

Engineering Research in Undergraduate Studies: Neural Engineering

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Summer 2005

Acknowledgements

- Would like to thank:
 - Dr. David Schneeweis, UIC Neuro-engineering
 - Sujata Sundara-Rajan, Graduate Student Mentor
 - Dr. Takoudis, Dr. Linninger; REU Program Directors
 - **NSF** EEC-0453432 Grant, Novel Materials and Processing in Chemical and Biomedical Engineering

Presentation Overview

- Overview of 10 week research
- Results with Images
- Final Conclusions
- Remaining Issues

Original Project Goals: Micropatterning

- Silicon Molds



- Rubber-like PDMS stamps with micro-sized features



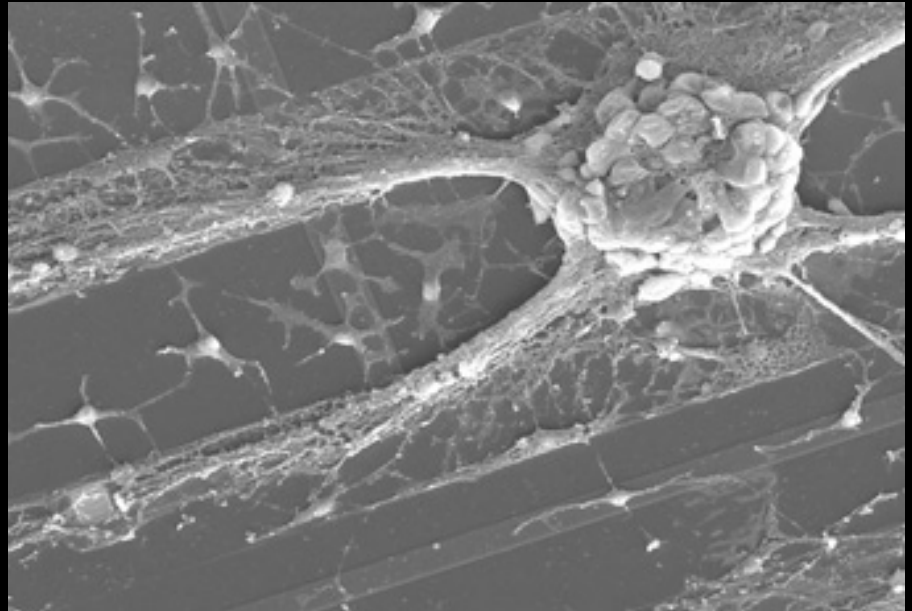
- Stamps "inked" with protein



- Patterns of protein on glass slides

Motivation for Research

- Develop substrate with micropatterned proteins
- Surface induces living neurons, cells to grow into and interact with substrate
- Optimizing “interface”



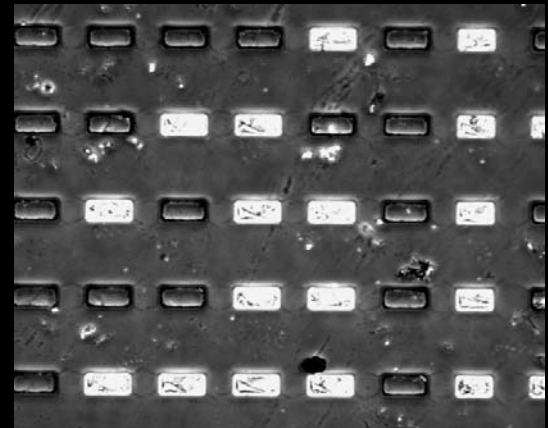
B. Wheeler.
<http://soma.npa.uiuc.edu/labs/wheeler/home.html>, 2005.

Obstacles Encountered

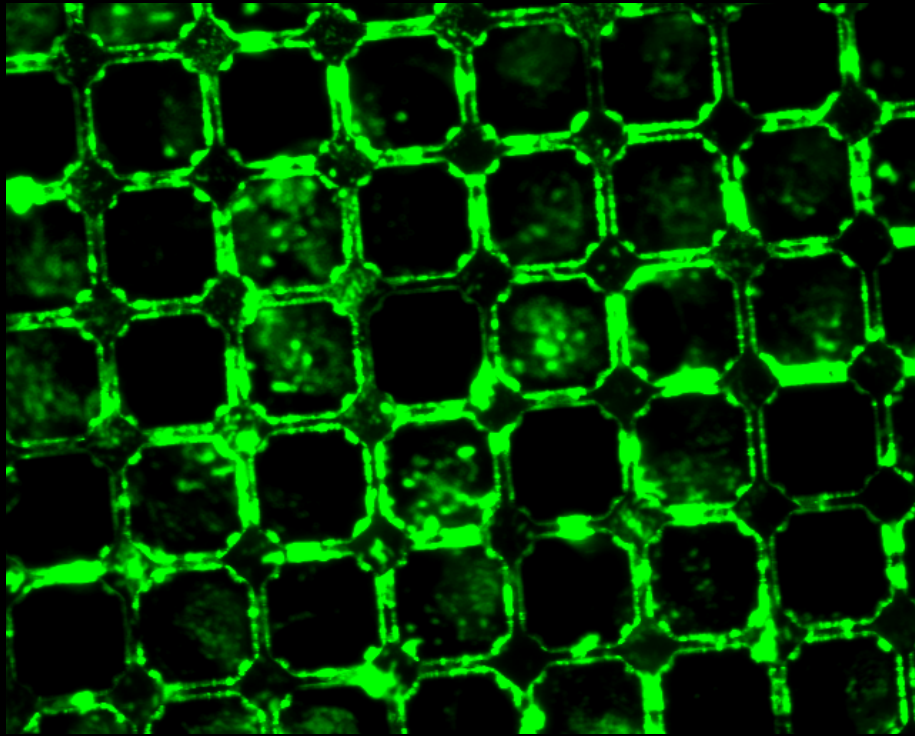
- Set out to find “optimal protocol” for
 - Making Stamps
 - Inking Stamps with Protein
 - Transferring protein to glass substrates
- Experienced difficulties at every step of the process

Obstacle: Making Stamps

- Air bubbles
 - Trapped at relief surface
 - Trapped within stamp body
- Missing Features
 - Pattern not complete
- Stamp surface unclean
 - Too much contamination



Obstacle: Inking Stamps



- Surface too hydrophobic to adsorb protein
- Protein adsorbs in inconsistent patches, uneven thickness

*10x magnification, 20 um features

Obstacle: Stamping Substrates

- Protein does not transfer to substrate in any visible quantity
 - No results can be shown

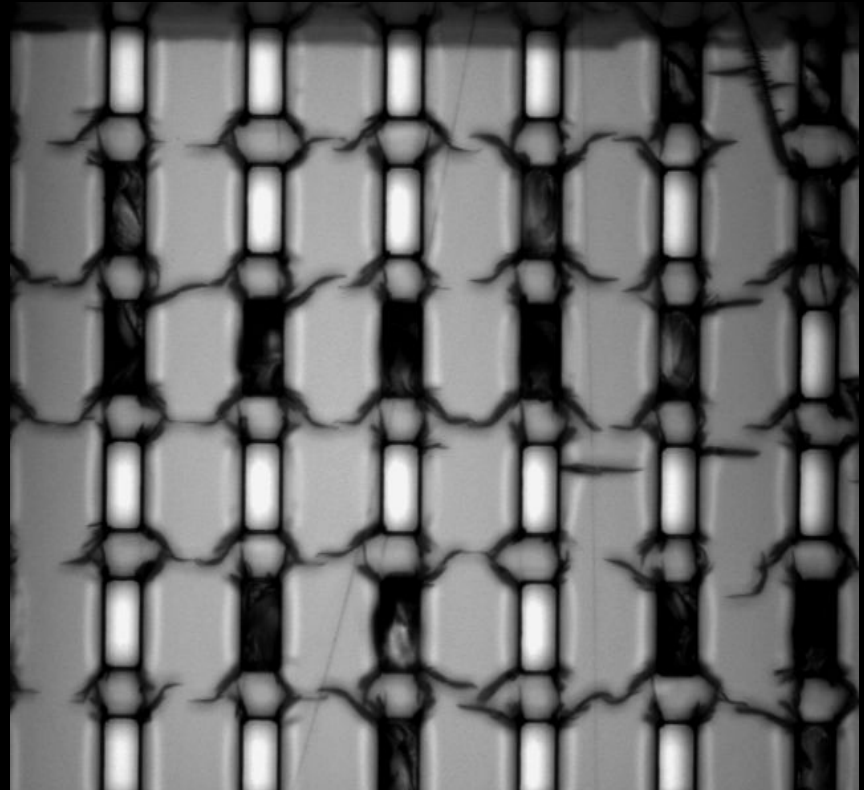
*Note: Have only limited trials with stamping

Obstacle: Most Problematic

- Stamp quality continued to decline, though were “improving” process
- Molds themselves were degraded with use
 - Unable to make new molds, hindered progress

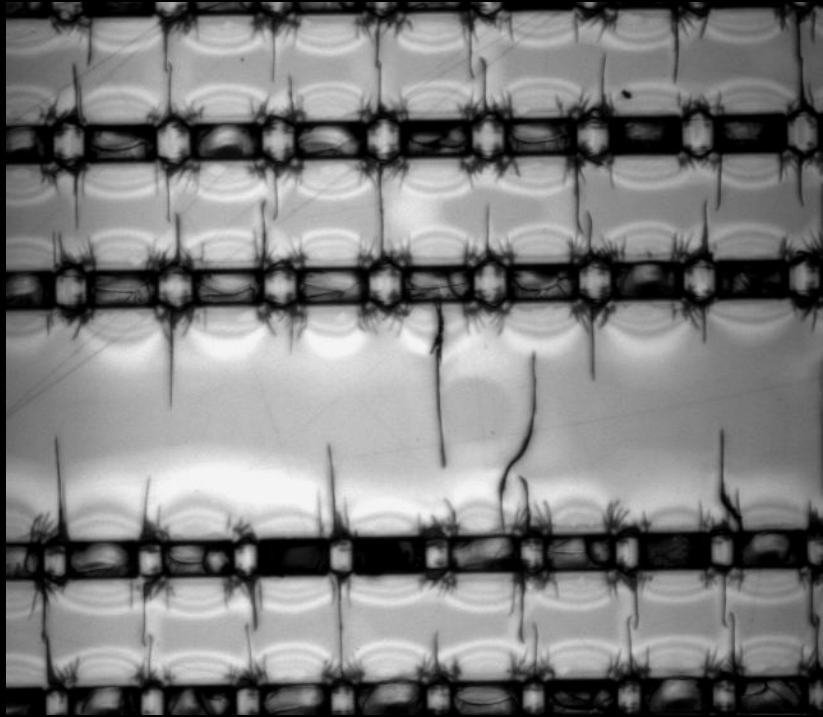
Obstacle: Mold Degradation

- Molds became “dirty” over time
 - Pattern depressions filled with unknown material
 - Could not be cleaned successfully



*10x magnification, 15 um features

Obstacle: Mold Degradation



- Features started "breaking"
 - Surface showed cracks, missing pieces

*10x magnification, 15 um features

Conclusions: Making Stamps

Air Bubbles:

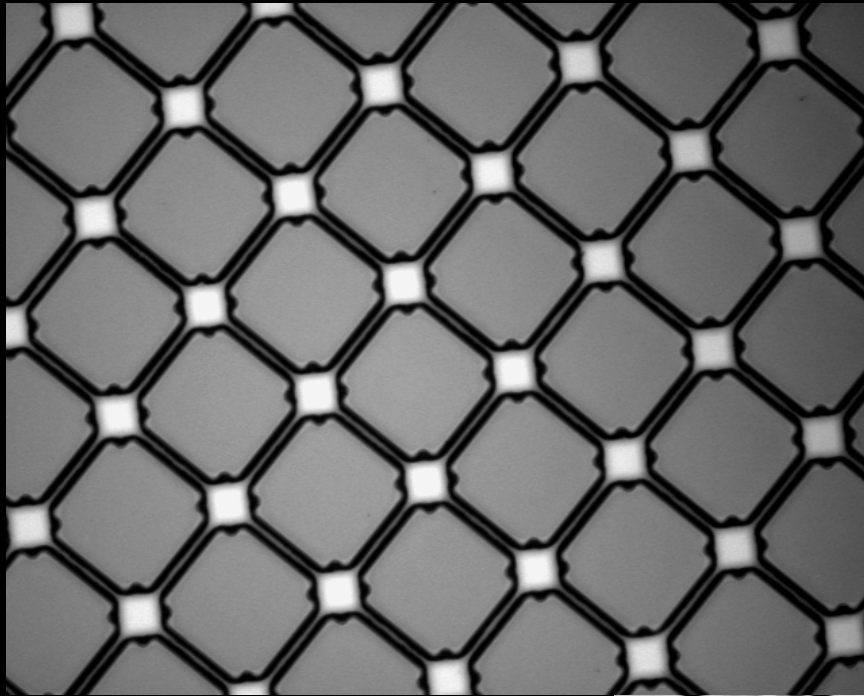
- Re-pressurize vacuum to eliminate surface air bubbles
- Heating stamps reduces curing time
 - Must make stamps thinner to prevent trapping bubbles

Conclusions: Making Stamps Con't

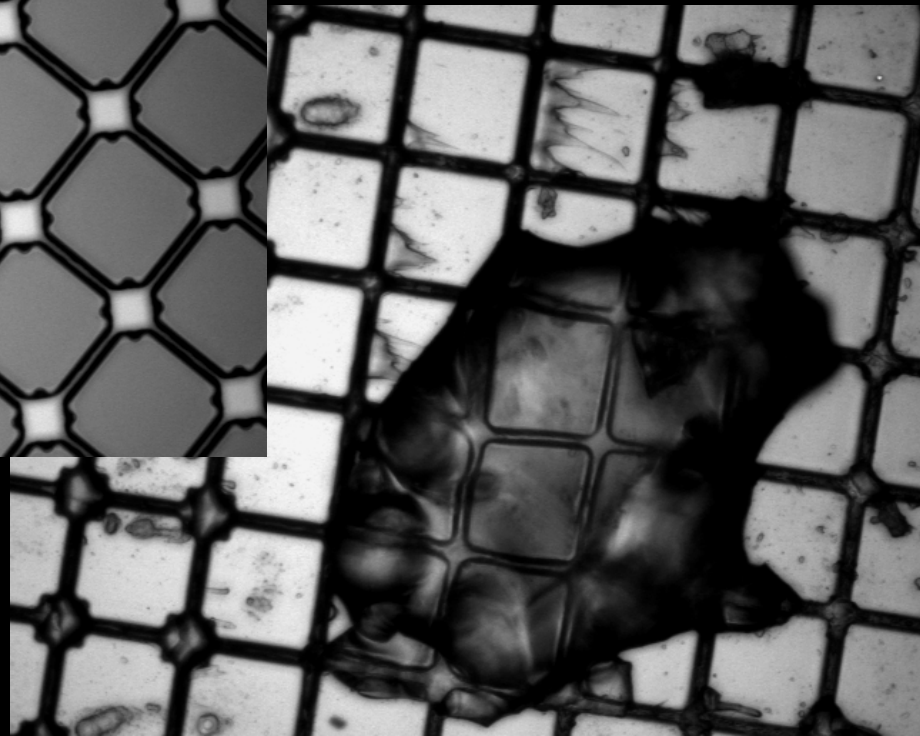
Missing Features:

- Best *not* to have other material between mold and PDMS
 - No Detergent: plain stamps have best quality
 - No silanization procedure
- Perfect mold required to make perfect stamps

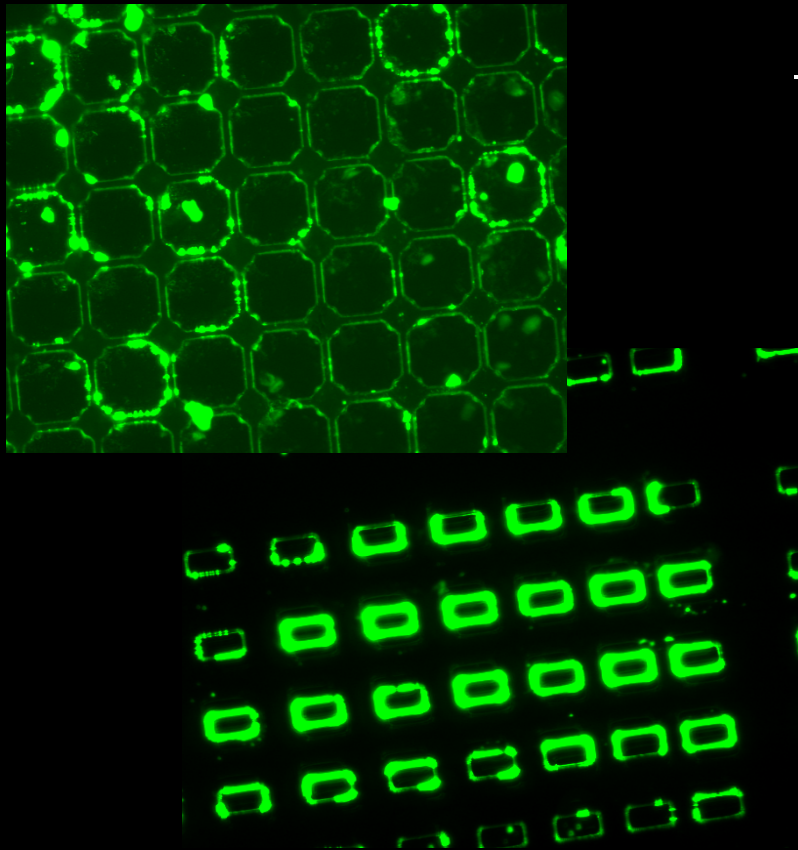
Differing Stamp Quality



*10x magnification, 20 um features



Conclusions: Inking Stamps



Inking time:

- Let protein sit on stamp at least 30 min
 - More time= no difference
 - Less time= less protein

*Note: Storing PDMS in deionized water for 4+ days before inking helped adsorb protein

*10x magnification, 15 um features

Conclusions: Stamping the Substrate

- Cannot produce good substrate printings without thick, even layer protein adsorbed on stamp
- Important to keep time between drying stamp and stamping substrate minimized

Proposed Future Work

- Questions still to address:
 - Understand why molds degrade
 - How to perfect stamp modification
 - Determine best stamping method
 - Culture cells onto protein-stamped substrates

References

- 1. H Kolb. How the retina works. American Scientist, Jan.-Feb. 2003.
- 2. J. Chang et al. A modified microstamping technique enhances polylysine transfer and neuronal cell patterning. Journal of Biomaterials, 2003.
- 3. D. Branch et al. Long-term stability of grafted polyethylene glycol surfaces for use with microstamped substrates in neuronal cell culture. Journal of Biomaterials, 2001.
- 4. D. Branch et al. Long-term maintenance of patterns of hippocampal pyramidal cells on substrates of polyethylene glycol and microstamped polylysine. IEEE Transactions on Biomedical Engineering, March 2000.
- 5. A. Bernard et al. Microcontact printing of proteins. Journal of Advanced Materials, July 2000.
- 6. B. Wheeler et al. Microcontact printing for precise control of nerve cell growth in culture. Journal of Biomechanical Engineering, February 1999.
- 7. R. Kane et. al. Patterning proteins and cells using soft lithography. Journal of Biomaterials, 1999.
- 8. D. Branch et al. Microstamp patterns of biomolecules for high-resolution neuronal networks. Medical & Biological Engineering & Computing, 1998.
- 9. B. Ujhelyi and K. Garsha, Patterned growth of neurons in vitro. Beckman Institute for Advanced Science and Technology, UIUC, 2003.
- 10. B. Wheeler et al., <http://soma.npa.uiuc.edu/labs/wheeler/home.html>. UIUC, 2005.

Thank You!

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- All RET/REU Colleagues
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