High-throughput single-cell whole transcriptome analysis of various blood cell types including neutrophils using a microwell-based system

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Abstract

Microfluidic technologies have been developed and used for single-cell analysis over the past decade, and devices have been developed to support higher throughput single-cell multiomics analysis. A microwell-based system that relies on gentle settling of cells in microwells was used for this study, which made it flexible to capture a wide range of cell sizes ranging from 5 µm to 20 µm as well as a magnitude of different cell types including fragile cells such as neutrophils. In this system, the microwell cartridge has eight lanes instead of one to increase the maximum throughput of cell capture. In this study, we tested the feasibility of the eight-lane cartridge for analysis of various cell types and compared the performance with the current single-lane cartridge. In brief, neutrophils, NK cells and T cells were isolated and loaded in two separate lanes of the eight-lane cartridge to show reproducibility of data from multiple lanes. Cartridge metrics showed a capture rate of >60% from viable cells loaded in the cartridge, which showed comparability to the current single-lane cartridge. In addition, whole transcriptome analysis of the same samples loaded in separate lanes showed high correlation (R^2 > 0.95) of gene expression detection. This study demonstrated the flexible application of the eight-lane cartridge in terms of the capture of various cell types. We also demonstrated feasibility of use for specific research designs based on individual researcher needs while keeping similar performance with the single lane. The consistency of results between multiple lanes also supports high-throughput single-cell analysis using the eight-lane cartridge without batch effect.

Methods

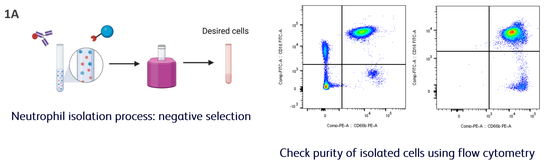


Figure 1. **(A)** Neutrophils in whole blood were isolated using a column-free immunomagnetic strategy and the purity was evaluated with a three-color flow cytometry panel using the BD FACSLyric™ Flow Cytometer. **(B)** T cells and Natural Killer (NK) cells were simultaneously sorted from frozen Peripheral Blood Mononuclear Cells (PBMC) with fluorescent antibodies using the BD FACSAria™ II Cell Sorter. **(C)** Single cells were loaded and captured using the BD Rhapsody™ HT Single-Cell Analysis System and libraries were generated for sequencing with the BD Rhapsody™ WTA Amplification Kit. Neutrophils were isolated and captured using an 8-lane cartridge previously used to capture T cells and Natural Killer cells on different lanes.

8-Lane Cartridge Capture of Fragile Cells and Whole Transcriptome Analysis

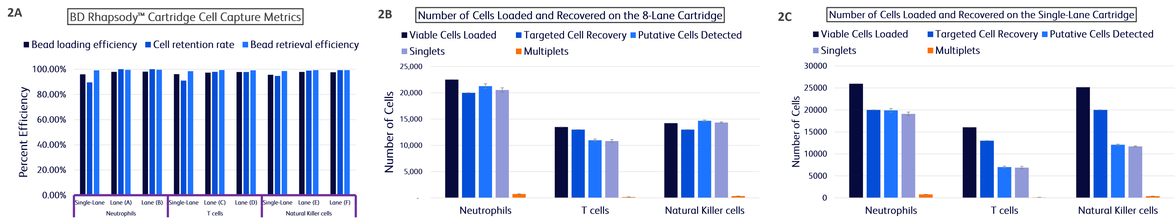
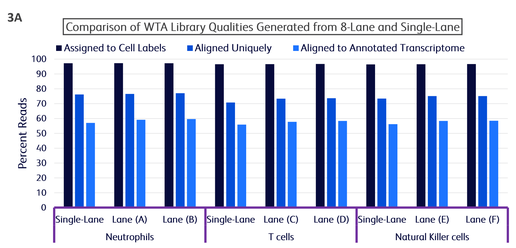


Figure 2. Two lanes each of the 8-lane cartridge were loaded to capture Neutrophils, T cells and NK cells. To demonstrate partial use of the 8-lane cartridge, T cells and NK cells were captured on the first use, and Neutrophils were captured subsequently after storage of the partially used cartridge. BD Rhapsody™ Cartridge cell capture metrics showed that the bead loading efficiency, cell retention rate and bead retrieval efficiency were comparable between the 8-lane cartridge and the current single-lane cartridge **(2A)**. A separate single-lane cartridge was used to capture each of the individual fragile cell type. In addition, the number of cells loaded and recovered on the 8-lane cartridge **(2B)** and the single-lane cartridge **(2C)** were also comparable. Data also showed that partial use and storage of the 8-lane cartridge did not affect cell capture performance.



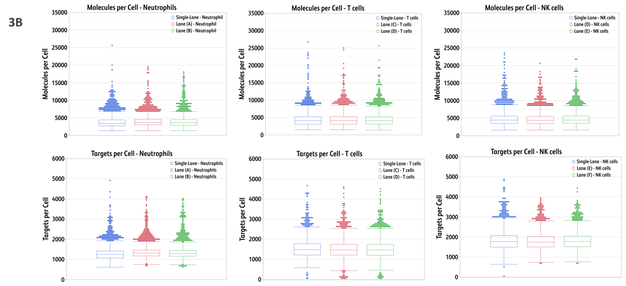


Figure 3. Libraries were constructed using the BD Rhapsody™ WTA Amplification Kit. WTA library qualities generated from the 8-lane cartridge were comparable to the single-lane cartridge **(3A)**. These include percent reads assigned to cell labels, reads aligned uniquely, and reads aligned to annotated transcriptome. At ~17,000 reads per cell, similar molecules and targets per cell were detected from the different cell lines captured using both types of cartridges **(3B)**.

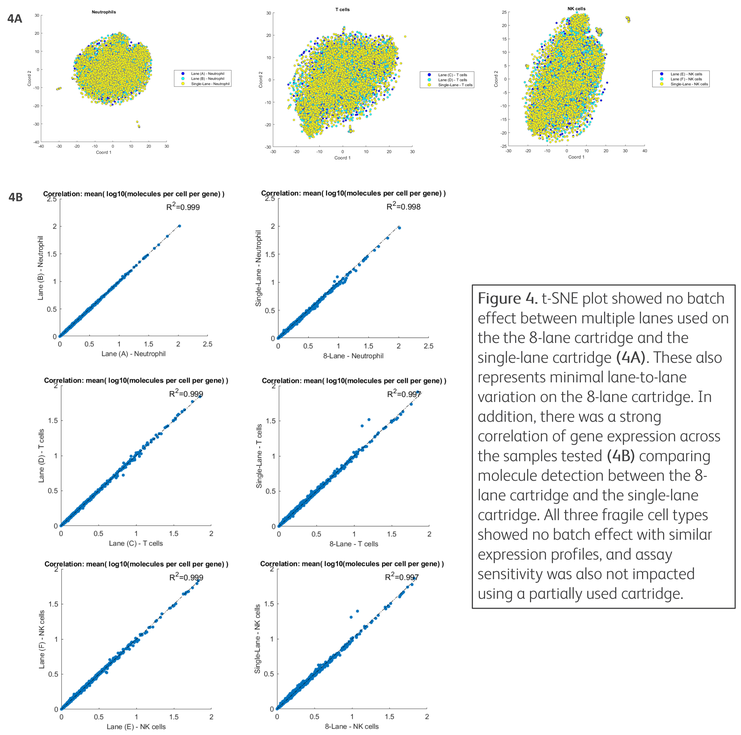


Figure 4. t-SNE plot showed no batch effect between multiple lanes used on the the 8-lane cartridge and the single-lane cartridge **(4A)**. These also represents minimal lane-to-lane variation on the 8-lane cartridge. In addition, there was a strong correlation of gene expression across the samples tested **(4B)** comparing molecule detection between the 8-lane cartridge and the single-lane cartridge. All three fragile cell types showed no batch effect with similar expression profiles, and assay sensitivity was also not impacted using a partially used cartridge.

Single-Cell Gene Expression Analysis from Captured Fragile Cells

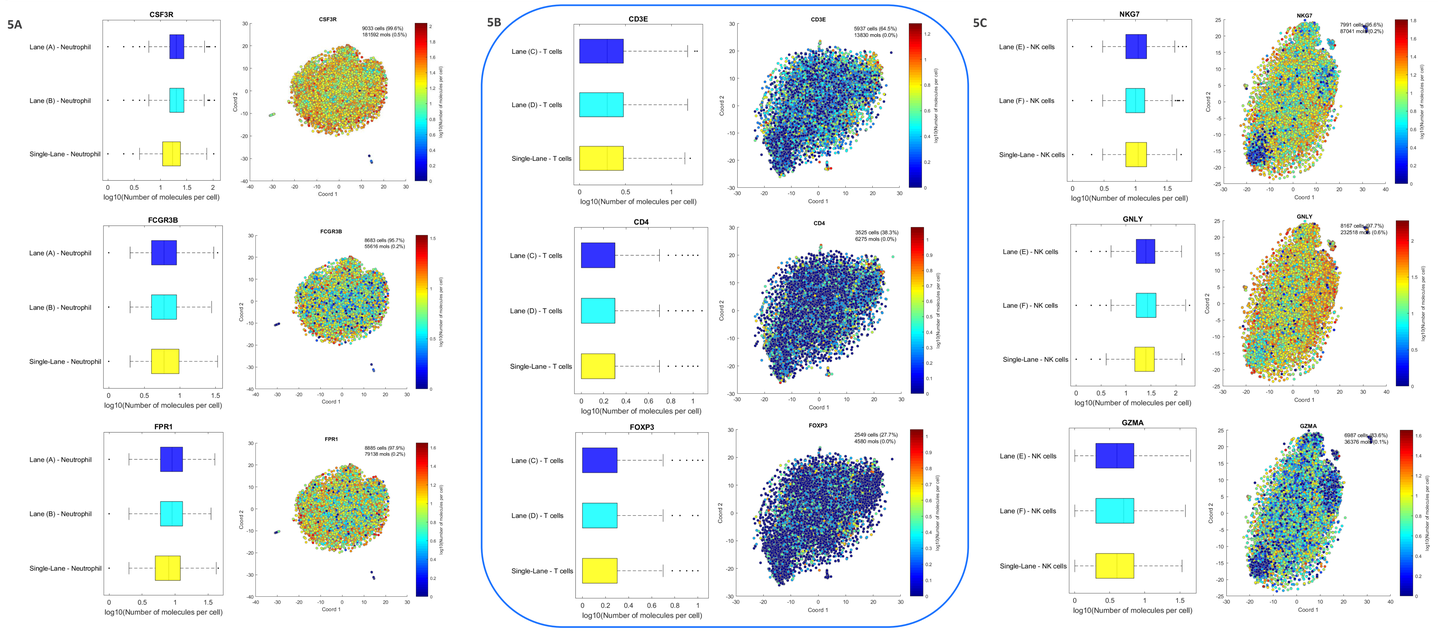


Figure 5. Neutrophil samples from both the 8-lane and single-lane cartridges had a high expression of gene expression such as CSF3R, FCGR3B and FPR1 suggesting successful cell enrichment and cell capture of Neutrophils **(5A)**. Gene expression detection on the T cell sample was consistent with the sorting strategy of regulatory T cells **(5B)** with detection of CD3E, CD4 and FOXP3 genes. NK cell-associated genes such as NKG7, GNLY and GZMA were detected on the NK sample **(4B)**. Box plots associated with gene expression also showed comparable detection between both cartridges and minimal lane-to-lane variability on the 8-lane cartridge.

Conclusions

- Fragile cells such as Neutrophils, T cells and NK cells were successfully isolated and captured using the BD Rhapsody™ 8-Lane and Single-Lane Cartridges.
- The number of cells loaded and recovered were comparable between the BD Rhapsody™ 8-Lane and Single-Lane Cartridges.
- Similar number of molecules and targets per cell from the WTA assay were detected using the BD Rhapsody™ 8-Lane and Single-Lane Cartridges.
- No batch effect was observed on the cell types tested, and correlation of gene expression was R^2 = 0.99.
- Detection of expressed genes from cells captured showed dependable capture and profiling of fragile cells on the BD Rhapsody™ HT Single-Cell Analysis System.
- Minimal lane-to-lane variability was observed on the 8-lane cartridge and performance was not impacted by partial-use storage.