

Effects of Microscope Objectives on Magnetic Field Exposures

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Distortions in magnetic field intensity generated by commonly used microscope objectives (1× to 100×) were characterized within a Helmholtz coil-based exposure system. Objectives from a variety of manufacturers distorted applied field intensities by up to 23% in the image plane. Components that contribute to distortions include (1) nickel-chrome plating of objective housings, (2) the presence of steel springs in objectives with compression collars, and (3) steel screws or studs used to hold together separately manufactured parts. Steel springs and screws produce radially asymmetric profiles, whereas distortions generated by nickel-chrome plating are typically radially symmetric. All components can produce spatial gradients in field intensity if objectives are not perfectly aligned with exposure systems or if placed in the earth's magnetic field. Alterations in the magnitude of magnetic field intensities as well as the production of spatial gradients might have an effect on biological responses. By maintaining optical glass components and replacing metallic components, functional objectives can be reconstructed that produce no measurable effects on magnetic flux densities. *Bioelectromagnetics* 20:387–395, 1999. © 1999 Wiley-Liss, Inc.

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INTRODUCTION

Light microscopy is a powerful tool to monitor intracellular and intercellular dynamics in the presence of electromagnetic fields [EMFs; Tenforde, 1992]. Bright-field and phase-contrast techniques have been used to observe cell viability, geometries, interconnections and growth patterns. More recently, fluorescent imaging has become an increasingly important tool to monitor cellular functions at more molecular levels in response to applied EMFs [for reviews, see Goodman et al., 1993; Callahan et al., 1993; Liburdy, 1994]. Although the functional components of microscope objectives used in these studies are made from glass, most structural components of commercially available microscope objectives are metallic. The presence of ferromagnetic elements in particular, in conjunction with other metallic components of microscope-based imaging systems, could lead to field distortions that may alter cellular responses [Liburdy, 1995; Lindström et al., 1995]. Therefore, it is important to understand the nature of field distortions and spatial gradients produced by microscope-based imaging systems during EMF exposures.

The goals of the present study were to measure magnetic field distortions produced by commonly used microscope objectives, identify the structural components of objectives that contribute to distortions, characterize the types of distortions produced, and generate a series of suggestions to maintain the optical functionality of imaging systems while minimizing EMF effects. Representative objectives were selected to encompass (1) a variety of manufacturers of scientific-grade microscope systems, (2) the magnification range (1× to 100×) commonly used at the tissue and cellular levels, and (3) different ferromagnetic components that furnish struc-

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tural support or mechanisms that aid in focusing. Particular attention has been given to identify situations in which spatial gradients in flux density are generated within the field-of-view (i.e., the region observed when viewed through the objective).

METHODS

During EMF exposures involving microscope-based imaging systems, commercially available objectives are a contributor to overall inductive losses and are the major factor in producing spatial gradients in magnetic flux density in the region of a biological preparation. This arises because, during use, objectives must be placed in close proximity to the preparation. An experimental setup was designed to isolate the effects of different microscope objectives and to accurately assess regional flux densities. In the present study, the stage and objectives were oriented in the “inverted microscope” configuration (objectives located beneath a dish, viewing upward); however, similar objectives (with similar working distances) are also commonly used in the noninverted (compound microscope) configuration.

Magnetic Field Exposure System

In general, the dimensions of an exposure system are constrained by the physical construction of the particular microscope used, but must be sufficiently large to produce a uniform magnetic field throughout the region containing biological materials (e.g., a 35-mm diameter Petri dish). In this study, custom-built Helmholtz coils (radius, $a = 5$ cm) consisting of 30 turns (N) of 19-gauge bifilar enameled copper wire (MWS Wire Industries, Westlake Village, CA) were designed to mount on a support structure (reconstructed microscope stage) made of linen base phenolic. Except where noted, results were obtained without mounting the phenolic stage onto an actual microscope. Objectives were aligned in the center of the Helmholtz coils by using an adjustable wooden support. Except for the microscope objective under investigation and the Hall effect probe, no metallic objects were present within the region between the coils or within 20 cm outside the coils. Extraneous fields were reduced by placing the exposure system in a custom-built, shield box (72 cm high \times 62 cm wide \times 57 cm deep) constructed from 0.5-mm-thick Co-netic AA perfection annealed alloy (similar to mu-metal) providing an attenuation of 30–40 dB according to manufacturer’s specifications (Magnetic Shield Corp., Bensenville, IL).

During the generation of constant (i.e., “DC”) magnetic fields, coils were driven by a regulated, constant-current power source (Lambda, model LP-531-FM). During the generation of sinusoidal time-varying (i.e.,

“AC”) magnetic fields, coils were driven by a programmable AC power source (Elgar, model 1001C). Unless otherwise stated, magnetic fields were created with a 1.1 A source current (I) that was continuously monitored (Fluke multimeter, model 77).

Magnetic Field Measurement System

Magnetic flux density was mapped by using a Walker Scientific (model MG-3D) gaussmeter and Hall effect, transverse probe (Walker Scientific, model HP-33S). In addition to factory calibration, the Hall effect probe was checked against a reference coil magnetic sensor (Electric Field Measurements, Model EFM-140) and flux densities computed according to the Helmholtz equation: $B = 2(\mu_0 I a^2 N) / [2(a^2 + d^2)^{1.5}]$, where $\mu_0 = 4\pi \times 10^{-7}$ H/m and $2d = a$. Over the range of currents used in this study and near the center of the coils, the three methods agreed to within 0.5 %.

The probe was mounted to a plastic arm which, in turn, was mounted to a two-dimensional micrometer stage (Klinger Instruments). This design allowed movements to be controlled to within 0.1 mm in each direction while keeping metallic micrometer components well away from the coils. In some cases, fields were measured across the top of an objective (i.e., X direction) and as a function of height above an objective (Z direction). During most scans, the stage and support apparatus were adjusted to obtain a map in the image (i.e., focal) plane of the objective (X-Y plane). The axial center of the objective at the tip of the outer-most lens was considered to be the origin (0 distance) for the X, Y, and Z axes (see Fig. 1 [A]).

RESULTS

In the absence of an objective, the exposure system produced a relatively uniform DC magnetic field in the central region between the coils (see Fig. 1 [B]). As predicted by Helmholtz theory [Reitz and Milford, 1960; Misakian et al., 1993], the magnitude of the radially symmetric field decreased somewhat with increased distance from the center (in the vicinity of the coils). This decrease in field intensity was less pronounced, and even reversed at increased distances above the image plane ($Z > 0$). The magnitude of the field was directly proportional to the applied current. A similar uniformity of fields was measured when time-varying signals (20 to 400 Hz sinusoidal waveform) were applied (data not shown).

Microscope Components That Contribute to DC Field Distortions

Figure 2 provides examples of distortions in magnetic flux density produced by “simple” objectives in-

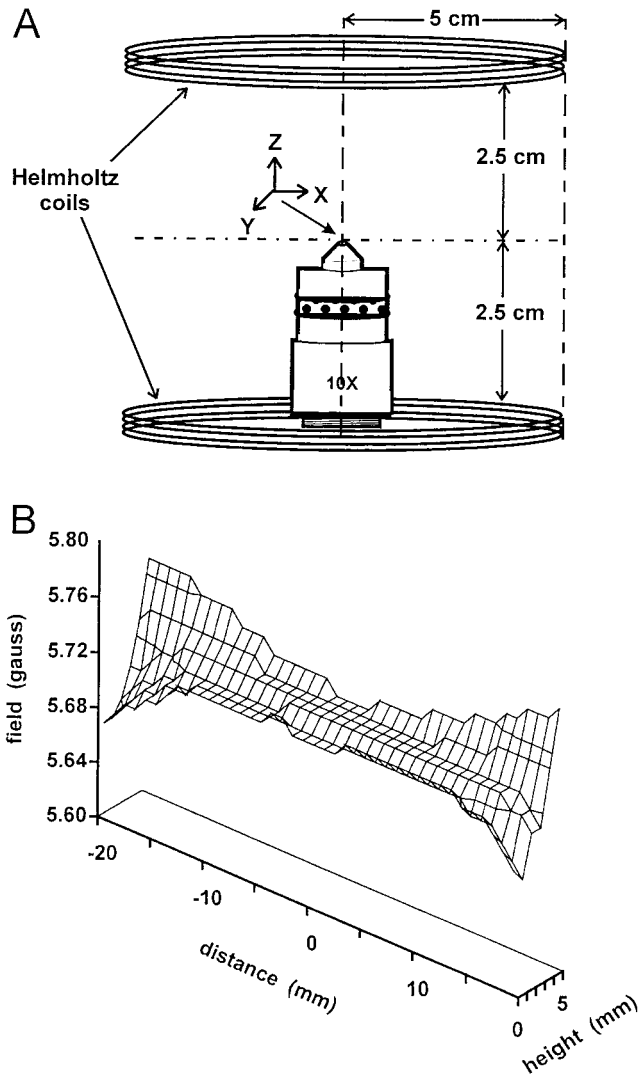


Fig. 1. Helmholtz coil-based exposure system. **(A)** The coordinate system used to map magnetic fields. **(B)** Scans of radially uniform magnetic flux density (X direction) in the absence of an objective at various heights (Z direction) above the midplane between the coils ($I = 1.06$ A).

cluding a 10 \times objective manufactured by Olympus (Fig. 2 [A]) and a 40 \times objective manufactured by Nikon (Fig. 2 [B]). In these objectives, no springs, steel screws, or other ferromagnetic parts could be found and none were indicated in specifications provided by the manufacturers. Internally, glass lenses were supported by structures (spacing rings and housing) manufactured primarily from brass. However, the outer surfaces of the objectives were generally coated with a nickel-chrome alloy, 4 ± 1 μm thick (Olympus manufacturer's specifications). This alloy appeared to be the major source of field distortions produced by these objectives. Distortions were radially

symmetric throughout the X-Y plane and decreased as a function of distance from the image plane (Z axis).

Most "simple" objectives suppressed magnetic flux density by up to 6% in the image plane of the objective (as illustrated in Fig. 2 [A]). Some older objective housings (manufactured before 1960) contain significant ferromagnetic content that generate larger distortions (data not shown). Modern-day objectives that depressed field intensities included those distributed by Olympus, Nikon, Zeiss, Leitz, and Edmund Scientific. Low optical power objectives often generate a slight elevation in flux density in the exact center of the field-of-view relative to the surrounding depressed field. This feature is particularly evident in the lowest power objectives (1 \times to 4 \times , data not shown) but can be seen in objectives up to 10 \times (as in Fig. 2 [A], see arrow). This effect is likely due to the lack of influence by the glass components that make up the objective's axial core.

Although most "simple" objectives suppressed magnetic flux density, some produced slight elevations in field intensity in the image plane. An example is shown in Figure 2 (B). The materials used to generate coatings as well as the exact geometry of the objective (particularly close to the image plane) influence the form of the distortions in field intensity. Objectives with a midrange of optical magnification (10 \times to 40 \times) such as the one used to generate Figure 2 (B) usually have a shape that draws to a point with a limited glass core region, producing elevations in field intensity.

In all measurements of magnetic flux density, care has been taken to align objectives precisely with the direction of the applied field (i.e., exactly perpendicular to the plane of the coils). Figure 2 (C) shows an example of field distortions produced by a "simple" objective that has been "tilted" by approximately 1 $^\circ$ away from the direction of the applied field. The Nikon 10 \times objective used in these scans produces radially symmetric distortions similar to those shown in Figure 2 (A) when aligned with the field. However, the 1 $^\circ$ tilt produced a characteristic "peak and valley" pattern in the field near the image plane. This effect decreased with increasing distance above the objective.

High-magnification, low working-distance microscope objectives often use a steel spring mechanism to facilitate adjustment of the outer objective lens during focusing procedures. Figure 3 (A) shows an example in which a ferromagnetic spring has been removed from an objective (Edmund Scientific, 20 \times) and positioned uncompressed within the exposure system as if the remainder of the microscope objective were present (i.e., focused at $Z = 0$). In this case, there is a pronounced increase in field intensity in the direction of the starting

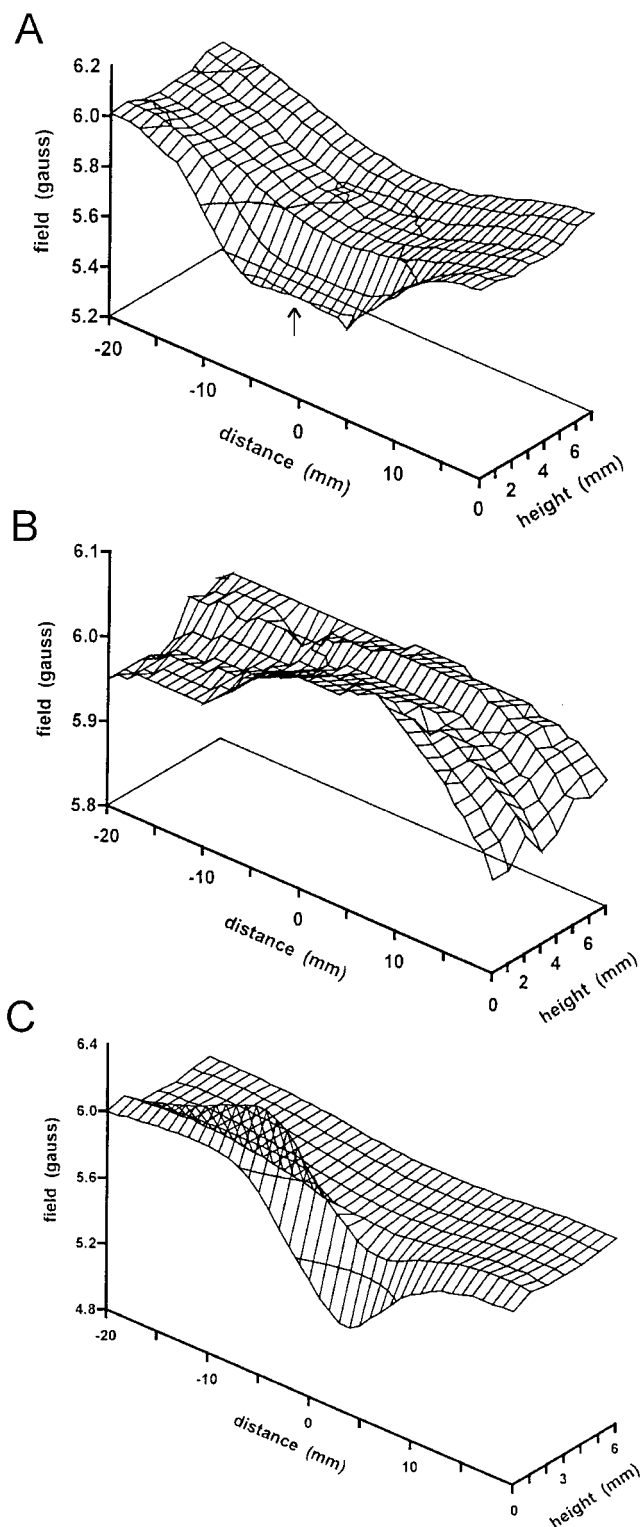


Fig. 2. DC field distortions produced by nickel-chrome plating of objective housings. Scans of DC magnetic flux density were measured (X direction) at various heights above mid-plane between the coils (Z direction). **(A)** A radially symmetric suppression of flux density produced by an Olympus 10 \times objective. **(B)**

point of the wire that made up the spring (oriented along the positive X axis).

Objectives may also contain one or more small screws or slugs (acting as rivets) to fasten together different components within the housing. Although at first glance an objective may appear to be axially symmetric (centered around the light path), distortion of magnetic fields can be axially asymmetric due to the presence of these embedded steel components. Figure 3 (B) shows distortions in magnetic flux density in the X-Z plane (measured at Z = 0) generated by an Olympus 100 \times objective containing both a spring and a small (3 mm head diameter \times 4 mm long) steel screw located 24 mm from the focal plane (Z direction). The positive X axis was aligned to correspond to the axial direction of the screw. The orientation of the spring within the objective was not determined. The spring appears to be the primary cause of the increase in field intensity shown within the negative-X, negative-Y quadrant of Figure 3 (B). The screw generated the large "peak" in the field along the positive X axis. Removal of the screw caused this "peak" to decrease significantly (data not shown).

AC Field Distortions

Much of the current interest in the biological effects of electromagnetic fields is centered on time-varying fields, more specifically sinusoidal fields in the 50–60 Hz (power-line frequency) range. Distortions produced by microscope objectives are frequency dependent, particularly in the extremely low frequency (ELF) range. Figure 4 (A) shows an example of AC distortions in the presence of the same Olympus 100 \times objective used to generate Figure 3 (B). The overall magnitude of field distortions was reduced compared with DC fields (see Fig. 3 [B]). Objective components (spring, screw, and nickel-chrome coating) appear to be sufficiently small and at an adequate distance from the image plane to not substantially alter the 60 Hz field, although there may also have been some effect due to the finite sample area sensed by the Hall effect probe. When the objective was replaced by a similarly sized nickel cylinder (2-mm-thick wall), large field gradients were observed in the vicinity of the cylinder. Larger gradients were also observed at other frequencies (100 to 400 Hz; data not shown).

Distortions of the Earth's Magnetic Field

Figure 4 (B) shows an example of field intensities in the image plane of an Olympus 100 \times objective re-

A slight elevation in flux density near the center of the field-of-view produced by a Nikon 40 \times (air) objective. **(C)** The characteristic "hill and valley" field distortions produced by a Nikon 10 \times microscope objective tilted less than 1 $^\circ$ away from the vertical orientation of the applied field.

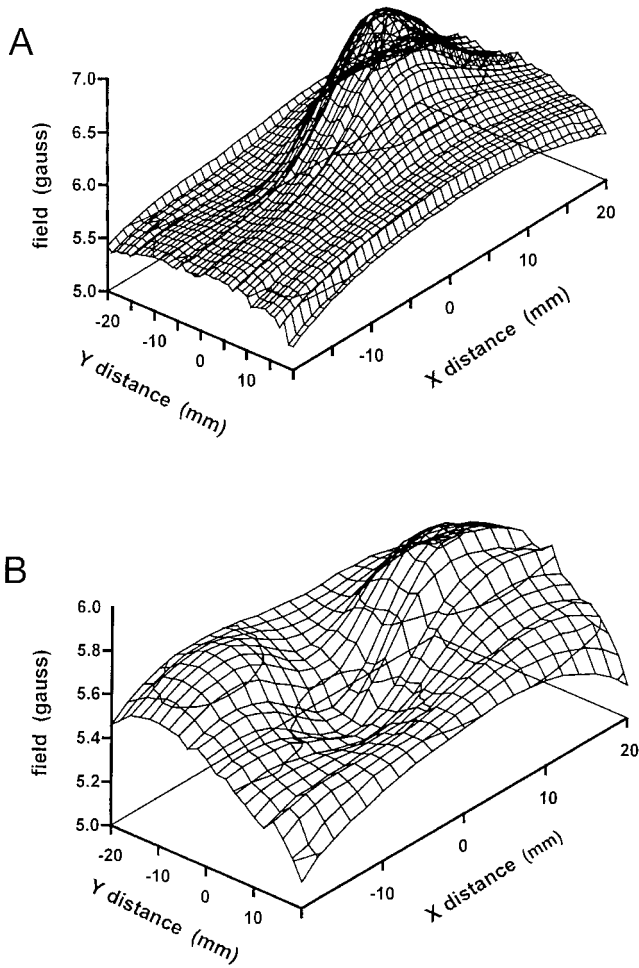


Fig. 3. DC field distortions in the field-of-view (X-Y plane at Z = 0) produced by objective components. **(A)** A steel spring (diameter 11 mm) was removed from an objective (Edmund Scientific 20×) and positioned uncompressed as if the remainder of the objective were present (see Fig. 1 [A]). The beginning and end of the wire that made up the spring were positioned along the positive X axis. **(B)** An Olympus 100× objective (outside diameter 24 mm, height 50 mm) containing both a steel spring and a steel screw was oriented with the long dimension of the screw along the positive X axis.

corded outside the shield enclosure. The earth’s magnetic field was oriented 47° from the vertical alignment of the objective. Radially asymmetric variations in field intensity were generated due to the objective’s nickel-chrome plating, and steel spring and screw. Even a “simple” cylindrical objective plated with a nickel-chrome surface produces asymmetric field distortions, because the earth’s field is not aligned with the axial direction of the objective during normal use.

Elimination of Field Distortions

The functional components (lenses) of microscope objectives are nonmetallic (glass). Thus, with appropriate

machining facilities, it is possible to begin with a commercially available objective and remanufacture a functional objective by using an opaque plastic housing and supports. Figure 5 (A) shows magnetic flux density in the presence of a reconstructed “plastic” objective. Radially symmetric field intensities were measured throughout. Compared with fields generated by Helmholtz coils in the absence of an objective, there were no significant differences in field intensities due to the presence of the objective within the coils (see Fig. 1 [B]).

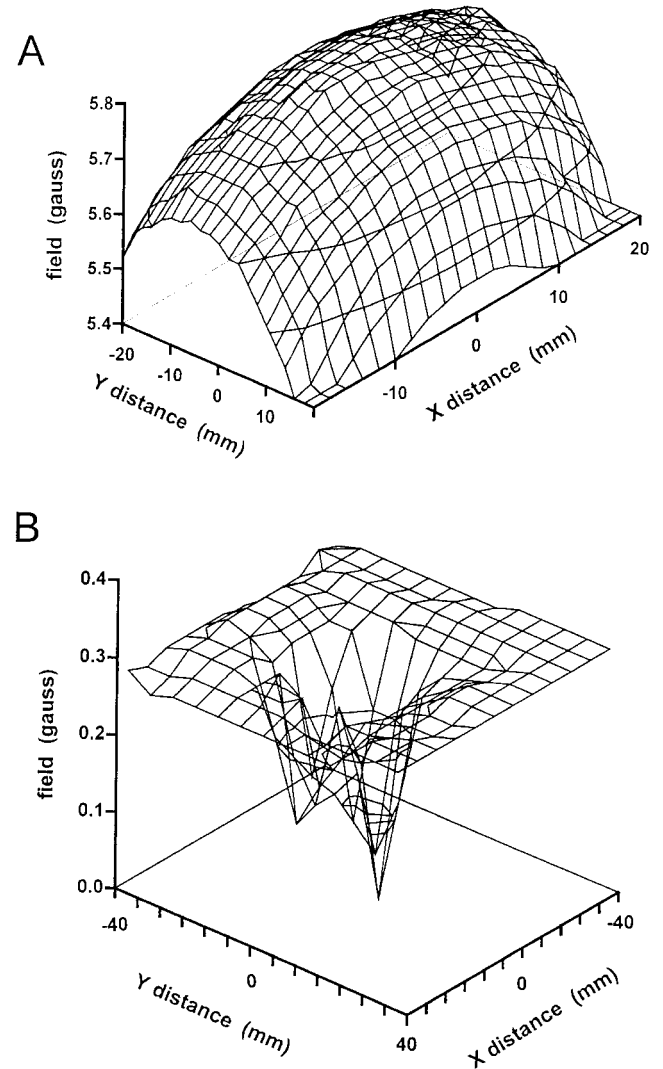


Fig. 4. Image plane distortions produced by AC exposure and the earth’s magnetic field. **(A)** The Helmholtz coils were driven by a 60 Hz sinusoidal wave form and RMS field intensities were measured in the presence of the same objective used in Figure 3 (B). **(B)** The complex field distortions of the earth’s magnetic field (absence of a shield enclosure) produced by the same objective. The earth’s field (0.3 gauss, spatially uniform in the absence of the objective) was oriented 47° from the Z axis of the objective placed in its typical (vertical) operational orientation.

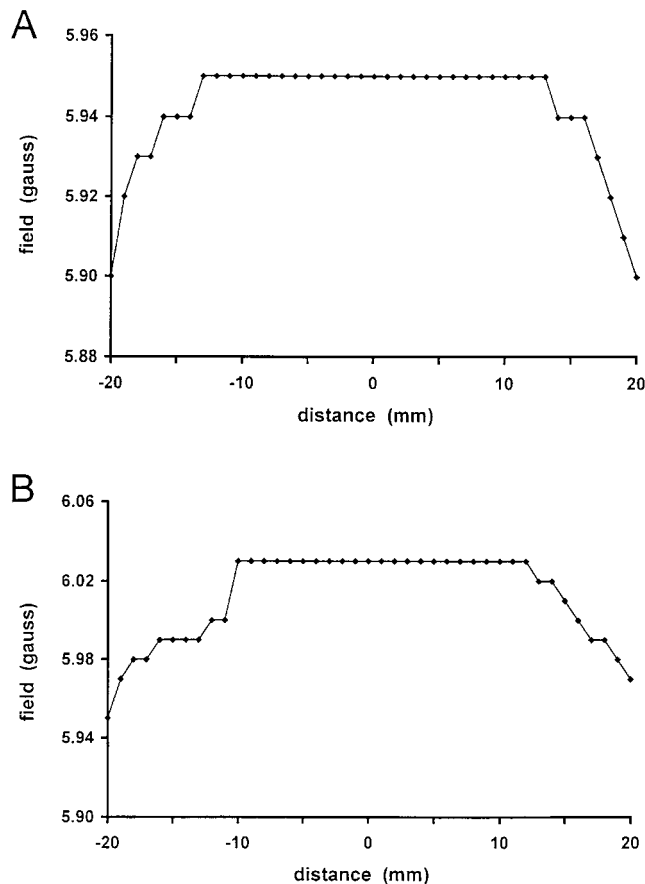


Fig. 5. Magnetic field profiles in the presence of a plastic objective. **(A)** The magnetic flux density (radially symmetric, scanned along the X axis at $Z = 0$) with a microscope objective in which metallic components have been remanufactured from polyimide (Dupont). **(B)** The fields were remeasured with a custom stage constructed from linen base phenolic and functional microscope (aluminum base with steel screws and fasteners, and epifluorescence light source) attached to the objective.

Figure 5 (B) shows a map of field intensities in the presence of a plastic objective, custom-built, nonmetallic stage and otherwise unmodified microscope base and illumination system. Although the majority of the microscope (Nikon, Diaphot) is constructed from aluminum, there are a number of steel components such as screws, fasteners, and collars. These were located sufficiently far away from the image plane in the center of the Helmholtz coils that no substantial differences in the profile of magnetic flux density were observed (Fig. 5 [A] vs. [B]). The slight change in the absolute value of the field was due to effects on flux density due to metallic components in the vicinity of the coils.

DISCUSSION

A great deal of attention is currently being given to the design of EMF exposure systems and to the complete

characterization of EMF exposure conditions [e.g., Valberg, 1995]. At the cellular level, investigators have pointed out the possibility of significant field distortions produced by commercially available microscope objectives [Liburdy, 1995; Lindström et al., 1995]; however, a detailed assessment of their effects has not been performed. Furthermore, no one has assessed the effectiveness of countermeasures to minimize field distortions produced by microscope-based imaging systems.

The present study demonstrates that most commercially available microscope objectives can produce significant distortion of EMFs, particularly at extremely low frequencies. In the presence of an objective, resultant fields are generally a spatially complex series of elevated and depressed flux densities in the field-of-view of the objective and in the surrounding region. Three common components that contribute to such field distortions are (1) the nickel-chrome plating of objective housings, (2) steel compression springs used to aid in focusing, and (3) screws or other fasteners used to hold separately manufactured parts together.

Table 1 summarizes many of the potential problems that might be encountered when using microscopes during EMF exposure studies. It also provides quantitative estimates of the maximal deviations that might be expected under these specific conditions. (1) As predicted by the Helmholtz equation, in the central plane between the coils, there is a decrease in field intensity with increasing distance away from the axial center. The magnitude of the decrease depends on the size of the coils compared with the size of the area under investigation. (This effect is not influenced by the presence of an objective or microscope.) (2) Away from the midplane between the coils, the decrease in field intensity is transformed to an increase with increasing distance away from the axial center of the coils. This might influence EMF exposures involving cell suspensions in deep dishes or in which dishes are stacked (whether in the presence of a microscope or not). (3) The nickel-chrome plating (in particular, the nickel component) on the surface of an objective generally produces a circular decrease in field intensity that extends over a region approximately the size of the diameter of the objective. (4) Some objectives with complex geometries near the nickel-chrome-coated tip (at $X = Y = Z = 0$, see Fig. 1 [A]) concentrate field lines to produce an increase in field intensity in the center of the field-of-view. Generally, the more complex the tip contour (in close proximity to the focal plane), the more complex the patterns of distortion in this region. (5) The effects of nickel-chrome plating on the side walls of an objective are dramatically increased if the objective is not precisely aligned with the coils in the exposure system. Tipping the objective increases the cross-sectional

TABLE 1. Measured Distortions Under Specific Conditions*

Measurement condition	Maximum deviation	See figure
1. No metallic objective, 35 mm diameter region	-0.4%	5(A)
2. No objective, 5 mm from mid-plane between coils	+1%	1(B)
3. Simple objective with nickel-chrome plating	-6%	2(A)
4. Simple objective with complex tip geometry	+2%	2(B)
5. 1° misalignment between coils and objective	+4% -9%	2(C)
6. Compression spring	+23%	3(A)
7. Complex objective (plating, spring, screw)	+4% -7%	3(B)
8. Complex objective in 60 Hz AC field	±2%	4(A)
9. Complex objective in earth's field	-87%	4(B)

* Maximum deviations in field intensities were measured relative to average flux densities in less disturbed regions around the distortion within the image plane. The sign indicates the direction of the deviation (positive indicating an increase in field intensity) compared with average values.

area for interaction with field lines (limited by the $\sim 4\text{-}\mu\text{m}$ thickness of the coating when not tipped). Unless specific steps are taken to align objectives with the exposure system, it is difficult to avoid the "hill and valley" profile illustrated in Figure 2 (C). The effect is increased with increased misalignment and might vary during an experiment due to seemingly inconsequential acts such as adding weight to the coil support apparatus. (6) An increase in field intensity is generated in the vicinity of the starting point of the wire (closest to the image plane) that makes up a spring. Because these terminations are generally at some distance from the optical path (i.e., axial center of the objective), distortions are usually radially asymmetric and gradients across the field-of-view are generated. Generally, spring orientation is not apparent in most objectives without disassembly and may change with the use of the objective over time. The degree of distortion is affected by the degree of compression (less compressed springs producing larger peaks of distortion). (7) Complex objectives can produce intensity profiles in which all regions of the image plane contain significant spatial field gradients. The influence of small steel screws or fasteners is greatly affected by their position (Z direction) relative to the image plane. The orientation of field distortions produced by these fasteners and springs can also be affected by seemingly inconsequential acts such as procedures to thread and tighten objectives into a microscope nose piece. (8) Time-varying (AC) field distortions at 60 Hz are reduced compared with DC effects due to the generation of induced currents which produce a magnetic field in opposition to the applied magnetic field (Lenz's law). (9) Extremely large spatial gradients in field intensity are generated if steps are not taken to shield the earth's magnetic field. These can be superimposed on exposure conditions during applied EMFs or generated by simply viewing cells in the absence of a shield enclosure.

Consequences of Field Distortions Produced by Microscope Objectives

Spatial distortion of magnetic fields generated by microscope objectives might generate significant biological effects or alter the apparent flux density where these effects occur. Most distortions generate spatial gradients in the electromagnetic field in the region of the microscope's field-of-view. According to Maxwell's equations, non-uniform spatial gradients generate induced currents that differ from those computed based on a constant magnetic field in a homogeneous conductive medium. Such field gradients coupled with inhomogeneous, anisotropic conductivities of cells or tissues under observation [Hart, 1996] can quickly generate extremely complex patterns for induced current flow. It has even been suggested [Ayrapetyan et al., 1994] that magnetic fields themselves can alter the electrical properties of physiological media, further complicating induced current patterns. The net result is that cells or tissue segments, even within a single field-of-view can be exposed to different exposure conditions.

Even during the use of an objective that generates minimal gradients in the field-of-view, the location of the objective relative to the center or sides of a dish may influence responses. In studies of growth patterns or cell motility, the locations of distortions might have a significant effect on directional spread. If cellular functions such as uptake or release are simultaneously monitored by sampling media, measurements might be influenced by cells throughout the dish exposed to a variety of field intensities. Optical magnification is often used during the preparation of cells and tissues. Unless steps are taken to attenuate the earth's field, significant flux densities and gradients in fields can be generated during these periods.

Steps to Reduce Field Distortions Produced by Imaging Systems

Small components that are magnetically permeable and well away from the region of the coils do not significantly alter the spatial uniformity of the field in the central region between the coils (see Fig. 5). Overall effects can be accounted for by simply measuring flux densities and establishing a (linear) correction factor that can be applied to the Helmholtz equation. Clearly, the microscope objective must be considered as the most significant potential source for "local" perturbations in the immediate vicinity of the image plane, with magnetically permeable components of the microscope stage a distant second. To reduce distortions produced by objectives, there is a balance between the degree of distortion generated, cost, and optical quality. The following provides a list of practical steps that can be considered to minimize distortions in microscope-based imaging systems.

In general, the more complex the construction of an objective (correction collars, compression sleeves, etc.) the more likely the presence of steel springs, screws, or studs. Based on simple visual inspection, it is often not possible to determine the alignment of springs and screws. Without disassembling an objective or relying on manufacturer's specifications, springs and large screws can be detected by the attraction of a strong permanent magnet. The smaller screws frequently used in higher magnification objectives are difficult to detect. Less complex objectives are often less costly, although optical quality may be compromised. An outer sleeve can be removed from some objectives without affecting optical performance. It is also possible to use a wire brush or lathe to remove nickel-chrome plating. Steel screws or studs can be replaced with ones made from nylon or epoxy resin.

In some cases, it may be sufficient to map field intensities generated by a metallic objective, perhaps modified as suggested above. Unfortunately, the wide range in styles of objectives make it necessary to map individual objectives whenever a new one is considered. In addition, in the case of objectives with asymmetrically located screws, springs, or studs, it is necessary to mark the orientation of the objective (and the microscope) to ensure that alignment is maintained to minimize variability.

A solution that we adopted to eliminate distortion was to disassemble a metallic objective and reconstruct it by using only plastic and glass components. A number of issues may need to be considered to apply this process to other objectives: (1) Many problems are avoided if an objective can be selected that does not contain steel springs or screws. (2) Precision machining is required for

some components, particularly those involved in spacing lenses. (3) Because components are sometimes glued in place, an appropriate solvent (e.g., xylene) might need to be selected to dissolve adhesives without harming lens coatings. (4) Lenses may be supported by beveled brass fixtures. If a brass support has no nickel-chrome plating, it can be re-used as is. Similarly, brass spacing rings between lenses can often be re-used without modification. (5) The reconstructed housing must be made of a material that is opaque, easily machined, and resistant to warping, particularly when heated by intense light sources. Polyimide (Dupont) is an example of a "plastic-like" material that meets these criteria. We have used a reconstructed lens in fluorescent imaging studies to monitor changes in intracellular calcium concentration in response to applied EMFs in freshly isolated chromaffin cells [Craviso et al., 1998]. Once focused, no degradation in optical quality was observed over the time course of experiments in these studies (typically lasting 30 min or more).

In summary, care must be taken during EMF-imaging studies to ensure that the apparatus used to image cellular function does not distort magnetic fields, whether applied or in the natural environment. Commercially available objectives can elevate or suppress the absolute magnitude of fields in regions within the field-of-view and, perhaps more importantly, produce spatial gradients in field intensity. Fortunately, a number of simple steps can be taken to minimize distortions produced by imaging systems.

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