Estimation of articular cartilage properties using multivariate analysis of optical coherence tomography signal

P.H. Puhakka †‡*, N.C.R. te Moller §, I.O. Afara †, J.T.A. Mäkelä †, V. Tiitu †, R.K. Korhonen †, H. Brommer §, T. Virén †¶, J.S. Jurvelin †, J. Töyräs ††

† Department of Applied Physics, University of Eastern Finland, Kuopio, Finland
‡ Department of Clinical Neurophysiology, Kuopio University Hospital, Kuopio, Finland
§ School of Medicine, Institute of Biomedicine, Anatomy, University of Eastern Finland, Kuopio, Finland
 parade of Equine Sciences, Utrecht University, Utrecht, Netherlands
¶ Cancer Center, Institute of Biomedicine, Anatomy, University of Eastern Finland, Kuopio, Finland

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SUMMARY
Objective: The aim was to investigate the applicability of multivariate analysis of optical coherence tomography (OCT) information for determining structural integrity, composition and mechanical properties of articular cartilage.

Design: Equine osteochondral samples (N = 65) were imaged with OCT, and their total attenuation and backscattering coefficients (μs and μb) were measured. Subsequently, the Mankin score, optical density (OD) describing the fixed charge density, light absorbance in amide I region (Aamide), collagen orientation, permeability, fibril network modulus (Ei) and non-fibrillar matrix modulus (Em) of the samples were determined. Partial least squares (PLS) regression model was calculated to predict tissue properties from the OCT signals of the samples.

Results: Significant correlations between the measured and predicted mean collagen orientation (R² = 0.75, P < 0.0001), permeability (R² = 0.74, P < 0.0001), mean OD (R² = 0.73, P < 0.0001), Mankin scores (R² = 0.70, P < 0.0001), Em (R² = 0.50, P < 0.0001), Ei (R² = 0.42, P < 0.0001), and Aamide (R² = 0.43, P < 0.0001) were obtained. Significant correlation was also found between μb and Ei (P = 0.280, P < 0.03), but not between μs and any of the determined properties of articular cartilage (P > 0.05).

Conclusion: Multivariate analysis of OCT signal provided good estimates for tissue structure, composition and mechanical properties. This technique may significantly enhance OCT evaluation of articular cartilage integrity, and could be applied, for example, in delineation of degenerated areas around cartilage injuries during arthroscopic repair surgery.

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Introduction

Articular cartilage injury can initiate development of osteoarthritis (OA) in both human and animal joints1,2. As a consequence to injury, chondrocytes of articular cartilage may die or get damaged, cartilage matrix disruption occurs, and proteoglycan (PG) content decreases, initiating the development of post-traumatic OA1. The superficial zone of cartilage is the initial zone in which degenerative changes are typically encountered. In the early stage of cartilage degeneration tissue water content increases and the collagen fibrils are disorganized3. These changes further lead to increased permeability and decreased stiffness of articular cartilage.

Several surgical techniques, (e.g., microfracturing and tissue and cell transplantation) are used to repair cartilage injuries and prevent the development of post-traumatic OA3. The choice for the optimal repair technique is based on the location and size of the lesion3, which are usually visually assessed during arthroscopy. However, the detection of early-stage degenerative changes in tissue surrounding the lesion, by means of arthroscopic examination, is difficult4,5. To improve treatment planning, it would be beneficial...
to have a more accurate, high-resolution arthroscopic technique for diagnosing early-stage degeneration around injuries and to delineate the optimal cartilage region for repair. Identification of early, potentially reversible changes in articular cartilage would also be essential for development of disease-modifying treatment methods.

Optical coherence tomography (OCT), an arthroscopically applicable technique, provides adequate resolution (~10–20 μm) for detection and assessment of articular cartilage lesions. Morphological features of cartilage surface observed in OCT images agree with the ones found in histological investigation. Furthermore, abnormal organization of collagen fibrils can be detected as a lack of birefringence using polarization sensitive OCT. OCT imaging is based on the measurement of intensity of the light backscattered from different depths of the tissue. The measured intensity depends on light attenuation in the tissue and, at each depth of the tissue, on the amount of light scattering directed to the detector. Microscopic changes in composition and structure of tissues lead to changes in attenuation and scattering properties. Early stage degenerative changes in articular cartilage, like depletion of PGs, could possibly be detected by measuring total attenuation ($\mu_t$) and backscattering ($\mu_b$) coefficients with OCT. To the authors’ knowledge, the differences in $\mu_t$ or $\mu_b$ between normal and degenerated articular cartilage have not been reported. It is known, though, that attenuation is weaker in repair tissue than in native cartilage. Further, the decrease in collagen and chondrocyte contents has been found to decrease light attenuation in agarose scaffolds.

Due to its layered nature, articular cartilage exhibits depth-dependent light backscattering and attenuation properties. Therefore, the $\mu_t$ and $\mu_b$ of the total cartilage thickness layer may not optimally represent this tissue and may not be sensitive enough to detect early stage degeneration. Thus, an alternative analysis approach for OCT data is required. In the present study, the applicability of multivariate partial least squares (PLS) regression to analyse OCT signal and predict cartilage degeneration is studied. This analytical technique has been applied for the analysis of cartilage spectroscopic data. The method is used to obtain those features of the multivariate input data (predictor variable) that explain most of the variation in the reference data (response variable) of the sample. We hypothesize that with PLS regression modelling we could obtain a more accurate approximation of articular cartilage properties as compared to measurement of bulk $\mu_t$ and $\mu_b$.

Methods

Sample preparation

Osteochondral samples were prepared from metacarpophalangeal joints of healthy, skeletally mature horses ($N = 13$) obtained from a slaughterhouse. Either left or right joint was obtained from eight horses and both from five horses. The horses had a variety of chondral lesions. Osteochondral samples ($N = 65$) were obtained from 1 to 6 anatomical locations within each joint, namely: the tips of the medial and lateral eminences of the first phalanx ($N = 34$), the opposing sites on the medial and lateral condyles ($N = 15$), and the sagittal ridge of the metacarpal bone ($N = 14$). The samples were cut into osteochondral blocks with a minimum surface area of $10 \times 10$ mm² and the area of interest (e.g., lesion) located in the center.

OCT imaging

The osteochondral samples were imaged using OCT (wavelength 1305 ± 55 nm, axial resolution <20 μm, lateral resolution 25–60 μm; Ilumien PCI Optimization System, St. Jude Medical, St. Paul, MN, USA). During the OCT imaging the samples were immersed in phosphate buffered saline (PBS). The OCT system has a rotating scanning geometry providing cross-sectional images (thickness = 0.1 mm). Each cross-sectional image consists of 504 radial scan lines obtained during one revolution (Fig. 1). The system measures the intensity of the light backscattered at different depths in each scanning direction. Five adjacent cross-sectional images were recorded from each sample. The cartilage surface was automatically detected from the cross-sections, while the cartilage—bone interface was manually determined (by PP). Cartilage thickness in the samples varied between 0.40 mm and 1.39 mm. An average depthwise intensity curve was calculated for an analysis window with a width of 21 scan lines and a height matching the cartilage thickness. Subsequently, average intensity curves of the five cross-sections in each sample were averaged. The first 5% of the curves were excluded during the analyses to avoid specular reflection at the articular surface.

Biomechanical properties

Biomechanical properties of the samples were determined by means of indentation testing. The test was conducted using a custom-made material testing system (resolution for force and deformation, 5 mN and 0.1 μm, respectively). Cartilage thickness was measured from the OCT image of the sample. The sample was submerged in PBS and a cylindrical plane-ended indenter with a diameter of 530 μm was driven into contact with the sample on the same location where the OCT imaging was conducted. The contact and full recovery of deformation were ensured by indenting the sample 5% of its thickness five times. Then, a stress-relaxation indentation test consisting of two 5% strain steps was performed. Strain rate was 100%/s relative to the thickness of the cartilage. Equilibrium was assumed to be achieved when the slope of relaxation rate was less than 10 Pa/min.

Abaqus (V6.10-1, Dassault Systèmes, Providence, RI, USA) and Matlab (2012a, The MathWorks Inc., Natick, MA, USA) were employed to calculate cartilage biomechanical parameters by fitting an axisymmetric fibril-reinforced poroelastic finite element model to the experimental stress-relaxation data. Cartilage was modelled using axisymmetric 4-node continuum pore pressure elements (CAX4P). An elastic fibrillar matrix represented the collagen network and non-fibrillar matrix represented primarily PGs and fluid. The fibrillar matrix was described with organized primary fibrils and randomly organized secondary fibrils. The indenter was modelled as rigid and the cartilage-indenter contact was assumed to be frictionless and impermeable. The cartilage—bone interface was fixed in all directions. The cartilage edge and

![OCT catheter](image)

Fig. 1. In the rotational scanning geometry, the OCT system obtains 504 radial scan lines during one 360° revolution to create a cross-sectional image of the sample. The imaging speed is 100 cross-sectional images per second.
the surface not in contact with the indenter were assumed to be fully permeable (zero pore pressure). Mechanical behaviour of the collagen network was expressed with the fibril network modulus \( (E_f) \), while non-fibrillar matrix modulus \( (E_m) \) and permeability represented the PG/fluid complex. Fluid fraction (80%) and the Poisson’s ratio of the non-fibrillar matrix \((0.42)\) were fixed in the model\(^{22,23}\), whereas \( E_f, E_m \) and permeability were obtained by minimizing the mean square error between the reaction forces in the experiment and finite element model. Since the first step was considered as a pre-strain, the optimization was performed only for the second step.

**Histology**

After indentation testing, the samples were stored in a freezer \((-20\, ^\circ\mathrm{C})\). For histological and spectroscopical analyses, the osteochondral samples were thawed, immersed in formalin for at least 48 h, and then decalcified in ethylenediaminetetraacetic acid. After further processing, three Safranin-O stained sections \((\text{thickness } = 3 \, \mu\text{m})\) for histological evaluation and three unstained sections \((\text{thickness } = 5 \, \mu\text{m})\) for spectroscopical analyses were prepared from the measurement site of each sample.

The stained sections were examined with a light microscope \((\text{Axio Imager M2}, \text{Carl Zeiss MicroImaging, Jena, Germany})\). Histological integrity of the samples were evaluated from their images by assigning Mankin scores\(^{24}\). The images of the three sections from each sample were blindly coded and scored by three investigators \((\text{NM, JT, and VT})\). The final score was calculated as an average of the scores rounded to the nearest integer. Based on Mankin scores, the samples were further divided into two groups by the severity of the degeneration; from no to mild degeneration \((\text{Mankin scores } 0–6)\) and from moderate to severe degeneration \((\text{Mankin scores } 7–14)\).\(^{25}\)

Depthwise PG distribution \((\text{fixed charge density})\) was estimated from optical density \((\text{OD})\) of the grayscale images of Safranin-O stained sections captured with a light microscope and a CCD camera \((\text{SenSys, Photometrics Inc., USA})\).\(^{26}\) The final OD distribution was obtained as the average OD distribution of the three sections.

**Fourier transform infrared spectroscopy and polarized light microscopy**

Light absorbance in amide I region \((\text{Amide}: 1594–720 \, \text{cm}^{-1})\), representative of the collagen content, was assessed as a mean absorbance distribution in the three unstained sections using Fourier transform infrared spectroscopy \((\text{FTIR}; \text{Spotlight 300 FTIRI, Perkin Elmer, Shelton, CT, USA})\).\(^{27}\) Collagen orientation with respect to cartilage surface direction was determined by means of polarized light microscopy \((\text{Ortholux II POL; Leitz Wetzlar, Wetzlar, Germany})\) based on Stokes parameters.\(^{28,29}\)

**OCT analysis: total attenuation and backscattering coefficient**

\( \mu_{t} \) and \( \mu_{b} \) were determined by fitting the mean depthwise OCT intensity curve, \( I(d) \), into the following equation\(^{16}\):

\[
I(d) \propto \sqrt{\mu_b} \frac{1}{\sqrt{\left( \frac{d - d_0}{Z_0} \right)^2 + 1}} \exp(-\mu_t d),
\]

\[(1)\]

where \( d \) is the probing depth in cartilage, \( d_0 \) beam focus position and \( Z_0 \) the apparent Rayleigh length. Prior to fitting, the OCT system was calibrated using suspension series of water and polystyrene spheres \((\text{diameter } = 5 \, \mu\text{m}; \text{Phosphores Inc., Hopkinton, MA, USA})\).\(^{15}\)

**OCT analysis: PLS regression multivariate analysis**

PLS regression models were developed to estimate cartilage properties based on depth-dependent OCT signal. Multivariate PLS regression is an analytical technique for relating potentially correlated and noisy predictor variables to one or several response variables by finding a linear regression between them in a new space\(^{18}\). Briefly, developed and validated PLS regression models that optimize the relationship between the predictor and response variables are used to predict the response variables of new samples from predictor variables. In the present study, the OCT intensity curves were smoothed using a fourth degree Savitzky-Golay filter and the second derivatives of the smoothed intensity curves were used as predictor variables in the PLS analyses. Mankin score, severity of degeneration, \( E_f, E_m \) permeability and averages of OD, \( A_{\text{amide}} \) and collagen orientation within the whole cartilage layer, the superficial zone \((10\% \text{ of cartilage thickness})\), the middle zone \((15\%)\) and the deep zone \((75\%)\) of the cartilage served as response variables.

Leave-one-out cross-validation was computed in order to determine the optimal number of PLS components of the models\(^{11}\). Too few components may result in under-fitting, while too many may yield over-fitted models. In leave-one-out cross-validation each sample is left out one by one and the other samples are used to predict the response property of the sample left out. The criteria for optimal model selection were based on the model with the highest coefficient of determination \((R^2)\) and the lowest root mean square error of cross-validation \((\text{RMSECV})\). Model performance was evaluated by predicting the cartilage properties of the samples from their OCT signals using the created model\(^{22,23}\). The root mean square error of prediction \((\text{RMSEP})\) was calculated from the deviation between the predicted and true response parameter values \((y' \text{ and } y \text{, respectively})\):

\[
\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - y'_i)^2}{n}}
\]

\[(2)\]

where \( n \) is the number of the samples. For data pre-processing and multivariate analyses, custom-written software utilizing the SIMPLS algorithm in Matlab \((2014a; \text{MathWorks, Inc., Natic, MA, USA})\) was used.

**Statistical analyses**

Relationships of \( \mu_t \) and \( \mu_b \) with other determined properties of articular cartilage were evaluated by calculating Spearman’s rank correlation coefficients \((\text{IBM SPSS Statistics 19, SPSS Inc., Chicago, USA})\). A monotonic relationship of \( \mu_t \) and \( \mu_b \) with the cartilage properties was assumed.

**Results**

Mean (standard deviation) for \( \mu_t \) and \( \mu_b \) of the samples were 2.2 mm\(^{-1} \) \((1.1 \, \text{mm}^{-1})\) and 13.4 mm\(^{-1} \) \((7.9 \, \text{mm}^{-1})\), respectively. Mean and standard deviation of Mankin score, permeability, \( E_m, E_f \) OD, \( A_{\text{amide}} \) and collagen orientation are presented in Table 1. A significant linear correlation was found between \( \mu_b \) and \( E_f \) but not between \( \mu_b \) and the Mankin score, \( k \), \( E_m, \text{OD, } A_{\text{amide}} \text{ or collagen orientation} \). The \( \mu_t \) values did not correlate significantly with any of the cartilage properties (Table II).

In the PLS analyses, three to seven PLS components were found to be optimal in the regression models created between OCT intensity curve and the different properties of articular cartilage. Correlations between the measured properties of articular cartilage and those predicted using PLS models were high for Mankin score.
(the predicted and measured bulk collagen orientation was good were found between the measured and predicted RMSEP = std of articular cartilage.

Changes in the composition, structure, and mechanical properties of OCT via multivariate analysis, in assessment of degenerative changes. The present study demonstrates the diagnostic potential of the articular cartilage samples (Table I). The relations between the predicted and measured bulk properties of the samples are presented in Fig. 2. Bland–Altman plots show the agreement between the measured and predicted properties of the samples (Fig. 3). Nine of all samples were initially grouped into the category of moderate to severe degeneration based on their Mankin scores. Six of those samples were similarly diagnosed when the severity of degeneration was predicted using the PLS model.

### Table I

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Zone</th>
<th>Mean of response variable</th>
<th>Std of response variable</th>
<th>Number of components</th>
<th>RMSEP</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mankin score</td>
<td>All</td>
<td>3.5</td>
<td>2.6</td>
<td>5</td>
<td>1.4</td>
<td>0.70</td>
</tr>
<tr>
<td>Permeability (× 10⁻¹⁵ m²N⁻¹s⁻¹)</td>
<td>All</td>
<td>5.5</td>
<td>8.7</td>
<td>5</td>
<td>4.4</td>
<td>0.74</td>
</tr>
<tr>
<td>Eₘ (MPa)</td>
<td>All</td>
<td>0.25</td>
<td>0.24</td>
<td>3</td>
<td>0.17</td>
<td>0.50</td>
</tr>
<tr>
<td>Eᵦ (MPa)</td>
<td>All</td>
<td>1.74</td>
<td>1.18</td>
<td>3</td>
<td>0.89</td>
<td>0.42</td>
</tr>
<tr>
<td>OD (arb. unit)</td>
<td>All</td>
<td>1.42</td>
<td>0.37</td>
<td>5</td>
<td>0.16</td>
<td>0.73</td>
</tr>
<tr>
<td>Aamide (arb. unit)</td>
<td>Superficial</td>
<td>0.67</td>
<td>0.40</td>
<td>6</td>
<td>0.18</td>
<td>0.79</td>
</tr>
<tr>
<td>Collagen orientation (deg)</td>
<td>Middle</td>
<td>1.20</td>
<td>0.55</td>
<td>6</td>
<td>0.25</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Deep</td>
<td>1.57</td>
<td>0.35</td>
<td>6</td>
<td>0.16</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>42.0</td>
<td>4.9</td>
<td>4</td>
<td>3.7</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Superficial</td>
<td>23.6</td>
<td>4.6</td>
<td>5</td>
<td>2.7</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>30.7</td>
<td>5.0</td>
<td>5</td>
<td>3.2</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Deep</td>
<td>46.7</td>
<td>5.3</td>
<td>5</td>
<td>8.8</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>66.2</td>
<td>14.4</td>
<td>7</td>
<td>7.1</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Superficial</td>
<td>35.9</td>
<td>17.0</td>
<td>4</td>
<td>11.3</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>58.7</td>
<td>16.1</td>
<td>4</td>
<td>10.6</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Deep</td>
<td>71.7</td>
<td>16.8</td>
<td>4</td>
<td>11.8</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Std = standard deviation, RMSEP = root mean square error in prediction, R² = coefficient of determination, Eₘ = non-fibrillar matrix modulus, Eᵦ = fibrill network modulus, OD = optical density, Aamide = FTIR absorbance in amide I region.

### Table II

Linear correlations between the measured compositional, structural and biomechanical properties of articular cartilage and its light attenuation and backscattering coefficients (µᵣ and µₛ, respectively) indicated by Spearman’s rank correlation coefficients (rho) and the corresponding P-values

<table>
<thead>
<tr>
<th>Mankin score</th>
<th>Permeability (× 10⁻¹⁵ m²N⁻¹s⁻¹)</th>
<th>Eₘ (MPa)</th>
<th>Eᵦ (MPa)</th>
<th>OD (arb. unit)</th>
<th>Aamide (arb. unit)</th>
<th>Collagen orientation (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>µᵣ (mm⁻¹)</td>
<td>rho = 0.19 (P = 0.88)</td>
<td>rho = 0.107 (P = 0.41)</td>
<td>rho = 0.093 (P = 0.47)</td>
<td>rho = 0.021 (P = 0.87)</td>
<td>rho = 0.071 (P = 0.38)</td>
<td>rho = 0.129 (P = 0.31)</td>
</tr>
<tr>
<td>µₛ (mm⁻¹)</td>
<td>rho = 0.043 (P = 0.74)</td>
<td>rho = 0.128 (P = 0.33)</td>
<td>rho = 0.166 (P = 0.20)</td>
<td>rho = 0.086 (P = 0.20)</td>
<td>rho = 0.241 (P = 0.06)</td>
<td>rho = 0.138 (P = 0.28)</td>
</tr>
</tbody>
</table>

Eₘ = non-fibrillar matrix modulus, Eᵦ = fibrill network modulus, OD = optical density, Aamide = FTIR absorbance in amide I region, µᵣ = total attenuation coefficient, µₛ = backscattering coefficient.

Based on the results, the bulk attenuation and backscattering parameters, determined from OCT signal, were not sensitive enough to detect small compositional changes in articular cartilage. Both collagen and chondrocytes scatter light and affect the OCT signal. However, possible changes in their content during degeneration were not revealed by measuring ρᵣ or ρₛ. The sensitivity of the bulk attenuation coefficient for early osteoarthritic changes in articular cartilage was also questioned by Nebeling et al. This supports the choice of multivariate analysis of the OCT signal for detailed diagnostic purposes.

The PLS models constructed based on the measured OCT signals provided high correlation especially between the measured and the predicted values of Mankin score, permeability, OD, and bulk collagen orientation. All the samples having no or mild degeneration were correctly classified by predicting their Mankin scores, whereas three out of nine samples were misclassified to have no or mild degeneration as opposed to moderate to severe degeneration. Permeability does not have direct effect on the optical properties of articular cartilage, but it is a good measure of many simultaneous changes in composition and structure of the tissue during degeneration. The permeability of articular cartilage relates to the PG content of the tissue. Therefore, the contribution of PGs to OCT signal may be the reason for the correlation between the measured and predicted permeability. In the present study, OD was used as the measure of PG content. Earlier, PGs have not been shown to affect the birefringence or surface reflection when measured by OCT. However, the present high correlation between measured OD and OD predicted by the PLS model might indicate a contribution of PGs to OCT light backscattering. The influence of PGs on OCT signal is of great interest as PG depletion, in addition to disorganization of collagen matrix, is one of the first signs of cartilage degeneration.

### Discussion

The diagnostic value of conventional arthroscopic evaluation is limited when assessing early stage cartilage degeneration due to subjectivity. The application of OCT under arthroscopic guidance provides more detailed images of the cartilage lesions and enhances the reproducibility of cartilage lesion scoring. In addition, OCT could also be used to investigate microstructural tissue changes. The present study demonstrates the diagnostic potential of OCT via multivariate analysis, in assessment of degenerative changes in the composition, structure, and mechanical properties of articular cartilage.
It is worth noting that the OCT system used in this study was not designed for measurement of birefringence. Hence, the significant relation between the OCT signal and collagen orientation may result, in part, from varying scattering properties of fibrils oriented at different angles as well as from other compositional and structural properties that change simultaneously with disorganization of the collagen network. However, the measurement of birefringence would be a valuable addition for identification of early...

Fig. 2. The measured bulk properties of articular cartilage and those predicted from OCT signal using PLS regression models. (a) Mankin score, (b) permeability, (c) non-fibrillar matrix modulus ($E_m$) (d) fibril network modulus ($E_f$) (e) bulk OD (f) bulk FTIR absorbance in amide I region ($A_{amide}$) and (g) bulk collagen orientation.
cartilage degeneration. Additionally, the use of an ultra-high resolution OCT system could improve the detection of the depthwise changes in the amount of small scattering components. The use of higher resolution would also increase the number of predictor variables in the model, and could possibly improve the accuracy of the PLS predictive model.

The relationship between the measured and predicted FTIR absorbance in amide I region (i.e., collagen content) was not strong.
Mechanical properties of articular cartilage are related to collagen matrix properties. Therefore, the relation of OCT signal to fibril network modulus may reflect its relation to collagen content and organization. In early stage of degeneration, the collagen content abides although the fibril organization changes. Therefore, the assessment of collagen content may be of minor importance when differentiating healthy cartilage from areas with signs of the earliest stage of degeneration.

Due to the limited light penetration, OCT imaging cannot be conducted non-invasively, but is a useful tool in arthroscopies. Presently, with thin equine cartilage, the prediction ability of the PLS models was similar for each of the three cartilage zones. In OCT analysis, the structural and compositional properties in the superficial zone of articular cartilage may have the highest diagnostic value. The limited penetration depth (1–2 mm) of the light might hinder the ability to analyse middle and deep zones of thick human articular cartilage. By choosing adequate central wavelength for the OCT light, the light penetration could be marginally improved. However, the optimal choice of wavelength is a sensitive balance between increase in water absorption and decrease of scattering. Further, doubling the sample set. The errors obtained in the present study should be evaluated. The approach needs to be further validated and optimized before it can be applied clinically. For example, test set validation, which uses a set of test samples independent of the training set, can be used to further validate the performance of the multivariate models developed in this study. Further, doubling the amount of samples in training of the PLS model may improve the accuracy of the assessment and would enable use of the test set validation. Adequate diagnostic accuracy and good intra- and inter-observer agreements in both model creation and prediction of cartilage properties are required, and they need to be thoroughly evaluated.

Quantitative OCT analysis could improve the evaluation of articular cartilage integrity. After careful calibration and validation of the PLS model, the use of the model for prediction of cartilage properties could be fast and feasible in clinical use. The present technique might be used in arthroscopic surgery in order to delineate degenerated areas around articular cartilage lesions and to aid the selection of the optimal treatment method.

Author contributions
Conception and design of the study: All authors. Acquisition of data: Puhakka, te Moller. Analysis and interpretation of data: All authors. Critical revision and approval of the final submitted version of the article: All authors. Responsibility for the integrity of the work as a whole: Puhakka, Töyrä.

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Competing interest statement
None of the authors have conflict of interest.

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