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**Bioelectromechanical Imaging by Scanning Probe Microscopy:
Galvani's Experiment on the Nanoscale**

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Strong coupling between electrical and mechanical behavior is a universal feature of biological systems. Indeed, the effect of electric current on muscle contraction in a frog leg was discovered as early as the 18th century by Galvani. In the last 50 years, it was shown that nearly all biological tissues based on polymers such as collagen, cellulose, or keratin, possess strong piezoelectric properties. It has been postulated that piezoelectricity is intrinsically related to the fundamental mechanisms of tissue development including bone growth and remodeling. However, macroscopic studies of the elastic and electromechanical properties in biological systems are inherently limited by the complex structure of these materials, thus necessitating studies of electromechanical and elastic properties of biological systems from mesoscopic to molecular levels. Here, we demonstrate a scanning probe microscopy (SPM) based approach for electromechanical imaging and spectroscopy of biological systems based on the combination of 3-dimensional Piezoresponse Force Microscopy and Ultrasonic Force Microscopy that allows local electromechanical and elastic imaging with nanometer resolution. This technique is used to address several biological systems, including hair, tooth and butterfly wings. 3D piezoelectric activity in butterfly wings is interpreted in terms of the relative orientation of chitin molecules and is correlated with the surface topography and elasticity map. In the human tooth, we observed a vector electromechanical response of a single collagen molecule bundle that allows the local molecule orientation to be reconstructed. This allows us to repeat Galvani's experiment on the nanoscale – 230 years later and with a million times higher resolution. The future opportunities of electromechanical SPM for characterization of complex biological systems are discussed.

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