Fast Imaging for Hyperpolarized MR Metabolic Imaging

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MRI with hyperpolarized carbon-13 agents has created a new type of noninvasive, in vivo metabolic imaging that can be applied in cell, animal, and human studies. The use of ^{13}C-labeled agents, primarily [1-^{13}C]pyruvate, enables monitoring of key metabolic pathways with the ability to image substrate and products based on their chemical shift. Over 10 sites worldwide are now performing human studies with this new approach for studies of cancer, heart disease, liver disease, and kidney disease. Hyperpolarized metabolic imaging studies must be performed within several minutes following creation of the hyperpolarized agent due to irreversible decay of the net magnetization back to equilibrium, so fast imaging methods are critical. The imaging methods must include multiple metabolites, separated based on their chemical shift, which are also undergoing rapid metabolic conversion (via label exchange), further exacerbating the challenges of fast imaging. This review describes the state-of-the-art in fast imaging methods for hyperpolarized metabolic imaging. This includes the approach and tradeoffs between three major categories of fast imaging methods—fast spectroscopic imaging, model-based strategies, and metabolite specific imaging—as well additional options of parallel imaging, compressed sensing, tailored RF flip angles, refocused imaging methods, and calibration methods that can improve the scan coverage, speed, signal-to-noise ratio (SNR), resolution, and/or robustness of these studies. To date, these approaches have produced extremely promising initial human imaging results. Improvements to fast hyperpolarized metabolic imaging methods will provide better coverage, SNR, resolution, and reproducibility for future human imaging studies.
Level of Evidence: 5
Technical Efficacy Stage: 1

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MAGNETIC RESONANCE IMAGING (MRI) with hyperpolarized carbon-13(^{13}C) agents has created a new type of noninvasive, in vivo metabolic imaging that can be applied in cell, animal, and human studies.[1] The use of ^{13}C-labeled agents, primarily [1-^{13}C]pyruvate, enables monitoring of key metabolic pathways with the ability to image substrate and products based on their chemical shift. [1-^{13}C]pyruvate has been especially successful because its properties are well suited for hyperpolarized imaging studies (eg, long T_1, high-concentration preparations) and because of pyruvates critical position in glycolysis, where it can either be used in tricarboxylic acid cycle (TCA) cycle metabolism or converted to lactate (Fig. 1). The conversion of pyruvate to lactate is preferentially upregulated in many types of cancers in a process referred to as the "Warburg effect," while normal differentiated cells prefer to use pyruvate in the TCA cycle.[2] In the heart, the relative conversion of pyruvate going into the TCA cycle vs. conversion to lactate is reflective of substrate selection between fatty acid and carbohydrate metabolism that is altered in many heart diseases. Altered pyruvate metabolism has also been studied preclinically in the context of traumatic brain injury,[3,4] multiple sclerosis,[5] liver disease,[6] and diabetes,[7] to name a few. Many other ^{13}C-labeled agents have been successfully developed in animal studies, including ^{13}C-urea as a measure of perfusion,[8,9] ^{13}C-fumarate as a measure of necrosis,[10] ^{13}C-alpha ketoglutarate as a measure of IDH status,[11] ^{13}C-bicarbonate as a measure of extracellular pH,[12] ^{13}C-butyrate as a measure of fatty acid metabolism,[13] ^{13}C-dehydroascorbate as a measure of redox potential,[14] and many more.[15]

Human hyperpolarized (HP) ^{13}C MRI studies have used dissolution dynamic nuclear polarization (DNP) to create a solution with up to a 100,000-fold increase in

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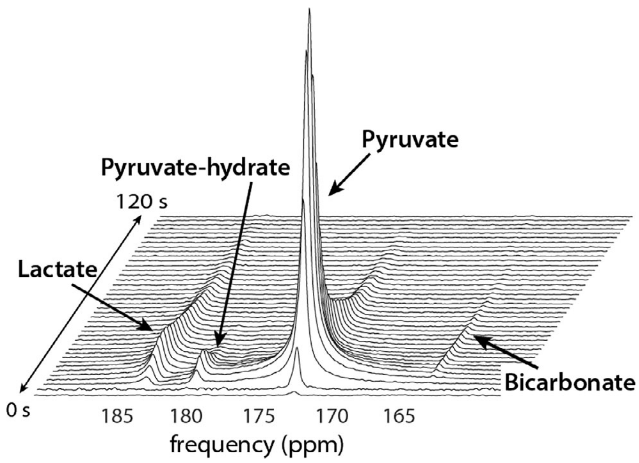

FIGURE 1: Representative dynamic HP-^{13}C spectra illustrates the metabolic conversion of the injected substrate ([1-^{13}C]pyruvate) into products of interest ([1-^{13}C]lactate, [^{13}C]bicarbonate, and [1-^{13}C]pyruvate-hydrate) in the human brain. Conversion to alanine is also observable within the timescale of the experiment in other organs. Their temporal profiles reflect the rapid enzymatic conversion of pyruvate throughout the timeframe of HP-^{13}C studies. Figure adapted from Ref. 32.

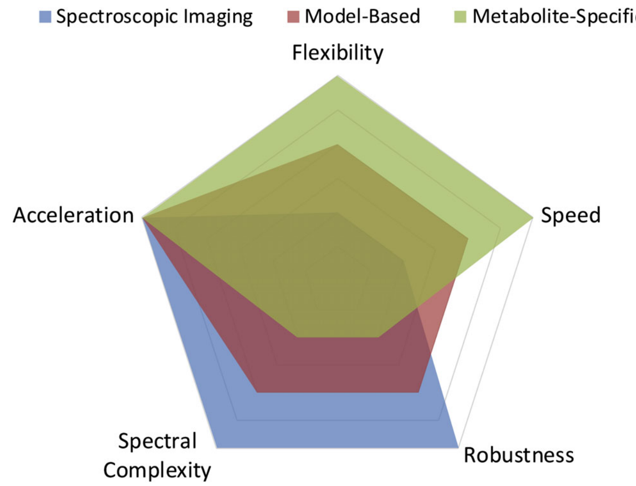

FIGURE 2: Overview of the main categories of fast hyperpolarized metabolic imaging methods and their corresponding advantages and disadvantages. Acceleration refers to compatibility with compressed sensing/parallel imaging; Flexibility refers to the available acquisition and tailored flip angle schemes; Speed refers to acquisition time; Robustness refers to sensitivity to bulk frequency errors and B_0 inhomogeneity; Spectral complexity refers to compatibility with substrates that have complicated spectra (ie, numerous resonances, poor spectral separation, j-coupling, etc.). The tradeoffs between the categories are described in greater detail in Table 1, as well as throughout the text.

polarization compared to thermal equilibrium at room temperature and clinical MRI field strengths.[16] In dissolution DNP, the ^{13}C-labeled substrate is mixed with an electron paramagnetic agent (EPA) and placed at low temperature (~1K) and at high magnetic field (typically ≥5T). Under these conditions, the free electron in the EPA is nearly 100% polarized, and microwave irradiation around the electron spin resonance frequency is applied to transfer this polarization to the ^{13}C nuclear spins to create a hyperpolarized state. After ~1–2 hours of microwave irradiation, the frozen mixture is rapidly dissolved with superheated water and neutralized to physiologic pH, creating an agent suitable for injection. However, the hyperpolarized state is transient and relaxes to thermal Boltzmann equilibrium with time-constant T_1 (eg, for [1-^{13}C]pyruvate $T_1 \approx 50$ seconds in solution, $T_1 \approx 30$ seconds in vivo). This T_1 decay, as well as the rapid metabolic conversion (via ^{13}C label exchange), necessitates fast imaging techniques that can capture rapidly evolving signals within 1–2 minutes following injection.

This review will discuss the current state-of-the-art in fast imaging techniques for HP ^{13}C MR metabolic imaging. While dissolution DNP has been the primary polarization mechanism for human applications, the imaging strategies discussed here are equally amenable to hyperpolarization via other processes, such as PHIP or SABRE.[17,18] These techniques can generally be grouped into three categories: 1) spectroscopic imaging; 2) model-based approaches; and 3) metabolite-specific imaging. The imaging speed typically increases from spectroscopic to metabolic-specific imaging, but with increasing constraints, and are summarized in Fig. 2 and Table 1. The review will also cover several sequence options that can be combined with these imaging techniques, including parallel imaging and compressed sensing acceleration, tailored flip angles, spin refocusing methods, and real-time calibrations (summarized in Table 2.).

TABLE 1. Strengths and Weaknesses of the Three Main Imaging Methods for Hyperpolarized ^{13}C MRI

Method category	Pros	Cons
Fast spectroscopic imaging	No prior knowledge of spectrum required. Insensitive to B0.	Slower than other methods.
Model-based/ hybrid imaging	Rapid imaging of complex spectra.	Requires prior knowledge and model of the spectrum.
Metabolite-specific imaging	Very fast and RF efficient. Robust to motion.	Requires a sparse spectrum. Sensitive to B0 inhomogeneities.

TABLE 2. Overview of Additional Sequence Options That Can Be Combined With the Main Categories of Imaging Techniques Described in Table 1

Sequence options	Pros	Cons
Parallel imaging	Accelerate acquisitions using multichannel receive arrays.	^{13}C coil sensitivities challenging to measure. g-factor SNR penalty.
Compressed sensing	Accelerate acquisitions based on data sparsity.	Tuning of regularization parameters. Excessive denoising possible.
Tailored flip angles	Metabolite-specific flip angles to improve metabolic product SNR. Variable flip angles to increase overall SNR.	Increased sensitivity to perfusion variability and flip angle miscalibration.
Refocused methods (spin-echoes, SSFP)	Improve SNR with spin refocusing.	Refocusing pulses introduce B_1+ sensitivity and saturation. Dynamic imaging is challenging.
Real-time calibration	More reproducible results with timing, B_0, and B_1 calibration. B_1+ maps improve SNR and accuracy of quantifications.	Utilizes HP magnetization (<5%) for calibration. Requires highly specialized pulse programs. Delays start of main acquisition.

Fast Spectroscopic Imaging

Magnetic resonance spectroscopic imaging (MRSI) can provide spatial and spectral encoding to localize and resolve HP ^{13}C-labeled metabolites. MRSI techniques have the advantage that they provide a continuous spectrum that can be analyzed to extract expected as well as unexpected resonances, making this approach very robust and the go-to method for exploratory HP studies. The most straightforward method is phase-encoded chemical shift imaging (CSI), which offers a large spectral bandwidth and high spectral resolution. However, the main challenge in performing hyperpolarized MRSI is imaging speed, and CSI is quite slow because it is a pure phase-encoded sequence. For example, CSI requires $N_x \times N_y$ RF excitations for a single slice, where $N_x \times N_y$ is the number of voxels in the 2D spatial array. Even a coarse 8×8 matrix requires 64 RF excitations, resulting in a long acquisition time (5+ seconds) for a single slice. It is most well-suited for preclinical studies with small fields of view (FOVs)[19,20] or for substrates with complicated spectra, such as [2-^{13}C] pyruvate,[21] as its poor temporal resolution can hamper measurements of metabolic conversion and precludes volumetric coverage.

Rapid spectroscopic imaging techniques employing switched time-dependent or echo-planar type gradients applied during acquisition can greatly reduce the scan time for HP experiments compared to phase-encoded CSI. Joint spectral and spatial encoding is accomplished by traversing *k*-space at multiple echo times (TEs), shifted in time by ΔTE (Fig. 3). By acquiring the same *k*-space point (k_x, k_y) at multiple TEs, a Fourier transform along the echo dimension produces a spectrum at each *k*-space point, reducing the scan time by the number of acquired points in the frequency-encoded dimension. The spectral and spatial encoding for rapid MRSI techniques with switched gradients can be achieved with numerous *k*-space trajectories, including echo-planar spectroscopic imaging (EPSI),[22] spiral,[23] radial,[24] and concentric rings,[25] all of which reduce the number of excitations and thus the scan time compared to phase-encoded CSI. It is important to note that by explicitly encoding the spectral dimension, non-Cartesian trajectories that might normally be sensitive to off-resonance artifacts are rendered amenable to MRSI. All of these non-Cartesian rapid MRSI trajectories can be reconstructed using similar algorithms applied to non-Cartesian MRI such as gridding[26] or the nonuniform fast Fourier transform (nuFFT[27]).

However, the resulting speed advantage with EPSI comes at a cost of a coarser spectral resolution and limited spectral bandwidth (SBW $\approx 1/\Delta$TE), and also results in tradeoffs between spectral bandwidth, spatial resolution, and signal-to-noise ratio (SNR) efficiency.[22] This tradeoff is compounded by the fact that the ^{13}C gyromagnetic ratio is roughly one-fourth that of ^{1}H, further increasing the demand on gradient strength and slew-rate and requiring the gradients to work four times as hard to achieve the same *k*-space coverage and ΔTE. This is particularly problematic at higher B_0 because the chemical shift difference (in Hz) between metabolites scales linearly with field strength, so that even higher bandwidths must be accommodated. To increase the spectral bandwidth, rapid spectroscopic imaging *k*-space trajectories, including echo-planar, spiral, and rings, can be interleaved by echo-shifting and/or rotating the trajectory, albeit at a cost of increased scan time. The tradeoff between several key parameters for different rapid MRSI strategies is summarized in Fig. 3.

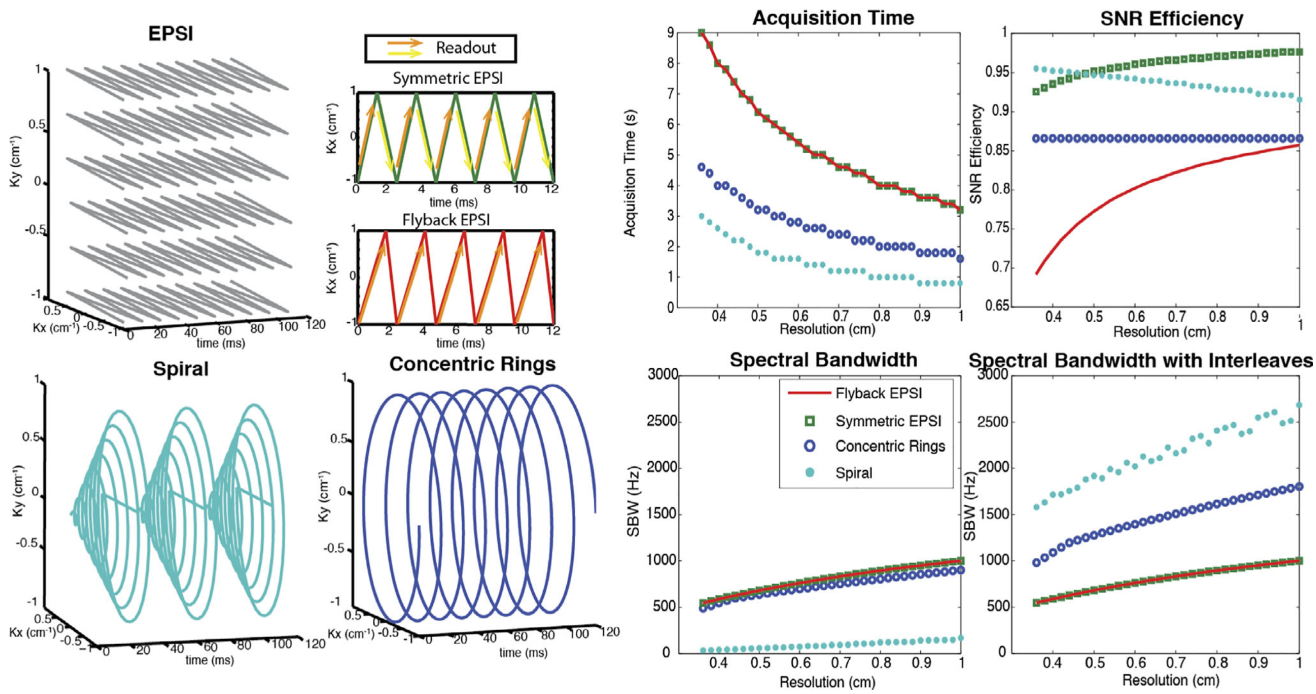

FIGURE 3: \Illustration and comparison of several rapid MRSI methods employing switched/time-dependent read-out gradients. Left two columns: *k*-space trajectories for EPSI (symmetric and flyback), spiral and concentric rings spectroscopic imaging. Right two columns: Design tradeoffs between spatial resolution, spectral bandwidth, acquisition time, and SNR efficiency, assuming typical clinical MRI system gradients with a maximum amplitude of 40 mT/m and maximum slew rate of 150 mT/m/ms. EPSI is the slowest, but symmetric EPSI trajectories have very high SNR efficiency. The concentric rings method requires half of the total acquisition time compared with the EPSI trajectories, offers about 87% SNR efficiency, and provides much wider spectral bandwidth than either flyback or symmetric EPSI. Although spirals are nominally the most efficient trajectories (offering the fastest acquisition time and highest spectral bandwidth while sacrificing the least SNR), they are limited by their sensitivity to gradient infidelities. Adapted from Ref. 25.

Alternatively, accelerated spectroscopic imaging can also be performed with spatiotemporal encoding (SPEN), which has additional requirements of frequency swept excitation pulses whose effects are demodulated in the reconstruction. Inspired by multidimensional spectroscopy, SPEN utilizes a frequency-chirped RF excitation to impart a spatially-dependent quadratic phase.[28] Gradients applied during the readout shift the position of this localized point through physical space rather than *k*-space to construct an image.[29] In SPEN, spatial information is recorded in the magnitude of the free induction decay (FID) and spectral information is inherently recorded in its phase. This eliminates the need to explicitly encode spectral information using an EPSI-like readout.

Since the location of the sample is not encoded into the phase and demixed with a Fourier transform, like in conventional MRI, the SPEN technique is especially robust to B_0 field inhomogeneities.[30] SPEN has recently been extended to dynamic and multislice spectroscopic imaging of hyperpolarized compounds in both healthy rats and in a murine lymphoma model, showing good spatial agreement when compared to 2D CSI and good temporal agreement when compared to 1D dynamic spectra.[31] With a temporal resolution on the order of 100 msec, SPEN will find utility when trying to acquire data with rapid reaction rates or where B_0 homogeneity is poor.

The fast MRSI approaches form the backbone of several important pilot studies of hyperpolarized ^{13}C in cancer patients. This includes malignancies such as brain cancer,[32,33] primary[34–36] and metastatic[37] prostate cancer, renal cell carcinoma,[38] and pancreatic adenocarcinoma[39] (Fig. 4). The ability to cover a continuous chemical-shift spectrum allows resolution of downstream metabolic products without a priori knowledge of their identities or chemical shifts. Such chemical resolution is essential for pilot patient studies investigating new diseases or organs of interest, drug targets, and metabolic pathway inhibition, or in the setting of HP probe development.

A potential limitation of fast spectroscopic imaging is the relatively long scan times vs. imaging-based strategies, owing to the need to encode the spectral dimension. Such a limitation can be partially offset by migrating to an imaging-based acquisition strategy (see the following section) in later phases of a clinical study where the ^{13}C substrate and products are assigned, and ΔB_0/susceptibility has been better characterized for the target of interest. Having said this, the fast MRSI approaches still retain important applications in those scenarios where quantitative accuracy and microenvironment characterization have priority over spatial coverage, for probe development when the metabolites are not yet known, or for when spatial localization is limited by the coil, such as prostate studies using an endorectal receive coil.

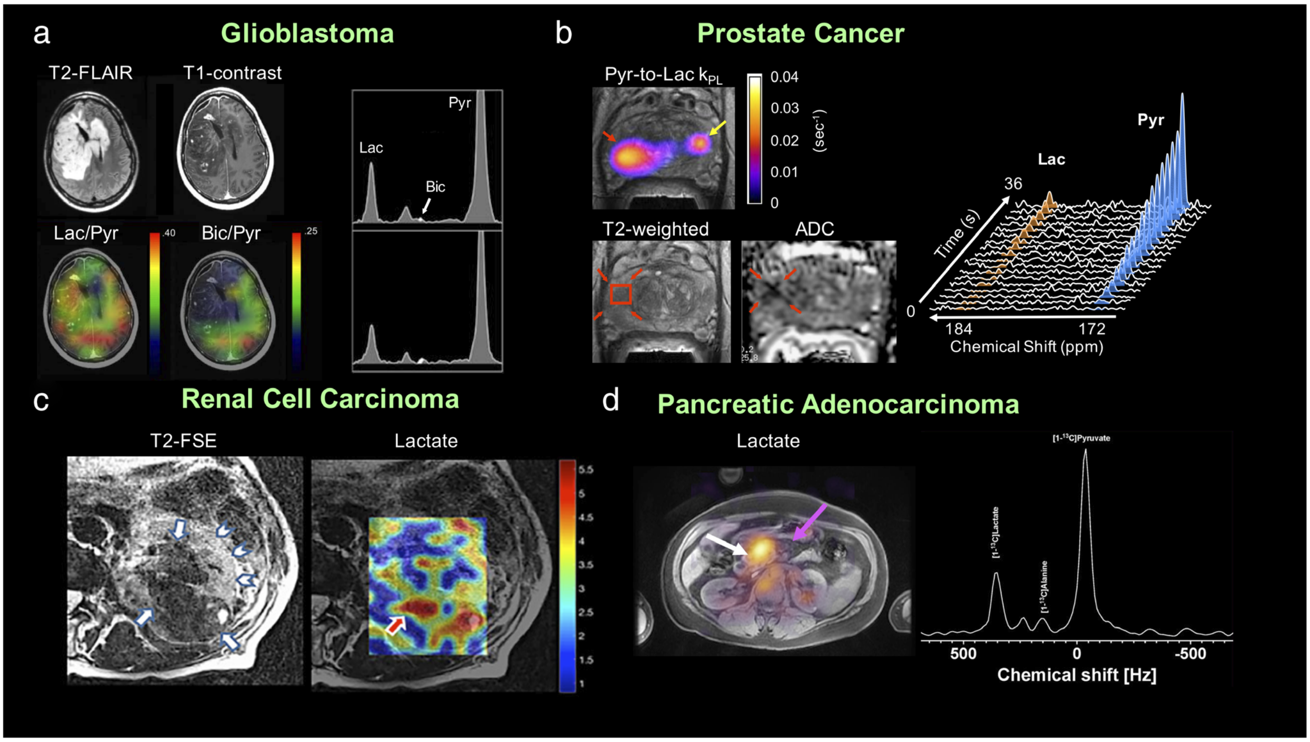

FIGURE 4: Proof-of-concept human studies based on fast spectroscopic imaging approaches applied in (a) brain tumor (b) primary prostate cancer (c) renal cell carcinoma, and (d) pancreatic cancer with example images and spectra, highlighting the ability to extract spatially-resolved information of metabolism without a priori knowledge of the product identities. Figure adapted from Refs. 32,36,38,39.

Model-Based and Hybrid Spectroscopic Approaches

Model-based approaches can be combined with spectroscopic imaging to reduce the scan time in a hyperpolarized experiment. As with fast spectroscopic imaging, data are acquired at multiple echo times to jointly encode the spatial and spectral information. By taking advantage of prior knowledge, fewer echoes need to be acquired, accelerating the acquisition. HP ^{13}C spectra are amenable to model-based techniques because of the readily available prior knowledge of the number and relative chemical shift of ^{13}C resonances. The prior knowledge is then used to aid in the reconstruction of the undersampled data. In this section, we discuss two types of model-based reconstructions: chemical shift encoding (CSE) and spectroscopic imaging by exploiting spatiospectral correlation (SPICE).

Chemical Shift Encoding

CSE methods, which include "Dixon" and IDEAL (iterative decomposition with echo asymmetry and least squares estimation),[40] assume a priori knowledge of the substrates and products. For example, after injection of hyperpolarized [1-^{13}C]pyruvate, [1-^{13}C]pyruvate-hydrate, and the metabolites [1-^{13}C]lactate, [1-^{13}C]alanine, and ^{13}C-bicarbonate can be observed (Fig. 1). Rather than having to satisfy the Nyquist sampling rate for accurate reconstruction of a spectrum, CSE can reconstruct the expected resonances from far fewer echoes than would otherwise be possible.

For N different types of molecules, the signal at location r and echo time TE_m after excitation for CSE is modeled as[41,42]:

$$S_r(TE_m) = \left(\sum_{n=1}^{N} \rho_n \left(\sum_{j=1}^{J_n} q_{n,j} e^{i2\pi \Delta f_{n,j} TE_m}\right)\right) \exp(i2\pi\psi_r TE_m)$$

where TE_m is the m_{th} echo time, and Ψ_r is the off-resonance due to the main magnetic field at location r. For the molecule type indexed by n, there are J_n spectral peaks with Larmor frequencies $\Delta f_{n,j}$ and relative intensities $q_{n,j}$ such that $q_{n,1} + q_{n,2} + \ldots q_{n,j} = 1$ for all n. Note that ρ_n is a complex number for all n. The field map Ψ can be determined from ^{1}H data and scaled to the ^{13}C frequency. The matrix inversion can occur in either k-space[42–44] or image-space[41] to extract images for each metabolite of interest (Fig. 5).

Model-based approaches have been used preclinically in the study of kidney,[45,46] cardiac,[47] and tumor metabolism,[42,48] and in healthy volunteers[49] to characterize brain metabolism. The advantage with CSE is that only N echoes are required to solve the system given the measured ^{1}H field map, reducing the total scan time compared to encoding the full spectrum with MRSI. However, the echo

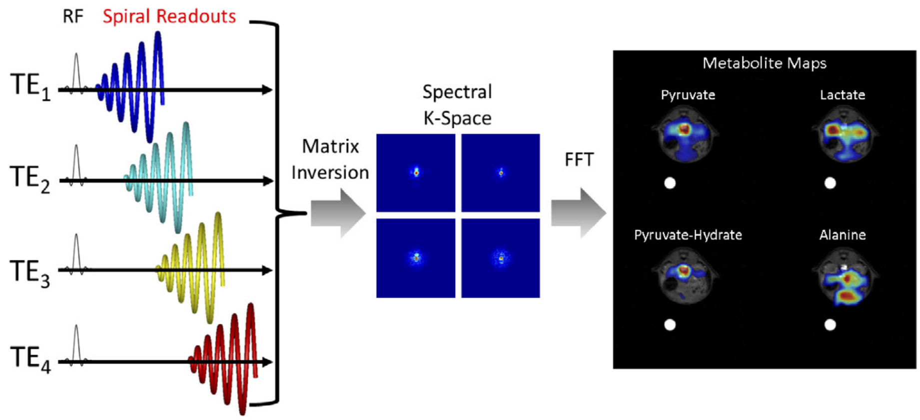

FIGURE 5: Schematic illustrating the acquisition and reconstruction of a model-based spiral pulse sequence. In this illustration, data are acquired with a long duration spiral readout that is spoiled at each TR. Each subsequent excitation is shifted in time by ΔTE. For non-Cartesian approaches, a matrix decomposition occurs in *k*-space, yielding *k*-space data for each metabolite. A subsequent gridding or nonuniform fast Fourier Transform (nuFFT) step yields spectral images for each hyperpolarized metabolite. (Reproduced with permission from Ref[95].)

spacing needs to be carefully chosen to avoid noise amplification in the matrix inversion. This can be optimized by calculating the effective number of signal averages (NSA) for each ^{13}C metabolite,[41] which is a measure of relative SNR for each metabolite and is a function of chemical shift and sampled echo-times. Choosing an optimal echo spacing becomes more difficult when many resonances are present, or when spectral peaks are poorly separated. Frequency swaps can also occur if the B_0 field map is unaccounted for or if it changes during the acquisition, potentially leading to quantification errors and introducing artifacts.

SPectroscopic Imaging by Exploiting Spatiospectral CorrElation (SPICE)

An alternative model-based method is SPectroscopic Imaging by Exploiting Spatiospectral CorrElation (SPICE). SPICE is a combined data acquisition protocol and image reconstruction algorithm that exploits the partial separability inherent in spectroscopic signals[50,51] to reconstruct high-resolution spectroscopic imagery. SPICE is comprised of two separate data collections: one is of low-spatial and high-temporal resolution (ie, high spectral bandwidth), while the other is of high-spatial and low-temporal resolution.[52] The data acquisition protocol is depicted in Fig. 6. The first acquisition is a phase-encoded CSI sequence used to estimate the spectral signatures of the molecules in the imaged sample. The reconstructed imagery is constrained so that each voxel generates a linear combination of these signatures. The second sequence is an EPSI sequence, used to attain a sparse sampling of high spatial-resolution data.

From the CSI acquisition, a 2D spatial Fourier transform reconstructs a high-temporal but low-spatial resolution dataset. It is assumed that each voxel is comprised of a small number of different molecules. In this case, the temporal signal exhibited by any voxel can be expressed as:

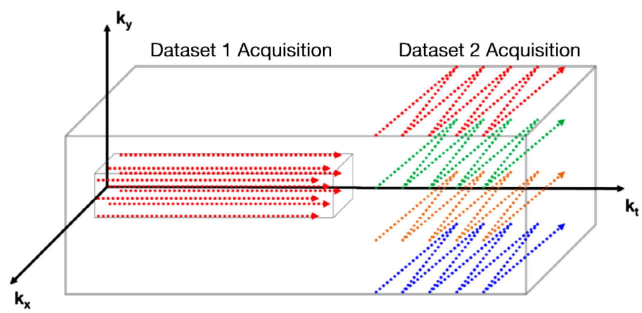

FIGURE 6: Graphical depiction of the SPICE acquisition. The acquisition is comprised of two stages. The first stage acquires a low spatial resolution / high spectral bandwidth dataset (D1) with a CSI sequence. The data of this stage are used to identify the spectral signals images. The second stage acquires a high spatial resolution / low spectral bandwidth dataset (D2) with an EPSI sequence. These two datasets are combined to generate spectroscopic images with high spatial and spectral resolution.

$$\rho(r,t) = \sum_{j=1}^{J(r)} c_j(r)\varphi_j(t)$$

where φ_j is the temporal signal exhibited by molecule j and c_j is the amount of molecule j at location r. By exploiting the partial separability of this model, the individual spectral signatures are identified.[50,51] SPICE next reconstructs high-resolution imagery by constraining the output of each voxel to be a linear combination of the known signals. SPICE reconstructs the high-resolution spectroscopic imagery by solving the follow optimization problem:

$$\text{minimize} \left(\frac{1}{2}\right) \|DF_B\Phi\|_{Fr}^2 + \lambda\Psi(\Phi C)$$

where $\|\cdot\|_{\mathrm{Fr}}$ represents the Frobenius norm, F_B is a transformation that performs a Discrete Fourier Transform and removes off-resonance due to B_0 field inhomogeneity, Φ is a matrix of the spectral signatures, Ψ is a regularization function, and $\lambda \geq 0$ is a regularization parameter. In,[53] Lam et al set $\Psi(\Phi C) = \|DC\Phi\|_{W,\mathrm{Fr}}$ where $\|\cdot\|_{W,\mathrm{Fr}}$ is a weighted Frobenius norm with weights W and D is a finite difference operator. This yields a least-squares problem, which can be solved with known algorithms.[54]

While SPICE has been used extensively in ^{1}H MRSI,[55–57] it has only recently been applied to hyperpolarized ^{13}C applications. Lee et al[52] applied SPICE at 9.4T to create spectroscopic images of mouse kidneys at high resolution (0.47 x 0.47 mm^2) for a single timepoint, showing that SPICE reconstruction outperformed a standard EPSI approach. Song et al[58] extended SPICE to a single-slice dynamic acquisition. The calibration dataset was acquired once, but 10 separate high-spatial resolution datasets were acquired every 2 seconds, with a reconstructed spatial resolution up to 1 x 1 mm^2. An important constraint to the dynamic SPICE scheme is choosing an appropriate scan timing for D1 in order to derive a complete basis of substrate and products. This may offset the subsequent D2 timing, missing the initial pyruvate-to-lactate dynamics and therefore deteriorating quantitative accuracy of disease metabolism.

Metabolite-Specific Imaging

Fast spectroscopic imaging and model-based imaging approaches acquire spectral and spatial information simultaneously, decoupling multiple metabolite signals using multi-echo readouts. Alternatively, metabolite-specific imaging uses a specialized RF pulse and a rapid imaging readout to encode the spectral and spatial domains in two distinct steps. In this approach, a single-band spectral-spatial RF pulse that is both frequency- and slice-selective performs the spectral encoding, exciting a single metabolite within a slice (or slab).[59] A rapid imaging readout trajectory, typically single-shot echo-planar[60,61] or spiral,[62] is then used to spatially encode the magnetization as a 2D multislice or 3D slab encoded dataset. The acquisition then cycles through the resonances of interest over time to acquire a volumetric dynamic dataset for each metabolite (Fig. 7). An example of metabolite-specific echo-planar imaging (EPI) can be seen in Fig. 8, showing pyruvate uptake and conversion to lactate and bicarbonate throughout the brain at a spatial resolution of 1.5 × 1.5 × 2.0 cm^3.[63] These total signal (area under the curve) images show that artifact-free data can be acquired with rapid imaging readouts in the clinical setting, enabling volumetric coverage of the whole brain with a temporal resolution (3 seconds in this study) equivalent to that of single slice EPSI. This type of approach is inherently more flexible than spectroscopic imaging or model-based sequences, as the metabolites of interest can be selectively excited and encoded, eliminating the need to encode the entire spectrum. The total acquisition time can also be significantly reduced if single-shot readouts are employed, greatly reducing the scan time and making the acquisition more robust to motion compared to an MRSI acquisition.

However, metabolite-specific imaging requires a sparse spectrum with well-separated resonances, which is dependent on the chemical shift of metabolites and the operating field strength. This approach is therefore not applicable to all substrates but is well suited to imaging of [1-^{13}C]pyruvate (Fig. 1), since all five resonances are separated by at least 3 ppm, or 93 Hz at 3T. Because each metabolite is excited and encoded separately, optimized flip angle strategies that

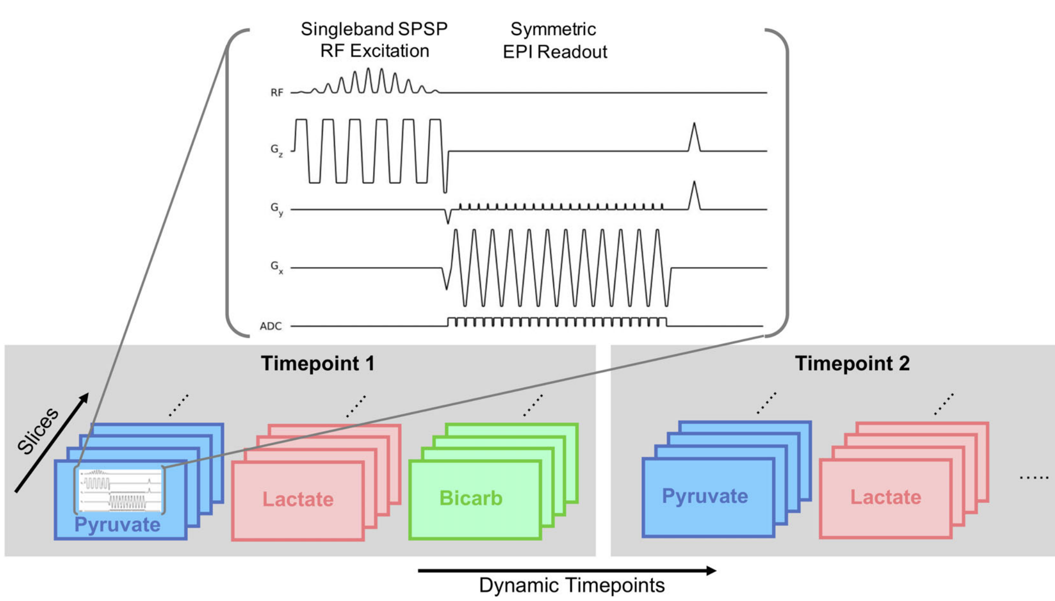

FIGURE 7: Depiction of a multislice metabolite-specific acquisition using EPI. The sequence provides volumetric coverage for all metabolites of interest by shifting the spectral–spatial (SPSP) passband to separately excite and encode each resonance. This is repeated through time to acquire a volumetric and dynamic dataset for all metabolites of interest. Figure adapted from Ref. 61.

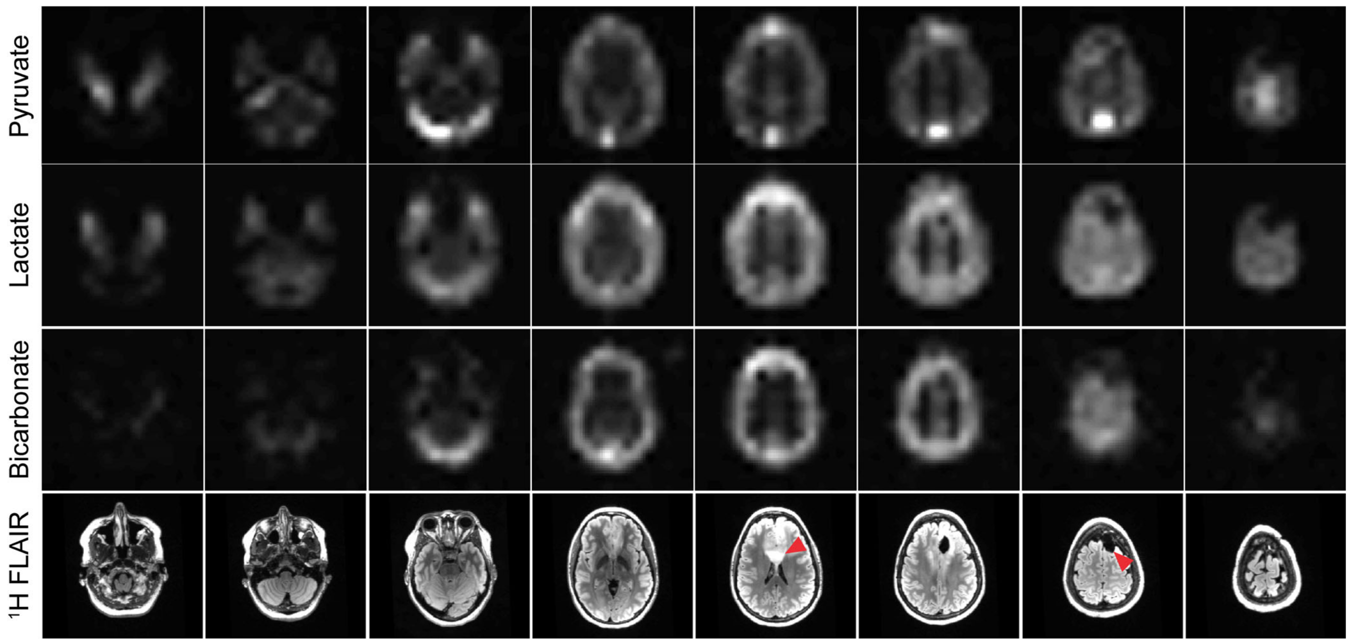

FIGURE 8: Area under the curve (sum through time) images of pyruvate, lactate, and bicarbonate for the eight slices covering the entire brain. In this experiment, a spectral–spatial RF pulse was used to separately excite each metabolite, which was then encoded with a single-shot echo-planar readout. Artifact-free data can be acquired with rapid imaging readouts in the clinical setting, enabling volumetric coverage of the whole brain with a temporal resolution (3 seconds) equivalent to that of single slice EPSI. Figure adapted from Ref. 63.

provide metabolite-specific flip angles can be easily integrated, which have been shown to increase SNR over a constant flip angle scheme.[64,65]

The rapid imaging readouts used in metabolite-specific imaging are also more sensitive to center frequency errors and B_0 field inhomogeneity when compared with spectroscopic or model-based approaches. This will manifest as geometric distortion or blurring in metabolite data acquired with echo-planar and spiral readouts, respectively, due to the accumulation of phase during the readout. These artifacts can be partially mitigated by shortening the readout duration, albeit at the cost of reduced SNR efficiency. They can also be reduced through off-resonance and distortion correction strategies developed for ^{1}H MRI that are also compatible with hyperpolarized ^{13}C MRI. For spiral, autofocus algorithms[66] have been used to correct for B_0-induced blurring. For EPI, an alternating blip strategy[67] or integrated dual-echo readout[68] can be used to estimate and correct for B_0 distortion. Symmetric echo-planar readouts additionally can suffer from Nyquist ghost artifacts due to inconsistencies between the phase encodings, which can be corrected for by estimating the phase coefficients from a ^{1}H reference scan using the ^{13}C waveform[61] or via an exhaustive search.[69]

Because the RF pulse performs the spectral encoding, proper frequency calibration is crucial. Miscalibration of the center frequency and the B_0 field inhomogeneity will reduce the applied flip angle due to the narrow passband of the spectral–spatial (SPSP) RF pulse (typically ± 2.5 ppm full-width at half-maximum (FWHM) for [1-^{13}C]pyruvate applications). The reduced flip angle will lead to overall reduced SNR and can potentially bias quantification if left unaccounted for.

The remaining sections will focus on additional pulse sequence options that are compatible with the acquisition methods discussed above. These sequence options can be used to improve scan coverage, speed, SNR, resolution, and/or robustness in a hyperpolarized experiment.

Refocused Imaging Methods

Because of the nonrenewable magnetization, the imaging methods discussed in the previous sections are all typically acquired as gradient echo sequences, where transverse magnetization is spoiled at the end of each relaxation time (TR). A potentially more efficient way to use nonrenewable hyperpolarized signals is to repetitively refocus transverse spins, which is especially valuable for imaging metabolites with long T_2 seconds such as [1-^{13}C]pyruvate, [1-^{13}C]lactate, and [^{13}C, $^{15}N_2$] urea.[70–74] Two types of sequences have been explored for spin refocusing of hyperpolarized substrates: the balanced steady-state free-precession (bSSFP) sequence and the fast spin echo (FSE) sequence.

bSSFP Sequences

In the bSSFP sequence, a train of refocusing pulses with angle θ is applied with alternating polarity and net gradient areas that are always zero between two neighboring refocusing pulses. Typically, each bSSFP timepoint is preceded by a $\theta/2$ preparation pulse TR/2 before the first imaging repetition to reduce transient state oscillations, and is ended by a $\theta/2$ tip back pulse to return magnetization to the longitudinal axis.[75] The $\theta/2$ preparation pulse can be replaced by a set of linear ramped preparation pulses to improve its robustness to off-resonance frequencies and power variations.[76]

The bSSFP sequence has an intrinsic periodic frequency response (Fig. 9a) at repetitions of 1/TR. The choice of flip angle of the excitation passband in the bSSFP sequence is a tradeoff between alleviating banding artifacts and preserving magnetization for dynamic imaging. A large flip angle (>100°) is favorable to reduce banding artifacts but limits the total available scan time because the majority of the magnetization is tipped into the transverse plane, leading to signal decay via T_2 rather than T_1 relaxation. However, to perform dynamic imaging in hyperpolarized studies, a small flip angle is necessary to preserve sufficient magnetization for subsequent timepoints. An intermediate flip angle (eg, 60°) can be used to achieve a compromise between the two factors. In addition to TR and flip angle, the spectral response of the RF excitation profile can also influence the bSSFP signal[73] and must also be taken into consideration to avoid off-resonance excitation.

Three bSSFP strategies have been used in hyperpolarized ^{13}C studies (Fig. 9b–d). The first strategy (Fig. 9b) utilized a broadband pulse to excite all metabolites and reconstructed the spectral information from multiecho readouts using CSE[70] or fitting of multiple acquisitions with variable phase advance.[77] This strategy is advantageous to acquire all metabolites at the same time, but it requires long TRs and limits the optimization of scan parameters (eg, flip angle, resolution) for individual compounds.

The second strategy (Fig. 9c) excites one metabolite at a time and has been applied for imaging metabolically inert agents with a single HP resonance such as urea,[8,72,74,75,78–80] or imaging multiple metabolites at ultrahigh field (eg, 14.1T)[73,81] (Fig. 10). At ultrahigh fields, frequency separation between metabolites is sufficiently large to enable spectrally selective RF pulses short enough to meet the short TR constraint in the bSSFP sequence. However, adapting this strategy to clinical field strength at 1.5T or 3T is challenging due to the long RF pulses needed to obtain spectral selectivity.

The third strategy (Fig. 9d) extends metabolite specific imaging with bSSFP to lower field, where the frequency separation between metabolites can hamper spectral selectivity, by using a chemical saturation pulse to suppress undesired signals at the beginning of each timepoint.[71] This strategy alleviates the constraint of RF pulse duration. However, the

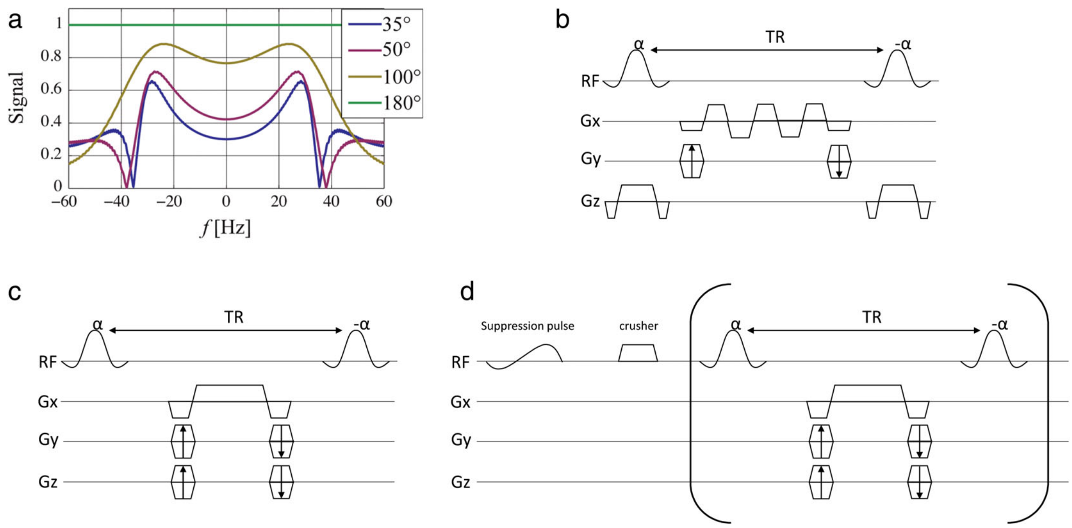

FIGURE 9: Simulated SSFP signal as a function of frequency and flip angle for TR = 14.3 msec (a). The choice of flip angle in the bSSFP sequence is a tradeoff between alleviating banding artifacts with high flip angles and preserving magnetization for dynamic imaging with lower flip angles. Pulse sequence diagrams for bSSFP, including (b) multiecho readouts to decompose spectral information, (c) metabolite specific acquisition, and (d) metabolite specific acquisition with spectral suppression of undesired signal at the beginning of the acquisition. Portions of this figure are adapted from Ref. 72.

presaturated signal may replenish due to inflow or metabolism and contaminate later data, and saturation performance also heavily depends on a homogeneous transmit B_1 profile.

FSE Sequences

FSE sequences, also known as turbo spin-echo (TSE) or rapid acquisition with relaxation enhancement (RARE), typically use 180° refocusing pulses surrounded by a pair of identical crusher gradients. Compared with bSSFP sequences, FSE sequences are substantially less sensitive to B_0 inhomogeneity, and have no constraint of short TR, which allows a longer RF excitation pulse to achieve a better metabolite selection. Refocusing pulse design can be either Shinnar–Le Roux (SLR) pulses or adiabatic inversion pulses.

SLR pulses are sensitive to B_1 variation, and thus susceptible to imperfect refocusing and loss of nonrenewable hyperpolarization, making them most suitable for single timepoint imaging. Sukumar et al[82] utilized a 3D FSE sequence with a 180° SLR pulse and EPI readouts to acquire 3D volumes from a single timepoint in copolarized studies with [1-^{13}C]pyruvate and [^{13}C]urea at 14.1T. Yen et al[83] applied a non-CPMG[76] echo train with SLR refocusing pulses to improve the realignment of longitudinal magnetization as well as to keep the transverse magnetization refocused.

Compared with SLR pulses, adiabatic pulses are insensitive to B_1 above the adiabatic threshold, which is crucial to preserve hyperpolarized magnetization. However, under the constraint of the specific absorption rate (SAR) limit, the high RF power of adiabatic pulses limits the density of these pulses, making it challenging for clinical translation. Wang et al developed a 3D FSE sequence using adiabatic pulses and stack of spiral readouts on a preclinical 7T system, with data acquisition either at only even echoes[84] or at all echoes.[85] In a later work,[85] the authors found the quadratic phase variation of transverse magnetization to be small (<0.02 rad) over a small range (±50 Hz) of frequency variation, and thus signals can be acquired at echoes formed by unpaired adiabatic pulses. However, the phase and magnitude profile of unpaired adiabatic pulses were not analyzed as a function of RF power, and phase mismatch between odd and even echoes could occur as a function of B_1 and lead to aliasing artifacts. Spatial B_1 inhomogeneity will also result in phase inconsistency across voxels, complicating the reconstruction. Therefore, the utility of signals at echoes formed by unpaired adiabatic pulses needs to be further evaluated.

Calibration Methods

Accurate RF calibration and variations in bolus timing between subjects presents significant challenges to acquiring robust and reproducible hyperpolarized datasets. Center frequency and RF power calibration is crucial to the success of all imaging approaches but is difficult to perform due to the lack of endogenous ^{13}C signal. One common strategy is to perform this calibration on an external ^{13}C-enriched phantom.[36,49,63] However, this approach does not fully account for the variability between subjects, or for variation in the B_1+ field of transmit RF coils. Susceptibility variations of tissues will contribute to spatial B_0 inhomogeneity, while subject loading causes a bulk RF power offset. As an alternative, center frequency calibration can be performed based on the measured water ^{1}H frequency and then converted to the ^{13}C operation frequency after accounting for differences in chemical shift.[86]

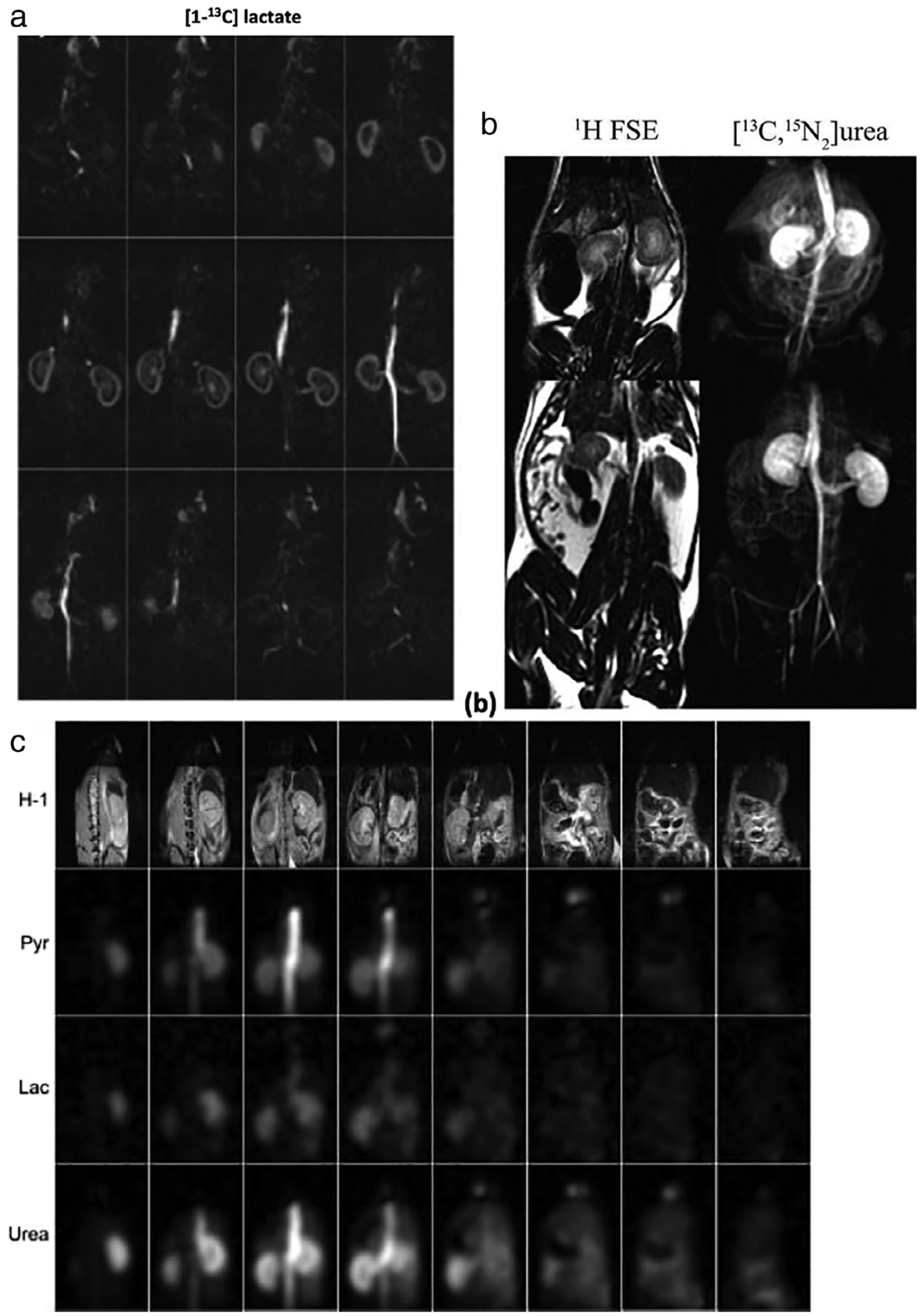

FIGURE 10: Representative bSSFP results from prior works. (a) Rat 3D kidney images of [1-^{13}C]lactate at 3T. (Reproduced with permission from Ref.[74]) (b) Rat kidney projection image of [^{13}C-, ^{15}N$_2$]urea at 3T. (Reproduced with permission from Ref.[72]) (c) 3D kidney images of copolarized [1-^{13}C]pyruvate and [^{13}C, ^{15}N$_2$]urea at 14T. (Reproduced with permission from Ref.[73])

Appropriate acquisition timing for hyperpolarized ^{13}C imaging is also important to avoid saturation of the non-recoverable hyperpolarized spins during bolus arrival, particularly near the transmitter conductive elements where the B_1^+ is high. Many variable flip angle schemes[87–90] also assume knowledge of the bolus shape and timing, but variability in bolus kinetics leads to quantification errors in the context of ratiometric measures.[91,92] A fixed delay between injection and acquisition have been employed in many hyperpolarized ^{13}C imaging studies[33,34,49,62] but the empirically determined delay time can be unreliable due to the inherent physiological variability between subjects, and in human cancers where the vascularization and perfusion are highly variable over subjects.[93] This is particularly problematic in human subjects in which timing differences of up to 12 seconds have been observed in hyperpolarized ^{13}C studies of prostate cancer patients.[34,35]

Variable perfusion characteristics can be resolved using a bolus tracking technique to trigger the acquisition upon pyruvate arrival, as demonstrated in work by Durst et al[91] and Tang et al.[94] Center frequency and RF power calibration using a Bloch–Siegert approach can also be incorporated into bolus tracking,[94] eliminating the challenges associated with RF calibration and variations in bolus timing. The primary disadvantage of using a real-time bolus tracking framework is the engineering efforts to address system compatibility issue and the potentially complicated analysis of bolus kinetics.

Tailored Flip Angles

In hyperpolarized metabolic imaging, the flip angles must be chosen carefully to efficiently utilize magnetization before the irreversible signal decay to thermal equilibrium and to measure the dynamic metabolic conversion occurring during the experiment. The irreversible signal decay occurs both due to T_1 relaxation and RF excitation. This limits the overall duration of the hyperpolarized experiment as well as the number of measurements that can be made. In order to observe metabolic conversion to downstream metabolites, which are typically SNR-limited, care must be taken to not saturate the magnetization of the injected metabolite substrate.

There are two main strategies used to choose the flip angles for hyperpolarized metabolic imaging experiments: variable flip angles, in which the flip angles are changed throughout the acquisition, and metabolite-specific flip angles, in which the flip angle is varied across metabolites. Variable flip angle schemes take into account the effect of prior RF pulses throughout the acquisition to efficiently use the hyperpolarized magnetization, regardless of whether these are used to encode a single image or across timepoints. In a single image that requires multiple excitations, using constant flip angles will result in varying signal amplitudes, leading to *k*-space modulations that can create blurring or other artifacts depending on the phase-encoding scheme used.[95] Variable flip angle schedules that account for prior RF pulses can be used to eliminate this modulation, theoretically providing constant signal amplitudes across excitations. When acquiring multiple timepoints to measure the conversion kinetics, variable flip angles can be designed to maximize total SNR or improve estimates of conversion rates.[90] The main challenge using variable flip angles is that they are more sensitive to the timing of the acquisition relative to the bolus characteristics and to inhomogeneities in the transmit B_1 field.[96] Thus, they will work best when paired with the advanced calibration methods described above.

Metabolite-specific flip angles aim to preserve the magnetization of the hyperpolarized substrate and improve the SNR of the downstream metabolic products[64,65] (Fig. 11). This is feasible because the injected substrate (eg, pyruvate) is typically present at a much higher concentration, and thus the flip angle can be reduced while still maintaining adequate substrate SNR. Meanwhile, reducing the substrate flip angle preserves more of this magnetization for metabolic conversion, and downstream products can be excited with a higher flip angle to improve their SNR. The implementation of this strategy is straightforward in metabolite-specific imaging since only one metabolite is excited at a time. Multiband spectral–spatial RF pulses can be designed to modulate the flip angle across metabolites in spectroscopic or hybrid methods, albeit within the constraints of the RF pulse design algorithm.[64]

When using tailored flip angles, it is important to include these effects in the data analysis. This is made more challenging by the fact that each excitation, particularly those on the substrate, affects all downstream metabolite measurements as well as later timepoints. In kinetic modeling, arbitrary flip angle and acquisition timings can be incorporated using a hybrid discrete-continuous model.[90,92,96,97]

Another often neglected consideration is the slice profile effects.[98] These arise from the imperfect excitation profile of slice-selective RF pulses. Cumulative RF effects in the transition regions of slice-selective RF pulses, where the flip angles are less than expected, leads to excess signal after repeated excitations. This is especially pronounced later in the acquisition or when larger flip angles are used, potentially leading to quantification errors if unaccounted for. Several simple solutions include using smaller flip angles or performing 3D encoding. More complex solutions include using gradient scaling or redesigned RF pulses[99,100] and models that simulate all locations across the slice.[90]

Acceleration Strategies

Because of the limited lifetime of hyperpolarized substrates, reducing the total scan time and increasing temporal resolution can improve SNR and image quality. Both parallel imaging and compressed-sensing are applicable to hyperpolarized ^{13}C MRI and can be applied to the aforementioned imaging sequences discussed above.

Parallel imaging can be used to improve volumetric coverage and/or reduce the scan time in hyperpolarized experiments that utilize multichannel arrays. The undersampled data can be reconstructed either in the image domain using known coil sensitivities or in the spatial frequency domain from autocalibrating signals in the fully-sampled center of *k*-space. Because of the limited magnetization, coil sensitivities are typically not directly measured with the hyperpolarized substrate. Instead, the sensitivity maps can be estimated via numerical simulation,[101] from a separate precalibration scan using a thermal (nonhyperpolarized) ^{13}C phantom,[102] or they can be estimated from the fully sampled central region of *k*-space.[25,103] Calibrationless parallel imaging approaches (such as SAKE[104]) are also well suited for hyperpolarized substrates, as they eliminate the need to explicitly estimate coil sensitivity maps or acquire a fully-sampled center of *k*-space. Calibrationless parallel imaging has been used in conjunction

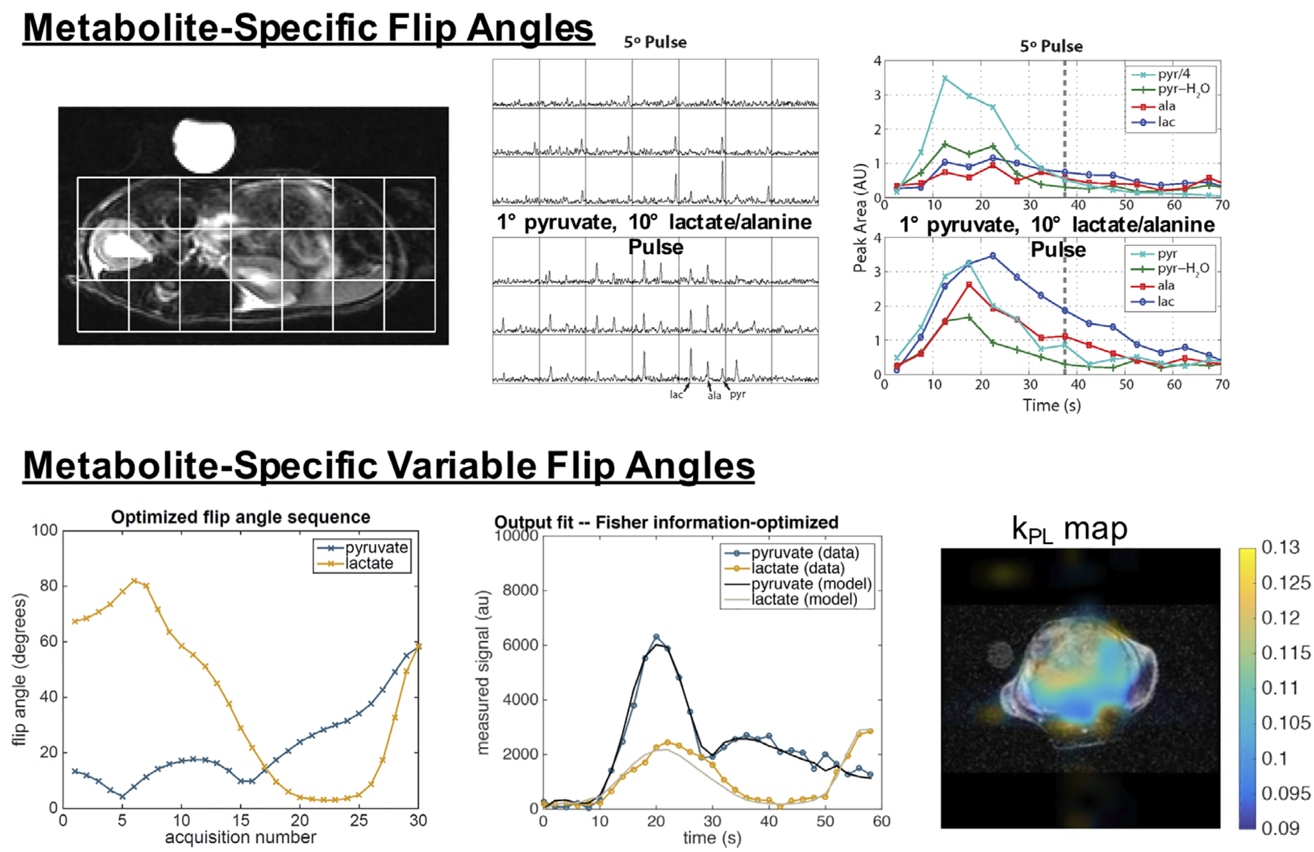

FIGURE 11: Top: Metabolite-specific flip angles, implemented in an MRSI acquisition using multiband spectral-spatial RF pulses, show substantial improvements over constant flip angle pulses. In this example, the multiband pulse applied a 1-degree flip angle to pyruvate (substrate) and 10-degree flip angle to the metabolic products of lactate and alanine to improve their SNR while maintaining adequate pyruvate SNR. (Adapted with permission from Ref.[64]) Bottom: A metabolite-specific variable flip angle schedule implemented in a metabolite-specific EPI acquisition, optimized for estimation of the pyruvate to lactate conversion rate, k_{PL}, for an expected set of experimental parameters. Fitting to data acquired with metabolite-specific imaging and the resulting k_{PL} map in a prostate tumor mouse show high-quality fits. (Adapted with permission from Ref.[90])

with a 4-fold undersampled 3D EPI acquisition to acquire cardiac and renal metabolic data in a single volumetric acquisition in a healthy human volunteer study using [1-^{13}C]pyruvate.[105] A simultaneous multislice acquisition with 2-fold acceleration and a large $48 \times 48 \times 24$ cm^3 FOV has also been successfully demonstrated for hyperpolarized ^{13}C imaging experiments using pigs.[106] However, it is important to note that parallel imaging can still reduce the overall SNR in a hyperpolarized experiment. While the acceleration penalty ($\sqrt{R}$) can be offset by increasing the flip angle to compensate for fewer RF excitations, g-factor noise amplification will still be present and will lead to an SNR penalty dependent on both the coil geometry[107] and sampling pattern.[25]

Acceleration can also be achieved through compressed sensing[108] and is particularly useful when multichannel arrays are not used. HP ^{13}C MRI is particularly amenable for compressed sensing because it is typically not limited by SNR but rather by encoding time, and the resulting spectra are typically sparse. Compressed sensing has been utilized in EPSI for human acquisitions using pseudorandom blip gradients that are applied during an EPSI readout and constraining the reconstruction based on sparsity in the spectral and dynamic imaging dimensions to achieve 3D MRSI with 2 seconds temporal resolution.[36,109,110] To overcome the bandwidth limitations of EPSI, blip gradients can be applied during the readout in phase-encoded 2D MRSI for both acceleration and high-bandwidth applications.[111] Compressed sensing has been demonstrated in 2D metabolic imaging of the rat heart using a single-shot echo-planar readout at a spatial resolution of $1 \times 1 \times 3.5$ mm^3 with up to 5-fold acceleration,[112] and in rat kidneys with a 3D EPI sequence using a psuedorandom blip scheme in the z-dimension, achieving up to 3-fold acceleration.[113] Compressed sensing can also be used in conjunction with model-based approaches to further accelerate the acquisition.[45,112] However, choosing the proper value for the regularization parameter λ requires numerical simulations or empirical studies to determine the tradeoff between data consistency and excessive denoising, which can impact quantification if not properly tuned.

Future Outlook

The current state-of-the-art for fast hyperpolarized metabolic imaging includes rapid spectroscopic imaging, model-based methods, and metabolite-specific imaging, which are summarized in terms of pros and cons in Table 1, as well as tradeoffs in terms of flexibility of acquisition, speed, robustness to field inhomogeneities, ability to capture complex spectral, and compatibility with acceleration methods are summarized in Fig. 2. The speed, SNR, and/or robustness of all of these approaches can be improved by the addition of parallel imaging, compressed sensing, tailored flip angle schemes, refocused imaging methods, and real-time calibrations. The pros and cons of these various sequence options are summarized in Table 2.

Our experience is that metabolite-specific imaging provides the best performance in terms of speed, coverage, and acquisition flexibility for human studies with [1-^{13}C] pyruvate, the most widely used hyperpolarized ^{13}C agent. The main failure mode of this approach is B_0 inhomogeneities, which has not been a major issue and is mitigated through ^{1}H-based shimming prior to ^{13}C studies. We have been using metabolite-specific flip angles, with lower flip angles on pyruvate and higher flip angles for the metabolic products, to improve the overall SNR. While we have used variable flip angles in past studies, we are not including them in the current studies due to their sensitivity to transmit B_1+ inhomogeneities and calibration errors, both of which have proven to be a limitation with current ^{13}C hardware. We have also been increasingly incorporating real-time calibrations of bolus timing, B_0, and B_1^+ to improve the reproducibility and SNR, and they also will facilitate the future use of variable flip angles to improve SNR and quantification.

Looking forward, we anticipate fast imaging improvements for other hyperpolarized agents, increasing spatial coverage, and improving SNR and/or resolution. For clinical applications of hyperpolarized agents beyond [1-^{13}C]pyruvate[21] or combinations of multiple agents, metabolite-specific imaging may no longer be an option due to increased spectral complexity and reduced spectral separation. For these studies, we anticipate that improved model-based approaches will provide fast imaging techniques. Future human studies will also likely have increased demands on spatial coverage (eg, for metastatic disease), which will require the use of parallel imaging and/or compressed sensing for acceleration. Refocused imaging offers incredible potential for improving the resolution and SNR of hyperpolarized studies several-fold due to the long T_2 relaxation times.[74,114] We are optimistic that these approaches will develop the selectivity and robustness needed for future human imaging studies.

In summary, current techniques for fast hyperpolarized metabolic imaging can provide rapid, volumetric, and dynamic imaging of metabolic conversion in human studies. These techniques will most certainly improve in terms of SNR, resolution, coverage, and reproducibility with additional hardware, pulse sequence, and reconstruction developments.

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