Novel Dual-Parameter Live Single Cell Detection, Sorting, and Isolation Platform

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Abstract. Access to single cells has had a large and quickly growing impact for biomedical research due to interest in studying heterogenous populations, including those found in clinical samples. Bulk analyses only provide averaged population data, and more specific, often critical information in relevant subpopulations can be lost. However, at present, isolation and access to live single cells is challenging. Nodexus’ NX One utilizes a dual-parameter approach consists of patented Node-Pore Sensing (“NPS”) to offer superior sensitivity in conjunction with low-shear microfluidic sorting and single-cell dispensing. The platform offers functional (e.g. maximized cell viability and marker expression density), workflow, and cost benefits in the rapidly-emerging single-cell analysis space while preserving sample sterility, viability, and minimizing contamination.

1. Introduction

Access to single cells has had a large and quickly growing impact for biomedical research due to interest in studying heterogenous populations, including those found in clinical samples. Bulk analyses only provide averaged population data, and more specific, often critical information in relevant subpopulations can be lost. For instance, cancer is a function of mutations, selection, and clonal expansion, which result in different subclones within a single tumor. Subtle genomic signals from such subclones can be masked during bulk study and often require significant sequencing/computation resources for analysis. Access to single cells from these subpopulations can provide a means of acquiring this information in a more efficient manner as well as a method to gain additional insight. The growing drive toward personalized medicine can be accelerated with the development of tools that enable access to single cells for such analysis. However, at present, isolation and access to live single cells is challenging. Specifically, numerous industrial (including major biopharmaceutical companies) and academic researchers have reported that they face challenges with the existing technology solutions including: (1) serial dilution (low efficiency of error-free handling, low reproducibility, and high hands-on time), (2) FACS sorting (low cell viability, challenges with single-cell isolation if sample is heterogeneous, high cost, and need for skilled technician to constantly monitor the run in a Core Facility), and (3) antibiotic resistance e.g. puromycin-based selection (higher cell toxicity, and the same issues faced in 1 & 2 for single-cell separation). Ultimately, failure to isolate single-cells efficiently leads to inconsistent results between experiments and time-consuming, expensive repetition to reproduce stable single-cell derived lines – causing numerous issues for both production-level biopharma sites and individual academic or clinical researchers.
The majority of techniques for single-cell enrichment currently rely on encoded fluorescence detection (FACS or single-cell “picking” under a fluorescent microscope) or antibiotic (e.g. puromycin), but the challenges described above lead to inefficient and expensive selection. Researchers looking to perform agnostic, size-based, and/or fluorescence-based single-cell isolation are confined to using very high-end sorting instruments that are overbooked, expensive, and require extensive tuning/training while still suffering from cell viability, contamination, and sterility challenges. We envision widespread adoption of single cell isolation and analysis by enabling every lab to isolate transfected, primary, and/or stem (e.g. iPS) cells using a compact footprint, highly sensitive instrument with sterile single-use microfluidic cartridges – all at a low price-point affordable even for individual labs and using a “one-click, hands-off” workflow for ease-of-use.

Nodexus’ NX One utilizes a dual-parameter approach consists of patented Node-Pore Sensing (“NPS”) to offer superior sensitivity in conjunction with low-shear microfluidic sorting and single-cell dispensing. The instrument with single-use disposable cartridges will enable an end-to-end solution for academic, industrial (e.g. biopharma), and clinical researchers to isolate single cells. The platform offers functional (e.g. maximized cell viability and marker expression density), workflow, and cost benefits in the rapidly-emerging single-cell analysis space while preserving sample sterility, viability, and minimizing contamination.

2. Methods

The NX One platform incorporates Nodexus’ dual-parameter detection and single-cell isolation functionality to address pain points felt by researchers who seek an isolation tool that enables single-cell studies – currently a challenge with technologies on the market or in development. The system provides individualized cells in microtiter well plates while also providing a sharp reduction in false positives and negatives, improved measurement sensitivity and reduced photobleaching, improved cell viability/minimized differentiation due to low shear rates and phototoxicity.

2.1. Workflow Design

The workflow is outlined in Figure 1. Nodexus’ patented and exclusively licensed NPS12-18 module performs single-cell absolute sizing (not relative as in forward scatter), detection and morphology analysis (which can further distinguish single-cells from doublets) using high dynamic range impedance-sensing (permitting high variation of cell sizes in heterogeneous sample inputs). In
conjunction, Nodexus has added on a dual-parameter sensing mechanism that uses both NPS and marker detection to solve pain points in single cell workflows while also providing unprecedented price points and ease-of-use for individual researchers. Module 2 of our integrated system involves using data inputs from the detection module(s) to inform fluidic sorting to isolate cells actively (as opposed to stochastic isolation performed by other microfluidic single-cell systems). Moreover, we have developed single-cell dispensing into standard 12-, 96-, or 384-well microtiter plate outputs (Module 3) as desired. Collectively, modules 1 – 3 take samples and individually isolate viable single cells – with indexed cell and population statistics – all in an easy-to-use, compact footprint, and low-cost system. These isolated cells are then available for desired downstream analysis. The disposable cartridge nature of the NX One system ensures that no sample contamination occurs (i.e. samples never contact regions that prior samples touched).

Protocol and System Details:

**Figure 2.** (A) Microchannel design used in NPS30. (B) Signal of MCF-7 cell; throughput is 250µL/minute. Unique electronic signatures allow for refined sensitivity/specificity (software “gating”).

Node-Pore Sensing Detection (NPS): NPS is based on patented microfluidics (Fig. 2A)12 that allows for highly sensitive absolute counting, sizing, and morphological measurements of cells. As a cell enters the microchannel “pore”, it blocks the flow of current, leading to a transient decrease in the pore’s electrical current (Fig. 2B, circled) that contains a unique detection signature. Highlighted data of MCF-7 carcinoma cells in media is shown in Fig. 2B, highlighting superior dynamic range of NPS. Unlike Coulter sensing, NPS utilizes unique geometries to enhance detection sensitivity.17,18 It has also been shown that cells are not damaged (the shear stresses present are at least 100x less than those in flow cytometry or sorting)15. Further, cell clumps and clusters (e.g. doublets, triplets) can be distinguished from single cells using size and shape/morphology criteria that can be measured using NPS20. All testing described herein has been performed at a flow rate of 250 µL/minute.

Low-infrastructure NPS-activated marker detection:

**Figure 3.** Detection of MCF7 cells. Output plotted from NI VirtualBench.

Nodexus has developed and patented a novel detection methodology to work in conjunction with NPS to detect absolute cell counts, sizes, shapes, and marker/protein expression. Traditional microfluidic flow cytometers are typically “always on”, which can lead to deleterious effects on the samples tested.19 Nodexus’ approach allows for a sharp reduction in false positive rates, sensitive
measurement of expression density (i.e. normalized by absolute cell volume instead of forward scatter) as well as the novel ability to perform NPS-activated triggering. Dual-parameter sensing as shown in Fig. 3 has the advantages of superior sensitivity with both absolute cell sizing and volume-normalized fluorescence readouts for every single cell, a drastic reduction in false positive and false negative rates including accurately discriminating single cells from doublets or triplets, and an increase in cell viability due to minimized photobleaching effects.

Active Isolation and Single-Cell Dispensing is Following the detection module, single cells enter an isolation module, which utilizes flow switching to steer cells into a collection flow stream. Single-cells are diverted into the collection channels and ready for single-cell dispensing into microtiter well plates.

3. Result and Discussion

Based on the workflow and module integration described above, we have demonstrated the ability of the NX One system to accept cell suspensions and even complex, heterogeneous samples such as whole blood and with minimal or no pre-processing screen and sort target cells of interest based on marker/protein expression or size-based criteria. Figure 4 below demonstrates the accuracy of NPS-based size selection of MCF-7 breast ductal carcinoma cells spiked into whole blood at physiologically-relevant concentrations (approximately 100 cells/mL of blood), and subsequent recovery of >98% of target cells. Viability testing was performed and ~99% viability of MCF-7 cells after detection and sorting within the NX One system was demonstrated.

Figure 4. (A) Demonstration of integrated NPS and AI stages shows excellent signal-to-noise and (B) counts and size distribution of the cells of interest (MCF7 breast cancer cells). 98-100% of MCF7 cells spiked into whole blood are actively isolated and recovered post-AI module.

Maintenance of cell viability following detection, isolation, and dispensing in the NX One system
Figure 5. (A) Maintenance of high (>95%) cell viability across human MCF-7, BC3, and Jurkat cell lines. Control samples were taken from the input sample to the NX One cartridge and viability evaluated using Trypan Blue of both experimental and control samples following sorting/dispensing runs. (B) Head-to-head benchmarking was performed against the gold-standard BD Influx FACS instrument within the LKS Core Facility at UC Berkeley. The results of cell viability and isolation efficiency are shown, with the NX One outperforming the BD Influx in terms of single cell deposition rates and cell viability maintenance.

Figure 5 above highlights repeatably high (>95-99%) cell viability (testing using Trypan Blue dye and imagebased counting) across three diverse cell lines after flow through the NX One system’s detection, sorting, and dispensing modules. The NX One’s performance in maintaining cell viability and single-cell isolation efficiency is clearly favorable compared to gold-standard instrumentation such as the BD Influx fluorescence-activated cell sorter. In terms of isolation efficiency, >85% of wells in a microtiter plate had a single target cell after dispensing by the NX One as opposed to ~65% on the Influx, and cell viability following operation of the NX One was nearly unchanged from the control samples (>99%), while the cells following the Influx were <75% viable. For the key performance metrics that are critical for a cell screening and isolation too, the NX One system excels while offering unprecedented cost and ease-of-use to even individual researchers.

4. Conclusion
Single-cell analysis is poised to become a cornerstone of biomedical research, clinical diagnostics, and truly personalized medicine. Access to single-cells is critical for insights into heterogeneous cancer biology, efficient drug targeting and development, and gene editing workflows (e.g. CRISPR engineering clonal isolation). The cell line development/gene editing, stem cell, tumor heterogeneity,
drug discovery, and immunotherapy markets have experienced rapid growth especially in the single-cell analysis sector.

With the cost and labor constraints faced by individual researchers, the purchase of a “personal” single cell isolation instrument is often not feasible – leaving only the option of overbooked and expensive Core Facilities, whose instruments often detrimentally impact cell viability, lead to contamination, and have issues with single-cell isolation on heterogeneous cell types. In addition, because the desired output is live single cells, it is not possible for these researchers to send out their samples to outsourced facilities, as shipping these precious samples is often impossible. We envision that our platform will allow even individual labs to purchase their own system, allowing institutional customers that previously had to rely on high-infrastructure FACS sorters with single-cell dispensing units (uncommon and very expensive), automated liquid handling systems, or manual serial dilution new options for improving their research efficiency and reproducibility. We foresee NPS-based detection, sorting, and dispensing becoming a powerful tool in the arsenal of cell biologists in the future.