Keysight Technologies
Elastic Modulus Mapping Using the 7500AFM

Application Note

Introduction

The atomic force microscope (AFM) [1] has become a very important tool for investigating samples on the nano-scale. It combines in a unique way high spatial resolution with very high force sensitivity, allowing for mapping of elastic properties with highest spatial resolution.

For quantitative determination of elasticity a force distance curve is obtained. From such a force distance curve the elastic modulus can be extracted. A mapping of local elastic properties is achieved by obtaining two-dimensional arrays of force distance curves. Such elasticity maps can be used to derive information on cellular processes. In particular, elasticity measurements provide valuable insights into various dynamic cellular processes such as cell migration and cell division.

Here, we show how the Keysight Technologies, Inc. 7500 AFM can be utilized to map the elastic modulus of endothelial cells.

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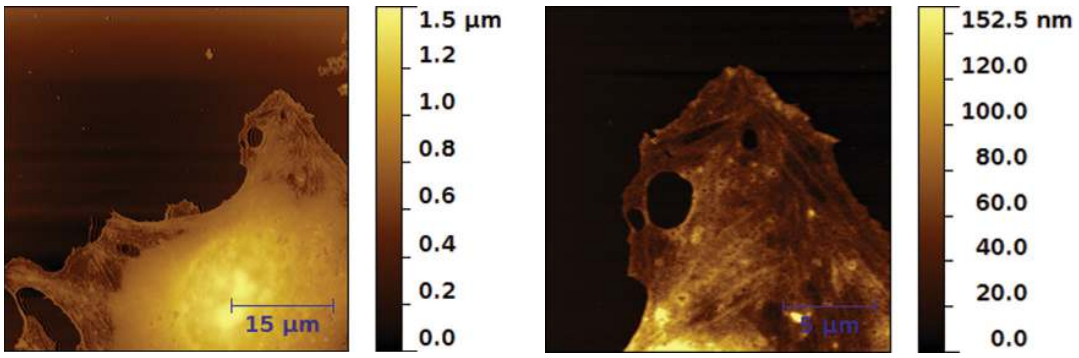

Figure 1. Topographical images. Contact mode images of a sample cell were made to characterize cell morphology. The left image shows an overview (50 x 50µm) of a cell including the nucleus (lower right) and flat parts (upper right and lower left). In the right image a zoom (18 x 18µm) of the flat area in the upper right corner is shown. Here, the actin network can be seen nicely.

Elasticity Nano Mapping

Mapping the elasticity of cells is a basic challenge in cell biology and microbiology, since the elastic properties of cells play an important role in critical cellular functions, including migration, division and growth. AFM force-distance curves measurements may be applied to map the cell elasticity at a resolution of a few tens of nanometers. Analysis of the obtained force-distance curves provides quantitative information on the elasticity[2] of the cell surface and of the underlying cytoskeleton. An elasticity map can be obtained by recording arrays of curves over the cell surface. This procedure was used on a variety of cells, including yeast cells, fibroblasts, platelets diatoms and metastatic cancer cells [3, 4, 5, 6, 7, 8, 9, 10].

This technology gives the possibility of monitoring changes in elasticity on incubation of the cells with drugs. For instance it was investigated how various drugs, which disrupt or stabilize actin or microtubule networks, affect the elasticity of cells. It was found that disaggregation of F-Actin resulted in a loss of cell rigidity. But treatments with drugs which destroy the microtubule network show no effect, leading to the conclusion that the actin network mainly determines the elastic properties of living cells [3]. Recently the stiffness of live metastatic cancer cells of patients with suspected lung, breast and pancreas cancer was measured [7]. It was found that cancer cells were substantially softer than benign cells, indicating that nanomechanical analyses can distinguish cancerous cells from normal ones even when they have similar morphologies. This shows the strong potential in biomedicine of AFM-based nanomechanical measurements.

As an example how the Keysight 7500 AFM can be used to investigate sample properties we show the results of spatial mapping of elasticity modulus of MyEnd cells. For this, cells were grown on glass slides and afterwards fixed with paraformaldehyde. After sample mounting, a cell of interest was identified using the top-down optics of the AFM. Next, it was further investigated by obtaining contact mode images (Figure 1). AFM imaging allowed to investigate the morphology. Typically MyEnd cells are organized into whirl-like formations and highly elongated. Around the nucleus the cell height is approximately 1.5µm, at the periphery flat regions with ~100nm height were found. Those flat

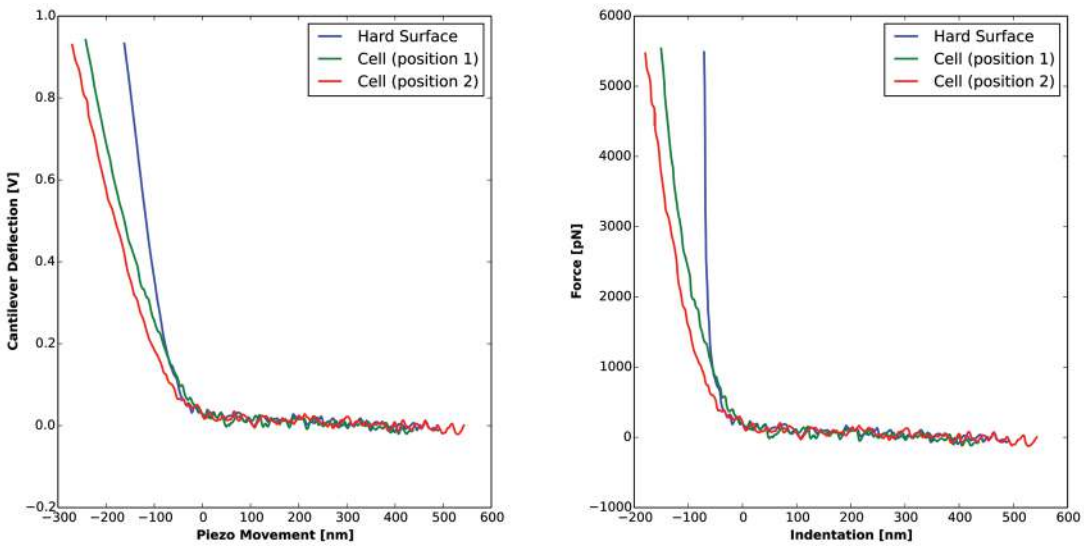

Figure 2. Force-distance curves on different substrates. The left panel shows the raw cantilever deflection as a function of the vertical piezo movement at 3 different places: glass slide (blue) and two different cell positions (green and red). The curve on the glass slide was used to determine the deflection sensitivity. The right panel shows the respective "Force versus Indentation" curve. As expected on the hard surface almost no indentation is visible, indicated by the vertical blue line. In contrast, on the cell surface the tip was roughly intended by 100nm. Those curves were used to determine the elasticity using the Hertz model for a spherical indenter. The calculated values were 308 and 345kPa for cell position 1 and 2, respectively.

regions allowed to observe the underlying actin network, which is responsible for the cell morphology.

Once a cell of interest was found, individual force distance curves at different sample locations were recorded (Figure 2). Force distance curves on the glass substrate showed the expected linear behavior during tip contact. Those curves were used to determine the deflection sensitivity. In contrast to that, force distance curves on a cell showed a curved behavior with a moderate slope compared to curves on glass. The moderate slope can be attributed to the fact that the tip is indenting the cell. The curvature can be explained by taking into account that during indentation the contact area between substrate and tip is increased. Since no adhesion between substrate and tip was observed a simple Hertzian contact mechanics model was used to extract the elasticity modulus, which was found to be several hundreds of kPa, which is in good agreement with literature values [11].

Finally, the elastic modulus was mapped by measuring two dimensional arrays of force distance curves. These arrays were converted into images by analyzing the individual force distance curve. For this the "Super plug-in package" was downloaded from www.pico-cafe.com and installed. Amongst others it contains a module allowing to determine the elasticity modulus during force curve acquisition. The resulting elasticity map is shown in Figure 3. The supporting glass substrate is stiffer than a fixed cells, visualized by the blue color. The elasticity of the cell weakly anti-correlates with the height of the cell itself. I.e. the cell is stiffer in flat regions and softer around the nucleus. Especially the stiffness is larger in the periphery in the upper left corner.

Conclusion

Force distance curve based AFM measurements are a powerful tool to quantify and map several parameters of samples with high spatial resolution. As an example we have shown how Keysight's 7500 AFM can be used to help to understand cell mechanics. In particular, the ability to making 2D arrays of force distance curves

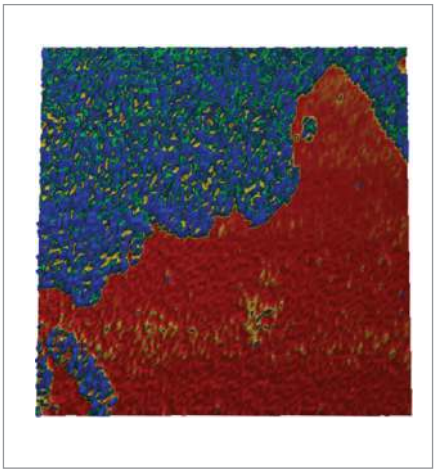

Figure 3. Elasticity map of a fixed MyEnd cell. An array of force distance curves were measured at the same position as shown in Figure 1. A map of elasticity modulus was constructed by analyzing the individual force distance curves. The image size is 40 x 50 µm. The color scale ranges from < 100 kPa (red) to > 2000 kPa (blue). The stiff glass substrate is indicated by the blue areas, while the softer cell is red. The flat region of the cell in the top part are slightly stiffer than the lower part of the cell.

combined with the possibility to analyze these data online, with a user-supplied function on-line, was used to map properties of samples on the nanoscale. For this, force distance curve maps of fixed cells grown on a glass slide were recorded. The individual curves were analyzed using a Hertzian model to extract the elastic modulus, resulting in an elasticity map. The extracted values agreed well with previously published values for fixed cells [11].

Materials and Methods

Immortalized microvascular endothelial cells from myocardium (MyEnd cells) were grown and prepared as previously described [12]. The cells were a kind gift of Professor Peter Hinterdorfer (Institute for Biophysics, University of Linz, Austria). Elastic moduli were derived from force distance curves. In order to measure the moduli quantitatively and laterally resolved the Keysight 7500 AFM was operated in the volume spectroscopy mode, i.e. two-dimensional arrays of force distance curves were recorded while raster-scanning the tip across the sample. Elastic moduli were calculated from force distance curves using the Hertzian model as described previously [13].

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