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Component Separation in NMR Imaging and Multidimensional Spectroscopy through Global Least-Squares Analysis, Based on Prior Knowledge

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Use of prior knowledge with regard to the number of components in an image or NMR data set makes possible a full analysis and separation of correlated sets of such data. It is demonstrated that a diffusional NMR microscopy image set can readily be separated into its components, with the extra benefit of a global least-squares fit over the whole image of the respective diffusional rates. As outlined, the computational approach (CORE processing) is also applicable to various multidimensional NMR data sets and is suggested as a potentially powerful tool in functional MRI. © 1998 Academic Press

General problems in spectroscopy and image processing are to achieve a separation of component spectra or images and to cope with noisy data. However, one often experiences a situation where the number of components in the underlying data is actually known beforehand. In case of correlated sets of data, one also often knows the functional form of the intensity variation of a particular component within the data set. Prior knowledge of this kind makes possible a full analysis of such data sets. The present communication specifically deals with diffusion images in NMR microscopy, but the principles are general and applicable to a larger family of data types, as further discussed below.

A spectroscopic diffusion measurement on a sample with n components, using the Hahn echo (*1*) and the FT-PGSE technique, is fully describable by a sum of n Stejskal–Tanner exponentials [1] (*2, 3*),

$$A_{\text{tot}}(2\tau) = \sum_{i=1,n} A_i(0)\exp(-2\tau/T_{2i}) \times \exp(-D_i(\gamma g\delta)^2(\Delta - \delta/3)). \quad [1]$$

Here the symbols have their usual meaning. It is important to note that the D_i-values are global to the data set and that therefore (under the condition of a fixed value of τ) Eq. [1] holds for any frequency in the spectrum. In other words, a given component bandshape will remain constant for all gradient settings of the FT-PGSE experiment (*3, 4*). This is the actual prior knowledge needed for separating such data into its components—i.e., the bandshapes (described by the respective $A_i(0)$-values at different frequencies) and the diffusion coefficients (D_i) of the constituents. A global analysis using such a strategy will also provide the best possible estimate of the diffusion coefficients of the components, since all available information will be accounted for. In practice, this will be a signal-averaging process where data for a large number of frequency channels are added. As a consequence, the effective signal/noise ratio of the experiment will increase in comparison with a simple peak height fitting analysis—generally by an order of magnitude or more.

Following the original qualitative suggestion of the potential use of FT-PGSE based "size-resolved NMR" (*3, 4*), several quantitative strategies for achieving the desired global analysis of such spectroscopic data have been described. These include the use (*5*) of a coupled inverse Laplace transform (ILT) approach name SPLMOD (*6, 7*), the use and development of multivariate statistical methods like NIPALS (*8*) and GRAM, DECRA (*9, 10*) and others similar (*11*) and also of a more general and direct (but very computer-intensive) global least-squares approach, named CORE (*co*mponent-*re*solved spectroscopy) (*12, 13*). In contrast to the latter methods, SPLMOD only partly accounts

FIG. 1. The six 5-mm NMR samples inside a 20-mm tube containing water. The image intensity displays are autoscaled with respect to the intensities within the individual image. (a) The full data set. The sample compositions are: (1) HDO in D_2O, (2) PEO 3400 in D_2O, (3) PEO 400 in D_2O, (4) PEO 400 + PEO 3400 in D_2O, (5) PEO 400 + HDO in D_2O, (6) PEO 3400 + HDO in D_2O (see text). (b) CORE fitted data using Eq. [1] with three components, after numerical elimination of the water signal between the tubes. (c) Component No. 1, D = 1.8 10^{-10} m^2 s^{-1} ("PEO 400"). (d) Component No. 2, D = 1.7 10^{-9} m^2 s^{-1} ("HDO"). (e) Component No. 3, D = 1.8 10^{-11} m^2 s^{-1} ("PEO 3400"). (f) The difference map.

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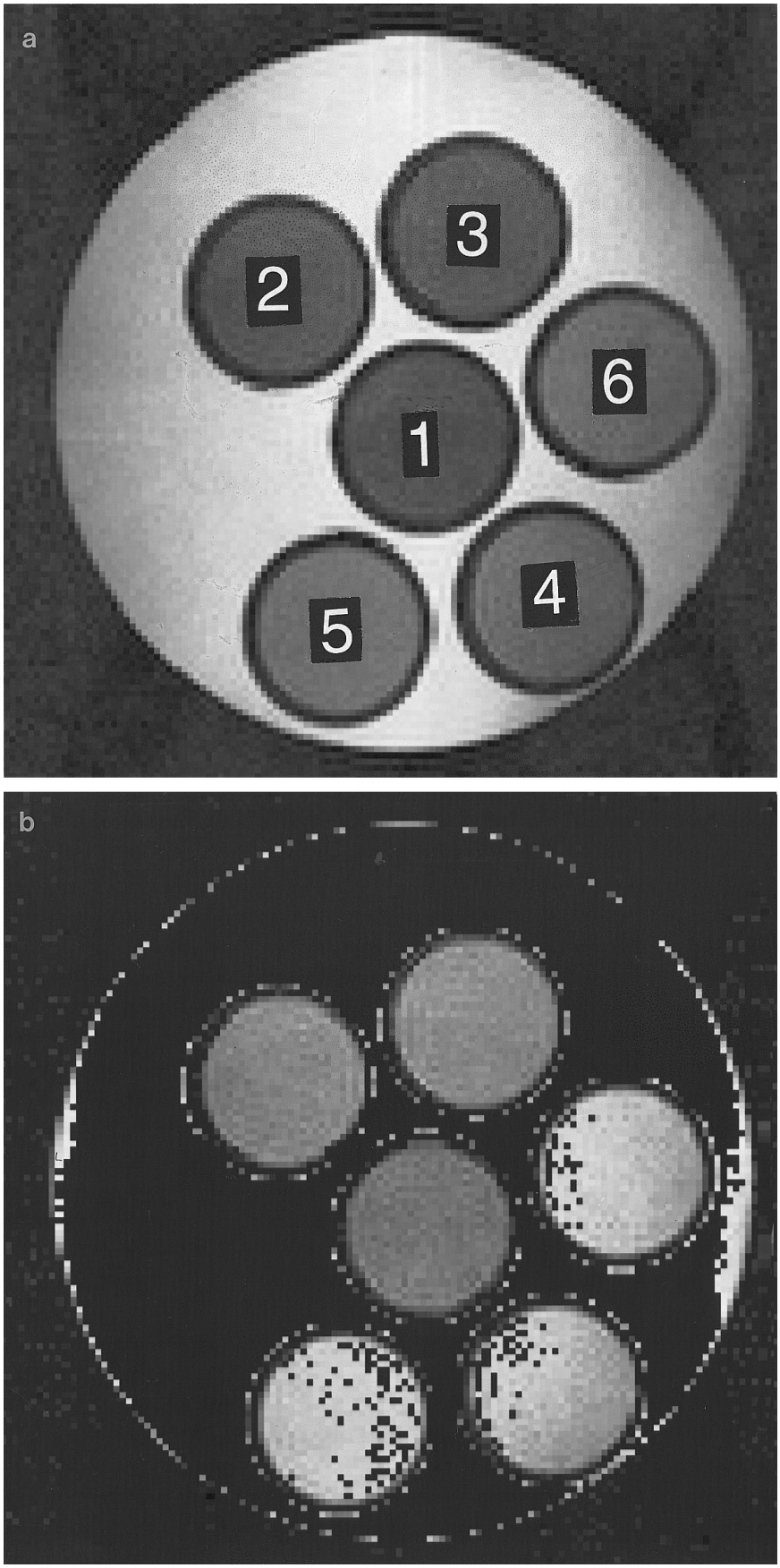
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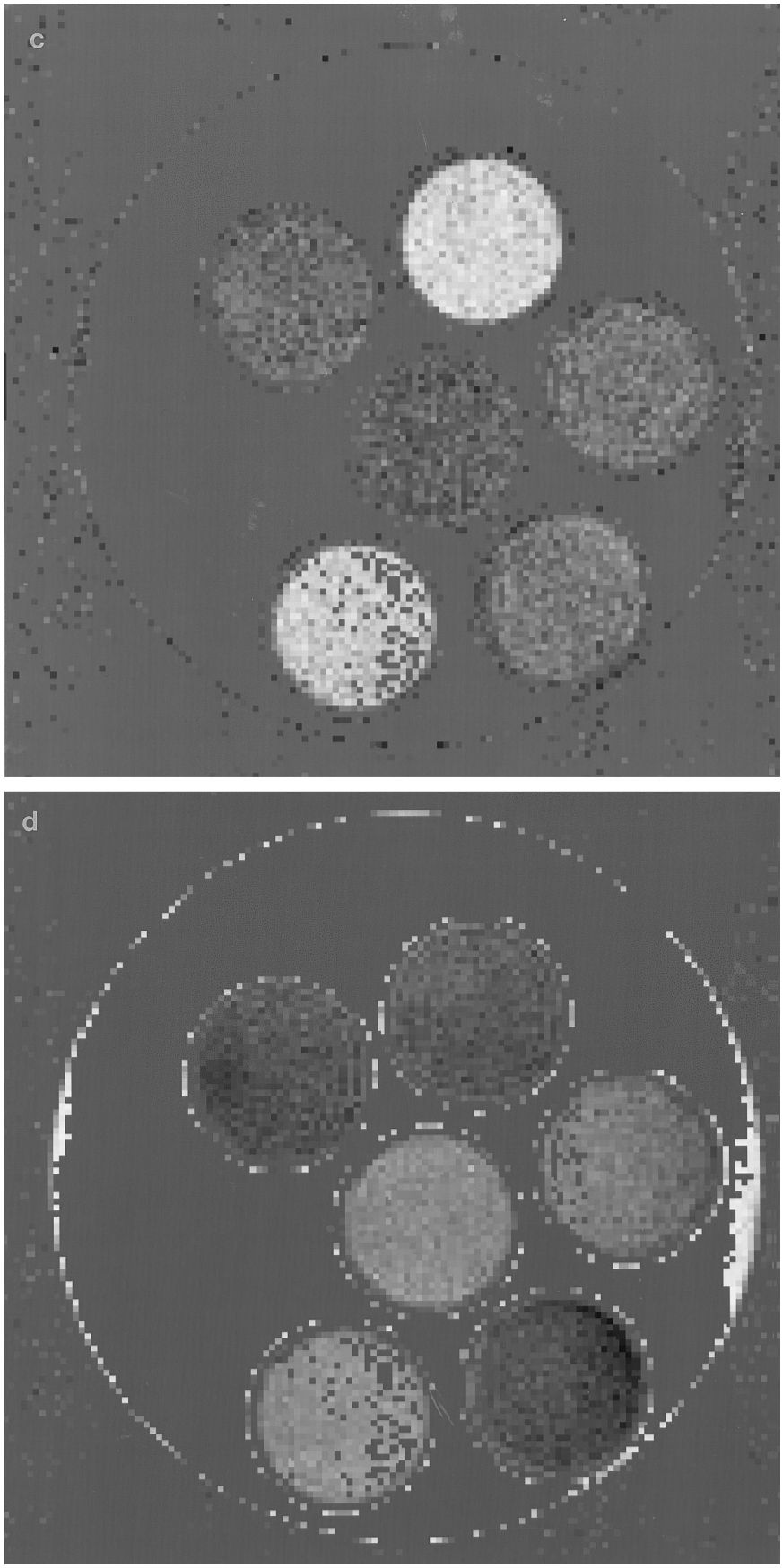

FIG. 1—*Continued*

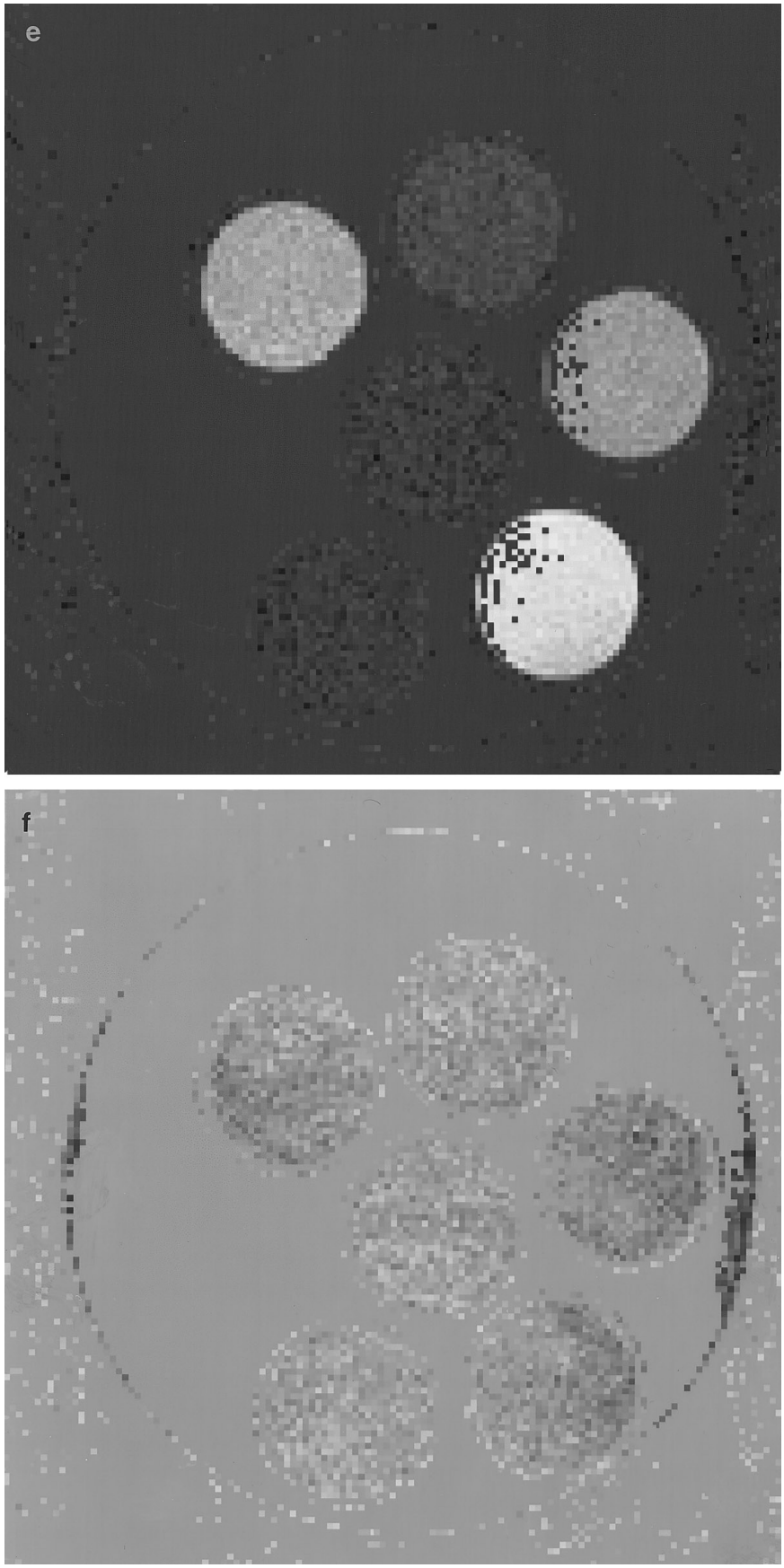

FIG. 1—*Continued*

for the information contained in the data set, since it can only deal with one limited frequency region at a time. When applying the multivariate methods mentioned, one concludes that all "components" have been found, when just noise remains. In a CORE analysis one instead tests models of increasing complexity (in the present application: more terms in Eq. [1]), unless the number of components is known beforehand.

A 2D-like display mode name DOSY (diffusion ordered spectroscopy) of processed multicomponent FT-PGSE data has also been introduced by Morris and Johnson (*5*) (chemical shift (x) vs diffusion coefficient (y) and intensity (z)). It has become quite popular and has been extended to 3D data, using a combination of the FT-PGSE experiment with various multidimensional NMR techniques like COSY, NOESY, and HMQC, primarily for the purpose of separating the bandshapes of different components in a mixture (*14–16*). It is important to note, however, that presently the vast majority of DOSY-like applications are not normally based on a *global* analysis approach of the previously mentioned kind, but rather on (interpolated) contour plots (or similar) of *unrelated* single-channel data.

NMR microscopic images constitute a special form of multidimensional spectral data sets, but the basic data analysis problem is the same. In the present paper we describe how NMR PGSE-based diffusion images can be efficiently analyzed using the prior knowledge that the intensity of each component in each pixel is fully described by Eq. [1].

Figure 1a illustrates the first slice of a phased absorption-mode PGSE image set, recorded at increasing gradient settings ranging from 0 to 96 G/cm, in 16 equidistant steps, using $\delta = 2$ ms and $\Delta = 50$ ms. The sample consisted of six 5-mm NMR tubes, immersed in normal water (for shimming purposes) in a 20-mm tube. The measurements were made at room temperature and 200 MHz in a vertical superwide-bore magnet. The samples (see Fig. 1) contained various combinations of (a) doped water, (b) PEO, (poly(ethylene oxide)), Mw = 400, and (c) PEO, Mw 3400, all dissolved in heavy water to a total concentration of 10–20%.

The raw experimental data (sixteen 128 * 128-pixel images) were then transformed row by row into sixteen "linear" data sets, each of length 16,384. Then, the more intense water background was removed by numerical selection, in order to limit the analysis to the actual samples in question. Unfortunately, this procedure did not work completely—there remains some "outer water" near the walls of the tubes, and particularly near the right-hand side of the image. This did not significantly disturb the analysis, however. The samples contain three components in total, so the transformed 16 * 16K data set was then simply fitted by the normal CORE procedures (*12, 13*) to Eq. [1], using $n = 3$, and then reassembled for display (Figs. 1b–1f). As seen, the procedure correctly finds the presence of the different components in the samples as well as their (global) D-values. By normal spectroscopic FT-PGSE all image diffusion data were confirmed and match almost perfectly.

It should be noted that traces of HDO were also naturally present in samples did not contain any added doped water. This shows up as a weak background signal (assigned to component 2) in all six tube images. Also, the results for "PEO 400" definitely look erroneous at first inspection—it shows up as component 3 in tube 4, rather than component 1. However, PEO 400 actually diffuses at a very much lower rate in this quite concentrated mixed PEO 400/PEO 3400 sample. Its diffusion rate is lowered about a factor of 10 from its "normal" aqueous solution value and is very close to that of PEO 3400. This was specially checked by spectroscopic FT-PGSE.

With regard to related image processing problems one should note that (i) the effective S/N of the experiment increases quite extensively by using this global data analysis approach, and (ii) when using the direct CORE least-squares analysis (rather than any of multivariate methods mentioned) one is not limited to exponential functional relations between the images in a set (like in Eq. [1]). CORE processing can be made with any functional form. One obvious candidate for further application is functional MRI, where a brain image is monitored under conditions of "on" or "off" of a certain stimulus. Subtle intensity changes result in certain areas of the brain images under on/off conditions. Some time-shifted and modified square-wave or cosine-like burst response may be expected to model this behaviour in CORE processing, and the matching image region will stand out in the displayed results. Work along such lines is in progress. Of course, the outlined computational procedure is directly applicable also to many types of multidimensional NMR data sets, where the component bandshapes remain constant with the experimental parameter(s), and the functional form of the bandshape intensity variation is known. A CORE-like global analysis using this prior knowledge, rather than making interpolated contour plots on unrelated data, allows extraction of the full information content of the experiment.

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