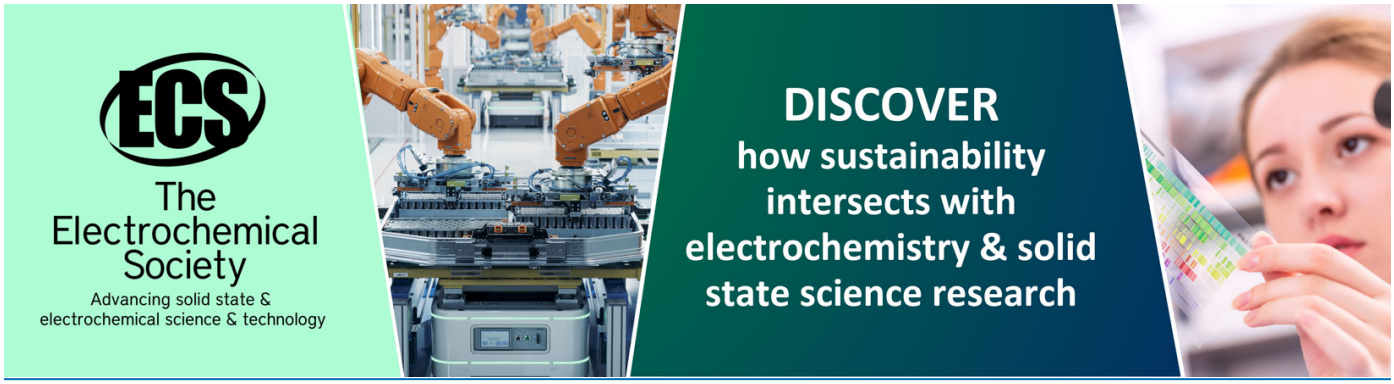
# Hybrid mode piezoresponse force microscopy for compositional electromechanical study of biopiezoelectrics





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# Hybrid mode piezoresponse force microscopy for compositional electromechanical study of biopiezoelectrics

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**Abstract.** We present our recent development of new approach for compositional study of topography, nanomechanical and piezoelectric properties with nanometer spatial resolution and demonstrate it in application to two types of biopiezoelectrics: type I collagen and diphenylalanine peptide nanotubes.

## 1. Introduction

Atomic force microscopy (AFM) is a powerful tool for surface imaging and examination of a material's local properties with nanometer-level spatial resolution. Since an AFM's working principle is based on the direct interaction between sharp tip and sample, a variety of unique AFM measurement techniques have been developed: quantitative nanomechanical measurements, conductivity mapping, local electromagnetic studies etc. One of these AFM techniques is Piezoresponse Force Microscopy (PFM) – where we explore the electromechanical performance of ferroelectric and piezoelectric materials in terms of their domain morphology with nanometer spatial resolution in different environments and various temperatures. Since the development of this technique by Guthner and Dransfeld in 1991 [1], PFM has become a widely used technique for ferro- and piezoelectric crystals research.

Piezoelectricity was also discovered for biological objects. This effect, which arises from the electromechanical coupling, was named biopiezoelectricity. Biopiezoelectricity was observed in certain types of muscular movement, the nervous system, ion transportation, amino acids etc. [2–8] and as a result, its detection has become important for nanomedicine and biomedical applications. This requires a new method for investigating of electromechanical coupling in life systems. PFM is great candidate for this purpose since it allows piezoresponse measurement with nanometer-level resolution. However, PFM being a contact mode technique, it is unsuitable for studying biological samples: the lateral tip-sample interaction arising from the constant contact of AFM tip with the surface can be significant enough to destroy or deform softer and fragile materials. We introduce a new approach for PFM investigation of such soft and fragile objects by utilizing the reduced lateral tip-sample interaction in HybriD Piezoresponse Force Microscopy mode (HD PFM)

## 2. Instrumental part

HD PFM is extension of recently introduced HybriD mode (HD mode) – scanning technique based on fast force-distance curves measurements with real-time processing of tip response (Figure 1).

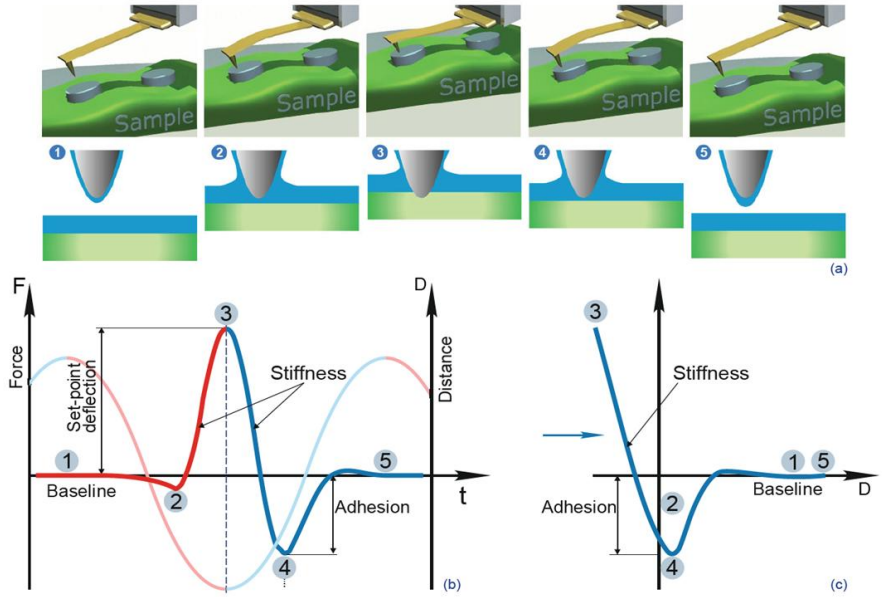


**Figure 1.** HD mode working principle: a) a model illustrating a performance of HD mode; b) an idealized temporal deflection curve during an oscillatory cycle; c) an idealized force-distance curve.

In HD mode, the sample or the tip is driven into a vertical oscillation by a Z piezoscanner with hundreds of Hertz frequency. As the result, each oscillatory cycle AFM tip goes through 5 main points: point 1 – away from the surface where only long-distance forces can be detected, point 2 – bends down in response to adhesive or capillary forces, point 3 – bending reverses upwards until it reaches the set-point level, point 4 – experiences adhesive interactions, point 5 – goes back to the baseline. The resultant vertical and lateral displacement curve of the tip is recorded and analysed to get: topography, Young's modulus, tip-surface adhesion value and long-distance electrostatic or magnetic forces map simultaneously.

One of the features of HD mode is ability of "time window" measurements: ability of switching on AC or DC voltage supply, signal recording and processing in the user-defined part of oscillatory cycle. This feature allowed for example to investigate conductivity of carbon nanotubes by allying DC voltage and detecting tip-sample current signal in the "time window" corresponded to the tip-sample contact (range 2-4 on the figure 1) [9].

HD PFM mode working principle also based on the "time window" approach (Figure 2). In the user-defined "time window" referred to the tip-sample contact the AC voltage applied between conductive coating of the tip and investigated object. AC voltage causes mechanical oscillations of the sample depending on its local polarization. Corresponding vertical (DFL siganl) and lateral (LF signal) motion of AFM tip is recorded in defined "time window" and processed to get amplitude and phase signals. Amplitude of DFL and LF signals characterize local piezoelectric coefficient of the material whereas the phase signals give information about local polarization direction. The full DFL(t) curve of each HybriD mode circle is also processed to calculate adhesion, E modulus, feedback input signals and long-distance electrostatic forces. Thus, HD PFM provides parallel study of different sample's electromechanical properties through a single measurement cycle, and at the same time, makes piezoresponse measurements nondestructive by retracting the tip from the sample at each HybriD oscillating cycle.

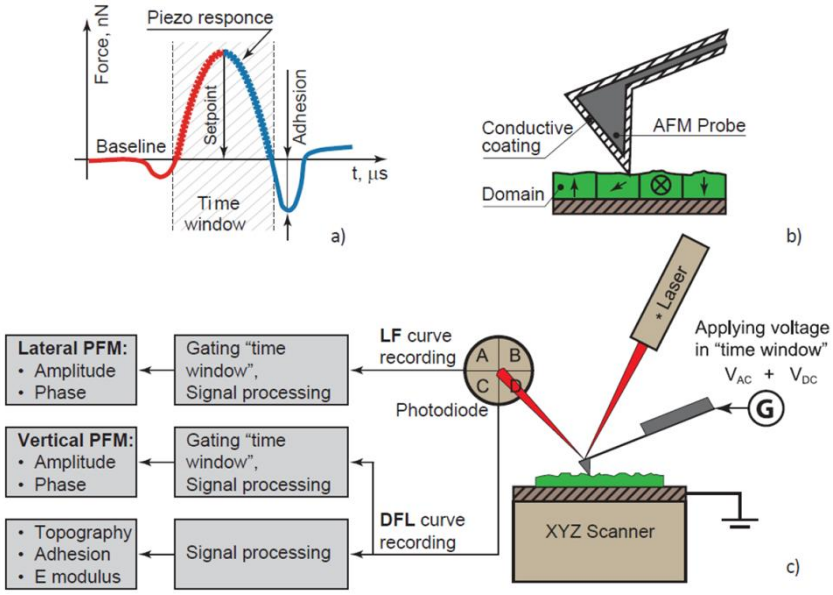


**Figure 2.** Working principle of HD PFM: a) an idealized temporal deflection curve during an oscillatory cycle; b) tip-sample interaction scheme; c) piezoresponse detection scheme.

## 3. Results and discussion

Above discussed HD PFM was implemented using Hybrid 2.0 control electronics (NT-MDT S.I., Moscow, Russia). Two types of biopiezoelectrics was studied: type I collagen matrix and diphenylalanine peptide nanotubes using VEGA atomic force microscope (NT-MDT S.I., Moscow, Russia)

### *3.1. Study of type I collagen matrix*

One of the most interesting biomaterials demonstrating piezoelectricity are collagen fibrils – the main building component of bones, teeth, corneal stroma and blood vessels. Collagen is composed of aligned polar protein molecules (fibrils) [6] that form a strong organic crystalline matrix. Piezoelectric properties of single collagen fibrils were recently studied with nanometer-level resolution [10,11]. Despite this advance, the scientific challenge of any piezoresponse study of collagen matrix is still tricky. The main issue for traditional contact PFM measurements of these structures is complicated by rough surfaces where there is height variation of almost one micrometer. This makes AFM tip to cling to the single fibrils, and vice versa, highly distorting the topography and piezoresponse images.

Due to its working principle, HD PFM mode was found to be a superior technique for studying collagen matrix. The samples were provided by Fibralign Corporation, where a unique technique of depositing animal collagen on glass substrate was developed [12]. Figure 3 illustrates results of HD PFM study of type I collagen matrix isolated from bovine corneas.

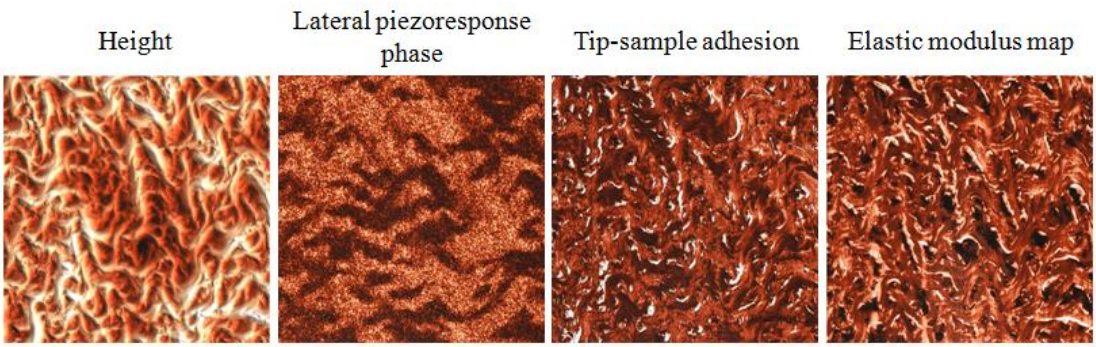


**Figure 3.** HD PFM images of animal collagen's matrix. Images size is 15×15 um. Obtained using NT-MDT S.I. Etalon HA_HR probes with force constant of 14N/m.

Measurements were performed with 280 kHz AC voltage of 8 V applied to conductive tip of Etalon HA_HR/W2C+ probe with force constant 14 N/m (NT-MDT S.I., Moscow, Russia). The contrast in height covers variation in 0-800 nm range. Lateral PFM phase demonstrates the domain distribution, darker and brighter areas correspond to the different direction of polarization. Adhesion demonstrates tip-sample adhesion force, brighter areas correspond to higher adhesion. E modulus in demonstrates collagen matrix E modulus distribution, brighter areas correspond to higher E modulus.

*3.2. Study of peptide nanotubes*

Peptide nanotubes (PNTs) self-assembled from diphenylalanine monomers were recently discovered to exhibit strong piezoelectric properties. Kholkin et al. demonstrated in-plane PFM contrast and high effective $d_{15}$ piezoelectric coefficient of at least 60 pm/V [13] (for tubes 200 nm in diameter) which is the highest value for known biopiezoelectrics. Together with intrinsic biocompatibility this makes PNTs to be very promising materials for developing piezonanodevices that are potentially compatible with human tissue.

PNTs are challenging samples for traditional contact PFM investigation due to their fragility and loose contact with a substrate. However, HD mode was recently demonstrated to be nondestructive method for investigation of thin PNTs (around 30 nm in diameter) in terms of obtaining information on topography, adhesion and Young's modulus [9]. Therefore, it was logical to apply HD PFM to these fragile samples in order to measure piezoresponse of PNTs to demonstrate advantages of this mode. Figure 4 demonstrates topography, lateral PFM phase, tip-sample adhesion force and long-distance electrostatic forces map.

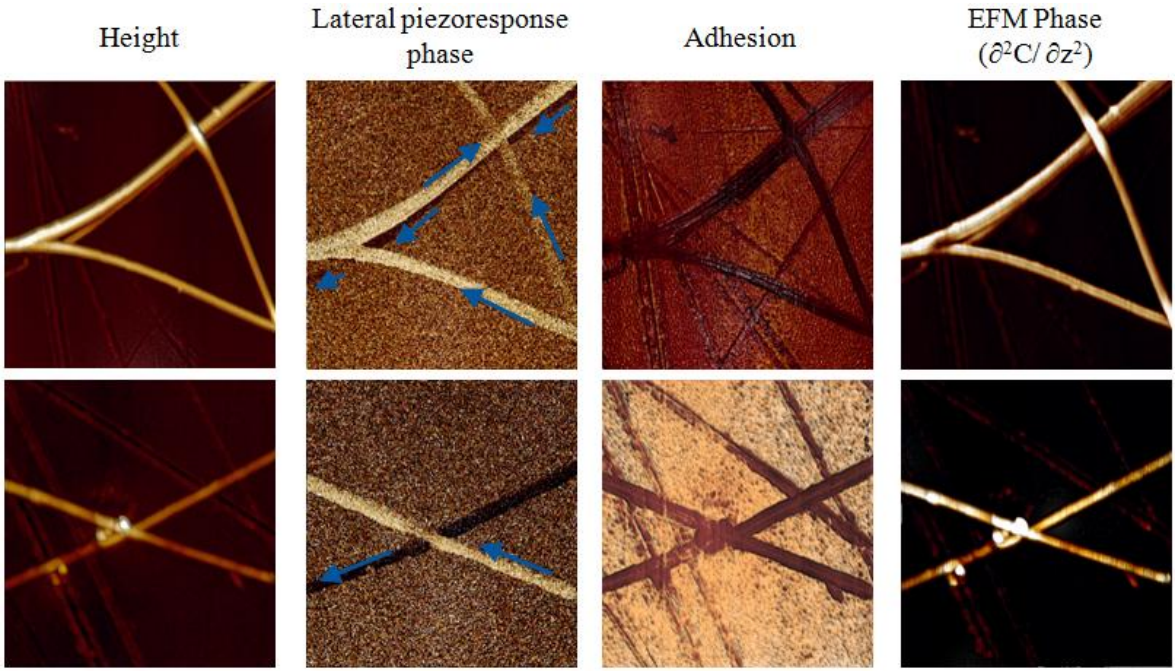


**Figure 4.** HD PFM images of peptide nanotubes. Arrows demonstrate the direction of polarization. Scan size 8×8 um (top row) and 7×7 um (bottom row). Sample courtesy: Prof. V.Y. Shur, S.G. Vasiliev UCSU "Modern Nanotechnologies" UFU; A.L. Kholkin, University of Aveiro.

Measurements were performed with 100 kHz AC voltage of 10 V applied on conductive tip coating of Etalon HA_FM/W2C+ probe with force constant of 3 N/m ((NT-MDT S.I., Moscow, Russia)). Lateral PFM phase also demonstrates PNTs with opposite polarization direction, that is marked by arrows.

## 4. Conclusion

The novel approach for non-destructive electromechanical studies of soft and fragile piezoelectrics is demonstrated. It has been successfully applied for simultaneous investigation of topography and local piezoresponsese, adhesion and E modulus of challenging biological samples – corneal stoma collage matrix and self-assembled diphenylalanine peptide nanotubes.

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