A Prototype CytoFLEX for High-Sensitivity, Multiparametric Nanoparticle Analysis

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Introduction

Extracellular vesicles (EV) are a rapidly growing area of biomedical research. They are generated in large numbers by living cells and carry surface markers that can trace them to their cell or tissue of origin. EVs are present in all types of bodily fluids, and their potential for use as biomarkers is the subject of active research in the fields of major therapeutic importance, such as cancer, cardiovascular and neurodegenerative diseases. In addition, since EVs are much more abundant than their cells of origin, they promise to provide a better way to detect minimal residual disease.

Flow cytometry may be uniquely suited to address the needs of the EV field. It has the potential to provide for quantitative, particle-by-particle, multiplexed phenotypic analyses of EVs, and the ability to sort specific populations for functional analyses. However, currently available flow cytometers have significant limitations for the analysis of particles of exosome size. Indeed, the light-scatter intensity generated by exosomes on most flow cytometers is too low to be discriminated from optical and electronic noise, resulting in the common notion that only "the tip of the iceberg" of the EV population can be detected by flow cytometry.

To address these issues, we have developed a prototype nanoparticle analyzer based on the technology of the CytoFLEX platform. Our current prototype can detect and resolve 30 nm-polystyrene and 50 nm-silica nanoparticles. It has enhanced fluorescence sensitivity due in part to modifications that were made to enhance size resolution. And, it has very minimal background noise due to enhancements in noise filtering and coincidence reduction. In this poster, we will demonstrate the VSSC-based size resolution and fluorescence sensitivity of our prototype using a variety of NIST-traceable size standards and fluorescent nanoparticles.

Materials

Item	Catalog #	Vendor
40nm PS NIST Size Standard	3040A	ThermoFisher
50nm PS NIST Size Standard	3050A	ThermoFisher
60nm PS NIST Size Standard	3060A	ThermoFisher
80nm PS NIST Size Standard	3080A	ThermoFisher
100nm PS NIST Size Standard	3100A	ThermoFisher
150nm PS NIST Size Standard	3150A	ThermoFisher
50nm Si NIST Size Standard	NS-0050A	MSP Corporation
100nm Si NIST Size Standard	NS-0100A	MSP Corporation
200nm Si NIST Size Standard	147040-10	Corpuscular, Inc.
20nm Yellow Green PS Beads	F8888	ThermoFisher
40nm Yellow Green PS Beads	F10720	ThermoFisher
110nm Dragon Green PS Beads	FCDG002	Bangs Laboratories, Inc.
Prototype CytoFLEX Nanoparticle Analyzer	Custom	Beckman Coulter
CytExpert Software v2.0	B49006	Beckman Coulter

Methods

1. Upon startup, the instrument was first primed and flushed.
2. The sample probe was cleaned using a standard cleanse panel: bleach, cleanse and 2x water for flushing. This panel was repeated twice.
3. The nanoparticles were triggered using VSSC-H, with the threshold set just below the noise peak (taking in all of the noise).
4. The APD gains were each set at their optimal detection levels: near the middle of the gain range for the prototype instrument.
5. Each nanoparticle sample was diluted in water and titrated to find the optimal working range.
6. Mixtures of nanoparticles were prepared based upon the optimal titrations.
7. Exosome samples were diluted in 1x PBS, titrated and acquired similar to the nanoparticles.
8. Each sample was acquired for 30 sec irrespective of the number of events collected.
9. Following acquisition, the data were analyzed using CytExpert Software v2.0.

Results

A) Resolution of Polystyrene Nanoparticles
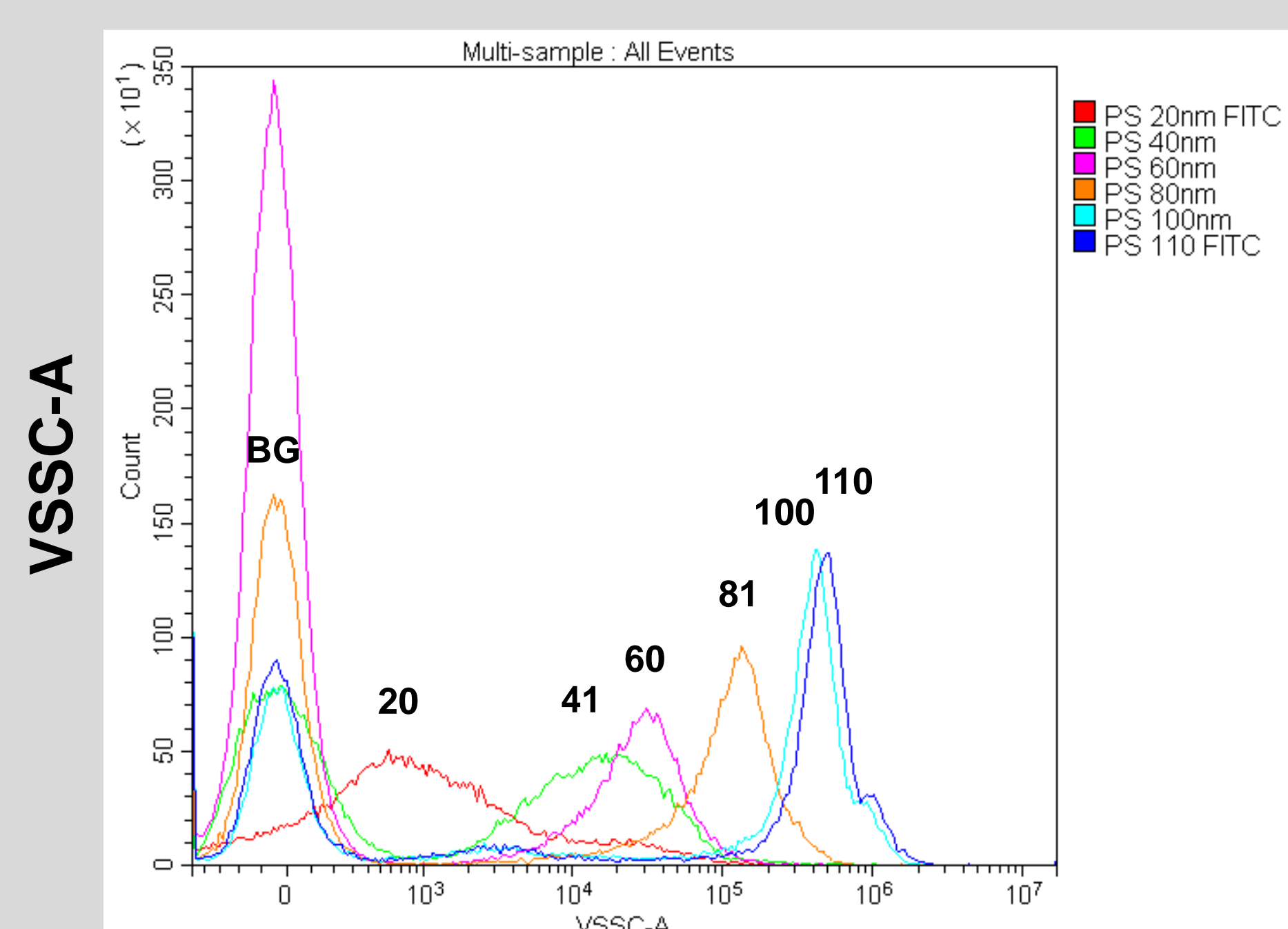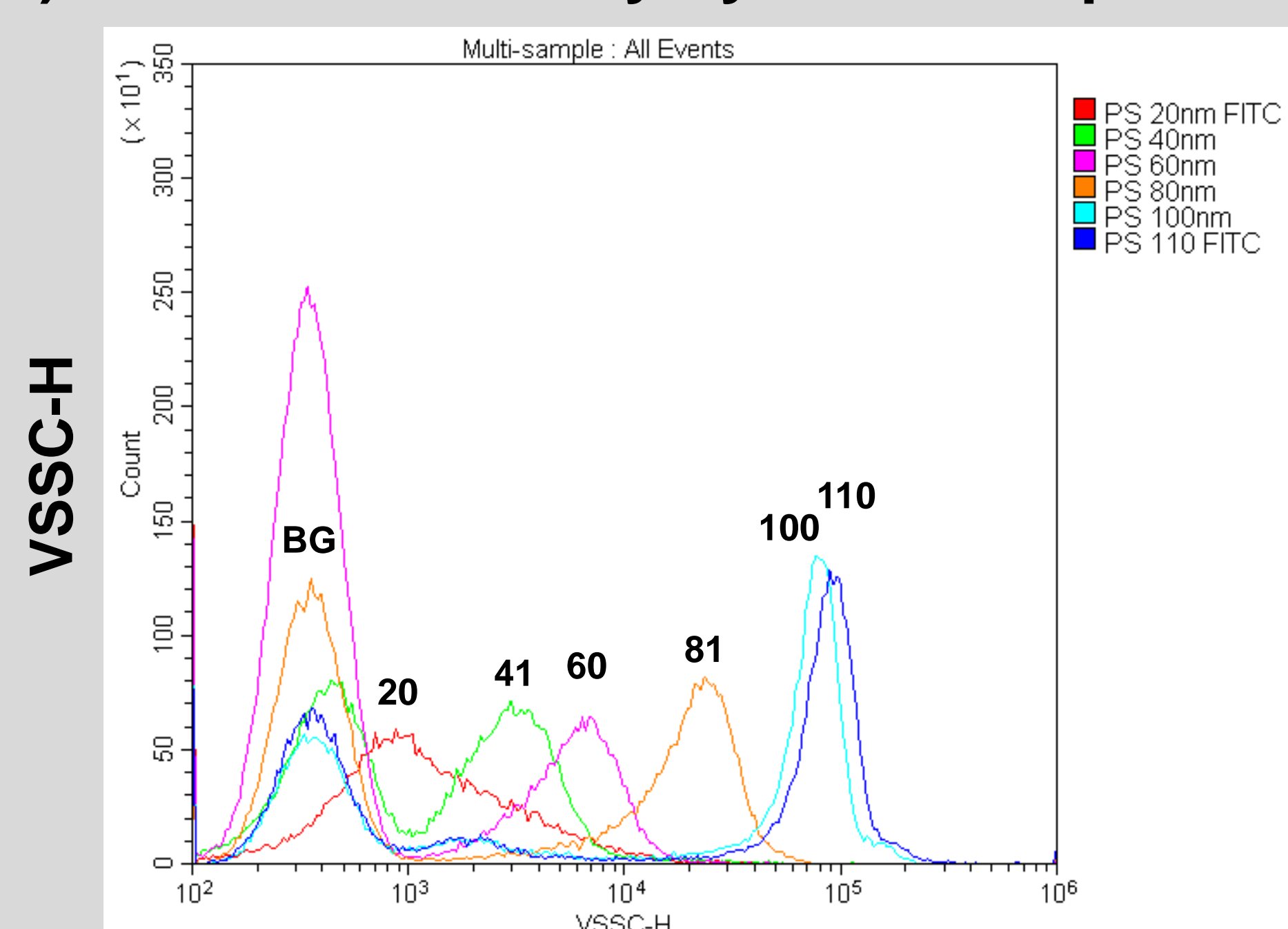
Each nanoparticle was triggered using VSSC-H except for the 20 nm FITC particles. The median of the 20 nm particles was right at the noise threshold, so these particles were triggered using FITC-H and displayed in order to reference the size range.
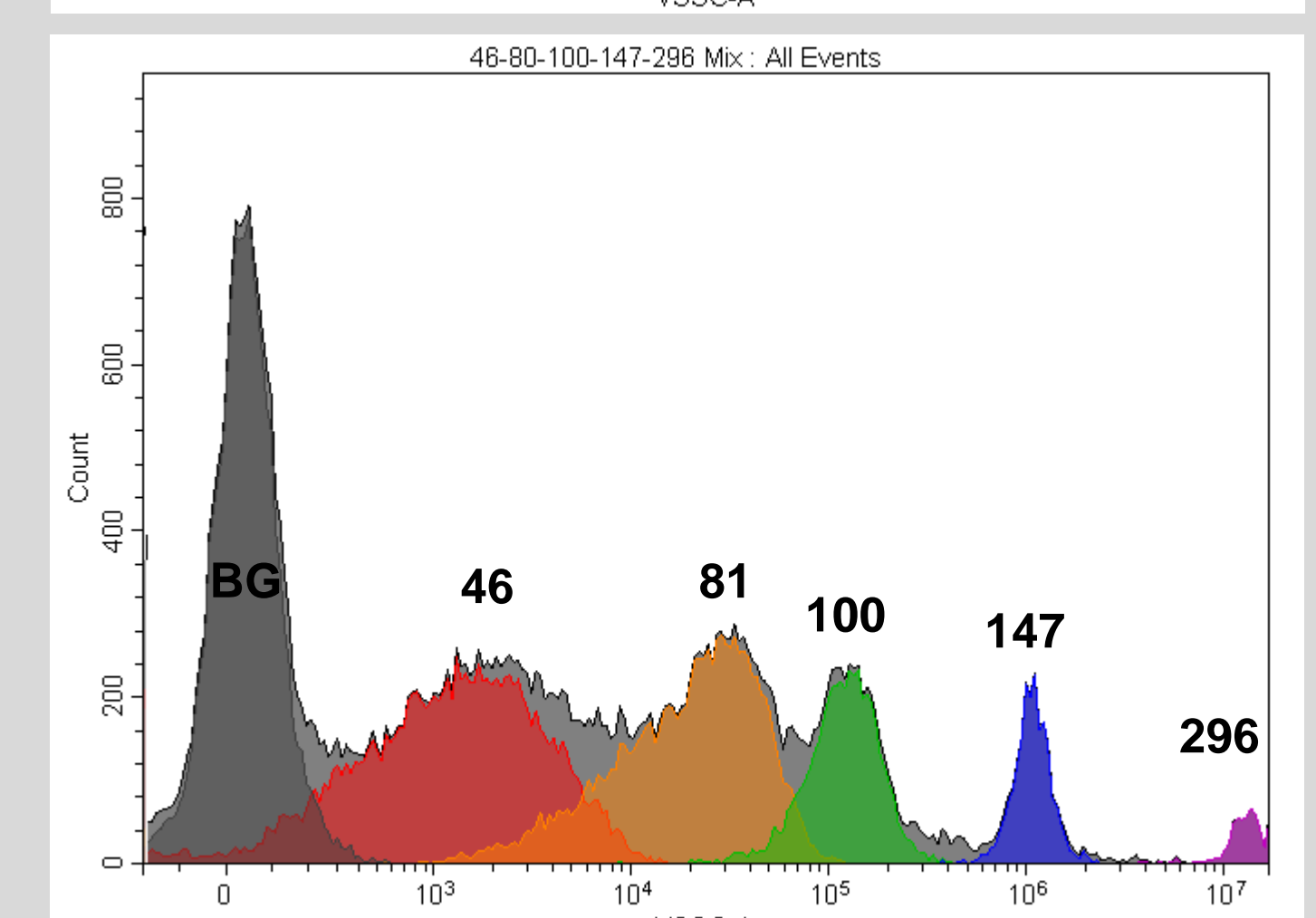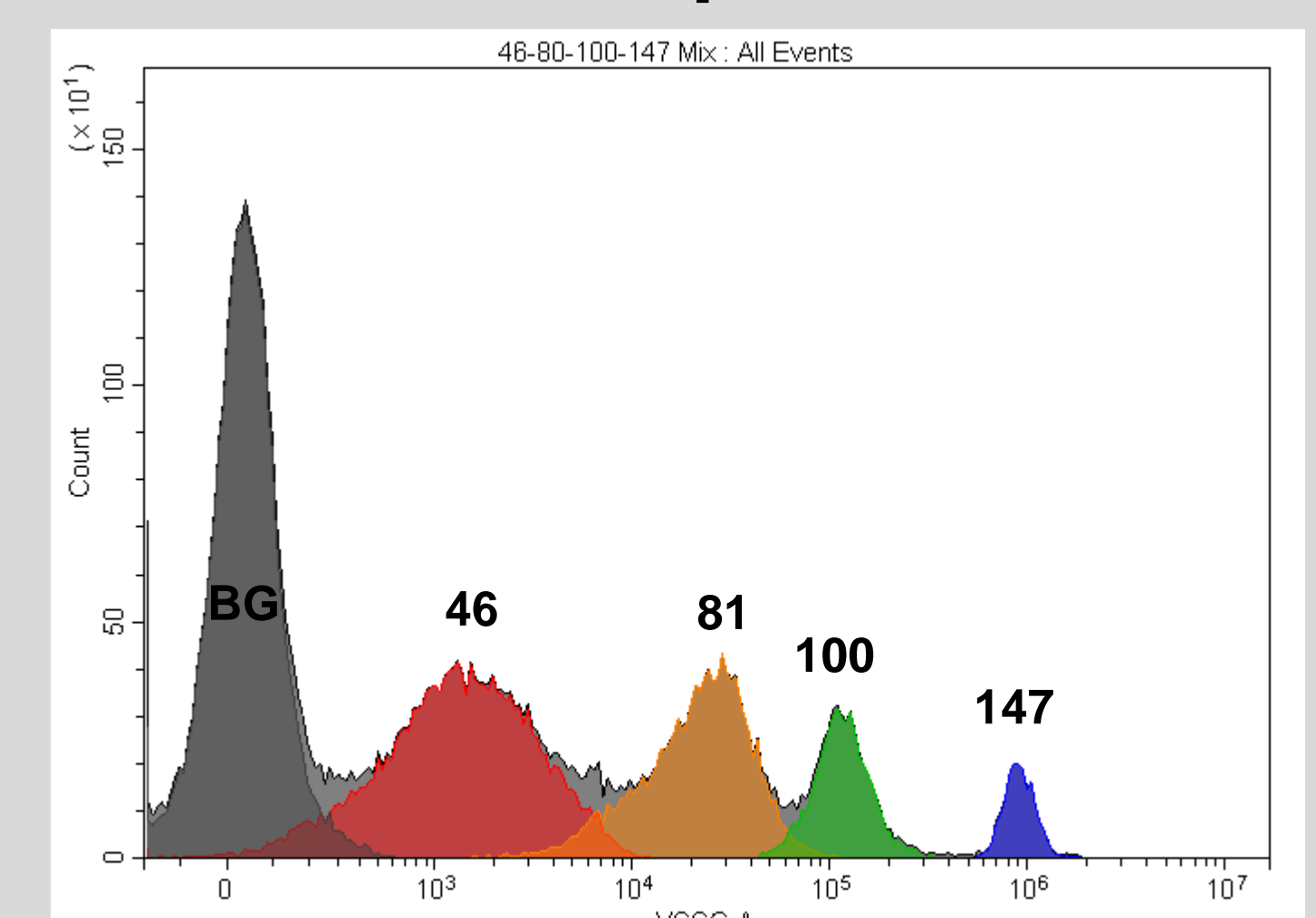
B) Resolution of Silica Nanoparticles
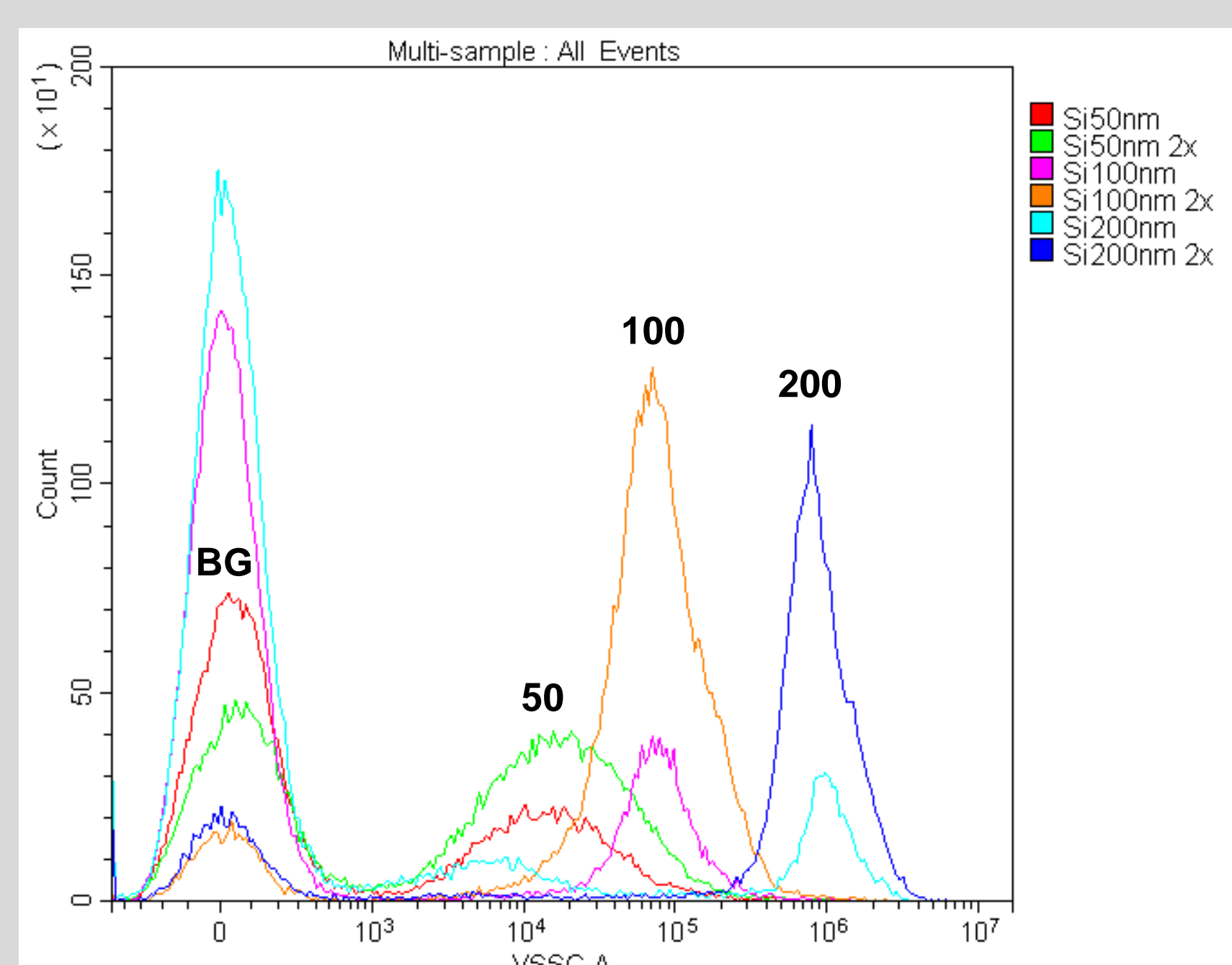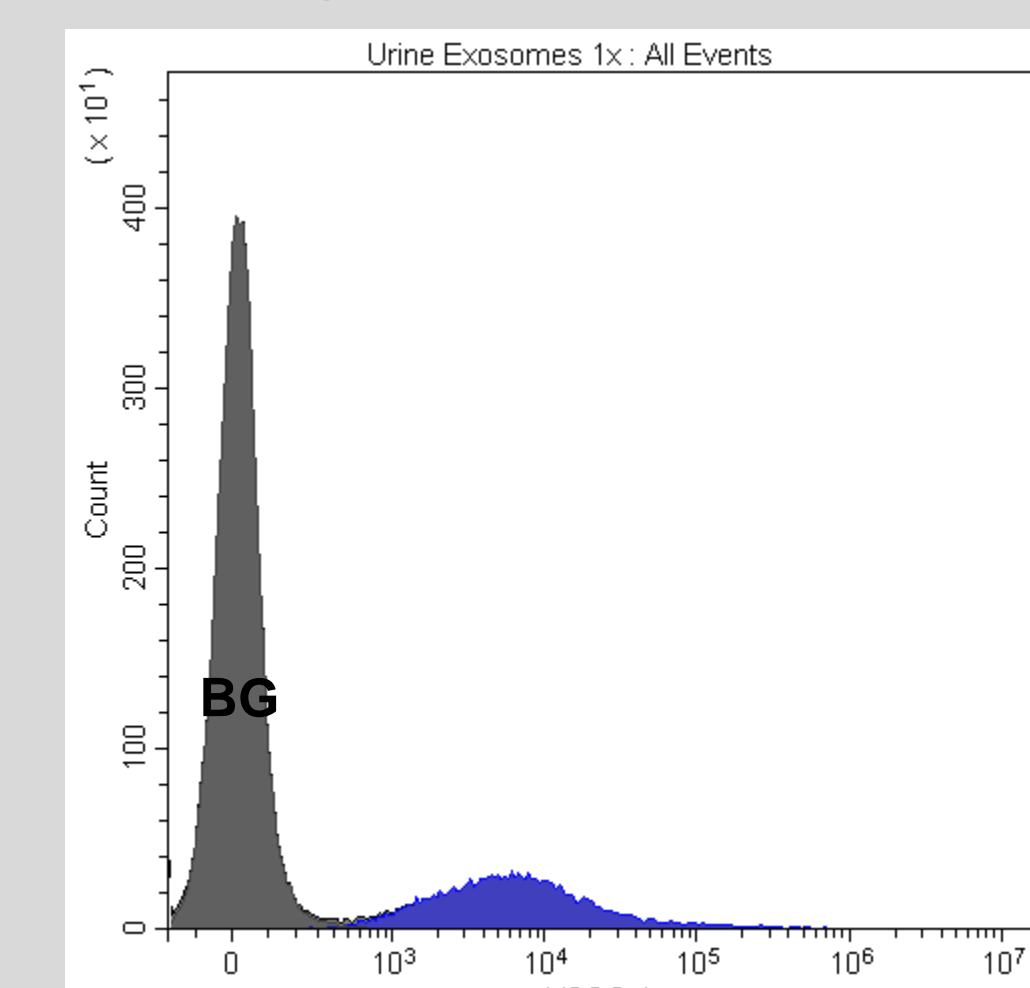

C) Resolution of Fluorescent Nanoparticles
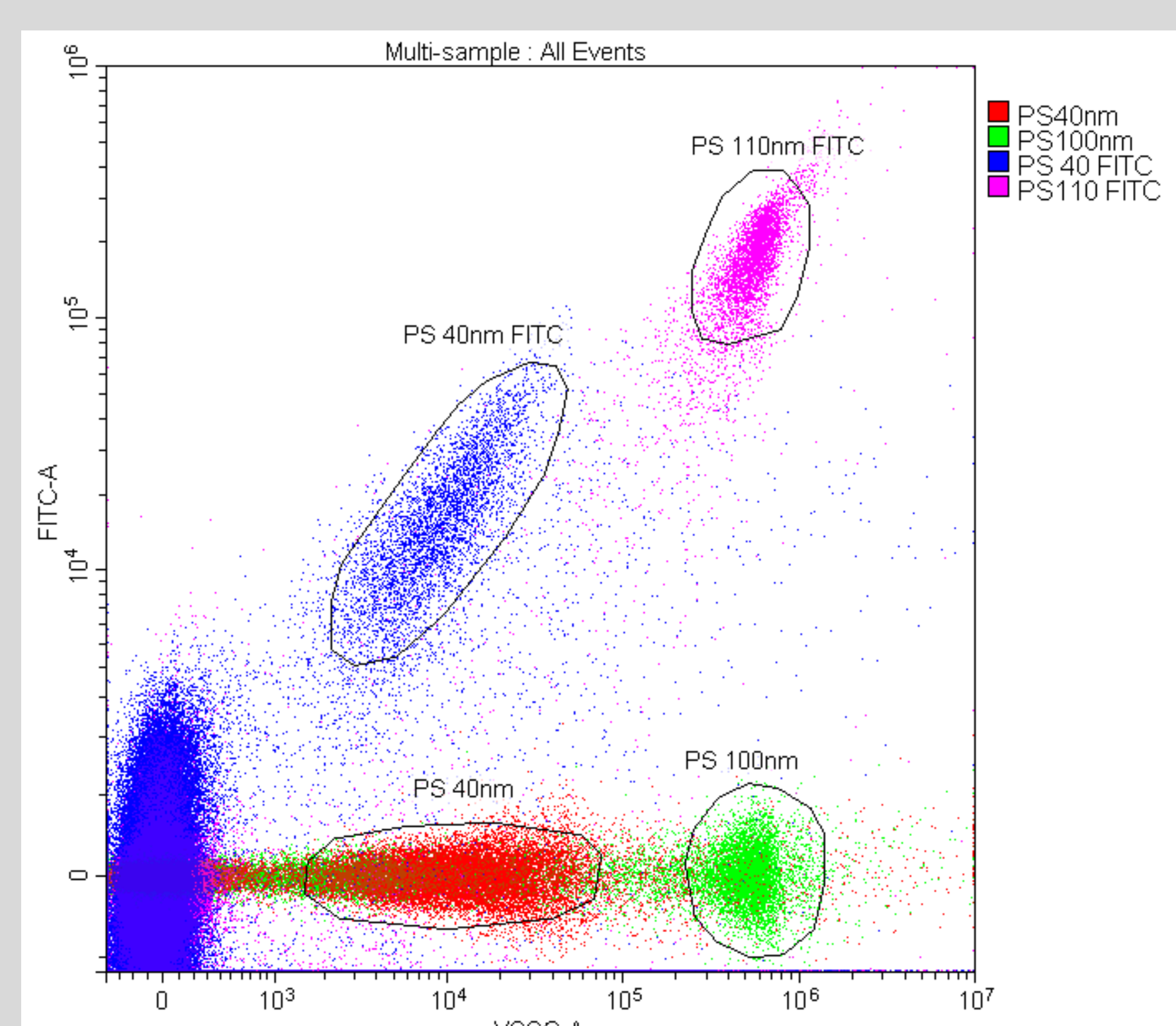
D) Resolution of Nanoparticle Mixtures
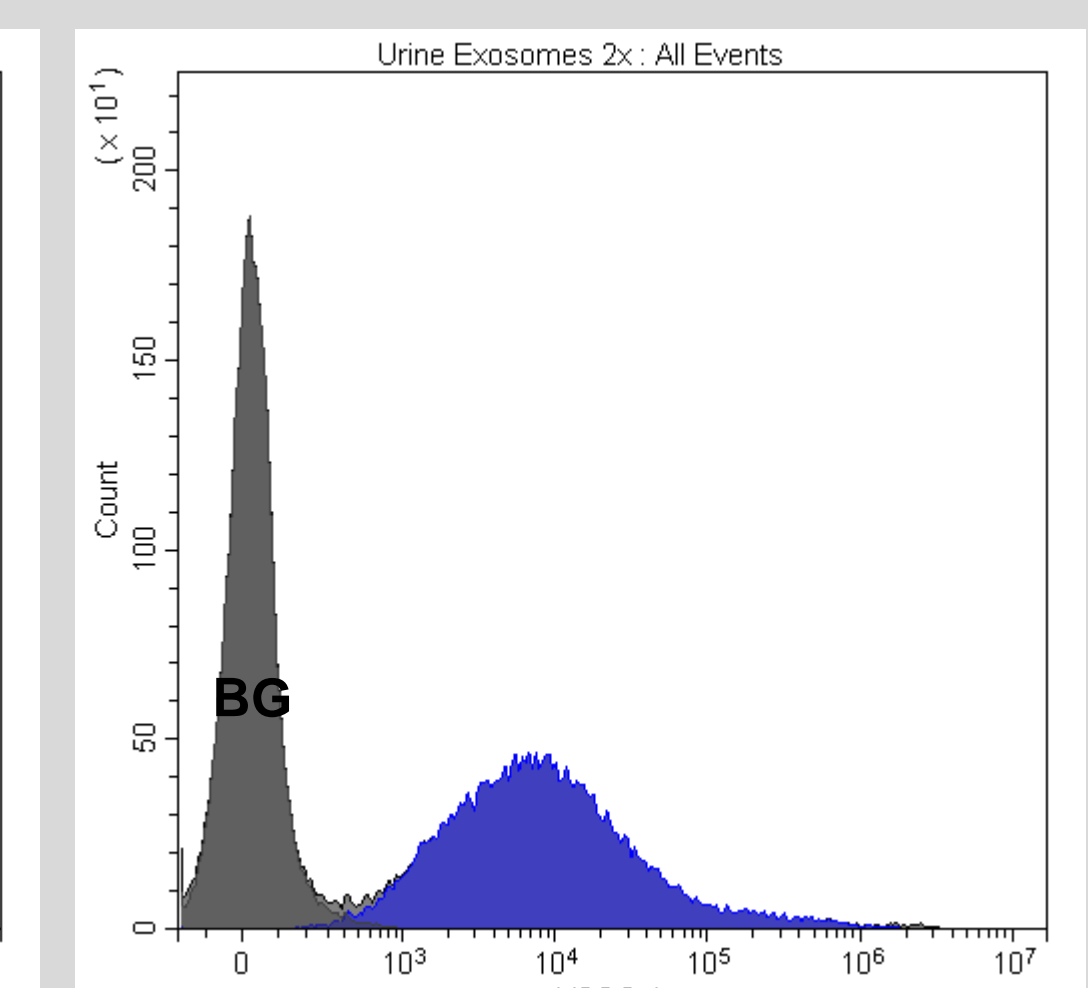

E) Resolution of Purified Exosomes

1x Urine Exosomes

2x Urine Exosomes

Discussion

In this poster, we have demonstrated that our current prototype nanoparticle analyzer can fully resolve nanoparticles > 20nm using a VSSC trigger, and has improved fluorescence sensitivity that enables decades of separation for fluorescently labeled nanoparticles. Moreover, this instrument retains the ability to analyze larger particles or cells using FSC and 488-SSC, unlike dedicated nano- and microparticle analyzers that confine the user to a very small dynamic range. Ultimately, we have optimized the already exquisite sensitivity of the CytoFLEX platform, and have made it more sensitive in order to provide the fields of EV and nanoparticle research with an easy-to-use flow cytometer that has the ability to effectively detect and resolve nanoparticles.

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