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The Fractal Geometry of Life

Lovingly dedicated to my Maîtres and Friends, Professor Benoît Mandelbrot, Boston (USA), Professor Theo F. Nonnenmacher, Ulm (D) and Professor Ewald R. Weibel, Berne (CH).

Keywords. Fractal geometry, fractal dimension FD, fractalomics, irregularity, form invariance, scaling window, membranes and subcellular components, biological tissues, tumours, tissue architecture and organization.

Abstract. *The extension of the concepts of Fractal Geometry (Mandelbrot [1983]) toward the life sciences has led to significant progress in understanding complex functional properties and architectural / morphological / structural features characterising cells and tissues during ontogenesis and both normal and pathological development processes. It has even been argued that fractal geometry could provide a coherent description of the design principles underlying living organisms (Weibel [1991]). Fractals fulfil a certain number of theoretical and methodological criteria including a high level of*

organization, shape irregularity, functional and morphological self-similarity, scale invariance, iterative pathways and a peculiar non-integer fractal dimension [FD]. Whereas mathematical objects are deterministic invariant or self-similar over an unlimited range of scales, biological components are statistically self-similar only within a fractal domain defined by upper and lower limits, called scaling window, in which the relationship between the scale of observation and the measured size or length of the object can be established (Losa and Nonnenmacher [1996]). Selected examples will contribute to depict complex biological shapes and structures as fractal entities, and also to show why the application of the fractal principle is valuable for measuring dimensional, geometrical and functional parameters of cells, tissues and organs occurring within the vegetal and animal realms. If the criteria for a strict description of natural fractals are met, then it follows that a Fractal Geometry of Life may be envisaged and all natural objects and biological systems exhibiting self-similar patterns and scaling properties may be considered as belonging to the new subdiscipline of "fractalomics".

1. INTRODUCTION

The Fractal Geometry of Nature, Benoît Mandelbrot's masterpiece evoking a new "*Weltanschauung*", has provided an innovative paradigm, a novel epistemological approach for interpreting the natural world and a more intelligent vision of life itself (in the etymological sense of the Latin word *intellegere*), even though it has given rise to and is still giving rise to controversial opinions in the scientific community while offering incentives to satisfy the curiosity of the public at large. This fractal geometry founded upon a body of well-defined laws and coherent principles, including those derived from chaos theory (Prigogine [1997]), allows the recognition and quantitative description of complex shapes, images and other figures usually created through unlimited iterations of a simple generator, often a mathematical motif, by means of computer-aided design (CAD). CAD figures which were indecipherable using classical geometry were called " Fractals" because of their peculiarity, which lies in the reproducibility of their shape over a range of scales and in a non-integer topological dimension called a "fractal dimension", from the Latin word *fractus.* Non-Euclidean iterated figures, now including fractals, have often been considered

to bear resemblance to pathological entities or mathematical monsters despite of, or owing to, their beauty, richness and fascinating shapes (Mandelbrot [2006]).

Nowadays, most of them have become explicable and even familiar since Mandelbrot's assertion that they can almost be considered as a general rule of nature, which led him to conclude that "clouds are not spheres, mountains are not cones, coastlines are not circles, and bark is not smooth, nor does lightning travel in a straight line". Afterwards, it was noticed that these virtual figures share some morphological traits and self-similar properties which could be encountered not only in elements of the inanimate world but also, though less evident, in complex forms, functions and shapes belonging to the plant and animal realms. Living forms develop according to organized morphological patterns correlated with a complex system of functional metabolic interactions which make the accomplishment of the adaptive response possible. Iteration, self-similarity, form invariance upon scaling, non-equilibrium thermodynamics, self-organization and energy dissipation are among the mechanisms reputed to sustain the emergence and maintenance of living forms, in contrast to those of homeostasis, linearity, smoothness, regularity and thermodynamic reversibility pertaining to a more traditional vision based upon the concepts and rules of Euclidean geometry and adequate for an ideal world (Losa [2002]). Over the past decade a large amount of experimental evidence has been accumulated showing that biological elements do indeed express statistical self-similar patterns and fractal properties within a defined interval of scales, called the "scaling window", in which a direct relationship between the observation scale and the measured size/length of an object or the frequency of a temporal event can be ascertained and in turn quantified by a peculiar fractal dimension FD (Losa and Nonnenmacher [1996]). In other words, the fractal dimension of a biological component remains constant within the scaling window and serves to quantify variations in length, area or volume with changes in the dimensions of the measuring scale. However, real "fractality" exists only when the experimental scaling range covers at least two orders of magnitude, although fractality over many orders of magnitude has

been observed in various natural fields (Mandelbrot [1998]).

Hence, defining a "scaling range" of length measurements appears to be an inescapable requisite for assessing the fractality of any biological element. Experimental evidence of a definite scale interval avoids any ambiguous assignment of objects or figures lacking that requirement and confirms Mandelbrot's assertion that "fractals are not a panacea; they are not everywhere" (Mandelbrot [1998]).

2. IRRUPTION OF FRACTAL GEOMETRY IN BIOLOGY AND MEDICINE

From the direct observation of nature it turns out that most cells, tissues, organs, in either the animal or vegetal worlds are systems in which component parts and unit fragments assemble with different levels of complexity and organization. This means that a single fragment or element may on various scales, reproduce the whole object from which it is derived, in other words it is self-similar, albeit in a statistical sense. Very few of these shapes can be analytically described or evaluated using Euclidean geometry, which was developed to trace regular and ideal geometrical forms practically unknown in natural and biological systems. Thus a Fractal Geometry of Life can be envisaged and the totality of biological elements, natural objects and physiopathologic processes carrying spatial or temporal self-similar properties, be gathered into "fractalomics". Fractalomics is proposed as a novel variety of "omics" comprehensive of biological systems by analogy with other established subdisciplines such as genomics, where the suffix "*omics*", derived from the Greek word "*ome*," refers to wholeness or to completion. Although the first coherent essay on fractal geometry was published in French more than 30 years ago (Mandelbrot [1975]), it may be worth considering exactly how and when the "heuristic introduction" of such an innovative discipline occurred or, more pregnantly stated, as when "the irruption of fractal geometry" into the life sciences such as biology and medicine actually took place (Belaubre [2006]). Although there is no precise date, it is generally agreed that its introduction occurred within

the "golden age" of cell biology, i.e., between the 1960s and 1990s. According to "the state of the art" there was a pressing need to consider the morphological complexity of cells and tissues using a systemic approach, and at the same time to develop instruments which could enable the accomplishment of that goal without introducing any shape approximation or smoothing, a condition which could not be satisfactorily achieved with conventional analytical methods.

Actually the latter, which rested on conventional disciplines such as morphometry and stereology (Weibel [1981]), yielded experimental data about the quantitative description of membranes which was usually controversial, leaving many questions unresolved, thus preventing a true consensus being reached amongst researchers (Loud [1968]; Weibel *et al.* [1969]; Losa *et al.* [1978]). To highlight the striking debate that led to turmoil within the community of biologists, it may suffice to report the original description, proffered by an outstanding scientist in the field (Weibel [1994]), of the first case study realized several years earlier (Paumgartner *et al.* [1981]). This related to the application of Fractal Geometry in cell biology, namely:

> the discovery that cellular membrane systems have fractal properties arose from the uncertainty of observation about the extent of such membranes. When the first studies on the morphometry of liver cell membranes were published the results did not match as we obtained much higher values than other groups. Long debates followed about which of the estimates was correct, whether the liver cells contained 6 or 11 m^2 of membranes per cm^3, a quite significant difference, and whether the stereological methods used were reliable since it appeared possible that the same method may yield different results if the measurements were done at different magnifications of the electron micrographs.

Indeed, the systematic study on measuring liver cell membranes revealed that "the estimates of surface density increased with increased resolution" (Weibel [1994]). Soon after the conclusion of the experimental phase of the study mentioned above, Mandelbrot suggested interpreting the results with the likely effect of the "resolution scale" in analogy with the "Coast of Britain effect" (Mandelbrot [1967]) which, if so, would have resolved

the estimate discrepancy and explained why measurements of irregular liver cell membranes at higher magnification yielded higher values than those obtained at lower magnification (Weibel [1994]).

It has to be stressed that the scaling effect applies mainly to cellular membranes with a folded surface or an indented profile such as the inner mitochondrial membrane. Its surface density estimate increased with increasing magnification yielding a fractal dimension consistently as high as the estimated value of 2.54, whereas the measurement of the surface density of the outer mitochondrial membrane, almost smooth, was only slightly affected by the resolution effect and, in fact, the estimated FD was about 2.09, rather close to the topological dimension of 2.0, as documented in figure 1 (Paumgartner *et al.* [1981]).

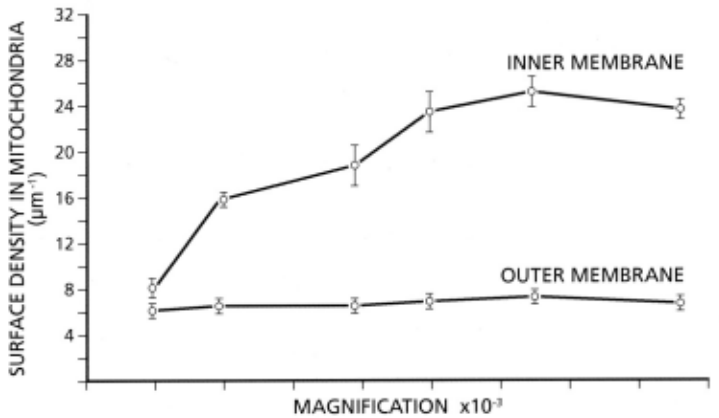

Figure 1 – Change of surface density estimate for outer and inner mitochondrial membranes with magnification (from Paumgartner *et al.* [1981]).

The second case study dealing with the gas exchange surface of the lung revealed that the measure of the alveolar surface area increased at increasing magnification with a slope yielding a fractal dimension of 2.24 (Gehr *et al.* [1978]). It has been reported that the inner lung surface consists of a hierarchy of successive struc-

tures, first alveoli, then capillaries in the alveolar wall and finally alveolar epithelial cells with wrinkled membranes, which are resolved at increasing magnification (figure 2). These different structures however can hardly be considered as self-similar structures of a unique type: "each has its own generator, is determined by its own constructive algorithm and, accordingly, we should search for at least two or three self-similar levels" (Weibel [1994]), which a successive systematic study could indeed confirm.

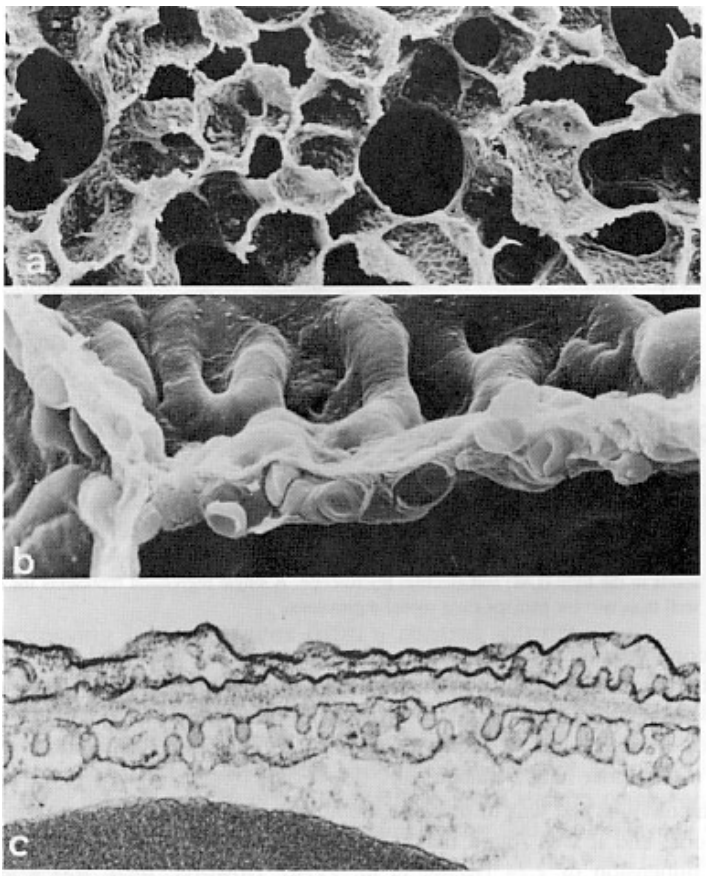

Figure 2 – Increasing microscopic resolution reveals a hierarchy of structures that form the inner lung surface: alveoli around the airways (a), capillaries in the alveolar walls with their imprints on the surface (b), and membrane folds of the epithelial cell forming the surface of the air-blood barrier (c). Scanning electron micrographs magnified 75 x (a) and 1000 x (b) c) thin section electron micrograph: 46,000 x (from Gehr *et al.* [1978]).

3. FRACTAL CRITERIA

Mandelbrot stated in his book that "A fractal set is a set in metric space for which the Hausdorff-Besicovitch dimension D is greater than the topological dimension Dt". In nature, a fractal object is defined by its structural properties, mainly by its lack of smoothness. Additional important properties of a fractal object are roughness or shape irregularity at every scale, high level of organization, iterative pattern, a peculiar non-integer fractal dimension [FD] and self-similarity or scale invariance. This means that an object is called self-similar if any part of it, after being scaled by an arbitrary factor, looks the same as the whole object (Losa and Nonnenmacher [1996]). The Richardson-Mandelbrot equation provides the mathematical basis for understanding geometrical and spatial fractal structures, and for measuring and interpreting them, namely:

$$L(\varepsilon) = N(\varepsilon)\cdot(\varepsilon) \tag{1}$$

where $L(\varepsilon)$ represents the contour (perimeter) length of the biological component under investigation, (ε) the unit length of measure and $N(\varepsilon)$ the number of unit lengths (ε) needed to cover the contour $L(\varepsilon)$. By substituting $N(\varepsilon)$ with $[l_0^D\ \varepsilon^{-D}]$ into (1), where l_0 is a reference scale without influence on the determination of D, the above equation can be transformed by logarithmic procedure and rewritten as:

$$\log[L(\varepsilon)/l_0] = (1 - D)\log[\varepsilon/l_0] \tag{2}$$

Equation (2) represents a dimensionless scaling power law indicating that the estimated contour, perimeter or curve length $L(\varepsilon)$ changes as a power function of the scale unit length (ε). The dimensional exponent D is the *fractal dimension* which defines the nature of the curve. Mathematical fractals are invariant over an unlimited range of scales, whereas biological components are statistically self-similar only within a fractal domain or *scaling window* delimited by upper and lower scaling bounds covering at least two orders of magnitude. This also implies that such a domain must be experimentally established for any biological element in-

vestigated and that a lowest scale of measure exists which can detect the smallest entity measurable, below which there is no physical sense to measurement. Only within region II on a log-log plot, can a straight line be drawn and its slope (1-D), as defined in the logarithmic equation log L(ε) = (1 – D) log (ε), be used to evaluate the numerical value of the fractal dimension D (figure 3) (Losa and Nonnenmacher [1996]).

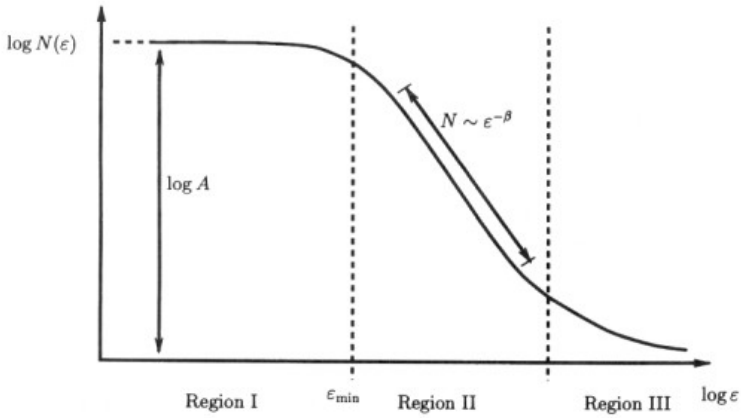

Figure 3 – The three typical regions of an asymptotic fractal. Asymptotic natural or biological fractals only show autosimilar scaling properties (fractality) within a fractal window, represented by the Region II, limited by a lower (ε_{min}) and an upper bound (ε_{max}) (dashed lines), where a straight line can be drawn and the fractal dimension calculated from its slope. The practical evaluation of the fractal dimension D could be easily obtained by the box counting method based on counting of the non empty boxes N(ε) of a grid with box side of varying length (ε), and by using the relation D = log N (ε) /log (1/ε) (from Dollinger *et al.* [1998]).

The fractal scaling power law is ubiquitous in nature (West *et al.* [1997]; Weibel [2002]) and can be applied to the study of a wide variety of biological problems including: allometric scaling growth (Dreyer and Puzio [2001]) allosteric enzyme kinetics (Savageau [1985], [1995]); metabolic rate in mammals (Weibel *et al.* [2004]); population genetics (Vlad *et al.* [2007]); tumour growth (Delsanto *et al.* [2008]; Guiot *et al.* [2008]); modelling of drug clearance (Pugno [2008]); ontogenic growth of human haemopoietic stem

cells (HSCs) (Dingli and Pacheco [2007]); cardiac function and body size (Dewey *et al.* [2008]) and many others as documented recently (West and Brown [2005]). Disregarding the theoretical and experimental framework of criteria which define inexact statistical fractals, such as biological and natural ones, could lead to a series of pitfalls in determining the fractal dimension, as pointed out by several authors (Rigaut [1984], [1989]; West and Deering [1994]; Smith *et al.* [1996]; Landini and Rigaut [1997]; Jelinek and Fernandez [1998]; Jelinek *et al.* [2005]; Milosevic and Ristanovich [2006]; Eke *et al.* [2002], [2006]). For a better understanding, in a certain number of reports the fractal dimension was evaluated from the slope of the straight line but without the scaling domain being established or, sometimes, with only the lower bound scale displayed on the log-log plot (corresponding to the maximal resolution scale) being checked. Often the straight line was drawn without the data being fitted by using an automatic procedure based on a least squares fit algorithm or other statistical method, thus preventing an objective slope estimation. The automatic procedure enabled searches for the widest interval within which the standard deviation of the estimated slope did not exceed a given limit corresponding to a confidence interval ranging from 95% to 99%, whereby the fractal dimension D of any shape profile examined could be estimated at a fixed standard deviation (Nonnenmacher [1994]). Apart from the *scaling window* principle there are two other criteria which must be fulfilled for achieving a reliable fractal analysis: the first requires the adoption of a correct sampling procedure while the second imposes that experimental data be evaluated through an adequate statistical methodology, which rests on the type of frequency distribution with which the collected data must fit. This said, here there will be neither specific criticism on specific reports, such as those cited in the references section, nor comparison of, or opinions expressed about, data concerning similar topics, such a task being beyond the scope of this study.

4. FRACTALOMICS

The fractal dimension is considered as a statistical measure which correlates the morphological structural complexity of cellular components and biological tissues (Nonnenmacher *et al.* [1994]). The fractal dimension FD is also a numerical descriptor which serves to measure qualitative morphological traits and self-similar properties shown by most biological elements at various levels, cellular, tissue or organic, as highlighted below using several examples. Immature feline oocytes with or without the *cumulus oophorus* [CO], known to affect their developmental potential, were investigated in order to verify whether distinct cytoplasmic components with irregular features have self-similar properties which could be described by fractal analysis (De Vico *et al.* [2005]). Original images of oocytes collected by ovariectomy and segmented by a grey threshold procedure revealed that the highest FD of 1.91 was measured on grey-dark profiles of cytoplasm elements characterized by a highly connected network of lipid droplets and intracellular membranes. Unexpectedly, fractal dimension values from the different oocyte elements were close to each other and not influenced by the presence or absence of CO. The fractal analysis provided an effective quantitative descriptor of the complex cytoplasm morphology which may contribute to an objective and reliable classification of the feline oocyte, without introducing any bias or shape approximation. Previous studies discussed the fractal nature of cytoplasm and revealed that the fractal architecture is a result of the iteration of an invariant simple pattern spanning several length scales and is organized into a percolation lattice with clusters emerging as fractal forms. Such a spatiotemporal cytoplasmic organization bestowed properties which amplify enzymatic activities and metabolic networks (Aon and Cortassa [1994]; Golberger *et al.* [2002]; Aon *et al.* [2004]). Particularly at the electron microscopy level, fractal analysis proved useful for an objective investigation of the fine cytoplasmic structure and the organization of various types of chromatin, nuclear components and other subcellular organelles, either in normal or pathological tissues and in cell cultures. For example, measuring the FD of euchromatin and heterochromatin nuclear domains helped to discriminate lymphoid cells found in

Mycosis fungoides from those in chronic dermatitis (Bianciardi *et al.* [2002]). External nuclear membrane envelope (ENM) and membrane-bound heterochromatin domains (NMBHC) of MCF-7 human breast cancer cells briefly triggered by steroid hormones, such as 17ß-estradiol or dexamethasone, underwent ultrastructural changes at the beginning of the growth which were quantified by their peculiar FDs. Indeed, after five minutes of treatment, the 17ß-estradiol (1nM) growth factor significantly enhanced the ultrastructural irregularity or the DNA unfolding of the NMBHC domain by increasing its FD, whereas dexamethasone (1nM), a growth antagonist, reduced it when compared to control MCF-7 cells. Neither steroid significantly modified the ENM ultrastructure (Losa *et al.* [1998]). This fractal tool has also 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 (programmed cell death) induced in human breast cancer SKBR-3 cells by the ionophore calcimycin. The ultrastructural changes which involved a loss in heterochromatin irregularity (or an increased condensation of it) as quantified by a lower FD, were evident well before the detection of conventional cell markers, which were only measurable during the active phases of apoptosis (Losa and Castelli [2005]). These and other quoted reports (Santoro *et al.* [2002]; Marinelli *et al.* [1998]; Weyn *et al.* [2002]; Nielsen *et al.* [2002]) indicated that the fractal analysis carried out on electron microscopic images is very efficient for the quantitative detection of cellular components and associated morphostructural changes, and confirm a pioneering study documenting that rat liver cells contain intracellular membranes with irregular and self-similar traits observed over several scales of measurement (Paumgartner *et al.* [1981]).

The first application of fractal morphometry in non-solid cancer came later, when human leukaemia cells of lymphoid and/or myeloid origin were characterized on electron microscopic images through the quantitative measurement of membrane surface properties which could be correlated with specific phenotype markers. Cells isolated *ex vivo* from the blood of humans with acute T-lymphoid leukaemia revealed pericellular membranes with a nearly smooth outline as documented by fractal dimension FD values sig-

nificantly lower than FDs evaluated on pericellular membranes of healthy blood cells. Healthy lymphocytes of B-cell lineage had an FD (1.20) significantly different from the FD of lymphocytes of T-cell lineage, i.e., CD4-T helper (1.17) and CD8-T suppressor (1.23) cells (figure 4).

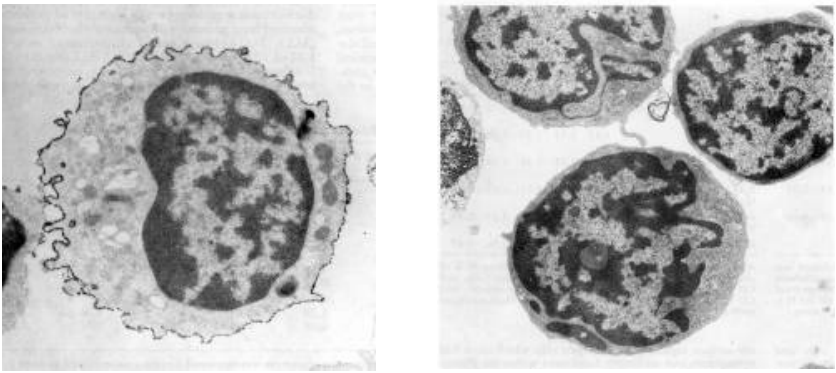

Figure 4 – Thin sections electron micrographs. On the left: view of an immune competent T helper blood lymphocyte (decorated with an anti-CD4 monoclonal antibody) showing a wrinkled cell surface membrane. On the right: some cells of an acute human lymphoblastic leukaemia of the T-cell lineage showing a plasma membrane rather smooth (magnification 8000x) (from Losa *et al.* [1992]).

Unexpectedly, strongly proliferating T-lymphoid leukemic cells were found to possess a plasma membrane characterized by a low FD value (1.10), close to the FD value measured on the plasma membrane of *in vitro* growing lymphoblasts derived from mature T-lymphocytes triggered by phytohaemagglutinin PHA, a mitogenic lectin (Losa *et al.* [1992]). About 80% of acute leukaemia subtypes of the B-cell lineage (c-ALL and pre-B undifferentiated phenotype) showed plasma membranes with FDs ranging from 1.12 to 1.17, below the FD of the plasma membrane of differentiated B-lymphocytes. The remaining cases (20%) of acute lymphoblastic B-leukaemias showed a more convoluted cell surface with FD values of up to 1.24. Cells from hairy-cell leukaemia, a chronic type of human leukaemia, with a highly convoluted plasma membrane morphology and a completely different surface pheno-

type displayed the highest FD, between 1.32-1.36 (Losa [1994]). The fractal dimension of scale-invariant self-similar chromatin was measured in nuclei of blasts isolated from patients suffering from acute leukaemia of the precursor B lymphoblastic type (B-ALL). The increase of the FD together with the accentuated coarseness of nuclear surface reflects significant changes in the DNA methylation pattern usually localized in heterochromatin nuclear regions and therefore was regarded as a bad prognostic factor for these patients (Adam *et al.* [2006]). The usefulness of fractal analysis to assess the haematological cell phenotype and to define a clinical group was confirmed about twenty years later (Mashiah *et al.* [2008]). These authors analysed on conventional slide preparations nuclei "contours" of cells belonging to the B lineage, i.e., normal and reactive lymphocytes and lymphoid cells isolated from patients with chronic lymphocytic leukaemia (CLL), follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). They found that the fractal dimensions of perinuclear membranes were significantly different between the groups and all correlated with their biological properties, i.e., that reactive lymphocytes (FD = 1.20) were situated between CLL (FD = 1.25) and normal cells (FD = 1.13), while aggressive lymphoma cells had a significantly higher fractal dimension ranging from 1.23 (FL) to 1.31 (DLBCL). By comparing data from the latter papers dealing with haematological malignancies it turned out that cells isolated from patients with different types of leukaemia and/or lymphoma have nuclear chromatin with roughness or complexity (high FD value) increasing with increasing degrees of aggressiveness and malignancy, whereas pericellular membranes acted inversely and looked smoother (low FD value) in cells having a high degree of malignancy. One could infer that haematological tumours did not undergo uniform neoplastic transformations but rather manifest a manifold of metabolic and phenotype changes, which implies either an increasing or a decreasing complexity of the morphological surface and an altered organization of cell components mainly dependent upon the cytotype under investigation. This contrasts with an apparent type of behaviour observed in several cell colonies of breast cancer origin and experimental tumours which were observed to obey the same dynamics of proliferation and growth and to display contours with

fractal self-similar features when submitted to scaling analysis (Brú *et al.* [2003]). Over the last two decades several papers have reviewed the heuristic importance of applying the fractal approach to quantitatively characterize cancer tissues in order to overcome the uncertainty in current practice which involves various systems of diagnosis mostly arising from subjective observations rather than from a quantitative procedure (Goldberger and West [1987]; Losa and Nonnenmacher [1996]; Cross [1994]; Baish and Jain [2000]; Landini *et al.* [2000]; Spillman *et al.* [2004]; Janecka [2007]). In routine histology and cytology the examination of cell nuclei and nuclear components by fractal morphometry has greatly improved the comprehension of cell behaviour and the assessment of diagnosis and prognosis for various disease states (Muniandy and Stanlas [2008]). The nuclear chromatin organization was properly quantified by fractal morphometry in order to evaluate malignancy degree in human breast cytology (Einstein *et al.* [1998]) while the fractal dimension served to discriminate cytology smears of breast and cervical lesions (Ohri *et al.* [2004]). More recent studies targeting cell nuclei periphery showed fractal properties which allowed classification of early ovarian cancers and even to distinguish normal from malignant liver cells (Nielsen *et al.* [2002], [2005]). Relevant fields for which fractal geometry may provide an original approach for investigation and where the fractal dimension represents more than an additional geometrical parameter, as claimed by followers of the "reductionistic view", include cell and tissue heterogeneity, architectural organization of organs, shape features, developmental, morphogenetic and growth processes in tissues and organs in healthy, pathological and tumour conditions. Cell heterogeneity, known to contribute in a decisive way to the histological grading of human breast cancer, has been examined using geostatistics and the Hurst fractal parameter (Sharifi-Salamatian *et al.* [2004]). As reported in a recent study, tumour grading (a measure of the degree of cellular differentiation) may be difficult to assess because tumours often consist of a heterogeneous mixture of cells with varying degrees of differentiation. Upon inspection of breast and prostate histology specimens, the authors observed that tumour structures deriving from poorly differentiated cell elements possess a greater complexity, as characterized by a higher degree of

irregularity. By measuring the fractal dimension they were able to quantify the degree of irregularity as well as local variations in cellular differentiation. Such a tool could aid pathologists in grading heterogeneity and in determining the spatial extent of poorly differentiated regions of tumours (Tambasco and Magliocco [2008]; Tambasco *et al.* [2008]). All examples reported above, as well as those below, seem to indicate that the occurrence of morphogenetic dynamics, the emergence of complex patterns and the architectural organization of active tissues and tumour masses may be driven by constructive mechanisms related to fractal principles, including deterministic and/or random iteration of constituent units with varying degrees of self-similarity, scaling properties and form conservation (Landini [2002]). The preservation of tissue architecture and the cell polarity of organs and the eventual restoration of organized traits in tumour tissues, deconstructed and deregulated at various levels, is an emerging field of interest since it has been observed that biological entities organise with their own degrees of structural and behavioural complexity and develop on different spatial and time scales (Russo *et al.* [2001]; Bissell *et al.* [2003]; Grizzi *et al.* [2005]; Nelson *et al.* [2005]). For a quantitative description of all the problems mentioned above which "take all levels of biological organization into consideration" (Soto and Sonnenschein [2005]), adequate tools of investigation are required based upon the principles of fractal geometry. This constitutes a novel way of understanding higher-level phenomena (form generation, tissue organization and development, carcinogenesis, cell proliferation, cell death) which could convey us toward the unifying frame of tissue organization field theory (TOFT) (Soto and Sonnenschein [2005]). For a long time the interactions between stroma, the extracellular matrix and epithelium have been extensively examined in various mammalian tissues because of their role in the architectural organization of tissues (Liotta *et al.* [1983]; Bissell and Hall [1987]; Bissell *et al.* [2003]; Iozzo and Cohen [1993]; Losa and Alini [1993]; Russo *et al.* [2001]; Wiseman and Werb [2002]; Maffini *et al.* [2003]; Sonnenschein and Soto [2004], [2008]; Ingber [2008]). Stromal tissue has a major role in the control and regulation of physiological processes and in supporting the tumorigenic process in the breast (Kim *et al.* [2005]; Provenzano

et al. [2006]; Beck *et al.* [2008]; Schnitt [2009]). Recently, well-defined three-dimensional (3D) models were developed to decipher stromal-epithelial interactions which mediate mammary gland development and the formation and progression of breast cancer (Krause *et al.* [2008]). The outline roughness and the internal irregularity of collagen extracellular matrix examined on biopsy specimens of livers affected by chronic diseases were evaluated using the fractal approach, which has yielded a reliable measure, extremely useful in describing these two qualitative properties of the liver matrix (Grizzi *et al.* [2001]). It has also been shown that a quantitative evaluation of the surface fractal dimension may allow not only the measurement of the complex geometrical architecture but also to model the development and growth of tumour neovascular systems and explore the morphological variability of vasculatures in nature, and in particular the microvasculature of normal and adenomatous pituitary tissue (Di Ieva *et al.* [2007]). Neuronal and glia cells from the brain, spinal cord neurons and retinal ganglion cells were found to show a fractal dimension which correlated with the increase of the morphological complexity revealing a progressive level of morphological maturity (Smith and Bejar [1994]; Bernard *et al.* [2001]; Milosevic *et al.* [2005]; Ristanovic *et al.* [2006]; Jelinek *et al.* [2008]). Several fractal and non-fractal parameters have been considered for the quantitative assessment of the vascular architecture, using a variety of test specimens and of computational tools. Fractal parameters have the advantage of being scale invariant, i.e., independent of the magnification and resolution of the images investigated, making it easier to compare different set-ups and experiments (Mancardi *et al.* [2008]). In the normal human retina, blood vessels or vascular trees exhibited a FD of 1.7, the same fractal dimension found for a diffusion-limited growth process, a finding which may have implications for the embryological development of the retinal vascular system (Masters [2004]). A considerable amount of fluctuation was present in both artery blood flow velocity (FV) and arterial blood pressure (ABP) after subarachnoid hemorrhage (SAH). Variability and fractal analysis provide valuable information regarding the complexity of the human organism: fluctuations are reduced in cerebral vasospasm with a decrease in variability suggesting a loss

of complexity associated with a less favourable outcome. The decomplexification theory of illness may therefore apply to SAH (Soehle *et al.* [2008]). Fractal dimensions have been used as characterization parameters of premalignant and malignant epithelial lesions of the floor of the mouth in humans (Landini and Rippin [1993]; Abu Eid and Landini [2003]). Architectural changes associated with aging of the normal oral buccal mucosa have been found to exist between tree main age ranges by measuring the global fractal dimension of the epithelial tissue interface and the fractal dimension of segmented epithelial cell borders (Abu Eid *et al.* [2008]). The onset of fundamental phenomena such as growth and cell death can be adequately investigated by fractal geometry: a recent report revealed that micro-architectural alterations of the uninvolved colonic mucosa, shown through an increased FD, occurred early during the experimental colon carcinogenesis and preceded the expression of conventional biomarkers of apoptosis and proliferation (Roy *et al.* [2004]). Fractal structures were also observed in animal diseases. The fractal dimension of dog kidney proximal convoluted tubuli established by means of the box-counting algorithm was used to automate its recognition in anatomy and pathology (Gil *et al.* [2006]). The discrimination between benign (fibroadenoma, FD = 1.09) and malignant (carcinoma, FD = 1.21) mammary tumours in dogs and cats was achieved by determining the fractal dimension of the inner surface of mammary ducts (De Vico *et al.* [2002]). Canine trichoblastomas constitute a class of "benign tumour derived from or reduplicating the primitive hair germ of embryonic follicular development" (Goldschmidt *et al.* [1998]) and represent about 25% of all epithelial skin neoplasms (Abramo *et al.* [1999]). They are classified into ribbon (RT), trabecular/granular cell (TT/GT) and spindle cell (ST) types (figure 5). Trichoblastomas are not exclusively epithelial tumours but heterogeneous biological systems arising from epithelial-mesenchymal interactions. In these neoplasms the epithelial component appears to be equivalent to the hair germ and the mesenchymal component to represent the dermal papilla, both essential for hair follicle development (Millar [2002]). In despite of the relevance of these aspects, the complex mesenchymal epithelial relationship and the links between molecular and morphogenetic cell signals which

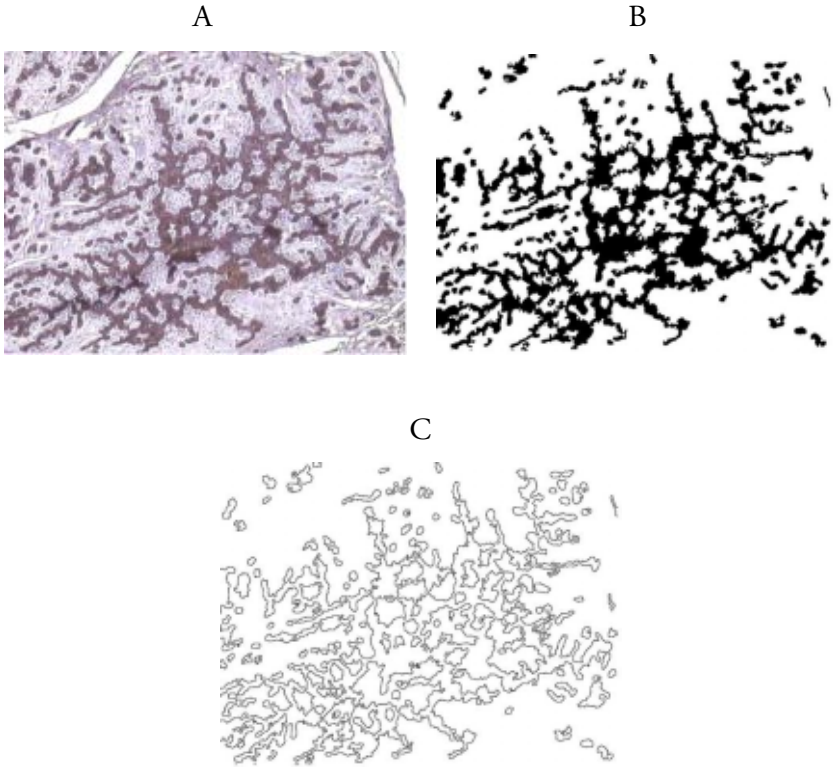

Fig. 5 – Microscopic view of canine Trichoblastoma of the ribbon type (RT). a) the epithelial component is positive for the marker cytokeratin. b) binary image obtained by grey tresholding the area occupied by the epithelial component and c) outline of the area occupied by the neoplastic epithelium. Magnification 40x (from Cataldi *et al.* [2008]).

may occur in trichoblastomas still remain poorly understood. Therefore, fractal morphometry was applied to these canine tumors for investigating the tissue organization in relation to the distribution patterns of epithelial/connective tissue components and the expression of β-catenin, a structural constituent between cells, anchoring the actin cytoskeleton, regulating normal cell growth and behaviour, and modulating epithelial architecture and the polarity of cells and tissues. Fractal analysis was performed on

masks and outlines of epithelial tumour components segmented from grey level threshold pictures which were randomly taken from histological trichoblastoma sections at 10x magnification, using a program described elsewhere (Dollinger *et al.* [1998]). Morphometric results indicated that the relative amount of the mesenchymal stroma expressed as $[V_v]_M$ was higher ($p < 0.001$) in the RT type, i.e., 46% ± 2% compared to the other types which accounted for 31% ± 5% and 33% ± 3% in ST and TT/GT, respectively. All the examined tumours showed epithelial components with irregular self-similar properties distinguished by characteristic values of their fractal dimension (FD). Tumour mask analysis unexpectedly revealed that RT trichoblastomas showed lower FD values (1.75 ± 0.01) significantly different ($p < 0.001$) from those of ST and TT/GC types with 1.78 ± 0.03 and 1.85 ± 0.02, respectively. On the contrary, trichoblastoma outlines showed absolute FD values lower than those obtained from tumour masks which were statistically inadequate for any histological discrimination. β-catenin occurred in the cytoplasm and in the nucleus of both mesenchymal and epithelial neoplastic cells, while its expression pattern (nucleus, cytoplasms, or both) was closely related to the growth pattern morphology and the type-specific architectural organization of trichoblastoma types, as documented by the FD which progressively increased on going from RT, ST up to TT/GT (Cataldi *et al.* [2008]).

Trichoblastoma of the dog could provide a reliable model to unravel the morphogenetic dynamics and organization underlying the neoplastic process and provide in quantitative terms useful information concerning the link between molecular, cellular and tissue changes during tumour development (Losa *et al.* [2009]). A highly promising approach appears to be the combination of fractal analysis, providing a quantitative description of shapes, with radiographic image techniques which may be able to discriminate malignant from benign tumour masses and from normal tissue structures as well (Penn and Loew [1997]; Penn *et al.* [2006]; Li *et al.* [2007]; Bocchi *et al.* [2004]; Kriege *et al.* [2004]; Chen *et al.* [1997]; Megalooikonomou *et al.* [2007]; Soares *et al.* [2007]). The fractal dimension of the contour of a mass may be computed either directly from the two-dimensional contour or

from a one-dimensional signature derived from the contour which can be useful for characterizing shape and grey-scale complexity which may vary between benign masses and malignant tumours in mammograms (Rangayyan *et al.* [2007]). Fractal theory provided the basis for a unique software platform program, developed for use in conjunction with magnetic resonance imaging (MRI) showing great promise in the early diagnosis and treatment of breast cancer. A recent study applying this advanced method documented that in over 30 percent of patients there were additional tumors in the same breast, and in almost 10 percent of the patients there were tumours in the other breast. These tumours had not been found using conventional mammography or ultrasound (Wiener *et al.* [2005]). Image analysis and non-Euclidean geometrical fractal analysis have been applied to describe changes in the actin cytoskeleton of neonatal cardiac fibroblasts responding to mechanical stress (Fuseler *et al.* [2007]). Fractals were used to test the similarity hypothesis in the human proteome by analysing human proteins for similarity profiles of two pentapeptide sets with different functional properties, which were quantified as a fractal dimension (Capone *et al.* [2008]).

6. EPILOGUE

Irregularity and self-similarity under scale changes are the main attributes of the morphological complexity of cells and tissues, either normal or pathological. In other words, the shape of a self-similar object does not change when the scales of measurement change because any part of it might be similar to the original object. Size and geometrical parameters of an irregular object, however, differ when inspected at increasing resolution, revealing greater detail. Significant progress has been made over the past three decades in understanding how to analyse irregular shapes and structures in the physical and biological sciences.

Dominant influences include the important discovery by 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

conventional Euclidean geometry developed for describing regular and ideal geometrical 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 biological tissues. Over the past decade, a large amount of experimental evidence has accumulated showing that even in the biological world 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 quantify qualitative peculiarities, such as form irregularity, to describe morphogenetic processes and to identify cell components, cell types and other tissues sharing different morphological traits and functional peculiarities. In contrast, mathematical statistical approaches such as the grey-level co-occurrence matrix (GLCM), polynomial equations, and Fourier analysis, amongst others, require a large number of parameters to do so. Through the use of several examples, borrowed from the recent literature, we have highlighted the application of the fractal approach in measuring irregular self-similar features in normal and pathological cells and tissues with a high degree of organized complexity and of plasticity (Buiatti and Buiatti [2008]), as well as its potential role in reassessing morphological information for a deeper insight into, and understanding of, the biology of normal tissues and tumour masses. Moreover, the fractal approach enables one, not only to avoid any approximation or simplification in analysing real shapes and functional behaviours and hence to describe irregular morphologic components and ultrastructural features as they are, but also through a quantitative comparison, to show every modification over time which the structural features and shapes in either normal, pathological or tumour stages may undergo. In conclusion, two questions must be posed: first of all, do the numerous examples presented here concur in explaining how self-similarity or form invariance on various scales and self-organization can govern different biological processes such as growth, morphogenesis, shape remodelling, architectural organization and form conservation which emerge in all living organisms in line with the assumptions of Systems Science? (Von Bertalanffy [1968]; Minati [2008]). If

so, then the hypothesis that the "morphogenesis of biostructures follows fractal principles " and that "fractal geometry is a design principle for living organisms", as recalled elsewhere (Weibel [1991], [1994]), may be envisaged for interpreting how biological phenomena and shapes come about, whilst being well aware that the true reality may remain undisclosed! The second question adumbrates the link of fractals to biological design which can be formulated as: "do genes contain fractal algorithms?" (Weibel [1994]). This appears much more interlocutory because genes are DNA entities codifying constructive units or templates, while fractal algorithms represent mechanisms (iteration, self-organization, environmental constraints, etc.) which nature may eventually adopt in order to assemble self-similar dynamic units into final shapes. Whether genes do or do not determine biological shape directly or by following stochastic and environmental effects (Honda [1999]), either through a selective activation driven by transcription factors (Nusslein-Volhard *et al.* [1987]) or by fractal mechanisms will likely be a matter of lively debate. It is worth noting here that while RNA-interference (RNAi) technology adapted to *Drosophila* cell culture has made it possible to screen systematically for genes controlling specific cell-biological processes, including those required to influence cytoskeletal organization and to generate distinct morphologies (Kiger *et al.* [2003]), very few genes known to control the shape of biological elements, such as in fruit and vegetables, have so far been cloned (Gonzalo and Van der Knapp [2008]).

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