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and

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PART I

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Part I contains papers of general interest, techniques, and physical applications.
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Part IV will contain papers on all topics which were processed after above parts were prepared.

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DATES OF FUTURE SEM MEETINGS:

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REVIEWING PROCEDURE
AND
DISCUSSION WITH REVIEWERS

Each paper in this volume contains a Discussion with Reviewers. This discussion follows the text and should be read with the paper. Each paper submitted to SEM, Inc. for publication is reviewed by at least three, up to an average of five, reviewers. The reviewers are asked to separate their comments from their questions. The comments are useful in determining the acceptability of the papers as submitted. Although the comments require no written response, in several cases, the authors have included responses to comments, or to questions phrased from, or based on, comments (either as a result of editorial suggestions or on the author's own initiative). Based on these comments approximately 15% of the submitted papers were not accepted for publication; while almost all of the others were asked to make changes involving from minor to major revisions.

The questions, for the most part, originate as a result of statements included in our cover letter accompanying each paper sent to the reviewers. The reviewers are asked to suppose they are attendees at a conference where this paper, as written, is being presented, and then ask relevant questions which would occur to them resulting from the presentation. From the questions so asked, some are not included with the published paper because the authors attended to them by text revisions. In some cases, editorial and/or space considerations may exclude inclusion of all questions asked by reviewers. The authors are asked to prepare their Discussion with Reviewers section in a camera-ready format in accordance with detailed instructions which they are sent by SEM, Inc. In some instances the authors edit the questions and/or combine several similar questions from different reviewers to provide one answer. While all efforts are made to check that the questions in the printed version faithfully follow the views of the specific reviewer, the editors apologize, if in some instances, the actual meaning and/or emphasis may have been changed by the author.

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Finally, readers are urged to be cautious regarding the weight they attach to the authors' replies, since the answers to the questions represent the authors' unchallenged views--except for minor editorial changes--the authors generally have the last word. Also, please consider that the questions were, in most all cases, relevant to the originally submitted paper, and they may not have the same significance for the revised paper published in this volume.

If you disagree with the results, conclusions or approaches in a paper, please send your comments, as a Letter to Editor, typed in a column format (each column is 4-1/8 inches wide and 11-1/2 inches long; i.e., 10.5 by 29.3 cm.). Your comments along with author's response will be published in a subsequent issue.

The editor gratefully thanks the authors and reviewers (see p. ix-xiv) for their contributions, invites your comments on ways to improve this procedure and seeks qualified volunteers to assist with reviewing papers in the future. (see p. xiv)

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*EXPLANATION OF THE TYPES OF PAPERS IN THIS VOLUME:

TUTORIAL: Presentation of established material in teaching format emphasizing techniques.

REVIEW: A review of the chosen subject with emphasis on author's own work, placing it in context with relevant literature and putting the topic in perspective.

Volunteers to prepare tutorial, review papers or bibliographies should contact Om Johari (see page ii).

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THE ANALYSIS OF ORGANIC SURFACES

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Abstract

The characterization of a surface involves more than just obtaining high resolution topographic images along the X and Y axes and the chemical identity of the surface layer of atoms. It should also include a co-ordinated in-depth analysis along the Z axis at as high a resolution as may be obtained with a particular form of instrumentation. Surface characterization techniques may be divided into those methods covering microtopography and those involved in chemical analysis. The former category is served by optical microscopy and scanning and transmission electron microscopy while the latter techniques are based on excitation of atoms and molecules at the surface by photons, electrons and ions. A surprisingly large number of techniques are available for surface analysis, although the properties of most organic samples prevents all these techniques being used. Optimal specimen preparation is critical in order that these surface characteristics give adequate information, and cryotechnology plays a central role in specimen preparation, examination and analysis.

KEY WORDS: Morphology, analysis, low temperatures, electrons, photons, ions, lasers, organic biological

Introduction

"We live in an age of surfaces" (Oscar Wilde).

Although we live in a complex, three dimensional world lit from above, our cognition of this environment takes place by a process of visual perception of interlocking two dimensional surfaces. Through experience, we can recognize patterns and shapes, and because we possess the remarkable facility of binocular vision, we are able to synthesize a series of planar surfaces into familiar three dimensional shapes. In many instances, and in particular in microscopy, we are able to codify and recognize new images which we have never seen before. No object is mysterious, the mystery is in our eyes. With a few exceptions, we are unable to see below the surface of natural objects, yet our experience tells us that many of the sub-surface features of an object are important in determining the surface characteristics which are presented to us. Organic surfaces, and in particular biological surfaces, are heterogeneous and generally more complex than inorganic surfaces. The surface of a block of metal, a crystal of an inorganic salt or a piece of concrete appear the same from whichever direction they are viewed, although important sub-microscopic and crystallographic differences do exist. If we were to section or fracture these homogeneous materials the internal surfaces we reveal are, at a macroscopic level, identical to the external surface. This is not true with many organic materials and much of their functional significance can be related to the internal surfaces which are normally hidden from our view.

An analysis of organic surfaces must necessarily look below the surface and it is important to consider some of the ways we can expose internal surfaces without introducing artefacts during the preparative procedures. These manipulations are particularly important in the examination of biological surfaces, and great care must be taken in converting or replicating the specimens into a form which is conducive to the alien environment of the systems used to image and abstract the information.

It is convenient to consider the properties of surfaces from the point of view of their morphology and their composition. The

morphological features of a surface may be divided into an examination and measurement of the micro-structural features (topography) and a quantitation of their inter-relationships (topology). This morphological information is essentially two dimensional and is frequently represented as a series of picture points along the X and Y coordinates. The real surface, as seen by our eyes, and images of the surface (photographs, drawings, maps etc) only becomes three dimensional because our previous experience in pattern recognition tells us that the various surface features are separated along an axis normal to the mean depth of the surface. This recognition is usually quite easy in the real world but may become problematic when we examine images of unfamiliar objects, for here the third dimension may not be directly observed but only perceived. In these circumstances it is essential to subject the planar surface projections to stereo-pair analysis, whereby it is possible to more easily perceive the distances between points on a non-planar surface. Stereogrammetric techniques are the only way in which accurate measurements may be made on non-planar surfaces.

The compositional analysis of a surface must necessarily delve below the surface although it could well be argued that the wavelength contrast (colour), albedo (solar reflectivity) and specular features (shininess) can give us compositional information about an object (the shiny yellow colour of gold is a good example). The depth analysis may be confined to the first few atomic layers of the surface, or penetrate several micrometres below the surface. The information which may be gained can be related to the elemental, molecular and even macromolecular composition of the surface.

Although it is convenient to consider morphology and composition separately, they are in reality very closely linked. This link sometimes puts very severe restraints on the effectiveness of the imaging systems for while one particular technique may give accurate compositional information the very act of quantitation may destroy the specimen. The obverse is also true, and in most instances the process of preserving and subsequently observing may only be achieved at the expense of the natural composition of the object. This paradox, "the very act of observing introduces artefacts", is alas one of the problems we have to accept in the microscopy and analysis of organic materials.

The discussion in this paper is limited to a consideration of the in situ analysis of organic samples, as they usually present greater problems in preparation, examination and analysis than do their inorganic counterparts. Biological specimens present the most severe challenge to the microscopist and analyst, because they are nearly always highly hydrated. The presence of water is less of a problem in the other types of organic samples, such as natural and artificial polymers, and elastomers, and the low water content and structural characteristics of some of the more robust plant and animal products (chitin, wood, bone, teeth etc) is such that they require little preparation prior to examination in the microscope.

Analysis of surface morphology

The structural features of the specimen surface may be readily localized using a beam of photons or electrons. Although other imaging systems exist, for example those based on sound waves, X-rays and ions, they do not at present give any more information than can be achieved using photons and electrons. An interesting exception to this may be found in ultra-soft X-ray microscopy, which although not a new technique, has recently become a subject of renewed interest (Parsons, 1980). The X-ray imaging depends on the differential absorption of X-rays in the wavelength range 1-10nm, by the various components in the specimen. Recent work by Rudolph et al (1980) has shown that it is possible to obtain high resolution (70nm) images of organic material using zone plate optics in conjunction with a high X-ray flux. Alternatively the image need not be viewed directly but a replica is formed on a photo-resist by the X-rays which pass through the specimen. In areas of high mass density the X-rays are absorbed and these regions appear in high relief after the photoresist is chemically developed and examined in an SEM. The spatial resolution varies but can be as good as 5nm. The advantages of the system are the reduced specimen damage and the ability to examine hydrated samples.

This procedure is quite distinct from direct X-ray microscopy which although giving information from wet specimens up to 5μm thick can only produce images up to a magnification of x200. The only advantage of acoustic microscopy, in which acoustic waves are reflected or deflected by variations in specimen density, is that it can only be carried out on unstained specimens immersed in water. The resolution is consequently no better than 2.0μm. By using cryogenic liquids (Rugar et al,1980) instead of water it has been possible to obtain a spatial resolution of about 100nm.

The limited resolution of light microscopes is the only restraint which can be put on this form of imaging system. If we use light in the visible range we are able to obtain information about the surfaces of living, physiologically intact specimens. The information is presented to the observer in both wavelength and amplitude contrast, albeit at somewhat limited spatial resolution (c. 100-200nm). The process of information transfer does not appear to damage or unduly perturbate the organic specimen and we assume we are observing the specimen close to its natural state.

In order to increase the spatial resolution of the imaging system, we must use an illuminating system of much reduced wavelength. High energy electrons are a convenient imaging system and although the spatial resolution is increased to a few tens of nanometres, the use of electron beams creates serious problems for many organic specimens. The short mean free