ACTA STEREOL 1987; 6/SUPPL II: 23—33
ISS COMMEMORATIVE-MEMORIAL VOLUME
DISCOURSE

IDEAS AND TOOLS: THE INVENTION AND DEVELOPMENT OF STEREOLOGY

Ewald R. Weibel Department of Anatomy,

University of Berne, Bühlstrasse 26,

CH-3000 Berne 9, Switzerland

ABSTRACT

This paper reviews the development of stereology and the impact that the foundation of the International Society for Stereology has had on this process. It also presents some anecdotal observations about the foundation of this Society and the role played by Hans Elias. The last two decades are described as phase III in the development of stereology. The main achievement of this phase was the consolidation of basic stereological methods; they were made both reliable and efficient. Much effort was devoted to develop the technology of automatic image analysis, but this has resulted in deceivingly few contributions to stereology and its practical usefulness. We are currently entering the fourth phase, and must face new challenges, because the "classical" stereological methods have neglected the objective analysis of form. Morphological features of form and design are however most important characteristics of living systems. The challenge for stereology is to devise sound methods by which form and design can be assessed.

Keywords: stereology, morphometry.

INTRODUCTION

A quarter of a century after its invention the term "stereology" cannot yet be found in any modern dictionary, nor is it an entry in any of the major listings of scientific literature - inspite of its old "history" (see Cruz-Orive, this issue). And yet, some articles which have developed stereological methods for practical use, e.g. in cell biology, are among the most extensively cited papers, which must mean that stereological methods are widely used - although the science on which they are based is practised by only a small group of active stereologists.

If one then looks at the state of the art in stereology as a science and confronts it with the state of the art in its broad application in biology, for example, one notes a further discrepancy: most of the stereological methods used in solving morphometric problems are still based on the art of nearly two decades ago; they make very little use of the enormous improvements of stereology that has come about in recent years. I have noted with great interest that one of my own papers on "stereology for morphometric cytology", published in 1964, was very heavily quoted as a major reference to stereological methods until a few years ago; it was replaced as a "citation classic" in 1982 by a review article from 1969(!) and not by any more recent papers or books, although they were not lacking (Weibel, 1986).

In this review - commemorating the 25th anniversary of "stereology" - I would like to examine some of the reasons for this strange delay in the transfer of methodology from theory to practical use by looking at the way stereology has developed in three phases up to the present. The basic insight will be that the problem is twofold: (1) the ideas behind stereology are difficult to grasp, and (2) the tools are perhaps too simple. I shall finally consider phase IV, perhaps the next decade, and the challenges for stereology ahead of us. The message will be that we should be more concerned with questions of form and design.

PHASE I: TOWARDS STEREOLOGY

The foundation of the International Society for Stereology by Hans Elias - as an idea in 1961, as a formal organization in 1963 - marked an important turning point in the development of the field that then became "stereology". The ideas behind it were, in fact, not new. Many morphologists had, for over a century, struggled with the problems of making measurements on sections and interpreting them in terms of the spatial structure from which the sections were cut. The best known case was that of the French geologist Auguste Delesse who, in 1847, had shown that the composition of rocks can be measured on sections by tracing the profiles of the components, cutting out the tracings and weighing them. In biology Harold Chalkley, a cancer researcher, had developed a point counting method allowing him to study the composition of tissues in 1943, not knowing that the same had been done some ten years before by geologists again (Thomson 1930; Glagolev 1933). When, in 1959, I was confronted with the problem of estimating the number of alveoli in the human lung, I developed my own pragmatic method (Weibel and Gomez, 1962), not knowing that De Hoff and Rhines (1961) were doing the same thing, and did it better!

The pattern, in fact, was consistent and typical for this first phase: problems of a "stereological nature" came up in many fields of applied science, and one tried to find a pragmatic solution. These solutions were very often of a more empirical nature and the "proofs" were based on simple model experiments. There were some notable exceptions. For example the very profound study of "the corpuscle problem" by Wicksell (1925/26) who solved the problem of estimating particle number and size distribution on sections; his papers remained unnoticed, perhaps because he published them in a biometric journal, perhaps also because the ideas he had developed were rather difficult to comprehend for microscopists. These methods were, in fact, rediscovered many times thereafter - but rarely developed as stringently as by Wicksell.

PHASE II: "STEREOLOGY" INVENTED

The "pragmatic phase" ended when the International Society for Stereology was founded 25 years ago by Hans Elias, who was the central figure and the catalyst, the person who pulled the whole thing through. Here are a few anecdotal remarks about some of the events preceeding the foundation of the ISS, as I experienced them.

I had got to know Hans Elias in 1958 in Boston at the meeting of the International Academy of Pathology where we were both presenting what one would now call a poster. Fate wanted it that our posters were adjacent. Hans explained to me his problems of interpreting the shape of kidney tubules from sections, but he also - successfully - charmed my young wife! Shortly thereafter we visited him in Chicago where he made me join in one of his artistic adventures with which he wanted to demonstrate to the world that abstract art was "monstrography". We then met again in 1960 at the Anatomy Congress in New York where a social event, a boat trip around Manhattan, should turn out to become fateful. On this boat trip I also found Herbert Haug (Fig. 1), and I introduced him to Hans Elias. The two took off together, deeply engaged in discussions, and I didn't see them again. But a year later, in summer 1961, Hans Elias came through New York and called me up. We had a long stroll up Fifth Avenue and ended up in a café on Central Park South, Hans sipping orange juice while I had a beer. I told him about my struggles with "morphometry" of the lung, and he told me that he returned from the Feldberg in Germany where he had invented the word "stereology" for what I was doing - this should be the beginning of a long, ardent but friendly debate about how "stereology" was fundamentally different from "morphometry"! But he also told me that he had founded the "International Society for Stereology", and I immediately joined.

These events were all very typical for Hans Elias: convinced of his ideas, dedicated to the cause, and grand visions: the society had to be "International", and the first gathering was called the "First International Congress for Stereology" - although it brought together only a handful of scientists (Haug, 1963). But it is this dedication, this grand vision that eventually led stereology to success, and for this we must be grateful to Hans Elias.

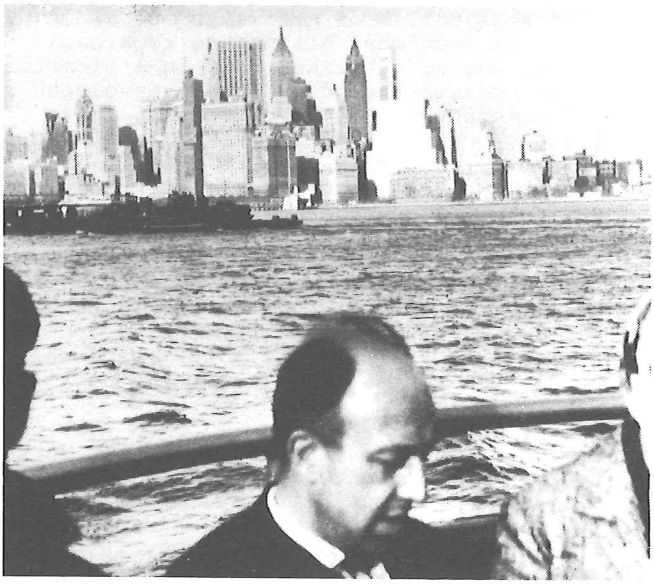

Fig. 1: Photograph of Herbert Haug on the boat trip around Manhattan in summer of 1960.

The chief effect of the foundation of an international society for stereology was that scientists from all parts of the world and from virtually all fields of microscopic investigation - metallurgy, geology, biology - now joined forces in order to solve problems of mutual concern. The invention of a new word -"stereology" - facilitated this process because people from "quantitative metallography" were just as attracted as those from biological "morphometry". And the mathematical connotation of the term attracted the interest of mathematicians concerned with geometric probability, integral geometry, stochastic geometry, and statistics. The idea behind stereology had caught on, and the progress was rapid: within a few years the pragmatic stereological methods were provided with solid theoretical foundations, and simple but reliable methods for their practical use were developed. As a consequence stereological methods became used more broadly, particularly in cell biology and histology where, in the 1960s, electron microscopy was making important contributions to understanding the structure of living matter.

PHASE III: DEVELOPING RELIABILITY AND EFFICIENCY

The period following the Second International Congress for Stereology, held in Chicago in 1967, was dominated, on the one hand, by efforts to consolidate and expand the existing methods, and to develop their application by improving sampling strategies and statistics; on the other hand, major efforts were made to improve their efficiency by developing suitable technologies. The ideas and the tools were developed along somewhat different routes, which has had significant effects on the evolution of stereology (Elias, 1967; Weibel et al., 1972; Weibel, 1979/80).

The theoretical development brought the insight that special precautions are needed to avoid bias in stereological estimates. This resulted particularly from the efforts of Roger Miles (1972, 1976, 1978) to establish general mathematical conditions for stereological methods: the term "random" - often used rather loosely when one means "arbitrary" - needs to be defined in order to obtain unbiased estimates (Cruz-Orive and Weibel, 1981). These new insights have allowed efficient and reliable sampling strategies to be developed, even when the "sections" are imperfect, i.e. slices of finite thickness. Deepening the mathematical foundations has also made it possible to get away from assumptions of randomness and to ask new questions. Randomness in a spatial sense implies isotropy, at least between section and objects. But many real structures exhibit preferred orientations or anisotropy to a greater or lesser extent, one example being muscle cells and their associated capillaries. Not only would it be more efficient to design sampling strategies by considering anisotropy (Cruz-Orive et al., 1985), it is often of considerable biological interest to assess the degree of orientation along with the basic quantitative characteristics of structure. Also the spatial distribution of cell or tissue constituents are of interest, but basic stereological methods do not allow such parameters to be estimated. The improved mathematical foundations of stereological procedures make it possible to develop such methods, as we shall see in the last section.

But let us first look at the second line of development, that of the tools for stereology, which played an important role in this phase. Hans Elias was one who advocated simple technology - and he was right because with good theoretical design of the approach stereological methods can, in general, be reduced to simple counting operations, counting with respect to well-defined test systems of points, lines and areas. It was also evident, however, that computers could be used to advantage in assisting the counting and computing operations, and the advent of minicomputers allowed on-line solutions which made stereological methods more efficient.

But the rapid increase in computer power led in another direction: automatic image analysis. The possibility to produce digitalized images by a video process and to then perform stereological operations on these images fully automatically was, indeed, a great challenge. It also held great promise that it could make stereological methods more efficient and more objective: the hopes went for a completely automatic stereological machine. The progress made was impressive (see e.g. Serra 1982), but still not adequate to make the dream come true. The problems of this approach are fundamental and intimately linked to the problems of stereology: the image presented on an electron micrograph, for example, is the result of (randomly) sectioning a three-dimensional object - usually unknown in its spatial complexity - so that the interpretation of such images requires considerable prior knowledge and judgement by experience.

The technological trend towards automatic image analysis has had unfortunate effects on the technological advancement of stereological tools. Too much of the manufacturers' resources was directed towards solving the most difficult but also least important problem: segmentation of a digitized image. The more important problem of implementing sound stereological methods in computer-based machines was almost totally neglected. As a consequence the market now offers a host of expensive image analyzers which do not make use of the best of stereology. The problem is a serious one: misdirected technology does not allow potential users of stereological methods to do the best possible job, not even in terms of efficiency (Mathieu et al., 1981). It is usually not realized that the ideas behind stereological methods are much more powerful than the tools available to apply them in practice. Thinking and planning is therefore more effective than labouring. And, in fact, considerations on sampling efficiency have shown that automation of image analysis attempts to improve the least critical of all the steps in a stereological analysis: the slogan "do more less well" means that the precision of the estimate is affected more by the number of sample images analysed than by the precision with which the single image is "measured" (Cruz-Orive and Weibel, 1981; Gundersen and Osterby, 1981).

PHASE IV: BEYOND CLASSICAL STEREOLOGY

At the Eighth International Congress of Anatomists, which followed the one in New York and was held in Wiesbaden (Germany) in 1965, Hans Elias and I were asked to organize a symposium on "Quantitative Methods in Morphology", this invitation being made a few months after the formal foundation and incorporation of the ISS in Vienna in 1963. The introductory chapter of the book based on the proceedings of this symposium (Weibel and Elias, 1967) deals with an "Introduction to stereology and morphometry" and exposes what we felt were the essential features of "stereology" as one approach to "morphometry" or "quantitative morphology" in a broad sense.

To study the internal structure of biological materials - organs, tissues, cells - necessitates their destruction in an orderly fashion: anatomical dissection, or sectioning with microtomes, the latter being the method of choice for microscopic studies. The main feature of sections is that "the spatial relationship between the different substructures is faithfully preserved at least in two of the three dimensions of space. However, the third dimension is lost in the process of tissue destruction... It is therefore the main objective of the field named 'stereology' to devise methods for compensating this loss of the third dimension by 'reconstruction' of spatial relationships on theoretical grounds". And then:

> "Stereology is a body of procedures, mainly geometrico-statistical, which have the aim of obtaining information about three-dimensional structure from two-dimensional, flat images. It can also be defined as extrapolation from two- to three-dimensional space". ... "The stereologist measures and counts the profiles of the cut tissue elements within a slice, and from the data thus obtained he draws conclusions about the geometrical properties of the original, three-dimensional objects".

In these definitions, drafted mostly by Hans Elias, his main concern for the correct spatial interpretation of forms - kidney tubules, liver cell "plates" rather than "strands" - is prominent, but most of the book really dealt with the "basic stereological principles" for measuring volumes, surfaces and lengths, or particle sizes from sections. Whereas Hans Elias was interested primarily in the precise morphology of well-defined structures, most of "classical stereology" dealt with reducing structures to simple numbers, quantifying aggregates of components with respect to a suitable frame of reference. No doubt this approach has made important contributions to biology: it has become possible to accurately estimate the inner surface of the lungs which serve the uptake of oxygen from the air, or the volume of mitochondria in muscle cells which determine the capacity of muscles to produce energy by combustion, to mention only two examples from our own work (Weibel, 1984). This type of work has contributed mostly to our understanding of the role of structures in determining functional processes; accordingly, this type of results is appreciated particularly by physiologists, and one will find numerous references to morphometric data obtained by stereological methods in textbooks of physiology.

On the other hand, morphologists find these results quite unattractive - and they are rarely taught in anatomy courses, nor are they found in textbooks of anatomy. The results of "classical stereology" lack the esthetic appeal of form which they, in fact, eliminate from consideration. Spatial relations are not truely assessed and described in their - relevant - complexity: stereology impoverishes morphology!

This criticism is justified, and it should be a challenge for stereology as it now enters its fourth phase of development. Biological structure is, indeed, more than the correct quantitative "mixture" of components, as well balanced as it may be in functional terms. The spatial arrangement of these components is a very pertinent and tightly controlled property of living systems. This is true also for any machine: my automobile is not adequately described by estimating its composition in terms of steel, aluminium or plastic; the design of the engine and the form of the hull - eminently morphological properties - are most important characteristics by which its performance and its appeal differ from other cars. These features stereology has neglected so far. I would like to illustrate this by three examples related to the design of the respiratory system.

(1) In the lung it is not enough to simply measure the total area of the alveolar gas exchange surface, or the mean linear intercepts in the air spaces. This surface is arranged about the last generations of a branching airway tree which has all the features of a fractal tree (Mandelbrot, 1983). Fig. 2 shows a cast of the peripheral units of the airways of a rat lung; the branched system of airways around which the alveoli are arranged is buried because the air spaces are packed into a space-filling system (Rodriguez et al., 1986). This spatial arrangement may have considerable importance, both physiologically for gas exchange, and morphologically in understanding how a lung is made and maintained. In such studies of form stereological methods fail so far, and one has to resort to anatomical dissection, or to serial section reconstruction, and it turns out to be very difficult to obtain accurate quantitative data.

(2) The blood capillaries around muscle cells form partially oriented networks whose degree of preferred orientation appears to be characteristic of the muscle type; the density of capillaries may also vary along the blood flow path from the arterial to the venous end of the network. Both the degree of orientation and possible differences in local density are very important, but difficult to measure features.

(3) The mitochondria of muscle cells are not uniformly or randomly distributed, but they rather appear concentrated towards the cell surface or even towards the capillaries, and such gradients are difficult to assess reliably with current methods, although they are of paramount importance for precisely defining the energetic pathways of the muscle cells (Kayar et al., 1986).

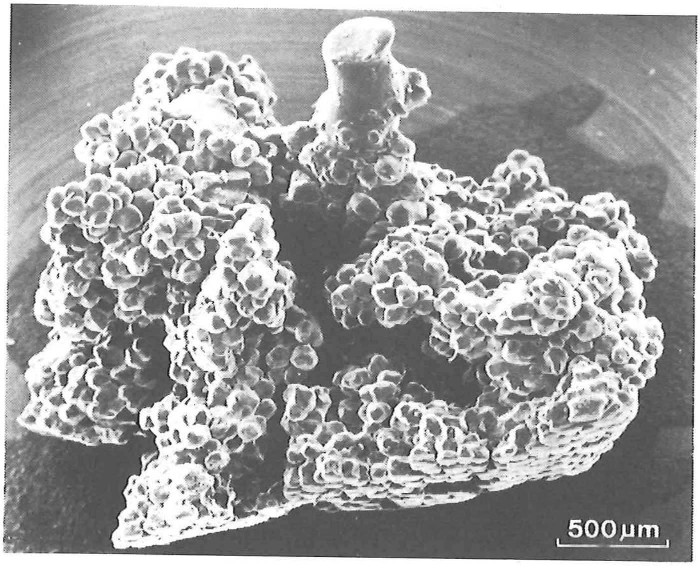

Fig. 2: Scanning electron micrograph of the cast of an acinus from a rat lung.

Problems of this nature which combine morphometric requirements on bulk parameters with parameters of form and design are very numerous, and it is to be hoped that the "stereology of tomorrow" will endeavour to develop the methods to solve them. Before such methods are available we cannot hope to understand the full significance of structural organization, the impact of morphology on life processes as a whole.

To add the old concerns of Hans Elias for forms and design to the classical and now well established stereological principles dealing with aggregates would constitute a significant step in extending the power of stereology.

REFERENCES

Chalkley, HW. Methods for quantitative morphological analysis of tissue. J Nat Cancer Inst 1943; 4:47.

Cruz-Orive LM, Weibel ER. Sampling designs for stereology. J Microsc 1981; 122: 235-257.

Cruz-Orive LM, Hoppeler H, Mathieu O, Weibel ER. Stereological analysis of anisotropic structures using directional statistics. J Appl Statist 1985; 34: 14-32.

De Hoff RT, Rhines FN. Determination of the number of particles per unit volume from measurements made on random plane sections: the general cylinder and the ellipsoid. Trans AIME 1961; 221: 975.

Delesse MA. Procédé mécanique pour déterminer la composition des roches. C R Acad sci (Paris) 1847; 25: 544.

Elias H. Stereology. Proceedings of the Second International Congress for Stereology. New York: Springer Verlag, 1967.

Glagolev AA. On the geometrical methods of quantitative mineralogic analysis of rocks. Trans Inst Econ Min (Moscow) 1933; 59: 1.

Gundersen HJ, Osterby R. Optimizing sampling efficiency of stereological studies in biology:or "Do more less well". J Microsc 1981; 121: 65-73.

Haug H. Proceedings First International Congress for Stereology Vienna. Congressprint, Wien, 1963.

Kayar SR, Claasen H, Hoppeler H, Weibel ER. Mitochondrial distribution in relation to changes in muscle metabolism in rat soleus. Respir Physiol 1986; 64: 1-11.

Mandelbrot BB. The Fractal Geometry of Nature. New York; Freeman and Company, 1983.

Mathieu O, Cruz-Orive LM, Hoppeler H, Weibel ER. Measuring error and sampling variation in stereology: comparison of the efficiency of various methods for planar image analysis. J Microsc 1981; 121: 75-88.

Miles RE. Multidimensional perspectives on stereology. J Microsc 1972; 95: 181-195.

Miles RE. Estimating aggregate and overall characteristics from thick sections by transmission microscopy. J Microsc 1976; 107: 227-233.

Miles RE. The sampling, by quadrats, of planar aggregates. J Microsc 1978; 113: 257-267.

Rodriguez, M, Bur S, Favre A, Weibel ER. The pulmonary acinus: geometry and morphometry of the peripheral airway system in rat and rabbit. Am J Anat (Hans Elias Memorial Issue) 1986 (in press).

Serra J. Image Analysis and Mathematical Morphology. London; Academic Press, 1982.

Thomson E. Quantitative microscopic analysis. J Geol 1930; 38: 193.

Weibel ER. Stereological Methods. Vols. I and II. London; Academic Press, 1979/80.

Weibel ER. The Pathway for Oxygen. Cambridge Mass.; Harvard University Press, 1984.

Weibel ER. Citation Classic. Current Content 1986; 10:20.

Weibel ER, Gomez DM. A principle for counting tissue structures on random sections. J Appl Physiol 1962; 17: 343-348.

Weibel ER, Elias H. Quantitative Methods in Morphology. Berlin-Heidelberg-New York; Springer Verlag, 1967.

Weibel ER, Meek G, Ralph B, Echlin P, Roos B. Stereology 3. Proceedings of the Third International Congress for Stereology. Oxford; Blackwell Scientific Publications, 1972.

Wicksell S. The corpuscle problem I. Biometrica 1925; 17: 84.

Wicksell S. The corpuscle problem II. Biometrica 1926; 18: 152.