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DEVELOPMENT OF A TUNING FORK BASED ATOMIC FORCE MICROSCOPY (FORK-AFM) FOR IMAGING ON ERYTHROCYTES

Vo Thanh Tung*

A.V. Luikov Heat and Mass Transfer Institute of National Academy of Sciences of Belarus, P. Brovki Str. 15, Minsk, Belarus. Email: votungbeo@gmail.com

We describe the development of Fork-AFM for investigating of human blood cell morphology, namely erythrocytes with high-resolution imaging. The measurements were compared with Fork-AFM using silicon cantilever tip on erythrocytes for two modes operation: shear-force and intermittent contact modes. We have glued directly the tips to the prong of tuning fork. The received results shown that the capabilities of the combination of AFM and tuning fork could quantitatively analyze the properties of surface of erythrocytes samples with high precision and resolution. Furthermore, it suggests that Fork-AFM can become a very useful and reliable tool in the study of biomolecules.

Introduction

Since its invention [1], atomic force microscopy (AFM) is increasingly becoming a tool for high-tech industrial applications. Nowadays, AFM development can be considered as a key point for advances in nanoscience and nanotechnology. Especially, AFM has proven to be a powerful tool for biological studies. Applications include imaging molecules [2–5], cells [6–8] tissues [9, 10] biomaterials [11–13], and measuring forces [14–18]. However, the use of a diode laser in AFM introduces several problems. First of all, it introduces noise in the setup due to thermal mode hopping of the laser and drift caused by heat dissipation of the laser diode. Second, the alignment of the laser beam on the cantilever and the position sensitive photo detector is an elaborate process. Third, for many types of measurement, illumination from the diode laser is a detriment. Finally, the need of a laser diode, an adjustable mirror, and a photo detector prevent the simplification of the scanning head.

Due to its high stability, precision and low power consumption, the quartz crystal tuning fork has become a valuable basic component for frequency measurements. For instance, since the late 1960s, mechanical pendulum or spring based watches have largely been replaced by crystal watches, which are sufficiently stable for most daily uses. The key component of these watches is mass produced at very low cost. Recently, tuning fork based shear force detection, as implemented in a large number of near-field scanning optical microscopes (SNOM), has proven to be an easy and reliable method by which to control the distance between the probe and sample by Karrai and Grober [19]. In following, Giessibl *et al.* [20] has employed them for atomic resolution AFM imaging. Tuning forks have been used as sensors at low temperatures and in high magnetic fields by Rychen *et al.* [21]. It is said that at this moment the applications of tuning fork are rather widespread.

Recently, D. V. Serebryakov *et al.* [22] reported the new principle for scanning with a tuning fork, which was able to achieve the high quality factor Q, simultaneously decrease the response time τ. They have used this transducer for SNOM with the fibre mounted in cover of tuning fork to increase the factor Q. However, the preparation of this system is not simple task and the factor Q of this system is not high. Based this transducer, the authors have developed

* Permanent address: Physics department, Hue University, 77 Nguyen Hue, Hue, Viet Nam.

an atomic force microscopy based on tuning fork (Fork-AFM) on two operation modes: tapping and shear force mode. In these studies, we have glued directly the tungsten tips and silicon cantilever tips to the prong of tuning fork [23]. The process of gluing these tips is not complicated. The quality factor of system is rather high, and in general it belongs to the gluing process. Especially, for the silicon tips, the radius of tips is rather small (~10nm), therefore it maybe achieved the high resolution of images of the samples. However the expansion of the applications of the using the Fork-AFM in investigation the properties of different materials, especially for the bio-material, is still limited.

The aim of our work was investigated of human blood cells morphology, namely erythrocytes, using a system of a combination between the above transducer and atomic force microscopy (AFM) NT-206 (Microtestmachines Co.,Belarus) [24] based tuning fork with the silicon cantilever tips on two operation regimes: i) intermittent contact mode and ii) shear-force mode. We discuss the advantages and disadvantages of these modes of operation and give wide possibility of analyzing the properties of surface of biological objects with high resolution.

Experimetal setup

We have used commercially available quartz tuning fork (type 74-530-04 of ELFA Company), having a resonance frequency 32757 Hz and quality factor Q of 14000 in air after uncover the packaging lid, as a force sensor (Fig. 1). The theoretical spring constant is obtained from the formula $k = \frac{E}{4} w \left(\frac{t}{l}\right)^3$ [25], where $E = 7,87.10^{10}\ N/m^2$ is the Young modulus of quartz. The length (L), thickness (T) and width (W) of the tuning fork used are 6.01, 0.35 and 0.61 mm, respectively. Using these parameters, we obtain k ≈ 7 kN/m, which agrees reasonably well with our experimental result. The tips used in our experiment were commercial Contact silicon cantilever CSC21/15 chips produced by MicroMasch that have six straight cantilevers of different lengths. The cantilever had silicon tips of different length and nominal radius 10 nm. The manufacturing process of these tips ensures that their properties are reproducible from chip to chip.

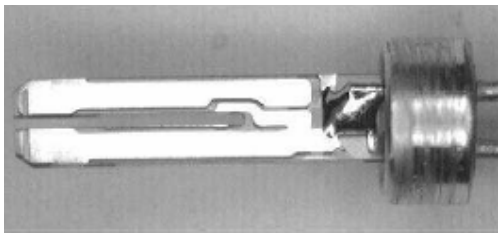
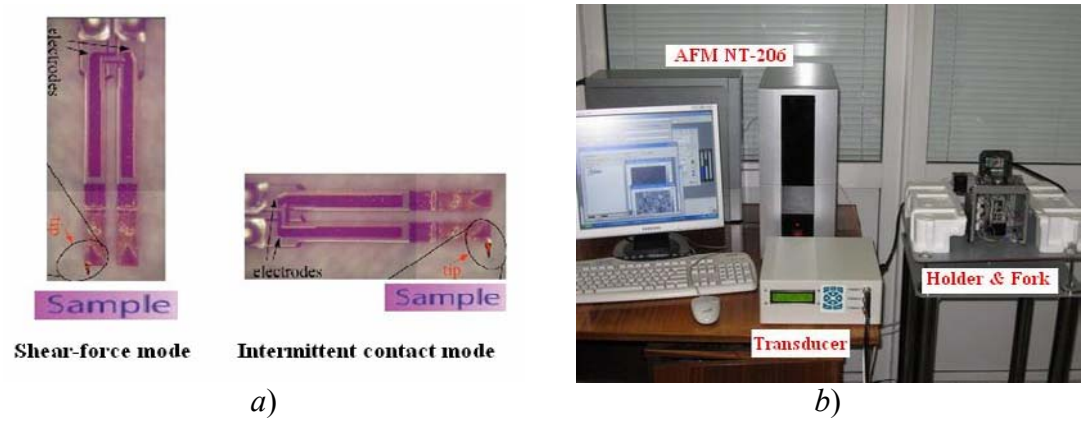


Fig. 1. Photograph of the commercially available quart tuning fork

To attach the tip to the quartz tuning fork, we glue a cantilever from chip to the end of the tuning fork with epoxy two systems glue. The cantilever tip was placed on the tuning fork using two optical microscopes equipped with a micropositioning stage. After the glue became dry, it was easy to break the tip from the rest of the cantilever chip by gently moving the chip up and down with respect to the tuning fork.

Figures 2 *a* illustrate two mode operations with tuning fork. For operation, a tip was glued to the end of the one prong of the tuning fork and that prong is oscillated parallel to the sam-

ple surface: shear force operation. Another option is to oscillate the prong perpendicular to the surface, as tapping mode operation. Moreover, hardware realization of this scheme was performed using mechanical and control electronics systems of atomic force microscope NT-206 (Microtestmachines Co.) [24]. For receiving the signal and controlling the oscillation from tuning fork, we have used the transducer instrument to connect the Fork-AFM (Fig. 2 *b*).



a) *b*)

Fig. 2. Principle of two operation modes: shear-force and intermittent contact mode (*a*). Photography of system AFM NT-206 using a quartz tuning fork (Fork-AFM). The signals from the tuning fork are connected to AFM by the transducer instrument (*b*)

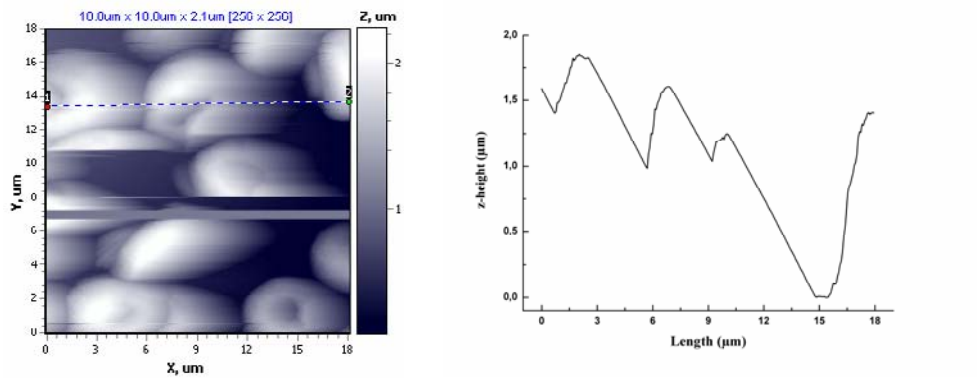
Erythrocutes samples were prepared by different ways. The first method is standard for clinical laboratory. The drop of fresh human blood (10-20 µl) was applied on the glass surface and smeared by the second glass. The thickness of the smear decreases along a direction of smearing. In the second method, some drops of fresh human capillary blood were fixed in 2 % aqueous glutaraldehyde solution. The cover slips were rinsed with a 2 % aqueous glutaraldehyde solution and washed with phosphate buffered saline (PBS). The freshly extracted blood was then diluted in PBS and this solution was then added again for 1 minute to rigidify the cell. Then the cells were washed with PBS. A preparation was dried up on air at room temperature during several hours.

Results & Discusions

Figures 3 and 4 show topographical images and line profile obtained in the shear force and intermittent contact mode, respectively, of erythrocytes samples in the air at room temperature. The scanning speed of two mode operations is 0.3 Hz per line, the current through the tuning fork is 3nA (the prong vibration amplitude is around 3.8 nm) for shear force mode and 2nA for intermittent contact mode. The entire image was obtained in about 15 min for resolution 256x256. The set point of the feedback circuit was set at 90 % maximum amplitude on resonance.

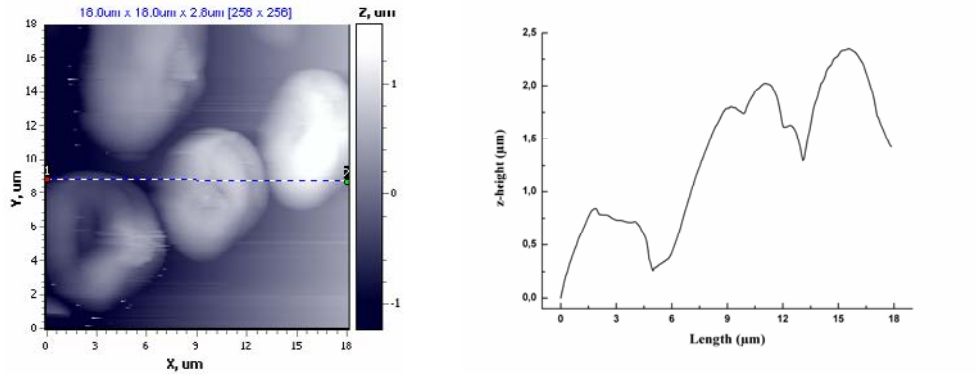
The topography of the erythrocytes sample is obtained using shear force detection in air in Fig. 3 *a*. Figure 3 *b* shows the averaged line profile. From this line profile, the maximum height of the feature indicated by an arrow in the image is about 1.8 µm. Here, we observed the abrupt change in the shear-mode image. To explaining for the abrupt change, we bring out some assumption for explanation in the following way: in this mode, because the tip oscillates

parallel to the surface of sample, the area contact between tip and sample is about 30-40 nm. Furthermore, the differential height of sample is rather large. Therefore, in this process, the instability such as signal drift or tip contamination maybe appear and influence the results scanning.



a) *b*)

Fig. 3. Shear force mode topographical images of erythrocytes. The dimension of images is noted in the figure (*a*). A line profile from 1 to 2 in (*a*) is show in figure (*b*)



a) *b*)

Fig. 4. Intermittent contact mode topographical images of erythrocytes. The dimension of images is noted in the figure (*a*). A line profile from 1 to 2 in (*a*) is show in figure (*b*)

On the contrary, figure 4 *a* shows the topography of erythrocytes in intermittent contact mode. The image in Fig. 4 *a* can be seen as more obviously than the result in Fig. 3 *a*. From this line profile (Fig. 4 *b*), the maximum height in the image is about 2.3 µm. Clearly, in this mode, the area contact between tip and sample is much smaller than shear force mode (about 10 nm). As a result, the region contact between tip and sample may achieve the atom interaction, thus it prevents sample damage, and we could obtain the images with high contrast resolution.

Conclusions

We have demonstrated a Fork-AFM with silicon cantilever tips operated in two modes operation in ambient conditions for investigating the erythrocytes sample. We have obtained images with a spatial resolution of not less than 1.5 μm in height after careful calibration. By comparing experimental results obtained in intermittent contact and shear mode we observed that intermittent contact mode operation gave a much optimum signal control. In addition to these results, one can also think of the combination of the tuning fork with the different type of tips that allows to inexpensively implement a variety of scanning probe micros copies for investigation the properties of biological materials.

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