

Aptamer-based Optically Induced Dielectrophoresis for High-Resolution Single-Cell Phenotyping

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Summary: Cellular heterogeneity is critical in oncology, where rare subpopulations drive tumor progression and therapy resistance. Here, we combine optically induced dielectrophoresis with receptor-specific aptamers to enhance single-cell resolution. Aptamers modulate membrane charge, amplifying sensitivity to receptor expression. By analyzing frequency-dependent cell dynamics using machine learning, this approach enables high-resolution phenotyping at the receptor level within cell subpopulations. The approach was validated on PC3 prostate cancer cells targeting $\alpha\beta3$ and EGFR receptors.

Keywords: optically-induced dielectrophoresis, aptamers, lab-on-chip, machine learning, single-cell analysis.

Background, Motivation and Objective

Cellular heterogeneity plays a critical role in oncology, particularly at the single-cell level, where it drives functional variability among individual cells that collectively determine tumor behavior and treatment response. Conventional bulk analysis cannot resolve this complexity, as it averages signals and masks rare, clinically relevant subpopulations [1]. To address these limitations, optically induced dielectrophoresis (ODEP) has emerged as a powerful, label-free platform for single-cell manipulation and analysis. ODEP exploits light-patterned virtual electrodes projected onto a photosensitive surface to generate dynamic, non-invasive electric fields, enabling flexible and parallel control of individual cells [2]. Compared to conventional dielectrophoresis systems with fixed microelectrodes or optical tweezers requiring high-intensity laser exposure, ODEP provides a low-power, scalable, and minimally invasive alternative with reduced photo-damage [2]. Recent advances combining ODEP with machine learning have enabled the extraction of single-cell dielectric fingerprints based on frequency-dependent electrokinetic behavior and cellular deformation, reflecting membrane structure and biochemical composition [3-4]. However, the discrimination of cellular subtypes remains challenging due to limited molecular specificity. Here, we propose an enhanced ODEP-based strategy based on the use of aptamers (artificially selected single-stranded oligonucleotides able to bind non-DNA targets) as

receptors to selectively bind target cell surface receptors. The binding with the selected target allows aptamers to modulate the cell surface charge, introducing negative charges for the phosphate backbone of DNA strands. This increases the sensitivity and specificity of ODEP measurements, enabling the detection and amplification of functionally distinct subpopulations within heterogeneous samples. The proposed approach preserves global phenotypic information while enhancing receptor-level resolution, allowing broad characterization and high-resolution discrimination of cellular subtypes. It was validated using the castration-resistant prostate cancer cell line (PC3), targeting $\alpha\beta3$ integrin and epidermal growth factor receptor (EGFR). Results demonstrate the capability of the system to resolve phenotypically distinct subpopulations, highlighting its potential for high-precision single-cell phenotyping and stratification at the single-receptor level.

Description of the New Method or Systems

Cells were detached and harvested, and aptamer binding was performed on cell suspensions in binding buffer [5]. A non-binding sequence was used as a negative control. After incubation, both targeted and control cells were washed with washing buffer [5] and subsequently resuspended in a low-conductivity buffer. Cells were then loaded into a lab-on-chip (LOC) device, where they were trapped and analyzed using the ODEP-based platform (see Fig.1a). An AC electric field was applied while

dynamic light patterns were projected onto a photosensitive (amorphous silicon) substrate, generating reconfigurable virtual electrodes. Cellular responses were evaluated using alternating rectangular light patterns at multiple frequencies (25–150 kHz) with a 10 Vpp amplitude. This enabled the acquisition of receptor-specific frequency-dependent response profiles, recorded via time-lapse microscopy (TLM) (see Fig.1b). Single-cell trajectories were extracted by tracking cell motion over time. Due to alternating electrode activation, cells exhibited frequency-dependent motion under ODEP. Both x- and y-direction components were used for spectral analysis. Feature extraction was performed using the wavelet scattering transform (WST), combined with machine learning algorithms for feature selection and classification, to identify the most discriminative patterns of single-cell dynamic (Fig.1c). To the best of our knowledge, this is the first platform integrating ODEP with selective aptamer-based functionalization of the cell membrane for high-resolution single-cell phenotyping. This approach enables probing of specific cell-surface receptor expression within a label-free electrokinetic framework. By combining receptor-targeted aptamer modulation with dielectric profiling, the proposed method enables the identification and amplification of distinct cellular subpopulations in heterogeneous samples, overcoming key limitations of conventional bulk assays and advancing single-cell analysis to receptor-level resolution.

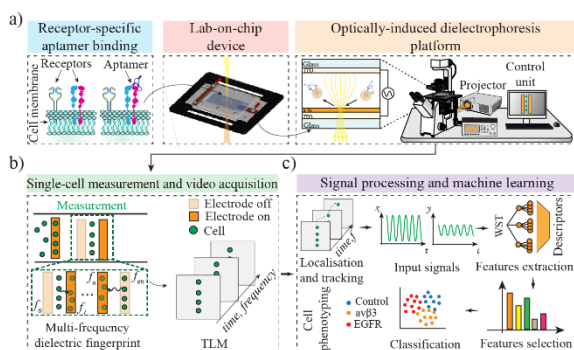


Fig. 1. Schematic representation of the proposed platform. (a) Aptamer-based cell targeting followed by re-suspension in ODEP buffer and loading into LOC device for analysis. (b) Multi-frequency single-cell measurements with video acquisition. (c) Extraction of features from DEP-induced cell motion, including WST-based parameters, was used to build the classification model.

Results

Fluorescence images of the target aptamers were acquired to assess their binding to the receptor. The aptamers receptors modified with a fluorophore at their 5'-end, allowed direct visualization of their localization, thereby confirming their specificity and proper interaction with the target receptor. As shown in Fig. 2a, control

aptamer does not exhibit specific binding and is effectively removed upon washing with the washing buffer. Differently, both aptamers, anti- $\alpha\text{v}\beta 3$ (Fig. 2b) and anti-EGFR (Fig. 2c), exhibit specific and stable binding to their respective targets. Fig. 2d shows the confusion matrix for the linear discriminant analysis (LDA) classification model, validated using Monte Carlo cross-validation (80% training and 20% test with 100 repetitions), across four classes, including the control group representing untreated cells without any specific or non-specific aptamer binding. As shown in the LDA scores plot in Fig. 2e, based on the first two discriminant directions, the two control groups appropriately overlap, whereas the two aptamer-treated groups are clearly separated. This result confirms the proposed approach's ability to recognize binding specificity and highlights its high-resolution capability.

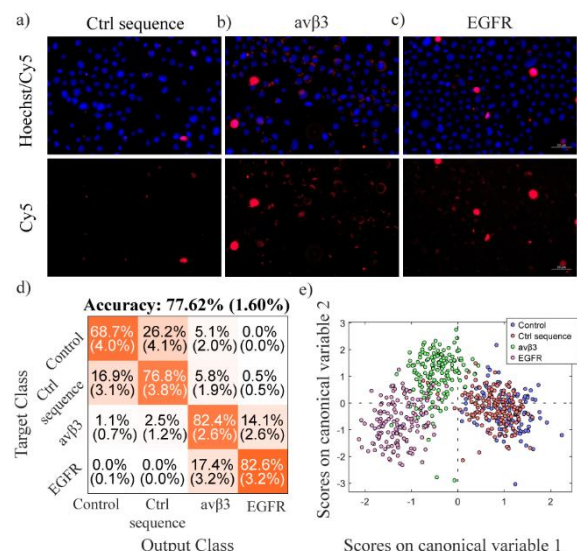


Fig. 2. Fluorescence images of PC3 cells treated with (a) control sequence, (b) anti- $\alpha\text{v}\beta 3$ aptamer, and (c) anti-EGFR aptamer. Nuclei were stained with Hoechst, and aptamers were labeled with Cy5. Magnification 20x, scale bar: 50 μm . (d) Confusion Matrix of the LDA classification model with average accuracy (and standard deviation). (e) Scores on the canonical variable of the LDA model.

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