Study of Cancer Cells Used Atomic Force Microscopy

K. Tomankova[*,1], H. Kolarova[1], M. Vujtek[2] and H. Zapletalova[2]

[1] Department of Medical Biophysics, Faculty of Medicine, Palacky University in Olomouc, Hnevotinska 3, 775 15 Olomouc, Czech Republic
[2] Department of Experimental Physics, Faculty of Nature Science, Palacky University in Olomouc, tr. Svobody 26, 771 46 Olomouc, Czech Republic

Cell-surface interaction plays a crucial role in the adhesion, motility, function of the cells. Hence the control of the topographical structuring of the substrate interacts directly on the tissue development. Atomic Force Microscopy (AFM) has been used to image the morphology of developing tumor cells and their processes. However, it is frequently reported that prior fixation is required for reliable imaging of cells with lower adhesive properties. The aim of the presented study is to picture the cell surface in air. A Dry Scanner in Non-contact or Tapping mode was used in the biological application of AFM. In our work we used the cell line A549 (human lung carcinoma cells) as a biological sample. We obtained two types of pictures: topography and phase image.

Keywords Atomic force microscopy; cancer cell

1. Introduction

The growth of AFM has been parallel to the revolution of computer technologies. In fact, computers have played a central role in the development of this technique significantly improving the data acquisition, control, image processing and data analysis. AFM has emerged into a technique capable of resolving molecular details of cell surface under ambient condition [1, 2]. Since 1986, AFM has become versatile tool in the field of biological science [3]. Enveloped cell biological membranes and many biomolecules, including proteins and nucleic acids [4, 5], have been extensively imaged [6]. Atomic force microscopy has emerged as only technique capable of real-time imaging of the surface of living cell at their native environment. This technique has found many applications in pharmacology, biotechnology, microbiology, structural and molecular biology, genetics and other biology-related fields [7].

Interactions between the cantilever tip and the cell surface are so complex, so there is no simple way to control tip-cell interactions and to eliminate the disruptive effect of scanning cantilever [8 - 10]. Visualization of biological material has greatly benefited from the development of AFM techniques at least for two main reasons. First, these samples are soft and are significantly deformed by forces applied in AFM. Second, these materials adhere weakly to the substrate and can easily be displaced or destroyed by the lateral forces that arise during the scanning of the tip in contact with the sample. Hence, the biological material is scanned in non-contact (NC-AFM) mode allowing the visualization of the delicate samples in air and other surroundings. In this mode the tip makes no contact with the surface of the sample, minimizing the destructive lateral forces [11]. Lateral resolution of the AFM on rigid surfaces can reach the atomic level, while scanning soft biological surfaces reach resolution of about 1 nm. Vertical resolution is mostly determined by AFM scanned sensitivity and typically is as high 0,01nm. [12]. Several studies have shown that there is a close relationship between cell shape and cell function. Cell shape can be influenced by the topography of the surface, where the cells are grown, both in cell culture and in an animal [13]. It was proven that the cell morphology and orientation is determined by the topography of the structured polymer substrates. The patterning of plastics using mechanical methods (embossing, molding, lithography) are shown to have excellent resolution for cell growing. The drying

[*] Corresponding author: e-mail: tomanko@tunw.upol.cz, Phone: +420 585 632 106

method used reflects morphology of the cells in stage closely before drying. The live undamaged cells have elongate shape in comparison with photodynamically damaged cells. The term "lives cell imaging" in this case is really not exact and therefore we replaced or removed the adjective from the term, even if the cells look like live on the images.

2. Materials and methods

2.1 Materials and instruments

In our experiments we used cell line A549 (human lung carcinoma cells) as a biological materials. Chemicals used were Dulbecco´s Modified Eagle Medium (DMEM), glutaraldehyde (GA, Sigma Aldrich), poly-L-lysine (PLL, Sigma Aldrich). Measurements were carried out on AFM *Explorer* with cover head (Veeco, USA), inverse fluorescent optical microscope Olympus IX70. Further we used sterile plastic microscope slides Thermanox® as substrates for cells and 35 mm Petri dishes for cultivation of the cell line.

2.2 Sample preparation and Atomic force microscopy

For the cell imaging we used PLL covered plastic disks Thermanox® (pH 7.4). The plastic disks were incubated in 0.1% aqueous PLL solution for1 h. After incubation, the disks were removed and allowed to dry overnight by standing on edge on a paper towel [14]. Samples of 20 µl aliquot of A549 cells were pippeted onto PLL treated disks and spreaded with the pipette tip to a diameter roughly 5 - 7 mm. Then samples were allowed stand for 20 minutes in DMEM at 37 °C and 5 % CO_2. Before the AFM experiments, the DMEM covering the cells on the slides was removed and several times optical microscopy was used to identify suitable cell candidate or cluster of cells for AFM examination in air. The cells were fixated with 1% glutaraldehyde in PBS for 20 minutes.

Scanning was performed at high dry level. We imaged the cell line by large dry scanner at size of 100 × 100 µm. The images were acquired by non-contact tip from antimony doped silicon, 125 µm long, 30 µm wide, 10 µm – 15 µm high with spring constant of 42 Nm^{-1}, and resonance frequency of 320 kHz (Veeco, USA). The radius of curvature of the sharpened probe was 20 nm. AFM scanner with hardware correction was needed to reduce non-linearity of the piezoelectric elements. All images were processed by WSxM software [15].

3. Results and discussion

Imaging of cell surface was earlier considered as an important biological application of the scanning probe microscopy and more particularly the atomic force microscopy. Among the identified factors that can affect the quality of imaging belongs the force applied during scanning. Low forces limit the deformation of the cell surface under the AFM tip and thereby reduce the influence of the rigid cytoskeleton submambraneous structures on the images contrast [18]. Using AFM we observed morphological features on A549 cells. Fig. 1A presents topographical parameters of complete cell, her body and invadopodia, and Fig. 1B presents phase image. Live cells adhered to substrate created clusters with cell extension (invadopodia) projecting toward other cells [23]. The size of invadopodia can be up to 100 µm long. Healthy culture was very vital and aggressive to growth. Invadopodias are characterized for cancer cell line. We can study number of invadopodias per cell, the length of invadopodias and the orientation of cells. Most of the cell are aligned parallel to the grooves and show the typical bipolar shape. We found that 70% of human lung carcinoma cell are bipolar but 30% contain one, three of more invadopodias.

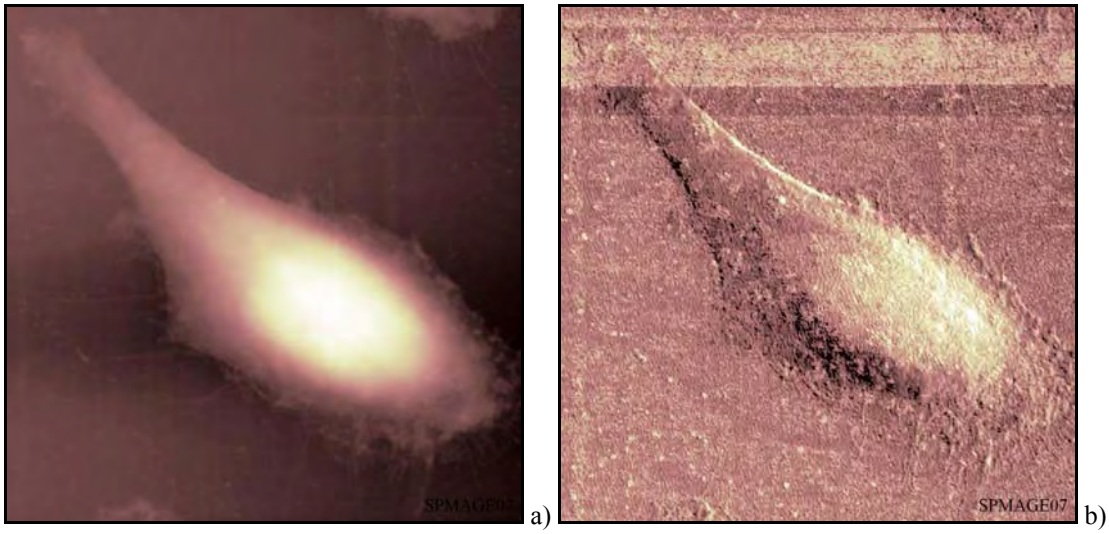


Fig. 1 One cell of A549 cell line. The image was obtained in non-contact topography mode (A) and phase mode (B). Parameters of the pictures are 52 μm × 52 μm, resolution 300 × 300 pixels, scan rate 50 μm/s. The height of the cell is expressed in color scale 0 (dark fields) – 2.06 μm (light fields).

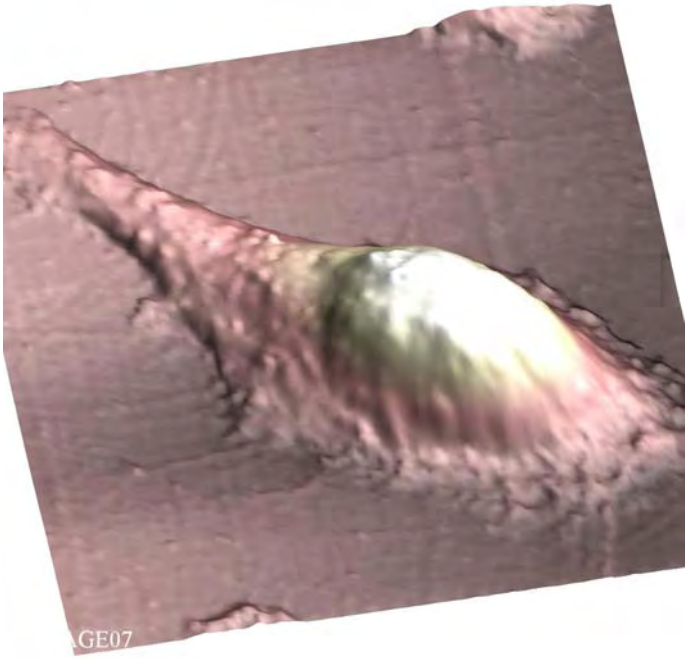
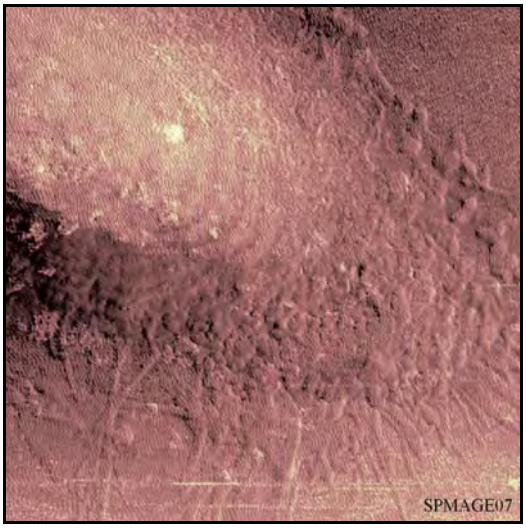
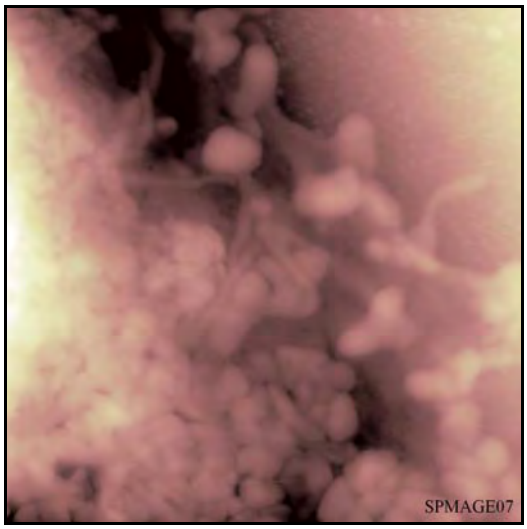


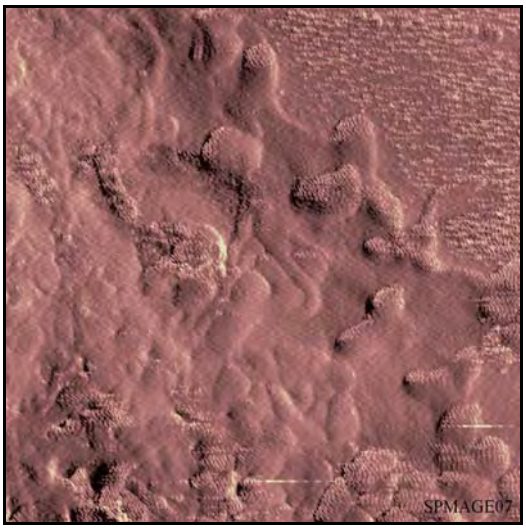
Fig. 2 One cell of A549 cell line. The image was obtained in non-contact 3D topography mode. In this processing we can see superficial structure of the cell and her smoothness or roughness in details. Parameters of the pictures are 52 μm × 52 μm, resolution 300 × 300 pixels, scan rate 50 μm/s. The height of the cell is expressed in color scale 0 (dark fields) – 2.06 μm (light fields).

a)


b)

Fig. 3 Detailed illustration the edge of the cell, which is adhered on poly-L-lysine substrate treated by glutaraldehyde. The image was obtained in non-contact topography mode (A) and phase mode (B). Parameters of the pictures are 20 μm × 20 μm, resolution 300 × 300 pixels, scan rate 22 μm/s. The height of the cell is expressed in colour scale 0 (dark fields) – 2.8 μm (light fields).



a)


b)

Fig. 4 Detailed illustration the edge of the cell, which is adhered on poly-L-lysine substrate treated by glutaraldehyde. The image was obtained in non-contact topography mode (A) and phase mode (B). Parameters of the pictures are 8 μm × 8 μm, resolution 300 × 300 pixels, scan rate 10 μm/s. The height of the cell is expressed in colour scale 0 (dark fields) – 440 nm (light fields).

AFM surface images were acquired in non-contact mode either as topographies that show height of contours or as phase images that highlight the fine of features or surface morphology [16].
Although some authors have used phase imaging as a complement to topographic imaging to provide information on sample heterogeneity, phase images are usually very difficult to interpret since many different factors can cause the phase shift [20].

3D topography gives us complex information about superficial structure of the cell and her smoothness or roughness in details. Cover of the cell is quite soft. However, an edge of the cell and part,

which is adhered to substrate, is roughness than upper part (see Fig. 2). Details adhered parts of the cell from Fig. 1 you can see in Fig. 3A in topographic mode and in Fig. 3B in phase images. On these pictures phase image give us detailed information, than topographical images. It is bring about high difference in high individual parts of the cell. Hence, we imaged more details of this adhered part and you can see in Fig. 4A (topography) and Fig 4B (phase image). The altitudinal difference in adhered parts of the cell is about 400 nm. You can see this in Fig. 5.

One scan 100 × 100 μm with resolution 300 pixels per line took between 5 and 10 min based on the scan rate of 0.4 – 1 lines per second. The identification of the optimum cantilever amplitude set point was critical in our experiments in respect to image the soft cell surface without damage. For optimum imaging the set point was found about at 50 % in lateral resolution [17]. Proximity of the cantilever tip to the surface was identified by reduction of oscillation amplitude, this is valuable for imaging of biological samples. AFM studies of soft materials are often conducted to increase cantilever damping and thus reduce the local damage caused to cells.

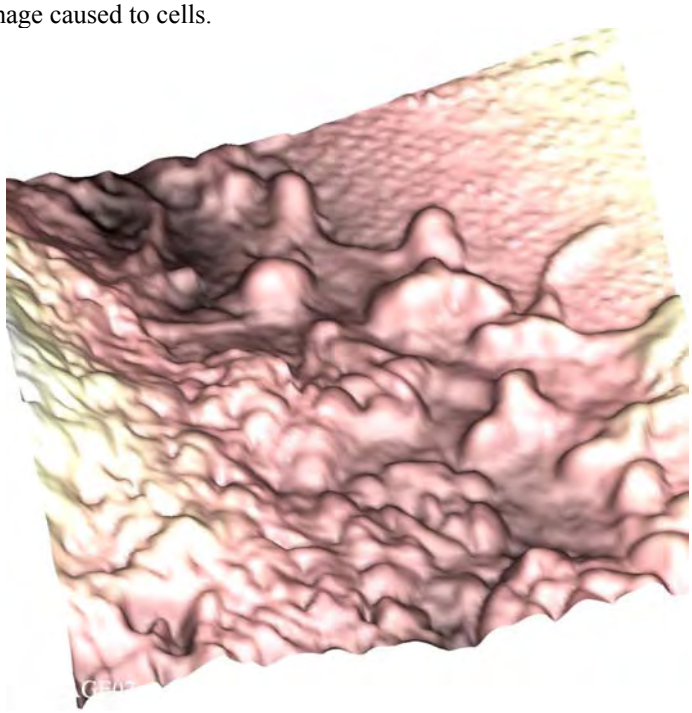


Fig. 5 Detail of one cell of A549 cell line. The image was obtained in non-contact 3D topography mode. In this processing we can see superficial structure the edge of the cell and her smoothness or roughness in details of the fixation by the combination poly-L-lysine and glutaraldehyde. Parameters of the pictures are 8 μm × 8 μm, resolution 300 × 300 pixels, scan rate 10 μm/s. The height of the cell is expressed in colour scale 0 (dark fields) – 440 nm (light fields).

The AFM does not require much in sample processing in fact. Its main requirement is that the sample has to be well-adhered to a substrate such that does not move around when the AFM probe scans it [19]. The high adhesion was observed on the film of poly-L-lysine (PLL). In addition, the cell adhesion properties can be tuned depending on the deposition pH of the polyelectrolyte solution [20].

NC-AFM mode was developed for improving imaging of soft samples by AFM. Difficulties in the proper adjustment of the scanning parameters are often encountered when using tapping–mode atomic force microscopy (TM-AFM) for imaging thick and soft materials, and particularly living cells in aqueous buffer. To increase quality of our images, we scanned cells in non-contact mode (NC-AFM) [18]. Constant force applied on the biological samples may damage the cell and thus change morphology. Considering this, various imaging modes have been developed such as resonance based

tapping mode, lift mode, force modulation imaging, nanoindenting, scratching and lateral force microscopy.

The effect of drying procedure may cause a certain degree of flattening in the nanostructure of the sample and this was taken into account during interpretation of the results. Drying may also increase the surface roughness of the sample [21]. However, the effect of fixation with glutaraldehyde (GA) on these samples brings the images morphologically more similar to the TEM images [19]. Used of GA fixation give us high quality of this images without artifact bring about drying process. For example work from Mendez-Vilaz has suggested that specific interaction play an important role in adhesion and the adhesive interaction. In contrast, a decrease of adhesion force in glutaraldehyde-treated cells was reported by [22] suggesting a limitation of the method in biological application using AFM. Nevertheless, our results did not prove the fact. We used 1 % GA in PBS and fixation for 20 minutes. Cell samples were dried about half an hour and the presented Figures showed typical shape of individual live cell.

AFM, in itself, has proved to be a powerful instrument in nanoscopic analysis of biological samples. Nevertheless more information with finer details may be achieved if combined with various other techniques such as optical microscope and optical tweezers as these technique allow direct manipulation of individual cell.

Acknowledgements This work was supported by the Grant Project MSM 6198959216.

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