DIRECTIONS IN SCANNING ELECTRON MICROSCOPY

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The famous French mathematician, Henri Poincaré, once said that it is by logic that we prove but by intuition that we discover. The scanning electron microscope (SEM) can be helpful to both processes.

As an analytic tool, the SEM can be applied in the logical, precise modes of classical science and as a device for imagery the SEM can provide us with the experiential contact that is necessary for intuitive response by the observer.

The SEM has developed under the needs and limitations found in light microscopy and conventional electron microscopy and it is useful to consider the SEM in an historical context in order to understand better its unique advantages.

The light microscope has been limited by resolution but possessed capabilities for very high information content imaging. With the development of the conventional electron microscope, the resolution was very much improved but some of the information content of the image was lost. Where visible light interacted with the specimen at the chemical bond level, electrons interacted at the elemental or atomic level. This meant that the basic chemical nature of the specimen was not revealed by electron microscopy (EM) and that the number of specific stains available for conventional EM was very much reduced as compared to light microscopy.

A second limitation of conventional EM is the result of the non-penetrating nature of the electron beam. The specimen for conventional EM must either

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be very tiny (macromolecules, viruses) or the specimen must be cut or sectioned into very thin slices.

The light microscope, at high resolution, was limited by optical sectioning (narrow depth of focus) but such optical sections could be reconstructed serially by changing the focus up and down through the specimen. In the conventional EM the sections are actual physical slices and their reconstruction to the three dimensional form of the specimen is a very difficult and time consuming process.

As long as the two functions of the microscope, localization and information transfer, are carried out by the same radiation, both resolution and information are necessarily linked to the physical characteristics of this radiation. Both the light microscope and the conventional electron microscope utilize such a coupled, spatially focused system. It is possible however, to design an image-forming system that allows resolution and information content to be uncoupled; that is, one form of radiation may be used to localize and another form used to carry information about that point. We may localize or address this point in time rather than in space and by this process allow a separation between the localizing or probing radiation and the signal or information radiation. This is essentially the basic characteristic of the image forming system of scanning electron microscopes and to a large extent accounts for the usefulness of the SEM. We may, for example, probe with an electron beam and use the visible light induced by this beam at each instant as the video signal. This mode of operation (cathodoluminescence) utilizes some of the higher resolution capabilities of electron optics plus some of the higher information capabilities of visible light

production at the specimen.

Instead then of either choosing between the light microscope with its high information and low resolution or the conventional EM with its high resolution but rather low information, the SEM can be placed somewhere in between with higher resolution than light microscopy and higher information content than conventional microscopy.

The SEM in no way replaces the other instruments and we should resist the temptation to think that a newer instrument is necessarily a better instrument. The SEM complements the other image forming systems and is not to be considered as being in competition with them.

It would be very desirable if the SEM could be available for personal use by the investigator in a manner similar to the availability of the light microscope. The difficulties associated with a remote operation where the researcher leaves his samples one day and picks up the micrographs a week later are obvious. But even the degree of lost contact associated with working "over-the-shoulder" of a technical operator can be quite serious. There is a considerable loss of time as the researcher tries to direct the operator towards the appropriate field, magnification, and other parameters associated with SEM viewing and photography. Also, remote operation reduces the degree of experiential contact between the researcher and the specimen under study and makes intuitive processing very difficult.

When the SEM was first introduced as a commercial instrument, there were several factors which reduced the possibility for direct contact between researcher and instrument. First, the price of the unit was quite high, nearly twice that of a conventional EM. Very often an individual program or even a

single department could not justify such a large outlay of funds particularly at a time when applications of these instruments were still in an experimental stage. Several departments, however, might pool their resources and purchase an SEM which would be operated on a shared basis. While such arrangements have been quite productive, operations through a shared, central facility tend to separate the individual researcher from direct contact with the instrument, at least for major portions of his time.

Besides the large initial cost, another economic consideration was the concern that in this rapidly developing field, the instrument purchased would become obsolete in six months and that up-dating might be very difficult.

A third factor which discouraged direct participation was the complexity of the instrument. Many researchers, particularly biologists, felt that these original instruments had been designed by physicists and electrical engineers and that you had to be practically an electrical engineer in order to operate one.

Along the same lines, there were questions in the minds of many concerning down-time and maintenance. Would the instrument produce reliably and if anything did go wrong, could it be fixed promptly without extensive in-house electronics facilities?

With all of these considerations, it is not surprising that personal contact with an SEM has not been very widespread.

One of the most important directions in SEM instrument design is to work to overcome these barriers. The goal is to produce an instrument which is economically feasible, technically excellent, and humanly attractive for large numbers of individual researchers.

Scanning electron microscopes are now available at prices much closer to the familiar range of conventional electron microscopes. In addition, modular design allows for up-dating and extension of operating capabilities at any time. These developments place the SEM within the reach of many more individual research programs.

In order to reduce operating complexity, many automatic features are now available. Contrast and brightness levels which are important both in viewing and recording, can be kept at appropriate levels automatically. Focus can be controlled dynamically and the entire photographic process can be automatic resulting in substantial savings of time and material.

It is often desirable to change accelerating voltage, particularly as it effects specimen charging artifacts. The relationships between accelerating voltage and focus, magnification (field) and other image parameters can also be automated allowing for easy and rapid changes to the appropriate potential.

All of these automatic features can help to reduce the complexity of operation and make the instrument available to the researcher with a minimum of specialized training. Combined with simplified alignment and improved console and stage controls, these features make the newer SEM instruments inviting rather than intimidating. Such an instrument becomes a positive motivation for productive research.

Another significant direction in SEM development is the extension in number of operating modes. The familiar secondary electron signal is now augmented by additional analytic signals which measure a variety of geometric, chemical and electrical properties of the specimen.

It is very convenient to be able to detect and display two or more analytic signals simultaneously and such correlated multiple analyses are a powerful tool for the characterization of the physical properties of the specimen.

The analytic, objective approach to gaining an understanding of the specimen remains the surest, most reliable channel of information transfer. The ability to abstract from the world of existence to the world of ideas where controlled manipulation is possible has been a proven asset to the scientist since the time of Plato. Although certain limitations to an exclusively objective approach will be discussed below, it is through careful application of analytic techniques that our foundation of proven knowledge is acquired.

The SEM offers a choice of information signals each of which can be related to a specific set of specimen parameters. We will consider secondary electron, visible light, x-ray, Auger electrons and specimen induced current as examples of the kinds of analytic signals which can be utilized by the SEM.

The most often used information signal is that of the low energy electrons leaving the surface of the specimen. The secondary electron signal is strongly influenced by the angle between the probing beam and the specimen surface at each instant and can thus be used to determine the shape distribution of the matter of the specimen. The ability to determine the morphology of the specimen is probably the most familiar SEM analytic technique with both topographic and topologic geometries utilized to assess stereometric data.

Information concerning the chemistry of the specimen can also be obtained by SEM techniques. At the atom or chemical element level, both characteristics-

x-rays and Auger electrons have been counted and used as the video signal. When the SEM is used in the x-ray mode, it performs in the same manner as the electron microprobe, which has been found so useful for elemental analysis particularly in the physical sciences. In either instrument, the two questions that arise are: what is the smallest volume which can be analyzed and what concentration of the element can be measured in this volume. If we are able to make the volume analyzed small enough, perhaps we may find high local concentrations of even the trace elements and this possibility is particularly attractive in biology.

The biological application of elemental analysis by SEM is also often concerned with the low atomic number elements where the characteristic x-ray yield is small. In this case, elemental analysis by Auger electron spectroscopy may provide an increased signal since this type of induced radiation is more suitable for analysis of the light elements. Auger spectroscopy also offers the possibility of very accurate depth determination of the location of the chemical elements. Surface chemistry in layers of less than 10 Å might be possible.

Some materials will produce visible light when bombarded by the probing beam of the SEM. Such cathodoluminescence can be counted and used as the video signal. In contrast to the characteristic x-ray or Auger signal, cathodoluminescence is not in general a function of atomic or elemental structure but is more a reflection of the molecular or solid state properties of the specimen. Cathodoluminescence offers a possibility of extending chemical analysis further than the determination of which chemical elements are present, to a picture of the chemical bonding which tells us how the atoms are

put together. While cathodoluminescence holds considerable promise for the future, certain technical limitations associated with excitation, permanence of fluorescence, specimen damage and resolution have made its use to date relatively minor.

The use of a modified current flowing in the specimen as the video signal has found many applications in electrical engineering studies of semiconductor device performance and circuitry. The current flowing in the specimen is a function of the particular spot being irradiated by the probing beam. This specimen induced current modulation can be used to present the electrical properties of the specimen as a picture or can be analyzed quantitatively.

Careful preparative procedures are often the secret of successful SEM analysis. Methods include dissection, fixation, dehydration (freeze-drying, critical point drying) and techniques associated with improving the specimen surface conductivity. A particularly attractive technique to biologists would be to retain specimen water in the frozen state and examine the specimens in the SEM using a low temperature stage.

Any of these preparative steps can introduce unwanted artifacts and careful attention at each stage of the process is essential. Recognition of artifact is sometimes aided by preparing the sample by at least two independent methods.

Micromanipulation in the column of the SEM is a kind of on-going preparation in that the specimen can be altered while observation is in progress. A micromanipulator utilizing two independently controlled piezoelectric needles has been developed by Pawley which can position the needles to within 0.1 microns over a range of 100 microns. The use of microdissection in

the column of the microscope to expose deeper laying layers of tissue or other material has been found to be of considerable value.

The analytic process can be extended to signal processing and display modes. Among processing modes that have been successfully utilized are derivative signal display, deflection modulation and color modulation. Each of these modes of signal processing can make analysis more readily available to the observer, and the modulation devices while clearly introducing an artificial parameter, have been utilized to improve the analytic performance of the SEM system under certain specialized requirements.

The final step of analysis is that analytic technique which occurs in the mind of the observer. The mathematical analyses of SEM data has progressed in the areas of metric geometry (topography), stereometric analysis, enumerative geometry (topology), and in the computational processing associated with pattern recognition and psychopictorics. The analysis of data from the image by mathematical techniques allows assimilation of the most important characteristics of the system in the mind of the observer. Such analysis has been particularly valuable where the parameters of metric geometry have been invoked.

Computational methods associated with pattern recognition are somewhat less well developed and may in general be qualified by the difficulty of computer pattern recognition. Although the computer's forte is not at the moment in the realm of total pattern imagery, the continued advances along the lines of psychopictorics as carried out by computational means point towards a more exact and objective method of analysis of even rather intricate relationships. Mathematical analyses of SEM image data is still a relatively new and growing field and one in which the promise over the next few years seems very encouraging.

The various modes of analytic operation of scanning electron microscopy have very great advantages. However, it is not always possible to apply total analysis to some of our most pressing problems. The analytic, objective approach while being the foundation for scientific progress, has not been without its critics. Perhaps the most eloquent is Kierkegaard, who commented on the limitations of the ~~subjective~~ objective approach to our understanding of reality. Thus if we are to be engaged in discoveries, we should make ourselves available to the kind of contact with the system under investigation that will yield intuitive responses.

If we are to bring our intuitive capabilities to bear on problems in microscopy, we must have instrumentation that provides the kind of contact with the specimen which imitates our contact with the large world around us. Intuitive response by its very nature is a subjective kind of interaction and depends very much on each individual rather than on the application of a system of ideas.

In the most frequently utilized mode of operation, that of secondary electron video signal, the SEM can provide us in a limited way with an extension of our visual senses. It is this experiential contact in part, that accounts for the impact of scanning electron micrographs and forms a major part of the advantage of this form of microscopy. At the same time such contact must also be looked at carefully as to its limitations. The scanning electron micrograph from a secondary electron signal utilizes only two of the four or more visual codes for depth and shape which we utilize when we view the world around us. In such a limited contact there are bound to be many instances of ambiguity and misinterpretation. As an example, if we eliminate the binocular code from

our macroscopic vision by placing a patch over one eye, we still can utilize many of the codes for depth and shape - we can recognize a chair, a friend, we can move about in our environment, we can even drive a car - but we have increased our chance for error and we have increased the number of cases where ambiguous information will be presented to the individual. If we extend this to the microscopic world we find that in utilizing the SEM (in spite of the realistic appearance of its images), there are only a very few of the total number of codes being utilized and therefore the possibilities for misinterpretation are very great.

A second extension of the senses can be gained by the introduction of micromanipulation needles as a part of the instrumentation in the SEM column. We can, to a small degree, carry out that basic urge to reach in and take something apart as we try to understand its form and function. Micromanipulation within the column of the instrument requires that we are able to visualize dynamic events and thus implies a scan rate which can be rapid enough for flicker-free presentation of the information. Thus a TV scan rate and TV recording system is necessary for the operation of micromanipulation or microdissection.

The final step in the imagery of the SEM is concerned with the perception of the observer. Rudolf Arnheim has suggested three types of perception.

First, the "peep-hole" or camera perception where the individual does not separate between the object and its context. Such perception could be represented in art by the approach that would mask out all of the surroundings except for a very small point and take the exact description of each point as a representation of the entire image. Each point looked at in this peep-hole

manner is completely separated from its context because there is no recognition of context as separate from the object itself. It is this kind of perception that is carried out by a camera, for example, where there is no distinction made between context and object and where context is assigned a position as part of the object under study.

The second kind of perception might be described as scientific or practical perception. In this kind of perception the observer attempts to strip off context as an unwanted part of the image and tries to extract the idea or essence from the image according to a pre-arranged set of standards. This kind of perception might be represented by the housewife who observes meat in the market under a known red light source. As she perceives the image she mentally strips away the red light because practically what she wishes to know is whether or not the meat's color would appear gray or washed out in normal white light. To the scientist, the traditional approach has been to reduce or abstract the essential qualities of the object and to try to eliminate (or at least control in a fixed manner) all environmental or contextual qualities associated with the object.

The third kind of perception might be described as artistic or contextual perception and in this form of interaction the observer welcomes the context of the object as a useful tool to be manipulated in order to reveal subtle qualities of the object under investigation. The artist has long utilized such manipulation and a particular example might be the Impressionist School of painting, which utilizes changing light as a contextual variable to reveal the essential characteristics of the scene being represented. Thus the Impressionist might paint the same cathedral in the morning, at noon and ~~at night~~ in the afternoon and in

this way try and reveal the nature of the specimen by consciously and willfully changing the contextual variables associated with it.

It is this third type of perception that can be useful in scanning electron micrograph interpretation but is often overlooked in favor of the second or classically scientific approach. However, the techniques of art should be recognized as an additional battery of tools that can aid man in his attempt to understand his surroundings.

Often it has been emphasized that it is important for the scientist as an individual to be culturally aware of the arts, but it is less often emphasized that he can utilize art in a fundamental way in the practice of his own profession of science. Art has sometimes been utilized as a kind of audio-visual window dressing for analytic data, but this kind of presentation cannot take the place of a fundamental application of artistic technique. Once data reduction has taken place - once the specimen has been reduced to a system of ideas, no amount of artistic presentation methods can ever replace the opportunity that has been lost. If we are to utilize the techniques of art, for example the Impressionist School, we must apply them before or in place of the objective, analytic techniques that we are so comfortable with. We must be willing to explore the value of a changing context; to explore the value of purposely deforming a specimen or an image in order to gain a reasonable assessment of whether or not such techniques may in fact allow us to gain a broader understanding of the specimen under study.

The scanning electron microscope is a powerful analytic tool and can also allow for a certain amount of intuitive, subjective contact with the microscopic specimen. In using the SEM we have the happy opportunity of engaging not only our systems of mathematics, physics, biology, etc., but also something of ourselves as persons.

Red blood cells, lyphocytes and platelets. 11,000X.
Photo: B. Wetzel and W.Lewis, NIH, Cancer Institute.

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Scanning Transmission of a 1μ section of anterior pituitary cut from epoxy embedded material, no staining was used except osmium fixation. 30 kV. 4,000X. Photo: B. Smith, Eli Lilly and Company.

Editor: please remove the Etec logo from the micrograph we are using, also the number 4.

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(A) Foraminifiera. 100X. (B - C) Coccolith on the surface of the foraminifera. 500X and 5,000X. Photo: H. Turnbull, Imperial Oil Ltd.

10,000 X

5000 X

2000 X

Porqupine Quill. Photo: W. Ward, U.S.D.A., Western Utilization, Research and Development Division, Albany, N.Y.