TECHNICAL NOTE

Sources of Variability in Electrical Imaging Data

INTRODUCTION

Variability in electrical imaging data can arise from two main sources: electronic noise generated by circuits and electrodes, and biological variability stemming from cells. Our system is equipped with robust built-in quality control measures, ensuring high accuracy in measurements.

This note showcases the robustness and low variance achieved by our impedance measurements. Furthermore, we demonstrate the value of real-time monitoring for early identification of outliers in experiments, effectively minimizing the biological variance in the data.

TECHNOLOGY

CytoTronics' 96-well R&D prototype plate[1] provides real-time, high-resolution, label-free electrical images of live cells. Each well contains 4,096 electrodes providing a spatial resolution of 25 µm. The electric field-based (vertical and lateral), multi-frequency (0.25, 1, 4, 16 kHz) measurement techniques capture more than 20 parameter images including tissue barrier, cell-surface attachment, cell flatness, and motility every 5-15 minutes throughout an experiment.

RESULTS

Assessment of Electronic Variance of Cytotronics' Microplates

CytoTronics microplates are constructed with semiconductor circuits, leveraging the high-yield of state-of-the-art manufacturing and performance. Circuit integrity calibration scans performed prior to plating cells measure a known electrode impedance within the circuit and allow us to evaluate the performance of amplifiers across the plate. Variance in this measurement may indicate instability in the function of the amplifiers. Calibration scans also provide information as to microplate sensitivity. Measurement of a high impedance signal indicates ability to measure a low current, i.e. high sensitivity. The calibration impedance is relatively high, enabling assessment of plate sensitivity prior to use.

Figure 1A shows an example circuit integrity calibration scan at 4 kHz vertical field (VF 4 kHz) across all 393,216 electrodes (4,096 electrodes per well x 96 wells). The tight distribution of values as shown in the histogram in Figure 1B, with a very low coefficient of variation (CV = 3.16%), demonstrates the consistency of impedance measurements across the entire circuit. Calibration scans are performed across the entire range of impedance measurements to evaluate the electronic uniformity of a plate.

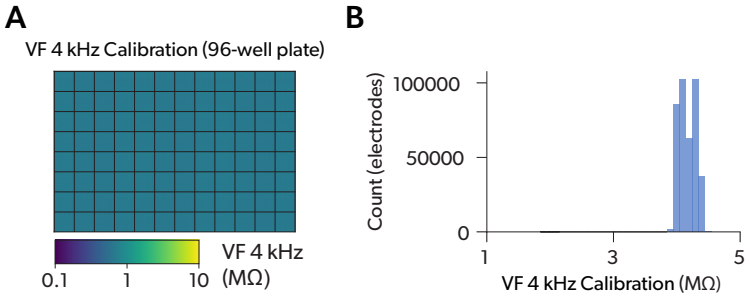

Figure 1. (A) Impedance map showing a circuit integrity calibration scan for the vertical field (VF) 4 kHz across a 96-well plate. (B) Histogram showing distribution of electrode impedance values for VF 4 kHz calibration across the 96-well plate shown in panel A.

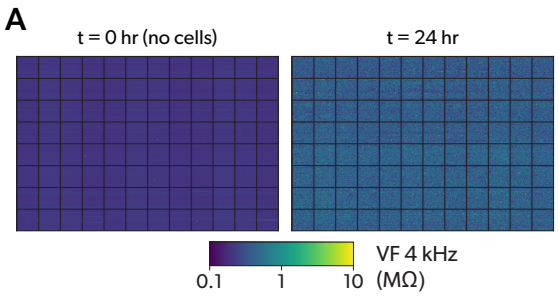

Figure 2. (A) Impedance map showing the vertical field (VF) 4 kHz measurement across a 96-well plate without cells (0 hr) 24 hours after plating A549 cells (24 hr). (B) Histogram showing distribution of well-median impedance values for the VF 4 kHz measurement across the 96-well plate shown in panel A.

Quantifying Cell-Specific Impedance Measurements Over Background

Another factor that contributes to robustness of measurements is signal-to-noise ratio. Figure 2A shows impedance images for wells with only media as a conducting solution (0 hr). This assesses the baseline impedance prior to plating cells. Impedance images taken 24 hours after plating, at sub-confluent cell densities show a clear increase in impedance, with identifiable regions with cells and without cells.

Figure 2B illustrates the median well impedance values at 0 hour (blank medium) and 24 hours (with cells). Wells containing cells are clearly separated from blank wells which have a low impedance. The overall variance of measurements is low, with the well-to-well coefficient of variation (CV) being 3.6 % at 0 hour and 5.3% at 24 hours. The increase in variance at 24 hours stems from the biological variability of cell growth across the wells.

It should be noted that for the 24 hour impedance measurements a cell mask was applied in order to assess the impedance of electrodes occupied by cells. The cell mask confers the additional advantage of improving the accuracy of measurements. Morphological measurements can be restricted to areas with cells, enabling comparison between wells with different confluencies. It further increases accuracy of spatially dependent properties such as tissue barrier.

Controlling for Biological Variance

The largest source of variance in biological systems is the cells themselves. Small differences in cell number, growth rate, or functional features can compound over time and cause large differences in observed effects, complicating data deconvolution and interpretation. The ability of our technology to perform real time monitoring across a range of morphological and functional parameters makes it possible to monitor and control for this variance in biological systems.

Figure 3 shows 24 hours of growth of A549 cells on a 96-well plate. 15,000 cells were plated in each well and allowed to grow for 24 hours. Cell properties within each well were measured

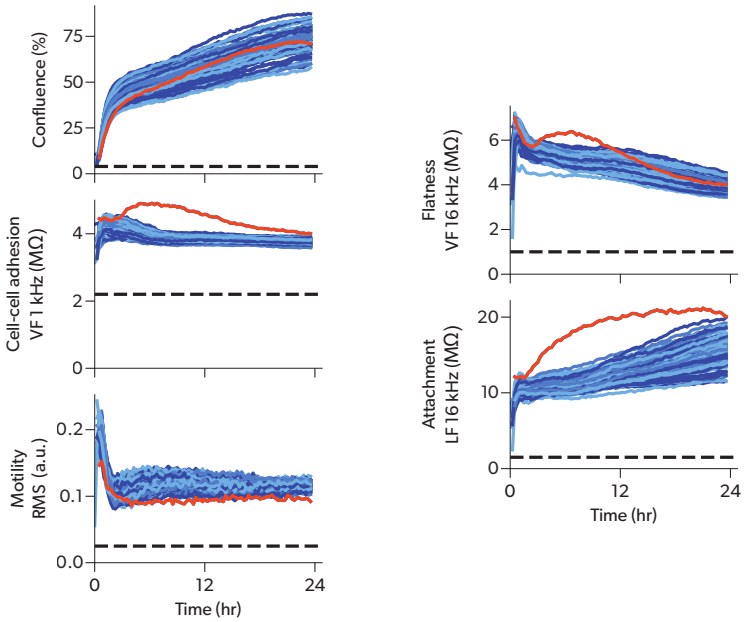

Figure 3. (A) Functional parameters (confluence, cell-cell adhesion, motility, flatness, and attachment) plotted individually for each well of a 96-well plate over time up to 24 hours post cell seeding in A549 cells (blue traces). The red trace is a well identified as an outlier in some, but not all measurements. The black dashed line in each plot represents the electrode background value with no cells present.

across a range of functional parameters and revealed an outlier well (red trace). While not an outlier in the confluence plot, this well had noticeably distinct adhesion (VF 1 kHz), attachment (LF 16 kHz), and flatness (VF 16 kHz) measurements. Our platform's ability for real-time, multi-parametric monitoring of cell state allows us to easily identify outlier wells such as this one prior to experimental interventions (i.e. compound addition). These wells can then be excluded from the experiment, thus controlling for the inherent biological variance in data and improving experimental reproducibility.

CONCLUSION

CytoTronics' 96-well microplates can measure a variety of impedance-based parameters at high-resolution and in real-time. In this note, we highlight the low electronic variance of the CytoTronics' platform (including circuitry and electrodes). We demonstrate that the ability to monitor cells non-invasively through the duration of an experiment can help assess and control for inherent biological variability in experiments ultimately providing a uniform starting state for complex biological assays.

METHODS

Cell Lines and Culture

A549 (CCL-185) cells were obtained from ATCC, cultured in DMEM supplemented with 10% FBS and 100 U/mL Penicillin-Streptomycin, and maintained in a humidified incubator at 37°C and 5% CO_2.

Measurement

Impedance measurements were taken inside a humidified incubator at 37°C and 5% CO_2 every 15 minutes throughout the experiment. A549 cells were seeded at 15k cells per well and grown for 24 hours.

Data Analysis

To assess variance at the circuit and electrode level, impedance measurements were taken for each electrode and its corresponding circuitry. To assess variance at the well level, the median value of all the electrodes (4,096) within each well was calculated and plotted as a distribution. A coefficient of variation was calculated as the ratio of the standard deviation to the mean of the distribution.

REFERENCES

1. Chitale, S. et al. A semiconductor 96-microplate platform for electrical-imaging based high-throughput phenotypic screening. *Nat Commun* **14**, 7576 (2023).

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