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Nanomechanical Measurements in Biological Atomic Force Microscopy (AFM)

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The atomic force microscope (AFM) has found broad use in the biological sciences largely due to its ability to acquire high resolution images of native structures (molecules, cells, tissues) under fluid in near-physiological conditions. The integration of the AFM with an inverted optical microscope allows the AFM to be combined with such advanced techniques as epifluorescence, confocal, FRET and TIRF. These combinations further enable researchers to use optical microscopy to precisely navigate the AFM probe to desired locations on the sample, acquire images of surface (or near-surface) features and then correlate them with labeled structures.

In addition to imaging, AFMs are commonly used as mechanical probes. The AFM can be used in concert with functional optical labels where the AFM probe is utilized to stimulate the sample. This provides valuable information on the structure-function relationship of biological samples under native conditions. Additionally, the AFM can measure the mechanical properties (Young's Modulus) of a sample. This aspect is important for biological research as our appreciation of the role of mechanics in biological structure and function has been steadily increasing over the past few decades. Understanding this role is especially pertinent in the field of cell biology. It has been found that the geometrical and mechanical properties of the extracellular microenvironment are important in such processes as cancer, cardiovascular disease, muscular dystrophy, and even the control of cell life and death. Indeed, as cell and tissue engineering technologies become central to regenerative medicine, control and quantification of these external geometrical and mechanical factors become key issues in the field. AFM provides novel insights to both cell function and cell-substrate interactions due to its ability to quantify the Modulus of a wide variety of biological samples.

As the application of AFM to these types of problems is widened, it is important to understand the performance envelope of the technique and its associated data analyses. Important issues that must be considered when mechanical models are applied to real-world data will be discussed. In our approach, an appropriate contact model must be first selected based on the data. Once a model has been designated, accurate values must be used for such parameters as tip geometry, contact point and sample Poisson ratio. Examples of the effect of different model assumptions on our understanding of the measured material properties will be shown.

Figure 1 shows one example to be presented of how an integrated AFM/optical microscope can be used to optically select a cell of interest before mechanically probing the cell surface (a). Using a technique commonly referred to as force volume or force map, a topographic map of the surface was acquired based on the trigger point of each force curve (b). Finally, the modulus map was calculated using the Hertz model (c). Figure 2 is an overlay of modulus on top of the 3D topography – the color is solely derived from the modulus data. Variability in moduli can be seen across the cell. The stiffer nucleus can be clearly resolved against the softer cytoplasm, as to be expected for fibroblast cells. Additional appropriate data will be presented.

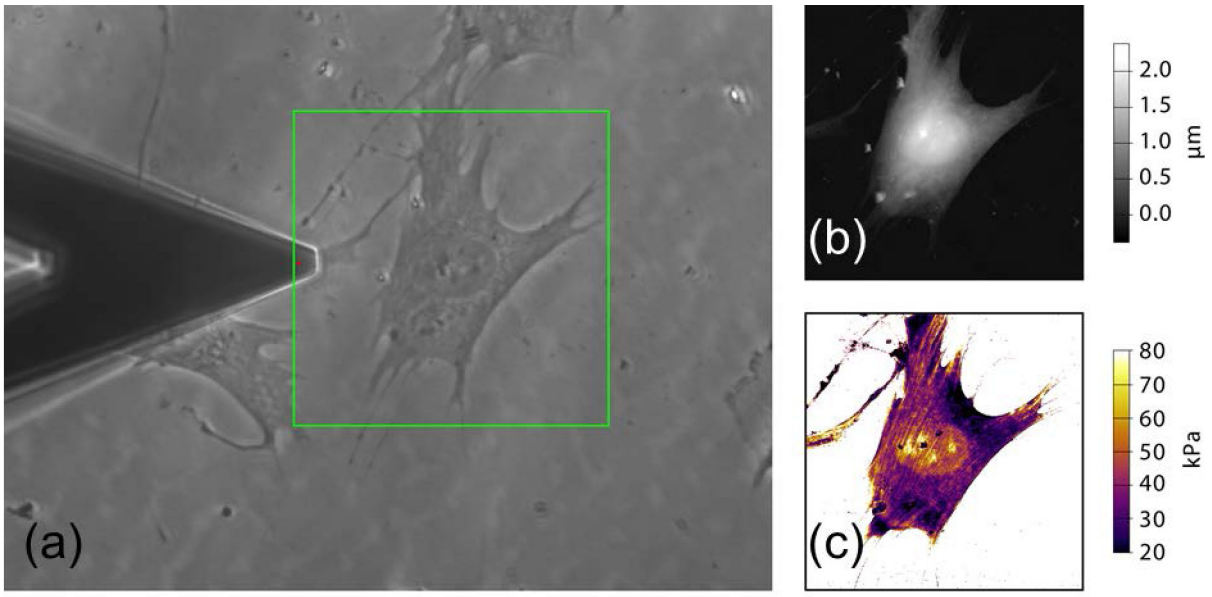

Figure 1. Image series of a lung fibroblast (MRC5 cell line). A single cell was identified using 40x optical phase contrast and the area to be probed with the AFM is outlined by the green box (a). A height image was created based on the trigger point of each force curve. A 512 x 512 force map was captured using a calibrated AFM cantilever. The corresponding modulus map was created after applying the Hertz Model to each force curve.

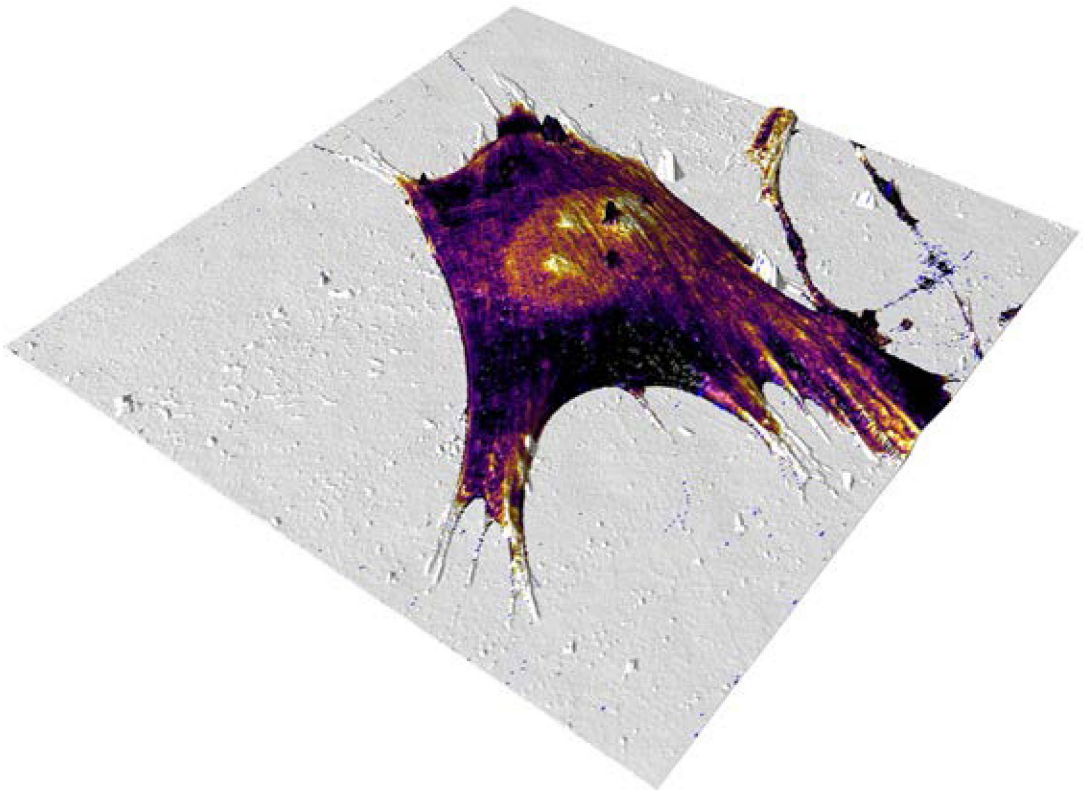

Figure 2. 3D rendering of modulus painted onto topography.

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