Human Feasibility Study of Fluorescence Spectroscopy Guided Optical Biopsy Needle for Prostate Cancer Diagnosis

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Abstract— **Current prostate biopsy cores have a very low diagnostic yield. These biopsies often fail to diagnose prostate cancer since 90% of cores are histopathologically classified as benign. The concentrations of endogenous fluorophores in prostate tissue vary with disease states. Thus, fluorescence spectroscopy could be utilized to quantify these variations for identification of malignant lesions. We investigated clinical feasibility of a 14 gauge (1.98 mm) optical biopsy needle guided by fluorescence spectroscopy for real-time *in vivo* prostate cancer diagnosis. Built-in optical sensor has 8x100µm fibers for tissue excitation and a single 200µm fiber to collect spectral data. Custom-made fluorometer has 2 light-emitting diodes at 290 and 340 nm and a spectrometer. User interface for fluorometer operation and data collection was developed using LabView software. Each spectral data acquisition required ~2 seconds. The *in vivo* biopsies were performed during radical retropubic prostatectomy surgery on the exposed prostate with blood flow to the gland intact. A tissue biopsy core was obtained from each biopsy site after acquisition of spectral data. Above procedure was repeated *ex vivo* after surgical excision of the prostate. Biopsy cores were histopathologically classified as either benign or malignant and correlated with corresponding spectral data. Partial Least Square analysis was performed to determine diagnostically significant principal components as potential classifiers. A linear support vector machine and leave-one-out cross validation method was employed for tissue classification. Thirteen patients were consented to the study. Histopathological analysis found cancer in 29/208 *in vivo* and 51/224 *ex vivo* viable biopsy cores. Study results show 72% sensitivity, 66% specificity, and 93% negative predictive value for *in vivo* and 75%, 80%, and 93%, respectively, for *ex vivo* malignant versus benign prostatic tissue classification. Optical biopsy needle has a very high negative predictive value to indicate benign tissue while sufficient sensitivity for targeting areas suspicious for cancer within the prostate gland. Hence, the optical biopsy needle can increase the diagnostic yield of prostate biopsies with consequent improvement in patient care.**

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I. Introduction

Prostate cancer is the most common cancer among American men other than skin cancer [1]. In 2015, an estimated 220,800 men will be diagnosed with prostate cancer and 27,540 are expected to die from this disease [1]. Prostate cancer is diagnosed by pathological examination of tissue obtained during prostate biopsy procedures from men suspected of having this disease due to elevated serum PSA or abnormal digital rectal exam [2]. Prostate biopsies are taken randomly from 10-12 locations of the prostate gland under the guidance of transrectal ultrasound (TRUS).

TRUS-guided biopsies taken with an 18-gauge needle are subjected to serious sampling errors and often fail to diagnose clinically significant prostate cancer [3].TRUS images cannot differentiate cancer lesions from benign tissue and only useful for locating anatomical boundary of the prostate gland to guide prostate biopsy. Pallwein *et al* found only 6% (130/2,300) of systematic biopsy cores had cancer [4]. Nelson *et al* showed only 13% (106/818) of sextant biopsy cores were useful for cancer diagnosis [5]. The main reason for very low diagnostic yield of TRUS biopsy cores is that they are taken randomly without any prior knowledge of whether the underlying tissue is malignant or not.

Fluorescence spectroscopy could be used to determine whether biological tissue is malignant or not [6]. We have prototyped an optical biopsy needle to obtain prostate biopsy *after* the optical characterization of underlying tissue [7]. For tissue classification, this needle utilized fluorescence spectra (FS) of endogenous tryptophan, collagen, and nicotinamide adenine dinucleotide (NADH) measured at five excitation wavelengths of 280, 290, 300, 330, 340, and 350 nm. Study results show 86% sensitivity, 87% specificity, 90% negative predictive value, and 83% positive predictive value for malignant versus benign prostate tissue classification from surgically excised radical prostatectomy specimens. Hence, the optical biopsy needle has the potential to overcome limitations of TRUS biopsies in clinical settings.

The standard laboratory fluorometer, Fluorolog-3 (JY HORIBA, New Jersey), used in the aforementioned study is *not suitable* in clinical settings due to a number of limitations such as having a large footprint, very slow data acquisition time, and being expensive [7]. Nevertheless, Zhu *et al* used this fluorometer to measure FS of breast tissues *in vivo* with a fiber optic probe at seven excitation wavelengths from 300 to 420 nm in 20 nm increments during percutaneous image-guided breast biopsy [8]. Study results show sensitivity and specificity of up to 81% and 87%, respectively, for malignant versus fibrous/benign breast tissue classification.

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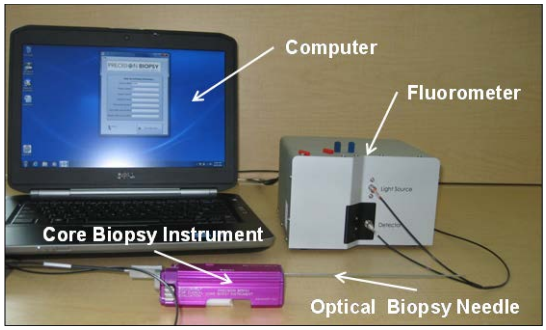

Figure 1: Optical biopsy needle, core biopsy instrument, fluorometer, and computer used in the human feasibility study

For human feasibility study of our optical biopsy needle, six fluorometers were custom-made using low power light emitting diodes (LED) as light sources and charge-coupled device (CCD) as detector. We also manufactured medical grade optical biopsy needles for this study. In Section II we present design and manufacturing of fluorometers and optical biopsy needles, human feasibility study design, and spectral data analyses for prostate tissue classification. In Section III, human feasibility study results are provided including sensitivity and specificity for prostate cancer diagnosis. Conclusions are presented in Section IV.

II. METHODOLOGY

A. Product Design, Development, Verification & Validation

Several custom-made products were used in the human feasibility study as shown in *Fig. 1*. These products include optical biopsy needle, core biopsy instrument (biopsy gun), fluorometer, and software for data collection and user interface. All designs were subjected to strict Quality Assurance and Quality Control protocols. Product requirement, specification, verification & validation documents were available for the regulatory agency audit.

We used the same optical biopsy needle design employed in our previous *ex vivo* study [7]. Briefly, optical biopsy needle consists of a 14-gauge (1.98 mm) outer needle and a slightly smaller diameter inner needle. The working length of the needle is 18 cm. The inner needle has an optical sensor at the tip with a 22 mm long sample notch to hold the biopsy tissue. The optical sensor consists of 8x100 µm source fibers with numerical aperture (NA) of 0.22 arranged in a circle and a single 200 µm center fiber with NA of 0.22 to read the fluorescence spectra. Tip of the needle was cut and polished at a 60 degree angle to facilitate tissue cutting. The backend of fibers are terminated with standard SMA connectors.

Optical biopsy needle interface with the core biopsy instrument and can cut 22 mm long tissue cores following optical characterization of underlying tissue. Core biopsy instrument is a modified BARD Urological MAGNUM® gun with a metal bracket at the back-end to support the biopsy needle and fiber optic source/detector splitter. The Device Company (TDC), A Vention Medical Company, Sunnyvale, CA was responsible for manufacturing and sterilization of optical biopsy needles and core biopsy instruments.

Figure 2: Custom-made fluorometer with internal components

To design a fluorometer suitable for clinical applications, we had to identify a minimum number of excitation wavelengths required for reliable tissue classification by tracking tryptophan, collagen, and NADH. We selected 290 nm for tracking tryptophan and 340 nm for tracking both collagen and NADH based on our previous *ex vivo* study results and commercial availability of LEDs [7]. We designed the fluorometer with two LEDs with central output peaks at 290 and 340 nm and a Hamamatsu CCD as detector. Ocean Optics Inc. (OOI), Dunedin, FL, built six fluorometer units for the clinical trial based on our specifications. *Fig. 2* shows the internal components of the fluorometer.

The two LEDs selected as light sources were rated at 10 mW output power with central output peaks at 290±2 and 338±2 nm. The bandwidth of the each LED was ≤10 nm, sufficiently small to minimize Rayleigh scattering and subsequent optical bleed through from the respective LED source into the emission detection. LEDs were mounted on heat sinks with a cooling fan. Internal optics was setup for each LED to deliver light to the same SMA output port which was connected to source fiber of the optical biopsy needle. The electronic circuitry controls on/off functions and output power of each LED via computer software. On/off function allows light from two different LEDs at independent times delivered to the SMA port.

The OOI Maya Pro 2000 spectrometer selected as the detector consists of a back-thinned S10420 Hamamatsu CCD, Ocean Optics Grating H2, and no entrance slit. In the absence of an entrance slit, the spectrometer resolution is established by the 200 µm read fiber connected to the input (measured to be 2.4 ± 0.6 nm FWHM – full width at half maximum). Maya Pro 2000 spectrometer uses an USB port for connecting to the computer and one SMA port to receive the fluorescence signal collected by the read fiber of the optical biopsy needle. Hamamatsu CCD detector has 2048 pixel elements and internally calibrated to wavelength range of 200-660nm.

Optical power and exposure times were conservatively set to meet the laser safety standards for human applications. Each LED was independently adjusted to output excitation power of 6±2 µW to tissue. The integration time of the CCD detector was set to125 ms. Background signal (noise) was obtained by turning off both LEDs prior to each spectral data acquisition. Spectral data acquisition time (background, 290, 340) was less than 2 second.

Length, width, and height of the fluorometer were 18.4, 19.4, and 11.1 cm, respectively, with a footprint (length x width) of 357 cm^2 and weight of 2.5 kg. It was powered by 5 V, 120 W power supply. The device was tested at ITC Engineering Services Inc., Sunol, CA, to meet regulatory requirements for safe operation in hospital environment.

Software for user interface and fluorometer operation were developed in-house using National Instruments LabView Version 2011 and OOI LabView drivers. Software was designed for Windows 7 Professional, 64-bit operating system on a laptop computer with an Intel Core i5-2410M, 2.30GHz, 4 GB SDRAM, and 320 GB hard drive. Pre-clinical system performance was evaluated using surgically excised prostates, canines, and human male cadavers.

B. Human Feasibility Study Design

Colorado Multiple Institutional Review Board (COMIRB) protocol #12-0740, "*Preliminary Assessment of Optical Spectroscopically-Guided Needle Biopsies for Diagnostic Accuracy of Prostatic Carcinoma: A Feasibility Study*," was designed to assess the initial feasibility of an optical biopsy needle, core biopsy instrument, fluorometer, and laptop with operating software. The study was a prospective, single center, non-randomized, feasibility study designed for a total of 30 male patients with prostate cancer scheduled to undergo radical retropubic prostatectomy surgery. After receiving the COMIRB approval, study was conducted at the University of Colorado Hospital in July-December, 2012. Thirteen patients consented to the study.

The primary study objective was to evaluate safety and effectiveness of the optical biopsy needle to acquire spectral data and correlative tissue biopsy cores for real-time *in vivo* prostate cancer diagnosis in clinical settings. Secondary objective was to evaluate the impact of interference from oxy- and deoxy hemoglobin (blood) on accuracy of tissue classification [9]. The *in vivo* optical biopsies were performed in the OR during radical prostatectomy surgery on the exposed prostate with blood flow to the gland intact. Multiple *in vivo* biopsy core samples and correlative spectral data were obtained from each patient within a 10-minute period. Multiple *ex vivo* biopsy core samples and spectral data were also obtained from each surgically excised prostate within a 90-minute period in the Pathology Lab [7].

Spectral data and corresponding tissue biopsy cores were obtained from different locations within each prostate specimen. The distal-end of each biopsy core was inked and put inside a specimen vial containing 10% neutral buffered formalin (NBF). Each specimen vial was labeled accordingly to correlate with spectral data. Following *in vivo* and *ex vivo* data collection, the prostate specimen was placed in a specimen container with 10% NBF.

C. Data Analyses

Standard hematoxylin and eosin (H&E) stain slides were prepared for histopathological classification from formalin-fixed biopsy cores. Spectral data was correlated with histopathology of tissue within a 0.5 mm wide *measurement window* located 1.7 mm from the inked-end of the biopsy core as shown in *Fig. 3* [7]. Tissue was classified as either

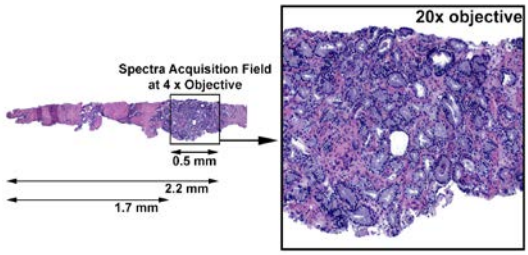

Figure 3: *Measurement window* on H&E stained biopsy core

benign or malignant. Benign category includes cores that did not show any evidence of malignancy within *measurement window*. In the *Fig. 3*, 80% of tissue within *measurement window* is cancer. Some biopsy cores were not included in the study due to either fragmentation or off-setting errors. Some cores were broken, i.e., fragmentation errors. Some cores were not correctly placed at the distal-end of the needle, i.e., off-setting errors. These cores were adequate for diagnostic purposes. However, histopathology of these cores could not be reliably correlated with corresponding spectral data and therefore excluded from the subsequent analysis.

Spectral and histopathology data were processed according to the steps shown in *Fig. 4*. Background signal was subtracted from spectral data. Signal-to-noise (S/N) ratio was determined as the ratio of peak amplitude of the signal to standard deviation of the background signal. Data was excluded from the analysis when S/N $\leq$6. Moving average was used to filter out high frequency noise modulated with the spectra. Standard interpolation technique was used for partitioning each spectrum into integer wavelengths at 2 nm intervals. Each spectrum was normalized to the area under the curve to eliminate inter- and intra-patient variations [8].

Partial least square analyses of tissue spectra was carried out to reduce data dimension and identify principal components (PC) that can used as potential classifiers for benign versus malignant tissue classification [10]. Wilcoxon rank-sum test was used to identify statistically significant PCs. Pearson correlation coefficient was used to determine whether any of the statistically significant PCs were correlated or not. Statistically significant ($P < 0.05$) and least correlated PCs ($R<0.4$) were used for tissue classification. Selected PCs were tested for their ability to differentiate between benign versus malignant tissue using support vector machine (SVM) with linear and non-linear (radial basis function) kernels [11]. The leave-one-out cross validation method and SVM learning were used to determine sensitivity (SE), specificity (SP), negative predictive value (NPV), and positive predictive value (PPV) for tissue classification.

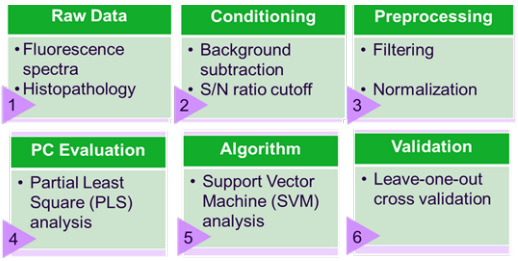

Figure 4: Steps involved in the optical biopsy needle data analysis

III. EXPERIMENTS AND RESULTS

The patient population consisted of 13 men with a mean age of 60.9±5.9 years (range: 49.2-71.0) and serum PSA of 6.5±2.7 ng/mL (range: 2.5-11.6). Histopathological analysis found cancer in 29/208 *in vivo* and 51/224 *ex vivo* viable biopsy cores. The mean length of biopsy cores were 11.8 ± 4.2 mm for *in vivo* and 13.2 ± 4.4 mm for *ex vivo*. Patients #13's *in vivo* (10 benign, 2 malignant) and *ex vivo* (10 benign, 13 malignant) biopsy cores were excluded as spectral data was collected from a defective biopsy needle with broken fiber. Patient #2's *in vivo* (19 benign) biopsy cores were excluded due to wavelength calibration errors of the fluorometer used. After exclusion of biopsy cores with S/N ≤6, final analyses included only 156 *in vivo* (25 malignant) and 174 *ex vivo* (32 malignant) biopsy cores.

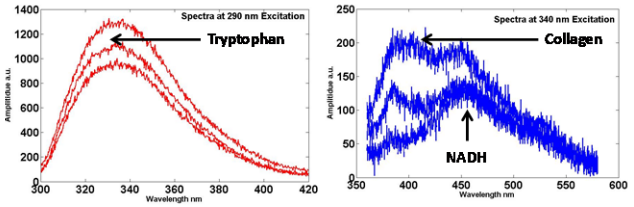

Figure 5: fluorescence spectra of prostate tissue

Fig. 5 shows background subtracted fluorescence spectra of prostate tissue at 290 and 340 nm excitations. Notable peaks are related to tryptophan, collagen, and NADH. *Fig. 6* illustrates the mean FS of benign and malignant prostate tissue. There was better separation between benign versus malignant *ex vivo* spectra compared to *in vivo* spectra because *in vivo* spectra were subjected more background noise due to interference from oxy- and deoxy hemoglobin on optical signals. This affects the accuracy of *in vivo* tissue classification.

SVM analysis with linear kernel function showed 56% SE, 70% SP, 89% NPV, and 26% PPV for *in vivo*, and 75% SE, 80% SP, 93% NPV, and 46% PPV for *ex vivo* malignant versus benign prostate tissue classification. Tissue classification results for *ex vivo* data were better than *in vivo* data due to lack of interference from blood as illustrated in *Fig. 6*. Spectral data were Z-score normalized (mean of zero with standard deviation of one) following area-under the curve normalization to further improve *in vivo* tissue classification. Ensuing SVM analysis provided 72% SE, 66% SP, 93% NPV, and 29% PPV for *in vivo* malignant versus benign prostate tissue classification. SVM analysis with non-linear kernel function also gave similar results.

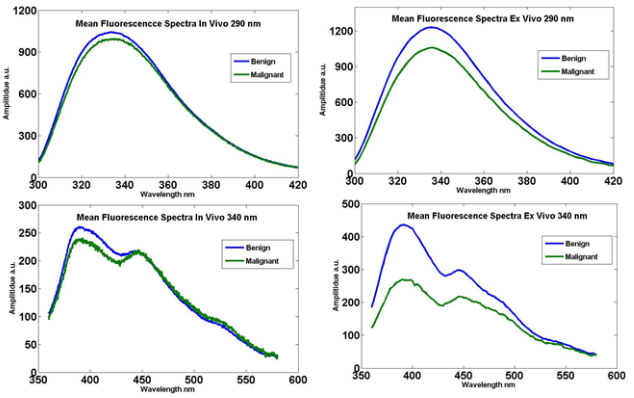

Figure 6: Mean fluorescence spectra of benign (blue) and malignant (green) prostate tissue at 290 nm (top) and 340 nm (bottom) excitations for *in vivo* (left) and *ex vivo*

IV. CONCLUSION

The human feasibility study demonstrated potential clinical application of an optical biopsy needle based on fluorescence spectroscopy for prostate cancer diagnosis. All custom-made products performed within specifications. High NPV value ensures benign tissue can be accurately identified and thereby reducing the number of benign biopsy cores. It also has sufficient SE to target areas suspicious for cancer. Optical power output of the fluorometer must be increased to overcome interference from blood while maintaining safe exposure levels. Hence, systematic use of TRUS-guided optical biopsy needle has the potential for accurate diagnosis of this disease for improved patient care.

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