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

Supplementary Figure 1. In the central injury chamber of the AoC, the strong flow of 1000 μL/min causes detachment and axotomy in most axons.

Rat hippocampal neurons cultured in AoC were transfected with GCaMP-6f on DIV6 and stressed by the 1000 μL/min-flux for 180 s on DIV8. **(a)** The dynamic axotomy process is caused by 1000 μL/min. Most axons detached and were rapidly removed by the medium flux (detached). Rarely some remain attached and then are partially removed (attached). Bar = 40 μm. **(b)** In 23 axotomized axons, the ratio of detached and attached axon types are quantified.

Supplementary Figure 2. Automatic detection of the FAS sites in live axons during the flux-induced mechanical stress in AoC.

Rat hippocampal neurons cultured in AoC were transfected with Lifeact-GFP on DIV 5, and on DIV 7, the axons in the central injury chamber were fluxed by culture medium at different flow speeds for 180 s to induce various levels of mechanical stress. **(a)** Main steps of automatic FAS detection. Raw live images of the axon were smoothed and converted to binary masks (middle, top). Then, based on the roundness and size of the masks, all FAS sites were automatically detected (middle, bottom). Finally, FAS sites were colour-coded, showing the difference in size. See also the Materials and Methods part for more detail. **(b)** The representative field shows the crossing axons in AoC before the flux. The flux direction is indicated with an arrow. Bar = 20 μm. **(c)** Amplification of the boxed region in **(b)** showing the FAS formation before, during and after the 50 μL/min flux for 180 s, with the top panels showing the raw images and the bottom panels showing automatically detected FAS area (magenta). Bar = 10 μm. **(d)** Amplified regions of the bracketed regions in **(c)** are further resolved temporally, with time-lapse images showing the flux-induced FAS sites are soon reversed after the flux (50 μL/min, 180 s). Bar = 5 μm (left).

Supplementary Material

Supplementary Movie 1. High-speed flux causes axotomy accompanied by Ca^{2+} elevation in AoC. Rat hippocampal neurons cultured in AoC were transfected with the genetic Ca^{2+} sensor GCaMP-6f. Axotomy was induced by injecting high-speed flow (1000 μL/min, 180 s) into the central injury chamber. Instant Ca^{2+} fluctuation during the axotomy was monitored using time-lapse microscopy. A representative movie shows the significant Ca^{2+} elevation accompanied by the axotomy, with the instant Ca^{2+}intensity colour-coded. The duration of 1000 μL/min flow injection was indicated with a yellow arrow. Bar = 50 μm.

Supplementary Movie 2. Automatic detection of FAS underlying the DAI induced by low-speed flux. Rat hippocampal neurons cultured in AoC were transfected with the F-actin marker Lifeact-GFP. DAI was induced by injecting high-speed flow (200 μL/min, 180 s) into the central injury chamber. Fast time-lapse images showed that flux-induced FAS formation is placed in the top panel. Boxed ROI is amplified in the bottom panels, which show the raw images and FAS automatic detections and area changes (colour-coded) of the same ROI. Bar = 30 μm.

Supplementary Movie 3. The DAI induced by weak flow is spatially restricted. DIV 9 rat hippocampal neurons expressing Lifeact-GFP were subjected to DAI caused by medium flux (200 μL/min, 180 s). The representative tiled images show the axonal parts in the soma chamber, axon channels and the injury chamber before the flux (left panel). In the right panel, time-lapse images were acquired from the soma chamber (blue box), axon channels (black box) and injury chamber (red box), respectively. The injection of 200 μL/min flow for 180 s is indicated with the white arrow. Bar is indicated in the frame.

Supplementary Movie 4. Rapid $[Ca^{2+}]_{axon}$ elevation accompanying FAS formation in axons subjected to flux-induced DAI. Rat hippocampal neurons expressing GCaMP-6f were subjected to flux-induced non-disruptive DAI on DIV 10. Time-lapse images were acquired using open-field microscopy in the central injury chamber. The injection of 200 μL/min flow was indicated with the yellow arrow. A Representative time-lapse image showing the injury process of transfected axons is shown, with $[Ca^{2+}]_{axon}$ level indicated by the fluorescent level of GCaMP-6f. Bar = 5 μm.