Electromagnetic Biology and Medicine, 28: 223–232, 2009
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ISSN 1536-8378 print/1536-8386 online
DOI: 10.1080/15368370802710807

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Measurement of Electrical Oscillations and Mechanical Vibrations of Yeast Cells Membrane Around 1 kHz

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Fröhlich postulated coherent polar oscillations as a fundamental biophysical property of biological systems. Recently, Pelling et al. (2004, 2005) detected mechanical vibrations of yeast cell membrane with atomic force microscope (AFM) and analyzed by Fourier analysis in the frequency range 0.5–2 kHz with amplitudes of the order of 1 nm. This article describes the measurement of electric activity of yeast cells in the acoustic frequency range and of mechanical vibrations of cell membrane. Spectrum analyzer and electrically and electromagnetically screened box with point sensor and amplifiers fed by batteries were used for measurement of synchronized and non synchronized tubulin mutants of yeast cells. We show that the electric activity of synchronized cells in the M phase is greater that of non synchronized cells. That corresponds to the findings of Pohl et al. (1981). Obtained results of measurement of cell electric activity are in good agreement with AFM findings.

Keywords Electromagnetic activity of cells; Fröhlich's hypothesis of coherence in biology; *Saccharomyces cerevisiae*; Detection of very weak signals.

Introduction

Living cells exhibit electromagnetic (EM) activity in a wide spectrum of frequencies, which is connected with biological processes inside cells (Pokorný et al., 2001; Beloussov et al., 2007). H. Fröhlich formulated theory of electrical polar vibrations in biological systems excited by metabolic energy supply with preferential condensation

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of energy in a vibration mode (Fröhlich, 1968, 1969, 1980). EM fields are supposed to be generated by electrically polar structures of the cytoskeleton.

Cytoskeleton is the main organizing structure of the eukaryotic cell (Alberts et al., 1994). It is composed of the three types of filaments: actin filaments, intermediate filaments, and microtubules. Microtubules are built from tubulin heterodimers, which are protein molecules of high electric dipole moment (of about 1,000 Debye, i.e., 10^{-26} cm). Tubulin heterodimers stack to form protofilaments during microtubule polymerization. Microtubules resemble hollow tubes emanating from centrosome and have radial orientation during the interphase of the cell. Their inner and outer diameter is 17 nm and 25 nm, respectively. Their length depends on the dimension of the cell.

Microtubules are electrically highly polar structures. Providing the vibrations are excited in microtubules, they will generate electromagnetic field. Wang et al. (2006) treated microtubule as an orthotropic hollow tube. Due to the interaction of longitudinal, torsional, and radial modes, frequencies of microtubule vibrations may span from kHz to GHz region. Since microtubules are electrically polar structures, frequencies of electrical oscillations are expected in the same frequency range as the vibration modes.

To excite vibration in microtubules, energy needs to be supplied. Possibilities for energy supply are the following:

- Energy released from hydrolysis of guanosine triphosphate (GTP) in processes of **dynamic instability of microtubules** is the energy supply dependent on internal microtubule process. β-tubulin bound GTP is hydrolyzed to guanosine diphospate (GDP) after the polymerization of tubulin heterodimer to microtubule. In the interphase the half lifetime of individual unstable microtubules (subject to dynamic instability) is about 10 min. Energy from hydrolysis of GTP stored in microtubule lattice is estimated to be 1.7 kcal/mole (7.1 kJ/mole) and power of about 10^{-14} W/cm (Pokorný, 2003). Assuming 400 microtubules per cell with average length of 2 μm, one obtains the power of about 10^{-16}–10^{-15} W in microtubular vibrations per cell. In the M phase, the dynamics of polymerization and depolymerization is greater (for instance, the treadmilling in the metaphase).
- Energy of **movement of motor proteins on microtubules** may also contribute to excitation of vibrations in microtubules. One ATP (adenosine triphosphate) molecule is hydrolyzed for each step of motor protein. One step of motor protein is ca. 8 nm long, corresponding to dimensions of tubulin heterodimer. Energy released during ATP hydrolysis under cellular ATP concentrations is ca. 58.2 kJ/mol (14 kcal/mol)—activation energy (Pelling et al., 2004). Motor proteins move with velocity ca. 0.1–2 μm/s (Alberts et al., 1994). Assuming 4 motor proteins on each of 100 membrane-bound microtubules, each motor protein moving with average velocity of 800 nm/s, power exerted by all motor proteins in a cell is about 4×10^{-15} W. Assuming that 1–25% of this power excites vibrations in microtubules, it corresponds to 4×10^{-17} W–1×10^{-15} W.
- Energy released from **mitochondria as wasted energy** may be the most significant energy source for excitations of microtubules. Whole metabolic turnover of *Saccharomyces cerevisae* cell is 0.1 pW (10^{-13} W) (Lamprecht, 1980). Mitochondria are "power plants" of the cell, extracting energy from acetyl CoA in the Krebs cycle. Less than 50% of energy is stored in ATP. The rest of the energy (wasted energy), i.e., more than 50%, is released to surroundings of mitochondria in the form of heat and

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emitted radiation. Corresponding wasted power may be 0.05 pW. Since mitochondria are located near microtubules, part of the wasted power, assume 20%, i.e., 10^{-14} W, may be involved in excitation of vibrations in microtubules.

Mitochondria have another important role except ATP production and thermal energy supply to its surroundings. Diffusion of protons from mitochondrial intermembrane space into cytosol creates a high static electric field with intensity of about 3×10^6 V/m around mitochondria (very likely intensity corresponding vacuum conditions). Intracellular static electric field was measured with nanoparticles containing voltage dependent fluorescent dyes (Tyner et al., 2007).

Recently, Pelling et al. (2004, 2005) measured mechanical vibrations of yeast cell membranes in the acoustic range of frequencies by atomic force microscope (AFM). They estimated the origin of these nanomechanical vibrations in the action of many proteins working in a concerted and cooperative manner inside the cell and related them to the metabolic activity of the cell. They proved dependence of excitation of vibrations on the function of mitochondria. After damage of the mitochondrial function mechanical vibrations disappeared. The authors explain extinction of vibrations by exhaustion of ATP resources.

Using our experience from previous EM activity measurements of yeast cells, we have tried to measure electrical oscillations of yeast cells in the acoustical range of frequencies and mechanical vibrations of their membranes. This article describes our measurement equipment and gives some results of measurement.

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Materials and Methods

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 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.

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

Laboratory Equipment

A schematic diagram of the measurement system is shown in Figure 1. An important part of the equipment is the facilities with a cuvette, a point sensor, and transformation

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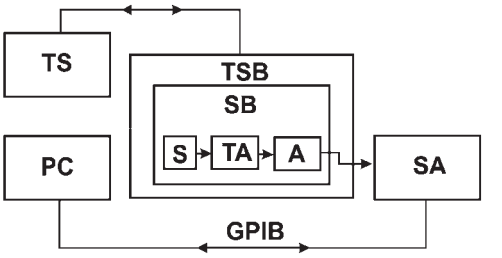


Figure 1. Schematic diagram of the laboratory equipment. SA—spectrum analyzer; TSB—temperature stabilized box; SB—threefold screened box; S—sensor; TA—transformation amplifier; A—amplifier; PC—computer; TS—temperature stabilizer.

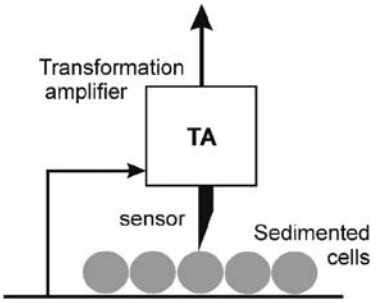


Figure 2. A schematic picture of point contact measurement of sedimented yeast cells.

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 50 nm point. The facilities allow to adjust mechanically the distance of the point sensor from the bottom of the cuvette to about 8 μm before each measurement cycle. The diameter of a yeast cell is of about 8 μm. Therefore, we can expect that the tip of the point sensor touches only one cell during the measurement; see Figure 2. 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.

Mechanical vibrations of the membrane of yeast cell were measured by the atomic force microscope MultiMode IV, Veeco. The scheme of measurement contained 512 measured points in the trace and in the retrace system with frequency 59.2 Hz.

Measurement

Suspension with synchronized cells was cultivated at the temperature below 14°C. Suspension with non synchronized cells was cultivated at the temperature about 30°C. Before measurement the test tubes with the suspensions were put into a water

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bath at 28°C for 3 min. Optical density (OD 600) of such suspension was 4.5, which corresponds to concentration of about 2×10^8 cells per milliliter. Afterwards, the carefully cleaned cuvette was filled with a given volume of suspension (approximately 0.06 ml). Measurement then immediately started.

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.

Data measured by AFM were analyzed by the Fourier transform. The noise level in the frequency range below and above 0.5 kHz was of the order of magnitude of 0.1 nm and of 0.01 nm or lower, respectively.

Results

Figure 3 shows mean electric power of synchronized yeast cells versus frequency evaluated from 10 sweeps at about 20 min after beginning of measurement. Data from 400 double sweeps (40 min) in the frequency range 400–1,600 Hz were recorded for each individual measurement and the electrical mean power was evaluated (after Pokorný et al., 2008). Figure 4 shows mean electrical power of individual measurements of the synchronized (crosses) and of the non synchronized (triangles) yeast cells evaluated from all values of each double sweep in the frequency range 400–1,600 Hz and 400 double sweeps. The full lines denote the mean values of all individual experiments. The electrical power of synchronized cells is about 10% greater than the electrical power of non synchronized cells (see Figure 5) with statistical significance of $p = 0.001$ determined from both two-group t-test and matched t-test.

For comparison, mechanical vibrations of the synchronized yeast cells were measured. Figure 6 shows a spectrogram of the synchronized yeast cells measured by AFM. Cells were measured in the sedimented layer at the temperature of 28–30°C. Figure 6 shows frequency versus time of cell membrane oscillations where amplitude is visualized in a scale shown in the upper-right corner of the figure. The amplitude of vibrations is about 0.8 nm. The frequencies of vibrations are in the same range as were the frequencies of electrical oscillations.

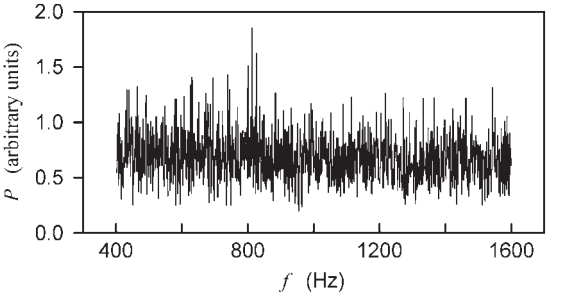


Figure 3. Mean electric power of synchronized yeast cells versus frequency evaluated from 10 sweeps (in 1 min) at about 20 min after beginning of measurement. After Pokorný (2008).

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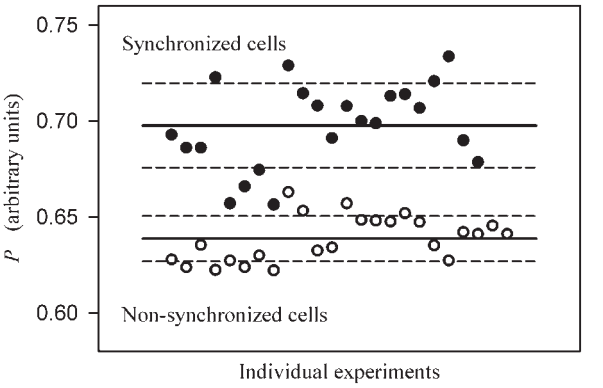


Figure 4. Mean power of individual experiments of yeast cells in the frequency range 400–1,600 Hz with 1 Hz RBW and VBW of the spectrum analyzer. Synchronized cells—the full circles; non synchronized cells—the circles. Dashed lines—mean errors of the mean values.

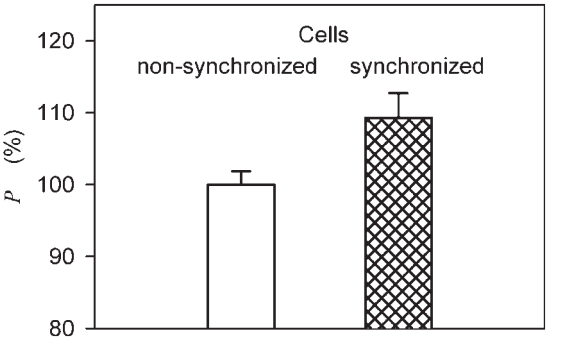


Figure 5. Electrical power (in %) of non synchronized and synchronized yeast cells—mean values and mean errors. Synchronized yeast cells—nearly 10% higher electrical power than non synchronized cells.

Discussion

The synchronized yeast cells in the M-phase display greater electrical activity than the non synchronized cells as follows from experimental results described in this contribution. It corresponds to the experimental findings of Pohl et al. (1981) based on dielectrophoretic attraction of dielectric particles to yeast cells in their non homogeneous electric field indicating that their electromagnetic activity is greatest in the M phase. Dielectrophoretic effects of living cells were studied also by Lamprecht and Hoelzel (1987) and Hölzel and Lamprecht (1994). They imaged by a microscope the dielectrophoretic collection of yeast cells near a pin like electrode (Figure 7) and the dielectrophoretic attraction of barium titanate particles by a long cell of the alga *Monoraphidium griffithii* (Figure 8). The strongly polarizable particles were collected at the cell—mainly at the sharp tips of elongated algae cells—in regions of the highest intensity of the electric field and its gradient.

In the M phase, the maximum (average) value of attracted particles with high and low permittivity is about 2 (1.5) times greater than in the interphase (determined

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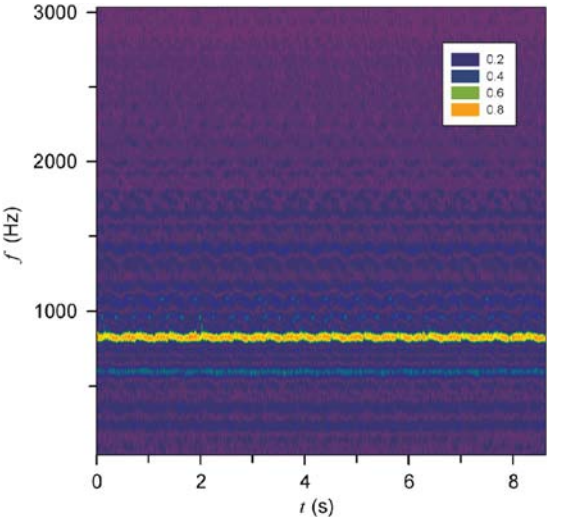


Figure 6. A spectrogram of yeast cell mechanical vibrations. Amplitude of vibrations is about 0.8 nm (see scale). Frequency—*f*; time—*t*.

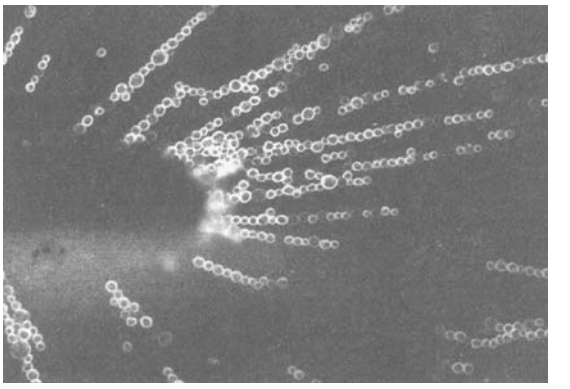


Figure 7. Dark field microscopy of dielectrophoretic collection of yeast cells near a pin-like electrode (left). The individual yeast cells form pearl chains due to mutual dielectrophoresis. Effect of external electic field. After Hölzel and Lamprecht (1994).

from Figure 9, the average values are evaluated from the grey areas). Assuming that gradient of the electric field is proportional to the intensity E of the electric field (i.e., geometrical changes of the space field structures during measurements are neglected), then the ratio of the number of attracted particles in the M phase to that in the interphase is proportional to the square of the ratio of corresponding intensities of the electric field $(E_M/E_I)^2$. Therefore, the maximum (average) value of E_M in the M phase is about 40% (20%) greater than in the interphase.

In the M phase, the average power of the signal measured by a tip electrode at the membrane is only about of 10% greater than that in the interphase, which would correspond to about 5% difference between corresponding intensities of the electric fields. However, the signal was measured only in a narrow frequency band (0.4–1.6 kHz) and also additional noise (whose power may be more than about one

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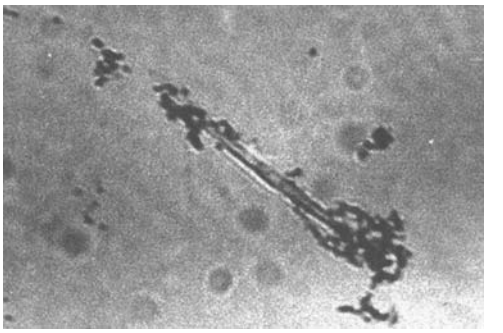


Figure 8. Microdielectrophoresis by the alga *Monoraphidium griffithii* exerted on barium titanate particles (black spots) with a diameter of approximately 2 μm. The length of the cell amounts to ~50 μm. Effect of biological electric field. After Hölzel and Lamprecht (1994).

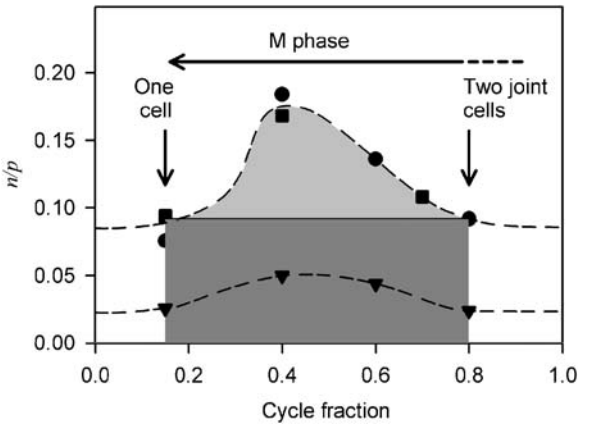


Figure 9. Dielectrophoretic effects of yeast cells *Saccharomyces cerevisiae*: average relative number of attracted dielectric particles as a function of the M phase evolution. (*n*—number of attracted particles; *p*—total number of particles around the cell). Squares—$NaNbO_3$; circles—$BaTiO_3$; triangles—$BaSO_4$. Investigation was performed in four subphases of the M phase: (a) "single cell"; (b) "small—budded" with a bud less than 1/3 cell width; (c) "medium-budded" with bud diameter greater than 1/3 and less than 2/3 cell diameter; (d) "double cell" with two cells joint (points at 0.15, 0.4, 0.6, and 0.8, respectively).

order of magnitude greater than the power of the cellular signal) shifts the power ratio to lower values.

If the source of oscillations is inside the cell, capacity of the cell membrane is likely to attenuate electric signals in lower-frequency (kHz) region. Depending on the cell cycle phase a significant number of microtubules is anchored to cell membrane. It may be that the vibrations of microtubules are transferred to the membrane, which contains transmembrane proteins. The majority of proteins have complex charge distribution, they are electric multipoles. They generate electromagnetic field if they are excited by vibrations.

Actin filaments form a mesh-like network beneath a cell membrane. They are also electrically polar, but less dynamic than microtubules. They also polymerize and

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depolymerize, what could provide energy for excitations of vibrations in them or vibrations may be transmitted from microtubules.

Strong static electric field in cytosol around mitochondria is very likely a necessary condition for nonlinear transformation of mitochondria wasted energy from incoherent to coherent form by the cytoskeleton filaments. Stable microtubules are bound to some structures at the membrane and form oscillation centers. Therefore, the cell membrane is a locus of two-dimensional superposition of oscillations excited by many centers. It needs to be emphasized that any sensor must have dimension of the detection point smaller than the half of the wavelength (the cantilever tip of AFM and the tip of a sensor for electrical measurements). It is likely that we detect electrical oscillations of single microtubule with the point sensor. Sensitivity and character of the sensor and measurement setup must be corresponding to this assumption. Estimated order magnitude of power in microtubular vibrations in the Introduction section is calculated for all microtubules in a cell. Power in vibrations in single microtubule will be by more than two orders of magnitude lower. It corresponds to the magnitude of the measured electrical signals, when we take into account probable imperfect impedance matching of the sensor to the measured source.

We assume that biological processes depend on biological electromagnetic field and that disturbances of the electromagnetic field cause some pathological states. Experimental data on the electromagnetic activity in living cells support the hypothesis, in particular that cancer transformation is connected with some changes of cellular biological electromagnetic field (Pokorný et al., 2008).

Conclusion

Electrical activity of the synchronized and non synchronized yeast cells *Saccharomyces cerevisiae* (of the β-tubulin mutant *tub2-401*) was measured in the frequency range 0.4–1.6 kHz. The synchronized yeast cells display greater electrical activity than non synchronized cells. The frequencies of electrical oscillations are in the same range as the mechanical vibrations. Atomic force microscope measurement shows mechanical oscillations in the acoustic frequency range too. The results confirm dielectrophoretic experimental findings of Pohl et al. (1981).

Acknowledgment

This work was supported under grant OC 281.001 of Ministry of Education, Youth, and Sports of the Czech Republic in the framework of COST 281. A part of the work was also supported by Internal Grant Agency of Czech Technical University in Prague, n. CTU0806413 and Grant Agency of the Czech Republic, n. 102/08/H081.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Alberts, B., et al. (1994). *Molecular Biology of the Cell* (3rd ed., pp. 38–39). New York: Garland Publishing.

Beloussov, L. V., Voeikov, V. L., Martynyuk, V. S. (2007). *Biophotonics and Coherent Systems in Biology*. New York: Springer.

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Fröhlich, H. (1968). Long-range coherence and energy storage in biological systems. *Int. J. Quant. Chem.* 2:641–649.

Fröhlich, H. (1969). Quantum mechanical concepts in biology. In: Marois, M., ed. *Theoretical Physics and Biology* (pp. 13–22). Amsterdam: North Holland.

Fröhlich, H. (1980). The biological effects of microwaves and related questions. *Adv. Electron. Electron Phys.* 53:85–152.

Hölzel, R., Lamprecht, I. (1994). Electromagnetic fields around biological cells. *Neur. Network World* 4:327–337.

Lamprecht, I. (1980). Growth and metabolism in yeasts. In: Beezer, A. E., ed. *Biological Microcalorimetry* (pp. 43–112). London: Academic Press.

Lamprecht, I., Hoelzel, R. (1987). Microdielectrophoretic effects of cells. In: Fiala, J., Pokorný, J., eds. *Biophysical Aspects of Cancer* (pp. 124–130). Prague: Charles University.

Pokorný, J. (2003). Viscous effects on polar vibrations in microtubules. *Electromag. Biol. Med.* 22:15–29.

Pelling, A. E., Sehati, S., Gralla, E. B., Gimzewski, J. K. (2005). Time dependence of the frequency and amplitude of the local nanomechanical motion of yeast. *Nanomed. Nanotechnol. Biol. Med.* 1:178–183.

Pelling, A. E., Sehati, S., Gralla, E. B., et al. (2004). Local nano- mechanical motion of the cell wall of *Saccharomyces cerevisiae*. *Science* 305:1147–1150.

Pohl, H. A., Braden, T., Robinson, S., et al. (1981). Life cycle alterations of the micro-dielectrophoretic effects of cells. *J. Biol. Phys.* 9:133–154.

Pokorný, J., Hašek, J., Jelínek, F., et al. (2001). Electromagnetic activity of yeast cells in the M phase. *Electro. Magnetobiol.* 20:371–396.

Pokorný, J., Hašek, J., Vaniš, J., Jelínek, F. (2008). Biophysical aspects of cancer electromagnetic mechanism. *Ind. J. Exper. Biol.* 46:310–321.

Tyner, K. M., Kopelman, R., Philbert, M. A. (2007). "Nanosized voltmeter" enables cellular-wide electric field mapping. *Biophys. J.* 93:1163–1174.

Wang, C. Y., Ru, C. Q., Mioduchowski, A. (2006). Vibration of microtubules as orthotropic elastic shells. *Physica E* 35:48–56.

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