AUTOMATED CELL CHARACTERIZATION PLATFORM: APPLICATION TO YEAST PROTOPLAST STUDY BY ELECTROROTATION

J. Laforêt[1], M. Frénéa-Robin[2], H. Cérémonie[1], F. Buret[1] and L. Nicolas[1]
[1]*AMPERE, UMR CNRS 5005, Ecole Centrale de Lyon, Ecully, France*

[2]*Université de Lyon, Lyon, F-69622, France ; AMPERE Villeurbanne, France*

Keywords: Dielectrophoresis, electrorotation, yeast cells, yeast protoplasts.

Abstract: This paper is about the development of a new automated platform dedicated to cell manipulation and characterization by dielectrophoretic methods. We illustrate its possibilities by studying yeast protoplasts and yeast cells electrorotation spectra, obtained using polynomial microelectrode structures powered by computer-controlled generators. Measurements were made over the frequency range 100 kHz to 80MHz, mostly in a suspending medium of conductivity 50 mS/m inside the rotation chamber. The rotation rate of yeast protoplasts was inferior to that of whole yeast cells. To understand such behavioral differences, yeast protoplasts were modelled as single-shell spheres in a first approach.

1 INTRODUCTION

The term dielectrophoresis (DEP) is used to describe the motion and orientation induced by a non-uniform electric field on polarizable particles, such as cells. In conventional-DEP (c-DEP), stationary fields of inhomogeneous strength are used to translate cells toward field minima or maxima. Electrorotation (ROT) relies on non-uniformities in the phase distribution of the applied field to induce cell rotation at constant velocities.

The effects of chemicals and environmental factors on the cell electric properties are more and more adressed. ROT technique enables the study of various organisms individually without physiological damage and cell characterization by angular velocity measurement. This type of microelectrodes system allows cell handling (separation, selection, electrofusion…) and transport and may be basic components to be integrated into lab-on-a-chips.

Yeasts are eukaryotic cells widely used as model organism in cell biology, mainly because they are quick and easy to grow. Preparation and regeneration of yeast protoplasts are important in fusion, transformation and cloning studies (Kofod and al., 1998). Protoplast fusion can be used to improve anti-bacterial and anti-fungi characteristics of bakery yeast. In this study, spectra are analysed according to a two-shell spherical model for whole yeast cells and single-shell for protoplasts in a first approach.

2 THEORY

2.1 Yeast Cell Model

In this paper, whole yeast cells are modelled by a two-shell spherical model. Cytoplasm, membrane and cell wall are considered as concentric spheres, according to the individual yeast cell model developed by Falokun (Falokun and al., 2006). The complex permittivity of cell interior and membrane are denoted ε_0^*, and ε_1^*.To replace the "smeared out" sphere, we used:

$$\varepsilon_{1eff}^* = \varepsilon_1^* \frac{\left(\frac{R_1}{R_0}\right)^3 + 2\left(\frac{\varepsilon_0^* - \varepsilon_1^*}{\varepsilon_0^* + 2\varepsilon_1^*}\right)}{\left(\frac{R_1}{R_0}\right)^3 - \left(\frac{\varepsilon_0^* - \varepsilon_1^*}{\varepsilon_0^* + 2\varepsilon_1^*}\right)} \qquad (1)$$

where R_i is the radius of the shell index i.

Laforêt J., Frénéa-Robin M., Cérémonie H., Buret F. and Nicolas L. (2008).
AUTOMATED CELL CHARACTERIZATION PLATFORM: APPLICATION TO YEAST PROTOPLAST STUDY BY ELECTROROTATION.
In *Proceedings of the First International Conference on Biomedical Electronics and Devices*, pages 190-193
DOI: 10.5220/0001053401900193
Copyright © SciTePress

Then, the complex permittivity of the equivalent homogeneous cell can be expressed as:

$$\varepsilon_p^* = \varepsilon_2^* \frac{\left(\frac{R_2}{R_1}\right)^3 + 2\left(\frac{\varepsilon_{1eff}^* - \varepsilon_2^*}{\varepsilon_{1eff}^* + 2\varepsilon_2^*}\right)}{\left(\frac{R_2}{R_1}\right)^3 - \left(\frac{\varepsilon_{1eff}^* - \varepsilon_2^*}{\varepsilon_{1eff}^* + 2\varepsilon_2^*}\right)} \quad (2)$$

Relative permittivity and conductivity of the suspension medium used in the experiments are 78 and 50 $mS.m^{-1}$. Yeast cells average electrical and geometrical parameters are stored below (Table 1).

Table 1: Properties of different cellular compartments of yeast cells (Falokun and al, 2006 & Zhou and al, 1996).

	Radius or thickness	Conductivity	Permittivity
0: cytoplasm	5 µm	2 $S.m^{-1}$	50
1: membrane	8 nm	9.10^{-6} $S.m^{-1}$	6
2: wall	150 nm	6.10^{-2} $S.m^{-1}$	60

2.2 Electrorotation Theory

The structure and properties of biological cells can be investigated by observing their ROT spectra (Gascoyne and al., 2004). Indeed, the rotation velocity of a spherical particle submitted to a constant rotating electric field can be expressed as:

$$R(\omega) = -\frac{\varepsilon_m \mathrm{Im}[K(\omega)] E^2}{2\eta} \quad (3)$$

where η is the solution viscosity, E and ω are the magnitude and angular frequency of the applied field.

$K(\omega)$ is the Clausius-Mossoti factor (CMF), depending on the particle and its immersion medium complex permittivities ε_p^* and ε_m^*:

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (4)$$

The value of ε_p^* varies according to cell type. Therefore, each cell type is characterized by a particular ROT spectrum (as Figures 3 and 4). When $\mathrm{Im}[K(\omega)] > 0$ (angle of the induced dipolar moment with respect to the electric field vector comprised between 0 and 180°), cells exhibit anti-field rotation ($R(\omega) < 0$). On the contrary case, cells share the same rotation sense as the field ($R(\omega) > 0$).

3 MATERIALS AND METHODS

3.1 Experimental Setup

The microelectrode structure used in the DEP and ROT experiments is composed of 4 polynomial electrodes (Au-Ti deposited on glass) disposed in a circular arrangement. Those electrodes are powered by 4 generators delivering sine-wave voltages up to 80 MHz. We simply switch from c-DEP to ROT according to the phase configuration of the 4 signals (Figure 1). An advantage is that cells may be concentrated at the centre of the s by negative DEP before a ROT experiment. Indeed, all the cells situated in this area will experiment the same constant rotating field when undergoing ROT. Only these cells must be taken into account in rotation measurements. In this constant field area (Hughes, 1998), cell translation is reduced during the course of a measurement.

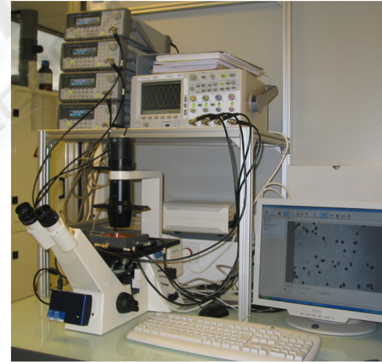

Figure 1: Experimental setup.

Visualization of the applied voltage and impedance matching are achieved thanks to a wide band oscilloscope, whose input impedance can be set to 50 Ohms. All these equipments are controlled by PC through GPIB interface using a software developed under LabView® which enables synchronized signal generation. Voltages are kept constants over the whole frequency range thanks to automatic gain control.

Cell motion is observed under an inverted microscope. Image sequences are captured by a high speed camera. Velocity depends on the electric field frequency and on the dielectric properties of the cell and its surrounding medium. Frequency-dependent rotation rates were first measured with a stopwatch

and then confirmed with a software under development. After an image processing in order to detect and label each isolated cell, the angular velocity is calculated with Matlab® by determining the orientation (angle between its main axis and horizontal axis) in each sequence of images.

3.2 Cell Preparation

Before experiments, the system was rinsed with distilled water, washed with ethanol and dried with an air jet. The samples were centrifuged and the cells were washed 2 times with a solution whose conductivity was adjusted to 50 $mS.m^{-1}$ by addition of KCl and directly measured with a conductivity meter. For protoplasts only, glucose was added to the solution (at a concentration of 30mM) to adjust the osmolarity.

Prior to experiments, a drop of cell suspension (60µL) was deposited onto the electrode system (gap: 400 µm), in a chamber fabricated with a self-adhesive silicone bond. Then, a lid was used to close it and prevent fluid circulation caused by evaporation.

3.3 Protoplast Forming

Yeast cells (*Saccharomyces Cerevisiae*) were suspended and incubated at 35°C during ten minutes in a pre-treatment solution. After centrifugation, they were resuspended two times in a buffer solution which contained 4.7 $g.l^{-1}$ sodium citrate, 10.8 $g.l^{-1}$ potassium dihydrogenophosphate and 21.8 $g.l^{-1}$ sorbitol.

In a second time, they were centrifuged and resuspended in a 500 $U.ml^{-1}$ solution of lyticase enzymes with the buffer solution. Enzymes digested the yeast cell wall during an one hour incubation at room temperature to generate protoplasts.

4 RESULTS AND DISCUSSION

4.1 Simulated ROT Spectra

To obtain the general appearance of the whole yeast cell spectrum, we use the two-shell model with the Table 1. The rotation rate is proportional to the imaginary part of the CMF (3), plotted on Figure 2:

$$R(\omega) = -\chi \operatorname{Im}\left[K(\omega)\right] \textit{ where } \chi = \frac{\varepsilon_m E^2}{2\eta} \quad (5)$$

In the case of protoplasts, we used the single-shell model, only taking into account the cytoplasm and membrane properties (Table 1), in a first approach.

4.2 Experimental ROT Spectra

First, we have collected typical ROT spectra exhibited by single viable yeast cells by measuring the induced rotational velocities at a medium conductivity of 50 $mS.m^{-1}$ under a constant voltage of 3 V_{pp} (Figure 3). The average ROT spectrum is in agreement with previously reported data (Hölzel, 1997).

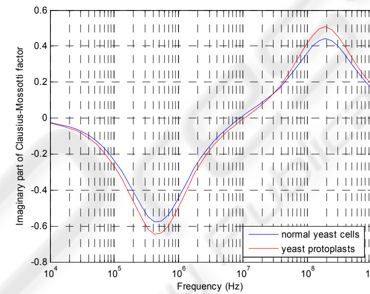

Figure 2: $-\operatorname{Im}\left[K(\omega)\right]$ at 50 $mS.m^{-1}$.

There are two ROT peaks of nearly the same amplitude. The rotation rate was calculated from about ten cells per point. Positive and negative values respectively indicate co-field and anti-field cell rotation. The results plotted Figures 3 and 4 were averaged across 4 experiments, vertical lines indicated amplitude between the minimal and maximal values.

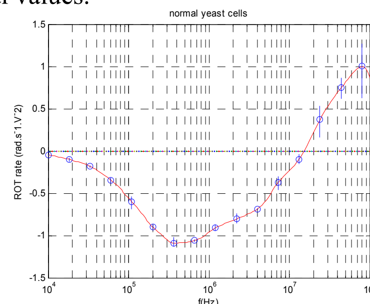

Figure 3: Experimental ROT spectra at 50 $mS.m^{-1}$.

Then, we have collected ROT spectra exhibited by yeast protoplasts for a conductivity of 50 $mS.m^{-1}$ under a 6 V_{pp} constant voltage (Figure 4). The zero crossing frequency was situated around 15 MHz and 11 MHz for normal yeast cells and protoplasts respectively.

The cells exhibited anti-field rotation at frequencies below the zero crossing and co-field rotation above.

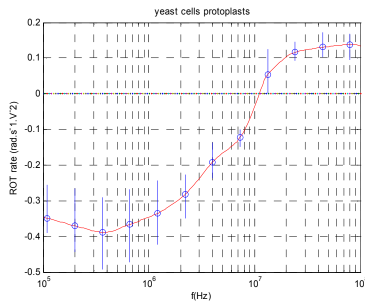

Figure 4: Experimental ROT spectra at 50 mS.m^{-1}.

4.3 Discussion

For whole yeast cells, the overall spectrum (Figure 3) is consistent with that obtained from simulation (Figure 2), up to a multiplicative scalar factor, which could be explained by the fact that the simulated data did not take into account the factor χ (5).

On both experimental spectra, the negative ROT peak happens near 500 kHz despite rotation rate attenuation for yeast protoplasts. The viscosity of the medium suspension, in the case of protoplasts, was almost 5% higher than water viscosity because of glucose (Easteal, 1989), which affects the value of χ. Nevertheless, more precise investigation is necessary to understand this rotation slowdown.

As can be seen from Table 1, the dielectric properties of cell wall are close to medium properties. This may explains the resemblance between yeast cells and yeast protoplast simulated spectra (Figure 2) obtained with our approach, consisting in switching from a model to another by suppressing the shell representing the cell wall.

The approach consisting in modelling protoplasts by the two most inside shells presents its limitations. Indeed, simulated result does not fit the experimental data well in the co-field rotation part of the spectra. For 1.1 mS.m^{-1}, simulation points out more differences between the two spectra (Figure 5).

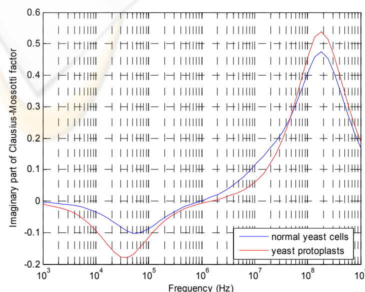

Figure 5: $-\mathrm{Im}\left[K(\omega)\right]$ at 1.1 mS.m^{-1}.

5 CONCLUSION

As further developments, we need to improve our experimental setup to cover a wider frequency range and obtain thereby a complete ROT spectrum, including the second peak. Future experiments performed in lower conductivity immersion media (1.1 mS.m^{-1}, for example) may bring more information about the cell wall influence (Figure 5).

Yeast protoplast and whole cell electric properties can be extracted from experimental ROT spectra by parameter identification thanks to a identification process under Matlab®. During this step, more sophisticated models could be used to describe cells, as for instance a N-shell ellipsoidal model. The measurement of cell properties is a step toward the modelling of electromagnetic field-tissue interaction using a bottom-up approach.

LabView® interface allows to realize several series of different experiments. Cell motion was successfully observed over a wide frequency range for yeast cells. Fabrication of microelectrodes enabling travelling-wave dielectrophoresis is the last part of our platform to be developed. Further, these cell manipulation techniques permit to study the effects of various treatments on cells such as response to toxicants for magnetic field exposure and to detect cell pathologies.

REFERENCES

Kofod, L.V., Andersen, L.N., Kauppinen, M.S., Christgau, N.S., Dalboge, H., 1998. Enzyme with P-1,6 endoglucanase activity, *United States Patent. Patent Number 5770406*.

Falokun, C.D., Markx, G.H., 2006. Electrorotation of beads of immobilized cells, *J. of Electrostatics*.

Zhou, X-F., Markx, G.H., Pethig, R., 1996. Effect of biocide concentration on electrorotation spectra of yeast cells, *Bioch. and Biophys. Acta*.

Gascoyne, P. R. C., Vykoukal, J. V., 2004. Dielectrophoresis-based sample handling in general-purpose programmable diagnostic instruments, *Proceedings of the IEEE*.

Hughes, M.P., 1998. Computer-aided analysis of conditions for optimizing pratical electrorotation, *Physics in Medecine and Biology*.

Hölzel, R., 1997. Electrorotation of Single Yeast Cells at Frequencies Between 100Hz and 1.6GHz, *Biophysical J.*

Easteal, A.J., 1989. Can. Tracer diffusion in aqueous sucrose and urea solutions, *J. Chem.*.