介電泳探討葉綠體定位與肌動蛋白重組
Investigating Chloroplast Positioning and Actin Configuration with Dielectrophoresis

李正和 陳政宏 陳恕行*
Cheng-Ho Li, JHeng-Hong Chen, Shu-Hsing Chen*

摘要

植物細胞會從結構上進行改變以適應環境，這些改變甚至是超過動物所能的。這些適應發生在細胞內部，例如有些植物細胞之葉綠體會改變位置以便調整吸光面積。介電泳可用來協助研究這些細胞內部結構的改變。介電泳是種非侵入式的微操控術。以接受兩種不同光照條件的水蘊草薄壁組織細胞進行實驗。在實施介電泳時，兩者的葉綠體呈現不同型態的運動。進一步實驗顯示論肌動蛋白組態的改變可能是造成不同運動型態的原因。
關鍵詞：葉綠體，介電泳，肌動蛋白

Abstract

Plants are capable of changing their structures, even more than animals, in response to environmental changes. Those adjustments take place within a cell. For instance, to adjust light absorbing ability, chloroplasts can change their positions within a cell. A non-intrusive micromanipulation technique, dielectrophoresis, can be used to assist the investigation of intracellular adjustment. Experiments were conducted on parenchyma cells of *Egeria densa* acclimated to two different lighting conditions. Chloroplasts of the two cases exhibit spatially different patterns of motion during dielectrophoresis. Closer looks at the cells reveal that the change of actin configuration is the possible cause of the different motion patterns.
Keywords: chloroplasts, dielectrophoresis, actin

I. INTRODUCTION

Dielectrophoresis is a technique of using electromagnetic fields to manipulate dielectric particles. Besides well-known applications in manipulating or separating cells and bacteria, many potential applications still wait to be realized. One of the potential applications is to use the non-intrusive nature of electromagnetic wave to poke the internal structure of viable cells. Organelles like chloroplasts and mitochondria, which consist of lipid bilayer membrane, are especially sensitive to electromagnetic fields.

Dielectrophoresis was first discovered during a study on the motion and precipitation of suspensoids in divergent electric fields (Herbert Pohl 1951 [1]). A large volume of literature concerning the theories and application of dielectrophoresis has been published since. A rotating electric field can produce a phenomenon known as electrorotation (Herbert Pohl 1982 [2]), which has become a technique for measuring the electric properties of cells. The electromagnetic effect was found to be able to affect the behavior of unicellular organisms (Teixeira-Pinto 1960 [3]). The term "dielectrophoretic manipulator" was used to indicate the potential of using dielectrophoresis as a manipulative tool (Batchelder 1983 [4]).

Applications of dielectrophoresis include separation of small particles suspended in liquid (Masuda et al 1987 [5]; Masuda et al 1988 [6]), manipulation and characterization of cells (Pethig 1991 [7]), single-cell experiments (Müller 1988 [8]), gene expression profiling (Huang et. al. 2002 [9]), and probing the relaxation time of a cell (Wong et. al 2005 [10]). Formulas for calculating dielectrophoretic forces are available in the literature (Li 2004 [11]).

In a number of plant cells, chloroplasts change their intracellular positions to optimize photosynthetic activity (Zurzycki 1980 [12]; Haupt and Scheuerlein 1990 [13]). Under low-intensity light, chloroplasts accumulate on the periclinal cell wall, optimizing their potential to harvest sufficient sunlight for photosynthesis. Under high-intensity light, the chloroplasts move away from the periclinal walls and toward the anticlinal walls, minimizing potential

崑山科技大學機械工程系
*Corresponding author. Email: chensh@mail.ksu.edu.tw
Mechanical Engineering, Kun Shan University, Tainan, Taiwan, R.O.C.
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photodamage (avoidance response). Organelle movement is important to various physiological processes and is closely related to the function of actin cytoskeleton (Patrick et al. 2006 [14]).

Though actin is a highly conserved protein, it has a large variety of functions through cooperation with a variety of actin binding proteins (ABP). A conserved protein has similar genetic and polypeptide sequences among different species. The ABPs regulate nucleation, bundling, filament capping, fragmentation, and monomer availability. The actin cytoskeleton can undergo rapid reorganization during cellular movement, phagocytosis, and cytokinesis (Diez 2005 [15]). From genetic studies, it has been identified that a unique gene, CHUP1, encodes a protein required for organelle positioning and movement in plant cells (Oikawa et al. 2003 [16]).

Using non-intrusive methods to poke chloroplasts for studying the properties of cytoskeleton has been attempted by using centrifugal force. A centrifuge microscope, consisting of a light microscope, a stroboscopic light source and a position sensor to control the camera, has been constructed for this type of investigation (Oiwa et al. 1990 [17]; Sugi1 and Chaen 2003 [18]). Reorganization of actin filaments has also been studied by centrifugation immediately followed by fixing with formaldehyde (Sakai et al 2005 [19]).

Comparing to centrifugal force, dielectrophoresis provides a much more straightforward way to poke the internal structure of a cell. The difference is that dielectrophoretic force mainly works on organelles with bilayer membrane structure. This article presents experimental results that show motions of chloroplasts under dielectrophoretic. Experiments were mostly conducted on parenchyma cells of *Egeria densa*.

II. ANALYSIS OF DIELECTROPHORESIS

Dielectrophoretic force is generated on uncharged particles as a result of polarization induced by an electromagnetic field. The magnitude of the induced dipole moment is proportional to the distance between their oppositely charged poles. As shown in Figure 1, the induced dipole moment arising from the two induced charges + and - located at r- and r+ respectively is given by

$$\vec{m} = q\vec{r} \tag{1}$$

where $\vec{r}$ is the vector from negative charge to positive charge.

The vector form of dipole moment $\vec{m}$ induced by the electric field is

$$\vec{m} = 4\pi\varepsilon_m r^3 f_{cm}\vec{E} \tag{2}$$

where ε_m is the permittivity of suspending medium which equals to the multiplication of relative permittivity and vacuum permittivity (8.8542×10-12 F/m), r is the radius of particle, and f_{CM} is the Clausius-Mossotti (CM) factor defined as

$$f_{cm} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{3}$$

where ε_p is the relative permittivity of particle and ε_m is the relative permittivity of medium. The asterisk sign, *, represents "complex". The relative permittivity is in complex form, with the real part representing permittivity, ε, and imaginary part representing conductivity, σ. The complex permittivity can be written as

$$\varepsilon^* = \varepsilon - j(\sigma/\omega) \tag{4}$$

where ω is the angular frequency of the alternative electric field.

The electric field will produce a force on the induced dipole moment:

$$\vec{F} = (\vec{m}\cdot\vec{\nabla})\vec{E} \tag{5}$$

The electric field can be divided into two parts: the real part corresponding to 0° phase angle and the imaginary part 90° phase angle. This can be written as

$$\vec{E} = \vec{E}_R + j\vec{E}_I \tag{6}$$

The induced dielectrophoretic force then consists of two components. The in-phase component, corresponding to the real part of the CM factor, is

$$\vec{F}_{in} = 4\pi\varepsilon_m r^3 \,\mathrm{Re}[f_{cm}]\{(\vec{E}_R\cdot\vec{\nabla})\vec{E}_R + (\vec{E}_I\cdot\vec{\nabla})\vec{E}_I\} \tag{7}$$

The out-of-phase component, corresponding to the imaginary part of the CM factor, is

$$\vec{F}_{out} = 4\pi\varepsilon_m r^3 \,\mathrm{Im}[f_{cm}]\vec{\nabla}\times(\vec{E}_R\times\vec{E}_I) \tag{8}$$

The out-of-phase force exists when the field or the particle is rotating.

The electric field is the gradient of electric potential, ϕ, as depicted by

$$\vec{E} = \vec{\nabla}\phi \tag{9}$$

The nature of electromagnetism is controlled by Maxwell's equations. For frequencies typically used in dielectrophoresis, the problem can be reduced to the quasi-electrostatic. At high frequencies, the impedance between the two electrodes is low enough that a displacement current, given by Equation (11), exists.

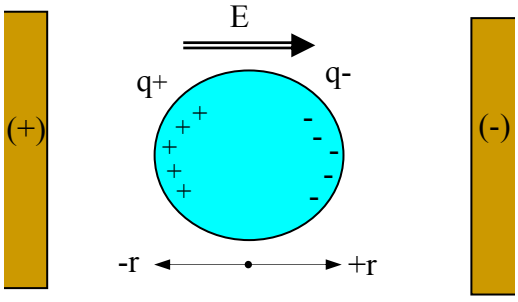


Fig. 1 Polarization induced by an electric field

$$\vec{J}_d = j\omega\vec{D} = j\omega\varepsilon\vec{E} \quad (10)$$

where $\vec{D}$ is the displacement vector.

The electric potentials are satisfied by the Laplace's equations as shown below:

$$\nabla^2\phi = 0 \quad (11)$$

The potential distributions can be numerically obtained by solving Equation (11). An appropriate boundary choice is to specify the Dirichlet condition for the electrodes and the Neumann condition for enclosing boundary.

III. EXPERIMENTAL METHODS

The basic setup of dielectrophoresis consists of two narrowly separated metal electrodes. An electromagnetic field is generated with an AC current source, as shown in Figure 2. The electrodes used in this work are made from copper stripes of 5 mm wide, 0.1 mm thick, and about 30 mm in length. The gap is controlled within 80 ± 10 μm. The experiments were observed with a transmission optical microscope (Leica DM IL). A function generator provides a sine AC current with frequency at 2 MHz and peak to peak voltage 20 V. The conductivity of the medium is 100 μS/cm.

Experiments were conducted on parenchyma cells of *Egeria densa* acclimated to two different lighting conditions. Dim light acclimatization was conduced in a dark cabin while the high light case was carried out with a 300 lux illumination provided by a fluorescent lamp. Acclimatization time was one hour. During dielectrophoresis, chloroplasts would be poked right after the power on but relax back to their original positions within 3-4 seconds. This is because the induced dipole moments only last for a short period of time. The dipole moment can be generated again after a resting period of 2-3 seconds. The current work is focused on the analysis of spatial motion pattern. The temporal behavior, though also containing useful information, is not considered in this work.

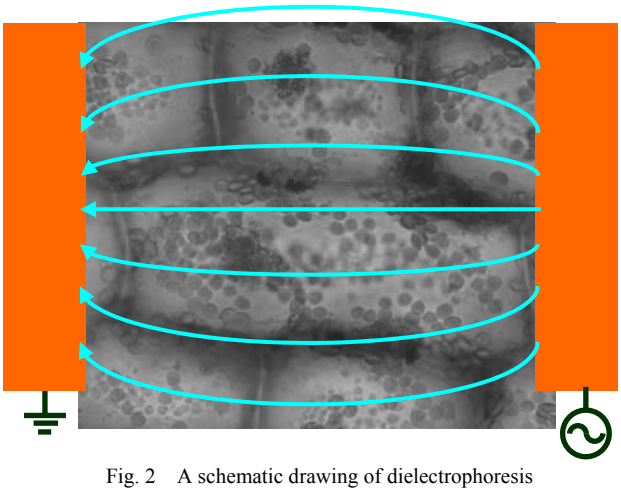


Fig. 2 A schematic drawing of dielectrophoresis

IV. RESULTS

Plot of the dielectrophoretic force and verification.

The dielectophoretic force is location-dependent. Assuming the displacement current is sufficiently large, a plot (Figure 3) has been generated to show the distribution of the dielectrophoretic force. The calculation is done by assuming that there are three particles with the CM factor of 0.5 and that the CM factor of the medium is 0.01. The three particles are indicated by the shaded areas. The plot predicts that the two particles at both sides would be tugged away from the center.

There is experimental proof of this analysis. In Figure 4, the center chloroplast remains at the same position while the other two move in opposite directions.

V. MOVEMENT OF CHLOROPLASTS UNDER DIFFERENT LIGHTING CONDITIONS

For cells acclimated to high-intensity light, chloroplasts accumulate on the anticlinal wall. In Figure 5, the triangular frame, with a fixed shape, is used as a reference. One of the internal chloroplasts is marked by a small diamond, and one of chloroplasts near the wall is marked by a circular dot. The movement of internal chloroplasts is larger than that of those near wall. Every frame is about 40 mini seconds apart.

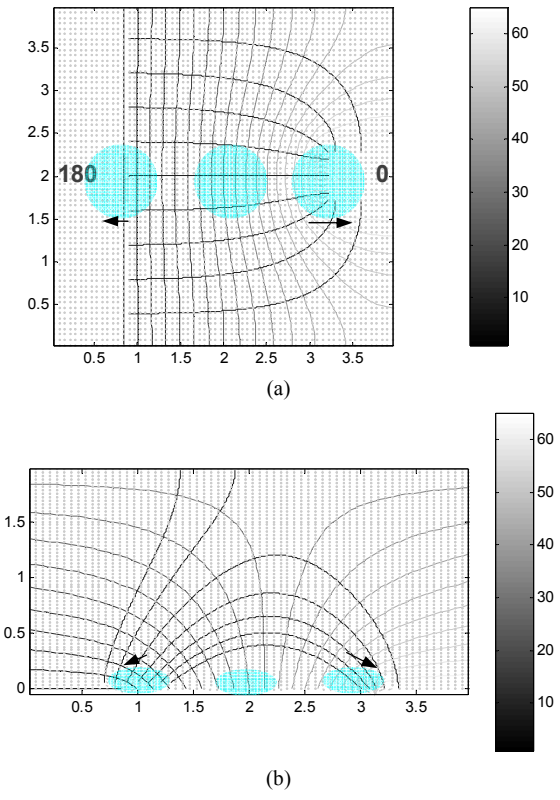
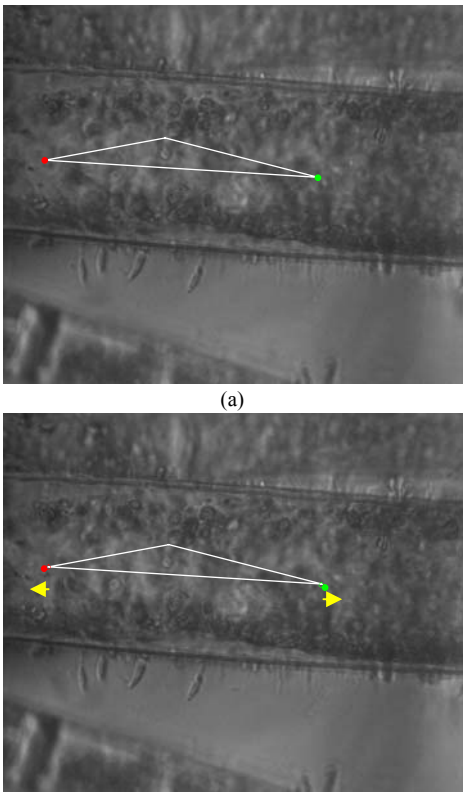


Fig. 3 Dielectrophoretic forces on three cells with CM factors other the medium, (a) in x-y plane z=0, (b) in x-z plane, y=0



(a) (b)

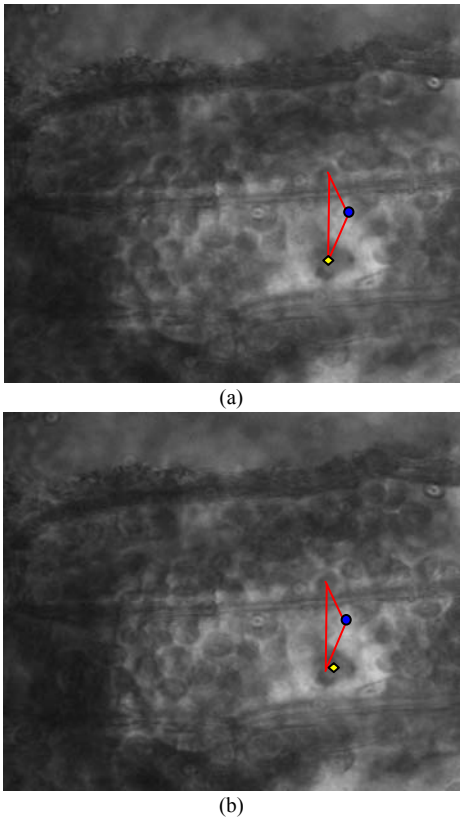
Fig. 4 Chloroplasts tugged toward opposite directions, (a) at rest, (b) power on (cells of green algae)

For cells acclimated to low light, chloroplasts evenly spread on the periclinal surface. During dielectrophoresis, the internal chloroplasts and chloroplasts near wall have the same amount of movement, as shown in Figure 6. Every frame is about 60 mini seconds apart.

It is expected that actin configuration of those cells has also been changed by light. Using an oil immersion ×100 objective lens, the change of actin configuration can be seen clearly. In Figure 7, an actin bundle quickly disbanded. The process lasted about 3 seconds. The actin bundle is a very dynamic structure. This actin bundle is visible under optical microscope. It is expected that both visible (barring using fluorescent dyes) and invisible changes of actin configuration exist to affect the motion pattern of chloroplasts during dielectrophoresis.

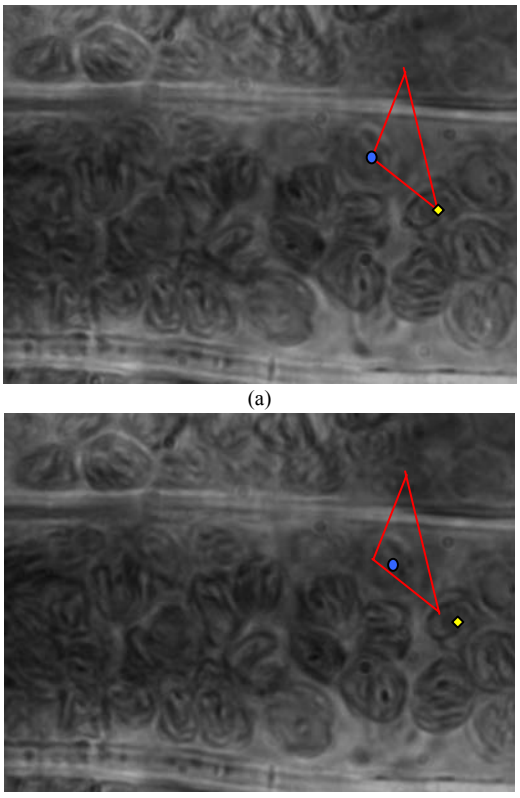
VI. CONCLUSION

Chloroplasts are sensitive to electromagnetic effect, and their motions are affected by the lighting condition in acclimation. Two basic rules can be inferred from the current study on *Egeria densa*. First, organelles which consist of lipid bilayer membrane are sensitive to electromagnetic fields. Second, actin cytoskeleton is sensitive to lighting condition. More applications based on these inferred rules of dielectrophoresis are expected.



(a) (b)

Fig. 5 For the high light case, the movement of internal chloroplast is larger than that of the outside one, (a) at rest, (b) power on



(a) (b)

Fig. 6 For the dim light case, the movements of internal and outside chloroplasts are the same, (a) at rest, (b) power on

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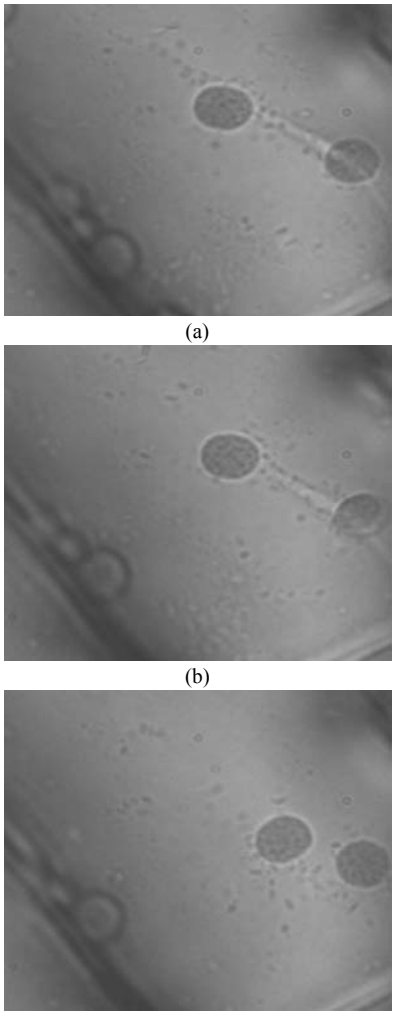

(a) (b) (c)

Fig. 7 A sequence of photos shows that an actin bundle formed and quickly disbanded

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