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Real Time Radio Frequency Exposure for Bio-Physical Data Acquisition

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1. Introduction

Focus of this chapter is the description of the exposure systems used for data acquisition during the exposure to radiofrequency (RF) electromagnetic (EM) fields in bioelectromagnetic investigations. Such a kind of system will be referred to as real-time and can be defined as an EM structure (waveguide or antenna) able to generate and control a known and reproducible EM field and suitable to be used in experiments where data acquisition has to be carried out simultaneously with the exposure (Paffi et al., 2010).

Common real-time applications are usually based on programs that function within a time frame that the user senses as immediate and which require what is called real-time computing (RTC). In biomedicine the real-time concept is applied to both fast calculation of some parameters of biomedical significance (Seong et al., 2011; Wang et al., 2011) and the experimental acquisition of physiological data simultaneously with a correlated event (Li et al., 2010; Voyvodic et al., 2011).

In this context, real-time exposure systems are used to acquire fast biological responses, typically in the order of milliseconds, simultaneously with the exposure to EM fields, in order to study possible health effects due to EM exposure. Usually the responses to be recorded are electrophysiological signals as cellular currents (mA) or membrane potentials (mV); they need to be acquired through a sophisticated instrumentation made of microscopes, patch-clamp recording electrodes, temperature sensors, which fix strict and defined requirements to the design and optimization of the exposure system.

The introduction of this kind of systems has been made necessary due to the need to better investigate the coupling of RF EM fields with learning and memory in both animal models and humans. Neurons, which are at the basis of brain functioning, are electrically active cells. Their electric fields are maintained and controlled by a wide variety of biochemical and metabolic processes. In neurons, fundamental functions such as neurotransmitter release, enzyme activation, intracellular signal transduction, and gene expression are critically dependent on electrical signals. Therefore it has been postulated several times a possible coupling of their electrical activity with a RF EM field.

In the past, several studies were carried out suggesting a possible interference of the EM fields with neurons, but with controversial results and unable to clearly state the molecular basis of this interaction (Sienkiewicz et al., 2000; Wang and Lai, 2000; Dubreuil et al., 2003; Preece et al., 2005). For a review on EM field effects on cognitive functions see (D'Andrea et al., 2003). This is not surprising: experimental investigation of the EM coupling with neurons, in fact, is rather complicated since it involves measurements of the electrical activity of neurons, i.e. the acquisition of electrophysiological recordings of the transmembrane voltage and of the ionic currents. Therefore, a prerequisite of well-posed experiments involving neuronal activity is the possibility to acquire the useful signals simultaneously with the exposure to the RF EM field. Hence, in the last years, there has been a pressing need to design real-time exposure systems.

Some of the most recent results achieved with the aid of ad hoc designed real-time systems state that there is no significant coupling between low intensity RF EM fields and specific membrane channels, i.e. biological membrane proteins which allow the passive movement of ions from the external to the internal of the cell and vice-versa (Marchionni et al., 2006; Platano et al., 2007). Other studies, investigating the effects of low intensity RF EM fields on electrical activity in rat hippocampus, one of the major component of brain, (Tattersall et al., 2001) described an effect on synaptic transmission; however the effect reported has been later explained by localised heating produced by interaction of the RF fields with the recording and stimulating electrodes. This contradiction emphasizes the fundamental issue related to the proper design of real-time systems.

Although real-time investigations have been gaining increasing interest in the last ten years, a complete and systematic framework on specific requirements of RF exposure systems when applied to real-time data acquisition is still lacking.

Aim of this chapter is to merge the well-assessed design procedure of RF exposure systems (Kuster and Schönborn, 2000; Paffi et al., 2010) with the requirements emerging from real-time investigations, thus providing a reference work on this segment of knowledge.

In the chapter, at first a description of what is an exposure system and which are the guidelines to optimally design it are given. Then, it is provided how to adapt the general rules for exposure system design to real-time systems ones, which, of course, have very specific requirements. Finally, a complete and updated review of the real-time systems available in literature is provided.

2. Basic requirements for the exposure systems

Since the early development of mobile telephony, concern aroused on the possibility of the exposure to EM signals in the RF range inducing hazardous effects on population health. Consequently, a number of experiments were carried out (Lin, 2004; Valberg et al., 2007) aiming at the evaluation of possible biological effects of RF EM fields. The obtained results were often conflicting and difficult to replicate, mostly due to inaccurate dosimetry and to a lack of well-characterized exposure conditions.

Since the 90s, the need of a common approach to the bioelectromagnetic research became evident, as pointed out by a number of workshops and publications yielded by the scientific community. In 1994, the Wireless Technology Research (WTR) held a workshop to highlight

the appropriate directions for the development of exposure systems (Carlo, 1998). In 1996, the EMF Project of the World Health Organization (WHO) fixed these concepts and emphasized the importance of an accurate dosimetry in all scientific studies (WHO, 1996). Such items, together with a deep discussion on quality assurance, were the main arguments of two European Cooperation in Science and Technology (COST) workshops: "Exposure systems and their dosimetry", in Zurich, in February 1999 (Schönborn et al., 1999; Bitz et al., 1999) and "Forum on future European research on mobile communication and health", in Bordeaux, in April 1999 (Kuster and Schönborn, 1999).

In 2000, recommended minimal requirements for exposure systems, in order to obtain reproducible and scientifically valuable results, were synthesized in (Kuster and Schönborn, 2000). Basically, two classes of requirements can be identified: biological and EM ones. Biological requirements are dictated by the laboratory equipment, the experimental procedures, and the environment; the EM ones define the exposure parameters (frequency, amplitude, modulation scheme of the signal), the characteristics of the induced EM field (polarization, intensity and homogeneity), and the "dose" at the location of the biological sample.

More in detail, to meet the biological requirements, the exposure system must allow for the exposure of the required number of samples or animals; in the meantime, the environmental conditions required by the specific experiment must be guaranteed (Kuster and Schönborn, 2000). As for the EM requirements, (i) the delivered signal must be precisely defined in terms of frequency, amplitude, and modulation scheme; (ii) the electric and magnetic field strength and polarization must be known at the location of the exposed biological target; (iii) the fields inside the sample should be homogeneous; (iv) any electromagnetic interference (EMI) and/or electromagnetic compatibility (EMC) issue must be avoided (Kuster and Schönborn, 2000). Moreover, the system should guarantee the monitoring of relevant parameters, such as the temperature and the delivered power, during the experiment and the possibility of carrying out sham exposures and blind experiments. In the sham exposure, a number of cell cultures or animals are subjected to environmental conditions identical to those of the group of the exposed subjects, except for the exposure. Data collected by the sham group are thus used as negative control. This is an unavoidable procedure to prevent the incorrect attribution to the RF exposure of an observed effect, which might be due to other factors, e.g. to the stress of the animals (Samaras et al., 2005). In blind experiments the exposure setup provides an automated procedure to assign the real and the sham exposure to two different groups of samples. In this way, experimenter polarization errors are removed, since he/she does not know which samples are really exposed.

Finally, the exposure system should be easy to be handled even by non-engineering personnel and its cost should be reasonable (Kuster and Schönborn, 2000). To maintain low the cost of the whole exposure setup, in particular of the signal amplifier of the generation chain, the EM structure should be designed to have the power efficiency as high as possible. The power efficiency is defined as the mean EM power absorbed by the unitary mass of sample per unitary input power feeding the EM structure.

Biological requirements are usually the most limiting ones on the exposure setups. As example, the kind of experiment may dictate the equipment needed and the environment; the overall duration of the experiment and the number of samples or animals necessary for statistical significance may strongly influence the choice of the EM structure employed as

exposure system. For this reason, a lot of different exposure systems have been published during the past ten years (Lovisolo et al., 2009; Paffi et al., 2010, 2011), according to the great variety of experimental endpoints and protocols.

Moreover, during the past years, several cooperative research programmes, e.g. the European projects: PERFORM A, PERFORM B, and RAMP2001 have been carried out. The necessity of conducting a coordinate research activity in laboratories of different countries has arisen the issue of whether standardized exposure systems and protocols should be used. This was one of the topics of the Workshop: "EMF health risk research lessons learned and recommendations for the future" held in Monte Verita in November 2005. The outcome of the work stated that, due to different endpoints and protocols used in bioelectromagnetic investigations, exposure setups could not be standardized. In the same time, strong quality control on dosimetry is mandatory to assure the repeatability and reproducibility of results even when different exposure systems are used (Samaras et al., 2005; Lovisolo et al., 2009). Although the concept of using a standardized exposure system for all types of studies is not possible, the choice, design, and characterization of the system can be standardized to obtain repeatable and reproducible results from biological experiments (Paffi et al., 2010).

3. Exposure systems for experimental investigations

Well-defined and characterized exposure conditions are necessary for health-risk assessments (WHO, 1996). Unless the "dose" is accurately known, the results of bioelectromagnetics studies will have little value for determining exposure thresholds for health risk or for developing exposure limits in standards; therefore, great research effort has been devoted to reproduce well-defined exposure conditions in the last twenty years.

For the EM exposure in the RF range, the "dose" is considered the incremental absorbed EM energy per unit mass (ICNIRP, 1998), given in terms of Specific Absorption Rate (SAR), which is defined as follows:

$$\text{SAR} = \frac{d}{dt} \left(\frac{dW}{dm} \right) = \frac{d}{dt} \left(\frac{dW}{\rho dV} \right) \quad (1)$$

where dW is the incremental energy dissipated in an incremental mass dm included in an incremental volume dV and ρ is the mass density. SAR (measured in W/kg) can be calculated directly from the electrical loss, which is proportional, through the conductivity σ , to the mean square of the electric field strength E locally induced in the tissue (ICNIRP, 1998), as follows:

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

To have a precise and accurate knowledge of the SAR distribution inside the exposed sample during the experiments, exposure systems have to be designed, fabricated, and characterized, in order to meet all the requirements discussed in Section 2. In fact one cannot employ commonly used EM sources, such as phone cells or microwave ovens, as done in several cases, especially in studies published prior to 1990s, since in these cases the dose absorbed by the target will be completely unpredictable.

The aim of an exposure system is to expose a biological target to a well-controlled and reproducible SAR, while maintaining appropriate environmental conditions and allowing the monitoring of some parameters during the exposure, such as temperature (Lin et al., 2009).

An exposure system is a complex structure used for allocating the biological samples during the exposure phase in a biological experiment. It encompasses:

1. the EM structure emitting the EM field;
2. the chain for the generation and control of the EM signal;
3. the system for maintaining the environmental parameters needed for the well-being of the exposed samples;
4. the software for the system management.

Although the exposure system is the complex structure, consisting of several parts, guaranteeing the desired exposure conditions of the sample, in the following, with the term exposure system, we will refer to its main part: the EM structure emitting the field, which can be an antenna, a waveguide, a resonator, as described in detail in Section 6.

Essentially it is possible to find exposure systems for *in vitro* and for *in vivo* biological experiments. In the first case, the biological target is constituted by cultured tissues or cells contained in sample holders such as flasks, tubes, Petri dishes, multiwells, and cuvettes. In the second case, the target is a specific animal or part of it, e.g. head, eyes, and ears.

In vitro experiments are an accepted method for determining, at cellular level, the SAR threshold for the onset of biological effects and damage (Guy et al., 1999). One of the main limitations of *in vitro* experiments is that the effects observed in cell cultures do not necessarily imply any impairment at physiological level. For this reason, *in vivo* experiments, carried out on living animals, such as mice, rats, and rabbits, are of great scientific interest.

In vivo exposure systems could be rather different from the *in vitro* ones (Lin et al., 2009). This is mainly due to the larger dimensions of animals with respect to the *in vitro* sample holders. Moreover, animals tend to move inside the exposure system, unless they are restrained inside special containers, as required in some experiments. Finally, requirements for the maintenance of the environment conditions depend on whether cells or animals have to be exposed. In the first case, generally an incubator is needed; in the second case, ventilation, food and water should be provided, especially for long-term exposures.

4. The concept of real-time acquisitions in bioelectromagnetic investigations

Real-time data acquisition in biomedicine is employed in a lot of different applications, both *in vitro* and *in vivo*. Real-time monitoring of chemical reactions (Shutes and Der, 2005), gene expression (Gubern et al., 2009), drug release from nanoparticles (Chouhan and Bajpai, 2009) are significant examples of *in vitro* applications. As for *in vivo* investigations, the real-time acquisition is usually based on non-invasive imaging techniques (Ohtani et al., 2010; Li et al., 2010; Voyvodic et al., 2011) suitable to be applied to living animals.

The common issue in both *in vitro* and *in vivo* real-time experiments for biomedicine is that the experimental acquisition of physiological data is performed simultaneously with the correlated event.

In bioelectromagnetic investigation the concept of real-time is applied to experimental acquisition of data, which have to be collected simultaneously with the EM exposure, as schematically reported in Fig. 1.

In real-time experiments, the time slot of data acquisition often starts before and ends after the exposure period. The data acquired before and after the exposure are compared with those collected during the exposure. The pre-exposure data are used as a negative control; the post-exposure ones are used to identify possible long term or permanent modifications induced by the RF field on the observed parameter. Conversely, in off-line experiments data acquisition is carried out at the end of the exposure (Fig. 1) and the negative control is obtained from a parallel session (sham exposure) where biological samples are subjected to environmental conditions identical to those of the group of the exposed ones, except for the exposure. On the contrary, in real-time experiments, the same sample, observed in different time slots, is used as its own control. This concept is better explained in Fig. 2, showing the current through an ionic channel recorded before (black trace on the left), during (red trace on the center) and after (black trace on the right) the exposure.

From Fig. 2, a clear effect of the RF field is observable only comparing the three segments of the recorded current. Being reversible, such an effect would not be detected in off-line experiments.

Therefore, the main advantage of real-time acquisition is that it enables the experimenter to identify possible reversible or cumulative effects, otherwise difficult or even impossible to be detected. Moreover real-time experiments permit time saving, since one does not have to wait for the end of the experiment before collecting and analyzing the biological data.

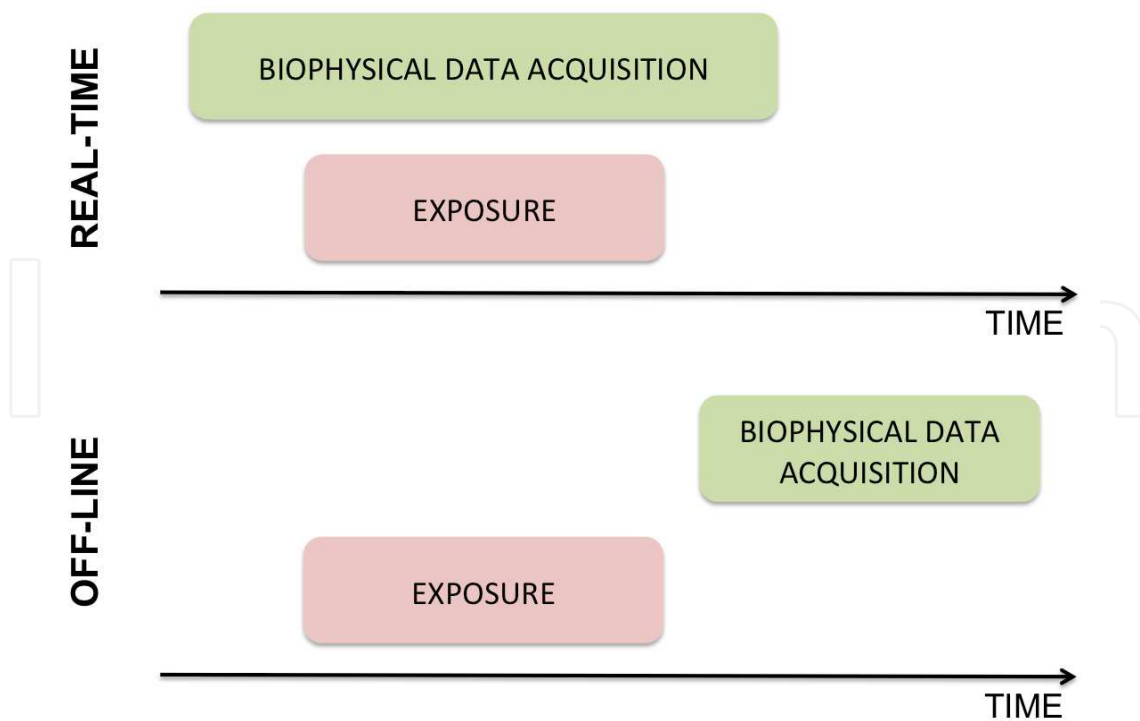


Fig. 1. Schematic representation of the time scheduling of exposure and data acquisition in real-time and off-line experiments



Fig. 2. Single channel current recorded before, during, and after the exposure to RF field

Real-time experiments have revealed a powerful tool especially in the investigation on possible modifications of the activity of neuronal cells or cell networks during the exposure, through electrophysiological recordings, as evident from Table 1, where information on published *in vitro* real-time experiments is summarized.

| SAMPLE | OBSERVABLE | EXPOSURE | TECHNIQUE/ INSTRUMENT |
|------------------|------------------------------------|--|-----------------------------|
| Neuronal cells | Ionic current; Membrane voltage | CW 900; 1800 MHz; GSM900 | Patch-clamp recording |
| Organ of Corti | Ionic current | GSM900; GSM1800; UMTS | Patch-clamp recording |
| Brain slices | Field potential | CW 700 MHz; Pulsed 9.2 GHz; CW 1.8 GHz | Extracellular recording |
| Heart slices | Field potential | Pulsed 9.2 GHz | Extracellular recording |
| Skeletal muscle | Muscle contraction | CW 0.75-1.12 GHz | Force transducer |
| Chromaffin cells | Catecholamine release | CW 0.75-1.12 GHz; CW 1-6 GHz | Electrochemical detector |
| Liposomes | Enzymatic activity | CW 2.45 GHz | Spectrophotometer |

Table 1. Schematic description of the *in vitro* real-time experiments in the literature, in terms of samples, observables, RF signals and techniques used for the experimental activity

The most used experimental samples are excitable cells and tissues since they are likely to be sensitive to EM stimulation and to display reversible effects. They include single neuronal cells (Liberti et al., 2004; Platano et al., 2006; Marchionni et al., 2005; Paffi et al., 2007), neuronal networks (Tattersall et al., 2001; Pakhomov et al., 2003; Koester et al., 2007; Merla et al., 2011), inner ear hair cells of the organ of Corti (El Ouardi et al., 2011), and muscles, both skeletal (Lambrecht et al., 2006) and cardiac (Pakhomov et al., 2000). The observables are mostly electrophysiological parameters, such as the ionic current and the membrane voltage at the level of single cell, or the field potential generated by a group of interconnected neuronal cells. This latter observable is largely used in the studies on synaptic plasticity, the basic mechanism underlying high cognitive functions, such as memory and learning. However, even chemical parameters can be analyzed, such as the enzymatic activity, through a spectrophotometer (Ramundo-Orlando et al., 2004), or the neurotransmitter release, through an electrochemical detector (Hagan et al., 2004; Yoon et al., 2006). The techniques employed for the acquisition of the electrophysiological traces are patch-clamp (Neher and Sakmann, 1998) and extracellular recordings. Both of them require the use of microelectrodes near the membrane cell (extracellular recordings) or in close contact with it (patch-clamp). The EM signals mostly used in the experiments are

continuous waves (CW) or modulated signals, especially at the frequencies typical of mobile communication. In one case (Pakhomov et al., 2003), the effects of a pulsed signal at 9 GHz on brain slices were investigated.

Due to the complexity of the experimental procedure, generally exposure systems for real-time experiments are more difficult to design since, beside the requirements typical of any exposure system (see Section 2), they have to meet additional and demanding requirements (see Section 5).

In particular, for *in vivo* experiments, only two real-time systems are present in the literature from 1999 until now (Testilier et al., 2002; Arima et al., 2011). Both of them are based on a simple EM structure (i.e. an antenna), while the real-time monitoring of the biological parameter is obtained through highly invasive techniques. In Testilier et al. (2002) neurotransmitter levels in a rat brain were monitored during the exposure through an invasive microdialysis technique; in Arima et al. (2011) the microcirculation of the rat brain during the exposure is observed through a cranial window on the skull.

The reason of such a reduced number of *in vivo* studies can be found in bioassays typically used to measure the effects of EM fields that require the employment of complex procedures and instrumentation. Biological data are often collected after the animal sacrifice and the subsequent removal of organs and tissues for the analysis.

5. Design procedure

In (Paffi et al., 2010) a standardized procedure was proposed for reaching the optimum exposure design. It consists of seven main steps, as schematically shown in Fig. 3.

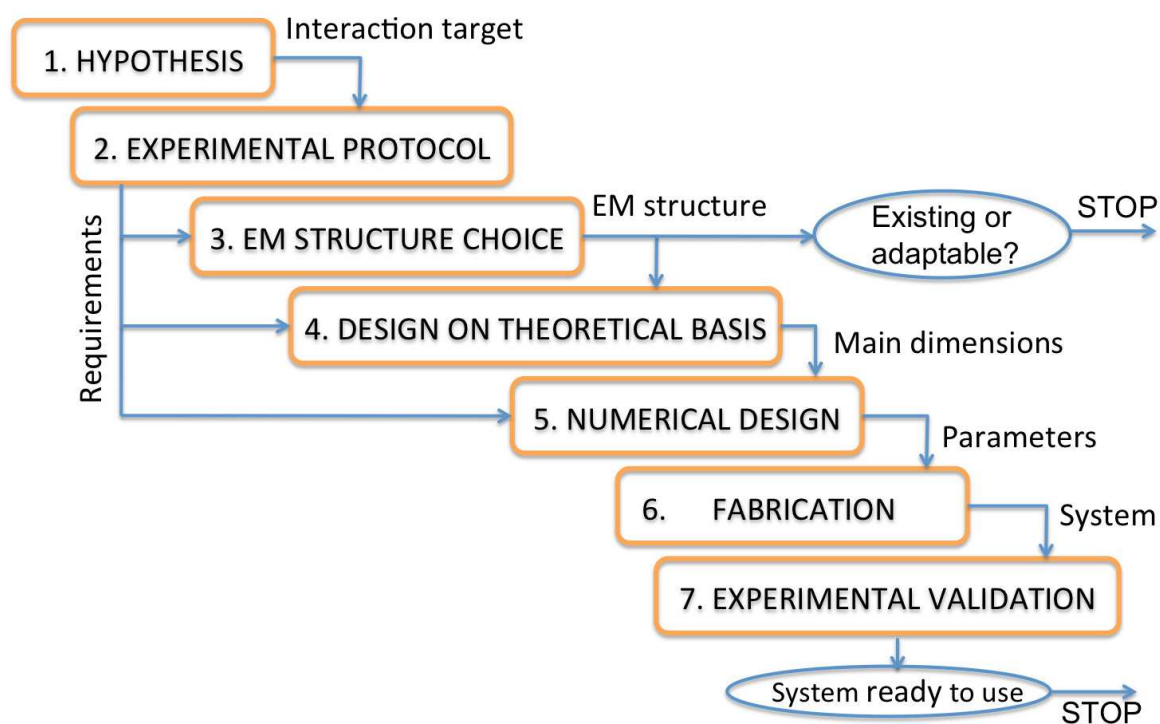


Fig. 3. Flow chart of the standardized procedure for the design and characterization of exposure systems

Before carrying out the experimental activity, a hypothesis is formulated (step 1), leading to the choice of an appropriate biological system to be exposed. It can be a particular kind of cell, a tissue, an organ, or a whole organism, as an animal. The experiment to test the hypothesis is then defined (step 2), including biological models, endpoints, techniques, and exposure parameters. The outcome of the analyses carried out during this step determines all the requirements, both biological and EM, of the exposure system. Moving from biological protocol and requirements on exposure parameters, the most suitable EM basic structure is chosen (step 3). If a system meeting all the requirements for the planned experiment is already present in the literature, one may decide to use that system as it is or to adapt it to new constraints; otherwise, the chosen EM structure must be first dimensioned on theoretical bases (step 4). Generally, the basic structure must be modified in order to meet all the biological and EM requirements. Thus, an iterative process of adaptation and optimization of the system (step 5) takes place using numerical tools, leading to the final design parameters (dimensions, materials, sample position, etc.). The next two steps are the manufacture (step 6) and the experimental validation (step 7) of the exposure system. Measurements should be conducted firstly with the empty structure; then it has to be loaded with the biological sample to validate the behavior of the system and to experimentally evaluate the dosimetry, i.e. the SAR distribution in the sample. If acceptable agreement between measurement and simulation is not achieved, one must return to steps 5, 6, or 7 depending on the hypothesized cause of mismatch.

As already noted, biological requirements represent a crucial point for the design of an exposure system since they could be the most limiting ones, especially when a particular equipment and protocol procedures are needed, e.g. in real-time experiments.

5.1 Special requirements for the design of real-time systems

Although the procedure (Paffi et al., 2010) for the design and characterization of the exposure system is the same regardless of the particular kind of analysis (real-time or off-line), real-time acquisitions impose additional biological requirements to the system. In particular, when dealing with the electrophysiological recordings, they can be summarized in the easy access to the sample (i.e. through the microscope, the electrodes, and the perfusion apparatus) and the minimum coupling between the EM field and the acquisition equipment, in order to avoid artifacts.

For the correct placement of the electrodes, the sample must be illuminated and observed through a microscope. Moreover, in some experiments, the biological sample, e.g. brain slices, has to be perfused to preserve its structure and function. For these reasons, in order to easily reach the sample, the exposure system must be either an open structure or a closed one modified with holes.

The microelectrodes for electrophysiological recordings are usually made of glass filled with a saline solution with a metallic wire inside. Being the biological cultures exposed to CW or modulated signals, especially at the frequencies typical of mobile communication, the real-time acquisition implies the possibility of EM coupling between the electrodes and the electric field, with consequent artifacts on the recorded trace. Another possible source of artifacts is the interference between the EM field generated by the system and the whole acquisition setup. To minimize the coupling between the field and the electrode, the direction of the electric field

must be almost orthogonal to the electrode. Moreover, to avoid interference of the field with the laboratory equipment, in principle, the EM field should be confined in a closed structure. However, even open structures can be used, provided that the electric and magnetic fields sharply decay with the distance from the system.

Finally, the presence of metallic and/or dielectric objects (microscope objective, lamp, electrodes, perfusion apparatus), placed very close to the region where the sample is exposed (see Figure 4), may modify the field distribution and thus the whole behavior of the system. This kind of coupling must be carefully taken into account during the numerical optimization of the system in order to minimize it.

The different solutions adopted to meet such demanding requirements will be described in Section 6.

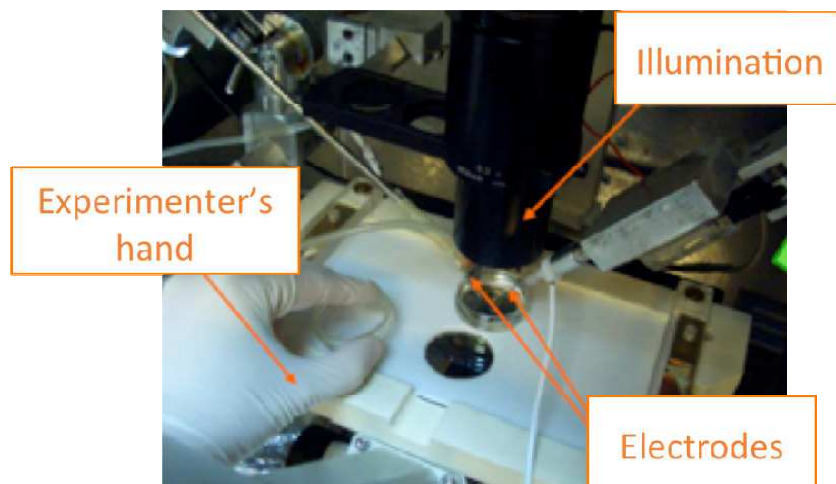


Fig. 4. Picture of a planar exposure system used for electrophysiological recordings from brain slices (Paffi et al., 2007). Arrows highlight the presence of different objects placed very close to the system surface during the experiments.

6. Classification of the exposure systems

6.1 General classification

Due to the great variety of biological targets and protocols, a number of different exposure systems have been adopted in the experimental investigation, both *in vivo* and *in vitro*.

Despite their peculiarities, the exposure systems can be classified on the basis of the employed EM structure into three main groups: radiating, propagating and resonant systems, as proposed in (Lovisolo et al., 2009; Paffi et al., 2010).

Conversely, for what concerns the biological experiment, the main classification is between the *in vitro* and *in vivo* setups, as already discussed in Section 3.

In turn, with respect to the experimental protocol, *in vivo* systems have been classified in two main classes, depending on whether the exposure involves the whole body or is focalized into a specific organ (Paffi et al., 2011). An additional criterion of organization is the possibility for the animals to freely move during the exposure (Paffi et al., 2011). In this case, animals are generally housed in their own cages, provided with food and water.

Otherwise animals are restrained inside plastic holders, to guarantee their relative position and orientation with respect to the electric and magnetic fields.

Conversely, *in vitro* exposure systems have been classified (Paffi et al., 2010) in two different groups: off-line and real-time, depending on the kind of data acquisition they have been designed for. This latter classification has not been adopted for the *in vivo* setups, since real-time acquisitions are very uncommon for *in vivo* experiments, as pointed out in Section 4.

Proposed classification is reported in Figure 5, together with the number of systems belonging to each category and published in international journals since 1999.


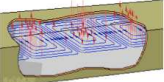
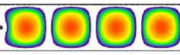
| <i>Experiment</i> | | | <i>EM structure</i> | | |
|-------------------|------------|---------------|--|--|--|
| | | |  radiating |  propagating |  resonant |
| <i>IN VIVO</i> | local | restrained | 19 | - | - |
| | | freely moving | | | |
| | whole body | restrained | 14 | 12 | 7 |
| | | freely moving | | | |
| <i>IN VITRO</i> | Off-line | | 5 | 24 | 12 |
| | Real-time | | 1 | 11 | 1 |

Fig. 5. Classification of the exposure systems. For each category the number of systems published from 1999 is reported

Among the 52 *in vivo* systems, those used for local exposure (19) are exclusively based on radiating structures. In particular, they are small antennas placed close to the target organ (brain, ear, eye) to induce significant and localized power absorption (Paffi et al., 2011). To reduce the cost of the experiment, a single antenna can be used to simultaneously expose several animals, if they are arranged in a sort of carousel around it (Schönborn et al., 2004). Generally, animals locally exposed are restrained within plastic holders to obtain a more accurate and precise SAR distribution, even though body-mounted antennas (Bahr et al., 2007) become necessary for the well being of animals when the exposure is prolonged.

For the 33 whole body exposure setups, the uniformity of SAR absorbed by animals of the same group and within each animal was a critical requirement (Paffi et al., 2011). This is particularly difficult to achieve especially for large-scale experiments. In this case, radiating structures (14 systems found in the literature) could be particularly suitable since a lot of bodies can be simultaneously exposed to a plane-wave equivalent field (Paffi et al., 2011).

However, the power efficiency is quite low (Paffi et al., 2011). Propagating systems are mainly based on rectangular or radial waveguides (Hansen et al., 1999), whereas resonant cavities are mainly obtained by shorting radial waveguides through metallic rods (Balzano et al., 2000). These structures are characterized by high volume efficiency, i.e. a lot of bodies (up to 65 animals) can be simultaneously exposed in a limited space. However, due to the importance of a correct body positioning, animals are restrained within plastic cylinders. Therefore, resonant cavities are suitable for chronic long-term (days or months) exposures only if the exposure is limited to a few hours per day, as done in some two years bioassay experiments (e.g. PERFORM A).

A separated mention must be made of reverberating chambers (Corona et al., 2001). Inside those systems a statistically uniform field can be obtained and a large number of animals can be simultaneously exposed in a habitat simulating the usual one of animals (Wu et al., 2010). These are the reasons why they are suitable for large-scale, long-term experiments.

Moving to the *in vitro* systems, among the 54 off-line setups found in the literature, the most used (24) are propagating structures (Paffi et al., 2010), such as Transverse Electromagnetic (TEM) cells and rectangular waveguides. The main advantages of propagating structures are the EM field uniformity inside the biological sample and versatility. With a proper dosimetric characterization, propagating systems have been used to expose different volumes of various kinds of cells inside sample holders, such as multiwells, Petri dishes, and flasks (Paffi et al., 2010). Also resonant systems have been largely used (12) in off-line *in vitro* experiments (Paffi et al., 2010). They are closed and compact structures, such as short-circuited waveguides; thus both the active and the sham systems easily fit inside a commercial incubator, often needed for maintaining the optimal environmental conditions (Schuderer et al., 2004). Due to the onset of a standing wave inside resonant systems, the power efficiency is generally high, but the positioning of the sample is critical, because of the extremely localized regions of field uniformity (Paffi et al., 2010). On the contrary, radiating systems, usually consisting of commercial or ad hoc fabricated antennas, present low power efficiency, but generally allow for the simultaneous exposure of a lot of samples. However, they need EM compatible arrangements due to the lack of enclosures confining the emitted field (Paffi et al., 2010).

Regarding the 13 real-time *in vitro* systems, they are mostly based on propagating structures, with the only exception of one resonant (Hagan et al., 2004) and one radiating system (Yoon et al., 2006). Indeed, propagating structures are generally the most versatile and adaptable to the additional constraints imposed by real-time acquisition.

To meet such requirements, two main solutions have been proposed in the literature (Paffi et al., 2010): closed structures modified with holes for sample observation and data recording and open systems specially designed to have the field confined in a small volume around the surface. This latter solution also implies high values of power efficiency (Liberti et al., 2004; Paffi et al., 2007).

A detailed description of different kinds of real-time systems, together with their main features, will be given in Section 6.2.

6.2 Real time systems in the literature

According to the classification described above, real-time *in vitro* systems published in international journals from 1999 up to now, have been organized in a schematic view in Table 2.

| EXPOSURE SYSTEM | | REFERENCE | |
|-----------------|---------------------|---------------------------------------|--|
| REAL-TIME | PROPAGATING | <i>Modified TEM cell</i> | (Linz et al., 1999); (Merla et al., 2011). |
| | | <i>Modified rectangular waveguide</i> | (Linz et al., 1999); (Pakhomov et al., 2000); (Lambrecht et al., 2006); (Koester et al., 2007). |
| | | <i>Parallel plates</i> | (Tattersall et al., 2001). |
| | | <i>Coplanar waveguide</i> | (Liberti et al., 2004); (Paffi et al., 2007). |
| | | <i>Modified stripline</i> | (Ramundo-Orlando et al., 2004). |
| | | <i>Fin-line</i> | (El Ouardi et al., 2011). |
| | RESONANT | <i>Modified rectangular waveguide</i> | (Hagan et al., 2004). |
| RADIATING | <i>Horn antenna</i> | (Yoon et al., 2006). | |

Table 2. Classification of the real-time *in vitro* systems published from 1999

6.2.1 Propagating structures

Propagating systems for real-time studies are generally closed structures modified with holes for sample observation and perfusion, and for online monitoring of biochemical or biophysical parameters.

They are mostly TEM cells and rectangular waveguides as described in (Linz et al., 1999), where the electrophysiological activity of myocyte cells was recorded, through the patch-clamp technique, during the exposure to 900 MHz and 1800 MHz CW. The systems were equipped with two holes: one in the top wall to insert the recording electrode and the other in the bottom to observe the sample with a microscope. The solution adopted to avoid interference between the electric field inside the guide and the metallic wire of the patch-clamp electrode was the elongation of the glass microelectrodes used for sealing the cell in order to place the wire outside the exposure device. The power efficiency was not bad: 1.66 (W/kg)/W at 900 MHz and 3.16 (W/kg)/W at 1800 MHz.

A rectangular waveguide (Lambrecht et al., 2006), operating between 0.75–1.12 GHz, was also used to measure, through a force transducer, the effects of the RF field on skeletal muscle inserted in a bath placed in the center of the waveguide. The waveguide was modified with slots in the walls to allow connection with measurement, control, and stimulating devices outside the system. The power efficiency was good (> 3 (W/kg)/W) and SAR homogeneity, around 79 %, was in line with minimal requirements for *in vitro* experiments (homogeneity > 70 %). A modified rectangular waveguide was used for delivering CW or Universal Mobile Telecommunications System (UMTS) signals to neuronal networks during electrophysiological recordings from multiple electrodes (Koester et al.,

2007). The neuronal network was cultivated on a microsensor chip fitted into a recess in the guide to avoid short circuiting the measuring probes while exposing the neuronal cells.

For a similar experiment at 1800 MHz, a different solution was proposed in (Merla et al., 2011). The exposure system consisted of a TEM cell open in correspondence of the lateral walls. The multiple electrode array used for the electrophysiological recordings was inserted in a circular hole in the TEM cell bottom plate. The power efficiency of 3.2 (W/kg)/W was comparable to that of the closed systems described above.

A waveguide terminated with an exposure cell containing the sample was used to expose heart slices (Pakhomov et al., 2000) and brain slices (Pakhomov et al., 2003) to high-power microwave pulses (repetition frequency 9.2 GHz). The system had an extremely high efficiency of 3.3 (kW/kg)/W which decreased about twofold per millimeter with distance from the waveguide aperture. In this case, the sample was used as the load of the guide; the impedance matching was achieved through a sapphire matching plate to maximize the power absorbed by the slices.

To record brain slices electrophysiological activity simultaneously with the exposure to a 700 MHz field, Tattersall *et al.* (Tattersall et al., 2001) utilized a waveguide made of two parallel plates. In this case, as in (Linz et al., 1999), the top and bottom plates of the guide had holes to observe the sample and insert both stimulating and recording electrodes. The coupling between the electric field and the electrodes was not avoided since the electrodes were placed at an angle of about 45° to the electric field (Misfud et al., 2007). The estimated efficiency value was lower than 0.03 (W/kg)/W.

A completely different solution, based on an open coplanar waveguide was adopted in studies involving patch-clamp recordings from neuronal cells (Liberti et al., 2004) and field potential recordings from brain slices (Paffi et al., 2007). Both systems operate in the 800–2000-MHz frequency band and differ from each other by the distance between the central and lateral conductors because of the different size of biological samples to be exposed to the EM fields. The open planar geometry and the transparent glass substrate allowed easy access to the samples through the microscope and the electrodes; the electric and magnetic fields were confined in a small volume around the surface that guaranteed the avoidance of interference with the data acquisition setup and high efficiency values (> 17 (W/kg)/W) at all frequencies.

To perform patch-clamp recordings from the ear hair cells of Corti organ, three exposure systems were described in (El Ouardi et al., 2011), operating at 900 MHz, 1.8 GHz and 2 GHz, respectively. All of them are based on the concept of the fin-line: a quasi-planar transmission line structure embedded in a metallic rectangular waveguide. In this case, the two fins were placed in the magnetic field plane of the waveguide. The chamber containing the samples was placed onto the two fins with a slot in between, where the exposure field concentrates, and inserted inside the guide through a circular opening in the top plate. This opening was also used to insert the electrodes and the microscope objective during the experiments. The efficiency was very high (> 40 (W/kg)/W for all systems), with a good homogeneity of 90 %. The drawback was the narrow band that imposed the fabrication of three different systems, one for each frequency band of interest (El Ouardi et al., 2011).

A modified stripline, where the sample holder (a cuvette) and the temperature regulating chamber were part of the dielectric substrate, was described in (Ramundo-Orlando et al., 2004). The system was placed inside a spectrophotometer for monitoring the enzymatic activity of ascorbate oxidase trapped in liposomes during the exposure to a CW at 2.45 GHz.

6.2.2 Resonant structures

Only one example of resonant structure used for real-time acquisition is presented in literature. The resonant system proposed by Hagan *et al.* (Hagan et al., 2004) was a short circuited rectangular waveguide designed to expose neural cells in the frequency range of 0.75–1.12 GHz while monitoring catecholamine release. The guide had slots on the plates to allow the communication between the cell perfusion apparatus, placed inside the waveguide, and the exterior.

6.2.3 Radiating structures

Even for radiating structures only one system has been published. A radiating system, based on a horn antenna, with the perfusion chamber placed in the far-field region, was used in (Yoon et al., 2006). The biological protocol was the same described in (Hagan et al., 2004), but the exposure frequencies were higher (1–6 GHz). For this higher frequency range, a standard waveguide is too small to accommodate the sample and the cell-perfusion apparatus and a radiating system is necessary. However, this solution required a special arrangement to avoid interference with the experimental equipment that was shielded in a conducting box behind the perfusion chamber and a layer of absorber material was used to prevent electric field reflection. The whole system was placed within an anechoic chamber.

7. Conclusion

Real-time data acquisition in biomedicine is employed in a lot of different applications, both *in vitro* and *in vivo*. Examples are given in monitoring of chemical reactions (Shutes and Der, 2005), gene expression (Gubern et al., 2009), and drug release (Chouhan and Bajpai, 2009). Concerning living animals, the real-time acquisition is usually based on non-invasive imaging techniques (Ohtani et al., 2010; Li et al., 2010; Voyvodic et al., 2011).

The common issue in both real-time *in vitro* and *in vivo* investigations for biomedicine is that the experimental acquisition of physiological data is performed simultaneously with the correlated event. This is also the case of bioelectromagnetic investigations.

Focus of this chapter was a review of the exposure systems used in real-time biological experiments, i.e. in those investigations where bio-chemical or bio-physical data are acquired from the sample simultaneously with the exposure to the EM field. Real-time investigation is widely spreading, especially in the study of the interaction between nervous system and RF EM fields through *in vitro* electrophysiological techniques.

Although real-time investigations have been gaining increasing interest in the last ten years, a complete and systematic framework on specific requirements of RF exposure systems when applied to real-time data acquisition is still lacking.

Aim of this chapter was to merge the well-assessed design procedure of RF exposure systems (Kuster & Schönborn, 2000; Paffi et al., 2010) with the requirements emerging from real-time investigations, thus providing a reference work on this segment of knowledge.

The exposure system, defined as the complex structure used for allocating the biological samples (cell cultures for the *in vitro* experiments, animals for the *in vivo* ones) during the exposure phase of a biological experiment, is a fundamental device in the whole experimental setup. Indeed, a well-designed and characterized exposure system is a prerequisite for obtaining reproducible and scientifically meaningful results useful in the process of health risk assessment. Therefore, moving from the analysis of both biological and EM requirements (Kuster et al., 2000), a standardized procedure for the design of the system should be used, as proposed in (Paffi et al., 2010).

Due to the great variety of biological protocols and exposure parameters (dose, duration, frequency and waveform of the EM signal), different exposure systems can be found in the literature, based on radiating (antennas), propagating (waveguides), and resonant (resonant cavities) structures. From an accurate review of them, it emerges that real-time systems, in almost all cases, were designed for *in vitro* investigations on the electrophysiological activity of excitable cells. Such systems generally require modifications of standard RF structures to allow the continuous monitoring of the sample while avoiding RF coupling and interference with the recording apparatus (Paffi et al., 2010). Different solutions can be found in the literature, almost all based on propagating structures. They are mostly open planar structures or semi-open and closed structures modified with holes to allow the access to the sample for the data acquisition. The proposed systems, tailored for the particular biological endpoint and protocol, often result from the trade-off between the two conflicting requirements: the easy access to the sample and the avoidance of interference with the laboratory equipment. Therefore, in these cases, guidelines to design the optimal and most efficient system are particularly significant.

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