Use of Fractal Dimension for the Analysis of Biological Effects of Electromagnetic Fields on the Moss P. Patens and the Nematode C. Elegans

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Abstract – The paper describes the use of Fractal dimension to analyze the biological effects of electric and magnetic fields on two well-known organisms, namely the moss *Physcomitrella patens*, and the nematode *Caenorhabditis elegans*. The two model organisms have been illuminated by low frequency magnetic fields ($16^{2/3}$, 50, and 400 Hz) using Helmoltz coils as well as by high frequency electromagnetic field (900 MHz) using TEM cells. The obtained results are compared with statistical data and it is shown that fractal dimension is a powerful tool to indicate variations in the moss growth and the nematode mobility resulting from the application of an electromagnetic field.

Summary

1. Introduction

No clear conclusion about possible effects of electromagnetic fields on health could be clearly put into evidence by epidemiological studies or in vitro experiments on tissues or cells. Recently, the Food and Drug Administration backed by the CTIA (Cellular Telephone Industry Association) emphasized the need to work on simple animal, plants and cellular systems [1]. The two biological organisms chosen for this research study are well suited in this respect: *Physcomitrella patens* is a moss whose development is very well known in biology and therefore is the object of several programs of functional genomic (e.g. [2,3]). On the other hand, the *Caenorhabditis elegans* is an extensively studied organism; it has been and is still the most well known chordate as far as genetics, developmental and behavioural biology are concerned. *Caenorhabditis elegans* has been the object of a recent study on non-thermal effects of microwaves [4].
Preliminary results on the effect of 50 Hz magnetic fields on *Physcomitrella patens* have been published in the last EMC Europe Symposium [5].
In this paper, we propose and validate a method based on fractal dimension to quantify the effect of electromagnetic field on the growth of *Physcomitrella patens* and on the mobility of *Caenorhabditis elegans*.

2. Cultivation Conditions and Field Exposure

For both organisms (*Physcomitrella patens* and *Caenorhabditis elegans*), already published basic cultivation conditions are used [6,7]. They have been exposed to the experimental conditions on agar medium in 5 cm plastic Petri dishes.

3. Exposure of *Physcomitrella patens* to Low Frequency Magnetic Fields

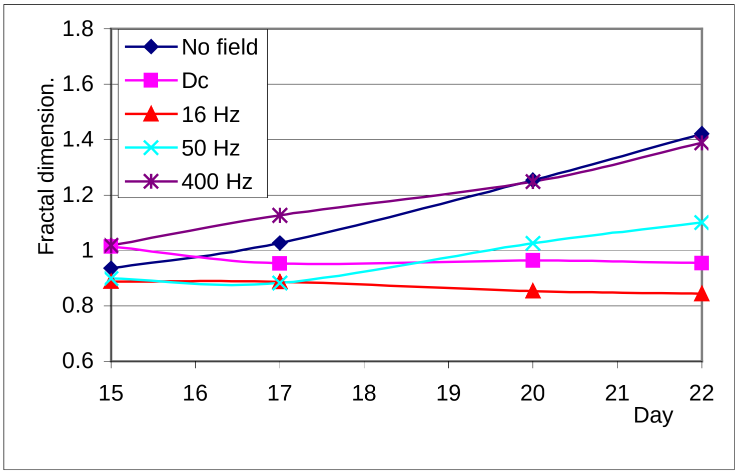
The behaviour of *Physcomitrella patens* is investigated under low frequency magnetic field exposure (DC, $16^{2/3}$, 50 Hz). A set of 5 Helmholtz coils has been installed in a shielded room in order to maintain the samples in controlled environment from the point of view of the magnetic field. Samples of moss in Petri dishes were arranged in the working volume where the same conditions of light and temperature as in the plant biology laboratory have been created. Samples of *Physcomitrella patens* grown from spores were exposed to the magnetic fields produced by the Helmholtz coils during different periods of time up to 4 weeks. The influence of field exposition on different parameters has been analyzed. Table 1 shows the average number of cells measured after exposure to low frequency magnetic fields of different frequencies (DC, $16^{2/3}$ and 50 Hz). These results suggest a certain effect on the moss development, for magnitudes of 1 mT (a value 10 times as large as the ICNIRP). More interestingly, the moss development appears to be even more negatively affected at $16^{2/3}$ Hz compared to 50 Hz. This result is in clear contradiction with the exposure limits set in the ICNIRP guidelines [8], which at the above-mentioned low frequencies decay as $1/f$.
In parallel, a morphological analysis of the moss is performed using fractal theory. The petri dishes were photographed using a digital camera (Leica DC 200) and a microscope (Diavert, Leitz). The digitized images are transferred to a PC and after some pre-processing, their fractal dimensions are computed using the box-counting technique [9]. Figs.1 show the evolution of fractal dimension for different exposures.
The interpretation using the fractal dimension is based on the following considerations. The fractal dimension of a point is zero, of a straight line 1 and of a surface 2. This means that the fractal dimension of an object with a complicated shape or with a larger surface will be close to 2, while non developed spores will tend to keep the shape of a point with a fractal dimension lower than 1 or so. It can clearly be seen that for the control sample and for the one exposed to a 400 Hz field the fractal dimension is increasing for a longer exposure. The samples exposed to DC have a dimension close to 1, while the ones exposed to a $16^{2/3}$ Hz field see their dimension

decreasing down to values lower than 1. This means that these spores die before achieving a high degree of development.
The results of Fig. 1 are in agreement with statistical results presented in Table 1.

Table 1 – Relative average number of cells per moss colony (from spore) after 3 weeks of cultivation.

Experiment #	Control (no field)	1 mT, DC	1 mT, $16^{2/3}$Hz	1 mT/50Hz	1.2 mT, 50Hz	1 mT, 400Hz
1	100%	82%	87%	93%	-	107%
2	100%	77%	91%	82%	-	99%
3	100%	98%	78%	138%	-	167%
4	100%	84%	75%	114%	-	131%
5	100%	44%	56%	58%	-	108%
6	100%	74%	74%	97%	-	119%
7	100%	80%	66%	112%	-	141%
8	100%	99%	65%	75%	77%	119%
9	100%	72%	81%	68%	69%	122%
10	100%	106%	75%	74%	75%	116%
11	100%	120%	81%	89%	80%	132%

No influence | Delayed growth | Accelerated growth



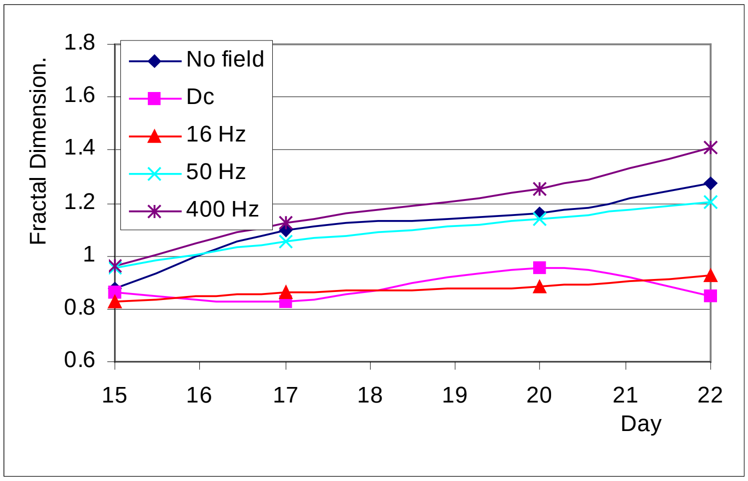


Fig. 1 – Temporal variation of Fractal dimension of moss colonies as a function of field exposure.

4. Exposure of *Caenorhabditis elegans* to High Frequency Electromagnetic Fields

Other preliminary tests were conducted on *Caenorhabditis elegans* exposed to a 900 MHz field produced by a TEM cell in order to test the use of fractal dimension for the analysis of the mobility of the nematodes. After 2 to 3 days of electromagnetic field exposures, the samples were extracted from the TEM cell and photos of the nematode movement were taken after 1, 2 and 3 minutes. Fig. 2 shows the trajectory of nematodes in the petri dish. The graph in Fig. 3 shows the evolution of the fractal dimension for different moments when photographs were taken. Higher fractal values indicate a more complicated trajectory of the nematode's movement. It can be seen that the exposed nematodes seem to be more excited than those who were not exposed to any field.
For the moment these are only preliminary results but they show that the fractal dimension can be successfully used for an evaluation of the influence of the electromagnetic field on the behaviour of the *Caenorhabditis elegans.*

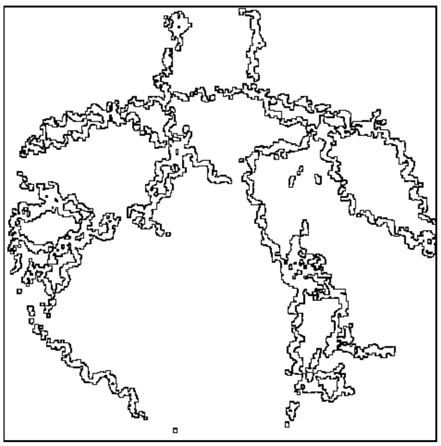


Fig. 2 - Trajectory of nematodes in the petri dish.

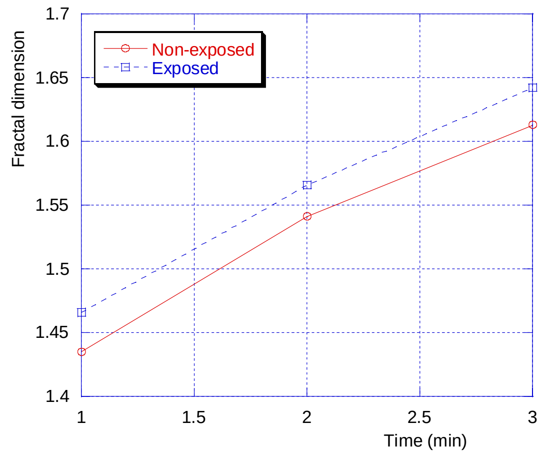


Fig. 3 – Time-variation of fractal dimension associated with the trajectory of nematodes.

5.Conclusion

The results presented in this paper shows that an analysis based on fractal dimension is a powerful tool to indicate variations in the moss *Physcomitrella patens* growth and in the nematode *Caenorhabditis elegans* mobility resulting from the application of an electromagnetic field. The use of fractals provides objective criteria for evaluating these effects.
As the behaviour of living organisms is a complex issue, more experiments are needed before a conclusion on biological or possible health effects can be drawn.

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