The Use of an Aurora Spectral Cytometer for Small Particle Analysis

Joanne Lannigan[1], Michael Solga[1], Vera Tang[2,3], and Marc-Andre Langlois[2,3]

[1] University of Virginia Flow Cytometry Core [2]University of Ottawa Flow Cytometry and Virometry Core [3]ViroFlow Technologies, Inc.

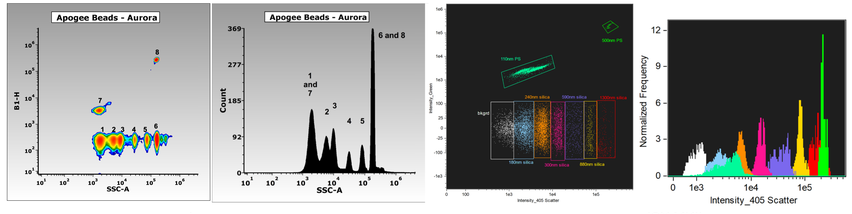
Introduction

There is an exploding interest in the use of flow cytometry to assess a variety of small particles such as Extracellular Vesicles (EVs), liposomes, and viruses. Many flow cytometry instruments have been shown to struggle with particles smaller than 300nm. Recent advances in flow cytometry instrumentation design has led to increased opportunities to measure particles less than 300nm. The Aurora spectral cytometer has multiple design attributes that should make this instrument sensitive enough to detect small particles in this range. In this study, we sought to evaluate whether this instrument has the ability to detect small particles in a size range that would be useful for typical biological studies.

Methods

Various fluorescent small particles (<300nm) with a variety of sizes and refractive indices were run on a Cytek™ 3 laser Aurora. These included apogee beads, viruses (90-250nm), liposomes (100nm), silica (180nm) and EVs (100-300nm). Instrument settings were optimized for either violet/blue SSC or fluorescence triggering so that the buffer only control contained 2-4 events per second (fluorescence triggering) or <40 events per second (scatter triggering). For fluorescence triggering, the detector wavelength closest to the maximum fluorescence emission was used. Parallel samples were run on an ImagestreamX® (ISX) imaging flow cytometer for comparison. All samples were collected for 2 minutes. Data was analyzed using either FCSExpress 6.06 or 7.0 software.

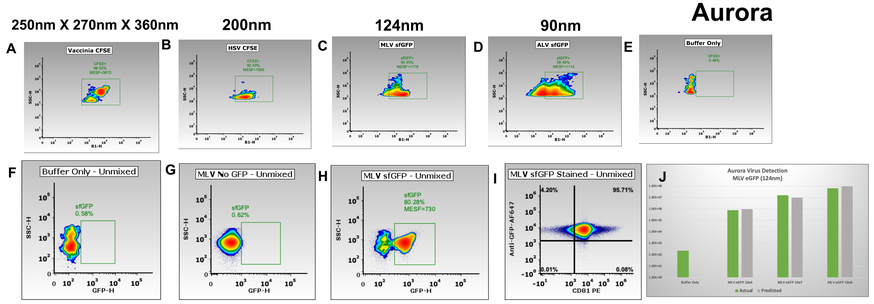
Results – Apogee Beads



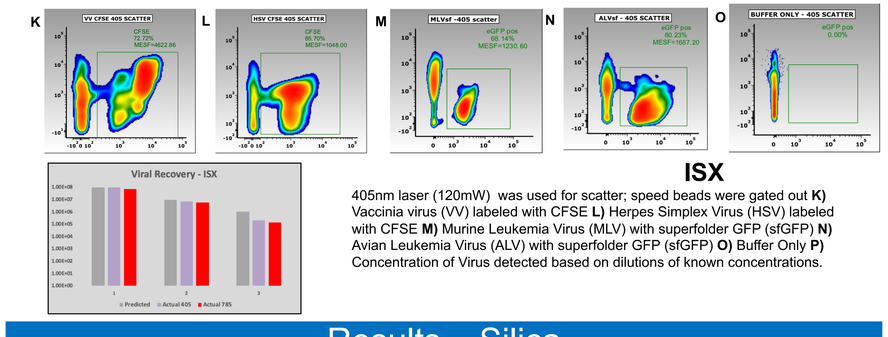
Aurora ISX MKII

Apogee Beads: 8 peaks: 2 fluorescent PS; **110 and 500nm**, 6 non-fluorescent silica; **180, 240, 300, 590, 880, 1300nm**
Aurora (left) Scatter 405nm 100mW ISX MKII (right) Scatter 405nm 120mW

Results – Virus



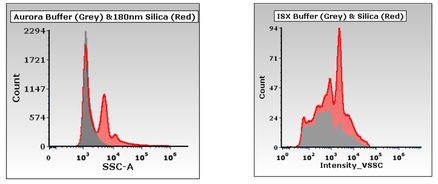
Violet SSC-H was used as a threshold trigger, raw data is displayed **(A-E) A)** Vaccinia virus labeled with CFSE **B)** Herpes Simplex Virus (HSV) labeled with CFSE **C)** Murine Leukemia Virus (MLV) with superfolder GFP (sfGFP) **D)** Avian Leukemia Virus (ALV) with superfolder GFP (sfGFP) **E)** Buffer Only.
Unmixed Data (F-H): B-SSC threshold, V-SSC displayed **F)** Unmixed buffer only **G)** Unmixed MLV No GFP **H)** Unmixed MLV sfGFP **I)** Unmixed stained with anti-GFP and CD81 MLV sfGFP gated on GFP **J)** Concentration of virus detected based on dilutions of known concentrations.
NOTE: These were different lots of virus run on different Aurora instruments



ISX

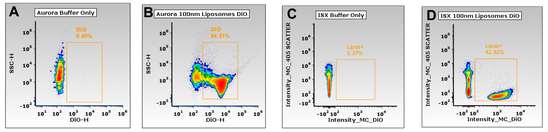
405nm laser (120mW) was used for scatter; speed beads were gated out **K)** Vaccinia virus (VV) labeled with CFSE **L)** Herpes Simplex Virus (HSV) labeled with CFSE **M)** Murine Leukemia Virus (MLV) with superfolder GFP (sfGFP) **N)** Avian Leukemia Virus (ALV) with superfolder GFP (sfGFP) **O)** Buffer Only **P)** Concentration of Virus detected based on dilutions of known concentrations.

Results – Silica

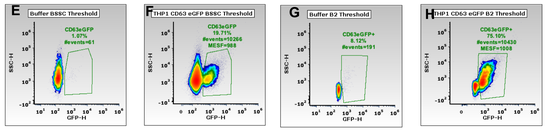


180nm non-fluorescent silica particles (Spherotech): Buffer (Grey) and Silica particles (Red); Aurora (left), ISX (right). ISX does not resolve above background well when there is no fluorescence.

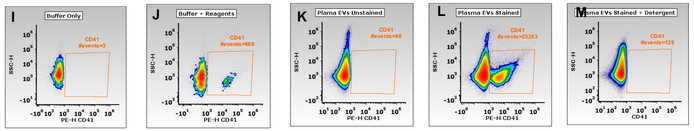
Results – Liposomes & EVs



100nm Liposomes stained with DiO: Aurora BSSC threshold **A)** Aurora Buffer only **B)** Aurora Liposomes; ISX 405nm scatter **C)** ISX Buffer only **D)** ISX Liposomes

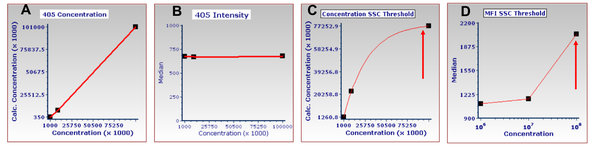


THP1 CD63eGFP EVs (150nm): **E-F** BSSC threshold; **G-H** B2 threshold



Plasma derived EVs (P20Kxg) BSSC threshold: **I)** Buffer only, **J)** Buffer + Reagents, **K)** Unstained EVs, **L)** Stained EVs, and **M)** Stained EVs + 0.5% SDS detergent

Results – Coincidence (Swarming)



Evaluation of Coincidence (Swarming): MLVsfGFP at 10^6, 10^7, 10^8/mL; **A-B** ISX concentration **(A)** and MFI **(B)**; **C-D** Aurora concentration **(C)** and MFI **(D)**. Aurora demonstrates coincidence at higher concentrations (red arrows).

Conclusions – References - Acknowledgements

- The Aurora has the sensitivity to detect biological particles as small as 100nm
- Both unmixed and raw (single channel) data demonstrate excellent sensitivity
- Both scatter and fluorescence thresholding provide comparable results
- Aurora can detect small particles even in the absence of fluorescence
- Coincidence is detected at higher concentrations (10^8)

MLV Reference: Engineered Retroviruses as Fluorescent Biological Reference Particles for Small Particle Flow Cytometry. Tang,et.al.**doi:** https://doi.org/10.1101/614461

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