# Monitoring of RF Effects on Cells Aided by Simulation Software

The Project:Establish a system that ensures a maximum and consistent electromagnetic field exposure and SAR for the cells within the waveguide.

The Solution:Utilize electromagnetic simulation software to optimize the overall effectiveness of the research model.

*By Jerry Fireman*

Finite difference time domain (FDTD) simulation played a key role in the development of a unique exposure system that provides online monitoring of nonthermal radio frequency (RF) effects on catecholamine release from cultured adrenal medullary chromaffin cells, a well-characterized *in vitro* model of neural-type cells. While RF effects on cultured cells have been studied in the past, the new exposure system is believed to be the first that provides online monitoring of bioactive molecules secreted from cells—in this case, catecholamines. This is accomplished by placing the cells within a cell perfusion apparatus inside a waveguide. Continuous superfusion of the cells, where the perfusate that exits the cell perfusion chamber reaches an electrochemical detector, allows for both basal and stimulated catecholamine release to be assessed during RF exposure of the cells. A critical aspect of these experiments is the need to ensure maximum and consistent electromagnetic field exposure and specific absorption rate (SAR) for the cells within the waveguide. To achieve this goal, FDTD electromagnetic simulation software was used both to optimize the design of the waveguide-based exposure system and to characterize fully the electromagnetic fields during exposure of the cells to RF fields in the 0.75-1.0 GHz frequency range.

### Biological Effects of RF Exposure

Many of the *in vivo* and *in vitro* biological effects of RF exposure that have been reported in the literature can be explained by tissue heating. On the other hand, some effects have been observed in the absence of heating, suggesting that RF fields can also cause effects by nonthermal mechanisms. For example, there may be RF electromagnetic field interactions with specific cellular membrane constituents, such as neurotransmitter receptors and ion channels. Investigating this intriguing possibility is at the forefront of efforts directed at developing novel bioelectromagnetic technologies.

### Providing Online Measurement

Studies in which cultured cells are exposed to RF radiation to examine how a particular biological response is affected by the exposure typically employ off-line measurement of the biological response being examined. This situation makes it difficult to determine the point at which changes occur, and in general, reduces the amount of information that could be obtained from each experiment.

**The actual** cell perfusion apparatus used for experiments.

In their studies investigating nonthermal effects of RF fields on catecholamine release from cultured chromaffin cells, University of Nevada, Reno researchers Todd Hagan, Indira Chatterjee, Dana McPherson, and Gale Craviso have overcome this problem by combining a waveguide with a cell perfusion apparatus that allows online monitoring.[1] Chromaffin cells are immobilized on a glass fiber filter within a plastic filter holder to allow continuous superfusion of the cells with a balanced salt solution (BSS). The BSS reaches an electrochemical detector, used in the amperometric mode, for detecting catecholamines and thus measuring release online.

The cell perfusion apparatus is constructed entirely of nonmetallic components and placed inside the waveguide. It includes a Millipore Swinnex filter holder, two injection fittings, silicone rubber tubing and a Plexiglas stand. Inside the filter holder are three nylon filters sandwiching a single glass fiber filter on which the chromaffin cells are located. The two halves of the filter holder, each with a silicone rubber gasket, screw into each other, compressing the filters. However, there is sufficient separation between the two sections of the filter holder so that once loaded onto the glass fiber filter, the cells are not crushed and the BSS spreads out uniformly across the entire filter during perfusion. Dental putty fills the dead space on either side of the filter during perfusion, leaving only a central flow channel having a diameter of 1.8 mm for the BSS to flow through.

**Experimental Procedure**

For experiments, four to eight million chromaffin cells are gravity fed onto the glass fiber filter located in the filter holder and superfused with temperature controlled BSS at a flow rate of 1.2 ml/min. A fraction of the effluent flows into the electrochemical detector and the remaining effluent flows into a fraction detector for later offline quantification of the amount of catecholamine release by high performance liquid chromatography. At all times during experiments, the temperature of the BSS entering and exiting the cell perfusion apparatus as well as the ambient temperature inside the waveguide is monitored and logged continuously by a fluoroptic thermometer interfaced to a computer.

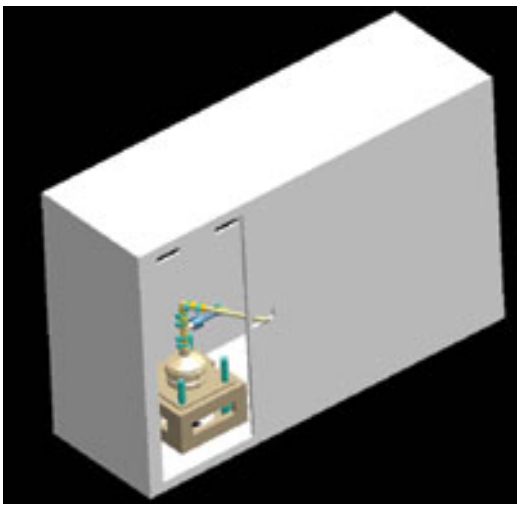

**Figure 1:** Geometry of the cell perfusion apparatus within the waveguide as created in SolidWorks and imported into XFDTD.

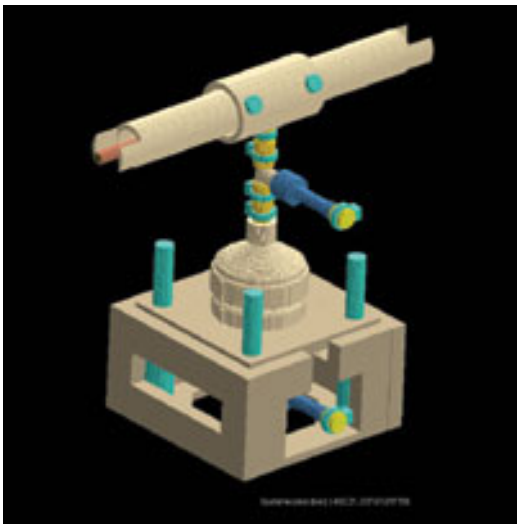

**Figure 2:** Numerical mesh of the cell perfusion apparatus created by XFDTD.

The exposure system is based on a WR-975 rectangular waveguide terminated with a shorting plate at one end to create a standing wave. The other end is connected to a computer controlled RF/MW signal generator and amplifier via a waveguide to coax adapter containing a probe launch. A circulator is inserted between the amplifier and waveguide to reroute the reflected power to a 50 Ohm load. A wattmeter provides an indication of both forward and reflected power.

#### Simulating SAR Exposure

Performing experiments such as these requires a high and consistent level of RF exposure in the area where the cells are located. In addition, it is important to know the SAR distribution. As a way to obtain this information in their experimental exposure setup, the researchers made the decision to simulate the RF fields using a commercially available FDTD software package called XFDTD from Remcom Inc. A key advantage of the FDTD method is that it can provide results for a wide band of frequencies in a single computation.

“The FDTD method is well suited to seeing changes over time, which is required in our experiments to quantify the SAR,” Hagan said. The researchers modeled the entire exposure system including both the cell perfusion apparatus and waveguide. The main grid covering the area outside the perfusion apparatus was composed of cubical Yee cells of dimension 1.524 mm. The smaller features of the cell perfusion apparatus as well as the regions in the areas of the waveguide top and bottom slots were represented by a subgrid of smaller cubical Yee cells of dimension 0.3048 mm, one-fifth the size of the main grid cells, in order to increase accuracy in these critical areas.

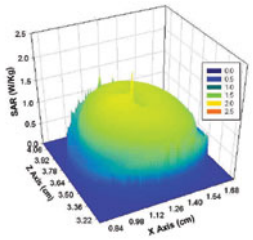


**Figure 3:**
Distribution of the SAR in W/kg in the region containing the biological cells. Here the biological cells were assumed to be evenly distributed over the entire glass fiber filter. Results are from XFDTD for an input power of 0.5W into the waveguide and a frequency of 1 GHz, and are imported into SigmaPlot for plotting and analysis.

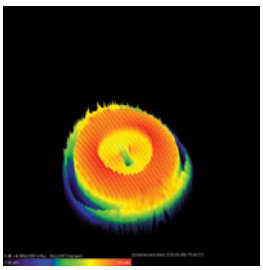

**Figure 4:**
Distribution of the SAR in W/kg in the region containing the biological cells. Here the same number of biological cells as used in generating the

results shown in Figure 3 were assumed to be evenly distributed over a small circular region of diameter 1 cm at the center of the glass fiber filter. Results are directly plotted in XFDTD for an input power of 0.5 W into the waveguide and a frequency of 1 GHz.

In the main grid, the brass probe launch and aluminum waveguide walls were modeled as perfect conductors. The probe launch was excited by a discrete sinusoidal RF source. The glass fiber filter has a very fine mesh and was hence approximated as a solid dielectric. The default time step was 2.0964 picoseconds in the main grid and 0.420 picoseconds in the subgrid. Each simulation ran for 9550 time steps.

**Validating the Model**

To test the validity of the XFDTD model, the numerically computed electric field (E) and magnetic flux density (B) within the waveguide, with the cell perfusion apparatus removed, were compared with well-known waveguide theory based on measured values of forward and reflected power. The magnitudes of E and B at their maxima in the standing wave pattern in the waveguide at a frequency of 1 GHz showed good agreement for forward powers in the range 0.5-5 W, with a maximum difference of 8.5%. This difference can likely be attributed to the fact that the computations based on waveguide theory utilized values of power measured by a wattmeter having a manufacturer specified accuracy of 5% and that the waveguide theory computations did not take into account field disturbances caused by the probe launch and waveguide slots.

**Determining Position and Orientation**

The next step was simulating the waveguide with the cell perfusion apparatus in order to determine a position and orientation for the RF exposure of the cells. The researchers first used XFDTD to predict SAR distribution with the cell perfusion chamber located at the maximum in B. This position with the E field perpendicular to the plane of the glass filter provided a very low SAR, on the order of 10-2 W/kg across the filter. The overall SAR on the filter is small because of the low value of the E field at the maximum of B in the standing wave pattern and the E field, being normal to the plane of the filter, provides little coupling. Computation of the SAR distribution across the filter with the E field parallel to the plane of the glass fiber showed higher SAR values but with relatively higher variation of SAR within the filter.

Researchers next changed the XFDTD model to position the cell perfusion chamber at the E field maximum. When the cell perfusion apparatus was oriented so that the filter was perpendicular to E, the SAR distribution showed dramatic differences that also precluded this configuration from being used in experiments. The parallel orientation, on the other hand, provides two orders of magnitude larger SAR than

when the filter is located at the B field maximum. This is because the E field is much stronger in this orientation and the E field is coupled into the filter according to the tangential boundary condition. In this orientation, homogeneity within 30% is achieved over 83% of the filter area. The simulation demonstrated that this configuration and this configuration alone provides the high and consistent SAR needed to evaluate the exposure of chromaffin cells for identifying RF field effects on catecholamine release. The simulation also provided detailed SAR values over the filter. Knowing the SAR distribution, together with ability to determine the location of cells on the glass fiber filter at the end of the experiment by staining the cells with a dye, provides the researchers with important information to interpret their experimental results.

[1]*Hagan, Chatterjee, McPherson, and Craviso, "A novel waveguide-based radiofrequency/microwave exposure system for studying nonthermal effects on neurotransmitter release – Finite-Difference Time-Domain Modeling" IEEE Transactions on Plasma Science. Vol. 32, August 2004. p. 1668-1676.*

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