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Stiffness mapping prostate biopsy samples using a tactile sensor

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Abstract– Previous studies have demonstrated that the stiffness of cancerous cells reflects their pathological stage and progression rates, with increased cancerous cell stiffness associated with increased aggressiveness. Therefore, the elasticity of the cancerous cells has the potential to be used as an indicator of the cancer's aggressiveness. However, the sensitivity and resolution of current palpation and imaging techniques are not sufficient to detect small cancerous tissues. In previous studies, we developed a tactile-based device to map with high resolution the stiffness of a tissue section. The purpose of this study is to evaluate this device using different tissues (BPH, Cancer and PZ) collected from human prostates. The preliminary results show that the tactile device is sensitive enough to tell the differences of the stiffness of different tissues. The results also disclosed the factors (humidity, temperature and tissue degradation) which could dramatically affect the results of stiffness mapping. The tactile technology described in this paper has the potential to help disclose the underlying mechanical mechanisms that lead to increased stiffness in prostate tumors.

***Index Terms*—Stiffness mapping, tactile sensor, Cancer, Biopsy**

I. Introduction

The wide spread use of prostate specific antigen (PSA) testing and transrectal ultrasound (TRUS)-guided needle biopsy dramatically improved the detection rate of early prostate cancer [1, 2]. The TRUS-guided 12-core biopsy technique has become the gold standard method for obtaining histological samples [2]. Two essential tasks in prostate cancer biopsies are locating any cancerous cells that are present and identifying the aggressiveness of cancer. The purpose of this study is to develop tools to help pathologists identify the aggressiveness of cancerous cells found in prostate cancer biopsies.

There are two physiological factors that could potentially provide this extra information. The first is metabolic activity. The second is the mechanical stiffness, which reflects the pathological stage and progression rates. Increased tissue stiffness is associated with increased cancer aggressiveness [3-5]. This property has already been used extensively for prostate screening, as palpation (during a digital rectal exam) has been an effective way to detect a tumor in vivo. This increased stiffness (or reduced elasticity) extends down to individual cancer cells[6, 7] and/or the extracellular matrix (ECM) which clusters and binds the cells together to form tissues [8], and can be used as an indicator of the prostate cancer aggressiveness. Advanced imaging technologies, including in vivo ultrasound and MRI elastography, can differentiate cancerous tissues that have elastic properties that are distinct from the normal tissues. However, the resolution and sensitivity of those imaging techniques are not sufficient to detect small islands of cancerous cells [9-12].

In this study, we evaluated a previously designed tactile mapping device using different tissues (BPH, Cancer and PZ) collected from human prostates. The preliminary results show that the tactile device is sensitive enough to tell the differences of the stiffness of different tissues. The results also disclosed the factors (humidity, temperature and tissue degradation) which could dramatically affect the results of stiffness mapping. The tactile technology described in this paper has the potential to help disclose the underlying mechanical mechanisms that lead to increased stiffness in prostate tumors.

II. Methods

A. Tactile device

As shown in Fig. 1, The tactile sensor consisted of a glass probe (5 μm, 10 μm, 20 μm or 100 μm) and a PZT sensor. When the glass probe touched the bio-specimen, the changes of the resonance frequency detected by the PZT sensor were measured to characterize the elasticity of the bio-specimen. The tactile sensor and a conventional CCD camera were mounted on a 3D manipulator that moves 1 μm per step for point-by-point 2D stiffness mapping. A sample chamber for sample holding was equipped with a heater and a temperature sensor. A LabVIEW program was developed to control the 3D manipulator and the heater and to read stiffness and temperature.

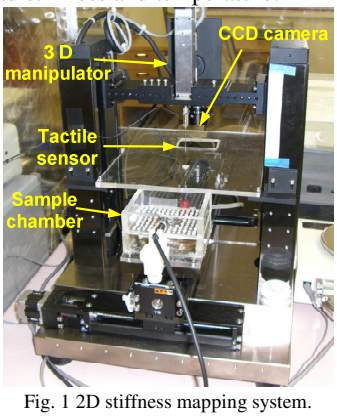

Fig. 1 2D stiffness mapping system.

B. Tissue sample preparation

In conventional biopsy procedure, the 18-gauge prostate needle specimens are harvested using standard TRUS guided 12-core biopsy. The specimens are cut into three 6mm segments and sliced longitudinally into thin 5~20μm slices (Fig. 2 a). Three slices are selected for formalin fixation and Hematoxylin and Eosin (H&E) stain.

Tissue slices acquired using conventional biopsy sectioning techniques are inevitably deformed and distorted. Our pilot experiments show that the deformation and distortion could make it very difficult to map the stiffness of the slices. In addition, it

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will be very difficult to register the stiffness images with the distorted H&E stained microscopic image accurately. We therefore adapted transverse slice technique as shown in Fig. 2 (b) to minimize the tissue deformation.

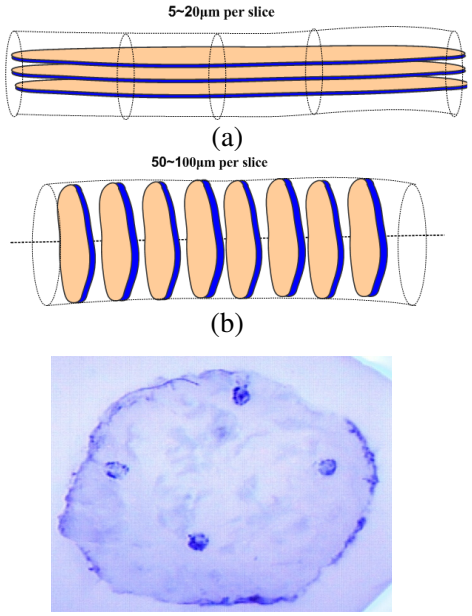


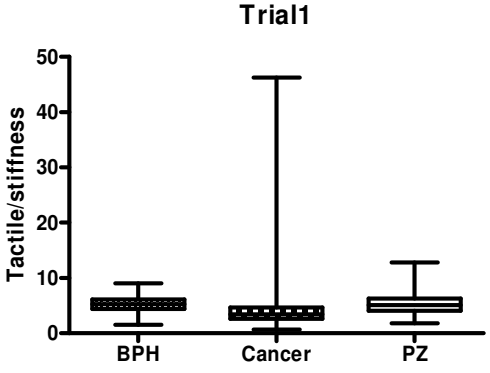
Fig. 2. (a) Conventional longitudinal sectioning. (c) Transverse sectioning will be used in this study. (d) A prostate sample with blue ink stained edge and four additional ink marks. Our pilot experiments showed that the blue ink remained after the formalin fixation and H&E stain.

In addition, we stained the edge of the transverse slices and put several ink marks in them (Fig. 2 c), in order to compare the stiffness images with the conventional optic microscopic images.

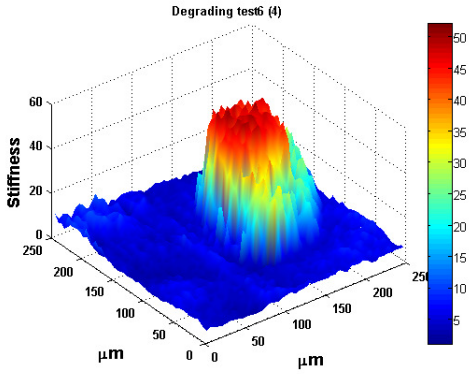
III. Results

A. *Stiffness of the different tissues (BPH, Cancer and PZ)*

Firstly, we did the coarse scanning of the samples with a lower resolution (20μm). The results show that the average stiffness of the BPH, cancer and PZ are very similar. However, the boxplot of the stiffness distribution of the cancer is very different from those of the BPH and PZ (Fig. 3), because of some hard nodules in the cancer sample. We then did some high-resolution scanning on the nodules (5μm/step and 10μm/step).



(a)



(b)

Fig. 3 (a) The box plot of the tactile/stiffness of the BPH, cancer and PZ. The step/resolution of the mapping is 20μm. (b) 3D views view of a typical nodule (resolution: 5μm and 10μm respectively). The diameter of the nodule is about 100μm.

B. *Effects of dehydration on the stiffness of the samples*

Then, an area in the cancer was scanned repeatedly to test effects of the dryness on the stiffness measurement. Figure 2 shows that the stiffness of the scanned area only has some small drifts in the first 74 minutes. The stiffness increased significantly after 74 minutes. One-way ANOVA with Tukey's multiple comparison test was performed to compare the differences between the stiffness of the last 7 trials (from 49 min to 102 min). The results show that there is no significant difference between the five trials from 49 min to 74 min. The stiffness increased significantly after 74 min ($P<0.001$). But the changes of the stiffness after 74 min are not very big (80min: ↑4.4; 102 min: ↑6.1).

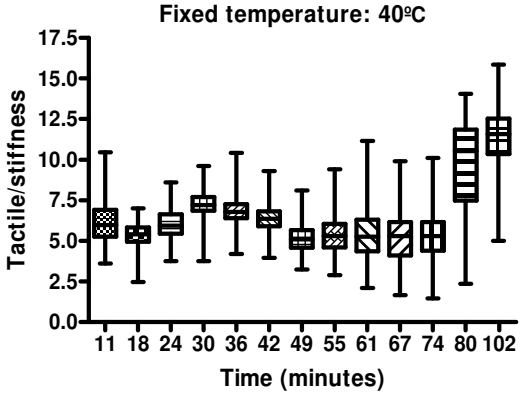
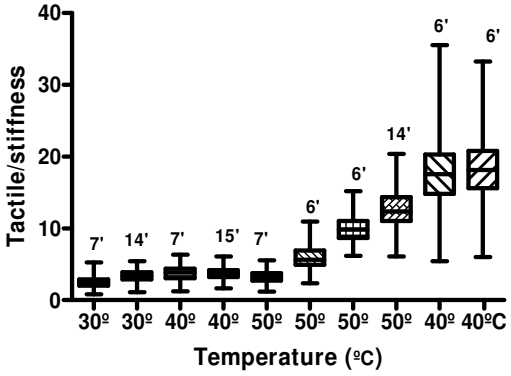


Figure 2 The effects of the dryness on the stiffness of the cancer. X-axis is the time. Y-axis is the boxplot of the stiffness in a small area (20×20 pixels, 20μm/pixel). The temperature was fixed to 40ºC during the test. The same area was matched exactly by the computer and measured repeatedly.

C. *Effects of temperature on the stiffness of the samples*

Then, we tested the changes of stiffness of the cancer under different temperatures. The results (Figure 4) show that the stiffness of the sample starts to increase when the temperature is increased to about 45ºC.



(a)

Figure 4 The effects of the temperature on the stiffness of the prostate cancer. X-axis is the temperature. Y-axis is the boxplot of the stiffness in a small area (20×20 pixels, 20μm/pixel). The same area was matched exactly by the computer and measured repeatedly. The time spent during each scanning/heating are marked on the top of each plot.

D. Effects of tissue degradation on the stiffness of the samples

Then, we tested the stiffness of BPH, cancer and PZ samples repeatedly with time intervals ranging from several hours to several days (Figure 3). We manually matched the same area for scanning each time. But the manual matching may not be very accurate.

Figure 3 shows that the stiffness of all the samples has a trend to increase with time. The decreases in the forth trial of BPH and the fifth trial of the cancer may caused by the inaccurate manual matching. Figure 3 also shows that the stiffness of the cancer has a bigger deviation than those of the BPH and PZ in the first four trials (cancer: 2.854, 1.522, 1.963, 3.015; BPH: 1.319, 1.563, 1.684, 1.551; PZ: 1.619, 1.759, 2.586, 3.165).

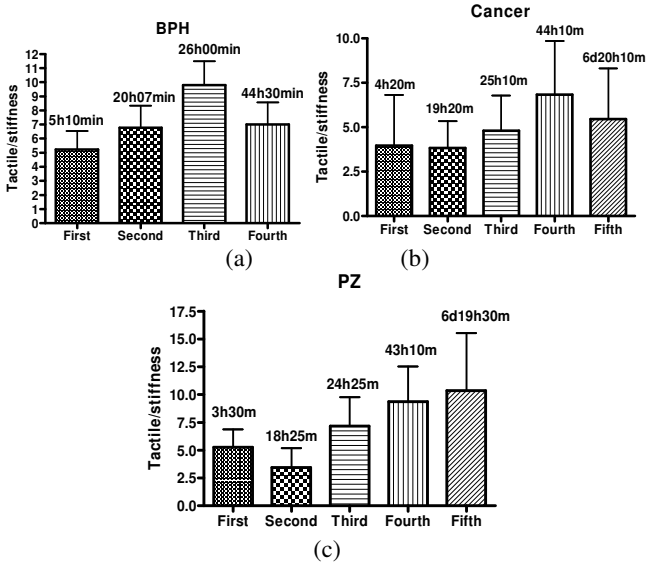


Figure 3 The effects of the degradation of the stiffness of (a) BPH, (b) cancer and (c) PZ samples. X-axis is the index of trails. Y-axis is the mean and STD of the stiffness in a bigger area (50×50 pixels, 20μm/pixel). The elapsed times were marked on the top of each bar plot.

IV. CONCLUSIONS AND DISCUSSION

Preliminary studies identified some technical problems that must be overcome. (1) Water on the surface of the sample can change the resonance frequency of the tactile sensor and cause measurement errors. One solution is to dry the surface of the sample using a 0.22 μm micro-pore filter paper. (2) To avoid damaging the fragile tactile probe, our LabVIEW program steers the 3D manipulator very carefully and slowly. It takes about 0.5 second to scan one single point and about 4 hours to scan a 1mm diameter area with a 5μm resolution. However, the thin tissue slice dehydrates very quickly when it is exposed to air. The pilot studies show that the stiffness of the tissue sample changed significantly after being exposed to 40 ºC air for 71 minutes.

To speed up the 2D stiffness mapping, we plan to rewrite the LabVIEW control program to optimize the 3D manipulator steering. We will also develop fast scanning algorithms by combining a 20μm resolution coarse scan and a 5 μm resolution fine scan. We will achieve a 10~20 times faster scanning speed using these methods. In addition, an environment control system with high temperature stability (< 0.05°C/24h) and zero air flow (SASAM® ECS, Kibero Inc.) will be adapted to slow down the dehydration of the samples.

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