

# Enhancing the Precision of Biophysical Force Spectroscopy Assays using Focused Ion Beam Modified Cantilevers

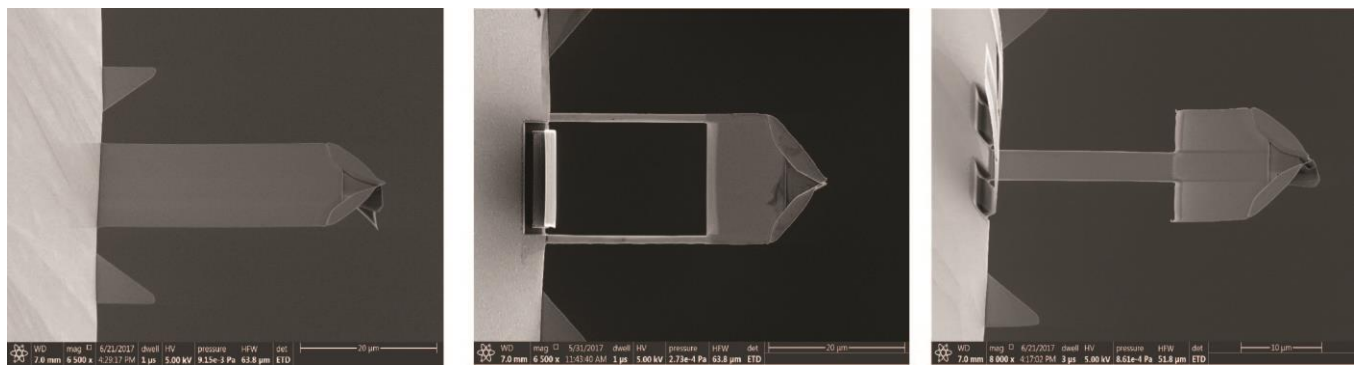
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State-of-the-art atomic force microscopy (AFM)-based force spectroscopy assays require cantilevers with dimensional control and modification at nanometer length scales. Such cantilevers have been used to unfold proteins with extremely high spatial and temporal precision. Our group has started to use a focused ion beam milling machine to modify the dimensions of commercial AFM cantilevers. The first modified cantilever shape was achieved by drilling out a rectangular portion of the BioLever Mini. The second shape, called the war-hammer, was achieved by drilling out the sides of the cantilever, leaving only a central support structure. In order to compare the modified and unmodified cantilevers, both the spring constants and the power spectral density (PSD) data were used. The PSD data shows that the modifications have changed the cantilever from an underdamped simple harmonic oscillator (SHO) to an overdamped SHO, ideal for force spectroscopy measurements. Before modification, the cantilevers had a spring constant of roughly 50 pN/nm or higher. After modifications, the first configuration had a spring constant of 10 pN/nm. The ‘war-hammer’ configuration had a spring constant of 7 pN/nm. The benefit of the lower spring constant is that the low frequency force drift is lower as compared to a stiffer cantilever. Also, according to the Fluctuation-Dissipation Theorem, which states that the force precision is proportional to the square root of the hydrodynamic drag, the modified cantilevers will have increased force precision. We plan on using these modified cantilevers to investigate peptide-lipid interactions with higher force precision and temporal stability than previously available in our lab.



**Figure 1.** From left to right SEM images of: commercially available BioLever Mini AFM cantilever; configuration one modified tip; configuration two, war-hammer, modified tip.