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Rapid Automated Sample Preparation for Biological Assays

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Disclaimer

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Title:
Rapid Automated Sample Preparation for Biological Assays

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Hardware Delivery Date: Phase II start + 9 months
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Program managers: Jeff Salyards, jeff.salyards@us.army.mil, (404) 469-5569

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: $552,000
Total Funds Spent: $175,000
Projected Month Money is Exhausted: November, 2011
Co-Workers (this reporting period): Maxim Shusteff (PI), Dietrich Dehlinger (postdoc), Matthew Farmer (UG student intern), Michelle Packard (PhD student collaborator, Michigan State Univ.)

III. TECHNICAL ACCOMPLISHMENTS

a. Approach

There are no changes in the technical approach from what was described in last month's report.

b. Design

All chips have been fabricated based on the designs laid out in last month's report. Additionally:

(1) ITP DNA extraction chip:

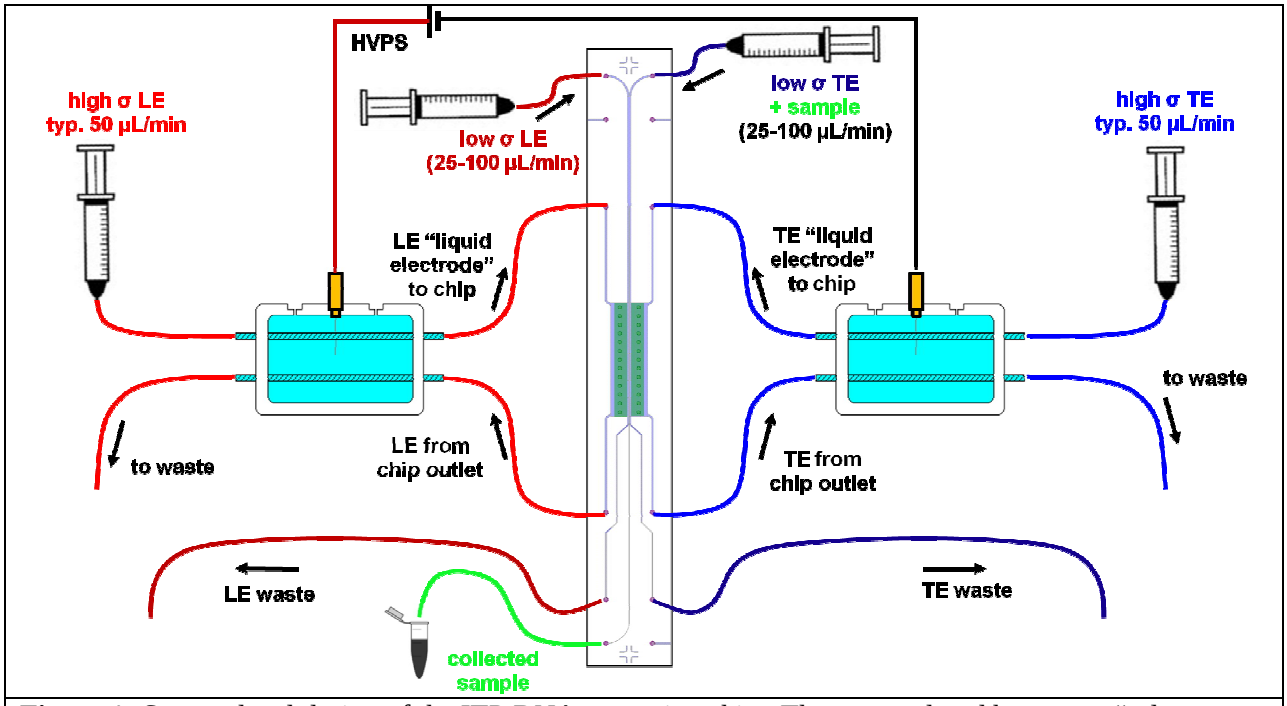


Figure 1: System-level design of the ITP DNA extraction chip. The cyan-colored boxes are "salt bridges" which are filled with an electrolyte to which the high voltage is applied by platinum wires. The TE and LE "liquid electrodes" flow through nafion ion-permeable tubing passing through these boxes, and conduct the applied potential to the separation chamber on the chip. ITP takes place perpendicular (here right-to-left) to the flow through the chip (top to bottom in this figure), with the concentrated band of nucleic acid exiting through the center outlet, with unwanted analytes (LE and TE waste) flanking it on the right and left.

c. System Testing

(1) Acoustic debris filter:

To properly choose the desired acoustic fiter size-cutoff between cells and contaminants for the contaminant-removal milestone, we performed flow cytometry measurements on the AZ Test Dust, the NIST Urban Dust, as well as the eukaryotic baby hamster kidney (BHK) cells. These measurements showed a significant overlap in size distribution between contaminants and cells (see Fig 2 at right). However, cytometry is a particle counting measurement, and larger particles tend to occupy a much larger volume-fraction (or mass-fraction) than they appear from cytometry data. We intend to proceed with using these samples as planned, and supplement our measurements with a mass- or volume-based particle quantitation method.

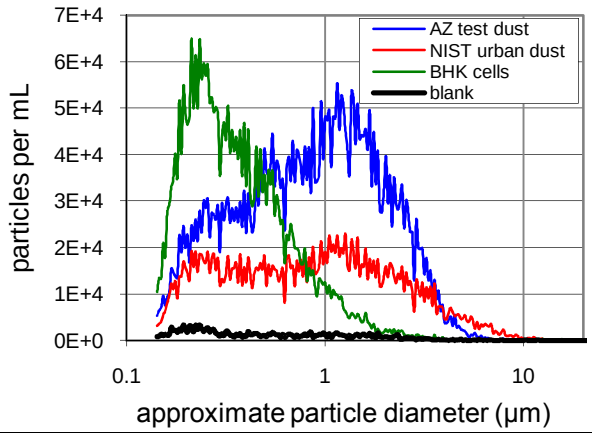
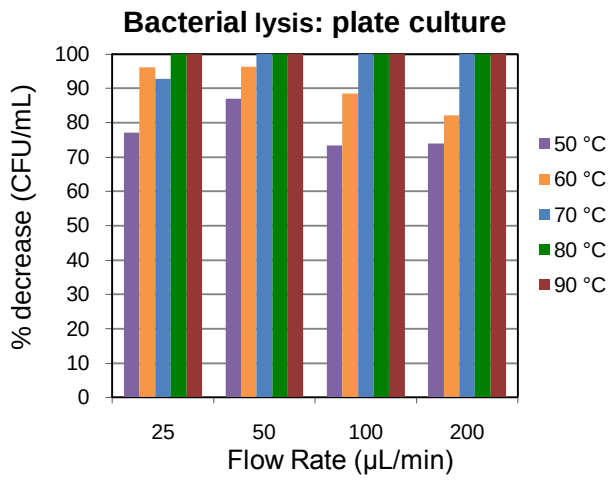


Figure 2: Flow cytometry data for the available contaminant dust samples as well as the cells. Note that the smallest particle fraction is "overstated" due to much larger numbers of small particles taking up equivalent volume to fewer large particles.

(2) Thermal lysis chip:

Michelle Packard, our collaborator at Michigan State has performed confirmatory on-chip lysis experiments using the chip design described in last month's report (previous preliminary data used off-chip benchtop protocols). The current set of experiments also used *E. coli* bacteria as before, due to the ready availability of both the bacterial samples, as well as well-characterized assays for quantitation. Figure 3 below shows the results of colony counting in culture plates as well as an ATP protein-release assay.

These results show excellent lysis performance at the highest flow rates, and we will move on to tests with BHK cells as better predictors of performance with other eukaryotic cells.



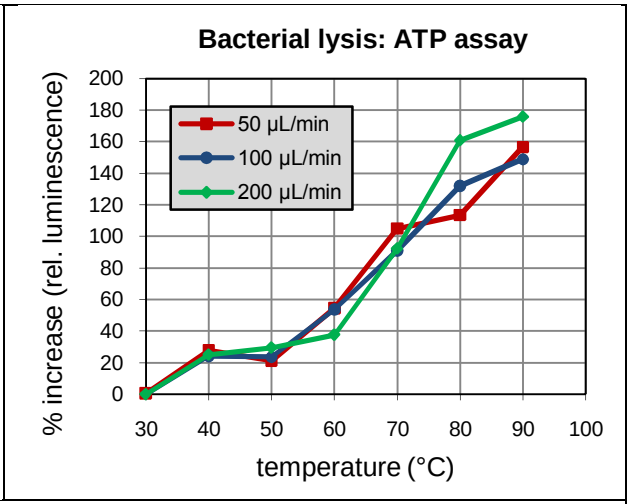


Figure 3: On-chip lysis data of *E. coli* bacteria.

(3) ITP DNA extraction chip:

In February, an undergrad. student intern joined us (Matthew Farmer, San Diego State Univ., Chemistry) and will be working with us through the spring term. The success of recent efforts by co-op student Mark Stambaugh to optimize the in-situ patterning of polyacrylamide gel electrodes now allows us to reliably make these in our chips. Matthew Farmer is testing the existing chips (see Fig. 4) to determine what bounds on sample conductivity and flow rate are imposed by the current device geometry.

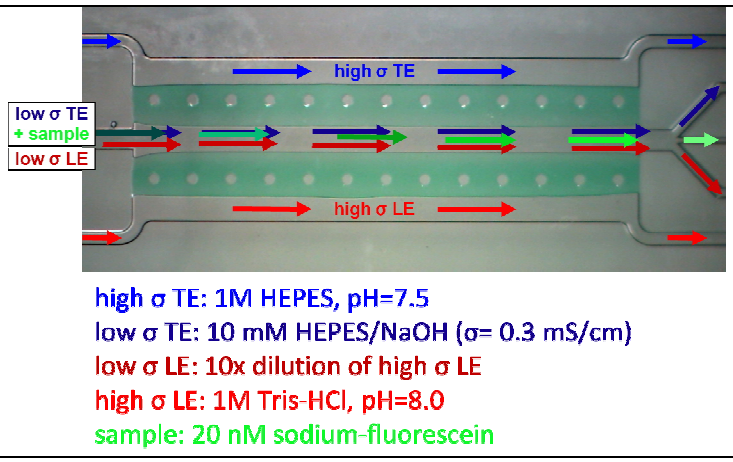


Figure 4: Photo of a 13 mm ITP DNA extraction chip, with different flow streams sketched and labeled in overlay.

We have likewise provided our collaborator Juan Santiago at Stanford University with chips for testing these experimental parameters (his research group has extensive experience with capillary-based ITP). We anticipate these tests will yield data to inform a modification of the design for the next chip generation to achieve greater electric field uniformity and strength.

IV. PROBLEM AREAS

(1) Acoustic debris filter: The main risk remains insufficient acoustic size-difference between the cells to be recovered and the contaminant to be removed. Since acoustics is a volumetric force, this is an inherent limitation of the technique.

(2) Thermal lysis chip: Risks/challenges remain as described in last month's report.

(3) ITP DNA extraction chip: Nonuniformity of the applied electric field is the most significant hurdle at the moment. Fortunately, the success of the recent efforts to reliably make the polyacrylamide gels suggests that we can now re-integrate metallic electrodes on-chip without bubbles, electrolysis, pH gradients, etc. interfering with sample flow. This will provide the desired uniform high-strength electric field, while reducing the complexity of the electrical system.

V. FUTURE EFFORTS

(1) Acoustic debris filter:
- Contaminant-removal milestone demonstration by filtration of spiked test dust from BHK cells.
- Continued testing of current chip generation with polymer microspheres based on quantitative metrics of focusing/separation.

(2) Thermal lysis chip:
- Continue lysing tests at a range of flow rates and temperature using eukaryotic cells.
- Develop metric for quantitation of lysate DNA.

(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

None.