"Smart" Microfluidics Instrumentation For Gentle & Accessible Cell Sorting

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Introduction

Fluorescence-Activated Cell Sorting (FACS) remains the industry standard for the analytical determination of cellular phenotype and sorting cells for further analysis or generating clonal populations; however, significant bottlenecks persist in these applications. Even for low-parameter workflows (*e.g.*, GFP⁺ single-cell sorting / dispensing into plates), high-end sorters are often the only automated option. Here, we present a commercial platform, Nodexus Inc.'s NX One, which is affordable, efficient, and offers functional benefits compared to traditional flow sorters and manual approaches (such as limiting dilution) for routine single-cell workflows.

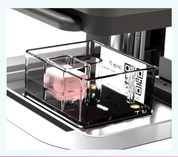
The NX One **maximizes cell viability through low-pressure microfluidics and offers contamination-free operation** through disposable cartridges. Sorter-Induced Cellular Stress (SICS) can be particularly harmful to fragile cells, often limiting the use of sorting technology to make workflows utilizing sensitive and fragile cells such as stem cells, nascent organoids, and even primary samples. In contrast to traditional electrostatic sorters, the NX One's microfluidic cartridge-based sorting approach is gentle on model cell types and sensitive samples alike. This advantage can be critical for monoclonal cell line and antibody development, as well as single-cell 'omics workflows, as fewer perturbations to the phenotype of the cells to be analyzed and even proliferated downstream are introduced.

This poster highlights the NX One's capabilities to uniformly, reproducibly, and accurately sort various single cells. **Using human induced pluripotent stem cells (iPSCs), we demonstrate that the NX One's microfluidic gentle sorting approach maintains the cell's intrinsic properties, such as pluripotency and ability to generate neuronal cell types**. Nodexus' cartridge-based approach offers contamination- and aerosol-free sorting for individual researchers and laboratories across the continuum of cell biology, ranging from **sorting and dispensing yeast (~2 µm) to nascent organoids (60 µm).**

NX One Platform Advantages

- Priming
- Automatic Stage Alignment
- Pressure, Flow Rate, and Drop Time Optimization

→ NX One **Auto-Tuning** **<2 minutes**



Traditional Sorter Setup & Clean >30 minutes

- Laser warmup: 30 min
- Fluidics Preparation: 5 min
- Optical Alignment and Calibration: 10 min
- Flow Rate, Drop Delay, and Sort Stream QC: 30 min
- Cleaning: 10 min

Consistent And Precise Sorting Across Various Conditions and Cell Types

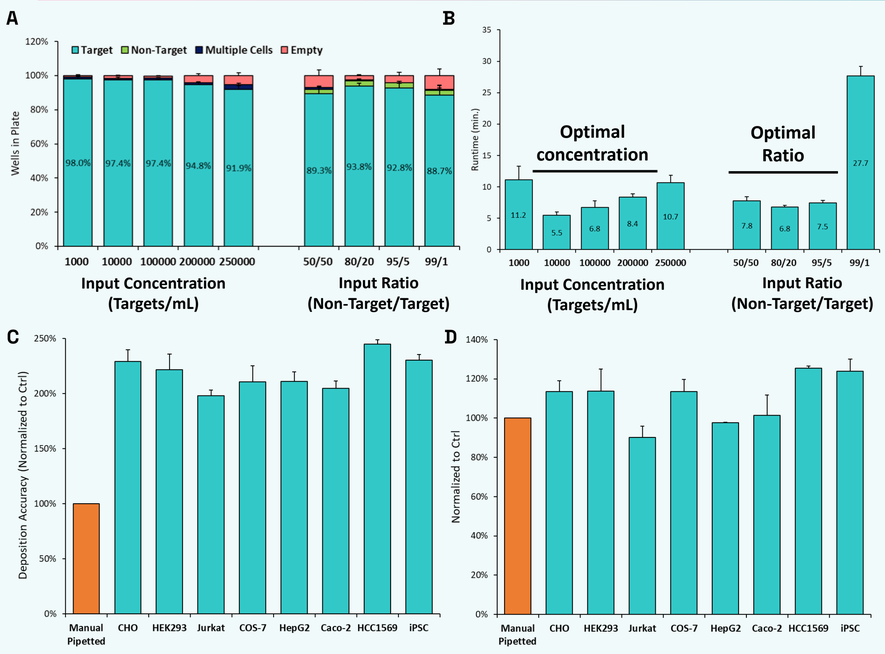
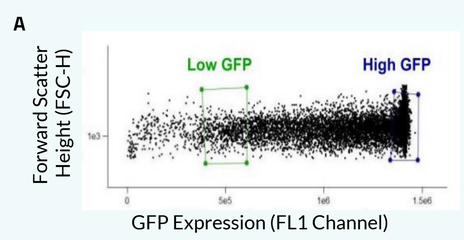


Figure 1. The NX One maintains superior sorting performance across varying concentrations and diverse cell types. (A-B) The NX One performs efficiently across input concentrations, ranging from 1,000 cells/mL to 250,000 cells/mL. The **optimal input concentration range is 10,000 cells/mL to 200,000 cells/mL**, based on high deposition accuracy (97.4%) and shorter duration (6.9 mins) required to finish the sort. Similarly, the NX One demonstrates precise sorting with varying input non-target-to-target ratios, ranging from 50:50 to 99:1.

(C) The NX One demonstrated **higher deposition accuracy (>2x)** when various cell types like adherent CHO, HEK293, COS7, HepG2, Caco-2, HCC1569, and iPSCs or suspension cells like Jurkat were sorted than the manual pipetted control. **(D)** The **gentle pressure of the NX One (<1 PSI sample)** enabled higher single-cell survival and colony formation, which were equal or superior to the manual pipetted control.

NX One Demonstrates Superior Performance in High-Growth Applications

Single-Cell Sort: Cell Line Development



30 ± 5 µm Spheroid Sort

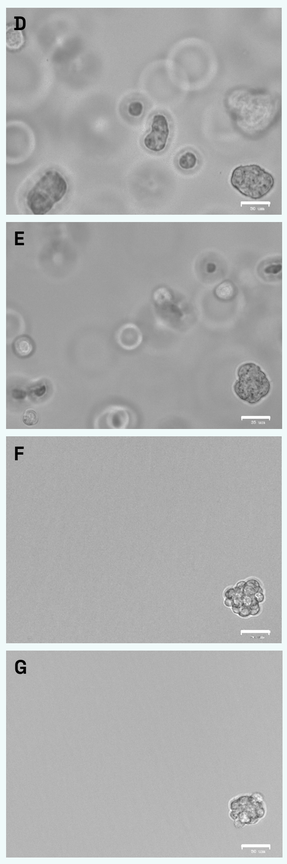


Figure 2. The NX One demonstrates versatility in sorting capabilities across various application areas. (A-B) Before sorting, the CHO-GFP cell line consisted of a heterogeneous population of cells based on the GFP expression profiles. Two sequential single-cell sorts targeting either high or low-GFP-expressing CHO cells resulted in **94% (B) and 83% monoclonal colony formation**, respectively. Cells were spiked into well A1 as an imaging control. **(C)** On DIV 15, colonies from each sort were selected based on their proliferation rate and expanded into daughter cell lines. Analyzing these developed cell lines on the NX One further validated the **generation of robust cell lines** based on GFP expression homogeneity. **(D-E)** 30 ± 5 µm spheroids were generated from the developed CHO-GFP cell line using VitroGel® HydroGel Matrix. **(F-G)** The gentle sorting of the NX One maintained the integrity of the spheroids even after the hydrogel matrix was dissolved, highlighting the **NX One's dynamic size detection range and large particle sorting capabilities**. Scale bar: 30 µm.

NX One Single-Cell Sorting Yields Significantly More iPSC Clones

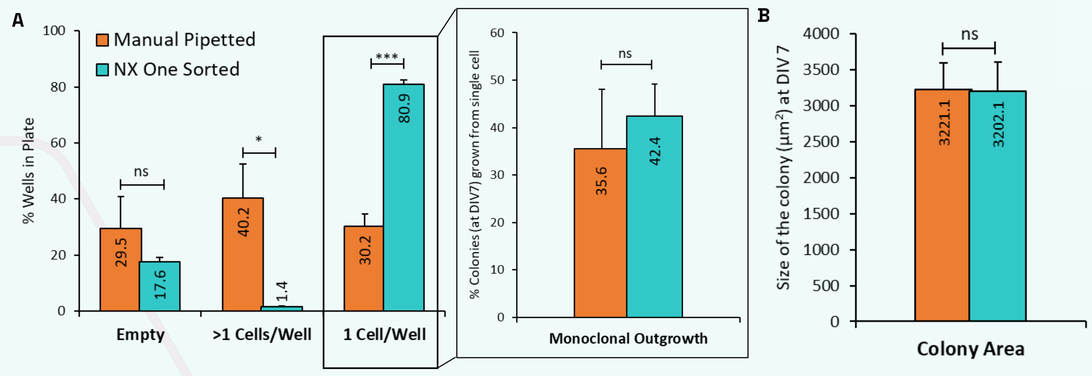
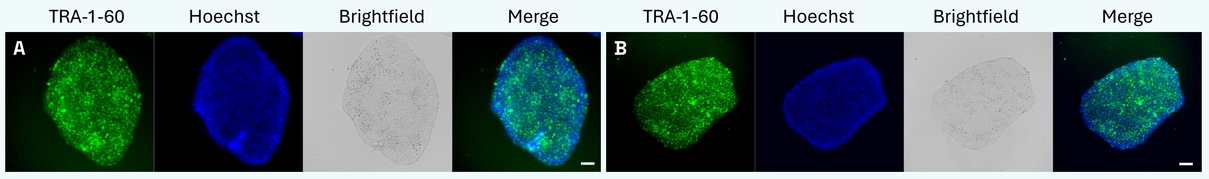


Figure 3. The NX One sorting workflow generates a significantly higher number of iPSC clones. (A) Undifferentiated iPSCs were labeled with the proliferation marker CytoTell UltraGreen before sorting into Matrigel-coated 96-well plates. The NX One demonstrated significantly **higher (2.6x) deposition accuracy** (80.9%) than the manual pipetted control (30.2%). Inset denotes the subsequent clonogenicity and outgrowth where the NX One is similar or superior to the manual pipetted control. **(B)** The gentle sorting approach of the NX One did not alter the proliferation potential of these sorted iPSCs, as a similar colony size was observed in both sorted and control conditions. Error Bars indicate SD; *p<0.05; ***p<0.0001; ns – not significant; two-way ANOVA.

NX One's Gentle Sorting Maintains Pluripotency in iPSCs



Cortical Neurons Differentiated from NX One-Sorted iPSCs

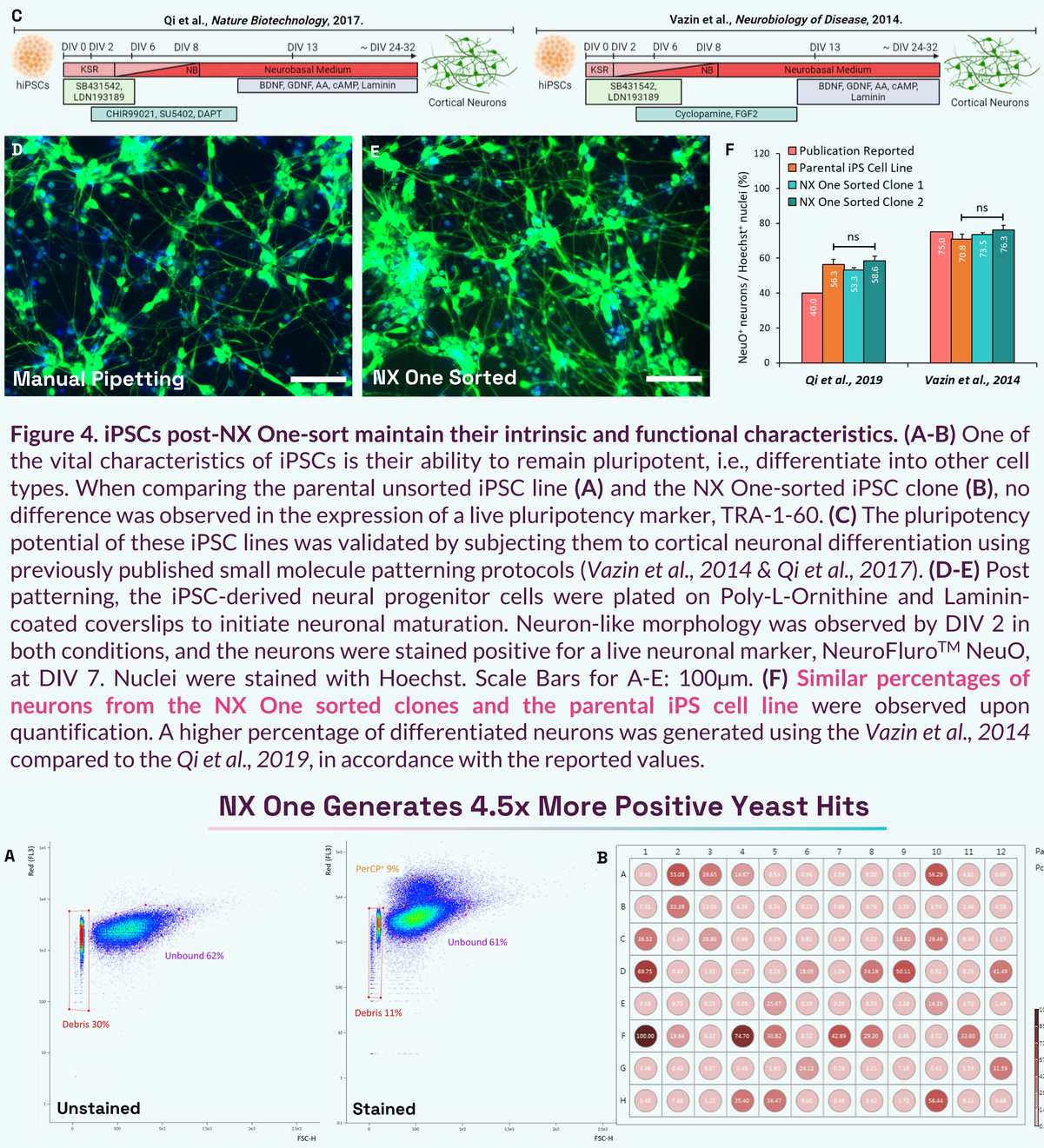


Figure 4. iPSCs post-NX One-sort maintain their intrinsic and functional characteristics. (A-B) One of the vital characteristics of iPSCs is their ability to remain pluripotent, i.e., differentiate into other cell types. When comparing the parental unsorted iPSC line **(A)** and the NX One-sorted iPSC clone **(B)**, no difference was observed in the expression of a live pluripotency marker, TRA-1-60. **(C)** The pluripotency potential of these iPSC lines was validated by subjecting them to cortical neuronal differentiation using previously published small molecule patterning protocols (*Vazin et al., 2014 & Qi et al., 2017*). **(D-E)** Post patterning, the iPSC-derived neural progenitor cells were plated on Poly-L-Ornithine and Laminin-coated coverslips to initiate neuronal maturation. Neuron-like morphology was observed by DIV 2 in both conditions, and the neurons were stained positive for a live neuronal marker, NeuroFluro™ NeuO, at DIV 7. Nuclei were stained with Hoechst. Scale Bars for A-E: 100µm. **(F)** **Similar percentages of neurons from the NX One sorted clones and the parental iPS cell line** were observed upon quantification. A higher percentage of differentiated neurons was generated using the *Vazin et al., 2014* compared to the *Qi et al., 2019*, in accordance with the reported values.

NX One Generates 4.5x More Positive Yeast Hits

Figure 5. The NX One efficiently sorts clones from yeast display libraries. (A) Yeast display is an effective method for screening protein binding affinity and is commonly used in antibody engineering. Conventional methods like colony picking and serial dilutions have low throughput, are highly labor intensive, and are nonspecific. The NX One accurately sorted individual PerCP-positive yeast clones into each well of a prefilled 96-well plate. **(B)** After DIV 14, 95% of the dispensed wells showed outgrowth. The post-screening protein binding assay demonstrated that 45% of the sorted clones expressed the construct of interest at varying degrees vs. a typical 12% positive using a random hand-picking method. This highlights the NX One system's **superior positive hit generation (4.5x) more positive clones per plate** compared to the conventional method, indicating the cost-effectiveness of the NX One by reducing the number of clones needed to be screened and generating more diverse clones.

Conclusion

- Plug-&-Play Sorting with "Smart" Auto-Tuning
- Gentle and Reliable Sorting Approach for Cell Lines, iPSCs, Yeast, & Large Particle Samples
- High Viability for Even Fragile Samples
- <10 min for 96-Well Plate Sorts Including Setup

References: Chambers et al., Nature Biotechnology, 2009; Mahajani et al., Cell Death & Disease, 2019; Qi et al., Nature Biotechnology, 2019; Vazin et al., Neurobiology of Disease, 2014.

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