Joey's Status Update

Weekly Worm Meeting

August 16th, 2006

What I'm Doing (Last Time)

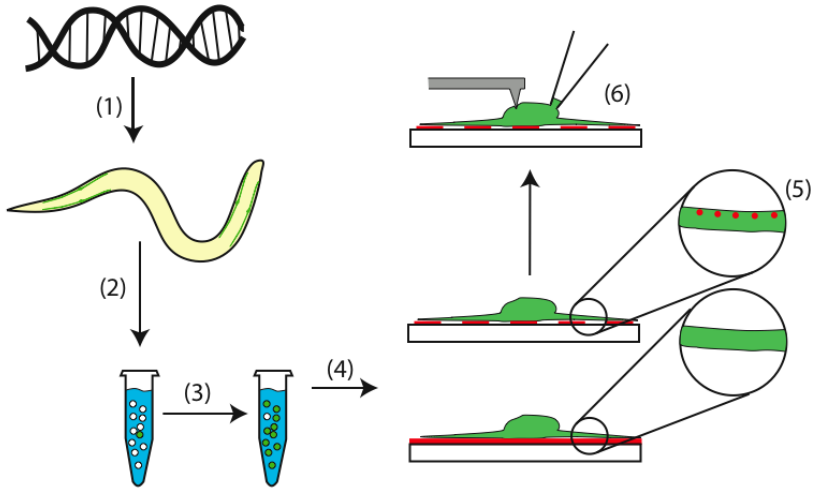
- Collagen for TRN Culture
  - Working with stamping right now, microfluidic channels soon
- *In vivo* imaging of collagen
  - On hold while An is in Belgium
- Real-Time Automatic Worm Tracking
  - Same as last time

What I'm Doing

- Microcontact printing of collagen
- Microfluidic patterning of collagen
- Remaking wafers for Shawn

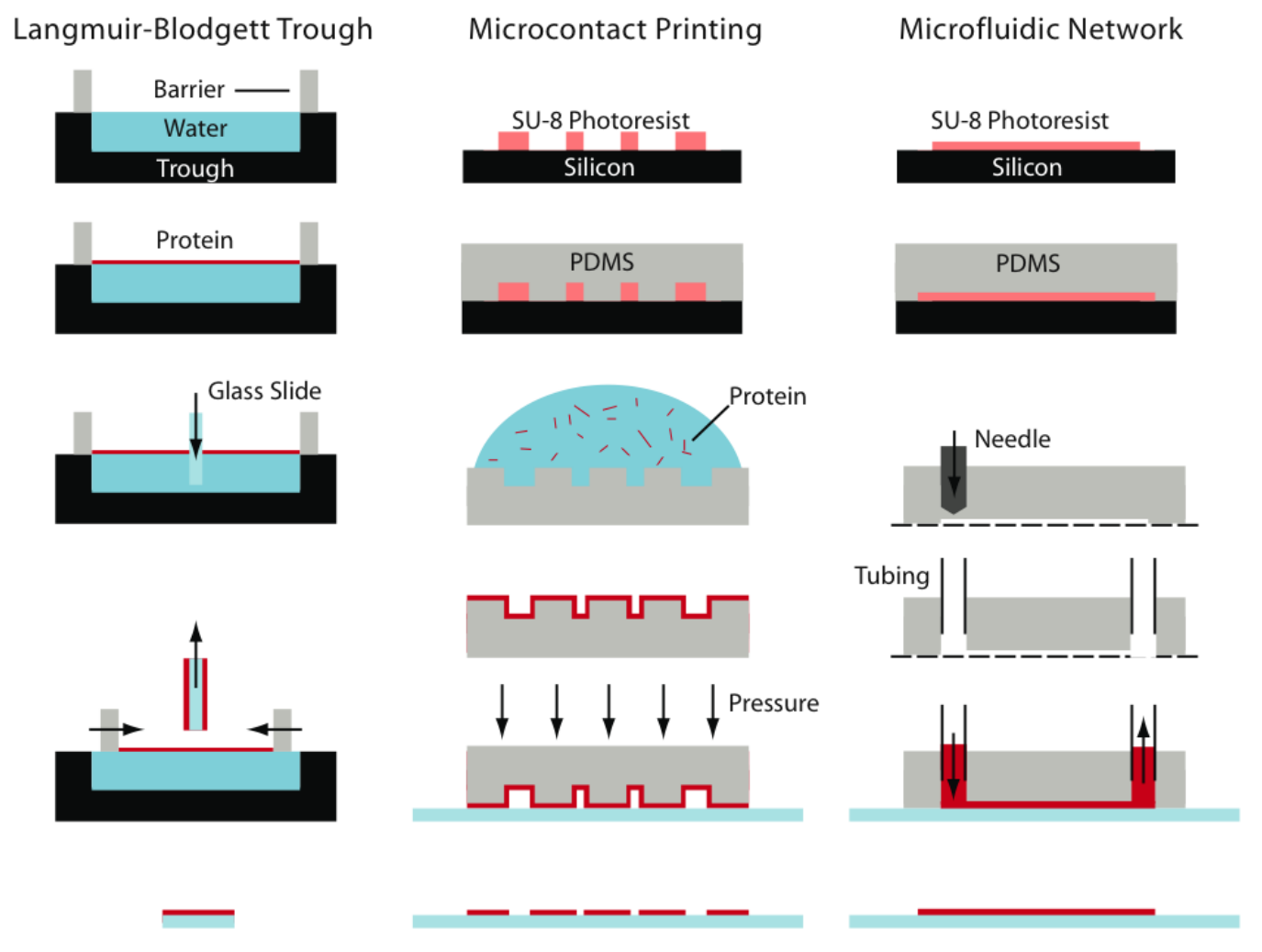
- Misc Stuff
  - Asher in clean shop works now
  - Asha-Dee has computer for simulations

In Vitro TRN Cell Culture

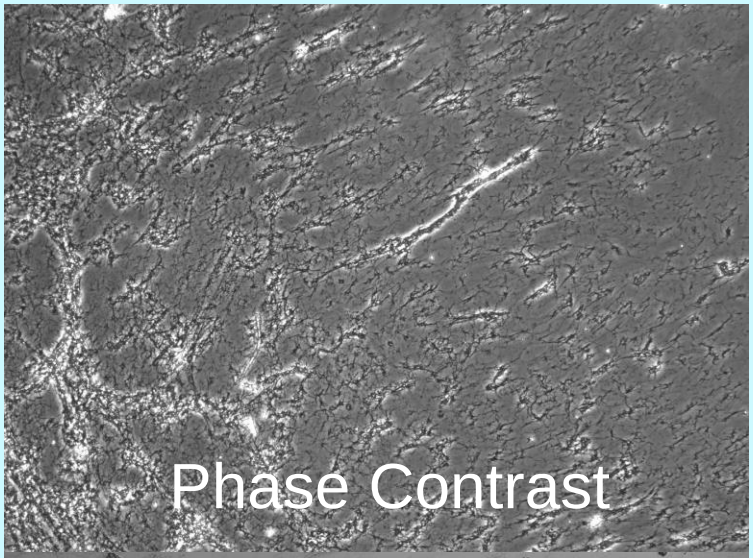


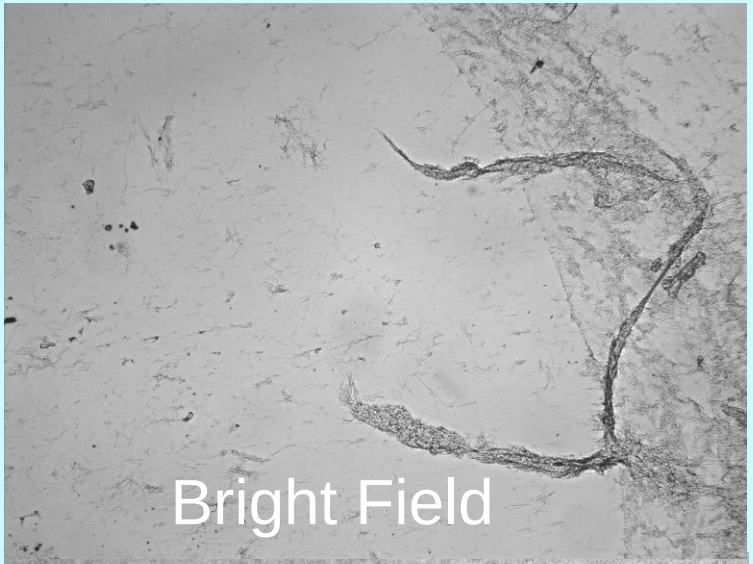
cDNA for CD8::GFP and MEC-4::mCherry will be created and transfected into C. elegans populations (1). The touch receptor neurons (TRNs) in these worms will express GFP labeled CD8 surface receptors and mCherry labeled MEC-4 ion channel complexes. The embryos from these worms will be extracted (2) and the embyrionic TRNs will be enriched by fluorescently activated cell sorting (FACS) or anti-CD8 functionalized magnetic bead sorting (3). ECM components will be patterned onto glass slides using microfabrication techniques for neuron cell culture to control gene expression (4). The ion channel complexes will be visualized with mCherry to verify expression and measure protein localization (5). *In vitro* TRN function will be measured using patch clamp and elecrode array techniques during mechanical stimulation and compared to *in vivo* results (6).

Methods for Protein Patterning



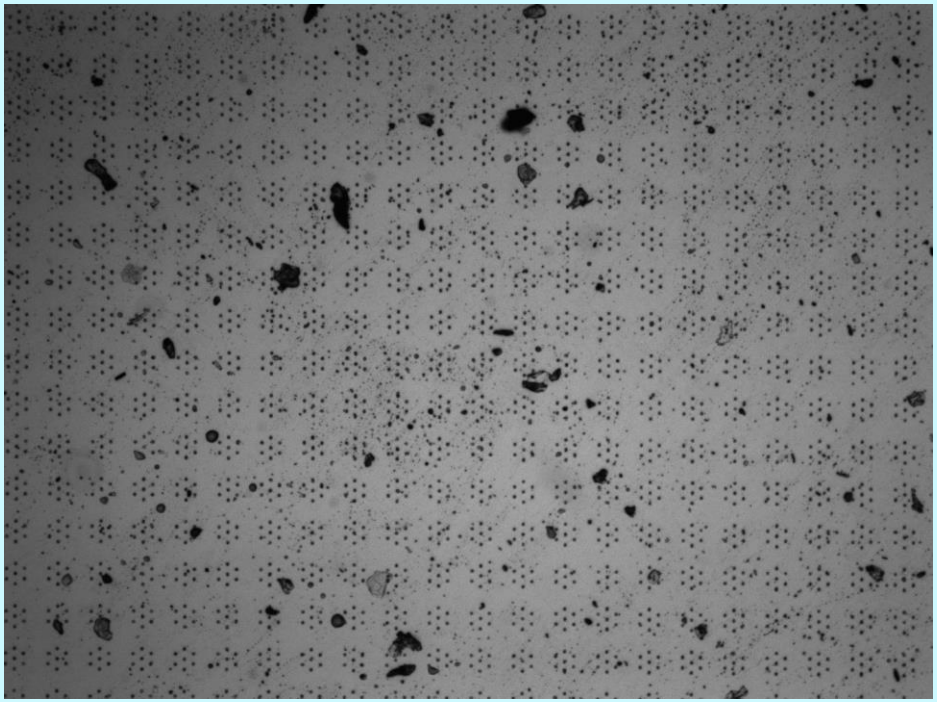
Collagen Type I

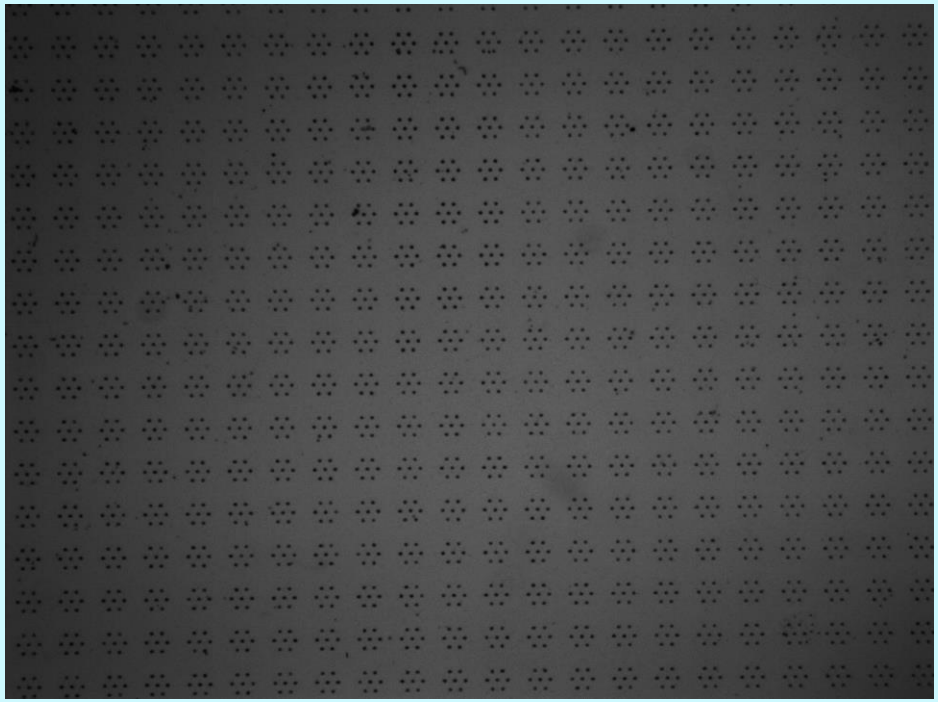




Stamp Cleaning

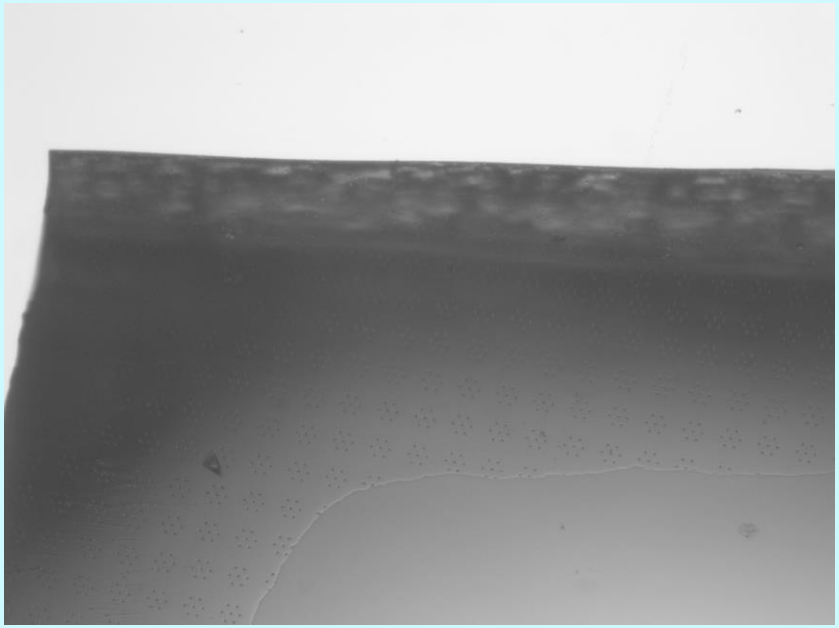
- 5-10 minutes of ultrasonication in ethanol works wonders

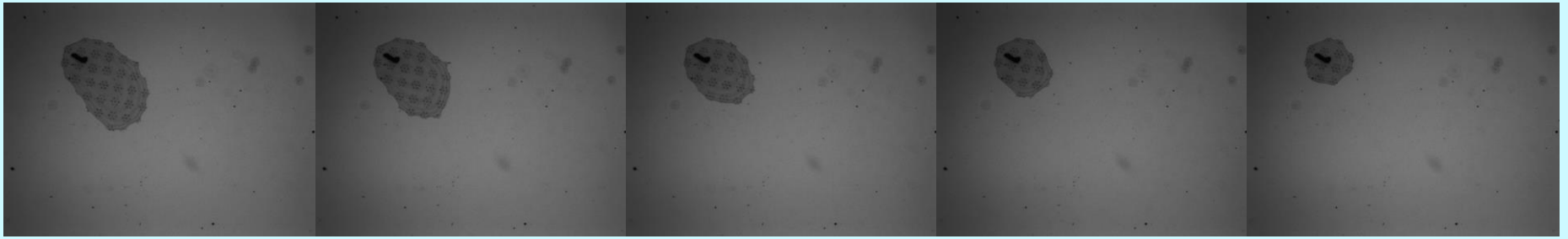




Stamp Collapsing

- Stamps are delicate
- Fixes
  - Use thinner SU-8
  - Increase PDMS area coverage (if possible)

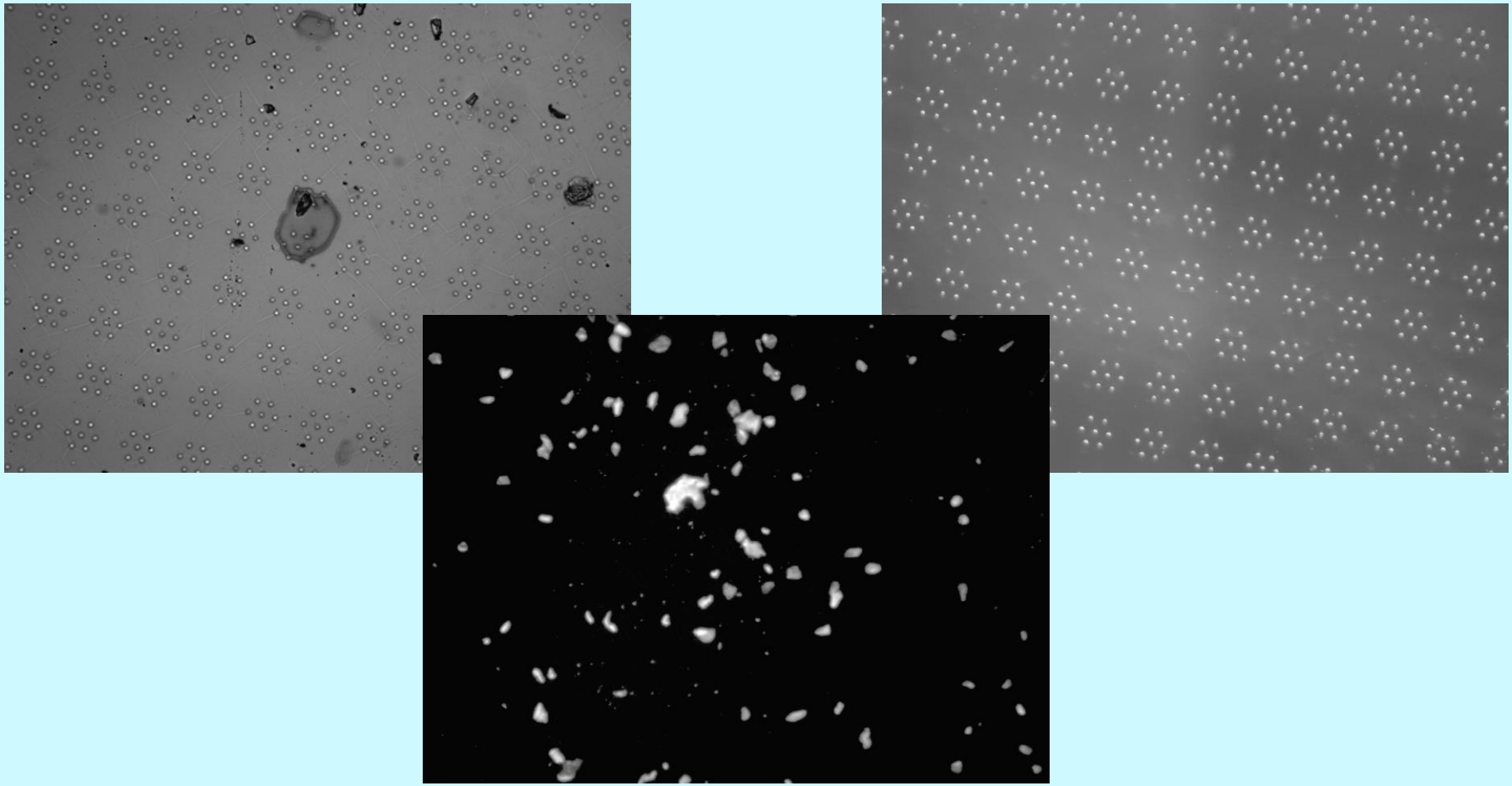




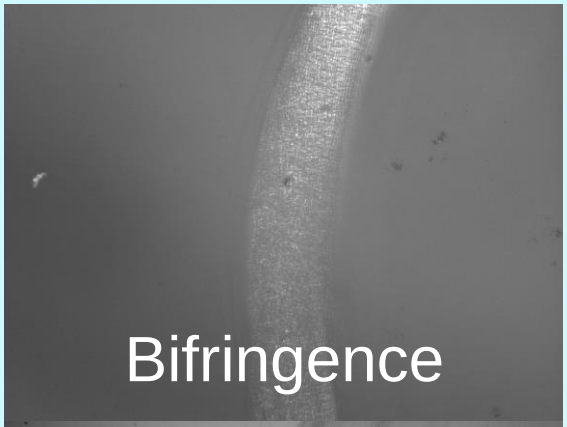
t = 0s

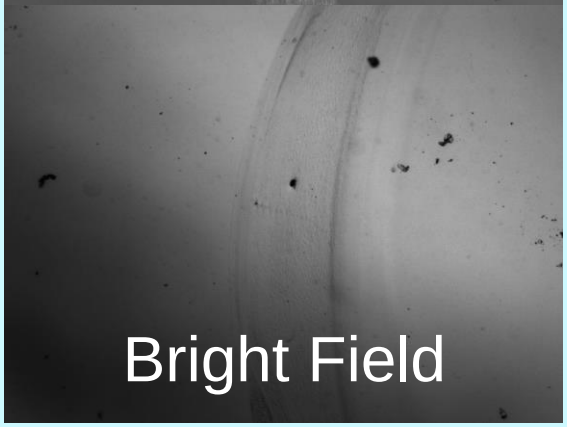
t = 5s

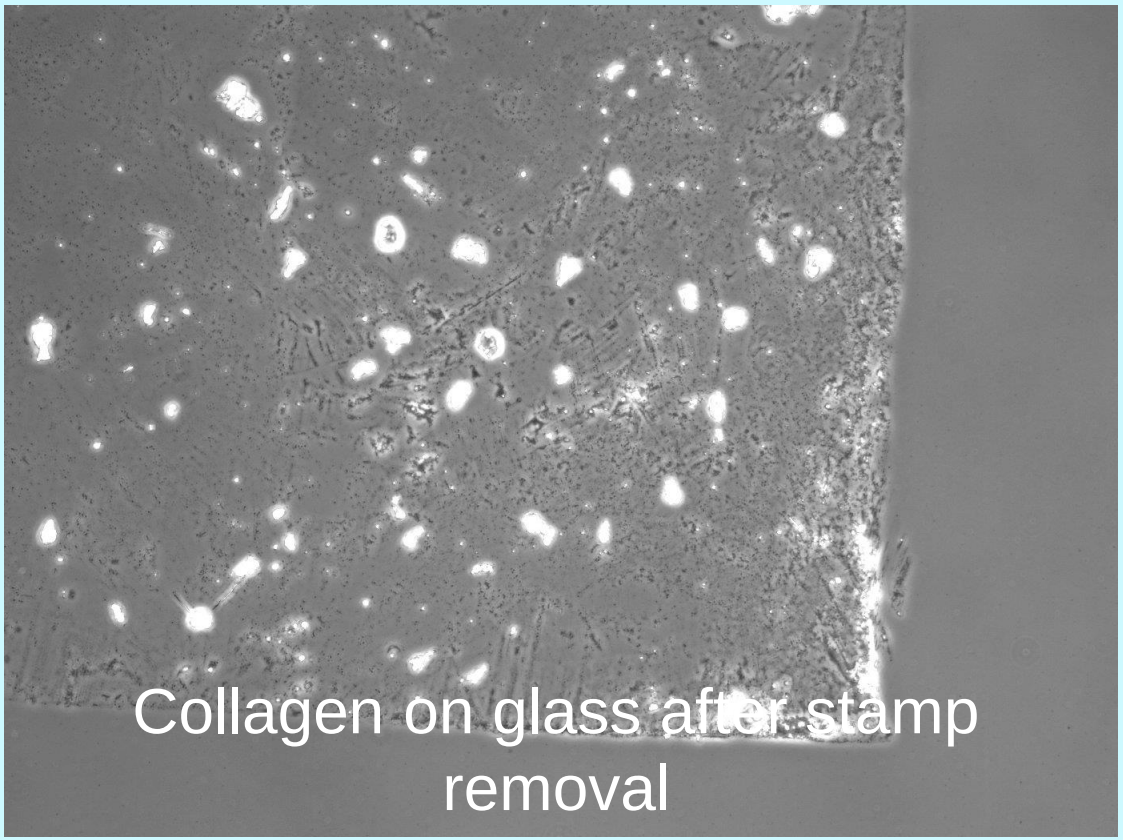
Rhodamine Stamp Inking



Collagen Stamps

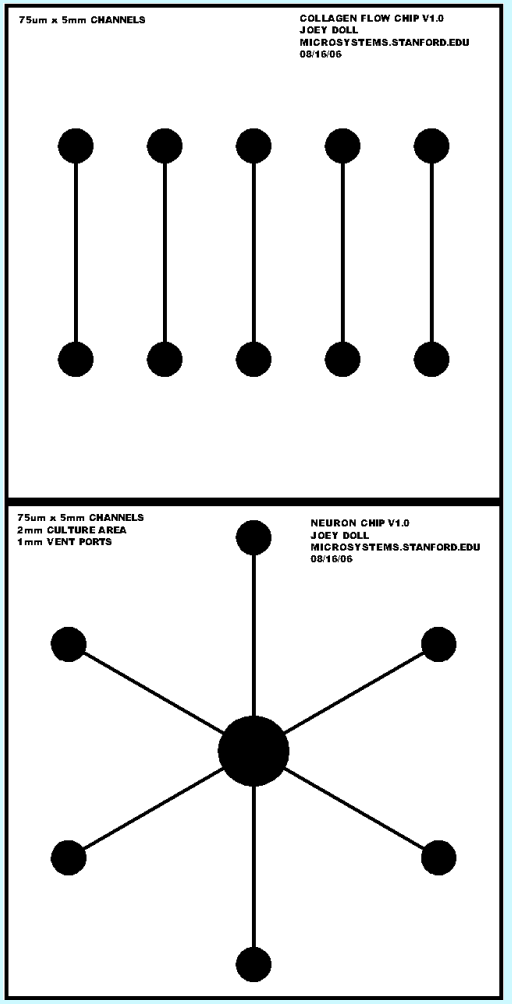






Neuron Stuff with Jay

- Control neuron process growth with micropatterned environments
- Apply to implanted devices
- Reduce scar tissue formation through use of aligned collagen
- Will try new transparency mask vendor: CAD/Art Services
  - 20k dpi, 10um feature size
- If 10um feature size (or smaller) is doable then make parallel microfluidic channels for TRN culture



What I'm Doing (Next)

- FACS User Orientation and Training
- Learn embryo and TRN extraction protocol from Ali and Juan
- Work on neuron patterning with Jay

- Cool movies from Shawn