

CHARACTERIZATION OF CELL PHENOTYPE USING DYNAMIC VISION SENSOR AND IMPEDANCE SPECTROSCOPE

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ABSTRACT

This paper reports on an improved method for characterizing single cells within a microfluidic channel, which combines the output of a Dynamic Vision Sensor camera with data from a differential impedance spectroscopy measurement. The combination of optical and impedance data allows the size, shape and position of the cells to be determined in addition to their dielectric properties. Here, we demonstrate the utility of the method by discriminating between normal and budding yeast cells.

KEYWORDS: Impedance spectroscopy, flow cytometry, single-cell analysis, Dynamic Vision Sensor

INTRODUCTION

Electrochemical impedance spectroscopy is a powerful method for label-free flow-through detection and analysis of individual biological cells in a lab-on-a-chip setup [1]. The method analyzes the cellular dielectric properties at different frequencies and provides quantitative information on membrane capacitance, membrane resistance and cytoplasmic resistance [2]. Enhancing the method with optical information aids in interpreting the results, but is impractical with a regular camera since a frame rate of more than 1000 frames-per-second is needed for normal flow rates. The retina-like DVS128 camera (iniLabs Ltd, Switzerland) only transmits changes in intensity of the individual pixels as opposed to full frames and is therefore better suited for detecting fast-moving objects [3], allowing it to be used as a tool for control/reference measurements.

THEORY

For differential impedance spectroscopy measurements, particles are dispersed in a liquid, which flows through a microfluidic channel with two pairs of planar electrodes patterned at top and bottom. An AC voltage is applied to the electrodes causing a current to flow between them. The current change upon passage of a particle between the electrodes is differentially measured and analyzed to determine the cellular dielectric properties [4].

The camera provides discrete events consisting of a timestamp, pixel position and a binary intensity change. When no movement is detected, the camera will output few events which are caused by noise, as illustrated in Figure 1 (a). When an object passes its field of view, a cascade of events is generated within a short time frame. For analysis, the data are assigned to frames consisting of events occurring within windows of 400 μ s. The resulting images are analyzed using MATLAB (The MathWorks, Inc.) to extract regions of connected pixels, for which the area and eccentricity is measured as shown in Figure 1 (b). Finally, data from successive frames are combined to detect the movement of the object as shown in Figure 1 (c).

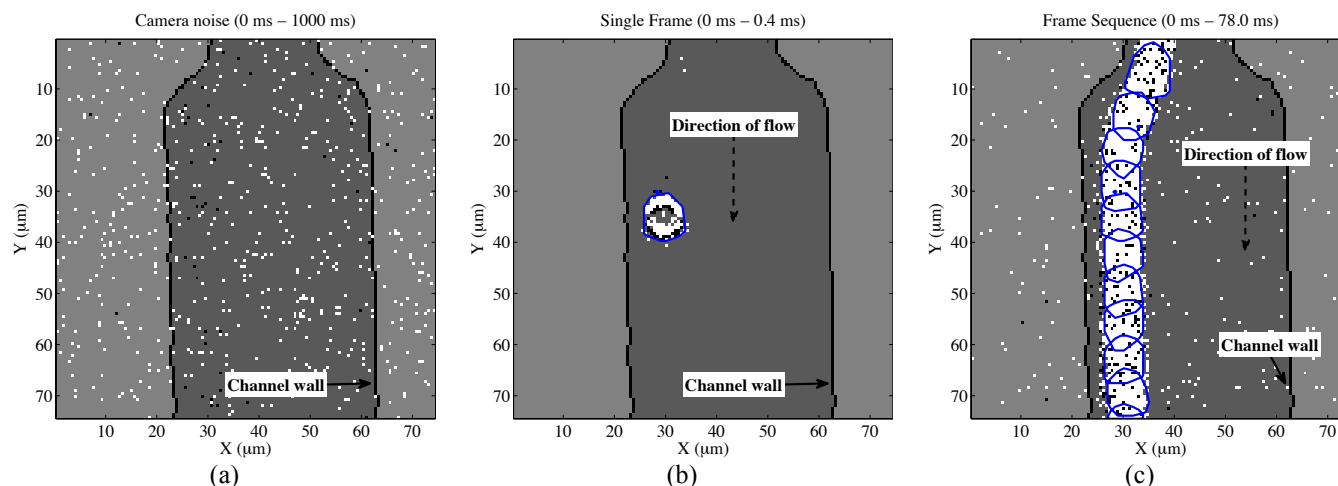


Figure 1: (a) Intensity events recorded by the DVS128 camera during a time frame of 1 second with no movement in the channel; white and black pixels correspond to an intensity increase/decrease respectively. (b) Events during a time frame of 400 μ s as a single yeast cell passes through the channel with the circumference of the detected connected region marked. (c) Overlay of multiple frames showing how the movement of a cell can be tracked by detecting an overlap between the connected regions of successive frames. The channel wall has been overlaid on the data from the camera for illustrative purposes.

EXPERIMENTAL

A schematic of the measurement setup is shown in Figure 2. The microfluidic device consists of two glass plates with 200-nm-thick and 20- μm -wide platinum electrodes. The two plates are bonded face-to-face using a 14- μm -thick lithographically structured dry film resist as spacer that defines the microfluidic structure, resulting in a final channel cross-section of $14 \times 20 \mu\text{m}^2$. An HF2IS impedance spectroscop (Zurich Instruments AG, Switzerland) generates the excitation signal and demodulates and filters the currents from the response electrodes, which are converted to voltages using a current amplifier. The DVS128 camera captures an area downstream of the electrodes with illumination provided by an incandescent light source.

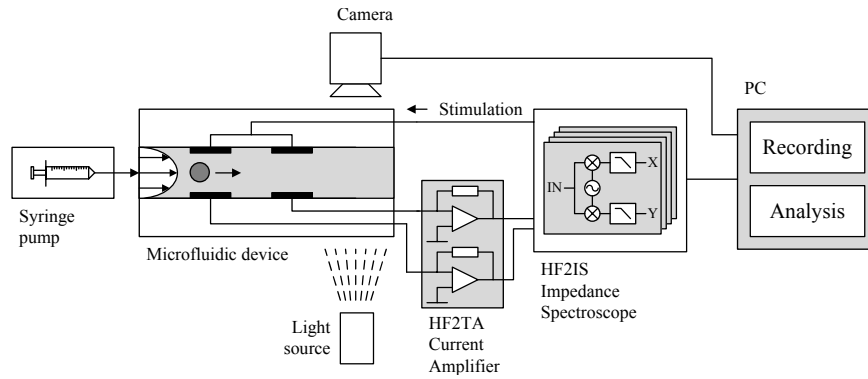


Figure 2: Measurement setup.

For the experiment, yeast cells (*S. cerevisiae*, BY4741 strain) were suspended in phosphate-buffered saline (Sigma-Aldrich Chemie GmbH) and driven through the channel by a syringe pump with an average flow velocity of 7 mm/s. The data from the camera were recorded together with the impedance data.

RESULTS AND DISCUSSION

Figure 3 shows examples of the detected events as a normal single yeast cell and a budding yeast cell pass through the channel. The asymmetrical shape of the budding yeast cell is clearly visible. These cells pass slightly outside the focus plane of the microscope and therefore appear somewhat larger than their true size.

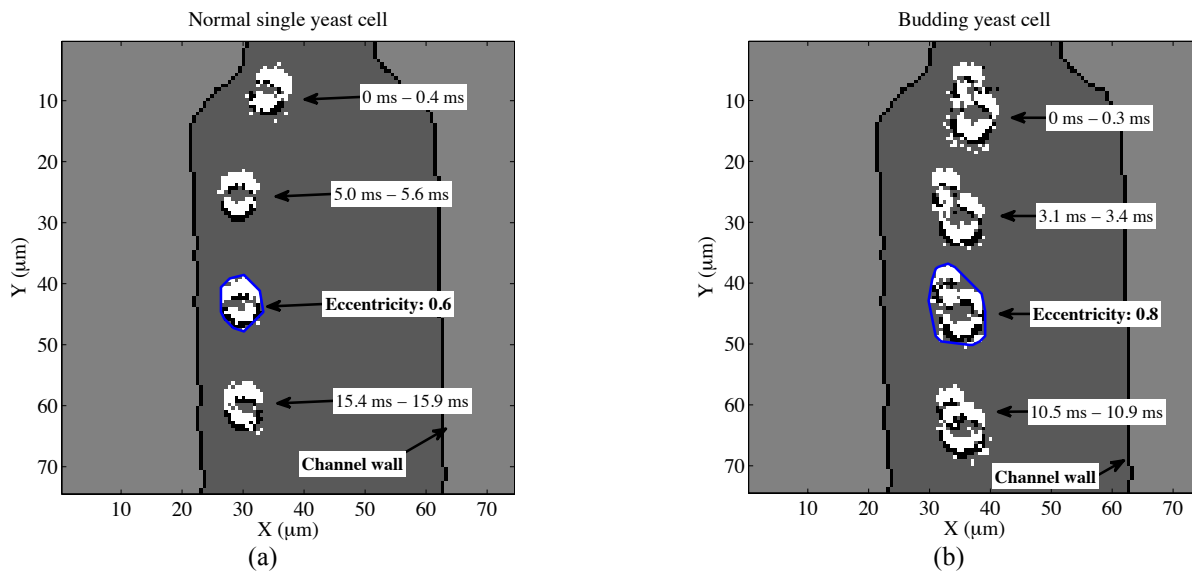


Figure 3: Intensity events recorded by the DVS128 camera for a normal single (a) and a budding (b) yeast cell passing through the channel. Only the events within the labeled time windows are shown. In (a), the cell is almost circular, giving an average eccentricity value of 0.6. In (b), the budding cell has an elongated shape resulting in a higher eccentricity value of 0.8.

Figure 4 (a) shows a histogram of the eccentricity values, defined as the ratio of the distance between the foci of the elliptical circumference of the detected object and its major axis length, which shows two peaks corresponding to normal single

yeast cells and budding yeast cells. Figure 4 (b) shows a scatter plot of the real and imaginary parts of the detected peak-to-peak voltage from the impedance measurement recorded at 3.8 MHz. Data points corresponding to budding yeast cells, which have a high (> 0.75) eccentricity value due to their asymmetrical shape, have been labeled and show a tendency towards having a more negative imaginary part. Since the measurement is made differentially, this is an indication that the budding yeast cells cause a larger change in capacitance of the channel compared to normal single yeast cells.

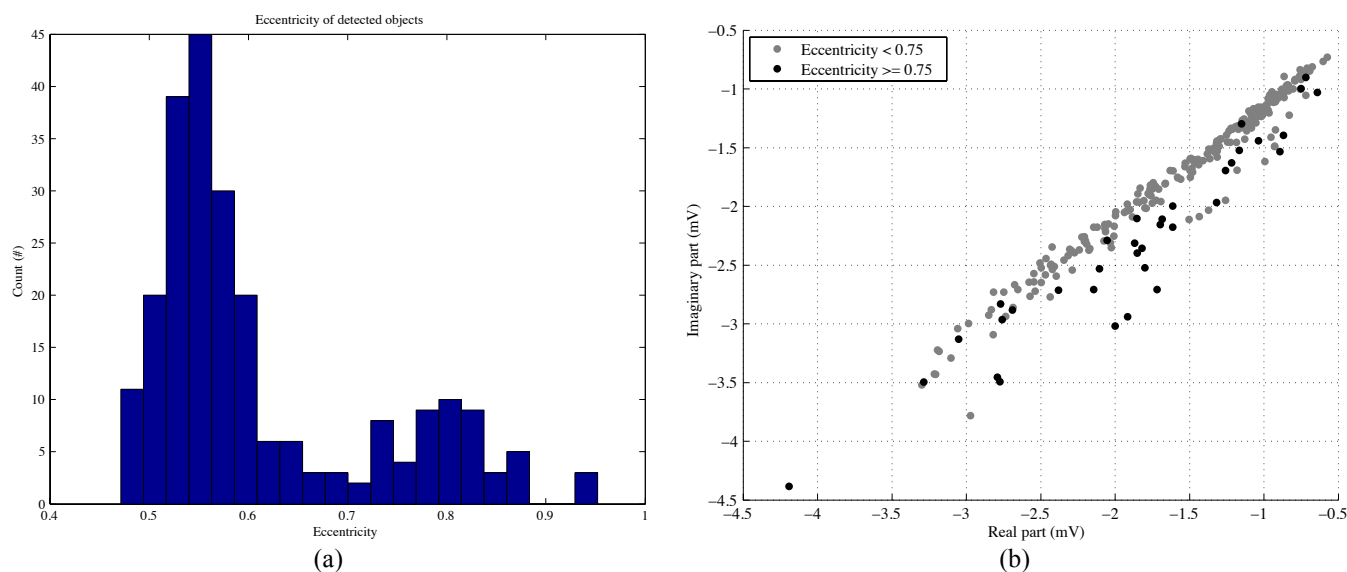


Figure 4: (a) Histogram of eccentricity showing the two populations corresponding to regular and budding yeast cells. Regular cells have a mean eccentricity of approximately 0.5 whereas the budding cells have a mean eccentricity of 0.8; (b) Scatter plot of the real and imaginary part of the peak-to-peak voltage signals for the individual cells measured at 3.7 MHz. The grey levels correspond to the eccentricity of the cell. High eccentricity values (> 0.75) correspond to asymmetrical cells. Some outliers can be explained by the cells being rotated such that the bud is out of sight of the camera.

CONCLUSION

Our experimental results show that it is possible to track the movement of cells within a microfluidic channel using the DVS128 camera, as well as to extract useful information from the recorded data. By combining the optical data with results from a differential impedance measurement, we have shown that budding yeast cells give rise to a larger change in capacitance of the microfluidic channel in comparison to normal single cells.

Current work is ongoing to improve the optical characterization algorithm to extract more details, such as the number of cells in a cluster, from the available data.

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