A Simple Method for Imaging DNA using SEM

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The intricate relationship between molecular structure and function is a common theme in molecular biology. Visualizing the structure of biological macromolecules through imaging is therefore useful in understanding their varied biological roles. The process is often complex; imaging in a high voltage Transmission Electron Microscope (TEM) involves extensive staining and freezing.[1] The aim of this experiment was to image DNA easily in a close to natural environment in a simple microscope. Samples were imaged using a Leo (Zeiss) 1550 Scanning Electron Microscope (SEM) with a Schottky field emitter in the 15-30kV range to reduce radiation damage. After imaging off-the-shelf DNA, two more DNA samples were dialyzed with RbCl and NaCl and imaged to elucidate what made the DNA visible. Rb^+ is very similar to Na^+ in its chemical interactions with negatively charged DNA, so the simulated environment is close to natural.[2]

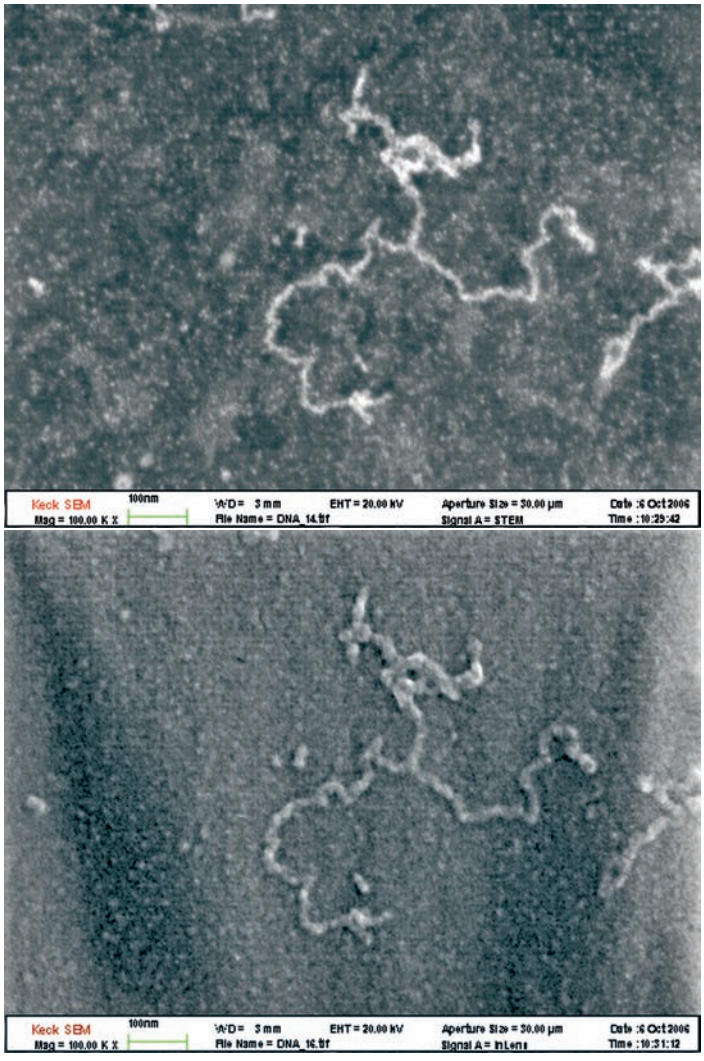

Fig 1a: *Off-the-shelf DNA imaged in ADF-STEM mode on a Leo (Zeiss) 1550 with Schottky emitter.* **b:** *The same molecule imaged in SE mode*

Inaga *et al.*[3] have also presented SEM images of uranyl acetate stained DNA.

The off-the-shelf sample was prepared using 16µm lambda DNA (#N3031L) from New England Biolabs. The DNA was not treated in any way, and was simply diluted from 50µM to 0.1µM using de-ionized water. There was no purification or staining. Three µL of solution were pipetted onto copper mesh grids with an ultra thin (2-3 nm) carbon film. The grids were then allowed to dry in air for one hour.

Fig 1 (a+b) shows images taken using both Annular Dark Field Scanning Transmission Electron Microscope (ADF-STEM) and Secondary Electron (SE) modes. ADF-STEM images were taken using a solid-state detector (K&E Developments). The use of a thin substrate reduced the specimen volume of secondary electron production in SE mode and allowed transmission imaging. This significantly enhances resolution in SE mode.

A second sample was prepared from the same 16µm lambda DNA used above, but was subsequently dialyzed with RbCl**.** One µL of DNA was first placed in a filter fine enough to block passage of the DNA. 400µL of 1M RbCl was then added, and the tube was spun in a centrifuge until the RbCl had passed through the filter (~20min). This was repeated 4 times. Next, 400 µL of de-ionized water was added and centrifuged through the filter. This was repeated 8 times. The filters were then inverted and the DNA spun out. The final DNA concentration was roughly 0.1µM, though this need not be precise. 0.1µM was selected to simplify the process of finding DNA on the carbon mesh grids. 3µL of solution were then pipetted onto each grid and allowed to dry in air for one hour.

A third sample was prepared using 1M NaCl in place of 1M RbCl. Although the Rb^+-treated DNA imaged easily, the Na^+-treated DNA did not. Both were imaged in dark field STEM mode only. Dark field STEM collects electrons scattered to high angles, which are much more likely from heavy atoms.

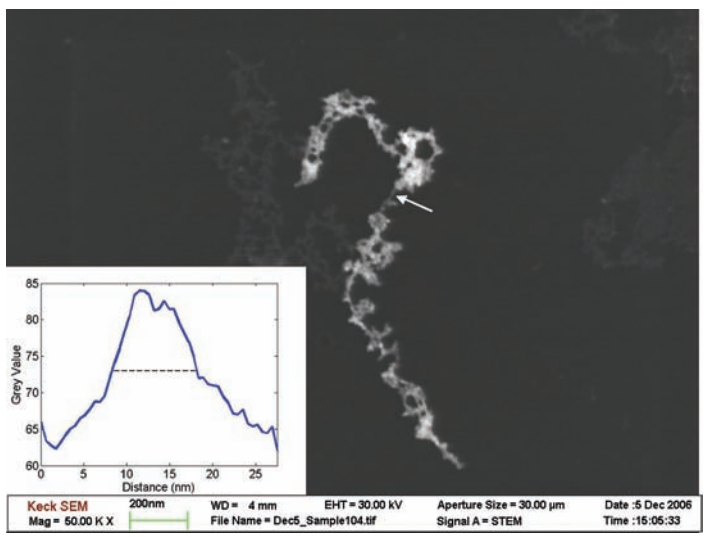

Fig 2: *ADF-STEM image of lambda Rb-DNA.* **Insert:** *Pixel value vs. length across a thin section of DNA (indicated by arrow). The full width at half maximum (dotted line) was measured to be 8.3nm. This is comparable to the width of a 2nm diameter strand of DNA surrounded by Rb ions.*

This produces a higher contrast for heavy atoms (the so called *Z*-contrast signal with limited chemical sensitivity). Fig 2 shows images of Rb^+-treated DNA.

Conclusions

Both off-the-shelf and the Rb^+-treated DNA were imaged with relative ease without complex preparation. The off-the-shelf DNA imaged in both the ADF-STEM and SE modes.

To understand the mechanism for imaging the DNA, DNA was then dialyzed with NaCl and RbCl to produce known sample conditions. While the Rb^+-treated sample was clearly visible, the Na treated sample was not, suggesting that the heavy Rb nucleus may act like a stain in the dark-field mode. This method provides a simple way to image DNA in a SEM without perturbing molecular interactions. ■

Acknowledgements

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References

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