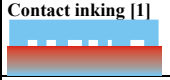
		under a stream of N_2.	
4a.2	**Drying**	Continue the flow of N_2 for 30 s after evident disappearance of the bulk drop to evaporate residual EtOH, use within 15 s.	
4b	**Contact inking [1]** 	Inking with an ink pad selectively directs the ink where it is needed.	*quality of monolayer is less dependent on pattern geometry, diffusion is minimized.*
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.	*conformal contact allows transfer of thiols. Inking times control amount of thiols transferred.*
5	**Printing**		
5.1	**Making Contact** 	Place stamp onto gold substrate, monitor formation of conformal contact optically.	*conformal contact is made by the stamps own weight.*
5.2	**Detaching**	Remove the stamp after 10-20 s.	*the longer the printing time, the fewer the defects in the printed monolayer, but the higher the ink diffusion.*
6	**Etching [3]**		
6.1	**Preparation of etch bath**	Prepare a ferric nitrate etch bath (20 mM $Fe(NO_3)_3 \cdot 9H_2O$ and 30 mM thiourea in DI water, adjusted to pH 2.0 using HCL)	*the concentration of the ferric and thiourea in solution determine the etch rate*
6.2	**Etching** 	The bath should be operated at 23-25 ºC with moderate stirring and has an etch rate of ~ 10 nm min^{-1}.	*the granularity of the gold substrate limits the edge resolution to the size of the gold grains (15-30 nm).*

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

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Process: microcontact lithography
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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:

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[3] A. Bernard *et al.*, *Langmuir* **1998**, *14*, 2225-2229.

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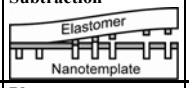
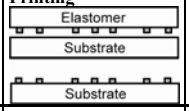
Protein Patterning

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)	*The nanotemplate material must be more hydrophilic than the elastomer material.*
2.	**Elastomer**		
2.1	**Mixing of elastomer**	Polydimethylsiloxane (PDMS) prepolymer prepared using SYLGARD® 184 (elastomer base to curing agent 10:1)	*Good mixing required for catalytic reaction. Premixed aliquots can be stored at -20 ºC for 1-3 months*
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 ºC 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_2O (20/80) solution for 5 mins. Rinse stamp in Millipore H_2O. 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.	*Protein must meet the requirement of adsorbing to hydrophobic surfaces from solution.*
3.2	**Ink solution**	Prepare dilute protein solution in phosphate buffered saline (PBS). Desired concentration ranges from 0.05 to 0.5 mg/mL	*Concentration is an important factor for producing patterns with complete protein coverage and high edge definition. Optimal concentration varies depending on the protein.*
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.	*The substrate must be more hydrophilic than the elastomer material.*
4.2	**Cleaning**	Place substrate in isopropanol/DI H_2O (20/80) solution. Ultrasound 5 mins. Rinse substrate in Millipore	

		H_2O. 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.	*Plasma treatment increases the hydrophilicity of the surface.*
5	**Inking** 		
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.	*For 5 × 5 mm^2 elastomer surface, use ~0.1 mL protein solution.*
5.2	**Incubation**	Incubate protein solution on stamp for 1 hour.	
6	**Subtraction** 		
6.1	**Plasma treatment**	Treat nanotemplate with O_2 plasma for 1 min.	*Plasma treatment increases the hydrophilicity of the surface. Complete this step shortly before subtraction.*
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.	*Under or over drying the protein monolayer on the stamp will affect the printed pattern quality.*
6.4	**Contact**	Bring inked surface of stamp into contact with nanotemplate for 15 s.	*Light pressure can be applied to assure conformal contact between stamp and nanotemplate.*
6.5	**Release**	Release stamp from nanotemplate.	
7	**Printing** 		
7.1	**Contact**	Place stamp onto substrate for 30s.	*Light pressure can be applied to assure conformal contact between stamp and nanotemplate.*
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: