Optimal regression method for near infrared spectroscopic evaluation of articular cartilage

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Abstract

Near infrared (NIR) spectroscopy has been successful in non-destructive assessment of biological tissue properties, such as stiffness of articular cartilage, and is proposed to be used in clinical arthroscopies. NIR spectroscopic data includes absorbance values from a broad wavelength region resulting in a large number of contributing factors. This broad spectrum includes information from potentially noisy variables, which may contribute to errors during regression analysis. We hypothesized that partial least squares regression (PLSR) is an optimal multivariate regression technique and requires application of variable selection methods to further improve the performance of NIR spectroscopy-based prediction of cartilage tissue properties, including instantaneous, equilibrium and dynamic moduli and cartilage thickness. To test this hypothesis, we conducted for the first time, a comparative analysis of multivariate regression techniques, which included, principal component regression (PCR), PLSR, ridge regression, least absolute shrinkage and selection operator (Lasso), and least square version of support vector machines (LS-SVM), on NIR spectral data of equine articular cartilage. Additionally, we evaluated the effect of variable selection methods, including Monte Carlo uninformative variable elimination (MC-UVE), competitive adaptive reweighted sampling (CARS), variable combination population analysis (VCPA), backward interval PLS (BiPLS), genetic algorithm (GA) and jack-knife, on the performance

of the optimal regression technique. PLSR technique was found as an optimal regression tool ($R^2_{\text{Tissue thickness}} = 75.6$, $R^2_{\text{Dynamic modulus}} = 64.9$) for cartilage NIR data; variable selection methods simplified the prediction models enabling the use of lesser number of regression components. However, the improvements in model performance with variable selection methods were found to be statistically insignificant. Thus, PLSR technique is recommended as the regression tool for multivariate analysis for prediction of articular cartilage properties from its NIR spectra.

Key terms

Uninformative variable elimination (UVE), arthroscopy, multivariate regression, cartilage, near infrared (NIR) spectroscopy

Introduction

Articular cartilage (AC) is a specialized type of hyaline cartilage found at the distal ends of bones providing smooth, low friction, load-bearing interfaces in joints. This cartilage comprises mainly of water (65-80%) and extra-cellular matrix (ECM) consisting primarily of collagen (10-30% w/w) and proteoglycans (PGs; 10-20% w/w).[1] Degenerative joint conditions, such as osteoarthritis (OA), are generally characterized by disruption of the superficial collagen network and loss of PGs. These changes in biochemical composition results in alteration of the biomechanical properties of AC.[2–5] Light-based imaging modalities, such as Fourier transform infrared (FTIR) spectroscopy,[6] near infrared (NIR) spectroscopy,[7–9] optical coherence tomography (OCT) and Raman spectroscopy have been proposed for arthroscopic evaluation of joint tissues.[10–12]

Recent cartilage studies have advocated the use of NIR spectroscopy for in vivo evaluation of articular cartilage integrity,[13–15] particularly due to its superior tissue depth penetration compared to other optical techniques. Furthermore, Afara et al. showed that the NIR diffuse reflectance spectrum of articular cartilage correlates with biomechanical,[16] biochemical and histological properties of non-calcified cartilage. NIR spectroscopy is a

vibrational spectroscopy with main contributing bonds in biological tissues being C-H, N-H, O-H and S-H.

Regression techniques enable the development of mathematical models relating NIR spectra with reference parameters (e.g. tissue composition) of the studied tissue (e.g. cartilage). Multivariate regression techniques are primarily used to extract information from NIR data,[17] due to non-specificity of the functional bands and overlapping overtones in NIR spectra unlike mid-IR,[18] a single wavelength represents multiple components. Additionally each property, such as thickness, is best described by partial contribution from multiple variables. Hence, multivariate techniques would be an appropriate choice.[19] The popular multivariate regression techniques utilized in NIR spectroscopy are principal component regression (PCR) and partial least squares regression (PLSR).[20,21] PLSR is the most common regression technique used in NIR spectroscopic studies of articular cartilage. However, the potential of regression shrinkage methods, such as ridge regression and least absolute shrinkage and selection operator (Lasso),[22] and least square version of support vector machines (LS-SVM) based regression,[23] for NIR evaluation of articular cartilage has not been investigated. A comparison of the aforementioned multivariate regression techniques was undertaken to determine the best technique applicable for NIR spectroscopic evaluation of cartilage. A summary of these common techniques is presented in Table 1.

Selecting optimal variables for regression models is an essential step as the spectra may contain noisy or irrelevant variables that hinder the analysis. Conventionally, variable selection is done by restricting the spectral wavelength based on experimental knowledge or known restrictions (manual wavelength selection), which may lead to inconsistent results and is prone to human error. Statistical studies conducted by Xiaobo et al.,[24] Westad et al. and Mehmood et al. have shown the significance of variable selection methods in multivariate regression techniques.[25,26] In general, variable selection in multivariate regression is based on the principle of either choosing the most contributing variables or eliminating the noncontributing variables.[24] Monte Carlo uninformative variable (MC-UVE), competitive adaptive reweighted sampling (CARS), variable combination population analysis (VCPA), interval selection methods (BiPLS), genetic algorithm (GA) and jack-knife are different variable selection methods available for analyzing NIR spectra.

In this study, multiple multivariate regression and variable selection methods are utilized to determine the most optimal algorithms for analyzing articular cartilage NIR spectra. PLSR has been successfully applied in spectroscopy applications, such as paper,[27] food and mineral industries.[28,29] Recent NIR and FTIR studies have demonstrated PLSR to be a capable technique for analysis of cartilage spectra.[30,31] Additionally, a FTIR study demonstrated that variable selection further improves PLSR models in case of composition

and compressive properties of cartilage.[32] Thus, we hypothesized that PLSR is an optimal regression technique for evaluation of cartilage NIR spectra, and its model performance may further be improved with variable selection. To test the hypothesis, calibration models relating the NIR spectra with reference properties of cartilage were developed and evaluated using independent group of areas of interest (AI).

Materials and methods

This study was conducted on NIR spectral data collected from equine cartilage, used in an earlier study.[33] Equine metacarpophalangeal joints (N = 5) were obtained from a slaughterhouse and areas of interest (AI, N = 44) of intact and damaged cartilage were selected by experienced veterinary surgeons. The blind coded AIs were evaluated twice arthroscopically under independent settings by the surgeons according to the international cartilage repair society (ICRS) scoring system to differentiate healthy (N = 19) and damaged (N = 25) AIs. The AI grids, 15 × 15 mm were outlined with a felt-tip marker, which did not interfere with NIR spectroscopy measurements. In each AI, 25 equally spaced locations at inter-distance spacing of 2.5 mm were measured. Fully eroded cartilage surface locations were excluded. In total, the 44 AIs yielded 869 locations which were measured by NIR spectroscopy.

Measurements were performed using a customized NIR spectroscopy diffuse reflectance instrument coupled with a fiber optic probe was utilized. The instrumentation consisted of a halogen light source (wavelength 360 – 2500 nm, power 5 W, optical power 239 μW in a d_{fiber} = 600 μm, Avantes BW, Apeldoorn, Netherlands), a spectrometer (wavelength 200 – 1160 nm, Avantes BW, Apeldoorn, Netherlands) and a fiber optic probe (d = 5 mm) with seven fibers (d_{fiber} = 600 μm) within the central window (d = 2 mm), six peripheral transmitting fibers and one central reflectance collector.

Near infrared (NIR) spectral measurements were performed three times (with realignment of the measurement probe) on all 869 points, and the three measurements were averaged for each point. The biomechanical measurements were conducted once due to long protocol times. Instantaneous modulus was measured on all 869 points, and the dynamic and equilibrium moduli were measured for 202 points, as the measurement protocol and sample geometry limited conducting these measurements for all locations. Equilibrium, dynamic and instantaneous moduli were obtained via indentation testing.[34] Additionally, cartilage thickness was determined using OCT.[35] During NIR spectral measurements, the probe was in contact with and oriented perpendicularly to the cartilage surface. The physiological conditions of the tissue were maintained by periodically spraying phosphate-buffered solution (PBS) on cartilage and placing PBS soaked cloths around the other measurement

locations. Each spectrum was an average of three spectral measurements that consisted of eight co-added spectral scans ($t_{eight\ scans}$ = 720 ms). NIR spectral measurements, thickness measurements and indentation testing were conducted on the same equidistant point locations within the AI grid. The central window of the NIR spectral probe is 2 mm within the 5 mm probe housing. The field of view of OCT (wavelength 1305 ± 55 nm) is 4 mm deep and the cartilage thickness was measured in the center of the image cross-section. In biomechanical indentation testing, a plane ended indenter (d = 0.53 mm) was utilized and both the thickness and indentation measurements were within the NIR measurement location.

The NIR spectral data was preprocessed by smoothing and filtering using a third degree Savitzky-Golay filter with 25 nm window to remove background noise. Subsequently, second derivative pretreatment was applied on the smoothed and filtered data to eliminate baseline offset and dominant linear terms, and to highlight the subtle absorption peaks. This study builds on Sarin et al. where the effect of multiple preprocessing methods on the performance of PLSR were investigated.[33] Therefore, further optimization with the preprocessing methods were not explored. However, changing the preprocessing methods will likely impact the prediction accuracy of the regression techniques and the selection of optimal method. The pretreatment of the dataset and subsequent splitting into training dataset and testing dataset were consistent with our earlier study. The first set (training set),

consisting of 41 AIs, was used for calibration model training, and the second set (test set), consisting of 3 AIs and independent of the training set, was used to evaluate the model performance.[36] Test set was designed to include maximum locations (N = 25) within each AI grid and corresponding reference parameters to lie within the range of the calibration test set. All spectral analyses were done using MATLAB R2014a (Mathworks Inc, Natick, MA).

Regression techniques

Partial least squares regression (PLSR), PCR, ridge, Lasso and LS-SVM techniques were employed for multivariate regression comparative analysis. In this study, the tuning parameters as indicated in Table 1 for each regression technique were varied from minimum to maximum values resulting in a series of models with each model using k-fold cross-validation (k = 10). The models were then tested on an independent test set. The model which performed the best, in terms of root mean square error of calibration (RMSEC), R^2_{Train} and R^2_{Test} from each series, was retained. Finally, the retained parameters of each model were compared. This protocol ascertained optimal settings for each regression technique.

Variable selection methods

Monte Carlo uninformative variable elimination (MC-UVE), CARS, VCPA, BiPLS, GA and jack-knife methods were used for variable selection to further optimize the best regression technique. The algorithms for MC-UVE and CARS were obtained from Integrated library for PLS and discriminant analysis,[37,38] VCPA from Variable Combination Population Analysis toolbox,[39] GA algorithm from PLS-Genetic algorithm toolbox and jack-knife algorithm was coded in-house. [40,41]

The MC-UVE (mcuvepls) variable selection was optimized by first calculating the reliability index of all the wavelengths and then determining the optimal threshold for reliability index by finding the maximum correlation with the training set. CARS (carspls) and VCPA (vcpa) did not require additional input, as the respective functions auto-handled the optimization protocol. In interval selection BiPLS method, the algorithm was optimized by eliminating uninformative intervals and the three intervals with the lowest RMSECV were retained. In GA algorithm, the effective number of evaluation and number of variables were first determined, using the gaplsopt function in the toolbox and the main function gaplssp was invoked to perform variable selections for spectral data. In jack-knife method, the student's t-statistics was used for variable selection by selecting variables with values less than the predefined threshold (t = 0.05).

Statistics and model comparison

The calibration models developed were analyzed based on the following key parameters: root mean square error of calibration (RMSEC), R^2 in training set, root mean square error of prediction (RMSEP), R^2 in test set, error percentage in the test dataset and run times in seconds. Additionally, for PCR and PLSR models the number of components were recorded.

In order to investigate statistically the difference in performance (correlation coefficient) of models developed with the different regression techniques, Zou's confidence interval test,[42] with dependent dataset condition, was conducted. The test was also applied to investigate the significance of variable selection methods on the performance of PLSR.

Results

The regression models were built and optimized for cartilage thickness, instantaneous modulus, equilibrium modulus and dynamic modulus. The statistical description of the dataset is given in Table 2. PLSR technique was found to be the best, with the highest R^2 in test set and the lowest RMSEP and error percentages, amongst the investigated regression

techniques in the cases of cartilage thickness, instantaneous modulus and equilibrium modulus (Table 3). In the case of dynamic modulus, Lasso was found to have the highest R^2 for the training set; however, it presented a higher percentage error than PLSR. The computational times in Table 3 suggests that PLSR is quicker when compared to other regression techniques. Zou's test showed that the differences between the best three regression techniques (with respect to test set R^2) were not statistically significant. Similarly, the improvement in model performance with variable selection was not statistically significant when compared with traditional PLSR technique.

PLSR models optimized using variable selection methods indicated improved model performance (Table 4). As evident from Table 4 in comparison with Table 3 the variable selection methods do not significantly add value to the calibration models in terms of R^2 but there is improvement in terms of simplicity of the model with reduced number of components. MC-UVE was found to improve the PLSR model performance consistently for all tissue parameters. The MC-UVE algorithm eliminated the wavelength variables (Figure 1) depending on the relative importance of each variable in the calibration model.

Discussion

The NIR spectra is affected by instrumentation noise (e.g. thermal noise and readout noise) and interference with the measurement environment. Longer acquisition times and averaging, and preprocessing methods, such as baseline correction, smoothing and normalizations are utilized to reduce the noise in the signal. In this study, for the first time a comparative analysis of multivariate regression technique for analysis of articular cartilage NIR spectral data is done.

First, comparison of different optimized multivariate regression techniques, namely PCR, PLSR, Lasso, ridge, and LS-SVM, using key statistical parameters, was conducted. PLSR was found to be the most optimal regression technique for evaluation of cartilage NIR data based on its consistent performance across all the reference parameters of cartilage. PLSR showed better consistency in comparison to the other regression techniques investigated, which is in agreement with the findings of Yeniay et al.[43] Second, the effect of variable selection methods on the performance of PLSR regression models was evaluated. MC-UVE algorithm, which was the best performing variable selection method in this study, consistently selected variables in the 730 to 780 nm (CH and OH bonds) and 925 to 980 nm (CH and OH bonds) spectral ranges retaining essential spectral information characteristic for AC. [24] The NIR absorption spectra in AC arise mainly from CH, NH, OH and SH bonds which form the molecular constituents of the cartilage matrix,[44] and thus, the information in

this region indicates the biochemical composition of the tissue. Cartilage NIR spectrum (Figure 1) in the region between 800 to 1100 nm is due to 3rd overtone CH and NH bond vibrations, associated with the tissue's solid matrix components (PGs and collagen), and absorption at 970 nm is due to the 3rd overtone OH bond vibrations resulting from the water content of the tissue.[45] Application of MC-UVE method not only improved the performance of the prediction models, but also preserved the essential spectral information indicating the tissue condition.

The PLSR technique has been shown to perform well with multicollinear data in NIR spectroscopy.[46] PLSR probably performed best because the algorithm decomposes the predictor into latent variables (maximum co-variance first) with respect to reference parameters. This intrinsic property of the algorithm maximizes the variance, and thus relationship, between the predictor and response variables. As shown by Afara et al.,[13] our results depict that the error associated with using NIR spectroscopy for predicting cartilage thickness are relatively low in comparison with the resolution of clinical MRI,[47,48] currently used in diagnosis of joint defects.

In the present study, cartilage thickness was predicted more efficiently (R^2 = 75 vs. 57 – 66) by PLSR in comparison to biomechanical properties of the tissue. This can be attributed to direct relationship between the NIR spectra and the tissue thickness since the

path length affects the light absorption as the rays traverse the tissue.[13] The regression comparison highlighted some limitations of sophisticated regression techniques in modelling cartilage NIR data, as LS-SVM and PCR seemed to suffer from overfitting and under fitting, respectively (Table 3). While it is difficult to speculate on the acceptable error limit for NIR predicted values of cartilage mechanical parameters with respect to clinical diagnostics, a motivation of the current study was to provide a quantitative approach that could be complementary and add more value to traditional tissue palpation. With prediction errors between 11 - 13 % (Table 4), diagnosis of cartilage health based on NIR predicted equilibrium or dynamic moduli values may not be optimal, but still better than current qualitative and subjective arthroscopic assessment. In practice, orthopedic surgeons assess the stiffness of cartilage by palpating it with a metallic hook. This method, however, is subjective and unreliable compared to the gold standard of indentation testing performed in a laboratory.[34,49]

Recent cartilage studies have favored projection regression techniques such as PLSR and PCR due to ease of implementation. However, the effect of variable selection methods has not been investigated. Following the previous study,[33] the wavelength region of the NIR spectra was limited to 700 - 1050 nm.[50] Thus, the variable selection methods had a relatively narrow spectrum of variables. Nonetheless, the results demonstrate the applicability of

variable selection methods in regression analysis, which is consistent with the findings of Abrahamsson et al. where NIR transmission spectroscopy was applied in intact tablets.[51] The present results indicate that variable selection improves model performance and enhances the results of PLSR. In particular, MC-UVE is well suited for NIR spectroscopy of cartilage.

Comparison of the present results with an earlier study on the corresponding equine data,[33] encourages the use of variable selection methods for analysis of cartilage thickness and dynamic modulus from its NIR spectra. MC-UVE prediction models displayed 7 to 8% improvement of the R^2 in the test set in thickness prediction and 15% lower RMSEP in dynamic modulus prediction. On the other hand, with instantaneous and equilibrium moduli, variable selection methods showed no improvements in R^2 or RMSEP over the standard PLSR models. This may be due to the limited spectral range used in this study. However, it is worth noting that variable selection based prediction models required lesser number of PLSR components than the standard PLSR models, which reduces the possibility of overfitting. Although the variable selection methods presented only marginal improvement in PLSR models, the simplification of the model and potential improvement in computational times could be considered as advantages.

Zou's test showed the statistical significance between regression models only based on their R^2. The test suggests that there are no significant differences between correlation

coefficients of the top performing regression techniques, and that variable selection methods did not significantly improve the performance of PLSR models. Nevertheless, a major drawback of the Zou's test in this comparison is that certain factors specific to the different regression techniques, such as number of components, in the case of PLSR and PCR, are not taken into consideration. In addition, models based on fewer variables are computationally efficient and better suited for real time applications.

Chemometrics in horticultural studies,[52] food engineering and fuel analytical studies found GA to be the most suitable variable selection method for PLSR.[53,54] However, in the current study on articular cartilage NIR spectra, MC-UVE surpassed GA and VCPA, contradicting the results of Yun et al. regarding VCPA.[39] The limited input wavelength range could have reduced the performance of the VCPA, as in a previous study VCPA performed better than other variable selection methods in multiple NIR datasets.[39] Likewise, the narrow wavelength band and the resolution of the NIR system probably limited the performance of the other variable selection methods, as there are less relevant variables. It is worth noting that besides PLSR the interaction effects of variable selection on the other regression techniques were not investigated in this study, as the purpose was to investigate the potential of variable selection in further improving the performance of the optimal regression technique. In conclusion, the results of the present study recommend PLSR technique as the

multivariate regression tool for prediction of articular cartilage properties from its NIR spectra. Application of variable selection methods simplified the models by reducing the number of spectral variables and components; however, the improvements in model performance were statistically insignificant.

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Contributions

Prakash M: Analysis and interpretation of data and the main writer of the manuscript.
Sarin J.K: Acquisition of data and drafting of manuscript.
Rieppo L: Study design and drafting of manuscript.
Afara I.O: Spectral measurements, supervision of statistical analyses and drafting of manuscript.
Töyräs J: Study conception and design, drafting of manuscript.
All authors contributed in the preparation and approval of the final submitted manuscript.

Conflict of Interest

The authors have no conflicts of interest in the execution of this study and preparation of the manuscript.

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Table 1: Summary of regression techniques utilized in this study.

Technique	Summary	Tuning Parameter(s) and Range utilized.	Advantage(s)	Disadvantage(s)	Matlab function(s)/ Toolbox utilized
PCR	Linear projection method, reduces the dimensionality of the data using only explanatory data, X, * into uncorrelated subspace. Ordinary least squares applied to regress.	Number of components:1 to 15	Dimensionality reduction, handles multicollinearity in X.	As latent variables are based only on explaining the variance X, they are not optimal for every problem.	pcrsse, pca
PLS	Linear regression technique based on dimensionality reduction method by projecting explanatory data, X, to subspace of latent components maximizing covariance between X and the response matrix, Y. **	Number of components:1 to 15	Dimensionality reduction, handles multicollinearity.	Output is a linear combination of input.	plsregress
Ridge	Shrinkage regression technique. Shrinks the dimensions with the least variance the most.	Shrinkage penalty: 0 to 1000.	Stable when p >> N.	Selects all predictors in the final model instead of subset of variables.	ridge
Lasso	Shrinkage regression technique by minimizing the sum of squared error and setting some to zero.	Step size = 0.01	Solution is sparse.	Covariate selection is arbitrarily done if the dataset is highly collinear.	lasso
LS-SVM	Least squares version of support vector variant. Creates model based on newly formed support vectors from the training dataset.	Lambda: 0	Can also model nonlinear relationships.	Lack of sparseness.	LS-SVM lab: initlssvm,tunelssvm and trainlssvm

* X is explanatory input data matrix containing NIR spectra.

** Y is response data matrix containing reference data.

Table 2: Reference data and NIR spectra. NIR wavelength in 700 – 1050 nm range was utilized.

Reference parameter	Training Dataset		Testing Dataset	
	Mean	Range	Mean	Range
Thickness (mm)	0.88	0.32 - 1.81	0.96	0.52 - 1.32
Instantaneous Modulus (MPa)	4.74	0.11 - 20.88	4.88	0.29 - 12.67
Equilibrium Modulus (MPa)	1.97	0.36- 5.38	2.96	0.30 - 4.96
Dynamic Modulus (MPa)	8.38	0.36 - 22.97	10.02	0.69 - 17.32

Table 3: Comparison of the different regression technique across different tissue parameters for equine articular cartilage. Data is arranged in descending order of the test set R^2 as highlighted in bold. The error percentage for prediction of the test set indicated in the table was calculated as the average error divided by the range of the respective reference variable.

	Cartilage Thickness (mm)					
Regression techniques	**Train Set**		**Test Set**		**Error Percentage**	**Computation Time (sec)****
	R^2	**RMSEC**	**R^2**	**RMSEP**		
PLSR (C* = 5)	70.28	0.13	**75.57**	0.11	5.94	2.50
RIDGE	73.02	0.12	**74.09**	0.11	6.17	1200
LASSO	72.90	0.12	**68.63**	0.12	6.90	170
LS-SVM	77.55	0.11	**67.87**	0.13	6.89	0.30
PCR (C= 13)	60.44	0.15	**67.38**	0.13	7.02	1.0
	Instantaneous Modulus (MPa)					
PLSR (C = 6)	42.88	2.60	**51.00**	2.46	10.04	2.0
RIDGE	46.69	2.51	**49.76**	2.49	10.24	1100
LASSO	41.21	2.97	**48.71**	2.52	10.20	5800
PCR (C = 5)	25.41	2.97	**44.29**	2.62	9.77	0.60
LS-SVM	99.98	0.04	**42.82**	2.66	11.16	0.25
	Equilibrium Modulus (MPa)					
PLSR (C = 5)	67.81	0.84	**68.58**	0.94	15.35	1.0
LASSO	80.27	0.65	**60.13**	1.06	17.91	96
LS-SVM	99.99	0.00	**54.22**	1.14	19.48	0.16
RIDGE	80.69	0.65	**54.12**	1.15	20.23	505
PCR (C = 15)	22.89	1.30	**32.60**	1.38	22.13	0.37
	Dynamic Modulus (MPa)					
LASSO	69.16	3.44	**66.35**	3.56	13.85	102
LS-SVM	99.69	0.34	**65.90**	3.58	14.67	0.15
PLSR (C = 2)	37.27	4.90	**64.88**	3.63	13.05	1.0
RIDGE	63.45	3.74	**61.30**	3.82	15.44	470
PCR	27.02	5.29	**60.34**	3.86	14.55	0.33

* Number of components for PLSR and PCR is indicated by C

** The computation times were calculated on Intel(R) Core(TM) i5-2400 CPU at 3.10 GHz, 64 bit Operating System.

Table 4: Comparison of variable selection methods. The data is presented in descending order of R^2 for the test set as highlighted in bold. The error percentage for prediction of the test set indicated in the table was calculated as the average error divided by the range of the respective reference variable.

	Cartilage Thickness (mm)					
Variable Selection method	**No. Of PLS Components**	**Training set**		**Test set**		**Error Percentage**
		R^2	**RMSEC**	**R^2**	**RMSEP**	
MC-UVE	4	70.61	0.14	**75.94**	0.10	5.95
None	5	70.28	0.13	**75.57**	0.11	5.94
GA	8	69.55	0.14	**74.86**	0.11	5.93
JK	1	59.64	0.15	**74.05**	0.11	6.13
BiPLS	12	59.05	0.15	**70.05**	0.12	6.58
CARS	5	72.33	0.13	**69.77**	0.12	6.68
VCPA	5	63.67	0.14	**65.59**	0.12	7.22
	Instantaneous Modulus (MPa)					
CARS	3	38.56	2.78	**51.85**	2.44	9.44
None	5	42.88	2.60	**51.00**	2.46	10.04
VCPA	4	34.90	2.81	**49.07**	2.51	9.57
MC-UVE	2	34.20	2.85	**48.35**	2.53	9.46
GA	4	36.69	2.81	**48.33**	2.53	9.94
BiPLS	7	28.92	2.90	**45.56**	2.59	9.83
JK	7	33.79	2.91	**45.43**	2.60	10.20
	Equilibrium Modulus (MPa)					
None	5	67.81	0.84	**68.58**	0.94	15.35
MC-UVE	5	70.95	1.27	**65.53**	0.99	16.30
VCPA	6	48.95	1.12	**54.76**	1.13	19.08
CARS	2	43.29	1.29	**54.16**	1.14	19.13
GA	3	50.06	1.22	**51.99**	1.17	19.18
JK	6	39.03	1.36	**43.05**	1.27	21.36
BiPLS	6	34.85	1.19	**38.29**	1.32	20.50
	Dynamic Modulus (MPa)					
CARS	3	61.36	4.69	**77.82**	2.89	11.04
MC-UVE	3	63.60	4.78	**73.92**	3.13	12.46
GA	3	50.66	4.85	**72.79**	3.50	13.49
JK	5	43.63	5.40	**67.47**	3.50	13.49
None	2	37.27	4.90	**64.88**	3.63	13.05
BiPLS	7	38.73	4.85	**55.98**	4.07	14.87
VCPA	1	37.85	5.04	**50.59**	4.31	17.12

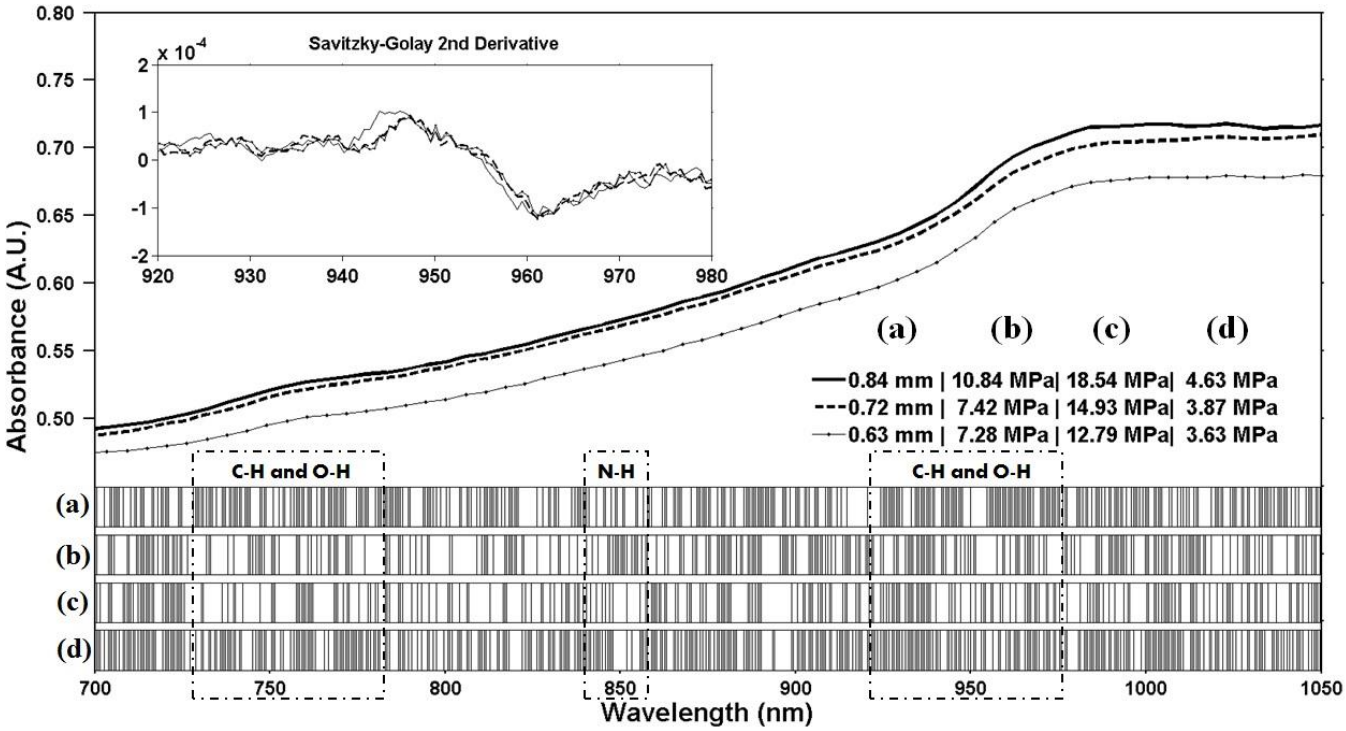

Figure 1: Representative absorbance spectra of articular cartilage with different **(A)** thickness values, **(B)** instantaneous modulus values, **(C)** dynamic modulus values (**D)** equilibrium modulus values and 2nd derivative preprocessed spectra (top inset). The MC-UVE selection ranges (bottom inset) shows regions of wavelength selected (black bars) and empty (white) spaces indicates the eliminated variables.