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Dosimetry of an in vitro exposure system for fluorescence measurements during 2.45 GHz microwave exposure

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An in vitro system for 2.45 GHz microwave (MW) exposure with real-time fluorescence measurements is proposed. This system is specifically designed for the measurement of those biophysical parameters of living cells or membrane models which can be quantified by spectrofluorometric methods (e.g. membrane generalized polarization (GP), membrane fluidity, membrane potential, etc.). The novelty of the system consists in the possibility to perform fluorescence measurements on the biological samples simultaneously with their exposure to MW. The MW applicator is an open ended coaxial antenna which is dipped into a cuvette. The distribution of electromagnetic field and specific absorption rate (SAR) in the cuvette are provided from a rigorous electromagnetic numerical analysis performed with a finite difference-time domain (FDTD) based tool. With this system, fluorescence measurements were used to calculate the membrane GP values of giant unilamellar vesicle suspensions that were acquired during exposure to a 1.2 W incident power. For this power, the SAR distribution and mean SAR value for the whole volume were calculated based on temperature measurements made at different positions inside the cuvette.

Keywords: Microwave exposure, FDTD, Generalized polarization, Fluorescence measurements

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I. INTRODUCTION

In order to obtain information about the effects of microwave (MW) on living cells, different types of exposure systems have been developed during the last two decades. These exposure systems are used for MW medical applications or studies of MW biological effects and mechanisms [1–6]. The difficulties in bioelectromagnetic studies arise from the control of the physical and biological parameters during exposure. Moreover, as bioelectromagnetic investigations involve different targets and protocols, exposure setups can hardly be standardized. Therefore, the design of each exposure device must be supported by numerical electromagnetic dosimetry and validated with experimental measurements.

In most exposure systems MW irradiation is performed prior to the biophysical analysis. For example, as presented in [3], multilamellar liposomes permeability and lipids' conformational changes were studied after 5 h exposure to 900 MHz MW. The liposome samples were placed in a coaxial-type glass tube applicator and exposed to a specific absorption rate (SAR) of 12 W/kg. Among existing applicators, antenna-based systems [4–6] have been developed for studying the radio frequency/MW fields' interaction with biological samples or for MW medical applications. All these systems present the same major disadvantage: the biophysical parameters are measured after the irradiation was stopped, which prevents the observation of any transient effect.

The system presented in this paper permits the acquisition of temperature and fluorescence measurements while the biological sample is simultaneously exposed to the MW signal through an open coaxial applicator. In the present experiments, a suspension of giant unilaminar vesicles (GUV) was used as a model of living cells and exposed to MW in a spectrofluorometric cuvette while the GUV generalized polarization (GP) was measured.

For a rigorous analysis of biological experiments, the entire systems must be electrically and thermally characterized. Complementary characterization can also be obtained with numerical analysis. In this study, accurate dosimetry of the proposed MW exposure system was performed. The dosimetry is based on electromagnetic simulations that permit the estimation of SAR levels in well-controlled numerical models.

This paper is organized as follows. Section II provides a description of the MW setup with the open coaxial applicator, the fluorescence measurement technique, and the exposure protocol. Section III details elements about the performed experimental and numerical electromagnetic characterization. The dosimetric results in terms of SAR distribution and temperature measurements are presented in Section IV. An example of GUV membrane's GP measurements is also given in Section IV.

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II. EXPOSURE SETUP AND PROTOCOLS

A) Open coaxial applicator system

The device is composed of a spectrofluorometer combined with a 2.45 GHz MW exposure system. The latter component comprises a generator (Sairem, France), a bidirectional coupler (Hewlett Packard 777D, CA, USA), an impedance matcher (Microlab, FXR SF-31N, NJ, USA), and an applicator placed in a holder which contains the biological sample. The generator delivers a 2.45 GHz MW signal with an adjustable output power (up to 120 W). As shown in Fig. 1, the generator is connected to the bidirectional coupler for incident and reflected powers measurements and to the impedance matcher. The latter permits the achievement of optimal impedance matching between the generator and the applicator. Once the impedance matching was performed after measuring the reflected power with a powermeter (Hewlett Packard 436 A, CA, USA) the experiment can be carried out.

The radiating element is an open coaxial cable ended by a central metallic pin conductor which is in direct contact with the biological suspension (Fig. 2(a)). The inner and outer diameters of the coaxial cable (0.8 and 2.75 mm, respectively) are designed for a 50 Ω impedance. The length of the pin (2 mm) is small compared to the wavelength λ (14.2 mm in the solution) and provides the best impedance matching at 2.45 GHz (Fig. 2). The location of the pin permits to deliver the MW power density in the center of the cuvette near the spectrofluorometer analysis area (light beam). The GUV suspension is placed in a 12 × 12 × 40 mm plastic cuvette (Fig. 2(a)). The thickness of the cuvette walls is 1 mm. The exposed solution volume is 10 × 10 × 23.5 mm which corresponds to 2.35 ml. As illustrated in Fig. 2(b), the spectrofluometric system (SPEX, Germany) is composed of a thermostated holder (TLC50, Quantum Northwest Inc., USA) which sustains the cuvette. Rectangular windows of 4 × 8 mm are found on three sides of the holder in order to allow fluorescence measurements (the fourth window at 180° with respect to the excitation light path is closed with an aluminum obturator). The Peltier elements of the holder are under computer control. A fiber optic temperature probe (Luxtron, Model One, CA, USA) immersed into the medium allows the temperature acquisition during the MW exposure. The tip of the fiber optic is located near to the applicator's pin (Fig. 2(a)). A magnetic stirrer placed at the bottom of the cuvette was also used during the experiments.

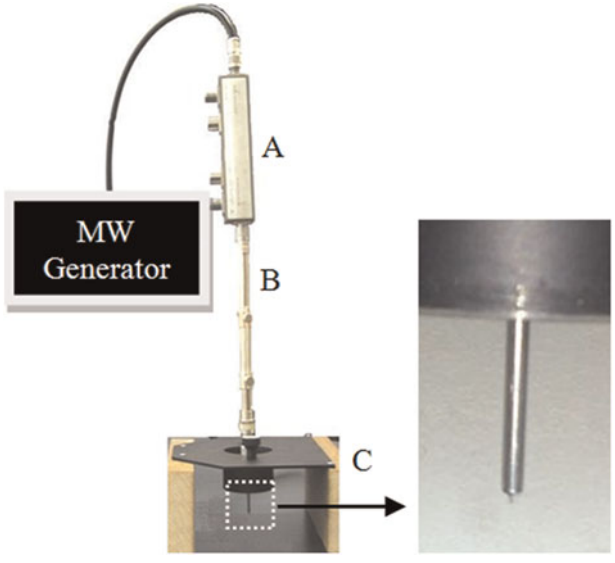

Fig. 1. Exposure setup: bidirectional coupler (a), impedance matcher (b), and open coaxial applicator (c).

B) Membrane GP measurements

Membrane GP is a parameter which accounts for the presence of small, mobile, polarizable molecules (e.g. water) within a biological membrane. It can be measured by fluorescence techniques, using a specific dye, Laurdan (6-dodecanoyl-2-dimethylamino-naphthalene) [7–9], which, in its spectroscopic ground state, is non-polar. The excited state of Laurdan (which has a high dipole moment) may interact with other dipolar molecules in the environment, shifting its emission spectrum toward lower energies (higher wavelengths) because part of the excitation energy is dissipated in the reorientation process of the dipoles. Basically, one has to measure the fluorescence intensity of Laurdan at two specific wavelengths (in our case 437 and 485 nm), called I_B and I_R, respectively. The numerical value of GP is given by the relation $GP = (I_B - I_R) / (I_B + I_R)$. If the membrane is destabilized by an external factor, i.e. MW exposure, water molecules penetrate into the lipid bilayer, and GP values become smaller. In our experiments, GP values are measured in real time during the MW exposure.

C) Chemicals and exposure protocol

GUVs labeled with 10 μM Laurdan (Molecular Probes, Oregon, USA) (excitation wavelength 364 nm) are prepared

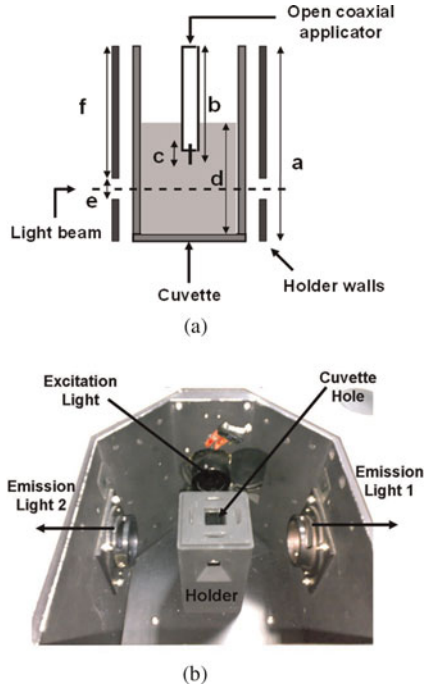

Fig. 2. Studied exposure applicator. (a) Spectrofluorometric cuvette containing the GUV suspension. The dimensions are: $a = 40$ mm, $b = 28$ mm, $c = 2$ mm, $d = 23.5$ mm, $e = 4$ mm, $f = 30$ mm. (b) Cuvette inside the thermostated holder and the fluorescence system.

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from DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) (Sigma–Aldrich GmbH, Germany) by the reversed phase evaporation method [10].

During MW exposure, the emission spectra of Laurdan are recorded and GP is calculated [7]. The GUV suspension is thermostated at an initial temperature of 15°C. Then, a MW signal is applied until a temperature of 40°C is reached inside the GUV suspension. The experiments are carried out at 1.2 W incident power. GP values are represented as a function of temperature.

III. ELECTROMAGNETIC CHARACTERIZATION

A) Numerical modeling analysis

In order to characterize the response of the biological sample to the 2.45 GHz MW exposure, numerical analysis is performed using in-house software based on the finite difference-time domain (FDTD) method [11–14]. The calculation requires a grid meshing of the exposure system geometry and takes into account the physical properties of the biological sample such as permittivity and conductivity. The numerically modeled geometry is composed of the electromagnetically relevant parts (Fig. 2(b)) which are, in this case, the open coaxial cable, the cuvette containing the GUV biological suspension, and the internal part of the holder (mostly metallic). The geometry is discretized with a uniform 0.2 × 0.2 × 0.2 mm grid. A 50 Ω localized EM excitation is placed in the open coaxial cable.

The FDTD method gives the EM fields inside the cuvette exposed to MW. In addition, it gives the SAR which is a commonly used unit in bioelectromagnetic studies [15–18]. The SAR quantifies the amount of absorbed power per unit mass (W/kg) in the exposed sample:

$$SAR = \frac{\sigma E^2}{2\rho}, \quad (1)$$

where σ (S/m) and ρ (kg/m^3) are, respectively, the electric conductivity and the mass density of the exposed biological medium. E (V/m) corresponds to the electric field magnitude. In our study, the considered electromagnetic properties of the biological medium at 2.45 GHz are $\varepsilon_r = 74$, $\sigma = 2.85$ S/m, and $\rho = 1000$ kg/m^3, in final suspension, at room temperature. The electromagnetic properties (permittivity and conductivity) of the biological medium are measured with a dielectric probe (85070E Dielectric probe kit, Agilent, USA) connected to a vector network analyzer (VNA 8753E, HP, USA). SAR values are then computed, as illustrated in Section IV, for each elementary cell of the discretized sample volume.

B) Thermal dependencies of electromagnetic properties

SAR values are obtained for electromagnetic properties of the biological medium at ambient temperature. But, as described in Section IIC, the GUV suspension's temperature increases during the experiments. Therefore, in order to estimate the SAR deviation, ε_r and σ values at different temperatures are measured with an open-ended dielectric probe with the same composition as the GUV suspension except for the lipids. During the measurements, the probe solution is heated using a thermostated water bath. Results show that at 2.45 GHz, a temperature increase from 21 to 43°C induces a variation of 7.8% for the permittivity and 6.1% for the conductivity (Fig. 3). Consequently, a comparable deviation, which is within an acceptable range, can also be assigned to the SAR distribution. With parameters at 21 and 43°C, the calculated mean SAR varies less than 5% from the SAR with the selected permittivity and conductivity.

IV. RESULTS

The mean SAR value calculated in the whole volume is 448 W/kg for 1 W incident power. The SAR distribution computed with the FDTD tool is shown in Fig. 4. The electromagnetic field values in the GUV suspension are higher near the open coaxial applicator (Fig. 4). As the SAR distribution is not homogenous, a 1 × 3 mm magnetic stirrer covered with PTFE (Cole-Parmer, Amex, Austria) is used to homogenize the temperature distribution in the biological medium. During the exposure, the MW incident power delivered to the biological sample is 1.2 W. For this power, the mean SAR value is 538 W/kg for the whole volume. This mean value is evaluated by averaging the SAR values computed in each voxel by FDTD simulation. In Fig. 4, only one vertical plane is represented to illustrate the numerical spatial distribution of the SAR.

Figure 5 presents the SAR distribution in the region where the light beams are crossing the GUV suspension. The SAR value presents an expected variation with a maximum placed in the center of the cuvette near the open ended coaxial. However, the mean value along the light beams (534 W/kg for 1.2 W incident power) is similar to the one obtained for the whole volume.

During the exposure protocol, temperature measurements are recorded. As shown in Fig. 6, the temperature reaches 40°C in 600 s. Using the formula $C\Delta T/\Delta t$, where C represents the medium-specific heat (4187 J/kg/K) and $\Delta T/\Delta t$ corresponds to the initial slope of the temperature versus time, an estimation of the mean SAR is equal to 537 W/kg (for 1.2 W incident power). The measured SAR matches well to the numerical result. The temperature-based SAR measurement neglects the heat loss at the boundary of the cuvette in

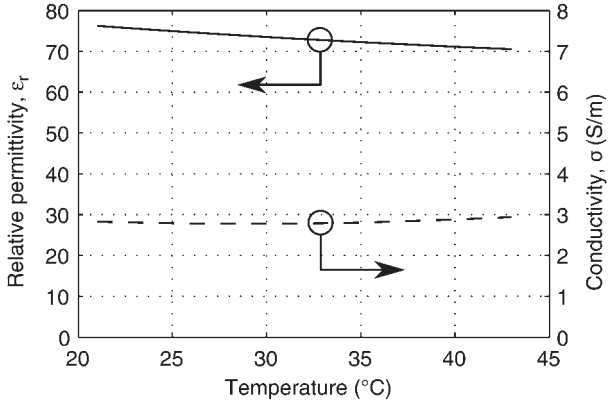

Fig. 3. Variation of the biological sample' relative permittivity and conductivity at 2.45 GHz versus temperature.

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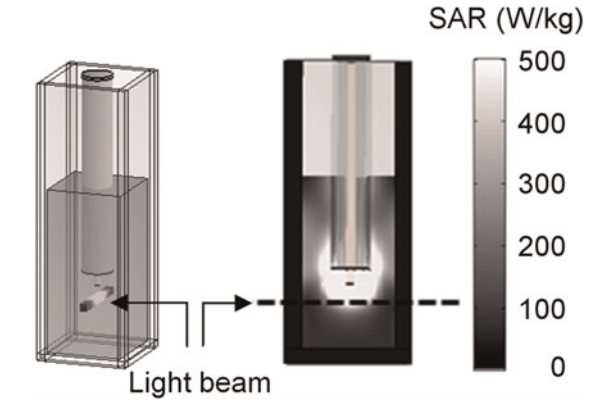

Fig. 4. SAR distribution at 2.45 GHz for 1 W incident power microwaves.

the initial period, which can account for the small differences between the experimental and computed SAR values.

As an illustration of the fluorescence measurements performed with this exposure system, Fig. 7 shows the GP evolution measured for an exposure to 1.2 W MW power. The GP curve presents regular values and variation as the temperature rises from 15 to 40° C.

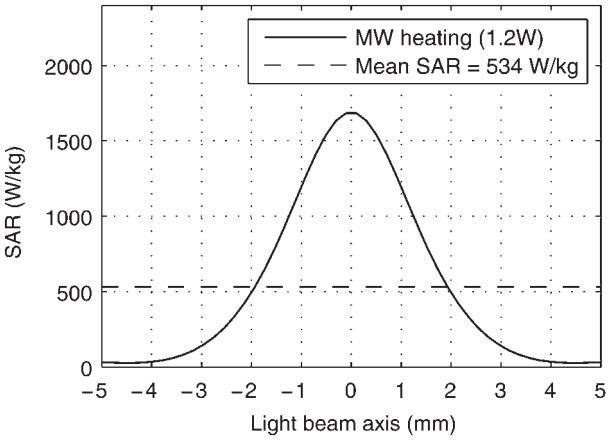

Fig. 5. SAR distribution along the light beam through the cuvette at 2.45 GHz for 1.2 W incident power microwaves.

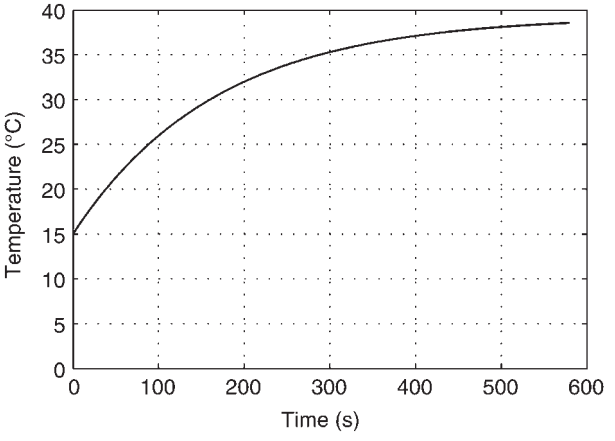

Fig. 6. Measured temperature in the GUV suspension heated by 1.2 W incident power microwaves.

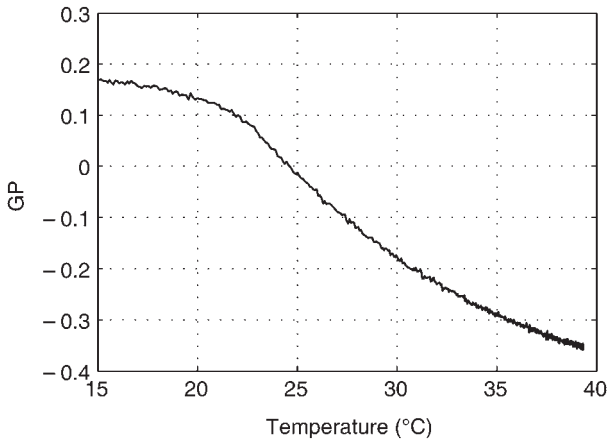

Fig. 7. General polarization of 1, 2-dimyristoyl-sn-glycero-3-phosphocholine GUV suspension heated by 1.2 W incident power microwaves.

This system allows the observation of the GP evolution versus temperature. In the current experiments, the temperature increase is induced by MW exposure. For a similar temperature evolution obtained with classical heating, the measured GP variation was slightly different from the one produced by MW exposure. However, due to the electromagnetic field in-homogeneity, it is difficult to state whether the GP variation is induced only by increase in temperature or also by the electromagnetic field. To determine the possible MW effects on the GP, experiments could be made at a constant temperature with a MW modulated signal that does not induce complementary heating.

V. CONCLUSION

In this work, we presented an exposure system for bioelectromagnetic studies involving real-time fluorescence and temperature measurements on biological media during MW exposure. The combination of a spectrofluorimeter with a coaxial exposure device is designed to study effects of 2.45 GHz MW on biological samples. Simultaneous measurements help to understand molecular mechanisms of the effects and dissociate thermal and non-thermal effects, but add up to the complexity of the exposure device as well as the dosimetry. We presented SAR and temperature evaluations of the 1.2 W MW exposure. The SAR is numerically characterized by the Gaussian-like inhomogeneous distribution strictly related to the position of the coaxial applicator. The SAR averaged in the entire sample volume is close to the 534 W/kg mean value along the fluorescence light beam. The experimentally measured temperature is in the range of 15–40°C and quantitatively compatible with the calculated SAR. The GUV GP is in a unilateral relation to the temperature. Both the SAR homogeneity and the temperature control of the exposure system need consideration in improved designs.

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REFERENCES

[1] De Vequi-Suplicy, C.C.; Benatti, C.R.; Lamy, M.T.: Laurdan in fluid bilayers: position and structural sensitivity. J. Fluoresc., **16** (3) (2006), 431–439.

[2] Deshan, Y. et al.: A floating sleeve antenna yields localized hepatic microwave ablation. IEEE Trans. Biomed. Eng., **53** (3) (2006), 533–537.

[3] Gaber, M.H.; El Halim, N.A.; Khalil, W.A.: Effect of microwave radiation on the biophysical properties of liposomes. Bioelectromagnetics, **26** (3) (2005), 194–200.

[4] Gerbaux, N.A.X.; Glowacki, J.; Ammann, M.J.; Thorne, V.P.: Investigation of the near-field behaviour and SAR for the resonant loop antenna operating at 434 MHz for medical applications. Microw. Opt. Technol. Lett., **47** (6) (2005), 597–599.

[5] Hurter, W.; Reinbold, F.; Lorenz, W.J.: A dipole antenna for interstitial microwave hyperthermia. IEEE Trans. Microw. Theory Tech., **39** (6) (1991), 1048–1054.

[6] Kuster, N.; Schönborn, F.: Recommended minimal requirements and development guidelines for exposure setups of bio-experiments addressing the health risk concern of wireless communications. Bioelectromagnetics, **21** (7) (2000), 508–514.

[7] Laval, L.; Leveque, P.; Jecko, B.: A new in vitro exposure device for the mobile frequency of 900 MHz. Bioelectromagnetics, **21** (4) (2000), 255–263.

[8] Leveque, P.; Dale, C.; Veyret, B.; Wiart, J.: Dosimetric analysis of a 900-MHz rat head exposure system. IEEE Trans. Microw. Theory Tech., **52** (8) (2004), 2076–2083.

[9] Leveque, P.; Reineix, A.; Jecko, B.: Modeling of dielectric losses in microstrip patch antennas – application of FDTD method. Electron. Lett., **28** (6) (1992), 539–541.

[10] Lucio, A.D.; Vequi-Suplicy, C.C.; Fernandez, R.M.; Lamy, M.T.: Laurdan spectrum decomposition as a tool for the analysis of surface bilayer structure and polarity: a study with DMPG, peptides and cholesterol. J. Fluoresc., **20** (2) (2010), 473–482.

[11] O'Connor, R.P.; Madison, S.D.; Leveque, P.; Roderick, H.L.; Bootman, M.D.: Exposure to GSM RF fields does not affect calcium homeostasis in human endothelial cells, rat pheocromocytoma cells or rat hippocampal neurons. PLoS ONE, **5** (7) (2010), 16.

[12] Pickard, W.F.; Straube, W.L.; Moros, E.G.: Experimental and numerical determination of SAR distributions within culture flasks in a dielectric loaded radial transmission line. IEEE Trans. Biomed. Eng., **47** (2) (2000), 202–208.

[13] Savopol, T.; Kovacs, E.; Iacob, N.; Martin, D., Bajenaru, L.; Miclaus, S.: Lipid phase transition temperature modifications by microwave irradiation of model membranes. Eur. Biophys. J. Biophys. Lett., **36**, Suppl. 1 (2007), S86–P135.

[14] Schuderer, J.; Spat, D.; Samaras, T.; Oesch, W.; Kuster, N.: In vitro exposure systems for RF exposures at 900 MHz. IEEE Trans. Microw. Theory Tech., **52** (8) (2004), 2067–2075.

[15] Szoka, F.; Papahadjopoulos, D.: Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc. Natl. Acad. Sci. USA, **75** (9) (1978), 4194–4198.

[16] Taflove, A.; Hagness, S.C.: Computational Electrodynamics: The Finite-difference Time-domain Method, 3rd ed., *Artech House*, Boston, 2005.

[17] Yee, K.: Numerical solution of initial boundary value problems involving maxwell's equations in isotropic media. IEEE Trans. Antennas Propag., **14** (3) (1966), 302–307.

[18] Zhao, J.: Improvement of MMW irradiation uniformity in culture dishes for experiments on MMW biological effects. Microw. Opt. Technol. Lett., **40** (3) (2004), 258–261.

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