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Technical aspects of measurement of cellular electromagnetic activity

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Abstract Electromagnetic fields generated by living cells have been experimentally investigated in the past 3 decades; however, the results are often inconsistent. In this paper we discuss some technical aspects of such challenging experiments, a brief review of which is also included. Special attention is paid to the sensor with respect to the power available from a cell and the power needed to excite the macroscopic measurement devices. We drew the conclusion that the nanoelectronic approach should be used.

Keywords Cellular biophysics · Electromagnetic activity of cell · Nanoelectronics · Weak power measurements

Introduction

Endogenous electromagnetic (EM) activity of living cells and tissues has been investigated by several authors for a long time. Despite a number of accomplished experiments and theoretical works, much still needs to be discovered about this challenging issue. However, we believe that the frontiers of technology have already reached the point where it is possible to construct sensors resolving biological electromagnetism once and for all.

This paper discusses technical conditions that must be satisfied for non-invasive measurement of biological EM activity, because most of experimental works carried out up to now do not seem to fulfill them. In the following, we skip widely known low-frequency electrophysiology topics since those are well understood and corresponding measurement techniques have been precisely developed. Moreover, we limit ourselves to the radiofrequency range (kHz ÷ GHz), because detection methods of the EM field (EMF) of frequencies beyond the THz gap are of a different nature.[1]

There are several theoretical and experimental works supporting the hypothesis of biological EMF. Indirect experimental evidence of cellular EMF is based on two different principles.

On the one hand, force effects of the biological EMF can be observed; Pohl (1985) discovered attraction (or repulsion) of small dielectric particles to (from) the surface of the living cell as a result of the dielectrophoretic effect.

On the other hand, external EMF, if applied, is supposed to interact resonantly with endogenous cellular vibration states. Grundler and Keilmann (1983) observed a nonthermal effect of 42-GHz microwaves on the growth rate of yeasts. Many other workers reported other nonthermal effects of microwaves on biological systems: an overview is given by Belyaev (2005). One of the theoretical elucidations of the origin of biological EMF resides in the

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[1] For instance, single photon counting in the optical region—which is widely available—is the cause of plenty of published work regarding cellular EM activity in the visible and UV part of the EMF spectrum.

mechanical vibration of bound charges of polar structures, such as proteins, amino acids, etc. Therefore, if one observes a resonant effect of external EMF on biological systems at a certain frequency, one can also expect that there are vibration states that can generate endogenous EMF of the same frequency under certain conditions (energy supply, etc.).

Fröhlich, Pohl, Tsong and others proposed several theories describing the generation of EMFs in living cells in terms of nonlinear physics, thermodynamics, quantum mechanics, etc. A comprehensive review of these theories is given in Cifra (2009).

Leaving aside the problem of the origin of biological EMFs, let us focus on the technical delicacy of its measurement. Hereafter we would like to:

- Present a brief review of the considerable experiments dealing with direct measurement of biological EMF.
- Assess fundamental technical requirements for direct cellular EMF measurement systems.
- Point out some technical imperfections of performed experiments with respect to given requirements.

Review of direct measurements

A short review of observations about the cellular radio frequency EMFs obtained by direct measurement methods is given in the following. A common feature of all methods is the structure of the measurement system, where the sensor followed by an amplifier—both preferably in a shielded enclosure—are connected to the spectrum analyzer (a typical measurement setup is depicted in Fig. 1).

Jelínek et al. (2009) used a platinum microwire forming a tip with a linear dimension of about 50 nm to measure electric vibrations of yeast cell membranes in kHz range. The tip was positioned above the layer of synchronized (in the M phase of the cell cycle) and nonsynchronized yeast *Saccharomyces cervisiae*. The total value of power of the synchronized yeast cells in the frequency range $400 \div 1600$ Hz was about 9% greater than that of the nonsynchronized cells with statistical significance better than 0.001. The authors suggested that the increased EM activity is caused by the raised energy supply to vibration states during cell division. This result is consistent with the experimental findings of Pohl et al. (1981) who found an increased number of attracted dielectric particles to yeast cells in the M phase.

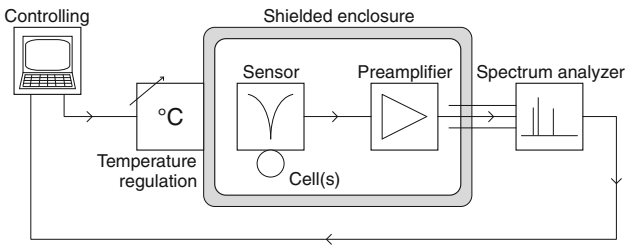

Fig. 1 Typical setup for measurement of the electromagnetic field of cells

Pohl (1985) reported measurement of electrical oscillations of alga *Netrium Digitus* with Pt electrodes as a sensor. Signals of fractions of μV in bands around 7 and 33 kHz have been detected.

Rather extensive analysis and description of measurement circuitry for detection of cellular radiofrequency electrical activity are given in Hölzel's thesis (Hölzel 1990), with some additional results published afterwards in Hölzel and Lamprecht (1995), Hölzel and Lamprecht (1994) and Hölzel (2001). Tungsten microelectrodes and electrodes formed by Pt-plated glass slides were prepared, and measurements of *Schizosaccharomyces pombe*, *Saccharomyces cervisiae* and *Monoraphidium griffithii* cells were performed. Accepting only signals that exceeded a probability of 99.9% of not being of noise origin, peaks of several frequencies in the MHz region were detected (Hölzel and Lamprecht 1994; Hölzel 1990).

Further measurements of yeast cells have been carried out by Pokorný et al. (2001). The threshold value used to detect the frequency peaks was 6 dB above the mean value of noise. Four maxima of power were detected in the distinct time periods of measurement. These four maxima were correlated with the microtubules reassembly into mitotic spindle, with binding of chromatids to kinetochore microtubules, and with elongation of mitotic spindle during anaphase A and B, each visualized by fluorescence microscopy.

Attempts have been made by Jelínek et al. to measure electromagnetic activity of yeast cells also in the GHz region (Jelínek et al. 2002, 2005, 2007), especially in the band around 42 GHz. This was inspired by the experiments of Grundler and Keilmann (1983) mentioned in the Introduction. Macroscopic detection systems such as fine-line sensors (Jelínek et al. 2002) and resonant cavity sensors (Jelínek et al. 2005), in spite of their high quality technical realization, were unsuitable for measurement of cellular EM activity.

Requirements of measurement systems

The measurement of the EMF of living cells needs to take into account several aspects, which are mutually interconnected and which were not fully considered in the accomplished experiments. In the following section we will discuss biophysical issues limiting and influencing the possibility of experimental devices to resolve the electromagnetic activity

of a single cell. Each biophysical aspect (BA) determines a technical requirement (TR) postulated consequently. Some of the BA are of general character, and some result from a particular theory of EMF generation.

BA 1

Overall power production of the living cell is of the order of magnitude of 10^{-13} W (determined for yeast cells with calorimetric measurements in Lamprecht 1980). About the same power may be delivered from mitochondria in the form of the "wasted energy" for excitation of the EM activity of the cell. However, power is further divided to assumed oscillation centers and distributed into the whole spectrum of possible modes. Power of the majority of modes remains at or near the thermodynamic equilibrium lever (i.e., $10^{-20} \div 10^{-18}$), but some of the modes may be strongly excited by energy condensation in the nonlinear oscillating system. We may admit that transiently the power production may increase by a few orders of magnitude, and it can excite individual modes at different frequencies. Energy channelled in the system is adequately raised. As the oscillation system is nonlinear, the high order nonlinear terms may stabilize the amount of condensed energy by increased energy leakage to the heat bath. On the other hand, if the energy supply is strongly diminished [as it is in cancer cells (Pokorný 2009)], the energy condensed in the system is also decreased, and consequently the generated electromagnetic field is decreased too.

TR 1

Sensitivity of the measurement setup must allow measurement of power of several orders of magnitude lower than overall power generated by the cell, i.e., $10^{-20} \div 10^{-18}$ W, almost on the level of thermal noise. Generally, the signal-to-noise ratio can be increased by, e.g., reduction of the resolution bandwidth of the measurement system or by employing lock-in techniques, etc. Nevertheless, an extremely low resolution bandwidth increases scanning time and thus deteriorates the time resolution (uncertainty principle). Lock-in techniques can be used only when the frequency of cellular EMF is precisely known, which is not the case. Moreover, it cannot be excluded that the frequency of the signal naturally fluctuates. Thus, our ignorance of parameters of the signal to be measured strongly limits the possibility of employing the signal processing methods to enhance the signal-to-noise ratio.

BA 2

The cellular EMF was calculated to have a strong electric field component that decays rapidly outside the cell within a micrometer (Pokorný and Wu 1998). Phenomenologically this is related to the fact that the cellular electrodynamics are expected to be of near-field nature. Thus, the radiative fraction will be very low, except for very few special phases in the cell life cycle. This is due to multipole distribution of charges of cellular EMF sources (see, e.g., Nakamura and Wada 1985) and a special symmetry of sources (Pokorný et al. 1991).

TR 2

Detector of the cellular EMF cannot be antenna designed for detection of far-field radiated waves. Detectors should be of a contact type coupled directly to the cellular near field. Detectors need to be in the close vicinity of the cell, essentially in contact with the cell membrane.

BA 3

A large number (about 10^2) of vibrational sources is to be expected in a cell and cellular membranes; consequently, the spatial field structure on the cytoplasmic membrane will be rather complex, resembling interference of tesseral spherical harmonics from many sources with distance of amplitude minima and maxima of possibly hundreds or tens of nanometres. It can be expected that the spatial resolution for detection of high frequency oscillations will have to be in the order of tens of nanometres or units of nanometres (Tsong et al. 1989).

TR 3

"Point" measurement (resolution) is necessary in order to resolve the spatial minima and maxima of electric intensity. The signal will be essentially proportional to an integral of the spatial distribution of detected intensity; see Fig. 2.

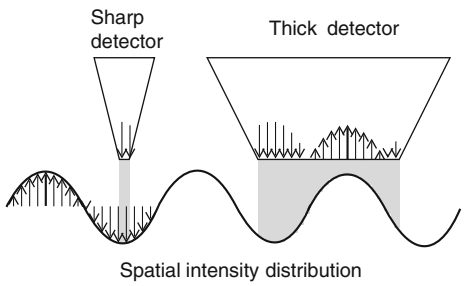

Fig. 2 Illustration of the point detector problem. Spatial intensity distribution is approximated by harmonic function. A thick detector will effectively sense the integral of the spatial intensity, the value of which approaches zero for the *integer multiple of the spatial period* (IMSP). Although the size of the detector will generally not be equal to the IMSP, the increased bulk of the detector, not corresponding to the waveform structure, will deteriorate the signal-to-noise ratio due to charge relaxation

BA 4

A cell is separated by a cytoplasmic membrane from the surrounding medium. The membrane is composed of lipids and proteins and is rather nonconductive for low frequencies under 1 MHz. Cytoplasmic membrane has relative permittivity of about 3 and conductivity of ca. 10 nS cm^{-1} (Hölzel 1997) for frequencies up to the MHz region. Equivalent impedance of cell is about 1 MΩ for the frequencies $\leq$1 MHz. However, for the higher frequencies the membrane capacitance becomes short circuited, and the equivalent cell impedance drops to the value governed by the content of ionic water (cytosol, ca. 75% of cellular wet weight) and the content of proteins and lipids (cell organelles, ca. 25% of cellular wet weight) in the cell.

TR 4

From the viewpoint of electrical engineering, sensors and amplifiers need to be matched to the source—the cell—in order to efficiently transfer the energy. Hence, typical 50 Ω sensors or transmission lines would not be suitable for measurement of electrical oscillations of cells under the GHz frequency region. Accordingly, high input impedance amplifiers have to be used. It is also necessary to reduce the length of the detector transmission line from the cell to amplifier as much as possible.

BA 5

Related to the previous aspect, the cell may be considered a very "soft" source. The measured power may quickly drop in the course of measurement while the cell is loaded by the measurement circuit.

TR 5

For lower frequencies around the MHz level, high input impedance of the sensor and first preamplifier is necessary. For the high frequencies (tens of GHz), the concept of "soft" source is not encountered in classical electronics. The point is to match the sensor in a way that minimal energy is drained from the cell, while the amplitude of the signal is high enough to excite the preamplifier.

BA 6

As mentioned above, cellular EMF-generated power, which should be detectable, is of the order of magnitude of $10^{-20} \div 10^{-18}$ W.

TR 6

To prevent external electromagnetic radiation from superposition to measured signals, it is necessary to shield the measurement box, the sensor, and the first steps of the amplification cascade. Feeding of amplifiers from the batteries is also recommended.

BA 7

Measurement setup must fulfill physiological conditions for measured cells. This is limiting not only for maintaining appropriate ranges of the osmotic pressure, pH, and temperature, but also for dielectric properties (complex permittivity) of the cell medium. Due to osmosis, the hypotonic (hypertonic) surrounding medium causes increased (decreased) water content in the cell; the cell may be swollen (crenated). The change of the water content in the cell may disturb intracellular functions including cellular electromagnetic activity.

TR 7

The medium where the cells are suspended may have non-negligible conductivity because of mobility of ions for lower frequencies. Dielectric losses become significant for the higher frequencies around the dielectric relaxation one of unbound water (ca. 20 GHz). Thus, the field intensity decays even more rapidly with increasing distance than it would in lossless medium. Intensity of the signal in lossy medium is proportional to $e^{-\alpha r}$, where r is the distance of the sensor from the cell surface, and α is the attenuation constant, which is proportional to the root of the medium conductivity. In order to obtain high signal, one must reduce αr. For example, using deionized water with low α as a medium would lead to a significant decrease of amplitude losses, but it will result in a large increase of the cytoplasmic volume because of the hypotonicity of the medium. Hypotonicity can be reduced by adding nonionic substances (e.g., sucrose), but they may influence cellular metabolism. Another way to obtain maximum signal amplitude is to reduce the distance, r. This is another reason for contact measurement electrodes that are in direct contact with the cell membrane. Technical realization must also take into account possible changes of physical properties of the buffer due to the cellular metabolism.

BA 8

Suitable cell type and conditions for measurement, reasonable size of the cell and cell division period are necessary. The cell type and strain used depend naturally on the focus of the research as well.

TR 8

It cannot be expected that the cell will generate the desired power of EMF if exhausted by lack of nutrients, poisoned, or dead.

BA 9

There is one more aspect, common to all biological experiments, which is connected to processing of the data measured. The objects of measurement are living cells, each of which is unique in a certain manner.

TR 9

The measurement device must be flexible enough to enable investigation of cells whose parameters differ within some interval.

Discussion on experiments

In this section, we discuss how experiments mentioned in "Review of direct measurements" are consistent with requirements postulated in "Requirements for measurement system."

Only measurement systems in Hölzel and Lamprecht (1995), Hölzel and Lamprecht (1994), Hölzel (2001), Pokorný et al. (2001), and Jelínek et al. (2002, 2005, 2007, 1999) fulfill the technical requirement TR 1 for power sensitivity of signals of the order of magnitude $10^{-20} \div 10^{-17}$ W, while the older systems (Pohl 1985; Jelínek et al. 1996; Jafary-Asl and Smith 1983) provided probably only threshold power sensitivity.

In our opinion, most controversial is the issue of spatial resolution and the impedance matching of the sensors used.

Discussing the spatial resolution, we have found that almost all sensors used in the experiments described in the review section, except for the tip used by Jelínek et al. (2009), do not satisfy TR 3.

The detector based on a resonant cavity (Jelínek et al. 2007) is not able to resolve the spatial distribution of the near field, since it is sensitive only to the radiative field component (not in accord with TR 2). Regarding metal stripes used as electrodes in Jelínek et al. (1999), their width is comparable to the size of the cell, again not fulfilling TR 3. Effective spatial resolution of tungsten microelectrodes used by Hölzel (1990), Hölzel and Lamprecht (1995), Hölzel and Lamprecht (1994), and Hölzel (2001) are expected to be at the threshold level (500 nm), while tips of his platinum film electrodes were probably too wide (500 nm × 600 μm).

The influence of impedance matching (TR 4, 5) can be shown in the experiment of Jafary-Asl and Smith (1983) who used electrodes directly connected to spectrum analyzer. Hölzel failed to reproduce their finding of a signal around 7 MHz using 100-fold more sensitive detector. He states that using a 50 Ω sensor, as was done by Jafary-Asl and Smith (1983), at this frequency range to detect the signal would require power in cellular EM activity of one order of magnitude greater than the total thermal power of the cell (Hölzel 1990). It is suspected that positive feedback could create an artifact. Other cited experiments used preamplifiers, which served for impedance matching of cells to the spectrum analyzer.

All described experiments (except some of the early experiments of Hölzel 1990) included a shielded box or enclosure (TR 6) in which the measurement system or its amplification part was located to prevent signal artifacts of technogenous origin.

Several authors considered measured spectral lines to be of a cellular origin if the probability of not being noise is greater than $x\%$. For applying such a method the noise distribution has to be known. One can use either the physical model based on a measurement system or the relevant control experiment. The first possibility can hardly include all the real influences effecting in the transmission and signal processing chain. However, using a nutrient medium only or inactive cells for the purpose of the control experiment may also lead to differences of physical parameters (such as permittivity, conductivity, etc.) compared to primary cell measurement; thereupon a noise distribution is affected. Therefore, the control experiment has to be designed carefully.

Conclusion

The issue of biological EMF measurement was discussed. We conclude that accurate measurement must be done in the immediate vicinity of the cell using a sensor making the low power signal from a soft nanoscale source accessible to the macroscopic measurement system.

On the basis of the facts mentioned above, we summarize the requirements for the measurement system. The device must be able to:

- Detect a signal of the order of magnitude of thermal noise.
- Achieve the spatial resolution required (hundreds of nm or better).
- Measure the near field component.
- Match the resistance and capacity of the nanoscale cellular source (high effective impedance for kHz and MHz frequencies).

– Shield itself from the various sources of noise.
– Form a transition link from nanoscale structures to macroscopic systems.

We discussed the experiments that have been carried out up to now with regard to the mentioned technical requirements. We found that each of the used sensors meets some of the requirements presented here, but none of them meet them entirely. Sensors used up to now could provide only some threshold conditions for measurement of biological EMF. In our opinion the results of these experiments are to be accepted with scepticism. Inasmuch as the most of the observations are predominantly of a statistical nature, using more sophisticated methods for interpretation of data measured, such as specially adapted correlation techniques taking into account biological conditions and time varying properties of the measured signal, artificial neural network analysis or causality analysis is recommended. In conclusion, the precise direct measurement of biological EMFs in the radiofrequency range has not been accomplished yet.

Considering the requirements with respect to the ineptitude of macroscopic concepts like impedance, etc., for this purpose, we may draw the conclusion that the nanoelectronics approach should be used. Further development of sensors in this direction is thus strongly encouraged, since the construction of sensors fulfilling the abovementioned requirements has become feasible because of the rapid progress of nanotechnology.

We believe that the accurate measurement of biological electromagnetic activity may deliver crucial information for elucidation of some biological processes in cellular energetics and electrochemistry. Intracellular transport, organization, and interactions may depend on the endogenous cellular electromagnetic field. Understanding of this field will also be very important for assessment of nonthermal effects of external electromagnetic fields on living organisms. Biological electromagnetism could also play a role in intra- and inter-cellular signalling pathways.

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