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Newsletter '80 in Stereology

G. Ondracek (editor) Institut für Material- und Festkörperforschung

Kernforschungszentrum Karlsruhe

KERNFORSCHUNGSZENTRUM KARLSRUHE

Institut für Material- und Festkörperforschung

KfK 3049B



Newsletter '80 in Stereology

edited by

Gerhard Ondracek

Secretary of the

International Society for Stereology Société internationale pour la Stéréologie Internationale Gesellschaft für Stereologie

Kernforschungszentrum Karlsruhe GmbH, Karlsruhe

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Preface and Summary

The Newsletter '80 in Stereology is subdivided in a first part devoted to the ISS history and a second part containing original papers on the subject.

Almost two decades have past, since the ISS was founded in 1961. Reason enough for looking back briefly. Hans Eckart Exner, elected president for the period 1980-1983, introduces the present Newsletter, followed by a report on the foundation by G. Bach in 1963 and a general information on goals and structure of the ISS. As required in a discussion during the last international congress of the ISS, the by-laws of the society as filed with the US registration of the society are reprinted. It should be mentioned here that the ISS is also registered in Germany, the by-laws are similar with exception of such items which are regulated by regional laws. Additional rules for running the society (e.g. definition of membership and statement of dues, rules for elections and additional information on duties of committee chairmen and regional representatives) have been or will be established on the basis of these by-laws. So in fact their republication is at least partly a look back into history as well as a view of the presence. Furthermore, the few available issues of "Stereologia" are reprinted in this issue. "Stereologia" served as the official bulletin of the ISS before it was substituted by the "Newsletter in Stereology" in the early seventieths. The reproduction of these issues seems to be worthwhile due to the fact that only a few copies have been printed and now are not available to a larger readership.

The original articles of the second part deal with topics of widespread interest due to the interdisciplinary character of stereology. The paper on the analysis of unflat surfaces is a contribution from the field of life sciences and is considered to stimulate interest in this problematic topic. The second paper is a report on the stereological analysis in granulometry and the third paper discusses the quantitative microstructural analysis of materials with respect to stereology and materials properties.

On occasion of that selection it may be declared for those accustomed to the "referee-style" of common journals, that the Newsletter is not based on these methods, but encourages everybody to discuss critically the articles published or re-published here by comments in the Newsletter itself.

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This Newsletter ends with an information on forthcoming events in stereology and contains also a review on books edited by or for the International Society for Stereology and other monographs of interest in the field, as well as an information on the second official publication of ISS, the Journal of Microscopy.

The Editor

Zusammenfassung

Der Newsletter '80 besteht aus zwei Teilen, deren erster der Geschichte der Internationalen Stereologischen Gesellschaft gewidmet ist, während der zweite Teil Originalmitteilungen enthält.

Fast zwei Jahrzehnte sind vergangen, seit im Jahre 1961 die "International Society of Stereology" gegründet wurde. Der 1980 gewählte Präsident Hans Eckart Exner leitet den Newsletter 1980 ein. Danach folgt G. Bach's ISS-Gründungsreport sowie ein Abdruck der damals definierten Ziele, Organisationsformen und Statuten. Auf dem letzten ISS-Kongreß in Salzburg 1980 wurde dieser Abdruck gewünscht. Es sei erwähnt, daß diese Statuten außer in den USA auch in Deutschland registriert sind und durch gegenwartsbezogene Aussagen zu wichtigen Dingen wie Mitgliedschaft, Pflichten der Präsidiumsmitglieder, Wahlen und dergleichen ergänzt werden sollen. Die "historischen" Statuten bilden daher auch die Basis für ISS-Gegenwart.

Bevor der wissenschaftliche "Newsletter in Stereologie" mit Beginn der 70'er Jahre herauskam, gab es das ISS-Bulletin. Auszüge aus diesem "Newsletter-Vorläufer" zusammen mit einem Überblick über stereologische Bücher und Artikel im Journal of Microscopy schließen den historischen Teil des Newsletter '80 ab.

Sein zweiter Teil beginnt mit der "astereologischen" Analyse nichtflacher Oberflächen, einem Beitrag aus dem Gebiet der Bio-Wissenschaften, wird fortgesetzt mit einem Beitrag über stereologische Methoden in der Granulometrie und abgeschlossen mit der Anwendung der Stereologie in der werkstoffkundlichen Gefügeanalyse. Diese über die verschiedenen Wissensgebiete breitgestreute Auswahl von Themen soll den interdisziplinären Charakter des Newsletters unterstreichen. Da stereologische Arbeiten zwangsläufig in den Fachzeitschriften sehr unterschiedlicher Disziplinen erscheinen und erscheinen müssen, soll der Newsletter eine sammelnde Informationsquelle für alle Stereologie sein, die den Blick über die Grenzen des Fachgebietes in Sachen Stereologie erleichtert. Die Auswahl seiner Themen erfolgt bewußt nicht nach dem - traditionell vielfach vertraulichen - Gutachterprinzip herkömmlicher Zeitschriften. Kritiker der Originalbeiträge sind vielmehr aufgefordert, ihre Kommentare im Newsletter selbst zu veröffentlichen und damit zur wissenschaftlichen Diskussion beizutragen.

Am Schluß des Newsletters sind stereologische Veranstaltungen der nächsten Jahre angekündigt.

Der Herausgeber

PRESIDENT'S NOTE

Since its foundation in 1961, the International Society for Stereology has been successful in gathering scientists of various disciplines who ware interested in the evaluation of the morphology of microstructures. Three-dimensional thinking rather than overestimating two-dimensional information has been well established and a common nomenclature has been adopted due to the efforts of our Society. Instrumental techniques for sample preparation, for data collection, and for data transfer as well as methods of geometrical statistics and picture processing provided a broad basis for the application of stereology which now has become an indespensible tool in the investigation of biological tissues, of minerals, as well as of ceramic and metallic materials.

Taking over the duties as President of the Society for Stereologiy is, first of all, a great honour and opportunity. As a member of our Society I have watched the progress achieved by my predecessors. Due to their enthusiasm, this Society became a stable and at the same time dynamic organisation. The membership has increased steadily worldwide. Regular meetings and publications provide means of communication. Cooperation with other scientific organisations has become a prominent feature. Our Society is flourishing throughout.

Nevertheless, there are still open problems and unsettled tasks. It is obvious that only a relatively small portion of those working and interested in the field of stereology have joined the society, and it is also obvious that a much larger number of people could use stereology as a useful means for research or routine work. There is still a lack in general knowledge of the field due do limited information and education, and there is still a gap between theoretical stereology and its practical application. These facts are a great challenge for our Society, and the help of each member is needed to make significant progress.

The first half year of presidency has shown to me that, due to the help offered by many friends and collegues, this Society has an excellent chance to continue its success. For the years between the International Congresses, a number of scientific meetings sponsored by the International Society for Stereology will take place in Europe (with the Third European Symposium in Ljubljana in June 1981 as a main event) and, hopefully, also in the United States as well as in other parts of the world. Courses will be organized by members for the Society (three have been held recently and several are scheduled). The ISS Newsletters in Stereology and the Journal of Microscopy will serve to inform membership in all aspects of stereology. In addition to the Nomeclature Committee working successfully for a couple of years, Committees on Education, Geometrical Statistics, Literature, Picture Processing, and Society Rules have been founded with others to follow.

Our Society depends on the active cooperation of individual members. I invite all of you to take part in the Society's activities. There is plenty to do, let's do it now, and let's do it together.

Stuttgart, Summer 1980

Hans Eckart Exner

(Dr. H.E. Exner)

Courtesy of:

Zeitschrift für wissenschaftliche Mikroskopie und mikroskopische Technik

S. Hirzel Verlag Stuttgart

Band 65, Heft 3 (1963)

Gründung einer internationalen Gesellschaft für Stereologie

Bericht von G. Bach

Am 11. und 12. Mai 1961 fand auf Anregung und unter Vorsitz von Herrn Professor Dr. Hans ELIAS (Chicago Medical School) im Hotel Feldberger Hof (Schwarzwald) ein Kolloquium über das Thema "Räumliche Deutung zweidimensionaler Bilder und Schnitte" statt. Teilnehmer waren Angehörige der Disziplinen: Anatomie, Histologie, Pathologie, Neuropathologie, Ingenieurwissenschaften und Mathematik.

In seiner Eröffnungsansprache ging Herr ELIAS kurz auf die Gründe ein, die ihn zur Einberufung des Kolloquiums bewogen haben. Er betonte besonders die von ihm als Hochschullehrer gemachten Beobachtungen, die auf ein immer stärker werdendes Sehfaul-Werden der Studenten schließen lassen und folgerte daraus die Notwendigkeit einer sorgfältigeren Schulung der Fähigkeit zur räumlichen Deutung von Schnitten und Projektionsbildern. Da außerdem verwandte Fragestellungen in so verschiedenen Disziplinen wie Biologie, Histologie, Kristallographie, Mineralogie, Metallographie, Astronomie und Kosmologie auftauchen, bestehe in steigendem Maße das Bedürfnis nach einer interdisziplinären Zusammenarbeit, die gegenseitige Anregungen ermöglichen und Doppelbearbeitungen vermeiden helfen soll.

Nach der Eröffnungsansprache wurden von einigen Teilnehmern Kurzreferate mit folgenden. Themen gehalten:

Professor Dr. med. H. HASELMANN über: Zur räumlichen Wahrnehmung und Deutung mikroskopischer Bilder.

Privatdozent Dr. med. H. HAUG über: Probleme bei der exakten Zellzählung im histologischen. Schnitt.

Dr.-Ing. A. HENNIG über: Länge eines räumlichen Linienzuges.

Dipl.-Math. Dr. G. BACH über: Die Vernachlässigung der Schnittdicke histologischer Präparate als Ursache für Fehlauswertungen.

In der den Vorträgen folgenden Diskussion kam zur Sprache, daß es für den Praktiker oft wünschenswert ist, neben der mathematisch exakten Lösung eines Problems, grobe Abschätzungsformeln zu besitzen, die häufig elementar ableitbar und außerdem leichter anzuwenden sind.

Im Anschluß an weitere Aussprachen der Teilnehmer wurde der Beschluß gefaßt, eine Gesellschaft zu gründen *). Die Namensgebung stieß auf einige Schwierigkeiten, da eine Widerspiegelung des Hauptanliegens der Gesellschaft (Räumliche Deutung von Schnitten und Projektionsbildern) durch ein einziges Wort naturgemäß nicht ganz einfach sein konnte. Schließlich einigte man sich auf das Wort STEREOLOGIE (als Zusammensetzung aus $\sigma\tau\epsilon\rho\epsilon\delta s$ und $\lambda\delta\gamma\sigma s$).

Nach der notariellen Gründung der Gesellschaft ging die Tagung mit der Vorführung eines Lehrfilmes von Herrn ELIAS zu Ende. Der Film mit dem Titel "Schau in die Tiefe" zeigt in instruktiver Weise einige Methoden der Stereologie und beginnt mit Anwendungen aus der Astronomie (Formen unzugänglicher Objekte). Dann wird die Geometrie des Schneidens vorgeführt, d. h. die Abhängigkeit der Schnittform vom Schnittwinkel wird mit Anwendungen aus der Histologie erläutert.

Über die Methode der Rekonstruktion der räumlichen Verhältnisse durch Serienschnitte führt der Film zur Schätzung von Volumenverhältnissen und Ermittlung der räumlichen Gestalt komplizierter Gebilde aus Schnittbildern. Die Gefahr von Trugschlüssen wird aufgezeigt.

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INTERNATIONAL SOCIETY FOR STEREOLOGY (ISS)

SOCIÉTÉ INTERNATIONALE POUR LA STÉRÉOLOGIE

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STEREOLOGY

GENERAL INFORMATION ON GOALS AND STRUCTURE OF THE ISS

INTERNATIONAL SOCIETY FOR STEREOLOGY

OFFICERS

- PRESIDENT Hans Elias, Professor of Anatomy Chicago Medical School 710 S.Wolcott Ave., Chicago 12, Illinois
- VICE PRESIDENT Herbert Haug, Associate Professor Anatomisches Institut der Universität Krankenhausstr. 9, Erlangen, Germany
- SECRETARY E wald R. Weibel, Associate Professor Anatomisches Institut der Universität Gloriastr. 19, Zürich, Switzerland
- TREASURER Ervin E. Underwood, Staff Scientis Lockheed Missiles and Space Corporation Palo Alto, California
- ASSISTANT Werner Treff, Research Associate SECRETARY Institut für Hirnforschung Dennenbergstr. l - 5 Neustadt im Schwarzwald, Germany

OBJECTS OF THE ISS

The purpose of the ISS is to facilitate the exchange of ideas among persons of various scientific disciplines who are interested in problems of three-dimensional interpretation of two-dimensional images of solids (sections or projections), to stimulate, promote, and coordinate their efforts, and to demonstrate to a wider community of related research workers the usefulness of their methodology.

WHAT IS "STEREOLOGY" ?

Stereology has been defined as the science of spatially interpreting two-dimensional "samples" of three-dimensional structures. Stereology thus deals with a body of methods which facilitate the investigation of (three-dimensional) solid bodies, when only (two-dimensional) sections or projections of these bodies are amenable to direct observation. Such problems occur in practically all morphological sciences. To give only a few examples: the histologist, the mineralogist, the petrographer, or the metallurgist are interested in the study of the internal structure of a tissue, a rock, or a metal alloy. These materials are usually mixtures of a large number of small solid substructures whose shape or spatial relationship needs to be known. Since most of these materials are opaque an analysis is only possible on sections, which are studied either by incident or by transmitted light, and mostly with a microscope. This method of preparation, together with the uni-axial mode of observation, has eliminated one (the third) dimension from the object. This yields a considerable gain in lateral resolution, but means also, that the substructures, or their images on the sections, have lost their solid character. And evidently, a two-dimensional sample of a solid cannot represent all its spatial properties.

The methods of stereology are, hence, designed to make possible a "reconstruction" of the artificially eliminated third dimension, or, in more general terms, to allow an extrapolation from two-dimensional to three-dimensional relations.

It might be necessary to mention the difference between "stereology", where conclusions are based on uni-axial viewing of the object, and "stereoscopy", where the third dimension is preserved by parallaxial observation.

OUTLINE OF SOME STEREOLOGICAL METHODS

There are two basic propositions in stereology: One is the deduction of shape. As an example, elliptic section images can result from cutting either a cylinder, an ellipsoid, a paraboloid, or a cone etc. Stereological methods should provide the means of differentiating between these cases. It may also be necessary to estimate the genus of a complex topological figure; or to determine the spatial interconnection of an aggregate of structures, and so on.

The second proposition is that of quantitative analysis of aggregates of solid figures from two-dimensional sections. This is also named "modal analysis", "quantitative or stereometric metallography", or "morphometry" in some texts. Suitable methods enable the investigator to infer linear dimensions, surface areas, volumes, and numbers of solid figures from simple measurements obtained on sections or on slices of the material. This part of stereology attracts most interest today, so that some general aspects of quantitative stereological principles shall be mentioned.

Principles for Volumetric analysis.

Most quantitative stereological principles refer to aggregates of randomly distributed "granular" structures. If these granules occupy a fraction V of the volume, a random section of this aggregate (tissue, alloy, rock etc.) will be covered by transsections of granules over a fraction S = V, provided the section size is large enough. This fundamental principle of quantitative stereology was developed in 1847 by the French geologist Delesse. Up to the end of last century it was used to determine the volumetric composition of materials by planimetric measurement of surface fractions on sections.

In further extension of this principle it could then be shown that, if a line crosses the material at random a fraction $L_{L} = V_{V}$ of this line will pass through the granules; this principle formed the basis for extensive volumetric work done with so-called linear integrating stages in geology, metallurgy and biology. - Furthermore, if a number of points are randomly distributed in the material- or randomly placed on a section of it - a fraction $N_N = V_V$ of these points will lie in the granules. By applying this last principle the volumetric composition of any material can now be determined in very simple fashion by differential counting of the location of a set of test points which are placed at random on sections of the material.

Principles for estimating surface areas.

The probability by which a straight random probing line will intercept a surface convoluted in space is well definable. From this a simple principle has been derived which allows an estimate of the area of a surface in space from counting the number of intercepts of a random probing line with this surface. This is most conveniently done by placing the line on a random section of the material containing the surface.

The size of granules.

If an aggregate of spheres of varying size is sectioned at random the distribution of radii of the sphere sections will not correspond to the distribution of sphere radii. Various stereological principles present means by which the latter can be inferred from some statistical measures of the distribution of section radii.

These few examples should only give a general idea of the possibilities of stereological methods of which the majority have not been mentioned. Others deal with the precise recognition of shape or configuration, or with the counting of structures on sections, etc. In general it can be said that the application of stereological principles yields <u>objective</u> and often precise information on a three-dimensional material, and thus stands in contrast to conventional descriptive morphology, where observations made on sections are spatially interpreted through subjective judgements.

WHO IS INTERESTED IN STEREOLOGY ?

Problems of stereological character occur in very diverse fields. Three-dimensional structures are studied on sections by geologists, petrographers, mineralgists, metallurgists, and biological morphologists. Various types of projection images of solid figures are investigated by astronomers, crystallographers, electron microscopists etc. In each of these fields the task arises to derive information on the configuration and dimensions of solid bodies from observations made on two-dimensional samples, and hence stereological methods have often proven useful.

The theoretical problems connected with the development of precise stereological methods have aroused the interest of mathematicians of varying orientation, who have contributed greatly to the establishment of sound approaches to our problems.

HOW FAR ARE WE ADVANCED ?

The various disciplines have become aware of the potentials of stereological research methods at different times. The first to introduce this approach were the mineralogists, with first fundamental efforts made around the middle of last century. Up to recent decades most of the initial progress, although slow, has been made in the field of geology. But in recent years the stereological approach has also been introduced in other fields. It is now extensively used in metallurgy and is also making its way into biological morphology. Largely independently, each field has been able to solve some of the problems which appear of primary concern to it, but it is quite obvious that there still remains a large number to be worked out. And no doubt, in this process, great benefit can be derived from cooperation and coordination of efforts between the various fields.

The ISS was founded to provide a common forum for such cooperation. The first International Congress for Stereology, which was held in Vienna in April 1963 has vividly demonstrated that such cooperation is well possible across the boundaries of very different branches of science. The Proceedings of this Congress present a revealing cross section of the broad spectrum of interests in the ISS; they have been printed and can be purchased at a price of \$ 6.- (\$ 9.- for non-members) from the Buchhandlung für Medizinische Literatur, BRAUMAPHARM G.m.b.H., Kolingasse 19, Wien IX.

STRUCTURES OF THE ISS

MEANS OF SCIENTIFIC COMMUNICATION

The chief means of scientific communication of the ISS are the International Congresses for Stereology which are organized by the ISS. They usually take place every three years and bring together stereologists from the various fields for the purpose of direct discussion. The Second International Congress for Stereology is scheduled to be held in spring 1966 at the University of Florida in Gainesville, Florida (Chairmanelect: Dr. Frederick N. Rhines, Metallurgical Research Laboratory, University of Florida, Gainesville, Florida, USA).

The ISS is periodically publishing a bulletin STEREOLOGIA which is sent free of charge to all members. Non-members, institutions, libraries etc. can subscribe at an annual rate of \$ 2.50. STEREOLOGIA publishes apstracts of papers of stereological interest from the various fields. This should help to make these papers accessible to workers in other disciplines. New members are invited to send a brief summary of their work in the field of stereology to one of the editors. This summary is also published in STEREOLOGIA for the orientation and benefit of the other members. This custom has already helped to establish numerous contacts between stereologists with similar interests.

The ISS is also compiling a reprint library of stereological papers. These reprints will be available on loan to the members of the society.

MEMBERS

Every scientist who is interested in stereology is eligible to active membership by applying to one of the officers of the ISS. The current annual membership dues, payable to the Treasurer, are \$ 3.- . Additional membership categories are "honorary members" and "sponsoring members". The General Assembly of the members takes place triannually at the occasion of the International Congress for Stereology.

INTERNATIONAL SOCIETY FOR STEREOLOGY

ADDRESSES

- SECRETARIAT Dr. Ewald R. Weibel Anatomisches Institut der Universität Gloriastr. 19, Zürich, Switzerland
- TREASURER Dr. Ervin E. Underwood Materials Sciences Laboratory, Bldg. 201 Lockheed Missiles and Space Corporation 3251 Hanover Street, Palo Alto, Calif., USA
- EDITOR OF Dr. Robert T. DeHoff STEREOLOGIA Metallurgical Research Laboratory College of Engineering, University of Florida Gainesville, Florida, USA
- ASSISTANT Dr. Günther Bach EDITOR Am Lehrstuhl für Mathematik German language Technische Hochschule Oppelnstr. 28, Braunschweig, Germany

REGIONAL REPRESENTATIVES

| SPANISH | Dr. Fernando Marin - Giron, Instituto Cajal |
|-------------------|---|
| SPEAKING | C.S. Investigaciones Cientificas |
| COUNTRIES | Velasquez 138, Madrid 6, Spain |
| EASTERN EUROPE | Dr. Miklos Palkovits Department of Anatomy, Medical Academy Budapest, Hungary |

New address of the Treasurer?

Dr. Ervin E. Underwood Research Center Malerial Sciences Laboratory Department 72-14, Zena 400 Lockheed - Georgia Company Marietta, Georgia 30061, USA

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BY-LAWS

OF

INTERNATIONAL SOCIETY FOR STEREOLOGY

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ARTICLE I

Purposes

The purposes of the corporation as stated in its certificate of incorporation are

To establish, maintain, and operate a society for the purpose of exchanging ideas amongst persons of various scientific disciplines and countries who are interested in the science of Stereology.

Stereology is the science of three-dimensional (spatial) interpretation of two-dimensional (flat) images.

The Society proposes to promote three-dimensional (spatial) thinking in the younger generation, especially among those young people who study natural science.

The corporation also has such powers as are now or may hereafter be granted by the General Not For Profit Corporation Act of the State of Illinois.

ARTICLE II

Offices

The corporation shall have and continuously maintain in this state a registered office and a registered agent whose office is identical with such registered office, and may have other offices within or without the State of Illinois as the board of directors may from time to time determine.

ARTICLE III

Members

SECTION 1. CLASSES OF MEMBERS. The corporation shall havel class of members. The designation of such class and the qualifications of the members of such class shall be as follows:

Only a natural or juridical person who is willing to permit) three-dimensional thinking, be it by scientific investigation, teaching or education or by contributions, either financial or other kinds, can become a member of the society. An application for membership should be addressed to the secretary and to the treasurer of the society simultaneously. The application sent to the treasurer should be accompanied by a remittance to cover membership dues for one year. The secretary shall then notify all directors of the pending applications for membership. SECTION 2. ELECTION OF MEMBERS. Members shall be elected by the board of directors. An affirmative vote of two-thirds of the directors shall be required for election.

SECTION 3. VOTING RIGHTS. Each member shall be entitled to one vote on each matter submitted to a vote of the members.

SECTION 4. TERMINATION OF MEMBERSHIP. The board of directors by affirmative vote of two-thirds of all of the members of the board may suspend or expel a member for cause after an appropriate hearing, and may, by a majority vote of those present at any regularly constituted meeting, terminate the membership of any member who becomes ineligible for membership, or suspend or expel any member who shall be in default in the payment of dues for the period fixed in Article XII of these by-laws.

SECTION 5. RESIGNATION. Any member may resign by filing a written resignation with the Secretary, but such resignation shall not relieve the member so resigning of the obligation to pay any dues, assessments or other charges theretofore accrued and unpaid.

SECTION 6. REINSTATEMENT. Upon written request signed by a former member and filed with the Secretary, the board of directors may by the affirmative vote of two-thirds of the members of the board reinstate such former member to membership upon such terms as the board of directors may deem appropriate.

SECTION 7. TRANSFER OF MEMBERSHIP. Membership in this corporation is not transferable or assignable.

ARTICLE IV

Meetings of Members

SECTION 1. ANNUAL MEETING. An annual meeting of the members shall be held on the 9 th day of $U_{PA,iE}$ in each year, beginning with the year 1964, at the hour of 10:00 o'clock, U_{i} . M., for the purpose of electing directors and for the transaction of such other business as may come before the meeting. If such day be a Sunday or a legal holiday, the meeting shall be held at the same hour on the next succeeding business day. If the election of directors shall not be held on the day designated herein for any annual meeting, or at any adjournment thereof, the board of directors shall cause the election to be held at a special meeting of the members called as soon thereafter as conveniently may be.

SECTION 2. SPECIAL MEETING. Special meetings of the members may be called either by the president, the board of directors, or not less than one-tenth of the members having voting rights.

SECTION 3. PLACE OF MEETING. The board of directors may designate any place, either within or without the State of Illinois, as the place of meeting for any annual meeting or for any special meeting called by the board of directors. If no designation is made or if a special meeting be otherwise called, the place of meeting shall be the registered office of the corporation in the State of Illinois, provided, however, that if all of the members shall meet at any time and place, either within or without the State of Illinois, and consent to the holding of a meeting, such meeting shall be valid without call or notice, and at such meeting any corporate action may be taken.

SECTION 4. NOTICE OF MEETINGS. Written or printed notice stating the place, day and hour of any meeting of members shall be delivered, either personally or by mail, to each member entitled to vote at such meeting, not less than five nor more than forty days before the date of such meeting, by or at the direction of the president, or the secretary, or the officers or persons calling the meeting. In case of a special meeting or when required by statute or by these by-laws, the purpose for which the meeting is called shall be stated in the notice. If mailed, the notice of a meeting shall be deemed delivered when deposited in the United States mail addressed to the member at his address as it appears on the records of the corporation, with postage thereon prepaid.

SECTION 5. INFORMAL ACTION BY MEMBERS. Any action required to be taken at a meeting of the members of the corporation, or any other action which may be taken at a meeting of members, may be taken without a meeting if a consent in writing, setting forth the action so taken, shall be signed by all of the members entitled to vote with respect to the subject matter thereof.

SECTION 6. QUORUM. The members holding one-tenth of the votes which may be cast at any meeting shall constitute a quorum at such meeting. If a quorum is not present at any meeting of members, a majority of the members present may adjourn the meeting from time to time without further notice.

SECTION 7. PROXIES. At any meeting of members, a member entitled to vote may vote either in person or by proxy executed in writing by the member or by his duly authorized attorney-in-fact. No proxy shall be valid after eleven months from the date of its execution unless otherwise provided in the proxy.

ARTICLE V

Board of Directors

SECTION 1. GENERAL POWERS. The affairs of the corporation shall be managed by its board of directors.

SECTION 2. NUMBER, TENURE AND QUALIFICATIONS. The number of directors shall be three . Each director shall hold office until the next annual meeting of members and until his successors shall have been elected and qualified. Directors need not be residents of Illinois or members of the corporation.

SECTION 3. REGULAR MEETINGS. A regular annual meeting of the board of directors shall be held without other notice than this by-law,

immediately after, and at the same place as, the annual meeting of members. The board of directors may provide by resolution the time and place, either within or without the State of Illinois, for the holding of additional regular meetings of the board without other notice than such resolution.

SECTION 4. SPECIAL MEETINGS. Special meetings of the board of directors may be called by or at the request of the president or any two directors. The person or persons authorized to call special meetings of the board may fix any place, either within or without the State of Illinois, as the place for holding any special meeting of the board called by them.

SECTION 5. NOTICE. Notice of any special meeting of the board of directors shall be given at least two days previously thereto by written notice delivered personally or sent by mail or telegram to each director at his address as shown by the records of the corpo-If mailed, such notice shall be deemed to be delivered when ration. deposited in the United States mail in a sealed envelope so addressed, with postage thereon prepaid. If notice be given by telegram, such notice shall be deemed to be delivered when the telegram is delivered to the telegraph company. Any director may waive notice of any The attendance of a director at any meeting shall constitute meeting. a waiver of notice of such meeting, except where a director attends a meeting for the express purpose of objecting to the transaction of any business because the meeting is not lawfully called or convened. Neither the business to be transacted at, nor the purpose of, any regular or special meeting of the board need be specified in the notice or waiver of notice of such meeting, unless specifically required by law or by these by-laws.

SECTION 6. QUORUM. A majority of the board of directors shall constitute a quorum for the transaction of business at any meeting of the board, provided, that if less than a majority of the directors are present at said meeting, a majority of the directors present may adjourn the meeting from time to time without further notice.

SECTION 7. MANNER OF ACTING. The act of a majority of the directors present at a meeting at which a quorum is present shall be the act of the board of directors, except where otherwise provided by law or by these by-laws.

SECTION 8. VACANCIES. Any vacancy occurring in the board of directors or any directorship to be filled by reason of an increase in the number of directors, shall be filled by the board of directors. A director elected to fill a vacancy shall be elected for the unexpired term of his predecessor in office.

SECTION 9. COMPENSATION. Directors as such shall not receive any stated salaries for their services, but by resolution of the board of directors, a fixed sum and expenses of attendance, if any, may be allowed for attendance at each regular or special meeting of the board; provided, that nothing herein contained shall be construed to preclude any director from serving the corporation in any other capacity and receiving compensation therefor.

ARTICLE VI

Officers

SECTION 1. OFFICERS. The officers of the corporation shall be a president, one or more vice presidents (the number thereof to be determined by the board of directors), a treasurer, a secretary and such other officers as may be elected in accordance with the provisions of this article. The board of directors may elect or appoint such other officers, including one or more assistant secretaries and one or more assistant treasurers, as it shall deem desirable, such officers to have the authority and perform the duties prescribed, from $t \neq m_c$ to time, by the board of directors. Any two or more offices may be held by the same person, except the offices of president and secretary.

SECTION 2. ELECTION AND TERM OF OFFICE. The officers of the corporation shall be elected annually by the board of directors at the regular annual meeting of the board of directors. If the election of officers shall not be held at such meeting, such election shall be held as soon thereafter as conveniently may be. Vacancies may be filled or new offices created and filled at any meeting of the board of directors. Each officer shall hold office until his successor shall have been duly elected and shall have qualified.

SECTION 3. REMOVAL. Any officer or agent elected or appointed by the board of directors may be removed by the board of directors whenever in its judgment the best interests of the corporation would be served thereby, but such removal shall be without prejudice to the contract rights, if any, of the person so removed.

SECTION 4. VACANCIES. A vacancy in any office because of death, resignation, removal, disqualification or otherwise, may be filled by the board of directors for the unexpired portion of the term.

SECTION 5. PRESIDENT. The president shall be the principal executive officer of the corporation and shall in general supervise and control all of the business and affairs of the corporation. He shall preside at all meetings of the members and of the board of directors. He may sign, with the secretary or any other proper officer of the corporation authorized by the board of directors, any deeds, mortgages, bonds, contracts, or other instruments which the board of directors have authorized to be executed, except in cases where the signing and execution thereof shall be expressly delegated by the board of directors or by these by-laws or by statute to some other officer or agent of the corporation; and in general shall perform all duties incident to the office of president and such other duties as may be prescribed by the board of directors from time to time.

SECTION 6. VICE PRESIDENT. In the absence of the president or in the event of his inability or refusal to act, the vice president (or in the event there be more than one vice president, the vice presidents, in the order designated, or in the absence of any designation, then in the order of their election) shall perform the duties of the president, and when so acting, shall have all the powers of and be subject to all the restrictions upon the president. Any vice president shall perform such other duties as from time to time may be assigned to him by the president or by the board of directors.

SECTION 7. TREASURER. If required by the board of directors, the treasurer shall give a bond for the faithful discharge of his duties in such sum and with such surety or sureties as the board of directors shall determine. He shall have charge and custody of and be responsible for all funds and securities of the corporation; receive and give receipts for moneys due and payable to the corporation from any source whatsoever, and deposit all such moneys in the name of the corporation in such banks, trust companies or other depositaries as shall be selected in accordance with the provisions of Article VIII of these by-laws; and in general perform all the duties incident to the office of treasurer and such other duties as from time to time may be assigned to him by the president or by the board of directors.

SECTION 8. SECRETARY. The secretary shall keep the minutes of the meetings of the members and of the board of directors in one or more books provided for that purpose; see that all notices are duly given in accordance with the provisions of these by-laws or as required by law; be custodian of the corporate records and of the seal of the corporation and see that the seal of the corporation is affixed to all documents, the execution of which on behalf of the corporation under its seal is duly authorized in accordance with the provisions of these by-laws; keep a register of the postoffice address of each member which shall be furnished to the secretary by such member; and in general perform all duties incident to the office of secretary and such other duties as from time to time may be assigned to him by the president or by the board of directors.

SECTION 9. ASSISTANT TREASURERS AND ASSISTANT SECRETARIES. If required by the board of directors, the assistant treasurers shall give bonds for the faithful discharge of their duties in such sums and with such sureties as the board of directors shall determine. The assistant treasurers and assistant secretaries, in general, shall perform such duties as shall be assigned to them by the treasurer or the secretary or by the president or the board of directors.

ARTICLE VII

Committees

SECTION 1. COMMITTEES OF DIRECTORS. The board of directors, by resolution adopted by a majority of the directors in office, may designate one or more committees, each of which shall consist of two or more directors, which committees, to the extent provided in said resolution, shall have and exercise the authority of the board of directors in the management of the corporation; but the designation of such committees and the delegation thereto of authority shall not operate to relieve the board of directors, or any individual director, of any responsibility imposed upon it or him by law.

SECTION 2. OTHER COMMITTEES. Other committees not having and exercising the authority of the board of directors in the management of the corporation may be designated by a resolution adopted by a majority of the directors present at a meeting at which a quorum is present. Except as otherwise provided in such resolution, members of each such committee shall be members of the corporation, and the president of the corporation shall appoint the members thereof. Any member thereof may be removed by the person or persons authorized to appoint such member whenever in their judgment the best interests of the corporation shall be served by such removal.

SECTION 3. TERM OF OFFICE. Each member of a committee shall continue as such until the next annual meeting of the members of the corporation and until his successor is appointed, unless the committee shall be sooner terminated, or unless such member be removed from succh committee, or unless such member shall cease to qualify as a member thereof.

SECTION 4. CHAIRMAN. One member of each committee shall be appointed chairman.

SECTION 5. VACANCIES. Vacancies in the membership of any committee may be filled by appointments made in the same manner as provided in the case of the original appointments.

SECTION 6. QUORUM. Unless otherwise provided in the resolution of the board of directors designating a committee, a majority of the whole committee shall constitute a quorum and the act of a majority of the members present at a meeting at which a quorum is present shall be the act of the committee.

SECTION 7. RULES. Each committee may adopt rules for its own government not inconsistent with these by-laws or with rules adopted by the board of directors.

ARTICLE VIII

Contracts, Checks, Deposits and Funds

SECTION 1. CONTRACTS. The board of directors may authorize any officer or officers, agent or agents of the corporation, in addition to the officers so authorized by these by-laws, to enter into any contract or execute and deliver any instrument in the name of and on bchalf of the corporation and such authority may be general or confined to specific instances.

SECTION 2. CHECKS, DRAFTS, ETC. All checks, drafts or other orders for the payment of money, notes or other evidences of indebtedness issued in the name of the corporation, shall be signed by such officer or officers, agent or agents of the corporation and in such manner as shall from time to time be determined by resolution of the board of directors. In the absence of such determination by the board of directors, such instruments shall be signed by the treasurer or an assistant treasurer and countersigned by the president or a vice president of the corporation. SECTION 3. DEPOSITS. All funds of the corporation shall be deposited from time to time to the credit of the corporation in such banks, trust companies or other depositaries as the board of directors may select.

SECTION 4. GIFTS. The board of directors may accept on behalf of the corporation any contribution, gift, bequest or devise for the general purposes or for any special purpose of the corporation.

ARTICLE IX

Certificates of Membership

SECTION 1. CERTIFICATES OF MEMBERSHIP. The board of directors may provide for the issuance of certificates evidencing membership in the corporation which shall be in such form as may be determined by the board. Such certificates shall be signed by the president or a vice president and by the secretary or an assistant secretary and shall be sealed with the seal of the corporation. All certificates evidencing membership of any class shall be consecutively numbered. The name and address of each member and the date of issuance of the certificate shall be entered on the records of the corporation. If any certificate shall become lost, mutilated or destroyed a new certificate may be issued therefor upon such terms and conditions as the board of directors may determine.

SECTION 2. ISSUANCE OF CERTIFICATES. When a member has been elected to membership and has paid any initiation fee and dues that may then be required, a certificate of membership shall be issued in his name and delivered to him by the secretary, if the board of directors shall have provided for the issuance of certificates of membership under the provisions of Section 1 of this article.

ARTICLE X

Books and Records

The corporation shall keep correct and complete books and records of account and shall also keep minutes of the proceedings of its members, board of directors and committees having any of the authority of the board of directors, and shall keep at the registered or principal office a record giving the names and addresses of the members entitled to vote. All books and records of the corporation may be inspected by any member, or his agent or attorney for any proper purpose at any reasonable time.

ARTICLE XI

Fiscal Year

The fiscal year of the corporation shall begin on the first day of January and end on the last day of December in each year.

ARTICLE XII

Dues

SECTION 1. ANNUAL DUES. The board of directors may determine from time to time the amount of initiation fee, if any, and annual dues payable to the corporation by members of each class.

SECTION 2. PAYMENT OF DUES. Dues shall be payable in advance on the first day of January in each year. Dues of a new member shall be prorated from the first day of the month in which such new member is elected to membership, for the remainder of the fiscal year of the corporation.

SECTION 3. DEFAULT AND TERMINATION OF MEMBERSHIP. When any member of any class shall be in default in the payment of dues for a period of twenty-four months from the beginning of the period for which such dues became payable, his membership may thereupon be terminated by the board of directors in the manner provided in Article III of these by-laws.

ARTICLE XIII

Seal

The board of directors shall provide a corporate seal which shall be in the form of a circle and shall have inscribed thereon the name of the corporation and the words "Corporate Seal, Illinois."

ARTICLE XIV

Waiver of Notice

Whenever any notice whatever is required to be given under the provisions of the General Not For Profit Corporation Act of Illinois or under the provisions of the articles of incorporation or the bylaws of the corporation, a waiver thereof in writing signed by the person or persons entitled to such notice, whether before or after the time stated therein, shall be deemed equivalent to the giving of such notice.

ARTICLE XV

Amendments to By-Laws

These by-laws may be altered, amended or repealed and new by-laws may be adopted by a majority of the directors present at any regular meeting or at any special meeting, provided that at least two days' written notice is given of intention to alter, amend or repeal or to adopt new by-laws at such meeting.

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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

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Dr. Francisco Abadia Fenoll Valle de Hebron 89-9-1 Barcelona, Spain

Professor Pedro Amat Muñoz Avendia Fernando el Católico, 66-1º—izq. Zaragoza, Spain

Dr. Günter Bach (Secretary)

Mathematisches Institut Technische Hochschule Braunschweig Braunschweig, Germany Mrs. Barbara G. Barnes

Mrs. Barbara G. Barnes 22 Grantchester Road Cambridge, England

Dr. Felix D. Bertalanffy Department of Anatomy Medical College University of Manitoba Winnipeg 3, Manitoba, Canada

Mr. Paolo Bianchi Instituto de Patologia della Università de Bari Bari, Italy

,

Profesor Adjunto de Anatomia

Pathological anatomy

Neuroendocrinology

Mathematics

Cancer research, electron microscopy

Associate Professor of Anatomy

Pathology

Dr. Franz Bierring Medicinsk-Anatomisk Institut Norre Alle Kopenhagen, Denmark

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Dr. Josef Bugl Battelle Memorial Institute 505 King Avenue Columbus 1, Ohio, U.S.A.

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Dr. Felix Chayes Geophysical Laboratory 2801 Upton Street, N.W. Washington 8, D.C., U.S.A. Anatomy, electron misroscopy, and surgery

Professor of Pathology

Physical chemistry

Mineralogy, reology

Quantitative metallography, transformations and thermodynamics of interfaces

Petrography

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Dr. Morris Cohen Massachusetts Institute of Technology Cambridge, Massachusetts, U.S.A.

Dr. Paolo Contu Faculty of Medicine Institute of Anatomy Porto Alegre, GRS, Brazil

Professor Ronald DeFord University St., Box 7652 Austin 12, Texas, U.S.A.

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Dr. Wihelm Harkmark Department of Anatomy University of Oslo Oslo, Norway Mətallurgy, in particular wəlding, mətallography

Physical Metallurgist

Professor of Metallurgy

Anatomy

Geology

Assistant Research Professor

Imperfections in crystals

Professor of Anatomy

Pre-med student at Stanford University

Modal rock analysis

Metallography

Physical metallurgy

Histology and physiology

Anatomy

Anatomy

X-ray crystallography, metal physics

Associate Professor, Physical Metallurgy

Anatomy and pathology

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Metallurgy, eutectic structures

Mathematics and anatomy

Physical metallurgy, quantitative metallography

General medical practice

Metallography, corrosion

Anatomy

Assistant Professor of Biology

Electro-optical circuits

Chemist, petrographer

Sterophotomi crography

Anatomy

X-ray and electron diffraction

Anatomy

Physical metallurgy, quantitative metallography

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Zoology

Professor of Anatomy

Histology

- 29 -

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Mathematics

Anatomy

Metal physics, crystallography

Metals joining, fracture

Astronomy

Quantitative metallography

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Pathology

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Histologic and cytologic topology

Kinetics and morphology of metallurgical transformations

Professor of Anatomy



THE BULLETIN OF THE INTERNATIONAL SOCIETY FOR STEREOLOGY

Editors

Dr. Ervin E. Underwood Research Metallurgist Battelle Memorial Institute Columbus 1, Ohio, U.S.A.

Dr. Günter Bach Mathematisches Institut Technische Hochschule Braunschweig Braunschweig, Germany



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, <u>Stereologia</u>, 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 <u>Stereologia</u>, 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 angioarchitectonics – 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 charakteristisschen 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 selfmade 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 pointmethod. 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).

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

- "Quantitative Untersuchungen an der Sehrinde", G. Thieme, Stuttgart 1958.
- "Die Treffermethode, ein Verfahren zur quantitativen Analyse im histologischen Schnitt", Z. Anat. 118, 302-312 (1955).
- "Remarks on the determination and significance of the gray cell coefficient", J. Comp. Neur. 104, 473-492 (1956).
- "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. - Iam 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 Mitteilungenüber 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 INTER-CONNECTED 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 attemperatures 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 myco-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).

- 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.
- R. T. DeHoff, "Determination of the Size Distribution of Ellipsoidal Particles from Measurements Made on Random Plane Sections", Accepted for publication, Trans. AIME, (1962).
- 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 INTERNATIONAL SOCIETY FOR STEREOLOGY June 18, 1962

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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 sketch 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

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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 Be0: 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 Be0 and other wurtzite compounds, 1962, with Carl F. Cline, in press.

QUANTITATIVE ANALYSIS OF SERIAL SECTIONS FOR STUDY OF PATTERN FORMATION IN THE CENTRAL NERV-OUS 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 Botcrenbrood 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 central 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 con-

figuration.

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.

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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

- 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 kikueszoeboeleeseenek 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.

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

A few years 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

Volume 2, Number 2. December, 1963

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 desquamate 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 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°) 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.

- BERTALANFFY, F. D., "Cell Renewal in the Gastointestinal Tract of Man." <u>Gastroenter-</u> <u>ology</u>, <u>43</u> (1962) 472-475.
- BERTALANFFY, F. D., "Cell Renewal as the Basis of Diagnostic Exfoliative Cytology." <u>Amer. J. Obst. Gyn.</u>, <u>85</u> (1963) 383-396.
- BERTALANFFY, F. D., and LAU, C., "Cell Renewal." Int. Rev. Cytology, 13 (1962) 357-366.

BERTALANFFY, F. D., and NAGY, K. P., "Mitotic Activity and Renewal Rate of the Epithelial Cells of Human Duodenum." <u>Acta</u> <u>anat.</u>, <u>45</u> (1961) 362-370.

CHODOSH, S., ZACCHEO, C. W., and SEGAL, M. S., "The Cytology and Histochemistry of Sputum Cells." Amer. Rev. Resp. Dis., 85 (1962) 635-648.

LEBLOND, C. P., and WALKER, B. E., "Renewal of Cell Populations." Physiol. Rev., 36 (1956) 255-276.

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, shadcw 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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- KOH, P. K., and DUNN, C. G.: "Primary Recrystallization Textures in Cold Rolled Si-Fe Crystals", Trans. AIME 206, (1956) 1017 and J. Metals (1956)
- 3. KOH, P. K. and DUNN, C. G.: "Simple Orientation Relationships for Secondary Recrystallization in Si-Fe", <u>Trans.</u> <u>AIME</u>, 209, (1957) 81 "Information on 'Nuclei' for Secondary Recrystallization in Si-Fe" <u>Trans. AIME</u> <u>212</u>, (1958) 80.
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- 5. KOH, P. K.: "Grain Growth in Silicon Iron", Trans. Met. Society AIME, 215, (1959) 1043.
- 6. KOH, P. K.: "Orientation Study of Ultra-Thin Molybdenum Permalloy Tape", <u>J.</u> <u>Applied Physics</u>, <u>29</u>, (1958) 636.
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- KOH, P. K.: "Cube Texture in Ultra-Thin Molybdenum Permalloy Tape", <u>Trans. Met.</u> <u>Society AIME</u>, 221 (1961) 50, and <u>J.</u> <u>Applied Physics</u>, <u>32</u>, (1961) 358.

QUANTITATIVE RESEARCH ON THE STRUCTURE OF THE BRAIN

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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 ob-tained 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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- JUNGKLAAβ, F. K., U. H. ORTHNER 1960: Uber quantitative Beziehungen im Stammhirn. Vorbericht. Dtsch.Zschr. für Nervenheilkunde <u>181</u>, 62-70.

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 timeconsuming 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
 - (b) in terms of spherical polar angles from which a rapid plot of the surface normals can be made.

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The following preprints have been received by the Lending Library, and are available to the members on request. Where possible, the Journal in which the article is published is included.

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- LOEB, A. L., "A Binary Algebra Describing Crystal Structure with Closely Packed Anions", <u>Acta. Cryst. 11</u> (1958) 469.

- 3. LOEB, A. L., and MORRIS, I. L., "A Binary Algebra Describing Crystal Structures with Closely Packed Anions. Part II: A Common System of Reference for Cubic and Hexagonal Structures", <u>Acta. Cryst., 13</u> (1960) 434.
- 4. MOORE, Geo. A., "Direct Quantitative Analysis of Microstructures by a Digital Computer", <u>NBA Report 8101</u>, (1963).
- 5. MOORE, Geo. A., "Survey of Factors Controlling the Desing of Automatic Systems for the Quantitative Analysis of Micrographs", <u>NBA</u> Report 8073, (1963).
- YUE, A. S., "Microstructure of Magnesium-Aluminum Eutectic", <u>Trans. of AIME 1010</u>, (1962) 224.

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-CuAl₂ 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 eufectic 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 solidliquid 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

Volume 5, Number 2, August, 1966

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. STEREO-LOGIA 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

6. 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. <u>Points</u>, 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, A line, when intercepted by a plane, yields a point. Fibers, fibrils, and fila-ments 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 \propto . 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 dia-phragm 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 micro-tome 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 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 super-ficial inspection, the long lines appear more numerous than they are because each occupies more space than several dots to-gether. 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. <u>Solids of various shapes</u>. Fortunately, most organs are con-structed of components of similar, threedimensional 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 <u>length</u> 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 <u>may</u> 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 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 = cosec α) (Fig. 7).





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 $\cot \alpha$ (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 (eggshaped) 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.



<u>Figure 8.</u> 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 base-

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.

Review of Books on Stereology

This review includes the most important books in the field of stereology. Forthcoming reviews will list recent stereological papers. Authors and readers are asked to help to compile these lists. Please send all information to Dr. Ondracek, Kernforschungszentrum, P.O.Box 3640, D-7500 Karlsruhe, Federal Republic of Germany

- 1. <u>Proceeding of International Congresses</u> of the International Society for Stereology (ICS).
- 1.1. First ICS, Vienna 1963. Reprint of ISS 1979, (US\$ 16,- or DM 35,- including postage. Contact Dr. H. Haug, Inst. f. Anatomie, Med. Hochschule, D-2400 Lübeck)
- 1.2. Second ICS, Chicago 1967. Ed. H. Elias. Springer Verlag, Berlin-Heidelberg-New York 1967 (contact Dr. H. Elias, 463 Marietta Drive, San Francisco, CA 94127, USA).
- 1.3. Third ICS, Bern 1971. Stereology 3. Eds. E. R. Weibel, Meek, Ralph, Echlin and R. Ross. Reprinted from Journal of Microscopy vol. 95, parts 1 and 2, 1972. Blackwell Scientific Publications, Oxford.
- 1.4. Fourth ICS, Gaitherburg, 1975. Eds. E. E. Underwood, R. de Wit and G. A. Moore. NBS Special Publication 431, US Government Printing Office, Washington 1976 (US\$ 15,or DM 27,- including postage. Contact Dr. H. E. Exner, Max-Planck-Institut, Seestr. 92, D-7000 Stuttgart 1, Federal Republic of Germany
- 1.5. Fifth ICS Salzburg 1979. To be published summer 1980. (Contact Dr. G. Bernroider, Zoologisches Institut, Akademiestr. 26, A-5020 Salzburg, Austria).
- 1.6. Stereology 4, Selected papers from the Fourth International Congress for Stereology, Journal of Microscopy vol 107, part 3, 1976. Blackwell Scientific Publications, Oxford.
- 1.7. Stereology 5. Selected papers from the Fifth International Congress for Stereology, Salzburg 1979. Journal of Microscopy, Blackwell Scientific Publishers, Oxford, in preparation. (Contact Dr. H. J. Gundersen, Aarhus Kommunehospital, Second University Clinic of Internal Medicine, DK-8000 Aarhus C, Denmark).

- 2. <u>Proceedings of European Symposia of the International</u> Society for Stereology (ESS)
- 2.1. First ESS, Leoben, 1974. Quantitative Analysis of Microstructures in Medicine, Biology and Material Development. Ed. H. E. Exner. Special Issue of Practical Metallography 5, Dr. Riederer-Verlag GmbH; Stuttgart 1975 (DM 45,-- plus postage, contact Dr. H. E. Exner).
- 2.2. Second ESS, Caen 1977. Quantitative Analysis of Microstructures in Biology, Material Sciences and Medicine. Ed. by J. L. Chermant. Special Issue of Practical Metallography 8, Dr. Riederer-Verlag GmbH: Stuttgart 1978. (DM 70,-- plus postage, contact Dr. H. E. Exner or Dr. J. L. Chermant).
- 3. <u>Proceedings of Other Events Organised by the Inter-</u> national Society for Stereology
- 3.1. Quantitative Methods in Stereology, Proceedings of a Symposium in Wiesbaden, Germany, 1965.Eds. E. R. Weibel and H. Elias, Springer-Verlag, Berlin-Heidelberg-New York 1967 (contact Dr. H. Elias).
- 3.2. Geometrical Probability and Biological Structures: Buffon's 200th Anniversary. R. E. Miles & J. Serra (Eds.) Lecture Notes in Biomathematics, No. 23, Springer-Verlag, Berlin, 1978. (Contact Dr. J. Serra, Centre de Geostatistique et de Morphology Mathematique, 35 rue de St. Honore, F-77305 Fontainebleau, France).
- 4. <u>Newsletters of the International Society for Stereo-</u> logy
- 4.1. Stereological Newsletter 1971A Bibliography for Stereology by H. E. Exner andH. F. Fischmeister (out of print, for Xerox-copies contact Dr. H. E. Exner)
- 4.2. Newsletter 1972 in Stereology : Quantitative Image Analysis by Electronic Image Analysers.
 Ed. G. Ondracek. KFK-Ext. 6/71-3.

- 4.3. Newsletter '73 in Stereology (1973). Ed. G. Ondracek, KfK-Ext. 6/73-2.
- 4.4. Newsletter '74 in Stereology (1974). Ed. G. Ondracek, KfK-Ext. 6/74-4.
- 4.5. Newsletter '75 in Stereology (1975). Ed. G. Ondracek, KfK-Ext. 6/75-3.
- 4.6. Newsletter '76 in Stereology (1976). Ed. G. Ondracek, KfK-Ext. 6/76-6.
- 4.7. Newsletter '77 in Stereology (1977). Ed. G. Ondracek, KfK-Ext. 6/77-3.
- 4.8. Newsletter '78/'79 in Stereology (1978) Ed. G. Ondracek, KfK 2769 B
- 4.9. Newsletter '80 in Stereology (1980). Ed. G. Ondracek, this volume.

For copies of Newsletters in Stereology contact KfK Library, Kernforschungszentrum Karlsruhe, P.O. Box 3640, D-7500 Karlsruhe,Literature Department,Federal Republic of Germany

- 5. <u>Other Books and Proceedings</u> (contact the publisher or your book dealer. The ISS has no further information on these books).
- 5.1. S. A. Saltykov: Stereometric Metallography
 - a: Russian Original, Third Edition, Metallurgisdat Moscow 1970.
 - b: German Edition, Ed.: H. J. Eckstein, VEB Deutscher Verlag für Grundstoffindustrie, Leipzig 1974.
 - c: English Translation of the Second Edition. Report MCL-905/1+2, 1961. MCLTD, Wright Patterson Air Force Base, Ohio, USA.
- 5.2. Quantitative Microscopy. Eds.: R. T. De Hoff and F. N. Rhines, McGraw-Hill, New York. 1968.
- 5.3. Underwood, E. E.: Quantitative Stereology. Addison-Wesley, Massachusetts, USA. 1970.
- 5.4. Special Issue 1 of Practical Metallography, Proceedings of the Third Quantimet Colloquium 1970, Dr. Riederer-Verlag, Stuttgart. Eds.: G. Petzow and H. E. Exner.

- 5.5. Advances in Quantitative Image Analysis (contact Cambridge Instruments Co., GmbH, D-4600 Dortmund, Harnackstr.35-43, Federal Republic of Germany
 - a: Imanco-Symposium, Frankfurt 1973.
 - b: Imanco-Symposium, Frankfurt, 1975.
- 5.6. Stereology and Quantitative Metallography, Eds.: G. E. Pellissier and S. M. Purdy, American Society for Testing and Materials, STP 504, Philadelphia 1972.
- 5.7. Special Image Analysis Issues of The Microscope (contact Editor, 2 Mc Crone Mews, Belsize Lane, London NW3 5BG, England)
 - a: Volume 16 No. 2, 1968.
 - b: Volume 19 No. 1, 1971.
 - c: Volume 22 No. 1, 1974.
- 5.8. Microstructural Analysis, Eds.; J. L. McCall and W. M. Mueller, Plenum Press, New York 1973.
- 5.9. Proceedings of the Colloquium on the Applications of Stereology and Computing in Earth Sciences, Metallography and Biological Research, Vysoké Tatry, Czecho slovakia, 1976. (Contact Dr. G. Timcák, Kosiče Tech. Univ., Kosiče, CSSR, or J. -L. Chermant, Univ. of Caen, F-14032, Caen, CEDEX, France.)
- 5.10. W. Rostoker and J. R. Dvorak; Interpretation of Metallographic Microstructures, Academic Press, New York-San Francisco-London, 1977.
- 5.11. J. L. Binet; La Cytologic Quantitative. Presses Universitaires de France, 1977 (contact Prof. J. L. Binet, Fac. Pitié-Salpêtière,91 Boulevard de L'Hôspital, F-75013 Paris, France).
- 5.13. Proceedings of an International Symposium on Quantitative Metallography in Florence, 1978. AIM, Milano 1978 (contact Associazione Italiana di Metallurgia, Piazzale R. Morandi, 1-20121 Milano, Italy).
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Stereological Methods

Volume 1 Practical Methods for Biological Morphometry



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In microscopical research the use of quantitative methods — the morphometric approach — is still not very popular. This is largely because the microscopist shies away from the relatively new stereological methods, which are mathematical in nature. The aim of this book is to show that these methods are in fact simple to use, and that their mathematical foundations can be understood even with limited knowledge of mathematics.

This first volume deals with the practical methods in stereology, which are introduced at an elementary level. The rules for both sampling and performing the measurements on sections, together with the subsequent calculations, are presented and discussed in great detail, ending in precise formulations of instructions and guide lines for practical applications of these methods. A number of specific examples, written by experienced investigators, concludes the volume.

The second volume will deal with the theoretical foundations of stereological principles in detail to provide the practician with a sound basis to enable him to design his own analytical schemes.

This book, uniquely comprehensive in the field of stereological methods for biologists, is both a reference work and an introduction to this important subject. It will be invaluable to biologists, medical researchers, anatomists, histologists, pathologists, electron microscopists, cell biologists, pharmacologists, physiologists, histochemists, and many other researchers in the materials sciences.

All prices, except in the UK, are in US dollars and are subject to change without notice.

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The JOURNAL OF MICROSCOPY is the Royal Microscopical Society's principal publication. It is an international journal with a distinguished editorial board; its scope is wide, covering all branches of microscopy and related sciences, with particular interest in the optical, mechanical and electronic features of design of all types of microscopes and accessories. In 1969 the JOURNAL also became the official journal of the International Society for Stereology. The quantitative dimensional analysis of solid objects from information in two dimensions obtained by studying sections has long been of great importance to microscopists. The International Society for Stereology was founded in 1961 to bring together scientists with a common interest in stereological methods from such diverse fields as mineralogy, metallurgy, materials science, biology and medicine. Their papers appear regularly in the JOURNAL OF MICROSCOPY.

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"ASTEREOLOGICAL" ANALYSIS OF UNFLAT SURFACES⁺

Miroslav Kališnik¹, Olga Vraspir-Porenta², Janez Šuštaršič³, Kristijan Jezernik⁴, Nada Pipan⁵, Marija Us-Krašovec⁶

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> "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."

Hans Elias, Honorary President, ISS

⁺Revised edition of a poster, presented at the 5th international congress for stereology, Salzburg, 3. - 8. September 1979.

Responsible for the methodological part¹ or the examples at macroscopic³, microscopic^{2,6} and electron microscopic^{4,5} levels.

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0. Summary

In some circumstances, merely unflat surfaces represent an easily accessible or interesting object for morphometric analysis. Projections of such surfaces on flat test areas can be quantitatively analyzed using modified stereological methods, but giving up a three-dimensional interpretation. Since the latter was essential for coining the term stereology, a neologism "astereology" has been proposed, meaning the areal (two-dimensional) analysis of the surface projections.

The article presents a coherent system of astereological equations for the estimation of areas, boundaries and numbers of structures, in both implicit and explicit forms. Next, formulas are given for estimating the relative and absolute values, with the indirect and direct method of determination for the latter.

Objects suitable for astereological analysis could be found in the life sciences as well as in the material sciences. Three practical examples are given from the biomedical field, from the electron microscopic, light microscopic and macroscopic levels respectively. These examples show that worthwile quantitative, comparative data can be obtained by this approach.

1. Methodological Part

The term "astereology" implies a set of methods using the standard stereological equipment, procedures and mathematical background, which make possible a morphometric analysis of <u>unflat surfaces</u> and their components without aspiring to a three-dimensional interpretation of data. Since the latter was essential for coining the term "stereology", we have proposed a neologism adding "alpha privativum" as a prefix designating a negation (Kališnik, 1977) in order to stress the restriction to two-dimensional space.

The basic symbols used in this paper are presented in Table 1.

Table 1

Interrelationship of Features, Reference Spaces and Densities with Corresponding Symbols and Dimensions

| | | | reference spaces | | | | | |
|--------|-------|-----|------------------|-------------|----------------|---|------------------|-----|
| | | | plane | ; | line | <u>, , , , , , , , , , , , , , , , , , , </u> | point | |
| | | | А | (2) | L | (1) | Р | (0) |
| | area | | areal | | inter | cept | point | |
| O S | A | (2) | AA | (0) | L _L | (0) | P _P | (0) |
| atur | bound | ary | bound | lary | inter | section | | |
| fee | L | (1) | LA | (-1) | PL | (-1) | | |
| | numbe | r | numer (area | rical 1) | | densi | Lie ⁵ | |
| | N | (0) | NA | (-2) | | | | |

A coherent system of astereological formulas has been partly complied and partly deduced (Table 2). These formulas aim at making it possible for a nonmathematician, e.g. biologist cr physician, to apply this quantification access.

Table 2

Coherent System of the Astereological Formulas, Used for Computation of Quantities, Measured on Projected Plane (Indicated by Primes)

| | explicitly | | | | | |
|---|--|---|---|--|--|--|
| implicitly | relatively | absolutely | | | | |
| | | indirectly | directly | | | |
| $A'_A = L'_L = P'_P$ | $A'_{Ak} = \frac{P'_{k}}{P_{O}}$ | $A_{k}' = \frac{P_{k}'}{P} A_{O}$ | $A_k' = P_k' \cdot a$ | | | |
| $L_{\rm A}^{\prime} = \frac{\pi}{2} P_{\rm L}^{\prime}$ | $L'_{Ak} = \frac{\pi}{2} \frac{P'_{k}}{L_{o}}$ | $L_{k}' = \frac{\pi}{2} \frac{P_{k}'}{L_{o}} A_{o}$ | $L_{k_{1}}^{+} = \frac{\pi}{2} P_{k}^{+} \cdot d$ | | | |
| N'A | $N'_{Ak} = \frac{N'_k}{A_o}$ | $N_{k}' = \frac{N_{k}'}{A_{o}} A_{o}$ | N ; | | | |

This formula⁺ is valid only if a test system composed solely of parallel lines is utilized. The following formulas are to be used for a grid of horizontal and vertical lines:

$$L'_{k_2} = \frac{\pi}{4} P'_{k}$$
 . d (1)

and for the multipurpose system:

$$L_{k_3}^{\prime} = \pi \cdot P_k^{\prime} \cdot d$$
 (2)

A special warning should be given, when the absolute boundary length L_k is estimated in a direct astereological manner. The formula presented in Table 2 is valid only when a test system composed solely of parallel lines is used. But if a grid of horizontal and vertical lines (test system A) is applied, the obtained number of intersections is two times greater and with the multipurpose test system (test system M) two times smaller, the same distance d between the test lines being supposed.

Some stereological methods have been developed for a three-dimensional interpretation of the data obtained on uneven surfaces, e.g. on the freeze-fracture preparations (Weibel et al., 1976) and scanning electron micrographs (Leblond and Shoucri, 1978). These methods, how-ever, are valid for a limited number of cases only.

In the material sciences these problems have been thoroughly studied by Underwood, who proposed the term "quantitative fractography", suitable for one type of objects (Underwood, 1972, 1974, 1979).

There are a great many cases where stereological methods, sensu strictu, cannot be applied or may even not be interesting. Such cases could be found in various scientific fields and on various magnification levels. This paper presents three examples from the biomedical field, of which one is for the electron microscopic, one for the light microscopic and one for the macroscopic level. In these three cases the quantification has been performed astereologically and its usefulness has been evaluated either by estimating various objects or comparing the astereologically obtained results with the directly morphometrically ascertained ones.

2. <u>Morphological Changes in Tight Junction Elements of the Exocrine</u> Pancreas During Embryonic Development

Ultrastructural Astereological Analysis of Frozen Fractured Material

2.0 Abstract

The development of tight junction elements in the cells of the exocrine pancreas in embryonic and adult mice was quantitatively analyzed by means of the freeze-fracture technique.

The density of the boundary length of junctional elements in the membrane area significantly increases from embryonic to adult cells. This indicates changes in the arrangement of membrane particles (i.e. membrane differentiation) following the embryonic development.

2.1 Introduction

The apical part of the lateral face of pancreas cells contains ridges in the <u>PF</u> faces and grooves in the <u>EF</u> faces, indicating the presence of zonula occludens - tight junction (Wade and Karnovsky, 1974). The difference in the morphology of the zonula occludens in freeze-fracture material between the cells of 17 embryonic day old and adult mice was examinated. It was found the embryonic zonula occludens consists of only a few junctional strands. By contrast, in the cells of adult animals there are more interconnected strands (Jezernik and Pipan,1978). In our work the morphological data from these cells were analyzed quantitatively.

2.2 Material and Methods

Fixation and Freeze-fracture

Pieces of the exocrine pancreas of embryonic and adult animals were fixed for 1 h in 2 % solution of glutaraldehyde and 4 % paraformaldehyde. They were then soaked for 2 h in 20 % glycerol solution in sucrose (for the cells of adult animals) or prolonged for 1 h in 30 % glycerol in the same solution (for the embryonic cells). Pieces of tissue were quickly frozen in Freon 22 cooled with liquid nitrogen. Fracturing was performed at $-loo^{\circ}$ C in a Balzers BSV 202 unit. Replicas were cleaned in 70 % H_2SO_4 and 40 % CrO_3 , washed in distilled water and recovered on coated copper grids. The replicas were then examinated in a Siemens electron microscope.

Morphometry

We examined two groups of animals. From each group twelve replicas were examined at a fixed magnification of 12 ooo. In all cases only the micrographs with intercellular junctions between two neighbourcells have been evaluated. The same method of sampling has been used by some other authors too (Humbert et al., 1976). The micrographs were examined without any knowledge of the experimental conditions and evaluated with the Merz test system. The number of intersections of the test lines with tight junction elements (ridges on <u>PF</u> faces and grooves on <u>EF</u> faces) was recordered and the density of the boundary lenght (L_A^{\prime}) of tight junction elements was analyzed according to the following formula:

$$L'_{A} = \frac{\pi}{2} \cdot \frac{P}{L_{T}}$$
(3)

where L_{A}^{\prime} is the density of the boundary length on the membrane area, <u>P'</u> is the number of intersections of the tight junctional elements, and L_{T} is the total length of the test lines. Student's test was used for the statistical analysis of the results.

2.3 Results

Tight junctional elements in the embryonic and adult cells of the exocrine pancreas are qualitatively and quantitatively different (Fig. 1,2). The quantitative changes are shown in Table 3.

Table 3

Density of Boundary Length of Junctional Elements in the Membrane Area

| Animal No | Embryonic | Adult | |
|--------------|-----------|-------|--------|
| 1 - 4 | 0.07 | 2.1 | |
| 5 - 8 | 0.23 | 0.95 | |
| 9 - 12 | 0.20 | 0.96 | |
| Mean for all | 0.17 | 1.00 | P<0.05 |

The density of the boundary length (L_{A}^{\prime}) of tight junction increased from the 17 th embryonic day $(L_{A}^{\prime} = 0.17)$ to adult animals $(L_{A}^{\prime} = 1.00)$. This difference is statistically significant (P<0.05).

2.4 Discussion

The reference area in our case was not the entire cell surface projection, but the surrounding of intercellular junctions between two cells, the extension being determined by the fixed magnification. This made possible a comparison of results between two groups of animals. A three-dimensional interpretation of the data would be difficult because the studied surface is not flat. We therefore used the astereological access (Kališnik, 1977). Tight junctions are oriented in a preferential direction, so we applied the Merz test system.

The increase in the tightness of the zonula occludens network represents a kind of membrane differentiation in embryonic development. This indicates qualitative and quantitative changes in the membrane plane, i.e. changes in the organization and rearrangement of particles (Friend



Fig. 1

PF face of luminal plasmalema in the cells of a 17-embryonic day-old mouse exocrine pancreas. Note the tight junction elements (arrows). x 40 000.



Fig. 2

PF face of luminal plasmalema in the cells of the exocrine pancreas of an adult mouse. Tight junction elements consist of many grooves and ridges (arrows). x 40 000.

and Gilula, 1972),

It was found that the zonula occludens in the cells of the "leaky" epithelium consists of only one or a few junctional strands. A large fraction of the passive transepithelial flux appears to follow the paracellular route. By contrast, in the "tight" epithelia (consisting of many junctional strands) the resistance of the paracellular pathway to the passive flux is greatly increased (Claude and Goodenough, 1973). On the basis of our results as well as according to data by Claude and Goodenough (1973), we can expect a reduction of the passive flux in embryonic cells of the exocrine pancreas following the differentiation process. Our results confirm observations of morphological developments of embryonic cells in the exocrine pancreas.

3. Morphometric Analysis of Mammary Fibroadenoma Nuclei

3.0 Abstract

In aspiration biopsy smears of mammary fibroadenomas, the area and boundary length of nuclei were estimated astereologically and their diameter computed. The results were compared with the values determined by a direct morphometrical method using an ocular micrometer. No statistically significant differences were obtained.

Astereological estimation of the cell nuclei is satisfactorily precise and less time consuming than direct measuring with an ocular micrometer.

3.1 Introduction

The aim of this study was to find out whether the diameters of the cell nuclei can be estimated astereologically with satisfactory precision, if the results are compared with values obtained directly with an ocular micrometer.

3.2 Material and Methods

The material was obtained with aspiration biopsy from 16 mammary fibroadenomas, dried on air and stained according to Giemsa (Fig. 3). Two




morphometric methods were applied for determinating the cell nuclei profiles diameter: The indirect astereological and direct method using an ocular micrometer. For the astereological estimation of cell nuclei profiles diameter, the area $(\underline{A'})$ and boundary length $(\underline{L'})$ were estimated and the diameter computed according to the following formulas:

$$2r_{1} = 2 \sqrt{\frac{A}{\pi}}$$
 (4)
$$2r_{2} = \frac{L}{\pi}$$
 (5)
$$\overline{2r} = (2r_{1} + 2r_{2})/2$$
 (6)

For the computation of diameters from the area and/or boundary length, the circle has been approximated for the form of nuclei projections.

Preliminary measurements showed that in each smear at least 68 nuclei were to be measured if the maximal error was not to exceed 10 % of the average at the 95 % confidence interval.

The astereologically estimated diameters were compared with the directly determined values.

3.3 Results

The data are presented in Graph 1 and Table 4.

Table 4

The Average (\bar{x}) , Standard Deviation (SD) and Standard Error (SE) for Area (A'), Boundary Length (L') and Diameter of Fibroadenoma Nuclei Profiles, Obtained Indirectly According to Formulas /(4),(5),(6)/ and Directly (d) in μm

| | Α' | L' | 2r1 | 2r2 | 2r | d |
|-----------|------|------|------|------|------|-----|
| x | 74.3 | 28.0 | 9.7 | 8,9 | 9.3 | 9.5 |
| SD | 11.3 | 2.2 | 0.8 | 0.7 | 0.8 | 0.7 |
| <u>SE</u> | 2.8 | 0.5 | 0.2 | 0.2 | 0.2 | 0.2 |
| Р | - | - | n.s. | n.s. | n.s. | - |



Graph 1

ī

Frequency distribution of the cell nuclei diameters estimated directly (hatched) and indirectly (white), in μ m.

The differences between astereologically estimated diameters and directly measured values were not significant.

3.4 Discussion

The differences between the values obtained by various estimation techniques are possibly due to the fact that the projections of nuclei are not only circles but also ellipses. It should be pointed out that the indirect astereological method is faster than the direct one.

4. The Use of the AstereologicalTechnique in Nuclear Medicine

4.0 Abstract

The astereological technique can be used for the analysis of the area and boundary length of scintigram.

4.1 Introduction

Thyroid, liver and spleen scintigraphy is daily performed in nuclear medicine departments and scintigrams are quantitatively analysed by means of diameters, planimeter, computer etc. A scintigram (area), a two-dimensional projection of the organ, correlates with its volume (three-dimensional), which is important from several viewpoints. Thyroid volume and/or weight is necessary for calculating a therapeutic dose of ¹³¹I in the treating hyperthyroidism. The speed of liver metastases growth has some prognostic value and the magnitude of the spleen indicates the therapeutic effect in leucosis. We use the astereological method (Kališnik, 1977) for the analysis of scintigrams.

4.2 Technique

The scintigram has to be completely covered with a transparent lattice test system. Using the simplest operation - counting - the absolute values of the area, boundary and number are established. A thyroid scintigram, taken with a Scintimat rectilinear scanner is given as an example (Fig. 4).



Fig. 4 Thyroid scintigram (d = 10,6 cm).

4.3 Results

Table 5 presents the values obtained by the astereological method (area and boundary) compared with those obtained directly morphometrically by means of a planimeter and a thread.

| Table | 5 |
|-------|---|
|-------|---|

Variables for the Thyroid Scintigram in Figure 4

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| | Method | astereological | direct |
|--------------|-----------------------------------|---------------------------------------|--------|
| Variable | | · · · · · · · · · · · · · · · · · · · | |
| Area | (A') cm ² | 83 | 81 |
| Boundary | (L') cm ^l | 45 | 43 |
| Shape factor | $\frac{L^{L}}{A}$ cm ^o | 24,4 | 22,8 |

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STEREOLOGICAL ANALYSIS AS ONE OF THE METHODS

IN MODERN GRANULOMETRY

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Summary

The paper points out the usefulness of stereological analysis, both when determining the geometrical characteristics of the structure of a rock /regarded as a grained material formed from crystals of one or more minerals/ subjected to crushing, and also when determining the geometrical characteristics of grains as a product of crushing. There are given sets of characteristics referring on the average to a unit volume of a rock and the results of their determination, taking as an example a set of cupriferous minerals of copper ore. There has been presented the mathematical basis for determining the total mean curvature and the surface area of single grains basing on the measurements made in projections. The equivalence of the total mean curvature of a grain and its Feret's diameter has been noted. The paper proposes to characterize the size and the shape of grains on the basis of the functionals of convex bodies as the principal quantitaties occuring in stereological analysis.

Introduction

Traditional granulometry developed the methods of determining geometrical characteristics of grains in loose materials, i.e. the products of the crushing of rocks. Modern granulometry has recently become more and more interested in the grained materials, i.e. solid bodies consisting of grains of one or more components. An example of such materials are the rocks composed of crystals of one or more minerals.

Though from geometrical point of view a single crystal may be regarded as a convex body, sets of crystals of one mineral surrounded in the rock by crystals of other minerals may frequently present a great number of spatial configurations. Our notions of the spatial structure of a rock are usually based in the observation of the mosaic visible on flat sections made from rock samples. By analogy we might say that the task of reconstructing the spatial structure on the basis of our knowledge of the mosaic visible on a flat section is similar to the task of reconstructing a flat mosaic on the basis of the knowledge of the chords along a secant lying on a plane.

Paper presented at: XIII International Mineral Processing Congress Warszawa, June 4-9, 1979 An attempt at a qualitative characteristics of the particular structural elements may have at least two aims: a) to supplement the qualitative mineralogical description of the rock with a quantitative one and b) to determine the effect of the geometrical structure of the rock on its mechanical properties, on the behaviour of the rock during its crushing and while liberating the grains of a particular mineral.

A quantitative characteristics of the particular elements of the spatial structure of a grained material and a geometrical characteristics of the individual grains and their collection, i.e. a loose material, are the subject of the stereological analysis. In the first case the analysis is based on measurements or calculations made on plane sections of the samples. In the other case - it is based on the measurements of the elements of projections of individual grains on a plane, i.e. their shadow. Stereological analysis may also be characterized as a method of three-dimensional interpretation of flat images, i.e. cross-section and shadows.

Numerical Characteristics of Rock Structure

To characterize a single grain as a geometrical body we assume its basic functionals: volume V, surface F and the total mean curvature M. Considering a small acquaintance with the last quantity we quote its definition /1, 17/. If the grain consitutes a body confined by a smooth surface F of C_2 class, then

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$$M = \frac{1}{2} \int_{F} \left(\frac{1}{R_{1}} + \frac{1}{R_{2}} \right) dF, \qquad (1)$$

where R_1 , R_2 are the radii of the main curvatures at a point of the surface F. If the grain is a convex polyhedron, then

$$M = \frac{1}{2} \sum_{i} l_{i} \mathcal{X}_{i}, \qquad (2)$$

where l_i - is the length of the i-th edge, and $\boldsymbol{\chi}_i$, O< $i^{<\pi}$ is the angle formed by the versors of the sides intersecting along the i-th edge. A grain confined by a smooth surface may be regarded as a limit of a sequence of polyhedron circumscribed on it in a proper way. It is proved in geometry that a sequence of values of mean total curvatures corresponding to a sequence of polyhedrons and calculated by means of formula (2) tends to the value given in formula (1). The total mean curvature of the sphere M = 4π R (R - sphere radius), total mean curvature of a rectangular paralleleliped M = π $(l_1 + l_2 + l_3)$ $(l_i - edge length)$. Formula (1) is valid for concave bodies. Formula (2) can be generalized for concave polyhedrons. Let us notice that the quantities (V, F, M) have the dimensions $(1^3, 1^2, 1^1)$, respectively.

Let us consider a piece of rock, in which we distinguish a single mineral. The remaining minerals are regarded as a matrix.The grains G_i (i = 1,...,N) of this mineral are sets of crystals confined by the contact surface with the matrix. Let us denote by V_r the volume of a piece of rock, and by N - the number of grains located in it. To each grain G_i we assign three numbers (V_i , F_i , M_i). As the quantities characterizing the grains of the distinguished mineral we assume the set of indices $(\overline{v}, \overline{F}, \overline{M}, \overline{N})$, whose names and definitions are given in Table 1, column 1 and 2, respectively. As follows from the definitions these are the mean value per a volume unit of the rock. Let us assume that a piece of rock has been cut through by r planes located uniformly in space. On the obtained plane sections with the areas f_j ($j = 1, \ldots, r$) the grains are visible as plane figures. Let us denote the area and the perimeter belonging to the i-th grain on the j-th section by f_{ij} and l_{ij} , respectively. The total number of grains visible on the j-th section will be denoted by m_j . As a result of the summation of the above quantities over all the sections we obtained Σf_{ij} , Σl_{ij} , Σm , Σf_j . These sums enable to calculate the values of the estimators \overline{v}_1 , \overline{F}_1 , \overline{M}_1 of the unknown accurate values \overline{v} , \overline{F} , \overline{M} according to the formulae in column 3, Table 1.

The measurements and calculations of the elements of the mosaic visible on plane sections may be also carried out along the lines with the length s_j , situated at random or regularly on those sections. Let us denote by s_{ij} the length of the chord formed by the j-th line with the i-th grain, and by n_j - the number of chords on the j-th line. After summing up these values on all lines we shall obtain Σs_{ij} , Σn_j , Σs_j , which, on the basis of formulae given in column 4, Table 1, provide the values of the estimators \overline{V}_2 , \overline{F}_2 of the unknown quantities \overline{V} , \overline{F} .

On the plane sections of a rock sample points may be projected at random or they may be situated in a systematic way. Let us denote by z_R the total number of projected points and by z_G - the number of points which have fallen on the grains,

| | | Estimators obtained from the | | | |
|--|--|---|--|--|--|
| Magnitude | Definition | plane | linear | point | |
| | | | analysis | | |
| 1 | 2 | 3 | 4 | 5 | |
| Specific volume V | $\frac{\sum V_{1}}{V_{R}} \left[\frac{1}{1^{3}}\right]$ | $\bar{v}_{1} = \frac{\sum_{j=1}^{f} ij}{\sum_{j=1}^{f} j} \left[\frac{1^{2}}{1^{2}} \right]$ | $\bar{v}_{2} = \frac{\sum s_{ij}}{\sum s_{j}} \left[\frac{1}{1}\right]$ | $\bar{v}_3 = \frac{z_G}{z_R} \left[\frac{1^\circ}{1^\circ} \right]$ | |
| Specific surface F | $\frac{\sum F_{i}}{V_{R}} \boxed{\frac{1^{2}}{1^{3}}}$ | $\overline{F}_{1} = \frac{4}{\pi} \frac{\sum l_{ij}}{\sum f_{j}} \left[\frac{1}{l^{2}} \right]$ | $\bar{F}_2 = 4 \frac{\sum n_j}{\sum s_j} \left[\frac{1^0}{1^1} \right]$ | | |
| Specific total mean \overline{M} curvature | $\frac{\sum_{i=1}^{M} \mathbb{I}}{V_{R}} \begin{bmatrix} \mathbb{I} \\ \mathbb{I} \\ \mathbb{I} \end{bmatrix}$ | $\bar{M}_{1} = 2\pi \frac{\sum_{j=1}^{m} j}{\sum_{j=1}^{m} j} \begin{bmatrix} 1^{o} \\ 1^{2} \end{bmatrix}$ | | | |
| Specific Ñ number Ñ | $\frac{N}{V_{R}} \begin{bmatrix} \frac{1}{0} \\ 1 \end{bmatrix}$ | | | | |

Table 1: List of estimators of specific volume, specific surfaces and specifiv total mean curvature

in column 1, Table 1, there has been given the estimator \overline{v}_3 of the specific volume \overline{v} .

Table 1 contains a list of the estimators obtained from plane, linear and point analyses, carried out on plane sections. Let us notice that the estimators should be regarded as random variables, the values of which tend asymptotically to the unknown $(\overline{V}, \overline{F}, \overline{M})$ with increasing representativeness of the measured population.

The problem connected with: a) methods of deriving the estimators of indices referring to a unit volume of the rock, b) the validity range of the formulae, c) definite methods of carrying out measurements and calculations on the cross-sections by means of various instruments, d) discussion of errors - have been widely discussed in literature. We shall mention here some studies based on original works, concerning the methods of deriving the estimators. Thus, estimator \overline{V}_1 has been supplied by Delesse /14/, estimator \overline{V}_2 by Rosiwal /24/. The estimators \overline{F}_1 and \overline{F}_2 have been given by Saltykov /25/, Smith and Guttman /26/ and by Hennig /18/. Finally, estimator \overline{M}_1 was given in the study /2/ as well as by Cahn /11/, De Hoff /13/ and Giger /16/. The formulae for the estimators - except \overline{M}_1 - are valid for arbitrary shapes of the grains. The extension of the validity of the estimator \overline{M}_1 on concave grains makes it necessary to provide an interpretation of the notion of \overline{M} for this class of bodies.

Up to now no method for determining the estimator for \overline{N} with equally general assumptions has been given. Saltykov /25/ has supplied the method of determining the specific number of spheres.De Hoff /12/ has derived the set of formulae

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for determining the specific number of grains, geometrically similar to each other, when simultaneously postulating the type of their size distribution. In the study /19/ there has been given and in the studies /8, 5/ there has been developed the secalled method of coupled sections for determining the specific number of convex grains when simultaneously postulating the type of their random distribution in the rock.

The finding of the method of determining N would enable a wider application of the characteristics reffered usually to a single grain. We are concerned here with the quantities:

$$\overline{\overline{V}} = \frac{\sum V_{i}}{N} = \frac{\overline{V}}{\overline{N}}; \quad \overline{\overline{F}} = \frac{\sum F_{i}}{N} = \frac{\overline{F}}{\overline{N}}; \quad \overline{\overline{M}} = \frac{\sum M_{i}}{N} = \frac{\overline{M}}{\overline{M}}$$
(3)

The set $(\overline{\overline{V}}, \overline{\overline{F}}, \overline{\overline{M}})$ characterizes the average size of a grain of a particular mineral in the rock and it corresponds to the set (V, F, M), which characterizes the size of a single grain.

Stereological Analysis of Copper Ore

A mining deposit is not a homogeneous formation, but is characterized by a variety of structure and physical properties. A values \overline{V} , \overline{F} , \overline{M} , \overline{N} defined in the preceding chapter are determined from measurements on thin sections or polished sections of samples taken from the deposit. The natural variability of the deposit is the reason for the fluctuation of the measured quantities which is caused by the random choice of the place from which the sample has been taken. The values of \overline{V} , \overline{F} , \overline{M} , \overline{N} obtained from measurements are random variables. In stereological analysis we usually give some numerical characteristics of these random variables (mean value, variance). A full probabilistic characteristics of a random variable is given by the distribution function or the distribution density. The knowledge of the distribution function or the density makes it possible to determine all the moments, as well as the probability of assuming by the random variable of a value from an arbitrary given interval.

To illustrate the application of the stereological analysis we shall present here the results of measurements of the specific volume \overline{V} , of the specific surface \overline{F} and of the specific total mean curvature \overline{M} of a set of cupriferous minerals found in the copper ore from the mine "Rudna".

Nine samples were taken from a piece of ore, weighing ca. 30 kp. From each sample there were prepared three rectangular, polished sections, of the size 24 x 36 mm, cut out in three directions, perpendicular to each other. The grains of cupriferous minerals were visible on the sections as bright, metallic figures. On account of a big contrast between those minerals and the sandstone matrix it was possible to use for the measurements an automatic device - a Quantimet 720. On each of the 27 microsections the measurements were made on 12 separate fields of the size 2,2 x 1.5 mm, situated regularly on the sections. Due to the fact that the distance between the measured fields was considerably greater than the size of the figures, the measurements of the particular field could be regarded as randomly independent. In each measured field there were determined: the specific volume, specific surface and the specific total mean curvature. Use was made of the estimators \overline{V}_1 , \overline{F}_1 , and \overline{F}_2 (specific surface was determined

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by two methods) and of \overline{M}_1 , which have been given in Table 1.

It should be noted here that the estimator \overline{M}_1 for the total mean curvature is valid only for convex bodies. The grains of cupriferous minerals in the examined ore are not all convex bodies, nevertheless this estimator was used in the present study for determining the specific total mean curvature.

Each set of results from 324 measurements, being the realization of the random variables \overline{V}_1 , \overline{F}_1 , \overline{F}_2 and \overline{M}_1 has been the subject of statistical elaboration. There have been determined the frequency distributions and the empirical distribution functions of those variables. The empirical distributions of all four variables were approximated with the continuous probability distribution "gamma" (Γ) /23/. The density of this distribution is defined by the formula:

$$g(x) = \begin{cases} 0 & \text{for } x \leq 0\\ \frac{b^{a}}{\Gamma(a)} & x^{a-1} & e^{-bx} & \text{for } x > 0 \end{cases}$$

and the distribution function by the formula:

$$G(x) = \begin{cases} 0 & \text{for } x \leq 0\\ \frac{b^{a}}{\Gamma(a)} & \int_{0}^{x} s^{a-1} e^{-bs} ds & \text{for } x > 0 \end{cases}$$

The constants a and b are the parameters of the distribution Γ . With these parameters the expected value m and the variance σ^2 are expressed by the formula:

$$m = \frac{a}{b}, \quad \sigma^2 = \frac{a}{b^2} \tag{4}$$

When approximating the empirical distributions using the distribution Γ , it was desired that the two first moments of the theoretical distribution (m and σ^2) were equal to those of the empirical distribution. This enabled to determine a and b.

In Figs.1, 2, 3, 4 there have been shown the distributions \overline{V}_1 , \overline{F}_1 , \overline{F}_2 and \overline{M}_1 obtained from measurements and approximated by the distributions Γ .

The assumption that the variables \overline{V}_1 , \overline{F}_1 , \overline{F}_2 and \overline{M}_1 have the probability distribution Γ was verified by means of two tests of the goodness of fit: the χ^2 test and the Kołmogorow's test. On the significance level $\alpha = 0.05$ none of the tests gave reasons for rejecting this assumption. The detailed results can be found in /10/.

As we have already mentioned the measurements results in the particular fields can be regarded as randomly independent. Thus the arithmetic means from all realizations are assumed as the estimators of the average random variables \overline{V}_1 , \overline{F}_1 , \overline{F}_2 , \overline{M}_1 and denoted by \overline{V}_{1s} , \overline{F}_{1s} , \overline{F}_{2s} , \overline{M}_{1s} , respectively. The question arises about the accuracy with which the arithmetic means approach the real average values. The repetition of the measurements on another sample taken from the same ore and the determination of the arithmetic means will in general give as results values different from those obtained by us. With this in mind we may regard the arithmetic means \overline{V}_{1s} , \overline{F}_{1s} , \overline{F}_{2s} , \overline{M}_{1s} as random variables and determine their distribution of probability.

It is easy to prove that the arithmetic mean of n independent random variables, each of which has the same probability distribution "gamma", has also the distribution "gamma".

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Fig.2. Distribution fo the specific surface \overline{F}_1 x empirical values of probability, + values of the empirical distribution function.

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Fig.3. Distribution of the specific surface F_2 x empirical values of probability, + values of empirical distribution function.



Fig.4. Distribution of the mean specific curvature \overline{M}_1 x empirical values of probability, + values of the empirical distribution function.

The parameter a₁ and b₁ of the arithmetic mean distribution are expressed by means of the parameters a and b of the distribution of a particular variable with the following formulae:

$$a_1 = na, \quad b_1 = nb$$

According to (4) for the first two moments m_1 and σ_1^2 of the arithmetic mean distribution we have:

$$m_{1} = \frac{a_{1}}{b_{1}} = \frac{na}{nb} = \frac{a}{b} = m,$$

$$\sigma_{1}^{2} = \frac{a_{1}}{b_{1}^{2}} = \frac{na}{n^{2}b^{2}} = \frac{a}{nb} = \frac{\sigma^{2}}{n}$$

i.e. the arithmetic mean has the same mean value as a single variable, and its variance is n times smaller.



Fig.5. Distribution of the arithmetic mean of the specific volume \overline{V}_{1s} .

In Fig.5 there has been shown by way of example the course of the distribution function and of the probability density of the arithmetic means \overline{V}_{1s} . The obtained distribution enable to determine the probability that the arithmetic means will be contained in an arbitrary chosen interval.

It should be noted that the three numerical characteristics \overline{V} , \overline{F} , \overline{M} of the geometrical structure of a rock are not, in general, randomly independent quantities. For a structure composed of similar bodies there exists a close functional relation between these quantities. The conditions under which a deposit is formed favour the formation of crystals and their complexes of similar shape. Hence the three mentioned quantitities are usually statistically correlated.



Fig.6. Correlation between the variables \overline{F}_1 and \overline{V}_1 .

In Fig.6, by way of example, there has been shown in double logarithmic system the correlation of the variables \overline{V}_1 , \overline{F}_1 , that has been obtained from measurements made on sections of copper ore.

Determination of the Surface and the Total Mean Curvature of a Single Grain Using Projection Method.

The surface area F and the total curvature M of convex grains can be determined from the measurements of perpendicular projections of the grains. A theoretical basis for this procedure is provided by Cauchy's projection formulae /17/:

$$F = \frac{1}{\pi} \int \sigma(n) dn$$
 (5)

$$M = \frac{1}{2} \int b(n) dn$$
 (6)

 $\sigma(n)$ denotes here the area of a perpendicular projection of a grain on a plane normal to the direction n, b(n) is the length of a perpendicular projection of a grain on a straight line with the direction n, i.e. Feret's diameter of this grain in the direction n. The integration domain in both integrals is the full solid angle equal to 4π stereoradians.

In practical application the determination of the surface area or the total mean curvature is based on replacing the integrals by approximate sums and on the measurements of projections in a few directions located at random or uniformly in space.

The accuracy of the result obtained in this way depends on the number of projection directions and on the grain shape. Thus for a sphere, a single measurements suffices to determine the accurate value of the surface or of the total mean curvature. The flatter or longer is the grain the greater is the dispersion of a single measurements of a projection in a direction chosen at random. We may consider theoretically the most disadvantageous extreme case when the grain degenerates at measurement of the shadow area into a plane fragment, and at the measurement of the total mean curvature - into a segment. In both these cases the measured values of a single projection have a uniform probability distribution /6, 7, 4/.

With a multiple projection we may distinguish two cases: when the projection directions are randomly independent and when they are coupled. In the first case the arithmetic mean from the perpendicular measurements is assumed as the magnitude of the area or the total mean curvature

$$F = \frac{4}{k} \sum_{i}^{k} \sigma(n_{i})$$

$$M = \frac{2\pi}{k} \sum_{i}^{k} b(n_{i})$$
(7)
(8)

where k is the number of measurements, $\sigma(n_i)$ - the area of the projection in the direction n_i , $b(n_i)$ - the length of the projection on a straight line having the direction n_i . When projection in independent directions the obtained values have a variance which is inversely proportional to the number of projection directions.

The measurements in coupled directions are more advantageous. With a right choice of directions, when they are uniformly located in space, the magnitude of the area or of the total mean curvature (measured, as well, according to formulae (7), (8)), has a considerably smaller variance than while projecting in equal number of independent directions.

Fig.7 shows the distribution functions of the magnitude

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Fig.7. The distribution function of the projection area of a plane fragment when projecting in one direction and in 3, 4 and 6 coupled directions.

of an area of a normal projection of a plane fragment when projecting in one direction (curve 1) and when projecting in k = 3, 4, 6 coupled directions which form normals to a cube, octa- and dodecahedrons (curves 2, 3, 4). The independent variable s is here the ratio of the projections area σ to the area of the projected fragment of a plane, σ_0 (s = $\frac{\sigma}{\sigma_0}$).

As it has been pointed out in /9/ these results can be used to obtain the distribution of the magnitude of an area, determined with a single projection, for a cube, octa- and dodecahedron. Between the variable s and the surface area F of these regular polyhedrons there exists the relation

$$F = 4 k \sigma_s$$

where σ_0 - is the area of a single side, k = 3, 4, 6, respectively.

Fig.8 shows the length distribution of a perpendicu-



Fig.8. The distribution function of the projection length of a segment, a tetrahedron, a cube, an octohedron and dodecahedron with a single projection in a straight line.

lar projection of a segment with the length 2 (curve 1) and that of a tetrahedron, cube, octa- and dodecahedron, in a randomly chosen direction (curves 2, 3, 4, 5). These polyhedrons are inscribed in a sphere with the radius 1.

Equivalence of Feret's Diameter and the Total Mean Curvature of a Convex Grain.

Feret /15/ has defined the grain size as the mean distance of tangents of a constant direction, drawn to the opposite sides of the grain contour (with a sufficient number of grains, approximately the same size).Thus Feret has not supplied a direct definition for the size of s single grain, but has defined it as the mean value for a collection of grains which are "approximately the same size". The grain size according to Feret, traditionally called Feret's diameter, has been widely discussed in literature. This notion is generally understood as the mean distance of the tangents drawn on a plane in a fixed direction to the contours of an orthogonal projection of the grains into this plane, on which - what is important - those grains are lying. Considering the fact that the grains on the plane usually lie in their most stable positions, the averaging is performed only within a narrow range of the likely orientations of the grains with respect to this plane. Strictly speaking, while formulating correctly the definition of the grain size, Feret failed to point out the method of realizing all possible spatial orientations of the grains in relation to the chosen direction.

Feret's diameter should be understood as the mean value of the distance between the planes supporting the grain, taken from all possible orientations of the grain within the full solid angle equal to 4π stereoradians. Then we have

$$D_{\rm Fe} = \frac{1}{4\pi} \int b(n) \, dn \tag{9}$$

where b(n) - the width of the grain in the direction n, i.e. the length of an orthogonal projection of the grain in a straight line with the direction n. It will be noted that from the above formula and from formula (6) there follows immediately the relation

$$D_{\rm Fe} = \frac{1}{2\pi} M \tag{10}$$

which expresses the proportionality of the mean total curvature of the grain and Feret's diameter. In the sence of formula (10) we may speak of the equivalence of these two notions for every convex grain.

It is possible to prove /3/ that Feret's mean diameter \overline{D}_{Fe}

and $\overline{\overline{M}}$ of the same collection of grains fulfill the relation

$$\overline{\overline{D}}_{\text{Fe}} = \frac{1}{2\pi} \frac{\overline{\overline{M}}}{\overline{N}} = \frac{1}{2\pi} = \overline{\overline{\overline{M}}}.$$
 (11)

It follows from this formula that the determination of $\overline{\overline{D}}_{Fe}$ may be based on the method of the analysis of plane sections and become independent of the analysis of projections on a plane.

A Natural Characteristics of the Size and Shape of a Grain

The quantities which in the stereological analysis characterize the size of a single grain, so to say in a natural way, are the values of its functionals

$$(V, F, M)$$
 or (V, F, D_{FO}) (12)

It should be stressed, however, that the assignement of a set of numbers (12) to a grain is not one-to-one correspondent. Te each grain we can assign precisely three numbers (12), whereas to the definite three numbers there may correspond one or more grains having different shapes, and it may also happen that no single convex grain corresponds to the selected three numbers. The characterization of a solid body through a set of numbers in a unique way requires the determination of an at least countable set of numbers. An attempt of this kind was undertaken by Meloy /22/.

Consequently, a natural approach to a numerical characterization of the shape of a grain is the use of its functionals (12). The realization of this tendency is the development of some concepts represented in literature, e.g. by McAdams /20, 21/. For this purpose we shall make use of Blaschke's coefficient and diagram /17, 4/.

Blaschke has derived a pair of coefficients

$$x = \frac{CF}{M^2}$$
; $y = \frac{3C^2V}{M^3}$ (13)

where $C = 4\pi$ is Gauss' total curvature of a solid body. The coefficients (x, y) satisfy the inequalities $0 \stackrel{<}{-} x \stackrel{<}{-} 1$, $0 \stackrel{<}{-} y \stackrel{<}{-} x^2$. To a collection of grains geometrically similar to each other there corresponds one pair of numbers (x, y).



Fig.9. Blaschke's diagram.

Fig.9 shows Blaschke's diagram and it illustrates the variation area (x, y) for convex grains. We shall give an interpretation of the boundary of this area. To the point A (0, 0) there corresponds a segment as a degenerated convex body. To the curve AC with the equation $y = x^2$ there cor-

respond extreme solid bodies, which, with given F, M, have the greatest possible volume V. To the point C (1, 1) there corresponds a sphere. To the curve CE (with an up to now unknown equation) there correspond extreme bodies, which, with given F. M, have the smallest possible volume V. To the segment AE there correspond plane convex figures (V = 0) as degenerated bodies. To the point $E(\frac{8}{\pi^2}, 0)$ there corresponds a circle.

A set of Blaschke's coefficients may be proposed as a set of coefficients of a convex grain shape. However, in this case as well, the correspondence is not one-to-one.

The determination of the magnitude and shape of a grain on the basis of its main functionals is characterized by a logical bond with the quantities determined in stereological analysis of sections and projections.

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Quantitative Gefügeanalyse, Stereologie und Eigenschaften von Werkstoffen

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Quantitative Probenpräparation, optisch-elektronische Messung und stereologische sowie weiterführende Rechenprogramme bilden zusammen die quantitative Gefügeanalyse. Ihre derzeitigen Genauigkeitsgrenzen und Einsatzmöglichkeiten – auch und insbesondere zur Eigenschaftsbestimmung – werden behandelt.

Quantitative microstructural analysis includes the preparation, optic-electronical measurement and stereologic as well as proceeding computer programmes. Its accuracy and performance – also and in particular to determine materials properties – are treated in this presentation.

L'analyse de microstructure quantitative est composée par la préparation d'échantillon, la mesure optique et électronique, la stéréologie ainsi que les programmes avancés pour des ordinateurs. La limite de précision actuelle ainsi que la performance de cette méthode seront traiteés dans l'article présenté.

1. Begriffe, Definitionen und experimentelle Grundlagen der quantitativen Bildanalyse

Aufgabe der quantitativen Gefügeanalyse ist es, das Gefüge von Werkstoffen und seine möglichen Veränderungen durch geeignete Parameter quantitativ zu charakterisieren. Das solcher Charakterisierung zugrunde liegende Experiment ist die Vermessung von Gefügebildern. Gefügebilder sind zweidimensionale Ansichten ebener Schnitte durch den dreidimensionalen Werkstoff. Sie werden so präpariert, daß die Gefügebestandteile nach mikroskopischer Vergrößerung visuell unterschieden, ihre Geometrie und geometrische Anordnung in der Bildebene also erkannt werden kann. Dabei ist zwischen Durchlicht- und Auflichtpräparaten zu unterscheiden. In der Werkstoffkunde überwiegen Auflichtpräparate, die für quantitative Messungen vielfach beispielsweise durch Gasätzen farbkontrastiert werden können. Mittels Farbfiltern können diese Farbkontraste in hell/ dunkel-Kontraste überführt werden und ergeben damit das quantitativ auswertbare zweidimensionale Gefügebild (12). Zur Vermessung solcher Gefügebilder kann direkt vom mikroskopischen Bild, von seiner fotografischen Aufnahme oder von einer elektronischen Nachbildung ausgegangen werden. Die Messung selbst erfolgt mit Hilfe von Meßpunkten (Punktanalyse), -linien (Linearanalyse) oder Meßflächen (Flächenanalyse) (2), (3), (6). Die entsprechenden Meßgeräte lassen sich einteilen in manuelle, halb- und vollautomatische Systeme, wobei die Übergänge fließend sind (4), (5), (6). Manuelle Systeme sind solche, bei denen die Entscheidung über die Zuordnung der Meßpunkte, -linien oder -flächen zu bestimmten Bildmerkmalen (Selektion) sowie alle folgenden Operationen, die zum Resultat der Bildanalyse führen, vom Beobachter getroffen werden (Beispiel: Zeiss-Meßokulare und -Strichplatten; Wild-Stichprobenmikroskop zur Mikrostereologie). Bei halbautomatischen Geräten übernimmt der Beobachter nur die Selektion, während alle anderen Operationen vom Gerät ausgeführt werden (Beispiel: Zeiss-TGZ, Kontron-MOP, Leitz-ASM). Vollautomatische Geräte schließlich werden lediglich auf das zu analysierende Bild justiert und arbeiten dann bis zum Bildanalysenergebnis unabhängig vom Beobachter.

Solche vollautomatischen Geräte messen nach zwei Meßprinzipien:

- das vom im Mikroskop beleuchteten ebenen Präparat kommende Lichtbild wird über eine Rasterblende in Bildpunkte zerlegt, deren Helligkeit über Fotometer oder Fotomultiplier registriert wird
- das vom im Mikroskop beleuchteten ebenen Pr

 p

 r

 p

 r

 aparat kommende Lichtbild wird
 über eine Fernsehr

 öhre auf einem Bildschirm elektro- nisch nachgebildet und mit Hilfe der elektroni-schen Bildpunkte vermessen.

Die mit all diesen Geräten meßbaren Daten stammen vom zweidimensionalen Bild. Um aus ihnen auf den räumlichen Aufbau des Präparates schließen zu können, werden die Meßdaten mit Hilfe von stereologischen Gleichungen in die dritte Dimension näherungsweise umgerechnet (2), (3), (8), (9), wobei entsprechend der Präparationsmethode zwischen stereologischen Gleichungen für Auflicht- und Durchlichtpräparate zu unterscheiden ist. Die Grundlage dieser Gleichungen ist die mathematische Statistik. Ihre Ergebnisse sind daher umso zuverlässiger, je mehr Meßdaten zur

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Ermittlung des Flächenverhältnisses der Phasen in einem Ag-Cu-Eutektikum mit verschiedenen, vollautomatischen Bildanalysatoren

Verfügung stehen und je geringer ihre Streuung ist. Diese Streuung hängt einmal ab von der Bildqualität und damit von der Güte der Präparation. zum anderen von der Meßgenauigkeit der Bildanalysatoren. Nach neueren Untersuchungen beträgt die Standardabweichung beim gegenwärtigen Entwicklungsstand der elektronischen Geräte etwa ± 10%. Dieser Richtwert für die statistische Streuung von quantitativen elektronischen Bildanalysen ergibt sich sowohl beim Vergleich zwischen Meßergebnissen, die einmal durch halbautomatische und zum anderen durch vollautomatische Messung gewonnen wurden, als auch beim Vergleich von Meßergebnissen, die mit verschiedenen vollautomatischen Geräten ermittelt wurden (Abb. 1). Er gilt

- bei unterschiedlicher Me
 ßproblematik wie Sehnenl
 ängenverteilung, mittlerer Sehnenl
 änge oder Phasenanteilen,
- als statistischer Fehler der Messungen einer Person,
- bei Messungen durch verschiedene Personen.

Obwohl gelegentlich bessere Reproduzierbarkeiten erreicht werden, sollte für quantitative Bildanalysen zur Zeit keine höhere Genauigkeit erwartet werden, zumal zu den präparations- und meßtechnisch bedingten Ungenauigkeiten noch diejenige bei der statistischen Umrechnung der Meßdaten über Stereologiegleichungen in Gefügeparameter hinzukommt.

2. Stereologie und Gefügeparameter

Nach den bisherigen Ausführungen besteht die quantitative Gefügeanalyse aus drei aufeinander folgenden Schritten

- Herstellung eines geeigneten Präparates (Präparation),
- seine mikroskopische, vergrößerte zweidimensionale Abbildung sowie eine fotografische und/oder elektronische Nachbildung, Nachvergrößerung und Vermessung (Quantitative Mikroskopie),
- die n\u00e4herungsweise Errechnung der seinen r\u00e4umlichen Aufbau charakterisierenden Daten \u00fcber stereologische Gleichungen bzw. Rechenprogramme (Stereologie).

Das Ergebnis einer quantitativen Gefügeanalyse sind Parameter, die das Gefüge quantitativ beschreiben. Für eine Reihe von Werkstoffproblemen ist dies bereits die Lösung. So ist beispielsweise die Messung von Sehnenlängen zur Beschreibung der Größe, diejenige von Flächenanteilen zur Beschreibung von Volumenanteilen geeignet. Besonders einfach ist der Parameter "mittlere Sehnenlänge", da sein im zweidimensionalen Bild ermittelter Wert direkt der Sehnenlänge im räumlichen Aggregat entspricht. Ebenso entspricht der mittlere Flächenanteil nach Delesse direkt dem Volumenanteil eines Bestandteils (Abb. 2). Die Größenverteilung oder mittlere Größe der Teilchen von Pulvern, aus denen Sinterwerkstoffe hergestellt werden, gibt dem Technologen jenes Kriterium an die Hand, nach dem er das technologische Rezept, wie Drücke, Temperaturen und Sinterdauer, festlegen kann. Die Messung der Porosität solcher Sinterprodukte ermöglicht ebenso wie die mittlere Größe oder Größenverteilung von Kristalliten in Stählen deren Qualitätskontrolle.

Die gefügeanalytische Ermittlung von Phasenanteilen in mehrphasigen stabilen Werkstoffen ist


die Grundlage für die Aufstellung ihrer Zustandsdiagramme, und die Größenbeschreibung von Gefügebestandteilen instabiler Werkstoffe über Sehnenlängen ermöglicht die quantitative Verfolgung kinetischer Vorgänge wie

- die Rekristallisationskinetik in kristallinen einphasigen Werkstoffen oder
- die Kornreifung in kristallinen mehrphasigen Werkstoffen.

Für die Beschreibung der Kinetik von Wechselwirkungsvorgängen wie der Reaktion zwischen den Komponenten eines instabilen Werkstoffs zu neuen Werkstoffphasen dagegen eignet sich die Messung des Anteils der neuen Phase. Schließlich sei in diesem Zusammenhang noch ein Beispiel aus der Kernforschung aufgeführt: Für die Endlagerung von hochaktivem Abfall ist dessen Einschmelzen in Glas vorgesehen. Dieses Glas soll eine möglichst hohe Beständigkeit gegen hydrolytische Auslaugung besitzen. Glaspulver werden daher auf ihre Auslaugbarkeit getestet. Die Ausmessung der Projektionsflächen (A') in Durchlichtaufnahmen solcher Pulver (Abb. 3) liefert über die stereologische Beziehung von Cauchy (S = 4A', s. Abb. 2), deren Oberfläche (S), die ihrerseits in die Bestimmung der Auslaugrate eingeht.

3. Eigenschaftsbestimmungen über Gefügeparameter

Die bis hierher erörterten Beispiele betrafen Fälle, in denen Gefügeparameter und deren stereologische Umrechnung für den dreidimensionalen Werkstoff bereits die erstrebte Lösung anstehender Werkstoffprobleme ergeben. Nun ist ein Werkstoff nicht nur durch seinen Aufbau, sondern auch durch seine Eigenschaften charakterisiert, die ihrerseits vom Werkstoffaufbau abhängig und mit den Zustandsbedingungen veränderlich sind. Wenn sich die Eigenschaften mit dem Aufbau eines Werkstoffs ändern, so muß auch ein Zusam-

Gemessene Größen

Projizierte Gesamlfläche : ΣA' Anzahl von Teilchen : N

Berechnete Größen

<u>Beispiel</u>

 $\frac{\Sigma A'}{\overline{A}'} = 3,78 \text{ mm}^2$ $\frac{\overline{A}'}{\overline{A}'} = 0,12 \text{ mm}^2$

- N = 32
- S_v = 0,48 mm²/Teilchen



Zur Ermittlung der spezifischen Oberfläche von Glasgranulaten durch quantitative Gefügeanalyse



menhang zwischen seiner Gefügestruktur und seinen Eigenschaften bestehen. Demnach müßte die quantitative Gefügeanalyse auch Aussagen über Eigenschaften und Eigenschaftsänderungen ermöglichen, ohne daß diese direkt gemessen werden.

Quantitative, empirische und halbempirische Zusammenhänge zwischen Eigenschaften und Gefügestruktur sind mehrfach nachgewiesen worden. So ist beispielsweise bekannt, daß

- bei Hartmetallen die Koerzitivfeldstärke und die Härte von der mittleren freien Weglänge zwischen den Hartstoffteilchen abhängen (3), (13) und die Bruchfestigkeit mit dem Verhältnis der spezifischen Korngrenzenfläche zur Summe der spezifischen inneren Grenzflächen (Phasengrenzen + Korngrenzen) linear abnimmt (4), (5),
- bei kristallinen einphasigen Werkstoffen die Streckgrenze (σ_s) mit der Kristallitgröße ($L_3 =$ mittlere Sehnenlänge der Kristallite) nach der Hall-Petch-Beziehung veränderlich ist (4):

$$\sigma_{\rm s} = \sigma_{\rm o} + {\rm K}/\sqrt{{\rm L}_3} \qquad [1]$$

(K,
$$\sigma_0$$
 = materialspezifische Konstanten)

bei einphasigen Eisenlegierungen die Zerreißfestigkeit (σ_B) von der spezifischen Korngrenzenfläche (S_v) linear abhängt (13)

$$\sigma_{\rm B} = 420 + {\rm const. S_V}$$
 [2]

- bei verschiedenen Metallen und einphasigen Legierungen die Brinell-Härte (HB) mit der spezifischen Korngrenzenfläche (S_v) linear zunimmt (Abb. 4) (13):

$$HB = HB_0 + const. \cdot S_v.$$
 [3]

Darüber hinaus ist es aber in letzter Zeit gelungen, ein System von Gleichungen abzuleiten, die den Gefüge-Eigenschafts-Zusammenhang für einige Eigenschaften quantitativ erfassen (8–11), (17). Sie gelten in ihrer derzeit vorliegenden Form für zweiphasige Werkstoffe, wobei der Fall des porösen Werkstoffes mit Poren als zweite Phase ein-

geschlossen ist (16). Ihre Ableitung geht aus von einer kontinuierlichen Matrixphase, in welche die Teilchen einer zweiten Phase diskontinuierlich eingebettet sind. Um die Geometrie und die geometrische Anordnung solcher Gefügestrukturen stereologisch zu beschreiben, werden die Teilchen der eingebetteten Phase durch Rotationsellipsoide substituiert, die das gleiche Volumen bei gleichem Verhältnis von Oberfläche zu Volumen haben wie die realen Teilchen. Dieses Modell hat den Vorteil, daß die Form über das Achsenverhältnis kontinuierlich variierbar ist. Dabei ergeben sich die zylindrische Nadel, die Kugel und die zvlindrische Scheibe als Sonderfälle des gestreckten und abgeplatteten Rotationsellipsoids. Dies sind Formen, die für Poren als besonders gute Näherung gelten dürfen (Abb. 5). Aber auch feste Einschlüsse zweiter Phasen lassen sich auf diese Weise für eine ingenieurmäßige Beschreibung hinreichend gut erfassen. - Ein weiterer grundlegender Vorteil des Modells liegt darin, daß die rotationsellipsoide Form aus Messungen im Gefügebild bestimmbar ist. Abb. 6 zeigt dieses Verfahren schematisch am Beispiel eines porösen Werkstoffes. Man mißt zunächst Fläche und Umfang eines Realteilchens und ersetzt dieses dann durch eine Schnittellipse, die das gleiche Verhältnis von Fläche und Umfang aufweist. Das Achsenverhältnis dieser Ellipse läßt sich auf statistischer Grundlage über stereologische Beziehungen in das mittlere Achsenverhältnis eines Rotationsellipsoids überführen, durch das die Poren des Werkstoffes substituiert werden. Mit dem Achsenverhältnis dieses Rotationsellipsoids, das dann ebenfalls bekannt ist (2), lassen sich jene Gefügeparameter bestimmen, die in Gefüge-Eigenschafts-Gleichungen auftreten. Ihre Ableitung ist an anderer Stelle beschrieben (7), (8), (14), (17). Sie ergibt, daß der Gefügeeinfluß auf die Eigenschaften durch drei Faktoren erfaßt wird:

den Formfaktor (F_D),

- den Orientierungsfaktor ($\cos^2 \alpha$) und
- den Phasenkonzentrationsfaktor (c_D).

In welcher Form diese Faktoren in Gefüge-Eigenschafts-Gleichungen erscheinen, ist in der folgenden Gleichung für die Leitfähigkeit poröser Werkstoffe gezeigt

$$\varphi_{\rm c} = \varphi_{\rm M} \left(1 - c_{\rm D}\right)^{\frac{\cos^2 \alpha - 1}{{\sf F} - 1}} + \frac{\cos^2 \alpha}{2{\sf F}} \qquad [4]$$



Formvariation des Rotationsellipsoids



Abb. 6 Die Schnittellipse eines Rotationsellipsoids (unten) und ihre Anpassung an reale Poren im Schnittbild (oben)

Handelt es sich beispielsweise um sphärische Poren, so vereinfacht sich die Gleichung dadurch, daß F = 0,33 und $\cos^2 \alpha$ = 0,33 gesetzt werden.

Die Gleichung heißt dann:

$$\emptyset_{c} = \emptyset_{M} (1-c_{D})^{-\frac{3}{2}}$$
 [5]

In Abb. 7 ist das Rotationsellipsoid-Einlagerungsmodell schematisch am Beispiel der Konzentrationsfunktion der Eigenschaften von zweiphasigen Werkstoffen wiedergegeben. Bei jeder Konzentration kann die Eigenschaft zwischen zwei äußeren Grenzkurven varijeren. Ihr tatsächlicher Wert hängt von der Gefügestruktur des Werkstoffes ab. Die äußeren Grenzkurven entsprechen extremen Gefügeanordnungen, in denen die beiden Phasen entweder parallel oder hintereinander angeordnet sind. Löst man gemäß Abb. 7 die hintereinander angeordneten Scheiben der einen Phase auf und lagert sie in die andere Phase als Matrixphase ein, wobei man die Orientierung beibehält, so ändert sich der Eigenschaftswert. Ändert man dann die Orientierung und die Form kontinuierlich über abgeplattete Rotationsellipsoide, Kugeln, gestreckte Rotationsellipsoide und nadelförmige Einschlüsse mit paralleler Orientierung, wie in bestimmten faserverstärkten Werkstoffen, so verschiebt sich die Eigenschaft von einer Grenzkurve bis hin zur anderen. Der Varia-



Modell der Gefüge-Eigenschafts-Abhängigkeit mit äußersten Grenzkurven I. Ordnung (8), (17)

tionsbereich zwischen den beiden Grenzkurven nimmt ab mit abnehmendem Unterschied zwischen den Eigenschaften der reinen Phasen (Fall B, Abb. 7). Für isotrope Werkstoffe sind inzwischen engere Grenzkurven abgeleitet worden (17), (18). Da in den Grenzkurven keine Form- und



Spezifische elektrische Widerstände von UO₂-Cermets mit Metallmatrix (8), (14)

Orientierungsfaktoren mehr erscheinen, ist ihre praktische Handhabung sehr einfach und insbesondere dann empfehlenswert, wenn die Grenzkurven relativ nahe beieinander liegen, sodaß die Ungenauigkeit gemessener Daten in dieselbe Größenordnung fallen würden. In Abb. 8 sind ge-



Quantitative Gefüge-Analyse =

Schematische Darstellung der quantitativen Gefügeanalyse

messene Werte des spezifisch-elektrischen Widerstandes von WC-Ag-Cermets mit den äußersten Grenzkurven und den Grenzkurven für den isotropen Werkstoff verglichen. Wie die Abbildung zeigt, werden die gemessenen Werte durch die Grenzkurven hinreichend gut eingebunden. Abb. 9 zeigt den Vergleich von experimentellen Werten des spezifisch-elektrischen Widerstandes für UO_2 -Cermets mit Metallmatrix mit derjenigen Kurve, die für sphärische UO_2 -Teilchen gilt (s. Gleichung [5]).

Abschließend sind in Abb. 10 die einzelnen

Schritte der quantitativen Gefügeanalyse bis hin zur Eigenschaftsbestimmung nochmals zusammengefaßt. Sie beginnt mit der Probennahme und Kontrastierung der zweidimensionalen Schnittbilder, ihrer optisch-elektronischen Vermessung und der Übergabe der Meßwerte in stereologische Rechenprogramme, aus denen die Gefügeparameter folgen. Mit ihnen ist die quantitative Beschreibung solcher Gefüge möglich. Sie sind so definiert, daß sie in einem weiteren Computerprogramm zur Eigenschaftsberechnung benutzt werden können.

Zusammenfassung

Die Anwendung der Bildanalyse in der Werkstoffforschung bildet die Grundlage für die quantitative Gefügeanalyse. Dabei ist unter quantitativer Gefügeanalyse die Kombination von Probenpräparation, optisch-elektronischer Messung und stereologischer Rechnung zu verstehen. In diesem Zusammenhang werden die Prinzipien der quantitativen Bildanalyse kurz beschrieben und ihre erreichbaren Genauigkeiten diskutiert. Aus den optisch-elektronischen Messungen können Gefügeparameter abgeleitet werden über stereologische Gleichungen, mit deren Hilfe die quantitative Charakterisierung der Werkstoffgefüge möglich ist. Diese Gefügeparameter gehen in Gefüge-Eigenschaftsgleichungen ein, welche die Berechnung von Werkstoffeigenschaften aus Gefügedaten ermöglichen und damit eine Erweiterung der Qualitätskontrolle durch quantitative Gefügeanalyse durch die Angabe von Eigenschaftsdaten darstellen.

Summary

The use of image analysis procedures in materials science forms the basis for quantitative microstructure analysis, where quantitative microstructure analysis means a combination of specimen preparation, microscopical measurement and stereological calculation. In that context image analyzing principles are described briefly and the nowadays obtained accuracy of microstructure analysis results is discussed. Direct and indirect microstructural parameters are described by stereological equations characterizing the material microstructural state. The indirect microstructural parameters provide calculations about the materials properties without measuring them directly thus increasing the efficiency of the quantitative microstructure analysis of engineering material. The present paper is a modified German version of the original in English in J. Chermant (ed), Quantitative Analyses of Microstructures in Materials Science, Biology and Medicine, Riederer Verlag GmbH, Stuttgart (1978), 103.

Résumé

L'emploi de l'analyse d'images dans la recherche sur les matériaux constitue la base de l'analyse quantitative de structure. On entend par analyse quantitative de structure la combinaison de la préparation des échantillons, de la microscopie optique et électronique et du calcul stéréologique. Dans cet ordre d'idées, on décrit les principes de l'analyse quantitative d'images et on discute la précision pouvant être ainsi obtenue. A partir des mesures optiques et électroniques, on peut calculer les paramêtres de structure en utilisant des équations stéréologiques permettant d'établir les caractéristiques de structure du matériau. Ces paramètres de structure sont introduits dans les équations de propriétés de structure permettant de calculer les propriétés des matériaux d'après les données sur la structure et d'élargir ainsi le contrôle de qualité grâce à l'analyse quantitative de structure et à l'indication de données sur les propriétés.

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EVENTS IN STEREOLOGY

Events sponsored by the International Society for Stereology

The following events have taken place since the last International Congress, Salzburg, September 1979:

- Course in Quantitative Microscopy, Department of Engineering, Chalmers University of Technology, Göteborg, Sweden, October 29 November 2, 1979 (H. Fischmeister and B. Karlsson).
- Symposium on Stereology during the Meeting of the American Association for the Advancement of Sience, San Francisco, USA, January 3-8, 1980 (H. Elias and E.E. Underwood).
- 3. Fourth International Course on Morphometry and Stereology, Kütai, Austria, January 12-19, 1980 (W. Pfaller and A. Reith).
- 4. <u>Course on Applied Mathematical Morphology</u>, Centre de Morphologie Mathématique, Fontainebleau, France, June 2-6, 1980 (J. Serra).

The following events are scheduled:

- Course on Quantitative Structure Analysis, Stuttgart, FR Germany, November 24-28, 1980, (Contact Dr. H.E. Exner, Max-Planck-Institut, Seestr. 92, D-7000 Stuttgart-1, Federal Republic of Germany
- Fifth Scandinavian Course on Morphometry and Stereology, Gausdal, Norway, January 19-23, 1981 (Contact Dr. R. Østerby, Anatomical Institute, Aarhus University, DK-8000 Aarhus, Denmark).
- <u>Third European Symposium for Stereology</u>, Ljubljana, Yugoslavia, June 22-27, 1981 (Contact Prof. M. Kalisnik, Institut for Histology and Embryology, P.O. Box 10, 61105 Ljubljana, Yugoslavia).
- Symposium "Stereology '82", July 5-7, 1982, Sheffield University S10 2TN UK, (Contact Dr. N. James).

More events are in the stage of planning (USA 1981, England 1982, USA 1983, various courses).

Other meetings of interest

The following events are scheduled by other Societies (Cooperation of the International Societies for Stereology is sought for):

- Image Analysis Techniques and Applications, Tuscon, Arizona, USA, January 6-9, 1981 (Contact Society of Photographic Scientists and Engineers, 1411 K Street, N.W., Suite 930, Washington DC 20005, USA).
- Sixth European Anatomical Congress (featuring special symposia on image analysis and morphometry), Hamburg, September 28 Oktober2, 1981 (Contact Prof. W. Lierse, P.O. Box 302360, D-2000 Hamburg 36, Federal Republic of Germany

ROUND TABLE MEETING OF THE COMPUTER IMPLEMENTATION GROUP.

- 1) It was agreed that there was a need for such a group in order to avoid reduplication of effort by the various interested members of ISS.
- 2) It was also agreed that members of the group should pool their various programs, with a view to combining the best features together to form a complete stereological package which would ideally contain
 - 1. Experimental optimization routines
 - 2. Counting routines, either manual and/or interfaced to digitizing equipment.
 - 3. A universally agreed format for data storage.
 - 4. Statistical routines.
- 3) Dr. Laurence Scales, who is a computer scientist, was appointed as coordinator. He was considered well suited because of his wide knowledge of programming language and associated implementation problems. An additional advantage is that Liverpool University has a fully equipped microprocessor laboratory. Thus there will be the opportunity to produce the final result on "burned" PROM chips.
- 4) All interested parties were requested to send details of their programs and hardware setup to Dr. L. Scales, The Computer Laboratory, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, U.K., by Christmas. This should be advertised in the Newsletter. This formation will then be made into a Register and recirculated. The best features of all the programs will be formed into an improved package design and sent to all interested members for comment and critisism by not later than 1980. It is intended that a working version of this package be produced by not later than September 1981 in time for a between congress meeting at a venue to be arranged.
- 5) It is intended that this work will be conducted in close collaboration with Dr. Grip from Uppsala and Dr. Hoppeler from Bern.
- 6) It was agreed that the official newsletter did not appear often enough to suit our purpose and that a bulletin should be sent to a readersship restricted to those directly involved. The cost of this could probably be met by the University.

Vyvyan Howard Laurence Scales Hans Hoppeler Sverker Griph