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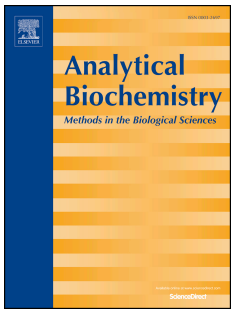

A multi-layer microchip for high-throughput single-cell gene expression profiling

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A MULTI-LAYER MICROCHIP FOR HIGH-THROUGHPUT SINGLE-CELL GENE EXPRESSION PROFILING

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ABSTRACT

Microfluidics or Bio-MEMS technology offers significant advantages for performing high-throughput screens and sensitive assays. While, the ability to correlate single-cell genetic information with cellular phenotypes is of great importance to biology and medicine, as it holds the potential to gain insight into disease pathways that is unavailable from ensemble measurements. Previously, we reported two kinds of prototypes for integrated on-chip gene expression profiling at the single-cell level and the throughput has been designed to be six. In this work, we present a five-layer microfluidic system for parallelized, rapid, quantitative analysis of RNA templates with low abundance at the single-cell level. The microchip contains two multiplexors and one partitioning valve group, and leverages a matrix (6×8) of Quantitative Reverse Transcription Polymerase Chain Reaction units formed by a set of parallel microchannels concurrently controlled by elastomeric pneumatic valves, thereby enabling parallelized handling and processing of biomolecules in a simplified operation procedure. A comprehensive metallic nanofilm with passivation layer is used to run PCR temperature cycles. To demonstrate the utility of the approach, artificial synthesized RNA templates (XenoRNA) and mRNA templates from single cells are employed to perform the 48-readout RT-qPCRs. The PCR products are imaged on a fluorescence microscope using a hydrolysis probe/primer set (TaqMan®). Fluorescent intensities of passive reference dye and a fluorescein amidite reporter dye are acquired and measured at the end of PCR cycles.

Keywords: Bio-MEMS; Integrated RT-qPCR; High Throughput Single-Cell Analysis

1. INTRODUCTION

One of the biggest challenges in gene expression research is the heterogeneity existing in most biological samples. Conventionally, gene expression measurements are performed on groups of cells from organs, tissues or cell cultures, under the assumption that all cells are similar and the expression profiles of individual cells are unique. Although, cells are often morphologically identical, recent studies reveal that gene expression levels of individual cells in a population varies dramatically according to environmental conditions, cell-division cycle, epigenetic and stochastic differences