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Near Real-Time Electrochemical Analysis of Carcinogenic Cell Lines Using Planar Microelectrodes

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Physical manipulation of polarizable particles generated by the transient force when placed in non-uniform electrical fields is termed dielectrophoresis. The electrophysiological make-up of the particle and the surrounding medium decides the magnitude of the force; hence specific DEP profile may be acquired for any polarizable particles based on the electrical properties of the membrane and cytoplasm alone. Any changes to these parameters may be detected, in turn, by observing the corresponding DEP spectra. The reason DEP is not applied widely in spite of its non-invasiveness primarily is that the process is time consuming. The proposed DEP-based lab-on-chip application of semi-automated cell electrophysiology characterization allowed concurrent DEP experiments to be conducted serially, which significantly reduced the required experiment time. This capability is achieved via the employment of a planar microelectrode with modified dot microarray geometry. The results showed that the system is capable of producing a complete DEP spectrum profile for K562 leukemic cells between the 1 kHz to 1 MHz frequency range in less than 10 minutes.

Keywords: Lab-on-a-chip; Dielectrophoresis; AC electrokinetics; Cancer

1. INTRODUCTION

Dielectrophoresis (DEP) is a phenomenon in which a transient force of specific strength is exerted on a dielectric particle when it is subjected to a non-uniform electric field. The magnitude of the generated transient force is dependent on the electrophysiological make-up of the surrounding media and the particle itself, both externally (membrane surface) and internally (internal compartments, if any). DEP is also typically grouped as part of AC electrokinetics, and has been used for manipulating various types of cells and particles since its description by Pohl in 1951 [1].

DEP is capable of producing specific cellular profiles based on the electrophysiological properties, hence DEP has been employed, among others, to separate live and dead yeast cells [2,3], various bacterial [4-9] and viral [10-14] strains, DNA molecules [15], spores [16,17], and algae [18]. Other types of molecules such as nano-sized latex spheres [19,20] and biopolymers [21] have also been the subject of DEP characterization studies. In addition, numerous studies focused on the use of DEP in characterizing the myriad of mammalian cells, including neurons [22-24], leukocytes [25-27], erythrocytes [28,29], platelets [30,31], and even human spermatozoa [32]. Although this in itself provides a much needed alternative method for cell detecting and sorting assays, a worthier cause of DEP usage would be in detecting and collecting abnormal particles from a given cell population. Similar studies on separating these cells have been conducted as early as the recognition of DEP as marker-less cell sorting technique in the 1980s. A study by Mischel *et al.* for example, had successfully separated malignant melanocytes from normal cell populations and showed that the electrical behavior of malignant melanocytes is markedly dependent upon the type of cell line, age, and drug treatment (e.g., chlorpromazine) and may be separated from normal cells using DEP [33].

In spite of the advantages, DEP is yet to be adopted by the biotechnology industry, because of two reasons, namely the time-consuming processes involved, and the lack of applications to conduct high volume cell assays [34]. Many studies have focused on reducing the time taken to conduct DEP experiments by automating some experimental processes (e.g. [35,36]), but were limited to recording DEP features on single particles. A semi-automated system well-based DEP system [34], was designed to address the high volume limitation and was successfully used in many DEP-based experimental studies (e.g. [17,34,37,38]). However, it still takes between 120-240 min to construct a statistically acceptable DEP spectrum for a given cell sample.

This study presents the method and development of a semi-automated, DEP application that is capable of conducting DEP studies and the subsequent analyses for carcinogenic cell samples of 1 x 10^7 cells per ml at a much lesser time. The data point resolution of the DEP spectra will be similar to those acquired when using the aforementioned well-based system. The developed application is also capable of completing DEP studies close to real-time, due to the speed in conducting DEP studies and analyzing the acquired data.

2. THEORY

When exerted on a homogeneous spherical particle, the DEP force (F_{DEP}) is typically expressed as:

$$\left\langle F_{DEP} \right\rangle = 2\pi r^3 \varepsilon_m \,\mathrm{Re}\left[K(\omega)\right] \nabla E^2 \qquad (1)$$

Where r is the radius of the particle, ε_m the permittivity of the surrounding medium, $K(\omega)$ the complex Clausius-Mossotti factor, and E the electric field strength expressed in root mean squared (RMS) value. The Clausius-Mossotti factor is a measure of the effective polarizability strength:

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \qquad (2)$$

where ε_p^* and ε_m^* are the complex permittivities of the particle and the medium, respectively. In addition,

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega} \qquad (3)$$

where σ is the conductivity, ε the permittivity, and ω the angular frequency of the electric field.

The analytical expressions mentioned above explain the DEP behavior of spheres in a given solution; however, it is considered to be too complex to be expanded in order for the dielectric properties to be directly calculated and correlated in a useful manner [39]. The common method of correlating the relevant cellular electrical parameters with the DEP behavior is by estimating the electrophysiological properties performed by best-fit numerical analysis [40], and has been successfully used in many DEP characterization studies (e.g. [37,38,41,42]). The estimation method has also been shown to be useful in characterizing multiple cell populations within a heterogeneous cell sample [43]. Figure 1 shows a typical DEP spectrum for a sample cell population using the said numerical analysis, with the characteristic 'crossover' occurring at about 40 kHz.

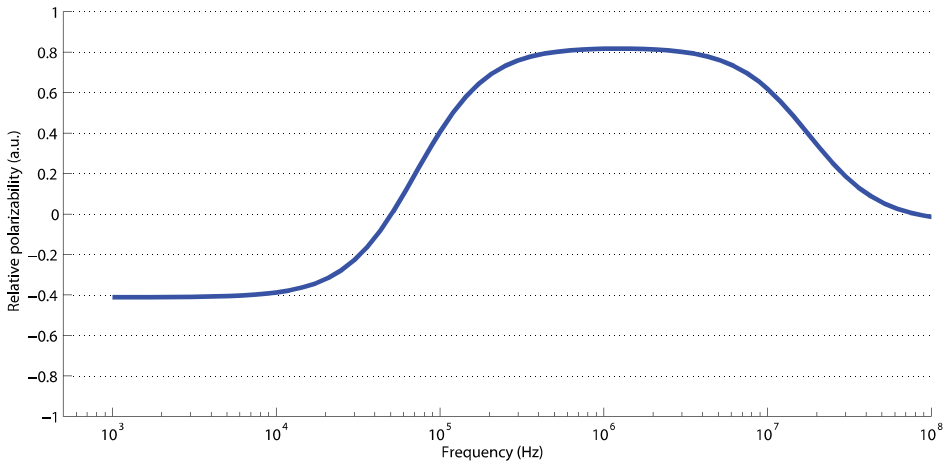

Figure 1. A typical shape of the DEP spectrum based on the single shell model

3. METHODS

3.1. Device assembly

The BioMEMS application assembly consisted of a DEP chamber, where the DEP effects may be observed and recorded, sandwiched between two conductive layers (Figure 2). The bottom

conductive layer was fabricated from Au-plated microscope glass slides, while the top conductive layer was made from indium tin oxide (ITO) coated glass slides of slightly smaller dimensions, and acted as the counter electrode. The DEP chamber was fabricated from UV-sensitive polyresin, and the thickness determined the height of the chamber, which is about 200 μm.

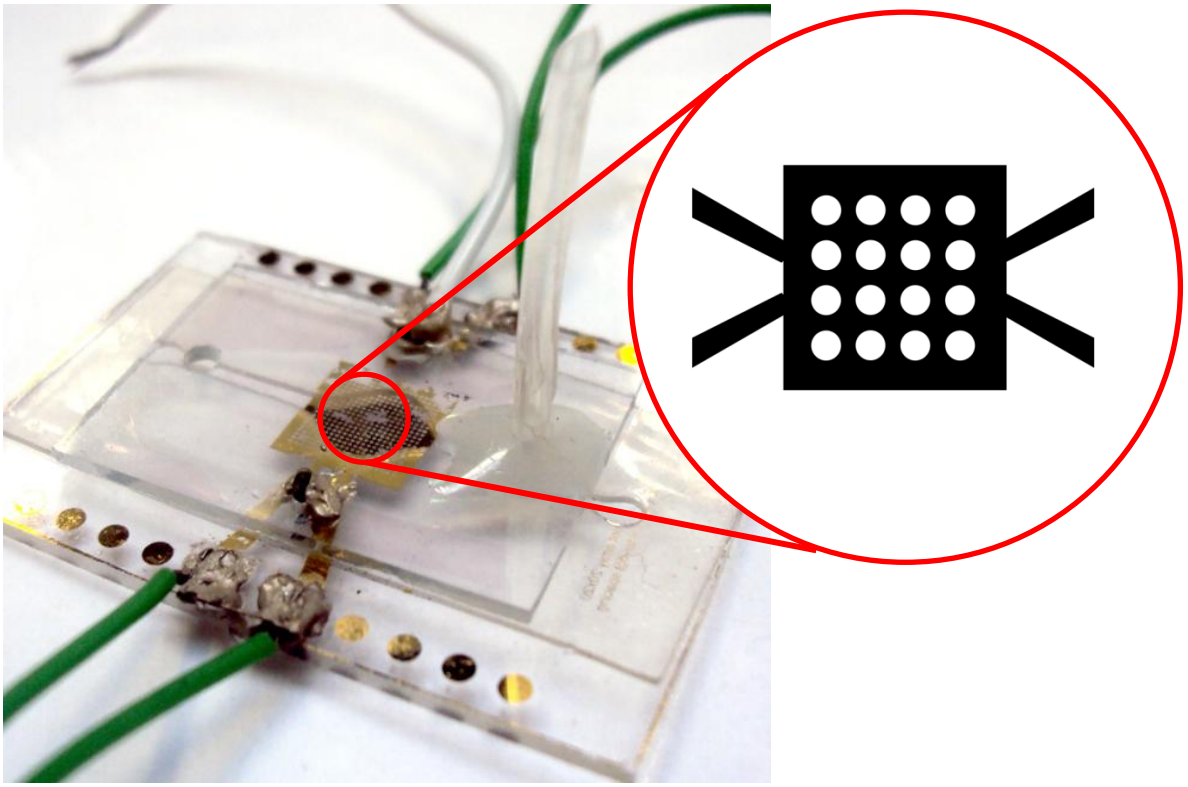

Figure 2. The BioMEMS application assembly, with the schematic design of the electrode overlaid

Using typical photolithography methods, a specific microarray design was patterned onto the Au-coated glass slides. The planar electrode geometry used was similar to the dot array design previously used by Fatoyinbo *et al.* [44], in order to produce axisymmetrical electrical field gradients over each of the dots. The DEP force generated should consequently be axisymmetrical as well, greater at the electrode edge and decreasing in magnitude towards the center of the dot. This should provide a correlation between particle motion and relative particle polarisability; which is determined based upon images captured from a digital camera attached to the microscope.

3.2. Preparation of cell samples

Human leukemic K562 cell lines used in the study were sourced from LGC Standards (Teddington, UK), and all reagents and solutions were from Sigma Aldrich Co., (St. Louis, USA), unless stated otherwise.

The cell culture medium were prepared using RPMI solution (GIBCO® RPMI Media 1640, Invitrogen Ltd., Paisley, UK), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (PAA, Pasching, Austria), 1% penicillin-streptomycin, and 1% L-glutamine. The cells were cultured at 37oC and 5% CO2, and may be used for experiments once confluence was reached. For DEP experiments, cells were transferred in conductive medium made of 8.5% sucrose and 0.3% dextrose, with the desired conductivity value adjusted using 150 mM KCl solution, verified by a conductivity meter. If the conductivity was set at 10 mS/m, the crossover frequency for K562 cells should lie between 10 to 100 kHz (based on previous findings by e.g. [38,45]). Cell samples were 'washed' twice by centrifugation at 180 g for 5 minutes, and subsequently resuspended in the DEP conductive medium. Cell sample concentration was adjusted to about 10 million cells per ml prior to the commencement of DEP experiments.

3.3. Experimental procedure and data analysis

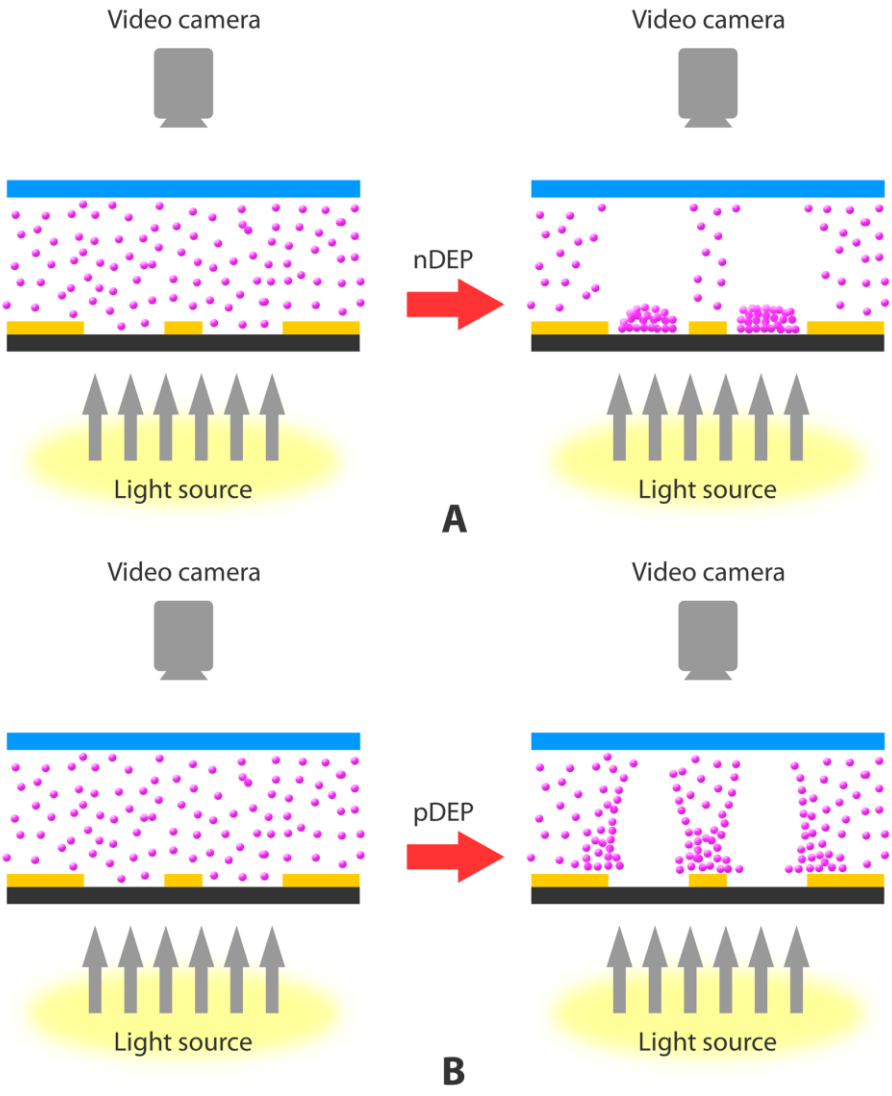

Figure 3. A schematic diagram of the movement of cells over the dots when experiencing a) negative and b) positive DEP

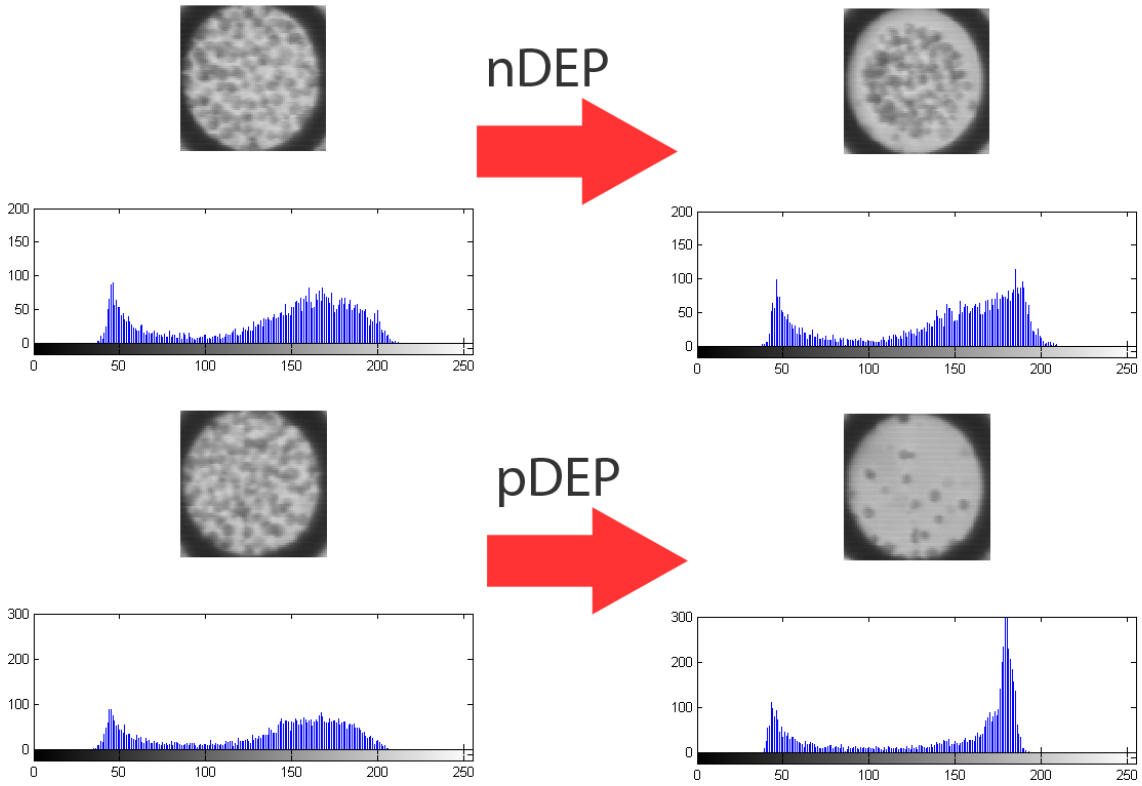

Figure 4. The captured images and the corresponding histogram plots for dots experiencing negative (top) and positive (bottom) DEP

About 15 µl of cell suspension was slowly delivered using a syringe through the inlet of the DEP chamber. If the chamber has been satisfactorily assembled, it should be filled with the solution uniformly via capillary action. The electrode and the ITO were connected to the positive and negative connectors of the signal generator, respectively. The AC signal was applied for 15 seconds at each chosen frequency value to produce the electric fields with the corresponding gradient strength.

To create the corresponding DEP spectrum, a correlation between the particle movement and its relative polarizability is required. This was done by analyzing the captured images of particle movement over each of the dots for the duration of signal application. Figure 3 shows the schematic diagram of cell movement as a result of applying electrical signal, which created negative DEP effects for the particles in use. On the other hand, positive DEP repels the particles towards the edges of the dot. The algorithm used was similar to the Cumulative Modal Intensity Shift (CMIS) used previously [44], whereby the changes in the peak values were determined, both before and after the application of the signal. A change in the negative direction indicated negative DEP effects, and vice versa. Electrophysiological properties, namely conductance and capacitance, of the membrane and inner compartments (if any) may be determined using a best-fit model constructed from Equation (1). Figure 4 shows the change in the histogram values for cells experiencing negative DEP, where a collection of cells was produced at the center of each of the dots.

4. RESULTS

The extent of normalized shifts in the pixel values, when plotted against the frequency at which the images were taken from, was used to construct the corresponding DEP spectrum for the K562 cell population used in the study (Figure 5). The plot showed the typical S-shape associated with DEP electrophysiological profile, which is similar to the previously published data for K562 cells in similar experimental conditions [38,45]. Notably, the crossover frequency occurs at the predicted 10 to 100 kHz range (about 40 kHz in this particular case). From the best-fit model constructed from Equation (1), it is inferred that the electrophysiological properties of the cellular membrane were close to the previously determined values for K562 cells of about 200 S and 7 mF/m^2 for conductance and capacitance, respectively.

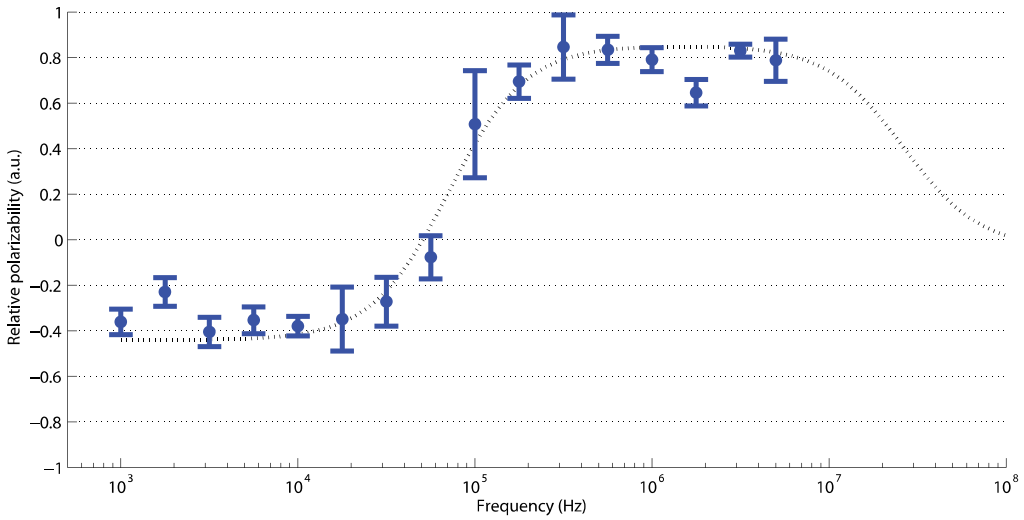

Figure 5. Typical DEP spectra for K562 cell population (cell concentration 1x10^7 cells per ml, electrical field 10 V_{p-p}, KCl conductive medium 10 mS/m). Dotted line indicates best fit model using the real part of the Clausius-Mossotti factor

5. DISCUSSION

As shown in Figure 5 each data points were constructed from eight dots having the same DEP effects at the same frequency. Since the electric field was generated for between 15-20 seconds, the whole DEP experiment for this particular carcinogenic cell line was completed in less than 10 minutes, including the time taken for manually transferring the cells through the inlet of the chamber. This is significant improvement over previously used DEP-based techniques (e.g. [17,34,37,38,46]), which may take anywhere from 1 to 3 hours to complete the necessary experimental procedures in constructing DEP plots of similar data points resolution. This enhancement will further improve the chances of DEP becoming a complementary diagnostic tool in cancer studies, since it is of importance

that a non-invasive technique be developed and capable of producing the desired outcome in the shortest time possible.

The BioMEMs application discussed in this study is capable of recording DEP events at a resolution of 8-by-n data points between the frequency range of 10 kHz and 1 MHz within 10 min. The hardware components should also be compatible with any lab-on-chip modules utilizing AC electrokinetics and microfluidics. The significant reduction in time to complete DEP experiments should encourage others to conduct real-time DEP experiments and analysis for any carcinogenic cell lines using currently available microengineering techniques. Figure 6 shows an example of the possibility in constructing real-time 3D DEP plots that would be highly beneficial in observing electrophysiological changes occurring in the cellular population, if any. This should subsequently open the possibility of, for example, real-time *in situ* observation of the effects of newly-developed cancer drug on the cellular membrane of cancerous cells without the need of additional chemical reagents.

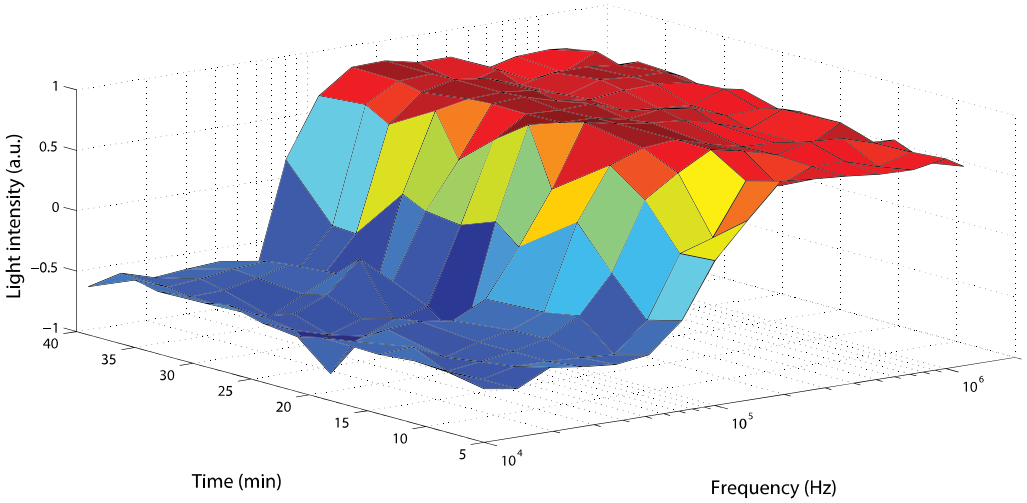

Figure 6. Preliminary 3D DEP spectra of K562 cells over 40 minutes, showing gradual changes in DEP profile over time

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