AxonQuant: a microfluidic chamber culture-coupled algorithm that allows high-throughput quantification of axonal damage

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Supplementary information

Supplementary Figure legends:
Figure S1. Critical dimensions of the microfluidic chambers.
Using microfluidic chambers to simultaneously image multiple neuronal growth cones. The growth cones of developing axons in microfluidic chambers were imaged using fluorescent microscopy following staining using phalloidin-Alexa Fluor 594. Microscopic images of growth cones were shown as red phalloidin-Alexa Fluor 594 signals and differential interference contrast (DIC) images. (a) Multi-microgroove images taken on a Zeiss LSM710 using a 63X oil objective (zoom factor 0.6). (b) Higher magnification images of growth cones (63X oil objective with zoom factor 2.0).
Fig. S3. Axonal continuity index was not influenced by different imaging intensity or cell density. (a) Fluorescent microscopic images of axonal bundles cultured in microfluidic chambers following immunostaining using anti-Tuj1 and secondary antibody conjugated to Cy3. Axons in peripheral axonal microgrooves (#1-#10) showed inconsistent results in supporting axonal growth, whereas those in the central axonal microgrooves (#131-#140) showed consistent results. (b) Fluorescent microscopic images of axonal bundles following culture at different cell densities, 7500 or 15000 cells per chamber. (c) Fluorescent microscopic images of axonal bundles taken at high or low fluorescent intensities. (d) Axonal continuity index obtained using AxonQuant1.0 was not affected by image signal intensity.
Fig. S4 Axonal continuity index was not influenced by different cell density when axons were severely damaged. (a). Fluorescent microscopic images of axons treated with 10uM MnCl2 for 12hrs with neurons cultured at three different cell densities: 7500, 15000 and 30000 cells per chamber. (b, c) Axonal continuity index of neurons was not affected by variations in cell densities.