# A WORLD-TO-DIGITAL MICROFLUIDIC INTERFACE FOR TOTAL RNA EXTRACTION FROM BLOOD SAMPLES

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## ABSTRACT

While clinical transcriptomics methods vary widely, a common characteristic is the need for chemical processing of extremely heterogeneous fluids (e.g., whole blood) prior to analysis. Here, we introduce the first world-to-digital microfluidic (DMF)-based method for chemical processing of RNA in blood. This sample-to-answer system has great potential for rapid characterization of novel and emerging pathogens from clinical samples.

**KEYWORDS:** Digital microfluidics, world-to-chip interface, sample preparation, RNA extraction, clinical transcriptomics

## INTRODUCTION

Clinical transcriptomics has emerged as an important discipline, promising the discovery of new biomarkers that will be useful for early diagnosis and prognosis of disease [1]. Here, we introduce the first world-to-digital microfluidic (DMF)-based method for chemical processing of RNA in blood. Methods were developed to extract total RNA (via magnetic beads) from real-world volumes of human blood samples, followed by purification of the extracted analytes. The new method is fast and automated relative to conventional methods (pipet, centrifuge, etc), inexpensive and multiplexed, and is capable of yielding high-quality total RNA (integrity number >7) which is essential in many downstream molecular biology applications (e.g., real-time-PCR, microarray analysis, RNA-Sequencing, etc.). This work is an important first step in our efforts to develop fully automated methods for biomarker discovery (i.e., clinical transcriptomics).

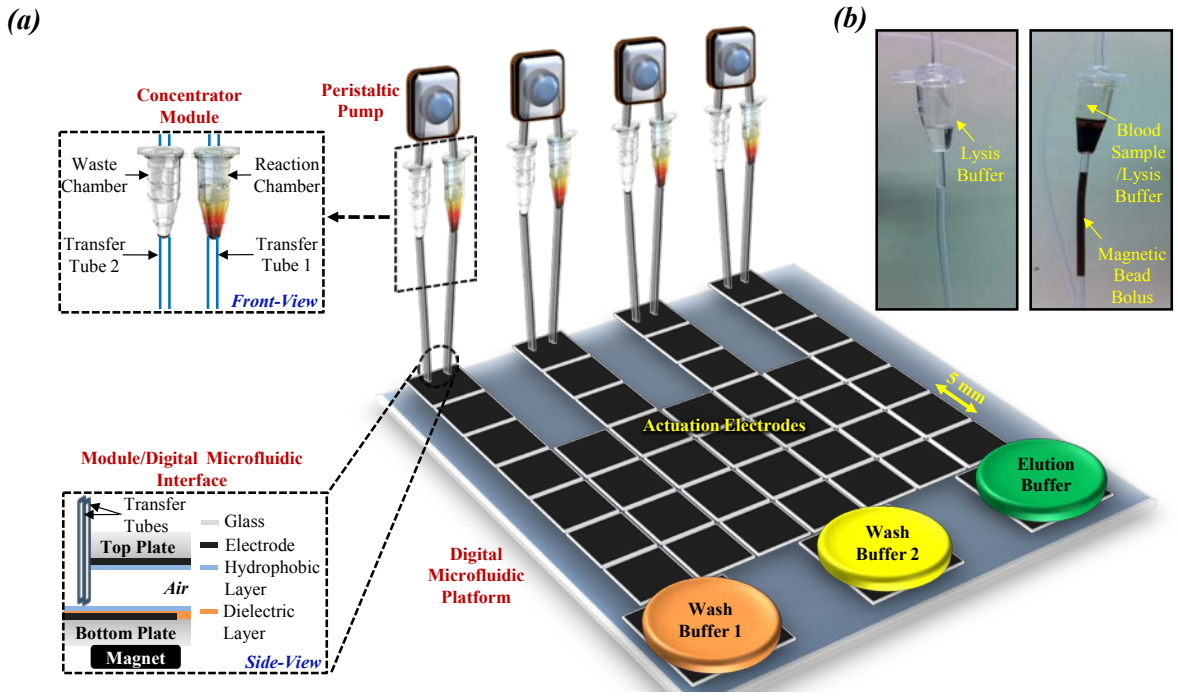
## EXPERIMENTAL

DMF devices were fabricated in the Sandia National Laboratories Applied Biosystems Laboratory cleanroom facility, using a transparent photomask printed at Photo Sciences (Torrance, CA). Glass devices bearing patterned chromium electrodes were formed by photolithography and etching as described previously [2], and were coated with 5 µm of Parylene-C and 50 nm of Teflon-AF.

For RNA analysis, Agilent 2100 Bioanalyzer System (Santa Clara, CA) was used with a high-sensitivity RNA kit. For RNA quantification, Thermo Scientific NanoDrop 2000 (Wilmington, DE) was used.

## RESULTS AND DISCUSSION

The world-to-chip method incorporates a digital microfluidic (DMF) platform mated to a peripheral macroscale module via the space between the two DMF plates as shown in Figure 1a.



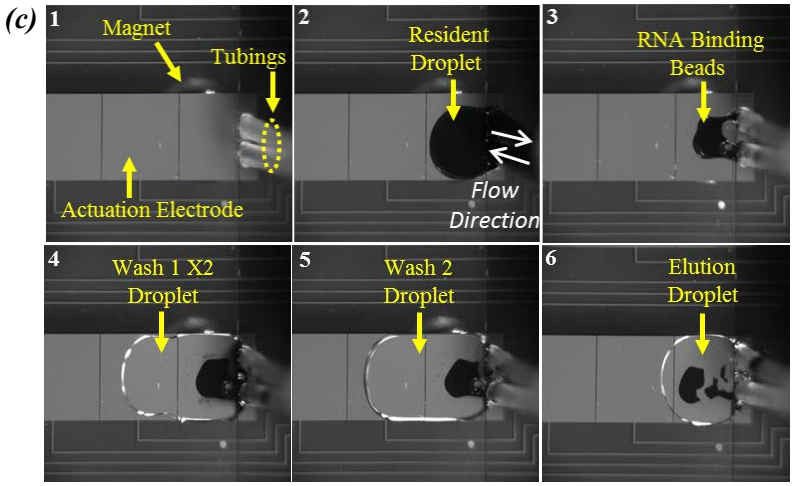



***Figure 1: Total RNA extraction from real-world volumes of blood samples using world-to-digital microfluidic (DMF) interface.*** ***(a)*** *Schematic of concentrator modules interfaced to a DMF device for RNA extraction from four blood samples simultaneously.* ***(b)*** *Images from a movie (front-view) depicting lysis of blood sample in reaction chamber of a concentrator module. (1,2) Blood sample (25 – 100 µL) is mixed with lysis buffer (130 µL) for 1 min, and reaction mixture is then mixed with capture magnetic bead solution (20 µL).* ***(c)*** *Sequence of frames from a movie (left-to-right) depicting several stages in RNA extraction on DMF device: (1) Module – DMF interface; (2) mixing was achieved by passing reaction mixture (back-and-forth) between the reaction and waste chambers (for 5 min) using DMF device as a bridging point; (3-6) RNA was isolated by immobilizing RNA-binding magnetic beads to DMF surface using magnet, aspirating supernatant to waste module, rinsing bead pellet with droplets of wash buffers 1 and 2 (10 µL ea.), and finally reconstituting RNA in a droplet (10 µL) of elution buffer for analysis off-chip.*

In DMF, discrete microliter-size droplets are controlled (i.e., moved, merged, mixed, and dispensed from reservoirs) by applying a series of electrical potentials to an array of patterned electrodes coated with a hydrophobic insulator [3,4]. DMF has recently become popular for biochemical applications [5] because of the benefits of reduced sample size and analysis time and the potential for multiplexed analysis. Figure 1b,c illustrates a series of key frames from a movie depicting the automated extraction of total RNA from human blood samples (25 – 100 µL). First, sample is added to lysis buffer and mixed in reaction chamber by moving mixture up-and-down using peristaltic pump. Second, RNA capture magnetic beads is added to the reaction and mixed by shuttling mixture back-and-forth between the reaction and waste chambers via a bridging droplet formed between the two DMF plates. Third, using an external magnet, the beads are focused into a pellet and immobilized on the surface of the DMF device, such that the pellet remained behind when the supernatant is displaced to the peripheral waste chamber. Fourth, a droplet of wash buffer 1 was dispensed by DMF device, actuated to pellet, moved back-and-forth across pellet to remove trace contaminants, and then aspirated to waste module. The same process was repeated with wash buffer 2. Finally, a droplet of elution buffer was dispensed and actuated to pellet in which RNA was eluted for analysis off-chip.

To qualitatively evaluate the effectiveness of our system for extracting RNA from blood samples, a Bioanalyzer instrument was used to assess the quality of RNA. As shown in Figure 2, Bioanalyzer gels reveal crisp 28S and 18S RNA bands with integrity numbers > 7 indicating high-quality RNA. To quantify the amount of RNA extracted by the new technique, a spectrophotometer was used. As shown in Figure 3, the new method seems to be similar to the conventional method, with the advantages of automation and shorter processing times (10 vs. 40 min).

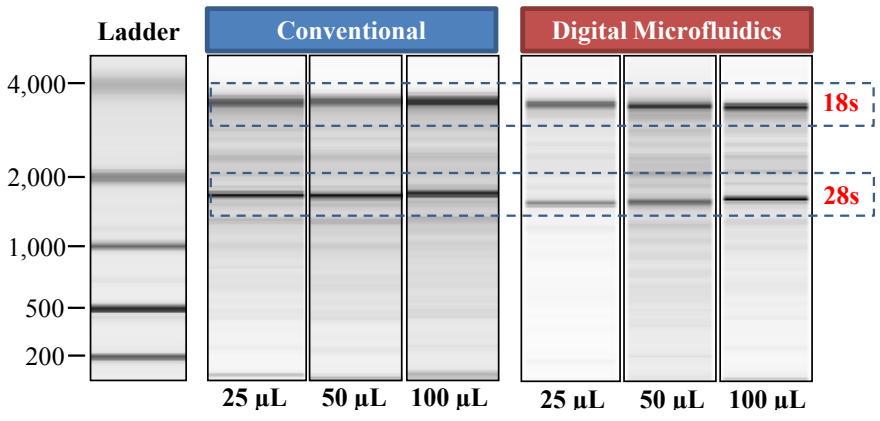



***Figure 2: Quality analysis of total RNA extracted from blood samples using conventional and DMF methods.*** *Bioanalyzer gels reveal 28S and 18S RNA bands.*

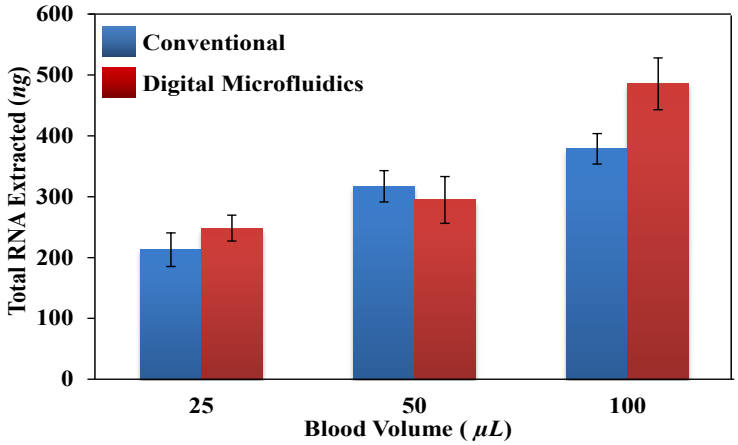



***Figure 3: Quantification of total RNA extracted from blood samples****. Bar graph of RNA extracted using DMF (red bars) and conventional methods (blue bars).*

## CONCLUSION

In conclusion, we present the first world-to-digital microfluidic interface for total RNA extraction. These results suggest great potential for DMF-driven method for automated clinical transcriptomic biomarker discovery.

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