Session Topic: Advances in Microfluidics

Presentation Type: Oral

A Fully Integrated Microfluidic System for microRNA Biomarker Identification

Background: MicroRNA biomarkers for breast cancer, particularly BRCA1, are found in trace concentrations in various intracellular fluids, and are gaining popularity as potential diagnostic markers for early disease detection. Their low concentrations require that they be isolated with maximum efficiency, labeled, and analyzed using a highly selective approach. This paper describes a microfluidic approach for extraction, isolation, labeling and analysis of microRNA from a variety of intracellular fluid surrogates.

Methods: An on-chip solid phase extraction (SPE) zone was implemented on a polycarbonate laser-etched platform by first filling a 3mm long zone with a slurry of 2μm silicate packing material. The material was laser sintered to anchor it in place, and subsequently flushed with a 5mM solution containing a pre-synthesized RNA complement strand for the target microRNA. Capture and concentration was performed by hybridization, allowing for a relatively large volume sample (10μL) to be applied. Subsequent thermal melting to release the target mRNA to a downstream reservoir was followed by in-situ hybridization with another complement strand, this one labeled with the fluorophore rhodamine-B. Pinched electrokinetic injection via an offset cruciform channel introduced the labeled sample to a 1cm electrophoretic separation channel terminating in a detection zone incorporating both an LED light source and an inexpensive photodiode for light collection. The system is depicted in Fig. 1.

Results: A total of 4 identical microfluidic microRNA analysis systems were constructed following an iterative development process. These optimized systems were utilized to collect 20 analytical datasets per device, for a total of 80 analyses. The surrogate cellular fluid samples. The samples consisted of a combinatorial array of >80 microRNA's, including the target sequence, in a bovine serum matrix. The resulting datasets were evaluated to reveal the selectivity of the custom-designed SPE sorbent, the resolving power of the electrophoretic separation, the sensitivity of the detection approach, and the within-chip and between-chip reproducibility. The results of the statistical analysis are shown in Fig. 2, and Fig. 3 shows a nested set of analytical runs for chip 3 – this set is representative of the performance achieved for all four devices.

Conclusion: A microfluidic system for extraction, isolation, labeling and analysis of microRNA from a variety of intracellular fluid surrogates was developed, consisting of an SPE zone, an extract reservoir and labeling system, a capillary electrophoretic separation channel, and a fluorescence detection zone. MicroRNA biomarkers for breast cancer, entrained in a surrogate intracellular fluid doped with >80 alternative microRNAs, were successfully analyzed at trace concentrations (<10pM), indicating that detection of clinically relevant concentrations of microRNA biomarkers in early stage cancer is feasible. A statistical analysis of reproducibility suggests that the system, if built in quantities needed for deployment, would function as needed in a clinical setting.

Word count: 448

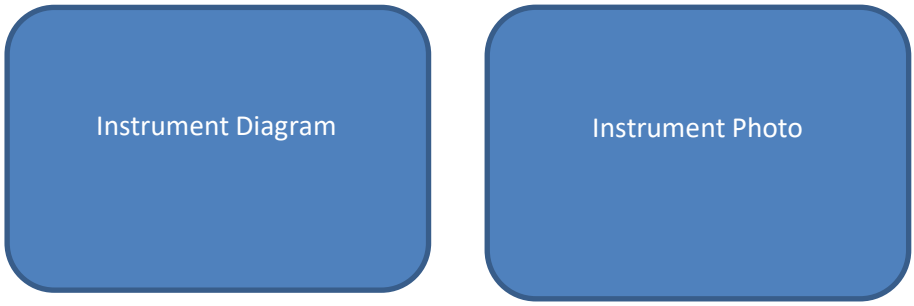

Figure 1. Schematic diagram of a fully integrated microRNA extraction, labeling, separation and detection system (left) and photograph of system on the bench (right).

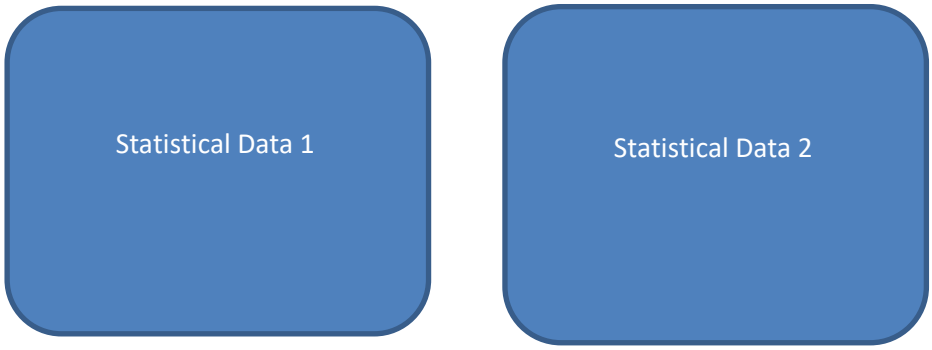

Figure 2. Statistical data indicating within-chip (left) and chip-to chip (right) qualitative and quantitative reproducibility.

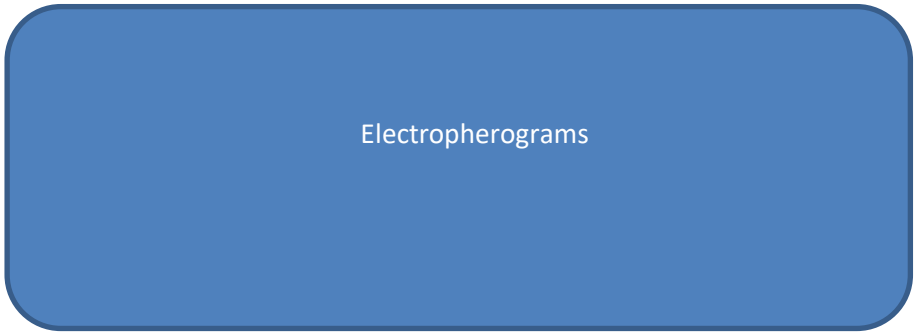

Figure 3. A nested set of 10 consecutive electropherograms for analysis of microRNA performed on chip 3, indicating achievable sensitivity, selectivity and reproducibility for the analysis of samples as described in the abstract.