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Inverse relationship between photon flux densities and nanotesla magnetic fields over cell aggregates: Quantitative evidence for energetic conservation

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ABSTRACT

The quantitative relationship between local changes in magnetic fields and photon emissions within ~2 mm of aggregates of 10^5 – 10^6 cells was explored experimentally. The vertical component of the earth's magnetic field as measured by different magnetometers was ~15 nT higher when plates of cells removed from incubation were measured compared to plates containing only medium. Additional experiments indicated an inverse relationship over the first ~45 min between changes in photon counts ($\sim 10^{-12}$ W·m⁻²) following removal from incubation and similar changes in magnetic field intensity. Calculations indicated that the energy within the aqueous volume containing the cells was equivalent for that associated with the flux densities of the magnetic fields and the photon emissions. For every approximately 1 nT increase in magnetic field intensity value there was a decrease of ~2 photons (equivalent of 10^{-18} J). These results complement correlation studies and suggest there may be a conservation of energy between expression as magnetic fields that are subtracted or added to the adjacent geomagnetic field and reciprocal changes in photon emissions when aggregates of cells within a specific volume of medium (water) adapt to new environments.

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A biophysical approach to the dynamics of aggregates of cells predicts that the magnitude of potential differences of the plasma membranes within the aqueous volume, the magnetic fields associated with these aggregated potentials and the bulk emissions of photons should be quantitatively related. Contemporary procedures in biomolecular science often involve 10^5 – 10^6 cells within a volume of ~5 cc. Over the decades, several groups of researchers have measured ultralow intensities of photons from cell or tissue preparations whose flux densities are in the order of 10^{-12} – 10^{-11} W·m⁻² [1–4].

Mouse melanoma cells, in particular, when removed from incubation and placed at room temperature exhibit marked increases in photon emissions as measured by photomultiplier tubes (PMTs) during the first hour [5]. The enhanced photon emissions continue for several hours with discrete shifts in the wavelengths as demonstrated by optical filters [6]. The calculated equivalent power (Watts) per cell was about 10^{-20} J·s⁻¹ which is the energy

associated with the force over the distance between the potassium ions that contribute to the membrane potential, the energy associated with base-stacking in RNA ribbons, and even the action potential of neurons [7]. We hypothesized that with such power densities the equivalent energies should be discernable by shifts in the proximal background of the geomagnetic magnetic field around these aggregate of cells as measured by magnetometers. Here we present evidence of quantitative convergence of the energy associated with changes in magnetic flux density and photon emissions from aggregates of mouse melanoma cells.

We reasoned that if the value for the bulk “energy” of aggregates of cells were known from photon counts, we should be able to calculate the associated magnetic field strength which should be reflected in direct measurements as additions or subtractions from the vertical component of the geomagnetic field that passes through the cross-sectional area of the dish. According to the classic association between magnetic field strength and energy:

$$B^2 = E \cdot 2\mu \cdot m^{-3}, \quad (1)$$

where B is the magnetic field strength, E is the energy, μ is the magnetic permeability constant ($4\pi \cdot 10^{-7}$ N·A⁻²) and m is the volume. Assuming the typical photon radiant flux density as measured by

Abbreviation: PMTs, photomultiplier tubes

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analogue and digital PMTs measured for these cells by Dotta et al. [6,8], i.e., $10^{-11} \text{ W}\cdot\text{m}^{-2}$, there should be $\sim 5 \cdot 10^{-16} \text{ J}\cdot\text{s}^{-1}$ from a plate of 10^5 – 10^6 melanoma cells within 5 cc ($5 \cdot 10^{-6} \text{ m}^3$). For this calculation we appreciate that the aperture for the digital PMT is 1 cm^2 compared to the area of the plate (28 cm^2) and hence only that portion of those cells would be directly measured. However the equivalent energy in magnetic field strength as measured by the relatively wider functional width of the magnetometer sensor when all of the cells within the volume are considered should be in the order of 15 nT. This is well within the range that can be reliably detected by flux-gate magnetometers which are sensitive to changes of 1 nT.

To test the validity of these calculations the changes in the ambient magnetic field strength within 1–2 mm from the bottom of standard plastic plates ($60 \times 15 \text{ mm}$) of melanoma cells that had achieved 90% confluence were measured at room temperature (21°C) for one hour starting about 15 min after removal from incubation (37°C) as described previously [5,6]. The passage number for the cells was between 15 and 20. The growth media was Dulbecco's Modified Essential Medium (DMEM, Hyclone, Logan UT) containing 10% fetal bovine serum, $100 \mu\text{g}$ per ml of streptomycin and 100 U of penicillin per ml (Invitrogen, Burlington, Ont). There were four replicates, each on separate days. A single plate of $\sim 10^6$ cells in 5 cc of medium was placed over the sensor marker of a SENSYS GmbH (FGM3D) fast frequency magnetometer (Germany, +49 (33631) 59650; www.sensy.de). For comparison single plates containing *only* the medium (no cells) were measured for the same duration (1 h). The sensor and the plates were placed in an acoustic chamber that was also a Faraday chamber (13 m^3) within which the intensity of the resultant geomagnetic field was reduced from 45,000 nT to about 20,000 nT.

The computer operating the measurement software and other components of the system were placed outside of the closed chamber. To ensure any effect was not related to sampling anomalies, the resultant magnetic field was measured at 450 and 3200 samples per second (2 of each for the cell and the medium only conditions; there was no difference in the results). After about 10 min when the field measurements had stabilized inside the chamber values were set automatically from the computer to zero so that only relative changes over time would be measured during the experiment. The results were very conspicuous as indicated by the means and standard deviations (Fig. 1) of the shifts that occurred in the total field during the subsequent 1 h when either the plates containing medium only or plates containing cells were measured. On average the magnetic field strength above the plate containing the cells was about 16 nT higher than the plates containing no cells [$F(1,6) = 15.86, p < .01$]. The presence or absence of the cells explained over 70% of the variance in the change in magnetic field strengths. This value is consistent with the predictions from the calculations.

We have shown over multiple experiments [5,6,8,9] that during the first hour after removal from incubation melanoma cells (and all cells, particularly cancer cells in general) display conspicuous increases in photon flux density ($10^{-12} \text{ W}\cdot\text{m}^{-2}$) compared to warmed, media only dishes. To ensure there was a temporally-coupled and dynamic relationship between the strength of the magnetic field and photon flux density directly associated with aggregates of these cells, plates were placed over the aperture of a Sens Tech LTD DM0090C photomultiplier unit (PMT). The spectral sensitivity was between 280 and 850 nm (peak around 400 nm). The dark counts for this PMT was ~ 10 per s. There were four replicates. The sensor for a second type of vector magnetometer (MEDA-FVM 400, still accurate to 1 nT variations) was then placed over the top (of the cover) of the plate of cells. Both sensors were maintained in a wooden black box covered with several layers of black thick terry cloth towels that was housed in a dull light

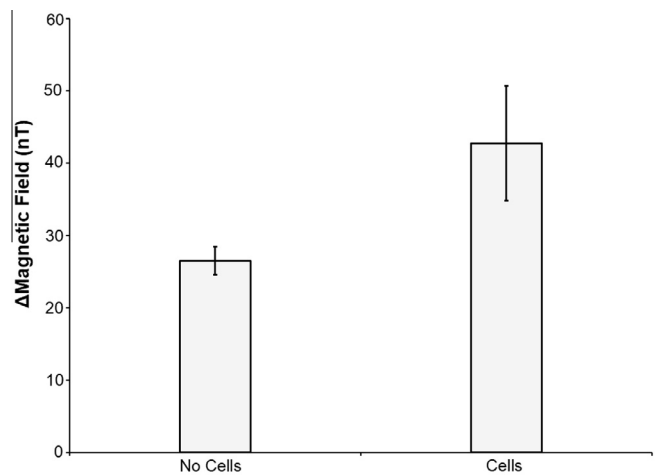


Fig. 1. Means and standard deviations for the changes in the surrounding magnetic field as measured by a fast frequency magnetometer for 60 min when either plates with medium only or plates with about 1 million cells were placed over the sensor.

environment within a basement laboratory (not the chamber used in the first part of the experiment) that was at least 20 m distance from any human movement in order to minimize variability in the magnetometer measurements.

Once the plate of cells had been placed (within 5–10 min of removal) from the incubator on the aperture of the PMT and the sensor for the magnetometer was placed upon the top of the dish containing the cells the software that controlled the magnetometer and PMT were activated. The mean numbers of photon counts per second for the dishes containing no cells (medium only) from other experiments using this PMT and the same procedure during this period and the dishes containing cells for the present analyses are shown in Fig. 2. The plates containing the cells displayed significantly [$F(1,7) = 41.60, p < .001; \eta^2 = 87\%$] more photon counts than the plates containing no cells. The data were sampled from both the PMT and magnetometer at 1 s intervals for about 3 h. Increases in photon counts were relative to reference conditions (empty dishes or dishes with only media). The major increase occurred during the first hour particularly during the first few minutes which comparable to our previous experiments that employed older, non-digital PMTs.

The data from the X, Y, and Z magnetic field components and the numbers of photon counts per s from the PMT were loaded into SPSS PC (16) for analyses. Because of the movement artifacts of the experimenters the data were analyzed starting 10 min after the experiments began (to allow time for the experimenters to leave) and emphasized the subsequent 30 min. Hence the real-time end of this component of the experiments was about 1 h after removal from the incubators. Graphic data indicate this was the duration of the largest change in photon counts and magnetic field strength. Both the magnetometer and the PMT had been operating continually for several weeks (for other experiments). Consequently potential drifts due to stabilization latencies for the equipment were unlikely.

The average increase in magnetic field (vertical) intensity over the plates of cells for the four experiments from the beginning to the end of the 30 min period was 15 nT (range = 9–23 nT) which is within 1 nT and not significantly different (considering the range) from that recorded by a different (fast frequency magnetometer) when cells were measured in the acoustic chamber in a different room during the first block of experiments. The range in mean photon counts s^{-1} for the four runs was 25 ($SD = 6$) for the experiment with lowest counts and 54 ($SD = 25$) for the experiment with the highest counts. This approximately factor 2

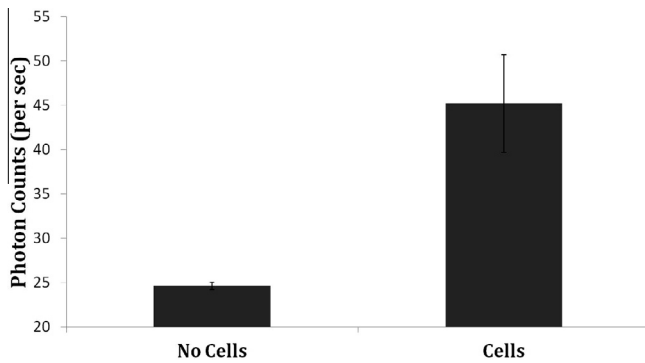


Fig. 2. Photon counts per second from all dishes containing either no cells (medium only) or cells. Vertical lines indicate standard deviations.

difference is not unusual for different batches of melanoma cells. Assuming the peak sensitivity of the PMT, 400 nm, and hence each photon displaying an energy of $\sim 5 \cdot 10^{-19}$ J, the mean values would have ranged from 1.3 to $2.7 \cdot 10^{-17}$ J·s⁻¹. Considering the area of the plate containing the plate relative to the area of the aperture of the digital PMT, the energy for all of the 10^5 – 10^6 cells would be between 3.8 and $7.6 \cdot 10^{-16}$ J·s⁻¹. This is within the same order of magnitude calculated from Eq. (1). The power density would be in the order of 10^{-12} W·m⁻² which is similar (when the total surface area and distance are considered) to that measured at aperture-cell plate by analogue [5] and digital [6] PMTs for these cells.

Regression analyses for the magnitudes of the photon counts per s and the magnetometer measurements per s consistently demonstrated an inverse relationship between the increased magnetic field intensity, particular within the vertical component (the strongest component, i.e., ranged between 58,450 and 58,939 nT for the different experiments) and the diminishment of photon flux densities. An example of this relationship is shown in Figs. 3 and 4. The rapid attenuation of photon flux density is typical of what we have found when these cells were measured by analogue and digital PMTs.

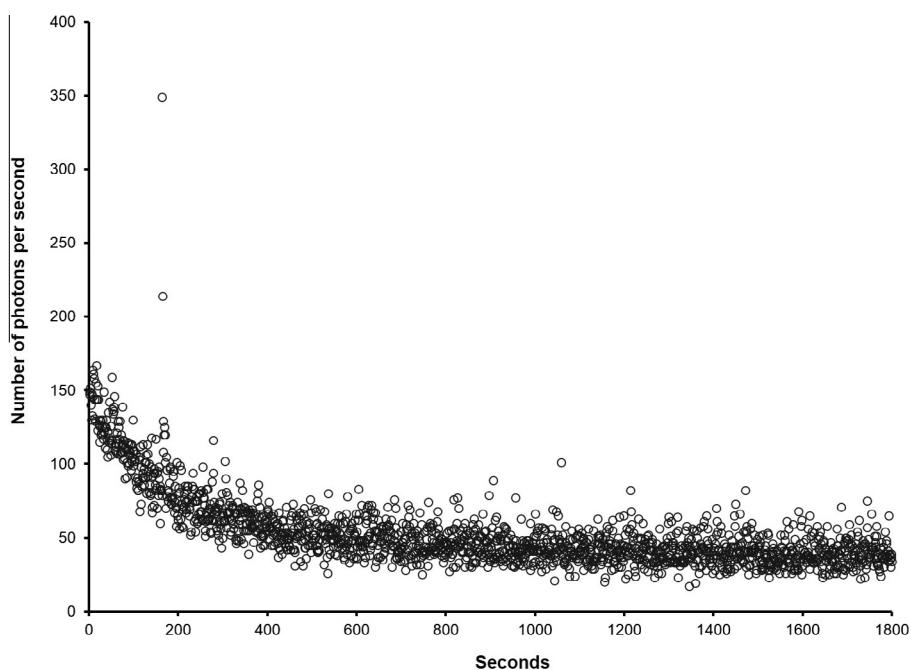


Fig. 3. Example of diminishment of photon counts for 30 min about 15 min after removal from incubation.

The average correlation for the 30 min interval between magnetic field intensity for the vertical component and photon counts was $r = -0.45$ (range = -0.12 to -0.62). Because the degrees of freedom (11,799) all of the correlations were strongly significant statistically. The average slope was -2.1 (range -1.6 to -5.1) which means that for every 1 nT increase in the strength of the field near the cells there was a decrease of 2.1 photon counts. Additional regression analysis where time (s) was the predictor indicated that between 33 and 200 s were required for the decrease of 1 photon and between 250 and 300 s were required for an increase of 1 nT.

To ensure there were no obvious artifacts of the measurement procedure correlations between the second-to-second magnetic field strength and photon counts were completed for the subsequent ~ 2 h when photon emissions have usually entered the asymptote stage. The absolute value (because there were both positive and negative values) of the average correlation was $r = 0.08$ (range 0.02–.22). The average slope was 0.17 (-0.04 to 0.66). Consequently there was no consistent or strong association between the photon counts and the changes in magnetic field strength during this period. This effect when combined with the early rapid decline in photon flux density but increased magnetic field intensities over about 2.7 h are shown in Figs. 5 and 6.

If the inverse correlation between the change in photon counts and change in local magnetic field intensities attributed to the presence of the cells were sharing the same source of variance, then standardized scores for each would be expected to exhibit similar vectors but in opposite direction. This was confirmed for each of the four trials and was most conspicuous in one of them. As seen in Fig. 7 the within measurement z-scores for photon and magnetic field changes over time were comparable and the time courses are mirror images of the other. The mutual inflections of the flux density curves in Fig. 7 would have occurred about 15 min after removal from incubation. In each of the other experiments similar shifts involving similar magnitudes occurred relatively quickly. In one experiment the sudden reciprocal shift occurred within about 2 min.

The inverse relationship between the power or flux density for proximal changes in the earth's magnetic field and photon emissions from living tissue has been measured several times by our research group [10,11]. Simultaneous measurements of photon emissions and the local magnetic field near the right hemisphere of people sitting in hyperdark conditions have shown an inverse correlation between the two energies quite reliably, even for special cases [12]. In those studies for every 1 nT increase in the measured magnetic field there was $\sim 1 \cdot 10^{-12} \text{ W} \cdot \text{m}^{-2}$ decrease in photon

emissions. Direct measurements of the power of the photon flux density and the geomagnetic change as a function of distance from the head indicate a conservation of energy.

The significance of the 1 nT increase in the geomagnetic field adjacent to the cells (and presumably produced by the aggregate) for every decrease in ~ 2 photons (or $\sim 10^{-18} \text{ J}$) could be revealing for the boundaries within which the convergence of energies was occurring. According to Eq. (1), a change of 1 nT within a single melanoma cell with a volume of $\sim 10^{-15} \text{ m}^3$

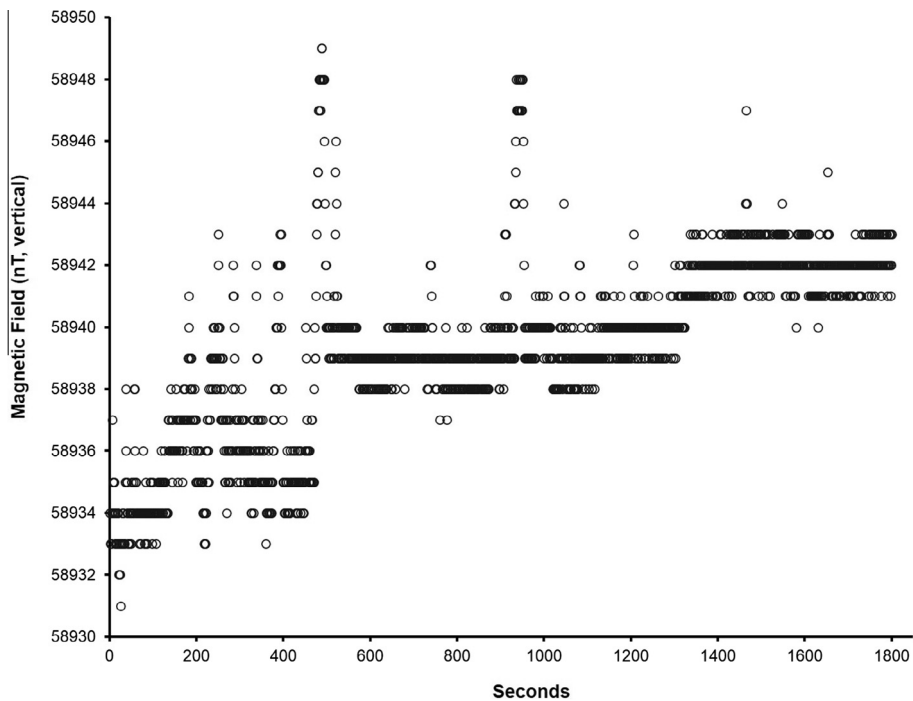


Fig. 4. Example of the increased intensity for the vertical component of the earth's magnetic field within 2 mm of the cells during the same period as Fig. 2.

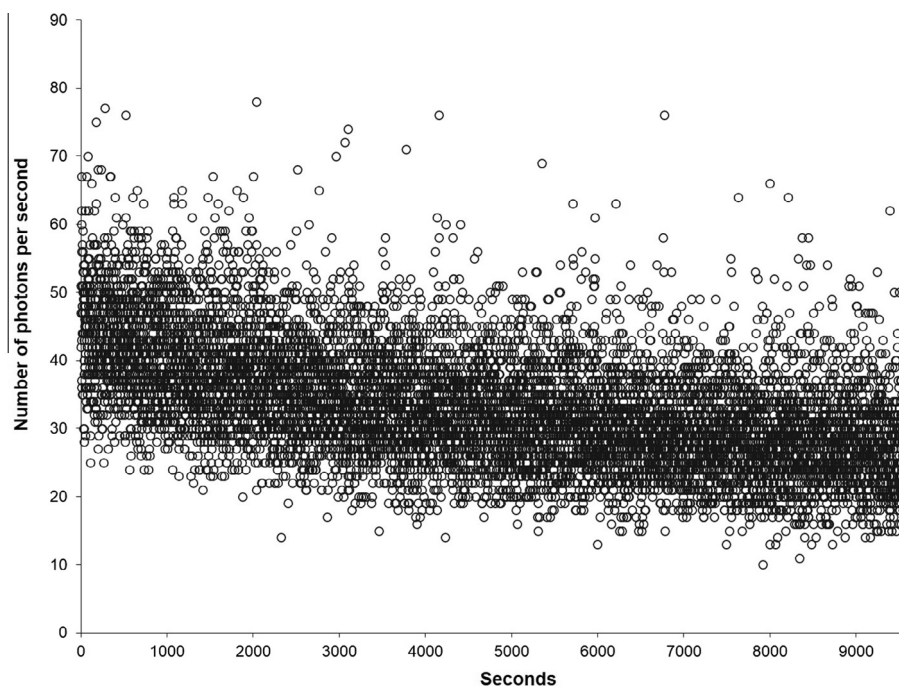


Fig. 5. Example of the numbers of photons per second measured over an aggregate of melanoma cells (about 1 million) over a period of 10,000 s (about 3 h).

would be associated with $0.4 \cdot 10^{-27}$ J and for 10^5 – 10^6 cells in the aggregate, $0.4 \cdot 10^{-22}$ – 10^{-21} J. Even if energy were summated over time required for the increase in 1 nT, which the slopes indicated to be about 200 s, the “magnetic energy” within each cell would only be about 10^{-26} – 10^{-25} J and the aggregate would be $\sim 10^{-20}$ – 10^{-19} J.

To obtain the equivalent relationship between the magnetic energy associated with an increase in 1 nT and a decrease of photon energy of $\sim 10^{-18}$ J, according to Eq. (1), the volume would be about 2 cc. This is the volume of media within which the cells were contained. This suggests an interesting possibility that has been developed by Pollack and his colleagues [13,14] and by Del

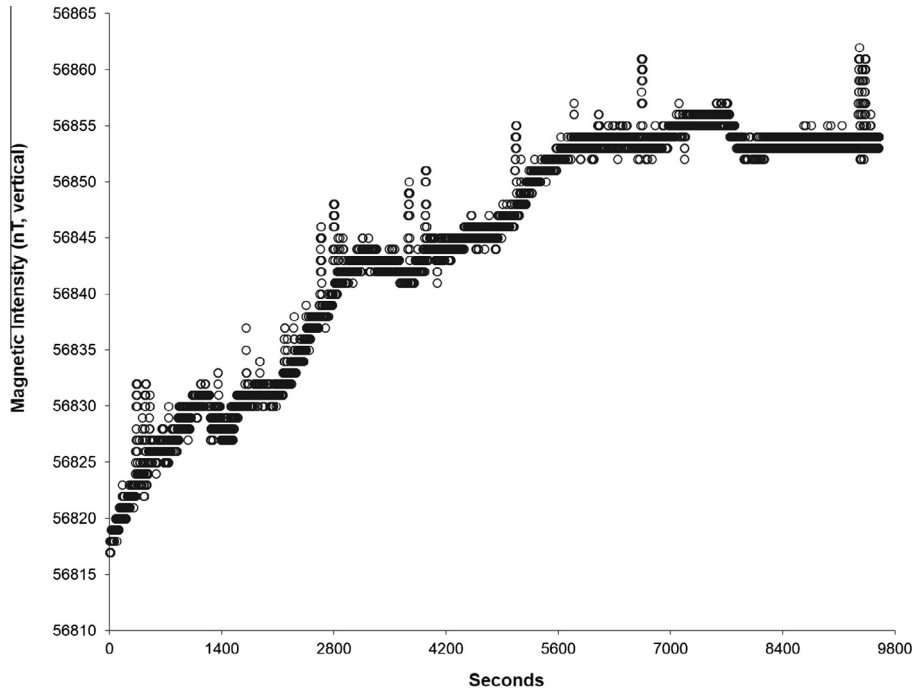


Fig. 6. Example of the change in intensity of the vertical component of the earth’s magnetic field over the same aggregate of cells noted in Fig. 4 during the same duration the photons counts were being measured.



Fig. 7. Z-scores of distributions for a single experiment (run) over the 30 min interval (about 15–20 min after removal from incubator and 10 min after activation of measurements) for photon emissions (green) and the vertical magnetic field strength (blue) measured simultaneously near an aggregate of about 1 million melanoma cells.

Giudice and Preparata [15] that interfacial water near surfaces, such as cell membranes, have the capacity to display coherent domains within which magnetic fields and photons can interface. The calculation of the energy associated with a magnetic field does not involve a temporal component. If the temporal slope (200 s) is relevant then the “cumulative” energy would be $\sim 2 \cdot 10^{-16}$ J. If “stored” within the intricate structure of water the release of this power per s over the 10^{-4} m² aperture of the PMT would be about 10^{-12} W·m⁻².

Most cancer cells display membrane potentials that are hypopolarized compared to normal cells. Persinger and Lafrenie [16], who averaged the data collected by Levin [17], showed that malignant cells as a population display resting membrane potentials that approach ~ 26 mV, the constant within the Nernst equation independent of intracellular–extracellular ratios of ions. We did not measure the plasma membrane potentials in the present experiments. However if one assumes the estimated average resting membrane potential for a melanoma cells to be about -35 mV, the equivalent energy by multiplying by the unit charge ($1.6 \cdot 10^{-19}$ A·s) would be $5.6 \cdot 10^{-21}$ J per cell or $\sim 5.6 \cdot 10^{-16}$ J for an aggregate of 10^5 cells. This value is within the order of magnitude for photon energy measured directly from aggregates of these cells. The equivalent magnetic field strength for this energy within 1 cc would be ~ 15 nT.

There have been several correlational studies involving bacteria that indicated associations between photon emissions and geomagnetic activity. However they involved relatively wide temporal intervals [18]. Recently we [9] found that injections of morphine, a compound that produces analgesia but can also encourage metastases, into plates of melanoma cells was associated with a conspicuous increase in bursts of photons for several hours. If photons emissions are correlated with inter-cell communication such large bursts could be significant and should be associated with compensatory diminishment of proximal magnetic field intensities. As aptly reiterated by Cifra [19], traditional applications of “kT boundary” arguments for cell systems may not be completely applicable and distant interactions between mammalian cells can occur through intrinsic electromagnetic coupling.

Although there is an accumulating scientific literature indicating the persistent display of ultraweak photons or biophotons from cell preparations, bacteria, and entire organisms, the transformation of these energies from and to other forms has not been examined experimentally. Helmholtz’ principle states that energy is neither created nor destroyed but only changes form. If it is applicable here then the significance of a local equilibrium of energy as either photon flux density or magnetic fields, such that as one increases the other decreases, may reveal additional processes by which cells interact with each other and the environment. They could be potential mechanisms to help explain results that suggest non-local effects in populations of cells that can be induced with specifically patterned, shared magnetic fields [20].

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