Measurement of Electromagnetic Activity of Yeast Cells at 42 GHz

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Abstract. *This paper discusses the possibility of using a device composed of a resonant cavity, preamplifiers, and a spectrum analyzer to detect electromagnetic emission of yeast cells at a frequency of about 42 GHz. Measurement in this frequency range is based on the Fröhlich's postulate of coherent polar oscillations as a fundamental biophysical property of biological systems and on the experiments of Grundler and Keilmann who disclosed effects of exposure to the electromagnetic field at 42 GHz on the growth rate of yeast cells. This article includes a detailed description of the laboratory equipment and the methods used to evaluate the obtained results.*

Keywords

Millimeter wave measurement, electromagnetic activity of cell, cellular biophysics.

1. Introduction

H. Fröhlich formulated theory of electrical polar vibrations in biological systems excited by metabolic energy supply with preferential condensation of energy in a vibration mode [1-3]. EM fields are supposed to be generated by polar structures of the cytoskeleton [4]. It is assumed, that the generated electromagnetic fields participate in the mechanisms forming and regulating order in biological systems [5].

Considerable experimental data prove existence of polar vibration states. Oscillating electric field around living cells attracts small dielectric particles by dielectrophoretic effect [6]. Rowlands investigated attraction forces between red blood cells [7] and oscillations of their membranes were measured by Levin and Korenstein [8]. Oscillations of the plasma membrane have a character of zonal harmonics. Albrecht-Buehler observed interaction between cells in the red or near infrared range [9]. Vos et al. measured coherent oscillation in proteins in the submillimeter and far infrared range [10]. Oscillations of yeast cell membranes in the acoustical range of frequencies were measured by atomic force microscope [11], [12]. Amplitudes of vibrations of several nm were observed. Grundler, Keilmann et al. [13] disclosed non thermal frequency dependent increased and decreased growth rate of yeast cells after exposure to electromagnetic waves in the 42 GHz region and explained the effects by resonance interaction of external electromagnetic field with internal oscillations in the cells. This paper describes measurement equipment in the present state of its development. Some previous results are in [14].

2. Materials and Methods

2.1 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 (non-synchronized cells) Evolution of the cells can be synchronized by decrease of temperature below the threshold of the restrictive temperature (14 °C) when microtubules cannot by formed. 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 certain time period evolution of all the mutant cells are 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. Time dependence of evolution of the M phase after warm-up above permis-

sive temperature is described in detail in [15].

We measured suspension of synchronized cells and of non-synchronized yeast cells (as a reference medium). The yeast cells were suspended in the aqueous sucrose solution. Therefore, sucrose solution was also used for control measurements.

2.2 Laboratory Equipment

A schematic diagram of the measurement system is shown in Fig. 1. The core of the system is represented by the sensor and by the preamplifiers, both of which are placed in a shielded temperature stabilized box. The sensor is formed by a rectangular waveguide resonator for frequency of about 42 GHz operating in TE_{102} mode (*Q-factor* is about 1000 and 100 without and with the cuvette containing cell suspension, respectively). A slot in the resonator wall is in the E plane in the place of the maximum value of the vector E. The cuvette including measured medium (suspension with yeast cells or aqueous sucrose solution) is inserted in the slot. Resonator is coupled to the low-noise preamplifier Spacek SL406-34-4 (*Frequency Range* 37 to 43 GHz, *Gain* 37 dB, *Noise Figure* 3.5 dB). Our previous measurements showed that the sensitivity of the system with this amplifier is not sufficient to detect cell signals immersed in noise [14]. Therefore, we inserted after the amplifier mentioned another one, namely Spacek SLQ15-4W (*Frequency Range* 33 – 50 GHz, *Gain* 24 dB, *Noise Figure* 3 dB). The total gain including losses in interconnections is 54 dB at 42 GHz. Both amplifiers are supplied by a battery placed also in the shielded box for minimizing spurious signals. Preamplifiers are followed by the spectrum analyzer Agilent E4448A (settings: *RBW* = *VBW* = 1 Hz, *MaxPeak* detector, *FFT* analysis, *Span* 10 kHz, logarithmical averaging), which is computer-controlled through GPIB interface. Temperature control (28 ± 0.2 °C) of measurement box is realized through NI PCI-4351 interface connected to control computer.

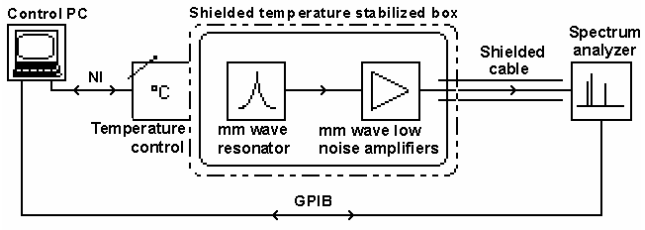

Fig. 1. Schematic diagram of the laboratory equipment.

Sensitivity of the measurement system is of the order of magnitude of 10^{-20} W, so at the level of thermal noise.

2.3 Measurement

Suspension with synchronous cells was cultivated at the temperature below 14 °C Suspension with non-synchronous 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 minutes and were stirred by horizontal shaker. Optical density (OD 600) of such suspension was 4.5 [15], which corresponds to concentration of about 2×10^8 cells per milliliter. Afterwards the cuvette was filled with a given volume of suspension (approximately 0.01 ml) and inserted into the resonator. Measurement started immediately after inserting the cuvette.

We performed the measurement in the frequency range 41.6975 ÷ 41.698 GHz (i.e. in the frequency band 500 kHz wide). The frequency band was divided into 50 successively scanned subbands whose width was 10 kHz; each scan yielded 601 digital samples. The frequency band 500 kHz was 13 times repeatedly scanned. Total time of measurement was approximately 65 minutes. Measured data were stored in the control computer for further analysis.

2.4 Statistical Evaluation

Mean values of power of spectral lines in the measured range are evaluated for each scan 500 kHz and plotted as a function of time. Number of spectral lines exceeding the threshold level (value of x % quantile of probability distribution or signal level higher than x dB above mean value of power) was evaluated as a function of time.

3. Results

Measured values of power are at the level of the noise of the system (about 10^{-20} W). In the previous experiments we did not prove correlation between the power of signal measured and the cell evolution in the M phase. High power spectral lines did not coincide in frequency and signal seemed to be random according to findings of statistical hypothesis tests.

Let us show some preliminary results obtained with the system with two preamplifiers with amplification 54 dB. Self calibration of the spectrum analyzer was performed before measurement and then was switched off. (When extremely weak signals are measured self calibration produces jumps of sensitivity and disturbs measured signal.) The depth of insertion of the cuvette was adjusted and the same cuvette was used for measurement of synchronous cells, non-synchronous cells, and for sucrose solution.

Fig. 2 shows number of spectral lines exceeding the threshold level 4 dB above mean value of power of the spectral lines in corresponding time interval as a function of time (each value is determined from one 500 kHz scan). Dots connected with solid lines represent suspension with synchronous cells (B), squares connected with dashed lines represent also synchronous cells (N), and triangles connected with dotted lines represent sucrose solution (S). The B and N curves display similar time dependences. In this case the N curve represents yeast cells that were cultivated

in the permissive temperature and then accidentally stored in cold. The obvious similarity of B and N plots is caused by synchronous start of the M phase and synchronous behavior of the yeast cells.

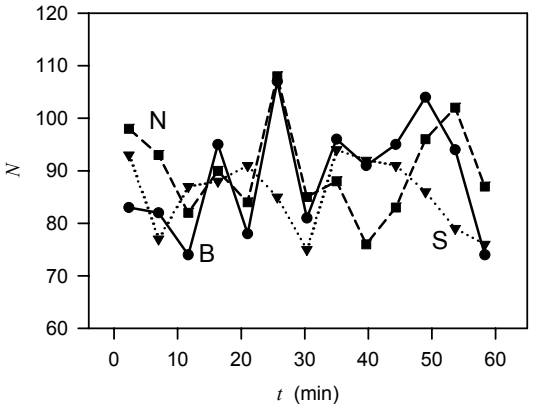

Fig. 2. Numbers of signal peaks exceeding the threshold level as a function of time. Figure is described in the text.

The time dependence of B and N curves displays evolution of the M phase: The maximum values correspond, respectively, to processes of mitotic spindle formation, metaphase, anaphase A, and anaphase B. Nevertheless, the noise level of the sucrose solution curve is too high and further increase of the sensitivity of the system must be done.

4. Discussion

Polar vibrations of the plasma membrane have to be excited by the cytoskeleton, in particular by microtubules as follows from theoretical analysis and experimental results. The microtubules are electrical polar structures whose vibrations can generate electromagnetic field. Some microtubules are connected to the plasma membrane proteins which transfer vibrations. Oscillations of the membrane lipid bilayer excite oscillations of the ionic charge layers at both sides of the membrane. The plasma membrane vibrations have tesseral structure and can be measured at small patches of linear dimensions smaller than about 500 nm. The mm wave cavity can be excited by vibrations in convenient direction.

Elastic vibrations display temperature frequency shifts. Frequency of oscillations of yeast cells membranes in the acoustical range is strongly dependent on temperature. Pelling et al. [11] measured frequency of about 1.6 and 1.1 kHz at the temperature 30 and 26 °C, respectively (i.e. the rate of frequency changes is about 10 % per 1 °C). The electromagnetic emission may depend on the cell cycle conditions too. Typical signal emitted by synchronous yeast cells cultures in the M phase display time shifts within 10 min or within even greater time intervals in the reproduced measurements as was observed in MHz frequency range [15]. These time shifts seem to be caused by different time evolutions of the M phase processes. Coherent vibrations in biological system have considerable random component [12] too. Biophysical mechanism of formation of biological coherent states (different mechanism from that of stimulated emission in lasers) is based on non-linear energy transfer between vibration modes [2], [3] and, therefore, large random components of all parameters are present. Random component of the detected signal depends on the measurement method too. Resonant cavity is excited by superposition of signals generated by oscillations of a large number of cells. Due to motion of cells causing changes of their position and orientation the emitted signal contains fluctuating component. Statistical evaluation should take into consideration all these facts and select important values within assessed range of parameters. To exclude motion effects, yeast cells measured by atomic force microscope are trapped in pores of a filter.

One of the main problems of the measurement is the available power regardless of the fact that the dipole moments of the heterodimers in microtubules as well as of the unit area of the membrane are considerably high. For instance, a patch of the membrane of the area of 0.25 μm^2 with the thickness of 5 nm and the relative permittivity ε = 2.5 has capacitance of the order of magnitude of 10^{-3} pF and the charge that could be released may correspond to this value. Macroscopic measurement system has several orders of magnitude greater input capacitance (of the cavity, of the waveguide, and of the input of the preamplifier) which has negative effect on the sensitivity of the system. A nanotechnology point detection and amplification system should be used to overcome the difficulties.

5. Conclusion

On the basis of our findings we may conclude that the system described in this paper could provide some threshold conditions for detection of the electromagnetic emission of the yeast cells. The noise fluctuations in the system are greater than or at least as large as the signal generated by cells.

For further investigation of the electromagnetic emission of living cells point measurement in the immediate vicinity of cell have to be realized. The cellular nano sources should be detected by nano scale sensors with power amplification forming transition link to macroscopic systems.

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