Review

Ke Xu, Weihang Sun, Yongjian Shao, Fanan Wei, Xiaoxian Zhang, Wei Wang and Peng Li*

Recent development of PeakForce Tapping mode atomic force microscopy and its applications on nanoscience

<https://doi.org/10.1515/ntrev-2018-0086>

Received May 13, 2018; accepted September 6, 2018; previously published online October 13, 2018

Keywords: force control; force curve; nanomechanical properties mapping; nanoscience; PeakForce Tapping mode.

Abstract: Nanoscience is a booming field incorporating some of the most fundamental questions concerning structure, function, and applications. The cutting-edge research in nanoscience requires access to advanced techniques and instrumentation capable of approaching these unanswered questions. Over the past few decades, atomic force microscopy (AFM) has been developed as a powerful platform, which enables *in situ* characterization of topological structures, local physical properties, and even manipulating samples at nanometer scale. Currently, an imaging mode called PeakForce Tapping (PFT) has attracted more and more attention due to its advantages of nondestructive characterization, high-resolution imaging, and concurrent quantitative property mapping. In this review, the origin, principle, and advantages of PFT on nanoscience are introduced in detail. Three typical applications of this technique, including high-resolution imaging of soft samples in liquid environment, quantitative nanomechanical property mapping, and electrical/electrochemical property measurement will be reviewed comprehensively. The future trends of PFT technique development will be discussed as well.

1 Introduction

Nanoscience is the study and application of structures and materials at the nanoscale in all science fields, such as physics, materials science, and chemistry. The rapid expansion of this field dramatically fueled the development of new advanced techniques and rigid methodologies to reveal the intrinsic but unknown issues in this field. Since its invention in 1986 by Bing et al., the atomic force microscope (AFM) has been a critical instrument in the development of nanoscience [1]. At the very beginning, contact mode was often applied, and the AFM tip is always in contact with the sample surface during the scanning process [2, 3], thus, inducing a large lateral force, which often causes damages to the tip or the samples. Consequently, soft samples, especially biological samples, are difficult to be characterized. To address this issue, the tapping mode (TM) AFM was proposed [4–6]. In the TM-AFM, the cantilever is driven to oscillate up and down near its resonant frequency, and the tip intermittently touches the sample surface. The frequency and amplitude of the driven signal are kept constant; however, the amplitude of the cantilever changes with the tip-sample interaction force exerted on the tip. This amplitude is always used as the input parameter of the feedback loop to obtain the topographic information of the sample. Meanwhile, the phase of the cantilever oscillation also changes during the scanning process, which reflects some properties of the sample [7–9]. However, the mechanical properties including elasticity, viscoelasticity, and adhesion, all contribute to the phase shift, so the explanation of the phase image remains elusive [10].

Except for TM, the non-contact mode [11] and multifrequency mode [12] were also developed. Considering that the cantilever oscillates close to its resonance

***Corresponding author: Peng Li**, CAS Key Laboratory of Standardization and Measurement for Nanotechnology, CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology, Beijing 100190, China, e-mail: lipeng@nanoctr.cn

Ke Xu, Weihang Sun and Yongjian Shao: School of Information and Control Engineering, Shenyang Jianzhu University, Shenyang, China

Fanan Wei: School of Mechanical Engineering and Automation, Fuzhou University, Fuzhou, China

Xiaoxian Zhang: CAS Key Laboratory of Standardization and Measurement for Nanotechnology, CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology, Beijing, China

Wei Wang: HIWING Technology Academy of CASIC, Beijing, China

frequency [11–13], all these three modes can be collectively called resonance mode. In the resonance mode, the motion of the cantilever is dynamical and highly nonlinear. In addition, the operator should tune the cantilever to find the resonance peak before imaging and optimize the various parameters including driving frequency, free vibration amplitude, setpoint, and feedback gains during imaging. Furthermore, the change in amplitude, phase, or frequency reflects the average force over multiple periods.

On the contrary, the off-resonance mode, in which the cantilever works at a frequency much lower than its resonant frequency, can be approximated to be a quasi-static process. Up to now, several off-resonance modes have been introduced, including force volume (FV) mode [14–16], digital pulse-force (DFT) mode [17, 18], Hybrid mode [19], QI mode [20], and PeakForce Tapping mode (PFT). In the off-resonance mode, the tip at the end of the cantilever moves toward the sample and then away from the sample. As the tip interacts with the sample, the deflection of the cantilever reflects the interaction force. Through controlling the maximum interaction force (peak force) in each pixel on the sample, the topography of the sample can be obtained. Because of the quasi-static process of the cantilever, the tip-sample interaction force can be measured and controlled precisely in each pixel at real time, and the operation is also simplified without the cantilever tuning process. Most importantly, the force-distance (FD) curve, which reflects the multiple nanomechanical properties of the sample, can be acquired as well. Thus, the off-resonance modes are widely used in biology [21, 22], materials science [23], and other fields recently [24].

The FV mode is the early-developed off-resonance mode [25, 26] and is still used nowadays [14]. However, in the FV mode, a sharp turnaround of the triangular driving signal makes the piezoelectric tube of the scanner oscillate, which can be visible in the image. In order to suppress the resonance of the piezoelectric tube, the vibration frequency of the scanner should be small (i.e. <100 Hz) [24]. Thus, it causes a slow scanning speed, which is usually several hours per frame [20]. Furthermore, when the setpoint (trigger force) is reached, the piezo changes its motion to drag the tip away from the sample; however, at this time, the force higher than the setpoint (overshoot) will be applied to the sample. The overloading causes the difficulty of maintaining low setpoints or imaging forces at higher frequencies. Using a sinusoidal wave as the driving signal, the subsequent pulsed-force mode relieves the low speed and the overloading [27–29]. With no sharp turnaround in the sinusoidal signal, the DFT mode is allowed to drive at significantly higher modulation frequency (about 1.5 kHz), permitting the improvement of the scan

speed limitation [28, 30, 31]. However, there are presently still several issues to be tackled in the DFT mode. First, the tip completely detaches from the sample, and then, the tip will freely oscillate above the sample surface. Meanwhile the deflection signal looks like a ringing signal. The trigger force in the DFT should be set to large (a few nanonewtons or more [32]) to distinguish the vertical maximum interaction force signal from the ringing signal. Otherwise, the parasitic motion (i.e. ringing of the tip) of the cantilever will dominate and cause feedback instability. In addition, the DFT AFM needs to wait for the ringing signal to be attenuated to get the baseline of deflection in each cycle, thus, bringing in the limit of modulation frequency.

The PFT mode, introduced by Su et al., effectively solves the above-mentioned problems [33, 34]. Figure 1 illustrates the schematic of the PFT AFM. There are many advantages of the PFT mode. First, similar to the DFT mode, the PFT also uses the sinusoidal wave as the driving signal, but the synchronization algorithm was employed. With the synchronization algorithm, the synchronization window is set at about half of the period to extract the actual vertical maximum interaction force (PeakForce); then, the vertical maximum interaction force signal is distinguished from the ringing signal, and thus, the setpoint can be set to small enough. Second, the background subtraction algorithm is adopted in the PFT mode to eliminate the effect of parasitic deflection signal, which occurs due to environmental damping or rough sample topography [35]. The parasitic deflection signal can be obtained as background signal when the tip lifts up to a certain height (several tens of nanometers) before each scan. Recently, a real-time curve fitting software method was also utilized, which does not require the previous lifting procedure and suits for parasitic deflection signal caused by the interaction forces between the cantilever and rough samples [35]. Finally, the synchronization algorithm separates the tip-sample interaction and non-interaction region effectively. Then, the data obtained from the non-interaction region is averaged to get a horizontal line as the baseline, which efficiently overcomes the impact of the ringing signal on the baseline fluctuations. This allows the PFT mode to start the next cycle without waiting for the ringing to be totally attenuated, thus, enabling a high scanning speed of the AFM. The performance comparison of the PFT with the other modes is summarized in Table 1. It can be seen that the PFT enables quantitative nanomechanical properties of the samples and faster scan rate with the lowest minimum peak force setting as well as the high-resolution mapping of the sample surface.

There were many reviews describing various imaging modes of AFM and their applications in the past two

Figure 1: Schematic diagram of the PFT.

Table 1: Comparison of PFT mode with other modes.

	Resonance mode	Off-resonance mode		
Scanning mode	TM	Force-volume (force mapping)	DFT	PFT
Minimum PeakForce	10 nN [36]	Several nN [37]	1 nN [32]	100 pN [32, 38]
Modulation signal	Sinusoid (100 kHz–300 kHz) [36]	Triangle (2 Hz–100 Hz) [24]	Sinusoid (100 Hz–1.6 kHz) [28, 30, 31]	Sinusoid (100 Hz–2 kHz) [32]
Quantitative nanomechanical measurement (QNM)	Phase image (mixed information) [39]	Adhesion, modulus, deformation, energy dissipation, separately [14, 40]	Adhesion, modulus, deformation, energy dissipation, separately [17, 41]	Adhesion, modulus, deformation, energy dissipation, separately [42, 43]
Easy to use	No	Yes	Yes	Yes
Company	Bruker [39, 44]; Oxford Instrument (amplitude modulation) [45]; JPK [46]	Bruker [47]; Oxford Instrument (force mapping mode) [48]; JPK (QI mode) [20, 49]	WITec [18, 50]; NT-MDT (hybrid mode) [19]	Bruker [42, 43]

decades [51–55]. In this review, we will only focus on the state-of-the-art applications and development of the PFT mode. Next, we will discuss the development of the PFT into a powerful tool in three applications. Then, the future development in this technique will also be discussed in detail.

2 High-resolution imaging of soft samples in liquid environment

The AFM was used to image various soft materials, especially delicate biological samples, such as DNA [56], virus [57], proteins [58], and fixed or live cells [59].

However, though a wide range of use, the AFM is not an ideal tool for imaging biological samples because it inevitably exerts vertical and lateral forces on samples that causes soft sample damage while scanning [60]. To avoid the deformations of the biological sample, the force is suggested to be less than 100 pN [55]. When the probe moves in the liquid environment, a hydrodynamic force will be generated and leads to a complex background signal and the distortion of the interaction force and, thus, affecting the image quality [38]. It is reported that the resolution of a live cell in the liquid environment is less than 50 nm [61]. The tapping mode is the most widely used imaging mode; however, it is not easy to operate it in liquid because there is always no single well-defined resonance but, instead, forest of

peaks, and the cantilever resonance is highly damped due to the addition of inertia and damping of the surrounding fluid. On the contrary, the PFT offers a simpler way to operate and also a subtle force control. There are several improvements of the PFT mode to operate in liquid. First, the PFT uses the background subtraction algorithm, extracts the actual peak force in a complex liquid environment, and lets the setpoint value settled to be small enough. Such small imaging force will avoid deformation and damage of the biological samples. Second, commercialized tailored probes (PeakForce QNM-Live Cell), which has a 45- μm -long cantilever with a 17- μm -long tip, can ensure that the cantilever is far from the sample surface and reduces the hydrodynamic force and background signal during scanning [38].

Fakhrullina et al. used the PFT AFM to characterize the *Caenorhabditis elegans* epicuticle cell at different processes of growth [62]. These epicuticle cells needed to be kept alive in liquid environment. As shown in Figure 2A, the width of the annuli increases with the age growth of the *C. elegans* (from L1 to L4), but the packing density of the annular rings per area unit decreases. Schillers et al. employed the PFT AFM to characterize the microvilli structure of the Madin-Darby canine kidney cells [38]. In the past, the microvilli structure of the living cells were too fragile to be imaged with the AFM and requires force-less imaging tools, for example, the scanning ion conductance microscope (SICM) [64–68]. As shown in Figure 2B, with a fine trigger force setpoint value of 100–130 pN, the individual microvilli starts to appear as cylindrical

Figure 2: Different biological samples with PFT mode. (A) AFM images of *C. elegans* at different processes of growth. (B) The images of the microvilli structure on an MDCK C11 cell with a vertical force of 100–130 pN setting. (C) The images of the microvilli structure on an MDCK C11 cell with a vertical force of 80–100 pN setting. (D) The image of the M immunoglobulin antibodies absorbed on mica in liquid environment. (E) High-resolution AFM topography of a single-molecule DNA. (A) Fakhrullina G, Akhatova F, Kibardina M, Fokin D, Fakhrullina R. Nanoscale imaging and characterization of *Caenorhabditis elegans* epicuticle using atomic force microscopy. *Nanomedicine* Feb 2017, 13, 483–491. Reproduced with permission from Ref. [62] by Elsevier. (B, C) Schillers H, Medalsy I, Hu S, Slade AL, Shaw JE. PFT resolves individual microvilli on living cells. *J. Mol. Recognit.* 2016, 29, 95–101. (D) Voss A, Dietz C, Stocker A, Stark RW. Quantitative measurement of the mechanical properties of human antibodies with sub-10-nm resolution in a liquid environment. *Nano Res.* 2015, 8, 1987–1996. Reproduced with permission from Ref. [63] by Springer Nature. (E) Pyne A, Thompson R, Leung C, Roy D, Hoogenboom BW. Single-molecule reconstruction of oligonucleotide secondary structure by atomic force microscopy. *Small* 2014, 10, 3257–3261.

structures. Some artifacts such as the wave structures indicate that there is still deformation and displacement of the microvillus. However, when the setpoint value decrease to 80–100 pN, those artifacts disappear, and the structures seems almost upright cylindrical, as displayed in Figure 2C.

As for the other biological samples in liquid, high-resolution images with the PFT mode are also obtained. As shown in Figure 2D, a sub-10-nm resolution image of the human IgM molecules adsorbed on mica depicts a homogeneous distribution. As shown in Figure 2E, with the fine force control (down to 50 pN) in the PFT mode, high-resolution image of the DNA plasmid molecule can be recognized with the lateral resolution down to several

nanometers. The recent application of the PFT mode in soft sample imaging is summarized in Table 2.

3 Quantitative nanomechanical measurement

As one kind of the off-resonance mode of the AFM, the PFT mode can also probe biophysical properties through recording a single FD curve when the AFM tip is approached to and retracted from the sample. As illustrated in Figure 3A, the approach FD curves (red line) and retraction FD curves (blue line) can quantify the height,

Table 2: Imaging soft samples with PFT mode in liquid.

Category	Sample	Setpoint	Resolution
Living cells	Fibroblasts [69]	150 pN	Pixel size of 15 nm
	Microvilli structure of Madin-Darby canine kidney [38]	80–100 pN	Pixel size of 26 nm
	Breast cancer cells [70]	700 pN–1 nN	20 nm
Molecular bio-imaging, including DNA, proteins, and membranes in liquid	M immunoglobulin antibodies [63]	300 pN	Sub-10-nm resolution
	DNA plasmid [71]	50 pN	Single-molecule resolution
	DNA and DNA-protein complexes [72]	100 pN	Sub-10-nm resolution

Figure 3: (A) Schematic illustration of the FD curve. The red line indicates the process of the tip approaching the sample, while the blue line indicates the process of the tip retracting from the sample. (B) The mechanical properties of the human IgM antibodies obtained using PFT QNM in a liquid environment. (B) Voss A, Dietz C, Stocker A, Stark RW. Quantitative measurement of the mechanical properties of human antibodies with sub-10-nm resolution in a liquid environment. *Nano Res.* 2015, 8, 1987–1996. Reproduced with permission from Ref. [63] by Springer Nature.

surface forces, and mechanical deformation of the sample, or derive its elastic modulus and energy dissipation, and also allow adhesion forces to be measured. The details are provided as follows.

Deformation: The maximum deformation can be gained by subtracting the position of the tip where the force is zero from the maximum normal interaction force (peak force point) position along the approach FD curve. This value reflects the deformability property of the sample. Figure 3B shows several property images of immunoglobulin antibodies adsorbed on the mica substrate [63]. It is hard to distinguish the antibodies from the mica substrate from the topography image, but from the deformation image, it is easy to distinguish those two parts and shows that the deformation of the antibodies is much larger than that of the mica substrate under the same applied force.

Adhesion: The maximum adhesion force can be gained by extracting the force of the tip as it detaches from the sample (the pull-off point) in the FD curve of the tip away from the sample. Adhesion includes a range of attractive forces such as Van der Waals forces, capillary forces, and electrostatic forces. As observed in Figure 3B, the adhesion of the immunoglobulins is smaller than that of the mica substrate.

Modulus: To obtain the Young's Modulus, the part of the retract FD curve where the sample and tip are in contact is fitted using the contact mechanics models. There are several models that are based on continuum mechanics already developed, including the Hertz model [73], Johnson-Kendall-Roberts (JKR) model [74], and Derjaguin-Muller-Toporov (DMT) model [75]. The Hertz model describes the non-adhesive contact between two elastic spheres. More realistic modes are DMT and JKR

models, which both account for adhesion effects. The difference between the two models is that the JKR mode is recommended for a soft sample with high energy of adhesion and a large tip radius. The suitable model can be selected according to a elasticity parameter, which is the ratio of the adhesion to elasticity [76]. With the equations listed in Table 3, the Young's modulus can be calculated by the FD curve fitting with a knowledge of the tip radius. The maximum adhesive force and deformation can be obtained from the FD curve. Thus, we can calculate the contact radius of the tip and finally get the effective Young's modulus of the tip sample interface E_{tot} . The relationship E_{tot} is defined as

$$E_{\text{tot}} = \frac{4}{3} \left(\frac{1 - V_{\text{tip}}^2}{E_{\text{tip}}} + \frac{1 - V_{\text{sample}}^2}{E_{\text{sample}}} \right)^{-1} \quad (1)$$

where V_{tip} is the Poisson's ratio of the tip, V_{sample} is the Poisson's ratio of the sample, E_{tip} is the tip's elastic modulus, and, E_{sample} is the sample's elastic modulus. With the tip modulus E_{tip} assumed to be infinitely large, and V_{sample} and V_{tip} are known, we can calculate the E_{sample} . In addition, for measuring samples quantitatively, the appropriate spring constant K of the probe should be selected. The K should neither be too large to get a sufficient deflection signal for providing accurate force measurement, nor too small to achieve enough sample indentation. As shown in Figure 3B, the modulus of the immunoglobulins is much smaller than that of the mica substrate.

Energy dissipation: The energy dissipation is calculated by the hysteresis area (yellow area) between the approaching and retracting processes, which characterizes the loss of the energy in one cycle. Usually, the work of adhesion

Table 3: Relevant results predicted by the Hertz, DMT, and JKR contact models [77].

	Hertz	DMT	JKR
Range of use	No adhesion; $a_{\text{Hertz}} \ll R_{\text{tip}}$	Low adhesion; small R_{tip}	High adhesion; large R_{tip}
Maximum adhesive force F_{ad}	0	$F_{\text{ad}}^{\text{DMT}} = 2\pi R_{\text{tip}} W_{132}$	$F_{\text{ad}}^{\text{JKR}} = \frac{3\pi}{2} R_{\text{tip}} W_{132}$
Deformation D	$D_{\text{Hertz}} = \frac{a_{\text{Hertz}}^2}{R_{\text{tip}}}$	$D_{\text{DMT}} = \frac{a_{\text{DMT}}^2}{R_{\text{tip}}}$	$D_{\text{JKR}} = \frac{a_{\text{JKR}}^2}{R_{\text{tip}}} - \frac{4}{3} \sqrt{\frac{F_{\text{ad}}^{\text{JKR}} a_{\text{JKR}}}{R_{\text{tip}} E_{\text{tot}}}}$
Contact radius a	$a_{\text{Hertz}} = \left(\frac{R_{\text{tip}} F}{E_{\text{tot}}} \right)^{\frac{1}{3}}$	$a_{\text{DMT}} = \left(\frac{R_{\text{tip}} [F + F_{\text{ad}}^{\text{DMT}}]}{E_{\text{tot}}} \right)^{\frac{1}{3}}$	$a_{\text{JKR}} = \left(\frac{R_{\text{tip}}}{E_{\text{tot}}} \left[\sqrt{F_{\text{ad}}^{\text{JKR}}} + \sqrt{F + F_{\text{ad}}^{\text{JKR}}} \right]^2 \right)^{\frac{1}{3}}$

R_{tip} , Radius of the tip; W_{132} , work of adhesion; F , applied force (tip-sample interaction force); E_{tot} , effective elastic modulus.

and viscous/plastic deformation contributes to the dissipation. In the FD curve, as shown in Figure 3, the yellow area between the red and blue lines represents the energy dissipation.

Using PFT-QNM AFM, Heu et al. studied the effects of glyphosate on the topography and mechanical properties of HaCaT keratinocytes [78] (Figure 4A). After the cell was treated with glyphosate, the cell membrane becomes flat, and the amount of protrusions decreased as illustrated from the topography image (Figure 4A3), while the cell turns to be stiffer as indicated from the modulus images (Figure 4A3), which explains a defense mechanism of the cell to glyphosate. In addition, Sababi et al. distinguished different components of the paperboard coating by analyzing the material properties using PFT-QNM AFM [42]. As shown in Figure 4B, the paperboard is coated with starch, latex, and clay composition in sequence. The latex particles are packed closely together except in some areas where some of the particles are absent from the starch layer. The clay composition is difficult to be distinguished from

the latex part in the topography; however, the modulus image shows the difference due the larger modulus of clay (arrow in Figure 4B). Chen et al. also used the PeakForce QNM to study the topography and mechanical properties of organic matter and clay minerals in the bitumen froth fine solids [79]. From the topography image as shown in Figure 4C1, the height of the two particles seems to be similar. Meanwhile, the adhesion force exhibits a significant difference: the upper particle has a lower adhesion force (10 nN) than the lower particle (75 nN) does, which means that the lower particle (circle region in Figure 4C2) is most likely organic in nature.

4 Electrical and electrochemical property measurement

The PFT can be also combined with the other techniques to characterize more properties of the sample, such as

Figure 4: (A) Topography and modulus mapping using PFT AFM of HaCaT cells with glyphosate-treated vs. untreated control. Topography image (A1) and modulus image (A2) of the untreated control HaCaT cells. Topography image (A3) and modulus image (A4) of the 6 h–15 mm glyphosate-treated cells. (B) The topography and modulus image of the paperboard with different material coatings. Topography (B1) and modulus image (B2) of the paperboard. (C) The topography and adhesion image of the bitumen froth fine solids. The topography (C1) and modulus image (C2) of the bitumen froth fine solids. (A) Heu C, Berquand A, Elie-Caille C, Nicod L. Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells. *J. Struct. Biol.* Apr 2012, 178, 1–7. Reproduced with permission from Ref. [78] by Elsevier. (B) Sababi M, Kettle J, Rautkoski H, Claesson PM, Thormann E. Structural and nanomechanical properties of paperboard coatings studied by peak force tapping atomic force microscopy. *ACS Appl. Mater. Interfaces* Oct 24 2012, 4, 5534–5541. Reproduced with permission from Ref. [42] by the American Chemical Society. (C) Chen Q, Liu J, Thundat T, Gray MR, Liu Q. Spatially resolved organic coating on clay minerals in bitumen froth revealed by atomic force microscopy adhesion mapping. *Fuel*, 2017, 191, 283–289. Reproduced with permission from Ref. [79] by Elsevier.

the electrical properties and electrochemical properties. The application of the PFT mode in electrical and electrochemical property mapping is summarized in Table 4. In addition, this section can be divided into three parts: conductivity measurement, Kelvin probe force microscope (KPFM), and electrochemistry measurement.

Initially, the contact mode was adopted to perform the conductivity measurement of different samples such as biological samples [90], material samples [91, 92], and chemistry samples [93], which is called the conductive AFM (CAFM). However, in the CAFM, the tip is easily contaminated or damaged in the scanning. The TMAFM can protect the tip; however, it needs a current amplifier with a large bandwidth (about dozens of kHz to hundreds of kHz) and high gain setting (up to 10^8), which is very challenging for the recent technology of electronics. The PFT provides a possible solution, PeakForce Tunneling (PF-TUNA), which allows the tip to intermittently touch the sample to extend the tip service life, but requires a smaller bandwidth (i.e. 10 kHz) of the current amplifier due to the lower modulation frequency. In addition, in the PF-TUNA, the tip oscillates at a fixed voltage toward the sample. The force-distance curve, which is used to extract the topography, modulus, adhesion, and other mechanical properties, is obtained in each tapping cycle with a controlled peak force. At the same time, the peak current (currents at the maximum contact force), contact current (currents averaged over the contact duration), and TUNA current (currents averaged over the whole tapping cycle) are all recorded and used for representing the electrical conductivity of the sample.

With the PF-TUNA, Wang et al. studied the effects of different environmental factors and different components on the conductivity of shale samples [80]. Pyrite and the organic in illite promote the electrical conductivity of a shale sample, while other components such as chlorite,

quartz, and carbonate do the opposite. The phenomena that conductivity increases with time were explained by the absorbed water molecules on the surface of illite. In addition, the quantitative nanoelectrical properties of nanostructured hybrid composites (ITO/PEDOT:PSS/P1:EG-C60) are measured through the PF-TUNA [81]. The increasing current information shows that the add material EG-C60 acts as a receiving material, facilitating the transfer of electrons and improving the conductivity of the sample ITO/PEDOT:PSS/P1. Moreover, the PF-TUNA also obtains the morphology and mechanical properties of the sample while probing the electrical properties [82], as shown in Figure 5A. The adhesion image reveals that the nanoparticles can be divided into two phases due to a different adhesion force, while those produce almost the same current value under applied bias voltages regardless of the sign.

The KPFM is another kind of electrical property-probing tool with a nanometer resolution [94, 95]. The KPFM can be used to map local electrostatic potential at sample surfaces to provide information about work function [96], trapped charges [97], doping level variations [98], and electronic structure [99]. The sensitivity of the KPFM is proportional to the value of the dividing quality factor Q to the spring constant K [100]. Using a cantilever with a large Q and a small K also can improve the KPFM sensitivity, but for the TM, Q cannot be very large, and k cannot be very small. The PFT mode has no Q and K limitation, thus, it has a higher sensitivity than the TM. Apart from this, the probe is scanned twice per line. In the first scan (main scan), the PFT can simultaneously obtain the topography, stiffness, and adhesion properties of the samples. In the second scan (lift scan), the tip lifts at a fixed user-defined distance above the sample surface to avoid mechanical cross-talk caused by the tip-sample interactions, then performs like the conventional frequency modulation KPFM

Table 4: Measurement of electrical and electrochemical properties using PFT-AFM.

Category	Sample	Environment	Measurement
PF-TUNA	Shale [80] and Polymer [81]	Air	Conductivity
	V:Ti/SEO nanocomposites [82]		QNM and conductivity
	Semiconductor/metal [83], HOPG [84], Pt surface [84]	Deionized H ₂ O	Conductivity
PF-KPFM	Graphene [85], Perovskite/Au [86], Duplex stainless steel [87]	Air	QNM and surface potential
PF-SECM	CH ₃ -thiol monolayer on Au substrate [83]	5 mM [Ru(NH ₃) ₆] ³⁺ and 0.1 M KNO ₃	QNM and electrochemical properties
	Gold electrodes on polydimethylsiloxane [88]	2.5 mM Fc(MeOH) ₂ and 0.1 M KCl	
	High pyrolytic graphite [84] and Pt/p Si electrodes [89]	10 mM [Ru(NH ₃) ₆] ³⁺ and 0.1 M KCl	QNM, local electrochemical activity and conductivity

Figure 5: (A) Topography image, adhesion image, and current images of the nanostructured hybrid composites obtained by PF-TUNA. (B) Surface potential image of multilayer, turbostratic graphene films obtained by chemical vapor deposition. (C) The topography and potential image of the perovskite/Au areas after positive poling. (C1) Topography of the perovskite/Au areas after positive poling; (C2) potential image of the perovskite/Au areas after positive poling. Topography image (D1), adhesion image (D2), and work function image (D3) of duplex stainless steel obtained by PF-TUNA. (A) Gutierrez J, Mondragon I, Tercjak A. Quantitative nanoelectrical and nanomechanical properties of nanostructured hybrid composites by PeakForce Tunneling atomic force microscopy. *J. Phys. Chem. C* 2014, 118, 1206–1212. Reproduced with permission from Ref. [91] by the American Chemical Society. (B) Robinson JT, Culbertson J, Berg M, Ohta T. Work function variations in twisted graphene layers. *Sci. Rep.* 2018, 8. (C) Xiao Z, Yuan Y, Shao Y, Wang Q, Dong Q, Bi C, Sharma P, Gruverman A, Huang J. Giant switchable photovoltaic effect in organometal trihalide perovskite devices. *Nat. Mater.* 2015, 14, 193–198. Reproduced with permission from Ref. [86] by Springer Nature. (D) Guo L, Hua G, Yang B, Lu H, Qiao L, Yan X, Li D. Electron work functions of ferrite and austenite phases in a duplex stainless steel and their adhesive forces with AFM silicon probe. *Sci. Rep.* 2016, 6, 20660.

to measure the work function difference between the tip and the sample.

Robinson et al. used the PF-KPFM to study the correlation between the layer orientation and graphene layer

thickness with surface potential [85]. It is found that the surface potential and G-peak Raman shifts are linearly correlated in different thickness and torsion angle regions, and surface potential increases with the twist

angle. Therefore, graphene with different thicknesses and twist angles can be distinguished by analyzing the surface potential, as presented in Figure 5B. In addition, Xiao et al. also used the PF-KPFM to distinguish perovskite from the Au region in the surface potential image (as shown in Figure 5C1 and C2), although the topography seems to show that there is no change in those two regions [86]. Similar to the PF-TUNA, the PF-KPFM can also obtain the surface topography and mechanical properties simultaneously. It was verified by the result of the duplex stainless steel, as shown in Figure 5D [87].

Local electrochemistry studies are useful in different areas, including chemical manufacturing [101–103], material development [104–106], energy research, biological systems [107, 108], and surface protection. Initially, scanning electrochemical microscopy (SECM) uses ultramicroelectrodes to probe electrochemical processes of the sample area underneath the probe. Classic SECM generally has limitations in poor spatial resolution due to the micrometer size of the probe, convolution between topography and electrochemistry, and inefficient control of the tip position. To address these limitations, AFM based on SECM was developed due to the state-of-the-art reliable batch probe fabrication technology [83]. As shown in Figure 6A, the SECM probe is fully insulated with SiO_2 and other dielectric sub-layers, except that the tip apex is exposed with a Pt coat. Using these probes, it is possible to map the electrical property in a liquid environment with AFM. For instance, Huang et al. used the PF-TUNA to study the conductivity of semiconductor/metal samples in liquid and air environment [83]. It is found that semiconducting/metal junctions exhibit unidirectional conduction characteristics in air, but bidirectional conductivity in liquids. Meanwhile, as shown in Figure 6B, contact current of HOPG in deionized H_2O was measured using PF-SECM.

Similar to the PF-KPFM, the tip performs to scan twice per line in the PF-SECM. In the main scan, the PFT captures most information including topography, mechanics, and electrical conductivity, while electrochemical activities are characterized in the lift scan. As for the electrochemical activity probing, a self-assembled monolayer of CH_3 -thiol on the Au substrate is studied. The topography shows that height variations about 1 nm is hardly distinguished. However, a donut-shaped structure is clearly observable by the quantitative adhesion force and current signal obtained through the PF-QNM and PF-SECM, respectively. Nellist et al. also adopted the PF-SECM to characterize high pyrolytic graphite with different environments [84]. Figure 6C shows the characterization of a high pyrolytic graphite sample in 10 mM of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ and 0.1 M

of KCl solution. The topography shows that the surface of the high pyrolytic graphite sample has a circular defect region, which is about 0.4 nm higher than the surrounding region. Meanwhile, the adhesion image shows that the adhesion of the defect area is smaller than the surrounding area. In addition, the electrochemical activity image also shows that the induced current of the tip in the defect area is smaller than that of the surrounding area.

Jiang et al. adopted the PF-SECM to study the electrical and electrochemical properties of Pt/p-Si and Pt/p⁺-Si electrodes [89]. Figure 6D is the PF-SECM image of the Pt particles, which were deposited on a Si substrate doped with p⁺. The images were obtained using a 700-pN image force in a solution of 10 mM of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ and 0.1 M of KCl. A low imaging force (700 pN) can minimize the movement of the particles. Some particles were deposited on a substrate to form the topography, while the contact current image shows that the particles in regions 1 and 2 have better conductivity than the particles in regions 3 and 4, and the electrochemical activity current image shows that the particles in regions 1 and 3 have a higher electrochemical activity. Thus, different particles can be distinguished by comparing the contact current and the electrochemical activity current.

5 Development of the PFT technology

A slow imaging speed strongly limits the use of the PFT AFM. However, several developments were introduced in different groups to improve the PFT mode recently. First, the modulation frequency of the scanner has to stay well (1–2 kHz) below the scanner frequency to avoid a distorted motion, which is the main reason for the PFT scan rate limitation. Fantner et al. introduced a fast-driving mechanism including a two-actuator design and a resonance suppression control to improve the scanning speed [109]. A secondary stack piezo actuator with higher resonance frequency, which is responsible for the high frequency but small amplitude component of the Z-axis displacement, is placed on top of the long-range standard scanner. Along with the first-order notch filter method, which is applied to suppress both the axial as well as the lateral resonance of the scanner, the authors were able to operate at 31.5-kHz modulation frequency, thus, obtaining large line rates (17.6 line/s), as shown in Figure 7A. Second, Dokukin et al. came up with a powerful extension of the PFT mode—ringing mode. The ringing mode is based on the ringing signal of defection, which is usually

Figure 6: (A) SEM image of special AFM probe used to obtain the electrical properties in the liquid environment. (B) The topography and contact current image of the HOPG in deionized H_2O obtained using PF-TUNA. (C) The topography, adhesion, and electrochemical current images of highly ordered pyrolytic graphite in the solution of 10 mM $[\text{Ru}(\text{NH}_3)_6]^{3+}$ and 0.1 M KCl. (D) PF-SECM imaging of Pt nanoparticles deposited onto a doped Si substrate and in contact with 10 mM $[\text{Ru}(\text{NH}_3)_6]^{3+}$ and 0.1 M KCl solution. (A) Huang Z, De Wolf P, Poddar R, Li C, Mark A, Nellist MR, Chen Y, Jiang J, Papastavrou G, Boettcher SW, Xiang C, Brunswig BS. PeakForce scanning electrochemical microscopy with nanoelectrode probes. *Micros. Today* 2016, 24, 18–25. Reproduced with permission from Ref. [83] by Cambridge University Press. (B, C) Nellist MR, Chen Y, Mark A, Godrich S, Stelling C, Jiang J, Poddar R, Li C, Kumar R, Papastavrou G, Retsch M, Brunswig BS, Huang Z, Xiang C, Boettcher SW. Atomic force microscopy with nanoelectrode tips for high resolution electrochemical, nanoadhesion and nanoelectrical imaging. *Nanotechnology* Mar 3 2017, 28, 095711. Reproduced with permission from Ref. [84] by IOPscience. (D) Jiang J, Huang Z, Xiang C, Poddar R, Lewerenz HJ, Papadantonakis KM, Lewis NS, Brunswig BS. Nanoelectrical and nanoelectrochemical imaging of Pt/p-Si and Pt/p(+)-Si electrodes. *ChemSusChem* 2017, 10, 4657–4663. Reproduced with permission from Ref. [89] by John Wiley and Sons.

treated as noise and always filtered out in the previous PFT mode, to get more compositional information and faster speed than the PFT mode [110]. Compared with the adhesion image of the PFT (Figure 7B1), the restored adhesion image (Figure 7B2) of the ringing mode is much clearer at the 5-Hz scanning rate. From Figure 3A, the

adhesion value in the PFT mode easily produces errors due to the hard identification of the instantaneous pull-off moment. So multiple scanning times are performed for each pixel to reduce the error by averaging the adhesion data. However, the multiple scanning makes the imaging time longer. Different from the PFT mode, the restored

chosen. Therefore, the PFT mode is not sufficient to characterize the samples that have elastic modulus values that varied with a range of two or more orders. As presented in Figure 7C, a magnetic bead attached on a soft cantilever is actuated by an alternating magnetic field in the Z direction to drive the cantilever up and down. When scanning a region with a large elastic modulus, the magnetic force of the magnetic beads is increased by adjusting the strength of the magnetic field to drive the tip to produce a sufficiently large effective sample indentation. Thus, using this method, with a soft cantilever, it can measure the elastic modulus ranging up to four orders of magnitude.

6 Summary and outlook

The PFT AFM offers a powerful tool to biology, medicine, materials science, and many other disciplines. It enables high-resolution imaging, fine force control, and concurrent mapping of mechanical, electrical, or other properties. Despite of the wide applications and significant process of the PFT mode in the past years, challenges, including further improvement of imaging speed, reliability of property characterization, and integration with other applications, still remain. Although efforts were made in the improvement of imaging speed, the PFT mode still lags behind the contact mode or TMs. The introduction of faster data acquisition systems, feedback loop probe with a high bandwidth, and advanced control schemes is expected to solve the problem. The PFT offers the ability to record thousands of force curves in each second automatically; however, by evaluating the physical properties from those force curves, it is possible that they will be distorted by artificial factors, such as improper spring constant or uncertain shape of probe, appropriate curve fitting mode, and incorrect calibration. A probe with certain shape and larger wear resistance, and automatic parameter calibration will help solve this problem. Due to the low wear and fine control of PFT, SECM and Scanning Microwave Impedance Microscopy both have already been integrated into PFT. What's more, there are still other AFM applications such as scanning probe lithography required to be integrated with PFT.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (Grant Nos. 61604048, 11604064, and 61504072), and the National Natural Science Foundation of Liaoning (Grant Nos. 20170540748 and 20170520444). The authors also thank Dr. Hao Sun from Bruker Scientific Technology Co. Ltd for his useful discussion and support.

References

- [1] Binnig G, Quate CF, Gerber C. Atomic force microscope. *Phys. Rev. Lett.* 1986, 56, 930–933.
- [2] Bloo ML, Haitjema H, Pril WO. Deformation and wear of pyramidal, silicon-nitride AFM tips scanning micrometre-size features in contact mode. *Measurement* 1999, 25, 203–211.
- [3] Kimura K, Kobayashi K, Yamada H, Horiuchi T, Ishida K, Matsushige K. Orientation control of ferroelectric polymer molecules using contact-mode AFM. *Eur. Polym. J.* 2004, 40, 933–938.
- [4] Zhong Q, Inniss D, Kjoller K, Elings VB. Fractured polymer/silica fiber surface studied by tapping mode atomic force microscopy. *Surf. Sci. Lett.* 1993, 290, L688–L692.
- [5] Magonov SN, Elings V, Whangbo MH. Phase imaging and stiffness in tapping-mode atomic force microscopy. *Surf. Sci.* 1997, 375, L385–L391.
- [6] Putman CA, van der Werf KO, de Grooth BG, van Hulst NF, Greve J. Viscoelasticity of living cells allows high resolution imaging by tapping mode atomic force microscopy. *Biophys. J.* 1994, 67, 1749–1753.
- [7] Bar G, Thomann Y, Brandsch R, Cantow HJ, Whangbo MH. Factors affecting the height and phase images in tapping mode atomic force microscopy. Study of phase-separated polymer blends of poly(ethene-co-styrene) and poly(2,6-dimethyl-1,4-phenylene oxide). *Langmuir* 1997, 13, 3807–3812.
- [8] McLean RS, Sauer BB. Tapping-mode AFM studies using phase detection for resolution of nanophases in segmented polyurethanes and other block copolymers. *Macromolecules* 1997, 30, 8314–8317.
- [9] Schmitz I, Schreiner M, Friedbacher G, Grasserbauer M. Phase imaging as an extension to tapping mode AFM for the identification of material properties on humidity-sensitive surfaces. *Appl. Surf. Sci.* 1997, 115, 190–198.
- [10] Cleveland JP, Anczykowski B, Schmid AE, Elings VB. Energy dissipation in tapping-mode atomic force microscopy. *Appl. Phys. Lett.* 1998, 72, 2613–2615.
- [11] Martin Y, Williams CC, Wickramasinghe HK. Atomic force microscope–force mapping and profiling on a sub 100-Å scale. *J. Appl. Phys.* 1987, 61, 4723–4729.
- [12] Garcia R, Herruzo ET. The emergence of multifrequency force microscopy. *Nat. Nanotechnol.* 2012, 7, 217–226.
- [13] Albrecht TR, Grütter P, Horne D, Rugar D. Frequency modulation detection using high-Q cantilevers for enhanced force microscope sensitivity. *J. Appl. Phys.* 1991, 69, 668–673.
- [14] Medalsy I, Hensen U, Muller DJ. Imaging and quantifying chemical and physical properties of native proteins at molecular resolution by force-volume AFM. *Angew. Chem. Int. Ed.* 2011, 50, 12103–12108.
- [15] Reynaud C, Sommer F, Quet C, El Bounia N, Duc T.M. Quantitative determination of Young's modulus on a biphasic polymer system using atomic force microscopy. *Surf. Interface Anal.* 2000, 30, 185–189.
- [16] Guz N, Dokukin M, Kalaparthi V, Sokolov I. If cell mechanics can be described by elastic modulus: study of different models and probes used in indentation experiments. *Biophys. J.* 2014, 107, 564–575.
- [17] Marti O, Holzwarth M, Beil M. Measuring the nanomechanical properties of cancer cells by digital pulsed force mode imaging. *Nanotechnology* 2008, 19, 384015.

- [18] Gigler A, Gnahm C, Marti O, Schimmel T, Walheim S. Towards quantitative materials characterization with digital pulsed force mode imaging. *J. Phys. Conf. Ser.* 2007, 61, 346–351.
- [19] Kaimaki D-M, Smith BE, Durkan C. On the use of nanomechanical atomic force microscopy to characterise oil-exposed surfaces. *RSC Adv.* 2018, 8, 6680–6689.
- [20] Chopinet L, Formosa C, Rols MP, Duval RE, Dague E. Imaging living cells surface and quantifying its properties at high resolution using AFM in QI mode. *Micron* 2013, 48, 26–33.
- [21] Beckwitt EC, Simon N, Carnaval I, Kisker C, Carell T, Van Houten B. Peakforce tapping AFM reveals that human XPA binds to DNA damage as a monomer producing a 60° bend. *Biophys. J.* 2018, 114, 93a.
- [22] Al Faouri R, Henry R, Biris AS, Sleezer R, Salamo GJ. Adhesive force between graphene nanoscale flakes and living biological cells. *J. Appl. Toxicol.* 2017, 37, 1346–1353.
- [23] Knittel P, Hibst N, Mizaikoff B, Strehle S, Kranz C. Focused ion beam-assisted fabrication of soft high-aspect ratio silicon nanowire atomic force microscopy probes. *Ultramicroscopy* 2017, 179, 24–32.
- [24] Sokolov I, Zorn G, Nichols JM. A study of molecular adsorption of a cationic surfactant on complex surfaces with atomic force microscopy. *Analyst* 2016, 141, 1017–1026.
- [25] Radmacher M, Cleveland JP, Fritz M, Hansma HG, Hansma PK. Mapping interaction forces with the atomic force microscope. *Biophys. J.* 1994, 66, 2159–2165.
- [26] Cleveland JP, Radmacher M, Hansma PK. In *Forces in Scanning Probe Methods*, Güntherodt, HJ, Anselmetti, D, Meyer, E, Eds., Springer, Kluwer: Dordrecht, 1995, pp. 543–549.
- [27] Krottil H-U, Stifter T, Waschipky H, Weishaupt K, Hild S, Marti O. Pulsed force mode: a new method for the investigation of surface properties. *Surf. Interface Anal.* 1999, 27, 336–340.
- [28] Rosa-Zeiser A, Weilandt E, Hild S, Marti O. The simultaneous measurement of elastic, electrostatic and adhesive properties by scanning force microscopy: pulsed-force mode operation. *Meas. Sci. Technol.* 1997, 8, 1333–1338.
- [29] Rezende CA, Lee LT, Galembeck F. Surface mechanical properties of thin polymer films investigated by AFM in pulsed force mode. *Langmuir* 2009, 25, 9938–9946.
- [30] Maiorana A, Papi M, Bugli F, Torelli R, Maulucci G, Cacaci M, Posteraro B, Sanguinetti M, De Spirito M. A fast and quantitative evaluation of the *Aspergillus fumigatus* biofilm adhesion properties by means of digital pulsed force mode. *Appl. Surf. Sci.* 2013, 279, 409–415.
- [31] Marti O, Stifter T, Waschipky H, Quintus M, Hild S. Scanning probe microscopy of heterogeneous polymers. *Colloids Surf. A Physicochem. Eng. Asp.* 1999, 154, 65–73.
- [32] Young TJ, Monclus MA, Burnett TL, Broughton WR, Ogin SL, Smith PA. The use of the PeakForce™ quantitative nanomechanical mapping AFM-based method for high-resolution Young's modulus measurement of polymers. *Meas. Sci. Technol.* 2011, 22, 125703.
- [33] Starikov A, Hu S, Mininni L, Hu Y, Erina N, Kindt J, Su C. High-speed atomic force microscopy and peak force tapping control. *Proceedings of the SPIE* 2012, 8324, 832410.
- [34] Chanmin Su JS, Hu Y, Hu S, Ma J. Method and apparatus of using peak force tapping mode to measure physical properties of a sample. US9291640B2, Bruker Nano Inc, 2008.
- [35] Gazze SA, Hallin I, Quinn G, Dudley E, Matthews GP, Rees P, van Keulen G, Doerr SH, Francis LW. Organic matter identifies the nano-mechanical properties of native soil aggregates. *Nanoscale* 2018, 10, 520–525.
- [36] Spatz JP, Sheiko S, Moller M, Winkler RG, Reineker P, Marti O. Forces affecting the substrate in resonant tapping force microscopy. *Nanotechnology* 1995, 6, 40–44.
- [37] Cappella B, Dietler G. Force-distance curves by atomic force microscopy. *Surf. Sci. Rep.* 1999, 34, 1–104.
- [38] Schillers H, Medalsy I, Hu S, Slade AL, Shaw JE. PeakForce Tapping resolves individual microvilli on living cells. *J. Mol. Recognit.* 2016, 29, 95–101.
- [39] Herzele S, Veigel S, Liebnert F, Zimmermann T, Gindl-Altmutter W. Reinforcement of polycaprolactone with microfibrillated lignocellulose. *Ind. Crops Prod.* 2016, 93, 302–308.
- [40] An H, Nussio MR, Huson MG, Voelcker NH, Shapter JG. Material properties of lipid microdomains: force-volume imaging study of the effect of cholesterol on lipid microdomain rigidity. *Biophys. J.* 2010, 99, 834–844.
- [41] Meincken M, Roux SP, Jacobs EP. Determination of the hydrophilic character of membranes by pulsed force mode atomic force microscopy. *Appl. Surf. Sci.* 2005, 252, 1772–1779.
- [42] Sababi M, Kettle J, Rautkoski H, Claesson PM, Thormann E. Structural and nanomechanical properties of paperboard coatings studied by peak force tapping atomic force microscopy. *ACS Appl. Mater. Interfaces* 2012, 4, 5534–5541.
- [43] Siniscalco D, Arnould O, Bourmaud A, Le Duigou A, Baley C. Monitoring temperature effects on flax cell-wall mechanical properties within a composite material using AFM. *Polym. Test.* 2018, 69, 91–99.
- [44] Comesano TA, Natan MJ, Logan BE. Observation of changes in bacterial cell morphology using tapping mode atomic force microscopy. *Langmuir* 2000, 16, 4563–4572.
- [45] Bensouici F, Bououdina M, Dakhel AA, Tala-Ighil R, Tounane M, Iratni A, Souier T, Liu S, Cai W. Optical, structural and photocatalysis properties of Cu-doped TiO₂ thin films. *Appl. Surf. Sci.* 2017, 395, 110–116.
- [46] Migliorini E, Greci G, Ban J, Pozzato A, Tormen M, Lazzarino M, Torre V, Ruaro ME. Acceleration of neuronal precursors differentiation induced by substrate nanotopography. *Biotechnol. Bioeng.* 2011, 108, 2736–2746.
- [47] Wang D, Fujinami S, Nakajima K, Inukai S, Ueki H, Magario A, Noguchi T, Endo M, Nishi T. Visualization of nanomechanical mapping on polymer nanocomposites by AFM force measurement. *Polymer* 2010, 51, 2455–2459.
- [48] Prabhune M, Belge G, Dotzauer A, Bullerdiel J, Radmacher M. Comparison of mechanical properties of normal and malignant thyroid cells. *Micron* 2012, 43, 1267–1272.
- [49] Smolyakov G, Formosa-Dague C, Severac C, Duval RE, Dague E. High speed indentation measures by FV, QI and QNM introduce a new understanding of bionanomechanical experiments. *Micron* 2016, 85, 8–14.
- [50] Balss KM, Fried GA, Bohn PW. Chemically selective force mapping of electrochemically generated two-component ω -substituted alkanethiol monolayer gradients by pulsed-force-mode atomic force microscopy. *J. Electrochem. Soc.* 2002, 149, C450.
- [51] García R. Dynamic atomic force microscopy methods. *Surf. Sci. Rep.* 2002, 47, 197–301.
- [52] Giessibl FJ. Advances in atomic force microscopy. *Rev. Mod. Phys.* 2003, 75, 949–983.

- [53] Jalili N, Laxminarayana K. A review of atomic force microscopy imaging systems: application to molecular metrology and biological sciences. *Mechatronics* 2004, 14, 907–945.
- [54] Butt H-J, Cappella B, Kappl M. Force measurements with the atomic force microscope: technique, interpretation and applications. *Surf. Sci. Rep.* 2005, 59, 1–152.
- [55] Dufrene YF, Ando T, Garcia R, Alsteens D, Martinez-Martin D, Engel A, Gerber C, Müller DJ. Imaging modes of atomic force microscopy for application in molecular and cell biology. *Nat. Nanotechnol.* 2017, 12, 295–307.
- [56] Hu J, Zhang Y, Gao HB, Li MQ, Hartmann U. Artificial DNA patterns by mechanical nanomanipulation. *Nano Lett.* 2002, 2, 55–57.
- [57] Kuznetsov YG, Malkin AJ, Lucas RW, Plomp M, McPherson A. Imaging of viruses by atomic force microscopy. *J. Gen. Virol.* 2001, 82, 2025–2034.
- [58] Goncalves RP, Scheuring S. Manipulating and imaging individual membrane proteins by AFM. *Surf. Interface Anal.* 2006, 38, 1413–1418.
- [59] Scheuring S, Casuso I, Rico F. Biological AFM: where we come from – where we are – where we may go. *J. Mol. Recognit.* 2011, 24, 406–413.
- [60] Engel A, Muller DJ. Observing single biomolecules at work with the atomic force microscope. *Nat. Struct. Biol.* 2000, 7, 715–718.
- [61] Muller DJ, Dufrene YF. Force nanoscopy of living cells. *Curr. Biol.* 2011, 21, R212–R216.
- [62] Fakhru'llina G, Akhatova F, Kibardina M, Fokin D, Fakhru'llin R. Nanoscale imaging and characterization of *Caenorhabditis elegans* epicuticle using atomic force microscopy. *Nanomedicine* 2017, 13, 483–491.
- [63] Voss A, Dietz C, Stocker A, Stark RW. Quantitative measurement of the mechanical properties of human antibodies with sub-10-nm resolution in a liquid environment. *Nano Res.* 2015, 8, 1987–1996.
- [64] Hansma P, Drake B, Marti O, Gould S, Prater C. The scanning ion-conductance microscope. *Science* 1989, 243, 641–643.
- [65] Korchev YE, Bashford CL, Milovanovic M, Vodyanoy I, Lab MJ. Scanning ion conductance microscopy of living cells. *Biophys. J.* 1997, 73, 653–658.
- [66] Chen CC, Baker LA. Effects of pipette modulation and imaging distances on ion currents measured with scanning ion conductance microscopy (SICM). *Analyst* 2011, 136, 90–97.
- [67] Korchev YE, Gorelik J, Lab MJ, Sviderskaya EV, Johnston CL, Coombes CR, Vodyanoy I, Edwards CR. Cell volume measurement using scanning ion conductance microscopy. *Biophys. J.* 2000, 78, 451–457.
- [68] Novak P, Li C, Shevchuk AI, Stepanyan R, Caldwell M, Hughes S, Smart TG, Gorelik J, Ostanin VP, Lab MJ, Moss GWJ, Frolenkov GI, Klenerman D, Korchev YE. Nanoscale live-cell imaging using hopping probe ion conductance microscopy. *Nat. Methods* 2009, 6, 279–281.
- [69] Eghiaian F, Rigato A, Scheuring S. Structural, mechanical, and dynamical variability of the actin cortex in living cells. *Biophys. J.* 2015, 108, 1330–1340.
- [70] Calzado-Martin A, Encinar M, Tamayo J, Calleja M, San Paulo A. Effect of actin organization on the stiffness of living breast cancer cells revealed by peak-force modulation atomic force microscopy. *ACS Nano* 2016, 10, 3365–3374.
- [71] Pyne A, Thompson R, Leung C, Roy D, Hoogenboom BW. Single-molecule reconstruction of oligonucleotide secondary structure by atomic force microscopy. *Small* 2014, 10, 3257–3261.
- [72] Murugesapillai D, Bouaziz S, Maher LJ, Israeloff NE, Cameron CE, Williams MC. Accurate nanoscale flexibility measurement of DNA and DNA-protein complexes by atomic force microscopy in liquid. *Nanoscale* 2017, 9, 11327–11337.
- [73] Hertz H. Ueber die Verdunstung der Flüssigkeiten, insbesondere des Quecksilbers, im luftleeren Raume. *Ann. Phys.* 1882, 253, 177–193.
- [74] Johnson KL, Kendall K, Roberts AD. Surface energy and the contact of elastic solids. *Proc. R. Soc. A Math. Phys. Eng. Sci.* 1971, 324, 301–313.
- [75] Derjaguin BV, Muller VM, Toporov YP. Effect of contact deformations on the adhesion of particles. *J. Colloid Interface Sci.* 1975, 53, 314–326.
- [76] Maugis D. Adhesion of spheres: the JKR-DMT transition using a Dugdale model. *J. Colloid Interface Sci.* 1992, 150, 243–269.
- [77] Reifengerger RG, Eds. *Fundamentals of Atomic Force Microscopy: Part I: Foundations*. World Scientific Publishing Company Pte Limited: Singapore, 2016.
- [78] Heu C, Berquand A, Elie-Caille C, Nicod L. Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells. *J. Struct. Biol.* 2012, 178, 1–7.
- [79] Chen Q, Liu J, Thundat T, Gray MR, Liu Q. Spatially resolved organic coating on clay minerals in bitumen froth revealed by atomic force microscopy adhesion mapping. *Fuel* 2017, 191, 283–289.
- [80] Wang W, Li J, Fan M, Abedi S. Characterization of electrical properties of organic-rich shales at nano/micro scales. *Mar. Pet. Geol.* 2017, 86, 563–572.
- [81] Pai RK, Pillai S, Ahipha TN. Microscopic analysis of polymer honeycomb thin film studied by PeakForce TUNA for organic solar cell application. *J. Renew. Sustain. Energy* 2016, 8, 023703.
- [82] Gutierrez J, Mondragon I, Tercjak A. Quantitative nanoelectrical and nanomechanical properties of nanostructured hybrid composites by PeakForce Tunneling atomic force microscopy. *J. Phys. Chem. C* 2014, 118, 1206–1212.
- [83] Huang Z, De Wolf P, Poddar R, Li C, Mark A, Nellist MR, Chen Y, Jiang J, Papastavrou G, Boettcher SW, Xiang C, Brunschwig BS. PeakForce scanning electrochemical microscopy with nano-electrode probes. *Micros. Today* 2016, 24, 18–25.
- [84] Nellist MR, Chen Y, Mark A, Godrich S, Stelling C, Jiang J, Poddar R, Li C, Kumar R, Papastavrou G, Retsch M, Brunschwig BS, Huang Z, Xiang C, Boettcher SW. Atomic force microscopy with nanoelectrode tips for high resolution electrochemical, nanoadhesion and nanoelectrical imaging. *Nanotechnology* 2017, 28, 095711.
- [85] Robinson JT, Culbertson J, Berg M, Ohta T. Work function variations in twisted graphene layers. *Sci. Rep.* 2018, 8, 19631.
- [86] Xiao Z, Yuan Y, Shao Y, Wang Q, Dong Q, Bi C, Sharma P, Gruverman A, Huang J. Giant switchable photovoltaic effect in organometal trihalide perovskite devices. *Nat. Mater.* 2015, 14, 193–198.
- [87] Guo L, Hua G, Yang B, Lu H, Qiao L, Yan X, Li D. Electron work functions of ferrite and austenite phases in a duplex stainless steel and their adhesive forces with AFM silicon probe. *Sci. Rep.* 2016, 6, 20660.
- [88] Knittel P, Mizaikoff B, Kranz C. Simultaneous nanomechanical and electrochemical mapping: combining Peak Force Tapping atomic force microscopy with scanning electrochemical microscopy. *Anal. Chem.* 2016, 88, 6174–6178.

- [89] Jiang J, Huang Z, Xiang C, Poddar R, Lewerenz HJ, Papadantonakis KM, Lewis NS, Brunschwig BS. Nanoelectrical and nanoelectrochemical imaging of Pt/p-Si and Pt/p(+)–Si electrodes. *ChemSusChem* 2017, 10, 4657–4663.
- [90] Cohen H, Nogues C, Ullien D, Daube S, Naaman R, Porath D. Electrical characterization of self-assembled single- and double-stranded DNA monolayers using conductive AFM. *Faraday Discuss.* 2006, 131, 367–376.
- [91] Pea M, Maiolo L, Giovine E, Rinaldi A, Araneo R, Notargiacomo A. Electrical characterization of FIB processed metal layers for reliable conductive-AFM on ZnO microstructures. *Appl. Surf. Sci.* 2016, 371, 83–90.
- [92] Sahoo PK, Mangamma G, Rajesh A, Kamruddin M, Dash S. Polarization induced conductive AFM on cobalt doped ZnO nanostructures. *AIP Conf. Proc.* 2017, 1832, 050170.
- [93] Ranjan A, Raghavan N, Molina J, O'Shea SJ, Shubhakar K, Pey KL. Analysis of quantum conductance, read disturb and switching statistics in HfO₂ RRAM using conductive AFM. *Microelectron. Reliab.* 2016, 64, 172–178.
- [94] Weaver JMR. High resolution atomic force microscopy potentiometry. *J. Vac. Sci. Technol. B Microelectron. Nanometer Struct.* 1991, 9, 1559.
- [95] Nonnenmacher M, O'Boyle MP, Wickramasinghe HK. Kelvin probe force microscopy. *Appl. Phys. Lett.* 1991, 58, 2921–2923.
- [96] O'Boyle MP, Hwang TT, Wickramasinghe HK. Atomic force microscopy of work functions on the nanometer scale. *Appl. Phys. Lett.* 1999, 74, 2641–2642.
- [97] Sun H, Chu H, Wang J, Ding L, Li Y. Kelvin probe force microscopy study on nanotriboelectrification. *Appl. Phys. Lett.* 2010, 96, 083112.
- [98] Goryl M, Kolodziej JJ, Krok F, Piatkowski P, Such B, Szymon-ski M. Epitaxial nanostructures assembled on InSb(001) by submonolayer deposition of gold. *Microelectron. Eng.* 2005, 81, 394–399.
- [99] Yamauchi T, Tabuchi M, Nakamura A. Size dependence of the work function in InAs quantum dots on GaAs(001) as studied by Kelvin force probe microscopy. *Appl. Phys. Lett.* 2004, 84, 3834–3836.
- [100] Fukuma T, Kobayashi K, Yamada H, Matsushige K. Surface potential measurements by the dissipative force modulation method. *Rev. Sci. Instrum.* 2004, 75, 4589–4594.
- [101] Ye H, Lee J, Jang JS, Bard AJ. Rapid screening of BiVO₄-based photocatalysts by scanning electrochemical microscopy (SECM) and studies of their photoelectrochemical properties. *J. Phys. Chem. C* 2010, 114, 13322–13328.
- [102] Zhu Z, Ye Z, Zhang Q, Zhang J, Cao F. Novel dual Pt-Pt/IrO_x ultramicroelectrode for pH imaging using SECM in both potentiometric and amperometric modes. *Electrochem. Commun.* 2018, 88, 47–51.
- [103] Chen X, Botz AJR, Masa J, Schuhmann W. Characterisation of bifunctional electrocatalysts for oxygen reduction and evolution by means of SECM. *J. Solid State Electrochem.* 2015, 20, 1019–1027.
- [104] Jamali SS, Moulton SE, Tallman DE, Forsyth M, Weber J, Wallace GG. Evaluating the corrosion behaviour of magnesium alloy in simulated biological fluid by using SECM to detect hydrogen evolution. *Electrochim. Acta* 2015, 152, 294–301.
- [105] Jamali SS, Moulton SE, Tallman DE, Forsyth M, Weber J, Wallace GG. Applications of scanning electrochemical microscopy (SECM) for local characterization of AZ31 surface during corrosion in a buffered media. *Corros. Sci.* 2014, 86, 93–100.
- [106] Xia D-H, Zhu R-K, Behnamian Y, Luo J-L, Lin C-J, Klimas S. Understanding the interaction of thiosulfate with Alloy 800 in aqueous chloride solutions using SECM. *J. Electroanal. Chem.* 2015, 744, 77–84.
- [107] Koley D, Bard AJ. Triton X-100 concentration effects on membrane permeability of a single HeLa cell by scanning electrochemical microscopy (SECM). *Proc. Natl. Acad. Sci. USA* 2010, 107, 16783–16787.
- [108] Chen B, Hu Q, Xiong Q, Zhang F, He P. An ultrasensitive scanning electrochemical microscopy (SECM)-based DNA biosensing platform amplified with the long self-assembled DNA concatemers. *Electrochim. Acta* 2016, 192, 127–132.
- [109] Nievergelt AP, Erickson BW, Hosseini N, Adams JD, Fantner GE. Studying biological membranes with extended range high-speed atomic force microscopy. *Sci. Rep.* 2015, 5, 11987.
- [110] Dokukin ME, Sokolov I. Nanoscale compositional mapping of cells, tissues, and polymers with ringing mode of atomic force microscopy. *Sci. Rep.* 2017, 7, 11828.
- [111] Meng X, Zhang H, Song J, Fan X, Sun L, Xie H. Broad modulus range nanomechanical mapping by magnetic-drive soft probes. *Nat. Commun.* 2017, 8, 1944.

Bionotes

Ke Xu

School of Information and Control Engineering, Shenyang Jianzhu University, Shenyang, China

Ke Xu, a PhD, is a Professor at the Shenyang Jianzhu University. He obtained his master degree in Control Theory and Control Engineering in March 2005 from Shenyang Jianzhu University, Shenyang, China. On March 2008, he joined the State Key Laboratory in Shenyang Institute of Automation (SIA), CAS, and obtained a PhD in Pattern Recognition and Intelligent System in July 2012. He finished his postdoctor research in Mechanical and Electrical Engineering, at the SIA, CAS, China, on January 2016. His e-mail address is xksky1234@163.com.

Weihang Sun

School of Information and Control Engineering, Shenyang Jianzhu University, Shenyang, China

Weihang Sun obtained his Bachelor's degree in Electrical Engineering and Automation in 2015 from Shenyang Jianzhu University,

China. After graduation, he went on to study for a Master's degree in Control Engineering from Shenyang Jianzhu University. His main research interests include the PeakForce Tapping mode atomic force microscope and its related applications.

Yongjian Shao
School of Information and Control
Engineering, Shenyang Jianzhu University,
Shenyang, China

Yongjian Shao obtained his Bachelor's degree in 2017 from Shenyang Jianzhu University. After graduation, he went on to study for a Master's degree at Shenyang Jianzhu University. His major is Control Science and Engineering. His research interests are the development of the four-probe atomic force microscope and its applications.

Fanan Wei
School of Mechanical Engineering and
Automation, Fuzhou University, Fuzhou,
China

Fanan Wei obtained his undergraduate degree in Automation on June 2009 from Huazhong University of Science and Technology, Wuhan, China. On September 2009, he joined the State Key Laboratory in Shenyang Institute of Automation, CAS, and obtained a PhD in Mechanical and Electrical Engineering on December 2015. Then, he joined the faculty at the School of Mechanical Engineering and Automation in Fuzhou University, Fujian, China.

Xiaoxian Zhang
CAS Key Laboratory of Standardization
and Measurement for Nanotechnology,
CAS Center for Excellence in Nanoscience,
National Center for Nanoscience and
Technology, Beijing, China

Xiaoxian Zhang received her PhD degree in Physical Electronics from Peking University in 2011 and, then, worked as a research fellow in the department of Chemistry at the University of Michigan, Ann Arbor. She is currently an associate professor at the National Center for Nanoscience and Technology (NCNST). Her current research includes the studies of the relationship between interfacial structure and properties, and the development of methodologies to achieve *in situ* observation of the working mechanism of

electronical, optoelectrical, and thermoelectrical devices using advanced scanning probe microscopy. Prof. Zhang has published more than 40 SCI peer-review papers, in *Nature Communications*, *JACS*, and *Nano Letters*, etc.

Wei Wang
HIWING Technology Academy of CASIC,
Beijing, China

Wei Wang obtained her undergraduate degree in Automation on June 2009 from Sichuan University, Chengdu, China. On September 2009, she joined the Northeastern University and received her Master's degree in Control Theory and Control Engineering on July 2011. Then, she joined HIWING Technology Academy of CASIC, Beijing, China.

Peng Li
CAS Key Laboratory of Standardization
and Measurement for Nanotechnology,
CAS Center for Excellence in Nanoscience,
National Center for Nanoscience and
Technology, Beijing 100190, China,
lipeng@nanoctr.cn

Peng Li obtained his undergraduate degree in Automation on June 2009 from Sichuan University, Chengdu, China. On September 2009, he joined the State Key Laboratory in Shenyang Institute of Automation, CAS, and received a PhD in Mechanical and Electrical Engineering on July 2015. Then, he joined the CAS Key Laboratory of Standardization and Measurement for Nanotechnology in National Center for Nanoscience and Technology (NCNST), Beijing, China. His current research interests include scanning probe lithography, multiple-tips scanning probe microscope, and advanced atomic force microscopy techniques.