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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our research is to lay the foundation for developing non-lethal stunning/immobilizing weaponry based on radiofrequency (RF)/ microwave(MW) radiation by identifying RF/MW parameters potentially capable of selectively altering exocytosis, the process underlying neurotransmitter release and hence nervous system functioning. Major accomplishments included 1) assembling, characterizing and optimizing a free-space MW exposure system for assessing effects of exposures in the 1 to 6 GHz frequency range on exocytosis, using neurosecretory adrenal chromaffin cells as an in vitro model. Other accomplishments included implementing experimental approaches that will allow us to distinguish between non-thermal versus thermal effects of the exposures. The research was presented at one international meeting and has culminated in one manuscript that is under review. Personnel on the project included a neurobiologist and an electrical engineer as principal investigators, an associate engineer, one research assistant and two graduate students. The research has been transitioned into AFOSR grant FA9550-05-1-0308.

15. SUBJECT TERMS

Microwave fields, non-thermal bioeffects, free-space exposure system, exocytosis, neurotransmitter release, adrenal chromaffin cells, FDTD numerical modeling

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Gale L. Craviso
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FINAL PERFORMANCE REPORT

Technical Proposal entitled: "Interdisciplinary research project to explore the potential for developing non-lethal weapons based on radiofrequency/microwave bioeffects"

Award Number: FA9550-04-1-0194

Start Date: 15 March 2004

Termination Date: 14 December 2005

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ABSTRACT

Our research is to lay the foundation for developing non-lethal stunning/immobilizing weaponry based on radiofrequency (RF)/microwave(MW) radiation. We are focusing specifically on identifying RF/MW parameters potentially capable of selectively altering exocytosis, the process underlying neurotransmitter release and hence nervous system functioning. Major accomplishments included 1) assembling, characterizing and optimizing a free-space MW exposure system for assessing effects of exposures in the 1-6 GHz frequency range on exocytosis, using neurosecretory adrenal chromaffin cells as an *in vitro* model. Other accomplishments included implementing complementary experimental approaches that will allow us to distinguish between non-thermal versus thermal effects of the exposures. The research has been presented at one international meeting and has culminated in one manuscript that is under review. Personnel involved in the project included a neurobiologist and an electrical engineer as principal investigators, an associate engineer, one research assistant and two graduate students. The research has been transitioned into AFOSR grant FA9550-05-1-0308.

SUMMARY

Objectives:

One of the major objectives of the research was to assemble, characterize and optimize a free-space, microwave (MW) exposure setup for exposing adrenal chromaffin cells inside an anechoic chamber to MW fields in the 1 to 6 GHz frequency range. Essential to our overall experimental approach was to have the capability of superfusing the cells with a balanced salt solution to allow on-line monitoring of catecholamine release from the cells under dynamic temperature control during MW exposure. To facilitate conducting the experiments, included in this effort was the establishment of a cell/tissue culture facility and biochemical laboratory in the Harry Reid Engineering Laboratory building where the anechoic chamber is located and hence, where the free-space exposure experiments will be carried out. Another major objective was to augment existing research capabilities to increase both efficiency and productivity and to allow for the implementation of additional methodologies relevant to the projects already underway.

Accomplishments:

1) **MW Exposures in the 1-6 GHz frequency range in free-space.** All instrumentation and components necessary for carrying out free-space exposures of chromaffin cells in the 1-6 GHz frequency range have been acquired and the basic setup designed, constructed and characterized. Included in the design are 1) a dynamic temperature feedback system to maintain temperature to within ±0.2°C of a setpoint and 2) having all exposure parameters computer controlled and continuously logged. The exposure system is in the final stages of being optimized using the Finite-Difference Time-Domain (FDTD) numerical modeling method. For the latter, we have maximized our capabilities for using the FDTD numerical modeling software, XFDTD.
2) **Infrastructure development.** We have established a fully equipped and operational cell/tissue culture facility and biochemistry laboratory in the Harry Reid Engineering Laboratory in a room next to the anechoic chamber.
3) **Development of complementary experimental approaches for assessing effects on catecholamine release due to heating.** We have made significant progress in developing a temperature control system that will allow us to examine the effects of rapid heating on catecholamine release.

New Findings:

With respect to the free-space exposure setup, we have determined that maintaining an acceptable level of homogeneity of the electric field and specific absorption rate for exposing cells microwave fields over the 1 to 6 GHz frequency range requires a different distribution of the cells within the cell perfusion apparatus for exposures carried out at the lower versus the higher end of this frequency range of interest. Ongoing efforts are to define better the optimal conditions for conducting experiments at discrete frequencies within this limited frequency range.

Publications:

Yoon, J., Chatterjee, I., McPherson, D. and Craviso, G.L. Design, characterization and optimization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells exposed to microwave radiation: Finite-Difference Time-Domain

technique. Submitted to IEEE Transactions on Plasma Science; revised manuscript in review.

Interactions/Transitions:

a) **Presentations**

Oral

Yoon, J., Chatterjee, I., McPherson, D. and Craviso, G.L. Design and characterization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells due to non-thermal levels of 1 – 6 GHz continuous and pulsed microwave radiation - Finite-Difference Time-Domain computations. 4th International Symposium on Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases (ElectroMed 2005, Portland, OR).

b) **Consultative and advisory functions:** None

c) **Transitions:** The research project that is to examine the effects of rapid, reversible increases in temperature on catecholamine release from chromaffin cells has already been transitioned into AFOSR grant FA9550-05-1-0308.

New Discoveries, Inventions or patent disclosures: None

Honors/Awards: None

Personnel Supported:

Gale L. Craviso, Ph.D. – Principal Investigator
Indira Chatterjee, Ph.D., Professor of Electrical Engineering
Dana McPherson, Associate Engineer, Dept. of Electrical Engineering
Jeffrey Quinn, Research Assistant
Bindya Dumpala, M.S. graduate student in Biomedical Engineering
Jihwan Yoon, Ph.D. graduate student in Electrical Engineering

COMPREHENSIVE TECHNICAL SUMMARY

Rationale

Although the United States Department of Defense is one of the world's largest developers and users of RF/MW-emitting systems for radar, communication and anti-electronic weaponry purposes, the use of RF/MW radiation as a non-lethal weapon *per se* has not yet been realized. Most likely this is because the effects of exposure of biological systems to RF/MW fields at levels that do not produce thermal effects are largely unknown. The overall objective of the research funded by this grant was to begin laying the foundation upon which RF/MW technology can be developed that would have an application for non-lethal weaponry purposes, such as stunning/immobilizing the enemy. To accomplish this goal, this proposal had as one of its objectives to assemble, characterize and optimize an exposure system that would allow us to undertake a carefully designed and controlled investigation of the MW exposure parameters in the 1 to 6 GHz frequency range that can alter exocytosis, the process underlying neurotransmitter

release. The experiments would use a well-characterized model for studying exocytosis, isolated adrenal medullary chromaffin cells. These cells synthesize, store and release the catecholamines epinephrine and norepinephrine.

Experimental Procedure

1) MW Exposures in the 1-6 GHz frequency range in free-space.

Figure 1 is a schematic diagram of the entire free-space exposure system that was assembled over the funding period. Major instrumentation for this work, which includes a RF/MW signal generator, broadband power amplifier, broad-band horn, high-power cables and a computer to control the instrumentation and experimental protocols, was acquired via a Defense University Research Instrumentation Program (DURIP) grant. Thus, we now have the capability of carrying out these experiments without having to interrupt the experiments being carried out in the waveguide-based system (0.7 – 1 GHz frequency range). All instrumentation has been set up in the screen room adjacent to the microwave anechoic chamber (located in the Harry Reid Engineering Laboratory building) where the broad-band horn antenna is placed (and hence where cell exposures will be carried out). In addition, all aspects of the exposure parameters are computer-controlled using locally written LabVIEW programs.

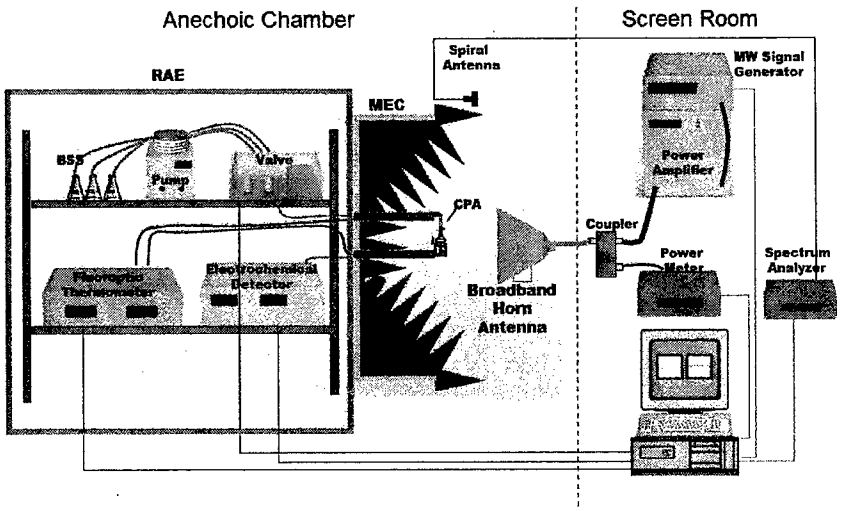


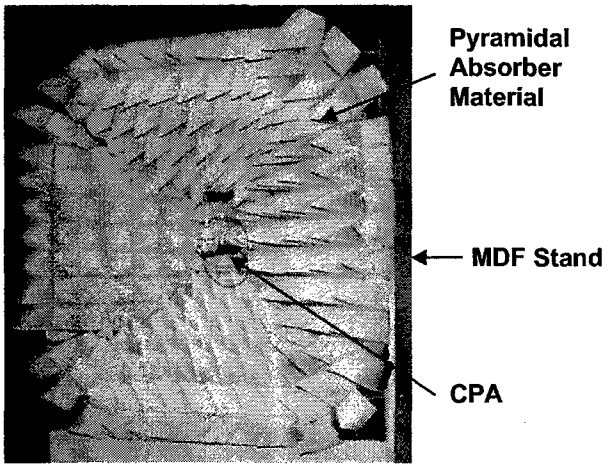
Figure 1. Schematic diagram of the entire free-space exposure system. RAE is rectangular aluminum enclosure; MEC is mini exposure chamber and CPA is cell perfusion apparatus.

The experimental approach for monitoring catecholamine release on-line from continuously perfused chromaffin cells for the free-space exposures in the 1 to 6 GHz frequency range is similar in concept to that used for our waveguide-based exposures for the 0.75 to 1 GHz frequency range. Essentially, the cells are immobilized on a glass fiber filter (GFF) inside a plastic filter holder that serves as a cell perfusion apparatus (CPA), and the balanced salt solution (BSS) that perfuses the cells reaches an electrochemical detector (ECD) that monitors in the amperometric mode the amount of catecholamine released. However, considerable

modifications were required to optimize the setup for MW exposures in the 1 to 6 GHz frequency range.

First, because it is essential to have the shortest lag time between catecholamine release and detection, the ECD has to be located as close as possible to the location of the cells and therefore in the anechoic chamber, rather than in the screen room which would be several feet away. This in turn means that the ECD needs to be shielded from the MW fields. The same consideration holds for any other equipment pertaining to the perfusion system that also should be near the location of the cells (e.g., peristaltic pump for superfusing the cells; fluoroptic thermometer for monitoring temperature). To get the appropriate shielding, we constructed a rectangular enclosure made out of aluminum (RAE) of dimensions 1 x 1 x 0.5 m that all necessary equipment pertaining to the cell perfusion system is placed in. Appropriately-sized openings in the aluminum enclosure allow tubing, temperature probes, etc., to reach the CPA that is placed inside a mini exposure chamber (MEC; described in the next section) located in front of the RAE.

Second, because the presence of the RAE in the anechoic chamber would cause large reflections of the MW field, a MEC was designed to house the CPA and minimize electric field reflections (Figure 2). The MEC was constructed out of MDF (medium density fiberboard and consists of a back wall (dimensions 1 x 1 m) and four side walls (dimensions 1 x 0.34 m) each making an angle of 45° with the back wall. The angle of 45° was chosen based on measured oblique reflectivities of a high-performance vented microwave pyramidal absorber material (AEP-8-V, Advanced ElectroMagnetics Inc.) that was glued to the inside of the five walls of the MEC. Figure 3 is a photograph of the actual exposure system within the anechoic chamber.



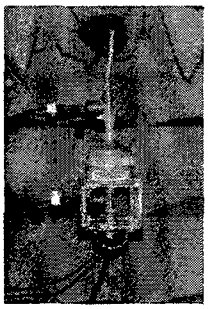


Figure 2. Left: Photograph of the CPA within the MEC; Right: Close-up view of the CPA.

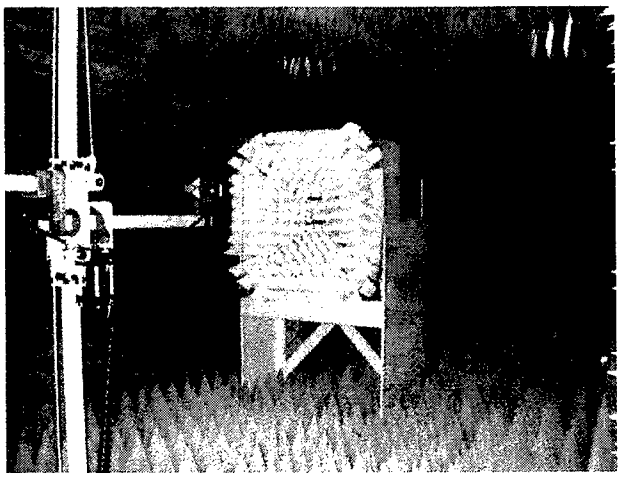


Figure 3. Photograph of the actual exposure system within the anechoic chamber.

The ECD is interfaced with a computer, using locally written programs in LabView. During cell perfusion, the temperature of the BSS entering and exiting the CPA is continuously monitored by fluoroptic temperature probes (Luxtron Model 790 Fluoroptic thermometer with model SFW-5 non-perturbing temperature probes) inserted into the BSS inlet and outlet tubing of the CPA. The temperature of the BSS entering the CPA is maintained at 36.5 ± 0.2°C by means of a glass inlet tube to the CPA around which is wound a 1 foot long piece of nichrome wire of radius 0.5 mm that serves as the heat source. Current through the nichrome wire is supplied by a constant voltage source (Astron RS-35A) that works in conjunction with a custom-built current controller utilizing a series of FETs (field effects transistors). The temperature of the BSS in the inlet measured by the Luxtron thermometer is used in a feedback loop to the computer. The set point temperature of 36.5oC is compared to the inlet temperature and is used to provide the control to the current controller. Because our system incorporates this dynamic temperature control, the temperature of the BSS entering the CPA is maintained at 36.5± 0.2oC both in the absence and presence of MW fields.

Because knowledge of the gain and far field radiation pattern of the horn antenna will be important for quantifying the incident field exposure on the cells, these parameters were measured and compared with Finite-Difference Time-Domain (FDTD) numerical modeling results, obtained using the software XFDTD. Overall agreement was good. A broadband spiral antenna that was fabricated in our laboratory is placed at a fixed location just outside the MEC for monitoring the electric field continuously during experiments.

FDTD numerical modeling was used throughout the design of the exposure system for characterization, optimization and refinement of experimental protocols. Because FDTD numerical modeling plays such a crucial role in all aspects of the research, we have spent a considerable amount of time maximizing the capabilities of the XFDTD software (purchased from Remcon) used for the numerical modeling. For example, on two computers that were newly acquired and assigned for the modeling, we have switched to a Linux operating system and have implemented dual processors in each system. We are now able to run far larger and

faster simulations than previously. We have been told by Remcon that we are the first of their customers to utilize the new 64-bit AMD dual processor desktop system to exceed the 2 GB RAM limitations of a 32-Bit system, without having to resort to an expensive and complicated multi-processor parallel system such as a Beowolf cluster.

Figure 4 shows the XFDTD model of the entire exposure system and Figure 5 shows the XFDTD model for the GFF on which the cells are immobilized. For all simulations, the FDTD models took into account the geometries and dielectric properties of all components of the exposure system.

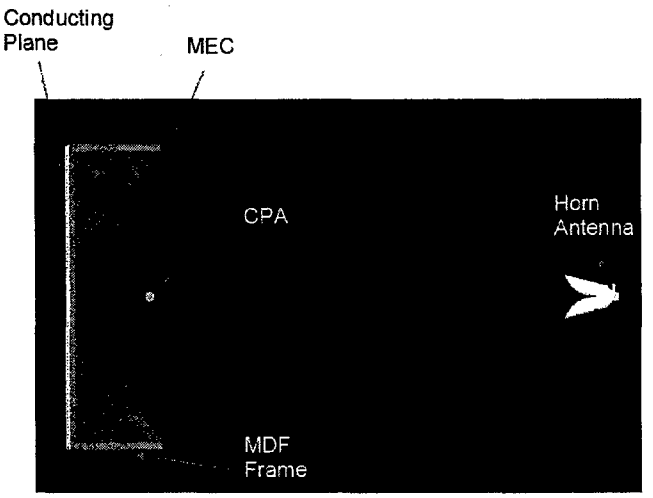


Figure 4. XFDTD model of the entire exposure system (cross-sectional view through the central horizontal plane).

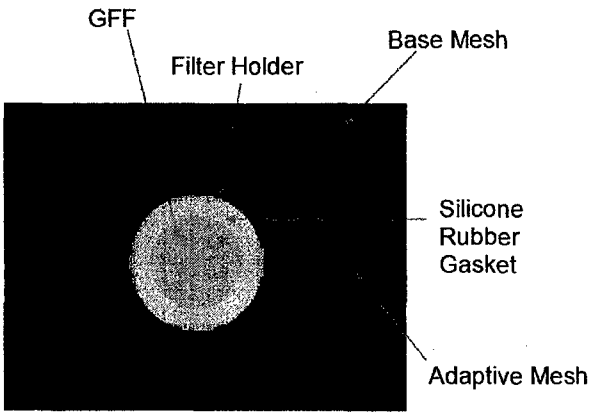


Figure 5. XFDTD cross sectional view of the GFF.

The results of the FDTD numerical computations were used to characterize and optimize the design of the exposure system so that it provides the maximum possible non-thermal level of

electric field as well as acceptable homogeneity (to within 30%) of the electric field and specific absorption rate in the region containing the cells. Highlights of the data obtained appear in the Appendix. Briefly, it was found that as frequency increases from 1 GHz to 6 GHz, the homogeneity of both the electric field and the specific absorption rate distribution decreases. Also shown is that this frequency effect can be counteracted by reducing the available area for cell distribution on the GFF. Based on these results to date, we now have good insight into how experimental protocols need to be adapted to enable us to perform experiments under well-defined and controlled conditions and thus allow us to obtain interpretable and reproducible results over the entire 1 to 6 GHz frequency range.

2) Infrastructure development.

To increase efficiency and productivity when carrying out the free-space exposure experiments in the Harry Reid Engineering Laboratory building, which is located at the opposite end of the UNR campus from Dr. Craviso's laboratory at the School of Medicine where the waveguide-based experiments are being carried out, we have established, as proposed, a fully equipped cell culture/tissue facility and biochemistry laboratory in the Harry Reid Engineering Laboratory building, as no such facilities existed in this building. Photographs of the facility are shown in Figure 7.

Figure 7. Photographs of the tissue culture facility in the room next to the anechoic chamber.

3) Development of complementary experimental approaches for assessing effects on catecholamine release due to heating.

We are very much aware of the need to be able to reliably distinguish between thermal versus non-thermal effects of RF/MW radiation. One way that we plan to be able to do this is by obtaining detailed information on how rapid heating affects catecholamine release from chromaffin cells. We will use a method that will allow us to examine, in real-time, the influence of rapid temperature changes above 36°-37°C, the temperature range at which experiments are conducted, on both the rate of secretion of catecholamines and the quantity of catecholamine released, both spontaneously (basally) and in response to a stimulus. The approach is an electrochemical amperometric technique that measures the release of catecholamines from individual cells via carbon fiber microelectrodes. While funds from the present grant have

allowed us to purchase several pieces of equipment for these studies (Figure 8) and to recruit a Biomedical Engineering graduate student into our group who will be carrying out the research as part of her Mater's thesis project, we were fortunate to obtain funds to fully support this research effort via AFOSR grant FA9550-05-1-0308.

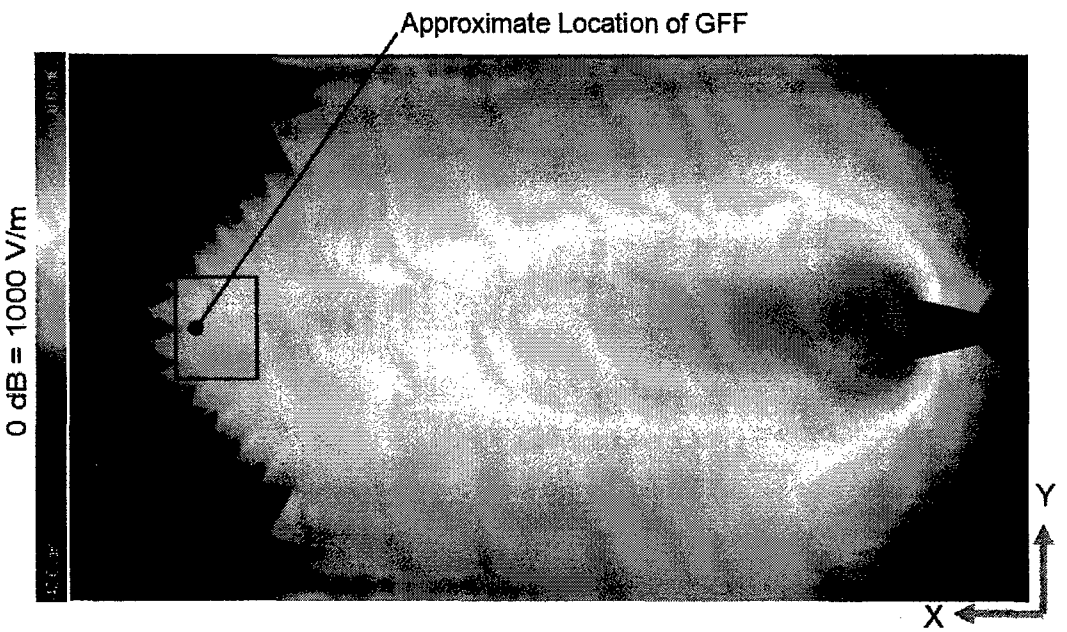
Figure 8. Photograph of the basic microscope setup and accompanying equipment for quantifying catecholamine release from individual cells by amperometry.

Ongoing work/future directions

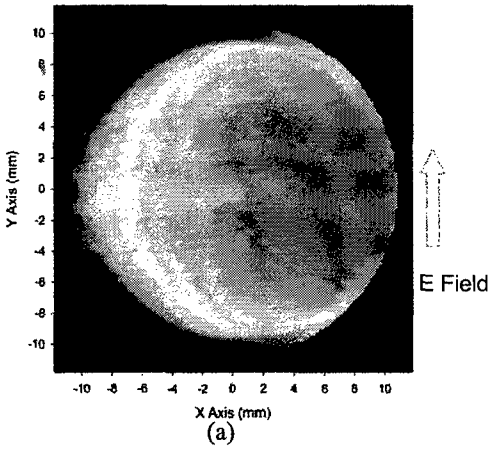
For the free-space exposure experiments, we still need to optimize conditions for working at each frequency. This will be done as experiments begin, which we anticipate will be very soon. To continue this work, funds will be obtained from AFOSR grant FA9550-05-1-0308 and any new grants that are funded. Because setting up the methodology to examine how rapid increases in temperature affect catecholamine release has already been transitioned into AFOSR grant FA9550-05-1-0308, we are actively continuing our efforts to implement fully this experimental approach. These experiments will be important since they will enable us to differentiate better between thermal and non-thermal effects of RF/MW exposure on catecholamine release.

Appendix

1) XFDTD contour plot of the electric field in the central horizontal plane that contains the GFF.



2) XFDTD computed electric field contour plot for 1 GHz (a), 3.5 GHz (c) and 6 GHz (e) and histogram and descriptive statistics of the electric field distribution for 1 GHz (b), 3.5 GHz (d) and 6 GHz (f) on the GFF having a region of radius 11 mm available to cells.



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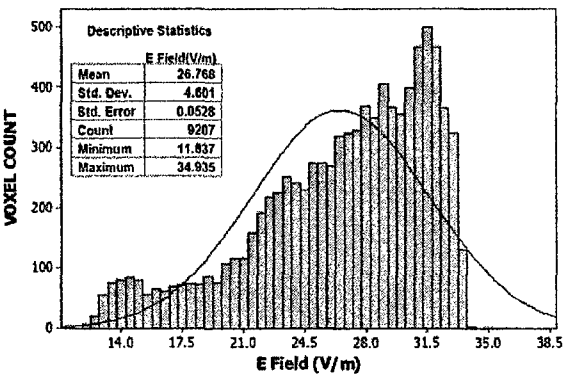

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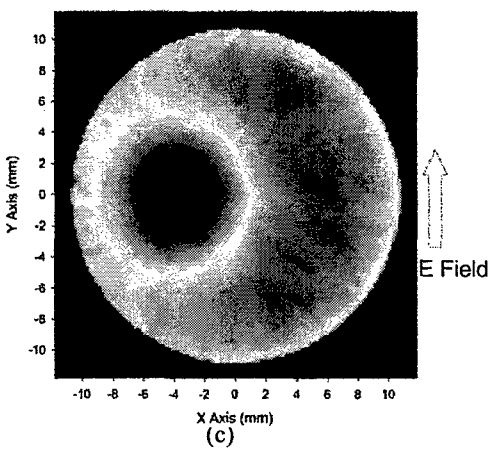

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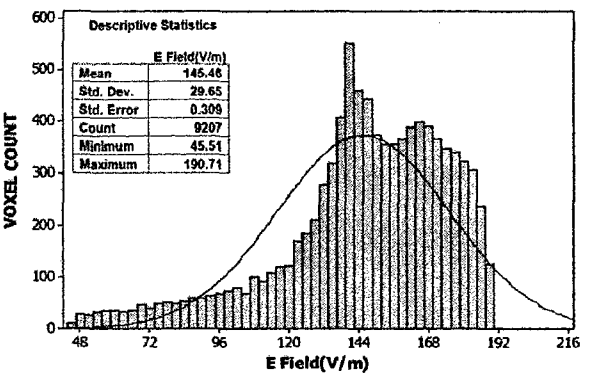

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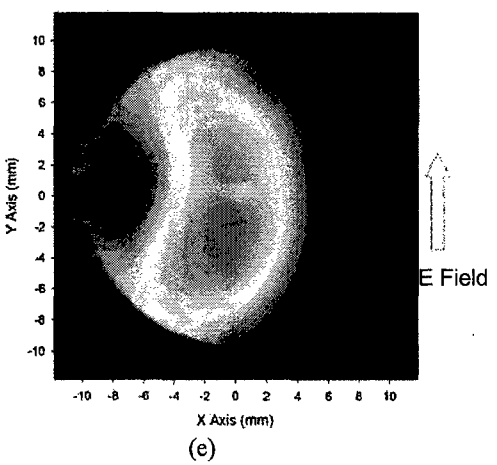

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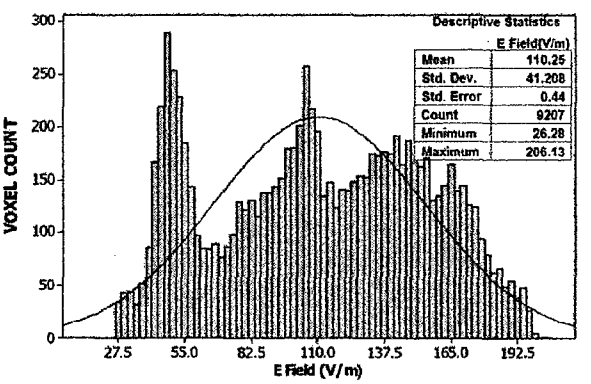
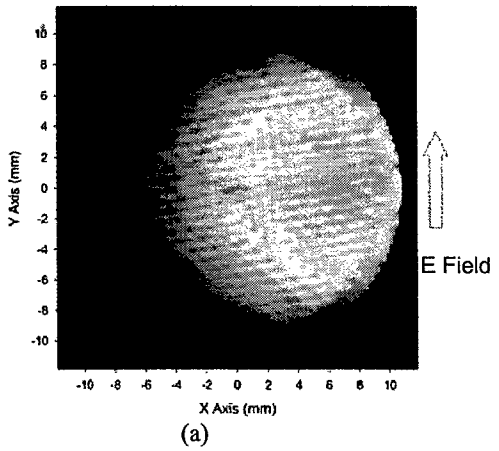


(f)

3) XFDTD computed specific absorption rate contour plot for 1 GHz (a), 3.5 GHz (c) and 6 GHz (e) and histogram and descriptive statistics of the specific absorption rate distribution for 1 GHz (b), 3.5 GHz (d) and 6 GHz (f) on the GFF having a region of radius 11 mm available to cells.



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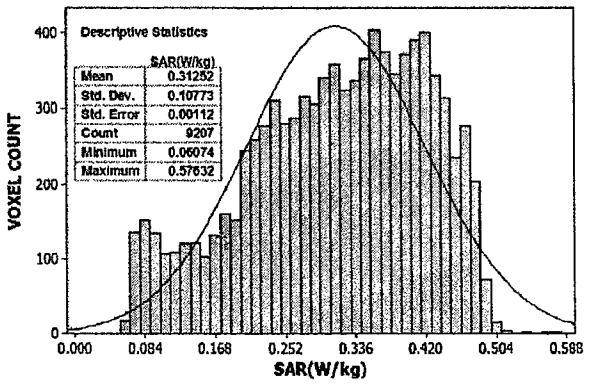

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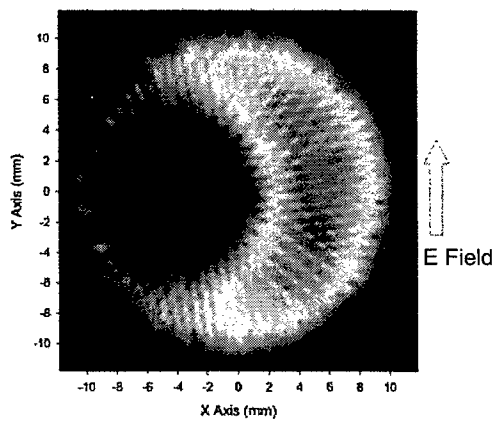

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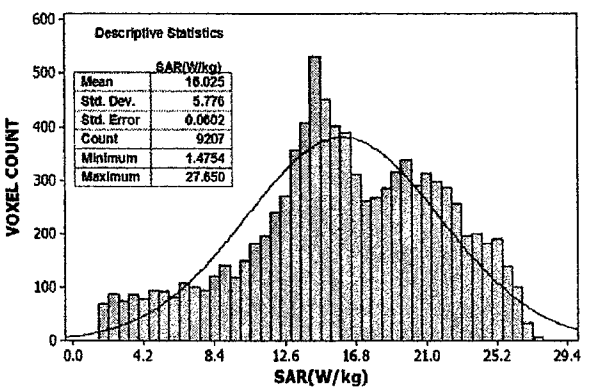

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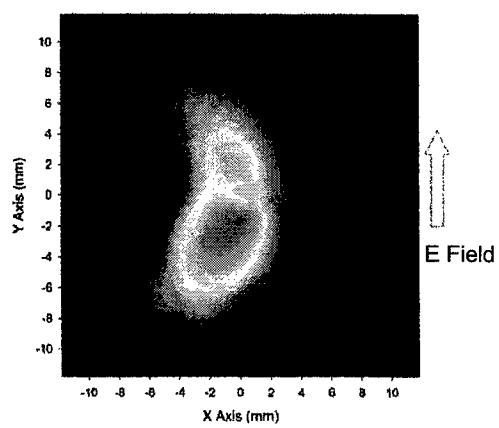

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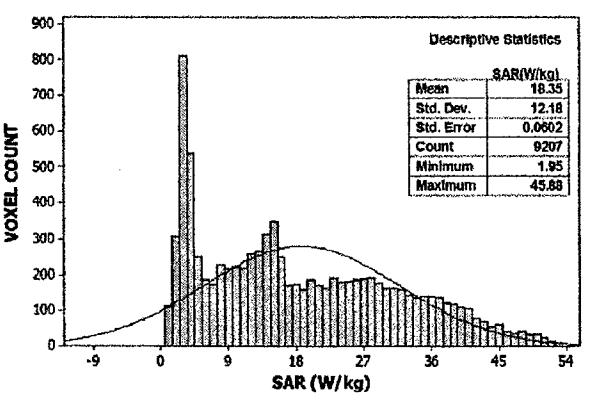
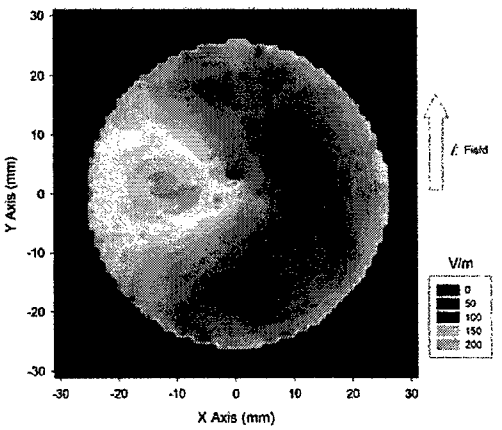


(f)

4) XFDTD computed contour plot of the electric field (a) and specific absorption rate (c), and histogram and descriptive statistics of the electric field (b) and specific absorption rate (d) for a GFF having a region of radius 7 mm available to cells at 6 GHz.



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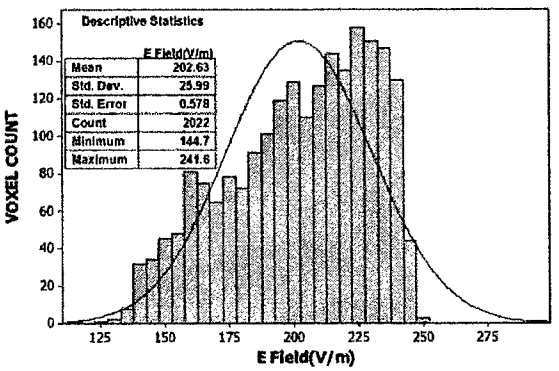

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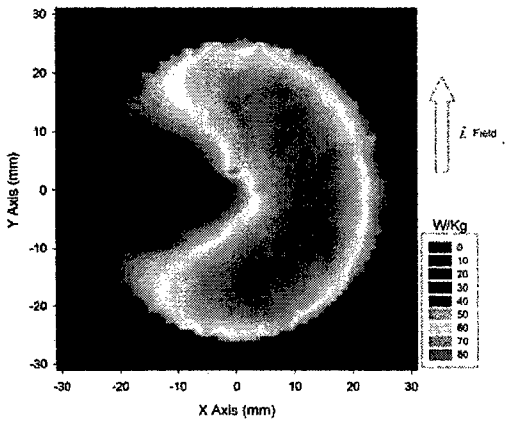

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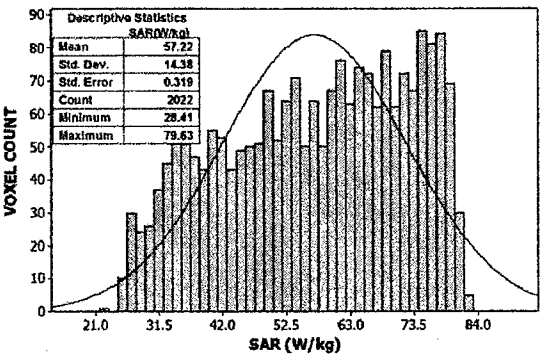

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