Bioelectromagnetics 25:27–32 (2004)

A Single Magnetic Field Exposure System for Sequential Investigation of Real Time and Downstream Cellular Responses

Raj R. Rao and William S. Kisaalita*

Cellular Bioengineering Laboratory, Biological & Agricultural Engineering Department, University of Georgia, Athens, Georgia

To be able to correlate real time membrane potential or ion flux changes with further downstream gene transcription responses due to extremely low frequency (ELF) electromagnetic field (EMF) exposure, we devised an experimental system consisting of a pair of symmetric circular coils. This system can be used on an inverted microscope stage (real time signaling) as well as inside controlled environment incubators (gene transcription end points). The system includes a unique, custom made switch box for blinding the experimental staff and a power amplifier. We report herein the design and characterization of the system with respect to parameters considered important in in vitro ELF–EMF exposure studies, including linear magnetic field distribution, compensation for microscope objective lens interference, heating effects of the coils, and harmonic content of the signals. Bioelectromagnetics 25: 27–32, 2004. © 2003 Wiley-Liss, Inc.

Key words: imaging; circular coils; microscope objective interference; harmonics

INTRODUCTION

Exposure systems have been designed to study the effects of extremely low frequency (ELF) electromagnetic fields (EMF) over a wide range of frequencies [Mullins et al., 1993] and also for the induction of uniform electric and magnetic fields [Bassen et al., 1992]. Several types of apparatus for generating uniform magnetic fields for in vitro studies have been described [Misakian et al., 1993]. The most commonly used system uses circular or rectangular loops of wire of many turns. The critical factors that experimenters have tended to address when subjecting field exposed and sham exposed cultures are temperature, atmosphere, lighting levels and cycles, and vibration from the field generating apparatus. In addition, design of experimental systems has focused on two criteria: first the magnetic fields must be well characterized, and second the apparatus should not exert any additional influence on the cells [Goodman et al., 1995]. A Helmholtz pair [Krause, 1984] approximation has been used extensively by many investigators. In gene transcription studies, ELF magnetic fields have been generated by Helmholtz coils [Goodman et al., 1989; Lin et al., 1996], solenoids [Harrison et al., 1997], and a double wound, square, four coil configuration [Saffer and Thurston, 1995]. Unfortunately, most exposure devices reported to date were either designed for use inside CO_2 incubators or on microscope stages and not for both applications. Use of the same device providing identically controlled ELF magnetic field exposure parameters, for measurements of real time signaling and gene transcription is needed to enable more exact correlation of real time signaling, e.g., resting membrane alterations, and downstream changes in gene transcription levels.

We describe here a low cost, custom made circular coil ELF magnetic field exposure system designed to serve the above dual purpose. The system has been characterized with respect to parameters commonly considered in ELF magnetic field exposure system evaluation for in vitro studies. These include linear magnetic field distribution, as well as changes in the nature of the distribution in the presence of a microscope objective lens, coil heating effects, and harmonic contents of the ELF magnetic field signals. Application of the system to real time membrane potential and gene

Grant sponsors: The Georgia Research Alliance; The University of Georgia Research Foundation.

*Correspondence to: William S. Kisaalita, Cellular Bioengineering Laboratory, Biological & Agricultural Engineering Department, Driftmier Engineering Center, The University of Georgia, Athens, GA 30602. E-mail: williamk@engr.uga.edu

Received for review 24 January 2003; Final revision received 30 April 2003

DOI 10.1002/bem.10151
Published online in Wiley InterScience (www.interscience.wiley.com).

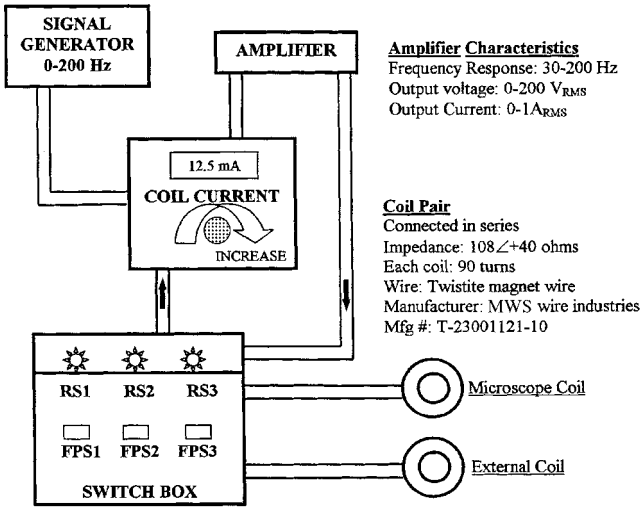

Fig. 1. Overall hardware schematic of the magnetic field exposure system.

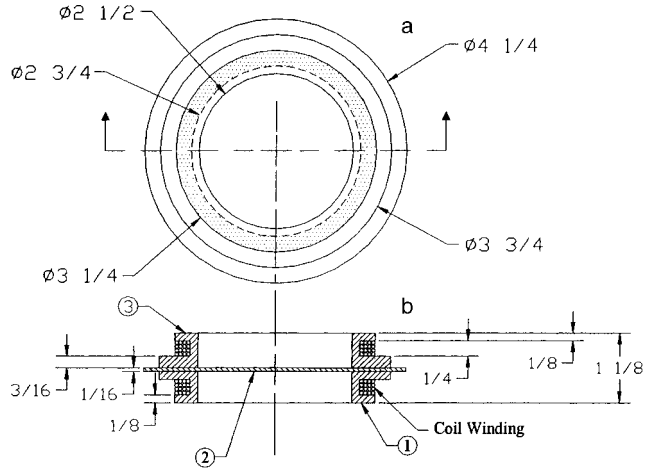

Fig. 2. Scale drawings of circular coils showing: (**a**) top view of coil; (**b**) side view of coil (ϕ, diameter; 1, bottom coil; 2, microscope stage plate; 3, top coil; all dimensions in inches). The stage plate and coil form were made from Plexglass and PVC, respectively.

transcription level changes have been published elsewhere [Rao et al., 2002].

SYSTEM DESIGN

The system schematic is shown in Figure 1. Briefly, the components of the system are a symmetric pair of coils in parallel, a signal generator, a power amplifier, and a custom made switch box. Detailed descriptions of each component are given below.

Coils

The details of the coils are given in Figure 2. Two coil pairs were required for blinding the experimental staff; the operator could energize either coil without knowing which coil was active. The coils had an inner diameter of 2.5 in. (63 mm) and an outer diameter of 3.25 in. (83 mm). The vertical distance between the coils was 0.3125 in. (0.80 mm). Each coil consisted of 90 turns of 30 gauge enameled, double twisted (red and green) copper wire (T2301121-10, 10 twists per inch, MWS Wire Industries (Westlake Village, CA). The diameter of the 1/16 inch (1.6 mm) plate (Fig. 2) was made such that the coils assembly dove-tailed in the microscope stage circular opening.

Switch Box

The switch box was composed of three eight pole, six position rotary (RS1, RS2, RS3) switches (Electroswitch Inc., Raleigh, CA; Model # C4D0806N-A). Three double-pole, double-throw front panel switch (FPS1, FPS2, FPS3) combinations were used to randomize exposure, sham, and external coil activations. Since many switch settings could make possible a given exposure/sham exposure combination, an operator is less likely to guess or reason out which is which. The RS and FPS labeling used in this paper is shown in Figure 3a. The schematic of the system circuitry linking the RS, FPS, coils, and the power amplifier are shown in Figure 3b,c,d. The switch settings shown in Figure 3b,c,d for the "exposed" condition (first setting of Table 1) where the microscope coil is energized to expose cells to the magnetic field generated.

Amplifier

The coils were driven by a sinusoidal signal from a function generator (LFG 1300S, Leader, Electronics Corp., Yokohama, Japan) and an in-house built class AB power amplifier. The amplifier had a frequency and current ranges of 5–100 Hz and 0–1 A, respectively. Current was varied through a rheostat.

Fig. 3. Detailed schematic of the custom made switch box: (**a**) label key for the rotary switch (RS), front panel switch (FPS), and Pole "common" connections; (**b**) circuit diagrams for RS1; (**c**) circuit diagram for RS2; and (**d**) circuit diagram for RS3. Different combinations of RS1 and RS2 defined the experimental settings (exposure or sham) of the microscope coil, while the setting of RS3 defined the inactivation/activation of the external coil.

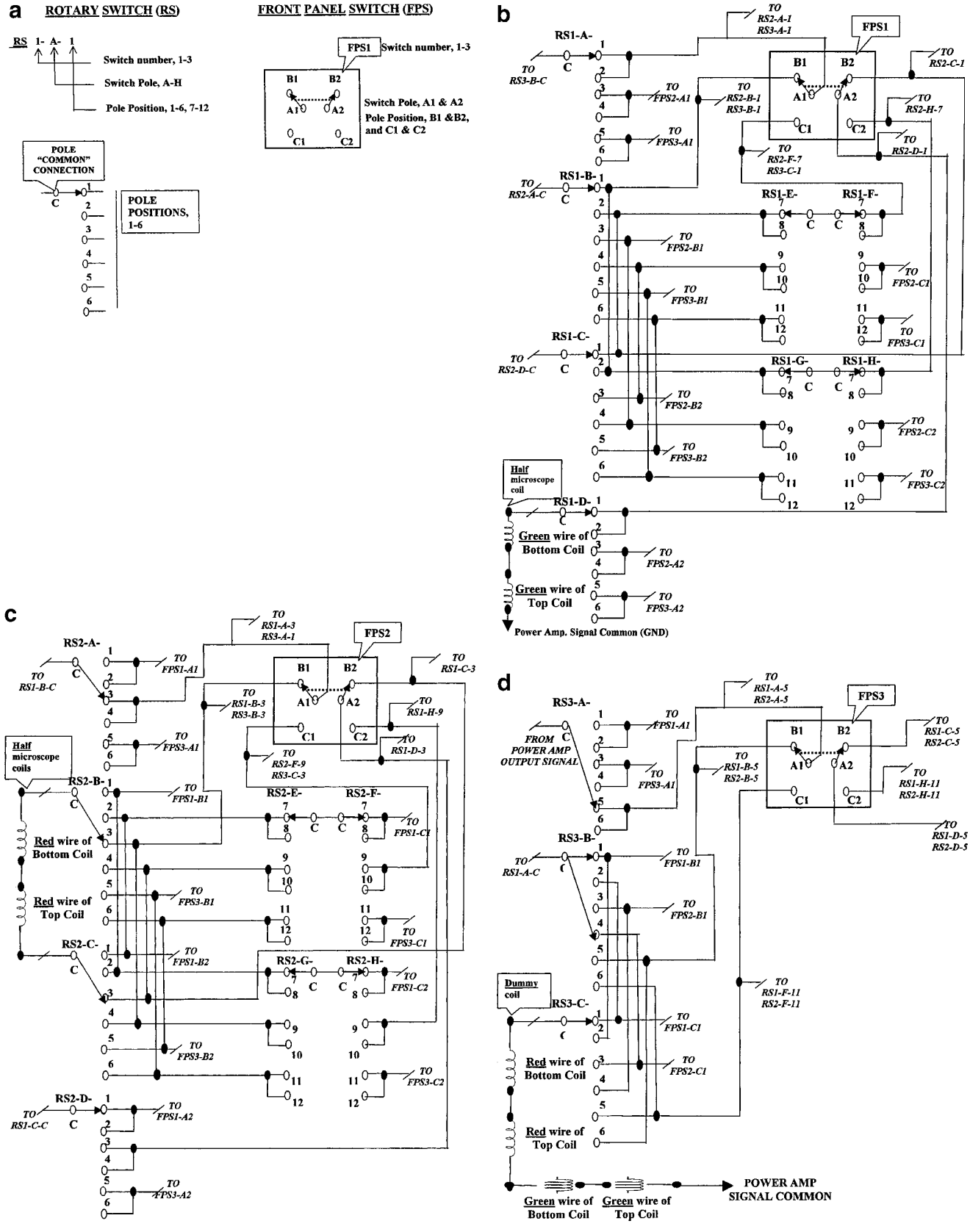
a
ROTARY SWITCH (RS)
RS 1- A- 1
Switch number, 1-3
Switch Pole, A-H
Pole Position, 1-6, 7-12
POLE "COMMON" CONNECTION
POLE POSITIONS, 1-6
FRONT PANEL SWITCH (FPS)
FPS1
Switch number, 1-3
Switch Pole, A1 & A2
Pole Position, B1 &B2, and C1 & C2
b
RS1-A-
RS1-B-
RS1-C-
RS1-D-
RS1-E-
RS1-F-
RS1-G-
RS1-H-
FPS1
Half microscope coil
Green wire of Bottom Coil
Green wire of Top Coil
Power Amp. Signal Common (GND)
c
RS2-A-
RS2-B-
RS2-C-
RS2-D-
RS2-E-
RS2-F-
RS2-G-
RS2-H-
FPS2
Half microscope coils
Red wire of Bottom Coil
Red wire of Top Coil
d
RS3-A-
RS3-B-
RS3-C-
FPS3
FROM POWER AMP OUTPUT SIGNAL
Dummy coil
Red wire of Bottom Coil
Red wire of Top Coil
Green wire of Bottom Coil
Green wire of Top Coil
POWER AMP SIGNAL COMMON

Fig. 3.

TABLE 1. Sample Output in Response to Different Combination of the Three Front Panel Switch Settings

FPS1	FPS2	FPS3	Result
0	0	0	MIC-EXP
0	1	0	MIC-SHAM
1	0	0	MIC-SHAM
1	1	0	MIC-EXP
X	X	1	EXT
X	X	1	EXT
X	X	1	EXT
X	X	1	EXT

The three rotary switches designated RS1, RS2, and RS3, controlled the ''meaning'' of the three front panel switches FPS1, FPS2, and FPS3. RS1 and RS2 controlled the polarity of the magnetic fields that were generated by the two coils. The various combinations of RS1 and RS2 yielded field exposure (EXP) and cancellation (SHAM). ''EXT'' sends current to external coil (see Fig. 1). The setting of RS3 determined which one of the three front panel switches controlled the ''Microscope (MIC)/External (EXT)'' coil state. FPS1, FPS2, and FPS3 could have three combinations, namely, 0, 1, or X (0/1). A manual for all possible switch combinations is available on request.

SYSTEM PERFORMANCE AND CHARACTERIZATION

Measurement of Ambient Magnetic Fields

The background AC magnetic field in the laboratory near the microscope and the background AC magnetic field inside the incubator were measured over a period of 4 h using the F.W. Bell gaussmeter (Model 9550, Orlando, FL) and probe (Model T-99-253). The probe was placed in the center of the incubator close to the location of the exposure device.

The maximum levels of the ambient AC magnetic field near the microscope and inside the incubator were 0.1 μT and were consistent with the required background levels measured by others [Davis et al., 1999]. The DC magnetic field in the laboratory and inside the incubator ranged between 30 and 40 μT. The DC magnetic field level in the laboratory was not considered as one of the exposure parameters due to its constant value both near the microscope and in the incubator. The absence of stray AC fields from the incubator was noted.

Linear Magnetic Field Mapping of the Exposure System

The magnetic field levels across the coil were measured with the F.W. Bell gaussmeter and probe. Since applied field intensity distortion by the microscope objective was expected [Publicover et al., 1999], experiments were carried out to map the magnetic field intensity in presence and absence of the microscope objective (Nikon, Inc., Melville, NY; Apo 60X, 1.40). The magnetic field levels across the stage plate were also measured with the coil system located off the microscope. The mapping was conducted at 60 Hz with input current levels of 50, 100, 200, 300, and 500 mA. The choice of 60 Hz frequency was patterned after previous studies focusing on power-frequency magnetic fields.

The results from the linear magnetic field mapping are presented in Figures 4 and 5. The presence of the objective elicited an increase in the magnetic field level around the edges of the objective and the relative difference increased with current. In the absence of the objective, the exposure system elicited a relatively uniform magnetic field across the coil stage plate (as shown in Fig. 4) in accordance with minor variation with increased distance from the center [Misakian et al., 1993]. As expected, the magnitude of the field was directly proportional to the applied current. The relative difference in magnetic field levels at the center of the coils due to the presence of the objectives were found to change by 7.1, 4.3, 5.3, 4.9, 4.8% in response to current values of 50, 100, 200, 300, and 500 mA, respectively. The results from the linear magnetic field mapping of the coil at different currents indicates that under normal conditions, the magnetic field across the coil measured on the bottom plate is a direct measure of the input current and the spatial distribution is maintained. The presence of the microscope objective disrupted the spatial distribution of the magnetic field intensity with the major increase seen around the edges of the objective. For example, at the center of the coil (see Fig. 6), the magnetic field difference in the presence and absence of the objective was linearly related to the input current. No differences in field intensity were observed at finer spatial resolution (0.1 cm), ruling out the likelihood of more severe distortions undetected at the higher resolution (0.5 cm). Also, lack of more severe distortions at finer spatial resolution in our case confirmed that the observed distortions were not due to objective coating as previously suggested by Publicover et al. [1999]. It is important to note that the results presented in Figure 6 can be used to determine the amount of current adjustment required to compensate for the presence of the objective at a specific location.

Monitoring of Heating Effects

The heating in the system (difference between coil and room temperatures) due to current passing through the coils was measured using a Cole-Parmer (Vernon Hills, IL) Temperature and Humidity Logger Model #91090-70. Measurements were done with thermocouples at linear positions of 0, 12.5, and 25 mm from the center of the coil along the stage plate. Temperature differences between room and plate were recorded at

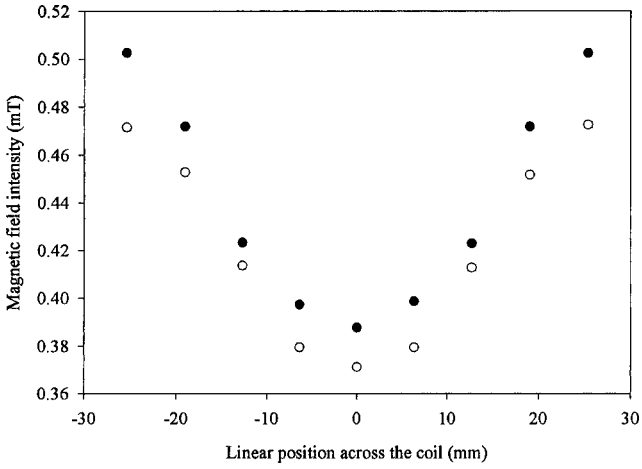

Fig. 4. A typical linear magnetic field distribution of the coil setup, with (●) and without (○) the microscope objective, at a frequency of 60 Hz and an input current of 100 mA.

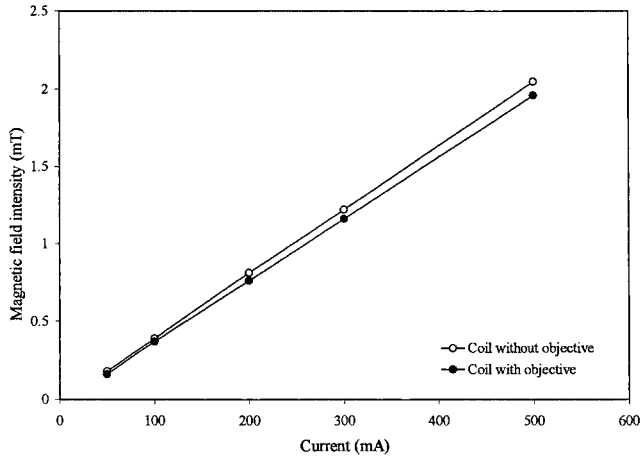

Fig. 6. Difference in magnetic field intensity at 60 Hz at the center of the coil at different input currents. The lines are almost identical suggesting a nonsignificant (maximum of 4.9%) disruption of the spatial magnetic field distribution by the microscope objective.

current levels of 50, 100, 300, and 500 mA, at 10 min intervals over a period of 4 h.

Figure 7 gives a summary of the temperature distribution with time at three different positions of the coil in response to different input currents. At an input current of 50 mA, there was no measurable temperature change. Temperature differences were observed in response to input currents of 100, 300, and 500 mA. It is, however, important to note that the magnetic field intensities in our experimental conditions were usually less or equal to 200 μT (corresponding to input currents less or equal to 50 mA).

Measurement of Harmonic Contents

The harmonic content of a 60 Hz signal from the function generator was measured with an Onosokki CF 360 Portable Dual Channel (Ono Sokki Technology, Inc., Yokohama, Japan) FFT Analyzer at 15 Hz intervals. Measurements were done over a 4-h period with input signals from the signal generator before and after amplification. The rationale for harmonic content characterization was based on the need to ensure that the system would expose cells to only one fundamental

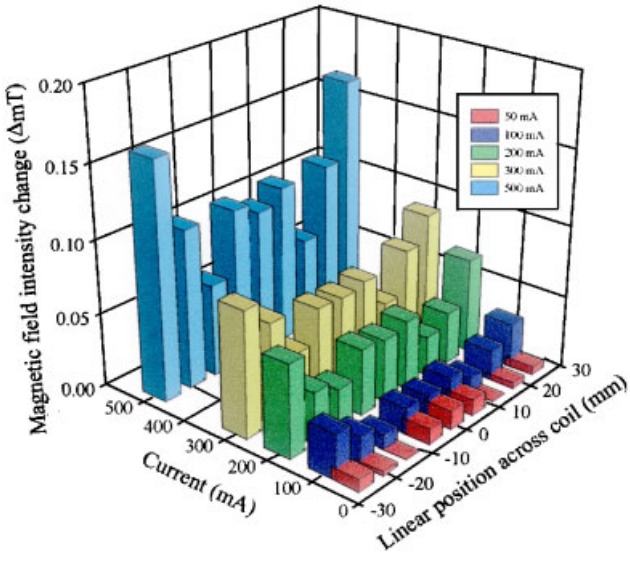

Fig. 5. Magnetic field change due to microscope objective interference across the coil at different input currents (60 Hz). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

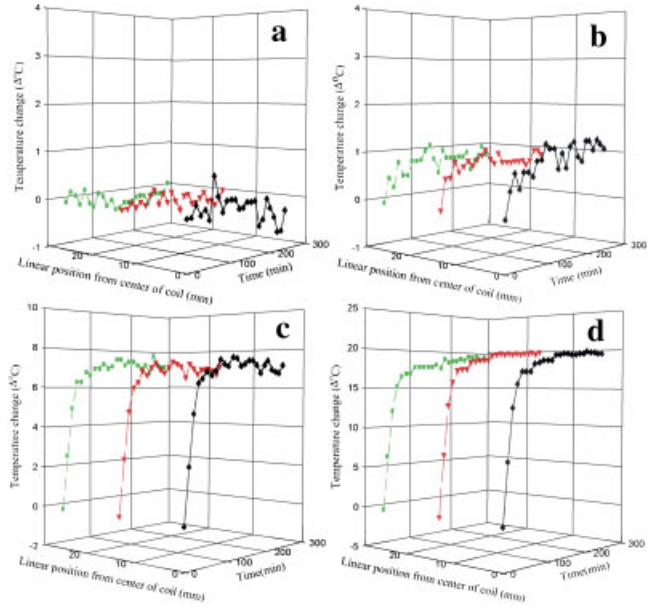

Fig. 7. Temperature difference (microscope stage plate vs. room) at different currents of 50 (**a**), 100 (**b**), 300 (**c**), and 500 (**d**) mA at a constant frequency of 60 Hz over a 4 h time period. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

frequency throughout the exposure period. Four-hour exposure period was based on the previous stress studies requiring several hours to maintain transcription levels [Rodeberg et al., 1999].

The amplitude of harmonics was expressed as percentages of the 60 Hz signal. Since most electric power is generated at a frequency of 60 Hz, we were interested in studying the effects of magnetic fields generated at that particular frequency. The fundamental frequency (f_o) was thus 60 Hz and harmonic signals (15, 30, 45, 90, 120 Hz) were analyzed from the output of both the amplifier and the signal generator. The results indicated that 60 Hz is the fundamental frequency in both the outputs from the amplifier and the signal generator. The amplitude of the harmonics was of the order of 0.1–0.2% of the fundamental frequency which was an indication that the signal generator and the amplifier were suitable for exposure studies at 60 Hz.

Other Factors

Blurring of edges of cells, which is a characteristic of severe vibration, was not observed at all magnetic field intensities studied, suggesting that vibrations were minimal. Magnetic field levels decreased sharply with increasing distance from the coils (data not shown), suggesting that control and exposure experiments could be conducted simultaneously in the same incubator. This eliminated the need for a second incubator normally used in magnetic field exposure studies with large coil setups.

SUMMARY

In conclusion, the exposure system devised and characterized in this study should be useful for measuring real time biological changes (e.g., membrane potential, ionic fluxes, pH) as well as gene transcription studies [Rao et al., 2002] that involve longer exposure times traditionally conducted in cell culture incubators.

ACKNOWLEDGMENTS

The authors thank Steve McDonald for the fabrication of the switch box and the amplifier. Mention of brand names is for information only and does not imply endorsement.

REFERENCES

Bassen H, Litovitz T, Penafiel M, Meister R. 1992. ELF in vitro exposure systems for inducing uniform electric and magnetic fields in cell culture media. Bioelectromagnetics 13: 183–198.

Davis CC, Barber I, Swicord ML. 1999. Food and drug administration low-level extremely-low frequency magnetic field exposure facility. Bioelectromagnetics 20:203–215.

Goodman R, Wei L-X, Xu JC, Henderson A. 1989. Exposure of human cells to low-frequency electromagnetic fields results in quantitative changes in transcripts. Biochim Biophys Acta 1009:216–220.

Goodman EM, Greenebaum B, Marron MT. 1995. Effects of electromagnetic fields on molecules and cells. Int Rev Cytol 158:279–338.

Harrison GH, Balcer-Kubiczek EK, Shi Z-M, Zhang Y-F, McCready WA, Davis CC. 1997. Kinetics of gene expression following exposure to 60 Hz, 2 mT magnetic fields in three human cell lines. Bioelectrochem Bioenerg 43:1–6.

Krause JD. 1984. Electromagnetics. 3rd edition. New York: McGraw Hill.

Lin H, Blank M, Jin M, Goodman R. 1996. Electromagnetic field stimulation of biosynthesis: Changes in c-myc transcript levels during continuous and intermittent exposures. Bioelectrochem Bioenerg 39:215–220.

Misakian M, Shephard AR, Krause D, Frazier ME, Miller DL. 1993. Biological, physical, and electrical parameters for in vitro studies with ELF magnetic and electric fields: A primer. Bioelectromag Suppl 2:1–73.

Mullins RD, Sisken JE, Hejase HN, Sisken B. 1993. Design and characterization of a system for exposure of cultured cells to extremely low frequency electric and magnetic fields over a wide range of field strengths. Bioelectromagnetics 14:173–186.

Publicover NG, Marsh CG, Vincze CA, Craviso GL, Chatterjee I. 1999. Effects of microscope objectives on magnetic field exposures. Bioelectromagnetics 20:387–395.

Rao RR, Halper J, Kisaalita WS. 2002. Effects of 60 Hz magnetic field exposure on APP695 transcription levels in differentiating human neuroblastoma cells. Bioelectrochemistry 57: 9–15.

Rodeberg DA, Meyer JG, Babcock GF. 1999. Heat shock response: Presence and effects in burn patient neutrophils. J Leukocyte Biol 66:773–780.

Saffer JD, Thurston SJ. 1995. Short exposures to 60 Hz magnetic fields do not alter MYC expression in HL60 or Daudi cells. Radiat Res 144:18–25.