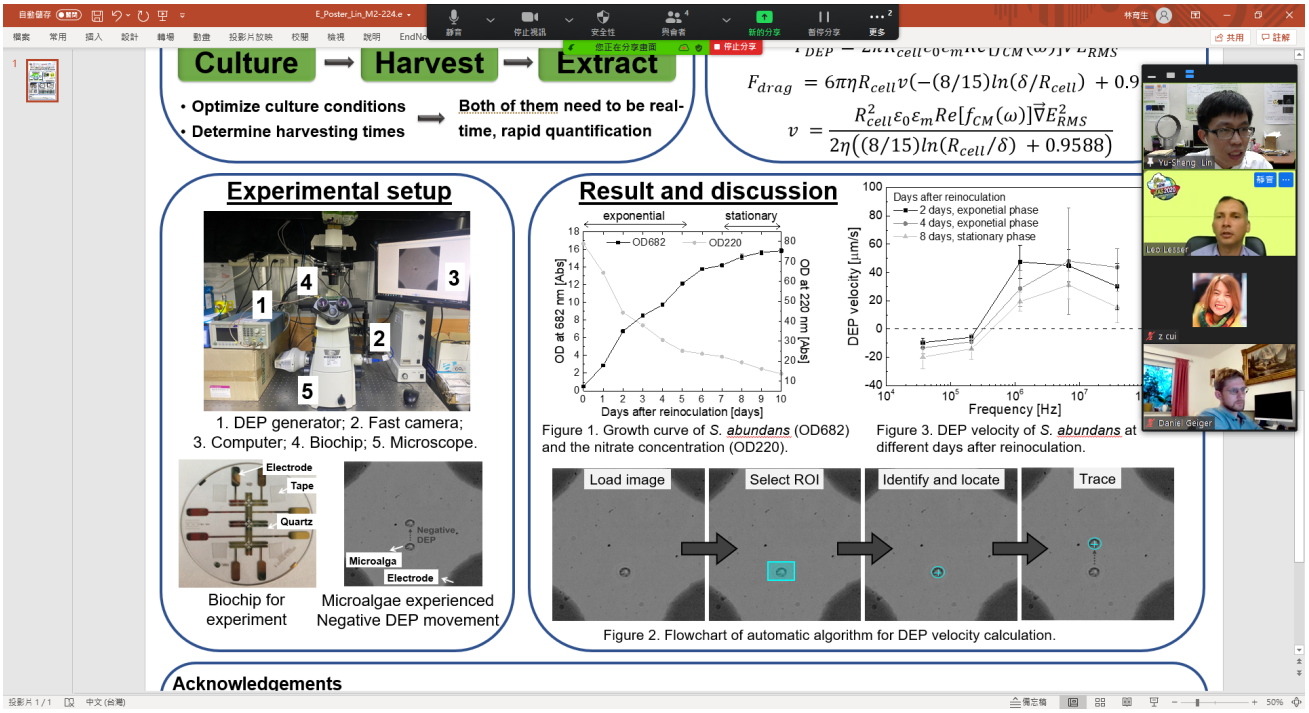
1、會議經過

本次會議的開場由日本東京大學的Fumihito Arai教授給的演講，演講的主題是發展晶片上機器人技術，結合感測器與傳感器，未來的世界會需要越來越多的晶片用於環境監測、老人醫療、運動管理等等，因此目前持續的發展各式感測器與傳感器的結合。聽完開場的演講後，我就開始準備即將到來的個人報告，報告的內容如下圖所示，右上是鏡頭前的我，下面三位是聽眾。在過程中，我依序介紹實驗目的、發展的技術、量測結果與分析方法，在呈現上以直版的投影片介紹為主，但後來發現其他人的報告是改成橫版的投影片介紹，這樣就可以不用將投影片上下滑動。大家所問的問題我有紀錄下來如投影片下方的呈現，主要分為結果的討論與分析的方法，有些問題是當下聽眾的提問我才發現的有趣結果！



Questions from floor.

1. Thao: It should be negative dielectrophoresis (type wrong).
2. The error bar for positive dielectrophoresis is bigger than the one for negative dielectrophoresis. Why is it?
3. The DEP velocity spectrum of day 2 is different from others especially in high frequency regions (The DEP velocity at 38.6 MHz decreases significantly). What is the meaning behind it?
4. The scale bar needs to be added in figures (lots of people ask the size of cell and the gap between electrodes).
5. How's the equation deduced? What's the amptuide of electric field square gradient? Can you do the experimental measurement and compare with theoretical calculation on the amptuide of electric field square gradient?
6. How's the image processing program working? It is similar to the work present by Dr. Daniel Geiger. (view poster of NOVEL IMAGING BASED HIGH-SPEED, HIGH-THROUGHPUT ANALYSIS AND CONTROL SYSTEM FOR MICROFLUIDICS from Daniel Geiger)

接著我參加許多與我研究相關的論文發表，如下面呈現。

1. 使用電旋轉技術來量測鼻竇鱗狀細胞肉瘤的特徵用於癌症的研究。在癌症不同的生長週期內，使用傳統的方法是相當難分別的，因此此研究應用非侵入式的電旋轉技術來量測不同時期的癌細胞，結果顯示不同時期的癌細胞有相當大的差異，其旋轉速度會有兩倍之差，此

差異來自於細胞內部成分的不同，細胞膜與細胞質的電導度跟介電常數都有可能造成此差異。

T3-301.a

CHARACTERIZING SINGLE SINONASAL SQUAMOUS CELL CARCINOMA USING DI-ELECTROPHORESIS AND ELECTROROTATION

Thao N.P. Mai[1*], Sakina Bensalem[1*], Bénédicte Thiebot[2], Philippe Manivet[3], Juan Pelta[2], and Bruno Le Pioufle[1]

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[2] Université Paris-Saclay, Université d'Evry, LAMBE, France

[3] Biobank Lariboisière, Assistance Publique-Hôpitaux de Paris, INSERM UMR1141, Université de Paris, Paris 10, France

Abstract

In this study, we describe methods and device for the capture and analysis of single cells, in particular human sinonasal squamous cell carcinomas (SCC), by the combination of di-electrophoresis (DEP) force and electrorotation. A set of 4 planar polynomial electrodes was used to perform nDEP trapping of two cancer cell lines with different invasivities (NC5 and NC7). Once captured at the center of the electrodes set, electrorotation was performed to extract the electro-mechanical signature of the single cell. From the electrorotation spectra, the electrophysiological properties of cells are estimated.

Keywords: Sinonasal squamous cell carcinomas, di-electrophoresis, electrorotation, single cell analysis

Introduction

Cancer cells can be found in various forms, at different stages of malignancy. Physical biomarkers discovery (in particular biomarkers of the metastatic form) is a challenge to develop diagnostic tools. In this context, single cell sensing by di-electrophoresis (DEP) and electrorotation methods, have demonstrated a potential for tumor cell characterization in lab-on-chip platforms [1]. In this work, we investigate and compare two cancer cell lines of sinonasal squamous cell carcinomas at different degrees of malignancy. The electrorotational spectra are different for these cells. This spectra depends on the cell dielectric characteristics, such as membrane capacitance, membrane permittivity, cytoplasm conductance and cytoplasm permittivity [1, 2].

Methods

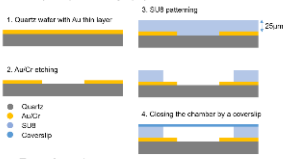
- **Cell preparation:**

The two cell lines NC5 and NC7 are used in our experiments, with a low conductivity buffer (8%wt of sucrose, KCl, $\sigma = 100mS/m$)

A cell suspension of 30µL is pipetted on the device surface, a coverslip is being used to close the chamber.

- **Device fabrication:**

The device is made of quartz on which polynomial gold electrodes are patterned (Cr sub-layer for attachment). A micro channel is patterned in SU8 resist by UV photolithography.



- **Experiment**

A voltage with 5V peak-to-peak amplitude is applied on the electrode array, at a frequency of 500kHz to trap cells by negative DEP force in the center of the electrodes set. The rotational signal is superposed using 90° phase-shifted voltages, at a fixed 2V amplitude and over a frequency range from 37kHz to 2MHz. Cell mechanical velocity is recorded with a fast camera (PHANTOM – 100fps) during 1 minute.

Results

After the image analysis, the rotational spectra is determined. Curve fitting between the experimental and theoretical spectra was then performed by the Genetic algorithm [3]. The spectra of NC5 and NC7 are indicated in Figure 1.

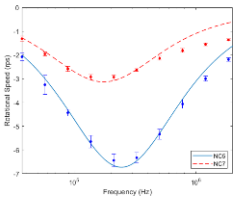


Figure 1: Electrorotational spectra of NC5 and NC7.

The electrophysiological properties of NC5, NC7, such as permittivity and conductivity of the membrane and cytoplasm, are estimated from the spectra (Table 1).

Table 1: Estimated electrophysiological parameters of cells with different level of malignancy.

	σ_{mem}(S/m)	ε_{mem}	σ_{cyto}(S/m)	ε_{cyto}
NC7	2.932×10^{-4}	29.929	0.094	75.248
NC5	2.229×10^{-4}	29.758	0.045	37.021

Conclusions

We propose a low-cost and simple method to trap and characterize single cancer cells. The electrorotational spectra shows that the maximum velocity decreases with the degree of invasiveness. This can be correlated to the change of conductivity and permittivity of both membrane and cytoplasm.

Electrorotation method can be used to characterize many types of cell.

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❖ **ACKNOWLEDGEMENTS**

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❖ **CONTACT**

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*These authors distributed equally to this work

2. 使用柱狀電極量測細胞的特徵用於阿茲海默症的研究。目前阿茲海默症的疾病機制尚未明朗，眾多的研究正在發展當中，此研究首先發展三維柱狀電極，此可以有效的執行電旋轉實驗，接著量測細胞的電旋轉頻譜，想要探討在毒化下細胞上細胞膜的影響。結果呈現可以使用量測細胞膜介電常數的改變來得知細胞膜團聚的現象，進而用於阿茲海默症的研究。

ELECTROROTATION FOR SINGLE CELL ANALYSIS OF MEMBRANE DAMAGE INDUCED BY TOXINS MIMICKING THE NEURODEGENERATIVE EFFECT OF AMYLOID BETA IN THE ALZHEIMER'S DISEASE

Till Ryser[1]*, Kevin Keim[1], Anne-Laure Mahul-Mellier[1], Hilal Lashuel[1] and Carlotta Guiducci[1]
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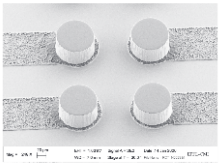
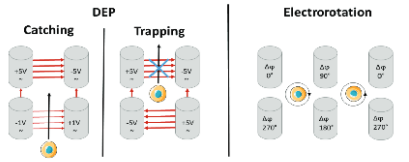
EPFL M1-144.e

INTRODUCTION

Toxic aggregates of the protein amyloid beta (Aβ) are hypothesized to be the major cause for the Alzheimer's disease (AD). Despite its high prevalence, the exact mechanism of neurotoxicity remains unclear. It is hypothesized that aggregates of Aβ disrupt the membrane of neuronal cells, e.g. by forming pores. A promising method that can give insights into the integrity of the cell membrane is electrorotation [1,2]. In our study, we use on-chip electrorotation to monitor changes in the membrane capacitance to assess the neurotoxicity of toxins related to the AD and aim for a diagnostic tool for the evaluation of membrane damage induced by Aβ.

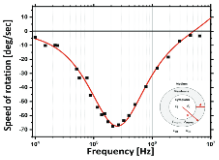


METHODS



Three different modes:
- Catching
- Trapping
- Electrorotation

DATA ANALYSIS

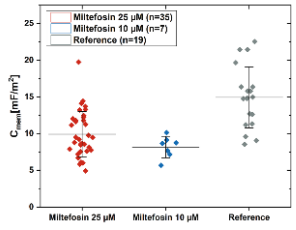


The obtained spectrum is fitted to single-shell model to obtain the membrane capacitance [3].

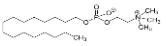
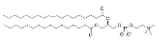
Speed of rotation:

$$\Omega_0 = -\frac{\varepsilon_m \, Im[CM^*(\omega)]E_0^2}{2\eta}$$

RESULTS



The obtained membrane capacitance gives insight into the membrane integrity of a single cell. When the cells are incubated with Miltefosine, a synthetic phosopholipid analogous of the main membrane constituent phosphatidylcholine, our data shows a decrease in membrane capacitance. This suggests that with our system we can detect membrane alterations caused by toxic substances. Viable cells are identified by fluorescent labeling of intracellular esterase activity and membrane permeability.

Miltefosine  Phosphatidylcholine 

CONCLUSION

The obtained results underline the potential of our system to detect membrane damage. In future experiments, cells will be incubated with Aβ to provide a better insight into the Alzheimer's disease.

References

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FNSNF
Swiss National Science Foundation

3. 使用新的成像系統進行電旋轉頻譜的採集。旨在解決諸如高通量之類的電旋轉問題，並且他們的目標是無透鏡成像不僅可以提供高分辨率，而且還可以提供大視野。這個研究在未來能結合電極矩陣來達到高通量的電旋轉頻譜量測，解決電轉轉技術最重要的問題。

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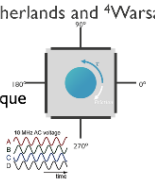
ANALYSIS OF ELECTRICAL PROPERTIES OF CELLS USING ELECTROROTATION AND LENS-FREE IMAGE DETECTION

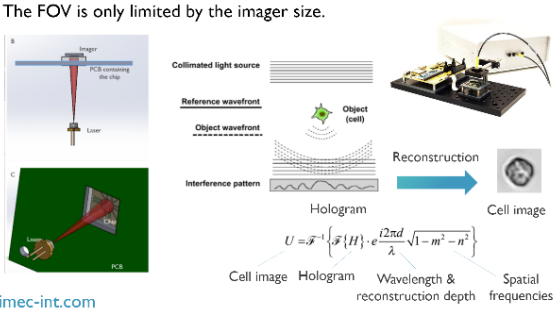
Camila D.M. Campos[1,2], Yuqian Li[1], Ziduo Lin[1], Geert Vanmeerbeeck[1], Pawel Barmuta[2,4], Tom Markovic[1,2], Rahul Yadav[1], Giovanni Mangraviti[1], Wim Van Roy[1], Ilja Ocket[1], Yao-Hong Liu[3], Tim Stakenborg[1], Richard Stahl[1], Liesbet Lagae[1,2], Jan Genoe[1,2] and Chengxun Liu[1,*]

[1]Imec, Belgium, [2]K.U. Leuven, Belgium, [3]imec, The Netherlands and [4]Warsaw University of Technology, Poland. *Chengxun.liu@imec.be

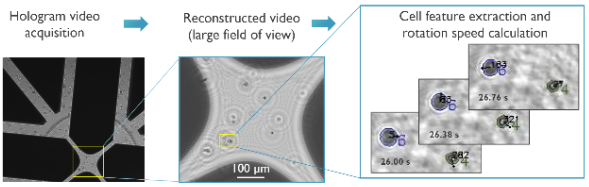
- Electrorotation for label-free cell assay
  - Cell electrorotation = a cell is electrically polarized in a rotating electric field and spins by the induced torque
  - Electrorotation indirectly measures cell electrical properties which reflects sub-micrometer cellular features (e.g. cell membrane roughness, conductivity)
  - Electrorotation opens the possibility of characterizing cellular features without optical labeling
  - However, electrorotation has not been successful in high throughput cellular assay partially because optical detection cannot achieve high resolution and large field of view (FOV) at the same time.



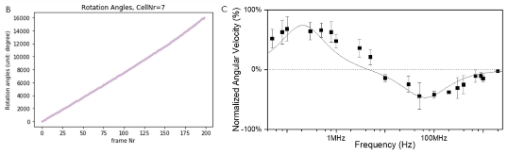
- Lens-free imaging for high throughput cell image tracking
  - Lens-free imaging (LFI) = acquisition of cell holographic pattern and reconstruction to cell image
  - Lens free imaging delivers sub-micrometer cell image resolution. The FOV is only limited by the imager size.



- LFI cell rotation images processing



- Electrorotation spectrum by real-time LFI measurement
  - Cell electrorotation spectrum from 100 kHz to 1 GHz.



Good linearity obtained by automated cell rotation measurements

The cell electrorotation spectrum obtained by repeating LFI-ROT for every electric field frequency

- Conclusion

We demonstrated the combination of cell electrorotation and lens-free imaging techniques. The high resolution in large field of view allows image tracking hence electrorotation pattern recognition for massive single cells. This technique paves the way for high throughput single cell analysis.

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2、心得

化學與生命科學之微型化系統國際研討會 (MicroTAS) 是全世界最大的微型系統研討會，每年與會人數多達1000人，發表論文也多達1000多篇，在各個不同的微型系統領域中，都能見到最新的研究成果。因為本次會議改為線上舉行，難免會擔心與實體會議有差距，所幸大家都相當捧場我的報告，所問的問題來自四面八方，包含我們發展的介電泳技術、影像辨識自動化程式等等，有相當多的討論。印象最深的是來自Dr. Daniel Geiger的問題，他問了我影像處理程式的運作方法，並分享說他也有做類似的相關產品並已經商業化使用，這讓我對微型系統能商業化成商品更加有概念，且能將研究成果變成商品。

此外，線上舉辦的會議還有一個好處是有留下紀錄檔，當兩個報告時間衝突時，可以在事後回去看紀錄檔兩者報告的紀錄檔，得知想聽報告的內容，但比較可惜的是沒有辦法認識到其他新的研究人員，頂多在報告期間做討論而已。在會議舉辦之前，大會還有請我們上傳一分鐘的影片介紹，並有影片人氣獎，得獎的影片都相當有創意，印象最深的是有一個影片是用唱歌的方式來呈現自己的研究，把自己的研究做成定格動畫的方式呈現，相當吸睛。

在會議過程中，我參加許多的報告跟講座，無論在專業領域的知識，或是報告的呈現方式、影片的製作等等，都讓我受益良多，同時也對微型系統的各種應用有更多的了解，目前台灣的主流發展產業是半導體產業，希望未來能藉由這樣的基礎，來發展生物科技，應用在更廣的領域，造福社會大眾。即便在國際疫情(COVID-19)非常嚴重的情況下，還是非常感謝這次的機會參加國際級的研討會議。

3、發表論文全文及摘要

MONITORING THE GROWTH PHASES OF MICROALGAE USING STRAIGHTFORWARD DIELECTROPHORESIS MEASUREMENTS

Yu-Sheng Lin[1,2], Bruno Le Piouffle[3], Hsiang-Yu Wang[1]

[1]Department of Engineering and System Science, National Tsing Hua University, Hsinchu, Taiwan
[2]ENS Paris Saclay, CNRS Institut d'Alembert, SATIE, Université Paris Saclay, Gif sur Yvette, France
[3]ENS Paris Saclay, CNRS Institut d'Alembert, LUMIN, Université Paris Saclay, Gif sur Yvette, France

ABSTRACT

This study demonstrates a straightforward method for differentiating single microalgal cells in different growth phases based on dielectrophoresis (DEP). The balance of DEP force and drag force leads to an easy-to-use linear relation between DEP velocity and the real-part of the Clausius Mossotti factor, which determines the velocity of DEP. The DEP velocity of microalgae in the stationary phase is slower than microalgae in the exponential phase, owing to the cellular compositions changed during growth. Microalgal cells in the stationary phase also showed a higher transition frequency than cells in the exponential phase.

KEYWORDS: Microalgae, Growth Phases, Dielectrophoresis Measurement

INTRODUCTION

Photosynthetic microalgae convert carbon dioxide into high-value metabolic products such as pigments, proteins, and lipids. Microalgae produce metabolic products such as pigments, proteins, lipids in different growth phases and the changes of cellular compositions can be considerable in a short period of time [1]. However, conventional analyses either are time-consuming or require fluorescent labels. Our previous study showed that microalgae cells have apparent differences in dielectrophoresis and electrorotation between generations and growth phases [2]. In this study, a straightforward method for identifying cellular status based on dielectrophoretic movements is developed and applied to the monitor of microalgae in different growth phases.

THEORY

Dielectrophoresis (DEP) force, as shown in equation (1), drives a cell to the highest (positive DEP) or lowest (negative DEP) electric field regions, depending on the polarizability of the cell compared to its medium. The direction of DEP force ($\vec{F}_{DEP}$) is determined by the real part of the Clausius Mossotti factor ($Re\left[f_{CM}(\omega)\right]$).

$$\vec{F}_{DEP} = 2\pi R_{cell}^{3}\varepsilon_0\varepsilon_m Re\left[f_{CM}(\omega)\right]\vec{\nabla}E_{RMS}^{2} \quad (1)$$

where ε_0 and ε_m respectively stand for the permittivities of the vacuum and the medium, R_{cell} is the radius of the cell and ωis the angular frequency of applied electric field E.

When the movement of a cell is at steady state, dielectrophoresis force is balanced with the viscous drag as shown in equation (2) [3]. In equation (2), v is the velocity of the cell and δ is the distance between the wall and the microalgae cell. The velocity v, shown in equation (3), thus can be obtained by balancing equation (1) and (2).

$$F_{drag} = 6\pi\eta R_{cell} v\left(-(8/15)\ln\left(\delta/R_{cell}\right) + 0.9588\right) \quad (2)$$

$$v = \frac{R_{cell}^{2}\varepsilon_0\varepsilon_m Re\left[f_{CM}(\omega)\right]\vec{\nabla}E_{RMS}^{2}}{2\eta\left((8/15)\ln\left(R_{cell}/\delta\right)+0.9588\right)} \quad (3)$$

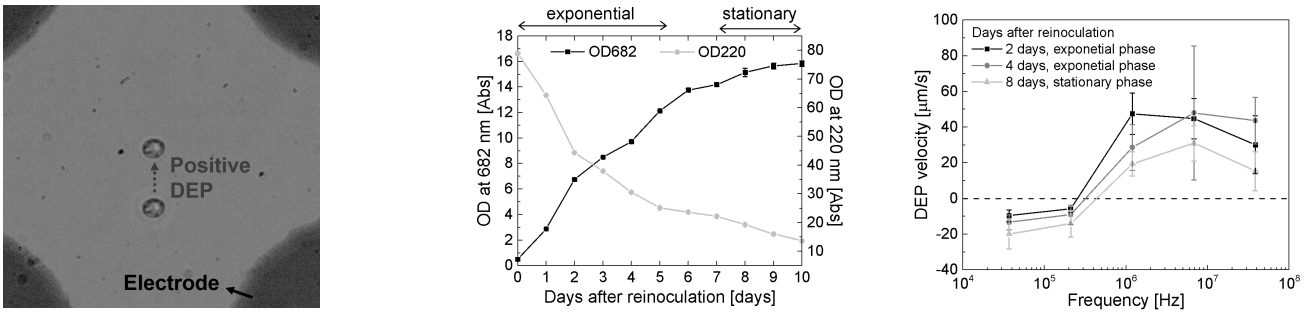
EXPERIMENTAL

The microalgae *Scenedesmus abundans* was cultured in BG-11 medium. The growth was monitored by UV/Visible spectrometry and dielectrophoretic movements. The growth of *S. abundans* was measured by a spectrophotometer at wavelengths of 682 nm (OD682) and 220 nm (OD220), which represented the total amount of chlorophylls and nitrate concentration, respectively. The dielectrophoretic movements were measured between 4 planar parabolic electrodes (Figure 1a) with a 75 μm gap on the quartz wafer. The electrodes were fabricated by conventional photolithography technology and the fluidic chamber was

constructed by 3M tapes. The conductivity of the medium was adjusted to 0.02 S/m for DEP measurement. The electrodes were then connected to two-channel function generators (AFG3102, Tektronix, USA) with electric signals of 3 Vpp, 180° phase shift, and 5 frequencies ranging from 37 kHz to 38.6 MHz. At each frequency, at least 10 movements of microalgal cells induced by DEP forces were recorded during 5 seconds by a high-speed camera with a frame rate of 100 fps.

RESULT AND DISCUSSION

The amount of chlorophylls and nitrate consumption of *S. abundans* were monitored daily to assess the growth conditions of microalgae as shown in Figure 1b. The OD682 (total chlorophylls) doubled and OD220 (nitrate) decreased by 68 % in the first fifth days. The rapid rates of chlorophylls increase and nitrate consumption are a characteric of the exponential phase of microalgae. The much slower increase in OD682 and decrease in OD220 after the 7th day of the reinoculation indicated the stationary phase had been reached. *S. abundans* cells were subjected to dielectrophoresis at the second, fourth and eighth days after the reinoculation.
The DEP velocities of microalgae shown in Figure 1c indicated that DEP velocity of microalgae in the stationary phase is slower than the one of microalgae in the exponential phase. Moreover, the movement of microalgae in the exponential phase changed from negative DEP velocity to positive DEP velocity (i. e. the transition frequency) at much lower frequency (253 kHz) compared with microalgae in the stationary phase (436 kHz). The differences in the above dielectric behavior were the results of changing cellular compositions during growth and can be applied to separate cells with different physiological status.



(a) (b) (c)

Figure 1: (a) Single microalgal cell trapped and moved to the center of the electrode set by positive DEP force. (b) Growth curve of S. abundans (OD682) and the nitrate concentration in BG-11 medium (OD220). (c) DEP velocity of S. abundans at different days after reinoculation.

CONCLUSION

This study demonstrated a straightforward method for discriminating microalgal cells in different growth phases using dielectrophoresis. When DEP force is balanced by the drag force, the variation in dielectric properties of microalgal cells at different physiological states lead to different DEP velocities. The DEP velocity of microalgal cells in the stationary phase are slower than those in the exponential phase, making DEP a straightforward method for separating microalgal cells in different growth phases.

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