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SHORT NOTES

STEREOLOGIA--THE FIRST NEWSLETTER OF THE I.S.S.

by

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The idea for "Stereologia" and its name originated with Hans Elias, the first President of I.S.S. in about 1960. Since I was Secretary-Treasurer at the time, Hans asked me to be the Editor and to find funding for it. He also suggested I choose a suitable logotype for the "Stereologia" heading. As an example, he sent me a drawing of a cube within which was placed a tetrahedron and a few toruses (for red corpuscles). I took him at his word and checked around for some other appropriate symbol, but ultimately decided in favor of the design Hans had submitted. He was very pleased when I told him I had selected the logo he had proposed in the beginning.

I was working for Battelle, in Columbus, Ohio at the time, and was successful in obtaining a grant of $200.00 from Battelle for art work, printing and mailing of the new I.S.S. newsletter. Accordingly, the first issues of "Stereologia" came from Columbus, Ohio until about 1962, when I changed jobs to go with Lockheed in California.

Since I felt my new responsibilities would not permit me to continue as Editor, I asked Bob DeHoff to take over, which he did until around 1965, when further publication was discontinued. Because I probably had the only complete set of "Stereologias", Miro Kališnik recently asked me for a set of copies for the Society archives. These copies are reproduced here for their historical significance and for their value in identifying the early stereologists and their activities. I think you will enjoy browsing through these pages of the early "Stereologias".

STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Volume 1, Number 1, April, 1962

With this, our first issue of Stereologia, the Bulletin of the International Society for Stereology, we inaugurate a series of monthly newsletters. In it we hope to acquaint you with the work that others are doing in the field of stereology. This will be done primarily by means of short personal communications from the members. Also, from time to time, we plan to include brief announcements, membership lists, and other Society business.

Many diverse scientific disciplines are embraced within this Society. Thus, it is desirable that we know of the work that other members are doing. Their problems and their solutions will be available to others, and duplication of effort will be avoided. By far the greatest benefit should come through the mutual stimulation afforded by the interplay of ideas generated in these Bulletins.

This issue of Stereologia should prove a memorable one. First, there is an exposition from our President, Professor Hans Elias, on the history and scope of the Society. Then, there are brief accounts by outstanding scientists of their work in metallography, petrology, and mathematics. --The Editors

STEREOLOGY: SCOPE AND LIMITS

Hans Elias, Professor of Anatomy, Chicago Medical School, President International Society for Stereology

The word "stereology" was coined by the charter members of this Society on May 11, 1961, on the Feldberg Mountain in the Black Forest. An informal meeting was held at that location of persons interested in the three-dimensional interpretation of flat images, such as sections and projections. On the evening of May 10, the members of the group decided that they should think up one word to take the place of the above lengthy expression. The word "stereology" was suggested and unanimously accepted.

Stereology, sensu stricto, deals with a body of methods for the exploration of three-dimensional space, when only two-dimensional sections through solid bodies or their projections on a surface are available. Thus, stereology could also be called extrapolation from two- to three-dimensional space.

The conclusions of stereology are based on uniaxial viewing. Stereology is, therefore, to be distinguished from stereoscopy, a method of space exploration based on parallax, i.e., biaxial viewing. In a sense, stereology is the opposite of photogrammetry, a body of methods which utilizes parallax, i.e., viewing from more than one direction to obtain flat maps.

Stereometry, also known as solid geometry, deals with the properties of solids of known shapes.

Descriptive geometry, of which perspective is an integral part, supplies methods by which projections of objects of known three-dimensional shape can be drawn on flat surfaces in such a manner that this three-dimensional shape is easily comprehended by a beholder.

In microscopic anatomy and histopathology, for example, the internal structure of bulky organs is explored by means of extremely thin slices, which are viewed uniaxially with the light microscope.

In geology, petrography, mineralogy, ceramics, and metallography we have sciences which deal with opaque masses often consisting of mixtures of various materials. Usually, in order to examine the internal structure, the mass is sectioned so that the two-dimensional patterns are revealed. From these patterns, conclusions are drawn as to the amount, shape, size, and arrangement in space of the component materials.

Also, thin sections of rocks are frequently prepared for viewing and analysis by polarized transmitted light. Recently, the internal structure of thin metallic films is being studied increasingly by transmission electron microscopy. In these latter examples, of course, we are dealing with projected images.

One of the earliest stereologists whose work has remained known until this day was Ptolemy who developed his well-known system of planetary motion as an attempt to interpret, in a meaningful manner, the apparently chaotic movements of the images of planets on the inner surface of a sphere.

Today, stereology is practiced in a number of sciences.

In astronomy, the observer is confronted with images of heavenly bodies projected, as it were, on the inner surface of a sphere around the observer. These images must be interpreted in such a way that the spatial distribution and the spatial motions of the objects from which light emanates can be understood. To estimate distances of nearby stars, the astronomer can still use a "stereoscopic" method, namely parallax from two opposing points of the earth's orbit. But beyond the realm of a small number of stars, stereology is the only available method of investigation.

Spatial interpretation of X-ray diffraction patterns is an important part of stereology. It is a complicated procedure to derive information on molecular and crystalline structures from flat, photographic images.

There are a variety of approaches to stereological problems.

The statistico-geometrical approach depends on measuring and classifying images. It is applicable when objects of similar shapes are randomly distributed in space. In such cases, a single section or projection, if only extensive enough to contain a statistically significant number of figures, may suffice to obtain valid results.

If objects of similar shape are arranged regularly in space, a great number of sectional planes or visual angles, or at least three of them which are perpendicular to each other, must be used.

In the case of objects of complicated shapes, reconstruction from serial sections is the method of choice. The same is true if only one or a few objects are available.

Finally, there are cases in which a small number of similar objects, but of complicated shapes, are available, and serial sectioning is impracticable. One depends, in these cases, on visual examination of a great number of random sections. From these pictures the investigator must create, mentally, a three-dimensional image of a typical object which may be modeled in clay, and then cut in various directions. If the sections of this model resemble those made with the microtome, the chances are that the model is representative of the type of objects under observation. In the case of distant objects, a number of random projections can be used; and, instead of sections through a model, its shadows can be checked against photograms. The result of this type of examination resembles an ideogram and, in the case of three dimensions, has been called "stereogram".

Except for the interpretation of X-ray diffraction patterns, we have, in essence, three basic stereological methods:

The statistico-geometrical approach gives information on a range of size and shape within a class of objects.

Reconstruction gives information about shape, size, and position of one specific, individual object.

A stereogram is a three-dimensional presentation of a type.

The primary concern of the stereologist is his endeavor to recognize three-dimensional structure. His secondary concern is to communicate his findings to his colleagues and to the public. It is in this communicative phase that descriptive geometry and illustration enter into the realm of stereology.

The International Society for Stereology, through its members, intends to promote three-dimensional thinking. The ever-increasing need to understand structure stands in contrast to a progressive degeneration of structural thinking among the younger generation. Neglect of geometry and of representational drawing in the elementry and secondary schools, as well as neglect of morphology in the colleges, is largely to blame for this state of affairs so noticeable among our students. The International Society for Stereology hereby appeals to its members to use their personal influence in their communities to strengthen the teaching of subjects dealing with shape and structure.

QUANTITATIVE METALLOGRAPHY; SPACE-FILLING RELATIONSHIPS; SECTIONING OF STRUCTURES IN SCIENCE AND THE DECORATIVE ARTS

Dr. Cyril Stanley Smith, Institute Professor, Massachusetts Institute of Technology

As a metallographer, I have studied the interplay between the local geometry resulting from surface-energy equilibrium and the long-range problems of space filling. Paper 1 below includes an analysis of the three-dimensional shape of grains which are inherently instable, as are all interface-determined structures which are not symmetrically stacked. The prevalence of pentagons has basic significance in nature. Lester Guttman and I developed basic relationships between measurements of linear features on sections and the three-dimensional ratios of surface to volume (Paper 2). In odd moments speculate on insoluble problems such as the basic nature of structure and role of structural relationships in perception and aesthetic appreciation.

1. "Grain Shapes and Other Metallurgical Applications of Topology," Metal Interfaces, American Society for Metals, Cleveland, (1952), 65-113.

2. With Lester Guttman, "Measurement of Internal Boundaries in Three-Dimensional Structures by Random Sectioning", Trans. AIME, 197, 81-87 (1953).

3. "Microstructure", Trans. ASM, 45, 533-575 (1952).

4. "Practical Indices of Grain Shape and Grain Size", Quantitative Metallography, (F. N. Rhines, editor), in press, McGraw-Hill Book Co., Inc.

COMMENTS ABOUT MY WORK

Dr. Felix Chayes, Geophysical Laboratory, Carnegie Institution of Washington

My principal interest is not inferences about form, but estimates of relative volumes from measurements of relative areas made in section. This is perhaps the oldest micrometric problem in petrography, and some excellent work on it actually predates the application of the microscope to our subject matter. Measurements of this type are of great interest because they provide the only potentially unbiased estimates of the relative abundances of the different minerals of which most rocks are formed, and in many petrographic problems, as well as in much petrographic taxonomy, these relative abundances are the key variables. Analyses of this kind are known in the trade as modal analyses, or, simply, modes. In addition to their intrinsic interest, they have the advantage of being relatively easy and quick to make. With the increasing interest in statistical petrography, this last property has greatly increased the popularity of modal analysis. In much recent work, to the horror of "chemical-petrologists" like myself, modal analyses seem to play about the same role as index fossils in stratigraphy.

Most of my methodological work in this subject is summarized in:

Chayes, F., Petrographic Modal Analysis (1956), John Wiley and Sons, Inc., N.Y.

For some practical applications, see

Chayes, F., "Composition of the Granites of Westerly and Bradford, R.I.", Am. J. Sci., 248, 378-407 (1950).

Chayes, F., "The Finer-Grained Calcalkaline Granite of New England", J. Geol., 60, 207-254 (1952).

Chayes, F., "Potash Feldspar as a By-Product of the Biotite-Chlorite Transformation", J. Geol., 63, 75-82 (1955).

A BRIEF SUMMARY OF MY WORK IN STEREOLOGY

Dr. August Hennig, Anatomisches Institut, Munich, Germany

My work in the Department of Anatomy, University of Munich, deals with the mathematical-physical problems of macroscopic and microscopic anatomy.

In 1954, I served as consultant to Mr. C. Elze, editor of a new edition of a compendium on Anatomy, on physical problems of the lungs. This experience acquainted me with the usual methods for determining the inner surface area of the lungs, as well as with the defects in these methods. I succeeded in developing a generally valid method for measuring any surface area from their traces in thin sections (see Publications Nos. 1 and 3), and gave special instructions for the proper use of the formulae.

Mr. R. Wetzstein, Munich, consulted me about the errors involved in measuring spheres in thick sections. Later, I found that the formula which was developed for him is known in American publications as the Holmes effect.

The inexact use of line sampling with the Leitz integration stage (for volume measurement) was common with biologists because of the lack of mathematically based directions for use. Thus, uncertain results which could not be controlled were the reward of time-wasting efforts. I supported my recommendation of the more suitable and modern point-counting technique with simple instructions for correct use, including a discussion of the errors. Following my recommendation, the firm Carl Zeiss built two integration eyepieces for the measurement of volumes and surfaces (see Publications Nos. 2 and 3).

Some day, I would like to determine the volume of tiny irregular bodies (such as organs of embryos of little animals) imbedded in slices. It is well known that such a volume is equal to the product of the sum of cross sections throughout the body times the (regular) distance separating the sections; however, no one knew the dependence of the errors on direction and number of these cross sections and on the shape of the bodies. For several simple forms and for the principal directions, I determined the necessary number of sections for a desired accuracy (see Publication No. 4).

Finally, I was quite pleased to make the acquaintance of Professor H. Elias, Chicago, through some critical notes concerning his "Geometry of Sectioning". Following his suggestion, I determined the distribution of shapes of sections made through irregularly straightened rotary ellipsoids and elliptical cylinders (see Publication No. 5). Further studies followed. One of them was communicated at the foundation meeting of the Society; I demonstrated how to determine the length of lines in space by counting the number of intersections with periodic sections of known spacing.

PUBLICATIONS

1. "Bestimmung der Oberfläche beliebig geformter Körper mit besonderer Anwendung auf Körperhaufen im mikroskopischen Bereich", Mikroskopie, 11, 1-20 (1956).

2. "Das Problem der Kernmessung. Eine Zusammenfassung und Erweiterung der mikroskopischen Messtechnik", Mikroskopie, 12, 174-204 (1957).

3. "A Critical Survey of Volume and Surface Measurement in Microscopy", Zeiss Werkzeitschrift No. 30 (1958). Also in German, French, and Spanish.

4. "Fehler der Volumbestimmung aus dem Flächeninhalt periodischer Schnitte", Z. mikr. anat. Forschung, 66, 515-530 (1960).

5. With H. Elias, "Theoretical and Experimental Investigations on Sections of Rotatory Ellipsoids", Z.wiss.Mikrosk., 65 (in print).

STEREOLOGIA
THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

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STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Volume 1, Number 2, May, 1962

In this second issue of Stereologia, we present the Membership List of the Society as of April 15, 1962. Members are listed alphabetically, followed by their special field(s) of interest, and their addresses.

It is interesting to note that the 96 members in this list represent 16 countries and more than 16 specific disciplines. And yet, the great bulk of the scientists who would profit from association in this Society have scarcely been reached. We urge each member to actively promote this Society among his colleagues, so that its true growth potential and scientific mission can be realized to the utmost.

We will continue in subsequent issues of the Bulletin with the autobiographical sketches from the members. If you have not already done so, please send your résumé to an Editor.

Thank you. -- The Editors

NOTICE TO MEMBERS

Please bring this Bulletin to the attention of your Institute Librarian. Annual subscriptions are available at the nominal price of $1.00 (plus postage). Requests can be made to either Editor.

PLANS FOR FIRST CONGRESS UNDERWAY

Arrangements for the First Congress of the International Society for Stereology are now well on their way. All members who wish to submit a paper at the First Congress are cordially urged to do so.

As of now, the following information is available:

Place: Vienna, Austria
Time: 17-20 April, 1963
Housing: Hotel Europa
Registration Fee: $10.00
Papers: Submitted at least 4 weeks in advance.

Inquiries and communications should be addressed to the Congress Chairman:

Dr. Herbert Haug, Vice President
International Society for Stereology
Krankenhausstrasse 9
Erlangen, Germany

MEMBERSHIP LIST
INTERNATIONAL SOCIETY FOR STEREOLOGY
April 15, 1962

Dr. Juan-Antonio Astruc-Franco Facultad de Medicina de Granada, Spain	Profesor Adjunto de Anatomia
Dr. Francisco Abadia Fenoll Valle de Hebron 89-91 Barcelona, Spain	Pathological anatomy
Professor Pedro Amat Muñoz Avendia Fernando el Católico, 66-1.°-izq. Zaragoza, Spain	Neuroendocrinology
Dr. Günter Bach (Secretary) Mathematisches Institut Technische Hochschule Braunschweig Braunschweig, Germany	Mathematics
Mrs. Barbara G. Barnes 22 Grantchester Road Cambridge, England	Cancer research, electron microscopy
Dr. Felix D. Bertalanffy Department of Anatomy Medical College University of Manitoba Winnipeg 3, Manitoba, Canada	Associate Professor of Anatomy
Mr. Paolo Bianchi Instituto de Patologia della Università de Bari Bari, Italy	Pathology
Dr. Franz Bierring Medicinsk-Anatomisk Institut Norre Alle Kopenhagen, Denmark	Anatomy, electron misroscopy, and surgery
Dr. Adalbert Bohle Pathologisches Institut des Katharinen-Hospitals Stuttgart, Germany	Professor of Pathology
Mr. Richard C. Bourke Battelle Memorial Institute 505 King Avenue Columbus 1, Ohio, U.S.A.	Physical chemistry
Dr. Josef Bugl Battelle Memorial Institute 505 King Avenue Columbus 1, Ohio, U.S.A.	Mineralogy, reology
Dr. John W. Cahn G. E. Research Laboratory P. O. Box 1088 Schenectady, New York, U.S.A.	Quantitative metallography, transformations and thermo-dynamics of interfaces
Dr. Felix Chayes Geophysical Laboratory 2801 Upton Street, N.W. Washington 8, D.C., U.S.A.	Petrography

Dr. Nils Christensen SINTEF Trondheim, Norway	Metallurgy, in particular welding, metallography
Mr. William P. Clancy Materials Research Laboratory Ordnance Materials Research Office Watertown 72, Massachusetts, U.S.A.	Physical Metallurgist
Dr. Morris Cohen Massachusetts Institute of Technology Cambridge, Massachusetts, U.S.A.	Professor of Metallurgy
Dr. Paolo Contu Faculty of Medicine Institute of Anatomy Porto Alegre, GRS, Brazil	Anatomy
Professor Ronald DeFord University St., Box 7652 Austin 12, Texas, U.S.A.	Geology
Dr. Robert T. DeHoff Metallurgical Research Laboratory College of Engineering University of Florida Gainesville, Florida, U.S.A.	Assistant Research Professor
Dr. Roland deWit Metal Physics Section National Bureau of Standards Washington 25, D.C., U.S.A.	Imperfections in crystals
Dr. Hans Elias (President) Chicago Medical School 710 South Wolcott Avenue Chicago 12, Illinois, U.S.A.	Professor of Anatomy
Mr. Peter M. Elias 2175 Williams Street Palo Alto, California, U.S.A.	Pre-med student at Stanford University
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Mr. Martin S. Farkas Battelle Memorial Institute 505 King Avenue Columbus 1, Ohio, U.S.A.	Metallography
Dr. Paul J. Fopiano Manlabs, Inc. 21 Erie Street Cambridge 39, Massachusetts, U.S.A.	Physical metallurgy
Professor H.S.D. Garven Institute of Physiology University of Glasgow Glasgow, Scotland	Histology and physiology
Dr. Margarethe Gihr Institut für Hirnforschung Dennenbergstrasse 1-5 Neustadt im Schwarzwald, Germany	Anatomy
Professor Muratori Giulio Università Degli Studi Ferrara Instituto Anatomico Via Fossato di Mortara, n. 66 Ferrara, Italy	Anatomy
Dr. Helmut J. Goldschmidt B.S.A. Group Research Centre Mackadown Lane, Kitts Green Birmingham 33, England	X-ray crystallography, metal physics
Dr. Joseph Gurland Division of Engineering Brown University Providence 12, Rhode Island, U.S.A.	Associate Professor, Physical Metallurgy
Dr. Wihelm Harkmark Department of Anatomy University of Oslo Oslo, Norway	Anatomy and pathology
Professor Helmut Haselmann Brahmsstrasse 20 Aalen/Wuertt., Germany	Anatomy and optics
Dr. Herbert Haug (Vice President) Krankenhausstrasse 9 Erlangen, Germany	Associate Professor of Anatomy
Dr. C. W. Haworth Department of Metallurgy St. George's Square Sheffield 1, England	Metallurgy, eutectic structures
Dr. August Henning Anatomisches Institut Pettenkoferstrasse 11 Muenchen 15, Germany	Mathematics and anatomy
Dr. John E. Hilliard G. E. Research Laboratory P. O. Box 1088 Schenectady, New York, U.S.A.	Physical metallurgy, quantitative metallography
Dr. Arne Hossmann Bekkelagsweien 15 Oslo, Norway	General medical practice
Mr. Earle C. Hoxie E. I. du Pont de Nemours & Co. Aiken, South Carolina, U.S.A.	Metallography, corrosion
Dr. Gabro Inke Tiergartstrasse 2 Halle/Salle, Germany, D.D.R.	Anatomy
Dr. Oscar C. Jaffee University of Buffalo Buffalo 12, New York, U.S.A.	Assistant Professor of Biology
Mr. Horace M. Joseph National Bureau of Standards Washington 25, D.C., U.S.A.	Electro-optical circuits
Mr. James S. Kahn Building 173B University of California P. O. Box 808 Livermore, California, U.S.A.	Chemist, petrographer
Mr. J. Ferdinand Kayser 14 Leigh Park Datchet, Buckinghamshire, England	Sterophotomicrography
Professor Ekkehard Kleiss Department of Anatomy Merida, Venezuela	Anatomy
Dr. P. K. Koh Research Department Bethlehem, Pennsylvania, U.S.A.	X-ray and electron diffraction
Dr. Tokuzo Kojima Department of Anatomy School of Medicine Nihon University Itabashi-Ku, Tokyo, Japan	Anatomy
Professor R. Wayne Kraft Department of Metallurgical Engineering Lehigh University Bethlehem, Pennsylvania, U.S.A.	Physical metallurgy, quantitative metallography
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Dr. Helmut Kulenkampff Koellikerstrasse 6 Wuerzburg, Germany	Professor of Anatomy
Professor Sten Lagerstedt Histological Institute Lund, Sweden	Histology

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Mr. Rolf Lorenz Bundesforschungsanstalt fuer Viruskrankheiten der Tiere Waldhaeuserhoehe Tuebingen, Germany	Mathematics
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Dr. Koichi Masubuchi Battelle Memorial Institute 505 King Avenue Columbus 1, Ohio, U.S.A.	Metals joining, fracture
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Dr. George A. Moore National Bureau of Standards 8.3 Washington 25, D.C., U.S.A.	Quantitative metallography
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Professor W. Rotter Direktor des Pathologisches Institutes der Universität Frankfurt Frankfurt am Main, Germany	Pathology
Professor Yutaka Sano Anatomisches Institut der Medizinischen Akademie Kyoto Kawaramachi-Hirokoji Kyoto, Japan	Anatomy
Professor Kurt Schwidefsky Director, Institut für Photogrammetrie und Topographie Technische Hochschule Karlsruhe Karlsruhe Englerstrasse 7, Germany	Photogrammetry and topography
Dr. W. F. Sheely Division of Research Metallurgy and Materials Programs U. S. Atomic Energy Commission Washington 25, D.C., U.S.A.	Physical metallurgy
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Dr. Mª del Carmen Smith Agreda San Vincente de Paúl, 8, Spain	Superconductivity, transport theory
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Dr. Heribert Thaler Sebastianplatz 7 Wien III, Austria	Histology of liver and kidney

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Dr. Fritz R. Winslow Battelle Memorial Institute 505 King Avenue Columbus 1, Ohio, U.S.A.	Kinetics and morphology of metallurgical transformations
Dr. Wolfgang Wuescher Grassistrasse 10 Leipzig, Germany, D.D.R.	Professor of Anatomy

STEREOLOGIA

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STEREOLOGIA
THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Volume 1, Number 3, June, 1962

Revisions to the Membership List which appeared in the May, 1962, Stereologia, are presented herewith. Please send in any further corrections to the Editors.

Do not forget to plan now for the First Congress of the International Society for Stereology, to be held in Vienna, 17-20 April, 1963. Contact the Congress Chairman, Dr. Herbert Haug, for further details, or see the announcement in Stereologia, May, 1962. – – The Editors

Prof. Dr. Wolfgang Wünscher, Director, Hirnforschungsinstitut der Universität Leipzig, Emilienstr. 12, Leipzig

Some years ago I set myself the task to improve the method of demonstrating angio-architectonics – developed by the then director of our Brain Research Institute – to such an extent as to enable the reliable identification and differentiation of the particular kinds of vessels. These investigations have been pursued by Mrs. Werner, D.Sc., and myself since 1958.

The Pfeifer-method has been modified insofar as to enable us to form distinct conceptions about the individual vessels of a given griseum and definite area, respectively, in a fully injected animal brain.

The next object tackled by us was to find out the number of vessels in a griseum and area, respectively, using biometrical methods among others. This method has been developed by Mrs. Werner, D.Sc., and taken as a basis for her report.

The subject of future investigations will be the application of the angio-architectural methods, as developed and set forth here, to researches concerning the ageing brain.

Preliminary Report by Günter Bach

Histological problems frequently deal with the following question: In measuring microtome sections one finds distribution of sizes of circular figures derived from sections of spheres. Technical difficulties associated with the measurement of smaller sections often yield a truncation of the distribution. Furthermore, in practice, samples are grouped, so that we have to estimate from the "truncated grouped distribution" the number of spheres in unit volume, their mean diameter, and the standard deviation.
In the case of very thin sections, the formulae are relative simple, but in case of thicker slices, the formulae include an auxiliary function. The values of this function must be read with the respective index off a curve.
For more details see: "Über die Bestimmung von charakteristischen Grössen einer Kugelverteilung aus der Verteilung der Schnittkreise." Zts. f. wiss. Mikroskopie (In print).

QUANTITATIVE ANGIOARCHITECTURAL EXAMINATIONS OF THE BRAIN STEM OF THE GUINEA PIG

Dr. Lisa Werner, Hirnforschungsinstitut der Universität Leipzig, Emilienstr. 14, Leipzig

After finishing qualitative examinations on angioarchitecture, a quantitative method was sought in order to obtain sufficient numerical results for statistics, and to compare with other parts of the brain tissue (e.g., nerve cells, glia cells, nerve fibers). The appropriate method for this purpose seemed to be the "Punktzählverfahren" (Treffermethode) (method of counting hits). The efficiency of this method was checked in an animal specimen as well as in a self-made model by means of ocular micrometers with different numbers of intersections. Mathematical help was given by Dipl.Math. Weissenburger and Grimm, at Jena.

The investigation has been finished by now and will be published soon. This will be my first publication on this problem.

STEREOLOGICAL PROBLEMS OF MICROSCOPICAL NEUROANATOMY

Priv.-Doz. Dr. med. Herbert Haug, Department of Anatomy, University of Erlangen, Germany West

In 1950 the counting of nerve cells in the cerebral cortex introduced me to problems which we call now "Stereology". I learned hereby that counting errors are greatly influenced by the cell-shape and thickness of the sections. I saw only one possibility to exclude errors in counting, and that was the combination of mathematical method with theoretical "stereological" considerations. The results obtained in solving this problem have been quite good and I could go on with examinations in cerebral cortex (No. 1).

In the following years I became acquainted with the point-sampling-method suggested by Chalkley. With the aid of this method I found an exponential correlation between the weight of the brain and the relative volume of the nerve cells in the cortex cerebri. We see this correlation in the development of the human brain as well as in a comparison of brains of adult mammals (No. 2).

Different results which have been published by other authors using the same method on the same subject forced me to look for the exact foundation and limits of the point-method. I found that the method is based on stringent rules. If these rules are not followed the results are most likely to be incorrect (Nos. 3 and 4).

I made the acquaintance of other scientists, who have worked on the same or similar problems, through publication of my results. I had discussions with Prof. H. Elias and was very pleased with his ideas to arrange a small meeting on the Feldberg in the Black Forest and to establish a Society for such problems.

My most recent papers contain practical instructions for cell counting and evaluation of the relative volume in biological microscopy and their theoretical development (Nos. 5 and 6).

1. "Der Grauzellkoeffizient des Stirnhirnes der Mammalia", Acta anat. 19, 60-100. 153-190. 239-270 (1953).

2. "Quantitative Untersuchungen an der Sehrinde", G. Thieme, Stuttgart 1958.

3. "Die Treffermethode, ein Verfahren zur quantitativen Analyse im histologischen Schnitt", Z. Anat. 118, 302-312 (1955).

4. "Remarks on the determination and significance of the gray cell coefficient", J. Comp. Neur. 104, 473-492 (1956).

5. "Bedeutung und Grenzen der quantitativen Messmethoden in der Histologie", Medizin. Grundlagenforsch. 4, 299-344 (1962).

6. "The quantitative microscopical research in the nervous system, possibilities and limits", in press.

THREE-DIMENSIONAL ARRANGEMENT OF COLLAGENOUS FIBERS IN THE WALLS OF BLOOD VESSELS; THREE DIMENSIONAL ARRANGEMENT OF BLOOD VESSELS IN THE KIDNEY

Kuegelgen, von, Alkmar, Dr. med., Professor of Anatomy, University of Kiel, Kiel, Neue Universität, West Germany

Anatomy is a matter of three dimensions or it is no anatomy at all. – It was my first problem to develop microscopical methods for visualizing the 3-d collagenous network in thick sections. It could be managed (1955) by synchronous movement of the analysor and the polarisor of the polarizing microscope: double refringent elements by that manoeuvre brightening one after another without moving the specimen, and therefore without losing orientation. – I am developing another method utilizing the "Universal-Drehtisch" of the polarizing microscope (which has been used till now by mineralogists only), to measure the angles of double refringent elements (muscles and collagenous structures) in thick sections, not only in the horizontal plane, but also three-dimensionally.

The 3-d arrangement of the fine blood vessels of the kidney cannot be recognized by analysis of sections. My coworkers and I (1959) have been dissecting injection and corrosion specimens in the classical manner under the binocular microscope.

I am now trying (1962) another way of exploring the 3-d arrangement, especially of the kidney capillaries, by combining quantitative analysis (with help of the integrating oculars of A. Hennig, Munich) of kidney structures in microscopical sections, with other information concerning kidney structures and functions. The leading concept of this work is a true stereological one: definite numerical relations and certain patterns of arrangement, especially of transverse sections of certain kidney structures, do allow - by means of some geometrical arguments - a stereological interpretation.

Publications:

KUEGELGEN, von, A. (1955), Über den Wandbau der Vena cava caudalis eines erwachsenen Finnwales, Ztschr. Zellforschung 41, 435-459.

KUEGELGEN, von, A. (1956), Weitere Mitteilungen über den Wandbau der groassen Venen des Menschen unter besonderer Berücksichtigung ihrer Kollagenstrukturen, Ztschr. Zellforschung 44, 121-174.

KUEGELGEN, von, A., B. KUHLO, W. KUHLO und Kl.-J. OTTO (1959), Die Gefässarchitektur der Niere (Zwanglose Abhandlungen a.d. Gebiet d. Normalen u. Pathol. Anat., Heft 5), Georg Thieme, Stuttgart.

KUEGELGEN, von, A. und B. BRAUNGER (1962), Quantitative Untersuchungen über Kapillaren und Tubuli der Hundeniere, Ztschr. Zellforschung 56 (in press).

MEASUREMENT OF NUMBER AND GENUS IN INTERCONNECTED BODIES

Robert T. DeHoff, Metallurgical Research Laboratory, University of Florida, Gainesville, Florida

Sintering is the process by which small solid particles agglomerate to form a solid mass at temperatures below the melting point. Geometrically, the process consists of the formation and growth of interparticle contacts, subsequent closure of intervoidal channels resulting in isolation of porosity, and finally, the shrinkage and disappearance of these isolated pores. The conviction that the geometry of the aggregate determines its behavior during sintering led my co-workers and me to an effort to develop means for describing and measuring the evolution of the complex geometry involved. It became important to find means for determining pore volume, surface area, number and size distribution of pores and interparticle contacts, genus, and surface curvature. Because the materials we used are opaque (copper, nickel, iron, etc.), the geometry had to be determined from measurements made on two-dimensional sections, or, at best, from microradiographs of thin sections. Accordingly, I have been actively engaged in the development of the fundamental relations of stereology, or as we call it, quantitative metallography, for five years or more. This had led to two publications on the number and size distribution of ellipsoidal particles[1,2] and culminated in a Conference on Quantitative Metallography held at the University of Florida in January of 1961. Dr. F. N. Rhines and myself are currently editing the proceedings of this conference, to be published later this year[3].

1. R. T. DeHoff and F. N. Rhines, "Determination of the Number of Particles Per Unit Volume from Measurements Made on Random Plane Sections: the General Cylinder and the Ellipsoid", Trans. AIME, 221 (1961) 975.

2. R. T. DeHoff, "Determination of the Size Distribution of Ellipsoidal Particles from Measurements Made on Random Plane Sections", Accepted for publication, Trans. AIME, (1962).

3. R. T. DeHoff and F. N. Rhines, Editors, "Quantitative Metallography", to be published by McGraw-Hill.
   a. Chapter 2, "Geometric Probabilities"
   b. Chapter 5, "Measurement of Number in Volume"
   c. Chapter 11, "Topology of Interconnected Phases"

ADDITIONS TO MEMBERSHIP LIST
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STEREOLOGIA
THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

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Volume 1, Number 4, July, August and September, 1962

Society News

Dr. Ervin E. Underwood, the Treasurer of the Society, has accepted a new position in California. His new address is: c/o Lockheed Missiles and Space Co., Materials Sciences Laboratory, Building 201, Palo Alto, California.

While Dr. Underwood will remain the Treasurer, he had to resign from the editorship of STEREOLOGIA. We are deeply grateful to him for the excellent first three issues. Fortunately, we have found a new editor: Dr. Robert T. DeHoff, Metallurgical Research Laboratory, University of Florida, Gainesville, Florida, U.S.A., who has ample experience as a scientific editor and whose autobiographical s k e t c h you have read in STEREOLOGIA, 1, 3:11.

We intend to open the pages of STEREOLOGIA to brief summaries and preliminary notes on your most recent research, not to exceed 500 words. The use of simple formulae which present no difficulty to the printer is permitted.

One diagram can also be included in each brief report of maximum dimensions 9x9 cm, *if* you send the cut (clichee) or pay $1 per square inch.

May we remind you again of our Congress, April 17-20, 1963 in Vienna.

Please urge every scientific library in your city to subscribe to STEREOLOGIA. The yearly subscription rate is $2.50. An order form will be found in the Bulletin. The subscription rate for new subscribers had to be raised because we must have STEREOLOGIA printed commercially from now on. But it will still be sent free to the members.

New Honorary member: Dr. Otto Roehm, Direktor, Roehm und Haas G.m.b.H., Darmstadt, Germany, Mainzer Str. 42. Special interest: Chemistry.

THREE-DIMENSIONAL GRAPHS

Arne SOLLBERGER, Department of Anatomy, Caroline Institute, Stockholm 60, Sweden

Though being an anatomist my chief interest is biostatistics and biological rhythms. The present problem arose in connection with the need for a spatial representation of the simultaneous correlation between three variables. Though many methods exist for preparing such graphs, they are usually bulky. I wanted them to be simple and to occupy as little space as possible since, at that time, a large number of them seemed to be required. Stereograms of various kinds were not acceptable since they do not yield the freedom to view the diagrams from different directions in the search for correlation surfaces.

I finally constructed a collapsible diagram which was hanging upside down and which was viewed in a mirror. The observer's head must be above and the mirror below the model level. After use the whole diagram may be put in an envelope. The x-y-z diagram is prepared as follows:

The bottom plate (x-y-plane) is first prepared as a x-y dot diagram. Holes are drilled through the plate at the dots. Small wooden pellets are glued to thin threads. A length on the threads proportional to the z-values is marked with a pencil. The thread is drawn through the holes in the bottom plate until the pencil mark is flush with the bottom plate surface and fixed with glue on its back side.

The hanging diagram may be viewed in a mirror (which enables one to obtain any angle of observation with ease) or it may be photographed from below. Threads may be chosen very thin and of the same colour as the bottom plate, pellets preferably of the complementary colour. The threads may thus be made fairly invisible, which aids in the search for correlation surfaces.

SOLLBERGER, A., Studies of temporal variations in biological variates, Suppl. Rep. 5th Conf. Soc. Biol. Rythm, Stockholm 1960.

COMMENTS ON MY WORK AND ITS RELATION TO STEREOLOGY

James Steven KAHN, Ceramics Group, Chemistry Division Lawrence Radiation Laboratory The University of California, Livermore, California, USA.

When I was a graduate student I became aware of the fact that the zoologist and botanist as well as the geologist look at thin sections of matter. I was unprepared for this revelation, simply because my training treated geological microscopy and its problems as being unique to geology. Although, I feel that my present outlook is less parochial than it was when I was a student, the above attitude still exists in many quarters today. The ceramic petrographer is generally unaware of the geological techniques, the geologist is ignorant of the metallographers problem, and the metallographer . . ., etc.

The major portion of my current work is concerned with the inter-relationships among the many parameters involved in the study of the microstructure of Ceramics. Of ultimate concern is the correlation of the microstructural properties with macrostructural properties as well as with atomic structure. By use of plane sections and the fields of view they present under the light and electron microscopes, attempts to bridge the gaps between the 2-dimensional plane and the three dimensional solid are being made.

Experimental procedures are discussed in:

Statistics for Geologists, 1962, John Wiley & Sons, Inc., (with Robert L. Miller, University of Chicago). Experimental results may be seen in:

Anisotropic sedimentary parameters, 1959, Trans. N.Y. Acad. Sci., Ser. II, v. 21, 5, p. 373.

Microstructure of BeO: Preferred orientation of extruded tubes, abs. paper to be presented at Fall, 1962 meeting of Pacific Coast Section of the American Ceramic Society (with Eugene Monroe UCRL-6927-T).

Microhardness of single crystals of BeO and other wurtzite compounds, 1962, with Carl F. Cline, in press.

QUANTITATIVE ANALYSIS OF SERIAL SECTIONS FOR STUDY OF PATTERN FORMATION IN THE CENTRAL NERVOUS SYSTEM IN AMPHIBIANS.

Pieter D. NIEUWKOOP, Hubrecht Laboratory, International Embryological Institute, Janskerkhof 2, Utrecht, Holland.

A new operation technique, by which long strips of competent gastrula ectoderm were attached to various points of the anlage of the central nervous system, enabled me to test the inductive capacities in the entire neural area. A more detailed study of the experimental material, however, called for a quantitative analysis of the material concerned. In drawings of successive serial sections the outlines of the various structures of the nervous system were determined and their surface areas measured planimetrically. The sum total of the surface areas of each of the structures, multiplied by the thickness of the sections and divided by the square of the linear magnification used for the drawings, yields the absolute volume of the individual structures of the central nervous system (Nieuwkoop 1958).

The same method has recently been applied by my co-worker Miss Boterenbrood to experimental material of very high complexity, which moreover could not be oriented before sectioning (Boterenbrood 1962).

Although this method is reliable, it is so time-consuming that the further analysis of pattern formation in the cenral nervous system is strongly hampered by the lack of a more suitable stereological technique.*

Publications

NIEUWKOOP, P. D., Neural competence of the gastrula ectoderm in Amblystoma mexicanum. An attempt at quantitative analysis in morphogenesis, Acta Embryol.Morph.Exp., 2: 13-53, 1958.

BOTERENBROOD, E. C., On pattern formation in the prosencephalon. An investigation on disaggregated and reaggregated presumptive prosencephalic material of neurulae of Triturus alpestris, Thesis, Utrecht, 1962.

**The method of counting "hits", i.e. points in a net which are superimposed on the microscopic image should facilitate your work greatly. See for example: Hennig, A., Kritische Betrachtungen zur Volumen-und Oberflaechen-Messung in der Mikroskopie. Zeiss - Werkzeitschrift Nr. 30. — Also: Hennig, A., Zwei neue Messokulare fuer die mikroskopische Volum-und Oberflaechenmessung, Forsch. Ing.-Wes. 23:71 — 73, 1957, Editor*

SOME STEREOLOGICAL METHODS USED IN EMBROLOGY.

Oscar Charles JAFFEE, Department of Biology, University of Buffalo, Buffalo 14, New York, U.S.A.

In studies of the embryonic kidney (pronephros), I found it necessary to obtain three dimensional concepts of this structure. Since this is a relatively simple organ with three tubules, I found the glass plate method satisfactory. This consists of projecting (with a camera lucida) cross sections on glass plates and then obtaining a three dimensional view by holding several glass plates together. Thus by viewing several plates at a time the organ can be reconstruced.

In more recent studies of cardiac development in which anomalous hearts are produced experimentally, dissection of such hearts, along with normals, is most helpful. Photographs of such dissections have been made in my laboratory with a stereographic camera mounted upon a dissecting (stereoscopic) microscope.

The classic method of Born for the reconstruction of organs from serial sections is still indispensible. I have used a variation of this method, which utilizes transparent plastic instead of wax.

Projections of sections are sketched on the plastic and marked and then cut out. It is possible to use thin plastic sheets, and compensate for the thickness of the sections with small plastic blocks. This allows the construction of a transparent model which allows a view of the internal structure in relation to the overall configuration.

Another useful method for making reconstructions has been developed by an art student, Mrs. Rebecca Convisor, who has been assisting me. This consists of sculpturing a solid model of a heart from cross sections in modelling clay. This is then cut in half longitudinally and the internal structure hollowed out, again with reference to the sections. This is an expedient method and especially useful for earlier, less complex, stages.

JAFFEE, Oscar Charles Morphogenesis of the pronephros of the Leopard frog (Rana pipiens). J. Morph 95:109-124, 1954.

JAFFEE, Oscar Charles Hemodynamics and Cardiogenesis I. The effects of altered vascular currents on cardiac development. Journal of Morphology 1962.

THE ROLE OF SPATIAL ARRANGEMENT OF NUCLEI IN KARYOMETRIC EXAMINATIONS

Miklos PALKOVITS, Department of Anatomy, University of Budapest, IX. Tuzolto-utca 58., Hungary.

For six years I have been working on methodological problems of karyometric statistical examinations. In cooperation with G. Inke I have elaborated histological, mathematical and statistical correction factors. We established the degree in which fixation, embedding, sectioning and staining influences karyometric results (1-2). We worked out mathematical formulae for the cubing of nuclei of different shapes. Nuclei can be regarded as ellipsoids of rotation and their cubic content can be determined with the aid of the widest diameter of the ellipsoid and the perpendicular normal bisector (3). Based on these formulae J. Fischer and G. Inke made nomograms, and with their aid I prepared a chart (4). From this chart the nuclear volumes can be obtained.

In practice — I made about 300.000 measurements — I found that although I considered all histological, mathematical and statistical coefficients of correction, greater deviation was experienced than I had anticipated. In the diverse tissue-structures the divergence was varying, therefore, measurements in tissue types were made with a nuclear excentricity of varying magnitude (5). The tissues of the organism can be classified into three main types: diffuse, columnar and aciniform nuclear arrangement. In cases of globular nuclei the spatial arrangement of the nuclei is indifferent. the direction of the sectional plane is likewise indifferent. In case of oval nuclei karyometric results are influenced by three factors: the excentricity of the nuclei, spatial arrangement and functional change in form, resulting from structural composition. In diffuse arrangement the excentricity of the nuclei is the dominating factor. If the nuclei are cut in some plane, the most varying sections are obtained. which, however, equalize each other during statistical evaluation. The greater the excentricity of the nuclei, the greater is the variability of measurements. The number of the various section planes will be higher, so the number of the measurements have to be increased in order that the values be equalized within the given significance limits. I succeeded to prepare a diagram from which the number of measurable nuclei of varying excentricity can be read. If the nuclei of the examined material show columnar formation, their stereoscopic placement in space and direction of the section plane is decisive. If the axial ratio of the nuclei is above 1 : 1.15, significantly different results appear on the very same material. In aciniform structure, apart from excentricity the functional state of the acini-forming cells is also of importance, since increase in function is followed by diminution in excentricity, functional diminution by increase in excentricity. This fact greatly enhances the measure of standard deviation. Therefore, the number of measurable nuclei in these tissues is much higher than in the former two cases. Since all tissues can be traced back to these three basic types, the above viewpoints must always be taken into consideration, and the correction data, — which I succeeded to establish in diagrams — should be applied.

The significance of these karyometric examinations, in my opinion lies in the very sensitive quantitative histological method, which is suitable also for the evaluation of slighter functional changes of nuclei. Nuclei are exceedingly responsive to metabolic cellular changes, their size alters, but is well measurable. I find this method suitable for the determination of target organs relative to certain chemicals, medicines and hormones and as a means of finding out whether the functional change in some organs influences others or not. The determination of the manner of action is naturally the task of another examination. This karyometric method was of great value to me in my endocrinological examinations.

Publications

(1) INKE, G. M. PALKOVITS, I., GYARFAS and A. BAJTAI, Ueber methodische Fragen der Kernvariationstatistik I-III, Acta biol. Acad. Sci. Hung., 8, 305-323,1958.

(2) INKE, G. M. PALKOVITS, I. GYARFAS and A. BAJTAI, Ueber methodische Fragen der Kernvariationstatistik IV-VI, Acta morph. Acad. Sci.Hung. 8, 233-271, 1958.

(3) PALKOVITS, Miklos, A sejtmagvariaecioes statisztikai eljaeraes moedszereenek egyszeruesiteese ees a meeree hibaforraesok kikueszoeboeleesenek felteetelei. Kiseerletes Orvostud. /Budapest/. In press.

(4) PALKOVITS, Mikloes, Angaben und Hilfsmittel zur Auswertung von Kernvariationsuntersuchungen, Z.mikrosk.anat. Forsch., 67, 343-355, 1961.

(5) PALKOVITS, Miklos and Janos FISCHER, Ueber methodische Fragen der Kernvariationstatistik IX. In press.

SIZE DISTRIBUTION OF SPHERES AS DERIVED FROM TRANSPARENT SECTIONS OF FINITE THICKNESS

Guenter BACH, Am Lehrstuhl fuer Mathematik der Technischen Hochschule, Braunschweig, Germany

A few year's ago, a histologist who consulted me awakened my interest in stereological problems. He brought to my attention the problem of "sliced tomatoes", i.e. the question of how the distribution of sizes of shperes could be derived from the distribution of the diameters of their slices. Since histologists depend on "sections" of finite thickness, the problem becomes rather complicated. I succeeded in developing an exact solution of the problem leading to a Volterra, Type II integral equation. In the publication cited below a few methods are given which can lead to practical results. A simpler approach is in press.

Several of the members of our society have struggled with the same problem and published papers about it. Among them, let us cite DeHoff, Elias, Lenz, Palkovits, Underwood — to mention only a few. The similarity of their results and of their illustrations is often striking.

Bach, G., Ueber die Groessenverteilung von Kugelschnitten in durchsichtigen Schnitten endlicher Dicke. Z. wiss. Mikrosk. 64: 265-270, 1959

STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY
Vol. 1, No. 4

STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Volume 1, Number 5, October, November, December, 1962

SOCIETY NEWS

This Society had its origins in the realization that many unrelated branches of science maintain a fundamental interest in the structure of things. The problem of the mathematical representation of structure, and its quantitative description from observations that are necessarily distorted or incomplete becuase of the manner in which the structure can be examined, was found to be an unusually common one. Examination of the literature of various fields revealed, and is still revealing, much duplication of effort. It became apparent that the formation of an organization which could provide a common ground for interaction of researchers in all fields could make an important contribution to each field, by eliminating duplication of effort, and by creating new ideas and approaches born of interdisciplinary interaction. STEREOLOGIA is this common ground.

Because I am cognizant of the importance of the contribution which this publication could make to many areas of science, I was duly flattered when Dr. Elias offerred me the priviledge of editing STEREOLOGIA. I hope that I can maintain the high standards which have preceded my acceptance of this office.

R. T. DeHoff, Editor

DOCENDO DISCIMUS (TEACHING, WE LEARN)

Hans ELIAS, Professor of Anatomy,
Chicago Medical School, Chicago, Illinois, U.S.A.

I am a teacher at heart. My subject is microscopic anatomy. The best way to impart understanding of the minute structure of human organs would be to take the students on a field trip through the body. Since such a field trip is physically impossible, I developed the habit of making three-dimensional illustrations of inside views of organs.

This exercise undertaken for the sake of teaching produced some unexpected discoveries concerning the structure of a few vertebrate organs. In the case of the avian proventriculus I found alleged tubular glands to be sulciform (slit-like). The liver, thought to be a network of irregularly curved cylinders revealed itself as a system of interconnected walls (a muralium). The renal glomerulus was found to be a branched sheet pierced by cylindrical blood channels.

In the cirrhotic liver, long, wavy lines evident in thin sections had been thought to be fibers all miraculously lying in the cutting plane. However, lines appearing in sections are usually traces of membranes.

These and similar findings were arrived at by common sense consideration and verified by reconstructions, until it dawned to me that the interpretation of sections is a geometrical problem. Common sense judgment on individual, structural problems had to be replaced by a systematic study of the geometry of sectioning involving all imaginable shapes and arrangements in space.

My co-workers and I published some of our results. But Dr. August Hennig, of the Anatomy Department in Munich, found mistakes in our papers. "Here is a man who understands this field better than I," I thought, and sought his collaboration. Through Hennig I learned that geologists and mineralogists had done much similar work. The establishment of this Society is largely due to my collaboration with August Hennig.

In the spring of 1961 when planning the Feldberg meeting, I invited Professor Felix Chayes, the Dean of Geological Stereologists, to attend. He expressed astonishment about the fact that an anatomist was interested in the geometry of sectioning.

All of a sudden, specialists encapsulated in our fields learned that we have very strong common interests. It is not only a method, but it is a way of thinking which binds us together.

Generally, I dislike journals and societies which are devoted to a method instead of being devoted to a subject. In our case it seems a bit different. To use the words of Professor Cyril Smith, what brings us together is "less the mathematics than the philosophy and aesthetic aspects of the interplay of dimensions, and the representation of multi-dimensional structures."

ELIAS, Hans, "A Re-examination of the Structure of the Mammalian Liver, I. Parenchymal Architecture." Am. J. Anat., 84 (1949) 311-334.

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ELIAS, Hans, "De Morphologia Carcinomatis Primarii Hepatis Humani et du Eius Contextu cum Evolutione Phylogenetica et Ontogenetica." Acta Hepatologica, 5 (1957) 1-18.

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CLASSIFICATION AND RECONSTRUCTION OF NERVE CELLS

Margarete GIHR, Institute fur Hirnforschung und allgemeine Biologie, Neustadt (Black Forest), Germany

For neurohistological and especially cytoarchitectonic studies mostly sections of an average thickness between 15-25 microns are used. A large part of the nerve cells in the section are dissected. Seeing them only from one direction one cannot realize their spatial form with certainty.

I first met with this problem when I was trying to calssify the so-called gigantopyramidal cells in area 4 (BRODMANN) of the human cerebral cortex. It was not possible to obtain an exact spatial picture on the basis of histological sections. Likewise extensive quantitative examinations and their statistical analysis did not give a distinct conception of all cell types. Therefore reconstruction of the cells seemed to be necessary. I developed a simple method which made it possible to reconstruct nerve cells with magnification of 2000 by using cresylviolett-stained paraffin sections at 3 microns. (The details may be taken from the publication cited below.)

The result of the reconstruction was surprising: The histological picture and the true shape of the nerve cells did not always correspond to each other. Thus it might be possible, that a cell which appeared pyramidal in a single section revealed itself as a spindle cell, upon reconstruction from serial sections or vice versa. This one example already shows that the histological pictures should be interpreted with great care. This must always be taken into consideration in qualitative and quantitative cytological examinations.

With the same method of reconstruction I am studying the nerve cells of the nucleus medialis dorsalis thalami of the human brain.

GIHR, Margarete, "Methode zur Rekonstruktion von Nervenzellen (aug der Basis des NISSL-Aequivalentbildes).", J. Hirnf., 5 (1962) 7-22.

INVESTIGATIONS OF THE DEPOSITIONS OF LIPOFUSCINE IN THE CELLS OF THE CENTRAL NERVOUS SYSTEM.

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At the suggestion of Prof. Wünscher I have for some time been concerned with examining the depositions of Lipofuscine in the cells of the central nervous system. In this connection the following questions have been studied: the deposition of Lipofuscine in certain areas of the human brain during the different ages of life; comparative anatomical observations on the deposition of Lipofuscine in some areas of the brain of different species of mammals; and histochemical examinations on the composition of Lipofuscine in different ages of life. In these investigations the arrangement of the conglomerates of Lipofuscine in the cells has been taken notice of as well as the morphology of the single granules of Lipofuscine. The observations will be evaluated quantitatively by means of statistical methods. The first paper will be finished in the course of this year. There are no publications as yet.

MORPHOMETRIC STUDIES ON THE LUNG AS PART OF A PROJECT ON CORRELATION OF PULMONARY STRUCTURE AND FUNCTION.

Ewald R. WEIBEL, M.D., Research Associate, The Rockefeller Institute, New York, New York, U.S.A.

Over the past few years I have been engaged, together with Dr. Domingo M. Gomez of Columbia University, in a project on correlation of structure and function of the human lung. It soon became obvious that the most significant morphological information could be gained through a rigorous quantitative analysis of pulmonary architecture. I have thus engaged in a systematic morphometric study on the normal human lung which involved at first an investigation of number, volume and surface of alveoli and alveolar capillaries, as well as a study of branching pattern and dimensions of the airway tree. I am presently pursuing this project at the level of resolution provided by the electron microscope in an attempt to analyze morphometrically the dimensions and composition of the alveolo-capillary air-blood barrier. The results of these studies are reported in communications 1-3 which are all in press; 1 and 2 should appear shortly.

In the course of these studies I became more and more interested in what we like to call morphometric principles. Needless to say that I first familiarized myself with the well-known works of several members of our society. The broad spectrum of our study, however, required some further work on additional methods and on adaptation of some of the existing principles to the particular problems. Our experiences with this line of work are presented in paper 5. In brief summary, I have been concerned with the preservation of pulmonary architecture, with the relation between distribution of structures and suitable sampling procedures, with a series of methods of random measurement on two-dimensional samples and with the effect of section thickness. As an original contribution we have devised a principle for counting "randomly distributed" structures on random sections; this is developed in paper 4. I am presently also working on some methods of random measurement which relate to my particular problems at the electron microscopic level of resolution.

In studying the conducting airway system I have also met with problems of systematic sampling and measurement of "non-randomly" distributed structures whose polar orientation is an important feature. They could be overcome by using a systematic stratification of the airway tree with respect to generations of branching.

These morphometric studies are summarized in a short monograph which is in press with Springer-Verlag (reference 3). They have finally led to the formulation of some quantitative models of the normal human lung which are presently being used by Dr. Gomez in his physico-mathematical analysis of function and structure relations in the lung.

[1]WEIBEL, Ewald R., and GOMEZ, Domingo M., "The Architecture of the Human Lung." Lead Article. Science (in press).

[2]WEIBEL, Ewald R., "Morphometrische Bestimmung von Zahl, Volumen and Oberfläche der Alveolen und Kapillaren der menschlichen Lunge." Z. Zellforsch. (in press).

[3]WEIBEL, Ewald R., "Morphometric Studies on the Human Lung." Monograph. Springer-Verlag. (in press).

[4]WEIBEL, Ewald R., and GOMEZ, Domingo M., "A Principle for Counting Tissue Structures on Random Sections." J. Applied Physiol. 17, (1962) 343-348.

[5]WEIBEL, Ewald R., "Principles and Methods for the Morphometric Study of the Lung and Other Organs." (submitted to Laboratory Investigation).

THE PHYSICAL PROPERTIES OF HETEROGENEOUS MATERIALS

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The bulk, or macroscopic, physical properties of heterogeneous materials are sensitive to their microstructures; that is to the three dimensional distribution of their phases. As a consequence, the prediction of the physical behavior of such a material requires some knowledge of this structure. One is thus led to the stereological problem of "adequately describing" a three dimensional structure from information obtained from sections and projections of the material.

My approach to the problem has been essentially statistico-geometrical. I have sought to describe structures of interest by stochastic-geometrical models(1,2). With a "sufficiently reliable model" the desired physical behavior can then, in principle, be predicted in the sense that one can say with what probability some property might exceed a given value. I have applied these techniques to the problem of predicting the behavior of neutrons diffusing through heterogeneous materials(4,5). It is my intention to consider more general physical processes.

Since the availability of large quantities of precision data is one of the keys to accurate prediction, rapid, automatic material scanning instruments are being developed, or evaluated. One such devise, an automated densitometer, is described in reference 3. Another, an electrostatic scanner developed by Dr. J. Hilliard, is now being modified so that it can feed data (at the rate of better than 5,000 readings per second) directly to a large computer for analysis. X-ray scanning equipment has also been considered, among others. The development of statistical techniques to process the resulting data is another area of interest. Here our purpose is to make the "most efficient" use of the available data to describe the scanned material. One relatively simple computer data processing program, MEAN is described in reference 3 and 5. An effort is now being made to develop a general purpose computer program to accomplish these ends.

The mathematical structure of the overall problem is also being investigated as an abstract algebraic system. The phrases "sufficiently reliable model" and "most efficient" have been used rather loosely. It is the purpose of this analysis to determine with some precision what data are in fact required to predict some property of a material with a given degree of confidence, and what in fact constitutes the most efficient use of the available data. None of this particular work has been published as yet. However, the preliminary results make it clear that mathematical structure is analogous to that of quantum mechanics. In view of the fact that we are in effect considering an algebra of observables this, in retrospect, is not too surprising.

[1]RANDALL, C. H., "The Central Problem of Dispersion Analysis," KAPL-M-CHR-4, Knolls Atomic Power Laboratory, (February 1961).

[2]RANDALL, C. H., "Stochastic Models for Heterogeneous Materials-I," KAPL-M-CHR-6, Knolls Atomic Power Laboratory, (August 1962).

[3]RANDALL, C. H., and ALLIO, R. J., "The Quantitative Analysis of Microstructure with Densitometer Data", Trans. AIME, 224 (1962) 221.

[4]RANDALL, C. H., "Particle Self-Sheilding", Naval Reactor Physics Handbook, Section 4.2.B, U.S. Atomic Energy Commission (to be published January 1963).

[5]RANDALL, C. H., "Microscopic Effects in Multiphase Mediums (Neutron Self-Shielding)", KAPL-M-CHR-2, Knolls Atomic Power Laboratory, (May 1960).

RESPIRATORY UNITS (ACINI)

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Investigating the respiratory tissue of the growing lung from birth to puberty it was soon obvious that routine histological procedure is unable to unravel the intricate acinar architecture. Sections usually cut the acinar elements into incoherent fragments, occasionally however larger portions of the acinus occur in a single section. Special methods had to be employed; serial sections, it turned out, are indispensable as a tool in my research. They allow the analysis of the elements that compose the acinar tuft. Acini are small formations; in the adult the volume amounts to approximately 150 cubic millimeters. This volume is that of a 7mm diameter sphere, the size of a pea. Every acinus is supplied by a special tube, the terminal bronchiolus. It divides into several generations of intra-acinar tubules which, finally, lead to the alveolated saccules, the very element in which the exchange of gases takes place.

In order to recognize the details of the acinar structure various components had to be investigated separately:

1. The system of intra-acinar tubules,
2. the alveolated saccules,
3. the acinar structure in its entirely,
4. the volume of acini and individual elements,
5. the respiratory surface.

1. The tubules were reconstructed (x 70 or 100). In large, three-dimensional models the system appeared much more complex than individual sections had suggested.

2. Reconstruction of alveolar saccules is not feasible as these are so close to each other that any reconstruction of an acinus would result in an almost solid block. The same is true of casts using Wood's metal or resin. Visual reconstruction by the help of many serial sections and camera-lucida tracings was the method of choice.

3. The combination of all elements into a functional acinus again was achieved by the help of serial camera-lucida tracings.

4. Volumes were calculated from suitable sections containing coherent parts of acini.

5. The respiratory surface could be calculated after the volume of the acinus or parts of it had been determined.

[1]ENGEL, S., The Child's Lung, Eward Arnold and Co., London, (1947).

[2]ENGEL, S., Lung Structure, C.C. Thomas, Springfield, Illinois, (1962).

STEREOLOGIA

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STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Volume 2, Number 1. January, February, March, and April, 1963.

The First International Congress for Stereology was held at the Allgeneine Krankenhaus in Vienna on April 18, 19, and 20. This edition of Stereologia constitutes a brief report of the proceedings of this first official meeting of the International Society for Stereology.

Some forty participants, about one third of the membership of the Society, attended the Congress, which consisted of three days of technical meetings, and two brief business meetings. Virtually all of the structurally interested branches of science were represented, and the participants came from many nations, giving this meeting a truly interdisciplinary and international flavor.

Dr. Herbert HAUG, the man chiefly responsible for organizing the technical program and making detailed preparations for the Congress, is to be congratulated for a difficult and tedious task well done. We expect that Dr. HAUG was gratified at the enthusiasium and interest which prevailed at the technical sessions.

THE TECHNICAL SESSIONS

The purpose of the technical sessions, and in fact, the purpose for the existence of this Society, is to provide a ready channel of communication among the various brances of science which share a quantitative interest in the structure of things. The provision of a vehicle for easy communication is expected to provide varying viewpoints on the same problem, and to reduce the excessive duplication of effort that has been found to be so prevalent in the field of stereology. This First Congress did much to further both of these ends. It also provided some insight into the branches of stereology which are of major concern in the various disciplines.

With a few very notable exceptions, papers presented from the medical-related sciences concerned themselves with the identification of the three dimensional <u>shape</u> of objects. This preoccupation with shape is probably largely due to the complexity of the shapes which are encountered in this general area of scientific enquiry. All of the papers on serial sectioning were chiefly concerned with the synthesis of three dimensional shape. Mathematical analyses of the triaxial ellipsoid exposed the distribution of shapes of sections through the particles, rather than, for example the distribution of sizes.

On the other hand, those disciplines which are concerned with inanimate structures in nature, such as geology, minerology, metallurgy, and astronomy, seem to be confronted with structures which have relatively simple shapes. As a result, researches in these areas have tended to concentrate upon more metric aspects of structure. Volume fraction analysis, surface area measurement, determination of size distribution, and other quantitative measurements dominated presentations in these fields.

This difference of emphasis in these two general branches of science does not imply that an interaction between the branches is not useful. With the advent of the electron microscope, the structures now being encountered in the life sciences are geometrically far simpler than are the shapes of whole organs, for example; furthermore, these structures are to some extent repetitive. Thus, the biophysicist and the anatomist are beginning to encounter with increasing frequency structures which satisfy the statistical conditions necessary for metric analysis of geometric properties. At the same time, the physical scientists are beginning to have interest in structures having shapes which were too complicated to be described mathematically with any clarity.

Thus, the emphasis shifts, and perhaps converges, in the study of structures in these two areas.

<u>The Inaugural Sessions</u>

The first technical presentation was a colored film entitled "Looking into Space, and Introduction to Stereology". This film, presented by Dr. Hans ELIAS, the President of the Society, was a clear, graphic presentation of the basic problems with which quantitative stereology concerns itself; projection or shadow evaluation, shapes and sizes of inaccessible bodies, relations of sections and projections to the three dimensional shapes they represent, and determination of volume ratios.

After Dr. ELIAS had laid the groundwork with his intorductory film, Mr. Buckminster FULLER, a distinguished architect and scientist, whose approach to structural design and analysis has led to such revolutionary innovations in architecture as the geodesic dome and the tensegra structural unit, presented the Inaugural Address. In a profusely illustrated presentation, Mr. FULLER related the story of the development of his structures, and showed evidence of the recurrence of his basic structural elements in the microcosm of nature, as, for example, in certain crystal structures, and in complex molecules. Mr. FULLER's lecture was one of the highlights of the Congress.

The afternoon sessions began with presentations by distinguished representatives of three fields in which stereological methods are widely used. Dr. August HENNIG, a mathematician with particular interest in applications to the life sciences, presented a general historical background of the development of the subject from the medical viewpoint. Dr. Felix CHAYES, a geophysicist, summarized the evolution of the volume fraction measurement from the original method of DELESSE to the most modern automatic and semiautomatic methods in use today. Dr. F. N. RHINES, a metallurgist, reviewed the development of the subject by metallurgists, emphasizing metallurgical applications. Prof. Dr. Jose ESCOLAR presented the introductory paper in the sessions which dealt with serial sectioning techniques. The history and development of the art and science of reconstruction of complex structures from a series of sections produced through the structure was summarized, including approximate methods for determining such metric properties as volume and surface area.

<u>The Analysis of Shape</u>

The concept of shape is very elusive. The shape of a particular body or structure can be described with three different degrees of accuracy.

If it is quite complex, as are most of the organs of the human body, for example, a mathematical description of shape is impossible, and the researcher must resort either to the qualitative realm of description words, such as "leaf-like", "like a bunch of grapes", "tufted", "feathery" and so on, or to direct reproductions of the structures. Certain simple shapes can be described at least approximately, by mathematical expressions; examples are "cylinder", "ellipsoid", "sphere", "needle", or the various polyhedra. Such shapes are adapted to quantitative analysis from observations made on a section, although this analysis may in many cases be tedious and time consuming. The third kind of description of shape ignores the details of convolutions of complex structures, and describes quantitatively topological aspects of shape, such as the number of separate parts of the structure, or its connectivity. Papers were presented at the Congress which dealt in detail with each of these three aspects of shape.

By far the most popular method for determination and description of complex shapes was the serial sectioning technique. In this approach, the structure is reconstructed from a series of successive sections prepared as usual, and observed under the microscope. Several interesting methods for presenting the three dimensional image thus obtained were demonstrated at the Congress.

Two examples of reproduction using time as the third dimension were presented. A study of cardiac development in embryonic frogs and chicks presented by O. C. JAFFEE applied this approach. Each successive sectioned is appropriately stained and photographed as a single frame on motion picture film. Projection of this movie film permits the mind to build up a three dimensional image of the heart as the sections progress through it. In his presentation, Dr. JAFFEE demonstrated that the shape of the embryonic heart is determined by the flow of blood through the heart. Environmental changes which produced changes in flow rate and pressure produced variations in shape, sometimes leading to permanent defects in the structure. S. N. POSTLETHWAIT reported upon the use of this technique in studying botanical anatomy; his paper emphasized technique.

Several papers pointed up the importance of interpretation when viewing an histological section. M. GIHR particularly emphasized this point in a paper dealing with the structure of nerve cells. The shape of the cell as it may appear on a single histological section depends upon the orientation and position of the section; for cells of complex shape, a single section may give an entirely erroneous impression of the true shape of the cell. Furthermore, the shape observed in the section is sensitive to the the thickness of the section.

Several of the papers dealing with the three dimensional shape of structures were concerned with the basic description of the structures involved. In these cases, the shapes are so complex, and the histological techniques so tedious, that more quantitative information would be neither accessible nor useful. J. AGREDA-SMITH presented two examples of the application of serial sectioning to structures falling into this catagory. The first of these was a study of the development of the human embryo, in which the evolution of the matrix zone in the nerve canals of the structure was followed by comparing two embryos of different sizes. A second paper described differences in the struc- of the pituitary vascular system in the human, feline, canine and rodent families, emphasizing the effect variations in the staining reagent may have upon the structure which is observed under the microscope. A somewhat similar approach was taken by Dr. P. AMAT in a paper describing the role of reconstruction from serial sections in the field of nuero anatomy. A three dimensional model of the brain of a cat was displayed and explained; the aim of this research was to gain further insight into the structure of the human brain by comparison with this model.

If the observer is not interested in the internal structure of a complex body, or if the body is transparent, the three dimensional shape may be observed stereoscopically. Two papers presented at the Congress dealt with this approach to the problem of three dimensional visualization. An excellent example of the application of stereomicroscope to a transparent structure was presented by E. BRUNNER and L. KUCSK. They developed a means for observing the detailed network of the blood vessels in the lung by injecting appropriate staining reagents into the blood supply. Thus, the structure of interest became stained, and observable in a transparent sample of the lung. The use of a stereoscopic microscope completed the development of a three dimensional image of the network. A useful extension of this technique was described by G. LUPACCIOLU in a general description of the uses of stereoscopic microradiography in stereological studies. The basic procedures for producing three dimensional images of structures which are opaque to the visible spectrum of radiation were outlined in detail, together with methods for projection. The presentation included a demonstration of projection of stereoscopic motion pictures.

The estimation of the shape, size, and volume of living structures existing in human subjects was the subject of a lecture persented by W. STÜRMER. A suitably opaque reagent is introduced into the system to provide contrast with the surroundings, and the structure of interest is examined radiographically. The three dimensional shape of the structure is obtained by making exposures from a series of slightly different orientations; this results in focusing at a series of depths in the structure, providing information which is very similar to that obtained in ordinary serial sectioning.

The quantitative analysis cf shape is possible only if the structure is simple enough so that the shape can be described mathematically. Further, all of the methods for the quantitative estimation of shape from sections taken through a structure require that all the particles in the structure be the same shape. The following papers dealing with shapes of sections through various three dimensional bodies must be considered in the light of these two quite limiting assumptions.

The basic approach to the quantitative determination of simple three dimensional shapes from two dimensional measurements is to derive the distribution of shapes of two dimensional sections through a particular kind of shape, such as a cylinder. Comparison with an experimentally determined distribution of section shapes permits the quantitative estimation of the shape of the body from which the sections were taken. The most general paper presented in this area was that of E. J. MEYERS, in which cylinders, rectangular parallelapipeds, tetrahedrons, octahedrons, rhombic dodecahedrons, tetrakiadecahedrons, and hexagonal prisms were all treated. Non-regular tetrahedra were examined in detail by H. KNESER; J. KLIMA reported results for sections through ellipsoids of revolution, and for circular and elliptical cylinders. The distribution of sections through triaxial ellipsoids was studied by two investigators: Dr. August HENNIG delivered a paper in collaboration with Dr. Hans ELIAS which contained a purely mathematical analysis of the problem for a small range of axial ratios; M. PALKOVITS was concerned with this problem as it applied to the three dimensional shape of cell nuclei in various environments. A. SCHWINK, et. al., reported studies of sections through periodic structures, i.e., a laminated stacking of several membranes encountered in electron microscope investigations of the structure of the brain.

A few of the basic concepts of simple topological theory were introduced into the analysis of very complex shapes in a paper presented by R. T. DEHOFF, dealing with an application of topology to structures encountered in the sintering of metal powders into useful metal parts.

Topology provides a new parameter, called the genus, which quantitatively describes the connectivity of an arbitrarily complex network.

Metric Analysis of Structures

The majority of papers presented at the Congress dealt with the determination of properties which monitor the quantity of material in a structure. These include volume fraction analysis, measurement of surface area, particle size, particle size distribution, and number of particles. Although the statistical problems are in some cases different for transparent structures, in which the projection of all the material in a thin section is viewed in transmitted light, and for opaque structures, which present a true two dimensional section for observation in reflected light, the methods of attack are similar for these two cases.

Estimates of Volume Fraction. It was apparently generally accepted that the most efficient method for determining the volume occupied by a specific structure, or part of a structure, is the so-called point count method, or method of hits. A grid of points is superimposed on the sample, and the fraction of the points in the grid which happen to lie on the constituent of interest is used to estimate the volume fraction of that constituent. However, this method is not well adapted to volume estimation in non-repetitive structures. Several applications of this method were reviewed in the papers presented: its historical development was thoroughly covered in the three introductory papers, Dr. HENNIG following its early development and application to the medical sciences; Dr. CHAYES relating its very broad application in geology; and Dr. RHINES tracing its history in metallurgy. E. WEIBEL gave an example of its use in electron microscopic studies of oxygen transfer in the lung.

Two papers dealt with volume estimation in structures which are too coarse, or are not sufficiently repetitive to permit valid statistical analysis. Dr. Hans ELIAS gave an example of volume estimation of adrenal glands from a single histological section, provided the section is carefully taken. A method for volume estimation, involving x-ray absorption of a structure viewed from several angles was presented by W. STURMER. Several of the papers on serial sectioning alluded to the possibility of volume estimation from progressive cross sectional areas and the section thickness. I. and P. MEYER also presented an analysis of the material content of a structure, their subject being the shape and size of the cortex of the human brain.

Estimates of Particle Size. Several papers dealt with the determination of the strongly interrelated parameters, number, size, and size distribution. E. E. UNDERWOOD, et. al., compared the various methods for estimating size distributions of spherical particles in metals. This problem was also investigated by Dr. Gunter BACH, in a theoretical treatment of size distribution determination both in two dimensional sections, and in thin films. W. TREFF exposed the effect of the thickness of an histological section upon the measured size of spherical particles contained within the structure . Dr. H. HAUG demonstrated that not only the section thickness, but also the particle size and shape effect the estimation of the number of particles in a structure from an histological section.

The papers in the proceeding paragraph dealt with fairly regular particles shapes. In his work on the human lung, E. WEIBEL obtained statistically precise estimates of the thickness of the oxygen diffusion zone. Dr. A. SCHWINK, et. al., presented a method for obtained metric information from measurements on periodic structures, their subject matter being the cerebral cortex. Dr. A. HENNIG presented a simple method for estimating the total length of a three dimensional linear tract from counting measurements made on a section. Several examples of structures in the life sciences were included in his presentation.

Orientation Analysis

Metallurgists have long been concerned with the problem of "preferred orientation" in polycrystalline metals; the crystalline axes of the tiny individual grains which make up most commercial materials tend to cluster in certain orientations, with a resulting effect upon important properties of the material. Dr. P. K. KOH presented an example of the determination of such orientation "textures" in magnetic tapes used in computers. A somewhat similar phenomenon was discussed by J. D. FORTUYN who was studying the orientation tendencies of nerve cells in the cerebral cortex, and throughout the nervous system. Still a third kind of orientation analysis was presented by R. H. ATKINSON: the orientation of individual crystals in polycrystalline metals, as determined by the measurement of angles between slip traces, markings produced on a metal surface when the metal is deformed.

Special Topics

The remaining papers dealt with problems which do not directly fall into any of the above catagories. For example, W. LINDEMANN presented a paper on the determination of crystal structures of metals from the pattern which develops as a result of the diffraction of monochromatic beam of x-rays by a fine powder sample of the material under study. A distinguished astronomer, Professor HOPMANN, presented an application of the quantitative evaluation of shadow lengths to the precise determination of the gross shape and fine topography of the surface of the moon. Measurements of this kind were conducted as part of research into the origin and evolution of the topography of the moon, and the nature of the lunar craters.

Stereoscopic electron microscopy was the subject of a paper by K. HUBNEY. A detailed mathematical analysis of the quantitative determination of the third dimension from stereoscopic reproductions of the structure was included in this paper.

The optical problem associated with developing a three dimensional image of a structure on a single photograph was approached by F. KISS, in a paper dealing with a new optical principle. A special adjustable condenser at the light source illuminates the specimen from several orientations, producing shading which gives the image a three dimensional character.

The general field of application of semi-automatic and fully automatic instrumentation to the measurements of the parameters of quantitative stereology was reviewed in a comprehensive paper by Dr. H. FISCHMEISTER. This paper was a very gratifying report on the progress being made in taking the tedium and boredom out of making stereological measurements. It also served to emphasize the point that the time is ripe for theoretical advances in measuring techniques which will permit more detailed descriptions of three dimensional structures.

THE BUSINESS MEETINGS

Two brief business meetings were held in the course of the Congress. The main purpose of these meetings were: to adopt the new Charter for the International Society for Stereology, which incorporates the Society as a non-profit organization in concord with the laws of the Federal Republic of Germany, and the State of Illinois, U.S.A.; to elect a new Board of Directors; and to tentatively set the date and place of the next International Congress. The results of the election of the Board of Directors were:

President	Hans ELIAS
Vice President	Herbert HAUG
First Secretary	Ewald WEIBEL
Second Secretary	Werner TREFF
Treasurer	Ervin UNDERWOOD

The newly elected officers are, for the most part, continuing in the positions they held before the official incorporation of the Society. In addition, the two Editors of the Bulletin were reappointed to those offices, Robert T. DE HOFF as Editor in Chief, and Gunter BACH as European Editor.

At the invitation of Dr. F. N. RHINES, the next International Congress for Stereology was tentatively set for the Spring of 1966, at the University of Florida, in Gainesville, Florida.

ANNOUNCEMENTS

The International Society for Stereology is inaugurating a lending Library with the help of its membership. We request each member to send an abstract and two copies or reprints of any paper which they publish that is pertinent to the field of quantitative stereology to either of the following addresses, whichever is more convenient:

Robert T. DE HOFF
Metallurgical Research Laboratory
University of Florida
Gainesville, Florida

or to Werner TREFF
Institut fur Hirnforschung
Dennenbergerstrasse 1-5
Neustadt im Schwarzwald
Germany

The abstract will be published in the Bulletin, and a copy of the paper will be retained at each of the above addresses. A copy may be borrowed from either of these locations by any member. If copies of your paper are not available, please send an abstract for publication in Stereologia.

The proceedings of the First International Congress for Stereology were published in time for the Vienna meeting. A few extra copies of this are currently available, and may be obtained from Mrs. Wiedemhaus c/o Vienna Medical Academy Vienna, Austria at a cost of $6.00 per copy for members and libraries, $9.00 for non-members.

In order to furnish funds for more frequent editions of the Bulletin, and in order to spread the burden of financial support more evenly over the membership, an increase in membership dues was approved at the Business Meetings of the Congress. The new annual dues will be $3.00; these dues include the Bulletin. All financial payments should be made payable to the Society, and sent to the Treasurer:

Ervin E. UNDERWOOD
Staff Scientist
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If you have not received and replied to a request for a brief biographical sketch emphasizing your activities in the field of stereology, please send a brief account of your work in the field to one of the editors for publication in Stereologia. It is hoped that a fairly complete picture of the wide variety of problems and approaches that now exist in this field can thus be compiled.

It has been suggested that an attempt be made to compile a complete bibliography for the general area of quantitative stereology. If you are interested, please send the Editor in Chief a list of those references in your particular area of interest which you feel make a contribution to the development of quantitative stereology. Please include the title in your listing, to help fit each reference into its proper catagory.

Robert T. DE HOFF

Editor in Chief

STEREOLOGIA

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STEREOLOGIA

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FROM THE EDITORS

Two new features are inaugurated in this issue. Abstracts of papers that have been received by the Lending Library of the Society (See Vol. 2, No. 1) appear for the first time at the end of this issue. The Bulletin now accepts problem and discussion papers, provided they are brief and of relatively wide interest. The first such paper to be published in the Bulletin, by F. D. BERTALANFFY, appears in this issue. Submissions of papers of this type, and submissions of preprints to the Lending Library or abstracts of papers published in other Journals, are welcomed by the Editors.

Those members who have not as yet contributed a brief biographical sketch of their stereological activities are urged to do so in the near future.

IS IT FEASIBLE TO ESTIMATE THE TOTAL NUMBER OF CELLS COMPOSING THE PARENCHYMA OF AN ORGAN?

Felix D. BERTALANFFY, Department of Anatomy, Faculties of Medicine and Dentistry, University of Manitoba, Winnipeg, Manitoba, Canada.

A problem is outlined that has intrigued me for some time, even though I myself am unable to propose how it could be tackled technically. It is well known that many tissues (epithelia in particular) undergo renewal of their constituent cells throughout life, and this often at rapid rates (e.g., LEBLOND and WALKER, 1956; BERTALANFFY and LAU, 1962). Cells continuously desquamate from such tissues and are replaced by new cells arising from mitosis. Inasmuch as cell addition does not normally occur in adult tissues, the number of cells formed by mitosis and simultaneously desquamated have to be identical. It is readily feasible to determine the percentages of cells that arise from mitosis during a 24-hour period, as well as the time required for the renewal of 100% of a cell population (the turnover time). To indicate the extent of cell formation in rat tissues it may be mentioned that daily 3-5% of new cells arise by mitosis in the epidermis, 2-4% in epithelia of trachea and bronchus, 10% in esophageal epithelium, and 13% in sebaceous glands; exemplifying turnover times, renewal of the entire epithelium of the colon requires 10 days, of corneal epithelium 7 days, of the oral cavity 4-5 days, of vaginal epithelium 4 days; it is most rapid of the epithelium in the small intestine, both in rat (1.3-1.6 days), and man (2 days; BERTALANFFY and NAGY, 1961; BERTALANFFY, 1962).

Whereas it is feasible to estimate the percentages of cells arising by mitosis, it is in most instances impossible to measure the numbers of cells that are extruded. Counting the individual cells that have desquamated is out of the question; it is infeasible to collect complete 24-hour exfoliated specimens from a significant number of individuals. Moreover, for example the study by number of epithelial cells, histiocytes, and leukocytes that are daily extruded with sputum, for instance, are in the millions.

If some method could be devised whereby the total number of cells of an epithelium, for instance, can be estimated, the absolute numbers (e.g. in 10^6) of cells newly formed each day in the particular tissue and extruded from it could be derived; it would simply correspond to that percentage of the total number of cells in the population determined quite accurately as being formed by mitosis during a 24-hour period.

The estimation of cells composing a cell population would presumably have to take into account the numbers of cell layers in an epithelium (if stratified), and this would further have to be correlated with the total surface of the epithelium. Is it feasible, however, to calculate an epithelial surface? This may prove particularly difficult if the surface is uneven, as it is in the intestine.

Inasmuch as the process of cell renewal facilitates cytological cancer diagnosis for instance (BERTALANFFY, 1963), utilizing the vast numbers of desquamated cells, estimates of their numbers would be considerable significance. Whether it is feasible to arrive at such estimates by the means outlined above is another question. Comments on this problem would be appreicated.

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SHADOWS ON THE MOON

James Quincy GANT, Jr., M. D., M. Sc.
Assistant Clinical Professor of Dermatology and Syphilology, George Washington University School of Medicine. Chief of Skin and Allergy Clinic, Washington Regional Office of the Veterans Administration. Private practice at 1801 Eye St., N. W., Washington 6, D. C.

I have been interested in astronomy, particularly the moon, since boyhood and began serious lunar study at the age of 14. Being equally interested in medicine, I became a physician, specilizing in Dermatology. After graduation from medical school I was a ship's doctor with the American Export Lines, a circumstance which added much to my knowledge and interest in astronomy, as well as navigation.

For 42 years I have been working on interpreting lunar formations. Color changes, shadow intensities, shadow formations with height of the sun above the lunar horizon and trying to interpret the character of the reflecting surface, and height of formations from the shadow lengths.

The moon's image as usually seen through a telescope and photographs of lunar surfaces through a telescope are flat. It is not until the shadows are correctly interpreted that the three dimensional reality can be visualized and measured.

In 1954 Lunar crater Archimedes A was renamed "GANT", by Dr. Hugh Percival WILKINS, then Director of the Lunar Section of the British Astronomical Association. Among several offices held in various astronomical organizations, the most important was that of President of the International Lunar Society 1959-1960. I am, at present, Secretary General of that organization. I have a private, well equipped observatory at Boyds, Maryland, as well as an extensive Astronomical Library, containing rare photographs, drawings and books.

X-RAY ORIENTATION STUDIES

KOH, P. K., Homer Research Laboratories, Bethlehem Steel Company, Bethlehem, Pennsylvania, U.S.A.

Physical measurements and mechanical testing have proven that x-ray diffraction serves as the only means of true orientation determination of a metallic or non-metallic crystal accurate to one stereographic degree. Standard methodology uses the variation of intensity of diffraction maxima produced by x-ray beams to determine the pole figures of definite atomic planes of either single crystals or preferred oriented polycrystalline materials. Transmission films are used for single crystal pole figures while quantitative diffractometer methods are used for polar stereographic plots of polycrystalline materials in terms of ideal poles. Practical applications include studies of nucleated grain growth, magnetic orientation, epitaxial and varied growth of thin films and mechanical anisotropy due to plastic flow of materials.

Recent work, sometimes with Dr. C. G. DUNN, involved study of various phases of preferred orientation in grain-oriented silicon steel including such topics as: cold rolled end orientations (1), primary recrystallization (2), secondary recrystallization (3), and grain growth behavior (4). Related work was devoted to preferred orientation in ultra-thin mobybdenum permalloy tape used in computer memory and switch-core circuits. The nature of hot rolled, cold rolled and annealed textures was established (6), variance between tapes of different production heats was studied (7), and inferior magnetic performance of some annealed tapes was found to be caused by magnetically unfavorably oriented cube texture (8).

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QUANTITATIVE RESEARCH ON THE STRUCTURE OF THE BRAIN

H. ORTHNER, Director of the Neuropathological Division, Universitätsklinik für psychische und Nervenkrankheiten, Göttingen, Germany.

My relationship to Stereology is primarily based upon the problem of quantitative research on the structure of the brain. Since the development of the Gottingen brain microtome.(JUNGKLAASS and ORTHNER, 1959, 1960) about 5 years ago, which permits an exact reproduction of the human brain from sections about 4 mm. thick, we have used this instrument on a routine basis in our work. We have ascertained the volume contained in the separate parts of the brain from planimetric measurements of photographs of sections obtained from the brain. The extent of microscopic structural elements (nerve cells,glia cells, mesenchym,the so-called intercellular substance) was determined from the measured areas with the methods of quantitative microscopy. An exact relationship between the brain and the inner volume of the cranium was developed to make possible a method for obtaining the inner cranial volume during a routine autopsy. (JUNGKLAASS, 1959).

Fundamentally, we determined the volume contained in the entire cerebrum, that in the three constituents of the cerebrum (cerebral cortex, medulla, ganglion stalks), that in a single cross section of the cerebral cortex and the ganglion, and the microscopic content of specific structural elements. The method established the average value and variability as a basis for investigations into cerebral constitutions and, among other things, set the limits to be investigated, whether and which relations exist between cerebral structure and corpuscular and solution constitution. The problems of the anatomy of the brain of not only especially gifted people (the so-called elite brain studies), of people of psychically negative variation (the so-called state of idiocy),but also of the endogenous psychosis, and various other diseases, for example, the system of hereditary atrophies, can become feasible with increasingly successful results. In addition, the study showed an exact basis for the chemical actions occurring in the brain.

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TABLES OF CUBIC CRYSTAL ORIENTATIONS FROM SURFACE TRACES OF OCTAHEDRAL PLANES.

by M. P. DRAZIN and H. M. OTTE (probably around $2.50; to appear Summer, 1964)

In crystallography, metallurgy, mineralogy, geology, chemistry, solid state physics and many other fileds, the problem of determining the orientation of a cubic crystal from traces of octahedral planes on the surface of a specimen is one that frequently arises. Whereas such determinations have previously involved laborious and time-consuming graphical methods, these Tables now provide the same information (and more, with greater accuracy) in a matter of seconds. They may be used together with, or in the place of, x-ray methods.

The octahedral planes are usually associated in face-centered-cubic metals and alloys with the active slip and twin planes in deformation and with the boundaries of annealing twins. A particularly important current application is the determination of orientations from surface traces in thin film electron transmission microscopy. Another application is to the determination of the orientation of a crystal that has transformed to a new structure but where the traces from the parent structure have been retained; x-ray methods clearly cannot be applied in such a situation.

The Tables are accompanied by an introduction giving detailed instructions for their use (with numerical examples) and cover all possible combinations of trace angles, tabulated at 1° intervals. The following information may be read off directly from the Tables:

(1) The complete specification (in terms of rotation matrices) of the possible orientations of crystal axes.
(2) The directions of the possible surface normals, tabulated
  (a) in terms of their direction cosines.
  (b) in terms of spherical polar angles from which a rapid plot of the surface normals can be made.

LENDING LIBRARY

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ABSTRACT SECTION

QUANTITATIVE METALLOGRAPHIC ANALYSIS OF LINEAR FEATURES IN ANISOTROPIC STRUCTURES. SUBSTRUCTURE OF LAMELLAR EUTECTIC ALLOY.

R. W. KRAFT, F. D. LEMKEY, and F. D. GEORGE

From a consideration of the geometrically possible ways in which an array of lines or linear features in three-dimensional space can depart from a statistically random arrangement a system was developed to describe and measure different types of anisotropic arrangements of lines in opaque solids. The technique was applied to a specimen of unidirectionally solidified Al-$CuAl_2$ eutectic which contained microstructural imperfections termed lamellar faults. Experimental data obtained from photomicrographs of different facets of a grain of this alloy were then analyzed using this system. It was deduced that the defects, which are essentially linear in nature, were predominantly parallel to one another but not parallel to the nominal growth direction probably because of some lateral heat flow during solidification.

LAMELLAR TILT BOUNDARY OF Mg-32 Wt Pot Al EUTECTIC

A. S. YUE, Materials Sciences Research Laboratory, Lockheed Missiles and Space Company, Palo Alto, California

Small-angle tilt boundaries have been observed in a lamellar eutectic structure. These boundaries are morphologically similar to a theoretical tilt boundary originally proposed by Burgers. The production of a small-angle tilt boundary in the lamellar eutectic structure is attributed to the formation of an indentation on a planar solid-liquid interface during growth. Constitutional supercooling due to the accumulation of impurity atoms ahead of the interface is primarily responsible for the generation of an indentation. The distance between two faults of like sign, designated as lamellar fault distance, is calcuated by the Burgers equation for small-angle boundaries. It was found that the lamellar fault distance decreases exponentially and the tilt angle increases exponentially with freezing rate.

STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

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FROM THE EDITORS

There appears in this issue of the Bulletin an authoritative reply to the question posed by Dr. BERTALANFFY in the last issue, concerning the very general problem of counting the number of three dimensional objects from measurements made on various kinds of laboratory sections. This kind of response is very heartening, exemplifying as it does the interest in our common area of endeavor.

The compilation of biographical sketches continues in this issue as do the Library and Abstract sections. Your support of all of these areas of the publication is the necessary ingredient which determines the success of the Bulletin, and we continue to seek it.

The next issue will constitute somewhat of a departure from procedure for this publication, in that virtually all of it will be devoted to the presentation of a proposal by E. E. UNDERWOOD for the adoption of a standard set of notation to be used in all areas of application of stereology. Also coming up in future issues is the presentation of an up to date membership listing, to permit cross-communication among members with common interests.

THE NUMBER OF CELLS PER UNIT VOLUME

Hans ELIAS, Department of Anatomy, Chicago Medical School

Dr. BERTALANFFY'S inquiry in Stereologia 2, 2: 25-26 can be answered in the positive. It is, indeed, feasible to estimate the total number of cells composing an organ.

The boundaries of cells are rarely distinct; but the nuclei are always sharply outlined in routine slides. Therefore, we count nuclei, rather than cells. Since pluri-nucleate cells are rare, we can usually make this substitution.

If all nuclei were spherical and of equal size, and if histological "section" were infinitely thin, the solution of the problem would be relatively simple. Since, however, our material is transparent and since even "thin" sections are very thick compared with the size of the nucleus (at best their thickness is one fifth of the diameter of a nucleus), further difficulties arise.

We must proceed as follows:

1) The average diameter of the typical nucleus must be estimated. BACH (1) has given us a procedure to arrive at this figure.

2) For spherical tissue components it is possible to estimate their number per unit volume, provided the average diameter is known (ELIAS, HENNIG and ELIAS, 2).

3) If the nuclei have the shape of rotatory ellipsoids, as is the case in the basal layer of stratified, squamous epithelia, and in the distal layers of pseudostratified epithelia, procedures for estimating their numbers are available, provided the axial ratio and the average size of the nuclei are known (HENNIG and ELIAS, 3).

Thus, it is possible, thought not easy, to obtain the information which Dr. BERTALANFFY desires. We hope, of course, that in a few years, this Society will have completed a comprehensive book of stereological procedures. Once this exists, sailing in stereological waters should become easier.

To our fellow stereologists active in metallurgy it might be of interest that one of the greatest difficulties for us biologists is the fact that our so-called sections are, indeed, transparent slices of finite thickness. We must always struggle with optical overlapping and with the very disturbing depth of focus which makes it difficult to decide where to take a measurement, if a structure is inclined toward the cutting plane. We envy you for the opacity of the metals which permits you to work with really two-dimensional, sectional planes, excluding all the ambiguity encountered in our material.

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THE FINE STRUCTURE OF HUMAN SKIN

Hermann PINKUS, M.D.(Berlin), M.S.(Michigan), Professor and Chairman,Department of Dermatology, Wayne State University College of Medicine, Detroit, Michigan, U. S. A.

I was introduced to problems of three-dimensional structure early through the work of my father, Felix PINKUS, who had developed to a fine point the method of wax-plate reconstruction of anatomical and histologic configurations. This old anatomical method later was replaced by easier cardboard or blotting paper models, and he practiced reconstruction of many details of normal and pathological skin to the end of his long life. I learned from him to think in three-dimensional terms when examining histologic sections and tried my hand a few times myself in the preparation of similar models. Several of my father's research results as well as my own have been made possible only through application of stereological methods. In the interpretation of the microscopic features of skin disease and experimental pathology, actually the fourth dimension of time also must be taken into consideration. Where many pathologists have been content to describe disease processes in two-dimensional terms based on thin paraffin sections, it is gratifying to see that now the electron-microscopist, because he is dealing with ultra-thin sections and truly two-dimensional shadowgraphs, is forced to stereological interpretation of his findings, and in this respect my early training is paying off when I now started work in the field of fine structure of cells and tissues.

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A MORPHOMETRIC STUDY ON THE THICKNESS OF THE PULMONARY AIR-BLOOD BARRIER

Ewald R. WEIBEL, M. D. and Bruce W. KNIGHT
Journal of Cell Biology, Vol. 20, 1964, in press.

A reliable knowledge of the thickness of the alveolocapillary "membrane" or air-blood barrier is of physiologic interest since it is intimately related to a quantitative estimation of such functional events as gas diffusion or tissue metabolism in the lung. The characteristic thickness of the air-blood barrier with respect to gas diffusion is its harmonic mean thickness, while the arithmetic mean thickness is related to the mass of tissue building the barrier and consuming oxygen in the lung. Two stereologic methods are proposed by which these two dimensions can be estimated from random measurements in the electron microscope in a reliable, simple, and efficient manner. By applying these methods to three rat lungs the arithmetic mean thickness of the barrier was found to measure 1.25μ, the harmonic mean thickness 0.43μ.

On the basis of these measurements a geometric model of the barrier in form of a corrugated membrane was derived. It showed close similarity to the dimensions of the natural barrier. This model analysis suggested furthermore that the gas conductance of the barrier is nearly optimal if one considers the mass of tissue and the minimal barrier thickness as fixed properties which are determined by other functional requirements on the alveolocapillary membrane.

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BIOGRAPHICAL SKETCH OF R. BUCKMINSTER FULLER

R. BUCKMINSTER FULLER, research professor of design science at Southern Illinois University, was born in Milton, Mass., July 12, 1895. He studied at Milton Academy and Harvard University and had honorary doctoral degrees conferred on him by the Universities of North Carolina, Michigan, Washington, and Southern Illinois and by Rollins College. He worked in many responsible positions in industry until World War II broke out, whereupon he served with the Board of the Foriegn Economics Administration.

After the war he became board chairman of Dymaxion Dwelling Machines and in 1955, president of Synergetics, Inc., Raleigh, N.C. Now, while serving as a research professor, FULLER continues to head, as president or board chairman, the Fuller Research Foundation, Wichita, Kan.; Geodesics, Inc., Raleigh, N. C.; Plydomes, Inc., Des Moines, Iowa; the Tetrahelix Corp., Hamilton, Ohio, and the Buckminster Fuller Institute at Carbondale.

FULLER'S inventions include a "Dymaxion House", a Dymaxion 3-wheeled automobile, a steel igloo, a stationary submarine, and a Dymaxion World Map which has been patented. He has received numerous awards from both here and abroad, and his work has been exhibited widely around the world. He was one of 13 artists and designers represented in the American Federation of Arts centennial traveling exhibit, "Form Givers of the 20th Century".

FULLER, inventor of the geodesic dome, has licensed some 125 commercial companies to produce prefabricated parts of the domes, which are being built of aluminum, steel, wood, reinforced concrete, plastics -- even paper. More than 2,000 Fuller domes have been built in 40 countries, including "Radomes" to house D.E.W. radar installations in the Arctic, trade pavillions for the U.S. international trade fair exhibits, restaurants, gymnasiums, airline terminals, and even churches. The Russian government purchased the Fuller dome used to house the U.S. trade fair exhibit in Moscow; it is now a permanent Moscow exhibition building.

FULLER published four books during 1963, including his autobiographical and philosophical "Ideas and Integrities" (Prentice Hall), and has six others on contract for publication in the next three years. For a report on his talk in Vienna, see Volume 2, No. 1, of Stereologia.

Farouk EL BAZ, Graduate Student, Missouri School of Mines, Rolla, Missouri, U. S. A.

For the last four years I have been working almost entirely for the understanding of mineral deposits. Besides being compositional, my approach is basically geometrical. It is the feeling that the geometry, at large and small scales, of mineral deposits has not been given due attention. The geometric nature is being studied now for its implication on the genetic pattern of mineral deposits.

A major part of my studies is finding the relations between the various ore minerals (especially sulphides) and the minerals of the enclosing facies. A portion of my work was presented to the International Mineralogical Congress, Washington, D. C., April 1962 and is entitled: "A Statistical Study of Bravoite Zoning".

Finally I would like to add that my approach to the problem depends thoroughly on the relations between the two-dimensional conventional methods for studying rocks and minerals, and the actual three-dimensional patterns.

LENDING LIBRARY

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YUE, A. S., "Decanted Interface Morphology of Mg-32 Wt Pct Al Eutectic", TRANS. Met. Soc. AIME., Vol. 227, February 1963.

ABSTRACT SECTION

R. T. DeHOFF, "The Determination of the Geometric Properties of Aggregates of Constant Size Particles from Counting Measurements Made on Random Plane Sections", Trans. AIME, 230 (1964) 764.

A general method for determining the geometric properties of structures composed of particles which are all the same shape and size is presented. The application of the method requires a knowledge of the qualitative shape of the particles in the structure, and a maximum of three simple counting measurements, which are made on a representative plane section taken through the structure. It is also shown that the number of different kinds of measurements necessary for the complete description of the structure can be decreased if some independent information about individual particles is available. The geometric properties that can be determined quantitatively from counting measurements for such structures are size, shape, number, surface area, volume, and such extensive properties as volume fraction and surface area per unit volume.

Gunter BACH, "Uber die Bestimmung der Anzahl driachsiger Ellipsoide aus der Anzahl ihrer Schnittellipsen in zufalligen Schnittebenen, Z. Angew Mat. Phy., 15 (1964) 205.

The basic problem in estimating the number of particles of a particular shape dispersed in a structure from measurements that can be made on a section through the structure is the calculation of the average distance between tangent planes for all orientations of the particle. In this paper, Dr. BACH succeeds in performing this calculation for the case of tri-axial ellipsoids. Combination of the resulting shape factors with the approach presented in the paper abstracted above permits the estimation of the number of particles of this shape, per unit volume.

R. T. DeHOFF, "The Estimation of Particle Size Distributions from Simple Counting Measurements Made on Random Plane Sections".

A new approach to the measurement of the size distribution of particulate structures imbedded in opaque bodies is developed. The method is based upon the assumption of a general two parameter distribution curve, and the estimation of these two parameters from three well-known counting measurements made upon metallographic sections. The total number of particles in the system is also a product of the analysis. Application of the method with the assumption of the logarithmico-normal distribution function is developed in detail.

STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

STEREOLOGIA
THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Volume 3, Number 2, December 1964

SOCIETY NEWS

In This Issue: A proposal for a standardized system of notation in Stereology, by E. E. UNDERWOOD, and a discussion of the use of counting measurements in Stereology, by R. T. DEHOFF. Dr. UNDERWOOD brings up an extremely important point, which was discussed at some length at the Vienna Congress. In the development of a field such as Stereology, which has broad application to virtually all scientific disciplines, the introduction of a wide diversity of notation for similar or identical concepts, as non-communicating researchers develop the field, is unavoidable. On the other hand, standardization of notation is clearly a desireable, and eventually expedient requirement. One has only to look at mathematics, music, or thermodynamics to appreciate this fact. I think that Dr. UNDERWOOD has provided a very useful and self-explanatory system of notation. Your comments, criticisms, and additions to his list are essential.

In his brief paper, Dr. DEHOFF emphasizes the utility of the three counting measurements which are basic to most Stereological measurements. Application to estimation of size distribution parameters, and to uses in both section and projection microscopy are also discussed.

In Coming Issues: STEREOLOGIA has appeared somewhat infrequently during the past year because we have attempted to adhere to the original format of this publication, which was largely to provide a vehicle for public communication of individual efforts by researchers in various disciplines. It has become apparent that a shift in emphasis is necessary to the continuation of this BULLETIN. Accordingly, a program is being instituted which will transform your BULLETIN into a reliable bibliography of Stereology. The scope of this bibliography will be both historical and current; an attempt will be made to include all disciplines in this compilation. Although specific members are being approached to aid in this collection, your support is also necessary. The program will be given an immediate impetus if each member will, upon reading this editorial, sit down and compile a short list of what you consider to be the most important (historical and current) papers on the development or application of the methods of stereology to your own particular area of investigation. Please include titles of papers.

The usual features of the BULLETIN, including the lending library, abstracts of current papers, biographies of new members, discussions of current research, and presentation of problems in application of the art, will be continued. Any contributions you may have will be welcomed. However, the emphasis will be shifted to the Bibliography section.

R. T. DEHOFF, Editor

A STANDARDIZED SYSTEM OF NOTATION FOR STEREOLOGISTS

by Ervin E. UNDERWOOD, Treasurer

Several items of business were proposed for Society action at the Vienna Congress. Among these proposals was that of adopting a standardized system of notation for the basic geometrical quantities. The system evolved by F. N. RHINES and R. T. DEHOFF[1] was proposed as a prototype, because their notation was simple, self-explanatory, and descriptive, and because the symbols had already some basis in previous literature.

A survey of the symbols used in a broad range of stereological literature reveals several essentially parallel systems. SALTYKOV,[2] the outstanding Russian pioneer in stereological matters, has adopted English letters for his system of notation. One of the more complicated systems was proposed by A. BRAUN[3] of Switzerland. Fortunately, his basic symbols agree, in general with those of previous workers. The important paper by C. S. SMITH and L. GUTTMAN[4] has helped set the system of symbols used frequently in the United States.

Two tables will be shown. The first is an attempt at a systematic, self-consistent set of symbols that embody the best features of previous usage.[5] Symbols for only the geometric elements are included. For special requirements, one can modify the basic symbols appropriately -- for example, by subscripts or superscripts. The second table compares several major systems of notation in terms of the proposed system. The gaps in the other systems become evident when compared in this way. However, some ideas of how to make special modifications of the basic symbols become apparent from this tabulation.

Without doubt, other symbols may be preferred by various workers instead of those proposed here. If there are any alternate schemes or suggestions please send them to the Treasurer of the Society. We, as a Society, are interested in taking the lead in proposing an international set of symbols for stereological workers. Only by taking the best suggestions from all members and distilling the residue into a scientifically consistent and acceptable set of basic symbols, will we come up with an enduring contribution to unity in a new field. Thus, stereological symbols, like music, like mathematics, can have a truly international acceptance and understanding.

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(1) Personal communication from Dr. Robert T. DEHOFF

(2) S. A. SALTYKOV, Stereometric Metallography, Metallurgizdat, Moscow (1958) (Professor SALTYKOV has made a copy of his book available for members of the Society.)

(3) A. BRAUN, "Considérations sur la micrographie quantitative" Revue Métallurgie Col. 52, No. 9 (1955) 676.

(4) C. S. SMITH and L. GUTTMAN, "Measurement of Internal Boundaries in Three-Dimensional Structures by Random Sectioning", Trans. AIME, Vol. 197 (1953) 81.

(5) E. E. UNDERWOOD, Quantitative Stereology, to be published by Addison-Wesley Co., Cambridge, Mass.

TABLE I

PROPOSED BASIC LIST

OF SYMBOLS AND THEIR DEFINITIONS

SYMBOL	DIMENSIONS	DEFINITIONS
P	0	Number of point elements or test points
P_P	0/0	Point fraction, number of points per test point
P_L	mm^{-1}	Number of points (intersections) per unit length of test line
P_A	mm^{-2}	Number of points per unit test area
P_V	mm^{-3}	Number of points per unit test volume
L	mm	Length of lineal elements, or test line
L_L	mm/mm	Lineal fraction, length of lineal intercepts per unit length of test line
L_A	mm/mm^2	Length of lineal elements per unit test area
L_V	mm/mm^3	Length of lineal elements per unit test volume
A	mm^2	Planar area of intercepted features or test area
S	mm^2	Surface or interface area (not necessarily planar)
A_A	mm^2/mm^2	Area fraction, area of intercepted features per unit test area
S_V	mm^2/mm^3	Surface area per unit test volume
V	mm^3	Volume of 3-dimensional features or test volume
V_V	mm^3/mm^3	Volume fraction, volume of features per unit test volume
N	0	Number of features (as opposed to point intersections)
N_L	mm^{-1}	Number of features intersected per unit length of test line
N_A	mm^{-2}	Number of features intersected per unit test area
N_V	mm^{-3}	Number of features per unit test volume
$\overline{L}$	mm	Average lineal intercept, L_L/N_L
$\overline{A}$	mm^2	Average areal intercept, A_A/N_A
$\overline{S}$	mm^2	Average surface area, S_V/N_V
$\overline{V}$	mm^3	Average volume, V_V/N_V

TABLE II

COMPARISON OF SYMBOLS

USED BY VARIOUS STEREOLOGISTS

UNDERWOOD	RHINES & DEHOFF	SALTYKOV*	BRAUN**	SMITH & GUTTMAN***
P				N
P_P	P_P			
P_L	N_L	m		
P_A	P_A	M		n
P_V	P_V			

L		L, ℓ, P	J, U, L	λ, ℓ, L
L_L	L_L			L_α/L
L_A	L_A	ΣP	$\overline{L}_2$	ℓ/A
L_V	L_V	ΣL	$\overline{L}_3, T_3$	λ/V
A(flat)		F	F	A
S(curved)		S	S	
A_A	A_A	ΣF		
S_V	S_V	ΣS	$\overline{S}_3$	S/V
V		V	V	
V_V	V_V	ΣV	V_3	
N				N/L
N_L	N_L		N_1	
N_A	N_A	n	N_2	
N_V	N_V	N	N_3	P/V
			(also N_3^L and N_3^S)	
$\overline{L}$	$\overline{L}$		$\overline{L}$	L/N
$\overline{A}$	$\overline{A}$		$\overline{S}$	A/M
$\overline{S}$	$\overline{S}$			
$\overline{V}$	$\overline{V}$		$\overline{V}$	V_p

* Distinguishes between individual and total quantities.
**Uses boldface for ensembles per unit volume, in some cases.
***Distinguishes between lines on the section and on the grid.

Symbols for diameters and intercepts of particles and sections of particles not given here. However, some authors use upper case letters (capitals) for objects in three-dimensions and lower case letters for planar features.

COUNTING MEASUREMENTS IN STEREOLOGY

Robert T. DEHOFF, Associate Professor of Metallurgy, Metallurgical Research Laboratory, University of Florida, Gainesville, Florida.

Measurements on a Two Dimensional Section.

The three counting measurements of stereology are so basic to the development of the field that they have been re-derived in each of the disciplines that use this tool. These three quantities, the point count, the line intercept count, and the number per area count, are designated, respectively, as P_P, $\mathbf{P}_L$, and N_A in the standard notation proposed by Dr. UNDERWOOD. They have the obvious advantage of simplicity of measurement, as well as accuracy, or, more precisely, unambiguity. Their usefulness stems from the fact, which has been demonstrated independently on several occasions, that they are simply related to certain geometric properties of the three dimensional structures on which they are determined:

$$P_P = V_V \quad (1)$$ [1]

$$\mathbf{P}_L = \frac{1}{2} S_V \quad (2)$$ [2]

$$P_A = \frac{1}{2} L_V \quad (3)$$ [2]

$$N_A = N_V \overline{D} \quad (4)$$ [3]

All of these quantities have been defined in the preceding paper except $\overline{D}$, which may be shown to be the average distance between tangent planes on all particles in the structure.[3] These relationships are precise, and involve no assumptions with regard to the shape and size of the constituent of the structure being measured. However, they are restricted to measurements made upon two dimensional sections through the structure, and further require that the observations are randomized with respect to the structure, or that the structure itself is random and repetitive. Equations (1) through (4) do not apply to directly projection microscopy, i.e., to thin section analysis, which is common in petrography, and in the life sciences.

These three relations provide the basis for the statistical estimation of the total geometric properties of a structure: the volume fraction, total interface area, and length of lineal feature. The product of the number of particles and the particle diameter can be determined, but these quantities cannot be separated.

It is possible to obtain further information about the structure from these counting measurements if certain assumptions are justified. For example, if the particles in the structure are the same size and shape, then it is possible to combine the counting measurements to give the number of particles, their size, and their quantitative shape.[4] In another approach, the size distribution of particles in the particulate structure (which catagory may include cell structures) may be estimated if it is possible to infer a mathematical form for the size distribution function.[5] A good, versatile form to choose is the logarithmico-normal distribution,[6] since this function has been found to approximate the tediously measured distributions of many particulate systems. The total number of particles may also be estimated in this case.

These two analyses are based upon an extension of equations (1), (2), and (4):

$$P_P = V_V = N_V \overline{V} \quad (5)$$

$$\mathbf{P}_L = \frac{1}{2} S_V = \frac{1}{2} N_V \overline{S} \quad (6)$$

$$N_A = N_V \overline{D} \quad (7)$$

where $\overline{V}$, $\overline{S}$, and $\overline{D}$, are respectively the average particle volume, surface area, and tangent diameter. These quantities are proportional to the first three moments of the size distribution function, and provide estimates of the mean and variance of the distribution, or of other appropriate distribution parameters.

Application to Projection Microscopy.

One of the more important theoretical problems in the application of stereological methods to projection microscopy is the extension of the counting measurements, which are precise only from a true two-dimensional section, to the analysis of thin petrographic or histological sections.

The image that is observed in thin section analysis includes contributions from the top and bottom faces of the slice, and from structural elements contained within the volume. Each of the surfaces contributes to the projected image the equivalent (in P_P, P_L, or N_A) of half of a two dimensional section of the same area. The contribution from the interior of the slice must be estimated for each case, and usually involves some assumptions about particle shape.(7) In structures in which the constituent of interest occupies a moderate or large fraction of the system, the problem of particle overlap in the projected image, which contributes a negative error to all three counting meaturements, must be dealt with.

On the other hand, in a system in which the particles are convex, and occupy only a small fraction of the volume, the relationship between the counting measurements as observed on a projection, and the true, two-dimensional counting measurements are relatively straightforward. The counts corresponding to those made on a section are

$$P_P(\text{proj.}) = P_P(\text{sect.}) + N_V t \bar{A}_P \qquad (8)$$

$$P_L(\text{proj.}) = P_L(\text{sect.}) + \frac{2}{\pi} N_V t \bar{L}_P \qquad (9)$$

$$N_A(\text{proj.}) = N_A(\text{sect.}) + N_V t \qquad (10)$$

where t is the thickness of the slice, and $\overline{A_P}$ and $\overline{L_P}$ are the average projected area and projected perimeter of particles in the structure. In each case, the second term on the right represents the contribution to the total projected count of the particles whose centers lie within the slice. It is also possible to manipulate these counting measurements, under the restriction that particle overlap is negligible, to estimate size distribution parameters, and quantitative particle shape.

Summary.

The application of the counting measurements of stereology to the estimation of what might be called intensive geometric properties of structures would appear to provide a reasonable short cut to the detailed analysis of structures. Until automatic scanning techniques have been perfected and become reasonably priced, the approach discussed in this paper is particularly attractive. Not only are the total properties of the structure rigorously determined, but simple manipulation of the counting data yields a relatively complete structural characterization.

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STEREOLOGIA

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STEREOLOGIA
THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

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SOCIETY NEWS

Plans for the SECOND INTERNATIONAL CONGRESS FOR STEREOLOGY are well under way at this writing. This important meeting will draw participants from many disciplines and from both hemispheres. It is scheduled for early March, 1966, and will be held at the University of Florida in Gainesville, Florida, U.S.A. A preliminary program will be issued in the next few months. Each session will begin with a keynote lecture, giving the underlying fundamentals of a particular kind of stereological measurement. This keynote talk will be followed by a series of papers applying the same measurement. Thus, the format of the Congress is designed to give basic instruction in fundamentals, and to present current research at the frontiers of the field. Plan now to attend and participate in your CONGRESS.

This issue of the BULLETIN presents the latest membership list for the Society. Of the 177 members currently on the rolls, 91 are from the European countries, 78 from North America, 3 from South America, 3 from Asia, and one each from Africa and Australia. They divide among the various disciplines as follows: Biology, 5; Geology, 7; Electrochemistry, 1; Electron Microscopy, 2; Mathematics, 2; Medicine, 58; Metallurgy, 29; and Zoology, 1. These brief statistics give graphic evidence that your Society is indeed International and Interdisciplinary.

We continue to solicit your contributions to the Bulletin, including short reports of current work, discussions of unsolved stereological problems, philosophical papers, and contributions to the Lending Library and Abstract Sections. Send them to either of the Editors listed on the back page.

ABSTRACT SECTION:

LOEB, A. L., "The Subdivision of the Hexagonal Net and the Systematic Generation of Crystal Structures," Acta Cryst., Vol 17, p. 179, 1964.

A classification of all crystal structures containing closely packed ions is based on the stacking of partially or totally occupied nets. It is shown that the symmetry of the hexagonal net is preserved only if the fraction of the net occupied is an integral multiple of $1/(k^2+kl+l^2)$, where k and l are integers. On this basis the plausibility of some existing structures and the non-existence of others is discussed.

MOORE, George A., "Direct Quantitative Analysis of Photomicrographs by a Digital Computer," Photographic Science and Engineering, Vol. 8, No. 3, May-June 1964.

A program on the National Bureau of Standards SEAC Computer directly accepts photomicrographs or other pictures as the information input. Commands in English format cause compilation of operational orders as required to carry out modifications or analyses of the pictures. The 28 operations now functional include lineal and area analyses of whole pictures, sorting out of individual coherent objects, and determining 15 parameters of each. Special photographic precautions are necessary to supply only truthful information to the computer. Studies of complex niobium-tin superconductors and dispersed particle structures are shown. Probable applicability to biological problems and to photographic emulsions is considered.

YUE, A. S., "Microstructure of Magnesium-Aluminum Eutectic," Trans. Met. Soc. AIME, Vol. 224, October 1962.

The morphology of the Mg-32 wt pct Al eutectic has been studied as a function of freezing rate and temperature gradient. At slow freezing rates a lamellar eutectic was formed; whereas, a rod-like eutectic was generated at fast rates. The interlamellar spacing increased as the freezing rate decreased in agreement with theoretical prediction. Lamellar faults, morphologically similar to edge dislocation models in crystals, were responsible for the subgrain structures in the eutectic mixture. A linear increase in fault density with freezing rate was observed. Fault concentrations of the order of 10^5 per sq cm for a range of freezing rates from 0.6 to 3.0 x 10^{-3} cm per sec were estimated. The transformation from lamellar to rod-like morphologies was determined experimentally to be dependent on the freezing rate and independent of the temperature gradient. Moreover, the number of rods formed per unit cross-sectional area increased exponentially with increasing freezing rate.

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STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

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STEREOLOGIA
THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

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SOCIETY NEWS

Preparations for the SECOND INTERNATIONAL CONGRESS FOR STEREOLOGY remains the most important current activity of the Society. Invitations have been sent to the keynote speakers, and affirmative replies are beginning to return. The subject areas which are tentatively proposed for coverage in this Congress are: Basic Counting Measurements; Concepts of Size; Size Distributions; Shape Determination; Applications of Topology to Stereology; Characterization of Orientation in Space; Reconstruction from Serial Sections; Transmission Stereology; and Automatic Measuring Devices. A session on Special Topics in Stereology will also be presented.

The Congress Committee, consisting of Dr. F. N. RHINES, Chairman, Dr. Hans ELIAS, Dr. E. E. UNDERWOOD, and Dr. R. T. DeHOFF has generated a program which is both broad and intensive. Plan now to attend and participate in your CONGRESS.

ON DETERMINATIONS OF THE RADII OF SPHERES BASED ON TWO-DIMENSIONAL MEASUREMENTS.

P. J. VAN MULLEM, Zoology Laboratory of the University of Leiden, The Netherlands

Several methods reported in the literature for the determination of the mean radius of cells in histological sections were tested for their applicability to egg cells from the ovary of the fish Gasterosteus aculeatus. A number of eggs almost ready for spawning, surgically removed from the ovary and heaped together, were subjected to routine histological treatment, sliced into 10μ sections, and stained with eosin.

The following methods of calculating the radii of these eggs were applied:
1) Numerical integration. In the projection plane at a magnification of 54 a planimeter was used to measure the surfaces of all sections (about 1100) containing the 11 eggs selected for study. The sum of the surfaces deriving from one egg, multiplied by the thickness of the sections and corrected for the HOLMES effect (HENNIG, 1963) and for the magnification at projection, gives the volume of the egg from which R can be calculated. For 11 eggs this gives $\overline{R}$*. This value was used for comparison with results obtained with the other methods.

For methods 2 through 7, and using random numbers (de JONGE, 1963), 11 mutually independent samples were taken from the 11 series of surface values. The mean measured radius of 11 eggs was found to be: r=411.5μ. Taking as a basis this mean value or the individual observations for the 11 samples, the following methods by which $\overline{R}$ is calculated from $\overline{r}$ were applied as described by the authors:
2) the method of BACH (1959),
3) the simple method of EBLING (1957),
4) the improved method of EBLING (1964; ADAMS 1962),
5) a method using the basic formula for the derivation of EBLING'S log equation (improved method):

$$A = \frac{(2/3\pi R^2 \cdot 2R) + (\pi R \cdot t)}{2R + t}$$

in which A is the mean surface of all the cross-sections of one egg. For t = 10μ (the thickness of the sections), this gives $\overline{R} = 1.222\ \overline{r}$.
6) the method of LENZ (1956), and
7) the method according to WEIBEL (1963, p. 29).
In addition, in a number of sections of the combined eggs the points of intersection of the chords of two different integration eyepieces with the external surface of the cell walls of the eggs were counted. According to HENNIG (1958), the relationship

$$U = \frac{\pi\ a\ p_m}{2}$$

applies here, in which U is the sum of the circumferences of all the cross-sections, a is the distance separating the chords as measured in the plane of the preparation, and p_m is the average number of intersections in a number of different positions of the eyepiece. Using the equation for the circumference of a circle we derive for the average radius

$$\overline{r} = \frac{a\ p_m}{2n}$$

in which n is the total number of cross-sections in the sections examined. For the calculation of $\overline{R}$ the method given by WEIBEL ($\frac{4}{\pi}$) was used. Counting was done with:
8) the Zeiss integration eyepiece I, the lines being used as chords,
9) an integration eyepiece made in this laboratory with approximately twice as many lines as that of the Zeiss integration eyepiece. The results are shown in the Table.

DATA AND RESULTS CONCERNING NINE METHODS FOR THE DETERMINATION OF THE MEAN RADIUS ($\overline{R}$)

Method for the determination of mean radius	Multiplication factor $\overline{R}/\overline{r}$	R in μ	For the calculation of $\overline{R}/\overline{r}$ the method takes into consideration t	$\overline{r}$	Applicability based on tests given by two authors
1) Numerical integration		513,6			
2) BACH	1,270	522,6	yes	yes	yes
3) EBLING (simple method)	1,225	504,1	no	no	
4) EBLING (improved method)	1,323	544,3	yes	yes	
5) Equation derived from EBLING'S basic formula	1,222	502,9	yes	no	
6) LENZ	1,266	520,9	no	yes	yes
7) WEIBEL	1,273	524,0	no	no	
8) Zeiss Integration eyepiece I	1,273	517,0	no	no	
9) Integration eyepiece (designed by us)	1,273	527,1	no	no	

t = thickness of section.

$\overline{r}$ = mean radius measured in section.

The data in the Table indicate that if we tolerate deviations of $\pm$ 2.6% from the values obtained by numerical integration, all but one of the methods may be applied. EBLING'S improved method must be excluded, as was to be expected in view of the fact that EBLING states that the upper limit of the volumes to be determined lies at $1000\mu^3$. The other seven methods approach the actual value of the mean radius of eggs which have been submitted to histological treatment to an extent satisfactory for many biological investigations.

*) The author is indebted to Mrs. L. G. van der STARRE-van der MOLEN for these measurements.

ABSTRACT SECTION

LOEB, A. L., "A Modular Algebra for the Description of Crystal Structures," Acta. Cryst., Vol. 15, p. 219, 1962.

Anticuprite, cadmium iodide, perovskite, cesium chloride and many other structures can be considered as stacked, partially occupied hexagonal nets. Algorithms are presented for a mathematical description of such structures. Whereas previously only quarter-, half-, and three-quarter full nets were considered, one-twelfth, one-sixth, one-third, and two-thirds full nets are also described, and the results applied to quartz and corundum. Certain formalisms of mathematical logic are introduced. Rutile is described as a structure made up of closely packed anion pairs with cations occupying the interstices. Plastic modules have been constructed for implementing the mathematical descriptions.

LOEB, A. L., "A Binary Algebra Describing Crystal Structures with Closely-Packed Anions," Acta Cryst., Vol. 11, Part 7, July 1958.

A description of structures with close-packed anions is given in terms of simple, interpenetrating component lattice arrays. Each component array is denoted by a set of binary digits, from which the spatial relationship of these arrays can be derived. For ferrimagnetic rocksalt-like structures the magnetic dipole-dipole energy is minimized subject to exchange restraints, and it is shown that dipole patterns so obtained match those found by neutron-diffraction, and are not necessarily parallel and anti-parallel to a single direction. Spinel and olivine structures are found to be ordered mixtures of some component arrays found in rocksalt structures and some found in sphalerite structures.

THE ESTIMATION OF CHARACTERISTIC PARTICLE SIZES IN A MICRO-STRUCTURE BY COUNTING ON RANDOM SECTIONS

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A method for determining the mean particle dimension, the variance, and the number of particles per unit volume by three simple counting measurements (made upon random plane sections taken through the multiphase structure) is presented.

The application of the method requires a knowledge of the geometric shape of the particles, which may be unequal in size.

A RAPID TECHNIQUE FOR OBSERVATION OF THREE-DIMENSIONAL MICROSTRUCTURES: APPLICATION TO ANALYSIS OF FAULTED STRUCTURE IN A EUTECTIC ALLOY.

Abstract of M.S. Thesis by RICHARD H. HOPKINS, Lehigh University, January, 1965.

Conventional metallographic techniques, although indispensable for studying the microstructure of alloys, only provide a two-dimensional picture of three-dimensional structures. Hence in the past conclusions regarding the spatial features of micro-constituents in opaque materials have been based upon inference from two-dimensional sections, upon three-dimensional models constructed by tedious sectioning procedures, or upon dissolution of one or more phases.

This paper describes a new technique which facilitates the rapid determination of the three-dimensional morphology of phases suspended in an opaque matrix. The rapidity of the technique is based upon the continuous cinephotomicrographic recording of specimen microstructure as the specimen surface is removed under controlled conditions in an electrolytic cell.

The advantages of a rapid technique for determining spatial morphologies in opaque materials are illustrated by application of this method to the study of the configuration of faults in a specimen of $CuAl_2$-Al eutectic alloy. (A fault is the term applied to termination of an extra lamella in the eutectic structure; faults are morphologically similar to models of edge dislocations in crystals.) A spatial model of the eutectic fault network deduced by the above technique has substantiated the facts that faults in this eutectic alloy tend to run parallel to one another in a direction close to that of the specimen growth axis, and that the majority of faults bridge lamellae rather than lie in a plane parallel to the average lamellar direction. For the specimen area studied, fault density decreased as growth proceeded.

"A Morphometric Study on the Thickness of the Pulmonary Air-Blood Barrier"

A reliable knowledge of the thickness of the alveolo-capillary "membrane" or air-blood barrier is of physiologic interest since it is intimately related to a quantitative estimation of such functional events as gas diffusion or tissue metabolism in the lung. The characteristic thickness of the air-blood barrier with respect to gas diffusion is its harmonic mean thickness, while the arithmetic mean thickness is related to the mass of tissue building the barrier and consuming oxygen in the lung. Two morphometric methods are proposed by which these two dimensions can be estimated from random measurements in the electron microscope in a reliable, simple, and efficient manner. By applying these methods to three rat lungs the arithmetic mean thickness of the barrier was found to measure 1.25 μ, the harmonic mean thickness, 0.57 μ. On the basis of these measurements a geometric model of the barrier in the form of a corrugated membrane was derived. Its dimensions showed close similarity to those of the natural barrier. This analysis suggested furthermore that the gas conductance of the barrier is nearly optimal if one considers the mass of tissue and the minimal barrier thickness as fixed properties which are determined by other functional requirements on the alveolo-capillary membrane.

WEIBEL, Ewald R., and Domingo M. GOMEZ, "Architecture of the Human Lung," Science, Vol. 137, No. 3530, pp. 577-585, August 1962.

WEIBEL, Ewald R., "Morphometrics of the Lung," Handbook of Physiology - Respiration I Printed in U.S.A.

LAMELLAR FAULT CONFIGURATION IN UNDIRECTIONALLY SOLIDIFIED Al-$CuAl_2$ EUTECTIC

SETH R. THOMAS and R. W. KRAFT

Microscopic defects called lamellar faults in a single-grained ingot of unidirectionally solidified Al-$CuAl_2$ eutectic alloy were analysed by means of quantitative metallographic methods to determine their geometric configuration within the ingot. It was found that the faults in a given sample were parallel to one another to a good approximation but that the fault direction in different samples varied in a systematic fashion as a function of sample location about the ingot centre axis. This was taken as a positive indication of rotational symmetry in the fault configuration about the growth direction and is postulated to be due to radial heat flow combined with the translatory motion of the solid-liquid interface. In addition, it was observed that the faults tend to cross lamellae rather than run parallel to them. This was interpreted to mean that individual lamellae can accommodate variations in growth conditions lying within the plane of the lamellae more readily than variations perpendicular to the lamellae.

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The zone-melting technique can be adapted for the determination of the eutectic composition in complex metal systems. The application of this method is demonstrated in a simple eutectic system, Mg-Al, in which the eutectic composition is known, and in a complex ternary system, Mg-Al-Zn, in which the literature is uncertain as to the composition of the ternary eutectic. The advantages and limitations of this unique approach for the determination of the eutectic composition are discussed.

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STEREOLOGIA

THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

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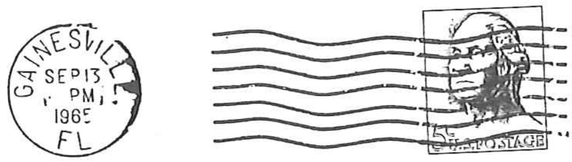

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STEREOLOGIA
THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Volume 5, Number 1, June, 1966

SOCIETY NEWS

The program for the Second International Congress for Stereology is evolving into a collection of presentations that you won't want to miss. Applications of the principles of Stereology to virtually all fields will be represented. This meeting will provide you with up to date information about the relationships and procedures on a truly interdisciplinary scale. Clearly, this is an unparalleled opportunity to exchange problems and solutions, and to absorb a complete spectrum of the current state of the art.

Plan now to attend your Congress.

The present issue of STEREOLOGIA is primarily devoted to the first half of a paper by Dr. Hans ELIAS, dealing in a general way with the over-all structure of the field. The article is presented from the point of view of the life scientist, so that it provides both a fairly complete review of the basic relationships in the field, and a broadening of viewpoint for our readers in the natural sciences.

The next issue of STEREOLOGIA will present the second half of Dr. ELIAS' paper. In addition, it will contain a brief statement of a new fundamental relationship in the field. Watch for it.

Interpretation of Sections*

HANS ELIAS, Chicago Medical School

The most frequently used method of microanatomy is the observation of "sections" which are really very thin slices cut through a three dimensional organ (Fig. 1). They are produced with a microtome after the organ has been hardened. Mounted on glass slides (Fig. 1D), they are usually stained to emphasize contrast. A true section would be a plane without thickness (Fig. 1B). True sections can be used in the study of metals and ceramic materials, because these

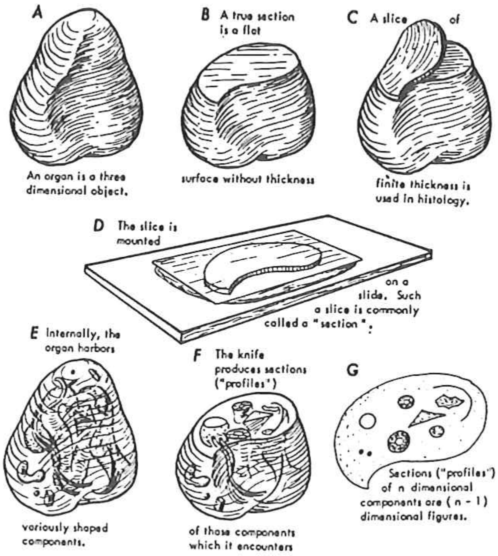

Figure 1. Illustration of a slice through a three dimensional organ.

substances are opaque. But the parts of living organisms (as well as rocks) are transparent enough so that the thickness of the slides must often be considered in histology as well as in geology. In histology, then, we deal with slices (Fig. 1C), but we call them "sections."

An organ (Fig. 1A) is composed of various parts (Fig. 1E). A section through an organ (Fig. 1F), produces a section of each part which the knife encounters (Fig. 1G). Therefore, the word "section" has more than one meaning. Electron microscopists have introduced the term "profile" for the shadow of a slice of a component part.

For a physiological evaluation of histological preparations, mere description does not suffice. Thus, the histophysiologist must attempt to learn about size, shape, and orientation of the parts within an organ. Also volume ratios and the number of parts per unit volume are often of interest.

*This article presents a pre-publication of one chapter of the third edition of ELIAS and PAULY, Human Microanatomy, to be published by F. A. Davis, Philadelphia.

In the following brief outline of quantitative stereology, procedures and formulae but not their derivations are given.

1. Volume ratios.

Organs are mixtures of various components. The parenchyma of a organ, for example, may consist of tubules lined by epithelium surrounding a lumen. Its stroma consists of interstitial connective tissue and its vasculature of blood vessels. The ratio of the volumes of these four components may be of interest.

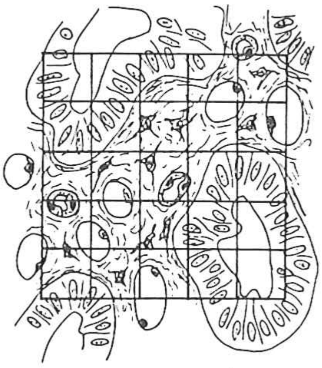

Figure 2. Method for estimating volume fraction of identifiable parts of an organ.

Let us take the papilla of the kidney as an example. Various states of dehydration and diuresis may express themselves in the relative volumes of the urine, the blood, the interstitium and the epithelium, both of papillary ducts and nephric loops.

To arrive at an estimate of the ratio of the volumes of Epithelium to Lumen to Interstitial connective tissue to Vasculature, all we must do is place a reticule of crossing lines on the diaphragm of a Huygens eyepiece and count the cross points of the reticule which happen to fall on each of the components (Fig. 2). A standard reticule divided into 25 squares has 36 crossing points. In the position shown in Fig. 2, 10 points fall on epithelium, 5 on tubular and loop lumina, 14 on interstitial tissue, and 7 on blood vessels. This count of "hits" is very rough. By repeating this process often enough, moving the slide in a random fashion, any desired degree of accuracy can be attained. Point ratios equal volume ratios. This mathematically proven relationship is expressed by stereologists by the formula $P_p = V_v$. In common language, this formula means: if so many points out of all points hit one kind of tissue, this tissue occupies the same percentage of space in the organ.

2. The problem of size.

Let us take the simplest solid, a sphere, as an example. Let spheres of equal size be distributed in an organ (as, for instance, fat cells in adipose tissue). Any section through a sphere is a circle whose radius r depends on the radius R of the sphere istelf and on the distance d of the cutting plane from the center of the sphere (Fig. 3) so that $r = R^2 - d^2$. Thus, a slice ("section") through a mass of spheres will show large and small circles. At first, we measure the diameter of the largest circle which can be assumed to pass approximately through the center of a sphere. Let this diameter equal D. Then, all spheres in the organ are of equal size, if 13.4% or less of

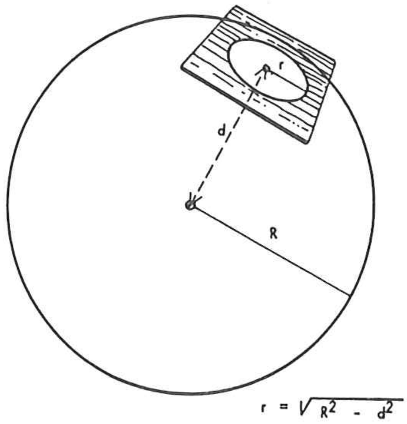

Figure 3. A plane cutting a spherical object produces a circle whose radius is, in general, smaller than the radius of the sphere.

all the circles in the "section" have diameters smaller than $\bar{D}/2$. If the number of smaller circles exceeds 13.4% we know that the spheres are of various sizes. This percentage is correct only for sections which are very thin when compared with the spheres.

3. Surface area per volume.

An organ may contain a great number of parts of the same kind (such as follicles, alveoli, or capillaries), and it may be necessary to estimate the total surface through which diffusion can take place. Let us take the sinusoids in the liver as an example. The total volume of the liver can be measured. If we knew the total surface of sinusoids in a very small fraction of the organ, say in a volume of 1 mm^3, we could compute the total sinusoidal surface in the entire liver.

Again we can use the same reticule which we have taken before; a square divided into 25 little squares. By means of a stage micrometer we determine the length of a side of the large square. In the case of Figure 4, it measures 180μ. All the lines together

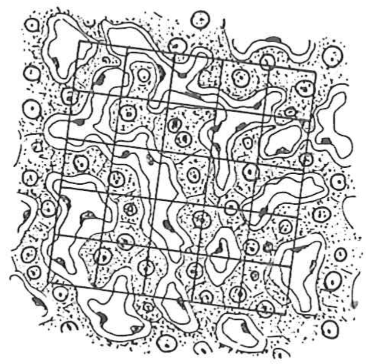

Figure 4. Method for estimating the area of interfaces between parts of an organ.

measure 2160μ or 2.16 mm. Then we count the number of intercepts of the 6 horizontal lines with traces of the sinusoidal walls. In the position shown in Fig. 4, we count 30 intercepts. Next we count 32 points of intersection with sinusoidal walls for the vertical lines in our pattern. We have obtained altogether 62 points of intersection along a stretch of 2.16 mm. In stereology, this finding is expressed thus:

$$N_L = \frac{62}{2.16}\ mm^{-1} = 22.8\ mm^{-1}.$$

S_V stands for surface per volume. The formula $S_V = 2\ N_L\ mm^2/mm^3$ should give us in our case a sinusoidal surface area of 55.4 mm^2 for one cubic millimeter of liver. If our intercept count were accurate, we could compute the total surface area of all the sinusoids in the liver, from the measured volume of the liver. Of course, to achieve accuracy, we must repeat the intercept count very often. Since the number of intercepts is proportional to the sine of the angle between the testline and the traces, we must rotate the eyepiece frequently in a random fashion.

In the case of the problem just presented, we should first obtain the volume fraction occupied by portal canals and by central and hepatic veins using the method for volume ratios described under heading 1 and a very low power objective. This fraction should be subtracted from the measured volume of the liver.

4. Number of parts per unit volume.

It will often be interesting to know the number of specific parts per unit volume of an organ, such as the number of alveoli per unit volume of the lung, the number of islands per unit volume of pancreas, etc. If these units of structure are approximately spherical and of approximately equal size, but much larger than the thickness of the slice, it is obvious that each will be seen in several slides. Most convenient for the determination of the number of spherical elements per cubic millimeter (N_V), is the use of the camera lucida. The image of the scale of the stage micrometer is projected on the working area at a convenient magnification, and a magnified paper replica of the scale is made. Then one draws on a piece of paper a circle which should have a diameter two to ten times larger than the mean distance between the centers of the profiles to be counted. For ease of calculation, the circle should have an area A of 1 mm^2, 10 mm^2, 0.1 mm^2, or 0.01 mm^2. The radius of this circle must be calculated from the well-known formula $A = \pi r^2$. Thus the sought radius for the counting circle is $r = \sqrt{\frac{A}{\pi}}$.

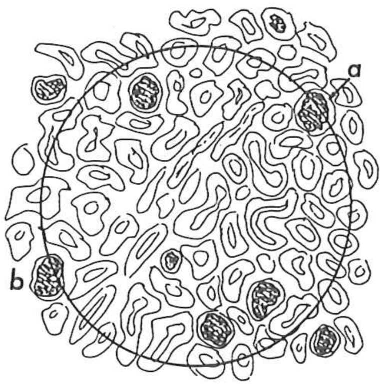

Figure 5. Method for estimating the number of separate parts per unit volume of some identifiable part of an organ.

Now, we superimpose the images of the slide and of the circle and count the number of profiles which fall into the circle (Fig. 5). If one of them is transsected by the perimeter, we count it as one; if, by eye estimate, its center lies within the circle (glomerulus a in Fig. 5). If the center appears to lie outside, we give it a value of zero (glomerulus b in Fig. 5). If we are undecided, we count it as one-half. This operation must be repeated very often. And the more frequently it is done the greater will be the accuracy.

Let $\overline{N}_A$ be the average number of profiles counted per square millimeter (arithmetic mean), and d the average diameter of a corpuscle, and t the thickness of the slice, then

$$N_V = \frac{\overline{N}_A}{(d+t)}$$

N_V is the number of corpuscles per cubic millimeter of that organ.

5. Length per unit volume.

It is often interesting to learn how long a tube or a fiber is of which only sections can be seen in slides. For example, the total length of the nephron, the combined length of the seminiferous tubules, or the combined length of capillaries in a bulky organ may present a problem of physiological interest. In former times, such problems were studied by maceration and teasing out of the long objects, stretching them out on a glass plate and measuring them with a yardstick. This method presents often insurmountable technical obstacles. However, using the technique of Alexander the Great, the histologist, instead of untying the Gordian knot, cuts it. Counting the number of intersected elements per unit area, he very easily obtains reliable results. Let us take as an example the length of the convoluted tubules per cubic millimeter of kidney cortex. We project an area of kidney on a paper on which a circle or a square of specified area is drawn and count the number of tubular sections in the circle. If the average number of sections through tubules within a square millimeter equals $\overline{P}_A$, then the formula

$$L_V = 2 \cdot \overline{P}_A$$

gives their total length in mm per cubic millimeter of cortex. Again, the greater the number of counts to obtain the average number of intersections, the greater the accuracy.

When using a square instead of a circle, we count all intersected profiles on one side as in, while profiles intersected on the opposite side are counted out.

STEREOLOGIA

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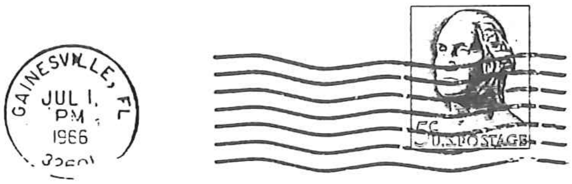

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STEREOLOGIA
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SOCIETY NEWS

This issue is primarily devoted to the completion of the article by Dr. ELIAS dealing with General Stereology, as seen from the viewpoint of the life scientist. This simultaneously supplies a fairly complete review of the fundamentals of the field, provides physical scientists with a biological viewpoint, develops some applications in that field, and lays the foundation for future basic articles to build upon.

YOUR specific contributions, whether they be in the form of an expansion of the techniques, or an application of a particular relationship, are earnestly sought for presentation in subsequent issues. STEREOLOGIA is your Bulletin, and its issues must be filled by YOUR problems, analyses, and solutions.

The new stereological relationship, promised in the last issue, must now await the next issue. There is simply not sufficient room for it in the present edition.

One of our graduate students recently brought to our attention a book entitled "GEOMETRIC PROBABILITIES," by KENDALL and MORAN. (Published by Hafner Publishing Co., 1963). A brief survey of this text reveals that it contains essentially all of the fundamentals of Quantitative Stereology. A fairly extensive, though incomplete, bibliography shows that most of these relationships have a longer history than most of us suspected. We were surprised to learn that one of the stereological problems solved in our doctoral dissertation had been previously solved by an astronomer, some thirty years earlier. Indeed, the basis for the "new" relationship referred to in the preceding paragraph was published in a mathematical journal in 1898.

This discussion serves to re-emphasize the need for the existence of our Society: the duplication of effort in the past has been phenomenal.

INTERPRETATION OF SECTIONS (Continued from last issue)

HANS ELIAS, Chicago Medical School

ć. Identification of shape.

Shape, often contemptuously considered a merely qualitative property and hence not worthy of a scientist's consideration, may be of great functional importance. Let us think of the difference between a fiber and a membrane. Let the fiber be represented by a rope attached to the ceiling and to the floor of a room. You can walk around it and hold on to it for support. The membrane may be represented by a sheet attached to ceiling, walls, and floor of a room. This sheet can divide the room into two parts, fully separated from one another, while the rope cannot divide it. In certain sections, a fiber may not be distinguishable from a membrane.

In a single section, a follicle may not be distinguishable from an alveolus or from a tubule. Yet these three shapes are functionally very different from each other. A follicle is closed on all sides. Only by diffusion or active transport can substances pass out of its lumen. An alveolus, however, opens into a duct, and its contents can flow away freely. A tubule, also open, offers its lining epithelium an opportunity to act upon the content while the content slowly streams from the dead end to the opening.

Thus we see that shape is physiologically as important as size and number. And we shall present some methods for the determination of shape.

a. Points, lines, and surfaces (granules, fibers, and membranes).

An n dimensional formation, when intercepted by a plane, yields an (n-1) dimensional figure. Conversely, an n-dimensional figure in a section indicates the presence in space of an (n+1) dimensional figure.

A point (granule) would not be cut at all by a mathematical plane. However, since in histology we deal with slices rather than with true sections, granules will be found within a slice, between its two cutting planes. A granule will be in focus at oil immersion with the condenser up and with the diaphragm open for a depth of about 1μ or at the most 2μ only. It can be identified as a granule, because it can be put out of focus within the slice.

A line, when intercepted by a plane, yields a point. Fibers, fibrils, and filaments have the quality of lines. Since we deal with slices of finite thickness, the slices contain a short segment of the fiber. When cut perpendicularly, this fiber segment will appear in the microscope as a point. This point will remain in focus throughout the thickness of the "section." Fiber segments inclined toward the cutting plane will appear as rods of various lengths according to the angle of inclination α. In a section of thickness t the "apparent length" of the fiber segment inclined against the cutting plane by the angle α; i.e., its projection p as observable in the microscope (with diaphragm narrow) is $p = t \cdot \cot \alpha$ (Fig. 6). Applying rules of probability it can be shown that, if a mass of fibers randomly running through space is cut with a microtome at thickness t, approximately 50% of fiber segments included in the slice ("section") will appear shorter than t; about 50% will appear longer. Very long lines will be very rare; i.e., about 1% of all fiber segments will appear longer than 7 t and only 1/2% longer than 10 t. Upon superficial inspection, the long lines appear more numerous than they are because each occupies more space than several dots together. Therefore, actual counts must be made to identify fibers correctly.

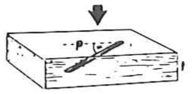

Figure 6. Illustration of an histological section containing a fiber.

Since fibers are often curved, they may wind within the slice, thus raising the number of longer segments above 50%. But when the number of lines longer than t exceeds 60%, it is probable that among the suspected fibers there are in reality some bonds.

A two-dimensional surface, when intercepted by a plane, yields a line, which is often called the "trace" of the surface. Membranes have the geometrical quality of surfaces. Hence, if one finds in a slide numerous lines but few dots and commas, it is probable that these lines are traces of membranes.

b. Solids of various shapes.

Fortunately, most organs are constructed of components of similar, three-dimensional shape. Homogeneous construction is a physiological advantage because only similar parts can act in unison. Because of their uniform architecture they yield themselves to geometrico-statistical analysis.

The shape of a section ("profile") of a solid depends on the three-dimensional shape of this solid and on the angle of cutting. A solid has three dimensions: length, width and height. A section through it has only two dimensions: length and width. We call the quotient $\frac{\text{length}}{\text{width}}$ its axial ratio Q. If many three-dimensional objects of equal shape, randomly distributed in space, are cut by a plane, the axial ratios of their sections ("profiles") will show a characteristic distribution.

Spheres

One hundred percent of sections through spheres will have an axial ratio of Q = 1; i.e., they are all circles. Thus, if one finds only circles in a section, these may have resulted from cutting many spheres.

Circular cylinders

Many circles in sections could also be "profiles" of parallel, circular cylinders cut transversely (as for example sections of nerve fascicles in a cross section through a nerve). If the plane of cutting is oblique to the longitudinal direction of these parallel circular cylinders, all their "profiles" will be ellipses of equal shape. Their common axial ratio will be equal to the cosecant of the angle which the cutting plane forms with the longitudinal direction of the cylinders ($Q = \operatorname{cosec} \alpha$) (Fig. 7).

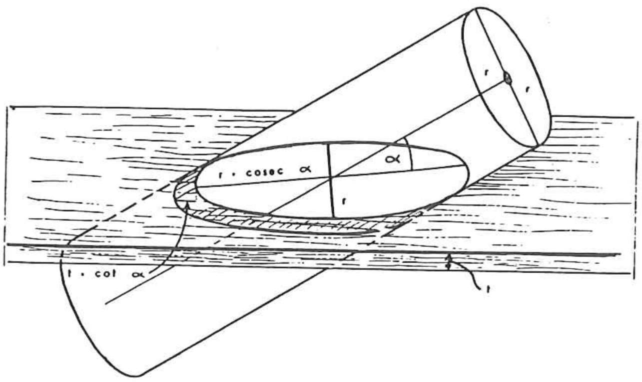

Figure 7. Illustration of a typical section through a cylindrical body.

When circular cylinders are randomly distributed in space (as for example the seminiferous tubules), exactly 75% of their sections will be "short" ellipses; i.e., their axial ratios, Q, will lie between 1 and 2 (1<Q<2). Exactly 25% will be oblong. In histological "sections" however, the long ellipses will appear just slightly more numerous than they would in cutting planes of thickness zero. In fact, their absolute length will be augmented by t · cot α (Fig. 7). Cylindrical tissue components (such as tubules, arteries, the gut as a whole) are confined to a restricted space. Therefore, they are twisted, convoluted, and curved. For this reason, the number of very long ellipses will be even lower than the value of 2% predicted for straight cylinders. Actually, if in the human organism a cylinder is straight, as for example certain large arteries and ducts, it will be alone (i.e., unaccompanied by structures of its own kind), except for nerves which are bundles of cylindrical fascicles. Randomly arranged cylinders in man are always crooked.

Rotatory ellipsoids

Shapes intermediate between the sphere and the circular cylinder are those of rotatory ellipsoids. These may be oblong (egg-shaped) as many nuclei in columnar epithelium, or oblate (lens-shaped) as nuclei of squamous epithelium. When sectioned in any direction, they yield ellipses. There is a distribution of the axial ratios of their sections characteristic for each shape of rotatory ellipsoid, as shown in Fig. 8, always assuming that the ellipsoids are equal in shape and randomly oriented; or that several sectional planes in random directions are used.

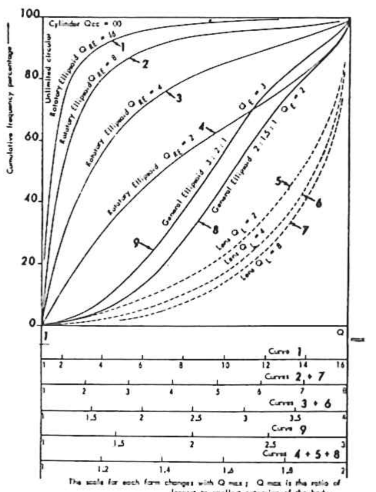

Figure 8. Distribution of axial ratios of sections through various shapes of objects.

Tri-axial ellipsoids

Tri-axial ellipsoids are oblong, flattened ovoids. Many endothelial nuclei exhibit this shape. Again, the sections of ellipsoids of specific degrees of flattening and elongation show characteristic distributions of axial ratios (Fig. 8).

Elliptic cylinders

In contrast to circular cylinders whose exact cross sections are circles, those of elliptic cylinders are ellipses, so that they approach the shape of bands. The taeniae coli have this shape; so do many veins and venous sinuses, as well as certain Platyhelminthes. Distribution of axial ratios for sections of elliptic cylinders of various flatnesses are shown in Fig. 9.

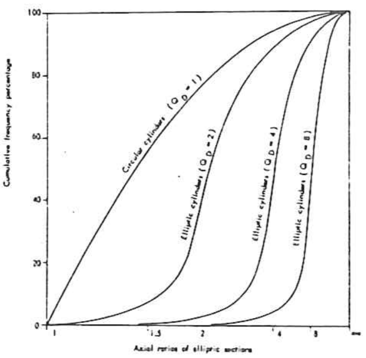

Figure 9. Distribution of axial ratios of sections through rotatory and triaxial ellipsoids.

Laminae

The greatest amount of flatness is possessed by a sheet, plate, or lamina of infinite extension and of finite thickness. An epithelium would have these properties. While a basement membrane appears in the light microscope almost as a surface without thickness, in electron microscopy it acquires the geometric properties of a lamina of measurable thickness. If such a flat object is said to be of infinite expansion, we mean that it reaches beyond the boundaries of the microscopic or electron-microscopic field. All sections of it are stripes of "infinite" length. The width of such a stripe varies as the cosecant of the angle of sectioning. Since such plates in the living organism are usually buckled, their sections exhibit various thicknesses from place to place. Let us visualize a lamina which is of equal thickness throughout and which is irregularly folded and let us assume that its real, constant thickness equals T. If we measure the width of its sectional stripe at regular intervals we find 86.6% of the places to have a width W, so that T < S < 2T, while at 13.4% of measured locations, the stripe is wider, so that W > 2T. The renal glomerular basement membrane is a lamina of finite thickness. Its sections exhibit various widths dependent on the angle of cutting. Since at this time no statistical measurements have been made of the widths of the sections, we do not yet know whether this particular membrane is of even or variable thickness.

Muralia

A muralium which consists of interconnected walls appears in sections as a maze of interconnected stripes without end in any direction (Fig. 4).

Branched sheets

Intermediate between a muralium and a very flat, oblate lens, is a branched lamina of finite extension, such as the lamina vasculosa glomeruli. Its sections are very long, branched stripes with an axial ratio distribution intermediate between that for a very flat, oblate lens and for a sheet of finite extension.

This account exhausts the list of shapes which had been analyzed stereologically by 1965. As new objects are observed, hitherto unknown shapes are likely to be discovered requiring further analysis. Until the middle of the 20th century, histologists used chiefly intuitive thinking for the elucidation of the three-dimensional properties of objects. Nowadays, we have at our disposal the methods of quantitative stereology by means of which we are able to put the interpretation of sections on a mathematically sound basis.

The methods of mathematical stereology are applicable only where a great number of similar objects are present in the tissue.

But if a structure is present in the singular only, and if it is of complicated shape, geometrico-statistical methods are of little use. For the determination of the shape of such objects, reconstruction from serial sections as practiced in embryology is the only, though very time consuming, method available at this time. Reconstruction depends on the production of large, uninterrupted series of very well-stretched slices of uniform thickness. These can be obtained only under optimal conditions by highly skilled technicians. In many cases, particularly in electron microscopy, this is possible of fulfillment only in fortunate accidents and if the object to be reconstructed is very small, compared to the size of an entire cell. Four hundred consecutive sections, fully visible, would be needed to reconstruct a single cell of average size, electron microscopically.

Mathematical stereology, however, within its field of application, provides efficient and easy methods which yield results of relatively high precision.

STEREOLOGIA

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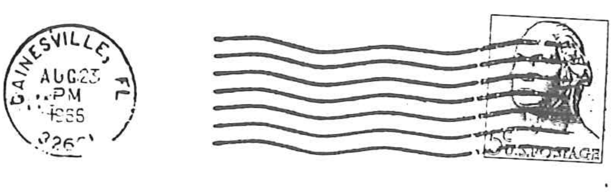

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