Dielectrophoresis and COMSOL simulation of cell entrapment at electrodes

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ABSTRACT

Dielectric properties of biological samples change with frequency of the interrogating electric field therefore frequency dependent response can be used to obtain information about structures as well as various kinetic, bio-physical and - chemical processes in the cells. We analyzed cells by impedance and capacitance/conductance measurements after trapping them directly on miniaturized electrodes using dielectrophoresis (DEP). The electrode structures with cells attached at their edges, as obtained experimentally, were simulated using Comsol Multiphysics 3.4. Dielectric behavior of the system under ac voltages of different frequencies was studied. Mathematical modeling was done. Both in design/fabrication of the structures and in simulations we took into account the polarization effect at the electrode/electrolyte interface, which at low frequency range introduces very significant errors.

Keywords: polarization, dielectric spectroscopy, Dielectrophoresis.

1 INTRODUCTION

Dielectric spectroscopy (DS) is a powerful characterization technique, which is frequently used in biotechnology for monitoring and studying living biosamples. It involves simple procedures of measuring capacitance and conductance or impedance in order to study both permittivity and dielectric losses of such samples as a function of frequency. If properly interpreted, results of such measurements can provide information on properties of biological samples including cells viability in various physiological conditions. Dielectric studies of biological samples give a wealth of information about behaviour and parameters of constituent matter [1] and from dielectric relaxation mechanisms of the system, the dynamic and structural properties can be understood. At low frequencies, it is largely influenced by charge accumulation around the cell membrane [2], thus DS can be a useful tool in studying membrane structure and properties like membrane potential, capacitance etc. Measurements, which are typically used on biological suspensions can be also done on single cells/organella if micro- or nanofluidics are integrated with miniaturized electrodes. Here, sequential monitoring of individual cells can be done, such as in impedance flow cytometers, or cells can be trapped and/or attached to the substrate electrodes for monitoring via operation of electron devices (capacitors, transistors etc.). Despite clearly obvious concept, unambiguous realization of DS measurements is very difficult. Equivalent circuit that represents the capacitive system has to be also correctly identified and include parasitic effects. At low frequencies, double layer forms due to charge accumulation at the electrode-solution interface and masks the actual sample capacitance behavior. Misinterpretation of measured quantities by electrode polarization effects has limited the frequency range employed in this technique for years. The very thin double layer (10 nm for water) formed on electrode surfaces has a large capacitance and various analytical and experimental methods have been used to overcome it.

Our aim is to study the dielectric properties of the cell membrane as a function of frequency including low ranges. Here we do this by incorporating nanogap capacitors (dielectric thicknesses of 17 nm and 150 nm) in which the double layers overlap and result in the sample capacitance being independent of the double layer capacitance [3, 4]. DEP was used to trap the cells preferentially at the electrode edges where the applied field can penetrate the membrane. DEP is an inexpensive, controllable and non-invasive method of aligning particles. By careful designing of electrodes, a non-uniform electric field can be induced which aligns particles in a desirable manner.

2 MATERIALS AND METHODS

The nanogap capacitor consists of a highly doped Si n+ layer as bottom electrode and a doped polysilicon top electrode. Silicon dioxide of desired thickness is grown between them by thermal oxidation. Patterning of electrodes is done by photolithography and oxide was laterally etched in some cases (Fig. 1). Here we grow oxides of thicknesses 17 nm and 150 nm using thermal oxidation since it has good control over thickness, enabling very thin dielectric layer which is vital for double layer elimination. The oxide was either etched laterally or left unetched to see dependence of E field on permittivity.

Capacitance and conductance as a function of frequency (1 Hz to 1 MHz) was measured across the nanogap capacitor using an HP LCR meter with a superimposed AC signal of amplitude 50 mV. DEP experiments were done using wave generator with sine wave of amplitude up to 20 V peak to peak, and a

frequency range of 1 – 15 MHz applied between paired electrodes. The electrode geometry used was to enhance the

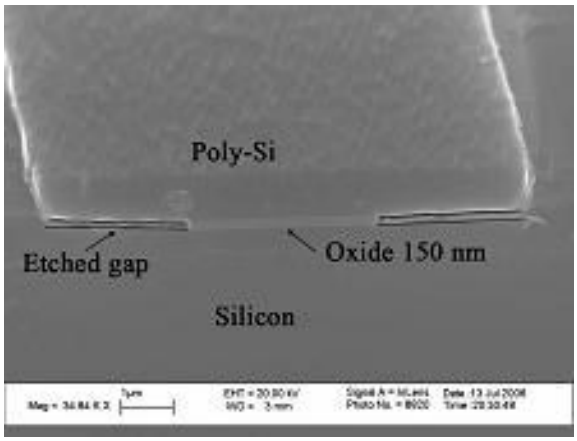

Figure 1: SEM image of the cross section of the electrode with a 150nm thick oxide layer etched for 30 minutes.

non-uniformity of the field for the DEP effect. The smallest width of the electrodes was 8 um. The DEP force depends on the square of the gradient of electric field (Eq. (4)), thus the electrode configuration is important. It can be designed to get desired non-uniformities in the field. Here the design was selected due to the small distance (8 um) between the electrodes, which increases the field and hence the DEP force is higher. The shape of the electrodes is almost symmetrical, hence finite element calculation of the field was much easier, by assuming square symmetry. The ground electrode here is much larger than the central measurement electrode, hence field strength was much higher at the central electrode than the outer one, to have greater DEP force. Yeast cells (Saccharomyces Cerevisiae and Schizosaccharomyces Pombe) in Phosphate buffer saline (PBS) were used.

3 EXPERIMENTAL AND SIMULATION RESULTS

3.1 Experiments

Capacitance measurements of S. Pombe cells at nanogap capacitors were taken for different oxide thicknesses. Obtained results show β-dispersion, as relaxation occurred at around 100 kHz. Obtained results correlate with theoretical results [5-8]. Beta-dispersion occurs due to the charging of the outer cell membrane, due to the Maxwell-Wagner effect, as the cell membrane is non-conducting and the internal cytoplasm and external media solutions are highly conducting. The frequency dependence of the permittivity in response to a step function of the type 1-exp (-t/T) can be given by the Debye equation [9]

$$\varepsilon = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + j\omega T}. \qquad (1)$$

On separating the real and imaginary parts, we get [5]

$$\varepsilon' = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + (\omega T)^2}, \quad \text{and} \qquad (2)$$

$$\kappa = \kappa_\infty \frac{(\omega T)^2}{1 + (\omega T)^2}. \qquad (3)$$

As $\varepsilon = \varepsilon' - j(\kappa / \omega\varepsilon_r)$

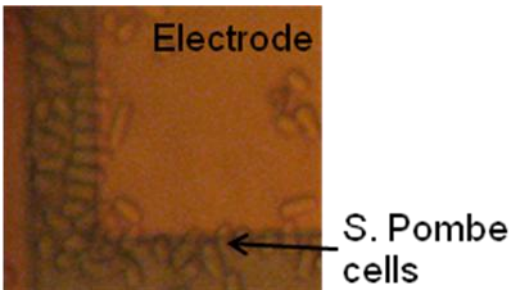

Figure 2: S. Pombe cells aligned along electrode periphery.

Trapped cells (Fig. 2) stayed long enough at the electrode perimeters to perform CV and GV measurements. Fig. 3 shows these curves for the S. Pombe cells for 170 A wafers. The unetched wafers have a higher capacitance due to the higher permittivity of the oxide. The curves for untrapped and YPD media show a different frequency dependence as compared to the trapped curves. As expected at high frequencies, capacitance increases due to well known changes of dielectric constant. At the same time, conductance increases with frequency indicating higher losses. It appears that the fringing electric field that probes accumulated cells is responsible for the capacitance behavior. The dependence of capacitance and conductivity on frequency for the 170A oxide thickness capacitor is shown below (Fig. 3) and here we can see that the relaxation for the trapped samples is different from untrapped samples and from the YPD media. Capacitance was also observed to be higher for the unetched wafers; hence there was not much penetration into the Nanogap for the trapped samples. The relative dielectric constant for cells is reported to go very high, about 1000 for low frequencies. The results show similar behavior as theoretically predicted [6].
Relatively small conductance values indicate losses as predicted but they do not degrade accuracy of capacitance measurements.

3.2 Simulation Results

Frequency dependence of capacitance and conductance was simulated using Comsol Multiphysics 3.4. Quasistatics mode in the AC/DC module was used to simulate the E fields. The electrodes were modeled

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according to the design employed and first the DEP force and magnitude was calculated.

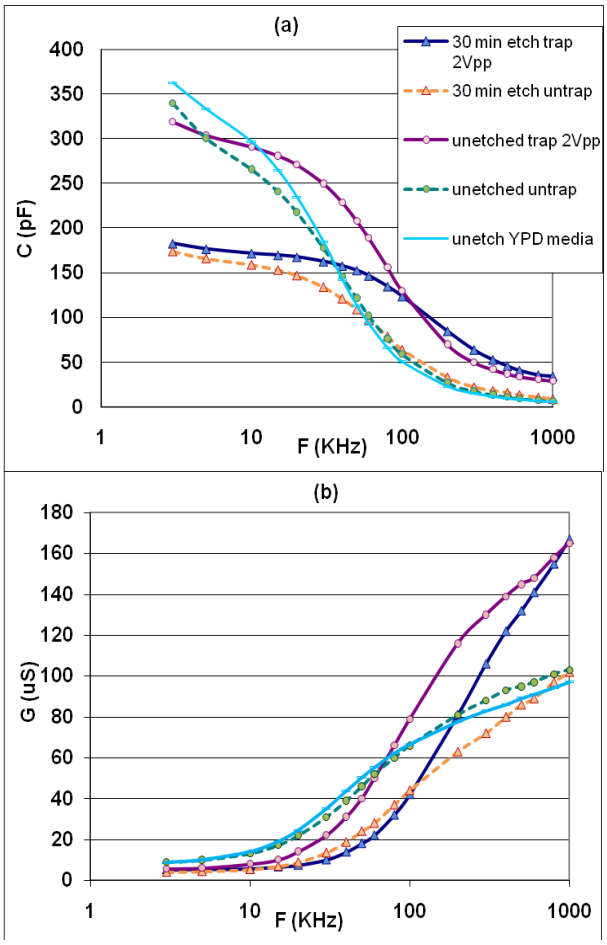

Figure 3: Capacitance (a) and Conductivity (b) dependence on frequency for 170A Oxide wafer for S. Pombe cells.

The DEP force acting on a lossless dielectric sphere of permittivity ε_2 and radius r under the influence of an electric field E_{rms} with a frequency ω in a medium of permittivity ε_1 is given by

$$F_{DEP} = 2\pi r^3 \nabla E_{rms}^2 K(j\omega), \qquad (4)$$

Where,

$$K(j\omega) = \frac{\varepsilon_2 - \varepsilon_1 - j(\sigma_2 - \sigma_1)/\omega}{\varepsilon_2 + 2\varepsilon_1 - j(\sigma_2 + 2\sigma_1)/\omega}. \qquad (5)$$

Where $K(j\omega)$ is the Clausius Mossotti factor. Thus particles with different radii and different combinations of complex permittivities will experience different magnitudes of the DEP force in similar electrode conditions and under similar applied electric fields [10]. Hence by varying the experimental conditions, such as frequency and solution conductivity, we can manipulate the particles to deliver them to specific sites of a test structure and separate particles of different properties and sizes [11].

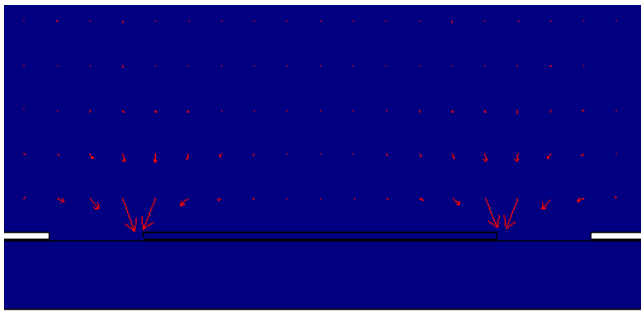

Figure 4: DEP force simulation showing its direction.

Literary values for the permittivity and conductivity of the cell interior and cell membrane were used. The cell was simulated as a sphere with a highly conductive inner region and a non-conductive membrane layer. This was based on a simple cell model where the cell membrane is included [12]. The sample medium was also highly conductive. Simulations of DEP force (Fig. 4) calculated at a supply frequency of 10 MHz show it pointing toward the electrode edge and having highest intensity along the edge. Hence cells get attracted and stay along the periphery of the electrode where they are subsequently probed.

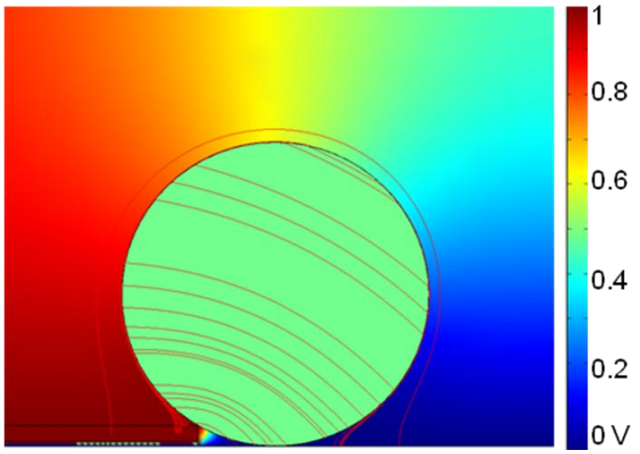

Figure 5: Comsol simulation of the potential profile with electric field lines (streamline plot) of a cell at the electrode edge at 10 kHz supply frequency.

The fringing field could be seen to penetrate into the cell (Fig 6). Frequency sweep of 1 kHz to 100MHz was done. As obtained from measurements, relaxation occurs after 100 kHz, thus at frequencies below that, the field does not penetrate the cell membrane (Fig 5). At higher frequencies, the field penetrates the cell and due to the Maxwell-Wagner mechanism, charging of the membrane occurs causing the relaxation (Fig 6). By using the lumped parameters inbuilt in the quasi statics application mode in the AC/DC module of Comsol, the frequency dependence of Capacitance was calculated. The results for the 1500 A Oxide wafer for trapped and untrapped cells are shown below. As obtained

experimentally, untrapped cells show a different frequency dependence and relax earlier than trapped cells.

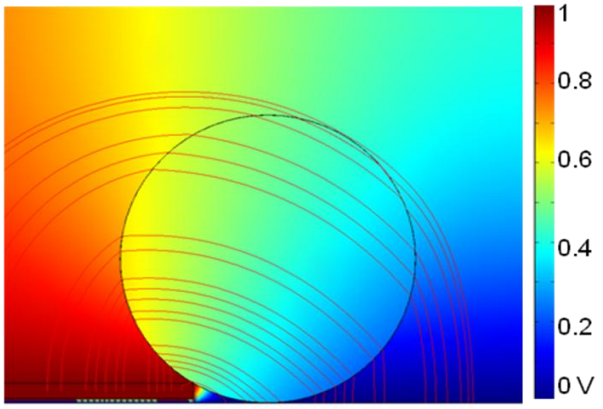

Figure 6: Potential profile at the electrode edge with electric field lines (streamline plot) at 1 MHz supply frequency.

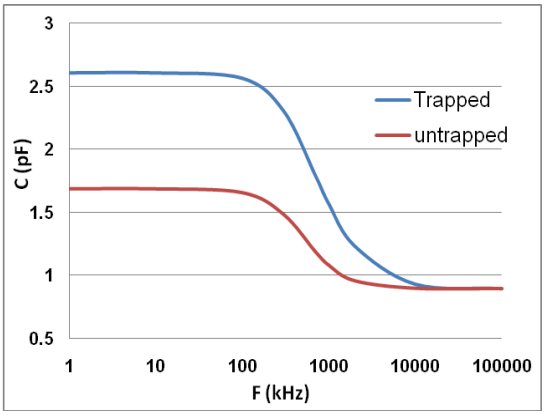

Figure 7: Comsol simulation of frequency dependence on capacitance.

3.3 Numerical Calculations

By taking values of ε_∞ and ε_0 from the experimental results, using (2), the permittivity was calculated. By curve fitting, the values of time constant T can be obtained. In fig 8 the permittivity dependence on frequency for the 170 A oxide wafer, from capacitance measurements done with S. Pombe is shown. Time constants ranged from 0.0000011 to 0.0000048, with higher values for the untrapped cell samples than the trapped samples.

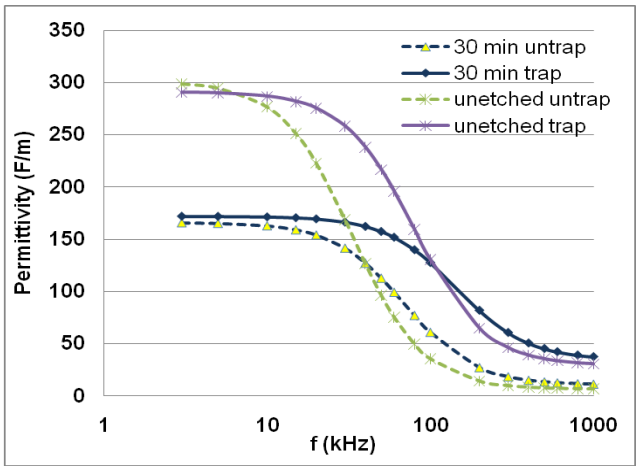

Figure 8: Frequency dependence of permittivity calculated.

We summarize that we designed and fabricated nanogap capacitors to eliminate electrode polarization effects. By careful electrode design, we preferentially aligned cells via DEP at the electrode edges. Fringing fields penetrate the cell membrane and capacitance measurements were taken. Dielectric spectroscopy results show relaxation across the cell membrane and this effect was also simulated in a similar environment using Comsol Multiphysics. The dielectric behavior depended on many factors like the presence of cells at the electrode edges, difference in capacitor permittivity etc. This methodology could prove as a useful tool in studying the dielectric properties of small particles more effectively, and without polarization errors.

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