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

Authors: Guan Xu, Janggun Jo, Javed Siddiqui, yunhao zhu, Linyu Ni, Sri-Rajasekhar Kothapalli, Scott Tomlins, John Wei, Evan Keller, Aaron Udager, Xueding Wang

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S1. Feasibility of assessing glandular architectures in mouse prostate using illumination at 266 nm

As shown in Fig. S1, the glandular architecture of mouse prostate is mostly tens of microns from the organ surface. As reported in previous studies, the penetration of focused optical energy (which is intrinsically limited in penetration [1]) at 266 nm can penetrate at least 100 μm into tissue [2]. Therefore, sensing the glandular architectures in prostates with wide field illumination is feasible.

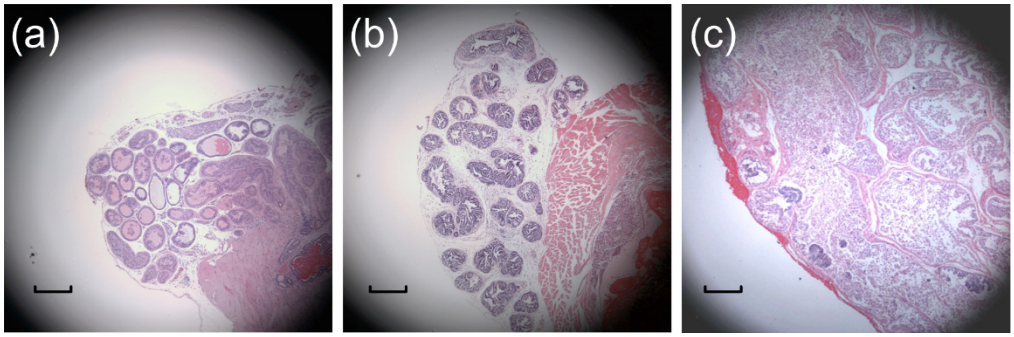

Fig. S1. Histology of mouse prostates at low magnification. (a) Prostate of a normal mouse. (b) Prostate of a TRAMP mouse at 12 weeks. (c) Prostate of a TRAMP mouse at 16 weeks. The glandular architectural heterogeneities are relatively uniform throughout the prostate and the architectures are superficial and reachable by light penetration at 100s μm. Scale bars: 300 μm.

S2. Experiment setup for 2D imaging in Fig. 1

The experiment setup has been described in our previous publications [3, 4] and is illustrated in Fig. S2. Purposed at avoiding water attenuation of optical energy at 266 nm, the samples were placed in a cup-shaped holder made of 10% porcine gelatin-water solution. A small amount of water was added to have the tissue sample barely submerged. Such setup minimized the water attenuation of optical energy and allowed for acoustic coupling through the side wall of the holder. Illumination at 266 nm generated by the fourth harmonics of an Nd:YAG laser (Surelite, Contimuum, Santa Clara, CA) was collimated to 10 mm in diameter and delivered from the top opening of the sample holder at an optical density of 3 mJ/cm^2 per pulse, which is the safety limit established by American National Standard Institute (ANSI) [5]. The PA signals were captured by a needle hydrophone with frequency response calibrated within 0.1-20MHz (HNC-1500 and preamplifer AH2010-025, ONDA corporation, Sunnyvale, CA). A rotational stage driven by a step motor positioned the hydrophone at 192 uniform steps around the sample holder. The hydrophone was aligned tangential to the sample surface for optimal signal reception as most of the signals were generated close to the surface of the sample. The PA signals were amplified by a low-noise amplifier (5072PR,

Panametrics, Waltham, MA) with a gain of 30 dB before being recorded by an oscilloscope at a sampling rate of 250 MHz.

The thin layers of coupling water and red blood cells in Fig. S1(c) both had large dimension in the tangential direction of the tissue surface and, therefore, produced low frequency signal components. High pass filtering in the data acquisition and processing excluded the contribution of these signal components in the spectral analysis.

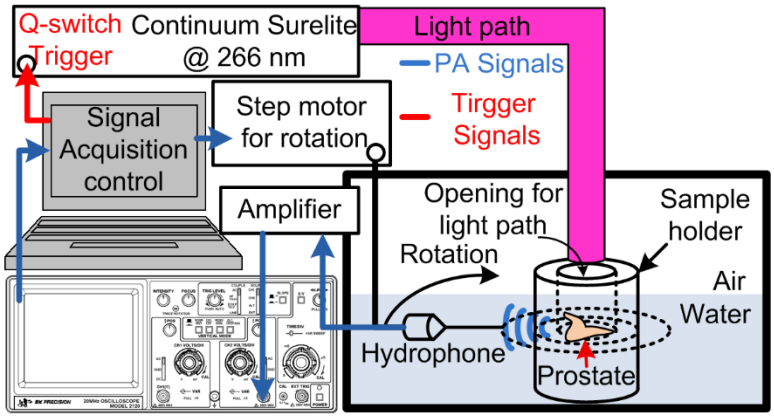

Fig. S2. Setup of the experiment in Fig. 1.

S3. Selection of window size in the 2D photoacoustic (PA) signal power spectra

The selection of the window size for calculating the pixel-wise spectral parameters in Fig. 1(g-i) is based on the conclusion in quantitative ultrasound studies that the signal length should be at least 10 wavelengths of the central frequency of the transducer bandwidth for consistency in statistics-based spectral analysis [6]. The hydrophone was calibrated within 0.1-20MHz. We empirically selected 12 wavelengths at 10MHz, i.e. 1800 μm.

S4. Experiment setup in Fig. 3(a)

For the purpose of acoustic coupling, the glass slide was barely submerged into a water tank and the surface where the tissue attached were positioned downward. The laser beam at 266-nm wavelength was collimated to 5 mm in diameter with 3 mJ/cm^2 per pulse optical density and projected perpendicular to the sample surface. The same needle hydrophone and data acquisition system in the animal studies collected the PA signals. The hydrophone was align as tangential to the tissue sample as possible for optimal signal reception.

S5. Frequency range selection for PA spectral analysis (PASA)

Frequency dependent acoustic attenuation between the sample and the hydrophone is difficult to compensate and introduces uncertainty to the quantitative PA measurement. In this study, instead of compensating for the attenuation, we fixed the relative locations of the illumination and the hydrophone in the each experiment setup. Therefore, the measurements within the same geometry were attenuated identically in frequency domain and comparable. This resulted in the selection of different frequency ranges in PASA for different measurement setups. We will search for and determine the optimal configuration for our interstitial needle PA probe for the ultimate clinical translation.

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