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REVIEW ARTICLE

FRACTAL ANALYSIS IN NEUROANATOMY AND NEUROHISTOLOGY

FRAKTALNA ANALIZA U NEUROANATOMIJI I NEUROHISTOLOGIJI

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ABSTRACT

For decades, in the field of neuroscience, research includes the use of different methods which allow us to visualize the elements within the nervous system, primarily nerve cells. An integral part of such research is the quantification of the analyzed image, which largely relies on the use of traditional mathematical methods, based on linear analysis. On the other hand, fractal analysis as a form of non-linear analysis, can give us more detailed information about the complexity of some anatomical or histological structures, which classical Euclidean geometry is not able to adequately describe and quantify. This review aims to show the possibilities of fractal analysis in neuroanatomy and neurohistology, i.e. in the analysis of images of different components of the nervous system and nervous tissue.

Key words:
fractal dimension;
brain;
neuron;
neuropathology

SAŽETAK

Istraživanja u oblasti neuronauka već decenijama koriste raznovrsne metode kojima na mnogobrojne načine mogu da se vizuelizuju različiti elementi u sastavu nervnog sistema, pre svega nervne ćelije. Sastavni deo ovakvih istraživanja podrazumeva kvantifikaciju analiziranih slika, koja se većim delom oslanja na upotrebu tradicionalnih matematičkih metoda zasnovanih na linearnoj analizi. S druge strane, fraktalna analiza, kao oblik nelinearne analize, može nam dati mnogo više podataka o kompleksnosti neke anatomske ili histološke strukture, koje klasična Euklidova geometrija nije u stanju da adekvatno opiše i kvantifikuje. Ovaj pregledni rad ima za cilj da prikaže mogućnosti upotrebe fraktalne analize u neuroanatomiji i neurohistologiji, tj. u tumačenju slika različitih komponenti nervnog sistema i nervnog tkiva.

Ključne reči:
fraktalna dimenzija;
mozak;
neuron;
neuropatologija

The authors declare no conflicts of interest.

UNIVERZITET U BEOGRADU MEDICINSKI FAKULTET
UNIVERSITY OF BELGRADE SCHOOL OF MEDICINE

doi:10.5937/mp67-12385
Editorial board: podmladak.med.bg@gmail.com
ISSN: 2466-5525

INTRODUCTION

In the past few decades, enormous advances have been achieved in the field of brain research. Technological breakthroughs in the fields of microscopy have allowed us to better visualize and understand the structure of nervous tissue and cells. By using different imaging techniques and neural computation methods, we are now even able to reconstruct and study the connectivity map of the brain, which has led to the creation of an entirely new research in the field of neuroscience, called connectomics (1). However, as new methods of imaging are being discovered, the expenses of such techniques are rapidly increasing. Thus, low-income countries are not able to follow the modern neuroscience research, which brings them in backlog compared to richer countries, which are able to finance such research. Because of this, a large number of researchers are using different mathematical methods for image analysis, which are inexpensive and in the same time can provide sufficient amount of information needed to draw certain conclusions.

One of the methods whose use is rapidly increasing in biomedical research is fractal analysis. Based upon non-Euclidean geometry, i.e. fractal geometry, this non-linear analysis method has found its broad use in neuroscience, where it is used for the quantitative descriptions of nervous tissue cells, as well as the nerve tissue as a whole, in physiological but also pathophysiological conditions (2).

FRACTAL GEOMETRY

Fractal geometry is a branch of mathematics dealing with the analysis of irregular patterns made of elements that are in some way similar to the whole (3). The fractal theory concept was developed by Benoit B. Mandelbrot (1924 – 2010) who coined the term fractal, derived from the Latin word *frangere* (to break or to fragment) (4). Fractal elements that can be found in nature have to fulfill a certain number of criteria, most notably characterized by four distinct properties: 1) irregularity of their shape, 2) self-similarity of their structures, 3) non-integer or fractional (fractal) dimension, and 4) scaling, which means that measured properties depend on the scale at which they are measured (2). Objects that can be found in nature, especially in the living organisms, have irregular and rough shapes that are responsible for the objects complexity and thus make it difficult to describe such structures using Euclidean geometry. By using fractal geometry and its main parameter fractal dimension (FD), we are able to describe the space-filling properties of irregularly-shaped objects (2). Thus, when we talk about the FD of a biological object, we are describing a statistical measure that correlates the morphological structural complexity of cellular components and biological tissues (4).

FRACTAL DIMENSION: THEORY AND CALCULATION

As previously mentioned, fractal analysis measures the complexity of a certain geometrical figure. The complexity is quantified by FD, which represents a basic parameter in fractal analysis (5). The FD value of an object depends on the ruggedness and irregularity of its borders and it is also used to describe the space-filling properties of a pattern (5, 6). This is particularly important in neuroscience research, since it indicates how densely the pattern occupies a portion of the metric space in which it is embedded (7). In this way, quantitative information about how a particular neuron occupies a certain portion of space, e.g. nervous tissue, is obtained.

There are several published techniques for calculating the FD (5, 8). One of the most commonly and widely used methods is the box-counting method, where a grid of square cells (with cell size r) is superimposed over the binary image. The total number of square cells (boxes) intersecting with the image pattern is counted and this step is further repeated with different cell sizes r, where the cell size is expressed as the number of pixels (**Figure 1**). The number of squares $N(r)$ needed to cover the image is given by a power law: $N(r) = \text{const} \cdot r^{-D_B}$,

where D_B is the box dimension, obtained as an absolute value of the slope of the log-log relationship between $N(r)$ and r (9).

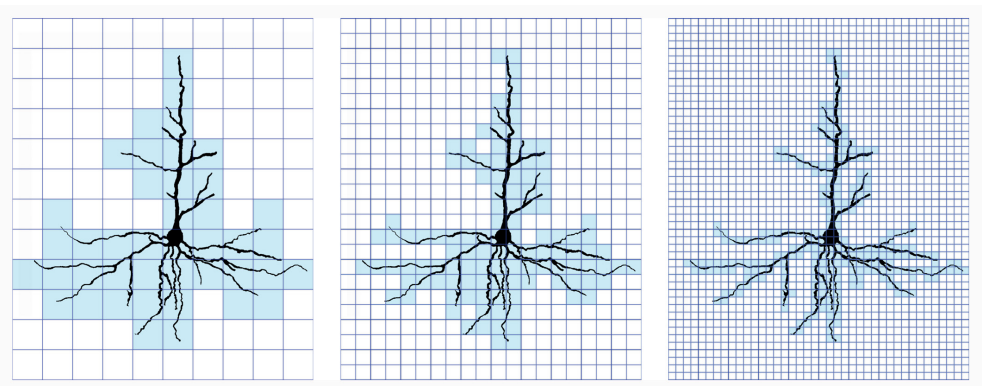

Figure 1. Box-counting method of fractal analysis. The figure represents an example of how the box-counting method works when applied on 2D histological reconstructions of neurons. An image of a superficial pyramidal neuron from rat cerebral cortex is covered with boxes, whereby in each of the next steps the procedure is repeated with boxes of different sizes. Only the boxes intersecting with the neuron (colored in blue) are counted when determining the FD of an object

One of the reasons why fractal analysis has gained such popularity in the past decade is that the calculation of box-counting FD is simple, easy and most importantly, it can be done automatically in different freeware software's. The most popular program in biomedical research used to calculate the FD is the *ImageJ* software (NIH, Bethesda, USA; free download available on http://rsbweb.nih.gov/ij), which has a built in command for doing the box-counting analysis. Researchers can also install a free *ImageJ* plugin called *FracLac* (built by Audrey Karperien; free download available on https://imagej.nih.gov/ij/plugins/fraclac/fraclac.html), which allows users a wider range of options and the ability to calculate additional useful parameters, such as lacunarity. The only prerequisite for analysis is that the images are previously converted to grayscale and "black-and-white" (binary) forms, which can also simply be done in the *ImageJ* software. Thus, calculating the FD of a certain object represents a simple task. However, what to analyze and what are the appropriate images or parts of the images that will be analyzed is the main question, which lies in front of every researcher.

WHAT TO ANALYZE AND HOW WITH FRACTAL ANALYSIS?

In order to apply the fractal analysis and calculate the FD of an object, an adequate capture and processing of digital images is required first. Thus, a neuroscience researcher should be aware of the possibilities, limitations and methodologies for applying the fractal analysis. One of the main things in which fractal analysis can be of a great use is the determination and quantification of neuron morphology, obtained from 2D histological images. The neurons can be visualized by using different immunohistochemical and histochemical techniques, such as Golgi's silver impregnation method, which is one of the oldest, but still the best method for visualizing the cell body and its neurites. By calculating the FD we are able to compare the changes in the complexity between different types of neurons subjected to various factors and conditions. Therefore, after obtaining digital photographs of cells, additional image processing is needed to isolate single neurons and/or their branches from the surrounding image area. This can be done automatically by certain programs or one can simply manually crop the desired object or erase everything except the object of interest, in any photo editing software. The automatic option, in addition to the advantage in speed, is also better since it preserves the ruggedness of the objects border, which may be more or less disturbed in the manual option and thus may affect the final FD value.

It has been reported previously (10) that the box-counting method applied on binary images of neurons depends on the way the image is processed for fractal analysis. The FD of a whole neuron or its parts can be calculated in three main forms and from three types of images: 1) whole "black-and-white" (binary) image; 2) binary-outlined image; 3) binary-skeletonized image (**Figure 2**). Each of these forms measures different parameters. The first method, measures the space-filling property of an object, while the second method assess the irregularity in the shape of an object (11). The third method is especially useful in calculating the FD of dendrites and summarizes the degree of dendrite aberrations from straight lines (7). Due to the different parameters that are calculated by these methods, it is sometimes necessary to calculate all of these values, in order to obtain relevant results.

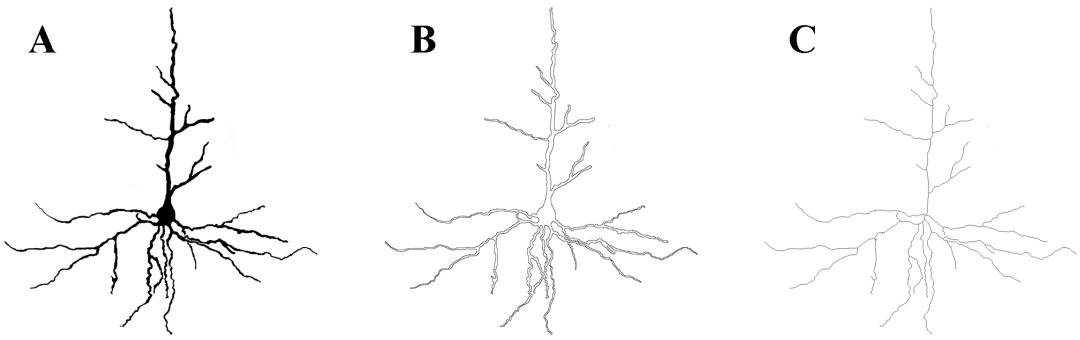

Figure 2. Three binary forms of 2D neuronal reconstructions used for calculating fractal dimension. The figure shows a binary reconstruction of a superficial pyramidal neuron from rat cerebral cortex in original binary form (A), outlined form (B) and skeletonized form (C). The outlined form represents the borders of the neuron, while the skeletonized form is obtained when the thickness of the neuronal branches is reduced to the width of a single pixel

Comparison of two or more groups of cells demands that the images of those cells are taken under the same conditions, i.e. image size and resolution, since the FD value is dependent on those two parameters (12). Additional problem in calculating the FD is the position and orientation of the object on the canvas. Namely, one of the most abundantly used methods to calculate the FD is the previously mentioned box-counting method. This method, although simple and efficient is dependent on the objects rotation. Thus, if the object of interest is rotated in different manners, the FD value will also change (13). One of the simple solutions for this is to place the object in the exact middle of the canvas and to rotate them so their longest diameter is parallel to the x or y axis. In this way, the influence of rotational variation on the FD value can be diminished. The other solution can be to rotate the object multiple times at different angles and then to calculate mean from all rotational degrees. The rotational vari-

ation is especially important when calculating the FD of non-stellate neurons (e.g. pyramidal neurons), which lack strong radial symmetry (13, 14).

Besides histological staining protocols used to visualize nervous tissue, different neuroimaging techniques also represent a valuable source of digital photographs that can be studied by fractal analysis. Magnetic resonance imaging of healthy human subjects has showed that cerebral cortex gray matter has a fractal-like organization and thus is suitable for this kind of mathematical analysis (15). Nowadays, the fractal-like property of the brain is widely accepted and proven not only for MRI images of the cerebral cortex (16), but also for other structures of the brain, such as the cerebellum (2, 17, 18). The fractal analysis of brain MRI photographs is also done automatically by a standard box-counting algorithm, but still requires a previous pre-processing of the obtained images. Given the fact, that the advances in modern imaging techniques are being developed on a daily basis, the image processing and fractal analysis are now mostly automated and several different mathematical algorithms, which perform these tasks, can be found (19). In recent years, fractal analysis of retinal blood vessels has also gained significant attention (20). Changes in the branching patterns and branching density of retinal vascular network, as well as the changes in its space-filling properties, can be described by FD (6). Since retinal blood vessels can be easily visualized by digital funds camera, the acquisition of such images thus represents a relatively simple task. However, the extraction of the binary images of retinal blood vessels may arise as a difficult task due to their complex nature, i.e. branching and tortuosity. Different approaches for automatic segmentation of retinal blood vessels are available (21-24), although manual segmentation still remains as an option, especially when the vessels are not clearly outlined by automatic-segmentation algorithms (25).

APPLICATION IN NEUROSCIENCE RESEARCH

As mentioned in the introduction, modern neuroscience research is dependent on the use of sophisticated and thus expensive cellular and molecular laboratory methods. Low income tax countries have limited resources they can direct to this biomedical field, which is why fractal analysis can take an important place in the study of nervous system. Besides its simplicity and sensitivity, it is more importantly fast and cheap and requires almost no financial investment in technical equipment (26).

Within the field of neuroscience research, fractal analysis can be applied on various neuroimaging techniques, in order to quantify the complexity of brain cells and nervous tissue, both in physiological and pathological conditions (2). One of the first studies, which showed the usefulness of fractal analysis in neuroscience research, dealt with the morphological aspects of glial development (27). Following this research, further studies have concluded that FD of neuron and glial cells corresponds with the increase in their morphological complexity during development and maturation (2, 28).

Fractal analysis has also been applied in distinguishing and supporting the classification of different types of neurons located in the retina, spinal cord and dentate nucleus (29-32). Thus, a long term hypothesis that large principal neurons from the human dentate nucleus can be classified into four distinct types was proven by analyzing their morphometric parameters, such as the FD of dendritic branching complexity (31). Similarly, by analyzing the apical dendritic arborization of pyramidal neurons from the rat cerebral cortex, it was shown that the FD of superficial and deep apical dendrites of these neurons differs, in terms of a larger FD in superficial neurons (14, 33). This result can be interpreted as a consequence of higher degree of complexity exhibited by the apical dendritic arborization of superficial pyramidal neurons, which corresponds to earlier findings in the monkey motor cortex, where pyramidal neurons from lamina II–III had higher FD than those from lamina V (34). Fractal analysis was also able to detect the changes in the dendritic arborization complexity of pyramidal neurons from the CA1 region of the hippocampus, after neonatal Bacille Calmette-Guérin (BCG) vaccination (35).

In addition to the analysis of single cells or their parts, such as dendrites, fractal analysis has also shown a great value in the analysis of certain brain areas and layers. Structural analysis of the hippocampus, an important brain area involved in memory formation, emotional processing and stress response (36), has shown that fractal analysis has high discriminatory ability in distinguishing two morphologically similar regions of the rat hippocampus: stratum lacunosum-moleculare and stratum radiatum (37). Also, it was shown that FD is a good indicator of axonal orientation in white matter regions, such as corpus callosum and cingulum (38).

Certainly the most important use of fractal analysis is in the quantification of brain tissue complexity changes in different neuropsychiatric diseases. Most of the conducted research used MRI of different brain regions to analyze their alterations in shape and space-filling pattern (18). Thus, changes in the brain volume and shape, both in gray and white matter, can be described and quantified by FD. In multiple sclerosis, a progressive demyelinating disease of the brain, FD of the brain white matter was found to be decreased even in patients who were in early phase of the disease (39). On the other hand, changes were also found in gray matter, where patients with multiple sclerosis had a significant increase in the FD of the gray matter, compared to controls (40). Changes in the brain white matter FD were also found in patients with amyotrophic lateral sclerosis (41), multiple system atrophy of the cerebellar type (42), but also age and gender related complexity alterations have been detected (43).

The possibilities of applying fractal analysis in the research of nervous system diseases is numerous and it is not only limited to the analysis of the entire brain regions or individual neuronal and glial cells. For example the

variations in the amyloid deposits, a hallmark of the Alzheimer's disease, can also been analyzed by fractal analysis and quantified by FD (44-46). The results of these studies have shown that FD is able to differentiate various plaque types, but can also be used to study the genotype–phenotype correlations in Alzheimer's disease (46). Microvasculature complexity of brain tumors can also be expressed through FD, which is of great importance since different tumors show diverse angiogenic patterns of branching (47). Fractal analysis of WHO grade II and WHO grade III gliomas, has shown that histopathological specimens of grade III gliomas are generally more vascularized than grade II gliomas (48). Since histopathology is still a golden standard in the diagnosis of brain tumors, the addition of complexity parameters such as FD, to the final analysis, could aid in the discrimination between grade II and grade III gliomas (49) and also other types of brain tumors, which show different microvasculature branching patterns.

Besides the fractal analysis of neuronal populations, microglia has also been analyzed by this mathematical method. Given the fact that microglia vary in size and shape as they cycle, migrate, wave, phagocytose, extend and retract their processes, it is clear that standard morphological measures cannot adequately describe and quantify such variability (50). Therefore, fractal analysis is imposed as the preferred method of choice, in the classification of these cells. Namely, FD is able to differentiate between protoplasmatic, fibrous and activated astrocytes (51). However, fractal analysis has now exceeded its use in a simple classification of microglia and has mostly been used for the quantification of their morphology, which varies in different pathological conditions of the nervous system (2, 50, 52). Although stroke and dementia are two pathological conditions with different underlying mechanism, changes in the morphology and function of astrocytes are common for both injury and neurodegeneration. Fractal analysis of these conditions has shown that astroglial transformation occurs in both diseases and that significant differences can be found when comparing the FD of astrocytes isolated from stroke patients and Alzheimer's disease patients (51). Thus, FD is able to quantify gliosis in different neurological disorders and could be used as a valuable method in neuropathology research.

As mentioned previously, quantification of the retinal vascular network complexity by FD, has gained significant attention in research dealing with blood vessel alterations, in numerous neurological diseases (20). Fractal analysis of retinal vasculature has found its research and diagnostic application in stroke (53-56) and Alzheimer's disease (57, 58), but also in non-neurological disorders such as hypertension (20, 59-61), diabetic retinopathy (25; 62-64), chronic kidney disease (65, 66) and HIV (67).

CONCLUSION

As it can be seen from the abovementioned examples, fractal analysis plays an important role in determining the complexity of biological objects in both physiological and pathological conditions. Thus, just by using one quantitative descriptor, i.e. FD, we are able to quantify the shape irregularity and self-similarity of nervous cells and tissue. In today's modern neuroscience research, different methods for examining the structure and function of the nervous system are constantly being developed. Although several decades old, non-linear and fractal analysis can still serve as excellent methods for data analysis, especially if we bear in mind that these methods can more precisely describe the brain complexity than traditional mathematical and computational methods, centered around linear and deterministic analysis (68). The need for non-linear analysis and fractal analysis will certainly be present in future research, especially in the analysis of neural networks, where it can help us to better understand the complexity and self-similarity underlying our brain organization.

ACKNOWLEDGMENT

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No. 175061

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