

IMAGING LACTATE METABOLISM IN BREAST CANCER WITH HYPERPOLARIZED ^{13}C
MAGNETIC RESONANCE IMAGING

by

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Abstract

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Breast cancer is a heterogeneous disease. Some patients attain prolonged control with first-line therapies, while others present with highly aggressive, treatment resistant malignancies. Second- and third-line treatments often carry the risk of severe, potentially life threatening side effects and only a minority patients ultimately live longer because of them. The difficulty of identifying which patients would be likely to benefit from which combination of drugs, as well as separating those whose disease is responding to their treatment from those whose is not, contributes to the provision of ineffective care. Hyperpolarized ^{13}C magnetic resonance imaging is an emerging modality that can detect lactate production associated with reprogrammed metabolism in cancer. Changes in ^{13}C lactate signal following treatment may be a valuable early indication of disease response and may help guide treatment decisions. While clinical translation of hyperpolarized ^{13}C MRI has been steadily progressing, there are currently no published studies assessing the value of imaging lactate with hyperpolarized ^{13}C MRI for better understanding and treating patients with breast cancer. In this dissertation, three technical works are presented to address the needs of robust ^{13}C imaging for clinical translation.

^{13}C MRI requires separate radio frequency transmit and receive hardware called RF coils that have been designed to operate at the ^{13}C rather than ^1H resonant frequency. RF coils must also conform to the anatomy under investigation. In order to perform ^{13}C imaging in breast, a ^{13}C breast coil system was devised and constructed.

While the speed afforded by echo-planar imaging makes it an attractive technique for meeting the strict constraints of ^{13}C MRI, off-resonance magnetization can lead to geometric distortion and spatial misregistration of imaging data. A dual-echo technique is proposed as an approach to account for sources of distortion.

Volumetric encoding is highly desirable in order to allow comparison of ^{13}C lactate signals from diseased and normal tissues, but larger regions require more time to encode and this can limit the temporal resolution of dynamic acquisitions. A pseudo-random undersampling scheme is proposed in

order to reduce encoding time requirements and improve coverage.

To Jess

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List of Abbreviations

^1H	proton
^{13}C	carbon-13
^{18}F -FDG	2-deoxy-2-[fluorine-18] fluoro-D-glucose
1D	one-dimensional
2D	two-dimensional
3D	three-dimensional
AUC	area-under-curve
ATP	adenosine triphosphate
BW	bandwidth
BW_{pp}	bandwidth-per-pixel
CAD	computer assisted design
CEST	chemical exchange saturation transfer
CS	compressed sensing
CT	computerized tomography
DC	direct current
DNA	deoxyribonucleic acid
DNP	dynamic nuclear polarization
DQ	double-quantum
EDTA	ethylenediaminetetraacetic acid
EPI	echo-planar imaging
ER	estrogen receptor
FID	free induction decay
FOV	field-of-view
FT	Fourier transform

HP	hyperpolarized
IDEAL	iterative decomposition of water and fat with echo asymmetry and least-squares estimation
imBI	induced metabolic bioluminescence imaging
ISMRM	International Society for Magnetic Resonance in Medicine
IV	intravenous
LABC	locally advanced breast cancer
LDH	lactate dehydrogenase
LNA	low-noise amplifier
MCT	monocarboxylate transporter
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MSE	mean-squared-error
NAC	neoadjuvant chemotherapy
NAD	nicotinamide adenine dinucleotide
NEX	number-of-excitations
NMR	nuclear magnetic resonance
PCB	printed circuit board
PDC	pyruvate dehydrogenase complex
QC	quality control
PET	positron emission tomography
RECIST	response evaluation criteria in solid tumors
RF	radio frequency
ROI	region-of-interest
SENSE	sensitivity encoding
S-D	Sørensen-Dice
SNR	signal-to-noise ratio
SSIM	structural similarity
ssRF	spectral-spatial radio frequency
T_1	longitudinal relaxation time constant
T_2	transverse relaxation time constant
T_2^*	field inhomogeneity-shortened transverse relaxation time constant
TE	echo time

TR	repetition time
TV	total variation
VEGF	vascular endothelial growth factor
ZF	zero-filled

Chapter 1

Introduction

1.1 Locally Advanced Breast Cancer

For Canadian women, breast cancer is the most common form of cancer and the second most common cause of cancer death. Owing to the past 60 years of evidence-based medicine, early detection and treatment of breast cancer have improved dramatically, and, for the majority of patients, this has led to substantial reductions in mortality and morbidity. Still, a small subset of new diagnoses (5-15%) are classified as locally advanced breast cancer (LABC), a class of pre-metastatic breast cancer characterized by regional lymphadenopathy and large invasive tumors (≥ 5 cm) that are often inoperable at the time of detection [4], and these cases continue to pose a significant clinical challenge.

The category of LABC comprises biologically heterogeneous malignancies, yet, since breast cancer patients with locally advanced disease exhibit poor locoregional control and an elevated risk of metastatic spread, women with LABC represent a distinct group and are thus, treated as clinically similar in regards to therapeutic strategies and expected outcomes [5]. As a case-in-point, the five-year survival rate in early stage breast cancer is almost 90% whereas the same outcome metric for LABC patients is approximately 50% [6].

1.1.1 Breast Cancer Treatment and Management

Once a suspected breast cancer has been confirmed by biopsy, patients undergo disease characterization and staging on the basis of molecular subtype, tumour extent, nodular status and incidence of distant metastases [7]. The standard-of-care for patients staged with LABC involves neoadjuvant chemotherapy to downstage the primary tumour, followed by surgical resection, and since clinical factors such as

tumour grade are associated with an increased risk of locoregional recurrence[8], radiation therapy is usually recommended in LABC.

Patients with positive cell-surface receptor status are candidates for adjuvant targeted therapies, and some of these medications are to be taken daily thereafter for up to 5 years post-treatment. At the time of surgery, response to therapy can be evaluated by the extent of residual invasive disease and patients who respond favourably to neoadjuvant chemotherapy have been shown to have significantly improved prognoses over non-responders [9, 10, 11].

Approximately 1 in 10 patients who undergo primary systemic therapy will achieve “pathological complete response,” which is an outcome that’s commonly defined as the absence of residual invasive disease according to a histopathological analysis of the surgically resected tissue [12]. Most patients, however, fall along a spectrum encompassing partial response, non-response and disease progression. Consequently, many patients endure the toxic and often agonizing side effects of chemotherapy for upwards of 3 months to half a year—only to be informed that their treatment was ultimately ineffective. Addressing this unfortunate reality of breast cancer management is an active area of research that spans numerous domains; one aspect of these efforts is distinguishing between responders and non-responders early in the course of treatment, so that management of the latter group might be improved.

1.1.2 Individualized Therapies and Response-based Care

To illustrate the potential of response-adaptive care, consider the following. If a patient could be reliably classified as non-responsive within just a few weeks into their months-long regimen, then simply terminating treatment would spare them the risk of any further unnecessary side effects—a net benefit to their overall well being. Furthermore, non-responsive patients, if identified early, may cross-over to alternative regimens or be enrolled in clinical trials with experimental therapies. This could help address a particular clinical problem in LABC since, for many women with locally advanced disease, their eligibility for breast-conserving surgery depends on whether or not their cancer responds to treatment.

The notion of monitoring early response to help physicians make more informed decisions on behalf of their patients is in harmony with the increasingly prevalent movement in clinical oncology towards personalized medicine: an aspirational paradigm that seeks to optimize treatment for each individual patient, given the unique and specific nature of their individual disease [13]. A major challenge of implementing a response-adaptive approach in the management of breast cancer and other pervasive malignancies is obtaining accurate and actionable patient data, in a manner that is clinically feasible, safe, and if possible, minimally or non-invasive.

1.1.3 Monitoring Response to Treatment

Various medical imaging technologies have been applied to probe the early response to therapy in LABC patients, and some attempts have sought to correlate reductions in tumour volume with drug efficacy. These efforts drew inspiration from the widely published set of guidelines known as RECIST, which stands for Response Evaluation Criteria In Solid Tumors and includes quantitative thresholds to help oncologists determine if a lesion improves, worsens or stabilizes in response to treatment [14, 15]. Studies considering magnetic resonance imaging, ultrasound, mammography, as well as clinical examination have led some authors to conclude that, even if reductions in tumour volume can be accurately measured, since gross morphological changes can require substantial periods of time to accumulate they cannot be reliably detected within the early stages of neoadjuvant therapy [16, 17]. Accordingly, response status is rarely assessed until after surgery, and this forgoes an opportunity to gain key insights into the patient's disease and attend to their evolving clinical needs.

While changes in tumour size can represent a macroscopic view of the interaction between anti-oncogenic drugs and the patient, the cascade of events that precede the ultimate disease response resides within the microscopic scale of individual cancer cells. Since any major structural changes associated with histopathological response are necessarily preceded by biological changes, scientists and engineers are investigating the use of functional imaging techniques for monitoring early treatment response in breast cancer. Successful imaging strategies must generate contrast and/or quanta that can serve as a marker for changes within the underlying cancer biology.

For example, quantitative ultrasound can be used to produce an echogenic assessment of the breast, which has been shown to depend on the presence of the molecular byproducts of cellular death [18]. Dynamic contrast-enhanced magnetic resonance imaging (MRI) is sensitive to locoregional tissue perfusion and can reveal the microvascular character of breast tumours—a known radiological correlate of malignancy and a potentially useful marker of response [19]. For a comprehensive survey of efforts that have applied functional imaging in probing the early response to therapy of breast cancer, refer to [20].

1.2 Metabolic Reprogramming in Cancer

Imaging biomarkers that report on cellular metabolism are particularly compelling for early treatment monitoring, since whatever the desired biological response to therapy may be, regardless of mechanism of action, it would almost certainly be accompanied by a disturbance in the uptake and consumption

of resources and energy—a consequence of the interconnected organization of metabolic networks in biological systems [21]. In fact, deregulated metabolism is a known hallmark of cancer [22, 23], thus, metabolic imaging biomarkers would offer an in situ report on cellular energetics of tumour tissues that are still in tact, relaying information that might help guide and individualize treatment.

1.2.1 Cellular Energetics in Transformed Cells

The link between aberrant metabolism and cancer was first noted in the 1920's by German physiologist, medical doctor, and Nobel laureate Otto Heinrich Warburg, who observed the tendency of cancer cells to meet the majority of their energy demands through high rates of glycolysis, followed by lactic acid fermentation [24]. This is in contrast to most normal cells, which predominantly rely on oxidative phosphorylation and use comparatively lower rates of glycolysis. Warburg found that increased lactate production persists even in the presence of oxygen—a phenomenon since termed the “Warburg Effect” [25]—and this provided the basis for some of the earliest attempts towards a biochemical mechanism of cancer [26]. Yet, in the grand scheme of cancer biology's storied history throughout the 20th century [27], often lauded for the revolutionary progress made in the areas of molecular genetics, cell signalling and human genomics [28], Warburg's work might appear inconspicuous in comparison. Recently, however, the study of cancer metabolism has seen a wide resurgence and there now exists a mounting body of evidence that implicates metabolic reprogramming as an early and potentially contingent step in tumourigenesis.

Cancer cells have evolved the ability to deregulate metabolism as a means to support tumour initiation and progression [29]. In vivo, malignant cells are afflicted with an assortment of metabolic challenges such as producing sufficient biosynthetic intermediates and energy for growth; surviving in oxygen and nutrient-depleted environments; and maintaining redox homeostasis while supporting a high metabolic rate [30]. In the face of these challenges, metabolic reprogramming allows cancer cells to survive and, thus, is considered an essential feature of malignant transformation. Deregulated metabolism is a result of mutations in oncogenes and tumour suppressors, which can rewire signalling pathways and modulate gene transcription to promote cellular growth and proliferation [31]. Furthermore, mutations in metabolic enzymes and transporters have been shown to influence gene expression and cell signalling directly, and therefore, might play an important role in tumourigenesis. Given the primacy of metabolic processes to all life and the distinct patterns of metabolic dysregulation in cancer, cellular metabolism makes a compelling target for functional imaging technology.

1.2.2 Imaging Cancer Metabolism

Positron emission tomography (PET) with the glucose analogue radiotracer 2-deoxy-2-[fluorine-18] fluoro-D-glucose (^{18}F -FDG) is an established functional imaging modality that reports on cellular metabolism. Due to increased glycolysis and elevated glucose uptake in many cancer cells, lesions can be detected in ^{18}F -FDG PET scans and then accurately localized by fusion with x-ray computerized tomography (CT) data from the same imaging session. ^{18}F -FDG PET/CT is an important tool in routine clinical oncology, and while its primary clinical use is for patient staging and recurrence monitoring, in certain cancers, it has been used to distinguish responders from non-responders prior to observing reductions in tumour size [32]. For example, in patients with gastrointestinal stromal tumours who were treated with imatinib, lesion enhancement on ^{18}F -FDG PET/CT was reduced as early as 24 hours post-treatment—weeks to months are usually needed to detect if drug-responsive tumours shrink [33].

Whether or not ^{18}F -FDG PET/CT alone can be used to predict response in breast cancer remains inconclusive. One meta-analysis of 19 studies, which included a total of 920 patients with biopsy confirmed breast cancer, found that although the pooled sensitivity and negative predictive value of ^{18}F -FDG PET/CT for predicting histopathological response to neoadjuvant therapy was 84% and 91%, respectively—that is, ^{18}F -FDG PET/CT accurately identified 84% of the true responders and correctly predicted non-response in 91% of the primary breast lesions that were classified as non-responsive—the pooled specificity and positive predictive value were comparatively low at 66% and 50%, respectively [34]. The authors drew attention to the heterogeneity of the experimental protocol among the included publications i.e. the timing and frequency of imaging, the threshold used to classify response, etc., and suggested that further prospective studies were warranted to assess the potential role of ^{18}F -FDG PET/CT for monitoring early treatment response in breast cancer.

1.3 Clinical Correlates of Tumour Lactate Metabolism

Increased glucose uptake is a strong correlate of malignancy and the *raison d'être* of ^{18}F -FDG as a radiotracer in clinical oncology; that said, it represents just a singular aspect of the near-universal up-regulation of glycolysis in cancer [35]. Since high lactate levels correlate with increased glycolysis, lactate-derived imaging biomarkers can, in principle, confer information on metabolic status and be used to monitor the “Warburg Effect” in vivo. For much of the past century, the lactate produced by cancer cells has been simply viewed as a byproduct of high rates of glycolysis, but such an innocuous portrait is no longer tenable; the evidence is suggesting that tumour lactate accumulation is closely

linked to the aggressiveness of cancer and that lactate itself is, in fact, a key metabolic player in carcinogenesis [36].

1.3.1 Cervical Cancer and Human Rectal Adenocarcinoma

The first clinical demonstration of the link between tumour lactate and malignancy came from a pilot study that quantified metabolite concentrations in biopsy specimens taken from 10 cervical cancer patients at the time of their initial diagnosis [37]. Steady-state lactate concentrations were found to be significantly higher in tumours from patients with metastatic spread than in those without incidence of metastasis. In a follow-up study, which increased the original cohort to a total of 35 patients and maintained an observation period for up to 8 years [38], the authors concluded that high tumour lactate levels predict the likelihood of metastasis, tumour recurrence, and restricted patient survival in human cervical cancers. Interestingly, it was noted that neither stage nor grade at presentation correlated with any of the measured metabolite concentrations and that ATP and glucose concentrations did not differ significantly between metastatic and nonmetastatic primary tumours. Similar findings were echoed in a 24-patient study of human rectal adenocarcinoma, which showed a statistically significant difference in lactate levels between metastatic and non-metastatic carcinomas and led the authors to speculate that measuring tumour lactate “...may serve as an early prognostic tool in the clinic with regard to formation of metastasis and patient survival.” [39]

1.3.2 Head and Neck Cancer

In a similar pilot study that instead examined steady-state metabolism in squamous cell carcinoma of the head and neck, biopsy specimens were obtained from 15 patients at first diagnosis and the concentrations of several metabolites, including lactate, were quantified [40]. Despite the low number of patients, the authors observed a statistically significant correlation between increased tumour lactate levels and incidence of metastasis. To address the relationship between pretreatment tumour lactate concentrations and subsequent development of metastatic disease, a larger study was performed in a population of 34 head-and-neck cancer patients who shared a more homogeneous presentation of the disease, and follow-up data was collected for as long as 3 years [41]. According to a median-split by tumour lactate concentration, patients who presented “low” lactate tumours had two-year overall and metastasis-free survival rates of 90%, while the corresponding survival rates for patients with “high” lactate primary tumours were 35% and 25%, respectively. The median lactate concentration for tumours that subsequently metastasized was significantly elevated compared to patients who showed

no evidence of disease during follow-up. The authors concluded that tumour lactate assessment may be clinically useful for predicting increased risk of metastasis in head-and-neck cancer.

An investigation into the possible role of tumour lactate in radioresistance and recurrence was presented in a recently published prospective study of 17 newly diagnosed head-and-neck cancer patients [42]. Disease staging was performed following surgery and all 17 patients were regarded as high-risk for locoregional tumour recurrence; consequently, all patients received adjuvant irradiation and clinical data was maintained within an observation period of up to 15 years. Although neither tumour glucose nor ATP concentrations predicted tumour recurrence, the authors reported a statistically significant correlation between recurrence and tumour lactate concentration. They also demonstrated that recurrence occurred significantly earlier and more frequently in “high” versus “low” lactate tumours. Based on their findings, the authors concluded that lactate plays a role in treatment resistance, and therefore, may be a reliable prognostic biomarker in adjuvant therapies such as radiation.

1.4 Tumour Lactate Mediation in Carcinogenesis

The consistent results across a diverse sampling of solid cancer cohorts i.e. cervical [37, 38], head and neck [40, 41] and rectal adenocarcinoma [39], continue to motivate investigation of biological mechanisms that would explain the adverse clinical correlates of high tumour lactate concentration. Increasingly, reprogrammed lactate metabolism has become the focus of a growing number of in vitro and pre-clinical studies in cancer biology, and to-date, these efforts have led to the identification of numerous lactate-mediated effects [36, 43, 44].

1.4.1 Metastasis and the Tumour-Associated Microenvironment

There have been several published studies that lend support to the conclusion that tumour lactate accumulation promotes metastasis by enhancing cellular migration. The addition of exogenous lactate has been shown to induce a concentration-dependent increase in the random migration of cells from various normal [45] and cancer [46] lines. Although a comprehensive biomolecular mechanism for lactate-enhanced metastasis remains to be described, lactate exposure is known to stimulate the production of several key mediators of cell proliferation. Lactate prompts endothelial cells to increase production of a signalling protein called VEGF, vascular endothelial growth factor, that stimulates the formation of new vessels from the existing vascular bed in a process known as angiogenesis—an essential biological process not only for growth and wound healing, but also the progression of malignant

tumours [47].

Additionally, lactate has been shown to stimulate fibroblasts to increase production of hyaluronic acid [48], which is a long-chain polymer of the extracellular matrix that creates openings in tissue spaces to allow proliferating cells to migrate through, and is thought to play a role in the regulation of cancer progression [49]. The fact that increased concentrations of tumour lactate have been observed in patients with metastatic disease corroborate an ability to promote stimulatory factors of cell migration and angiogenesis.

Lactate is also known to take part in shaping the makeup of the tumour-associated microenvironment: a dynamic milieu that emerges throughout tumour development as a result of tumour-host interactions, currently receiving considerable attention for its multifaceted role in disease initiation and progression. In order to orchestrate molecular and cellular events within the surrounding tissues, tumours direct and regulate the activities and contents of their tumour-associated microenvironment, comprised of proliferating tumour cells; the tumour stroma; blood vessels; infiltrating inflammatory cells; and various associated tissue cells [50]. A typical characteristic of the tumour microenvironment is that it is acidic—a feature that has been shown to drive local invasion in solid cancers [51]—and lactic acid (lactate anion with H^+) is the major constituent responsible for metabolic acidosis of the extracellular space[52].

1.4.2 Immune Response Evasion

The combination of high lactate and low pH within the tumour-associated microenvironment can impair the anti-tumoral response of the host immune system, thereby linking lactate to immune escape—an essential condition for sustained tumourigenesis [53]. One way in which lactate has been shown to inhibit the host immune response is by inactivating the release of signalling proteins from dendritic cells—a type of differentiated white blood cell—that would have otherwise identified proliferating tumour cells for destruction [54]. Lactate has also been found to inhibit cytotoxic or “cell killing” elements of innate immunity known as natural killer cells; lactate can interfere with their cytolytic function—the process of inducing an osmotic imbalance that leads to membrane rupture—and prevent natural killer cells from responding to tumour formation [55].

Furthermore, activated T cells, the central elements of cell-mediated immunity, happen to use glycolysis as their main energy source and thus rely on efficient secretion of lactate in order to prevent disruption of their metabolism due to intracellular lactate accumulation. Since the transport of lactate across the plasma membrane depends on the presence of an intra-to-extracellular gradient, high

lactate concentrations within the tumour-associated microenvironment can block T cells from ridding themselves of their own lactate, and this can impair their cytotoxic activity and even initiate apoptosis; therefore, lactate inhibits the anti-tumoural response of activated T cells and thereby assists tumours in evading elimination by the host immune system[56].

1.4.3 Radiation Therapy Resistance

Interestingly, there is also a suspected biochemical capacity of tumour lactate to promote resistance to radiation therapy. Given that the anti-tumoural action of ionizing radiation arises from the localized generation of DNA-damaging reactive oxygen species, it has been hypothesized that an accumulation of antioxidant compounds such as lactate can interfere with the fixation of damage to malignant DNA and that lactate can induce and/or enhance resistance to radiation therapy [57].

This hypothesis was explored through a pre-clinical study using murine tumour xenografts, which were derived from 10 different human head and neck squamous cell carcinoma lines and irradiated in 30 graded fractions over a period of 6 weeks [58]. For each of the xenograft groups, it was observed that the 50% control dose—that is, the radiation dose necessary to “control” i.e. kill the specified fraction of tumour cells, which can be empirically determined by irradiating tumours of uniform sizes with various doses and then observing for local control or recurrence [59]—was strongly correlated with the average tumour lactate concentration. In other words, the cell lines that produced tumours with the most lactate required a higher radiation dose, and vice versa. This led the authors to conclude that tumour lactate might be predictive of response to radiotherapy and that pharmacologically inhibiting glycolysis may provide promising avenues for translational pre-clinical and clinical studies.

1.5 Lactate Homeostasis and Targeting for Novel Cancer Therapeutics

As noted in a recent review, lactate, which is elevated in glycolytic cancers and implicated in promoting angiogenesis, cell migration, immune escape, and metastasis, appears to be the only organic compound that is involved in all the major steps in carcinogenesis [44]. While efforts to further characterize the link between lactate and malignancy remain ongoing, some researchers have already begun investigating the prospects of pharmacologically inhibiting lactate metabolism as part of the next-generation of molecularly targeted therapeutics [43].

1.5.1 Targeting *HER2/neu* Overexpression in Breast Cancer

Targeted therapy represents the state-of-the-art in cancer treatments, and, in fact, its earliest demonstration happened to be in breast cancer patients; particularly, individuals who were screened and tested positive for overexpression of an oncogene called *HER2/neu*. Also known as *ERBB2*, *HER2/neu* codes for a protein called human epidermal growth factor receptor 2 (HER2): a type of plasma membrane-bound receptor that plays a role in multiple signal transduction pathways for regulating cell growth, survival, differentiation and proliferation [60]. Amplification of *HER2/neu*, present in approximately 15-30% of breast cancers, has been shown to be "...associated with an aggressive clinical phenotype that includes high-grade tumors, increased growth rates, early systemic metastasis, and decreased rates of disease-free and overall survival." [61]

Trastuzumab, commonly marketed as Herceptin[®], is an antibody that was specifically developed for its high-affinity for HER2; when administered in *HER2/neu*-positive patients, trastuzumab can bind to the extracellular domains of the overexpressed HER2 plasma membrane receptors, and attenuate cell signalling, as well as induce antibody-dependent cellular cytotoxicity. Since its first approval by Health Canada in 1999 [62], trastuzumab has significantly improved survival rates of individuals with *HER2/neu-positive* breast cancer, and, as a successful demonstration of the rational development of molecularly targeted therapies, it signified a revolutionary step forward in the treatment of cancer.

1.5.2 Cytosolic Production of Lactate

In general, targeted therapies are first conceived by identifying biomolecular features that are both distinct to and complicit in certain malignant phenotypes. Thus, lactate homeostasis has been identified as a promising therapeutic target[43]. Two main lines of attack have been proposed: inhibition of lactate production; and suppression of lactate transport across the plasma membrane. In virtually all life-forms, lactate is enzymatically produced from another metabolite called pyruvate—the anionic form of pyruvic acid—and direct interconversion is possible given their nearly identical chemical structures; both contain three carbons and one carboxyl group, but in place of lactate’s hydroxyl group, pyruvate instead has a ketone. Pyruvate is the end product of glycolysis and since it can be metabolized into a variety of products, such as carbohydrates, fatty acids, or precursors for energy synthesis, pyruvate is an important biochemical compound that unites several key metabolic processes. In the context of cellular respiration, glycolytically-derived pyruvate is the bifurcation point between two major pathways: oxidative phosphorylation, used by most cell types in the presence of oxygen; and lactic acid fermentation, used by normal cells under anaerobic conditions, as well many cancer cells, but regardless of

oxygen tension i.e. the “Warburg Effect.”

Bidirectional conversion of lactate to pyruvate (and vice versa) is catalyzed by the enzyme lactate dehydrogenase (LDH). LDH has a quaternary structure made up of four subunits. The four subunits are each made up of a single protein, which is found in two isoforms, LDHA and LDHB; therefore, given all possible combinations of LDHA and LDHB subunits in a single LDH tetramer, five unique isozymes exist, namely LDH1 through LDH5. While all LDH isozymes can mediate interconversion between lactate and pyruvate, LDH5, comprised entirely of LDHA subunits, has the highest affinity for binding pyruvate, rather than lactate, and the highest maximum rate of pyruvate-to-lactate conversion i.e. lactic acid fermentation; accordingly, LDH5 is the predominant isoform found in highly glycolytic tissues.

It's long been observed that the concentration of LDHA found in tumour extracts is markedly higher than in normal tissues [63]. Moreover, upregulation of LDHA has been shown to connote poor prognosis in several human malignancies such as non-small-cell lung cancer [64]; colorectal cancer [65]; and squamous cell carcinoma of the head and neck [66]. As the enzyme responsible for producing lactate, LDHA—or more precisely, the LDH5 isoform of lactate dehydrogenase—plays a crucial role in metabolic reprogramming and tumourigenesis; therefore, targeting LDHA expression might inhibit tumour cell maintenance and survival. Interestingly, in humans, LDHA is generally considered to be a safe therapeutic target, since individuals who have inherited a loss-of-function mutation in LDHA [67] exhibit only relatively mild symptoms of exertional myopathy, which, under normal conditions, is otherwise absent [68]. Although numerous inhibitors of LDHA have been identified, many of which have garnered encouraging results in pre-clinical studies, to date, none have been approved for clinical use [69]. Nevertheless, LDHA retains its high placement on the list of most promising drug targets. The current status of targeting lactate production for cancer therapy, including a comprehensive summary of all the known small molecules that have been shown to have an inhibitory effect on LDHA activity, have been reviewed in [70] and [71].

1.5.3 Transport of Lactate Across the Cell Membrane

In addition to LDH activity, cellular lactate homeostasis is primarily determined by the transport of lactic acid across the plasma membrane. Since both glycolytic tumour cells and rapidly proliferating normal cells divert much of their glucose carbon to biosynthesis instead of aerobic respiration, they rely on lactic acid fermentation to support consistently high rates of glycolysis. In addition to producing ATP, the LDH catalyzed reduction of pyruvate to lactate i.e. lactic acid fermentation, is concomitant

with the replenishment of an essential glycolytic precursor, the oxidized form of the coenzyme nicotinamide adenine dinucleotide (NAD^+) from its reduced form (NADH)—in the presence of oxygen, the high-energy electron carrier NADH could have been recycled in the mitochondria during oxidative phosphorylation—therefore, lactic acid fermentation enables rapid cellular growth by supporting redox homeostasis during periods of sustained glycolysis. Given the high amounts of lactate that are produced in association with high rates of glycolysis, efficient lactate secretion is necessary in order to prevent lactic acid build-up inside the cell, which, if not removed, would lead to potentially lethal acidosis. Accordingly, even though the precise role and clinical relevance of lactate transporters in solid tumours is still poorly understood, they are considered promising targets for cancer therapy [72]

The bidirectional, proton-linked passage of lactate through the plasma membrane is mediated by a family of integral proteins known as monocarboxylate transporters (MCT)—also referred to as Solute Carrier Family 16—that is made up of fourteen related proteins; of these, MCT1-MCT4 are responsible for the efflux of lactic acid into the extracellular space [73]. The clinicopathological significance of MCT1 expression in breast cancer has been highlighted in one analysis of 249 primary breast carcinomas [74], which found that MCT1 was significantly increased in tumour versus normal tissue and, also, that MCT1 was associated with poor prognostic variables such as high tumour grade and the aggressive “triple-negative” phenotype—that is, a molecular subtype of breast tumours, which lack expression of all three of the commonly targeted cell receptors and are thus resistant to first-line targeted therapies. To date, several authors have demonstrated the importance of MCT inhibition in cancer using both in vitro and in vivo models [72], and it has been recently demonstrated that the in vivo growth of breast tumours can be disrupted by targeting lactate transporters, emphasizing the role of MCTs in breast tumour initiation and progression [75].

1.6 Biopsy-derived and Noninvasive Measurement of Tumour Lactate

On the basis of findings such as these, lactate metabolism is considered a promising target for novel therapeutics, but, as with any experimental therapy, its real clinical value for treating breast cancer will ultimately be decided by randomized controlled trials. Just as recipients of trastuzumab were screened for *HER2/neu* status, screening for expression of a lactate-producing or “lactagenic” phenotype would likely be an important aspect of implementing human trials for drugs that are designed to target lactate metabolism. For such a study, not only would tumour lactate measurement be useful in identifying

eligible participants, but it could also be used in follow up to determine if the drug had in fact lowered tumour lactate levels and if there were any attendant clinical benefits.

1.6.1 Induced Metabolic Bioluminescence

Unlike the assignment of molecular subtype, a routine aspect of breast cancer diagnosis where pathologists use immunohistochemistry and to identify unique molecular biomarkers—hormone and growth factor receptors expressed on the surface of breast cancer cells—there exists no “gold standard” technique for assessing tumour lactate metabolism in patients. In the aforementioned publications that correlated clinical parameters with lactate levels [37, 40, 38, 41, 39], results were derived from snap-frozen biopsy samples, and although several methods are capable of measuring the distribution of lactate in such substrates, it has been noted that “...approaches to a direct quantification of metabolites in microdissected tissue samples seem to be rare in the literature.” [76] In all of these clinical studies, the concentration of lactate, among other metabolites, was measured using the technique of induced metabolic bioluminescence imaging (imBI). Briefly, imBI operates on individual cryo-sections of snap-frozen tissue by first halting any endogenous enzyme activity through heat-inactivation, and then immediately treating each slice with a cocktail of metabolite-specific enzymes and cofactors. These serve as metabolite “detectors” by coupling the activity of an exogenous enzyme to the emission of light in a separate bioluminescence reaction and, given specific conditions, the total light emitted is proportional to the initial metabolite concentration. A detailed review of imBI is provided in [77].

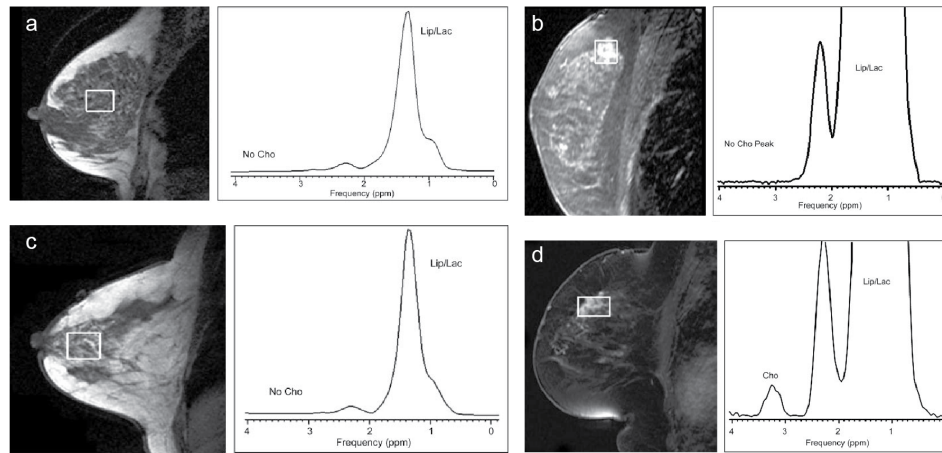
One group has reported the use of imBI to quantify the concentration of tumour lactate from patients with LABC [78]. Prior to therapy, 25 core biopsy samples were obtained from 23 patients and clinical data was collected for up to 5 years. Unfortunately, the size of the sample set was too small to derive any statistical significance between the lactate levels measured in LABC biopsies and clinical parameters; nevertheless, the authors were able to glean some insight into the nature of lactate metabolism in breast cancer tumours. Since a few of the samples came from more than one biopsy site, an analysis of variance suggested that lactate levels were less heterogeneous within a single biopsy than between biopsies; thus, indicating that the amount of lactate can vary significantly among different areas of the same tumour. Considering the dimensions of breast core needle biopsies in relation to the large tumours that might be encountered—for example, 11-16 gauge needles have diameters of 0.3-1.7 mm, whereas locally advanced breast tumours are commonly greater than 50 mm in their longest dimension—spatial heterogeneity of tumour lactate may have important implications for investigating lactate metabolism in breast cancers. Accordingly, noninvasive sampling methods that permit lactate measurement across

anatomical regions-of-interest may be necessary to avoid missing vital information [79].

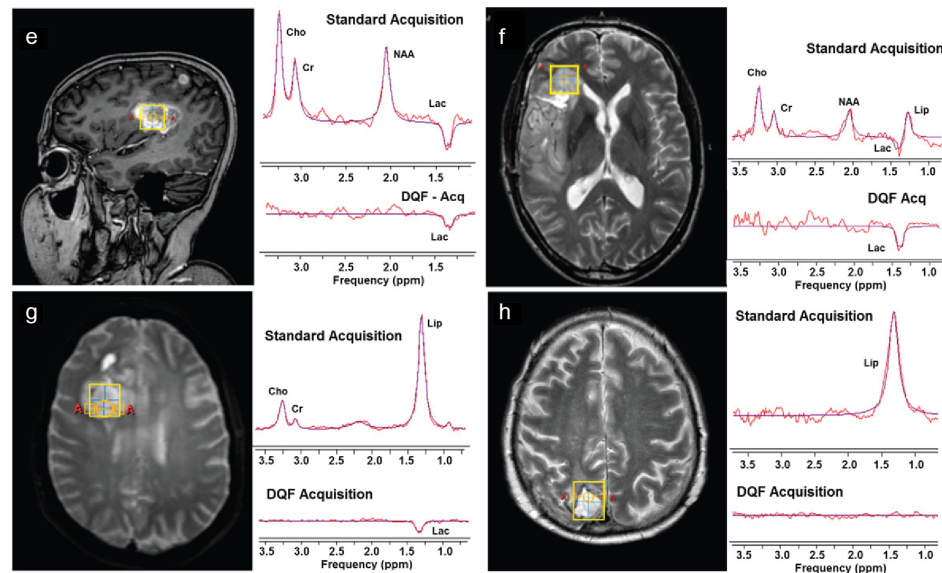
1.6.2 ^1H Magnetic Resonance Spectroscopy

Currently, very few noninvasive methods are available for measuring tumour lactate in vivo and, among them, demonstrations in breast have been rare. The most studied method for obtaining in vivo lactate measurements is magnetic resonance spectroscopy (MRS), in which the subject lies in a magnetic resonance imaging (MRI) scanner and the proton (^1H) nuclei within a region of their body—some of which are covalently bound in endogenous lactate molecules—are probed with radio frequency (RF) electromagnetic waves. While all endogenous protons can emit RF signals, by far, the most abundant sources of ^1H nuclei within the body are water and fat; the former comprises the molecular basis of diagnostic MRI and the latter, although not replete of clinical utility, is generally considered a source of nuisance signals. The value of ^1H MRS in breast cancer has been typically based on its ability to detect increased levels of choline—choline being a marker of active tumours—since it's been shown to assist in the differentiation of benign from malignant breast lesions, and, as an adjunct to MR imaging, it can improve the positive predictive value of MRI-based biopsy recommendations and potentially reduce the number of benign biopsies [80].

Whereas the aggregate signal owing to choline bound protons has a characteristic frequency that lies in a remarkably uncluttered region of the in vivo ^1H MR spectrum, the dominant signal frequencies arising from lactate happen to coincide with those from multiple lipids; accordingly, most published studies have only considered lactate in brain tumours, where, lipid contamination is known to be relatively low—one of several major technical differences between MRS inside the brain as compared to elsewhere in the body [81]. Since ^1H lactate MRS can be severely hampered by spectral overlap from intense lipid signals and that breast tissue is largely comprised of adipose cells, ^1H lactate MRS in the breast is especially challenging. Numerous advanced MRS techniques have been developed in order to address this particular limitation [82, 83, 84, 85, 86, 87] and efforts to measure tumour lactate with ^1H MRS are ongoing; still, choline detection remains the primary clinical use of ^1H MRS in breast cancer.



Bartella, Lia, and Wei Huang. "Proton (^1H) MR spectroscopy of the breast." *Radiographics* 27 (2007): S241-S252.



Payne, Geoffrey S., L. M. Harris, G. S. Cairns, C. Messiou, N. M. deSouza, A. Macdonald, F. Saran, and M. O. Leach. "Validating a robust double-quantum-filtered ^1H MRS lactate measurement method in high-grade brain tumours." *NMR in Biomedicine* 29, no. 10 (2016): 1420-1426.

Figure 1.1: (a-d) Examples of standard single voxel-spectra in breast tissue, adapted from Bartella, Lia, and Wei Huang. "Proton (^1H) MR spectroscopy of the breast." *Radiographics* 27 (2007): S241-S252. Sagittal MR images from two women in their mid-fifties (a, c) show normal glandular parenchyma and spectra illustrate high lipid (*Lip*) but no choline (*Cho*). *Lac* = lactate. (b) Non-mass-enhancing lesion on contrasted-enhanced MR showed no choline (a-c are "true-negatives"). (d) Palpable, mammographically detected, biopsy-proved invasive lobular carcinoma with early enhancement show detectable choline. (e-h) Examples of standard and double-quantum- (DQ) filtered single-voxel spectra in glioblastoma, adapted from Payne, Geoffrey S., et al. "Validating a robust double-quantum-filtered ^1H MRS lactate measurement method in high-grade brain tumours." *NMR in Biomedicine* 29.10 (2016): 1420-1426. (e) Example with no apparent lipid overlying the lactate. (f) Example showing significant overlap between lactate and lipid, with consequent partial signal cancellation. (g), Example showing a dominant lipid peak in the standard acquisition but lactate detected in the DQ-filtered spectrum. (h), Example of a dominant lipid resonance in which no lactate was present (below detection threshold in the DQ-filtered spectrum). Only in (e) can a reasonable estimate of lactate concentration be obtained from the standard acquisition spectrum.

1.6.3 Chemical Exchange Saturation Transfer

Another ^1H MR-based technique that can measure in vivo lactate is Chemical Exchange Saturation Transfer (CEST). Like MRS, CEST relies on the MR signal from protons to probe molecular compounds and these can also be spatially resolved by incorporating MR imaging schemes; in contrast, whereas the ^1H signals measured in MRS arise from the individual molecules in which they are bound—for instance, the ^1H MR lactate spectrum contains 6 distinct peaks that are attributed to its methyl and hydroxyl protons—CEST operates by modulating the ^1H MR signal of water as a function of its local biochemical environment. CEST relies on indirect detection of molecules with protons that can readily exchange with water and, since water is a highly abundant solvent in vivo ($>50\text{ M}$), this fact can be exploited to greatly amplify the detection limit of certain endogenous compounds. Numerous reviews of CEST have been written to provide in-depth treatments of its physical mechanisms and application in molecular imaging [88, 89, 90].

The first in vivo imaging study that used the CEST technique to exploit the exchange of bulk water with lactate's hydroxyl protons was recently featured in an open access scientific report [91]. The method was validated by measuring the lactate CEST changes in lymphoma tumors pre- and post-infusion of pyruvate and correlating them with measurements from lactate-optimized proton MRS. Additionally, dynamic changes in lactate CEST measured in exercising human calf muscle were also correlated with dynamic lactate ^1H MRS. Compared to MRS, lactate CEST offers over two orders of magnitude increased sensitivity; however, a major drawback of lactate CEST stems from the fact that the resonance frequency of the lactate hydroxyl proton is in close proximity to that of water protons and thus, since the resolvability of spectral peaks is linearly proportional to the applied magnetic field, MRI scanners with field strengths greater than 4T are required. In order to make lactate CEST tractable, human data was acquired using a 7T whole-body scanner and pre-clinical data was acquired with a small animal system at field strength of 9.4T.

Ultra high-field systems ($\geq 7\text{T}$) are scarcely available, with approximately 30 whole-body human 7T MRI scanners currently deployed at research centers around the world [92]. Since the majority of “workhorse” systems that are routinely used in the clinic are MRI scanners at field strengths of 3T or lower, this represents a significant hurdle to wide translation of lactate CEST for clinical applications in human cancers. To mitigate the challenge of resolvability by means other than increasing the strength of the applied static magnetic field, one group has proposed the use of a CEST contrast agent that is capable of shifting the resonance frequency of lactate's hydroxyl proton far away from bulk water [93, 94]. The proposed contrast agent is a type of paramagnetic shift reagent called EuDO3A, which

forms a complex with lactate and creates a new CEST peak, shifted far away from the usual location of lactate's hydroxyl resonance of unbound lactate, thus, providing greatly improved resolvability from water while retaining the CEST properties of lactate itself. In vitro validation was performed using multiple sequestrations of cell culture media that were harvested from lung cancer cells after variable periods of incubation; placed in separate vessels; pH normalized; combined with EuDO3A and then imaged with CEST MRI. In addition, an in vivo imaging result was presented, which demonstrated the detection of endogenous lactate from within the bladder of an anesthetized mouse. Since the shift reagent is a hydrophilic molecule, it cannot enter cells and is confined to the extracellular space and, thus, may one day be useful for in vivo monitoring of lactate export from tumours.

1.7 Hyperpolarized ^{13}C Magnetic Resonance Imaging with $[1-^{13}\text{C}]\text{Pyruvate}$

In absence of additional qualifiers, the terms MRI, MRS and CEST are all understood to mean techniques that exploit the magnetic properties of ^1H nuclei; which is to say that, unless otherwise stated, the specifier ^1H , “hydrogen-one,” or “proton,” is implied. The nuclei of hydrogen atoms carry an intrinsic form of angular momentum known as spin, and nuclear spin is what gives rise to the magnetic moment that can be observed in a proton nuclear magnetic resonance (NMR) experiment. Atomic nuclei that possess a magnetic moment can be induced to precess about an applied magnetic field and, in turn, emit a detectable NMR signal at its characteristic Larmor frequency:

$$\omega = \gamma B \quad (1.1)$$

where ω is angular frequency ($\text{rad}\cdot\text{s}^{-1}$); B is the magnitude of the applied field (T); and γ is the gyromagnetic ratio, which is a nuclei-specific constant that relates angular frequency to the applied field ($\text{rad}^{-1}\cdot\text{s}^{-1}\cdot\text{T}^{-1}$). NMR spectrometers, as well as MRI scanners, operate by external application of a static magnetic field, often termed B_0 , which forms the dominant contribution to the applied field, B . The actual magnetic field experienced by an individual atomic nucleus is influenced by its local electronic environment—that is, the magnetic fields of its associated electron cloud can subtract from the externally applied field—and, thus, the Larmor frequency can be rewritten as:

$$\omega = \gamma(1 - \sigma)B_0 \quad (1.2)$$

where σ is a dimensionless shielding parameter. The relative electronic shielding experienced by a given nucleus gives rise to the phenomenon known as “chemical shift”, and imparts upon NMR spec-

troscopy its unparalleled ability to resolve complex chemical structures, making it an indispensable tool for analytical chemistry.

1.7.1 ^{13}C Magnetic Resonance Spectroscopy

The presence of nuclear spin is determined by the contents of the nucleus i.e. numbers of protons and neutrons, and although net spin for the majority of atomic nuclei is typically zero, there are several stable isotopes that contain odd numbers of protons and/or neutrons, which gives rise to non-zero nuclear spin and, thus, are amenable to observation with NMR spectroscopy. Carbon-13 (^{13}C) is a stable, naturally occurring isotope of carbon, which, unlike the main carbon isotope, ^{12}C , has a non-zero nuclear spin, and, in fact, shares similar magnetic field properties with ^1H . Given the dependence of organic compounds on carbon atoms, ^{13}C NMR is an important tool for elucidating the structural identity of molecules in organic chemistry. Furthermore, biological processes and disease states have long been studied with ^{13}C NMR, either through natural abundance experiments, or through addition of ^{13}C -enriched compounds [95]. One of the main challenges of ^{13}C NMR, which is not a factor for ^1H spectroscopy, is the fact that the natural abundance of the ^{13}C isotope is just 1.1%, meaning that only a small fraction of naturally occurring carbon nuclei can be observed in an NMR experiment; therefore, all else equal, since the natural abundance of ^1H is $> 99.9\%$, ^{13}C NMR is nearly 2 orders of magnitude less sensitive than ^1H NMR.

Issues of ^{13}C natural abundance aside, NMR is known as an intrinsically insensitive technique due to the low magnetic energy of nuclear spins compared to thermal energy at room temperature [96]. This can be understood in terms of the extent of nuclear polarization under thermal equilibrium conditions: in relation to an applied magnetic field, both ^1H and ^{13}C , as well as any other so-called “spin-1/2” nuclei such as ^{31}P and ^{19}F , exist in either high or low energy orientations, and the amplitude of the observable NMR signal is directly proportional to the difference in the number of spins that occupy these two distinct populations. In other words, spin polarization is necessary to observe NMR signals. Because the actual energy difference between the two polar states is exceedingly low, the degree of nuclear polarization that is generally achieved in NMR experiments is on the order of 1-100 parts per million (ppm), and this gives rise to its inherently low sensitivity.

Despite being an intrinsically weak signal, it is by virtue of the sheer number of water-bound protons within tissue that makes MRI a valuable tool for assessing soft tissue morphology. On the other hand, in vivo ^1H MRS has not seen widespread clinical use and this is largely due to challenges associated with its limited sensitivity. The concentrations of the metabolites observed with in vivo ^1H MRS

(<50 mM) are significantly less than water (>50 M), so in order to acquire data with adequate sensitivity, long scan times (> 10 minutes) are usually required, and, as a trade-off for improving signal-to-noise ratio (SNR), spatially localized, multi-voxel MRS, is acquired at coarse resolution. Therefore, considering its inherent insensitivity, as well as its low natural abundance, in vivo ^{13}C MRS at thermal equilibrium is not a practical technique for imaging carbon metabolism in the clinic [95].

1.7.2 Dynamic Nuclear Polarization and $[1-^{13}\text{C}]\text{Pyruvate}$

In order to overcome the inherent sensitivity limitations of utilizing ^{13}C -labelled molecules as injectable probes for studying metabolism, several methods have been proposed for enhancing nuclear polarization. These are referred to as hyperpolarization techniques, as the number of polarized spins can be made to greatly exceed those in a state of thermal equilibrium. By far, the most influential approach for achieving hyperpolarization is dissolution dynamic nuclear polarization (DNP). Dissolution DNP can dramatically (> 10,000-fold) increase the sensitivity of liquid-state ^{13}C MRS and, thus, has enabled the use of magnetic resonance imaging technology for making images of enzyme-mediated metabolic conversion in in vivo [97]. Briefly, DNP makes use of the fact that at temperatures approaching absolute zero ($\sim 1\text{ K}$), the spin polarization of electrons under a strong magnetic field approaches 100%, and with microwave irradiation, this can be partially transferred to nuclear spins. In the case of ^{13}C nuclei, DNP can attain a maximum degree of polarization of 50% [95]. Following dissolution, after the frozen sample is rapidly heated and dissolved, the hyperpolarized state is short-lived and the level of nuclear polarization rapidly decays toward thermal equilibrium. Although one of the advantages of DNP comes from the variety of ^{13}C -labelled molecules that can be hyperpolarized, the chemical properties of the ^{13}C substrate constrain both the achievable degree of polarization, as well as the longevity of the hyperpolarized state; therefore, there are a limited number of hyperpolarized ^{13}C -labelled tracers that are considered clinically relevant for metabolic ^{13}C MRI.

Hyperpolarized (HP) $[1-^{13}\text{C}]\text{pyruvate}$ is the single most studied substrate for metabolic ^{13}C MRI, and the reasons for this are several fold. $[1-^{13}\text{C}]\text{pyruvic acid}$ is relatively unique among biologically interesting metabolites because it can be easily polarized without the need of adding a glassing agent, such as glycerol, since the glass state is required for homogenous distribution of polarized electron source throughout the sample, and this simplifies the process of substrate preparation. Additionally, the first label position of pyruvic acid is a carboxyl carbon with a relatively long T_1 relaxation time, which is the rate constant used to describe the exponential decay of the hyperpolarized state after dissolution. In vivo, the T_1 relaxation time of $[1-^{13}\text{C}]\text{pyruvate}$ is 30-60 seconds and this is sufficiently

long as to allow for substrate delivery and uptake in cells. The fact that pyruvic acid is highly soluble in water is also important, as this prevents undesirable dilution of the hyperpolarized sample after dissolution [98].

Pyruvate, as discussed previously, is a biologically important metabolite and aside from the numerous physical properties that make $[1-^{13}\text{C}]$ pyruvic acid remarkably well suited for dissolution DNP, it has favourable physiological and kinetic properties for noninvasive metabolic imaging. When administered intravenously, HP $[1-^{13}\text{C}]$ pyruvate can freely enter tissues and is rapidly transported across the cell membrane by facilitated transport through MCT1 symporters. Once inside the cell, pyruvate's HP ^{13}C label has several fates. One possibility is exchange with endogenous alanine, an amino acid that can be produced from pyruvate in the reversible reaction catalyzed by amino-transferase. Also, $[1-^{13}\text{C}]$ pyruvate can be taken up by the mitochondria to enter the tricarboxylic acid cycle, which gives rise to the irreversible formation of $[1-^{13}\text{C}]$ bicarbonate. Finally, through the catalytic activity of LDH and cofactor NADH, the HP label of $[1-^{13}\text{C}]$ pyruvate rapidly exchanges with endogenous lactate.

The in vivo chemical shifts of $[1-^{13}\text{C}]$ pyruvate and its downstream metabolic products, $[1-^{13}\text{C}]$ alanine, $[1-^{13}\text{C}]$ bicarbonate and $[1-^{13}\text{C}]$ lactate, are easily resolved at clinical field strengths, and thus, the HP ^{13}C label can simultaneously confer regional uptake along with chemical information. This is a remarkable feature, which ought be appreciated in relation to other prominent metabolic imaging tools, such as PET, that are based on radionuclides: although a radioactively labelled substrate might be metabolized into various downstream molecular products, with potential flux of radioisotopes through multiple biologically interesting pathways, the local chemical neighbourhood of the radioactive label does not confer any information to the emitted photons and, thus, the particular molecular source of the in vivo imaging data cannot be determined. In other words, the clinical interest garnered by HP $[1-^{13}\text{C}]$ pyruvate magnetic resonance imaging is because it allows monitoring of not just substrate uptake, but also of real-time metabolic exchange in several important biochemical pathways.

1.7.3 Metabolic Imaging of Cancer with Hyperpolarized $[1-^{13}\text{C}]$ Pyruvate

Numerous excellent reviews surveying hyperpolarized ^{13}C media have been written, with great attention paid to the clinical utility of HP $[1-^{13}\text{C}]$ pyruvate and other HP ^{13}C agents for understanding normal and pathological metabolic processes in vivo [99, 95, 98]. As a noteworthy example, the flux of the HP ^{13}C label from $[1-^{13}\text{C}]$ pyruvate to $[1-^{13}\text{C}]$ bicarbonate is related to activity of the pyruvate dehydrogenase complex (PDC) and deficiencies in PDC flux have been linked to diseases of the human heart [100, 101, 102]. In 2016, our group published the first-in-man study of HP $[1-^{13}\text{C}]$ pyruvate cardiac

MRI in healthy volunteers, and we were able to demonstrate safety and feasibility of the technology with a clinical 3T MRI scanner [103].

To date, the most developed application of hyperpolarized ^{13}C MRS using $[1-^{13}\text{C}]$ pyruvate is in pre-clinical studies of tumour metabolism and, while label exchange with $[1-^{13}\text{C}]$ alanine and flux to $[1-^{13}\text{C}]$ bicarbonate in tumours has been reported [104], the primary focus has been on monitoring rapid label exchange between $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate in vivo. It's been shown [105, 106] that within cells, lactate dehydrogenase catalyzes a rapid exchange reaction at or near-equilibrium according to:



where E refers to the enzyme LDH from within the cytosol and NAD^+/NADH to the oxidized and reduced forms of the coenzyme nicotinamide adenine dinucleotide, respectively. This means that given adequate delivery, transport, enzyme and cofactor availability, the injected hyperpolarized $[1-^{13}\text{C}]$ pyruvate substrate effectively labels endogenous pools of lactate; accordingly, increased lactate labelling has been observed in tumours relative to normal tissue [107]. The kinetics of pyruvate-to-lactate conversion have been studied in vitro and in vivo in order to understand the dynamics of label exchange in time-resolved ^{13}C MRS experiments, and the apparent exchange velocity of the HP ^{13}C label between pyruvate and lactate has been shown to be function of substrate delivery to the tumour; membrane transport by MCT1; and the kinetics of LDH, which in turn depend on intracellular pH as well as the steady-state concentrations of each substrate (lactate/pyruvate/ NAD^+/NADH); therefore, monitoring of the HP label exchange between $[1-^{13}\text{C}]$ lactate and $[1-^{13}\text{C}]$ pyruvate can serve as a biomarker of reprogrammed metabolism and the “Warburg Effect” in vivo [108, 107].

There is evidence from a range of pre-clinical models that suggests in vivo HP $[1-^{13}\text{C}]$ pyruvate MRS can detect early response to therapy [109, 110, 111, 112]. One study, which involved experiments with a human breast adenocarcinoma tumour model (MDA-MB-231), revealed a significant reduction in HP pyruvate-to-lactate exchange 24 hours after treatment with doxorubicin—an anthracycline-based, DNA damaging agent and first-line systemic therapy for LABC patients [108]. After 48 hours, the reduction was further pronounced, but no significant change in tumour size was measured during the experiments. To investigate the feasibility of using HP $[1-^{13}\text{C}]$ pyruvate MRI to probe the early response to radiation therapy, our group collaborated in a study that used xenografts grown from the same human breast cancer line [113]. It was demonstrated that a significant decrease in pyruvate-to-lactate ^{13}C -label exchange in MDA-MB-231 tumours can be observed 96 hours following a single dose of ionizing radiation. In a recent HP ^{13}C MRS study with a transgenic murine model of breast cancer recurrence, which used modified xenografts with a diet-controlled and, thus, externally “switchable”

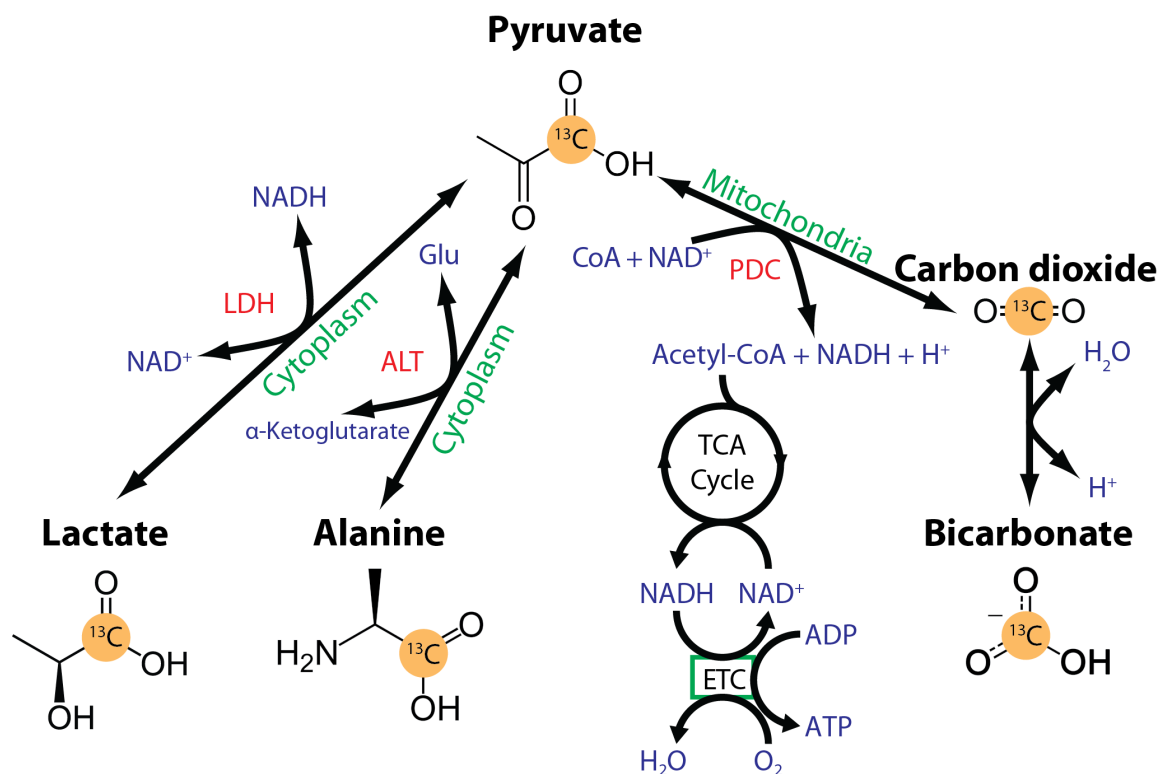


Figure 1.2: Isotopic labelling of the C₁ position with ^{13}C can reveal some of the key metabolic fates of pyruvate. LDH: lactate dehydrogenase, ALT: alanine transaminase, PDC: pyruvate dehydrogenase complex, PC: pyruvate carboxylase, ETC: electron transport chain

oncogene, the authors found increased pyruvate-to-lactate labelling in tumour-bearing mice following the controlled induction of recurrence and attendant tumour regrowth [114]. The authors also noted that from a serialized case study involving a single specimen, an increase in HP label exchange could be observed earlier than an increase in tumour volume, offering further support to the notion that glycolytic alterations precede tumour formation in cancer relapse and progression.

Encouraging results such as these are a demonstration of the potential utility of hyperpolarized ^{13}C MRI with $[1-^{13}\text{C}]\text{pyruvate}$ for understanding how tumour metabolism changes during treatment, as well as monitoring early response to therapy, and have contributed to the excitement for translating the technology to studies in patients. The first report of hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ MRI in humans was published in 2013 and was performed in prostate cancer patients to demonstrate the safety and feasibility of HP ^{13}C MRI in a clinical setting [1]. Recently, the same group presented the first application of HP $[1-^{13}\text{C}]\text{pyruvate}$ metabolic imaging in patients with brain tumours [2].

1.8 Potential Role of Hyperpolarized [1-¹³C]Pyruvate MRI in Breast Cancer

The standard approach to diagnosing breast cancer is with tissue biopsy and histopathology at a single point in time. As is the case with many other solid cancers, once a diagnosis has been established, management decisions typically rely on a combination of clinical evaluation and radiological imaging [98]. Diagnostic breast MRI is routinely used to direct patient staging, planning and follow-up; accordingly, given the present understanding of metabolic reprogramming in carcinogenesis, including its clinical significance as a driver of malignancy, the prospect of obtaining metabolic imaging scans as an adjunct to existing MRI protocols has tremendous potential to help oncologists provide more personalized care to patients. Hyperpolarized ¹³C MRI is an emerging molecular imaging technology that can be applied to monitor cellular metabolism and, with a minimally invasive intravenous (IV) administration of prepolarized [1-¹³C]pyruvate, a non-toxic, endogenous compound, it can be used to probe the biochemical mechanisms that give rise to increased tumour lactate production and the “Warburg effect” in vivo. There are two major areas where metabolic breast MRI with HP [1-¹³C]pyruvate can make a contribution to current clinical practice: early treatment response; and metabolic readouts for the assessment of novel targeted therapies.

1.8.1 Monitoring the Early Response to Chemotherapy

As discussed previously, breast cancer patients who are offered neoadjuvant chemotherapy to down-stage locally advanced disease prior to surgery have the possibility to undergo smaller lumpectomies and, thus, the chance to be able to conserve more breast tissue, while, hopefully, still presenting negative surgical margins. Despite that only 1 in 10 cases involve complete pathological response, nearly 90% of patients are offered the same cytotoxic chemotherapy regimen; this means that under current guidelines, medical oncologists have limited options for tailoring systemic therapies for each individual. Given that treatment response is a strong prognostic indicator of outcome in LABC, it can be argued that such patients, who are also known to present increased risk for locoregional recurrence and metastatic progression, could especially benefit from abandoning ineffective treatments at an early stage and then selecting alternative ones. If enacted with the requisite timing and precision, response-adaptive management strategies such as these would have attendant welfare benefits for the patient, as well as cost benefits for the health-care system [115].

On the basis of numerous pre-clinical results with genotoxic agents [109, 110, 116], including the

common breast cancer drug doxorubicin [108], hyperpolarized ¹³C MRI with [1-¹³C]pyruvate has a potential role in monitoring the early response to neoadjuvant chemotherapy in locally advanced breast cancer. A decrease in pyruvate-to-lactate HP ¹³C-label exchange between scans obtained prior to starting chemotherapy with those after a few days of treatment could be used as an early indication that the tumour is responding and might predict a benefit to completing the prescribed course of treatment [106].

One of the advantages of HP ¹³C-labelled tracers as injectable contrast agents is that they make use of non-radioactive isotopes and are observed with non-ionizing electromagnetic radiation; notably, by the very same physical operating principles that make diagnostic MRI an essentially harmless technology, associated with only minor biological side-effects and few serious contraindications such as metallic implants, pacemakers, stents etc. This means that HP [1-¹³C]pyruvate MRI could be used to non-invasively probe changes in [1-¹³C]lactate labelling across the entire volume of a breast lesion, repeatedly over time, without the risk of radiation exposure. This feature of HP [1-¹³C]pyruvate compares favourably with some of the important contemporary metabolic imaging tools that use radionuclide-based contrast agents, since repeated exposure to ionizing radiation carries risks that make them undesirable for longitudinal studies and can prohibit their use in certain patients.

1.8.2 Non-invasive Metabolic Phenotyping of Breast Cancer

The other foreseeable role of hyperpolarized ¹³C MRI in the clinical management of breast cancer is as part of the rapidly maturing, breast MRI toolkit that's available to physicians to perform what is essentially "radiological phenotyping" of disease [117, 118]. The substrate [1-¹³C]pyruvate is a proven agent for labelling endogenous lactate pools in vivo, which makes HP ¹³C MRI the only currently available imaging method for noninvasively monitoring tumour lactate metabolism in breast cancer. As discussed in section 1.5, elevated lactate production is thought to be a promoter of tumourigenesis and metastatic spread in numerous solid cancers; accordingly, the next generation of targeted therapies include pharmacological treatments that exploit the known switch to glycolytic metabolism by disrupting lactate homeostasis, either through small molecule inhibition of LDH to reduce the rate of pyruvate-to-lactate conversion in the cytosol, or by inhibiting activity of MCT1/4 and preventing the export of lactate into the tumour associated microenvironment [119, 120, 121]. Upon injection of prepolarized [1-¹³C]pyruvate, the amount of [1-¹³C]lactate labeling is known to be related to several factors, which, among others, includes both MCT1 expression and LDH activity; therefore, HP ¹³C MRI could be used to identify patients who present "lactagenic" radiological phenotypes, as well as monitor the

biochemical response to novel targeted therapies.

A powerful feature of metabolic imaging with hyperpolarized ¹³C-labelled substrates comes from the fact that, with a few hardware upgrades, it can be performed on conventional clinical MRI scanners. One of the implications for this, which is analogous to the way that multi-modal fusion is achieved with ¹⁸F-FDG PET/CT systems, is that standard diagnostic breast MRI scans acquired in the same session can be used to provide accurate anatomical localization for the [1-¹³C]pyruvate and [1-¹³C]lactate images. Additionally, since the irreversible decay of the hyperpolarized state limits the duration of the data acquisition window to less than 3 minutes, the extra time that would be needed to obtain HP ¹³C metabolic imaging scans, including the time required for ¹³C-specific setup and calibration, would add only 5-10 minutes to the duration of the MRI exam, which usually takes a total of 35-55 minutes. From the perspective of the patient, since it's likely that they would already be required to have an intravenous catheter inserted in their forearm for administering the gadolinium chelate contrast agent during the dynamic contrast-enhanced portion of their exam, the addition of a HP ¹³C scan into an existing breast MRI workup could be incorporated seamlessly, with little change to their overall patient experience.

1.8.3 Technical Considerations for Clinical Translation

While it's true that there are no fundamental limitations preventing widespread clinical translation of HP ¹³C metabolic imaging to enhance current radiological practices for breast cancer, ¹³C MRI entails the use of specialized hardware, which is not typically found in diagnostic imaging suites. In order to perform a hyperpolarized ¹³C MRI experiment on a clinical scanner, there are at least three hardware components that, while irrelevant for proton scanning, are strictly indispensable. First and foremost, a hyperpolarizer is required for hyperpolarized ¹³C imaging. The method-of-choice for producing hyperpolarized [1-¹³C]pyruvate from ¹³C-labelled pyruvic acid is DNP. DNP polarizers are now commercially available and our group is one of a few early adopters of GE Healthcare's prototype DNP polarizer: the SPINlabTM. Importantly, the SPINlabTM can be integrated with a separate quality control (QC) module that carries the dissolved sample through a series of safety checks, which must be passed before it can be approved for IV administration. It should be noted that if intended for human application, as opposed to pre-clinical, current Health Canada guidelines mandate the use of sterile fluid paths for transportation of hyperpolarized media; consequently, [1-¹³C]pyruvic acid must be both prepared and sealed from within the confines of an accredited clean room. Most major hospitals with cancer centres should be well equipped to negotiate the provision of trained technologists and materials in

order to comply with the relatively straightforward regulations issued by the federal government.

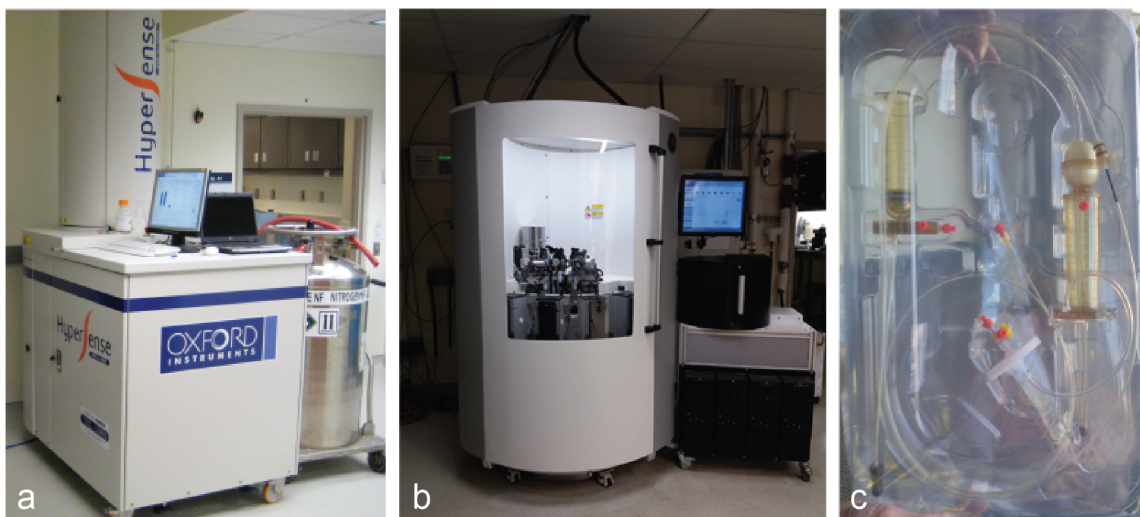


Figure 1.3: Polarizer hardware at Sunnybrook Research Institute. The Oxford HyperSense (a) was decommissioned in 2013 and replaced with the GE SpinLab (b), with adjoining QA/QC module in black with silver handle. The design of the sterile fluid paths (c) allows for contactless polarization in the SpinLab and up to 4 doses of investigational product can be prepared simultaneously.

The remaining hardware upgrades for clinical MRI scanners are not necessarily unique to specific applications with hyperpolarized [1-¹³C]pyruvate; in fact, they are technical requirements of any type of ¹³C-based imaging, regardless of the compounds bearing ¹³C-labels or whether or not the nuclei are even hyperpolarized. The first of these hardware components is “broadband” radio frequency (RF) electronics: equipment used for the various stages of filtering, digitization, amplification and digital-to-analog conversion of the RF signals that are sent and received during an MRI experiment. Here, the term broadband is used in reference to the notion that for a fixed field strength, the operating frequency of an observable RF signal, according to the Larmor equation (Eqn. 1.1), is determined by its nuclei-specific gyromagnetic ratio. In conventional MRI scans, this pertains to the narrow range of frequencies around the proton resonance, meaning that clinical RF equipment tends to be optimized for highest performance within a particular “narrowband” of the RF spectrum corresponding to the ¹H nuclei of endogenous water molecules. In order to produce and sample RF signals at rate corresponding to the ¹³C Larmor frequency, which is approximately 4-fold lower than ¹H, specialized RF electronics with broadband capabilities are required. Since most of the major commercial providers of clinical MRI systems currently sell RF equipment for broadband applications, and that each vendor would likely orchestrate the details of hardware design and system integration, this is currently feasible.

1.8.4 ^{13}C Hardware for Imaging the Human Breast

In addition to the DNP polarizer and broadband RF electronics, the third hardware component needed for clinical MRI scanners to perform metabolic imaging with HP ^{13}C in breast cancer patients is a dedicated ^{13}C RF coil system that conforms to the anatomy of the female breast. RF coil systems must fulfill two tasks: RF reception and RF transmission. With regards to constructing ^{13}C receiver coils for breast imaging—apart from the large difference in operating frequency and, thus, incompatibility with ^1H coils for ^{13}C nuclei and vice versa—the fact that the examined nuclei is ^{13}C holds little bearing on the design principles that, with respect to the pursuit of optimal MR signal reception in a target anatomy, are virtually independent of the specific nuclei at clinical field strengths. In other words, a ^{13}C RF coil that is optimized for high fidelity signal reception from a specific anatomical region i.e. breast, would likely share the same overall look and design as an analogous ^1H receiver coil.

The situation is slightly different in regards to the development of ^{13}C coils for RF transmission. This is not because of some fundamental disparity in coil design principles, rather, it is due to concerns of practicality. Transmit coils fulfill the task of MR signal excitation within the body, and they accomplish this by conversion of electrical power into an oscillating magnetic RF field. Optimal transmit coils maximize efficiency of power transfer to the subject, while minimizing spatial heterogeneity of its excitatory magnetic field. For nearly every clinical MRI exam, RF excitation is performed by what's usually termed the “body coil”—a large, volumetric transmit coil that, in typical systems, resides within the housing of the MRI scanner. One of the practical concerns for constructing ^{13}C transmit coils is the limited space inside the scanner bore, which must be shared with the other essential entities: the ^{13}C receiver coils; some type of patient-support structure; and, of course, the human subject. Accordingly, unless the intended use is in particularly wide-bore scanners, the scarcity of space is an important practical concern for designing dedicated ^{13}C RF coil systems for breast imaging.

Of these upgradeable hardware components—DNP polarizer (~\$2M); broadband RF electronics(~\$300k); dedicated ^{13}C RF coils for breast imaging(~\$100k)—the latter presents perhaps the most significant challenge to rapid and widespread translation. Unlike breast coils for conventional MRI, for which there are many products currently competing on the open market, “off-the-shelf” coils for ^{13}C breast imaging are only beginning to become available. Nevertheless, with access to a DNP polarizer, broadband RF electronics, and a ^{13}C breast coil system, clinical MRI scanners can be transformed into multi-modal platforms for monitoring tumour metabolism in breast cancer.

1.8.5 Hypothetical HP [1-¹³C]Pyruvate MRI Exam

To understand what is actually entailed by “monitoring” ¹³C signals in vivo, consider a hypothetical experiment involving an IV injection of prepolarized [1-¹³C]pyruvate into the forearm of a breast patient at time $t = t_0$. At some point prior to t_0 , the patient is given an IV catheter, secured and localized inside of the MRI scanner; patient-dependent calibration of the ¹³C RF coil system is performed; and a clinical dose of [1-¹³C]pyruvic acid has reached optimal polarization inside the nearby DNP polarizer. At t_0 minus ~ 40 seconds, the frozen sample of hyperpolarized [1-¹³C]pyruvic acid is rapidly heated and dissolved with hot, pressurized water. Next, the hyperpolarized pyruvic acid solution is neutralized with NaOH in a Tris buffer to reach a physiological $\sim$ pH of 7.4. Immediately following dissolution, the sample is delivered to an adjacent QC module, where several important parameters i.e. concentration, pH, sample purity, etc., are measured as quickly as possible. Since the QC module takes a total of 20-30 seconds to complete its safety tests—samples that, for any reason, fall outside acceptable limits are rejected and cannot be administered—it is important to consider that the hyperpolarized state begins its irreversible decay towards thermal equilibrium the moment that dissolution begins. At t_0 minus ~ 10 seconds, the QC module releases the loaded sterile fluid path, which is handled by a pharmacy technician and passed through a dedicated aperture to an MR technologist, standing-by inside the scanner room, who then loads the sample into a clinical grade power-injector. Finally, at time $t = t_0$, from inside the control room, a qualified physician arms the power-injector and the patient receives the dose of hyperpolarized [1-¹³C]pyruvate at $4 \text{ mL} \cdot \text{s}^{-1}$, followed by a 25 mL saline flush.

1.8.6 Physical Considerations of Metabolic Imaging with HP [1-¹³C]Pyruvate

The end of the flush marks the beginning of the 2-3 minute imaging window, beyond which, due to T_1 relaxation, the polarization of the ¹³C-labelled metabolites inevitably decays to thermal equilibrium levels, rendering them undetectable. In order to observe hyperpolarized compounds using magnetic resonance, nuclear spins must first become energized or “excited,” which causes them to emit a detectable MR signal in the form of an oscillating magnetic field. To reiterate, the task of excitation is accomplished by ¹³C RF transmit coils, while the task of detection is performed by ¹³C receiver coils. It is important to consider that after dissolution, T_1 relaxation occurs whether or not any subsequent magnetic resonance imaging or spectroscopy is performed at all. In fact, as one of its more nuanced idiosyncrasies, the very act of converting the stored energy of HP nuclei into an observable RF signal causes irreversible consumption of the hyperpolarized state. This is to say that it is technically possible to convert the entirety of the hyperpolarized ¹³C magnetization into an observable signal after applying

a single excitation; however, such an experiment would prematurely accelerate the reestablishment of thermal equilibrium and, thus, close the finite imaging window after just one measurement. Therefore, this means that the impact of every measurement must carefully be considered during the development of data acquisition methods for HP ¹³C MRI. Despite purporting to measure the same biochemical phenomena, the fact that there have been so many published reports on data acquisition methodology for HP [1-¹³C]pyruvate MRI stands as a testament to the inherent technical challenges of “monitoring” HP ¹³C-labelled substrates in vivo.

After exciting the spins within the subject by playing an “RF pulse”—a finite duration (< 100 ms) electrical waveform that is modulated to the Larmor frequency, amplified and delivered as an RF magnetic field by the transmit coil—the observable signal can be measured as a voltage induced across the terminals of each RF receiver coil, which is subsequently amplified, filtered, demodulated, digitized by the broadband RF electronics and, finally, stored in memory. Ignoring the contribution from T_1 relaxation, as well as the RF coils themselves, the sampled metabolic signal $s[n]$ can be modelled as:

$$s[n] = \sum_{m=1}^M \rho_m(x, y, z, n\Delta t) e^{i2\pi\Delta f_m n\Delta t} e^{-n\Delta t/T_2^*} \quad (1.4)$$

where the index $m = 1 : M$ refers to the individual metabolites i.e. pyruvate, lactate, etc.; the index $n = 1 : N$ is the sample number; Δt is the sampling period (s); Δf_m is the m^{th} chemical shift (Hz); T_2^* is the decay rate of the excited signal (s^{-1}); and $\rho_m(x, y, z, n\Delta t)$ is spatio-temporal profile of the m^{th} metabolite. Physically, ρ represents ensembles of spins—in this case, ¹³C nuclei—that together, under the influence of an external magnetic field, produce a measurable net magnetization. The complex exponential term $e^{i2\pi\Delta f_m n\Delta t}$ can also be expressed in the equivalent form $\cos(2\pi\Delta f_m n\Delta t) + i \cdot \sin(2\pi\Delta f_m n\Delta t)$, which makes clear the sinusoidal, or oscillatory, nature of MR signals.

The objective of an MRS experiment is to decode the parameters under investigation, ρ_m , from measured signals like $s[n]$. This highly simplified model describes the response of a system to an RF pulse, which is called a “free induction decay” or FID. The Fourier transform (FT)—a ubiquitous analysis tool that is used to uncover a signal’s underlying spectral components—can be applied to the digitized FID, $s[n]$, to examine its spectrum and, thereby, resolve the M separate signals. The rudimentary experiment described here i.e. an RF pulse followed by the acquisition of an FID, is sometimes termed “pulse-acquire” and it embodies the essence of FT-NMR spectroscopy.

1.8.7 Technical Considerations of Metabolic Imaging with HP [1-¹³C]Pyruvate

Returning to our hypothetical patient experiment—once the hyperpolarized [1-¹³C]pyruvate has been taken up by cells into the cytosol, through the enzymatic processes described in section 1.7, the ¹³C label may be found in [1-¹³C]lactate, [1-¹³C]alanine, and [1-¹³C]bicarbonate, as well as one other: [1-¹³C]pyruvate-hydrate, which itself is not a unique metabolite but, simply, a water-bound form of pyruvate that's found in $\sim 1:10$ equilibrium with free [1-¹³C]pyruvate in solution. Accordingly, $\rho_m(x, y, z, n\Delta t)$ here corresponds to the spatio-temporal profiles of these $M = 6$ excited metabolites. Since the objective of performing hyperpolarized ¹³C MRI in breast cancer is to observe the kinetics of ¹³C-label exchange between pyruvate and lactate in vivo, unless numerous a priori assumptions about the data are made, a pulse-acquire MRS experiment, even with multiple repetitions to generate a dynamic MRS dataset i.e. $s_j[n] \in \{s_1[n], s_2[n], s_3[n], \dots, s_J[n]\}$, is highly underdetermined for fitting all parameters of Eqn. 1.4—namely, resolving spatial information. It should be noted that, although it's true a certain degree of spatial localization is achieved with simple pulse-acquire MRS—this is by virtue of the “sensitivity profile” of RF receiver coils, which limits signal detection to a finite region that's within close proximity to its conductive elements—a ¹³C spectrum alone can't relay the relative contributions of metabolic signals arising from different tissue compartments i.e. normal-versus-tumour, and this severely hampers interpretability; therefore, spatial encoding is a highly desirable feature of hyperpolarized ¹³C data acquisition schemes.

The task of spatial localization in “magnetic resonance imaging” is performed by the concerted action of three linear magnetic field gradient coils: G_x , G_y and G_z . As the name implies, gradient coils impose a linear magnetic field ramp or gradient along a prescribed direction, and this serves to slightly add or take away from the MRI scanner's main magnetic field. By manipulating the total magnetic field experienced by nuclear spins within the body, the gradient coils, referred to throughout as “gradients”, can be used to impart a spatially dependent change in precessional phase. The relationship between the gradients and phase can be expressed as follows, considering only a single spatial dimension for clarity:

$$\frac{d}{dt}\theta(x, t) \equiv \omega(x, t) = \gamma B(x, t) = \gamma B_0 + \gamma G_x(t)x \quad (1.5)$$

$$\theta(x, t) = \int_0^t \omega(x, \tau) d\tau = \gamma B_0 t + \int_0^t \gamma G_x(\tau)x d\tau \quad (1.6)$$

$$\theta_0(x, t) = \theta(x, t) - \gamma B_0 t = x \int_0^t \gamma G_x(\tau) d\tau \quad (1.7)$$

where $\omega(x, t)$ is the precessional angular frequency ($\text{rad} \cdot \text{s}^{-1}$); γ is the gyromagnetic ratio ($\text{rad}^{-1} \cdot \text{s}^{-1}$); $B(x, t)$ is the total applied field (T); B_0 is the static field of the scanner (T); $G_x(t)$ is the amplitude of the x-directed field gradient ($\text{T} \cdot \text{m}^{-1}$); $\theta(x, t)$ is the instantaneous precessional phase (rad); and $\theta_0(x, t)$ represents the phase after demodulation to baseband. The takeaway point is that by manipulating the amplitude of the total applied field, the phase of the excited spins, located at position x , after time t , can be controlled by a linear field gradient. This expression can be easily generalized to 3D space:

$$\theta_0(x, y, z, t) = x \int_0^t \gamma G_x(\tau) d\tau + y \int_0^t \gamma G_y(\tau) d\tau + z \int_0^t \gamma G_z(\tau) d\tau \quad (1.8)$$

The specific history of the gradient waveforms after an RF excitation at $t=0$ can be used to establish spatial distributions or phase patterns across the subject, and this is described using the k-space formalism:

$$k(t) = \int_0^t \gamma G(\tau) d\tau \quad (1.9)$$

$$\theta_0(x, y, z, t) = x \cdot k_x(t) + y \cdot k_y(t) + z \cdot k_z(t)$$

Therefore, the effect of the gradients on a sample, $\rho(x, y, z)$, can be written mathematically as:

$$\rho(x, y, z) \cdot e^{i\theta_0(x, y, z, t)} = \rho(x, y, z) \cdot e^{ix \cdot k_x(t)} \cdot e^{iy \cdot k_y(t)} \cdot e^{iz \cdot k_z(t)} \quad (1.10)$$

Finally, considering the contributions from each of the nuclear spins across the full extent of the excited volume, the received signal at time t can be written as:

$$s(t) \approx \iiint \rho(x, y, z) \cdot C_j(x, y, z) \cdot e^{ix \cdot k_x(t)} \cdot e^{iy \cdot k_y(t)} \cdot e^{iz \cdot k_z(t)} dx dy dz \quad (1.11)$$

where $C_j(x, y, z)$ is the spatial sensitivity profile of the j^{th} receiver coil. Through inspection, $s(t)$ can be viewed as relating to a mapping or projection of $\rho(x, y, z)$ onto a domain that is not parameterized by x , y and z , but, rather, by k_x , k_y and k_z . In an MRI experiment, the object under investigation, $\rho(x, y, z)$, is imaged by collecting a set of “spatially encoded” measurements that directly correspond to its physical representation in an inverse-distance domain, $P(k_x, k_y, k_z)$, otherwise referred to as “k-

space". Once a complete set of k-space measurements have been acquired, the "x-space" or "image-space" representation of the raw k-space data can be reconstructed by application of the inverse Fourier transform:

$$\rho(x, y, z) \approx \iiint P(k_x, k_y, k_z) \cdot e^{-ix \cdot k_x} \cdot e^{-iy \cdot k_y} \cdot e^{-iz \cdot k_z} dk_x dk_y dz \quad (1.12)$$

This astounding result implies that an MRI scanner can be thought of as a sort of Fourier transform machine, which takes pictures of the inside of the body by measuring its spatial-frequency components. The determinant of whether or not a set of k-space measurements is "complete" depends on the desired imaging resolution and field-of-view. By simultaneously applying a time-varying gradient waveform while sampling the received signal, a quick succession of k-space measurements can be made along a predetermined path called a "k-space trajectory". A prescription for the durations, amplitudes and shapes of the RF pulses and gradient waveforms used in an MRI experiment to perform the task of image formation is called a pulse sequence design.

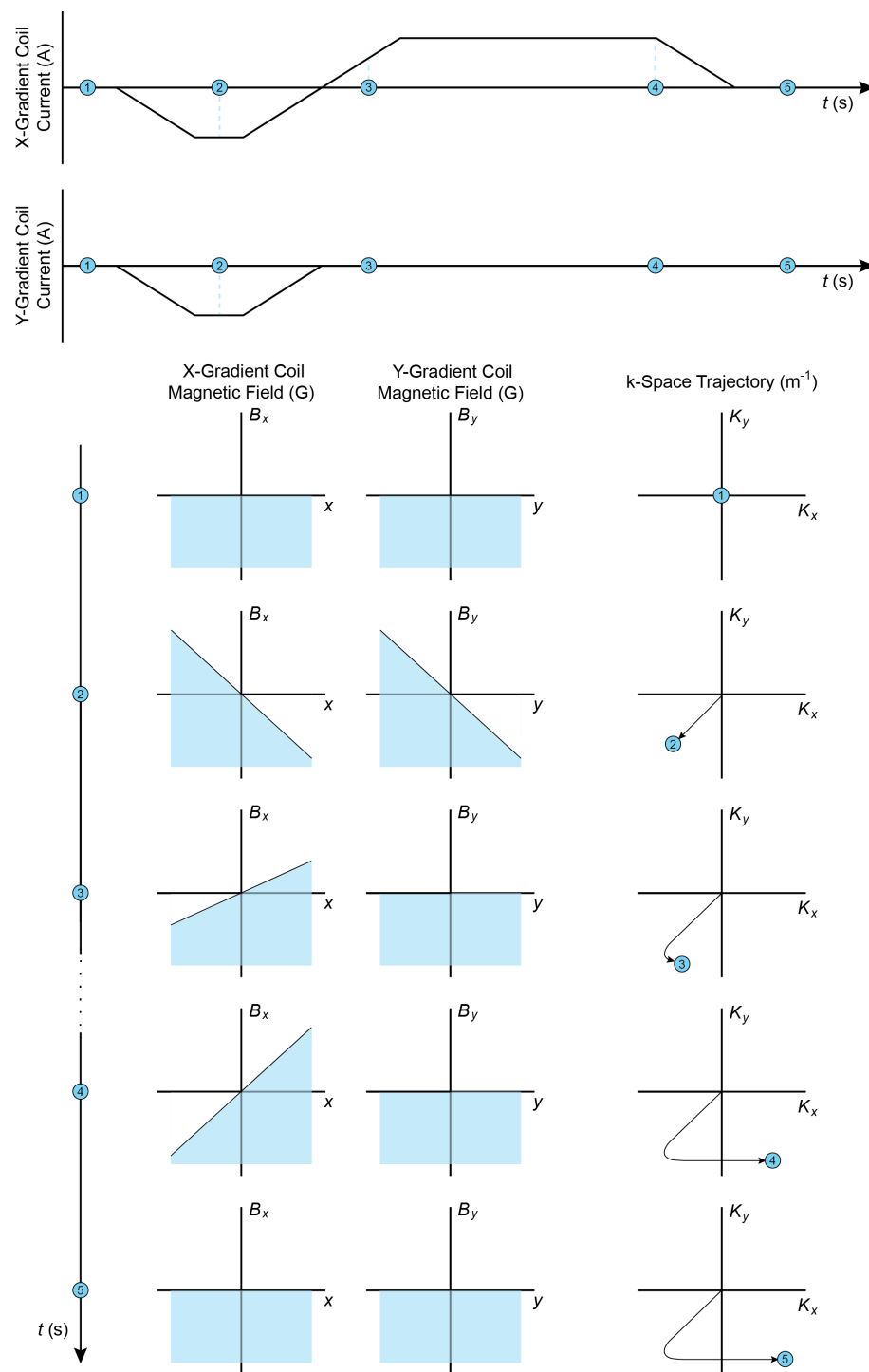


Figure 1.4: Pulse sequence diagram (PSD) illustrating the relation between timing of gradient amplitude and k-space traversal. Before applying any gradients (1) the signals from different regions of the sample all have the same frequency, determined by the external field. The current travelling through the X- and Y-gradient coils generates magnetic fields that adds and subtracts from the main magnetic field to impose a linear spread Larmor frequency, the steepness of which is controlled by the gradient amplitude. As the gradients are held on, the non-uniform rate of precession causes the phase of some signals to lag behind and others to advance ahead, which winds a periodic spatial wave of signal phases. The particular spatial wave created is identified by its location in k-space.

What makes the task of developing data acquisition schemes for hyperpolarized ^{13}C imaging with $[1-^{13}\text{C}]\text{pyruvate}$ technically challenging is the inherent multi-dimensionality of the underlying signal. The metabolite profiles in vivo have not only spatial dimensions, but also a temporal dimension that is modulated by its chemical shift, molecular kinetics and T_1 decay. This means that competing pulse sequence designs may offer trade-offs that must be carefully considered in each application. For instance, in the first-in-man-study that investigated HP $[1-^{13}\text{C}]\text{pyruvate}$, 3 different pulse sequence designs were tested in order to assess feasibility: 1D spatially localized dynamic MRS; 2D localized dynamic MRS; and 3D localized single time point MRS. The 3D k-space matrix could not be acquired in a time-resolved fashion since the pulse sequence used was prohibitively slow, thus, the volume could only be covered once during the finite imaging window. While a single time point or static, volumetric MRS scan, can, in principle, be used to estimate pyruvate-to-lactate ^{13}C label exchange, data fidelity can be highly sensitive to the timing and kinetics of substrate delivery [122]. Accordingly, the focus has been on developing data acquisition methods that are capable of providing volumetric coverage, spectral decomposition or resolution, and a temporal dimension in order to capture important signal dynamics. Many advanced imaging sequences have been designed to fulfill these objectives and have been described in multiple recent reviews [123, 124, 125].

1.9 Outline of thesis

This thesis describes the design, development and validation of a ^{13}C magnetic resonance imaging platform that can enable studies with hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ and $[1-^{13}\text{C}]\text{lactate}$ as potential metabolic biomarkers in breast cancer. The contributions presented herein are motivated by overlapping but technically independent challenges, which, taken together, constitute a tool kit that is capable of not only demonstrating feasibility of HP ^{13}C metabolic MRI in breast cancer patients, but also of allowing investigations into the role of hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ for personalized care.

In Chapter 2, a ^{13}C RF coil system for ipsilateral breast imaging is described. The system is comprised of a low-profile ^{13}C RF transmit coil and 2-channel ^{13}C RF receiver array, and was built with the dual-objectives of high fidelity and maximal comfort. In vitro studies validate the system performance and a study MRI without injection was conducted in a group of healthy volunteers to assess the overall workflow.

In Chapter 3, a data acquisition scheme is presented that, in addition to meeting the technical challenges of collecting time-resolved, volumetric images of $[1-^{13}\text{C}]\text{pyruvate}$ and $[1-^{13}\text{C}]\text{lactate}$, addresses a known limitation of the prior art by allowing data-driven correction of geometric distortion. The

method is a “dual-echo” method that trades off a 50% reduction in the size of the imaging matrix i.e. field-of-view or resolution, for encoding important phase information, which can be used to correct off-resonance artifacts. Experiments with healthy Sprague Dawley rats, as a pre-clinical model for in vivo $[1-^{13}\text{C}]$ lactate labelling, were performed to validate the technique of a clinical 3T scanner.

In Chapter 4, an imaging methodology for accelerating data acquisition in hyperpolarized ^{13}C MRI with $[1-^{13}\text{C}]$ pyruvate is presented. The method has since been dubbed by another author as a “pseudo-random raster” technique that has favourable off-resonance performance. Up to two 3-fold acceleration was demonstrated in vivo, and this reduction in encoding time can be used to increase the allowable size of the imaging volume or increase the temporal resolution in time-resolved studies.

In Chapter 5, the prospects and limitations of HP ^{13}C MRI for use in breast cancer are examined and outlines of conceivable studies are provided. Future directions for improving the hardware and imaging strategies are discussed.

Chapter 2

Radio Frequency Coil System for ^{13}C Magnetic Resonance Imaging in Breast

2.1 Introduction

The magnetic resonance (MR) transceiver system is an essential component of a magnetic resonance imaging (MRI) scanner and performs two tasks: *excitation*—to generate observable MR signal inside the body; and *reception*—to measure said signal. Both tasks are carried out by radio frequency (RF) “coils” that serve as interfaces between RF magnetic fields propagating through space and electrical voltage in metal conductors. Although in principle, a device that can receive RF signals would then also be capable of functioning as an RF transmitter, and *vice versa* [126], the use of dual-purpose, “transceive” RF coils in contemporary MR imaging has been marginalized in favour of separate, task-specific circuits.

For RF transmission, virtually all MRI examinations make use of the scanner’s built-in “body coil,” engineered specifically to provide spatially uniform MR signal excitation across large regions of the body. In fact, the design principles that make body coils versatile RF transmitters, in turn, make them fairly lousy general-purpose RF receivers. Since the achievable signal-to-noise ratio (SNR) of MR images is limited by the sensitivity of the MR detection circuit, RF receiver performance is an important consideration in MR imaging experiments. For instance, in MRI scans, $\text{SNR} \propto \sqrt{t}$ i.e. time spent measuring data, and $\text{SNR} \propto \Delta x \Delta y \Delta z$, so it follows that any gains to sensitivity afforded by one RF coil relative to another could be traded-off to reduce the length of scan times and/or the size of the imaging voxels i.e increase resolution.

RF receive coils are designed to conform closely to the target anatomy in order to improve inductive coupling of their conductive elements with the magnetic nuclei within those tissues; this, in effect, boosts the strength of nearby MR signals. In contrast to body coils, which are large structures that completely surround the subject, most RF receivers are relatively small “surface coils” that trade-off the extent of their detection field for the amount of noise coupled i.e. similar signal with less noise. Modern MR receiver systems offset this trade-off through the use of “phased arrays” of surface coils; by combining the data from multiple independent receivers, anatomical coverage can be extended without compromising sensitivity [127]. At field strengths of 3T or less, state-of-the-art MR transceiver systems for diagnostic MRI comprise the body-coil, for uniformly exciting water-bound ^1H nuclei within the anatomy under investigation, along with receive arrays, specialized for use in a particular anatomical region i.e. neuro-vascular array for imaging the brain, cardiac array for imaging the heart, breast coils for breast imaging, etc.

There is growing interest in developing MR transceiver systems capable of imaging ^{13}C nuclei in humans in an effort to evaluate the clinical utility of hyperpolarized ^{13}C MRI. However, at present, only a small number of centres have the requisite physical and institutional means for conducting HP ^{13}C MRI experiments in human subjects. A review of some ^{13}C coils that have been used to collect human data are presented in the following section.

2.1.1 ^{13}C RF Coils for Imaging in Humans

The first-in-man demonstration of HP [$1\text{-}^{13}\text{C}$]pyruvate imaging was published in 2013 with the aim of showing safety and feasibility of HP technology in prostate cancer patients [1]. For RF transmission, the authors used a bore-insertable ^{13}C volume coil, which was hinged like a “clamshell” in order to facilitate patient entry, and a $^1\text{H}/^{13}\text{C}$ endorectal coil was used for reception (figure 2.1.1). Patients were positioned face up, with their pelvis centred in the clamshell, and a separate support stand (not shown) was used as an anchor for stabilizing the physician-guided, transrectal insertion of the endorectal coil. These same coils were also used in other preliminary studies of HP ^{13}C MRI in patients with prostate cancer: two technically-oriented conference proceedings [128, 129] and one case-study of early response to androgen ablation therapy [130].

Clamshell coils have been used as ^{13}C RF transmitters in various other human studies. As described in a recent pilot study from our group [103], the first HP ^{13}C cardiac images in humans were obtained by positioning healthy volunteers inside a clamshell coil and then placing a 4-channel phased array ^{13}C RF receiver on top of their chests, directly above their hearts. The design of this ^{13}C RF receiver was first

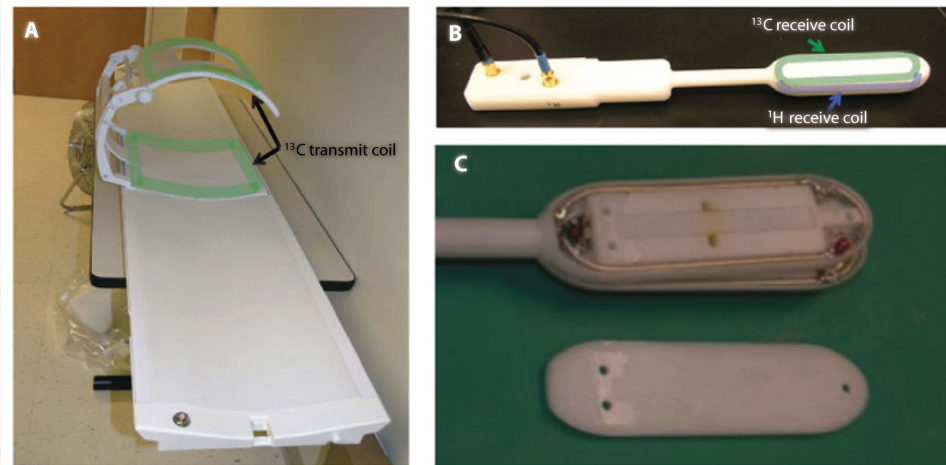


Fig. 1. ^{13}C coil setup

(A to C) ^{13}C transmit coil (A) and endorectal $^1\text{H}/^{13}\text{C}$ receiver coil (B) used for acquiring data. The location of the coils is outlined on (A) and (B), with the layers inside the endorectal coil being seen in (C). The dimensions of the elements in the endorectal coil were 4 inches $\times$ 1 inches, with the total length of the coil being 12 inches.

Figure 2.1: The “clamshell” ^{13}C transmit coil and endorectal $^1\text{H}/^{13}\text{C}$ receive coils used in [1]. Adapted from Nelson, Sarah J., et al. “Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized $[1-^{13}\text{C}]$ pyruvate.” *Science Translational Medicine* (2013)

described in [131] and is made up of two separate phased arrays—sometimes referred to as “paddle coils” on account of their particular shape—each with 4 individual receiver elements (see figure 2.1.1a). The recent demonstration of HP ^{13}C MRI in brain tumours [2] made use of the same hardware, but instead, placed the paddles bilaterally with respect to the head (figure 2.1.1c).

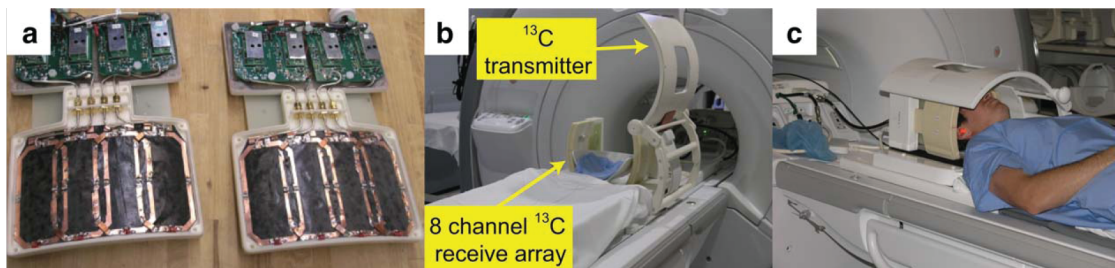


FIG. 1. ^{13}C RF coil configurations developed for human brain study. (a) Eight-channel ^{13}C phased-array coils. (b) Clamshell volumetric ^{13}C transmit coil and bilateral eight-channel phased-array receive coils. (c) A picture of ^{13}C RF coil setup with a volunteer.

Figure 2.2: Depiction of the 8-channel ^{13}C paddle and clamshell coil setup used in [2]. Adapted from Park, Ilwoo et al. “Development of Methods and Feasibility of Using Hyperpolarized Carbon-13 Imaging Data for Evaluating Brain Metabolism in Patient Studies.” *Magnetic Resonance in Medicine* (2018)

During the proceedings of the 24th annual meeting of the International Society for Magnetic Resonance in Medicine (ISMRM) in 2016, there was a presentation of preliminary results from the first-in-woman study of in vivo breast cancer metabolism using HP $[1-^{13}\text{C}]$ pyruvate [132]. The study included

3 patients (age = 58 ± 16 yrs) with biopsy confirmed lesions (>2 cm) who were scanned prior to treatment and, just as in the human ^{13}C studies in heart [103] and brain [2], the authors made use of the clamshell and paddle coils. Their ^{13}C RF coil setup for breast imaging had the patients positioned face down in a clamshell coil, laying on top of a single paddle (4-channels) so that the receiver was located anterior to the breast.

Other recent demonstrations of HP ^{13}C MRI in humans used ^{13}C coils that differed from the previously described hardware and were presented at the 25th annual meeting of the ISMRM in 2017. One involved healthy volunteers (age = 46 ± 5 yrs) and investigated cardiac metabolism under fasted and glucose loaded conditions [133]; unfortunately, although it was noted that multi-channel data was collected, the details of the RF coils used in the study were not provided. Another other study was performed in a group of 8 patients with liver metastases (age = 56 ± 10 yrs). Uniquely, the authors made use of a prototype “figure-8” transmit/receive coil, which they secured anterior to liver (figure 2.3).

2.1.2 Integrating ^{13}C RF Coils with Conventional ^1H MRI

One of the most compelling features of HP ^{13}C imaging is that the metabolic data can be readily co-registered or “fused” with proton MRI scans from the same imaging session, thus, creating the possibility of adding a metabolic dimension to the existing diagnostic capacity of MR technology. Since safety and feasibility were the primary aims of the HP ^{13}C human studies mentioned, multi-modal i.e. $^1\text{H}/^{13}\text{C}$, data integration was not a critical objective. As a consequence, most of the studies performed ^1H MR signal reception with the body-coil.

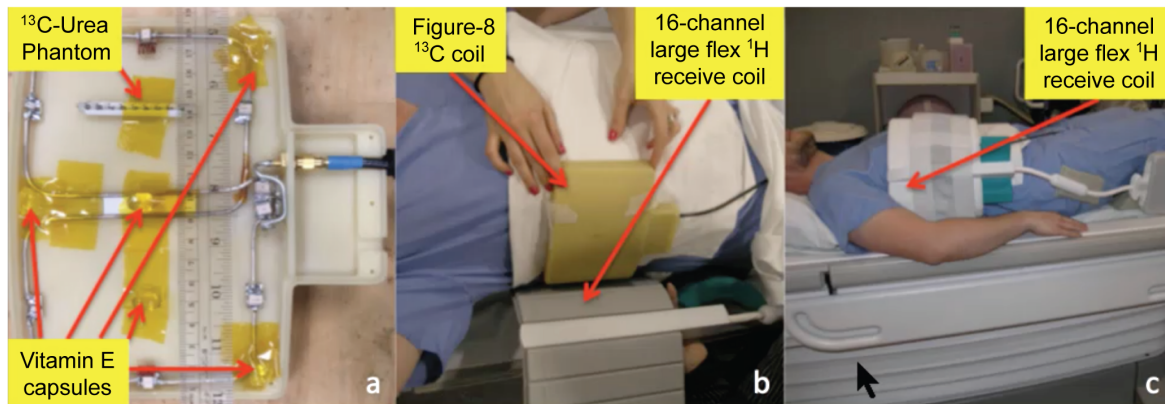


Figure 2.3: The figure-8 ^{13}C transmit/receive coil (a) and subject positioning (b-c) used in [3]. Adapted from Zhu, Zihan et al. “Hyperpolarized ^{13}C Dynamic Breath-held Molecular Imaging to Detect Targeted Therapy Response in Patients with Liver Metastases.” *In Proc 25th Annual Meeting ISMRM; Honolulu, HI, USA* (2017)

There are a few notable exceptions to this trend. The first comes from the endorectal coil used in the prostate imaging studies [1, 128, 129, 130], which contained individual ^{13}C and ^1H receiver elements (figure 2.1.1b). Given the close proximity of the coils to the prostate gland afforded by the transrectal approach, the sensitivity of the single ^1H channel may out-perform that of an exterior, multi-channel ^1H pelvic array. Another exception comes from the metastatic liver cancer study, where the authors made use of a commercially available flexible 16-channel phased array for ^1H reception (figure 2.3c).

There are both advantages and disadvantages to using the body coil as the ^1H receiver for acquiring anatomical images as part of HP ^{13}C MRI experiments. The advantages stem from the logistical simplicity of using a coil that is already contained within the structure of the MRI scanner itself. This approach provides the maximum amount of space for accommodating the ^{13}C RF coils and allows unimpeded positioning of the ^{13}C receivers. This is especially important for studies attempting to utilize a bulky clamshell coil, which, in fact, was not originally intended for applications beyond the prostate. The most important disadvantage of using the body coil for ^1H reception is relatively poor image quality that, as discussed previously, stems from its low sensitivity in comparison to dedicated RF receivers.

The disadvantage of poor image quality obtained with the body coil can be offset by fusing the data with high-quality diagnostic ^1H MRI scans from a separate examination. Still, this particular remedy fails to address the added costs of acquiring in two exams, what could have been feasibly acquired in a single session. There are no fundamental barriers to the integration of ^{13}C RF coils with ^1H receivers in patient studies, but, as is the case with all MR development, the best technical solutions will most certainly vary in accordance with each unique clinical application.

The objective of this work was to develop a ^{13}C RF coil system that is dedicated to performing HP ^{13}C MRI in breast cancer patients. In addition to its ^{13}C imaging capabilities, the proposed system was designed to permit the use of commercially available proton MRI breast coils for obtaining conventional diagnostic MRI scans in the same session.

2.2 Methods

The unilateral imaging system operates on the basis of “coil swapping” and relies on an independent, rigid support structure that holds the receivers as closely as possible to the breast. The ability to keep a patient secured while physicians and/or operators manipulate devices inside the scanner room is one of the marketed features of the SentinelleTM breast MRI coil system (Invivo, Gainesville, FL, USA), which, notably, has been used to perform MR-guided interventions in breast cancer patients [134]. In particular, their proprietary Variable Coil GeometryTM technology (figure 2.4b) was engineered to en-

able customizable coil positioning, serving a range of patient sizes, and is compatible with multiple removable coils that can be used in different applications, such as an 8-channel phased array for diagnostic imaging or a pair of single loop coils for unilateral MR-guided biopsy (figure 2.4c-d). Such system could be used as a base for obtaining a set of breast MRI scans of different nuclei i.e. ^1H , ^{13}C , etc., without moving the patient, offering intrinsic multi-modal co-registration within a single exam.

Therefore, the ^{13}C RF coil system consisted of separate ^{13}C transmit and receive coils that were designed to integrate with an existing SentinelleTM breast MRI coil system—referred to throughout as the SentinelleTM breast support. All hardware was developed for and tested on a clinical-grade MRI scanner, the DiscoveryTM MR750 3.0T (GE Healthcare, Waukesha, WI, USA).

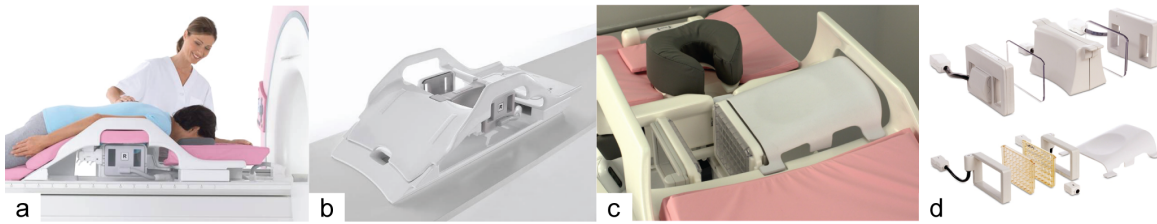


Figure 2.4: SentinelleTM breast MRI coil system (Invivo, Gainesville, FL, USA). (a) demonstration of fully assembled setup with operator and patient. (b) Variable Coil GeometryTM support structure enables device modularity. (c) assembly for unilateral biopsy, with biopsy needle grid, single loop receiver coils, contralateral breast support for medial access and padding for patient comfort. (d) 8-channel receiver coils for high quality diagnostic breast imaging and 2-channel, single loop setup for interventions. Adapted from *Sentinelle Coils Marketing Kit Presentation*. Retrieved from <http://www.invivocorp.com/wp-content/uploads/2016/10/Sentinelle-Coils-Marketing-Kit-Presentation.pptx>.

2.2.1 ^{13}C RF Transmit Coil Design

A low-profile ^{13}C transmit coil was devised to accommodate the space requirements of an existing SentinelleTM breast support. The design is analogous to modifying the previously described clamshell by removing its upper-half, theoretically giving up both some transmit-field homogeneity and power transfer efficiency in exchange for freeing up much needed room inside the scanner’s 60 cm bore. A schematic of the proposed RF transmit circuit is shown in figure 2.5 with dashed lines to delineate its individual functional units.

Practically speaking, since RF coils are resonant RLC circuits—resistive (R), inductive (L) and capacitive (C)—designed to interact with magnetic rather than electric fields, the most important functional unit for this transmitter design is the large conductive loop that serves as the principle inductive element (figure 2.5c). Resonance can be achieved at the desired ^{13}C frequency, i.e. 32.14 MHz at 3T, by balancing the inductance of the large loop with tuning capacitors (C_t). In order to provide efficient

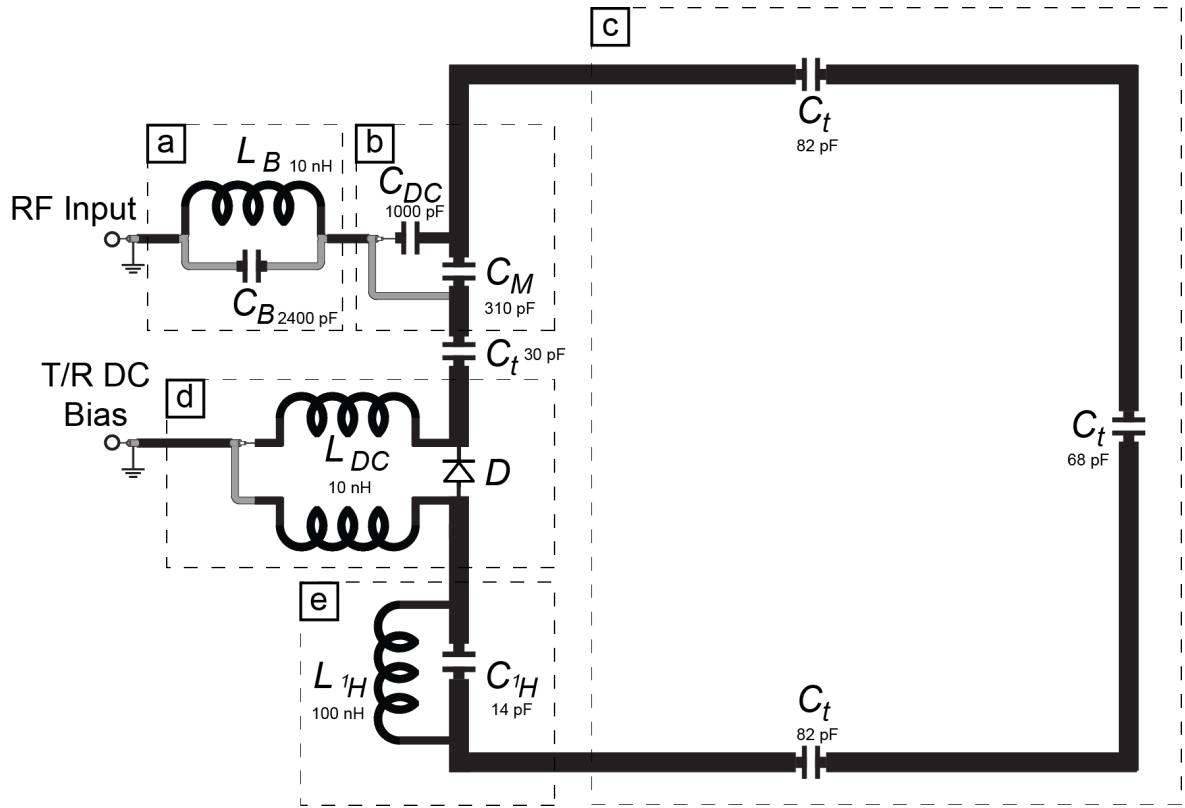


Figure 2.5: RF transmit circuit. a) Balun or “cable-trap” used to isolate the coil from the up-stream amplifier electronics. b) Matching circuit comprised of parallel matching capacitor (C_m) and DC blocking capacitor (C_{DC}). c) main inductive loop with 3 segmented tuning capacitors (C_t). d) Active blocking network, consisting of PIN diode (D) and “RF choke” inductors (L_{DC}). e) Proton blocking circuit that presents a high impedance in the coil at the ^1H resonant frequency.

power transfer from the RF amplifier to the transmit coil and, ultimately, the subject, the electrical impedance of the total circuit is transformed to $50\ \Omega$ by a matching capacitor (C_m) at the coil terminals (figure 2.5b).

The circuit has two independent inputs: one for applying electrical power in the form of RF current; and the other for applying a logical signal in the form of a DC bias voltage. The latter is used to control an active blocking network (figure 2.5d), a critical component of transmit-only coils, which prevents the flow of current through the coil except when it is being used to transmit RF power. The main purpose of this is to make the transmit coil inactive while the receiver coils are measuring signal. When a 15 V DC signal is applied to the “T/R Bias” input in figure 2.5, the PIN diode (D) is reversed biased ($-15\ \text{V}$) and behaves as an open circuit, effectively blocking current flow in the coil. Alternatively, upon applying a $-2\ \text{V}$ DC signal, the PIN diode is fully forward biased and behaves as a short circuit. This type of binary logical control is commonly used to switch between the transmit and receive phases of MR imaging experiments and is sometimes referred to as the “T/R bias”.

The final two circuit components are the “balun” (figure 2.5a) and the “proton trap” (figure 2.5e). The balun is a tuned element that blocks the flow of current along the outer-shield of the co-axial cable connecting the coil to the RF amplifier, and serves to isolate the transmit coil from the rest of the cables and electronics. The proton trap is also a tuned element that is instead designed to present large impedance at the resonance frequency of ^1H i.e. 128.1 MHz at 3T, and, thus, helps to prevent the coil from absorbing RF power during ^1H transmission by “trapping” current flow.

2.2.2 ^{13}C RF Receive Coil Design

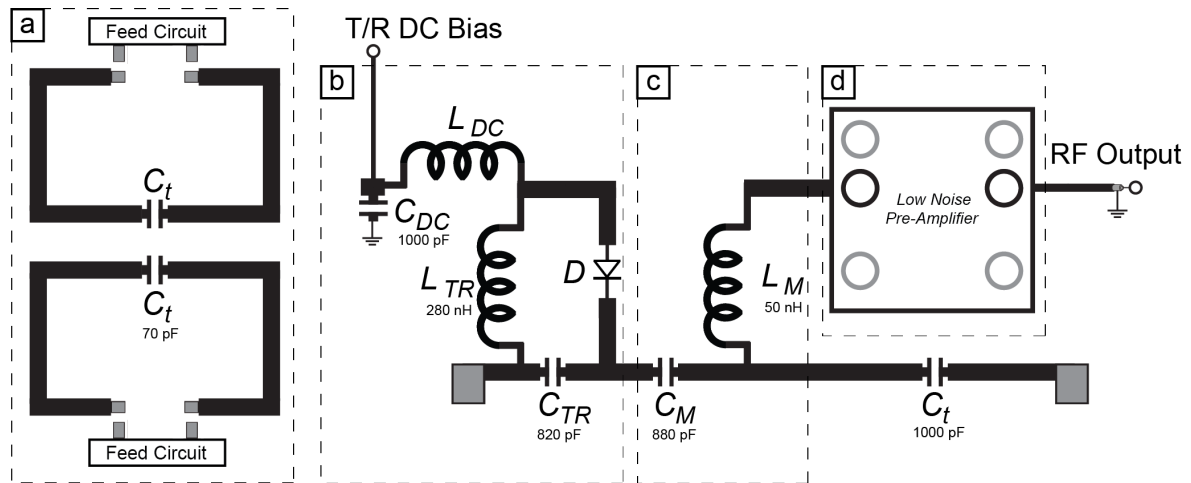


Figure 2.6: RF receive circuit. a) schematic of the two single loops with feed circuits, segmented by a series tuning capacitor (C_t). b) active blocking network, with RF choke elements (L_{DC} , C_{DC}), PIN diode (D) and tuned L-C tank (L_{TR} , C_{TR}). c) matching circuit configuration for transforming the impedance of the coil. d) Low noise, low input impedance pre-amplifier (WMA32C, WanTCom, Chanhassen, MN, USA).

The strategy for devising the ^{13}C receive coils was to replicate the geometry of the single loop ^1H receivers of the Sentinelle breast MRI system (figure 2.4d). The proposed system involves two single loop ^{13}C RF coils for which a schematic of the receive circuitry is shown in 2.6. The inductive loops seen in figure 2.6a are completed by identical feed circuits. The feed circuits comprise an active blocking network (figure 2.6b), a matching network (figure 2.6c) and a low-input impedance, low-noise pre-amplifier (LNA, figure 2.6d). The active blocking network serves a similar purpose as described previously and is important for preventing the direct transfer of power from the transmit coil to the receiver, which can be damaging and a potential safety hazard.

The matching network shown in figure 2.6c serves two purposes: first, it transforms the electrical impedance looking into the terminals of the coil to $50\ \Omega$; second, in conjunction with the low-input impedance LNA (figure 2.6d), this network performs an impedance transformation at the coil terminals

that limits current flow [127]. The former serves to match the circuit to the pre-amplifier for optimal noise performance and the latter is used for multi-channel decoupling. Since the MR signal is received as a voltage at the coil terminals, current flow is not required for signal measurement. Any currents flowing are actually detrimental because they can induce signal into nearby coils, which, in the extreme case, can cause a set of coils to behave as a single, large receiver with attendant penalties to sensitivity.

2.2.3 Circuit Layout and Housing

The design for the receive coils (figure 2.6) was used to generate printed circuit board (PCB) layouts (figure 2.7) with the computer assisted drawing tool EAGLE (Autodesk, San Rafael, CA, USA). The layouts were ordered for printing through an external company (AP Circuits, Calgary, AB, CAN). The choice was made to use separate PCBs for the “feed board” and the “loop” so that replacement parts could be made available in case of any damages during assembly. In contrast, the layout of the transmit coil was not formed from PCB but rather, was laid with 1 cm wide copper strips, 1 mm thick.

The housing for the transmit coil was 3D printed to conform to the GE MR750 scanner bed, such that when the bed’s covering is removed, it fits into the cutouts and is mechanically secured (figure 2.8a). Additionally, housings for the receive coils were also 3D printed in order to achieve mechanical compatibility with the SentinelleTM breast support. The design and construction of the 3D printed materials, as well as the details of integrating the coils with the GE MR, was handled by a private contractor (LT Imaging Inc., Toronto, ON, CAN).

2.3 Results

A photograph of the fully assembled coil setup is shown in figure 2.8. The imaging phantom was a 500 mL bottle that contained 1 M of 99% enriched ¹³C-urea. Although solutions up to 10 M can be prepared, the cost for the desired volume would have been prohibitive. A portable vector network analyzer (Keysight Technologies, Mississauga, ON, CAN) was used to fine-tune and impedance match the coils.

2.3.1 ¹³C RF Transmit Coil Performance

Due to the large size of the transmit coil ($44 \times 40 \text{ cm}^2$), there were significant interactions with the proton body coil that needed to be accounted for in order to achieve resonance at the ¹³C resonant frequency (32.13 MHz at 3T). As a consequence, network analyzer readings were obtained while the transmit coil

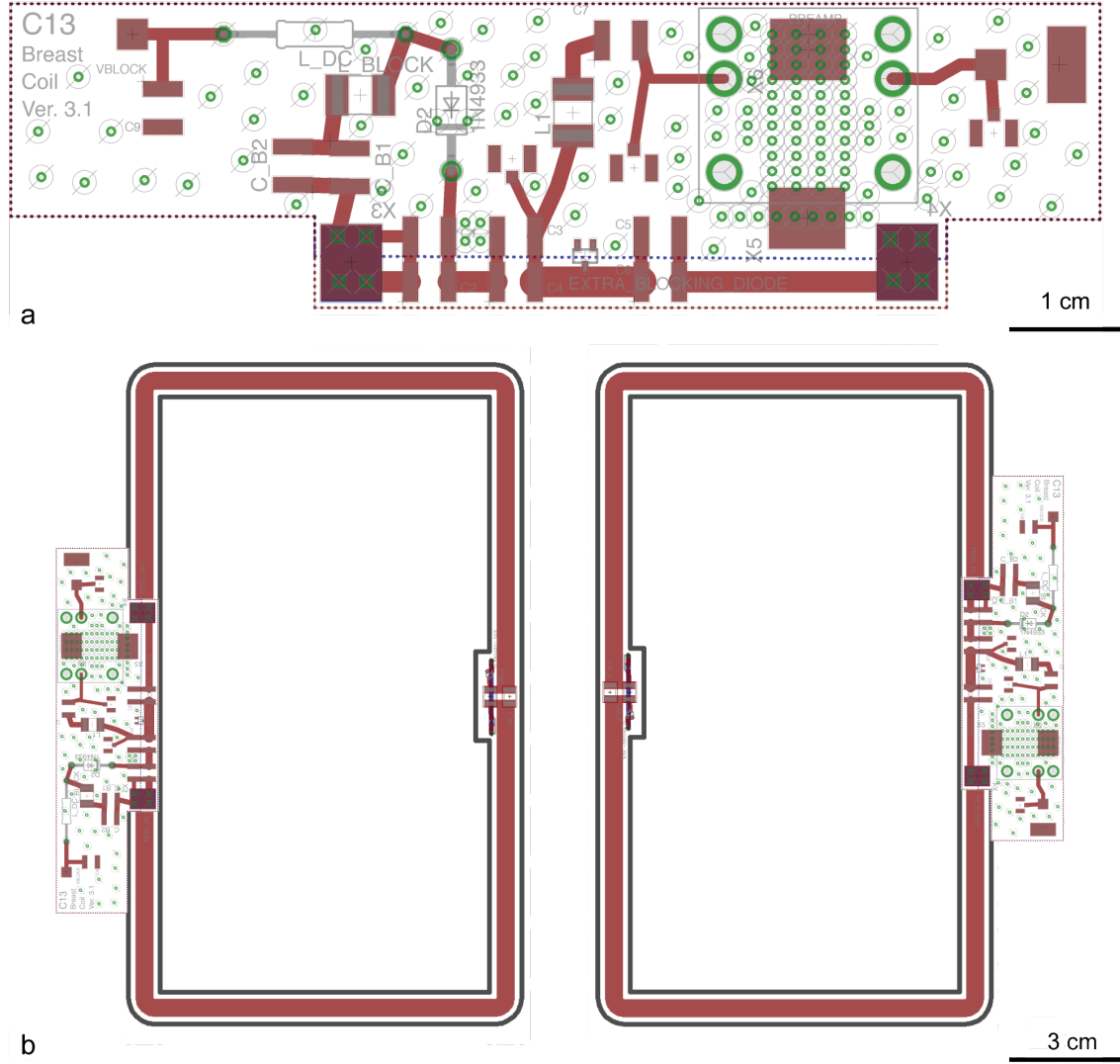


Figure 2.7: EAGLE CAD PCB design for feed board (a) and entire coils (b).

was in the magnet bore at iso-centre, and these were used to guide the iterative tuning and matching procedure. Once the tune and match were nearly 32.13 MHz and $\sim 50 \Omega$, respectively, a volunteer laid on top of the coil to provide realistic loading conditions so that final adjustments could be made.

The spatial profile of the transmit coil's excitation field was investigated by acquiring a set of B_1 maps according to the double-angle method [135]. Imaging was performed using a slice-selective 2D chemical shift imaging sequence (TR/TE = 5000/100 ms, 64×64 matrix, $2.5 \times 2.5 \text{ mm}^2$ in-plane resolution, $16 \times 16 \text{ cm}^2$ FOV, 256 points, $\pm 2.5 \text{ KHz}$ BW, 1.8 ms soft pulse). The slice thickness was 5 cm and 4 averages were acquired to obtain sufficient sensitivity in the thermally polarized ^{13}C urea phantom. Maps were acquired with the same phantom in 3 configurations: left axial; right axial; and central sagittal. A composite of the results from the 3 separate experiments is shown in figure 2.9.

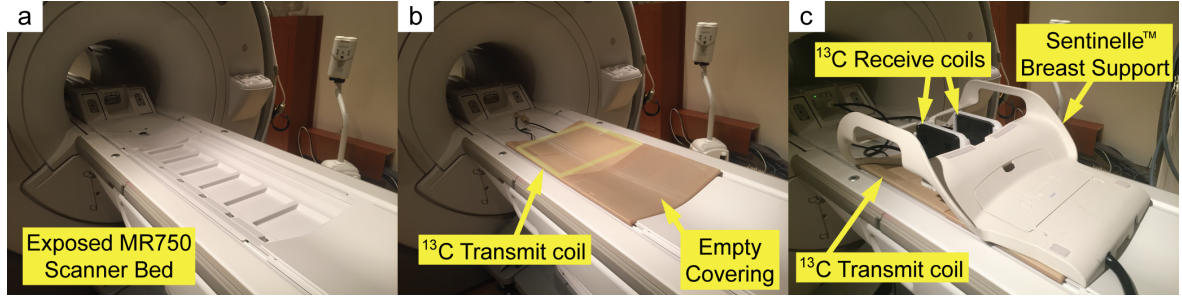


Figure 2.8: Setup of the proposed ^{13}C coil system. a) exposed GE MR750 MRI scanner bed. b) the proposed transmit coil is nearly square shaped and fills approximately half of the exposed space. A second 3D printed filler piece is placed to complete the covering. c) the Sentinel[™] breast support with the proposed ^{13}C receive coils.

Transmit gain (TG) was manually calibrated by identifying the power level necessary to obtain a signal null in a slice-selective pulse-acquire readout, as this serves as an estimate of the TG necessary for a producing a flip angle of 180° (although the flip angle varies over the volume, as seen in figure 2.9). For double-angle B_1 mapping, TG was then scaled to obtain images with flip angles of 120° and 60° . For a perfectly calibrated, perfectly homogeneous RF transmitter, all of the pixels in the B_1 maps would indicate a measured flip angle in perfect accordance with the prescribed excitation i.e. 60° . Given the planar geometry of the transmit coil here, spatial heterogeneity is expected.

The extent of heterogeneity can be read from figure 2.9. For the axial plane (figure 2.9 *above*), there is an approximately linear change in flip along the anterior/posterior direction, moving away from the plane of the coil. When calibrating TG across an entire volume, over-tipping occurs closer to the coil, and under tipping occurs further from the coil. The maximum flip angle observed was over 85° —more than 25° higher than the calibrated flip angle. These regions of over-tipping correspond to those nearest the transmit coils conductors, indicated in figure 2.9 as circles and crosses. Histograms for the actual distribution of flip angles measured over the phantom is shown in figure 2.10.

2.3.2 ^{13}C RF Receiver Coil Performance

On a bench-top setup, the receiver coils were separately assembled and tuned to resonate at 32.13 MHz. Each coil was connected to a WMA32C low-noise preamplifier (WanTCom, Chanhassen, MN, USA). The values of the components in the matching networks (figure 2.6c) were iteratively selected in order to satisfy two specifications: the input impedance of the coil as measured from the terminals at the input of the pre-amplifier matches the manufacturer stated noise impedance of the device ($50\ \Omega$); and the impedance of the matching network plus pre-amplifier as measured from the terminals of the coil appears as an open circuit [127, 136]. The latter specification is intended to reduce inter-coil

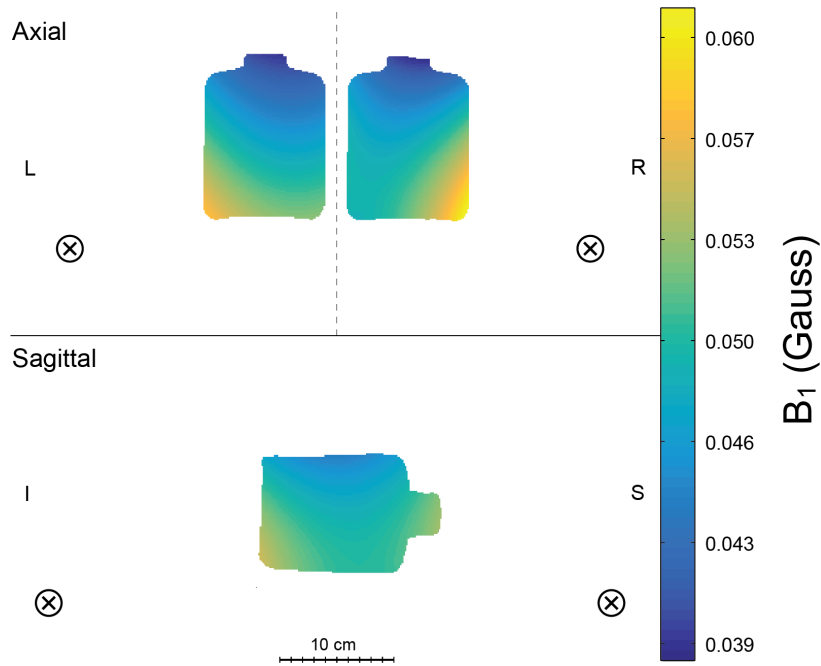


Figure 2.9: B_1 maps acquired with the proposed ^{13}C RF coil system. The figure is a composite of three separate B_1 mapping acquisitions to visualize the spatial heterogeneity of the transmit RF field. The crosses indicate where the conductors of the transmit coil intersect the imaging plane.

coupling by limiting the flow of current in the coils.

The measured decoupling performance of the matching network in each coil is shown in figure 2.11 and a description of the experimental setup is provided in the caption. The important result can be read off the plots as the difference in measured power transfer when the coils are connected to the pre-amplifier. For both receiver channels, the plots indicate that the amount of current passing the detection circuit at the resonance frequency of ^{13}C is reduced by ~ 24 dB or around 16-fold.

Imaging experiments were conducted to assess the spatial profile of the ^{13}C receiver coils, both in isolation, and with the lateral and medial channels combined. The coils and phantom were secured inside the SentinelleTM breast support and imaging was performed with a slice-selective 2D chemical shift imaging sequence (TR/TE = 80/0.9 ms, 64×64 matrix, $2.5 \times 2.5 \text{ mm}^2$ in-plane resolution, $16 \times 16 \text{ cm}^2$ FOV, 256 points, $\pm 5 \text{ KHz}$ BW, 1.8 ms soft pulse, 5 cm slice thickness, 2 averages). In order to obtain images that relayed the performance of each coil in electromagnetic isolation from each other, images were acquired for each coil while the opposing channel was actively de-tuned (figure 2.12a-b). A third set of images was acquired with both channels simultaneously active (figure 2.12d-f).

The resulting imaging data shown in figure 2.12 was converted into units of SNR by dividing each individual magnitude image by its apparent noise variance, which was estimated from a region of background signal in each image. The plane of the image is the axial plane, and the two ^{13}C receiver

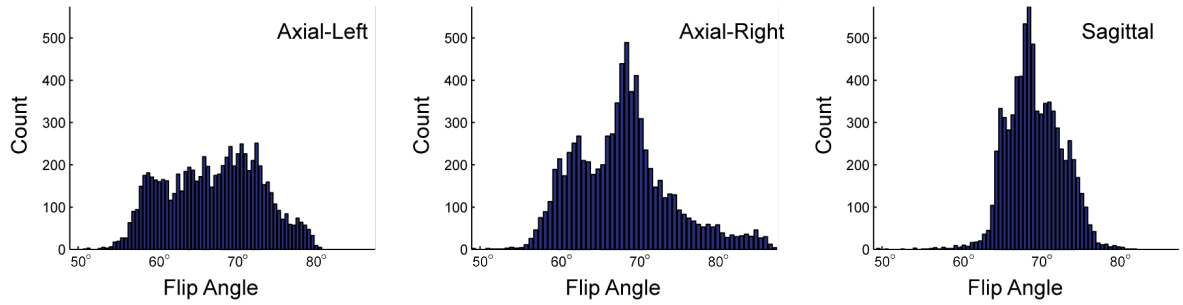


Figure 2.10: Distribution of flip angle across the voxels within the phantom for all three experiments ($N \sim 7600$). The median flip angle was 68° . For a perfectly homogeneous transmitter, all pixels in the distribution should collapse to a single flip angle, which in this case was 60° .

coils were placed on either side (left/right) of the phantom, such that the cross-sectional areas formed by their co-axial loops are parallel to the sagittal plane. The coils were placed 14 cm apart as an example of a typical spacing that would be used in practice. For scale, the phantom is approximately 10 cm wide. Single rows of pixels were taken at the locations indicated by the white arrows and these were overlaid on a single SNR-versus-position plot in figure 2.12c. The image in figure 2.12f is result of a sum-of-squares combination of both channels—the standard approach to combining multi-channel data.

The isolated receiver profiles (figure 2.12a-c) exhibit the typical sensitivity-distance performance of surface coils, as SNR drops by nearly a factor of 8 across the width of the homogeneous ^{13}C -urea phantom. According to figure 2.12, the coils have almost twice the maximum SNR when working independently. In contrast, the individual coil images obtained when both were active (figure 2.12d-e) exhibit lower maximum SNR and spatial profiles that vary more slowly. It should be noted that in all of the raw images, the maximum pixel intensity is approximately equivalent, but in the non-isolated case (figure 2.12d-e), the noise power was increased, causing the observed degradation in SNR. It was verified that the increase in noise power is inversely proportional to the separation distance between the coils and is thus attributable to insufficient decoupling. This is a known consequence of the strong magnetic and electrical interactions between two such relatively large receivers [137].

2.4 Discussion

A dedicated ^{13}C RF coil system for MRI in breast was developed for a clinical-grade 3T MRI scanner. Comprised of separate transmit-only and receive-only ^{13}C RF hardware, the coil system was designed to integrate with the medically licensed and commercially available SentinelleTM breast MRI coil system (Invivo, Gainesville, FL, USA). Specifically, the 3D printed housings of the ^{13}C receivers permits mechanical compatibility with the proprietary SentinelleTM Variable Coil GeometryTM technology which,

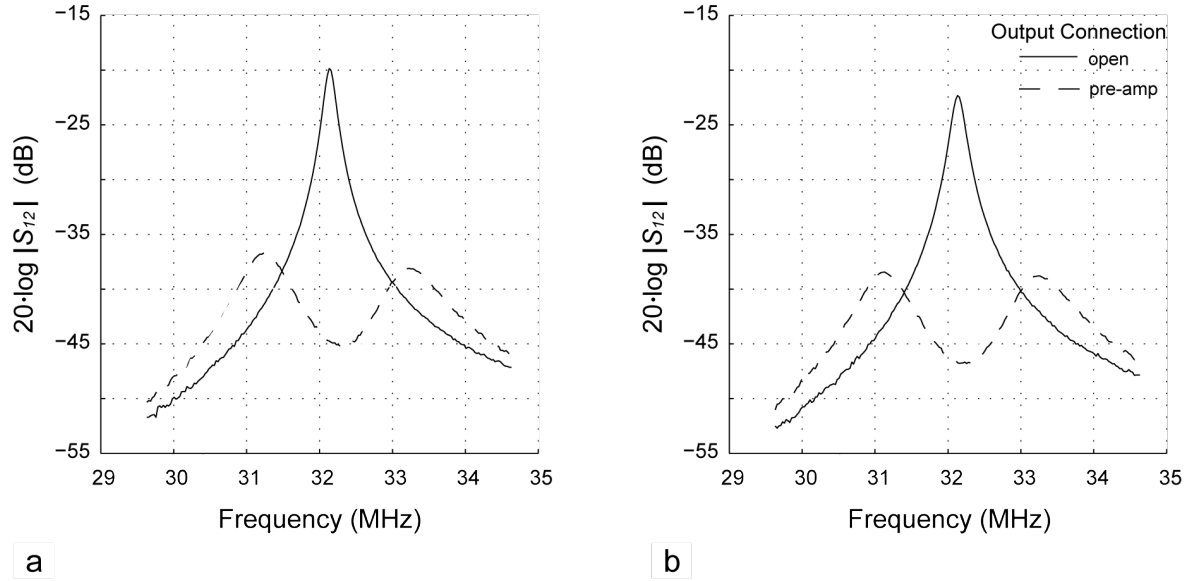


Figure 2.11: S_{12} measurements of channel 1 (*left*) and channel 2 (*right*). A pair of probes were fixed to a stand approximately 10 cm apart such that the amount of signal coupled between the coils, as measured by an S_{12} parameter estimation, was on the order of noise i.e. decoupled probes. To measure the amount of current that travels in the coil, in turn, each coil was placed on top of the two probes. For the solid trace, the output of the coil was left disconnected to the input of the pre-amplifier, exhibiting the maximum amount of RF power picked up and transmitted via the coil between the two probes at 32.1 MHz. The dashed trace corresponds to the same measurement but with the coil connected to the pre-amplifier. The reduction in current is on the order of -24 dB.

in addition to facilitating customizable coil positioning for unilateral breast imaging, enables sequential ^{13}C and ^1H imaging in a single exam without moving the patient. The open access design of the SentinelTM system facilitates the proposed “coil swapping” approach and is therefore an indispensable aspect of the proposed receiver design.

The ^{13}C receiver hardware consisted of two identical channels that share a similar overall design and structure as the ^1H SentinelTM coils used for MR-guided interventions (figure 2.4c-d). The decision to utilize separate receivers for ^{13}C and ^1H imaging allows for independent optimization of the respective systems, affording the use of conventional (single frequency) designs. Here, the single-loop ^{13}C coils represent a simple approach to constructing two-channel receiver hardware for unilateral ^{13}C breast imaging.

The strengths and weaknesses of the proposed design can be seen in figure 2.12. The profiles of the isolated coils (figure 2.12a-b) show that they have large sensitive regions extending along the A/P (top-to-bottom) direction that remain high at distances of 8-10 cm away from the coils in the L/R direction, parallel to the axes of the loops. For imaging volumes that have a smaller width than this distance, the addition of a second independent receiver would offer up to a factor of $\sqrt{2}$ increase in

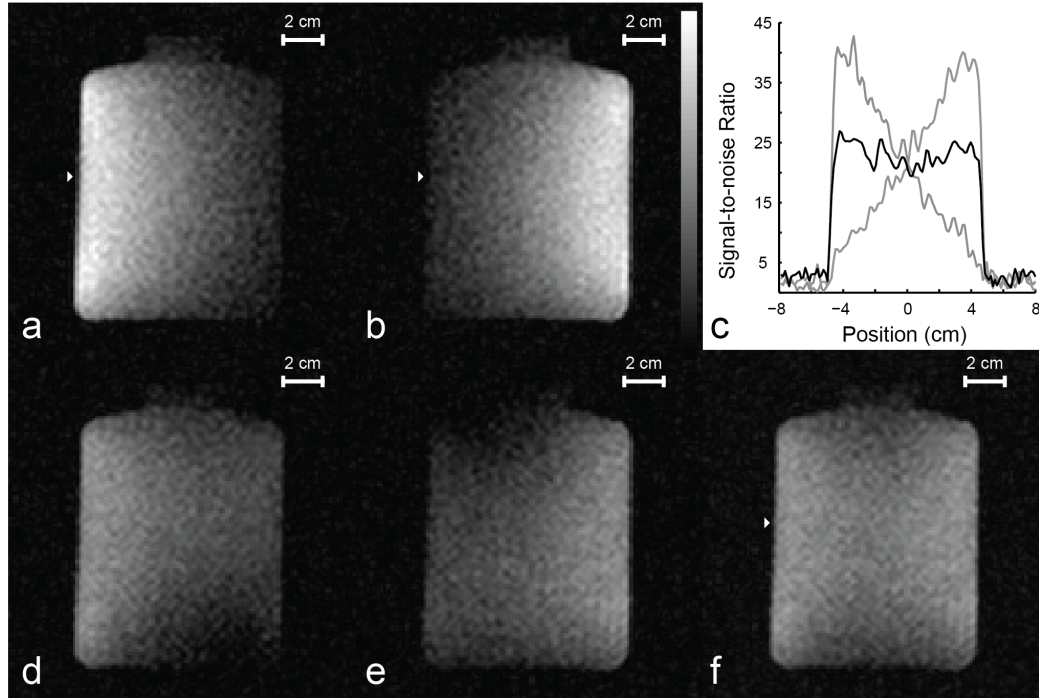


Figure 2.12: Receiver profile for the 2-channel ^{13}C coils. Profiles for channel 1 (a) and channel 2 (b) in isolation of each other. Profiles for channel 1 (d) and channel 2 (e) with both coils simultaneously active. f) the sum-of-squares combination of the two channels. The white arrows in a), b) and f) indicate the location where a single row of pixels was extracted and compared in c). The reduced SNR is due to an increase in noise power rather than a reduction in signal amplitude.

SNR across such a volume. Since the coils are large relative to most breast sizes, both coils should couple to most of a single breast and so the benefit in SNR offered by two independent coils rather than one is achieved by effectively measuring the same imaging signals twice. For breast sizes that are larger than the high-sensitivity regions of the receivers, then the advantage of a second receiver is the mutual accommodation for the drop in sensitivity away from the coils (figure 2.12c), giving a spatially varying SNR benefit.

The images in figure 2.12d-e indicate the presence of strong inductive coupling between the two receivers, which manifests as receiver profile corruption and as sensitivity degradation i.e. noise amplification; therefore, channel decoupling clearly has not been achieved for these coils. The co-axial geometry of the receivers is highly problematic for inductive coupling between two loops; in fact, the geometry is similar to that of a transformer. Reducing the sizes of the loops at the cost of a smaller detection field is a straight-forward approach for reducing the amount of coupling between two such coils. In this case, a reduction in coil size would need to be combined with the addition of more receiver channels in order to cover the same volume and, thus, there would be a concomitant increase in

hardware complexity.

Another approach for reducing coupling would be to improve the effectiveness of the pre-amplifier decoupling network, evaluated in figure 2.11. While alternative configurations of the matching circuit are possible [136], the input impedance of the pre-amplifier plays a central role in the effectiveness of the overall network. Currently, the WMA32C pre-amplifier made by WanTCom is the only commercially available non-magnetic pre-amplifier, designed for use in MR imaging receivers at 32.1 MHz. The resistive input impedance of the WMA32C is approximately $2.5\ \Omega$, whereas the classical configuration used for decoupling assumes negligible resistance [127]. It was recently shown that improved decoupling for ^{13}C coil arrays could be achieved through the use of non-conventional matching and preamplifier impedance [138]. Custom-built pre-amplifiers with inductive rather than resistive input impedances were shown to provide greatly reduced coupling in a 2-channel array.

In contrast to the design of the receivers, the proposed ^{13}C transmit RF coil is specific only to the GE MR750 scanner and has no other structural dependencies (figure 2.8b). The proposed transmit coil could be easily integrated with alternative breast MRI systems besides the SentinelleTM platform and, in fact, given its low-profile design, has potential as a multi-purpose ^{13}C RF transmitter for use in a variety of anatomical regions. The primary trade-off encountered in opting for a planar loop/surface coil-style transmit coil is its compact size at the expense of spatial heterogeneity of its RF excitation field. The extent to which local deviations in the transmit field can be deemed as falling within acceptable limits is influenced by the size of the region-of-interest. In other words, the impact of excitation heterogeneity on the utility of the proposed ^{13}C transmit coil will be less severe for imaging smaller rather than large volumes.

B_1 -maps were acquired in order to investigate the spatial profile of the transmit coil's RF excitation field across a region that roughly corresponded to the volume filled by the human breast. For the purpose of visualization, the B_1 -maps shown in figure 2.9 were processed by applying a 2D polynomial fit (2nd degree) to the computed double-angle data. The histograms shown in figure 2.10 provide a detailed description of the B_1 -heterogeneity of the transmit coil as measured in each orientation. In the axial plane, approximately 88% of the voxels within the phantom fell within $\pm 10^\circ$ of the mode of the distribution ($\sim 68^\circ$) and approximately 56% were within $\pm 5^\circ$ (interquartile range 8.6°). Reduced heterogeneity was observed within the sagittal plane, with $\pm 10^\circ$ and $\pm 5^\circ$ contained within 99% and 85% of the voxels, respectively (interquartile range 4.8°). Assuming an inverse-square law decay for magnetic field of the planar transmit coil, this apparent discrepancy in the transmit coil's excitation field heterogeneity for the two planes can be attributed to the relative distances of the phantom to its left-right and anterior-posterior conductive elements, respectively.

2.5 Conclusions

Hyperpolarized ^{13}C MRI is an emerging metabolic imaging technology that is currently in the early stages of translation for use in humans. The objective of this work was to construct an RF coil system to enable ^{13}C MRI in breast. The proposed design integrates with the SentinelleTM breast MRI coil system and allows the swapping of coils between proton and ^{13}C image acquisitions.

The low-profile ^{13}C transmit coil design is well-suited to imaging with the SentinelleTM system. The main drawback of the single-loop design chosen for the transmit coil is the issue of B_1 inhomogeneity. In cases with large breasts, multiple foci and/or extensive disease, inhomogeneity of transmit B_1 across space could impair interpretation of HP ^{13}C images. The B_1 field is much stronger near the conductive elements and so care must be taken when devising the power limits for device use; despite active decoupling, power transfer from the transmit coil to the receivers is unavoidable.

The two channel design is a straightforward approach that can improved in future iterations by introducing more elements. Given that the current decoupling method is insufficient for the preventing noise amplification, reducing the size of each coil could limit the interaction between co-axial loops, thus, improve decoupling. Multi-channel arrays can also be exploited for accelerating imaging acquisition, and this has already proved useful for helping to address the need for rapid encoding in HP ^{13}C MRI. The following chapter is concerned with how one rapid encoding scheme—echo-planar imaging—can be made more robust to misregistration.

Chapter 3

Addressing Geometric Distortion in Hyperpolarized ^{13}C Echo-Planar Imaging

This chapter was published as Geraghty, Benjamin J., Justin YC Lau, Albert P. Chen, and Charles H. Cunningham. “Dual-Echo EPI sequence for integrated distortion correction in 3D time-resolved hyperpolarized ^{13}C MRI.” *Magnetic Resonance in Medicine* 79, no. 2 (2018): 643-653. It was also the topic of a patent application, Geraghty, Benjamin J., and Charles H. Cunningham. “System and Method for Producing Distortion Free Magnetic Resonance Images Using Dual-Echo Echo-Planar Imaging.” U.S. Patent Application 15/495,256, filed October 26, 2017.

3.1 Introduction

Given that the hyperpolarized state is so short-lived in solution [122], rapid acquisition schemes are necessary to permit both time resolved and volumetric imaging [123]. For HP ^{13}C substrates that give rise sparse metabolic spectra, such as $[1-^{13}\text{C}]\text{pyruvate}$, frequency-selective excitation schemes may be leveraged to avoid the need for direct chemical shift encoding [139, 140] and dramatically reduce the number of acquisitions required per volume. Once a single resonance has been excited, single-shot spatial encoding techniques can be employed to rapidly sample k-space. Echo-planar imaging (EPI) sequences achieve this by rastering through k-space along a Cartesian grid, using a bipolar readout gradient train to encode one spatial dimension while a blipped gradient is typically used to encode the

other [141].

A powerful feature of HP ^{13}C MRI is the implicit registration of patient data obtained from conventional diagnostic MR with metabolic HP ^{13}C exams, enabling trivial multi-modal data fusion as seen in hybrid platforms such as PET/CT or MR/PET. However, if EPI is used in image formation, then the inherent susceptibility of EPI to geometric distortion must be considered. In contrast to spectroscopic HP ^{13}C MRI techniques, where the direct encoding of metabolic frequency information confers some insensitivity to off-resonance phenomena, off-resonance sources encountered in HP ^{13}C EPI cause linear phase accrual across both dimensions of each k-space plane. Linear phase ramps in k-space manifest as spatial shifts in the image domain according to the Fourier relation. Since the effective readout bandwidth in the blipped direction is typically orders of magnitude smaller than the bandwidth used to sample each k_x line, the spatial misregistration in the image domain associated with off-resonance magnetization can be well approximated [142, 143] by considering only the bandwidth per pixel in the blip encoded direction: $y' = y \pm \Delta f(y)/BW_{pp}$ where y is the pixel location in undistorted coordinates, $\Delta f(y)$ is the effective resonance frequency at location y , BW_{pp} is the bandwidth-per-pixel in the blipped direction and y' is the resulting pixel location in distorted coordinates. The sign of the $\pm \Delta f(y)/BW_{pp}$ term depends on the polarity of the blip gradient used.

Static B_0 field mapping methods represent the gold standard approach for correcting EPI distortion in conventional proton MRI: provided an estimate of $\Delta f(y)$, an off-resonance corrected EPI image can be reconstructed by effectively resampling the distorted image data onto the undistorted coordinate space [143]. Within the frequency-selective HP ^{13}C imaging regime however, the true in vivo ^{13}C center frequency is unavailable before the HP injection and performing a separate field mapping acquisition at the ^{13}C frequency consumes precious, non-renewable magnetization. Off-resonance ^{13}C signals, unaccounted for by proton derived estimates of $\Delta f(y)$, induce distortion in EPI metabolic maps and can compromise their spatial registration with respect to the underlying anatomy.

To address this, Cunningham et al. [144] proposed to alternate the polarity of the blip gradient across successive time points in a dynamic HP ^{13}C EPI acquisition, in order to generate two data sets: images distorted according to the positive form of $\pm \Delta f(y)/BW_{pp}$, and images distorted according to its negative form. The 0th order off-resonance term Δf_0 can be estimated by finding the value which accounts for the “best” alignment of the two image sets according to a mutual information metric. The so-called “blip reversal” method was shown to perform well [145, 146] when the signal dynamics of the $[1-^{13}\text{C}]\text{pyruvate}$ substrate are adequately sampled (i.e. successive time-resolved volumes are spatially similar); however, this requirement is not always met, particularly when encoding larger fields-of-view, which take longer.

In this work, a dual-echo 3D EPI pulse sequence was developed that encodes the 0th order off-resonance term within the data from a time-resolved HP [1- ^{13}C]pyruvate scan. Multiple sources of EPI distortions, including static field inhomogeneity and readout gradient eddy currents, are separately encoded using a multi-echo ^1H EPI reference scan. These are combined in the reconstruction pipeline to provide registered, distortion corrected images from the ^{13}C dual-echo 3D EPI data. Corrected and uncorrected [1- ^{13}C]pyruvate and [1- ^{13}C]lactate images obtained in phantoms and healthy rats are presented. The proposed acquisition and reconstruction scheme provides improved spatial registration without any loss in SNR efficiency.

3.2 Theory

Dual-echo EPI was first proposed by Yang et al. to provide a simple and effective method for eliminating Nyquist or $N/2$ ghost artifacts, which arise due to time-reversal asymmetry between odd and even echoes in bidirectional EPI readouts [147]. The dual-echo EPI sequence (figure 3.1) traverses each readout line twice before blipping to successive k_y locations, encoding two k_x - k_y planes within a single TR. Although this penalizes the dual-echo matrix size by a factor of 2 relative to a bidirectional EPI sequence with the same readout duration, dual-echo EPI images are robust to $N/2$ ghosting and retain approximately equivalent SNR [147]. Additionally, since the two images obtained from a dual-echo EPI readout are effectively separated in time by the echo spacing ΔTE , off-resonance signals are encoded as phase evolution between the odd and even imaging data. This has been exploited in application to dynamic distortion correction of functional MRI data, since each temporal volume encodes a distorted dual-echo EPI phase map [148, 149].

While each of the dynamic functional MR images within a single exam are expected to provide consistent SNR during the acquisition, directly translating the dynamic dual-echo distortion correction approach to HP [1- ^{13}C]pyruvate imaging is limited by the highly variable SNR of each time resolved volume. Early [1- ^{13}C]pyruvate time points may lack sufficient signal due to the kinetics of substrate delivery, and late time points are penalized by both T1 relaxation and non-renewable magnetization consumption. Instead, high SNR ^1H phase maps can be obtained either prior to or after the HP [1- ^{13}C]pyruvate imaging exam and used to perform geometric distortion correction in the ^{13}C metabolic maps. If the imaged object was not moved between acquisitions, and the ^{13}C transmit centre-frequency is on-resonance, then the ^1H phase maps should account for most major sources of EPI distortion in the ^{13}C images.

Since the exact in vivo ^{13}C resonance frequency cannot be determined until the injection, small

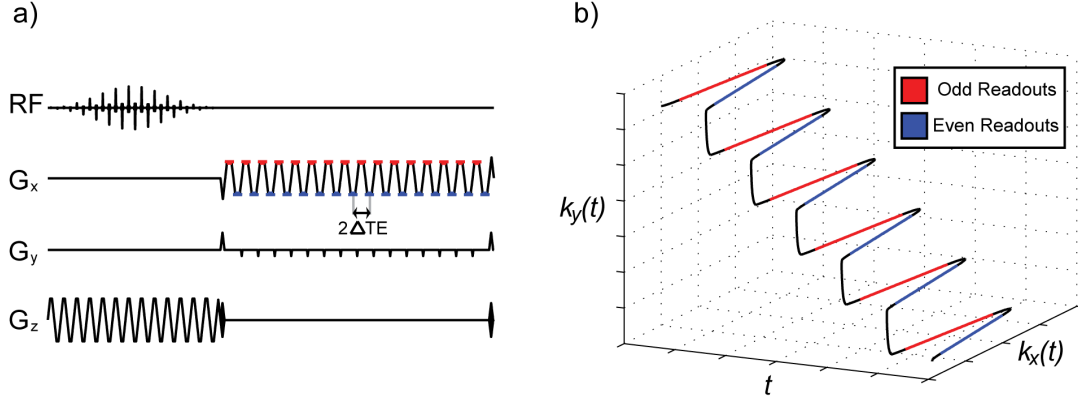


Figure 3.1: Proposed dual-echo 3D EPI pulse sequence diagram **(a)** and in-plane k-space trajectory **(b)**. Volume selection and chemical shift separation is performed with a tailored 18.8 ms spectral-spatial RF pulse. In plane spatial encoding is achieved with a dual-echo EPI readout, and volumetric imaging is attained with a through-plane phase encoding table along G_z . Odd (red) and even (blue) gradient-echoes are separated by $\Delta\text{TE} = 0.892$ ms, which encodes off-resonance information as the phase evolution between the pair of images.

errors in centre-frequency calibration are expected; therefore, I proposed to estimate the 0th order off-resonance from time averaged, dynamic HP [1- ^{13}C]pyruvate dual-echo 3D EPI data to complement the ^1H phase maps. In the following section, the signal equations for the dual-echo 3D EPI sequence and corresponding ^1H multi-echo reference scan are defined, and the procedure of distortion correction through multi-echo demodulation is described. The full reconstruction pipeline is detailed under Methods, which addresses implementation of the waveforms, rectification of ^1H reference data with the ^{13}C images, as well as estimation of the ^{13}C centre-frequency offset.

3.2.1 Dual-Echo 3D EPI Signal Equation and Multi-Echo Reference Scan

For brevity, the signal equation for one of the two 3D EPI k-space echo data $S'_{1,2}$ and its corresponding proton reference scans $R_{1,2}$ are described. The prime notation indicates when images are defined in distorted rather than undistorted coordinates. Ignoring any time-dependence of the imaged object $\rho(x, y, z) \approx \rho(x, y, z, t)$, the acquired k-space data for the first echo image $S'_1(m\Delta k_x, n\Delta k_y, l\Delta k_z)$, with matrix size $N_x \times N_y \times N_z$, can be expressed as:

$$S'_1(m\Delta k_x, n\Delta k_y, l\Delta k_z) \approx \iiint \rho(x, y, z) \exp(i(m\Delta k_x x + n\Delta k_y y + l\Delta k_z z)) \exp(i\omega_1(x, y, z, t)t) dx dy dz \quad (3.1)$$

$$-N_x/2 < m < N_x/2, \quad -N_y/2 < n < N_y/2, \quad -N_z/2 < l < N_z/2,$$

where $\rho(x, y, z)$ is spin density, Δk_x , Δk_y and Δk_z are the k-space increments for each axis, and

$\omega_1(x, y, z, t)$ is the EPI phase error term. $\omega_1(x, y, z, t)$ contains the spatial distribution of all off-resonance factors including main field inhomogeneity, RF pulse frequency offset and the time-varying eddy current effects resulting from gradient switching. Provided prior knowledge of the phase error term $\omega_1(x, y, z, t)$, geometrical distortions in EPI can be corrected through phase demodulation.

Due to the relatively small amplitude of the gradient blips and the low duty cycle of the slice encoding gradients, their contribution to $\omega_1(x, y, z, t)$ is typically dominated by other off-resonance factors. Previous literature has shown that most geometrical distortions can be corrected without accounting for slice and blip gradient eddy currents [150, 151]; therefore, it is useful to approximate $S'_1(m\Delta k_x, n\Delta k_y, l\Delta k_z)$ with a simplified phase error term $\Omega_1(x, y, z, \Delta t_n)$:

$$\begin{aligned} S'_1(m\Delta k_x, n\Delta k_y, l\Delta k_z) &\approx \iiint \rho(x, y, z) \exp(i(m\Delta k_x x + n\Delta k_y y + l\Delta k_z z)) \\ &\quad \exp(i\Omega_1(x, y, z, \Delta t_n) \Delta t_n) dx dy dz \\ 0 < \Delta t_n &< (N_y - 1)\Delta T_{blip} \end{aligned} \quad (3.2)$$

where $\Delta T_{blip} = 2\Delta\text{TE}$ is the period between successive k_y lines. $\Omega_1(x, y, z, \Delta t_n)$ describes the average frequency over the time Δt_n , and can be estimated from a multi-echo reference scan $R_1(m\Delta k_x, n\Delta k_y, l\Delta k_z, \Delta t_n)$ with matrix size $N_x \times N_y \times N_z \times N_t$ where $N_t = N_y$:

$$\begin{aligned} R_1(m\Delta k_x, n\Delta k_y, l\Delta k_z, \Delta t_n) &\approx \iiint \rho(x, y, z) \exp(i(m\Delta k_x x + n\Delta k_y y + l\Delta k_z z)) \\ &\quad \exp(i\Omega_1(x, y, z, \Delta t_n) \Delta t_n) dx dy dz \end{aligned} \quad (3.3)$$

R_1 is acquired at $N_t = N_y$ successive echo-times that correspond to the relative acquisition times for each gradient-echo in the readout train. The multi-echo reference scan is effectively a low spectral resolution, 3D echo-planar spectroscopic imaging sequence that can be implemented by removing the blip gradients from the readout train and using conventional phase-encoding gradients in the y -direction; therefore, the phase error that would have accumulated across the blip direction in k -space during a single-shot EPI readout can be measured and accounted for. The following section describes the extraction of the phase error term from the reference data, and how it is applied to correct geometric distortions in EPI. It is assumed that the imaged object is not moved between the multi-echo reference scan and the dual-echo 3D EPI acquisition.

3.2.2 Multi-Echo Modulation and Demodulation

Let $r_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_n)$ be the inverse spatial Fourier transform of $R_1(m\Delta k_x, n\Delta k_y, l\Delta k_z, \Delta t_n)$ where Δx , Δy and Δz are the spatial increments for each axis. r_1 is used to compute a set of phase maps

$M_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_n)$, which track differential phase accumulation across the blip direction in k-space due to off-resonance sources. The phase map for the n^{th} pair of gradient-echoes is computed as the angle of the complex quotient of the n^{th} reference image to the first:

$$M_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_n) = \exp\left(i \frac{r_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_n)}{r_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_1)}\right) \quad (3.4)$$

The phase map matrix M_1 in Eq. 3.4 is explicitly cast here as a unit-magnitude phasor. Given a set of phase maps M_1 , the method of multi-echo modulation can transform an undistorted image into distorted coordinates by introducing phase accrual across the blip direction of k-space. For example, let $r_{11}(m\Delta x, n\Delta y, l\Delta z, \Delta t_n)$ be a single image from the set of N_t undistorted reference images r_1 , and $M_{\Delta t_n} = M_1(\dots, \Delta t_n)$ be the n^{th} phase map. The product $r_{11} \cdot M_{\Delta t_n}$ has added phase, which accounts for the accumulation of off-resonance terms after the n^{th} pair of gradient-echoes. The Fourier transform of $r_{11} \cdot M_{\Delta t_n}$ provides the n^{th} readout plane of the distorted k-space data matrix $R'_{11}(m\Delta k_x, n\Delta k_y, l\Delta k_z)$:

$$R'_{11}(m\Delta k_x, n\Delta k_y, l\Delta k_z) = \mathcal{F}\{ r_{11} \cdot M_{\Delta t_n} \} |_{m\Delta k_x, n\Delta k_y, l\Delta k_z} \quad (3.5)$$

Using Eq. 3.5, each of the N_y readout planes of R'_{11} are separately derived with their corresponding phase maps. Inverse Fourier transform of the resulting matrix gives the final image r'_{11} in distorted coordinates. This forward process of image transformation in Eq. 3.5 is referred to throughout as multi-echo modulation.

Off-resonance artefacts in EPI data S'_1 can be corrected with the inverse procedure, multi-echo demodulation, provided a set of phase maps M'_1 that are defined in the same distorted EPI coordinate space. In order to compute M'_1 , a set of distorted reference scan data R'_1 is obtained by separately applying multi-echo modulation Eq. 3.5 on each of the N_t undistorted reference images r_1 . The resulting images r'_1 are subsequently used to produce conjugate phase maps M'_1 in distorted coordinates:

$$M'_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_n) = \exp\left(-i \frac{r'_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_n)}{r'_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_1)}\right) \quad (3.6)$$

The corrected EPI k-space data S_1 is reconstructed through multi-echo demodulation using the distorted phase maps M'_1 from Eq. 3.6:

$$S_1(m\Delta k_x, n\Delta k_y, l\Delta k_z) = \mathcal{F}\{ s'_1 \cdot M'_{\Delta t_n} \} |_{m\Delta k_x, n\Delta k_y, l\Delta k_z} \quad (3.7)$$

where s'_1 is the inverse Fourier transform of S'_1 . The final images $s_{1,2}$ are separately reconstructed with phase maps computed from their respective reference scans $R_{1,2}$. The methods of multi-echo

modulation and demodulation are depicted schematically in figure 3.2.

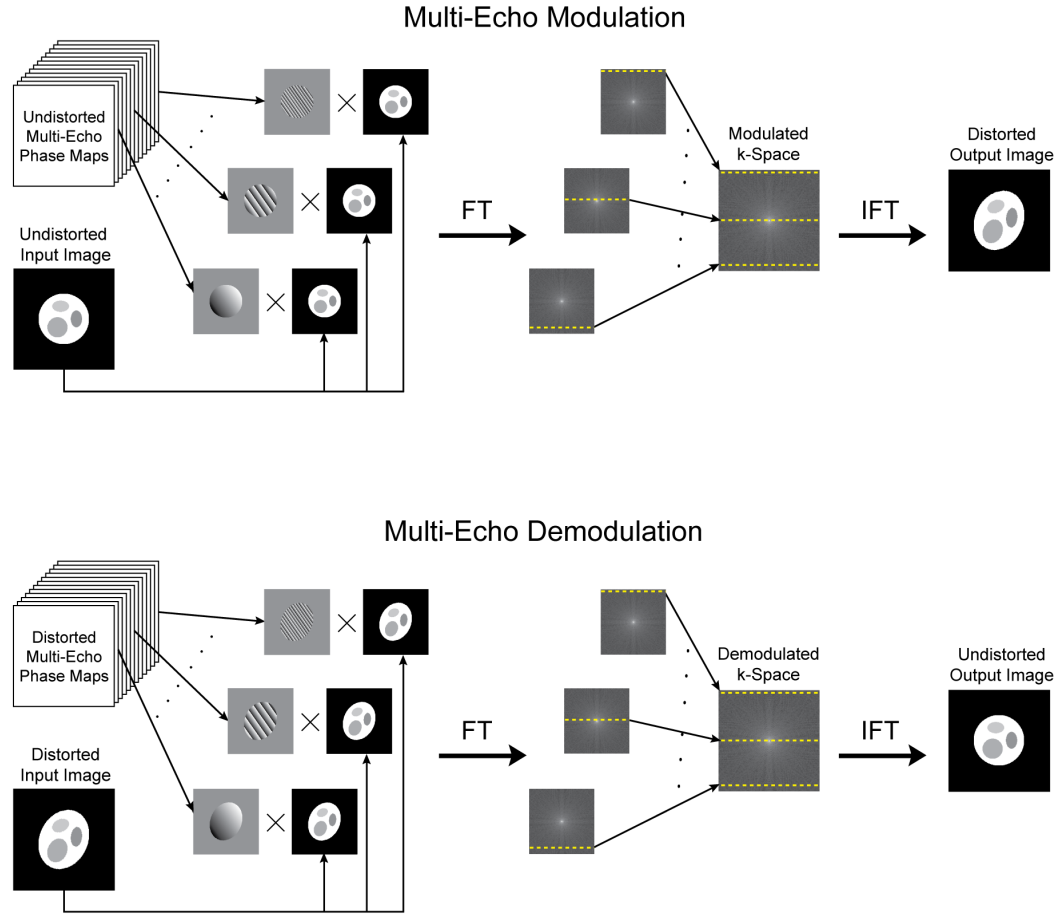


Figure 3.2: Multi-echo modulation and demodulation. Given an image and set of phase maps defined in undistorted coordinates, a distorted image can be generated by separately modulating the input image with each phase map and collecting each modulated readout line. The inverse procedure operates under the same principle, except that the input data is defined in distorted coordinates to produce an undistorted output image.

The preceding theoretical treatment described how a multi-echo reference scan can be used to reconstruct off-resonance corrected dual-echo 3D EPI images $s'_{1,2}(m\Delta x, n\Delta y, l\Delta z)$. In this work, it was assumed that with the exception of the 0th order term owing to the a priori uncertainty of the experimental in vivo ^{13}C resonance-frequency, most major sources of distortion in a HP ^{13}C EPI acquisition can be accounted for by data acquired from a ^1H multi-echo reference scan. Implementation details for rectifying proton acquired phase maps with ^{13}C dual-echo 3D EPI images, and estimating the ^{13}C centre-frequency offset are provided under Methods.

3.3 Methods

RF/gradient waveform design, image reconstruction and data analysis were performed in Matlab (The MathWorks Inc., Massachusetts). Phantom and in vivo imaging experiments were acquired using a dual-tuned transmit/receive ^1H - ^{13}C rat coil on a GE MR750 3T MR ($G_{\max} = 50 \text{ mT/m}$, $S_{\max} = 200 \text{ T/m/s}$, GE Healthcare, Waukesha, WI). ^{13}C images were acquired with the proposed dual-echo 3D EPI readout. ^1H Multi-echo reference images were acquired and processed via multi-echo modulation to account for phase evolution that would arise from ^{13}C sources in distorted coordinates. The ^{13}C dual-echo 3D EPI images were compensated with an odd and even echo asymmetry factor measured from the reference data, and then used to produce ^{13}C centre-frequency offset estimates. The ^{13}C frequency offsets were combined with their respective phase maps and the final distortion corrected ^{13}C images were reconstructed through multi-echo demodulation.

3.3.1 Frequency-Selective ^{13}C Dual-Echo 3D EPI Sequence

The dual-echo 3D EPI sequence was implemented with a 29.6 ms symmetric EPI readout, comprised of 32 gradient echoes, to provide 64 cm FOV in the readout direction at 5 mm resolution. The length of the readout window was chosen based on an estimated in vivo $[1\text{-}^{13}\text{C}]\text{lactate } T_2^*$ of 32 ms [152, 153]. Blip gradients were used to encode the orthogonal in-plane direction with an 8 cm FOV at 5 mm resolution by playing out during the ramp phase of every other gradient-echo. A volumetric FOV of $64 \times 8 \times 6 \text{ cm}^3$ at $5 \times 5 \times 5 \text{ mm}^3$ nominal resolution was achieved using conventional through-plane phase-encoding. The inter-echo spacing ΔTE between odd and even readouts was $892 \mu\text{s}$, providing a spectral width $\Delta f_{\max} = (2\Delta\text{TE})^{-1}$ of 560 Hz. Sampling was restricted to the readout gradient plateaus, resulting in a gradient SNR efficiency of 74% [154].

Spectral and volume selection was achieved with a tailored 18.8 ms spectral-spatial RF pulse [139] consisting of 26 sub-lobes, each with $720 \mu\text{s}$ duration, enabling a passband of 120 Hz (full-width-at-half-max) and a stopband of 1.1 KHz ($< -60 \text{ dB}$). The acquisition time per volume was 0.7 s, allowing sequential time-resolved sampling of $[1\text{-}^{13}\text{C}]\text{pyruvate}$ and $[1\text{-}^{13}\text{C}]\text{lactate}$ at a maximum temporal resolution of 1.4 s ($\text{TR}/\text{TE} = 56/26 \text{ ms}$, $128 \times 16 \times 12$ matrix).

3.3.2 ^1H Multi-Echo Reference Scan

The dual-echo G_x readout waveform, designed with specifications for ^{13}C imaging, was used in the ^1H multi-echo reference scan. Despite the nearly 4-fold increase of $\gamma^{^1\text{H}}$ relative to $\gamma^{^{13}\text{C}}$, the same G_x gradi-

ent amplitude was deliberately used for both the ^1H reference scans and ^{13}C imaging. This permitted characterization of off-resonance sources due to both field inhomogeneity as well as eddy currents from the same gradient waveform used during ^{13}C imaging. Although the concomitant reduction in FOV_x by the factor $\gamma^{13\text{C}}/\gamma^{1\text{H}}$ limits the readout dimension of the reference scan to 16 cm, this was tolerable since the initial readout FOV was greatly oversampled relative to the blip and slice directions.

Conventional phase encoding gradients provided $16 \times 8 \times 6 \text{ cm}^3$ coverage at $1.25 \times 5 \times 5 \text{ mm}^3$ resolution. In vivo ^1H multi-echo reference scans were acquired using the aforementioned spectral-spatial RF pulse tailored for $[1-^{13}\text{C}]\text{pyruvate}$ imaging. The RF pulse allowed selective excitation of water, while omitting confounding phase contributions from fat. With a TR of 1.5 s, the duration of the reference scan was ~ 3 minutes.

3.3.3 Phantom Study

The dual-echo 3D EPI sequence was validated with a spherical ^{13}C -bicarbonate phantom (30 mm diameter, 1.0 M concentration, doped with 0.02% OmniScan (GE Healthcare)). A $500 \mu\text{s}$ hard pulse was used to eliminate potential flip angle modulation across the phantom due to static field inhomogeneity. The first of two sets of experiments were conducted using the manufacturer's automated shimming scheme, while the second set included a strong linear shim, which was manually applied to induce non-uniform distortion in the EPI images.

For both shim conditions, three dual-echo 3D EPI data sets were acquired, each with a separate transmit centre-frequency offset Δf of either 0 Hz, -50 Hz or 75 Hz. 16 averages were obtained in each experiment to cope with low signal from the thermally polarized phantom. Two ^1H multi-echo reference scans were acquired, one for each of the shim conditions. In order to provide an undistorted ground truth ^{13}C image, the ^1H multi-echo reference sequence was modified to acquire images that matched the FOV of the ^{13}C dual-echo 3D EPI data.

3.3.4 In Vivo Data Acquisition

One of two Sprague-Dawley rats (weight = 510/450 g, Harlan Laboratories) was used during each imaging session. In vivo imaging was conducted in accordance with a protocol approved by the local institutional animal care and use committee. Coronal fast-spin echo images were acquired for localizing the rat kidneys. Centre-frequency and transmit gain calibration were performed using an external ^{13}C urea reference phantom. A constant flip angle scheme was used for RF excitation, and transmit gain was adjusted for providing a net flip angle per volume of 10° for $[1-^{13}\text{C}]\text{pyruvate}$, and 80° for $[1-^{13}\text{C}]\text{lactate}$.

The inter-volume dwell time in the dynamic acquisition was padded to give 5 s temporal resolution.

30 μL of pyruvic acid doped with 15 mM of OX63 radical (Oxford Instruments, Tubney Woods, UK) was polarized using a SPINlab 5T DNP polarizer (GE) for each experiment [155]. The hyperpolarized pyruvic acid mixture was dissolved using heated/pressurized H_2O (100 mg/L EDTA) and neutralized in the receiver with a 720 mM NaOH / 400 mM TRIS buffer solution to yield ~ 4.8 mL of 80 mM hyperpolarized pyruvate in solution. The polarization of $[1-^{13}\text{C}]$ pyruvate achieved in solution was not measured in these experiments; however, previous tests measured $\sim 40\%$ polarization using the same materials and protocol.

3 mL of the 80 mM pre-polarized $[1-^{13}\text{C}]$ pyruvate solution was injected over 12 seconds through a tail vein catheter at the start of each acquisition. Each rat was imaged twice and consecutive hyperpolarized ^{13}C scans were acquired 30 minutes apart ($N_{\text{total}} = 4$). Based on the ^{13}C urea reference spectra acquired during pre-scanning, the best guess of in vivo ^{13}C centre-frequency was calculated for each pair of experiments. Experiment 2 included an intentional transmit frequency offset of -50 Hz, while both experiments 3 and 4 used a 50 Hz offset. Additionally, a manually applied shim was used in experiment 4 to disrupt the field homogeneity. Axial T_2 weighted fast spin-echo images were acquired for anatomical reference ($\text{TR}/\text{TE} = 5000/83$ ms, 8×8 cm 2 FOV, 128×128 matrix, NEX = 4).

3.3.5 Image Reconstruction

Odd and even readouts from both the dual-echo 3D EPI data and reference scans were separated and individually processed on an offline workstation. Using the notation defined previously, the initial undistorted phase maps $M_{1,2}$ were computed from the ^1H multi-echo data $R_{1,2}$ according to Eq. 3.4. Since the resulting phase maps contained off-resonance phase accrual measured from ^1H sources, the phase from each voxel in $M_{1,2}$ was first one-dimensionally unwrapped with respect to Δt_n , and then scaled by the factor $\gamma^{13\text{C}}/\gamma^{1\text{H}}$ in order to describe any distortion that would have been observed from ^{13}C EPI images.

Distorted reference data $R'_{1,2}$ was generated via multi-echo modulation according to Eq. 3.5, using the γ -scaled phase maps as input. Distorted phase maps $M'_{1,2}$ were then computed according to Eq. 3.6, and the process of unwrapping and γ -scaling was repeated to generate distorted, ^{13}C converted phase maps. Finally, each of the N_t phase maps $M'_{1,2}$ were spatially resampled to match the ^{13}C FOV and then convolved with a 3D gaussian kernel to regularize potential phase errors.

It was hypothesized that the 0th order ^{13}C frequency-offset could be estimated by computing that mean phase difference between odd and even dual-echo 3D EPI images over the period ΔTE . In practice

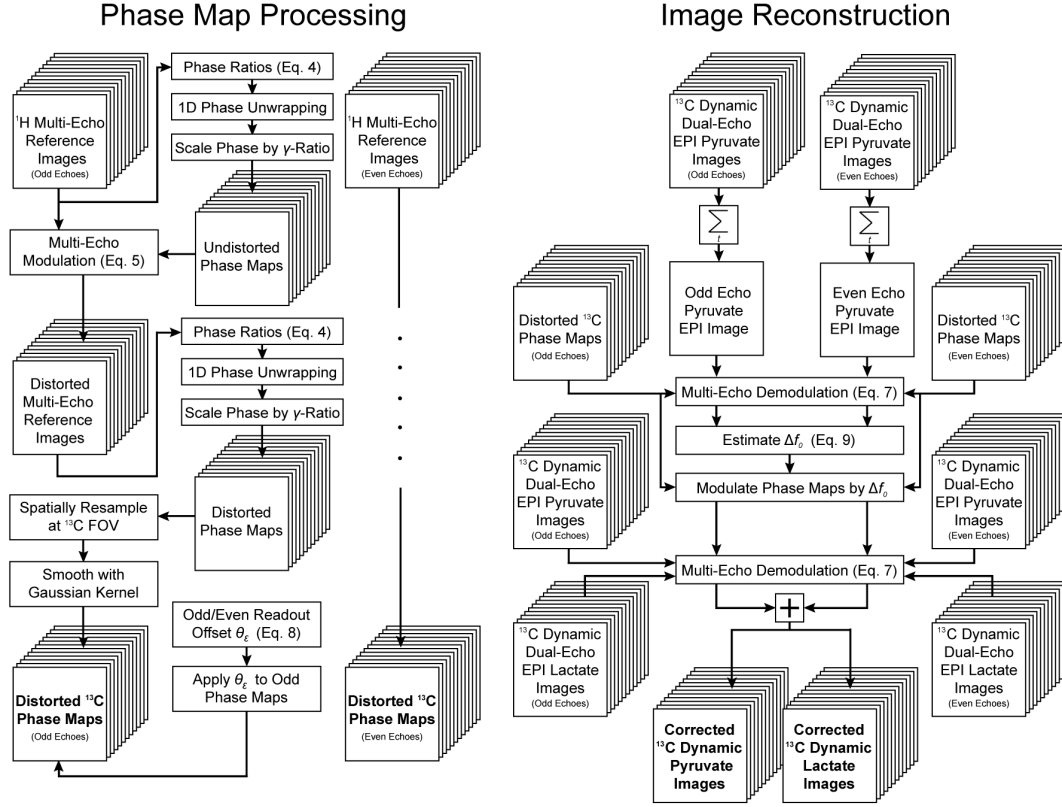


Figure 3.3: Dual-Echo EPI image reconstruction pipeline. Undistorted ^1H Multi-echo reference images are first used to generate a set of undistorted phase maps that describe phase evolution that would arise from ^{13}C sources. The resulting phase maps are used to generate distorted reference images via multi-echo modulation. These are subsequently processed to produce the final distorted ^{13}C phase maps. The ^{13}C frequency offset is estimated from the multi-echo demodulated $[1-^{13}\text{C}]$ pyruvate images. The offset is included into the phase maps, and the final images are generated through multi-echo demodulation.

however, it was observed that incidental phase between the opposing readouts leads to erroneous estimates of the experimental ^{13}C centre-frequency. In order to account for echo asymmetry, the phase offset θ_e was estimated by computing a dual-echo 3D EPI phase map from the ^1H reference data $r_{1,2}$, and taking the mean value according to Eq. 3.8:

$$\theta_e = \frac{\gamma^{13\text{C}} / \gamma^{1\text{H}}}{N_t \cdot N_{ROI}} \sum_m \sum_n \sum_l \sum_{\Delta t_n} \frac{r_2(m\Delta x, n\Delta y, l\Delta z, \Delta t_n)}{r_1(m\Delta x, n\Delta y, l\Delta z, \Delta t_n)} \cdot ROI(m\Delta x, n\Delta y, l\Delta z) \quad (3.8)$$

The binary mask $ROI(m\Delta x, n\Delta y, l\Delta z)$ included N_{ROI} voxels that were automatically selected by thresholding the ^1H reference images at an SNR level of 50. Selection of this threshold level is discussed in section 3.5. Eq. 3.8 is motivated by the assumption that if the ^1H centre-frequency is on-resonance, then 0th order phase difference between odd and even reference images should be negligible. How-

ever, echo asymmetry, a common source of ghosting artefacts in conventional bidirectional EPI imaging, gives rise to a residual phase distribution that was approximated here with a bulk offset θ_ϵ . The factor $\exp(i\theta_\epsilon)$ was multiplied with the distorted phase maps M'_1 so that the resulting multi-echo demodulated data were corrected for the erroneous phase offset between odd and even images.

Image reconstruction was conducted in two stages: off-resonance correction and 0th order frequency Δf_0 estimation. First, the dual-echo 3D EPI $[1-^{13}\text{C}]$ pyruvate data $S'_{1,2}$ were used as input to multi-echo demodulation in Eq. 3.7 with their respective phase maps $M'_{1,2}$, to produce the set $s_{1,2}$. Subsequently, the residual 0th order offset was estimated according to Eq. 3.9:

$$\Delta f_0 = \frac{1}{2\pi\Delta TE \cdot N_{ROI}} \sum_m^{N_x} \sum_n^{N_y} \sum_l^{N_z} \left/ \frac{s_2(m\Delta x, n\Delta y, l\Delta z)}{s_1(m\Delta x, n\Delta y, l\Delta z)} \right. \cdot ROI(m\Delta x, n\Delta y, l\Delta z) \quad (3.9)$$

where $ROI(m\Delta x, n\Delta y, l\Delta z)$ is the binary mask computed from the dual-echo 3D EPI images at an SNR threshold of 15. To account for bulk off-resonance, the phase maps were modulated by Δf_0 . The original time resolved $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate images were then corrected via multi-echo modulation with the echo-asymmetry and bulk off-resonance compensated phase maps, producing odd and even reconstructed images. The final reconstruction was taken as the complex sum of the two corrected images $s_{1,2}$. A flowchart describing the complete image reconstruction pipeline, including computation of distorted phase maps, is depicted in figure 3.3.

3.4 Results

Shim Condition	Transmit Offset Δf	Uncorrected $S-D_{indices}$	Corrected $S-D_{indices}$	Estimated Offset Δf_0	Error: $ \Delta f - \Delta f_0 $	Error: Δ px	Error: Δ mm
Automated	0 Hz	0.97	0.97	4.10 Hz	4.10 Hz	0.12 px	0.59 mm
	-50 Hz	0.74	0.95	-41.3 Hz	8.70 Hz	0.25 px	1.2 mm
	75 Hz	0.57	0.96	79.4 Hz	4.40 Hz	0.13 px	0.64 mm
Disrupted	0 Hz	0.90	0.97	2.90 Hz	2.90 Hz	0.082 px	0.41 mm
	-50 Hz	0.84	0.95	-50.4 Hz	0.400 Hz	-0.011 px	-0.057 mm
	75 Hz	0.97	0.96	81.5 Hz	6.50 Hz	0.19 px	0.93 mm

Table 3.1: Phantom study Sørensen-Dice results and frequency offset estimates. The disrupted shim condition included a strong linear shim. Errors are provided in units of absolute frequency difference and the equivalent pixel shifts (Δ px) and spatial shifts (Δ mm). The bold numbers indicate where the proposed reconstruction improved registration. The maximum error in frequency offset estimation was less than 10 Hz, corresponding to less than one third of a pixel, given the bandwidth per pixel of the dual-echo EPI sequence used in this study.

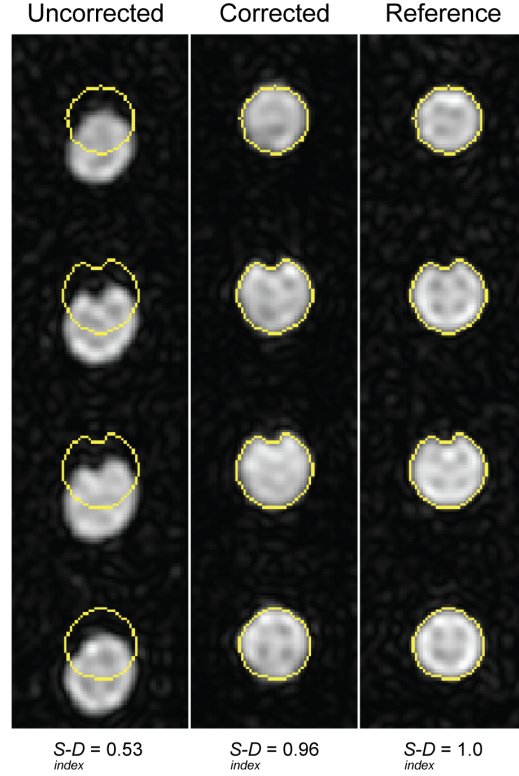


Figure 3.4: Corrected and uncorrected phantom images. Four of the twelve slices taken from the centre of the phantom are shown. The Sørensen-Dice indices were calculated from all slices. The experimental conditions included a strong linear shim and frequency offset of 75 Hz. The undistorted reference images were acquired using a modified version of the multi-echo reference scan. The proposed reconstruction provides superior image registration for all off-resonance experiments.

3.4.1 Phantom Study

The performance of the proposed image reconstruction was evaluated on the phantom data using the Sørensen-Dice index given in Eq. 3.10:

$$S-D_{index}(A[n], B[n]) = \frac{\sum_{n=1}^N A[n] \cdot B[n]}{\sum_{n=1}^N A[n] + B[n]} \quad (3.10)$$

$A[n]$ and $B[n]$ are the vectorized, binary masks of the test and ground truth images, respectively. The Sørensen-Dice index is defined as a dimensionless fraction taking values between 0 and 1, to be interpreted here as ranging from the absence of overlapping pixels to perfect spatial alignment.

For each experiment, corrected, uncorrected and ground truth 3D images were reshaped into N -dimensional vectors, where $N = N_x \times N_y \times N_z$. To produce the set of binary masks, all vectorized images were thresholded at the same SNR level, which was manually selected at a level that suppressed

the background signal of the ground truth images. The Sørensen-Dice indices were computed for each experiment according to Eq. 3.10 and is presented in table 3.1. Images and Sørensen-Dice indices for a single experiment are depicted in figure 3.4 (strong linear shim, $\Delta f = 75$ Hz). The outline delineating the spatial profile of the phantom was generated from the ground truth binary mask, and overlaid on each image for comparison.

3.4.2 In Vivo Imaging

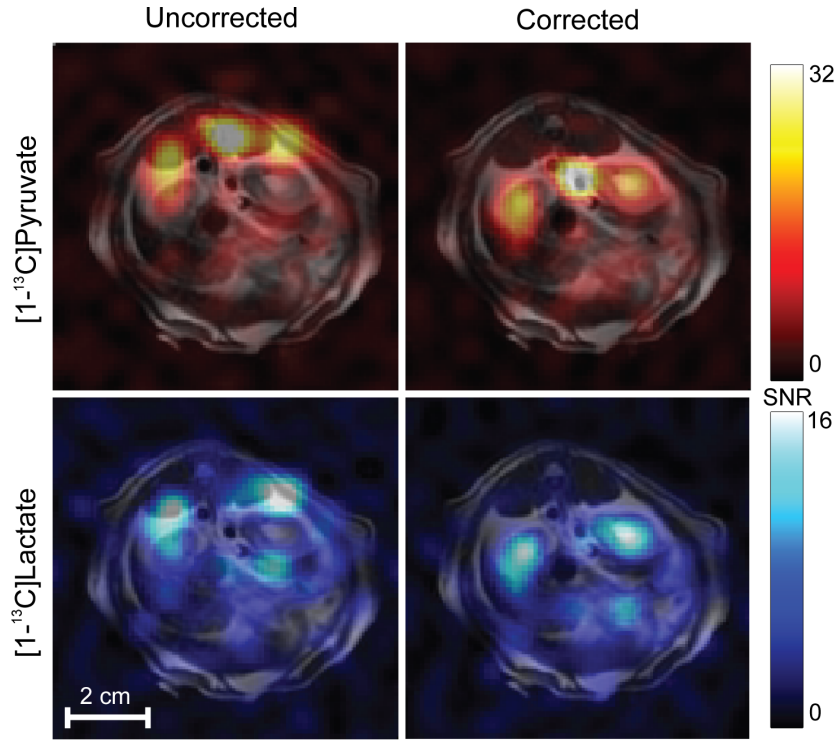


Figure 3.5: Corrected and uncorrected in vivo ^{13}C images. The experiment included an intentional frequency offset of -50 Hz. While pyruvate images were summed across all time points for visualization of the tissue signal distribution, only the later time points were included in the lactate images to minimize the appearance of the pyruvate-hydrate contamination. Superior agreement between the metabolic images and underlying anatomy is observed.

Prior to image reconstruction, the dynamic dual-echo 3D EPI images were summed across the temporal dimension to increase signal. Images were reconstructed according to the proposed method. Corrected (right) and uncorrected (left) $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate images are shown in figure 3.5. The ^{13}C metabolic images were taken within a slice intersecting the kidneys, 4X zero-padded in k-space, and then overlaid on the corresponding fast-spin echo images. The centre-frequency estimates

Experiment	Transmit Offset Δf	Estimated Offset Δf_0	Single-Echo SNR (Lac, Pyr)	Dual-Echo SNR (Lac, Pyr)	SNR Increase (Lac, Pyr)
1	0 Hz	-6.3 Hz	16.6, 36.2	22.6, 51.1	1.26, 1.41
2	-50 Hz	-55 Hz	11.0, 15.6	16.5, 22.4	1.50, 1.44
3	50 Hz	43 Hz	18.7, 31.7	26.2, 44.1	1.39, 1.40
4*	50 Hz	69 Hz	16.5, 22.8	23.1, 31.9	1.39, 1.36

*Strong Linear Shim Added

Table 3.2: In vivo ^{13}C frequency offset estimates and single versus dual-echo SNR. In experiments 1-3, excellent agreement between the transmit offset and estimated offset was observed. It should be noted that the offset provided here does not necessarily reflect the deviation from the true in vivo ^{13}C centre frequency, as this is unknown prior to the experiment. Experiment 4 suggests that the proposed reconstruction overestimated the frequency offset; however, it is feasible that the combination of a transmit offset and strong shim limit the available voxels for frequency estimation, and that the estimate still produces accurate shift correction for the excited signal.

for each reconstruction are given in table 3.2.

The intense $[1-^{13}\text{C}]\text{pyruvate}$ signal bolus can be seen in the rat aorta, between the two kidneys. The aorta can be visualized in the fast-spin echo images as a circular signal void. Note the alignment of the bolus signal with respect to the aorta before and after the proposed reconstruction, indicating appropriate off-resonance correction. Subtle higher order off-resonance correction can be observed, most strikingly in the right kidney of the $[1-^{13}\text{C}]\text{lactate}$ images. The outline of the kidneys in the corrected metabolic maps conform well to the underlying anatomy.

To demonstrate the boost in SNR provided by the dual-echo 3D EPI sequence relative to a single-echo acquisition, as in fly-back EPI, corrected images from the on-resonance experiment are shown in figure 3.6. Single-echo data were simulated from this dataset to represent the standard fly-back EPI sequence used for this purpose. The left image shows the data corresponding to a single echo image, while the right image shows the complex sum of the two reconstructed echoes. The single echo image data was generated by including only the odd echo data from the dual-echo reconstructed images. Colourbars in all metabolic overlays are in units of SNR. The relative SNR values for corrected images with either one or two echoes is provided in table 3.2.

3.5 Discussion

Early into the development phase, considerable effort was expended attempting to correct geometric distortions on the basis of field maps that were calculated directly from the ^{13}C dual-echo 3D EPI data. Phase mismatch between the dual-echo images, which was attributed to echo asymmetry, resulted in what appeared to be bulk shifts in the blip direction of the corrected images. The multi-echo reference

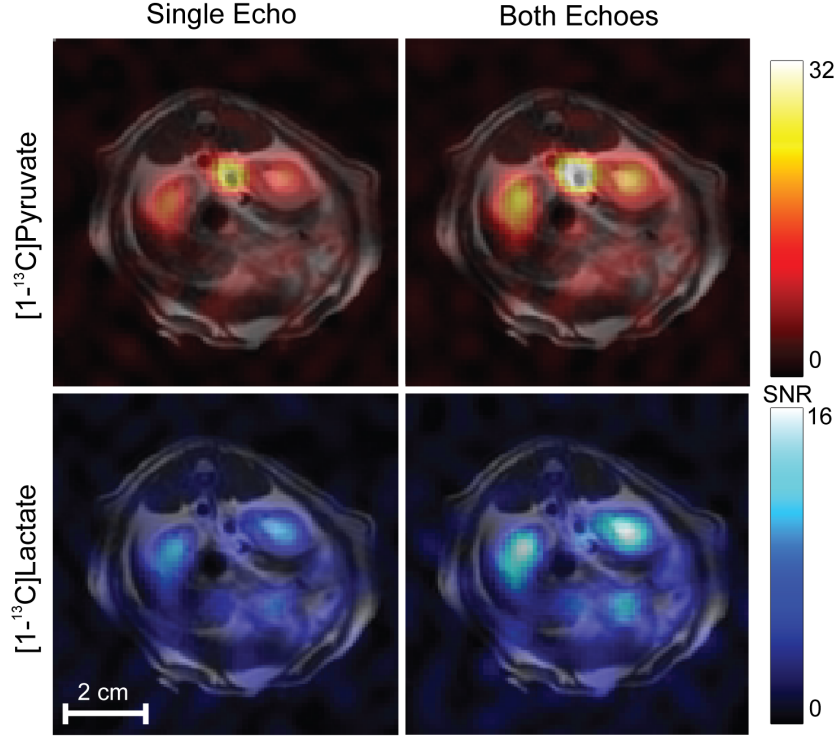


Figure 3.6: Corrected in vivo ^{13}C images with one versus two echoes. The figure demonstrates the approximately $\sqrt{2}$ increase in SNR afforded by the dual-echo sequence relative to a single-echo readout, such as fly-back EPI.

scan was originally implemented as an attempt to characterize the nature of the phase error between odd and even echoes. It was observed that the incidental phase offset between images depended on factors that may vary between experiments, such as field homogeneity; therefore, this precluded the possibility of using a single phantom experiment for correcting subsequent acquisitions.

The originally proposed scheme involved fitting a polynomial surface to distorted ^{13}C field maps calculated from the $[1-^{13}\text{C}]\text{pyruvate}$ dual-echo 3D EPI images, in order to correct for ^{13}C field inhomogeneity. Due to low SNR, the resulting ^{13}C field maps were seen to be highly sensitive to the choice of parameters for thresholding, smoothing, and polynomial degree. Even with higher SNR, the method would still have required an odd/even correction factor obtained from a separate reference scan. By correcting various ^{13}C independent off-resonance sources through multi-echo demodulation with high SNR, ^1H derived phase maps, this reduced the required contribution from the dual-echo images to only the 0th order offset term, which is more amenable to direct estimation from the relatively low SNR ^{13}C data.

The choice of SNR threshold during the frequency-offset estimation was selected on the basis of the

appearance of the in vivo images, and then carried over for the phantom reconstructions. Although tuneable parameters such as this are often selected on the basis of well controlled, phantom experiments, and carried on to subsequent data sets, in this work, it was observed that the phantom data provided similar estimates for a much wider range of SNR thresholds than the in vivo HP ^{13}C data. This is unsurprising, since the signal distribution in the phantom images is much more uniform than in $[1-^{13}\text{C}]$ pyruvate images, which vary widely due to the high signal intensity of the bolus relative to the tissue.

Large values of the threshold tended to provide inaccurate estimates of the frequency-offset, likely due to the estimation of the 0th order term from a small number of voxels. In contrast, small values of the threshold also lead to poor estimates, owing to the inclusion of background signal. A balance was found by identifying the lowest SNR threshold that provided appropriate background suppression, without excluding viable, albeit lower signal voxels. The final 0th order estimates were shown to converge to slightly different values, depending on the SNR threshold used; however, most reasonable threshold levels produced estimates that agreed within ± 10 Hz, which corresponds to an equivalent shift range of approximately ± 0.3 pixels or ± 1.4 mm. An SNR threshold of 15 was used for all in vivo reconstructions in this study. Future work will investigate data driven approaches which will seek to select the best threshold automatically from a given set of HP ^{13}C images.

The proposed dual-echo 3D EPI sequence offers clear advantages over an equivalent fly-back EPI sequence. For example, using the same gradient hardware specifications and waveform generation algorithm, a fly-back EPI readout can satisfy the same resolution requirements with a readout length of 27.1 ms, representing a potential speedup over the proposed sequence of only 2.5 ms per TR. This can be attributed to the low gyromagnetic ratio of ^{13}C , which effectively reduces gradient performance relative to proton by approximately 4-fold. With a negligible difference in TR, the dual-echo 3D EPI sequence spends exactly twice as long acquiring signal, providing a theoretical $\sqrt{2}$ increase in SNR. HP ^{13}C fly-back EPI is attractive as it is robust to echo asymmetry, thereby mitigating against a primary source of ghosting artifacts that are typically encountered with symmetric EPI readouts. Granted a minor increase in readout time, the dual-echo 3D EPI sequence retains the benefit of echo asymmetry insensitivity, while improving readout efficiency by nearly 20%. Asymmetry between odd and even echoes is accounted for in the reconstruction pipeline in order to maximize the coherent addition of the echoes.

A limitation of the proposed method is the assumption that there is no motion of the imaged object between the ^1H multi-echo reference scan and the ^{13}C dual-echo 3D EPI acquisition. This limitation is not a unique feature of this study, as reference scan driven methods, from distortion correction to

coil sensitivity estimation in conventional MR, also carry the burden of such an assumption. While some methods explore the use of navigator signals built into the encoding scheme to combat motion, these are generally not applicable to ^{13}C studies due to severely limited SNR and the fact that the HP magnetization is non-renewable. Detecting subject motion during a ^{13}C exam remains an open problem that will likely be addressed in the traditional manner of using adequate patient restraint.

3.6 Conclusion

We have demonstrated a novel HP ^{13}C dual-echo 3D EPI acquisition that combines proton derived reference scans with off-resonance information obtained from the phase evolution between echoes. The method was shown to provide superior alignment in the presence of off-resonance source according to a phantom study. In vivo images depicted good agreement with the underlying anatomy. In all cases, the proposed method was shown to account for off-resonance distortion and preserve SNR efficiency. ^{13}C dual-echo 3D EPI is an attractive method for adhering to the physical constraints of HP ^{13}C imaging while ensuring accurate spatial registration in the presence of off-resonance magnetization. Whereas the dual-echo trajectory is concerned with encoding in-plane, the following chapter is focused on speeding up the through-plane encoding time of 3D EPI.

Chapter 4

Accelerated Encoding for Hyperpolarized ^{13}C Echo-Planar Imaging

4.1 Introduction

Hyperpolarized ^{13}C imaging studies involving time-resolved rather than static imaging permit extraction of kinetic model parameters [106], which may provide more detailed quantitative metabolic information [107]. In addition to providing richer data time-resolved imaging sequences are arguably more robust than their static counterparts since both SNR and image quality [122] are less sensitive to the precise timing of the acquisition window with respect to the substrate injection and perfusion. Furthermore, since the pharmacokinetics of tracer delivery and ^{13}C label exchange have yet to be characterized beyond prostate cancer patients [1], time-resolved acquisitions are ideal for investigating metabolism with hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ in novel cohorts.

Due to strict technical demands, only a subset of existing hyperpolarized ^{13}C imaging sequences [123] are capable of achieving time-resolved, volumetric coverage. Spiral chemical shift imaging [156, 157] couples spectral and in-plane spatial encoding to enable dynamic spectroscopic acquisitions. Time-resolved IDEAL spiral chemical shift imaging [158] exploits prior knowledge of the precise chemical shifts to facilitate spectral separation via a sequence of echo-time shifted spiral imaging readouts. Frequency selective methods [100, 140] exploit the spectral sparsity of $[1-^{13}\text{C}]\text{pyruvate}$ and its downstream

metabolic products to perform spectral encoding “up front”, permitting the use of rapid spatial encoding schemes such as spiral or echo-planar imaging (EPI) for time-resolved acquisitions.

Since the acquisition time per volume increases linearly with the number of acquired slices, adapting any imaging technique for larger, clinically relevant, volumes places constraints on the achievable temporal resolution. This is compounded by the requirement for additional spatial interleaves to increase in-plane coverage in multi-echo and spectroscopic imaging sequences [159].

Though few time-resolved techniques have been demonstrated, several acceleration schemes have been proposed to address the challenge of implementing volumetric hyperpolarized ^{13}C imaging for large FOV applications. Tropp et al. utilized a 3-channel array to achieve 2-fold SENSE acceleration in 3D echo-planar spectroscopic imaging [160]. This work was extended by Ohliger et al. who used an 8-channel array with a phase constrained reconstruction to increase the acceleration factor up to 3.75-fold [161]. Hu and Larson et al. exploited the spatio-temporal sparsity of the dynamic 3D echo-planar spectroscopic imaging signal to achieve 18-fold acceleration by use of a pseudo-randomly undersampled k-space trajectory and a non-linear compressed sensing reconstruction [162, 163, 164].

In this chapter, I present a novel technique for accelerating time-resolved, 3D echo-planar imaging in vivo with hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$. The essential features include a spectral-spatial pulse [139] for simultaneously resolving metabolite signals and efficiently utilizing the hyperpolarized magnetization [165], a randomized 3D EPI readout and a non-linear reconstruction [166] with compressed sensing acceleration in two spatial dimensions. The results herein support the feasibility of compressed sensing 3D EPI for accelerating dynamic hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ and $[1-^{13}\text{C}]\text{lactate}$ imaging.

4.2 Theory

Compressed sensing (CS) has now become a well-known acceleration method in MRI, but is briefly summarized here for completeness. CS is a sub-Nyquist sampling framework that provides probabilistic bounds for the recovery of sparse signals from fewer measurements than would be required for linear reconstruction [167, 168, 169]. The rationale is that sparse signals are constrained by far fewer degrees of freedom than the number of measurements required by Nyquist sampling theorem. In order to exploit signal sparsity in CS accelerated data acquisitions, the acquired set of linear measurements must give rise to incoherent aliasing within the transform domain for which the underlying signal admits a sparse representation. Incoherent aliasing can be qualitatively described as a noise-like appearance of the transform point spread function resulting from the measurement matrix [166]. CS acceleration has been successfully demonstrated in various MRI applications that exhibit exploitable image sparsity, as

the condition for incoherent aliasing of undersampling artifacts is almost always satisfied for random Fourier measurements [167, 166].

Randomly undersampled signals that admit a sparse representation in some transform domain can be recovered through non-linear minimization of $\phi(x)$:

$$\phi(x) = \|\mathcal{F}_u x - y\|_2^2 + \lambda_1 \|x\|_1 + \lambda_2 TV(x) \quad (4.1)$$

where x is the desired image; y are the set of linear k-space measurements; $\mathcal{F}_u$ is a random subset of the Fourier measurement matrix; $\|x\|_1$ is the l_1 norm of x ; $TV(x)$ is the total-variation norm of image x ; λ_1 and λ_2 are regularization parameters. The image x can be iteratively recovered in Eq. 4.1 through non-linear optimization by enforcing finite-difference sparsity and data consistency.

The number of random Fourier measurements m that are required to recover x with high probability is linearly dependent on the number of sparse coefficients k and logarithmically dependent on the signal dimension N :

$$k \geq \text{Const} \cdot m \log N \quad (4.2)$$

In reality, images are not strictly k -sparse but may be k -compressible, meaning that the k -term approximation of the images faithfully captures meaningful signal information. It has been shown empirically that $k \sim 3m - 5m$ gives enough measurements for sparse recovery [162]. In previous work, Hu and Larson et al, achieved 18-fold acceleration by exploiting the total-variation and wavelet-in-time compressibility of the time-resolved $[1-^{13}\text{C}]$ pyruvate spectroscopic imaging signal.

In contrast to this previous work, here the CS framework is applied to frequency-selective imaging data, with a signal dimensionality N necessarily much lower than spectroscopic imaging data. Only spatial finite-difference sparsity was exploited in this work, and this constrains the achievable acceleration factor. Archived $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate EPI data from previous studies in rats was used to estimate image compressibility, and showed that acceleration factors up to 3-fold would provide a sufficient number of measurements for CS reconstruction provided that the requirement for incoherent undersampling artifacts could be met by the pulse sequence design described in Methods.

4.3 Methods

4.3.1 Time-resolved EPI Sequence

A 34 ms symmetric EPI readout comprised of 36 gradient echoes was designed for in-plane spatial encoding across a $72 \times 18 \text{ cm}^2$ FOV at $5 \times 5 \text{ mm}^2$ resolution. Sampling was restricted to the readout gradient plateaus, resulting in a gradient SNR efficiency [154] of 78%. The $\pm 125 \text{ kHz}$ readout bandwidth was chosen to increase the FOV in the readout direction at no SNR penalty [170]. Spectral and volume selection was achieved with a tailored 18.8 ms spectral-spatial RF pulse [139] consisting of 26 sub-lobes, each with $720 \mu\text{s}$ duration, enabling a passband of 120 Hz (full-width-at-half-max) and a stopband of 1.1 KHz ($< -60 \text{ dB}$). For the fully sampled acquisitions, conventional slice-encoding was used to provide $72 \times 18 \times 18 \text{ cm}^3$ volumetric coverage at $5 \times 5 \times 5 \text{ mm}^3$ nominal resolution (TR/TE = 56/26 ms, $144 \times 36 \times 36$ matrix). The acquisition time per volume was 2 s, allowing sequential time-resolved sampling of $[1\text{-}^{13}\text{C}]\text{pyruvate}$ and $[1\text{-}^{13}\text{C}]\text{lactate}$ at a maximum temporal resolution of 4 s.

4.3.2 Incoherent k_y - k_z Undersampling

The proposed undersampling scheme involves the use of blipped z-gradients during the ramp portion of the EPI readout to skip readout lines and generate incoherent aliasing artifacts in linear image reconstructions. The single degree of freedom afforded by z-blipped acceleration was utilized for direct k_z undersampling and indirect k_y undersampling. The k_x (frequency encoded) readout direction was fully sampled for all acquisitions. A pulse sequence timing diagram and the accompanying 3D sampling mask for 2-fold acceleration are illustrated in figure 4.1.

The k_y - k_z sampling masks, dictating which specific k_y - k_z points were acquired and which were skipped, were generated by incorporating variable-density sampling [166] with respect to k_z . Each k_y row of a k_y - k_z mask was independently filled by randomly selecting N_z/R samples without replacement from a Gaussian weighted probability distribution; where R is the acceleration factor and N_z is the number of Nyquist samples. 100 sets of masks for each $R = 2$ and $R = 3$ were generated, and the sampling patterns that exhibited the minimum peak-to-side-lobe ratio for their respective transform-point-spread-functions [166] were selected for waveform design.

The selected masks were subsequently converted to acquisition schedules that defined which sequence of k_y - k_z readout locations would be sampled during a single TR, serving both as an input to the z-blip gradient waveform design and as a mapping for each measurement in the raw k-space data files. The acquisition schedules were derived by scanning through each k_y row of the sampling masks, from

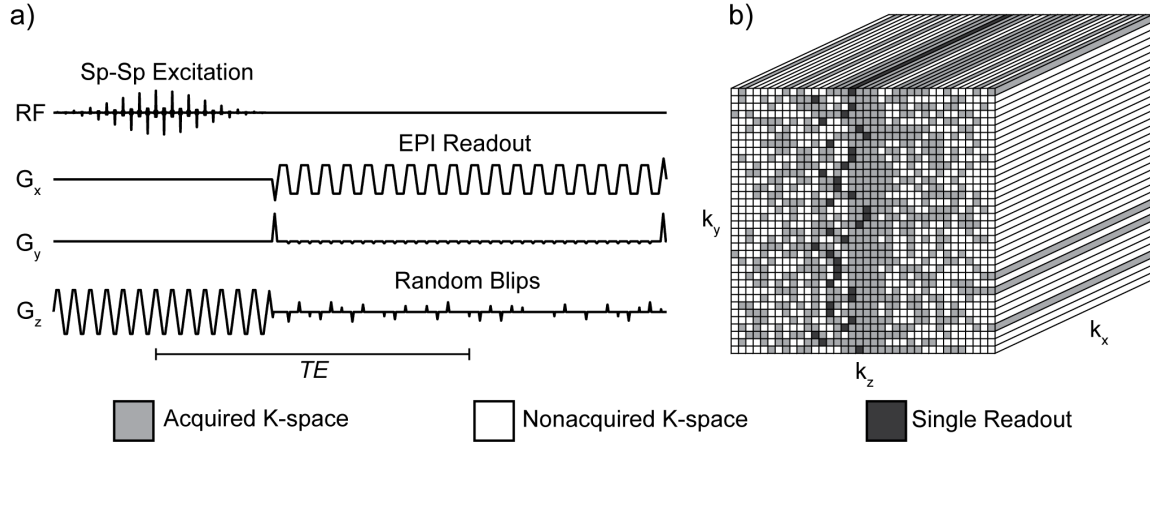


Figure 4.1: Blipped scheme for k_y - k_z undersampling. a) Frequency selective 3D echo-planar imaging sequence timing diagram. The spectral-spatial RF pulse consists of 26 sub-lobes, each with $720\ \mu\text{s}$ duration, giving a 120 Hz passband (full-width-at-half-max) and a 1144 Hz stopband. A symmetric, 56 ms EPI readout consisting of 36 gradient echoes is used for in-plane spatial encoding. Acceleration is achieved through random G_z blips which play out during the ramps of the EPI readout gradient. b) 3D k-space sampling schedule for 2-fold acceleration. A single readout corresponding to the G_z waveform shown in a) is highlighted in black. White and grey readout lines correspond to non-acquired and acquired k-space, respectively.

$-k_z$ to $+k_z$, until a sample was located. Once the k_y - k_z index of a sample was stored in the acquisition schedule, its corresponding location was removed from the mask and scanning resumed on the next row; this ensures that each sample is only acquired once. Scanning terminated when $N_y = 36\ k_y$ - k_z sampling locations had been assigned to a single echo-planar readout. This process was iterated until all k_y - k_z locations within the sampling masks were mapped to their acquisition schedules for a total of N_z/R readouts.

The derivative of the acquisition schedules with respect to k_z provided the required discrete k-space displacements at each readout. The maximum displacements were converted to physical units and used to design prototype trapezoidal z-blip gradient waveforms. Prototype trapezoids for $R = 2$ and $R = 3$ were subsequently scaled for each required k_z displacement, and inserted to align with the centres of the EPI readout ramps. Finally, dephaser trapezoids were designed to produce the requisite initial displacement for each readout waveform, and rephaser trapezoids were designed to null their respective 0^{th} moments.

4.3.3 Phantom Study

All imaging experiments were performed with a dual-tuned transmit/receive ^1H - ^{13}C rat coil on a GE MR750 3T MR ($G_{\max} = 50 \text{ mT/m}$, $S_{\max} = 200 \text{ T/m/s}$, GE Healthcare, Waukesha, WI). The pseudo-random k-space trajectory design was validated with a spherical ^{13}C -bicarbonate phantom (30 mm diameter, 1.0 M concentration, doped with 0.02% OmniScan (GE Healthcare)). Dyun's method [171] was used to measure the empirical k-space trajectories for the z-axis gradient waveforms. Briefly, Dyun's method works by exciting a thin slice off isocentre, parallel to the gradient direction under investigation, and then acquiring data for each axis separately. Assuming no effect of the slice selection gradient, the phase of the MR signal can be approximated by $\phi_r(t) = \int_0^t \gamma \cdot G_r(t) \cdot D_r \cdot dt = D_r \cdot k_r(t)$, where γ is the gyromagnetic ratio; $G_r(t)$ is the spatial encoding gradient amplitude; r is the encoding direction (x , y , or z); and D_r is the distance of the slice to gradient isocentre. The k-space trajectory $k_r(t)$ can be obtained by normalizing $\phi_r(t)$ to D_r . No significant deviations from the target trajectories were observed (see figure 4.2). Fully sampled ($R = 1$), $R = 2$ and $R = 3$ undersampled images were acquired using conventional slice-encoding and the proposed z-blip encoding scheme, respectively ($\text{TR}/\text{TE} = 1500/12$, $\text{FA} = 10^\circ$, $\text{NEX} = 8$). A $500 \mu\text{s}$ hard pulse was used for constant flip angle RF excitation.

4.3.4 Data Acquisition

One of two Sprague-Dawley rats (weight = 510/450 g, Harlan Laboratories) was used during each imaging session. In vivo imaging was conducted in accordance with a protocol approved by the local institutional animal care and use committee. Coronal fast-spin echo images were acquired for localizing the rat kidneys. Centre frequency and transmit gain calibrations were performed using an external ^{13}C urea reference phantom. A constant flip angle scheme was used for RF excitation to achieve a net flip angle per volume of 10° for $[1\text{-}^{13}\text{C}]\text{pyruvate}$ and 80° for $[1\text{-}^{13}\text{C}]\text{lactate}$. The constant flip angle was increased for the prospectively undersampled acquisitions in order to maintain the same net flip angle per volume across all experiments. For example, the lactate flip angle per excitation was 17.7° and 30.2° for $R = 1$ ($N_z = 36$) and $R = 3$ ($N_z = 12$), respectively. The inter-volume dwell time was set for each acceleration factor to retain 5 s temporal resolution in all ^{13}C acquisitions.

30 μL of pyruvic acid doped with 15 mM of OX63 radical (Oxford Instruments, Tubney Woods, UK) and 1 mM of Prohance (Bracco Diagnostics, Princeton, NJ) was polarized using a SPINlabTM DNP polarizer (GE Healthcare) [155] for each experiment. The hyperpolarized pyruvic acid mixture was dissolved using heated/pressurized H_2O (100 mg/L EDTA) and neutralized in the receiver with a 720 mM NaOH / 400 mM TRIS buffer solution to yield $\sim 4.8 \text{ mL}$ of 80 mM hyperpolarized pyruvate in

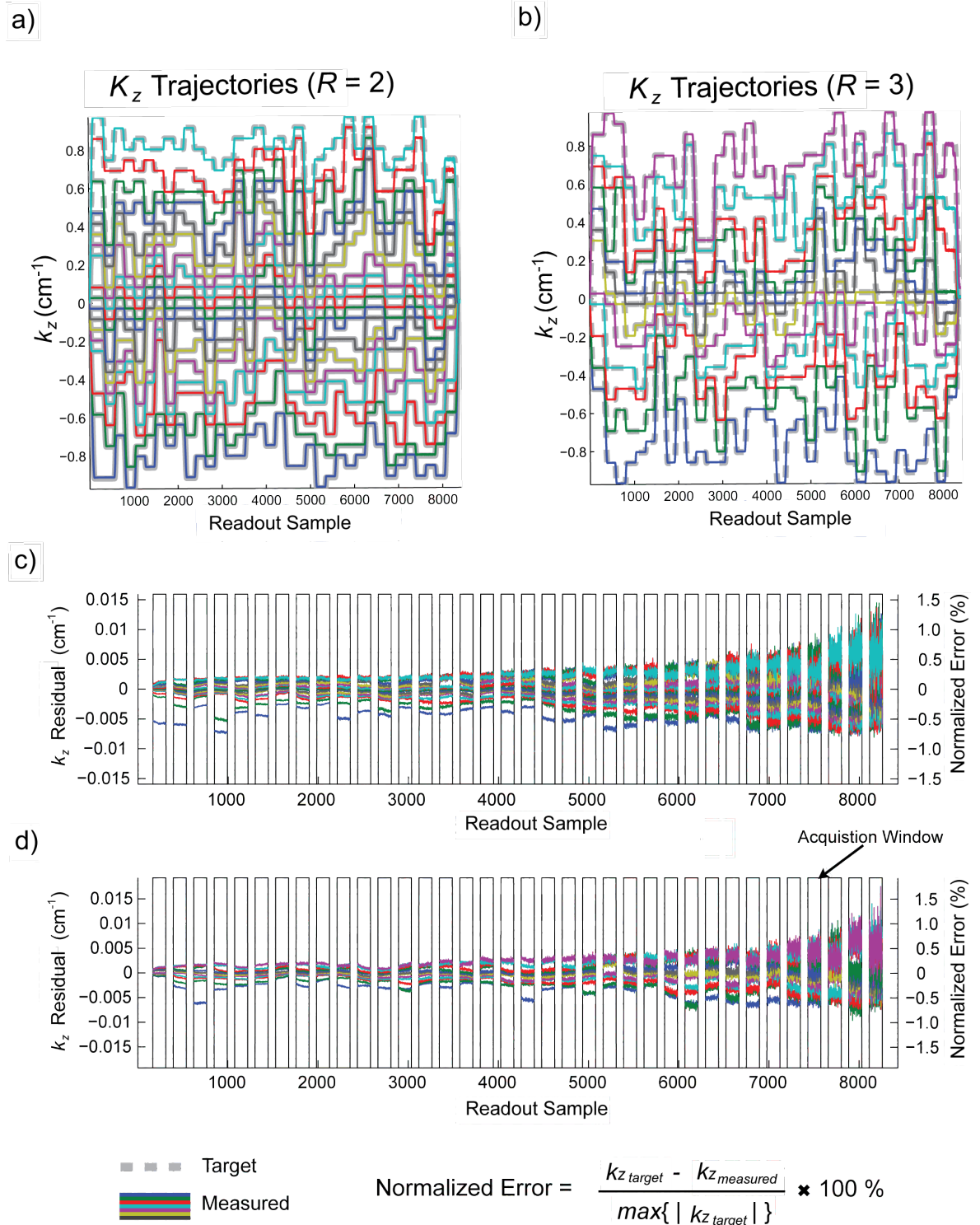


Figure 4.2: Overlays of target and measured k-space trajectories for $R = 2$ a) and $R = 3$ b). Trajectory residuals for $R = 2$ c) and $R = 3$ d) sequences are shown for points within the acquisition window. The measured deviations in the k-space trajectory are on the order of 1-2% of the maximum target spatial frequency. Dephasing during the readout contributes to loss of SNR in later samples of the trajectory measurement.

solution. The polarization of $[1-^{13}\text{C}]$ pyruvate achieved in solution was not measured in these experiments; however, previous tests measured $\sim 40\%$ polarization using the same materials and protocol. 3 mL of the 80 mM pre-polarized $[1-^{13}\text{C}]$ pyruvate solution was injected over 12 seconds through a tail vein catheter at the start of each acquisition. All prospectively undersampled data were obtained from a single rat over two separate imaging sessions with 1 injection for each of the three R factors. Consecutive hyperpolarized ^{13}C scans were acquired 30 minutes apart for sessions with multiple injections. Axial and sagittal fast spin-echo images were acquired for anatomical reference (TR/TE = 5000/83, 18 cm FOV, 192×192 matrix, 4 NEX).

4.3.5 Image Reconstruction

Image reconstruction, analysis and RF/gradient waveform design were implemented in Matlab (The MathWorks Inc., Massachusetts). Prospectively undersampled in vivo data were relocated within zero-filled matrices according to the acquisition schedules for each trajectory. $R = 2$ and $R = 3$ retrospectively undersampled data sets were generated by multiplying the fully sampled 3D matrices with the respective sampling masks. Image reconstruction was applied to each time-resolved volume independently. Ghost correction was performed for each slice with N.K. Chen's image based, 2D phase cycled algorithm [172]. Briefly, the algorithm generates a series of reconstructed images by cycling through different possible phase errors and among these, the image with the lowest signal level within a pre-defined region is selected as the final ghosted corrected image. Each undersampled volume was scaled relative to its maximum pixel signal intensity to allow reuse of the same regularization parameters across all experiments [166]. The non-linear conjugate gradient CS reconstruction was performed using the SparseMRI toolbox (<http://www.eecs.berkeley.edu/~mlustig/Software.html>), and the regularization parameters were manually set to 0.01 for both the data fidelity and total-variation constraints.

4.3.6 Data Analysis

The recovery of spatial features in retrospective CS images was assessed with a mean structural similarity index (*SSIM*). *SSIM* is an objective image quality metric that provides a measure of the similarity between two images, a and b , by comparing their luminance $l(a, b)$, contrast $c(a, b)$ and structure $s(a, b)$. These properties are estimated by computing local image statistics for signal intensity mean, variance, and cross-correlation [173], respectively. Reconstructed data were temporally summed to increase SNR, and sagittal slices corresponding to the incoherently sampled dimensions were concatenated into respective $N_y \times (N_z \times N_x)$ 2D images. The expression in Eq. 4.4 was used for generating *SSIM* maps

among the concatenated fully sampled (a) and retrospective CS (b) images:

$$S(a, b) = l(a, b) \cdot c(a, b) \cdot s(a, b) \quad (4.3)$$

$$SSIM(a, b) = \frac{(2\mu_a\mu_b + C_1)(2\sigma_{ab} + C_2)}{(\mu_a^2 + \mu_b^2 + C_1)(\sigma_a^2 + \sigma_b^2 + C_2)} \quad (4.4)$$

where μ_a and μ_b are the mean signal intensities; σ_a^2 and σ_b^2 are the signal intensity standard deviations; σ_{ab} is the correlation coefficient between image vectors [173]; and C_1 / C_2 are stabilizing constants. Local image statistics were calculated over a 3×3 gaussian weighted window. The stabilizing parameters C_1 and C_2 were set to the default values of 0.01 and 0.03, respectively [173]. The reported $SSIM$ index for each reconstruction was calculated by taking the mean values within high SNR regions (threshold $\geq 50\%$ max SNR) of each $SSIM$ map.

To evaluate the fidelity of parametric estimates from both prospective and retrospective CS reconstructions, the total lactate-to-pyruvate area-under-the-curve ratio ($L:P_{AUC}$) was calculated according to Eq. 4.5:

$$L:P_{AUC} = \frac{\sum_{t=1}^N \sum_{i \in ROI} |L[i, t]|}{\sum_{t=1}^N \sum_{i \in ROI} |P[i, t]|} \quad (4.5)$$

where N is the number of time-points; $L[i, t]$ and $P[i, t]$ are the time-resolved $[1-^{13}\text{C}]\text{lactate}$ and $[1-^{13}\text{C}]\text{pyruvate}$ imaging data, respectively; and ROI is the 3D region-of-interest corresponding to the rat kidneys. A region-of-interest was generated for each imaging session by manually segmenting both right and left kidneys from the high-resolution ^1H axial spin-echo images, and resampling the selected regions to the digital resolution of the ^{13}C imaging data.

4.4 Results

Retrospectively undersampled data were generated by multiplying the $R = 1$ phantom acquisition with the 3D sampling masks for each $R = 2$ and $R = 3$ times acceleration. Zero-filled (ZF) images were generated by taking the inverse Fourier transform of the zero-filled data. Figure 4.3 a) shows ZF images within the central slice for the retrospectively (column 1) and prospectively undersampled data (column 2). The third column shows the corresponding difference images. The difference between the prospective and retrospective ZF images indicate that the incoherent aliasing artifacts resulting from the

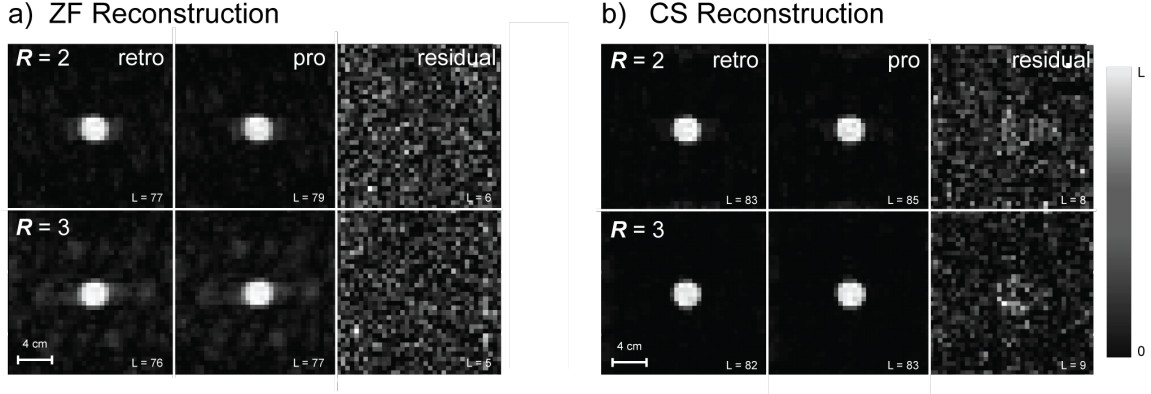


Figure 4.3: Phantom study for $ky-kz$ undersampling validation. Retrospective (retro) data were generated by multiplying fully sampled ^{13}C bicarbonate phantom data with the $R = 2$ and $R = 3$ masks. Prospectively (pro) undersampled data were acquired using the proposed sequence. a) Incoherent aliasing for $R = 2$ (top) and $R = 3$ (bottom) resulting from direct Fourier transform of retrospectively (left) and prospectively (middle) undersampled data, denoted ZF for zero-filled. b) CS reconstruction for $R = 2$ (top) and $R = 3$ (bottom) of retrospectively (left) and prospectively (middle) undersampled phantom data. Residual images between retrospective and prospective undersampling (right) for $R = 2$ and $R = 3$ are shown for both reconstructions. The peak intensity within the residual images is $\sim 10\%$ of the maximum signal in the fully sampled data. All images were extracted from a central sagittal slice corresponding to the incoherently sampled spatial dimensions. Colourbar is in arbitrary units.

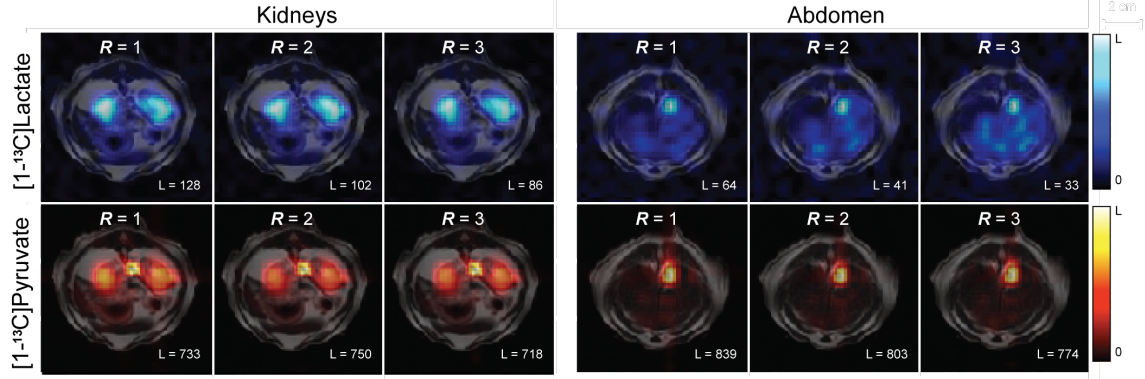
ideal undersampled trajectories are similarly generated by the prospectively undersampled trajectory. The peak difference in the ZF images is $\sim 10\%$ of the peak pixel intensity taken within the fully sampled phantom image, which is on the order of noise.

CS image reconstructions corresponding to the same slice for the retrospectively (column 1) and prospectively undersampled data (column 2) are shown in figure 4.3 b). The respective difference images are shown in column 3. The difference images between the prospective and retrospective CS reconstructions provides further evidence that the deviations between the ideal and actual pseudo-random k-space trajectory are negligible. While the phantom study results are encouraging, success of CS reconstructions rely heavily on the exploitable sparsity of the underlying image. Accordingly, it is difficult to extrapolate the results derived from a homogeneous spherical ^{13}C -bicarbonate phantom to arbitrary image sources; the total-variation compressibility of the phantom images can not predict the sparsity of in vivo ^{13}C images. Nevertheless, the results suggest that the proposed trajectory can reliably induce incoherent aliasing required for CS reconstructions of data acquired on clinical hardware.

Representative axial $[1-^{13}\text{C}]\text{pyruvate}$ and $[1-^{13}\text{C}]\text{lactate}$ CS reconstructions for $R = 2$ and $R = 3$ prospective acceleration are shown in figure 4.4, along with the corresponding fully sampled images. Images were generated by summing the time-resolved series over a contiguous window, which was automatically selected for each data set in order to maximize the measured $[1-^{13}\text{C}]\text{lactate}$ SNR within

the rat kidneys. Temporally summed images were overlaid on the corresponding axial fast spin echo images for anatomical reference. The ^{13}C overlay colourbar is displayed in arbitrary units and each image is displayed across its respective full dynamic range.

a) Retrospective CS



b) Prospective CS

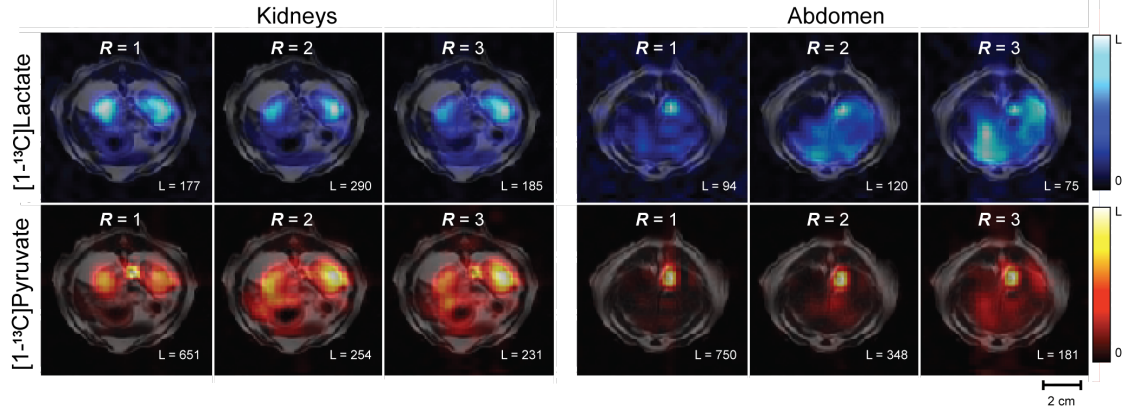


Figure 4.4: Representative image reconstructions corresponding to fully sampled and prospective $R = 2$ and $R = 3$ fold accelerated data acquired with the proposed sequence. Time-resolved $[1-^{13}\text{C}]\text{pyruvate}$ and $[1-^{13}\text{C}]\text{lactate}$ reconstructed volumes were temporally summed to increase SNR. Axial slices corresponding to the rat kidneys (*left*) and abdomen (*right*) were extracted from the summed volumes and overlaid on the respective fast spin echo images. Colourbars are in arbitrary units.

The data shown in figure 4.4 was acquired using 3 separate injections in a single rat (weight = 510 g) during a single imaging session (30-40 minutes between acquisitions). The relative spatial distribution of $[1-^{13}\text{C}]\text{pyruvate}$ and $[1-^{13}\text{C}]\text{lactate}$ appears similar for both CS reconstructions as compared to the fully sampled images, with high signal contrast seen within the kidneys. The distribution of $[1-^{13}\text{C}]\text{lactate}$ in all images is primarily localized to the kidneys, heart and gut. No artifacts or loss of resolution are evident in the CS reconstructed images acquired with the proposed sequence. The CS images exhibit increased apparent SNR compared to the fully sampled images by a factor of 2-3 fold. This is likely due to the combined effect of using a larger flip angle and the denoising properties of the

non-linear reconstruction [162, 163, 164, 166].

R_{lac}	Zero-Fill $_{lac}$	Low-Resolution $_{lac}$	CS $_{lac}$
2	0.85 ± 0.03	0.91 ± 0.03	0.96 ± 0.02
3	0.61 ± 0.05	0.80 ± 0.06	0.91 ± 0.05

R_{pyr}	Zero-Fill $_{pyr}$	Low-Resolution $_{pyr}$	CS $_{pyr}$
2	0.83 ± 0.05	0.87 ± 0.08	0.99 ± 0.01
3	0.58 ± 0.06	0.78 ± 0.07	0.96 ± 0.02

Table 4.1: Mean $\pm$ standard deviation of the *SSIM* indices for zero-filled, low-resolution and CS reconstruction. The measured *SSIM* indices for the CS reconstructions indicate the best recovery of spatial features. While the *SSIM* indices for $R = 2$ fall within a similar range in all cases, increased dissimilarity was measured for $R = 3$ for the linear acceleration schemes

Results for the *SSIM* analysis for assessing the recovery of structural features in retrospectively undersampled in vivo data is presented in table 4.1. Each of the fully sampled data sets ($N=6$) was retrospectively undersampled for $R = 2$ and $R = 3$ times acceleration and reconstructed images were temporally summed using the method previously described. The *SSIM* index takes values between 0 and 1, with the value of 1 being uniquely defined for the case of flawless reconstruction [173]. *SSIM* indices greater than 0.9 indicate excellent reconstruction fidelity [173, 174]. Low resolution retrospectively undersampled data were generated by setting the high k_z spatial frequencies to zero for hypothetical $R = 2$ and $R = 3$ acceleration. For visual reference, sagittal overlays for Nyquist, zero-filled, low resolution and CS reconstruction are shown in figure 4.5. The mean *SSIM* values for each of the images are in good agreement with a qualitative assessment of reconstruction fidelity granted by each method.

The mean $\pm \sigma$ *SSIM* index for the $R = 2$ and $R = 3$ times undersampled retrospective CS [$1-^{13}\text{C}$]lactate reconstructions was 0.96 ± 0.02 and 0.91 ± 0.05 , respectively. Corresponding [$1-^{13}\text{C}$]pyruvate *SSIM* indices were 0.99 ± 0.005 for $R = 2$ and 0.96 ± 0.02 for $R = 3$. The mean *SSIM* indices suggest good to excellent recovery of structural features in all retrospective CS reconstructions. Higher mean *SSIM* values were measured in the $R = 2$ times undersampled images as compared to $R = 3$. This is likely due to the combination of the $\sqrt{R}$ input SNR penalty incurred by the retrospective undersampling process and the use of fewer measurements in the $R = 3$ data sets. The mean $\pm \sigma$ factor increase in apparent [$1-^{13}\text{C}$]lactate SNR measured in the kidneys was 1.34 ± 0.05 and 1.43 ± 0.07 for $R = 2$ and $R = 3$, respectively. The increase in apparent SNR is due to the denoising properties of the non-linear CS reconstruction, and depends heavily on the choice of regularization parameters used [163, 164].

The relative $L:P_{AUC}$ values for all images is shown in figure 5. The mean $\pm \sigma$ % difference in $L:P_{AUC}$ between the fully sampled and $R = 2$ retrospectively undersampled images was 2.6 ± 1.6 %. The corresponding % difference for the retrospective $R = 3$ images was 4.3 ± 2.3 %. For the prospectively

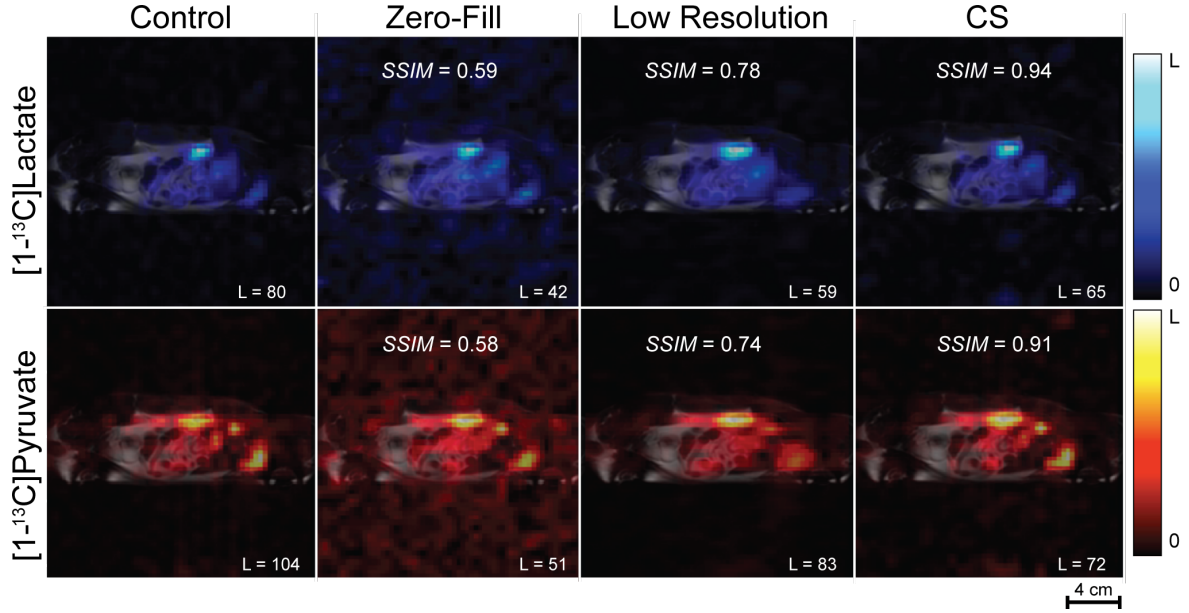


Figure 4.5: Representative $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate images reconstructed with (from left to right) fully sampled, zero-filled, low resolution sampling and compressed sensing for $R = 3$. The mean SSIM was calculated from high SNR regions of the full maps. ^{13}C data were resampled to align with the corresponding sagittal fast spin echo images. The SSIM indices for each reconstruction method agree qualitatively with image artifacts and loss of resolution. Colourbars are in arbitrary units.

undersampled images ($N = 2$ for each R), the % differences in $L:P_{AUC}$ was 7.3 and 11 % for $R = 2$ and 5.6 and 4.8 % for $R = 3$. On the basis of previous data, the reported $L:P_{AUC}$ differences are on the order of the experimental variance. The combined % difference for all reconstructions of $R = 3$ times undersampled data was approximately 2% higher than for $R = 2$. A possible explanation for this trend is that the non-linear CS reconstruction algorithm is known to underestimate intensity of low signal pixels [163, 164]. Despite this bias, the modest differences in the measured $L:P_{AUC}$ values suggest that the proposed acquisition encodes metabolic information on par with fully sampled acquisitions.

4.5 Discussion

We have demonstrated a novel z-blip acquisition sequence for compressed sensing accelerated hyperpolarized ^{13}C 3D EPI with $[1-^{13}\text{C}]$ pyruvate. Analysis of the structural similarity between retrospectively undersampled and control in vivo data indicate that the signal intensity distribution in CS reconstructed $[1-^{13}\text{C}]$ lactate and $[1-^{13}\text{C}]$ pyruvate can accurately recover spatial features in metabolic images evaluated. The close agreement in $L:P_{AUC}$ estimates from both retrospectively and prospectively undersampled CS reconstructions provide further evidence that pertinent metabolic information is preserved through acceleration.

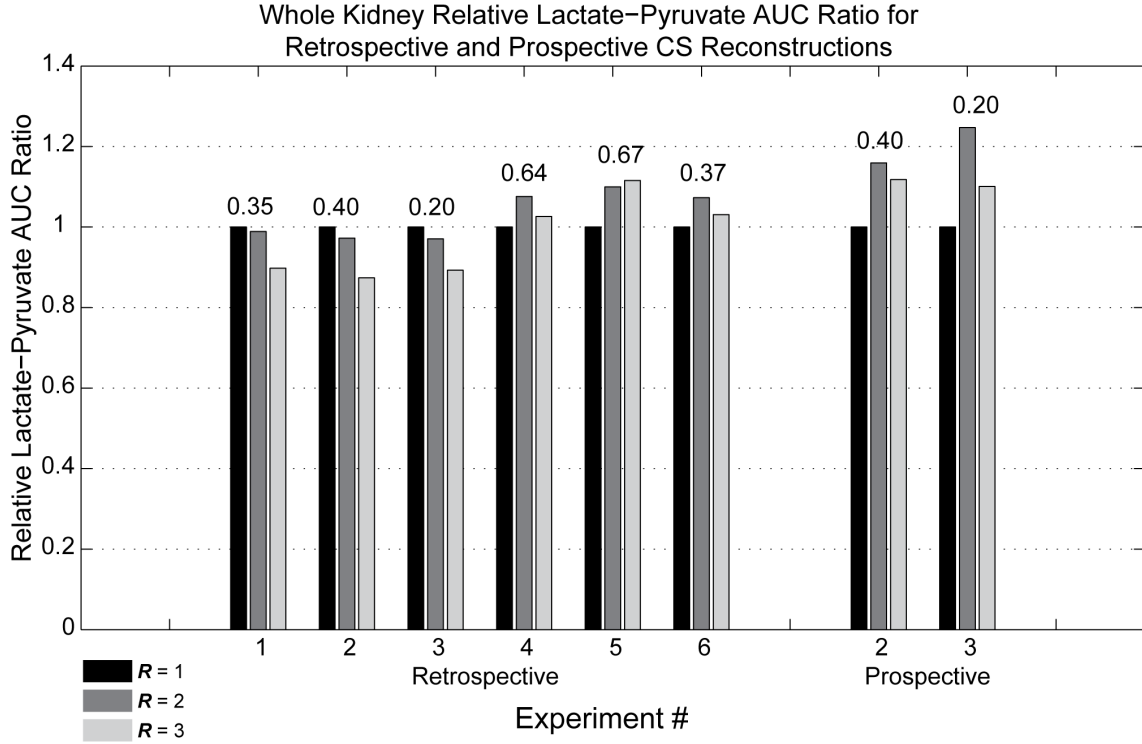


Figure 4.6: Relative whole kidney lactate-to-pyruvate area under the curve (AUC) ratio for retrospective (*left*) and prospective (*right*) CS reconstructions. Ratios were normalized by the estimate corresponding to $R = 1$ in each case. Lactate-to-pyruvate ratios for each $R = 1$ data set are displayed above each experiment group. Close agreement in parametric estimates were observed in CS images with respect to fully sampled controls. Variation between experiments is much greater than the variance within reconstructed sets.

The measured lactate-to-pyruvate estimates varied considerably between experiments, ranging from 0.20 to 0.67 across all fully sampled data. Closer inspection of the individual time courses within the selected ROI revealed that the majority of the variance in lactate-to-pyruvate ratio is due to inter-experimental variance in pyruvate signal. The coefficient of variation (σ/μ) across experiments for total pyruvate and total lactate within the ROI was 0.43 and 0.12, respectively. The large variance in pyruvate signal relative to lactate between experiments is likely due to unavoidable variations in dose delivery, heart rate and anesthesia.

The use of mean *SSIM* rather than the ubiquitous mean-squared-error (*MSE*) for analyzing retrospective CS data was motivated by the absence of noiseless ground truth images. Even with perfect signal reconstruction, *MSE* can only detect the Euclidean energy of the residual image that ultimately arises due to the difference between the two input noise vectors; therefore, is heavily dependent on SNR. This critique of *MSE* as an objective image quality metric has been noted in the literature [173, 175]. To provide context for the mean *SSIM* values reported for CS reconstructed data, the mean

SSIM was evaluated in both zero-filled low-resolution reconstructions (table 4.1). As expected, the similarity estimates following linear reconstruction were markedly lower, which was in good agreement with the apparent image degradation through either incoherent aliasing or apparent loss of resolution (figure 4.5).

A limitation of the *SSIM* analysis as implemented here is the requirement for thresholding the images on the basis of SNR. Since large regions of the image are void of signal, mean *SSIM* estimates taken across the entire volume are dominated by the lack of structural similarity within noise. We observed that once the majority of the noise background was omitted through an appropriate threshold, the *SSIM* estimates were relatively stable and were therefore insensitive to the precise threshold level used. This region-of-interest based analysis was noted as a potential application of *SSIM* in its original publication [175] and inspired its usage here.

It must be noted that due to erroneous centre frequency calibration, images from one of the experimental data sets were manually shifted to align with the anatomically derived regions-of-interest. Fortunately, this did not impede the objective of this study, which was a proof-of-concept demonstrating that the proposed sequence can be used to accelerate data acquisition. Chapter 2 addressed this limitation of frequency-selective echo-planar imaging techniques—that centre frequency mismatch can lead to mis-registration of the metabolic images with respect to the underlying anatomy. Since dual-echo trajectories give up half of their matrix size compared to bidirectional trajectories, bidirectional EPI was used in this work to demonstrate a method for encoding large matrices. An alternative approach that would have preserved the matrix size is applying blip reversal across the time-resolved acquisition [144]; however, for simplicity, blip reversal was not incorporated in this study, as it would require separate z-gradient waveforms for blip reversed acquisitions to ensure that the same data is acquired for each time-point.

The relevance of the proposed encoding scheme for alternative hyperpolarized tracers is limited by the spectral dispersion and SNR of the metabolic products. Since the method is frequency selective, only tracers admitting sufficient spectral sparsity for single resonance excitation are applicable. This is a limitation of spectral-spatial pulse design rather than the undersampling scheme utilized in the proposed method. Nevertheless, the proposed technique could be integrated into multi-echo spectral decomposition schemes in order to reduce the number of required slices for full volumetric coverage.

Independent to hardware constraints, the achievable acceleration factor is constrained by exploitable image sparsity [166]. Since compressibility can be difficult to assess a priori, the authors suggest that wherever possible, fully sampled data sets should be acquired prior to attempting accelerated data acquisitions. This may reduce or remove temporal resolution for prohibitively large FOVs, but should

provide the essential data for designing an undersampled acquisition sequence. Additionally, the maximum acceleration factor that can be achieved with the proposed sequence is ultimately limited by the gradient hardware. The pulse width of the prototype blip gradients used in this study for prospective undersampling is constrained by the duration of the EPI ramp; entailing that the largest allowable k_z displacement is sequence limited. Lengthening the EPI ramp can allow larger displacements/acceleration factors; however, this ultimately prolongs the readout and can undermine the technique as an acceleration scheme. Nevertheless, the degree of acceleration demonstrated in this work could allow time-resolved acquisition across volumes that are prohibitively large for non-static temporal encoding.

Here, the same slice-blipped EPI pattern was used to undersample each time point, thus, the temporal dimension was fully resolved. Alternatively, a different pattern could be used for each time point, which could spread incoherence through the temporal dimension and potentially allow more aggressive undersampling; however, the marginal utility of transforming the encoding problem from that of spatial to spatio-temporal is limited by the low dimensionality along the time dimension i.e. we only acquire 8-16 points. That said, such an extension warrants investigation in future studies.

The regularization parameters used in this work were selected manually. The most clear avenue for improvement would be optimization of the regularization parameters, either on the basis of the quality metrics used here, or conventional *MSE*. We observed considerable sensitivity to the choice of parameters. Values ranging over 3 orders of magnitude were explored qualitatively. Large values tended to produce blurred images with an unnatural appearance, while small values tended to produce images which were largely indistinguishable from direct Fourier transform of the zero-filled data. It is likely that optimal parameters for pyruvate images will differ from lactate images due to the difference in SNR, but the same parameters were used for both metabolites in this study.

The proposed method can be flexibly extended for multi-channel acquisitions to make use of the increased SNR afforded by surface coils. Individual coil data can be separately reconstructed and subsequently combined to yield sum-of-squares images. It has been shown that strategies which simultaneously exploit image sparsity and implicit coil based encoding can provide superior reconstructions compared to CS or parallel imaging alone [176, 177]. Auto-calibrated parallel imaging schemes such as l_1 -SPiRiT [178] that promote sparsity during image reconstruction are well suited for the proposed gradient waveform design. The added redundancy of multi-channel data may be used to stabilize the reconstruction, potentially allowing higher acceleration factors required for large FOV time-resolved hyperpolarized ^{13}C imaging applications.

4.6 Conclusion

A novel z-blip acquisition sequence for compressed sensing accelerated hyperpolarized ^{13}C 3D EPI was developed and demonstrated. Analysis of the structural similarity between retrospectively undersampled and control in vivo data showed that the signal intensity distribution in CS reconstructed $[1-^{13}\text{C}]\text{lactate}$ and $[1-^{13}\text{C}]\text{pyruvate}$ images is accurately recovered. The close agreement in lactate-to-pyruvate ratios from both retrospectively and prospectively undersampled data from rats shows that metabolic information is preserved with acceleration factors up to 3-fold with the developed method. While the encoding time for $[1-^{13}\text{C}]\text{lactate}$ and $[1-^{13}\text{C}]\text{pyruvate}$ imaging using 3D EPI across a slab thickness suitable for unilateral breast scanning (18-24 cm) should not be prohibitive in this application, extending the region of interest to include the chest wall, axilla, and/or full bilateral scanning would require additional time and would benefit from this z-blipped approach.

Chapter 5

Thesis Discussion

In August 2013, after a decade of research and development following the dissemination of dissolution DNP, the first-in-man report of HP ^{13}C MRI showed that molecular imaging with ^{13}C -labelled pyruvate was a feasible non-invasive approach for investigating lactate metabolism in solid tumours [1]. This seminal work was published just weeks prior to the beginning of this work and has served as the standard for our group and others who are seeking to translate HP ^{13}C MRI from pre-clinical studies to phase I clinical trials. Advancements in the field continue to depend critically on customization of MRI systems and pulse sequences for optimized detection of HP ^{13}C signals [179]—hardware and software innovations for which the best solutions will inevitably vary by clinical application and target anatomy. The contributions of the current thesis add to this research by providing a hardware system capable of exciting and receiving HP ^{13}C signals in breast cancer patients as well as imaging sequences appropriate for robust data acquisition and improved speed. The following section provides a brief synopsis of the main contributions, followed by a discussion on potential clinical implications and recommendation for future directions for breast cancer imaging studies with HP ^{13}C .

5.1 Summary and Conclusions

The contributions from chapter 2 include the design, prototype construction and validation of an ergonomic hardware system for HP ^{13}C breast imaging and is comprised of two separate components: a transmit coil and two identical receivers. The ^{13}C receive coils mechanically integrate with the clinically approved SentinelleTM ^1H breast MRI coil system (Invivo, Gainesville, FL, USA) to enhance patient comfort and facilitate sequential $^{13}\text{C}/^1\text{H}$ imaging. The ^{13}C transmit coil is a low profile design that allows the use of the SentinelleTM system. To our knowledge, this is the only existing hardware platform

dedicated to HP ^{13}C breast MRI.

The contribution of chapter 3 is the development and validation of a multi-nuclear workflow i.e. ^1H and ^{13}C to measure and correct for the predominant sources of geometric distortion in HP ^{13}C EPI data. An existing methodology for acquiring and using ^1H reference scans for distortion correction was adopted and extended to apply to ^{13}C imaging data obtained during the same imaging session. A dual-echo EPI sequence is used to account for ^{13}C -specific phase errors that can arise if the prescribed ^{13}C centre frequency does not match the as yet unmeasured in vivo frequency. Since the difficulties in obtaining magnetic field homogeneity in female breast are well known, and that EPI is especially sensitive to off-resonance phenomena, the ability to measure and account for geometric distortion in HP ^{13}C EPI is crucial for interpreting $[1-^{13}\text{C}]\text{pyruvate}$ and $[^{13}\text{C}]\text{lactate}$ imaging data and integrating it with the patients' existing diagnostic breast images.

Chapter 4 presents a novel pulse sequence design for reducing the time needed to encode a single volume in HP ^{13}C MRI using 3D EPI. Encoding time is an important parameter in HP MRI experiments since it defines the maximum achievable temporal resolution in time-resolved exams; for 3D EPI encoding time increases linearly with matrix size i.e. number of slices in a volume. My proposed sequence—dubbed elsewhere as a “pseudo-random raster” trajectory—performs undersampling to achieve increased matrix sizes and/or reduced encoding times for 3D EPI. By design, the undersampling artefacts in these undersampled MR images appear noise-like/incoherent and can be greatly suppressed during image reconstruction. Two- and three-fold acceleration factors were achieved in phantoms and in a pre-clinical HP $[1-^{13}\text{C}]\text{pyruvate}$ and $[^{13}\text{C}]\text{lactate}$ imaging study. Importantly, this particular sampling scheme is known to have favourable off-resonance behaviour in so far as it is no more sensitive to off-resonance artifacts than conventional 3D EPI. Extending FOV without compromising temporal resolution or slice thickness will be crucial for moving from unilateral primary breast tumour imaging to scanning the axilla, chest wall or full bilateral examinations.

5.2 Pathway to Clinical Impact

Today, our understanding of how breast cancer manifests, progresses and ultimately responds (or not) to treatment still relies on notions of disease burden i.e. tumour size, invasion, axillary node metastases, but increasingly, prognostic and predictive measures are based in biology since “[b]reast cancer is not a single disease but a series of genetically distinct entities.” [180] This is apparent in the sorts of clinical questions that are being asked, as today's researches are not concerned with the outcomes of treatments for “breast cancer” *per se* but, rather, for patients with “*HER2/neu*-overexpressing breast

cancer” or “triple-negative breast cancer,” for example. These biological distinctions are eminently visible in routine medical oncology practice, where the decision making guidelines for offering systemic therapies depend on the assessment of risk factors obtained from pathology and/or genetic screening.

In contrast, the techniques and guidelines for managing local disease i.e. surgery and radio therapy, which have improved patient outcomes as compared to before they became the standard-of-care, were developed in the mid 1970s to early 1980s—meaning they were conceived in a time when very little of what is known about breast cancer biology was in place. Looking ahead, rather than engaging in serial episodes of care by doctors of different specialties, clinicians who aim for the ideals of integrated multidisciplinary care are seeking to enhance patient outcomes as well as minimize the morbidities associated with the totality of therapeutics used in patients with breast cancer. This is an essential component of realizing personalized medicine in breast cancer so that the right treatments at the right times are offered to the right patients, and at the centre of that bullseye lies biology; this means finding more specific targets, more potent drugs, fewer harmful side effects, as well as earlier and less invasive methods of diagnosis and response monitoring.

5.2.1 Ongoing Clinical Trial in LABC Patients

Our group is actively recruiting a total of 13 participants for a clinical trial (ClinicalTrials.gov Identifier: NCT03121989) to evaluate the feasibility of HP [1-¹³C]pyruvate imaging in breast cancer patients receiving neoadjuvant chemotherapy (NAC). The overall study design is a pilot prospective, single-institution study in volunteer participants between 18 and 80 years of age with pathology- and imaging-confirmed breast cancer requiring NAC. Imaging confirmation involves highly suspicious masses on mammography and/or ultrasound and pathology confirmation involves image-guided core biopsy of the suspicious mass.

Each participant will be receive one HP ¹³C MRI scan and this visit will occur separately from the patient’s clinical dynamic contrast enhanced (DCE) MRI. The HP ¹³C MRI exam will be scheduled before the initiation of NAC, and within 5 days prior to, or following, the clinical MRI scan. The secondary objectives of the study include: determining the diagnostic accuracy of time-resolved, 3D HP [1-¹³C]pyruvate MRI for differentiating breast cancer from normal breast parenchyma; spatially correlating lactate seen on HP ¹³C MR with conventional anatomical MR images; and, ultimately, acquiring statistical information to help design, fund, and power future clinical studies with larger patient populations.

5.2.2 Potential Role in Anti-Cancer Drug Trials

If a newly developed experimental therapy was shown to provide complete pathological response in less than 5% of patients, then the question of determining whether or not to proceed to more comprehensive and resource intensive studies is rife with value judgements; if, however, there existed a diagnostic test that could identify the minority of breast cancers who have a high likelihood of responding to said treatment, then suddenly, the experimental therapy, while no different, is considered a home-run. Lactate imaging may some day be used as such a test, by identifying breast cancers with certain in vivo metabolic phenotypes.

There is growing evidence that differences in metabolic changes exist between the various molecular subtypes of breast cancer [181]. For example, one study found that as compared to ER-positive tumours, triple-negative breast cancers had an elevated level of almost all glycolytic intermediates along with strong lactate accumulation [182]. Triple-negative breast cancer is of particular interest for pharmaceutical research since thus far, these tumours have no effective targets for treatment. Therefore, if targets are devised that will undercut the metabolic dependencies of certain breast cancers, then lactate imaging could be used as confirmation that the drug has hit its target.

5.2.3 Potential Applications in Breast Cancer Beyond LABC

It is difficult to speculate on the value of HP [1-¹³C]pyruvate for measuring lactate metabolism in breast cancers that are not considered locally advanced, the reason being that it depends entirely on what the elusive *actual* SNR of the experiment may be. Smaller lesions obviously occupy smaller volumes so, other things equal, it would be expected that relatively fewer HP ¹³C-labelled molecules would be present for imaging, which, in the limiting case, falls below the threshold of detection. In MR imaging, the limit of detection ultimately depends on the sheer number of contributing nuclei; however, it is also coupled to the imaging resolution—a parameter that specifies data acquisition. Lowering imaging resolution i.e. making the imaging voxels larger, will improve the detection limit, but this comes at the cost of spatial accuracy. At present, there have been no reports of human imaging studies with HP [1-¹³C]pyruvate where the voxel was less than 1 cc. Therefore, time-resolved, 3D imaging with [1-¹³C]pyruvate will not likely play a role in diagnosing or studying early stage breast cancer.

That said, it may very well be possible that the value of tumour lactate as a biomarker can be leveraged without the need for performing 3D multi-voxel localization of the signal. Instead, time-resolved spectroscopic readouts could be used to measure lactate in slices through the breast, allowing comparison of signals from slices containing tumour with slices in the surrounding normal tissue. Additionally,

bilateral spectroscopy could be incorporated, with the task of left/right localization performed by the limited receptive fields of each receiver coil. In exchange for the ability to spatially discriminate among signals, non-localized approaches provide the maximum possible SNR and this may make it more suitable for smaller lesions.

5.3 Limitations and Future Directions

Before addressing some limitations that are specific to the work put forward in this thesis, it is important to note how its scope covers narrow range of possible strategies for HP ^{13}C image formation; namely, spectral-spatial RF excitation with “single shot” echo-planar readouts. While there are indeed many areas for improvement, as will be shown, some of the disadvantages under this regime are non-issues for alternative methodologies. For example, standard chemical shift imaging effectively encodes a complete MR spectrum within each voxel and, thus, is insensitive to many off-resonance based artifacts. The pros and cons of the various approaches have been reviewed elsewhere. That said, there appears to be a growing consensus for performing HP $[1-^{13}\text{C}]$ pyruvate MRI with strategies that are similar to those described in this thesis.

The fact that the ^{13}C hardware presented in Chapter 2 was designed to take advantage of an existing platform—the SentinelleTM ^1H breast MRI coil system—implies another major limitation of scope that has also served to heavily constrain the final product. Many diverse breast MRI solutions are available on the free market; either a different platform could have been chosen or the support itself could have been included as a part of the design. A minor drawback of the current system is the requirement for patients to enter the magnet bore head first, whereas some systems allow patients to be placed in feet first; a seemingly small difference that might help with feelings of anxiety or claustrophobia in the scanner. Furthermore, the hardware was designed with the combined aims of safety, ergonomics and robustness i.e. stability and durability. This means that simplicity was favoured over complexity and noise performance was not held as the main priority; still, the current system will serve as a useful benchmark for measuring any real gains sought through subsequent hardware improvements, such as increasing the number of receive channels.

5.3.1 Pyruvate-Hydrate Contamination

It can be argued that from a data acquisition perspective, the fundamental technical challenge of HP ^{13}C imaging with $[1-^{13}\text{C}]$ pyruvate is the need for separating out substrate signals from those of downstream

metabolites i.e. $[1-^{13}\text{C}]\text{lactate}$, ^{13}C -bicarbonate etc and doing so quickly. Spectral-spatial RF (ssRF) pulses address this by changing the problem from being the separation of multiple excited signals to that of selective excitation; imaging becomes massively simplified if only a single resonance is present. The ssRF pulses used in this thesis were designed to excite a gaussian-shaped frequency band with a full-width-at-half-max of approximately 70 Hz—metabolites more than $\pm 35\text{Hz}$ off-resonance will be excited less than half as much as on resonance signals.

In cases of severe B_0 inhomogeneity, this means that some areas within the region-of-interest might be excited more than others, which leads to non-physiological signal modulation that may hamper the task of data analysis and interpretation. Furthermore, at physiological pH, pyruvate is found in $\sim 20:1$ equilibrium with its hydrated form, pyruvate-hydrate [183], and so for HP ^{13}C -labelled experiments, this creates an additional spectral peak only $\sim 120\text{ Hz}$ away from $[1-^{13}\text{C}]\text{lactate}$. For certain amounts of off-resonance, partial excitation of $[1-^{13}\text{C}]\text{pyruvate-hydrate}$ can cause contamination in $[1-^{13}\text{C}]\text{lactate}$ images (figure 5.1b). Such contamination cannot be removed through post-processing methods and, therefore, must be either avoided or taken into account during the data acquisition stage.

A possible strategy for the latter is to acquire the same volume multiple times while incrementing the echo-time by a specific amount, which is a well-known method for decoding multiple signals given a priori knowledge of the underlying spectrum. This was the subject of a recently published study from our group, which used a modified version of the sequence described in Chapter 3 and the data was reconstructed according to the scheme in figure 5.1d. In the first stage of the reconstruction, a phase map is generated using an iterative least squares algorithm in a pixel-independent manner i.e. without spatial regularization. The phase map is then used to remove background phase accrual from the acquired images and then each is Fourier transformed back into k-space. Finally, spectral decomposition is performed on the processed k-space data while accounting for phase evolution during the readout, which, as revealed upon inverse Fourier transform, corrects for chemical shift artefacts, such as misregistration in the blip direction for EPI data.

One of the limitations of the study was that complete signal separation failed when the temporal resolution used during the acquisition was insufficient to capture the underlying signal dynamics; this occurred because the model used to reconstruct separated images from multi-echo data assumed that the only difference between the inputs was phase accrual. Although a fundamental limitation of multi-echo imaging, in practice, if sampling is much faster than the underlying process, then this assumption holds. It turns out that the temporal resolution between echoes could have been significantly higher; however, this would have required potentially drastic overhauls to a pre-existing and well validated imaging sequence.

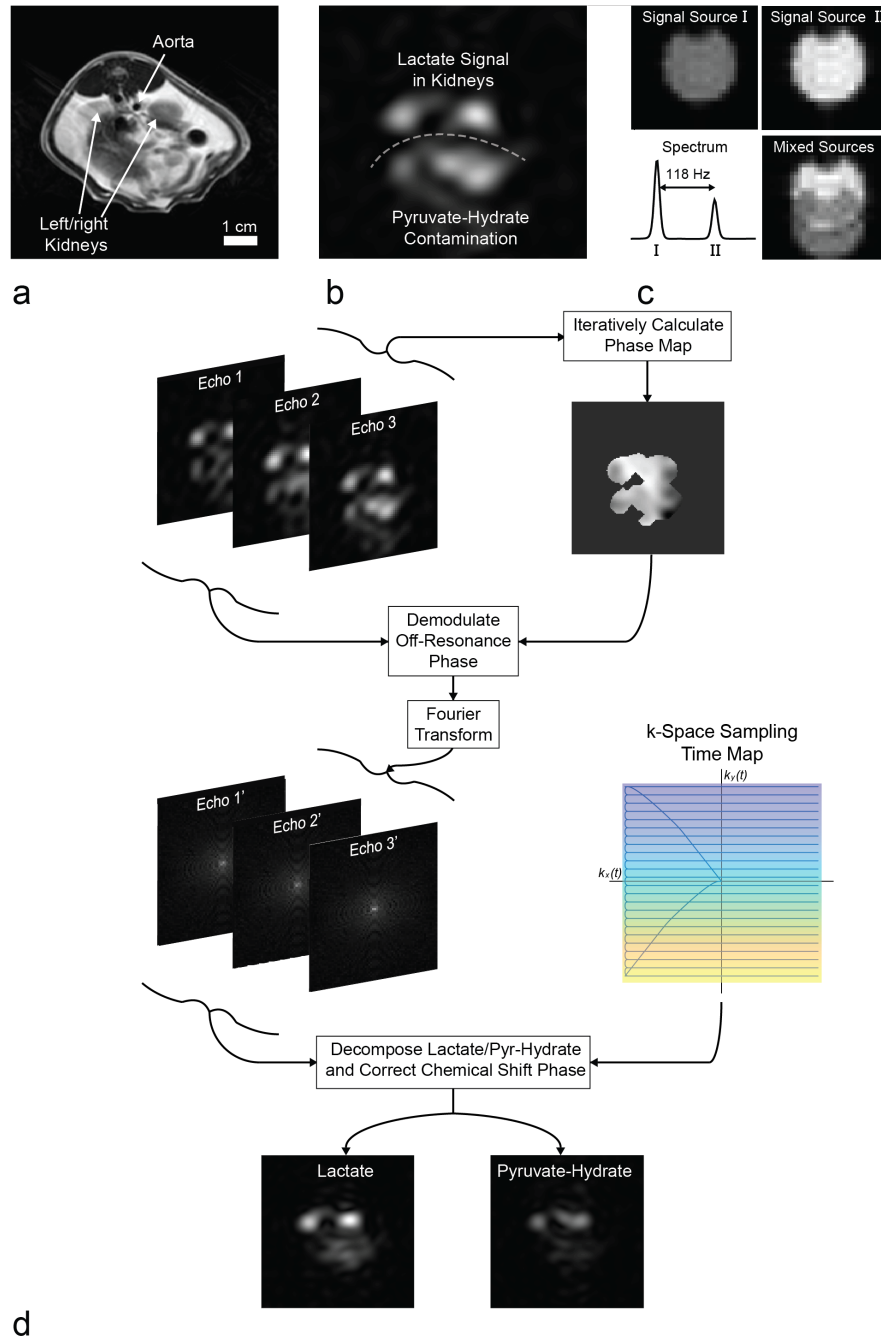


Figure 5.1: Pyruvate-Hydrate Contamination with Spectral-spatial RF and EPI. Axial T_2 weighted image of rat (a) and HP $[1-^{13}\text{C}]$ lactate images with contamination from $[1-^{13}\text{C}]$ pyruvate-hydrate (a). When multiple signal sources are excited and spatially encoded using EPI, a mixed image is produced with a shift between image sources proportional to the difference in frequency (c). Generalized k-space decomposition with chemical shift correction algorithm for separating lactate and pyruvate-hydrate signals (d). In the first step, an off-resonance phase map is iteratively generated from the input data and then used to correct for background i.e. non-chemical shift based, phase accrual in image-space. In the second step, lactate and pyruvate-hydrate images are non-iteratively decomposed, using the k-space sampling time map as input to correct for chemical shift artifact i.e. shifting, in the final images.

In future work, the multi-echo approach should be considered in breast imaging because of its increased robustness against off-resonance. Wider spectral excitation bands means less chance of *under-exciting* $[1-^{13}\text{C}]\text{lactate}$. The possibility of $[1-^{13}\text{C}]\text{pyruvate-hydrate}$ contamination is greatly reduced and this will be essential for interpreting the first sets of patient images. Acquiring the lactate volume multiple times inevitably increases the encoding time and thus constrain the size of volumes that may be measured dynamically. That said, it might be possible to forgo exciting the injected substrate $[1-^{13}\text{C}]\text{pyruvate}$ in a separate imaging interleave altogether in lieu of using the $[1-^{13}\text{C}]\text{pyruvate-hydrate}$ as a means to normalize the $[1-^{13}\text{C}]\text{lactate}$ images, which would then shorten and simplify data acquisition.

5.3.2 Signal Loss During Imaging Readouts

When spectral-spatial RF (ssRF) excitation is used in conjunction with rapid spatial encoding schemes like EPI, the durations of these types of RF pulses can be on the order of tens of milliseconds; this increases the minimum achievable echo-time (TE) in comparison to other HP ^{13}C imaging approaches that utilize non-selective RF excitation. Longer TE entails heavier T_2^* weighting—irreversible loss of signal by dephasing—and this can lead to degradation of SNR. For a fixed RF pulse length, the minimum TE for ssRF with 3D EPI can be reduced considerably with partial sampling along the blipped direction in k-space.

To explore SNR performance of asymmetric vs symmetric k-space sampling in EPI, an experimental paradigm was used, which toggles between two different spatial encoding strategies during a dynamic HP $[1-^{13}\text{C}]\text{pyruvate}$ acquisition. By interleaving the two readouts within a single injection, as opposed to comparing than between successive experiments, potentially confounding variables such as polarization, perfusion, injection timing and metabolism can be arguably eliminated. The following pre-clinical results compare asymmetric vs symmetric EPI obtained with the HP ^{13}C breast imaging platform.

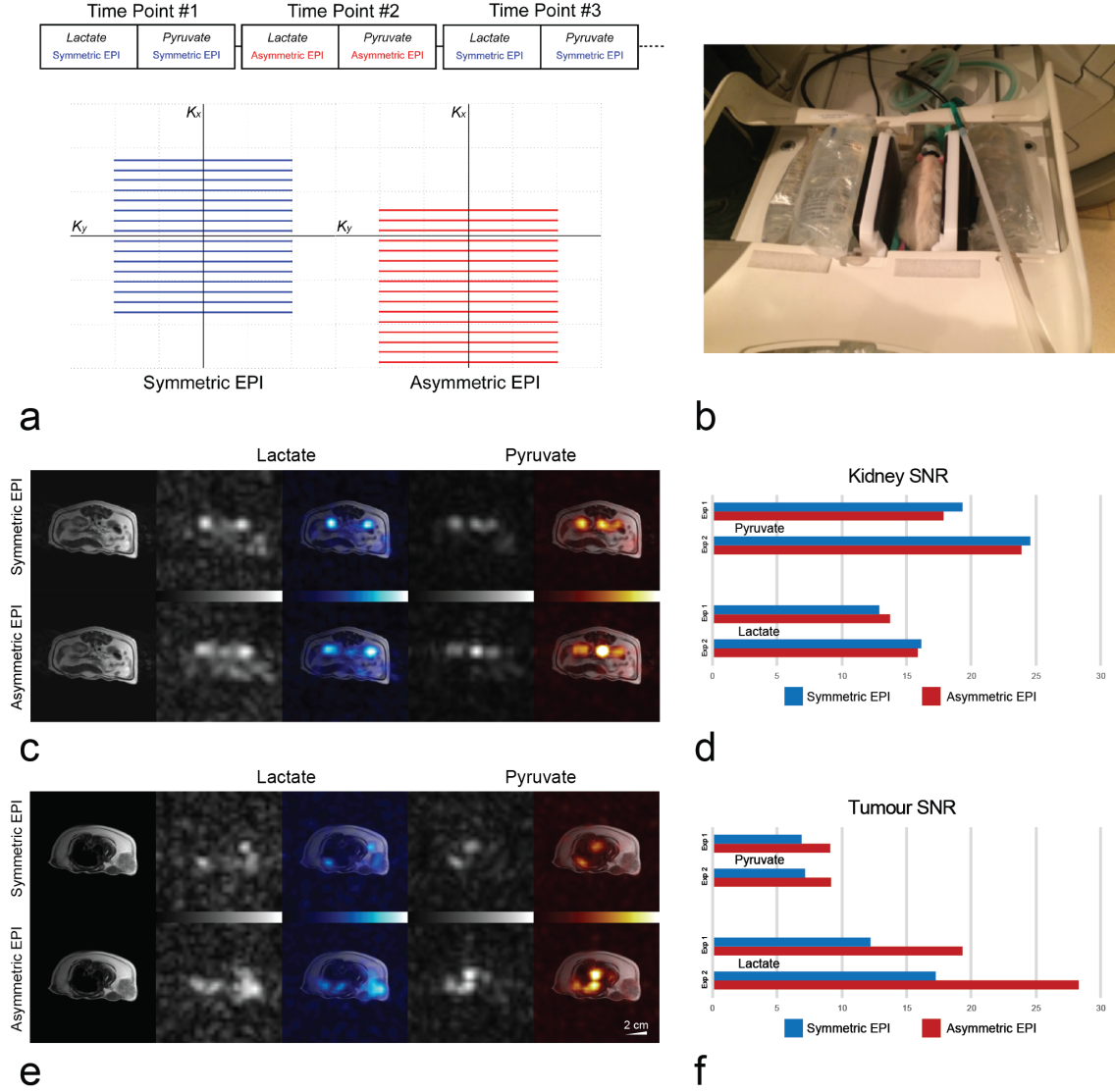


Figure 5.2: Comparison of EPI k-Space Sampling in $HP^{13}C$ MRI. Schematic of interleaved experimental protocol (a). $HP^{13}C$ breast imaging hardware with anesthetized rat (b). Time-integrated images and overlays for slice containing the tumour. Colourbars are in units of SNR.

Symmetric EPI gradient waveforms were designed for $96 \times 12 \text{ cm}^2$ field-of-view at 7.5 mm in-plane resolution. For asymmetric EPI, the dephasing and rephasing trapezoids for the blipped gradient were redesigned to shift the sampling window in k-space by the equivalent of 5 lines, reducing minimum TE by $\sim 7.6 \text{ ms}$. Phase encoding was used for covering a 24 cm slab with 10 mm slice thickness. A tailored 18.8 ms ssRF pulse¹³ was used for excitation. Waveforms were incorporated into a 3D gradient-echo sequence and were toggled according to figure 5.2a. Odd and even time points were encoded with symmetric and asymmetric EPI, respectively. Imaging was performed on a GE MR750 3T MR scanner (GE Healthcare, Waukesha, WI) using the previously described 2-channel HP ^{13}C breast imaging platform (Chapter 2). A sprague-Dawley rat with a spontaneous endogenous tumour was handled in accordance with our institutional animal care and use committee (figure 5.2b).

Time-integrated $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate overlays are displayed in figure 5.2c. In both experiments, the SNR within the kidneys was apparently similar between the two EPI encoding methods (*data not shown*); however, there was a dramatic difference within the tumour, with elevated SNR in the volumes encoded by asymmetric EPI. A region-of-interest (ROI) analysis for SNR was performed in the tumour is illustrated in figure 5.2d.

Asymmetric EPI is a viable method for shortening minimum TE in order to mitigate T_2^* related SNR reductions in the ssRF regime and for the case studied, the SNR advantage of asymmetric-vs-symmetric EPI was up to 65%. A potential drawback of asymmetric-vs-symmetric EPI sampling is ringing artifacts, which were not observed here. On the basis of this study, our group is employing asymmetric EPI in our ongoing human trials.

5.3.3 Matrix Size Penalty of Dual-Echo EPI

The dual-echo methodology for EPI (Chapter 3) was shown to have decisive advantages over a single-echo/“flyback” approach. As compared to bidirectional acquisition, however, the fact that the dual-echo necessarily sacrifices 50% of spatial encoding efficiency cannot be ignored when weighing the merits of either method for a given application; recall that for a fixed readout duration, bidirectional EPI can produce images that have twice the resolution *or* FOV as dual-echo. Assuming equivalent SNR—valid under ideal conditions, since $\text{SNR} \propto \sqrt{t}$ —the clear benefit of dual-echo over bidirectional EPI lies in its ability to encode phase information, which can add robustness against geometric distortion from off-resonance phenomena. For cases where FOV or resolution are not limiting i.e. adequate volumes can be encoded without impeding temporal resolution, then the robustness afforded by dual-echo EPI

makes it the clear choice; therefore, in cases where such constraints are an issue, then facing some trade-offs becomes unavoidable.

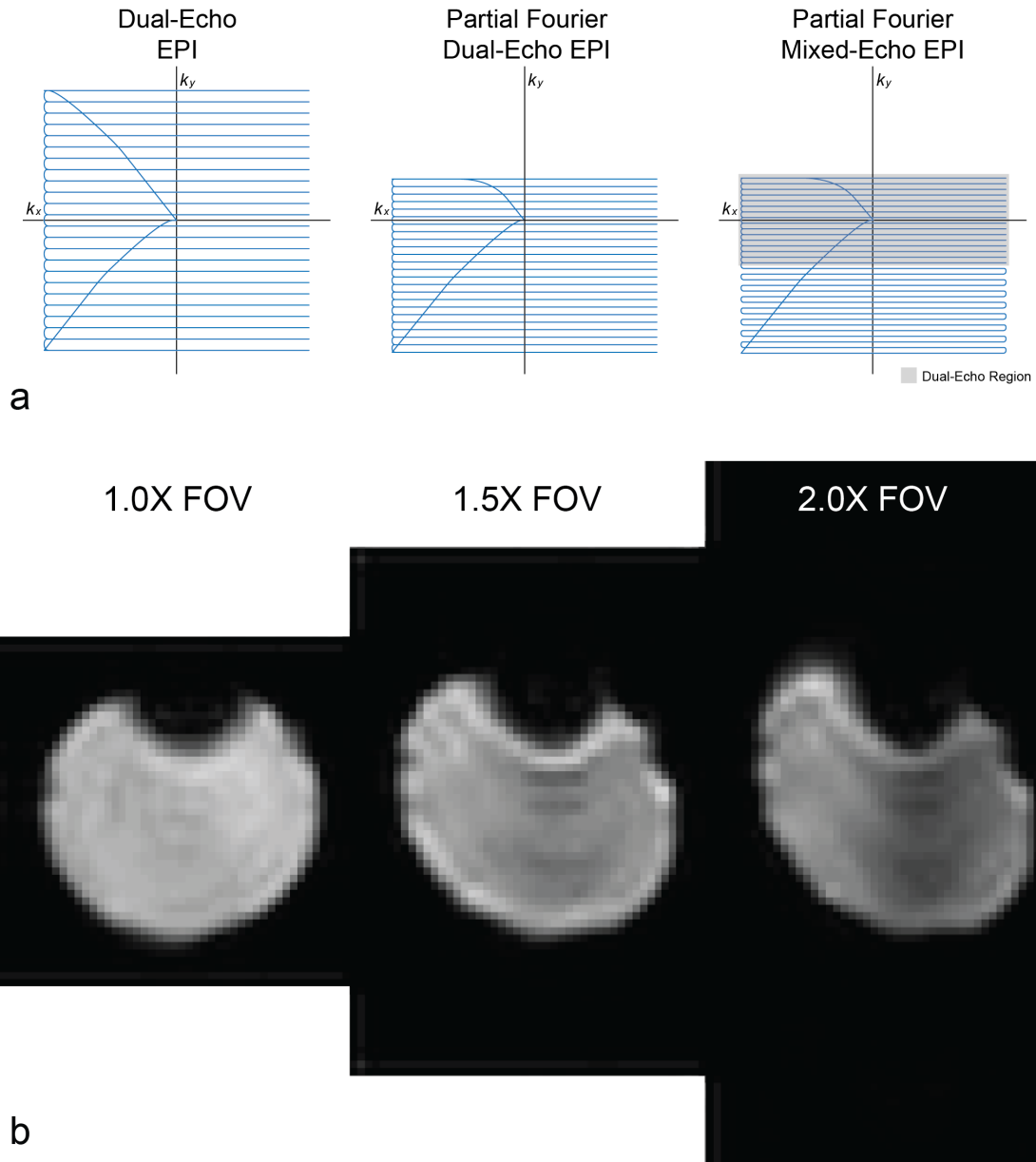


Figure 5.3: Partial Fourier Mixed-Echo EPI Sequence (a). k-Space trajectories for a dual-echo EPI readout (*left*), partial Fourier dual-echo EPI readout (*centre*) and the proposed partial Fourier mixed-echo EPI readout ((a)). All three trajectories include the same total number of measurements and all complete them in the same amount of time. ^1H phantom results for each of the above sampling trajectories (b). The partial Fourier dual-echo EPI trajectory offers a 50% increase in FOV while the increase afforded by the proposed mixed-echo trajectory is 100%. The increased sensitivity to off-resonance geometric artefacts can also be appreciated and is a trade-off offered by this approach.

Partial Fourier (PF) encoding is one possible strategy that could be used for improving the encoding efficiency of dual-echo EPI (figure 5.3a). Since the spatial-frequency representation of natural objects is highly redundant i.e. k-space is data approximately symmetric, images can be formed just over 50% of the measurements in “fully sampled” data. The reason that undersampling in k-space must be less than 2-fold is because in reality, complete symmetry of k-space data is broken whenever the phase in different voxels is not the same. This proximate cause of asymmetry in k-space, however, tends to vary slowly across images, and thus, can be estimated from just a low-resolution subset of the total data. Various PF reconstruction algorithms exist and commonly seek to synthesize unmeasured data from a partial sampling of k-space data that includes a symmetrically sampled region at the centre of k-space.

In order to enhance encoding efficiency further, a “mixed-echo” sampling strategy is proposed, in which a symmetric region of low-resolution k-space measurements is acquired twice (dual-echo region figure 5.3a), and the remaining measurements are acquired along a bidirectional trajectory. Preliminary ^1H data comparing the 3 encoding schemes is shown in figure 5.3b. It is important to note that the acquisition matrix and thus acquisition time for each image is identical. The difference between the data sets is the k-space density of the acquisition matrix and increased final matrix size and FOV.

Since the bandwidth-per-pixel is effectively reduced, the price of increasing the encoding efficiency in this way is a proportional increase in sensitivity to off-resonance artifacts. Also, EPI data acquired with bidirectional sampling must be corrected for odd/even-echo asymmetries that can give rise to ghosting artefacts; however, since only the high-resolution region of k-space is sampled this way, the appearance of ghosting is greatly diminished without correction. Further studies are warranted to approach optimal sampling strategies for HP ^{13}C .

5.4 Conclusions

With a growing number of centres from across the world both granted and currently seeking regulatory approval for conducting studies with injections of DNP media in humans, the ^{13}C research community has gained considerable momentum and is nearing the end of its early days. There will soon come a point of critical mass for which sufficient data generation will allow researchers to ask deeper questions that go beyond flat, albeit essential, studies concerned with demonstrating safety and feasibility. In order to pursue clinical ends, robust methodology is paramount; generating reproducible, biologically meaningful measurements proceeds all else. The technologies developed in this thesis aim to accomplish this in the context of breast cancer and can be used as a platform for performing HP ^{13}C MRI exams in breast cancer patients in a manner that is both robust and ergonomic.

It is important to note, however, that this work could also be extended and applied to other clinical imaging studies with HP [1-¹³C]pyruvate. Namely, the dual-echo EPI sequence and imaging workflow (Chapter 3) is applicable to other regions of the body, as the encoded phase information can provide valuable context for interpreting in vivo images. Also, the low-profile design of the ¹³C transmit coil (Chapter 2) can be taken advantage of when space within the scanner bore becomes limiting and may serve as a general purpose ¹³C RF transmitter in other patient studies. Innovations in ¹³C RF hardware, such as large flexible arrays incorporated into wearable vests, would allow HP [1-¹³C]pyruvate studies that require extensive anatomical coverage i.e. whole torso scanning, and “pseudo-random rastered” 3D EPI (Chapter 4) could be used to reduce the attendant increase in time spent encoding larger volumes, thus, preserving temporal resolution. Attributable perhaps to a consistent commitment to robustness and simplicity, the technologies developed in this thesis have a strong likelihood of being adopted in other patient studies and may prove useful in the wider advancement the clinical translation of HP [1-¹³C]pyruvate MRI.

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