nodexus

APPLICATION NOTE

nx one

Plug-and-Play Sorting for Large Particles: 60µm and Beyond

Introduction

Size-based particle analysis and sorting are crucial in biological applications as the function, origin, or type of biological particles can be traced using cell size as a metric. For traditional fluorescence-activated cell sorting systems, processing large (>30 µm diameter) particles or heterogeneous samples containing a mixture of particle sizes requires laborious laser and fluidic stream alignment, precise droplet break-off optimization, and can often result in poor target recovery and low purity. Here, we highlight the Nodexus NX One benchtop single-cell sorting and dispensing system's ability to seamlessly analyze, sort, and dispense particles of a diverse size range spanning 3 - 60µm (**Fig. 1**).

Methods

The NX One's dynamic particle size range was evaluated using a mixture of microsphere beads with 6 different diameters viz. 3 µm, 5 µm, 10 µm, 16 µm, 43 µm, and 60 µm (Spherotech Inc. and Cospheric LLC). To achieve a homogeneous suspension, the beads were mixed in PBST (phosphate-buffered saline, 0.1% Tween-20) in the sample loading chamber of the NX One microfluidic cartridge, which was then inserted into the NX One to categorize the corresponding size of each bead subpopulation (the NX One utilizes Axial Light Loss for particle size and doublet determination). Both 60µm beads as well as Chinese Hamster Ovary (CHO) spheroids of various sizes, generated using VitroGel Hydrogel Matrix (VHM01), were sorted and dispensed to demonstrate the gentle large particle sorting capability of the NX One.

All gating and visualization shown are using the NX One's customer-facing software interface.

Results

The NX One has previously shown detection of the entire Flow Cytometry Size Standard Kit range (Spherotech PPS-6K). Here, we show expanded detection dynamic range to include 43 µm and 60 µm beads sourced from Cospheric LLC (**Fig. 2**). Beads were mixed in PBS (for non-hydrophobic beads) or PBST in the sample loading chamber of the cartridges.

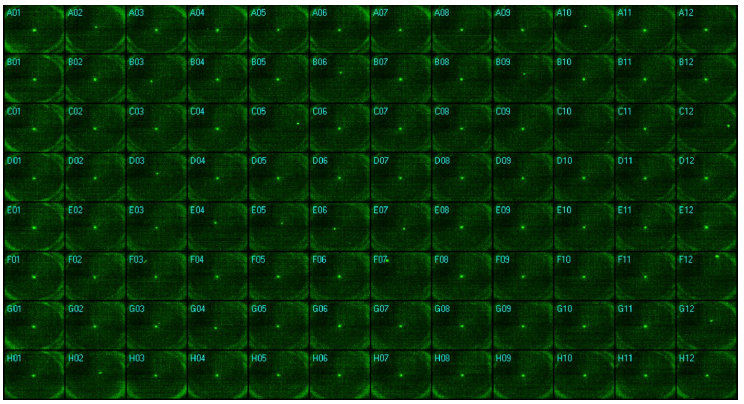

Figure 1. Representative image of single-bead dispensing of 60 µm beads on a 96 well-plate. Whole well imaging performed using ImageXpress Micro

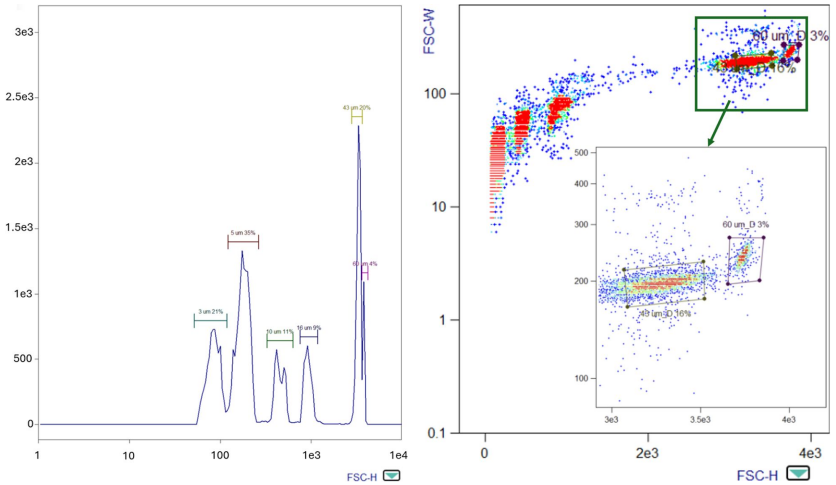

Figure 2. Real-time visualization showcasing the NX One's size dynamic range capabilities. Left panel: a histogram of FSC-H showing the distribution of forward light scattering (measured via occlusion or axial light loss) for beads with different diameters. Right panel: the light scattering width vs height density plots which can be used to discriminate particles that are close to the maximum limit of sensitivity

Results

96 well-plate single-bead dispensing results indicate that the NX One is capable of depositing single 15 µm, 43 µm, and 60 µm beads with high accuracy (**Fig. 3**). Although the experiments shown here tested beads up to 60 µm in diameter, the NX One can support sorting even larger particles beyond the detection limit (i.e., when the FSC-H signal becomes saturated).

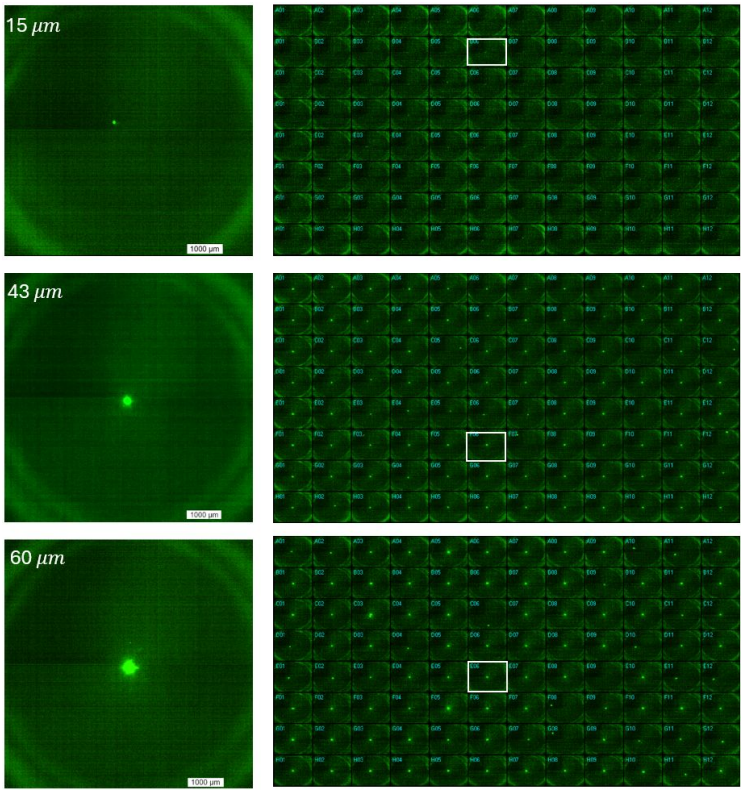

Figure 3. *Representative images of single-bead dispensing into 96 well-plates for 15 µm (top), 43 µm (middle), and 60 µm (bottom) beads.Note the actual bead size is smaller than the ones shown in the figure due to the merging of images.*

Using a CHO cell line, we generated spheroids following the protocol recommendation for the use of VitroGel® Hydrogel matrix. The spheroids were sorted after DIV 4 and were 30 ± 5 µm in diameter. The gentle sorting of the NX One maintained the integrity of the spheroids even after the hydrogel was dissolved (**Fig. 4**).

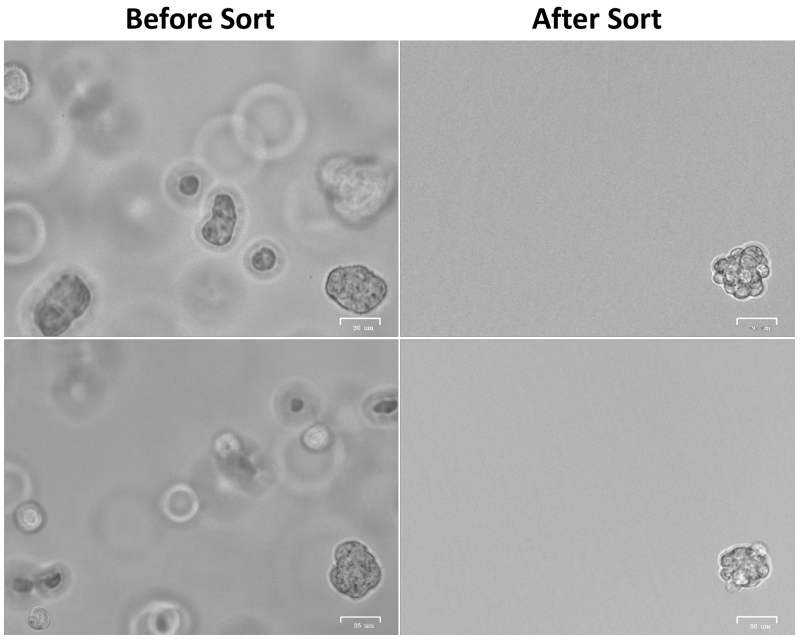

Figure 4. *Representative images of the spheroids before sorting and after sorting on the Nodexus NX One. The presence of hydrogel gives the spheroids shape as they are embedded in a matrix. The hydrogel is dissolved before sorting. Scale Bar : 30µm.*

Conclusion

Ultimately, the Nodexus NX One benchtop cell sorting and dispensing platform shows robust size-based particle analysis and sorting capability. On a single cartridge, particles with sizes between 3µm to 60µm can be clearly identified and selected using a single plot. The NX One also demonstrated its ability to accurately deposit large particles into microtiter well plates (up to and including 384 well plate formats) without the use of any secondary accessories and with no user interaction. Cell-based validation using spheroids highlighted the ability of the NX One to perform high throughput, gentle, and efficient sorting of large cell clusters and nascent spheroids/organoids/tumoroids.

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