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MASTER

TECHNICAL PROGRESS REPORT

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Covering

Electron Microscope Studies

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TECHNICAL PROGRESS REPORT

A. Progress on the 5 Å Microscope

At the time of submission of the last progress report we were heavily engaged in studying the visibility of single atoms. Let us briefly review the method that was used in these experiments.

It had already been shown that the resolution of the microscope is about 5 Å, which is close to our calculated theoretical limit of 4.6 Å at the operating voltage of 30 kilovolts. The 5 Å resolution represents the size of the focused spot of electrons as it scans across the specimen. As the electrons leave the specimen they can be thought of as consisting of three distinct groups.

The first group consists of electrons which have not been scattered at all by the specimen and therefore carry no information.

The second group consists of electrons which are elastically scattered by the atoms in the specimen. These electrons are scattered through angles which are large compared to the incident cone of illumination. Specifically the characteristic angle of the angular distribution is of the order of 50 milliradians for carbon and 100 milliradians for uranium, while the incident cone of illumination subtends an angle of about 10 milliradians. One can conclude, therefore, that 90 to 95 per cent of the elastically scattered electrons are scattered outside the region which contains the unscattered electrons. To obtain the number of electrons which are scattered we used the theoretical results of Lenz to derive an equation for the fraction of

electrons which are elastically scattered as

$$f_e = \frac{I_e}{I_o} = \text{fraction scattered} = \frac{46.5\ Z^{4/3}\ n}{V\ \sigma_b}$$

where I_e is the intensity of the scattered electron beam, I_o is the intensity of the incident beam of electrons, Z is the atomic number, n is the number of atoms contained within the incident beam, V is the accelerating voltage in volts, and σ_b the area of the focused spot of electrons in square angstroms.

The third group of electrons consists of those electrons which have lost energy in passing through the specimen--that is, they have been inelastically scattered. In this case the characteristic angle of scatterjng is very small, being characterized by the ratio of the energy loss to the total energy. As the average energy loss is about 20 volts and the incident energy is about 30,000 volts, this characteristic angle is less than one millIradian, which means that the majority of inelastically scattered electrons are contained within the incident cone of illumination. The fraction of electrons which have been inelastically scattered can be obtained theoretically and is

$$f_I = \frac{I_e}{I_o} = \frac{880\ Z^{1/3}\ n}{V\ \sigma_b} \quad .$$

The use of the scanning microscope allows one allows one to physically separate these three groups of electrons in a way which cannot be done in the conventional electron microscope. In order to detect the elastically scattered electrons we place an annular detector below the specimen whose dimensions are chosen so that the central hole in the detector allows the unscattered and the inelastically scattered

electrons to pass through. The outer diameter of the annular detector subtends an angle of several hundred millliradians so that this detector then produces a signal which, with only a few per cent error, represents the fraction of electrons which are elastically scattered.

Electrons which pass through the hole in the annular detector are then allowed to pass through an energy selecting spectrometer which physically separates the inelastically scattered electrons from the electrons which are unscattered and therefore retain the full energy of the incident beam. A suitably placed detector will then produce a signal which is within a few per cent error equal to the fraction of the electrons which have been inelastically scattered. In order to eliminate the problem of having to determine the exact value of the intensity of the incident electron beam a normalization procedure is used whereby during the "fly back" of the scanning system the voltage on the spectrometer is readjusted so that the unscattered electron beam falls for a brief period of time on the inelastic electron detector, and this signal is used for normalization so that the two detectors, the elastically scattered detector and the inelastically scattered detector, produce a signal which is exactly proportional to the fraction of the scattered electrons rather than an absolute number.

The ability of the scanning microscope to produce these two quantitative measurements in the microscope specimen leads to a number of very important applications of the electron microscope which may very well place a new perspective to the kinds of quantitative measurements which can be made on biological specimens.

We are now in a position to make two specific measurements on every point on the specimen. Looking at the equations which these measurements represent, one can see that there are just two unknowns in those two equations, namely the atomic number and the number of atoms. We therefore have two equations with two unknowns that can be solved either digitally or with an analog device, to provide us with the average value of Z at every point on the specimen and the average value of N at every point on the specimen. We note that if we know Z and assume that the atomic weight A is equal to 2Z, which is surely valid for virtually all biological specimens, then we are in a position to measure directly the molecular weight per resolution element in the microscope and by suitable integration procedures we are then able to measure the molecular weight of an object in the electron microscope. It should be perfectly possible to provide a display on the electron microscope which represents either the average value of Z everywhere on the specimen or the molecular weight. In other words, we can now raise electron microscopy to a new level of quantitative importance. Except in a few instances electron microscopes are almost exclusively used for pictorial information which is qualitative in nature, but now we are in a position to make it quantitative. It is precisely these aspects of the electron microscope which we have been investigating during the last year.

(1) Investigation of the visibility of single atoms.

We are extremely interested in the use of single heavy atom markers for use in studies of DNA, RNA, proteins, etc., and at the time of submission of the last proposal, we had succeeded in showing

that uranium and thorium atoms were visible in our scanning microscope. The calculations of the expected visibility rely upon the equations given above, and the one perturbing feature at that time was the fact that the visibility of the atoms was approximately twice the expected value. Since that time we have spent a considerable amount of time investigating the experimental procedures and the theoretical foundations of this single atom visibility. The problem finally resolved itself when we discovered that the theoretical formula given above for elastic scattering is wrong in the case of very heavy atoms. This is because an approximate wave function was used for the electron distribution in the atom, whereas the correct wave function can be obtained by the Fermi-Thomas method. The Fermi-Thomas yields an expression for the elastic scattering cross section which is almost exactly twice that given above; specifically, the constant 46.5 is replaced by a value of 88 in the Fermi-Thomas theory. All other evidence points to the fact that the Fermi-Thomas approach is the correct one for heavy elements, so that the experimental factor of two discrepancy is now fully explained. We intend to complete this work by measuring the visibility of other heavy atoms, hopefully as low in atomic number as Mercury. We will attempt to measure the scattering cross sections for these single atoms in order to compare them with the Fermi-Thomas theory.

One of the graduate students (Langmore) is pursuing this line of work by cooperating with a member of our faculty, Dr. Cozzarelli, in preparing single atom stains for DNA. At the moment it looks

possible to provide reasonably specific staining for the various bases which would thereby allow us to begin the work on DNA base sequencing. A great deal of time during the last year has been spent on preparing very clean carbon films with a minimum of heavy atom contamination and devising methods for removing residual heavy atom stains on the DNA.

(2) Viruses.

The other student involved in the 5 Å microscope (Wall) is now in the process of completing his thesis on the study of the fd phage. This is a small filamentous virus some 8800 Å long and is thought to consist of a double strand of DNA in the form of a loop with a protein coat. The molecular weight is about 11 million Daltons. This is an ideal virus for our purposes because it allows us to test our ability to measure molecular weights and to confirm or otherwise the structure of the virus. The virus can be obtained in a purified form with very little residual salt bound to it. Experiments so far have shown that the virus can easily be seen, the molecular weight as measured in our microscope agrees with the molecular weight from ultra-centrifuge measurements, and the DNA from the virus can also be readily seen. However, we also see virus-like particles whose length is the same as the FD phage but whose molecular weight is one-half that of the phage, indicating that the phage has divided into two equal sections. This is somewhat difficult to explain unless there is a mechanism whereby the DNA is split into two single strands. This aspect will be studied within the next few weeks.

(3) Additional work in progress on the 5 A microscope is concerned with the use of the scanning microscope for the study of thick specimens. It seems clear from our analysis that the scanning microscope should be able to look at specimens which are considerably thicker than those which can be used in the conventional microscope. We are at the moment studying this aspect both theoretically and experimentally. This work may be of considerable importance because if it should happen that we can indeed utilize much thicker specimens than can be used in a conventional microscope there would be less pressure on the current vogue of using very high voltage electron microscopes.

B. The Gun Microscope

The work during the last year has been concerned almost exclusively with the study of electron energy losses in the various DNA bases. Accurate energy loss spectra have now been obtained both in the low energy region and in the region near the carbon X-ray line and the nitrogen X-ray line.

In the low energy loss region between 0 and 40 v there is considerable energy loss structure from the bases. This will be compared with existing ultraviolet observation data and in addition we hope to utilize the synchrotron radiation of the Wisconsin accelerator to extend the known ultraviolet observation data down into the region several tens of volts. We will then be able to compare the energy loss data with the ultraviolet observation data.

The energy loss spectra in the region of the carbon and nitrogen X-ray lines shows a considerable amount of detailed structure which as yet is not fully understood. We will of course attempt to interpret this data in the near future.

This type of data is also being used for the study of radiation damage by the electron beam. This type of study is of great importance in electron microscopy because we need to understand the stability of the specimens which we are looking at. We study the radiation damage by observing the decrease in intensity and ultimate disappearance of the detailed structure of the energy loss spectrum. These results are of considerable interest because they show that the bases vary considerably in their ability to withstand the radiation damage caused by the electron beam. This work at the moment has been attempted at room temperature and at liquid nitrogen temperatures. In the near future we hope to extend the work to liquid helium temperatures. The two students (Isaacson and Johnson) who are involved in this work are currently completing their theses and should then be in a position to write several papers on this subject.

C. Electron Holography

This work has been pursued by one student (Saxon) and has now progressed to the point where electron holograms have been obtained. The holograms are made by taking the electron beam which emerges from a field emission gun and dividing it into two beams with the aid of an electrically charged wire. The specimen is placed in one of the beams, and the two beams are then allowed to interfere. The resulting holograms

contain several hundred interference fringes and have been successfully reconstructed. The resolution of the system, however, is not very high, principally because the lenses which are used are not of very short focal length. The aim of this work is to determine whether or not the spherical aberration of the lenses can be corrected in the subsequent optical reconstruction. A scheme has been devised whereby an electron hologram will be produced and will contain a significant component due to spherical aberration. This hologram will then be optically reconstructed and the spherical aberration removed during this reconstruction process. If we can show that this is possible it should then be possible to extend this work to very high resolution. The principal difficulty in extending this work to very high resolution will be that of providing very good magnetic shielding from the stray AC magnetic fields in our laboratory.

D. 100 kv Microscope

We have been heavily engaged in constructing the 100 kv microscope which should have a resolution of 3 Å or less. The current state of this microscope is that the electron gun has been constructed and vacuum obtained in that gun, and it has successfully withstood the application of the full high voltage. This is a non-trivial success, because the ultra high vacuum which exists in the electron gun makes it much more difficult to apply high voltage. However, the technology which was acquired in the other microscopes has proved successful. At the moment the objective lens is being constructed in our machine shops together with the specimen stage. When this lens and stage has been completed it will be attached to the microscope and we will operate the microscope

using elastically scattered electrons. A subsequent step will involve placing a spectrometer below the objective lens in order to be able to utilize inelastically scattered electrons. The many current and voltage supplies associated with this microscope have been built and tested, and perhaps one of the major achievements of this work is that all these supplies are operating at a stability level of one part per million for 30 minutes. This should be more than adequate to obtain resolution of 3 Å.

E. Display System

In our last proposal we discussed the possibility of using a new kind of display system in the microscope. This would involve storing the elastic and inelastic information on magnetic tape during the scan and then manipulating this data with the aid of a small computer in order to provide the various kinds of output which are possible. In addition to this we needed to construct a new display system for the 100 kv microscope and wanted to avoid the use of the very expensive storage oscilloscopes which are now used on the other two microscopes. This system is under construction and is almost completed. The system utilizes a small storage device marketed by Princeton Electronics. It consists of a small oscilloscope tube which will store an image, and this image can be read at TV rates and displayed on a TV screen. We have experimented with the idea of storing both the elastic and the inelastic images on the same storage tube so that they can be displayed on the TV set. (Zenith Radio Corporation generously donated a color TV set for this purpose.)

It is not clear, however, whether this system will be successful because of the difficulty of registering the two images in two different colors exactly. It may be necessary to acquire a second Princeton unit for this purpose.

A tape recorder and computer system has been ordered and will for the moment consist of a single tape unit and a small Supernova computer. This system should be delivered in May. In its current configuration this system would allow us to store the image on the tape and then manipulate this image with the aid of the computer to produce a final improved image. Expansion of this system to a more complete one will be discussed later.

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