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# Rhodamine B Temperature Dosimetry of Biological Samples Interacting with Electromagnetic Fields in Macrosystems

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**Abstract**—Exposing living cells to a certain level of Electromagnetic Field (EMF) might induce some biological effects including temperature elevation. In this paper, we show the dosimetry of exposure systems such as an Open Transverse Electro-Magnetic (TEM) cell allowing the study of the effect of EMF on biological samples exposed to 1.8 GHz signals. Temperature measurements are carried out with a fluoroptic probe to extract specific absorption rate (SAR) values that are compared to numerical dosimetry, based on a FDTD method. To investigate dosimetry at a microscopic level the fluorescence of the temperature dependent dye Rhodamine B was measured with fluorescence microscopy. The results are confirmed by measurements and simulations with a SAR of 13.9 and 11.8 W/kg for 1 W incident power, respectively. Results evidence that the objective working distance of the microscope strongly influence SAR values. After calibration, the fluorescence fits well with the temperature variation measured by the probe.

**Keywords**—dosimetry, fluorescence, exposure systems, microelectrodes, microfluidics.

## I. INTRODUCTION

The interaction of electromagnetic fields (EMF) with living entities has been the subject of a considerable amount of research in the past three decades. The studies concerned a broad panel of topics ranging, non-exhaustively, from the effects of telecommunications systems on health [1], to biomedical applications, such as hyperthermia, electroporation [2], nanosecond pulsed electric [3] fields, as well as microfluidic [4] and lab-on-a-chip devices. To reach rigorous conclusions, these studies require the accurate assessment of EMF parameters such as electric field, temperature and specific absorption rate (SAR) [5]. However, due to the lack of appropriate instrumentation, assessing these exposure parameters is challenging, in particular at microscopic scales.

This paper aims to investigate dosimetry at a microscopic level and further contribute to the characterization of several exposure systems. The microdosimetry is based on the fluorescence intensity of the dye, Rhodamine B (RhodB), that is temperature dependent. To our knowledge, only two papers by Bermingham, Wood et al. have studied Rhodamine B temperature measurements in the context of 900-MHz, tissue exposure in a specific custom-made device [6]. Research and advances on the characterization of exposure systems and the dosimetric technique are presented here, based on the preliminary results of [7]. Biological cells are exposed in this study to different EMF incident powers of 1.8 GHz frequency.

A first complete study is carried out in an open transverse electromagnetic (TEM) cell exposure system that contains a FluoroDish. Although TEM cells have been used as a macrosystem in bioelectromagnetic studies for *in vitro* experiments [1, 8, 9], real-time microscopic observations studies with FluoroDishes are rather uncommon.

In recent years, microsystems, such as thin interdigitated arrays of electrodes with  $\mu\text{m}$  gaps, have been used for impedancemetry real-time monitoring of cellular changes caused by exposure to EMF [10]. In such microsystems, numerical simulations evidenced a significant difference between the SAR values of the whole volume and local SAR at the electrodes and cellular level. Microfluidic and lab-on-chip technologies are currently the subjects of an important research effort. By reducing analyte and reagent volumes and allowing for multiplexing, they have a large range of potential applications in chemical, bio-medical or environmental fields. In most lab-on-chip applications, effective control of temperature is a key factor in the optimization of the chip's performance, especially when biological samples are exposed to EMF [11, 12]. Temperature measurements in a microfluidic channel, with very limited volume of analysis that include biological materials, requires the development of specific protocols as conventional physical probes are not suitable [13]. Although challenging, microdosimetric measurements using the temperature dependent dye, RhodB, at the electrodes/cells level would be highly useful in assessing local exposure parameters.

## II. MATERIALS AND METHODS

### A. EMF Exposure setup

The setup for exposures to EMF of biological samples is shown in Fig. 1. It is composed of a continuous wave (CW) RF signal generator (HP8648B, Hewlett-Packard, USA) connected to a 44-dB gain power amplifier (M.19.40.50, Nuclétudes, France). The amplifier is connected to a bidirectional coupler that monitors incident and reflected powers at the exposure systems input. Depending on the exposure system (e.g. TEM cell), a 50- $\Omega$  high power load can be connected to the output port to absorb transmitted power and match the impedance.

### B. Macro- and micro- exposure systems

Experiments on temperature variation of biological samples exposed to EMF were first conducted with an open TEM cell exposure system (Fig. 1a). This exposure system served as a

reference and for exploring the limitations and defining optimal conditions for the RhodB-based temperature calibration. The TEM cell containing the FluoroDish design and dimensions are detailed in Fig. 2. A 20-mm aperture on the TEM lower plate allows real-time microscopic fluorescence measurements. Fig. 1b illustrates the microsystem based on thin, small gap array of electrodes that was setup for dosimetric characterizations. The microsystem is based on a E-plate that is composed of 16 circular wells with 2x25 interdigitated of 80  $\mu\text{m}$  wide electrodes with 40  $\mu\text{m}$  interelectrodes gap. EMF signals are applied through coaxial cables connected directly on electrodes pins allowing exposures of biological cells placed on the wells bottom between the electrodes. Fig. 1c shows an example microfluidic chip allowing to apply EMF fields in the microwave range that will be investigated with the proposed RhodB technique.

### C. Biological sample labelling

The human glioblastoma cell line U87-MG (brain cancer cells), was used for these experiments. Cells were grown in complete culture medium (MEM supplemented with 10% fetal bovine serum, glutamine, 100 units/ml penicillin, and 100  $\mu\text{g/ml}$  streptomycin; Invitrogen) in an incubator at 37°C and 5%  $\text{CO}_2$ . Cells were subcultured onto plastic FluoroDishes (WPI) at a density of 1–2  $\times 10^6$  cells per ml and allowed to adhere and grow for 48 h prior to experiments. Before imaging cells were loaded with rhodamine B (RhodB)(Sigma) by incubating with 50  $\mu\text{M}$  RhodB in HEPES-buffered salt solution (HBSS) (NaCl 121 mM, KCl 5.4 mM,  $\text{MgCl}_2$  0.8 mM,  $\text{NaHCO}_3$  6 mM, D-glucose 5.5 mM, HEPES 25 mM,  $\text{CaCl}_2$  1.8 mM, pH 7.3) for 1 h at 37°C. Following this incubation, cells were washed with HBSS to remove excess unloaded dye. A volume of 3 ml of HBSS was used for imaging.

### D. Rhodamine B Fluorescence measurements and analysis

RhodB (excitation: 553 nm, emission: 627 nm) was observed by epifluorescence using a solid-state light engine (Spectra 7, Lumencor), coupled to the microscope (DMI6000, Leica) by a 1-mm quartz fiber. Emitted light was separated from excitation with a dichroic beam-splitter (89100BS, Chroma) and selected by emission filters controlled by a filter wheel (MAC 6000, Ludl). Images were captured on an electron-multiplying charge-coupled device camera (EMCCD Evolve 512, Roper) with 512 x 512 pixels. RhodB fluorescence was either measured in solution, by adding 3 ml of RhodB 50  $\mu\text{M}$  in HBSS to a FluoroDish, or in RhodB loaded cells. Images were collected every 15 seconds. ImageJ was used for image analysis. For cell free experiments, fluorescence intensity was measured using a region of interest that covered the whole imaged area. For experiments using adherent cells, images were background corrected and fluorescence intensity was measured in regions of interest manually drawn around cells.

### E. Exposure protocol

For reference and calibration purposes, two optical fiber temperature probes (Luxtron One, Lumasense Technologies, CA, USA) were used (Fig. 1a). The probes were placed in the center of the FluoroDish containing the biological solution and

outside the exposure system, respectively. Temperature variation following exposure to 1.8 GHz EMF at different incident powers was simultaneously measured by the two probes and by the RhodB fluorescence. Images for measuring fluorescence intensity were collected using two microscope objectives, a 63x objective for experiments with cells and a 20x objective for experiments without cells

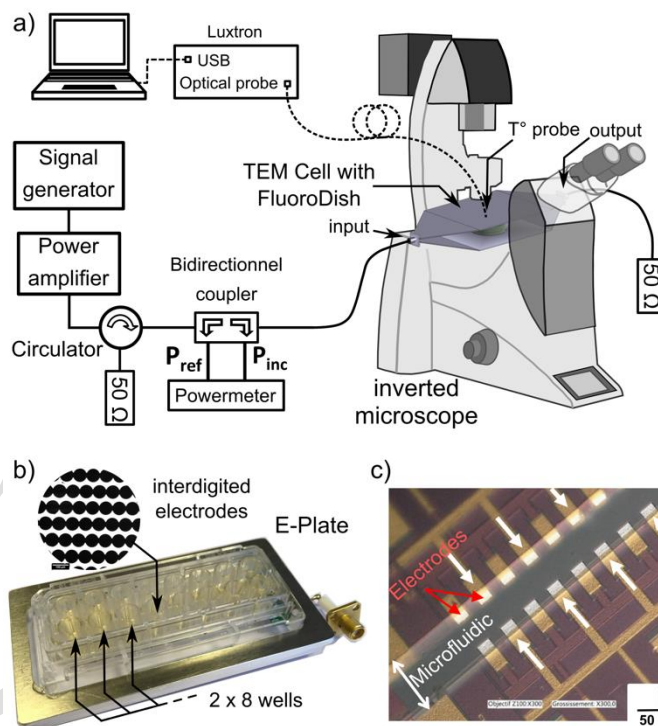


Fig. 1. Biological sample EMF exposure, temperature measurements setup and real-time microscopic measurements of Rhodamine B fluorescence. a) TEM cell with FluoroDish. b) Microsystem with thin interdigitated array electrodes c) Microfluidic chip.

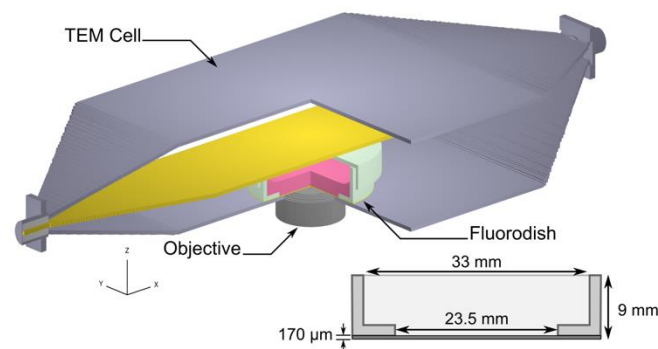


Fig. 2. TEM cell with a FluoroDish that contains the biological sample (numerical model extracted from FDTD software, FluoroDish dimensions).

### F. FluoroDish Numerical modelling and simulations

The numerical dosimetry was performed with a 3D electromagnetic software based on the FDTD method. The TEM and the FluoroDish, with the biological sample, were

modeled as illustrated in Fig. 2. Perfect electric conductors were assigned to model the metallic plates of the TEM cell. 50- $\Omega$  impedance ports were used for the input feed source and the output load. The FluoroDish glass and plastic permittivities were set to 7.5 and 2.5, respectively. The biological buffer solution containing the RhodB was modelled with the following dielectric properties at 1.8 GHz: conductivity of 2.1 S/m, relative permittivity of 76 and mass density of 1000 kg/m<sup>3</sup>. These properties were measured at room temperature using a dielectric probe immersed in the buffer solution (85070E Dielectric Probe, Agilent, USA). The simulations were performed at a frequency of 1.8 GHz. The TEM structure was meshed with non-uniform mesh size. For increased accuracy, the biological sample and the FluoroDish glass bottom were meshed with a grid mesh of 100  $\mu$ m. The top of the microscope objective is also considered in the simulations as a perfect conductor with a lens modelled as a glass with a relative permittivity of 7.5.

### G. Dosimetric assessments

Along with the electric field and temperature, the SAR is a commonly determined parameter to assess exposure of biological samples to EMF. The SAR was extracted from measurements and simulations using the following equations:

$$SAR = C \left. \frac{\partial T}{\partial t} \right|_{t=t_0} \quad (\text{W/kg}) \quad (1)$$

where C is the specific heat capacity of the biological sample, 4186 J/(kg·K) and  $\partial T/\partial t$  is the initial slope of the temperature increase versus time and

$$SAR = \frac{\sigma E^2}{2\rho} \quad (\text{W/kg}) \quad (2)$$

where E is the electric field amplitude (V/m),  $\rho$  is the mass density (kg/m<sup>3</sup>) and  $\sigma$  is the electrical conductivity (S/m) of the biological sample.

## III. RESULTS

### A. Dosimetry of the TEM cell and FluoroDish

Fig. 3 shows the numerical simulations results in terms of SAR distribution (a transversal cut along the centre of the FluoroDish is shown). All SAR values are normalized per 1 W incident power. The SAR value averaged over the whole biological solution volume is 34.2 W/kg. The SAR probe, averaged over a cylinder of 1 mm diameter and height corresponding to the Luxtron probe measurement volume, is equal to 11.8 W/kg. The inhomogeneous SAR distribution within the whole volume is not critical as we conduct temperature measurements and fluorescence acquisitions over small volumes and thin imaging slices. Fig. 4 presents SAR values (whole volume, bottom layer at the cells level, Luxtron temperature probe) versus the microscope objective position. The SAR values considerably increase with the microscope objective position. Thus, the objective working distance must be taken into account for an accurate dosimetry.

Table 1 shows the SAR probe obtained from simulations and measurements for two microscope objectives 63x and 20x placed in contact and at 100  $\mu$ m, respectively. Results show a good agreement between simulations and measurements for both configurations.

### B. Experimental temperature measurements

Fig. 5 shows a typical temperature variation measured with a Luxtron probe and the Rhod B fluorescence intensity. The values of the Rhod B are inverted as the fluorescence intensity decreases with the temperature elevation.

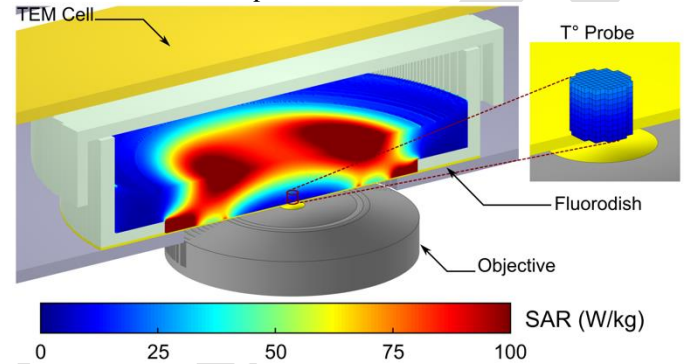


Fig. 3. SAR spatial distribution within the biological sample inside the FluoroDish placed in the TEM cell. In the inset, the SAR Probe 1-mm cylinder for comparisons with Luxtron probe measured SAR. SAR values are normalized per 1 W incident power.

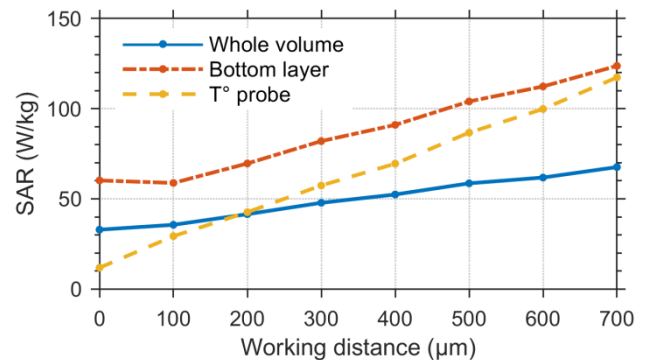


Fig. 4. Variation of SAR values (whole volume, bottom layer at the cells level, Luxtron temperature probe) versus the microscope objective position (0  $\mu$ m and 100  $\mu$ m correspond to the working distance of the 63x and 20x objectives, respectively). SAR values are normalized per 1 W incident power.

Table 1. Localized and simulated SAR probe at 1.8 GHz. SAR values are normalized per 1 W incident power.

Fluoro dish	SAR (W/kg)	
	Measurements	Simulation
Objective 63x (0 $\mu$ m)	13.9 $\pm$ 2.0	11.8 $\pm$ 4.6
Objective 20x (100 $\mu$ m)	31.5 $\pm$ 1.5	29.3 $\pm$ 8.6

The measurements were performed before, during and after the exposure and the biological samples were exposed twice to

the EMF signal with a duration of 15 minutes each. After calibration, the Rhod B fluorescence fits well with the temperature variation measured by the probe.

Experiments were conducted and repeated for several incident powers ranging from 0.5 W to 20 W allowing to calibrate the fluorescence with the temperature probe measurements. For 20 W power, the temperature increases by nearly 10°C and the fluorescence decreases by about 12.5% per °C. Fig. 6 shows a typical fluorescence image of adherent cells labelled with Rhodamine B for temperature measurements.

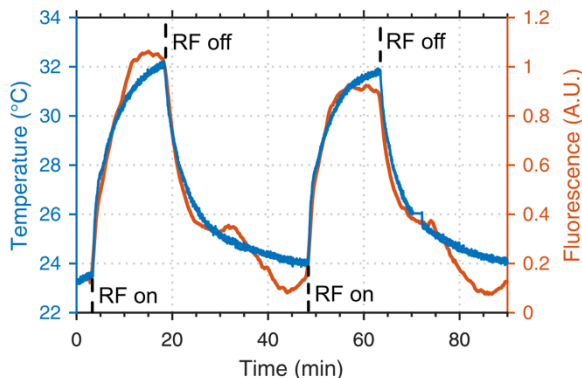


Fig. 5. Experimental results of temperature versus Rhodamine B fluorescence variation obtained with the 20x objective. The biological samples (3ml of 50  $\mu$ M Rhod B in HBSS) are exposed to 1.8 GHz, 20 W incident power for twice a duration of 15 minutes.

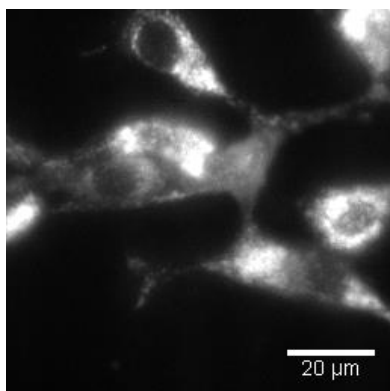


Fig. 6. Fluorescence image of Rhodamine B labelled U87 adherent cells.

### C. Optimizations of the RhodB-based dosimetric technique

RhodB fluorescence measurements at the level of biological cells or thin imaging slices is a rather complex process. We observed that the control of the room temperature is important. For instance, the air-conditioning can cause  $\pm 1^\circ\text{C}$  on room temperature. Also, high temperature variations can modify the microscope focus. The setup of RhodB measurements technique thus requires simultaneous fine tuning and optimization of different parameters related to the EMF

exposure system, the imaging fluorescence acquisitions and biological samples preparation.

### IV. CONCLUSION

In this study, a temperature dosimetric technique based on rhodamine B fluorescence variation was setup and optimized. The technique serves to complete numerical dosimetry results for EMF exposure systems. The exposure setup was based on a FluoroDish, containing 3 ml of HBSS with cells, inserted into the TEM cell. The dosimetry assessment was carried out both numerically, with the FDTD method to obtain SAR distribution values, and experimentally, using a Luxtron probe and Rhod B for temperature measurement. The results are confirmed by measurements and simulations with a SAR of  $13.9 \pm 2.0$  and  $11.8 \pm 4.6$  W/kg for 1 W incident power, respectively. A RhodB fluorescence decrease of about 12.5% per °C was measured. Further investigations that will be presented at the conference, are currently continuing on microelectrodes systems dosimetry such as interdigitated electrodes and microfluidic chips.

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