ABSTRACT

Flow cytometry is an indispensable tool for identification, classification and enumeration of single cells/particles in biomedical applications like pathology, immunology, gene sequencing, chromosome sorting, biodefence and disease diagnosis. Flow cytometers represent a classic example of multidisciplinary instrumentation involving integrated optical, mechanical and electronic modules in synchronous operation, and are indeed a gold standard for chronic disease diagnosis. However, the bulkiness and high cost limit their use in state-of-the-art research laboratories and high-end clinical settings by skilled personnel. Recent advances in solid-state optics and durable micro-fabrication techniques have inspired scientists and engineers to design micro-flow cytometers that are portable, easy-to-operate and cost effective. A conventional flow cytometer comprises of a nozzle system that aligns the input sample cells/particles into a focused stream of single-cells/particles surrounded by a stream of sheath fluid. Sample focusing by this method occurs as a result of differential pressure between the sample and the sheath stream. This pressure-driven particle focusing technique is termed as sheath flow-assisted hydrodynamic focusing. Most miniature flow cytometers also incorporate the same technique for focusing of cells/particles in a microfluidic channel wherein the sample is directed through the main channel and the surrounding sheath fluids are directed into the main channel through inlets on either side of the main channel. This method narrows down the sample stream width, but it requires additional modifications such as changes in the microfluidic channel geometry or active sources, for achieving a single-line focused stream of cells in the outlet channel. Thus, multiple inlet and outlet ports add complexity to the microfluidic set-up and limits reproducibility. Moreover, the presence of sheath fluid results in sample dilution and reduction of the overall throughput of the lab-on-a-chip flow cytometer. The second hydrodynamic focusing technique used for particle focusing in microfluidic channels is based on inertial forces. This sheathless particle focusing technique usually employs a single inlet and single outlet microfluidic channel design. Nevertheless, it requires hybrid channel geometries to reduce multiple equilibrium positions to a single position in three dimensions. Thus, the first objective of the work presented in this thesis is to design a novel microfluidic channel that is able to focus randomly distributed cells/particles at the inlet to a single equilibrium position at the outlet using the sheathless, inertial forces-based hydrodynamic focusing technique. The proposed microfluidic channel geometry is a uniform square cross-section, single

inlet and single outlet microchannel, comprising a series of radially increasing semi-arcs interleaved by linear sections, which is used to focus randomly fed cells/particles at the inlet to a single equilibrium position at the outlet. The entire geometry is symmetric from the midline of the microchannel. To our knowledge, this is the first sheathless microfluidic chip with a uniform cross-section throughout the microchannel length to achieve a stable single position focusing for spherical particles/cells with diameters from 10 µm to 30 µm at fast flow rates (up to 700 µl/min). Single position focusing for spherical particles/cells that satisfy the condition $0.1875 < a/w < 0.9$ (where, a – diameter of the spherical particle/cell and w – width of the proposed microchannel) for $10 < Re < 150$ is achieved. We present a lab-on-a-chip based miniature flow cytometer that incorporates our proposed microfluidic chip integrated with tapered optical fibers for interrogating the focused cells/particles.

Nanaoassays are imperative to extract any morphological or biological information from flow cytometers. In the context of disease diagnosis, nanoassays are extensively used for providing and enhancing the signals from biomolecules. Drug delivery, disease progression and prognosis is studied using nanoassays designed using fluorescent beads, organic dyes, quantum dots, variable sized-colored beads, etc. However, these assays cannot be used as simultaneous carrier (biomolecules/drug/gene delivery) and signal providers (output/fluorescence signal). Thus, the second objective of this work is to develop alternative non-toxic, stable and biocompatible nanoassays that can be used for both biomolecule carriers and signals analysis for cancer diagnosis and therapy. We have investigated the feasibility of using gold nanorods (AuNRs) and biodegradable charged polyester-based vectors (BCPVs) as gene delivery nanovehicles and signal providers in pancreatic cancer cells and chronic myeloid leukemia (CML) cells, respectively. Following this, flow cytometer has been used to the measure the performance indicators- gene transfection efficiency and cell apoptosis, and evaluate the effectiveness of the proposed nanoassay. The excellent gene knockdown performance (over 81%) of the proposed model support *in vivo* trials for RNAi-based cancer theranostics. Post transfection with AuNRs-siRNA nanoprobes, the 14-16 µm pancreatic cancer cells can be readily focused in our engineered portable flow cytometry set-up, and the transfection efficiency can be determined by measuring the output fluorescent signal. The lab-on-a-chip flow cytometer offers distinct advantages when compared with the conventional bulky flow cytometer: lower volume of samples is needed, fewer sample preparation steps, cartridge-type one-off use chip, easier handling and operation, and low cost.