

Reliable Cell Concentration Prediction Using a Temperature-Compensated Four-Electrode Impedance Sensor with Machine Learning

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Summary:

This work presents a compact PCB-based four-electrode impedance sensor for reliable live-cell concentration prediction under electrode polarization and temperature drift. The four-electrode configuration suppresses double-layer capacitance, while temperature compensation stabilizes impedance features. Based on compensated spectra, machine learning enables robust prediction under small-sample conditions. Experiments demonstrate improved stability and accurate prediction, with LASSO achieving the best performance, showing the system's potential for broader biosensing applications.

Keywords: Electrical impedance spectroscopy (EIS), Four-electrode configuration, Temperature compensation, Cell concentration prediction, Machine learning

Background

Electrical impedance spectroscopy (EIS) is widely used for label-free live-cell analysis [1]. However, its practical application is limited by electrode polarization and temperature-induced impedance drift, which degrade feature reliability and hinder quantitative analysis. At low frequencies, double-layer capacitance masks cellular signals [2], while temperature variations introduce additional fluctuations in impedance measurements. Although differential schemes can mitigate these effects [3], they increase system complexity and limit real-time applicability. Therefore, achieving stable and reliable impedance-based cell concentration prediction under practical conditions remains a challenge.

Description of the System

A compact PCB-based four-electrode impedance sensing system is developed for cell concentration monitoring (Fig. 1). A sinusoidal excitation signal from 10 kHz to 10 MHz is applied, and impedance and temperature are simultaneously recorded. The four-electrode configuration suppresses electrode polarization and enhances low-frequency sensitivity. Temperature compensation is performed to correct impedance drift and stabilize feature behavior. The compensated multi-frequency impedance features are then processed using PCA and used to train

regression models for concentration prediction, enabling robust and real-time analysis.

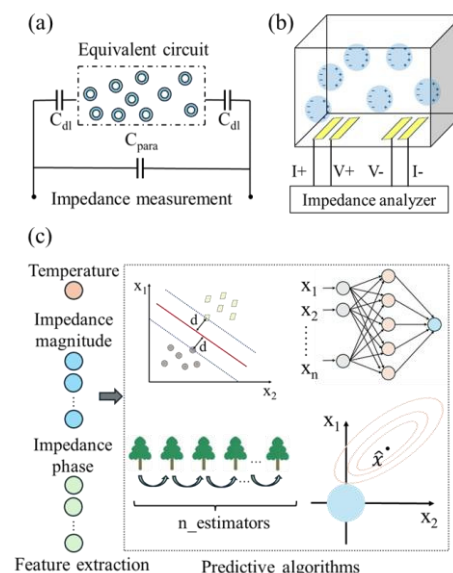


Fig. 1. Overview of the cell concentration prediction system. (a) Equivalent circuit representation of the impedance measurement. (b) Impedance spectra are acquired using an impedance analyzer (E4990A, Keysight, USA). (c) Measured impedance and temperature data are processed to extract features for training predictive models of cell concentration.

Results

Figure 2 shows that the four-electrode design effectively suppresses the double-layer capacitance seen in two-electrode systems, thereby revealing cellular polarization features at low frequencies.

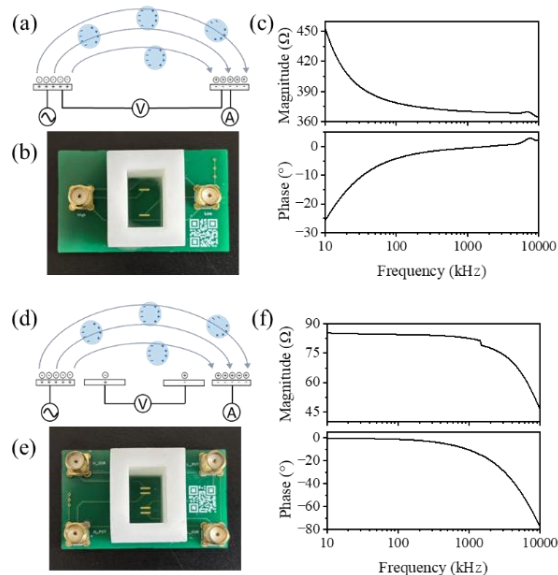


Fig. 2. Suppression of double-layer capacitance by the four-electrode configuration. Schematics, PCB structures, and impedance results of (a)–(c) two-electrode and (d)–(f) four-electrode.

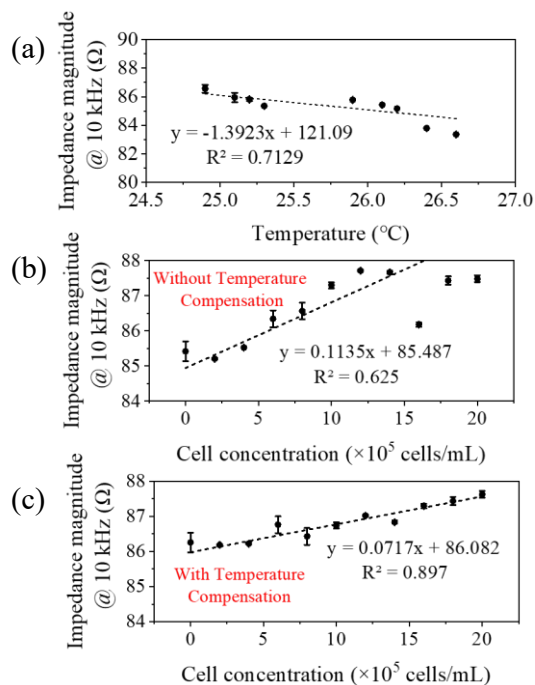


Fig. 3. Temperature dependence and compensation of impedance. (a) Low-frequency impedance shows a temperature coefficient of $\sim -1.39 \Omega/^{\circ}\text{C}$. (b)–(c) Fitting results before and after compensation, showing improved monotonic and consistent impedance–concentration relationship.

Figure 3 further demonstrates that the low-frequency impedance magnitude is strongly temperature-dependent, with a temperature coefficient of $-1.39 \Omega/^{\circ}\text{C}$, and that temperature compensation improves its monotonic correlation with cell concentration. Based on the compensated multi-frequency spectra, machine learning models were trained for concentration prediction. As summarized in Table 1 and Figure 4, LASSO achieved the best performance under small-sample, high-dimensional conditions, indicating that feature-selective linear models can be highly effective in constrained biosensing tasks.

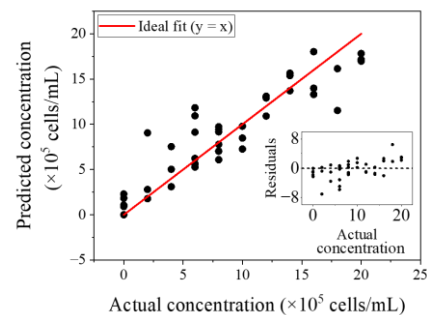


Fig. 4. LASSO fitting curve and residual distribution for cell concentration prediction.

Tab. 1: Performance comparison of different regression models in cell concentration prediction.

Predictive Model	RMSE	MAE	R ²
SVM	3.15	2.44	0.770
FNN	2.86	1.91	0.811
XGBoost	2.78	1.44	0.821
LASSO	2.28	1.60	0.880

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