A three-dimensional electron spin resonance microscope

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An electron spin resonance (ESR) imaging system, capable of acquiring three-dimensional (3D) images with a resolution of \(~10 \times 10 \times 30 \ \mu m\) in a few minutes of acquisition, is presented. This ESR microscope employs a commercial continuous wave ESR spectrometer, working at 9.1 GHz, in conjunction with a miniature imaging probe (resonator+gradient coils), gradient current drivers, and control software. The system can acquire the image of a small \((\sim 1.5 \times 1.5 \times 0.25 \ mm)\) sample either by the modulated field gradient method, the projection reconstruction method, or by a combination of the two. A short discussion regarding the resolution of the modulated field gradient method in two-dimensional (2D) and 3D imaging is given. Detailed descriptions of the various system components are provided, along with several examples of 2D and 3D images that demonstrate the capabilities of the system. © 2004 American Institute of Physics.

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I. INTRODUCTION

Magnetic resonance is one of the most useful imaging methodologies in materials science, biology, and medicine.\(^1\) While “traditionally” most of the applications of this technique have been associated with nuclear magnetic resonance (NMR) imaging, some of the more recent investigations have been carried out by electron spin resonance (ESR) imaging. The main ESR imaging (ESRI) efforts have been directed towards the observation of large biological objects and the determination of their radical and oxygen concentrations (by their effect on the radical linewidth).\(^3\) Such experiments, conducted in vivo, employ low fields of \(\sim 10 \ mT\) at low rf frequencies (which results in relatively low spin sensitivity), in order that the rf energy will penetrate deeply into the biological object. Consequently, a typical voxel resolution in low frequency ESR experiments is \(\sim [2 \ mm]^3\). A different approach attempts to examine much smaller objects, with better spatial resolution. This type of ESRI, directed towards microscopy (analogous to a NMR microscope\(^6\)), can be employed at higher frequencies with improved sensitivity. Previous efforts in ESR microscopy (ESRM) are scarce, and have resulted in an achievable resolution of \(\sim 25–100 \ \mu m\) for two-dimensional (2D) and three-dimensional (3D) imaging.\(^9\) We recently achieved 2D images with a resolution of \(\sim [10 \ \mu m]^2\) by employing continuous wave (cw) ESR imaging utilizing a unique probe design.\(^13\)

At present, ESRM is still far less developed (mainly due to technological issues) than NMR microscopy, for which commercial instruments can provide 3D resolution of \(\sim 10–20 \ \mu m\) in small biological samples.\(^8\) Nevertheless, ESR has many virtues compared to NMR, which should make it the technique of choice with respect to magnetic resonance imaging in many microscopic applications. For example, the signal per spin in ESR is much greater than in NMR,\(^14\) diffusion does not limit the resolution in the short time scales \((T_1,T_2 \ s \leq 10 \ \mu s)\) of the ESR measurements, unlike NMR,\(^15\) ESR microresonators have a quality factor \((Q)\) of \(\sim 1000\) compared to a \(Q \sim 10\) of the NMR microcoils,\(^5\) and the ESR line shape is more sensitive to dynamic effects—leading to richer information.\(^20\) An additional factor is the lower cost of electromagnets used in ESR as compared to the expensive superconducting magnets of NMR. These fundamental advantages, along with recent advances in ESR resonators, ESR spectrometer hardware, and paramagnetic contrast solutions, warrant the development of a micron resolution ESR-based microscope to overcome the resolution limitations of NMR microscopy and to provide complementary information to optical imaging modalities.

Our recent publication\(^13\) described several potential applications for ESRM. It discussed in detail the theory and the limiting factors of current ESRM technology, and it presented some initial cw 2D imaging results performed with a high-permittivity miniature X-band (9.1 GHz) imaging probe. In the present work we discuss in detail the 3D cw microscope, which we have now developed. It is based on an improved microstrip-fed high permittivity resonator, a more efficient 3D imaging gradient coils set, and improved control software. The sophisticated hardware and software design of the microscope enables one to acquire the image either by the modulated field gradient (MFG) method\(^22\) or through the projection reconstruction (PR) imaging method.\(^4\) The imaging system, suitable for use as an accessory with many pre-existing X-band cw ESR spectrometers, provides magnetic resonance imaging capability with a voxel resolution down to \(\sim 10 \times 10 \times 30 \ \mu m\) in a few minutes of acquisition. To demonstrate the capability of the system, we show some imaging results for a solid sample of LiPc (Lithium Phthalocyanine radical) and for a solid form and a liquid

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suspension of the synthesized LiNc–BuO (lithium octa-
n-butoxy-substituted naphthalocyanine radical) micropar-

ticulates. 25

II. IMAGING METHOD

As noted above, the system can employ two different imaging methods to obtain the ESR image, within the framework of cw acquisition. Some of the results presented in this article were obtained with the PR method, which is probably the most common method used to acquire in vivo cw ESR images. This tomographic imaging technique has been de-

scribed previously, 13, 22, 27 with discussions of the im-

tageous for microscopic applications. The MFG method has

been described previously, 13, 22, 27 with discussions of the image acquisition technique, image signal-to-noise-ratio (SNR), and gradient coil requirements. Nevertheless, we include here, for clarity, a short outline of this method, and also present a discussion of image resolution in 2D and 3D imaging, as a function of the modulated field gradient ampi-

tude, since this subject was not treated in previous publica-

tions.

The idea behind the MFG method is to over-modulate the entire imaged sample, apart from a single voxel, from which the ESR signal is obtained. 22 The over-modulation of the sample is achieved by a set of gradient coils excited by a low frequency periodic current. These coils have a null field point that can be swept in space by changing the relative current amplitude in each coil pair that produces the gradient field. Let us analyze more quantitatively the imaging scenario and obtain the image resolution for various cases. The time domain cw ESR signal in the case of conventional field modulation is given by 14

\[ S(t,B_\delta) = S_0 \left( \frac{\Delta B_{1/2}^z}{2} \right)^2 \frac{1}{(B_\delta + B_m \sin \omega_m t)^2}, \]  

(1)

where \( \Delta B_{1/2}^z \) is the full width half maximum (FWHM) of the ESR line; \( B_\delta = (B-B_0) \), where \( B_0 \) is the center of the line; \( B_m \) is the modulation field amplitude (at a frequency of, e.g., 100 kHz). The addition of sinusoidal modulation fields, whose amplitudes depend both on time and the spatial location, results in the following spatial/time domain signal

\[
S(x,y,z,t,B_\delta) = S_0 \left( \frac{\Delta B_{1/2}^z}{2} \right)^2 \frac{1}{(B_\delta + B_m \sin \omega_m t)^2} \left[ B_\delta + B_m \sin \omega_m t + B_m^x(x) \sin \omega_x t + B_m^y(y) \sin (\omega_y t + \varphi_y) + B_m^z(z) \sin (\omega_z t + \varphi_z) \right]^2.
\]  

(2)

The modulated field gradients for the X, Y, and Z axes \(-B_m^x, B_m^y, B_m^z,\) and of course the main modulation field, \( B_m \), are all in the direction of the laboratory Z axis (determined by \( B_0 \)). The field \( B_m \) is assumed to be homogeneous over the entire sample volume (i.e., without any spatial dependence). The modulated field gradients are employed at much lower frequency (e.g., 10–1000 Hz), and have the following spatial dependence:

\[
B_m^x(x) = xG_x, \quad B_m^y(y) = yG_y, \quad B_m^z(z) = zG_z.
\]  

(3)

It is thus clear that at the origin, where \((x,y,z)=(0,0,0)\), Eq. (2) simplifies to Eq. (1). At other locations however, the ESR signal is greatly attenuated, due to the over-modulation induced by the modulated gradients, and this attenuation increases as the voxel is more distant from the origin. As mentioned above, the origin (null field point) can be moved by changing the ratio of the currents in the pairs of coils, which generate the modulated field gradients. The entire image is obtained by electronically scanning the imaged voxels within the sample volume.

We shall now address the issue of image resolution. Qualitatively speaking, it is clear that the image resolution should be finer as \( G_x, G_y, \) and \( G_z \) increase. In addition, the gradient modulation frequencies \( \omega_x, \omega_y, \omega_z \), the relative phases between the gradient modulation (\( \varphi_y, \varphi_z \)), and also the time constant of the cw ESR signal acquisition affects the image resolution. In order to provide quantitative analysis of these factors, we examine the ESR signal harmonics (with respect to the main modulation frequency). These harmonics are detected by the cw ESR spectrometer and are given (for the \( p \)th harmonic) by 14

\[
a_p = \int_{t_0}^T S(x,y,z,t,B_\delta) \sin(p\omega_m t) dt.
\]  

(4)

If \( B_m^x=B_m^y=B_m^z=0 \), then each harmonic signal has a specific field, \( B_\delta=\ell B_m^x \), for which it is maximal (for example, for the second harmonic signal, \( B_m^y=0 \)). As one increases \( B_m^x, B_m^y, \) and/or \( B_m^z \), the amplitude of \( a_p \) at \( B_m^y \) will decrease and quickly reach zero. 23 To calculate the image resolution, for a specific signal harmonic, we first find the field \( B_m^y \) and then increase \( B_m^x, B_m^y, \) and/or \( B_m^z \) (depending on the dimensionality), in our numerical calculations of \( a_p \), until \( a_p \) at \( B_m^y \) becomes zero. The values of \( B_m^x, B_m^y, \) and/or \( B_m^z \) for which \( a_p=0 \), divided by the applied gradient [Eq. (3)], provide us with the image resolution. This resolution criterion is analogous to the Rayleigh criterion for resolution in optics. 28
The calculations of the resolution were performed numerically for several representative cases, and the results are shown in Fig. 1. We assumed for purposes of this analysis that \( \omega_x = \omega_y = \omega_z \) (although the system can support different frequencies for the \( X, Y, \) and \( Z \) gradient coils, see below). Also \( G_x = G_y = G_z = G \), and the integration time, \( T \), in Eq. (4) was taken as the period time of \( \omega_z \). The relative phases, \( \varphi_x \), and \( \varphi_y \), are determined according to the dimensionality of the problem: When increasing the dimensionality of the image (e.g., from 1D to 2D), every point in space experiences the added fields of more than one coil pair. This can lead to “interference effects” as shown for the 2D example in Figs. 2(a) and 2(b), which show how strong image artifacts are created if \( \varphi_y = 0 \), since the resolution depends on the direction taken from the null point. To avoid such artifacts, one should apply a phase difference between the modulated fields of the different axes. In the 2D calculation of Fig. 1, we employed an optimal \( \varphi_y = 90^\circ \), and in the 3D example we applied \( \varphi_y = 120^\circ, \varphi_z = 240^\circ \). This approach tends to minimize the spatial dependence of the resolution (i.e., image artifacts) by averaging out the positive and negative interference effects [cf. Figs. 2(c) and 2(d)]. Similar artifact cancellation can be achieved by employing different modulated gradient frequencies for each axis. Both methods (i.e., phase and/or frequency variation among the imaging axes) can be employed in our imaging system (see below).

III. CONTINUOUS WAVE ESR MICROSCOPE

The cw ESR microscope has the following components (Fig. 3): (a) Conventional cw ESR spectrometer; (b) dedicated computer which controls the imaging process and acquires the ESR signal; (c) current drivers for the gradient coils; (d) base band (up to \( \sim 250 \) kHz) amplifier and filter unit (signal conditioner); (e) control unit; (f) imaging probe that includes the microwave resonator, mechanical fixture for holding the sample, and gradient coils. We shall first describe an overview of the imaging procedure and the manner by which the microscope is operated. This provides the overall perspective before describing, in detail, the functionality and characteristics of each of the main components of the microscope system.

To image a sample, one must first attach the imaging probe (Fig. 4) to the cw ESR spectrometer (by replacing the conventional resonator). The imaging probe is connected to the spectrometer through the microwave (MW) input/output of the spectrometer and the modulation coils line (similar to any cw-ESR resonator). In addition, the probe is connected to the gradient current drivers and to air and water cooling lines. The water and air cooling lines are essential to maintain a stable resonance frequency of the high permittivity resonator [Fig. 4(b)]. The investigated sample should be sealed (under normal or deoxygenated atmosphere) between two flat glass cover slips and inserted, with the help of the sample holder, to an exact position in the center of the resonator. Following this, the ESR spectrometer is set to acquire the signal of the sample at the required conditions of MW power, dc magnetic field, and main modulation. After fixing the spectrometer on the maximum of the ESR signal, the computer controlled imaging procedure is initiated, and the gradient coils are activated to obtain the image (see below). The ESR microscope incorporates a field frequency lock (FFL) system that adjusts the dc magnetic field by biasing the modulation coils of the imaging probe, and maintains the on-resonance condition throughout the period of the imaging experiment. Thus, the basic commercial cw ESR spectrometer is completely “unaware” of the imaging procedure, with the interfaces between the spectrometer and the imaging probe/system being kept to a minimum. The image is displayed on the computer in real time during the acquisition process and then can be saved and/or manipulated as necessary.

Following this introductory description, we now relate more details of the individual components of the 3D cw ESR imaging system:

(a) Continuous wave ESR spectrometer. As mentioned above, most commercial cw ESR spectrometers (e.g., from Bruker, Varian, or JEOL) can be used as a basis for the imaging module. The spectrometer serves as a good stable, amplitude controlled, MW source that is independently frequency locked on the resonance frequency of the imaging probe, by means of the spectrometer automatic frequency control (AFC). The spectrometer also provides a current drive for the regular modulation coils of the imaging probe and should allow for the operator to fix the external dc magnetic field close to the resonance field of the imaged sample.
The MW ESR signal returning from the imaging probe is detected and preamplified at the spectrometer MW bridge. In our spectrometer (Varian E-12), we inserted prior to the diode detection of the ESR signal from the resonator a low noise X-band preamplifier (Miteq AFS3-08001200-14-ULN). This amplifier improves the SNR of the spectrometer by a factor of 5, and its amplification gain enables the AFC of the Varian bridge to lock on the returning signal even for low MW powers, 1 mW, that is common in our imaging experiments (see below). The diode detected base band signal is directly fed from the bridge preamplifier (similar to the case of time resolved ESR measurements) to a signal conditioning unit and then goes to the PC for sampling and further analysis (see below).

(b) Control computer and imaging software: The entire imaging process is controlled by a standard PC equipped with two analog input+ digital input/output (I/O) (National Instruments 6023E) and analog output (National Instruments 6713) cards. These cards enable arbitrary wave form generation and fast sampling of signals up to several hundred kHz. The digital analysis of the sampled diode detected ESR signal supersedes the need to employ a conventional lock-in amplifier while simultaneously obtaining all the ESR signal harmonics, in the correct phase, with respect to the main modulation current. The current software version of the system is capable of acquiring 2D images at any given z location (3D slice selection, see below). It should be noted that the hardware (probe+ current drivers) also supports 3D imaging with projection reconstruction and 4D spectral-spatial projection reconstruction that can be pursued in the future by updating the imaging control software. The control software (based on LABVIEW) obtains the imaging parameters from the user. These parameters include, for example, the number of pixels in the image (x and y), the current amplitude in the gradient coils, the wave form and frequency used in the modulated gradient coils (e.g., sine, serrasoid, etc.), the image extent in mm, and parameters related to the functionality of the FFL system. The software can also acquire the normal ESR signal (first and second harmonics), by sweeping the magnetic dc field.

FIG. 2. (a) The effect of two modulated field gradients applied for the x and y axes simultaneously. The fields due to the x coil pair are marked with ± without circles and the fields due to the y coil pair are marked with ± with surrounding circles. It can be seen from the figure that if \( \phi_y = 0 \), then the modulated fields will be stronger in the first and third quadrants, while tending to cancel each other in the second and fourth quadrants. (b) Image of a point target with infinitely small size situated at the origin of axes (point spread function) for 2D MFG, assuming \( \phi_y = 0 \). Parameters used in this calculation are: linewidth of 0.01 mT, \( B = 0.01 \) mT, \( G_x = G_y = 1 \) T/m. It is obvious that such a point spread function will result in bad artifacts in the image. (c) The same as (b), but with \( \phi_y = 90^\circ \). This phase difference between the x and y gradients leads to the minimization of the artifacts in the point spread function. (d) A 2D cut through the 3D point spread function, which involved the simultaneous application of x, y, and z gradients. The phase difference of 120° between each axis minimizes the image artifacts but still causes an appreciable anisotropy in the point spread function, which corresponds to some unavoidable anisotropic artifacts in the 3D MFG method.

FIG. 3. Block diagram of the 3D cw ESR microscope.
field with the probe’s gradient coils. At the end of the imaging process the data can be saved and/or further processed with the aid of MATLAB software. Such postprocessing includes, for example, in the case of image acquisition through the PR method, de-convolution of the projections, and inverse Radon transformation to obtain the image.

(c) Current drivers for the gradient coils: The gradient coils are driven by six programmable current sources (one for each coil) each capable of supplying up to 3 A of arbitrary wave form current, in the dc-10 kHz range. Each current driver is based on a simple feedback loop, and its electronic schematic is shown in Fig. 5. In practice, the coils in the present system do not require more than 1 A to generate high enough gradient fields. The low current consumption of the system greatly simplifies the design and space requirements for the driver unit.

(d) Base band amplifier and filter unit (signal conditioner): In most ESR spectrometers, the ESR signal, detected by the MW diode, goes through a base band preamplifier that is part of the commercial bridge. The signal level after this preamp is not large enough to be sampled directly by the analog/digital (A/D) card in the computer. To facilitate proper A/D sampling we employ a signal conditioning unit comprised of bandpass filters and a high gain amplifier. The dual bandpass filter transfers only signal at the regular modulation frequency generated by the cw spectrometer, and its second harmonic. For example, in our present system we have a filter that transfers 25 and 50 kHz and the spectrometer is operated at 25 kHz modulation frequency. The high gain amplifier (Tektronix AM502) that follows the dual bandpass filter produces variable gain (manually controlled) in the range of 40–100 dB.

(e) Control unit: The current wave form in the gradient coils can be generated either directly at the computer analog output card or in the control unit. This unit receives commands from the computer via the 8 bit digital I/O bus of the 6023E card and consequently synthesizes the required wave forms. The control unit is limited in the complexity of the wave form it can generate (as compared to the arbitrary wave form generator of the analog output card), but for most imaging tasks it has sufficient capabilities. Its important virtue
is that it reduces the overhead time in the imaging procedure related to the calculation and generation of the different wave forms in the computer and thus shortens the actual acquisition time by a factor of \( \sim 2 \).

(f) Imaging probe: The imaging probe is the heart of the cw imaging system. A schematic drawing of the imaging probe is given in Fig. 4, and a photo of the actual probe is in Fig. 6. The probe is based on a double stacked ring resonator, 13,19,32 machined from SrTiO\(_3\) single crystal (Commercial Crystals, FL). This crystal has permittivity of \( \sim 300 \) at room temperature and \( \tan \delta \sim 5 \times 10^{-4} \). Each one of the resonator rings is 0.55 mm in height with outer diameter of 2.31 mm and inner diameter of 0.81 mm. When the two rings were placed at a separation of \( \sim 0.4 \) mm, the fundamental resonance frequency of this structure was found to be \( \sim 9.15 \) GHz (in the center of the working frequency of the Varian E-12 ESR spectrometer). The resonator is excited by a microstrip transmission line on a low-loss substrate (Göge Electromaterials GTEK ML200D Epoxy/Polyphenylene Oxide resin). The excitation geometry and the calculated fields of this resonator at the resonance frequency (CST Microwave Studio) are shown in Fig. 7. The “effective volume” of the resonator, 13 as derived from the calculated magnetic fields is \( \sim 3 \) mm\(^3\) for ring separation of 0.4 mm. The two high permittivity rings are held by a Delrin part at a fixed distance [Fig. 4(b)]. This Delrin spacer also holds the flat sample and enables exact positioning of the imaged object in the center of the resonator. Variable coupling is achieved by changing the distance between the resonator rings and the microstrip line using a linear 1D stage (nonmagnetic model MDE 255 from Elliot Scientific, GB). In addition, the vertical position of the rings with respect to the microstrip line can be varied slightly by moving the Delrin part up and down and/or adjusting the vertical microstrip line position. This variability enables optimal control of the rings’ coupling for a wide variety of samples.

The resonator rings are surrounded by a thin (0.12 mm thick) hollowed brass cylinder that shields the resonator at the microwave frequencies, but is transparent to the low-frequency magnetic field gradients (Fig. 8). The regular modulation of the cw spectrometers (commonly at 25–100 kHz) penetrates through the shield opening along the cylinder axis. The loaded \( Q \) of the resonator was found to degrade slightly, from \( \sim 1200 \) to \( \sim 900 \), when inserting it into the brass shield. The gradient \( X \), \( Y \), and \( Z \) coils are arranged around the cylindrical shield, along with the regular modulation coils (Fig. 8). The gradient coils are made from bondable 38 AWG copper magnet wire, and the modulation coils are made from bondable 32 AWG wire. The innermost coils are the \( Y \) and \( Z \) gradient coils, respectively, which are based on a “Golay” design. 33 The \( X \) gradient coils (standard Maxwell pair33), are positioned in the outermost layer of the gradient coils, followed by the main modulation Helmholtz pair (not shown in Fig. 8). Gradient efficiency is 4, 5, and 2 T/(m \( \times \) A), and coil resistance is 9, 8.5, and 4.4 \( \Omega \) for the \( X \), \( Y \), and \( Z \), coils, respectively. These figures significantly improve upon our

![FIG. 6. Photo of the cw imaging probe. The resonator rings and the microstrip excitation line are not visible, since the rings are inside the gradient coils shield and the excitation line is facing down. The X gradient coils are right below the regular modulations coils and the Y gradient coils are below the Z gradient coils.](image1)

![FIG. 7. (Color online) The calculated microwave magnetic (\( H_1 \)) and electric (\( E_1 \)) fields of the resonating mode of the high-permittivity resonator, for the case of microstrip excitation.](image2)

![FIG. 8. The geometry of the gradient coils in the three axes. Dimensions are in mm. Main modulation coils (not shown) are a Helmholtz pair separated by 5 mm with a radius of 6 mm.](image3)
The spin sensitivity of the imaging probe was measured with a 1 mM deoxygenated water solution of protonated trityl radical. A SNR of $1.6 \times 10^4$ was obtained for a 0.06 μL sample, with 1 s time constant and $\sim 0.2$ G field modulation. This SNR is in good agreement with the results of our recent publication (cf. Fig. 1 therein), which predict a SNR of $1.8 \times 10^4$ for such a trityl sample. The measured SNR corresponds to a spin sensitivity of $\sim 2.2 \times 10^8$ spins/√Hz, and a concentration sensitivity of $\sim 6.2 \times 10^{-8}$ M/√Hz, for the flat sample considered here. The combination of high spin and concentration sensitivities, for samples of small volume, is essential to facilitate high resolution imaging capability.

**Imaging procedure:** At the beginning of this section we outlined the imaging process from the perspective of the operator of the system. The actual processes that occur “behind the scenes” are more involved and warrant some discussion. Two representative examples are described schematically for the MFG and the PR imaging methods in the form of time lines (Figs. 9 and 10). These examples, which are detailed in the figure captions, represent the flexibility and capability of the present system. As noted above, the hardware and soft-
ware support any arbitrary excitation of the gradient coils (within the bandwidth of up to ~10 kHz). Thus, further progress in the direction of, for example, spectral-spatial imaging or 3D projection reconstruction may be pursued in the future with just software updates.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

We now describe and discuss some representative experimental results of images acquired with the microscope system. These experiments, performed with solid and liquid samples, enable us to quantify the resolution, SNR, and image quality obtained in 2D and 3D measurements with the MFG and the PR methods. Imaging of the same samples with the two methods provides a good basis for comparison and discussion about their different advantages and disadvantages. Whenever applicable, the experimental results are compared to prior estimations of image SNR and resolution obtained by cw ESR imaging.13,26

(a) LiPc phantom: As a first example we measured a high spin concentration sample of solid LiPc crystal (the same crystal that was measured in our recent publication). Figure 11 provides the measured 2D and 3D images of this crystal. The 2D images (Figs. 11(a) and 11(b)), were acquired with both the PR and the MFG methods. The image SNR (max signal from a voxel divided by the rms of the noise in areas of the image where no radicals are present) was found to be ~110 and ~240 for the MFG and PR methods, respectively. These figures can be compared with our theoretical estimates of image SNR, and the spin sensitivity of the probe described above. The radical concentration is ~1020 spins in 1 cm3 (provided that the material density is ~1), which implies that there are ~8×1011 spins in an image voxel of 10×10×80 μm (see below). We know that Q~1000, and the resonator active volume is ~3 mm3 (see above) and we also assume T1 and T2 values similar to that of 1 mM trityl radical in water solution.13 All these parameters result in an estimated SNR of ~150 for the MFG method [employing Eq. (5) in our recent publication,13 and considering the second harmonic signal]. The PR method devotes longer acquisition time to each pixel (128 projections with sampling time of 20 ms as compared to 0.5 s per pixel in the MFG).26 It also uses the stronger first harmonic signal (~two times larger than the second harmonic signal14), to simplify the de-convolution process.26 These two factors should provide a SNR of the PR method, which is ~4.4[=2×(128×0.02/0.5)1/2] times larger than that of the MFG method. In practice we obtained a PR SNR that is 2.2 times larger, probably due to image artifacts (see below) that contribute to the PR image apparent noise. Accurate absolute predictions of the image SNR (and the ESR signal in general) are somewhat problematic and the present results provide relatively good agreement with the theoretical predictions, both for the absolute SNR values, and the relative SNR between MFG and PR methods. These SNR results are also compatible with the measured probe spin sensitivity described in the previous section.

In terms of image resolution, analyzing the ESR images by taking a 1D cut at certain locations reveals that the resolution is ~10×10 μm for the 2D images acquired with both methods, and ~30 μm for the z slice separation in the 3D images (the latter number is less reliable due to the difficulty of accurately measuring the crystal z dimension, which is estimated to be ~80 μm).38 The theoretical resolution of the MFG method for 2D imaging was discussed above (cf. Fig. 1). The image in Fig. 11(a) involves gradients of 1 T/m, radical linewidth of 0.01 mT, and main modulation field of 0.015 mT, which results in the calculated 2D resolution of 9.5 μm (for the second harmonic signal). The PR method, which observes the first derivative signal, requires gradients that are about two times stronger to obtain a similar resolution.26,39 In the present case, we obtained for the PR image a similar resolution (10 μm) to that of the MFG method with gradients of just 1.5 T/m, thanks to the use of de-convolution methods26 to further sharpen the im-
One additional important issue that should be discussed is related to image artifacts. In the MFG method image artifacts are mainly due to the negative wings of the point spread function [Figs. 2(c) and 2(d)] associated with this method and result in a negative signal surrounding the edges of the object. For the PR method, image artifacts tend to create “lobes” coming out of the object,\(^2\) which contribute more significantly to the overall effective noise of the image.

Notice that the 2D ESR images do not correspond exactly to the optical image. This may be caused by (1) an inhomogeneous distribution of spins within the phantom (corresponding to the weak signal on the right side). In addition, (2) application of cyanoacrylate glue, during sample preparation, destroyed some of the radicals in the lower part of the phantom. The very similar results using both ESR imaging methods reinforces our belief that the ESR images are correct, and it shows that the optical image and ESR image do not have to be the same (as one would expect), since they measure different properties.

(b) LiNc–BuO radical.\(^2\) This material, termed lithium octa-n-butoxy-substituted naphthalocyanine radical (LiNc–BuO), can be applied to biological samples in vivo/in vitro as a high concentration microparticulate liquid suspension.\(^2\) The linewidth of this radical increases significantly with in-
creasing oxygen concentration. Figure 12 presents 2D and 3D ESR images, acquired with the MFG and PR methods, of a test sample, which contains these radicals in a solid form. The sample was prepared in the following manner: A UV laser ($\lambda=193$ nm, Lumonics PM-848k excimer laser) was used to drill three small pits in a thin (150 $\mu$m) glass cover slip. The position of the center of the pits can be controlled to a high degree of accuracy ($\approx 0.2$ $\mu$m) with an XY table. However, the geometry of the pits depends on the shape of the laser beam, which tends to be oval rather than perfectly round, and also changes along the pit depth. A high resolution optical microscope (Olympus) was used to measure the typical pit dimensions ($\approx 25–40$ $\mu$m, depending on the position along the depth), and to estimate the depth of the pits [as marked on Fig. 12(f)]. The glass with the three pits was placed in a 10 mM LiNc–BuO suspension and then was taken out and its surface was cleaned with a sharp knife.

The 2D images of this sample [Figs. 12(a) and 12(b)], acquired with the PR and MFG methods, show good agreement with one another and also correspond well to the optical image [Fig. 12(f)]. The apparent increase in image...
“noise” [as compared to the 3D images in Figs. 12(c)–12(e)] is probably due to the signal from residual radicals left on both sides of the glass (that are largely eliminated in the 3D image). One important issue that is evident from these 2D images is that the PR image has fewer pixels than the MFG image. This is due to the fact that in the PR method one has to acquire information from the entire sample and cannot collect information from only a small part of the sample (as the MFG can). In the present case, there were residual signals from the glass surface and the edges of the glass. These signals had to be collected with the PR method, which resulted in a rather large image of \( \sim 2 \times 2 \text{ mm} \), with only 90 \( \times 90 \) pixels (after inverse Radon transform of the 128 projections, each with 128 samples\(^{26}\)). This is a disadvantage for PR in microscopy applications. The 3D ESR image of the upper side of the glass [Fig. 12(c)] follows the optical image closely, but with an additional small signal that is probably due to some residual radicals that were not removed from the glass surface. Figures 12(d) and 12(e) show the gradual disappearance of the signal from two out of the three pits, due to their different depths [Fig. 12(f)], as the imaging slices observe different parts of the glass. Again, some residual signal may arise from radicals left on the upper and lower surfaces of the glass.

It is difficult to supply meaningful quantitative analysis of image SNR in this case due to the lack of a good assessment of spin density in the pits and the problem of residual radicals on the glass surface that create effective “noise.” However, it is clear that this radical provides the possibility of looking at very fine details on the order of \( \sim 30 \mu \text{ m} \) in a short acquisition time of several minutes. In terms of image resolution, the theoretical resolution for this radical, that (for the present sample) has a linewidth of \( \sim 0.03 \text{ mT} \), is \( \sim 36 \mu \text{ m} \). This rather low resolution is apparent in the broadening of the signal from the small pits, but still enables good separation between the pits that are separated \( \sim 70 \mu \text{ m} \) apart (from edge to edge).

Another imaging example, which is more relevant to
biological applications, involves a liquid suspension of the same LiNc–BuO radical. Figure 13 shows the 2D ESR image obtained with the PR method for such a suspension (10 mM concentration) embedded within a fine nylon mesh (obtained from Goodfellow: mesh aperture 50 μm, and wire size 39 μm). The PR is the method of choice when one requires fast imaging of the entire sample in order to obtain, for example, morphological information. Following the acquisition of the PR image one can optionally observe in more detail some specific voxels within the sample (employing the MFG). The ESR image clearly shows: (1) the separation of the compartments in the mesh, and (2) that the signal is obtained only from the active area of the resonator (cf. Fig. 7). An interesting point that should be noted is that such radical suspensions are usually employed in in vivo imaging studies with ~1 mm resolution, and in this scale the suspension is rather uniform. However, at the 10 μm scale, the suspension is not uniform and some of the areas contain larger grains than other areas (as can also be seen in the optical image). This nonuniformity is manifested in the ESR microimage.

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38 Determining the resolution in magnetic resonance microscopy is far from trivial. Ideally one would like to look at a test sample consisting of two infinitesimally small points and to determine the minimal distance between the points that can still be resolved (i.e., the resolution). In practice, in contrast to optical methods, the signal of point-like sample will be too low to be detectable, and the actual fabrication of such a sample is a demanding task in itself. Instead, we follow here the conventional approach of NMR microscopy (Ref. 37), which observes objects of well defined finite size, where one may estimate the resolution in the image by measuring how sharp is the falloff of the signal at the sample edges, which are determined by the high resolution optical image of the same object.