

Life Sciences

THE SPM DESIGN AND METHODES FOR BIOLOGICAL APPLICATION

SPM is widely used device for biological investigations now. The main advantage of SPM is possibility to measure biological objects in liquid with molecular resolution. Application of the SPM to biology leads to some demands to the SPM construction. There are two main classes of NT-MDT SPMs that differ by scanning method: scanning by sample ([SOLVER P47](#)) and scanning by probe (SMENA head). Both types of devices have possibility of measurements in liquid in both contact and semicontact modes. Possibility of measurements in liquid 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 (Fig.1). SMENA has various possibilities for measurements in liquid: in the drop (Fig. 2), in the Petri dish, in the closed cell. P47 works in liquid only with the closed cell. Transition from scanning in air to scanning in liquid with SMENA head is carried out by changing of the magnetic cantilever holder (Fig.1). Such construction is very easy to use and allows easy cleaning of the cantilever holder.

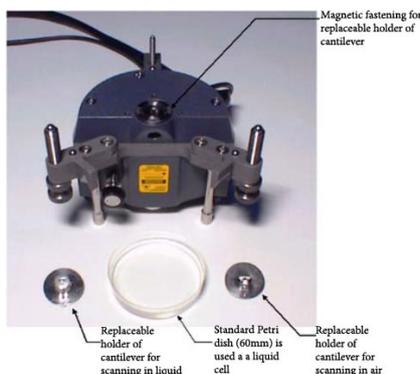


Fig. 1 SMENA head for measurements in liquid.

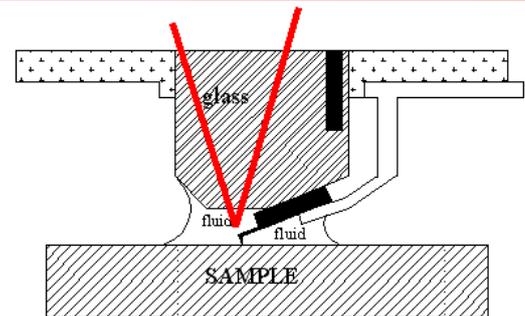


Fig. 2 Schematic view of scanning in the liquid drop with SMENA for liquid.

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. The closed cell is also intended for measurements in variable solution. Measurements in the closed cell can be carried out not only in liquid but also in gas. The using of helium as an environment enables increasing quality factor of the cantilever and decreasing adsorption on the sample surface.

■ There are two different configuration of NT-MDT SPMs based on SMENA head for liquid that are intended for biological measurements:

- SPM for single molecules, viruses and other nanometer-size objects.

Investigations of these objects at molecular scale require improving noise of device. Standard configuration of NT-MDT SPMs [SOLVER P47H](#), [SOLVER P47](#) is equipped with suspended vibroprotection system (Fig. 3) providing minimum noise during scanning. SOLVER P47H with SMENA head for liquid is more suitable for biological investigation with molecular resolution in both air and liquid.



Fig. 3 Passive vibroprotection system, SMENA head and automatic approach system (SOLVER P47H).

The minimal noise also can be achieved by using the scanner with the decreased Z-range that leads to decreasing the noise in Z-direction. Scanners being produced by NT-MDT with XYZ range up to $50\mu\text{m} \times 50\mu\text{m} \times 2.5\mu\text{m}$ is more suitable for molecular-scale 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.4a) and globular molecules (e.g. vegetable ribosome-activating toxin ML1, Fig. 4b). 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.

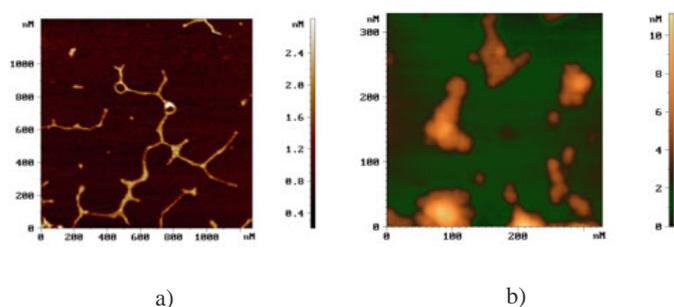


Fig.4. All images were got in semicontact mode.a) aggregation of DNA molecules. Image was obtained in alcohol,

b) vegetable ribosome-activating toxin ML1 on mica imaged in air. Single molecules are seen.

- SPM for cells, tissues, bacteria.

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. For such big objects NT-MDT has developed combination of inverted optical microscope (Olympus or Biolam) and SPM with SMENA head (Fig. 5). This combined device ([Solver BIO](#)) provides simultaneous measurements with high-resolution SPM and widely used optical techniques such as bright-field, phase contrast, fluorescence.



Fig. 5 SOLVER BIO: combination of the optical microscope and the SPM SMENA for liquid.

Example of big object is 3T3 mouse fibroblast fixed by formalin and imaged in phosphate buffer solution (Fig. 6). SPM for such objects requires 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 $90\mu\text{m} \times 90\mu\text{m}$ Z-range is about $10\mu\text{m}$ with this electronics.

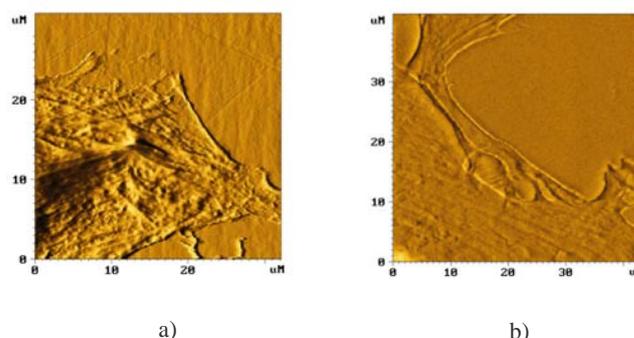


Fig. 6 Mouse fibroblast imaged in phosphate buffer solution. Cytoskeleton is seen on left image. Right image shows the folds that are caused by cell moving.

Inverted optical microscope is also compatible with Solver Scanning Near-Field Optical Microscope

(SNOM), which enables to use traditional for biologists optical methods with resolution better than 100nm (Fig. 6). This device can get both topography and spectral characteristics of the sample simultaneously in both air and liquid. For example information about distribution of marked molecules over sample surface is available.

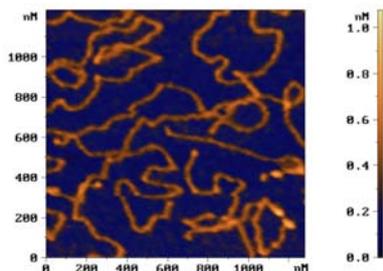


Fig. 7 Shear-force image of DNA obtained in air by SOLVER SNOM.

The combined optical-SPM (or optical-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 (Force Volume, FV). This is widespread technique in biology now (see FV analysis of collagen fibers). If the tip is coated by some certain kind of molecules then the map of tip-sample interaction can be obtained. For example, investigation of the antigen-antibody reactions can be done in such a way.

■ Acknowledgments:

Authors would like to thank A.S. Elkady (Moscow State University), M.M. Moisenovich, A.G. Tonevitskii (Institute for Transplantation, Moscow), A.A. Manykin (Institute for Virology, Moscow) for the samples used in this work.

CONTACT DETAILS

Building 167, Zelenograd, 124460, Moscow, Russia

Tel: +7(095)535-0305, 913-5736

Fax: +7(095) 535-6410, 913-5739

e-mail: spm@ntmdt.ru; <http://www.ntmdt.ru>