DOI 10.5162/EUROSENSORS2025/T5.2.2

An Optically-Induced Dielectrophoresis-Based Platform for Enhanced Identification of Prostate Cancer Cells with Different Grades of Malignancy

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Summary:

Understanding phenotypic heterogeneity at the single-cell level is essential for precise diagnosis and treatment evaluation. The integration of optically-induced dielectrophoresis (ODEP) with microfluidics, live-cell imaging, and machine learning offers a rapid, label-free, and non-invasive method for cell manipulation and analysis. By studying the defocusing dynamics of individual cells under varying DEP-induced frequencies, this platform generates unique phenotypic signatures to distinguish between cell types presenting different tumorigenicity levels.

Keywords: optically-induced dielectrophoresis, lab-on-chip, machine learning, single-cell analysis, prostate cancer

Background, Motivation an Objective

The ability to distinguish and classify individual cells within a heterogeneous population remains a major technological bottleneck in biomedical research, especially in cancer diagnostics [1]. Conventional cell classification methods often rely on biochemical markers or morphological features, which can be invasive, time-consuming, or limited in sensitivity. In this context, we propose a novel platform based on defocused optical analysis integrated with optically-induced dielectrophoresis (ODEP) [2] to stratify cancer cell types in a label-free and high-throughput manner. This approach exploits the subtle phase and contrast changes observed when cells are slightly out of focus, providing a unique optical fingerprint that can be used to differentiate subpopulations at the single-cell level. This technological innovation holds particular relevance for prostate cancer (PCa), the second most common malignancy among men worldwide. The disease is characterized by extreme phenotypic and molecular heterogeneity, complicating diagnosis, prognosis, and therapy selection [1]. Addressing this complexity requires tools capable of resolving single-cell variability efficiently and cost-effectively. In previous works, ODEP has been revealed to be a powerful method for single-cell dielectric characterization by evaluating individual cell responses across a frequency spectrum [3,4,5]. Here, we introduce a novel platform that integrates ODEP with microfluidics, live-cell imaging, and machine learning to extract dynamic information along the z-axis, a dimension typically overlooked in cell classification. By analyzing the depth-dependent optical signatures generated during DEP-induced motion, the system captures previously untapped features that reveal intrinsic phenotypic differences.

Description of the Method

The proposed approach exploits a novel DEP-induced dynamic to extract novel information content for better single-cell stratification. In detail, PCa cells were suspended in a low-conductivity buffer, loaded into a lab-on-chip device,

trapped, and measured with the ODEP-based platform, as shown in Figure 1. In particular, we considered hundreds of PC3 (human prostate cancer) cells, representative of castration-resistant cancer with high tumorigenicity, and LNCaP (Lymph Node Carcinoma of the Prostate) as androgen-sensitive cancer with low tumorigenicity (see Fig. 1a).

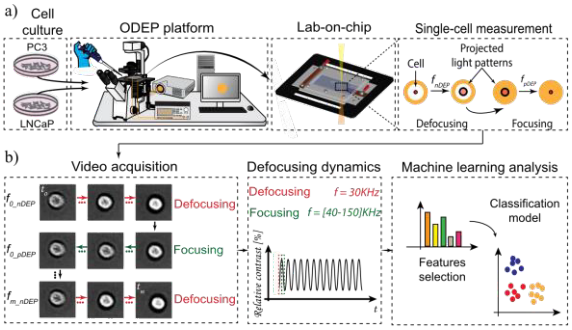

Fig. 1. General overview of the platform for the single-cell analysis. a) Measurement procedure. Cells were loaded into the LOC device for measurement. b) Analysis. TLM videos were acquired and analyzed. The features extracted and selected from the defocusing dynamic allow to build the classification model.

An AC signal was applied to the device, and light patterns were projected on the photosensitive substrate (amorphous silicon). The novel measurement approach involves multiple stimulation cycles to induce defocusing dynamics on the single cell. We considered an amplitude of 7 Vpp and varying frequencies, producing two different cell motions induced by the dielectrophoretic (DEP) force: attractive (pDEP) and repulsive (nDEP). A doughnut shape pattern was projected at 30 kHz to induce nDEP along the z-axis, alternating with a circular pattern at different attractive frequencies in range [40-150] kHz with step 10 kHz (see Fig. 1a). We acquired videos of cell responses recorded via time-lapse microscopy (TLM) (see Fig. 1b). For each video, a region of interest including the dynamic responses of a single cell was automatically extracted, after appropriate normalizations. The contrast was evaluated frame by frame with the Michelson formula (see Eq. (1)).

$$\boldsymbol{contrast(t)} = \frac{\max_{x,y}[I(x,y,t)] - \min_{x,y}[I(x,y,t)]}{\max_{x,y}[I(x,y,t)] + \min_{x,y}[I(x,y,t)]} \qquad (1)$$

A smoothed signal of the defocusing dynamic, in terms of variations relative to the first frame, was obtained for each cell as a unique signature of each cell type. After performing feature extraction and selection, an LDA classification model was built using the most automatically selected features (see Fig.1b).

Results

The stimulation cycles change the relative contrast of the single cell (with respect to the first frame). For example, Figures 2a and 2b show signals representing the percentage of relative contrast for a PC3 and a LNCaP cell, respectively. A number of 24 descriptors were extracted from the smoothed signals. The selected features were used to build an LDA classification model. The average accuracy, obtained with 10-fold cross-validation, increases with the number of considered stimulation cycles, as shown in Figure 2c. On average, the maximum value is 88.2%, with a standard deviation of 7.6% in correspondence of the 9th cycle. Figure 2d shows the confusion matrix of the classification model.

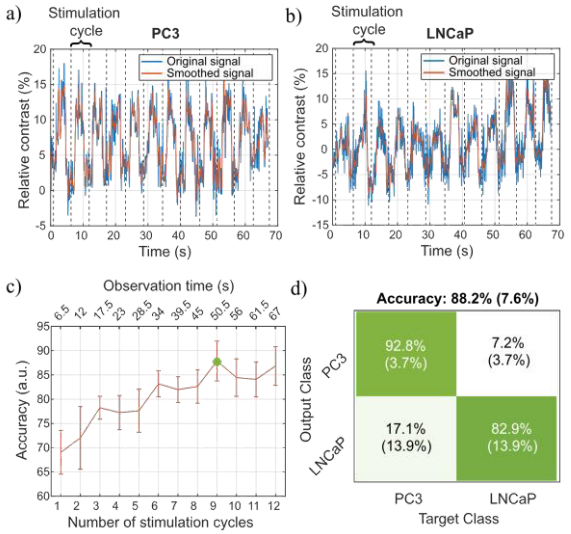

Fig. 2. a) Percentage of relative contrast for PC3 and (b) LNCaP cells. c) Classification accuracy as a function of the number of stimulation cycles. The green dot identifies the maximum value corresponding to the 9th cycle. d) Confusion matrix of the LDA classification model.

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