

4a.2	Drying	under a stream of N ₂ . Continue the flow of N ₂ for 30 s after evident disappearance of the bulk drop to evaporate residual EtOH, use within 15 s.	
4b	Contact inking [1]	Inking with an ink pad selectively directs the ink where it is needed.	<i>quality of monolayer is less dependent on pattern geometry, diffusion is minimized.</i>
4b.1	Ink pad fabrication	Prepare small blocks (~2 cm ² and 4mm thick) of cured PDMS as ink pads.	
4b.2	Impregnation	Immerse the ink pad in the thiol-solution for at least 12 h.	
4b.3	Drying and storage	Withdraw from the solution, dry in a stream of N ₂ for 10 s and store in a small glass flask.	
4b.4	Inking	Place the patterned stamp on the ink pad without applying pressure for 40s.	<i>conformal contact allows transfer of thiols. Inking times control amount of thiols transferred.</i>
5	Printing		
5.1	Making Contact	Place stamp onto gold substrate, monitor formation of conformal contact optically.	<i>conformal contact is made by the stamps own weight.</i>
5.2	Detaching	Remove the stamp after 10-20 s.	<i>the longer the printing time, the fewer the defects in the printed monolayer, but the higher the ink diffusion.</i>
6	Etching [3]		
6.1	Preparation of etch bath	Prepare a ferric nitrate etch bath (20 mM Fe(NO ₃) ₃ •9H ₂ O and 30 mM thiourea in DI water, adjusted to pH 2.0 using HCL)	<i>the concentration of the ferric and thiourea in solution determine the etch rate</i>
6.2	Etching	The bath should be operated at 23-25 °C with moderate stirring and has an etch rate of ~ 10 nm min ⁻¹ .	<i>the granularity of the gold substrate limits the edge resolution to the size of the gold grains (15-30 nm).</i>

4.2 Protein Patterning

Fabrication of high resolution protein patterns

Process: microcontact printing lithography



Figure:

Casting PDMS (silicone) precursor onto a structured template in a Petri dish.

Process:

Casting PDMS (silicone) precursor (elastomer base and curing agent) onto a structured template in a Petri dish. Curing (hardening) by heat (60°C, 12-24 h).

Application:

Microfluidic devices
Photonic crystals

Keywords: microcontact lithography, soft lithography, protein patterning, PDMS

Project leader: IBM Research Laboratory
Address: CH-8803 Rueschlikon / Switzerland
Web-Address: <http://www.zurich.ibm.com/>

Process: microcontact lithography
Responsible: Heiko Wolf
E-mail:

Process description: Subtractive Printing of High Resolution Protein Nanopatterns

Purpose: The Ink-Subtract-Print strategy is described in which an inked elastomer is patterned by subtracting proteins from the surface using a nanotemplate followed by printing from the elastomer to a final substrate.

Major advantages: This technique is designed to produce high resolution patterns of single or multiple proteins with intrinsic alignment. Other advantages include: easy to use, high throughput pattern production, large area patterns, and no stamp collapse.

General:

References:

- [1] S. R. Coyer, A. J. Garcia, E. Delamarche, *Angew. Chem. Int. Ed.* **2007**, *46*, 6837-6840.
- [2] J. L. Tan, J. Tien, C. S. Chen, *Langmuir* **2002**, *18*, 519-523.
- [3] A. Bernard *et al.*, *Langmuir* **1998**, *14*, 2225-2229.

Contact information:

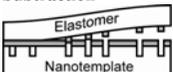
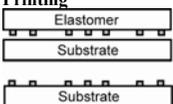
IBM Research Laboratory
Saeumerstrasse 4
CH-8803 Rueschlikon / Switzerland
<http://www.zurich.ibm.com/>

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Protein Patterning

Process: microcontact printing lithography

	Process	Technical Parameters	Remarks
1.	 Nanotemplate		
1.1	Fabrication	Fabricate patterned silicon nanotemplate using a system capable of nanoscale features (E-beam lithography, nanoimprint, nanoparticle patterning)	<i>The nanotemplate material must be more hydrophilic than the elastomer material.</i>
2.	Elastomer		
2.1	Mixing of elastomer	Polydimethylsiloxane (PDMS) prepolymer prepared using SYLGARD® 184 (elastomer base to curing agent 10:1)	<i>Good mixing required for catalytic reaction. Premixed aliquots can be stored at -20 °C for 1-3 months</i>
2.2	Degasing	Degas mixture to remove air bubbles in stamp. This can be completed by leaving the poured dishes at room temperature for ~20 mins. or by placing the dishes in a vacuum.	
2.3	Elastomer curing	Pour liquid prepolymer onto flat polystyrene petri dish and cure at 60 °C for 24 hours	
2.4	Stamp work-up	Cut elastomer into desired stamp size. Mark the surface of the stamp that is not in contact with the petri dish. Peel stamp off petri dish. Ultrasound stamp in isopropanol/DI H ₂ O (20/80) solution for 5 mins. Rinse stamp in Millipore H ₂ O. Rinse stamp with EtOH. Dry under a flow of N ₂ for 30 s.	
3	Ink		
3.1	Protein as ink	Chose a protein, e.g. anti-IgG, streptavidin. Available protein labels include fluorophores and gold conjugates.	<i>Protein must meet the requirement of adsorbing to hydrophobic surfaces from solution.</i>
3.2	Ink solution	Prepare dilute protein solution in phosphate buffered saline (PBS). Desired concentration ranges from 0.05 to 0.5 mg/mL	<i>Concentration is an important factor for producing patterns with complete protein coverage and high edge definition. Optimal concentration varies depending on the protein.</i>
3.3	Storage	Use fresh protein solution when available. If necessary, store solution at 4 °C for up to one week.	
4	Substrate		
4.1	Substrate selection	For atomic force microscopy (AFM), silicon is used for its low surface roughness. For fluorescence, glass is used because it does not quench the fluorophore.	<i>The substrate must be more hydrophilic than the elastomer material.</i>
4.2	Cleaning	Place substrate in isopropanol/DI H ₂ O (20/80) solution. Ultrasound 5 mins. Rinse substrate in Millipore	

		H ₂ O. Rinse stamp with EtOH. Dry under a flow of N ₂ for 30 s.	
4.3	Plasma treatment	Treat cleaned substrate with O ₂ plasma for 1 min.	<i>Plasma treatment increases the hydrophilicity of the surface.</i>
5	 Inking Elastomer		
5.1	Immersion inking	Place ink solution on surface of stamp that was in contact with the petri dish. Coat the entire surface with a droplet of solution.	<i>For 5 × 5 mm² elastomer surface, use ~0.1 mL protein solution.</i>
5.2	Incubation	Incubate protein solution on stamp for 1 hour.	
6	 Subtraction Elastomer Nanotemplate		
6.1	Plasma treatment	Treat nanotemplate with O ₂ plasma for 1 min.	<i>Plasma treatment increases the hydrophilicity of the surface. Complete this step shortly before subtraction.</i>
6.2	Rinsing	Wash ink solution off of stamp using hand pipette. PBS 3 × 1mL. Millipore 1 × 1mL.	
6.3	Drying	Dry stamp in N ₂ flow for 15 s.	<i>Under or over drying the protein monolayer on the stamp will affect the printed pattern quality.</i>
6.4	Contact	Bring inked surface of stamp into contact with nanotemplate for 15 s.	<i>Light pressure can be applied to assure conformal contact between stamp and nanotemplate.</i>
6.5	Release	Release stamp from nanotemplate.	
7	 Printing Elastomer Substrate Substrate		
7.1	Contact	Place stamp onto substrate for 30s.	<i>Light pressure can be applied to assure conformal contact between stamp and nanotemplate.</i>
7.2	Release	Release stamp from substrate.	
8	Repeat Ink-Subtract-Print		
8.1	Additional steps	Patterns of multiple proteins can be produced by repeating the ink-subtract-print steps. Also, the individual steps can be rearranged to produce a variety of protein patterns. The steps ink-subtract-ink-subtract-print using two different protein inks allows printing of multiple proteins at the same time with intrinsic alignment.	

General remarks: