Dependence of dielectrophoretic forces on membrane proteins

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Introduction

Dynamic response characteristics are a cornerstone of system design and control yet related tools are lacking. Time-lapse microscopy of individual cells expressing synthetic reporter proteins represents one of the only tools capable of making dynamic measurements. Disadvantages of time-lapse microscopy are limited throughput and photobleaching. Herein we explore changes in dielectrophoretic forces caused by changes in expression of membrane proteins in S. cerevisiae. Dielectrophoresis is proven in medium throughput experiments and is not subject to protein damage permitting longer exposure times and more sensitive measurements. Hence, the described changes potentially enable dynamic measurements of gene expression and subsequently the development of tools that better meet the engineering needs. Theoretical and FEM models were simulated and the results were investigated in detail. Linear dependence of the first crossover frequency in the dielectrophoretic response on membrane protein concentration was revealed. Several microfluidic experiments were designed to confirm the theoretical expectations.

Dielectrophoresis (DEP)

A charge neutral particle placed in an electric field is acted on by dielectrophoretic forces. These forces manifest themselves through polarization of the particle and its surrounding medium. Their magnitudes and directions are complex functions that depend on the dielectric properties of the entire molecular composition.

The time averaged dielectrophoretic force acting on a spherical particle in a non-uniform electric field is given by the following equation:

$$F_{DEP} = 2\pi R^3 \epsilon_0 \epsilon_{med} Re[CM_f(\omega)] \nabla E^2$$

In this expression R stands for the radius of the particle, ε_0 stands for the permittivity of the vacuum, ε_{med} stands for the relative permittivity of the surrounding medium, $Re[CM_f(\omega)]$ stands for the real part of the frequency dependent Clausius-Mossotti factor, $\nabla[E^2]$ stands for the gradient of the square of the electric field.

$$CM_f = \frac{\epsilon_1^* - \epsilon_2^*}{2\epsilon_2^* + \epsilon_1^*}$$

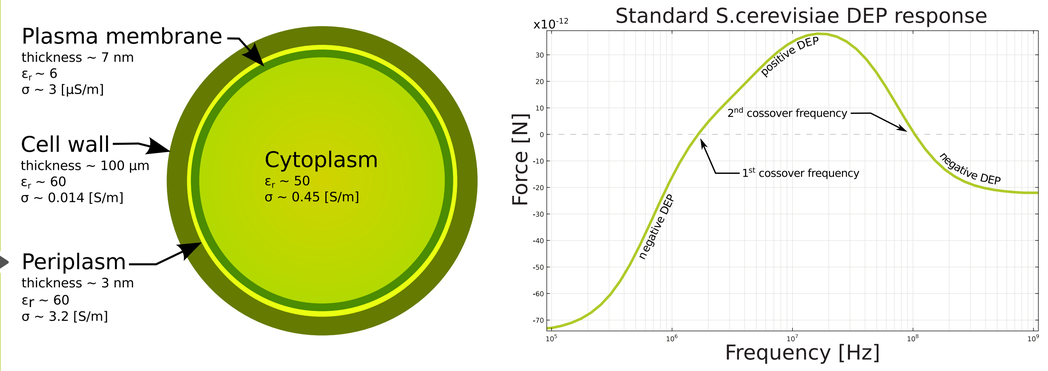
The permittivities ε* describing the Clausius-Mossotti factor are frequency dependent complex variables. They are dependent on the dielectric properties of the corresponding material and can be expressed as:

$$\epsilon^* = \epsilon_0 \epsilon_r - j\frac{\sigma}{\omega},$$

where ε_r is the relative permittivity of the material, σ is the electrical conductivity of the material and ω is the angular frequency of the applied electric field.

Model of Sacharomyces cerevisiae

Saccharomyces cerevisiae are fungi cells with roughly spherical shape 5 to 8 microns in diameter. They consist of a cytoplasmic space containing intracellular organelles and a cell envelope. The envelope is made of a thin plasma membrane and a thick cell wall separated by a thin periplasmic space.



The standard dielectrophoretic response of a Saccharomyces cerevisiae cell shows the magnitude and the direction of the induced force is controllable through electric field frequency modulation. Hypothesized dielectric reporter proteins are polypeptide molecules that significantly change the dielectrophoretic response. This change can be harnessed for cell sorting according to reporter protein concentrations.

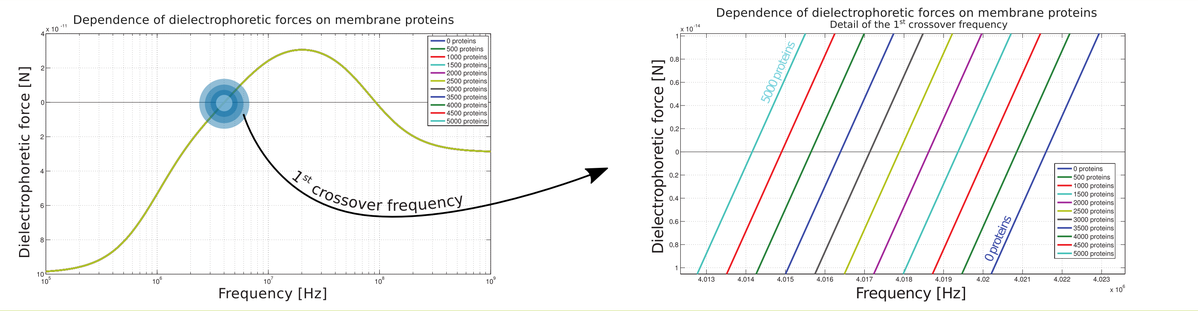
Dielectric reporter proteins (DRP)

Preliminary sensitivity analysis indicates cell wall proteins have the potential to sufficiently change the cell dielectrophoretic response. Several categories of dielectric reporter proteins are proposed. The DRP category is identified by the location of the DRP. Possible protein categories include ion channels, receptor proteins, intracellular membrane proteins.

Deposition of a new protein with a significant ectodomain creates an additional dielectric layer contributing to the dielectrophoresis of the cell. The embedded proteins and their ectodomains change the overall permeability of the cell wall affecting the ion conductivity.

FEM/analytical simulation

The above multishell Saccharomyces cerevisiae cell model is used to predict the cell behavior in a non-uniform electric field with various concentrations of channel forming DRP in the cell wall. An analytical solution and finite element analysis was used. Simulation results denote a shift of the first crossover frequency in the DEP response. This shift is proportional to the DRP concentration.



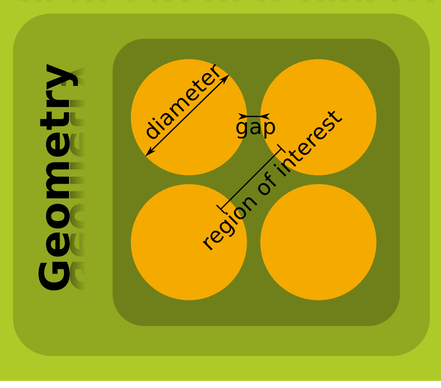
Forces computed in silico are sufficient to send cells with different DRP concentrations in different directions at rates well above the rate of diffusion.

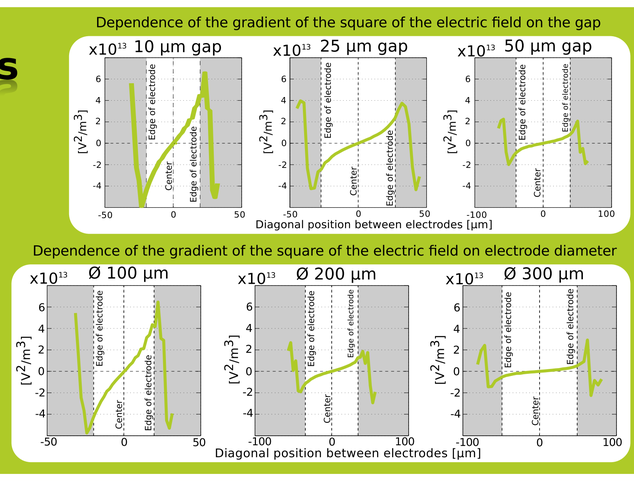
Optimization of electrode topology

The amplitude of the dielectrophoretic force around the crossover frequency is small. To produce sufficiently high DEP forces in this frequency range, optimization of the electrode topology is necessary. The electrode shape defines the electric field distribution. The optimal topology balances the tradeoff between the electric field gradient and its active domain to generate high forces for long periods of time. The topological parameter space of one planar electrode configuration suitable for live cells DEP dependence was characterized.

This parametric study focuses on the influence of the gap between the circular electrodes as well as on the electrode diameter. It is possible to increase the gradient of the electric field by decreasing the gap between the electrodes. Nevertheless, there is a limit value of the electric field. Once this value is reached, the transmembrane voltage increases above the permitted value (typically 0.5-1.0 V) which leads to electroporation or even cell death caused by the dielectric breakdown of the plasma membrane.

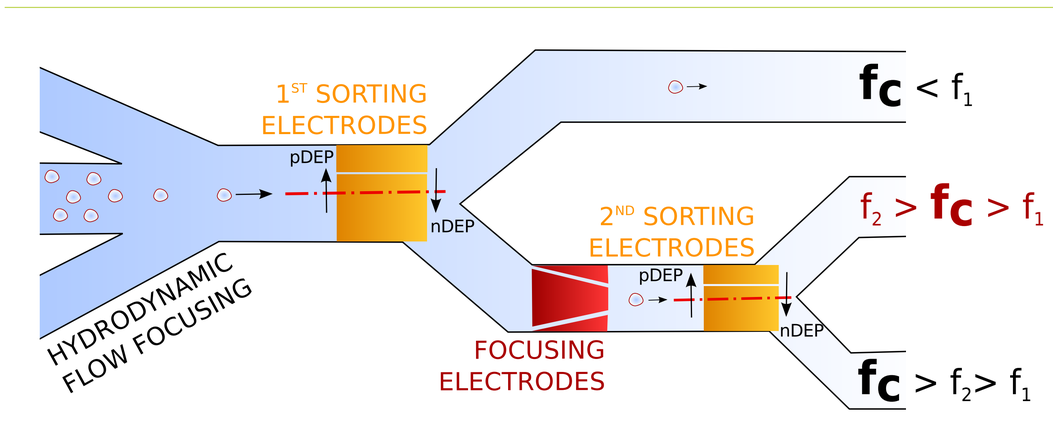
Array of circular electrodes

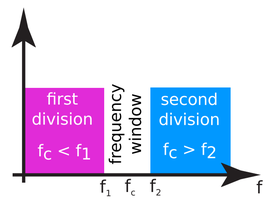




Experimental setup

New microfluidic device was designed and fabricated to measure the first crossover frequency in the dielectrophoretic response of Saccharomyces cerevisiae. The device is supplemented with hydrodynamic flow focusing for cell centering across the channel. This step ensures the cells enter the sorting stage at the same initial position in the microchannel. In the first sorting step, all cells with the first crossover frequency lower than the frequency of the applied electric field are discarded. A second cell focusing stage follows to again re-center the cells. Finally, all cells with the first crossover frequency higher than the frequency of the applied electric field are discarded in the second sorting step.

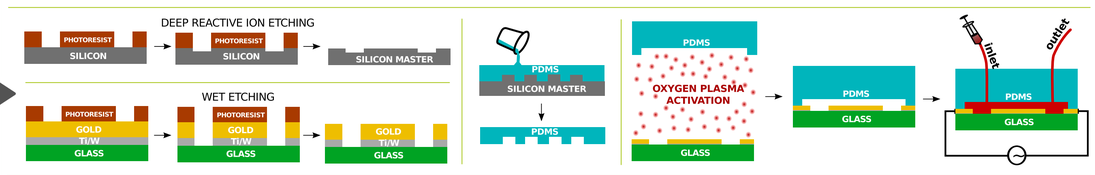




Cells tracing the middle path have the first crossover frequency in the designated frequency window (between the electric field frequency at the first sorting step and the second sorting step). The width of the frequency window is only limited by the sorting sensitivity. Different flow rates yield different sorting sensitivities. The active domain of the electric field gradient determines the absolute lower limit on the frequency window.

Fabrication process

Microfluidic channels were formed in PDMS by soft litography processes. Silicon master has been processed for this purpose. Planar gold electrodes were sputtered on glass substrate with the help of thin Ti/W adhesion layer. PDMS mold is bonded to the glass substrate after short exposure to the oxygen plasma to ensure proper bonding.



Another fabrication process is considered to avoid the PDMS misalignment. The channel walls may be formed on top of the glass substrate by layer of the SU-8 photoresist. Then, the channel can be sealed by flat piece of PDMS. Before sealing, SU-8 and PDMS surfaces must be exposed to oxygen plasma to ensure proper sealing as in the case of previous fabrication process.

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