FRACTAL DIMENSION: A METHOD FOR THE ANALYSIS OF THE BIOLOGICAL EFFECTS OF ELECTROMAGNETIC FIELD

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Abstract. The paper describes the use of fractal dimension to analyse the biological effect of electromagnetic fields on the mobility of the nematode *Caenorhabditis elegans.* Nematodes have been exposed to high frequency field (900 MHz) using TEM cells. Preliminary results show that fractal dimension is a powerful tool to measure variations in the nematode mobility resulting from the exposure to an electromagnetic field.

Introduction

The possible effects of low or high frequncy electromagnetic fields on living beings are highly debated; nevertheless no clear conclusion can be drawn from epidemiological studies or in vitro experiments on tissues and cells. Recently, the Food and Drug Administration backed by the CTIA (Cellular Telephone Industry Association) emphasized the need to work on simple animals, plants and cellular systems [1]. We decided to choose the nematode *Caenorhabditis elegans* as biological test organism for our research study on the EMF effects on biological systems.

Caenorhabditis elegans has been and is still the most well known chordate as far as developmental, behavioral and genetics biology are concerned. The genome of this small organism (growing to about 1 mm in length) has been completely sequenced; all 959 somatic cells of its transparent body are visible with a microscope, and its average life span is a mere 2-3 weeks. *C. elegans* is easily handled and maintained in the laboratory, thus it provides the researcher with the ideal compromise between complexity and tractability.

Fractal dimension as a measurement system for *C. elegans* mobility

When studying stressor effects on a biological organism, one of the problems is to find easy tools, parameters and measure methodologies with scientific validity. In biology there are several techniques of measure giving numeric values. *C. elegans* has been the object of recent studies on non-thermal effects of microwaves, based on the analysis of easy detectable reporter products, synthesized in stress conditions [2,3,4,5].

In developmental and behavioral biology it is often difficult to obtain quantifiable measures of critical parameters. In our work we have use a semi-automatic data acquisition method in order to minimize human errors due to the operator subjectivity, furthermore the use of fractal mathematic gives us the possibility to "quantify" the behavior of the subject organism.

As evaluation parameter of the biological effects of EMF exposure we measure the mobility of the nematode, supposingly related to the organism vitality. A fractal model is used to analyze the time evolution of nematodes traces complexity.

Cultivation conditions and field exposure

Nematodes are cultivated at 25°C on "Nematode Growth Medium" in 5 cm plastic Petri dishes. The medium is seeded with the OP50 *E. coli* strain as a food source. Already published basic cultivation conditions are used [6].

Nematodes are exposed to a high frequency electromagnetic field (900 MHz) produced by a TEM cell (Type TEM 1000) at 100 V/m field intensity. The cell is connected with a 50 Ω matching impedance.

Nematodes used as controls are put inside another identical TEM cell without electromagnetic field. Both TEM cells are located in an incubator (accurate to ±0,1°C). The incubator allows us to make experiences at different temperatures and at the same time guaranties temperature stability during the experience.

Images acquisition

After the exposure, several samples are extracted from each Petri dish and transferred on new agar medium. Nematodes start moving on the intact surface, furrowing the substrate and so letting traces of their passage. *C. elegans* moves by means of four longitudinal bands of muscle paired sub-dorsally and sub-ventrally. Alternative flexing and relaxation generates dorsal-ventral waves along the body, propelling the animal along. The sinusoidal trace resulting presents a fractal characteristic. Photos of the nematode movement are taken every 2,5 minutes for 10 minutes. The complexity of the traces image on the medium surface is related to the speed at which the nematode is moving.

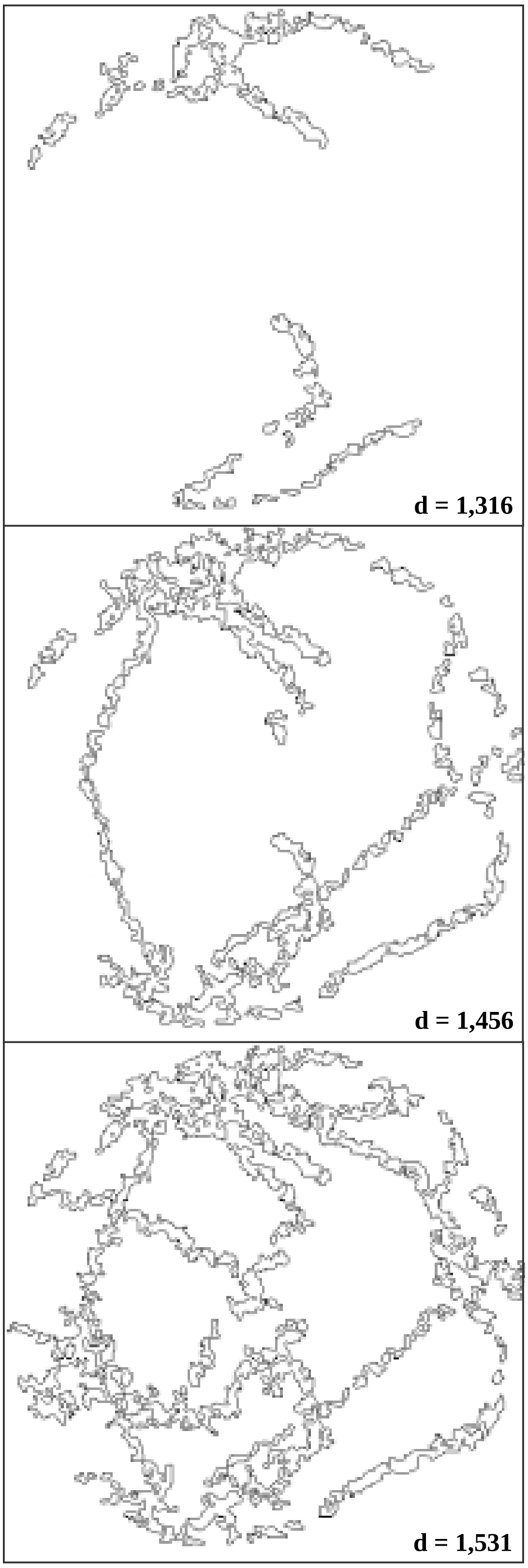

Fig.1 Nematodes trajectories: the elapsed time between two successive pictures is 2,5 minutes; each sample is constituted of 5 worms. The fractal dimension (d) of the first image is 1,316; it changes to 1,456 in the second image and finally rises up to 1,531 in the last image.

Traces pictures are obtained through with a digital camera (JVC GC-X3) connected to a microscope, using always the same magnification.

Images treatment and fractal dimension calculation

Each picture is submitted at local light equalization that increases the contrast, evidencing the traces. The first image of the series is subtracted from each successive image, in order to study the traces image complexity evolution in time, referred to a "zero time". The subtraction procedure allows also the removal of most of the image-noise. Images are converted to binary black and white, for the calculation of fractal dimension. Bidimensional images have a fractal dimension between 0 and 2; higher fractal values indicate a more complex trajectory of the nematode movement.

Fig. 1 shows the evolution in the time of nematodes trajectories.

Conclusions

Preliminary results based on three independents experiments indicate that the fractal dimension of traces is a good quantitative indicator of *Caenorhabditis elegans* mobility.

Fig. 2 shows the evolution of the fractal dimension with time, in the experiment realized on 7th may 2003. During the first 5 minutes of measurement the fractal dimension value increases in the same way for both exposed and control nematodes, then non exposed nematodes continue moving constantly, while exposed nematodes will slow down. We cannot preclude a possible heat effect dues to the high voltage used.

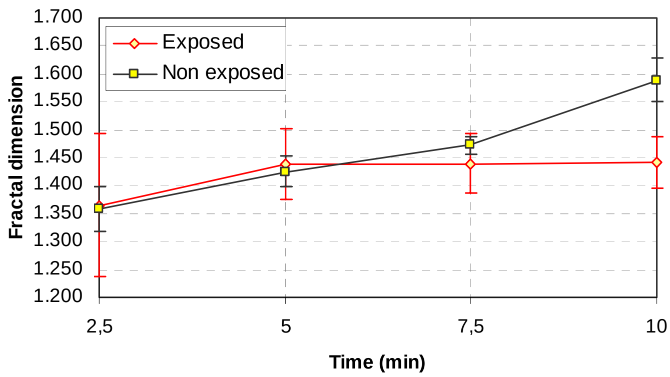

Fig.2 Time-variation of fractal dimension associated with the trajectory of nematodes.

The fractal dimension values in the graphic are the average of three values representing three different samples, coming from the same Petri dish. Each sample is constituted of 5 nematodes. The experience has been conducted at 100 V/m field intensity, for two days at 25°C.

Table 1 shows fractal dimension values of the reported experience.

	Fractal dimension	
t [min]	**Exposed**	**Non exposed**
2,5	1,365 ± 0,128	1,359 ± 0,040
5,0	1,439 ± 0,063	1,425 ± 0,028
7,5	1,439 ± 0,053	1,473 ± 0,016
10,0	1,442 ± 0,045	1,588 ± 0,039

Table 1. Fractal dimension values after two days of exposure at 900 MHz, 100 V/m and 25°C.

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