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

Autonomous Electrochemical Biosensing of Glial Fibrillary Acidic Protein for Point-of-Care Detection of Central Nervous System Injuries

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Figs. S1 to S7
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Other Supplementary Materials for this manuscript include the following:

Video S1

Table S1. Optimized parameters of the carbon dioxide (CO_2) laser cutter for patterning of different layer materials of the microfluidic device.

Materials	Laser power (W)	Irradiation Speed (m/s)	Irradiation Pass
PMMA (1.5 mm)	85	10	10
PMMA (6 mm)	95	3	20
PSA	59	30	6
Double-sided adhesive	59	45	6
NC fiber	38	40	3
VW tissue	36	50	2

Laminar flow field equations

For unsteady two-phase flow analysis, immiscible gas and liquid phases are considered, and both fluids behave as incompressible, with no phase change.[1] Under these assumptions, the governing equations for the two-phase Level Set model contain three conservation equations of continuity, level set function, and momentum:

The mass conservation equation is defined as:

$$\nabla.u = 0 \qquad \textbf{(S1)}$$

where u (m/s) represents gas/liquid velocity vector.

Phase conservation for the level set variable is defined as:

$$\frac{\partial \phi}{\partial t} + u.\nabla\phi = \gamma\nabla.\left(\varepsilon\nabla\phi - \phi(1-\phi)\frac{\nabla\phi}{|\nabla\phi|}\right)\frac{\partial \phi}{\partial t} + u.\nabla\phi = \gamma\nabla.\left(\varepsilon\nabla\phi - \phi(1-\phi)\frac{\nabla\phi}{|\nabla\phi|}\right) \qquad \textbf{(S2)}$$

where, ϕ denotes the volume fraction of the liquid phase, with $\phi = 0$ in the air and $\phi = 1$ in the water. The thickness of the interface is determined by ε parameter. γ is the reinitialization parameter which is equal to the maximum velocity magnitude occurring in the model.

Momentum equation:

$$\rho\left(\frac{\partial u}{\partial t} + u.\nabla u\right) =- \nabla p + \mu\nabla^2 u + \rho g + \sigma\kappa\nabla\phi\rho\left(\frac{\partial u}{\partial t} + u.\nabla u\right) =- \nabla p + \mu\nabla^2 u + \rho g + \sigma\kappa\nabla\phi \qquad \textbf{(S3)}$$

where ρ(kg/m^3) and μ(Pa.s) denote the density and viscosity of the liquid/gas, respectively. p(Pa) is pressure, σ(N/m) is surface tension coefficient, κ presents mean curvature of the phase interface and g(m/s^2) represents the gravity vector as a body force. Moreover, for two-phase flow, the density and viscosity physical properties are determined as a function of the volume fraction of the water and air using the following equation:

$$\rho = \rho_a + (\rho_w - \rho_a)\phi \qquad \textbf{(S4)}$$

$\mu = \mu_a + (\mu_w - \mu_a)\phi$

The water and air physical properties are specified with the subtitles w and a, respectively.

Equations governing the transport of diluted species

The diffusion–convection transport equation is utilized for simulating the transport of species through a liquid [1].

$$\frac{\partial c}{\partial t} + \nabla.(- D\nabla c) + u.\nabla c = 0D\nabla c) + u.\nabla c = 0 \quad \textbf{(S5)}$$

where c (mol/m^3) and D (m^2/s) are the concentration and diffusion coefficient of redox species, respectively. u(m/s) is the velocity vector of the flow field and obtained from laminar two-phase flow analysis. The no flux ($- n.(- D\nabla c) = 0$) and specific concentration ($c = c_0$) are suitable boundary conditions for the walls in contact with the liquid.

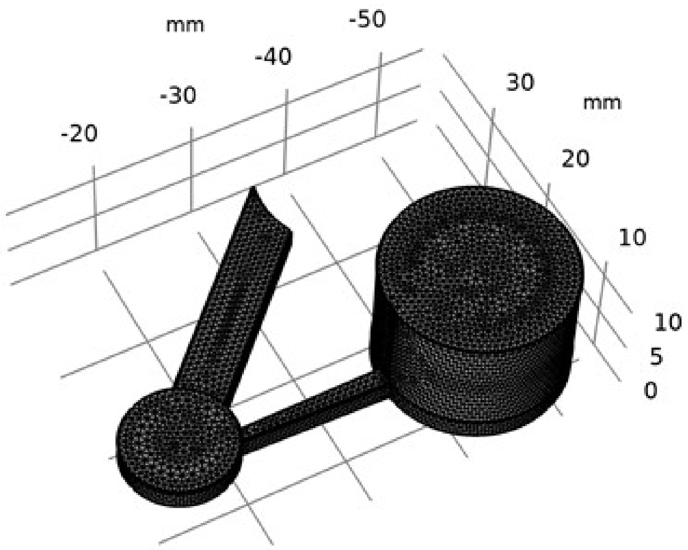

Fig. S1 Grid distribution in COMSOL Multiphysics for the microfluidic design with straight side-channel.

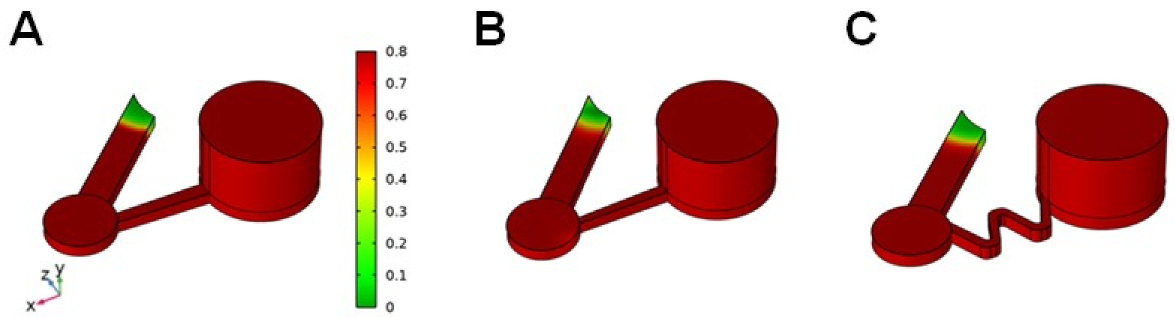

Fig. S2 Level set variable surface for the two-phase flow modeled inside different microfluidic designs for side-channel optimization. **(A)** design #1 (D1) with 2 mm width straight side channel. **(B)** D2 with 1 mm width straight side channel. **(C)** D3 with zigzag side channel.

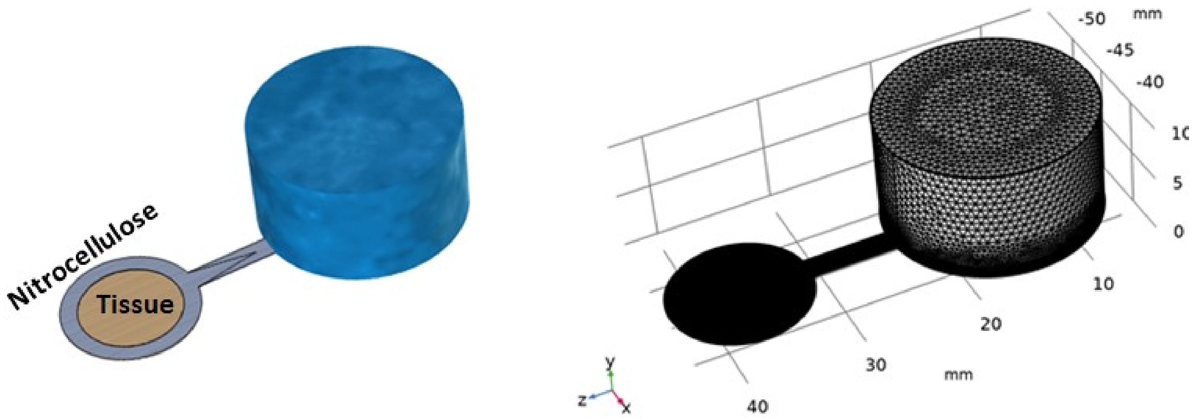

Fig. S3 Grid distribution in COMSOL Multiphysics for two-phase flow over the sensing electrode.

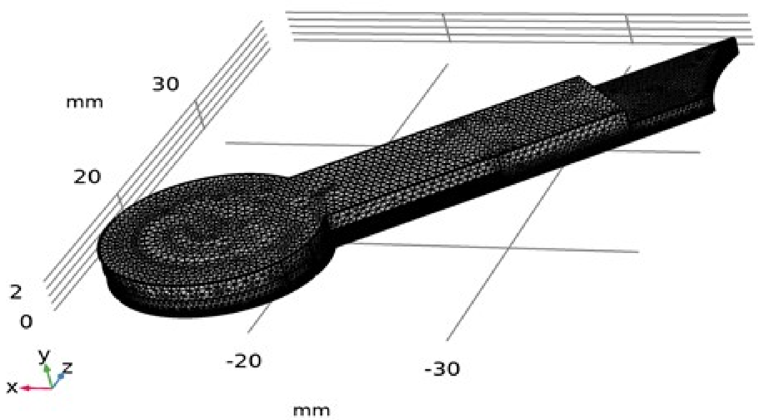

Fig. S4 Grid distribution in COMSOL Multiphysics for concentration distribution in the redox mixing channel.

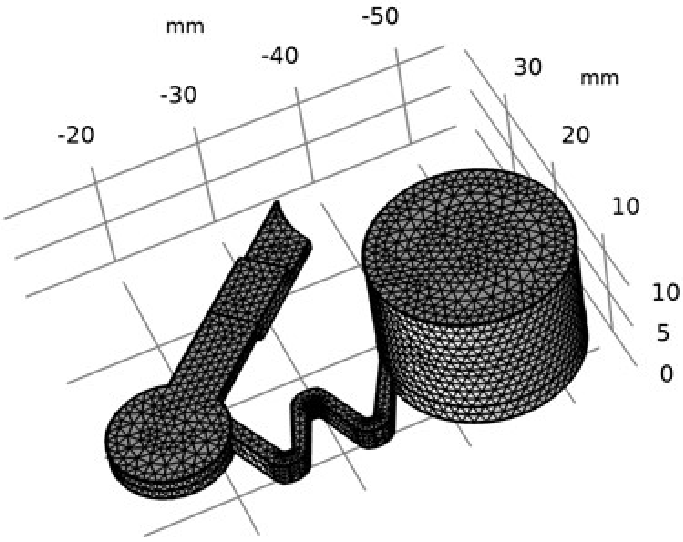

Fig. S5 Grid distribution in COMSOL Multiphysics for the design with zigzag side channel and a step feature designed in the redox mixing channel.

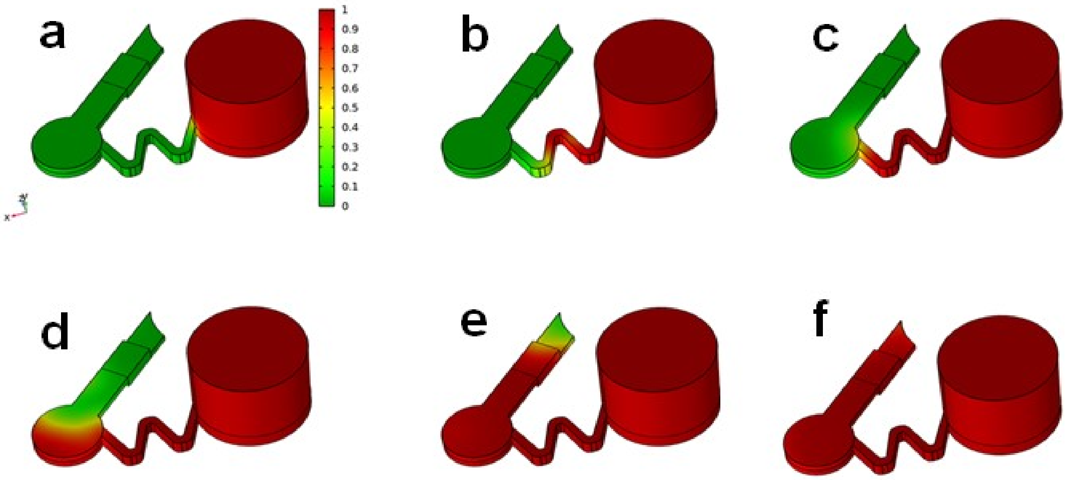

Fig. S6 Level set variable surface for the two-phase flow in the final design D11 with the zigzag side channel and a step feature within the redox mixing channel.

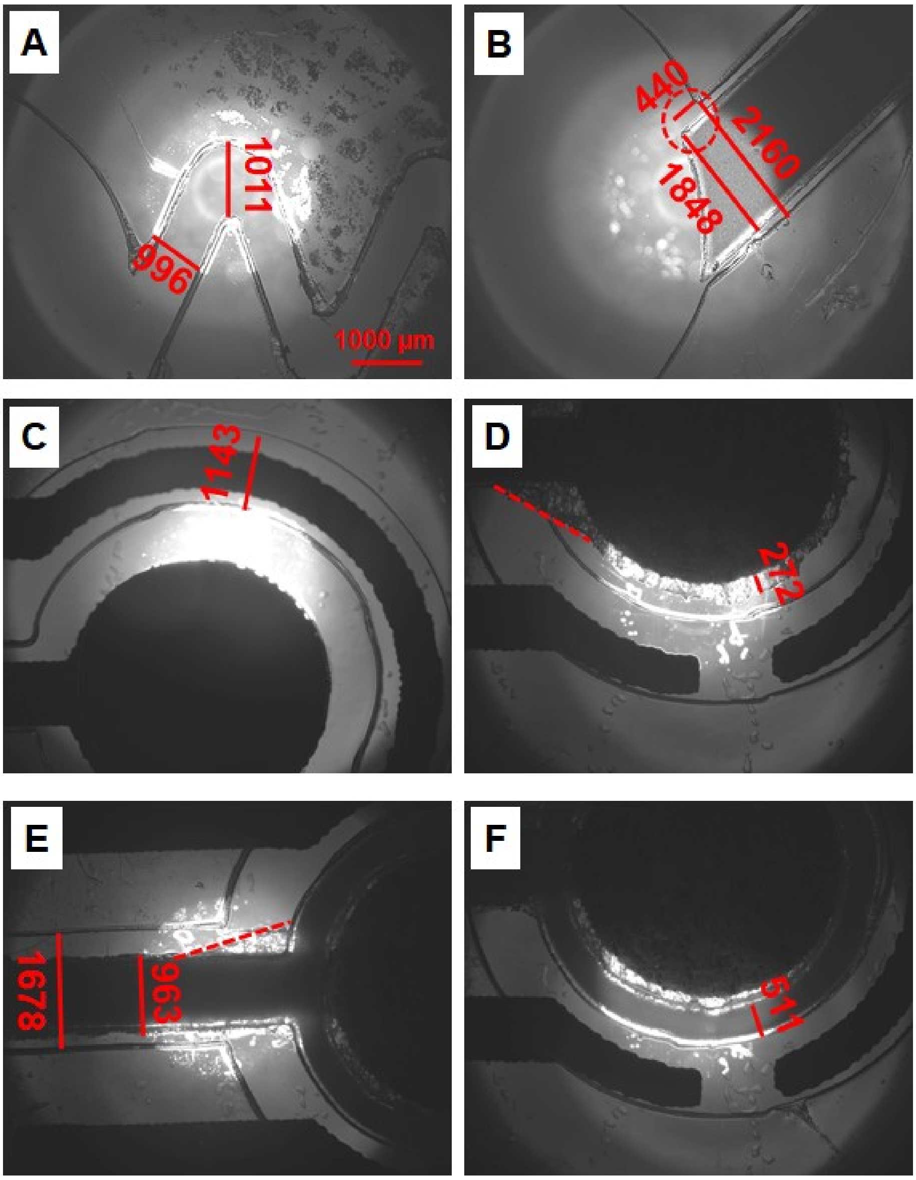

Fig. S7 Optical microscopy characterization of various laser-cut microfluidic layers. (A) The zigzag channel cut inside the double-sided adhesive layer. **(B)** The interface of the redox channel and the sensing area, representing the connection edge of redox-soaked Nitrocellulose (NC) with the sensing area. **(C)** Ring-shape channel cut inside the double-sided adhesive layer between the Working Electrode (WE) and Counter Electrode (CE) as an adhesive for attaching the Virgin Wood (VW) and NC. **(D)** The overlap of VW tissue positioned on top of WE and its connection with the inlet channel (indicated with diagonal line). **(E)** Dimensions of the inlet channel and NC positioned inside it. **(F)** Dimension of the NC ring positioned around the VW tissue on top of the WE. The soft edges of the cut sheet illustrate the appropriateness of the laser power, speed, and irradiation passes (Scale bar: 1000 µm).

Table S2. The comparison between the dimensions measured by optical microscopy and extracted from CAD files.

Materials	Nominal size (μm)	Fabricated size (μm)	Relative error (%)
Zigzag channel width	700	996	0.42
Zigzag channel bend	900	1011	0.12
Redox channel width	2000	2160	0.08
Redox NC width	1900	1848	-0.03
Channel around WE width	1000	1143	0.14
NC around WE width	7500	511	-0.93
Channel toward WE width	1520	1678	0.10
NC toward WE width	1200	963	-0.20

References

1. S. Mehraji and M. Saadatmand, *Physics of Fluids*, 2021, **33**, 072009.