The photograph in Fig. 1a shows the temperature-stable cabinet, which accommodates the NT-MDT microscopes: TITANIUM, NEXT and NTEGRA Prima. Inside the cabinet the microscope is placed either on the vibration isolation structure held by 4 bungee cords or on the vibration isolation table installed on the cabinet floor. For controlling the temperature several heaters are placed inside the cabinet and a convectional air circulation creates a locally stable thermal environment. Temperatures of the room (RT) and a sample are used for a feedback, which adjusts power to the heaters to keep the sample temperature at the set-point value slightly above RT. As RT changes in response to air-conditioning, the sample temperature varies only in the 0.005 °C range after initial stabilization, Fig. 1b.

A change of the sample and probe causes the temperature drop for 10-15 minutes prior to the return to the set-point value. The low thermal drift (~0.2 nm/min) of AFM imaging in the cabinet is illustrated by 100-nm sequential images of fluoroalkane self-assemblys (Fig. 2), in which a defect at the top right is “immobile”.

**Fig 1a-b. (a) A thermal cabinet. (b) Temporal graphs of the room and sample temperatures.**

**Fig 2. Height images of fluoroalkane self-assemblies on graphite collected in sequential scans with 1 Hz scanning rate.**
The AFM images of two layered materials, which were recorded in contact mode, are shown in Figs. 3a-c. The height image of mica (Fig. 3a) shows the periodical corrugations mimicking the structure of the silicate mineral. The image lattice is characterized by a main period of 0.52 nm that matches the crystallographic data shown in the insert.

The lateral force image (Fig. 3b) is different and the elevations coincide with the centers of honeycombs of the height image. The height image of MoSe$_2$ layered crystal (Fig. 3c) reproduces the structure of its ab plane with the spacing of 0.32 nm.

The images of layered materials were obtained with Si probes with a 40 N/m stiffness (NSG-30A). A height image of polydiacetylene (PDA) shows its crystallographic bc facet with polymer fibrils split from the crystal, Fig. 4a.

The facet structure is formed of the phenyl stacks aligned in the b direction (Fig. 4b), and the unit cell (0.53 nm × 1.62 nm) includes molecules of neighboring rows. The contact mode image of PDA crystal reproduces its crystallographic structure well, Fig. 4c.

The study of the same crystal in AM mode reveals at the 20-nm scale (Fig. 5a) and unit cells are clearly seen in the image at high magnification (Fig. 5b). A rubbed layer of poly(tetrafluoroethylene) – PTFE on glass, which is often used for aligning liquid crystalline materials and for epitaxial crystallization of low and high molecular weight compounds, is a good test sample for visualization of individual polymer chains.

The oriented PTFE layer on a glass substrate was examined in different AFM modes. AM height image of one of the polymer locations (Fig. 6a) shows the flat molecular layers with close chain packing, which is interrupted by various defects.

The surface is formed of multiple ribbons, which are aligned along the rubbing direction. The smallest steps between the neighboring ribbons (~ 0.5 nm) hint that they consist of single molecule PTFE sheets.

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Inside the thermal cabinet, the atomic and molecular scale imaging has been routinely achieved in contact and oscillatory modes (Amplitude Modulation and Hybrid mode)

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Fig 3 a-c. Contact mode images of mica (a-b) and MoSe$_2$ crystal(c).

Fig 4 a-c. Contact mode images of PDA (a), (c) and molecular structure of PDA on the bc facet
The 10-nm image of the surface location nearby one of the surface defects (the insert in Fig. 6a) exhibits the well-oriented linear features with separation close to the inter-chain distance of 0.56 nm, which was found in crystalline PTFE by TEM, X-ray analysis. Therefore, the observed pattern represents the close packing of PTFE chains.

A similar pattern has been recorded in other flat locations of the sample in the 10-nm images obtained in AM and contact modes, Figs. 6b-c. The same PTFE sample was also examined with Hybrid mode, Figs. 7a-c. The vertically oriented strands, which are visualized in the 1-micron height image (Fig. 7a), are separated into extended blocks. The smaller 90-nm image shows an extended crystalline block surrounded by protrusions with multiple steps, Fig. 7b.

The imaging of the flat surface of this block revealed the aligned polymer chains with the inter-chain distance of 0.56 nm, Fig. 7c.
The molecular ordering in adsorbates on different substrates is one of AFM research areas, and we performed a study of dodecanol order in its adsorbate on MoS$_2$ in AM, Hybrid and contact modes.

The AFM images of dodecanol on MoS$_2$ were first collected in AM mode, Figs. 8a-c. The left part of the height image (~ 400-nm surface area) is covered by a featureless layer, which is ~ 0.5 nm in height, Fig. 8a. This overlayer and its few separate patches can be assigned to the top molecular layer without a noticeable in-plane order. The darker areas on the right represent the bottom layer, in which traces of lamellae with spacing of ~ 3.3 nm are distinguished in the phase image (Fig. 8b). The top layer is weakly bonded and the shape of its domains is influenced by the tip force during scanning. As a result of competition between the tip-sample force interactions and the self-assembly of dodecanol molecules this layer seems to be “mobile”. This explains a change of the surface morphology near the particles marked with a blue circle in Figs. 8a-c. The 200-nm phase image in Fig. 8c more clearly shows the lamellar structures of the bottom layer. When a similar area is examined with Hybrid mode using the same probe as in the AM study, the top layer was not noticed and only lamellar structures were seen, Figs. 8d-e. Furthermore, the imaging of the dodecanol adsorbate with contact mode using the same probe revealed the molecular structure of MoS$_2$ with the hexagonal symmetry and main spacing of 0.32 nm, Fig. 8f.

In other words, the probe operating in contact mode brushed away the organic adsorbate and the tip interacts with the substrate. This example demonstrates that a combined use of different AFM modes, which are characterized by a dissimilar level of the probe-sample forces, brings complimentary information about the sample organization.

Figure 8 a-f. (a) - (c) Height and phase images obtained in the AM mode on surface of dodecanol adsorbate on MoS$_2$. A reference location is marked with a blue circle. (d) - (e) Height images obtained in the Hybrid mode on surface of dodecanol adsorbate. (f) Height image obtained on the same sample as in (a) - (e) in the contact mode.