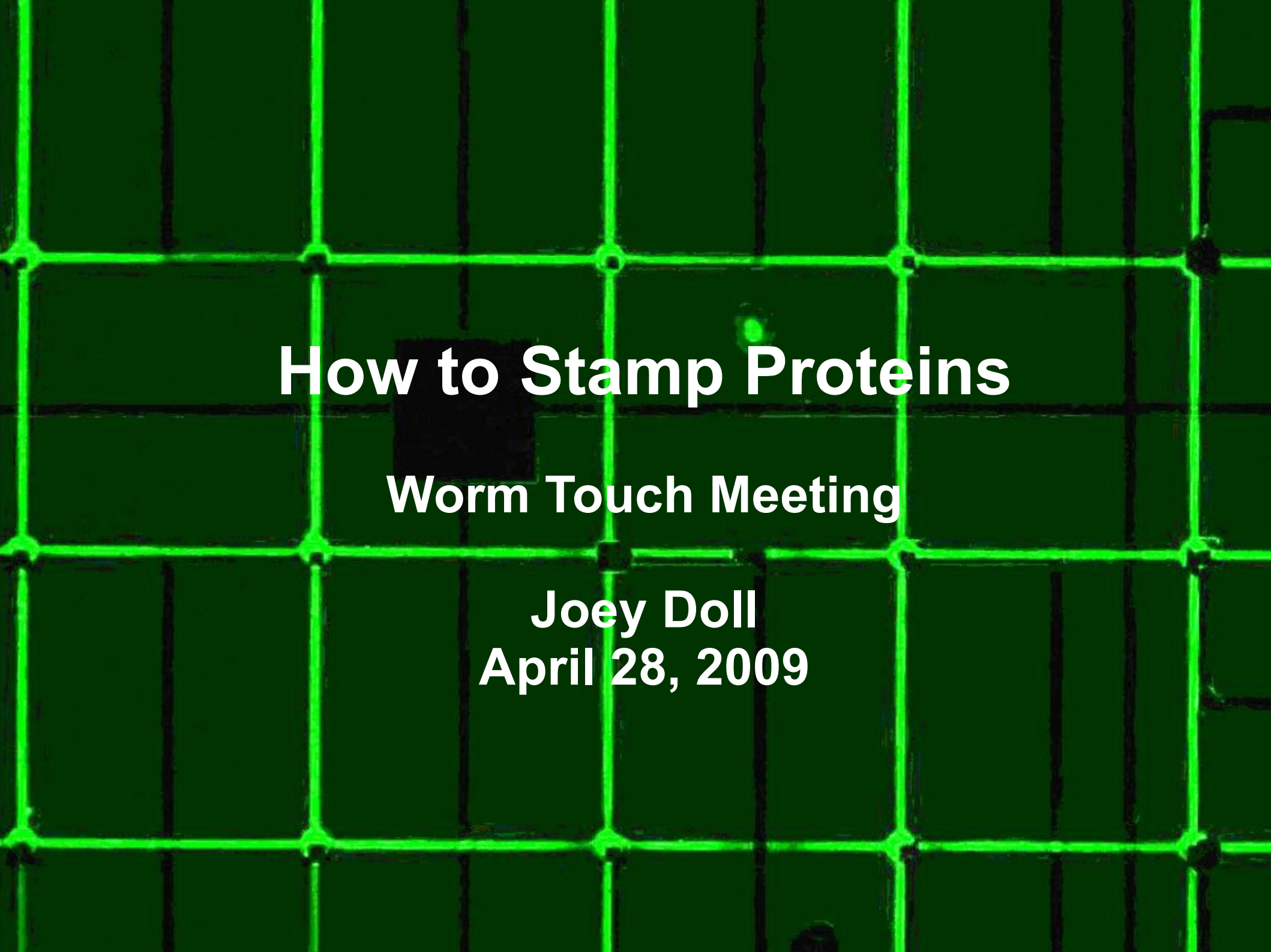


How to Stamp Proteins

Worm Touch Meeting

Joey Doll

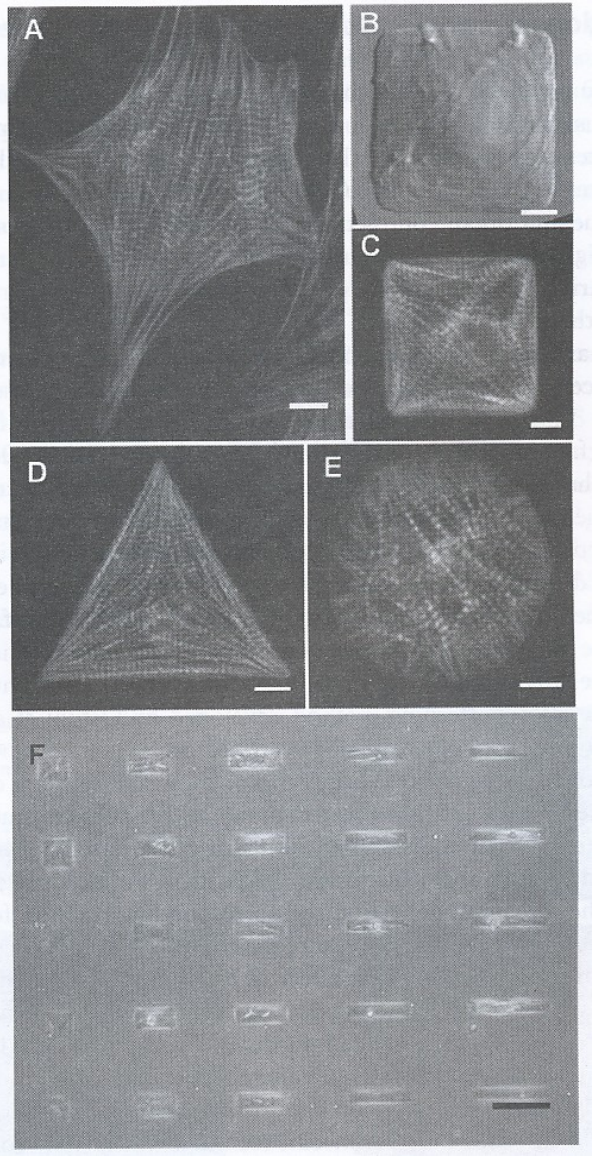
April 28, 2009

Papers

- Patterned Protein Layers on Solid Substrates by Thin Stamp Microcontact Printing
  - James, Craighead et al, Langmuir (1998)
- Micropatterning Approaches for Cardiac Biology
  - Geisse et al, from Micro and Nanoengineering of the Cell Microenvironment (2008)
- Microfabrication meets Microbiology
  - Weibel, DiLuzio, Whitesides, Nature Reviews (2007)
- Recent advances in microcontact printing
  - Quist et al, Anal. Bioannal. Chem (2005)
- Shrink-Dink microfluidics: rapid generation of deep and rounded patterns
  - Grimes, Lee, Khine, Lab on a Chip (2007)

Motivation for Micropatterning

- Control boundary conditions: relationship between structure and function
- Mimic in vivo environment
  - Cardiac myocytes seeded on PDMS (1 Mpa) survive longer than those cultured on polystyrene (3 GPa) or glass (70 GPa)
- Soft lithography tools, microcontact printing (uCP) and others



Motivation for Micropatterning

Microfabrication isn't necessary to create alignment features

(just if you want to do it repeatably)

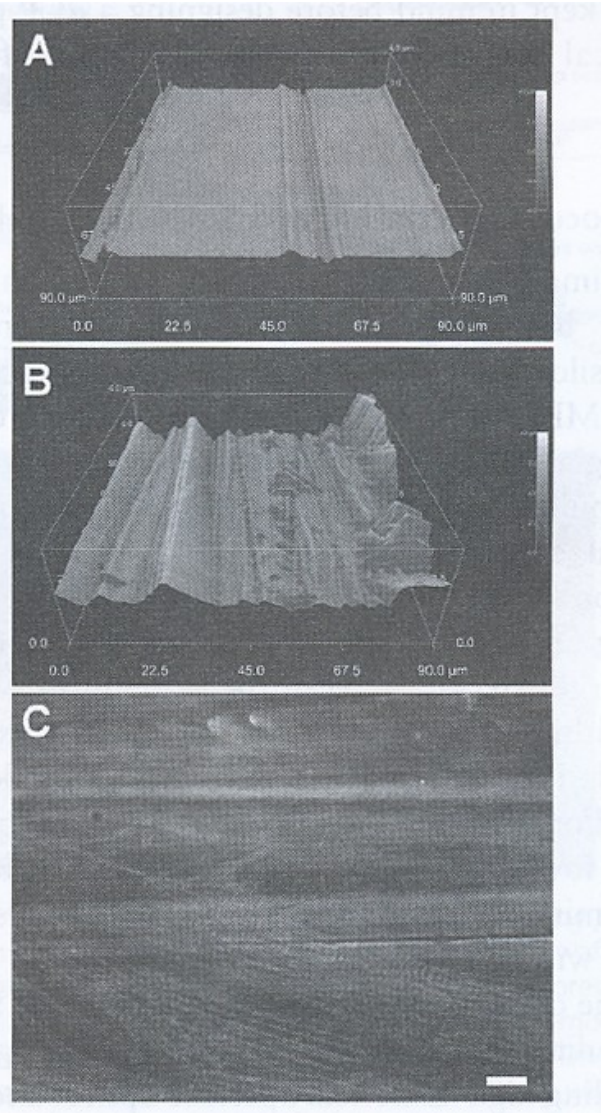


Figure 17.2 *Microabrasion of the cardiac myocyte culture substrate.* Atomic force micrographs of microabraded PVC coverslips show geometrical cues in the direction of the abrasion. (a) Single-pass microabrasion creates small, individual trenches across the surface that are approximately 0.5 µm deep. (b) Multiple-pass microabrasion results in a completely rough culture substrate with trenches in the direction of the abrasion. Height variation across this substrate is 3.0 to 4.0 µm. c) Cardiac myocytes cultured on these substrates and stained for actin filaments, sarcomeric α-actinin, and the nucleus show myofibrillar alignment along the direction of the microabrasions, visualized as the dark horizontal line. Scale bar = 10 μm.

The World of Soft Lithography

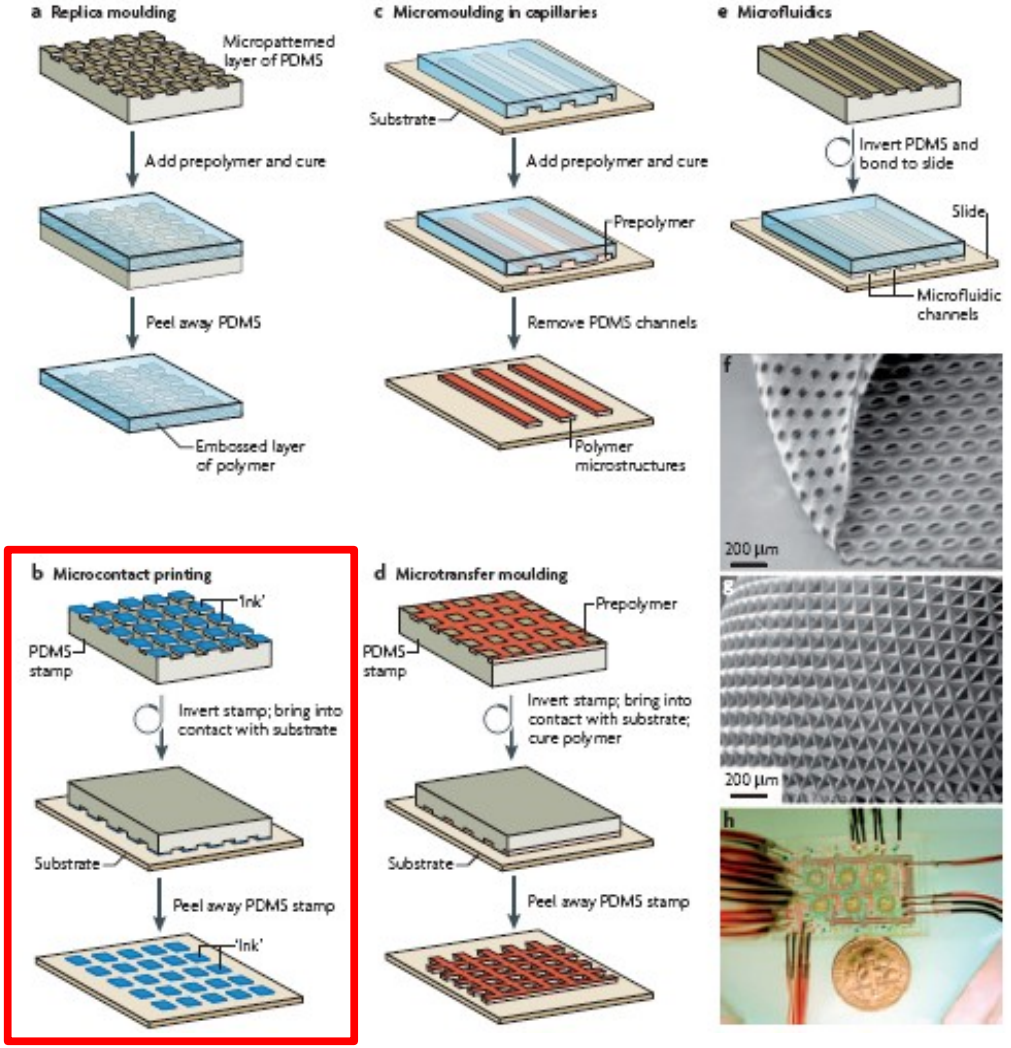


Figure 2 | **The core techniques of soft lithography.** The key stages of each of the following techniques are shown: **a** | replica moulding; **b** | microcontact printing; **c** | micromoulding in capillaries; **d** | microtransfer moulding; and **e** | microfluidics. **f** | A PDMS membrane with microfabricated holes created by replica moulding from a master with circular posts. **g** | A curved layer of micropatterned polyurethane created by bending a micropatterned layer of PDMS and then replica moulding against it. **h** | A microfluidic chemostat for the growth and culture of microbial cultures. The device incorporates six reactors with an intricate network of plumbing, in a footprint that is approximatley 5 cm². PDMS, poly(dimethylsiloxane). Part (**f**) reproduced with permission from REF. 74 © (2000) American Chemical Society. Part (**g**) reproduced with permission from REF. 1 © (1998) Geselleschaft Deutscher Chmiker. Part (**h**) reproduced with permission from REF. 59 © (2005) American Association for the Advancement of Science.

Early Problems with uCP

- Making stamps biofriendly (SAMs → proteins)
- Problems
  - Hydrophobic PDMS (for some proteins)
  - Sagging
- Solutions
  - Plasma treat PDMS
  - Thin stamps with rigid backing

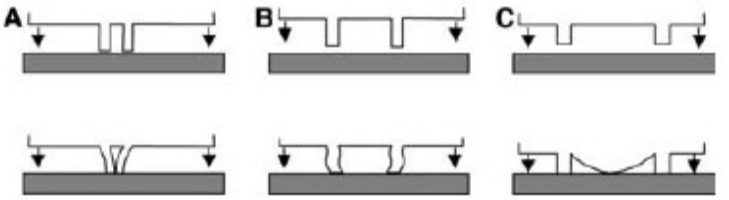
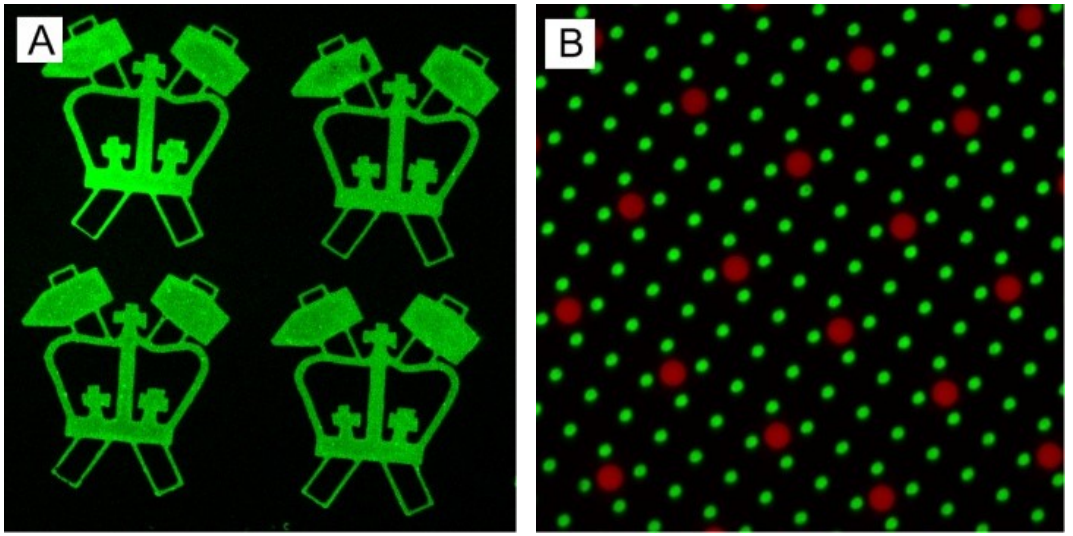
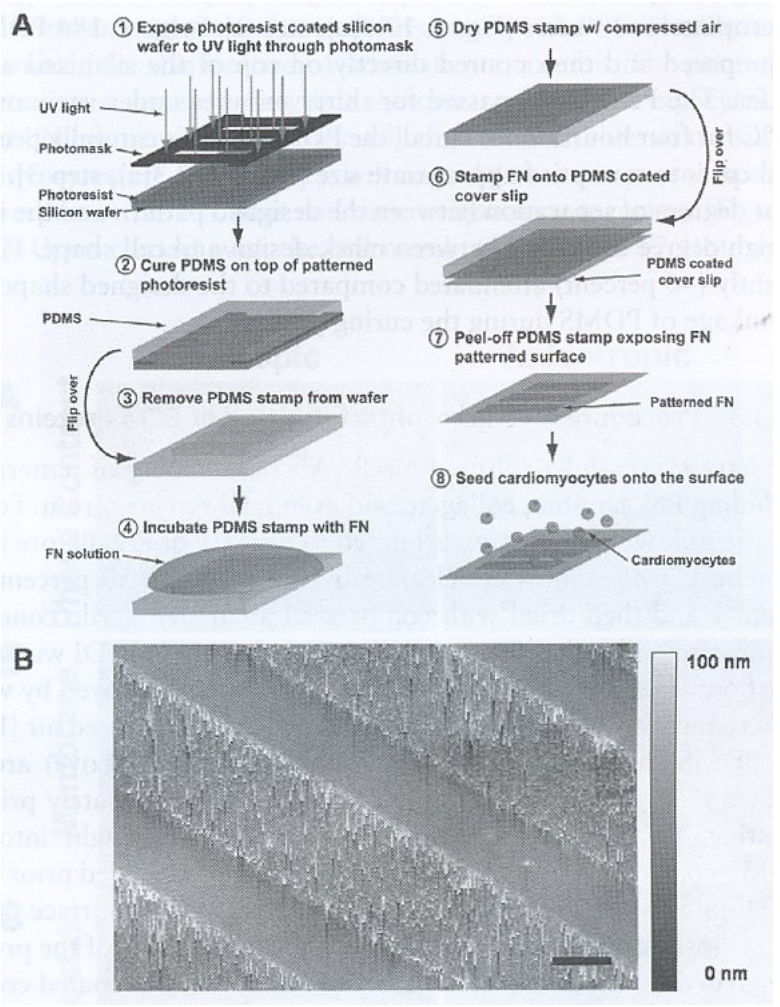


Fig. 2 The most commonly observed stamp deformations: **a** pairing, **b** buckling, and **c** roof collapse

Stamping Process

Figure 17.3 *Schematic of the stamp fabrication and microcontact printing process for ECM proteins on to PDMS coated coverslips*: (a) Overview of the process: (1) A silicon wafer is coated in photoresist and photolithographically patterned by exposing the photoresist with ultraviolet light passed through a photomask. (2) The photoresist is developed, thus removing the ultraviolet-exposed regions, and the remaining topography is copied by casting PDMS prepolymer on top. (3) The cured PDMS is peeled off of the silicon wafer, creating a stamp with microtopography. (4) The PDMS stamp is inked by incubation with an ECM protein solution for one hour. (5) The ECM protein solution is rinsed off in ddH_2O and then dried under compressed air. (6) The inked PDMS stamp is placed pattern-side down on the PDMS-coated coverslip, transferring the ECM protein to the substrate in defined patterns in a process adapted from [27]. (8) Once the ECM patterned coverslip is washed in buffer, myocytes are seeded. (b) Atomic force deflection micrograph of 20 μm–wide micropatterned lines of FN reveal a thin, ~3 nm layer of protein on the PDMS surface.





Images and instructional video @
http://www.jove.com/index/details.stp?id=1065

Example Stamping Procedure

- Make mold and cast PDMS
- Clean PDMS stamps (sonicate in 50% ethanol for 30 minutes, blow dry)
- Ink the stamps, incubate for 1 hour
- Wash the stamps (DI water) and blow dry
- Bring stamp into contact with substrate for 2 minutes
- Incubate substrate in another solution (e.g. Pluronics) for 15 minutes to fill in the line gaps, wash
- Store substrate in PBS for up to 48 hours, seed cells

Making Soft Lithography Molds

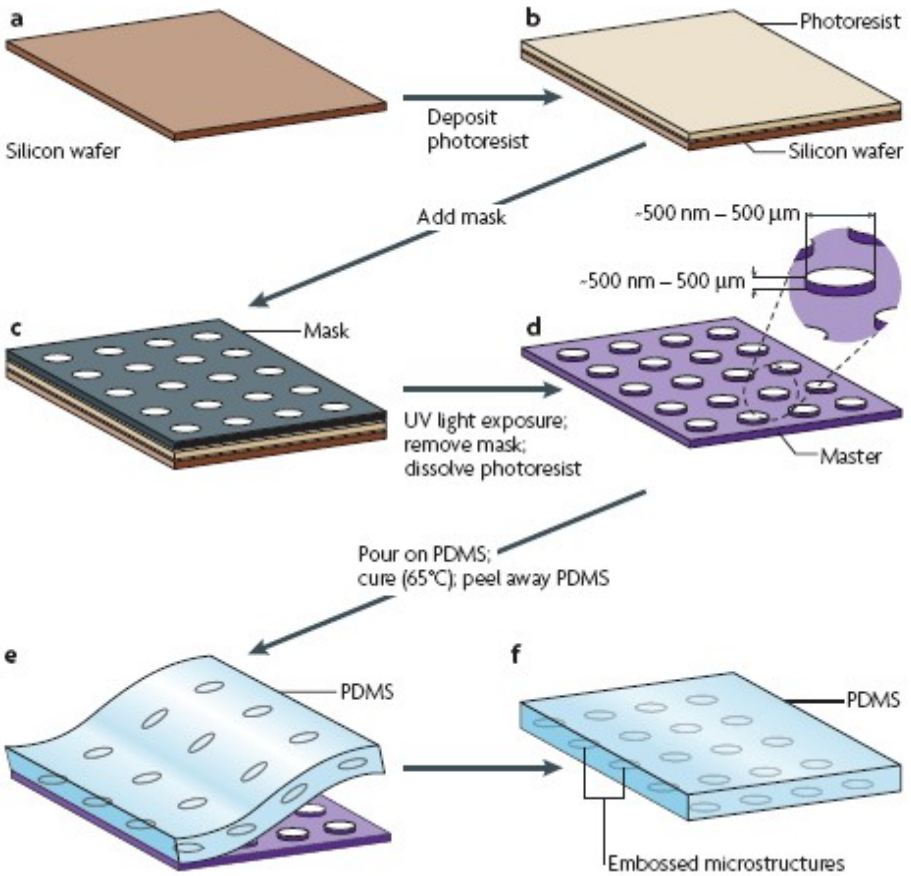


Figure 1 | **The fabrication of micropatterned slabs of PDMS. a–b** | Photoresist is spin-coated on a silicon wafer. **c** | A mask is placed in contact with the layer of photoresist. **d** | The photoresist is illuminated with ultraviolet (UV) light through the mask. An organic solvent dissolves and removes photoresist that is not crosslinked. The master consists of a silicon wafer with features of photoresist in bas-relief. An expanded view of one of the microfabricated structures with its characteristic critical dimensions is shown. **e** | PDMS is poured on the master, cured thermally and peeled away. **f** | The resulting layer of PDMS has microstructures embossed in its surface. PDMS, poly(dimethylsiloxane).

Alternative: Shrinky Dinks

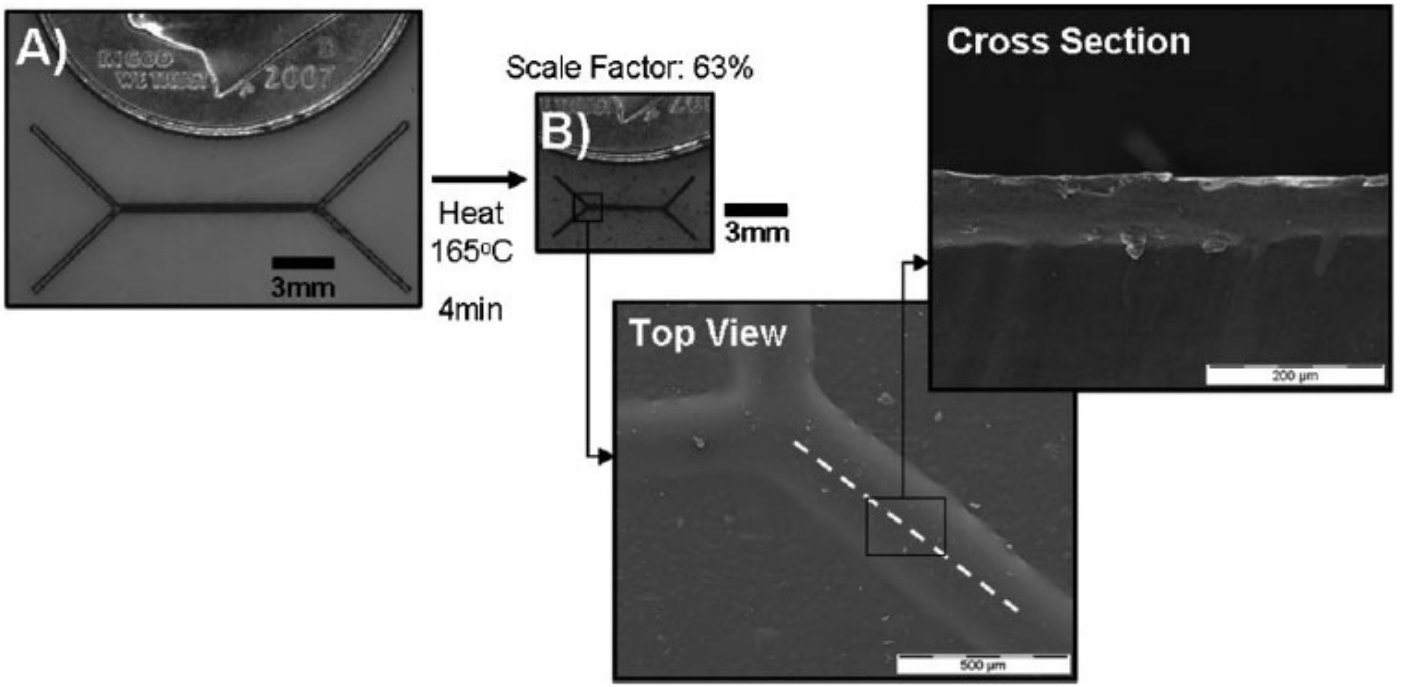


Fig. 1 ***Shrinky Dinks mold generation***: (A) Unshrunken Shrinky Dink master with laser printed master pattern. (B) The same master after being baked. Masters shrink from 800 to 300 µm in width. The original height of the ink (as measured on transparencies instead of unshrunk thermoplastic because the thermoplastic heats up and shrinks when prepared for the SEM) was approximately 10 µm but was difficult to measure precisely on the SEM (see Fig. 3(b)). Insets: SEM of channel top view and cross-section through a shrunken channel, illustrating a height of 80 µm. Heights varied depending on the printer used. For this 80 µm channel, we double printed with transparency mode on the Hewlett-Packard LaserJet 2200D.

Shrinky Dink Characterization

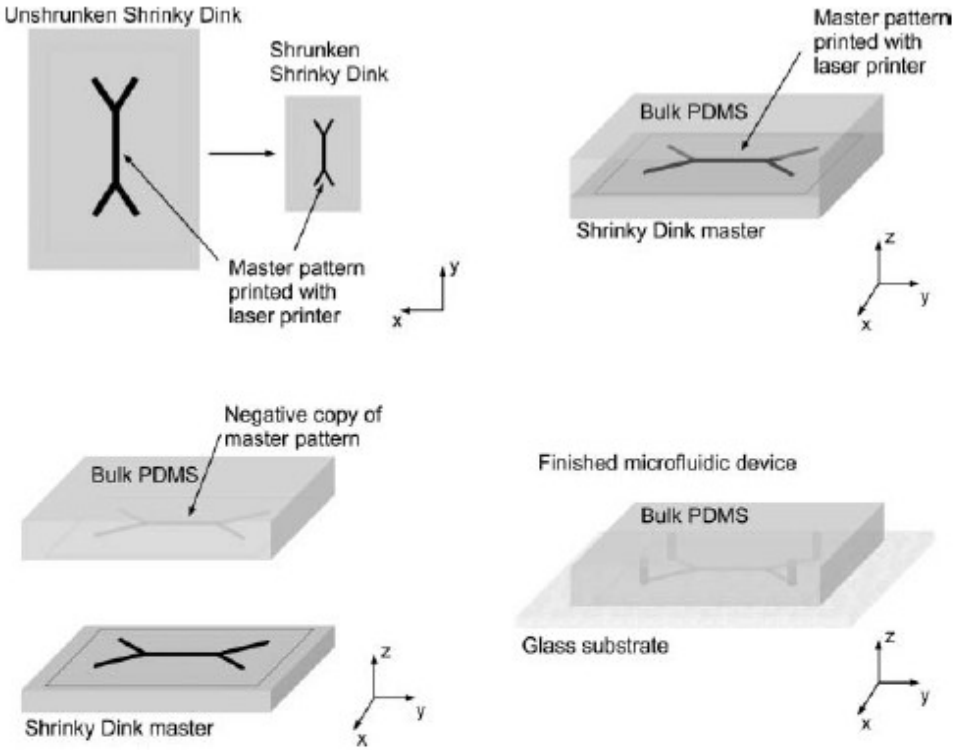


Fig. 2 ***Process flow***: (A) The master pattern is produced using CAD software and printed directly onto the Shrinky Dink. After baking, the pattern shrinks by 62.5%. (B) PDMS is poured over the master. (C) After curing, the PDMS is peeled off, containing a negative copy of the master pattern. (D) The molded PDMS is bonded to a glass slide to form microchannels and the finished microdevice.

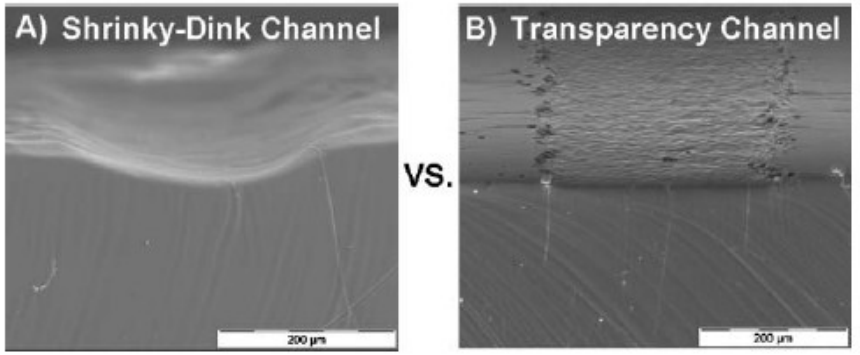


Fig. 3 ***Scanning electron micrographs (SEM) of channels made from our approach (A) compared to those made using transparencies (B)***: (A) Perspective SEM of channel made using Shrinky-Dink mold *vs.* (B) transparency. The difference in height and shape is apparent.

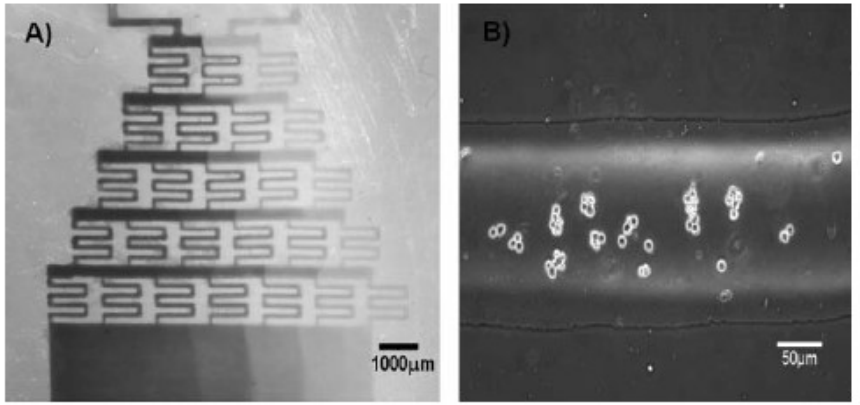


Fig. 4 ***Functional devices***: (A) Bonded PDMS gradient generator with food dye. (B) Bright field image of CHO cells flowing through channel.

Neuron Process Guidance

- Patterned substrate with laminin, blocked with PEG
- Incorporation with cell culture environments, chemical gradients is possible (e.g. Heilshorn group)

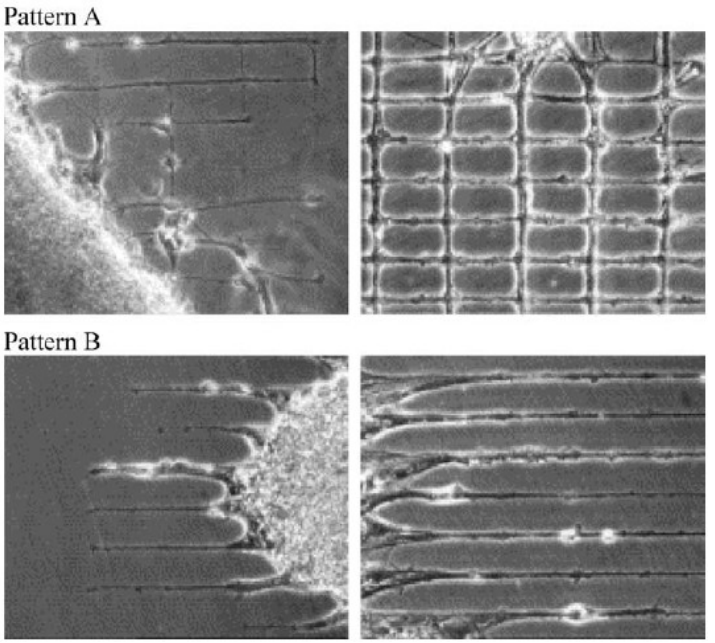


Fig. 8 Evaluation of a variety of extracellular matrix protein patterns. During the early days of the culture (left, day 3), the migrated neurons and growing processes are clearly visible with little overlapping on the patterns. These cultures continued to mature, and by ten days or more (*right*) the original shapes of the laminin stamped patterns were identifiable (reprinted from Yeung et al [143], with permission from Elsevier)

Patterning Other Things

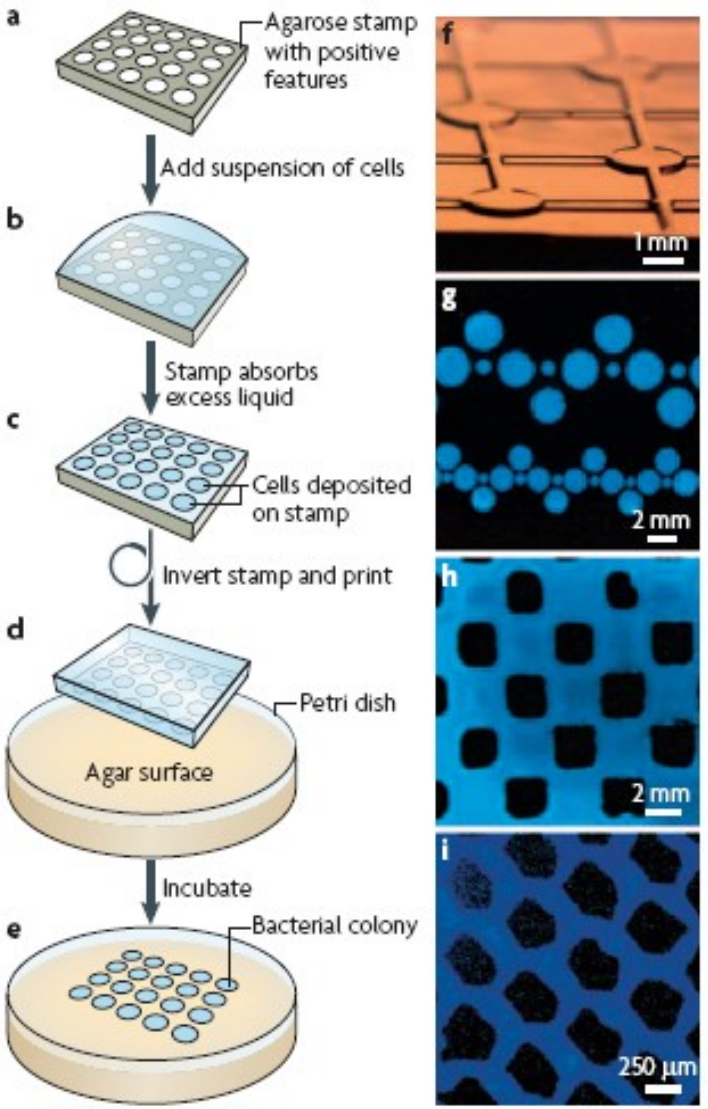
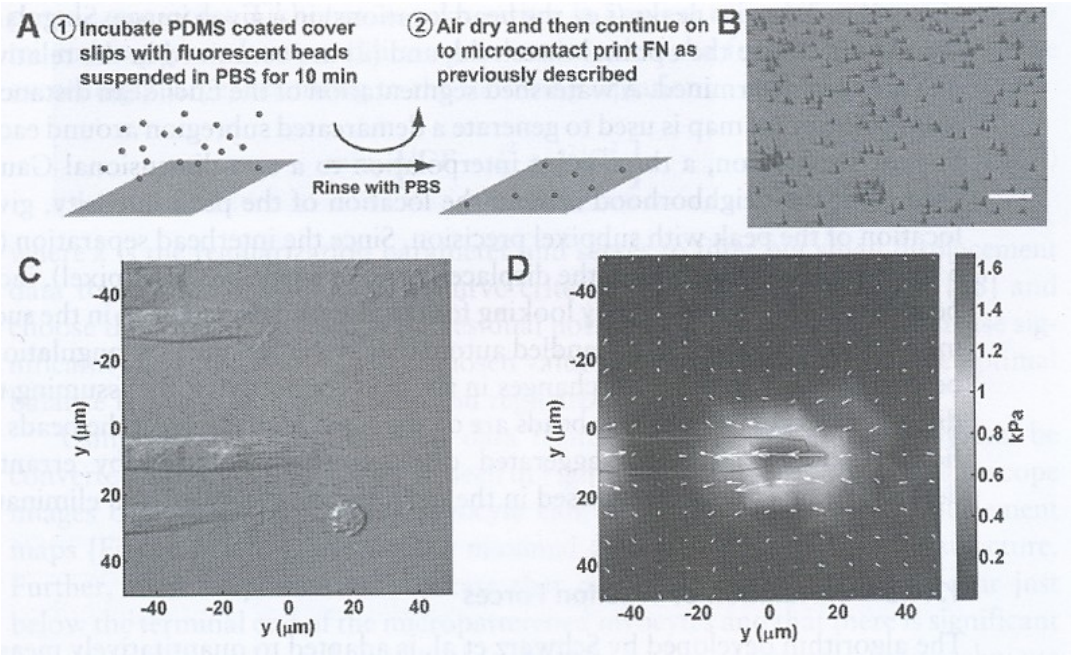


Figure 3 | **A method for microcontact printing patterns of bacteria using agarose stamps. a** | A cartoon of a stamp. **b** | The stamp is 'inked' with a suspension of bacterial cells. **c** | The stamp absorbs the excess liquid leaving bacteria on the surface. **d** | The stamp is inverted and brought into contact with agar in a Petri dish (or other surface). Cells are only transferred from the raised features of the stamp. **e** | Incubating the plate produces a pattern of bacteria. Hundreds of replica patterns can be produced from a single stamp. **f** | An image of a stamp. **g–i** | Patterns of *Vibrio fischeri* imaged by recording luminescence. Reproduced with permission from REF. 27 © (2005) American Chemical Society.

Related: Measuring Forces

- Micropost array
  - Cells seeded on array of posts
  - Measure force from post deflection
  - Works for motile cells
- Traction force microscopy
  - Flat, compliant cell culture substrate
  - Add markers, calculate force from their movement



Final Points

- PDMS stamps can be used to generate protein patterns with resolution from 100nm to 100um+
- Process Outline
  - Draw pattern (e.g. Adobe Illustrator, AutoCAD, L-Edit)
  - Print mask (transparency mask or quartz)
  - Make mold (e.g. SU-8 mold on Si, inkjet on shrinky dink)
  - Cast PDMS to mold
  - Stamp protein
- Resolution
  - Quartz mask and SU-8 or Si: 500nm+
  - Transparency and SU-8: 10+ microns
  - Shrinky dink: 60+ microns

Thresholds

- Easy
  - 10 micron+, transparency mask on SU-8/Si in MERL
  - Week, $100
- Medium
  - 2 micron+, quartz mask on SU-8/Si in MERL
  - 2 weeks, $1000
- Hard
  - 500 nm+, quartz mask on Si in SNF
  - Month, $3000
- Easy
  - 100-300nm lines, squares using premade PDMS stamps
- Blocking and washing is probably critical
  - PEG, Pluronics, silane are possible blockers