

FACILE IMAGE-BASED CELL SORTING USING OPTO-FLUCS (OPTO-FLUIDIC CELL SORTING)

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Abstract

We present a new approach for sorting cells based upon visual information, termed opto-fluidic cell sorting (OPTO-FluCS). This technique uses a combination of hydrodynamic and optical scattering forces to array, image, and then sort many individual cells. Our device combines the strengths of microscopy—imaging in space and time—with the ability to array cell populations and isolate cells of interest in an intuitive, user-friendly fashion. We successfully demonstrate image-based sorting based on fluorescence localization, a sort unavailable with flow-assisted cell sorting.

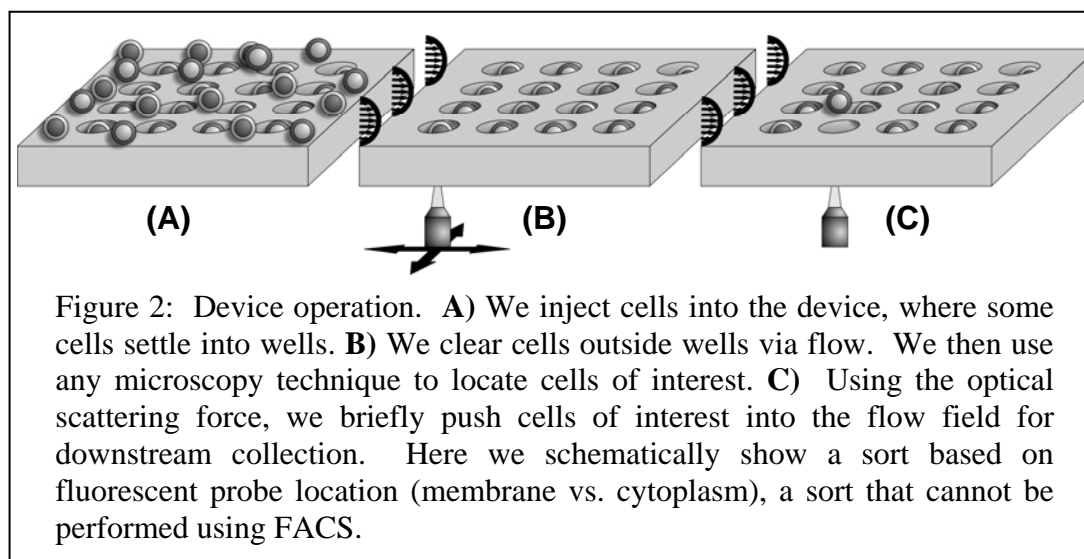
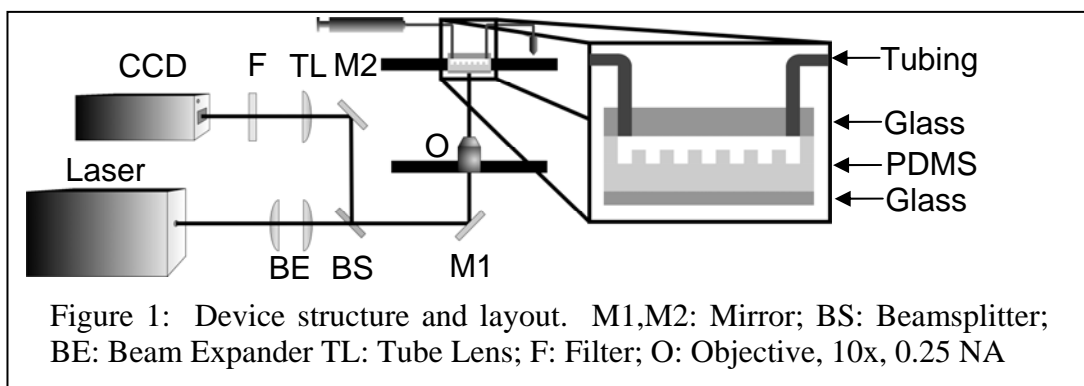
Keywords: Cell sorting, microwell array, optical manipulation, optofluidics

1. Introduction

We have developed technology to sort cells based upon visual information. Our opto-fluidic architecture obtains sorting information through microscopy, predicating sorts on temporal and spatial behavior of cells. While flow-assisted cell sorting (FACS) allows higher throughput, it bases sorts on whole-cell fluorescence at a single timepoint. Our approach scales effortlessly, requiring no electrical interconnects or support electronics to address cells [1]. Sorting of a single cell takes seconds, allowing for collection of larger numbers of cells than is practical with micropipette/micromanipulator systems [2]. While laser capture microdissection (LCM) has been used to sort non-viable cells from microwell arrays [3], its live-cell sorts require the use of proprietary films. Our opto-fluidic array architecture scales effortlessly to array sizes in excess of 20,000 sites, contains no interconnects, allows for diverse surface functionalization, requires seconds to remove a cell of interest, and provides for simple retrieval of released viable cells.

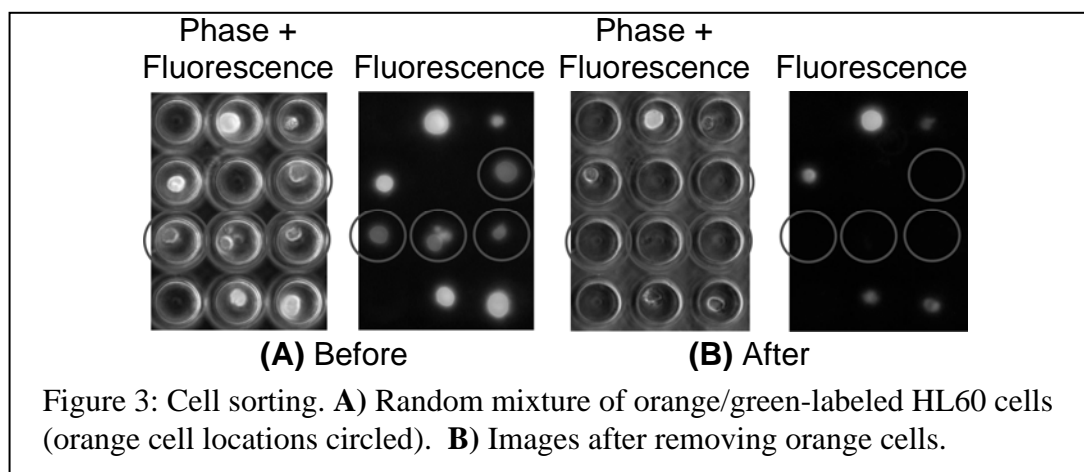
2. Architecture Design

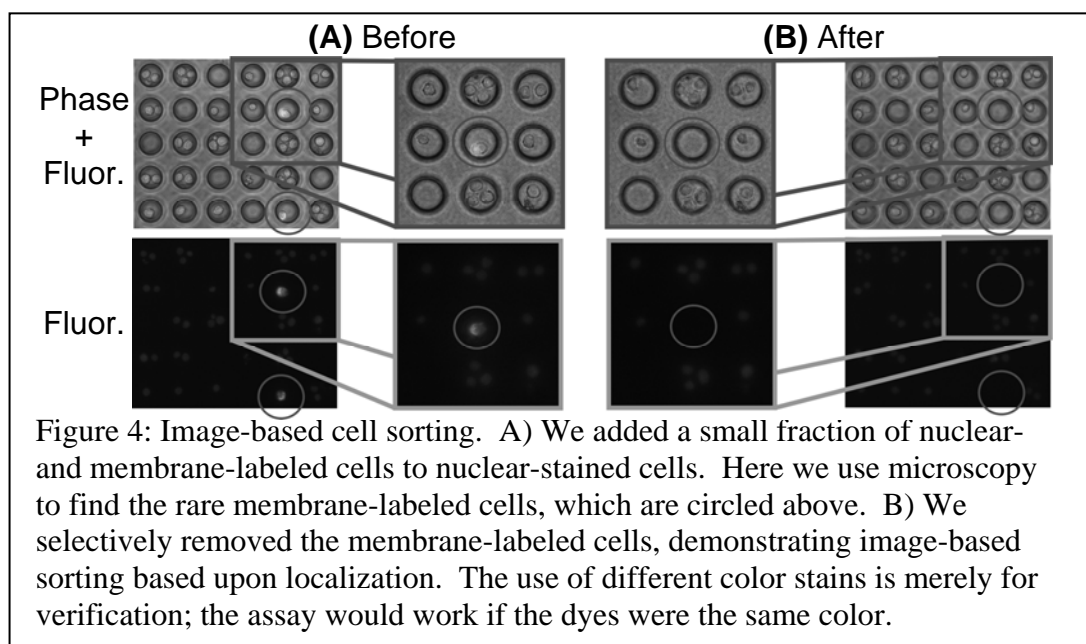
There are two important elements to our system. First is a microfluidic chamber (Figure 1) with a PDMS well array that we use for simple passive cell loading via sedimentation and subsequent flushing (Figure 2A-B). After visualizing cells using any desired microscopy technique (phase, fluorescence, etc.), we note the positions of cells of interest. Second, we use a straightforward optical system to couple a laser onto the imaging axis. We remove a target cell by focusing the laser through an inexpensive objective onto the cell; the optical scattering force pushes the cell into the flow (Figure 2C). The optical intensity and energy-density levels that we apply to cells are orders of magnitude below those found harmful in conventional, high-numerical aperture optical tweezers, and thus our approach is unlikely to damage cells [4].



3. Results and Discussion

As a first demonstration, we loaded orange- and green-labeled HL60 cells randomly into the device, imaged them, and then sorted out the orange-labeled cells (Figure 3).





We demonstrated true image-based sorting by loading the device with nuclear-stained cells and a small fraction of nuclear- and membrane-stained cells (Figure 4). Using microscopy, we identified the cells with membrane stain and sorted them from the cells only labeled with nuclear stain. This demonstrates image-based sorting based upon localization, an assay that cannot be performed using flow cytometry.

4. Conclusions

Our results demonstrate the feasibility of sorting cells via microscopy in a user-friendly fashion. The simplicity of the optical system allows for straightforward incorporation of the system into widely used microscopes. Using this novel architecture, we aim to sort cells predicated on complex phenotypes in realms where traditional flow-assisted cell sorting (FACS) falls short.

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References

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