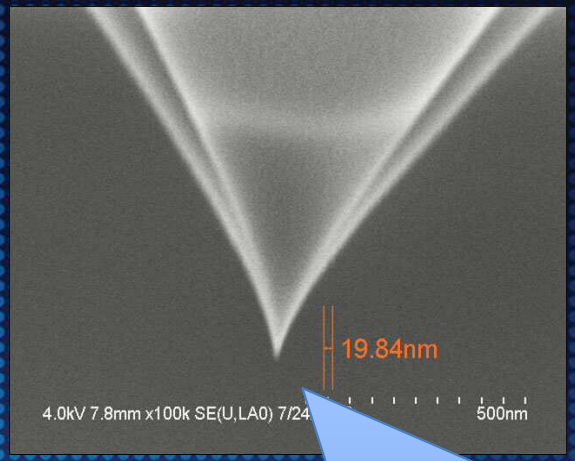


nanoINK INC®

NanoFabrication Systems

"Science at the Sharp End"

Recent Advances in Tip-based Nanolithography

Robert J. Stokes

Application Development Director EMEA,
Nanoink Inc. USA

NanoInk corporate overview

NanoFabrication Systems

- Nanolithography tools for researchers

Nano BioDiscovery

- Nanoscale Protein Arrays

NanoProfessor

- Hands-on Nanotechnology Education

NanoGuardian

- On-Dose NanoEncryption™ Technology for securing the global drug supply chain

Headquarters: Illinois Science + Technology Park, north of Chicago

MEMS Facility: Campbell, CA

NanoInk currently has over 250 patents and applications filed worldwide and licensing agreements with Northwestern University, Stanford University, University of Strathclyde, University of Liverpool, California Institute of Technology and the University of Illinois at Urbana-Champaign

Outline

- Background to tip-based nanolithography
- Ink types and tools

  - 1) “Bottom-up” - Assembly to templates.
  - 2) “Top-down” - Fabrication / etching
  - 3) “Top-down” - Direct writing- polymers
  - 4) “Top down” - Localised nanochemistry

Techniques

  - 5) Deposition of biomaterials for diagnostics.
  - 6) Depositions registered to existing structures.
  - 7) Biomimetic structures for cell biology.

Example Applications

Outline

- Background to tip-based nanolithography
- Ink types and tools

Pt 1: "What can it do?"

  - 1) "Bottom-up" - Assembly to templates.
  - 2) "Top-down" - Fabrication / etching
  - 3) "Top-down" - Direct writing- polymers
  - 4) "Top down" - Localised nanochemistry

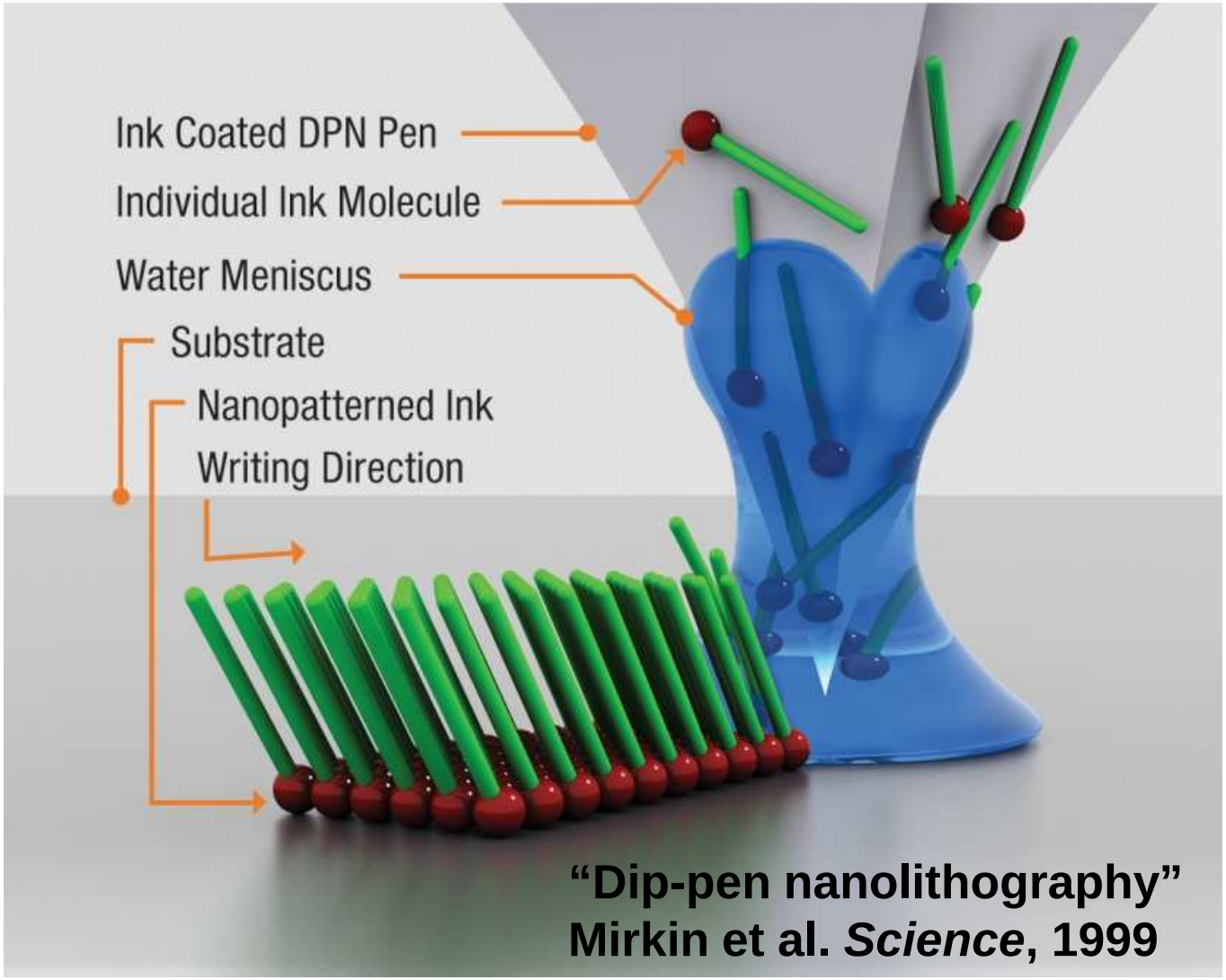
Techniques

  - 5) Deposition of biomaterials for diagnostics.
  - 6) Depositions registered to existing structures.
  - 7) Biomimetic structures for cell biology.

Example Applications

Pt 2: "What you can do with it"

What is Tip-based lithography and “DPN”?



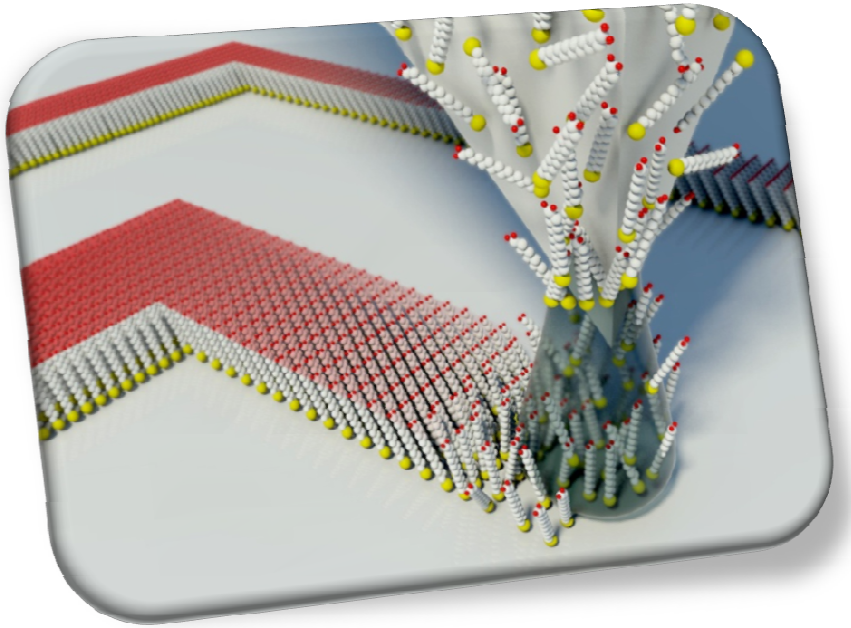
“Dip-pen nanolithography”
Mirkin et al. *Science*, 1999

D. Ginger *et al* Angew. Chem. Int. Ed. **2004**, 43, 30– 45

Ink Categories

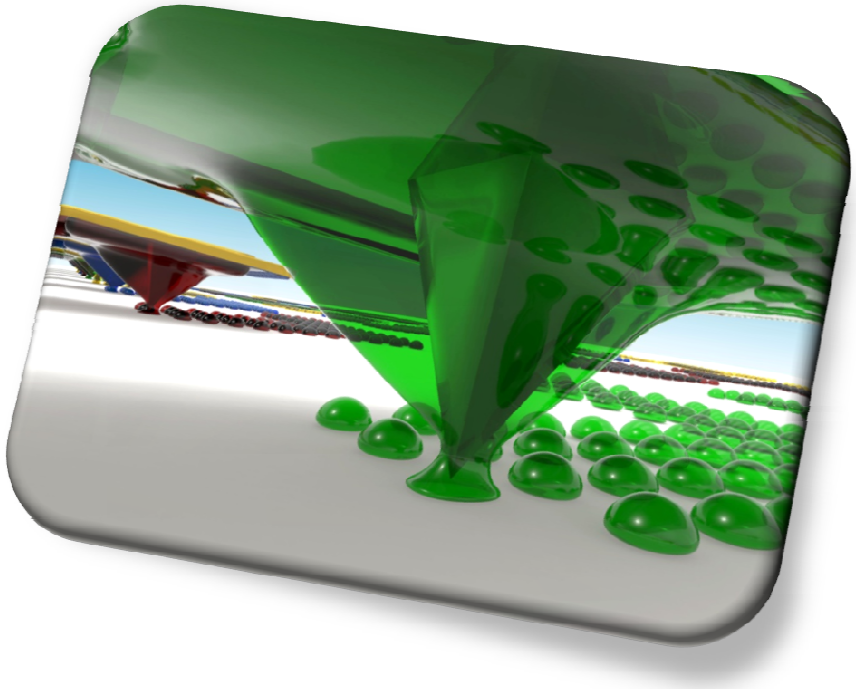
“Molecular inks”

Early DPN work… 1999 to present



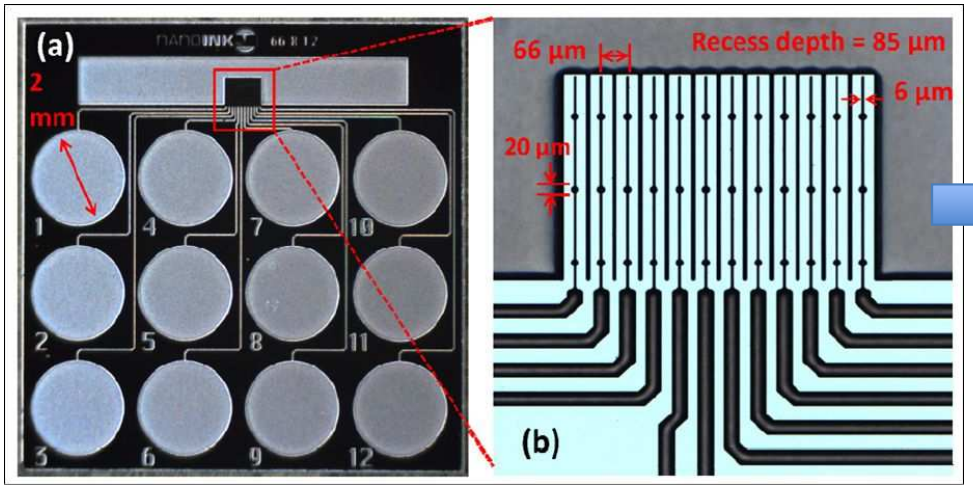
“Liquid inks”

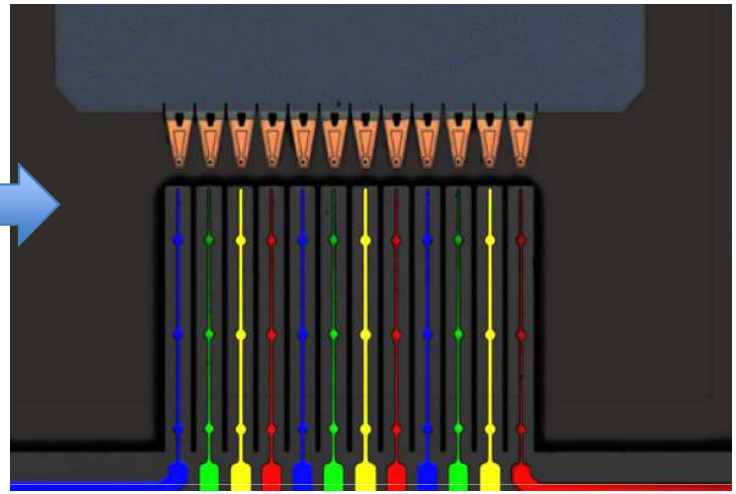
Biomaterials work… 2010 +



Nano tools

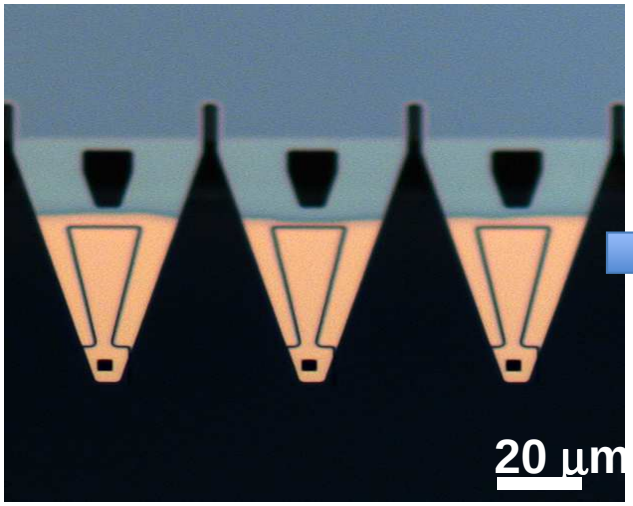
‘M-type’ inkwells

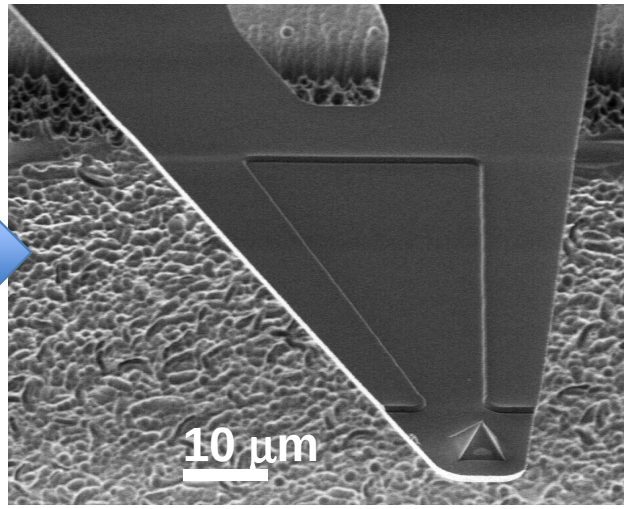




Side M-1

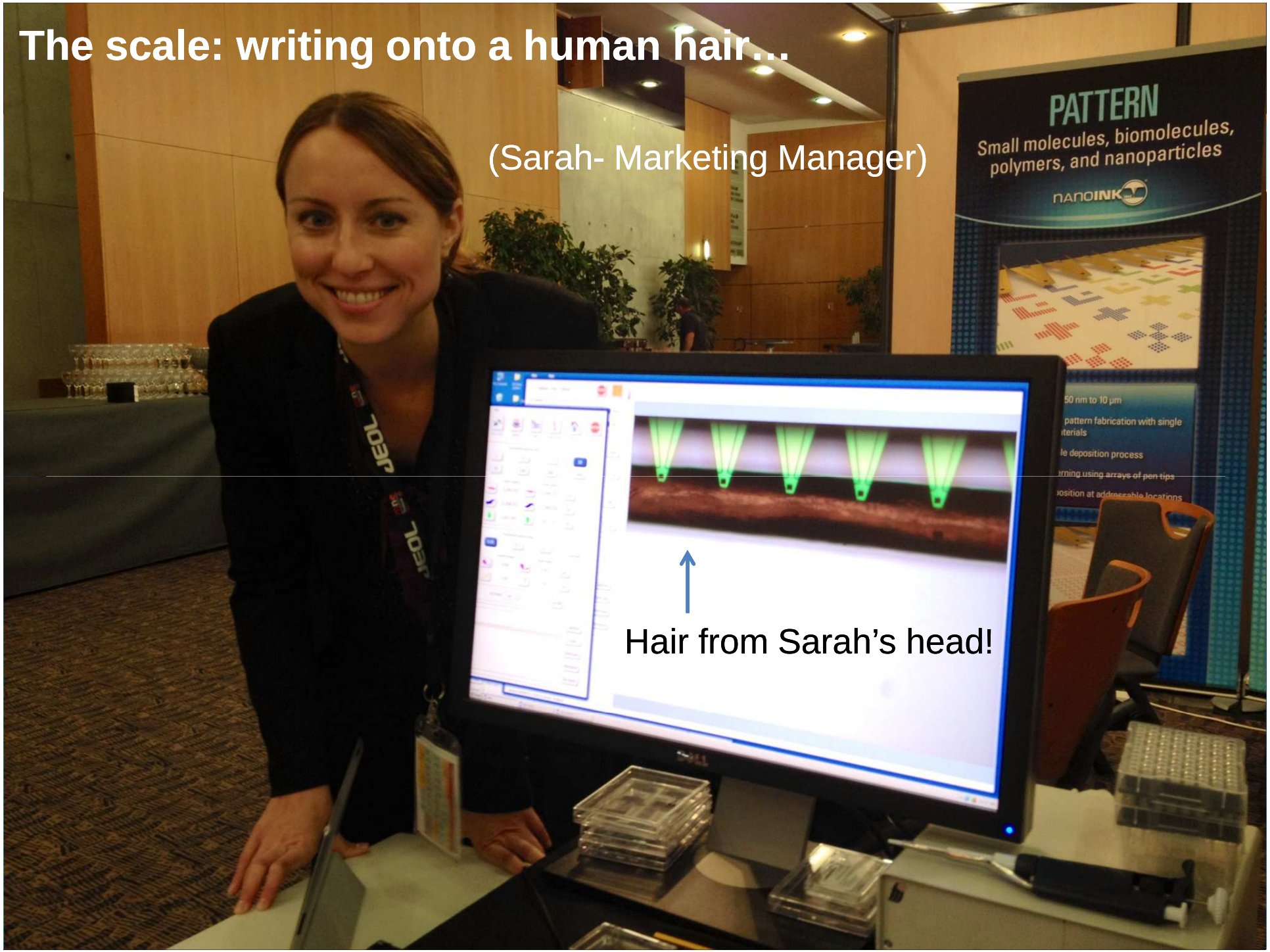
Side M-2





‘M-type’ pens

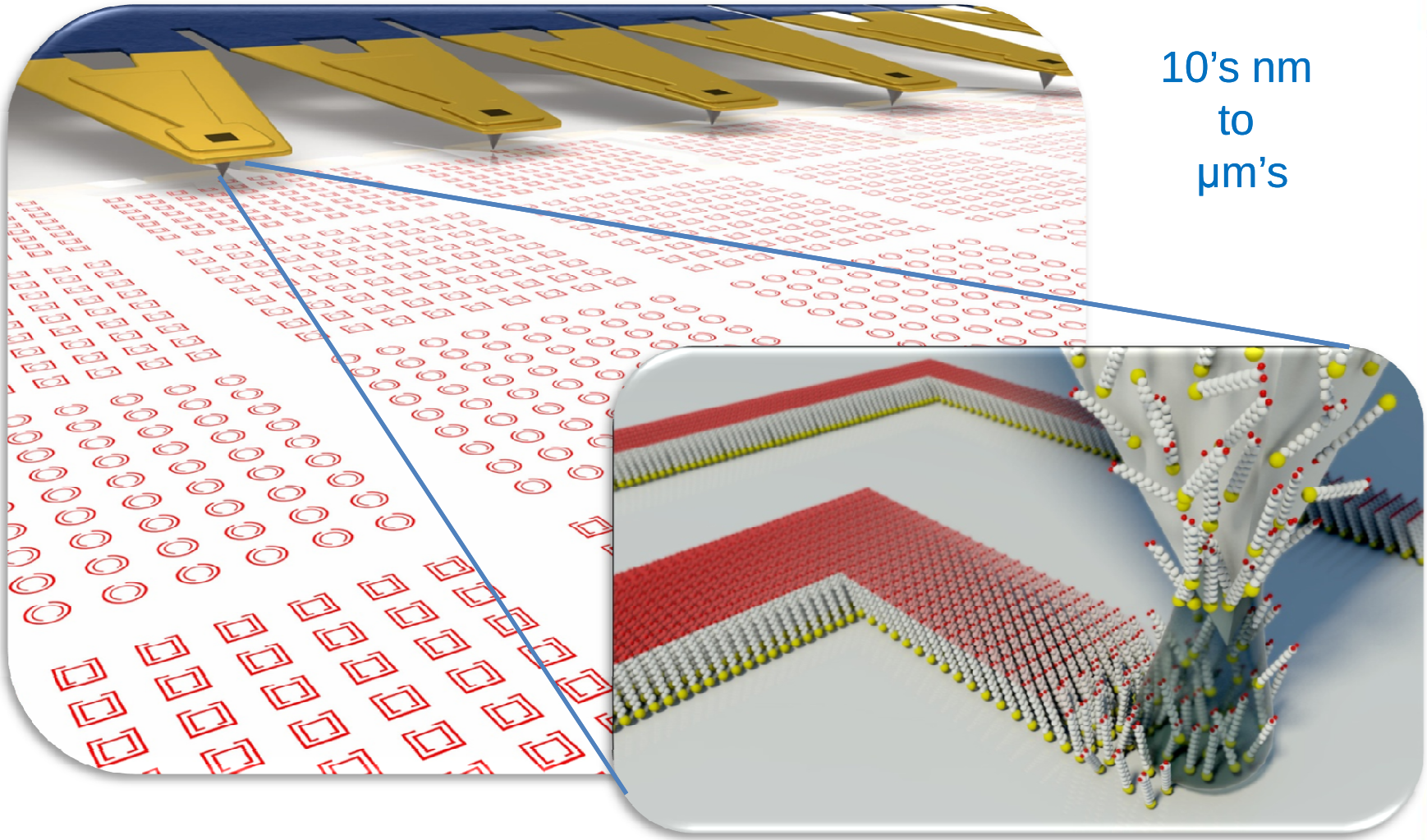
The scale: writing onto a human hair…



Molecular Inks

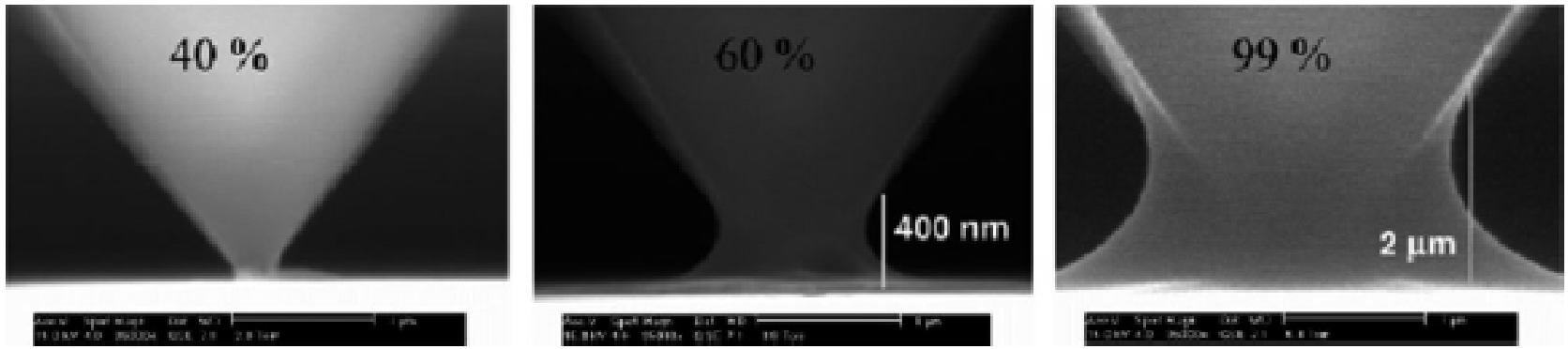
Molecular Inks

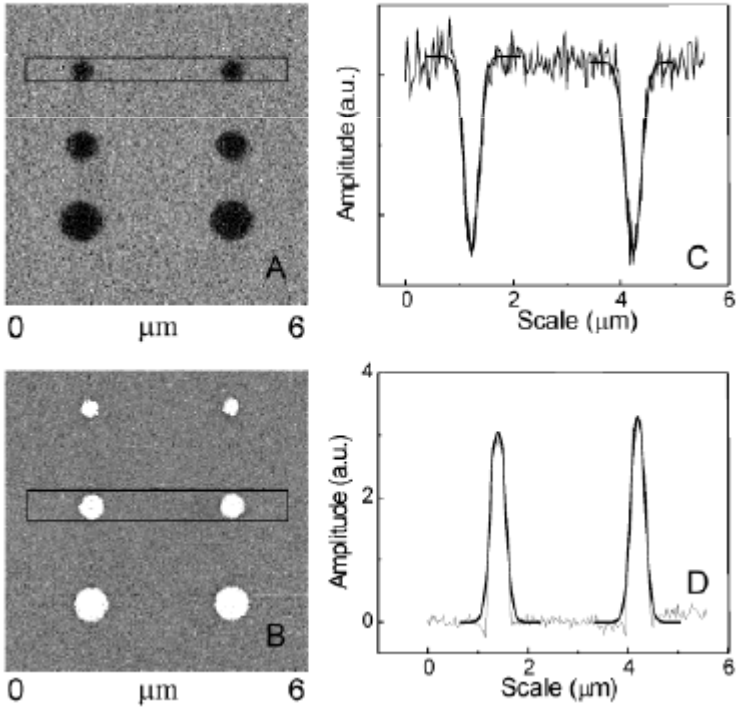
- Alkanethiols
- Silanes



10's nm
to
µm's

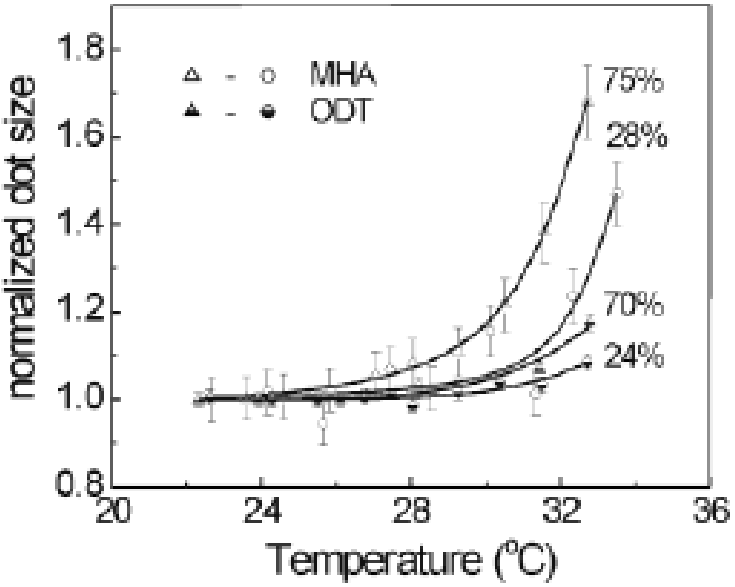
Control of molecular ink deposition





• Dwell time

• Humidity

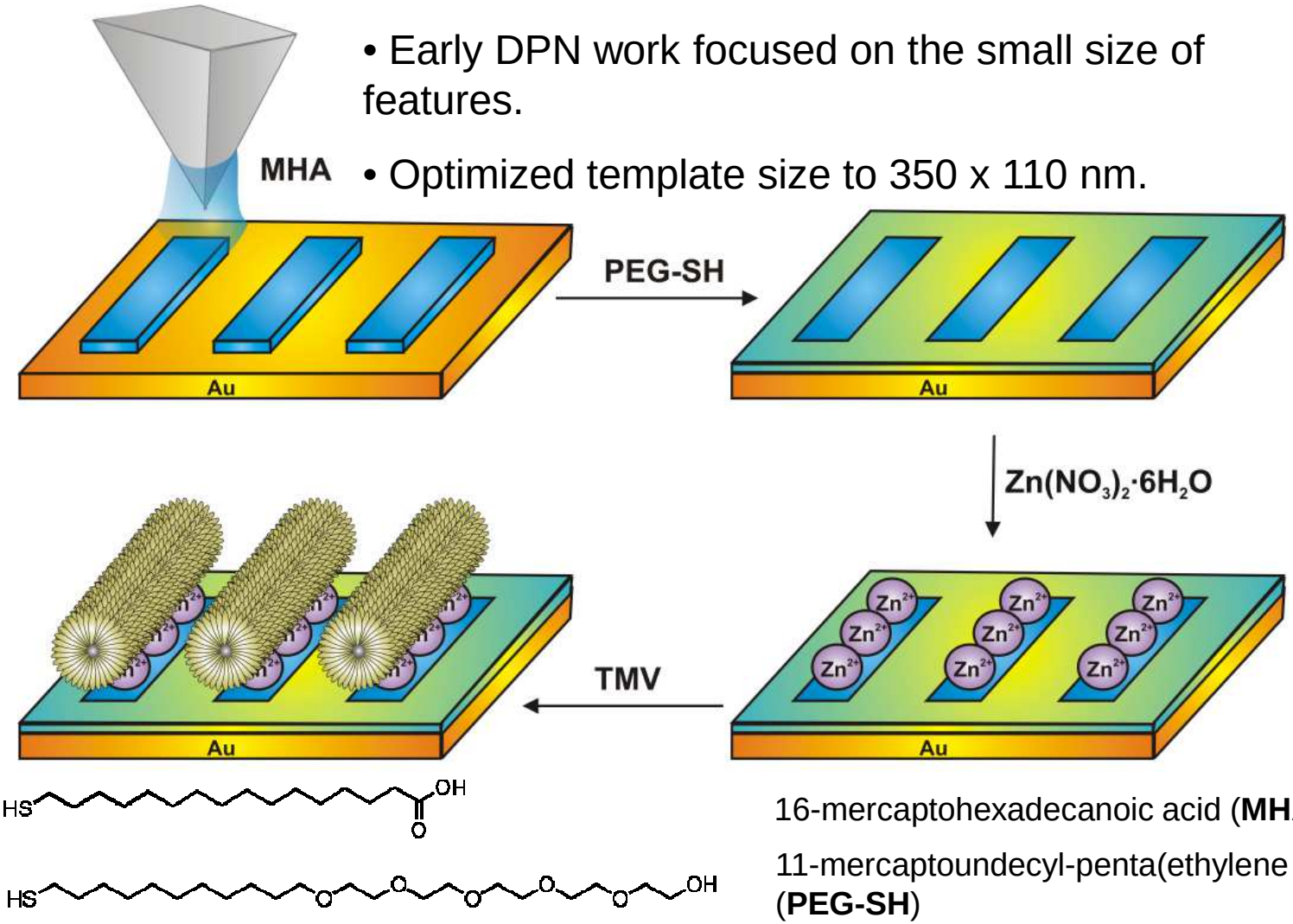


•Temperature

1) “Bottom up”

“Template approach”
(molecular ink)

Using DPN to create templates for virus deposition…



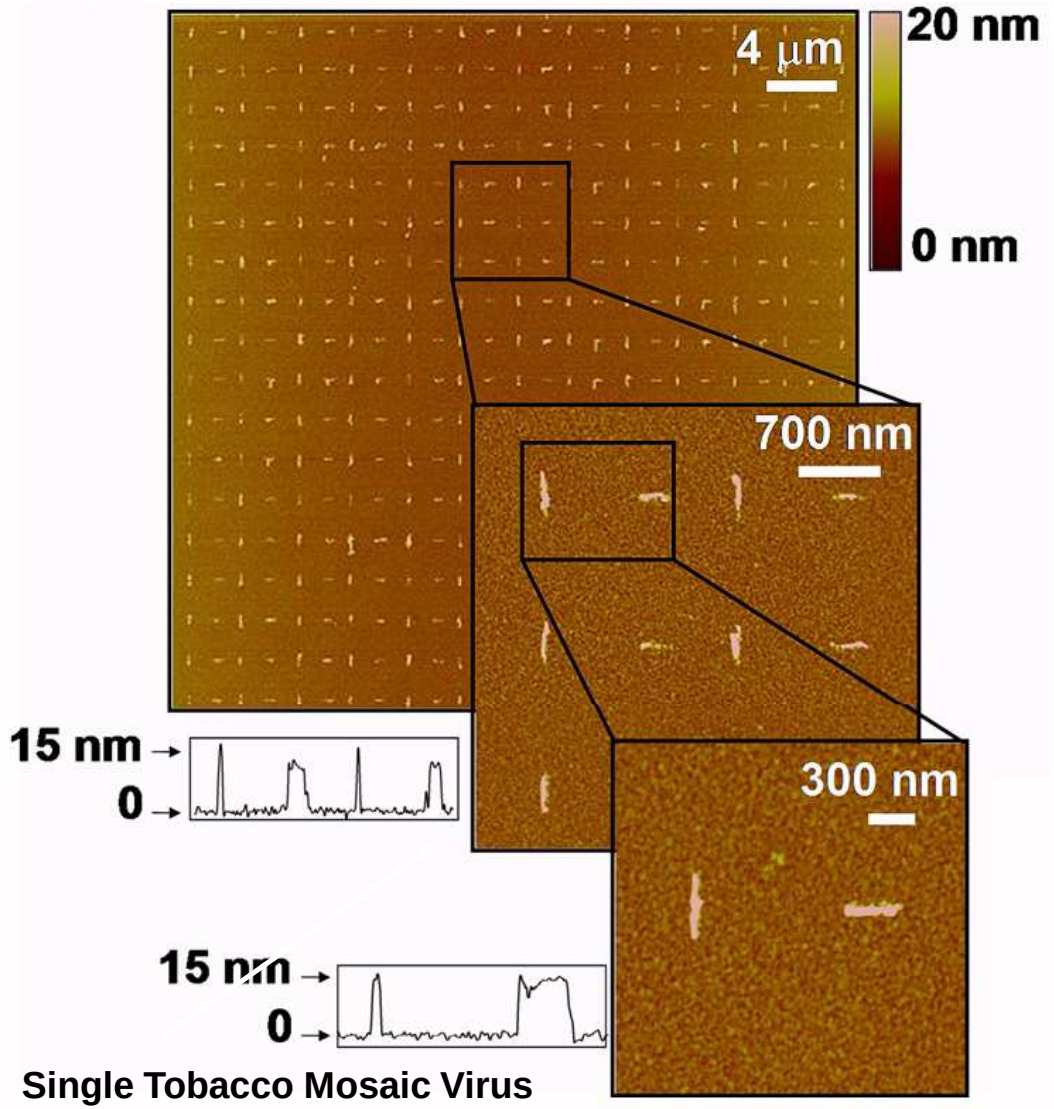
- Early DPN work focused on the small size of features.
- Optimized template size to 350 x 110 nm.

16-mercaptohexadecanoic acid (**MHA**)

11-mercaptoundecyl-penta(ethylene glycol) (**PEG-SH**)

Vega, R.A., et al. Angew. Chem. Int. Ed., 2005. **44**: p. 6013-6015.

Using DPN to create templates for virus deposition…

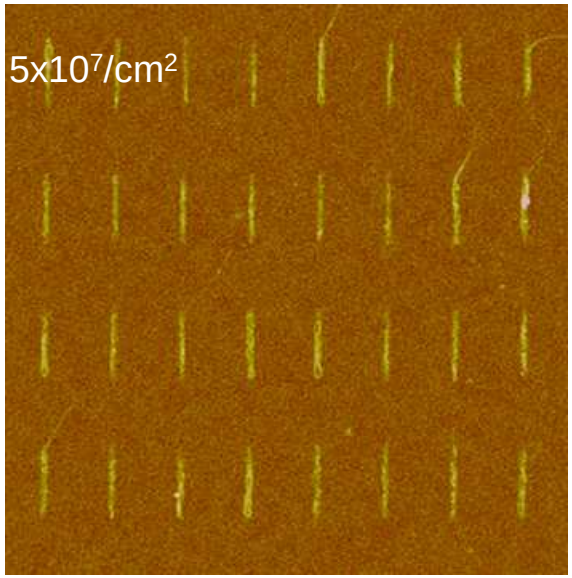


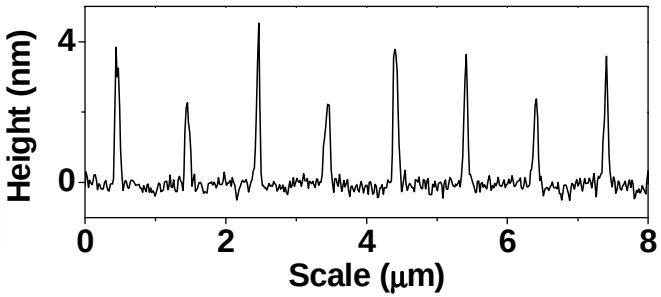
Single Tobacco Mosaic Virus

Vega, R.A., et al. Angew. Chem. Int. Ed., 2005. **44**: p. 6013-6015.

Templates for CNT deposition and organisation

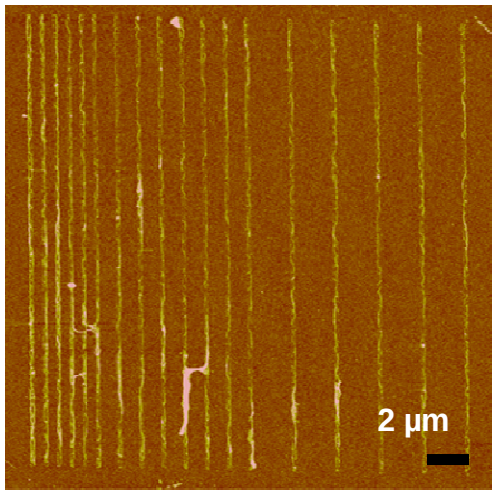
SWNT Architectures

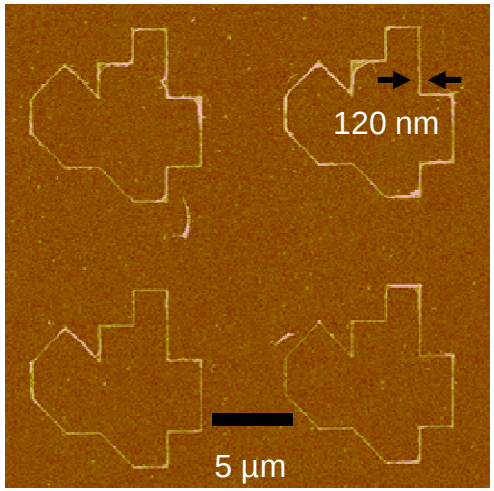




AC mode topography images of SWNTs attached to MHA affinity templates

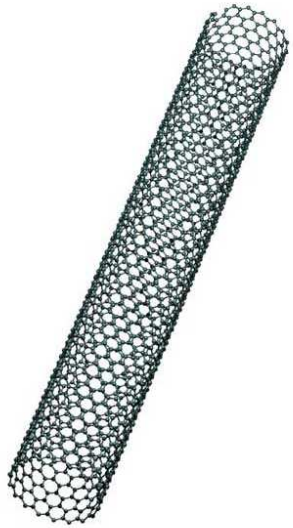
Continuous, Flexible Nanoropes





CNT manipulation precisely controls:

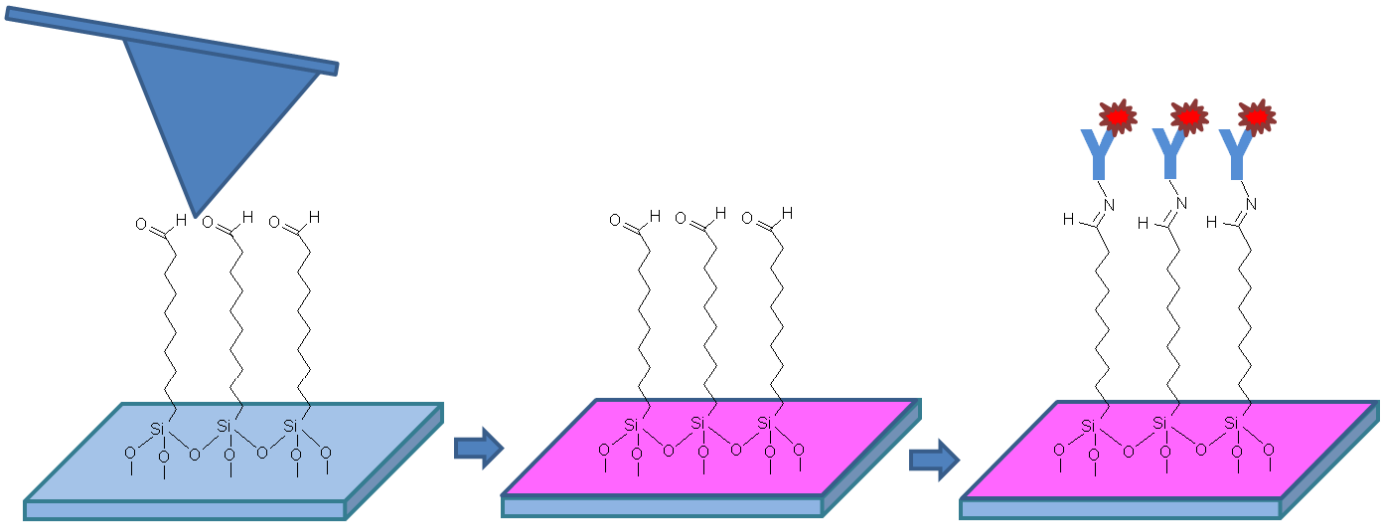
- placement
- orientation
- shape



Wang et al. *PNAS* **2006**,*103*, 2026-2031.

Silane patterning for protein immobilisaiton

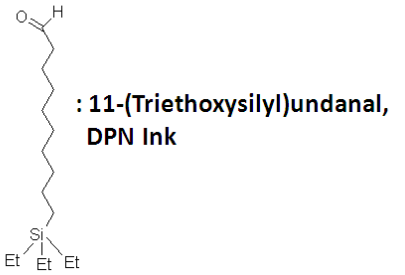
Glass and silicon / silicon oxide substrates



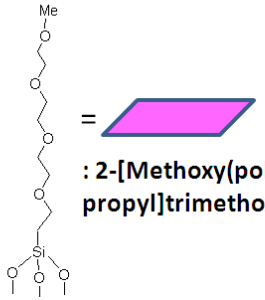
DPN on SiO_2 or glass substrate

PEG-silane passivation

Reaction with proteins



: 11-(Triethoxysilyl)undanal, DPN Ink



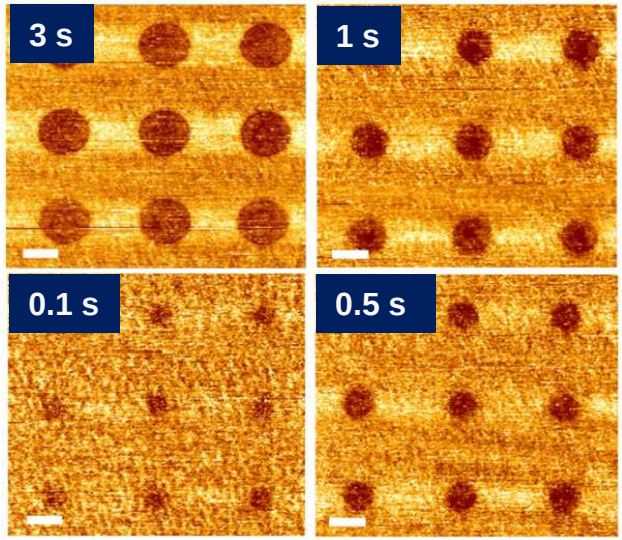
= : 2-[Methoxy(polyethyleneoxy) propyl]trimethoxysilane

: Fluorescence label tagged IgG

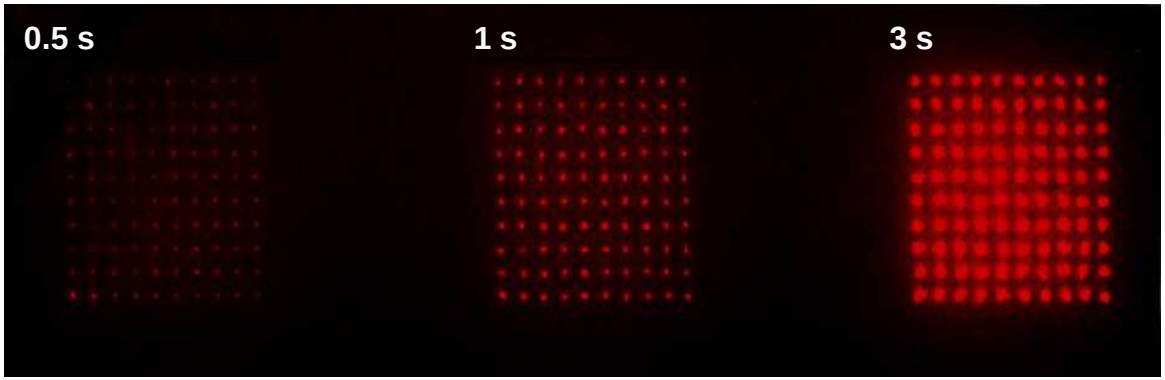
Silane patterning for protein immobilisation

Dwell Time Dependence

LFM image of silane spots

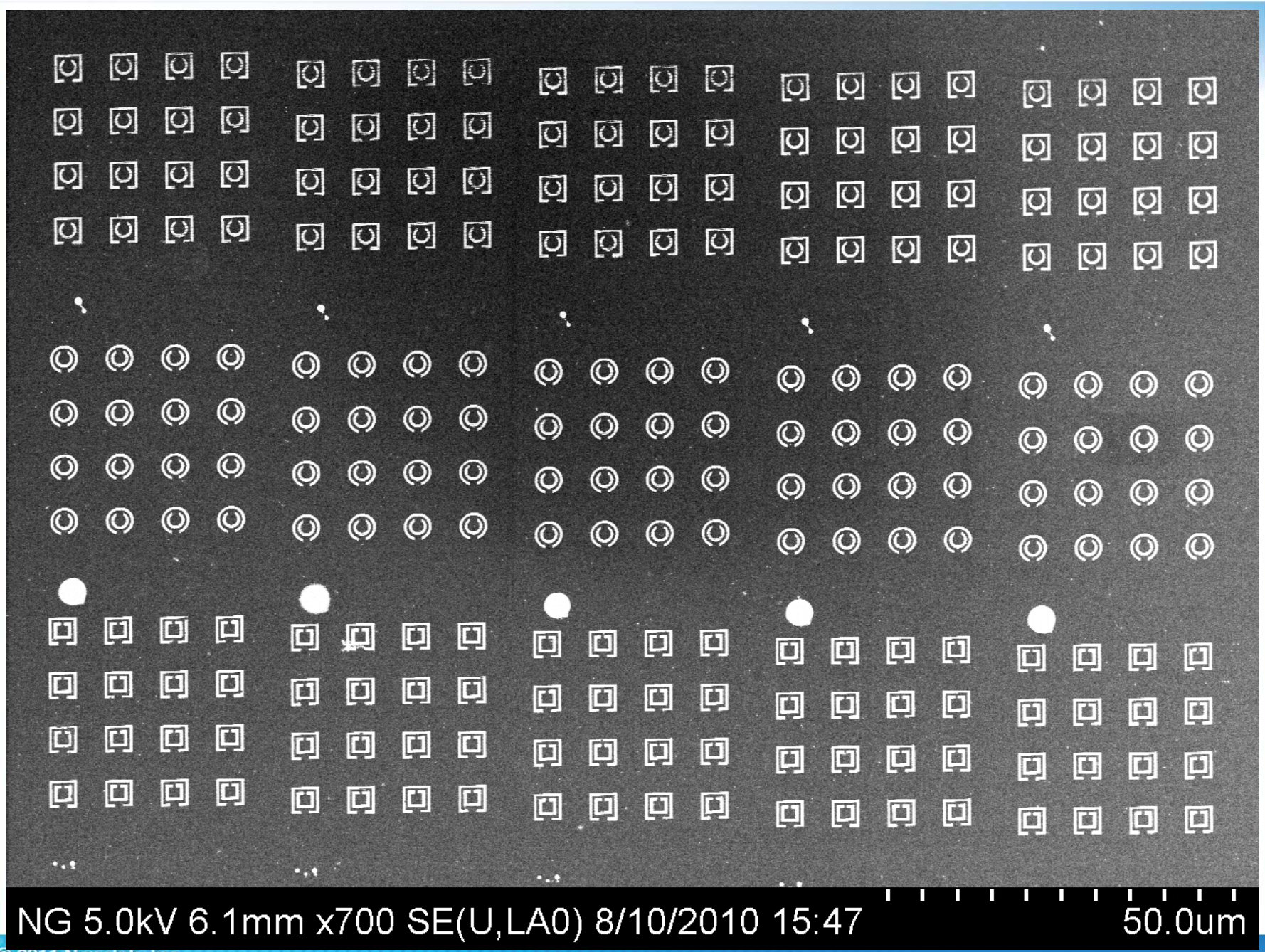


Fluorescence image of protein bound silane spots

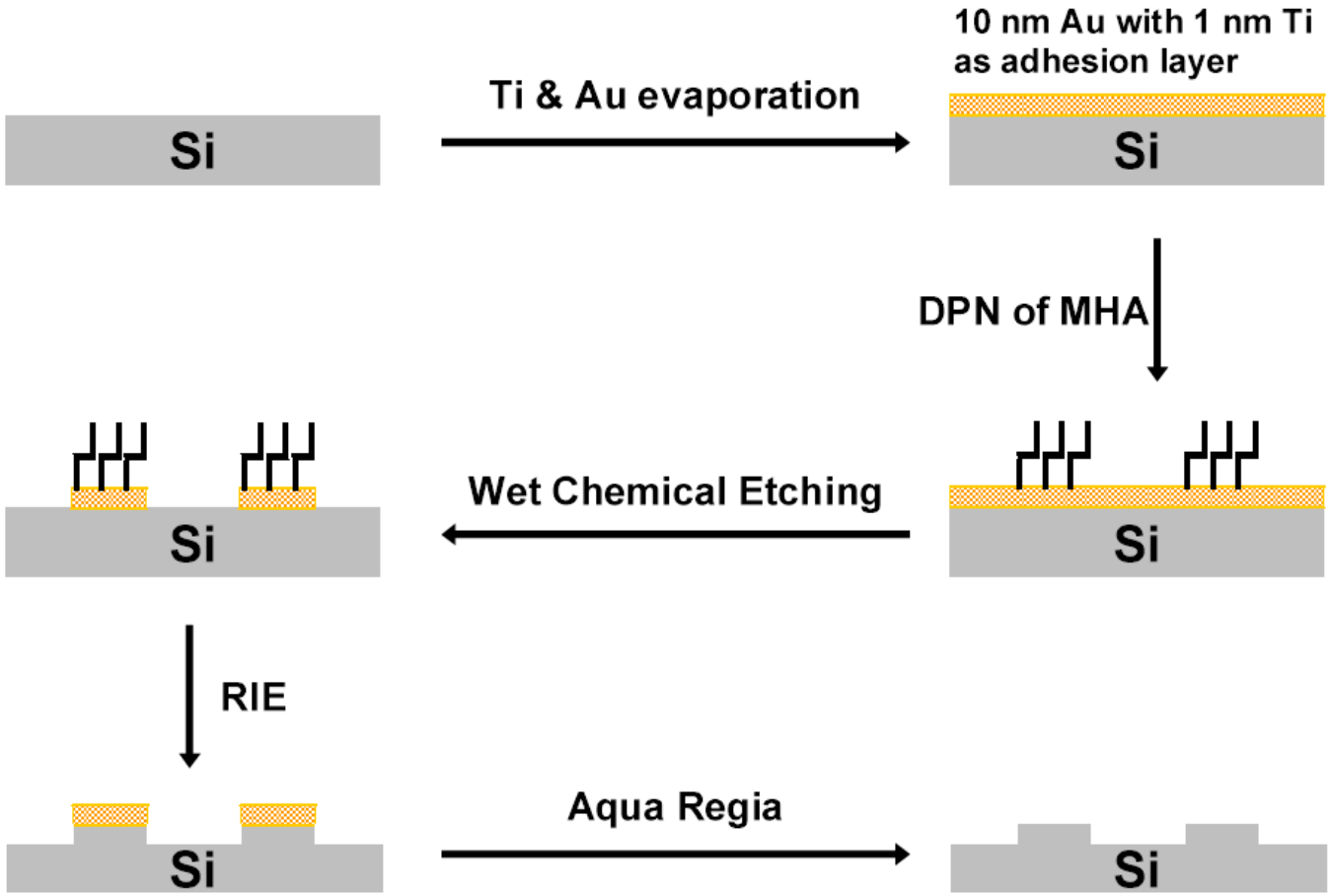


2) “Top-down”

Nanofabrication using etching (molecular inks as etch resists)

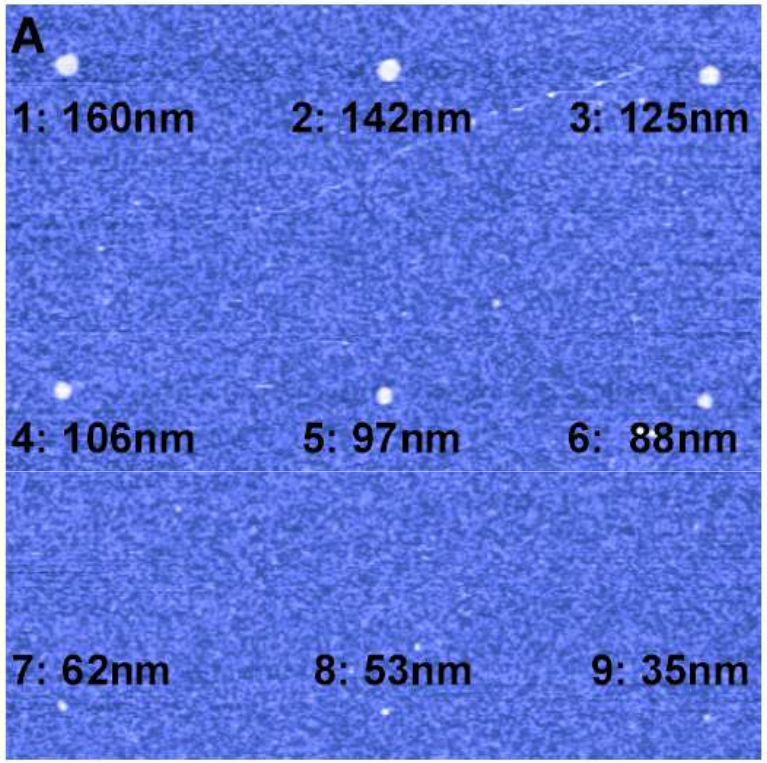


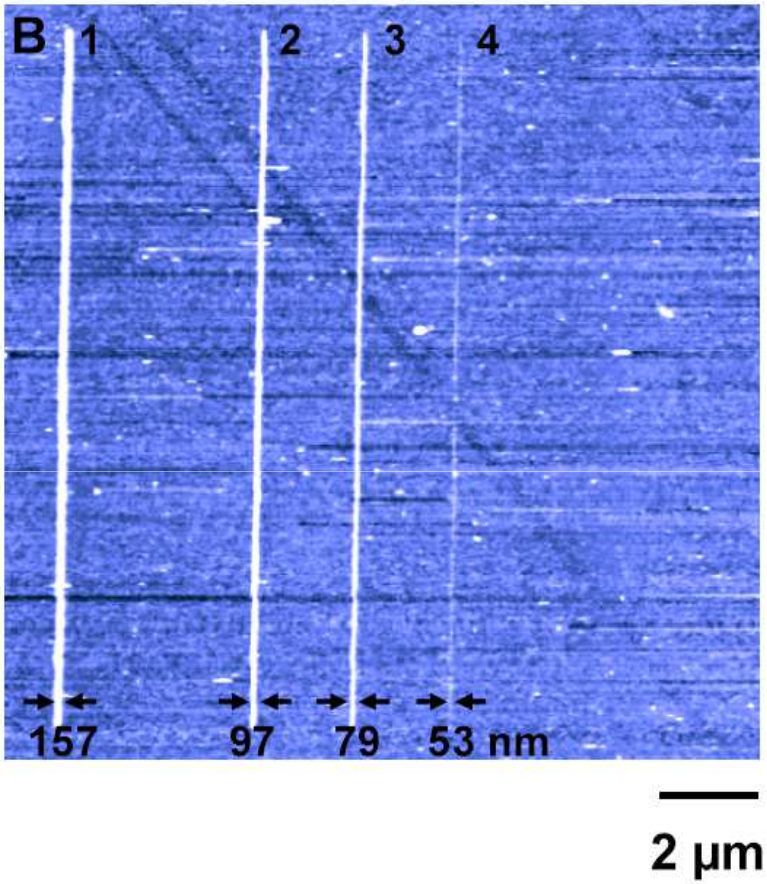
Creating structures in Si or Au



Zhang et al. Small, 2007. **3(1)**: p. 81-85.

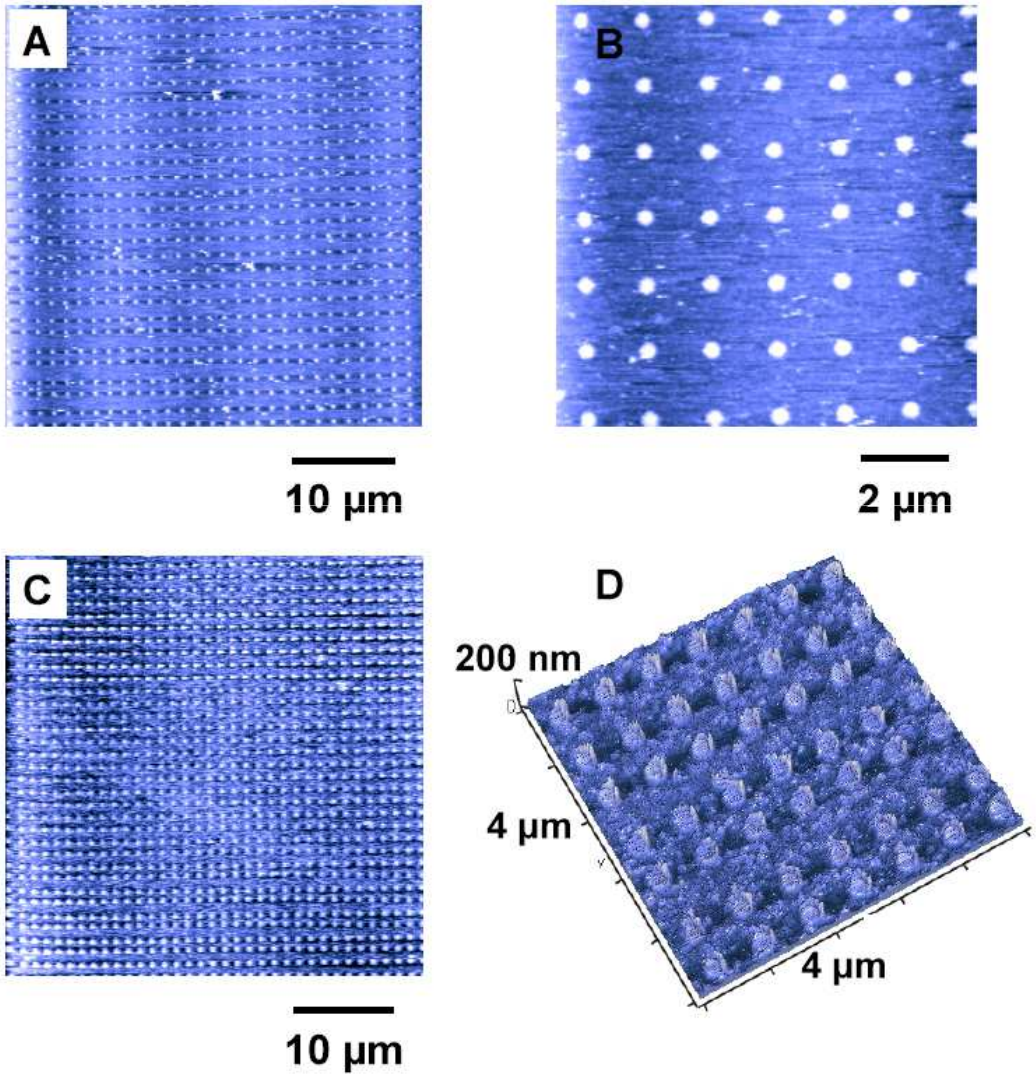
Example structures in Si or Au





Zhang et al. Small, 2007. **3(1)**: p. 81-85.

Example structures in Si or Au



- Scalable over larger areas.
- Sharp or angled features depending on the process / substrate.
- Nb. Can add further DPN steps to build up a complex nanostructure (e.g. polymers or biomolecules).

Zhang et al. Small, 2007. **3(1)**: p. 81-85.

tDPN Mask Writing: Graphene structures

NANO LETTERS

LETTER

pubs.acs.org/NanoLett

Chemically Isolated Graphene Nanoribbons Reversibly Formed in Fluorographene Using Polymer Nanowire Masks

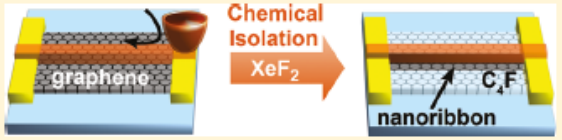
Woo-Kyung Lee,[†] Jeremy T. Robinson,[†] Daniel Gunlycke,[†] Rory R. Stine,[†] Cy R. Tamanaha,[†] William P. King,[‡] and Paul E. Sheehan*[,†]

[†]U.S. Naval Research Laboratory, Washington, D.C. 20375, United States

[‡]Department of Mechanical Science and Engineering University of Illinois at Urbana−Champaign, Urbana Illinois 61801, United States

Supporting Information

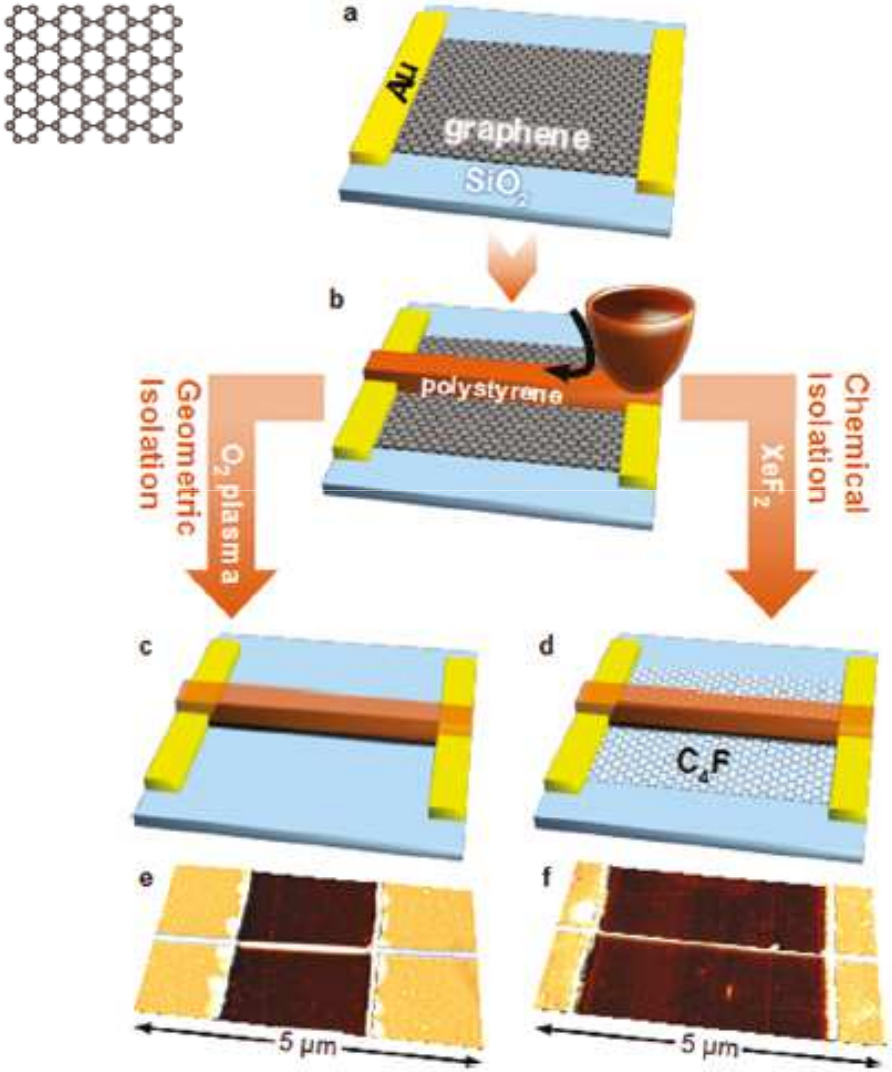
ABSTRACT: We demonstrated the fabrication of graphene nanoribbons (GNRs) as narrow as 35 nm created using scanning probe lithography to deposit a polymer mask[1−3] and then fluorinating the sample to isolate the masked graphene from the surrounding wide band gap fluorographene. The polymer protected the GNR from atmospheric adsorbates while the adjacent fluorographene stably p-doped the GNRs which had electron mobilities of ~2700 $cm^2/(V \cdot s)$. Chemical isolation of the GNR enabled resetting the device to nearly pristine graphene.



KEYWORDS: Graphene nanoribbons, thermal dip-pen nanolithography, fluorographene

Sheehan et. al. Nano Lett. **2011,** 11, 5461–5464

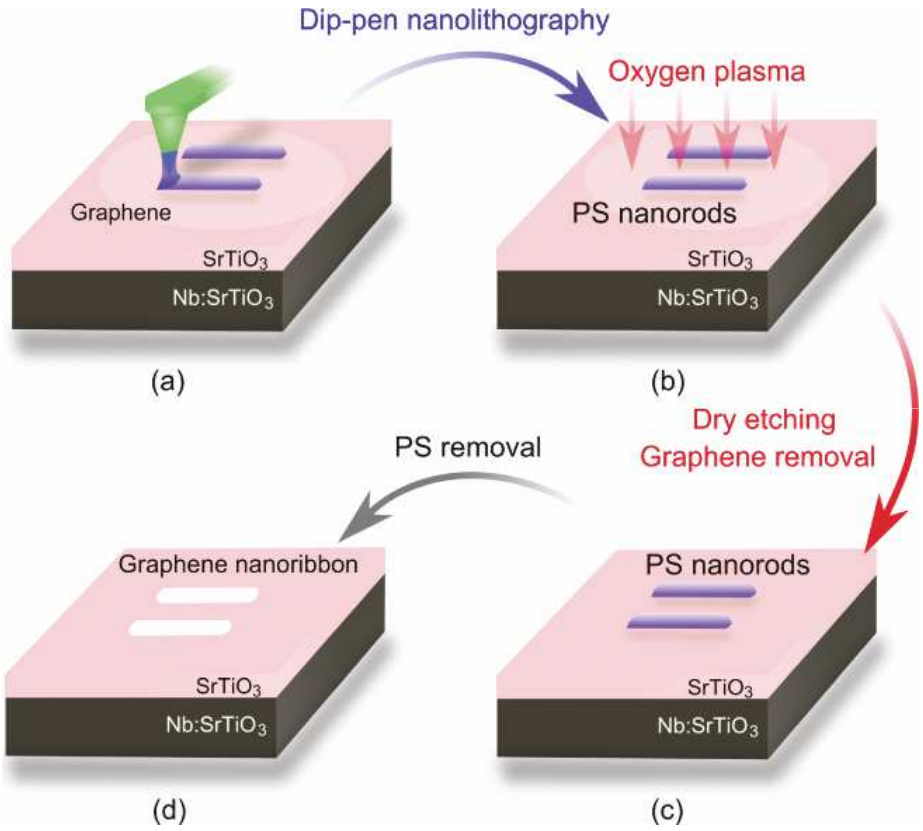
tDPN Mask Writing: Graphene structures

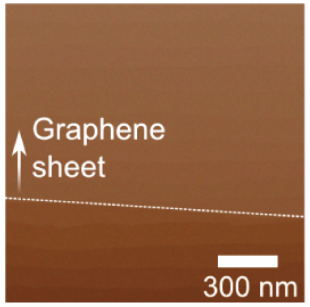


- In t-DPN the solid ink (polystyrene) deposits directly to the surface.
- Polystyrene by tDPN adheres strongly to single layer graphene.
- Lines from 300 to 35 nm.
- Tip speed up to 40 $\mu m.s^{-1}$
- Ordering process during deposition increasing performance of the mask.

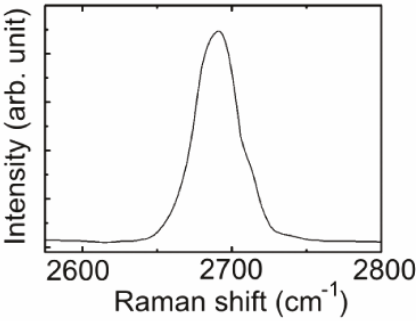
Sheehan et. al. Nano Lett. **2011,** 11, 5461–5464

Graphene Nanoribbons

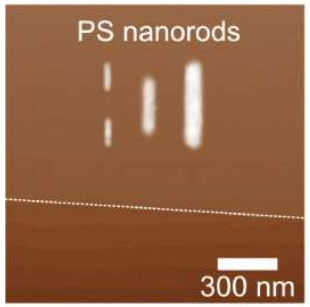




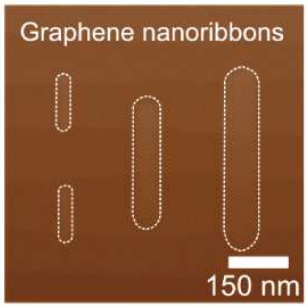
(a)



(b)



(c)



(d)

Yun-Sok Shin et.al., J. Am. Chem. Soc. 2011, 133, 5623–5625

Making a graphene device using DPN

nanoINK

ARTICLE

Dip-Pen Nanolithography of Electrical Contacts to Single Graphene Flakes

Wechung Maria Wang,[†] Nimrod Stander,[‡] Randall M. Stoltenberg,[§] David Goldhaber-Gordon,[‡] and Zhenan Bao[†,*]

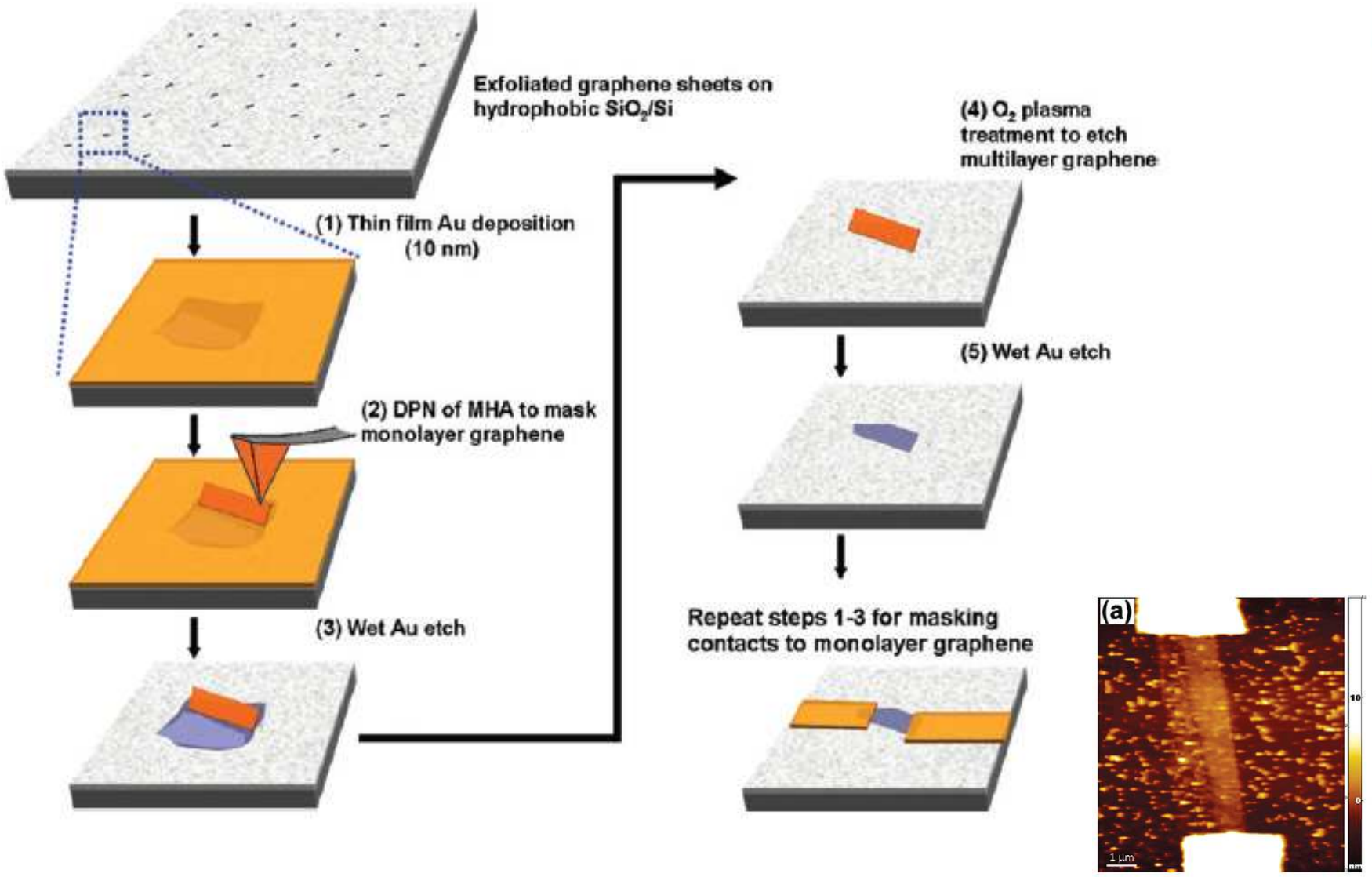
[†]Department of Chemical Engineering, [‡]Department of Physics, and [§]Department of Chemistry, Stanford University, Stanford, California 94305, United States

Scaling issues with Si field-effect transistors, particularly charge leakage due to quantum mechanical tunneling,[1] motivate the search for alternative semiconductors that are compatible with nanoscale Si technology platforms and that provide higher carrier mobilities. A material that holds much potential for next-generation nanoelectronics is graphene, which exhibits room-temperature mobility of up to ~10 000 $cm^2/V \cdot s$.[2] Moreover, when graphene is patterned into nanoribbons, the carriers are confined to a quasi-1D system, thereby opening a band gap that in-

ABSTRACT This study evaluates an alternative to electron-beam lithography for fabricating nanoscale graphene devices. Dip-pen nanolithography is used for defining monolayer graphene flakes and for patterning of gold electrodes through writing of an alkylthiol on thin films of gold evaporated onto graphene flakes. A wet gold etching step was used to form the individual devices. The sheet resistances of these monolayer graphene devices are comparable to reported literature values. This alternative technique for making electrical contact to 2D nanostructures provides a platform for fundamental studies of nanomaterial properties. The merits of using dip-pen nanolithography include lack of electron-beam irradiation damage and targeted patterning of individual devices with imaging and writing conducted in the same instrument under ambient conditions.

KEYWORDS: gold electrode · patterning · graphene · scanning probe lithography · dip-pen nanolithography · nanofabrication

Making a graphene device using DPN

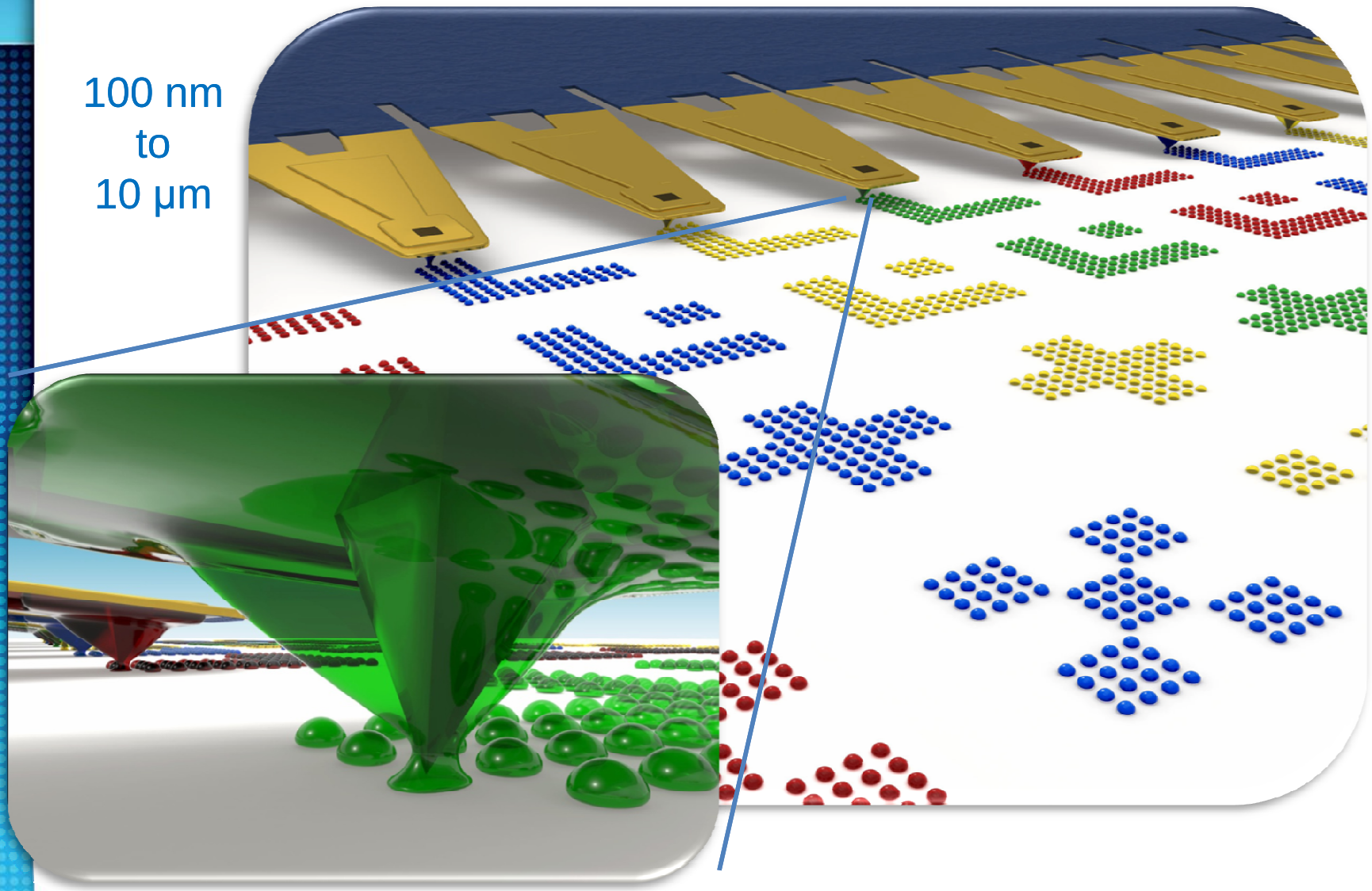


Liquid Inks

Liquid Inks

- Polymers or any viscous chemical…
- Protein, DNA, lipid solutions…

100 nm
to
10 µm

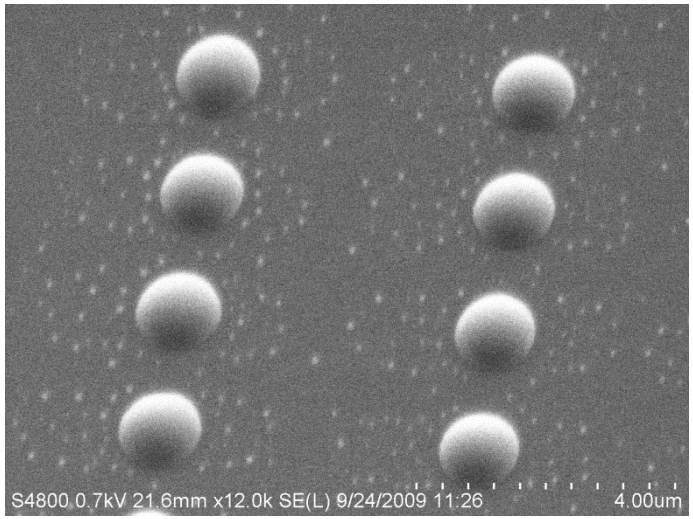


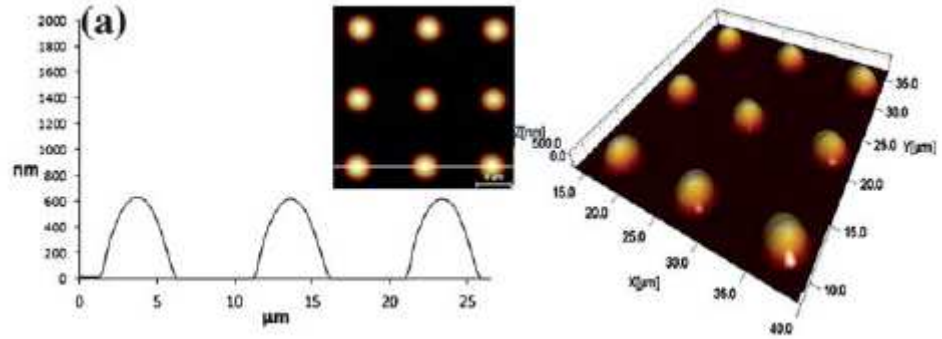
3) “ Top Down” Direct deposition:

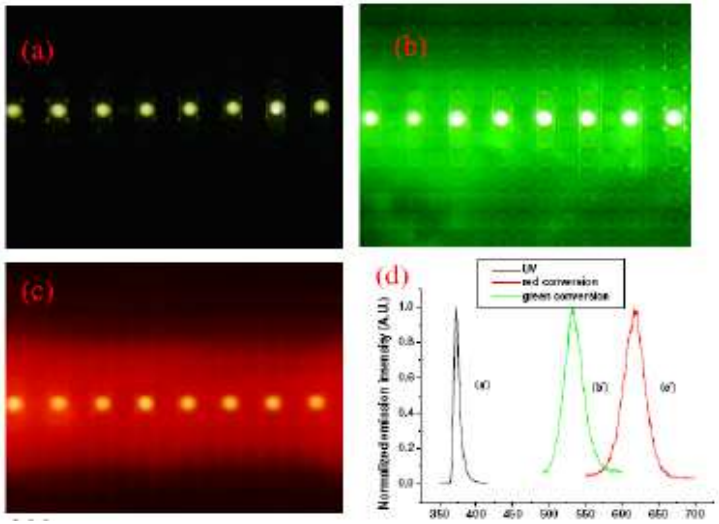
Liquid Inks (polymers)

Example of direct deposition: polymer inks

- Hydrogels
- Acrylic polymers
- Conductive polymers
- Biopolymers
- Optically active materials (e.g. light converting polymer), near field sources.



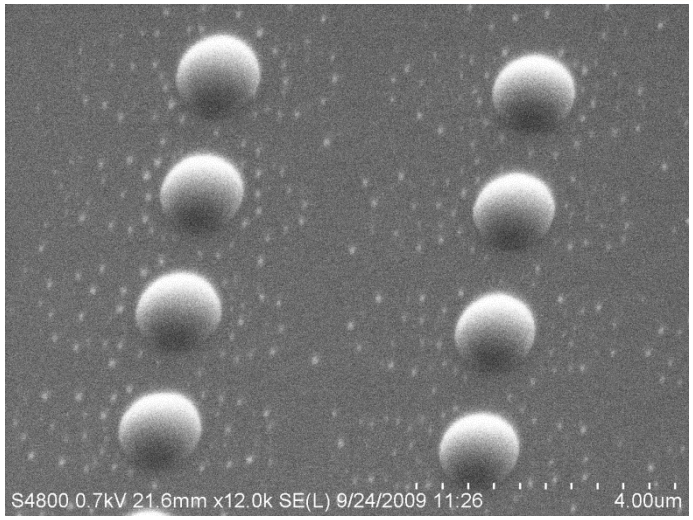


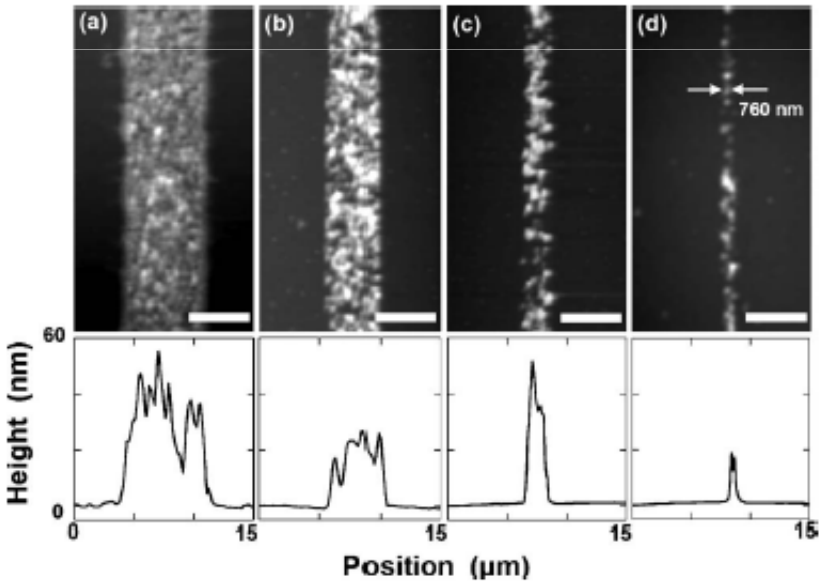


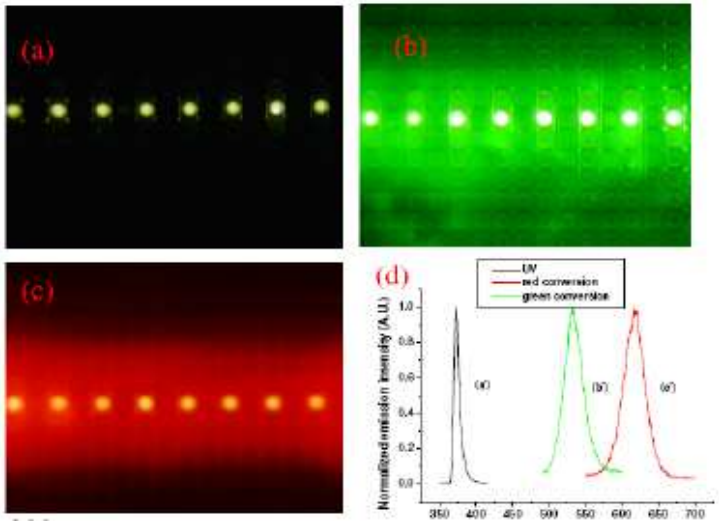
Truxenes: A. Hernandez-Santana et. al. J. Mat Chem. 2011.
PDMS: D Graham et. Al, Chemical Science 2011.

Example of direct deposition: polymer inks

- Polymers
- Nanoparticle solutions*
- Composite nanomaterials
- Biopolymers
- Optically active materials**



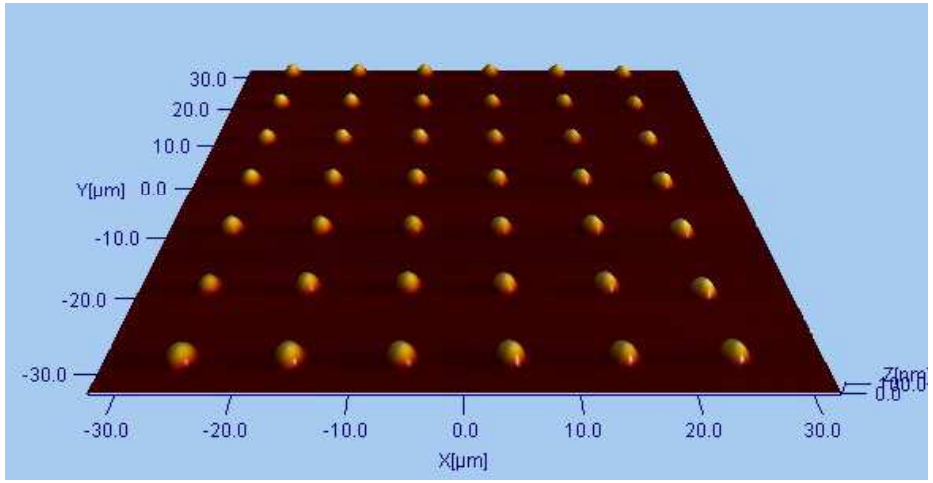




Conductive ink: Wang et al., APL 93, 143105, 2008

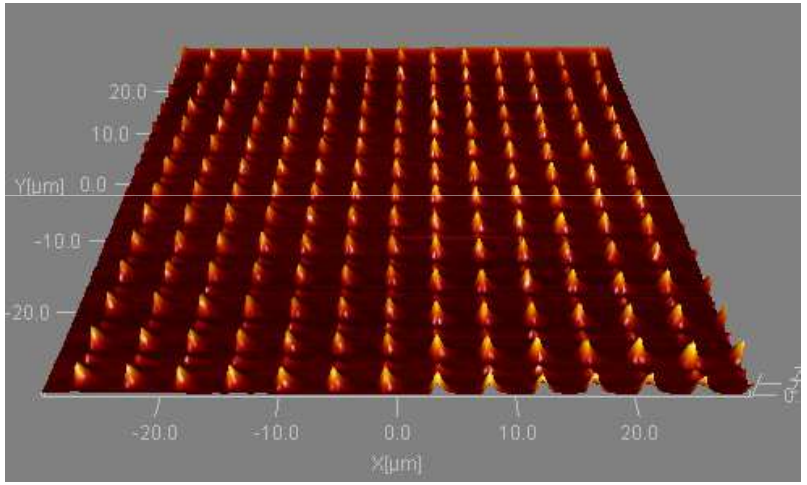
UV Curable polymer writing

On glass slide



Diameter: 2.5 µm
Height: 118 nm

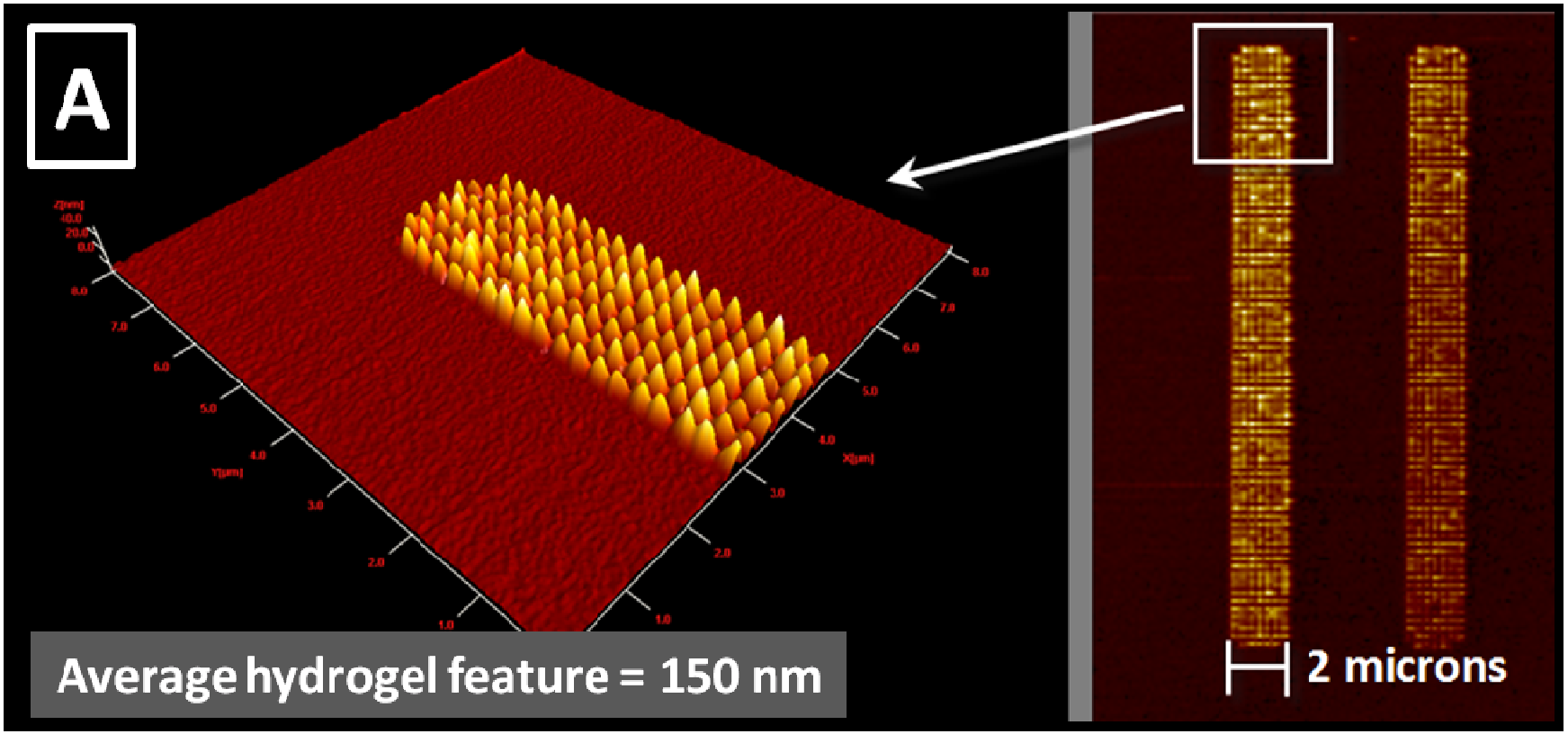
On silicon oxide



Diameter: 1.2 µm
Height: 32 nm

Smaller structures created by liquid inks

Average height = 37 nm
Average peak width = 90 nm
Average base width = 200 nm



- Higher viscosity results in smaller features.

Conductive polymer features

Langmuir

ARTICLE

pubs.acs.org/Langmuir

Liquid Deposition Patterning of Conducting Polymer Ink onto Hard and Soft Flexible Substrates via Dip-Pen Nanolithography

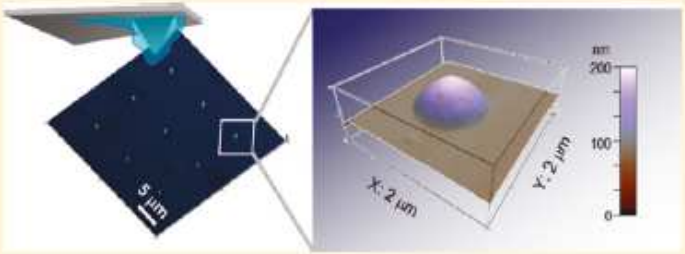
Hiroshi Nakashima,*[†,‡] Michael J. Higgins,[†] Cathal O'Connell,[†] Keiichi Torimitsu,[‡] and Gordon G. Wallace*[†]

[†]ARC Centre of Excellence for Electromaterials Science, Intelligent Polymer Research Institute, AIIM Facility, Innovation Campus, University of Wollongong, Wollongong, NSW 2522, Australia

[‡]Materials Science Research Laboratory, NTT Basic Research Laboratories, NTT Corporation, 3-1, Morinosato Wakamiya, Atsugi, Kanagawa 243-0198, Japan

Supporting Information

ABSTRACT: Ink formulations and protocols that enable the deposition and patterning of a conducting polymer (PEDOT:PSS) in the nanodomain have been developed. Significantly, we demonstrated the ability to pattern onto soft substrates such as silicone gum and polyethylene terephthalate (PET), which are materials of interest for low cost, flexible electronics. The deposition process and dimensions of the polymer patterns are found to be critically dependent on a number of parameters, including the pen design, ink properties, time after inking the pen, dwell time of the pen on the surface, and the nature of material substrate. By assessing these different parameters, an improved understanding of the ability to control the dimensions of individual PEDOT:PSS structures down to 600 nm in width and 10−80 nm in height within patterned arrays was obtained. This applicability of DPN for simple and nonreactive liquid deposition patterning of conducting polymers can lead to the fabrication of organic nanoelectronics or biosensors and complement the efforts of existing printing techniques such as inkjet and extrusion printing by scaling down conductive components to submicrometer and nanoscale dimensions.



Nakashima et. al. Langmuir 2012

Conductive polymer features

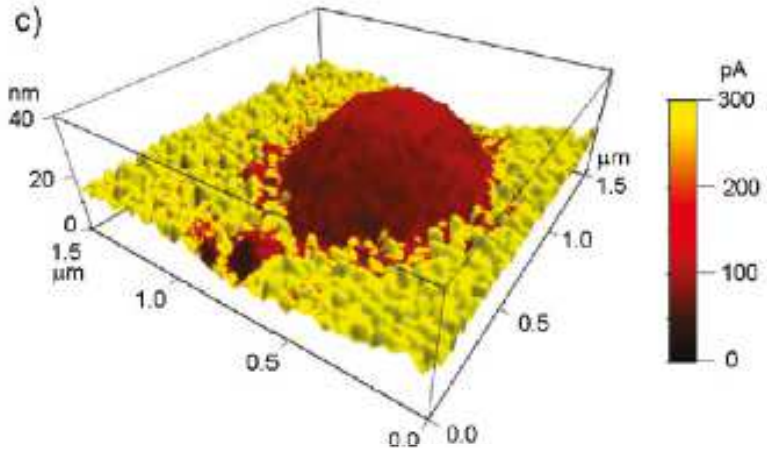
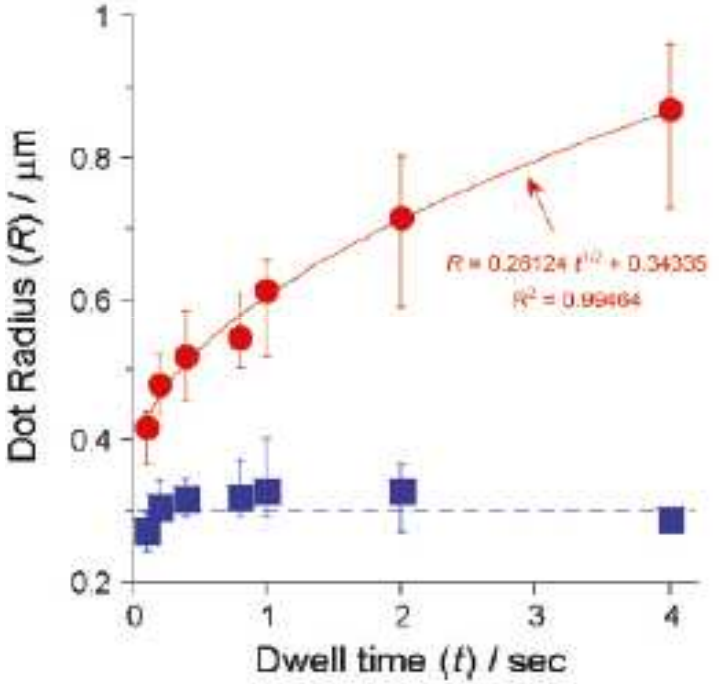


Table 1. Surface Properties of Various Substrates and the Size of the Polymer Dots Patterned on the Substrates

substrate	static water contact angle (deg)	rms roughness[g] (nm)	dot diameter[h] (µm)	dot height[h] (nm)
Si[a]	24.6	0.6	1.09 ± 0.14	15.9 ± 3.2
SiO_2[a]	22.6	3.7	1.24 ± 0.19	8.5 ± 3.5
ITO[b,c]	21.2	2.1	2.64 ± 0.58	8.0 ± 3.5
Gold[b,d]	88.5	2.2	1.96 ± 0.47	27.5 ± 5.6
PET film[e]	100.4	15.0	1.15 ± 0.27	81.9 ± 16.7
Silicone gum[e]	114.2	21.0	0.62 ± 0.15	53.1 ± 38.1
PDMS (hydrophobic)[e]	117.7	4.0		
PDMS (hydrophilic)[f]	12.9	2.2		



poly(3,4-ethylenedioxythiophene)

Nakashima et. al. Langmiur 2012

tDPN: Nanoparticle-polymer composites

NANO LETTERS

pubs.acs.org/NanoLett

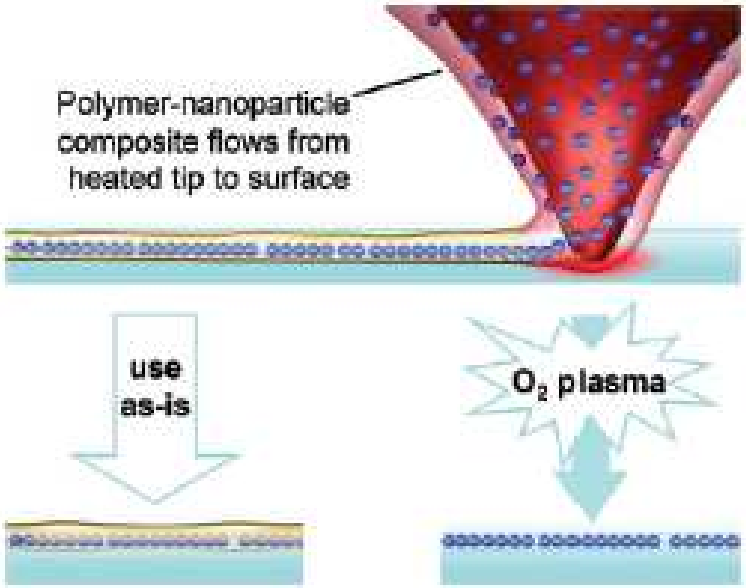
Maskless Nanoscale Writing of Nanoparticle–Polymer Composites and Nanoparticle Assemblies using Thermal Nanoprobes

Woo Kyung Lee,† Zhenting Dai,‡ William P. Ki

†Code 6177, Chemistry Division, U.S. Naval Research Lab
Mechanical Science and Engineering, University of Illinois

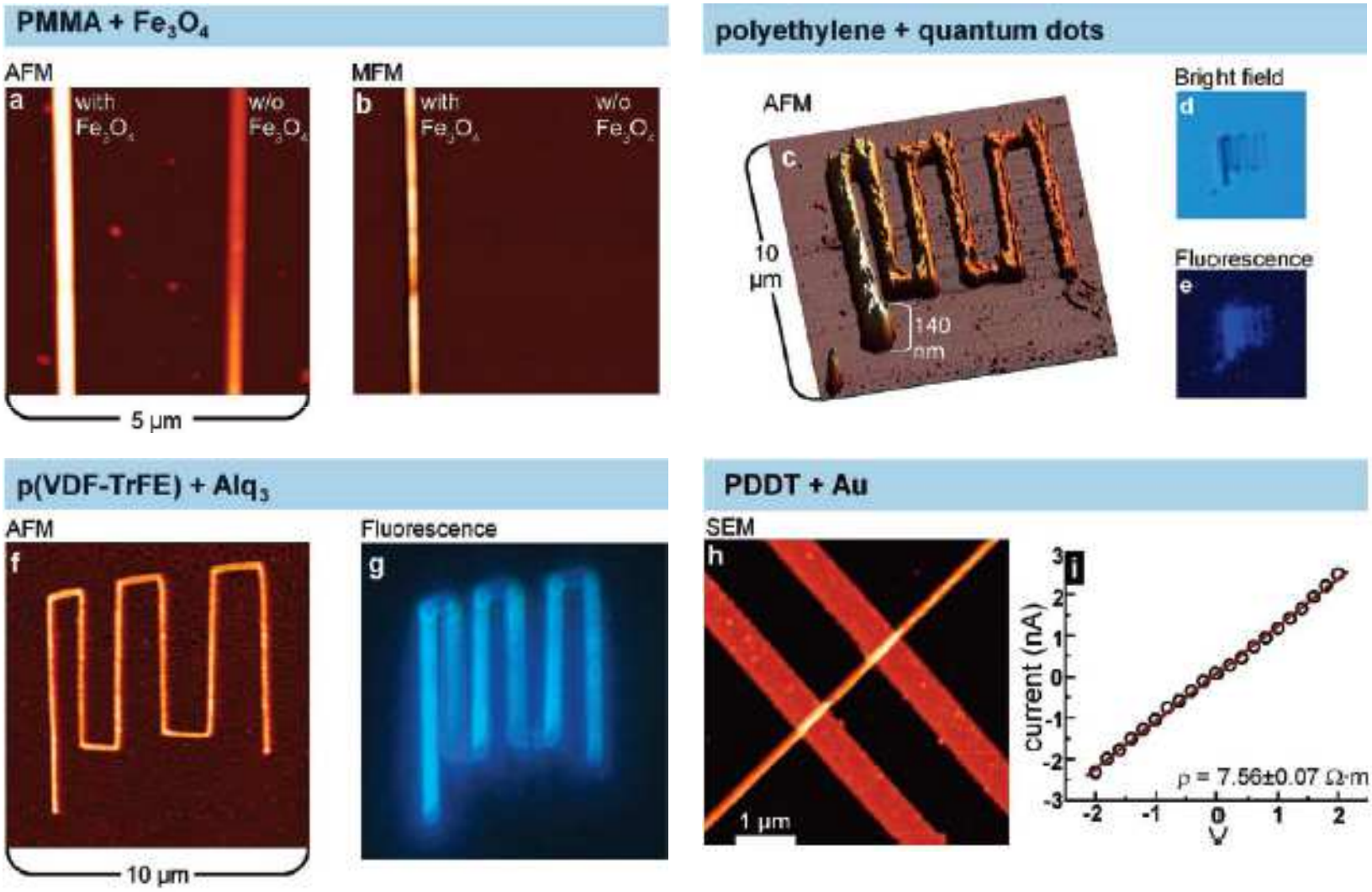
ABSTRACT Nanoparticle polymer composites containing metal,
deposited onto multiple substrates from a heatable atomic force r
deposited or could be etched with an oxygen plasma, revealing sin
can be patterned with the same technique, without the need to t

KEYWORDS Nanolithography, nanocomposites, atomic force m



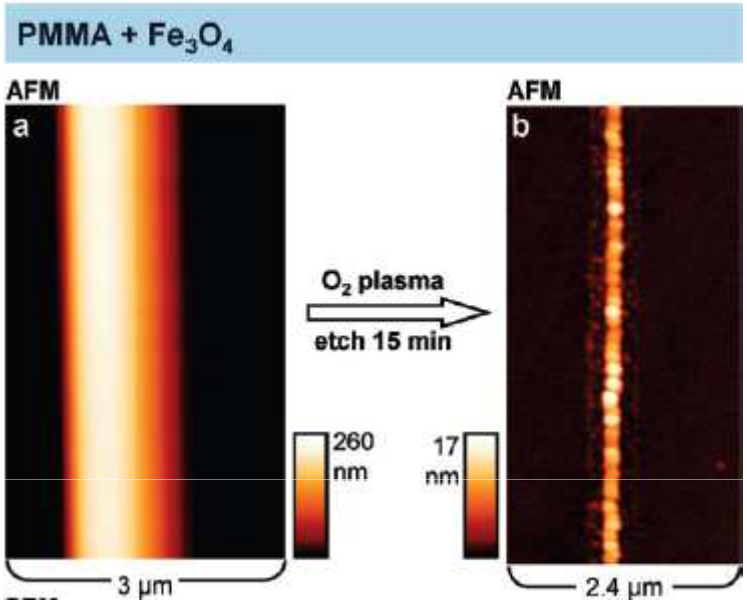
Sheehan et. al. Nano Lett. **2010, 10, 129-133**

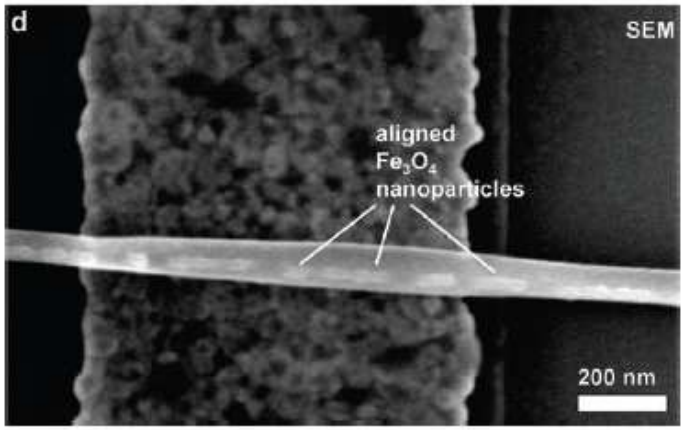
Nanoparticle-polymer composites

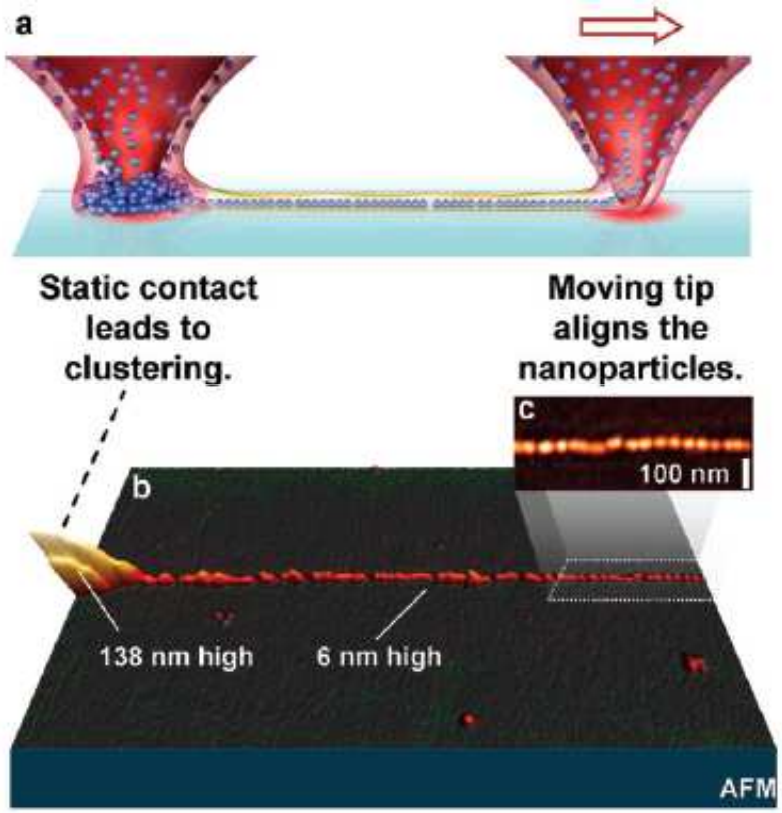


Sheehan et. al. Nano Lett. **2010, 10, 129-133**

Ordering processes during composite deposition



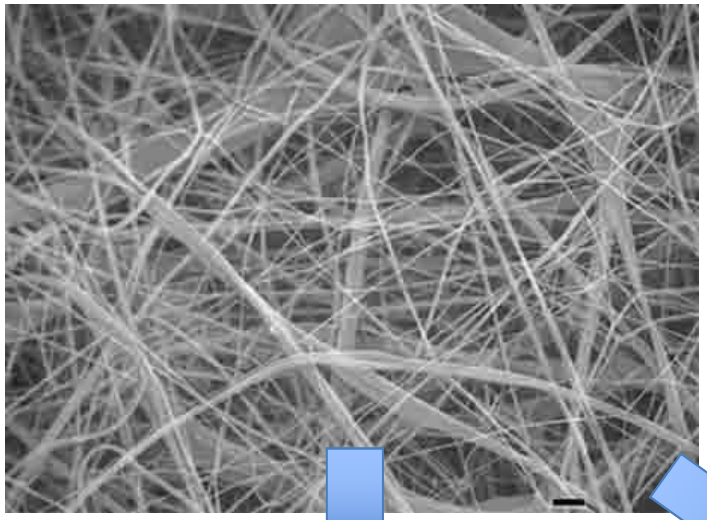




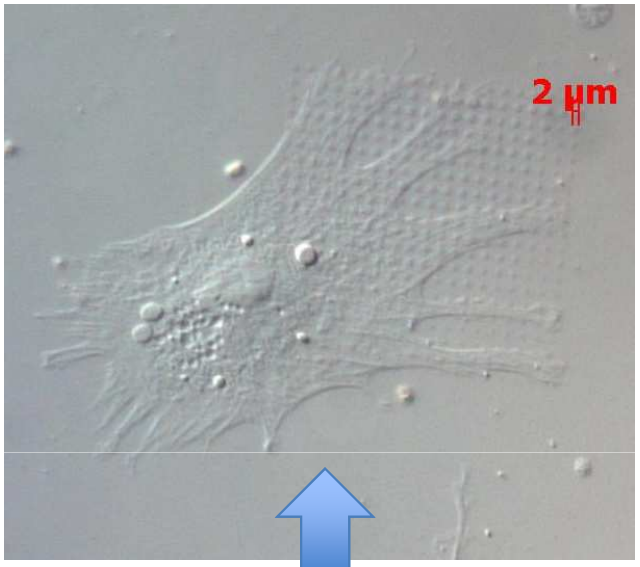
Sheehan et. al. Nano Lett. **2010, 10, 129-133**

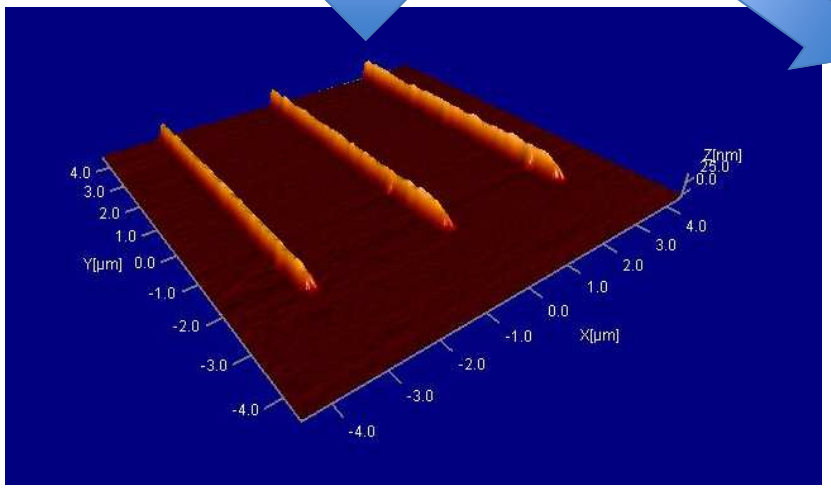
Model scaffolds for bioengineering

Random electrospun polymer scaffold

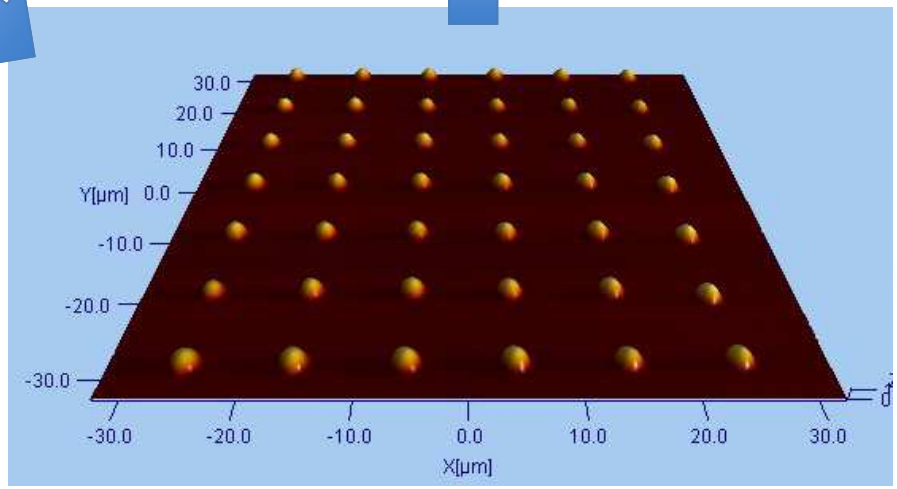


Example: MSC on PDMS dot array (DPN)





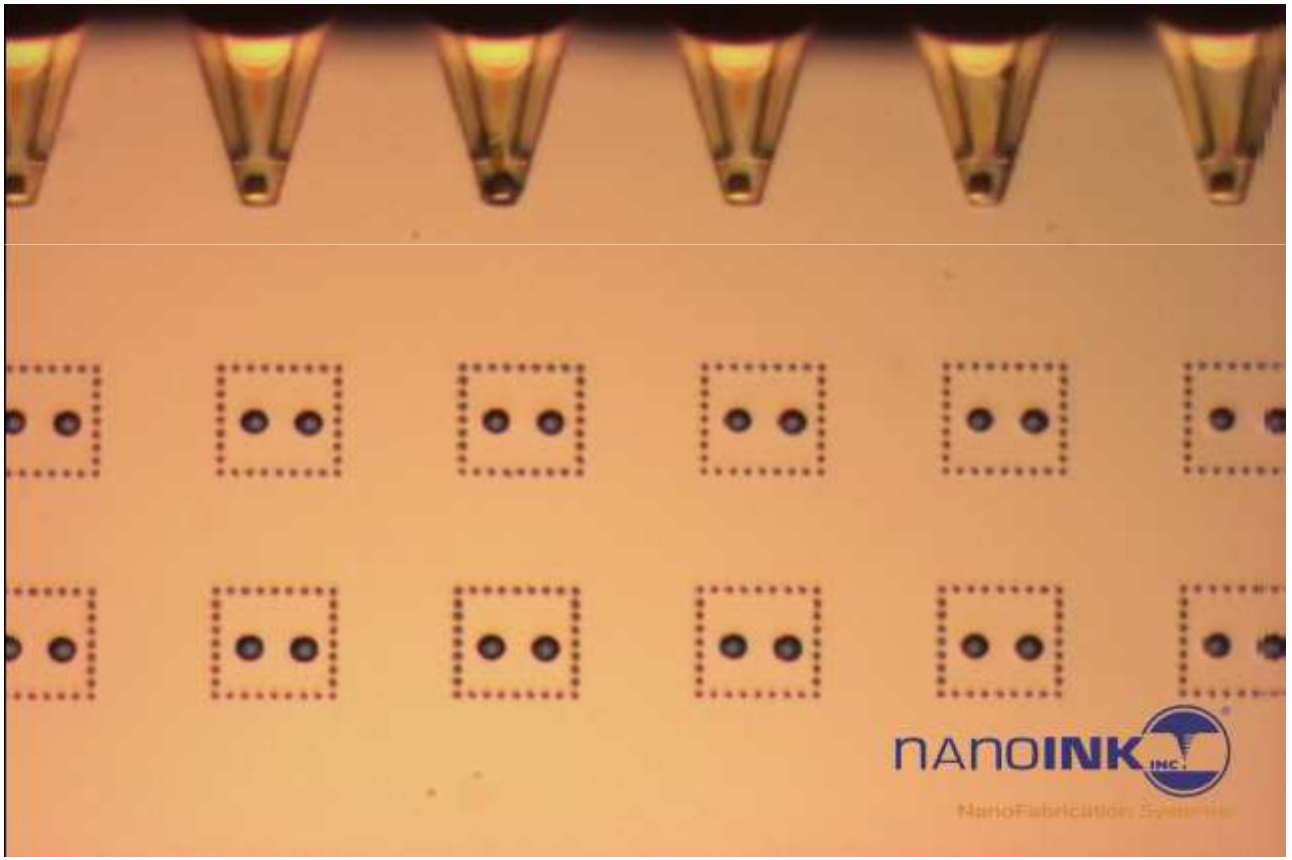
Hydrogel lines: DPN



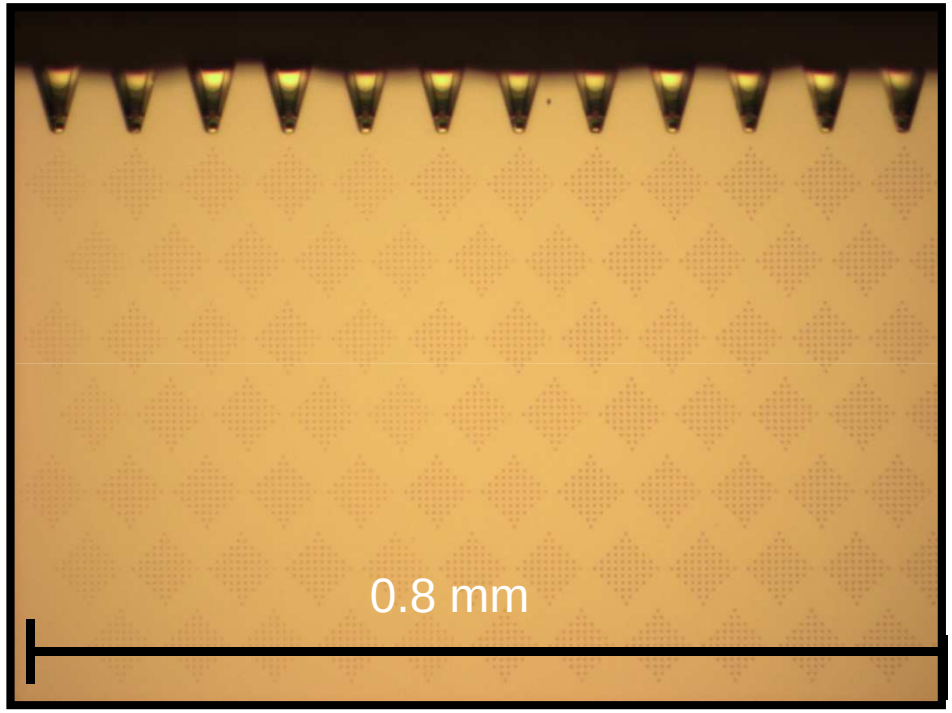
Acrylic polymer dots: DPN

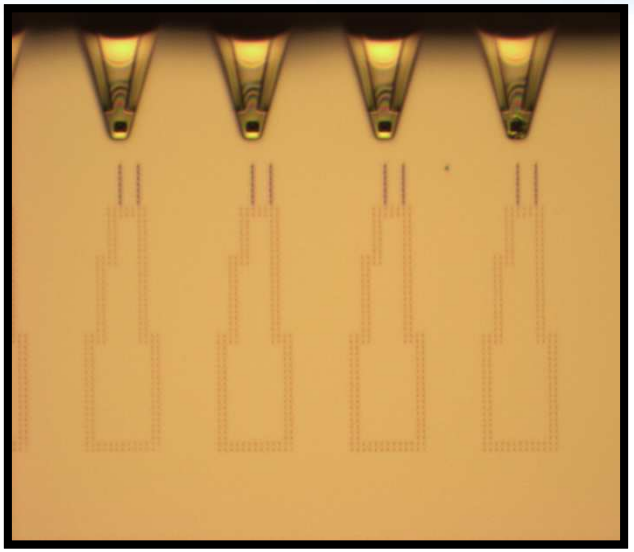
Polymer Printing

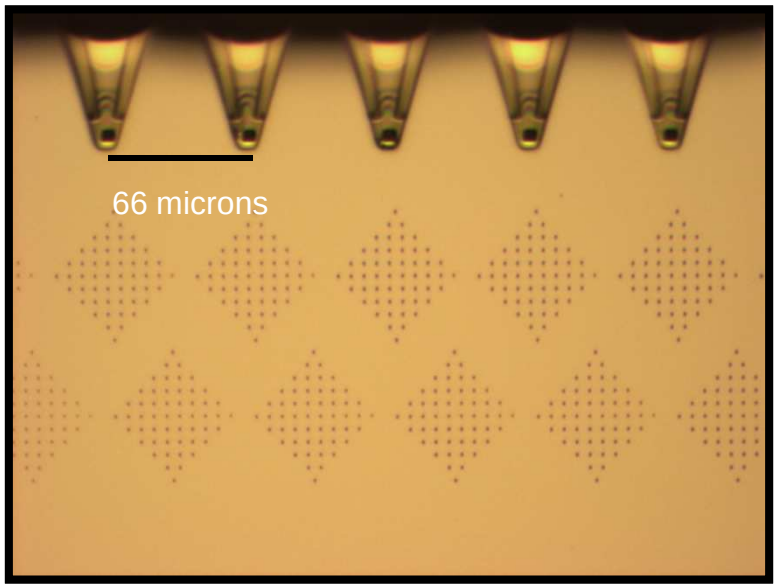
- UV curable polymer printing to silicon oxide surface.
- Dwell time principally controls feature size.
- ** Multiple polymers / compositions / levels of doping on each tip **



Polymer Printing: flexibility



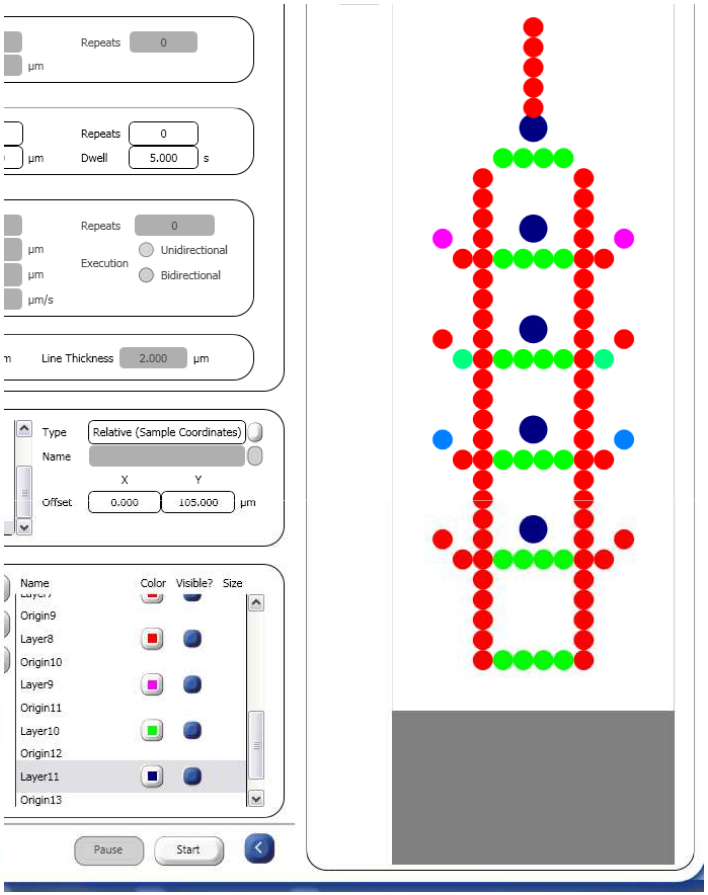


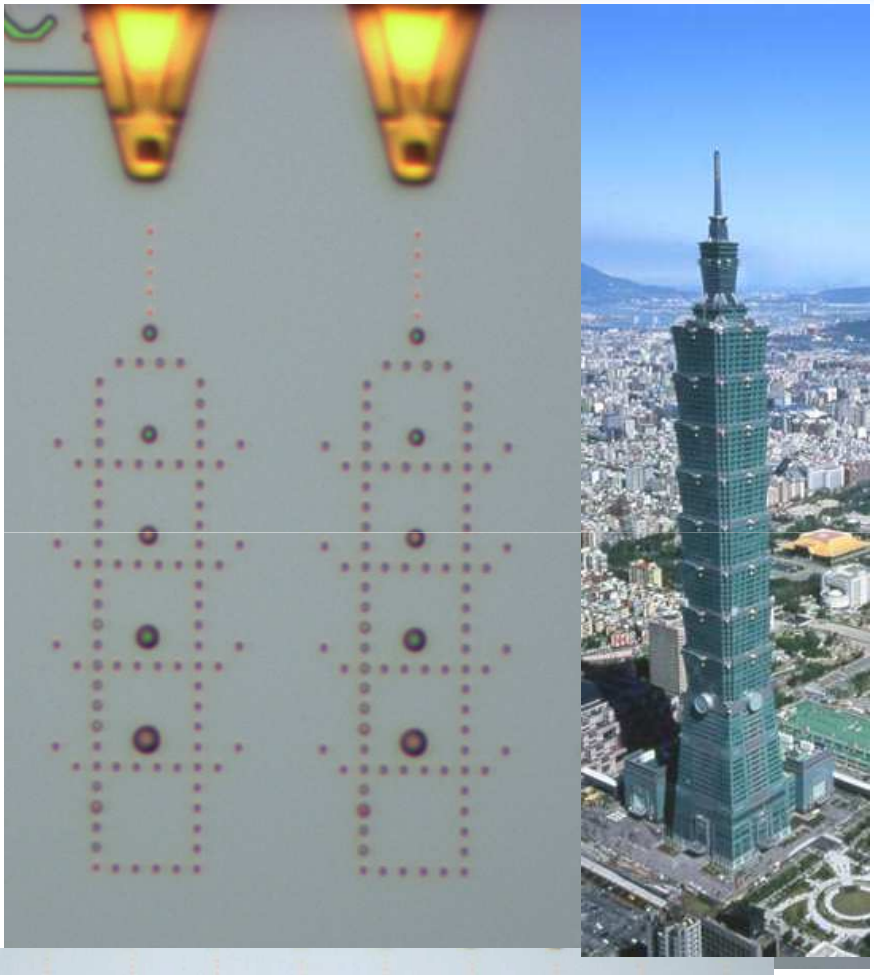


- The versatility of the technique allows rapid prototyping of almost any structure.

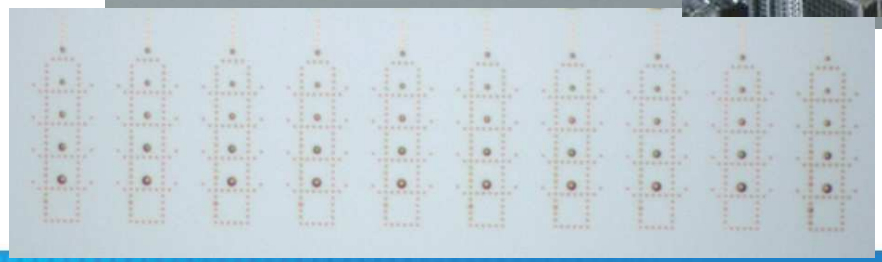
- This can be almost any liquid material.

Polymer Printing: Tapei 101 Tower….



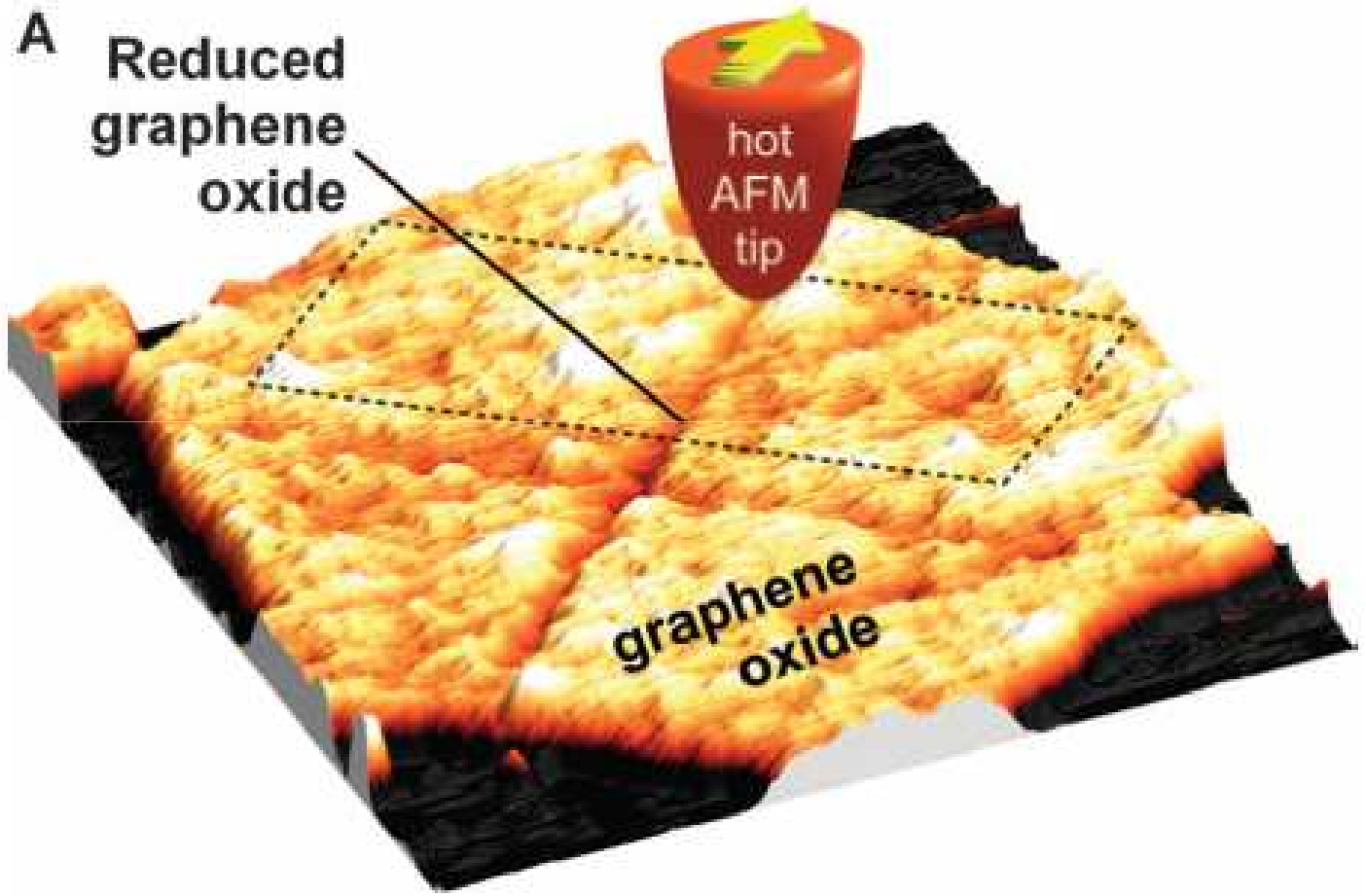


NLP : very simple to operate.



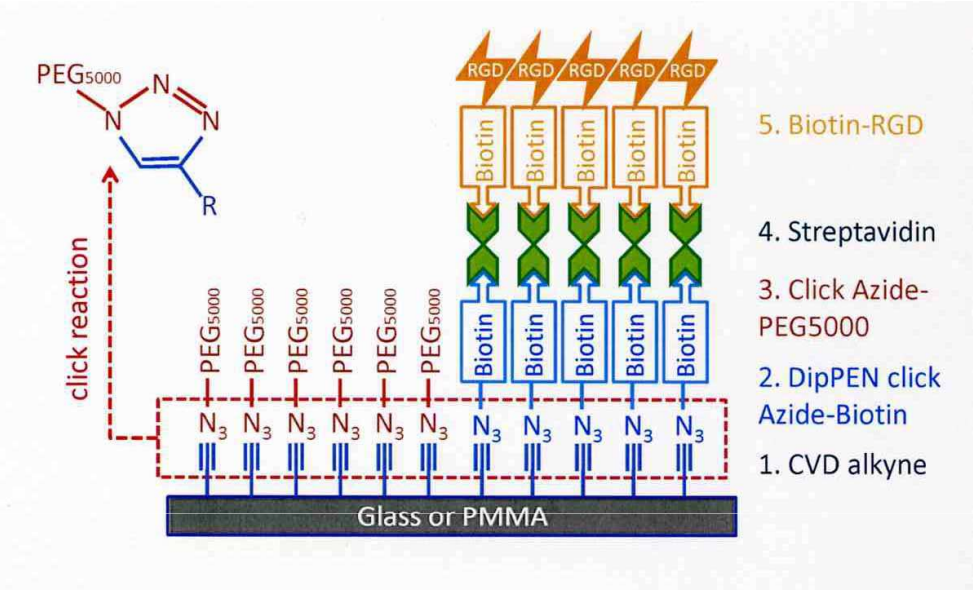
3) Liquid inks for in-situ Nanochemistry

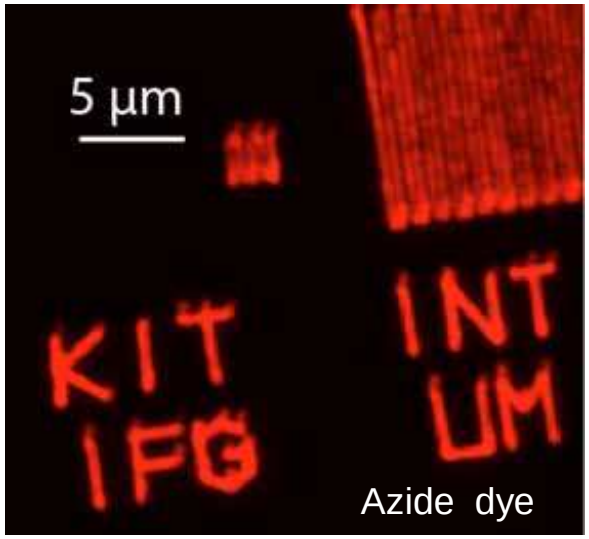
Graphene processing

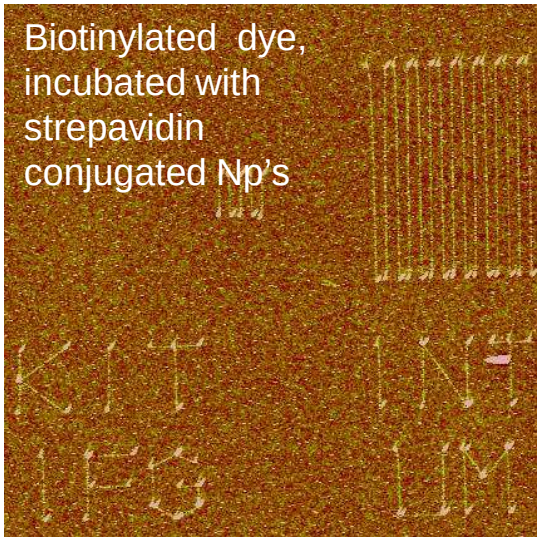


Sheehan, King, et al, **Science** 2010

In Situ “Click” chemistry



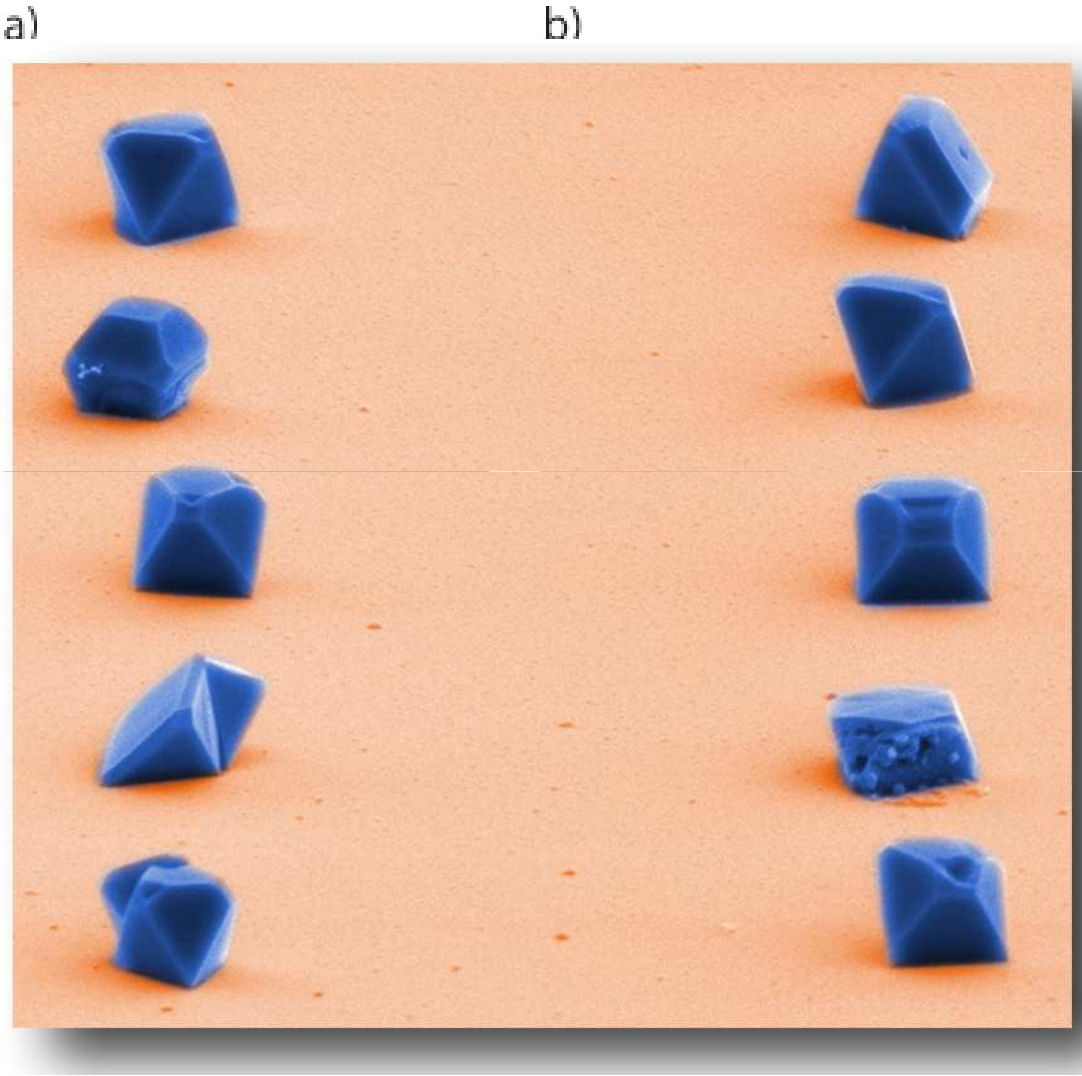




- Substrate independent lithography. Flexible chemistry allows deposition onto almost any surface.
- Surface prepared by CVD [chemical vapor deposition] as little as 10 nm thickness.
- Local chemistry is directed by DPN.
- Liquid inks enable multiplexed depositions.

Hsien-Yeh Chen, Michael Hirtz, *et. al*. JACS, 51(132), 18023-5, 2010.

Metal organic frameworks



- Deposit small amount f reaction material.

Perform localised eactions and rocesses.

Small volume critical.

DPN allows smaller rystals to be achieved- otentially…

Generic method could e applied to almost any naterial. e.g. sol-gels. (e.g. Mirkin 2002)

Carbonell et al. JACS 2011. 133: p. 2144-2147.

Au nanoparticle deposition by block copolymer

PNAS PNAS

Scanning probe block copolymer lithography

Jinan Chai[a,b,1], Fengwei Huo[a,b,1,2], Zijian Zheng[a,b,3], Louise R. Giam[b,c], Wooyoung Shim[b,c], and Chad A. Mirkin[a,b,c,4]

[a]Department of Chemistry, [b]International Institute for Nanotechnology, and [c]Department of Materials Science and Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208

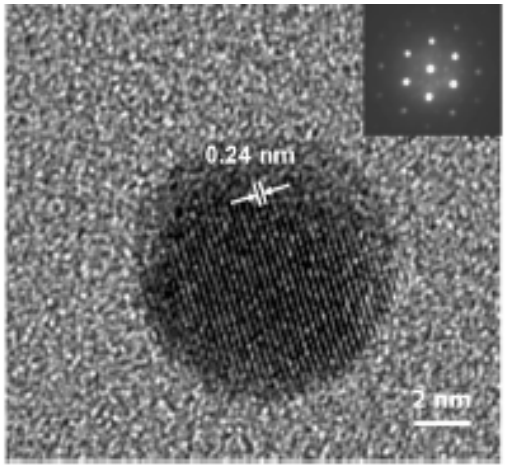
Contributed by Chad A. Mirkin, October 5, 2010 (sent for review September 3, 2010)

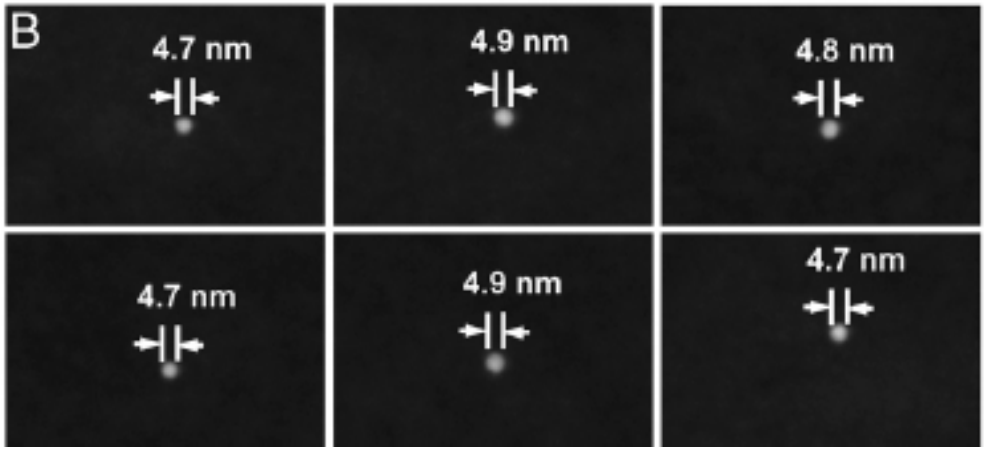
Integration of individual nanoparticles into desired spatial arrangements over large areas is a prerequisite for exploiting their unique electrical, optical, and chemical properties. However, positioning single sub-10-nm nanoparticles in a specific location individually on a substrate remains challenging. Herein we have developed a unique approach, termed scanning probe block copolymer lithography, which enables one to control the growth and position of individual nanoparticles in situ. This technique relies on either dip-pen nanolithography (DPN) or polymer pen lithography (PPL) to transfer phase-separating block copolymer inks in the form of 100 or more nanometer features on an underlying substrate. Reduction of the metal ions via plasma results in the high-yield formation of single crystal nanoparticles per block copolymer feature. Because the size of each feature controls the number of metal atoms within it, the DPN or PPL step can be used to control precisely the size of each nanocrystal down to 4.8 ± 0.2 nm.

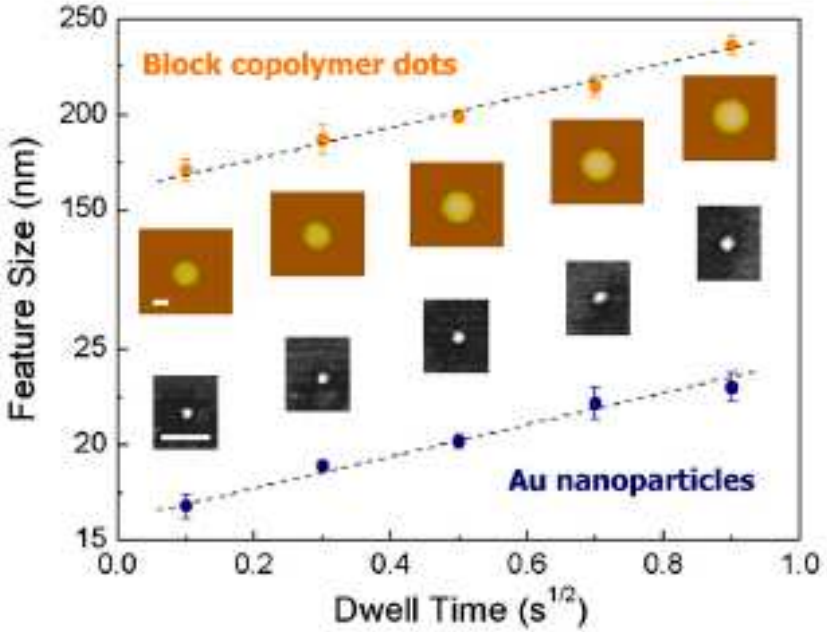
scanning probe lithography | block copolymer micelles | single particle synthesis | nanopatterning

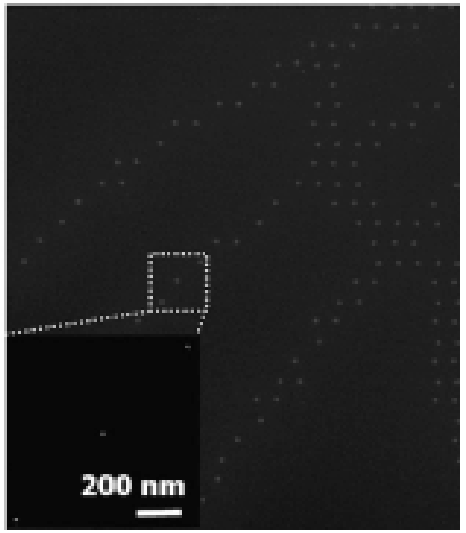
Nanoparticles exhibit size-dependent photonic, electronic, and chemical properties that could lead to a new generation of catalysts and nanodevices, including single electron transistors, photonics, and biomedical sensors (1–3). In order to realize many of these targeted applications, researchers need ways of synthesizing monodisperse particles while controlling individual particle as thin film templates for the synthesis of nanoparticle arrays in mass without control over individual particle position or dimensions. In this work, however, we demonstrate addressable and size-controllable single nanoparticle synthesis using a tip-based approach where the block copolymer acts as a delivery matrix for facile ink transfer and as a synthetic nanoreactor for forming single nanoparticles. With this PEO-*b*-P2VP block copolymer, the P2VP is responsible for concentrating nanomaterial precursors through metal ion association for subsequent in situ chemical synthesis (19, 20), whereas PEO acts as a delivery block to facilitate ink transport when used in a scanning probe experiment. Pure PEO is known to be a good ink matrix material for DPN (21), whereas P2VP alone is not a good transport matrix because of its low solubility in water at neutral pH. The block copolymer separates into nanoscale micelles, which not only localizes the metal ions, but also causes the amount of metal ion in each feature to be substantially lower than if the feature was made from pure metal ion ink. Moreover, the time-dependent ink transport characteristics of DPN and PPL determine the volume of transferred composite ink, which effectively controls the final feature size of the nanomaterials formed inside the polymer micelles. Indeed, the final dimensions of the metal nanoparticles that result from plasma reduction of the metal ions in the block copolymer features are smaller than those which define the original features. Importantly, feature size reduction beyond physical tip geometry constraints is achieved via this approach. It is worth noting that

Au nanoparticle deposition by block copolymer





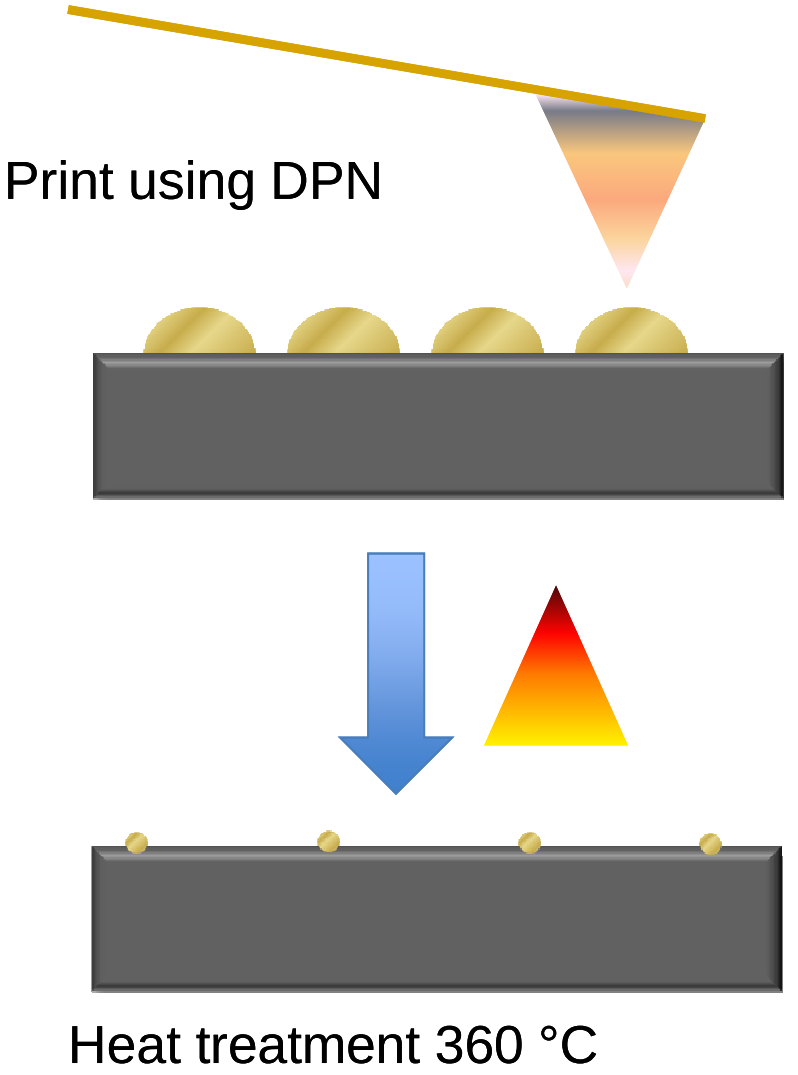




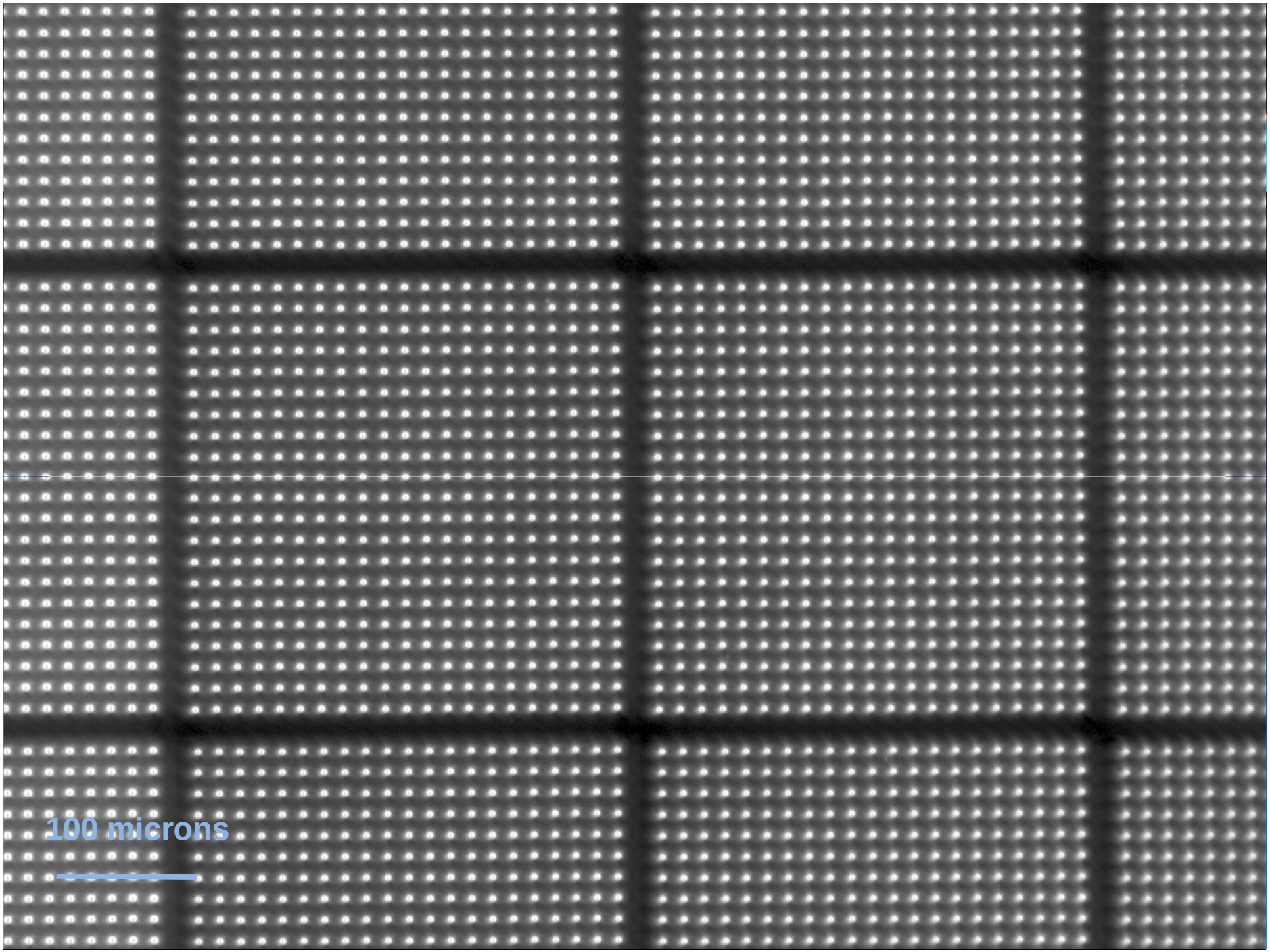
Fabrication *in situ* using block co-polymer

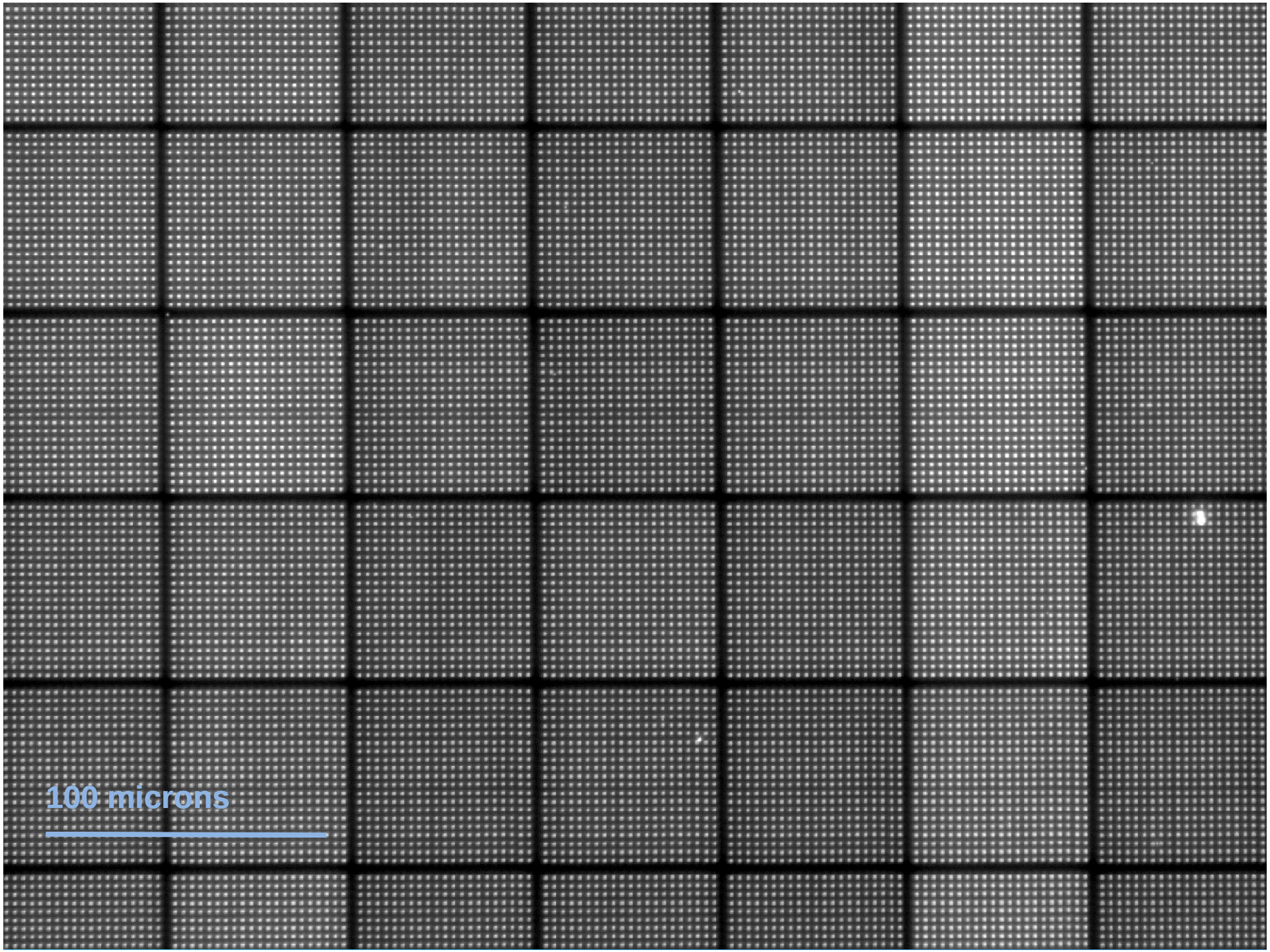
- Printed solution is a P_2VP-PEO block co-polymer with dissolved transition metal salt: $AuCl_3$, $IrCl_3$, $Fe(NO_3)_2$, $Ni(NO_3)_2$,$CrCl_2$, $C_4K_2O_9Ti$.

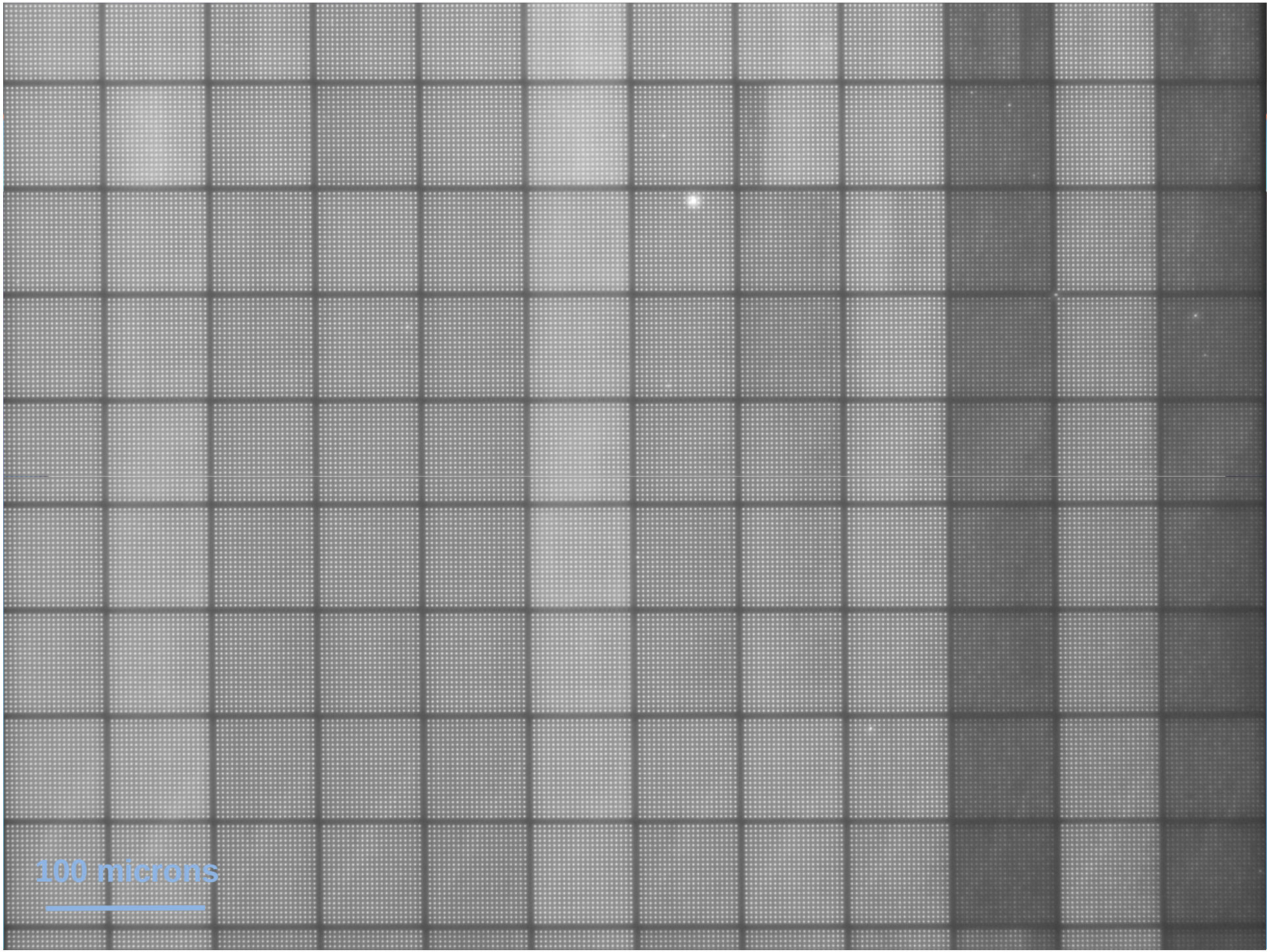
- Upon heat treatment in air the polymer is decomposed and the metal ions are either reduced (Au or Ir) or combined with O_2 to form an oxide (Fe or Ni).



Internal NanoInk data (under review J. Mat Chem.)


100 microns


100 microns

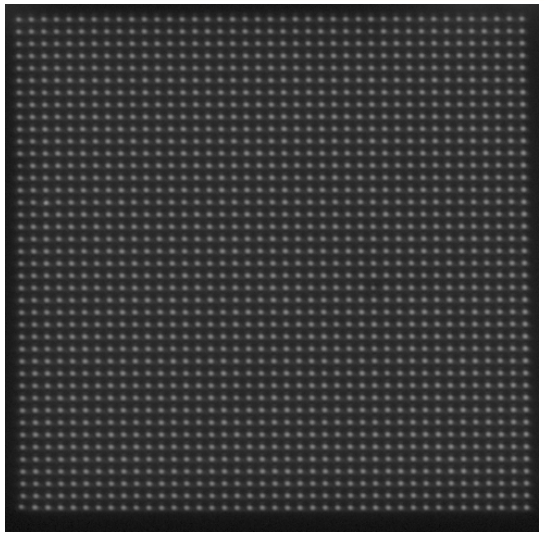

100 microns

One example of particle size distribution (Au)

0.00015 M concentration

As printed polymer/Au^{3+} dot
0.02 second dwell time

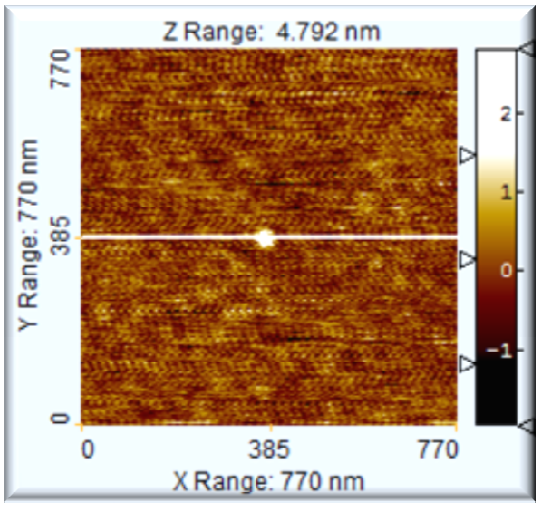
	Diameter (nm)
Minimum	578.33
Maximum	861.37
Mean	**761.64**
Std. Dev.	39.33
CV	**5.2%**



Imaging by dark field microscopy

After heat treatment

	Diameter (nm)
Median	38.56
Minimum	32.87
Maximum	42.82
Mean	**38.44**
Standard Deviation	2.34
CV	**6.1%**

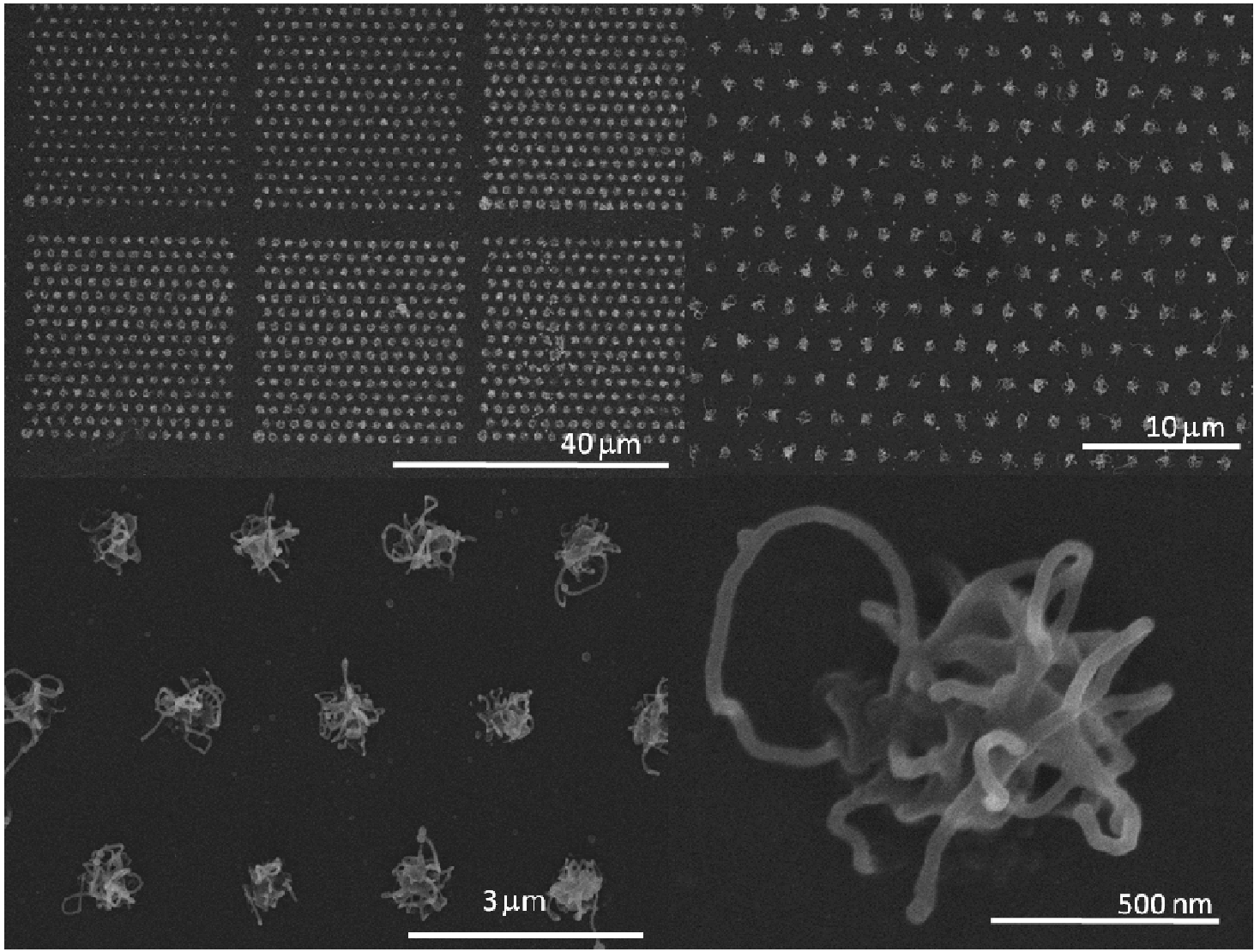


Imaging by AFM

95% Reduction in size

Internal NanoInk data (under review J. Mat. Chem).

Carbon Nanotube growth from Cobalt centers

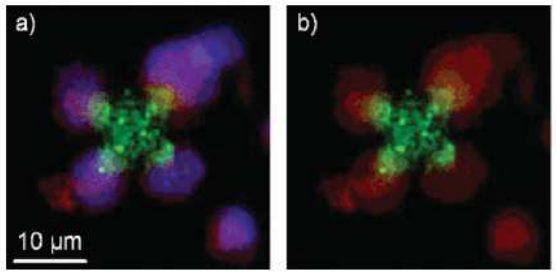


Internal NanoInk data (under review J. Mat. Chem).

5) Direct deposition of biological materials

- Lipids (direct)
- Proteins (via carrier ink)
- DNA (via carrier ink)

DPN using Lipids



Multiplexed Lipid Dip-Pen Nanolithography

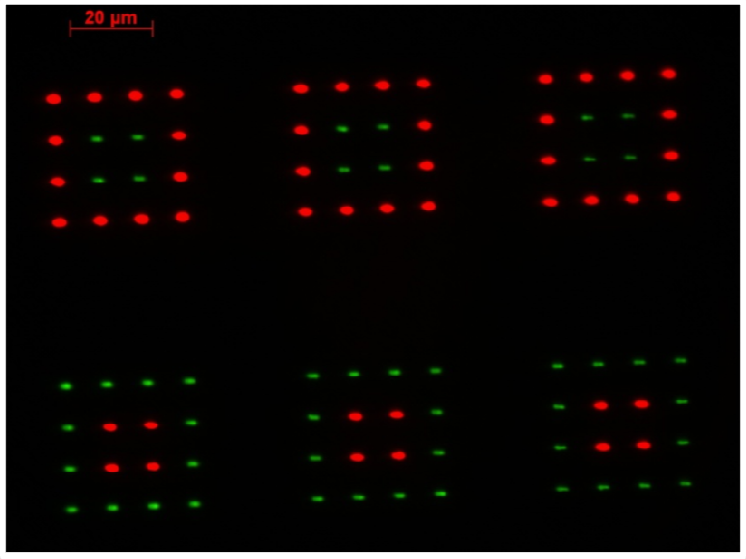
small

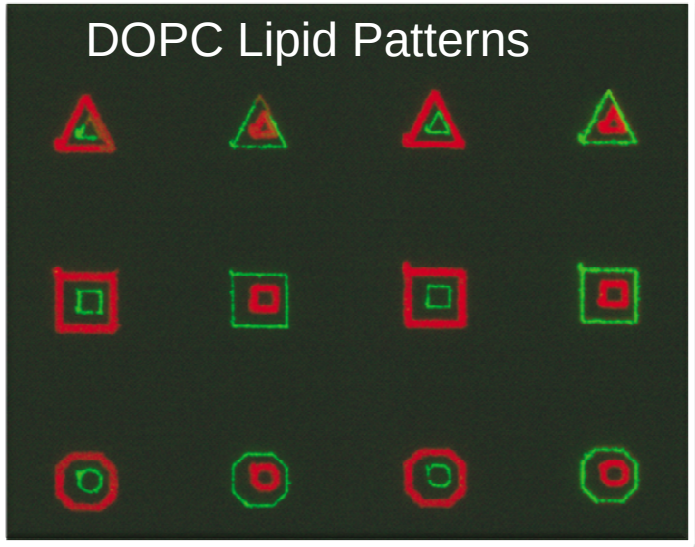
Dip-Pen Nanolithography

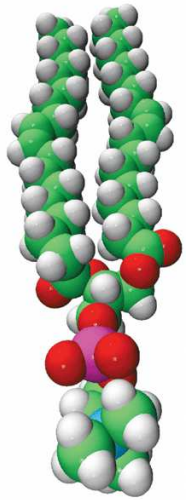
DOI: 10.1002/smll.200800949

Multiplexed Lipid Dip-Pen Nanolithography on Subcellular Scales for the Templating of Functional Proteins and Cell Culture**

*Sylwia Sekula, Jeanette Fuchs, Susanne Weg-Remers, Peter Nagel, Stefan Schuppler, Joe Fragala, Nora Theilacker, Matthias Franzreb, Christer Wingren, Peter Ellmark, Carl A. K. Borrebaeck, Chad A. Mirkin, Harald Fuchs, and Steven Lenhert**

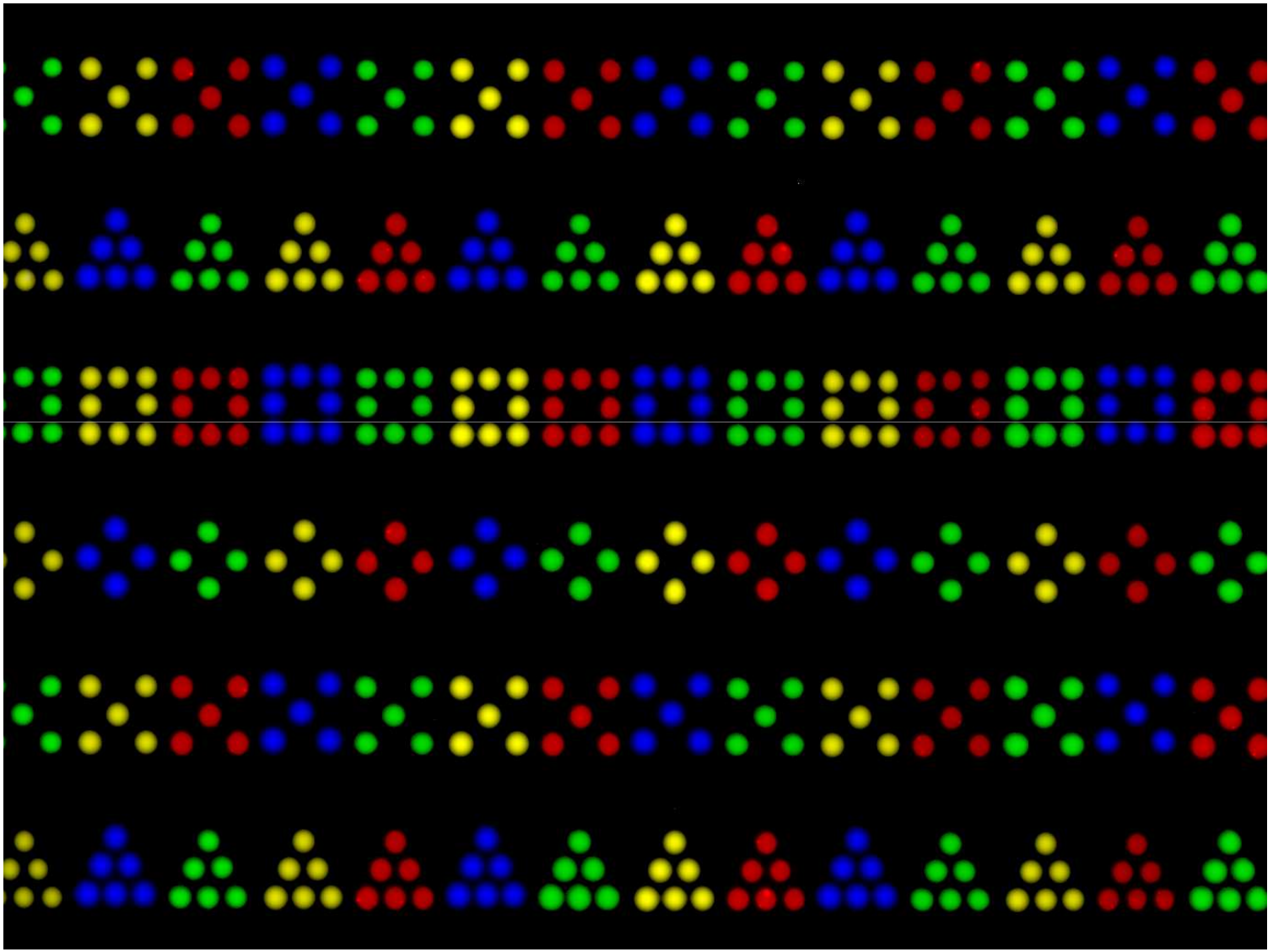






Steven Lenhert et. al. Small 2008, 4, No. 10, 1785–1793

Direct deposition of multiple proteins from liquid ‘ink’



Controlling the number of proteins deposited

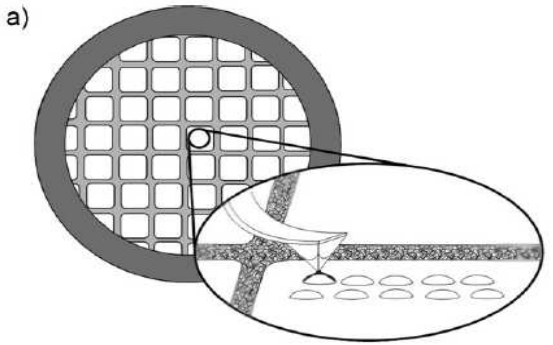
COMMUNICATION

ADVANCED MATERIALS www.advmat.de

Controlling the Number of Proteins with Dip-Pen Nanolithography

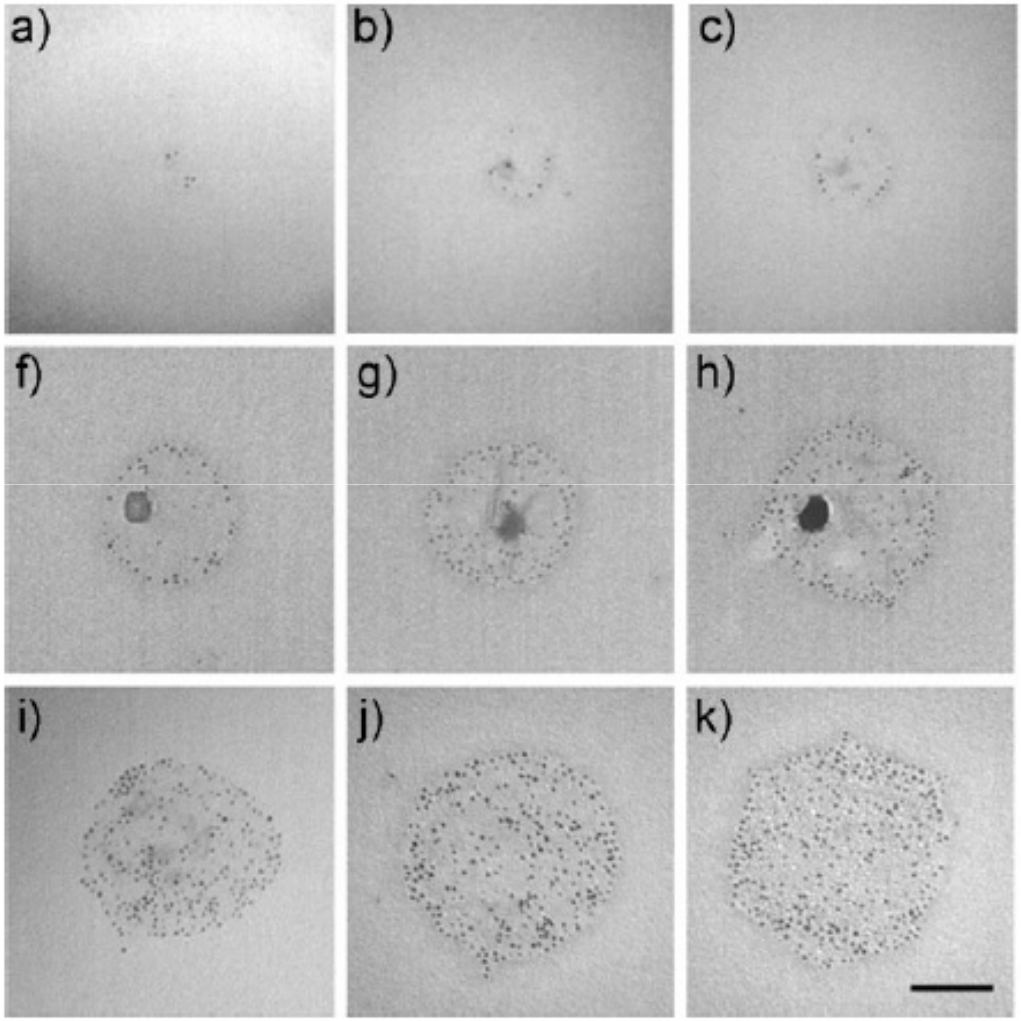
By *Elena Bellido, Rocío de Miguel, Daniel Ruiz-Molina, Anabel Lostao,* and *Daniel Maspoch**

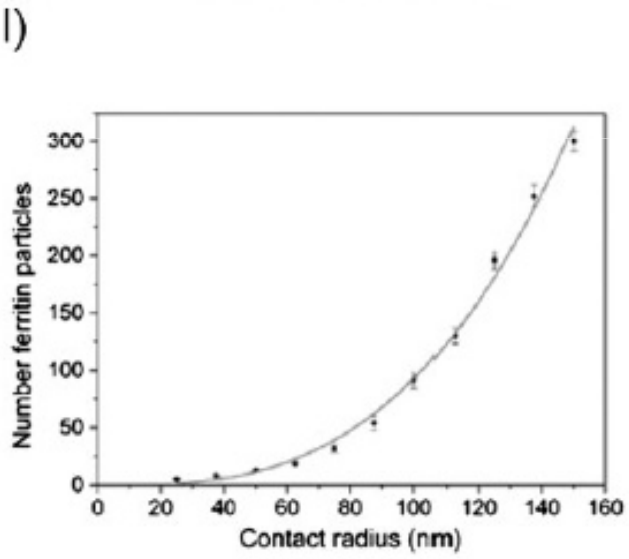
The controlled immobilization of biological entities on surfaces at the nanometer length scale is important in many areas of medical and biological research, opening up avenues for the functional and structural study of biomolecular interactions, the fabrication of nanobiosensors and biocompatible materials, and the development of denser combinatorial libraries for screening biological systems.[1–5] To date, a variety of scanning probe lithographies (SPLs), such as dip-pen nanolithography (DPN),[6,7] nanografting,[8,9] and local oxidation nanolithography,[10,11] have been used to immobilize biomolecules at this length scale. Among them, DPN has emerged as a particularly attractive tool since it allows the direct transfer of biomolecules onto surfaces with a high registration and resolution while preserving their biological activity. For example, functional proteins, antibodies, phospholipids, and DNA molecules have been successfully positioned on surfaces in the context of large arrays, to achieve dot-like features that have been miniaturized down to 50 nm in diameter.[12–21] With these direct-write capabilities, DPN provides the potential not only to fabricate more and more miniaturized and complex bioarrays but also to control the number of biomolecules immobilized at specific locations on surfaces. Once this can be done, researchers will start to envisage the fabrication of arrays in which the number of biological entities deposited on each feature will be controlled at the single-particle level. This capacity will certainly open up exciting opportunities, for model system because of its size and central inorganic core of hydrated iron(III) oxide,[22] which allows its visualization by TEM, and therefore, its individual identification on the surface of the TEM grids.[23] The data show that this is a versatile way to quantify the number of ferritin particles written by DPN, and that this number can be controlled by adjusting the protein concentration used to coat the atomic force microscopy (AFM) tips and the dimensions of the dot-like features fabricated by DPN.



Maspoch et al. *Adv Mater. (2010)*, 352-355

Controlling the number of proteins deposited

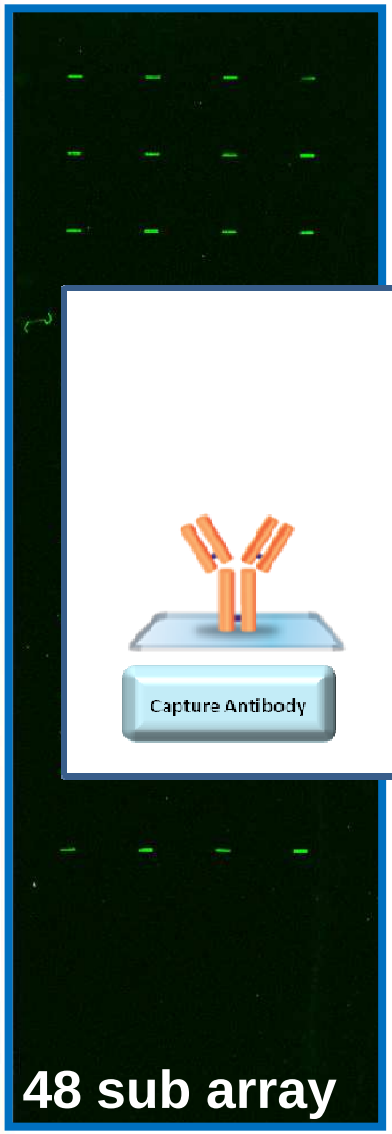


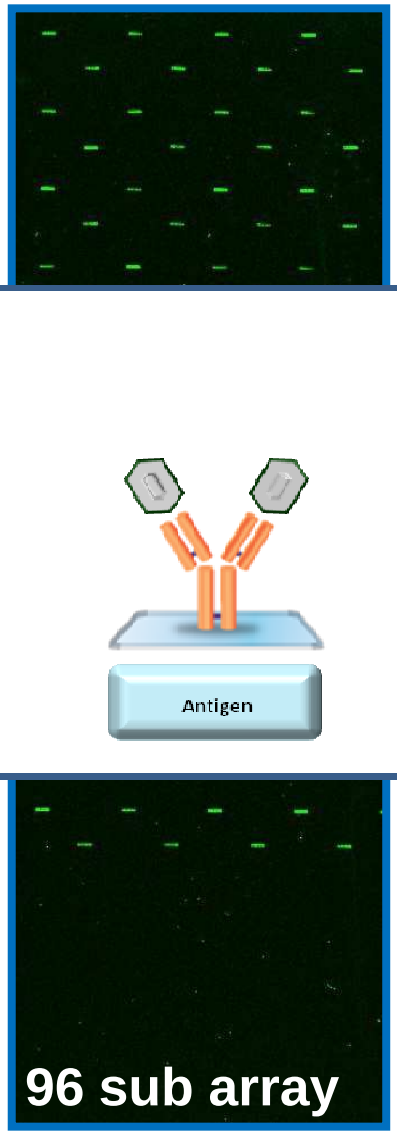


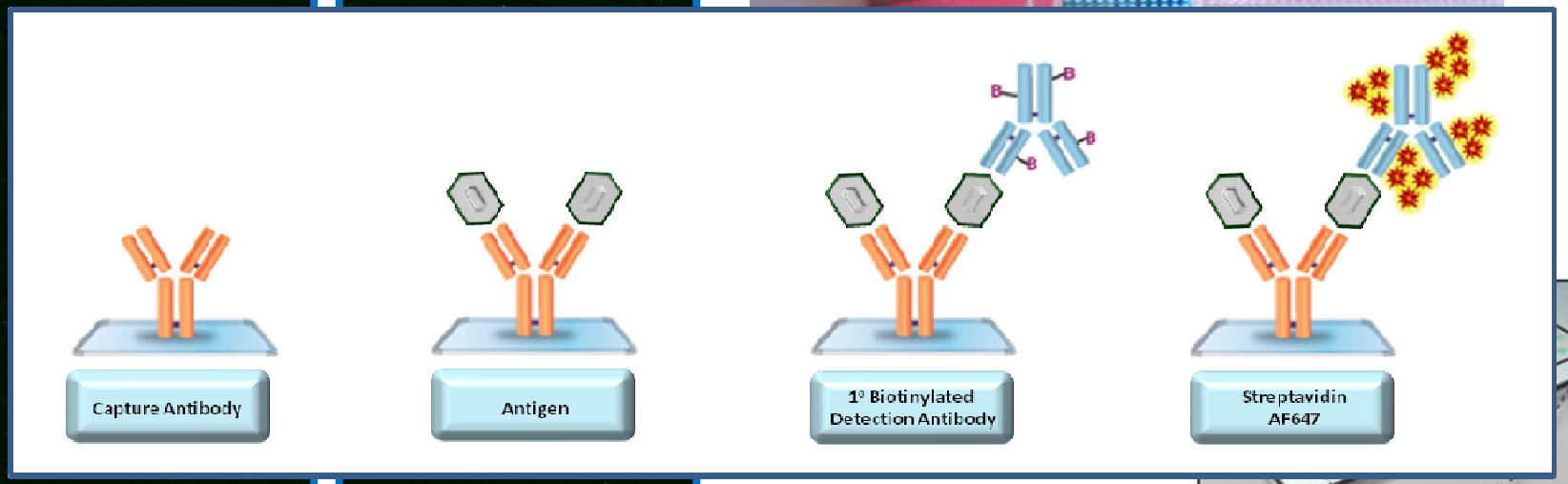
55 g.L $^{-1}$

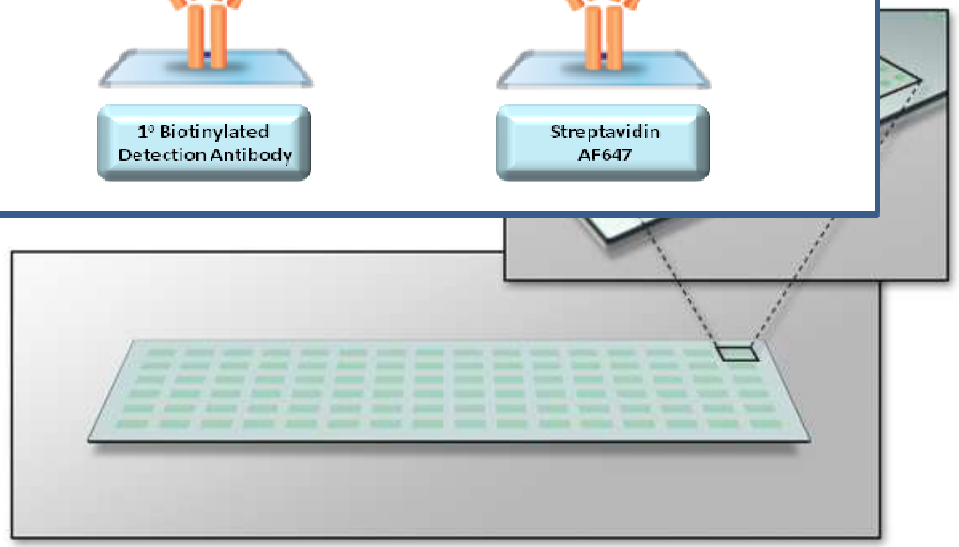
Bellido et al. *Adv Mater.* (2010), 352-355

NanoArrays for diagnostics (ELISA type)







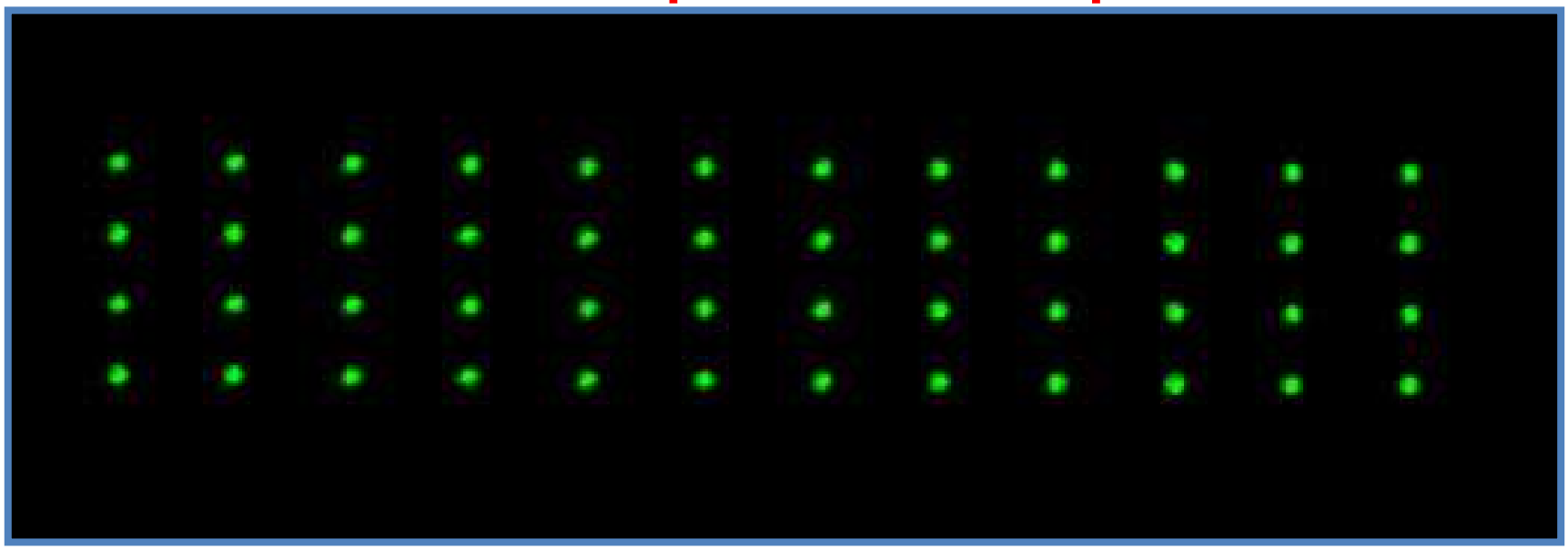


Nanoink test format

nanoINK INC.
Nano BioDiscovery

Sample volumes….

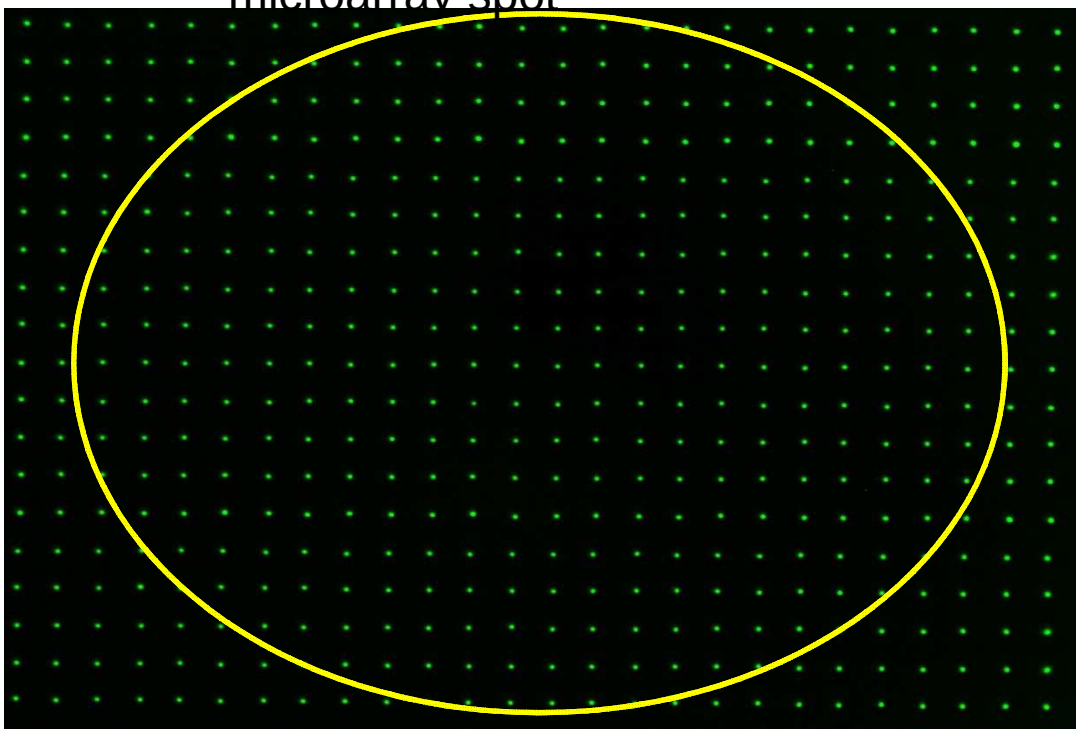
Low Sample Volume 1-2 µL



Close-up view of one of 96 sub-arrays

Each sub-array can contain up to 48 capture proteins . Only 1 to 2 uL of sample is needed for each of the 96 sub-arrays. The entire assay can be performed in 6 hours.

Benefits of scale difference

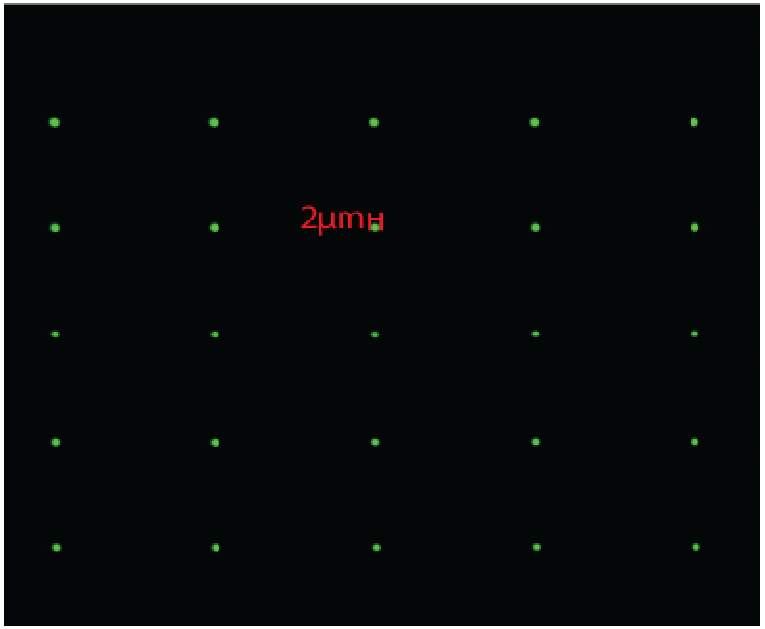


Approximately 250 nano-scale features (6.6µm apart) within a conventional 100µm spot

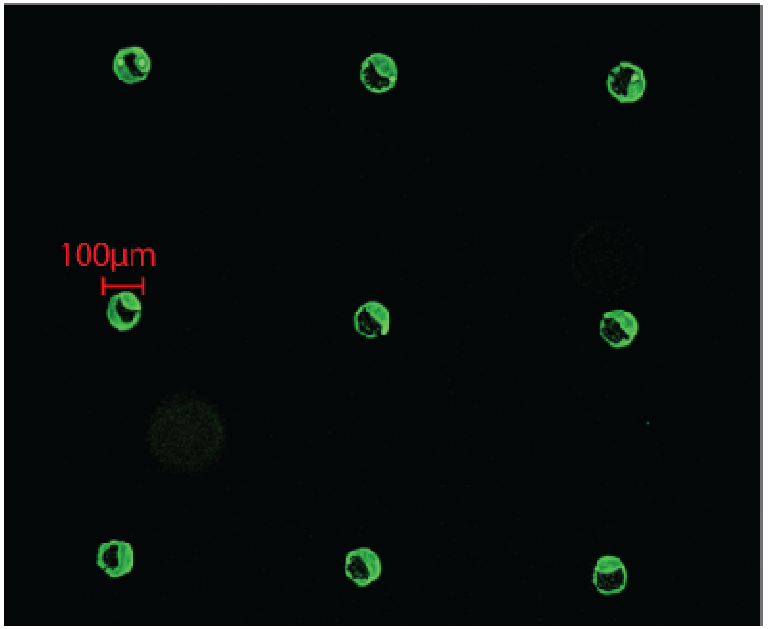
- Highly reproducible features
- Improved spot morphology
- Less sample requirements
- Low non-specific binding
- Faster reaction kinetics
- Standard protocols
- Higher Sensitivity

Microarrays v Nanoarrays:

High Resolution nano-scale immunoarray printed with NanoInk's NLP 2000 (50X)

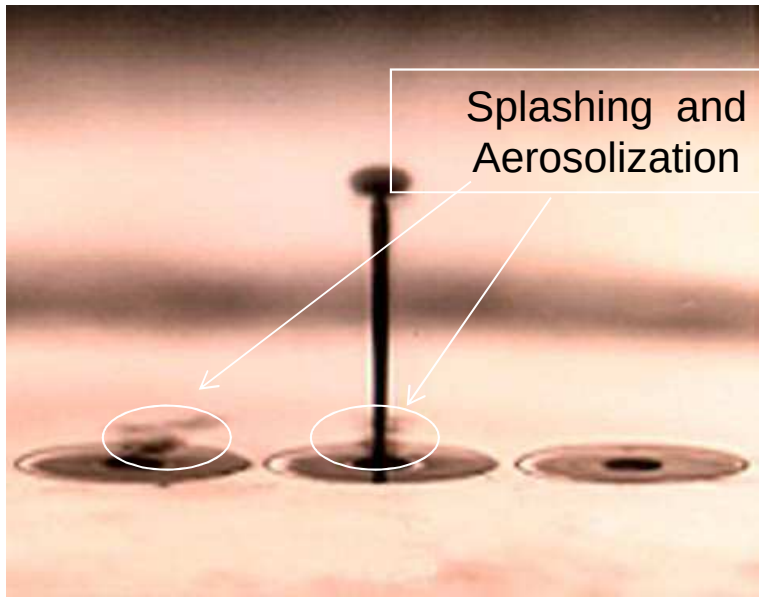


Microscale immunoarray printed with conventional contact printer (5X)

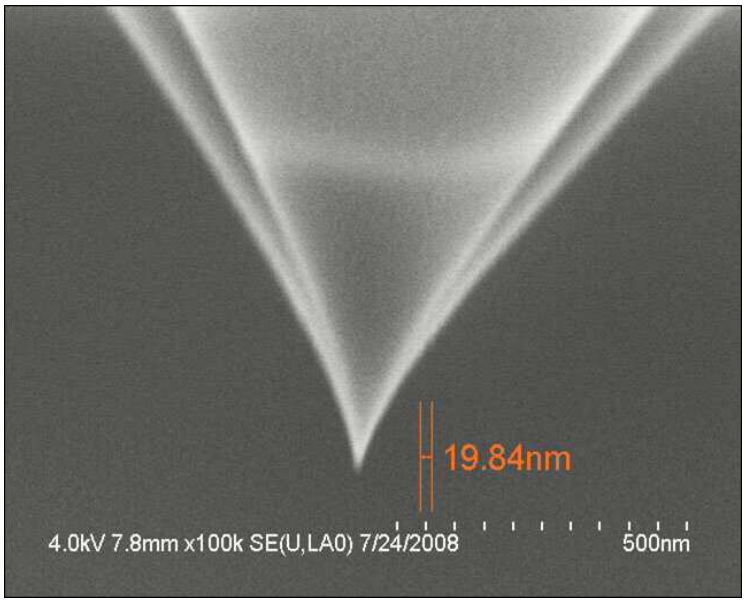


Nanoscale array versus conventional microarray

Materials handling: Micro v Nanoarrays…



Force (Inkjetting)

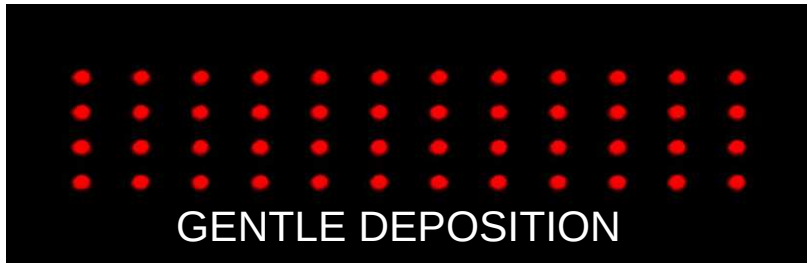


Gentle Deposition (DPN)

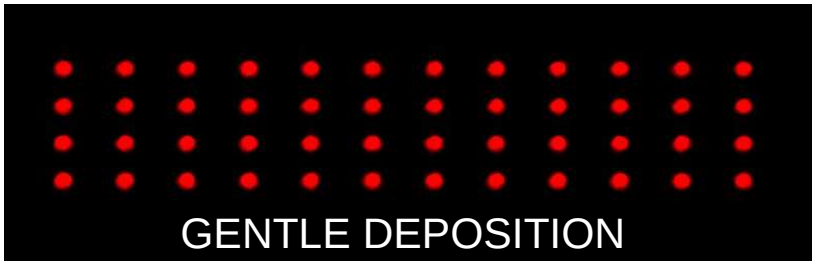
NanoArrays maintain conformational integrity for most biological structures

Microarrays v Nanoarrays: effect of force

- NANO ARRAYS 5 µm Features (10 mg/ml) – Volumes are in Femto/Atto Litre



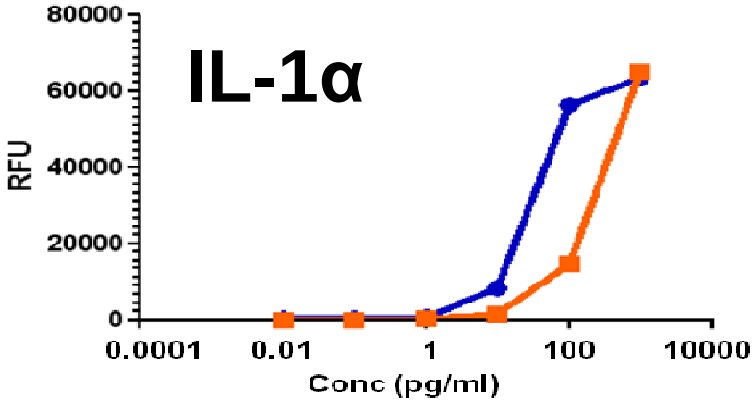
Intra Array CV <5%

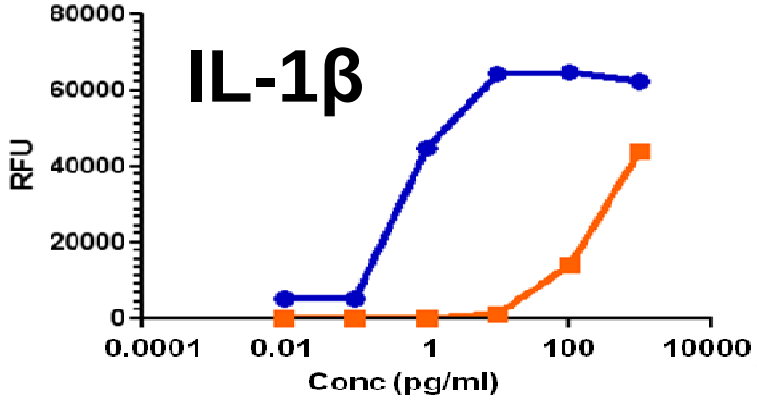


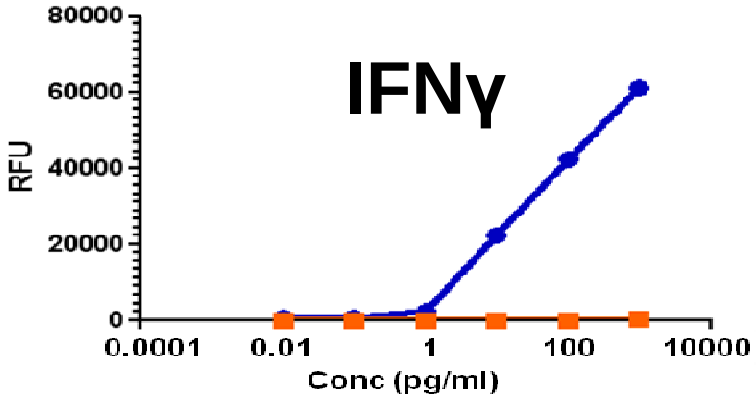
Inter Array CV% <8%

Microarrays v Nanoarrays: SENSITIVITY

Comparison of Nanoarrays ( ) to Microarrays ( )



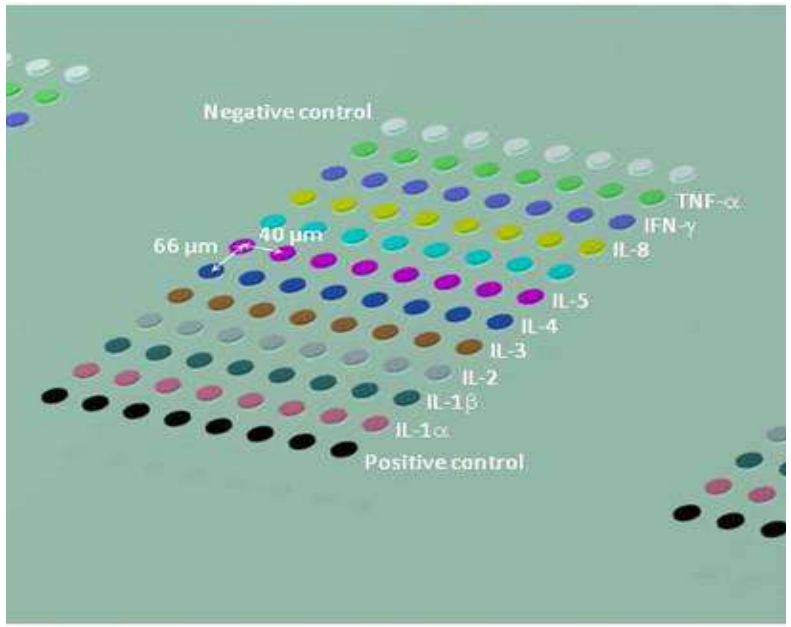




NanoArray Vs Micro Array – Piezo-controlled Ink Jet (vendor)

Cytokine kit: SENSITIVITY AND MULTIPLEX

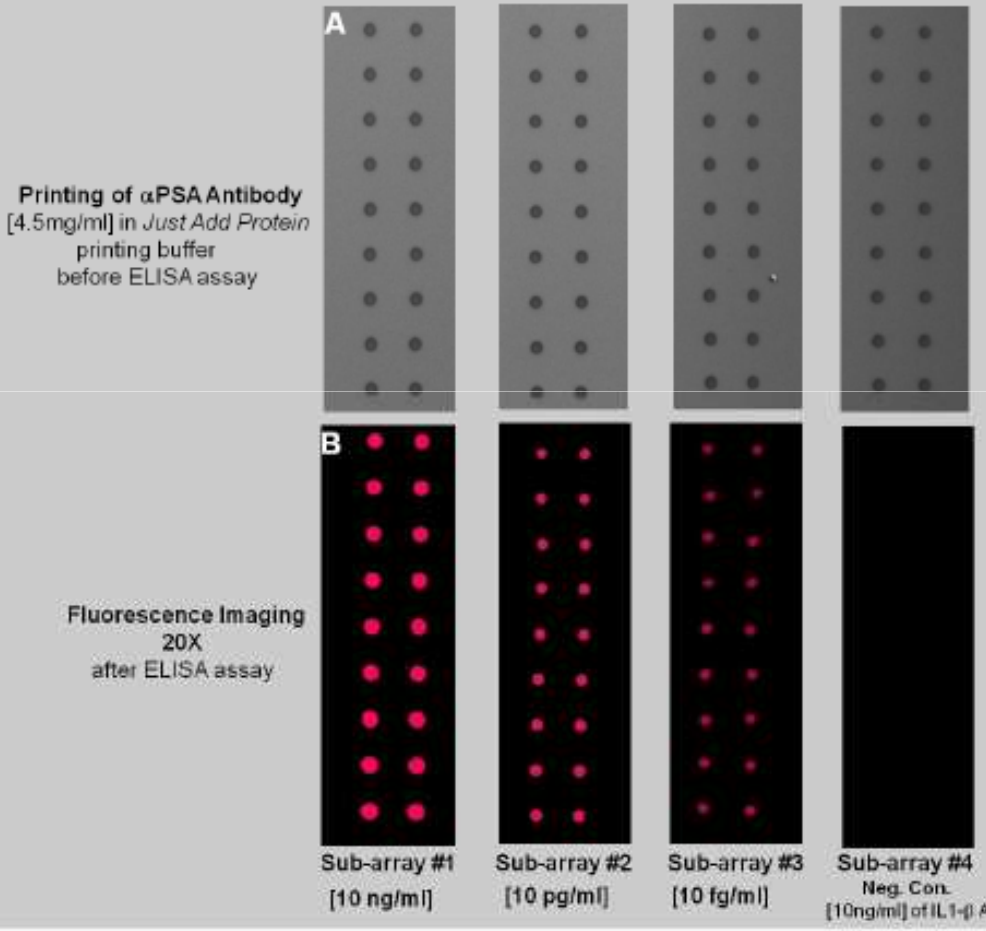
Cytokine	NanoInk Assay LLOQ (pg/ml)	Luminex Assay LLOQ (pg/ml)
IL 1 β	0.20	11
IL 2	1.85	10
IL 4	0.79	2
IL 5	0.30	1
IL 6	0.38	5
IL 8	0.47	4.1
TNF α	1.23	3.6
IFN γ	4.21	6



•Versus bead based- assay (University of Illinois)

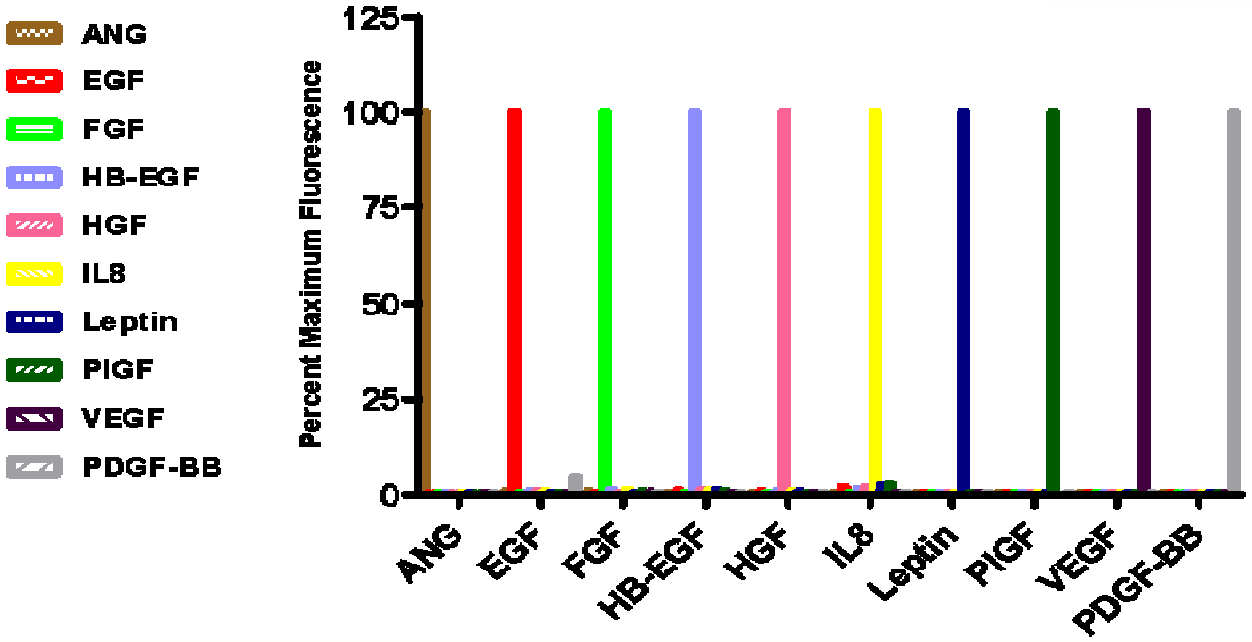
PSA detection- SENSITIVITY

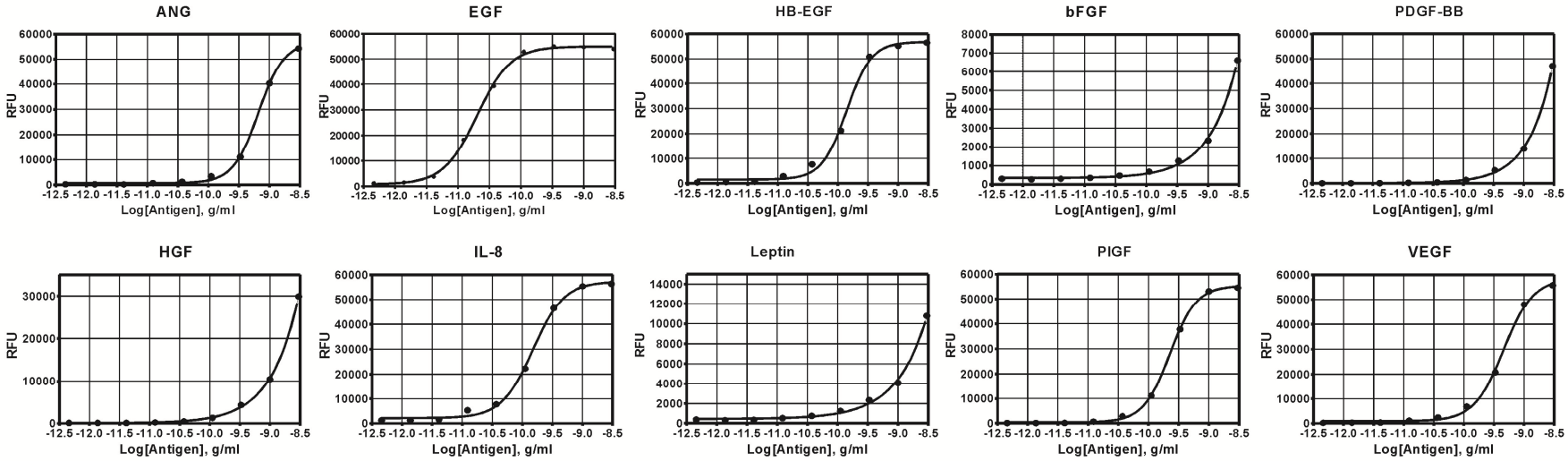
High sensitivity detection of recombinant prostate specific antigen (PSA)



Arrays scanned using high resolution microarray scanner (resolution ≤ 0.5 µm)

Angiogensis kit: SPECIFICITY



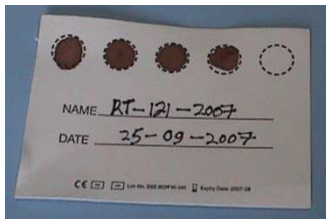


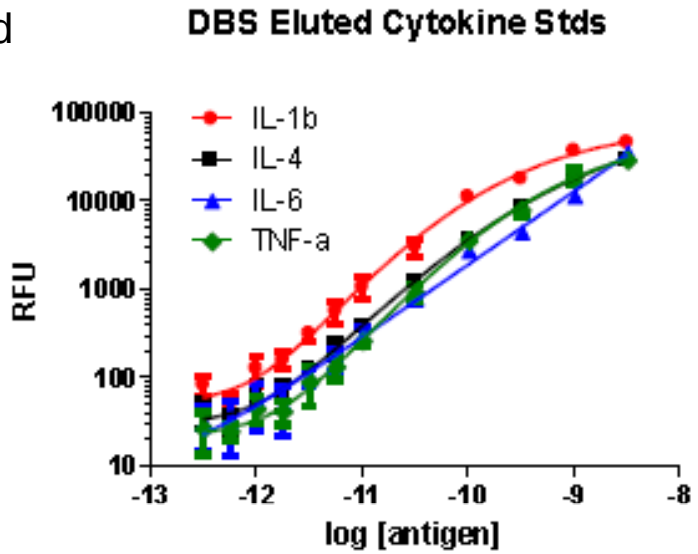
Dried blood spots: DIFFICULT SAMPLE

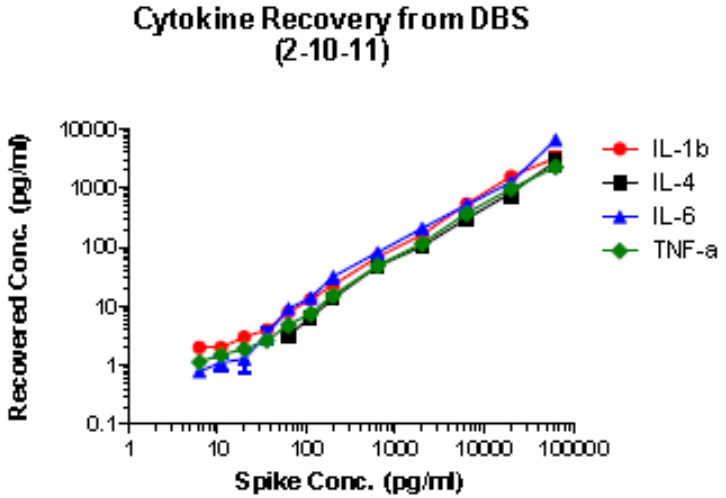
- DBS collects only 50 µL per sample compared to 10 mL for large blood draws (3 mm punch of blood which must be eluted for analysis).

- DBS can also be used for patient populations where sample size is problematic such as neonates, tissue lysates.

- The recoveries and CVs are difficult because of small sample size.



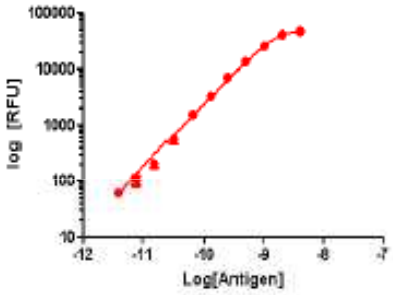


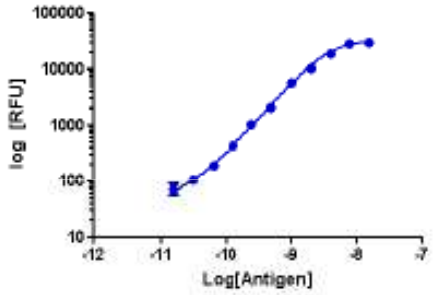


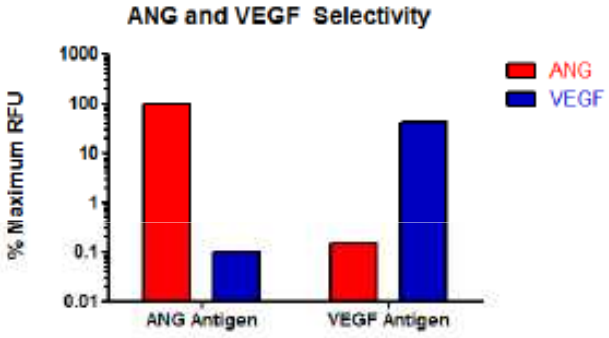
Xenograft mouse tumor lysates: LOW VOL

•A major limitation of screening for tumor lysates is the limited quantity of sample available.

• Each sample well required only 4 µL of sample and provided 48 data points per well. LLOQ of 12.03 pg/mL for VEGF and 3.02 pg/mL for Angiogenin.







ACCURACY					PRECISION				
ANG					ANG				
Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec	Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec
750	877	48	5	117	750	838	67	8	112
375	374	11	3	100	375	367	15	4	98
188	204	17	8	109	188	204	11	5	109
23	25	1	4	106	23	24	2	8	104
VEGF					VEGF				
Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec	Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec
750	771	38	5	103	750	697	26	4	93
375	363	23	6	97	375	316	8	3	84
188	202	17	8	108	188	179	6	3	95
23	25	3	12	108	23	25	4	16	108

LINEARITY					%RECOVERY				
ANG					ANG				
Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec	Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec
500	602	117	19	120	900	879	121	14	98
100	112	13	12	112	100	108	7	7	108
30	32	2	6	108	33	40	1	3	120
VEGF					VEGF				
Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec	Conc. (pg/mL)	Obs Conc. (pg/mL)*	SD*	%CV	%Rec
500	596	90	15	119	900	626	77	12	70
100	100	8	8	100	100	99	3	3	99
30	36	5	14	119	33	36	2	6	109

Kits developed by NanoInk

1. **10- Plex Human Inflammation Cytokine Kit -**
   - IL-1α, IL-1β, IL-2, IL-3, IL-4,IL-5,IL-6, IL-8, IFN-γ and TNF- α

2. **Angiogenesis Kit - Available**
   - bFGF, HB-EGF, EGF, PIGF, VEGF, HGF, IL-8, and TNF-α

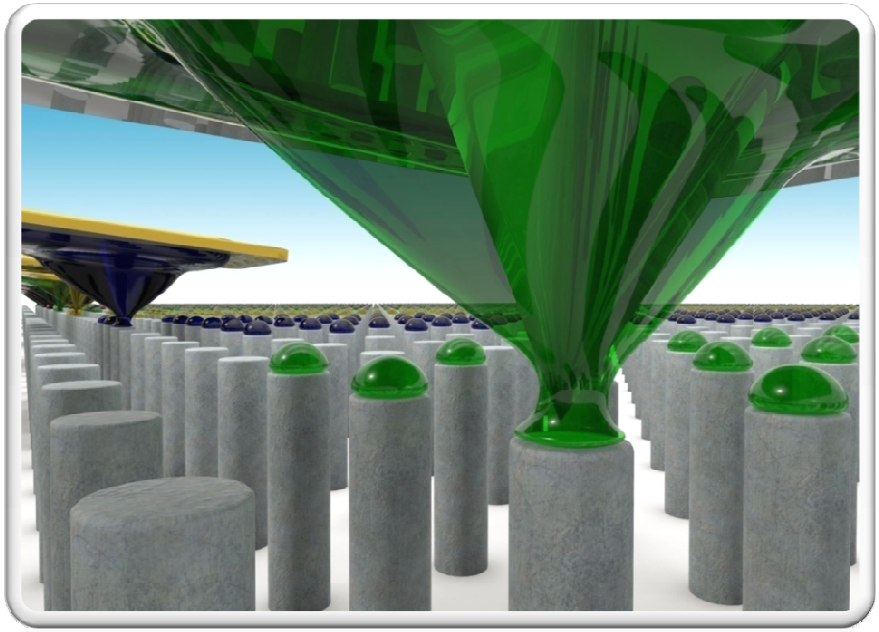
3. **Renal Toxicity Kit**
   - KIM-1, Albumin, β2-macroglobulin, Cystatin C, Clusterin and Trefoil Factor-3

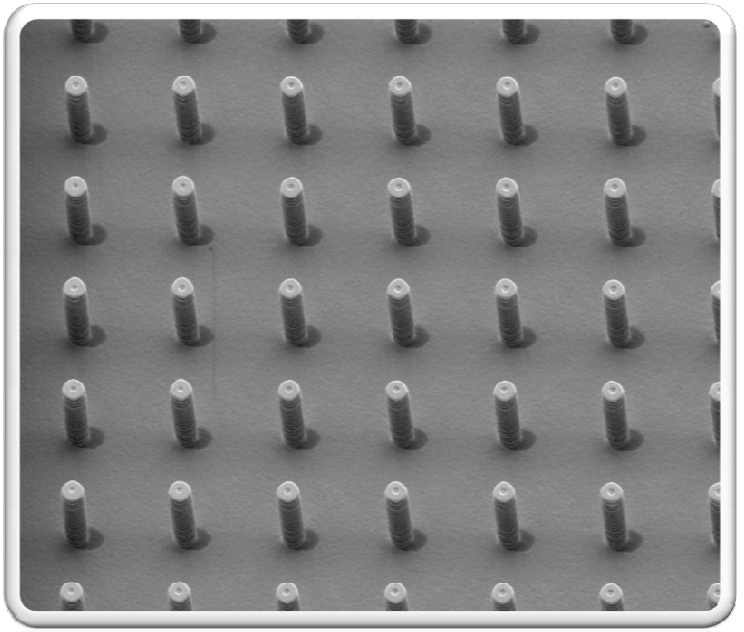
Validated tests developed by NanoInk

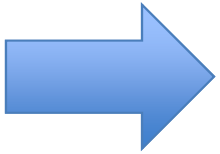
- Human Serum
- Human Tumor Needle Aspirates
- Breast Fluid Cancer Biomarkers (2.5µl of sample)
- Human Tumor Lysates from Mouse Xenograft models
- Mouse Serum
- Rodent Urine Toxicology Analysis (< 10 µl of sample)
- Dried Blood Spot Samples (1.7 µl of serum)
- Tissue Culture Media
- Tears samples (1- 2 µl of sample)

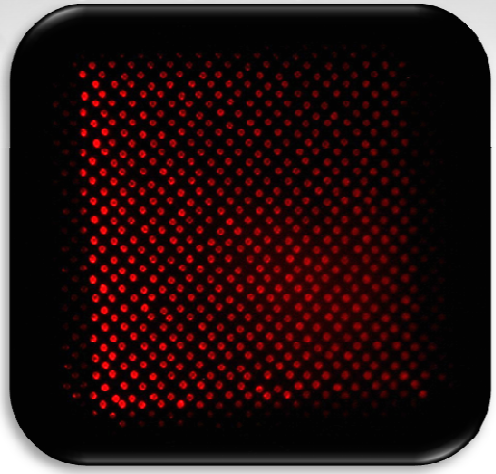
6) Deposition into existing chips/structures

SOFT STRUCTURES: Printing to pillars

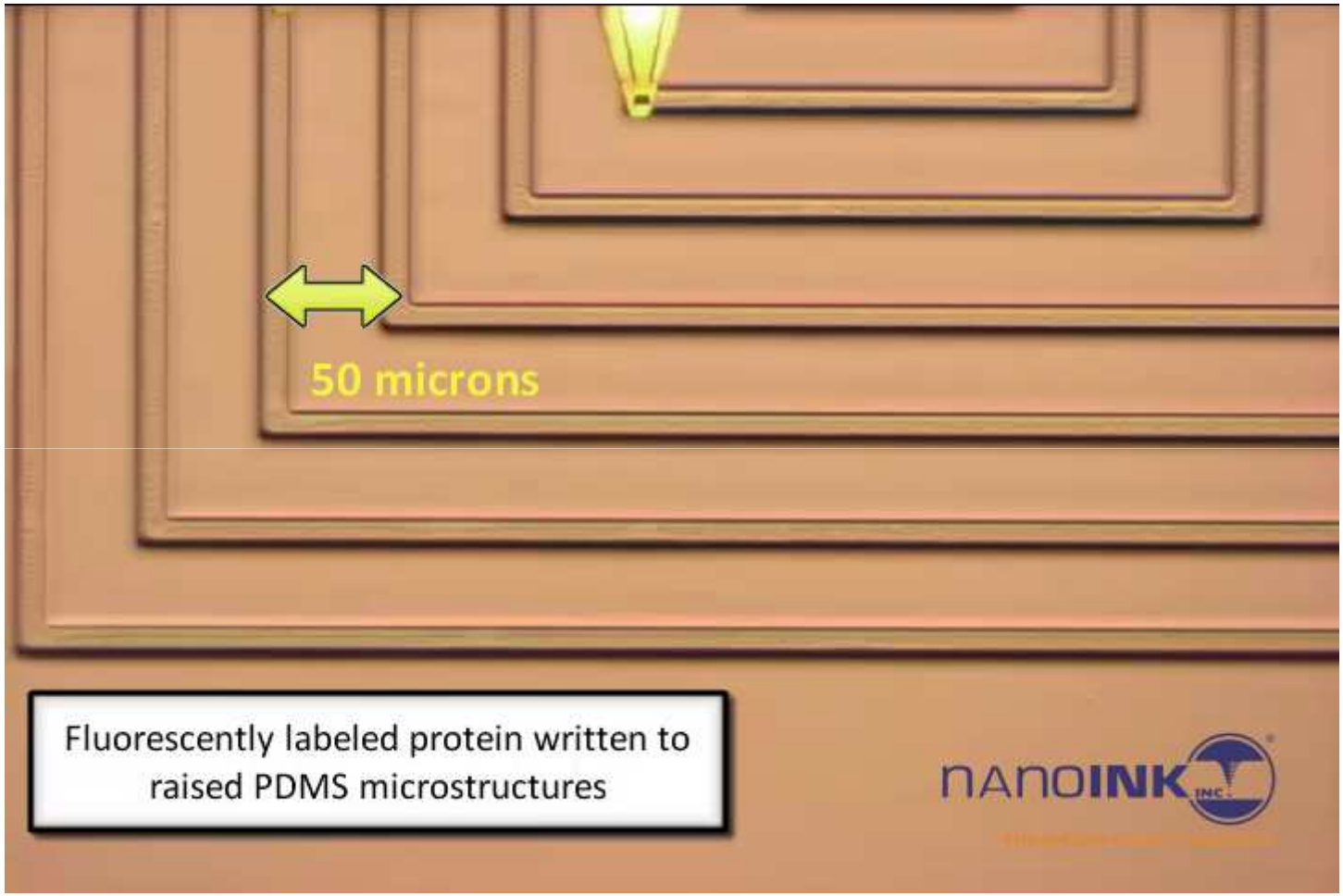






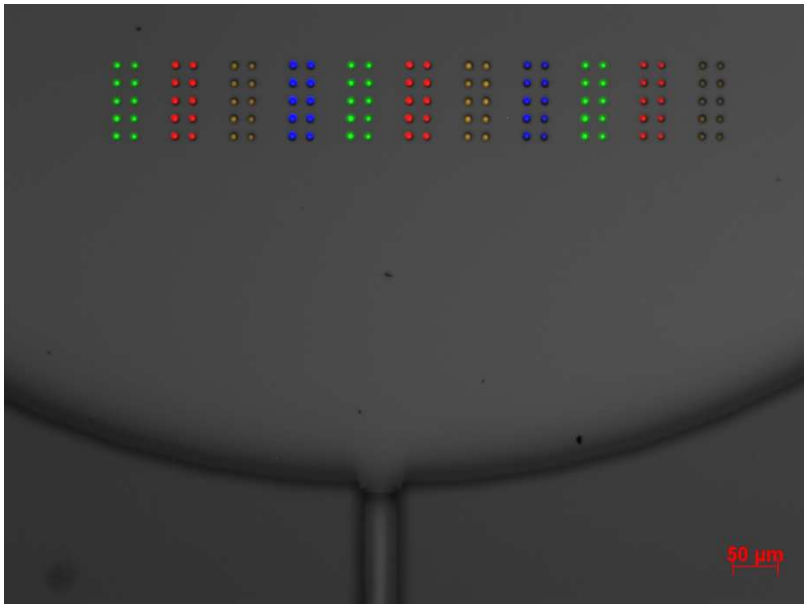


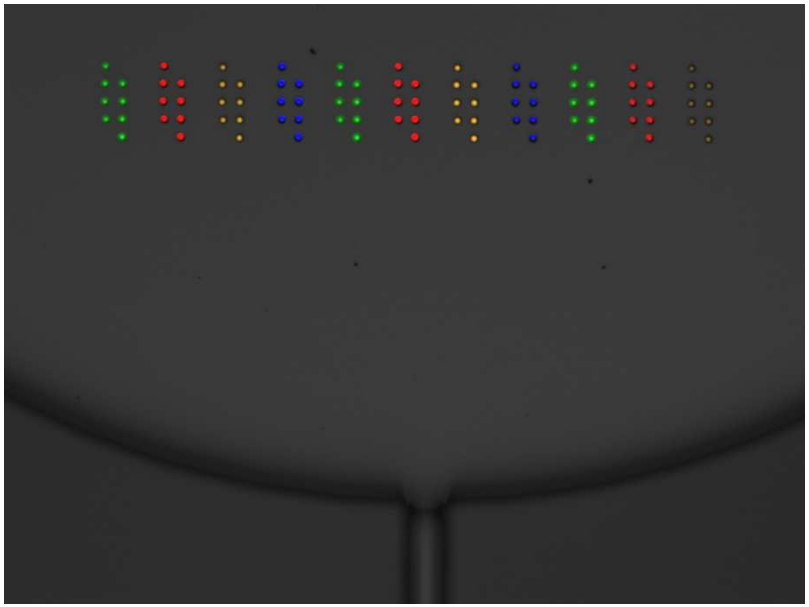
SOFT STRUCTURES: PDMS pattern



MICROFLUIDICS

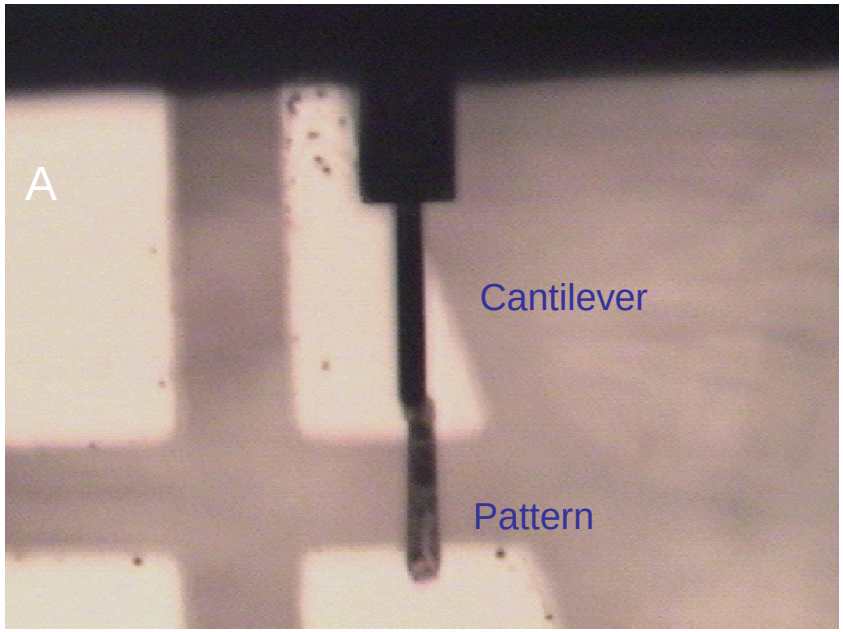
- Faster kinetics = more rapid time to result in assay.
- DPN allows more complexity to be printed into limited area.
- Allows analysis of very small volumes = greater sensitivity.



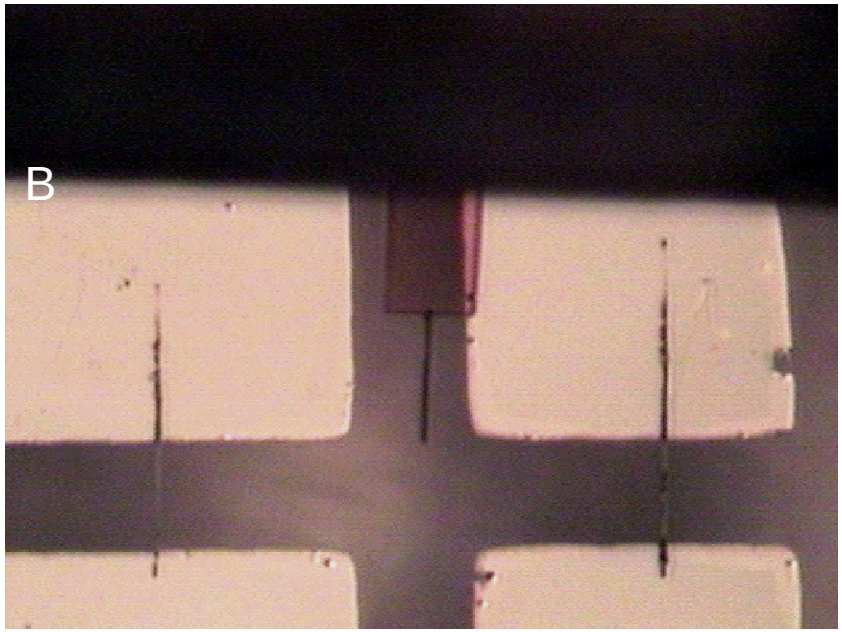


ELECTRONICS

Writing with 10 micron cantilever



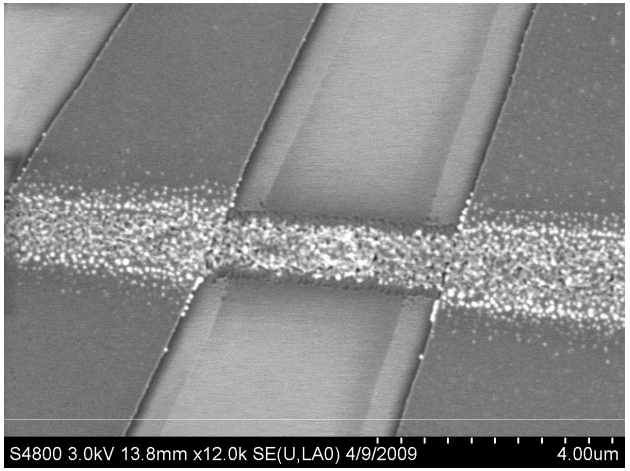
Writing with 2 micron cantilever

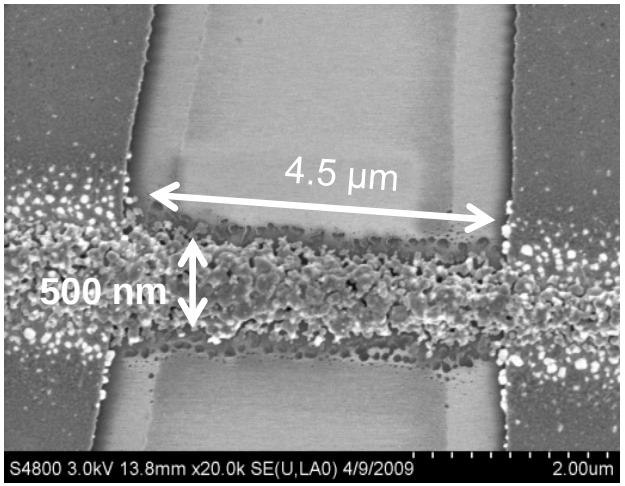


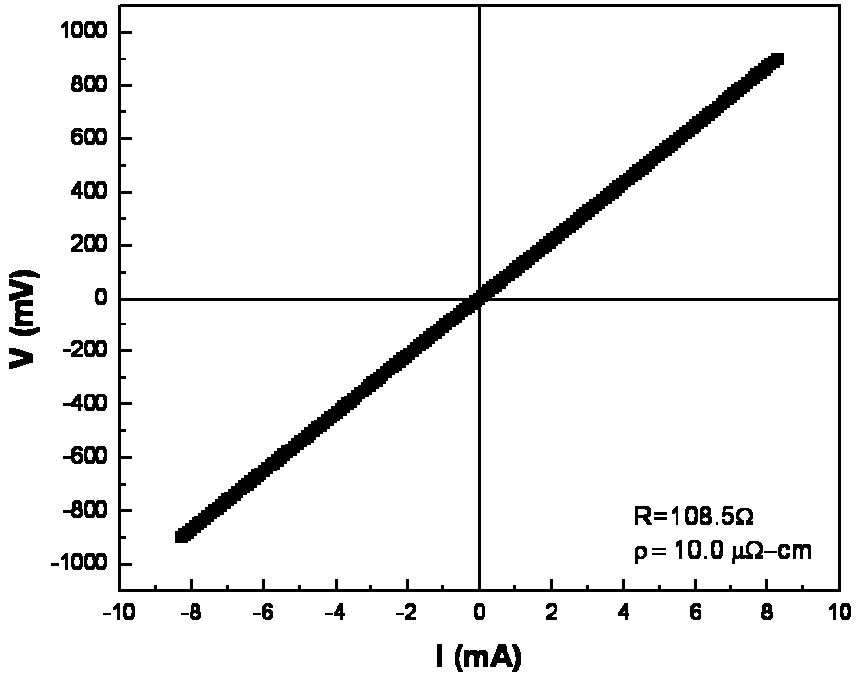
General method:

- Pattern metal salt sol-gel (e.g. Au/Pt)
- Heat (200-300˚C) or reduce in situ using borane methyl sulfide.

ELECTRONICS:
Accurate deposition of conductive traces

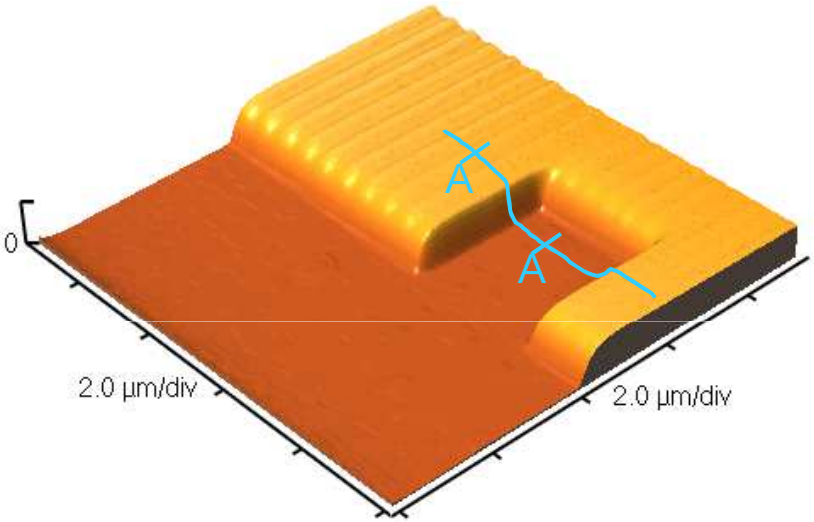


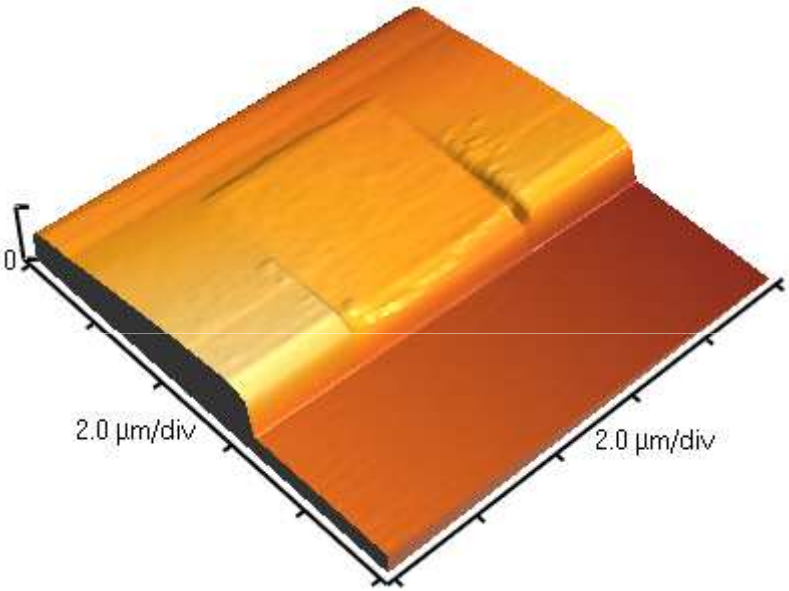


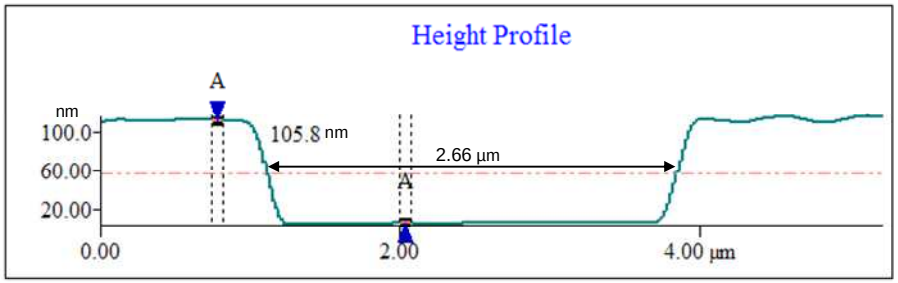


Photomask repair

3D AFM Topographic Image of a Defect Before and After Repair

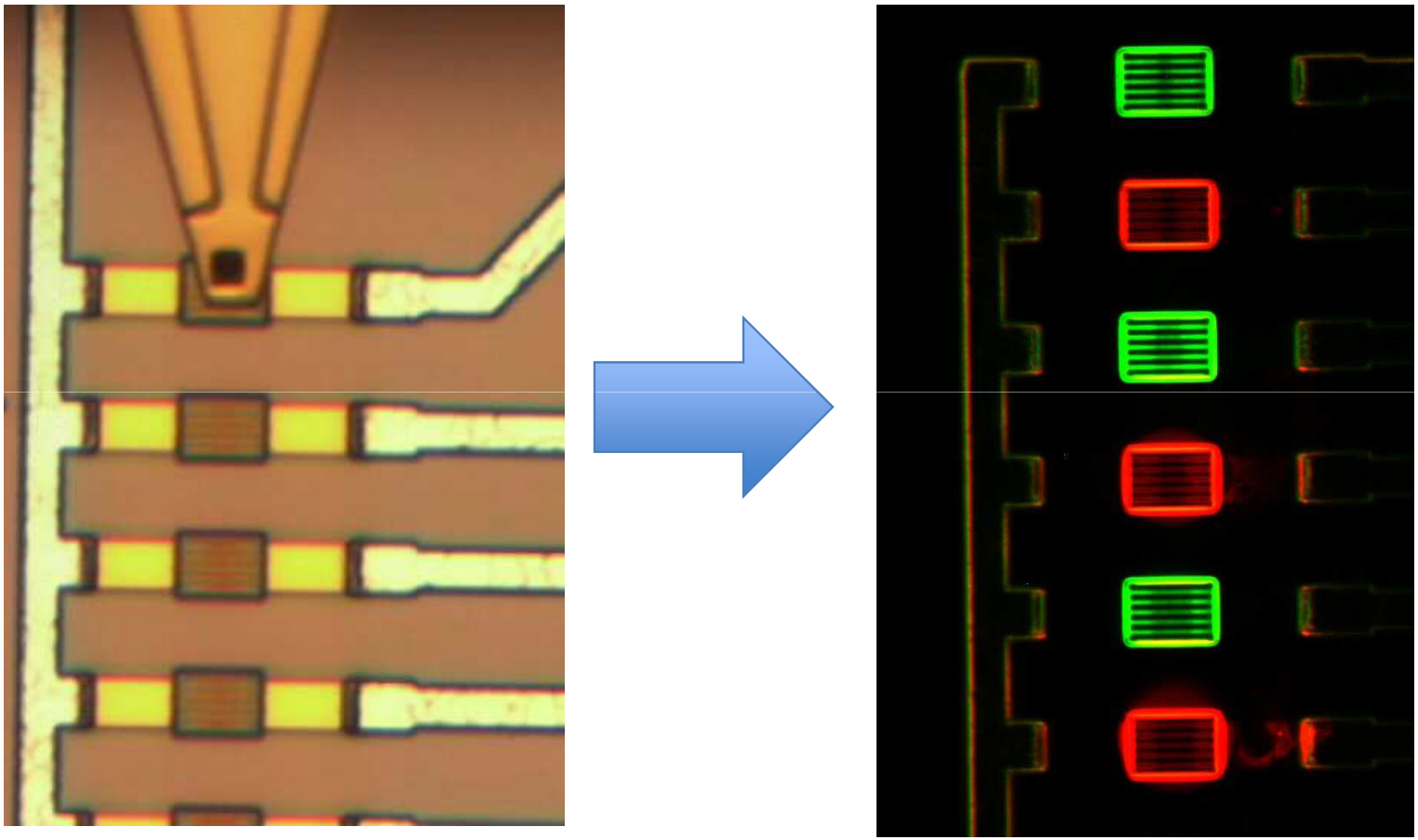




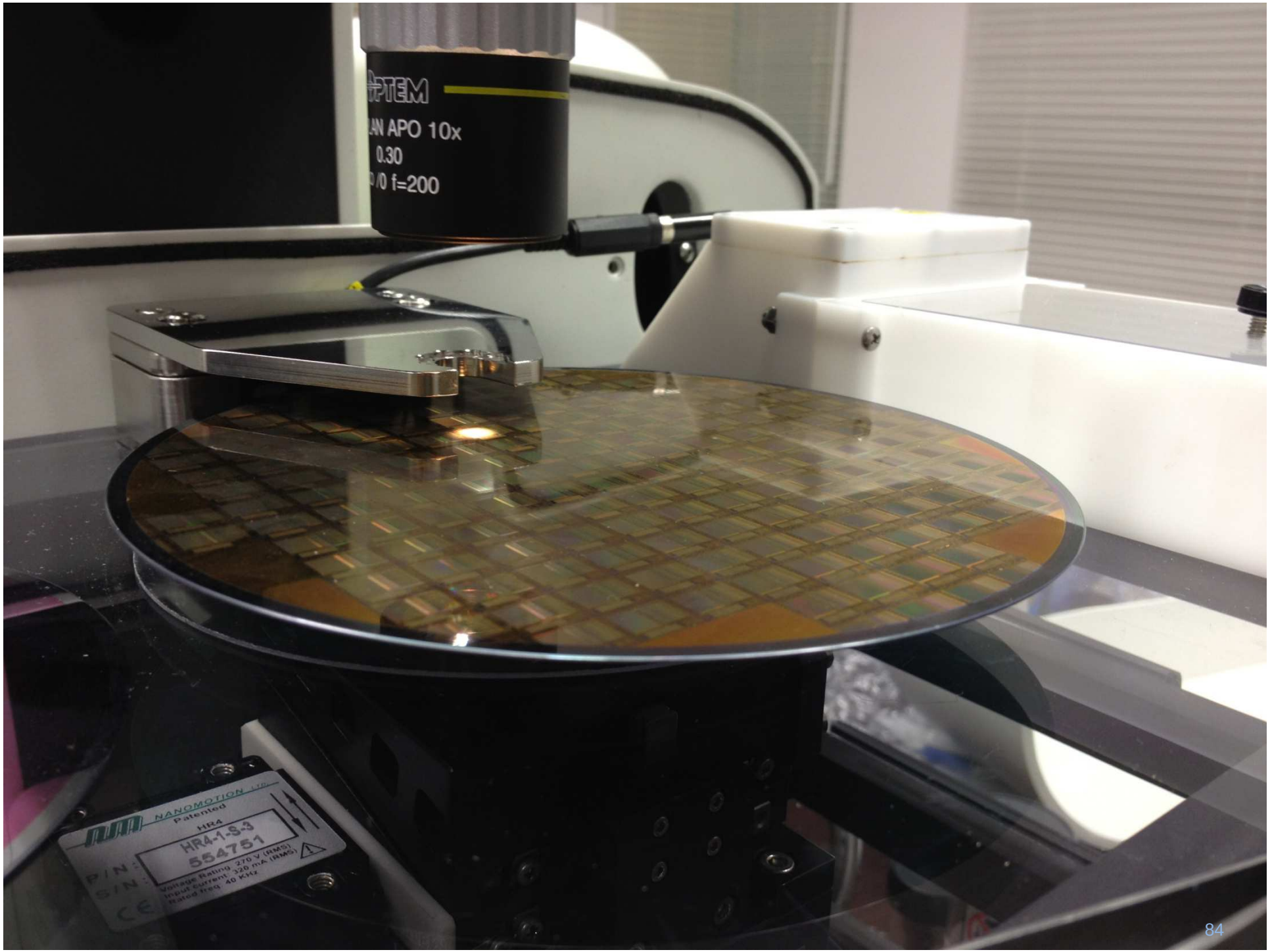


- Precise alignment to very small features.
- Single ink.

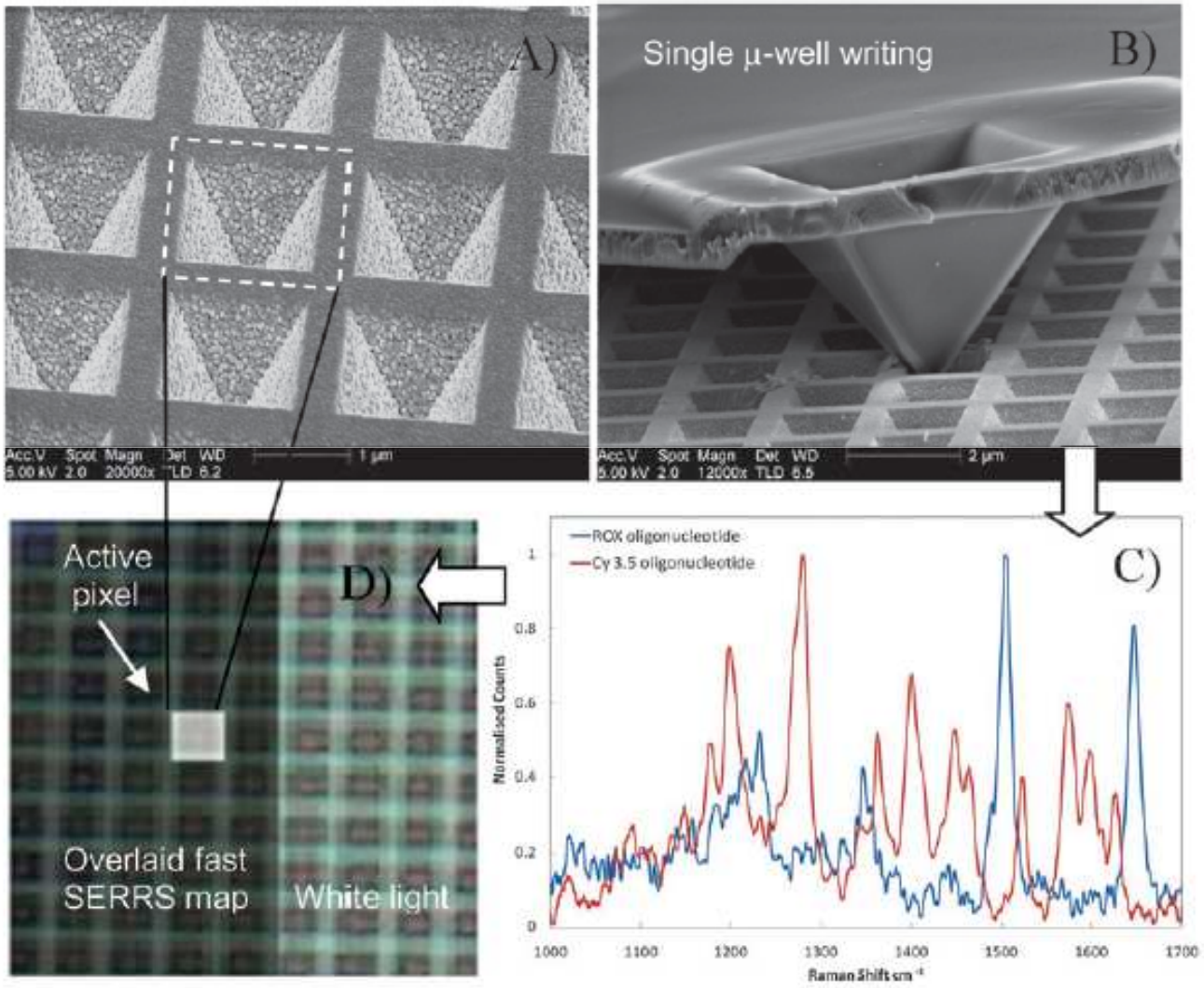
Direct deposition into nanowire biosensor chip



- Direct patterning of Ab to nanowire sensor device.

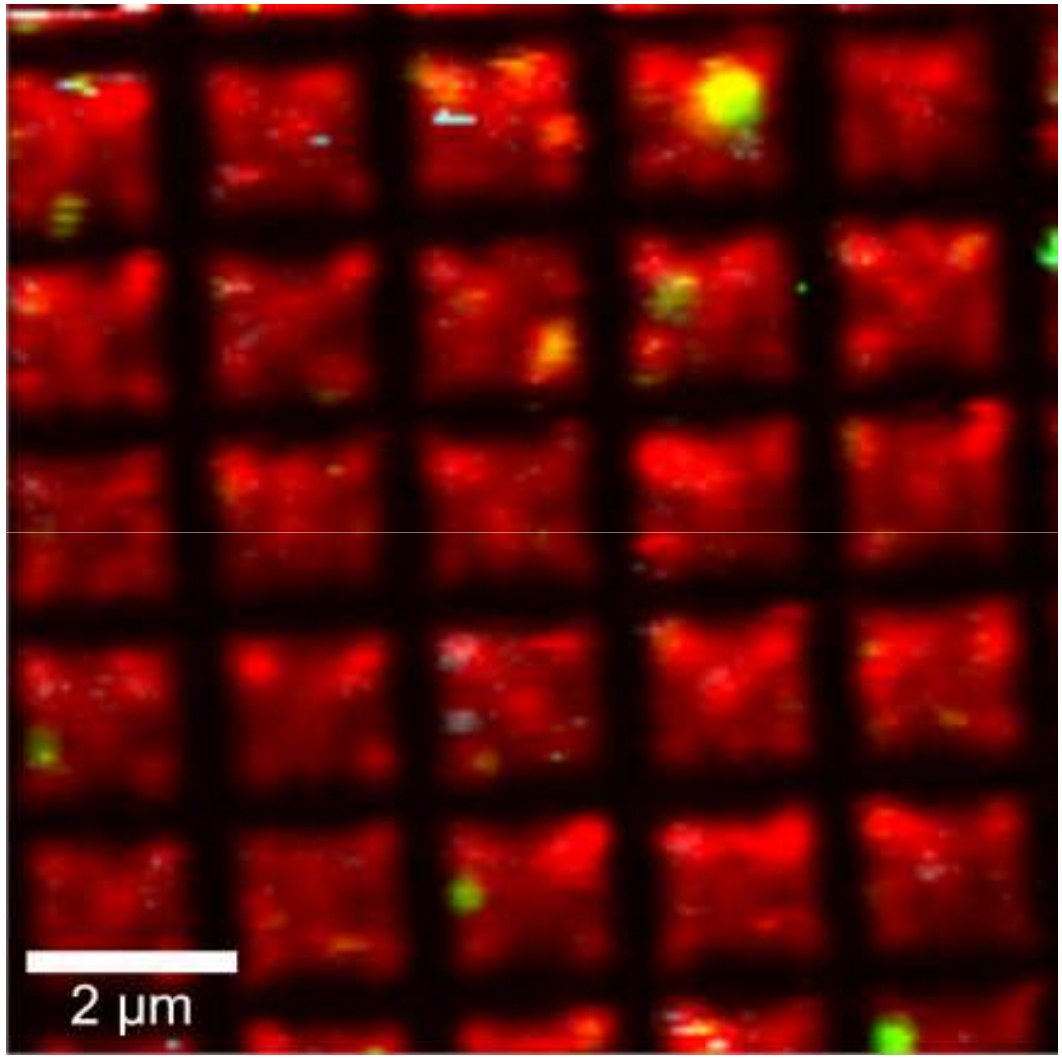

APTEM
AN APO 10x
0.30
0 f=200
NANOMOTION
Patented
HR4
HR4-1-S-3
554751
Voltage Rating: 270 V (RMS)
Input current: 320 mA (RMS)
Rated freq: 40 KHz

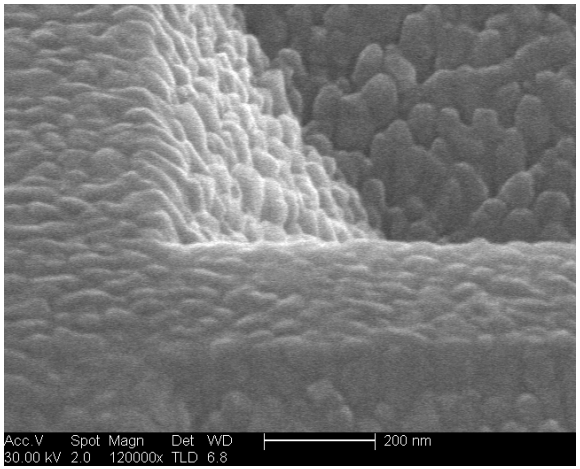
DPN used to functionalise a plasmonic microstructure

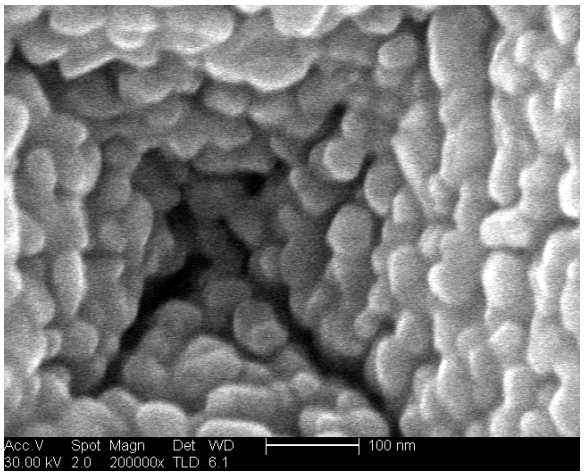


Stokes et al. *Chem. Commun.* (2008), 5734-5736

DPN used to functionalise a plasmonic microstructure







7) Patterns for cell biology

Controlling Cell Behavior with Nanopatterns

J|A|C|S COMMUNICATIONS

Published on Web 02/22/2008

Asymmetric Peptide Nanoarray Surfaces for Studies of Single Cell Polarization

Diana K. Hoover, Eugene W. L. Chan, and Muhammad N. Yousaf*

Department of Chemistry and the Carolina Center for Genome Science, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3290

Received December 11, 2007; E-mail: mnyousaf@email.unc.edu

Cell polarity is the ability to establish spatial, temporal, and functional asymmetry throughout the cell in response to cues from the local environment. This complex phenomenon plays a critical role in determining directed cell migration, ultimately impacting a wide range of biological processes including embryonic development, tissue repair, and the immune response.[1]

In particular, cell polarity derived from cell adhesion through cell−cell and cell−extracellular matrix (ECM) interactions regulates many cell and tissue functions. Polarity within a cell can be generated through cell−cell interactions mediated by cell surface cadherins, which induce protein asymmetry at the intersecting membranes. Cell adhesion to ECM may also cause cell polarity by the nonuniform distribution of interactions between cell surface integrin receptors and ligands on the ECM.[2] These sites of external contact then recruit cytoskeletal and signaling proteins that in turn serve as a scaffold to recruit additional signaling proteins to generate an anisotropic subcellular nanoarchitecture that estabilishes cell polarity.[3]

In this report we develop a combined nanolithography, fluorescence microscopy of key organelles, and electroactive immobilization surface strategy to examine how the surface nanoadhesive environment directs the polarity of an adherent cell without the

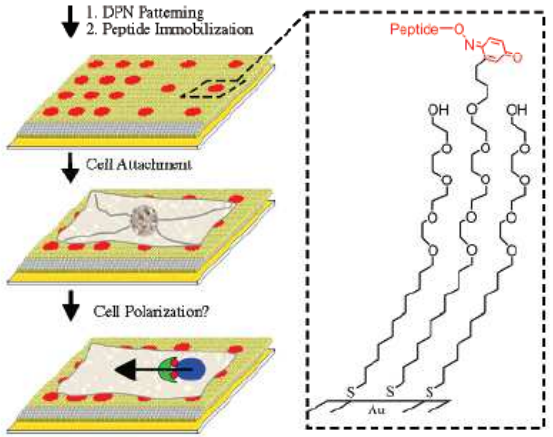
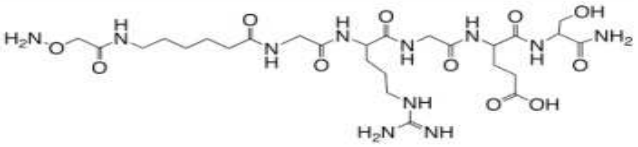


Figure 1. A schematic depiction of the production of asymmetric electroactive nanoarray patterns. DPN is used to pattern hydroquinone terminated alkanethiol in nanometer-sized spots. The remaining bare gold region is then backfilled with an inert tetra(ethylene glycol) terminated alkanethiol. Following electrochemical oxidation of the hydroquinone groups and chemoselective peptide immobilization, cells are seeded onto the surface. After a set period of time, the polarization of the cell is evaluated using fluorescent dyes targeting the nucleus (blue), centrosome (red), and golgi apparatus (green).

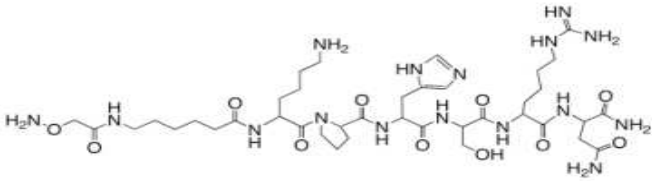
Hoover et. al. J. AM. CHEM. SOC. **2008**, 130, 3280-3281

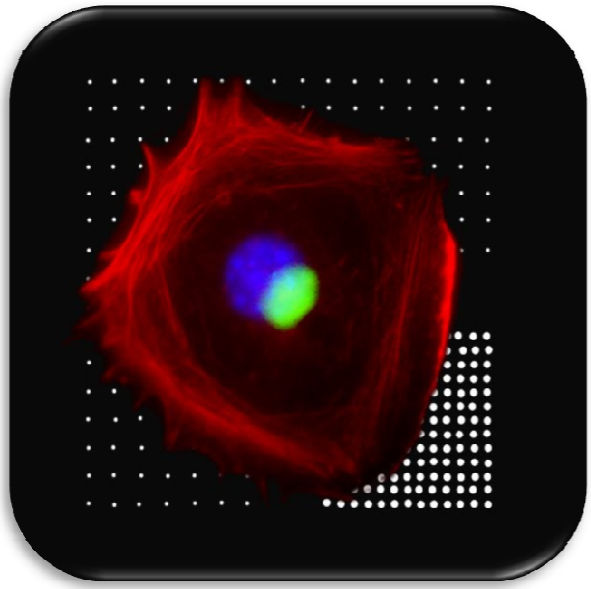
Creating RGD (peptide) patterns for cell polarisation:

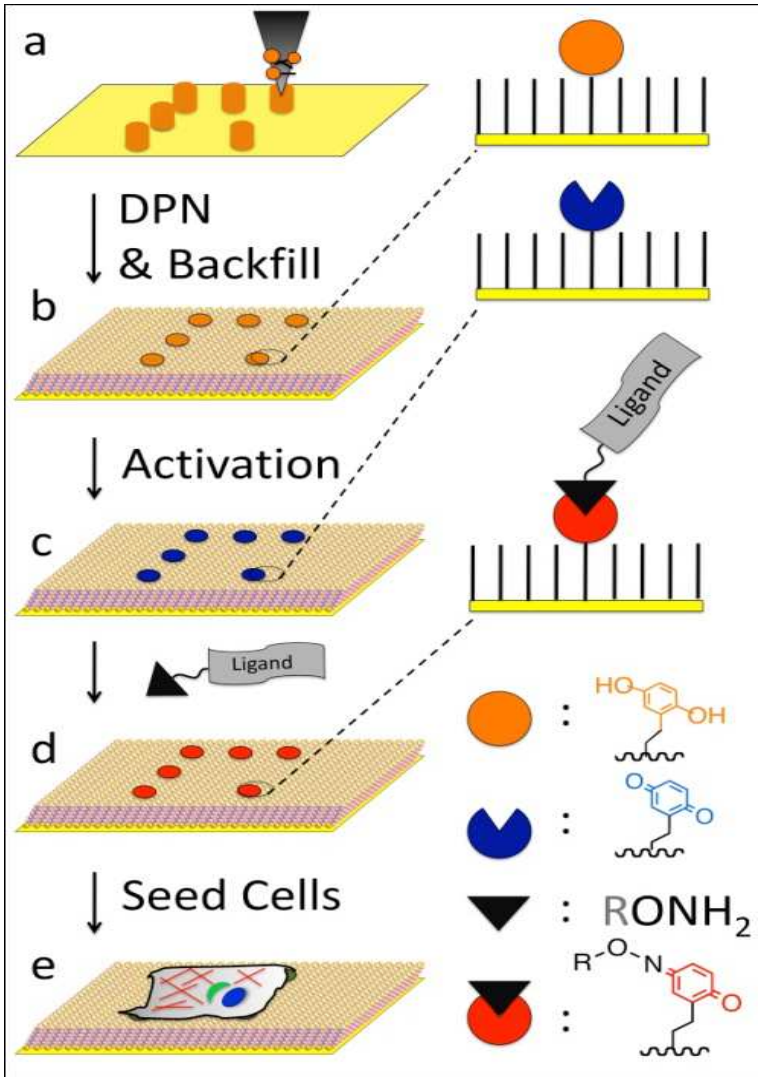
RGD:



PHSRN:

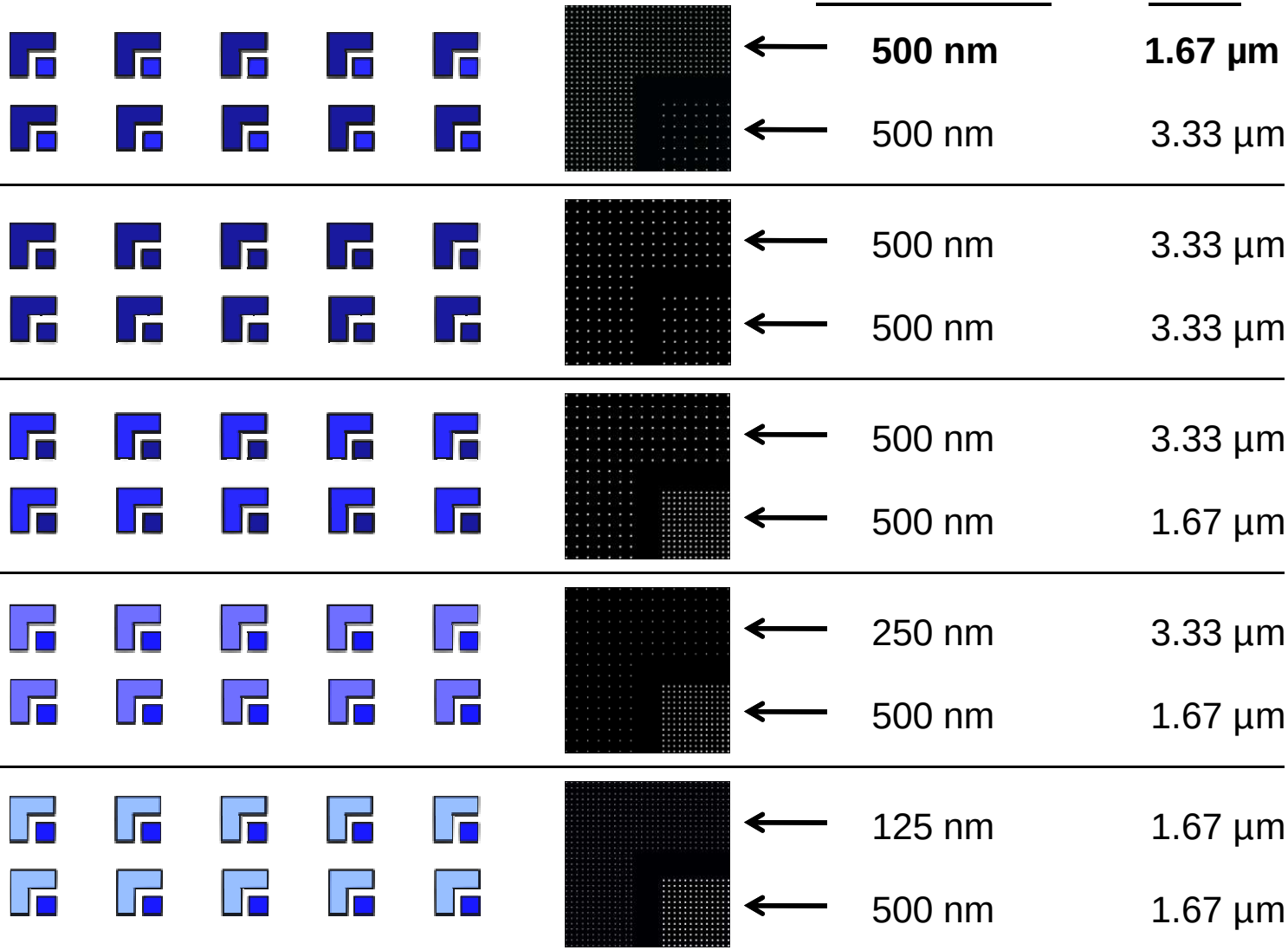






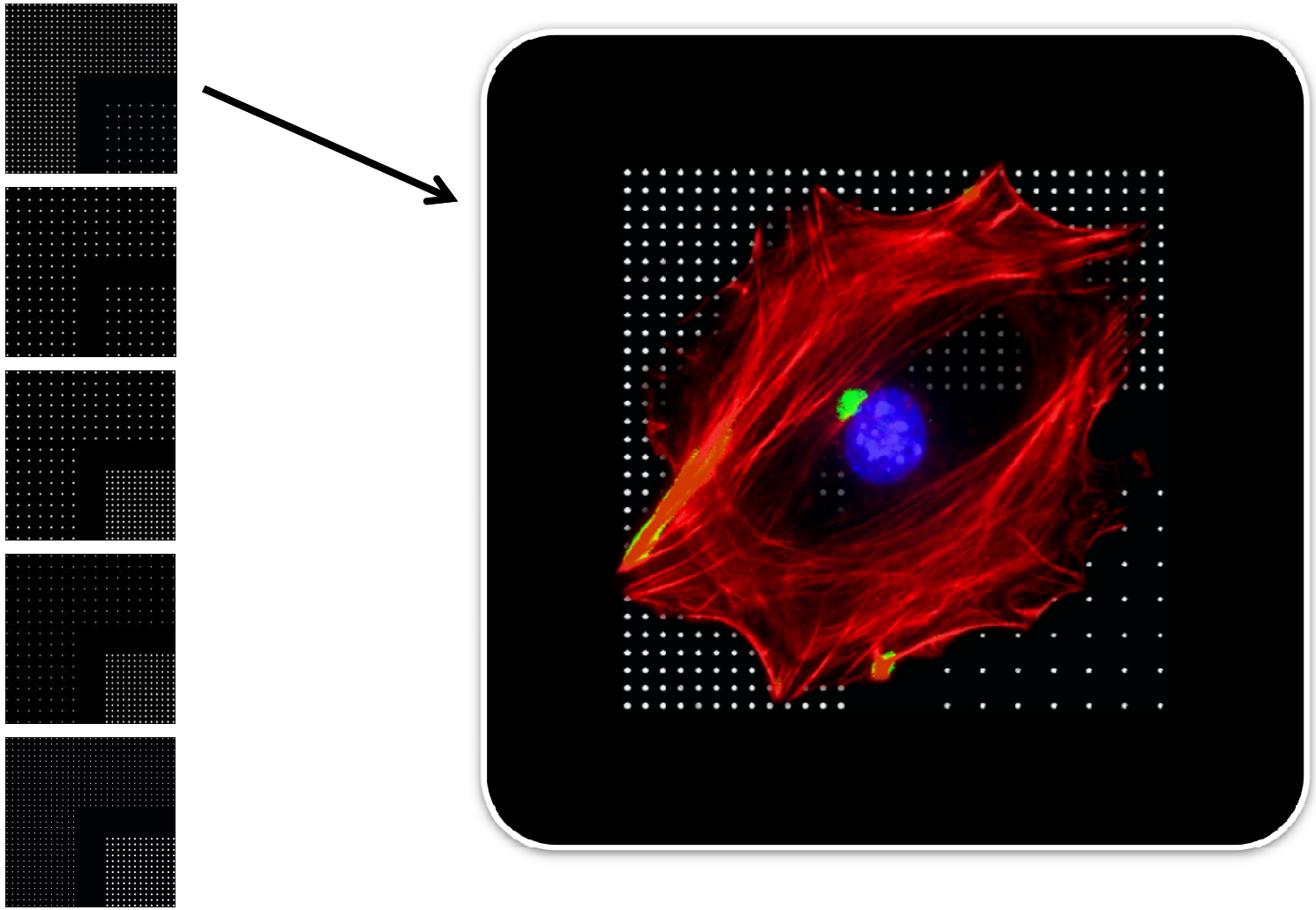
Hoover et. al. J. AM. CHEM. SOC. **2008**, 130, 3280-3281

Pattern Resolution and Pitch

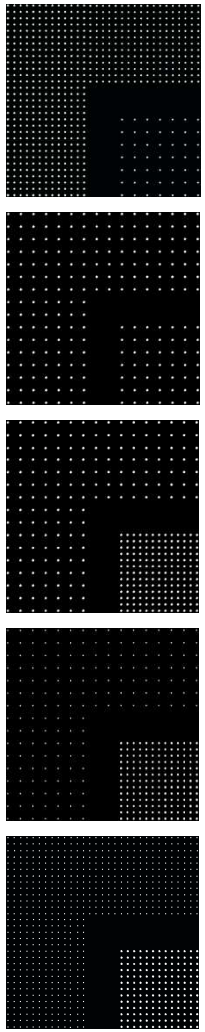


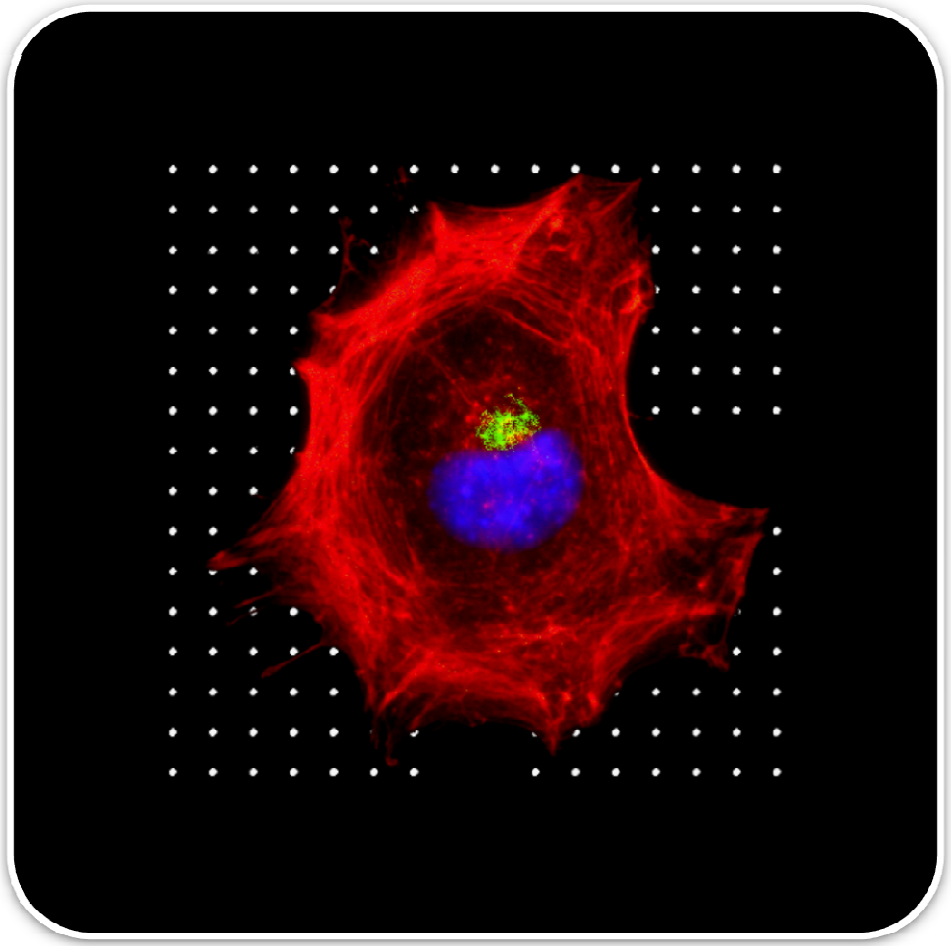
Feature Size	Pitch
500 nm	**1.67 µm**
500 nm	3.33 µm
500 nm	3.33 µm
500 nm	3.33 µm
500 nm	1.67 µm
250 nm	3.33 µm
500 nm	1.67 µm
125 nm	1.67 µm
500 nm	1.67 µm

Pattern 1

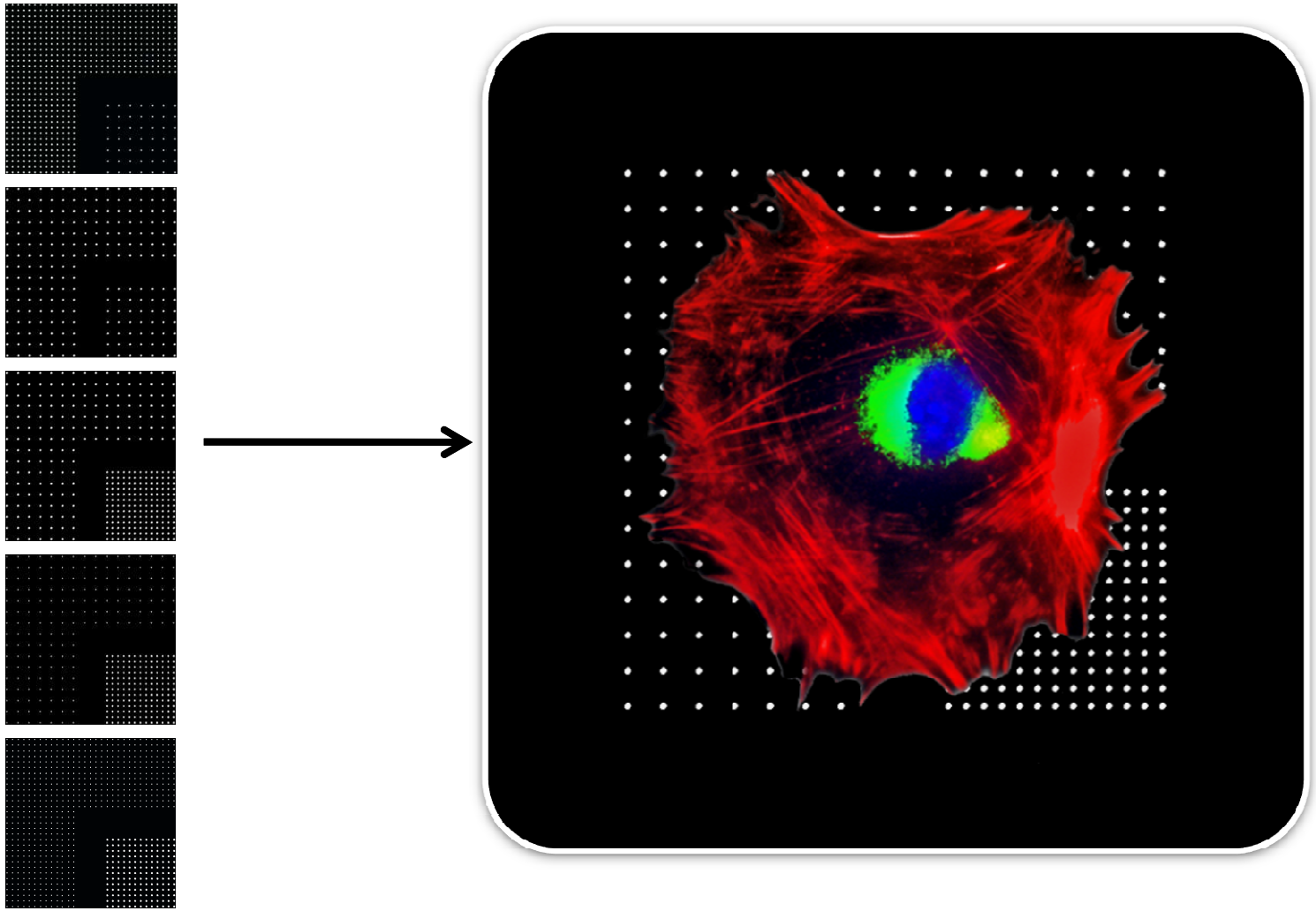


Pattern 2

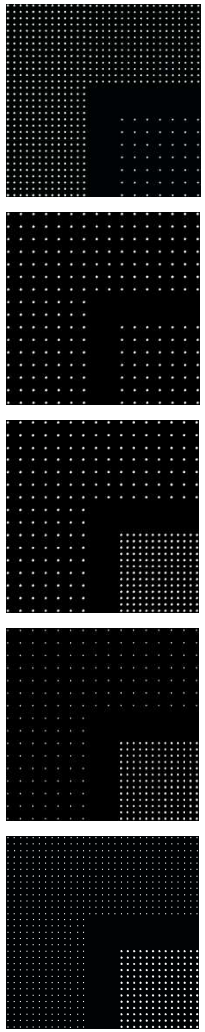


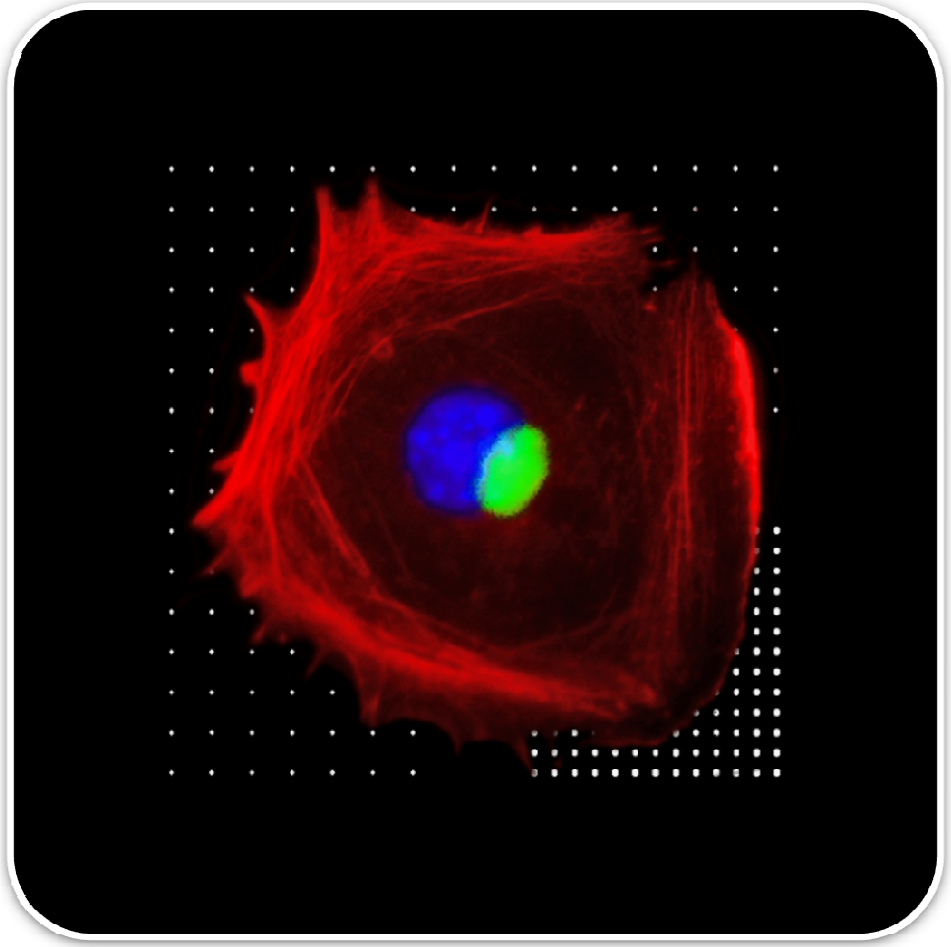


Pattern 3

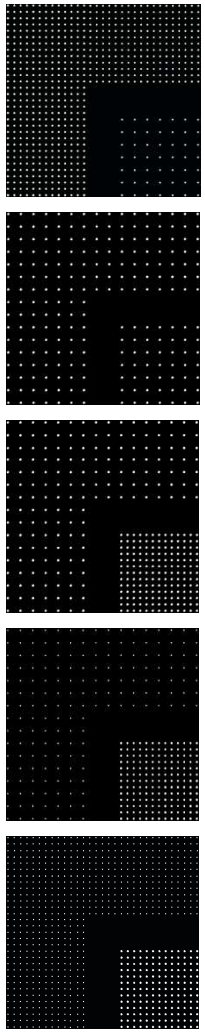


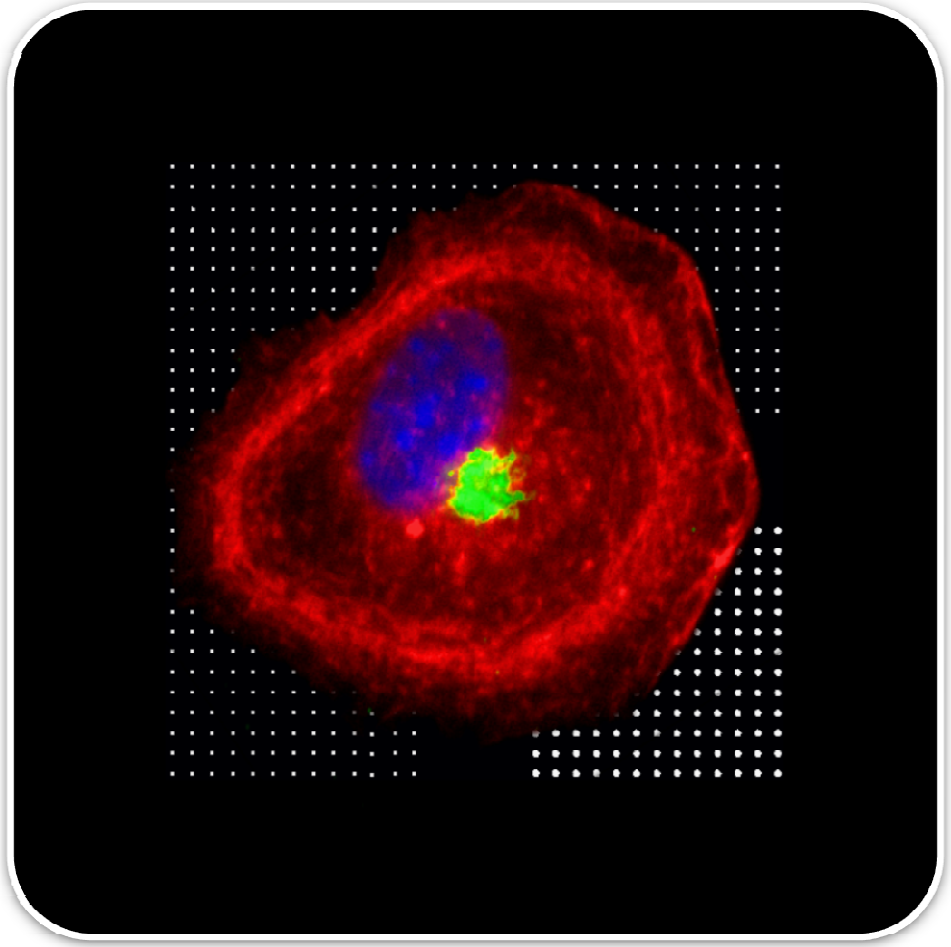
Pattern 4





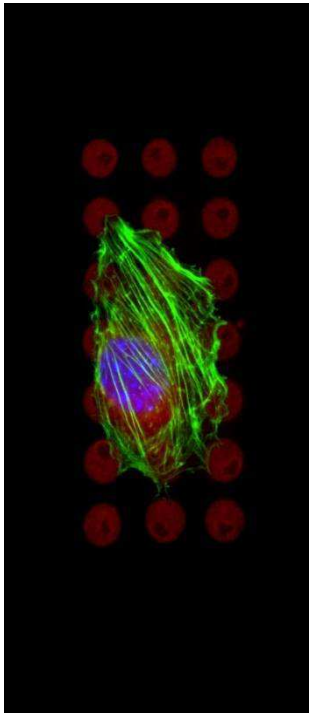
Pattern 5

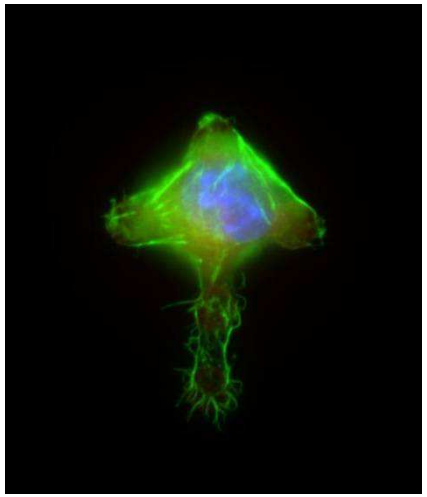


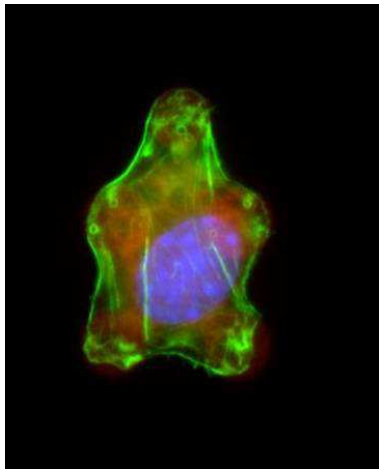


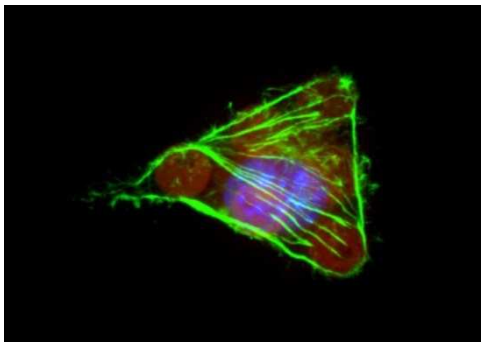
Ultramicroscale Cell Binding Patterns

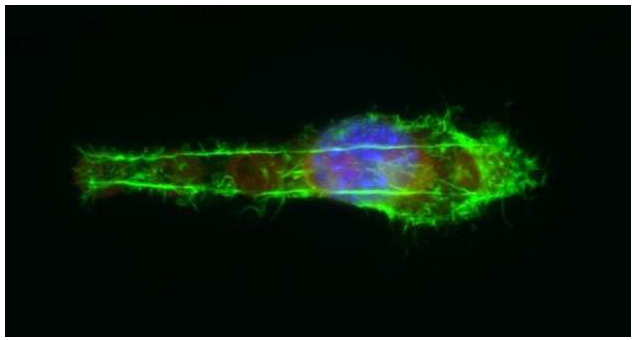
Direct patterning of revelvant materials (liquid ink)



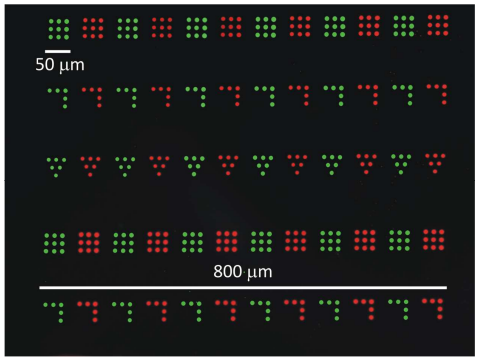




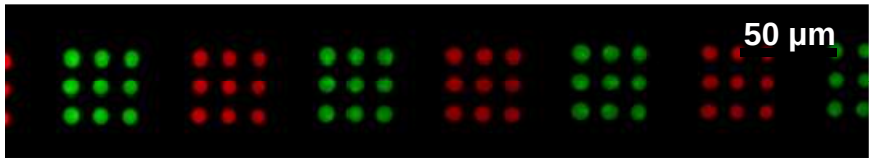




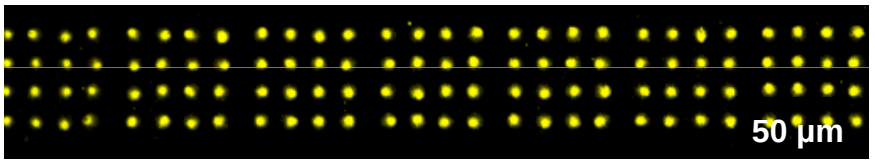
Expanding ECM Protein Library



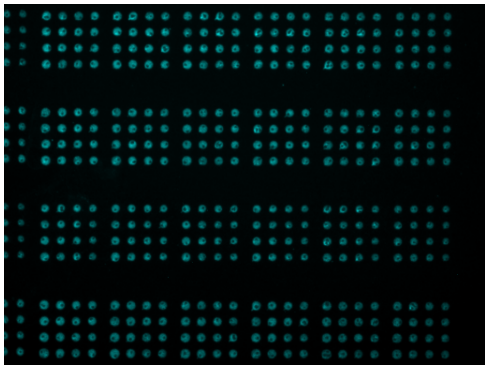
** Enables co-culture **

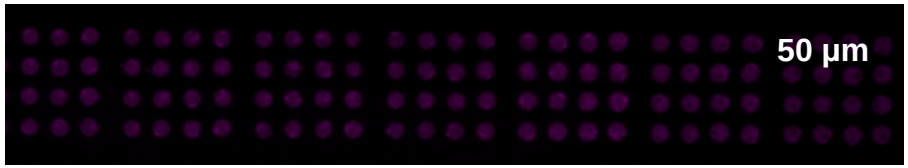


Laminin – Green (900 kDa) – [0.71 mg/ml]
Fibronectin – Red (440 kDa) – [1 mg/ml]



Tenascin C – Yellow (280-300 kDa) – [0.1 mg/ml]



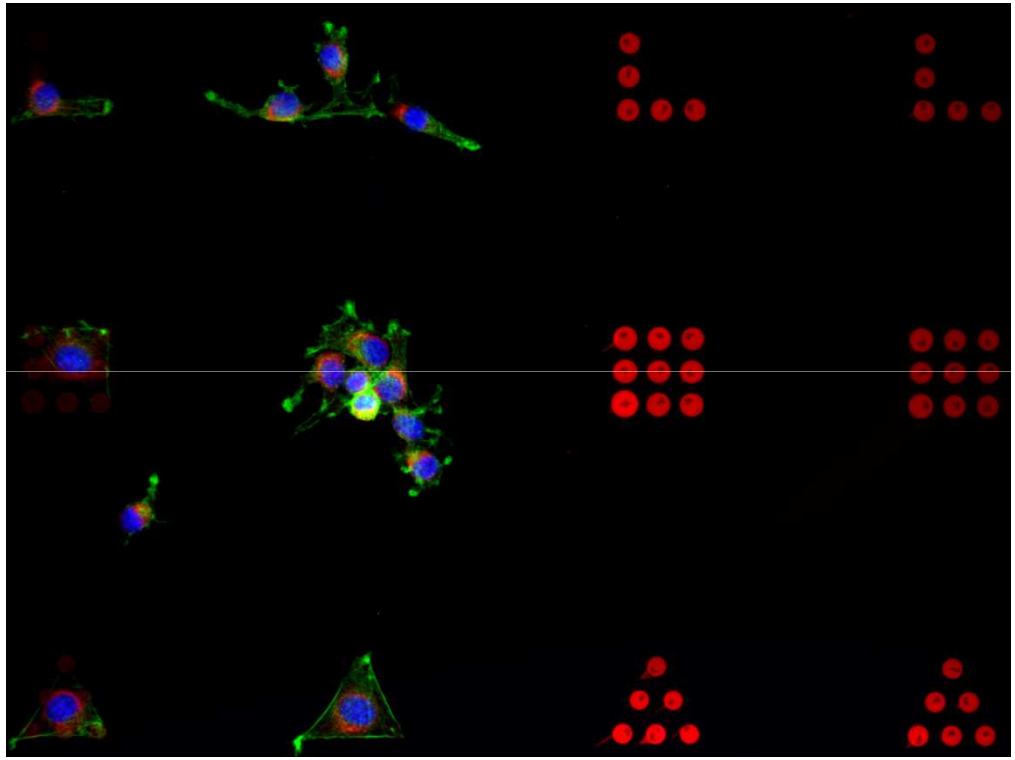


Vitronectin – Magenta (65 - 75 kDa) – [1 mg/ml]

Single Cell Micropatterning

Differential binding affinity enables co-culture

Fibronectin Laminin



3T3 fibroblasts

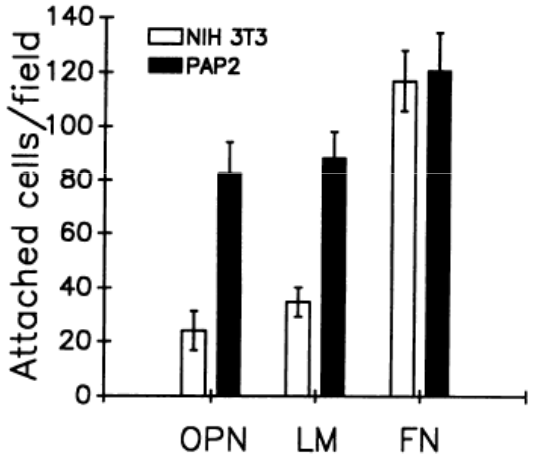


Fig. 1. Adhesion of cells to coated surfaces. NIH 3T3 and PAP2 cells were added to 96-well dishes that had been coated with osteopontin (OPN; 10 μg/ml), laminin (*LM*; 40 μg/ml), or fibronectin (*FN*; 10 μg/ml). Cells were allowed to adhere for 1 h, and plates were washed, fixed, stained, and counted as described in "Materials and Methods." Attached cells per microscope field (area, 0.13 mm^2) were counted; *columns*, mean values for 3 wells per condition; *bars*, SD.

[CANCER RESEARCH 53, 701–706, February 1, 1993]

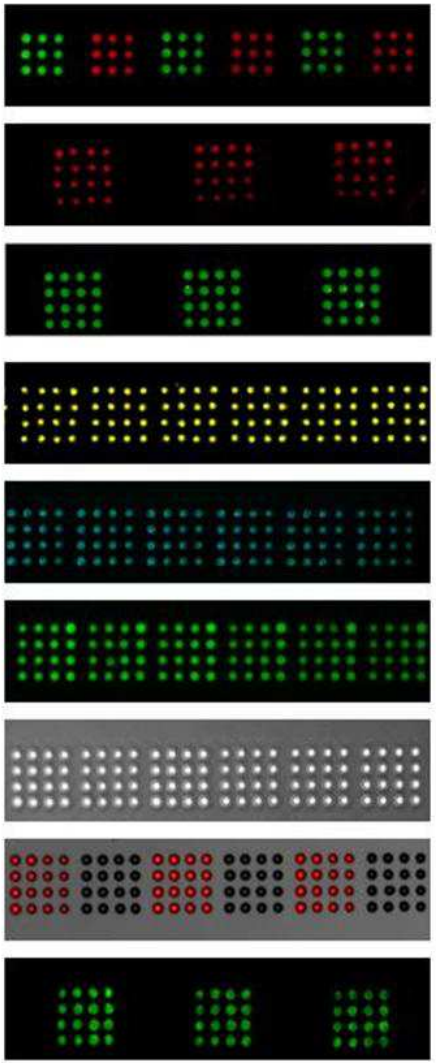
Adhesion of Metastatic, *ras*-transformed NIH 3T3 Cells to Osteopontin, Fibronectin, and Laminin[1]

Ann F. Chambers,[2] Charulata Hota, and Charles W. Prince

London Regional Cancer Centre [A. F. C., C. H.] and Departments of Oncology, and Microbiology and Immunology, University of Western Ontario, [A. F. C.], London, Ontario, Canada, and Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama 35294 [C. W. P.]

Collins and Nettikadan, *Analytical Biochemistry*, 2011 (in press)

Biomaterials library



LAMININ / FIBRONECTIN

COLLAGEN I

COLLAGEN IV

TENACIN C

VITRONECTIN

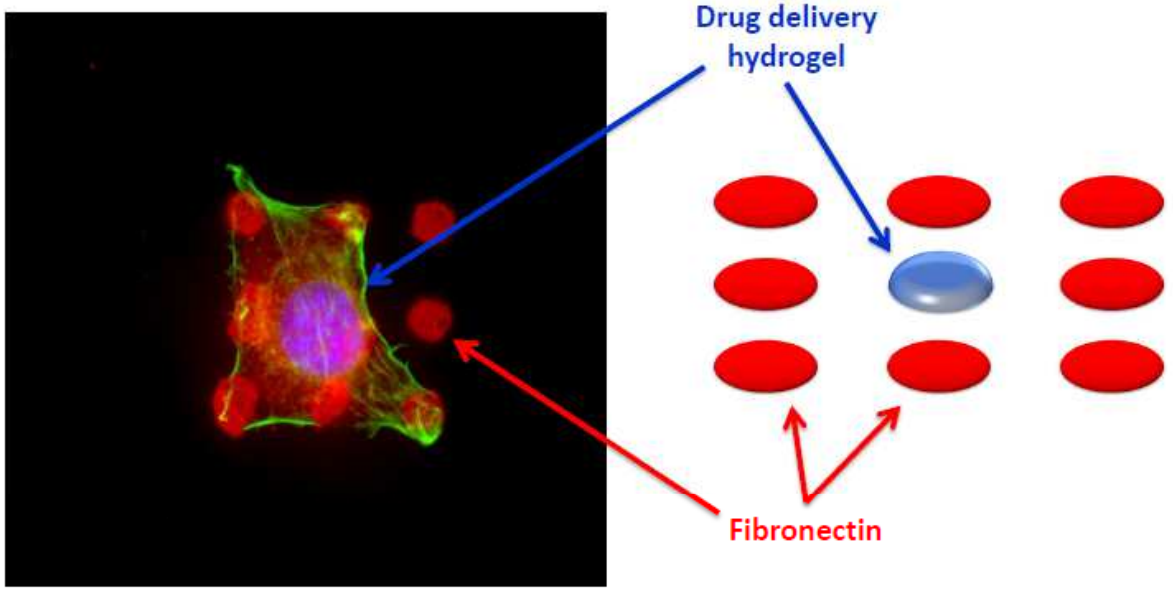
BD HUMAN ECM

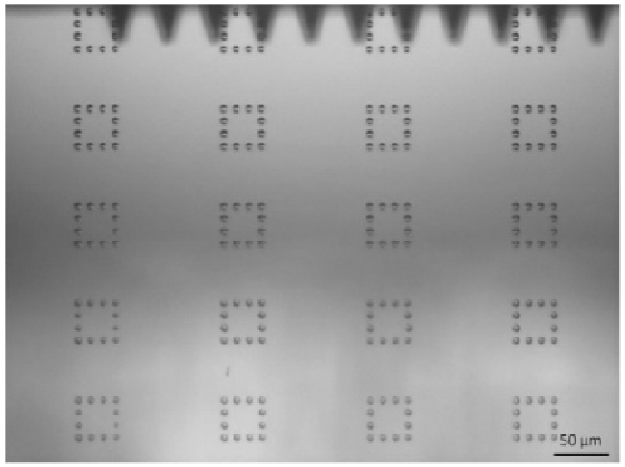
BD MATRIGEL

GELATIN WITH AND WITHOUT RHODAMINE

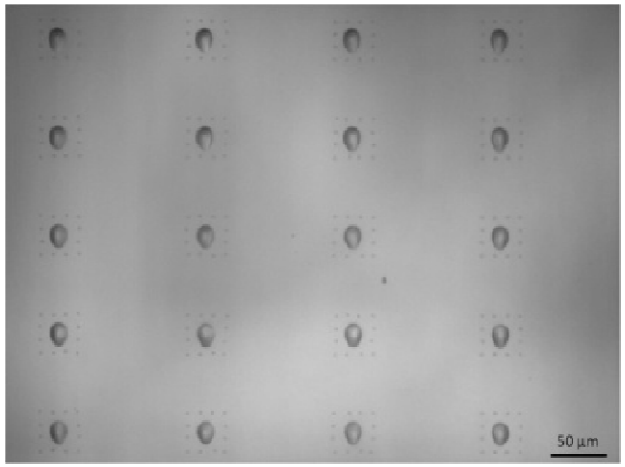
POLY-L-LYSINE

Live single cell arrays: basic concept



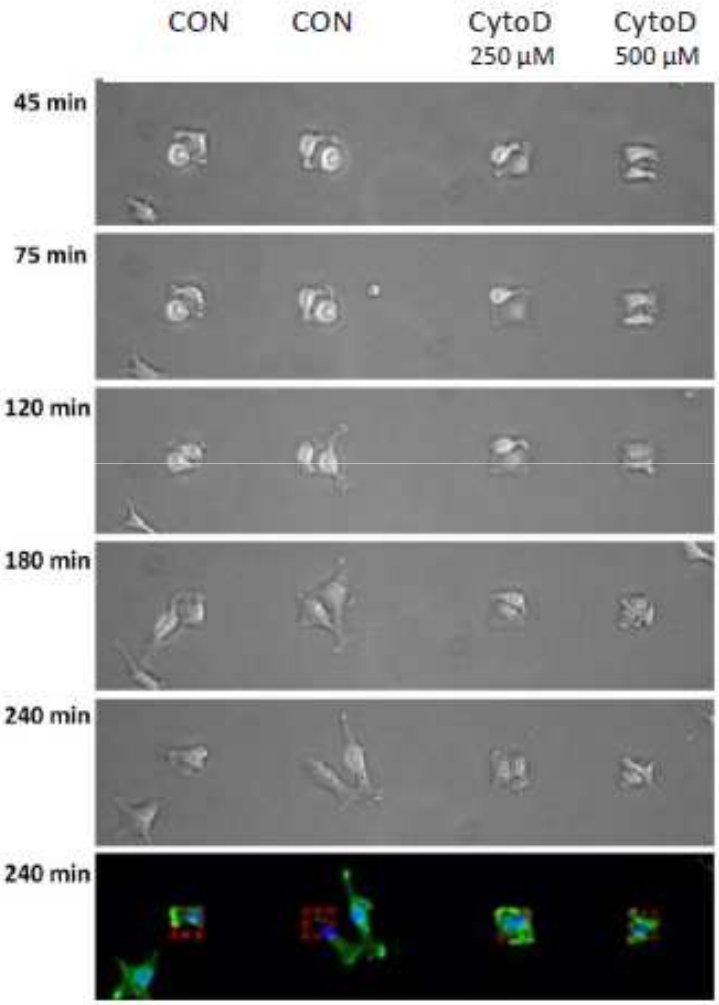


1) Print Fibronectin

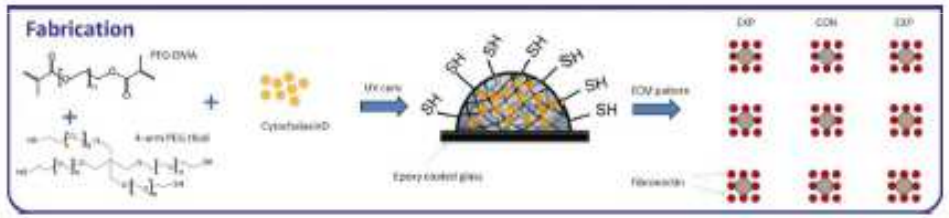


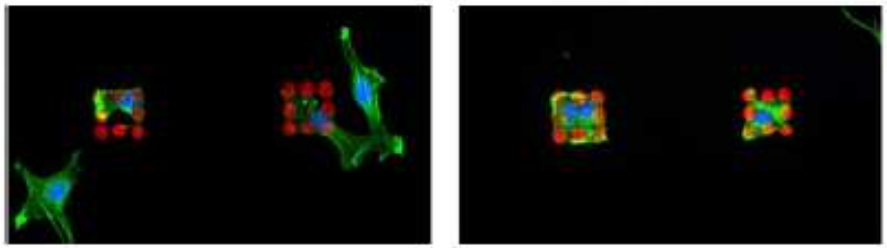
2) Print gel/carrier

Exposing single cells to varying concentrations of the Actin filament inhibitor Cytoclaisin D.

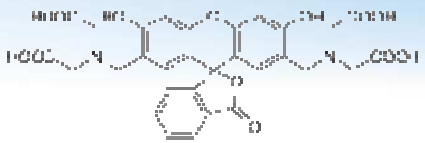


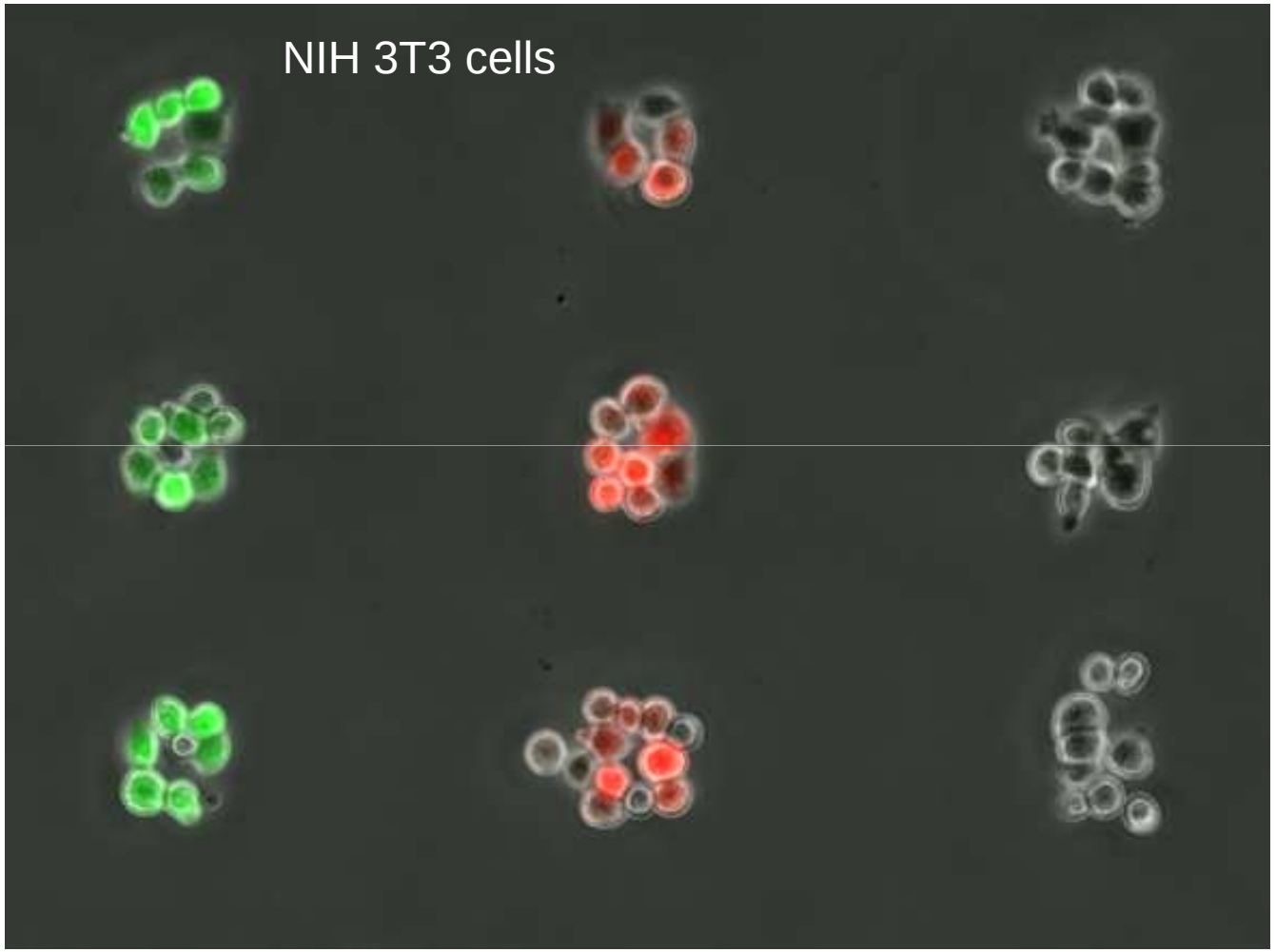
The cells are bound to a fibronectin box with a small PEG based hydrogel placed directly in the middle. The hydrogel can be loaded with either varying concentrations of a growth factor, inhibitor, small molecule, or nothing at all. The geometry of the pattern ensures that each bound cell is exposed to the material found in the hydrogel within the ECM protein box.





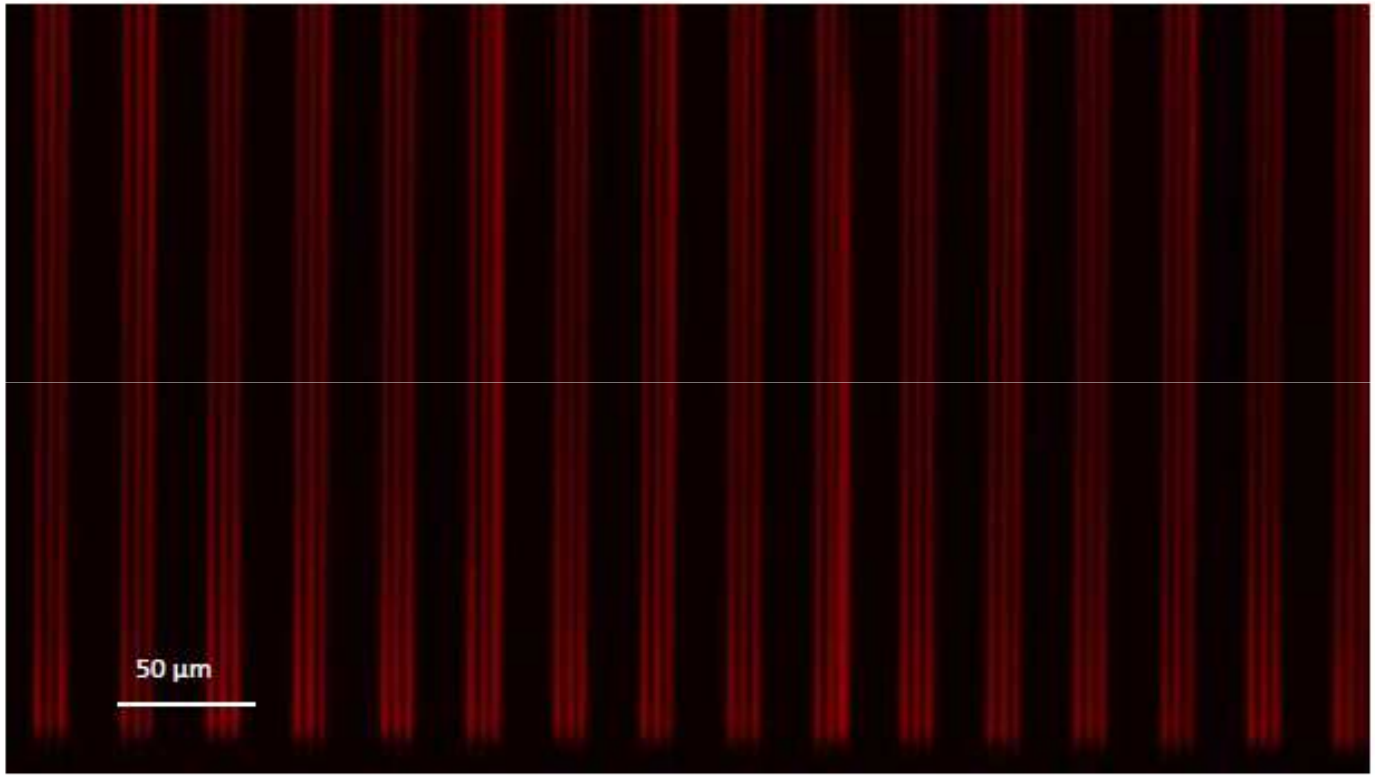
Targeted delivery- example





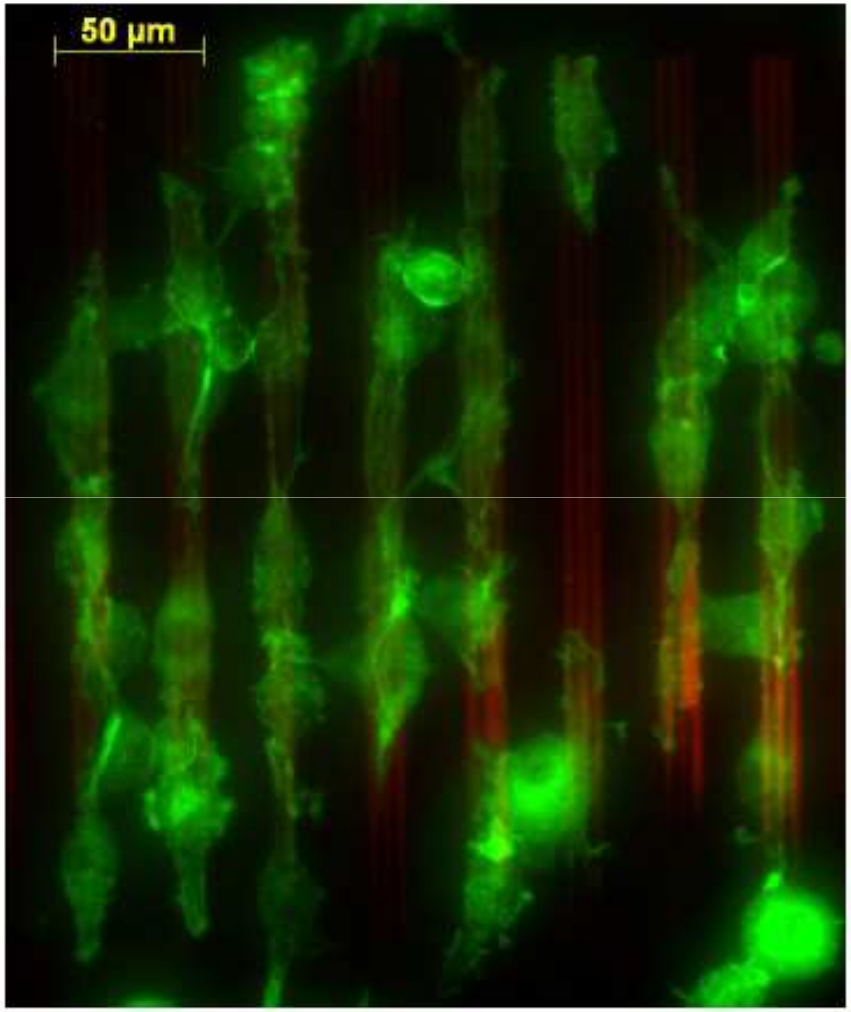
Gelatin- Calcein-AM Calcein-AM-Red DMSO control

Cell culture- create topography



The sets of lines (stained red) are composed of a non-interacting adhesive polymer and were directly written to a glass substrate.

Cell culture- topography

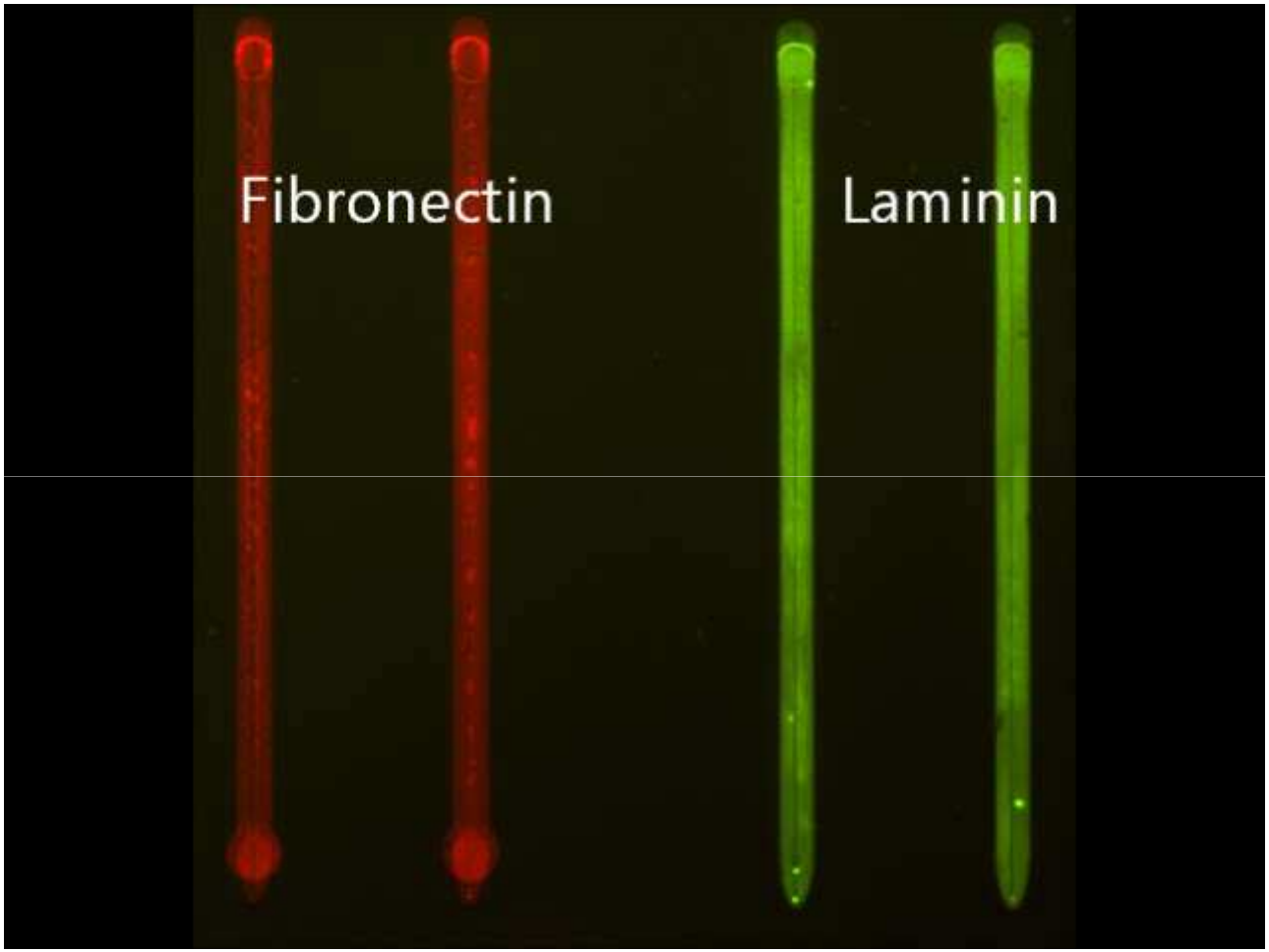


Cell aligned and elongated on top of the NLP 2000 constructed microenvironment

Cells are stained green and the polymer lines are stained red

*** DPN potentially allows control of topography AND chemistry… ***

Cell motility on DPN generated tracks



Fibronectin and Laminin tracks are 500 micron long, 20 micron wide and spaced approximately 120 micron from each other.

Summary

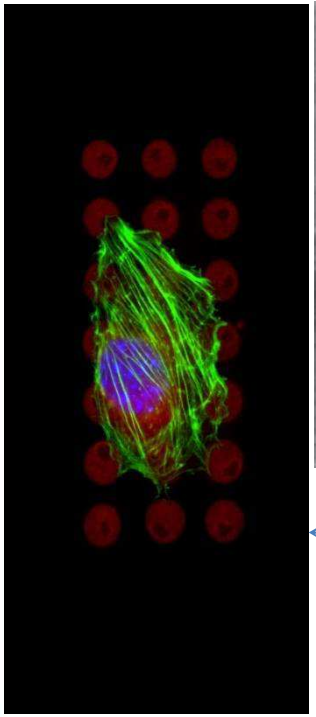
- Feature sizes: **10’s nm to 10 µm scale**
- Wide range of inks: e.g. nanomaterials, polymers, etch materials, protein solutions, DNA solutions.
- Pattern to almost any surface, SiN, Glass, epoxy, nitrocellulose.
- **Biocompatible** (soft process- in physical terms)
- **Multiplexing** possible with nanoscale registry.
- **Scalable** over large areas, quickly.
- **Addressable** patterning- chips, sensors, microchannels

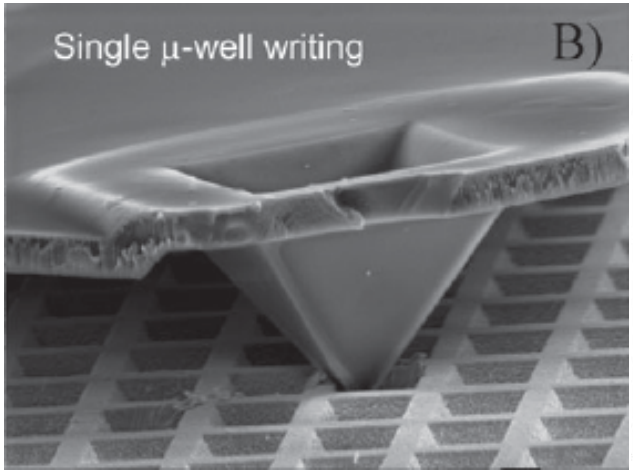
Precise control of cellular microenvironment

- **SINGLE CELL COCULTURE**
  - Cell-cell signaling, Cell-matrix effects
    - Mechanism of progenitor cell differentiation
    - Model of various disease states

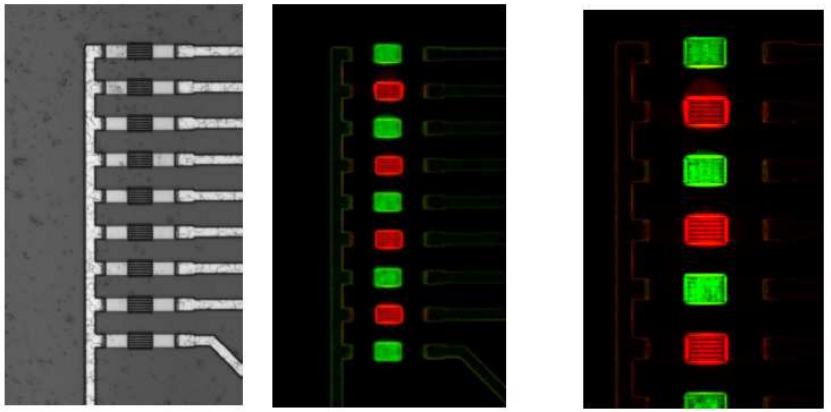
- **TARGETED DELIVERY**
  - Cytotoxicity (LD_{50})
  - Inhibitory effect (IC_{50})
  - Gene or protein expression
  - Targeted transfection.

Diverse applications…



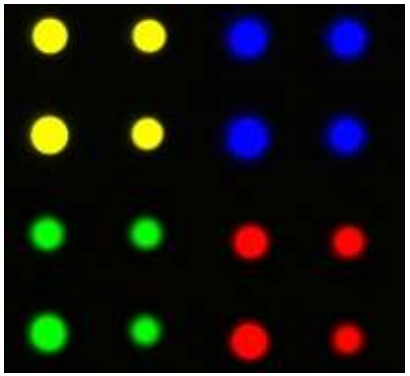


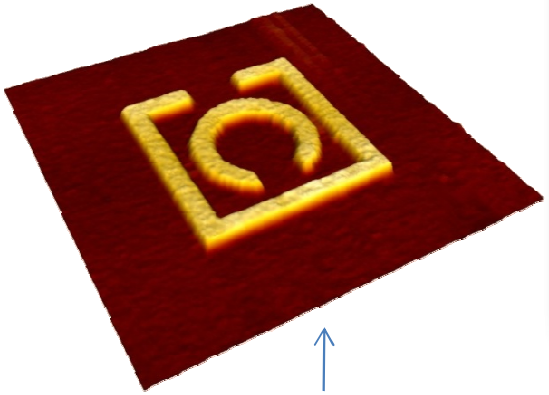
Deposition to existing structures and chips



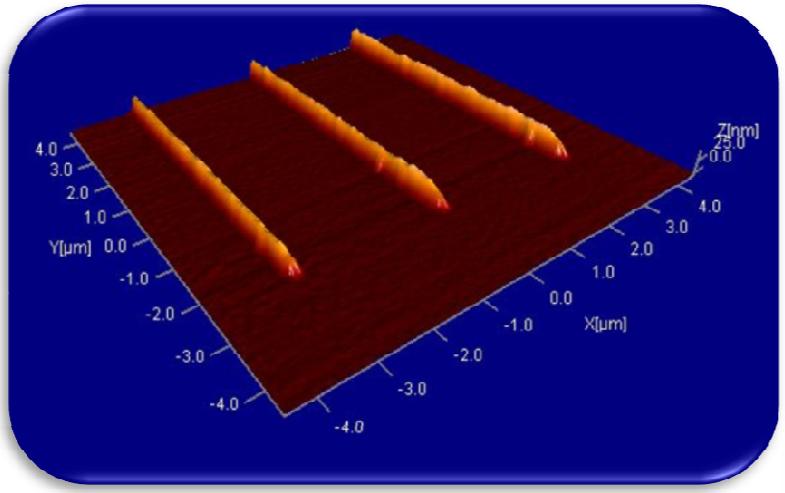
← Patterning for cell biology

Multiplexed depositions





Photonic/electronics structures



Direct deposition of polymers

Thanks for your attention!

Technical enquiries: Robert Stokes: rstokes@nanoink.net

Sales, Europe: Robert Marchmont: rmarchmont@nanoink.net

Turkey distributor: Veysel Ozkapici: bilgi@bnmfabrika.com
BNM Fabrika www.bnmfabrika.com
+(0) 533 214 49 77

NANOINK INC.
NanoFabrication Systems

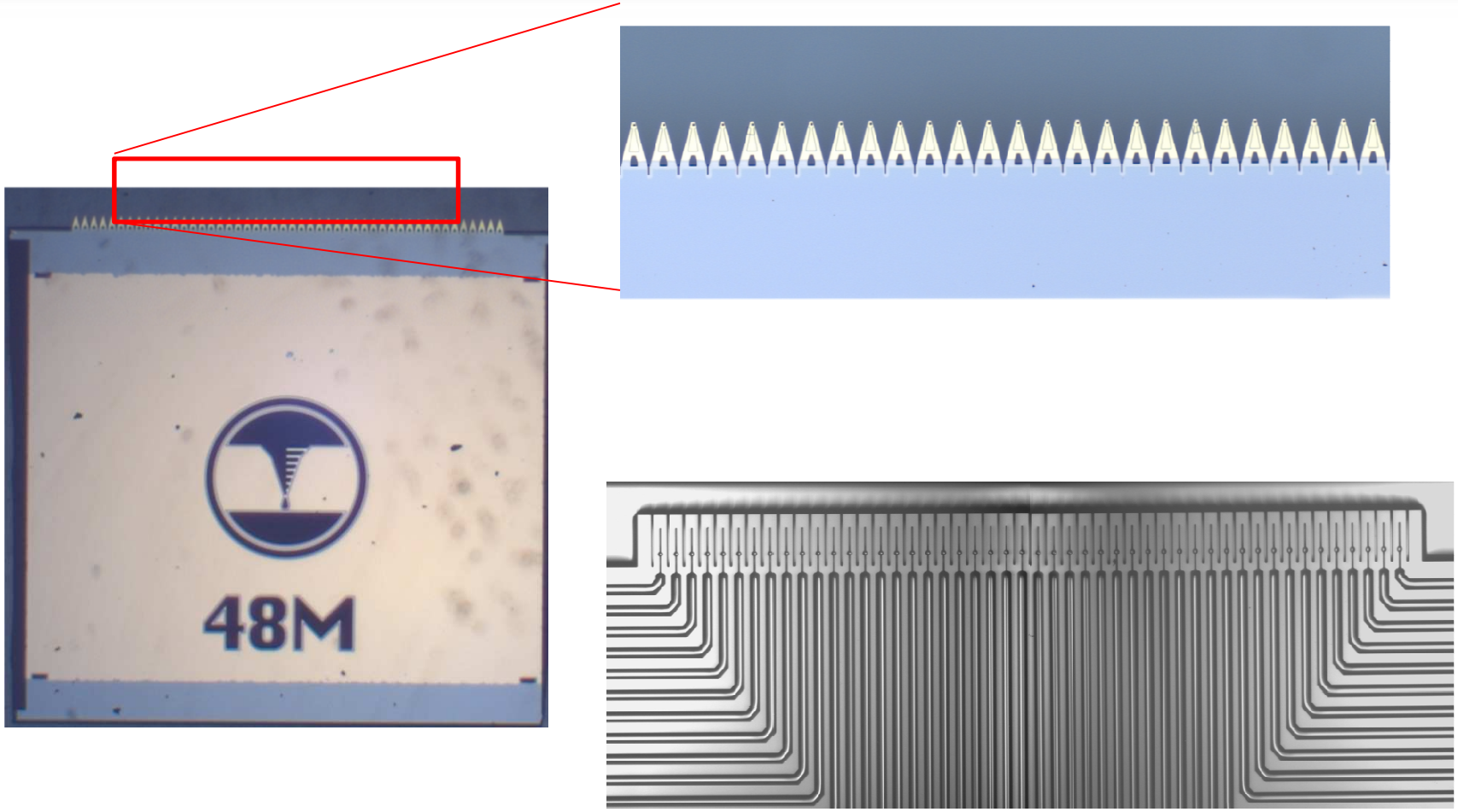
Bio Nano Micro Fabrication

Application notes and information:

www.nanoink.net

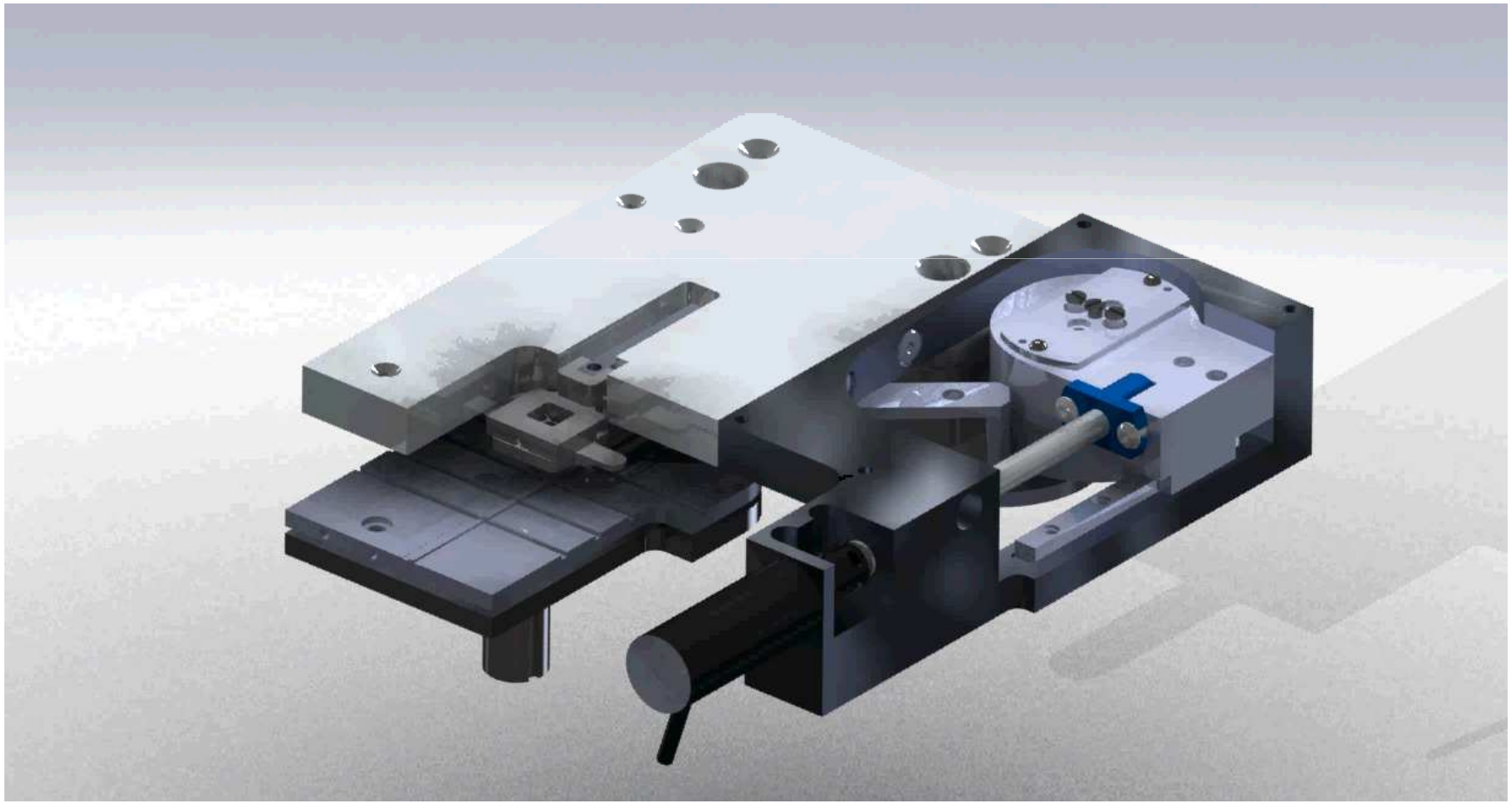
Tools for multiplexing

November 22, 2012



1D and 2D Automated Leveling Apparatus

- Capable of leveling 1D or 2D Tip Arrays of up to several million tips
- Leveling accuracy of 30 nm across a 5 square MILLIMETER array
- Automated leveling in under 2 minutes



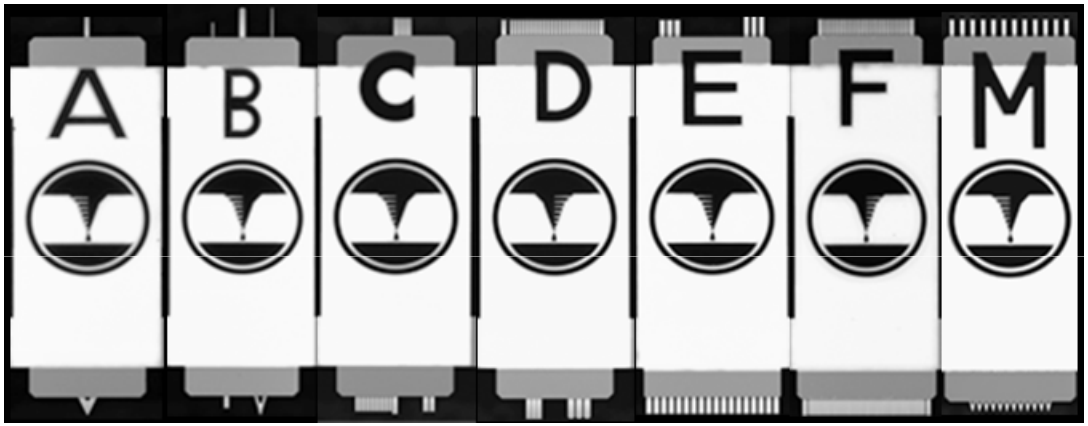
NLP 2000: Nano Lithography Platform

Features of the NLP 2000

- High quality optics for sub-micron resolution
- Environmental chamber for temperature and humidty control
- High speed 5-axis nano-positioning stage with 5 nm steps over 40 mm range
- Uses NanoInk's proven DPN technology and wide range of MEMs based ink delivery solutions
- Simplified user interface
- Integrated vibration isolation
- Feature sizes from less than 100 nm to greater than 10 µm

NanoInk's MEMS

DPN® Pens and Pen Arrays



Ink Material	Pen Type
MHA/ODT	A, B, C, D, E, F
Proteins	M
DNA	A,E,F,M
UV-Curable Polymers	M
Block Co-Polymer	M
Gelatin	M
PEG Polymers	M

NanoInk's MEMS

DPN® Pens and Pen Arrays: Nominal specifications

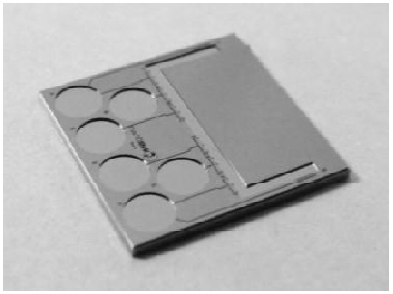
Pen	# Writer Pens	Pitch (µm)	Reader k (N/m)	Reader Length (µm)	Reader Width (µm)	Writer k (N/m)	Writer Length (µm)	Writer Width (µm)
A-1	1	--				0.04	200	45
A-2	1	--				0.1	200	200 @base
B-1a	1	--				0.004	300	15
B-1b	1	--				0.02	300	60
B-1c	1	--				0.03	150	15
B-2a	1	--				0.05	200	25
B-2b	1	--				0.2	120	45
C-1	3	30	0.06	175	45	0.03	175	25
C-2a	2	60				0.1	150	45
C-2b	10	35	0.06	175	45	0.05	150	25
D-1	24	35	0.1	150	45	0.05	150	25
D-2a	3	50				0.04	200	45
D-2b	3	75				0.06	200	60
E-1a	3	70				0.1	150	50
E-1b	3	70				0.04	200	45
E-2	18	70				0.1	150	50
F-1	24	35	0.097	150	45	0.05	150	25
F-2	50	23	0.097	150	45	<0.03	150	20
M-1	12	100				0.4	150	40
M-2	12	66				2.6	107	22
M-2 No Au	12	66				0.6	107	22
48 Bio M	48	66				0.6	107	22

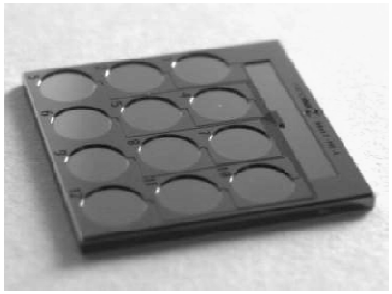
NanoInk's MEMS

DPN® Inkwell Arrays

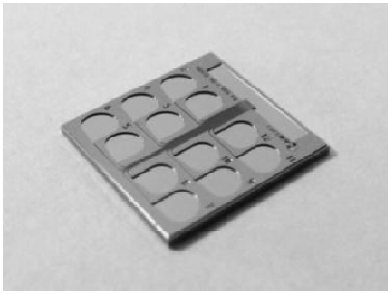
12 Reservoirs
66 um
69 um
70 um
100 um

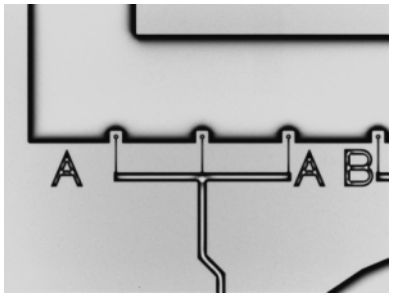
Universal

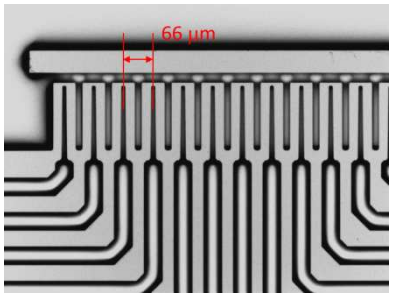


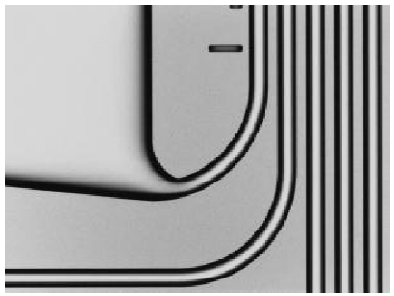


12 Reservoirs central channels





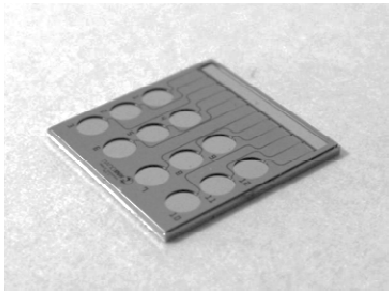




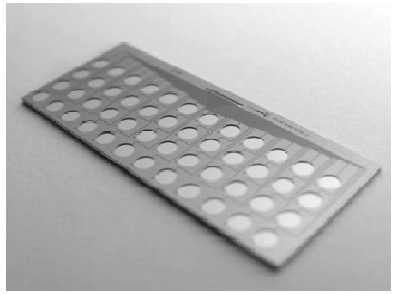
NanoInk's MEMS

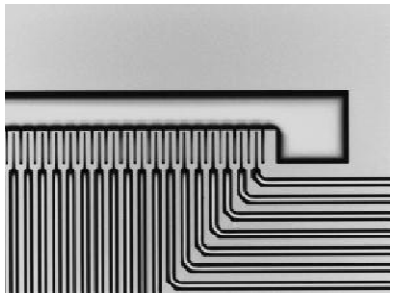
DPN® Inkwell Arrays

12 Reservoirs Universal
780 um



48 Reservoirs, 66 um



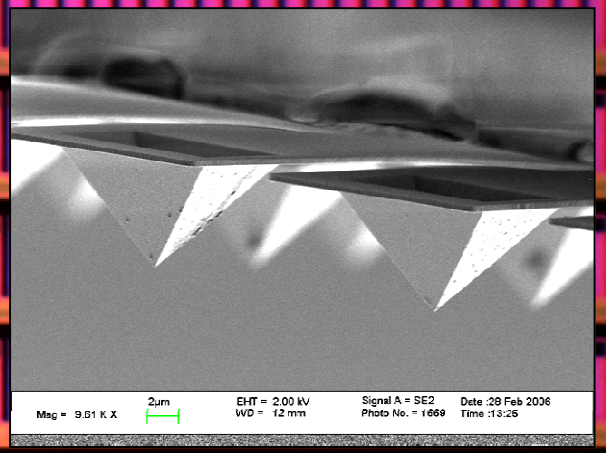


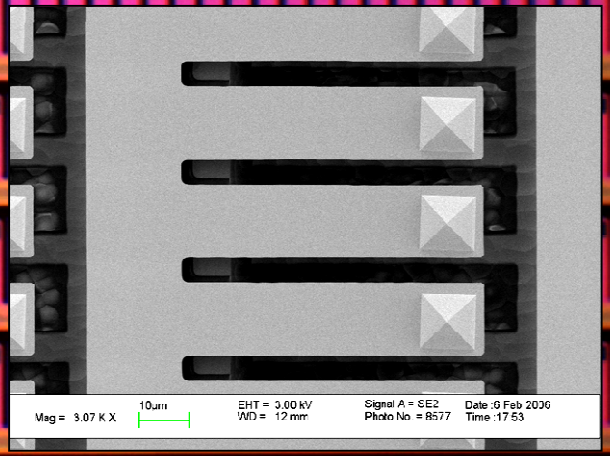
NanoInk's MEMS

DPN® Inkwell Arrays: Nominal Specifications

Loading dock	Reservoirs	Microwells	Pitch (µm)	Single ink	Multiple inks
Universal - Main	6	3	400	✓	
Universal - Side	2	24	35	✓	
66x12	12	12	66		✓
66x12-CC	12	12	66		✓
69x12	12	12	69		✓
70x12	12	12	70		✓
100x12	12	12	100		✓
48 Bio M	48	48	66		✓
780x12	12	12	780		✓

2D nano PrintArray 55,000 Tips!





20×90 µm

1.5 mm