Nanostamp optimization for single-molecule DNA/protein array studies

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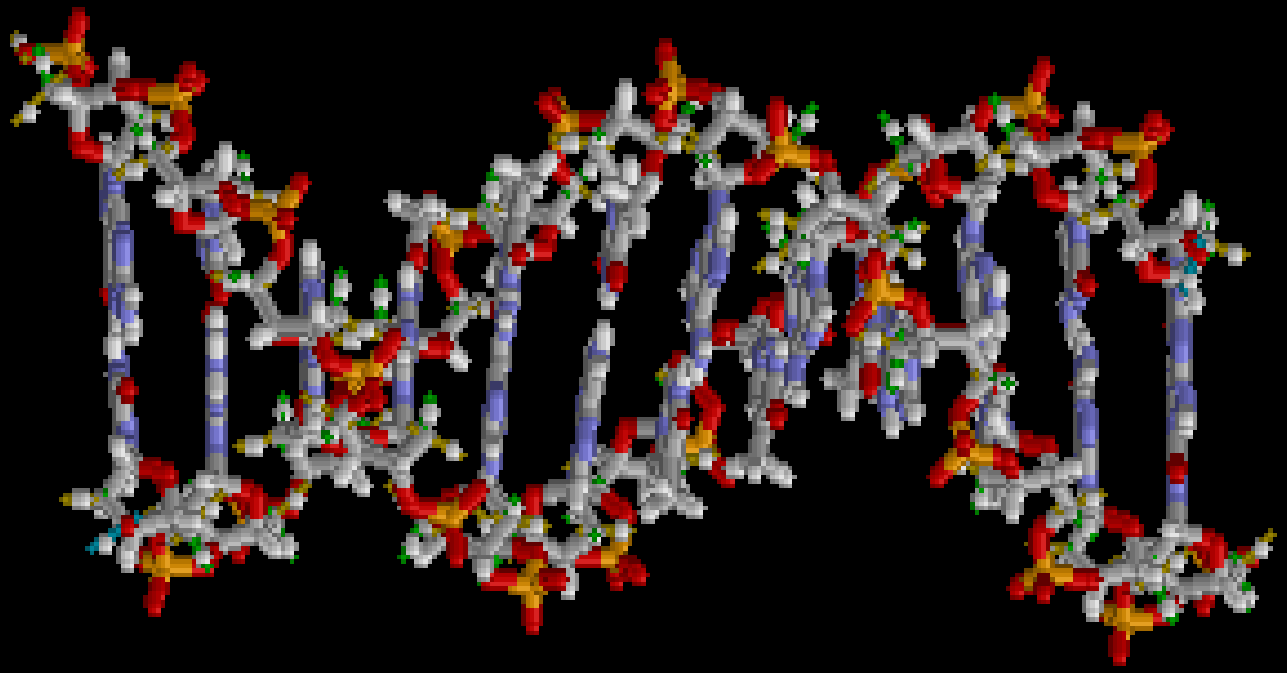
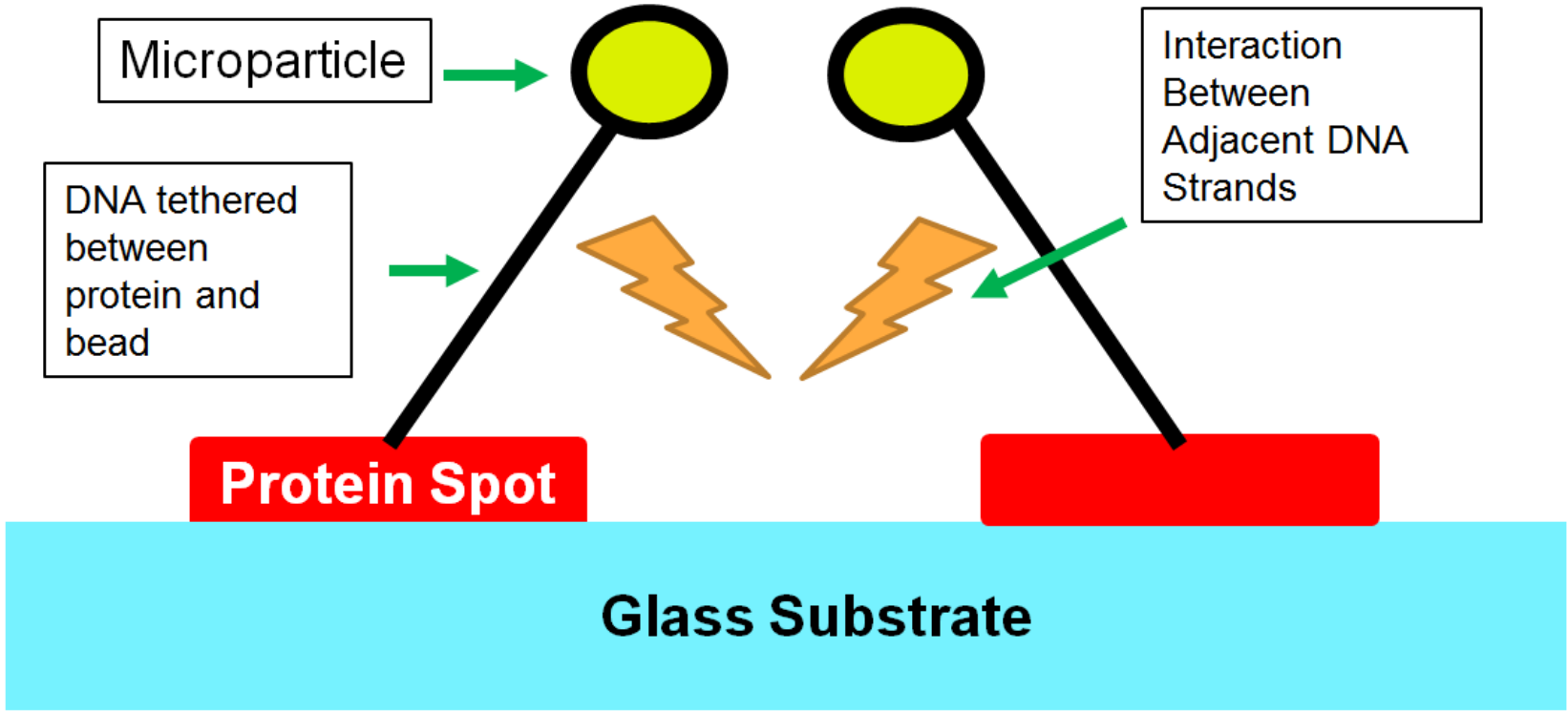
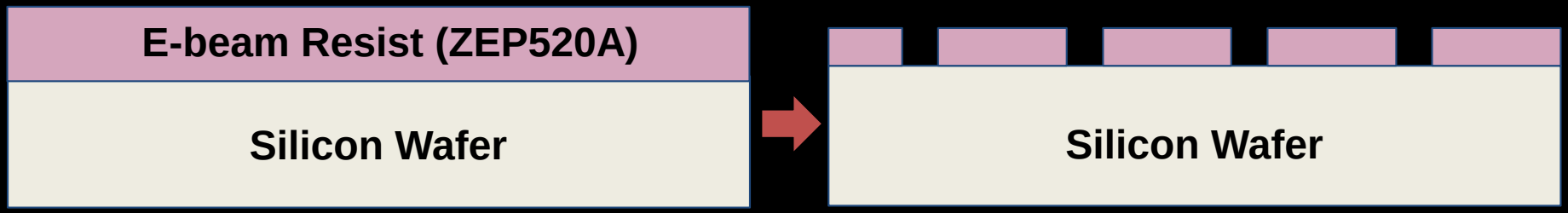
Science Motivation



Wang Lab Application



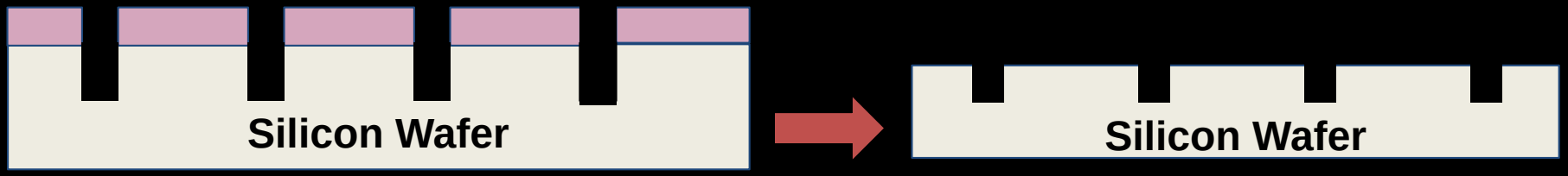
Spin coating and exposing Ebeam resist



E-beam resist: ZEP520a (300nm thick)

- Features: 100nm wide circle hexagonal array
- Tool: Electron Beam

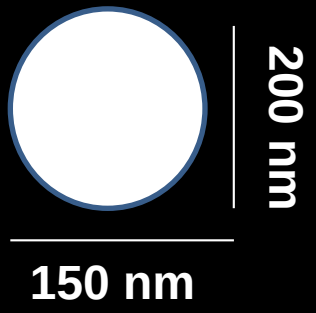
Etching Si template and removing Ebeam resist

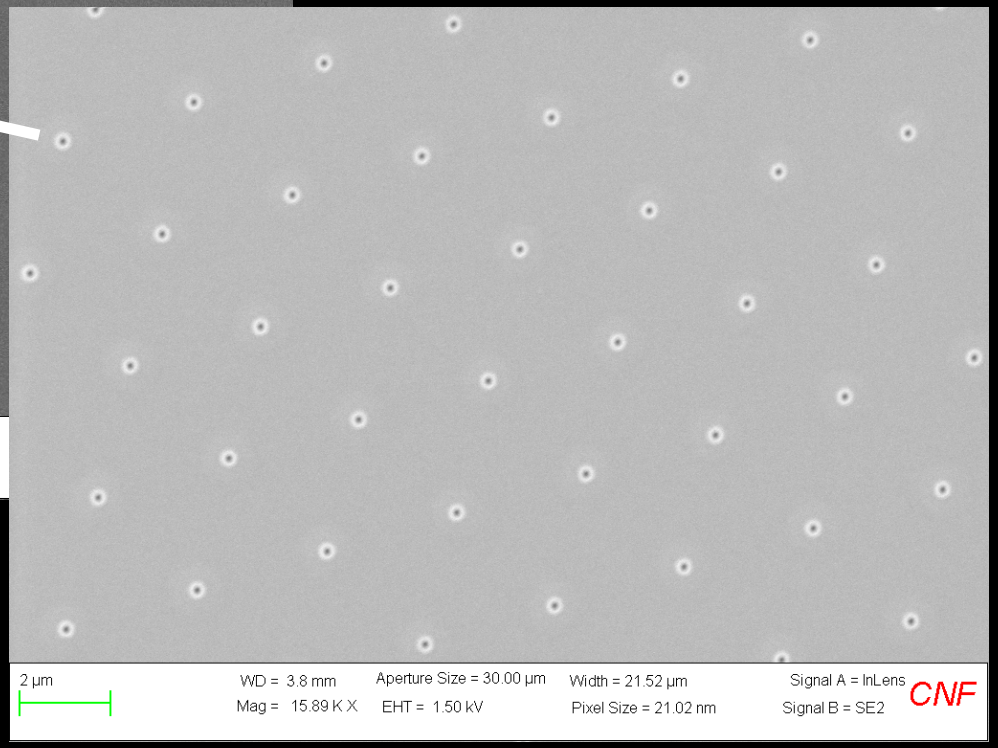


Etch Si with HBr/Ar plasma

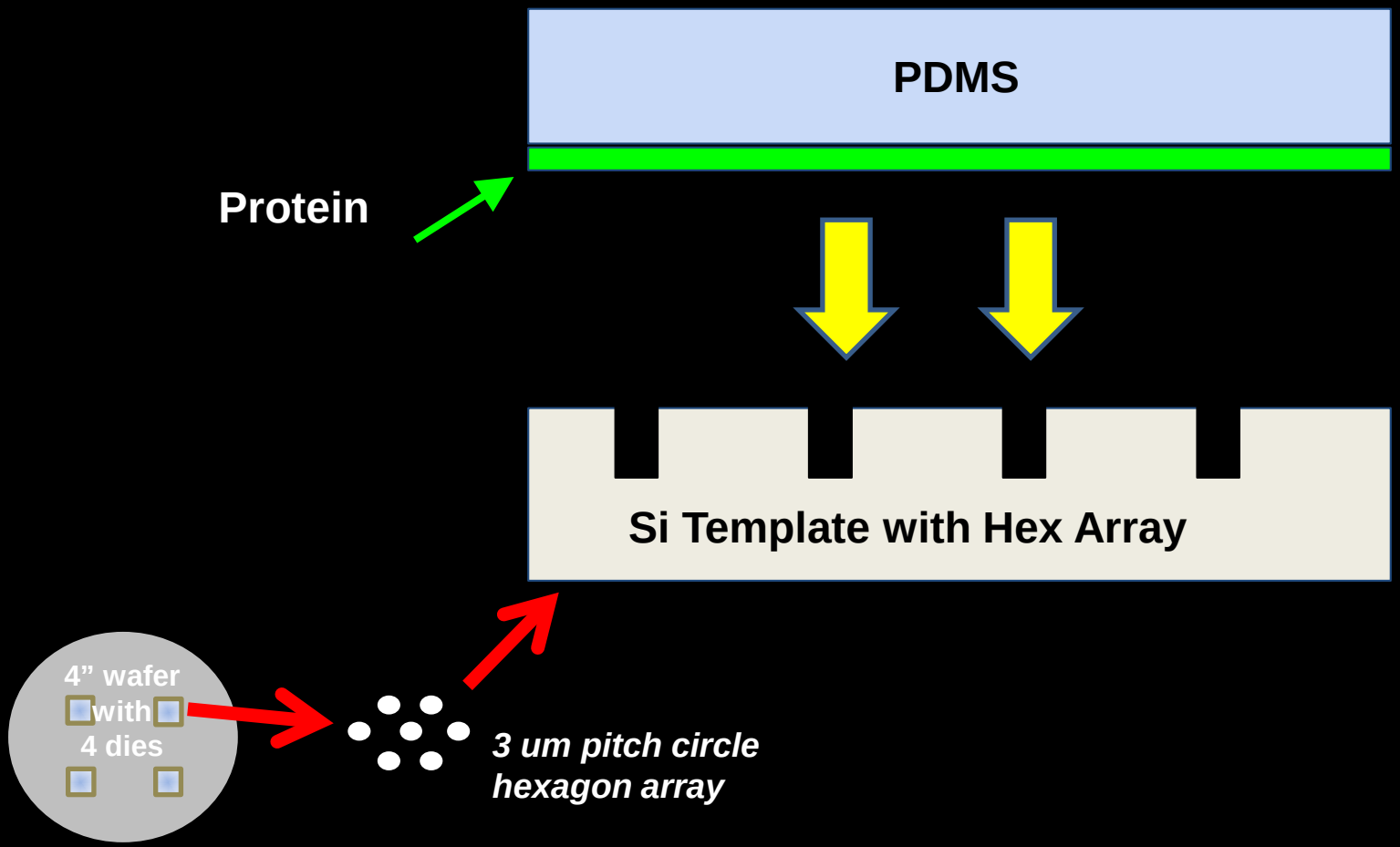
Stripper 1165 to remove Ebeam resist

Electron Beam Pattern Results

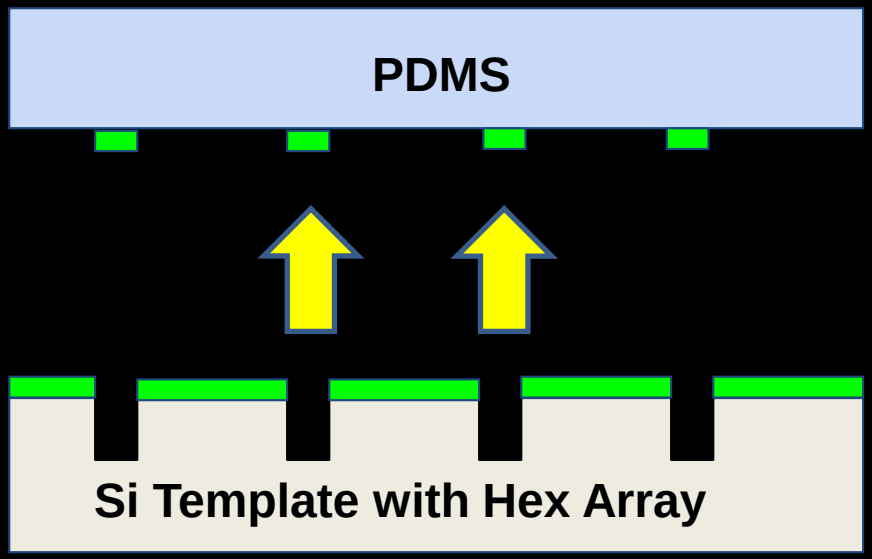




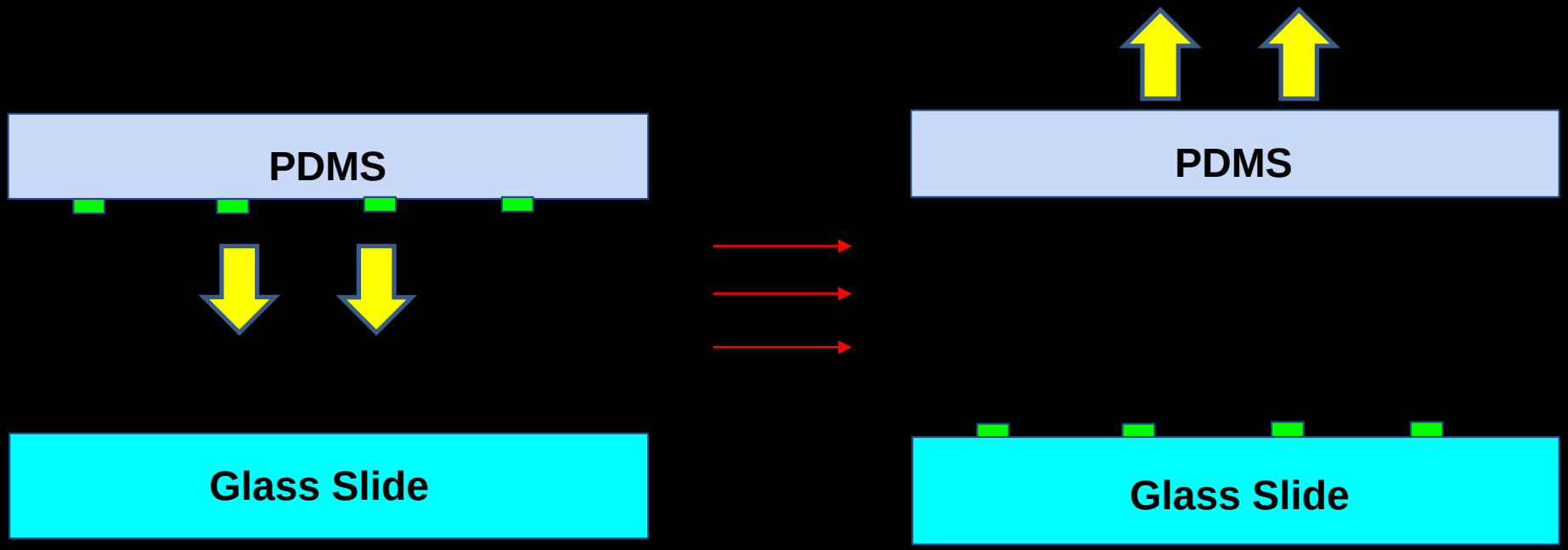
Ink Subtract Print Stamping Method



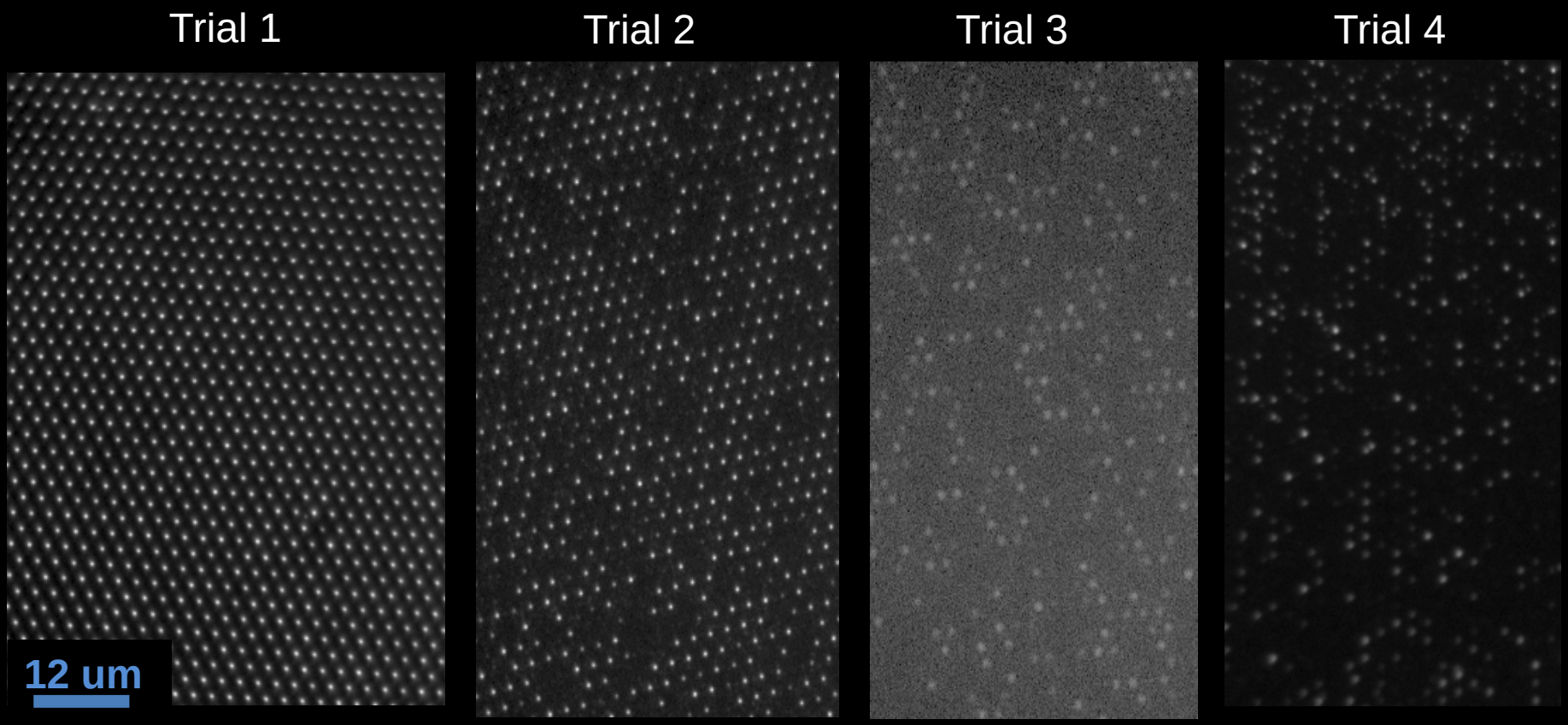
Ink Subtract Print Stamping Method



Ink Substract Print Stamping method

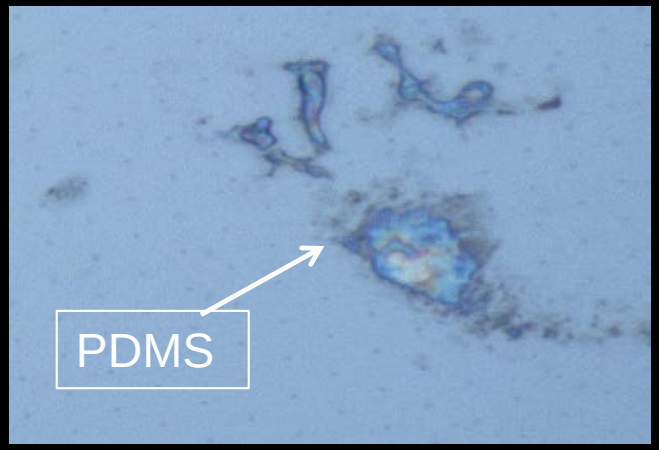


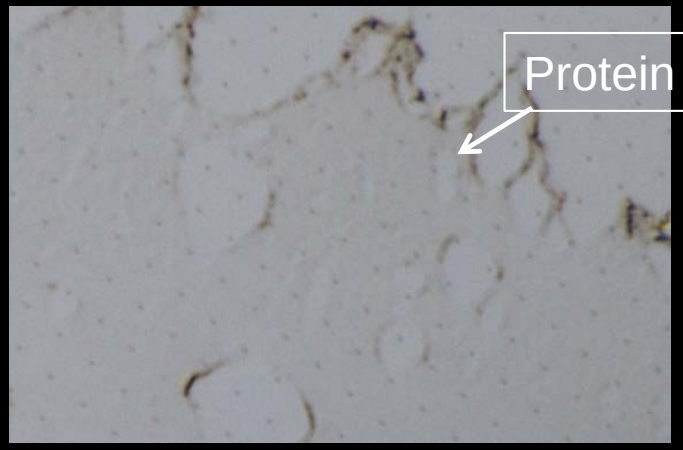
Successive Template Use



Fluorescent microscope images of stamped protein spots

Post-Stamp PDMS/Protein Cleaning Protocol

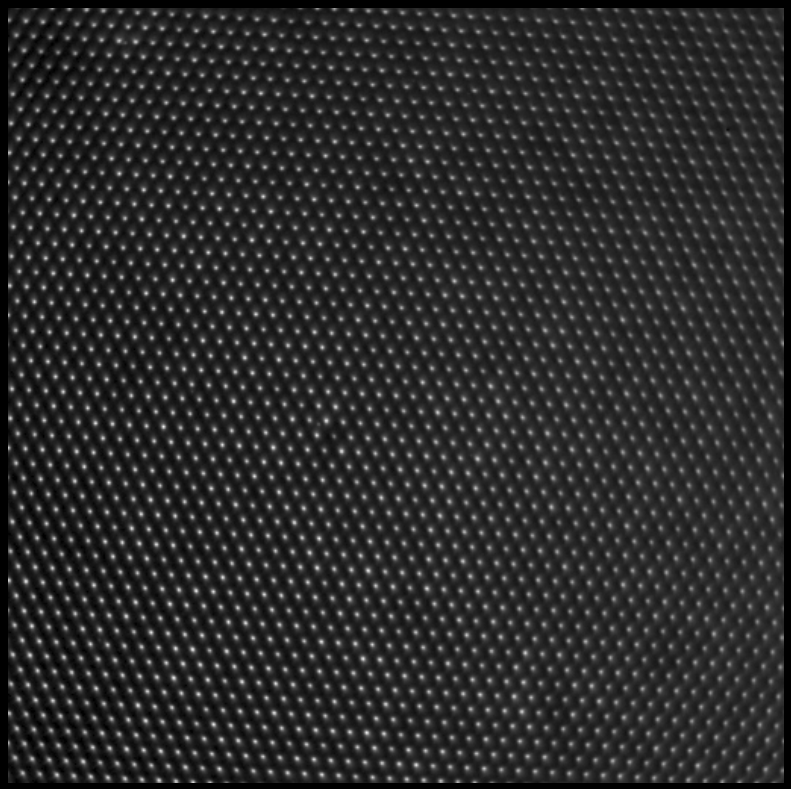


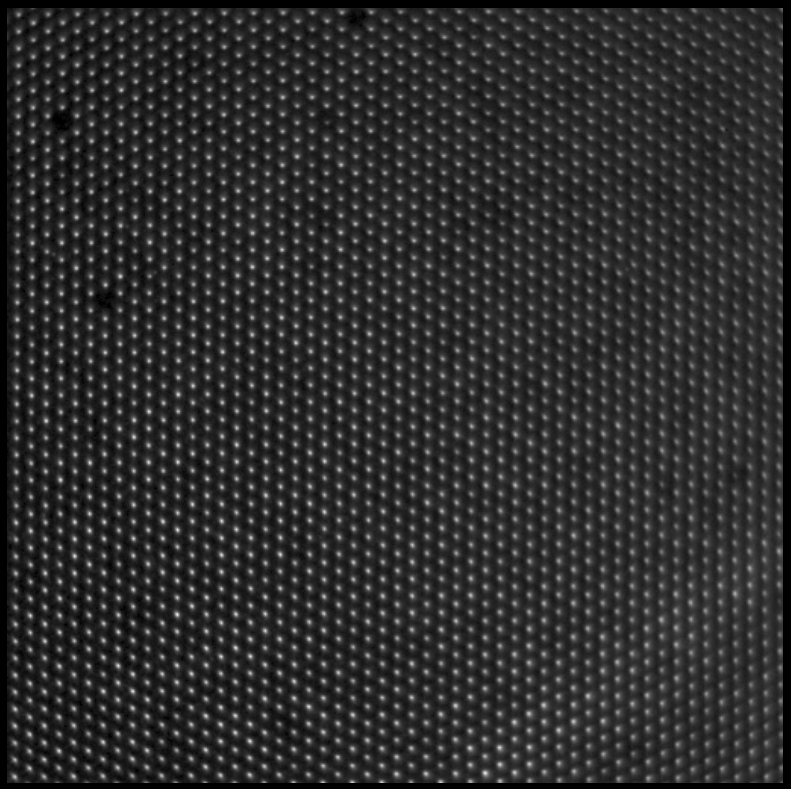


Cleaned protein with acetone/piranha, but no Buffer Oxide Etch(BOE)

Cleaned PDMS with BOE, but did not clean protein.

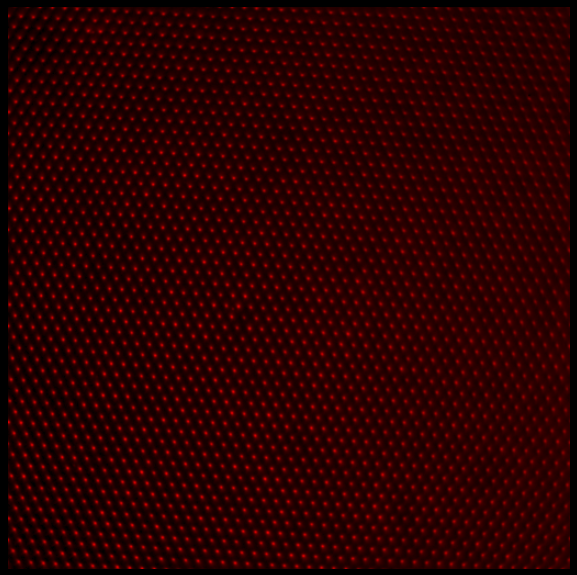
Post-cleaning stamping results

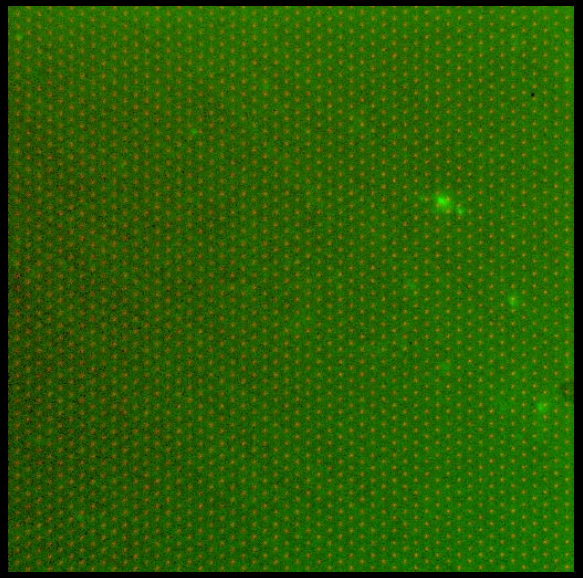


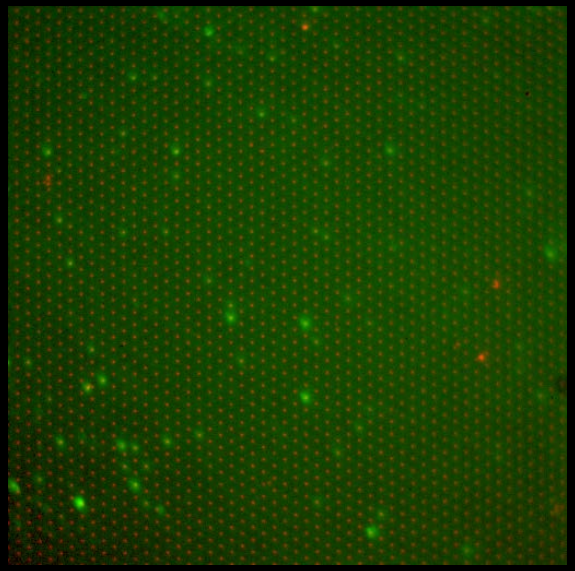


DNA tethering with protein

Patterned-Protein Spots (no DNA yet)	After 4 hours of DNA incubation time	After 17 hours of DNA incubation time

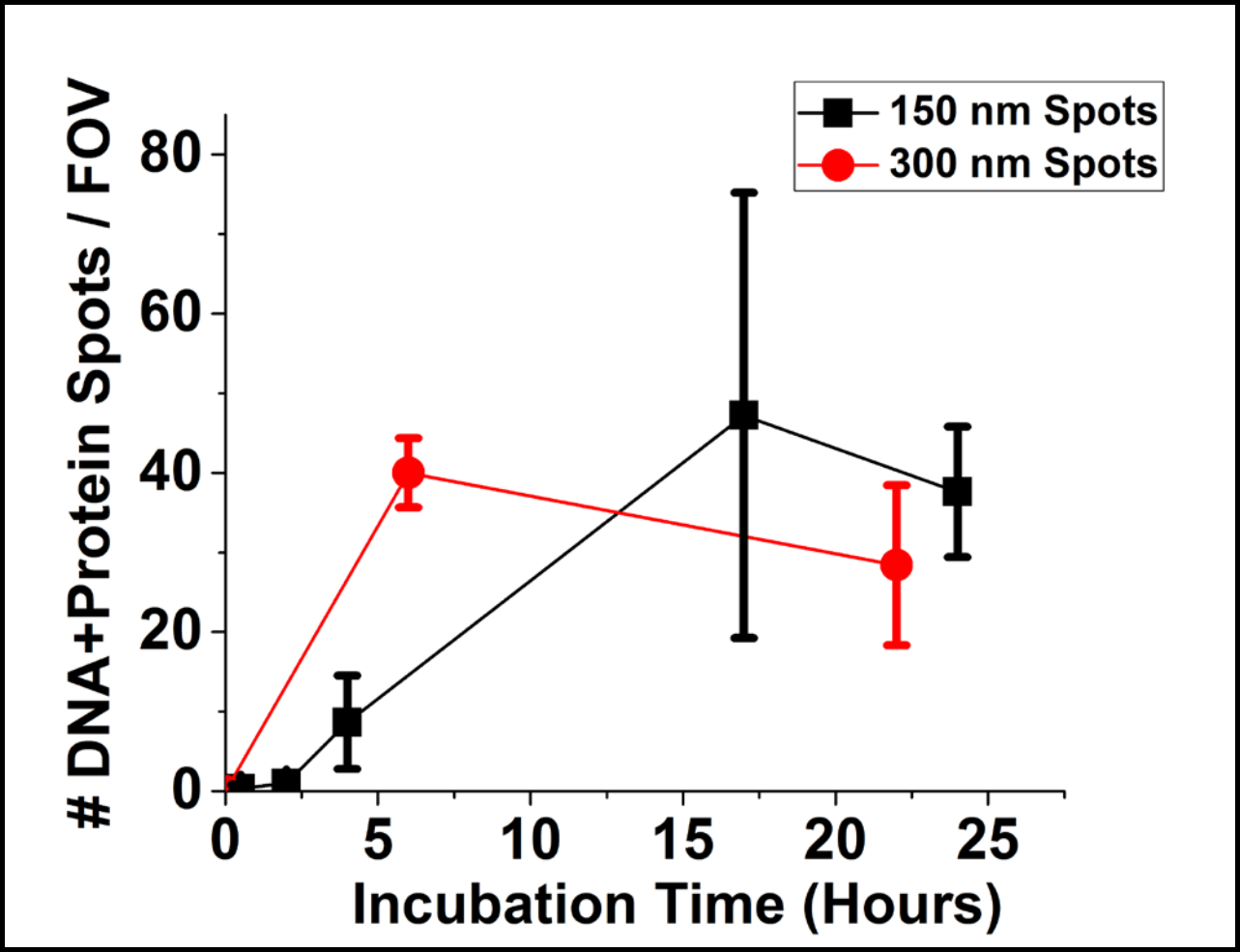






Green dots = DNA
Red dots = protein spots

Analysis of Incubation Time and Amount of DNA Tethers



- 13.7 Kb DNA
- Error bar in each data point is calculated from 5 different fields of view.

Moving Forward

- **There are several ways to improve the number of DNA tethers in the future:**

(1) Test larger stamp circles, still < 500nm
(2) Tether DNA to magnetic bead and "pull-down" beads to glass surface to increase chance of tethers forming
(3) Increase DNA concentration

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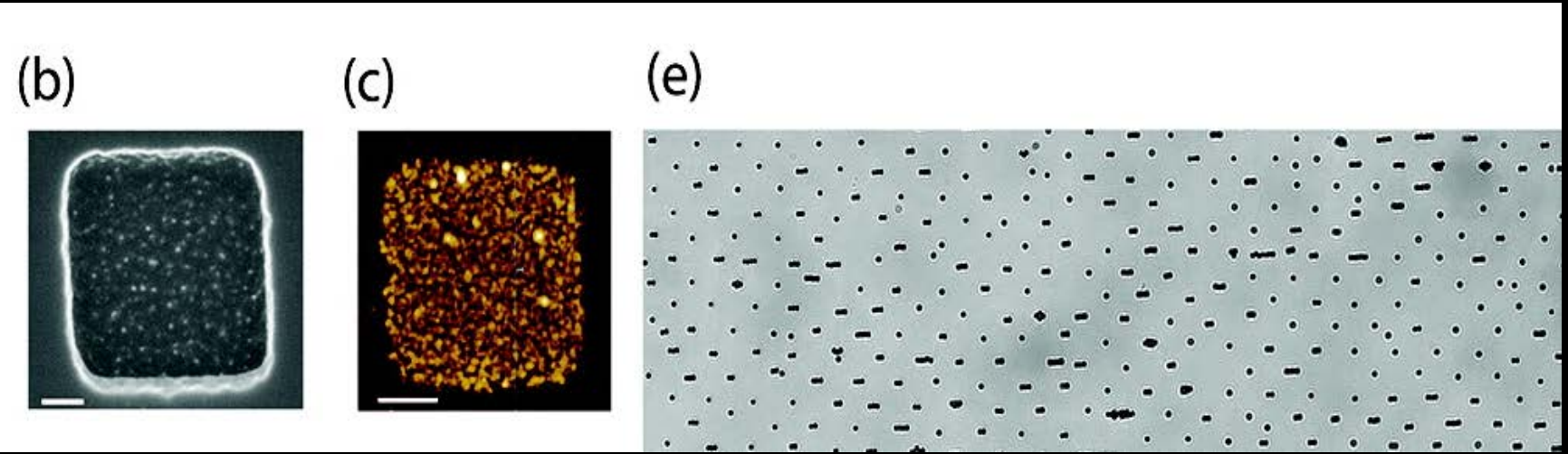
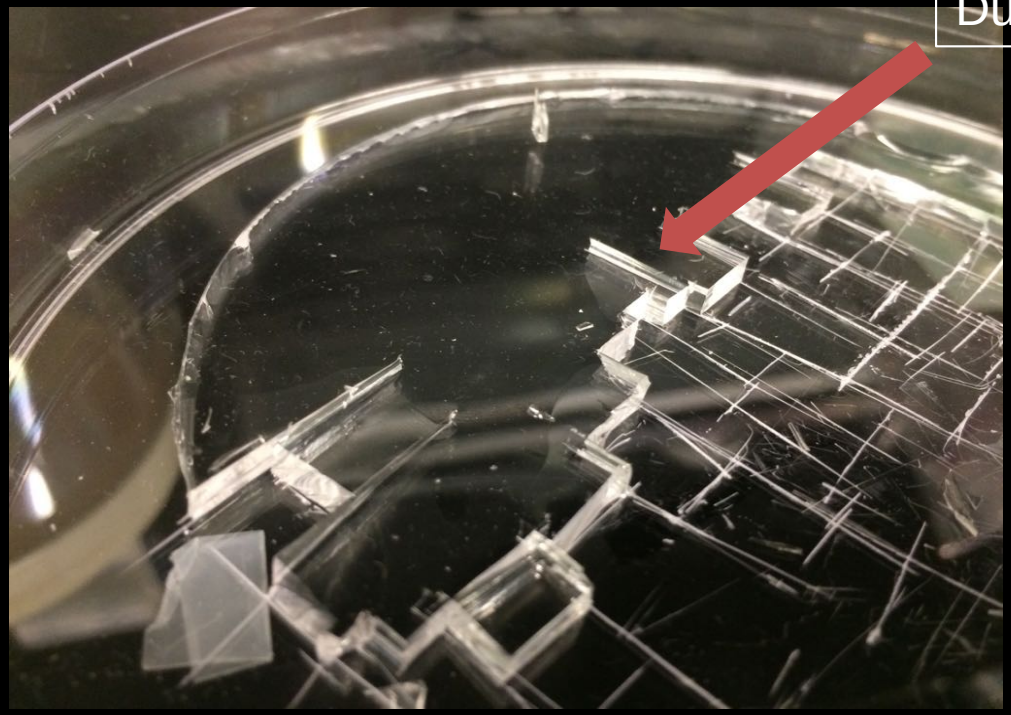
Our Spot Size Compared to Dekker Lab



--They make squares instead of circles, the scale bar in the SEM in (b) is 100 nm, so the spot is around 500 nm. Scale bar is 200 nm in (c)

Reference: De Vlaminck, Iwijn, et al. "Highly parallel magnetic tweezers by targeted DNA tethering." Nano letters 11.12 (2011): 5489-5493.

Never stamp with dirty PDMS



---PDMS left around for 1 month was used that was visibly dirty, this left hard to remove residues over the template.

---The templates cost ~$900, so use fresh, clean PDMS to avoid permanent damage to the template.

Clean Note

The Clark Hall clean failed. Whoever is going to use these stamps should probably get trained to use the general chemistry hood in CNF to do the effective clean we established there.

It is very easy to do and does not require much training.