#### A fast flexible inkjet method for patterned neuron culture Neville E. Sanjana<sup>1,\*</sup> and Sawyer B. Fuller<sup>2,\*</sup> <sup>1</sup>Dept. of Brain & Cognitive Science, <sup>2</sup>Dept. of Mechanical Engineering and Center for Biomedical Engineering, Massachusetts Institute of Technology

\* Authors contributed equally.

## Introduction

We present an ink-jet printing technique that allows precise micropatterning of cell adhesion substrates. Compared with traditional photolithography or recent soft lithographic techniques like PDMS stamping, our ink-jet method allows faster and less expensive fabrication of new patterns.

Disassociated rat hippocampal culture grown at low densities on our inkjet-printed surfaces show:

- Excellent adherence to the pattern over extended time periods
- Normal electrophysiological properties
- Similar distributions of immocytochemical markers for synapses and inhibitory cells as found in unpatterned, low-density controls

# Background

- Patterned cell culture can be used to study small, isolated networks, developmental cues, or single cell properties.
- Previous patterning work, such as photolithography (Kleinfeld 1988, Wyart 2002), microcontact printing (Wheeler et al., 1999), laser ablation (Corey et al., 1991) and other methods (Martinoia et al., 1999), relies upon an unalterable master pattern. We use inkjet printing, as it is a programmable and more flexible technique to rapidly print different patterns with minimal expense and time investment.

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## Methods

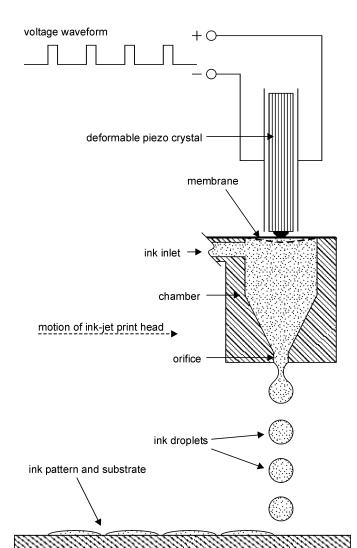
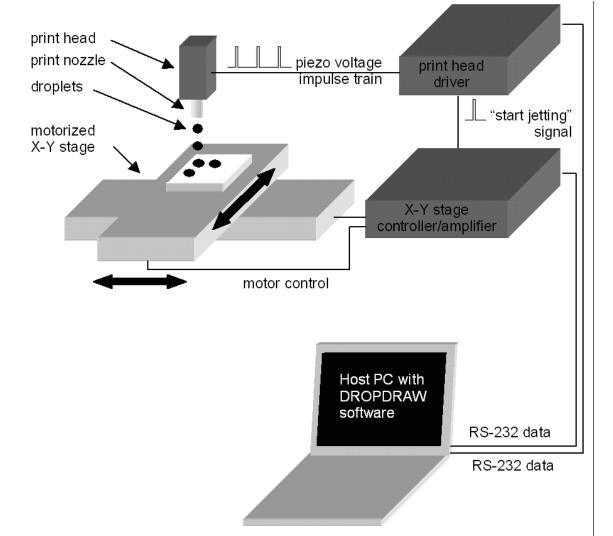
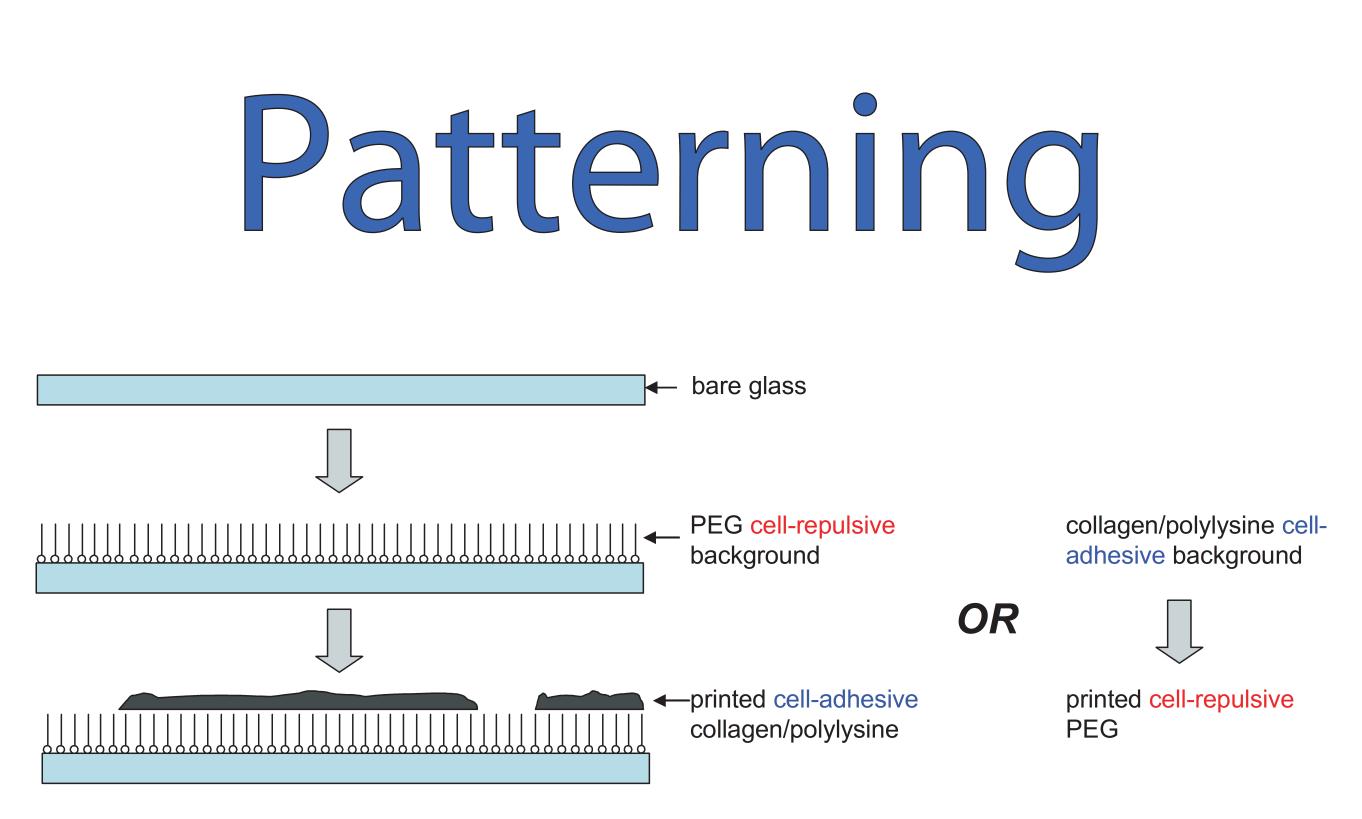


Diagram of the operation of the ink-jet printer. A pressure impulse delivered by a piezo crystal ejects single droplets by computer control as the print head is moved over the substrate.

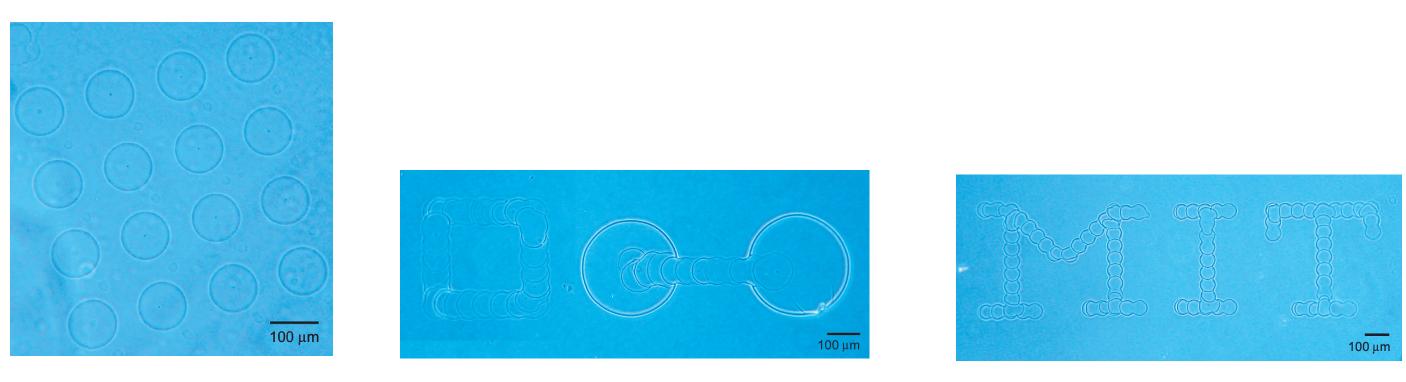
(a) printing hardware PC and software piezo crystal generator



The printer is mounted onto a cart for easy mobility.

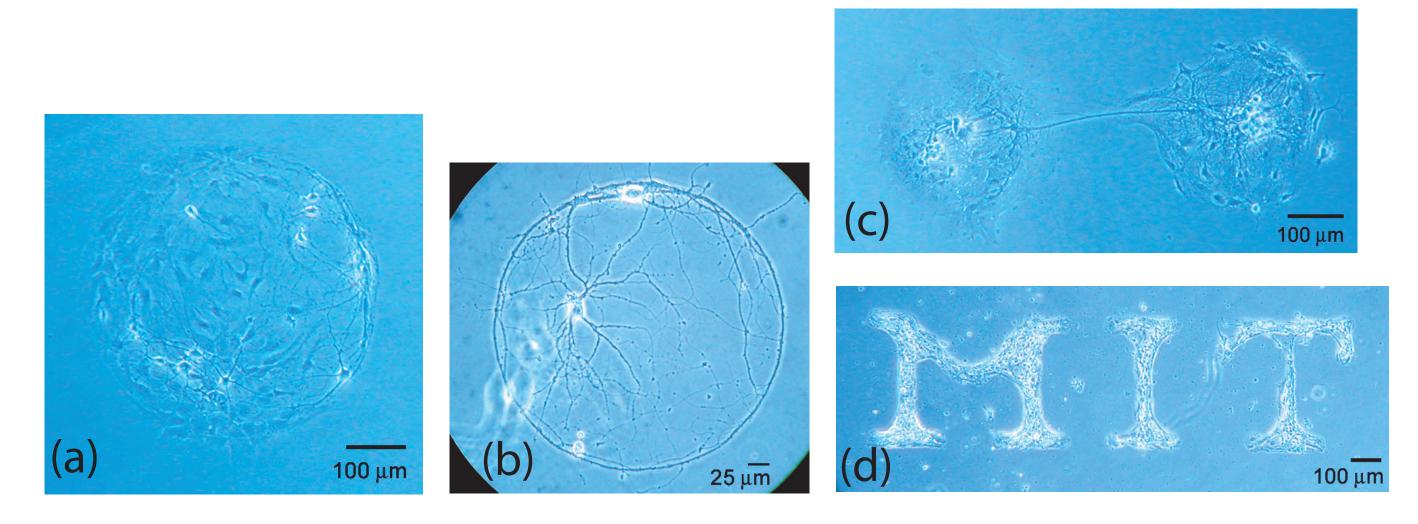


The flexible surface chemistry allows for printing in either positive or negative relief. This flexibility is possible because the adhesion and repulsion molecules have the same functional/reactive group.

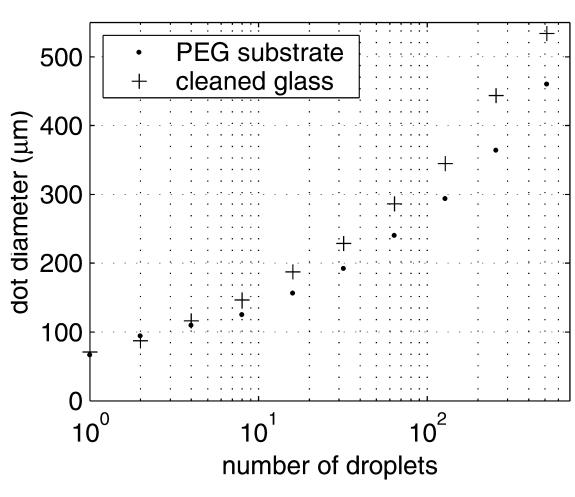


Printed patterns can include micro-islands (left) and connected micro-islands and arbitrary shapes (middle and right).

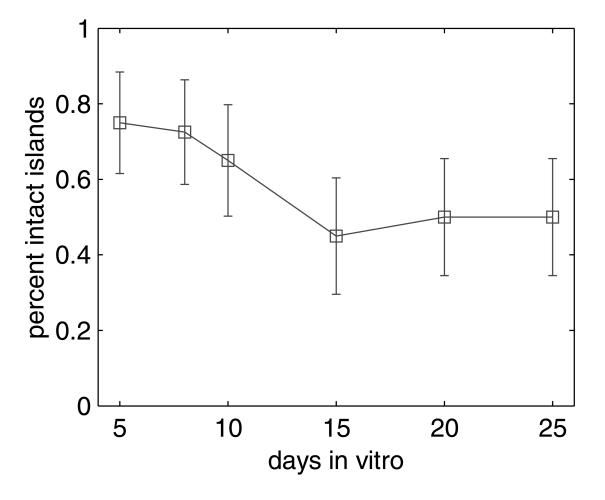
### Results



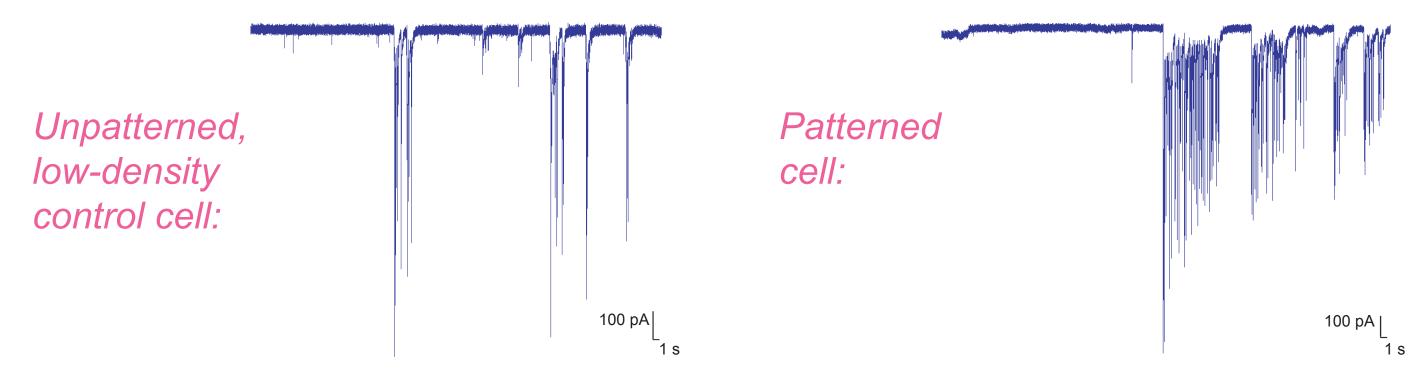
P1 hippocampal neurons patterned on micro-islands with glia (a), without glia (b), connected micro-islands (c), and arbitrary shapes (d).



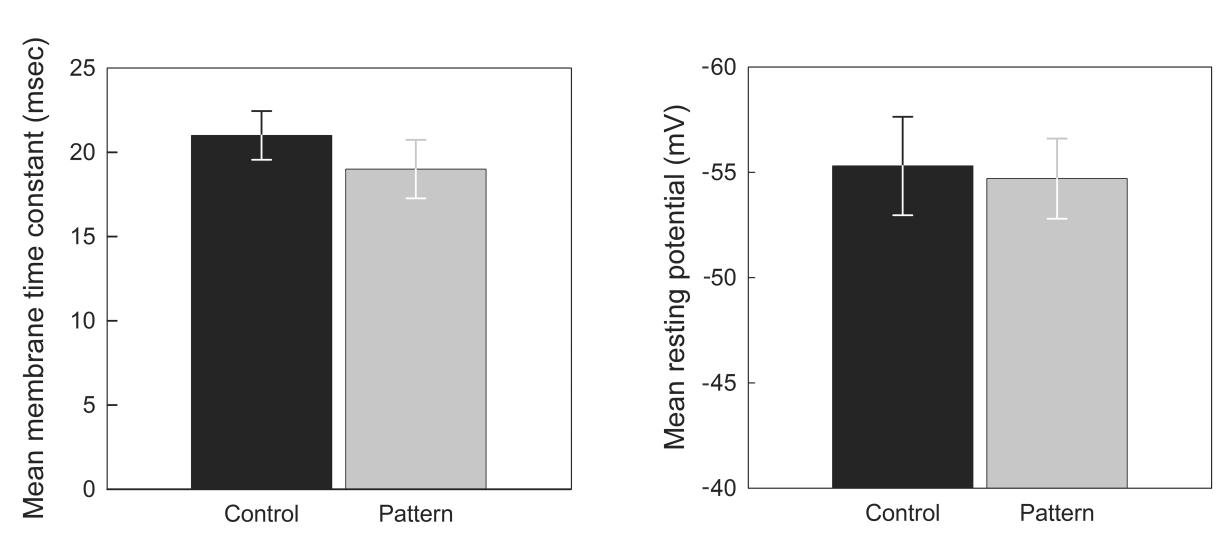
Micro-islands can be sized according to the number of droplets deposited.



50% of islands remain intact after 25 days *in vitro* (DIV).

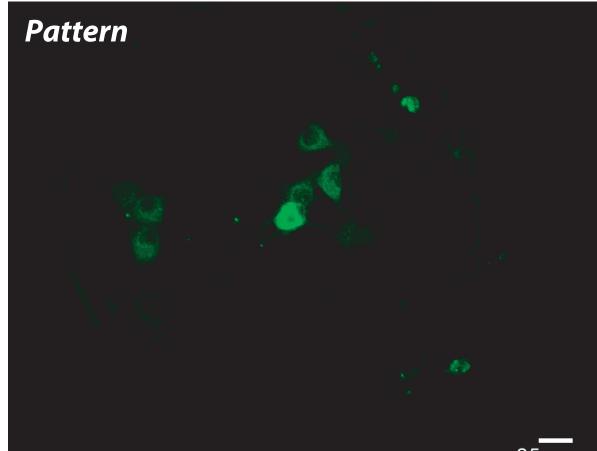


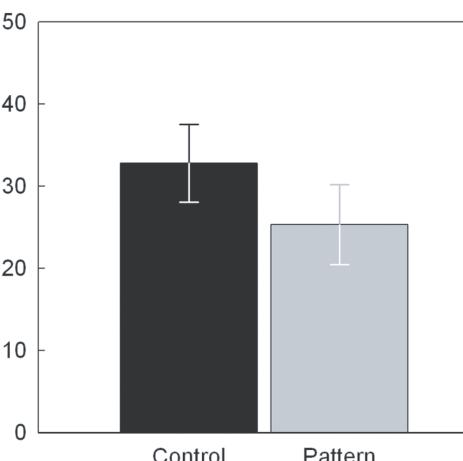
Synaptic activity traces from voltage-clamp recordings at DIV 10-12.



Similar passive membrane properties and resting potentials are found in patterned and unpatterned low-density control cells.

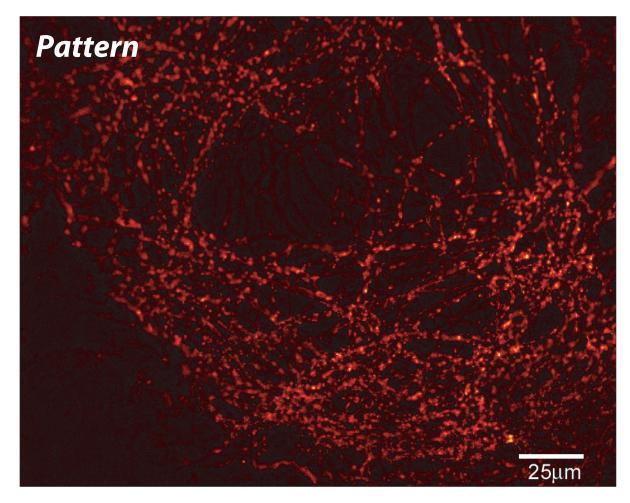
Immunostain for inhibitory cells (GABA):

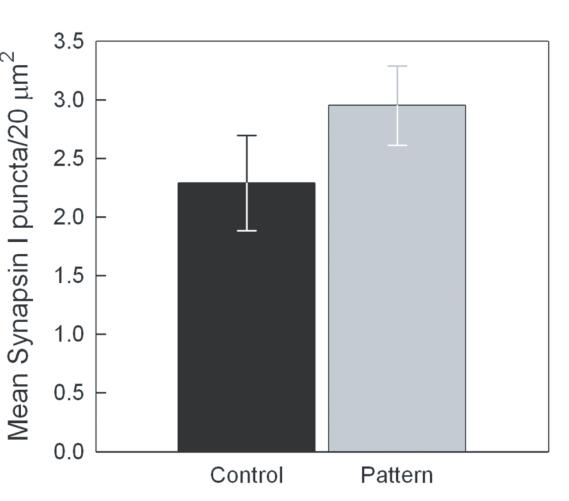




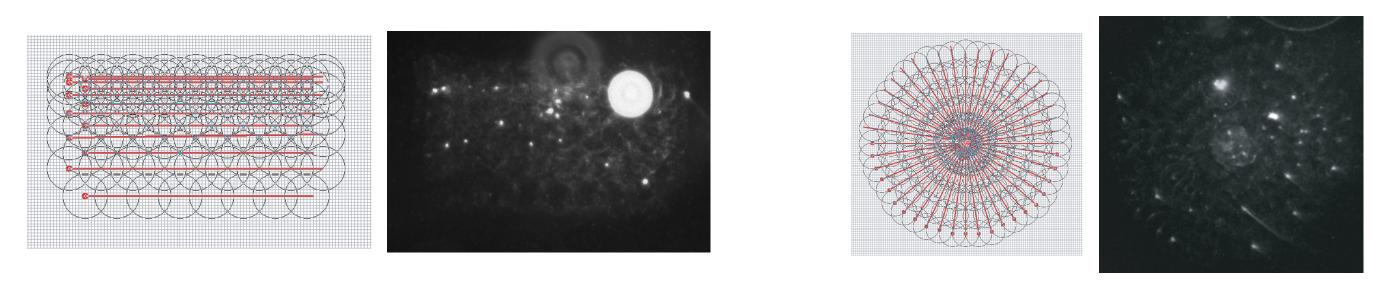
Patterned cultures show a lower percentage of inhibitory cells (25%) than low-density control cultures (33%) but are not significantly different (p<0.05).

• Immunostain for excitatory and inhibitory synapses (Synapsin I):





Synaptic density is not significantly different (p<0.05) between patterned cultures (2.95 synapses/20um<sup>2</sup>) and low-density control cultures (2.29 synapses/20um<sup>2</sup>).



FUTURE WORK: Gradients of laminin for developmental study of axon guidance. Linear gradient (left) and circular gradient (right) shown by laminin immunostain adjacent to CAD software representation.



- We designed an ink-jet printer to programmably arrange chemical factors.
- Patterned neurons exhibited:
  - healthy electrophysiologal properties
  - spontaneous activity
  - adherence to patterns for extended periods of time
  - normal immunocytochemical characteristics
- The ability to print gradients and other shapes suggest the use of ink-jet printing for further studies in neuron-substrate interaction and arrangement of neural circuits.