Novel microfluidic developments for biomedical research applications

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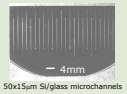
Microfluidic Devices: An Overview

We present a number of current developments in microfluidic systems for biomedical applications. The small length scale of microfluidic systems permits the use of smaller sample sizes, leading to decreased reagent costs, as well as faster analysis times. Several commercial microfluidic products have recently become available; however, there are ample opportunities in the biomedical research environment for improvements to existing technology as well as specialized applications.



50x15μm Si/glass microchannels

Microfluidic devices can incorporate extraction, purification, amplification, labeling, separation, and detection of analytes in a complex matrix *all on one small platform*.

Advantages of Microfluidics

- Sub-microliter sample volumes
- Rapid separation and analysis
- Numerous analytical steps can be performed in one small-footprint device
- Potentially less expensive than macro-sized counterparts:
  - -less reagent consumption
  - -multiplexing and mass-production easier

Materials for Microfluidic Devices

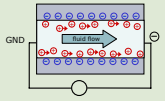
Silicon/glass
Chemically and physically robust
Directly patterned with photolithography

Poly(dimethylsiloxane) (PDMS)
Transparent elastomer
Good for rapid prototyping
Molded onto silicon templates

Polymers
Hot imprinted or injection molded
Facile thermal bonding
Good for single-use devices

Technological challenges

Controlling fluid flow

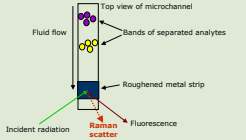


Electroosmosis is the primary method of flow control in microchannels

Requires charges on channel walls

Developing Alternate Detection Schemes

Currently, most microfluidic systems use fluorescence for detection
- requires fluorescent labeling of analytes
- no information about molecular structure

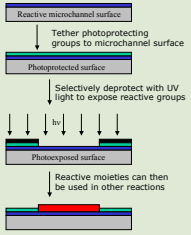


Other detection possibilities:
- Electrochemical detection
- Spectroscopic techniques
  - Absorption
  - **Surface-enhanced Raman scattering**

Photodirected Tethering of Charged Species in Microfluidic Devices for Fluid Flow Control

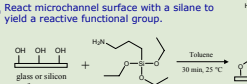
In making multifunctional devices, there is a need to create spatial variations of the surface properties along the length of a sealed microchannel. The ability to tailor these properties could lead to a variety of applications, such as improved electroosmotic pumping from highly charged channel regions, or the targeted patterning of capture ligands. In the present study, we wish to tether moieties with an additional charged species to the microchannel.

Patterning inside a microchannel: Monolayer photolithography

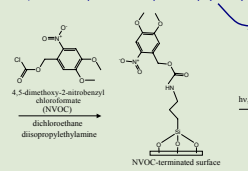


Detailed chemistry

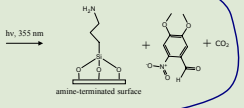
1. React microchannel surface with a silane to yield a reactive functional group.



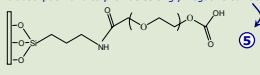
2. Attach photoprotecting group: 4,5-dimethoxy-2-nitrobenzyl carbonyl (NVOC)



3. Selectively expose to 355 nm light to get spatially patterned reactive primary amines.

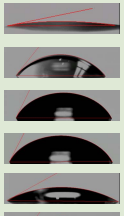


4. Attach carboxylated poly(ethylene glycol) (PEG) to minimize non-specific adsorption and to provide strongly negative surfaces for EOF

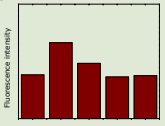


5. After tethering PEG to sections of the microchannel, deprotect other portions of the microchannel for further reactions.

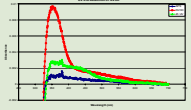
Contact angle goniometry



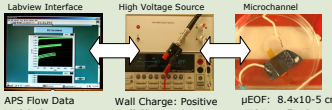
Fluorescence microscopy studies of glass surfaces terminated in various organic moieties



UV-visible spectroscopy studies of glass surfaces terminated in various organic moieties



Electroosmotic flow studies



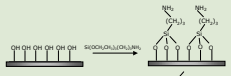
APS Flow Data	Wall Charge: Positive	μEOF: 8.4x10-5 cm2/V s
NVOC Flow Data	Wall Charge: Negative	μEOF: 1.5x10-4 cm2/v s
PEG Flow Data	Wall Charge: Negative	μEOF: 1.6x10-4 cm2/v s

Surface-Enhance Raman Spectroscopy as a Detection Method in Microfluidic Devices

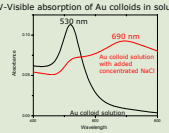
Currently, the most common detection method used in microfluidic systems is fluorescence detection. While fluorescence offers high sensitivity, chemical labeling of analytes with fluorophores is generally required. In addition, non-specific labeling and autofluorescence is often a problem. We present recent efforts to use patterned Au colloids in a microdevice for surface-enhanced Raman spectroscopy (SERS) detection. This detection technique offers direct measurement of biomolecules in aqueous solution .

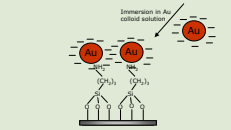
Synthesis and characterization of Au colloids and Au colloid-terminated glass

Au colloids were synthesized using $HAuCl_4$ and sodium citrate. The resulting citrate-stabilized Au colloids were 13 nm in diameter, with a negatively-charged shell.

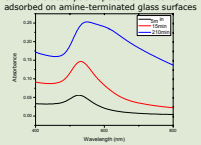


UV-Visible absorption of Au colloids in solution



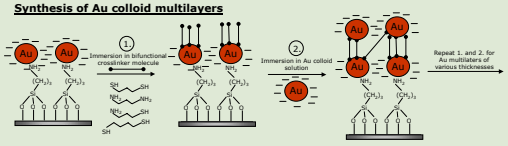


UV-Visible absorption spectra of Au colloids adsorbed on amine-terminated glass surfaces

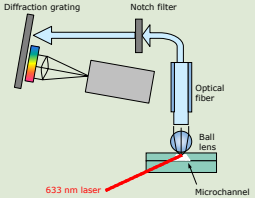


- Au colloids in solution have an absorption maximum at ~530 nm
- Au colloids adsorbed on amine-terminated surfaces have an absorption maximum similar to the colloids in solution
- The absorption maximum of the Au colloid monolayers shifts after immersion in the Au colloids solution for long periods

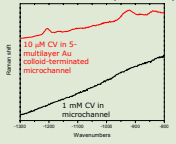
Synthesis of Au colloid multilayers



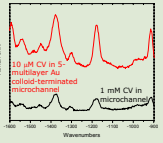
Schematic of Optical Detection System



Raman spectrum of crystal violet (CV) solution in microchannel (633 nm laser)

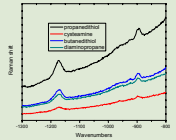


Raman spectrum of crystal violet (CV) solution in microchannel (532 nm laser)



Data shows factor of 300 enhancement of Raman signal using Au-colloid multilayers.

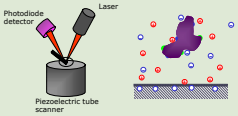
Raman spectrum of crystal violet (CV) solution in Au colloid multilayer-terminated microchannels (633 nm laser)



- multilayers were fabricated with a variety of bifunctional crosslinkers
- dithiol crosslinkers appear to produce multilayers that give greatest enhancement

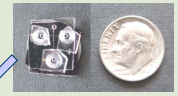
Rapid tuning of protein deposition conditions for AFM

Elastomeric microchannels are used to temporarily create a microdevice on an atomic force microscopy (AFM) substrate. Because flow in microchannels is laminar, all mixing is diffusive, which allows us to create a well-controlled buffer gradient, either in ionic strength or pH, over a few hundred microns. This permits a rapid sampling of buffer conditions for protein deposition on AFM substrates.



In microchannels, fluid flow is laminar. Diffusive mixing of two dissimilar streams can generate well-established buffer gradients over lengths useful for AFM (a few hundred microns).

Atomic force microscopy is widely used to image proteins and other biomolecules. However, the organization of proteins on the AFM substrate depends strongly on the pH and ionic strength of the buffer during deposition.

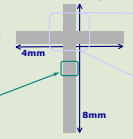


A PDMS cross-shaped microchannel

Top view: two side arms used as inlets for protein solutions in different buffers

First, the top of cross is used as the outlet, in order to balance flow from the sides before exposing lower part of device to protein.

Target area for imaging is in bottom of cross



Pipette reservoirs used to control pressure/flow rate

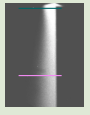
Flow monitored with fluorescent tracer

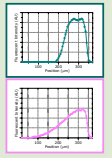
After the flow from the two side arms is equalized, the bottom reservoir is emptied.

As seen below, the solutions flow into the bottom of the cross, and protein deposition in the target area begins.

Channel cross-section: 300μm wide at top, 28μm deep

Device Operation: Salt concentration gradient





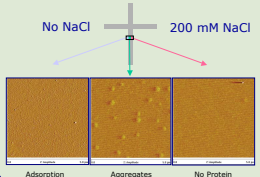
diffusion of Sulforhodamine B

Cytochrome C: salt gradient

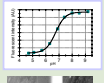
13kDa, ~1x3nm

As the concentration of NaCl increases across the channel, the protein deposition decreases.

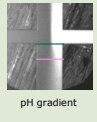
10 μg/mL Cytochrome c in 50mM $NaPO_4$



Device Operation : pH gradient



Fluorescein serves as a pH indicator

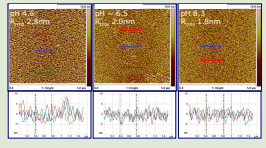


pH gradient

Bovine Serum Albumin: pH gradient

66kDa, ~4x8nm
Isoelectric point: 4.9

50 μg/mL BSA in 200mM phosphate



On the left side (pH 4.6), where BSA is near neutral, multilayered adsorption occurs