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Photoacoustic spectral analysis at ultraviolet wavelengths for characterizing the Gleason grades of prostate cancer

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Abstract

The diagnosis of aggressive prostate cancer (PCa) has relied on the microscopic architectures, namely Gleason patterns, of tissues extracted through core biopsies. Technology capable of assessing the tissue architecture without tissue extraction will reduce the invasiveness of PCa diagnosis and improve diagnostic accuracy by allowing for more sampling locations. Our recently developed photoacoustic spectral analysis (PASA) has achieved quantification of tissue architectural heterogeneity interstitially. Taking advantage of the unique optical absorption of cell nuclei at ultraviolet (UV) wavelengths, this study investigated PASA at 266-nm for quantifying the tissue architecture heterogeneity in prostates. The results have shown significant differences among the normal, early cancer, and late cancer stages in mouse prostates *ex vivo* and *in vivo* (n=20, p<0.05). The study with human samples *ex vivo* has shown a correlation of 0.80 (n=11, p<0.05) between PASA quantification and pathologic diagnosis.

Prostate cancer (PCa) has been the second cause of cancer death and has the highest incident rate in American men for the past three decades [1]. The diagnosis of PCa has relied on core biopsies guided by transrectal ultrasound (TRUS). The architectures of the extracted tissue samples are revealed by histological procedures and interpreted by pathologists under a

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Disclosures

The authors declare no conflicts of interest.

See Supplement 1 for supporting content.

Gleason grading system [2]. The grading system describes the increased tissue heterogeneity due to the destruction of normal glandular architectures. A Gleason score (GS) equal or larger than 7 is considered clinically significant and aggressive PCa. Due to the limited sensitivity of TRUS to PCa, less than 10% of the sampled cores are clinically significant [3], and the core extractions are frequently accompanied by post-procedure complications [4].

Photoacoustic (PA) spectral analysis (PASA) [5-7] is a recently developed technology that combines the established methodologies of quantitative US [8] and optical spectroscopy [9]. By assessing the power spectra of PA signals generated at specific laser wavelengths, PASA can quantify the architectural heterogeneity formed by the targeted molecular components [6, 7]. The method has been investigated in quantifying the progression of non-alcoholic fatty liver disease [5, 10], differentiating intraocular tumor types [11], determining the red blood cell aggregation [12], and characterizing the diseased conditions in lymph nodes [13].

PA imaging has shown the capability of delineating the malignancies in prostates [14]. Considering that the pathological diagnosis of PCa is founded on tissue architecture, we and other research groups [15, 16] have attempted implementing PASA to assessing the architecture within prostate tissue. PASA has identified aggressive PCa in *ex vivo* human tissues using the endogenous [15, 16] and exogenous [17] optical contrasts. Aimed at integrating PASA into a standard biopsy procedure, we have also developed an interstitial needle PA probe [15] that can assess a small tissue volume surrounding the probe. The needle PA probe was preliminary examined in intact human prostates *ex vivo* [15]. However, all of these studies used near infrared wavelengths, which produce positive contrast of tissue components such as hemoglobin, lipid and collagen, instead of the cancer cells forming the clustering patterns in the Gleason system [2]. Taking advantage of the positive optical contrast of cell nuclei over other tissue components, previous studies has shown that PA imaging at ultraviolet (UV) wavelengths can produce images comparable to histology [18-20]. PASA at UV wavelengths, therefore, may find its unique role in quantifying the architectural heterogeneity within prostate tissue. This study investigates PASA working at 266-nm wavelength for characterizing the progression of PCa in mouse prostates *ex vivo* and *in vivo*, and human prostate tissues *ex vivo*.

The laboratory animal protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Michigan, Ann Arbor. We used a transgenic mouse strain, TRAMP (008215, Jackson Laboratory, Bar Harbor, ME), that spontaneously develops PCa [21] with microscopic architecture close to the organ surface and comparable to that in human, as shown in histology images in Supplemental Document Section S1. C57BL/6J mice (000664, Jackson Laboratory, Bar Harbor, ME) were used as normal control. Human tissue procurement protocol for this study were approved by the internal review board for human subject study at the University of Michigan, Ann Arbor.

2D PASA method [5] was implemented at 266-nm to explicitly show the difference in architectural heterogeneity between normal and cancerous prostates *ex vivo*. We harvested prostates from normal mice, TRAMP mice at the age of 12 weeks with early stage cancer, and TRAMP mice at the age of 16 weeks with late stage cancer, as shown in Fig. 1(a-c). The samples were scanned in a conventional rotational tomographic PA imaging system [5]. The

scanning procedures have been described in detail in Supplemental Document Section S2. Illumination at 266-nm was generated by the fourth harmonics of an Nd:YAG laser (Surelite, Continuum, Santa Clara, CA) was collimated to 10 mm for covering the sampling at 3 mJ/cm² per pulse, which is the safety limit established by American National Standard Institute (ANSI) [22]. The PA signals were captured by a needle hydrophone with frequency response calibrated within 0.1-20MHz (HNC-1500 and preamplifier AH2010-025, ONDA corporation, Sunnyvale, CA) at a sampling rate of 250 MHz. Fig. 1(d-f) show the PA images by beamforming with delay-and-sum method [5]. A 2D sliding window with dimensions of 1800 x 1800 μm^2 scanned over the beamformed PA signals at a step size of 11 μm . At each step, the 2D power spectrum of the beamformed PA signals was calculated. The 2D power spectrum within the frequency range 1-7 MHz was fitted into a plane. The justifications of the window size and frequency range selection are included in the Supplemental Document Section S3. The slope along the diagonal of the fitted plane was quantified as the PASA slope. High slopes indicate more high frequency components in the signals and more heterogeneous tissue architecture [5-7]. The slope values derived at all sliding window locations were assembled to form the pixel-wise PASA maps in Fig. 1(g-i). Fig. 1(j-k) show the histology. The prostate with early stage cancer in Fig. 1(b) enlarged in size compared to the normal prostate in Fig. 1(a). The surface of the late stage cancer prostate in Fig. 1(c) appeared less smooth compared to the other two conditions, indicating the increased architectural heterogeneity. The PA images in Fig. 1(d-f) also show increasingly heterogeneous texture along with the progressive cancer stages. The heterogeneous textures were quantified by the pixel-wise PASA slope maps in Fig. 1(g-i). The imaging observations agree with the histology. The needle hydrophone with limited bandwidth cannot resolve the thin layer of epithelial cells in normal prostate shown in Fig. 1(j) and, therefore, shows mostly low PASA slopes. The early stage cancerous prostate has enlarged gland architectures due to the invasion of cancer cells in dark purple and shows increased heterogeneity and higher slope values. The late stage cancer lost the gland structure and shows highest heterogeneity as reflected by highest PASA slopes.

We further investigated PASA at 266-nm in mice *in vivo*. The mice were anesthetized with 3% isoflurane during the procedures. Since mouse prostates have small dimensions and are difficult to locate transabdominally, we exposed the prostates through laparotomy, as shown in Fig. 2(a). Due to the complicated abdominal anatomy, 2D imaging using the broadband needle hydrophone is difficult. Therefore, one dimensional measurements of the prostates were acquired using our needle PA probe [15]. As illustrated in Fig. 2(a), the needle PA probe consists of a 600 μm fiber optic diffuser with an emission end of 5 mm [Fig. 2(b)] and the needle hydrophone in Fig. 1. Such setup allows for the generation of sufficient signal intensity and length at the tissue/fiber interface for statistics-based spectral analysis. Due to the small volume and superficial glandular architecture of mouse prostate, the needle PA probe was attached to the surface of the prostate instead of being inserted as in [15]. US gel was applied for optimal acoustic coupling. The same laser and data acquisition system in the *ex vivo* study was used. The optical energy density at the surface of the fiber optic diffuser was maintained at 3 mJ/cm². As illustrated in Fig. 2(c), three PA measurements were taken from each prostate along either side lobe of the prostate, or across both lobes. Since 266-nm has limited penetration in biological tissues [18-20], the measurements within the same

animal has minimal overlapping and are considered independent. The animals were euthanized after the scanning by overdosed isoflurane. The prostates were harvested for histology. TRAMP mice at the ages of 12 and 16 weeks were used as experiment groups, and C57BL/6J mice at the same ages were used as control groups. Each group included 5 animals, making a total measurement number of 60 ($=4 \text{ groups} \times 5 \text{ animals} \times 3 \text{ measurements}$). The representative PA measurements were displayed in Fig. 2(d-e). Fig. 2(f) shows the power spectra of the PA signals in Fig. 2(d-e) calculated using Welch method [5]. As marked by the red dashed circles in Fig. 2 (f), the power spectra contain large low frequency components, which were generated due to the close distance between the needle hydrophone surface and the fiber optic diffuser. The PASA slopes were quantified by fitting the power spectra within 1.5-10 MHz to linear models to exclude the low frequency components. Fig. 2(g) shows the statistics of the PASA slopes. Significant differences were found between 1) normal and cancer groups at 12 weeks ($n=30$, $p=0.021$); 2) normal and cancer groups at 16 weeks ($n=30$, $p=5.8 \times 10^{-5}$); and 3) cancer groups at 12 and 16 weeks ($n=30$, $p=4.4 \times 10^{-3}$). The normal groups at 12 and 16 weeks did not show significant difference.

Since mouse PCa does not reflect the full spectrum of architectural changes in human PCa as described by GS system, we moved on to human prostate tissues with the purpose of finding the correlation between the PASA quantifications and the GS evaluated by a genitourinary pathologist. Fresh frozen normal prostate tissues and PCa tissues with GS 6, 7 (3+4 and 4+3) and 8 were sliced at $50 \mu\text{m}$ in thickness and approximately 15 mm by 15 mm in area. The samples were fixed on glass slides and scanned in the geometry in Fig. 3(a) with 3 mJ/cm^2 per pulse optical density. The detailed experiment setup is described in Supplemental Document Section S4. Fig. 3(b) shows the representative signals. Due to the limited illumination area and reception angle of the hydrophone, multiple scans were performed for each sample to cover the whole sample area. The signal power spectra at all locations in the same sample were averaged for PASA. We included a wider frequency range (0.1-10 MHz) compared to the mouse study as the needle hydrophone surface was positioned away from the illumination. In addition to the slopes, we also found that the area under the power spectra, namely the total spectra magnitudes, have correlations with the GS of the samples, as illustrated in Fig. 3. Representative H&E histology of the scanned tissues in Fig. 3(d-g) shows the progressive loss of the normal architecture and the increased architectural heterogeneity formed by the dark cancer cells as the GS increases.

Fig. 4 shows the statistics of the PASA parameters. Fig. 4(a-b) shows the differences between the non-aggressive (GS = 6) and aggressive (GS = 7) PCa. Similar to mouse prostate samples, human samples with aggressive PCa shows significantly higher slope values compared to the non-aggressive PCa ($n=11$, $p=4.4 \times 10^{-4}$). The increase of cancer cells with large cell nuclei produced larger spectral magnitude in higher-grade cancers ($n=11$, $p=0.022$). Since benign prostate tissue does not have a GS and we wish to include all the samples for analysis, we introduced the recently developed Gleason Grade Grouping (GGG) System [23], which takes into consideration of the regional difference in each sample. In the GGG system, GS = 6, GS=7(3+4), GS=7(4+3) and GS=8 are designated as GGG 1 to 4 respectively. We also designated the benign groups as GGG 0. In Fig. 4(c-d), The PASA slopes and spectral magnitudes have shown correlations of 0.78 ($n=11$, $p=0.0046$) and 0.60

($n=11$, $p=0.051$) with the GGG, respectively. The relatively low correlations are due to a sample containing only 10% cancerous area, as marked by red “x” in Fig. 4(c-d), whereas other samples have 60-70% cancerous regions, as quantified by our genitourinary pathologist. Excluding the sample will improve the correlation values to 0.84 ($n=10$, $p=2.5 \times 10^{-3}$) and 0.63 ($n=10$, $p=0.053$) for slopes and spectral magnitudes, respectively. We calculated the correlation between the PASA quantifications and the GGG weighted by the percentage areas of the cancerous regions. The weighted GGG have correlations of 0.80 ($n=11$, $p=2.9 \times 10^{-3}$) and 0.63 ($n=11$, $p=0.037$) with the PASA slopes and total spectral magnitudes, respectively.

The major challenge to the PASA quantification in this study is the averaging effect between the normal and cancerous regions in the samples. The wide illumination and the irregular shape of cancerous regions in the human prostate samples limited the quantification accuracy. Assisted with histology information and GGG system, we were able to compensate for the variation of the cancerous area in the samples. Pixel-wise PASA may provide a solution when translating to clinics. Considering the limited penetration of UV wavelengths in biological tissue, the PA signals acquired by the needle PA probe are close to 1 dimensional measurements. PASA within a sliding window along the fiber diffuser may provide localized tissue heterogeneity information.

Taking advantage of the strong optical absorption of cell nuclei and their positive optical contrast over other tissue components at UV wavelengths [18-20], we have achieved decent signal-to-noise ratio (SNR) following the ANSI safety limit (3 mJ/cm^2). We will further investigate the trade-offs among the optical density, SNR and safety for PASA at UV wavelengths. This will help us determine the diameter of fiber optic diffuser for miniaturization of the interstitial needle PA probe [15] and for future clinical translation.

The bandwidth and sensitivity of the hydrophone in this study limited the smallest detectable architectures. For example, the thin layer of epithelial cells in the normal prostate should produce high frequency signal components which, however, cannot be detected by our hydrophone. We will consider expanding our detection bandwidth and sensitivity in the future for more comprehensive architecture assessment. We expect nonlinearity in the signal spectra in wider bandwidth and will update the quantification approaches. As another pitfall, the selection of frequency range for PASA varies among measurement setups, further discussion is included in Supplemental Document Section S5. The optimal configuration with safety considerations for implementing UV PASA to human prostate will be reported in future works.

In summary, PASA at UV wavelengths has shown the capability of quantifying the microscopic tissue architectures in mouse prostates *ex vivo* and *in vivo*, and in human prostate samples *ex vivo*. Significant difference among the progressive cancer stages within the mouse and human prostates are found. The correlations between the PASA quantifications and the GGG are also found. Future works will focus on improving the signal processing approaches and translating the needle PA probe based measurements to human subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Experiment with mouse prostates *ex vivo*. (a-c) Gross pathology. (d-f) PA images of the prostates with the contours delineated according to the photos in (a-c). (g-i) Pixel-wise PASA slopes [5] overlaid on top of the prostate contours. (j-l) Hematoxylin and eosin (H&E) histology of the same prostates in (a-i). Scale bars in (a-i) and (j-l) represent 5 mm and 100 μm , respectively.

Fig. 2.

PASA of PCa in mouse *in vivo* using a needle PA probe [15]. (a) A photo of the exposed abdominal anatomies with the needle PA probe attached to the prostate. VG: vesicular gland. BL: bladder. PR: prostate. FOD: fiber optic diffuser. EE: emission end. NH: needle hydrophone. (b) The fiber optics diffuser coupled with visible light showing the emitting pattern. (c) Illustration of the measurement locations. The needle probe was positioned along the red dashed lines. (d-e) Representative PA signals. (f) Power spectra of the signals in (d-e) and the illustration of PASA slope, i.e. $\tan\theta$. (g) Statistics of the PASA slopes acquired from the 4 groups of mice. N1 and C1 are normal and cancer groups at 12 weeks. N2 and C2 are those at 16 weeks.

Fig. 3.

PASA of sliced human prostate tissues. (a) Scanning geometry. (b) representative PA signals of prostate samples with non-aggressive (GS 6) and aggressive (GS 7) cancers. (c) PA power spectra of the signals in (b). PASA slope is defined as $\tan \theta$ and total power spectral magnitude is defined as the shaded area under the spectral curve. (d-g) Representative H&E histology of normal prostate tissues and cancerous tissues with varied GS scanned in this study.

Fig. 4.

Statistics of the PASA slopes and total spectral magnitudes derived from the human prostate samples. (a-b) Difference between non-aggressive (NA) and aggressive (A) samples. (c-d) Linear regressions between the GGG and the PASA parameters. (e-f) Linear regressions between the GGG weighted by the percentage areas of the cancers within the tissue samples.