Minimizing Variations in Fiber Optic Visible-NIR Spectral Data Collection in Arthroscopic Settings

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INTRODUCTION: While current diagnostic arthroscopy techniques enable joint tissues to be visually assessed and mechanically evaluated, the information obtained is generally qualitative. Application of visible-near infrared (VNIR) spectroscopy offers the potential to obtain quantitative compositional data related to the tissues of interest. Previously, VNIR spectroscopy using a fiber optic probe has been used for nondestructive assessment of musculoskeletal tissues *in vitro* and *ex vivo*, but limited *in vivo* studies have been carried out. Some of the challenges of implementing this analytical technique for *in vivo* use include spatial limitations of the joint anatomy and interference of absorbances from the irrigation fluid (saline) used during the arthroscopy procedure. The objective of this work is to identify an optimized approach for data collection and analysis of tissues in a hydrated environment that could be applied for future *in vivo* studies, including use of a fiber optic probe with a 90 degree bend at the tip, designed for arthroscopic data collection.

METHODS: Samples: Porcine patellas were acquired from Animal Technologies, Inc. A 4 mm diameter biopsy punch was used to obtain tissue segments (plugs) for spectral analysis. Cartilage, bone, and osteochondral plugs (n=6 for each) were obtained from the patella and kept hydrated until use. Spectroscopy: An ASD Labspec 4 spectrometer was used for spectral collection coupled with a fiber optic probe. The fiber optic probe contained a 90 degree bend at the tip and a 1.5 mm thick glass spacer between the contact surface of the probe and the end of the fibers. A Spectralon standard was used for both the baseline collection and as the background for data collection. The spectra were collected in diffuse-reflectance mode with 50 co-added scans per spectrum over the visible (350 – 700 nm) and NIR (12,500 – 4,000 cm^{-1}) regions. For each of the tissue types, spectra were collected under several conditions (**Table 1**). These conditions were selected to both determine the optimized probe distance from the sample for spectra collection and to simulate potential situations that could occur during arthroscopic data collection related to the presence of saline. Spectral data analysis: All raw spectra were processed using the Unscrambler X software (CAMO) for obtaining the 2nd derivative spectra (Savitzky-Golay filter, 45 smoothing points) to resolve contributions to the broad absorbances, and computing principal component analysis (PCA) for each of the tissue groups. Statistical analysis: PCA was performed separately using the spectra collected from each tissue type to assess the impact of the presence of additional saline during the data collection with and without probe-tissue contact. Outcomes were assessed by evaluation of scores plots.

RESULTS: The NIR spectral region contained more information to assess the contribution of saline to the spectra compared to the visible region. Osteochondral samples: Averaged raw and inverted 2nd derivative spectra (inverted to have peaks positive) (**Figure 1**) show subtle differences in absorbances based on whether saline is present, and if the probe is in contact with the tissue (0 mm distance), or not in contact (1 or 2 mm distance). The scores plot from PCA of the 2nd derivative in the NIR region (**Figure 2**), specifically 12,500 – 5,000 cm^{-1}, shows separation of the spectra collected from the different experimental conditions. The spectra collected with the probe in contact with the tissue, both with and without environmental saline, are shown separated from those collected with saline in between the probe tip and sample, primarily along PC-1 (72%). However, the scores for the spectra collected with additional saline with the probe in tissue contact were more similar to the scores for the probe in contact with the tissue with no saline present. Cartilage samples: PCA for the cartilage experiments also demonstrated separation of the data collection groups along PC-1 (61%), however the samples collected with probe contact, with and without environmental saline were difficult to distinguish from each other. Bone samples: PCA for the bone samples over the same spectral region showed similar results to those of the osteochondral and cartilage samples. The groups of spectra collected were separated primarily along the PC-1 plot (70%), but the spectra collected with the probe in contact with the bone samples, both with and without additional saline, were not separated into distinct groups of scores.

DISCUSSION: As demonstrated through the PCA for each of the tissue types, maintaining the probe in contact with the tissue during data collection minimized the contribution of the surrounding saline on the spectra. Additionally, spectra were able to be separated based on the environmental condition (amount of saline between the probe tip and tissue sample), indicating potential for model development for assessing whether spectra are being collected with appropriate tissue contact arthroscopically. Because maintaining probe contact with the sample has demonstrated a reduction of the external saline interference with the spectral data, this supports further development of a data collection strategy that confirms probe-tissue contact *in situ*.

SIGNIFICANCE/CLINICAL RELEVANCE: Current diagnostic arthroscopy techniques have limitations, such as the qualitative nature of the tissue assessment. Application of fiber optic VNIR spectroscopy offers potential to complement arthroscopic tissue assessment by providing compositional tissue analysis, improving overall diagnostic capability.

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Table 1

Hydration Condition	Distance between probe tip and tissue surface (mm)
No additional hydration	0
No additional hydration	1
100 µL of additional saline covering tissue	0
100 µL of additional saline covering tissue	1
100 µL of additional saline covering tissue	2

Figure 1

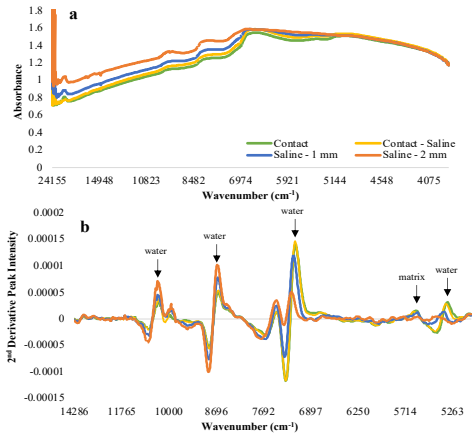

Figure 2

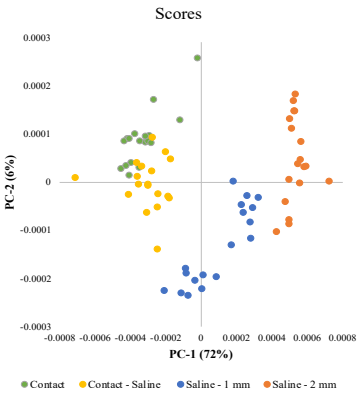

Table 1: Summary of experimental conditions for spectra collection
Figure 1: Averaged raw (b) and second derivative (b) spectra from osteochondral samples grouped by experimental condition, Tissue water absorbances are the most prominent spectral features.
Figure 2: PCA scores plot for 2nd derivative spectra of osteochondral samples from 12500 cm^{-1} – 5000 cm^{-1}