Thermal-Fluidic System for Manipulating Biomolecules and Viruses

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We are developing a reconfigurable fluidic system that demonstrates the ability to *simultaneously* perform separations, concentrations, and purifications of biomolecules and viruses. This microfluidic technology is an equilibrium gradient version of capillary electrophoresis (CE) that allows for the stationary fractionation and concentration (up to 10,000 x) of target analytes on the dimension of bulk or free solution electrophoretic mobility. In this technique, a delicate balance is achieved in a microchannel between a net fluid flow and an opposing electrophoretic velocity gradient to capture charged analytes at a specific location (see Fig. 1). The analytes are then separated based on their free solution electrophoretic mobility, which is related to the analyte's surface charge (zeta potential) and hydrodynamic drag (shape and size).

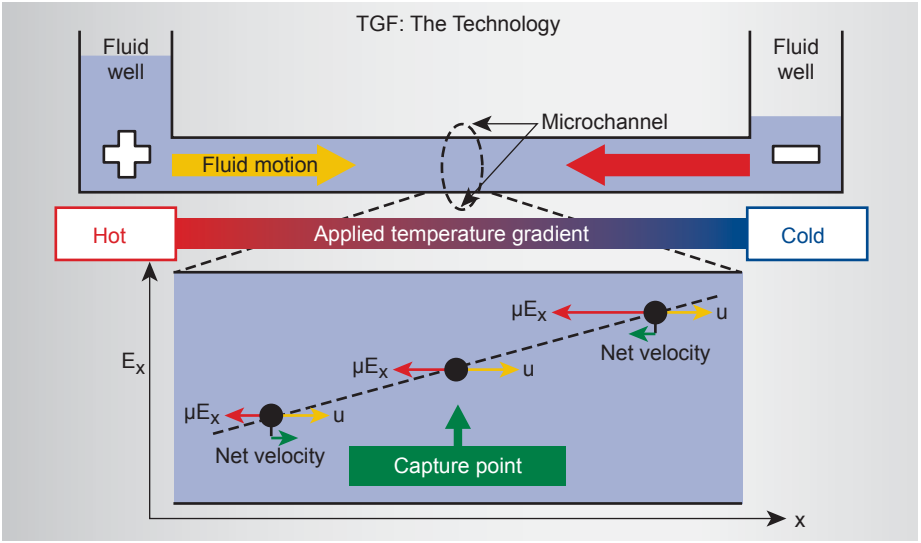

Figure 1. Schematic of temperature gradient focusing. Bulk fluid motion (yellow arrow) is balanced by an opposing electrophoretic velocity (red arrow) to capture analytes at a unique spatial location within a specific electrophoretic mobility range.

Project Goals

The project goal is to develop an automated temperature gradient focusing (TGF) instrument to improve the separation resolution and throughput when applied to front-end sample processing of biological samples. Two specific application areas are identified to demonstrate the novel sample manipulation capabilities inherent to TGF: 1) the purification and separation of different virus strains in complex samples; and 2) protein concentration and separations for *in vitro* transcription/translation (IVT) protein expressions.

Relevance to LLNL Mission

TGF specifically addresses needs of the bio-security program at LLNL, for the detection of biomolecules, viruses, or cells at low concentrations through

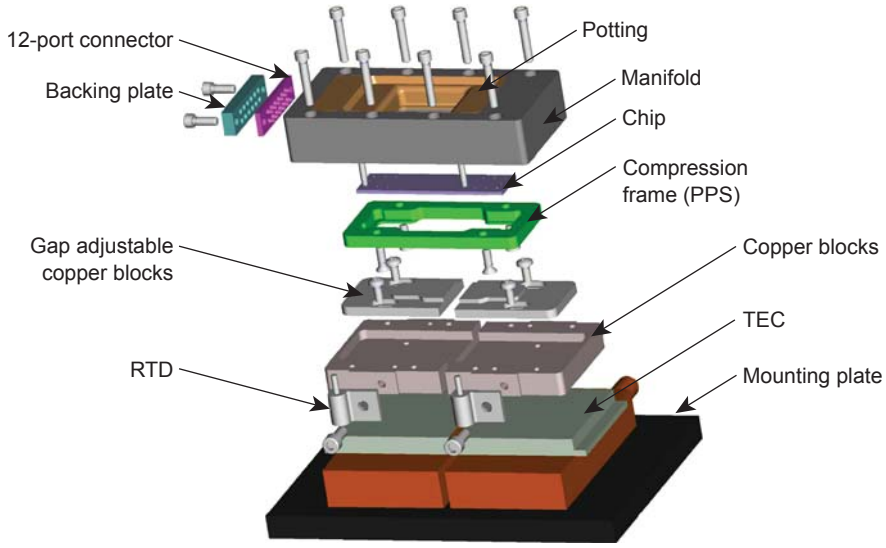

Figure 2. Exploded view of microfluidic package. The microfluidic package is composed of a fluidic manifold, a compression frame, and two independently controlled thermal regulation surfaces.

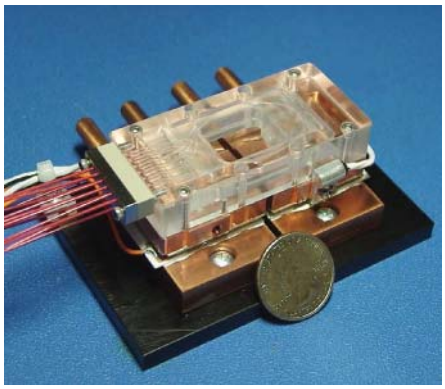

Figure 3. Photograph of the assembled package. The microfluidic package once assembled is compact and very robust. The chip can be loaded directly into the fluidic manifold and does not require the thermal module to form a fluidic seal.

enhanced collection, separation, and purification strategies. This is facilitated by performing the necessary front-end sample preparation through concentration procedures and removing noisy background signals/contaminants. This project supplies LLNL with a novel capability to perform biomolecular, viral, and cellular control in a flexible format to address a wide range of programmatic assay conditions.

FY2008 Accomplishments and Results

A thermal-fluidic package was developed and successfully tested to reproducibly control fluid flow, temperature gradients, and electric fields in microfabricated glass chips. The packaging allows for fluorescent optical access with an adjustable mounting surface to modify the temperature gradient length. Figure 2 shows an expanded view of the package and fluidic manifold for a 12-port chip with a 1/32-in. quick connect and a compression frame for repeatable leak-free fluidic connections. Once assembled, the package was very stable and required no further maintenance or monitoring. Figure 3 shows a camera image of an assembled package.

Previous analysis determined the need for thicker walled glass chips, a more robust thermal mounting to the heat source, and improved pressure stability. We were able to successfully demonstrate the capture and concentration of a small fluorescent dye molecule in the newly constructed package (Fig. 3). The package demonstrated a significant improvement in robustness over previous packaging by reducing common failure modes such as liquid leaks, air bubbles, clogging, temperature profile linearity, and overall pressure stability.

To help guide the experimental testing, a 2-D numerical modeling effort was developed that captures the relevant physics during the electrokinetic capture process. For the initial modeling of the relevant field variables (temperature, velocity, and voltage) we used a commercially available finite element modeling (FEM) package (COMSOL Multiphysics). To solve for the important analyte concentration profiles in the system, these known field variables (from FEM) were fed into a Monte Carlo simulation. Figure 4 shows good agreement between the experimental data (Fig. 4a) and simulation results (Fig. 4b), which validates the numerical modeling approach.

From here specific metrics, such as peak height, peak width, throughput, and limit of detection, can be extracted to determine the efficiency and resolving power for a particular set of capture conditions. Design guidelines and rules (geometric and operating conditions) were constructed for specific applications to aid in the design and testing of devices.

FY2009 Proposed Work

Starting from the manually controlled proof-of-principle TGF system, we propose to develop a more robust system to perform the following: 1) concentrate and separate different virus strains directly; and 2) perform protein purification and separation for IVT protein expressions. Although seemingly very different, both applications are possible because the assays themselves are used as a test platform to characterize and optimize the system performance.

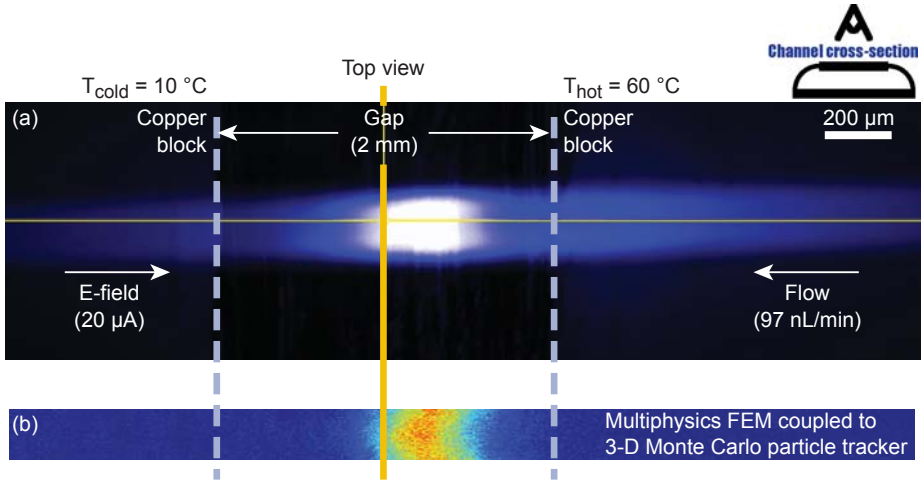

Figure 4. Numerical modeling validation: (a) experimental capture and concentration of a fluorescent dye using a rectangular channel (20 µm x 200 µm) as observed with fluorescent microscopy; (b) Monte Carlo simulations, showing good agreement with experimental data. Curvature in the focused analyte band is observed and predicted due to the high-aspect-ratio rectangular channel used.