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High-Density Nanopore Array for Selective Biomolecule Transport

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High-Density Nanopore Array for Selective Biomolecule Transport

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Abstract

Development of sophisticated tools capable of manipulating molecules at their own length scale enables new methods for chemical synthesis and detection. Although nanoscale devices have been developed to perform individual tasks, little work has been done on developing a truly scalable platform: a system that combines multiple components for sequential processing, as well as simultaneously processing and identifying the millions of potential species that may be present in a biological sample. The development of a scalable micro- nanofluidic device is limited in part by the ability to combine different materials (polymers, metals, semiconductors) onto a single chip, and the challenges with locally controlling the chemical, electrical, and mechanical properties within a micro or nanochannel.

We have developed a unique construct known as a molecular gate: a multilayered polymer-based device that combines microscale fluid channels with nanofluidic interconnects. Molecular gates have been demonstrated to selectively transport molecules between channels based on size or charge. In order to fully utilize these structures, we need to develop methods to actively control transport and identify species inside a nanopore. While previous work has been limited to creating electrical connections off-channel or metallizing the entire nanopore wall, we now have the ability to create multiple, separate conductive connections at the interior surface of a nanopore. These interior electrodes will be used for direct sensing of biological molecules, probing the electrical potential and charge distribution at the surface, and to actively turn on and off electrically driven transport of molecules through nanopores.

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The authors would like to acknowledge Mark Shannon, Director of the Center of Advanced Materials for the Purification of Water with Systems Micro-Nano-Mechanical Systems Laboratory at the Department of Mechanical Science and Engineering (MechSE) University of Illinois at Urbana-Champaign. Mark was instrumental in mentoring and guiding Adam Sawyer, Strategic Partnership fellowship recipient, in his reaching his daily research objectives and project goals.

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NOMENCLATURE

UICU	University of Illinois at Champagne Urbana
SNL	Sandia National Laboratories
PMMA	poly-methylmethacrylate
FIB	focused ion beam
PCR	Polymerase Chain Reaction
TAMARA	Carboxytetramethylrhodamine dye
DOE	Department of Energy
µm	micron
nm	nanometer

1. Introduction

The tools needed to rapidly separate, identify, and/or synthesize a huge array of biomolecules remains a grand, yet unsolved challenge. The goal is to simultaneously process huge numbers (>> millions) of biomolelcues in small volumes (<< nanoliters per sample). Simply put, we need to be able to transport and control biomolecules within a lab-on-a-chip analogous to the way electrons are routed and controlled in chips. Nanoscience and nanoengineering has been touted as the means to create the needed tools, but the technologies based on nanoscale components does not yet exist. A central problem facing nanotechnology is how to fabricate structures at sizes smaller than can be seen (read or transduced) and/or manipulated (written or transcribed). A nano-system is now possible that can selectively add and subtract incredibly small *attoliter* volumes (10^{-21} m^3) of different specific biomolecules from biological fluids. Moreover, this can be done in massive arrays to build true 3-D systems, thereby giving us the ability to control molecules millions at a time. The key discovery made at University of Illinois at Champagne Urbana (UICU) that enables such a technology to become reality is the molecular gate**.** Akin to the transistor, the molecular gate can digitally deliver and control attoliters of material, much like diodes and electronic switches deliver and control electrons, but with more function because molecules can undergo chemical reactions. The network of 3-D nanofluidic and electronic elements can be viewed as an array of multi-functional nanoscale chemical reactors simultaneously creating and delivering chemical compounds to spatial locations where needed. The proposed collaborative research between UICU and Sandia National Laboratories would expand our understanding of biomolecular interaction within nanofluidic constraints with the potential to explore new methods of *in-situ* identification of molecules within a nanopore and is the focus of this research report

Development of sophisticated tools capable of manipulating molecules at their own length scale enables new methods for chemical synthesis and detection. Although nanoscale devices have been developed to perform individual tasks, little work has been done on developing a truly scalable platform : a system that combines multiple

components for sequential processing, as well as simultaneously processing and identifying the millions of potential species that may be present in a biological sample. The development of a scalable micro- nanofluidic device is limited in part by the ability to combine different materials (polymers, metals, semiconductors) onto a single chip, and the challenges with locally controlling the chemical, electrical, and mechanical properties within a micro or nanochannel.

We have developed a unique construct known as a molecular gate: a multilayered polymer-based device that combines microscale fluid channels with nanofluidic interconnects. The molecular gate differs from other nanofluidic systems in that microfluidic and nanofluidic elements are assembled in a 3-dimensional construct where the nanofluidic connections are made with a nanoporous membrane, as opposed to single etched nanochannels. This allows for selective control of chemical and mechanical properties of each layer, and enables solution properties such as pH and ionic strength to be independently maintained in each layer. By utilizing dense arrays, nanofluidic transport phenomena can be exploited while maintaining high throughput. Molecular gates have been demonstrated to selectively transport molecules between channels based on size or charge. Processes have also been developed to metallize and modify the surface chemistry of the nanopores, changing the electrokinetic transport characteristics. Current research is focused on individually addressable nanopores for developing a scalable system to efficiently utilize nanoscale transport.

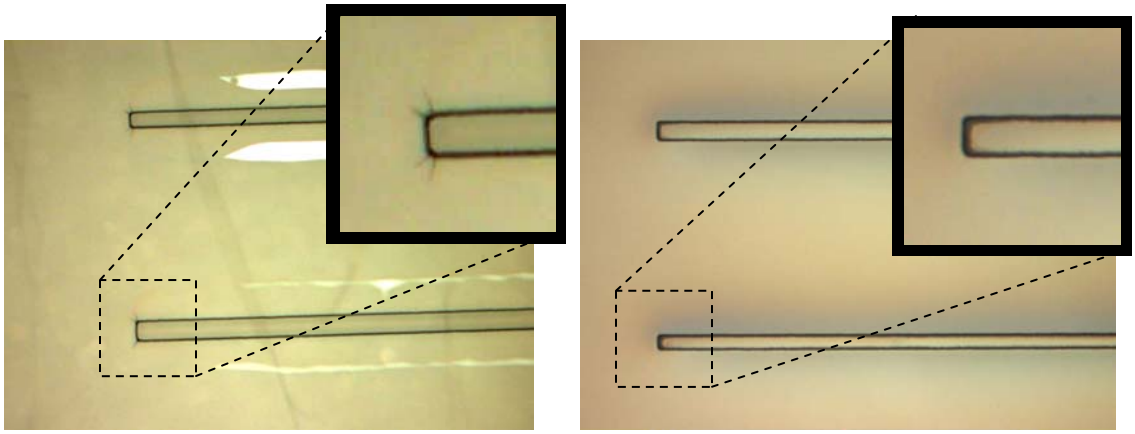
1.1. Development of the molecular gate system

The initial research focus in this project for the development of molecular gate system was to (1) Investigate new materials for device fabrication to reduce thermal stresses in layers and increase electrical isolation, and (2) develop a molecular gate based platform that includes multiple inlet and outlet microchannels, a central reaction chamber, and an on-chip microheater.

Previous designs for the molecular gate devices consisted of poly- methylmethacrylate (PMMA) channel layers separated by polycarbonate nanoporous membranes. Integrating thin metal layers into the devices proved challenging due to the large thermal stresses

produced from the required processing temperatures (200°C) and mismatch in coefficient of thermal expansion (~3ppm for metals, ~50ppm for PMMA). This thermal stress produced cracks in the metal layers. Electrical current leakage was also observed between metal layers separated by PMMA.

To address these problems, a new fabrication process was developed using SU-8, a photodefinable epoxy, for the primary device layers. Since SU-8 can be patterned and crosslinked at only 90°C, the overall thermal stress in the layers can be significantly reduced. The original SU-8 layers were processed on a temporary glass substrate, which still produced cracks in the layers due to thermal mismatch. By switching to a hybrid PDMS/glass substrate, thermal stresses could be further reduced, leading to crack free layers shown in Figure 1. Electrical measurements indicate leakage currents two orders of magnitude lower for SU-8 layers when compared to PMMA. A new patternable adhesive recipe for bonding layers together was also created to maintain low processing temperatures. An all SU-8 device with thin evaporated palladium electrodes is shown in Figure 2.



(a) (b)

Figure 1: Microscope images of a) cracks forming when SU8 is processed directly onto glass and b) a crack-free layer of SU8 when it is processed on custom substrate.

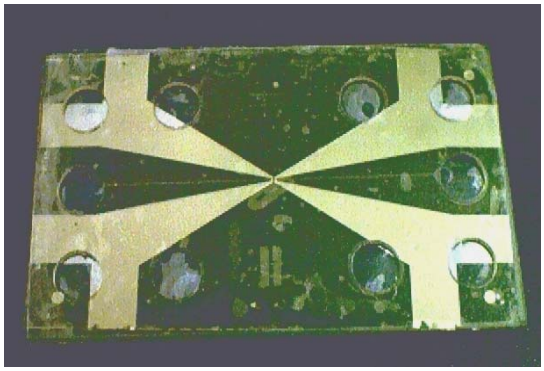


Figure 2 Molecular gate device with SU-8 microchannel layers and evaporated palladium electrodes

Devices designed for PCR or related biological tests were fabricated that included multiple inlet and outlet microchannels, a central reaction chamber, and an on-chip microheater (see Figure 3). Initial evaluation tests showed a tendency for uneven fluid flow through the reaction chamber, caused by increased capillary forces at the walls of the device and the physical deformation of the thin outer layer. These problems were overcome by using an externally applied pressure to control the deformation of the outer membrane, shown in Figure 4. The influence of shallow (3µm deep) surface microstructures such as grooves and holes on flow uniformity was also studied. It was found that grooves could direct the initial filling of the chambers, but had little effect during steady flow through the device. A LabVIEW based controller was used to control the temperature of the on-chip controller, although the actual temperature of the solution within the device still needs to be verified.

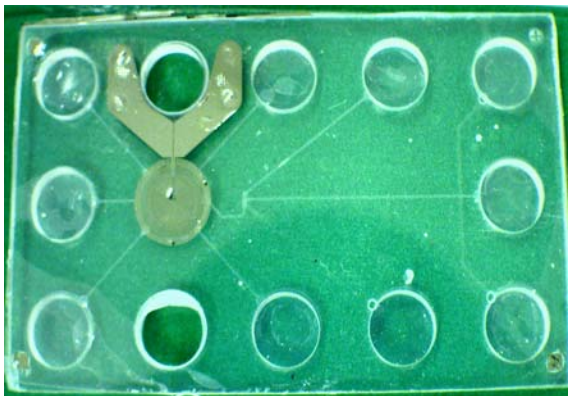


Figure 3. Molecular Gate device for PCR. A central reaction chamber with an on-chip heater is fed by inlet and outlet channels. Nanofluidic interconnects exist between the channels and the chamber, and at the crossing channels on the right side.

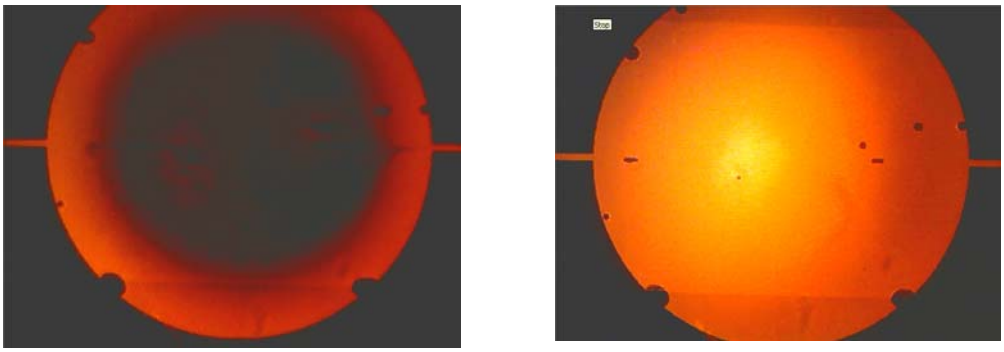


Figure 4. Fluorescent image of TAMRA dye injected into a chamber a) with thin outer layer and no external pressure and b) with external vacuum applied to the chamber

1.2. Nanochannel fabrication

One of the major accomplishments during this project was the successful fabrication of nanochannels with multiple addressable electrodes inside the channels. The previous approach to fabricating these nanochannels involved stacking alternating layers of metal and polymer (PMMA) layers and etching a nanopore using a focused ion beam (FIB). This FIB processes was believed to change the surface chemistry of the polymer nanopore, and electrical leakage was frequently observed between layers. In order to successfully integrate metal electrodes into nanochannels, a new device design was created. Sequential patterns of gold and copper were deposited on glass substrates and coated with a photodefinable polymer. The copper layer was then dissolved in a copper etching solution, leaving a nanoscale channel spanning over gold electrodes. Since normal chemical etching is limited by diffusion of reactants, creating a long aspect ratio channel will take several days. In order to accelerate the etch process, a potential is applied to the sacrificial electrode to assist in dissolution. This allows channels with over 1000:1 aspect ratios to be fabricated in several minutes.

Initial tests on the anodic dissolution of copper showed residue formed in the channel could inhibit complete etching. Bubble formation also occurred, which could increase the overall etch rate but also risked permanently clogging the nanochannel. By optimizing the applied potential, etchant solution composition, and device geometry, open nanochannels could be created with multiple gold electrodes spanning the channel surface.

Integrating addressable electrodes into the surface of a nanopore requires new methods of fabrication. Although traditional techniques involving wet chemical etching or plasma etching of silicon can be used to create nanoscale channels, patterning electrodes and complete sealing of the channels is challenging. The new fabrication approach involves patterning sequential layers of copper, gold, and SU-8, a photodefinable epoxy, on a glass substrate. An assembled device is shown in Figure 5. The copper acts as a sacrificial layer and the gold is used to create the in-channel electrodes. The SU-8 forms microchannels that are used to bring fluid to the entrance of the nanochannel. The removal of copper during the etch process is limited by the diffusion of copper ions, and can take up to several days without an external driving force. In order to accelerate the process, an external voltage was applied to the center channel, increasing the reaction kinetics and continuously driving fresh solution to the etch front. Complete etching of a 250 micron long nanochannel can be accomplished in approximately 10 minutes.

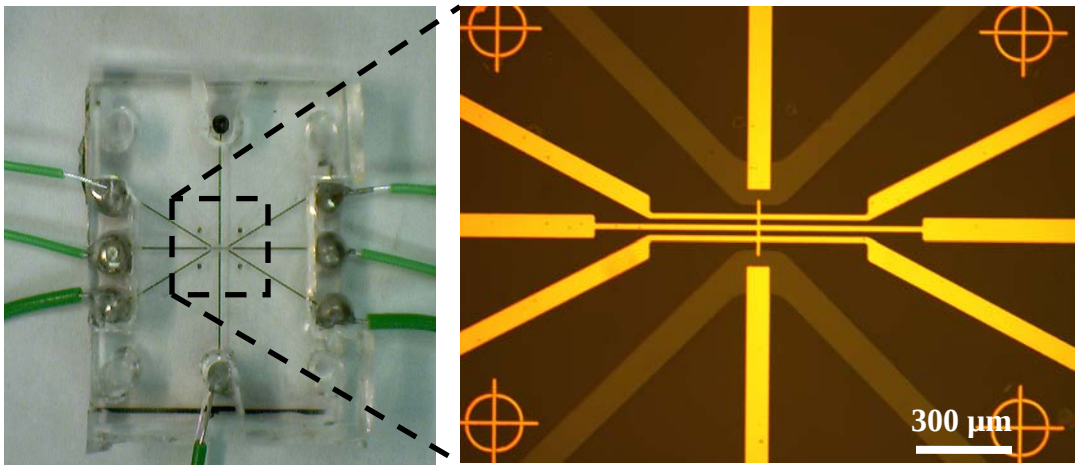


Figure 5. Assembled device with microchannels in SU-8, a sacrificial copper, and 3 gold cross electrodes. The cross section of the copper is 10µm x 100nm, and spans 250 microns.

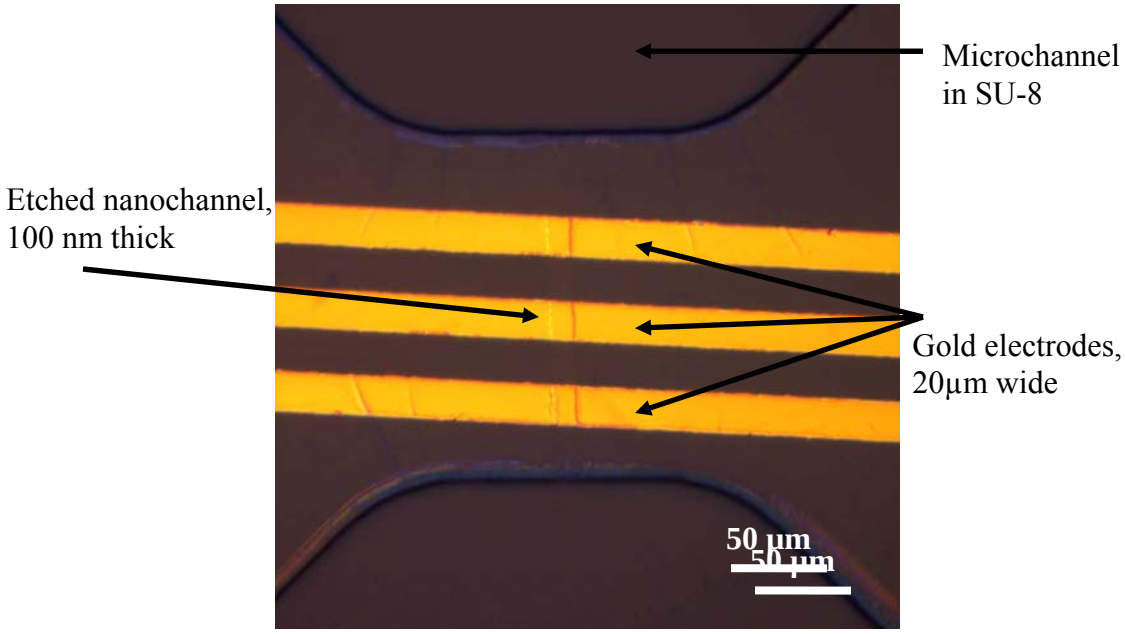


Figure 6. Micro-nanochannel device with gold electrodes

Figure 6 shows a completely etched channel. Creating an open nanochannel with intact electrodes proved challenging since local changes in pH and ion concentration can occur quickly inside the channel, leading to the formation of a copper hydroxide precipitate. Localized fields across the surface of the gold cross channels also lead to bubble formation, which can clog the channel and inhibit etching. Etching conditions were optimized to avoid these issues while maintaining fast etch rates. Initial results show that a rhodamine based fluorescent dye can be transported through the nanochannel, and bright spots appear where the rhodamine has apparently attached to the gold cross electrodes (Figure 7). Additionally, more work is necessary to focus on selectively attaching and removing thiolated flurophores to the gold surface, and utilizing these molecules to quantify the electrical potential inside the channel.

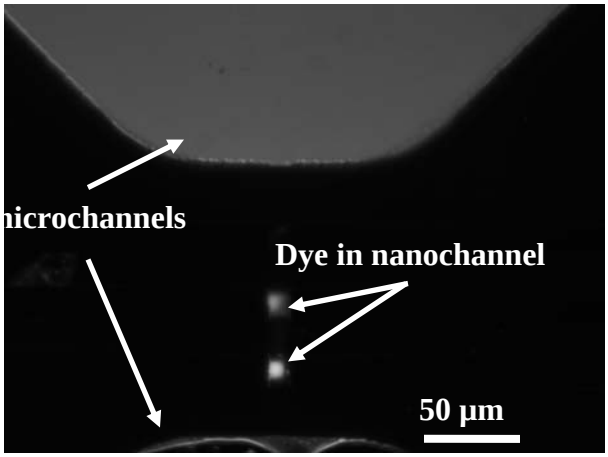


Figure 7. Rhodamine dye attached to gold cross electrodes in fabricated nanopore

1.3. Future work and application to biomolecules

Since it was demonstrated nanochannels with widths 25-100 nm can be created using a sacrificial metal etching process, with multiple gold electrodes patterned within the channel, our goals was to performed fluorescence and electrochemical measurements to demonstrate thiolated molecules (BODIPY derivatives and decanethiol) can be attached to a gold surface inside a microchannel. Future work is needed to verify thiol binding in a nanochannel to test and described sacrificial metal etching process for nanochannels as a function of etchant concentration, channel size, applied voltage and etchant pH.

Towards our end goal, it possible to adapt the Molecular Gate design to utilize glass/nanoporous silicon as a structural material instead of PMMA and polycarbonate membranes to dramatically reduce band broadening during electrophoretic separations, allowing for higher precision in detection. With this functional concept, this micro-nanofluidic structure can be tailored for detection and identification of specific proteins and other biological molecules. Now, these structures in arrays of nanopores (diameters <100nm) that connect two or more microfluidic channels in a three-dimensional networks of channels, rather than conventional single plane microfluidic devices where channels confined to a single plane enables is possible. High-density arrays of nanopores allow nanoscale transport phenomena to be exploited while maintaining high throughput. Furthermore, specialization of each layer for a single task, for example the surface chemistry of channels in an individual layer, can be modified without interfering with other layers. The multilayer design also enables simultaneous parallel processing between layers. For

example, a simple electrophoretic separation may be performed in one channel, while individual bands are extracted and undergo a chiral separation in another layer. Although molecular gates can be used for a variety of chemistry and biological studies, we believe the greatest utility of molecular gates is on the transport and detection of proteins. Research by Han and others has demonstrated how overlapping electrical double layers in a nanochannel can result in million-fold preconcentration of proteins. The ability to separate, concentrate, and detect molecules on a single device will enable the discovery of proteins at extremely low concentrations (picomolar or femtomolar) that are normally masked by more common proteins at high concentrations. In order to use fluorescent measurements or electrochemical methods for detection, local concentrations of species must be in the nano- micromolar concentration range. This can be achieved by multiple sample injections and repeated concentrations, which is why it is important to reduce band broadening. Silicon and glass channels should be an improvement over polymers, but it is unclear what the actual detection limit for these devices will be. To verification results and demonstrate performance both for separation/detection studies and for electrical measurements inside nanopores, atomic force microscopy and scanning probe microscopy can be used to measure electrical potential near a surface. However, it important to understand these measurements do not necessarily reflect phenomena that occur in an enclosed nanopore. Separation and detection of proteins can easily be compared with conventional electrophoresis techniques for common, high concentration molecules, but any newly discovered molecules will be difficult to verify.

2. Conclusions

Despite this prevalence of nanostructured materials in numerous applications, relatively little is now known about the fundamental behavior of fluids and charged species within the confinement of nanoscale structures having dimensions ranging from a few to several tens of nanometers. This works will benefit many cross-cutting investment areas by providing improved understanding of transport processes not just within a single nanopore but a network. This work has enabled the understanding and aid the rational design of storage and method to selectively transport molecules relevant to Sandia's mission. Development of molecular gates as a scalable architecture for controlling molecules and nanotransport is a vital part of DOE's mission to advance scientific understanding of nanoscale phenomena. Tools to help understand nanoscale transport will ultimately lead to the development of new diagnostic devices and methods to explore alternatives in biosciences and behavior of biomolecules, environmentally sound energy sources, and new methods for purification of water.

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