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A Brief Overview of Stereology and Morphometry Method in Histology and Biology

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ABSTRACT

Quantitative analyses in biological science are especially important in terms of determining and comparing the geometric properties of biological structures. Stereology and morphometry are two important complementary methods frequently used in this field. Stereology refers to the quantitative analysis of the three-dimensional geometric properties of biological structures. In particular, it is used to determine the criteria such as volume, surface area and length of many cells, organelles and tissues with microscopic properties. In addition, this method allows to obtain information about three-dimensional structures by measurements made on randomly selected sections. Thanks to these techniques, accurate estimates of the general structure can be made with data obtained from certain sections instead of examining biological samples completely. Morphometry, on the other hand, is suitable for examining biological structures in terms of shape and size. It is a suitable method for determining the shape changes of organisms and structural elements. Morphometry digitizes the data by making measurements in the digital environment and performs statistical analysis on these data. Measurements are made more quantitative by volume fraction analysis. The importance of stereology and morphometry in quantitative morphology enables the objective realization of biological structures in quantitative analysis in both methods. These methods thus allow the examination of the material at hand, which is mathematical and statistical. In addition to biology, tissue science Quantitative biology has a special place in three-dimensional studies in histology. This review is particularly concerned with stereology and morphometry, and the aim of the review is to give dimension to a specific topic under investigation, thus providing a good background for diagnostic decision making by strengthening traditional approaches, and to address the contributions of these methods in scientific studies.

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Introduction

Stereological Science and Morphometry

The term morphometry was first coined by combining the Greek words “morphe” (shape, form) and “metria” (measurement) (Mitteroecker & Gunz, 2009). Many of the questions that arise when working with a material are about the shape. These questions are; “What is the average shape of the bone/organ /structure in a population?”, “What is the pattern of variation in the population around this average shape?”, “How do groups differ in shape?” and “What is the functional significance of these differences?” such as. Morphometry is an area where statistical analysis is used to suggested addresses such questions (Slice, 2007). Microscopic qualitative observations may not directly represent the quantitative characteristics of a tissue or organ. Stereology is used to overcome this difficulty. Using point counting, three-dimensional features are simplified to a zero-dimensional format. Hilliard and Cahn (1998) demonstrated that using point counting to assess volume percentages resulted in a lower overall error compared to other methods. The outer surface area of three-dimensional structures within organs is reduced proportionally to their volume during tissue sectioning. Measurement accuracy depends on the number of points used and the bulk density of the structures (Baak et al., 1977). This method works with counts based on images where randomly selected points are inside or outside specific components. These counts are used to determine the volume of a particular ingredient within the overall volume. This is called volume fraction (Weibel, 1969). The volume fraction is based on the geometric probability that allows properties in three dimensions to be measured from two-dimensional images. Morphometric volume fraction measurement offers a variety of techniques that allow for the unbiased measurement of tissue, cellular, and subcellular characters.

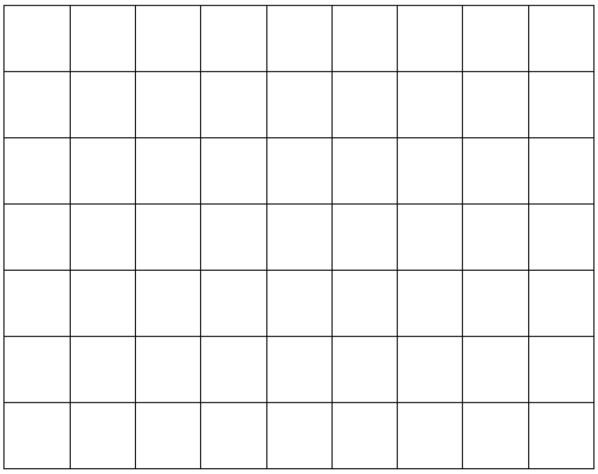

Figure 1. A. Caged Counting Grid with 1 cm spacing

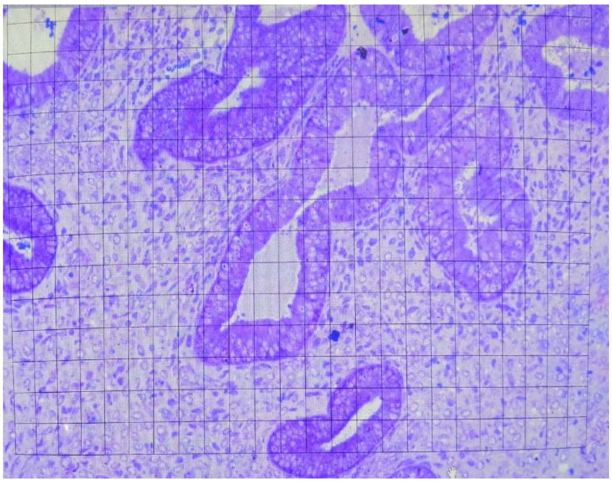

Figure 2. B. Overlay of the histological image on the gray (Dağdeviren,2024).

These techniques involve counting points or intersections using carefully designed counting grids and require precisely controlled tissue sampling for specific measurements. This can be achieved by photocopying the appropriate counting grid onto an acetate sheet, which can then be superimposed on photographs. Measurement helps us improve our interpretation of morphological change and understand the relationship between genotype and phenotype in more quantitative terms in disease processes. Volume fraction analysis is performed by superimposing the histological image with the counting grid with a certain intersection range (Figure 1-2), (Weibel, 1969).

Stereology holds a unique position in the three-dimensional studies of histology and quantitative biology. Consequently, the application of suitable stereological techniques is crucial. This method employs mathematics and statistics to estimate parameters such as volumes, dimensions, components, and their quantities. Although measuring parameters like length, weight, and count in histology and histopathology might seem straightforward, it becomes inefficient when analyzed microscopically. Direct measurement or routine techniques are not viable for determining these parameters under microscopic conditions (Gundersen et al., 1988). Stereology, on the other hand, is a technique for obtaining three-dimensional data from two-dimensional images. It employs geometric-statistical reasoning to derive quantitative properties. (Weibel, 1969). The size of a cell can be naturally expressed in pm, not by the area in pm ^{2} occupied by its profile on a section. Therefore, it can be a simple, fast and reliable method for quantifying two-dimensional structures. Thus, it is possible to understand tissues, cells, organelle, other cellular structures in an eastern way (Sagheb & Moudi, 2014). Microscopic sections are needed for light and electron microscopic studies of tissues. These sections are thin enough to be viewed as two-dimensional representations of a three-dimensional structure. During tissue processing, size reduction occurs, which significantly affects the quantitative properties of the structures within the sample (Weibel & Elias, 1967; Weibel, 1969). This is because the measurements in the microscopic sections do not directly represent the quantitative characteristics of the organ. Stereology is needed to overcome this challenge. The necessary condition for the stereological analysis of tissue sections is the random orientation of the structures according to the cross-sectional plane (Weibel, 1969; Cruz-Orive, 1997).

Stereological Formulas

The stereological formulas developed by many authors are the gold standard used in calculation (Weibel & Elias 1967; Underwood, 1973). Counting points reduces a three-dimensional feature to a point-like representation. Hilliard and Cahn (1998) showed that using point counting to assess volume percentages generally yields lower errors than other methods, underscoring its practical advantages. The accuracy of these measurements depends on the number of points used and the bulk density of the structures. During tissue sectioning, the size of the outer surface of three-dimensional structures in organs is reduced in the same way as their volume (Baak et al., 1977). If the tissues or structures whose volume is to be calculated have a macroscopic structure that will be isolated from other surrounding organs or structures such as, for example, the spleen, lung, liver, direct measurement can be made here instead of calculating volume. In such cases, a frequently used method is to measure the increased amount of water by immersing it in a graduated cylinder filled with water. However, if the organ of interest contains cavities such as the lung, volume measurement should be performed after the cavities of the organ are blocked in a waterproof manner to apply the fluid displacement method. Because the actual volume can be measured smaller with the filling of water from the organ cavities (Cruz-Orive et al., 1990; Canan et al., 2002). Bonaventura Cavalieri (1598-1647), an Italian mathematician and astronomer, is known for pioneering the estimation of volumes of biological objects in tissue sections through the use of their surface areas. In his 1635 book “Geometria Indivisibilibus Continuorum: Nova Quadam Ratione Promota,” he introduced what is now known as Cavalieri's principle and reinforced the concept of indivisibility. The probe can display a line, lines can represent regions, and regions can represent the entire image. In this context, a line is made up of an infinite number of points, a plane is composed of an infinite number of lines, and a solid comprises an infinite number of planes. Each indivisible element contributes to the continuity of the next higher dimension through continuous motion (Ghosh, 1998). Stereology was initially developed by the French geologist August Delesse in 1847, who suggested that the properties of mineral components observed in two-dimensional sections are directly proportional to their three-dimensional volumes (Casteleyn et al., 2014). He then showed that, using the Delesse principle, it was possible to determine the volume fraction of a test component. In this method, a mesh or dot grid is placed on the test component and the dots on this grid are considered to lie on the test component. Through this counting process, it becomes possible to estimate the volume fraction of the test component. For measuring the surface density of a test component, a test line system placed on the sample sections can be employed. This method enables the estimation of the surface density of the test component by counting the intersections formed by the membrane traces of the test body (Delesse, 1847). In the early years of stereology (1961-1971), biologists extensively used the "Needle Problem" along with the principles of Cavalieri and Delesse. However, due to the non-conformity of biological structures to classical geometric models such as Euclidean formulas for simple shapes like spheres, biologists turned to stochastic geometry, probability theory, and unbiased sampling strategies between 1971 and 1981. These approaches enabled precise quantification of structures such as number, length, surface area, and volume without requiring prior knowledge of object size, shape, or orientation. By the 1980s, a significant challenge was ensuring accurate counts (Bolender, 1992; Mouton, 2005). A notable advantage of modern stereology in histology is its capacity to streamline workloads through effective and reliable sampling, facilitating the extrapolation of findings to the entire structure (Gundersen et al., 1988). Biologists contend that stereology, when applied without bias, is the most effective practical method for quantitative histology. It plays a vital role in validating and refuting experimental hypotheses in biological research. With this technique, we can answer questions such as: To what extent is a tissue affected by a particular factor? In contemporary medical sciences, stereology explores changes in tissue volume, variations in blood vessel length, and differences in cell numbers between experimental and control groups. It employs advanced microscopic calculations to investigate the spatial distribution of tissues, cells, and organelles during disease and treatment.

Researching the volume, surface area, length, and number of biological objects significantly contributes to advancing scientific understanding in histology concerning health and disease (Sagheb & Moudi, 2014). In recent years, stereology has become a cornerstone in cell biology and medical image analysis for quantitative analysis, particularly in studying cancer cells, tumor grading, and patient prognosis. This approach allows for detailed examination of tumor characteristics such as size, cell density, and the three-dimensional structure of cancer cells and nuclei (Ladekarl, 1988; Sagheb & Moudi, 2014). Quantitative analyses in radiology utilize stereological principles applied to radiographic images. Key considerations include proper sampling, image segmentation, data recording, database searches, 3D reconstruction, non-invasive techniques, finite resolution, and ensuring high image quality, all vital for reliable quantitative assessments.These factors enhance comprehension of the structure and function of the human body and support more objective disease diagnosis, as well as evaluation of treatment response. These goals can be achieved by stereological methods (Roberts et al., 2000; Hu et al., 2012). For instance, in certain tissues like the endometrium, researchers used suitable morphometric methods to comprehensively describe the morphology of the human endometrium (Weibel, 1979). Since hormonal changes are reflected in morphology, there is a close relationship between structure and function in the endometrium (Dallenbach-Hellweg,1981). Quantitative microscopy methods have proven highly beneficial in histopathology. For instance, morphometric analysis of endometriosis offers detailed quantitative insights at the cellular and tissue levels, examining the microscopic properties of the diseased tissue. This analysis generated comprehensive data on the size, shape, density, and other measurements of endometriosis lesions. Thus, it plays an important role in determining the stage of the disease, planning treatment strategies, monitoring the development of the disease. Thanks to morphometric analysis, the differences of the tissue with endometriosis from the normal endometrial tissue are determined quantitatively. As a result, morphometric analysis of endometriosis deepens the understanding of the disease and contributes to the determination of more sensitive, effective treatment strategies (Dağdeviren, 2024).

Conclusion

Numerical morphological values are one of the important parameters that contribute to the establishment of the relationship between structure and function. Stereology offers a cost-effective yet highly effective method of measurement in histopathology and is indispensable for obtaining unbiased morphometric data. Stereology and morphometry show promise in detecting differences that cannot be assessed by both objective and subjective methods. While new molecular techniques in histopathology hold potential impact, morphology will remain the cornerstone of disease diagnosis and grading. Furthermore, advancements in tissue processing methods are essential for successful studies. Stereology, though not inherently complex, requires meticulous planning for sampling, data collection, and statistical analysis.

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