Holographic imaging flow cytometry using three-dimensional microfluidic hydrodynamic focusing

Abstract

Holographic flow cytometry (HFC) is a cell-analysis technique that employs digital holographic microscopy (DHM) to image and classify cells flowing in a microfluidic device. The current HFC techniques are based on phase reconstruction followed by numerical refocusing as pre-processing steps. The numerical refocusing method to retrieve the phase images is computationally expensive and, hence, limits the speed of current HFC techniques. Moreover, while applying numerical refocusing algorithms, if an individual object is far from the nominal plane that is in focus while recording the digital hologram, the corresponding object field may be highly blurred, and there is a possibility of missing such objects completely. As a result, the accuracy of classification and quantification of cells is affected. The accurate and rapid classification and quantification in HFC require the randomly suspended cells in the inlet stream to be three-dimensionally focused into a single file arrangement. Current three-dimensional (3D) sheathing devices focus the cells at the midplane of microchannels, necessitating the fabrication of devices with smaller depths through precise methods to keep cells within the working distance of the microscope objective. Furthermore, existing approaches for 3D hydrodynamic focusing using sheath flows require microfluidic devices with multi-layer construction, introducing complexity to the fabrication process and escalating device costs. As microfluidic devices for HFC are discarded after a single use to prevent contamination, it is imperative to lower the cost of 3D sheathing devices through uncomplicated designs and low-cost fabrication techniques without affecting functionality.

Firstly, we demonstrate the design, fabrication, characterization, and testing of a sheath-flow based microfluidic device for 3D hydrodynamic focusing of cells. Our device hydrodynamically focuses the cells in a single-file near the bottom wall of the microchannel which allows imaging cells with high magnification and low working distance objectives, without the need for small device dimensions. The relatively large dimensions of the microchannels enable easy fabrication using less-precise fabrication techniques, and the simplicity of device design avoids the need for tedious alignment of various layers. We have characterized the performance of the device with 3D numerical simulations and validated these simulations with experiments of hydrodynamic focusing of a fluorescently dyed sample fluid. The simulations show that the width and the height of the 3D focused sample stream can be controlled independently by varying the heights of the main and side channels of the device and the flow rates of sample and sheath fluids. Based on simulations, we provide useful guidelines for choosing the device dimensions and flow rates for focusing cells of a particular size. Thereafter, we demonstrate the applicability of our device for imaging a large number of red blood cells using brightfield microscopy.

Next, we demonstrate an HFC technique using 3D hydrodynamic focusing for accurate visualization, classification, and quantification of the cells from a mixture. Our HFC approach uses high-resolution, single-shot digital holographic microscopy to image flowing cells in a microfluidic device that orders the cells in a single file close to the bottom wall of the channel. The integration of a 3D focusing device with DHM allows high-magnification holographic imaging without the need for numerical refocusing. Moreover, the specially designed 3D sheathing device prevents the clustering of cells. To demonstrate the efficacy of our HFC method, we consider a challenging case of classification from a mixture of unstained red blood cells and polystyrene particles, which are otherwise indistinguishable in brightfield and phase-contrast microscopy. Through experiments with cell-particle mixtures with varying proportions, we show that our HFC technique can precisely count and classify the cells and particles based on their reconstructed phase values while avoiding computationally expensive and time-consuming numerical refocusing. We also discuss the choice of the region of interest and camera frame rate so as to image each cell and particle individually in our device.

Lastly, we demonstrate the application of our HFC approach for specific biological studies. We commence with presenting our HFC method for the classification of unstained trypsinized flowing MDA-MB-231 and ZR-75-1 breast cancer cells in suspension. The classification of MDA-MB-231 and ZR-75-1 cells serves as a challenging problem to showcase the capability of our HFC approach because these cells are indistinguishable under brightfield and phase-contrast microscopy without staining. Furthermore, we demonstrate the capability of our HFC approach for drug-susceptibility testing of cancer cells while avoiding numerical refocusing and staining. This is in contrast to conventional approaches for drug susceptibility assessment, wherein numerical refocusing and staining of cancer cells is necessary to visualize and estimate the proportion of live and dead cells in suspension. The findings of the work presented in this thesis can address a major limitation of HFC to compete with other high-throughput cell imaging and classification techniques. Our HFC method of cell classification opens the arena for its usage in various disciplines, including intelligent healthcare, medicine, and biophysics.