Device for Controlling the Electric, Magnetic and Electromagnetic Fields in Biological Incubators

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Device for Controlling the Electric, Magnetic and Electromagnetic Fields in Biological Incubators

by

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Device for Controlling the Electric, Magnetic And Electromagnetic Fields in Biological Incubators
written by Lucas Agustín Portelli
has been approved for the Department of Electrical, Computer and Energy Engineering

__________________________  __________________________
Frank Barnes  

__________________________  _______________________
Dejan Filipovic  

Date ________________

The final copy of this thesis has been examined by the signatories, and we Find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
This manuscript is divided in two parts. The extensive survey in biological incubators presented in PART I reveals how the background magnetic field can vary by orders of magnitude within and between incubators. These variations can be observed within the same incubator in locations that are centimeters apart from each other as well as between incubators that are identical and located in the same laboratory. Additionally, the values measured were frequently outside of the range of magnitudes found naturally on Earth’s surface or ordinary habitation spaces. Exposure to such altered environments has been shown experimentally to be sufficient to cause numerous effects in cell cultures. Examples of the effects reported span from differential generation of free radicals and heat shock proteins to differences in cellular proliferation, differentiation and death. Although the effects are not well established and the molecular mechanism of action is currently under debate, these observations alone support the notion that the inhomogeneity of the background magnetic field in biological incubators is a potential confounding source of the variability and irreproducibility for studies performed on cell cultures. More specifically, the existence of this uncontrolled factor would be especially counterproductive when investigating the biological effects of exposure to magnetic fields of comparable characteristics as the ones measured in this study or when studying small biological effects in general. PART II utilizes the set of measurements obtained in PART 1 to study the possibility of engineering a practical solution to ameliorate this problem. This maturates into a fully characterized prototype which provides a controlled background electric, magnetic and electromagnetic environment for cell or microbiological culture upon being placed inside a standard biological incubator. The design, in part, consists of a novel high permeability shield architecture which does not include doors or lids which was found to be comparable to “lidded” designs used extensively in preceding Bioelectromagnetics research. Its performance was verified by simulating the residual magnetic fields in its interior after being inserted into the 21 incubators surveyed in PART 1. The
device’s thermal characteristics and the tools and methods implemented to test this design are also fully characterized.
Dedication

To my family, Stella, Jorge, Nacho– who gave me all they had and what they didn’t have. We made it!

To my professor and friend, Frank Barnes. Without his patience, vision, and exceptional amount of support and confidence none of this would have been possible.
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This project relied on the willingness of professionals in charge of laboratories in the departments of Molecular, Cellular and Developmental Biology, Mechanical Engineering, Biochemistry and Civil, Environmental and Architectural Engineering at the University of Colorado at Boulder, to allow measurements in their cell culture incubators. Their kindness and courtesy is greatly appreciated. I would like also to thank the Electrical, Computer and Energy Engineering Department for its extended support to our projects over the years.

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Son, this is the secret I used to win:

When everybody was sleeping, I was training.

When everybody was resting, I was training.

When everybody was eating, I was training.

And all this time, I was also thinking about them and how I was going to beat them.

-Luis Jorge Portelli –my father
Chapter I

Introduction:

Many experiments on cell biology, microbiology and molecular biology are almost unavoidably performed in incubators as they serve to create homogeneous and controlled physical environments. Biologically relevant environmental parameters commonly controlled include temperature, humidity, CO$_2$ and O$_2$ concentrations. In these uniform conditions, groups of cellular and microbiological cultures can be grown and maintained with reasonably predictable outcomes. This makes incubators one of the most fundamental laboratory tools necessary to achieve experimental reproducibility. The immediate downside of not having such control over these parameters spans from increases in the amount of experimental resources needed to achieve statistical significance to obtaining and reporting misleading results. Therefore, careful control of these parameters becomes increasingly important as the experiments involved study small or exquisite biological effects. Occasionally, biological experiments which are well known and established are proven to be hard to reproduce. Unfortunately, it is not rare for this to happen even in the same laboratory. Common causes for this occurrence include variations in traditional factors of chemical, biochemical, biological, physical and procedural nature which are well known to have significant effects in biological experimental outcomes. Examples of these span from the origin and composition of medium, additives, disposables and reactants, to the specific techniques applied for culture, to name a few [Mather et al., 1998]. Putting these known factors aside, we show that the background magnetic field (BMF) in biological incubators is a potentially relevant variable that is often ignored in
conventional biological research. Our measurements reveal that magnetic fields are highly variable and inhomogeneous in standard CO₂ biological incubators. These inhomogeneities are large enough that these magnetic fields can be deemed at least in part responsible for “biological variability” inherent to in vitro models [Jayaraman and Hahn, 2009] which burdens even the most carefully designed and executed biological experiment. In this regard, there is a growing cohort of experimental data showing compelling effects of magnetic fields in cell cultures, some of which are presented later in this document. Furthermore, the variability and inhomogeneity in BMF’s is also of special interest to the careful researcher in Bioelectromagnetics, as it encompasses extra hurdles to overcome in order to achieve controlled or “clean” magnetic field environments to investigate the biological effects of magnetic fields.

**The biological incubator’s background magnetic field:** In incubators and all other experimental setups, the BMF exists as a consequence of the combination of two magnetic field sources: (i) the naturally occurring magnetic field (geomagnetic field or GMF) and (ii) the artificially-generated magnetic field (AMF). The GMF and AMF are individually comprised of both a static magnetic field (SMF) component (0 Hz) and a time-varying magnetic field (TVMF) component (> 0 Hz). Their general characteristics are known. The magnitude of the SMF component of the GMF currently ranges between ~23 and ~65 µT over the planet’s surface [Finlay et al., 2010]. The amplitude of its TVMF component is typically less than 0.1 µT, except during the rare cases of large magnetic storms when it can grow up to 1 µT for short periods. It is composed of frequencies ranging from fractions to several hundred Hertz and its direction depends on the location of the source (terrestrial or extraterrestrial) [Hansson Mild and Greenebaum, 2006; Zhang et al., 2007]. On the other hand, the TVMF component of the AMFs
in habitable areas of industrialized countries is primarily a consequence of electrical power
distribution and usage. For this reason, its amplitude is highly variable, but, in general, not
greater than 0.4 μT as 99.2% of the population resides in homes with a TVMF less than or equal
to this value [Ahlbom et al., 2000; Greenland et al., 2000]. Also for this reason, the peak
amplitude in the spectrum of such fields is typically at 60 Hz (or 50 Hz depending on the power
distribution convention of each geographic region) and its harmonics [Hansson Mild and
Greenebaum, 2006]. The SMF component of the AMF is typically generated by the presence of
magnetically relevant materials and static electrical currents, both common occurrences in
laboratory settings and equipment. Additionally, these can considerably alter both the magnitude
and direction of the existing GMF. This includes biological incubators simply because their
construction, in the most basic form, typically amounts to a metal box. Also, the relocation or
replacement of common accessories made of these materials that are put near; on top or lean
against biological incubators (such as gas tanks) have the ability to modify such fields.
Furthermore, both SMF and TVMF are generated by other essential parts of incubators such as
heating elements, fans and control circuitry, and their locations and electromagnetic
characteristics depend on the specific design of each incubator, a factor that is not standardized
in the industry. In addition, it is not uncommon to find electrically powered equipment placed
permanently on top of incubators. These devices could generate magnetic fields of magnitudes
sufficient to affect the BMF inside the incubator in a relevant way. Similarly, an incubator’s
BMF is susceptible to events such as relocation of other electrically powered laboratory
equipment and spatial or temporal changes in power consumption by equipment inside or outside
the laboratory. These events would inadvertently change the laboratory AMF on which the BMF
inside incubators depends. In this regard, since scientific facilities, on average, consume
significantly more (5 to 10 times) electrical energy per unit area than a regular habitation facility [2008], it is reasonable to expect that the AMF in a laboratory is likely a factor of relevance significantly contributing to the incubators’ BMF.

All these factors combined entail an intrinsic uncertainty in the BMF exposure parameters for cell culture experiments in conventional biological laboratory environments that require the use of an incubator. Such factors are usually outside the awareness or control of the researcher. Previous studies have pointed out this problem by giving a glimpse at the TVMF present in biological incubators [Hansson Mild et al., 2003; Moriyama et al., 2005; Hansson Mild et al., 2009]. However, a complete characterization of the nature of the BMF in incubators, which can only be assessed by systematic and careful direct measurements, has not been attempted before. Such a survey is a fundamental starting point needed to assess and, if necessary, address the issue. With this in mind, a set of complementary magnetic probes were designed, built and calibrated in order to measure the BMF in incubators by measuring their SMF and TVMF components.
Chapter II

**Materials and methods:**

Both SMFs and TVMFs were measured for all 3 axes at 27 locations in each of 21 incubators, resulting in a thorough spatial survey. These data allowed for analysis of the typical variation within a single incubator and across multiple incubators.

**Type of biological incubators measured:** Brand name, c.a. 160 liters, CO₂ tissue culture incubators were measured. A total of 7 different and widely utilized brands were surveyed (see Table 1). All incubators were powered and regulated at 37 ± 1°C and 90% humidity at the time of start of measurements. The incubators had metallic shelves, with the exception of 2 which had plastic shelves. All incubators in this study were owned by laboratories at the University of Colorado (Boulder, Colorado, USA). Incubators were located in 8 different laboratories on floors 1, 2, 3 and basement of four different buildings on campus. This diverse set was selected to best represent a typical incubator and laboratory setting.
Table 1. Brands and locations of incubators. Incubators were located in 4 different floors (basement to 3rd) and 4 different buildings.

**Measurement locations:** Both SMF and TVMF components were measured for all 3 axes in 27 locations of each incubator to obtain a spatial sample of the entire incubator cavity. A diagram of the measurement locations is shown in Figure 1. Data were collected for 3 shelves per incubator (near top, middle, near bottom) in nine locations on each shelf. One location was in the middle of each shelf (Location 1) while the remaining eight were positioned approximately 3-5 cm from the perimeter of each shelf, surrounding the middle location and numbered in a counterclockwise manner. The three shelves were approximately evenly spaced in the interior of the incubator (12 +/- 2 cm). Measurement locations were kept consistent for all incubators.
Measurements were made at the lowest height above the shelves permitted by the design of the probes which are described for each sensor below.

**SMF measurement system:** SMF probes were constructed from identical, miniature hall-effect sensors (M/N 912-039, Magnetic Instrumentation, Indianapolis, IN, USA). Each probe consisted of a sensor affixed in the bottom (inside of) a Petri dish (diameter: 5.15 cm, height: 17 mm, plastic thickness: 1.25 mm). This design allowed for the measurements to be made as close as possible to the adherent-cell and medium-height of typical mammalian cell cultures. The probes were affixed semi-permanently to the Petri dish to reduce error from pressure of physical stress on the sensor and its leads, which could generate false readings. To obtain the resultant magnitude for a SMF measurement, perpendicular SMF measurements in all 3 axes were required. Since each probe’s measurement capability was one-dimensional, it was necessary to have two such probes, one with the sensor flat on the bottom (vertical static magnetic field probe) and the other orthogonally vertical (horizontal static magnetic field probe). The

![Diagram of measurement locations within each incubator](image_url)
The combination of these two probes was used to measure the SMF in each location in all three axes. The active dimensions of the sensors were 3.05 x 1.52 mm. The centers of the SMF sensors when the probe was placed on an incubator shelf were above the shelf by 1.50 mm for the vertical probe and 1.85 mm for the horizontal probe. Figure 2 shows a diagram of the SMF probes. The Hall-effect sensors were factory calibrated by Magnetic Instrumentation (NIST certification numbers: vertical probe sensor (20100940-1), horizontal probe sensor (20100901-1)). The SMF sensors were connected to a Gauss-meter (model number: 912, Magnetic Instrumentation). Calibration of the whole SMF measurement system was performed in our laboratory as described below.

![Figure 2. SMF probe diagram.](image)

(1) Hall-effect sensor; (2) Insulation putty; (3) Sensor leads; (4) Petry dish; (5) Temperature sensor; (6) Sensor leads going to measuring instruments.
**Perpendicularity of the SMF measurements:** The horizontal probe had to be used twice for each SMF measurement (x and y components). A tool was built which allowed for the consistent perpendicular placement of the horizontal SMF probe in each measurement position to mitigate this potential source of error. It consisted of a plastic ring in which the SMF probes fit tightly into in two perpendicular positions only with a maximum variation of 5° between the axes. However, the measurement error introduced by this misplacement would vary with a cosine of the angle. Consequently, by using this tool, the maximum error introduced by inaccurate probe perpendicularity was less than 1%.

**Temperature compensation:** Magnetic field measurements with Hall-effect sensors are temperature dependent. Since the measurements were performed in heated incubators, care was taken to assess and regulate the temperature change of the sensors. A type-K thermocouple (Part number: 5TC-TT-K-40-72, Omega Engineering, Stanford, CT, USA) was placed inside each Petri dish in a location where it did not alter the magnetic field measurement. The petri dishes were then sealed to air flow and this served as a temperature buffer for the sensors and to protect them from damage, wear and shock. The temperature sensors were connected to a temperature meter (model number: HH506RA, Omega Engineering). The recorded temperature was then correlated to the change in SMF reading from the Gauss-meter. In addition, the behavior of the probes under thermal changes was studied. Magnetic field reading was found to change with temperature (tested in a range from 22 to 42 °C (± 0.1°C)) by a maximum of 1 μT/°C. During the SMF measurement sessions, the probes’ temperatures were continuously monitored and were only allowed to vary by a maximum of ± 3 °C (± 0.1°C). Therefore, ± 3 μT is the largest
uncertainty possible introduced by this factor. Figure 3 shows a diagram of the connections between sensors and meters in the SMF probe.

![Figure 3. Schematic of the connections for the SMF measuring system.](image)

**Calibration:** The SMF measurement system was calibrated to 0 µT (± 0.5 µT) at room temperature (23 ± 2 °C) by using a cylinder made of helicoidally wound µ-metal (inner diameter = 57.15 mm; length = 343 mm; attenuation = 99.87% or 58.12 dB at the center) (5-foot strip of AD-MU 80 .01" Thick x 4” Wide, Ad-Vance Magnetics, Rochester, IN, USA) which was placed perpendicular to the ambient SMF by relative inspection. Zeroing was accomplished by iteratively rotating the probe within the cylinder and setting the average of the extreme values recorded to zero. The largest uncertainty introduced by zeroing was found to be ±1.10 µT.

A known magnetic field was generated as described later in this section. A calibration curve obtained at 22.2 ±0.1°C (range = ±200 µT, 15 points) found that the SMF measurement system had no discrepancy between the theoretical and measured SMF with an uncertainty of ±0.16 µT ±1%.
**Resolution:** Adding in quadrature, the uncertainties due to perpendicularity of the horizontal probes, temperature variations, definition of zero magnetic field magnitude and the dynamic range calibration system, the uncertainty of the SMF measurement system was found to be ±3.98 μT ±2.94% for the resultant field magnitude. The placement of the probes in the measurement locations in the incubator shelves were allowed to vary a maximum of 2 cm radially. The overall discrepancy between the incubator axes and the magnetic field axes for SMF and TVMF was estimated to be less than 10°.

**TVMF measurement system:** The uncertainty introduced by the Hall-effect sensors made them inappropriate for measuring TVMF of magnitudes below 1 μT which were of potential interest to this study. This prompted the design and construction of a TVMF induction sensor. The advantages of this sensor design were linear frequency dependence of signal amplitude, simultaneous tri-axial measurement, no temperature dependence in the range of interest and that its cubic geometry makes it easy to align with the incubator geometry. The TVMF probe consisted of a set of mutually perpendicular square induction coils. Each coil was comprised of 200 +/- 5 turns of 34 AWG enamel coated copper magnet wire. The thickness of the coils was 2 x 2 mm. The coils were nested, sharing the same geometrical center and had different areas as follows: Area 1 = 9.92 x 10^{-4} m², Area 2 = 7.56 x 10^{-4} m², Area 3 = 5.52 x 10^{-4} m². The areas were calculated by taking the perimeter of each coil as passing through the center of the thickness of each coil. The coils were wound on 2 x 2 mm channels in the surface of a plastic cube (side length = 25.4 mm). The center of the TVMF sensor, when placed on an incubator shelf, was 12.7 mm from the shelf. The probe was not temperature dependent in the relevant range; therefore, temperature calibration, monitoring and compensation were not needed. The coils were fed in
twisted pairs by the same copper wire used for the coils (1.5 m long). The twisted pairs coming from each coil were connected via Bayonet Neill-Concelman (BNC) connectors to a manual multiplexer used to select the individual signal to be amplified by a high impedance differential amplifier (model number: OSP-1, Advanced Research Instruments, Golden, CO, USA) and filtered with a 10 KHz low-pass filter incorporated into the amplifier. A digital oscilloscope (model number: TDS2014B, Tektronix, Beaverton, OR, USA) followed the amplifier and was used to calculate the discrete Fourier transform (DFT) of the signal (Resolution = 0.49321 Hz, Bandwidth = 0-500 Hz, Hanning windowing). All circuitry was grounded to avoid capacitive-coupling related noise. Figure 4 shows a simple diagram of the TVMF probe. Figure 5 shows a diagram of the configuration of the TVMF measurement system.

![Figure 4. TVMF probe diagram. A set of square mutually perpendicular and nested induction coils.](image)

**Calibration:** A known magnetic field was generated as described later in this section and the error introduced by this system was ±0.02 μT ±1%. Calibration curves were generated at field amplitudes of 1, 3 and 5 μT and measurements made every 20 Hz (from 20 to 500 Hz) and every 10 Hz near 60 Hz. These curves compared the known applied magnetic field to the measured magnetic field, in spectrum. Calibration constants were obtained for each of the nested square
induction coils. The maximum uncertainty introduced by the system was primarily due to the frequency resolution of the DFT and the windowing of the time signal and was shown to be ±14%. The inherent noise in the system was determined by placing the TVMF probe inside of the μ-metal cylinder described above which revealed a baseline noise level of 0.03 μT.

![Schematic of the connections for the TVMF measuring system.](image)

**Resolution:** Adding in quadrature the errors described above, the error of the TVMF sensing system was found to be ±0.05 μT ±24.2% for the resultant field magnitude. The placement of the probes in the measurement locations in the incubator shelves was found to vary a maximum of 2 cm radially. The overall discrepancy between the incubator axes and the magnetic field axes for SMF and TVMF was estimated to be less than 10°.

**Measurement protocol:** Measurements were made with the voluntary approval of the owner or person in charge of each incubator. Measurement equipment was powered and allowed to warm up for at least 30 min before starting the measurements. Measurement sessions were comprised of the complete measurement of each incubator’s SMF or TVMF at one time. Data were always recorded after both incubator doors (glass (interior) and metallic (exterior)) were closed. This was done making sure the contact of the interior glass door with the sensor leads did not disrupt the probe position and orientation. Probes were aligned with the incubator geometry by sight.
Precautionary aseptic techniques were practiced at all times during the measurement sessions to protect the measurer from harmful biological exposure as well as the incubator from contamination. As part of this practice, SMF and TVMF probes were sprayed with 70% ethanol before being placed inside of the incubator. This practice also had a cooling effect on the probes which was purposely utilized in the case of the SMF measurements to regulate the temperature of the SMF probe (maximum variation = ±3 ±0.1°C). Also, in order to minimize the error in zeroing, data were collected for the whole incubator, with one of the SMF probes first (horizontal or vertical) and then with the other. At the beginning of each measurement session, the SMF system was always zeroed at the site of the incubator to be measured by using the μ-metal cylinder described above.

In the case of the TVMF measurements, zeroing and temperature control were not necessary and data for all 3 axes were recorded with the same probe. Also, signals from the TVMF probe were monitored in real time to occupy 2/3 of the maximum allowed amplitude in the oscilloscope screen before obtaining the spectra, thus avoiding chopping of the signal and introduction of unwanted artifacts.

Total magnitude SMF or TVMF in each location was calculated by vector summation of the magnitudes extracted from the spectrum obtained. Data were collected in 21 incubators. Therefore, the total number of locations measured in this study amounts to 567 in which SMF and TVMF spectra up to 500 Hz were recorded. Data manipulation and statistical analysis were done with Microsoft Office Excel 2007 (Redmond, WA, USA) and MATLAB R2007a.
Uncertainty was reported as one standard deviation which corresponds to 68.1% of the maximum value possible, assuming a Gaussian distribution.

**Magnetic field generation system for sensor calibration:** A coaxially fed pair of square coils in Helmholtz configuration (square Helmholtz coil - SHC) (side length (L) = 30.53 cm, number of turns (N) = 10, wire gauge = 24 AWG, separation between coils (D) = 16.31 cm, thickness of the coils = (axial = 6 mm), (radial = 0.5 mm)) was built. The dimensions of the SHC were within ± 1%. A plastic structure for supporting the sensor was aligned with the coils within 1% of level. These variations along with the coil design were capable of generating a uniform field (in direction and magnitude) in the volume occupied by the probes with a maximum relative error of 1%. The SHC system was located at least 1.5 m away from walls or metal objects. It was not necessary to shield the system from ambient magnetic fields since the calibrations made with this system were relative. A diagram of the SHC system is shown in Figure 6. For the calibration of the SMF measurement system, a known magnetic field was generated by injecting a direct current to the SHC and measured with a calibrated meter (NIST certification number: 1383235) (model number: 179, John Fluke MFG, Everett, WA, USA). The inherent uncertainty associated with this device propagated to ±0.16 μT ±1% when connected to the SCH. For the calibration of the TVMF measurement system, a known magnetic field was generated by injecting a sinusoidal current to the SHC. The current was assessed by measuring the potential drop across a high precision decade resistor (type number: 607-F, General Radio, Cambridge, Massachusetts, USA) with a high impedance oscilloscope (model number = 54622A, Agilent Technologies, Santa Clara, CA, USA) and compared against the previously described calibrated meter with static
current. Confounding error factors like temperature change in the resistor were proven to be negligible. The uncertainty for the generated TVMF was $\pm 0.02 \, \mu T \pm 1\%$.

Figure 6. Diagram of the SHC setup for sensor calibration. 1) Cubic acrylic frame; 2) Square coils; 3) Flat and leveled platform for sensor placement; 4) Plastic height adjustment screws for leveling the SHC; 5) Flat plastic platform for the SHC setup; 6) Plastic height adjustment screws for leveling the sensor platform.
Results and Discussion:

For the TVMF component, 60 Hz was continually found to be the primary peak when analyzing the spectra measured in every point of the 21 incubators (see Figure 7). This is an expected outcome since 60 Hz is the frequency of the power supplied to all the incubators. Similarly, 50 Hz can be observed in incubators fed with electrical power supplied at that frequency [Moriyama et al., 2005; Hansson Mild et al., 2009]. Because of this, all the data presented in this report for TVMF analysis are only the 60 Hz amplitudes.

Figure 7. An example of a typical magnetic field spectrum measured in a CO₂ controlled incubator. Notice how 60 Hz is the dominant frequency while the other prominent peaks correspond to its harmonics. The vertical scale is presented in μT • Hz to facilitate the observation of the harmonics.
Figure 8 shows the minimum and maximum values for 60 Hz found in each incubator surveyed. All incubators tested had points that exceeded natural and habitation values by at least a factor of three. These data also revealed that TVMF can vary as much as nearly 240 μT both between incubators and within a single incubator. This value exceeds habitation environments by a factor of 600 [Ahlbom et al., 2000; Greenland et al., 2000; Hansson Mild and Greenebaum, 2006]. As mentioned before, most of the time, the magnitudes found in habitation environments at 60 Hz exceed those found in nature by orders of magnitude.

Figure 8. Maximum and minimum TVMF amplitudes measured (at 60 Hz) in each of 21 CO$_2$ cell culture incubators. As a comparison, the TVMF in habitation environments is typically less than or equal to 0.4 μT at 50 or 60 Hz as depicted by the shaded area and light arrow. The black arrow represents magnitudes outside of this range. Error bars represent ±0.05 μT ±24.2% and correspond to error associated with the measurement system and protocol.
TVMF magnitudes such as the ones measured have been reported in the literature to affect cell cultures when compared to cells cultured in conditions close to those found in nature (TVMF < 0.1 µT). A brief review of the most recent data is here presented. In the case of human breast cancer cells (MCF-7) effects have been found to occur for magnitudes as small as 1.2 µT. These experiments were independently replicated several times. For instance, exposures as short as 24 h (at 60 Hz), have been identified to cause resistance to melatonin- and tamoxifen-induced growth inhibition [Blackman et al., 2001; Girgert et al., 2005], up-regulation of the plasminogen activator system [Girgert et al., 2009], alternation of the expression of estrogen receptor cofactors [Girgert et al., 2008] and disruption of one of the melatonin receptors (MT1) [Girgert et al., 2010]. Greater TVMF magnitudes have also been shown to induce other defensive effects on other cancer cell lines. For example, exposure of human melanoma (M14 and A375) to 38 µT (at 50 Hz) for 5 h has been shown to raise the levels of BAG3, a protein associated with anti-apoptosis [Basile et al., 2011]. Similarly, human myeloid leukemia cells (HL-60) were shown to express several heat shock genes after short (30 min) exposures to magnitudes as low as 10 µT (at 50 Hz). Most significantly, these cells expressed HSP70 (A, B and C), presenting maximum induction between 60 and 80 µT [Tokalov and Gutzeit, 2004]. Also, superoxide radical anion formation and HSP70 induction were shown to be affected in human Erythroleukemia cells (K562) when exposed to 25 and 100 µT (at 50 Hz) for 1 hour [Mannerling et al., 2010]. This data collection supports the possibility that altered TVMF conditions such as the ones measured in this study are extreme enough to cause abnormal cell behavior. Effects in other mammalian cell cultures analogous to the ones described are therefore a reasonable concern. Consequently, an issue of relevance is that nearly 93% of all the 567 locations measured in all 21 incubators present magnetic fields that fall above 0.4 µT (see Figure 9). As a comparison, habitation
environments exhibit TVMF primarily at 50 or 60 Hz which magnitudes are less than or equal to 0.4 $\mu$T.

![Habitation environment range](image)

**Figure 9. Distribution of all the TVMF amplitudes measured (at 60 Hz) in all 21 CO$_2$ cell culture incubators.** As a comparison, the TVMF in habitation environments is less than or equal to 0.4 $\mu$T at 50 or 60 Hz as depicted by the shaded area and light arrow. The black arrow represents magnitudes outside of this range.

In a similar way, the dataset obtained for the SMF component shows variability and extreme values. Although the static component of the Earth’s magnetic field in Boulder, Colorado was approximately 52.7 $\mu$T at the time of the measurements\(^1\), Figure 10 reveals that it is common to find locations with SMFs outside the natural GMF range (23-65 $\mu$T). Furthermore, differences in the SMF have been found nearly as big as 450 $\mu$T within the same incubator and

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\(^1\) According to data from the Boulder Geomagnetic Observatory (National Geophysical Data Center at the National Oceanic and Atmospheric Administration, USA).
between incubators; more than 600% the maximum SMF found naturally on Earth’s surface [Hansson Mild and Greenebaum, 2006; Finlay et al., 2010]. Interestingly, cells of various types have been found to behave differently when exposed to SMF outside vs. inside of the natural GMF range, especially when it is reduced below 23 μT. In the reviewed literature, these environments have been referred to as “Magnetic vacuum”, “Zero fields”, “Near-Zero fields”, “weakened GMF”, “Perturbed Geomagnetic fields” and “Low Level Fields” to name a few. For example, exposure to these reduced BMF conditions for times as short as 20 minutes have been shown to affect the kinetics of the conformation of chromatin in human fibroblasts (VH-10) and lymphocytes [Belyaev et al., 1997].

**Figure 10.** Maximum and minimum SMF magnitude measured in each of 21 CO$_2$ cell culture incubators. As a comparison, the shaded area represents the normal SMF magnitudes found naturally on Earth’s surface. The black arrows represent magnitudes outside of this range. Error bars represent ±3.98 μT ±2.94% and correspond to error associated with the measurement system and protocol.
Also, the self-assembly of tubulin, a key eukaryotic cytoskeletal constituent, was shown to result in amorphous oligomers instead of microtubule-like filaments in a similar time frame [Wang et al., 2008]. Other studies have shown this kind of exposure to affect growth rates of cancer (fibrosarcoma HT1080, colorectal HCT116) and primary human umbilical vein endothelial cells (HUVECS), bovine pulmonary artery endothelial cells (PAEC) cell lines, [Martino et al., 2010a,b; Martino, 2011] for exposures as short as 24 h. Our study shows that nearly 40% of all the points tested in the 21 incubators fall outside of the natural GMF range (see Figure 11), emphasizing the SMF component in biological incubators as another factor of concern.

![Figure 11. Distribution of all the SMF magnitudes measured in all 21 CO\textsubscript{2} cell culture incubators. As a comparison, the shaded area and light arrow represent the normal and most extreme SMF magnitudes found naturally on Earth’s surface respectively. The black arrows represent magnitudes outside of this range.](image)
Some more extensive studies have also revealed that exposure to altered BMF conditions (both for the SMF and TVMF components) exhibit dose-, time-, and cell type-dependent responses. For example, dose-dependent modulation of chemically induced differentiation was observed for Friend Erythroleukemia cells (i.e., 40% inhibition for 4 µT and 50% proliferation for 100 µT, both at 60 Hz) [Chen et al., 2000]. Similarly, magnitudes between 30 and 120 µT (at 60 Hz) have been shown to affect proliferation of human astrocytoma cells when exposed for between 6 and 72 h, presenting a time and dose-dependent effect [Wei et al., 2000]. Additionally, the same group reported that no effect was observed on rat cortical astrocytes (similar, but non-transformed cells) under the same exposure conditions. This is an example of differential effects occurring depending on cell type. Another such example shows human lymphoblastoid cells’ death rate to be differentially affected by exposure to 60 µT (50 Hz) depending on the cells’ origin; normal cells responded differently than cells from genetic instability syndromes [Mangiacasale et al., 2001]. Similarly, decreased DNA repair rates and protection from heat-induced apoptosis were observed to occur after exposure to 150 µT (60 Hz) for time periods of 4-24 h in two different human leukemia (HL-60, HL60R) and lymphoma cell lines (Raji). Also, in this case, both were reported to be dependent on cell type and time of exposure [Robison et al., 2002].

It has been suggested that the diversity of effects observed for exposure to altered BMF environments in different cell types and in different time frames may be due to the cell type specific redox status [Simko, 2007]. Exposure to these environments appears to modulate cellular redox balance by either enhancing oxidative intermediates or reducing antioxidants. Supporting evidence has been found for this hypothesis both for SMFs and TVMFs. For
example, exposures to SMFs below 23 μT have been shown to modulate hydrogen peroxide (H₂O₂) concentrations in human pancreatic cancer (AsPC-1) and HT1080 cells [Martino and Castello, 2011]. Similarly, free oxygen radicals concentrations have also been shown to be affected by 40 μT at 50 Hz in rat lymphocytes [Zmyslony et al., 2004].

![Figure 12. Example of the intermittence occasionally measured for TVMF in CO₂ incubators.](image)

In this case, the 60 Hz TVMF signal observed was modulated by a frequency close to 0.5 Hz. As a result, a substantial change in amplitude is observed. These data were taken on a single axis only (vertical) for one of the incubators measured and it is representative of the typical intermittence occasionally observed. Some incubators also exhibited changes in the amplitude of the 60 Hz signal either in a continuous way or in discrete steps.

Although 50 and 60 Hz have been of preferential interest in the data presented, other frequencies have shown to be biologically relevant in very selective ways. TVMF exposure of 2.5 μT at 7 but not 18 Hz for 3 to 5 days have been shown to modulate intracellular calcium
concentration, metabolic activity, proliferation and differentiation of human adult cardiac stem cells [Gaetani et al., 2009]. Also, the concentration of reactive oxygen species and nitric oxide in human neutrophils, macrophages and cancer cells (HT1080) have been shown to be susceptible to the electric fields of small magnitudes ($10^{-4}$ V/m) which were induced by pulsating magnetic fields of specific sub-Hertz frequencies. The effects observed were able to cause DNA damage in short timeframes (5 min) under conditions of resonance with the cellular metabolic oscillations [Rosenspire et al., 2005]. These experiments are of particular interest since our study has revealed, in some cases, intermittence in the TVMF generated by the incubators in similar frequency range which can induce electric fields with similar characteristics in cell cultures (see Figure 12). The magnitudes of the induced electric fields depend on the frequency components of the TVMF and on the geometries of the containers in which the cell cultures are grown, such as Petri dishes (round). The frequency components of the TVMF depend on the specific characteristics of the switching circuitry that feeds the devices generating the magnetic fields, such as heaters, electromechanical valves, etc. and on the electrical characteristics of the devices themselves. Furthermore, since these devices are usually part of a feedback loop, the frequencies at which the switching occurs also depend on the current state of the parameter being modified by the magnetic field-generating device. If the device is a heater, for example, the heater will be in the “on” state an increasing amount of time as the incubator temperature falls below the incubator’s nominal temperature. All these factors create a very complex set of electric and magnetic signals to which cell cultures are exposed which is hard to predict without direct measurements.
In addition to the variability of the BMFs in time, magnitude and frequency already discussed, the BMFs measured were inhomogeneous within the same incubator, increasing the level of complexity of the possible biological effects induced. Figures 13 and 14 show the TVMF and SMF magnitudes grouped by shelf and relative position on the shelf. These data point to the fact that in the absence of direct measurements, there is little room for educated guesses about the “best” location to perform experiments in a specific incubator. In this regard, notice how for SMF, locations found near the front of the incubator were, on average, the most variable, while the ones in the far back, especially on the bottom shelf, were the least. In contrast, for the TVMF, the smallest and least variable TVMFs were found, on average, at the front of the incubator. Additionally, since BMF variations were found in same brand incubators but in different locations, it was not possible to recommend a particular brand. Instead, this data indicates that BMF must be monitored and controlled on a per experiment basis.

There are strong classical and fundamental reasons why magnetic fields of such characteristics should not affect cellular biological systems [Weaver et al., 1999; Adair, 2003; Vaughan and Weaver, 2005] leaving the molecular mechanism of action that explains these effects currently open for debate. However, in view of the effects observed, several other possibilities have been explored theoretically and experimentally. Namely, these include the “ion cyclotron resonance model” and the “ion parametric resonance model” [Adair, 1998; Belova and Panchelyuga, 2010], the “molecular gyroscope model” [Binhi et al., 2001], the “Lorentz models” [Muehsam and Pilla, 2009], the “DNA-antenna model” [Blank and Goodman, 2011] the “radical pair model” [Timmel and Henbest, 2004] and other modifications and combinations of these. Currently, a promising molecular mechanism is based on the “radical pair model”. This theory
appears to have the fewest fundamental contradictions, as it does not encompass coupling with the thermal bath and several experiments have in some way supported this theory on cell-free assays [Engström, 2007]. Additionally, it suggests that the concentration of free radicals can be both decreased and increased by changing the magnitude of the magnetic field [Rodgers et al., 2007]. This, in turn, can be expected to both inhibit and accelerate biological processes such as growth rates with dependency on cell type and provide a possible explanation for some of the conflicting results in the literature.

![Figure 13. Average TVMF per location (at 60 Hz). Refer to Figure 1 for a diagram of the actual measurement location within each incubator. The error bars represent the standard deviation of the sample. Measurement error is not shown for clarity but was ±0.05 μT ±24.2%.](image-url)
The incubators measured in this study were all brand name CO\textsubscript{2} tissue culture incubators, but the results here presented are likely to be also relevant to incubators designed for support of non-mammalian cells since basic features and components are typically conserved on these devices. In this regards, evidence has also been presented supporting the sensitivity of pathogenic bacteria to exposure to altered BMF environments. For example, some \textit{Pseudomonas}, \textit{Enterobacter} and \textit{E. coli} strains’ resistance to antibiotics have been suggested to be influenced when reducing the static component of the BMF below 2 \(\mu\text{T}\) [Poiata et al., 2003; Creanga et al., 2004]. In a more extensive study, changes in the genome conformational state of \textit{E. coli} have been recorded for SMFs below 23 \(\mu\text{T}\), suggesting a maximum in the effects as the field magnitude approached 0 \(\mu\text{T}\) as well as 100 \(\mu\text{T}\) [Binhi et al., 2001]. Also, exposures as short as 20 minutes to 100 \(\mu\text{T}\) (60 Hz) have been shown to affect \textit{E. coli} morphotype and viability indicating stress [Cellini et al., 2008], while long exposures (58 h) to 100 \(\mu\text{T}\) (60 Hz) have been shown to affect \textit{E. coli} transposition activity in a dose dependent manner [Del Re et al., 2003].

Altered BMF environments have also been observed in incubators dedicated fruit flies and effects have also been reported for these insects when exposed to some altered BMF environments [Portelli et al., 2012]. In this regard, there has been also evidence of multicellular organisms being affected in physiologically relevant ways. Prolonged exposure to reduced SMF environments has been shown to affect regeneration of planarians [Novikov et al., 2007, 2008] as well as to induce developmental abnormalities in the Japanese newt [Asashima et al., 1991]. In plants, a large body of experimental data demonstrates in most cases suppression of growth processes, cell division and differentiation as well as significant changes at the cellular and subcellular level, alter ionic balance, enzymes activities and several metabolic processes.
Extensive effects have also been observed in warm-blooded animals exposed to these environments. In chicks, it was found that when incubated in a reduced SMF environment, significantly more exogenous noradrenalin was needed for memory consolidation than the control [Xiao et al., 2009]. Similarly, day-old chicks hatching from an altered BMF environment displayed long-term memory impairment in one-trial passive avoidance task [Wang et al., 2003]. In mice, reduced SMF exposure caused marked disturbances of blood and lymph circulation in the myocardium [Nepomnyashchikh et al., 1997], as well as induction of early embryogenesis in vitro, effects on their reproduction capacity in vivo [Fesenko et al., 2010], and analgesia [Prato et al., 2005; Koziak et al., 2006; Robertson et al., 2007; Prato et al., 2009]. The immune system of rats has also been shown to be susceptible to these exposures by diminishing the ability of macrophages to release nitric oxide (NO) and to synthesize superoxide anion $O_2^-$ [Roman and Tombarkiewicz, 2009]. Rabbits that passed through entire embryogenesis and grew to an age of one month in a reduced SMF environment were found to exhibit degenerative disturbances in the liver, myocardium, gastrointestinal tract, motor activity and insufficiency of the neuromuscular apparatus, as well as a higher mortality rate [Kopanev et al., 1979]. In golden hamsters, the noradrenergic activities in the brainstem were shown to be affected as both the content of noradrenaline and the density of noradrenaline immunopositive neurons in the tissue decreased significantly after exposure [Zhang et al., 2007]. In guinea pigs, the long action of reduced BMF has been shown to depress reserve potential of the sympathoadrenal system as well as decrease the organism’s ability to adapt to the conditions of combined action with other physical stresses [Podkovkin, 1992]. In men, circadian rhythmicity has been suggested to be affected (lengthened) by exposure to reduced BMF environments [Wever, 1970]. Lastly, in an insect model
*Drosophila Melanogaster* (DM) has been shown to become completely amnesiac after 10 generations in a reduced BMF environment [Zhang et al., 2004] and in similar conditions, it has been shown in our laboratory how such exposures affect susceptibility to ionizing radiation on the same organism [Portelli et al., 2012].

**Figure 14. Average SMF per location.** Refer to Figure 1 for a diagram of the actual measurement location within each incubator. The error bars represent the standard deviation of the sample. Measurement error is not shown for clarity but was ±3.98 μT ±2.94%.

In view of this evidence, it is reasonable to speculate that effects similar to those observed in cellular and microbiological cultures may be also of some relevance to other, more complex biological systems which also require incubators for the control of other environmental parameters.
Finally, since some of the TVMF signals recorded inside biological incubators were found to be intermittent instead of a continuous wave, the value reported for the amplitude of the 60 Hz component will be less than its actual peak value. This is because the data presented has been extracted from the Fourier transform of the signals obtained for each vectorial component of the total TVMF. Therefore, it is worthwhile to notice that data shown in Figures 3 and 4 represent a lower bound of the existing TVMF magnitudes. Also, all the measurements made for TVMF and SMF components correspond to a spatial discretization of the actual BMF in the incubator. Therefore, higher or lower SMF or TVMF values may exist in between the 27 locations surveyed in each incubator.

The cohort of experimental data supporting the various effects of exposure to altered BMF environments on microbial and eukaryotic cell cultures suggests this to be a potentially relevant environmental factor, eliciting effects analog to environmental stressors of physical or chemical origin. Therefore, the inhomogeneity and high variability of magnetic fields which are commonplace in biological incubators are variables which can potentially affect many of the experiments performed in eukaryotic or microbial cultures for which this factor has not been purposely controlled. Because of this, a revision of the design of this widely used and fundamental research tool should be considered. A device to control this parameter is presented in Part 2 of this document, which would aid this situation in view of the existing stock of biological incubators currently in use.
PART 2

Do all the good you can,
To all the people you can,
Whenever you can.

-Stella Maris Ramis de Portelli –my mother
Chapter IV

Introduction:

Consistency in the experimental methods and materials utilized is an indisputable need in scientific research. In view of the inhomogeneity and variability reported in PART 1, a system analogous or complementary to an incubator which provides controlled and homogeneous BMF environments for in vitro experimentation is needed. In view the already existing stock of biological incubators in the research community, a device which can add this feature is favored.

According to the body of literature surveyed, a set of essential specifications for this BMF equalization system (or BMFES) are: (i) to reduce TVMFs from NMFS or WMFS below 1 μT (at 60 Hz) (ii) to sustain a SMF within the natural range found naturally on Earth’s surface (23-65 μT) (iii) to maintain an equal or improved level of control over other physical variables controlled by the incubator, and (iv) to provide the greater possible area for gas exchange and instrumentation insertion from the incubator.

Based on these basic specifications, an essential function of the BMFES is to decouple or “shield” the culture designated volume (CDV) from the incubators BMFs. In this regard, magnetic shielding is an active field of research [Umurkan et al., 2010]. In general, there are 3 ways in which MFs (both SMFs and TVMFs) can be attenuated in a designated volume, namely: compensation, shielding or their combination [Reta-Hernández and Karady, 1998]. Compensation refers to the use of a feedback system to generate MFs in inverse direction to the
ones existing in the volume of interest, causing the resultant MF to be null. The magnitude and
direction of the MF generated by the compensation system is determined by sensors which
measure the fields in a designated location of the volume of interest, therefore completing the
feedback loop. The sensors don’t have to be a permanent part of the feedback loop only in the
case that the environmental fields are known and static. This is sometimes the case for
homogeneous SMFs in found in incubators. Previous experiments have taken advantage of this
fact by using triaxial square coils in Helmholtz configuration in order to compensate the existing
SMFs in the incubators to generate the desired BMF in a designated culture volume [Martino et
al., 2010]. In the case of TVMFs, sensors are necessary since the counteracting MF generated
needs to be in-phase with the environmental MF for complete attenuation to occur. However,
active shielding is most appropriate for when the MF sources characteristics in time and space
are known. In the case they are unknown; it is possible to attenuate the MFs coming from WMFS
with reasonable accuracy. This is because a homogeneous MF can be characterized by only one
measurement and several coil configurations exist which permit the generation of homogeneous
MFs in 3 dimensions to counteract those measured. However, in practice, often is not possible to
shield the MFs coming from NMFS with the desired level of accuracy with such simple
configuration (better than 10%). In the case that more complex sets of coils are available for
compensation, information about the location or morphology of the NFMSs is not always
available. This usually translates into some approximate solution to a complex inverse problem
requiring time and computing power. Also, in order for the problem to be solvable with
reasonable accuracy, it is necessary to spread a fair number of sensors through the volume of
interest to minimally characterize the sources. This is not only resource costly, but it also
dramatically reduces the volume available and the accessibility to the CDV. Also, a number of
Coils need to be arranged around the volume of interest to generate MFs to counteract the ones from NMFSs once an approximation of the environmental MF’s has been made. As more spatial uniformity is needed in the CDV, an active system requires increasing numbers of coils and precision current sources to drive those coils. Some attempts at calculating the general properties of such systems have shown that exponential amounts of energy are needed as levels of spatial accuracy increase in the volume of interest [Reta-Hernández and Karady, 1998]. Furthermore, the amount of energy consumed by the coil system is converted to heat which rises the coil system’s temperature. This heat can be conducted or radiated into the CDV prompting the need of an extra system to actively control for the temperature, adding more complexity to the system.

The combination of passive shielding and active compensation has been utilized successfully in cases where the space available for sensors and actuating coils is reasonably smaller than the volume of interest and for reducing MFs generated in WMFS [Kato et al.; Malmivuo et al., 1987; Bryś et al., 2005]. However, the cost reduction and cost increase must be balanced and are mostly adequate for large rooms. For incubators, this approach is inadequate, given the volume limitations imposed by its preexisting CDV.

Unlike the active compensation approach, shielding is passive so feedback systems are not necessary if the maximum magnitudes of the MF sources are known. Soft magnetic materials with high magnetic permeability can concentrate high magnetic flux densities at static and low-frequency MF environments. Therefore, an assembly consisting of a geometrically closed surface made of such material will concentrate the impinging MF into the alloy, bypassing or “shielding” its interior. Also, in general, shielding alloys are conductive. Therefore, induced eddy
current reflection makes these materials also able to shield incident MFs more efficiently as their frequency increases [1998]. For a more comprehensive explanation of ferromagnetic behavior, the reader is referred to the list of references at the end of this manuscript.

Unfortunately, this structure’s shielding ability is greatly diminished if the structure it is not completely closed. However, periodic access to the CDV is indispensable in order to service the cell cultures and perform experiments in general. Smaller permanent connections with the interior of the shield are also necessary for gas exchange and instrumentation connectivity. However, the size of these access points is usually minimized. The resulting restricted connectivity between the host incubator and the interior of the shield imposes clear limitations.

In view of this caveat, a generally resourced architecture amounts to a 5-sided box (body) with its 6th side acting as a lid or door to provide access to the CDV [Blackman et al., 2001; Schuderer et al., 2004]. Additionally, the lids or doors must be supplemented with flanges (several cm long) which overlap on the body of the shield. This increased contact area lowers the reluctance introduced by the interrupted structure in order to regain some of the lost shielding ability of the door or lid.

Unfortunately, all the corners, edges and other features of the shield which are hard to reach increase the susceptibility of the shield itself and of the host incubator for contamination. Contamination by opportunistic microorganisms is a widely known annoyance in biological incubators as goal of the environmental conditions generated in its interior is to promote biological system thriving. Deleterious substances (i.e. Ethanol, Bleach, UV light, Ozone),
radiation, dissecation, heat, etc. are used routinely for their suppression. Some funguses and other airborne pathogens are particularly hard to remove from the CDVs [Lincoln and Gabridge, 1998]. Contamination occasionally causes loss of time and materials to every cell culture user. For this reason, surfaces on the interior of biological incubators are typically made of materials which withstand chemical insults. Additionally, incubator interiors present a minimal amount of gaps, openings, angles and other structures which are difficultly reached for disinfection as it is known that these organisms use such locations as reservoirs for later recolonization of the incubator after a failed sterilization.

Some solutions which present the “lidded” architecture have been designed in a custom way over the years for the study of effects of electric and magnetic fields in biological systems CDV [Blackman et al., 2001; Schuderer et al., 2004]. These and other exposure systems [Davis et al., 1999] were not designed for minimizing the variability of the BMF (and perhaps of cell cultures) in general, but to isolate the biological systems according to the Bioelectromagnetics specific phenomenon studied. In part, this was due to the fact that the effects of low-level magnetic field effects are still not an established or explained satisfactorily.

With special attention to the multiple restrictions previously described, this document explores the possibility of designing a BMFES with an alternative to the “lidded” architecture based on: (i) the need for a structure that is designed based on systematic data obtained about the BMF conditions inside of a biological incubator that can be widely used by the general biological research community which can attenuate those BMFs to levels below biological significance, (ii) the need for a more “cleanable” design which has no lids, hinges, holes, angles,
which help, persist and opportunistic microbial growth; (iii) the need for less restricted amount of space for instrumentation and aeration.

Small temperature inhomogeneities have been long known to cause deleterious effects on *in vitro* biological experimentation [Weaver et al., 1999]. Therefore, a complete study of temperature homogeneity in static and dynamic conditions and how these compare to the performance of a standard tissue culture incubator were also performed.
Chapter V

Materials and Methods:

The worst-case BMF conditions were extracted from the measurements performed in the 21 tissue culture incubators described in PART I (see Tables 2 and 3). Tools were designed, built and calibrated to reproduce such conditions in at will in order to design and test the suggested BMFES prototype.

Identification of types of existing BMF sources: Tables 2 and 3 show the maximum magnitudes measured for SMF and TVMF by location in a typical incubator. Notice that in both tables, the standard deviation is generally of comparable size or greater than the mean. These MFs must be generated outside of the culture area of the incubator (i.e. in the interior of the wall of the incubator or beyond). With this in mind, and with the addition of some empirical surveying, the existence of two different kinds of MF sources were identified, namely Wide Magnetic Field Sources (WMFS) and Narrow Magnetic Field Sources (NMFS). WMFS group all sources which magnetic fields are quasi-homogeneous to the incubator. This happens, in part, because the dimensions of such sources are greater than the incubator’s. Examples of these fields are the ones produced by the Earth, power lines, and devices relatively far away from the incubator; therefore, fields generated by WMFS tend to be rather homogeneous inside the incubator. On the other hand, NMFS refer to those that generate MFs which have significant gradients due to the fact that its dimensions are comparable (usually smaller) than the incubators
size. These include sensors, devices with motors such as fans and pumps, devices with inductive and resistive loads such as heaters and valves, permanent magnets, etc.

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<th>Std. dev. (μT)</th>
<th>Max magnitude (μT)</th>
<th>Error (μT)</th>
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<td>187.34</td>
<td>28.26</td>
<td>219.60</td>
</tr>
<tr>
<td>Side walls of middle shell (5)</td>
<td>1,2,3,4,5,6,7,8,9</td>
<td>top</td>
<td>45.20</td>
<td>47.55</td>
<td>460.06</td>
<td>64.44</td>
<td>524.56</td>
</tr>
<tr>
<td></td>
<td>1,2,3,4,5,6,7,8,9</td>
<td>middle</td>
<td>50.07</td>
<td>52.08</td>
<td>460.06</td>
<td>64.44</td>
<td>524.56</td>
</tr>
<tr>
<td></td>
<td>1,2,3,4,5,6,7,8,9</td>
<td>bottom</td>
<td>35.16</td>
<td>38.10</td>
<td>274.05</td>
<td>38.40</td>
<td>312.50</td>
</tr>
</tbody>
</table>

Table 2. Maximum SMF magnitudes found in incubators. Refer to Figure 1 for the relation of location numbers and shelves within a typical incubator.

<table>
<thead>
<tr>
<th>Location</th>
<th>Numbers</th>
<th>Shelves</th>
<th>Mean magnitude (μT)</th>
<th>Std. dev. (μT)</th>
<th>Max magnitude (μT)</th>
<th>Error (μT)</th>
<th>Max magn. possible (max err.) (μT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corners (all)</td>
<td>4,4,6,8</td>
<td>top</td>
<td>7.69</td>
<td>11.51</td>
<td>78.93</td>
<td>11.08</td>
<td>90.01</td>
</tr>
<tr>
<td></td>
<td>2,4,6,8,9</td>
<td>middle</td>
<td>10.74</td>
<td>16.24</td>
<td>78.93</td>
<td>11.08</td>
<td>90.01</td>
</tr>
<tr>
<td></td>
<td>2,4,6,8,9</td>
<td>bottom</td>
<td>10.74</td>
<td>16.24</td>
<td>78.93</td>
<td>11.08</td>
<td>90.01</td>
</tr>
<tr>
<td>Corners of middle shell (4)</td>
<td>2,4,6,8</td>
<td>top</td>
<td>5.20</td>
<td>7.65</td>
<td>47.60</td>
<td>6.31</td>
<td>54.91</td>
</tr>
<tr>
<td></td>
<td>3,5,7,9</td>
<td>middle</td>
<td>6.75</td>
<td>9.36</td>
<td>47.60</td>
<td>6.31</td>
<td>54.91</td>
</tr>
<tr>
<td></td>
<td>3,5,7,9</td>
<td>bottom</td>
<td>4.60</td>
<td>6.20</td>
<td>47.60</td>
<td>6.31</td>
<td>54.91</td>
</tr>
<tr>
<td>Side walls of top shell (5)</td>
<td>3,5,7,8</td>
<td>top</td>
<td>10.36</td>
<td>15.61</td>
<td>243.74</td>
<td>34.15</td>
<td>277.89</td>
</tr>
<tr>
<td></td>
<td>3,5,7,8</td>
<td>middle</td>
<td>16.20</td>
<td>21.50</td>
<td>243.74</td>
<td>34.15</td>
<td>277.89</td>
</tr>
<tr>
<td></td>
<td>3,5,7,8</td>
<td>bottom</td>
<td>8.72</td>
<td>13.22</td>
<td>243.74</td>
<td>34.15</td>
<td>277.89</td>
</tr>
<tr>
<td>Side walls of middle shell (5)</td>
<td>1,2,3,4,5,6,7,8,9</td>
<td>top</td>
<td>15.26</td>
<td>20.40</td>
<td>243.74</td>
<td>34.15</td>
<td>277.89</td>
</tr>
<tr>
<td></td>
<td>1,2,3,4,5,6,7,8,9</td>
<td>middle</td>
<td>20.00</td>
<td>25.20</td>
<td>243.74</td>
<td>34.15</td>
<td>277.89</td>
</tr>
<tr>
<td></td>
<td>1,2,3,4,5,6,7,8,9</td>
<td>bottom</td>
<td>11.17</td>
<td>16.37</td>
<td>243.74</td>
<td>34.15</td>
<td>277.89</td>
</tr>
</tbody>
</table>

Table 3. Maximum TVMF magnitudes found in incubators. Refer to Figure 1 for the relation of location numbers and shelves within a typical incubator.

**Incubator sizes:** Table 4 shows the dimensions of the interior as well as of the door of the incubators tested for this study. The BMFES and the tools to generate the MF to test its attenuation ability were designed and built based in these dimensions. Notice that although only incubators which sizes approached more to 160 L were included in this table, their SMF and TVMF measurements were used for all calculations for this study.

**Extraction of the WMFS worst-case magnitudes:** Although the metal structures around the incubator and the incubator itself will deflect some of the imposed field by a WMFS, the best
location of the measurements performed to estimate its maximum value possible is in the center of the incubator. This location, as all the others, corresponds to the summation of an unknown number of WMFS and NMFS. However, since the locations of such sources are, in general, also unknown, their contribution at this point is minimal and making it the most adequate location to estimate such magnitude. Therefore, according to Tables 2 and 3, the maximum possible magnitude for SMF and TVMF that can be extracted from the data obtained are 108.75 μT and 17.73 μT (at 60 Hz) respectively. A more thorough reexamination of the incubators in which the maximum TVMF magnitudes were obtained revealed that the source of such fields was continuous, and therefore the values recorded were not scaled down by action of the Fourier transform of an intermittent signal.

<table>
<thead>
<tr>
<th>Number</th>
<th>Brand</th>
<th>Incubator location</th>
<th>Incubator dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Floor</td>
<td>Building</td>
</tr>
<tr>
<td>1</td>
<td>VWR</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Revco</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Revco</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4*</td>
<td>Forma Scientific</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5*</td>
<td>Fisher Scientific</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Fisher Scientific</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Nuaire</td>
<td>basement</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>Nuaire</td>
<td>basement</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>Forma Scientific</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>Forma Scientific</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>Thermo Scientific</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>Thermo Scientific</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>13*</td>
<td>VWR</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>Forma Scientific</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>Thermo Scientific</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>Forma Scientific</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>Binder</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>Binder</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td>Forma Scientific</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>Binder</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>Forma Scientific</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table 4. Incubator shelf and interior dimensions and statistics.** The asterisk (*) denotes incubators that were not included in the statistics at the bottom of the table since their volume differed from the standard (c.a. 160 L).
Device for the generation of WMFS-like exposure conditions: A coaxially-fed pair of square coils in Helmholtz configuration (test square Helmholtz coil or TSCH) was built to generate homogeneous SMF and TVMF with variable magnitudes over volumes comparable to the incubator interiors (see Table 3). These magnitudes included the worst-case values obtained in the previous section. Figure 15 shows a diagram of the TSHC. Dimensions and relevant characteristics follow: side length (L) = 152.4 cm, number of turns in each coil (N) = 126, wire gauge = 18 AWG, separation between coils (D) = 84.50 cm, thickness of the coils = (axial = 19.05 mm), (radial = 25 mm)). All the dimensions of the TSHC were within ± 2%. The structure was built with wood and plastic bolts in order to eliminate artifacts induced by metallic parts and to allow its dismantlement and transportation. The TSCH structure was suspended 74 cm from the ground by wood and plastic supports and was separated from all MF generating equipment by at least 1.5 m. Each 126-turn coil was divided in 3 independent windings consisting of 42 turns each. For all data presented here, these coils were connected in series. An adaptable plastic structure was built in order to align and support the test bed in the geometrical center of the TSHC. A system of plastic suspension wires were tensed diagonally from the corners of each coil and supplemented with plastic plumb-bobs which allowed for the subsequent alignment of the test bed and the TSHC. This allowed the placement of the BMFES to be tested within ±1% of level. These variations along with the coil design were capable of generating a homogeneous magnetic field with a maximum theoretical relative error of 4% in a spherical volume with up to 35 cm radius [Frix et al., 1994]. However, winding defects, insulation in the coil windings and its magnetostatic properties, thermal expansion along with geometric imperfections in the fabrication have been demonstrated to nearly double the field non-uniformity near the center of SHC systems [Crosser et al., 2010]. All this factors combined approximate an estimate of the
Figure 15. TSHC system. The diagram is not to scale.
error in this same spherical volume near the 10% range and below 2% for the MF magnitudes reported. In this case, the sensors to TSHC diameters ratio was below 0.02. This was 10 times better than with the calibration system used before, which had a ratio of approximately 0.2. This set the maximum error generated by such calibration to magnitudes below 0.1% [Nissen and Paulsson, 1996]. Therefore, the specifications described for the TSHC allowed for the generation of homogeneous magnetic fields for testing a square shield with dimensions of up to 40 cm side with a maximum variability of 10 % over the spherical volume containing it. The use of the TSHC system for such purposes is described later in this document.

![Figure 16. MF measurement locations on incubator fans.](image)

The measurements were made on the plane parallel to the fan propeller. See Figure 18 for the magnitudes obtained for a group of fans.

**Characterization of the devices acting as NMFS:** As part of this investigation, several incubators were dismantled and examined closely to identify and study some of the NMFS
sources. Because of its repeated prevalence, a complete characterization of the field generated by fans and heaters was performed. Figures 16 and 17 show the layout utilized as part of the procedure to gather such data. Measurements were done in a room where a volume with a radius of at least 1 m was cleared from all electrically powered equipment which could generate extraneous components. Baseline noise measurements of such environment were made with the unpowered devices and found to be below ±0.15 μT.

![Diagram](image)

**Figure 17. MF measurement locations on incubator heaters.** The measurements were made directly on the surface of the heater and also vertically from this surface where one of the magnitude peaks was found. See Figures 10 and 11 for the MF magnitudes obtained.

Figure 18 shows the measurements for typical fans found in the incubators. The figure shows the maximum resultant TVMF for each fan. Coincidently, this maximum intensity was found to be focused in the same direction as the airflow of the fan for all cases. Therefore, the measurement that has the least distance from the rotor (1.65 cm) correspond approximately to the magnitude that would exist at the wall of the incubator that this fan was part of. An empirical survey revealed, in general, a dipole-like field magnitude configuration for the fans measured. To
perform the measurements, fans were powered as labeled by the manufacturer. Both AC fans had spectrums which showed a maximum at 60 Hz, while the DC fan was at 100Hz.

![Graph of TVMF measurement in incubator fans](image)

**Figure 18. TVMF measurement in incubator fans.** Measurements were made as close as possible to the fan propeller and also only on the “x” axis along the fan’s propeller axis. Magnitudes measured correspond to the peak values of a 60 Hz (AC fan #1 and 2) or 100 Hz (DC fan) TVMF signal.

Figure 19 shows the measurements done on a typical incubator heater surface. The supply current was kept stable within ±10% of 1.9 A, the temperature was kept within ±12.5% of 45 °C and was monitored with the temperature measurement system (TMS) described later in this document. During the horizontal survey, a maximum intensity location was found in the surface of the heater. Consequently, a perpendicular survey was performed at that location and is shown in Figure 11. Dismantling the heater showed that these high MF locations corresponded to heater
wires which were not in proximity to any return wire. The heater wires which were in proximity to return wires had a much more reduced MF, as expected.

Figure 19. TVMF measurement in incubator heaters in the horizontal plane. Error bars are not shown for clarity but were ±0.05 μT ±24.2%. An analysis of point (5,3) in the vertical direction is shown in Figure 20.

Other sources of TVMF were devices like solenoids, switches, pumps but their appearance was more sporadic and the fields generated were typically of less magnitude than the fans or heaters. Sources of SMF typically included permanent magnets which were part of devices like valves, motors or the door gasket. Only empirical surveys were performed in these cases since their appearance was more sporadic and the fields resembled dipoles, as expected.
Device for the generation of NMFS-like exposure conditions: This device was designed to recreate the worst-case NMFS conditions observed in fans and heaters. Particularly, AC powered fans showed the greatest magnitudes and the lowest decay rate over other NMFS generating devices (see AC fan # 1 in Figure 18). In the extreme case of AC fan # 1, its MF decay rate resembled that of a dipole. Such MF distribution can be recreated with a simple round coil. Figure 21 shows how the MF magnitude generated along the axis of AC fan # 1 in Figure 18, coincides with the magnitudes generated along the axis of a round coil when their properties are scaled in a normalized magnitude plot. However, a round coil has two MF magnitude maximums in the plane parallel to its axis when in proximity to its center (see Figure 22). Therefore, in the final design, the axial distance at which the coil was used to test the BMFES shielding ability
was chosen to be greater or equal to one coil radius where there is only one distinguishable maximum along its axis.

![Graph](image)

**Figure 21.** A properly scaled round coil mimics the MF magnitude along the axis of the worst-case AC fan shown in Figure 18.

The parameters which were optimized for the final coil design were (i) the dipole size, (radius of the coil) on which the MF magnitude decay rate depends and (ii) wire gauge, which determined the number of turns and current needed to achieve the maximum MF magnitudes needed. In general, the more turns utilized, the less current needed, but the more spatial inaccuracy and noise amplification in the field generated. The device built consisted of coaxially fed coil (Radius = 2.5 cm, number of turns = 24, wire gauge = 18 AWG). Figure 23 shows a
diagram of the coil. All dimensions were within ±2%. Windings were done in 3 layers of 8 turns each. The coil was supplemented with a spacer with an axial distance of 1.24 times its radius (3.1 cm) so only one MF maximum is observed along the diagonal of such coil (see Figure 22). In this way, the maximum MF imposed in the surface of the BMFES being tested coincides with the coil’s geometric center and resembling the typical MF generated by a biological incubator fan. This spacer allowed placing the coil perpendicular and at a constant distance over the flat surface of the shield to be tested. Also, the coil spacer was supplemented with a hole in its center. This allowed for the testing of the corners of the shield. The coil was held by a metallic clamp from the plastic shaft protruding at its opposite end. This clamp was put at least 1 m away from the windings.

![Figure 22. MF magnitude along the plane parallel to a round coil axis.](image)
Extraction of the NMFS worst-case magnitudes: According to Tables 2 and 3, the maximum possible magnitude for SMF and TVMF that can be extracted from the data obtained are 524.50 μT and 277.89 μT (at 60 Hz) respectively. A more thorough reexamination of the incubators in which these maximum magnitudes were obtained was performed. Unlike the survey in PART 1, the reexamination was not spatially limited, allowing for a closer elucidation of the properties and MF magnitudes of the sources.

![Diagram](image)

**Figure 23. Diagram of the round coil designed and built as a NMFS.** Diagram is not to scale.

The results obtained were not surprisingly different. The maximum MF magnitude difference found in the reexamination exceeded the values in the survey by less than 20%. Also, although the magnitudes reported in the survey correspond to the summation of several sources, the reexamination determined that singular sources were responsible for all the maximum magnitudes recorded. Also, dismantling the incubators of interest showed that permanent magnets were the cause for the highest SMFs and that fans were for TVMFs. This implies that, the maximum TVMF magnitudes recorded in Part 1 were not scaled down by action of the
Fourier transform of an intermittent signal, since the signals emitting from fans were continuous.

On the other hand, this might not be true for small and intermediate TVMF magnitudes recorded.

![Diagram of spatial discretization](image)

**Figure 24. Reduction of the worst-case MF magnitudes due to spatial discretization.** In the case that the NMFS (K) causing most of the MF magnitudes measured at two sampling points (E and F) is found somewhere between those two points behind the incubator wall (C), then points between the two sampling points (G) could have considerably greater magnitudes than the points sampled along the same line parallel to the incubator wall (D). (I) corresponds to the line across the axis of the NMFS and its distance to (D) is less than through (H). (A) corresponds to the distance of the NMFS to the incubator wall and (B) from the incubator wall to the sampling points. (J) is the distance from the axis of the NMFS to the sampling points (E and F).

Also, the possibility of such magnitudes being scaled down by the spatial discretization effect imposed by the sampling was considered. Figure 24 shows how the highest magnitude measured at one point and due to a single NMFS could be below the actual maximum MF magnitude to which cells could be exposed in the same line surveyed. For the dimensions of the incubators measured the worst-case scenario (A = 3 cm, B = 5 cm, J = 10 cm) making K-to-E = 12.80 cm and K-to-G = 8 cm. Assuming that the worst-case magnitudes were observed (SMF = 524.50 µT and TVMF = 277.89 µT (at 60 Hz)) and a NMFS made up of a simple round coil
[Robinson, 1983] then the corresponding NMFS and TVMF at the point (G) would be 1733.4 $\mu$T and 918.4 $\mu$T (at 60 Hz) respectively. The reexamination performed showed that such underestimation by spatial discretization did not occur for the magnitudes measured on the incubators with the highest MFs. However; this could be the case for some of the medium and small magnitudes measured in other incubators. For the case of TVMF magnitudes measured in the fans extracted from incubators and shown in Figure 18, values between 364.9 $\mu$T and 177.2 $\mu$T were measured at distances at which a measurement could have been made inside of the incubator which originally contained the fan. For SMF, the reexamination showed values that were no higher than the ones reported. Therefore, the values surveyed (SMF = 524.50 $\mu$T and TVMF = 277.89 $\mu$T (at 60 Hz)) are good estimates of the actual maximum magnitudes to be found in a typical biological incubator.

**Static and time-varying power supplies for the NMFS and WMFS generating devices:** For producing MFs at 60 Hz, both NMFS- and WMFS-generating devices were powered by a variable autotransformer (Type WIOMT Variac, General Radio Company, Concord, MA, USA) which was connected directly to the power supply of the laboratory building. A multiplicity of time-dependent harmonics where observed as this power distribution system had multiple and variable loads. Therefore, an LC filter was built to diminish the noise and obtain a “clean” 60 Hz waveform. The filter was connected between the autotransformer and the WMFS and NMFS sources and a diagram is shown in Figure 25. The parameters of the filter (capacitor = 40 $\mu$F (Part. No. 2W1046TN, CSI Technologies, Vista, CA, USA); Inductor = 15.3 mH (500 turns 18 AWG magnet wire, diameter = 8.5 cm, length = 8.5 cm)) were chosen based on the current needed to generate worst-case MFs and on the properties of the WMFS and NMFS. For the
WMFS, the electrical impedance was $20.8 + 75.4j \, \Omega$ (at 60 Hz) and was obtained by the voltage division with a known resistor technique. The NMFS electrical impedance was theoretically calculated to be $83.9 + 16.1j \, m\Omega$ (at 60 Hz) [2011]. Due to the radical difference between the loads of the WMFS and NMFS, the capacitor of the filter was only utilized for the WMFS. The filtered current magnitude was obtained with a calibrated meter (NIST certification number: 1383235) (model number: 179, John Fluke MFG, Everett, WA, USA) or a AC/DC current probe (model number: 1146A, Hewlett Packard, Palo Alto, CA, USA) connected to an oscilloscope (54602B, Hewlett Packard, Palo Alto, CA, USA) depending on the magnitude range. Both measurement systems were calibrated and the error between them was less than $\pm 2.40\%$ in magnitude ranges where overlapping of measurement capabilities occurred (i.e. between 1 and 5 A). The errors in construction of both sources introduced an error below $\pm 2\%$ [Crosser et al., 2010]. Therefore, the error in the MF generated by the WMFS and NMFS and measured by these systems was below $\pm 3.12\%$ of the reported values.

![Figure 25. LC filter for powering NMFS and WMFS. Switching between sources was done manually.](image)

SMFs were generated with cascaded regulated DC power supplies (model number: 1210S, Power Designs, Danbury, CT, USA), (model number: 6024A, Hewlett Packard, Palo Alto, CA, USA) and (model number: 6050A, Power Designs, Danbury, CT, USA).
Attenuation factor measurements: Attenuation factor reported refers to the ratio between the MF magnitude measurements obtained in the absence (generated or incident MF) and presence (residual MF) of the high permeability material tested making use of MFs with known characteristics generated by the NMFS- and WMFS-generating devices described earlier. TVMF’s were measured by the induction sensor earlier described. The SMF was measured by either the SMF measurement system described earlier or by a fluxgate magnetometer (FGM-4D2N, Walker scientific, Worcester, MA, USA) depending on the space availability to perform the measurement. Total magnitudes reported for this device were calculated by vector summation of the magnitudes of each spatial component (x,y,z) which introduced an uncertainty of ±0.19 μT. The TVMF measurements were all done at 60 Hz, obtaining measurements simultaneously in all three dimensions. This is necessary since gradients are expected on homogeneous and gradient fields by the effect of proximity to a ferromagnetic soft material. Also, although the spectrums up to 500 Hz were obtained, measurements reported are based only on the time-domain 60 Hz signal. In this way, since the DFT is not necessary, the error introduced by the TVMF sensor was reduced to ±0.05 μT ±17.1%. As stated before, the error in MF generation was below 3.2%. Therefore, the error on the attenuation measurements is ±0.05 μT ±17.4%, obtained by adding these errors in quadrature. Error bars are not shown in some figures for clarity.

The MF magnitudes utilized for obtaining the attenuation values were chosen based on limitations imposed by the measurement techniques and equipment. The main limitation was that, in order to obtain realistic attenuation factor estimations, residual MF magnitudes obtained within the high permeability structures tested must be above the noise floor of the SMF and
TVMF sensing systems described before. At the same time, reliable measurements could only be made when saturation was not evident in the waveform obtained since in order to avoid energy dispersion from the 60 Hz component. Therefore, the values chosen complied with causing no saturation for the high permeability material thicknesses tested and, at the same time, yield values above the noise floor of the sensing systems described before.

**Attenuation factor testing approach:** A 60 Hz MF signal was used for the determination of the attenuation factor for the prototypes tested. High permittivity materials shielding factor increase with frequency, especially in the decade from 10 to 100 Hz to progressively stabilize at higher frequencies. However, a wider range (± 10 μT) is permitted to be left for SMFs and therefore less attenuation is necessary.

![Temperature probe diagram](image)

**Figure 26. Temperature probe diagram.** (1) 60 x 15 mm polypropylene Petri dish; (2) Petri dish lid; (3) Thin K-type thermocouple; (4) cyanoacrylate thin layer; (5) Thermocouple connection to the data acquisition system.
**Temperature measurement system:** or TMS, was based on precision fine-wire (diameter = 76.2 μm) and Teflon-insulated, K-type thermocouples (Part number: 5TC-TT-K-40-72, Omega, Stamford, CT, USA) which outputs were fed into a data acquisition system (serial number: 151F95A, Part number: NI9211, National Instruments, Austin, TX, USA). The temperature measurement probes consisted of these thermocouples affixed at the bottom of lidded 60 x 15 mm polypropylene Petri dishes with a thin layer of cyanoacrylate (see Figure 26). All measurements were taken with the petri dishes containing 6 ml of saline solution. The temperature real-time monitoring, control and storage was written in LabView 2009 (National Instruments, Austin, TX, USA). Figure 27 shows a diagram of the TMS. Data acquisition was performed with cold-junction compensation at a rate of 2 Hz per thermocouple and a 60 seconds (120 samples) moving-average filter implemented to reduce the quantification noise introduced by the data acquisition system.

![Figure 27. Schematic of the connections for the TMS.](image)

**Calibration:** TMS calibration was performed by placing all thermocouples simultaneously in environment-isolated water baths which had the same temperature (0 (ice melting point), 22.0 and 42.0 °C) for at least one hour to eliminate all transients. With this method, the maximum absolute discrepancy between probes was below 0.17, 0.12 and 0.13 °C (for 0, 22 and 42 °C
respectively). The maximum variability between any probe and the average of the group of probes was below ±0.094, ±0.071, ±0.070 °C (for 0, 22 and 42 °C respectively). Figure 28 shows the distribution of the maximum absolute difference between all probes while in water baths at 0, 22 and 42 °C obtained for 5000 samples. Notice how the distributions for 22 and 42 °C are all below 0.14 °C.

Figure 28. Distribution of discrepancy between 4 probes of the TMS. This figure represents 5000 temperature samples for each temperature tested (0, 22 and 42 °C). Samples were taken after 1 hour of stabilization of temperature transients.
Chapter VI

Results and Discussion:

Several configurations were explored based on the restrictions detailed in the introduction (see Figure 29). The BMFES final design consists of an internal and an external component. The external component (BMFES-shield) is comprised of a high permittivity material enclosure which decouples the CDV from the real biological incubators preexisting BMFs. The BMFES-shield, a high permeability enclosure, will generate an Hypogeomangetic field environment. Consequently, the interior part (BMFES-SHC) reverses this effect by generating a SMF of the same order what is naturally found on Earth’s surface (23-65 μT) by means of a pair of square coils in Helmholtz configuration.

![Figure 29. Incubator BMF equalization system.](image)

The diagram shows the basic component layout as well as their general relation with the environment.

This section presents a detailed description of the BMFES-shield design process and rationale. Additionally, simulations were performed to assess its attenuation ability when
inserted in real biological incubators tested in PART 1. These simulations yielded a group of locations in the interior of the BMFES which were predicted to have the worst-case residual BMFs when placed in specific real incubators. Direct BMF measurements in these locations were performed in order to validate the simulation results. Complementarily, a comparison of the attenuation ability of the BMFES-shield is made with a pseudo-standard BMF shielding solution used extensively in previously published Bioelectromagnetics research. Also, the BMFES-SHC design and the BMF homogeneity in its interior, temperature homogeneity, transients and contributions from the BMFES components are presented in a full characterization of the final design.

**High permeability material:** Because of its versatility, soft ferromagnetic materials were chosen as an integral part of the BMFES. AD-MU 80 (.01" thick and 15” wide sheet, Ad-Vance Magnetics, Rochester, IN, USA) was used for all the experiments because of extensive experience with this material. The material magnetic, electrical, physical and mechanical properties have been described in detail [Arentz et al.].

**Design of the BMFES-shield components:** A cubic shape denoted the general architecture of the high permeability shield, mimicking the horizontal plane of the interior of a typical incubator (See Table 4 and Figure 30). From this basic specification, alternative architectures were explored in an effort to override the need for flanged lids. Initial experiments led to construct prototypes with flangeless but multilayered lids with negative results.

The final BMFES-shield design consisted of two μ-metal cylinders of square profile as fundamental units, namely an inner shield (IS) and an outer shield (OS). The dimensions of the
IS (interior side length = 34 cm) allowed for its placement in the interior of the OS (side length = 38 cm) forming a cubic enclosure as shown in Figure 31. The sides of the cube were round (radius = 0.5 cm) in order to eliminate wedges in which microorganisms could easily proliferate. This cubic design for the µ-metal exterior of the BMFES resulted in a maximized CDV.

![Figure 30. Attenuation values obtained for two concentric cylinders of square profile with a range of possible separation distances for their two surfaces.](image)

Data was obtained for WMFS-generated MFs at 60 Hz. For all values tested, attenuation factor enhancement remained below 30% sacrificing 52% volume in between the layers. Procedure = (A cylindrical shield of square profile (AD-MU 80, Ad-Vance Magnetics) was built on the inside of an acrylic enclosure (inside diameter = 32 cm, thickness = 0.9525 cm, corners radius = 0.5 cm). A second cylindrical shield with square profile was built on the outside of the acrylic enclosure forming a two layer shield. The outside of the acrylic enclosure thickness was increased by the thickness of the material sequentially until reaching 5 times its original thickness. Notice how for all MF magnitudes tested, a marginal 30% increase in the attenuation is observed as a result of a decrease of roughly 10% in the ratio between the internal and external diameters of the shielding system. However, this apparently small ratio change results in a volume differential of approximately 52%. Although it is not shown in this figure, the total volume occupied by the shielding system grows from 32,768 cm³ for the smallest thickness measured (0.9525 cm) to 47,674 cm³ for the thickest (4.76 cm). As an alternative, the use of several layers in the same volume was also explored by way of adding an AD-MU 80 layer in between every extra plastic layer added to the original 32 cm side structure resulting in a maximum attenuation factor marginally smaller than the one obtained for this configuration.)

It is well known that the use of more than one layer (with enough separation in between them) may enhance the shielding ability of a high permeability enclosure when the necessary
specifications are met [1998] reducing the amount of resources in its construction. However, the need to maximize the CDV prompted the minimization of the unutilized volume sacrificed in between the $\mu$-metal layers. At the same time, the maximum volume that could be inserted inside a 160 L incubator did not allow the BMFES total volume to grow further. Because of this, the possibility of multiple spaced $\mu$-metal layers was investigated for the restrictions on the practical dimensions of this project. Figure 30 shows how marginal gains in attenuation factor required impermissible large volume sacrifices which reduced the CDV significantly, proving this architecture approach inappropriate for the BMFES design. As a result, both IS and OS consisted of a single layer of 0.254 mm thick $\mu$-metal sheet (AD-MU 80, Ad-Vance Magnetics) wound multiple times on a supporting acrylic structure (acrylic thickness = 0.9525 cm) which function was to keep the desired shape of the metal during experimentation.

Figure 31. Formation of a cubic shield by two cylinders with square profile. The figure is divided in two parts exemplifying the placement of one shield inside the other.
Determination of the IS and OS minimum thickness: A study was performed to estimate the minimum $\mu$-metal thickness of the IS and OS that would allow the BMFES-shield to attenuate the inherent BMFs in biological incubators below specification levels ($23 \, \mu T \leq \text{SMF} \leq 65 \, \mu T$; and TVMF (at 60 Hz) < 1 $\mu$T). Basic performance measurements of the IS under NMFS and WMFS exposures as a function of $\mu$-metal thickness were performed as shown in Figures 32 and 33. These measurements were all done at 60 Hz and at 3 different magnitudes for each source. Figure 34 shows the IS radial surveys performed for attenuation and residual MF magnitudes for WMFS-generated MFs incident perpendicularly to its axis for different thicknesses of $\mu$-metal. In these figures, it is evident that the point with the smallest attenuation factor, in the radial direction, is located in the center of the shield. Complementarily, Figure 35 shows a spatial survey at points along all 3 axes, located at a distance equal to 30% the side length from the center. Notice that a measurement in the geometric center of the shield represents the maximum axial and the minimum radial attenuation factors possible for this geometry. Likewise, Figure 36 shows the radial surveys performed for attenuation and MF magnitudes for NMFS-generated MFs incident perpendicularly to its axis and Figure 37 show a spatial survey of the same kind as Figure 35. Sliding the axis of the NFMS on the surface of the square profile cylinder until the side of the coil coincided with the edge of the cylinder yielded no significant changes in the magnitudes reported.
Figure 32. Surveying protocol for IS and OS under WMFS-generated MFs. Broken lines on the square cylinder of the top view denote a surface made transparent for clarity. Measurements for the IS and OS were made relative to the IS internal diameter (30 cm) and presented as a percentage of this dimension. A more accurate depiction of the WMFS is found on Figure 15.
Figure 33. Surveying protocol for IS and OS under NMFS-generated MFs. Broken lines on the square cylinder of the top view denote a surface made transparent for clarity. Measurements for the IS and OS were made relative to the IS internal diameter (30 cm) and presented as a percentage of this dimension. A more accurate depiction of the NMFS is found on Figure 23.
Figure 34. Radial surveys of the IS under WMFS-generated MFs (at 60 Hz). The configuration selected to perform these measurements is shown in Figure 32. Attenuation factors and residual MF magnitudes are found on the right and left columns respectively. Every curve represents a different total thickness in the \(\mu\)-metal material (0.762 to 1.524 cm). The incident MF magnitude at the wall of the shield was 41.52 \(\mu\)T for the top row, 85.71 \(\mu\)T for the middle row and 136.57 \(\mu\)T for the bottom row. All magnitudes reported were obtained as the vectorial sum of the components in all three axes of the MF at each location with a volumetric resolution of 2 cm radius. The relative position to the center of the shield is scaled to the IS internal diameter (30 cm). All measurements were performed without observing any evident saturation in the material. Error bars are not shown for clarity but the maximum deviation possible amounts to \(\pm 0.05 \mu T \pm 17.1\%\) for the MF measurements and 17.5 \% for the attenuation measurements.
Figure 35. Volumetric surveys of the IS under WMFS-generated MFs (at 60 Hz). The configuration selected to perform these measurements is shown in Figure 32. Attenuation values are found and residual MF magnitudes are found on the right and left columns respectively. For this measurements, only points along all three axes and located at 30% of the IS center (relative to the IS inner side length) are shown. Every curve represents a different total thickness in the \( \mu \)-metal material (0.762 to 1.524 cm). The incident MF magnitude at the wall of the shield was 41.52 \( \mu \)T for the top row, 85.71 \( \mu \)T for the middle row and 136.57 \( \mu \)T for the bottom row. All magnitudes reported were obtained as the vectorial sum of the components in all three axes of the MF at each location with a volumetric resolution of 2 cm radius. The relative position to the center of the shield is scaled to the IS internal diameter (30 cm). All measurements were performed without observing any evident saturation in the material. Error bars are not shown for clarity but the maximum deviation possible amounts to \( \pm 0.05 \mu T \pm 17.1\% \) for the MF measurements and 17.5 % for the attenuation measurements.
Figure 36. Radial surveys of the IS under NMFS-generated MFs (at 60 Hz). The configuration selected to perform these measurements is shown in Figure 33. Attenuation values are found and MF magnitudes are found on the right and left columns respectively. Every curve represents a different total thickness in the μ-metal material (0.762 to 1.524 cm). The incident MF magnitude at the wall of the shield was 658.32 μT for the top row, 2677.07 μT for the middle row and 5385.02 μT for the bottom row. All magnitudes reported were obtained as the vectorial sum of the components in all three axes of the residual MF at each location with a volumetric resolution of 2 cm radius. The relative position to the center of the shield is scaled to the IS internal diameter (30 cm). All measurements were performed without observing any evident saturation in the material. Error bars are not shown for clarity but the maximum deviation possible amounts to ±0.05 μT ±17.1% for the MF measurements and 17.5% for the attenuation measurements. Residual MF magnitudes measured for the positive positions relative to the center (farther away from the NMFS source) were small and close to the noise floor, propagating its error significantly when calculating their respective attenuation value. Therefore, attenuation factors are presented only for the center and negative positions (closer to the NMFS source).
Figure 37. Volumetric surveys of the IS under NMFS-generated MFs (at 60 Hz). The configuration selected to perform these measurements is shown in Figure 33. Attenuation values are found and residual MF magnitudes are found on the right and left columns respectively. For this measurements, only points along all three axes and located at 30% of the IS center (relative to the IS inner side length) are shown. Every curve represents a different total thickness in the μ-metal material (0.762 to 1.524 cm). The incident MF magnitude at the wall of the shield was 658.32 μT for the top row, 2677.07 μT for the middle row and 5385.02 μT for the bottom row. All magnitudes reported were obtained as the vectorial sum of the components in all three axes of the residual MF at each location with a volumetric resolution of 2 cm radius. The relative position to the center of the shield is scaled to the IS internal diameter (30 cm). All measurements were performed without observing any evident saturation in the material. Error bars are not shown for clarity but the maximum deviation possible amounts to ±0.05 μT ±17.1% for the MF measurements and 17.5 % for the attenuation measurements.
Two locations were chosen for obtaining attenuation values which describe the performance of the IS, namely (i) at the center of the IS for WMFS-generated MFs and (ii) at the +30% location in the radial (horizontal) direction from the center of the IS for NMFS-generated MFs. As a comparison, Figure 26 (top row) shows the variation for the WMFS and NMFS attenuation factors in relation to the material thickness at these locations respectively. Notice how the attenuation factor increases almost linearly within the magnitude range tested for the incident WMFS-generated MFs. Because of their architectural resemblance, a similar attenuation curve is observed for the OS at these points (See Figure 26 (middle row)). The only difference is an expected attenuation factor marginal decrease for the OS (of less than 7%) for both NMFS and WMFS-generated incident MFs.

Both IS and OS exhibited poor axial attenuation capacity when tested separately. However, Figure 38 (bottom row) shows how this dramatically improved when the combined into the BMFES final form (see Figure 31). Attenuation factor gains greater than 3 and 2.5 were observed for WMFS and NMFS-generated incident MFs respectively. Also, the same figure shows how attenuation factor variations of nearly ±13 % and ±9% are to be expected for the range of NMFS and WMFS magnitudes tested (respectively). Similarly, this figure shows how attenuation factor variations of ±24 % and ±17% for NMFS and WMFS (respectively) are to be expected for the combination of both shields.

Based on these observations, the IS and OS μ-metal thickness utilized for the final BMFES design was 1.524 cm (1 layer wound with 6 turns of 0.254 mm-thick AD-MU 80 sheet).
Figure 38. Comparison of the attenuation factors for the Inner and Outer Shields and their combination for WMFS- and NMFS-generated MFs (at 60 Hz). The configuration selected to perform these measurements is shown in Figures 32 and 33. WMFS- and NMFS-generated MF attenuation factors are shown in the left and right columns respectively. For the WMFS-generated MF attenuation factor values (right column), only measurements at the center of the shield are presented. For NMFS-generated MF attenuation factor values (left column) only measurements done on points located at 30% of the shield along the radial (horizontal) axis and closer to the NMFS source are shown. Every curve represents a different incident MF magnitude at the wall of the shield with respect to the total thickness in the μ-metal material (0.762 to 1.524 cm). The incident WMFS-generated MF magnitude at the wall of the shield was 658.32, 2677.07 and 5385.02 μT for the left column while for the NMFS-generated MF magnitude was 41.52, 85.71 and 136.57 μT for the right column. The top row contains measurements made on the IS. The middle row contains the comparison of the measurements made on the OS with the ones on the first row. The bottom row contains the comparison of the measurements made on the combination of IS and OS as shown in Figure 19 and those made on the OS. All MFs were generated and at 60 Hz. All magnitudes reported were obtained as the vectorial sum of the components in all three axes of the MF at each location with a volumetric resolution of 2 cm radius. The relative position to the center of the shield is scaled to the IS internal diameter (30 cm). All measurements were performed without observing any evident saturation in the material. Error bars are not shown for clarity but the maximum deviation possible amounts to ±0.05 μT ±17.1% for the MF measurements and 17.5 % for the attenuation measurements.
**Determination of the BMFES attenuation factors:** Unlike the exploratory IS and OS tests performed, the situation under consideration for the final BMFES-shield was more complex. The interior of a real biological incubator presents contributions from multiple NMFS and WMFS producing a BMF which characteristics are only partially known (see PART 1). Additionally, subjecting the BMFES-shield to exposures from individual NMFSs and WMFSs considerably affected all its interior volume in a way which was not easily summarizable. Consequently, a definition for attenuation was needed which allowed asking the question: What would be the resultant magnetic flux density (B-field) in the inside of the BMFES-shield when it is placed in an incubator with preexisting background B-fields? Therefore, in analogy to this situation, the attenuation factor ($Att$) utilized to characterize the BMFES-shield performance was defined as the B-field generated in free space by the NMFS or the WMFS ($B_{gen}$) divided by the B-field measured in each location in the interior of the BMFES-shield ($B_{meas}$) as follows:

$$Att(i) = \frac{B_{gen}}{B_{meas}(i)}$$

The reason that $B_{gen}$ is defined as the B-field generated by the NMFS or WMFS in free space is that its magnitude and direction are unavoidably perturbed by high permittivity material. Therefore, $B_{gen}$ is deemed to change as a result of positioning the BMFES-shield near the NMFS or WMFS from its value in free space in a not easily predictable way. More specifically, $B_{gen}$ was defined as the MF magnitude at the center of the WMFS and as the MF magnitude 3.1 cm from the center of the NMFS in its axial direction respectively.
Additionally, a special nomenclature was adopted to facilitate the characterization of the BMFES-shield attenuation capability. Figure 39 shows the names given to all 6 faces, 8 corners and 12 edges of the BMFES exterior and Figure 40 shows the names given to all 27 measurement points in the BMFES interior and how they relate its exterior. The 27 measurement locations were divided in 3 layers which define a cubic volume of 18 cm side. Notice how the CDV is contained by the cube defined by the 27 measurement locations. Therefore, the “i” index corresponds to one those 27 locations being measured inside the BMFES.

Based on this nomenclature and on the worst-case BMF conditions found in the Methods section of PART 2 (NMFS = 658.32, WMFS = 136.57), a complete survey of the BMFES exterior (6 faces, 8 corners and 12 edges) for each of the 27 positions inside the BMFES was performed (see Figure 42). Table 5 shows the 27 x 27 attenuation matrix obtained for NMFS and WMFS exposures. Here it can be observed how all 27 measurement locations inside the BMFES are affected by all NMFS and WMFS exposures made to each portion of the BMFES exterior. All surveys were performed by using a TVMF signal (at 60 Hz) since our laboratory ambient proved to be “cleaner” of TVMF signals in comparison to SMF signals for magnitudes of the order of those used to test the BMFES. Namely, the ambient TVMF were recorded to be below 0.1 µT while the SMF (mainly a product of GMF) was below 39.0 µT. Therefore, NMFS and WMFS measurements were taken with the TVMF measurement system described in PART 1. In order to obtain a lower limit for the attenuation factor matrix generated for the BMFES, NMFS and WMFS surveying techniques were implemented:
Figure 39. Nomenclature adopted for the BMFES exterior. The BMFES is rotated clockwise in its C1-C2 axis. Each column shows a top and a side view of the BMFES as it rotates 45° from its previous position. The dotted lines mark the position of the IS when its hidden below the OS. The 6 Sides are described by a two-digit code (e.g. A1), the 12 edges by a 4-digit code (A1B1) and the 8 corners by a 3-digit code (A1B1C1) totaling 26 fundamental components. The composition of the BMFES is shown in Figure 31. This figure is commentary to Figure 40.

Figure 40. Nomenclature adopted for the BMFES interior (measurement locations). The first column shows the location of the measurement point grid with relation to the BMFES center. The grid consists of a cube made of 27 points (3x3x3) which are the corners of 8 cubes of 9 cm side. In each row, the plane seen progresses inside of the cube along the normal to the plane corresponding to that row (Axial, Sagittal or Coronal) in 9-cm steps. The nomenclature adopted for the 27 points inside the BMFES consists of a 2 digit code (T1...T9, C1..C9, B1...B9). The first digit differentiates 3 axial planes (T = top, C = center, B = bottom). The second digit follows the nomenclature shown in Figure 1, i.e. “1” corresponds to the center of the plane while “2” to “9” are arranged in a counterclockwise manner around “1” in the same plane. The dotted lines mark the position of the IS when its hidden below the OS. This figure is complementary to Figure 39.
Table 5. **WMFS and NMFS attenuation matrices.** Each column corresponds to one of the 27 points in the interior of the BMFES described in Figure 40. Each row corresponds to all 26 different exposure directions of the BMFE exterior.
**NMFS surveying technique (The “ironing” method):** NMFS surveys were made by manually sliding the source on the entire BMFES exterior surface being tested (face, edge or corner). Special care was taken in positioning the axis of the source in all the angles possible in order to find the greater magnitude that could be detected with the TVMF measurement system placed inside the BMFES. This included pointing the NMFS axis directly to the point being tested. Data was recorded once the worst-case magnitude was found, which translated into the *minimum attenuation possible due to a worst-case exposure.*

![Diagram](image.png)

**Figure 41. “Ironing” NMFS survey method.** The NMFS is slided over the surface, edge or corner of the BMFES to be surveyed and it is also rotated on the plane perpendicular to the NMFS axis in order to find the worst-case attenuation factors for the x, y and z components of the TVMF measurement made inside the BMFES.

This method was informally named “ironing” because of its resemblance to ironing clothes (see Figures 41 and 42). Additionally, this method was made stricter by repeating the same survey for each TVMF vectorial component (x,y,z) and by recording the worst-case magnitudes for each component even if the location where these values were found did not coincide. This was possible since the TVMF measurement system could display the TVMF magnitudes for each axis by separate.
**WMFS surveying technique:** WMFS survey included the entire exterior of the BMFES in the same way as for the NMFS survey. Measurements were performed by placing the BMFES inside the WMFS and aligning its axes and diagonals with the source axis according to the face, edge or corner being tested. This required specially designed holders and a variable height test bed in order to maintain its position during measurements (see Figure 15). In this case, the physical size of the WMFS did not allow for the “ironing” method to be utilized. Instead, in an analogous way, WMFS matrix in Table 5 was transformed into only one vector by taking the minimum attenuation found for each column. The rationale for this transformation is that in the case that multiple WMFS exist in free space; these would be ultimately combined into only one in by vector summation before being incident in the BMFES. This vector was then incorporated to the NMFS matrix in Table 5 generating the matrix in Table 6.

![Diagram](image.png)

**Figure 42. Example of the NMFS survey (Coronal Plane).** Only 8 of the 26 positions are shown in this cross sectional plane as an example. The WMFS survey is performed in the same manner.
Table 6. Worst-case NMFS and WMFS attenuation matrix. This final version combines NMFS and WMFS attenuations matrices in Table 5. In this version, the WMFS attenuation was reduced to a single vector containing the minimum attenuation measured for each interior location, regardless of the direction of exposure to the exterior of the BMFES. Each column corresponds to one of the 27 points in the interior of the BMFES described in Figure 40. Each row corresponds to a NMFS or WMFS exposure of the BMFES exterior.

The BMFES-shield cubic architecture presented several symmetries including radial, axial and sagittal. This allowed for only 8 locations of the 27 in the interior of the BMFES (1C, 2C, 3C, 9C, 1T, 2T, 3T, 9T) to be completely surveyed to obtain a complete attenuation matrix. This reduced the total number of measurements taken to 624 (instead of 2106).

Simulation of the insertion of BMFES-shield in real biological incubators: The simulation was based on using the 54 measurements (27 TVMF and 27 SMF) made in each of the 21 biological incubators surveyed in PART 1 and applying the worst-case attenuation matrix in Table 6. In this way, an estimation of the upper-limit for the residual fields inside the BMFES-shield in the event of being placed inside a real biological incubator was obtained.
Figure 43. Example of the difference between simulation modalities. The diagram exemplifies how test 1 and test 2 utilize a real biological incubator data to estimate an upper-limit to the residual BMF inside the BMFES as it is inserted inside of the real biological incubator. Table 7 provides a full description of how real data is assigned to each attenuation coefficient in Table 6.
As mentioned before, the measurements made in real biological incubators in PART 1 are spatial samples of MFs which sources are unknown. Therefore, to ameliorate potential error introduced by this uncertainty on the estimation of the residual field in the BMFES-shield interior, two distinct simulation variations (Test 1 and Test 2) were implemented. In Test 1 the SMFs and TVMFs measured in the edges and corners of the real biological incubators (top shelf = 2, 4, 6, 8, and bottom shelf = 2, 4, 6, 8) contributed to the residual MF inside the BMFES-shield by ways of the edge and corner attenuation coefficients (the ones described by 4 and 6 digit codes i.e. A1B1, A2B2C1) respectively (see Table 8).

Table 7. Attenuation coefficient assignment table. Each row describes how the attenuation coefficient vector in Table 6 is assigned to each measurement obtained in real biological incubators (SMF or TVMF).

<table>
<thead>
<tr>
<th>Biological Incubator data (MF or TVM)</th>
<th>BMFES exterior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mode I</td>
</tr>
<tr>
<td>Top shelf</td>
<td>Test 1</td>
</tr>
<tr>
<td>1</td>
<td>A1</td>
</tr>
<tr>
<td>2</td>
<td>A1B2C3</td>
</tr>
<tr>
<td>3</td>
<td>A5</td>
</tr>
<tr>
<td>4</td>
<td>A1B2C3</td>
</tr>
<tr>
<td>5</td>
<td>A5</td>
</tr>
<tr>
<td>Middle shelf</td>
<td>Test 1</td>
</tr>
<tr>
<td>1</td>
<td>A1</td>
</tr>
<tr>
<td>2</td>
<td>A1B2C3</td>
</tr>
<tr>
<td>3</td>
<td>A5</td>
</tr>
<tr>
<td>4</td>
<td>A1B2C3</td>
</tr>
<tr>
<td>5</td>
<td>A5</td>
</tr>
<tr>
<td>Bottom shelf</td>
<td>Test 1</td>
</tr>
<tr>
<td>1</td>
<td>A1</td>
</tr>
<tr>
<td>2</td>
<td>A1B2C3</td>
</tr>
<tr>
<td>3</td>
<td>A5</td>
</tr>
<tr>
<td>4</td>
<td>A1B2C3</td>
</tr>
<tr>
<td>5</td>
<td>A5</td>
</tr>
</tbody>
</table>

In contrast, in Test 2, these contribute to the residual MF inside the BMFES only by ways of the face attenuation coefficients (the ones described by 2 digit codes i.e. 1C). As a result, Test 2 entails using the face attenuation coefficients for more than one measurement made in real biological incubators per simulation (3 faces per corner and 2 faces per edge). For clarity, configurations and differences between Test 1 and Test 2 are also described in Figure 43 and
Table 7. Based, in part, on an analysis of the dimensions of the incubators tested, both simulation modalities assumed that the data from real biological incubators correspond to NMFSs which center is placed at 3 cm from the surface of the BMFES and pointing in the worst-case direction. Incident WMFS was also assumed to be pointing in the worst-case direction. Therefore, both methods were designed to moderately overestimate (and therefore define an upper-limit) the residual MFs measured in the interior of the BMFES in the event of being inserted in a real biological incubator.

Figure 44. Modes of insertion of the BMFES. Even modes are obtained by rotating the BMFES from the previous (odd) mode in a CCW direction relative to its vertical axis (shown in the top view). The door is not shown for the front view diagram. Figure 39 shows how the nomenclature shown in this Figure translates to the BMFES.

Each simulation variation (Test 1 and Test 2) was performed for the data obtained for all 21 incubators (567 points), producing a new set of data for those 21 incubators, but now, corresponding to the residual MFs measured in the inside of the BMFES (567 points for Test 1 and 567 points for Test 2) (see Figure 40). Also, Figure 44 makes clear that, because of its geometric properties, the BMFES can be inserted in an incubator in six different ways (mode I through VI). Therefore, Test 1 and Test 2 were repeated for each of these modes (6 times) totaling 6804 points between the two tests. Finally, since this whole process was done for both for the
SMF and TVMF measurements made on real biological incubators, this number was doubled to 13608.

For both simulation variations, two probable sources of significant uncertainty were identified, namely (i) the accurate estimation of the BMFES attenuation coefficients based on their dependence on temperature, frequency and magnitude of the incident MF and (ii) the way in which the WMFS was estimated. Temperature of the BMFES was discarded as a source of significant uncertainty as direct measurements (see Figure 45) showed no significant attenuation ability dependence on the temperature range of interest (22 to 42 °C).

![Figure 45: BMFES attenuation dependence on temperature](image)

**Figure 45. BMFES attenuation dependence on temperature.** To assure that all parts of the BMFES were as close as possible to the same temperature during testing, temperature was simultaneously monitored at all times in the center of the BMFES with the TMS described before (inherent absolute uncertainty = ±2.2 °C) and on the outside of the BMFES with a calibrated infrared thermometer (Calibration number CF7381) (model number: 61, John Fluke MFG, Everett, WA, USA). The inherent absolute uncertainty associated with this device was ± 2°C. Measurements were taken after temperature was stable and within ± 0.5 °C. NMFS (NMFS = 658.32 μT at 60 Hz) was placed on all 3 faces (A, B, C) as described before and the TVMF sensor was placed at the center of the BMFES. Error bars are not shown for clarity but amount to ±0.05 μT ±17.1%.
Likewise, higher frequency (than 60 Hz) was proven to be of little importance as the attenuation factor increases as frequency is increased. On the other hand, the attenuation factor is diminished for lower frequencies.

![Graph showing BMFES attenuation dependence on temperature.](image)

**Figure 46. BMFES attenuation dependence on temperature.** To assure that all parts of the BMFES were as close as possible to the same temperature during testing, temperature was simultaneously monitored at all times in the center of the BMFES with the TMS described before (inherent absolute uncertainty = ±2.2 °C) and on the outside of the BMFES with a calibrated infrared thermometer (Calibration number CF7381) (model number: 61, John Fluke MFG, Everett, WA, USA). The inherent absolute uncertainty associated with this device was ± 2°C.

However, DC attenuation factors showed to be acceptable (see Figure 46). However, unlike temperature and frequency, magnitude was proven to be a factor of importance to the simulation. Figure 47 shows the attenuation as a function of the MF magnitude. Unfortunately, it was not possible to obtain accurate attenuation data at lower MF levels than the ones shown. The reason was that for such levels, the incident MF in the BMFES-shield was attenuated (in its center) to magnitudes that were close to the TVMF measurement system inherent noise. Therefore, the pervasiveness of the μ-metal linear dependence of the attenuation coefficients on the incident MF magnitude was not clearly defined due to the absence of direct measurements.
for the magnitudes found in real biological incubators. Measurements were taken after
temperature was stable and within ± 0.5 °C. NMFS (NMFS = 658.32 μT at 60 Hz) was placed on
all 3 faces (A, B, C) as described before and the TVMF sensor was placed at the center of the
BMFES. Error bars are not shown for clarity but amount to ±0.05 μT ±17.1%.

Because of these sources of uncertainty, two approaches were taken in the simulations
performed. The first possibility explored left the attenuation matrix in Table 6 unchanged. In
contrast, the second possibility used a function to predict the attenuation corresponding to
smaller incident magnitudes to the ones tested. This function was constructed paying attention to
the linear behavior observed in the data of Figure 47:

\[
\text{Att}(B_{gen}) = M_B (B_{off} - B_{gen}) - \text{Att}
\]

Where \( M_B \) corresponds to the attenuation slopes obtained from the sides of the BMFES
presenting the with the worst attenuation factors in Figure 47 (NMFS = 0.3273, WMFS =
0.0846, see Table 8), and \( B_{off} \) corresponds to the offset necessary to fit this function to the
attenuation curves shown in Figure 47 (NMFS = 658.32, WMFS = 136.57). Therefore, the
second possibility tested in the simulations was based on adjusting the attenuation values on
Table 6 with this function. In the results for these simulation, these two possibilities are referred
to as “attenuation correction = no” when Table 6 has been left unchanged and “attenuation
correction = yes” when the attenuation prediction function was utilized.
Figure 47. BMFES attenuation coefficient dependence on incident NMFS and WMFS magnitude. Measurements of the residual MF inside the BMFES were obtained at 60 Hz by placing the TVMF sensor in the center of the BMFES. WMFS (top) and NMFS (bottom) were done at magnitudes where saturation was not evident. Additionally, measurements reported fell above the inherent noise of the TVMF sensor. Error bars are not shown for clarity but are ±0.05 µT ±17.1%. Table 8 shows the linear slope obtained for each curve here shown.
Table 8. Linear slopes from BMFES attenuation coefficient dependence on incident NMFS and WMFS magnitude. Figure 47 shows curves from which these values were obtained.

<table>
<thead>
<tr>
<th></th>
<th>NMFS</th>
<th>WMFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.3273</td>
<td>0.0846</td>
</tr>
<tr>
<td>A2</td>
<td>0.3273</td>
<td>0.0846</td>
</tr>
<tr>
<td>B1</td>
<td>0.7124</td>
<td>0.1261</td>
</tr>
<tr>
<td>B2</td>
<td>0.7124</td>
<td>0.1261</td>
</tr>
<tr>
<td>C1</td>
<td>3.1007</td>
<td>0.1998</td>
</tr>
<tr>
<td>C2</td>
<td>3.1007</td>
<td>0.1998</td>
</tr>
<tr>
<td>A1B1</td>
<td>0.6164</td>
<td>0.1024</td>
</tr>
<tr>
<td>A1B2</td>
<td>0.6164</td>
<td>0.1024</td>
</tr>
<tr>
<td>A1C1</td>
<td>0.8531</td>
<td>0.1023</td>
</tr>
<tr>
<td>A1C2</td>
<td>0.8531</td>
<td>0.1023</td>
</tr>
<tr>
<td>A2B1</td>
<td>0.6164</td>
<td>0.1024</td>
</tr>
<tr>
<td>A2B2</td>
<td>0.6164</td>
<td>0.1024</td>
</tr>
<tr>
<td>A2C1</td>
<td>0.8531</td>
<td>0.1023</td>
</tr>
<tr>
<td>A2C2</td>
<td>0.8531</td>
<td>0.1023</td>
</tr>
<tr>
<td>C1B1</td>
<td>1.5786</td>
<td>0.1865</td>
</tr>
<tr>
<td>C1B2</td>
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<td>0.1865</td>
</tr>
<tr>
<td>C2B1</td>
<td>1.5786</td>
<td>0.1865</td>
</tr>
<tr>
<td>C2B2</td>
<td>1.5786</td>
<td>0.1865</td>
</tr>
<tr>
<td>A1C1B1</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
<tr>
<td>A1C1B2</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
<tr>
<td>A1C2B1</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
<tr>
<td>A1C2B2</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
<tr>
<td>A2C1B1</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
<tr>
<td>A2C1B2</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
<tr>
<td>A2C2B1</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
<tr>
<td>A2C2B2</td>
<td>0.7886</td>
<td>0.1146</td>
</tr>
</tbody>
</table>

Similarly, the way in which the incident WMFS was estimated had the potential to introduce significant amounts of error into the simulation. Also, in this case, two possibilities were identified for obtaining the most realistic estimation possible of the WMFS magnitudes found in real biological incubators. The first one assigned the value measured in the center of the incubator (Middle shelf-1, Figure 1) as the WMFS contribution. The rationale behind this
approach was that this is the value that is the least perturbed by NMFS contributions as it is the location that is furthest away from the incubator walls, behind which NMFS must be located. The limitation of this approach is that the center of the incubator will still have contributions from NMFS and this might be of a magnitude comparable to the real WMFS contribution. Additionally, the second possibility calculated the minimum SMF or TVMF value measured in the incubator as the WMFS contribution. In this case, the rationale was that since the WMFS affects the entire incubator, the “cleaner” sample of the incident WMFS will be found at the location with the least perturbation by NMFS and, therefore, will present the minimum value found in the incubator. The limitation of this possibility was the assumption that all sources share the same phase. In the results for these simulation, these two possibilities are referred to as “WMFS = center” and “WMFS = minimum” respectively.

All simulations for obtaining the residual BMF inside the BMFES were preformed based on permutations of these variables (attenuation correction and WMFS), generating a cohort of four possible residual BMF magnitudes for each simulation variation (Test 1 and Test 2). These results are presented in a condensed way in Figure 48 which shows the maximum TVMF magnitudes calculated inside of each BMFES for each of the 6 six different modes of insertion for all 21 real biological incubators of PART 1. In the same way, Figure 49 shows the maximum SMF magnitudes calculated for same incubators. Rows in Figures 48 and 49 correspond to four distinct simulation sets which were generated based on combinations of the simulation variables. The results obtained combine the contribution of both NMFS and WMFS.
Figure 48. Compilation of the maximum residual TVMF magnitudes inside the BMFES calculated with Test 1 and Test 2 simulation modalities. Results for Test 1 are shown in the right column and for Test 2 in the left column. Exposure data for the BMFES was obtained from 21 real biological incubators surveyed as shown in Figure 1. Attenuation values are shown in Table 6 and the insertion modes in Figure 44. Only the maximum values obtained for each mode in each incubator are shown. Because of this uncertainties in the simulation parameters, 4 different simulation sets are shown (1 per row) which correspond the possible combinations for these variables (Row 1 = (WMFS = center, Attenuation correction = yes), Row 2 = (WMFS = minimum, Attenuation correction = yes), Row 3 = (WMFS = minimum, Attenuation correction = no), Row 4 = (WMFS = center, Attenuation correction = no)).
Figure 49. Compilation of the maximum residual SMF magnitudes inside the BMFES calculated with Test 1 and Test 2 simulation modalities. Results for Test 1 are shown in the right column and for Test 2 in the left column. Exposure data for the BMFES was obtained from 21 real biological incubators surveyed as shown in Figure 1. Attenuation values are shown in Table 6 and the insertion modes in Figure 44. Only the maximum values obtained for each mode in each incubator are shown. Because of this uncertainties in the simulation parameters, 4 different simulation sets are shown (1 per row) which correspond the possible combinations for these variables (Row 1 = (WMFS = center, Attenuation correction = yes), Row 2 = (WMFS = minimum, Attenuation correction = yes), Row 3 = (WMFS = minimum, Attenuation correction = no), Row 4 = (WMFS = center, Attenuation correction = no)).
**Validating the simulation results:** Figures 48 and 49 show how multiple estimates were obtained for the residual TVMFs and SMFs inside the BMFES as a result of unavoidable uncertainties in the models in which the simulations were based on. After consideration of the absolute and relative magnitudes obtained for the residual MFs on all the simulations, special interest was taken in three incubators from the SMF simulations (6, 7 and 8) and three from the TVMF simulations (4, 9 and 14). These incubators were representative of the rest as they had most consistently shown to induce the worst-case MF magnitudes inside the BMFES in all variations of the model simulated.

Direct measurements were made inside the BMFES-shield after placing it inside these incubators in an effort to validate the predictions obtained from the simulations. These measurements were made at specific locations and insertion modes which yielded the worst-case residual TVMF and SMFs in the simulations performed. Tables 9 and 10 specify (i) the locations inside the BMFES (see Figure 40) and (ii) the mode (see Figure 44) in which the BMEFS has to be inserted into each real biological incubators for measuring the maximum TVMF or SMF magnitudes possible when the BMFES is inserted inside of a real incubator (of the real incubator collection surveyed). Table 11 (obtained from Tables 9 and 10) summarizes the internal location and mode of insertion of the BMFES on the set of real biological incubators of interest were the measurements were made.
Table 9. Location of the maximum TVMF values inside the BMFES calculated by Test 1 and Test 2. This table refers to Figure 48. Three incubators (4, 9, 14) were chosen as the ones that more consistently either surpassed the maximum limit set for TVMF values tolerated in the CDV (1.0 μT) and are delineated in the table by a border. Locations which surpassed the maximum TVMF or SMF magnitudes tolerated are shown in bold-type. The “X” marks the maximum value in that specific incubator for each testing modality. In some cases, more than one value was marked as a maximum value due to its insignificant difference with the maximum marked. The final list of values of interest is presented in Table 11.
Table 10. Location of the maximum SMF values inside the BMFES calculated by Test 1 and Test 2. This table refers to Figure 49. Three incubators (6, 7, 8) were chosen as the ones that more consistently either surpassed the maximum limit set for SMF values tolerated in the CDV (5 μT) and are delineated in the table by a border. Locations which surpassed the maximum TVMF or SMF magnitudes tolerated are shown in bold-type. The “X” marks the maximum value in that specific incubator for each testing modality. In some cases, more than one value was marked as a maximum value due to its insignificant difference with the maximum marked. The final list of values of interest is presented in Table 11.
Table 11. Representative TVMF and SMF values inside the BMFES calculated by Test 1 and Test 2. This table refers to Figures 48 and 49. Six real biological incubators (4, 6, 7, 8, 9 and 14) were identified as providing BMFs which would generate the worst-case TVMF and SMF conditions inside the BMFES when tested under the conditions shown in this table.

<table>
<thead>
<tr>
<th>Incubator 4</th>
<th>Incubator 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4 (Mode II)</td>
<td>C4 (Mode VI)</td>
</tr>
<tr>
<td>B4 (Mode II)</td>
<td>C2 (Mode II)</td>
</tr>
<tr>
<td>C4 (Mode I)</td>
<td>C4 (Mode II)</td>
</tr>
<tr>
<td>C9 (Mode I)</td>
<td>B2 (Mode II)</td>
</tr>
<tr>
<td>B4 (Mode I)</td>
<td>C5 (Mode IV)</td>
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<tr>
<td>C5 (Mode III)</td>
<td></td>
</tr>
<tr>
<td>C6 (Mode III)</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Incubator 9</th>
<th>Incubator 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4 (Mode II)</td>
<td>C4 (Mode VI)</td>
</tr>
<tr>
<td>B4 (Mode I)</td>
<td>B2 (Mode VI)</td>
</tr>
<tr>
<td>C6 (Mode IV)</td>
<td>C2 (Mode II)</td>
</tr>
<tr>
<td>B6 (Mode IV)</td>
<td>C9 (Mode I)</td>
</tr>
<tr>
<td>C6 (Mode III)</td>
<td>B6 (Mode III)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incubator 14</th>
<th>Incubator 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4 (Mode II)</td>
<td>C4 (Mode VI)</td>
</tr>
<tr>
<td>B4 (Mode I)</td>
<td>B2 (Mode VI)</td>
</tr>
<tr>
<td>C6 (Mode IV)</td>
<td>C2 (Mode II)</td>
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<tr>
<td>B6 (Mode IV)</td>
<td>C4 (Mode II)</td>
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<tr>
<td>C6 (Mode III)</td>
<td>B2 (Mode II)</td>
</tr>
<tr>
<td>C6 (Mode III)</td>
<td>B6 (Mode III)</td>
</tr>
</tbody>
</table>

Using this information, direct measurements were performed in the same fashion as the survey performed on real incubators in PART 1 and with the TVMF and SMF measurement systems described before (triaxial coil and Fluxgate respectively) in all 3 axes. A removable acrylic enclosure was built in order to protect the BMFES-shield and measurement devices in the process of loading and unloading from the host incubator for testing. Incubators were fully functional and allowed to stabilize $37 \pm 1 \, ^\circ\text{C}$ for at least an hour before the measurements were
performed. The door of the incubator was closed before the measurement was performed. The BMFES was positioned on a shelf in such way that it was centered in the interior of each incubator.

Figure 50 shows the TVMF and SMF magnitudes measured for the locations and modes of insertion proposed on Table 11 in comparison with the magnitudes predicted by the simulations performed. A total of 36 locations were measured, each consisting of measurements in all 3 axes. Notice how all estimations of Test 2 are superior to those of Test 1 in setting an upper-limit for the measurements obtained in a real situation. From this figure, the maximum residual MF magnitudes measured in the worst-case set of incubators are $0.74 \pm 0.18 \mu T$ (at 60 Hz) for TVMF and $4.57 \pm 0.19 \mu T$ for SMF. These values set an upper-limit to the possible TVMFs and SMFs measured inside the BMFES when placed inside of any of the set of the real incubators tested.

**Comparison of the BMFES-shield with previously existing BMF shielding systems:** The performance of the BMFES was compared to pseudo-standard BMF shielding equipment (BMF-shield) which has been extensively utilized in preceding Bioelectromagnetics research by Blackman et al. [Blackman et al., 1993; Blackman et al., 2001]. Two BMF-shield units were generously donated by Dr. Carl Blackman (Environmental Protection Agency, North Carolina, USA). Briefly, the BMF-shields consists of a cubic enclosure (side = 40.64 cm) made of 1.27 mm thick Co-nectic metal obtained from (Magnetic Shield Corp, Bensenville, IL, USA). A metallic hinge allows one of its faces to become a lid. The lid was supplemented with a flange (2.54 cm) which overlaps on all edges of the BMF-shield except for the edge to which the hinge
Figure 50. Validation of the worst-case SMF and TVMF magnitudes inside the BMFES calculated by Test 1 and Test 2. This figure corresponds to measurements proposed in Table 11. Left column corresponds to SMF measurements (incubators 4, 9 and 14, from top to bottom respectively). Right column corresponds to TVMF measurements (incubators 6, 7 and 8, from top to bottom respectively). Abbreviations correspond to Test 1 and Test 2 variations calculated (C = WMFS estimation is made from the value measured at the center of the real biological incubators, WMFS estimation is made from the minimum value measured in real biological incubators, Y = attenuation magnitude correction is used, N = attenuation magnitude correction is not used, also see Figures 48 and 49 and Tables 9 and 10).
is attached. The lid is held in the closed position by 3 metallic butterfly locks which are equally spaced along the hinge opposite edge. The BMF-shield is complemented in its interior by metallic sheets (6.35 x 40.45 x 0.127 cm) placed on the top and bottom edges near the lid. Also, the BMF shield has two non-magnetic metallic pieces (2.54 x 5.08 x 38.1 cm) bolted to its exterior (bottom) to serve as feet.

Figure 51. Performance comparison between BMF-shield and the BMFES. All enclosures were exposed to the same 60 Hz TVMF levels (NMFS = 658.32, WMFS = 136.57) as previously described in this document. Blackman et al. #1 and #2 were identical with the exception that #2 was previously exposed to standard biological incubator conditions while #1 was not. The TVMF sensor was placed in the center of both systems. Error bars are not shown for clarity, but they correspond to the TVMF measurement system error (±0.05 μT ±17.1%).
Figure 51 shows how the BMFES-shield and BMF-shield performances compare under the same exposure conditions (NMFS = 658.32, WMFS = 136.57). The methods utilized for measuring its performance were described earlier in this document. Notice how the residual TVMFs are less for the BMFES than for the tested BMF-shields for NMFS generated MFs while the opposite is observed for WMFS. Although both shields performance is comparable, several differences are distinguished from these two designs. First, unlike the BMFES-shield, the BMF-shield presents no symmetry. Because of this reason, a the performance comparison was done by placing the TVMF in the center of both systems and surveying all faces, edges and corners with both WMFS and NMFS-generated TVMFs (at 60 Hz). Also, in the case of the BMF-shield, 8 protruding tubes (diameter = 2.54 cm, height = 2.54 cm, thickness = 1.27 cm) which centers are located 2.54 cm from each edge on the top (4) and bottom (4) of the BMF-shield create a connection to the incubator allowing for gas exchange and instrumentation connections to reach its interior in a clearance area of only 40 cm$^2$. In contrast, the BMFES-shield had a nearly 1-cm gap between both of their fundamental parts’ (IS and OS) surfaces and edges which allowed for a clearance area close to 120 cm$^2$ between the interior of the BMFES-shield and the incubator. This exceeded the BMF-shield area by a factor of 3. Also, the BMF-shield architecture necessitates many right angles and corners hard to reach for cleaning purposes. In contrary, the BMFES-shield has all rounded shapes and also no moving parts (hinges, locks, etc).

**BMFES internal SMF generation:** To generate a SMF within the range naturally found on Earth’s surface in the CDV, a coaxially-fed pair of square coils (BMFES-SHC) in Helmholtz configuration (Side length = 30.00 cm, number of turns = 120 each, wire gauge = 24 AWG, separation between coils = 16.35 cm, thickness of the coils = 10 mm (axial) and 3.5 mm (radial))
Figure 52. Localization of the Square Helmholtz coil pair in the BMFES. Notice how the axis of the pair is parallel to that of the IS. The two-digit numbers representing each point represent its row and column starting at the A2-B2 corner which are also referred to in Table 12. Additionally, the measurement points presented in this figure corresponds to a spatial oversampling of the measurement points presented in Figure 40 in the sense that a instead of having a cube of 27 samples (3x3x3) in a 9 cm grid, it consists of a cube of 125 samples (5x5x5) in a 4.5 cm grid. Therefore all the 27 samples of the original cube coincide with 27 samples of the 125-sample cube (e.g. Top(C1)-11 = T8, Bottom(C2)-55 = B4). The dimensions of presented in this figure were within ± 1%.
was wound on the outside of a square acrylic cylinder (side length = 30 cm) which was placed inside of the IS as shown in Figure 52. Coils were coated with weather resistant metallic coated tape which was connected to ground of the system in order to shield the CDV from electric fields generated by the presence of charges at different potentials as electrical current flows through the coil wires. The attenuation factor of the BMFES-shield (see Table 6) did not show to be affected perceivably when injecting up to 2 A in the BMFES-SHC. The methods for the generation of homogeneous magnetic fields are diverse and can entail many different coil configurations. Although greater uniformities can be achieved with an increased number of coils [Robinson, 1983], the stray fields from this type of coil to generate any homogeneous MF inside a limited volume are the greatest that can be produced by any number of coils greater than 2 [Cacak and Craig, 1969]. Therefore, the use of this coil will not compromise the shielding ability if another coil design is to be used instead.

**Final BMFES specifications (Isolation from external BMF and homogeneity of internal BMF):** The BMFES was formed by placing the BMFES-SHC inside the BMFES-shield in a way that the axis of the SHC and the IS coincide. A thorough survey was performed on the locations defined in Figure 52 to determine the variability of the SMF generated by this system on the CDV of the BMFES. Although this survey was performed with increased definition (125 points-5x5x5) than for the BMFES-shield (27 points 3x3x3), these measurement locations coincided for both surveys. The MF magnitudes obtained are presented in Table 12. This table also compares the BMFES-SHC performance with that of an SHC in free space. Additionally, with the purpose of summarizing this data, Figure 53 shows how much these values deviate from the MF generated at the center of both coil systems (45 μT) for all the axial, sagittal and coronal planes.
Table 12. Comparison of the Homogeneity of the MF generated by square coils in Helmholtz configuration in free space versus inside the BMFES. The survey consists of 125 samples arranged on a 5x5x5 cube (4.5 cm grid) centered on the geometric center of the Helmholtz coil pair (as shown on Figure 52). Data in each cell is presented in the form “Helmholtz coil inside BMFES / Helmholtz coil outside BMFES”. MFs were generated by injecting a sinusoidal (60 Hz) signal of 0.0410 A and 0.0512 A respectively into the coils with a function generator (model number: 33120A, Hewlett Packard, Palo Alto, CA, USA) (Being inside of the BMFES reduced the current necessary to achieve 45 µT in the geometric center of the Helmholtz pair). Current was measured with a calibrated meter previously described in the text (model number: 179, John Fluke MFG). For the measurements made to the Helmholtz pair outside the BMFES, the pair was placed at least 1.5 m away from walls and metal objects. The noise in the sensor was described in the TVMF section of this document ±0.05 µT ±17.1%. The right side of the table shows the resultant TVMF amplitudes measured at 60 Hz. Data is presented in 5 slices parallel to the axial plane (Top (C1), Middle (C1), Center (C1-C2), Middle (C2) and Bottom (C2)). Each slice contains 25 measurements (11, 12, 13,…55). The left side of the table shows the absolute percentage deviation from the measurement made at the center of the pair for each case, and data is grouped in the same fashion as on the right side.
Figure 53. Absolute % deviation from the MF magnitude at the center of the BMFES. The location of the coronal, sagittal and axial measurement planes are shown in Figure 52. Error bars are not shown but correspond to the error presented by the TVMF sensor.
surveyed. Similarly, Figure 54 shows a distribution of the values presented in Table 12. For these figures, notice how the overall BMFES-SHC homogeneity is better than 28% for the CDV. Also, notice and how the SHC homogeneity is markedly improved by placing it inside de BMFES as compared to an SHC in free space.

![Graph](image)

**Figure 54. MF magnitude distribution inside the BMFES.** All points measured are included in this figure. Notice the marginal improvement obtained by placing the square Helmholtz coil inside of the BMFES.

Given that the upper limit measured for the possible SMF residual magnitudes inside the BMFES amounts to $4.57 \pm 0.19 \, \mu T$, then the maximum SMF possible is $4.76 \, \mu T$ ($4.57 + 0.19 \, \mu T$) due to the maximum uncertainty associated with the SMF measurement system. The requirement is that the SMF inside the CDV be within the range found naturally on Earth’s (23
to 65 μT) or 45 ± 20μT inside the CDV (SMF). The maximum variability presented by the BMFES-SHC is less than ±12.50 μT (or ±28%); therefore, the total maximum possible variability when the BMES is placed inside of any of the real biological incubators tested should be less than 17.26 μT (12.50 + 4.76 μT) even in the worst-case incident SMFs. This is shown in a more explicit way in Figure 55 where the hypothetical distribution of the maximum absolute deviation from 45 μT at the center of the CDV is presented.

![Figure 55](image)

**Figure 55.** SMF distribution inside the CDV due to worst-case external SMF incident in the BMFES. Notice how all SMF deviations from 45 μT remain below ±40% (or ±18μT).

Similarly, given that the upper limit measured for the possible TVMF residual magnitudes measured inside the BMFES amounts to 0.74 ± 0.18 μT (at 60 Hz), then, the
maximum TVMF possible is 0.92 μT (0.74 + 0.18 μT) due to the maximum uncertainty associated with the TVMF measurement system. Additionally, the amplification of the 60 Hz ripple noise was 775.86 μT/A due to the number of turns and geometrical characteristics of the BMFES-SHC. Table 13 shows the ripple TVMF (at 60 Hz) generated by this setup based on the input current. Given that the requirement that TVFM inside the CDV should be less than 1 μT (at 60 Hz), then, the maximum ripple MF allowed is less than 0.08 μT (at 60 Hz) which allows for 0.103 mA (or 0.18%) of the feeding ripple current. Therefore, the power supply feeding the BMFES-SHC must have an attenuation of at least 55 dB from the 60 Hz power distribution system.

<table>
<thead>
<tr>
<th>Input ripple current</th>
<th>Noise level at 60 Hz (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pA</td>
<td>7.76E-10</td>
</tr>
<tr>
<td>0.01 nA</td>
<td>7.76E-09</td>
</tr>
<tr>
<td>0.1 nA</td>
<td>7.76E-08</td>
</tr>
<tr>
<td>1 nA</td>
<td>7.76E-07</td>
</tr>
<tr>
<td>0.01 μA</td>
<td>7.76E-06</td>
</tr>
<tr>
<td>0.1 μA</td>
<td>7.76E-05</td>
</tr>
<tr>
<td>1 μA</td>
<td>7.76E-04</td>
</tr>
<tr>
<td>0.01 mA</td>
<td>7.76E-03</td>
</tr>
<tr>
<td>0.1 mA</td>
<td>7.76E-02</td>
</tr>
<tr>
<td>1 mA</td>
<td>7.76E-01</td>
</tr>
<tr>
<td>0.01 A</td>
<td>7.76E+00</td>
</tr>
<tr>
<td>0.1 A</td>
<td>7.76E+01</td>
</tr>
<tr>
<td>1 A</td>
<td>7.76E+02</td>
</tr>
</tbody>
</table>

Table 13. TVMF noise levels generated by the square coil system in Helmholtz configuration inside of the BMFES as a function of the input current.

Finally, with this set of specifications described, the whole CDV is guaranteed to be exposed to residual TVMFs below 1 μT (at 60 Hz) and the SMF at its center (45 μT) will show homogeneity better than ±38% which will fall within the range naturally found on Earth’s surface (23-65 μT).
BMFES thermal characteristics: In order to explore the thermal characteristics of the final BMFES design, the BMFES-SHC acrylic cylinder was supplemented with 6.35 mm thick shelves in which measurements for temperature homogeneity and transients were performed. These prototype shelves coincided with the axial planes of Figure 40 in order to perform measurements in the same locations which defined the CDV.

BMFES temperature transients and homogeneity: The single-shelf temperature homogeneity of the BMFES was measured by placing 4 identical temperature probes on the on positions C2, C4, C6 and C8 (See Figure 40). Under static temperature conditions (fixed BMFES temperature for more than 1 h) the maximum absolute discrepancy between probes was below 0.23 °C and the maximum variability between any probe and the average of the group of probes was below ± 0.16 °C for data taken at 5 °C intervals between 22 and 42 °C. On the other hand, under dynamic conditions (transient shield temperature), the maximum absolute discrepancy between probes was below 0.45 °C and the maximum variability between any probe and the average of the group of probes was below ± 0.29 °C for data taken at the same temperature intervals.

The time it took for the probes’ temperature to reach the temperature of the BMFES preheated at 42 °C when coming from room temperature (22 °C) (or for the temperature transients to disappear) for dishes placed at the center any of the 3 shelves tested was 300 ± 10 m (5 h). In this case, the static and dynamic maximum absolute discrepancy between probes and the maximum variability between any probe and the average of the group remained the same for all 3 CDV shelves (see Figure 40). However, the maximum discrepancy between shelves was of 3.4
°C, being the top shelf the warmest. As a comparison, the 3 incubator shelves spaced in the same way as the ones in the BMFES where tested following the same protocol. Transients disappeared in $120 \pm 10$ m (2 h) and the maximum discrepancy between shelves was of 2.5 °C. These results were not different when using aluminum or acrylic shelves. This difference between the shielded and unshielded incubator temperature transients is then attributed to the reduced air circulation imposed by the BMFES in the particular incubator tested. However, a finished product is expected to perform much closed to the host incubator as it would not have an acrylic frame as the BMFES prototype used in this study did. In its present form, the acrylic reduces the air circulation clearance to more than half its maximum area. Also, water-jacketed shelves would get rid of this problem effectively. A similar solution has been explored in one of our previous publications [Portelli et al., 2012] reaching temperature homogeneities better than 0.2 °C.

**BMFES-SHC temperature contribution:** A peak current of 0.041A was needed to generate 45 $\mu$T in the BMFES-SHC center. Since the resistance of the SHC = 19.30 Ω (Resistivity of copper =0.0 67 Ω/m, length = (240 turns)(4 sides)(0.30 m) = 288 m). Then, the power dissipated by the SHC is 0.032W. Assuming no heat loss, the rate at which this volume of copper can gain temperature is 0.56 °C/h ((24 AWG cross-sectional area = 0.205 mm²; Volume of 240 turns of 24 AWG wire = 59.04 cm³; volumetric heat capacity of copper = 3.45 J/cm³•K). This value is insignificant, given that the copper wire area exposed to humidified air is 0.46 m² and humid air thermal conductivity is 0.027 W/m•K. This was tested by supplying 0.050A of current to the BMF-SHC for two hours causing a differential in temperature of less than 0.2 °C as measured
with the IR-TMS and the TMS previously described. Measurements were done at 21.4 and an atmosphere with 40 % relative humidity.

**BMFES physical characteristics:** The prototype weight was 41 Kg. However, most of that weight corresponds to the acrylic supporting structure. The weight corresponding to the $\mu$-metal (density $= 8.75 \text{ g/cm}^3$) amounted to 7.91 and 6.35 Kg for the OS and IS respectively. In the case of the BMF-SHC copper (density $= 8.93 \text{ g/cm}^3$) it amounted to 0.63 Kg. These made a total of 14.89 Kg or 36% of the total prototype weight. Its total volume amounted to 54.87 L ($38.5 \times 38.5 \times 38.5 \text{ cm}$) while the interior volume available for culture was 5.83 L ($18.0 \times 18.0 \times 18.0 \text{ cm}$).
Chapter VII

Conclusions:

Following the specifications described in PART 1 of this document, it is possible to build a BMFES with no moving parts, sharp corners or edges which can protect a reasonable sized culture volume from BMFs existing in standard cell culture incubators without utilizing a “lidded” architecture. A static magnetic field within the range found naturally on Earth’s can be generated for this culture volume surface with a pair of square coils in Helmholtz configuration without affecting the BMFES shielding ability. This device could show similar performance when inserted in other kinds of biological incubators since the general set of basic features and components are typically similar to those of cell culture incubators. Also, this device could be utilized outside of an incubator if the biological system requires it so or with help of more localized environmental controls which may utilize water jackets or forced convection. In addition, this document describes a set of materials and methods which can be used to consistently assess the shielding ability of shielding devices.
Future work:

By utilizing the full experimental description of the BMFES properties here presented, further study of the behavior BMFES should look to reconcile the measurements performed with numerical methods in order to further optimize its performance. Given that the thickness values were tested in discrete steps, optimization of these values is still possible through computer modeling. A final prototype should consist of one single piece instead of wound from a thin sheet of high permittivity material, therefore increasing its shielding ability.

Future work also includes changing the shape of the shield to rectangular in an effort to reclaim the remaining space in the incubator. Additionally, smaller versions of the same BMFES can be built with less high permeability material given that its shielding ability increases as the diameter of the square cylinder decreases to perform experiments to study differential magnetic field effects.

Covering the BMFES with copper is recommended to reduce even further its contamination potential. Shelves should be made of a non-magnetic material with good thermal transfer properties.
Finally, further experimentation should be performed in order to corroborate that the reduced BMF variation produced inside the BMFES reduces biological variability when compared to a regular biological incubator.
**Bibliography:**


Arentz RF, Johnson MH, Magnetics A-V. Magnetic Shielding In a Cryogenic Environment.


