

# REPORT DOCUMENTATION PAGE

AFRL-SR-AR-TR-05-

0184

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instruction data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 12 4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display this OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

<b>1. REPORT DATE (DD-MM-YYYY)</b> 04/29/05		<b>2. REPORT TYPE</b> Final Report		<b>3. DATES COVERED (From - To)</b> February 2002 - January 2005	
<b>4. TITLE AND SUBTITLE</b> Biofluidic Transport and Molecular Recognition in Polymeric Microdevices				<b>5a. CONTRACT NUMBER</b> F49620-02-1-0042	
				<b>5b. GRANT NUMBER</b>	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Davis, Robert H. Anseth, Kristi S. Bowman, Christopher N.				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> Department of Chemical and Biological Engineering University of Colorado, 424 UCB Boulder, CO 80309-0424				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> DOD Air Force Office of Scientific Research Dr. Hugh C. DeLong Program Manager, Biomimetics, Biomaterials, and Biointerfacial Sciences 4015 Wilson Blvd., Room 713 Arlington, VA 22203-1954				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approve for Public Release: Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> Polymeric microfluidic components were fabricated using living-radical photopolymerization (LRPP), including three-dimensional geometries, modified surfaces by grafting, microporous mixers/filters, micropumps, wires, and movable parts. LRPP enables controlled geometry and surface chemistry for fluid control, biomolecular and cell detection, and particle sorting. Key accomplishments include specific cell adhesion and cytocompatibility demonstrated with grafted surfaces, a fluid-responsive polymer micropump integrated on a device and characterized, a porous polymer plug fabricated within a microfluidic channel and used to mix adjacent streams, analyses and verification of wall effects on particle motion in narrow channels, a polymerizable resistance heater as an electrolysis pump, cell detection with fluorescence when cells adhere to a modified surface, rapid antigen/antibody detection at <1 pM, hybrid silicon/polymer microfluidic devices, flexible membranes and septums for pumping and sample injection, and development of microfluidic devices with arrays of wells and cells.					
<b>15. SUBJECT TERMS</b> microfluidics, photopolymerization					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b>
<b>a. REPORT</b>	<b>b. ABSTRACT</b>	<b>c. THIS PAGE</b>			Robert H. Davis
U	U	U	UU	5	<b>19b. TELEPHONE NUMBER (include area code)</b> (303) 492-7314

# **Technical Summary of Work Accomplished on Biofluidic Transport and Molecular Recognition in Polymeric Microdevices**

*Final Report on AFOSR Contract F49620-02-10042*

*Prepared on 4/25/05 by*

**Robert H. Davis, Dean and Patten Professor  
Department of Chemical and Biological Engineering  
University of Colorado  
Boulder, CO 80309-0424  
303-492-7314, robert.davis@colorado.edu**

## **1. Objective and Goals**

The overall objective of the completed work was the development and characterization of polymeric microfluidic components and devices fabricated by living-radical photopolymerization (LRPP) to impart desired geometries, material properties, and chemical functionalities. The unique advantages of LRPP include the ability to fabricate complex three-dimensional (3D) geometries with high aspect ratios and with covalently bonded layers, the ability to modify surfaces selectively with chemical grafts of different functionalities, the ability to form large numbers of devices by parallel processing, and the ability to pattern spatially desired patterns for biofluidic transport and molecular recognition applications. Specific goals of the project included 3D microfluidic networks, fluid pumps and mixers, patterned cell adhesion, and biomolecular detection, all with polymeric microdevices.

## **2. Status Summary**

This project was supported for the period 2/1/02-1/31/05, and is now complete with the objective and goals met. Polymeric 3D devices were constructed with covalently bonded layers using liquid polymer precursors and a new methodology called Contact Liquid Photolithographic Polymerization (CLiPP). CLiPP enables high aspect ratios and subsequent surface modification of devices for fluid control, selective reactions, cell adhesion, biomolecular detection, and/or sorting of particles in an analyte stream. The individual layer thicknesses are controlled using an enclosed curing environment where the top consists of a mask in contact with the liquid polymer precursor, sides are defined by a stainless steel frame, and the horizontal base is movable in the vertical direction. By using sacrificial layers, enclosed channels are made without the need for spacer materials, thus allowing for homogeneous channels with chemically identical enclosing surfaces. Sacrificial material is also used to prevent covalent bonding of subsequent layers where so desired, allowing for unattached, dynamic movable structures, such as cogwheels, in the device.

## **3. Accomplishments**

### *3.1 Task 1 – Polymeric Component Design and Fabrication*

The first task of this research was focused on developing the LRPP fabrication method and using it to control polymer geometry and chemistry, so that 3D components and devices could be fabricated. This task was completed, and listed below are the key accomplishments:

- A. The basic LRPP fabrication method for patterning a surface with different polymer chemistries covalently bound to the surface is shown in Figure 1. An example of high-aspect-ratio channels made by this method is shown in Figure 2.
- B. A schematic of the LRPP fabrication method for making a 3D polymeric microfluidic network is shown in Figure 3. A wax is used to temporarily fill a channel while the next layer is formed, and then the wax is dissolved and removed with a solvent to form an enclosed channel. An example of a 3D flow network, with channels that have underpasses and overpasses, is shown in Figure 4.
- C. Electrically conducting “wires” were photocured on the polymeric microdevices using pastes of fine metal particles dispersed in a monomer. Figure 5 shows an example of a circuit made on a polymeric device.
- D. Unattached, moving parts were fabricated using the LRPP method with removable wax. An example cogwheel is shown in Figure 6.
- E. Porous polymer plugs (as filters, mixers or scaffolds) were fabricated directly into microfluidic channels by including fine salt crystals in the monomer mixture and then later dissolving the salt crystals to leave behind pores. An example for mixing two adjacent streams is shown in Figure 7.
- F. Responsive polymers were grafted onto surfaces for use as valves, pumps and detectors. Examples of hydrophilic and pH-sensitive polymers grafted onto a hydrophobic surface are shown in Figure 8.

### 3.2 Task 2 – Biofluidic Transport Modeling and Characterization

The second task of this research involved modeling and characterization of fluid pumping, mixing and cell and reagent transport in polymeric microfluidic devices. This task was completed, and listed below are the key accomplishments:

- A. A hydrogel micropump was fabricated and characterized by both theory and experiment. Upon wetting, hydrogel particles swell and push reagent or wash fluids from an adjacent reservoir. Good agreement between theory and experiment is demonstrated in Figure 9 for a test device. Hydrogel micropumps have also been integrated onto a polymeric microdevice, with a flexible membrane separating the particles and reservoir.
- B. Using photopolymerizable wires, an electrolysis pump was fabricated on a microdevice. It is powered by a low-voltage battery and pushes fluid from a reservoir by the generation of gas bubbles. Good agreement between theory and experiment was obtained (see Figure 10), and flow-rate control is accomplished by adjusting the current.
- C. A gas-generating micropump was also developed and characterized based on the effervescent reaction of carbonic acid and an organic acid. The pumping rate can be controlled by choice of organic acid, particle size, and amount of reagents.

- D. In-line, microporous mixers were characterized for their effective dispersivity as a function of flow rate and pore-size distribution using modeling (Figure 11) combined with experiments (Figure 7).
- E. Reagent and cell or particle transport in narrow microfluidic channels was characterized by theory and experiment. Figure 12 shows that larger particles are slowed by interactions with the channel walls and that an optimum ratio of particle diameter to channel height of 0.8 best keeps the particles and reagents together. This work has been extended to nonspherical and deformable particles, and to particles in contact with a channel wall.
- F. Electroosmotic pumping was also modeled, including patterned flows to promote mixing with step changes in channel geometry or wall surface potential. A recirculating side loop was also introduced to provide hydrodynamic focusing and enhanced mixing without constricting the channel (such as would be important if cells or other particulates were present).
- G. A microfluidic valve was demonstrated using a surface-grafted hydrogel that swells when wetted (Figure 13), with control possible via pH changes (Figure 8).

### 3.3 Task 3 – Molecular Recognition and Microfluidic Interfacing

The third task of this research was focused on developing several molecules and biological recognition or detection methods and integrating these methods with polymeric microfluidic devices. This task was also completed, though some of the applications are different than originally envisioned when the proposal was written four years ago. Key accomplishments are listed below:

- A. Antigen detection was accomplished by grafting the approximate antibody or sensing compound via acrylation and polymerization to the surface. Figure 14 shows a schematic of this technology and its demonstration with prostate-specific antigen (PSA).
- B. Antigen concentrations of less than 1 pM ( $10^{-12}$  M) were detected with assay times of approximately 10 minutes. Figure 15 shows detection data for a compound (glucagon) that is impossible to detect by standard techniques, due to its short half-life.
- C. The antigen-antimer detection strategy was integrated and demonstrated on a microfluidic device (Figure 16).
- D. A detection strategy for nucleic acids and other biomolecules was designed for very dilute solutions. As shown in Figure 17, a capture sequence is grafted to the polymer substrate, and then a genomic target binds to it along with a macroinitiator. After washing, the surface is exposed to light, causing a fluorescent monomer to polymerize and allowing detection/quantification of the genomic target. As many as  $10^9$  monomers are polymerized per binding event, allowing for oligonucleotide detection levels well below the standard of 100 nM ( $10^{-7}$  M) using conventional techniques.

- E. Surface modification was demonstrated for controlling cell adhesion and seeding on polymer surfaces. Figure 18 shows that cells grown on the hydrophobic substrate but not on the hydrophilic graft. Cytocompatibility was also demonstrated.
- F. A new technique for detecting individual cells and quantifying dilute cell concentrations was developed by patterning a substrate surface with a fluorescent marker on a cleavable moiety. When a cell deposits on the surface, it internalizes the moiety to produce fluorescence (Figure 19).
- F. Microfluidic devices were fabricated for cell-screening and tissue-engineering studies. Figure 20 shows fibroblast cells cultured in a  $4 \times 4$  array on porous polymer networks. Figure 21 shows an implantable microdevice capable of delivering nutrients or drugs to specific sites in brain tissue. Figure 22 demonstrates an array of tissue wells on a polymeric microdevice; the wells are seeded with liver cells for detection of viral pathogens and evaluations of various chemical agents.

### 3.4 Deliverables and Impact

This project resulted in an entirely new research effort in microfluidics at the University of Colorado. It is expected to lead to substantial spinoff research and further development, both at the University of Colorado and by partner institutions. The key deliverables and impact are listed below:

- A. *What impact did the funding have on the PIs and the institution?* The funding from this SIMBIOSYS grant established a collaboration between Professors Anseth (biological engineering), Bowman (polymer engineering), and Davis (fluid mechanics) at the University of Colorado. As noted in Section 4, it partially supported 10 PhD students (six of whom have completed their dissertations) and two postdocs. The collaboration has been highly successful, leading (so far) to 24 reviewed publications, 27 conference presentations, and eight patent applications or invention disclosures. In addition, the three PIs and three of the students formed a spinoff company, CLP Microtechnologies, to commercialize the CLiPP process.
- B. *What impact did the PIs and the institution have on the nano/micro/bio and microfluidic communities?* As listed in Section 10, collaborations were established with six other universities, four federal laboratories and five companies. These collaborations have varied from sharing research results to making devices or prototypes for testing in trial applications.
- C. *How has the DoD benefited from this work?* The work, which started from scratch, has not matured to the point of having a device or technology used by the DoD in the field. However, some of the technologies for cell or biomolecular detection may be of use in the future, and the work with Sandia to interface polymeric and silicon microcomponents may have future military applications.
- D. *What are the transferable technologies and products that have resulted from this work?* The basic living-radical photopolymerization (LRPP) technology has been licensed to Optical Associates, Inc. The ability to tailor-make desired geometries and surface chemistries is a transferable technology for a variety of applications, and several

examples are listed in Section 10. The new methods for detection and quantification of cells and biological molecules are expected to be transferable to military and civilian sectors, but products have not yet been developed. Finally, the modeling results, such as predictions of the speed at which a particle will translate through a narrow channel, are also transferable and may be of interest for microdevice design.

- E. *What future plans do the PIs and the institution have for further development of these technologies?* Commercial development will be pursued by the spinoff company, CLP Microtechnologies, and its partners. Basic research spawned by this project is being pursued by the PIs at the University of Colorado in areas such as multiphase flow through confined geometries such as porous media (now funded by NASA and ACS-PRF), applications of flexible polymers (funded by NIH), and use of polymeric microfluidic devices for rapid cell screening and tissue engineering (funded by NIH and HHMI).

#### **4. Personnel**

This work was undertaken by the following members of the Department of Chemical and Biological Engineering at the University of Colorado at Boulder.

##### *4.1 Faculty*

Kristi S. Anseth: Tisone Professor and HHMI Investigator  
Christopher N. Bowman: Gillespie Professor and Chair  
Robert H. Davis: Patten Professor and Dean

##### *4.2 Postdoctoral Research Associates*

Hadley Sikes  
Alexander Zinchenko

##### *4.3 PhD Students*

Chris Brotherton (2006 anticipated completion)  
Brian Good (2005 completion)  
Andrew Griggs (2007 anticipated completion)  
Tommy Harraldson (2005 completion)  
Brian Hutchison (2003 completion)  
Jeffrey Knutsen (2005 completion)  
Sirish Reddy (2004 completion)  
Bobby Sebra (2006 anticipated completion)  
Helen Simms (2007 anticipated completion)  
Michelle Staben (2005 completion)

#### **5. Reviewed Publications**

Sebra, R.P., Masters, K., Bowman, C.N., and K.S. Anseth. "Rapid antigen detection in a biologically complex fluid using photografted whole antibodies," *Nature Materials* (submitted).

Knutsen, J.S. and Davis, R.H., "Deposition of Foulant Particles During Tangential Flow Filtration," *Journal of Membrane Science* (under review).

- Haraldsson, K.T., Hutchison, J.B., Sebra, R.P., Good, B.T., Anseth, K.S., and Bowman, C.N., "3D Polymeric Microfluidic Device Fabrication via Contact Liquid Photolithographic Polymerization (CLiPP)," *Sensors and Actuators Part B: Chemical* (under review).
- Good, B.T., Bowman, C.N., and Davis, R.H., "Portable Fluid-responsive Polymer Micropumps," *Journal of Microelectromechanical Systems* (under review).
- Good, B.T., Reddy, S., Davis, R.H., and Bowman, C.N., "Tailorable, Low-Modulus Thiol-ene Materials for Microfluidic Applications," *Journal of Polymer Science Part A - Polymer Chemistry* (under review).
- Good, B.T., Bowman, C.N., and Davis, R.H., "An Effervescent Reaction Micropump for Portable Microfluidic Systems," *Lab-on-a-Chip* (under review).
- Good, B.T., Brotherton, C.M., Smirnova, D.S., Bowman, C.B., and Davis, R.H., "Integration of Conductive Materials into Polymeric Devices for Portable Microfluidic Applications," *Lab-on-a-Chip* (under review).
- Staben, M.E., Zinchenko, Z.Z., and Davis, R.H., "Dynamic Simulation of a Spheroid between Two Parallel Plane Walls in Low-Reynolds-Number Poiseuille Flow," *Journal of Fluid Mechanics* (under review).
- Staben, M.E., Galvin, K.P., and Davis, R.H., "Low-Reynolds-Number Motion of a Heavy Sphere between Two Parallel Plane Walls," *Chemical Engineering Science* (under review).
- Griggs, A., Zinchenko, A.Z., and Davis, R.H., "Low-Reynolds Number Motion of a Deformable Drop between Two Parallel Walls," *Journal of Fluid Mechanics* (under review).
- Staben, M.E., and Davis, R.H., "Particle Transport in Poiseuille Flow in Narrow Channels," *International Journal of Multiphase Flow* (accepted).
- Reddy, S.K., Sebra, R.P., Anseth, K.S. and Bowman, C.N., "Living Radical Photopolymerization Induced Grafting on Thiol-Ene Based Substrates," *Journal of Polymer Science, Part A. Polymer Chemistry* **43**, 2134-2144 (2005).
- Simms, H.M., Brotherton, C.M., Good, B.T., Anseth, K.S., Davis, R.H., and Bowman, C.N., "In situ Fabrication of Macroporous Polymer Networks by Living Radical Photo Polymerization and Leaching within Microfluidic Devices," *Lab-on-a-Chip* **5**, 151-157 (2005).
- Good, B.T., Bowman, C.N., and Davis, R.H., "Modeling and Verification of Fluid-responsive Polymer Pumps for Microfluidic Systems," *Chemical Engineering Science* **59**, 5967-5974 (2004).
- Brotherton, C. M. and Davis, R.H., "Electroosmotic Flow in Channels with Step Changes in Zeta Potential and Cross Section," *Journal of Colloid Interface Science* **270**, 242-246 (2004).
- Hutchison, J.B., Haraldsson, K.T., Good, B.T., Sebra, R.P., Luo, N., Anseth, K.S., and Bowman, C.N., "Robust polymer microfluidic device fabrication via contact liquid photolithographic polymerization (CLiPP)," *Lab-on-a-Chip* **4**, 658-662 (2004).
- Cramer, N.B., Reddy, S.K., Lu, H., Cross, T., Raj, R., and Bowman, C.N., "Thiol-Ene Photopolymerization of Polymer Derived Ceramic Precursors," *Journal of Polymer Science, Part A. Polymer Chemistry* **42**, 1752-1757 (2004).
- Staben, M. E., A. Z. Zinchenko, and Davis, R.H., "Motion of A Particle Between Two Parallel Walls in Low Reynolds Number Poiseuille Flow," *Physics of Fluids* **15**, 1711-1733 (2003).
- Luo, N. A. T. Metters, J. B. Hutchison, K. S. Anseth and Bowman, C.N., "A Methacrylated Photoiniferter as a Chemical Basis for Microlithography: Micropatterning Based on Photografting Polymerization," *Macromolecules* **36**, 6739-6745 (2003).
- Reddy, S.K., Cramer, N.B., Cross, T., Raj, R., and Bowman, C.N., "Polymer Derived Ceramics from Thiol-Ene Photopolymerizations," *Chemistry of Materials* **15**, 4257-4261 (2003).

- Luo, N., J. B. Hutchison, K. S. Anseth and Bowman, C.N., "Synthesis of a Novel Methacrylic Monomer Iniferter and Its Application in Surface Photografting on Crosslinked Polymer Substrates," *Journal of Polymer Science Part A-Polymer Chemistry* **40**, 1885-1891 (2002).
- Luo, N., Hutchison, J.B., Anseth, K.S., and Bowman, C.N., "Surface-initiated Photopolymerization of Poly(ethylene glycol) Methyl Ether Methacrylate on a Diethyldithiocarbamate-mediated Polymer Substrate," *Macromolecules* **35**, 2487-2493 (2002).
- Bowman, C. N., Sebra, R.P., Haraldsson, K.T., Luo, N., Hutchison, J.B., and Anseth, K.S., "3D-Microfluidic Devices using Liquid Polymer Precursors," *ACS POLY Preprints* **224**, 731 (2002).
- Hutchison, J.B., Haraldsson, K.T., Hawker, C.J., Bowman, C.N., and Anseth, K.S., "Living Radicalphotopolymerizations for Microfluidic Devices: Novel Methods for Fabrication and Modification of Polymeric Components," *ACS PMSE Proceedings* **224**, 003 (2002).
- Hutchison, J.B., Hawker, C.J., and Anseth, K.S., "Modifying Photocrosslinked Networks with Living Free Radical Initiators," *ACS POLY Preprints* **223**, 243 (2002).

## 6. PhD Dissertations

- Good, Brian T. 2005. Development of Flow Control Elements for Portable Polymeric Microfluidic Devices.
- Haraldsson, Tommy. 2005. Contact Liquid PhotoLithographic Polymerization (CLiPP) as a Method for the Fabrication of Fully Polymeric Microfluidic Devices. (tentative title)
- Hutchison, J. Brian. 2003. Crosslinked Network Synthesis by Radical Chain Photopolymerization: Natural and Directed Evolution of Macromolecular Structure.
- Knutsen, Jeffrey S. 2005. Membrane Bioseparations: Cellulase Recovery, Particle Deposition, and Second Osmotic Virial Coefficients.
- Reddy, Sirish. 2004. Mechanistic Modeling, Network Evolution, and Advanced Applications of Novel Thiol-Vinyl Systems.
- Staben, Michelle E. 2005. Low-Reynolds-Number Particle Transport in Narrow Channels for Microfluidics and Other Applications.

Dissertations may be obtained from UMI/Proquest Digital Dissertations. Phone number: 1-800-521-0600, website: <http://www.umi.com/umi/dissertations/>.

## 7. Conference Presentations

### *Poster Presentations:*

- C.M. Brotherton, B.T. Good, T.K. Haraldsson, J.B. Hutchison, S. Reddy, R.P. Sebra, M.E. Staben, K.S. Anseth, C.N. Bowman, R.H. Davis, Living Radical Photopolymerization of 3D Microfluidic Devices for Biofluidic Transport, 2002 BioExpo, Fitzsimmons Bioscience Park, Aurora, CO, December 4, 2002.
- M.E. Staben, B.T. Good, A.Z. Zinchenko, K.S. Anseth, C.N. Bowman, R.H. Davis, Modeling and Experimental Analysis of Particle Transport in Microfluidic Devices, 2003 AAAS Annual Meeting, Denver, CO, February 15, 2003.
- M.E. Staben, A.Z. Zinchenko, R.H. Davis, K.S. Anseth, Modeling and Experimental Analysis of Particle Transport in Microfluidic Channels, Gordon Research Conference on The Physics and Chemistry of Microfluidics, Big Sky Resort, Big Sky, MT, August 24-29, 2003.

- D. Smirnova, B.T. Good, C.N. Bowman, R.H. Davis, Optimization of an Electrochemical Pump for Microfluidic Applications, American Institute of Chemical Engineers, Austin, TX, November, 2004.
- S.M. Naski, B.T. Good, C.N. Bowman, R.H. Davis, Gas-generating Micropumps for Microfluidic Devices, American Institute of Chemical Engineers, San Francisco, CA, November, 2003.
- H.M. Simms, C.N. Bowman, K.S. Anseth, Interfacing Biomaterials and Microfluidics for Tissue Engineering Applications, American Institute of Chemical Engineers Fall Annual Meeting, Austin, TX, November 7-12, 2004.
- H.M. Simms, C.N. Bowman, K.S. Anseth, Microfluidic Device Design and Fabrication for On-Device Cell Culture and Analysis, Analytical Chemistry Gordon Research Conference, Roscoff, France, 2005 (accepted).
- R.P. Sebra, C.N. Bowman, K.S. Anseth, A Rapid Antigen Detection Assay Using Photografted Whole Antibodies", Analytical Chemistry Gordon Research Conference, Roscoff, France, 2005 (accepted).
- R.P. Sebra, K.S. Masters, C.N. Bowman, K.S. Anseth, A Rapid Antigen Detection Assay Using Photografted Whole Antibodies, Spring MRS Conference, San Francisco CA, 2005.
- R.P. Sebra, J.B. Hutchison, K.S. Anseth, C.N. Bowman, Living Radical Photopolymerizations to Modify Surfaces in Microfluidic Devices for Biological Applications, ACS Meeting, New Orleans LA, Spring 2003.
- R.P. Sebra, K.S. Anseth, C.N. Bowman, Surface Modification of 3D Microfluidic Devices using LRPP Chemistries, AAAS Meeting, Denver CO, Winter 2003.
- R.P. Sebra, T.K. Haraldsson, K.S. Anseth, C.N. Bowman, 3D Polymeric Microfluidic Devices, Photopolymerization Fundamentals 2002 Conference, Breckenridge CO, Spring 2002.
- B.T. Good, T.K. Haraldsson, J.B. Hutchison, R.P. Sebra, H.M. Simms, R.H. Davis, K.S. Anseth, C.N. Bowman, Fabrication of Photopolymeric Microfluidic Devices and Components, Australasian Polymer Symposium, Noosa, Australia, July, 2003.
- B.T. Good, R.H. Davis, C.N. Bowman, Hydrogel Micropump for Microfluidic Devices, Material Research Society Conference, Boston, MA, December, 2002.
- B.T. Good, R.H. Davis, C.N. Bowman, Hydrogel Micropump for Microfluidic Devices, Gordon Research Conference on Gravitational Effects in Physico-chemical Systems, New London, N.H., July, 2001.

*Oral presentations:*

- M.E. Staben, A.Z. Zinchenko, R.H. Davis, Modeling of Particle Transport in Microfluidic Devices, Student Annual Research Symposium, Department of Chemical Engineering, University of Colorado, Boulder, CO, April 15-16, 2002.
- M.E. Staben, A.Z. Zinchenko, R.H. Davis, Modeling of Particle Transport in Narrow Microfluidic Channels, Low Reynolds Number Hydrodynamics Session of the 2002 American Institute of Chemical Engineers Fall Annual Meeting, Indianapolis, IN, November 3-8, 2002.
- M.E. Staben, A.Z. Zinchenko, R.H. Davis, Dynamic Simulation of Particle Transport in Narrow Microfluidic Channels, Low-Reynolds-Number and Microfluidic Flows Session of the 2003 American Institute of Chemical Engineers Fall Annual Meeting, San Francisco, CA, November 16-21, 2003.
- M.E. Staben, R.H. Davis, Particle Transport in Poiseuille Flow in Narrow Microfluidic Channels, Microscale Flows Session of the 2004 American Institute of Chemical Engineers Fall Annual Meeting, Austin, TX, November 7-12, 2004.

- A. Griggs, A.Z. Zinchenko, R.H. Davis, The low-Reynolds Number Motion of a Drop between Two Parallel Walls, International Congress on Theoretical & Applied Mechanics, Warsaw, Poland, August 2004.
- R.P. Sebra, K.S. Masters, C.N. Bowman, K.S. Anseth, A Rapid Antigen Detection Assay Using Photografted Whole Antibodies, Spring MRS Conference, San Francisco, 2005.
- R.P. Sebra, K.S. Masters, C.N. Bowman, K.S. Anseth, A Rapid Antigen Detection Assay Using Photografted Whole Antibodies, Annual AIChE Conference, Austin TX, 2004.
- R.P. Sebra, K.S. Masters, C.N. Bowman, K.S. Anseth, Synthesis of Antimers for Photografting on Polymeric Surfaces using Living Radical Photopolymerization Techniques, Society for Biomaterials 7<sup>th</sup> World Congress, Sydney Australia, 2004.
- R.P. Sebra, K.S. Anseth, C.N. Bowman, Living Radical Photopolymerizations (LRPP) for Constructing Polymeric 3D Microfluidic Devices with Spatially Controlled Grafted Functionalities, Annual AIChE Meeting, San Francisco, CA, Fall 2003.
- B.T. Good, C.N. Bowman, R.H. Davis, Fabrication of Hydrogel Components on Photopolymeric Microfluidic Devices, Australasian Polymer Symposium, Noosa, Australia, July, 2003.
- B.T. Good, R.H. Davis, C.N. Bowman, Fabrication of Polymeric Microfluidic Devices, University of Colorado Chemical Engineering Student Annual Research Symposium, Boulder, CO, February, 2003.
- B.T. Good, R.H. Davis, C.N. Bowman, Hydrogel Micropump for Microfluidic Delivery, University of Colorado Chemical Engineering Student Annual Research Symposium, Boulder, CO, April, 2002.

## 8. Patents and Invention Disclosures

- H. Sikes, K. Rowlen, J. Birks, and C.N. Bowman, "On Chip Signal Amplification by Photopolymerization," U.S. Patent Application filed September, 2003.
- C. Bowman, N. Cramer, and S. Reddy, "Novel Photopolymerization Schemes to Form Polymer Derived Ceramic Materials," U.S. Patent Application filed August, 2003. Technology License to DSM Desotech.
- T. Haraldsson, B. Hutchison, C. Bowman, and K. Anseth, "Fabrication of 3D Photopolymeric Devices," U.S. Patent Application filed July, 2003. Technology Licensed to Optical Associates Incorporated.
- R. Sebra, K. Masters, K. Anseth, and C. Bowman, "Facile Surface Modification for Rapid Biological Assays," Invention Disclosure, August, 2004.
- C.N. Bowman, N. Cramer, S. Reddy, and R. Sebra, "Photopolymerization Techniques for Enhanced Photopatterning of Polymeric Devices," Provisional Patent Application filed December, 2004. Exclusive option agreement to CLP MicroTechnologies, Inc.
- C.N. Bowman, R.H. Davis, B.T. Good, S. Reddy, D. Smirnova, "Photopolymerizable Septum for Microfluidic Devices," Provisional Patent Application filed February, 2005. Exclusive option agreement to CLP MicroTechnologies, Inc.
- H. Sikes, R. Hansen, C. Bowman, R. Kuchta, "Detection of Molecular Recognition Without the Use of Instrumentation," Provisional Patent Application filed March, 2005
- T. Haraldsson, H. Sikes, C. Bowman, S. George, M. Groner, "Detection of Molecular Recognition on a Surface-Passivated Microfluidic Device" Invention Disclosure, February, 2005.

## 9. Awards and Honors

### 9.1 Faculty

Kristi Anseth            2002 College of Engineering and Applied Science Hutchinson Teaching Award  
2003 ASEE McGraw Research Award  
2003 AIChE Colburn Award  
2004 Kalpana Chawla Recent Alumni Award  
2004 Boulder Faculty Assembly Research and Creative Award  
2004 NSF Alan T. Waterman Award  
2004 College of Engineering and Applied Science Research Award  
2004 Tisone Professorship

Chris Bowman:        2002 Boulder Faculty Assembly Research and Creative Work Award  
2002 College of Engineering and Applied Science Peebles Teaching Award  
2003 University of Colorado Physical Sciences Inventor of the Year Award  
2004 College of Engineering and Applied Science Service Award  
2005 Clemson Award for Literature Contributions on Dental Materials

Robert Davis         2002 U.C. Davis Joe and Essie Smith Distinguished Lecturer  
2002 ASEE Dow Lectureship Award  
2003 Boulder Faculty Assembly Outstanding Service Award

### 9.2 Students

Chris Brotherton     2004 Sandia National Laboratories Graduate Fellowship

Tommy Haraldson     2002 First Place in BioMEMS and Biomedical Nanotechnology\ World  
Poster Competition, Columbus, OH

Brian Hutchison     2002 First Place Materials Poster Session Award, American Institute of Chemical Engineers Annual Meeting, Indianapolis, IN

Bobby Sebra         2005 Graduate Student Gold Award at the Materials Research Society Spring 2005 Conference  
2003 Honorable mention in Student Poster Competition at the 2003 Annual Meeting of the American Association for the Advancement of Science, Denver, CO

Michelle Staben      2002-2003 National Science Foundation Graduate Research Fellowship  
2003-present NASA Graduate Student Researchers Program (GSRP) Fellowship  
2003 Honorable mention in Student Poster Competition at the 2003 Annual Meeting of the American Association for the Advancement of Science, Denver, CO

## 10. Technology Transitions

*University of Pennsylvania* – A comparison of results from their LaGrangian-Eulerian technique and our boundary-integral method for translational and rotational velocities of a sphere in a narrow channel under pressure-driven flow. Contacts - Haim Bau (215-898-8363, bau@seas.upenn.edu), and Howard Hu (215-898-8504, hhu@seas.upenn.edu).

*Lawrence Livermore Laboratory* – A comparison of results from their Lattice-Boltzmann simulations and our boundary-integral method for translational velocities of a sphere in a narrow channel under pressure-driven flow. Contact – David Clague (925-424-9770).

*Sandia National Laboratories* – A collaboration to investigate the effects of step changes in zeta potential and channel cross section on electroosmotic flow. Contact – Amy Sun (505-284-5861, acsun@sandia.gov). In addition, polymeric materials were developed that reversibly bond to silicon. Contact – Paul Galambos (505-844-1542, pcgalam@sandia.gov).

*Massachusetts Institute of Technology* – We fabricated three-dimensional microwell arrays integrated with filters for seeding cells to detect viral pathogens. Contact – Karel Domansky (617-258-9571, domansky@mit.edu).

*John Hopkins University* – We fabricated microfluidic channel arrays to investigate the effects of nutrients or drugs to specific regions in brain tissue. Contact – Philippe Passeraub (philippe.passeraub@ieee.org).

*Optical Associates Incorporated* – OAI has licensed the CLiPP fabrication technology and we have worked together to develop a commercial mask alignment system with CLiPP process tooling. Contact – Charlie Turk (408-232-0600, cturk@oainet.com).

*CLP MicroTechnologies, Inc.* – A start-up company founded to commercialize the CLiPP fabrication process and other research endeavors started from the CU-SIMBIOSYS program. Contact – Chris Bowman (303-492-3247, christopher.bowman@colorado.edu)

*Stanford University* – A collaboration to investigate polymers for microfluidic device applications with low-swelling characteristics in a variety of solvents. Contact - Steve Quake (quake@stanford.edu).

*Thermo Electron* – A collaboration to investigate the use of on chip signal amplification by photopolymerization for their point-of-care diagnostic products. Contact – Rob Jenison (800-558-9115).

*Caliper Life Sciences* – The goal of this project was to determine of the distribution of particle and cell velocities in narrow microfluidic channels. Contact – Andrea Chow (andrea.chow@caliperLS.com).

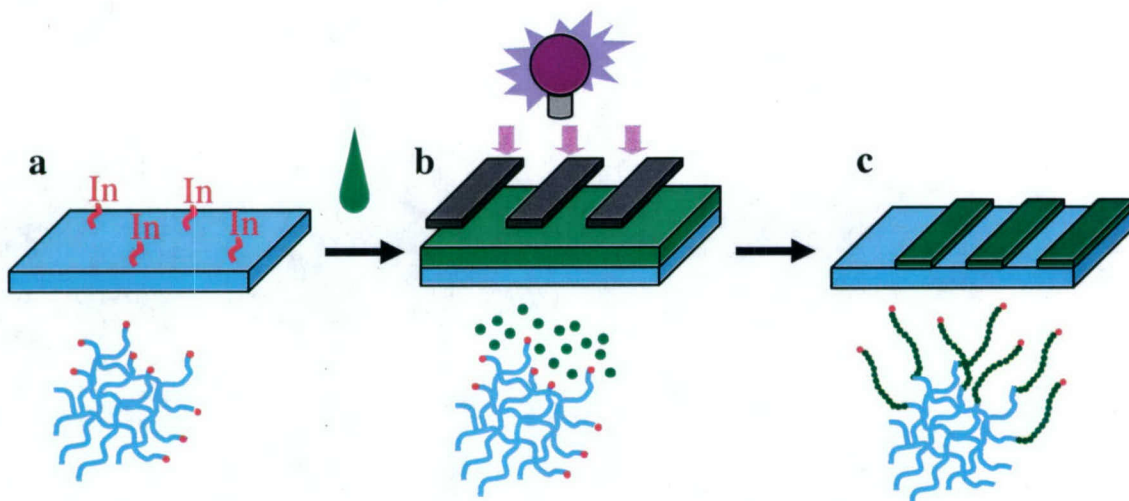
*Clemson University* – They purchased a mask alignment system with CLiPP tooling from OAI and we provided start-up support and know-how. Contact – Andrew Metters (864-656-3055, metters@clemson.edu).

*ALD NanoSolutions, Inc.* – This project is a collaboration to integrate their nanocoating technology with our fabrication method to passivate the microdevice surface. Contact – Karen Buechler (303-318-4145, buechler@ALDNanoSolutions.com).

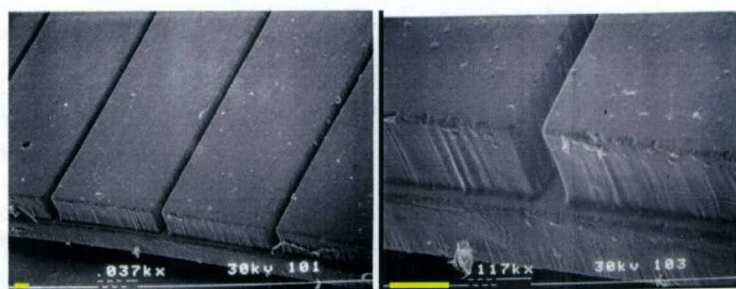
*Advanced MicroLabs/Colorado State University* – They would like to overcome difficulties in fabricating polymeric microdevices by using the CLiPP method. Contact – Chuck Henry (970-491-2852, cshenry@lamar.colostate.edu).

*NIST* – They are looking for a rapid-prototyping process to fabricate polymeric devices with low fluorescence. Prototype devices have been fabricated and are being analyzed by them. Contact – Brian Hutchison (301-975-3187).

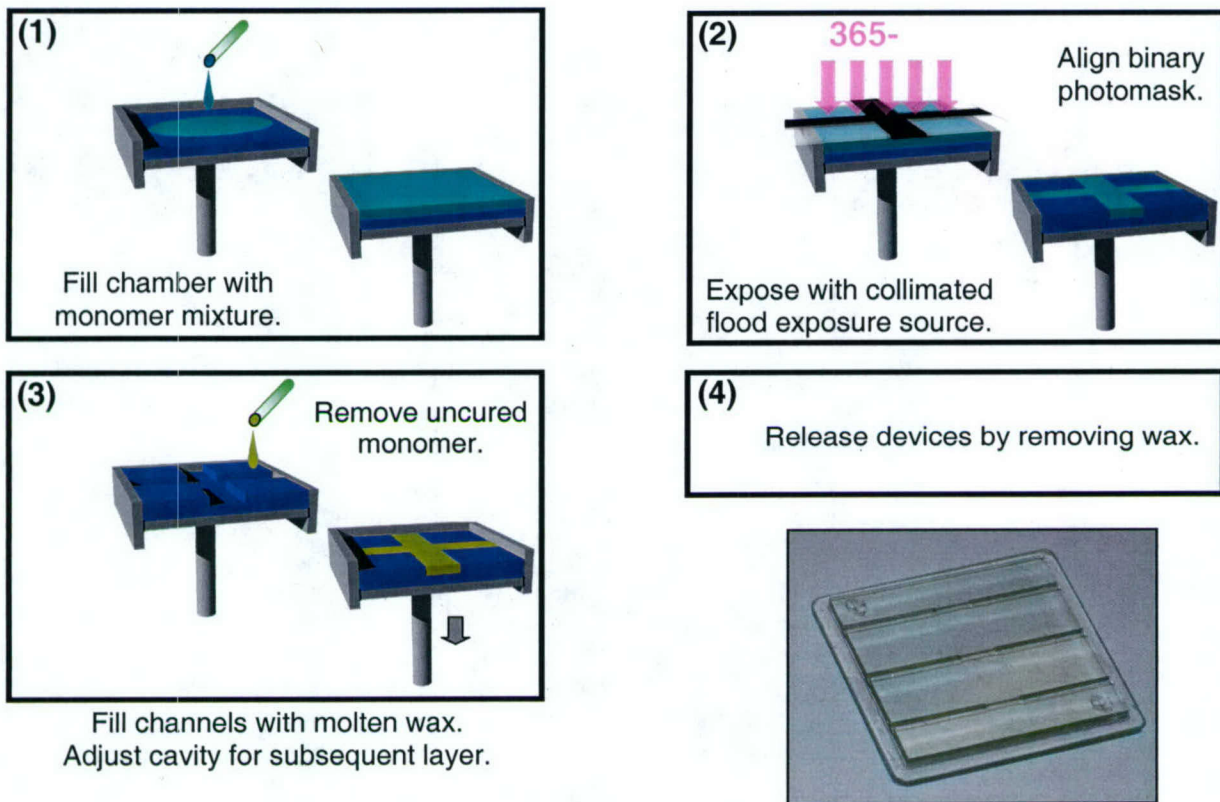
*NASA* – They are interested in a rapid-prototyping process to fabricate polymeric devices to test cell growth in microgravity environments. Contact – Tony Ricco (408-460-5666).



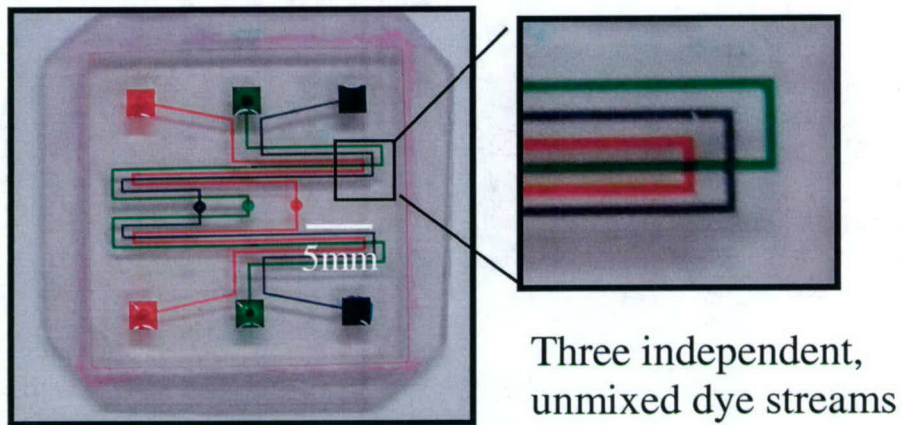
**Figure 1.** Schematic of the living-radical photopolymerization process for making multilayered polymeric microfluidic devices. After subsequent layers are polymerized on top of the first layer, the wax is removed to yield a 3D device with enclosed channels.



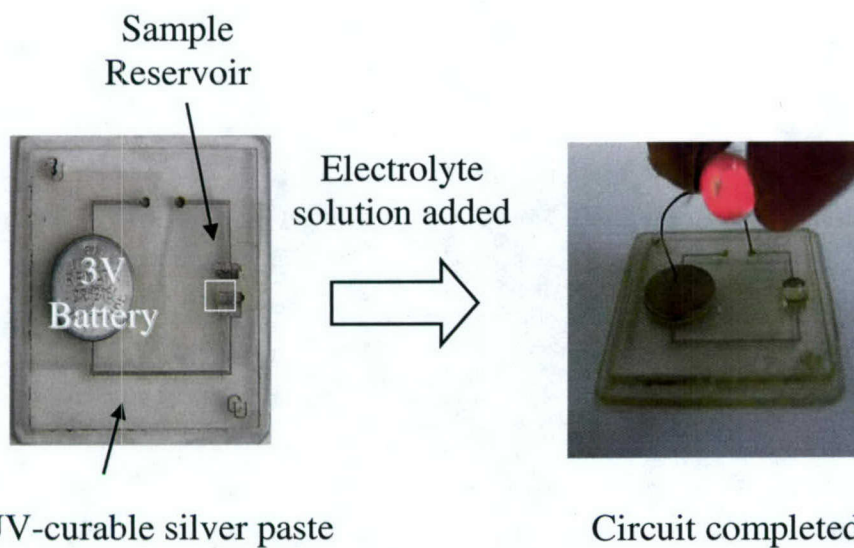
**Figure 2.** These scanning electron micrographs show that deep channels with high aspect ratios and good resolution can be achieved by LRPP. The figure on the left shows channels of varying width (60, 70, and 80  $\mu\text{m}$ , left to right), each 240  $\mu\text{m}$  tall, shown at a 50-degree tilt. A close-up of the 60  $\mu\text{m}$  channel at a 50-degree tilt is shown on the right (Yellow bars = 100  $\mu\text{m}$ ). Note: The lines on the edge of the polymer blocks are due to the limitation of the dot size on the transparency masks and not a limitation of the fabrication method.



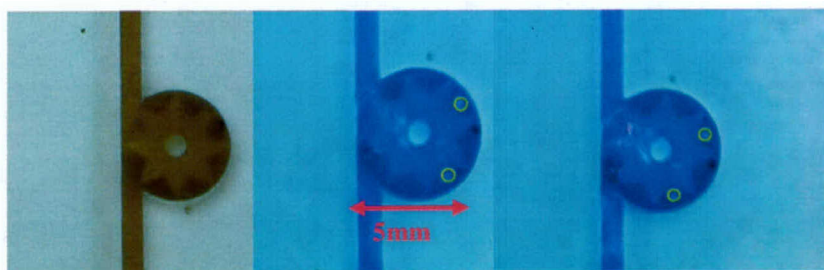
**Figure 3.** Schematic of the Contact Liquid Photolithographic Polymerization (CLiPP) method for making multilayer polymeric microdevices.



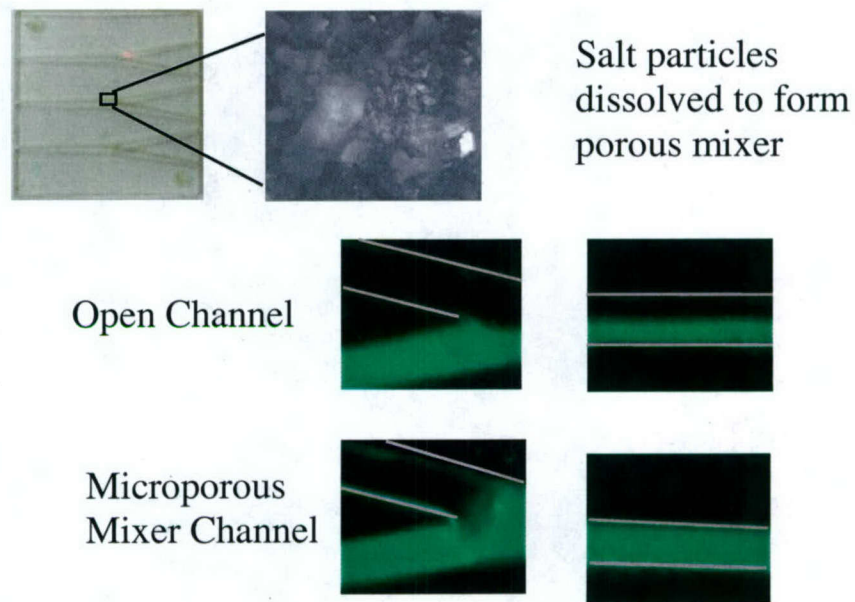
**Figure 4.** The figure on the left shows a five-layer, 3D device with 400  $\mu\text{m}$  wide channels that traverse multiple planes. The three channels are independent and unmixed, as shown in the inset. The LRPP fabrication technique enables complex, three-dimensional devices to be fabricated.



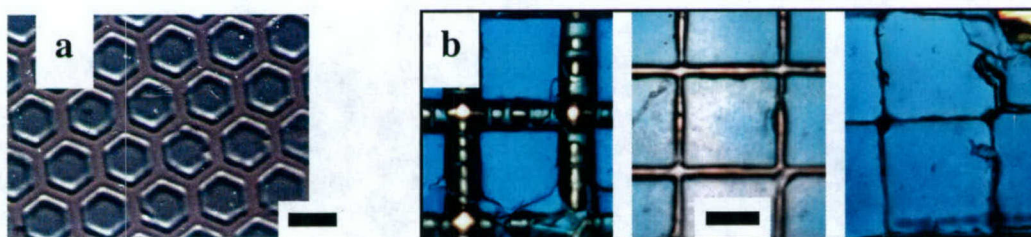
**Figure 5.** The figure on the left shows a device with an on-chip circuit fabricated from UV-curable silver paste polymerized in channels 50  $\mu\text{m}$  deep and 500  $\mu\text{m}$  wide. The circuit becomes conductive when an electrolyte solution (saturated ammonium chloride) is added to the reservoir and illuminates a jumbo LED bulb.



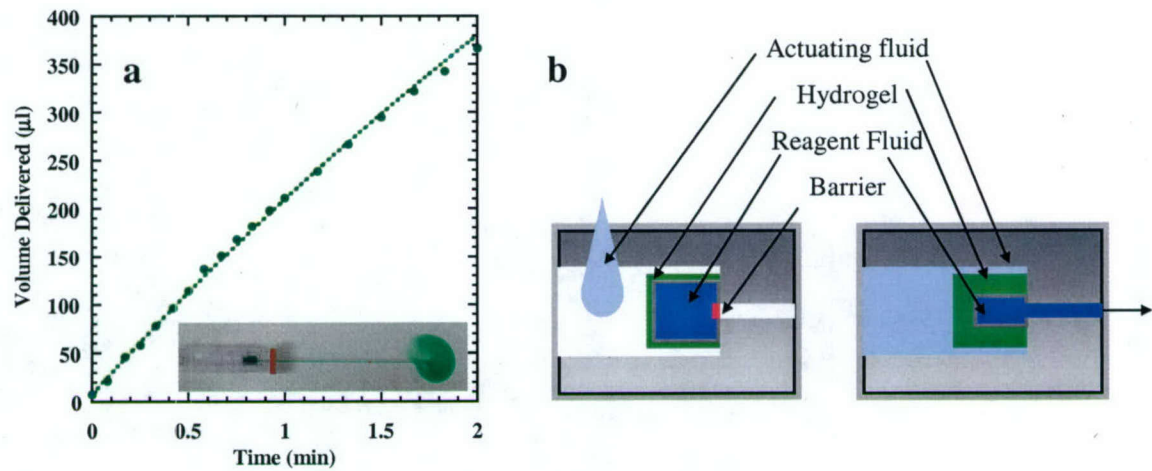
**Figure 6.** Unattached, movable structure fabricated using a sacrificial wax shown in the channel (left), as well with two distinct cogwheel positions (note position of outlined bubbles) that illustrate rotation due to fluid flow.



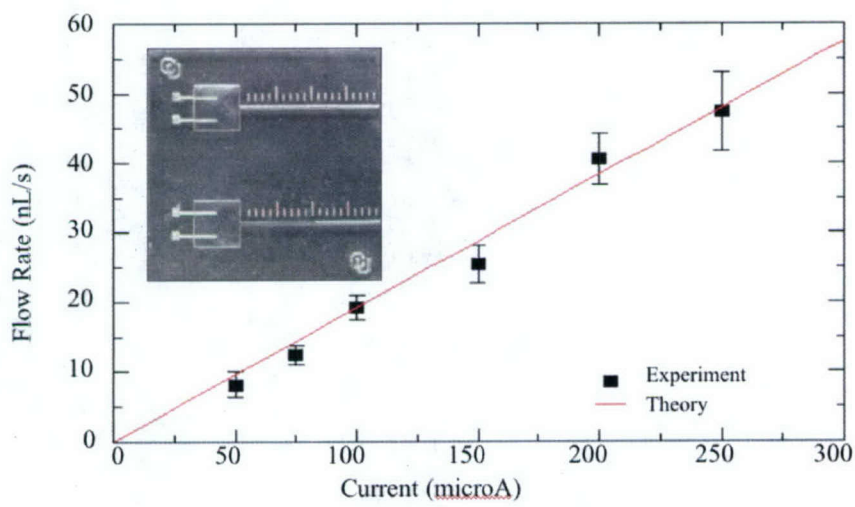
**Figure 7.** The top figures show a static porous mixer (3-4 mm long) polymerized in a  $400\ \mu\text{m} \times 400\ \mu\text{m}$  channel. The mixer is fabricated by adding a monomer solution with salt particles into the channel, polymerizing the monomer, and then dissolving out the salt particles to leave a porous structure. The lower figures show a comparison of mixing fluorescein-water with pure water in an open channel and in a channel with a microporous mixer (salt particles less than  $45\ \mu\text{m}$  in size were used). The efficiency of the mixer is a function of the size of the salt particles used to form the porous polymer plug.



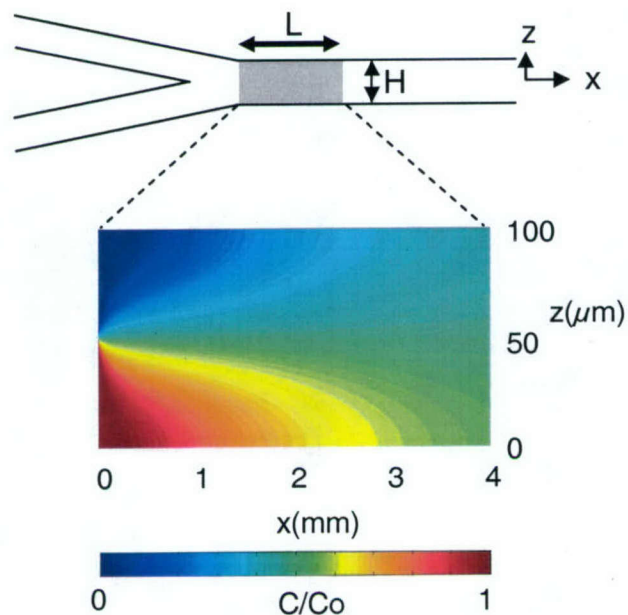
**Figure 8.** Polymer surfaces modified by photoiniferter-mediated grafting. (a) Hydrophilic PEG monomer grafted to a hydrophobic surface (scale bar:  $100\ \mu\text{m}$ ). (b) pH-sensitive poly(methacrylic acid) grafted to a hydrophobic substrate exhibits different swelling in response to pH variations (left: pH2; middle: pH7; right: pH10; scale bar:  $250\ \mu\text{m}$ ).



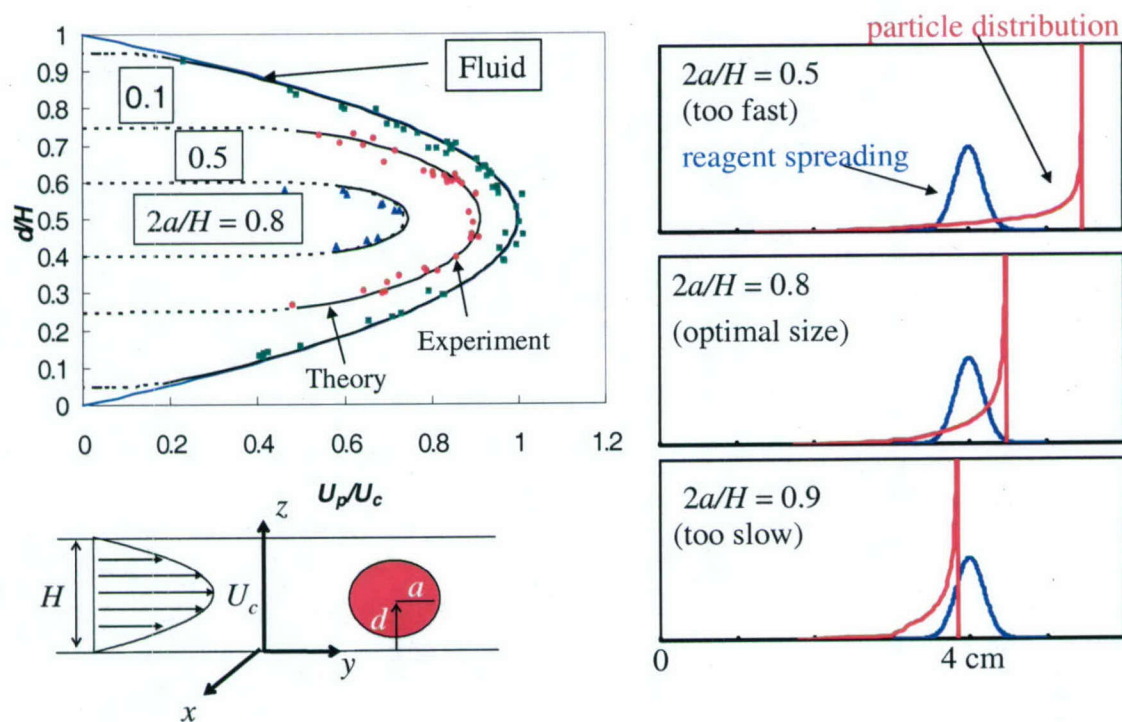
**Figure 9.** Fluid pumping actuated by hydrogel swelling. (a) Dynamic response of poly(acrylic acid) particles swollen in water to deliver fluid from a reservoir (the symbols are experimental data and the curve is a model prediction). (b) The design for a hydrogel pump fabricated by LRP includes a depot for the actuating fluid. The swelling hydrogel then constricts a reservoir containing the reagent fluid to be pumped through the channel. The barrier (which may be physical or a simple hydrophobic patch) yields due to pressure from the swelling hydrogel.



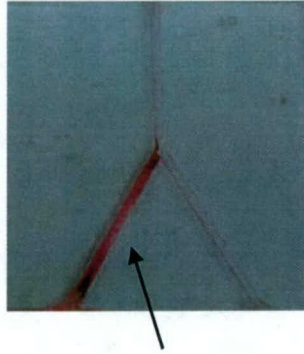
**Figure 10.** The inset figure shows two electrolysis pumps fabricated using living-radical photopolymerization combined with the integration of conductive carbon electrodes. The flow rate produced from these pumps is directly proportional to the applied current.



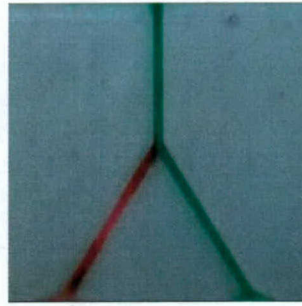
**Figure 11.** Predicted concentration profile for the mixing of two adjacent streams when passing through a porous plug.



**Figure 12.** Motion of particles and reagent in narrow channels during parabolic flow. The top left shows the particle velocity (normalized by the centerplane fluid velocity) as a function of particle center location across the channel for three different particle sizes. The figures on the right show the distances traveled by the particles and reagent molecules after a given time.

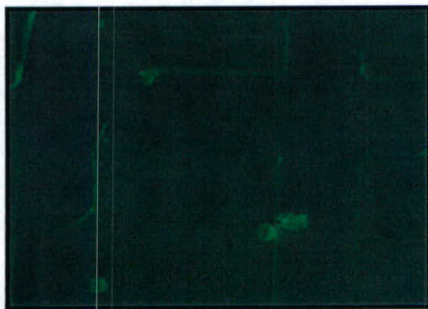
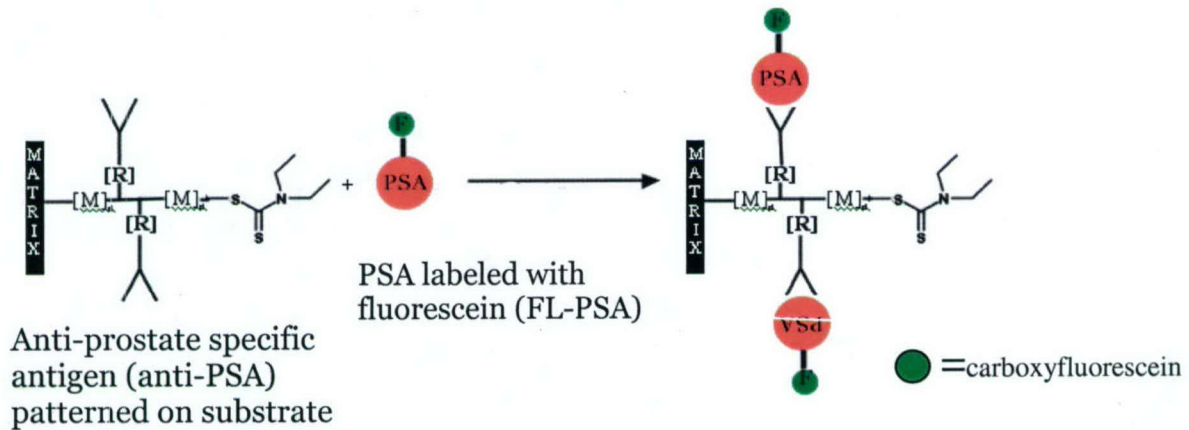


Grafted region reacts and becomes stained

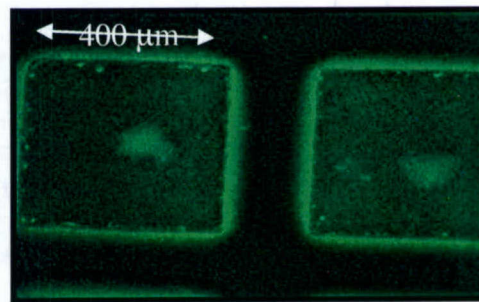


Grafted region swells in water and prevents flow of dyed water

**Figure 13.** This figure shows an 800  $\mu\text{m}$  wide channel that is surface-modified with a high molecular weight methacrylated chondroitin sulfate hydrogel that reacts with a Safranin O stain (red). The hydrogel acts as a valve when it is exposed to water (swells almost instantly) and prevents the flow of dyed water (green).

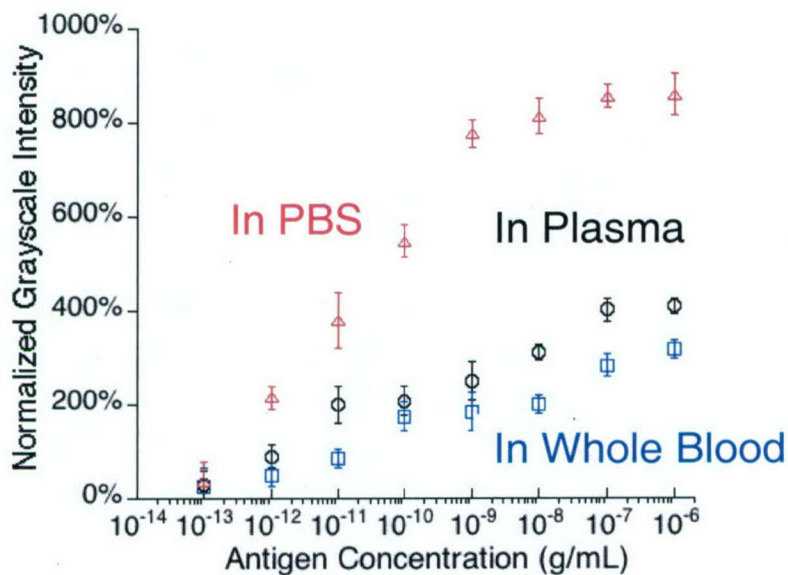


Negative Control: No PSA present

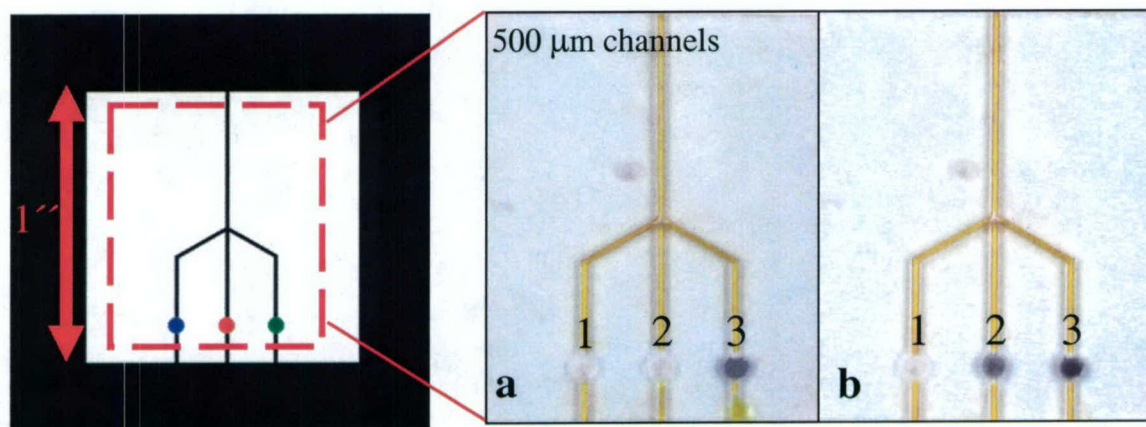


Positive Control: Contact with FL-PSA

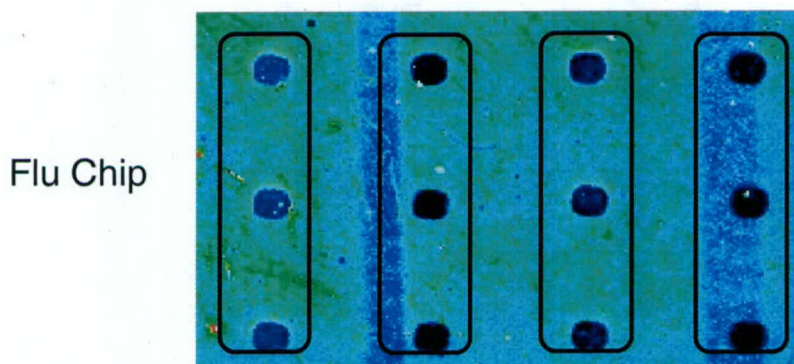
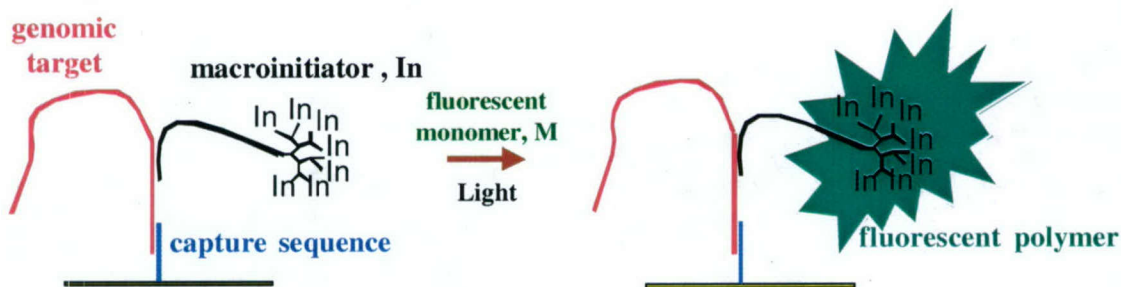
**Figure 14.** Detection of PSA on a polymeric surface by grafting the antimer onto the surface and including a fluorescent label with PSA.



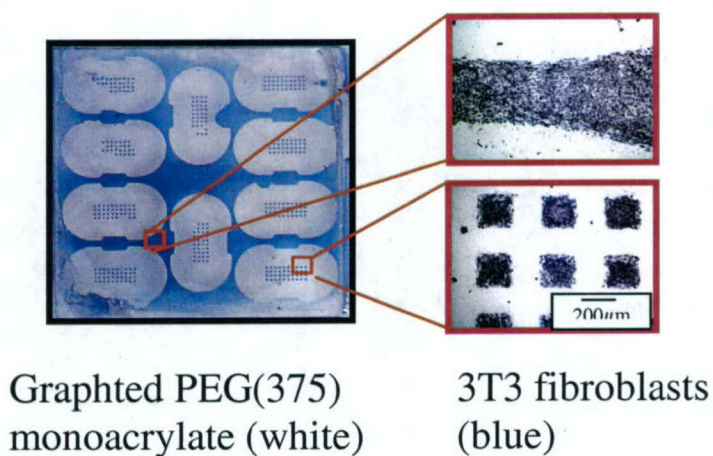
**Figure 15.** Chromatic intensity as a function of glucagon concentration in phosphate buffer solution, 20% plasma, and 20% blood analyte. The anti-glucagon antimer was immobilized on a polymer substrate.



**Figure 16.** Schematic of a 3-well, parallel microfluidic detection device. Blue well is a control PEG acrylate-grafted well, red is grafted with anti-goat antimer for detection of GAM-HRP, and green is grafted with anti-rabbit antimer for detection of RAM-HRP. a. Shows the chromogenic response using Vector VIP when well 3 detects GAM antigen. b. The response when well 2 detects RAM antigen (post GAM testing).

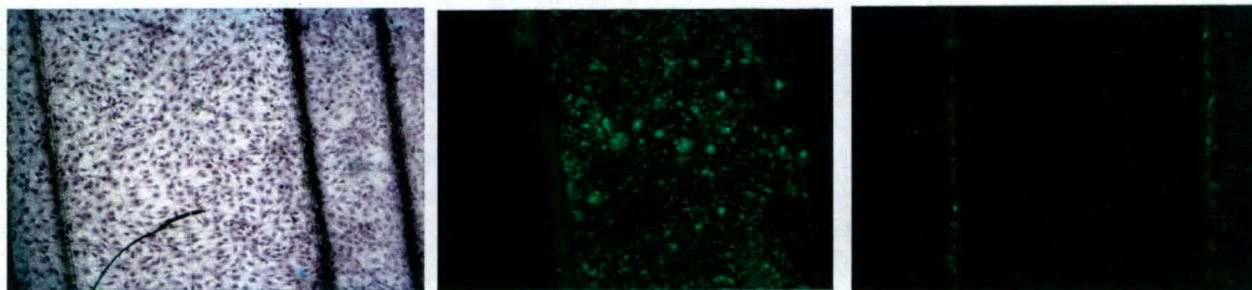
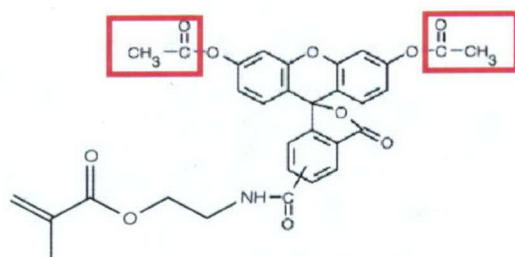


**Figure 17.** Schematic (top) for detection of dilute samples of DNA or RNA by hybridization, with amplification (below) detection of RNA influenza virus. The first column is a positive control, and the other columns are for flu types C, B, and A (left to right). Blue denotes areas of high fluorescence.



**Figure 18.** These figures demonstrate the absence of non-specific cell adhesion to the poly (ethylene glycol) (PEG) grafted regions. The white regions are grafted with PEG (375) monoacrylate. The blue regions are 3T3 fibroblasts seeded onto the device after 48 hours of swelling in ethanol/media solution.

## Fluoromer

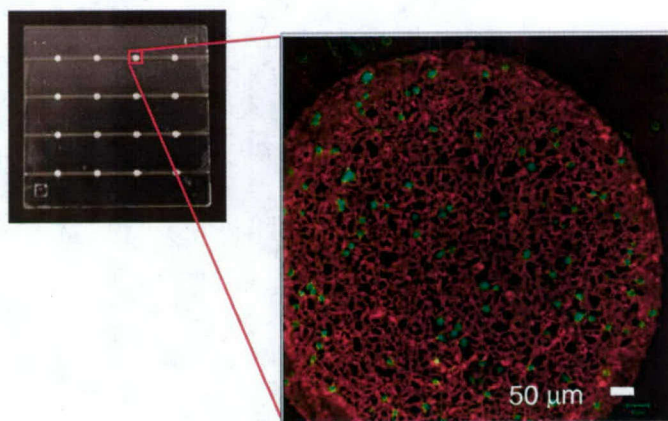


Attached cells

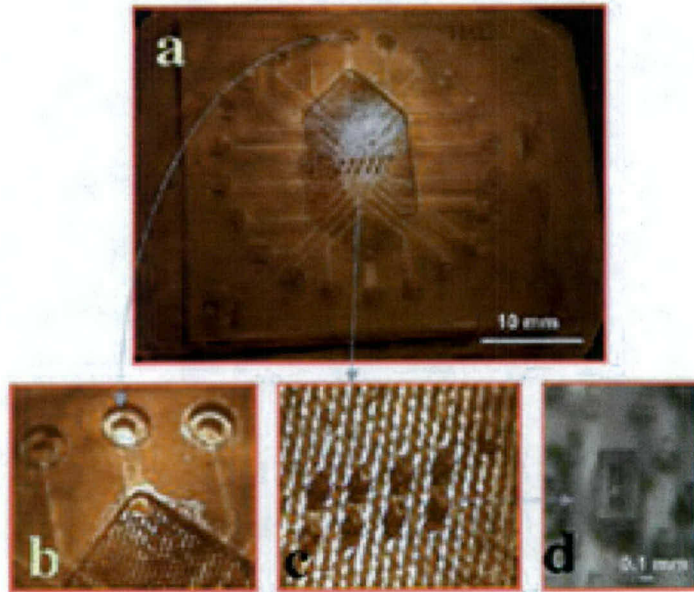
CFDA + cells

CFDA, no cells

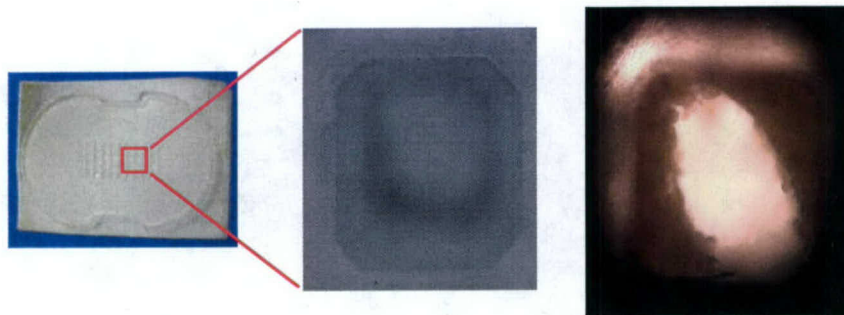
**Figure 19.** Substrate with patterned surface that has been selectively modified with CFDA-MA (carboxyfluorescein diacetate methacrylate, shown above the pictures). The cells internalize CFDA groups to produce fluorescences.



**Figure 20.** Microarray device with 3T3 fibroblasts cultured in porous networks for 24 hours. The goal is to dynamically address each well individually for cell screening and cell migration studies.



**Figure 21.** (a) Interfaces surrounding the device chamber enable connection of pumps that carry nutrients or drugs to specific sites in the brain tissue. (b) Inlet for nutrient flow, which is separated from the drug channels by a polymeric layer. (c) Supporting post structures and drug outlets. Posts are 100 x 100 x 400  $\mu\text{m}$ . (d) Two outlet holes are used for pushing the drug into and out of the slice, to ensure that only one point is affected.



**Figure 22.** The image on the left shows a filter (Durapore, 5 micron pore size) integrated into a device with 300  $\mu\text{m}$  wells. This device is used for seeding rat liver cells for the detection of viral pathogens. The middle image shows a close-up of one of these wells, and the image on the right shows the growth of cells in a well 4 days after seeding. This work was done in collaboration with MIT.