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Electromagnetic characterization of biological cells

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Abstract — This paper presents the most commonly used method to characterize individual biological cells on a dielectric point of view. It is a force based technique which lays on dielectrophoresis and/or electrorotation. First the principle of these phenomena are described and analyzed with an extension to magnetic forces at the micrometric scale level. Secondly we present an experimental setup which permits to acquire the dielectrophoretic spectrum which is a dielectric signature of a cell. The main dielectric parameters can be deduced by fitting the theoretical response of the cell issued from a dielectric model and the experimental data. At the end we present an improved fitting method which takes advantage of a sensitivity analysis based on a probabilistic approach.

I. INTRODUCTION

The effects of electromagnetic fields on the human being has become a big concern for the public opinion who suspect most often some bad consequences on the health. This dark side is brought to the fore while some positive aspects of these interactions are kept hidden. Besides medical imagery which is commonly used and accepted by people, electromagnetic fields can be an instrument of sophisticated technologies for clinical or biotechnological applications.

This point can be illustrated by the phenomenon called electropermeabilization (often called electroporation). Indeed the permeability of the cell membrane towards molecules can be transiently increased when a micro or millisecond external electric field pulse is applied on a cell. Under suitable conditions depending mainly on the pulse parameters, the viability of the cell can be preserved. It is an elegant way to gain access to the cytoplasm and to introduce chosen foreign molecules, without irreversibly damaging the cell. For example, the microbiologists commonly use the electropermeabilization phenomenon in order to perform gene transfers into bacteria by introducing plasmid into the cytoplasm. Now this method is also proposed as an efficient way for drug, oligonucleotides, antibodies and plasmids delivery *in vivo*. Thus if the microbiologists use electropermeabilization in a rough way where only the efficiency of the gene transfer is taken into account without any consideration to the rate of lethal effect, it is crucial to monitor and characterize the effect of an applied transient electric field when considering medicinal or veterinary *in vivo* applications.

Owing to the fact that the permeabilization of cells takes place only where the local field reaches a critical value through the membrane, we are facing a key question: how can we locally control the field, and what happens on both membrane and cellular scales, when an electromagnetic field is applied at the tissue level?

To answer this question, we have to face a large range of sub-questions, some of which being:

- the dielectric characteristic of tissues at the macroscopic level,
- a relevant model of a cell with regard to the problem,
- the dielectric characteristics of the different part of a cell.

The problems mentioned in the non-exhaustive list above are strongly connected and each of them is a scientific issue for researchers involved in the study of the interaction between electromagnetic fields and living matter. Among these issues, the understanding and the control of this interaction at the cell scale can be seen as the basic requirement to understand also what happens at a higher scale.

This paper is mainly focused on one aspect of the electric field interaction with living cell. It concerns a method which can be implemented to extract the dielectric parameters of a single cell.

II. THE CELL

A. Basis

Cells are the elementary units of living creatures. They can be classified into two main categories:

- prokaryotic cells which have no nucleus containing the genetic material. Their size is in the range of a few micrometers. Bacteria belong to this category.
- eukaryotic cells which have a nucleus. They are typically 10 times larger than prokaryotic cells. Tissues, organs of animals, fungi and plants are built with this kind of cell.

B. Structure

Of course the different cell species can present a large variety of shape and structure but these structures have always two common points: the cytoplasm and the membrane.

- The definition of the cytoplasm is slightly different between eukaryotic and prokaryotic cell but it does not matter in the present context. The cytoplasm is a jelly-like substance where water represents up to 70% of the cell mass. It contains the organelles and the nucleus in the case of an eukaryotic cell (Fig. 1).

- The membrane is the external envelop of the cell surrounding the cytoplasm. It acts as a selectively permeable barrier, keeping foreign entities out of the cell and its contents inside the cell (the cytoplasm), allowing in the same time selected materials to pass in and out of a cell. It is a structure consisting mainly of phospholipid molecules

organized in a double layer, and proteins which control ion exchanges through the membrane. This membrane is thin compared with the size of the cell. Its thickness is in the 2-9 nanometers range (Fig. 2). This contrast in term of size is a well known problem in numerical modeling [1].

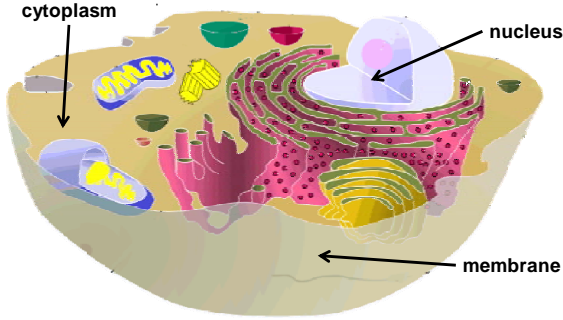


Fig 1. Schematic of a typical animal cell 1 nucleus; 2 cytoplasm; 3 membrane

From http://en.wikipedia.org/wiki/File:Biological_cell.svg

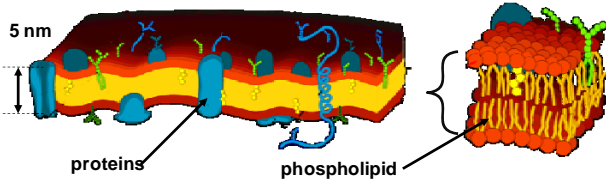


Fig. 2. Schematic of the membrane structure. From http://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_3.svg

C. Magnitude of electromagnetic characteristics

Most of biological cells are diamagnetic with a volume magnetic susceptibility χ which corresponds more or less to the value of water near -10^{-5} ($\chi = \mu_r - 1$ where μ_r is the relative permeability). If the red blood cells have a higher susceptibility value ($-6.5 \cdot 10^{-6}$) due to the small amount of iron that they contain, there are also cells which have intrinsic magnetization. For instance *Magnetospirillum Magnetactitum* is a bacterial specie which synthesizes chains of magnetic nanoparticles in the cytoplasm. The level of magnetization is sufficient to make the bacteria sensitive to the Earth's magnetic field.

Concerning cell dielectric properties, the description by a constant permeability and a constant conductivity is relevant in the range from 1000 Hz to 10^7 Hz [2]. The two main domains (cytoplasm and membrane) of a cell present a high contrast (Table 1) and play the most significant roles in the global dielectric behavior of the cell. It is the reason why attention is paid to these two regions in terms of modeling in a first approach. Indeed even if the volume of the membrane is negligible compared to the total volume of the cell, this latter has a great influence on the dielectric behavior.

TABLE I
DIELECTRIC CHARACTERISTICS

Cell type	cytoplasm		membrane	
	$\epsilon_{r_{\text{cyt}}}$	σ_{cyt} (S.m ⁻¹)	$\epsilon_{r_{\text{men}}}$	σ_{men} (S.m ⁻¹)
Red blood cell	59	0.3	4.4	$< 10^{-6}$
Jurkat	45	0.4	6	$3 \cdot 10^{-6}$

The almost high values of relative permittivity $\epsilon_{r_{\text{cyt}}}$ and conductivity σ_{cyt} in the cytoplasm are related to the content in water and the presence of ionic species.

III. INFLUENCE OF ELECTROMAGNETIC FIELDS ON A CELL.

As we want to characterize individual cell, we have to measure effects due to electromagnetic fields at the scale of the cell. This fact implies the use of micro technology. The characterization methods which will be presented below are based on the production of forces on a cell when it is submitted to a magnetic or electric field.

A. Magnetophoresis

A particle of volume V_p and of susceptibility χ_p immersed in a medium of susceptibility χ_m is submitted to a force given by the expression [3]:

$$\vec{F} = \frac{(\chi_p - \chi_m)}{2\mu_0} V_p \vec{\nabla} B^2 \quad (1)$$

where B is the modulus of the induction field vector and μ_0 the permeability of vacuum ($4\pi \cdot 10^{-7}$ H.m⁻¹).

The difference between the susceptibilities ($\Delta\chi = \chi_p - \chi_m$) can be positive or negative:

- if $\Delta\chi$ is positive (positive magnetophoresis), the particle is attracted towards the maximum field region.
- if $\Delta\chi$ is negative (negative magnetophoresis), the particle is attracted towards the minimal field region,

Equation (1) points out also that (i) the force is independent of the direction of the induction B, (ii) the gradient of B is more important than the magnitude of B. This last point is fundamental in the context of the cell. Indeed it is usually difficult to expect significant value of a force with diamagnetic material. At the micrometric scale, high values of field gradients can be obtained with a permanent micromagnet array. We can get an order of magnitude of the force on the basis of the following elements ($\Delta\chi = 10^{-6}$; $B_{\text{max}} = 0.25$ T; $B_{\text{min}} = 0$ T; typical length $l = 10 \mu\text{m}$): $F \approx 10^4 V_p$ (SI units).

By assuming that the main constituent of the particle is water, the value above can be compared to the weight which is in the same range: $P \approx 10^4 V_p$ (SI units).

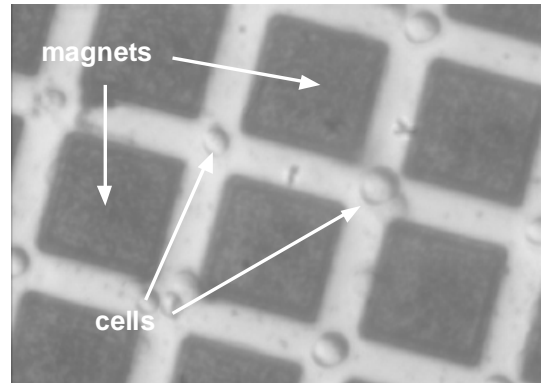


Fig. 3. Yeast cell submitted to negative magnetophoresis. Matrix of square magnets (CoPt plots, thickness : 8 μm , side length: 25 μm) separated by 10 μm gaps). Suspension medium: Aqueous solution containing 20 mM Gd-DTPA [4].

The susceptibility contrast can be also increased by adding paramagnetic ions (Gd-DTPA: Gadolinium-diethylenetriaminepenta-acetic acid is a paramagnetic complex used as a MRI contrast agent). The susceptibility of the medium can be measured thanks to a magnetic balance and thus we have a method to evaluate indirectly the susceptibility of a particle (cell) by measuring the velocity of the particle.

Nevertheless it must be noticed that the micromagnet array presented Fig.3 is not well suited for the measurement of the susceptibility and is more likely to be used in cell positioning applications. The technique of cell and particle tracking velocimetry for cell magnetization measurement was carefully described by Moore[5] and Häfeli [6].

The use of permanent magnets instead of coils and currents takes advantage of there is no heating of the medium and the cell. Nevertheless, in this case the only free parameter is the susceptibility (χ_m) of the medium.

B. Dielectrophoresis (DEP)

We consider a spherical particle (radius R, permittivity ϵ_p and conductivity σ_p) immersed in a medium (permittivity ϵ_m and conductivity σ_m) and exposed to a non-uniform electric field E. It is submitted to a force given by the expression [7]:

$$\vec{F} = 2\pi \epsilon_m R^3 \text{Re}[K(\omega)] \vec{\nabla} E_{rms}^2 \quad (2)$$

where K is the Clausius-Mosotti factor which depends on the complex permittivities and is expressed as:

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (3)$$

with

$$\epsilon^*(\omega) = \epsilon - j \frac{\sigma}{\omega} \quad (4)$$

where ω is the pulsation of the electric field.

Equation (1) is similar to (2) and the main remarks which have been pointed out for magnetophoresis are still pertinent:

- insensitivity of the force to the direction of the electric field.
- the field gradient is the main parameter.

We can also define (Fig. 4):

- positive dielectrophoresis when $\text{Re}[K(\omega)]$ is positive. The particle is attracted toward regions with high field values. In this case the global polarization of the particle has more or less the same direction as the electric field.

- negative dielectrophoresis when $\text{Re}[K(\omega)]$ is negative. The force is directed towards low field region and the direction of the particle polarization is opposite to the field.

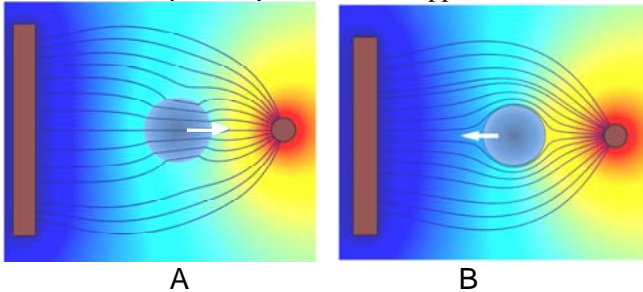


Fig 4. Positive (A) and negative (B) dielectrophoresis. The arrows indicate the direction of the force.

Practically, high gradient value of E can be produced by rectangular interdigitated electrodes (Fig. 5). It must be noticed that electric field values up to 10^5 V.m^{-1} can be obtained easily with an applied voltage of 10 volts and a gap in the range of $100 \mu\text{m}$. AC operation, which is possible due to the dependence of the force on the square of the field modulus, avoid electrolysis of the medium and

electrophoretic effects.

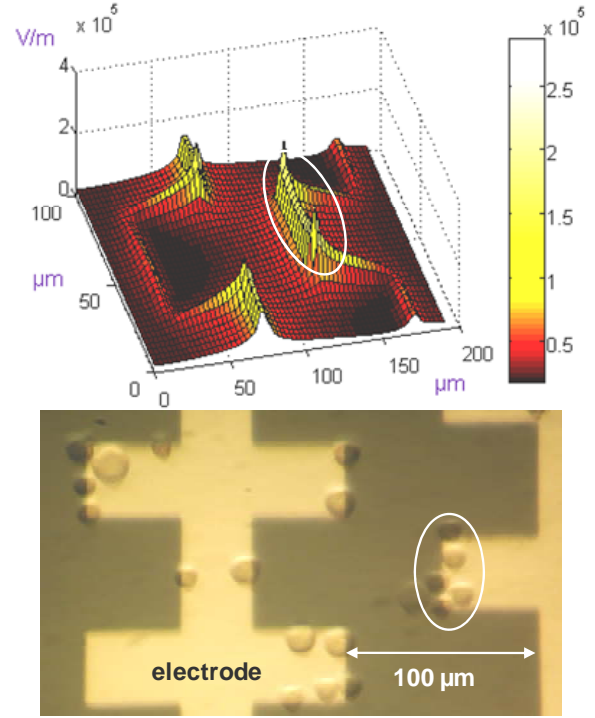


Fig. 5. Modulus of the electric field for one motif of the electrodes (up) and positive dielectrophoresis for Jurkat cells (down). High field region is circled.

C. Electrorotation (ROT)

Besides dielectrophoresis described above which is more precisely named conventional dielectrophoresis (c-DEP), another polarization effect, called electro-rotation can be obtained in a uniform rotating electric field.

When a rotating field with a constant modulus is applied to a particle, this latter is submitted to a torque which is given by the following expression [8]:

$$\vec{\Gamma} = -4\pi \epsilon_m R^3 \text{Im}[K(\omega)] E^2 \quad (5)$$

where ω is the rotation speed of the field.

The expression of the torque shows that electro-rotation only exists if at least one of the two materials (medium or cell) presents energy losses. The sign of the torque can be positive (co-ROT) or negative (anti-ROT) with reference to the rotation of the electric field.

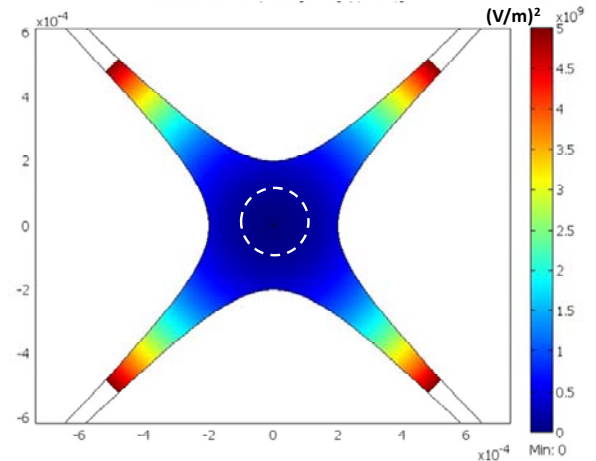


Fig. 6. Map of the square of the field modulus for polynomial electrodes.

The rotation field can be produced by a system of four electrodes fed with an equilibrated quadri-phase voltage.

The field map on Fig. 6 shows that the modulus is almost constant around the center of the electrode system (white circle).

This system also permits to perform c-DEP in the region between two electrodes by simply changing the feeding mode of the electrodes: for example, top and down electrodes to + V and left and right electrodes to -V (Fig. 7).

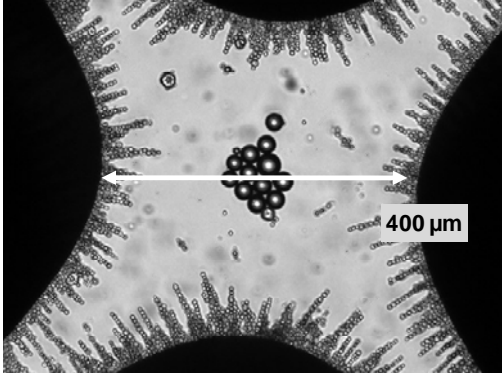


Fig. 7. Experiment of C-DEP in polynomial electrodes.

In Fig. 7 we see latex particles ($R = 12 \mu\text{m}$) submitted to a negative dielectrophoresis and which are grouped in the low field domain in the center of the electrodes while yeast cells ($R = 3 \mu\text{m}$) are attracted in the high field regions.

IV. CHARACTERIZATION METHOD USING C-DEP/ROT

A. Principle

As we stated before, a biological cell can be reasonably described by the dielectric properties of the two main domains (cytoplasm and membrane). From (4) it can be deduced that each of these domains has a transition frequency f_t defined by:

$$f_t = \frac{\sigma}{2\pi\epsilon} \quad (6)$$

Above f_t these material can be considered as an insulating medium while below this frequency the medium is mainly conductive. Considering the values given above in table I, the transition frequency of the membrane is much lower than the one of the cytoplasm. This fact has two consequences. On a fundamental point of view, the Maxwell-Wagner interfacial polarization is important which allows getting high value of the dipolar moment and then facilitates the creation of efficient forces. On a practical point of view, the behavior of the global cell varies with the frequency of the applied field.

To link the dielectric property of a biological cell to the expression of the force and the torque established for a homogeneous particle, we need to use a model.

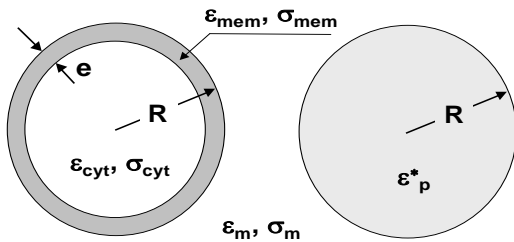


Fig. 8. Dielectric model of a single cell.

The simplified model of a biological cell presented on Fig. 8 (left) is equivalent to a homogeneous sphere with a

complex permittivity [9].

$$\epsilon_p^* = \frac{R \epsilon_{men}^* \epsilon_{cyt}^*}{R \epsilon_{men}^* + e \epsilon_{cyt}^*} \quad (7)$$

At low frequency the dielectric property are governed by the membrane ($\epsilon_p^* \approx \frac{R}{e} \epsilon_{men}^*$) while at high frequency the cytoplasm is dominant ($\epsilon_p^* \approx \epsilon_{cyt}^*$). It must be noticed that with the usual range of the ratio R/e (R/e about 1000), the apparent relative permittivity can be very high (several thousands) which renders a high polarization level of the cell as mentioned above.

The theoretical dielectrophoretic spectrum which corresponds to the variation of the Clausius-Mosotti factor $K(\omega)$ versus the frequency can be evaluated.

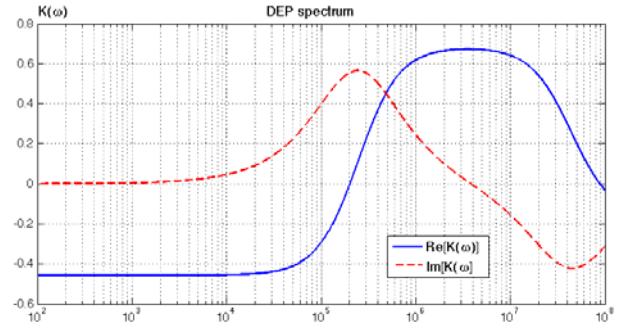


Fig. 9. Theoretical dielectrophoresis spectrum of a Jurkat cell, real and imaginary part. See Table I for the dielectric properties of the cell; $\sigma_m = 50 \text{ mS.m}^{-1}$ and $\epsilon_m = 80 \epsilon_0$ for the medium. $R = 5 \mu\text{m}$, $e = 5 \text{ nm}$.

The spectrum drawn on Fig. 9 shows that different types of behaviors can be obtained by varying the frequency. The ROT spectrum exhibits both anti-field and co-field resonances. At low frequency we have negative c-DEP and anti-ROT then above 200 kHz positive c-DEP and finally above 4 MHz co-ROT. The shape of this spectrum depends obviously of the dielectric parameter σ_{cyt} , ϵ_{cyt} , σ_{men} , ϵ_{men} and the geometrical parameters e , R . Inversely these parameters can be deduced from the experimental acquisition of the dielectrophoresis spectrum. It must not be forgotten that the spectrum is also dependent of the dielectric characteristics of the medium. These characteristics are an adjustment parameter in the range where the media stay compatible with cell viability.

This spectrum can be obtained though measurement of the speed of a cell in c-DEP and/or ROT experiment. Assuming that the viscosity of the medium is η

$$\text{Re}(K(\omega)) = \frac{3\eta v(\omega)}{\epsilon_m R^2 |\nabla E_{rms}^2|} \quad (8)$$

$$\text{Im}(K(\omega)) = -\frac{2\eta \Omega(\omega)}{\epsilon_m E^2} \quad (9)$$

where $v(\omega)$ and $\Omega(\omega)$ are respectively the measured velocity and the measured rotation speed.

B. Experimental setup

We have developed a platform to acquire the dielectrophoretic spectrum.

The microelectrode structure used in the c-DEP or ROT experiments is composed of four polynomial electrodes

disposed in a circular arrangement (Fig. 10).

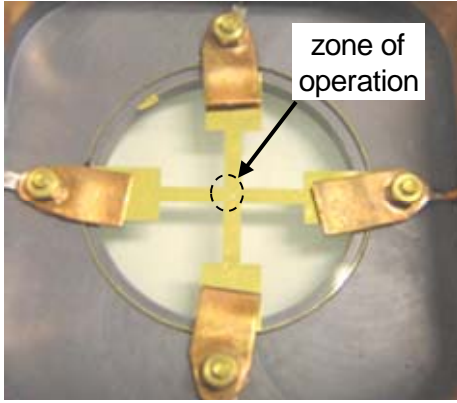


Fig. 10. Photo of the microsystem.

Those electrodes are powered by 4 generators which can deliver sine-wave voltages from a few Hz to 80 MHz with appropriate phases. Visualization of the applied voltage is achieved thanks to a wide band oscilloscope whose input impedance can be set to 50 Ohms for impedance matching. Cell motion is observed under an inverted microscope and image acquisition is performed by a high speed camera.

These different functions are PC-controlled via GPIB interfaces (Fig. 11). This permits in particular to maintain constant the applied voltage over the whole frequency range, despite the variations of the micro device impedance.

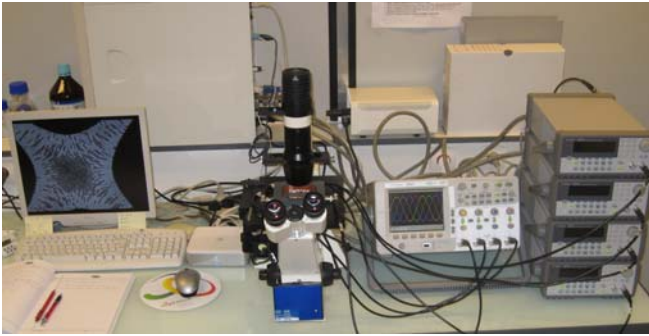


Fig. 11. Platform for dielectrophoretic spectrum acquisition.

The acquisition of a dielectrophoretic spectrum by measuring the speeds with a stopwatch is a tedious work. We have developed a software that analyzes the video performed with the high speed camera to extract automatically the speeds.

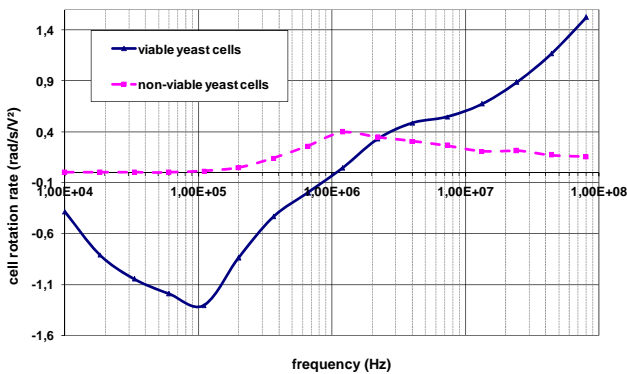


Fig. 12: Experimental ROT spectrum for yeast cell (*Saccharomyces cerevisiae*). Medium: $\epsilon_m = 80$, $\sigma_m = 1.1 \text{ mS.m}^{-1}$.

Fig. 12 presents the variation of the rotation velocity for viable and non viable cells.

C. Extraction of the parameters

Attention is more focused on the ROT spectrum the shape of which is complex. Thus this spectrum can give more information on the dielectric parameters than the c-DEP spectrum.

On a practical point of view, the rotation velocity is measured in a frequency range wide enough to take into account the interesting zones of the ROT spectrum: the two resonances and the transition frequency.

We are facing a problem with 8 parameters: σ_{cyt} , ϵ_{cyt} , σ_{men} , ϵ_{men} , e , R , E and η . The typical dimension R of the cell can be acquired by direct visualization (microscopy). Even if the electric field E can be measured, the evaluation of the viscosity η requires dedicated apparatus. Generally people work directly on the $\Omega(\omega)$ curve which is assimilated to the dielectrophoretic spectrum by misuse of language.

$$\Omega(\omega) = -\beta_{\text{ROT}} \epsilon_m \text{Im}(K(\omega)) \quad (10)$$

where β_{ROT} integrates the dependence for E and η . This term has no direct interest for the dielectric model but has a direct influence on the measured values $\Omega_{\text{exp}}(\omega)$.

Equation (7) shows also that σ_{men} , ϵ_{men} and e cannot be determined separately but only the ratio σ_{men}/e and ϵ_{men}/e .

Finally the mathematic problem consists in extracting 5 parameters: σ_{cyt} , ϵ_{cyt} , σ_{men}/e , ϵ_{men}/e , and β_{ROT} . The basic method to achieve this goal has been proposed in [10]. The experimental ROT spectrum Ω_{exp} is fitted with a simulated one Ω_{sim} calculated thanks to (9). This is done by minimizing the distance between experimental and simulated data at the different frequency points f_i (pulsation $\omega_i = 2\pi f_i$). This discrete least-square minimization problem can be summarized as follows:

$$\min \left\{ \sum_i \left| \Omega_{\text{exp}}(\omega_i) - \Omega_{\text{sim}}(\omega_i) \right|^2 \right\} \quad (11)$$

with each parameter included in their respective prospective range of values.

In the work of Gascogne and al.[10] the minimization procedure was achieved by using the Nelder-Mead simplex optimization method. Nevertheless tests have given evidence that the reliability of this method is somewhat weak.

We have proposed an improvement of this method by [11]:

- weighting the summation terms in (11) by an appropriate frequency-dependent coefficient α_i .

$$\min \left\{ \sum_i \alpha_i \left| \Omega_{\text{exp}}(\omega_i) - \Omega_{\text{sim}}(\omega_i) \right|^2 \right\} \quad (12)$$

- applying gradually the minimization process to the different parameters, using for each of them the suitable weighted spectrum instead of extracting all the parameter in one single step.

The underlying idea of this improved approach is that each parameter has an influence on the spectrum which varies with frequency as we have mentioned several time before. To evaluate this influence we have performed a sensitivity analysis (variance based) in order to ascertain how much the ROT spectrum depends on the different

parameters.

Fig. 13 and Fig. 14 illustrate this point. Fig. 13 represents the dispersion of the spectrum ($\Omega_i = \Omega(\omega_i)$) when parameters vary in their physical intervals. We observe that the dispersion is more important around the peak and that the position of the peaks is not sensitive.

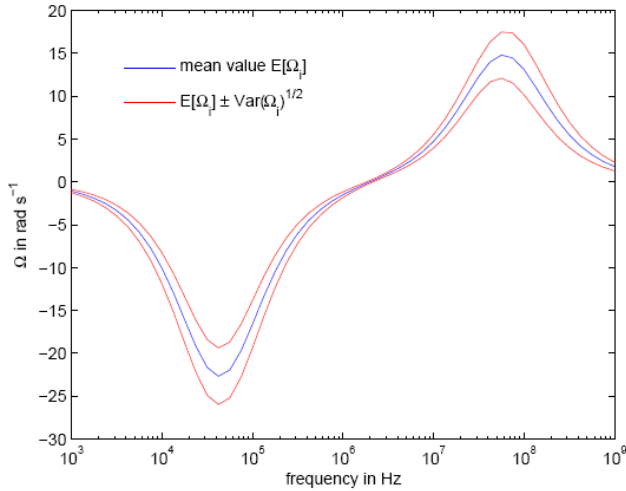


Fig. 13. Mean value of the spectrum and its dispersion (standard deviation) $\sigma_{\text{cyt}} \in [0.6, 0.9] \text{ S.m}^{-1}$, $\epsilon_{\text{rcyt}} \in [60, 140]$, $\sigma_{\text{mem}}/e \in [800, 1000] \text{ S.m}^{-2}$, $\epsilon_{\text{mem}} \in [20, 30] \text{ F.m}^{-2}$, $\beta_{\text{ROT}} \in [30, 50] \text{ rad.s}^{-1}$, $R = 15 \mu\text{m}$, $\epsilon_{\text{m}} = 80$, $\sigma_{\text{m}} = 50 \text{ mS.m}^{-1}$.

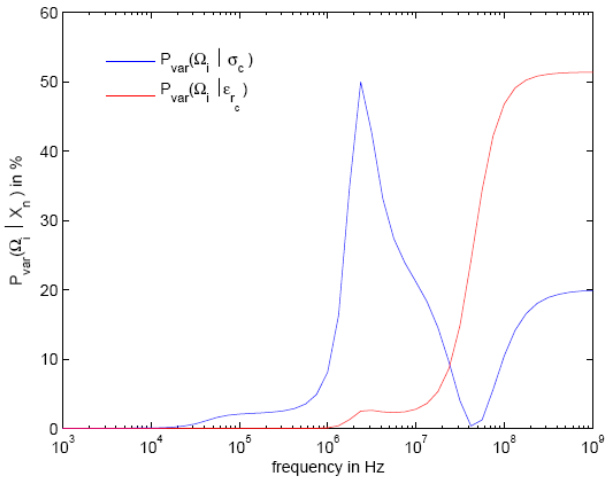


Fig. 14. Relative partial variance $P_{\text{var}}(\Omega_i | \sigma_c)$ and $P_{\text{var}}(\Omega_i | \epsilon_{\text{rc}})$ due to σ_{cyt} and ϵ_{rcyt} . (Partial variance divided by the variance at the considered frequency)

Fig. 14 shows that as expected the dielectric properties of the cytoplasm have an impact mainly in the high frequency range and how much σ_{cyt} and ϵ_{rcyt} impact the ROT spectrum. When the extraction of one of the parameters is under consideration, the relative partial variance of this parameter is a good candidate to be the weight term α_i in (12).

The parameters are extracted in 4 successive steps with regards to the partial variances where they are involved.

In order to evaluate the efficiency of this approach a numerical experiment has been developed. From (10) 200 samples of spectra have been built choosing randomly the parameters in the bounded interval (Fig. 13). The parameters are then extracted using the weighted and the unweighted minimization. It appears that the weighted minimization gives more accurate results. For example the value of ϵ_{rcyt} has been recovered with a confidence limit around 8% (weighted) and 15% (unweighted).

V. CONCLUSION

The characterization of biological cells is still a domain in development which needs the implementation of sophisticated experimental techniques associated to judicious numerical methods than can be still widely improved.

As we stated in the introduction a better knowledge of the behavior of a cell is one way (but not the only one) to understand what happens at the tissue level. But now we have the possibility to go deeper in the dimension scale. Indeed simulations in molecular dynamics permit to study what happens at the membrane level when this one is submitted to an electric constraint. Among all the information we can get from these simulations, the permittivity and the conductivity of the membrane can be extracted.

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