Wednesday 20 July
1530-1630

Thursday 21 July
1115-1215

Flow cytometric detection and sorting of EVs: analysis and characterization of background noise sources that may impact EV fluorescence or scatter detection

Introduction:

EV detection via flow cytometry in fluorescence or scatter reaches the limits of current flow cytometry. Measurements of Q and B using an LED pulser indicates scatter and detection limits on the flow cytometer and is useful in understanding cytometer capabilities. However, understanding how electronic and optical noise sources may individually contribute to the system instrument noise pinpoints specific areas of instrument improvement for better EV detection. With high resolution electronics at and below the PMT dynamic range for the system, the noise background may be fully visualized, characterized and reduced. This poster describes both optical and electronic noise sources that may impact EV detection, characterization and sorting.

Methods: A MoFlo Astrios EQ (BEC) with a quantiFlash LED pulser (APE) installed were used to study noise sources such as: electronic (ADC, Preamp and PMT) and optical (laser, stream, nozzle tip distance, autofluorescence, nozzle tip sizes 25-100 um, and drop drive frequency and amplitude).

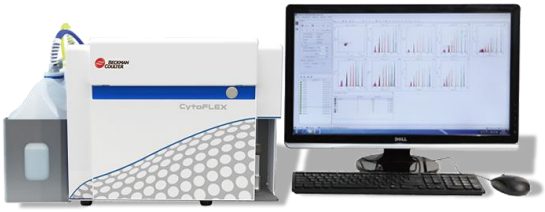
Results:

Optical and electrical noise determined the noise floor. The fluidic stream added 5-10% optical noise in fluorescent channels. Larger nozzle tip sizes reduced drop drive noise, whereas smaller nozzle tips reduced sort volume and optical noise but require higher pressure. About 5-10% of the optical noise is due to laser cross-talk.

Conclusions:

Characterization and analysis of the sorter noise background provided insight into instrument EV characterization capabilities. Improvements to drop drive amplitude and frequency, laser selection and location and nozzle tip will reduce the noise floor. Optimal EV detection, isolation and characterization depends on measuring not only the Q value, but the B and true noise floor of the flow cytometer.

CytoFLEX - Ultra flexible performance and resolution



The CytoFLEX system brings you easily upgradeable detection capabilities for up to 4 lasers and 13 colour research flow cytometry right on your bench top. Providing quality and performance at any configuration, the CytoFLEX system provides powerful sensitivity and resolution for the simple to the most challenging applications. CytoFLEX delivers and surpasses capabilities expected in top tier analysers, with excellent performance and nanoparticle resolution. The CytoFLEX system is easy to learn, easy to operate and easy to maintain.

A compact, reliable design fits right on your benchtop ($^{approx.}$ 23Kg, 45cm x 45cm x 33cm). Future-proof system with easy upgrade options. Versatile plug-n-play filters (bandpass-only light collection). Integrated optics for alignment stability. Accommodates 12x75mm tubes, Eppendorf tubes and 96 well microtitre plates.

When equipped with a violet laser, the CytoFLEX can be set up to measure Side Scatter from 405nm for enhanced nanoparticle detection. Mie theory predicts that the scattering cross section of a particle, and thus its scattering intensity, depends on the wavelength of light, the angle of collection, and the size, shape, and refractive index of the particle. All other factors being equal, using a shorter illumination wavelength will result in an increase in scattering cross section, and thus more scattered light. The CytoFLEX offers light scattering parameter measurements from the 488nm laser light, and optionally from the 405nm laser to enhance small particle detection. Increased sensitivity using an intelligent patent-pending optical design optimizes excitation from up-to three lasers (488nm; 638nm; 405nm) and light collection efficiency. With unique flow cell design and integrated optics, the innovative Wavelength Division Multiplexing (WDM) detection module includes solid-state, high efficiency, low-noise detectors for excellent performance. The WDM's bandpass-only design guarantees flexibility with easily changeable filters and 4-13 configurations.