G1 peak was narrow (<4% CV) with a G2 peak easily observed.*

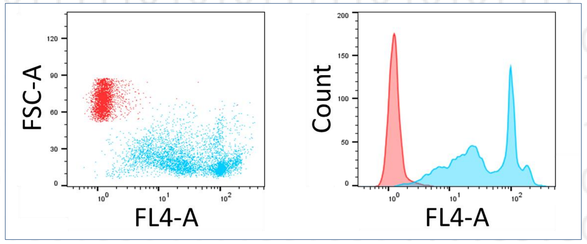

Figure 6. *A cell viability assay on the triple UV/violet* Potomac *using DAPI-stained SH-SY5Y cells (375-nm excitation). Panel A shows a dot plot that easily distinguishes live (red) and dead (blue) cell populations. Panel B is a histogram of the same data, with live cells (red) excluding DAPI.*

CONCLUSION

We have designed, built, and delivered a completely customized commercial flow cytometry solution that enables researchers at IREA-CNR to perform label-free excitation of algae and bacteria for water quality analysis. The system includes only those features required to meet the end-user's unique needs, with built-in room to grow.

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