Proceedings of the

29th INTERNATIONAL FIELD EMISSION SYMPOSIUM

held at
Chalmers University of Technology, Göteborg, Sweden
August 9–13, 1982

Edited by

H-O. Andrén and H. Nordén

ALMQVIST & WIKSELL INTERNATIONAL
STOCKHOLM/SWEDEN

ON THE FEASIBILITY OF IMAGING UNSTAINED DNA BY FIELD-ION TOMOGRAPHY

J. A. Panitz

Sandia National Laboratories
Albuquerque, New Mexico 87185

INTRODUCTION

Recently, we described a high field imaging technique which can be used to obtain contour slice images of unstained macromolecules placed on the apex of a field-emitter tip.[1] From a series of contour slice images, a three-dimensional reconstruction of molecule morphology can be obtained.[2] We call this technique "Field-Ion Tomography" since field-ion techniques are used to obtain a series of contour slice images. Unlike Computer Assisted Tomography[3] (which uses x-rays to probe the interior of an object) Field-Ion Tomography can only be used to reconstruct its exterior morphology.

In order to apply field-ion techniques, an object must be placed on the apex of a field-emitter tip within an area close to the tip axis.[1] Last year, we described an aqueous deposition procedure which can be used to place biomolecules within the area of the tip apex accessible to imaging.[4] By avoiding traversals thru an air-liquid interface which contained denatured protein, reproducible, submonolayer coverages of ferritin were obtained. Ferritin is an iron containing protein found in the liver and spleen of all mammals (including man).[5]

Although the procedure developed for ferritin can be used to place other biomolecules on to field-emitter

tips,[6] the procedure is difficult to apply unless there is an ample supply of the molecule of interest. Typically, several milliliters of sample are required for each deposition. A sample concentration of ≈10 μg/ml is needed to insure that an adequate number of molecules will be deposited on to the tip surface in a reasonable length of time (2-5 minutes). In order to work with DNA, we found it necessary to scale down the dosing parameters. To this end, we have developed a new dosing procedure which requires only 10λ (ten microliters) of sample at a concentration of ≈5 μg/ml.

MOLECULE DEPOSITION

In order to insure that molecules are successfully deposited onto field-emitter tips, a set of empirical "rules" have evolved:[4] 1) A field-emitter tip of large apex radius (120-250 nm) should be used. 2) The emitter-tip must only traverse a molecule-free, air-liquid interface. 3) The emitter tip and its support must be made of the same material if the tip and its support are both immersed into the deposition volume. The first "rule" minimizes surface tension problems associated with traversing a liquid-air interface. The second insures that molecules are not deposited onto the tip surface by the Blodget-Langmuir effect.[7] The third "rule" prevents galvanic activity from changing the distribution of molecules on the tip surface during deposition.[8]

A small volume doser has been designed which meets the deposition requirements outlined above. A solid teflon block is machined to contain a small dosing cavity whose base forms the seat of a valve placed in series with a supply of pure water. A drop (≈10λ) of suitable buffer is placed into the cavity with the valve closed. The tip is immersed into the droplet, and then ≈10λ of dosing solution is added at a concentration of ≈10 μg/ml This step in the deposition process is shown in Figure 1.

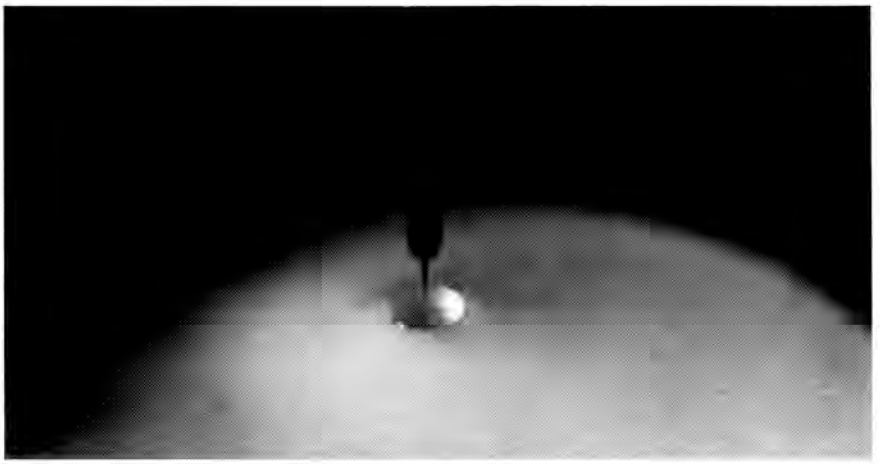

Figure 1. DNA deposition on to a field-emitter tip.

At this point, the concentration of molecules in solution is reduced to ~5μg/ml by the 2:1 increase in dosing volume. Since protein molecules will not generally adsorb on to teflon, the number of molecules deposited on to the tip surface will be determined by their concentration in solution and the deposition time.

After a suitable deposition time has passed (as predetermined by an idependent visual test of protein adsorption[9]), the valve at the base of the dosing cavity is opened. By carefully opening the valve, the flow of pure water into the dosing volume can be controlled. If the dilution process is observed with a microscope, concentration gradients during dilution can be seen. When these disappear, the concentration of molecules within the dosing volume will be negligible, and the tip can be safely withdrawn through a molecule-free, air-liquid interface. Since the tip support is not immersed into the dosing volume, galvanic effects[8] are eliminated. This means that the tip and its support can be made of different materials. This greatly simplifies tip preparation. Unlike the previous deposition method,[4] a preetched tip can be spotwelded to a prefabricated support.

Using the new dosing procedure, a synthetic DNA copolymer[10] was deposited on to several tungsten field-emitter tips. Of eight tips examined by Field-Ion Tomography, only one gave reproducible and structured images. The details of the imaging process will not be reviewed here, since they have been extensively discussed elsewhere.[1,2,6] Figures 2A-2E show contour slice images of the DNA distribution at decreasing elevation from a tungsten tip surface. Figure 2F shows a digital reconstruction[11] of adsorbate morphology made from these contour slice images. Figure 3 is a stereo pair image generated from the individual contours by a digital processing technique.[11]

The central portion of the large feature in Figures 2F and 3 appears to be a double helix, a structure never seen in Field-Ion Tomographic images of other molecules.[6] The size and the shape of the small feature (indicated by an arrow in Figure 2F) suggests that it is a single DNA molecule. If this interpretation is correct, the large feature in the image may be supercoiled DNA, a structure well documented in the literature.[12] A more precise knowledge of image magnification (and many more images) will be needed before more definitive conclusions can be reached. Nevertheless, these preliminary experiments seem to demonstrate the feasibility of imaging unstained DNA by Field-Ion Tomography.

We are currently examining various types of DNA in an attempt to select the form of the molecule which is most suitable for Tomographic imaging. In addition, we are attempting to define optimum buffer conditions which will lead to more successful depositions. We already know that ammonium acetate (a buffer often used by electron microscopists) is unsuitable because it tends to etch our tungsten specimens. For a similar reason, we suspect that EDTA is unsuitable.

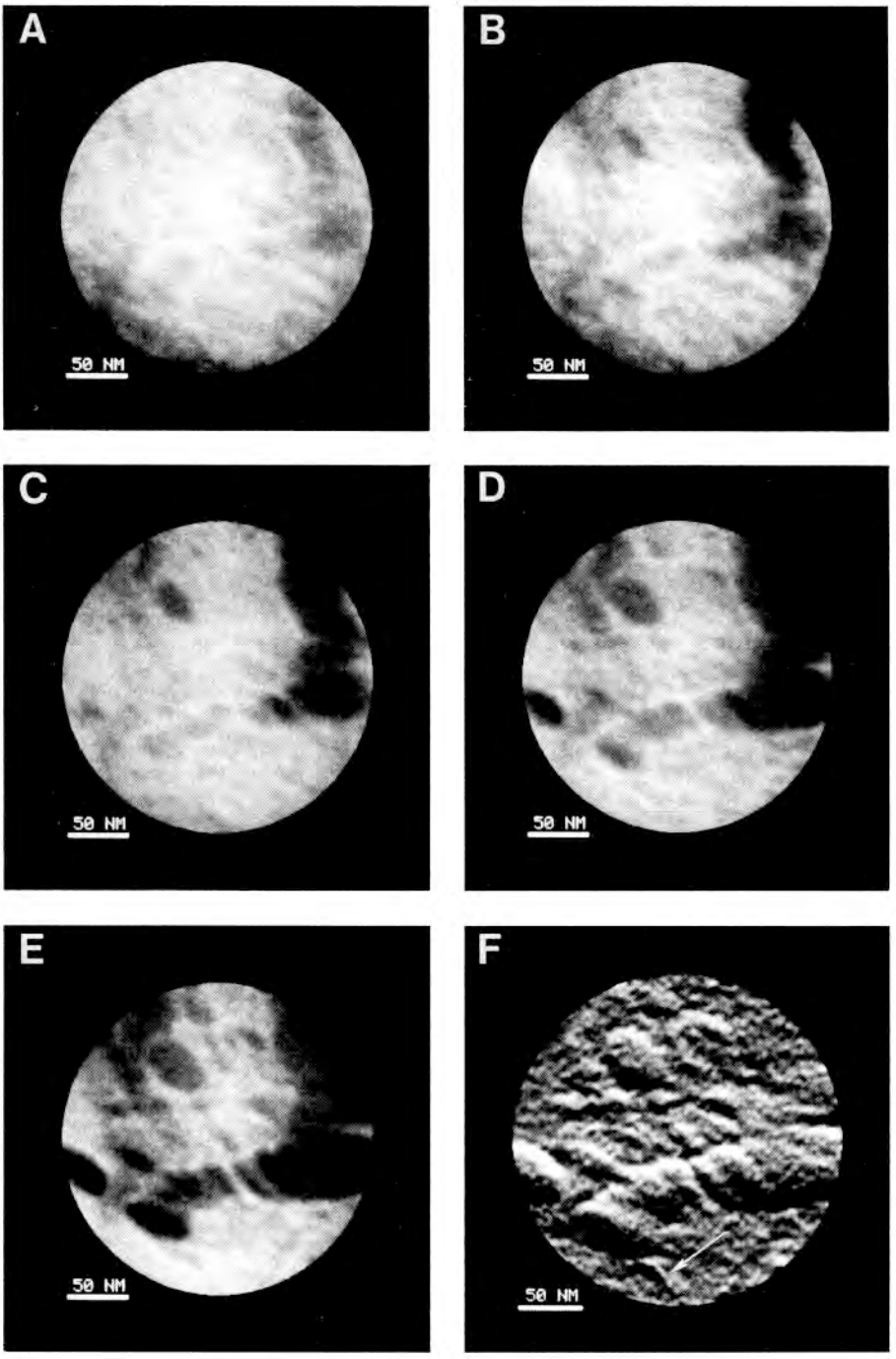

Figure 2. Field-Ion Tomography of Unstained DNA.

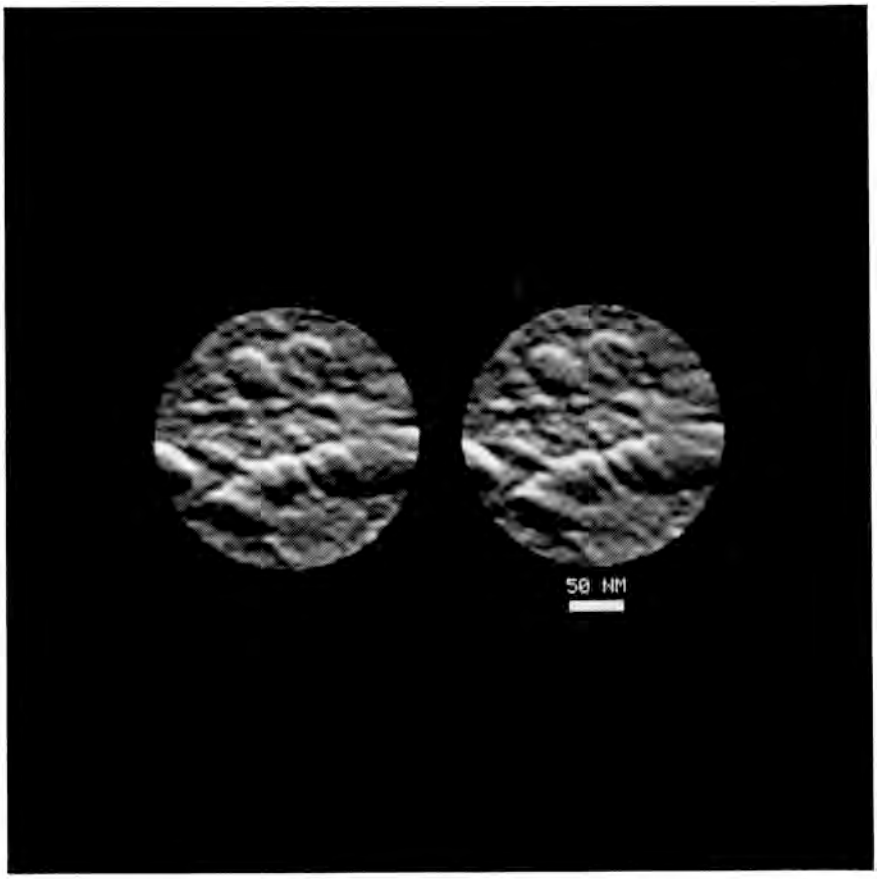

Figure 3. A stereo pair image of unstained DNA on tungsten. The image was digitally reconstructed from a series of contour slice images (Figure 2A-2E).

ACKNOWLEDGEMENT

The author gratefully acknowledges the advice and assistance of Ivar Giaever (the General Electric Research and Development Center, Schnectady, NY) who participated in the imaging experiments. Gerry Fowler, as always, provided expert technical assistance and was responsible for constructing the new doser described in this paper. The author also wishes to thank Dennis Ghiglia for his advice and assistance in preparing the digital images presented in this paper.

REFERENCES

1. J. A. Panitz, J. Microscopy 125, 3 (1982).
2. J. A. Panitz, Ultramicroscopy 7, 241 (1982).
3. See for example: R. W. Redington and W. H. Berninger Physics Today 34, 36 (1981).
4. J. A. Panitz and I. Giaever, Ultramicroscopy 6, 3 (1981).
5. R. R. Crichton, New England J. Med. 284, 1413 (1971).
6. J. A. Panitz, "Point-Projection Microscopy" in The Analysis of Organic and Biological Surfaces, ed. P. Echlin (Wiley Interscience, NY), in press.
7. K. B. Blodgett, J. Am. Chem. Soc. 57, 1007 (1935).
8. J. A. Panitz and I. Giaever, Surf. Sci. 97, 25 (1980).
9. I. Giaever, J. Immunology 110, 1424 (1973); 116, 766 (1976).
10. Sigma Chemical Company (Saint Louis, Mo) Cat. #P9764.
11. D. C. Ghiglia and M. Flickner, Optics Letts. 7, 116 (1982).
12. W. R. Bauer, F.H.C. Crick and J. H. White, Scientific American 243, 118 (1980).

*This work performed at Sandia National Laboratories.