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# A Comprehensive Analysis of MALDI-TOF Spectrometry Data

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## 1. Introduction

Today, biology and medicine need developed technologies and bioinformatics methods. Effective methods of analysis combine different technologies and can operate on many levels. Multi-step analysis needs to be performed to get information helpful in diagnosis or medical treatment tasks. All those processing needs the informatics approach to bioinformatics, proteomics and knowledge discovery methods.

Scientists find proteomic data difficult to analyse. On the other hand, the proteomic analysis of tissues like blood, plasma and urine might have an invaluable contribution to biological and medical research. They seem to be an alternative way of searching for new diagnostic methods, medical treatment and drug development. For example, typical analytical methods have problems with dealing with cancer diseases. Proteomics is a promising approach to those issues.

Proteomic signals carry an enormous amount of data. They reflect whole sequences of proteins responsible for various life processes of the organism. This diversity of data makes it hard to find specific information about, for example, the severity of the cancer. To discover interesting knowledge researchers need to combine a variety of techniques. One of the basic methods of tissue analysis is mass spectrometry. This technique measures the mass-to-charge ratio of charged particles.

There are various types of mass spectrometry techniques. They differ in the types of ion source and mass analysers. The MALDI-TOF (Coombes et al., 2007) is a technique widely applicable in proteomic research. The MALDI (Matrix - Assisted Laser Desorption / Ionisation) is a soft ionisation technique and the TOF (time of flight) is a detector determining the mass of ions. Samples are mixed with a highly absorbent matrix and bombarded with a laser. The matrix stimulates the process of transforming laser energy into excitation energy (Morris et al., 2005). After this process analyte molecules are sputtered and spared. The mass of ions is determined on the basis of time particular ions take to drift through the spectrometer. Velocities and intensities of ions obtained in such a way (Morris et. al., 2005) are proportional to the mass-to-charge ( $m/z$ ) ratio.

The analysis of mass spectrometry data is a complex task (Plechawska 2008a; Plechawska 2008b). The process of gaining biological information and knowledge from raw data is composed of several steps. A proper mass spectrometry data analysis requires creating and

solving a model and estimating its parameters (Plechawska 2008a). There are many types of models and methods which might be used. All of them, however, include mass spectrum preprocessing, which need to be done before the general analysis. Preprocessing methods need be adjusted to data. Spectra have some noise levels which need to be removed. Denoising and baseline corrections are done to get rid of the noise which might be caused by spectrometer inaccuracy or by sample contamination. Also, normalisation should be performed. After these steps, peak detection and quantification can be done. Successful preprocessing is a condition of reliable mass spectrometry data analysis (Coombes et. al., 2007). All elements of the mass spectral analysis are closely related. Any performed operation has an influence on the further quality of results. Not only the set of methods and parameters is important. The proper order of methods also matters.

There is an extensive literature on mass spectrum analysis problems (Plechawska et al., 2011). One can find several techniques of peak detection and identification. A very popular approach is to use local maxima and minima. Such methods (Morris et. al., 2005; Yasui et. al., 2003; Tibshirani et. al., 2004) usually compare local maxima with noise level. There are also methods (Zhang et al., 2007) considering the signal to noise ratio. This ratio needs to be high enough to identify a true peak with a local maximum. Such methods choose peaks with the highest intensities. Similar ideas (Mantini et al., 2007; Mantini et al., 2008) consider using predefined thresholds depending on the noise level. Peak detection is usually done on denoised spectra. Moreover, intervals based on local maxima and minima are calculated. Constituent intervals have differences between the height of the maxima and minima found. In addition, using the mean spectrum was proposed (Coombes et. al., 2007). Other methods (Fung & Enderwick, 2002) use regions which are determined to enable easier peak detection based on the signal to noise ratio. Peaks need to have an large enough area and appropriate width, which depends on starting and ending points of peaks and valleys on both sides of the apex. Peaks may be also considered a continuous range of points where intensities are high enough (Eidhammer et al., 2007). Another approach is using peak clusters to find peaks of the highest intensities (Zhang et al., 2007). There are also methods which try to distinguish true peaks from noise and contaminants. Du et al. (Du et al., 2006) for example use the shape of peaks. Some methods consider the mass spectrometer resolution. There are also methods turning spectrum decomposition into the sum of their constituent functions (Randolph et al., 2005) or the sum of the Levy processes (Zhang et al., 2007).

## 2. Mass spectrum modelling

Before the main decomposition, preprocessing needs to be performed. In our analysis we apply the following methods:

- Trimming is the cutting of the lower and/or upper parts of spectra according to specified boundaries designated by the type of analysis.
- Binning with a defined mask is a technique reducing the number of data points in a single spectrum. The researcher has to keep in mind that this process additionally gives noise reduction. This is optional method. It should be used if the number of the spectrum data points is too large to perform efficient calculations.
- Interpolation is a process which may be defined as the unification of measurements points along the  $m/z$  axes. It is needed in the case of dealing with a data set of spectra. Unification is obligatory if all spectra are to be analysed simultaneously.

- Baseline correction is an essential part of preprocessing. Baseline is a special type of noise which needs to be removed. It represents a systematic artifact formed by a cloud of matrix molecules hitting the detector (Morris et al., 2005). This noise is seen in the early part of the spectrum. Among typical methods of baseline correction one can find a simple frame with fixed sizes and quantiles. Our experience shows that a simple frame with the appropriate size is good enough.
- Smoothing and noise reduction might be performed in several ways. One can use wavelet transformation (for example the widely-used undecimated discrete-wavelet transformation, UDWT), the least-squares digital polynomial filter (Savitzky and Golay filters) or nonparametric smoothing (locally-weighted linear regression with specified window size and type of kernel). In our analysis we usually make use of a polynomial filter. However it is also possible to skip noise reduction due to the specificity of the decomposition method.
- Normalisation is an important preprocessing method consisting in minimising differences between spectra and their peak intensities. The most popular methods are scaling all spectra to total ion current (TIC) value or to constant noise. We found the TIC value appropriate for our analysis. It is calculated as the area under the curve, usually using the trapezoidal method.
- The mean spectrum calculation is useful in analysing data sets containing many mass spectra of the same type. The mean spectrum facilitating the simultaneous analysis of all spectra. Even small peaks are usually detected during mean spectrum analysis. Finding peaks in the mean spectrum are regarded as even more sensitive (Morris et al., 2005).

Most preprocessing steps need to be conducted under the supervision of the user. The parameters of the baseline correction especially need to be adjusted to the data. The order of operations is fixed. Many research studies were conducted in this area and this order has become a standard over the past few years. Some of operations might be skipped - but it should be depended on the data.

## 2.1 Gaussian mixture model decomposition

Our method of spectrum analysis is based on Gaussian Mixture decomposition. The Gaussian Mixture Model (GMM) (Everitt & Hand, 1981) with the appropriate number of components is suitable for spectrum modelling because they also might be used for noise modelling and determining. The idea of using GMM is that one peak is represented by a single distribution (Plechawska-Wojcik, 2011a). All peaks and the noise are represented by the mixture model. A mixture model is a combination of a finite number of distributions. The number of components might be estimated by the Bayesian Information Criterion (BIC).

The fitting is done with the Expectation-Maximisation algorithm (EM) performing maximising the likelihood function. A typical mixture model is a combination of a finite number of probability distributions (eq. 1).

$$f^{mix}(x, \alpha_1, \dots, \alpha_K, p_1, \dots, p_K) = \sum_{k=1}^K \alpha_k f_k(x, p_k) \quad (1)$$

where  $K$  is the number of components in the mixture and  $\alpha_k, k = 1, 2, \dots, K$  are weights of the particular component,  $\sum_{k=1}^K \alpha_k = 1$ . The Gaussian distribution is given with two parameters: mean  $\mu_k$  and standard deviation  $\sigma_k$ .

The Expectation-Maximisation (EM) algorithm (Dempster et al., 1977) is a nonlinear method and is composed of two main steps performed in a loop. The expectation step (E) consists of the calculation of the distribution of hidden variables (eq. 2).

$$p(k | x_n, p^{old}) = \frac{\alpha_k^{old} f_k(x_n, p^{old})}{\sum_{k=1}^K \alpha_k^{old} f_k(x_n, p^{old})} \quad (2)$$

The maximisation step (M) calculates new mixture parameter values. Formulas adjusted to mass spectrometry data are given by (eq. 3).

$$\begin{aligned} \mu_k^{new} &= \frac{\sum_{n=1}^N x_n y_n p(k | x_n, p_{old})}{\sum_{n=1}^N p(k | x_n, p_{old}) y_n}, k = 1, 2, \dots, K \\ (\sigma_k^{new})^2 &= \frac{\sum_{n=1}^N (x_n - \mu_k^{new})^2 p(k | x_n, p_{old})}{\sum_{n=1}^N p(k | x_n, p_{old}) y_n}, k = 1, 2, \dots, K \\ \alpha_k^{new} &= \frac{\sum_{n=1}^N p(k | x_n, p_{old}) y_n}{N} \end{aligned} \quad (3)$$

The calculated means represent the M/Z values of peaks, whereas standard deviations indicate the widths of peaks. Weights determine the shares of particular peaks in the spectrum. This method may be applied to individual spectra or to the mean spectrum calculated from the data set. In the case of the mean spectrum, the obtained means and standard deviations are treated as, respectively, M/Z values and widths of peaks in every single spectrum of the data set. The weights are calculated separately for each spectrum. The simple least-squares method might be used to obtain those weights.

Examples of a mass spectra collection analysis are presented in Fig.1. Fig.1a,c present the results of our calculations for single spectra with 40 components and Fig.1b,d presents the results with the use of the mean spectrum. The mean spectrum is presented in Fig.2.

## 2.2 Parameters of the decomposition process

There are several aspects which need to be considered before the decomposition. The first is the number of components which needs to be known before carrying out the analysis. The best solution is to use one of the available criteria. These are BIC (the Bayesian Information Criterion), AIC (the Akaike Information Criterion), ICOMP (the Information Complexity Criterion), AWE (the Approximate Weight of Evidence), MIR (the Minimum Information Ratio) and NEC (the Normalised Entropy Criterion). The proper number of components should minimise (or for some criteria maximise) the value of the chosen criterion. Most of

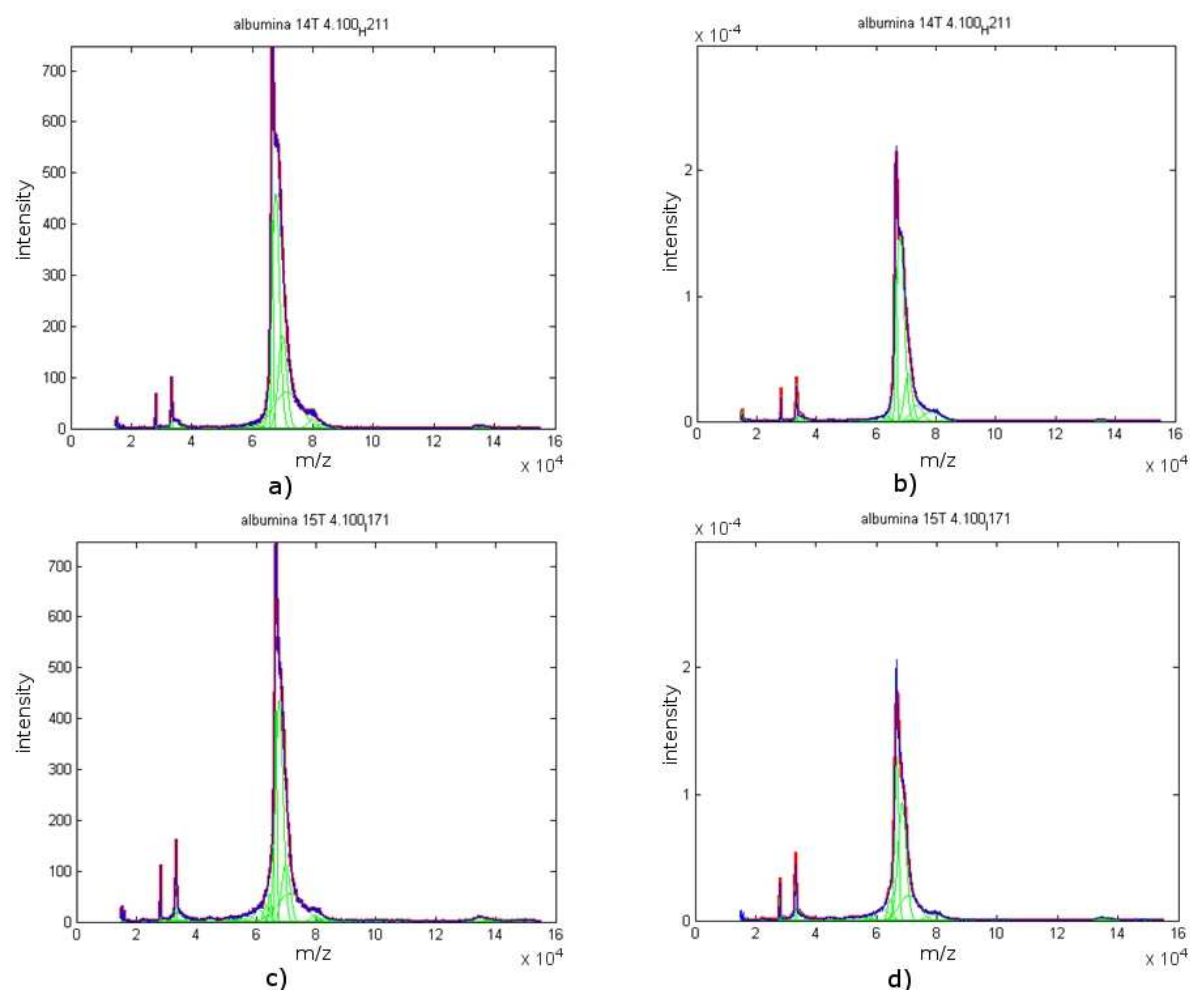


Fig. 1. A comparison of results obtained with and without the mean spectrum.

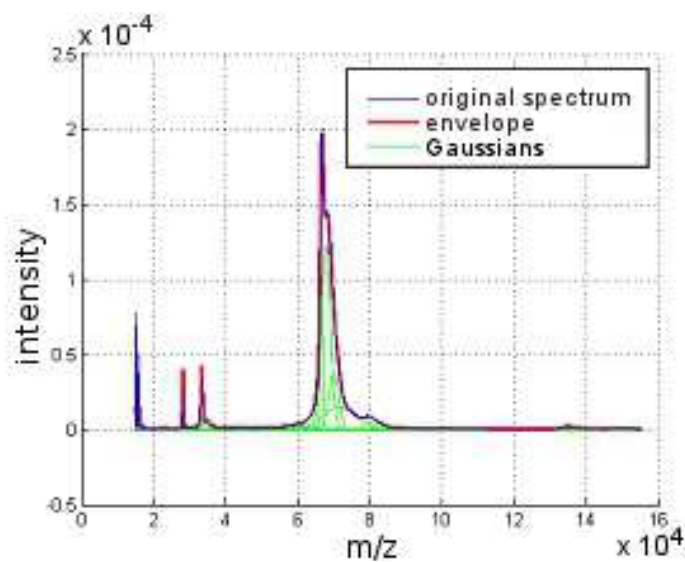


Fig. 2. The mean spectrum.



the mentioned criteria are based on the likelihood function. We chose the BIC criterion because it is easy to calculate and it considers such parameters as the size of the sample and the value of the likelihood function. Formulas defining the mentioned criteria are presented in Tab1. In the presented formulas models the parameters are marked as  $\psi$ .

The main disadvantage of using criteria to estimate the number of components is the fact that it is a time-consuming method. A single use of each criterion gives a result for the single number of components. To obtain reliable results the calculations need to be repeated many times for each single number of components. The example of using the BIC criterion for data presented in Fig.1 and Fig.2 is shown in Fig.3. According to Fig.3, the BIC criterion needs to be maximised. Results stabilise for 40 components, so this number was considered to be appropriate for the further analysis.

There are also different ways of dealing with the unknown number of the components problem. It is possible to use different, simple method of peak detection. Such methods work fast, because they are based on local maxima and minima. However, it is a reliable method only in the case of spectra which do not have many overlapped peaks.

Criteria	Formulas
BIC (the Bayesian Information Criterion) (Schwarz, 1978)	$BIC(g) = 2\log L(\hat{\psi}) - d\log n$
AIC (the Akaike Information Criterion) (Akaike, 1974)	$AIC(g) = -2\log L(\hat{\psi}) + 2d$
ICOMP (the Information Complexity Criterion) (Bozdogan, 1993; Bozdogan, 1990)	$ICOMP(g) = -2\log L(\hat{\psi}) + C_1 - C_2$ $C_1 = d\log[d^{-1} \sum_{i=1}^g \{\pi_i^{-1}tr \hat{\Sigma}_i + \frac{1}{2}tr(\hat{\Sigma}_i \hat{\Sigma}) + \frac{1}{2}tr(\hat{\Sigma}_i)^2 + \sum_{v=1}^P (\hat{\Sigma}_i)_{vv}^2\}]$ $C_2 = (p+2) \sum_{i=1}^g \log( \hat{\Sigma}_i  - p \sum_{v=1}^P \log(n\hat{\pi}_i) + gp\log(2n))$ $d = gp + \frac{1}{2}gp(p+1)$
AWE (the Approximate Weight of Evidence) (Banfield & Raftery, 1993)	$AWE(g) = -2\log L_C + 2d(3/2 + \log n)$
MIR (the Minimum Information Ratio) (Windham & Cutler, 1993)	$MIR(g) = 1 - \ \psi^{m+1} - \psi^m\  / \ \psi^m - \psi^{m-1}\ $
NEC (the Normalized Entropy Criterion) (Celeux & Soromenho, 1996)	$NEC(g) = \frac{EN(\hat{r})}{\log L(\hat{\psi}) - \log L(\hat{\psi}^*)}$

Table 1. Criteria used to estimate the number of components.

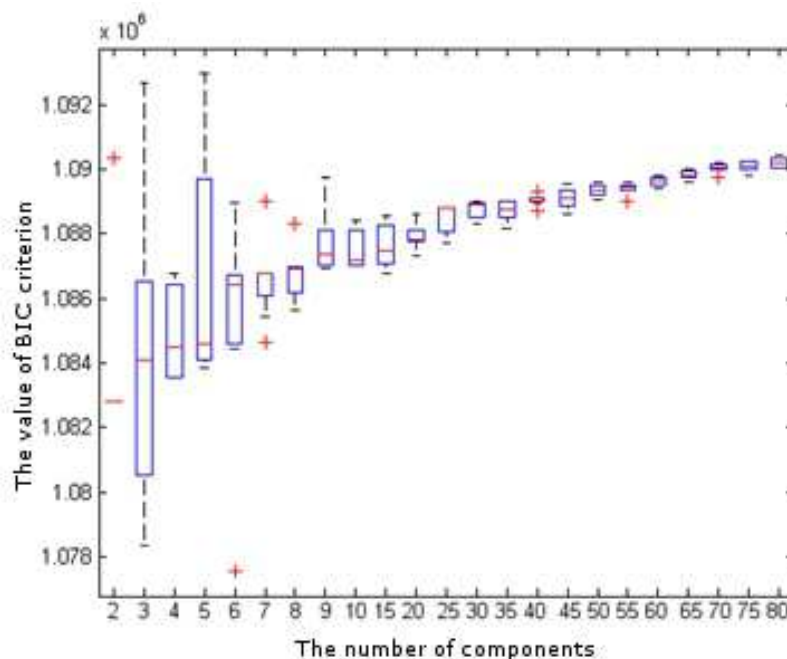


Fig. 3. The estimation of the number of components using BIC criterion.

It is also possible to reduce the number of components during calculations. This correction is based on the values of probabilities calculated during the M step of the EM procedure. If they are too small (very close to 0) it usually means that the number of components is overstated. Essential support is also given by the model testing procedure. EM calculations might be suspended after a few iterations. The researcher can check the so-far obtained weights and means indicating the peak localisations. If he/she finds many very small weights, or means are found to be located very close to each other, it usually means that the number of specified components is too large. Suspending the EM procedure makes sense because of the characteristic of the algorithm. It converges very fast at the beginning and after that it slows down. That is why the checking of the results after 10-20 calculations fairly well illustrates the quality of the modelling.

The other aspect which needs to be considered is the generation of initial parameters values. The EM algorithm is sensitive to the initial values. If they are poorly chosen, the quality of calculations might not be reliable. One option is to randomise them from the appropriate distribution. The better one, however, is to use the simple method of peak detection. This method gives less biased, more reliable results. The important thing is to add small Gaussian arousals to the results obtained from the peak-detection method.

The next decomposition aspect to be mentioned is the stop criterion. According our simulations one good idea is to use a stop criterion based on the likelihood value (eq. 4) and the maximum likelihood rule (eq. 5). The maximum likelihood rule states that the higher value of the likelihood function, the better the parameters estimation can be gained. Using the maximum likelihood rule gives the certainty of stability because of monotonicity of the likelihood function (Fig. 4).



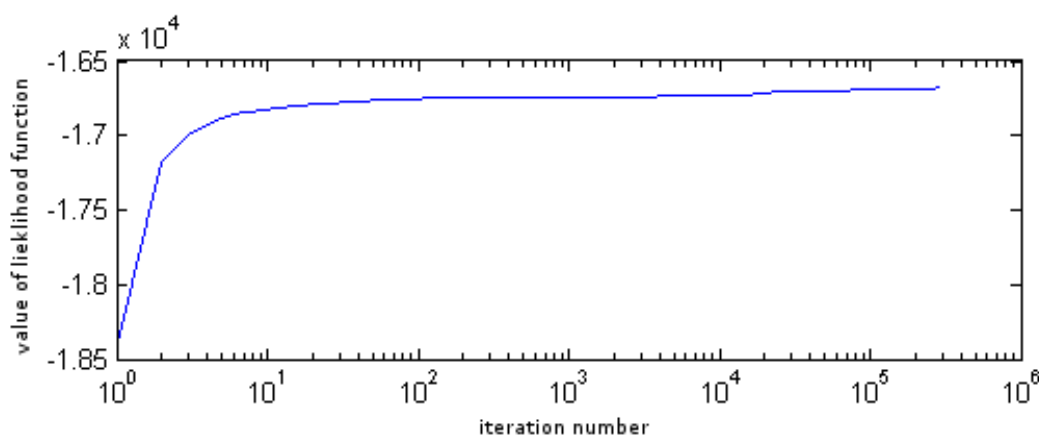


Fig. 4. The monotonicity of the likelihood rule.

$$L(p, x) = L(p) = f(x_1, x_2, \dots, x_N, p) = \prod_{n=1}^N f(x_n, p) \quad (4)$$

$$\hat{p} = \arg \max \prod_{n=1}^N f(x_n, p) \quad (5)$$

Maximum likelihood itself is an efficient method of parameters estimation. However, it cannot be used in the problem of spectral decomposition. The problem consists in the fact that we do not know the assignment of the Gaussians to the respective peaks. The EM algorithm deals with it using hidden variables. The probabilities of assignment are calculated in each iteration and finally the right assignment is found.

The decomposition with the EM algorithm is slower than using simple methods based on local minima and maxima. However, it copes better with spectra containing overlapped peaks. There are many examples of spectra which cannot be solved by such typical methods. Examples of decomposed spectra obtained from different methods are presented in Fig. 5.

The next argument for Gaussian decomposition is that using an EM algorithm and a mean spectrum eliminates the necessity for alignment procedure processing. This operation is done to align detected peaks among all spectra in the dataset. Those mismatches are due to measurement errors. The alignment procedure needs to be performed on most of peak processing procedures. It is a hard and difficult process. EM decomposition is based on the assumption that peaks are covered with Gaussians defined by means and standard deviations. That is why the  $m/z$  values do not need to match exactly to Gaussians means – we accept slight differences between peaks among different spectra in the dataset.

The method discussed in this chapter is based on Gaussian distributions. However, it is also possible to use different distributions, like Poisson, log-normal or beta. High-resolution spectra contain asymmetric peaks with the right skewedness. In such cases it is a good idea to use log-normal or beta distributions (Guindani et al., 2006). MALDI-ToF spectra are low-resolution and there is no skewedness seen. That is why Gaussian distributions are more appropriate to use. The second reason we use Gaussian distributions is connected with the error of the spectrometer measurement. This noise can be modelled in a natural way with Gaussians.

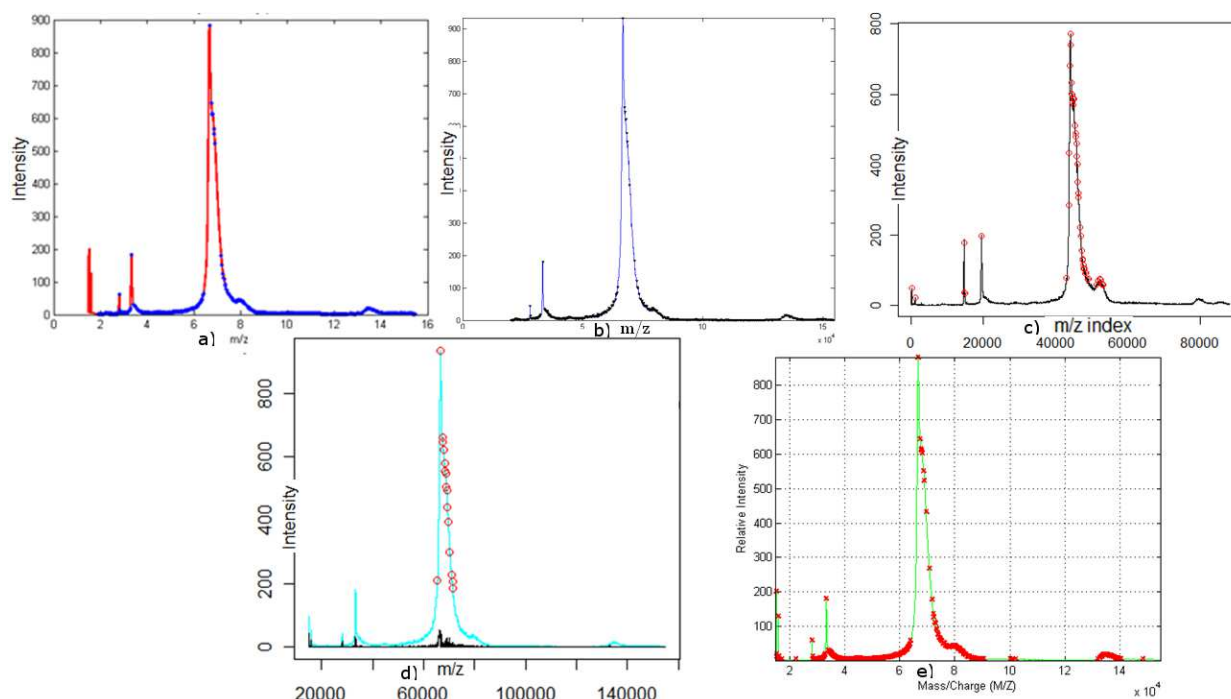


Fig. 5. Results of spectra decomposed with various methods and tools: a) the Cromwell package b) the PrepMS tool c) the MassSpec Wavelet tool d) the PROcess package e) the mspeaks function (Matlab).

Single peaks are, in fact, modelled with single Gaussians. The appropriate choice of distribution parameters allows the representation of peak shapes and measurement errors. It is also easy to write the model. It is worth paying attention to the fact that single Gaussians might be used only in the case of perfectly-separated peaks. In practice, the analysis of the real spectra is done using mixtures of Gaussian distributions. Using a mixture of Gaussian distributions instead of single Gaussian distributions additionally takes account of interactions between closely-located peaks. Mass spectra reflect a number of processes occurring in an organism. Those processes are usually correlated with each other. They have their representation in the characterising spectra, especially in the lack of separability among its particular peaks. This fact needs to be considered during the analysis. The application of mixture models allows the considering of dependences between spectral peaks. It also facilitates the modelling of overlapped measuring errors placed in adjacent regions of the spectrum. Separate-peak identification could be a case of the incorrect assessment of the individual Gaussians variances, because it is not possible to completely separate them. Mixture modelling makes it possible to detect all peaks simultaneously and correct measurement inaccuracies. However, mixture model parameter solving is a complicated task that needs the determination of many properties like the number of components, the type of stop criterion or calculation accuracy.

### 3. Data classification

Preprocessing steps and decomposition are the first steps in the analysis. The second is classification, which might be used in the process of significant peak determination. Classification allows the search for the distinction between ill and healthy patients. It is also

possible to look for the stage of disease progression or to check reactions (positive or negative) to medical treatment.

Classification of mass spectra collection is an essential but also difficult task, because of the specificity of the data. The most common classification tasks are based on the supervised learning. It usually consists of categorising data into two groups (for example ill and healthy). There are also attempts to classify data into three or more groups. Such classification tasks are, however, more complicated and they are not included in this chapter.

Classified objects are usually represented by vectors of observed, measured or calculated features. Supervised learning classification assumes that the unknown function  $\Phi$  is to be assigned to each object of population  $O$  as a label of one class. The classification process is based on the learning set  $U$  which is a subset of the whole data set  $O$ . Each element  $o_i$  of the learning set is composed of the object representation and a class label. This object representation is an observation vector of the features. The whole set is divided into  $c$  separated subsets and one-subset observations are numbered among one of the  $c$  classes. Such supervised learning is widely used in biomedical applications.

### 3.1 Classifiers construction

The construction of classifiers is based on several rules. Multiple different classifiers might be constructed on the basis of one single learning set. The ideal situation would be to choose the proper classifier on the basis of the number of misclassifications of the new, random observation. However, in reality bad classification probabilities are unknown. They might be estimated from a validation probe, which is a random sample, independent of the learning probe, where objects' belonging to classes are unknown. The misclassification probability of a specific classifier is estimated with mistakes done by the classifier on the validation probe. Classifier evaluation should be done using observations independent of those from the learning probe. In other cases the classifier will be biased.

The ultimate classifier evaluation is done with a test probe. It needs to be independent of other probes and it needs to have information about objects' membership of classes. If only one classifier is to be tested or basis size of the set is small, the validation probe might be omitted. In practice, the usually-chosen proportion is the division into 50% on the learning probe and 25% each for the validation and test probes (Cwik & Koronacki, 2008). However, in practice, the division depends on the specificity of the data set.

The classifier makes the decision about the belonging to classes on the basis of the learning probe. However, the trained classifier will need to operate on large datasets. These datasets are larger than sets used for classifier training. It makes non-zero the probability of a wrong decision (Stapor, 2005). The classifier is used for data other than those for which it was constructed. That is why the classifier quality depends on its generalisation ability. In practice it means that the learning properties need to be representative of all the population. On the other hand, nonessential properties should be omitted, because they only constitute features of the specific learning set.

The most popular measures of classification quality are classification accuracy (a proportion of correctly-classified sets) and error rate (a proportion of misclassified sets). Important rates are also TP (True Positives) – the number of correctly-classified positive sets, TN (True

Negatives) – the number of correctly-classified negative sets, FP (False Positives) – the number of incorrectly-classified positive sets, FN (False Negatives) – the number of incorrectly-classified negative sets.

Among useful measures one can also find sensitivity and specificity. This sensitivity is defined as a proportion of truly positive and false negative results (eq. 6). It is interpreted as ability of a classifier to identify the phenomenon if it really exists.

$$\text{sensitivity} = \frac{TP}{FN + TP} \quad (6)$$

On the other hand the specificity is a proportion of truly negative results and the sum of truly negative and truly positive results (eq. 7). The specificity is interpreted as the ability to reject truly false results.

$$\text{specificity} = \frac{TN}{TN + FP} \quad (7)$$

Sensitivity and specificity are opposed values – an increase in the one causes a decrease in the other.

The significant tool characterising a classifier's features is the receiver-operating-characteristic curve – known as the ROC curve. It is a chart of dependency between values: 1-specificity and sensitivity. Such a curve is created for a specific structure of the classifier (specified type, parameters, number of input features). The total error of the classifier remains unchanged. However, its division into values FP and FN is changed, because the ROC curve examines the proportion between FP and FN. In the case of the random division of objects, the ROC curve takes the shape of a curve going from the bottom left to the upper right corner. The better the classification results are, the more concave the curve is. The ideal situation will make the ROC curve go through the upper left corner of the chart.

An important factor in the classifier's quality is the curve under the ROC curve, the so-called AUC. The closer to the value 1 AUC is, the better are the classification results. An example of ROC is presented in Fig 6.

### 3.2 Dealing with high dimensionality

Mass spectrometry data are characterised by high dimensionality. The number of observations is significantly lower than the number of features. Each patient has a few thousand data points or even more, whereas a typical dataset contains dozens or hundreds of spectra. Typical classification and data mining techniques are designed to handle low-dimensional data, such as sales or economic indicators. Low-dimensional datasets contain many observations and just only a few, usually uncorrelated, features. Such data might be analysed using any type of method, including graphical interpretation and unsupervised learning. Dealing with high-dimensional data is much more difficult. The main problem is the correlation of features which always occur in high-dimensional data. In fact, to obtain statistical significance the number of observations should grow exponentially with the dimensionality. The existence of dependent features prevents or hinders the classification

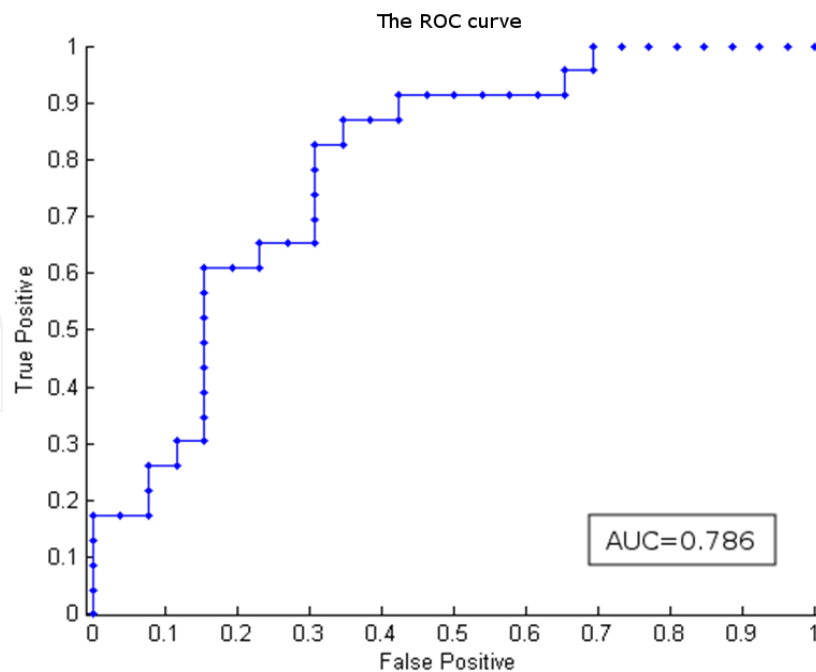


Fig. 6. Example of the ROC curve.

using typical, widely-known methods. Moreover, a large number of correlated features has a bad influence on the quality of the classification. This makes analysis difficult and diversification is hard to obtain (Stapor, 2005). A large number of features causes also large number of classifier parameters. It increases its complexity and susceptibility to over-learning and decreases its flexibility. The existence of the curse of dimensionality (Mao et al., 2000) proves that the complexity of the classifier has an impact on the classification quality. The more complex the classifier is, the higher should be the proportion between the number of observations and the number of features (Stapor, 2005). That is why high-dimensional data must be properly processed, including the application of dimension-reduction techniques. This task determines the success of the classification because of specificity of mass spectral data.

Another problem of dealing with bio-medical data is signal strength. A typical signal of mass spectrometry data carries information concerning the functions of the whole organism. Moreover, the development of medical, diagnostic and prevention programmes gives results for significantly less patients diagnosed with late-stage diseases. For example, cancer diseases are usually diagnosed at the first or second clinical level. Such signals are difficult to identify and to extract among the many different signals of the organism. Blood, serum or urine contain proteins responsible for the range of typical, vital functions of the body. One needs to notice that those proteins are much stronger than the signals of diseases.

One of the most frequently-used classifiers for mass spectrometry data is the Support Vectors Machines (SVM) proposed by V.N. Vapnik (Vapnik et al., 1992; Vapnik, 1995; Vapnik, 1998). The idea of this method is a classification using an appropriately-designated discriminant hyperplane. Searching for such a hyperplane is performed by the Mercer theorem and the optimisation of the quadratic objective function, with linear restrictions. The SVM idea is based on searching for two parallel hyperplanes. If classification groups are



linearly separated, those hyperplanes should delimit the widest possible area which contain no elements of the probe. The hyperplanes need to be based on so-called support vectors. If learning sub-sets are not linearly separated, a penalty is introduced. The best separation result is obtained for a higher dimensional space.

The SVM rule is presented in Eq. 8.

$$f(x) = \text{sgn}\left(\sum_{\text{sup. vect.}} y_i \alpha_i^0 (x_i x) + b^0\right) \quad (8)$$

where  $\alpha$  are Lagrange's coefficients and  $b$  is a constant value. For inseparable classes the additional restrictions take the form of Eq. 9.

$$\begin{aligned} x_i w + b &\geq 1 - \xi_i, y_i = 1 \\ x_i w + b &\geq -1 + \xi_i, y_i = -1 \end{aligned} \quad (9)$$

where  $\xi_i$  is a constant value  $\xi_i \geq 0$

Classifiers used in bioinformatics applications are solved with use of kernel functions. Such a construction enables one to obtain non-linear shapes of discriminant hyperplanes. One of the most popular kernel functions is the radial kernel (Eq. 10).

$$f(x) = \text{sgn}\left(\sum_{\text{sup. vect.}} y_i \alpha_i^0 K(x_i x) + b^0\right) \quad (10)$$

Before the main classification of mass spectrometry data, dimension reduction needs to be performed. Input data-sets for classification usually contain several hundreds or even thousands of features. From the statistical point of view, using such a number of features is unreasonable. Reduction might be carried out in two-stages. The first is spectrum decomposition, which reduces dimensionality from thousands of features to hundreds. The second step is applying feature reduction or selection techniques.

The first step in dimension reduction is based on applying the decomposition results. These results are used as a Gaussian mask, which is put on every single spectrum of the data set. This gives new values consisting of all spectra. Dimensions of mass spectrometry data decrease to the value of the GMM components number. The resultant matrix obtained after these steps is  $n \times k$ , where  $n$  denoted the number of spectra and  $k$  the number of components. The resultant matrix was the input data to the further dimension reduction and classification.

There are many reduction and selection techniques available. They attempt to find the smallest data sub-set chosen with defined criteria among the whole data set. Too large a number of features has an adverse impact on the classification results. A large number of features causes an increase in computational complexity and lengthen calculation time. There are two types of dimension reduction methods:

- feature extraction – data are subjected to certain transformation – a new data set is obtained



- feature selection – a subset of the most optimal data is chosen.

One of commonly-known features extraction methods is the Partial Least Squares (PLS) method (Barnhill et al., 2002). The method also facilitates classification. Feature selection in the PLS method is performed with use of both X and Y data, so it considers the whole structure of the learning set. The idea of the PLS method is to find latent vectors. Using latent vectors allows simultaneous analysis and the decomposition of X and Y, including a covariance between X and Y. Such an approach makes PLS a special case of Principal Component Analysis (PCA) (Mao et al., 2000). The original version of PLS is a regression method dealing with continuous values. Classification of mass spectrometry data usually consists of assigning data to one of two groups. So, the matrix of dependent features (Y) is composed of only two values. It is possible to directly apply PLS to mass spectrometry or microarray data. However, it is better to use one of the few PLS modifications dedicated to binary classification. The original PLS classification components are a linear combination of predictor variables. Weights, however, are a nonlinear combination of predictor and response variables (Nguyen & Rockeb, 2004). There are approaches (Liu & Rayens, 2007; Boulesteix & Strimmer, 2006; Fort & Lambert-Lacroix, 2005; Nguyen & Rockeb, 2004; Man et al., 2004; Huang et al., 2005) applying the original PLS to categorical, binary responses. However, research confirms that it is better to use PLS procedures adjusted to binary responses (Nguyen & Rockeb, 2002). One can use a hybrid-PLS method based on singular-value decomposition. Another approach is the hybrid-PLS method based on logistic regression predictors, where the PLS components are calculated as weighted averages of the original predictor/explanatory variables. Also, weights are dependent on sample predictor variances and the partial correlation coefficient (Garthwaite, 1994; Nguyen & Rockeb, 2004). PLS is also used in conjunction with Linear Discriminant Analysis (LDA) (Boulesteix & Strimmer, 2006; Boulesteix, 2004; Liu & Rayens, 2007). Fort and Lambert-Lacroix (Fort & Lambert-Lacroix, 2005) proposed a combination of the PLS and Ridge penalty.

Among the most popular features selection method one can find the SVM-RFE and traditional T test. The SVM-RFE (Support Vector Machine Recursive Feature Elimination) (Wold, 1996) method is a features-selection method. Features selection is performed with the propagation-backward method. The procedure starts with a full range of input features and features are successively removed. Only one feature is removed at a time. As a range criterion SVM weights coefficients are used. Therefore the SVM-RFE method is closely related to the SVM classification. The T test is a very common technique of feature selection. The most significant features are chosen according the T test. For each feature a T-test range is calculated. This statistics treat all features as independent and this assumption is usually not met. However, the T test is successfully used for protein data classification.

### 3.3 Learning the classifier

After applying dimension reduction, supervised classification is preformed with the SVM method. Our results (Plechawska-Wójcik, 2011) show that the best results can be obtained using linear SVM and SVM with the Gaussian Radial Basis Function kernel. However, before the learning process, proper classification parameters need to be estimated. Such an estimation is usually performed experimentally, for example using the Multiple Random Validation method.

Tests of classification and reduction performance need to be done for different values of the SVM parameters and the number of selected features. To find the most accurate values, the dataset must be divided into testing and learning subsets. Classification calculations need to be repeated several hundred times. The classification analysis should be performed separately for all used dimension reduction techniques. Each of them can have a different number of obtained features. Besides the total error, False Negatives and False Positives should be also checked.

The SVM parameters are the value of box constraints (C) for the soft margin and the scaling factor (sigma). The results of multiple repetitions of SVM for different sigma values are presented in Fig. 7. The classification was done with SVM classifier with a radial kernel. All calculations were done in the Matlab environment.

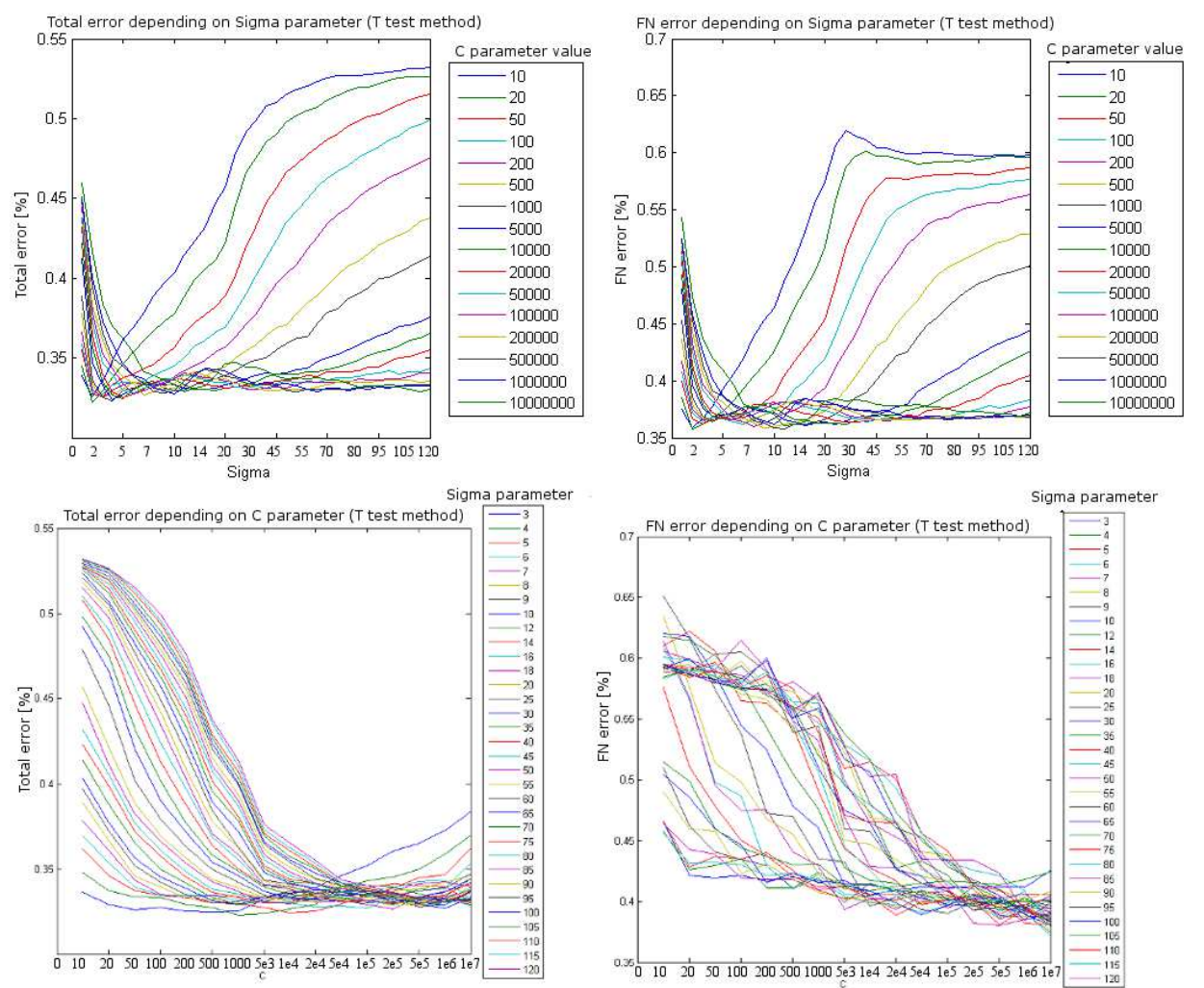


Fig. 7. Examples of the estimation of classification parameters.

If parameters are already known, there is a necessity to find the optimal number of features. For example, if there is a 50-element learning-data set, number of features shouldn't be larger than 10. The results for all three types of dimension-reduction techniques are presented in Fig. 8. The middle line is the obtained ratio and the upper and lower denotes the confidence interval. Similar results are obtained for the FN and FP values.

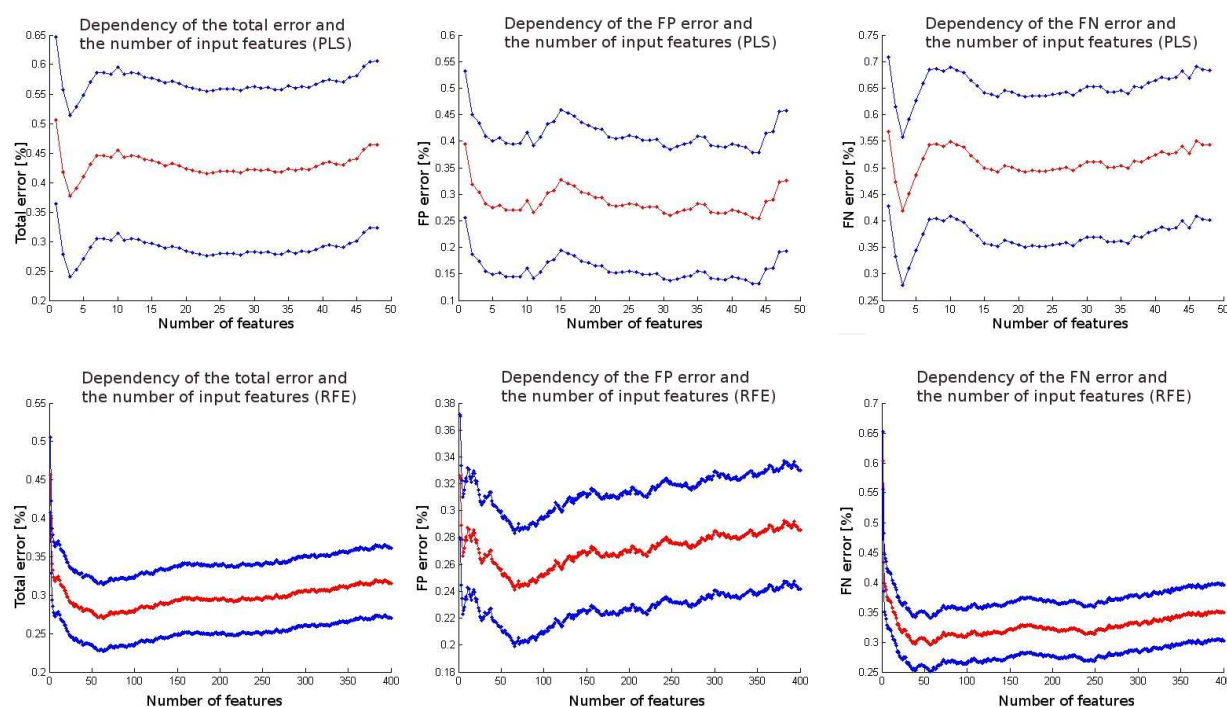


Fig. 8. Examples of the estimation of the features number.

#### 4. The biological interpretation of mass spectrometry data

Another important issue in mass spectrometry data analysis is the supporting of biological interpretation. The ability to check the details of those components is of great importance to biologists. Biological interpretation tries to determine peptides and proteins on the basis of the  $m/z$  values list. After this identification further analysis can be performed. The application gives characteristics of found peptides and proteins. It is also able to find characteristics of genes coding the proteins and to give access to genes paths. The biological context module is integrated with four biological databases available online.

At level0 the user is able to load data and give detailed search criteria. These criteria include accuracy, species, the MS platform, and the possibility of double and triple charges. Searching is based on  $M/Z$  values, which are transferred from classification module.

Level1 is based on the EPO-KB (Empirical Proteomic Ontology Knowledge Base) database (Lustgarten et al., 2008; Lustgarten et al., 2009). The names of proteins and peptides are found on the basis of given  $M/Z$  values with a specified percentage tolerance. The user can also see the original results in the EPO-KB service.

Level2 is a protein level and data presented here are obtained from an UniProt (Jain et al., 2009) database. The displayed results contain detailed information about proteins, such as

entry name, status of reviewing process, organism, gene names and identifiers, features and GO annotations. It is also possible to see the original results returned by the database.

Level3 is a genes level and it gives information about genes coding a particular protein chosen at a previous level2. Presented data are based on NCBI service (Wheeler, 2009). Searching is based on a gene identifier and it returns precise information about a particular gene, its role, status, lineage and related data. Level4 is based on gene pathways data. It is integrated with the KEGG database (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa, 2008). Level 4 gives details about gene pathways, structures, sequences, and references to other databases.

An example of biological results obtained at the level of proteins is presented in Fig. 10. More results of the analysis performed on real data are presented in (Plechawska-Wojcik, 2011a).

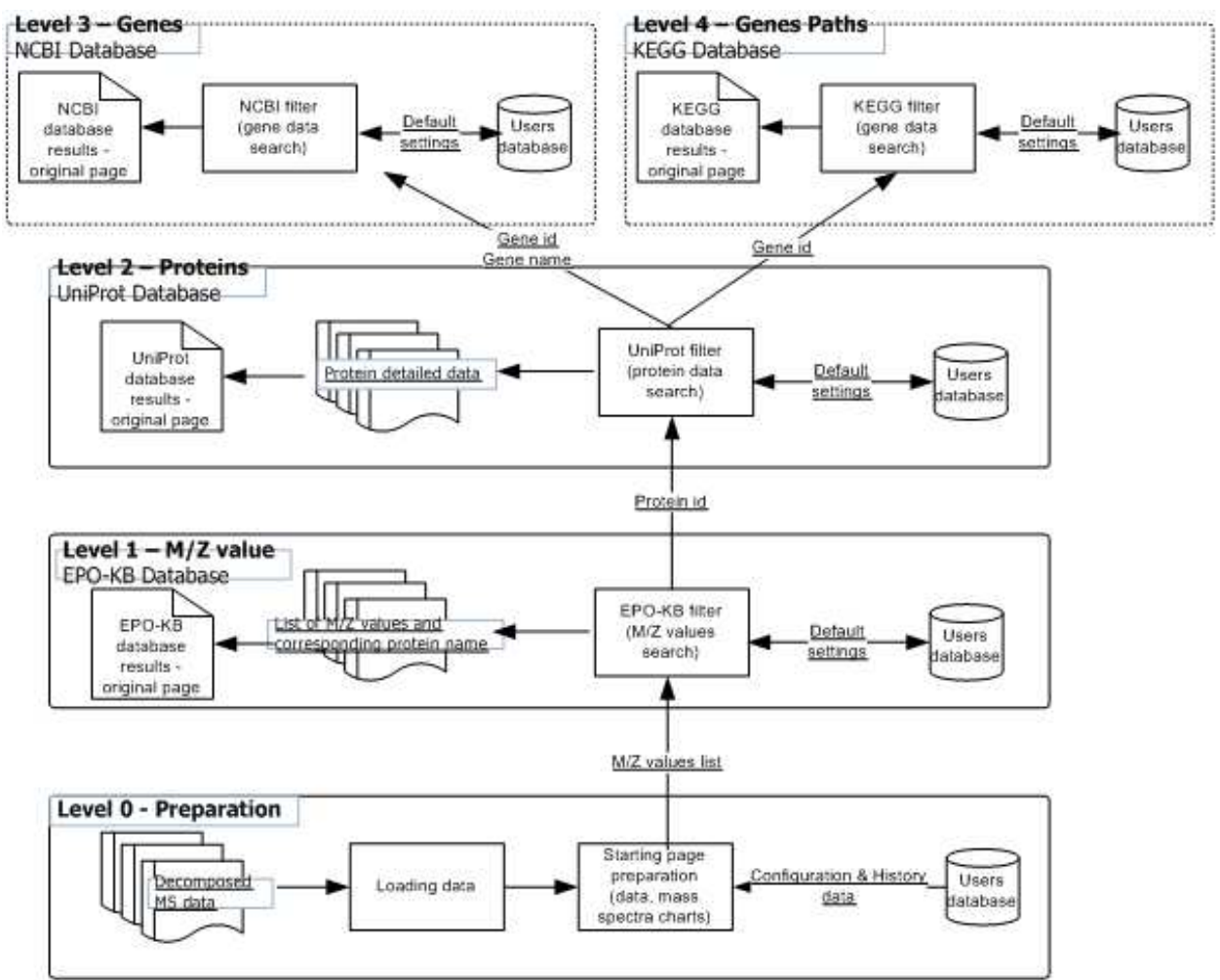


Fig. 9. Schema of a biological-interpretation module.



Accession	EntryName	Status	ProteinNames	GeneNames	Organism	Length	Szczegóły
<a href="#">P02656</a>	APOC3_HUMAN	reviewed	Apolipoprotein C-III (Apo-CIII) (ApoC-III) (Apolipoprotein C3)	APOC3	Homo sapiens (Human)	99	<a href="#">Więcej</a>
<a href="#">P02647</a>	APOA1_HUMAN	reviewed	Apolipoprotein A-I (Apo-AI) (ApoA-I) (Apolipoprotein A1) [Cleaved into: Apolipoprotein A-I(1-242)]	APOA1	Homo sapiens (Human)	267	<a href="#">Więcej</a>
<a href="#">P06727</a>	APOA4_HUMAN	reviewed	Apolipoprotein A-IV (Apo-AIV) (ApoA-IV) (Apolipoprotein A4)	APOA4	Homo sapiens (Human)	396	<a href="#">Więcej</a>
<a href="#">A3KPE2</a>	A3KPE2_HUMAN	unreviewed	Apolipoprotein C-III (Apolipoprotein C-III variant 2) (Apolipoprotein C-III variant 3) (Apolipoprotein C-III, isoform CRA_a)	APOC3 hCG_41334	Homo sapiens (Human)	99	<a href="#">Więcej</a>
<a href="#">Q6Q788</a>	APOA5_HUMAN	reviewed	Apolipoprotein A-V (Apo-AV) (ApoA-V) (Apolipoprotein A5) (Regeneration- associated protein 3)	APOA5 RAP3 UNQ411/PRO773	Homo sapiens (Human)	366	<a href="#">Więcej</a>
<a href="#">B0YIW2</a>	B0YIW2_HUMAN	unreviewed	Apolipoprotein C-III variant 1	APOC3	Homo sapiens (Human)	117	<a href="#">Więcej</a>

Fig. 10. An example of biological analysis at the protein level.

5. Conclusion

The presented project is a comprehensive bioinformatics approach enabling spectra pre-processing and analysing. The use of the Gaussian Mixture Model decomposition facilitates particular work with different types of spectra, especially complex, containing overlapped peaks. Before the analysis, one needs to choose the proper settings adjusted to the specificity of data. It is a condition of successful analysis. To minimise the risk of improper parameters selection, a parameters test should be performed.

All elements of the mass spectrometry data analysis process are closely related. Each performed operation has an influence on the further quality of results. Preprocessing analysis is especially essential for the final results. That is why it is necessary to perform it in the correct order and using the proper parameter set. Some operations, however, are performed optionally and are chosen by the user.

We found the peak-detection method based on Gaussian Mixture Models slower than common spectra analysis techniques based on local maxima and minima. However, it can deal with different kinds of data including spectra with overlapped peaks.

Mixture model parameters are estimated using the Expectation-Maximisation algorithm with appropriately-selected parameters. It enables to obtain reproducible, reliable results. The decomposition carried out in this way allows the detection of peaks which can be subjected to further analysis, like protein and peptide identification, biomarker detection and the allocation of the tested sample to one of target groups.

Classification allows the initial indication of the power of the predictive model and the functional analysis of detected peaks. It is, however, a difficult task, due to the high dimensionality and feature correlation. Many thousands of features and tens of objects require two-step dimensionality reduction. The first one is based on the Gaussian mask, imposed on all spectra of the dataset. The second is the separation of the most informative features, conducted by the dimensionality-reduction techniques. Due to the high correlation degree, the classification should be based on features. Before the classification procedure the classifier parameters need to be specified.

The last step of the analysis is the biological interpretation. Biological databases integration facilitates the verification of the results. This test is important because of the possible False Discovery Rate obtained during the raw spectra analysis and classification. Such verification gives the possibility to verify the analysis from another angle. Biological analysis based on several external databases gives reliable functional analysis. Source databases are updated frequently. This ensures reliable, actual results.

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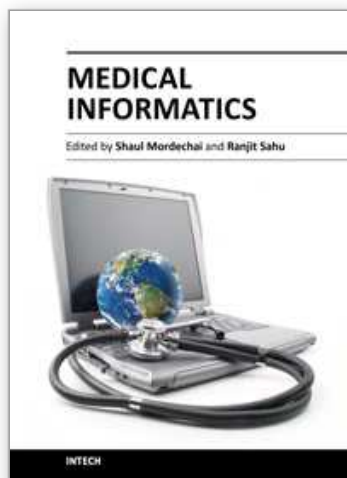
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