

## Titanium-Based Biomolecular Manipulation Tools

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### 1. Objectives

The objectives of the proposed research are to design, simulate and fabricate unique micro/nanofluidic devices for the pumping, trapping, concentrating, separating, mixing, and synthesis of biomolecules and bio-structures. The micro/nanofluidic devices will be fabricated, for the first time, using bulk Titanium-MEMS/NEMS processes. Ti-MEMS/NEMS take full advantage of both the silicon processing and Titanium processing infrastructures<sup>i</sup>. The new technology allows fabrication of multiple-level 3-D fluidic channel/well structures with electrically isolated planar and 3-D electrode structures to create custom-engineered electrical fields, for highly selective electrokinetic phenomena in micro/nanofluidic devices. With Ti processing, we can create nanoscale electrodes down to 50 nm in size. The nanometer scaling of Ti-MEMS/NEMS structures can be used to create highly-focused electrical fields, not previously achievable in Bio-MEMS. We propose to use nanoscale titanium-oxide structures to trap, filter and position molecules and cells<sup>ii</sup>.

Over the past ten years significant progress has been made in using MEMS technology to develop bio-analytical systems, to analyze DNA, proteins and cells. However, much work remains to be done. Dc electrokinetics (electrophoresis & electroosmosis) has been used to separate DNA molecules and to pump fluids. Typical voltages in these systems are of order 1-2 kV. Dc electrokinetics suffers from gas formation due to electrolysis. Ac electrokinetics (dielectrophoresis) has been commonly used to capture large DNA molecules or to manipulate cells<sup>iii,iv,v,vi</sup>. Dielectrophoresis has been restricted commonly to particles of order one micron or larger.

We are developing Ti-based microfluidic devices that are low in power, easy to fabricate, and can be used to mix, stir, focus, and concentrate bio-molecules and cells. Specifically, we are building ac electrokinetic pumps for fluid delivery and control, DEP filtering devices, electrokinetic concentrators, traveling-wave DEP cell separators, electrokinetic mixers, and protein & cell positioning devices. Theoretical models of ac electrokinetics are being developed and verified experimentally using micro-resolution particle image velocimetry.

### 2. Titanium Microfluidic Networks for Protein Self-Assembly Studies

The research combines state-of-the-art micro-channel fabrication technology, with modern synchrotron x-ray scattering and optical imaging methods, for developing methods to detect and study protein-protein interactions. These studies will contribute significantly to the rapidly emerging field of proteomics, which aims to elucidate all protein-protein interactions, in particular, for both healthy and altered states of protein complexes. The model systems we will use to demonstrate the new micro-channel-based method of studying protein-protein interactions will come from the cell cytoskeleton including, assemblies of filamentous-actin, microtubules and their associated proteins [<sup>vii</sup>, <sup>viii</sup>, <sup>ix</sup>].

In one scheme the micro-channel devices were designed to have two large reservoirs, connected by an array of narrow channels. These channels were varied in width and length. We used channels 5, 10, and 20  $\mu\text{m}$  wide, 10 $\mu\text{m}$  deep and 800  $\mu\text{m}$  or 1600  $\mu\text{m}$  long (Figure 1). The

devices were etched into polycrystalline titanium foil or silicon wafers and scanning electron microscope images of the channels opening out onto a reservoir area can be seen in figure 1b and 1c for both titanium and silicon respectively. Titanium channels were prepared where the backside of the titanium foil was deep etched to reduce the x-ray attenuation. Figure 1d shows examples of 20 $\mu\text{m}$  wide, 10 $\mu\text{m}$  deep titanium channels filled with channel induced oriented  $\alpha$ -actinin/actin bundles.

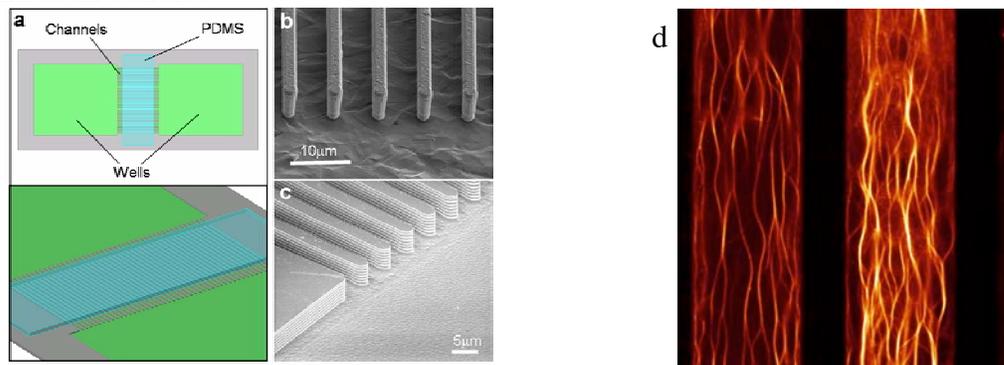


Figure 1 – (a) A schematic of the microchannel device and SEM images of the channel ends in (b) titanium and (c) silicon. These micro-channel devices have been utilized to confine and align the biopolymers including, filamentous actin bundles for structure studies (d).

### 3. Dielectrophoretic-based Particle Concentration and Mixing

Recently, a dielectrophoresis device using bulk titanium thin foil technology has been developed and characterized. This development included the exploration of novel manufacturing techniques for titanium microfluidic device fabrication.

The current DEP device design allows for particle motion detection in the x-y plane only. In order to visualize the z-direction, or out-of-plane, particle and fluid flow patterns for fundamental dielectrophoresis studies, a three-dimensional electrode structure is being developed. Bulk titanium is especially suited for this application due to its high conductivity.

### 4. Electrokinetic Pumps & Instability

An electrokinetic micropump for driving high conductivity biological fluids through microchannels has been designed, fabricated and shown to work for different conductivities. The pump utilizes very low DC voltages (5-15 volts) to generate significant flow velocities (20-150  $\mu\text{m}/\text{s}$ ). The velocity is found to be an increasing function of applied DC voltage and the electrical conductivity of the working fluid. Velocities were measured by using micron resolution particle image velocimetry.

The main components of the micropump chip consist of a microchannel and a gold wire meandering through the channel in a serpentine fashion. The package size of the chip is 35 mm by 15 mm by 1 mm (approx). The bottom substrate of the chip is 500  $\mu\text{m}$  thick pyrex glass. The microchannel walls are made of polyimide (HD 4010, HD Microsystems, Parlin, NJ). The serpentine gold wire is laid down in the bottom of the channel (i.e. on top of the pyrex wafer). The cross section of the wire is approximately 50  $\mu\text{m}$  (width) by 200 nm (height). The ends of the channel are connected to two cylindrical reservoirs of diameter 2 mm. The channels are covered on the top by another pyrex wafer. The top wafer has holes for accessing the end reservoirs. Figure 2 shows the layout of the pump as it is imaged by using an epi-fluorescent microscope lens.

Micron resolution particle image velocimetry ( $\mu$ PIV) was used for estimating the fluid velocities in the channel. Micro-PIV is a well established technique for quantitatively measuring spatially resolved velocity fields at micro scale. This technique determines fluid velocity by measuring the velocity of fluorescent tracing particles suspended in the transparent fluid. It is assumed that the particles follow the flow faithfully. The average fluid velocity as a function of voltage was measured for two different conductivities:  $5.55 \times 10^{-6}$  and  $0.0765$  S/m (see Fig. 3). This plot shows that the velocity is an increasing function of DC voltage. For DI water, the pumping velocity at 15 volts was found to be  $95 \mu\text{m/s}$ .

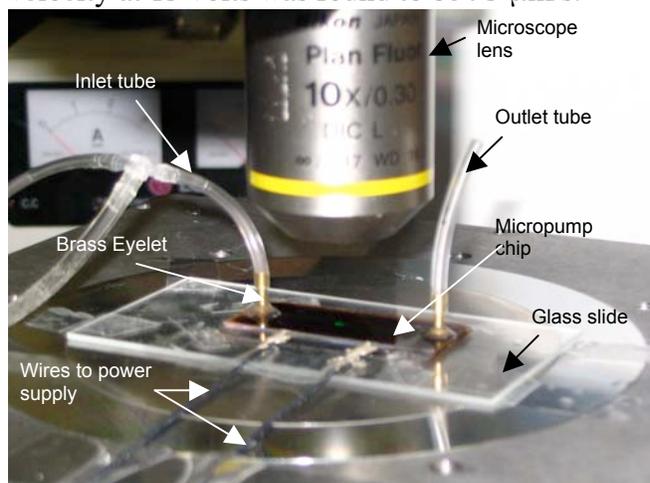


Figure 2: Partial photograph of the experimental setup. Inlet and outlet tubes, the chip, wires to power supply and a microscope lens are shown. The chip was affixed to a bigger glass slide for stability. Please note that the lens shown in this picture is a 10x lens whereas a 20x lens was used for PIV measurements.

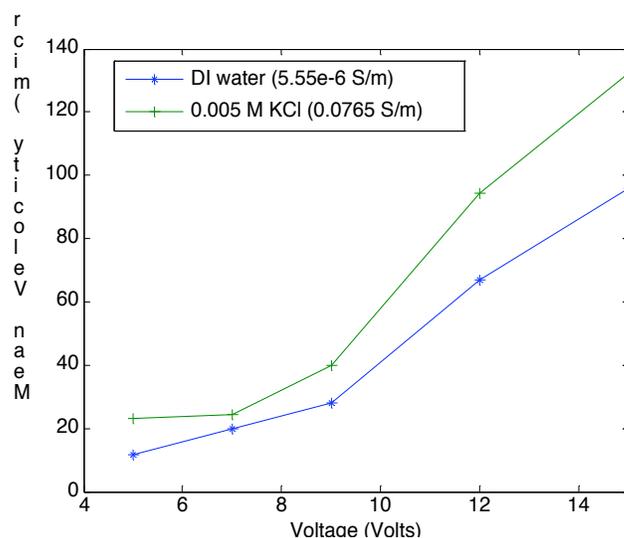


Figure 3: Experimental results of velocity measurements as a function of DC voltage and solution conductivity

## References

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