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Multivariate data analysis techniques as tools for analyzing force spectrometric data sets of multi-component systems

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Introduction

Multivariate data analysis techniques play an important role in visualization and interpretation of complex data sets where information is not clearly observable. These methods are widely used for different applications in e.g. quality management, market research and scientific research. Among the high variety of multivariate data analysis techniques, principal component analysis (PCA) and clustering methods are mainly used for the various fields.

Force Spectrometry has been recently used to examine biological samples in order to obtain directly observable information such as bond strength, protein folding and cell adhesion. Using the laterally scanned force-distance measurements, a sub-10nm resolution is possible prior to any optical methods. Such high resolution is similar to what can be obtained by atomic force microscopy (AFM).

Akin to the above mentioned applications of force spectrometry, force-distance measurements provide an information rich set of data which lack straightforward features for a direct chemical assignment. At first glance all force-distance curves look similar with only small observable differences, but nevertheless, contain information which are directly related to the material and, thus, allow a chemical assignment of the surface.

In this study multivariate data analysis methods are for the first time applied to force spectrometric data sets.[1]

Experimental

All experiments were performed with a commercially available AFM (NanoWizard, JPK AG, Berlin, Germany) with contact silicon nitride tips (MLCT-AUHW, Veeco, Camarillo, USA, tip diameter: 20 nm, force constant 0.5 N m^{-1}). For PCA and clustering, the Statistics Toolbox from the Matlab software suite (Matlab 7.0, MathWorks, USA) was used. To remove dominating topographical information from the multivariate analysis results, the raw data were corrected by offset correction in the y-direction for a force equal to zero and overlaid in the x-direction at the snap in point.

For preparation of the two-component system, 1 µl of a millimolar cytochrome c solution (Sigma , Germany) was applied to freshly cleaved mica (BAL-Tec, Liechtenstein) and dried under argon atmosphere.

As for the three-component system, 20 nm of gold were sputtered on the half of a coverslide using shadow mask method. In a second step pattern of avidin proteins as 20 µm-squared were applied with micro-contact printing (µCP) following the modified protocol by Bernard et al.[2]

Results and Discussion

To apply multivariate data analysis methods to force spectrometry data, a two-component system consisting of cytochrome c molecules attached to a mica surface was used. For a quick overview and an easier comparison to the chemometric data, the topography of the sample was recorded.

An agglomeration of cytochrome c molecules (100 nm in diameter, 9 nm in height) was observed in the topographical image. A grid of 32 x 32 equidistant points was applied and force-distance-curves were performed at each point.

After pretreatment to remove topographical information, the data set was assessed to PCA. As a result, the first principal component (PC 1) contains 40.8% of the information whereas PC 2 contains additional 24.7%. Transforming the results of PCA into a diagram comparable to the topography, a clear distinction between the mica surface and cytochrome c can be observed (see fig 1). The same data set was used for analyzing with hierarchical clustering. At first the distances of each object was calculated using Euclidian distances. After that, different methods were used to group the objects into cluster. Here the clustering using Ward's method produced the best results and was therefore used for further experiments. A clustering threshold of nine clusters was used to direct the multivariate treatment. This treatment produces a strong correlation of the clustering data to the topographical image and provides a clear distinction between mica and cytochrome c.

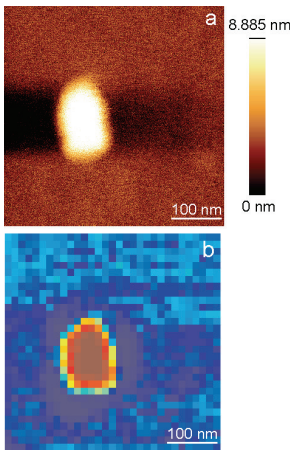
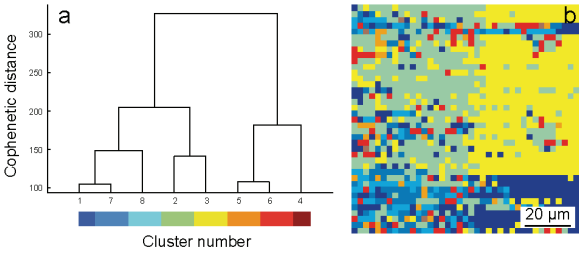


Fig. 1: Topographical image (a) and coresponding PC 1 (b).

These results demonstrate the feasibility of applying PCA and hierarchical clustering to force spectrometric data sets. Although both methods produce similar results PCA is preferred because of the danger of overclustering using hierarchical clustering.

After this proof of principle, a system of avidin islands on a gold/ glass surface was used to apply PCA and hierarchical clustering to a more complex system.

The topographical image shows clearly the avidin islands while the distinction between gold and glass is not possible. 40 x 40 force distance measurements on equidistant points were taken and assessed to PCA. As a result, PC 1 contains 56.5% and PC 2 additional 20.6% of the information. Displaying PC 1 in a comparable manner, the avidin-islands can be clearly distinguished, whereas a distinction of glass and gold could not be obtained. In contrast, the same set of data used for hierarchical clustering with Ward's method showed an improved result (see fig. 2). After treating the data with a cluster threshold of eight cluster a distinction between gold and glass on the surface as well as the avidin islands is possible. Therefore, Ward's method can be used for discrimination of material properties which are hidden in the complex data.



Dendrogram of the clusteranalysis (a) and the corresponding image (b).

In conclusion, PCA and hierarchical clustering are suitable for processing force spectrometric data sets and, in combination, can enrich the possibilities of both. Nevertheless, data pretreatment for removing topographical information and the use of cluster thresholds to avoid overinterpretation is necessary.

This high resolution approach to assign chemical information can lead to screening and characterizing of complex biological surfaces and therefore enhance the knowledge about tissues or living cells.

References

[1] C. Budich, J. West, P. Lampen, V. Deckert: Force microscopy analysis using chemometric

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[2] A. Bernard, E. Delamarche, H. Schmid, B. Michel, H.R. Bosshard, H. Biebuyck, Langmuir, (1998), 2225–2229