Nanofluidic Networks for Single Molecule Protein Analysis

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Overview

A robust, low cost, and reproducible process for polymer nanochannel fabrication based on thermomechanical deformation is being developed, with application to nanofluidic biomolecular separations followed by single molecule detection. A critical issue in single-molecule analysis is the ability to spatially constrain molecules of interest within a nanoscale confinement zone. Spatial localization serves to reduce Brownian motion of low molecular weight analytes, and to provide a fixed location for interrogation of the molecule of interest. A variety of early approaches to this goal have been reported, including the use of suspended microdroplets (2) and trapping molecules within nanoscale porous gels (3). More recently, confining molecules within submicron silica capillaries has received attention. For example, in an early demonstration by Nie and Lyon, submicron detection channels fabricated from silica capillaries were used to extend the observation time of single molecules (4). The nanoscale capillaries were fabricated by using a CO_2 laser to locally heat a standard capillary while pulling along the capillary length, thus necking down the detection channel, with final inner diameters of around 500 nm. However, capillary nanochannels cannot readily support the interconnection of multiple nanofluidic elements, or the integration of nanochannels with larger microfluidic networks capable of providing system-level functionality such as analyte separation and sorting, reagent and analyte delivery, and integrated sensing. Thus, to avoid the limitations of capillary nanofluidics, lab-on-a-chip technology using nanochannels fabricated on planar substrates is of interest for the development of system-level analytical platforms.

The thermomechanical nanochannel process employs thermoplastic preforms containing channels with relatively large (tens of microns) critical dimensions. By deforming a preform above its glass transition temperature using a uniaxial load oriented parallel to the channel length, the cross-sectional dimensions may be controllably reduced by several orders of magnitude through the Poisson effect. Unlike previously established methods, this nanofabrication technique offers several unique advantages. Because the initial substrate contains relatively large features, standard lithography with low-resolution optical masks may be used for preform fabrication. Furthermore, since only a portion of the initial substrate is deformed, the final device can contain both microscale and nanoscale channels in the same chip. This allows the fabrication of microscale features for system-level functionality such as fluid delivery, with a minimal dead-volume interface to the nanochannels, and with smooth and continuous transitions between microscale and nanoscale channels. The deformation process results in nanochannels with elliptical or circular cross-sections, which can improve the uniformity of flow and electric field distributions while also reducing flow resistance compared with channel cross-sections containing sharp corners. Nanochannel geometry may be controlled in a

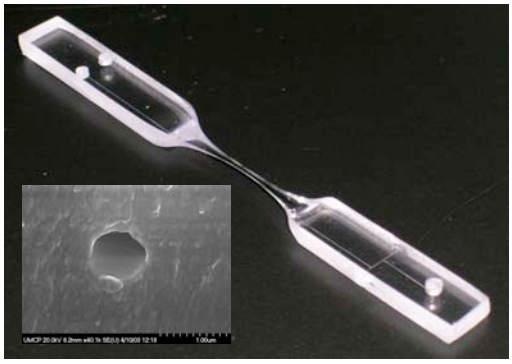
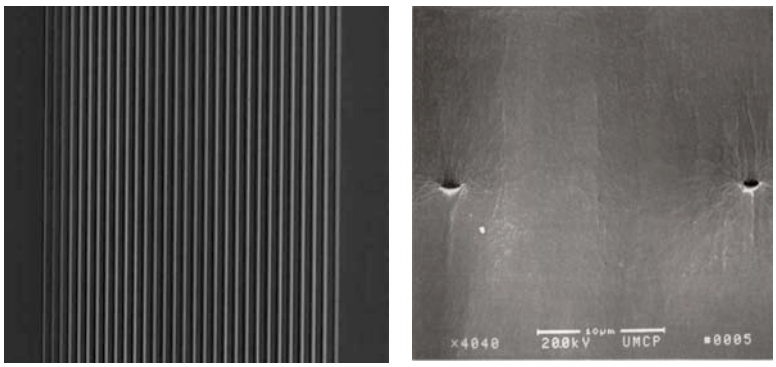


Fig. 1 Typical chip containing two 700 nm diameter nanochannels.

robust manner, and channels with widely-varying cross-sections may be prepared on the same chip by choosing different initial channel widths and locations relative to the preform edge. The process requires minimal fabrication equipment, is exceptionally high-throughput and cost-effective, and is applicable to a wide range of thermoplastics, enabling the fabrication of nanochannels which benefit from a wealth of polymer chemistries.

Fully enclosed channels with diameters ranging from several microns down to 200 nm have been realized. A typical nanofluidic chip fabricated using the thermomechanical deformation method is shown in Fig. 1. This particular chip contains two 700 nm diameter circular channels drawn down from initial preform channels with trapezoidal cross-sections of 30 μm depth and 20 μm width at half-depth. Large numbers of nanochannels are readily fabricated in a single substrate, such as the 30-channel array shown in Fig. 2. Channel geometry may be controlled using a number of process parameters, with an order-of-magnitude variation in diameter possible between different channels on a single chip.



(a) (b)

Fig. 2 (a) Nanochannel array, and (b) electron micrograph showing two adjacent channels.

Single Molecule Detection

To evaluate the utility of the nanochannel fabrication process, the detection of single bovine serum albumin (BSA) molecules has been demonstrated in a nanofluidic network consisting of adjacent detection and reference channels. This effort was initiated through new collaboration with Prof. Doug English at the Univ. of Maryland enabled by the NIRT project. Both the reference channel and detection channel were prefilled with isopropanol. The reference channel was next filled with a high concentration solution of 40 nm diameter fluorescent labeled polystyrene beads (FluoSpheres®, 505 nm excitation and 515 nm emission, Molecular Probes) suspended in deionized water with 1% v/v of Triton X-100 surfactant as a suppressing agent for dye adsorption on the channel walls. The fluorescent beads in the reference channel act as a bright marker allowing the detection channel position to be readily located. After preparing the reference channel, the detection channel was then filled with a solution containing BSA

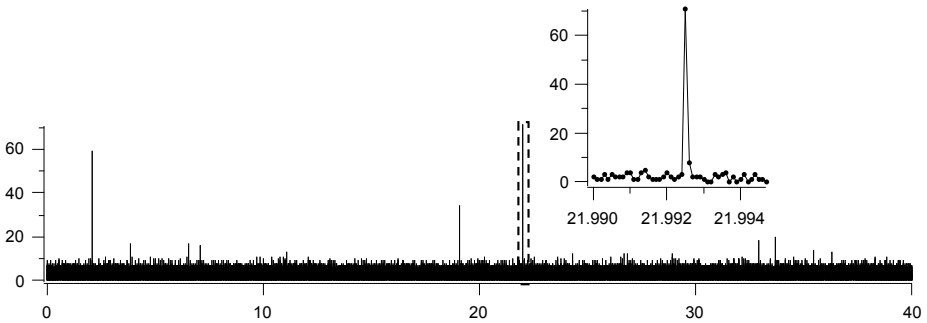


Fig. 3 Fluorescent measurements for labeled BSA (pH 8).

conjugated with Alexa Fluor dye (497 nm excitation and 520 nm emission). Measurements using two BSA concentrations were performed using 90% v/v isopropanol in 2 mM Tris HCl with a protein concentration of 15 nM. Measurements were performed on an inverted microscope (Carl Zeiss Axiovert 200) modified for sample-scanning laser confocal imaging. Typical transient measurements are shown in Fig. 3. The acquisition time for each point is 0.1 ms, with a measured average burst width of 0.22 ±0.13 ms. The variation in peak height in Fig. 3 is expected due to molecules passing through the detection region along longitudinal paths with different radial positions, resulting in different excitation energy and photon capture efficiency.

Nanochannel Protein Separations

Current research is focused on modeling and characterizing the performance of biomolecular separations in nanochannel networks, coupled with single molecule detection. We are currently investigating the performance of isoelectric focusing (IEF) of proteins in nanochannels, and plan to extend the concept to full 2-dimensional separations combining IEF and nanochannel electrophoresis with single molecule detection in an integrated platform for single-cell proteomic studies. Initial IEF results coupled with single molecule detection using a mixture of several model proteins have been obtained, using a chemical mobilization method to stream separated species past the fixed confocal detection window. A numerical model is under development to predict the formation of pH gradients within ampholyte solutions in nanochannels. Initial results using a 5-peptide ampholyte solution are shown in Fig. 4. Of particular interest in ongoing modeling studies are limitations imposed by proton and hydroxyl transport between macro-scale reservoirs, micro-scale delivery channels, and nano-scale separation channels.

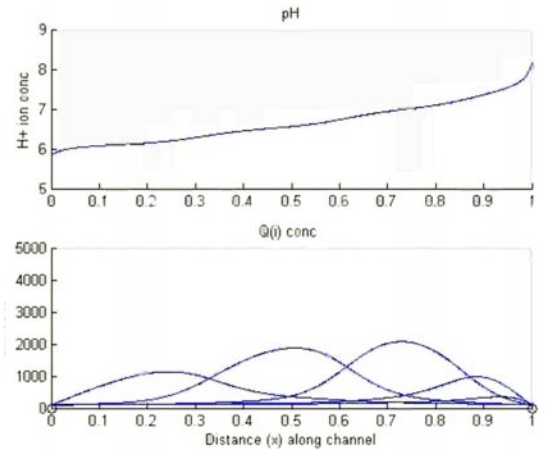


Fig. 4 Numerical model results for pH gradient formation during IEF.

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