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## Detection of biomarkers by MRS requires error analysis

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### Abstract

Using MRS imaging and singular value decomposition (SVD), Manganas et al. (*Science* **318**, 980, 2007) reported a nuclear magnetic resonance diagnostic for neural progenitor cells. Characteristics of MRS experiments are known to be problematic for SVD-based methods. Absent detection using alternate methods of spectrum analysis or controls to quantify the false discovery rate, little significance can be attached to their result.

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Manganas et al. (1) reported a <sup>1</sup>H nuclear magnetic resonance diagnostic for neural progenitor cells. While potentially a significant development for neuroscience, the experiment design contains serious shortcomings that render highly premature the conclusion that the resonance reflects a metabolite characteristic of neural progenitor cells, and that the resonance can be quantified by MRS imaging. Most importantly, it relies on an *ad hoc* combination of spectral processing methods for which meaningful error analysis is difficult. The spectrum analysis method at the heart of their protocol has known deficiencies under precisely the circumstances that prevail in <sup>1</sup>H MRS experiments. Their protocol would also be difficult for other laboratories to systematically reproduce.

The study uses Hankel singular value decomposition (HSVD, (2)), a non-Fourier method of spectrum analysis, to compute frequency spectra from time-domain MRS data. HSVD and closely related methods such as linear prediction (LP) extrapolation, LPSVD, maximum likelihood (MLM), and filter diagonalization method (FDM) explicitly or implicitly model the signal as a sum of exponentially decaying sine waves, or sinusoids, and implicitly model noise as randomly distributed (3). The assumption of exponential decay of the time domain signal is equivalent to modeling signals as Lorentzian lines in the frequency domain. Matrix methods (SVD in the case of HSVD, LP extrapolation, and LPSVD, matrix diagonalization in the case of FDM) are used to determine the values of the model parameters (amplitude, frequency, phase and decay rate for each sinusoid in the model) that result in optimal agreement between the measured data and the model. In very low signal-to-noise (S/N) regimes or where the signal decay is not exponential (e. g. due to magnetic field inhomogeneity), these assumptions do not hold. Methods that model the signal as Lorentzian lines are prone to false positives (4), mainly because they have no way to characterize noise except as an exponentially decaying sinusoid. Furthermore, they require a prior estimate of the number of signal components. A common phenomenon with these methods is “spontaneous splitting”, in which a peak characterized as a single exponential decay for one value of the number of sinusoids in the model becomes two decaying sinusoids when the number of sinusoids in the model is increased (5). When the number of sinusoids is underestimated, or the decay is not exponential, frequency errors can result (3). The false positives and frequency error can be highly reproducible, and are often associated with other signals or imperfect subtraction, so that they can exhibit an apparent mass dependence.

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Consequently the use of SVD-based signal processing, especially at low S/N, demands extraordinarily careful controls and error analysis (6).

Spontaneous splitting in SVD methods becomes especially problematic for data exhibiting high dynamic range, containing components with widely different amplitudes. In these circumstances many sinusoids may be required to represent large amplitude components to account for slight deviations from exponential decay. Such deviations are common for  $^1\text{H}$  MRS data due to magnetic and RF field homogeneity and radiation damping of the water signal. Weak components may be missed altogether unless a very large number of sinusoids are included in the model.

In an attempt to avoid these problems, Manganas et al. iteratively applied HSVD to determine a model for the strong water resonance, which may involve multiple sinusoids, and subtracted that model from the experimental data. The data was subsequently multiplied by an exponentially decaying function (to suppress noise at the expense of broadening the signal resonances) prior to a final HSVD analysis to determine model parameters for the remaining signal components. They report a statistical analysis of the variance of the signal parameters, which does not address the possibility that reproducible systematic errors due to non-exponential behavior of the signals or the residual water signal could lead to fictitious signal components. The use of synthetic exponentially decaying signals for error analysis, as previously reported for HSVD-based analysis of MRS data (7), does not account for deviations from ideal behavior expected for real data. Without a demonstration that the putative biomarker signal can be detected using alternative methods of spectrum analysis that do not share the vulnerabilities of SVD-based methods, and appropriate controls to elucidate the false discovery rate, one cannot assign confidence to the results.

As the seductive appeal of  $^1\text{H}$  NMR for identifying specific cell states has tempted others to reach premature conclusions (8–10), we believe that such reports should be viewed as extraordinary claims demanding extraordinary justification. The report by Manganas et al., while exciting, does not meet this standard.

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