Technique Imaging Single Isolated Cancer Cells By Advanced Automatic Atomic Force Microscopy Using Matlab

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Abstract - A research on the paper was optimal curing condition for image single automatic force microscopy technique-3.This report analyzes the worldwide market in us by the product of (optical microscope, charged Particle microscope, scanning probe microscope) and by the application (Life science, NANO technology).The before experimental results with the solidified with 4% paraformaldehyde for 10mins were obtained structure future report will be based on the results that outcomes from the research.

I. INTRODUCTION

Particular cancer is one of the most common malignant tumors today in the world. The amount of deaths per year more than 1.7 million are expected to be diagnosed in 2019. Many diagnosis methods have been develop one of the most common malignant tumors today in the world. The amount of deaths per year is nearly more than 1.7 million. More than 1.7 million new cancer cases d in recent years. The method of sections needs to be performed under high resolution microscopes. The Tosca series notably pools premium technology withtime depentent , making this AFM a perfect nanotechnology analysis tool for scientists and industrial user alike. Choose between two different models. To scale down 400, large quantity , finest AFM scale down 200 ,an AFM for medium- sized samples and limitations. Both provide the same level of matinee, flexibility and quality. AFMs have been widely used in the mileage and evocation of biological macromolecules, cells and DNAs , which are implemented by simple sample preparation and can be used to study living cells directly .

With the utilization of AFM, images of cancer cells can be obtained. The method like the elastic modulus measurement could be used for the diagnosis of cancer and the screening of anti-cancer drugs . The AFAM was invented on the basis of AFM and it was exployed to study thin film materials, moduli of elasticity and subsurface structures . It could also be used to detect defects such as internal cavity, particles and microcracks. Tetard et al. imaged phagocytes of particulars after inhalation of SiO2 nanoparticles in mice. In acoustic images, SiO2 particles in cells could be clearly observe.The cytoskeleton plays an important role in the functions of support for the shapes of cells, the transportation of substances inside cells and the transmission of information. After being fixed by hardener, cell structures can keep the statuses in physiology and cellular staining experiments can be conducted. After the treatment, the cells can be stored for long time. Thus, it is necessary to study on the curing conditions of different cells.

II. METHODS AND EXPERIMENT

A . EXSITING METHOD

1) CELL CULTURE

Particular cancer cells A549 (procured from the cell bank at the Chinese Academy of Sciences) were cultured in an environment with a 5% carbon dioxide concentration, and a temperature of 37?. The cells grew on the surface of silicone slice (10mm×10mm) and were cultured in the solution of 10% fetal bovine serum (HyClone) RPMI-1640media (HyClone). The cells were cultured for 24 hours till they adhered to the surface.

2) METHOD CURING

Paraformaldehyde and methanol are often used to solidify mono-layer cells at room temperature. In the work, methanol was used for cell curing for 15min, and 4% concentration of para formaldehyde was used for cell curing for 10min, 30min and 60min, and 2% and 3% paraformaldehyde was used for cell curing for 30min.

3) EXPRIMENT SYSTEM

In the experiment, acoustic and morphological images were obtained simultaneously, as shown in Fig. 1. The system frequency range was from 10kHz to 50kHz. The contact resonance frequency of cantilever beam and sample was 36.6kHz. The probe used in the experiment was the Tap300AL-G cantilever In the experiment, acoustic and morphological images were obtained simultaneously with a nominal stiffness of 40N/m and a resonant frequency of 300 kHz. Vaseline was used as the coupling agent

B) RESEARCH ON COMPONENTS

1) OPTICAL MICROSCOPES

The optical microscope, often referred to as the microscope, is a type of microscope that commonly uses visible light and a system of lenses to magnify images of small objects. Moreover its used extensively in microelectronics, nanophysics, biotechnology, pharmaceutic research, mineralogy and microbiology. In addition to this is ,the main advantages of fast and adaptable to all kinds of sample system ,in any shapes or geometries . However it is a low resolution usually down to only sub – micron or a few hundres of nanometers mainly due to the light diffraction limit .

2) CHARGED PARTICLE MICROSCOPE

The charged parcticle microscopy play a valuable role for applications that require high resolution image and small focused probe sizes –well beyond what can be achived with tradition light microscopes.Furthemore their advantages versus the photon arises from their having an appreciably smaller wavelength compared to lyphical photons.however the advantages is ,expensive piece of equipment.

3) SCANNING PROBE MICROSCOPE

Scanning probe microscope (SMPs) is used to create image of nanoscale surface and structures (or)manipulate atoms to move them in specific patterns. They are very powerfull and can have high resolution up to a nanometer. furthermore ,the specialzed probes improvements and modifications to scanning instruments continues to provide faster more efficient and revealing specimen image with minor effect and modification.In addition to this is unfortunately one of the downside of scanning prob microscope is that image are prouduced in black and white can in some circumstances exaggerate a specimens actual shape and size.

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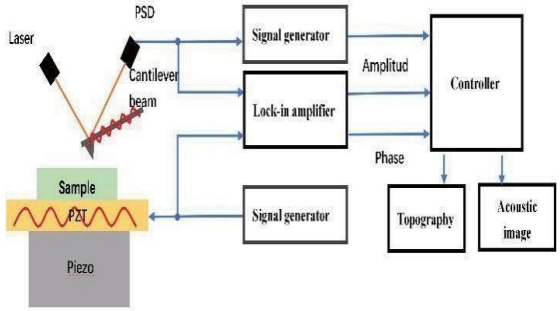

Fig. 1. AFAM system diagram

III. DISSCUSION

Fig. 2 shows the morphological image and acoustic image of single layer A549 cells cured by methanol for 15 min. It can be seen in both the morphological and acoustic images that the cell morphology is basically cured. In the acoustic image, the bulging small particles could lead to a large acoustic signal, displayed as a bright spot. Figs. 2(c) and 2(d) show the partially enlarged images of Figs. 2(a) and 2(b).

Fig. 3 shows the morphological and acoustic images of A549 cells cured with 4% paraformaldehyde for 10min. It can be seen that the cellular morphology is not completely fixed. The cell membrane collapses severely, and the cell height is reduced. Because of the collapse of the cell membrane, the cytoskeleton under the cell membrane is more clearly presented in the acoustic image. Figs (c) and 3(d) are the partially enlarged images of Figs. 3(a) and 3(b). The cell subsurface structures in the acoustic image are clearly shown.

Fig. 4 shows the morphological and acoustic images of A549 cells cured with 4% paraformaldehyde for 30min. The morphology of the cells is basically fixed and relatively complete. Compared with the cells cured for 10min, the cell surface is smoother, and the cell height is increased.

Fig. 5 shows the morphological and acoustic images of A549 cells cured with 4% paraformaldehyde for 60min. The morphology of the cells is basically fixed and relatively completed. The cytoskeletal filament can be clearly observed in the image

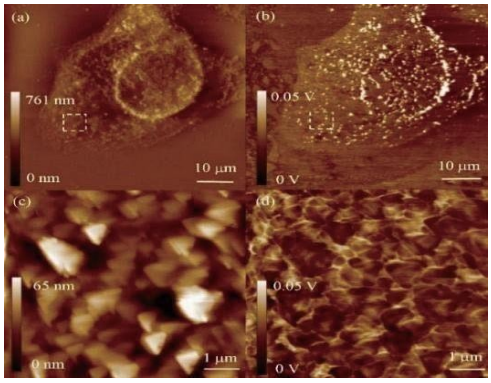

Fig. 2. Methanol cured particular cancer cells for 15 min; (a) morphological image, (b) acoustic image, (c) and (d) partially enlarged images of (a) and(b)

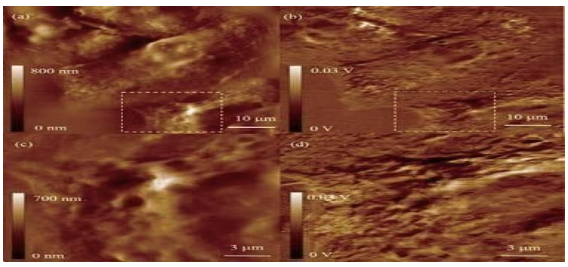

Fig. 3. Images of A549 cells cured with the 4% concentration of paraformaldehyde for 10 min; (a) morphological image, (b) acoustic image, (c) and(d) partially enlarged images of (a) and (b).

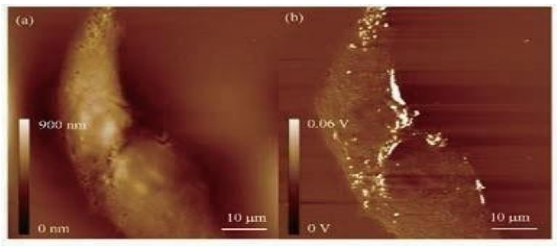

Fig. 4. Images of A549 cells cured with the 4% concentration of paraformaldehyde for 30 min, (a) morphological image, and (b) acoustic image

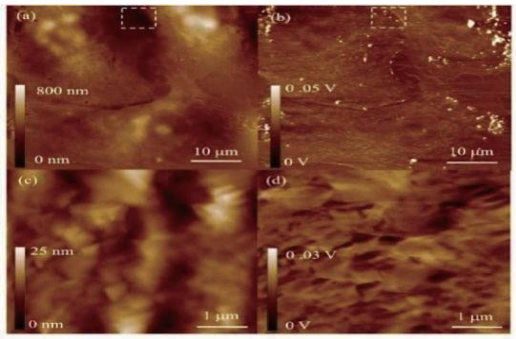

Fig 5. Images of A549 cells cured with 4% concentration of paraformaldehyde for 60 min ,(a)morphological image (b) acoustic image,(c) and partially enlarged images of (a) and (b).

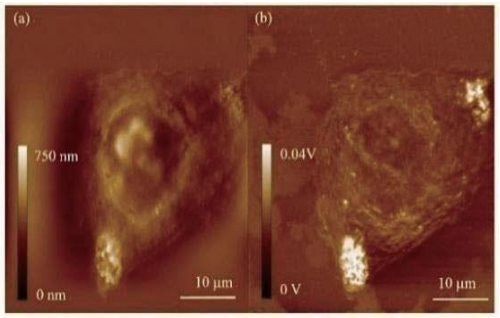

Fig. 6. Images of A549 cells cured with the 3% concentration of paraformaldehyde for 60 min.

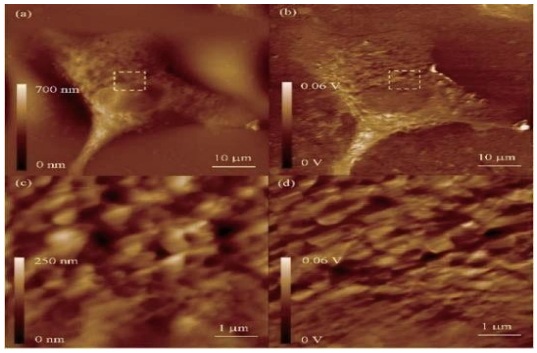

Fig 7.Images of A549 cells cured with 2% concentration of paraformaldehyde for 60 min, (a)morphological image,

(b)acoustic image (c)and (d)partially enlarged images of (a) and (b).

Fig. 6 shows the morphological and acoustic images of A549 cells cured with 3% paraformaldehyde for 60 min. The cell surface is rougher than those solidified with 4% paraformaldehyde. Fig. 7 shows the morphological and acoustic images of A549 cells cured with 2% paraformaldehyde for 60 min. Due to the curing time is too long and the concentration of curing agent is too low, the cells are in a dehydration state. Fig. 8 shows the changes in the height of paraformaldehyde-cured cells at different time periods. Fig. 9 shows the changes in the surface roughness of paraformaldehyde-cured cells at different time periods. Fig. 10 shows the changes in the height of paraformaldehyde-cured cells at different concentrations. Fig. 11 shows the changes in the surface roughness of paraformaldehyde-cured cells at different concentrations.

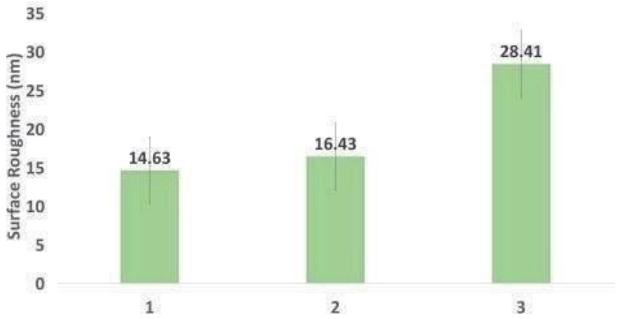

Fig. 8. Changes in the height of paraformaldehyde- cured cells at different time periods. Group 1 was cured for 10 min; Group 2 was cured for 30 min; Group 3 was cured for 60 min. 50 cells were measured in each group.

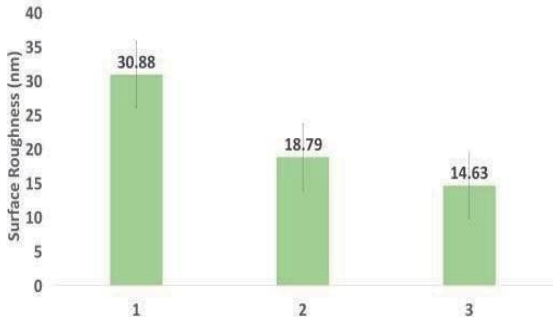

Fig. 9. Changes in the surface roughness of paraformaldehyde-cured cells at different time periods. Group 1 was cured for 10 min; Group 2 was cured for 30 min; Group 3 was cured for 60 min. 50 cells were measured in each group

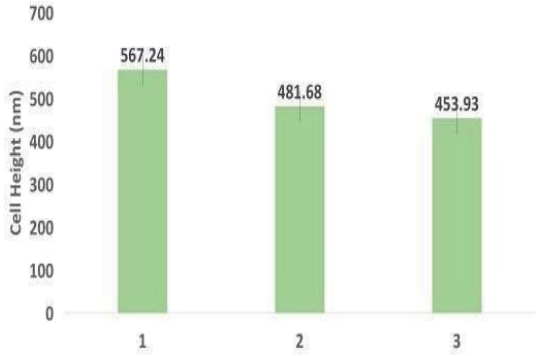

Fig. 10. Changes in the height of paraformaldehyde-cured cells at different concentrations. Group 1 was cured for 4%; Group 2 was cured for 3%; Group 3 was cured for 2%. 50 cells were measured in each group.

. Fig. 11. Changes in the surface roughness of paraformaldehyde-cured cells at different concentrations. Group 1 was cured for 4%. Group 2 was cured for 3%; Group 3 was cured for 2%. 50 cells were measured in each group.

Fig. 12 shows the morphological and acoustic images of A549 cells. In comparison with A549 cells, liver cancer cells SMMC-7721 were also used in the experiments. Fig.13 shows the morphological and acoustic images of SMMC-7721 cells that cured under the same conditions. There is no significant difference between the two types of cells in the morphological features. It can be clearly seen that the structures of skeleton of the two types of cells are obviously different in the acoustic images. The skeleton of A549 cells was arranged more loosely than 7721 cells. In Fig. 9, it can be seen that in the two cell-connected parts, microtubules are distributed in a network.

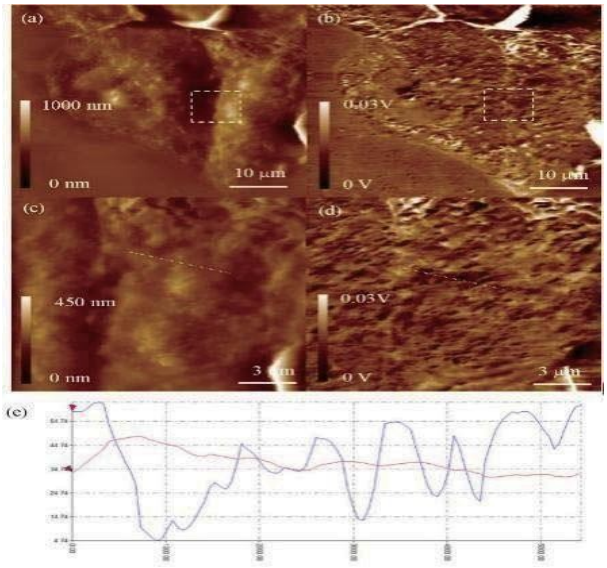
(a)
1000 nm
0 nm
10 µm
(b)
0.03V
0 V
10 µm
(c)
450 nm
0 nm
3 µm
(d)
0.03V
0 V
3 µm
(e)

Fig 12. paraformaldehyde for 10 min; (a) morphological image, (b) acoustic image, (c) and (d) partially enlarged images of (a) and (b). The sectional curvesrepresent the heights of the dashed lines in the morphological and acoustic images; The blue line represents the acoustic and the red line represents the morphological image.

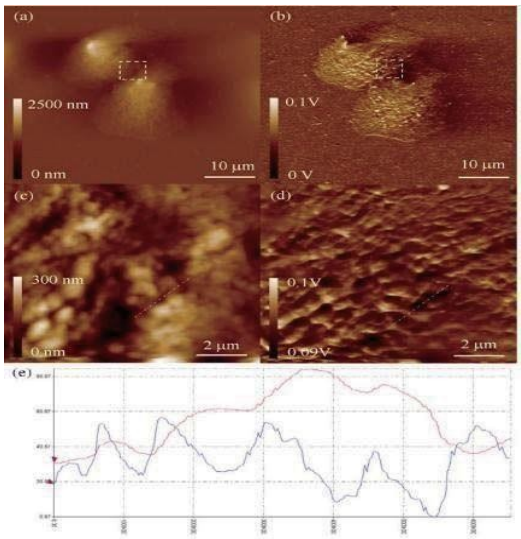

Fig. 13. Images of SMMC-7721 cells cured with the 4% concentration of paraformaldehyde for 10 min, (a) morphological image, (b) acoustic image, (c) and (d) partially enlarged images of (a) and (b). The sectional curves (e) represent the heights of the dashed lines in morphological and acoustic images; The blue line represents the acoustic and the red line represents the morphological image.

III. END USE APPLICATION OF AFM TECHNIQUIES III

A) LIFE SCIENCE

Life science has a candid ally in uplifting the quirk of life for all of mankind. advances in this domain have innumerable applications in the healthy pharmaceutical and agricultural industries. Research using park AFM in life science can acquire the NANO scale morphology of biological samples accurately and easily moreover this techniques allow for the characterization of biological material along such physical properties as stiffness adhesion and even it young's modules with sub – Nn level precision. Furthermore park AFM has developed an innovative in-liquid imaging technology ,scanning on conductance microscopy (SICM) .This techniques has enabled research to study complicated physiological phonomeno in liquied directly. The life science is used to technical methods on cell biology , micro–molecular , electrophysiology .

B) NANO TECHNOLOGY

NANO technology is the one of the latest research fields that rely on manipulation resulting from nanostructure breakthough have brough numerous technological advances in both research and industry. Moreover these days using NANO structure Fabirication methods in various way has become standard research in improving product performances irrespective of industry.the research have been utilizing AFM to find practical solution since the development of the first commercial AFM from park system. Additionally park system prime afm know how and a self –developed and notably strict rebuttal control system in their emptors to both image and manipulate nanostructure with increased accuracy with higher productivity.

IV. RESULT

We are taking brain cancer cell shows in the Fig.14.

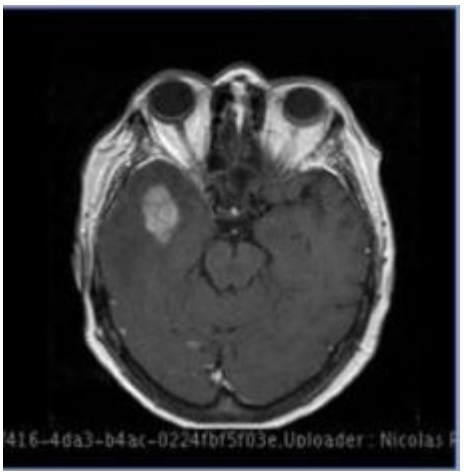

Fig 14. Brain cancer cell

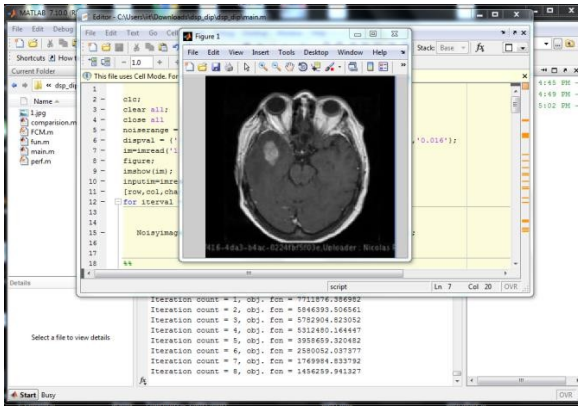

Fig.14.a. Represents the matlab process

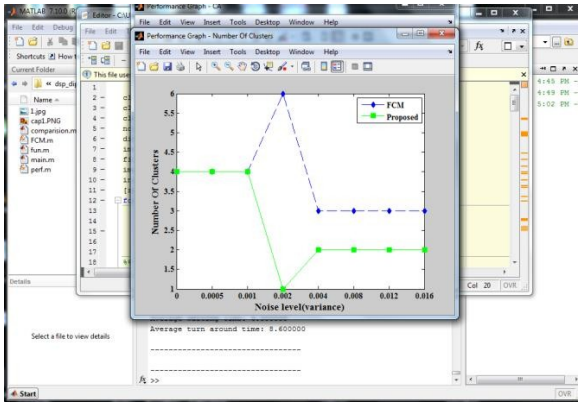

Fig.14.b.Graphs represents the number of clusters Vs number of noise level

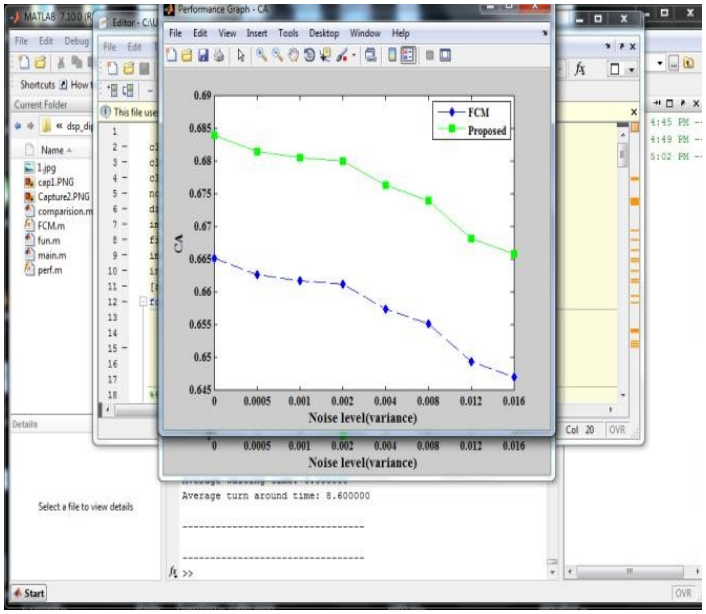

Fig.14.c.Represents the graph of CV Vs the noise level.

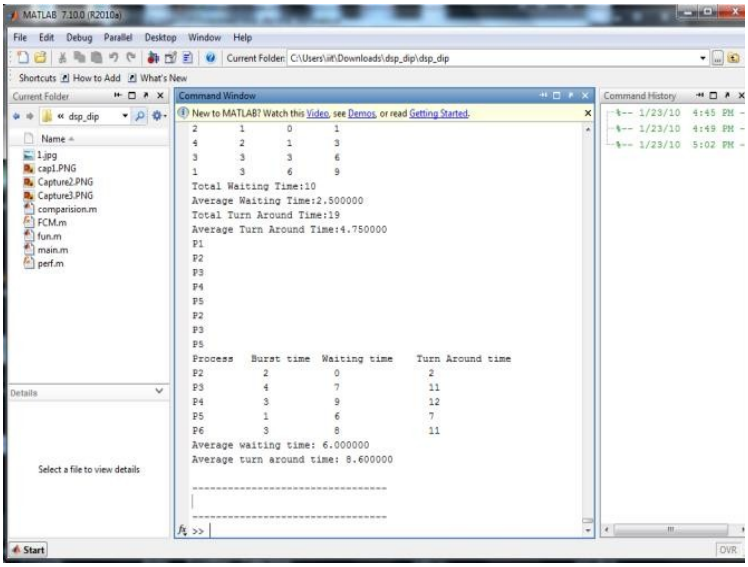

Fig.14.d. Represents the time taking at average

V. CONCLUSION

For the imaging of solified cells,the acoustic images will be concave and convex structure of the surface of the cells, compared with the morphological images. The cells treated with different concentrations of paraformaldehyde and method and durations will observe using an advanced automatic force microscopy technique-III. This experimental results will be showed that ,if a549 cells are solified with 4% paraformaldehyde for the period of may be reduced to

below 30minutes in the living cells .The cells that will be solifie with the same percent 10 minutes for subsurface structures.

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