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High Resolution Scanning Microscopy of Biological Specimens

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High resolution scanning microscopy of biological specimens

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[Plates 11 and 12]

A scanning microscope is described which is capable of a point resolution of 0.5 nm. We have demonstrated that it can produce the same kinds of micrographs as a conventional microscope. However, by using several detectors to produce signals corresponding to different groups of transmitted electrons we are able to produce an image contrast closely proportional to Z, the atomic number. Such a system is very promising for the study of stained molecules.

Introduction

During the past few years we have been involved in the development of a new type of electron microscope, namely a high resolution scanning microscope using a field emission source of electrons. The particular purpose of this programme is to exploit such a microscope for any advantages it may have in biological studies. The work has now reached a point where serious biological research programmes are just beginning and where it appears that the microscope has several advantages over commercial high resolution microscopes.

It has long been recognized that there are two separate methods for building an electron microscope.

The first of these consists of emulating the optical microscope as closely as possible (Knoll & Ruska 1932; von Borries & Ruska 1939). Electrons from a hot filament are accelerated to the required potential and pass through a system of condenser lenses in order that a collimated electron beam can be formed to illuminate a specimen. Electrons are scattered or diffracted by the specimen and some of them pass through a defining aperture and a system of magnifying lenses to produce an image on a fluorescent screen or photographic plate.

The current state of the art of conventional electron microscopes is that point resolutions of 0.24 nm can be achieved with the very best instruments and techniques (F. Thon 1969, personal communication). Such an instrument can also provide lattice resolutions of close to 0.1 nm. Contrast in the micrographs is provided by removing unwanted electrons from the image-forming process. These electrons are normally removed on a defining aperture.

The second method consists of producing a small focused spot of electrons and then scanning this focused spot across the specimen in the form of a miniature rectangular raster. Any physical effect caused by the incident beam can be detected and displayed as an intensity variation on a synchronously scanned display oscilloscope. Such systems are called scanning microscopes (Knoll 1935), and various commercial machines are available.

The commercial scanning microscope is now a well-established scientific tool which is best developed in those instruments which use secondary electrons as the contrast mechanism (Smith & Oatley 1955; Oatley, Nixon & Pease 1965). The focused spot of high-energy electrons produces slow secondary electrons when it strikes a surface, and these electrons can be collected efficiently to serve as the modulation signal for the display oscilloscope. The current state of the art is that a resolution of 10 nm can be achieved (Pease & Nixon 1965), and the instrument is

particularly suitable for studying surface detail. The contrast can be made very high by coating the specimen with a thin layer of gold to increase the number of secondary electrons.

As they have evolved, then, the two types of instruments are very different in their capabilities. The conventional microscope is useful for the examination of thin specimens at high magnification. The scanning microscope is useful for the examination of the surface of thick specimens at somewhat lower magnification because secondary electrons can be easily collected and because the inherent resolving power of the instrument is low.

The problem of resolving power

If one examines the reason for the difference in resolving power between the two types of microscope it becomes clear that the reason for the comparatively poor performance of the

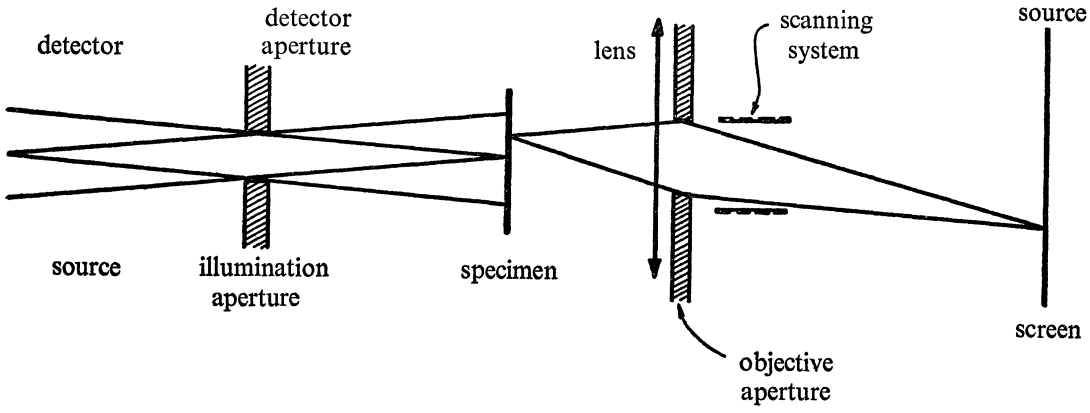

Figure 1. Schematic diagram of conventional and scanning electron microscopes. For the conventional microscope, read the diagram from left to right. We have an electron source providing illumination through an aperture onto the specimen. The specimen is then imaged by a lens through the objective aperture onto the screen. For the scanning microscope, we read the diagram from right to left. The electron source is imaged by a lens through an aperture onto the specimen. This image is scanned across the specimen by a scanning system. Electrons transmitted through the specimen pass through an aperture onto a detector. (Appeared previously in *Optik* and *Q. Rev. Biophys.*)

scanning microscope is that a hot tungsten filament is neither small enough nor bright enough to allow a higher resolution. A hot filament source has an inherent brightness of about 10^5 A cm^{-2} sr^{-1}. This brightness cannot be increased with the use of additional lenses and in general it will be slightly decreased. The resolving power (that is, the spot size) of a scanning microscope is therefore intimately connected with the available beam current in the spot. A detailed analysis of this problem leads to the conclusion that spot sizes less than 10 nm entail a beam current which is inadequate to form a picture in any reasonable time.

The reason for this asymmetry between the two types of microscope can be seen from figure 1. This diagram gives the basic electron optics of the two types of microscope. Reading from left to right we have the optics of a conventional microscope, and reading from right to left, we have the optics of the scanning microscope. The difference between the two is that in the case of the conventional microscope all image points are produced simultaneously, while in the scanning microscope they are produced sequentially. Therefore, the source brightness for the scanning microscope must be greater than that in the conventional microscope if they are to

have the same resolution. The multiplying factor must be of the order of the number of picture elements—about 10^6 for a useful machine.

We conclude, then, that if we wish to have a scanning microscope with comparable resolving power to a conventional microscope, we must have an electron source which has a brightness of at least 10^{11} A cm^{-2} sr^{-1}. Such a source exists in the field emission from a tungsten point.

The advantages of field emission as an electron source were recognized by Cosslett & Haine some time ago (1954), but their attempts to produce a workable source were not successful because the vacuum conditions in use at the time were inadequate. Now, however, suitable high vacuum equipment is readily available.

We have described the fabrication and performance of tungsten tips as field emission sources in previous publications (Crewe, Eggenberger, Wall & Welter 1968), and we need only point out here that at its maximum performance such a tungsten tip can emit 500 μA with an apparent source diameter of 4 to 5 nm into a solid angle of less than 0.1 sr. This corresponds to a brightness of about 40 GA cm^{-2} sr^{-1} which is more than adequate.

We designed and built a new type of electron gun for use with this small source. It consists of two specially shaped anodes which constitute an accelerating system with a spherical aberration constant which is as low as we know how to make it. The details of this gun have been published (Crewe *et al.* 1968), and we need only say here that it has been fully checked and it has first- and third-order optical properties which are exactly calculable.

For the purposes of the present discussion we need only say that the combination of a field emission source and this electron gun provides a beam of electrons which, from the point of view of the final resolving power, is essentially coherent. That is to say, when an auxiliary lens is used to refocus the electrons on to a specimen, the size of the focused spot is almost entirely due to diffraction and the aberrations of that lens. The defects in the source are demagnified by such a lens and the electron source can be considered to be perfect.

Our present instrument uses only one auxiliary lens which is an objective lens with a theoretical resolution of 0.465 nm at 25 kV. The best performance of the microscope to date is an experimentally determined resolution of 0.5 nm (Crewe & Wall 1970*a*, *b*).

Another feature of the microscope which is important in the present context is that we have installed an energy analyser in order to analyse the energy of the electrons which pass through the specimen. It is capable of an energy resolution of 0.3 V, but of greater interest so far is the ability which it provides to separate the energy-loss electrons from the no-loss electrons. A schematic diagram of the microscope is shown in figure 2.

It is convenient to discuss the operation of this microscope in terms of two verifiable statements.

1. *The scanning microscope is capable of producing all the various images of which a conventional microscope is capable.*

The theoretical justification for this statement is perhaps best obtained by inspection of figure 1. It is possible to show from that figure that from the point of view of electron optics the two instruments can be made identical. We have seen in the previous section that the resolution of the two instruments can be identical. It remains to examine the details of the various contrast mechanisms which can be obtained in a conventional microscope to determine whether or not they can be duplicated in the scanning microscope.

The essential argument here is that the optics is reversible. Whatever optical-ray construction

is used to describe a particular type of contrast, it is always possible to reverse the directions of all rays and obtain the same effect.

We give only one example here, that of phase contrast from a periodic object such as a crystal lattice.

Figure 3 shows a ray diagram which is used to explain the existence of this type of contrast. Reading from left to right we have the optics of a conventional microscope. A highly collimated beam of electrons is incident on the specimen which consists of a periodic phase structure such

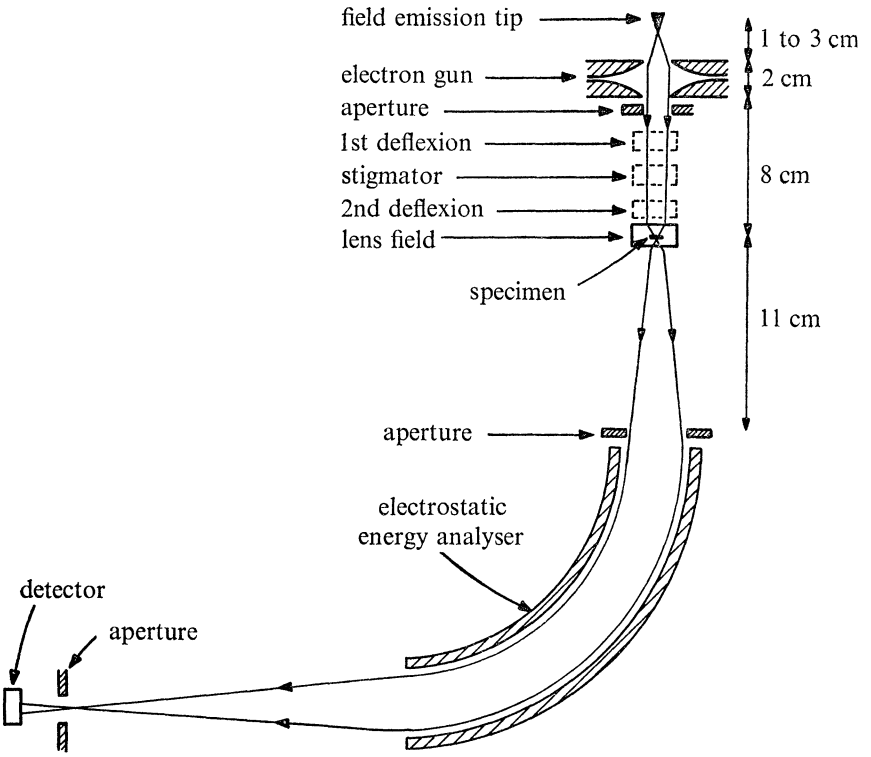

FIGURE 2. A schematic diagram of the high resolution scanning microscope. (Appeared previously in *Q. Rev. Biophys.*)

as a crystal lattice. We consider three beams which emerge from the specimen, the undisturbed beam and the two first-order diffracted beams. These interfere in a region close to the specimen to produce interference fringes which form an intensity distribution with the same period as the original phase lattice. If the lens is focused on this region we can obtain an image of this intensity distribution.

Experimentally it is better to under-focus the lens rather than over-focus because the aberration defect produced by the defocus error tends to compensate the aberration defect produced by the spherical aberration of the lens.

In order to observe small spacings it is generally necessary to increase the size of the lens aperture. This tends to decrease the point (scattering) resolution but increase the lattice resolution. For this reason it is generally possible to observe lattice spacings which are smaller than the point resolution of the instrument.

A further gain in resolution can be obtained by tilting the illumination system so that only the zero-order beam and one first-order beam can pass through the aperture. Such a system

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produces a two-beam interference pattern which contains the minimum amount of information, namely, a sine wave modulation at the correct spatial frequency.

These effects can all be duplicated in a scanning microscope. We simply reverse the diagram. In this case we provide the zero-order and two first-order beams in the illumination system (other beams are present but can be ignored). The zero-order beam passes through the specimen and proceeds along the axis to the detector. The first-order beams can be diffracted so that they also proceed along the axis and interfere with the zero-order beam. The analysis of this situation is precisely the same as in the case of the conventional microscope because the interference depends only upon path differences, not the direction.

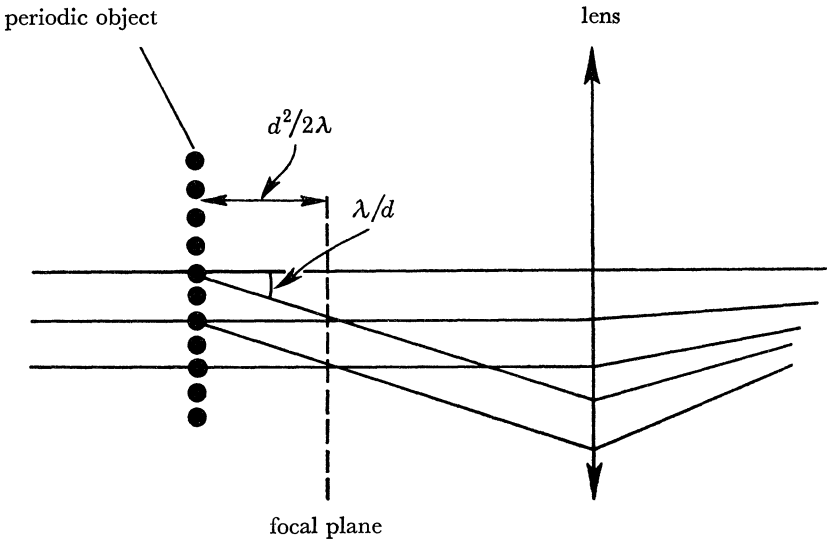

FIGURE 3. Illustration of the formation of phase-contrast images of periodic lattice. For a conventional microscope, read the diagram from left to right. The first-order diffracted beam interferes with the zero-order beam in the focal plane of the lens so that an image is formed of the interference pattern on the screen. For the scanning microscope, read the diagram from right to left. As all the path differences are the same, we should observe interference fringes as we scan the electron beam across the focal plane. (Appeared previously in *Optik* and *Q. Rev. Biophys.*)

Therefore as the beam is scanned across the specimen these interference effects produce a modulation in the signal generated by the detector. This duplicates the interference effects seen in a conventional microscope.

Similar explanations can be given of all the other kinds of contrast mechanisms which can be obtained in a conventional microscope. In each case one merely reverses the ray diagram.

These arguments make it clear that the close analogy between the two types of microscope which is illustrated in figure 1 can be interpreted exactly. In other words, we can make numerical statements about the two microscopes. For example, the use of highly collimated illumination systems in modern conventional microscopes (10^{-4} to 10^{-6} rad) corresponds to the use of a very small axial detector in the scanning microscope (subtending 10^{-4} to 10^{-6} rad at the specimen).

By following this recipe we have been able to duplicate several of the modes of operation of a conventional microscope (Crewe & Wall 1970*a*) such as: (*a*) scattering contrast—in focus operation; (*b*) lattice images—phase contrast of periodic object; (*c*) Fresnel fringes; (*d*) diffraction contrast—dislocations in metal films; (*e*) extinction contours. In view of the success of these attempts and the exact correspondence between the calculations of the necessary operating

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conditions and their experimental verification we have no reason to believe that all other forms of electron microscope contrast cannot be achieved.

A very complete mathematical analysis of this problem has been conducted by Zeitler & Thomson (1970) who also conclude that the two types of instrument can produce identical micrographs.

2. *New contrast methods are available in the scanning microscope which cannot be achieved in the conventional microscope.*

This is important to us, of course, because otherwise the scanning and conventional microscopes would be completely identical and there would be no point pursuing the scanning microscope.

To demonstrate that the second theorem is true, we will consider one example of a contrast mechanism in the scanning microscope which cannot be duplicated in the conventional microscope.

We will examine the fate of electrons which pass through a very thin specimen. These electrons can be divided into three categories:

(*a*) *Elastically scattered electrons.* These electrons have almost the same energy as the incoming electrons. The difference in energy is undetectable.

The number of such electrons can be calculated from the value for the atomic elastic cross-section.

$$\sigma_e = 0.465 Z^{\frac{4}{3}}/V \text{ nm}^2.$$

This elastic scattering is characterized by a very wide scattering distribution which is proportional to

$$1/(\theta^2+\theta_0^2)^2,$$

where θ is the scattering angle and θ_0 a constant (the screening angle) which in our case is of the order of 50 to 100 mrad.

Most of these electrons, therefore, are scattered outside the cone of the incident illumination (10 to 20 mrad). These electrons can be most readily detected by means of an annular detector of such a size that the unscattered electrons just pass through the hole in the detector.

(*b*) *Inelastically scattered electrons.* These are the electrons which lose energy in the specimen. They can lose any amount of energy, but the vast majority of them fall into a group such that $1V < \Delta V < 100$.

The total cross-section for this process is

$$\sigma_i = 8.68\ Z^{\frac{1}{3}}/V \text{ nm}^2.$$

The angular distribution is characterized by a narrow angular distribution

$$1/(\theta^2+\theta'^2),$$

where

$$\theta' \approx \Delta V/2V \approx 1 \text{ mrad}.$$

Most of these electrons, therefore, will be within the cone of incident illumination and will pass through the hole in an annular detector.

(*c*) *No-loss electrons.* By this we mean electrons which pass through the specimen without any interaction. Therefore

$$N = N_e + N_i + N_0,$$

where N is the number of incident electrons, N_e is the number of elastically scattered electrons, N_i is the number of inelastically scattered electrons, N_0 is the number of no-loss electrons.

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The no-loss electrons can easily be separated from the inelastically scattered electrons with the aid of the energy analysing spectrometer. It is therefore a simple matter to arrange the scanning microscope to give three simultaneous signals as the beam scans across a specimen. These three signals correspond to the three groups of electrons. It is the ability to acquire simultaneous signals corresponding to different groups of electrons which allows the scanning microscope to produce micrographs which cannot be obtained in a conventional microscope. These signals can be used in a variety of ways, but two illustrations will suffice here.

(1) N_e/N_0. This is a normalized elastic scattering signal which is formally equivalent to the use of dark-field illumination in a conventional microscope with the important exception that the detector can subtend an angle of 250 mrad or more, thereby providing a large signal. Strictly speaking, this ratio should be N_e/N but for thin specimens N_e and N_i are small (a few per cent) so that $N_0 \approx N$.

(2) N_e/N_i. We call this our 'Z' contrast signal because it can be easily verified that $N_e/N_i \approx Z/19$. In other words, this ratio provides a signal which is proportional to Z, the atomic number. In turn this means that a micrograph can be obtained where the intensity is proportional to Z.

This mode of contrast cannot be achieved in a conventional microscope. Very few high resolution microscopes have been fitted with energy-analysing equipment. Even where this has been done the resolution deteriorates because of the electron-optical problems.

While it may be possible to extract the two pieces of information N_0 and N_i with difficulty from a conventional microscope, it does not appear feasible to extract them simultaneously.

The 'Z' contrast method, then, is capable of producing an image which responds to high Z materials in a predictable and quantitative way. Specifically the calculations indicate that single atoms of $Z > 70$ should be visible under present operating conditions.

This is of importance to biology because macromolecules such as DNA and RNA can be selectively stained with heavy atoms. In such cases the sites of the heavy atom should be discernable and one can begin to examine structures at high magnification. The only limit to the resolution is the spot size itself which, at the moment, is 0.5 nm.

It is important to emphasize that the scanning microscope produces electronic signals which have a precise, numerical meaning. One signal (N_e) gives the instantaneous elastic scattering cross-section and another (N_i) gives the instantaneous inelastic cross-section. These are *numbers* with a definite physical meaning and are inherently capable of yielding quantitative information about the specimen.

For example, suppose the specimen (stained DNA on carbon, for example) consists of an assembly of two types of atom a and b.

Then

$$\frac{N_e}{N} = \frac{n_a\sigma_e^a + n_b\sigma_e^b}{\sigma_b},$$

$$\frac{N_i}{N} = \frac{n_a\sigma_i^a + n_b\sigma_i^b}{\sigma_b},$$

$$N_0 = N - N_e - N_i,$$

where σ_b is the cross-sectional area of the focused spot.

In addition, we know

$$\frac{\sigma_e^a}{\sigma_i^a} = \frac{Z_a}{19}\frac{\sigma_e^n}{\sigma_i^b} = \frac{Z_b}{19}.$$

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These equations can be solved for any of the variables. For example

$$\frac{n_a}{n_b} = \frac{Z_b^{\frac{4}{3}}}{Z_a^{\frac{4}{3}}}\left[\frac{1-(N_e/N_i)\ 19/Z_b}{(N_e/N_i)19/Z_a - 1}\right],$$

It is perfectly possible to build an electronic box which will perform the operation on the right-hand side of this equation. In this case a micrograph is obtained with an intensity proportional to n_a/n_b.

Alternatively one can ask for the meaning of the ratio n_e/N_i and we obtain

$$\frac{N_e}{N_i} = \frac{Z_a}{19}\left[\frac{1+(n_b/n_a\ (Z_b/Z_a)^{\frac{4}{3}}}{1+(n_b/n_a\ (Z_b/Z_a)^{\frac{1}{3}}}\right].$$

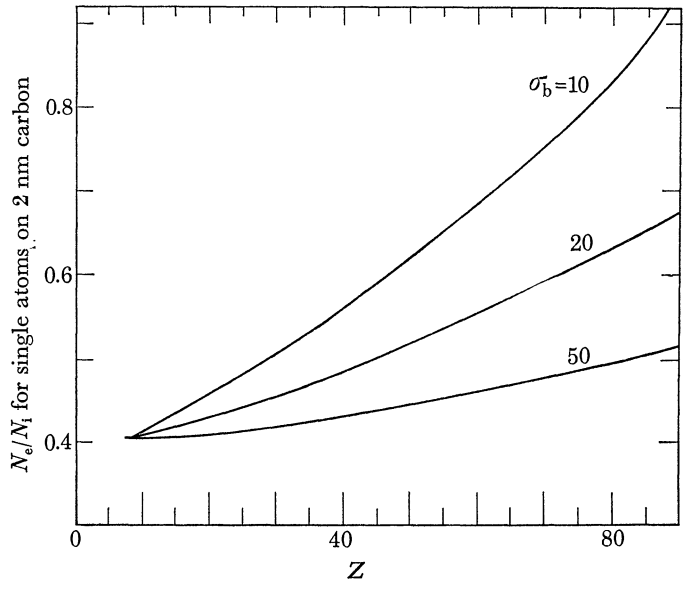

FIGURE 4. The value of the ratio of elastic to inelastic signal obtained for a single atom of the atomic number Z resting on top of a carbon film 2 nm thick. The three curves show the expected visibility of the atom as a function of the cross-sectional area of the focused beam.

The function N_e/N_i is plotted in figure 4 for the case of a single atom resting on a carbon film 2 nm thick. In this calculation we assumed $V = 25$ kV and the number of carbon atoms

$$n_b = \sigma_b t.0.11,$$

where $t = 2$ nm.

We have used this type of signal to study DNA strands on a carbon substrate. Some representative micrographs are shown in figures 5 to 7, plates 11 and 12. It can be seen that adequate contrast can be obtained even on unstained DNA.

We have also used the signal N_e/N_i to search for individual heavy atoms. Figure 8, plate 12, shows a micrograph containing pairs of white spots. The specimen consists of a thin carbon film which was sprayed with a dilute solution of benzene-tetracarboxylic acid reacted with uranyl acetate. The expected reaction product consists of two uranium atoms separated by 1 to 2 nm. The micrograph appears to represent such a molecule.

It is perhaps obvious that a micrograph can never contain all the information which is produced by the microscope. In the example above, the output of one scan of the microscope consists of about 10^6 separate, meaningful numbers. Furthermore, the equation above is not

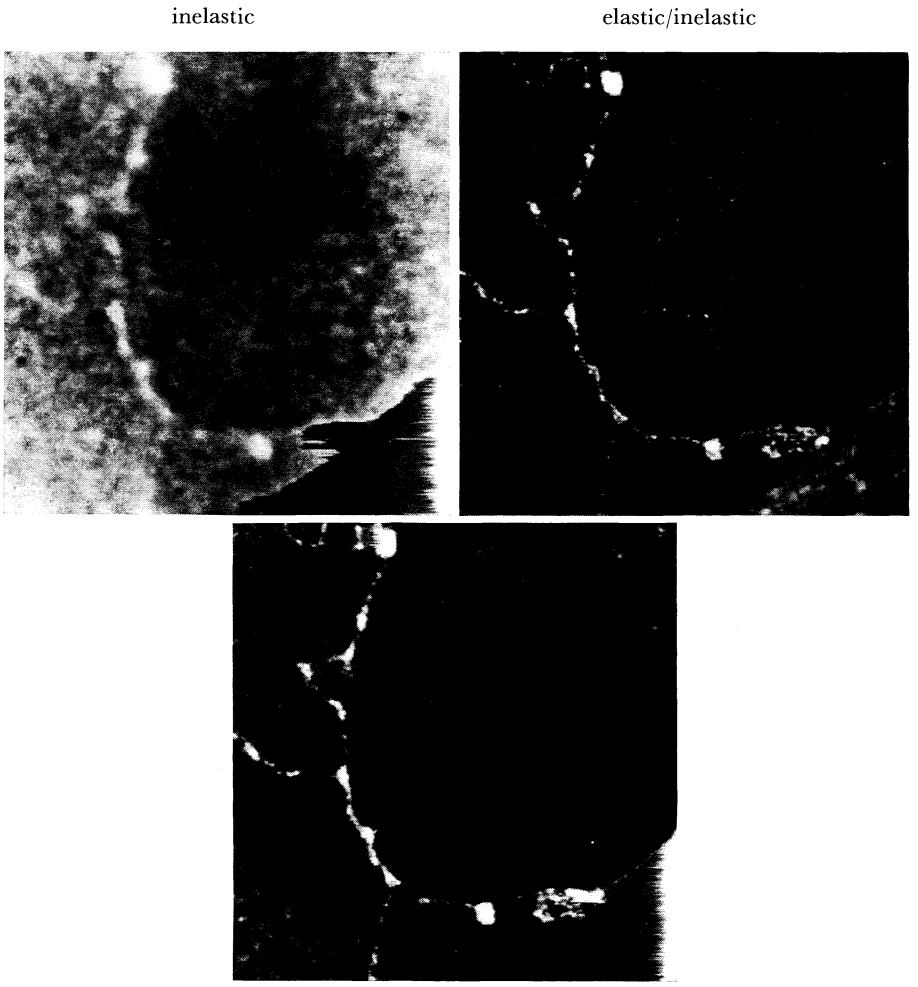

FIGURE 5. DNA stained with 10^{-2} mol l^{-1} T1 and washed in acetone (from M. Beer). 300 nm full scale. The apparent Z for the strand is 50; for the background 10. The thickness of the strand is 0.3 nm; of the background 1.5 nm.

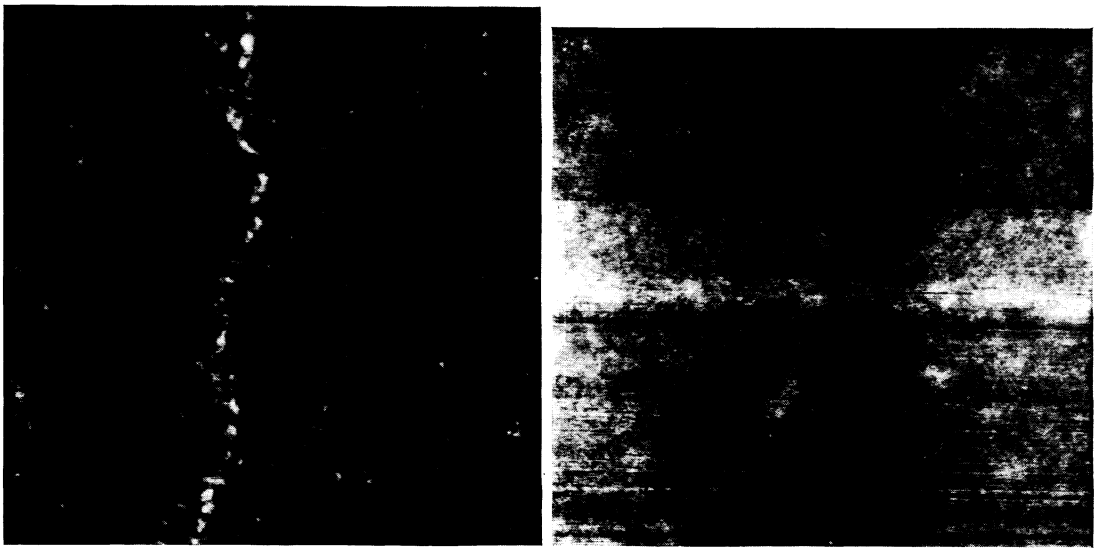

FIGURE 6. DNA stained with 10^{-4} mol l^{-1} uranyl acetate. Bright spots correspond to aggregates of $\sim$ 7 uranium atoms spaced on the average of 2 nm along strand. Background is 3 nm carbon covered with $\sim$ 0.3 nm uranium.

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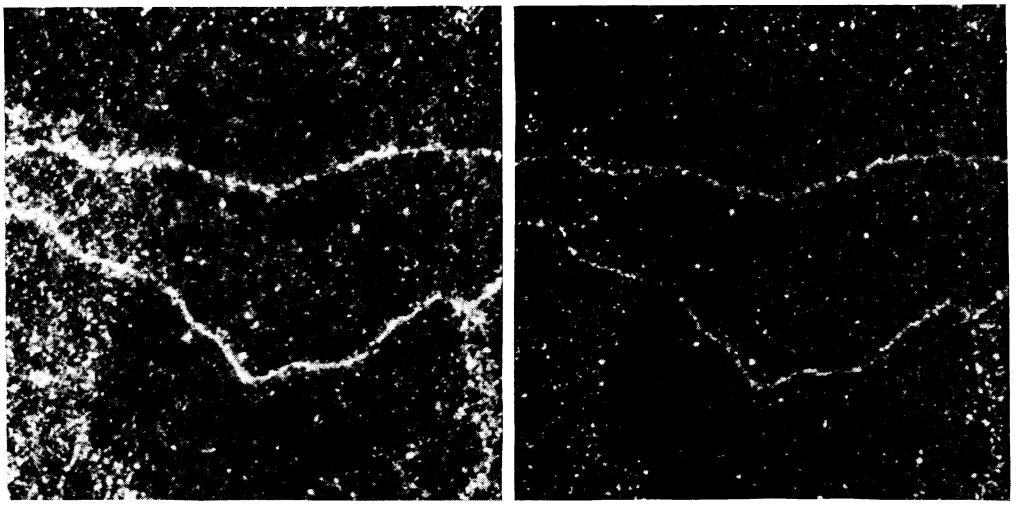

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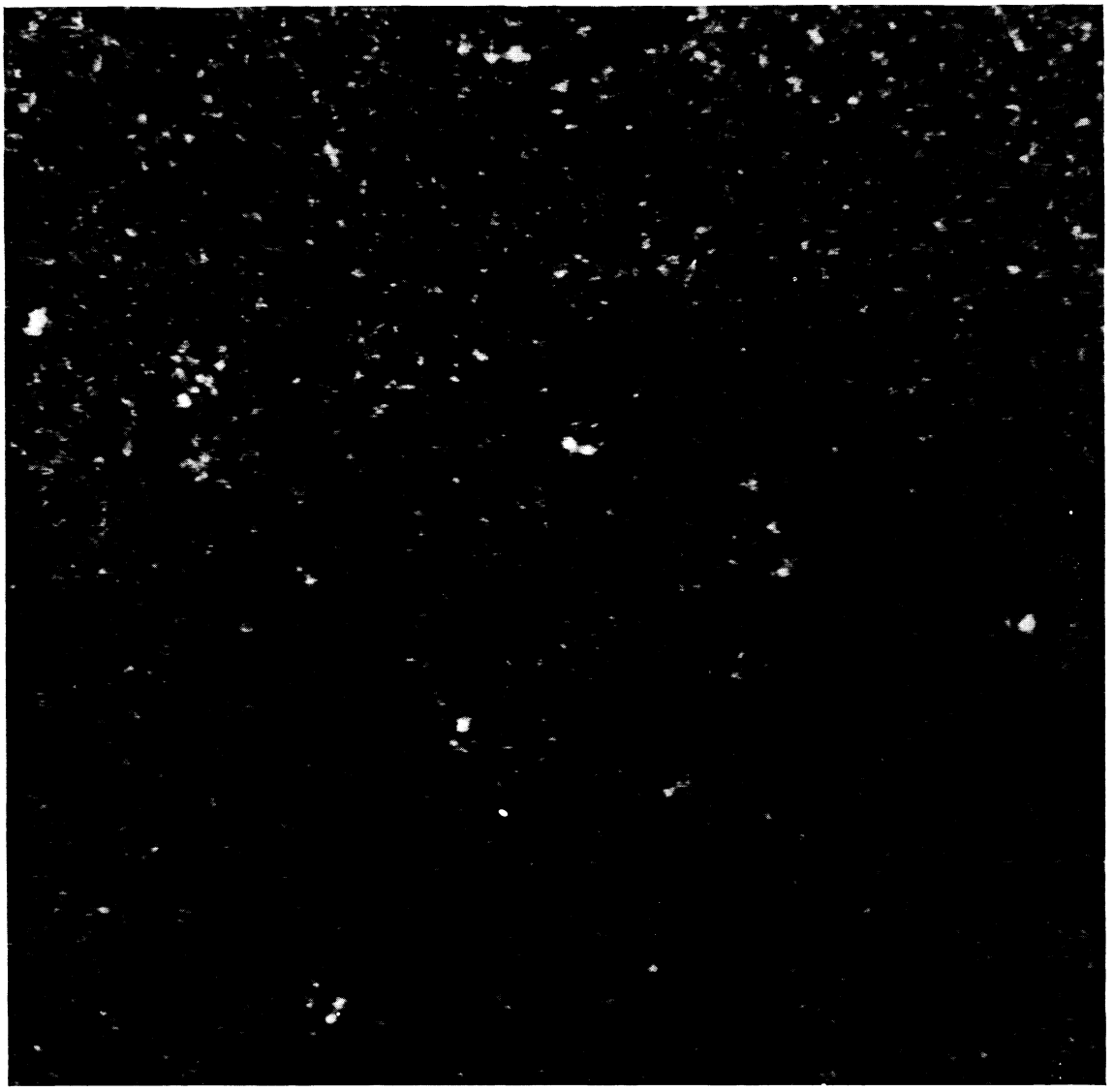

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FIGURE 7. T_4 DNA in 10^{-3} mol l^{-1} sodium, 300 nm full scale (*a*) elastic and (*b*) ratio of elastic to inelastic electrons. Note that the background is reduced when taking the ratio of elastic to inelastic electrons. This is because such a signal is independent of the thickness of the film and the background noise is now due to statistical variations in the two signals. The apparent Z for the strand is 10 to 20; for the background 10. The thickness of the strand 0.5 nm; of the background 2.3 nm.

FIGURE 8. Micrograph of a thin carbon film which has been coated with a dilute solution of uranyl acetate reacted with benzene tetracarboxylic acid. The expected appearance of the uranium atoms is that they will appear in pairs separated by 13, 23, 33, etc. nm. This picture was taken using the ratio N_e/N_i. Several pairs of bright spots can be seen. These are presumably uranium atoms. 210 nm full scale.

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the only way to combine the signals N_e, N_1 and N_0. For example one might want to solve for Z_a or there may be three types of atom which must be distinguished.

It would be possible to build several 'black boxes' which would form the required combination of signals. However, it is apparent that this is not the best way to proceed. The additive and multiplicative constants in the equations are not exactly known and there are noise problems. This means that many trial scans would be required before the best information was obtained. This is quite a dangerous procedure because we do not have any information on the radiation damage problem at this level. It seems apparent that one should attempt to obtain the maximum information from one scan before making a second scan on the same area.

Finally, the information is in the form of a micrograph which would then have to be examined. Microdensitometry would be ridiculous because the appropriate numbers are available in the original signal.

We are therefore considering the following system for obtaining information and micrographs.

The three possible electrical signals will be combined to give two signals

$$N_e/N_0 = A \quad \text{and} \quad N_1/N_0 = B.$$

These two signals will be processed by an analogue-digital converter and stored as numbers on a magnetic drum or disk. This requires the storage of 10^6 to 10^7 bits in 10 s, which is well within the state of the art.

The information in storage can then be interrogated in a variety of ways by means of an electrical interrogation system which will be either an analogue system of switches, patchboards, etc., or a very small computer system. Examples of the kinds of information we wish to extract are:

(*a*) $A - aB$ (background subtraction),

(*b*) $\dfrac{A-aB}{A+B}$ (normalized subtraction),

(*c*) $\dfrac{A-aB}{A-bB}$ ('Z' signal type),

(*d*) A/B ('Z' type signal),

(*e*) A alone,

(*f*) B alone.

In addition, the algebraic expressions we have given here and elsewhere are only correct in special cases. In order to obtain more accurate information it is necessary to correct the values of A and B for geometrical and other effects. In other words an operation $A \rightarrow A'$ and $B \rightarrow B'$ must be performed. These operations are mathematically simple but tedious.

Any of the above signals (or others) can then be preserved as micrographs by playing the signal through a camera system.

As one final example of the value of such a system, it can be seen that it would be a simple matter to obtain an intensity contour map of a specimen. This can be done by playing the appropriately chosen signal through a 'gate' such that signal values terminating inside the 'gate' would be displayed as full white and all other signals, both less than and greater than the gate, would be displayed as black. Successive micrographs can be obtained using the same basic stored data with the 'gate' in different positions so that a complete map can be obtained. The advantages of performing this operation on the same basic data rather than successive scans is perhaps obvious.

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Conclusions

The immediate future of our microscope appears to be best used for the study of very thin biological specimens which have been carefully and specifically stained. Both DNA and RNA are of the appropriate thickness and they can be stained. We expect that these structures can be explored down to the 0.5 nm level. The amount of information which can be obtained from each specimen is considerable, so that we expect to learn a great deal more about the problems of specimen preparation.

The use of multiple detectors in the scanning microscope shows promise of becoming an important technique, particularly at the highest possible resolution, and appears to be capable, in principle, of providing a great deal of exact information at the molecular level – for example the precise location of individual atoms of high Z.

The author wishes to express his gratitude to the many people who have contributed to this work, but in particular to J. Wall who was responsible for the micrographs shown here and who has contributed immeasurably to the ideas which are expressed here.

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References (Crewe)

Cosslett, V. C. & Haine, M. E. 1954 *Proc. 3rd. Int. Conf. on E.M., London*, p. 639.
Crewe, A. V., Eggenberger, E. N., Wall, J. & Welter, L. M. 1968 *Rev. scient. Instrum.* **39**, 576.
Crewe, A. V. & Wall, J. 1970*a* *Optik* **30**, 461.
Crewe, A. V. & Wall, J. 1970*b* *J. molec. Biol.* **48**, 375.
Knoll, M. 1935 *Z. tech. Phys.* **16**, 467.
Knoll, M. & Ruska, E. 1932 *Annln Phys.* **12**, 607.
Oatley, C. W., Nixon, W. C. & Pease, R. F. W. 1965 *Adv. Electronics Electron Phys.* **21**, 18.
Pease, R. F. W. & Nixon, W. C. 1965 *J. scient. Instrum.* **42**, 81.
Smith, K. C. A. & Oatley, C. W. 1955 *Br. J. appl. Phys.* **6**, 391.
von Borries, B. & Ruska, E. 1939 *Z. wiss. Mikrosk.* **56**, 317.
Zeitler, E. & Thompson, M. G. R. 1970 *Optik* **31**, 269, 319.

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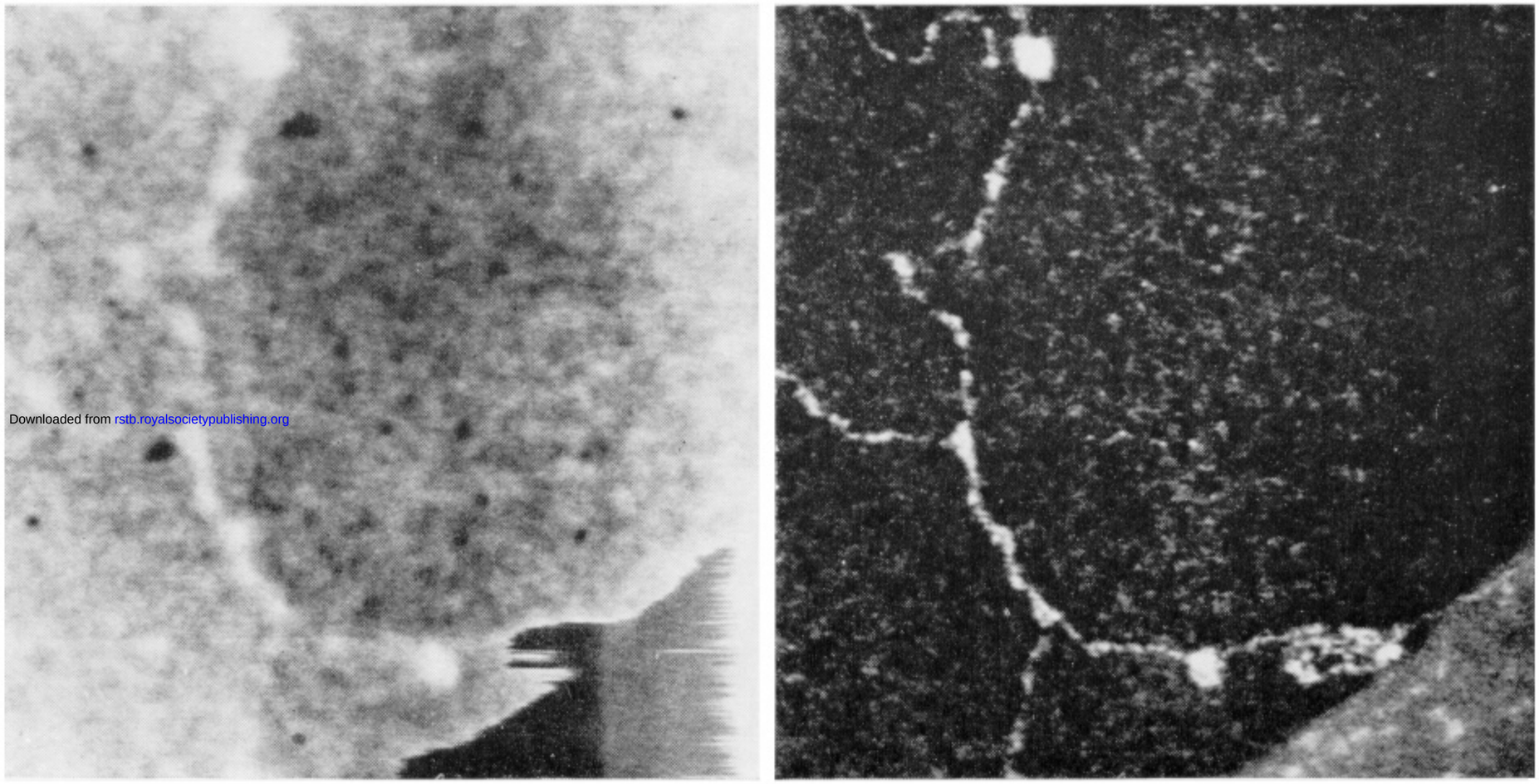

elastic

FIGURE 5. DNA stained with 10^{-2} mol l^{-1} T1 and washed in acetone (from M. Beer). 300 nm full scale. The apparent Z for the strand is 50; for the background 10. The thickness of the strand is 0.3 nm; of the background 1.5 nm.

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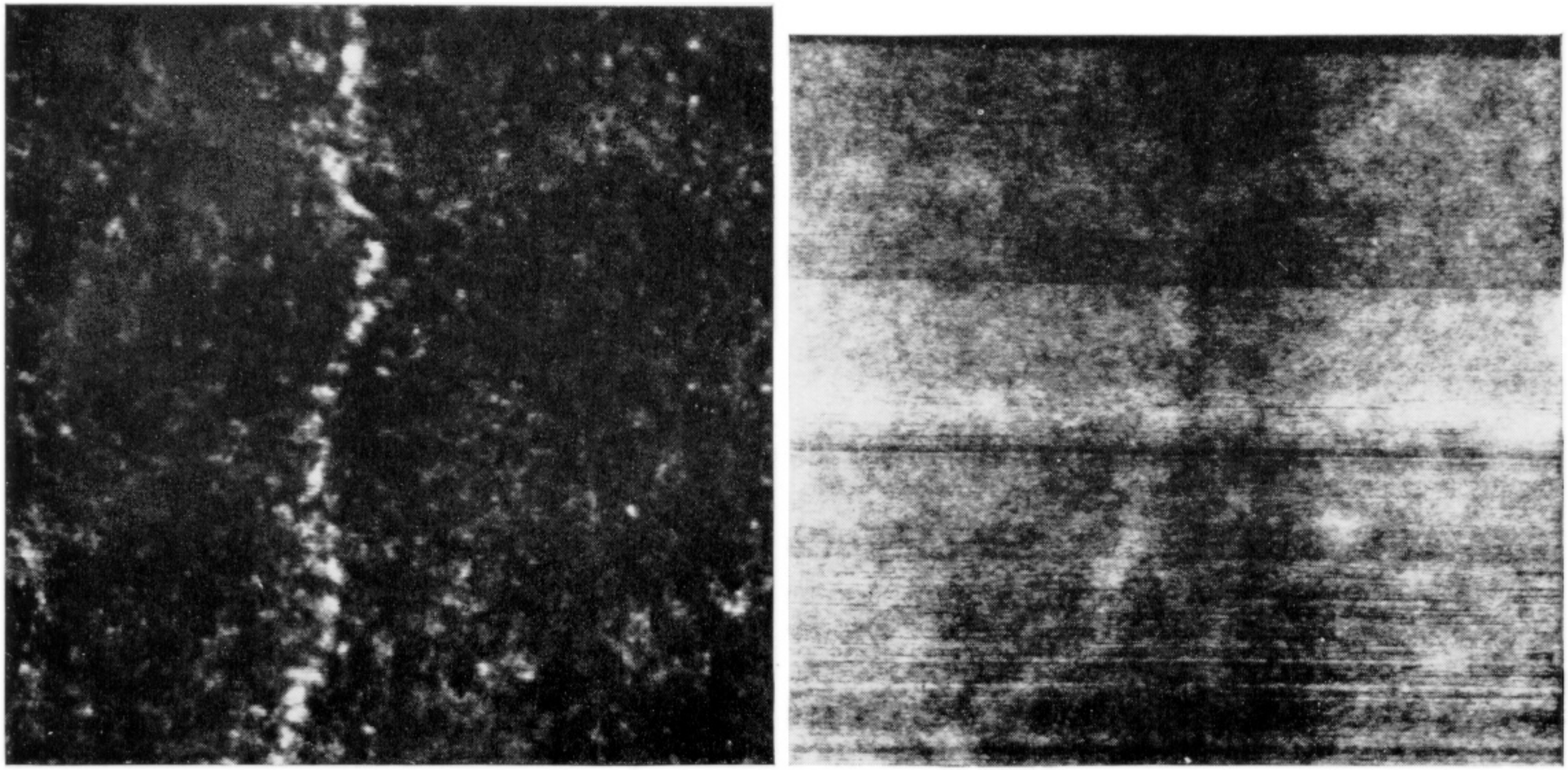
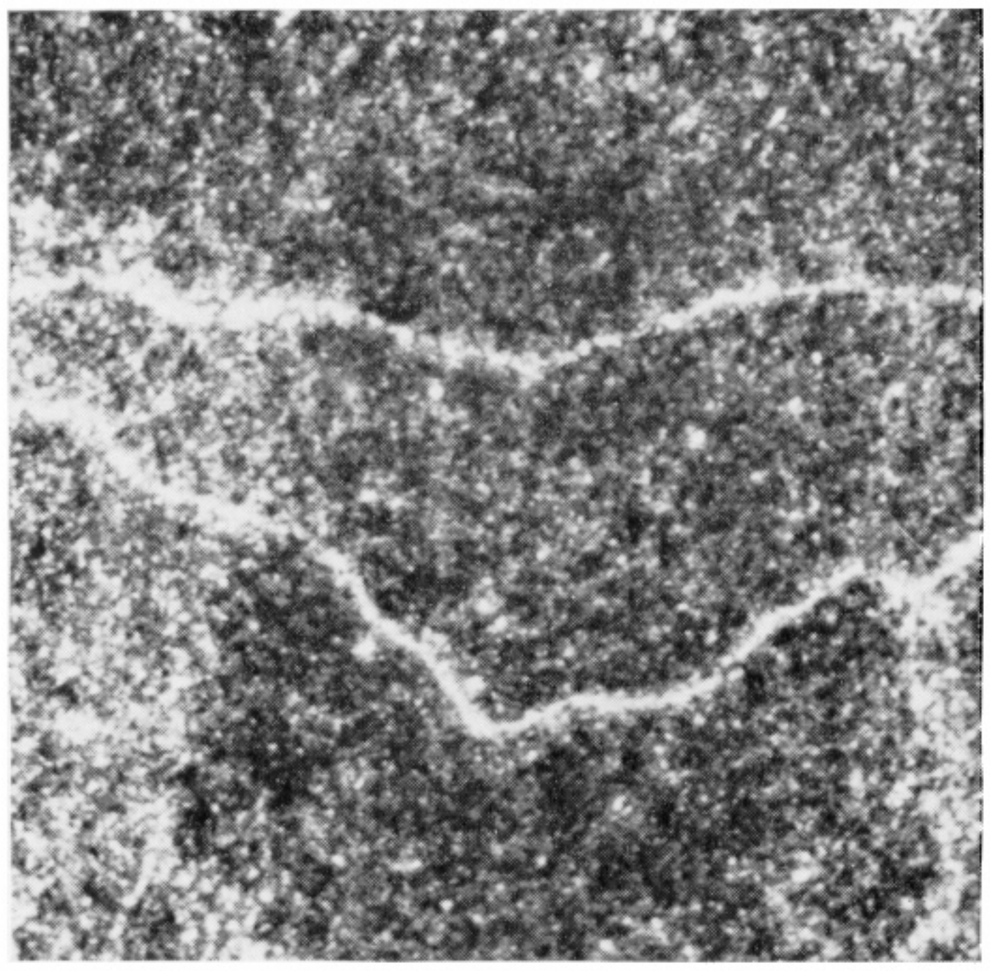


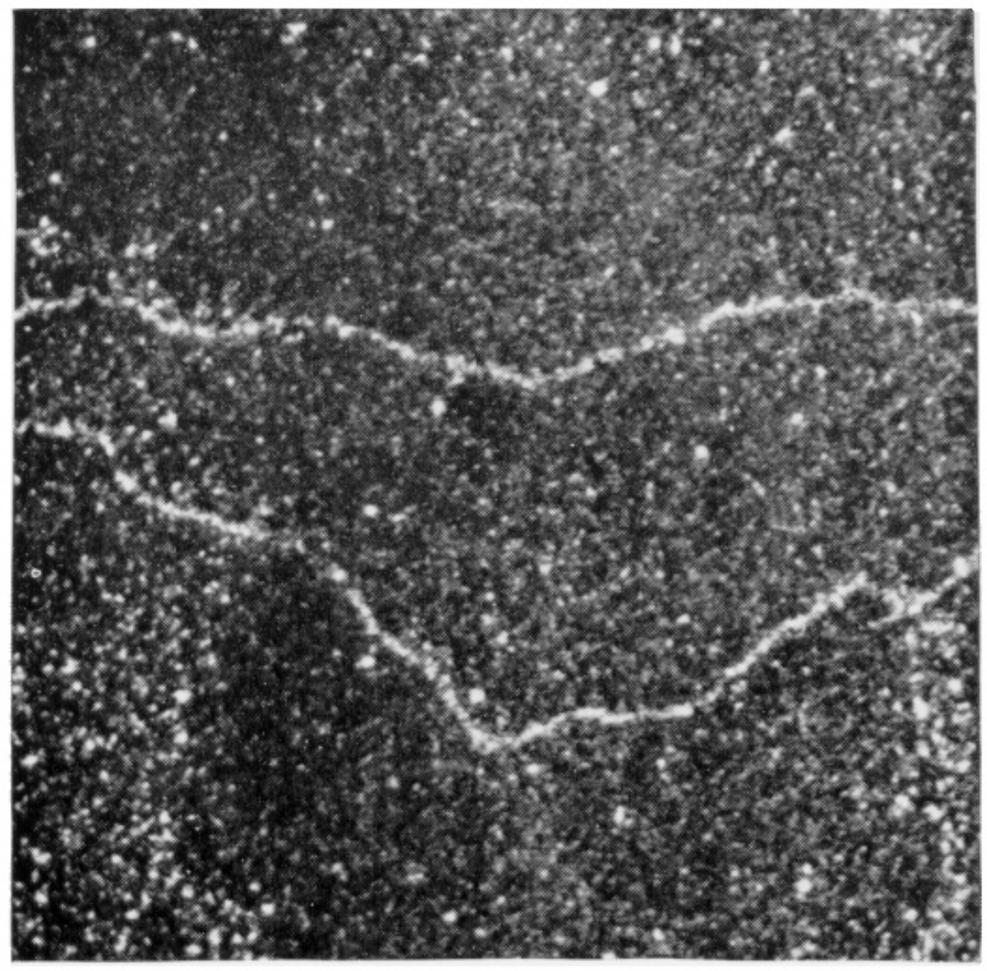
elastic inelastic

FIGURE 6. DNA stained with 10^{-4} mol l^{-1} uranyl acetate. Bright spots correspond to aggregates of ~ 7 uranium atoms spaced on the average of 2 nm along strand. Background is 3 nm carbon covered with ~ 0.3 nm uranium.

7 *a*



7 *b*



8

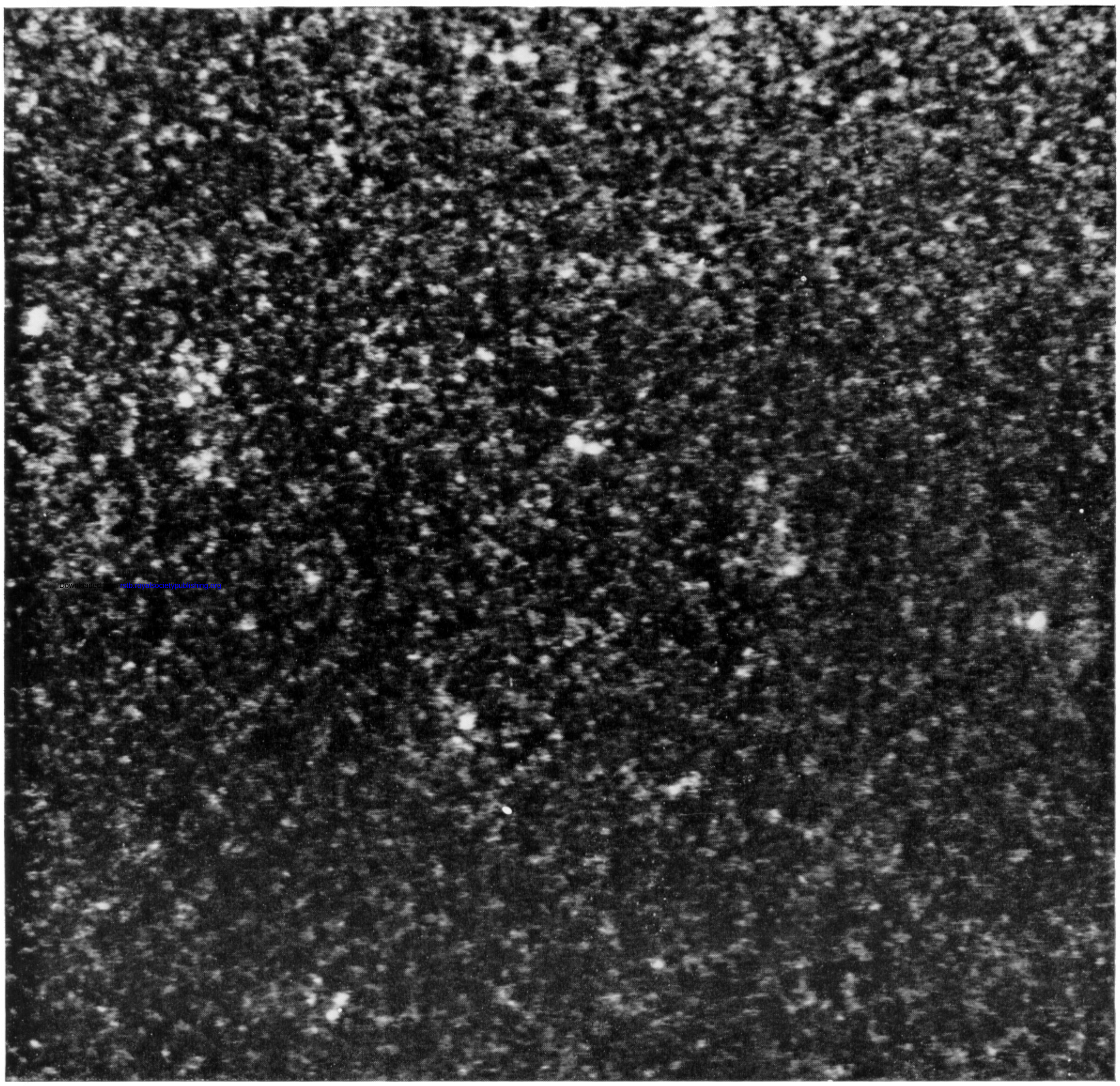


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FIGURE 7. T_4 DNA in 10^{-3} mol l^{-1} sodium, 300 nm full scale (*a*) elastic and (*b*) ratio of elastic to inelastic electrons. Note that the background is reduced when taking the ratio of elastic to inelastic electrons. This is because such a signal is independent of the thickness of the film and the background noise is now due to statistical variations in the two signals. The apparent Z for the strand is 10 to 20; for the background 10. The thickness of the strand 0.5 nm; of the background 2.3 nm.

FIGURE 8. Micrograph of a thin carbon film which has been coated with a dilute solution of uranyl acetate reacted with benzene tetracarboxylic acid. The expected appearance of the uranium atoms is that they will appear in pairs separated by 13, 23, 33, etc. nm. This picture was taken using the ratio N_e/N_i. Several pairs of bright spots can be seen. These are presumably uranium atoms. 210 nm full scale.