Measurement of Electrical Oscillations of Yeast Cells Membrane at Acoustic Frequencies

Michal Cifra[1,*], František Jelínek[1], Jiří Pokorný[1], Radek Janča[2], Jaroslav Šaroch[1], Jiří Hašek[3], Lenka Nováková[3]

[1]Institute of Photonics and Electronics, Academy of Sciences of the Czech Republic,
Prague, Czech Republic
Tel: +420-284680260; Fax: +420-284680222; E-mail: cifra@ufe.cz
[2]Department of Circuit Theory, Czech Technical University,
Prague, Czech Republic
Tel: +420-224351111; Fax: +420-224352016; E-mail: jancar1@fel.cvut.cz
[3]Institute of Microbiology, Academy of Sciences of the Czech Republic,
Prague, Czech Republic
Tel: +420-241062503; Fax: +420-241062501; E-mail: hasek@biomed.cas.cz

***Abstract*-Fröhlich postulated coherent polar oscillations as a fundamental biophysical property of biological systems. This paper describes the measurement of electric activity of yeast cells in the acoustic frequency range. It is a continuation of the article of the authors published in Electromagnetic Biology and Medicine in 2009. This paper describes briefly a development of a new point sensor and new analysis of the data obtained with previous sensor. The sensor with amplifiers fed by batteries are placed in an electromagnetically screened box. Detected signals of synchronized and nonsynchronized mutants of yeast cells were measured by a spectrum analyzer and stored in a computer memory. The power analysis of measured values was performed. Additional data mining by multi-layer neural network was designed. The evaluation of measured values showed that the electric activity of synchronized cells in the M phase is greater than of non synchronized cells.**

***Index Terms*- Detection of very weak signals, electromagnetic activity of cells, Fröhlich's hypothesis of coherence in biology.**

I. INTRODUCTION

Quasi-coherent electrodynamic activity of living systems have been predicted already more than 40 years ago by Fröhlich [1]. Theoretical mechanisms for generation of electrodynamic activity on cellular level have been studied, some proposals put cell skeleton, namely microtubules as main structures responsible for its generation [2]. Also various approaches for the measurement of electrodynamic activity on cellular level have been attempted [3]. This paper describes briefly a development of a new point sensor for measurement of cellular electrodynamic activity of yeasts and new neural network-based analysis of the data obtained with previous sensor.

II. MATERIALS AND METHODS

A. Yeast cells

We used β-tubulin mutant *tub2-401* of yeast cells *Saccharomyces cerevisiae* (strain CUY67 Mata *tub2-401 ura3-52 ade2-101*). The mutant is cultivated above the threshold of the permissive temperature (25°C) when microtubules are polymerized. The mutant cells are in different phases of the cell cycle (nonsynchronized cells). Evolution of the cells can be synchronized by decrease of temperature below the threshold of the restrictive temperature (14°C) when microtubules cannot be polymerized. The mutant cells continue in their cell cycle development up to the point before entering the M phase whose processes depend on microtubules. Thus, after a certain time period evolution of all the mutant cells is stopped at the same point. When the temperature increases above the threshold of the permissive temperature (25°C) microtubules are reassembled and the mitotic spindle is formed. Therefore, start of the M phase in the cells cultivated at the restrictive temperature is triggered by the temperature increase above 25°C. Thus, the cells are synchronized. Nonsynchronized yeast cells were measured too.

A part of the prepared yeast cell suspension was cultivated at the temperature of 30°C. The nonsynchronized cells were measured as the reference system. The cells were suspended in the aqueous sucrose solution.

B. Laboratory equipment

A schematic diagram of the measurement system is shown in Fig. 1. An important part of the equipment is the facilities with a cuvette, a point sensor, and transformation amplifier placed in a three-fold shielded box inside a temperature stabilized box. The suspension with yeast cells is inserted in the cuvette. The sensor is made up by a platinum wire whose tip is obliquely cut to form a submicrometer point, or probably (according to experiences with usage of this procedure in Atomic Force Microscopy tips) even much sharper (~ 50 nm) but such a small radius is not verifiable under optical microscope. With this sensor, which is the same as described in [3], it is possible to touch only one cell from above (Fig. 2.). But a large part of the platinum wire is immersed in the suspension with cells, so the whole cell suspension impedance contributes to the background noise. After the transformation amplifier another amplifier is inserted. The total power gain of both amplifiers is of about 110 dB at 1 kHz. Both amplifiers are supplied by a battery placed also in the electrically and magnetically screened box for minimizing spurious signals. A temperature stabilization unit keeps the temperature inside the box at about 28±0.5°C. The output of the amplifier is connected with a semirigid coaxial cable to the spectrum analyzer Agilent E4448A, which is computer controlled through GPIB interface. Sensitivity of the measurement system is of the order of magnitude of 10^{-18}-10^{-19} W, so almost at the level of thermal noise.

III. MEASUREMENT

Suspension with synchronized cells was cultivated at the temperature below 14°C. Suspension with nonsynchronized cells was cultivated at the temperature about 30°C. Before measurement the test tubes with the suspensions were put into a water bath at 28°C for 3 min. Optical density (OD 600) of such suspension was 4.5, which corresponds to concentration of about $2x10^8$ cells per milliliter. Afterwards, the carefully cleaned cuvette was filled with a given volume of suspension (approximately 0.06 ml). Measurement then immediately started.

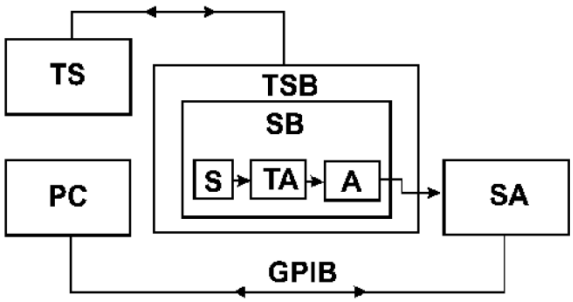


Fig.1. Schematic diagram of the laboratory equipment. SA-spectrum analyzer, TSB-temperature stabilized box, SB-threefold screened box, S-sensor, TA-transformation amplifier, A-amplifier, PC-computer, TS-temperature stabilizer

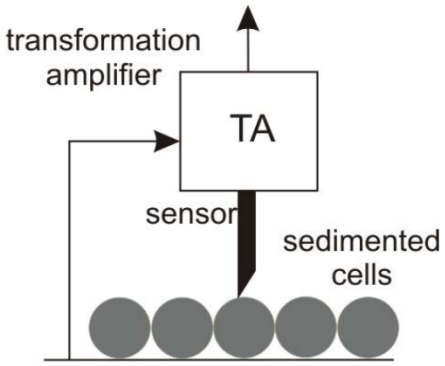


Fig.2. A schematic picture of point contact measurement of sedimented yeast cells

Electrical oscillations in the frequency range 400-1,600 Hz were measured by a spectrum analyzer. Reference level was -80 dBm, and the resolution (RBW) and the video (VBW) bandwidths were 1 Hz. The investigated frequency range (0.4-1.6 kHz) was examined by two sweeps, each of 600 Hz bandwidth. The total time of both sweep times and of the time for transfer of the trace data into the PC memory was 6 s. At least 400 double sweeps were measured (the total measured time was 40 min). The measured data stored in the PC memory were analyzed.

IV. DEVELOPMENT OF NEW SENSOR

We designed a new configuration of the point sensor in which the tip of the point sensor touches cells from below during the measurement, see Fig. 3. This configuration has great advantage in minimization of suspension impedance influence on the background noise signal. However, not all technical difficulties have been overcome yet and statistically useful results haven't been obtained yet due to insufficient number of measurements.

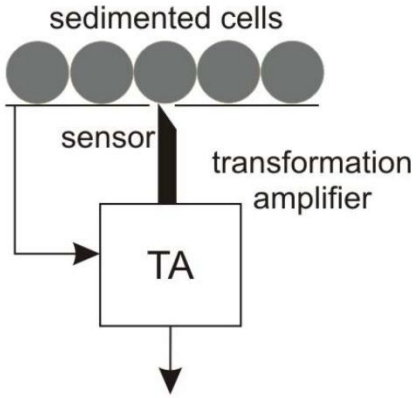


Fig.3. A schematic picture of point contact measurement of sedimented yeast cells. Point contact is realized through the hole in the ground surface electrode

V. DATA ANALYSES

The data obtained from the measurement described have a structure of spectral lines developing in time. For one set of the yeast cells 400 power spectra were obtained. The spectra have a noise character and they are superposed on an isoline that is caused by a nonideal transfer function of the amplifier. A shape of the isoline depends on the impedance of the measured suspension. The shape can be determined by polynomial approximation of degree 3 of mean spectra. After subtraction of the isoline from the spectra, the spectra are independent on frequency. For the analyses measured data of 23 synchronized and 26 nonsynchronized cell sets were used.

A. Power analysis

Pohl described a process of an increasing electrodynamic activity of yeasts in the metaphase and anaphase [4]. This increases the total spectral power. Fig. 4 shows the normalized total spectral power in time from all sets. The sets of the synchronized cells have about 7 % greater mean power then nonsynchronized cells. Between 5^{th} and 20^{th} minute the power is markedly increased. However, the growth of yeasts during the mitotic process changes the impedance of the measured suspense. The power of a thermal noise depends on the impedance. So the visible increase has not to be caused by electrodynamic process only.

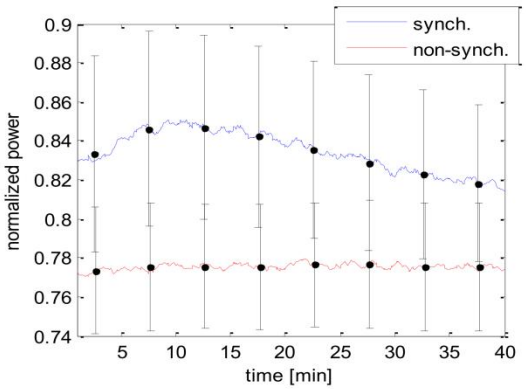


Fig. 4. Normalized spectral power of all sets versus time

B. Frequency analysis

As was described, the spectra have a noise character without high spectral lines. Standard methods didn't find significant difference between discrete frequencies. Therefore the method of data mining based on multi-layer neural network classifier (MLNN) was developed. The first approaches have been developed in [5]. The MLNN was trained by the spectrum without any information of a mean power to classify data sets into classes synchronized and nonsynchronized. After training, from the weights of the network inputs the importance of input data can be recognized. The nets topology was 1201-1600-200-2 with a logarithmic-sigmoid transfer function of neurons in hidden layers. For training the net a learning

function *learngdm* which prevents over-learn, was used. The validation of classification has been above 75 %. The MLNN was realized in the Matlab 7.9 with The Neural Network Toolbox [6]. Fig. 5 shows the weights of the network inputs corresponded with frequencies in the spectra. The high weights indicate frequencies with information of a difference between the synchronized and nonsynchronized sets. The probability density of noise is different for frequencies with the high and low weights. The frequencies with the high weights have more spectral lines of higher power.

Fig. 6 shows the mean cumulative distribution function (CDF) of 10 frequencies with the low and high weight. Here 90% percentiles ($k_{0.9}$) are shown which significantly present differences between discrete frequencies. Among important frequencies 588, 616, 721, 844, 935 and 1244 Hz were found.

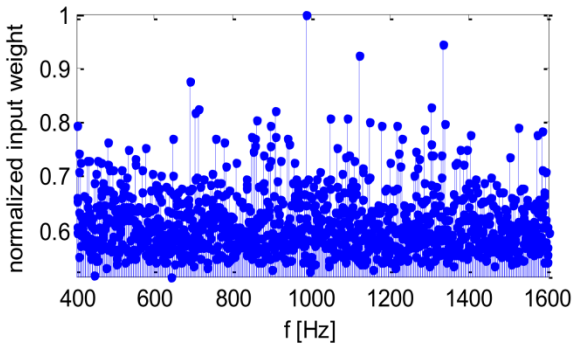


Fig. 5. Input weights as function of frequency

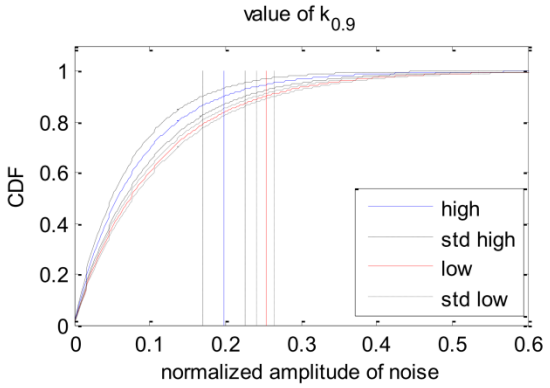


Fig. 6. Mean cumulative distribution function (CDF) versus noise amplitude

VI. DISCUSSION & CONCLUSION

Neural network-based analysis of the data obtained from the measurement of electrodynamic activity of the yeast cells indicate difference between the spectra of synchronized and nonsynchronized cells. Underlying mechanism of difference includes the mere fact that the synchronized cells enter mitosis simultaneously. During mitosis, more energy is supplied to microtubules which are probable generators of cellular electrodynamic activity. Higher input rate of energy, according to Fröhlich's hypothesis will, under certain conditions [1], increase the energy and coherence in certain spectral bands. Also the experimental procedure itself causes that synchronized cells have more energy available at moment when the measurement starts.

We also showed few steps in the development of the new configuration of the sensor. It is necessary to minimize the influence of suspension impedance and contacting only single cell. We are currently developing nanoelectrode based sensor for this purpose.

ACKNOWLEDGEMENT

This work was supported under grants No. 102/11/0649 and No. 102/10/P454 of the Czech Science Foundation.

REFERENCES

[1] H. Fröhlich, "Long-range coherence and energy storage in biological systems", *Int. J. Quant. Chem.*, Vol. 2, pp. 641-649, 1968.

[2] M. Cifra, et al., "Electric field generated by axial longitudinal vibration modes of microtubule", *BioSystems*, Vol. 100, pp. 122-131, 2010.

[3] F. Jelínek, et al., "Measurement of electrical oscillations and mechanical vibrations of yeast cells membrane around 1kHz", *Electromag. Biol. Med.*, Vol. 28, pp. 223-232, 2009.

[4] H.A. Pohl, "Oscillating fields about growing cells", *Int. J. Quant. Chem. Biol. Symp.*, Vol. 7, pp. 411-431, 1980.

[5] R. Janča, *Measurement of electromagnetic oscillations of cells (in Czech, Měření elektromagentických oscilací buněk)* Master thesis, 2010, Faculty of Electrical Engineering, Czech Technical University in Prague.

[6] *Neural Network Toolbox. MATLAB documentation,* The MathWorks, Inc., © 2008-2010.