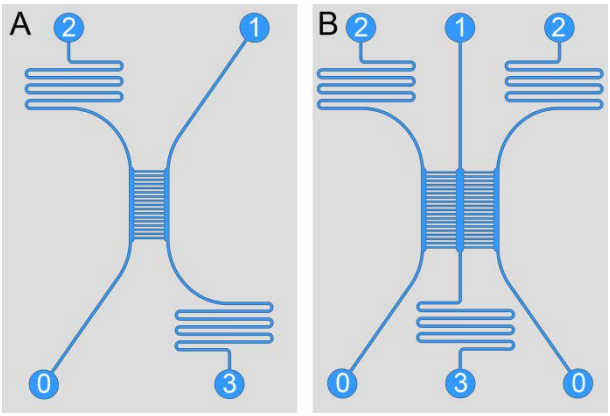
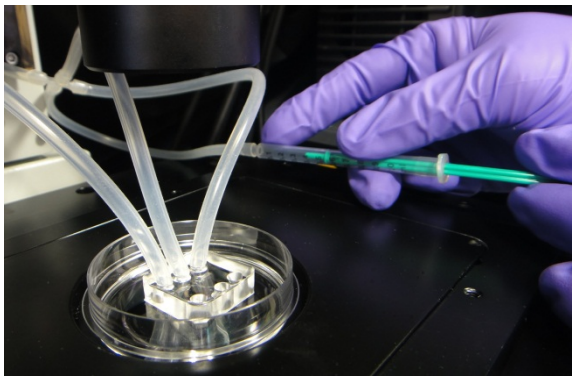
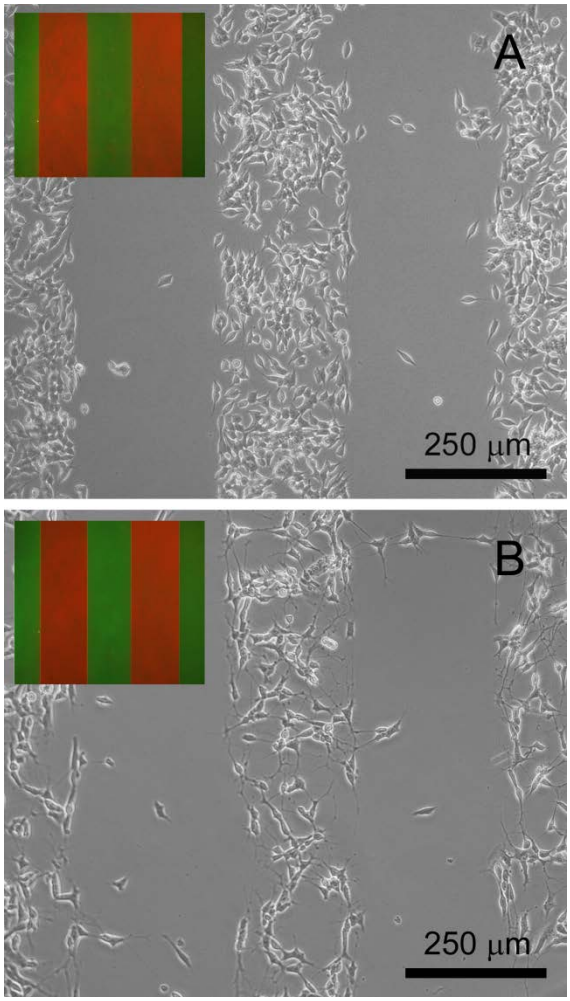


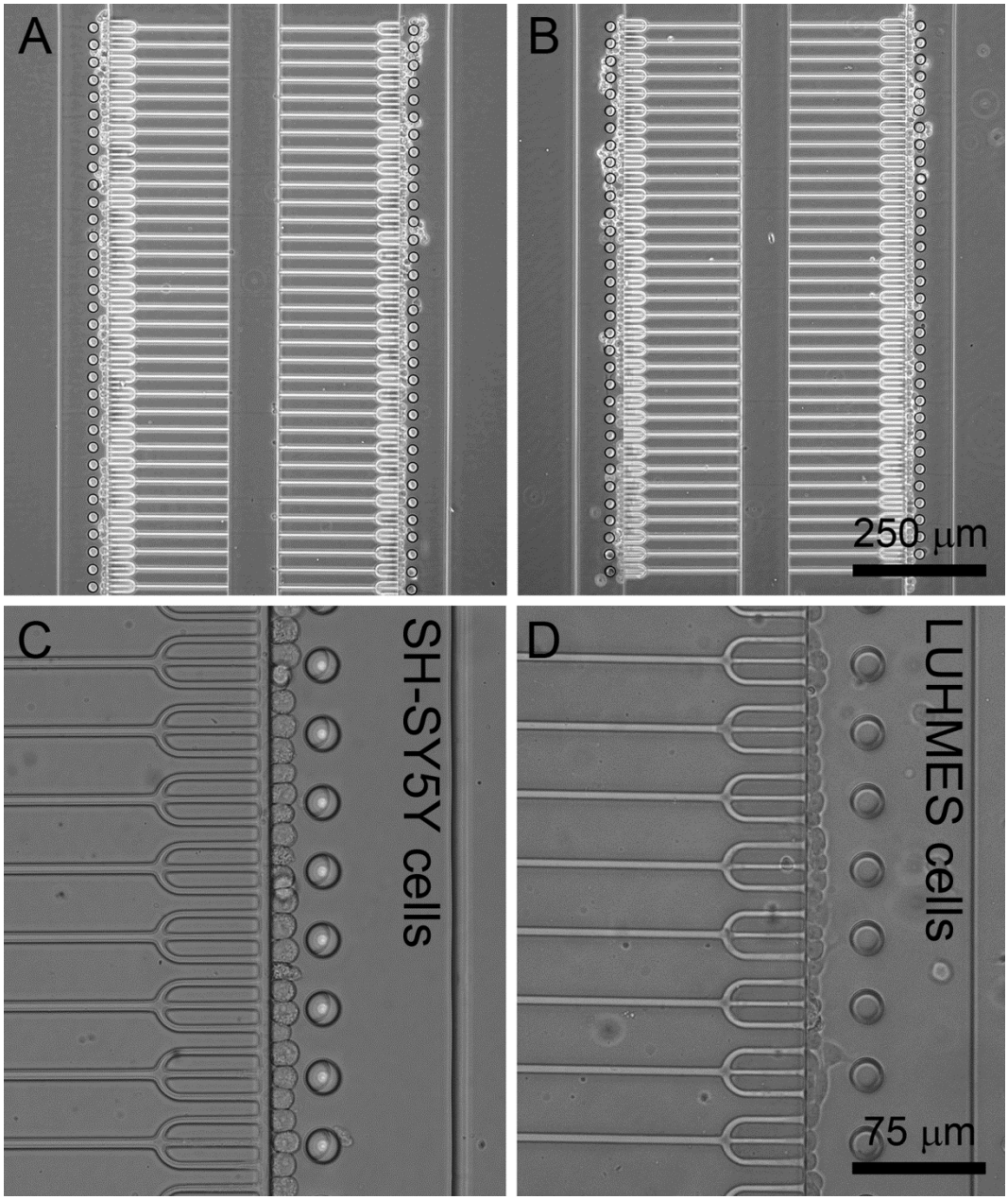
ESI Figure 1. Alternative CNA circuits with continuous neurite guidance channels. A 2-compartment circuit (A): The path 0→1 has a lower fluidic resistance than the serpentine path, 0→2, to direct neuron arraying. Following cellular valving (adhesion and flattening) of the first population, a second cell population can be arrayed, from top to bottom, using path 1→0, with path 1→3 serving as the bypass channel. A 3-compartment system (B): Neurons can be arrayed in the flanking compartments using paths 0→1, with the paths 0→2 serving as bypass channels. Following cellular valving neurons can be arrayed in the central compartment using paths 1→0, with path 1→3 serving as the bypass channel. Neuron arraying can also be achieved in the reverse order.



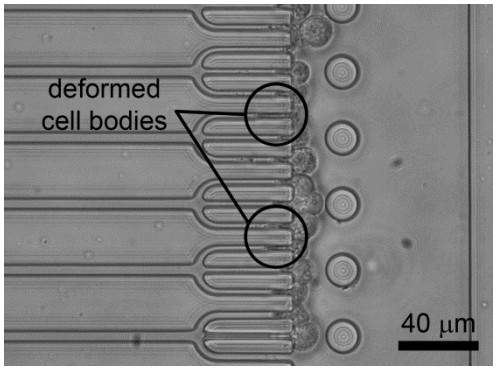
ESI Figure 2. A syringe was interfaced to the upper three ports using a cross union to trifurcate tubing sections of equal length for uniform neuron arraying.



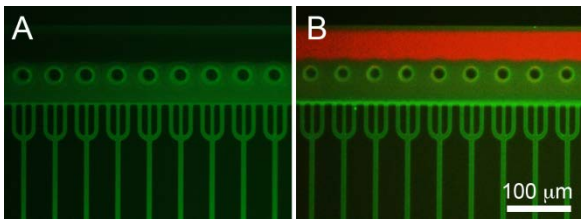
ESI Figure 3. Undifferentiated (A) and 4 day RA-differentiated (B) SH-SY5Y neurons cultured on planar patterns (inset) of FITC-labelled PLL (green) and TRITC-labelled PLL-g-PEG (red). Images recorded following 5 days of patterned culture. Undifferentiated SH-SY5Y cells had an Epatt of 94% and differentiated SH-SY5Y cells had an Epatt of 87%. Patterns were prepared on cleaned and plasma-treated glass substrates incubated with fluorescein-conjugated PL (PL–FITC, 100 µg/mL, Sigma-Aldrich) in PBS buffer for 5 minutes and rinsed in water. A PDMS stencil with 250-µm-wide and spaced microchannel features was used for 1 minute oxygen plasma patterning, followed by a 5 minute incubation for back-filling with rhodamine-conjugated PLL-g-PEG (PLL-g-PEG–TRITC, 100 µg/mL in PBS, Surface Solutions, Switzerland) followed by a PBS and water wash sequence. In this experiment, a 1 mL suspension of 200,000 SH-SY5Y cells (passage 18) were seeded overnight, and the following day washed with PBS and replenished with fresh media.



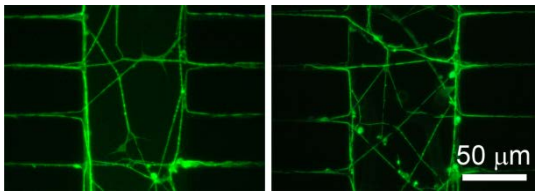
ESI Figure 4. Larger images of arrayed SH-SY5Y neuron-like cells in the CNA device (A,B). The meniscus-pinning micropillars have been outlined to distinguish these from the single neurons. Higher magnification image of optimally arrayed SH-SY5Y cells (C) and LUHMES cells (D) imaged 4 hours after arraying.



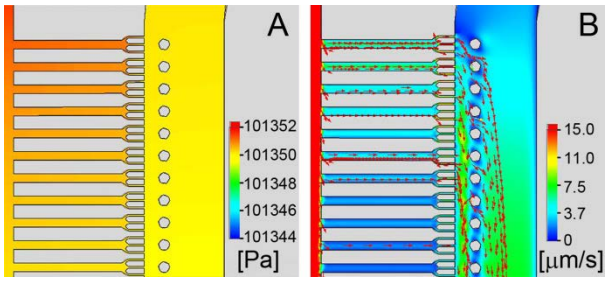
ESI Figure 5. Cell body deformation resulting from prolonged (30 minutes), low pressure microfluidic arraying by gravity-driven feed. All neurons are deformed with notable examples highlighted.



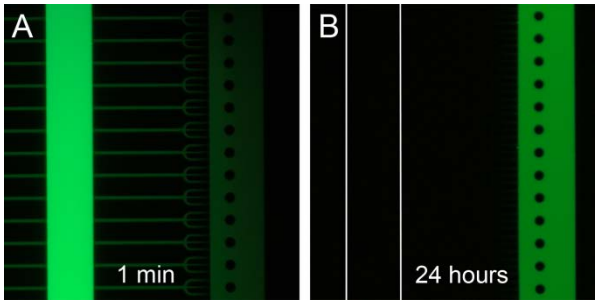
ESI Figure 6. Sub-optimal poly-lysine patterning by plasma stencilling with a water mask (A). PL-FITC remains intact on the outer wall of the microchannel, which prevents coating with PLL-g-PEG-TRITC (B).



ESI Figure 7. Examples of unguided neurite outgrowths bridging the central channel.



ESI Figure 8. Simulation of pressure (A) and velocity (B) distribution during balanced hydrostatic driven flow. Although the pressure difference between central and flanking channels is small (<2 Pa) this generates outward flows through the neurite outgrowth channels (~5 μm/s).



ESI Figure 9. HDF treatment of the central channel contaminates the flanking channels within 1 minute (A). The flanking channels remain fluidically isolated for ≥24 hours (B) when using a hydrostatic-driven flow. The boundaries of the central channel are highlighted using white lines. The treatment flow was doped with 10 µM FITC.