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Resonators for In Vivo Imaging: Practical Experience

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Abstract

Resonators for preclinical electron paramagnetic resonance imaging have been designed primarily for rodents and rabbits and have internal diameters between 16 and 51 mm. Lumped circuit resonators include loop-gap, Alderman-Grant, and saddle coil topologies and surface coils. Bimodal resonators are useful for isolating the detected signal from incident power and reducing dead time in pulse experiments. Resonators for continuous wave, rapid scan, and pulse experiments are described. Experience at the University of Chicago and University of Denver in design of resonators for in vivo imaging is summarized.

1. Introduction

A wide variety of resonators have been used for in vivo EPR imaging and other low-frequency EPR spectroscopy and relaxation measurements. Early low-frequency spectrometers have been comprehensively reviewed [1]. The focus of this paper is on the practical experience in the Chicago and Denver laboratories of the Center for EPR Imaging In Vivo Physiology <https://epri.uchicago.edu/> and <http://epr-center.du.edu/>. Examples of resonators that have been used for low frequency imaging and physiology studies in other labs include a 221 MHz saddle coil with 13.5 mm diameter and 23.5 mm long [2]; a 300 MHz parallel coil resonator with ca. 25 mm diameter, 50 mm long [3, 4] or 25 mm long [5]; a 55 mm diameter loop at 300 MHz [6]; a 15 mm diameter surface coil at 750 MHz [7]; a 1.1 GHz 33 mm diameter, 5 mm long loop gap resonator (LGR) [8]; and a 1.3 GHz 33 mm diameter 24 mm long LGR [9]. A 22 mm diameter 18 mm long 670 MHz multi-coil parallel gap EPR resonator was part of combined EPR/NMR co-registration imaging system [10], and a similar 22 mm diameter 34 mm long resonator was used in a 650 MHz imager [11]. A 590 MHz modified Alderman-Grant style resonator was built for proton-electron double resonance imaging (PEDRI) [12]. A Bruker L-band 23-mm reentrant resonator (E540R23) has been used for heart imaging [13]. Several resonators have been built for in vivo or isolated heart studies at 1.1 to 1.2 GHz, including a 42 mm diameter 48 mm long 16 gap resonator [14]; an analogous resonator in a hybrid EPR/NMR co-imaging system [15] and a 22 mm diameter 19 mm long 1-loop-2-gap resonator [14]. Resonators designed for application to the surface of an animal rather than to contain the animal include a 1.2 GHz 4.5 mm single turn coil [16]; 2.2 GHz bridged LGR [17]; and a segmented surface coil at 1.1 GHz [18].

The wide variety of resonator designs available, and the wide range of parameters accessible for each type, result in such a large parameter space that it is valuable to summarize our experience with resonators that have been used in our labs, which may guide future developments. Our labs have worked jointly on multiple aspects of in vivo imaging, and the relative roles are illustrated by differences in resonator designs. The Chicago resonators were designed specifically for 250 MHz animal imaging. The Denver designs usually were to test instrument design concepts and to develop new methods. The large number and type of design criteria should caution one against describing any resonator as “optimized.” Conversely, there is enormous opportunity for innovation. In this paper we report a few types of resonator designs that have been useful for one or another low-frequency EPR experiment that is inspired by the goal of preclinical in vivo imaging of trityl and nitroxide radicals.

2. Resonator design criteria

EPR signal intensity is proportional to the product of the filling factor, η , and resonator Q (Eq. (1) in Ref. [19]). Since the cost of the microwave or rf source increases with increasing power, it is important to use source power as efficiently as possible. Therefore, regardless of application, resonator design criteria include maximizing (a) resonator efficiency ($\Lambda = B_1/\text{watt}$) [20], (b) filling factor, (c) separation of magnetic and electric fields to minimize electric field interaction with the sample, and (d) stability of coupling and tuning. In addition, the resonator Q and bandwidth need to be matched to the type of EPR experiment:

high Q for CW, low Q and large bandwidth for pulse and rapid scan. The type of EPR experiment also dictates materials used in the resonator design: solid metal resonators can be used for pulsed EPR, but penetration of rapidly changing magnetic fields are essential for CW and rapid scan. Dielectric resonators provide high Q and minimum conducting material near the sample, which is advantageous for many EPR measurements.

Other concerns about materials of construction relate to experimental conditions. For example, CW or pulse measurements at low temperature encounter interference from the signals in surface oxides that are inherent in resonators that have copper or brass components. Silver is a better choice. Silver oxide formation can be reduced with a thin gold plating. Even the best available dielectric resonators and quartz Dewars exhibit signals that are easily observable in modern X-band spectrometers. For in vivo applications at low frequency and near room temperature these interferences are not of concern. However, Pyrex and analogous glasses do have iron signals that can interfere with wide magnetic field scans.

In vivo measurements usually require maintaining the animal at ca. 37 C. The air temperature, and heat from gradient coils and magnet coils result in heating the resonator, so it is important to design the resonator to not change frequency and coupling much with change in temperature.

2.1 Advantages of lumped-circuit resonators for low-frequency spectroscopy and imaging

Cavity-type resonators have dimensions that are some multiple of wavelength (various rf magnetic field modes are possible). At the low frequencies used for in vivo imaging, this is not practical, and lumped-circuit and other small resonators are used. Early examples of such resonators include dielectric [21], slotted tube [22], loop-gap [23], spiral [24], folded half-wave [25], and crossed loop resonator (CLR) [26]. These structures are smaller than cavity resonators, providing higher filling factors, higher efficiency Λ , and lower Q. Lower Q yields less sensitivity of the dispersion signal to source phase noise [27], faster power ring-down and hence shorter dead time after a pulse, and less sensitivity of the EPR signal to animal motion [7].

2.2 Microwave field patterns in resonators

The B- and E-field vectors for RF/microwave radiation in several cavity types are displayed in the review by Webb [28]. The B_1 in a resonator can be estimated fairly accurately based on the size and the conductivity of the materials of construction, as shown in [29] and [30]. Zweier and coworkers imaged the signal intensity distribution in an aqueous solution of trityl using a 1 GHz loop resonator [31]. The variation of B_1 in the resonator can be mapped using EPR imaging. For B_1 mapping at 250 MHz, a trityl phantom with deoxygenated 2 mM deuterated OX063 was installed in the resonator (Fig. 1). The phantom had 19 mm diameter, 8 cm length and was filled with trityl solution without head space. The two pulse imaging echo sequence used was: $t_\alpha - \tau - t_\pi - \tau - \text{echo}$; with t_α a pulse with variable flip angle. The duration of the π -pulse, t_π , was determined prior to imaging. 6 dB smaller RF power was applied for a t_α pulse; with t_α flip angle close to $\pi/2$. A number of images with different t_α was taken. The imaging methodology is similar to the one used for relaxation mapping [32]. The image voxel intensity is proportional to $\sin(\alpha)$, where α is the flip angle of t_α pulse [33].

at this voxel position. The B_1 image was obtained by fitting voxel amplitude dependence on t_a with $\sin(\gamma B_1 t_a)$ function, where γ is the gyromagnetic ratio of the electron, 1.7608×10^{11} rad/s $^{-1}$ T $^{-1}$.

2.3 Coupling to Resonator

The impedance looking into a typical resonator is complex. Depending on parallel- or series-resonance, the impedance will have a very low or very high real part. For a reflection resonator the coupling to the resonator must be close to critical, i.e. the resonator impedance as seen by the transmission line, at resonance, must be equal to its characteristic impedance, Z_0 , to minimize reflected source power that can saturate the detector. A lossless network is normally required to transform the resonator impedance to that required to match the line; typically, $Z_0 = 50 \Omega$ [34]. There are two general types of coupling networks, inductive or capacitive. For example, for a series-resonant resonator, a shunt reactance converts the resonator impedance to $Z = R \pm iX$ at some frequency f_0 . Then a capacitive or inductive series reactance is used to cancel the imaginary part of Z at the same frequency; $R = Z_0$ if critical coupling is required. In many cases, either type of coupling can be used, but some resonators are better suited to a particular coupling structure. Inductive coupling can be provided by a simple loop, arranged to permit variation of the loop-to-resonator mutual inductance by adjusting the spacing from the resonator. This coupling is suitable for many different resonators, such as LGRs, dielectric resonators and birdcage resonators. However, the mechanical nature of this coupling makes it less stable, due to vibrations in the coupling loop induced by scanning field and other external factor. A capacitive coupling structure can usually be made to be more stable.

For a bimodal resonator, critical coupling is not required for the source (or detection) resonator, since the reflected power does not reach the detector. Coupling that ensures the highest detected signal amplitude can be used. For example, the driven portion of a CLR can be over-coupled if a lower Q is required for the experiment.

When coupling loops are used, one has to avoid dimensions that are significant fractions of a wavelength. In some LGR applications, especially for animal imaging, a coupling loop near the resonator entrance obstructs access to the animal, and the loop may be subject to damage during heavy use. Capacitive coupling is typically applied to one of the resonator's resonating capacitors and is more robust from the mechanical point of view. Typically a scheme with a serial tank circuit resonance and matching capacitor in series with the resonator is used (Figure 2).

For animal resonators a balanced coupling scheme is preferable. This scheme avoids currents in the ground of the coupling elements and transmission lines and is less affected by changes in the resonator environment. A variety of balun (balanced-unbalanced) transformers have been used to convert unbalanced lines from the RF source and detection arm to the resonator built using a balanced scheme. Some baluns are available commercially. "Bazooka" style baluns can be fabricated, for example by inserting a small diameter coax into a larger diameter coax.

In many cases location of the adjustable elements directly on the resonator is impossible due to space limitations. In this case a remote coupling scheme can be used in which part of the resonance and matching capacitances can be separated from the resonator by a transmission line with a length equal to half of the wavelength at the resonance frequency. The wavelength in the transmission line may be considerably different from the wavelength in vacuum due to group delay imposed by the design of the transmission line. It should be noted that locating all of the resonant capacitances into a remote position is not advisable due to increased losses that reduce resonator quality factor. Figure 2 also includes an example of the use of voltage controlled capacitors such as varactors to maintain constant coupling in spite of animal motion. These are attractive, because they can be integrated into automatic frequency and matching (AFC, ATC) networks. However, varactors are sensitive to changes in the magnetic field, so if they are close to the resonator the varactor itself can contribute to background signal unless the magnetic field is constant.

2.4 Specifications for resonators for in vivo imaging

Dimensions of resonators for in vivo spectroscopy and imaging are dictated by the size of the specimen. As a practical matter, most preclinical imaging is performed using mice or excised organs, such as a rat heart. These define the resonator size. Resonators that include the specimen, sometimes called volume resonators, need to be ca. 16–19 mm diameter to include a mouse leg, 25 mm for full mouse body or a rat limb, 50 mm for rat body or rabbit limb. In addition to accommodating the animal, there is need for space for the animal support structure, for fiducial samples, and for general physiological support of the animal. Support of animals requires that the resonator have an open structure, such as a LGR, and not an enclosed cavity of the type commonly used for small spectroscopy samples at X-band. These requirements have major implications for the size of the resonator, RF/microwave shield, gradient coils, and support structure. Beyond about 50 mm diameter, volume resonators are impractical, and surface coils are the method of choice. Except for skin studies, surface coils need to have diameter larger than about 10 mm, because the penetration depth increases with diameter of the resonator. Although small diameter coils can provide good sensitivity for small samples, the penetration depth would be too shallow for most in vivo applications.

Higher Q would provide larger EPR signal, but would interfere with measuring FID and spin echo decay rates that are essential to trityl oximetry, so maximizing resonator Q is not a priority for resonator design. A CLR, including Q-switched CLR, permit some optimization of Q, and is commonly used in phantom measurements in the Denver lab, but convenience of a reflection resonator has led to preference for it for animal imaging in the Chicago lab [35]. Larger bandwidth (lower Q) needed for pulsed imaging and for rapid scan is more important for in vivo imaging than is the greater sensitivity that can be achieved with higher Q. For particular experiments some optimization tradeoff will guide resonator design. For CW imaging, however, higher Q is always desirable. The specimen may dominate the loss and hence lower the Q considerably relative to the Q of the empty resonator. For example, for a 19 mm diameter 250 MHz LGR the empty Q = 290 and the Q with an animal is ~90. For a 25 mm LGR the Q is reduced from 290 to close to 40. Similarly, a Q for a 52 mm LGR

reduced from 300 to about 12. The lower Q with an animal in the resonator requires higher incident pulse power for the same turning angle.

2.5 Resonator Characteristics

2.5.1 Filling factor—Resonator Q is usually low inherently because of animal loss, and purposefully to increase bandwidth for pulse and rapid scan EPR. Consequently, η becomes an important design parameter. Resonators are designed to match the portion of the animal to be imaged, with allowance for animal support and for fiducial samples to help quantitative comparisons and overlays of images obtained by multiple methods (e.g., EPR, MRI, CT). The filling factor is also determined by the resonator shield. The larger the shield diameter relative to the resonator diameter, the higher η , but the shield diameter is also constrained by the magnet gap. In addition, access to the animal requires that shields be incomplete and open. The EPR signal may be from a small volume of tissue such that the EPR sample filling factor is rather small, limiting signal-to-noise.

2.5.2 Dead time—The dead time after a pulse is defined as the time in which the signal amplitude cannot be defined with fidelity. If the resonator ring down and miscellaneous switching transients are reproducible, and these plus the EPR signal do not overload the digitizer, then it should be possible to subtract the non-EPR parts of the signal and obtain the correct EPR signal. It is important to note that dead time is relative to signal amplitude. The stronger the EPR signal, the shorter the dead time for a given amplitude of resonator ring down and switching transients. Conversely, the weaker the EPR signal, the longer the effective dead time. The EPR signal will be affected by the pulse power, but in the following we assume that the power and pulse length has been optimized to obtain the largest FID or spin echo for the resonator Q. Hence, we only compare other detected signals to an optimum EPR signal.

Power Ring Down Following a Pulse: Although commonly we express Q in terms of frequency bandwidth, it is also fundamental to express it in terms of the ringing time after a pulse. The decay of power at time zero, P_0 , to power P at time t, is a function of the resonator Q and the RF frequency, ν (Eq. 1). The higher the Q and the lower the frequency, the longer the power ring down time.

$$P = P_0 \exp\left(\frac{-2\pi\nu t}{Q}\right) \quad (1)$$

For a crystal detector, with the power in the range where the output voltage is less than ca. 15–20 mV, the voltage output is linear in microwave power incident on the crystal.

$$Q_L = 2\pi\nu\tau \quad (2)$$

where τ is the ring down time constant and ν is the resonator frequency.

For a double balanced mixer (DBM) biased on the LO side to ca. 10 mW (+10dBm), the output signals are linear in voltage, not power. For this case, the measurement of Q is:

$$Q_L = \pi \nu \tau \quad (3)$$

If the EPR signal is detected under conditions where the measurement is of voltage (Eq. 3), the ring down time constant that is relevant is given by Eq. (4)

$$\tau = \frac{Q_L}{\pi \nu} \quad (4)$$

For example, for Q = 20 at 250 MHz, $\tau = 25$ ns. A 2 kW pulse into a 50 Ω load corresponds to a peak voltage of

$$V_{\text{peak}} = \sqrt{2} \sqrt{50} \sqrt{2000} = 447 \text{ V} \quad (5)$$

The EPR signal, before amplification, is of the order of microvolts. Thus, the pulse voltage has to decay by many time constants before the EPR signal can be detected above the resonator ring down voltage. Standard NMR and EPR texts usually quote about 21 time constants for the ring down dead time. The 447 V peak would decay to about 1 microvolt after 20 time constants. For the case of Q = 20 and 250 MHz, 21 time constants amounts to 535 ns, which is within factors of two of common experience for pulsed VHF EPR.

Switching Transients: The switching transients may or may not depend on pulse power. Some of the transients are “video leak-through” of the control signals to switches. Depending on which switch is involved, these transients may be amplified either in the pulse power path or in the signal detection path.

Effect of CLR on Dead Time: A bimodal CLR decreases the dead time caused by resonator ring down proportional to the isolation between the two loops. The dead time is reduced by n time constants, where n is given by the formula

$$n = \frac{dB_{\text{isolation}}}{8.7} \quad (6)$$

Thus, for 60 dB isolation in the CLR, the dead time is shortened by 7 resonator time constants. In addition, each half of the CLR can be optimized and Q-switched to improve S/N and further reduce the dead time [36]. The Q-switching dissipates the power in the resonator very rapidly, so that the ring down contribution to the dead time becomes very short.

Amplifier saturation: Careful power and gain balance is required for spectrometer design. Microwave amplifiers typically recover from saturation much more rapidly than do video amplifiers, so it is important to avoid saturating the video amplifier. Since animal motion can cause fluctuating signal amplitudes, the signal detection mixer (downconverting to dc) should have high dynamic range.

2.5. Power requirements for pulsed EPR

Resonators for in vivo imaging inherently have low Q because they are designed to have high filling factor for the lossy animal. To estimate required RF pump power for pulsed EPR for resonators of different sizes consider two cases: one in which the Q = 12 resulting from the resonator construction materials and the loss due to the animal. As an alternative we consider a case in which the resonator Q = 40, but then we overcouple to achieve Q = 12 (Table 1) [37]. For Q = 12 at 250 MHz the resonator bandwidth is 21 MHz. Mims' criterion is to match half the resonator bandwidth (BW/2) with the 3 dB point in the roll-off of the frequency spectrum of the excitation pulse. For a rectangular pulse this means that $t_p = 0.886/BW = 42.5$ ns, where t_p is the pulse length. For a $\pi/2$ pulse the relationship between pulse length and B_1 is given by Eq. (7)

$$\frac{\pi}{2} = \gamma B_1 t_p \quad (7)$$

where γ is the gyromagnetic ratio.

The 42.5 ns pulse length corresponds to a B_1 of 2.1 G (Eq. 7). For this example we assume that the resonator efficiency $\Lambda = 0.15$ for the 16 mm diameter resonator. If the construction materials are the same, the efficiency of a resonator is proportional to $1/\sqrt{dl}$ where d is diameter and l is length (see eq. 6 in [19]), which gives $\Lambda = 0.096$ and 0.048 for the two larger resonators, respectively (Table 1). The ratio of power required for a resonator overcoupled to a given Q (P_{oc}) to that for a critically coupled resonator with the same Q (P_{cc}) is $P_{oc}/P_{cc} = 1/(2-q)$, where q is the ratio of the Q of the overcoupled resonator to what it would be if it were critically coupled [37]. This ratio is 12/40 for the example shown. Less power is required to achieve a $\pi/2$ pulse with the over-coupled resonator than the critically coupled resonator (Table 1). For resonators that are big enough to contain larger animals, very high pulse power is required (Table 1), which is the incentive to use surface coils instead of resonators that contain the animal.

2.6 Resonator Bandwidth for pulsed EPR

For pulse imaging measurements, resonator bandwidth is defined by the requirement of exciting spins subjected to the magnetic field gradients. The minimum bandwidth can be approximated by $BW = \gamma/G_{max}L_{max}$, where γ is the gyromagnetic ratio, G_{max} is the maximum applied gradient and L_{max} is the maximum extent of the sample. Since resonator bandwidth is inversely proportional to Q, this defines the highest Q that can be used for an image. In many cases the Q has to be reduced to rather small values, i.e. Q = 14 [38]. Under

these circumstances, for resonator comparison it is informative to define a normalized resonator efficiency Λ_{norm} that is independent of Q and resonator volume V (Eq. 8).

$$\Lambda_{\text{norm}} = \Lambda \frac{\sqrt{V}}{\sqrt{Q}}. \quad (8)$$

This parameter allows the comparison between different resonator types with different volumes and different Q . Table 2 summarizes data on many 250 MHz resonators.

3. Design and construction of resonators for EPR imaging

3.1 Imaging loop-gap resonators used in Chicago at 250 MHz

The main volume (or, to contain) imaging resonators currently used in Chicago for 250 MHz pulse and CW imagers are split-top loop-gap (LGR) resonators with 16 mm to 25 mm sample diameters [39]. Those resonators were initially designed for in situ radiation but proved to be very robust for every-day use due to their construction simplicity, high efficiency, fast installation and ease of animal access. Resonators are installed in a large 146 mm diameter RF shield and mounted using two screws that allow quick resonator replacement. The shield is mounted on the resonator platform (Figure 3) and the platform is installed into a pre-defined position of the imaging magnet, guided by directional pins. Other resonator types have a similar mounting platform that allows fast resonator replacement and switching from one imaging modality to another.

The split-top one loop - one gap resonators (Figure 4) were built from three ABS plastic segments (parts I, II and III). The assembled segments form a circular slab of 140 mm outer diameter with a central hole. The conductive surfaces a-b-c-d-e on part I, a'-b'-c'-d'-e' on part II and f-g-g'-f', on part III form an inductive loop, the resonating capacitance and part of the matching capacitors. The resonating capacitor is formed between the conductive surfaces b-c of part I and b'-c' of part II. The segments I and II are mechanically attached to each other using two screws (IV) through holes (3) and are separated by a 5–10 mils PTFE dielectric film. The thickness of the spacer defines the capacitance. For fine capacitance adjustment an additional 1–30 pF variable capacitor Johanson 5601 (Johanson Manufacturing Corporation, Boonton, NJ 07005, USA) was installed in parallel to the gap capacitor. Two swivel bolts (V) with knurled nuts (VI) on both sides of the resonator part III hold the upper and the bottom halves of the resonator together and can be easily fastened and released.

Conducting surfaces were fabricated using two technologies: electroplating or copper adhesive tape. For electroplating 12.5 μm silver (approximately 3 skin depths at 250 MHz) were deposited and covered with a 2 μm flash of gold for protection. The surfaces created with tape used adhesive 1245 copper tape (0.04 mm copper thickness, 3M, St. Paul, MN).

The coupling to the resonator is built using a balanced capacitive impedance transformer. The output of the balun (7) through 100 Ohm transmission line (8) is connected to the metal plates (6) that together with conductive surfaces b-c and b'-c' form coupling capacitors.

The mechanism allows adjusting metal plates position relative to $b-c$ and $b'-c'$ surfaces and thus modifying the coupling capacitors.

The previous generation of resonators (Figure 5) used smaller 4 inch RF shields and resonators that could not be disassembled in the way shown in Figure 4 [38, 40]. The construction of the resonators was otherwise similar to split top resonators. The resonator body was formed from two semi-circular slabs cut along capacitive gap. These resonators used the same coupling system as shown for the split top resonators. Resonators with up to 50.8 mm sample diameter [41] were used. The length of the resonators was 15 mm for 16 mm and 19 mm resonators, 25 mm resonators had 15 mm length for local measurements, 32 mm lengths for a partial abdomen imaging and 75 mm lengths for full body mouse resonator.

3M copper adhesive tape has been a main construction material for Chicago resonators. Machined or 3D printed dielectric bodies covered with the tape form the resonator. Chicago is moving away from mechanical coupling mechanisms in most current designs. This reduces production cost and complexity, but has the disadvantage of higher losses in compact capacitors relative to air gap capacitors that were used earlier. For higher frequencies surface mount device elements will be used, because component leads create unwanted inductances, and multiple wires form stray capacitances.

3.2 Cross-loop and other orthogonal resonators

Efficient isolation of the detection circuitry from the incident RF power can be achieved with a resonator topology involving separate resonators for detection and excitation. The CLR technology, initially developed at S-band [26] extends easily to 250 MHz [29]. The resonator designed for animal imaging is a result of practical compromises to ensure adequate access to the mouse. Although better field homogeneity can be achieved with longer resonators, the resonator is usually made with length not exceeding the diameter. This compromise almost requires violating many routine engineering design principles. All commercial cavity resonators at high microwave frequencies incorporate a restricted cross section (waveguide stack beyond cutoff) to prevent microwave leakage. One can see this in the 250 MHz CLR described in Ref. [29] for test-tube-type phantoms. The sample loop of the CLR described in Ref. [29] is 25.8 mm in diameter and the cross loop is 19 mm wide by 36 mm long. It has a 19 mm wide by 17 mm long rectangular section in the center and 19 mm semicircular ends. The version of this resonator that is used for mouse imaging in Chicago (Figure 6) does not use the stacks, in order to improve access to the mouse.

The large diameter/length aspect ratio can be compensated significantly with an adequate RF shield to define the RF field return path. A solid shield provides optimum shielding, but would have large eddy currents in any field-modulated CW or rapid scan measurement. The shield could have patterns of slots intended to interrupt eddy currents, or it can be made of wire wound around the appropriate form (often cylindrical). Painting with silver paint is also helpful.

For some experiments it has been convenient to be able to change the Q of the 250 MHz solid metal CLR. The CLR was originally constructed to be inductively-coupled using a

movable loop near the loop of each resonator. The loops were designed to be fed with a balun, so each end of the loop is brought out by the center conductor of a short piece of semirigid coax. When not used with a balun, one of the coaxes at the end of the loop was shorted and the other connected to the source or detector. When the short was removed (the coupling then becomes series capacitive) the coupling increased dramatically. A 5 kOhm pot was installed in parallel with the gap of each resonator. This allows the Q of the resonator to be adjusted at critical coupling from nearly its design value down to about 10. The CLR becomes a dial-a- Q resonator.

Since the power ring-down time of a resonator with a particular Q value is inversely proportional to the frequency, many low-frequency pulse measurements would not be feasible at the values of resonator Q commonly used in pulsed EPR at X-band and higher frequencies. For relaxation time studies, it is convenient to use resonator Q -switching, as described in [36]. If the resonator filling factor is high, the animal usually lowers the Q to the extent that the ring-down time is not limiting for pulsed EPR imaging of trityl radicals, but nitroxide relaxation is so short (less than a μ s) that pulsed imaging of nitroxide probes is a challenge [42], and rapid scan is the method of choice for imaging nitroxides [43, 44].

Other combinations of resonators achieve similar goals, so long as the B_1 fields are orthogonal. One such combination that has been useful for small animal imaging in Chicago combines an Alderman-Grant-type resonator with a LGR. The LGR can be placed inside or outside an Alderman-Grant-type resonator. One 250 MHz implementation designed for pulse EPR imaging that has been used in the Chicago lab has a 19 mm single-loop-single-gap slotted resonator placed inside, as shown in Figures 7 and 8 [45]. The equivalent circuit diagram for this type of resonator is included in Figure 7.

This resonator is constructed from two independent resonators of cylindrical shape, housed coaxially one inside another. The inner single-loop single-gap resonator is slotted for better B_1 penetration from the outer resonator. This resonator has a coaxial magnetic field orientation and is used for signal detection. Excitation is provided by the outer Alderman-Grant resonator with the field direction orthogonal to the resonator axes. The inner resonator support is attached to the shield while the AGR is rotated around the axes under control of a precise thread-based mechanism. This enables fine resonator isolation adjustment by alteration of the angle between the magnetic fields in the resonators. The combination of 40 dB isolation and good efficiency of the detection resonator allowed achieving higher signal to noise and considerable suppression of baseline artifacts. The LGR has inner diameter 19 mm, outer diameter 21 mm, and is 15 mm long. The Alderman-Grant (AGR) type resonator has inner diameter 23 mm, outer diameter 25.4 mm and is 40 mm long, including the end rings. The active region is 22 mm long. The sample occupies 0.82 of the LGR and 0.46 of the AGR. In this version, the end-rings of AGR are too large and obstruct the animal access. Newer versions of the resonator feature reduced wings. The LGR was tuned to 250 MHz. An additional mode was observed at 279 MHz. The AGR was tuned at 250 MHz. An additional mode was observed at 330 MHz. Resonator parameters are summarized in the Table 3.

3.3 Surface coil

One recent application of a small (10 mm) diameter surface coil is imaging of the breast of a mouse (Figure 9).

3.4 700 MHz CLR

Most of the resonators used for in vivo imaging in Chicago have been designed for about 250 MHz. Recently, we have been exploring higher frequencies for applications restricted to mouse studies. Resonators in use in Denver at ca. 700 MHz and in Chicago at ca. 720 MHz are steps toward implementation of preclinical imaging at frequencies designed specifically for mouse studies. To study the frequency region up to about 1 GHz, we designed a resonator for which the sample and filling factor could remain constant as the frequency was changed from about 400 MHz to about 1 GHz by changing the capacitance. This resonator and its applications were described in [46–48]. A rapid scan and pulse resonator for 700 MHz is shown in Figure 10.

3.5 Rapid scan resonators

Most of the resonators discussed in prior sections of this paper were designed for pulse applications, using trityl relaxation to measure pO_2 via its effect on T_1 . A wide range of applications of EPR imaging use nitroxide radicals, for which pulsed EPR is less widely useful [42] than is rapid scan EPR. Rapid scan EPR [44] can use much higher incident power than can be used in CW EPR without saturating the spin system. The isolation of the detection system from the incident power achieved by the CLR makes the CLR advantageous over a reflection resonator for rapid scan imaging.

A 16 mm diameter, 15 mm long resonator designed for rapid scan at 250 MHz was described in Ref. [30]. This resonator was made of thin wire to minimize eddy currents caused by the rapid magnetic field scans. An improved design 16 mm diameter CLR was used for imaging of nitroxides [49]. This resonator also used 0.1 mm copper wires for the 16 mm sample resonator. The orthogonal resonator was a 25 mm saddle coil.

A modified version of the wire CLR [30] was applied to in vivo rapid-scan imaging of dinitroxides [35]. In this resonator a shield was placed between the resonator and the rapid scan coils. The shield was wound using 20 gauge wire to decrease induced eddy currents, and painted with silver paint to improve RF shielding.

3.6 Differences in the excitation and signal detection pathways for different type resonators

A reflection resonator, such as an LGR, requires a circulator and a way to protect the sensitive components in the signal detection pathway from reflected high-power pulses. This protection can be a passive limiter, an active switch, or a transmit/receive switch such as described in [50].

A unique feature of a CLR, or other bimodal resonator such as an Alderman-Grant resonator combined with a loop gap or coil resonator is that the spectrometer does not require a circulator, because the resonator operates in transmission mode. This style resonator can be

used with a spectrometer that has a circulator if appropriate switching pathways are added. Isolation between the two resonators in such systems prevents high pulse power from reaching the signal detection system. The isolation also substantially reduces the dead time after the pulse. Since there are two resonators for which the frequency and coupling have to be separately adjusted, the spectrometer has to include switching paths that permit one to observe each resonator in reflection mode, and then the pair of resonators in transmission mode to observe while adjusting the isolation. Since the individual resonators of a CLR require reflection mode tuning, either directional couplers or a circulator can be used for this purpose. Since a circulator is not needed when the CLR is in normal operation, the use of a CLR can avoid the leakage signal through the circulator (commonly ca. -20 dB) that adds to the EPR signal in standard reflection resonator spectrometers.

CW imaging requires magnetic field modulation and lock-in detection at the modulation frequency. The modulated magnetic field requires that the resonator and shield be designed to transmit the modulated field at the desired frequency. Typically, magnetic field modulation for imaging, especially using trityl radicals, is performed at lower frequencies (e.g., 5 kHz) than the 100 kHz commonly used for X-band CWEPR, because the anoxic trityl linewidths can be narrow enough to be distorted by 100 kHz modulation. Although it has not been used for CW in vivo EPR imaging, S/N advantage can be attained by acquiring CW spectra with larger detection bandwidth and constructing the spectra using multiple harmonics [51]. Commercial CW EPR spectrometers use crystal detection. Quadrature detection would permit post-acquisition phasing.

Rapid scan resonator systems also require good penetration by the rapidly-scanned magnetic field, but they do not require lock-in detection. However, optimum use of rapid scan requires quadrature detection, and digital acquisition of multiple scan cycles to be able to take advantage of all available information in the data [52].

3.7 Resonator efficiency comparison

Most of the loop-gap resonators tested (Table 2) show Λ_{norm} close to $0.24 \text{ Gcm}^{3/2}/\text{W}$. Alderman-grant resonators follow closely with $\sim 0.20 \text{ Gcm}^{3/2}/\text{W}$, but other resonators show considerably lower efficiency. In comparison, the Λ_{norm} of Helmholtz pair resonators was considerably lower, about $0.08 \text{ Gcm}^{3/2}/\text{W}$. Therefore, it is not surprising that for pulse imaging simple one-loop one-gap resonators are commonly used. Considering the available power sources, the maximum size of the resonator for 250 MHz pulse measurements is 50 – 60 mm in diameter.

4. Summary

Current designs reveal practical solutions to problems, but are a work in progress. The design goals listed in the introduction to this paper continue to drive innovations. Crucial improvements will focus on making resonator frequency and coupling less sensitive to animal motion and other environmental effects, and on decreasing background signals in rapid scan resonators.

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Figure 1. B₁ map of a trityl-containing phantom 19 mm diameter, and longer than the resonator. This B₁ map is from the detection LGR used in a bimodal Alderman-Grant resonator (AGR), with the AGR grounded.

Figure 2.

(a) Example circuit for local coupling using a balun and step-up capacitive impedance transformer. A damping resistor can be used to adjust the resonator Q for pulse operation.

(b) The diagram also shows a way to achieve coupling ($\lambda/2$ away from the resonator) using a transmission line and varactors for automatic coupling (matching) and frequency (tuning) control (ATC/AFC).

Figure 3.
Magnet bench with guide pins and RF shield, showing coupling elements.

Figure 4.

19 mm split top resonator. A: The drawing of the resonator structure. B: The assembled resonator. The capacitor and shunt resistor across the gap are visible above the loop. The plastic clamps are used for added mechanical stability. C: Mouse leg installed into the resonator; the top part of the resonator is removed. D. RF shield and coupling element. RF shield surface is slotted for better modulation field penetration. Parts A – C are reproduced from Ref. [53].

Figure 5.

250 MHz resonators. A. “general-purpose” LGR 25 mm diameter and 32 mm long is housed in a 4” diameter shield. B. The 25 mm diameter and 75 mm long resonator used for pulse imaging experiments, with the 500 Ω equivalent shunt resistor (two 1 K Ω resistors in parallel) installed across the resonator gap. Once installed in the magnet, the capacitor plates on top provide coupling to the excitation/detection system. C. Tumor bearing leg in a single-loop-single-gap resonator. The inner diameter was 50 mm, the length 57.4 mm, and the volume 112 ml. Both CW and ESE measurements were performed in the same resonator. The resonator was critically coupled at a frequency of 239.3 MHz with a loaded Q of 11.7 [54].

Figure 6. 250 MHz CLR used for mouse imaging. Note the horizontal orientation and the removal of the stacks, relative to the resonator in figure 1 of Ref [29].

Figure 7. Sketch of the orthogonal resonator constructed with a LGR inside an Alderman-Grant type resonator and the equivalent circuit [45].

Figure 8.

An assembled version of the resonator is sketched in Figure 7. The LGR is attached to the RF shield while the AGR rocks around the axis orthogonal to B_1^{LGR} and B_1^{AGR} , which provides the isolation adjustment [45].

Figure 9.

(A) Oximetry of a mouse breast has been performed using a 10 mm diameter surface coil to detect trityl OX63 at 250 MHz. (B) Structure of the surface coil.

Figure 10.

700 MHz CLR with 25 mm diameter for rapid scan and pulse. Both capacitively coupled saddle-coil resonators are made from copper tape and chip capacitors attached to the outside of the support tube. Conductors are 6.35 mm wide. Coupling is fixed near critical coupling and the frequency of resonator 1 is adjustable. B_1 is perpendicular to the axis of the sample tube. The inside of the PVC RF shield is painted with conductive silver paint.

Table 1

Power requirements for $\pi/2$ pulse for critically-coupled and over-coupled resonators

Diameter (mm)	Length (mm)	ΔEfficiency, Watt G/	Power (W) for critical coupling with Q = 12	Power (W) for over-coupling from Q = 40 to 12
16	15	0.15	196	115
25	23.4	0.096	477	280
50	46.9	0.048	1910	1120

Table 2Comparison of normalized efficiencies for different 250 MHz resonator types with cylindrical geometries.^a

Resonator type	Description	Arrow shows orientation of B_1 field	Λ_{norm}
Loop-gap resonator	$\varnothing=16\text{mm}$, length=15mm small shield, air-gap capacitor coupling		0.19
Loop-gap resonator	$\varnothing=19\text{mm}$, length=15mm large shield, air-gap capacitor coupling		0.24
Loop-gap resonator	$\varnothing=25\text{mm}$, length=32mm small shield, air-gap capacitor coupling		0.24
Loop-gap resonator	$\varnothing=25\text{mm}$, length=76mm small shield, air-gap capacitor coupling		0.25
Loop-gap resonator	$\varnothing=51\text{mm}$, length=57mm small shield, air-gap capacitor coupling.		0.24
Alderman-Grant resonator	$\varnothing=25\text{mm}$, length=15mm small shield, air-gap capacitor coupling		0.20
Saddle coil	$\varnothing=25\text{mm}$, length=15mm small shield, air-gap capacitor coupling		0.13
Helmholtz pair	$\varnothing=16\text{mm}$, length=15mm small shield, air-gap capacitor coupling		0.08

^aThe efficiency was measured using Rabi oscillations in pulse mode.

Table 3

Parameters for resonator shown in Figure 7 and 8

	Method	Q	Power [W]	$[G/W]_{\alpha}$
Loop gap LGR19	Pulse, ESE, maximum signal	36	126	0.3
AGR (excitation)	Pulse, ESE, maximum signal	11	250	0.12