A Comment on AFM vs. Replicas for High Resolution Imaging

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High-resolution Pt/C shadowing and replication provided important insights into the size and shape of polymers beginning over 40 years ago. The first images of DNA molecules were made this way. However, in my opinion, this methodology has largely been supplanted by the use of Atomic Force Microscopy (AFM), both for direct height measurements (available in Pt/C replicas by measuring shadow lengths when the coating is deposited from one direction only) and for imaging molecular contours.

As an everyday example, consider that making a magnetic read and write head for a hard disk drive requires controlling the relative heights of several different regions to a tolerance of about 1 nm. AFM supports production by providing a rapid means of offline analysis, far faster and more precise than any replica method could be.

In the biopolymer area, for more than 10 years it has been relatively easy to prepare dispersions of molecules on smooth surfaces like mica for AFM images. Collagen and DNA are good examples of this.

One way to characterize materials such as collagen is by using Atomic Force Microscopy. Individual molecules can be seen and measured, and whether the molecule is a fragment, monomer, dimer, or higher oligomer can be determined from the length and geometry of the molecule. Monomers are nominally 280 nm long. Fragments will be shorter. Dimers and higher oligomers may be linear, branched or looped.

Collagen for medical use is commonly produced by digestion of bovine skin. This soluble collagen consists of individual "monomers," longer chains (dimers and higher oligomers) and fragments. In the USA, federal regulations require that the consistency of this mix be documented and controlled. One medical use of purified collagen monomers is to plump up the skin (remove wrinkles). By using AFM to take images of batches of collagen molecules and measuring the molecules within them, one can provide an independent check on other quality assurance techniques, ensure compliance with Federal regulations, and produce better quality materials, figure 1.

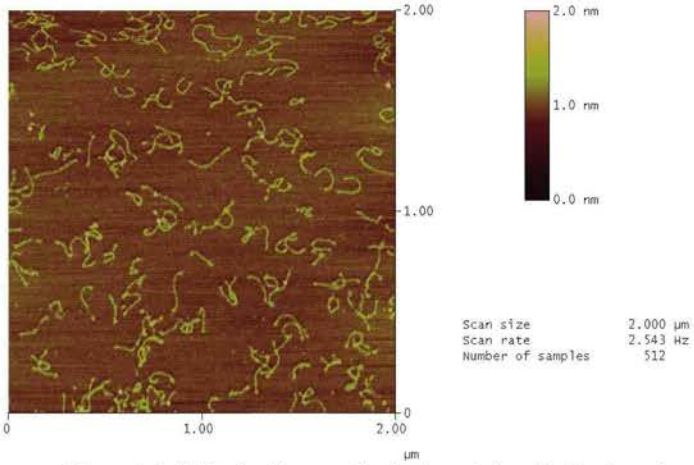

Figure 1: Individual collagen molecules deposited on freshly cleaved mica from an aqueous suspension form wormlike patterns. 2 µm scan.

Analysis of DNA is important in medicine and biology. New drugs, new treatments and new understanding of life come from better knowledge of DNA function. This in turn depends on the characteristics of the higher-order structures formed by DNA double helices. Whereas chemical and enzymatic probes yield only the average behavior of an ensemble of DNA molecules, direct observation of individual molecules provides specificity that sharpens insight.

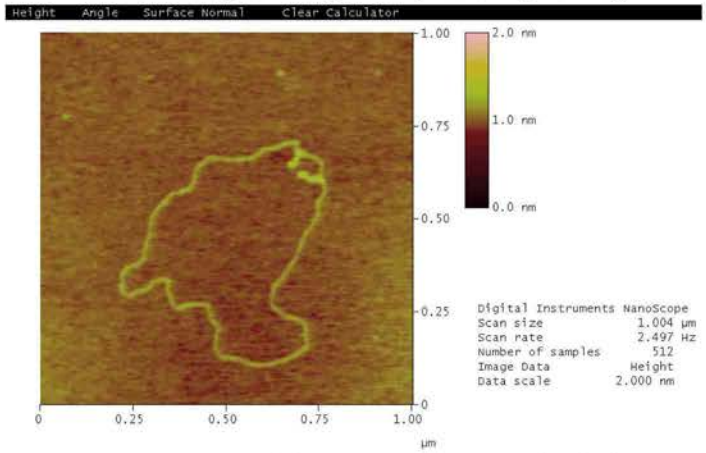

Figure 2: Tapping Mode height image of a single molecule of DNA, dried on a mica surface. Note the bright branch-like segments near the upper right. These thicker, coiled regions are present because the molecule is supercoiled. 1 µm scan.

DNA usually has a rod-like conformation that makes it ideal for AFM studies of molecular weight and conformation. Molecular weight is proportional to the contour length of the molecule. The presence of supercoiling in the circular DNA plasmid molecule shown in Figure 2 is evident from the presence of short branch-like features near the upper right corner. The branches are brighter in the height image than other parts of the molecule, which means they are thicker. Such regions are interpreted as coiling of the double helix. The coiling relieves stress and is analogous to the spontaneous coiling of a telephone handset cord.

Sample preparation is fairly straightforward, consisting of preparing dilute solutions of DNA in buffer, applying a drop to a mica surface, rinsing and drying. We usually image using Tapping Mode in air, but liquid tapping is also possible.

Samples for such AFM images are more easily and quickly produced, and provide more accurate dimensional information than can be achieved with replicas.

Disclaimer: ASM provides analytical services using AFM and benefits from increasing the demand for AFM data. ■

Embedding Cultured Cells Grown in Well Plates

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For years, I have used a hybrid Epon-analog resin to embed in culture dishes. I use a standard Epon formula but utilize the following components: LX-112 and DMP-30 from Ladd Research Industries DDSA and NMA from Electron Microscopy Sciences I know it seems weird, but years ago I tried all sorts things, from the "straight" formulations from each vendor to a bunch of mixtures. This one has