NANOFLUIDICS FOR SELECTIVE PROTEIN TRAPPING IN BIO-FLUIDS

Kuo-Tang Liao[1], Vasudha Chaurey[1], Mikiyas Tsegaye[1], Chia-Fu Chou[2], and Nathan S. Swami[1,*]

[1]Charles L. Brown Department of Electrical and Computer Engineering,
University of Virginia, Charlottesville, VA 22904, USA
[2]Institute of Physics, Academia Sinica, Taipei, Taiwan 11529, ROC

ABSTRACT

The development of clinical diagnostic systems of enhanced speed, sensitivity and selectivity requires methodologies for sensing rare numbers of protein biomarker against a background of high concentration of other matrix proteins. We address this challenge through selective protein pre-concentration in a nanofluidic device. Specifically, we focus on nanofluidic device design for selective dielectrophoresis pre-concentration of functional proteins within media of high ionic strength.

KEYWORDS: Protein Biomarkers, Nanofluidics, Dielectrophoresis, Electrophoresis

INTRODUCTION

The challenge of sensing rare numbers of cancer biomarker proteins against a background of high concentration of other matrix proteins directly within biological fluid media such as serum or saliva can be addressed through selective biomarker pre-concentration in proximity of the sensor. While prior approaches have applied ion exclusion-enrichment effects that are caused by electrical double layer overlap at the micro-to-nanofluidic interface [1-3], the resulting protein pre-concentration at 10^3−10^6-fold levels require tens of minutes to several hours, and this method cannot effectively separate target biomarker proteins against a background of numerous similar proteins in patient samples of bodily fluids. Dielectrophoresis (DEP) enables highly selective trapping of bio-particles based on the characteristic frequency response of the dielectric permittivity of the bio-particle versus that of the medium, and it has been extensively applied towards sorting of somewhat similar sized biological cells with differing dielectric frequency response. However, dielectrophoresis trapping of sub-50 nm bio-particles in highly conductive bio-fluid media is a challenge since dissipative electrothermal forces that arise due to Joule heating can dominate over dielectrophoresis trapping forces. In this presentation, we aim to elucidate device designs for protein preconcentration, so that DEP trapping is not hindered by dissipative electrothermal flow, while the sampled fluid volume is enhanced through applying DEP in conjunction with electrophoresis.

THEORY

Dielectrophoresis trapping forces fall as the cube of bio-particle radius, hence, trapping of successively smaller sized particles requires micro- and nanoscale constriction-based devices to enhance field gradients (∇E^2) [4-6]. However, trapping of protein biomarkers in high conductivity saline media, which is required to maintain their functionality, causes significant levels of dissipative electrothermal flow at the constriction region due to localized Joule heating arising from the high electric field. Since DEP forces scale as the product of the field and its gradient ($E.dE/dx$ or ∇E^2), while electrothermal forces scale as the second exponential power of electric field (σE^2), with no dependence on the electric field gradient, we seek designs that enhance electric field gradients over the net electric field intensity, to enhance DEP trapping forces over dissipative electrothemal forces. This leads us to examine nanofluidic device designs for selective protein dielectrophoresis preconcentration.

EXPERIMENTAL

The fabrication of nanofluidic devices with "molecular traps" for ultrafast protein preconcentration is based on our previously developed insulator constriction-based dielecrophoresis device [5-7]. First, a 3-μm deep microfluidic channel was constructed by photolithography on a fused silica substrate. Following this, electron beam lithography was applied to nanofabricate multiple constrictions of varying sizes (15 nm to 125 nm) within parallel channels of 200 nm depth, as confirmed by scanning electron microscopy. The whole device, with integrated micro- and nanofluidic channels were completed at room-temperature using a low-pressure sealing process recently developed by our group [8]. This device was subsequently applied towards ultrafast protein trapping for positive DEP at ~300 Vpp across ~ 1 cm at 100 kHz and negative DEP at ~300 Vpp across ~1 cm at 1 MHz, using a FLC voltage amplifier. Fluorescently labeled streptavidin protein samples at ~ng/mL concentration levels were imaged on an inverted Zeiss Observer microscope with a Hammatsu EM-CCD to enhance sensitivity.

RESULTS AND DISCUSSION

Need for nanofluidic devices for protein dielectrophoresis: While micron scale constrictions are sufficient to enable effective DEP trapping of double-stranded (ds-) DNA, as well as single-stranded (ss-) DNA fragments, they are unable to trap proteins. The primary reason is that since DEP trapping forces fall as the cube of equivalent spherical radius of the bio-particle, other disruptive forces such as electrothermal flow that can occur due to Joule heating as a result of current flow in conductive media, can overwhelm DEP trapping forces. As shown in **Figure 1a**, as a result of the enhanced electric field at the constriction, DEP force (F_{DEP}) is pointed towards the constriction edges. A 500-fold constriction (500 μm channel to 1 μm constriction) is sufficient to

trap ss-DNA fragments of equivalent spherical radius of ~50 nm. This is apparent from F_{DEP} of ~10^{-11} N within a large region of the constriction trap, which is far higher than the magnitude of the disruptive forces due to electrothermal flow of ~ 5 x 10^{-13} N that opposes F_{DEP}, as shown in Figure 1a and c.

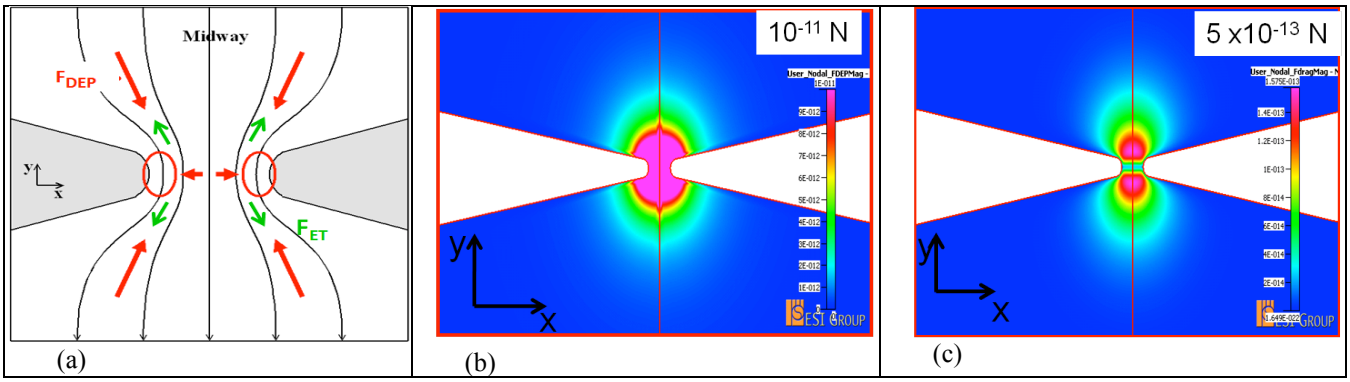

Figure 1: (a) Direction of positive dielectrophoresis trapping forces (F_{DEP}) versus dissipative electrothermal forces (F_{ET}); magnitude of F_{DEP} (b) versus that of F_{ETH} (c), at the floor of constriction device. This simulation was done for ss-DNA (50 nm size bio-particles) for channels constriction from 500 µm to 1 µm. Similar analysis will be presented for successively smaller bio-particle sizes, as well as constriction sizes and ratios for systems under positive and negative dielectrophoresis trapping.

Challenges for protein trapping under positive dielectrophoresis: For protein bio-particles, such as streptavidin, of equivalent spherical radius of ~5 nm, the F_{DEP} falls to <10^{-12} N, as shown in **Figure 2a**. This will likely be overwhelmed by the net electro-thermal flow due to Joule heating that is of comparable magnitude. Upon scaling the constriction to 5000-fold (500 µm channel to 100 nm constriction), the F_{DEP} can be enhanced to ~10^{-11} N to enable effective DEP trapping over electrothermal flow, as shown in **Figure 2b**. However, the Joule heating causes a localized high temperature region or "hot-spot" in the constriction at the midway depth through the channel. While this does not prevent effective DEP trapping, a temperature rise of ~55 C can cause conformation changes to the protein molecules.

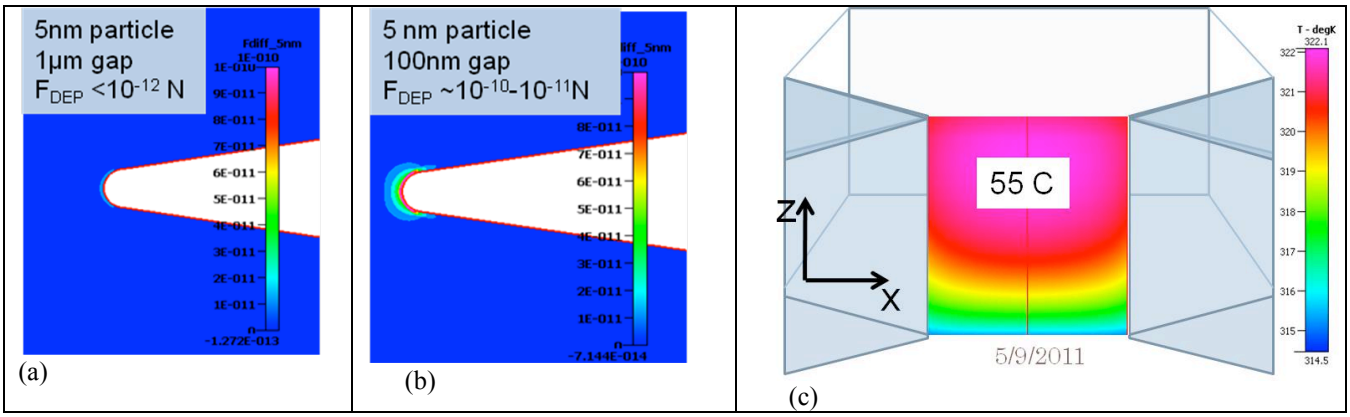

Figure 2: Magnitude of FDEP for a 5 nm particle using: (a) 500-fold constriction; (b) 5000-fold constriction; (c) Temperature rise in the hot-spot region due to a 500-fold constriction due to Joule heating within conductive media (σ_m ~ 1S/m).

Applying the nano-constriction as a dam to electrophoresis flow: **Figure 3a** shows the final nanofluidic device with a mcro-fluidic chamber that is connected to nanofluidic channels to implement a net 6000-fold constriction in the x, y, and z planes. This device was applied under positive DEP (~50-100 kHz) and negative DEP conditions, as shown in **Figure 3b** and **c**. To enable effective protein trapping without significant damage due to the temperature rise upon Joule heating in conductive media (σ_m ~ 1S/m), we carry out protein trapping away from the constriction hot-spot region where the localized temperature rise can damage the protein through inducing conformation changes. This is accomplished by applying negative dielectrphoresis in conjunction with a DC bias for electrophoresis flow to enable bio-particle trapping in the region at the interface of the mi-crofluidic channel and the nanofluidic constriction, away from high electric field of the constriction, where the temperature rise occurs. In this manner, the nanoscale constriction is applied as a "dam" for the electrophoretic flow of negatively charged proteins, to enable its pre-concentration away from the constriction, where there is no significant temperature rise.

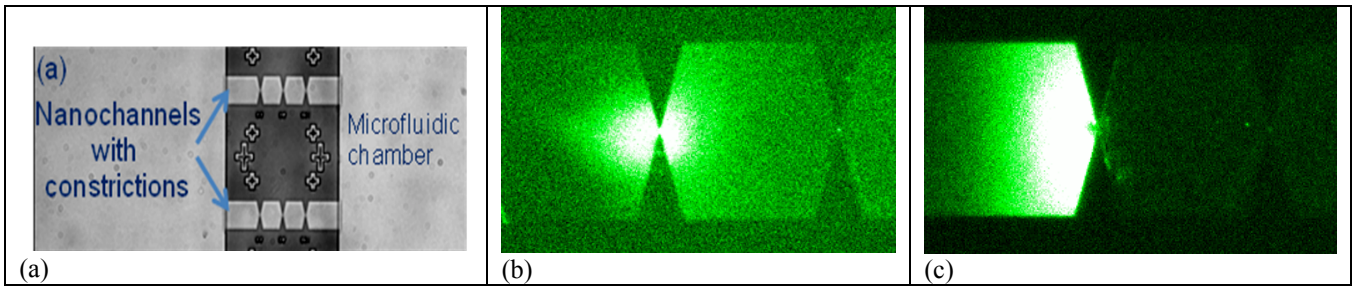

Figure 3. (a) Optical micrograph (top view) of trapping device with micro- and nanofluidic channels fabricated in fused silica containing three constrictions (30 μm to ~30 nm in width and to 200 nm in depth). (b) Protein (Alexa-488 labeled streptavidin) trapping with positive DEP at 50 kHz. (c) At 1 MHz, negative DEP in conjunction with electrophoresis results.

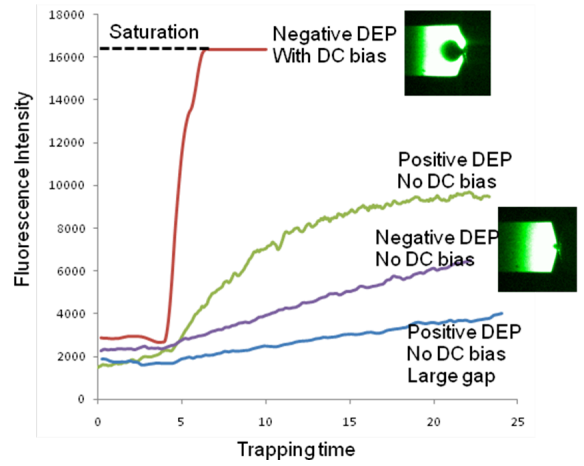

Figure 4: Negative dielectrophoresis under DC bias causes saturation of the protein signal, indicating 10^5-fold protein pre-concentration in 10 seconds. The same cannot be achieved in the absence of DC bias or with positive DEP or with negative DEP at a larger constriction gap.

Figure 4 shows that this implementation methodology, of negative dielectrophoresis with a DC bias causes >10^5-fold protein concentration within 20 seconds, which is 2~3 orders of magnitude faster than previous studies [1-3]. We will present a detailed transport analysis from the force balance of the particle mobilities due to dielectrophoresis, electrophoresis and electrothermal flow for varying nanofluidic constriction designs.

CONCLUSION

Protein trapping by dielectrophoresis in conductive bio-fluid media requires nanofluidic device designs with ~5000-fold to enhance DEP trapping forces over electrothermal flow. However, positive dielectrophoresis trapping can cause localized temperature rise within the hot-spot at the constriction to cause damage to the protein molecules through inducing conformation changes. Hence, we implement negative dielectrophoresis in conjunction with electrophoresis flow under DC to enable protein trapping away from the hot-spot regions of the constrictions.

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CONTACT

*N.S. Swami, tel: +1-434-9241390; nswami@virginia.edu