Published in final edited form as:

J Magn Reson. 2020 October ; 319: 106812. doi:10.1016/j.jmr.2020.106812.

Algebraic reconstruction of 3D spatial EPR images from high numbers of noisy projections: An improved image reconstruction technique for high resolution fast scan EPR imaging

Denis A. Komarov¹, Alexandre Samouilov¹, Rizwan Ahmad², Jay L. Zweier^{1,2,*}

¹Department of Internal Medicine, Division of Cardiovascular Medicine, and the EPR Center, Davis Heart & Lung Research Institute, College of Medicine, The Ohio State University, Columbus, Ohio 43210, USA.

²Department of Biomedical Engineering and the EPR Center, College of Engineering, The Ohio State University, Columbus, Ohio 43210, USA.

Abstract

A novel method for reconstructing 3D spatial EPR images from large numbers of noisy projections was developed that minimizes mean square error between the experimental projections and those from the reconstructed image. The method utilizes raw projection data and zero gradient spectrum to account for EPR line shape and hyperfine structure of the paramagnetic probe without the need for deconvolution techniques that are poorly suited for processing of high noise projections. A numerical phantom was reconstructed for method validation. Reconstruction time for the matrix of 128^3 voxels and 16,384 noiseless projections was 4.6 min for a single iteration. The algorithm converged quickly, reaching $R^2 \sim 0.99975$ after the very first iteration. An experimental phantom sample with nitroxyl radical was measured. With 16,384 projections and a field gradient of 8 G/cm, resolutions of 0.4 mm were achieved for a cubical area of $25 \times 25 \times 25$ mm³. Reconstruction was sufficiently fast and memory efficient making it suitable for applications with large 3D matrices and fully determined system of equations. The developed algorithm can be used with any gradient distribution and does not require adjustable filter parameters that makes for simple application. A thorough analysis of the strengths and limitations of this method for 3D spatial EPR imaging is provided.

Graphical abstract

*Correspondence to JLZ (jay.zweier@osumc.edu), Davis Heart & Lung Research Institute, The Ohio State University, 420 West 12th Ave, Columbus, OH 43210.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords

EPR imaging; image reconstruction; spectral deconvolution; least-squares problem; nitroxide radicals

Introduction

Electron paramagnetic resonance (EPR) imaging is a technique that allows visualization of the distribution of paramagnetic molecules inside an object and to perform spatially resolved spectral measurements [1-6]. In a simple case, when the EPR spectrum is invariant within an examined object, it is possible to run a purely spatial EPR imaging experiment [7; 8]. For doing this, the EPR signal is recorded in the presence of a magnetic field gradient of constant magnitude and oriented at different angles to cover the whole three-dimensional (3D) space. An EPR projection recorded in the presence of a gradient is effectively a cross correlation of the paramagnetic probe EPR spectrum and its single dimensional (1D) distribution profile in the sample (Eq. 4 in Theory). Most commonly used approaches for reconstruction of spatial EPR images begin with expelling spectral information from the projections by deconvolving them with a zero-gradient spectrum [9; 10]. The deconvolved projections are expected to reflect spin distribution along the gradient direction[†] and are used to produce an EPR image by an implementation of inverse Radon transform. Although this appears to be a straightforward method for reconstruction of purely spatial EPR images, its reliance on spatial projections that must be computed explicitly through deconvolution constitutes a major weakness. Spectral deconvolution is a poorly conditioned inverse problem that may corrupt spatial projections and the resulting image. This problem is particularly severe in the case of noisy projections.

With recent advances in the development of fast scan EPR imaging instrumentation, it is now possible to obtain image projections in milliseconds rather than the seconds previously required [11-13]. This has a number of advantages including higher sampling, decreased biological noise, and higher possible image resolution in a given time as well as the potential for greatly accelerated acquisitions. These fast scan projections; however, intrinsically have lower signal to noise than conventional slow projections. For example with 1000-fold acceleration the signal to noise ratio (SNR) would be predicted to be ~32-fold lower although in practice due to reduction in periodic biological noise this decline may only be

[†]For the reasons that will be explained below, the resultant projections will be flipped.

about 8 to 10-fold. Never the less, the low signal to noise projections typically obtained with fast scan EPRI acquisition, with SNR of 2 to 5 are commonly encountered and make the process of spectral deconvolution problematic.

A typical EPR spectral line lacks high frequency information due to homogeneous and inhomogeneous broadening. For this reason, small image details and sharp edges are smoothed on EPR projections that effectively causes the loss of resolution. Deconvolution of noisy experimental data with an EPR spectrum leads to a “division-by-zero” problem and drastically amplifies high frequency noise [9; 10]. This necessitates utilization of low-pass filters that leads to undesirable blurring of the images, sacrificing their spatial resolution even further and may also cause ringing artifacts for non-Gaussian filters.

An additional problem is encountered when the paramagnetic probe utilized exhibits an EPR spectrum with multiple lines due to hyperfine coupling, as with the triplet spectrum of nitroxyl radicals. In order to reduce image acquisition time, the spectral window in EPR imaging experiments is usually limited to a single peak. However, in the presence of the magnetic field gradient, EPR signals from other peaks may also contribute to the measured projections. In such cases, it becomes necessary to remove the superimposed signal from the experimental projections before doing deconvolution to prevent the formation of image artifacts [14; 15]. Subtraction procedures for overlapping signals impair signal-to-noise ratio of the projections but cannot guarantee an artifact-free image if the probe has asymmetric hyperfine structure or hyperfine splitting constant was not precisely defined.

A variety of approaches for extracting spatial information from EPR projections has been reported including: methods based on Fourier deconvolution with application of different low-pass filters with subjective choice of parameters or automatic detection of cut off frequency [16; 17]; iterative deconvolution techniques [18-20]; and several different procedures for hyperfine splitting correction [14; 15]. However, spectral deconvolution remains a poorly conditioned inverse problem that inevitably leads to the loss of information and may cause inconsistencies in the projections that subsequently corrupts the reconstruction of the image. For these reasons, it would be advantageous to avoid the need to compute deconvolved projections explicitly and proceed to the image reconstruction directly using the raw projection data. We have previously reported possible solutions to this problem in the context of applying compressive sensing to accelerated 3D spatial EPR imaging [21; 22]. These reconstruction approaches aim to solve a regularized least-squares problem using a gradient descent algorithm in conjunction with L1 norm minimization of the image itself or its wavelet transform coefficients and total variation minimization. More recently, a different group have proposed using curvelets regularization for 2D spatial EPR imaging [23]. However, these regularization-based reconstruction methods are computationally intensive and not well suited for large 3D matrices with high number of projections.

In the present paper, we introduce a novel algebraic reconstruction algorithm optimized for 3D spatial EPR image reconstruction from a high number of noisy projections with simplified procedure for solving the least square problem. The method is fast and memory efficient making it suitable for large 3D matrices and full sets of EPR projections. The

developed algorithm does not rely on precomputed deconvolved projections, inherently accounts for the hyperfine structure, requires no filtering and can be used with noisy data. The algorithm is suitable for any arbitrary choice of gradients and has no adjustable parameter that makes it easy to use in practical applications.

Theory

Assuming that the EPR spectrum does not change within a sample, the EPR projection for a given gradient of the magnetic field can be expressed as the following integral over 3D space:

$$p(\vec{G}, B) = \int \int \int_{\mathbb{R}^3} f(\vec{x}) s(B + \vec{G} \cdot \vec{x}) d\vec{x} \quad (1),$$

where $\vec{G} = \left(\frac{\partial B}{\partial x}, \frac{\partial B}{\partial y}, \frac{\partial B}{\partial z} \right)$ is the gradient, B – magnetic field at the origin, $f(\vec{x})$ – spin distribution function, $\vec{x} = (x, y, z)$ – spatial coordinate, and $s(B)$ is the EPR spectrum. The corresponding spatial projection of the sample can be written as:

$$t(\vec{n}, r) = \int \int \int_{\mathbb{R}^3} f(\vec{x}) \delta(\vec{n} \cdot \vec{x} - r) d\vec{x} \quad (2),$$

where $\vec{n} = \frac{\vec{G}}{\|\vec{G}\|}$ is normalized vector of the gradient, $\delta(r)$ – Dirac delta function, and r is the distance from the origin. If we take the gradient vector out of the spectral function in (1) we will have:

$$p(\vec{G}, B) = \int \int \int_{\mathbb{R}^3} \int_{-\infty}^{\infty} f(\vec{x}) s(B + \beta) \delta(\vec{G} \cdot \vec{x} - \beta) d\beta d\vec{x} \quad (3),$$

and using the equation (2) we finally get:

$$p(\vec{G}, B) = \int_{-\infty}^{\infty} t(\vec{G}, \beta) s(B + \beta) d\beta \quad (4).$$

That is, an EPR projection is effectively a cross-correlation of the object spatial projection and the probe EPR spectrum. Historically, it has been assumed that stationary magnetic field B and the shift caused by the applied magnetic field gradient, $r \text{ grad}_r B$, have opposite signs [24]. For this reason, EPR projections measured in the presence of an external field gradient are most commonly referred as “convolution” rather than “cross-correlation”. To avoid the confusion in the formulas, in this paper we will follow strict mathematical notation and consider the gradient positive if magnetic field increases along the specified axis. The physical meaning of this is quite transparent: EPR signals from the parts of the sample that are located in the higher magnetic field region appear earlier on experimental projections.

By changing the sign of the dummy variable β in the Eq. 4, this can be rewritten as:

$$p(\vec{G}, B) = \int_{-\infty}^{\infty} \tilde{t}(\vec{G}, \beta) s(B - \beta) d\beta \quad (5),$$

where $\tilde{t}(\vec{G}, \beta)$ is the flipped spatial projection. Under these terms, the EPR projection can be conceived as the convolution of the flipped spatial projection with the EPR spectrum. We will stick to this definition in the subsequent equations.

For the discrete case, let us assume that f_k is an estimation of 3D image obtained after k iterations and reshaped as a column vector. For the first iteration, f_0 can be set all zeros. Then, if A_j is the operator which produces the projection for the gradient $\vec{G}_j$, we have:

$$t_{j,k} = A_j f_k \quad (6).$$

According to the Eq. 5, the corresponding EPR projection will be:

$$p_{j,k} = \tilde{t}_{j,k} * s \quad (7),$$

where tilde "~" designates the flipped projection, and star "*" is used to denote the convolution operator. If we now want to apply a correction term to the image to approach the experimental projection, the gradient descend step towards the least square solution will be:

$$\Delta \tilde{t}_{j,k} = c_{j,k} (p_j^{exp} - p_{j,k}) * \tilde{s} \quad (8),$$

where p_j^{exp} is the experimental projection and $c_{j,k}$ is the step size which we will find next.

Cross-correlation with the EPR spectrum, written here as the convolution with the flipped spectrum $\tilde{s}$, is an operator which is adjoint to the forward convolution procedure as required by the gradient descend algorithm. Note that in the cases when the EPR spectrum has components that reside outside the experimental field sweep, $p_{j,k}$ can be computed using the full spectrum and then cropped to the size of the experimental projections.

If we backproject the correction term $\Delta \tilde{t}_{j,k}$ to the image the new projection will become:

$$p_{j,k+1} = p_{j,k} + c_{j,k} \Delta p_{j,k} * \tilde{s} * s \quad (9),$$

where $\Delta p_{j,k}$ is the difference between the experimentally measured and the calculated projection:

$$\Delta p_{j,k} = p_j^{exp} - p_{j,k} \quad (10).$$

We now want to find the optimal coefficient $c_{j,k}$ which will minimize the difference between p_j^{exp} and $p_{j,k+1}$:

$$c_{j,k} = \underset{c}{argmin} \|\Delta p_{j,k} - c \Delta p_{j,k} * \tilde{s} * s\|_2^2 \quad (11).$$

The last equation is a simple linear regression and the optimal $c_{j,k}$ can be found as:

$$c_{j,k} = \frac{\Delta p_{j,k} \cdot (\Delta p_{j,k} * \tilde{s} * s)}{\|\Delta p_{j,k} * \tilde{s} * s\|_2^2} \quad (12).$$

To apply the correction to the image we need to do:

$$f_{k+1} = f_k + A_j^T \text{diag}(w_j)^{-1} \Delta t_{j,k} \quad (13),$$

where w_j is a weight function, which indicates how many times each single point from $t_{j,k}$ will be placed on the image by the backprojection operator A_j^T . For square-shaped or rectangular image matrices, w_j depends on gradient orientation. In order to simplify the computations, we can use the upper estimation of w_j computed for the circumscribed spherical volume:

$$\hat{w} = \frac{\pi}{\|a \dot{\mathbf{G}}\|} \left(\frac{3}{4} m^2 - \frac{\iota^2}{\|a \dot{\mathbf{G}}\|^2} \right) \quad (14),$$

where $a = \frac{n \text{FOV}}{\Delta B \ m}$ is a coefficient to make the gradients dimensionless, n is the number of data points in the projections, B is the magnetic field sweep, FOV is the field-of-view, m is the linear size of the image matrix and ι (“iota”) is an index vector. Using $\hat{w}$ instead of w_j slightly decreases the rate of algorithm convergence, but may gain computation speed due to the simplification.

The image can be reconstructed by executing equations 6, 8 and 13 in an iterative cycle. We will consider one iteration completed when image matrix has been corrected for each available gradient and experimental projection once. The proposed reconstruction algorithm is a modification of the algebraic reconstruction technique [25] that intrinsically accounts for the probe EPR spectrum. It can be viewed also as a special case of gradient descent method with the step size of $c_{j,k} \text{diag}(w_j)^{-1}$. Analytical solution for the coefficient $c_{j,k}$ ensures a high rate of algorithm convergence and simplifies its usage, because no manually-tunable parameters are then required.

Methods

Computational environment

All computations were performed on a custom-built computer equipped with AMD Ryzen 7 2700X processor, 32 GiB of memory, running Debian GNU/Linux 10. The reconstruction algorithm and supplemental functions were developed in GNU Octave. Main computational part of the algorithm was written in C language for speed and compiled with GCC 8.3.0 as an Octave executable function. Forward projection operator and its adjoint were implemented implicitly using the approach similar to that described previously [26]. The method is fast and memory efficient allowing computation of 16,384 projections from a matrix of 128^3 voxels in 150 seconds (~ 9 ms per projection) and taking only 500 MiB of system memory. Convolutions were performed by pointwise multiplication in Fourier

domain using FFTW 3.3.8 library to execute the Fourier transforms [27]. The reconstruction algorithm with an example of its application is available for download at <https://gitlab.com/dkomarov/art3dx/> under the terms of General Public License.

Numerical phantom

A 3D numerical phantom modeling a threaded screw with hexagonal nut was generated (Fig. 1a). We have intentionally chosen the phantom with threaded elements, because of its small sharp details that provide a good insight on the resolution of the imaging technique. Matrix size of the phantom was 512^3 voxels, simulated field-of-view $25 \times 25 \times 25 \text{ mm}^3$. The model parameters were as follows: screw length 3/4 inch, thread size 3/8-16 UNC, nut size 9/16 inch. Relative intensities of the EPR signal for the screw and the nut were 1 and 1.5, respectively. An artificially generated zero gradient spectrum was used to produce EPR projections (Fig. 1b). The spectrum comprised of three peak components computed as the first derivatives of Voigt profile. The peaks were located at -0.5 , $+0.5$ and $+15 \text{ G}$ with respect to the central magnetic field and had equal integral intensities. Lorentzian line width for all of the peaks was 0.3 G , Gaussian widths were 0.6 , 0.3 and 0.6 G from low to high magnetic field, respectively. EPR projections were computed for 16,384 homogeneously distributed spherical gradients generated by 3D golden means algorithm [28]. Magnetic field sweep for the projections was 24 G , gradient strength 8 G/cm . The zero gradient spectrum and a few examples of the projections are presented in Fig. 1b and 1c, respectively.

Two additional sets of projections for the numerical phantom were generated using the EPR spectrum of 3-methoxycarbonyl-2,2,5,5-tetra-(D_3)-methyl-1-pyrrolidin(3,4,4- D_3)-1-oxyl nitroxyl radical (dMCP, see Fig. 4a for the structure). The projections were computed for gradient magnitudes of 8 G/cm and 16 G/cm . Magnetic field sweep for the two cases was 24 G and 48 G , respectively. The resultant projections were added with white Gaussian noise making their final signal-to-noise ratio (SNR, Eq. 16) 1.0 , 2.2 and 4.7 .

Imaging instrumentation

All experiments were performed on a laboratory built EPR imaging system that was previously described in detail [13]. In brief, the magnetic field is generated by an air-core resistive spherical solenoid magnet. The magnet is equipped with 3D cylindrical gradient coils and a field sweeping coil. Microwave part of the system consists of a radio frequency source, reflection type resonator, band pass filter, Schottky diode detector and automatic frequency control circuit. The signal is detected by envelop detection principle, with no reference arm used. A segmented cylindrical resonator with inner diameter of 18 mm , 20 mm length and resonant frequency at 1.25 GHz was utilized in this work. The resonator is equipped with a Hemholtz coil that is used for the magnetic field modulation. The EPR signal is demodulated by a lock-in amplifier and digitized. The main magnet, gradient coils, field sweeping coil and data acquisition are controlled from a computer using National Instruments multifunctional input-output devices and home developed C++ software. To minimize image acquisition time, the magnetic field sweep is driven with a triangular function and data are recorded for both forward and reverse scans.

Radical solution imaging

To construct an experimental positive phantom sample, a plastic mold of a hexagonal nut was printed using a 3D printer. Model dimensions were the same as for the numerical phantom. The mold was filled with 1.5 mM solution of dMCP in 10% phosphate buffered saline. EPR projections were recorded using the following instrument settings: magnetic field sweep 24 G, sweep time 4 ms, modulation amplitude 0.35 G, number of data points 1800, number of projections 16,384, number of scans for each projection 10, gradient strength 8 G/cm, total image acquisition time 11 min. The gradients were generated by 3D golden means algorithm [28] and sorted in spiral order to minimize the load on the power supplies and to ensure their best fidelity during fast projection scanning. Zero gradient projection was recorded with the field sweep of 48 G in order to include low-field and high-field components of the probe EPR spectrum.

Results and discussion

Initial validation of the proposed reconstruction algorithm was performed with a numerical phantom. EPR projections were generated using an artificial EPR spectrum that is presented in Fig. 1b. The spectrum does not correspond to any real paramagnetic molecule and was chosen with primarily goal to test the reconstruction algorithm in a difficult case when the EPR spectrum has overlapping components and an admixing signal from outside the spectral window. The total number of generated projections, 16,384, was sufficient to furnish us with a fully determined system of equations for an image of 128^3 voxels, so that image resolution would not be limited by the lack of projection data.

Reconstruction results for the numerical phantom are presented in Figure 2. Computation time for a single iteration was 4.6 min, reconstruction was stopped after the 12th iteration. The algorithm converged quickly and after the very first iteration with the determination coefficient measured between the original projections and those from the resultant image of 0.99975. Subsequent iterations gradually increased this value to 0.99998 (Fig 2d, blue line). The 3D surface rendered image is shown in Fig. 2a, with transverse and a longitudinal slices of the image are presented in Fig. 2b and 2c, respectively. Despite the very high convergence degree, the resultant image has some blurring. To estimate the achieved spatial resolution, we fitted edges of the visualized object with an error function similar to the procedures described previously [19; 29], Spatial resolution was determined as full width at half maximum of the corresponding Gaussian function:

$$Res = 2\sqrt{2 \ln(2)} \sigma \simeq 2.35 \sigma \quad (15),$$

where σ is standard deviation determined by the fitting. The red line in Fig. 2d shows how the resolution changed during reconstruction. After the first iteration it was 0.68 mm and improved to 0.41 mm at the end. Apparently, the limiting factor for the resolution in this case was the deficiency of high frequencies in the zero-gradient spectrum.

To study the effect of noise and gradient strength on image resolution, we reconstructed a series of images of the numerical phantom from the sets of EPR projections with the gradient strength of 8 and 16 G/cm. For this experiment, EPR projections were generated

using the real spectrum of nitroxyl radical dMCP (see Fig. 4 for the structure and EPR spectrum). White Gaussian noise was added to the projections to study the effect of SNR of input data on quality of the final images. SNR was defined according to the formula:

$$SNR = \frac{\hat{S}_{pp}}{2\sqrt{2}SD_{noise}} \quad (16),$$

where $\hat{S}_{pp}$ is the mean value of peak-to-peak signal intensity for all of the EPR projections and SD_{noise} is the standard deviation of noise. We should note here, that definition of SNR provided by the Eq. 16 is not standard and was used on a mere purpose to make a quick estimation of SNR of the experimental projections. For the white Gaussian noise, $2\sqrt{2}SD_{noise}$ will encompass 84.3 % of the noise signal and we suppose it is close to what a researcher may estimate as a peak-to-peak value of the noise.

The images were reconstructed on a matrix of $128 \times 128 \times 128$ voxels, reconstruction was stopped after 12 iterations (Fig. 3). As it could be expected the images obtained with the gradient of 8 G/cm look blurrier compared to those reconstructed from the projections with 16 G/cm. Both sets of images start to degrade significantly at $SNR \sim 1.0$, though images obtained from the projections with the gradient of 16 G/cm look somewhat cleaner. This can be explained by taking into account the fact that peak-to-peak intensity of the projections is inversely proportional to the square of the gradient. Thus, for the numerically generated data, EPR projections with the gradient of 16 G/cm have effectively 4 times more EPR signal than those with 8 G/cm at the same level of noise. In practice, it would require a 4-fold increase in probe concentration or ~16-fold increase in signal acquisition time to keep the SNR at the same level.

To demonstrate an application of the reconstruction technique for real measurements, we performed EPR imaging of the hex nut phantom sample filled with radical solution. Nitroxyl radical dMCP (Fig. 4a) was used as the paramagnetic probe for the measurements. The radical has a typical EPR spectrum of a nitroxyl radical with three lines with hyperfine splitting constant of 16.2 G. Under air-saturated conditions, the central spectral line has Gaussian width 0.23 G and Lorentzian width 0.36 G as determined by the fitting with Voigt function, peak-to-peak linewidth is 0.72 G. EPR spectrum of the radical and examples of experimental projections are presented in Fig. 4b and 4c, respectively. Mean SNR of the measured projections was 4.7 as defined by the Eq. 16.

The reconstructed 3D image of the sample is presented in Fig. 5. The image accurately reproduces the shape of the original sample. An appendix in the right top corner of the visualized object is a 1 mm hole in the plastic mold which was used to fill it with radical solution. Estimated resolution of the image is 0.42 mm. The image was obtained in 4 iterations with final $R^2 = 0.932$. Additional iterations did not improve the coefficient of determination, but the image appeared somewhat noisier. Simultaneously, estimated resolution increased with the number of iterations (Fig. 5d). Figure 6 shows the full picture of image progression during the reconstruction. The image looks blurry at the beginning, but resolution quickly improves during the first few iterations. The visualized object reaches final shape after 4 – 6 iterations, simultaneously the background noise starts to increase.

These observations made us conclude that image resolution in this case is limited by SNR of the experimental projections and the ratio of the probe line width and the gradient.

It should be noted here, that overfitting is typical for a least square solution without regularization. The algorithm has no grounds to differentiate between the signal and the noise, so it transfers all the data to the image. To constrain the least square problem, it is usually proposed to use a regularization technique [21-23; 30; 31]. The regularization parameters need to be chosen and adjusted for each particular data set by a researcher based on his own sense of image quality. As an alternative, we may exploit the fact that the noise on the experimental projections is uncorrelated and it is introduced to the image during the iterative reconstruction process more slowly than the signal. Therefore, it is possible to interrupt the reconstruction process whenever there is no visual improvements to the image occur but the noise starts to increase. This simple approach allows to use the reconstruction algorithm without any additional complications and helps to save the computation time.

There are three major factors limiting resolution of an EPR imaging experiment. Of those, we only include theoretical limitations leaving out technical issues such as homogeneity of the magnetic field, linearity of the sweep and the gradients. The first and probably the most important limitation is the number of experimental projections for the given field of view. We will discuss below, how this affects the resolution of an EPR imaging experiment, but here we need to mention that the EPR images in Fig. 2 and 5 were reconstructed from the set of experimental projections that was sufficient for a fully determined system of equations so that resolution of the images was not limited by this factor. The two other parameters that affect the resolution of an EPR imaging experiment are the ratio of EPR line width and absolute value of the gradient and signal to noise ratio (SNR). We will consider these factors together as they are interconnected. The following discussion will primarily address the case of a consistent system, when image matrix size corresponds to the number of experimental projections. For an overdetermined system the projections may sum up together effectively improving their SNR. An additional note here is that we will describe the case for an EPR absorption line instead of the derivative signal that is commonly detected in continuous wave EPR experiments. This is because solving the equation for the resolution limit with a derivative signal would require use of Lambert W function that gives a solution to the equation $\omega e^{\omega} = z$ and cannot be expressed in elementary functions. For the absorption profile a relatively simple analytical formula can be derived.

An EPR line can be described as a convolution of Lorentzian and Gaussian functions, i.e. Voigt profile. Homogeneous broadening caused by relaxation processes results in a Lorentzian line shape, while unresolved hyperfine structure and other sources of inhomogeneous broadening are commonly described with an effective Gaussian. In Fourier domain, the Lorentzian function becomes an exponent and Gaussian transfers to another Gaussian, so that the Fourier transform of the corresponding Voigt profile can be written as:

$$FT[V(\beta, \gamma, \sigma)] = e^{-(2\pi\xi)^2 \frac{\sigma^2}{2\Delta B^2} - 2\pi|\xi| \frac{\gamma}{\Delta B}} \quad (17),$$

where B is the sweep of the magnetic field, γ and σ are Lorentzian and Gaussian widths, respectively. We may suppose that in the presence of white Gaussian noise, image

information is effectively lost when spectral density of the signal becomes lower than the standard deviation of the noise similar to that described in Ref. [10]. Solving Eq. 17 for a given SNR (Eq. 16), we can find the critical frequency as:

$$2\pi\xi_{crit} = \frac{\sqrt{\gamma^2 + 2\sigma^2 \ln(SNR \cdot \Omega)} - \gamma}{\sigma^2} \Delta B \quad (18),$$

where Ω is a coefficient that depends on object geometry and spectral characteristics of the noise. In a trivial case, when instrument noise is characterized by the time constant $t_{const} = RC$ ("pink noise") and the object has homogeneous concentration of the paramagnetic probe, the coefficient Ω can be estimated as:

$$\Omega \sim \frac{4}{3} \frac{d_{avg}}{FOV} \sqrt{\frac{t_{scan}}{t_{const}}} \quad (19),$$

where d_{avg} is an averaged size of the object and t_{scan} is scan duration. Note that peak intensity of EPR signal on a projection is inversely proportional to the object size. Simultaneously, the standard deviation of the pink noise decreases proportionally to the square root of t_{const} . Thus, $(SNR \cdot \Omega)$ remain constant and final image resolution will not depend on object dimensions or time constant of the instrument.

From Eq. 18, the resolution limit of an EPR imaging experiment can be found as:

$$Res \simeq 2.35 \frac{1}{\|\vec{G}\|} \frac{\sigma^2}{\sqrt{\gamma^2 + 2\sigma^2 \ln(SNR \cdot \Omega)} - \gamma} \quad (20).$$

For a pure Lorentzian line the last formula can be simplified as:

$$Res \simeq \frac{2.35}{\ln(SNR \cdot \Omega)} \frac{\gamma}{\|\vec{G}\|} \quad (21).$$

The same for a Gaussian line will be:

$$Res \simeq \frac{2.35}{\sqrt{2 \ln(SNR \cdot \Omega)}} \frac{\sigma}{\|\vec{G}\|} \quad (22).$$

It can now be seen that the ratio of EPR line width and absolute value of the gradient remains the limiting factor of the resolution of an EPR imaging experiment even for the projections with high SNR. Inhomogeneously broadened signals affect image resolution to a greater extent than pure Lorentzian lines.

Using formula 20, the resolution limit for the image presented in Fig. 5 can be estimated as 0.46 mm. Any image details smaller than this size are likely to originate from noise in the experimental data. As we can see now, this number is in a good agreement with the actual results obtained after the four reconstruction cycles and also gives us an explanation why the increase in the number of iterations did not improve the image quality but only increased its

noise. For the noiseless numerical phantom presented in Fig. 2, the actual image resolution was limited by reconstruction time rather than by SNR of the input data. This conclusion is confirmed by the fact that increasing the number of iterations kept improving the coefficient of determination and the resolution (Fig. 2d). Low contribution of high frequencies in the original projections hampers the algorithm from drawing sharp edges and fine image details slowing down its overall convergence. In practice, we would expect that SNR of EPR projections will never exceed 10,000. Putting this number into the formula 19, we may estimate the asymptotic limit of resolution for the numerical phantom in Fig. 2 as 0.23 mm. To verify this number, we ran the reconstruction for 15 hours. This estimated resolution was achieved in 200 iterations (data not shown).

To discuss how the number of projections affects the resolution of an EPR imaging experiment, let us first consider a two-dimensional case. In a typical continuous wave EPR experiment number of data points on a single projection is greater than the number of gradients for each independent spatial coordinate. If we have a sinogram of k projections organized as matrix columns and each projection has n data points, so that $n > k$, the dimension of the matrix column space will be less or equal k , meaning that rows of the sinogram are linearly dependent and increasing the sampling rate would not provide any additional information to the system. Thus, for the two-dimensional case, we have a system of k^2 independent equations that would allow us to reconstruct a circular area of k^2 elements. Similar rationale can be applied for the 3D EPR imaging. For the spherically distributed gradients, there are two independent coordinates on the surface of the sphere. The most common choice for these is the pair of latitude and longitude, but it could be any other pair of independent orthogonal coordinates depending on the design of EPR imaging experiment. Considering now that we have k^2 projections measured for the gradients homogeneously distributed over a spherical volume, so that k projections have been acquired for each of the two independent coordinates, column space of the corresponding 3D sinogram will have k dimensions, providing us with data to reconstruct a spherical volume of k^3 elements. With this in mind and using the definition of resolution from Eq. 15, we can estimate the resolution limit for the 3D cubical image as:

$$Res \simeq 0.523 \frac{FOV}{k} \quad (23).$$

The proportionality constant in Eq. 23 may differ depending on the particular definition of resolution [32]. For practical applications, matrix size for reconstitution has to be chosen large enough to sustain all image details that could be possibly resolved. A matrix with linear size of $m = 2 k$ elements could be an optimal choice.

Until recently, the number of experimental projections remained a major factor limiting the resolution of EPR imaging experiments. The lack of experimental data led to development of reconstruction methods that rely on various regularization techniques such as L1 norm or total variation minimization [21-23; 30; 31]. Those methods can produce decent images even for the very limited data sets. However, they should be used with special caution, as regularization may cause deceptive images with filtering out important details or making sharp edges where they do not exist.

Recent advances in development of fast scan EPR imaging instrumentation made it feasible to scan thousands of projections in a matter of several minutes [13; 33; 34]. Thus, we finally are able to acquire sufficient projections for a fully determined system of equations that is necessary for obtaining unambiguous images. Large data sets require an efficient reconstruction algorithm that would allow one to reconstruct the images in a reasonable amount of time. Filtered backprojection (FBP) remains the one of the most commonly used image reconstruction algorithms. The method gives a result in a single stage and also permits parallelization [35]. Among its major drawbacks for EPR imaging is its reliance on deconvolved projections and also the requirement for the use of a ramp filter which may increase high frequency noise or smooth image details. Compared to the FBP method, the reconstruction approach described in this work is based on the algebraic reconstruction technique and it requires first to compute a projection (Eq. 6) and then to backproject the correction term (Eq. 13). Computation time for the Fourier convolutions in Eq. 7, 8 and 12 is not that significant. Thus, a single iteration of the method takes twice as much time as would be required by FBP. If the final image can be obtained in ~ 4 iterations, we may say that our method takes 8-fold more computer time than the conventional FBP. In this work, we acquired 16384 independent projections that is the most for any EPR imaging experiment published so far. With the algorithm developed in this work, the reconstruction time was 4.6 min per iteration for the image of 128^3 voxels and only a few iterations were required for the algorithm to converge with real data. This number would be suitable for the most applications of EPR imaging and is more than 10-fold faster than our previously published approaches [21, 22].

In summary, we have developed a novel algorithm that reconstructs 3D spatial EPR images using the raw projections and the zero gradient spectrum. In contrast to other reconstruction approaches, our method does not rely on spectral deconvolution and needs no data filtering, thus preserving information contained in the original projections. For EPR probes with multi-component EPR spectra, the zero gradient projection can be measured with a broader field sweep in order to account for the peaks that do not fit into the spectral window but may contribute to the experimental projections without the need for hyperfine splitting correction. The developed algorithm has no adjustable parameters, requires no tuning and can be used with any gradient distribution and this simplifies its application for any 3D spatial EPR imaging experiment.

Acknowledgments

This work was supported by NIH grants HL135648, HL131941, and EB016096. Authors are grateful to Dr. Kirilyuk (Novosibirsk Institute of Organic Chemistry, Novosibirsk, Russia) for providing the dMCP radical.

References

- [1]. Kuppusamy P; Chzhan M; Vij K; Shteynbuk M; Lefer DJ; Giannella E and Zweier JL (1994). Three-dimensional spectral-spatial EPR imaging of free radicals in the heart: a technique for imaging tissue metabolism and oxygenation. *Proceedings of the National Academy of Sciences* 91: 3388–3392.
- [2]. Elas M; Williams BB; Parasca A; Mailer C; Pelizzari CA; Lewis MA; River JN; Karczmar GS; Barth ED and Halpern HJ (2003). Quantitative tumor oxymetric images from 4D electron

paramagnetic resonance imaging (EPRI): Methodology and comparison with blood oxygen level-dependent (BOLD) MRI, *Magnetic Resonance in Medicine* 49: 682–691. [PubMed: 12652539]

- [3]. Matsumoto K.-i.; Subramanian S; Devasahayam N; Aravalluvan T; Murugesan R; Cook JA; Mitchell JB and Krishna MC (2006). Electron paramagnetic resonance imaging of tumor hypoxia: Enhanced spatial and temporal resolution for in vivo pO₂ determination, *Magnetic Resonance in Medicine* 55: 1157–1163. [PubMed: 16596636]
- [4]. Epel B; Sundramoorthy SV; Krzykawska-Serda M; Maggio MC; Tseytlin M; Eaton GR; Eaton SS; Rosen GM; Kao JPY and Halpern HJ (2017). Imaging thiol redox status in murine tumors in vivo with rapid-scan electron paramagnetic resonance, *Journal of Magnetic Resonance* 276: 31–36. [PubMed: 28092786]
- [5]. Komarov DA; Ichikawa Y; Yamamoto K; Stewart NJ; Matsumoto S; Yasui H; Kirilyuk IA; Khramtsov VV; Inanami O and Hirata H (2018). In Vivo Extracellular pH Mapping of Tumors Using Electron Paramagnetic Resonance., *Analytical chemistry* 90: 13938–13945. [PubMed: 30372035]
- [6]. Taguchi A; DeVience S; Driesschaert B; Khramtsov VV and Hirata H (2020). In vitro simultaneous mapping of the partial pressure of oxygen, pH and inorganic phosphate using electron paramagnetic resonance, *The Analyst* 145: 3236–3244. [PubMed: 32134072]
- [7]. Kuppusamy P; Wang P and Zweier JL (1995). Three-dimensional spatial EPR imaging of the rat heart, *Magnetic Resonance in Medicine* 34: 99–105. [PubMed: 7674904]
- [8]. Emoto MC; Sato-Akaba H; Hirata H and Fujii HG (2014). Dynamic changes in the distribution and time course of blood–brain barrier-permeative nitroxides in the mouse head with EPR imaging: visualization of blood flow in a mouse model of ischemia, *Free Radical Biology and Medicine* 74: 222–228. [PubMed: 25014567]
- [9]. Ewert U; Thiessenhusen K-U; Eaton G; Eaton S and Ohno K (1991). Deconvolution for the stationary-gradient method In: Eaton G; Eaton S and Ohno K (Ed.). *EPR imaging and in vivo EPR*, CRC Press Boca Raton: 119–26.
- [10]. Momo F; Colacicchi S and Sotgiu A (1993). Limits of deconvolution in enhancing the resolution in EPR imaging experiments, *Measurement Science and Technology* 4: 60–64.
- [11]. Sato-Akaba H; Fujii H and Hirata H (2008). Improvement of temporal resolution for three-dimensional continuous-wave electron paramagnetic resonance imaging, 79: 123701.
- [12]. Chen Z; Reyes LA; Johnson DH; Velayutham M; Yang C; Samouilov A and Zweier JL (2013). Fast gated EPR imaging of the beating heart: Spatiotemporally resolved 3D imaging of free-radical distribution during the cardiac cycle, 69: 594–601.
- [13]. Samouilov A; Ahmad R; Boslett J; Liu X; Petryakov S and Zweier JL (2019). Development of a fast-scan EPR imaging system for highly accelerated free radical imaging, *Magnetic Resonance in Medicine* 82: 842–853. [PubMed: 31020713]
- [14]. Sotgiu A; Gazzillo D and Momo F (1987). ESR imaging: spatial deconvolution in the presence of an asymmetric hyperfine structure, *Journal of Physics C: Solid State Physics* 20: 6297–6304.
- [15]. Kuppusamy P and Zweier JL (1996). A forward-subtraction procedure for removing hyperfine artifacts in electron paramagnetic resonance imaging, *Magnetic Resonance in Medicine* 35: 316–322. [PubMed: 8699942]
- [16]. Deng Y; He G; Kuppusamy P and Zweier JL (2003). Deconvolution algorithm based on automatic cutoff frequency selection for EPR imaging, *Magnetic Resonance in Medicine* 50: 444–448. [PubMed: 12876725]
- [17]. Hirata H; Itoh T; Hosokawa K; Deng Y and Susaki H (2005). Systematic approach to cutoff frequency selection in continuous-wave electron paramagnetic resonance imaging, *Journal of Magnetic Resonance* 175: 177–184. [PubMed: 15882954]
- [18]. Ahmad R; Clymer B; Vikram DS; Deng Y; Hirata H; Zweier JL and Kuppusamy P (2007). Enhanced resolution for EPR imaging by two-step deblurring, *Journal of Magnetic Resonance* 184: 246–257. [PubMed: 17113800]
- [19]. Ikebata Y; Sato-Akaba H; Aoyama T; Fujii H; Itoh K and Hirata H (2009). Resolution-recovery for EPR imaging of free radical molecules in mice, *Magnetic Resonance in Medicine* 62: 788–795. [PubMed: 19623620]

- [20]. Yokoyama T; Taguchi A; Kubota H; Stewart NJ; Matsumoto S; Kirilyuk IA and Hirata H (2019). Simultaneous T2* mapping of 14N- and 15N-labeled dicarboxy-PROXYLs using CW-EPR-based single-point imaging, 305: 122–130.
- [21]. Johnson DH; Ahmad R; He G; Samouilov A and Zweier JL (2014). Compressed sensing of spatial electron paramagnetic resonance imaging, *Magnetic Resonance in Medicine* 72: 893–901. [PubMed: 24123102]
- [22]. Ahmad R; Samouilov A and Zweier JL (2016). Accelerated dynamic EPR imaging using fast acquisition and compressive recovery, *Journal of Magnetic Resonance* 273: 105–112. [PubMed: 27821290]
- [23]. Durand S; Frapart Y-M and Kerebel M (2017). Electron paramagnetic resonance image reconstruction with total variation and curvelets regularization, *Inverse Problems* 33: 114002.
- [24]. Ewert U and Herrling T (1985). Numerical analysis in EPR zeugmatography with modulated gradient, *Journal of Magnetic Resonance* (1969) 61: 11–21.
- [25]. Gordon R (1974). A tutorial on art (algebraic reconstruction techniques), *IEEE Transactions on Nuclear Science* 21: 78–93.
- [26]. Komarov DA and Hirata H (2017). Fast backprojection-based reconstruction of spectral-spatial EPR images from projections with the constant sweep of a magnetic field, *Journal of Magnetic Resonance* 281: 44–50. [PubMed: 28549338]
- [27]. Frigo M and Johnson SG (2005). The Design and Implementation of FFTW3, *Proceedings of the IEEE* 93: 216–231.
- [28]. Chan RW; Ramsay EA; Cunningham CH and Plewes DB (2009). Temporal stability of adaptive 3D radial MRI using multidimensional golden means, *Magnetic Resonance in Medicine* 61: 354–363. [PubMed: 19165897]
- [29]. Ahn K-H and Halpern HJ (2007). Spatially uniform sampling in 4-D EPR spectral-spatial imaging, *Journal of Magnetic Resonance* 185: 152–158. [PubMed: 17197215]
- [30]. Qiao Z; Redler G; Epel B; Qian Y and Halpern H (2015). 3D pulse EPR imaging from sparse-view projections via constrained, total variation minimization, *Journal of Magnetic Resonance* 258: 49–57. [PubMed: 26225440]
- [31]. Qiao Z; Liang D; Tang S and Halpern H (2019). Optimization-Based Image Reconstruction from Fast-Scanned, Noisy Projections in EPR Imaging, *IEEE Access* 7: 19590–19601.
- [32]. Hoch MJ and Ewert U (1991). Resolution in EPR imaging, in: Eaton G; Eaton S and Ohno K (Ed.). *EPR imaging and in vivo EPR*, CRC Press Boca Raton: 153–9.
- [33]. Kubota H; Komarov DA; Yasui H; Matsumoto S; Inanami O; Kirilyuk IA; Khramtsov VV and Hirata H (2017). Feasibility of in vivo three-dimensional T2* mapping using dicarboxy-PROXYL and CW-EPR-based single-point imaging, *Magnetic Resonance Materials in Physics, Biology and Medicine* 30: 291–298.
- [34]. Tseytlin O; Guggilapu P; Bobko AA; AlAhmad H; Xu X; Epel B; O'Connell R; Hoblitzell EH; Eubank TD; Khramtsov VV; Driesschaert B; Kazkaz E and Tseytlin M (2019). Modular imaging system: Rapid scan EPR at 800 MHz, *Journal of Magnetic Resonance* 305: 94–103. [PubMed: 31238278]
- [35]. Qiao Z; Redler G; Epel B; Qian Y and Halpern H (2015). Implementation of GPU-accelerated back projection for EPR imaging, *Journal of X-Ray Science and Technology* 23: 423–433. [PubMed: 26410654]

Highlights:

- A novel reconstruction method for 3D spatial EPR imaging was developed
- The method converges towards least-squares solution by gradient descent algorithm
- It is suitable for noisy projections and probes with complex EPR spectra
- No spectral deconvolution or hyperfine splitting correction is required
- The method is easy-to-use and takes no manually adjusted parameters

Fig. 1. Numerical phantom used for validation of the reconstruction algorithm. (A) 3D surface-rendered image of the sample. Relative intensities of the threaded stud and the hex nut are 1 and 1.5, respectively. (B) Simulated EPR spectrum that was used to generate projections. (C) Examples of the projections. Totally 16,384 projections were generated for the spherically distributed gradients with magnitude of 8 G/cm.

Fig. 2.

Reconstructed image of the numerical phantom. Image matrix size is $128 \times 128 \times 128$ voxels, reconstruction was stopped after 12 iterations. (A) 3D-rendered surface computed for 25% of maximum signal intensity, (B) longitudinal and (C) transverse slice of the 3D image. (D) Blue line, coefficient of determination measured between the original projections and those from the reconstructed image. (D) Red line, image resolution estimated by fitting the edge of the visualized object with error function.

Fig. 3.

Images of the numerical phantom reconstructed from 16,384 simulated EPR projections with different SNR and gradient strength. Top row: gradient strength 8 G/cm, sweep width 24 G Bottom row: gradient strength 16 G/cm, sweep width 48 G. EPR projections were generated using the real experimental spectrum of dMCP radical (Fig. 4b), white Gaussian noise was added thereafter. Mean SNR of the projections is indicated in the top right corner of each particular image. Images were reconstructed on a matrix of $128 \times 128 \times 128$ voxels, reconstruction was stopped after 12 iterations.

Fig. 4.

(A) Chemical structure of the radical used to prepare the real phantom. (B) Zero gradient EPR spectrum of the radical solution and (C) examples of the experimental projections. Radical concentration was 1.5 mM. In total, 16,384 projections were acquired for the spherically distributed gradients with magnitude of 8 G/cm, magnetic field scan duration 4 ms, each projection was scanned 10 times, full data acquisition time 11 min.

Fig. 5.

Reconstructed image of the radical solution sample. (A) 3D surface-rendered image of the sample calculated with 25% intensity threshold. (B) Transverse and (C) longitudinal slices of the 3D image. Image matrix size is $128 \times 128 \times 128$ voxels, reconstruction was stopped after 4 iterations. (D) Blue line, coefficient of determination measured between the experimental projections and those from the reconstructed image. (D) Red line, image resolution estimated by fitting the edge of the visualized object with error function.

Fig. 6.

Image progression during reconstruction of the radical solution sample from Fig. 5. Number of iterations is shown in the top left corner of each particular image. Image matrix size is $128 \times 128 \times 128$ voxels, field-of-view $25 \times 25 \times 25$ mm³, total number of experimental projections 16,384, gradient strength 8 G/cm.