

A Microwell-Based 3D Electrorotation Platform for High-Throughput, Label-Free Single-Cell Analysis

Masato Suzuki^{1,2}, Ichiha Miya¹, Megumi Bando¹, Yushi Isozaki^{1,2}, Tomoyuki Yasukawa^{1,2}

¹ Graduate School of Science, University of Hyogo, 3-2-1, Koto, Kamigori, Ako-Gun, Hyogo, Japan

² Advanced Medical Engineering Research Institute, University of Hyogo, 3-264, Kamiya, Himeji, Hyogo, Japan

*suzuki@sci.u-hyogo.ac.jp

Recent advances in single-cell analysis have revealed substantial heterogeneity even within cell populations previously regarded as homogeneous. Therefore, non-invasive analytical methods for evaluating cellular states and functions are increasingly important in cell therapy, drug discovery, and basic biology.

Electrorotation (ROT) is a label-free method for characterizing the dielectric properties of single cells. When a cell is exposed to a rotating electric field, the cell is polarized and rotates about its own axis. Measurement of the rotation rate enables non-invasive evaluation of the dielectric properties (membrane capacitance and cytoplasm conductivity). However, conventional ROT measurements have limited throughput, and maintaining cells at stable positions during rotation is difficult, hampering time-resolved analysis of the same cells.

To overcome these limitations, we developed a microwell-based ROT device containing 1,521 microwells for single-cell trapping (Fig. 1)¹. Each microwell incorporates four electrodes positioned on its upper and lower surfaces of the microwells. Four AC signals with successive 90° phase shifts generate a rotating electric field within each microwell, allowing a trapped cell to rotate while remaining confined to the well. This configuration suppresses cell displacement, enables time-course measurements of individual cells, and supports parallel ROT analysis.

The device was evaluated using Jurkat and K562 cells. Their rotation rates varied with the applied frequency, and the resulting profiles reflected differences in dielectric properties between the two cell types (Fig. 2A). Furthermore, after the addition of ionomycin, a calcium ionophore, the rotation rate of Jurkat cells gradually decreased and reached approximately 90% of the initial value within 30 s (Fig. 2B). This response is consistent with an ionomycin-induced increase in membrane capacitance associated with Ca²⁺ influx and concomitant changes in cellular state². These results demonstrate that the microwell-based ROT device provides a useful platform for label-free characterization of single-cell dielectric properties and real-time detection of dynamic cellular responses to chemical stimulation.

Keywords: Electrorotation; Single Cell Analysis; Microelectrodes; Microwell

References

- [1] M. Suzuki, S. Kawai, C.-F. Shee, R. Yamada, S. Uchida, T. Yasukawa, *Lab Chip*, **23**, 692-701 (2023).
- [2] M. Suzuki, R. Yamada, Y. Imou, Y. Isozaki, T. Yasukawa, *Electrophoresis*, **46**, 1226-1236 (2025).

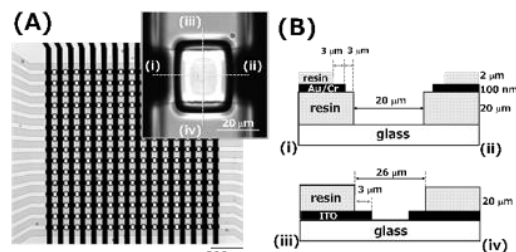


Figure 1. (A) Microscopic image of the microwell-based ROT device. (B) Sectional views of the device at line (i)-(ii) and (iii)-(iv).

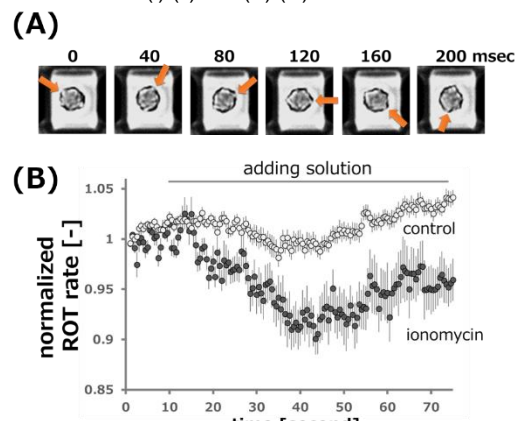


Figure 2. (A) Time-resolved images of a Jurkat cell rotating in the device. (B) Time course of normalized ROT rate obtained by dividing each ROT rate by the ROT rate at 0 seconds.