Microwave Exposure System for *In Vitro* and *In Vivo* Studies

C. T. Nadovich[1,2*], W.D. Jemison[2], J. A. Stoute[3], C. Spadafora[4]

[1]Lafayette College, Easton, PA, USA, [2]Clarkson University, Potsdam, NY, USA, [3]Penn State University, Hershey, PA, USA, [4]INDICASAT AIP, Ciudad del Saber, Panama

*Corresponding author: AEC 420, Lafayette College, Easton, PA 18042 USA, nadovicc@lafayette.edu

Abstract: A computer controlled microwave exposure system and specialized applicators were constructed for facilitating accurate observations of microwave radiation effects on uninfected and infected biological tissue *in vitro* and *in vivo* under different electromagnetic modalities and exposure configurations. Modeling of these new applicator designs was done using COMSOL Multiphysics®. To address diverse experimental requirements, three different applicators were developed: a broadband TEM chamber for either *in vivo* or *in vitro*, a narrowband TE_{101} waveguide and PTFE sample holder resonant at 2.45 GHz only for standing wave *in vitro*, and an innovative broadband quasi-TEM microstrip device for irradiating microscope slide mounted samples with high microwave fields simultaneously as they are being studied under a microscope (our so-called M^3 fixture). For *in vivo* modeling of mice in a TEM chamber applicator, the 3D Digimouse [2] image and tissue atlas defined tissue properties at 100 um voxel resolution through a LiveLINK™ for MATLAB®.

Keywords: absorption, microwave, biological, tissue, MATLAB.

1. Introduction

Many researchers have studied non-thermal microwave exposure effects including the possibility of treating diseased tissue with microwaves [1]. Duration of exposure, along with Specific Absorption Rate (SAR), determines the dosimetry in total energy absorbed per unit mass. As energy absorption increases, one would expect more "effects" of all kinds. Yet Figure 1, which was plotted from the extensive data in [1], shows no clear relationship.

There are a variety of hypothetical explanations for this surprising lack of correlation between non-thermal biological effects and energy absorption, including the following two possibilities.

1. Non-thermal mechanism(s) by which microwaves affect biological tissue are not a simple function of power, time, or total energy absorption.

2. Experiments looking for biological effects of non-thermal microwave radiation exposure suffer from a large uncertainty in

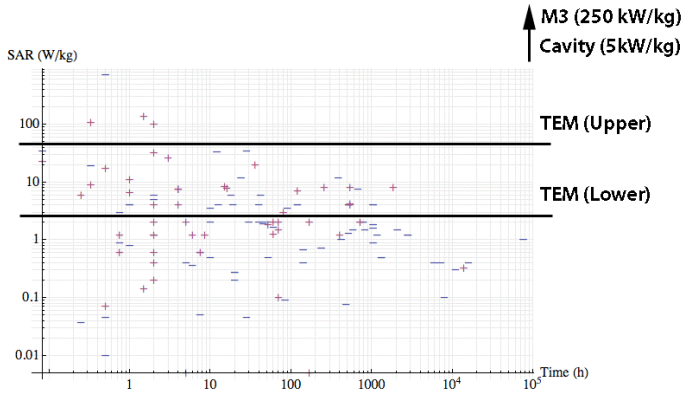

Fig 1. Outcomes of 130 different animal studies [1] on non-thermal microwave effects. Absorption and duration axes seem unrelated to positive or negative results. SAR capability lines are shown for applicators in this system

what power or energy is exactly absorbed by the individual sub-components of biological system under study. This uncertainty masks any functional relationship between effects and energy absorption.

Either or both of these hypotheses may be true, or neither may be the case, but it is no doubt critical for all experimenters to reduce absorption uncertainty as much as possible so as to best rule out the second hypothesis. Knowing the exact microwave fields at every point within the organism, along with the exact constitutive properties of the tissue at every point would permit accurately inferring absorption at every point. Unfortunately, experimenters typically only know bulk microwave *exposure* with any accuracy. And if any absorption measure is known accurately, it's often just the full body average absorption inferred by bulk bolometric methods.

Figure 1 also suggests that flexible time and power (as well as frequency) dosimetry is necessary for any thorough investigation of non-thermal effects. One can't assume that non-thermal effects will most likely be found in any given region of the SAR-time-frequency space. Finally, specially focused *in vitro* and *in vivo* studies demand specialized microwave applicators compatible with the practical aspects of biological experimentation, a requirement that is often at odds with the fundamental need to minimize uncertainty in absorption.

1.1 Overall System Design

To meet this fundamental need and allow observations of microwave radiation effects with low uncertainty in absorption, a computer controlled microwave exposure system and specialized applicators were designed, optimized and constructed with the aid of computer modelling. The system provides accurate dosimetry both in exposure and absorption. Samples can be uninfected and infected biological tissue *in vitro* and *in vivo* subjected to different electromagnetic modalities and exposure configurations.

A block diagram of the overall system is shown in Figure 2. The system consists of three main subsystems: controller, RF generation, and a set of multi-mode applicators.

The system controller is implemented with a compact PC running a custom GUI application written in Java that automates RF exposure experiments and data acquisition. The software manages RF power leveling, thermostatic temperature control, and orchestrates unattended automatic exposure sessions over variable length durations with logging.

Temperature control is critical for performing experiments at higher SAR. For use with any of the applicators is a four-channel fiber optic thermometer. Thermometry data is tightly integrated into the controller software for multi-channel thermal monitoring, as well as closed loop "thermostat mode" temperature control. In addition, thermochromic pigment verifies temperature rises in the sample.

The RF generation subsystem operates from 20 MHz to beyond 4 GHz with leveled incident power levels adjustable over a 150 dB range with computer logged forward and reverse power up to 50 watts. Broadband components with computer automation allows flexible, safe and efficient short or long term trials with dosimetry that spans the whole gamut.

TEM, cavity, and M^3 applicators with the

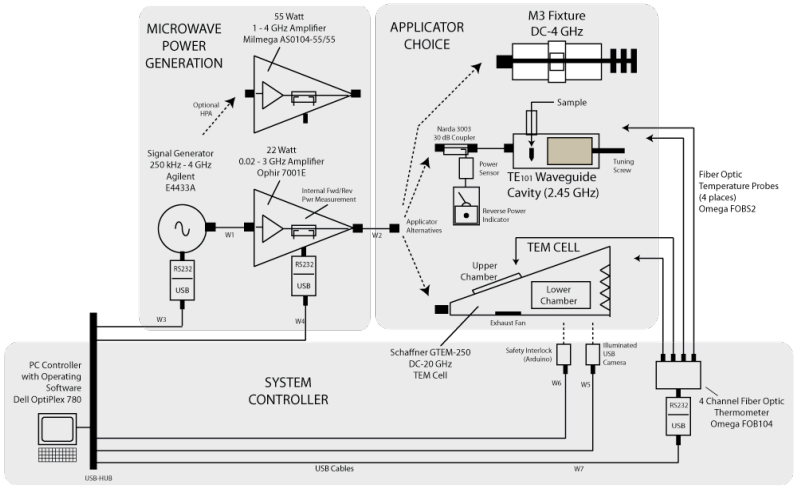

Fig 2. System block diagram illustrating the three main subsystems.

system described herein are capable of dosimetry that covers the entire range employed in Figure 1, and beyond, with CW, short pulse, and unattended operation over long periods of time. The M^3 applicator can achieve a SAR of over 250 kW/kg sustained without excess heating in a microscope viewable region of 75 ul samples. The cavity can work with larger samples at 5 kW/kg SAR, although cooling two orders of magnitude worse than the same SAR in M^3. The TEM applicator can expose live animals for weeks.

1.2 Applicator Details

Observing very different electromagnetic modalities requires variations in wave impedance, TEM, TE, and TM exposure. Work with live animals requires a sizable exposure volume, with ventilation, and animal safety features. Handling *in vitro* biological samples that optionally contain pathogens requires sterile sealed labware, such as polyethylene microcentrifuge tubes or polycarbonate flasks.

To address these requirements, three different applicators were developed: a broadband TEM chamber for either *in vivo* or *in vitro*, a narrowband TE_{101} waveguide and PTFE sample holder resonant at 2.45 GHz only for standing wave *in vitro*.

The TEM cell chamber has two regions for exposing samples, a lower chamber with 250 mm septum height, and an upper chamber with 45 mm height. Figure 3 shows a mouse loaded into the upper chamber.

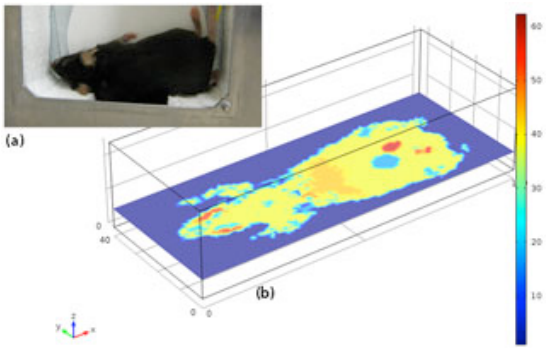

Fig 3. (a) Live mouse in HKE position loaded in upper TEM chamber with rectal temperature probe. H-plane modeling cut of relative permittivity within mouse based on Digimouse [2] atlas.

The most innovative applicator is a broadband quasi-TEM microstrip device for irradiating glass slide mounted samples with high microwave fields as they are being studied under a microscope (our so-called M^3 fixture). Shown in Figure 4, this device cools the sample so intimately that a 75 ul blood sample shows a steady state temperature rise less than 1 ºC per kW/kg of SAR. Compare this with the cavity applicator where a 150 ul sample cooks to over 160 ºC per kW/kg of SAR in steady state. A high exposure with effective cooling is one of the main advantages of M^3; another is the ability to observe live cells and microscale structures under intense RF irradiation without bulk temperature rise.

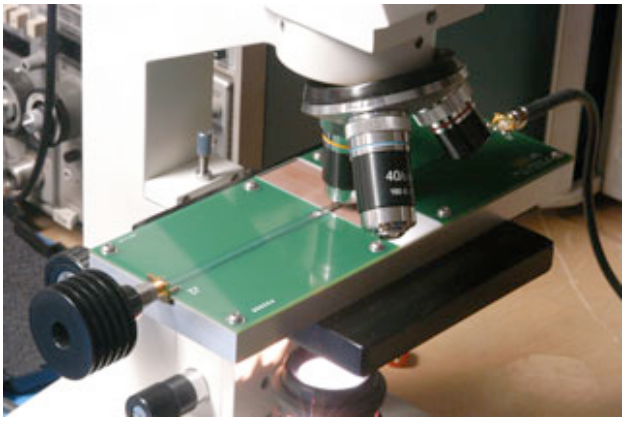

Fig 4. Microscope observation of sample in M^3 device during RF exposure. Note the thick aluminum baseplate that aids cooling.

3. Use of COMSOL Multiphysics

In support of the applicator designs and to minimize the uncertainty in absorption, modeling was done COMSOL Multiphysics®. For *in vivo* modeling of mice in the TEM chamber applicator, the 3D Digimouse [2] image and tissue atlas defined tissue properties (permittivity, loss tangent, density, heat capacity, etc…) at 100 um voxel resolution through a LiveLINK™ for MATLAB® function interface.

To model the scenario shown in Figure 3, the mouse was placed on and under perfectly conducting surfaces spaced and driven as the

septum and floor of the TEM cell. Perfectly Matched Layer absorbing boundaries were used around the edges. Given the posture of the animal in the Digimouse [2] data, the HKE orientation (Nose pointing in H-field direction; dorsal side in E-field direction; HKE per [3]) was modeled extensively.

The cavity and M^3 applicators were also modeled extensively. Dual physics microwave heating studies were conducted, exploring SAR and heating of alternative holder configurations for 200 ul sample tubes. Predicted S_{11} of the cavity and M^3 were verified with network analyzer measurements. Thermal effects in the sample were verified empirically with thermometers and thermochromic pigment.

4. Results

4.1 TEM Modeling

The TEM modeling of the detailed Digimouse structure revealed an interesting "double hump" resonance in the HKE animal orientation shown in Figure 5. Digimouse voxel extrema are 8.86 x 3.28 x 1.97 cm, as compared with 12 x 3 x 2 cm ellipsoid diameters that give a LMS best fit to the low frequency absorption of a HKE oriented homogeneous ellipsoid [3]. Since the longest axis is H-aligned, one would not expect a well defined resonance when this axis equals 0.4 λ per Lin [4]. The double resonance could be diffraction or ground plane reflection effect.

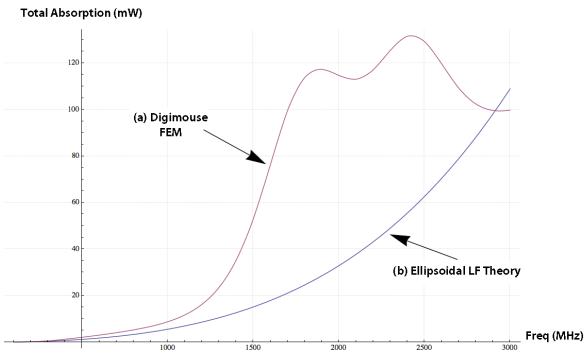

Fig 5. Whole body microwave power absorption of detailed Digimouse model (a) showing double resonance. (b) Low frequency ellipsoidal model.

4.2 Cavity Modelling

COMSOL Multiphysics® modeling of the cavity applicator irradiating tubes oriented upright (sample settled in the conical bottom) showed problematic non-uniformity of the electric field within the sample. The peak absorption rate at the bottom tip of the tube, and at the front and back surfaces normal to the field is more than 10 times higher than the rate at the top surface of the sample. Consider Figure 6 that shows Specific Absorption Rate (SAR) for a 3 watt incident exposure at four horizontal cuts through a 200 ul tube in the cavity applicator.

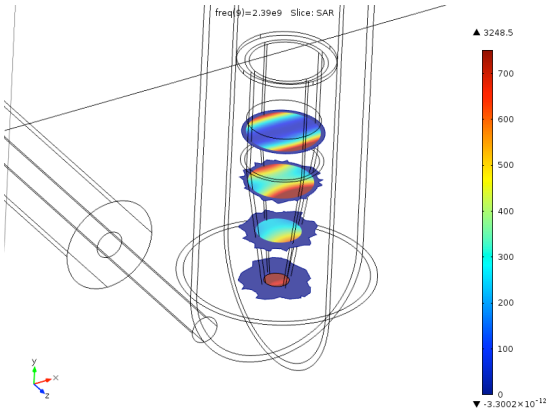

Fig 6. Cavity applicator SAR (W/kg) at four horizontal cuts through upright microcentrifuge tube containing blood. The front-back, and tube-tip non-uniformity of exposure is evident. Cavity drive probe and outer support tube shown in outline. Incident power was 3 watts.

Based on the problematic heating distribution for upright samples, an improved PTFE holder (Figure 7) was developed to allow inverted sample tubes. This holder reduced the SAR non-uniformity and improved cooling to reduced temperature rise.

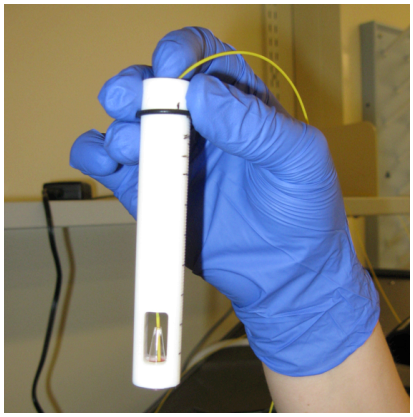

Fig 7. Improved PTFE holder with inverted micro centrifuge tube and temperature probe inserted through a hole in the tip.

The M^3 can induce high fields in the sample with very little heating. The steady state temperature distribution after RF exposure in M^3 is shown in Figure 8.

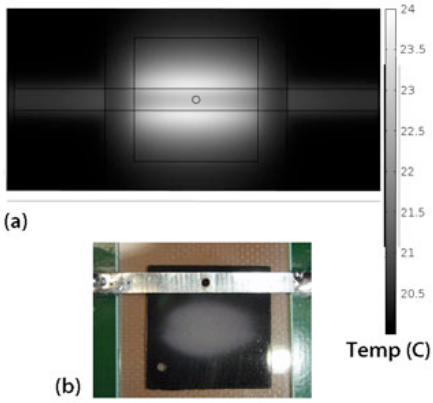

Fig 8. M^3 applicator with 1 watt at 2.45 GHz on 1% by mass saline sample. (a) RF heating COMSOL Multiphysics® simulation of temperature at $t = \infty$. (b) thermochromic pigment used to directly observe temperature rise. Slide was moved downward after exposure for photo.

Because of the small sample size and relatively large thermal mass in close proximity, COMSOL Multiphysics® modeling predicts a steady state temperature rise of around 3.8 ºC per incident watt in the center of a 250 um thick wet mount sample. This is unusually low heating for the very high peak SAR of 5500 W/kg. COMSOL Multiphysics® simulation results shown in Figure 8a match well with direct observation using thermochromic pigment in 8b.

Another fact revealed by the modeling is that for relatively lossy tissue (e.g. blood) the peak E-field occurs along the edges of the strip, uncovered by the metal. Under the strip, the wave is mostly H-field. Figure 9 shows this effect.

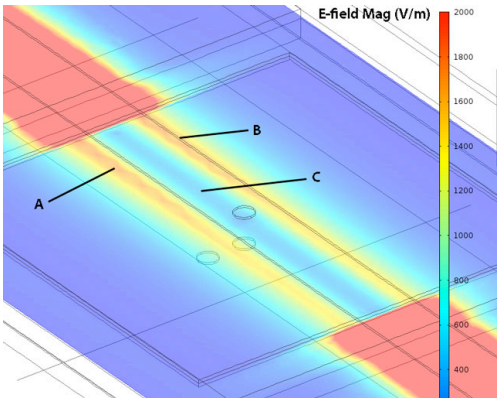

Fig 9. E-Field Magnitude Evaluated in plane of blood sample under coverslip. Wave travels from upper left to lower right. In the sample, strong E-field occurs outside the edges of the microstrip (at A and B) with mainly H-field underneath (at C).

5. Conclusions

This new system facilitates study of microwave radiation effects on biological tissues, pathogens, and other micro or nano scale structures. Direct observation is possible over a wide range of frequencies and field intensities with accurate classical dosimetry and thermal control. Central to the design, optimization, and operation of the system applicators was the use of COMSOL Multiphysics® modeling, which primarily served to reduce the uncertainty in absorption dosimetry.

6. References

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7. Acknowledgements

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