Image Processing of Finite Size Rat Retinal Ganglion Cells Using Multifractal and Local Connected Fractal Analysis.

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Abstract. Automated image processing aids in classification of biological images. Many natural structures such as neurons may be multifractal and therefore not analyzable using current methods. The multifractal spectrum proposed here may mitigate this, Here we report the outcome of applying three methods that elucidate the variation within 16 rat retinal ganglion cells using the local connected fractal dimension (LCFD), mass-radius (MR) and maximum likelihood multifractal (MLM) analyses. Our results based on LCFD indicate that the neurons studied are possibly multifractal. However utilizing the MR method provided inconclusive results due to the finite size of the cells and the density variation throughout their structure. This has been addressed by utilizing a novel unbiased method - the MLM method. To improve the our results we are now aiming to use AI algorithms to optimize the selection of parameter values associated with the MLM method.

1. Introduction

Fractal analysis is a useful tool in automated image processing, as it provides objective, quantitative measures that can help to characterize complex shapes. The aim of this work is to use such measures to increase our knowledge about the structure of neurons. Neurons are known to fall into several types, but distinguishing these types is a continuing problem and can be approached from an AI perspective.[1, 2] As neurons possess fractal structure, the global fractal dimension has been suggested as a useful measure.[3] Calculating the global fractal dimension of rat retinal ganglion cells has provided valuable additional data for classification of these cells and elucidating functional relationships.[4] Our work on rat RGCs suggests that the structure of such tissue is complex, and that there is great benefit to be obtained by applying a more sophisticated analysis than the global fractal dimension such as

multifractal analysis. However, the presence of multifractal features has been demonstrated in the physical sciences such as ecology, but in biology and especially in neuroscience, establishing whether or not neurons are multifractal remains elusive.[3,5] The problem is to determine if the branching pattern of neurons represents one or more developmental processes at work. The images studied in this work did not conform to the expected monofractal or multifractal attributes using traditional fractal analysis. This anomaly prompted us to apply a novel unbiased multifractal analysis method - the Maximum Likelihood Multifractal method (MLM).

1 Fractal analysis

The fractal dimension is a measure of the complexity and self-similarity of an image, and is becoming accepted as a feature for automated classification of images having branching structures. A characteristic of fractal geometry is that the length of an object depends on the resolution or the scale at which the object is measured.[3] This dependence of the measured length on the measuring resolution is expressed as the fractal dimension (D) of the object applicable when structures have a homogeneous fractal pattern distribution (Equation 1).

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where r is the scaling factor and $N(r)$ is the number of subsets for the scaling factor.

Many biological structures such as the dendritic pattern of neurons are not homogeneous (Figure 1) with the periphery being less dense compared to the central parts near the cell body.

Fig. 1. Example of dendritic pattern of rat retinal ganglion cell.

To ascertain the complexity of any number of components within an object, two methods can be used: 1) the local fractal dimension (D_{local}) or the local connected fractal dimension (D_{conn}) and 2) determination of the multifractal specrum.[6,7] D_{conn} has been extensively used in histopathology.[6] However D_{conn} does not indicate

multifractality. The box-counting method has been intensely investigated for use in multifractal analysis in biology but has several limitations.[7] The main problem with this method is its sensitivity to the extent the boxes are filled and is manifested for $q < 0$ where the $D(q)$ function increases monotonically rather than decreases.[8] The determination of the dimension spectrum using the mass-radius method has attempted to address this problem and $D(q)$ spectra decreasing monotonically with increasing q have been obtained for images other than neurons.[9]

To eliminate biases such as a low number of data points and finite size effects, Roberts and Cronin have proposed a Maximum Likelihood Multifractal (MLM) analysis that compares characteristics of the data to artificially constructed multifractals based on a binary multiplicative process.[10] By maximizing the likelihood that the characteristics of the image to be analysed are the same as a multifractal distribution, the multifractal nature of the data may be modeled by the characteristics of the artificial multifractal with the same number of data points as the data set.

2 Methods

Drawings of 16 rat retinal ganglion cells (RGCs) were analysed as binary images We performed a local connected fractal dimension analysis, estimated the multifractal spectrum using the mass-radius method and performed the MLM analysis to show the superior results of this method.

2.1 Local Connected Fractal Dimension

For a particular pixel P in the set, the pixels locally connected to P within a window of set side size (the analyzing window) is computed. Next, the "mass" or number of pixels $N(\varepsilon)$ in increasingly large sub-windows of size ε (all the odd values from 1 to maximum size) always centered at P is counted. This is repeated for all possible (non-empty) locations of the image. The dependence of the number of pixels on a particular window size is a scaling relation, D_{conn} that may be estimated by the linear regression of the logarithm of the mass in a box of size ε on the logarithm of ε. Values of D_{conn} describe the local complexity of the locally connected set.[6]

2.2 Mass-Radius Multifractal Analysis

The mass-radius method is a measure of mass distribution. Consider all circles of radius r that have their centre on the object. Let $M_i(r)$ be the mass within the *ith* circle and the total number of circles of radius r be $N(r)$. Then $Z(q,r)$, which is a density measure where q acts as a filter is defined as

$$(= (|) | =) . \quad (2)$$

The multifractal dimension *D(q)* is given by

$$(=) / (. \quad (3)$$

2.3 Quaternary Maximum Likelihood Multifractal Analysis

For each image, the inter-point distances of all data points was determined to estimate the partition function *Z(q,r)* as a function of length scale *r*. Plotting *log Z* versus *log r* a curve is fitted and any changes in the slope are identified. The kink in the slope separates the data into small and large-scale measures. Our analysis was restricted to the larger, relative coarser, length scales consisting of a few hundred data points to reduce the processing time. For the large-scale analysis Approximately 400 data points were retained from each image. For the multifractal analysis, the program fits a multiplicative quaternary multifractal to the inter-point distance information summarized in the correlation density function *Z(q,r)*. From the parameters of the best fit we determine any multifractal properties, such as the appropriate generalized dimension curves.

3 Results

3.1 Local Connected Fractal Dimension

Figure 1a depicts the distribution of the local connected fractal dimensions for all sixteen cells. Notice that one cell stands out as very different from the rest. For D_{conn} *~1.35* there is more than one order of magnitude difference in relative frequency of local connected dimension counts between this cell and the other cells. This analysis confirms that all the cells examined, with the possible exception of one, may be considered multifractal and warrant further analysis.

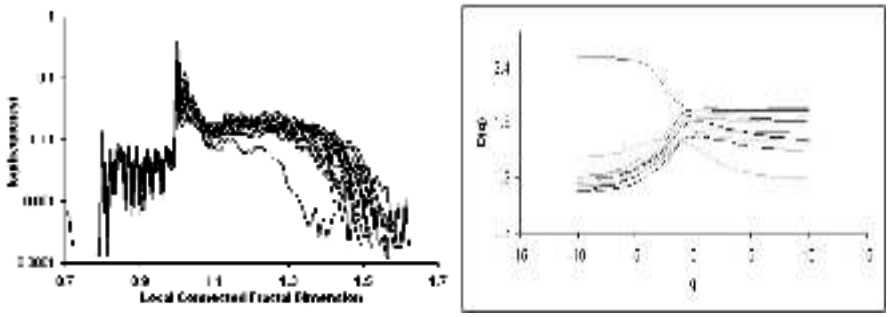

Fig. 1a. The distribution of local connected fractal dimension for the 16 rat ganglion cells. **1 b.** Mass-radius method multifractal analysis D(q) spectrum for all 16 images.

3.2 Mass-Radius Multifractal analysis

The results of the multifractal analysis using the mass-radius method are shown in Figure 1b. For the images studied, the *D(q)* spectrum is monotonically increasing for negative *q* apart for one cell image.

3.3 Quaternary Maximum Likelihood Multifractal Analysis

Figure 2 shows the multifractal spectra for all 16 cells analyzed using the MLM analysis. Note the difference in slope between $q = -1$ and $q = 3$, clearly indicating that the majority of cells are based on a multifractal construction. Of particular interest is the cell indicated above with the LCFD analysis (Figure 1a), which in this graph stands out, with a very low value (1.5) for D(q) when q = 3. Unlike the results of the mass-radius method, the MLM Analysis has confirmed that the cell identified as different from the others by LCFD analysis is indeed multifractal.

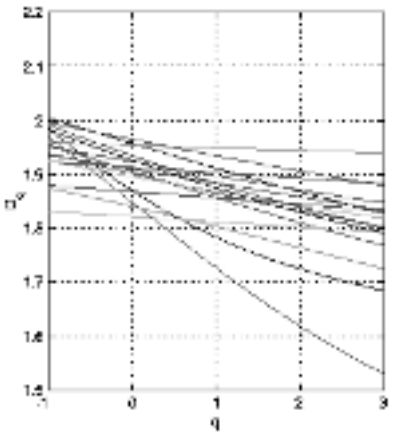

Fig. 2. Estimated generalized dimensions for the large scale branching structure of each of the cell images using the maximum likelihood multifractal analysis.

6 Discussion

Applying MLM analysis, provides quantitative evidence of the multifractal nature of neurons. Various approaches have been investigated that identify morphological differences at a local level. Our results clearly indicate a range of LCFDs associated with rat RGCs. Although the mass-radius method indicates some heterogeneity of the images the results are anomalous due to the increasing D(q) spectrum.[3] The MLM method is ideal as it uses less processed data and allows analysis of finite-size images. In addition the number of points used in the analysis can be selected and thus the scaling region. We suggest that the MLM is an improvement on existing methods for the multifractal analysis of biological material. This is because the method depends on the image data being superimposed on the quaternary multiplicative process. The results from this method pertaining to a range of q values and combined with other morphological parameters such as circularity, density or area provide the basis for AI methods to be applied optimally for cell classification paradigms.[1]

Acknowledgements

The authors like to thank Leo Peichl from the Max Plank Institute for Brain Research who provided the cell drawings and Cherryl Kolbe for technical assistance.

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