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Determination of Cell Dielectric Properties Using Dielectrophoretic Technique

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ABSTRACT Experiments were carried out on four cell types: *Chlorella* sp., *Tetraselmis* sp., and mesophyll protoplasts of *Dendrobium* and *Lilium longiflorum*. The speed of cell translation in between a pair of cylindrical electrodes was measured at different solution conductivities (σ_s). For each field frequency, the speed was plotted against the gradient of electric field squared to obtain the real part Re[f(ω)] of complex permittivity. Conductivity (σ) and permittivity (ε) of the cytoplasm and cell membrane were obtained by iterative method using a spherical single shell model. It was found that membrane permittivity (ε_m) of plankton cells was fairly large compared to that of mesophyll protoplasts, whereas the reverse was true for membrane conductivity (σ_m). The permittivity of cytoplasm (ε_c) varied from $105\varepsilon_o$ to $150\varepsilon_o$ for plankton and from $83\varepsilon_o$ to $105\varepsilon_o$ for the protoplasts. The estimated values for σ_c ranged from 8 to 40 $mS.m^{-1}$ for plankton and from 1 to 20 $mS.m^{-1}$ for the protoplasts. The cytoplasmic conductivity (σ_c) of the latter was the same as that of the external medium implying that these mesophyll protoplasts behave like lossy dielectric at σ_s between 1 $mS.m^{-1}$ to 20 $mS.m^{-1}$. Possible specific membrane capacitance (C_m) varied from 6 to 12 $mF.m^{-2}$ for plankton and from 2 to 31 $mF.m^{-2}$ for the protoplasts.

KEYWORDS: dielectrophoresis, dielectric property, spherical single shell model, plankton, plant protoplast.

INTRODUCTION

Single cells behave as dielectric particles in an Ac electric field. When being polarized, the dipole moment (μ) of the cell interior interacts with the field gradient (∇E). The resulting dielectrophoretic force (F_{DEP}) causes cell translation. Its direction is either towards or away from the higher field region depending on the relative complex dielectric values between the external solution and the cell interior. This force was first described by Pohl[1] and was studied extensively later by many researchers.[2-5] F_{DEP} was described as $(\mu \bullet \nabla)E$ or $2\pi\varepsilon_s R^3\, Re[f(\omega)]\nabla(E^2)$, where R and ε_s represent cell radius and the permittivity of the external solution, respectively. Re[f(ω)] is the real part of a complex function $Re[(\varepsilon'_{eff} - \varepsilon'_s)/(\varepsilon'_{eff} + 2\varepsilon'_s)]$ where ε'_{eff} and ε'_s are the frequency dependent complex permittivity of the cell and the solution, respectively. The model used was a spherical single shell model, which described a cell as a heterogeneous system. Determination of dielectric values using the model relates to the bulk properties for each cell compartment. Tests were made in plant protoplasts[2,3], mammalian cells[4,5], and yeast.[6] The differences among these cells were the internal composition and the membrane: with and without chloroplasts and the cell wall.

A non-uniform field, as required for dielectrophoresis (DEP), can be obtained by using concentric cylindrical electrodes[2], cone-plate[3], sheet-wire[4], or cylindrical parallel electrodes.[5] With a pair of cylindrical electrodes, the Re[f(ω)] has been modified in terms of permittivity (ε) and conductivity (σ) of the external solution, the cell membrane and the cytoplasm. The mathematical model and the electric field for such electrode geometry are sufficiently simple to allow easy calculation and experimentation. This laboratory has previously studied DEP of *Tetraselmis* sp. and *Chlorell* sp. using cylindrical parallel wires[7], since it is cheap and simple to begin. The results have shown that it was possible to manage a stronger positive F_{DEP} acting on the former, with a weaker force on the latter. It was noted that the stronger force was partly due to larger cell radius and partly to the difference in the membrane and the cell interior. The cell shape of these cells was different too: one was ellipsoidal and the other was spherical. These differences would affect the force in the same field and might be shown by cell dielectric parameters. The DEP technique has been

recently modified and used simultaneously with a multiple electrode system, making cell tweezers available[8].

This study measures cell speed between two cylindrical wires in a non-uniform electric field so that the Re[f(ω)] could be obtained and plotted in accord with frequency used, between 100 kHz and 15 MHz. Dielectric parameters for each cell species are predicted by an iterative method. The study is also extended to protoplasts of plant mesophyll for a comparison and to test for the single shell model.

Theoretical Concepts

When being induced in an electric field, a cell in between a pair of cylindrical electrodes at position z = 0 (see Fig 1) moves upward from the container bottom due to a resultant vector field between +y and either –z or +z direction. At the electrode focal plane, it moves mainly due to the horizontal field in z-direction towards an electrode. As shown by Mahaworasilpa et al[5], the cell movement is mainly due to the resultant of these two fields and only slightly affected by y-position, especially when z is increased. Their study described the field amplitude as E_Z, $\nabla(E^2)$, which is determined by the resultant field can be described by equation (1). The cell speed (v) relates to the real part Re[f(ω)] of the relative complex permittivity, as being a function of field frequency. The relation between v, Re[f(ω)] and $\nabla(E^2)$ is given by equation (2).

$$\nabla(\vec{E}^2) = \left[\frac{Vd}{\mathrm{In}(\frac{d-a}{a})}\right]^2 \left[\frac{z}{(\frac{d^2}{4}-z^2)^3}\hat{z}\,\frac{y}{(\frac{d^2}{4}-y^2)^3}\right] \quad (1)$$

$$v = \frac{\varepsilon_s R^2\ \mathrm{Re}[f(\omega)]\nabla(E^2)}{3\eta} \quad (2)$$

When plotting $(3\eta/\varepsilon_s R^2)v$ against $\nabla(E^2)$, as shown in Fig 2, the Re[f(ω)] values for each frequency used can be estimated from the slope of the graph. With the same method, Re[f(ω)] values for the whole frequency spectrum can be determined. Throughout this study, ε_s is assumed to be $80\varepsilon_o$[3, 4], where ε_o is the permittivity of a vacuum (8.85×10^{-12} F.m^{-1}). Cell radius R and the solution viscosity η can be obtained from the measurements.

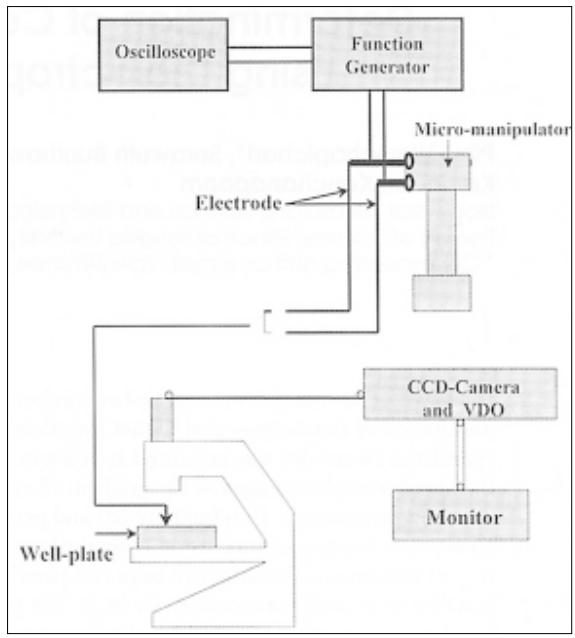

Fig 1. (a) Equipment set up for dielectrophoresis study.

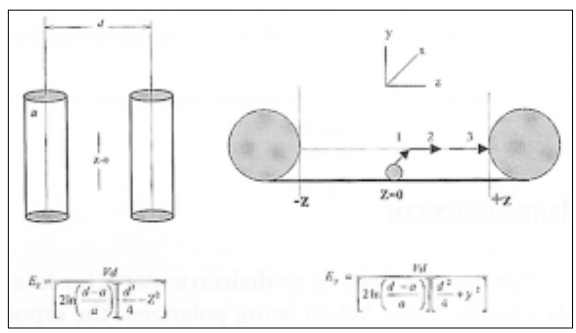

Fig 1. (b) Top view of the electrode geometry and the side view showing cell movement according to a resultant field between E_y and E_z (*for details see Mahaworasilpa* et al.[5])

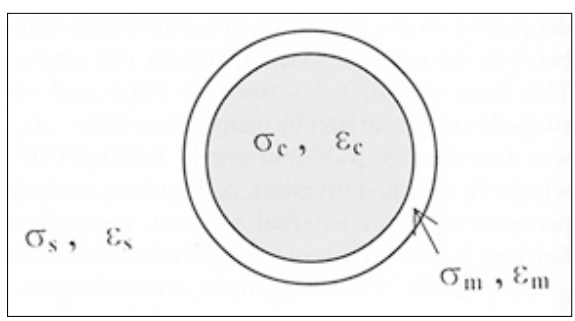

Fig 1. (c) Single shell model of a spherical cell. The dielectric permittivity, ε and electric conductivity, σ, in three phases are specified. The subscripts in s, m, and c denote the solution, the membrane and the cytoplasm, respectively.

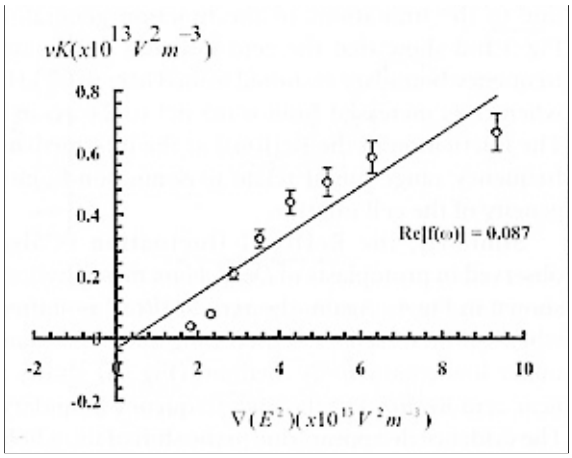

Fig 2. An example of plots between vk and $\nabla(E^2)$ at corresponding z positions for *Tetraselmis* sp. at MHz, σs=6 mS.m-1, where K is $3\eta/\varepsilon_s R^2$, and η is 1.67 mN.s.m^{-2}.

Materials and Methods

Cell preparation

Plankton cells were supplied by the National Institute for Coastal Aqua-culture. The cells were centrifuged twice at 1.8 g for 5 min, then re-suspended in a 0.5 M sucrose solution. Average cell size was 2.0 μm for *Chlorella* sp. and 3.5x9 μm for *Tetraselmis* sp. Plant leaves (3-4 cm in length) were used as source of explant. They were cut 0.5 cm at distal and proximal end and preplasmolysed in 0.7 M mannitol solution for 15 min. They were then chopped into strips about 1-2 mm in width before being transferred to the enzyme solution. The enzyme for preparing *Dendobium* protoplasts contained 2% Cellulase Onozuka R-10, 1% Driselase and 1% Marcerozyme R-10 (Yakult Honsha Co Ltd, Japan), whereas the Cellulase and Driselase for preparing *Lilium longiflorum* protoplasts were diluted 50%. Approximately 1 g of explants was used per 10 ml of solution. All enzymes were dissolved in 0.7 M mannitol at pH 5.7. The leaf-enzyme mixture was placed on a rotary shaker with the agitation speed of 50 rpm, 28 °C in darkness. After 4-h incubation, protoplasts were sieved through a 141 μm mesh stainless steel screen to remove any clumps of undigested cells and debris. The filtered protoplasts were centrifuged at 40 g for 5 min. The filtrate was removed using a Pasteur pipette and washed twice in 0.7 M mannitol. Finally the pellet material was transferred to the top of a 15-ml screw capped centrifuge tube, which contained 6 ml of 0.6 M sucrose solution. Sucrose density gradient centrifugation was performed at 50g for 10 min and protoplasts were observed on the surface of the sucrose solution. While the remaining cells and debris were sediment to the bottom of the tube, protoplasts were gently collected and washed 2-3 times with 0.5 M mannitol. The selected protoplast size of *Dendobium* was 80±4 μm and of *Lilium longiflorum* was 50±6 μm. The conductivity of cell external solution was measured by using a conductivity meter (Tetracon 325, LF 318).

Electric induction

A signal generator (DS 340, Standford Research System, USA) with frequency ranging between 1kHz and 15 MHz and with 10 V maximum voltage. A pair of cylindrical nickel alloy (California fine wire company, Grover City USA) electrodes, 125 μm in diameter, were bent in to an L-shape, and gradually lowered into an experimental well, filled with the low conducting medium. The study was made under an inverted microscope (LX 700, Olympus, Japan) with 200x to 400X magnification. The equipment setup is shown in Fig 1. A selected cell was placed in the middle of the electrode pair and an electric field was applied with a chosen frequency. Cell translation occurred due to E_y and E_z interaction with the cell induced dipole, soon after the cell was lifted from the container bottom. After the translation to an electrode was complete, the applied field was then turned off, the cell was re-arranged, and field frequency was changed to pre-selected values between 5kHz and 15 MHz. Cell translation was recorded using a CCD camera (Sony SSC-DC18P, Japan) and stored on a video recorder (Sony SLV-KH7, Japan), to be displayed later in order to estimate the translation speed on a TV monitor, with a scale of ± 1.2 μm accuracy. The field strength used for *Chlorella* sp., *Tetraselmis* sp., *Dendobium*, and *Lilium longiflorum* protoplasts were 116 kV.m^{-1}, 85 kV.m^{-1}, 13 kV.m^{-1}, and 23 kV.m^{-1}, respectively.

The solution viscosity was determined by using a capillary viscometer (model 516 10, Schott Gerate, Hofheim, Germany). It was 1.67 ±0.06 mNs.m^{-2} for 0.5M sucrose solution and 1.35 ±0.03 mNs.m^{-2} for 0.5M mannitol solution. Changes in the solution conductivity (s_s) were simply made by adding KCl solution.[2]

Results

The dependence of real function on solution conductivity

Re[f(ω)] values of *Chlorella* sp. and *Tetraselmis* sp. were plotted against frequency spectrum as shown in Fig 3 (a-d). It was observed through an

inverted microscope that cell speed was significantly high in the MHz range, which coincides with the finding of higher Re[f(ω)] values. In Fig 3 a and b, zero Re[f(ω)] can be observed at the low frequency boundary near 30 kHz for *Chlorella* sp. and 8 kHz for *Tetraselmis* sp. The zero Re[f(ω)] value at the upper frequency boundary could not be observed due to the limitations of the function generator. Fig 3 b-d show that the zero Re[f(ω)] at the low frequency boundary is shifted from 8 kHz to 90 kHz when σ_s is increased from 6 mS.m^{-1} to 24 mS.m^{-1}. The fluctuation of the Re[f(ω)] at the intermediate frequency range might relate to some non-homogeneity of the cell interior.

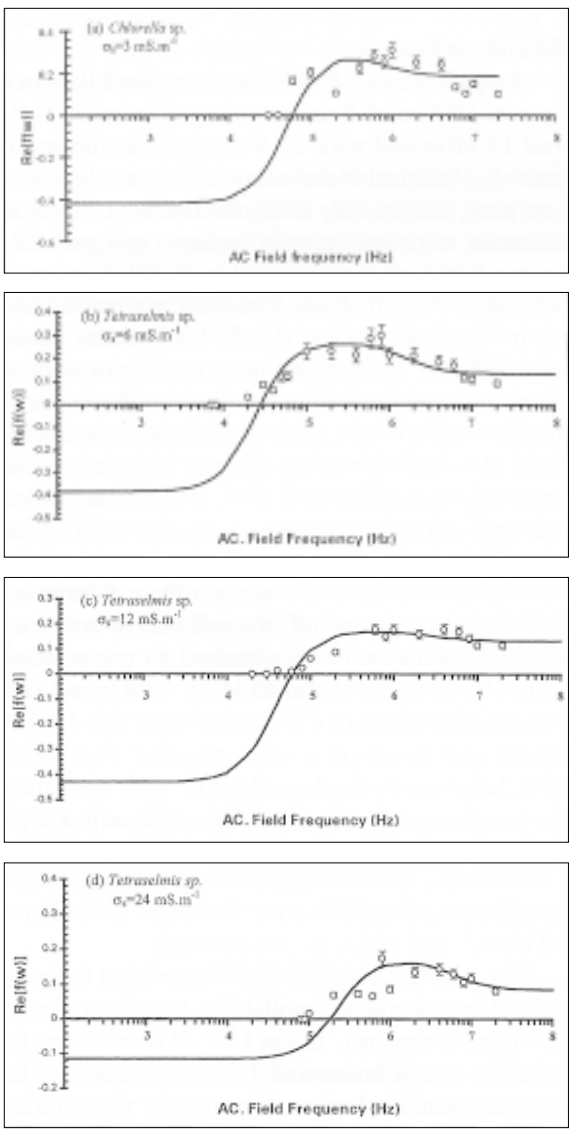

Fig. 3 Comparing Re[f(w)], which is obtained from the measurements (circles) and theoretical plots (line) by the iterative method for *Chlorella* sp. and *Tetraselmis* sp. The estimated parameters are;
(a) $\varepsilon_m = 70\varepsilon_o$, $\varepsilon_c = 150\varepsilon_o$, $\sigma_m = 0.020$ mS.m^{-1}, $\sigma_c = 8$ mS.m^{-1} and $\delta = 50$ nm.
(b) $\varepsilon_m = 40\varepsilon_o$, $\varepsilon_c = 120\varepsilon_o$, $\sigma_m = 0.008$ mS.m^{-1}, $\sigma_c = 13$ mS.m^{-1} and $\delta = 40$ nm.
(c) $\varepsilon_m = 22\varepsilon_o$, $\varepsilon_c = 120\varepsilon_o$, $\sigma_m = 0.005$ mS.m^{-1}, $\sigma_c = 20$ mS.m^{-1} and $\delta = 21$ nm.
(d) $\varepsilon_m = 14\varepsilon_o$, $\varepsilon_c = 105\varepsilon_o$, $\sigma_m = 0.100$ mS.m^{-1}, $\sigma_c = 40$ mS.m^{-1} and $\delta = 21$ nm.

Similarly, the Re[f(ω)] fluctuation is also observed in protoplasts of *Dendobium* mesophyll, as shown in Fig 4. Again, the zero Re[f(ω)] is shifted when s_s is increased. It is interesting that *Dendobium* under low conductivity medium (Fig 4a) shows a near zero Re[f(ω)] at the high frequency boundary. The evidence disappears due to the shift of the whole Re[f(ω)] spectrum with increased s_s (Fig 4b). This explains why the Re[f(ω)] for *Lilium longiflorum* reaches its maximum at a frequency close to 10 MHz (Fig 5) when σ_s of 20 mS.m^{-1} is used. It should be pointed out that Re[f(ω)] of both protoplasts are a factor of 10 lower than those of plankton cells, and the values decrease when σ_s is increased. Taking those of *Tetraselmis* sp. as an example, it is reduced from 0.30 to 0.17 when σ_s is increased from 6 mS.m^{-1}

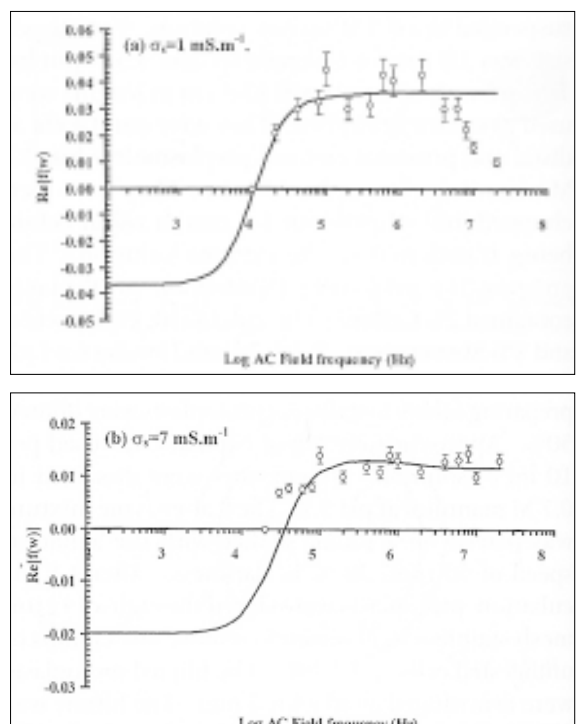

Fig. 4 Plots of Re[f(ω)] against field frequencies (circles) and theoretical plots for possible dielectric properties (line) of *Dendobium* protoplasts. For δ =18 nm;
(a) $\varepsilon_m = 4\varepsilon_o$, $\varepsilon_c = 89\varepsilon_o$, $\sigma_c = 1.12$ mS.m^{-1}, and $\sigma_m = 0.002$ mS.m^{-1}.
(b) $\varepsilon_m = 20\varepsilon_o$, $\varepsilon_c = 83\varepsilon_o$, $\sigma_c = 7.3$ mS.m^{-1}, and $\sigma_m = 0.03$ mS.m^{-1}.

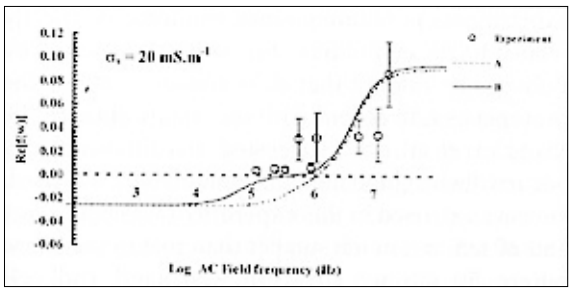

Fig. 5 Plots of Re[f(ω)] against field frequencies (circles) and theoretical plots for possible dielectric properties (lines) of *Lilium longiflorum* protoplasts.
A: δ = 10nm, $\varepsilon_c = 105\varepsilon_o$, and $\varepsilon_m = 5\varepsilon_o$ and
B: δ = 10nm, $\varepsilon_c = 105\varepsilon_o$, and $\varepsilon_m = 35\varepsilon_o$. Both lines are obtained using σ_m = 0.01 mS.m^{-1}, and σ_c = 20 mS.m^{-1}.

to 12 mS.m^{-1}. Similarly, it is decreased from 0.04 to 0.01 for *Dendobium* when s_s goes up from 1 mS.m^{-1} to 7 mS.m^{-1}, at a frequency around 1 MHz.

Estimations of dielectric parameters

Theoretical plots (lines) of Re[f(ω)] for plankton and mesophyll protoplasts are shown in Fig 3, Fig 4 and Fig 5. To obtain the best fit lines, several e and s values for the membrane and the cell interior have been tested. It was found that the dependence of Re[f(ω)] on changes in ε and σ is the same as described by Mahaworasilpa et al[5]. Fig 5 shows two possibilities of ε_m that affects the lower DEP boundary while ε_c affects the upper DEP boundary. Membrane thickness (δ) was chosen so that the line fits the experimental data in the high frequency range. Summary of these parameters is shown in Table 1. The σ_c of *Chlorella* is about $3\sigma_s$ while that of *Tetraselmis* is about $2\sigma_s$. Surprisingly, the σ_c of plant mesophyll has about the same value as σ_s. Note that in all cases, $\varepsilon_m < \varepsilon_s < \varepsilon_c$ and $\sigma_m << \sigma_c$. It is also interesting to point out that membrane thickness of plankton (with cell wall) is much greater than that of mesophyll protoplasts, particularly when σ_s is low.

As shown in Table 1, the membrane specific capacitance (C_m) varies from 6 mF.m^{-2} to 12 mF.m^{-2} for plankton, and from 2 mF.m^{-2} to 31 mF.m^{-2} for mesophyll protoplasts. Since it depends very much on the ratio between ε_m and δ, that of *Lilium* mesophyll varies in a wider range according to possible ε_m values. Smaller C_m of *Tetraselmis* compared to that of *Chlorella* might relate to the larger cell size, since larger membrane area is responsible for a smaller charge density.

Discussion

In a non-uniform field, the cell at a position near the center between two electrodes is first more strongly influenced by E_y and later by E_z, causing a lag in the movement at the beginning, as shown in Fig 2. After uniform movement, the cell is accelerated towards an electrode. The assumption of uniform cell speed is, therefore, an approximation which makes the estimated slope between $(3\eta/\varepsilon_s R^2)v$ and $\nabla(E^2)$ in the figure somewhat uncertain. However, it is reasonable for the present study since the iterative method is also an approximation. This study shows that Re[f(ω)] is sensitive to medium conductivity changes. The zero value is shifted towards higher frequency and Re[f(ω)] is decreased when σ_s is increased. This is consistent with the report by Jutiporn et al.[7] on the shift of zero dielectrophoretic forces acting on pearl chains.

Along the frequency spectrum, the fluctuation of Re[f(ω)] at the intermediate frequency range between 0.1 and 1.0 MHz seems to be the case for all cell species. Note that since the same cell was exposed to the whole frequency spectrum, it is

Table 1. Showing estimated parameters obtained by the iterative method for Re(f(ω)) of four different plant cell types. Membrane specific capacitance (C_m) was estimated from ε_m/δ.

Parameters	Phytoplankton				Mesophyll protoplasts		
	Chlorella		*Tetraselmis*		*Dendobium*		*Lilium longiflorum*
σ_s (mS.m^{-1})	**3**	**6**	**12**	**24**	**1**	**7**	**20**
$\varepsilon_s/\varepsilon_o$	80	80	80	80	80	80	80
$\varepsilon_m/\varepsilon_o$	70	40	22	14	4	20	5-35
$\varepsilon_c/\varepsilon_o$	150	120	120	105	89	83	105
σ_m (mS m^{-1})	0.02	0.01	0.01	0.10	0.002*	0.03	0.1
σ_c (mS m^{-1})	8	13	20	40	1.1	7.3	20
δ (nm)	50	40	21	21	18	18	10
C_m (mF.m^{-2})	12.4	8.8	9.3	5.9	2.0	9.8	4.4 –31.0

* the same value as for *Neurospora crassa* slime cells[4].

therefore likely that the Re[f(ω)] fluctuation describes a non-homogeneity of the cell system. This might be characteristic of Re[f(ω)] for cells containing chloroplasts and nuclei. If this is the case, fine changes in field frequency yield better resolution of Re[f(ω)]. Using feedback-controlled levitation technique, a finding of an anomalous peak of DEP response between 1 kHz and 50 MHz in *Canola* protoplasts and ligament fibroblasts was reported by Kaler and Jones[3] at a higher frequency near 20 MHz. On the smaller Re[f(ω)] values of the protoplasts, it is explained as being due to smaller field strength used and, hence, smaller DEP force interacting. In a preliminary study, it was found that experiments for *Dendobium* protoplasts must be carried out with the field less than 17 $kV.m^{-1}$; otherwise protoplast elongation would occur. This field strength was, however, not large enough to cause plankton cells to translate. Much higher field strength in plankton experiments is required since the force is directly related to the cell radius ($F_{DEP} \alpha R^3$) as reported by Pohl.[1] The finding that σ_c is similar to σ_s for the protoplasts is in accord with the results obtained by Marszlek et al[4], who suggested that this similarity occurred when high medium conductivity was used. However, σ_s used in this experiment was between 1 and 20 $mS.m^{-1}$, much smaller than that in their case where 50 $mS.m^{-1}$ solution was used and cell polarization was small. This evidence implies that the protoplast under the studied conditions behaves like a lossy dielectric at field frequencies of about 10 kHz onwards. For *Lilium longiflorum*, the protoplast surface is quite sticky, which might be related to some cytoplasm leakage under the induced electric field. Moreover, its membrane is very brittle and the protoplast could burst with little elongation (see Fig 6). Although this study finds a surprisingly large C_m of *Lilium longiflorum*, values as high as 15

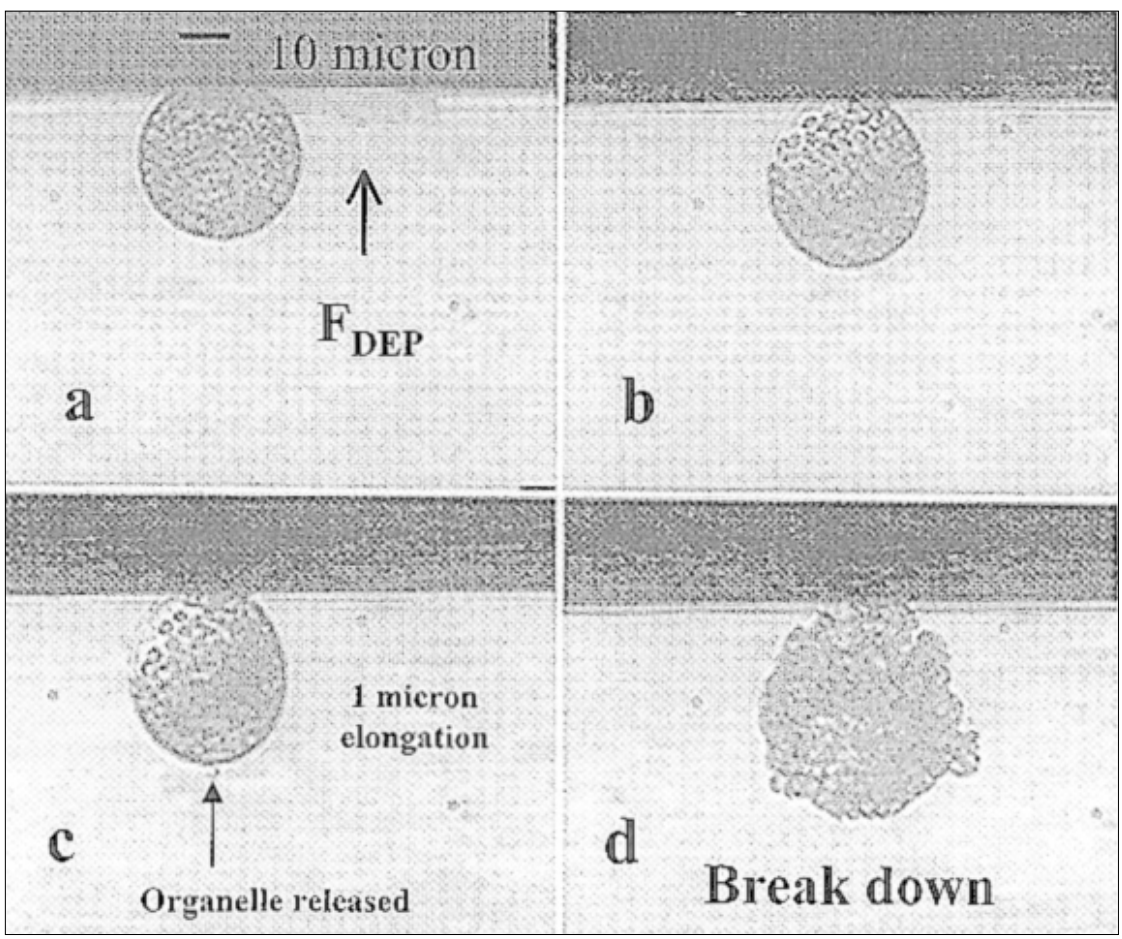

Fig. 6 Showing four step changes introducing to a *Lilium longoflorum* protoplast:
(a) F_{DEP} attracts the protoplast to an electrode,
(b) the protoplast is slightly elongated under 25.4 $kV.m^{-1}$ at 200 kHz,
(c) a release of some internal organelle when the field is increased to 85 $kV.m^{-1}$, and
(d) back to 25.4 $kV.m^{-1}$, membrane break down occurs at 100 kHz.

mF.m^{-2} or 20 mF.m^{-2} were reported in protoplasts of *Canola* leaves[3] and in infected kidney fibroblasts[11], respectively. Compared to yeast[6], the value reported for plankton cells are reasonable. On Maxwell-Wagner dielectric dispersion, Bonincontro et al[9] worked it out theoretically and suggested that the extra thickness of membrane, known as Debye's screening radius, could possibly occur due to cell volume polarization. If this is the case, the rather large membrane thickness of plankton cells, up to 50 nm, could be due to the accumulation of diffused counter ions towards the membrane surface, particularly under low σ_s.

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