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

Electronic Supplementary Information

Performance-enhanced clogging-free viscous sheath constriction impedance flow cytometry

Junwen Zhu[1], Yongxiang Feng[1], Huichao Chai[1], Fei Liang[1], Zhen Cheng[2], Wenhui Wang[1]*

[1]State Key Laboratory of Precision Measurement Technology and Instrument, Department of Precision Instrument, Tsinghua University, Beijing, 100084, P. R. China.

[2]Department of Automation, Tsinghua University, Beijing, 100084, P. R. China.

Supplementary Note 1

For viscosity characterization of PEG at different concentrations and other liquids, we use a classical functional relationship for Newtonian liquid[1, 2]

$$\frac{Q_{\mathrm{sam}}}{Q_{\mathrm{sh}}} \cdot \frac{\eta_{\mathrm{sam}}}{\eta_{\mathrm{sh}}} = \frac{w_{\mathrm{ap}}}{w_{\mathrm{ch}} - w_{\mathrm{ap}}}, \tag{S1}$$

where Q_{sam} and Q_{sh} are the flow rate of sample and sheath fluid, η_{sam} and η_{sh} are the corresponding viscosity, and w_{ap} and w_{ch} are the width of SA and whole flow channel, as shown in Figure S1. In this study, we used octyl alcohol with known viscosity (η = 8.9 mPa·s) as the sheath fluid and the liquid with viscosity to be measured as the sample fluid. In eqn. (S1), Q_{sam} and Q_{sh} are given by the syringe pumps, w_{ch} is set according to the device design, and w_{ap} is measured through microscope. In this way, viscosity of the liquids was obtained (Table S2).

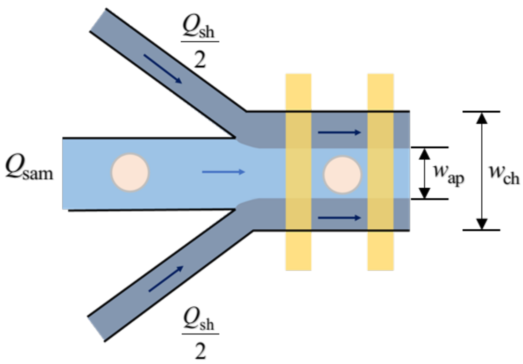

Figure S1. Schematic diagram of microfluidic flow channel structure under SC.

Supplementary Note 2

The electrical properties of the mixture, where the cell is centered, can be represented by the properties of the individual cell and the suspending medium through Maxwell's mixture theory (MMT)[3]. The complex impedance of the mixture can be given by

$$Z_{mix}^{*} = \frac{1}{j\omega\varepsilon_{mix}^{*} l\kappa}, \tag{S2}$$

where κ is the cell constant dependent on the geometrical parameters of the sensing zone, and l denotes the electrode length. $\varepsilon_{\mathrm{mix}}^{*} = \varepsilon_{\mathrm{mix}} + j\frac{\sigma_{\mathrm{mix}}}{\omega}$ indicates the complex permittivity of the mixture, with the conductivity σ_{mix}, the permittivity $\varepsilon_{\mathrm{mix}}$ and the angular frequency ω, which can be calculated as

$$\varepsilon_{\mathrm{mix}}^{*} = \varepsilon_{\mathrm{m}}^{*} \cdot \frac{1+2\delta \cdot f_{\mathrm{CM}}^{*}}{1-\delta \cdot f_{\mathrm{CM}}^{*}}, \tag{S3}$$

where $\varepsilon^{*}_{\mathrm{m}}$ is the complex permittivity of the medium. f^{*}_{CM} is the Clausius–Mossotti coefficient, given by $f_{\mathrm{CM}}^{*} = \frac{\varepsilon_{\mathrm{c}}^{*} - \varepsilon_{\mathrm{m}}^{*}}{\varepsilon_{\mathrm{c}}^{*} + 2\varepsilon_{\mathrm{m}}^{*}}$, where $\varepsilon^{*}_{\mathrm{c}}$ is the complex permittivity of the cell. δ represents the cell volume fraction, so δ increases by reducing SA. When measuring the impedance with the medium alone (i.e., Z^{*}_{base}), its complex permittivity is $\varepsilon^{*}_{\mathrm{m}}$.[4] Therefore, the impedance variation due to the presence of a cell can be calculated as

$$\Delta Z = Z_{\mathrm{mix}}^{*} - Z_{\mathrm{base}}^{*} = \frac{1}{j\omega\varepsilon_{\mathrm{m}}^{*} l\kappa} \cdot \frac{-3\delta \cdot f_{\mathrm{CM}}^{*}}{1+2\delta \cdot f_{\mathrm{CM}}^{*}}. \tag{S4}$$

Eqn. (S4) means $|\Delta Z| \propto \delta$. Sensitivity is defined as $|\Delta Z|/|Z_{\mathrm{base}}|$ in this study. Thus, reducing SA can increase δ, thus increase sensitivity.

On the other hand, SNR is calculated by $20\,lg(V_{\mathrm{s}}/V_{\mathrm{n}})$, where V_{s} and V_{n} represent the difference of signal and noise relative to the baseline voltage, respectively. Thus, $V_{\mathrm{s}} \propto |\Delta Z|$, and reducing SA can also increase SNR.

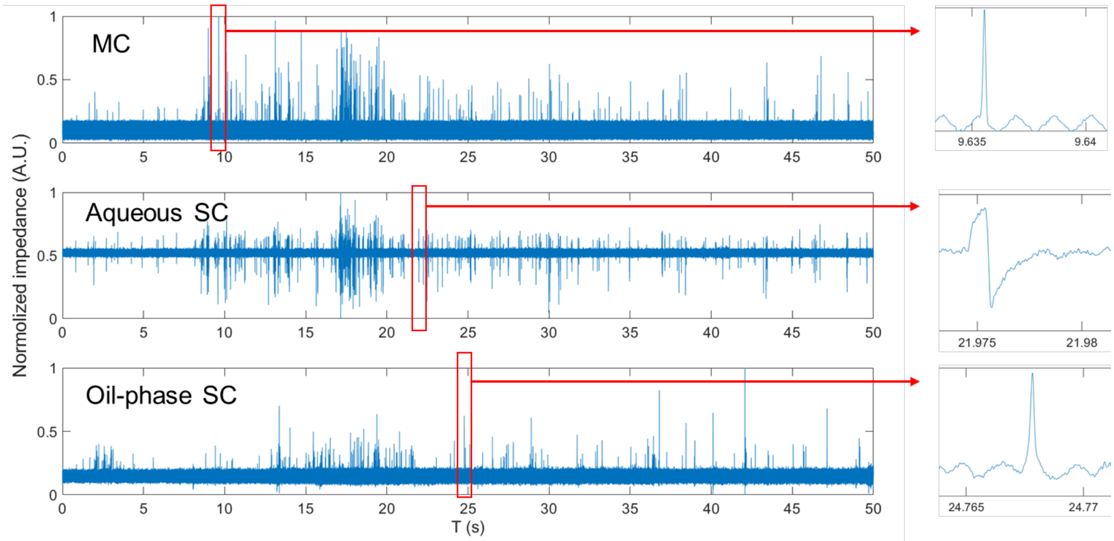

Figure S2. One example segment of impedance signals detected by the coplanar double electrodes with MC, aqueous SC, and oil-phase SC.

Table S1. Pre-determined values of C_S and C_{DL}.

Liquid type	**Viscosity**
C_S	0.96 pF
C_{DL} in MC with SA of 25 μm	70 pF
C_{DL} in MC with SA of 30 μm	101 pF
C_{DL} in MC with SA of 35 μm	126 pF
C_{DL} in SC with SA of 25 μm	104 pF
C_{DL} in SC with SA of 30 μm	119 pF
C_{DL} in SC with SA of 35 μm	135 pF

Table S2. Viscosity of different liquids in this study.

Liquid type	**Viscosity**
PBS	1.5 mPa·s
Deionized water	1.0 mPa·s
Octyl Alcohol	8.9 mPa·s
5 mM PEG	2.1 mPa·s
40 mM PEG	14.8 mPa·s
50 mM PEG	21.0 mPa·s
75 mM PEG	46.7 mPa·s
90 mM PEG	69.5 mPa·s

References

1. M. H. Panhwar, F. Czerwinski, V. A. S. Dabbiru, Y. Komaragiri, B. Fregin, D. Biedenweg, P. Nestler, R. H. Pires and O. Otto, *Nature Communications*, 2020, **11**.
2. J. B. Knight, A. Vishwanath, J. P. Brody and R. H. Austin, *Physical Review Letters*, 1998, **80**, 3863-3866.
3. T. Sun, D. Holmes, S. Gawad, N. G. Green and H. Morgan, *Lab Chip*, 2007, **7**, 1034-1040.
4. T. B. a. P. R. Ana Valero, Lab Chip, 2010, 10, 2216-2225.