Peculiarities of the SPM design and methods for biological application

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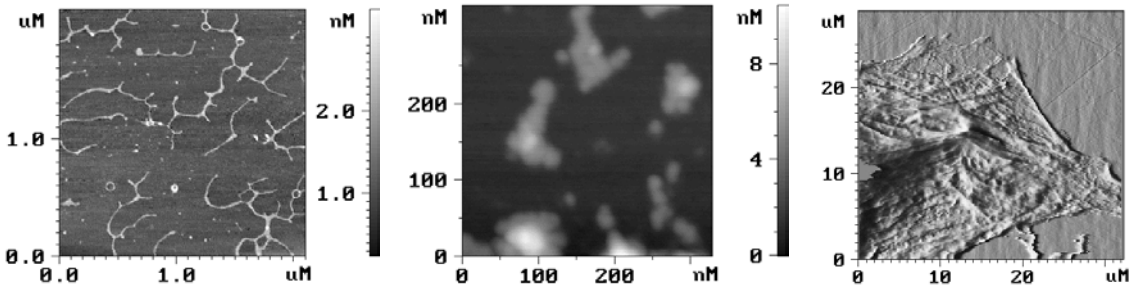
Application of the SPM to biology leads to some demands to the SPM construction. Here we describe different SPM constructions that were specially designed by NT-MDT Co for measurements of biological objects.

The most common features of the SPM follow from object type to be investigated. It is possible to distinguish two main sample types for biological investigation by SPM that produce quite different requirements to device. The objects with small height like single molecules or viruses relates to the first type. Investigations of these samples at molecular scale require improving noise of device. This can be achieved by using the scanner with the decreased Z-range that leads to decreasing the RMS in Z-direction. Scanners being produced by NT-MDT with XYZ range up to 50μm x 50μm x 2.5μm is more suitable for these samples. There are two different classes of small objects that have different lateral size. For example, molecules have different conformation: long molecules (e.g. DNA, fig.1a) and globular molecules (e.g. vegetable ribosome-activating toxin ML1, fig.1b). Measurements of DNA require big XY range up to several tens micrometers, but measurements of globular molecules can be carried out on relatively small area that enable to use scanner with small XY range. Smaller XY range enables more accurate XY positioning of the scanner that also leads to improving resolution.



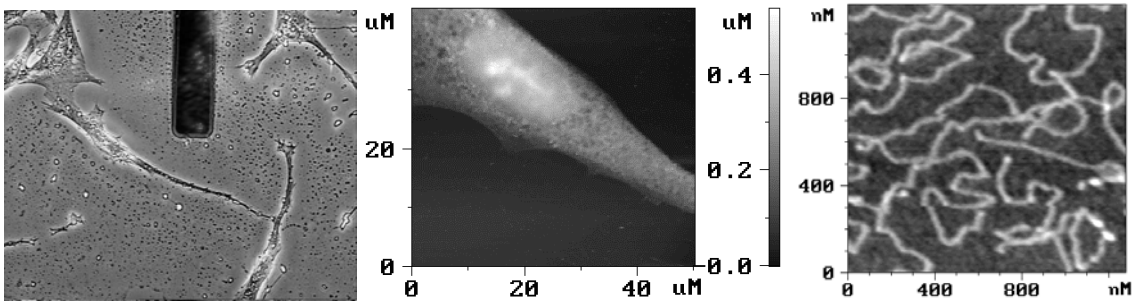
a) b) c)

Fig.1. a) molecules of DNA imaged in alcohol, b) vegetable ribosome-activating toxin ML1 on mica imaged in air. Single molecules are seen, c) mouse fibroblast imaged in phosphate buffer solution. All images were got in semicontact mode.

Big objects like cells, tissues are the second type of biology objects that have area of interest up to several tens microns with the height variations from several to tens microns. Example of

such object is 3T3 mouse fibroblast fixed by formalin and imaged in phosphate buffer solution (Fig1c). SPM for such objects require scanner with the big range in XYZ direction. The special electronic block provides increasing of the Z range in more than 2 times compare with standard electronics. For scanner with XY range of 90μm x 90μm Z range is about 10μm with this electronics.

There are two main classes of "Solver" line SPMs that differ by scanning method: scanning by sample (P47) and scanning by cantilever (SMENA head). Both types of devices have possibility of measurements in liquid in both contact and semicontact modes, which is important feature of the biological application. Acoustic excitation of cantilever is used for semicontact mode in liquid. SMENA head has most convenient design for biology samples owing to simpler principle of scanning in liquid and possibility of using the standard Petri dishes as an open cell. P47 has only closed cell [1]. Transition from scanning in air to scanning in liquid with SMENA head is carried out by changing of the magnetic cantilever holder [2]. Such construction is very easy to use and allows easy cleaning of the cantilever holder.



a) b) c)

Fig.2. a) optical image of human fibroblast, b) SPM image of part of a (inside white square) obtained in air, semicontact mode, c) SNOM image of DNA.

Possibility of measurements of the living objects is very important for biologists. During measurements of the living object the temperature must be kept constant (usually nearly 37°C). There are two different ways for providing such experiments with SMENA: 1) the use of the closed cell with temperature control, 2) the use of the isolation hood, which covers the whole instruments and is equipped with heating system. The first way is more convenient because of faster heating–cooling processes and better access to the instrument. But the second method provides more stable temperature during experiments. Closed cell is also intended for measurements in variable solution. Measurements in closed cell can be carried out not only in liquid but also in gas. The using of helium as an environment enables

increasing of quality factor of the cantilever and decreasing of the adsorption on the sample surface.

Any SPM measurements require good noise protection. Standard configuration of NT-MDT SPM is equipped with special antivibration protection provided minimum noise. This design is more suitable for molecular resolution. For big objects like cell NT-MDT has developed combination of inverted optical microscope (Olympus IX-70 or Biolam) and SPM with SMENA head [3]. This combined device (Solver BIO) provides simultaneous measurements with high-resolution SPM and such widely used optical techniques as bright-field, phase contrast, fluorescence. Fig.2a shows optical image (phase contrast) of human fibroblast cells and cantilever (top central part). Fig.2b demonstrates SPM topography of marked area of Fig.2a obtained in air.

Inverted optical microscope is also compatible with Solver Scanning Near-Field Optical Microscope (SNOM), which enables to use traditional for biologists optical methods with high resolution (better than 100nm, Fig.2c). This device can get both topography and spectral characteristics of the sample simultaneously. For example information about distribution of marked molecules over sample surface is available.

The combined optical-SPM (or SNOM) microscope can be equipped with a closed-loop scanning stage. This stage provides high linearity, accuracy and repeatability of scanning.

All NT-MDT SPMs are operated under software that has wide possibilities of the tip manipulating. It is possible to measure force-distance curves in each point of scan and then to analyze obtained data. Similar analysis of collagen fibers is discussed. If the tip is coated by some certain kind of molecules then the map of tip-sample affinity can be obtained. For example, investigation of the antigen-antibody reactions can be done in such a way.

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References

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