PRFFECT[☆]: A Versatile Tool for Spectroscopists

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Abstract

PRFFECT is a computer program to aid with spectral preprocessing and the development of classification models. Via a simple text interface, PRFFECT allows users to select wavenumber ranges, perform spectral preprocessing, carry out data partitioning (into training and testing datasets), run a Random Forest classification, compute statistical results, and identify important descriptors for the classification. The preprocessing options provided fall into four categories: binning, smoothing, normalisation, and baseline correction. The program outputs a wide-variety of useful data, including classification metrics and graphs showing the importance of individual wavenumbers to the classification models. As proof-of-concept, PRFFECT has been benchmarked on preprocessing and classification of four food analysis datasets. Sensitivities and specificities above 0.92 were obtained in all cases. The results show that different preprocessing procedures are optimal for different datasets. The PRFFECT software is available freely to the community via GitHub. Link: https://github.com/Palmer-Lab/PRFFECT.

Keywords: FTIR spectroscopy, Random Forest, software, machine learning, spectral diagnostics, food testing

[☆]PRFFECT: Pre-processing & Random Forest Feature Extraction Combination Tester.

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1. Introduction

Spectral diagnostics is an important and growing area of research. It involves the collection of spectral data with the aim of building a model to detect changes in samples, then using that model to classify new spectral samples. It is used in many diverse fields. For example, Ellis *et. al.* used Fourier transform infrared (FTIR) spectroscopy combined with genetic algorithms and regression models in order to detect spoilage of meats[1]. Raman spectroscopy combined with Neural Networks and k-nearest neighbour models have been used to detect illicit substances such as cocaine[2]. Near infrared (NIR) spectroscopy in combination with Least-Squares Support Vector Machines was used to develop a method of detecting acidity levels in grapes[3]. Recent work in our groups has used FTIR and Raman spectroscopy in combination with various types of machine learning, applying these to diverse tasks such as diagnosing cancerous disease states from serum samples, rapid discrimination of maggots, segregation of brain tumour cell lines, and discriminating biological warfare simulants.[4, 5, 6, 7, 8, 9]

The best choice of spectral preprocessing is an unresolved problem in the spectral diagnostic community. Often, preprocessing is done by a set routine used by research groups across all datasets. However, the choice of the particular methods and parameters are not finely tuned to particular dataset types. With PRFFECT (Pre-processing & Random Forest Feature Extraction Combination Tester) we provide a robust methodology to make it easier to test different pre-processing routines in the development of spectral diagnostic models. Finding reliable methods and parameters is important in order to build a strong routine for translation into clinical and other settings. PRFFECT makes this possible by offering a large array of user-settable preprocessing methods. The classification and feature extraction stages after preprocessing are accomplished by Random Forest.

The Random Forest (RF) machine learning algorithm is widely used in many different fields of research, including cheminformatics,[10, 11] bioinformatics, [12], and ecology,[13]. Within the field of biomedical spectroscopy, RF has been

used in the annotation of lung cancer subtypes [14] and in the diagnosis of non-small cell lung carcinoma, [15] urinary bladder cancer, [16], hyperlipidemia [17], and brain tumours [7]. RF has proven to be a robust and accurate technique for developing spectral diagnostic models, giving excellent classification results without over-fitting. It also includes built-in routines for variable selection and for assessing the importance of each variable to the model.

2. Materials and Methods

2.1. Programming

The PRFFECT program is written for the R programming language, which is an open-source and freely available environment for statistical computing[18] that can be installed on all common operating systems. The aim was to provide a comprehensive and user-friendly system for the preprocessing needs of spectroscopists. Since it was not feasible to implement all available preprocessing routines, only the most commonly used methods were included in the current release of the program, including vector normalisation, binning, Savitzky-Golay (S-G) filter smoothing, numerical derivatives, etc. Several other preprocessing routines have already been considered for future releases.[19, 20] All of the methods in the current version of PRFFECT are described in detail in the sections below. Many combinations of these methods can be used in conjunction with one another. The preprocessing options are arranged into sections. The order of these follows the most commonly encountered preprocessing workflow observed in biospectroscopy research: binning, smoothing, normalisation, baseline correction. The code does not currently include a routine to correct for resonant scattering effects, which the user may wish to consider before running PRFFECT.

Inspiration was drawn from a few existing R packages. In particular, Claudia Beleites' excellent *hyperSpec* package[21]. We build on the work that has previously been done in this field by providing a simple interface, several types of preprocessing, classification, feature extraction and statistical analysis all in

one program.

2.2. Binning

Binning is the practice of increasing the wavenumber spacing (δ) by averaging a number of adjacent datapoints, thereby reducing the complexity of hte dataset. Binning is often carried out on biospectra. Binning can sometimes increase signal-to-noise ratio by decreasing spectral resolution. It is also useful for reducing the dimensionality of a spectral dataset. The implementation provided here simply takes mean values of intensities and wavenumbers, then replaces the spectrum with these values at intermediate mean wavenumbers. The settable variable *bin_factor* decides the magnitude of the binning. For example, with a *bin_factor* of 4, the mean of every 4 data points will be calculated. The mean of the 4 associated wavenumbers of the spectrum will also be calculated and placed alongside the new binned data.

Table 1: Example of binning a spectrum by factor of 4

Input Data		Binned Data	
Wavenumber	Intensity	Wavenumber	Intensity
1000	0.125		
1001	0.13		
1002	0.12	1001.5	0.12125
1003	0.11		
1004	0.15		
1005	0.16		
1006	0.155	1005.5	0.15125
1007	0.14		

2.3. Smoothing and Smoothing Parameters

The options for smoothing include the most widely used in the field. The smoothing algorithms included in this section are Savitzky-Golay filtering, wavelet

denoising, and local polynomial fitting with Gaussian weighting. The purpose of these methods is to increase the signal-to-noise ratio without greatly distorting the signal.

Wavelet denoising is very good at improving the quality of spectra that already have a reasonably high signal to noise ratio[22]. It also improves the visual quality of these spectra. For datasets with a very low signal to noise ratio, wavelet denoising is on a par with other less sophisticated denoising methods. It has also been reported that this type of denoising improves spectral resolution and information recovery in IR spectroscopy[23]. PRFFECT includes an implementation of the discrete wavelet transform based on the work of Daubechies.[24] The smoothing parameter option in PRFFECT adjusts the length of the Daubechies' filters (i.e. the number of coefficients) and must always be an even number. The estimated noise-free signal is returned as output.

The algorithm for the discrete wavelet transform operates by iterating through the input spectral data in steps of two. At each iteration, the function used to estimate the smoothed data depends on both the current data point and a small number of following data points. The last iteration presents a problem, however, as some of the input data is not defined. This is overcome in our wavelet implementation by simply padding zeroes to the input data. This returns transformed input data elements of the same length and resolution as the original spectral data.

Savitzky-Golay filtering is especially good at removing high-frequency noise from input signals. It is a well established smoothing method for preprocessing of biospectra.[25, 26] The smoothing method is based upon local least-squares polynomial approximation. Savitzky and Golay showed that least-squares smoothing reduces noise while maintaining the height and shape of waveform peaks.[25] The algorithm fits a polynomial to a fixed number of input data points, within a moving window of fixed size. The smoothed data point is evaluated from the fitted polynomial at each step of the algorithm. In the PRFFECT implementation of this method, the degree of the polynomial can be

set by the user.

Local polynomial fitting with Gaussian weighting is another smoothing option included in the package. It is useful for general denoising and smoothing of data. The smoothing function works by estimating and fitting Gaussian curves for each part of the spectrum. The user-defined variable for this method is the bandwidth, which controls the amount of smoothing by modulating the Gaussian kernel density estimator.[26]

2.4. Normalisation choices

The normalisation choices are simpler than the smoothing choices, in that they do not have any parameters to be set.

Scaling of each spectrum between 0 and 1 can bring spectral datasets with varying overall intensity into compatibility. In a spectrum where x_{min} represents the minimum intensity, and x_{max} represents the maximum intensity, each element, x_i of the vector of absorption intensities $\boldsymbol{x}$ can be scaled to give $\tilde{x_i}$ in which $\tilde{x}_{min} = 0$ and $\tilde{x}_{max} = 1$ (Equation 1).

$$\tilde{x_i} = \frac{x_i - x_{min}}{x_{max} - x_{min}} \tag{1}$$

Vector normalisation is a widely-used method for spectral normalisation. Rather than a simple scaling operation, it ensures that all spectra within a dataset have the same vector length of 1 (i.e. unit vectors). The vector of absorption intensities $\boldsymbol{x}$ can be converted to its corresponding unit vector $\hat{\boldsymbol{x}}$ by dividing each element, x_i, by the vector length $||\boldsymbol{x}||$.

$$\hat{x_i} = \frac{x_i}{||\boldsymbol{x}||} \tag{2}$$

Normalisation to Amide I band is another offered normalisation procedure. Each point in the spectrum is scaled by the maximum intensity of the Amide I band (1600 - 1700cm^{-1}). The vector of Amide I band-scaled intensities

$\hat{\boldsymbol{x}}$ is found by dividing each element, x_i, in the vector of absorption intensities $\boldsymbol{x}$ by the maximum intensity within the Amide I band x_A (Equation 3).

$$\hat{x_i} = \frac{x_i}{x_A} \tag{3}$$

2.5. Baseline Correction choices and parameters

First and second derivative spectra are often used together with machine learning algorithms as spectral resolution can be increased this way. These methods are implemented in PRFFECT as numerical differentiation to give both first and second derivatives of the absorption intensities with respect to wavenumber. As these operations are straightforward mathematical transforms, no input parameters are necessary.

Rubberband baseline correction is offered. This is a somewhat uncommon method, intended to be used when other methods are not appropriate due to unusual background conditions. This is especially true when the background noise is non-linear, having a greater effect at one end of the spectrum than another. In this situation, spectra will appear to be very convex with respect to the baseline at one end but not the other. This method works by fitting smoothing splines through supporting points. These supporting points are chosen by an algorithm which finds a convex hull under the spectrum. The spectrum is then "pulled down" at the more convex areas to sit on the baseline along with the rest of the spectrum. The convex hull underneath the spectrum is defined with the aid of the base R function "chull". The user input for this type of baseline correction is the noise cut-off level. This is set in the user-defined input file.

Polynomial baseline correction is provided. This option is intended to be used when distortion of the spectra due to differentiation is undesired.[27] The method is based upon a least-squares polynomial curve-fitting function. The degree of the polynomial, k, is modulated in the user input. Equation 4 shows the generalised polynomial formula, where M is the number of terms associated with the polynomial at a particular degree. The measured spectrum y^{exp} with number of datapoints n, becomes the fitted spectrum y^{fitted}. a repre-

sents the constant, and x represents the indeterminate of the polynomial. The residual sum-of-squares (RSS) between these two is given by Equation 5. These equations show a very standard polynomial least-squares fitting. The implementation however becomes a more useful baseline correction approach by iterating the smoothing 100 times (by default), and having a tolerance for change in RSS between iterations of 0.001 by default.

$$y_j^{fitted} = \sum_{k=0}^{M} a_k x_j^k \tag{4}$$

$$RSS = \sum_{i=1}^{n} [y_i^{fitted} - y_i^{exp}]^2 \tag{5}$$

2.6. Random Forest

Classification models can be built in PRFFECT using the Random Forest algorithm introduced by Breiman and Cutler.[28] A Random Forest is an ensemble of decision trees grown from separate bootstrap samples of the training data using the CART algorithm.[28] The branches in each tree continue to be subdivided during training while the minimum number of observations in each leaf is greater than a predetermined value. The descriptor selected for branch splitting at any fork in any tree is not selected from the full set of possible descriptors but from a randomly selected subset of predetermined size. There are three possible training parameters for Random Forest: ntree - the number of trees in the Forest; mtry - the number of different descriptors tried at each split; and nodesize - the minimum node size below which leaves are not further subdivided. There is extensive evidence in the literature that the Random Forest algorithm is insensitive to small changes in the training parameters around their default values ($ntree = 500$, $mtry = \sqrt{N}$, $nodesize = 5$). [10, 29, 30, 31] The implementation of Random Forest in PRFFECT is based on the randomForest package by Liaw and Wiener.[32].

2.7. Random Forest Feature Importance

The importance of particular spectral features to the Random Forest classification can be ascertained using the combined mean decrease in Gini coefficient, with respect to wavenumbers. The Gini impurity of a node is dependent on the probability of each possible outcome. For a single node τ in the RF classification, the Gini impurity is found by Equation 6 below, where $g(\tau)$ is the impurity of node τ, n is the total number of spectra at the node while n_A and n_B are the number of spectra belonging to class A or B respectively.

$$g(\tau) = 1 - \left(\frac{n_A}{n}\right)^2 - \left(\frac{n_B}{n}\right)^2 \tag{6}$$

Every time a node is split on a predictor (wavenumber), the Gini impurity for the two child nodes is less than the parent node. This is because the dataset is gradually being sorted into predicted classes, and becoming more homogeneous with respect to the proportion of classes A or B. When node τ is split, resulting in two child nodes υ and ϕ, the change in Gini (Δg) is found by Equation 7 where n_υ and n_ϕ are the number of spectra in nodes υ and ϕ respectively. The value of Δg is larger when a greater change in impurity occurs after the split, thus allowing for the decrease in Gini to be used as a measure of importance of a certain wavenumber.

$$\Delta g = g(\tau) - \left(\frac{n_\upsilon}{n}\right) g(\upsilon) - \left(\frac{n_\phi}{n}\right) g(\phi) \tag{7}$$

The overall Gini importance (G) of a particular spectral feature θ is found by the sum across all nodes of each tree ψ, and across all trees in forest ω (Equation 8).

$$G(\theta) = \sum_{i=1}^{\psi} \sum_{j=1}^{\omega} \Delta g_{i,j} \tag{8}$$

2.8. Classification Metrics

A selection of statistical metrics are automatically generated by PRFFECT to give an in-depth analysis of the accuracy and reliability of each classification.

These are defined from the number of true positive(TP), true negative(TN), false positive(FP) and false negative(FN) predictions as well as "real"(actual number of positives and negatives in the dataset) positives(P) and negatives(N). The abbreviation MCC stands for Matthews Correlation Coefficient. Each statistic is generated for both cross-validation on the training data and for prediction of the test set.

$$Number\ of\ Positives\ (P) = TP + FN \tag{9}$$

$$Number\ of\ Negatives\ (N) = TN + FP \tag{10}$$

$$Sensitivity = \frac{TP}{P} = \frac{TP}{(TP + FN)} \tag{11}$$

$$Specificity = \frac{TN}{N} = \frac{TN}{(TN + FP)} \tag{12}$$

$$Positive\ Precision = \frac{TP}{(TP + FP)} \tag{13}$$

$$Negative\ Precision = \frac{TN}{(TN + FN)} \tag{14}$$

$$Accuracy = \frac{(TP + TN)}{(TP + FP + FN + TN)} \tag{15}$$

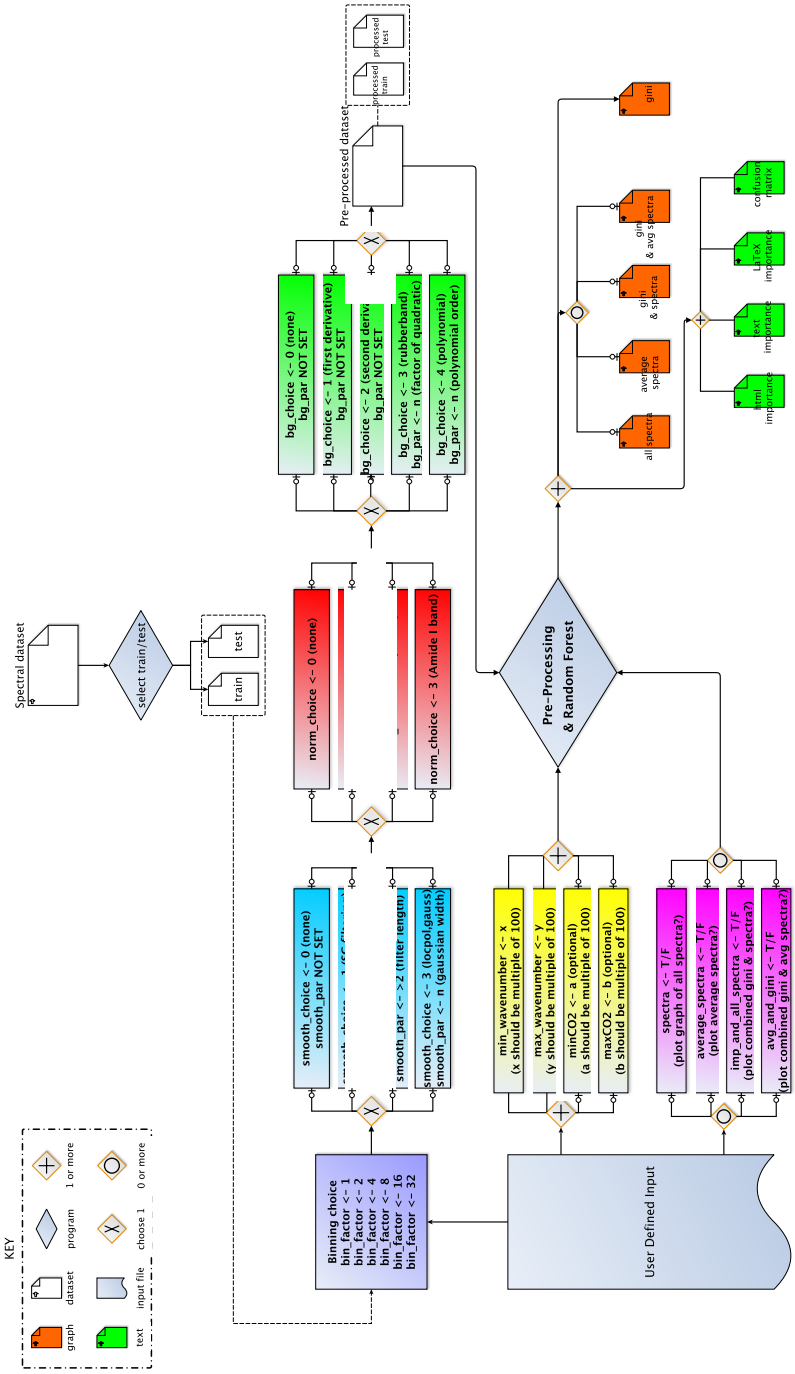
$$MCC = \frac{(TP * TN) - (FP * FN)}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}} \tag{16}$$

2.9. Operation of the program

The program can be set to use any combination of the preprocessing methods outlined above. There is a "none" option for each category. The categories being binning, smoothing, normalisation and baseline correction. The user-defined parameters are different depending on particular methods chosen. All of these options are to be set in input file "user_defined_input.R". Below is a flowchart

showing the available options and parameters in each category. See the online manual for PRFFECT for further detail.[33]

Flowchart 1: PRFFECT workflow. One option is chosen from each main preprocessing block (dark blue, light blue, red, green.) Auxiliary options for wavenumber range selection (yellow) and output graphs (pink) are also set in the user-defined input file.



2.9.1. Example of operation

The following is an example of setting up and running the program in an R environment. The user in this scenario wanted a binning of 4δ, S-G smoothing of order 2, vector normalisation and polynomial baseline correction of degree 3. They also wanted a wavenumber region of 900-1900 cm^{-1}, and wished to plot all available graphs. The descriptor being predicted was "disease/no disease", which is a column with a 1 to denote a patient with the disease, and 2 for a patient without the disease. The desired split between training and test sets was two thirds training - one third test. A script to do the splitting is described in the Supporting Information. This script will by default split a single CSV file into two files with training (input_training_dataset.ssv) and testing (input_testing_dataset.ssv) data respectively. The user intends to carry out a 5-fold cross-validation of the training data. In this example scenario, there are multiple spectra per patient, and the column denoting patient ID is called "Patient_ID". The file user_defined_input.R would be set up according to Table 2.

Table 2: Example scenario settings

Variable	Value
infile_tr	"input_training_dataset.ssv"
infile_te	"input_testing_dataset.ssv"
k	5
bin_factor	4
smooth_choice	1
smooth_par	2
norm_choice	2
bg_choice	4
bg_par	3
spectra	TRUE
average_spectra	TRUE
imp_and_all_spectra	TRUE
avg_and_gini	TRUE
yvariables	disease/no disease
min_wavenumber	900
max_wavenumber	1900
patients	"Patient_ID"

At the R command line, the user would then issue the command *source("PRFFECTv1.R)"*. The program would then run, and pick up all user defined variables. The results

and graphs would then be output as files into the current working directory. See the flowchart for a list of the output files.

2.10. Example Data

As an example of the operation of the program, four datasets have been analysed to find the best preprocessing parameters. These are all open-source datasets available via the internet. The datasets were all FTIR spectra of food items[34, 35, 36, 37]. The FTIR methods used included attenuated total reflection (ATR) and diffuse reflectance infrared Fourier transform (DRIFT).

2.10.1. Fruit Puree Example Dataset

The original fruit puree study[34] involved a PLS regression to predict adulteration of strawberry purees with various adulterants of other fruits or sugars. The overall binary classes of the study were strawberry and non-strawberry, with the adulterated samples being in the non-strawberry category. The study used a training, tuning and test set for the regression. The classification was then carried out by plotting the output of the PLS model, and cut-offs set by 95% confidence intervals. Using this methodology, overall results (mean of sensitivity and specificity) were 96.5%, 93.5% and 94.3% for the training, tuning and test sets respectively. There were 983 spectra in total, of which 351 were pure strawberry.

2.10.2. Meats Example Dataset

The meat authenticity study[35] used ATR FTIR spectra of three different types of meat. Chicken, pork and turkey samples were minced and the spectra directly taken by placing the mince on an ATR crystal. There were two spectra each of 20 samples, giving a total of 120 spectra. The classes were split equally, giving 40 per meat type. The study used PLS regression to attempt to detect varying levels of contamination of each meat into others. For example, a regression was carried out to detect levels of turkey mixed with chicken. The standard deviation of prediction errors (actual vs predicted composition) in that experiment was 14.8%. In pork contaminated turkey, the value was 13.8%.

2.10.3. Olive Oil Example Dataset

The olive oils study[36] combined multivariate analysis with ATR-FTIR, to attempt to distinguish olive oils from different countries. The analysis methods used were LDA based upon the scores from PLS, and a GA to determine variate selection followed by LDA on the subset. The method of statistical analysis and result collection was via a leave-one-out cross validation. The PLS-LDA achieved a 96% success rate, and the GA-LDA achieved a 100% cross-validation success rate.

The dataset itself consisted of 120 spectra, with 2 spectra per sample. Of the samples, there were 10 from Greece, 17 from Italy, 8 from Portugal and 25 from Spain. The region of the IR spectrum used was 799-1897cm^{-1}.

2.10.4. Coffee Example Dataset

The coffee study[37] focussed on the discrimination of two common types of instant freeze-dried coffee, *Arabica* and *Robusta*. The original article explores whether these types of coffee can be authenticated alone; and quantified in mixtures of the two. The spectra were collected via DRIFTS and ATR techniques. LDA, PCA and regression were then employed as the data analysis methods. LDA of the PCA scores yielded 100% accuracy of classification between the two species. This classification was run with a roughly 4:1 training:test set split. For the regression of differing proportions of each species, an R^2 value of 0.99 and standard error of 1.2% were reported using internal cross-validation.

The available dataset (raw data) consisted of 56 samples (29 *Arabica*, 27 *Robusta*), collected in the 800-1900cm^{-1} region, using the DRIFTS technique. The data had a nominal resolution of around 3.85cm^{-1}.

3. Results and Discussion

Grid searches were carried out to find best combinations of preprocessing. The fitness evaluation function was the prediction accuracy statistic for the cross-validation of the training set in all cases. In conjunction with the grid

search, every permutation of methods/parameters was tested on 96 randomly selected training and test set splits. This was done to ensure a fair and accurate portrayal of the abilities of the program. Results are given for best combinations of preprocessing found for the data described above. In each subsection, tables of the top 12 combinations are shown. A key to the table headings is given below.

Table 3: Key to results

Symbol	Explanation	Parameter	Explanation
B	Binning factor		
1	No binning		
2	δ*2		
4	δ*4		
8	δ*8		
16	δ*16		
32	δ*32		
S	Smoothing choice	Sp	S parameter
0	none	-	-
1	S-G filter	**1,2,3,4,5,6**	Filter order
2	Wavelet denoising	2*(**2,3,4,5,6**)	Filter length
3	Local polynomial fit with Gaussian	**1,2,3,4,5,6**	Width of Gaussian
N	Normalisation choice		
0	none		
1	min/max to 0-1 scaling		
2	Vector normalisation		
3	Normalise to Amide I band		
BL	Baseline Correction choice	BLp	BL parameter
0	none	-	-
1	First derivative	-	-
2	Second derivative	-	-
3	Rubberband baseline correction	RBp*(**1,2,3,4,5,6**)	Noise cut-off level
4	Polynomial baseline correction	**1,2,3,4,5,6**	Polynomial degree

Table 4: Key to notation for statistical metrics

Notation	Description
pacCV	Prediction Accuracy for training set
sensCV	Sensitivity for training set
specCV	Specificity for training set
pacTS	Prediction Accuracy for test set
sensTS	Sensitivity for test set
specTS	Specificity for test set

The statistical metrics used were sensitivity (true positive rate), specificity (true negative rate) and prediction accuracy. The first two of these metrics are widely used and have been defined elsewhere.[7]. Prediction accuracy, which is defined in Equation 15, was used as the fitness function to identify the optimal combinations of preprocessing routines.

3.1. Fruit Example Dataset

Table 5 shows the results for the binary classification of strawberry vs non-strawberry purees. The results are on a par with the original study[34]. The procedure in the original article is quite different from the procedure used to generate our results. In this work, we used a direct classification with output of important features. The original methodology was to use a PLS regression both to find optimum number of PLS scores and to do a classification. Since PRFFECT can directly produce a classification and report the best features, a standard classification methodology was used by us. The original study used three data partitions: training, tuning and test sets. As these were a third of the data each, and RF training parameters are set to default values, we used two thirds as a training set, and one third as a test set. A tuning set was not required because RF is insensitive to small changes in its adjustable parameters and is not prone to over-fitting.[7, 10, 38, 29] Default RF parameters were used for all classifications reported in this article (500 trees per Random Forest, $\sqrt{ndesc.}$ descriptors considered at each fork in each tree, terminal nodes all contain no

more than 5 samples). Presented in Table 6 below is a direct comparison of our best results from the top row of Table 5, and those of the original study.

The best combinations of preprocessing for this dataset always included a binning factor of 4 or 8. When a factor of 8 was found to be preferable, it was always followed with smoothing type 1 (S-G filter) with a smoothing parameter of 5 (filter order). Smoothing types 1 and 3, S-G filter and local polynomial Gaussian fit respectively, were the only two types to appear in the top dozen results. The dataset shows an insensitivity to normalisation type, with all four options appearing among the best classifiers. When it came to baseline correction, every time the best choice was first derivative.

Overall, the results are very consistent across the measured metrics and CV and test sets. The lowest statistic for both sets was specificity (equivalent to "Non-strawberry" column in Table 6 below). However, our results were still slightly in excess of the original study. Figure 1 shows the most important wavenumber descriptors for the classification of the fruit purees.

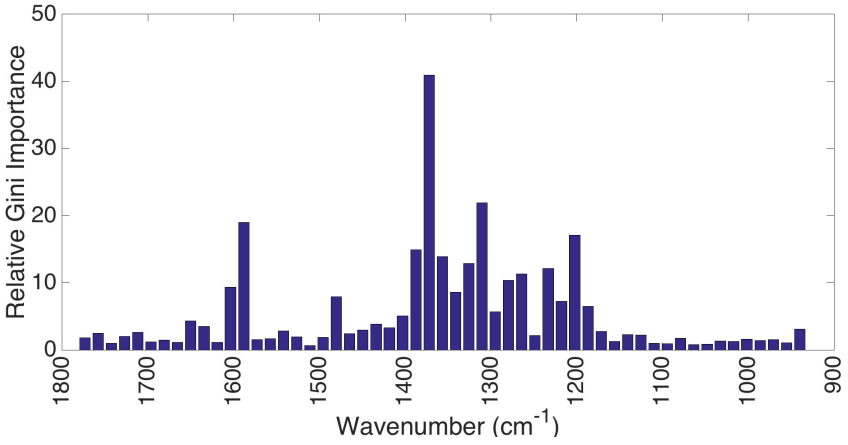
Table 5: Results for fruit dataset

B	S	Sp	N	BL	BLp	pacCV	sensCV	specCV	pacTS	sensTS	specTS
4	1	2	2	1	-	0.970	0.977	0.958	0.971	0.979	0.957
4	3	5	3	1	-	0.970	0.974	0.962	0.970	0.974	0.964
4	1	3	3	1	-	0.970	0.972	0.964	0.970	0.975	0.962
4	1	1	3	1	-	0.969	0.973	0.963	0.971	0.976	0.964
4	1	4	2	1	-	0.969	0.978	0.954	0.971	0.981	0.955
8	1	5	2	1	-	0.969	0.975	0.959	0.973	0.980	0.961
4	3	6	3	1	-	0.969	0.973	0.962	0.972	0.976	0.964
4	3	4	3	1	-	0.969	0.973	0.962	0.971	0.976	0.963
8	1	5	1	1	-	0.969	0.976	0.958	0.971	0.979	0.959
4	1	2	0	1	-	0.969	0.975	0.959	0.970	0.978	0.955
4	3	6	0	1	-	0.969	0.976	0.957	0.970	0.978	0.957
4	3	5	0	1	-	0.969	0.975	0.957	0.971	0.979	0.956

Table 6: Comparison of original results to this work, % correct classifications

		% Correct		
		Strawberry	Non-strawberry	Overall
Original study	Training	97.5	95.4	96.5
	Tuning	93.2	93.8	93.5
	Test	94.8	94.1	94.3
This work	Training	97.7	95.8	96.8
	Test	97.9	95.7	96.8

Figure 1: Gini Importance plot for best classification - Fruit purees



3.2. Meats Example Dataset

Since this dataset included 3 classes, metering the best possible preprocessing was more cumbersome than with a binary classification. This is because there are three separate sets of statistics which make up one result. Here, we have simply averaged the results across all 3 meat types to find the best combinations. A full database of all results obtained in this work can be found in the supporting information. All found best combinations shown in Table 7 gave very strong classification results. These are of course average results across pork, turkey and chicken meats. As might be expected, pork was the easier meat to identify, with many perfect results. The two bird meats were more difficult to separate from one another, but overall the classifications performed very well.

The best combinations for this particular 3-way classification are very interesting, especially the insensitivity to binning factor. All explored binning factors are present in the top 12 results. The best combinations also appear to be insensitive to smoothing choice and smoothing parameters. Again, all possible smoothing types are present in the top 12. As for normalisations, 0 (no normalisation) was the preferred option. For baseline correction, option 4 (polynomial baseline correction) was seen in all but one of the top dozen. The favoured baseline parameter (BLp) values were towards the higher end of the

parameter space, with 6 appearing the most often.

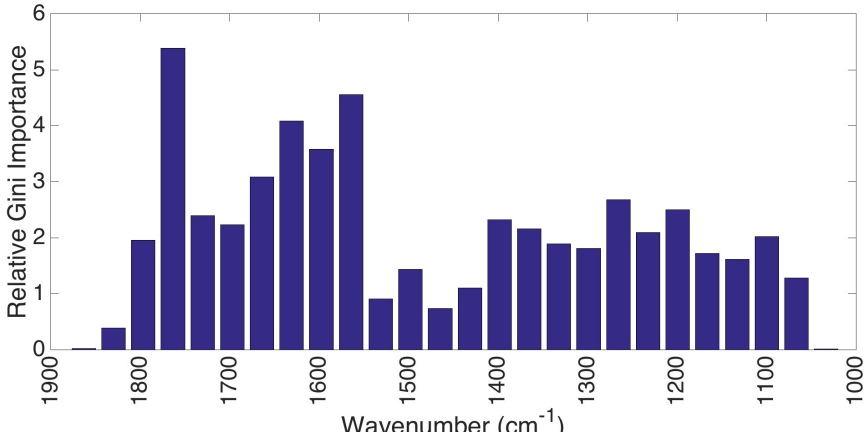
Of all the results displayed in Table 7, specificity is the highest overall. This could be due to the ease of which pork is separated from the two bird meats. The slightly lower sensitivity values (although still very high) are probably due to the difficulty of separating the turkey from the chicken meat. This gave a very good test for the robustness of the random forest algorithm as a whole, in that one class stood very far out from the others.

The main focus of the original paper[35] was to perform a regression of varying compositions of meat mixtures. The spectra of the mixtures were not available at the time of writing. Therefore, we have focussed on the classification of different meats from the dataset. It is shown in the original article that PCA was carried out on the whole meat data, and the loadings examined. The loadings described show the strongest features in the 1550-1650 cm^{-1} protein region. There was also a smaller feature at 1740 cm^{-1} (lipid region). Figure 2 shows that this assessment fits well with what was found in this study. Of course, it ought to be borne in mind that our best preprocessing combination had a heavy binning factor of 16. Therefore, exact matches to wavenumber values should not be expected.

Table 7: Results for meat dataset - average across 3 meat types

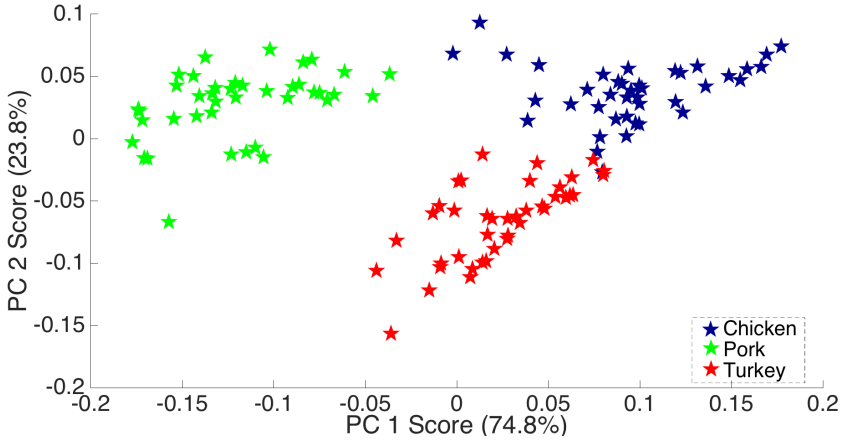
B	S	Sp	N	BL	BLp	pacCV	sensCV	specCV	pacTS	sensTS	specTS
16	2	2	0	4	1	0.984	0.975	0.988	0.986	0.978	0.990
16	2	4	3	4	2	0.980	0.969	0.985	0.988	0.978	0.990
2	1	1	0	4	6	0.978	0.966	0.983	0.974	0.954	0.978
16	2	2	1	0	-	0.976	0.966	0.982	0.982	0.972	0.986
8	1	3	0	4	6	0.976	0.964	0.982	0.970	0.948	0.975
8	0	-	0	4	6	0.976	0.964	0.982	0.983	0.970	0.985
32	1	4	0	4	3	0.976	0.963	0.982	0.980	0.968	0.985
8	2	6	0	4	4	0.975	0.964	0.981	0.980	0.965	0.984
2	3	2	0	4	6	0.975	0.962	0.981	0.976	0.959	0.980
2	2	2	0	4	6	0.975	0.962	0.981	0.976	0.958	0.980
1	2	2	0	4	6	0.975	0.961	0.981	0.974	0.954	0.978
4	3	4	0	4	6	0.975	0.963	0.981	0.976	0.958	0.979

Figure 2: Gini Importance plot for best classification - Meats



375 Figure 3 shows a PCA plot of the meat dataset, after being pre-processed according to the first row in Table 7. It shows an improvement over the PCA

Figure 3: PCA plot for best preprocessing - Meats



plot present in the original study. Since the separation is better, it can be concluded that the pre-processed data offers an improved classification.

3.3. Olive Oil Example Dataset

Similar to the meat example dataset, the olive oils dataset was a multi-class classification. In total there were four classes which represented country of origin. Therefore Table 8 represents results averaged across these four classes. A full database of results can again be found in the supporting information. The results shown are for a 66:33 training:test split, as it is common to all example datasets studied. The results were good overall, with specificity being the strongest statistic. This may be expected since there were four classes involved in the classification. Again, CV and TS results were very close.

Binning factors of 4 and 8 were preferred. The dataset appeared to be relatively insensitive to smoothing choice and smoothing parameters. Normalisation choice was similar, with normalisation to Amide I (option 3) being the only one not appearing. It was clear however that baseline choice 1 (first derivative) was strongly preferred.

Since the original study[36] employed a Leave-Two-Out Cross Validation (LTOCV), we have repeated the classification with that methodology, using processed data according to the best preprocessing combination from Table 8.

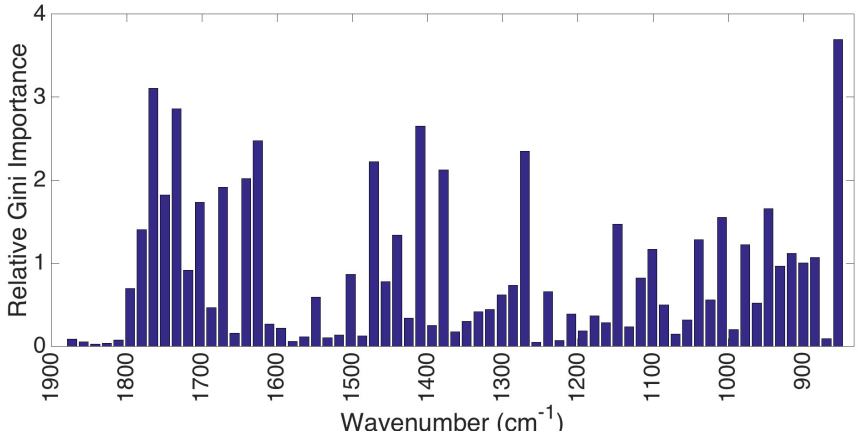
A LTOCV was employed because there were two spectra per sample. While the original study reported 100% correct classifications, our method misclassified a total of 7 spectra from the 120 in the dataset. Three Spanish samples were misclassified as Italian, two Spanish samples were misclassified as Portuguese and two Portuguese samples misclassified as Italian. This meant a 94.2% correct classification rate for our methodology. Although this is lower than the original study, it is on a par with the overall results shown in Table 8.

Figure 4 shows the most important wavenumber descriptors for the four-way classification. Some similarities can be seen between the histogram of the genetic algorithm in the original paper (Figure 5a) and the Gini plot in our Figure 4. It may be expected that these plots would show similar features, although they were arrived at in very different ways.

Table 8: Results for olive oil dataset - average across 4 oil origins

B	S	Sp	N	BL	BLp	pacCV	sensCV	specCV	pacTS	sensTS	specTS
8	0	-	1	1	-	0.964	0.928	0.975	0.965	0.927	0.975
4	3	6	2	1	-	0.963	0.929	0.974	0.963	0.927	0.973
4	3	6	1	1	-	0.963	0.929	0.974	0.958	0.923	0.970
4	1	2	2	1	-	0.962	0.927	0.974	0.963	0.934	0.973
4	3	5	2	1	-	0.962	0.924	0.973	0.963	0.931	0.974
4	3	4	2	1	-	0.961	0.923	0.972	0.965	0.937	0.975
8	0	-	2	1	-	0.961	0.916	0.973	0.969	0.940	0.978
4	1	6	0	1	-	0.960	0.924	0.972	0.966	0.939	0.976
4	1	3	0	2	-	0.960	0.927	0.972	0.964	0.930	0.974
4	3	4	1	1	-	0.960	0.922	0.972	0.961	0.927	0.972
4	1	4	1	1	-	0.960	0.921	0.972	0.971	0.947	0.979
4	0	-	1	1	-	0.960	0.916	0.972	0.960	0.923	0.972

Figure 4: Gini Importance plot for best classification - Olive Oils



3.4. Coffee Example Dataset

Table 9 shows the classification results for the coffee dataset. This shows clearly that the program performs just as well as the original study, even with (effectively) a much smaller training set. Another interesting comparison to make would be the areas of spectral distinction between the two species, as identified in this work, and that of the original authors. This was originally identified by examining PCA loadings. The Gini importance metric built into our program should also show similar regions. The original study[37] found areas of interest in the following regions: A large peak at around 1760 cm^{-1}, several loadings in the 1550-1750 cm^{-1} region, and several smaller features in the 1150-1300 cm^{-1} region. Figure 5 shows the Gini plot for the best combination of preprocessing parameters. As can be seen, the largest peak in the Gini is in the same area as the original study's PCA loadings. The other features described by Briandet et. al. also match up rather well. Our best classification happened to have a binning factor of 8 applied to the spectra. Therefore it should be borne in mind that such heavily binned spectra by definition have fewer datapoints overall.

It can be seen from Table 9 that the classification of *Arabica* vs *Robusta* was extremely successful overall. The top 12 preprocessing combinations show near-

perfect classifications. The reason there are small decreases in some statistics is because these results were generated from averages of 96 separate training/test set selections to ensure an un-biased test of the program. Binning factors of 8 and 4 were favourable. This translates to roughly a 30.8 cm^{-1} and 15.4 cm^{-1} effective resolution respectively. The most favourable smoothing type was wavelet, with a filter length of 4 (actual filter length is found by 2*Sp, as shown in Table 3.) The best normalisation procedures were found to be min/max scaling and vector normalisation. Interestingly, whenever min/max scaling was used, the best baseline correction was always first derivative. Conversely, whenever vector normalisation was used, the best baseline correction was the rubberband technique. The rubberband technique appeared to be insensitive to the baseline parameter, BLp (noise cut off level) for this dataset.

Overall, the program performed as well as the original study and showed similar important regions.

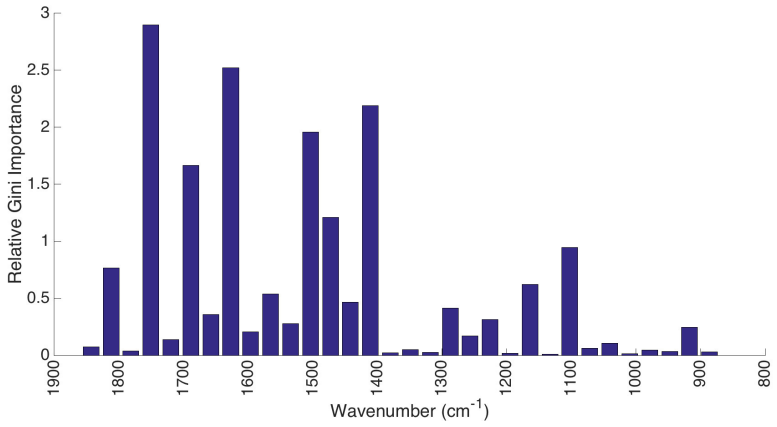
Table 9: Classification Results for *Arabica* vs *Robusta*

B	S	Sp	N	BL	BLp	pacCV	sensCV	specCV	pacTS	sensTS	specTS
8	1	3	1	1	-	1	1	1	1	1	1
8	1	5	1	1	-	1.000	1	0.999	1	1	1
8	2	2	2	3	3	0.999	0.999	1	0.997	0.998	0.997
8	2	2	2	3	5	0.999	1	0.998	0.998	0.998	0.999
8	2	2	2	3	6	0.999	1.000	0.998	1	1	1
8	2	2	2	3	1	0.999	0.999	0.999	0.999	0.999	1
8	2	2	2	3	2	0.998	0.998	0.998	1	1	1
4	1	4	1	1	-	0.998	0.997	0.999	1	1	1
4	0	-	1	1	-	0.998	0.996	1	0.996	0.993	1
8	2	2	2	3	4	0.998	0.997	0.999	1	1	1
8	1	1	1	1	-	0.998	0.999	0.997	0.999	1	0.999
4	1	2	1	1	-	0.998	0.997	0.999	0.998	0.997	1

Table 10: Results for coffee dataset - Comparison of methodologies

Source	Correct Classifications
Original LDA classifier	100%
This work RF classifier	100%

Figure 5: Gini Importance plot for best classification



4. Conclusions

Using PRFFECT, we were generally able to match or exceed the results from the example studies. It was also a more streamlined process, and provided more information in some cases via the Gini Importance metric. It was also possible to generate classification statistics automatically via the built-in algorithms. PRFFECT shows great potential as a tool for spectroscopists who wish to carry out classifications and explore various types of preprocessing. Our group has already used it with success for diagnosing brain cancer from serum FTIR samples.[7] It is particularly attractive since it is an all-in-one package, no external preprocessing or statistical analysis of results is required. Although PRFFECT dramatically simplifies the development of spectral classification models, it is not intended to be operated completely blindly. The user must still make their own decisions about the reliability of the input data, including whether the classes assigned to each sample are sufficiently well defined. Using the process-only version, it is possible to produce a dataset which has been subject to several complex preprocessing procedures in one step. We envision PRFFECT as being a popular and useful tool for spectroscopists of all fields.

5. Independent testing

The software was independently tested by Dr John Mitchell of the Department of Chemistry at the University of St. Andrews in the UK. At the time of testing, the list of software dependencies in the manual was incomplete, but this problem has now been fixed, as noted below. His comments in full:

"Thank you for giving me the opportunity to use your PRFFECT code. I think it's going to be a very useful resource for researchers looking to get extra value from spectroscopic data at the interface between spectroscopy and machine learning. I was aware of it from a poster at the ScotChem meeting in Glasgow.

Having looked at the code, the manual is extensive and very helpful. I was able to run the example datasets provided and they worked as expected. We initially encountered some issues identifying which packages were to be pre-installed. Thanks to your group for providing a definitive list of the R packages required. I'm glad to see that the manual has been updated accordingly. I think the current version of the manual is excellent and provides a clear and thorough guide to the usage and capabilities of your excellent software.

I can confirm that the software runs as expected and that I expect it to prove a valuable resource for the scientific research community."

Acknowledgements

We thank Holly Butler and Robby Fritzsch for useful discussions. DSP and BRS are grateful for use of the EPSRC funded ARCHIE- WeSt High Performance Computer (http://www.archie-west.ac.uk, EPSRC Grant No. EP/K000586/1). MJB and DSP would like to thank Scottish Enterprise for funding. MJB is grateful for funding from EPSRC, Dstl, Rosemere Cancer Foundation, Brain Tumour North West, and the Sydney Driscoll Neuro-science Foundation.

References

[1] D. I. Ellis, D. Broadhurst, D. B. Kell, J. J. Rowland, R. Goodacre, Rapid and quantitative detection of the microbial spoilage of meat by fourier transform infrared spectroscopy and machine learning, Appl. Environ. Microbiol. 68 (6) (2002) 2822–2828.

[2] M. G. Madden, A. G. Ryder, Machine learning methods for quantitative analysis of raman spectroscopy data, in: OPTO Ireland, International Society for Optics and Photonics, 2003, pp. 1130–1139.

[3] F. Chauchard, R. Cogdill, S. Roussel, J. Roger, V. Bellon-Maurel, Application of ls-svm to non-linear phenomena in nir spectroscopy: development of a robust and portable sensor for acidity prediction in grapes, Chemometr. Intell. Lab 71 (2) (2004) 141–150.

[4] J. R. Hands, G. Clemens, R. Stables, K. Ashton, A. Brodbelt, C. Davis, T. P. Dawson, M. D. Jenkinson, R. W. Lea, C. Walker, et al., Brain tumour differentiation: rapid stratified serum diagnostics via attenuated total reflection fourier-transform infrared spectroscopy, J. Neurooncol 127 (3) (2016) 463–472.

[5] C. L. Pickering, J. R. Hands, L. M. Fullwood, J. A. Smith, M. J. Baker, Rapid discrimination of maggots utilising atr-ftir spectroscopy, Forensic Sci. Int. 249 (2015) 189–196.

[6] T. Mudariki, R. Lea, G. Clemens, M. Baker, P59diagnostic application of vibrational spectroscopy for the segregation of brain tumour cell lines coupled with multivariate statistics, Neuro-oncology 16 (suppl 6) (2014) vi10.

[7] B. R. Smith, K. M. Ashton, A. Brodbelt, T. Dawson, M. D. Jenkinson, N. T. Hunt, D. S. Palmer, M. J. Baker, Combining random forest and 2d correlation analysis to identify serum spectral signatures for neuro-oncology, Analyst 141 (12) (2016) 3668–3678.

[8] J. R. Hands, K. M. Dorling, P. Abel, K. M. Ashton, A. Brodbelt, C. Davis, T. Dawson, M. D. Jenkinson, R. W. Lea, C. Walker, et al., Attenuated total reflection fourier transform infrared (atr-ftir) spectral discrimination of brain tumour severity from serum samples, J. Biophotonics 7 (3-4) (2014) 189–199.

[9] S. J. Barrington, H. Bird, D. Hurst, A. J. McIntosh, P. Spencer, S. H. Pelfrey, M. J. Baker, Spectroscopic investigations of surface deposited biological warfare simulants, in: SPIE Defense, Security, and Sensing, International Society for Optics and Photonics, 2012, pp. 83580E–83580E.

[10] D. S. Palmer, N. M. O'Boyle, R. C. Glen, J. B. Mitchell, Random forest models to predict aqueous solubility, J. Chem. Inf. Model 47 (1) (2007) 150–158.

[11] J. L. McDonagh, D. S. Palmer, T. v. Mourik, J. B. Mitchell, Are the sublimation thermodynamics of organic molecules predictable?, J. Chem. Inf. Model. 56 (11) (2016) 2162–2179.

[12] R. Diaz-Uriarte, S. de Andres, Gene selection and classification of microarray data using random forest, BMC Bioinformatics 7. doi:{10.1186/1471-2105-7-3}.

[13] D. R. Cutler, T. C. Edwards, Jr., K. H. Beard, A. Cutler, K. T. Hess, Random forests for classification in ecology, Ecology 88 (2007) 2783–2792. doi:{10.1890/07-0539.1}.

[14] F. Grosserueschkamp, A. Kallenbach-Thieltges, T. Behrens, T. Bruening, M. Altmayer, G. Stamatis, D. Theegarten, K. Gerwert, Marker-free automated histopathological annotation of lung tumour subtypes by FTIR imaging, Analyst 140 (7) (2015) 2114–2120. doi:10.1039/c4an01978d.

[15] J. Ollesch, D. Theegarten, M. Altmayer, K. Darwiche, T. Hager, G. Stamatis, K. Gerwert, An infrared spectroscopic blood test for non-small cell lung carcinoma and subtyping into pulmonary squamous cell carcinoma or

adenocarcinoma, Biomedical Spectroscopy and Imaging 5 (2016) 129–144. doi:10.3233/BSI-160144.

[16] J. Ollesch, M. Heinze, H. M. Heise, T. Behrens, T. Brüning, K. Gerwert, It's in your blood: spectral biomarker candidates for urinary bladder cancer from automated FTIR spectroscopy, Journal of Biophotonics 7 (2014) 210–221.

[17] H.-Z. Chen, G.-Q. Tang, W. Ai, L.-L. Xu, K. Cai, Use of Random Forest in FTIR Analysis of LDL Cholesterol and Tri-Glycerides for Hyperlipidemia, Biotechnology Progress 31 (6) (2015) 1693–1702.

[18] R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0 (2008).
URL http://www.R-project.org

[19] A. Kohler, J. Sulé-Suso, G. D. Sockalingum, M. Tobin, F. Bahrami, Y. Yang, J. Pijanka, P. Dumas, M. Cotte, D. G. van Pittius, G. Parkes, H. Martens, Estimating and Correcting Mie Scattering in Synchrotron-Based Microscopic Fourier Transform Infrared Spectra by Extended Multiplicative Signal Correction, Appl. Spectrosc. 62 (3) (2008) 259–266.

[20] L. Kerr, B. Hennelly, A multivariate statistical investigation of background subtraction algorithms for raman spectra of cytology samples recorded on glass slides, Chemometr. Intell. Lab. Syst. 158 (Supplement C) (2016) 61 – 68.

[21] C. Beleites, V. Sergo, hyperspec: a package to handle hyperspectral data sets in r, R package v. 0.98-20120725.

[22] B. K. Alsberg, A. M. Woodward, M. K. Winson, J. Rowland, D. B. Kell, Wavelet denoising of infrared spectra, Analyst 122 (7) (1997) 645–652.

[23] D. Zhan, S. Sun, Q. Zhou, Z. Qin, Wavelet denoising and optimization of two-dimensional correlation ir spectroscopy, Spectrosc. Spect. Anal. 24 (12) (2004) 1549–1552.

[24] I. Daubechies, Orthonormal bases of compactly supported wavelets, Comm. Pure Appl. Math 41 (7) (1988) 909–996.

[25] A. Savitzky, M. J. Golay, Smoothing and differentiation of data by simplified least squares procedures., Anal. Chem. 36 (8) (1964) 1627–1639.

[26] M. J. Baker, J. Trevisan, P. Bassan, R. Bhargava, H. J. Butler, K. M. Dorling, P. R. Fielden, S. W. Fogarty, N. J. Fullwood, K. A. Heys, et al., Using fourier transform ir spectroscopy to analyze biological materials, Nat. Protoc. 9 (8) (2014) 1771–1791.

[27] C. A. Lieber, A. Mahadevan-Jansen, Automated method for subtraction of fluorescence from biological raman spectra, Appl. Spectrosc. 57 (11) (2003) 1363–1367.

[28] L. Breiman, Random forests, Mach. Learn. 45 (1) (2001) 5–32.

[29] L. D. Hughes, D. S. Palmer, F. Nigsch, J. B. O. Mitchell, Why are some properties more difficult to predict than others? A study of QSPR models of solubility, melting point, and log P, J. Chem. Inf. Model. 48 (2008) 220–232.

[30] D. S. Palmer, M. Mišin, M. V. Fedorov, A. Llinas, Fast and general method to predict the physicochemical properties of druglike molecules using the integral equation theory of molecular liquids, Mol. Pharm. 12 (9) (2015) 3420–3432.

[31] E. O. Cannon, A. Bender, D. S. Palmer, J. B. O. Mitchell, Chemoinformatics-based classification of prohibited substances employed for doping in sport, J. Chem. Inf. Model. 46 (6) (2006) 2369–2380.

[32] A. Liaw, M. Wiener, Classification and regression by randomforest, R news 2 (3) (2002) 18–22.

[33] B. R. Smith, M. J. Baker, D. S. Palmer, PRFFECT User Manual, University of Strathclyde, Glasgow, UK (2017).
URL https://github.com/Palmer-Lab/PRFFECT

[34] J. Holland, E. Kemsley, R. Wilson, Use of fourier transform infrared spectroscopy and partial least squares regression for the detection of adulteration of strawberry purees, J. Sci. Food Agr. 76 (2) (1998) 263–269.

[35] O. Al-Jowder, E. Kemsley, R. Wilson, Mid-infrared spectroscopy and authenticity problems in selected meats: a feasibility study, Food Chem. 59 (2) (1997) 195–201.

[36] H. S. Tapp, M. Defernez, E. K. Kemsley, Ftir spectroscopy and multivariate analysis can distinguish the geographic origin of extra virgin olive oils, J. Agr. Food Chem. 51 (21) (2003) 6110–6115.

[37] R. Briandet, E. K. Kemsley, R. H. Wilson, Discrimination of arabica and robusta in instant coffee by fourier transform infrared spectroscopy and chemometrics, J. Agr. Food Chem. 44 (1) (1996) 170–174.

[38] D. S. Palmer, J. B. O. Mitchell, Is experimental data quality the limiting factor in predicting the aqueous solubility of druglike molecules?, Mol. Pharmaceutics 11 (8) (2014) 2962–2972. doi:10.1021/mp500103r.