Reports

Is a Scanning Ion Microscope Feasible?

Abstract. *Atomic collisions of high-energy heavy ions produce large yields of x-rays. The small de Broglie wavelength of massive ions leads to an estimate of 0.2 angstrom for the resolution of a microscope utilizing nitrogen ions with energies of 14 million electron volts. Estimates of the yield of x-rays relative to molecular radiation damage of the bases in DNA are made.*

Although the scanning microscope has been brought to a high level of development with beams of electrons, little thought appears to have been given to the possibility of using beams of high-energy heavy ions. Heavy ions have the theoretical advantage of having a smaller de Broglie wavelength than electrons, so that the limit on resolution associated with diffraction of matter waves in the aperture of a microscope is smaller (*1*). The wavelength of a 14-Mev nitrogen ion, for example, is about 2×10^{-13} cm, which is much smaller than the wavelength of 1×10^{-10} cm of the 1-Mev electrons in large electron microscopes.

An approach to the theoretical resolution involves a major developmental effort. Indeed, in the scanning electron microscope the point of optimum resolution, at which lens aperture aberrations and diffraction contribute equally to resolution, has only recently been achieved (*2*). In this design a field emission source with an extremely high emittance (particles per unit solid angle) was used, so that a sufficient number of electrons could be focused into the spot of optimum resolution. In the case of heavy ions, the most recent sources (*3*) appear to have emittances as low as 3×10^{-4} [in units of (cm)(mradian)(amu-Mev)$^{1/2}$], and a long magnetic quadrupole lens appears to have aperture aberration coefficients smaller than 3×10^{-3} cm (*4*). By using these figures it can be estimated that a source emitting 1 μa will provide about 10^4 ions per second in a spot 2 Å in radius, and 250 ions per second in a spot of the theoretical optimum radius of 0.2 Å for 14-Mev nitrogen ions.

Probably the reason heavy ions have been neglected in microscopy considerations is the degree of radiation damage that they create in a specimen. Breedlove and Trammel (*5*) concluded that neither electrons, protons, neutrons, helium ions, nor mu mesons could be used for such a purpose, and advocated the use of heavy ions with very low energies, which would scatter from the surfaces of biological molecules without damaging them. However, it is possible that highly charged heavy ions may cause copious emission of x-rays from the inner shells of atoms in a specimen, and in this way reduce the dose required for visibility down to a level at which it does not produce intolerable rearrangement in a large specimen molecule (*6*).

Consider first the yield of x-rays from heavy ion excitation. Figure 1 presents the available experimental data on the cross sections for K x-ray production in aluminum and copper. The cross section is plotted as a function of the energy per unit mass of the projectile, in other words as a function of the square of its velocity (except for the relativistic electrons). The data appear to follow the rule that a maximum occurs when the velocity of the ion is equal to the electron velocity in the shell emitting x-rays (*7*), and hence at a lower velocity for specimens of lower atomic number. Figure 1 also shows an increase of two orders of magnitude when oxygen ions rather than protons are used to excite aluminum at the optimum velocity.

For carbon, the atom of greatest biophysical interest, the maximum should occur at about 1 Mev/amu, and a similar or larger increase in cross section for heavy ions may occur. Although the maximum proton cross section of 2×10^{-21} cm^2 is not much larger than in aluminum (*8, 9*), and although the measured cross sections for heavy ions at velocities below the maximum are generally less than 10^{-20} cm^2 (*8, 9*), this increase may occur because of an increase in the fluorescence yield (the number of x-rays emitted per atomic vacancy) for heavy ions. Ions with velocities near 1 Mev/amu are known to produce multiple atomic vacancies (*10*), which cause a marked increase in the fluorescence yield. In fact, an increase of several orders of magnitude is theoretically predicted in the extreme case of atoms with whole shells vacant (*11*). Since multiple atomic vacancies are presumably produced most effectively by highly charged projectiles, it may be supposed that highly charged ions will cause the largest cross sections, perhaps as large as 10^{-17} cm^2 for carbon. Additional advantages of highly charged ions are that they may have a vacant level of greater binding energy than that of the shell producing x-rays, causing enhanced interaction (*12*) even at high velocities (*13*), and that they require less powerful focusing magnets. Measurements of x-ray cross sections for highly charged projectiles with high velocities are needed.

Some confusion may exist in regard to the charge of ions as they enter collisions which produce x-rays. In typical solids, ions reach charge-state equilibrium (*14*). In a microscope, ion beams of known charge can be made to impinge on specimen molecules, and since the cross sections for charge exchange are 10^{-16} to 10^{-17} cm^2 (*15*), their mean free paths will be 10 to 100 Å before they change charge. This is enough to traverse many molecules of biophysical interest.

Consider now the extent of molecular radiation damage produced by heavy ions. Damage is produced both through displacements of atomic nuclei in the specimen and through excitation and ionization of electrons. If an x-ray cross section of 10^{-17} cm^2 is assumed, it may be computed that a carbon, nitrogen, or oxygen atom bound by 10 ev will emit ten x-rays before being displaced by a 14-Mev nitrogen ion (*16*). For comparison, in the case of protons, which have a maximum cross section for x-ray production of 2×10^{-21} cm^2 (*9*), some 500 displacements would occur before the emission of one x-ray.

Electronic damage is more serious. On the basis of experiments on electron

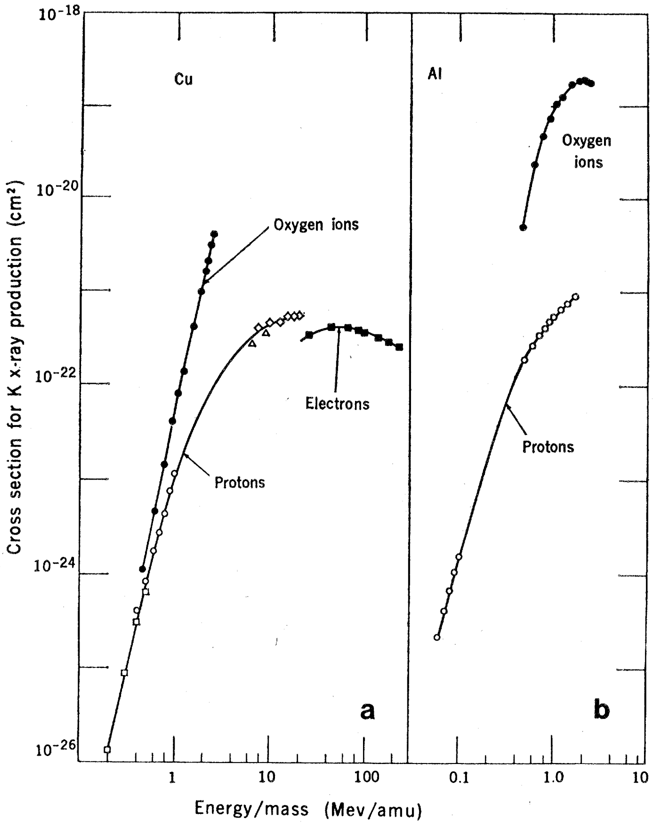

Fig. 1. Cross sections for K x-ray production in thin foils of (a) copper and (b) aluminum bombarded with energetic electrons, protons, and oxygen ions. (a) The sources of the data are: filled squares (*24*); open circles (*25*); open squares (*26*); open diamonds (*27*)—the experimental cross sections are divided by 4 [figure 6 in (*27*)]; open triangles (*28*); filled circles (*29*). (b) The sources of the data are: open circles (*9*); filled circles (*29*).

bombardment of thin films of adenine, it may be estimated that 1.4 kev of electronic excitation are required to disintegrate one molecule (*17*). This figure may be unduly large because of bulk plasmon production in the films of adenine (*18*), but a similarly large value may possibly be found for adenine in DNA because of the analogous process of exciton formation (*19*). Experimental measurements are needed to determine this number for the heavy ion irradiation of dry DNA. If the figure of 1.4 kev is used together with the tabulated rates of energy loss for heavy ions (*20*), an adenine molecule in DNA may be estimated to disintegrate five times before emitting an x-ray when it is bombarded by 14-Mev nitrogen ions, and to disintegrate 6000 times before emitting when bombarded with protons.

While these estimates indicate that the chemical integrity of a specimen will be disrupted before it can emit x-rays, there are factors which may each improve the emission-to-damage ratio by a factor of 10. Exciton-phonon scattering in molecules such as DNA is presumed to decrease at low temperatures, causing propagation of energy to sites where it can be dissipated without damage, at a rate greater than that of "internal conversion" from excitons to dissociative molecular states (*21*). This suggests that specimens should be held at low temperatures. Experimentally, fluorescence and phosphorescence measurements on DNA give no evidence of the fast singlet excitons at room temperature, but are consistent with exciton propagation at 77°K (*22*). More directly, the amount of damage to DNA irradiated with ultraviolet light is reduced five- to ninefold at 77°K (*23*).

Surprisingly, the emission-to-damage ratio may also be improved by about a factor of 10 if the energy per unit mass of the ions is increased from 1 to 10 Mev/amu. This is because the cross sections for both nuclear displacements and electronic excitation fall off as the inverse square of the velocity in this range, while the x-ray cross sections may decrease slowly with velocity above their maximums. Ions with 1 Mev/amu can be obtained at Van de Graaff accelerators, which are no longer in demand for research in nuclear physics. Ions can be accelerated up to 10 Mev/amu in large cyclotrons, although the large expense, the relatively poor beam emittance of cyclotrons, and the difficulty of focusing high-velocity ions pose severe practical problems.

While the above estimates of radiation damage by heavy ions are uncertain in regard to satisfactory definition of bases in DNA, they indicate the overwhelming advantage of heavy ions over protons if x-ray detection is used. Heavy ions possess a similar advantage over electrons, as may be seen from the similar maximum cross sections for electrons and protons in Fig. 1, and have the additional advantage of many orders of magnitude less bremsstrahlung than electrons.

If the yield-to-damage ratio for heavy ions is found to be sufficiently high to make them useful probing particles in a scanning microscope, their large mass should make possible better resolution than that of the best electron microscopes.

F. W. MARTIN

Department of Physics and Astronomy,
University of Maryland,
College Park 20742

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6. Even soft x-rays have mean free paths of the order of 1000 Å (several orders of magnitude larger than Auger electrons), so that all points within a biological molecule could be investigated with such a technique. In contrast, methods based on scattering low-energy ions or on detection of Auger electrons excited by any means will provide a description of the surface of a large molecule.
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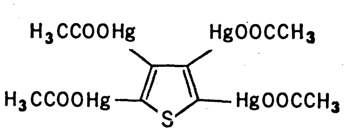
Image of a Sulfur Atom

Abstract. *Two-dimensional signal averaging has been applied to dark field electron micrographs of molecules of 2,3,4,5-tetraacetoxymercurithiophene. Only the mercury atom images are seen in single micrographs. However, in the composite image, resulting from photographic superposition of 64 individual images, the sulfur atom in the molecule is clearly revealed.*

Images of single atoms have now been obtained by four different techniques: field ion microscopy (*1*), scanning electron microscopy (*2*), and bright and dark field transmission electron microscopy (*3, 4*). All of the techniques image heavy atoms such as uranium (atomic number $Z = 92$), thorium ($Z = 90$), mercury ($Z = 80$), or tungsten ($Z = 74$). Lighter atoms such as iodine ($Z = 53$) or palladium ($Z = 46$) have been imaged with certainty only by the dark field technique (*4*). Even lighter substructures of molecules, substructures in which the heaviest atom was arsenic ($Z = 33$), have been revealed by signal averaging of their dark field images (*5*). Our results show that image processing of dark field electron micrographs of the molecule 2,3,4,5-tetraacetoxymercurithiophene can reveal an atom at least as light as sulfur ($Z = 16$).

The thiophene compound [made according to Palmer (*6*)] is planar in



structure. A chemical analysis of the molecule (*6*) yielded a ratio of mercury to sulfur atoms of 3.94 ± 0.09, very close to the expected ratio of 4. The location of the four mercury atoms in the structure of the molecule uniquely defines the position of the sulfur atom in the thiophene ring, even if the signal of the sulfur in the images of the molecule is completely masked by background noise. From model building with a Hg-C bond length of 2.07 Å and the experimentally determined structure of thiophene (*7*), the Hg-S distance should be 4.6 Å from the upper pair of mercury atoms (attached to C_2 and C_3) and 3.4 Å from the other two mercury atoms. Since the distance from the sulfur atom to each of the four mercury atoms is fixed, the restriction on the possible placement or misplacement of the sulfur atom in the superposition of any two images is very severe, making the molecule ideally suited for such a signal averaging process.

Dark field electron micrographs of the molecule were obtained on a Philips EM300 electron microscope at 80 kv and a magnification of 102,000. The objective aperture corresponded to a semiangular opening of 8.6×10^{-3} radian. Dark field conditions were obtained by tilting the incident beam about 0.75 degrees. Further details of the technique are given elsewhere (*4*).

Individual micrographs of the molecule are shown in Fig. 1, a to h. These images were chosen for the characteristic configuration and spacing of the four dark dots corresponding to the mercury atoms, as well as for the relative isolation of the group of dots from any neighboring configurations. Slight differences in configuration from one image to the next are easily accounted for by movement of the mercury atoms of as little as half an atomic diameter under the electron beam. Nevertheless, the constraint on the possible position of the sulfur atom is not lifted significantly. About 80 percent of the images showed configurations in which the distortion due to misplacement or movement of mercury atoms was more severe or in which the structure could be interpreted as a result of a tilted position of the planar molecule on the carbon support. Such images were not considered in the analysis, since the encroachment into the expected region of the sulfur atom of the rather strong image of a misplaced mercury atom could have biased the result.

Photographic superposition of four individual images (Fig. 1i) resulted in a marked relative decrease in the intensity of the background mottle around the molecule (*8*). Although no definite structure has emerged in the middle of the cluster of mercury atom dots, there is already some retention of signal in this region relative to the general background. This effect is enhanced when 16 individual images are superposed (Fig. 1j). Moreover, the reflection symmetry expected from the molecule is becoming more apparent in the image the greater the number of superpositions.

Since in the individual single images any observed asymmetry is thought to be due to displacement of atoms under the electron beam, rather than real structural asymmetry, the reflection symmetry of the molecule was used to increase the effective number of the image superposition with relatively little effort. The result is shown in Fig. 1k for 64 superpositions (32 individual images plus a reflection). In this image the background mottle is virtually absent while a definite signal has emerged at the position of the sulfur atom in the molecule.

In retrospect, the signal was already evident at 16 superpositions (Fig. 1j) and at 32 superpositions (not shown) without reflection. It is therefore not the result of a spurious effect of the symmetry operation. The reflection symmetry does appear to reinforce the fine detail suggesting further details in the image of the molecule. Such detail is, however, below the stated theoretical limit of resolution of the lens of the electron microscope and so should not be interpreted as representing structures of the molecule. It will be eliminated later (Fig. 1m).

Since the best superposition of the images had been chosen by visually aligning the marker mercury atom images, a check on this process was carried out by computer. The four images at the stage of 16 superpositions were digitalized in an array of 32 by 32 square elements, each element equivalent to an area of 0.25 Å². The photographic optical density range in each