Fractal Analysis: Pitfalls and Revelations in Neuroscience

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Summary. Fractal analysis has become a popular method in all branches of scientific investigation including ecology, physics and medicine. The method is often used to determine effects such as impact of cattle grazing, the distribution of stars within a galaxy or whether tissue is pathological. However several aspects of fractal analysis are not often considered when interpreting results communicated in the literature. These include the concept that no presentation of any pattern on a computer, even for an ideal fractal, is truly fractal. Pre-processing that is also required, such as scanning of images and resizing play a role in the variation of the final fractal dimension. In addition *D* is also a function of the fractal analysis method used and how the final fractal dimension is determined. To obtain a better overview of the effects of the steps involved in fractal analysis and the utility of this method, this chapter describes, using biological material from neuroscience, a non fractal based method, Sholl analysis and continues by discussing various processing options and the results obtained using fractal analysis. The effect of different fractal analysis methods, different computer applications of the same method, scale and resolution as well as regression analysis, which is for most methods the final step in determining *D* are discussed. This provides a platform for a better understanding of fractal analysis in research fields other than physics and mathematics and a more meaningful interpretation of results.

1 Introduction

What is a fractal? A simple definition provided by Mandelbrot states that a fractal structure is one where the structure is invariant under a number of transformations and the structure has no characteristic length.[1] The seemingly simple procedures involved in fractal analysis combined with the suggestion that the fractal dimension (*D*) describes the natural world 'better' than any other parameter, has led to its popularity in analysing natural objects but has also led to some misconceptions that require clarification. The problems in the field of fractal analysis lie in the fact that many experts, being confined within a specific linguistic boundary referred here as fractal literacy, communicate within this domain and therefore do not always provide the necessary information to researchers in different research fields with different subject literacy. This has led to misinterpretations of results due to the apparent lack of a sound description of fractal theory and its relationship to the associated analysis procedures.[2] As an example consider the question ”Are biological forms fractal?”.[3] Strictly speaking, the term fractal can apply only to forms that are strictly self-similar and infinite. Natural objects, are thus better described as prefractals.[4]

Can we then use fractal analysis to discuss forms in nature? As the magnitude of published literature indicates, many seem to think this is possible. Several practical methods based on the mathematics of complex geometry are now in use, including the calliper, box-counting, dilation and mass-radius methods.[5] Descriptions of these methodologies can be found in the literature.[6-8] Of interest here are practical

considerations when applying fractal analysis such as differences in results due to different applications of the same method and determination of the final fractal dimension.[9]

2 Fractal Dimension in Neuroscience

Sholl analysis is a commonly used method to analyse dendritic branching patterns of neurons or certain types of neuron support tissue. [10-12] Fractal analysis however can provide additional data not obtainable by Sholl analysis. Figure 1 illustrates using two hypothetical cells, one with simple and one with a complex branching pattern how fractal analysis differentiated between the two cells, whereas Sholl analysis did not.

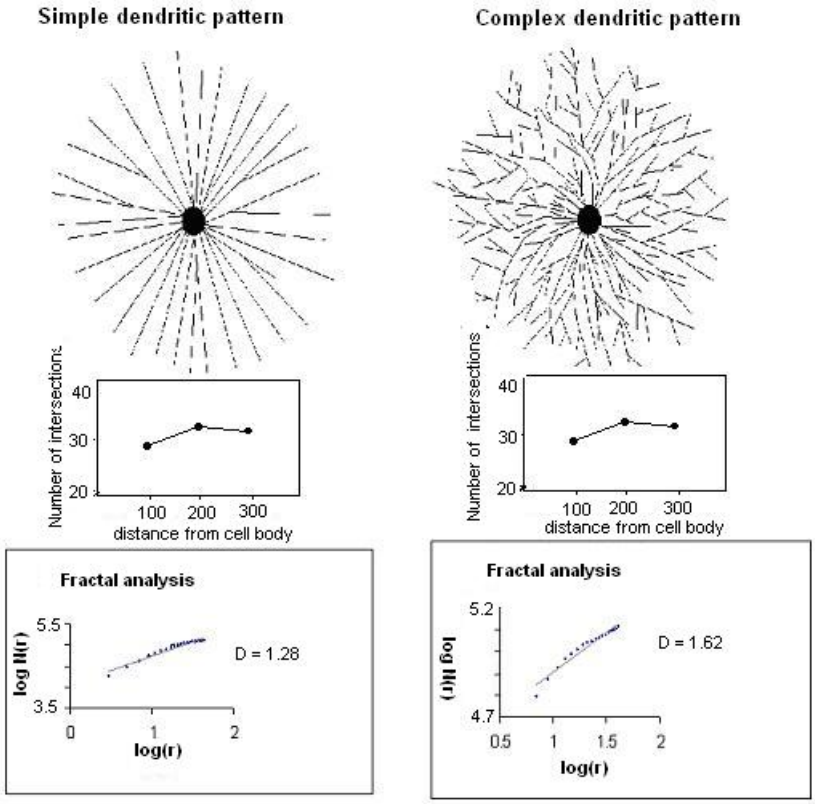

Figure 1: Simple and complex branching pattern analysed using dilation method.

Elston and co-workers have shown using Sholl analysis that the dendritic arbours of layer III pyramidal cells in the primate visual processing pathways increases from low level visual processing areas such as V1 to higher more complex processing areas such

as mediotemporal (MT) area.[13, 14] Figure 2 shows a simplified diagram of the visual processing pathways.[15]

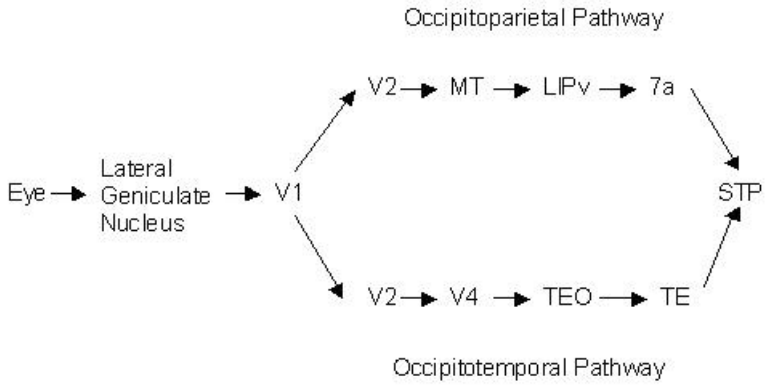

Figure 2: Simplified visual processing pathways. Abbreviations from [15].

Sholl analysis provided the first important insights into differences in dendritic branching patterns from low level to higher level processing in the macaque visual cortex.[12] A summary of the findings for both pathways is shown in Table 1.

a)

	V1i	**V1b**	**V2**	**V4**
V1b	*			
2	*	*		
V4	*	*	*	
TEO	*	*	*	*

b)

	V1	**V2**	**MT**	**LIPv**
V2	n.s.			
MT	*	*		
LIPv	*	*	n.s.	
7a	*	*	n.s.	n.s.

n.s. non-significant difference, * significant difference $p < 0.05$

Table 1: Sholl analysis of a) occipitotemporal pathway, b) occipitoparietal pathway.

As shown in Table 1 not all differences between areas were statistically significant. These results prompted fractal analysis to ascertain whether there were differences in the branching patterns not identifiable through Sholl analysis.

Implementing fractal analysis for cortical layer III pyramidal cells, fractal analysis differentiated between V1 and V2 and showed a trend for increasing *D* except for area 7a in the occipitoparietal pathway. It also differentiated between cells in different sublamina of V1 and between functional subregions in V2 (thin and thick cytochrome oxidase-rich bands). The occipitotemporal pathway showed a systematic increase in *D* corresponding to the position of the cells with lowest *D* in V1, the lowest station in visual processing to TEO/TE, a higher station in the visual processing pathway (Figure2, Table 2).[14, 16]

Area	Mean ± sd
V1 (1)	1.23 ± 0.9
V1 (2)	1.31 ± 0.4
V2 (3)	1.27 ± 0.9
V2 (4)	1.31 ± 0.9
V4	1.29 ± 0.8
TEO	1.39 ± 0.7
TE	1.42 ± 0.7
MT	1.4 ± 0.5
LIPv	1.42 ± 0.5
7a	1.34 ± 0.9
STP	1.44 ± 0.5

(1) middle and upper layer III
(2) layer IIIc
(3) cytchrome oxidase-rich thin bands
(4) cytochrome oxidase-rich thick bands

Table 2: Fractal dimension of cells in occipitoparietal and occipitotemporal visual pathways.

However, despite these findings and other interesting results reported in the literature, comparison of fractal data from diverse studies that utilize different methodologies remains difficult unless the methodologies are clearly outlined. Sources of variation can occur at several steps when applying fractal analysis, including image collection (resolution, image manipulation and scale), choice of fractal method (box-counting, dilation) and determination of the final fractal dimension. The following section discusses these issues using results from neuroscience.

3 Methodological Considerations

Strictly, if it is assumed that the image does not reflect an ideal fractal in a statistical sense (this is the case for biological images), then interpreting the image using *D* is meaningless. The fractal dimension may still be useful though by using it as a quantitative parameter like the dendritic field diameter or surface area that indicates complexity or the scale dependence of a pattern (Kenkel and Walker, 1996). *D* can be used for categorizing images representing morphologically complex objects such as neurons and thus *D* is not intended to indicate that the object is fractal.[3, 17, 18] This fundamental controversy has led to limited but important research into the utility of fractal analysis. Results of this research has suggested that variations in sampling and preparing images for analysis and the analysis procedure can have non-trivial effects on the estimation and interpretation of *D*.

3.1 Scaling

Theoretically images of identical objects at different sizes should not influence the magnitude of *D*. However drawings of the same sample of neurons from V1 of the owl

monkey at two different sizes but at the same resolution of 72 dpi influenced their fractal values. 22 cells were scanned at a standardised absolute scale of 100µm = 3cm on the page and saved at 100%. These images were compared to *D* obtained from images scanned into the computer from their original drawing size on A4 paper and then resized to either 400 x 400 pixels or 600 x 600 pixels. The *D* values returned for the very same cells differed as a result of scaling introduced during image capture and preparation (Figure 3).

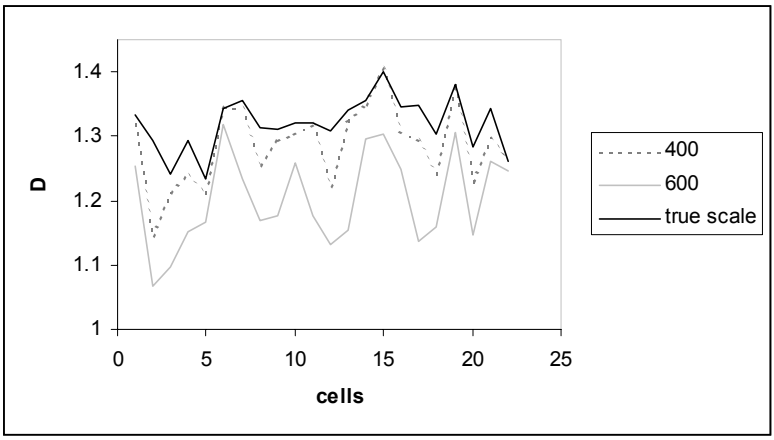

Figure 3: Fractal values of owl monkey V1 pyramidal cells scanned at 72 dpi and analysed at different scales.

An ANOVA indicated a significant difference between the groups ($p < 0.0001$) with cells with the standardised absolute scale (100µm = 3cm) having higher *D* values (mean + S.D.: 1.32 ± 0.04) compared to the 400 x 400 pixels group (1.29 ± 0.06) or 600 x 600 pixels group (1.2 ± 0.07). The standardised absolute scale also had the smallest variance. In Elston and Jelinek's early work they indicated differences observed between visual areas in the macaque when cells that were too large were rescaled to fit the computer screen at a width of 400 pixels.[14, 16] In later work these cells were reanalysed using the standardised absolute scale. *D*s for the cell sample previously analysed differed for some cells more than others, however identical conclusions were drawn in terms of significant differences observed between visual areas.[19] This latter result indicates that, provided the methodology is consistent meaningful conclusions can be drawn. In addition it needs to be noted that the effect on *D* associated with the resizing may not be related to the size *per se* but rather to the computer processing. As such, increasing the size of an image leads to insertion of interpolated (Euclidean) information along the boundaries and therefore changes the value of *D*.

3.2 Resolution

Scanning the same cells at different resolutions (72 dpi and 150 dpi) returned different *D* values, even when all other parameters are kept constant as shown for cells from area V1 (Figure 4). Scanning the drawings with standardized scale at 72 dpi resulted in less variance in *D*. Cells scanned at 72 dpi had, with one exception, higher *D* values than

those obtained at 150 dpi. A student t-test showed a significant difference between the groups ($p < 0.01$). The mean and standard deviation of the 72 dpi and 150 dpi group were 1.31 ± 0.04 and 1.27 ± 0.06 respectively. An ANOVA comparing V1, V2, ITc, ITr and PFC indicated that the p value for the 72 dpi was lower than for the 150 dpi showing a greater likelihood of identifying a difference when using the 72 dpi scanning resolution.

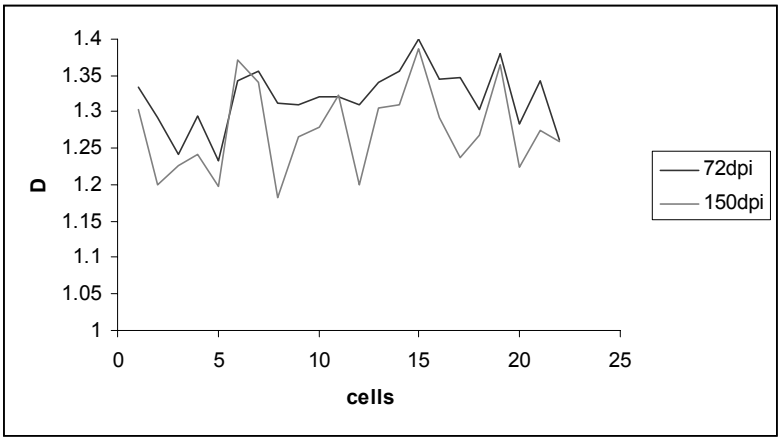

Figure 4: Fractal dimensions of cells from V1 of the owl monkey scanned at 72 and 150 dpi.

3.3 Comparison of Binary, Outlined and Skeletonised Images

Digitised images can be presented as binary, skeletonised or outlines of images. All the work with primate pyramidal cells involved skeletonised images, as we were primarily interested in the branching pattern. However many researchers use different processed images including binary silhouettes or the outline of the images.[20, 21] Jelinek and Fernandez investigated the effect of image presentation as a pre-processing step using more than 200 neurons from cat retina.[18] Binary images, independently of the method used to compute D, showed higher fractal values than outlined and skeletonised images. An analysis of the variance showed statistically significant differences ($p < 0.001$) in their fractal values, which was associated with the much smaller D values of the skeletonised images. When calculating D using complete binary images there may be a space filling effect that can lead to a higher D or a D of 2, depending on the relationship between the internal area and the contour.[22] However, previous results from our laboratory, have demonstrated no significant difference between the estimated D of binary images, binary images with cell body and axon removed or border only images of cat retinal ganglion cells as long as the dendrites are thin with respect to the cell body.

3.4 Fractal Method

It is well known that different fractal methods may return different fractal values for a given object.[6] The dilation method that is discussed here is based on the Minkowski-

Bouligand dimension.[23] A common form of this algorithm, was devised by Flook has been implemented in various laboratories.[24] This approximate dilation method replaces each pixel of the border by a circle/square whose diameter varies within a selected range. Applying a convolution procedure (see NIH macros) structures smaller than the current diameter of the circle/square can be filtered out. The length of the border for each respective diameter is then determined by dividing the area of the outline by the diameter. *D* is estimated from the slope of the log-log plot of length/area against diameter. An alternative dilation method introduced by Costa is the exact distance method. The exact distance method considers for dilation only those distances allowed in the orthogonal lattice underlying digital images.[25]

Here we re-examined the issue of how different applications of the same basic dimension analysis, the Minkowski-Bouligand dimension differ in their estimates of *D* using our samples of cortical pyramidal cells. Drawings of seventy-five neurons sampled from owl monkey V2 and IT cortex were scanned at a standardized scale (3cm = 100μm) and resolution of 72 dpi and analysed using the approximate method and 2) exact distance methods. Our results indicated (means ± s.d; $p > 0.001$) a significant difference between the exact distance (1.361 ± 0.07) and approximate methods (1.429 ± 0.07). A more inclusive analysis of the effect of fractal analysis methods involving 8 different methods and using 192 cat retinal ganglion cells (five box-counting, two mass radius, approximate dilation and one cumulative-intersection) has also indicated significant differences between methods.[18] Even methods that in theory are measuring the same type of dimension (i.e. the box counting procedures from NIH, or from the University of Otago) showed statistical differences in their measured *D* values ($p < 0.001$). However all the results were consistent in that the cells with the highest fractal values had always higher values, and the cells with intermediate or lowest average values independently of the method used, always had intermediate or the lowest average values. These results showed that it is important to distinguish between the precision or reproducibility of the measurement and the absolute accuracy. They also indicate the importance of using the same methodology in order to compare different data sets.

3.5 Regression Analysis

Different authors have used different methods to determine *D* from log-log values because of the limited scale-invariance of neurons. The simplest method of obtaining *D* is to fit a regression line to all data points and determine the slope of this line. The linear region can also be calculated by determining the local slopes. One method for this, described by Caserta for the mass-radius method, is to calculate the n-point local slopes, as the difference in log N(r) divided by log (r) for every *n* successive points. The region in which the local slopes are constant is then taken as the linear region.[26] An extension of this method uses wavelets and the derivative to determine the linear portion of the graph.[27] The use of a hierarchical cluster analysis to compute particular subsets of the log-log values that achieve the best linear fittings has also been reported.[28] This technique allows the detection of changes in *D* at different scales of measurement and compensates for the finite size effects induced by the limited resolution of the images.

When this method results in multiple values of D, it is suggested to use the value with the longest linear range.

Using the results obtained from the approximate and exact dilation methods discussed above, we determined the final fractal dimension by either 1) removing small disks until a predetermined cut-off is reached (r^2 value of 0.996 or greater), 2) find the range of best fit based on minimising the error in y of the regression analysis to 0.0086 or less and 3) a derivative method applied over a polynomial interpolation of the log-log graph of area.[25] The derivative method, known as multiscale fractal dimension, generated data for three parameters: 3a) maximum fractal dimension, 3b) mean fractal dimension and 3c) median fractal dimension. Thus fractal dimension values were generated using five different applications of determining the line of best fit, providing 35 pair wise comparisons. Of all 35 possible pair wise comparisons, a Bonferroni *post hoc* analysis obtained a significant difference between 33 of these ($p > 0.001$). Restricting the pair wise comparisons to within each of the two methods, we found that eight were significantly different within the exact distance method and six within the approximate method ($p > 0.001$). Table 3 shows the probability values obtained from the student t-tests for each alternative with respect to the two dilation methods.

Determination of *D*	**Exact Dilation Method**	**Approximate Dilation Method**
Maximum	2.7×10^{-5}	1.8×10^{-7}
Subtract	8.1×10^{-5}	5.8×10^{-7}
Median	0.00019	3.6×10^{-5}
Best fit	0.00023	2.2×10^{-5}
Mean	0.0013	0.00058

Table 3: P values obtained from student t-test for the 2 dilation methods and 5 regression methods.

The subtract and best fit methods to determine the final D based on a simple rejection rule each perform very well combined with either of the two dilation methods. The maximum method performed optimal but requires some subjective decisions associated with the polynomial fit required as part of determining the derivative. This makes this method not very suitable for use by different investigators. However all methods differentiate between the two groups suggesting that even though absolute values differ between methods the outcome and more importantly the conclusions that can be drawn from the results do not.

4 Conclusion

A fractal analysis is an ideal method for quantification of the branching patterns of dendritic trees, returning data not available by other methods that are based on Euclidean geometry. Fractal analysis can have three separate goals. 1. determination whether or not neurons are fractal, 2. classification of cells, 3. identification of biological meaning associated with D other than inherent in the notion of fractality. However, how these methods are implemented determines the final estimate of the

fractal dimension, *D*.[29] Several methodological criteria need to be considered when applying fractal analysis to avoid unexplainable sources of variation.

Notwithstanding the limitations outlined in this paper, it remains that in many situations a single number, the fractal dimension, summarises concisely the amount of detail and complexity of neurons. More importantly the relative differences observed between cell groups are in most instances identical for different applications of the same method. However differences between methods may be observed as a linear-based method such as dilation measures different attributes of the image compared to a mass-based method such as mass-radius. Thus our results show that different algorithms, and even the same algorithm performed by different computer programs and/or experimenters may give different but consistent numerical values. All described methods demonstrated their suitability for classifying neurons into distinct groups. Our results reinforce the idea that comparison of measurements of different profiles using the same measurement method may be useful and valid even if an exact numeric value of the dimension is not realised in practice.

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