Do Complex Cell Structures Share a Fractal-like Organization ?

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Abstract : The extension of the concepts of the Fractal Geometry [Mandelbrot 1983] toward the life sciences has brought a significant progress in understanding complex functional properties and morphological features that characterise cells and tissues. It has even be argued that fractal geometry might provide a coherent description of the design principle underlying living organisms. Fractals fulfil a certain number of theoretic and methodological criteria including an high level of organization, shape irregularity, functional and morphological auto-similarity, scale invariance, iterative pathways and a non-integer peculiar fractal dimension. Through selected examples we will show that cell membranes and organelles at different functional states are fractals according to the above mentioned criteria and that the application of the fractal principle is much valuable for measuring various dimensional parameters of these complex irregular structures.

Introduction

The extension of the concepts of the Fractal Geometry [Mandelbrot, 1983] toward the life sciences has brought a significant progress in understanding complex functional properties and morphological features that characterise cells and tissues. It has even be argued that fractal geometry might provide a coherent description of the design principle underlying living organisms [Weibel, 1991].

Fractal Criteria

Fractals fulfil a certain number of theoretic and methodological criteria including an high level of organization, shape irregularity, functional and morphological auto-similarity, scale invariance, iterative pathways and a peculiar non-integer fractal dimension [FD]. Whereas mathematical fractals are invariant over an unlimited range of scales, biological components are statistically self-similar only within a fractal domain or <scaling window> with upper and lower scaling limits spreading at least two orders of magnitude, which must be experimentally established. A straight line can be drawn on a log-log plot and its slope (1-D) used to evaluate the FD according to the logarithmic equation < $\log L(\varepsilon) = (1\text{-}D) \log (\varepsilon)$ >, which describes a power law (Fig 1) [Losa and Nonnenmacher, 1996].

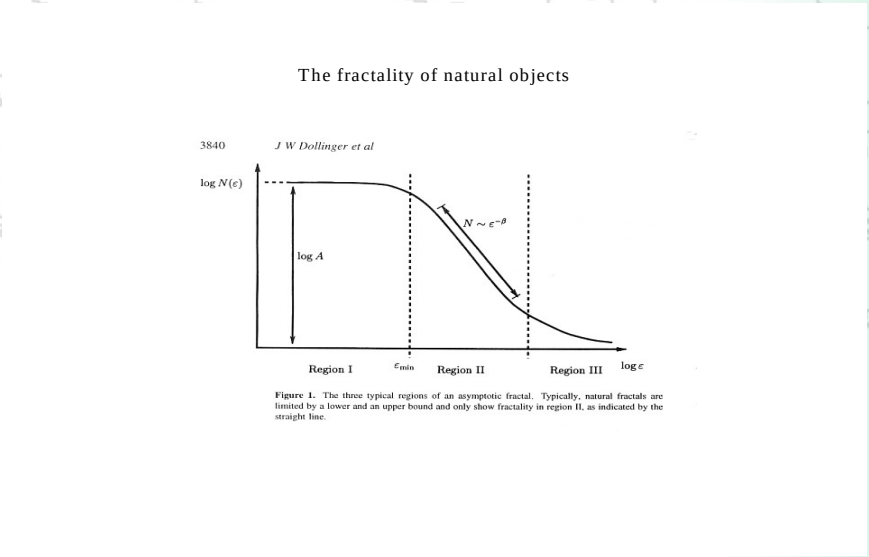


Figure 1:

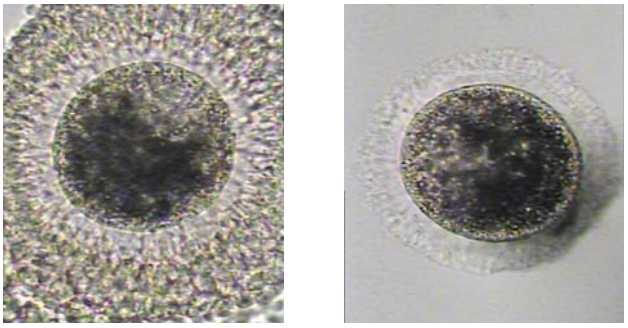
Cell membranes and organelles of tissues at different functional or pathological states, as well as extracellular elements are fractals according to the above-mentioned criteria. Hence, the application of the fractal principle is much valuable for measuring various dimensional parameters of complex irregular biological structures.

Results

Distinct cytoplasm features revealed in feline oocytes with or without the cumulus oophorus (CO), which has been shown to affect their developmental potential, were found to be self-similar elements with a fractal organization. However, the fractal dimension values of the respective oocyte elements were close and not influenced either by the presence or absence of CO (Fig 2) [De Vico et al, 2005].

Figure 2

Oocyte with and without Cumulus Oophorus [COC]



The fractal morphometry has been employed to analyse electron microscopic images of pericellular membranes, revealing higher FDs in healthy immune competent B (1.20) or CD4-T, CD8-T lymphocytes (1.17-1.23) than in lymphoblasts isolated from humans with acute lymphoid leukaemia (T-ALL), these cancer cells being characterized by a plasma membrane nearly smooth (1.10) (Fig 3a, 3b: table 1).

Figure 3a

CD-8 T-cell

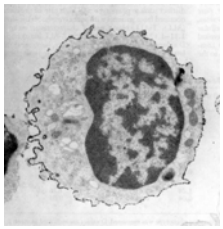
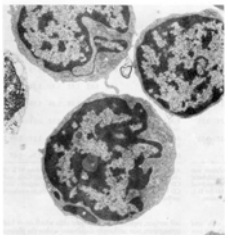


Figure 3b

T-ALL



Cells from hairy-cell leukaemia with highly convoluted morphology displayed the highest FD comprised between 1.32-1.36, as reported in table 1 [Losa et al, 1992].

Table 1

Fractal dimension of lymphocytes and leukemic cells

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Table 1. Fractal dimensions (five moments) of pericellular membranes from resting and lectin activated lymphocytes

Cells	Fractal dimensions D 1	D 2	D 3	D 4	D 5	SD ±
T-lymphocytes (n.d)	1.19	1.20	1.21	1.21	1.22	0.04
T-lymphocytes (CD 8+)	1.22	1.23	1.23	1.23	1.23	0.04
T-lymphocytes (CD 4+)	1.17	1.16	1.16	1.16	1.17	0.04
PBMN (CD 3+)	1.20	1.20	1.20	1.20	1.21	0.05
PHA-stimulated PBMN (n.d.)	1.11	1.11	1.12	1.12	1.12	0.03

The profile of twenty elements for each cell population was measured. PHA: phytohemagglutinin. PBMN: human peripheral blood mononuclear cells. n.d.: not decorated with monoclonal antibodies. D values are given as the mean ± 1 SD. SD for D 1, D 2, D 3, D 4, D 5 were similar for a given cell population.

Table 2. Fractal dimensions (five moments) of pericellular membranes from lymphoblastic leukemia

Leukemia	Fractal dimensions D 1	D 2	D 3	D 4	D 5	SD ±
T-ALL (n.d.)	1.10	1.11	1.11	1.11	1.11	0.03
T-ALL (CD 2+)	1.12	1.12	1.12	1.12	1.12	0.02
B-ALL (early) (n.d.)	1.14	1.14	1.15	1.16	1.18	0.02
B-ALL (n.d.)	1.13	1.13	1.13	1.12	1.12	0.03
B-ALL (CD 19+)	1.19	1.19	1.19	1.19	1.19	0.03
HAIRY	1.32	1.33	1.34	1.35	1.36	0.03

ALL = Acute lymphoblastic leukemia with more than 95% of circulating blasts. Early B-ALL blasts were found Calla and CD 19 positive. B-ALL blasts were found Calla negative and CD 19, CD 20 positive. n.d.: not decorated with monoclonal antibodies unless indicated (CD+).

This type of methodological approach has enabled to measure euchromatin and heterochromatin domains in nuclei of lymphoid cells [Marinelli et al, 2000] and of normal and malignant liver cells [Nielsen et al, 2002], and also to discriminate lymphoid cells found in Mycosis fungoides from chronic dermatitis by means of the FD measured on the respective nuclear membranes [Bianciardi et al, 2002]. Methods based on grey-level density discrimination have been applied to histological sections to measure the FD of the chromatin texture of nuclei in normal and malignant cells from breast tissues [Einstein et al, 1998]. Nuclear membrane envelopes (ENM) and membrane-bound heterochromatin domains (NMBHC) of human breast cancer MCF-7 cells triggered by steroid hormones, such as 17ß-estradiol or dexamethasone, underwent ultrastructural changes at the beginning of growth that have been quantified by the FD. Indeed, after five minutes of treatment, nanomolar 17ß-estradiol enhanced the ultrastructural irregularity of NMBHC by increasing its FD, whereas nanomolar dexamethasone reduced it when compared to MCF-7 control cells. Neither steroid significantly modified ENM ultrastructure (Fig 4 a, b) [Losa et al, 1999].

Figure 4a, 4b: fractal dimension values (fig 4b) were estimated on nuclear membrane envelope (ENM) and nuclear membrane-bound heterochromatin (NMBHC) (fig 4a) segmented from breast cancer MCF-7 cells treated with steroid hormones.

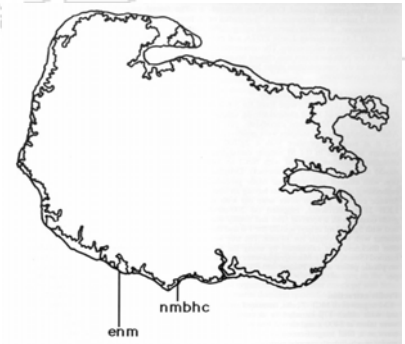

Figure 4a

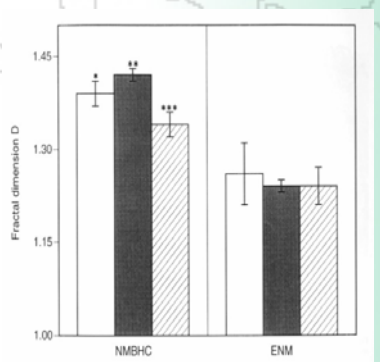

Figure 4b

By performing the fractal analysis on electron microscopic images of distinct internal organelles, it has become evident that liver cells contained a rough endoplasmic reticulum with a FD (1.72) higher than its smooth counterpart and mitochondria whose inner membrane was found more complex than the outer membrane, as documented by the fractal dimension (1.54 versus 1.09) reported in Fig 5 [Paumgartner et al, 1981].

Resolution scale

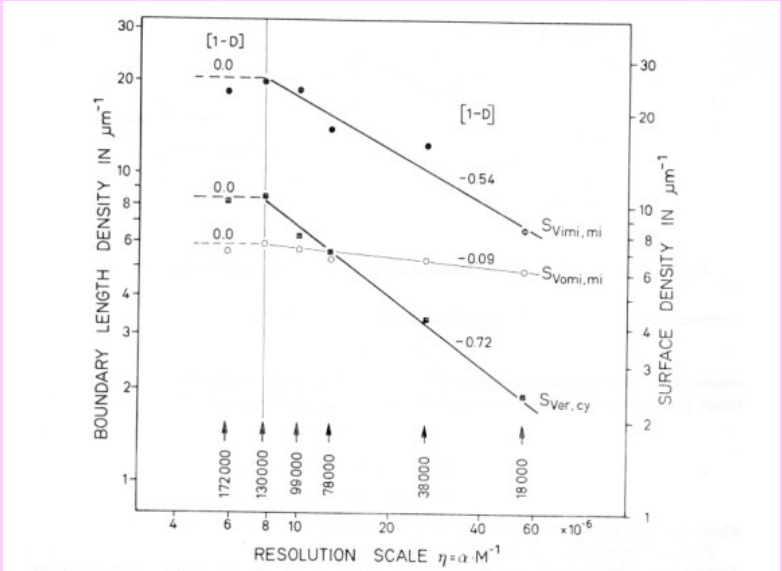

Fig. 9. Double-logarithmic plot of estimates of boundary length density against resolution scale (M^{-1}). Slope of regression line in the range $\{\times 18{,}000 < M < \times 130{,}000\}$ allows estimation of fractal dimension D. Note that $S_V = (4/\pi) \cdot B_A$.

Figure 5: [1-D] slope of regression line. The fractal dimension is D=1.54 for the inner mitochondrial membrane, D=1.09 for the external mitochondrial membrane and D=1.72 for the rough endoplasmic reticulum.

The outline roughness and the internal irregularity of the extracellular matrix collagen seen on liver biopsy specimens during chronic diseases has been evaluated by the fractal approach, thus yielding a reliable measure of these two qualitative properties of the liver matrix (Figs 6, 7) [Dioguardi and Grizzi,2002].

Figure 6

Collagen deposition in liver chronic disease

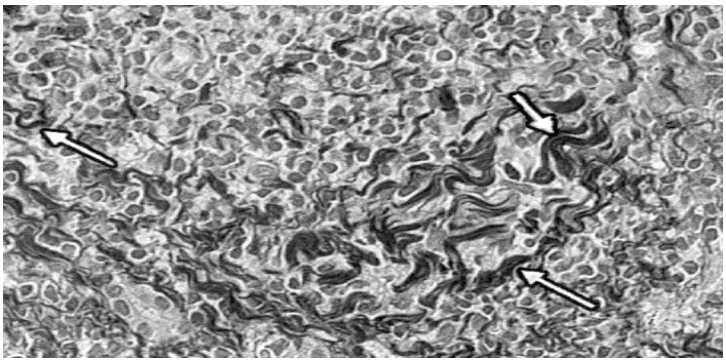

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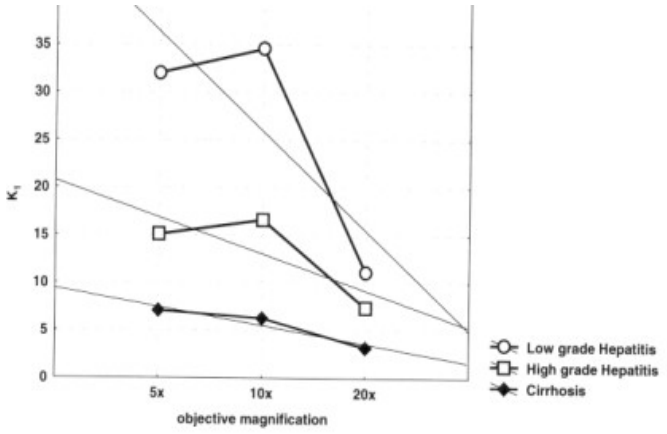
Figure 7

External rugosity of collagenic area



The graph shows the boundary (external) rugosity behaviours evaluated in the case-list considered in the preliminary study. K_1 was identified at three objective magnifications (5x, 10x, 20x) and at three different states of severity of the liver disease.

The fractal dimension has been used as a characterization parameter of premalignant and malignant epithelial lesions of the floor of the mouth in humans [Landini and Rippin,1993]. Recently, the fractal approach has been employed to document the feasibility of using

ultrastructural changes in cell surface and nuclear inter(eu)chromatin to assess the early phases of apoptosis in human breast cancer SKBR-3 cells treated with calcimycin for 12-24 h (table 2), namely prior to changes in conventional cell markers, which were only measurable during the active phases of apoptotic cell death manifest only after 60 h of culture (table 3) [Castelli and Losa, 2001; Losa and Castelli, 2005].

Table 2

FD of SK-BR-3 cell components

Table 2

Fractal dimension (FD) of plasma and nuclear membrane outlines in SK-BR-3 cells, untreated (control) and treated with 1 μM calcimycin for 24 h. Results are median values ± standard error on the median. Ranges of values are in the lower row. No significant difference between untreated and treated cells. *n*: number of examined cells

	Fractal Dimension (FD)	
	untreated cells ($n = 14$)	treated cells ($n = 15$)
FD plasma membrane	1.143 ± 0.013	1.119 ± 0.012
	1.066–1.169	1.059–1.179
FD nuclear membrane	1.027 ± 0.005	1.012 ± 0.008
	0.995–1.046	0.996–1.103

ical complexity of the perinuclear membranes in control (median FD = 1.027 ± 0.005) and treated (1.012 ± 0.008) cells confirmed the tendency towards a loss of

Table 3

Fractal dimension (FD) of the interchromatin space (IC) and of intranuclear chromatin regions of interest (ROIs) segmented by grey level slice density approach. Cell treatment: 1 μM calcimycin for 24 h. Results are median values ± standard error on the median. Ranges of values are in the lower row. *n*: number of examined images. *p*: significance evaluated by the Mann–Whitney test

	Fractal Dimension (FD)		
	untreated cells	treated cells	*p*
IC	1.714 ± 0.0087	1.679 ± 0.0152	0.0073
	1.652–1.745	1.543–1.727	
	($n = 14$)	($n = 14$)	
IC w/o nucleolus	1.716 ± 0.0097	1.677 ± 0.0152	0.0049
	1.648–1.745	1.542–1.727	
	($n = 14$)	($n = 15$)	
ROIs	1.591 ± 0.0071	1.547 ± 0.0078	<0.0001
	1.473–1.645	1.446–1.650	
	($n = 42$)	($n = 44$)	

Table 3

Apoptotic markers

Table 1

Time–course expression of membrane and nuclear apoptotic markers in SKBR-3 cells, untreated and treated with 1 μM A23187 Calcimycin

Hours	Plasma membrane markers			
	PI permeability (%)		Annexin-V (%)	
	untreated cells	treated cells	untreated cells	treated cells
0	8.4 ± 2.4	8.4 ± 2.4	4.1 ± 1.8	4.1 ± 1.8
24	13.7 ± 9.2	9.1 ± 0.8	7.3 ± 0.3	8.6 ± 6.6
48	10.3 ± 0.6	27.0 ± 4.3*	5.3 ± 3.2	8.5 ± 6.0
72	6.5 ± 1.0	52.7 ± 13.3**	5.2 ± 4.6	27.9 ± 9.1**
96	10.0 ± 5.2	84.8 ± 3.2**	5.7 ± 0.6	60.1 ± 7.2**

Hours	Nuclear markers			
	TUNEL (%)		Sub G_0/G_1 peak (%)	
	untreated cells	treated cells	untreated cells	treated cells
0	2.6 ± 0.6	2.6 ± 0.6	5.1 ± 1.8	5.1 ± 1.8
24	2.8 ± 0.9	10.4 ± 2.1	3.2 ± 0.4	4.4 ± 1.1
48	2.1 ± 0.5	22.7 ± 3.1**	5.7 ± 0.6	15.4 ± 6.6*
72	2.2 ± 1.3	57.1 ± 6.4**	3.8 ± 0.1	49.7 ± 7.2**
96	3.4 ± 1.0	58.1 ± 8.3**	7.5 ± 3.0	75.3 ± 2.7**

Results (%) are means ± one SD of three separate experiments.

*; ** Significant different at $p < 0.01$ and 0.001, respectively, between untreated and treated cells.

In this context it has been reported that micro architectural alterations of the uninvolved colonic mucosa, as determined through increased FD, occurred early in experimental colon carcinogenesis preceding the expression of conventional biomarkers of apoptosis and proliferation. [Roy et al, 2004]. Lastly, it has been shown that the quantitative evaluation of the surface fractal dimension (2-D) may allow not only to measure the complex geometrical

architecture but also to model the development and growth of tumour neo vascular systems and explore the morphological variability of vasculatures produced in nature [Grizzi et al, 2005].

Conclusions

The irregularity and self-similarity under scale changes are the main attributes of the morphologic complexity of cells and tissues, either normal or pathologic. In other words, the shape of a self-similar object does not change when scales of measure change because any part of it might be similar to the original object. Size and geometric parameters of an irregular object, however, differ when inspected at increasing resolution, which reveals more details. Significant progress has been made over the past three decades in understanding how to analyse irregular shapes and structures in the physical and biologic sciences. Dominant influences have been the discovery by B.B. Mandelbrot of a practical geometry of nature called fractal geometry, and the continuous improvements in computational capabilities. The application of the principles of fractal geometry, unlike the conventional Euclidean geometry developed for describing regular and ideal geometric shapes practically unknown in nature, enables one to measure the fractal dimension, contour length, surface area, and other dimensional parameters of almost all irregular and complex biologic tissues. During the past decade, a large amount of experimental evidence has accumulated showing that even in biomedical sciences fractal patterns could be observed within a <scaling window>, a condition to be experimentally established for each tissue element. The fractal dimension is a quantitative descriptor that can be used alone to identify the chromatin organisation and the ultrastructural changes of distinct cell components and other tissues sharing different morphological traits and functional peculiarities. In contrast, the mathematical statistical approach such as the grey-level co-occurrence matrix (CLCM) requires a large number of parameters to do so [Haraklick et al, 1973]. Through several examples borrowed from the recent literature, we focus on the application of the fractal approach to measuring irregular and complex features of pathologic cells and tissues and also on its potential role in the understanding of tumour biology and for reassessing the morphological information .

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