Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Influence of Microchannel Geometry on Device Performance and Electrophysiological Recording Fidelity During Long-Term Studies of Connected Neural Populations

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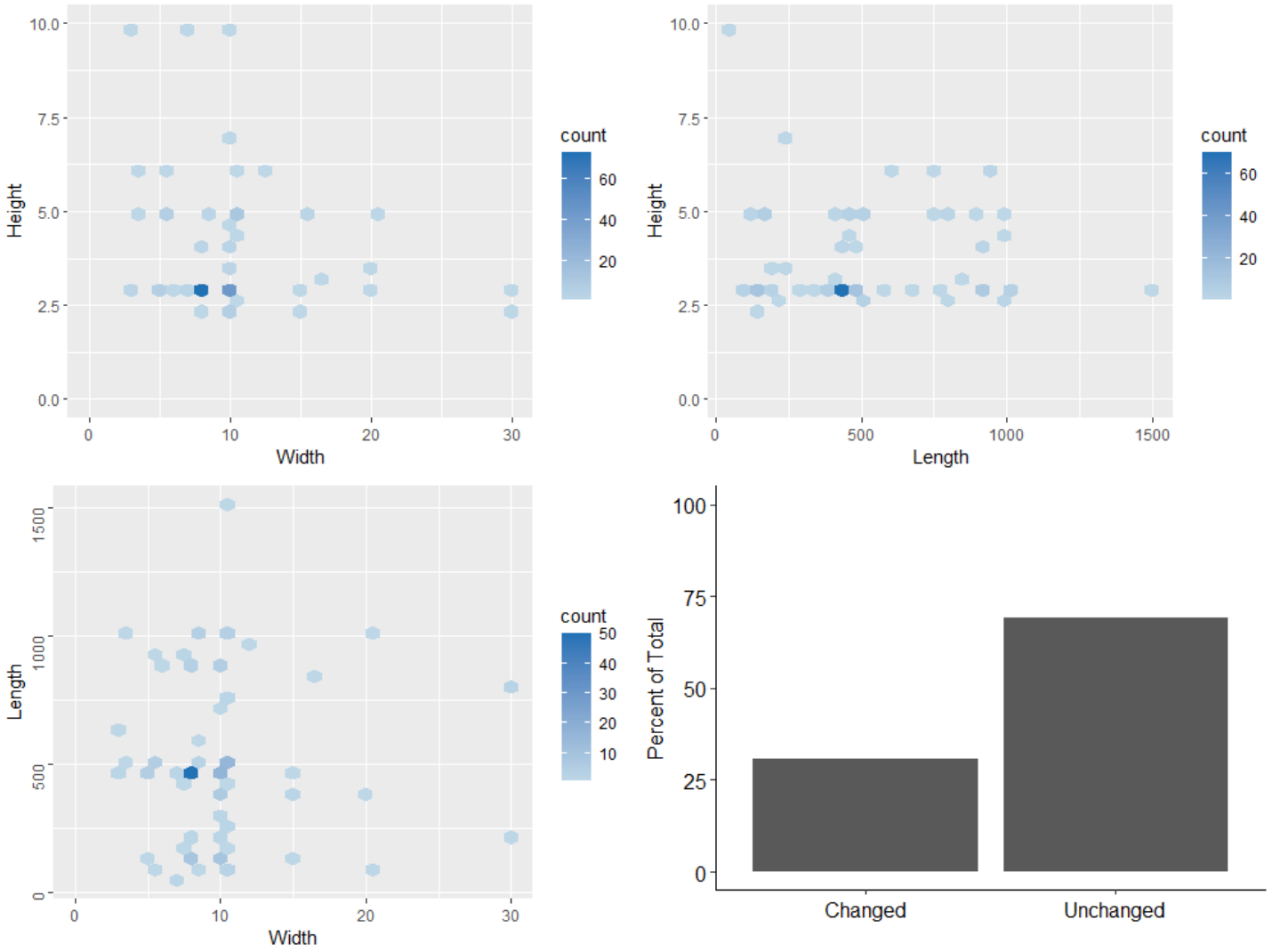

Figure S1: Analysis of different microchannel dimensions reported in publications using “axon compartmentalization” style microfluidic devices. Most common microchannel dimensions were 3 µm x 8 µm x 450 µm (H x W x L), which are the original dimensions reported by Taylor *et al* and are the dimensions of commercially available devices (Xona Microfluidics), with ~70% of all studies use microchannel dimensions of 3 µm x 8 µm x 450-1000 µm. The bottom-right plot illustrates the percentage of the cited papers that use the same original dimensions or different dimensions than the original. (A full list of references used to generate this data is listed at the end of the Supporting Information).

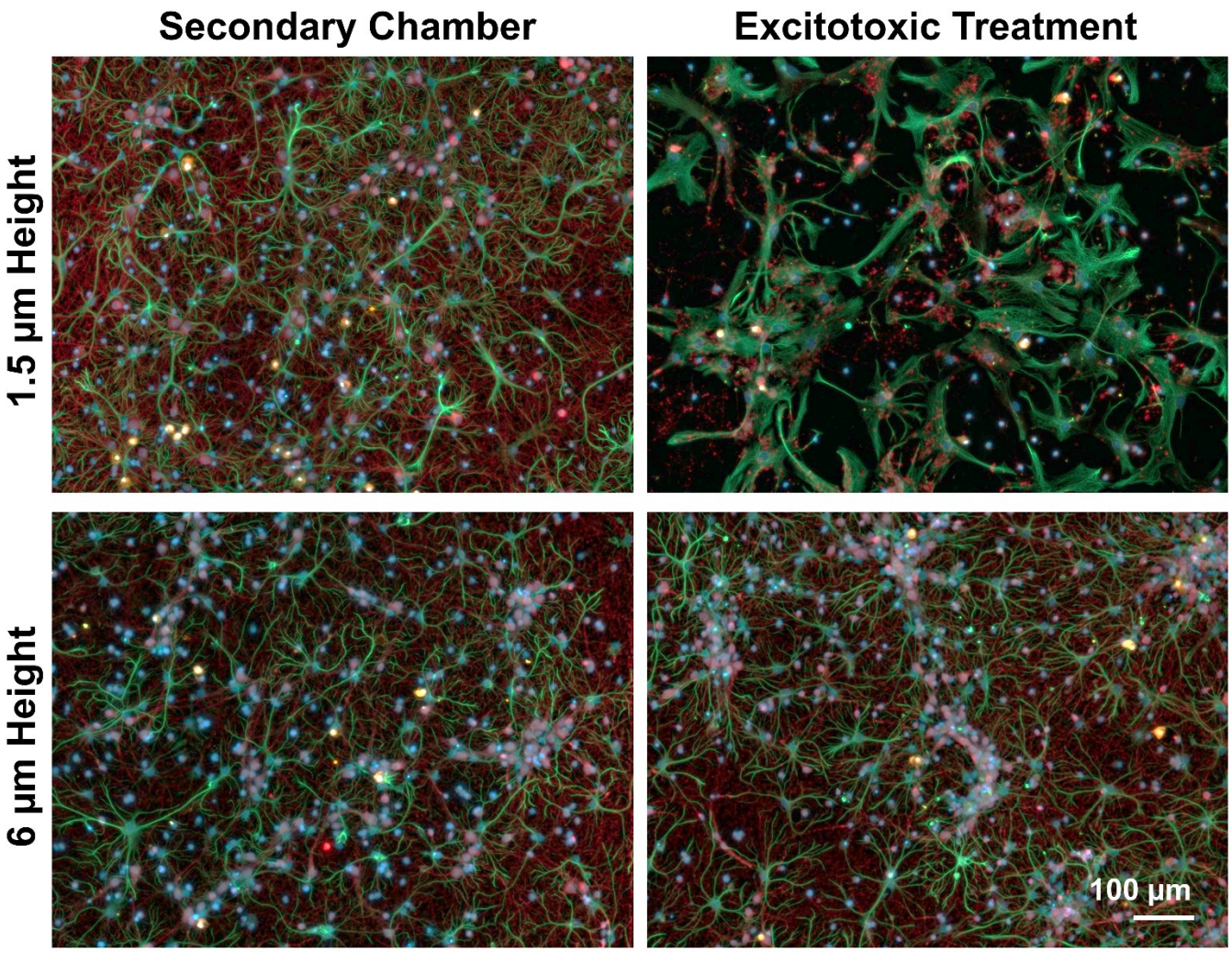

Figure S2: Representative fluorescence images from the different cell culture chambers following a 4-day excitotoxic treatment (100 µM glutamate). These cultures were maintained using a previously described tri-culture medium,[1] which maintains a small number of microglia in addition to neurons and astrocytes within the culture. For the device with 1.5 µm-high channels, the flow from the secondary chamber to the treatment chamber did not dilute the added glutamate in the treatment chamber; hence, excitotoxic injury was observed (e.g., severe neural death and astrocyte hypertrophy). However, for the device with the 6 µm-high channels, the excessive flow from the secondary chamber to the treatment chamber diluted the glutamate to the extent that no excitotoxic effect was observed in the treatment culture. The cultures were immunostained for neurons – anti-βIII-tubulin (red), astrocytes – anti-GFAP (green), microglia – anti-Iba1 (orange), and the general nuclear stain DAPI (blue).

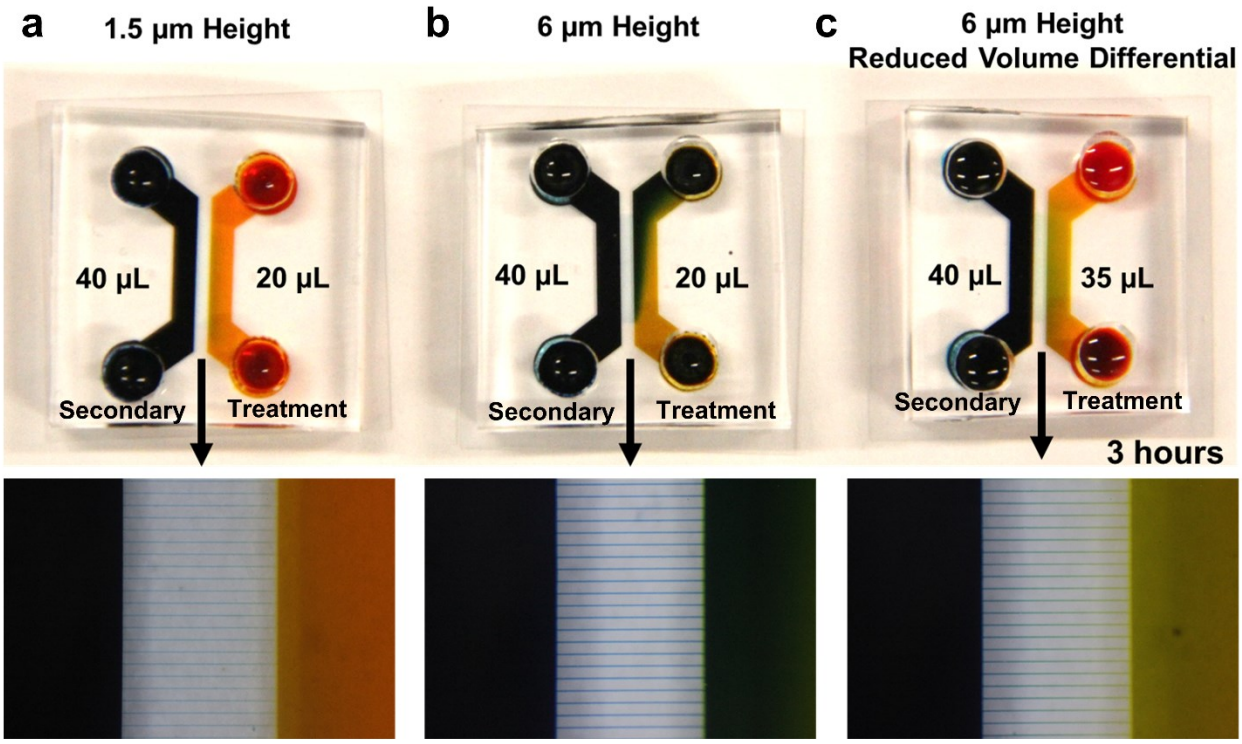

Figure S3: Comparing the fluid flow and fluidic isolation of different devices after 3 hours. In these devices a volume differential of 20 µL equated to a height differential of ~2.83 mm, while a volume differential of 5 µL equated to a height differential of ~0.71 mm. (a) For 1.5 µm height devices with a 20 µL volume differential we see both good fluidic isolation (negligible diffusive transport) and minimal flow between the chambers. (b) For the 6 µm height device and 20 µL volume differential there was good fluidic isolation, but significant flow between the chambers. (c) When the volume differential was reduced, the flow between the chambers was also reduced, but some of the dye from the isolated chamber (yellow) can be seen diffusing into the microchannels.

Supplemental Methods: Analytical and Computational Models.

The analytical model was based on the "full" style device with 101 microchannels with a channel length of 1000 μm and a channel width of 10 μm. The following electric circuit analogy with the four wells modeled as capacitors (C1-C4) and the interconnecting microchannels modeled as resistors (MC1-MC101).

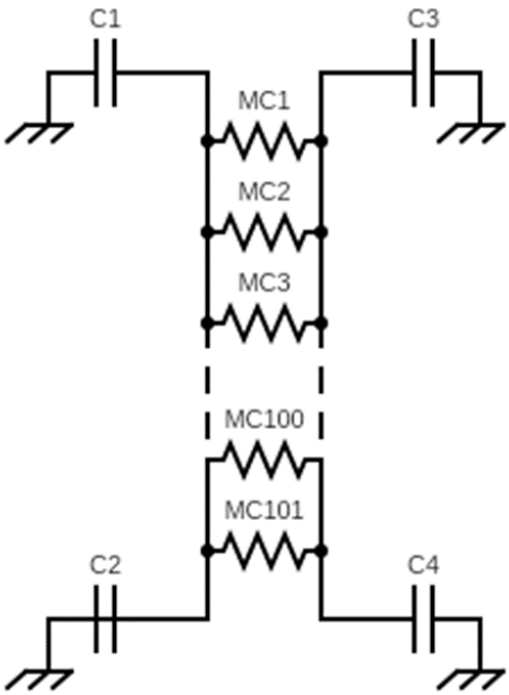

Figure S4: Electric circuit analogy used to estimate the fluid flow through the microchannels.

The change in volume between the chambers over time can be found using the following equation

$$V(t) = \frac{\Delta V}{2}\left(1 - e^{-\frac{2t}{RC}}\right)$$

Where *ΔV* is the initial volume differential between the chamber, *R* is the hydraulic resistance through the microchannels, and *C* is the "capacitance" of the wells. The hydraulic resistance through rectangular microchannels can be estimated using the following equation

$$R_H = \frac{12\mu L}{wh^3\left(1 - 0.630\left[\frac{h}{w}\right]\right)}$$

Where *w*, *L*, and *h* are the width, length, and height of the microchannel respectively, and *μ* is the dynamic viscosity. *C* is defined as the change in stored volumetric flow rate due to change in pressure and can be calculated for an open well as

$$C = \frac{\Delta V}{\Delta P} \propto Area_{Well}$$

Computational modeling was done in COMSOL Multiphysics 5.5 solving for the Navier-Stokes equations to model the pressure driven flow through the microchannels in a full 3D model of the "full" device with the following geometry: Chamber height: 75 μm, Channel length: 1000 μm, Channel width: 10 μm, Channel height: variable, 101 channels.

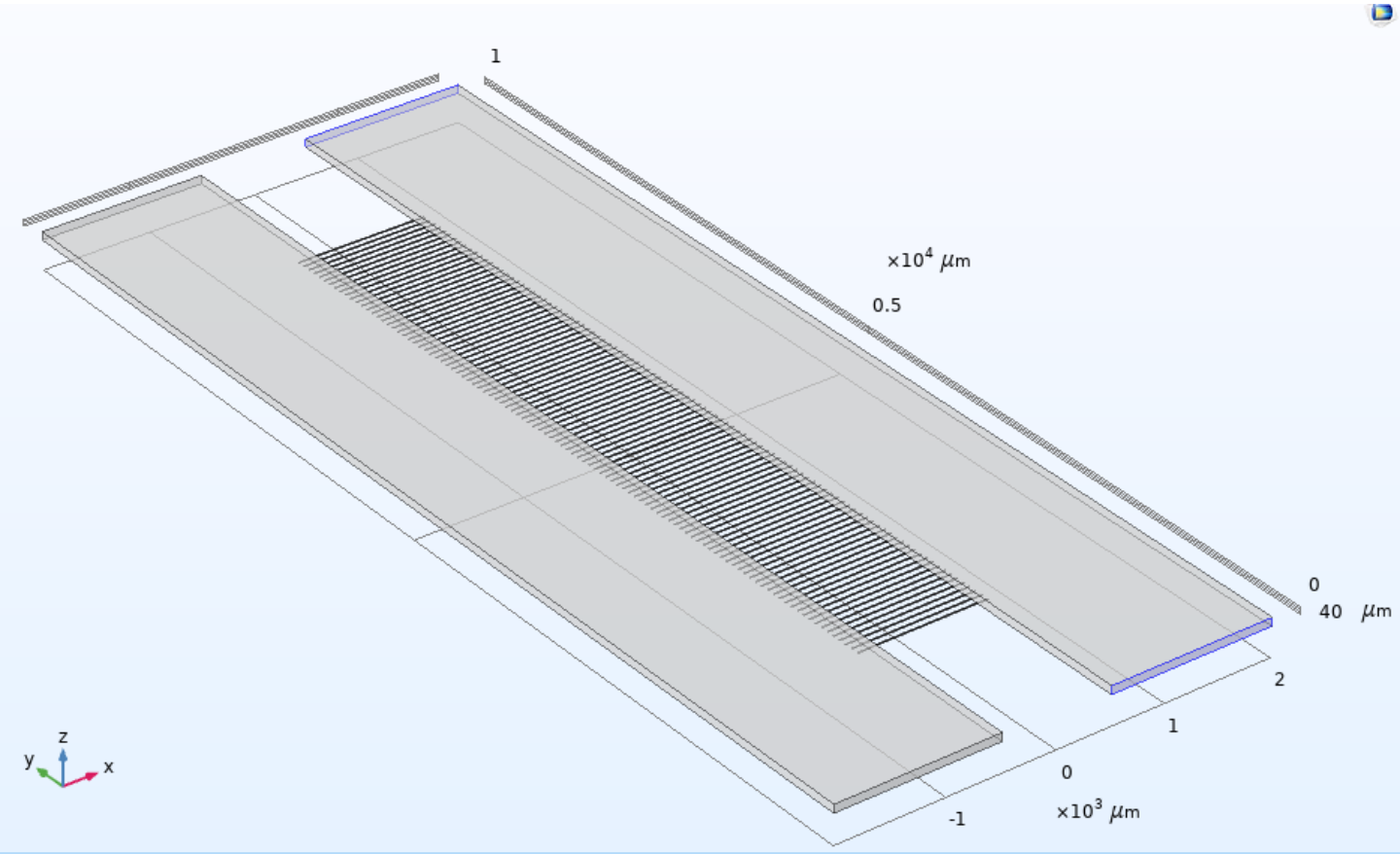

Figure S5: 3D geometry of the COMSOL model

The boundary conditions were set as follows: Inflow (Blue) with a set pressure equal to the volume differential of 150 µL per chamber (75 µL per well), Outflow (opposing “openings”), No Flux (all other surfaces). The model was solved for steady state, and the volumetric flow rate was determined by taking the average velocity at the outflow multiplied by the surface area of the outflow boundaries.

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