Supporting Information

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SI Text

Data analysis of 1D and 2D correlation spectra is given below. The 1D correlation coefficient between 2 time-dependent intensities at 2 wavelengths λ_1 and λ_2, $y(\lambda_1, t_i)$ and $y(\lambda_2, t_i)$ is given by

$$r(\lambda_1, \lambda_2) = \frac{\frac{1}{n}\sum_{i=1}^{n}(y(\lambda_1, t_i) - \bar{y}(\lambda_1))\cdot(y(\lambda_2, t_i) - \bar{y}(\lambda_2))}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y(\lambda_1, t_i) - \bar{y}(\lambda_1))^2}\cdot\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y(\lambda_2, t_i) - \bar{y}(\lambda_2))^2} \quad \text{[s1]}$$

with the average spectra $\bar{y}(\lambda_j) = (1/n)\Sigma_{i=1}^{n} y(\lambda_j, t_i)$.

The 2-dimensional synchronous correlation spectrum (2D-SCS) of a 2-dimensional data array $y(\lambda_j, t_i)$, e.g., of wavelength λ and time t, is given by the expression (1):

$$\Phi(\lambda_1, \lambda_2) = \frac{1}{m-1}\sum_{i=1}^{m}\hat{y}(\lambda_1, t_i)\cdot\hat{y}(\lambda_2, t_i), \quad \text{[s2]}$$

where $\hat{y}(\lambda_j, t_i) = y(\lambda_j, t_i) - \bar{y}(\lambda_j)$ describes the dynamical part of the spectrum. For sets of spectra taken as a function of time, the 2D-SCS represents correlated changes of intensities measured at wavelengths λ_1 and λ_2 during data collection. The 2D-SCS is a symmetric spectrum with respect to the diagonal $\lambda_1 = \lambda_2$. Peaks appearing on the diagonal and off-diagonal positions are referred to as autopeaks and cross-peaks, respectively. Autopeaks of a symmetric spectral band possess a circular shape in the 2D-SCS representation. Bands that undergo large intensity variations during the time of data collection show strong autopeaks, whereas bands that undergo no intensity variations show vanishing autopeaks. Cross-peaks represent a correlated change of intensity at 2 different spectral positions λ_1 and λ_2. The sign of the cross-peak specifies whether the changes are correlated (positive sign) or anticorrelated (negative sign). Example series of simulated spectra and their corresponding 2D-SCS are shown in Fig. S1. In the top row of Fig. S1, 3 types of time-dependent spectra are given; each of them has 3 contributions at the wavelengths λ_1, λ_2, and λ_3. In Fig. S1*A*), these 3 contributions undergo either a linear decrease (λ_1, λ_3) or a linear increase (λ_2). In Fig. S1*B*, the time order of the spectra shown in A was changed randomly; and in Fig. S1*C*, noise was added to the spectra shown in *B*. The middle row of Fig. S1 shows the contour representation of the spectra, and in the third row the corresponding 2D-SCS are given. The autopeaks are labeled in the left 2D-SCS as a1, a2, and a3 and the 3 cross-peaks as c12, c23, and c13.

The cross-peaks c13 connect the intensity at position a1 with the intensity at a3. The positive sign of the cross-peak indicates the correlated intensity change. Negative signs for the cross-peaks are found in the case of c23 and c12 formed between a1 and a2 and between a2 and a3, as their intensity changes are anticorrelated. The 2D-SCS spectra shown in Fig. S1 *B* and *C* show the same features as in A, irrespective of the random time order of the spectra and the added noise. With the 2D-SCS, it is therefore possible to unravel correlated behavior in single-molecule spectra that might be hidden in the time-dependent spectra due to random variations and limited signal–noise ratio.

1. Noda I, Dowrey AE, Marcott C, Story GM, Ozaki Y (2000) Generalized two-dimensional correlation spectroscopy. *Appl Spectrosc* 54:236A–248A.

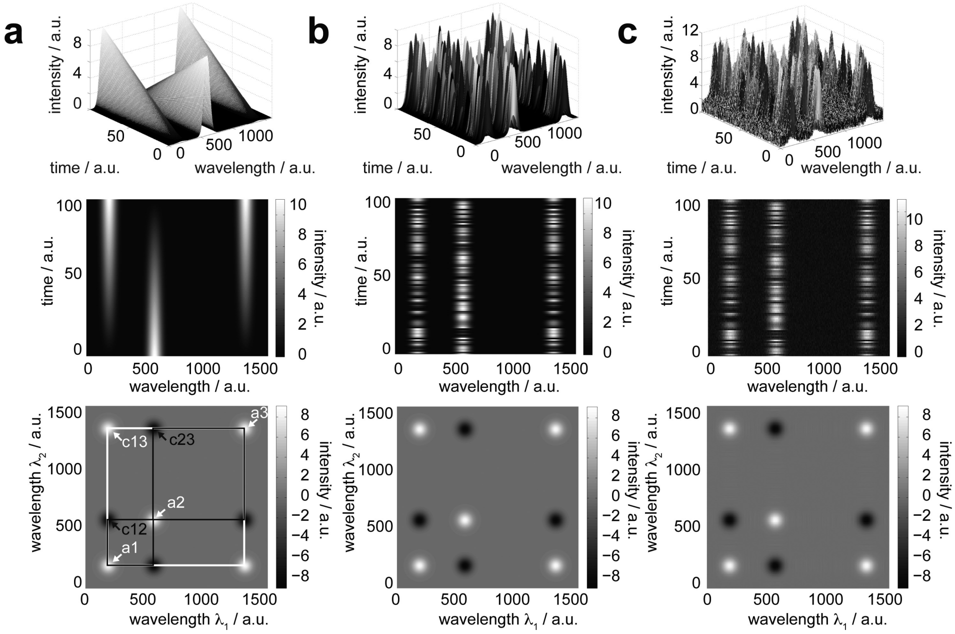

Fig. S1. Simulated spectra to illustrate the information gained by calculation of the 2D-SCS from series of spectra. Each of the series of spectra given in the top row show 3 contributions that change in dependence of an external parameter, here, the time. (*a*) The contributions undergo a linear increase or decrease in their intensity. (*b*) The same spectra as in *a* are shown, but the time order of the spectra was changed stochastically. (*c*) Noise was added to the spectra shown in *b*. The middle row shows the contour representation of the spectra shown above. The bottom row shows the resulting 2D-SCS spectra of the above spectra.