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Rapid Automated Sample Preparation for Biological Assays

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Rapid Automated Sample Preparation for Biological Assays

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I. INTRODUCTION/TASKS

Our technology utilizes acoustic, thermal, and electric fields to separate out contaminants such as debris or pollen from environmental samples, lyse open cells, and extract the DNA from the lysate. The objective of the project is to optimize the system described above for a forensic sample, and demonstrate its performance for integration with downstream assay platforms (e.g. MIT-LL's ANDE). We intend to increase the quantity of DNA recovered from the sample beyond the current ~80% achieved using solid phase extraction methods.

Task 1: Develop and test an acoustic filter for cell extraction
- Task 1.1: Fabricate acoustic devices using current LLNL design
- Task 1.2: Test separation of contaminates from cells

Task 2: Develop and test lysis chip
- Task 2.1: Fabricate lysis device using existing design
- Task 2.2: Develop lysis protocol for specific cell target

Task 3: Develop and test DNA extraction chip
- Task 3.1: Fabricate DNA extraction chip using existing design
- Task 3.2: Optimize extraction of DNA from cell lysate

II. FINANCIALS

Total Funds Received: Total funding to date: $252,000
Total Funds Spent: $135,000
Projected Month Money is Exhausted: April, 2011
Co-Workers (this reporting period): Maxim Shusteff (PI), Dietrich Dehlinger (postdoc), Mark Stambaugh (co-op MS student)

III. TECHNICAL ACCOMPLISHMENTS

a. Approach

(1) Acoustic debris filter: In contrast to acoustic devices demonstrated by other researchers, which most often focus the target particles to the centerline of the microchannel, our chip is designed for asymmetric focusing, in order to move the focused contaminant particles fully out of the input sample stream (see Fig. 1, left). Using analytical modeling, we can predict the position of the acoustic node, and compare it to experimental results.

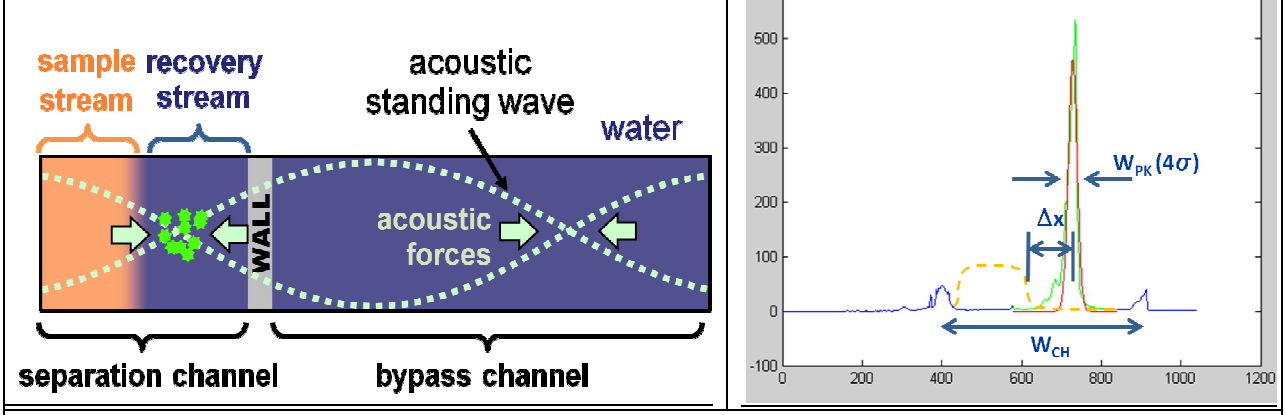

Figure 1: At left is a sketch of the acoustic channel cross-section, showing the full-wavelength (two-node) standing wave in the channel, and a thin wall (10-20 μm) subdividing the channel. The wall is acoustically transparent, such that the full channel cross-section, including the "bypass" channel participates in setting up the standing wave, but the sample to be analyzed only flows in the separation channel. At right is a plot showing a peak of focused particles within a separation channel, used to evaluate the chip performance. Key metrics include Δx, the separation between the edge of the input stream and the focused peak, and the concentration enhancement factor (C_{ENH}), comparing particle content in the peak vs. the initial particle distribution.

(2) Thermal lysis chip: Our approach for lysing cells is to flow the sample through a high-temperature region, heated by a kapton-encapsulated resistive heater. We plan to examine the influence of chip temperature, and residence time on lysis product. Preliminary data (not shown) suggests that temperatures greater than 65° C and residence times as short as 3-5 minutes may be sufficient. However, a more rigorous study is required.

To quantitate lysis results, we are evaluating a number of measurement techniques. Some will be a measure of cell death, an indirect method, whereas others will directly measure the quantity of DNA released into free solution. Once a method has been selected, we will be able to optimize the lysis chips for flow rate and temperature.

(3) Isotachophoresis (ITP) DNA extraction chip: To achieve high-yield DNA extraction, we intend to concentrate (post-lysis) free-solution DNA by means of isotachophoresis (ITP). Our ITP chip is a transverse-mode device, in which the electric field is applied perpendicular to the flow direction, to enable continuous flow-through separation at high flow rate. Current efforts are aimed at achieving a uniform stable electric field of sufficient strength perpendicular to the separation channel.

b. Design

We have laid out photomasks and fabricated the first generation of fluidic chips for all three modules (see Fig. 2 below).

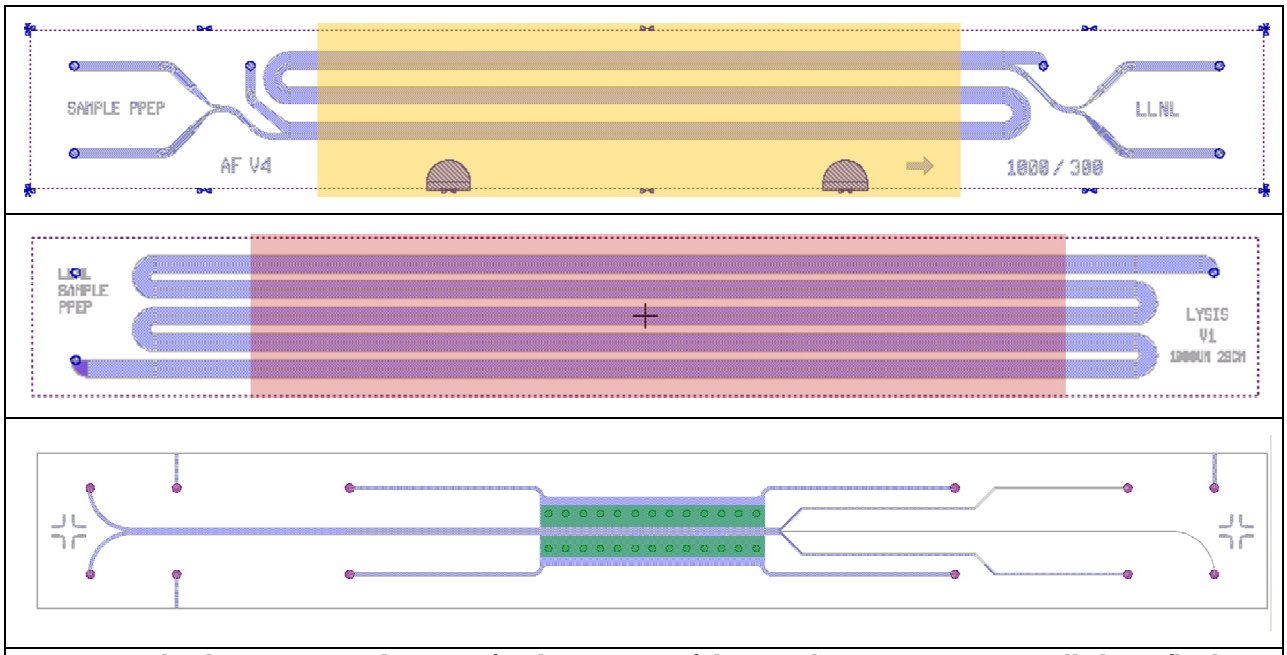

Figure 2: Chip layouts currently in use for three stages of the sample prep system. For all chips, fluid flow is from left to right. The chips are, from top to bottom:

The acoustic device (top), with the yellow rectangle indicating the piezoceramic transducer. The two parallel inlets and outlets are for sample and recovery streams, with the third inlet and outlet is for the "bypass" fluid, which flows "behind the wall" and has no fluidic connection with the separation channel.

The lysis chip (middle), has only one inlet and outlet, and the heated region is shown in pink.

The ITP chip layout (bottom), which incorporates a separation channel down the center, flanked by two hydrogels indicated in green (the circles are pillars that anchor the gel). On the outside of the gel regions, high-conductivity electrolytes flow through and act as "liquid electrodes" to bring the applied electric potential from off-chip connections to the separation chamber.

The channels for the acoustic device are dry-etched in silicon, and anodically bonded to a glass lid, for optical access. The channel passes the piezo transducer multiple times to increase particle residence time within the acoustic field, and allow tighter focusing at high flow rates.

The lysis chip channels are similarly dry-etched into silicon and anodically bonded to glass. Here, the channel is laid out to allow maximal residence time in the heated region for a given flow rate. A typical channel is 1 mm wide, 200-300 μm deep, and 25 cm long, for an on-chip time of 30-50 seconds.

The fluid channels for our current DNA extraction are wet-etched into glass, and the chip is sealed by thermal fusion bonding to a second glass wafer. The separation chamber has polyacrylamide hydrogels segregating the separation channel from the electrodes that apply the electric field.

c. System Testing

(1) Acoustic debris filter: We are currently characterizing the performance of our acoustic chips using variously-sized fluorescent microspheres. We have developed quantitative metrics for evaluating chip performance (Fig. 1, right), using fluorescent imaging of the sample channel – we have direct measurements of focused-particle peak position, width, separation from the input sample, and concentration enhancement. This enables us to evaluate a number of design variations and choose the optimal design for achieving the required contaminant removal.

We anticipate being able to run experiments to meet the acoustic contaminant removal milestone within the coming month.

PLANNED TEST SAMPLE FOR ACOUSTIC CONTAMINANT REMOVAL MILESTONE: We will spike baby hamster kidney (BHK) cells, a fibroblast-like eukaryotic cell, into a suspension of NIST SRM 1649a urban dust or NIST RM 8631a medium test dust. We anticipate that this will serve as a suitable proxy for pulling cellular material from sand/soil contaminated desert samples from the OIF/OEF theater. We would welcome guidance/input from the sponsor on our choice of test sample (see Section VI. Actions Requested).

(2) Thermal lysis chip: Imminently-planned tests will use *E. coli* bacteria as a sample cell type to characterize the lysis device performance. We are testing a number of cell-death based methods including a BacLight live/dead assay, flow cytometry, and direct fluorescence imaging, as well as quantitation of DNA content in the lysate by direct fluorescent DNA staining, qPCR, and capillary electrophoresis techniques. Once a method (or two complementary methods) is chosen for the most useful data and reasonable work-flow, we will test the parameter space of lysis temperatures and flow rates using that method to select our best parameters.

"REAL" LYSIS TEST SAMPLE: Guidance is requested from the sponsor for choosing an appropriate test sample and/or cell type relevant to sponsor's goals for this technology (see Section VI). *E. coli* bacteria may be of limited utility here.

(3) ITP DNA extraction chip: The most recent testing efforts with ITP chips have been aimed at reliably fabricating robust gels of the desired geometry to flank the separation channel. Experiments with varied acrylamide monomer, cross-linker, and photoinitiator formulations, as well as different light sources and exposure parameters have resulted in consistent gels that have enabled us to focus fluorescein dye using ITP in low-conductivity (0.3 mS/cm) samples.

IV. PROBLEM AREAS

(1) Acoustic debris filter: No significant problems are anticipated, since these chips are our most mature development effort. The main risks are (a) insufficient acoustic size-difference between the cells to be recovered and the contaminant to be removed, and (b) insufficient on-chip energy

density to achieve the target flow rate of 100 μL/min. To mitigate risk (a) we can run our size-tunable acoustic filter twice, selecting for particles first larger, then smaller than the target sells. To mitigate risk (b), a number of design elements to improve energy transfer can be explored, including inertial clamping for chip mounting, and deliberate selection of piezo transducer resonance.

(2) Thermal lysis chip: The key challenge that we anticipate will be to develop an understanding of the correspondence between evidence of cell death and DNA content in the lysate. Since DNA for forensic analysis is the relevant analyte at the system output, this will be the ultimate determinant of chip performance.

A secondary potential risk is that thermal lysis proves insufficient for extracting relevant DNA content, and that chemical or mechanical lysis methods will need to be brought in. While this will complicate chip design, we anticipate that the system can be easily adapted if this becomes necessary. As mentioned earlier, guidance is requested from the sponsor for choosing an appropriate cell type for making this determination.

(3) ITP DNA extraction chip: Currently, there is significant non-uniformity in the applied transverse electric field, especially in chips with a longer separation channel, due to "field sag" along the length of the liquid electrodes. This severely impacts the efficiency of separation/extraction, as well as the maximum usable sample conductivity, and the next design revision will need to address this. Pending experiments will enable us to make appropriate design decisions.

V. FUTURE EFFORTS

(1) Acoustic debris filter:
- Testing of current chip generation based on quantitative metrics of focusing/separtaion.
- Experiments to demonstrate contaminant-removal milestone.

(2) Thermal lysis chip:
- Develop metric for quantitation of lysate DNA.
- Measure lysing efficiency based on a range of flow rate and temperature parameters.

(3) ITP DNA extraction chip:
- Test limitations on sample conductivity and ITP field uniformity using current devices.
- Design and fabricate next chip generation to overcome these limitations.

VI. ACTIONS REQUIRED BY THE COTR

The contractor requests the sponsor's guidance in the following areas:

1. Input /comment on the choice of BHK cells and NIST standardized dust samples for demonstrating acoustic filter performance.
2. Preferred cell type for lysis chip testing and guidance on whether nucleic acid content aside from genomic DNA is relevant to the current project (e.g. mtDNA)