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Rapid-Scan EPR Imaging

Sandra S. Eaton¹, Yilin Shi¹, Lukas Woodcock¹, Laura A. Buchanan¹, Joseph McPeak¹, Richard W. Quine², George A. Rinard², Boris Epel³, Howard J. Halpern³, and Gareth R. Eaton^{1,*}

¹Department of Chemistry and Biochemistry and Center for EPR Imaging In Vivo Physiology, University of Denver, Denver, CO 80210

²School of Engineering and Computer Science and Center for EPR Imaging In Vivo Physiology, University of Denver, Denver, CO 80210

³Department of Radiation and Cellular Oncology and Center for EPR Imaging In Vivo Physiology, University of Chicago, Chicago, IL 60637

Abstract

In rapid-scan EPR the magnetic field or frequency is repeatedly scanned through the spectrum at rates that are much faster than in conventional continuous wave EPR. The signal is directly-detected with a mixer at the source frequency. Rapid-scan EPR is particularly advantageous when the scan rate through resonance is fast relative to electron spin relaxation rates. In such scans, there may be oscillations on the trailing edge of the spectrum. These oscillations can be removed by mathematical deconvolution to recover the slow-scan absorption spectrum. In cases of inhomogeneous broadening, the oscillations may interfere destructively to the extent that they are not visible. The deconvolution can be used even when it is not required, so spectra can be obtained in which some portions of the spectrum are in the rapid-scan regime and some are not. The technology developed for rapid-scan EPR can be applied generally so long as spectra are obtained in the linear response region. The detection of the full spectrum in each scan, the ability to use higher microwave power without saturation, and the noise filtering inherent in coherent averaging results in substantial improvement in signal-to-noise relative to conventional continuous wave spectroscopy, which is particularly advantageous for low-frequency EPR imaging. This overview describes the principles of rapid-scan EPR and the hardware used to generate the spectra. Examples are provided of its application to imaging of nitroxide radicals, diradicals, and spin-trapped radicals at a Larmor frequency of ca. 250 MHz.

Graphical abstract

Corresponding author: Gareth R. Eaton, Department of Chemistry and Biochemistry, University of Denver, Denver, CO 80210, Phone: 303-871-2980, geaton@du.edu.

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1. Introduction

In conventional continuous wave (CW) EPR spectroscopy the main magnetic field is scanned slowly through resonance. Superimposed on that slowly-varying field is magnetic-field modulation, often at a frequency of 100 kHz. Phase sensitive detection at the modulation frequency records the signal that is in phase with the field modulation (Fig. 1A). Provided that the amplitude of the field modulation is small relative to the linewidth of the spectrum, the detected signal is proportional to the slope of the absorption spectrum [1]. The first-derivative spectrum is recorded point-by-point by stepping the main field and recording the slope at each magnetic field point. Phase sensitive detection at the modulation frequency, which has been used since the early days of EPR, is very effective in suppressing noise. However, there are disadvantages. (i) If the amplitude of the field modulation is larger than about $\frac{1}{4}$ of the line width, the detected signal is not proportional to the slope at that field point and the spectrum is broadened. (ii) The amplitude of the signal that is detected is determined by the magnitude of the field modulation and is substantially less than the full signal amplitude.

What happens if you modulate the magnetic field with amplitudes that are large relative to the EPR line width, and use direct detection of the EPR signal instead of using a lock-in amplifier tuned to the modulation frequency? This is the experimental basis for the new type of EPR that we call rapid-scan EPR (Fig. 1B). In this technique the scan frequencies are of the order of kHz and the full signal amplitude is recorded both as the field is swept up-field and down-field through resonance [2]. In such scans, there may be oscillations on the trailing edge of the spectrum, as was first observed in NMR by Bloembergen et al. [3] and in EPR by Beeler [4]. Examples of the rapid-scan oscillations for a narrow lithium phthalocyanine signal as a function of scan rate are shown in Fig. 2 [5]. For the linear (triangular) scans the scan rate is $a_t = 2 f_s B_m$ where f_s is the scan frequency and B_m is the scan width. As the scan rate increases, the number of oscillations and the depth of the oscillations increase (Fig. 2A). The 'normal' slow-scan spectrum can be recovered by Fourier deconvolution of the rapid-scan signal (Fig. 2B). The deconvoluted rapid-scan signals are in good agreement with the slow-scan spectrum. The deconvolution procedure to remove rapid-scan oscillations was first demonstrated in NMR by Dadok [6] and Gupta [7, 8] and was the basis for what was known as Correlation NMR Spectroscopy.

The FID-like oscillations on the trailing edge of the signal are related conceptually to the FID in a pulsed experiment [9]. In a rapid-scan experiment the spins are on resonance for very short periods as the field (or frequency) is swept, similar to pulsed EPR where the microwave power is turned on and off while the field and frequency are kept constant. The oscillations in the rapid-scan signal do not have a constant oscillation frequency, because the field is changing, but otherwise there is considerable similarity between pulse and rapid scan. The fact that the spins are on resonance for a short time means that the turning angle is smaller for a given incident power than for CW. If B_1 is held constant as the scan rate is increased, signal amplitude decreases (Fig. 3). However, if B_1 is optimized to maximize signal amplitude, higher signal amplitude can be achieved at higher scan rates. Hence higher incident powers can be used and larger signals can be obtained before saturating the rapid-scan signals [10–12].

The rapid-scan oscillations are examples of ‘passage effects’. Passage phenomena in EPR were reported by Portis [13] and by Hyde [14], and a wide range of passage phenomena were discussed by Weger [15]. Weger [15] and others have given special meaning to rapid, fast, etc., and our initial papers defined rapid-scan relative to T_2 [5, 11]. Hyde and co-workers proposed the designation non-adiabatic rapid scan (NARS) for similar experiments in which the scans were wider than spectral features and in which direct detection was used, but scan rates were slow enough that rapid-scan oscillations were not observed [16–20]. However, as the technique becomes more generally applied we propose to use rapid-scan to describe the detection method rather than a particular scan rate regime. For many spin systems, there will be a relaxation-rate dependent response that is achievable by selection of scan rate and microwave power. Although the focus of this article is on rapid field scans, the same principles apply to rapid frequency scans [21, 22].

The transient responses (‘wiggles’) may be obscured by overlapping signals in spectra that are inhomogeneously broadened due to unresolved nuclear hyperfine splitting or by a magnetic field gradient as shown in Fig. 4. For two specks of LiPc the rapid-scan oscillations are observed at low gradient, but not at higher gradients (Fig. 4). It is important that the post-processing described for rapid scans [5, 23] can be applied regardless of the rate of passage through resonance and does not have deleterious effects on spectra in which the transient responses are not observed. Thus, superimposed spectra with mixtures of slow and fast relaxation or broad and sharp lines can be studied.

2. Instrumentation for rapid scans

A block diagram for a rapid-scan imaging system is shown in Fig. 5. The signal detection path for rapid scan is more like that in a pulse or transient bridge such as the Bruker E500T [10, 12] than in a CW bridge. After low-noise amplification, the microwave signal is detected by a quadrature mixer and digitized. The bandwidth of the signal path must be large because of the need to preserve high frequency components of the signal, including the rapid-scan oscillations. Quadrature detection is advantageous for the deconvolution procedures, permits phasing in postprocessing, and can improve signal-to-noise by combining the signals from the two channels [23–25].

Rapid-scan EPR requires uniform magnetic field for the scanning field, just as for the main (Zeeman) field, which has implications both for the scan coils and the coil driver. To obtain unbroadened spectral lines the scan coils have to be large enough to provide a magnetic field that is uniform over the dimensions of the sample to be studied. Rapid scan can be implemented on a commercial Bruker pulsed or time-domain EPR spectrometer by acquiring the EPR signal without magnetic field modulation and phase sensitive detection at the modulation frequency. The magnetic field modulation driver can be the rapid field scan driver, and the digitizer can be triggered at the start of each scan. Since the modulation systems are designed for up to about 40 G sinusoidal scans at 100 kHz, the maximum scan rate can be as high as $1.2 \times 10^7 \text{ G s}^{-1}$. However, because of the small size of the modulation coils the modulation field has a very small region of uniformity, so the magnetic field scan rate will be uniform over only very small samples. Nevertheless, this is an approach that can permit implementation of rapid scan in standard metal cryostats, and/or in many labs that have pulsed or time-domain EPR spectrometers. We have also used ENDOR coils, rotated 90° , to scan the field at frequencies well above 100 kHz.

To drive a current rapidly through coils that are large enough for rapid scan with extended samples requires a driver that has an appropriate amplifier and circuitry to create the desired time-dependence of the scanned fields. The simplest deconvolution procedure is for responses to linear (triangular) field scans [5], but linear scans are the most demanding of the driver [26]. The driver needs a feedback system to create a linearly changing current, not voltage, across the coils. To be sufficiently linear over about 90% of the scan (there is rounding at the turning points), the driver has to support at least the 7th harmonic of the scan frequency. Faster, wider, scans can be achieved by resonating the coils. The sinusoidal scans are less demanding of the scan driver, but the scan rate is different for each point in the scan, which requires different mathematical analysis of the rapid-scan response [23] than for linear scans. For example, we use ca. 75 mm diameter coils constructed with Litz wire to create uniform fields that can be driven at high scan rates by the sinusoidal drivers of the type described in [27].

In CW EPR, the inherent background signals are mitigated by lock-in detection at the modulation frequency (long ago, it was called base-line stabilization). The serious challenge for rapid-scan EPR is that some of the background problems look like EPR signals – that is, they are magnetic-field-dependent. Resonators must be designed and constructed to minimize background signals. Mechanical vibrations caused by eddy currents are decreased by minimizing the amount of metal exposed to the scanning magnetic field. Cross-loop resonators constructed with thin wire [28], and dielectric resonators [28] have been found to be effective. Even with improvements in hardware, the background signal is significant, especially for weak signals. Methods have been developed to remove the background signal [25, 29].

The maximum rapid magnetic field scan width that has been achieved in our lab for extended-length samples in 4 mm OD EPR tubes is 170 to 180 G peak-to-peak, for sinusoidal scans with scan frequencies of 6 to 15 kHz and ca. 8.8 cm diameter Litz wire coils [30, 31]. This is adequate for almost all organic radicals. For a sinusoidal scan the rate is maximum in the center of the scan and is equal to $\pi f_s B_m$ [2]. A key constraint is that as

the scan rate increases, the voltage across the resonating capacitor and scan coils increases dramatically, and the amplitude of the background signal induced by the rapidly changing magnetic field also increases. Wider scans can be obtained with slower scan frequencies.

To be generally applicable to transition metals and lanthanoids, scans of thousands of G are needed. This problem has been approached in two ways. The Hyde lab has collected Cu(II) spectra in several segments of about 20 G each and assembled them post-acquisition [18, 19]. Yu et al. collected Cu(II), vanadyl, and Mn(II) spectra over as much as 6200 G in a method called field-stepped direct detection rapid scan [32] (Fig. 6). In this direct-detection method the signal typically is oversampled in the field axis to assist in combining segments. S/N can be improved by averaging points and/or smoothing in post-processing. The background signal is much lower for the individual small segment scans than it would be for the full wide spectrum. Furthermore, the background signals for the individual scans are not coherent with the combined spectrum and tend to average out, analogous to noise.

3. Improved signal-to-noise for rapid scan relative to CW

Rapid-scan EPR at X-band (~ 9.5 GHz) has been shown to yield improved S/N per unit time relative to CW EPR by a factor of 2 for rapidly-tumbling nitroxides in fluid solution [10], by factors of 6 to 30 for immobilized nitroxides [33], by factors of 10 to 40 for spin-trapped superoxide [34], by a factor of 8 for the E' center in irradiated fused quartz [35], by more than a factor of 250 for amorphous hydrogenated silicon [12], by a factor of 25 for $N@C_{60}$ diluted in C_{60} [12], and by more than a factor of 140 for the neutral single substitutional nitrogen centers (N_S^0) in diamond [12]. Improved S/N has also been observed for irradiated teeth [36] and irradiated clipped fingernails [37], which have potential applications for radiation dosimetry. At L-band the S/N for radicals observed in γ -irradiated solids is improved by factors of 20 to 35 [30]. At 250 MHz rapid scan improves S/N relative to CW by about a factor of 10 for EPR imaging [38, 39]. Examples for a wide range of samples are discussed in prior reviews [2, 40].

Several factors contribute to the improved S/N in rapid scan relative to normal CW EPR. (i) Direct detection measures the full signal amplitude in each scan, not just a small segment as in normal magnetic field modulation (Fig. 1). This advantage is present independent of whether the scans are fast enough to be in the range where rapid-scan oscillations are observed. (ii) In the regime where scan rates are fast enough that higher B_1 can be used (Fig. 3), a larger signal is detected. (iii) Noise is reduced by averaging the signal thousands of times. Although the large bandwidth of the signal detection pathway includes more noise in the raw data, the signal after deconvolution has the same lineshape as the conventional CW spectrum so the filter bandwidth that can be applied in the final stage of post-processing is the same as for a slow-scan spectrum. (iv) averaging of a periodic signal is equivalent to a comb filter in the frequency domain [41]. The width of the comb is inversely proportional to the number of scans averaged, which makes it a much narrower filter bandwidth than is used in CW experiments. Coherent averaging of the periodic rapid-scan signal offsets the signal in the frequency domain to the region of the noise spectrum where the noise spectrum is approximately frequency independent. This principle was demonstrated by Klein and Barton in 1963 [42]. (v) When data are acquired with quadrature detection the signals from the two

channels can be combined [24]. (vi) The phase-sensitive detection at the modulation frequency that is used in CW experiments acquires only the signal that is in phase with the modulation, which is called the first harmonic. As the ratio of modulation amplitude to linewidth is increased, the amplitudes of signals at higher harmonics of the modulation frequency increase [43, 44]. These higher harmonics are rejected by the phase sensitive detection. The S/N of a CW spectrum can be increased substantially by using higher modulation amplitudes and reconstruction of the spectrum using digital phase-sensitive detection and inclusion of the higher harmonics [43, 44]. Rapid-scan EPR can be viewed as the extreme case of overmodulation. All harmonics that are within the bandwidth of the resonator are acquired and are included automatically in the deconvolved signal, thereby improving S/N relative to acquisition of only the first harmonic.

4. Advantages of rapid scan for imaging

Several characteristics of rapid-scan data are advantageous for imaging. (i) Improved S/N relative to CW spectra substantially decreases the time required for imaging or improves image quality for the same data acquisition time. (ii) The deconvolution of the transient response yields the absorption and dispersion spectra rather than the traditional first-derivative spectra [5, 23]. The absorption signal is especially advantageous for EPR imaging [5] because most image reconstruction algorithms are based on the absorption spectrum. Processing for first-derivative CW spectra requires an integration step that can introduce low-frequency noise [41]. That step is not needed when the native data are the absorption spectra. (iii) The magnetic field gradients that are applied to encode spatial information broaden signals. In CW spectroscopy, the amplitude of the first derivative signal decreases approximately quadratically with the field gradient. However, for the absorption spectrum obtained by rapid scan the signal amplitude decreases approximately linearly with gradient [5]. Unless additional signal averaging is performed, noise at constant bandwidth is independent of gradient, so a decrease in signal causes a proportional decrease in S/N . The weaker dependence of signal amplitude on gradient for the rapid-scan absorption signals than for conventional first-derivative spectra is a major advantage. For example, projections for spectral-spatial images are recorded at a series of gradients and the quality of the reconstructed images is strongly dependent on the S/N for the projections at highest gradients.

5. Application to low-frequency EPR imaging

Early EPR imaging was performed primarily at X-band [45, 46]. In vivo EPR imaging developed more recently at RF or low microwave frequencies and magnetic fields where depth of penetration is greater for lossy samples [47]. A paper by Epel and Halpern in this issue brings review of in vivo EPR imaging to the state of the art. Nitroxide radicals of many varieties have been developed for understanding physical systems from polymers to animals [48]. Imaging has played a major role, particularly when there is a spectral dimension in the image. At X-band, the entire nitroxide spectrum has been imaged, but there is a problem transferring the X-band imaging methodology to in vivo applications. To include a spectral dimension, projections must be acquired at a series of gradients and the sweep width for the projections increases with gradient [49]. At X-band, the nitroxide spectral width, and the

magnetic field gradient magnitudes and scan widths required for the common filtered back projection method of spectral-spatial image creation are small enough relative to the Zeeman field that the method works well.

For low-frequency in vivo imaging, with RF frequencies of 250 to 300 MHz and corresponding magnetic fields of 90 to 108 G (9 to 10.8 mT), acquiring sets of projections that are needed to create a spectral-spatial image by filtered back projection with a spectral dimension that encompassed the entire nitroxide spectrum is not feasible because the scan widths required are as large as the Zeeman field. The initial solution to this problem was to image only one line of the three-line nitroxide spectrum. A comparison of rapid-scan and CW 2D spectral-spatial imaging at 250 MHz for one line of a nitroxide spectrum was performed for a phantom composed of three tubes, each containing a 0.5 mM solution of a different nitroxide [38]. The volume of each solution was about 100 μ L. For the same data acquisition time, rapid-scan EPR provided projections with significantly improved S/N , particularly at higher magnetic field gradients. The improved S/N in the projections resulted in improved accuracy in linewidths calculated from slices through spectral-spatial images [38].

In the filtered back projection method for reconstruction of spectral-spatial images, the angles for the projections must be spaced equally in the spectral-spatial plane [49]. The requirements for projections that are used in iterative reconstruction methods for spectral-spatial imaging are more flexible than for filtered back-projection. Tseitlin et al. [50] demonstrated reconstruction of a spectral-spatial image of two 6 mm tubes containing ^{14}N -perdeuterated tempone (^{14}N -PDT) and ^{15}N -PDT at 250 MHz. Eighteen projections were acquired with a 3.5 kHz sinusoidal scan frequency, 65 G scan widths, and gradients equally spaced between -10 to $+10$ G/cm. The acquisition time per projection was 60 s. The reconstructed image (Fig. 7) accurately reflects the lineshapes of the signals for the two nitroxides and the spatial separation of the two tubes. This iterative algorithm has the advantage that the projections may be arbitrarily spaced, with selection based on prior knowledge of the object, or iteratively during data acquisition. Images acquired so far using the new rapid-scan projection acquisition and image reconstruction methods have been only two-dimensional. Extensive future development will be required to efficiently combine projections to reconstruct a full 4D (3 spatial dimensions and a spectral dimension) image.

Redox status is crucial for characterization of tumor physiology and can be monitored via thiol/disulfide equilibria [51]. EPR spectra of disulfide-linked dinitroxides are readily distinguished from those of the corresponding monoradicals that are formed by cleavage of the disulfide linkage by free thiols. EPR spectra can thus be used to monitor the rate of cleavage and the thiol redox status. This application of EPR imaging requires acquisition of the full nitroxide or dinitroxide spectra because of the overlapping signals from the two species. Reaction of disulfide dinitroxide **I** with glutathione produces the reduced species **Ia** [51]. Two spectral-spatial images of ^2H , ^{15}N -dinitroxide **I** in a two-compartment phantom are shown in Fig. 8. The images were obtained by rapid scan with 3.01 kHz sinusoidal scan frequency, 40 projections at gradients equally spaced between -20 and 20 G/cm, and 53 s/projection. In one image (Fig. 8A), both compartments of the phantom were filled with **I**. In the second image (Fig. 8B) one compartment contained **I**, and the other compartment

contained monoradical **Ia** obtained by reaction with glutathione (Fig. 8B). Cleavage of the disulfide linkage removes the nitroxide-nitroxide spin-spin exchange and eliminates the characteristic broad signal that is intermediate between the two hyperfine lines of the monoradical. The two images represent the extremes of the redox reaction. An *in vivo* environment would have a mixture of **I** and **Ia**. The image and slices through the image clearly display the changes in relative concentrations of mono and diradical.

These methods were developed to facilitate *in vivo* imaging of nitroxides. Fig. 9 presents an example of a rapid-scan *in vivo* study using the dinitroxide **I** at 250 MHz. Deuterated ^{15}N disulfide-linked dinitroxide was cleaved in a tumor grown on the leg of a mouse. The cleavage kinetics of the spin probe allow assessment of the concentration of glutathione in cells and thus assessment of redox balance. For this study the nitroxide was directly injected into a leg-borne tumor of a mouse and a series of 35s long 3D images of monoradical products **Ia** were taken to observe the signal dynamics that are governed by the combined rates of diradical cleavage and clearance of monoradical from the mouse [52].

Image quality can be further improved by fitting spectral slices, based on known characteristics of the species in the sample as shown in Fig. 10 [39]. Two compartments, separated by a 3-mm-thick barrier, contained the same spin trapping solution. Hydroxide radical was generated by irradiation of an H_2O_2 solution [53], to achieve a BMPO-OH (hydroxyl radical trapped with 5-*tert*-butoxycarbonyl-5-methyl-1-pyrroline-*N*-oxide) concentration of about 5 μM . This concentration of spin adduct is about two orders of magnitude lower than the concentrations of ^{15}N and deuterium substituted nitroxides that have been imaged previously at 250 MHz [54]. The frequency of the rapid sinusoidal scans was 3.054 kHz. The diameter of the rapid-scan coils is about 8.9 cm to ensure uniformity of the oscillating field over the sample. The data acquisition time was about 44 sec for each of 21 projections with equally spaced gradients between -10 and $+10$ G/cm. The rapid-scan signals were deconvolved to obtain the absorption spectrum for each projection [23]. Since the goal of the imaging experiment is to define the spatial distribution of the BMPO-OH, comparison of Fourier transforms of spectral slices and simulated spectra followed by thresholding were used as the final step in image processing. The resulting image is shown in Fig. 10. Slices parallel to the x axis of the image are spectral slices though the sample at particular positions in the sample. At 250 MHz the nuclear hyperfine splitting is a significant fraction of the external magnetic field, so the hyperfine lines are not equally spaced [39]. Slices parallel to the y axis represent the spatial distribution of signal. In the resulting image, the 3-mm spacer between the two compartments is well defined as a dark region in the spatial distribution.

To avoid the need to wait for gradients to settle after changes, continuously varying gradients (rotating gradients) have been used. Subramanian et al. demonstrated rapid scan at 300 MHz

for phantoms composed of N-methyl pyridinium tetracyanomethane or the trityl radical OX63 [55]. The magnetic field gradients were varied sinusoidal at a rate of 1 kHz and the sinusoidal rapid magnetic field scan rate was 0.33 kHz. This scan rate is slow enough that signal deconvolution was not required. To ensure linearity of the field scans only the central 35% of the sinusoidal scans was used. The factor of three difference in rates for the gradient and magnetic field scans provided a full sweep of the gradients during the time for one sinusoidal scan of the central 35% of the field scan. The data points were shuffled to generate projections for creation of 2D spatial images by filtered back projection. Czechowski and colleagues have demonstrated rapid-scan EPR imaging at 280 MHz for phantoms composed of two samples of LiPc with different linewidths [56, 57]. To generate a spectral-spatial image the magnetic field gradients varied slowly such that it was approximately constant during the time required for a full 2 kHz sinusoidal scan [56]. The maximum gradient was 7.32 G/cm. Since the central portion of a sinusoidal scan is approximately linear, a linear deconvolution algorithm was used. The field sweep width was constant (7.83 G) so variable-length segments of the scan were used for image reconstruction to satisfy the requirement for spectral-spatial imaging. Seven iterations of the Ordered Subset Expectation Maximization method were used to create images with 1.0 cm by 0.5 G dimensions. Projections for a spatial-spatial image were acquired with 4 kHz sinusoidal scans and the magnetic field gradient varied at a rate of 100 Hz [57]. For a sample with $\sim 10^{17}$ spins/tube the time for a 2D image was about 20 ms. For samples with fewer spins, signal averaging would be required, which would increase the imaging time.

6. Limitations and challenges

All types of EPR acquisition suffer background problems, including from impurities and defects in the resonator or sample holder, instrumental artifacts such as switching transients, magnetic field modulation eddy currents in the resonator, and many other such phenomena. All of these problems potentially impact rapid-scan spectra also. The most significant of the problems is an oscillating background signal that appears to be predominantly due to eddy currents induced in metallic components of the resonator. Resonator design and construction needs to minimize metal exposed to the rapidly scanning magnetic field. Some examples of these styles of resonators have been used in the measurements mentioned above.

Resonator bandwidth (Q) filters the rapid-scan spectra [58] in much the same way that detector bandwidth filters CW and pulsed EPR. The signal bandwidth increases with scan rate so resonator bandwidth may be a limitation on scan rate, particularly for signals with very narrow linewidths [12]. For in vivo experiments resonator Q typically is low because of the large high-loss samples, so Q is not likely to limit scan rates for these experiments. Detector bandwidth also has to be large, as in pulsed EPR, with attendant decrease in signal-to-noise. Post-acquisition processing can be used to optimally filter the spectrum after deconvolution, at which point the signal bandwidth is much lower than during acquisition.

Although it should be obvious from the above discussion, it is worth emphasizing that the deconvolution of rapid-scan spectra assumes that they were obtained in the linear response region. Saturated rapid-scan spectra will be distorted analogous to the distortion in saturated CW spectra.

Low temperature rapid scan

Since many paramagnetic species exhibit fast spin relaxation at room temperature, it is common to study these species at cryogenic temperatures. Common cryostats used for CW and pulsed EPR are constructed of glass or metal. As described in section 2, with small samples it is possible to use the normal modulation coils as rapid-scan coils, and in this way perform rapid scan in a cryostat. However, this severely limits the size of the sample and hence loses some of the S/N advantage of rapid scan. If rapid scans are performed with scan coils outside the metal walls of the cryostats that are used for the Bruker flexline series resonators, large eddy currents are induced and there is little penetration of the scan field through the cryostat walls. Implementation of rapid scan for cryogenic temperatures with flexline style resonators will require development of a non-metal cryostat system.

7. Prognosis for rapid scan

The combined description of rapid scan as simultaneously CW and pulse promises many future opportunities. The incorporation of rapid scan into increasingly digital spectrometers is promising [59]. As rapid-scan technology has evolved to solve problems, it looks increasingly that rapid scan could replace the CW EPR that has been the standard for seventy years.

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Highlights

- The full EPR signal is directly detected upon rapid scan through resonance.
- Rapid scan signal-to-noise is substantially enhanced relative to conventional CW.
- Images have been obtained in phantoms for nitroxides, dinitroxides, and spin-trapped radicals.
- Rapid scan enables in vivo imaging of thiol redox status in a mouse.

Fig. 1.

Schematic comparison of CW and rapid scans for a single-line EPR spectrum. A) At each point in a CW first-derivative spectrum the signal is encoded by phase-sensitive detection at the modulation frequency. B) In a rapid-scan experiment the center field is held constant and an additional field is rapidly varied across the full spectrum. The full amplitude of the spectrum is detected in each sweep. The example shows two full cycles of the rapid scan after deconvolution, which is comprised of two up-scans and two down-scans through the single peak.

Figure 2A

Figure 2B

Fig. 2.

(A) Rapid-scan signals (solid lines) for a LiPc sample, obtained at RF frequency = 248 MHz and $B_1 = 3.6$ mG at various scan frequencies and the signals obtained by deconvolving these signals (dashed lines). The signals were obtained at various scan frequencies and a scan width of 2.16 G. The central segment of each scan is plotted. The corresponding scan rates (a–e) are 4.32, 8.64, 21.6, 38.8, and 43.2 kG/s. (B) Deconvolved spectra from part A superimposed on the deconvolution of the spectrum recorded with 1 kHz scan frequency. Reproduced with permission from Ref. [5].

Fig. 3.

Relative intensities for the LiPc signal at the center of the scan as a function of scan rate at constant radiofrequency B_1 of 6.5×10^{-3} G (◆) and at the B_1 that gave the maximum signal amplitude (●). The number of sinusoidal scans was held constant. The relative signal amplitudes were scaled to 1.0 for the signal at constant B_1 of 6.5×10^{-3} G collected at the scan rate of 1.3×10^3 G/s (scan width of 0.42 G and scan frequency of 1 kHz). The solid lines connect points that were calculated by numerical integration of the Bloch equations using the parameters for LiPc and the experimental scan widths and frequencies. Reproduced with permission from Ref. [11].

Fig. 4. Rapid-scan signals for two LiPc samples separated by ~ 5 mm, obtained in the presence of magnetic field gradients with RF frequency = 248 MHz, $B_1 = 2.6$ mG, and a frequency of 4 kHz for triangular scans. The solid lines are the experimental data and the dashed lines are deconvolved spectra. Reproduced with permission from Ref. [5].

Fig. 5.

Block diagram for a rapid-scan imaging system. The rapid-scan driver triggers the digitizer to acquire signal coherent with the triangular or sinusoidal scans.

Fig. 6.

A) Spectrum of an oriented crystal of Mn^{2+} in $\text{Mg}(\text{acac})_2(\text{H}_2\text{O})_2$ obtained by field-stepped direct detection in 6201 steps over 6200 G. B) CW spectrum obtained with a modulation amplitude of 1.1 G and field resolution of 1.0 G. The B_1 was 174 mG for both spectra. Reproduced with permission from Ref. [32].

Fig. 7.
 A) 2D spectral-spatial image of phantom consisting of 0.2 mM ^{14}N -PDT and 0.53 mM ^{15}N -PDT in deoxygenated aqueous solution at 250 MHz. The samples were in 6 mm OD tubes with the edges separated by 2 mm. B) Comparison of spectral slices (^{14}N in green and ^{15}N in blue) through the image with the zero-gradient spectrum. Reproduced with permission from Ref. [50].

Fig. 8.

2D spectral-spatial images of **I** and **Ia** in a two-compartment phantom with a 10 mm spacer between compartments. A) Left: both compartments contain 0.5 mM diradical **I**; right: slices through the upper (blue) and lower (red) compartments of the image. B) Left: the upper compartment contains 0.5 mM **I** and the lower compartment contains 1 mM **Ia**, generated by the reaction of 0.5 mM **I** with 1 mM glutathione; right: slices through the upper (blue) and lower (red) compartments of the image. Reproduced with permission from [51].

Fig. 9.

Sagittal slice of overlapped MRI and rapid scan images of tumor-bearing mouse leg. A co-registered MRI image, shown in gray scale, was used to identify the location of the tumor that is outlined in magenta. An image of the cleavage rate for dinitroxide **1**, k^{obs} , is shown with the color scale. The cleavage rates were obtained by fitting the time dependence of the peaks for monoradical **2** in a series of images [52].

Fig. 10.

2-D spectral-spatial image of BMPO-OH in the phantom sample (A), a spectral slice through the image (B) and the error function (C) that was used in the spectral slice fitting routine to distinguish regions that contain BMPO-OH from noise-containing baseline regions. Reproduced with permission from [39].