

# Observations of liver cancer cells in scanning probe acoustic microscope: A preliminary study

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## Abstract

Scanning probe acoustic microscope (SPAM) can be used to acquire the morphology image as well as the non-destructive internal structures acoustic image. However, the observations of the morphology image as well as the internal structures acoustic image of liver cancer cells in SPAM are few. In this paper, we cultured 4 different types of liver cancer cells on the silicon wafer and coverslip to observe their morphology images as well as acoustic images in SPAM, and made a preliminary study of the 8 types of cells specimens (hereinafter referred to as the silicon specimens and coverslips specimens). The experimental measurement results showed that some cellular pseudopodium were observed in the morphology images of the coverslip specimens while no such cellular pseudopodium were appeared in the morphology images of the silicon specimens, which concluded that the living liver cancer cells were less likely to grow on the silicon wafer. SPAM provides a rapid and sensitive visual method for studying the morphology and internal structures of the cancer cells. The proposed method can be also used to obtain the morphology and internal information in both solid and soft material wafers, such as silicon and cells, with the resolution of nanometer scale.

**Key word:** Liver cancer cells; Scanning probe acoustic microscope; Acoustic image;

## I. INTRODUCTION

The acquiring the non-destructive internal structure acoustic images as well as morphology images of the materials, components, or biological cells in nanoscale level is critical for the numerous applications in material science, electronics and clinical. Scanning probe acoustic microscope (SPAM) can be used to acquire the morphology image as well as the non-destructive cellular structures acoustic image of living cells. However, the observations of the morphology image as well as the internal structures acoustic image of liver cancer cells in SPAM are few. SPAM provides a rapid and sensitive visual method for studying the morphology and internal structures of the cancer cells. It is

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possible to identify the morphology and internal information of different types of liver cancer cells by using SPAM.

By the second half of the 19th century, Magnus Retzius, Santiago Ramon y Cajal and Camillo Golgi were completing anatomical description of the cell by using the microscope<sup>1</sup>. Meanwhile, in the 1870s, Ernst Abbe's classical diffraction theory set the theoretical resolution limits for the optical microscope<sup>2</sup>, making it difficult to detect the features in nanoscale level by using optical microscope and making it inadequate for studying cellular structure. The breakthrough occurred in the early 1930s, when the transmission electron microscope(TEM)<sup>3</sup>, built by Ernst Ruska, extended the resolution to the nanometer scale, making it possible for studying cellular structure. As we all known, TEM has a nanometer resolution but usually have to be worked under the vacuum condition. Scanning tunneling microscopy (STM) is nondestructive<sup>4</sup> but can only be used to detect the conductive materials. The conventional approach for detecting buried defects non-destructively is scanning probe microscopy (SPM)<sup>5-7</sup>, but it is sensitive only to the surface or subsurface features. Similarly, high-resolution optical microscopy<sup>8-9</sup> is also unable to image the optically opaque or deeply buried structures.

Scanning probe acoustic microscopy (SPAM) is developed on the basis of the atomic force microscope(AFM), and combines the noninvasive penetration nature of ultrasound waves<sup>10</sup> as well as the high-spatial resolution of near-field imaging approach in SPM<sup>11</sup>. This technique offers a nanometer spatial resolution of the topography images as well as the buried defects, and is amenable both for the hard materials such as silicon wafer, soft materials like cells<sup>12</sup>, or hybrid structures. Therefore, high resolution imaging of cellular ultrastructure as well as the morphology of different types of liver cancer cells in SPAM is of great significance.

In this paper, we cultured 4 different types of living liver cancer cells on the silicon wafer and coverslip to observe their morphology images as well as acoustic images in SPAM and made a preliminary study of the differences of the 4 types of cells with SPAM. This research has a great significance in the visual morphology studies of different types of liver cancer cells, as well as in the studies of the cellular structures of the liver cancer cells. The proposed method can be also used to obtain both the morphology and internal information in solid or soft materials with the resolution of nanometer scale.

## II. Methodology

### 2.1. Reagents and materials

HepG2 stem cells with cPLA2 $\alpha$  expressed over, HepG2 stem cells with cPLA2 $\alpha$  expressed down, HepG2 ordinary adherent cells with cPLA2 $\alpha$  expressed over and HepG2 ordinary adherent cells with cPLA2 $\alpha$  expressed down were all provided by Tianjin Medical University Cancer Institute and Hospital. Dulbecco's modified Eagle medium(DMEM) and fetal bovine serum (FBS) were from Tianjin Medical University. The silicon wafer were purchased from Hefei Kejing Materials and Technology Co., Ltd., China. All solvents and other chemical materials were purchased from local commercial suppliers.

### 2.2. Cell culture

HepG2 stem cells with cPLA2 $\alpha$  expressed over, HepG2 stem cells with cPLA2 $\alpha$  expressed down, HepG2 ordinary adherent cells with cPLA2 $\alpha$  expressed over and HepG2 ordinary adherent cells with cPLA2 $\alpha$  expressed down were cultured with DMEM supplemented with 10% fetal bovine serum(FBS), in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. Trypsinization was stopped by the addition of fresh supplemented DMEM. The numbers of cells were counted with a hemacytometer.

### 2.3. Preparation of SPAM imaging specimens

Cells at a density of  $1 \times 10^5$  cells/mL were inoculated on the silicon and coverslip in 24 well culture plates (1 mL/well) and incubated in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. For SPAM imaging, HepG2 ordinary adherent cells specimens were taken out after 4 hours while HepG2 stem cells were taken out after 18 hours and washed 3 times with phosphate-buffered saline (PBS), fixed with 4% glutaraldehyde solution in PBS for 10 min at room temperature, and then again washed 3 times with PBS. All specimens were rapid dying before used.

### 2.4. SPAM imaging

The cell SPAM imaging was conducted in the atmospheric environment using contact mode. A 26 $\mu$ m AFM scanner (CSPM5500, Guangzhou origin nano Co., Ltd, Guangzhou, China) and Silicon tip (Guangzhou origin nano Co., Ltd, Guangzhou, China) were employed for cell scanning in this study. The tip cantilever length was  $450 \pm 10$   $\mu$ m, width was  $50 \pm 5$   $\mu$ m, thickness was  $2 \pm 1$   $\mu$ m, set back was  $15 \pm 5$   $\mu$ m, resonance frequency was  $13 \pm 4$  kHz and force constant was 0.07–0.4 N/m, and the tip radius of the probe was less than 10 nm. The sampling points were set at 512 and the scanning speed was 0.5–2 kHz, where the scanning speed was determined by the scanning area. At least two independent imaging scans for each type of cell were carried out to obtain a reliable observation. It is necessary to clean the cantilever holder with ethanol before the experiments, which helps to limit the contamination during the AFM measurement<sup>13</sup>.

## III. RESULTS AND DISCUSSIONS

The analysis on the collected images are implemented on the SPAM (CSPM5500). Figure 1 showed the morphology images of the 4 types of liver cancer cells in optical microscope in order to compare with the morphology images collected from SPAM. Figure 1(a) was the morphology image of HepG2 stem cell with cPLA2 $\alpha$  expressed over, and Figure 1(b) was the morphology image of HepG2 stem cell with cPLA2 $\alpha$  expressed down, and Figure 1(c) was the morphology image of HepG2 ordinary adherent cell with cPLA2 $\alpha$  expressed over, and Figure 1(d) was the morphology image of HepG2 ordinary adherent cell with cPLA2 $\alpha$  expressed down and all images were at a 400 $\times$  magnification.

Figure 2 showed the morphology images, acoustic images and section lines of single HepG2 ordinary adherent cell cultured on the silicon wafer. Figure 2(a) and 2(b) showed the morphology of single HepG2 ordinary adherent cell cultured on the silicon wafer in SPAM while Figures 2(c) and 2(d) showed the acoustic images. Figures 2(e) and 2(f) showed that the long axis of single living liver cancer cell is about 5.28 $\mu$ m and the short one is about 2.85 $\mu$ m. Figure 2(g) showed the three-dimensional image of the cell. And Figure 3 was the morphology images and acoustic images of the HepG2 stem cells cultured on the silicon wafer. Figure 3(a) and 3(b) were morphology images while Figure 3(c) and 3(d) were acoustic images. Figure 4 was the morphology images and acoustic images of the HepG2 stem cells cultured on the silicon wafer after the specimen was moved to the right.

Figure 5 showed the morphology images and acoustic images of the HepG2 ordinary adherent cell cultured on the coverslips. Figures 5(a) and 4(b) were morphology images while Figures 5(c) and 5(d) were acoustic images. There were some cellular pseudopodium in the morphology images of the coverslip specimens and there was the drift phenomenon in the scanning process. Figure 6 showed the morphology images and acoustic images of the HepG2 ordinary adherent cell cultured on the silicon. Figures 6(a) and 6(b) were morphology images while Figures 6(c) and 6(d) were acoustic images and showed that the cell was a little shrinkage. And the scan area was 14 $\mu$ m $\times$ 14 $\mu$ m.

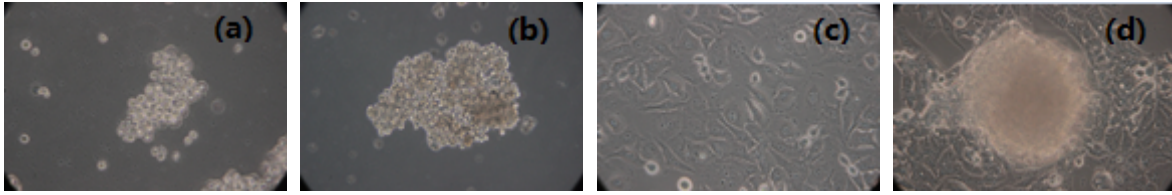


Fig.1. Optical microscope images of the 4 types of liver cancer cells. Figure (a) was the morphology image of HepG2 stem cell with cPLA2  $\alpha$  expressed over, Figure (b) was the morphology image of HepG2 stem cell with cPLA2  $\alpha$  expressed down, Figure (c) was the morphology image of HepG2 ordinary adherent cell with cPLA2  $\alpha$  expressed over, Figure (d) was the morphology image of HepG2 ordinary adherent cell with cPLA2  $\alpha$  expressed down. All images were at a 400 $\times$  magnification.

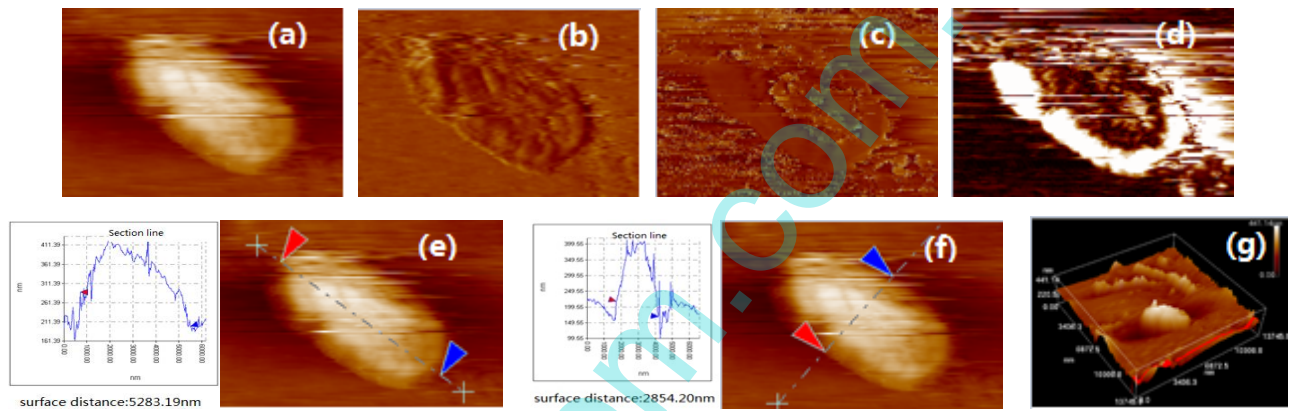


Fig.2. The morphology images, acoustic images and section lines of single HepG2 ordinary adherent cell cultured on the silicon wafer. (a) and (b) were morphology images while (c) and (d) were acoustic images. (e) and (f) were long axis section line and short axis section line, respectively. (g) was the three-dimensional image of the cell. The scan area was 14 $\mu\text{m}\times 14\mu\text{m}$ .

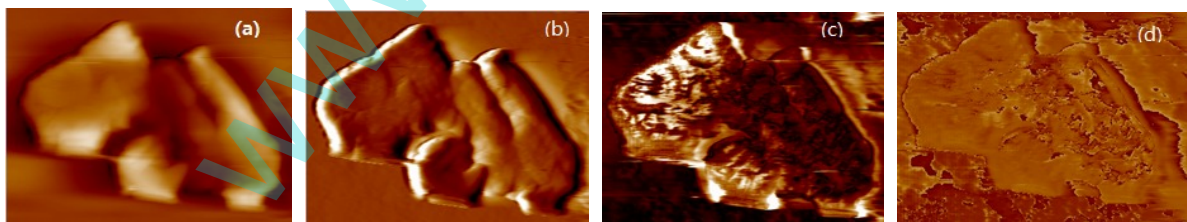


Fig.3. The morphology images and acoustic images of the HepG2 stem cells cultured on the silicon wafer. (a) and (b) were morphology images while (c) and (d) were acoustic images. The scan area was 14 $\mu\text{m}\times 14\mu\text{m}$ .

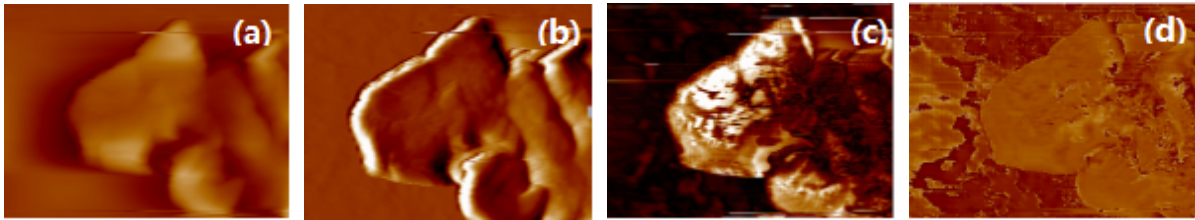


Fig.4. The morphology images and acoustic images of the HepG2 stem cells cultured on the silicon wafer after the specimen was moved to the right. (a) and (b) were morphology images while (c) and (d) were acoustic images. The scan area was  $14\mu\text{m} \times 14\mu\text{m}$ .

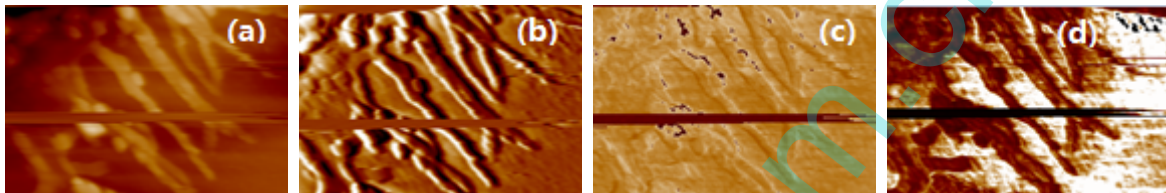


Fig.5. The morphology images and acoustic images of the HepG2 ordinary adherent cell cultured on the coverslips. (a) and (b) were morphology images while (c) and (d) were acoustic images. The scan area was  $14\mu\text{m} \times 14\mu\text{m}$ .

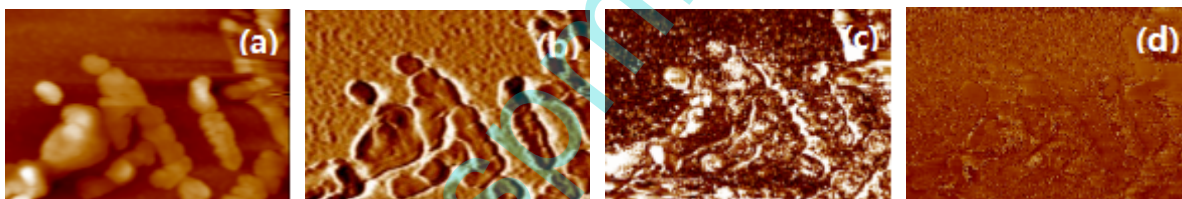


Fig.6. The morphology images and acoustic images of the HepG2 ordinary adherent cell cultured on the silicon. (a) and (b) were morphology images while (c) and (d) were acoustic images. The scan area was  $14\mu\text{m} \times 14\mu\text{m}$ .

#### IV. CONCLUSION

There were some cellular pseudopodium in the morphology images of the coverslip specimens while no such cellular pseudopodium were observed in the morphology images of the silicon specimens, and the morphology images of different types of cells collected by SPAM were different. So the preliminary conclusion was obtained that the living liver cancer cells were less likely to grow on the silicon wafer. Furthermore, the attained results indicated that the long axis of single living liver cancer cell is about  $5.28\mu\text{m}$  while the short one is about  $2.85\mu\text{m}$ . It is illustrated that, in the corresponding positions of the morphology image, there were the same details in acoustic image and the same phenomenon can be observed with the movement of samples. Therefore, we got the preliminary conclusion that SPAM provides a rapid and sensitive visual method for studying the morphology and the internal structures of the cancer cells. The proposed method may be used for verifying the internal information of liver cancer cells if the resolution of the acoustic images is high enough.

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## REFERENCES

- [1] Bustamante C, Keller D. Scanning force microscopy in biology[J]. *Physics Today*, 1995, 48(12):32-38.
- [2] Shekhawat G S, Avasthy S, Srivastava A K, et al. Probing buried defects in extreme ultraviolet multilayer blanks using ultrasound holography[J]. *Nanotechnology, IEEE Transactions on*, 2010, 9(6): 671-674.
- [3] Kunze U. Invited Review Nanoscale devices fabricated by dynamic ploughing with an atomic force microscope[J]. *Superlattices and microstructures*, 2002, 31(1): 3-17.
- [4] Chen C H, Deen M J. A general procedure for high-frequency noise parameter de-embedding of MOSFETs by taking the capacitive effects of metal interconnections into account[C]//*Microelectronic Test Structures*, 2001. ICMTS 2001. Proceedings of the 2001 International Conference on. IEEE, 2001: 109-114..
- [5] Somekh M G, Bertoni H L, Briggs G A D, et al. A two-dimensional imaging theory of surface discontinuities with the scanning acoustic microscope[J]. *Proceedings of the Royal Society of London. A. Mathematical and Physical Sciences*, 1985, 401(1820): 29-51.
- [6] Binnig G, Gerber C, Stoll E, et al. Atomic resolution with atomic force microscope[J]. *EPL (Europhysics Letters)*, 1987, 3(12): 1281.
- [7] Twerdowski E, von Buttlar M, Razek N, et al. Combined surface-focused acoustic microscopy in transmission and scanning ultrasonic holography[J]. *Ultrasonics*, 2006, 44: e1301-e1305.
- [8] Dickson W, Takahashi S, Pollard R, et al. High-resolution optical imaging of magnetic-domain structures[J]. *Nanotechnology, IEEE Transactions on*, 2005, 4(2): 229-237.
- [9] Denyer M, Micheletto R, Nakajima K, et al. Biological imaging with a near-field optical setup[J]. *Journal of nanoscience and nanotechnology*, 2003, 3(6): 496-502.
- [10] Shekhawat G, Srivastava A, Avasthy S, et al. Ultrasound holography for noninvasive imaging of buried defects and interfaces for advanced interconnect architectures[J]. *Applied physics letters*, 2009, 95(26): 263101.
- [11] Shekhawat G S, Dravid V P. Nanoscale imaging of buried structures via scanning near-field ultrasound holography[J]. *Science*, 2005, 310(5745): 89-92.
- [12] Shekhawat G S, Chand A, Sharma S, et al. High resolution atomic force microscopy imaging of molecular self assembly in liquids using thermal drift corrected cantilevers[J]. *Applied Physics Letters*, 2009, 95(23): 233114.
- [13] Thomas G, Burnham N A, Camesano T A, et al. Measuring the mechanical properties of living cells using atomic force microscopy[J]. *JoVE (Journal of Visualized Experiments)*, 2013 (76): e50497-e50497.