Feasibility of minimally-invasive fiber-based evaluation of chondrodystrophoid canine intervertebral discs by light absorption and scattering spectroscopy

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ABSTRACT

Extrusion or protrusion of an intervertebral disc is a common, frequently debilitating, painful, and sometimes fatal neurologic disease in the chondrodystrophic dog (dachshund, Pekingese, etc.). A similar condition of intervertebral disc degeneration with extrusion/protrusion is also a relatively common neurologic condition in human patients. Degeneration of the relatively avascular chondrodystrophoid intervertebral disc is associated with loss of water content, increased collagen, and deposits of calcified mineral in the nucleus pulposus. Current diagnostic methods have many limitations for providing accurate information regarding disc composition in situ prior to surgical intervention. Disc composition (i.e., mineralization), can influence the type of treatment regime and potentially prognosis and recurrence rates. The objective of this study is to investigate the feasibility of using a fiber-needle spectroscopy sensor to analyze the changes of tissue compositions involved in the chondrodystrophoid condition of the canine intervertebral disc. The nucleous pulposus, in which the metaplastic process / degeneration develops, is approximately 2mm thick and 5mm in diameter in the dachshund-sized dog. It lies in the center of the disc, surrounded by the annulus fibrosis and is enclosed by cartilaginous vertebral endplates cranially and caudally. This “shallow-and-small-slab” geometry limits the configuration of a fiber probe to sense the disc tissue volume without interference from the vertebrae. A single-fiber sensor is inserted into a 20 gauge myelographic spinal needle for insertion into the disc in situ and connected via a bifurcated fiber to the light source and a spectrometer. A tungsten light source and a 940nm light-emitting-diode are combined for spectral illumination covering VIS/NIR with expected improved sensitivity to water. Analysis of the reflectance spectra is expected to provide information of scattering and absorption compositions of tissue in proximity to the fiber-tip. Preliminary measurements on cadaveric canine intervertebral discs indicated significant reduction of scattering constituents and possible diminishment of water content after percutaneous laser disc ablation (PLDA). This fiber-needle based sensing configuration may be feasible for integrating the evaluation of calcification and water content into the work-flow of holmium:YAG laser disc ablation for pre-operative in-line detection and post-operative evaluation of therapeutic interventions regarding the chondrodystrophic disc.

Key words: intervertebral disc, degeneration, reflectance spectroscopy, scattering, percutaneous laser disc ablation.

1. INTRODUCTION

Intervertebral disc disease is a common, frequently debilitating, painful and sometimes fatal neurologic disease that occurs in chondrodystrophic dog breeds (dachshund, Pekingese, etc.). The disease occurs in dogs of any age [1], but it has an increased occurrence in middle-age dogs.
(4 – 7 years). It is a unique age-related process of disc degeneration that can affect the entire length of the vertebral column. A similar condition of intervertebral disc degeneration with extrusion/protrusion is also a relatively common neurologic condition in human patients.

The underlying etiology for most disc degeneration seems to be associated with dehydration of the gelatinous nucleus pulposus, a metaplastic process that involves fibrosis and deposition of a calcified mineral component within the nucleus itself. Typically, it involves multiple discs within the same dog. Figure 1 shows the anatomical position of the disc and its internal structure [2]. The dog’s spinal column consists of the spinal cord and surrounding vertebrae. Intervertebral discs are structures located between the vertebrae that act as “shock absorbing cushions” [2]. Each disc consists of a tough outer fibrous lamellar layer, the annulus fibrosus, and a gelatinous inner core, the nucleus pulposus. The principal component of the nucleus pulposus is water, making up to 80% to 88% of its content [3]. Water is confined to the disc in the form of proteoglycan, making the nucleus mostly transparent and appearing as a visco-elastic gel. When disc degeneration develops, water content within the nucleus pulposus decreases, and calcification often occurs. As the degenerative process continues, the material in the nucleus pulposus resembles and has a cottage cheese consistency, which radiographically is shown as opacification of the disc space [4, 5]. Extrusion or protrusion of degenerative nucleus pulposus through the annulus fibrosus results in serious pain and can proceed to paralysis and myelomalacia. A recommended prophylactic procedure for reducing risk of the recurrence in dogs with thoracolumbar disc disease is percutaneous laser disc ablation (PLDA), a minimally invasive procedure that uses a holmium yttrium aluminum garnet (Ho:YAG) laser [6]. PLDA is reported to reduce the volume and stabilize the nucleus pulposus, thereby reducing the risk of neurologic recurrence to approximately 3.4% [6].

Intervertebral disc degeneration is commonly diagnosed by radiographic examination. Radiography is reported to have a sensitivity of 60% and specificity of 100% when using histopathology as the gold standard [5]. MRI and CT have also been used for the diagnosis of intervertebral disc degeneration [4, 5, 7]. There are also recent studies of using polarization-sensitive optical coherence tomography to diagnose degenerated intervertebral discs in bovine and equine species [8][9]. The contrast differences using optical coherence tomography for imaging intervertebral disc degeneration results primarily from structural alterations of the outer annulus fibrosus. As the structural alteration may be preceded by bio-chemical changes,
sensitivity to the tissue microscopic constituents may render earlier and more accurate diagnosis of intervertebral disc degeneration.

Near-infrared reflectance spectroscopy (NIRS) is extensively employed for noninvasive or minimally invasive qualification or quantification of tissue optical properties, which are determined by tissue micro-architecture and bio-chemical compositions. In terms of fiber-needle based configuration, differential path length spectroscopy \cite{10, 11} was implemented to measure the local optical properties. Single fiber reflectance \cite{12, 13} geometry was also investigated in staging lung cancer through examination of mediastinal lymph nodes \cite{14}. The current investigation introduces an approach for minimally invasive single-fiber based evaluation of chondrodystrophoid canine intervertebral discs by light absorption and scattering spectroscopy.

This study differs from previous works in that the tissue being studied is relatively avascular, in other words, the major absorber of NIR light in the intervertebral disc is chromopore content other than hemoglobin as in many other non-cutaneous tissues. The study was proposed based on the following hypotheses: (1) the degenerated intervertebral discs will likely have increased optical scattering as a result of calcification; (2) the degenerated intervertebral discs will be associated with a decrease in optical absorption due to water, specifically at the spectral peaks sensitive to water content. Both mechanisms are expected to contribute to increasing the spectral reflectance intensity in a single-fiber configuration. Differentiating the contribution to the increased spectral reflectance by the mineralization from that by the loss of water entails decoupling the effect of increased scattering and decreased absorption of the spectral reflectance.

The current work involves primarily the construction of a single-fiber based reflectance spectroscopy system and calibration of the system. Preliminary analysis of the baseline data acquired from cadaveric canine intervertebral discs prior to and after percutaneous laser disc ablation (PLDA) demonstrated that the changes of diffuse reflectance spectra ranging from 550nm to 950nm correlate to the expected changes in scattering and absorption contents of the intervertebral disc.

2. MATERIALS AND METHODS

2.1 Fiber-needle reflectance spectroscopy system
The configuration of the needle-based signal-fiber reflectance spectroscopy system is illustrated in Fig. 2. The VIS/NIR light was generated by a compact deuterium tungsten light source (L10671, Hamamatsu Photonics, Japan) coupled with a 940nm light-emitting-diode (LED) (M940L2, Thorlabs, NJ, USA). Each of the two light sources was coupled to one branch of a bifurcated fiber A of 200µm core-diameter (BIF200-VIS/NIR, Ocean Optics Inc, USA) to integrate the spectra of the two light sources at the combining branch. The combining branch of fiber bundle A was coupled to one branch of a 400µm core-diameter bifurcated fiber B (BIF400-VIS/NIR, Ocean Optics Inc, USA), the other branch of whom was connected to a spectrometer (VIS-NIR Hyperspectral USB Spectrometer, NT58-303, Edmund Optics Inc, NJ, USA). The combining branch of the fiber bundle B was connected to a standard PLDA fiber of 320µm in core-diameter fiber (fiber C shown in Fig.2, H320R, New Star Lasers, Inc, CA, USA). The fiber
C was placed in the tissue by inserting through a 20 gauge myelegraphic spinal needle. The tip of fiber C was polished to an angle of 15 degrees \cite{13} to minimize the back-reflection. Despite angle-polishing of the fiber tip, there were reflections from the fiber tip as well as multiple stages of fiber connections in the light pass from the source to the tissue. The internal-reflections and fiber-air reflection resulted in a base-line spectrum, which after applying a Savitzky-Golay smoothing filter (SG filter) \cite{15} is like the one shown in Fig. 3 The spectrometer responses to a spectral range of 350-1050nm, however the integrated source has low overall illumination below 550nm, and above 950nm.

![Fig. 2. Experimental setup of needle-based single fiber reflectance spectrascopy](image)

2.2 Imaging protocol

The imaging protocol on canine species has been approved by the Institutional Animal Care and Use Committee (IACUC) of Oklahoma State University. For this study, however, only cadaveric dogs were used for base-line testing to discover the relationship between the changes of tissue reflection spectra with the expected changes of disc tissue contents. The single-fiber optical reflectance spectroscopy system is compatible to and readily integratable in the PLDA procedure. The discs being evaluated include T (Thoracic) 9-10 to L (Lumber) 5-6. A 20 gauge myelographic spinal needle was positioned in the nucleus of the intervertebral disc under fluoroscopic guidance. After retracting the stylet of the needle, the fiber sensor was introduced to the middle of the nuclues of the disc, as shown in Fig. 4, and was positioned approximately 1mm distal to the tip of the spinal needle by a marker placed on the proximal end of the fiber. For each disc, a total of 5 repeated spectral measurements were taken at integration time of 2 seconds per measurement, a time scale that is limited by the weak illumination of the compact Tungsten light.
source. After preoperative measurements, 40 seconds of PLDA laser ablation procedure was performed on each disc using a 2W Ho:YAG laser. Post-ablation reflectance spectra were then measured following the same procedure for preoperative measurements. The measurements from per-operative and post-operative discs were compared against those from air and water, as will be described afterwards. The total time necessary for spectral data collection for 10 discs adds approximately 10 minutes to the overall PLDA procedure. The measured spectra were displayed in realtime and stored automatically, but the analysis of data was performed off-line.

![Fig. 4. Procedure of inserting the sensing fiber to the intervertebral disc via spinal needle (a), the position of which in the disc (nucleus pulposus) is visually confirmed by fluoroscopy.](https://www.spiedigitallibrary.org/conference-proceedings-of-spie)

2.3 Cadeveric canine subject involved in this preliminary study

Five cadaveric canines were tested and the summary of disc data collected from canine cadaver specimens were shown in Table 1. Before the tests, radiographs were performed to locate the suspicious degenerated discs. Discs L2-3 and L3-4 in dog M were considered mineralized according to the radiograph. Spectra were collected on disc specimens located from T (Thoracic) 9-10 to L (Lumber) 5-6 on each dogs and then laser ablation was carried out on most discs being studied. After the laser ablation, the spectra of discs that has been previous measured were recollected.

<table>
<thead>
<tr>
<th>Canine Cadaver ID</th>
<th>Mineralized Discs on Radiographs</th>
<th>Spectroscopy Performed (pre and post ablation)</th>
<th>Laser Ablated Discs</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>L2-3, L3-4</td>
<td>T10-11, T11-12, T13-L1 thru L5-6</td>
<td>T13-L1 thru L3-4</td>
</tr>
<tr>
<td>Q</td>
<td>none</td>
<td>T7-8, T9-10 thru L5-6</td>
<td>T10-11 thru L3-4</td>
</tr>
<tr>
<td>Y</td>
<td>none</td>
<td>T8-9, T10-11 thru L5-6</td>
<td>T10-11 thru L4-5</td>
</tr>
<tr>
<td>Z</td>
<td>none</td>
<td>T12-13, L1-2, L3-4 thru L5-6</td>
<td>T12-13, L1-2, L3-4, L4-5</td>
</tr>
</tbody>
</table>

3. CALIBRATION METHODS

3.1 Analysis of the photon path-length and sampling volume
For this study, since the intervertebral disc is less than one centimeters in diameter (depending on the size of dogs) and a couple of millimeters in thickness, the nucleus pulposus within the intervertebral disc forms a “shallow-and-small-slab” geometry that is potentially challenging for the fiber-needle measurement. It is therefore imperative to estimate if the surrounding annulus fibrosus or vertebrae have significant effect on the measurement of the nucleus pulposus. The evaluation is relative to the path length of photon and the sampling volume in the given geometry.

For single-fiber based reflectance spectroscopy measurement, Kanick, et al. used Monte Carlo modeling to analyze the reflectance path length and sampling depth \([12]\). They proposed an empirical model, in the spectra range of 550nm up to 800nm, for photon path length \(L\) and sampling depth \(Z\) \([13]\) for single-fiber reflectance spectroscopy, which is adopted in this study as:

\[
L = \frac{1.34d e^{0.17d}}{(u_s'd)^{0.23}(0.52 + (u_a'd)^{0.52})}
\]

(1)

\[
Z = \frac{0.38e^{-0.06u_a'd}}{(u_s'd)^{0.12}} L
\]

(2)

![Fig. 5 Path length and sampling depth of signal fiber spectroscopy](image)

Where \(d\) is the diameter of the fiber, and \(u_a, u_s\) is the absorption coefficient and the reduced scattering coefficient, respectively. This empirical model was implemented in [13] for the parameters of \(u_a = [0.1-3] mm^{-1}, u_s' = [0.2-4] mm^{-1}\), and \(d = [0.2-2] mm\). In this study this empirical model given in Eqs. (1) and (2) was evaluated for the parameters of \(u_a = [0.001-1] mm^{-1}\), \(u_s' = [0.1-2] mm^{-1}\), and \(d = 0.32 mm\) for the estimation of the photon path-length as well as the sampling depth, as shown in Fig. 5. The indications in Figure 5 agree with the physical fact that light penetrates deeper and experiences longer path-length as the scattering and absorption coefficients decrease. It is estimated from Fig. 5 that when the reduced scattering and absorption...
coefficients are 0.5 mm$^{-1}$ and 0.001 mm$^{-1}$, the sampling depth and photon path-length for a 0.32 mm fiber is approximately 0.6 mm and 1.3 mm, respectively. This implies that if the fiber is placed in the middle of the nucleus, the interference of the surrounding annulus fibrosus and vertebra on the measurement is small.

### 3.2 Analysis of the reflection spectra

The raw reflectance spectra acquired in single-fiber configuration contain reflectance at the fiber/medium interface and some internal reflections. The raw spectra measured from a tissue sample, denoted as $R(\lambda)$, is normalized by

$$R_{\text{norm}}(\lambda) = \frac{R(\lambda) - R_{\text{water}}(\lambda)}{R_{\text{air}}(\lambda) - R_{\text{water}}(\lambda)}$$

(3)

with respect to the spectra measured from water $R_{\text{water}}(\lambda)$, and air $R_{\text{air}}(\lambda)$. The reflectance spectral from a scattering-dominant medium is modeled by

$$R(\lambda) = (a_1u'_s(\lambda) + a_{10})e^{-\mu_a(\lambda)L}\phi(\lambda) + \eta \times \phi(\lambda)$$

(4)

where $\phi(\lambda)$ is the native spectra profile of the source, $\eta$ represents the inter-fiber reflectance, $L$ is the path length of the light from leaving the fiber to returning to the fiber after scattering through the tissue. For non-scattering medium such as water and air, the reflectance spectra is modeled differently from Equ. (4), due to the dominant specular reflection, as

$$R_{\text{water}}(\lambda) = \eta_{\text{waterfiber}} \phi(\lambda) + \eta \times \phi(\lambda)$$

(5)

$$R_{\text{air}}(\lambda) = \eta_{\text{airfiber}} \phi(\lambda) + \eta \times \phi(\lambda)$$

(6)

where $\eta_{\text{waterfiber}}$ and $\eta_{\text{airfiber}}$ are the water/fiber and air/fiber index mismatch, respectively.

Substitute Eqs. (5)-(6) into Equ. (1), we have

$$R_{\text{norm}}(\lambda) = \frac{R(\lambda) - R_{\text{water}}(\lambda)}{R_{\text{air}}(\lambda) - R_{\text{water}}(\lambda)}$$

$$= \frac{(a_1u'_s(\lambda) + a_{10})e^{-\mu_a(\lambda)L}\phi(\lambda) + \eta \times \phi(\lambda) - \eta_{\text{waterfiber}} \phi(\lambda) + \eta \times \phi(\lambda)}{\eta_{\text{airfiber}} - \eta_{\text{waterfiber}}}$$

(7)

from which the effect of non-uniform source profile and fiber-transmission could be suppressed.

The validity of this normalization was examined using different concentrations of Intralipid solution. For Intralipid within the spectral range of 550 nm to 800 nm as in [13, 14], by neglecting small $\mu_a$ of water for a millimeter-scale path-length, Equ. (7) reduces to

$$R_{\text{norm}}(\lambda) = \frac{R(\lambda) - R_{\text{water}}(\lambda)}{R_{\text{air}} - R_{\text{water}}} = \frac{(a_1u'_s(\lambda) + a_{10}) - \eta_{\text{waterfiber}}}{\eta_{\text{airfiber}} - \eta_{\text{waterfiber}}} = \alpha u'_s(\lambda) + \beta$$

(8)

which indicates that the reflectance spectra can be considered linearly proportional to $u'_s$. Mie theory also indicates that
\[ R_{\text{norm}}(\lambda) = \alpha u_s'(\lambda) + \beta = \alpha A\lambda^{-b} + \beta \] (9)

For spectral range spanning from 800 to 950nm, the reflectance spectra of intralipid will be a combined effect of scattering as in (9) with the absorption by water and lipid droplets. Figure 6 shows the reflectance spectra, processed by use of Equ. (3), of intralipid at different concentrations within wavelength 550 nm to 950nm. The concentration of the intralipid ranged from 0.1% to 10%, representing \( u_s' \) changing approximately from 0.1mm\(^{-1} \) to 10mm\(^{-1} \). In order to verify the consistancy of the results, six rounds of tests were performed. For each round of testing, the air and water spectrum were measured and then the intralipid spectrum were obtained from 0.1% to 10%, all of which were performed in dark container to eliminate the influence of ambient light. Moving averaging over one-hundred points was applied to further smooth the spectra profile. For the wavelength range lower than 800nm, scattering dominated and the reflectance intensity has a near-linear relationship to the scattering coefficient of Intralipid as indicated in Fig. 7. The power-law fit given in Equ. (9) (red line) is applied to the spectra of 10% intralipid in the 550nm-800nm range in Fig. 8, which showed good match. The extropolation of that power-law fit to the spectra beyond 800nm deviates from the measurement, indicating the increased contribution of water or other chromophores to the reflectance spectra.

![Fig. 6 The reflectance spectra of Intralipid, after applying the analysis given above, measured at different concentrations.](image-url)
4. PRELIMINARY RESULTS OF BASE-LINE TESTING ON CADEVERIC DOGS

From the preliminary tests on canine cadaveric intervetal disc in situ, changes of reflectance spectra prior to and after disc laser ablation were observed. The average spectra acquired prior to PLDA (red solid line) and after PLDA (green dashed line) of the four dogs are compared in Fig. 9. The common feature of the 4 measurements is that the post-PLDA spectra are wavelength-independent in the range of 650—950nm, whereas the pre-PLDA spectra were all similar to the intralipid spectra in terms of the decreasing trend as the wavelength increases. For three out of four dogs, the average reflectance spectra of post-PLDA measurement was lower than that of pre-PLDA measurement in spectral range below 800nm, however, the data have significant variance. Nevertheless, the flatness and reduction of intensity of the post-PLDA spectra agree with the PLDA process that destroyed the tissue cellular structures thereby removing the scattering particles.
Fig. 10 showed the average spectrum intensity of the pre-PLDA discs and post-PLDA discs of Dog Y together with the intralipid spectrum. The pre-PLDA spectra are close to the spectra of 1% intralipid at below 700nm, but decreased toward the increasing wavelength. This difference in spectral profile may be related to the difference in average size of the scatterer as well as the size distribution of the scatter, between of the intralipid and of the disc tissue. The shorter-wavelength side of the reflectance spectra obtained from the tissue is seen with some variations that maintain after the PLDA. When the post-PLDA spectra were deducted from the pre-PLDA spectra, the resulting spectra, as shown in Fig. 11, is fitted by Equ. (9). The outcome of the fitting in Fig. 11 resembles that in Fig. 8, but with higher scattering power than that fitted for 10% intralipid. The spectra acquired from the radiographically indicated mineralized discs Dog M, however, did not show significant difference with respect to the other radiographically indicated normal discs, which is subject to comparing with pending histopathology results.
This work proposed a fiber-needle based sensing configuration for evaluating the changes of scattering in canine intervertebral disc in response to laser disc ablation, toward detecting the mineralization and water-loss of the intervertebral disc due to degeneration. A simple empirical model was introduced to account for the intensity changes of the single-fiber reflectance spectra due to changes of reduced scattering coefficients in the sub-800nm range. The measurements on cadaveric canine intervertebral discs indicated significant reduction of scattering constituents after laser ablation of the disc. More works are to be done to quantify the changes of single-fiber reflectance spectra

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References