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Modern methods of neuroanatomical and neurophysiological research

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REVIEW HIGHLIGHTS

- The article demonstrates the authors' research in the field of neuroanatomy using modern methods.
- In addition to their research, the authors who are also clinical neurologists, emphasize the connections between neuroanatomy and neurophysiology and the clinical significance of their long-term research in the article.
- Their research incorporates mathematical elements such as fractal analysis and utilizes advanced computer and information technology techniques in anatomy.

ARTICLE INFO

Method name:
Specific combination of modern methods of neuroanatomical and neurophysiological research: injection techniques, infrared and ultraviolet analysis, Pickworth method, scanning microscope, computer image processing, fractal analysis, metrological analysis using image analysis methods.

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Cranial base development
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ABSTRACT

Our article explores a variety of modern research techniques employed in neuroanatomy and neurophysiology. We highlight the use of computer technologies, image analysis methods, and innovative approaches that expand our understanding of anatomical structures. The techniques we discuss include fractal analysis, the Pickworth method, scanning microscopy, and advanced computer image processing systems. Fractal analysis, in particular, offers a unique perspective on brain structures and functions and is a key tool in neuroanatomical research. We also focus on its application in neuroanatomical studies, particularly in cases of Alzheimer's disease and epilepsy.

These modern research methods not only enhance our knowledge but also have significant clinical potential. Their use in diagnosing neurological diseases like Alzheimer's and epilepsy promises faster and more accurate diagnoses. We emphasize the combination of multiple methods for improved quality of anatomical structure imaging.

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Specifications table

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Name of your method:	Specific combination of modern methods of neuroanatomical and neurophysiological research: injection techniques, infrared and ultraviolet analysis, Pickworth method, scanning microscope, computer image processing, fractal analysis, metrological analysis using image analysis methods.
Name of the reviewed methodology:	Modern methods of neuroanatomical and neurophysiological research: computer image analysis of anatomical structures; fractal analysis and metrological analysis.
Keywords:	injection techniques; infrared and ultraviolet analysis; Pickworth method; scanning microscope; computer image processing systems; anatomical structures; fractal analysis; metrological analysis; cranial base development; Alzheimer's disease
Resource availability:	N. A
Review question:	Can modern image processing and analysis techniques bring new information to the description of human anatomy, in particular its nervous system? Can anatomical research using modern methods have clinical significance in neurology? What state-of-the-art research methods are particularly suitable for studying anatomical structures?

Background

In the vast landscape of biological sciences, anatomy stands as a cornerstone with roots extending deep into antiquity. Its role is pivotal in modern medicine, providing the foundation for precise diagnosis, surgical planning, and therapeutic advancements. Recent years have witnessed a transformative breakthrough in anatomy propelled by modern technologies, particularly computers. This evolution has ushered in a new era of anatomical research, enabling more advanced and precise analyses of anatomical structures.

The advent of modern research techniques, including advanced imaging methods, three-dimensional data analysis, and computer technologies, has significantly contributed to this paradigm shift. The research focus extends to the fetal development of the human brain and skull, utilizing these techniques to unravel the intricate processes shaping anatomical structures from their earliest stages.

A critical realm where modern anatomical technologies play a pivotal role is in the study of the brains of individuals afflicted by Alzheimer's disease. Through in-depth structural analyses supported by computer image processing techniques, researchers delve into the intricacies of this neurodegenerative disease. This not only facilitates an understanding of anatomical changes but also unveils potential avenues for therapeutic exploration.

The article delves into a plethora of modern research techniques employed in anatomical studies, ranging from injection techniques, infrared and ultraviolet analysis to the Pickworth method, scanning microscopy, and advanced computer image processing systems. The synergy of these tools allows for the exploration of microscopic details and precise examination of anatomical structures. Emphasis is placed on the importance of combining multiple methods to enhance imaging quality while exercising caution to prevent distortion.

The work is driven by fundamental questions:

Can modern image processing and analysis techniques provide novel insights into human anatomy, particularly the nervous system?

Does anatomical research utilizing modern methods hold clinical significance in neurology?

Which state-of-the-art research methods are particularly adept at studying anatomical structures?

The conclusions drawn from the study underscore the transformative impact of modern research techniques on understanding anatomical structures. These methods not only expand the scope of research but also harbor significant clinical potential. Their application in diagnosing neurological diseases like Alzheimer's and epilepsy promises expedited and more precise diagnoses, fostering collaboration between science and medicine to enhance healthcare and deepen our comprehension of human anatomy. Modern research techniques emerge as a cornerstone of progress in anatomy and medical sciences.

Specific findings, such as the significant differences in the fractal dimension of the cerebral cortex in Alzheimer's patients, position fractal analysis as a promising diagnostic measure for early detection of pathological changes. The insights gained from the analysis of electrical brain activity in epilepsy patients contribute significantly to knowledge about the disease, advocating for advanced methods in computer EEG analysis.

The integration of fractal analysis into the diagnosis of nervous system diseases represents a breakthrough, offering a more accurate understanding of structural and functional changes. This precision is pivotal for effective treatment and patient monitoring, establishing fractal analysis as a valuable tool in unraveling the complexities of the nervous system.

In essence, the work presented underscores the intrinsic connection between modern research techniques, clinical applications, and the advancement of anatomical understanding, portraying them as indispensable elements propelling progress in anatomy and medical sciences.

Method details

Anatomy, an integral part of biological sciences, has a long and fascinating history whose roots date back to antiquity. Anatomical knowledge is the unquestionable basis of modern medicine, enabling precise diagnosis, planning of surgery and development of therapy.

In recent years, there has been a breakthrough in the field of anatomy thanks to modern technologies, especially computers. This evolution opened new horizons in anatomical research, enabling more advanced and precise analysis of anatomical structures.

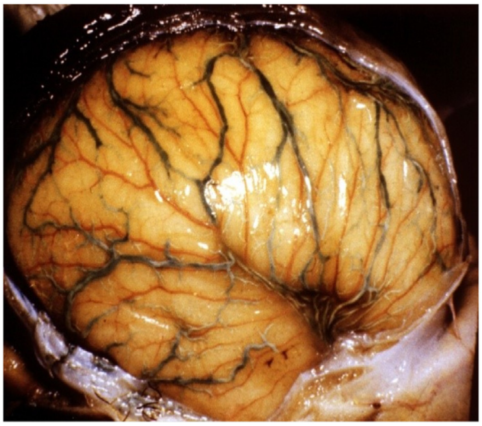

Fig. 1. Lateral surface of the brain of a 6-month-old fetus. 3022 latex dyed with pigments.

Examples of modern research techniques in anatomy include advanced imaging methods, three-dimensional data analysis, and computer technologies. In our research, we use these methods to conduct research on the fetal development of the human brain and skull, which has enabled us to better understand the processes of shaping anatomical structures from the earliest stages of development [1].

One of the areas in which modern anatomical technologies play a key role is research on the brains of people suffering from Alzheimer's disease. In-depth structural analyses, supported by computer image processing techniques, allow us to explore the secrets of this neurodegenerative disease. This makes it possible not only to understand anatomical changes, but also to search for new therapeutic paths [2–5].

In this article, we will focus on discussing the variety of modern research techniques that we use in our anatomical studies. We will present the use of computer technologies, image analysis methods and innovative approaches that contribute to expanding the boundaries of our knowledge about anatomical structures. Let's discover the secrets of modern research in anatomy and their importance for modern medical science.

A variety of modern anatomical research methods are revolutionizing our understanding of the anatomical structures of the human body. Including injection techniques, infrared and ultraviolet analysis, the Pickworth method, a scanning microscope, and advanced computer image processing systems, we will present how these tools enable the exploration of microscopic details and the precise examination of anatomical structures. It is important to combine several methods to improve the quality of images of anatomical structures. However, when combining different methods, you must be careful not to distort the image.

In our article, we present the modern research methods we use in neuroanatomical and neurophysiological research: injection techniques, infrared and ultraviolet analysis, Pickworth method, scanning microscope, computer image processing, fractal analysis in neuroanatomical research, metrological analysis using image analysis methods.

While each of these methods has already been used separately in various fields of biology, their specific combination has allowed us in recent years to make significant progress in the analysis of several dozen thousand images obtained during the dissection of several hundred brains. The particular application of the combination of methods we have presented has allowed for unique studies of fetal brains and has provided us with new insights into the prenatal development of the human nervous system. In this area, it is worth emphasizing the difficulties in obtaining material for research and the delicacy of the sectional material, which was easily damaged. For this reason, we had to develop non-contact measurement methods on a scale unprecedented in the anatomy of the "adult nervous system" and, in particular, combine the various methods we mentioned. The interpretation of the results obtained in this way, resulting from the combination of several methods, was complicated, and we were unable to obtain help despite searching the available literature. For example, infrared neuroanatomical images (saved on a glass plate and loaded by FEAG) turned out to be particularly difficult to interpret. The research was time-consuming and technically difficult, and there were no ready-made algorithms for conducting such research (Figs. 1–16).

1. **Injection techniques:** Injection techniques are commonly used in anatomical studies to introduce staining or contrasting substances. These methods are particularly useful when examining the circulatory system [6].
2. **Infrared and ultraviolet analysis:** The use of infrared and ultraviolet analysis in anatomical examinations allows for the visualization of structures that may be difficult to see in visible light. These methods provide information about various properties of the examined tissues, which is important in structural analysis. The use of infrared in anatomy is tedious and labor-intensive.
3. **Pickworth method:** The Pickworth (benzidine) method is a special method of preparing and analyzing microscopic specimens, used in anatomical studies. It allows for obtaining detailed images of microangioarchitecture [7,8]. It is extremely precise - single erythrocytes are stained in the examined blood vessels.

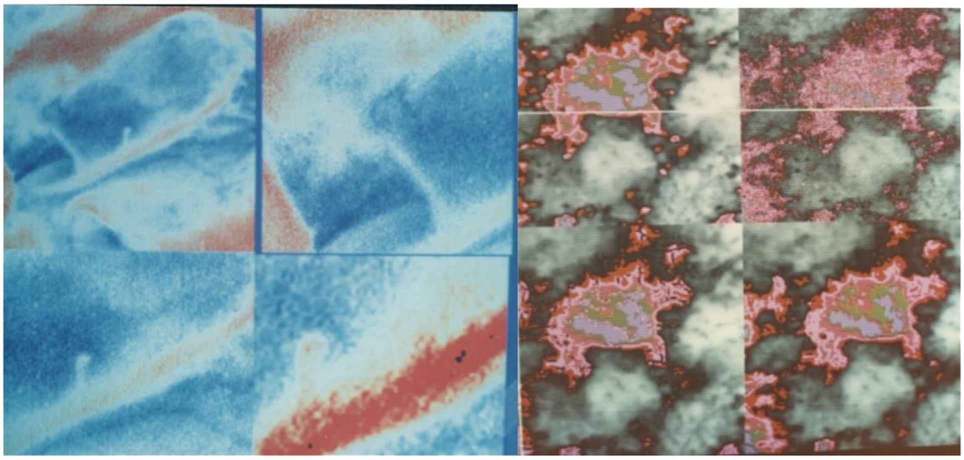

Fig. 2. Arachnoid granules, photo taken in infrared and processed by FEAG 200 laser digital image sensor manufactured by VEB Carl Zeiss Jena.

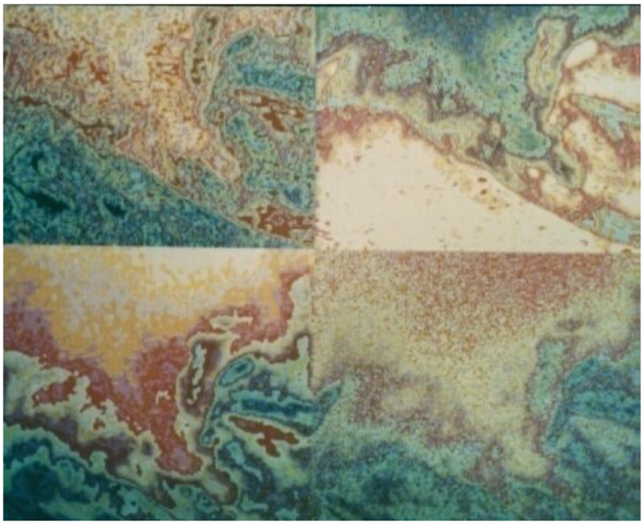

Fig. 3. Infrared image processed by FEAG, showing different sections of arachnoid granules.

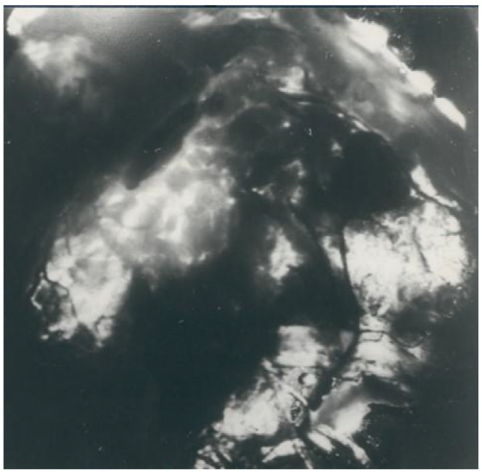

Fig. 4. Infrared image of the choroid plexus.

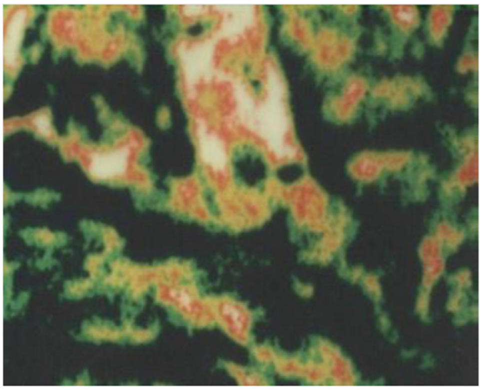

Fig. 5. Infrared image of the choroid plexus processed by FEAG.

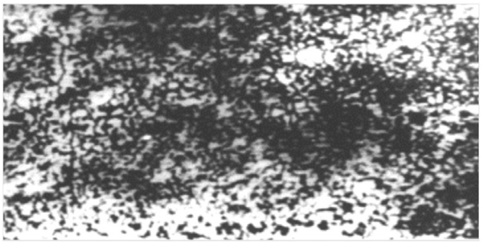

Fig. 6. Ultraviolet photo of the choroid plexus.

Fig. 7. Binary image of the choroid plexus, fractal dimension= 1.80.

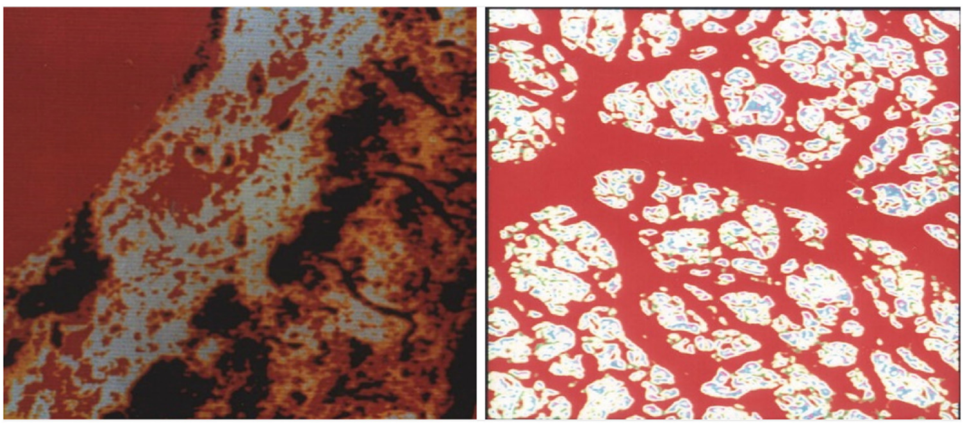

Fig. 8. Vessels of the capsule of subdural hematoma (left) Cortical vessels in the fetus, Pickworth method, and Scion for Windows image processing system (right).

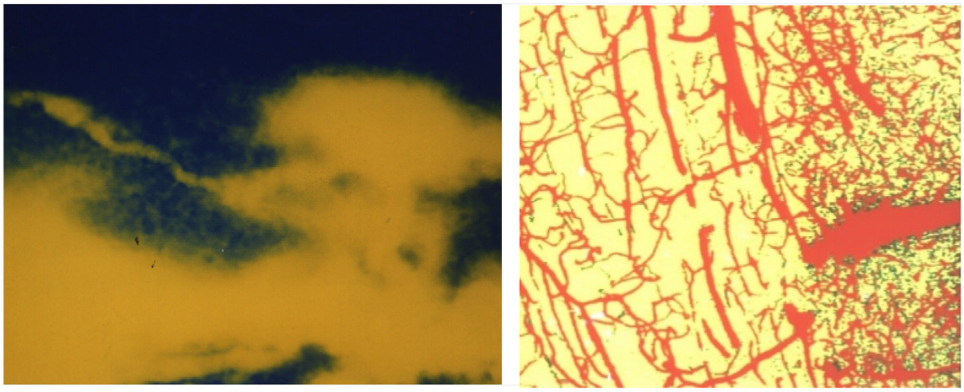

Fig. 9. Periventricular matrix vessel (left) Cerebral cortex and white matter vessels, Pickworth method, and Scion for Windows 98 image processing system (right).

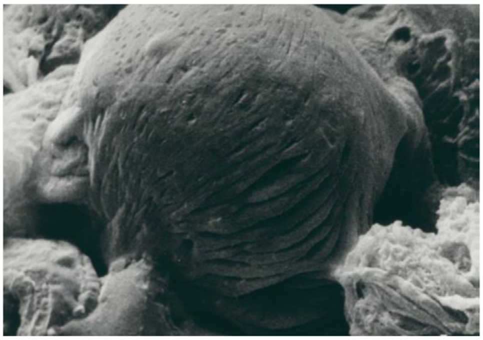

Fig. 10. Scanning microscope photo, arachnoid granulation capsule, magnification 3000 x.

Fig. 11. Scanning microscope photo, arachnoid granular villi, magnification 1000 x.

4. **Scanning microscope:** A scanning microscope is an advanced tool enabling the analysis of structures at the microscopic level. With this technology, researchers can obtain three-dimensional images of cells and tissues, which is crucial for neuroanatomical studies such as the architecture of the dura mater.
5. **Computer Image Processing:** Modern image processing systems such as BVS 6471, BVS 6472, Imtron 2000, Scion for Windows 98, Image J are a key part of modern anatomical research. Computer image analysis allows for precise measurements, visualization, and statistical analysis of anatomical structures.
   - *Scion for Windows 98:* (https://scion-image.software.informer.com/download/) This program offers a wide range of measurement, filter, and image transformation options. Its flexibility allows for advanced analysis and adjustment of images using a variety of tools. (http://mesonpi.cat.cbpf.br/e2002/cursos/NotasAula/ScnImage.pdf)
   - *Elf v. 4.2:* ELF runs in a DOS environment and was used to load camera images, enabling filtering, processing, and various measurement procedures [9–13].

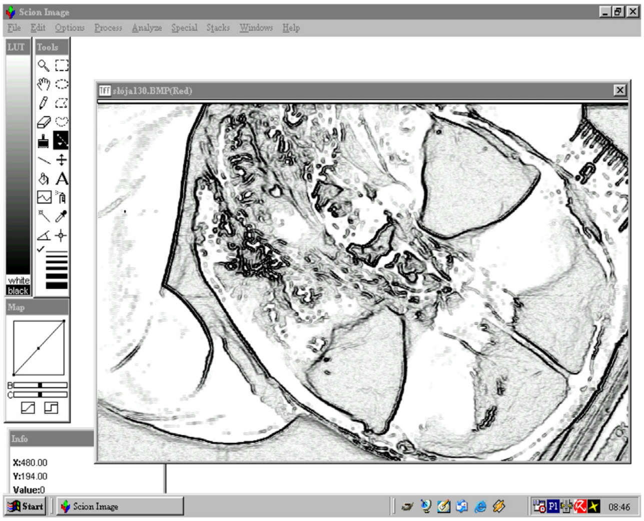

Fig. 12. Basic windows of the SCION for Windows 98 program, in the main window an image of the base of the fetal skull, view from above; an edge finding filter was used.

Fig. 13. Image of the fetal skull base cut using Bezier curves - GIMP program; fetus with a crown-rump length, (CRL) of 205 mm.

- *Graphics Programs:* Graphics programs such as GIMP 2.0.2 and 602 PC Suite were used to process and analyze the obtained photos, which allowed for obtaining detailed data and precise measurements.
  In image analysis, fractal assessment of vascular areas turned out to be an innovative technique enabling the assessment of the degree of complexity of the examined vascular areas. Studies of fractal dimensions of various anatomical structures have provided new data on brain development and brain atrophy.
  As a result, image processing systems, scanning microscopes and advanced computer technologies are the key to exploring anatomical secrets, and their use allows for achieving accurate measurements and analysis of structures with unprecedented precision. These discoveries contribute to expanding the boundaries of our knowledge of the anatomical complexity of the human body.

6. **Fractal analysis in neuroanatomical research**
   Fractal analysis, one of the modern research tools, plays a key role in neuroanatomical research, enabling an innovative look at brain structures and their functions. In this chapter, we will focus on the use of fractal analysis in neuroanatomical research, with particular emphasis on the cases of patients with Alzheimer's disease and epilepsy [2,14–17].

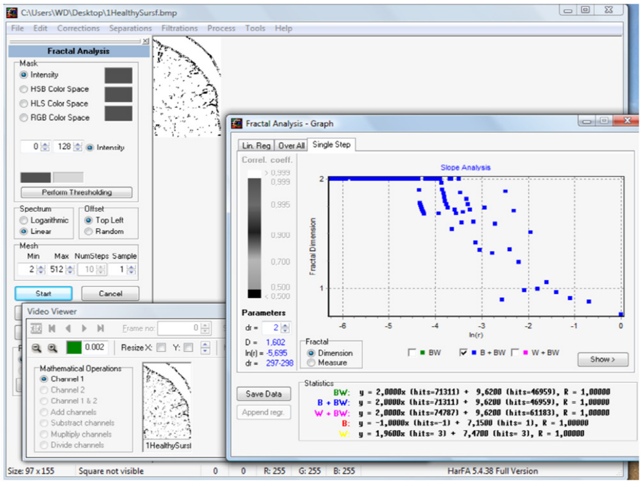

Fig. 14. Analysis of the fractal dimension of the cerebral cortex in the healthy control group.

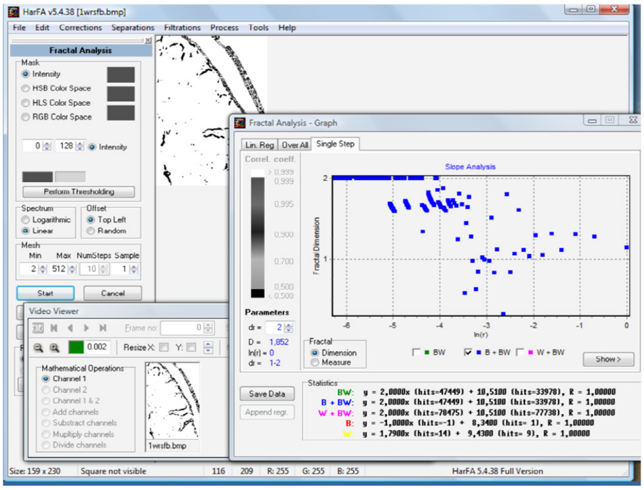

Fig. 15. Fractal analysis of the image of the cerebral cortex of a patient with Alzheimer's disease.

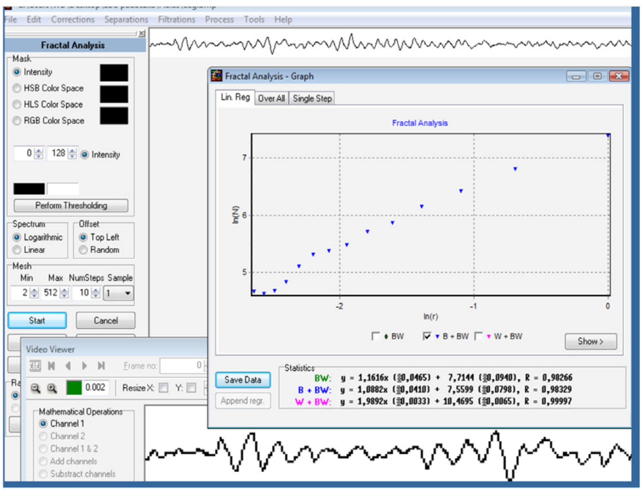

Fig. 16. Fractal analysis of the EEG curve.

Challenges and opportunities of fractal analysis in neuroanatomical research

Fractal analysis in neuroanatomical research poses both challenges and opens new opportunities for scientists. In the case of examining the fractal dimensions of the cerebral cortex of patients with Alzheimer's disease and epilepsy, Fractalyse 2.4.1 and Harfa software were used. The results of these studies were based on the analysis of EEG recordings and images of the cerebral cortex obtained in computed tomography studies.

Application of fractal analysis in research on Alzheimer's disease

Alzheimer's disease, one of the most common causes of dementia, is an area of particular interest in neuroanatomical research. In fractal analysis, the fractal dimension of the cerebral cortex of Alzheimer's disease patients differed from the fractal dimension of the cerebral cortex of healthy people. These studies confirm that fractal analysis can be a useful diagnostic tool that allows distinguishing the population of healthy people from patients with Alzheimer's disease. However, as noted, there are some limitations to this method, such as the influence of the skull on the shape of the cerebral cortex, which requires further research and improvement of techniques.

Fractal analysis and electroencephalographic diagnostics

Research on the fractal dimension of the cerebral cortex in patients with Alzheimer's disease provides valuable conclusions, but EEG analyzes are equally important. Comprehensive frequency analysis of EEG recordings from sick and healthy people allows for an in-depth understanding of the electrical activity of the brain. It was found that fractal analysis allows for more accurate diagnosis of diseases of the nervous system, especially in their initial stages. Therefore, the use of more sophisticated methods of computer EEG analysis is encouraged to expand the diagnostic scope [18,19].

Benefits of using fractal analysis in neuroanatomical research

1. Differentiation between sick and healthy populations: Fractal analysis of the cerebral cortex allows distinguishing fractal dimensions of sick people from healthy people, which may be of key importance in the diagnosis of neurological diseases.
2. Enriching knowledge about the electrical activity of the brain: The results of fractal analysis of the cerebral cortex and EEG recordings enrich our knowledge about the electrical activity of the brain, especially in the case of epilepsy.
3. Application in early diagnosis of nervous system diseases: Fractal analysis allows for more precise diagnosis of nervous system diseases, even in the initial stages, which may contribute to more effective treatment.

The conclusions from the conducted research emphasize the role of fractal analysis as a promising tool in neuroanatomical research. Despite some challenges, this method opens new perspectives for the diagnosis and understanding of diseases of the nervous system, which may have a significant impact on the development of more effective therapeutic strategies.

7. Metrological analysis using image analysis methods

Now we will discuss the results of metrological analysis using the example of our own research on the anterior cranial fossa in the prenatal period [20–23]. These studies were conducted on material consisting of seventy-seven human fetuses aged 10 to 27 weeks of fetal life, whose atlantoaxial dimensions ranged from 55 to 260 mm. The formalin-fixed fetuses were sectioned, and then the anterior cranial fossae were analyzed using computer image analysis.

The fetuses subjected to the study were prepared by dissection, which included separating the cranial vault, removing the brain, and then positioning the anterior cranial fossa specimen in a special stand. The images were then loaded into a computer using a Sony video camera and an ELF system image processing card. The SCION IMAGE for WINDOWS 98 and ELF v. 4.2 programs were used for image analysis.

SCION IMAGE for WINDOWS offers numerous options for measuring distances, areas, curves, and centroids. It allows you to transform images, adjust brightness and contrast, and create three-dimensional reconstructions. The ELF v. 4.2 program, working in the DOS environment, enables the registration and processing of black-and-white and pseudo-color images, offering various measurement, filtering, and histogram display procedures.

Computer programs used for measurements:

Scion Image for Windows 98:

- A program that allows you to measure distances, areas, curves, and points of gravity.
- Allows you to transform images, adjust brightness, contrast, and convert to pseudo colors.
- Convenient generation of results in the form of tables, histograms, and charts.

Elf v. 4.2:

- Image analysis program working in the DOS environment, recording, and processing black-and-white and pseudo-color images.
- Offers procedures for measuring, filtering, drawing, and displaying histograms.
- Useful for loading images from a camera and quickly processing hundreds of images.

Graphic programs:

- GIMP 2.0.2: For processing the obtained photos, tools for retouching, drawing, and editing images.
- 602 PC Suite: An alternative free office suite for processing Office files.

Statistical analysis and results:

The STATISTICA program was used for statistical analysis, comparing histograms of measurement features with a normal distribution. Sexual dimorphism was found in the anterior cranial fossa, where the angle of the anterior fossa was greater in male fetuses and the height of the rooster crest of the ethmoid bone was greater in female fetuses.

1. Shape changes during development:
   - The anterior fossa angle decreased at the expense of an increase in the middle fossa angle.
   - Development occurred with symmetry relative to the midplane of the body.
2. Sexual dimorphism:
   - In the prenatal period, sexual dimorphism was visible, with male fetuses having a larger anterior fossa angle and female fetuses having a higher bantam crest of the ethmoid bone.
3. Dimensional changes in various planes:
   - In the frontal plane, the angle between the lesser wings of the sphenoid bone decreased.
   - In the midline plane, the depth of the posterior cranial fossa increased, accompanied by a decrease in the skull base angle.
4. Mechanical behavior of the skull:
   - The skull, from a mechanical point of view, behaved like a raised drop-shaped reservoir, related to its function as a reservoir of cerebrospinal fluid and highly hydrated brain tissues.

So, metrological analysis provides valuable information on morphometric changes in the human skull during the prenatal period, which may contribute to a better understanding of developmental processes and the evolution of the skull structure. Sexual dimorphism and other developmental features are essential elements of this study, contributing to a more complete understanding of the dynamics of human skull development before birth [24–27], and in future the fractal analysis will become the valuable tool in understanding complex processes occurring in the nervous system [28].

Conclusion

Modern anatomical research techniques are changing our understanding of anatomical structures. These innovative methods reveal previously unseen details, significantly broadening research horizons and offering novel insights into the functions of examined structures. Beyond expanding knowledge, these techniques hold great clinical potential. Their application in diagnosing neurological diseases, such as Alzheimer's and epilepsy, promises quicker and more precise diagnoses. This collaboration between science and medicine enhances healthcare and deepens our grasp of human anatomy, marking modern research techniques as crucial contributors to progress in anatomy and medical sciences.

Fractal analysis of the cerebral cortex in Alzheimer's patients revealed significant differences compared to healthy individuals. This groundbreaking discovery positions fractal dimension as a promising diagnostic tool, capable of early detection of pathological changes in brain structure. In the realm of neurological disease diagnosis, fractal analysis opens new avenues for understanding and early detection, particularly in the initial stages of diseases.

Analysis of electrical brain activity in epilepsy patients provides valuable insights into the disease. Advocating for an advanced approach to electroencephalographic diagnostics, it emphasizes the need for sophisticated methods in computer EEG analysis. This analysis extends beyond visible graphical representation, paving the way for intricate and precise research into both basic and advanced brain activities.

The integration of fractal analysis into the diagnosis of nervous system diseases, especially in their early stages, represents a breakthrough. These tests enable more accurate diagnosis of structural and functional changes, crucial for effective treatment and patient monitoring. Fractal analysis emerges as a valuable tool, facilitating a deeper understanding of intricate processes within the nervous system.

Ethics statements

The Bioethics Committee of the Medical University of Wroclaw agreed to conduct our research - consent number: KB-540/2000.

CRediT author statement

Alicja Kędzia: Conceptualization, Methodology, Data curation, Writing- Original draft preparation, Visualization, Investigation, Supervision. **Wojciech Derkowski:** Writing- Original draft preparation and Reviewing and Editing, Conceptualization, Methodology, Data curation, Visualization, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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