

On the Use of Ion Bombardment and Implantation in the Study of Biological Material

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Abstract

The possible use of ion bombardment and implantation in the study of biological material is considered. Especially the use of ion implantation to enhance contrast in electron microscopy is discussed.

Introduction

Ion bombardment and implantation have been found to be of importance in studies of the structure of simple crystals (2). It is possible that the ion technique may also be of importance in probing more complex structures such as biological material. In fact, some preliminary investigations have shown hopeful signs.

Ions entering matter react with its constituents in a complex way and cause various effects which are characteristic of the material under exposure. In this connection the following subjects are of importance to consider briefly: Ion ranges, sputtering, radiation damage and ion implantation.

Ion Ranges

The penetration power or range of energetic ions has been studied extensively for non-biological material (2). For modest ion energies (≤ 100 kev) the range is of the order of 100 Å or less depending partly upon the nature of the ions and partly upon the target material. In general there is a certain spread in the ion range. For instance, for Rn 222 ions of energy 2 kev directed into Aluminum the range is about 50 Å with 50% of the ions stopped in a layer of about 20 Å thickness (2). For biological material range measurements have not been performed to any extent but it is probable that existing data for non-biological material are representatives also for biological material to a first approximation.

Under certain circumstances the ranges of ions in matter are considerably longer than those mentioned above. This happens if the incoming ions have a direction that coincide with a special preferred direction in the target crystal. This so called channeling effect has been used extensively to probe crystal structures. It is possible that similar effects may exist in biological material. For instance the laminar structure of membranes may give rise to preferred directions.

Sputtering and Radiation Damage

The target material is not unaffected by the incoming ions. Some target atoms are knocked out (sputtered) from the surface. This

process has been found to be useful in structure studies in many cases since the sputtering yield is dependent upon the characteristic properties of the target material. Although no direct measurements exist for biological material it is probable that similar effects exist there.

The occurrence of sputtering means that a certain amount of radiation damage is caused by the incoming ions. The amount of damage depends again upon the type of ion and target material. Sometimes the damage is so severe that the ion beam can be used for etching of surfaces. Recently, the damaging effect of ions has also been suggested to be used to erode parts of micro-organisms to observe its inner structure (2). This could be an interesting application of ion beam technique to biological structure research.

Ion Implantation

In contrast to X-ray and electron diffraction experiments an ion bombardment implants a certain amount of foreign material in the substance under study. This may sometimes be a disadvantage. However, under certain circumstances it may be useful. An example is ion implantation in biological material to enhance the contrast in electron microscopy. It has been shown recently that bacteria cells of *Escherichia coli B* exposed to 25 kev lead ions for a short interval show an enhanced contrast when viewed by an electron microscope compared to unexposed bacteria (1). The advantage of using ion implantation in this case is mainly that the depth of the contrast material can be regulated from the accelerating voltage of the ions. A low voltage of a few hundred volts will deposit the ions on the surface; a larger voltage will deposit the contrast material under the surface at a suitable level.

The above method is illustrated in Figure 1. This shows a part of a bacterium *Escherichia coli B* which has been exposed to 25 kev lead ions. Figure 2 shows a bacterium not bombarded by lead ions. It is obvious that a contrast enhancement has occurred for certain parts of the cell. Further studies of this method is required in order to determine its usefulness.

The electron micrographs (Figs. 1 and 2) were prepared in the following manner. Bacterial cells of *Escherichia coli B* (ATTC 11303) were grown at 37°C in nutrient broth with continuous aeration into late logarithmic phase. The bacteria were subjected to low speed centrifugation (10,000 $\times$ g) and resuspended into 0.1 M ammonium acetate solution at the same cell titer. A drop of the suspension was placed in a copper grid on which had been layered formvar and shadowed with carbon in a vacuum evaporator using conventional techniques. The drop of liquid was aspirated from the grid by touching it with the edge of a sheet of filter paper. The grid was then allowed to dry and subjected to lead ion implantation (ion current 10 μ amp, exposure time 10 min, incident angle 45°). The pictures were taken with Hitachi 11E transmission electron microscope operating at 75 kv accelerating potential.



FIGURE 1. *Electron micrograph of part of cell Escherichia coli B exposed to lead ions of 25 kev energy to enhance contrast. For details of preparation see Reference 1. Magnification X 58000.*

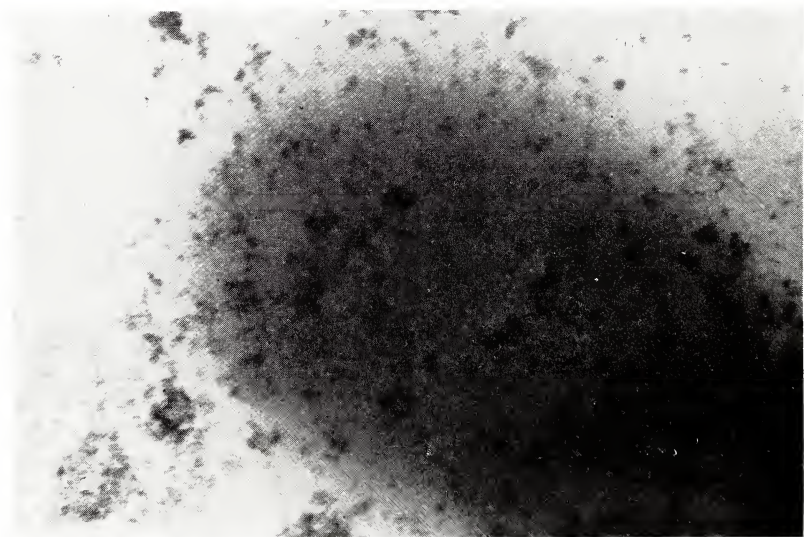


FIGURE 2. *Electron micrograph of control cell of Escherichia coli B not exposed to ion beam. Magnification X 58000.*

Conclusion

The few experiments using ion bombardment to study biological structure show certain promising results. A comparison with the data obtained by ion technique to investigate structure of non-biological material seems to indicate that a wider application of ion bombardment in the biological domain may be useful. A systematic study of the subject is therefore of interest.

Literature Cited

1. ALVÄGER, TORSTEN, and JOHN SWEZ. 1970. Contrast Enhancement of Biological Structure by Ion Implantation. *J. Appl. Phys.* (In press).
2. DEARNALEY, G., 1969. Ion Bombardment and Implantation. *Rep. on Prog. in Phys.* **32**(2):405.