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Potential pitfalls concerning visualization of the 2D results

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Abstract

Contrary to many advanced applications of the two-dimensional correlation spectroscopy that have been presented at the 2DCOS-3 conference we have focused on some basic aspects concerning visualization of the 2D results. After introducing the generalized 2D correlation, when the simple analytical expression replaced the complex Fourier transforms, the number of new users has been substantially increasing. In many cases it was revealed that analysis of a studied system could be essentially improved whenever the 2DCOS method is applied. A lot of useful information is available from the 2D spectra when analyzed according to the guidelines that are popularly known as Noda's rule. One of the most important information provided is the sequence order of events observed by the spectroscopic technique along the external perturbation. It finds excellent application in many studies, e.g., the investigation of the evolution of the unfolding mechanism of proteins. For such complicated systems most 2D features can be interpreted in a frame of the unfolding process. However, this information is not easily verified by other techniques. Therefore, we have to be very careful that this stage of 2D correlation is performed and interpreted correctly.

We will present a problem that is often overlooked in the visualization of the 2D data and that can lead to a misinterpretation of results, concerning orientation and labeling of the axes of 2D contour plots. In certain cases the orientation of 2D plots may be altered and obtained peaks could have a false sign, resulting in incorrect interpretation. On simulated data we will demonstrate these potential pitfalls that lurk for new 2D users and can finally lead to an inversion of sequence order of events.

Keywords: Two-dimensional correlation spectroscopy; Contour map; 2DCOS; Noda's rule; Sequential order

1. Introduction

Noda in the third comprehensive review [1] of the state of perturbation-based generalized two-dimensional correlation spectroscopy has shown that there is continuous progress in the application of 2D correlation spectroscopy since the first review in 1993 [2]. Both a free-domain as well as commercial version of the 2DCOS software that are available from Prof. Ozaki's web-site [3] and from the Thermo Electron Corporation [4], respectively, make available the 2DCOS method to many researchers. The commercial version has excellent visualization facilities. Both the zooming and scaling menu options help greatly in interpretation of the 2D synchronous and asynchronous spectra. In both versions the 1D data subjected to the 2DCOS calculations can be simply opened to the calculations. All the features result in that many new 2D users to prefer to use the easily accessible software instead of making the effort to write their own program for the calculations. However, it should be remembered that real profits gained from the application of the 2DCOS to the analysis of different kind of multivariate data do not concern only their visualization as a colorful 3D image or/and as a contour map. We would like to repeat after Czarnecki a question: Interpretation of Two-Dimensional Correlation Spectra: Science of Art? [5] Czarnecki in extensive simulated studies has revealed that although one can easily calculate 2D spectra, their interpretation is not always straightforward [5,6]. During last years the problem of interpretation of the patterns observed in 2D correlation

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synchronous and asynchronous spectra has been extensively studied by the groups of Dluhy [7,8], Ozaki [9,10], Pèzolet [11,12], and Painter [13]. It has become almost standard practice to generate a model that mimics different spectral evolution. The aim of the simulation was to seek profile of spectral changes for that the 2D pattern closely resembles the pattern achieved from experimental spectra. The simulation-added analyses support the interpretation of studied process.

Through the extensive modeled studies it was revealed that features observed in 2D maps could be caused by frequency shifts, changes in bandwidth, the evolution of band shape, and differences in the rate of absorption changes. This problem is of particularly great importance because for many cases the asynchronous peaks arising from spectral effects could be mistakenly assigned to normal modes of specific functional groups. We want to demonstrate another problem that until now has not been reported and that has fundamental meaning for the discussion of the 2D features obtained both for the simulated and experimental spectra. Because this problem concerns properties of axes in the contour plot and does not depend on the character of analyzed spectral variances it will be demonstrated only for modeled data. Depending on the software used for visualization the sign of peaks comprising the 2D maps could contain false positives or false negatives due to the specific orientation of the axes. The sign of peaks determines the order on intensity changes that provides important information about processes developed at molecular level. Consequently, it is essential for 2D correlation analysis that the correct signs of peaks are interpreted. On simulated data we will reveal possible pitfalls that lurk for unwary 2D users whenever one follows the Noda's rule [14] without taking care of the orientation and labeling of the axes of the 2D plot.

2. Calculations

In the simulations three Lorentzian bands A, B, and C were used, their positions, bandwidths, and initial absorption values are detailed in Table 1. Their absorptions were changed according to a linear (A), hyperbolic (B), and exponential (C) fashion in 20 steps as shown in Fig. 1A. Use of the three different functions has produced three groups of spectra presenting more or less uncorrelated absorption variations. Such changes guarantee that both synchronous and asynchronous spectra will be observed. In the case of simulated data the order of absorption changes can be evaluated prior to synchronous and asynchronous maps being calculated. This can be achieved by normalizing the range of the intensity changes for each group of bands to a common range varying from 0 to 1. The normalized values plotted against the perturbation steps are presented in Fig. 1B. Their analysis reveals that the absorption changes at 1641 cm^{-1} are ahead of those at 1655 cm^{-1}, whereas the changes at 1630 cm^{-1} are more delayed in course of the perturbation developed in 12 steps. In a real word system the very important sequence information is not known previously and can be obtained by 2DCOS method. Using the simulated data we will show where the pitfalls leading to inappropriate sequence analysis of absorption changes are hidden.

Table 1
Parameters of simulated bands presenting well separation

ν_{max} (cm^{-1})	$\Delta\nu_{1/2}$ (cm^{-1})	Absorption changes
1665 (A)	12	0.04 ⏩ 0.80
1641 (B)	10	0.05 ⏩ 0.76
1616 (C)	12	0.05 ⏩ 0.70

Calculations of the synchronous (Φ) and asynchronous (Ψ) spectra from discrete data arranged in a set of dynamic spectra ($\tilde{Y}$) are realized by multiplying them together in an outer product, respectively:

$$\Phi = \frac{1}{k-1}\tilde{Y}^{T}\,\tilde{Y} \tag{1}$$

$$\Psi = \frac{1}{k-1}\tilde{Y}^{T}\mathbf{N}\tilde{Y} \tag{2}$$

where k relates to the number of analyzed spectra (i.e., measured or simulated) and $\mathbf{N}$ is the Hilbert–Noda transformation matrix. It can be very easily calculated by MATLAB™ [15] functions that allow for easy integration of computation with visualization. Fig. 1D presents a synchronous spectrum corresponding to the simulated data shown in Fig. 1C. As a reference spectrum the averaged spectrum was selected. Analyzing the 2D synchronous map the following facts can be easily stated. First, there are exclusively positive cross peaks as a consequence of the same way data change for the simulated peaks (A, B, and C). Second, the cross peak at (1665, 1616 cm^{-1}) is much more intense than the cross peak at (1641, 1616) cm^{-1} seen from the pattern of contours. This result is due to the fact that the three functions used for modulating the peaks A, B, and C significantly differ from each other. Thus, the exponentially (C) and linearly (A) increasing changes are more similar than increases due to the exponential (C) and hyperbolical (B) functions. Looking at Fig. 1D one can see that by convention the x- and y-axis wavenumber values decrease from the left to the right and from the bottom to the top, respectively. Moreover, the same text label is displayed next to x- and y-axes, e.g., wavenumbers. Such uniform labeling should be avoided for asynchronous 2D correlation spectrum as their analysis requires more caution.

Fig. 2A presents the asynchronous correlation spectrum for the simulated spectra from Fig. 1C. As one can see, the x- and y-axes have increasing orientation due to the default MATLAB property automatically set for each 2D plot. However, the Property Editor that provides access to many properties of graphics objects, including axes, facilitates reversion of the x- and y-axes through one of the following methods:

(1) view (−90, −90); (2) view (180, 090); (3) Default 2D-view, i.e., view (0, 90) plus "Reverse" option from the Property Editor – Axes.