

Fractal analysis: A powerful tool to perform differential diagnosis and prognosis in cancer and other malignancies

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ABSTRACT

Fractal analysis is a technique that has become very useful in the understanding of many phenomena in various fields. In medicine we have one of the most important fields of application of the fractal approach, obtaining interesting results in medical imaging and pathology. Here we review and present a fractal approach based on box-counting techniques that has been successfully applied also by us to perform differential diagnosis, prognosis of the patient and to follow anti-cancer therapies.

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INTRODUCTION

Years ago, on Nature, West et al. described a "general model for tissue growth". The paper was based on the premise that the tendency of natural selection to optimise energy transport has led to the evolution of a "fractal-like distribution network"^[1]. Guiot et al.^[2] have investigated the extension of this model to the growth of solid malignant tumors. Their results "support the notion that tumor growth follows such a universal law" and its "relevance for tumor metastasis, recurrence, cell turnover rates, angiogenesis and invasion" parameters are directly related to prognosis^[1,2].

What is that universal law? Fractal analysis is a technique that has become very useful in the understanding of many phenomena in various fields, such as astrophysics, economics, agriculture and in biology and medicine. In medicine we have, in effect, one of the most important fields of application of the fractal approach, obtaining interesting results in bacteriology, medical imaging, pathology and so on^[3-7], also in our hands^[8-10,27,29,32,33]. Mandelbrot's concept of fractal geometry^[11] has produced the ability to quantitatively char-

acterize a lot of natural structures. Structures that follow new geometric laws, not in common with the classic rules. The term fractal is a geometric concept related to highly irregular shapes, with non-integer, or fractional, dimensions, and a property known as self-similarity. Unlike a smooth Euclidean line, a theoretical fractal line, which has a dimension between 1 and 2, is irregular or wrinkly. Examination of these wrinkles with the lens of a microscope reveals smaller wrinkles on the larger ones. Further magnification shows yet smaller wrinkles and so on. A natural fractal, an object composed of subunits that resembles the larger scale structure, so maintaining the same, at least statistically, fractal dimension (self-similarity or scale-invariant property), is present in a variety of biological structures: cardiopulmonary structures, the ramifying tracheo-bronchial tree, the His-Purkinje network, the cardiac muscle bundles^[12], as well as the placenta's arterial tree^[13]. The meaning of these fractal structures in the human body is profound. The self-similar tracheo-bronchial tree provides an enormous surface area for exchange of gases at the vascular-alveolar interface, coupling pulmonary and cardiac functions^[11]. For the vasculature, fractal

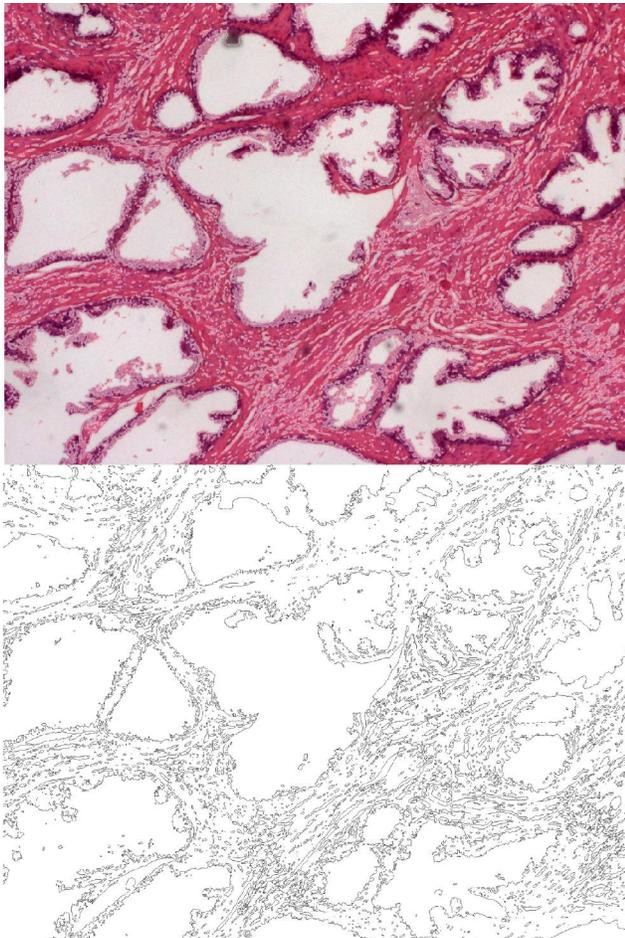


Figure 1 : Top: Prostate. Histological section of hematoxylin-eosin stained normal tissue. Bottom: Binary image after segmentation. Magnification, x40

branching provide a rich network for distribution of nutrients and oxygen, as well for the collection of metabolic waste products^[3]. A variety of other organ systems contain fractal structures that serve function, e.g., the fractal organization of connective tissue in the aortic leaflets that relates to the efficient distribution of mechanical forces^[14].

In effect, network structures and scaling laws developed in broad, quantitative, mathematical approaches must to be characterized in medicine to understand health and disease^[15]. If we follow the strict criteria of a fractal window, where biological components are statistically self-similar, the so-called “scaling windows”, or, in other words, within upper and limits of magnification, we can depict complex biological shapes as fractal entities characterized by a nonlinear behavior^[16].

In effect, fractal analysis is emerging as a powerful tool to perform differential diagnosis and prognosis of

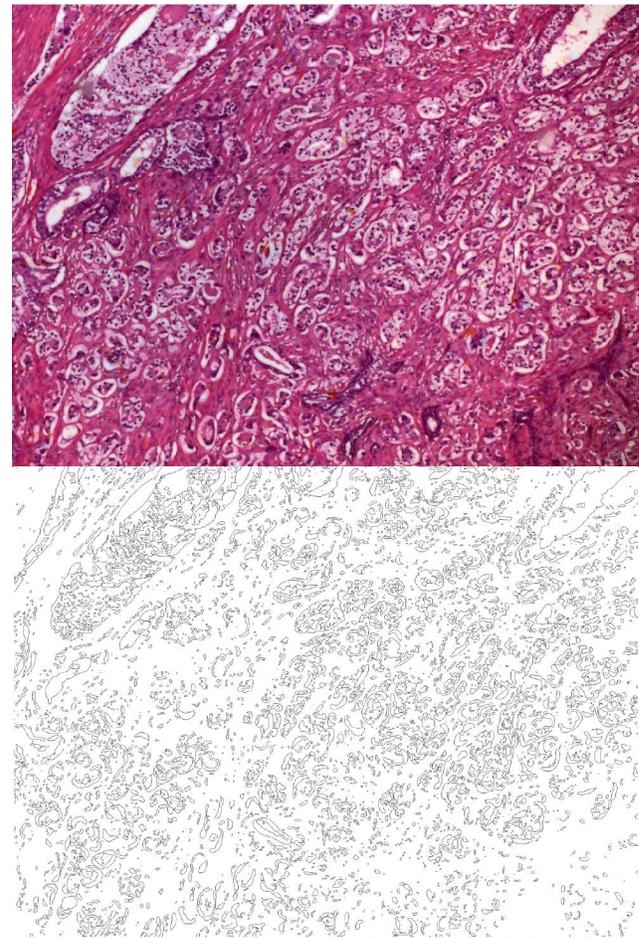


Figure 2 : Top: Prostate, malignant cancer. Histological section of hematoxylin-eosin stained tissue, Gleason score = 4. Bottom: Binary image after segmentation. Magnification, x40

the patients in cancer and other malignancies. Thus, in this paper we will present and discuss the use of fractal analysis to analyze histological specimen of tumors.

IMAGE ANALYSIS

Images are digitized, aperture settings and conditions of illumination and magnification are kept constant. By grey level threshold segmentation, single pixel outlines of the contours of the image are automatically obtained (Figure 1-2).

Fractal analysis

When dealing with random fractals D may be easily calculated on the skeletonized images by box-counting methods.

Box-counting methods have been preliminarily used to calculate fractal dimensions in biological objects and images, such as bronchi^[17], retina^[18] and

Review

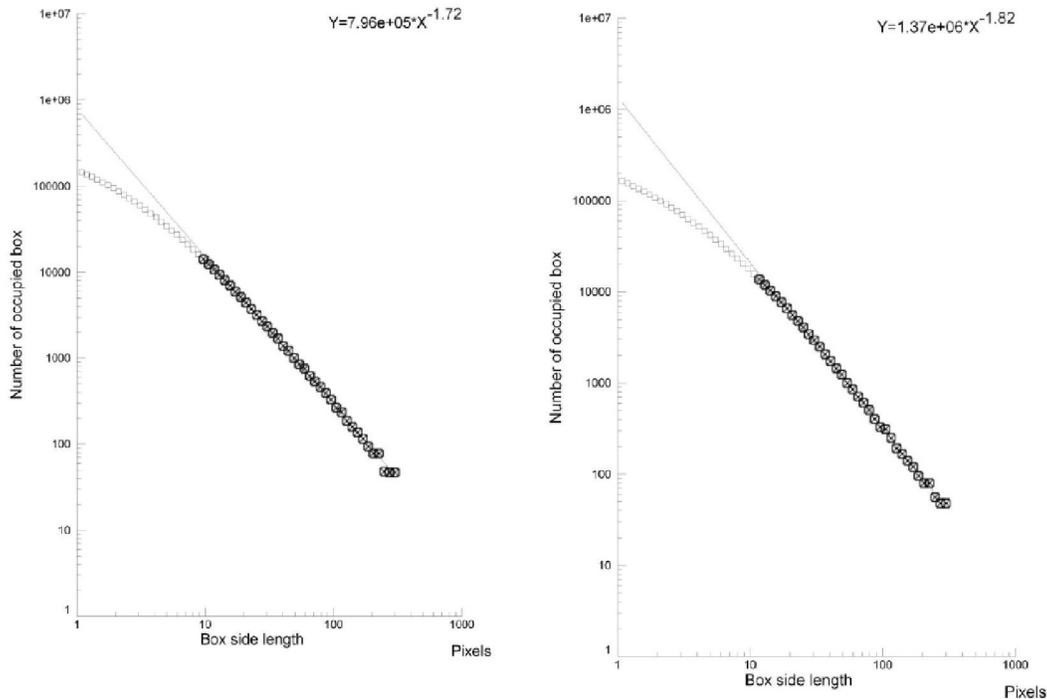


Figure 3 : Log-log plots of the single-pixels outlines of the same samples of prostate as in Figure 1 & 2 (normal features, left; malignant cancer (right) in order to determine the geometric complexity: The histological features are fractals (a straight line is present in the log-log plot: the structure is statistically self-similar), their slopes are the fractal dimension at the chosen scale. The malignancy present a higher fractal dimension than the normal tissue. Original data from an our work in progress

mammograms^[49]. The dimension derived by this methods is calculated by first covering the object (island, cell or tissue for example) by squares or “boxes” with a side length of $1/L$. Geometric complexity and Entropy can be derived.

Geometric complexity (D0)

Briefly, each image is covered by nets of square boxes and the amount of boxes containing any part of the outline is counted. A log-log graph is plotted of the side length of the square against the number of outline-containing squares. If our image is fractal a linear segment appears: the slope of the linear segment of the graph represents the local fractal dimension of the image (Figure 3).

In our example, a prostatic cancer, the log-log plot used to calculate the local fractal dimension shows one line (from 300 to 10 pixels; 1 pixel = 1 micrometer) with high correlation coefficient, always above a value equal to 0.99, thus justifying our fractal analysis. 300 - 10 pixels is our fractal window^[16].

Entropy (Information Dimension, D1)

To evaluate the information (entropy) present in the patterns, information dimension, D1, a robust estimate

from a finite amount of data that gives the probability of finding a point in the image, is calculated. The set is covered with boxes of linear size, d (as above), but now keeping track of the mass, m_i (the amount of pixels) in each box, and calculated the information entropy $I(d)$ from the summation of the number of points in the i -th box divided by the total number of points in the set multiplied for its logarithm. The slope of the log-log plot of Information entropy vs. $1/\text{box side length}$ represented the information dimension of the distribution. The log-log plot used to calculate the information dimensions shows a line from 300 to 10 pixels with high correlation coefficients, always above a value equal to 0.99 (Figure 3), thus justifying our fractal approach.

The methodologies are always validated by measuring computer generated Euclidean and fractal shapes of known fractal dimensions. In our experience inter- and intra-observers errors of the entire procedure are $< 3\%$.

DIFFERENTIAL DIAGNOSIS AND PROGNOSIS BY FRACTAL ANALYSIS

The power of fractal analysis in differential diagno-

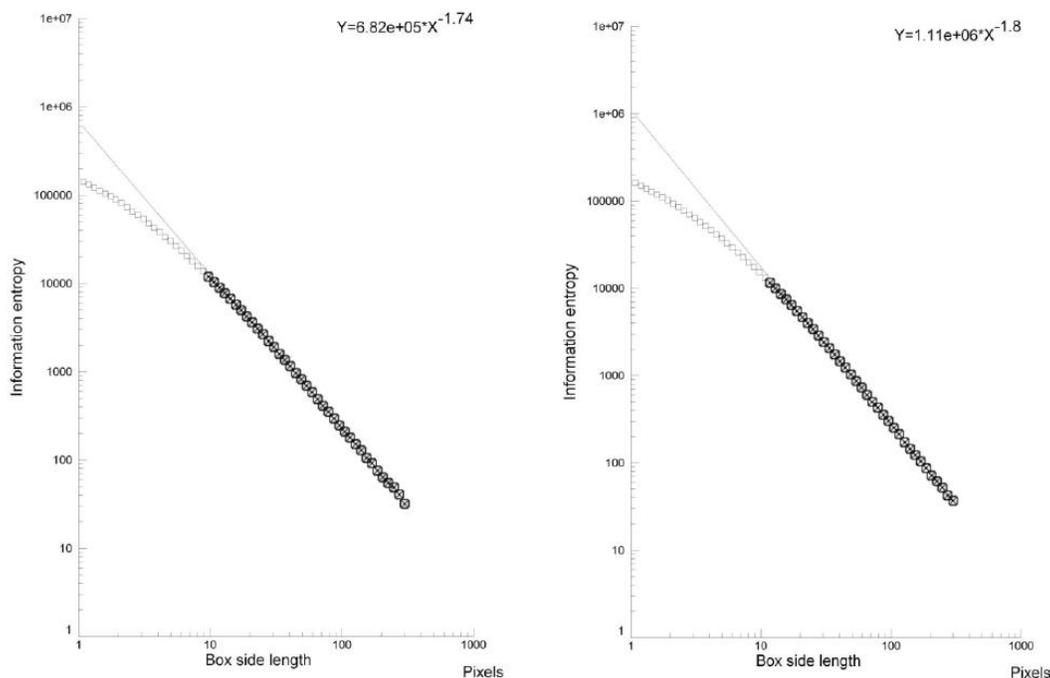


Figure 4 : Log-log plots of the single-pixels outlines of the same samples of prostate as in Figure 1 & 2 (normal features, left; malignant cancer (right) in order to determine the entropy of the image: The histological features are fractals (a straight line is present in the log-log plot: the structure is statistically self-similar), their slopes are the local entropy at the chosen scale. The malignancy present a higher entropy than the normal tissue. Original data from an our work in progress

TABLE 1 : Basal cell carcinoma. Predicted group membership by fractal analysis (geometric complexity of the epithelial-connective tissue interface, light microscopy)

Actual Group	No. of cases	Predicted group membership	
		Low risk	High risk
Group1, Low FD	108	108 100%	0 0%
Group 2, High FD	70	0 0%	70 100%

Percent of grouped cases correctly classified by fractal dimension, according to low risk (NBCC) or high risk (IBCC + BBCC): 100% (ratio between the number of correctly classified cases and all cases), p <0.001

sis in cancer and other malignancies is high Since more than ten years, neoplasms in organs have been studied by fractal analysis for demonstrating differences between normal, dysplastic and neoplastic cells^[20-22] or tissues^[23-24], also by X-ray^[25-27], estimating angiogenesis^[28,29], evaluating the response of anticancer therapy^[30] as well as in the prognosis of the patient (for example, in the squamous cell carcinomas of the larynx, the first work that used FD to perform diagnosis^[31]). In effect, also in our hands, the fractal approach is able to distinguish neoplastic condition till 100% of correct classification. Below, the results we have obtained in basal cell carcinoma (histology^[32]) and in early mycosis fungoides (cytology, transmission electron microscopy of the nucleus border of lymphocyte cells^[33])

TABLE 2 : Early mycosis fungoides. Predicted group membership by fractal analysis (geometric complexity of the nucleus, transmission electron microscopy)

Actual Group	No. of cases	Predicted group membership	
		Dermatitis	Early MF
Group1, Low FD	10	10 100%	0 0%
Group 2, High FD	17	0 0%	17 100%

Percent of grouped cases correctly classified by Fractal Dimension, according to flogosis (chronic dermatitis) or early cancer: 100% (ratio between the number of correctly classified cases and all cases), p <0.001

CONCLUSION

The BB Mandelbrot’s concept of random fractal^[34], a structure which is statistically self-similar, has disclosed new worlds in biology and medicine. Each part of a random fractal has the same statistical properties of the whole^[35]. A typical random fractal is in biology as well in the “coastline of Britain”, as announced by the seminal work by B.B. Mandelbrot^[36]: each randomly selected segment of the coastline possesses the same statistical properties over all scales of magnification^[17]. Just as a coastline, the perimeter of the surface of human gastrointestinal tract reveals more details over numerous magnifications. So, an exact calculation of the pe-

Review

rimeter of an island or a tissue is impossible since it is dependent on the scale of magnification used. That is, if one uses a certain scale to estimate the perimeter, the same calculation for a lower scale would give a different result, since more details are revealed. Instead, a random fractal such as a tissue or a coastline is characterized by its fractal dimension, FD, which is as independent of the scale of magnification, FD.

Diagnosis and prognosis based on morphometric criteria of specific tumors of patients could lead to more precise information on the pattern of tumor spreading, which would emphasize more individualized prognosis factors and, thus, appropriate modality selection. Since the pattern of tumor cells is irregular compared to the cells of the normal epithelium, one could assume that calculating the nonlinear spreading and heterogeneity of neoplastic tissue within the healthy cells will provide an estimate of the tumor's aggressiveness. Presuming that D describes such an irregularity, it can be used for an independent objective measurement of cancer spreading.

Based on accumulating clinical data, it is becoming increasingly clear that fractal analysis is able to exactly and objectively quantify and classify neoplastic lesions. Potent mathematical models based on fractal analysis are growing in order to perform diagnosis and predict responses to therapies of cancer^[37]. Changes in complexity in which fractal analysis is emerging as a reliable model to quantify and describe morphological aspects with potential implications in future clinical and surgical applications, as well as to follow anti-cancer therapy in pituitary adenoma, glioblastoma multiforme and other brain malignant tumors^[38,39]. The analysis is inexpensive and not time consuming (1 minute to skeletonize the image, 10 seconds to perform the fractal dimension analysis by a PC). It may also be performed using cheaper ready-made fractal analysis software, such as Benoit 1.3^[40] and image analysis may also be performed using free software such as JMicroVision^[41]

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