Stereology: Promise of a More Quantitative Microscopy

For the most part, microscopy has been a descriptive, rather than a quantitative, science. At the same time, however, a body of knowledge now known as stereology that deals with the problem of abstracting quantitative details of a three-dimensional structure from two-dimensional sections or projections has been built up over the last 125 years by geologists, mineralogists, metallurgists, biologists, and medical scientists, usually independently of one another. Stereology is not necessarily confined to microscopy. Astronomers, for example, have to deal with a two-dimensional projection of the sky. In the last decade, roughly since the formation of an interdisciplinary International Society for Stereology in 1961, developments in the topological analysis of structure, in mathematics and probability as applied to stereology, and in instrumentation have shown that microscopy can be more quantitative than it has been.

Metallurgists (most of whom are nowadays included in the discipline of materials science) have long been interested in quantifying the relation between the structure of a material and its properties. In 1848, the French geologist A. Delesse showed that the fraction of the total volume of a sample occupied by a constituent is equal to the fraction of the cross-sectional area occupied by that constituent. Since then, metallurgists and others using only a suitable number of measurements on two-dimensional sections have discovered how to determine this volume fraction, the surface area of particles in a sample, the total curvature of particles, the length of lines, and measures of particle size and spacing. Today metallurgical textbooks are filled with empirical correlations between properties, such as tensile strength or ductility, and stereological parameters, such as the volume fraction of a dispersed phase in a metal matrix.

Biological specimens are more complex than metallurgical ones, but are nonetheless susceptible to quantitative studies. For example, E. R. Weibel of the University of Bern in Switzerland, measured the surface area of oxygen exchange tissue in the lung and related it to maximum lung performance. And, according to A. V. Loud of the New York Medical College, Valhalla, New York, if a drug has the effect of quantitatively changing some aspect of cell structure, such as the surface area of a membrane, then stereological techniques can be used in conjunction with an electron microscope to monitor the action of the drug.

Empirical correlations between a metallurgical property and some stereological parameter stop short of fully elucidating the quantitative relation between structure and property that the metallurgist seeks. R. T. DeHoff and F. N. Rhines (and their students) at the University of Florida, Gainesville, have been combining the study of topological properties of metallurgical microstructures and stereology in order to develop such quantitative relationships. Measurement of topological parameters, such as the connectivity of a multiply connected structure, is tedious and time-consuming, because examination of serial sections is required. Thus the technique has not yet been widely applied.

High Temperature Grain Growth

So far, the Florida group has made such studies in connection with the sintering of metal powders and with grain growth in metals. (A metal is made up of crystalline regions of different orientations called grains. The grains are separated by grain boundaries.) In grain growth, which is detrimental to the high temperature strength of metals, a linear relation was observed between the average grain volume of aluminum and time by measuring (by serial sectioning) the average grain volume in samples that were heat treated for varying intervals at the same temperature. This linear relation was then derived theoretically from topological considerations of what must happen when one grain grows at the expense of another and of the effect of surface tension of the grain boundaries on the shape of the grains. Fitting theory to experiment required measurement of stereological parameters, such as total grain boundary curvature (techniques for these measurements were independently developed at Florida, and by J. W. Cahn at the Massachusetts Institute of Technology, Cambridge) and grain boundary surface area. DeHoff would like to apply similar techniques to biological problems, such as the progression of emphysema in the lung.

The accuracy and representativeness of stereological measurements depends on statistics. Several measurements are made in a microscopic field on a presumably randomly selected section (or projection in the case of a sample for transmission electron microscopy or some biological specimen slices), and several fields are measured. Researchers have found that the accuracy usually depends more on the number of fields measured than on the number of measurements in a given field. In recent years, increasing attention has been given to problems in stereology by mathematicians and statisticians. The work of one of these, R. E. Miles of the Australian National University, Canberra, is seen by some to have profound implications on the meaning of stereological measurements.

Miles astounded stereologists 3 years ago when he showed that all the basic stereology equations were special cases of equations in a generalized n-dimensional space. But beyond providing a kind of theoretical unity to what were previously separate relationships, Miles's work shows the critical role of randomization in the selection of sections for analysis, at least according to W. L. Nicholson of Battelle Northwest Laboratories, Richland, Washington. Nicholson, who is a mathematician interested in using stereology to study the properties of distributions of voids in nuclear fuel elements, maintains that Miles's rigorous treatment of randomization procedures indicates that stereology, as often practiced, can lead to badly biased results, particularly when only a few sections are observed.

In the early 1960's, automated machines began to appear with the promise of permitting the accumulation of a large amount of stereological data in a short time (thus relieving the drudgery of making rather monotonous measurements and presenting an opportunity for studies that had been too cumbersome because of insufficient time and lack of personnel). Unfortunately, this promise has been only partially kept. The largest single difficulty continues to be that of discriminating between objects in the field of view.

Machines discriminate by means of what is called the gray scale—that is, the difference between the intensity of light reflected or transmitted from different parts of the image. Since most samples are not well-defined composites of one light and one dark phase, and since machines cannot yet discriminate between objects as the human eye does (by features such as shape, orientation, and relative positions), the objects to be measured often must be identified by the researcher before the machine can be put to use freely. Nevertheless, sophisticated automated image analyzing equipment (both commercial and custom-built) is being designed and used.

In the latter category, for example, at Northwestern University, Evanston, Illinois, J. E. Hilliard is completing an automated system for studying metallurgical specimens. Machines such as Hilliard's typically are built up from commercially available components, including microscopes, electronic image analyzing units, programmable minicomputers, television-type displays, keyboards, and magnetic tape storage units. At Northwestern, a movable stage permits scanning the brightness of the reflected light as a function of position on the specimen. The brightness is measured by a photodetector. The system provides for interaction between the machine and the scientist. If the constituents of a sample cannot be discriminated by the photodetector because their reflectivities overlap on the gray scale, the researcher can signal the identity of the constituent by means of switches. The inexperienced operator can, on request, receive instructions (via a high-speed video display) for operating the instrument. All the information needed to begin data-taking is elicited by means of a dialogue with the operator. The machine can then accumulate data and compute stereological parameters, such as volume fraction, boundary surface areas, line lengths, particle size distributions, and orientation distributions. In addition, a running estimate of the statistical variance is printed out, so that data-taking can be terminated when the results are sufficiently accurate.

At the National Cancer Institute, Bethesda, Maryland, L. E. Lipkin and his associates have built an automated system for analyzing autoradiographs. These preparations provide a measure of the magnitude and rate of nucleic acid synthesis in cells (cell kinetics). When a nucleic acid precursor (thymidine) is labeled with tritium, beta decay of the tritium exposes individual grains of a photographic film which overlays the specimen on a glass slide. Lipkin's group uses a television-type camera to scan the microscope image. The Cancer Institute experimenters also designed their machine to focus the microscope successively at different depths within the film emulsion (optical serial sectioning). And the system is programmed to discriminate between exposed film grains due to beta decay in the cell nucleus and other randomly exposed grains (background). However, the operator must still manually select the cell to be examined. Extensive in-house modifications of hardware components and about 80 percent of the system software are directed toward the goals of user convenience and acceptance.

For the most part, stereology is a tool science which is used in a rather routine manner by researchers in various disciplines whenever they have a problem requiring quantitative measurement of structure in three dimensions from two-dimensional samples. A few investigators, however, are pushing the present limits of stereology into nonroutine applications and into new theoretical and instrumental capabilities.—ARTHUR L. ROBINSON

Additional Reading

1. *Journal of Microscopy* **95**, Nos. 1 and 2 (1972). These issues comprise the Proceedings of the Third International Congress for Stereology.
2. E. E. Underwood, *Quantitative Stereology* (Addison-Wesley, New York, 1970).

Undersea Storms: Experiment in the Atlantic

Water in the upper part of the North Atlantic circulates clockwise around the ocean basin, driven by winds. The flow in at least some deeper reaches of the ocean is thought to move in the opposite direction, driven by temperature differences. But if the overall pattern of motion is fairly well understood, relatively little has been known about more local oceanic phenomena, particularly those of the open ocean, until a recent experiment that for the first time mapped an undersea storm or eddy.

The effort, called the Mid-Ocean Dynamic Experiment (MODE), involved oceanographers from a dozen universities in the United States and Britain, half a dozen research vessels, and a new generation of oceanographic research instruments (see box). In addition to providing what is in essence the first three-dimensional weather map of a patch of ocean—data that is proving useful to oceanographers who are attempting to construct numerical models of the oceans—the experiment has established the importance of eddies in ocean dynamics. Indeed, MODE scientists believe that the eddies play a central role in determining the circulation of the open oceans comparable to atmospheric cyclones and anticyclones (low and high pressure systems) in determining weather patterns. If so, then understanding how eddies transport heat, momentum, and trace constituents of seawater will be essential to predicting such things as climatic change and the dispersal of pollutants in the oceans.

The eddy that fortuitously appeared in the MODE experimental area (Fig. 1) last year is the only one to have been studied in detail, but its general characteristics are believed to apply to eddies in other parts of the ocean. Eddies are roughly circular flows about 200 kilometers in diameter. They extend vertically throughout the entire depth of the ocean, although the motion is three or five times as strong above the thermocline—the region of large temperature gradients that effectively divides the ocean into a warm, salty layer overlying a cooler and less salty body of water.

With velocities of 3 to 5 centimeters per second in deep water and 15 to 30 centimeters per second nearer the surface, an eddy has more kinetic energy than an atmospheric storm and lasts many times longer—60 to 80 days, or even much longer, some oceanographers believe. (The time and also the space scales of oceanic and atmospheric eddies are more nearly comparable in nondimensional units that take ac-