Supplemental Information.

Materials and Methods.

Device fabrication: A master pattern was created using contact lithography with a negative photoresist (SU8 2000.5, Microchem, final thickness ~1 μm). After exposure and development following the manufacturers recommendations, the photoresist was hardbaked at 125°C for 5 min. PDMS (Sylgard 184, Dow Corning, Midland, MI) was mixed according to manufacturer specifications and poured onto the mold. After curing at 65°C for 4 hours, the PDMS was demolded, punched (Technical Innovations, Brazoria, TX), and cut into appropriately sized chips (approximately 22 mm x 30 mm) with a razor blade (each master had 8 individual devices arrayed onto a 4" silicon wafer). German glass cover slips (22 mm x 30 mm, Carolina Biological Supply, Burlington, NC) were sonicated in acetone, then isopropanol, and finally rinsed and stored under DI water until use. The PDMS chips were rinsed with isopropanol, dried with compressed nitrogen, and treated with Scotch tape (3M, St. Paul, MN) to remove any remaining dust. The PDMS parts were reversibly adhered via conformal contact to freshly dried glass slides with the patterned features against the glass.

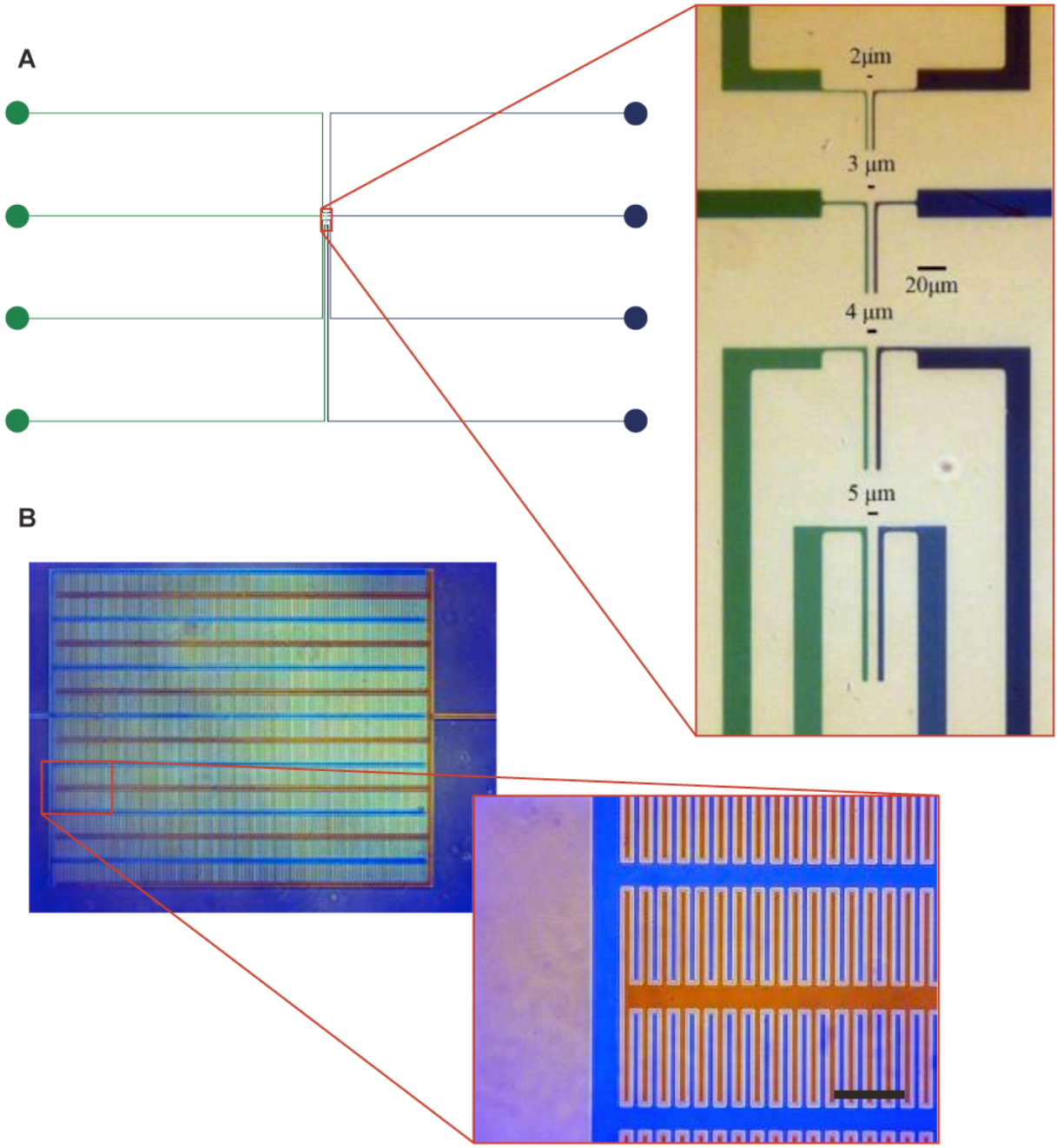
Pattern formation: To fill the channels of the device, the PDMS/glass sandwich was first placed in a vacuum chamber and pumped down to 1 mTorr for at least 25 minutes. Upon removal from vacuum, 2 μl of solution was quickly dropped on each of the channel inlets and outlets using a micropipette. Alternatively, a piece of Scotch tape can be used to seal the outlet channels and solution can be used to seal the inlet channels (Figure 1A). The device was left overnight at room temperature in an airtight 35 mm Petri dish. Filling of the full channel length (a volume of 11 nL) was typically completed within 30 minutes.

Because of the high surface area to volume ratio found in channels such as these, the solution concentration can be depleted due to surface adsorption during channel filling. Typically, the front of the liquid moving through the channel will have a lower concentration than the bulk of fluid following it. This may result in a gradient in concentration of adsorbed molecules on the surface, which for some applications may be beneficial. When this type of gradient is not desired, then the patterning solution concentrations and the channel geometry must be optimized to maintain a uniform concentration. For our experiments, the laminin patterning solution consisted of 5 μg/ml laminin (Invitrogen) mixed with 10 μg/ml AlexaFluor545-labelled bovine serum albumin (BSA, Invitrogen) for visualization in phosphate buffered saline. For multi-component patterns, the following reagents were dissolved in deionized water: MP-cAMP (20 μM, Alexa Fluor-conjugated 8-[6-aminohexyl] aminoadenosine 3',5'-cyclic monophosphate; Invitrogen, Carlsbad, CA), MP-cGMP (20 μM, 8-[(2-[(fluoresceinylthioureido)amino]ethyl]thio] guanosine-3',5'-cyclic monophosphate, Biolog, Bremen, Germany), poly-L-lysine (PLL, Sigma, St. Louis, MO), bovine serum albumin (BSA, Sigma). Three separate solutions were used to fill the three individual channels: a cell body solution (PLL/MP-cGMP), an axonal guidance solution (BSA/MP-cAMP), and a negative control solution (BSA/MP-cGMP). Coverslips were incubated overnight with the mold to create striped regions of small hydrophobic molecules physically entrapped within the BSA or PLL coatings.

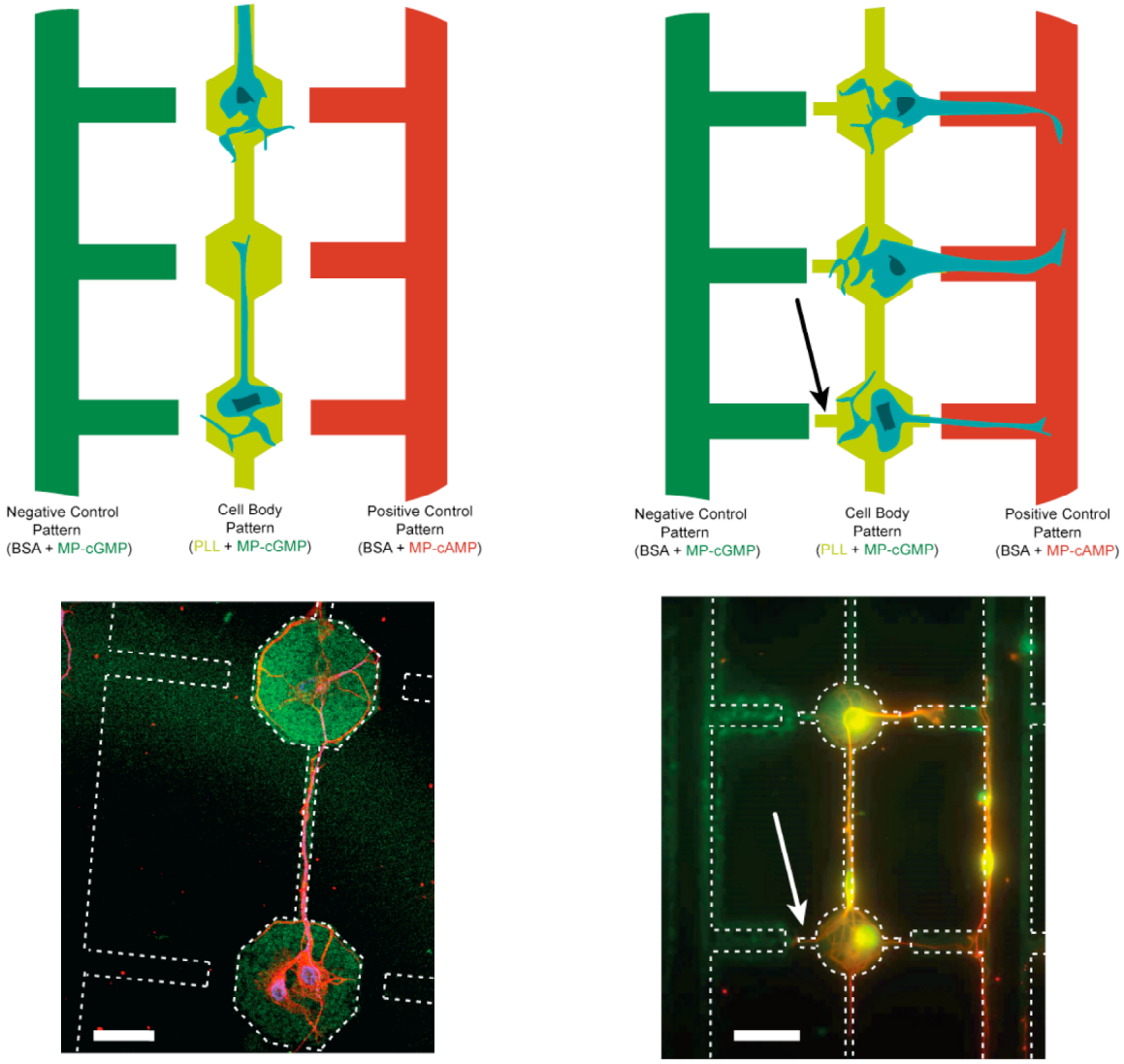
After incubation, the PDMS parts were peeled away from the glass coverslips and saved for re-use. The patterned slides were rinsed 3 times with sterile DI water to remove any unbound molecules. The water from the final rinse was left on the slide, and the submerged slide was sterilized under a germicidal UV lamp for 4-12 hours.

Cell culture and immunostaining: Dorsal root ganglion were isolated from E9 chicks as whole explants and cultured in DMEM (Invitrogen) supplemented with 10% FBS, 1% pen/strep, and 50 ng/mL NGF (Peprotech). Rat hippocampal cells were isolated from E18 rats and treated with trypsin (0.3 mg/ml, Sigma) for 15 min at 37°C, followed by washing and gentle trituration. The Neurobasal culture medium (Invitrogen) was supplemented with glutamine (4.0 mM, Sigma) and B27 (2%, Invitrogen). Cells were seeded onto patterned or control glass substrates at a density of about 200 cells/mm^2 contained within a PLL-coated tissue-culture polystyrene dish. PLL-coated dishes were prepared by adding 0.1 mg/ml PLL, incubating for 4-12 hours, and rinsing with sterile water. Cultures were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.1% Triton X-100 in PBS (PBST) for 15 min, and blocked with 10% normal goat serum (Jackson Immunoresearch, West Grove, PA) in PBST for at least 2 hr. Primary antibodies used were neuron-specific (polyclonal anti-β-tubulin, Tuj1, 1:1000, Covance, Berkeley, CA) and axon-specific (Smi312, 1:500, Covance). The fluorescent secondary antibodies used were anti-mouse AlexaFluor 488-conjugated, anti-rabbit AlexaFluor 546-conjugated, anti-mouse AlexaFluor 647-conjugated (all at 1:1000, Invitrogen). Incubation with primary or secondary antibody was both performed in 5% normal goat serum in PBST. Slides were mounted with Prolong Gold (Invitrogen) and imaged using a Leica DM IRBE confocal microscope.

Supplemental Video. Images recorded during filling of a chip. The video speed is real time. The solutions used are the same as described in the experimental section for filling of the multi-component device.



Supplemental Figure 1. A) (Left) CAD drawing of a design with dead-end channels. (Right) Photograph of a dye-filled channel demonstrating 2 µm resolution in channel width and spacing. B) Photographs of a dye-filled device demonstrating complete multiple, long, interdigitated, dead-end channels can be filled reliably using this technique. Scale bar is 100 µm.



Supplemental Figure 2. Several configurations of the multi-component design were tested to identify geometries that yielded reproducible neuronal polarization. (Top) Schematics of two designs. The negative and positive control patterns are located 2 μm away from each cell body pattern, which requires a motile growth cone (the 5-10 μm structure at the tip of an extending neurite) to momentarily contact the non-adhesive, plain glass substrate. To increase the probability that each cell would extend a neurite that samples the BSA/MP-cGMP and BSA/MP-cAMP patterns, small PLL/MP-cGMP "protrusions" (see arrow) were added to each cell body island to orient immature neurites toward the BSA/MP-cAMP and BSA/MP-cGMP patterns. (Bottom left) Without these patterned protrusions, all neurites (axons and dendrites) remained on the cell body pattern and did not explore the negative or positive control patterns. (Bottom right) With protrusions, neurites explored both the negative and positive control patterns. Neurites contacting the positive control pattern were observed to elongate, develop into axons, and follow the guidance path. Scale bars are 35 μm. Note that the first design (Bottom left) had larger PLL islands than the second design (Bottom right). The smaller islands were chosen to restrict a single cell body to each island.