Electronic Supplementary Information

Neural Network Enhanced Real-Time Impedance Flow Cytometry for Single-Cell Intrinsic Characterization

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Device Fabrication

The fabrication process of the microfluidic device is described in detail in our previous study.[1] Briefly, 10 nm Cr and 100 nm Au are patterned onto glass substrate through lift-off technique, and microchannel is fabricated using soft-lithography technique with a thickness of ~20 μm. The electrode layer and the microchannel layer are bonded together firmly after treating with oxygen plasma and baking. Ultimately, a printed circuit board is customized to match the electrode pattern for signal excitation and measurement. The fabricated device is shown in Fig. S1.

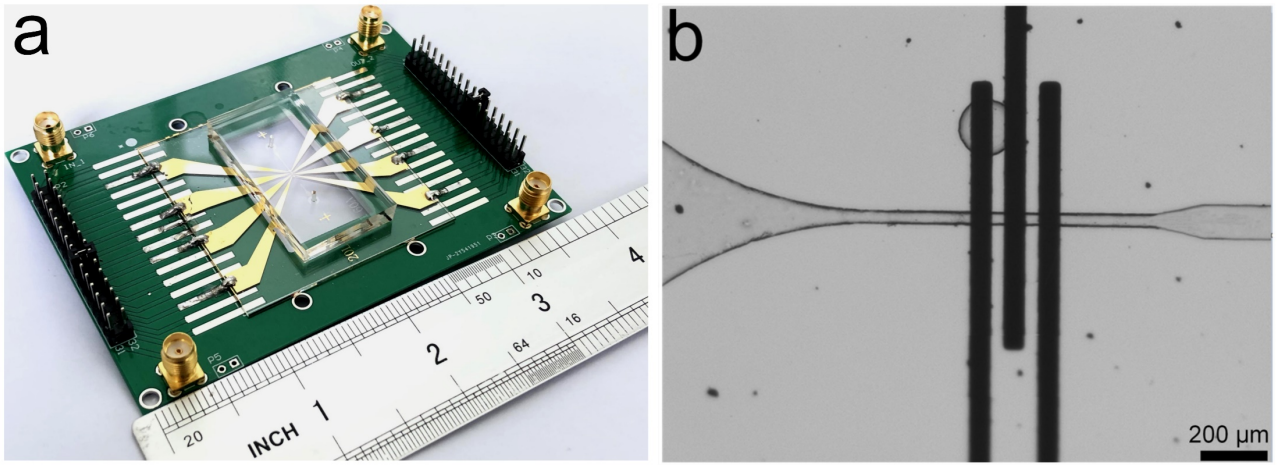

Figure S1. Overview of the fabricated device and corresponding microscopic image. (a) The assembled microfluidic device composed of a PDMS top layer and a glass bottom layer patterned with electrodes which are wired out through a customized printed circuit board. (b) Optical micrograph showing the sensing area of the chip, note that the three-electrodes design is to improve the success rate of chip fabrication, only two of them are used for the measurement.

Measuring System Configuration

When a cell passes through the coplanar electrodes, it replaces an equal volume of medium and perturbs the electrical field, leading to a change to the output signal of the measuring circuit. The signal resembles a Gaussian curve, in which the peak corresponds to the cell impedance at specific frequency. By transferring the current signal to voltage signal through transimpedance amplifier (TIA) and detecting the resultant weak difference of the response signals with or without cells via lock-in amplifier (LIA), IFC can measure the impedance of the cell. The measuring system is responsible to generate excitation signal, demodulate the response signal and acquire the output DC signal. Based on our previous work [1], the home-made LIA system is improved to simultaneously measure four-frequency signal. The four-frequency signal generator is built with AD9958 (ADI, USA), the TIA is chosen as

OPA657 (TI, USA) with 50 kΩ feedback resistor, the demodulators is set to four-channel parallel with AD835 (ADI, USA) and the OPA227 (TI, USA) is used as low-pass filter and amplifier with 5 kHz cut-off frequency. The final output DC signal is sampled by a data acquisition board (NI, PCI-6289) at 20 kHz. The graphical user interface (GUI) software system is responsible to real-time process, display and record the data.

Table S1. Times for each processing step in the workflow

Process	Time
Communication of LabView-Matlab	4 ms
Data preprocessing in Matlab script	0.5 ms
Communication of LabView-Python	0.4 ms
Deduction of the neural network in	0.3 ms

Table S2. Electrical model parameters for electrical property extraction based on model fitting

Model parameters	Values
double layer capacitance C_{DL}	60 pF
stray capacitance C_s	0.5 pF
medium conductivity σ_{med}	1.6 S/m
medium relative permittivity ε_{med}	78
cytoplasm relative permittivity ε_i	60
membrane thickness d	10 nm

Table S3. Accuracy of the NN models in training dataset, test dataset and novel dataset

Dataset			**Regression**			**Classification**
Data	Source	Number	r	σ_i	C_{sm}	Classification
Trainset	70% of dataset	7000	0.24 μm	0.02 S/m	0.71 mF/m^2	94.7%
Testset	30% of dataset	3000	0.28 μm	0.02 S/m	0.78 mF/m^2	93.2%
Novelset	New measurement	9751	0.33 μm	0.03 S/m	0.95 mF/m^2	91.5%

Table S4. NN-calculated intrinsic electrical properties for five types of cells

Cell type	***r* (μm)**	**σ_i (S/m)**	**C_{sm} (mF/m^2)**
MCF-7	7.51 ± 0.67	0.302 ± 0.029	20.08 ± 0.98
A549	6.79 ± 0.59	0.333 ± 0.035	22.69 ± 1.60
HeLa	7.20 ± 0.31	0.446 ± 0.047	18.56 ± 1.27
HL60	5.71 ± 0.38	0.545 ± 0.063	13.69 ± 1.71
GM12878	6.09 ± 0.41	0.496 ± 0.058	24.22 ± 1.35

Table S5. Proliferate number and rate of cultured cells

	1st day	**2nd day (24h)**	**Proliferate rate**
Control	453	791	74.6%
Experiment	569	968	70.1%

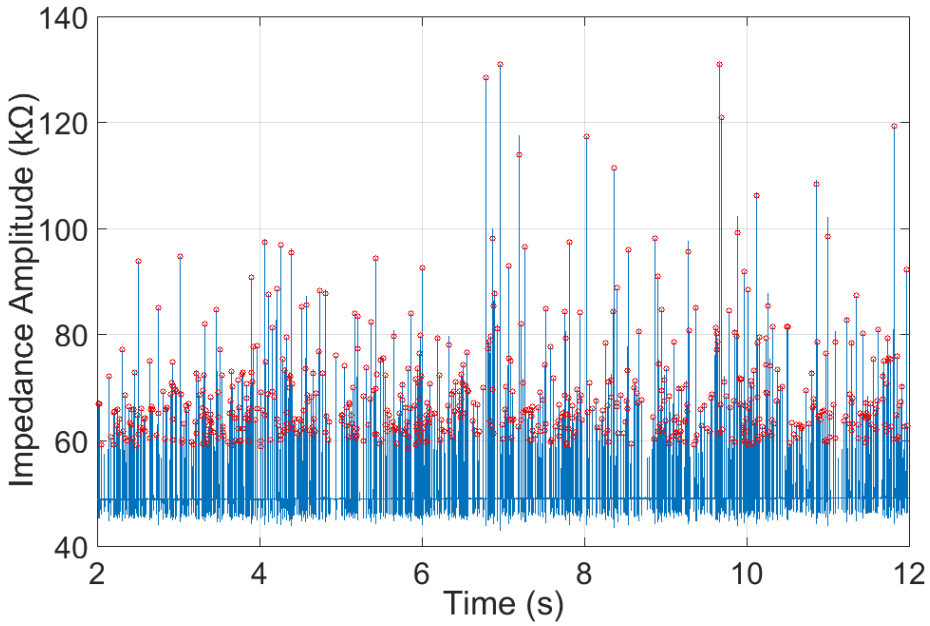

Figure S2. Instantaneous time-varying pulse signals measured for continuous cell transit events.

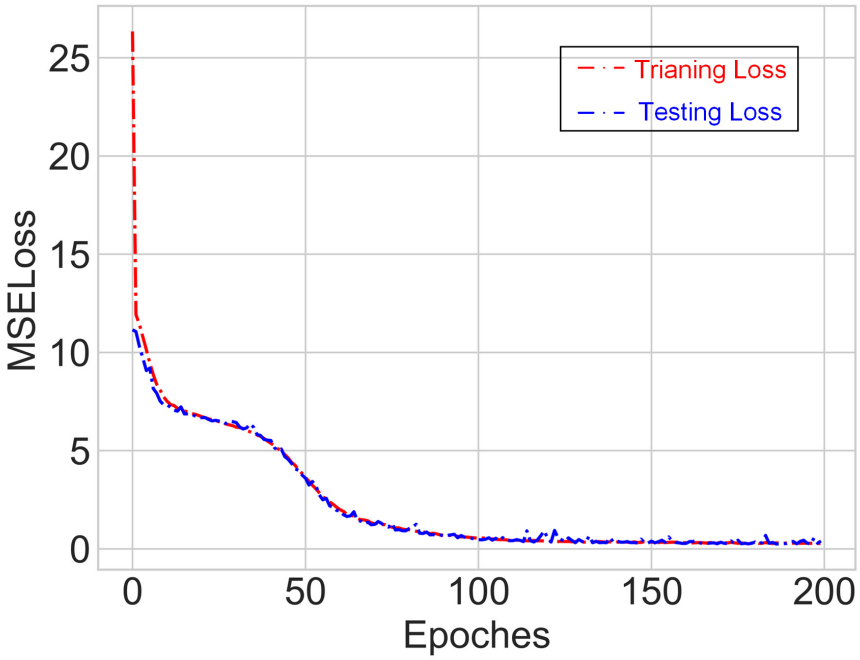

Figure S3. Training and testing results for the FCN.

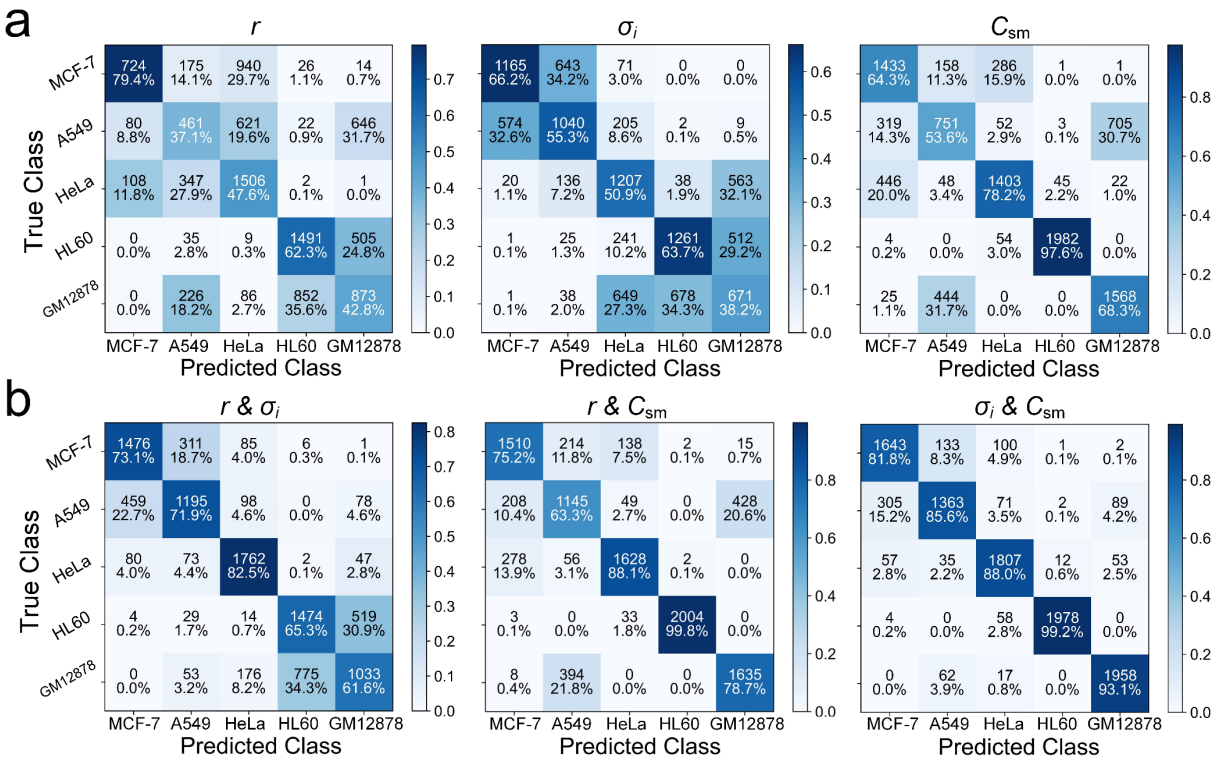

Figure S4. Confusion matrix for (a) one intrinsic parameter case of r, σ_i and C_{sm} and (b) two intrinsic parameters case of $r\&\sigma_i$, $r\&C_{sm}$ and $\sigma_i\&C_{sm}$ used for cell classification respectively.

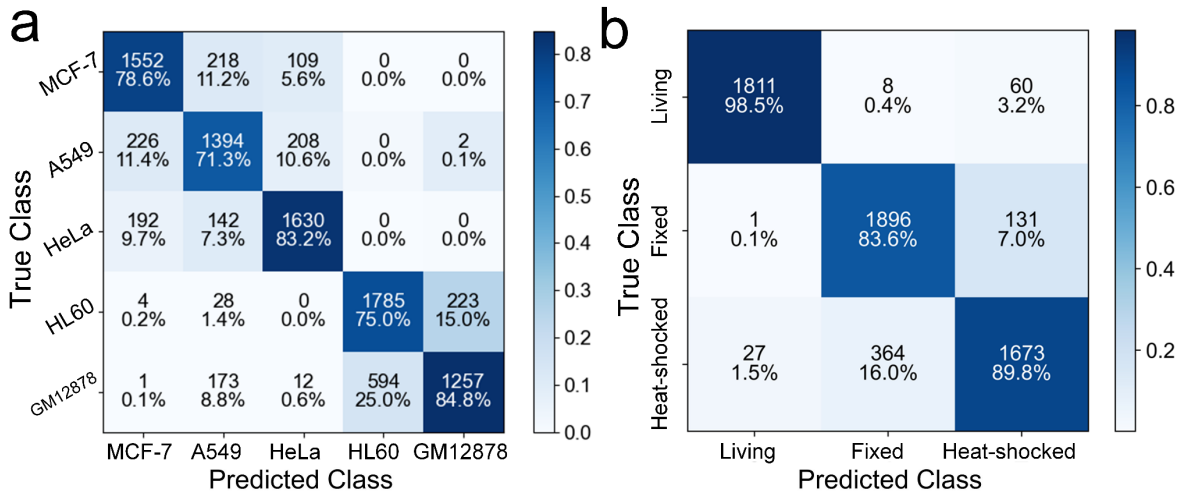

Figure S5. Confusion matrix for (a) three phenomenological metrics (i.e., Impedance amplitude and phase at 1.2 MHz, and impedance opacity at 1.2 MHz/0.25 MHz) of five cell types and (b) three phenomenological metrics of living and dead cells used for cell classification respectively.

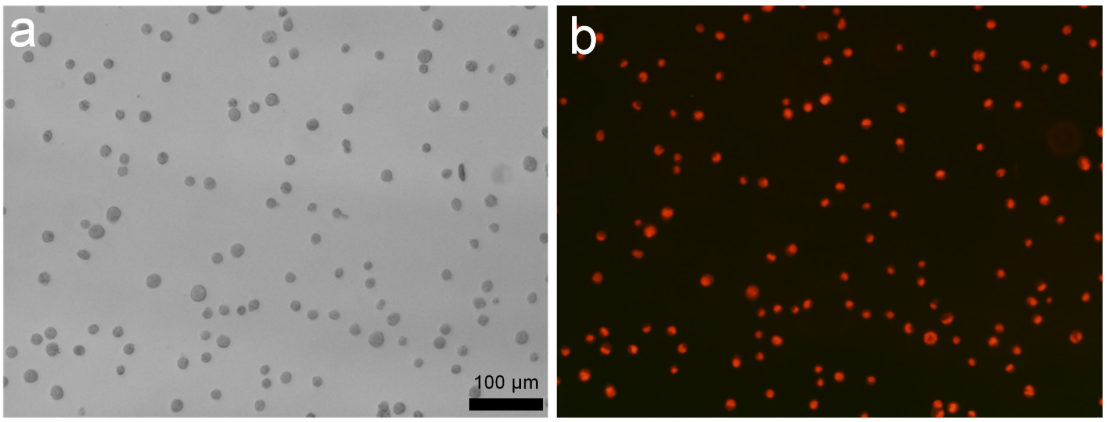

Figure S6. Microscopic images of brightfield (a) and PI-stained fluorescence (b) of heat-shocked cells, which indicates the death and loss of membrane permeability of the treated cell.

REFERENCE

1. Y. Feng, L. Huang, P. Zhao, F. Liang and W. Wang, *Anal. Chem.*, 2019, **91**, 15204-15212.