Microfluidic Probes for the Life Sciences

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Learning from History

Most relevant discoveries have been achieved at the intersection of disciplines!

- <u>The airplane</u>, which combined *aerodynamics*, *structural mechanics and propulsion*
- <u>The engine</u>, which emerged at the boundaries of *thermodynamics, combustion and mechanics*
- <u>The Atomic Force Microscope</u>, which combined *mechanics, materials, electronics*



The Atomic Force Microscope



AFM schematic: In this configuration the sample is mounted on the piezoelectric tube. **Resolution:** cantilever deflection +/- 0.02 nm; force 1 pN. *As a reference consider chemical bond forces: 0.1 mN for ionic bond; 10 pN for hydrogen bond.*



Patterning Molecular Inks at the Nanoscale



Dip-Pen Nanolithography (DPN)

- Direct-write nanopatterning technique based on AFM*.
- Alkanethiolate molecules locally deposited on a Au surface.



- AFM tip "nib" is used to deliver molecules "ink" to a surface "paper" via a solvent meniscus.
- Feature size: ~10 nm microns



*AFM: atomic force microscopy

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REF: Science **283**, 661 (1999)

Bio Applications Present and Future



DNA Arrays -- Gene Chip -- Biochips

APPLICATIONS

- Genetic Profiling (drug side effects, allergies)
- Mutations indicative of diseases
- Cancer detection and differentiation
- Drug Development
- Toxicology Experiments
- Cell Functionality





How Does It Work?



Making Chips to Probe Genes, IEEE Spectrum, 2001



Identification of Active Genes

Affimetrix



Commercially Available!



Surface Processing Techniques

- Patterns or spots of biological/chemical substances are deposited on surfaces to achieve a functionalized area for further processing (a bottom up approach).
- Most current surface patterning methods are based on tools that are built using the top-down approach:
 - Ink-jet
 - Microcantilever spotters (miniaturized robot spotting)
 - Scanning probe-based techniques
 - Soft lithography



Biosensing with high "selectivity and sensitivity"



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Cell Adhesion Studies

A: Diagram describing the cell adhesion experiment on the DPN-generated pattern. The total patterned area is 6400 μ m². The alignment marks were generated by scratching a circle onto the backside of the Au-coated glass substrate.

C: Large scale optical microscope image showing the localization of cells in the nanopatterned area



In the Lab!



Scaling Up the Patterning of Molecular Inks at the Nanoscale



Contact-Based Surface Processing

Robot spotter



REF: Reese *et al., Genome. Res.* **13**, 2348 (2003)

Feature size: > ~200 µm

Apertured AFM tip





REF: a) Belaubre *et al.*, *Appl. Phys. Lett.* **2003**, *14*, 1472;
b) Belaubre *et al.*, *Sens. Actuators A* **2004**, 110, 130

Feature size: ~10 μ m to >100 μ m

<u>Nanopipette</u>





REF: Lewis *et al.*, *Appl. Phys.Lett.* **75**, 2689 (1999); Bruckbauer *et al.*, *JACS* **124**, 8810 (2002)

Feature size: ~300 nm to 10 µm

Fork-arm microcantilevers



REF: Biomedical Microdevices 6, 117 (2004)

Feature size: 2~3 µm

<u>DPN</u>



Feature size: <100 nm



REF: Meister *et al.*, *Microelectron. Eng.* **67-68**, 644 (2003)





Objective



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Three Evolutions of the NFP chip



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NFP2 Features that Needed Improvement



- Backside Si₃N₄ too weak
- Channel sealing
- Inconvenient bending of cantilevers
- Cantilevers' stiff
- Channels height: larger (nanoparticles)
- More robust processes and enhanced yield





How it was improved: SOI



ORTHWESTERN UNIVERSITY Thermal Oxidation, 0.5 µm

Litho M1 trench on backside + KOH (not shown)

Litho M2

Pyramid etch (KOH)

NHA etch

 SiO_2 removal (only 0.5 μ m)

Litho M2' (new mask)

SOI wafer (continued)









 SiO_2 for sealing, PECVD, 2µm

Pattern sealing SiO₂

Thick PR, backside litho, M6 (for lower contact) RIE to etch nitride Glue wafer onto another

DRIE: stop in SiO₂

Remove glue (Nanostrip)



Second Generation NFP Chip





- 1 reservoir, 2 microchannels,
- 3 volcano tip
- Linear array of 12 cantilevers
- Double-ink reservoirs
- Elimination of sealing problems
- Deep RIE release of chips (more chips per wafer)















Flow Test



Reservoir area

• Water-soluble fluorescent dye





Comparison of Writing Mechanisms



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Ink Feeding to Volcano Tip

Ink transport from a reservoir to the volcano tip.



Integrating microchannels



Surface micromachining

I. Papautsky et al., IEEE Trans. on Biomed. Eng. 47, 812 (2000)



Microfluidic Simulations

- Simulate capillary driven flow from reservoir to the tip
- Select optimal materials and suitable tip shapes
- Determine stable fluid-air interface for DPN





Microfluidic Simulations

- Fluid moves by capillary action from a reservoir to the end of a tip
- Good control of the equilibrium in fluid-air interface is obtained when the tip material is hydrophilic and the shell material is hydrophobic



 C_t : contact angle on tip; C_s : contact angle on shell



Patterning with NFP1 Alkanethiolate molecules (MHA*) on a gold surface





NFP2: Double-Ink Patterning

 Saturated solutions in acetonitrile of two different types of alkanethiols



MHA

PFT



DNAs or proteins can be used as ink.

PFT (1H,1H,2H,2H-Perfluorododecane-1-thiol) $CF_3-CF_2-(CF_2)_{10}-CH_2-CH_2-SH$

To appear JMM, 2006



DNA Direct Delivery and Writing



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Hybridization Detection



kk_122105_dna_1207sample.016m

Zoom: 2:1

Cen line: Off

off

Cursor: fixed



Hybridization Detection





Dip-pen nanolithography (DPN)



- Tip pretreated with 3'-aminopropyltrimethoxysilane was dipped into a DNA solution
- Hexanethiol-modified oligonucleotides patterned on gold substrates => detected by tapping mode AFM



 Oligonucleotides with 5'-terminal acrylamide on 3'mercaptopropyltrimethoxysilane coated Si/SiO_x substrates => detected through hybridiziation scheme

NORTHWESTERN UNIVERSITY 1.5 um



Surface patterning tool (SPT)

REF: Biomedical Microdevices 6, 117 (2004)

20 un

- SPP pre-treated with UV/O₃.
- Cy3-streptavidin sample solution loaded into the reservoir by micropipette
- SPT mounted on the NanoArrayer (dedicated instrument)
- Spots patterned on a dithiobis-succinimidyl undecanoate (DSU) monolayer coated gold surface => Detection by fluorescence microscopy

Nano Fountain Probe (NFP)



REF: Small 1, 632 (2005)





	DPN	SPT	NFP
Resolution	~50 nm	2-3 µm	~200 nm
Microfabrication	Commercial tips	Developed steps	Developed steps
Continuous delivery	No	Yes	Yes

20 µm

NFP Applications

- Patterning of biomolecules (DNA, proteins) and proteins (biosensors)
- Patterning of inorganic materials (sol-gels, metal nanoparticles, conducting polymer)
- High throughput nanolithography
- Direct paterning of nanoparticles and catalysts
- Fabrication of nanodevices by directed self-assembly
- Repair of interference masks with nanometer features



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Patterning with Diamond Nanoparticles





Photo No.= 2051 Detector=InLess DHT= 3.00 kV WD= 6mm Aug = 365X 30µm Date :11 No.555 Stage at T = 0.0 Get Son Speed + 8 Collector Biss 30 V

Patterns obtained with NFP-2



Ref: Xiao et al., Adv. Mater. 17, 1496 (2005)

Potential application:





2. Array of patterned diamond particle and Fe catalysts



3. Aligned hybrid CNT/UNCD structures

CNT-based memory



Ref: Science 289, 94 (2000)



NEMS Switches and Biosensors

Directed assembly of CNTs for mass production of NEMS



- Pattern MHA on Au with DPN (NFP)
- Flush ODT
- Self assemble CNTs

Ref: Y. Wang et. al. *PNAS* **103** (7), 2026 (2006)

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Ref: *Appl. Phys. Lett.* **85**, 681 (2004)

CNT-based NEMS switch

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Our preliminary results (DPN) for integration in a device



1-D Array with Multiple Inks





Linear NFP Array

Single-tip feedback control

- The precise tilting stage of the conventional instruments is enough to ensure the contact of individual tips.
- Multiple-tip, double-ink patterning in a simultaneous fashion.





 SEM images of a microfabricated 1-D array with 12 pens and twin on-chip reservoirs



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Twin reservoir



History of the Pen (revisited)



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PZT Actuation of FPN Chip

- Extensive characterization of the PZT actuated probes.
- Integration onto nano fountain probe chips.



PZT actuator integrated on the cantilevers without a microfluidic system



The actuation module can be easily incorporated by deposition/patterning of PZT film because currently developed cantilevers have been designed in such a manner that many possible improvements and changes can be easily accommodated:

Collaborator: O. Auciello (ANL)



Parallelization

- One-dimensional array integrated with independent actuators and multiple reservoirs, to realize fountain-pen mode patterning with various inks in a simultaneous fashion.
- Evaluation of the sensing capability of PZT probes. It will potentially remove the necessity of the optical deflection detection scheme adopted in AFM techniques, which will benefit the operation of arrayed probes.

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* SEM image artificially modified to show equal length cantilevers



PZT Actuation

Integration of PZT actuators with AFM cantilevers without microfluidic system.



Optical image of the cantilevers with PZT actuators and electrodes prior to release



PZT Actuation



Pulse train applied with 5 Vpp.



Bending Profile of the Cantilevers

• Quantitative evaluation with phase shift technology.





Tip Wear Test

Si₃N₄ tip on Au substrates

Trade-off between the fabrication effort (cost) and writing capability of the chips are being examined.

before

Si tip on Si substrates



(512 pix by 512 pix) • 70 scans • 80 µm/s (4 Hz) ~72 cm long • ~2.5 hr scanning 200 nm 200 nm • F=~60 nN Si₃N₄ tip on diamond substrates • 10 µm by 10 µm (256 pix by 256 pix) 200 nm 200 nm • 60 scans Diamond tip on diamond substrates Electrostatic force • 80 µm/s (4 Hz) • ~31 cm long • ~1 hr scanning • F=~50 nN 200 nm 200 nm

after

J. Vac. Sci. Technol. 14, 2456 (1996)

applied between

the tip and the

20 nN for 5V

sample

Electrostatic Force (arb.)

-20 -15 -10 -5 0 5 10 15 20 Tip Voltage (V)

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• 10 µm by 10 µm

Diamond AFM Probe





images of a microfabricated probes

*UNCD: Ultrananocrystalline Diamond, grain size 2-5 nm



Fabrication of Diamond Tips





DPN Compatibility

• DPN compatibility has been examined with diamond tips.



LFM image of patterned letters by the diamond probe



Conduction Map





Topography

Conduction Map



UNCD Tip Characterization



AFM Potentiometry



Diamond Tip Integration with NFP Tip

UNCD film to be integrated on NFP



Current version of a volcano tip with Si₃N₄ core







