Quantitative imaging from single molecules to (bio)polymers

Introduction

Scanning force microscopy (SFM), also known as atomic force microscopy (AFM), is a surface imaging technique based on a purely mechanical imaging process. During its development over the last 20 years, SFM has become a key instrument in nanoscience and nanotechnology. In AFM, a very sharp tip attached to a cantilever is brought into very close proximity to the surface and then scanned line by line to probe the surface texture of the sample.

AFM has become an important tool for the field of material science for probing sample surfaces and determining the material properties at the nano scale. The technique gives fast reliable results in many aspects of the work of physicists, chemists, engineers and material scientists. AFMs have enhanced much of the technological progress of the last three decades.
The applications of polymers range from materials for the photovoltaic industry, research in automotive industry to the fields of medicine and life sciences.
Polymers are very diverse and their properties are widely spread. Polymers under the influence of temperature changes may show phase changes or melt. Also in liquid and solvents, they can change their behavior drastically due to swelling.
To characterize polymers on a nanometer scale, a high resolution instrument is required which enables the user to probe different aspects of the sample in question.
Traditional imaging modes of AFMs have well known drawbacks for challenging samples that exhibit steep edges or that are soft, sticky or loosely attached to the surface [1][2]. The most common modes such as contact mode or intermittent-contact (AC) mode introduce unwanted forces that might damage or compress the sample. Loosely attached objects might be moved due to lateral forces. The force applied when using AC mode is hard to control and might change when measuring soft biological samples.
JPK Instruments has released the new imaging mode Quantitative Imaging (QI™) which works without applying any lateral force and allows the user to control the vertical force at each pixel.

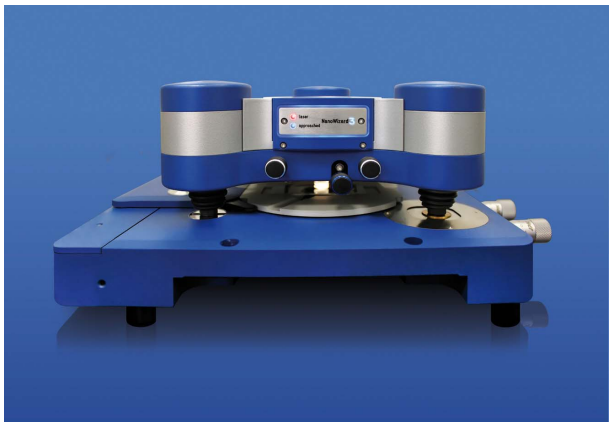


Fig. 1 *JPK NanoWizard® 3 head. Featuring closed loop operation in all three axes with scan ranges 100x100x15 µm (XYZ).*

Working Principle

QI™ is a force curve based imaging mode that ensures that not more than a set force is applied to the sample. The working principle allows easy imaging of soft materials such as hydrogels or biomolecules, sticky samples such as polymers or bacteria, loosely attached samples such as nanotubes or virus particles in fluid or samples with steep edges such as powders or MEMS structures.
QI™ can be used without limitations on the sample geometry, in any environment and does not require special cantilevers.
A novel tip movement algorithm records a complete force curve at every pixel while only performing lateral movement in between pixels. That way, a complete map with high spatial resolution is recorded with standard imaging speed and can be analyzed to extract different material properties.
The force that is applied to the sample can be exactly controlled and the resulting force curves can be saved along with the created image.

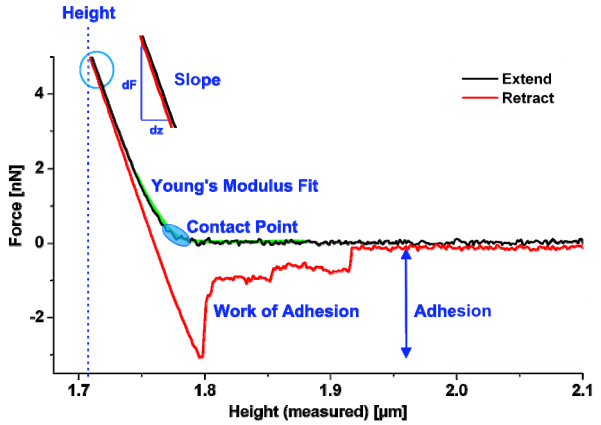


Fig. 2 *A typical force distance curve from which quantitative data like slope, adhesion and others can be extracted. All force curves can be saved together with the image and used to calculate e.g. the Young's Modulus or the work of adhesion*

By adjusting the imaging speed, the rate of deformation of the sample can easily be altered. The dependence of the Young's modulus with respect to this parameter can thus be investigated with ease.
In AC mode, the phase image represents stiffness contrast. However, it has always been hard to interpret these images regarding the extraction of quantitative data. QI™ gives the possibility to use the acquired data to calculate the properties in question using any fit algorithm. Parameters such as the tip shape which greatly influence the outcome of the fit can be freely chosen by the user.
Several parameters can be calculated such as the work of adhesion, the acting adhesion and the contact point height. Specific adhesion events can be detected and the Young's modulus of the sample can be calculated using contact mechanical models.
Further, the contact point height can be used to create a zero force image that most accurately resembles the real surface of the sample without indentation. Please see also the product note "The new JPK Contact Point Imaging (CPI) option based on QI™ mode" available on the JPK website.

Quantitative Imaging comes as standard with all models of the NanoWizard® 3 family making imaging even the toughest samples as easy as never before.

The add-on Advanced QI™ allows saving and post-processing of all recorded data and provides freely selectable online channels.

The challenges that are offered by many bulk and structured polymer samples are most diverse. High and steep edges, big height differences across the sample, temperature dependence and a great range of elasticity create high demands on the instrument. The following application examples give an impression of how powerful the new QI™ mode is.

Examples

Celgard®

Celgard® is a commercially available microporous membrane that is applied in rechargeable batteries as separator. This polypropylene monolayer is produced by a special dry-stretch process. The most important macroscopic properties are determined by its fiber thickness as well as the size of its pores.
In **Fig. 3** shows an example of a QI™ measurement of a

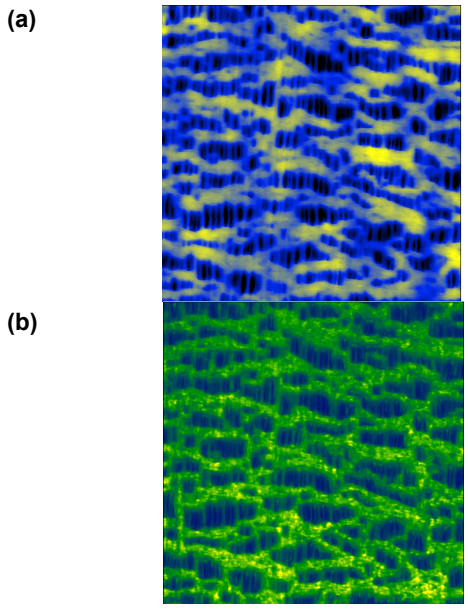


Fig. 3 (a) *Height image of Celgard® structure showing the individual fibres. Scan size: 3x3 µm, color scale: 160 nm*
(b) *Corresponding Young's modulus as calculated using the Hertz model. Color scale: 50-200 MPa*

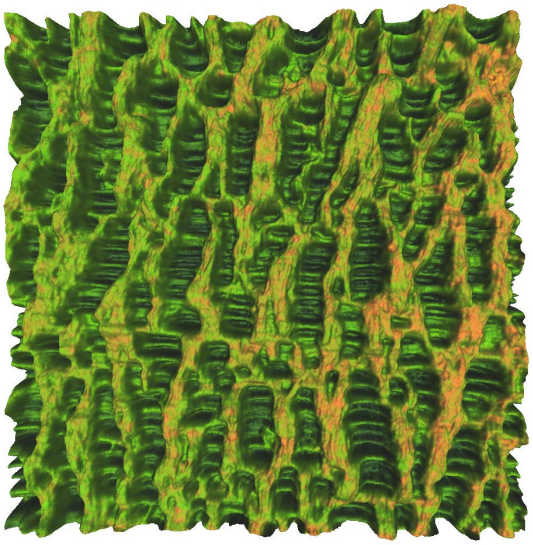


Fig. 4 *Overlay of 3D topography with elasticity information, color scale: 50-200 MPa*

Celgard® sample. The individual fibers can be clearly resolved in the height image (figure 3a). Due to the unique scanning procedure of Quantitative Imaging, no obstructions are present as may be observed in AC mode.

Analysis of the recorded data with the Hertzian model of elasticity yields the corresponding Young's modulus distribution as depicted in **Fig. 3(b)**.

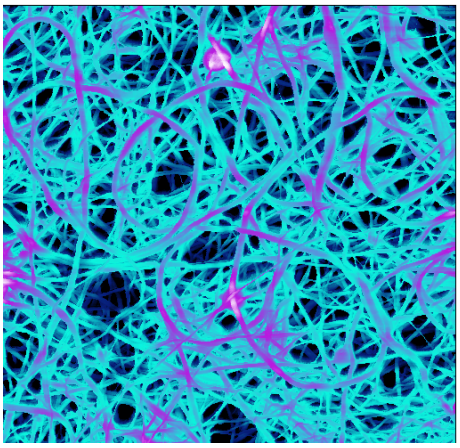
By overlaying the elasticity information over the 3D topography representation (**Fig. 4**), any inhomogeneities can be readily discovered.

Nanofibers

Nanofibrous materials are used in a wide range of applications in fields ranging from drug delivery, filtration and nanocomposites to regenerative medicine and responsive devices.

These kinds of materials show exceptional properties through their high surface to volume ratio and significant fiber interconnectivity. AFM is used to study the fiber density, network structure, micro scale interstitial spaces and fiber thickness. The challenges in imaging nanofibers with conventional intermittent-contact mode, such as its softness and deep trenches are overcome by QI™. The

(a)



(b)

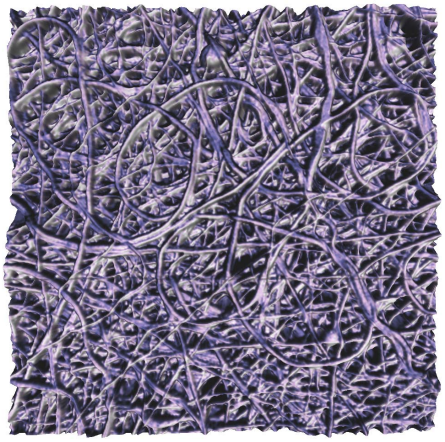


Fig. 5 (a) *Height image of nanofibers, deep trenches can be resolved with tip-limited resolution. Scan size: 10x10 µm, color scale: 710 nm*

(b) *Corresponding overlay of 3D topography with elasticity information, color scale: 2-8 MPa*

force that is applied to the individual fibers can be controlled with high precision.

The nanofiber network shown in **Fig. 5** was produced by electrospinning a solution of 11wt% PCL in acetic acid with 2wt% of TEA. Coiled nanofibers with different bending angles are randomly ordered in this structure. Areas with higher fiber density exhibit higher elasticity values which can be seen in the 3D image.

Polytetrafluoroethylene

Polytetrafluoroethylene (PTFE), better known under the brand name Teflon®, is a synthetic fluoropolymer with remarkable properties. Due to its hydrophobic character [3] and resistance to Van der Waals forces, it is used as

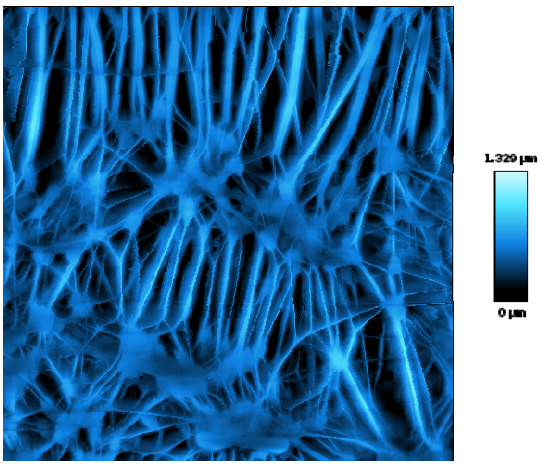


Fig. 6 *PTFE membrane, also known as Teflon® in a 10x10 µm scan. Note the individual free hanging filaments on which no lateral forces are applied during imaging.*

a non-stick coating. Further, PTFE is highly non-reactive which makes it an ideal material for reactive environments of acids and other corrosive materials.
AFM-studies have shown that the hydrophobic properties of PTFE can be minimized by surface modification using poly(ethylene glycol) methacrylate in order to increase resistance to biofouling [5]. This could reduce drawbacks in the manufacturing of biomaterials using PTFE.
Most of the previously mentioned properties stem from the electronegativity of fluorine as well as the strong fluorine-carbon bonds. The applications, however, require a detailed knowledge of the pore size, stiffness and fiber density. A scan with quantitative imaging reveals the structure of a PTFE-membrane sample (**Fig. 6**). The free-hanging fibers which are free to move are a serious drawback to conventional contact- or AC mode imaging. This can lead to unstable imaging conditions which are only overcome by exact force control in the vertical and lateral direction.

Hexacontane

Quantitative Imaging is also able to resolve small samples with great resolution. Hexacontane, an alkane with a non-branched chain of 60 carbon atoms, was spin-coated as a layer on HOPG and then imaged under ambient conditions (**Fig. 7**).

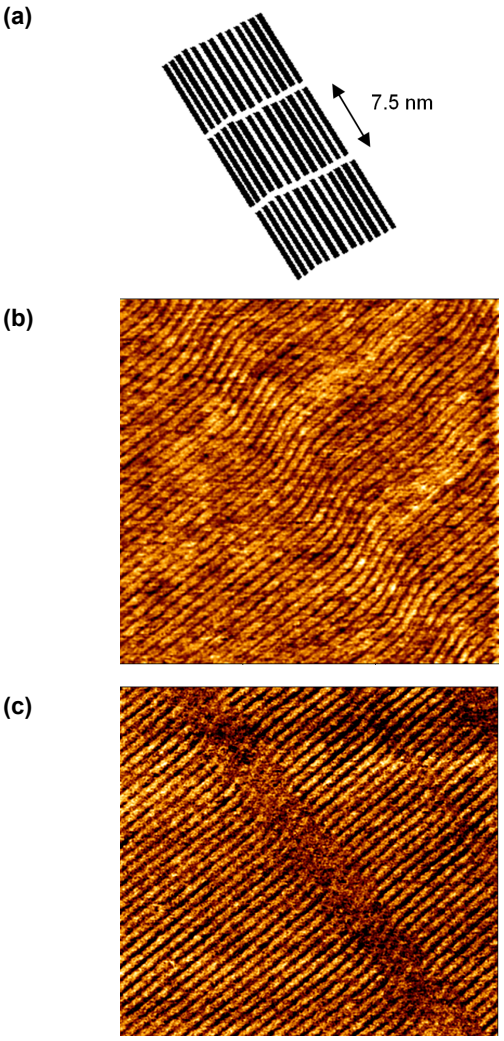


Fig. 7 (a) *Self-assembly of hexacontane molecules on HOPG leads to lamellar structures of 7.5 nm width.*
(b) *Hexacontane C60H122 in a 250x250 nm scan, color scale: 300 pm*
(c) *Hexacontane stiffness image, measured with a Si cantilever (f=75 kHz, k=2 N/m), color scale: 1.9-2.1 N/m*

Before spin-coating, the sample was diluted in warm xylene to a concentration of 0.001 mg/ml.
The width of one stripe in the image corresponds to the length of the hexacontane molecule (**Fig. 7 (a)**). The striped patterns are formed by lateral self-assembly.

The slope channel which is available during imaging gives a first impression on the stiffness of the sample. The area between the molecules is softer as the molecule ends are freely moveable and are possibly displaced by the cantilever.

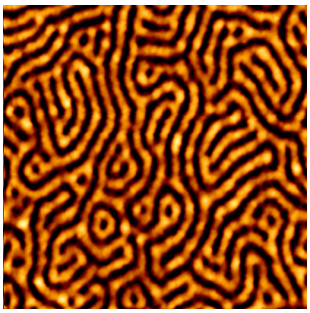
Block-copolymers

Another group of polymers, which can be investigated by AFM are so called block-copolymers. A block-copolymer is made up of two homo-polymer molecules covalently bound at one end. This link results in their unique properties . Especially when cooling a block-copolymer below a certain transition temperature, a so called self-assembled microphase separated structure is formed, which results from the segregation of the two blocks. Each phase reflects the properties of the corresponding homo-polymer, while the structure being formed only depends on the length ratio of the linked homo-polymers.

Fig. 8 (a) and **(b)** show the height and adhesion image of a microphase separated polystyrene-block-polybutadiene measured under ambient conditions. The sample was prepared by spin coating from solution on a silicon wafer. The two phases can be clearly distinguished in the height and adhesion image.

Additionally, the Young's modulus image (**Fig. 8 (c)**) indicates a strong difference in the stiffness of both phases. This difference in the Young's Modulus can be explained by the properties of the corresponding homo-polymers, as polystyrene is in a glassy state at room temperature, while polybutadiene is in a melt like state.

(a)



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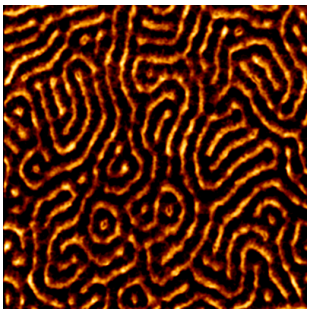


(c)

Fig. 8 (a) *Block-copolymer height image*
(b) *Adhesion image*
(c) *Young's Modulus image*

Lipid Bilayers

In an effort to find a suitable model which closely mimics biomembranes, amphiphilic block copolymers have drawn the interest of the scientific community. Those self-organized supramolecular structures are able to enclose liquid in a bilayer of spherical shape. Known as polymersomes, these are advantageous over e.g. liposomes through their increased stability and rigidity of their membranes [4].

Fig. 9 shows several poly(dimethylsiloxane)-block-poly(2-methyloxazoline) (PDMS-b-PMOXA) vesicles adsorbed to a mica surface. It can be seen that the size of the individual vesicles vary. While some vesicles are round, others show terrace like structure with different number of terraces. Corresponding cross sections of intact and terrace-like structures are shown in **Fig. 9**. The latter indicates a ruptured or collapsed vesicle. The rupture might be caused by the relatively high surface energy of mica.

Different numbers of terraces are a stacking of bilayers on top of each other. High structures of more than three layers result from the rupture of so called "pregnant" vesicles. One possible explanation of their origin is that one vesicle is enclosed in another vesicle. If the outer shell layer fuses with the solid support, the inner shell is

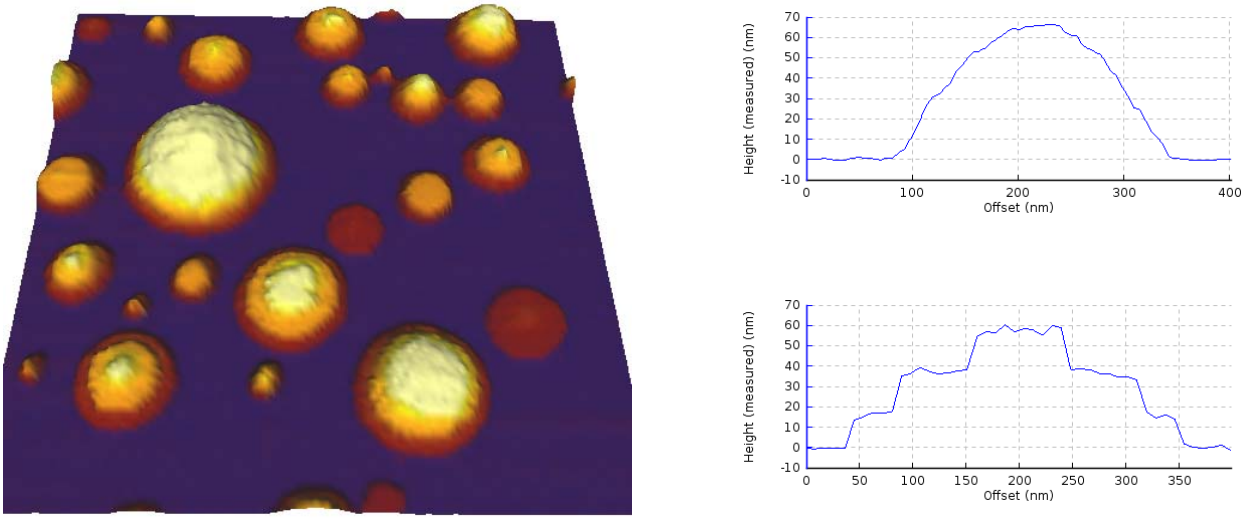


Fig. 9 *Overview scan showing different vesicles. Some of the vesicles are multi-layered. Intact as well as ruptured vesicles can be seen. Height image, dimensions: 1.7 µm x 1.7 µm x 65 nm*

embedded between two bilayers. Those "pregnant" polymersomes may form a fraction of 5% of all vesicles in the solution [4].

Whether or not a vesicle has opened up can not only be seen in the topography image but alsoby looking at the elasticity information. Intact vesicles exhibit a much lower stiffness and appear darker in the image (**Fig. 10**).

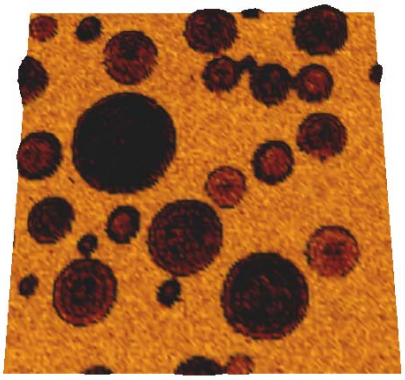


Fig. 10 *Different vesicle in 3D height image, with elasticity overlaid, scan size: 1.7x1.7 µm, typical elasticity on top of vesicle is ~15MPa*

Dendronized Polymers

Dendronized polymers are linear polymer chains (backbone) with regularly branched side chains (dendrons) attached to every repeat unit. Larger dendrons wrap the backbone so that the polymer can be viewed as a nanocylinder. The interior and surface of the nanocylinder can be functionalized. This property can be used to engineer molecular nanoconstructions, e.g. polymeric light emitters with an aggregation inhibitor or synthetic light-harvesting antennas.

The sample shown in **Fig. 12** consists of a polystyrol backbone with dendritic side chains of third generation. **Fig. 11** shows the structure of the repeat unit.

After spin coating the sample onto freshly cleaved mica, it was measured with QI™ mode under ambient

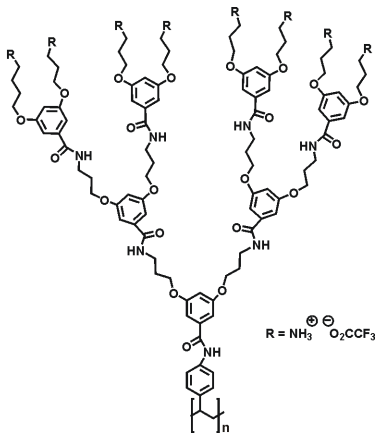


Fig. 11 *Schematic of the repeat unit of the dendronized polymer sample*

conditions.

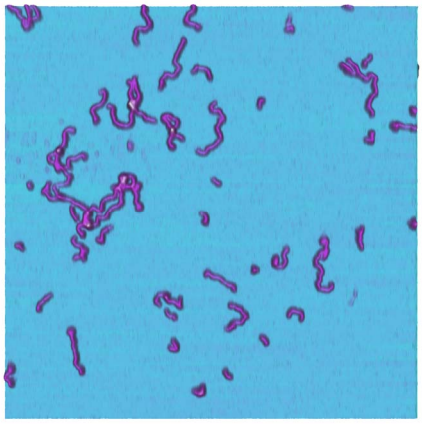
The preparation of the dendronized polymers on the substrate can be easily checked with the fast and reproducible scanning procedure offered by the NanoWizard® 3 system. This offers not only the possibility to calculate the stiffness (**Fig. 12 (b)**), but also to manipulate the individual polymer strands [6], [7].

Bacteriorhodopsin

Cellular membranes have drawn the interest of the scientific community in the past decades. They are thought to consist of different protein patches that determine different membranes functions. Bacteriorhodopsin is a protein found in membranes of Archaea where it acts as a proton pump and hence allows energy storage in a cell. In an effort to find new methods to characterize cellular membrane patches, bacteriorhodopsin was employed as a marker for purple membrane patches to identify them on bacterial surfaces [8] using Tip-enhanced Raman spectroscopy. Other studies employed conductive AFM [9] or force spectroscopy [10] to investigate bacteriorhodopsin patches.

Here we show images of a bacteriorhodopsin patch (**Fig. 13**) that was adsorbed on mica and imaged in buffer solution. Single defects prove the single molecule resolution of Quantitative Imaging in liquid. A Fourier transform image shows the periodicity of the structure (**Fig. 13 (c)**).

(a)



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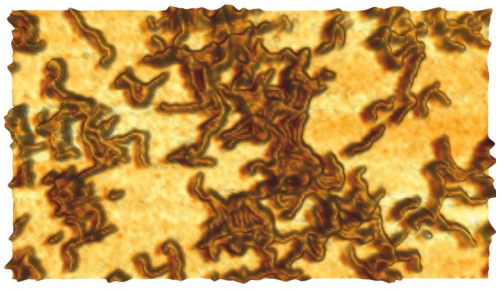

Fig. 12 (a) *Dendronized polymers 3D height image, Scan size: 1.3x1.3 µm, color scale: 2 nm*
(b) *Detail scan of different region with calculated Young's modulus overlaid. Scan size: 1.1x0.55 µm, color scale: 1-2 MPa*

(a)

(b)

(c)

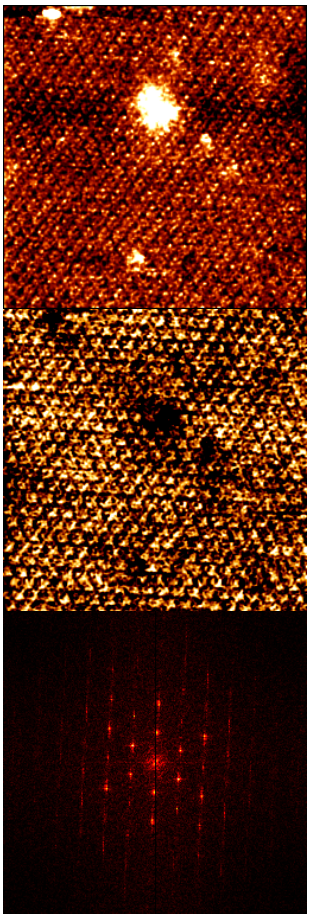

Fig. 13 (a) *Height and stiffness image of bacteriorhodopsin. Scan size: 120x120 nm, color scale: 480 pm*
(b)*Stiffness image, color scale: 72 nN/µm*
(c) *Fourier Transform image of stiffness data showing periodicity of the structure.*

Available Accessories

Polymer structure and crystallization is a rich field for AFM measurements with a wide variety of surface and structures and material property differences that can be seen under different conditions. For imaging polymers, the AFM instrument needs to be capable of high resolution and stability.

Fig. 14 *temperature control stages from JPK*
Left: Heating Cooling Stage (HCS™), 0-100 °C
Right: High Temperature Heating Stage (HTHS™), ambient-250 °C

JPK offers especially designed heating stages that enable high resolution scanning with minimal lateral or vertical drift during heating (Fig. 14). The High Temperature Heating Stage is particularly suited for crystallization studies because of the large temperature range available.

Acknowledgements

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