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Photoacoustic tomography of intact human prostates and vascular texture analysis identify prostate cancer biopsy targets

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ABSTRACT

Prostate cancer is poorly visualized on ultrasonography (US) so that current biopsy requires either a templated technique or guidance after fusion of US with magnetic resonance imaging. Here we determined the ability for photoacoustic tomography (PAT) and US followed by texture-based image processing to identify prostate biopsy targets. K-means clustering feature learning and testing was performed on separate datasets comprised of 1064 and 1197 nm PAT and US images of intact, *ex vivo* human prostates. 1197 nm PAT was found to not contribute to the feature learning, and thus, only 1064 nm PAT and US images were used for final feature testing. Biopsy targets, determined by the tumor-assigned pixels' center of mass, located 100% of the primary lesions and 67% of the secondary lesions. In conclusion, 1064 nm PAT and US texture-based feature analysis provided successful prostate biopsy targets.

1. Introduction

Prostate cancer (PCa) is the most incident, visceral cancer in USA men. An estimated 164,690 new prostate cancer cases are predicted to occur in 2018, which is 9.5% of all estimated 2018 cancer occurrences [1]. The current overall 5-year survival rate is 97.7%, especially when PCa is discovered at a local stage, but this drops to 30% if the PCa has metastasized prior to diagnosis [2]. In order to ensure that diagnosis occurs at the local stage while limiting harm to the patient, serum prostate-specific antigen (PSA) measurement is recommended as a screening tool for PCa depending on factors, such as age, family history and the patient's preference [3,4]. PSA is produced exclusively by prostate epithelial cells and can be influenced by benign conditions including: bacterial prostatitis [5], ejaculation [6], and benign prostatic hyperplasia [7]. Thus, false positive results from PCa serum PSA screening commonly occur, which makes a follow-up, confirmatory test necessary.

Currently to confirm the presence of PCa, histopathology analysis with Gleason grading must be performed on biopsy samples acquired from the prostate in order to guide clinical decision making [8]. Gleason grading is based on the microscopic tissue architecture, and the two major Gleason grades are added to give the Gleason score [9]. The current clinical standard for acquiring biopsy samples is to perform a 12-core transrectal ultrasound (TRUS)-guided biopsy (TRUS-GB), which entails following a template to systematically acquire 12 tissue samples from the prostate [10]. To follow the template protocol, a TRUS probe guides the biopsy procedure by allowing visualization of the anatomical locations within the prostate [10,11]. Even with optimization of the TRUS-GB, false negative results occur in approximately 15–34% of initial biopsy procedures due to the limited, untargeted sampling of the prostate [12,13].

The combination of the PSA and the TRUS-GB is considered to be the major contributor to the overtreatment problem for PCa [3]. Since the biopsied tissue, and not the PSA, currently provides the diagnostic information to aid in therapeutic decision making [9], the biopsy procedure needs improvement due to its low sensitivity [14]. As previously

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mentioned, the current gold standard for performing the biopsy is a systematic approach based on a template [10]. Therefore, providing a target for the prostate biopsy may help to improve the sensitivity of the procedure.

The most notable clinical advancement for targeting the prostate biopsy is the magnetic resonance imaging-fusion biopsy (MRI-FB), which is currently recommended for patients undergoing repeat biopsy following an initial negative biopsy [15,16]. For biopsy-naïve patients, recent conflicting evidence exists regarding the PCa detection rate when using the MRI-FB compared to TRUS-GB [17–22]. Overall, these clinical studies show that the MRI-FB alone can reduce the number of cores needed to achieve the same PCa detection rates as the TRUS-GB [17,20,22]. Additionally, the MRI-FB has been shown to miss fewer clinically significant PCa tumors [22]. This reduction in cores needed and detection of clinically significant PCa tumors can reduce the risk of side effects and the need for repeat biopsy. However, many pitfalls exist with this method. Careful calibration is needed to fuse the real-time US and previously acquired, annotated multiparametric MRI (mpMRI). If the patient moves after alignment, the calibration must be completed again. In addition, the mpMRI images are static, and manual pressure on the prostate during biopsy can distort the tissue compared to the mpMRI [23]. Other pitfalls include added costs for the mpMRI [24] and the injected contrast agents, which may be contraindicated in some patients [25], used in the procedure. Thus, an ideal solution for targeting the PCa biopsy includes endogenous contrast and real-time, co-incident imaging and analysis.

Since the prostate biopsy is TRUS-guided, photoacoustic tomography (PAT), which uses traditional ultrasound (US) transducer arrays for signal collection [26], is a potential tool to apply clinically in order to improve the prostate biopsy. In contrast to MRI-FB, PAT has inherent co-registration with the US imaging channel as the PAT and US images are sequentially acquired using the same US transducer array. MRI-FB does have an advantage in imaging resolution and difference in biomarker type compared to PAT for prostate biopsy targeting. For PAT, the imaging resolution is dependent on the US transducer's imaging resolution [26]. Since the TRUS probe used for prostate biopsy is typically a low frequency US transducer with central frequency at approximately 7 MHz [27], the axial resolution is approximately two to three times lower than the resolution of the mpMRI sequences used for the MRI-FB [28]. Another potential major difference is that the recommended mpMRI utilizes two functional imaging sequences out of the three total sequences as biomarkers [28], while PAT approaches can be based on biomarker content [29,30] and/or functional alterations [31]. Overall, PAT should be investigated as an alternative to MRI-FB for the purpose of targeting the prostate biopsy.

The photoacoustic signal detected during PAT results when an absorber interacts with pulsed light in such a way that the energy is converted to heat, and the resultant local thermodynamic expansion releases an acoustic wave, which is detectable via an US transducer [26]. Compared to traditional optical-only imaging techniques, this allows for deeper imaging of major endogenous absorbers, such as deoxygenated and oxygenated hemoglobin, lipid, and water [26,30]. A few examples of the applications in which these endogenous photoacoustic contrast agents have been used are intravascular imaging of atherosclerotic plaques [32], breast cancer tumor margin assessment [33], and PCa [34] and breast cancer [35] vascularity. Since PCa is known to involve angiogenic processes [36], PAT, with hemoglobin as the endogenous contrast agent [26,30], may be able to identify targets for the prostate biopsy. Thus, we utilized the 1064 nm output from our previously published barium nitrite Raman laser [37] to image hemoglobin in human prostates. Unfortunately, angiogenesis in the prostate is not specific to PCa [36], while increasing cholesteryl ester, i.e. lipid, storage has been shown to be a specific biomarker to increasingly aggressive PCa [38]. Therefore, PAT was also performed at 1197 nm, which is an absorption peak for lipid [30,37].

Recent studies have begun applying PAT to the identification of PCa in human prostates [34,39–41]. Unfortunately, a method of identifying targets for the prostate biopsy has yet to be achieved without manual selection of regions of interest (ROI) that rely on intensity-based thresholding [34,39] or the use of multispectral PAT analysis [39–41] that would decrease the frame rate. Out of these studies, Rajanna et al. used deep neural networks to learn features and then identify pixels representing PCa. This work was completed using a previously published PAT dataset of *ex vivo* human prostates that were sliced into axial sections prior to five wavelength PAT imaging [39]. The imaging method ensures uniform light fluence over the anterior-posterior axis of the tissue, which is currently not possible for prostate PAT in the clinical setting [34]. Additionally, the feature learning method used is based on feature learning of gene expression profiles, which can have hundreds of features [42]. Here, we minimize the PAT channels to 1064 and 1197 nm and acquire the standard US channel. Since feature learning typically involves 10 s–100 s of features [40,42], we utilize the "off-the-shelf" K-means clustering feature learning of texture patches, which has been shown to be effective in single-layer networks [43], for the purpose of identifying targets for PCa biopsy.

2. Materials and methods

2.1. Prostate specimen inclusion and handling

All work performed followed the approved Institution Review Board protocol (IUSCC-0581). A total of 9 prostate specimens were imaged in a room near the Indiana University Hospital surgical suite directly following radical prostatectomy (Fig. 1). After 10 sterile saline washes of the external surface, prostates were immobilized using an agar bed and imaged with PAT and US as described below. Formalin fixation and whole mount histopathological analysis was then performed by urogenital pathologist (L.C.) as previously described [44]. De-identified pathology reports were also provided in addition to the annotated whole mount histopathology slides.

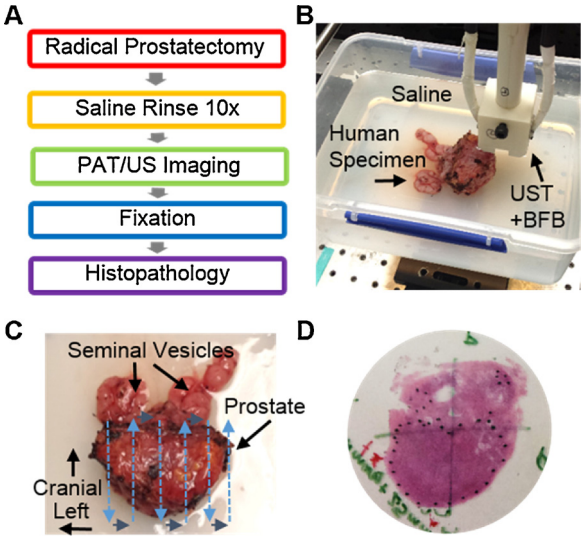

Fig. 1. Prostate Specimen Handling During Data Collection. (**A**) Prostate specimen handling procedure from radical prostatectomy to whole mount histopathology. (**B**) Image of prostate specimen during PAT and US imaging. UST + BFB: ultrasound transducer with bifurcated fiber bundle. (**C**) Image of prostate specimen depicting position during imaging and the raster scanning pathway. (**D**) Representative whole mount histopathology slide. An experienced urogenital pathologist marked the tumor margins and completed the corresponding histopathology report. These slides are considered ground truth for image analysis.