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Ferritin–EGFP Chimera as an Endogenous Dual-Reporter for Both Fluorescence and Magnetic Resonance Imaging in Human Glioma U251 Cells

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Key Words: ferritin, EGFP, dual-reporter human glioma U251 cells, tetracycline-regulated system, MRI

Abbreviations: Enhanced green fluorescent protein (EGFP), magnetic resonance imaging (MRI), Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide dimethyl sulfoxide (MTT), ferric citrate ammonium (FAC), inductively coupled plasma mass spectroscopy (ICP-MS), tetracycline repressor protein (TetR), tetracycline (Tet)

A unique hybrid protein ferritin–enhanced green fluorescent protein (EGFP) was built to serve as an endogenous dual reporter for both fluorescence and magnetic resonance imaging (MRI). It consists of a human ferritin heavy chain (an iron-storage protein) at the N terminus, a flexible polypeptide in the middle as a linker, and an EGFP at the C terminus. Through antibiotic screening, we established stable human glioma U251 cell strains that expressed ferritin–EGFP under the control of tetracycline. These cells emitted bright green fluorescence and were easily detected by a fluorescent microscope. Ferritin–EGFP overexpression proved effective in triggering obvious intracellular iron accumulation as shown by Prussian blue staining and by MRI. Further, we found that ferritin–EGFP overexpression did not cause proliferation differences between experimental and control group cells when ferritin–EGFP was expressed for \( \frac{96}{H11021} \) hours. Application of this novel ferritin–EGFP chimera has a promising future for combined optical and MRI approaches to study in vivo imaging at a cellular level.

INTRODUCTION

Glioma is among one of the most malignant tumors and is characterized by high levels of mortality and recurrence (1). Further, glioma cells show infiltrative growth and have no obvious boundaries with surrounding normal tissues. Precise noninvasive imaging is of great importance in tumor localization, metastasis detection, and subsequent therapy. Fluorescence imaging can provide noninvasive real-time dynamic observation of tumors. However, fluorescence has poor penetration capability, limiting its usage in deep-seated tumors. In contrast, magnetic resonance imaging (MRI) is not affected by the depth of target tissues and has high spatial resolution. However, the sensitivity of MRI is not high enough for performing cellular or molecular imaging. Therefore, to solve this problem, MRI contrast agents were invented using substances such as iron or gadolinium (2, 3). For example, iron oxide nanoparticles are often used in labeling cells (4–6). However, with the division of cells, the exogenous reporter is diluted, leading to a loss of signal change over time. Subsequently, several MRI reporter genes have been introduced into cells by either plasmids or slow virus transfection to serve as endogenous reporters (7, 8).

Our focus will be on the use of ferritin as part of a reporter gene. Ferritin is an extensively studied iron-storage protein in the human body and plays an important role in maintaining the balance of iron metabolism (9, 10). Ferritin is composed of 2 types of subunits, both H and L subunits, namely, the heavy and the light chains. H subunits are the core subunits of iron storage in ferritin and can work as preferable endogenous MRI reporters (11, 12). In recent years, dual reporters that combine the advantages of fluorescence imaging and MRI have gradually become hotspots for noninvasive imaging studies. A dual reporter is usually composed of a fluorescent protein that is used for fluorescence imaging and a ferritin protein that is used for MRI (13–16). However, transgenic ferritin has usually been expressed separately from fluorescent protein and thus is not directly observed by fluorescence detection. There is also some controversy about the effects of ferritin overexpression on cells and in the body (11, 17–20). The reason behind these contradictory facts may be the various ferritin expression levels and different cell types that have been applied in those studies.

Here, we propose an improved dual-reporter ferritin–enhanced green fluorescent protein (EGFP) chimera, with a human
ferritin heavy chain at the N terminus, an EGFP at the C terminus, and a special polypeptide as a linker in the middle that is expected to improve the fluorescence intensity and stability of EGFP (21). By building stable human glioma U251 cell strains that express ferritin–EGFP under the control of tetracycline (Figure 1), we could realize cellular imaging with both fluorescence imaging and MRI techniques.

**METHODOLOGY**

**Gene Constructs**

The gene of human ferritin heavy chain (NCBI Reference Sequence: NM_002032.2) with a polypeptide (LEGGGGSGGGTGGSAGGGA) at the C-terminus was synthesized with a Hind III restriction site at the 5'-terminus and a Kas I restriction site at the 3'-terminus (Biosune, Shanghai, China). The stop codon of the ferritin gene was deleted. The EGFP gene was generated by polymerase chain reaction amplification using pcDNA3.1-3'-EGFP as templates, with the Kas I restriction site at the 5'-terminus and a BamH I restriction site at the 3'-terminus. pCEP4-ferritin–EGFP was built by insertion of the EGFP gene into pCEP4-ferritin. The above ferritin–EGFP gene was ligated into a modified pcDNA4/TO-neo vector (zeocin-resistance gene of pcDNA4/TO was replaced by neomycin-resistance gene) and inserted with the ferritin heavy chain–EGFP fusion gene ferritin–EGFP at multiple cloning sites-transfected U251–TetR polyclonal cells and obtained U251–TetR–ferritin–EGFP polyclonal cells, which were further separated to create U251–TetR–ferritin–EGFP monoclonal cells that expressed tetracycline-inducible ferritin–EGFP at a uniformly high level.

**Establishment of U251–TetR–Ferritin–EGFP Cell Strains**

All transgene constructs were transformed into Escherichia coli strain Top10 (Self-made) and transformants with ampicillin resistance. After amplification in E. coli, plasmids were extracted by an Axygen mini-preparation kit (Axygen, Hangzhou, China), pCEP4-ferritin–EGFP plasmids were transfected by a self-made polyetherimide-based reagent into HeLa cells to check the ferritin–EGFP expression. Then, pcDNA6/TR vectors (Thermo Fisher Scientific, Shanghai, China) were transfected into human glioma U251 cells (Cell Resource Center of Shanghai Academy of Sciences, Chinese Academy of Sciences, Shanghai, China). Stable U251 cells that expressed tetracycline repressor protein (TetR) were selected using blasticidin (6 µg/mL) (Thermo Fisher Scientific, Shanghai, China) and named U251–TetR cells, which then served as host cells for pcDNA4/TO-neo."
ferritin–EGFP plasmids transfection. Several U251–TetR–ferritin–EGFP cell strains were generated by G418 screening (1 mg/mL) (Thermo Fisher Scientific, Shanghai, China) and then analyzed by Western blot and fluorescence imaging. Finally, a successful U251–TetR–Ferritin–EGFP cell strain was created that showed stable high-level expression of Ferritin–EGFP under tetracycline regulation. The cell culture medium was Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Shanghai, China).

Western Blot Analysis
Cells were grown on a 6-well cell culture plate (Corning, Shanghai, China) with the same seeding density. Ferritin–EGFP expression was started by adding tetracycline (2 μg/mL) (Aladdin, Shanghai, China) in the culture medium. Then cells were harvested and lysed on ice in a lysis buffer (50 mM Tris HCl, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 10% (v/v) glycerol, 1% (v/v) Triton X-100, and 1 mM phenylmethylsulfonyl fluoride [pH 7.4]) (Aladdin, Shanghai, China). Samples were washed using a 2× protein loading buffer (Self-made) for 10 minutes at 100°C, and then separated on 10% sodium dodecyl sulfate polyacrylamide gels (self-made) and later transferred to m polyvinylidene fluoride membranes (Millipore, Shanghai, China) for Western blot analysis. Membranes were blocked in the blocking buffer (5% skimmed milk powder in TBST buffer: 0.8% (w/v) NaCl, 0.02% (w/v) KCl, 0.3% Tris base, and 0.05% (v/v) Tween-20, (pH 7.4), for 2 hours at room temperature. Further, membranes were incubated with primary antibodies (CWBIO, Beijing, China) for 1 hour at room temperature, and then with secondary antibodies (CWBIO, Beijing, China) for 1 hour at room temperature. The protein was eventually imaged with Kodak films (Kodak, State of New York, United States) using ECL detection reagent (CWBIO, Beijing, China). Primary antibodies included anti-EGFP polyclonal rabbit antibody (Zen BioScience) and anti-β-actin monoclonal mouse antibody (CWBIO, Beijing, China). Secondary antibodies were horseradish peroxidase-conjugated Goat Anti-Rabbit antibody (CWBIO, Beijing, China) and horseradish peroxidase-conjugated Goat Anti-Mouse antibody (CWBIO, Beijing, China).

Fluorescence Imaging
Cells were cultured in a 6-well cell culture plate in DMEM (10% FBS). Then, DMEM was discarded and cells were washed by phosphate-buffered saline (PBS) (Self-made) 3 times. All fluorescence imaging was performed in the 6-well cell culture plate using a Leica fluorescent microscope (DM4000B, Leica, Solms, Germany). The green fluorescence of EGFP was excited by a blue laser.

Cell Proliferation Assay
Cells were seeded at the same density and cultured in 96-well cell culture plates (Corning, Shanghai, China); 2 groups of cells were tested in this experiment. The first group of cells (U251–TetR–ferritin–EGFP glioma cells) was used to check the effect of ferritin–EGFP expression on cell proliferation. Ferritin–EGFP expression was either started or turned off by adding or withdrawing tetracycline (2 μg/mL) into the cell culture medium. The second group of cells (normal U251 glioma cells) was used to check the effect of tetracycline on cell growth. The experimental group was labeled as tetracycline+ (“Tet+”) with 2 μg/mL tetracycline added to the cell culture medium, and the control group was labeled as “Tet−” without any tetracycline in the medium.

Cells were detected every 24 hour as follows (n = 6). Standard 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide dimethyl sulfoxide (MTT) detections were conducted to check cells’ proliferation, which mainly included the following procedures. First, 20 μL of 0.5% MTT (in PBS) (Aladdin, Shanghai, China) was added to each well. After 4-hour incubation in 37°C, the suspension medium was discarded. Then, 150 μL of DMSO (Aladdin, Shanghai, China) was added to each well. Finally, the absorbance of each well was measured using light of wavelength of 490 nm. Significant differences were examined using a t-test from SPSS 16.0 software. Differences were considered significant when P < .05.

Prussian Blue Staining
Cells were iron-loaded by growing them in supplemented medium that contained 2 mM ferric citrate ammonium (FAC) (Macklin, Shanghai, China) for 48 hours. Then, the cells were washed using PBS 3 times and were fixed in 4% paraformaldehyde (Macklin, Shanghai, China) for 15 minutes. Cells were washed using deionized water several times. Iron staining was performed using a Prussian blue staining assay kit (Solarbio, Shanghai, China) following standard procedures. Cells were stained for 30 minutes by potassium ferrocyanide in hydrochloric acid and then washed with deionized water several times and counterstained with Fast Red for 10 minutes. Digital pictures were taken using a Leica fluorescent microscope (DM4000B, Leica, Solms, Germany) under bright field conditions.

Iron Measurements by Inductively Coupled Plasma Mass Spectroscopy
Cellular iron content was detected by inductively coupled plasma mass spectroscopy (ICP-MS) (i CAP Q, Thermo Fisher Scientific, Shanghai, China). First, a cell pellet was dissolved in 3 mL HNO3/H2O2 (4:1) solution. Then, the clear sample solution was tested by ICP-MS following standard procedures.

MRI Experiments
For phantom preparation, cells (6 × 10⁶/group) were uniformly suspended in 0.1 cc of 1% agarose in the middle of long glass tubes. Except for the cell layer, both the upper and lower regions of the tubes were filled with 1% agarose gel. Three tubes were prepared in total; the first tube was filled with glioma cells with ferritin–EGFP expression (labeled as “+”), the second tube was filled with glioma cells with no ferritin–EGFP expression (labeled as “−”), and the last one was filled with pure 1% agarose (labeled as “0”). Note that all cells were incubated with 2 mM FAC for 48 hours. The 3 tubes were evenly inserted into 2% agarose in a disk-like 7 × 10-cm (height × diameter) container. Similarly, different concentrations of FAC tubes were prepared in 1% agarose in the long glass tubes. A multiecho, gradient echo technique was applied to estimate R2* (1/T2*), as iron content can be estimated from the change in R2* (∆R2* = R2’ − R2) between the gel with iron and that without iron. Data were collected on a 3 T scanner (MAGNETOM Trio, Siemens Healthcare, Erlangen, Germany) equipped with a standard 12-channel
head coil. The imaging parameters for the 7 multiecho, gradient echo sequences were as follows: repetition time = 80 milliseconds, echo time = 10–70 milliseconds in increments of 10 milliseconds, flip angle = 25°, resolution = 0.27 x 0.27 mm, bandwidth = 120 Hertz/pixel, and sections = 80. R2* values were measured above, below, and in the region of iron content in the tubes containing cells using a rectangular region of 5228 pixels after zooming by a factor of 8 (roughly 82 pixels in the original unzoomed images).

RESULTS
Expression of Ferritin–EGFP in HeLa Cells and Glioma U251 Cells
The ferritin–EGFP produced here was a fusion protein and was composed of an EGFP (27 kD), a ferritin heavy chain (21 kD), and a polypeptide linker (1.3 kD). Thus, its molecular weight was expected to be 49 kD. Our Western blot result (Figure 2A) showed that ferritin–EGFP was successfully detected by anti-EGFP antibodies and showed a molecular weight much larger than that of EGFP, approaching 50 kD (protein marker was not shown). This proved that ferritin–EGFP was successfully expressed in cancer cells. Fluorescence detection showed that cells that expressed ferritin–EGFP emitted bright green fluorescence (Figure 2B), which indicated that the EGFP functioned well and was not impaired when linked with heavy-chain ferritin. Both of the above results show that ferritin–EGFP was successfully expressed in both HeLa cells and glioma U251 cells.

Establishment of a Tetracycline-Inducible U251–TetR–Ferritin–EGFP Monoclonal Cell Strain
A tetracycline-inducible U251–TetR–ferritin–EGFP monoclonal cell strain was successfully built through plasmid transfection and antibiotics screening. The tetracycline regulation system worked through the following mechanism: U251–TetR–ferritin–EGFP monoclonal cell strains could stably express both TetR and ferritin–EGFP. TetR bonded with tetracycline operator sequences (TetO2) and thus suppressed the expression of the downstream gene (ferritin–EGFP gene in this case). But when tetracycline was present, TetR bonded with tetracycline and was structurally changed and detached from TetO2, because of which, ferritin–EGFP suppression was relieved. When tetracycline was added (2 μg/mL, 48 hours), the ferritin–EGFP expression showed obvious protein expression as shown by both bright green fluorescence (Figure 2B, Tet+) and Western blot detection (Figure 2C, Tet+). When tetracycline was absent in the cell culture medium, the ferritin–EGFP expression was suppressed (Figure 2, B and C, Tet−). Usually, some low-level basal protein expression existed in tetracycline regulation systems; however, it could not be detected by Western blotting (Figure 2C, Tet−). Nevertheless, this low-level basal protein expression was successfully detected by fluorescence (Figure 2B, Tet−) and showed a weak, sparse green fluorescence signal (as indicated by the white arrow), which indicated that fluorescence detection was more sensitive than Western blotting. These interesting results

![Figure 2. Ferritin–EGFP expressed in cells. Western blot detection of ferritin–EGFP. Left—ferritin–EGFP: HeLa cells' lysate transfected with pCEP4-ferritin–EGFP plasmids. Right—EGFP: HeLa cells' lysate transfected with pcDNA3.1-3′-EGFP plasmids (A). Fluorescence detection of tetracycline-inducible U251–TetR–ferritin–EGFP monoclonal cell strain (B). Upper—Tet+ group: ferritin–EGFP expression in U251–TetR–ferritin–EGFP monoclonal cells was started by adding tetracycline (2 μg/mL, 48 hours) into the cell culture medium. Lower—Tet− group: control groups, U251–TetR–ferritin–EGFP monoclonal cells cultured without any tetracycline. Scale = 50 μm. Western blot detection of U251–TetR–ferritin–EGFP monoclonal cell strain. Left—Tet− group: U251–TetR–ferritin–EGFP monoclonal cells lysate that was cultured without any tetracycline. Right—Tet+ group: U251–TetR–ferritin–EGFP monoclonal cells lysate, in which the ferritin–EGFP expression was started by adding tetracycline (2 μg/mL, 48 hours) into the cell culture medium (C). Note: Presence of anti-EGFP means the protein was detected by anti-EGFP antibodies, and presence of anti-β-actin means the protein was detected by anti-β-actin antibodies.](image-url)
revealed the high fluorescent sensitivity of ferritin–EGFP. In other words, we successfully established tetracycline-inducible U251–TetR–ferritin–EGFP monoclonal cell strain, and this cell strain worked stably even after 15 passages.

**Cellular Iron Intake Observed by Prussian Blue Staining**

In our experiments, both control group cells (that did not express any ferritin–EGFP) and experimental group cells (that stably expressed high levels of ferritin–EGFP) were iron-loaded with 2 mM FAC for 48 hours. Prussian blue staining (Figure 3) detected limited iron intake in the control group cells but detected obvious iron intake (blue particles shown by the red arrow) in the experimental group. Without FAC supplements, neither showed Prussian blue staining (data not shown). Because a small amount of native ferritin (compared with the large quantity of transgenic ferritin–EGFP) also existed in the cells (13), it was not surprising to observe some low-level iron intake in the control group with iron loading. However, the much higher level of iron intake in the experimental group indicated that the expression of ferritin–EGFP worked well as an iron-storage protein.

**Effects of Ferritin–EGFP Overexpression on Cell Proliferation**

Both control group cells (Tet−, without ferritin–EGFP expression) and experimental group cells (Tet+, with ferritin–EGFP expression) were evaluated every 24 hours to examine whether the ferritin–EGFP overexpression affects cell proliferation (Figure 4). The cell growth was monitored for 96 hours. A longer growth time may lead to an overgrowth of cells, causing large uncertainties in MTT detection. Significant differences were considered when $P < .05$. There were no significant differences between the 2 groups of cells when ferritin–EGFP expressed for 24, 48, 72 and 96 hours. Because the ferritin–EGFP expression was started by adding tetracycline (2 $\mu$g/mL for 48 hours) in the culture medium, the effects of tetracycline on cell proliferation were also examined through MTT detection. Results showed that tetracycline had no effect on cell growth, as no significant differences were observed between the experimental and control groups (Figure 5).

**Cellular Iron Measurements by ICP-MS**

The ICP-MS measurements (Figure 6) showed that the addition of iron (FAC) into the culture medium significantly increased cellular iron intake. Without iron supplement, limited iron was detected (about 0.11 pg/cell), whether or not the cells had ferritin–EGFP expression. However, with iron supplement, high levels of cellular iron were observed, particularly, measurements showed 5.1 pg/cell for the “Ferritin+” group and 3.9 pg/cell for the “Ferritin−” group. That is to say, ferritin expression promoted cellular iron intake by 31% compared with the control group (this was consistent with the Prussian blue results). However, the Prussian blue results showed less iron than the control group.
group as shown by ICP-MS. This apparent difference could be caused by the detection sensitivities of the 2 methods. ICP-MS is capable of detecting lower levels of iron because it is much more sensitive than Prussian blue staining.

**R2* Measurements and Estimation of Iron Content**

Echo times of 10, 20, 30, and 40 milliseconds were used, as these provided the best image quality. R2* values were measured for 2 sections of cells in each tube (for visualization of the iron cell layers in the tubes themselves and the 40-millisecond image, see Figure 7). In each tube, 2 sections were evaluated. For the “Ferritin+/Fe+” group, results for R2* were $35.3 \pm 0.8$ s in the iron-containing cell regions and $13.4 \pm 0.2$ s in the regions above and below the cells, yielding an R2* of $21.9 \pm 0.8$ s. For the “Ferritin−/Fe−” group, R2* was $27.5 \pm 0.4$ s in the iron-containing cell regions and $14.8 \pm 0.1$ s in the regions above and below the cells, yielding an R2* of $12.7 \pm 0.4$ s. (All errors quoted are standard error of the mean over the ROI used.) Using the relationship $R2* = 2.2 + 50 \times [Fe]$ (mg Fe/g wet tissue) (22), giving 0.39 mg Fe/g wet tissue ($6 \times 10^6$ cells) for the “Ferritin+” group and 0.21 mg Fe/g wet tissue for the “Ferritin−” group. These values predict $6.5 \pm 0.1$ pg Fe/cell and $3.5 \pm 0.02$ pg Fe/cell for “Ferritin+” and “Ferritin−” groups, respectively.

**DISCUSSION**

This study aimed to establish an endogenous dual reporter for both fluorescence imaging and MRI by building a novel hybrid protein ferritin–EGFP. Fluorescence detection proved that ferritin–EGFP functioned well as a fluorescence reporter by emitting bright green fluorescence. The ferritin–EGFP expression led to a higher cellular iron content as shown by the results of Prussian blue staining, ICP-MS, and MRI measurements, all of
which indicated that ferritin–EGFP expression was effective as an MRI reporter.

Before this study, several dual reporters were successfully established, in which ferritin and fluorescent proteins were separately expressed, such as myc-ferritin and green fluorescent protein (13), ferritin and red fluorescent protein (23), and ferritin and EGFP (15). Nevertheless, fluorescent protein-fused ferritin was more favorable, as it was distinguishable from native ferritin inside cells. Ono et al. (24) found lower DsRed fluorescence in a DsRed–ferritin fusion protein, and they speculated that DsRed’s structure or stability may be affected. In our study, a special 18-aminoo-acid-long polypeptide was added between ferritin and EGFP to avoid potential interferences between them. This polypeptide was expected to improve the performance of the EGFP reporter gene. When Kim et al. (21) studied the application of fluorescent ferritin nanoparticles to the aptamer sensor, they found that when EGFP was linked to the C terminal of heavy-chain ferritin by a flexible glycine-rich peptide, the emission intensity and stability of EGFP were both greatly improved because of the aggregation nature of the heavy-chain ferritin. Despite the fact that our EGFP was linked to a modified polypeptide, it still showed high fluorescent sensitivity, and its expression was successfully regulated by tetracycline.

The effects of ferritin overexpression have been controversial. Some studies found that ferritin may be an effective therapy for prevention and treatment of Parkinson disease by reducing reactive iron (25). Further, Ziv et al. (11) reported the follow-up of a transgenic mice that overexpressed H-ferritin in liver hepatocytes for 2 years, and found that ferritin overexpression was safe for the mice. However, there was also evidence showing damage caused by ferritin overexpression, such as cell growth inhibition (18) or progressive age-related neurodegeneration (26, 27). These findings may be caused by various ferritin expression levels. Therefore, we created a tetracycline-regulated ferritin–EGFP expression system to avoid potential harm and investigate the effects of ferritin at different expression levels.

Literture (14, 16) reported that ferritin overexpression is supposed to increase net iron uptake by improved transferrin receptor or intracellular iron redistribution even without iron supplements. However, as shown in our study, no obvious iron intake was observed (neither by Prussian blue staining nor by ICP-MS measurements) if there was no iron supplement in the culture medium. Instead, FAC supplement seems to be effective in increasing cellular iron intake. Ferritin expression enhanced this effect.

Although the estimates of iron content measured by MRI and ICP-MS are not in perfect agreement, they are often slightly different, and the relative values of each are similar. For the MR R² estimates, as the 2 sections had equal volumes, a simple average of the values in the 2 sections was taken. The thickness of the cell layers and resolution of only 1-mm-thick sections made it difficult to incorporate partial volume effects, so we considered just the central 2 sections with clear signal changes. The iron in the “Ferritin-” group appears to have settled, giving a different R² values for each layer, but the average R² still represents the correct concentration of iron.

One can estimate iron loading and effective susceptibility from these measurements. Using 6 pg Fe/cell for 6 million cells gives 6.4 × 10¹⁰ atoms/cell. If there are 100 million ferritin proteins/cell, this predicts 640 iron atoms/ferritin. This lies in the loading range of 0–4500 iron atoms/ferritin known in the literature (10). One can also estimate the susceptibility in every cell using the following formula: 

\[ R_2^* = k \lambda \Delta \chi \rho \text{Bo} \]

where \( k \) = 0.4 for point dipoles, and here, \( \text{Bo} = 3T \). Iron in the ferritin may take the form of \( 5FeO_2:9H_2O \), \( Fe_2O_3 \), or \( Fe_3O_4 \) (10). Using \( Fe_2O_3 \), the fact that the iron sits in 0.1 cc and the density of iron is 5.18 g/cc, the volume fraction of iron is estimated to be \( \lambda = 6.95 \times 10^{-6} \). This yields a \( \Delta \chi = 9.9 \times 10^4 \) ppm or a \( \Delta \chi / \text{cell} = 1.65 \times 10^{-4} \) ppm. This value is close to the nanoparticles’ susceptibility (7.5 × 10⁴ ppm at 3 T) used by Shen et al. (28). At 3 T, the noise in measuring \( \Delta \chi \) using quantitative susceptibility mapping (29) with a 3-dimensional sequence covering the whole brain is about 20 ppb for a 1-mm³ voxel size depending on the imaging parameters and imaging time. This suggests that it is possible to measure the presence of ~ 100 cells with a signal-to-noise ratio of ~ 8:1.

In summary, we successfully established a tetracycline-inducible ferritin–EGFP chimera. Our results confirm the potential to use this chimera as an endogenous dual reporter for both fluorescence imaging and MRI for cellular levels of ferritin–EGFP.

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MRI-Guided Stereotactic Biopsy of Murine GBM for Spatiotemporal Molecular Genomic Assessment

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Key Words: MRI-guided biopsy, murine studies, genomic analysis, glioma
Abbreviations: Glioblastoma (GBM), magnetic resonance imaging (MRI), gadolinium (Gd), magnetic resonance (MR), false discovery rate (FDR), ingenuity pathway analysis (IPA), 3-dimensional (3D), RNA sequencing (RNA-Seq)

Brain tumor biopsies that are routinely performed in clinical settings significantly aid in diagnosis and staging. The aim of this study is to develop and evaluate a methodological image-guided approach that would allow for routine sampling of glioma tissue from orthotopic mouse brain tumor models. A magnetic resonance imaging-guided biopsy method is presented to allow for spatially precise stereotaxic sampling of a murine glioma coupled with genome-scale technology to provide unbiased characterization of intra- and intertumoral clonal heterogeneity. Longitudinal and multiregional sampling of intracranial tumors allows for successful collection of tumor biopsy samples, thus allowing for a pathway-enrichment analysis and a transcriptional profiling of RNA sequencing data. Spatiotemporal gene expression pattern variations revealing genomic heterogeneity were found.

INTRODUCTION

Glioblastoma (GBM) is the most common and aggressive intracranial tumor in adults. Despite decades of research, survival remains dismal, with a mean survival time of 15 months and a mean 5-year survival rate of <10% (1-3). The standard therapy consists of maximal safe surgical resection, followed by radiation and then chemotherapy with temozolomide, and this protocol has changed very little since its inception (2). Although many patients initially respond, GBM inevitably recurs in most patients (1).

Given its poor prognosis, GBM is a perfect candidate for targeted therapy using precision medicine; thus, understanding the genetic profile of GBM is vital. However, known spatial and temporal tumor heterogeneity is recognized as a significant obstacle for accurate diagnosis impeding the use of targeted therapies. GBM is among the first tumor types for which in-depth genomic analyses were performed (4), and integrated genomic analyses performed in a large cohort of patients uncovered complex clonal heterogeneity (4), intertumoral heterogeneity (5-10), and intratumoral heterogeneity (11-14). Recent studies have revealed that customized treatments targeting specific mutations may not be efficacious in altering patient outcomes (15). Although in this case the cause of treatment failure is unclear, previous studies have shown that between 67% and 91% of posttreatment failures no longer harbored the target mutation (16, 17). This is probably because of clonal expansion of cells outside the targeted mutation. GBM is also known to change its molecular signature with treatment (18). These findings reflect the highly dynamic genetic evolution present in GBM, confounding the elucidation of its complete genetic landscape.

Multiregional tumor sampling is ideal in GBM and other tumor types, but it is often impractical and potentially risky for routine clinical application (19, 20). Furthermore, tumor sampling during different treatment phases is often clinically unindicated. However, precise mechanisms underlying tumor heterogeneity and its functional significance can be evaluated in preclinical models for discovery of driver mutations, discovery of mechanisms of drug resistance, identification of genetic and imaging biomarkers, and development of clone-specific cancer therapies.

Here, we devised and successfully showed a magnetic resonance imaging (MRI)-guided stereotactic biopsy method for isolation and functional profiling of tumoral heterogeneity to
assess temporal and geographical genomic variations during disease progression in an intact murine GBM model. Longitudinal tumor biopsy samples were obtained at different stages of tumor growth and in spatially distinct locations. RNA sequencing (RNA-Seq) of biopsy tissue showed unique temporal and spatial gene expression patterns.

**METHODS**

**Mouse Strains**

The p53 mutant GBM mouse model was obtained from Dr. Yuan Zhu Children’s National Medical Center (Washington DC). The genotype and maintenance of the strain have been described previously (21). Animal experiments were approved by the University Committee on the “Use and Care of Animals” (UCUCA) at the University of Michigan.

**MRI Acquisition**

Mice were anesthetized using a 1%-2% isoflurane-air mixture throughout the duration of the MRI experiment. Then, 5 minutes before anesthesia administration, 50 μL of 0.5M gadolinium (Gd)–diethylenetriaminepentaacetic acid (DTPA) (Magnevist; Bayer Healthcare Pharmaceuticals) was injected intraperitoneally. Once anesthetized, mice were securely fastened in the animal handling MRI cradle with a bite bar and stereotactic bilateral ear bars. An external fiducial marker consisting of Gd contrast agent was secured to the coil ~1 cm left of the mouse skull. MRI scanning was performed using a 7 T Direct Drive MR system (Agilent, CA) using a volume radiofrequency transmit coil and a 2-channel fixed-tuned actively decoupled surface receive coil. The mouse handling cradle including bilateral ear bars and a bite bar for securing the mouse head (Rapid MR International). During MRI, heated air was introduced into the magnet bore (World Precision Instruments) to maintain animal body temperature. T1-weighted spin echo images were acquired for each animal using a spin echo sequence with the following parameters: repetition time/echo time = 510/15 milliseconds, field of view = 20 × 20 mm², matrix size = 128 × 128, slice thickness = 500 μm, number of slices = 25 interleaved sections, and 2 signal averages per phase encode step.

**MRI Biopsy Validation Study**

Phantoms were constructed using agarose gel blocks. Bubbles with varying volumes of air (5, 10, and 20 μL) were injected into the gel before it solidified using a Hamilton syringe. The MRI radiofrequency coil bed with an associated fiducial marker was modified to allow for secure attachment to a Kopf stereotactic device to provide for a consistent and reproducible coordinate system. MRI-guided biopsy was performed on the gel blocks as described below to validate targeting accuracy. Instead of extracting “tissue” (eg, gel), we injected a 1:10 dilution of 0.5M Gd–DTPA (Magnevist) into the agarose gel at the calculated location of the intended target volume (eg, air bubble). If the air bubble was successfully filled with the contrast agent, the air would be displaced and a bright signal in the T1 magnetic resonance (MR) image would be used to verify success versus failure. Gel-based target biopsies were performed on 15, 12, and 12 bubbles with 5-, 10-, and 20-μL volumes, respectively. The accuracy of the method was determined as follows: (success)/(total number of target volumes) × 100%. Success was determined as the ability of the biopsy needle to completely fill the target volume as determined by MRI.

**Intracranial Biopsy Procedure**

After acquisition of prebiopsy MR images, the mouse was anesthetized with a ketamine/xylazine (0.1/0.02 mg/kg) mixture. The tumor was localized with a 3-dimensional (3D) Cartesian coordinate system on the basis of the distance from the external fiducial marker. The coil was secured to a coil–holder with an attached Kopf stereotactic coordinate system. The mouse was prepped for surgical biopsy and the eyes were protected using eye lubrication. The scalp was sterilized with a topical antiseptic solution and allowed to dry. A 1.5-cm longitudinal incision was made across the midline beginning just posterior to the nasion. The scalp and pericranium were gently retracted and the skull was exposed with cotton-tip applicators. A drill was attached to the stereotactic system (Foreedom K-1070 with MRI-170 drill with stereotactic attachment-Model 1474; with Kyocera #70 105-0280.400 drill bits). The attached drill was used to localize the skull burr hole site based on the MRI-determined Cartesian coordinates. After the burr hole was made, a fine aspiration needle (Inrad Model 54722) was secured to the stereotactic instrument and placed into the burr hole. The needle was then advanced to the MRI-determined depth at the proximal/superior edge of the tumor. The inner cannula was removed and the needle was advanced toward the center of the tumor. The needle was attached to a mini-bore extension set (Abbott 32072) connected using a locking aspiration syringe. A syringe was used to withdraw with 2.5 mL of pressure for 3 seconds before the pressure was re–equalized, and the needle was withdrawn from the skull. The biopsy sample was collected in a sterile 2-mL centrifuge tube. Samples were either snap frozen or manually dissociated and cultured at 37°C.

After the biopsy, the burr hole was covered with bone wax (#W810, Ethicon), and the incisions were approximated and closed with Vetbond (#1469SB, Vetbond). To minimize pain, 100 μL (1 mg/mL) of Rimadyl (Pfizer Animal Health) was subcutaneously administered following completion of the surgical procedure. The mouse was kept secured in the coil holder and the MRI was repeated. T1-weighted images were acquired as described above to confirm the accuracy of the biopsy and to rule out significant complications.

A second late-stage biopsy was performed when the tumor volumes reached ~100 μL. Two distinct biopsy locations were identified and similar biopsy procedures were carried out as described above. The notation Mx[y] was used to denote mouse number [x] and tumor biopsy sample number [y]. At the conclusion of the final biopsy and MR scans to confirm biopsy locations, the animals were sacrificed.

**RNA-Seq**

Tumor biopsy tissues were used for RNA extraction. Total RNA was extracted using TRizol™ (#10296010; Life Technologies) and ethanol precipitation as per the manufacturer’s instructions. RNA-Seq library preparation and sequencing was completed by DNA sequencing core at the University of Michigan. Specifically, the RNA library was prepared with NEBNext® rRNA De-
pletion Kit (#E6310L, NEB). Further, 30–45 million 50nt-single-end reads were obtained from each sample with an estimated 20–30× sequencing depth.

RNA-Seq Data Analysis

Single-end reads of RNA-Seq data were mapped to the mouse transcriptome (GRCm38.p4/mm10) using BWA, duplicate reads were marked by Picard, and base quality scores were recalibrated using GATK, by referring to the PRADA pipeline (22), followed by proceeding with the mapped reads to calculate the read counts and RPKM (reads per kilobase of transcript per million mapped reads) values for 39,017 expressed genes (Ensembl Gene ID) using UCSC/mm10/ensGene transcript database, with associated gene names annotated based on Ensembl genome assembly (Biomart). Differential expression of genes was calculated by applying quantile-adjusted conditional maximum likelihood method that fits read counts with negative binomial models and obtains dispersion estimates, followed by an exact test to determine differential expressions based on false discovery rate (FDR ~ adjusted P value) (23). Genes were considered to be differentially expressed when the change was greater than 1.5-fold, and the adjusted P value (FDR) was <0.05%.

Pathway and network analyses of differentially expressed genes in early- versus late-stage biopsies were performed using ingenuity pathway analysis (IPA) software (Qiagen). The molecule activity predictor in IPA was used to predict the upstream or downstream activation or inhibition of a given pathway. The P value of the enrichment score was used to evaluate the significance of the overlap between observed and predicted gene sets. All statistical analyses were performed and graphs were obtained under R (version 3.2.3) environment.

Tumor purity was evaluated on RPKM (reads per kilobase of transcript per million mapped reads) values of gene expression profiling using ESTIMATE, based on the enrichment of gene signatures in stromal and immune cells (24). The molecular classification of samples was performed using ssGSEA.

RESULTS

To enable temporal and geographical multisampling of tumor tissue in a murine GBM model, we devised a biopsy apparatus (Figure 1A). For tumor localization, a 3D Cartesian coordinate diagram with external fiducial markers was used to guide biopsy (Figure 1B). To validate the stereotactic biopsy efficiency and accuracy, an MR phantom consisting of agar gels was developed. Three different volumes of air bubbles (void spaces) were used as representative tumor sizes. Once the locations of the voids were determined by MRI within the 3D coordinate system on the basis of the distance from the external fiducial marker, Gd was stereotactically injected into spatially distinct voids via biopsy needles. MRI T1 images were taken before and after Gd injection to determine the accuracy of the biopsy method (Figure 1C). Further, 12–15 mock biopsies were performed for each size, and the accuracy was shown to be 100, 92, and 73% at volumes of 20, 10, and 5 µL, respectively (Figure 1D). These results show that sampling of tumors as small as 5 µL can be routinely achieved with a high degree of accuracy using this MRI-guided biopsy method.

Next, we demonstrated this method for stereotactic MRI-guided multiregional sampling of spontaneous murine p53 mutant GBM tumors with tumor volumes of 10–20 µL. Animals were secured in a stereotactic biopsy station (Figure 1A), and the tumor was localized on the basis of the distance from the
external fiducial marker (Figure 1B) using MRI. MR images were easily acquired both before and after stereotactic biopsy sampling (as depicted in Figure 2A) by transferring the MRI animal cradle to a holder with an attached stereotactic coordinate system. After surgical preparation, the Cartesian coordinate system was used to localize the tumor, and a fine aspiration needle attached to an articulating apparatus was used to withdraw the biopsy tissue specimen. The tissue was either snap frozen for RNA extraction or manually dissociated for cell culture (data not shown). T1-weighted MRI was repeated after biopsy to confirm the accuracy of the biopsy and the sampling location. Three p53 mutant GBM animals were used to perform an early-stage biopsy. Further, 2 geographically distinct locations were sampled during the late-stage biopsies for each animal at a tumor volume of ~100 μL (Figure 2, B and C) to investigate intertumoral genomic heterogeneity.

The analysis of spatiotemporal genomic heterogeneity was accomplished by RNA-Seq-based expression profiling of early- and late-stage biopsies, which revealed distinct temporal and spatial gene expression patterns. We compared both early- and late-stage biopsies of 3 individual mice, and we depicted the top 10 genes upregulated (Figure 3A) and downregulated (Figure 3B) in late-stage biopsies. Pathway-enrichment analysis of differentially expressed genes during tumor progression further identified unique molecular patterns. Upregulated and downregulated pathways in late- versus early-stage biopsies are depicted (Figure 3C). Overall, the pathway-enrichment analysis of differentially expressed genes in early- versus late-stage biopsy samples showed significant differences in molecular patterns during tumor progression. Moreover, spatially varying differences in expressed genes were also observed in late-stage tumors, revealing that intertumoral heterogeneity in this GBM mouse model could be detected using this method.

Transcriptional profiling of GBM has been previously performed in large-scale clinical studies, and heterogeneous genotypes consisting of subclones with varying tumorigenic, proliferative, differentiation, and drug responsiveness have been shown (4, 5, 18). To identify whether such spatial (geographical) gene expression patterns were present in a preclinical p53 mutant GBM model, 2 spatially distinct locations of late-stage tumors using the aforementioned criteria and IPA pathway analysis were compared (Figure 3D). Interestingly, transcription factor OTX2 was differentially regulated between the 2 spatially distinct regions. OTX2 has been implicated as an oncogene in medulloblastoma where it is highly expressed and amplified in a subset of these tumors (25). OTX2 regulates cell cycle and differentiation, and its regulated genes may serve as potential therapeutic targets (25). Differentially regulated pathways between regions are depicted (Figure 3E). Up- and/or downregulated genes were found, and although detailed studies need to be conducted to investigate the roles of each gene in tumor growth and survival, some genes identified have been indicated to play a role in overall patient survival and tumorigenesis. For example, Igf1 has been shown to affect overall survival in GBM patients, indicating that it may be associated with more aggressive tumor characteristics in the late stages of tumor growth.

**DISCUSSION**

Molecular characterization of tumors to guide treatment selection is hampered by intratumor heterogeneity (19, 26). Longitudinal and multifocal monitoring of clonal dynamics may provide for improved patient care (19). In this study, we devised an experimental approach for image-guided tumor sample acquisition from isolated GBM biopsies for sequencing to enable...
Figure 3. RNA-sequencing (RNA-Seq)-based expression profiling of early- and late-stage biopsies reveals distinct temporal and spatial gene expression patterns. The top 10 genes (A) upregulated and downregulated (B) in late-stage biopsies are displayed. Differential expression of genes was calculated. Pathway and network analyses of differentially expressed genes performed using ingenuity pathway analysis (IPA) software (C). The molecule activity predictor in IPA was used to predict the upstream or downstream activation or inhibition of a given pathway. Spatial gene expression patterns were identified by comparing 2 different locations of late-stage tumors (location 1 vs. 2) using the aforementioned criteria (D) and IPA pathway analysis (E). Nomenclature: Mx[y] denotes mouse number [x] and tumor biopsy sample number [y].
comparative analyses of gene expression patterns and tumor growth. This method allowed for longitudinal tissue sampling over time and in geometrically specific regions to gain further insight into the mechanisms of heterogeneity, thus allowing for linking phenotypic and genomic characteristics.

Although phenotypic analyses are not shown here, neurosphere cultures may also be established from GBM biopsies (data not shown). Single-cell expansion and functional assays may be performed from these biopsies and are a topic for future investigations. Although cell to cell differences were not investigated in this study, this approach would also allow for single-cell isolation and investigation of clonal heterogeneity. These studies can easily be expanded to include DNA extraction and the analysis of genetic variations by exome or deep sequencing approaches. We believe that these types of sequencing analyses may aid in the identification of genetic and imaging biomarkers.

Currently, MRI-based stereotactic intracranial procedures are routine in clinical practice. However, an accurate intracranial biopsy technique has not been shown in mouse models. Here, we established an innovative and accurate stereotactic image-guided murine biopsy method that is capable of accurate and repeated biopsy collection from an individual animal. Tissues collected can be used to investigate genomic dynamics during disease progression and modulation during treatment intervention. Spatial and temporal variations in the molecular landscape of preclinical models may provide unique insights into the development and interrogation of novel therapeutic strategies. This information provides opportunities for evaluating genomic responses during treatment to gain a further biological understanding of drug targeting and mechanisms of drug resistance and clonal evolution. Elucidation of the precise mechanisms of existing or emerging tumor heterogeneity and its functional significance in preclinical models provides opportunities to discover driver mutations, emergence of drug resistance, identification of generic and or imaging biomarkers, and development of clone-specific cancer therapies. Here, we devised a method to isolate and functionally profile intratumoral heterogeneity by using MRI-guided biopsies to assess temporal and geographical differences during disease progression in a murine GBM model. We successfully collected tumor biopsy samples at different stages of tumor growth and individual locations. RNA-Seq results showed unique temporal and spatial gene expression patterns in GBM. This approach may allow for new opportunities for assessing tumor tissue samples from discrete spatial locations over time to improve our understanding of the role of spatial heterogeneity and genomic instability in adaptation mechanisms and therapy resistance. In the future, this approach may be adapted to investigate treatment-induced changes and adapted to study other primary and metastatic tumor types. In summary, the combination of MRI-guided tumor sampling with advanced genome sequencing can facilitate identification of mechanisms leading to heterogeneity and development of clinically useful measures of heterogeneity, which may guide identification of new therapeutic strategies.

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Quantitative Analysis of the Spatial Distribution of Metastatic Brain Lesions

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Key Words: brain metastases, MRI, breast cancer, lung cancer

Abstract:
Brain metastases (BMs) are the most common intracranial malignancy and afflict ~10%–20% of patients with cancer. BMs tend to present at the boundaries of gray and white matter because of the distribution of small vessels. In addition, metastases may not be randomly distributed across gross anatomical regions of the brain, but this has not previously been quantified. We retrospectively analyzed a series of 28 patients with recurrent BMs with a total of 150 lesions. Each lesion was manually defined based on T1 gadolinium-enhanced imaging. Standard brain atlases were used to identify the anatomical brain region affected by each BM and the frequency of metastases in each region was compared with the expected probability, which was assumed to be a random distribution based on the brain volume. After correction for multiple comparisons, the paracingulate gyrus was found to have a statistically significant increase (P = 4.731 × 10⁻⁶) in the rate of BMs relative to the random spatial distribution. A nonstochastic spatial distribution of metastases may be used to guide partial brain radiotherapy with risk-adapted dose delivery and reduce the risk of neurotoxicity due to overtreatment.

INTRODUCTION
Whole-brain radiotherapy (WBRT) for patients with brain metastases (BMs) is a commonly used technique to treat both visible and subclinical disease. Neurotoxicity is a major concern and protocols to reduce the volume of brain receiving a full dose are currently being tested in clinical trials. However, there are currently no available methods to risk-stratify regions of the brain on the basis of the probability of developing a BM. Accurate segmentation of the brain based on BM risk would permit the radiation dose to be spatially tailored to improve disease control in high-risk regions and spare neurotoxicity by reducing dose to low-risk regions.

More accurate radiation delivery has the potential of affecting numerous patients because BMs are the most common intracranial malignancy, with an annual incidence of >150,000 in the USA (1), and are diagnosed in ~10%–20% of patients with cancer (2,3). Patients who have previously been treated for BMs or who are at an increased risk for developing BMs often undergo surveillance imaging with serial magnetic resonance imaging (MRI) scans, and treatment may involve focal radiotherapy, such as with stereotactic radiosurgery, or WBRT. Although WBRT remains the standard of care for many patients with BMs, improvements in systemic therapy have led to gains in survival for patients with metastatic disease, and the long-term neurocognitive toxicities associated with WBRT must now be weighed against the benefits of treatment. Therefore, several lines of investigation are now directed toward mitigating long-term toxicities associated with full-dose irradiation to the entire brain.

Partial brain techniques have recently been tested in an effort to reduce late neurocognitive decline that is associated with WBRT. RTOG 09-33 was a phase II study using intensity-modulated radiation therapy (IMRT) to selectively spare the hippocampi bilaterally while delivering a full dose to the remainder of the brain (4). Results showed that neurocognitive toxicities improved with IMRT relative to standard WBRT. In sparing the hippocampi from radiation toxicity, one would assume that patients would then be at an increased risk of developing metastatic foci in regions receiving a low radiation dose. On the contrary, local control appears to be maintained with IMRT, and extensive interrogation of the hippocampi revealed that it is at a very low risk for developing BMs (5). The finding that a critical brain structure can be safely spared the damaging effects of high-dose irradiation without compromising efficacy is intriguing and motivates investigation into other areas that might be at a low risk of developing BMs. Some studies have reported that the distribution of BMs may be based on the vasculature (6-8), but others have found that the pattern of spread is influenced by other factors, such as disease histology (9). The majority of the published work has relied on broad classifications of the brain, and there are currently no accepted atlases that segregate brain regions based on the risk of developing BMs.

Here, we hypothesized that discrete brain regions are involved with metastatic lesions beyond that which would be expected by chance. To test this, we identified 150 BMs in 28 patients treated at a single institution and compared the BMs’
spatial distribution with a computer model of randomly distributed BMs. All BMs were coded by their voxel coordinates, and these were mapped onto 52 anatomical locations based on a common brain atlas to determine if particular regions were at increased risk of harboring metastatic disease.

**METHODOLOGY**

All procedures described in this study were approved by the institutional review board of Columbia University. We searched a database of patients with BMs from a primary diagnosis of either lung or breast cancer treated at our institution between December 2008 and January 2016 and selected those who met the following criteria: (1) were treated for at least 1 recurrent BM, (2) review of radiology reports determined that the BM recurrence was not definitively seen on a prior scan, and (3) all MRI data were available for analysis. Imaging data were obtained on either a 1.5 T or 3 T magnetic resonance scanner (GE Medical Systems Waukesha, Wisconsin) or a 1.5 T or 3 T magnetic resonance scanner (Philips Healthcare, Best, The Netherlands). Image processing was performed with the Functional MRI of the Brain Software Library (10) (FSL; Oxford, UK) and Matlab (MathWorks, Natick, Massachusetts). Brain extraction was performed for each imaging sequence. All brain lesions were manually contoured based on T1 contrast-enhanced scans.

T1 contrast-enhanced scans were coregistered using an affine registration (12 degrees of freedom) to the Montreal Neurological Institute (MNI) standard brain. Registration matrices were then applied to each manually contoured BM, and the center of gravity was computed to identify the centroid voxel, which was used as the “origin” of the BM. All voxel coordinates were maintained in MNI-space. The Harvard–Oxford cortical and subcortical (http://www.cma.mgh.harvard.edu/) atlases were used to determine the anatomical structures corresponding to each metastatic centroid. After removing ventricular spaces, broad anatomic boundaries that overlapped with other areas (eg, “cerebral cortex”), and combining subdivisions of particular regions (eg, “anterior” and “posterior” divisions), 52 anatomically defined regions of interest (ROIs) were included in our analyses (Figure 1).

Coordinates for each of the 150 observed BM centroids were mapped onto the atlas, and the frequency of involvement for all 52 ROIs was computed. This observed value was compared with the expected value, which assumed that each voxel within the brain was at an equal risk of being involved with a BM. Therefore, the probability of 1 of the 52 ROI harboring a BM was a function of that region’s volume relative to the total volume of all 52 regions. The observed and expected rates of BMs for each ROI were compared with proportional 2-tailed hypothesis testing, which is presented as follows:

\[
Z = \frac{\hat{p} - p_0}{\sqrt{\frac{p_0(1-p_0)}{n}}}
\]

where:
- \(Z\): z-score
- \(\hat{p}\): proportion of observed BMs
- \(p_0\): proportion of expected BMs
- \(n\): number of observed BMs

Raw z-scores and P-values are reported, but we subjected the interpretation of all results to a Bonferroni correction to adjust for multiple comparisons across the 52 anatomic ROIs. There-

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**Figure 1.** The Harvard–Oxford cortical brain atlas overlaying the subcortical atlas and standard Montreal Neurological Institute (MNI) brain. In total, 52 distinct brain regions from the atlas were included in the analysis.

---

**Table 1.** Patient Characteristics and Additional Information for the 150 BMs Detected in this Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.5</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 (39)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (61)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>19 (67.9)</td>
</tr>
<tr>
<td>EGFR+</td>
<td>5 (26.3)</td>
</tr>
<tr>
<td>KRAS+</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>ALK+</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Breast</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td>ER+, PR+, Her2–</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>ER+, PR+, Her2+</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>ER+, PR–, Her2+</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>ER–, PR+, Her2–</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>ER–, PR–, Her2–</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Lesions per patient</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
</tr>
<tr>
<td>Range</td>
<td>1–19</td>
</tr>
<tr>
<td>Lesion volume (cc)</td>
<td>1.196</td>
</tr>
<tr>
<td>Mean</td>
<td>0.128–18.368</td>
</tr>
<tr>
<td>Initial treatment</td>
<td></td>
</tr>
<tr>
<td>Resection and adjuvant SRS</td>
<td>14 (50)</td>
</tr>
<tr>
<td>SRS alone</td>
<td>11 (39.3)</td>
</tr>
<tr>
<td>WBRT</td>
<td>3 (10.7)</td>
</tr>
</tbody>
</table>

Abbreviations: eGFR, estimated glomerular filtration rate; SRS, stereotactic surgery; WBRT, whole-body radiation therapy; No., number.
Therefore, the $P$-value needed to reach statistical significance was $P \leq 9.804 \times 10^{-4}$.

**RESULTS**

There were 11 (39%) male and 17 (61%) female patients identified with a primary cancer diagnosis of non-small cell lung cancer in 19 (68%) and breast cancer in 9 (32%) patients (Table 1). The mean age of all patients was 59.5 years (range, 38–89 years). The mean volume of all BMs was 1.196 cc (range, 0.128–18.368 cc). Several representative BMs are depicted in Figure 2 from 4 patients included in the analysis.

To visually inspect all BMs, a 3-dimensional rendering was created with all 150 lesions projected onto the MNI brain and dilated to the median BM diameter (see Supplemental Video). The 3-dimensional rendering provides a qualitative evaluation of spatial variations in BM distribution. To test for a non-stochastic spatial distribution, we compared the observed with the expected rate of BMs for each ROI. In this approach, the null hypothesis is that the spatial distribution of BMs is stochastic and the probability of detecting a BM within a brain region is a function of that region’s volume.

For each of the 52 brain regions, 2 parcellations were performed. First, each atlas-based region was coded by its proportional volume relative to all 52 ROIs (Figure 3). Second, within each region, voxels were coded by the number of BMs observed in the

Figure 2. Representative axial T1 contrast-enhanced images from 4 patients were included in the analysis. A large ring-enhancing lesion in the right middle temporal lobe in the posterior insular cortex represents metastatic breast cancer (A). In another patient, a small enhancing metastatic focus from non-small cell lung cancer (NSCLC) can be appreciated in the medial left cerebellum (B). A patient with multifocal recurrent BMs from a primary NSCLC is also shown where 2 large ring-enhancing lesions are seen on the same axial section (C). Finally, a small enhancing nodule from a metastatic breast cancer is visualized in the left frontal lobe (D).

Figure 3. Classification of all 52 regions of interest (ROIs) included in the analysis by their proportional volume relative to the entire area at risk for developing a BM. Each region’s color corresponds to the percent of the total volume as labeled by the colorbar (bottom right). For example, the frontal pole (*) contained the largest number of voxels, making up 11% of the total volume for all 52 ROIs. The paracingulate cortex (x, light blue) consists of a relatively small proportional volume at 1.7% of the total volume analyzed.

Figure 4. Cortical and subcortical anatomic brain regions as defined by the Harvard–Oxford atlas are color coded by the number of brain metastases detected within the anatomic boundaries from the 150 lesions analyzed. In total, 20 lesions fell outside the bounds of any of the 52 ROIs, leaving 130 BMs to be analyzed. The area with the highest frequency of involvement was the precentral gyrus (*, bright yellow), which was affected by 15 metastases, which was not significantly increased above the predicted value after correction for multiple comparisons. The paracingulate gyrus (x, red) contained 11 lesions, which was the only ROI that was significantly increased above the expected rate after Bonferroni correction.
### Table 2. Results from the Analysis for all 52 ROIs

<table>
<thead>
<tr>
<th>Atlas Region</th>
<th>Volume (cc)</th>
<th>% Volume at Risk</th>
<th>No. Observed</th>
<th>Observed Rate</th>
<th>z-Score</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angular gyrus</td>
<td>29.624</td>
<td>0.016</td>
<td>3</td>
<td>0.023</td>
<td>0.671</td>
<td>.502</td>
</tr>
<tr>
<td>Brain stem</td>
<td>72.376</td>
<td>0.039</td>
<td>5</td>
<td>0.039</td>
<td>-0.001</td>
<td>.999</td>
</tr>
<tr>
<td>Central opercular cortex</td>
<td>20.624</td>
<td>0.011</td>
<td>1</td>
<td>0.008</td>
<td>-0.358</td>
<td>.720</td>
</tr>
<tr>
<td>Cingulate gyrus</td>
<td>70.496</td>
<td>0.038</td>
<td>6</td>
<td>0.046</td>
<td>0.521</td>
<td>.603</td>
</tr>
<tr>
<td>Cuneal cortex</td>
<td>13.944</td>
<td>0.007</td>
<td>0</td>
<td>0</td>
<td>-0.985</td>
<td>.324</td>
</tr>
<tr>
<td>Frontal medial cortex</td>
<td>12.312</td>
<td>0.007</td>
<td>0</td>
<td>0</td>
<td>-0.926</td>
<td>.355</td>
</tr>
<tr>
<td>Frontal operculum cortex</td>
<td>8.496</td>
<td>0.005</td>
<td>0</td>
<td>0</td>
<td>-0.768</td>
<td>.442</td>
</tr>
<tr>
<td>Frontal orbital cortex</td>
<td>41.504</td>
<td>0.022</td>
<td>6</td>
<td>0.046</td>
<td>1.869</td>
<td>.062</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>207.200</td>
<td>0.110</td>
<td>5</td>
<td>0.039</td>
<td>-2.611</td>
<td>.009</td>
</tr>
<tr>
<td>Heschl’s gyrus</td>
<td>6.288</td>
<td>0.003</td>
<td>0</td>
<td>0</td>
<td>-0.660</td>
<td>.509</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>37.216</td>
<td>0.020</td>
<td>3</td>
<td>0.023</td>
<td>0.269</td>
<td>.788</td>
</tr>
<tr>
<td>Inferior temporal gyrus</td>
<td>64.240</td>
<td>0.034</td>
<td>3</td>
<td>0.023</td>
<td>-0.696</td>
<td>.487</td>
</tr>
<tr>
<td>Insular cortex</td>
<td>28.904</td>
<td>0.015</td>
<td>3</td>
<td>0.023</td>
<td>0.715</td>
<td>.475</td>
</tr>
<tr>
<td>Intracalcarine cortex</td>
<td>17.688</td>
<td>0.009</td>
<td>0</td>
<td>0</td>
<td>-1.111</td>
<td>.267</td>
</tr>
<tr>
<td>Lateral occipital cortex</td>
<td>175.792</td>
<td>0.094</td>
<td>5</td>
<td>0.039</td>
<td>-2.155</td>
<td>.031</td>
</tr>
<tr>
<td>Left accumbens</td>
<td>0.712</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>-0.222</td>
<td>.824</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>2.656</td>
<td>0.001</td>
<td>0</td>
<td>0</td>
<td>-0.429</td>
<td>.668</td>
</tr>
<tr>
<td>Left caudate</td>
<td>3.896</td>
<td>0.002</td>
<td>1</td>
<td>0.008</td>
<td>1.410</td>
<td>.159</td>
</tr>
<tr>
<td>Left hippocampus</td>
<td>6.120</td>
<td>0.003</td>
<td>0</td>
<td>0</td>
<td>-0.652</td>
<td>.515</td>
</tr>
<tr>
<td>Left pallidum</td>
<td>2.136</td>
<td>0.001</td>
<td>0</td>
<td>0</td>
<td>-0.385</td>
<td>.701</td>
</tr>
<tr>
<td>Left putamen</td>
<td>6.392</td>
<td>0.003</td>
<td>1</td>
<td>0.008</td>
<td>0.841</td>
<td>.400</td>
</tr>
<tr>
<td>Left thalamus</td>
<td>11.760</td>
<td>0.006</td>
<td>2</td>
<td>0.015</td>
<td>1.321</td>
<td>.187</td>
</tr>
<tr>
<td>Lingual gyrus</td>
<td>43.104</td>
<td>0.023</td>
<td>2</td>
<td>0.015</td>
<td>-0.574</td>
<td>.566</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>67.368</td>
<td>0.036</td>
<td>9</td>
<td>0.069</td>
<td>2.050</td>
<td>.040</td>
</tr>
<tr>
<td>Middle temporal gyrus</td>
<td>70.664</td>
<td>0.038</td>
<td>2</td>
<td>0.015</td>
<td>-1.330</td>
<td>.183</td>
</tr>
<tr>
<td>Occipital fusiform gyrus</td>
<td>28.696</td>
<td>0.015</td>
<td>6</td>
<td>0.046</td>
<td>2.874</td>
<td>.004</td>
</tr>
<tr>
<td>Occipital pole</td>
<td>77.264</td>
<td>0.041</td>
<td>8</td>
<td>0.062</td>
<td>1.175</td>
<td>.240</td>
</tr>
<tr>
<td>Paracingulate gyrus</td>
<td>32.760</td>
<td>0.017</td>
<td>11</td>
<td>0.085</td>
<td>5.856</td>
<td>4.731 × 10⁻⁹</td>
</tr>
<tr>
<td>Parahippocampal gyrus</td>
<td>42.616</td>
<td>0.023</td>
<td>1</td>
<td>0.008</td>
<td>-1.147</td>
<td>.252</td>
</tr>
<tr>
<td>Parietal operculum cortex</td>
<td>13.472</td>
<td>0.007</td>
<td>2</td>
<td>0.015</td>
<td>1.112</td>
<td>.266</td>
</tr>
<tr>
<td>Planum polare</td>
<td>9.680</td>
<td>0.005</td>
<td>0</td>
<td>0</td>
<td>-0.820</td>
<td>.412</td>
</tr>
<tr>
<td>Planum temporale</td>
<td>11.536</td>
<td>0.006</td>
<td>0</td>
<td>0</td>
<td>-0.896</td>
<td>.370</td>
</tr>
<tr>
<td>Postcentral gyrus</td>
<td>85.104</td>
<td>0.045</td>
<td>6</td>
<td>0.046</td>
<td>0.050</td>
<td>.960</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>117.736</td>
<td>0.059</td>
<td>15</td>
<td>0.115</td>
<td>2.700</td>
<td>.007</td>
</tr>
<tr>
<td>Precuneus cortex</td>
<td>62.752</td>
<td>0.033</td>
<td>3</td>
<td>0.023</td>
<td>-0.653</td>
<td>.514</td>
</tr>
<tr>
<td>Right accumbens</td>
<td>0.672</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>-0.216</td>
<td>.829</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>3.304</td>
<td>0.002</td>
<td>0</td>
<td>0</td>
<td>-0.478</td>
<td>.632</td>
</tr>
<tr>
<td>Right caudate</td>
<td>4.088</td>
<td>0.002</td>
<td>1</td>
<td>0.008</td>
<td>1.351</td>
<td>.177</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>6.120</td>
<td>0.003</td>
<td>0</td>
<td>0</td>
<td>-0.652</td>
<td>.515</td>
</tr>
<tr>
<td>Right pallidum</td>
<td>2.080</td>
<td>0.001</td>
<td>0</td>
<td>0</td>
<td>-0.379</td>
<td>.704</td>
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<tr>
<td>Right putamen</td>
<td>6.368</td>
<td>0.003</td>
<td>0</td>
<td>0</td>
<td>-0.665</td>
<td>.506</td>
</tr>
<tr>
<td>Right thalamus</td>
<td>11.208</td>
<td>0.006</td>
<td>3</td>
<td>0.023</td>
<td>2.536</td>
<td>.011</td>
</tr>
<tr>
<td>Subcallosal cortex</td>
<td>17.408</td>
<td>0.009</td>
<td>0</td>
<td>0</td>
<td>-1.102</td>
<td>.270</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>70.888</td>
<td>0.038</td>
<td>3</td>
<td>0.023</td>
<td>-0.875</td>
<td>.382</td>
</tr>
</tbody>
</table>
cohort of 150 lesions that fell within that ROI (Figure 4). We noted that 21 lesions fell outside the bounds of any ROI identified in the atlas. Therefore, the final analysis consisted of 130 observed BMs. An increased frequency of BMs can be appreciated along the major branches of the anterior cerebral artery in the region of the paracingulate (ie, precingulate) gyrus. There also appears to be a general increase in the frequency of BMs in superficial rather than deep brain structures.

The frequency of BMs observed was significantly different from the expected value in 10 ROIs at \( P \leq .05 \) and 4 ROIs at \( P \leq .01 \) (Table 2). Only the paracingulate gyrus reached significance after a Bonferroni correction for multiple comparisons (Figure 5). Four regions: frontal pole, lateral occipital cortex, supramarginal gyrus, and temporal pole, had fewer observed BMs that were predicted by the simulation, with \( P \)-values of .009, .031, .046, and .032, respectively. The possibility that this is an artifact of having a relatively small sample size of observed lesions cannot be ruled out from these data. Similarly, of the 52 anatomically defined brain regions in the atlas, 20 were not observed to have a BM, and this was likely influenced by the small sample size. We note that, consistent with the low rate of observed metastases in these ROIs, these 20 regions made up a small fraction of the total volume at risk (13.35%) with an average proportional volume of 0.67% (range, 0.04%–3.42%).

Finally, because the hippocampus has been the subject of prior investigations quantifying the frequency of BM involvement, we specifically reviewed results for this ROI. A review of the bilateral hippocampi and parahippocampal gyrus revealed that only 1 of the 150 total BMs in the cohort could be identified in the area. This is consistent with the literature, which has shown a near-zero rate of BM involvement with this structure (5, 6, 11).

**DISCUSSION**

Metastatic disease may develop anywhere within brain, but it may have a nonrandom distribution that has not previously been quantified. Here, we hypothesized that the spatial frequency of BMs follows a nonstochastic distribution. To test this, we identified 150 BMs in 28 patients treated at a single institution and used the major divisions of the Harvard–Oxford cortical and subcortical atlases to identify anatomic regions that were involved. We then compared these values with the expected rates under the assumption that all voxels were at equal risk and that the probability of a brain region being affected by a metastatic focus was a function of the region’s volume. Ten regions within the atlas showed a significantly different number of BMs that would be expected by chance, and the paracingulate cortex maintained significance after correction for multiple comparisons.

These results may be used to develop automated techniques to identify BMs where the nonuniform spatial distribution of these lesions could be used as a priori information to improve search algorithms. Of more immediate clinical relevance is the influence of our results on partial brain radiotherapy.

Prior investigations have shown a tendency for metastases to develop at terminal branches of arteries at gray–white boundaries...
and possibly at watershed areas (11, 12). Additional work has revealed variations in BM distribution across the supratentorial brain. In 1 study, ~70%–80% of lesions were identified in the cerebral hemispheres, 15%–20% in the cerebellum, and ≤3% in the brainstem, which are thought to roughly follow patterns of blood flow (6-8). Contrary to these results, a detailed autopsy analysis identified nearly equivalent rates of BM involvement in the cerebellum as compared with the cerebrum (9). One explanation for the varying results may be differential patterns of BM distribution based on the primary histology or may be an artifact of how finely the cerebrum is segmented.

Bender and Tome provided the first analysis to help inform this debate by using atlas-based brain segmentation and stratifying BMs by the histology of the primary cancer (13). This revealed that lung and breast cancer might have a predilection for the cerebellum, which aligns with prior autopsy results (9). The authors further show the potential for these data to influence BM treatment, where a nonuniform dose distribution may improve tumor control probability. These studies have provided insight into the pathophysiology of BMs, but have provided a broad description of BM distribution without an analysis of individual cortical and subcortical regions. A more granular understanding of BM distribution may permit sparing of additional brain regions with partial brain radiotherapy to further improve the therapeutic window.

A recently completed national clinical trial, RTOG 09-33 (NCT01227954), showed a reduction in neurocognitive toxicities associated with whole-brain irradiation for BM when the hippocampi were avoided (4). The rationale for sparing the hippocampus to spare side effects is bolstered by the finding that the area is at relatively low risk for developing BMs. Detailed analyses have been performed to assess the risk for failure within the hippocampus plus a margin. These studies have estimated that between 0% and 0.4% of BMs are located within the hippocampus and ~3% are within 5 mm of the hippocampus (5, 6, 14). This is considered an acceptably low risk and has allowed for IMRT to be used. In contrast to traditional whole-brain irradiation, IMRT allows for variable dose prescriptions to be delivered across the brain.

The success of RTOG 0933 has motivated additional studies, and partial brain techniques to spare the hippocampi are now being tested in numerous clinical trials. These include NRG-CC001, a phase II/III trial evaluating the role of memantine and brain irradiation with or without hippocampal avoidance (NCT02360215), and NRG-CC003 (NCT02635009), a phase II/III trial also comparing whole-brain treatment with or without hippocampal sparing in the setting of prophylactic cranial irradiation for small cell lung cancer. In addition to sparing particular brain areas, IMRT has the potential to ensure that high-risk regions receive adequate coverage or dose-escalated radiation. However, to the best of our knowledge, no ongoing trial uses any form of advanced radiation planning for this purpose. Tools to segment brain regions based on the rate of BM development would allow risk-based IMRT dosing to further improve the therapeutic window.

In the current study, patients with recurrent BMs were chosen because this is the population that may benefit from risk-adapted treatment with WBRT. For example, if a patient with BMs requires treatment for a low volume of disease and there is an opportunity to treat the brain partially, radiation may be tailored to avoid regions that are at a low risk of developing subsequent lesions. An additional rationale for analyzing patients with recurrent BMs is that this population is under close imaging surveillance, which typically identifies BMs when they are small and can be correlated to a specific anatomical focus. The selection of this patient population excludes those who have a cancer diagnosis, but do not have known brain involvement. Therefore, there may be limited application of these findings to patients being considered for prophylactic cranial irradiation, such as select patients with small cell lung cancer.

Another limitation of this study is the relatively small sample size of the clinically defined BMs. Although this does not challenge the interpretation that the paracingulate gyrus is an at increased risk of BM involvement, this may subject the study to more type II error. Further, the use of a stringent Bonferroni correction may further increase the false negative rate, but it strengthens the claim regarding the paracingulate gyrus. Therefore, it is likely that other brain regions are also at a relatively increased risk of developing BMs, and our data suggest that the paracingulate gyrus is among the most at-risk regions. To validate the findings of this study, reduce type II error, and improve applicability to other patient populations, future work should include a greater number of BMs and other histologies.

**Supplemental Materials**

Supplemental Video: http://dx.doi.org/10.18383/j.tom.2016.00268.vid.01

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**REFERENCES**


DCE-MRI Texture Features for Early Prediction of Breast Cancer Therapy Response

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Key Words: breast cancer, DCE-MRI, neoadjuvant chemotherapy, early prediction, 3D textural features, statistical matrices, residual cancer burden

Abbreviations: Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), neoadjuvant chemotherapy (NAC), pharmacokinetic (PK), residual cancer burden (RCB), locally advanced breast cancer (LABC), pathologic complete response (pCR), 2-dimensional (2D), 3-dimensional (3D), contrast agent (CA), regions of interest (ROIs), standard Tofts model (SM), shutter-speed model (SSM), area under the curve (AUC), gray-level cooccurrence matrix (GLCM), run length matrix (RLM), size zone matrix (SZM), local binary pattern (LBP), pattern spectrum (PS), pathologic nonresponse (pNR)

This study investigates the effectiveness of hundreds of texture features extracted from voxel-based dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) parametric maps for early prediction of breast cancer response to neoadjuvant chemotherapy (NAC). In total, 38 patients with breast cancer underwent DCE-MRI before (baseline) and after the first of the 6–8 NAC cycles. Quantitative pharmacokinetic (PK) parameters and semiquantitative metrics were estimated from DCE-MRI time-course data. The residual cancer burden (RCB) index value was computed based on pathological analysis of surgical specimens after NAC completion. In total, 1043 texture features were extracted from each of the 13 parametric maps of quantitative PK or semiquantitative metric, and their capabilities for early prediction of RCB were examined by correlating feature changes between the 2 MRI studies with RCB. There were 1069 pairs of feature–map combinations that showed effectiveness for response prediction with 4 correlation coefficients \( r > 0.7 \). The 3-dimensional gray-level cooccurrence matrix was the most effective feature extraction method for therapy response prediction, and, in general, the statistical features describing texture heterogeneity were the most effective features. Quantitative PK parameters, particularly those estimated with the shutter-speed model, were more likely to generate effective features for prediction response compared with the semiquantitative metrics. The best feature–map pair could predict pathologic complete response with 100% sensitivity and 100% specificity using our cohort. In conclusion, breast tumor heterogeneity in microvasculature as measured by texture features of voxel-based DCE-MRI parametric maps could be a useful biomarker for early prediction of NAC response.

INTRODUCTION

Neoadjuvant (preoperative) chemotherapy (NAC) was introduced in the 1970s, and over the past 2 decades, it has been established as a standard of care for patients with locally advanced breast cancer (LABC) for both initially operable and inoperable tumors (1–3). Compared with adjuvant (postoperative) chemotherapy, NAC has been shown to increase the breast-conserving surgery rate. Furthermore, the pathologic complete response (pCR) to NAC or minimal post-NAC residual disease has been found to be clearly correlated with disease-free and overall survival rates (1, 4–9). However, patients undergoing NAC do not always achieve pCR, and the pCR rate is reported to vary in the range of 6%–45% depending on breast cancer subtypes and treatment regimens (10–13). In the emerging era of precision medicine, early prediction of NAC response may allow rapid, personalized treatment regimen alterations for nonresponding patients with breast cancer, and spare them from potential short- and long-term toxicities associated with ineffective therapies. In addition, accurate evaluation of residual disease after NAC is vital for surgical decision-making and could result in surgical treatment plans that are more tailored to individual patients.

As a noninvasive imaging method for in vivo measurement of tissue microvascular perfusion and permeability, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is
increasingly used in clinical trial and research settings to assess breast cancer response to NAC (14). Changes in tumor size observed on the basis of DCE-MRI images are routinely used in clinical trials to assess tumor response to treatment. However, size change in response to therapy is often found to manifest later compared with changes in underlying tumor functions (15-18), such as vascularization and vascular permeability, cellularity, and metabolism. There is substantial literature showing that semiquantitative analysis (19-24) or quantitative pharmacokinetic (PK) analysis (25-35) of DCE-MRI time-course data can provide better early prediction of breast cancer pathologic response to NAC than image tumor size changes after 1–2 NAC cycles. We have recently shown (25, 36) that changes in tumor mean PK parameters after only 1 NAC cycle are good early predictors of pCR versus non-pCR.

However, the approaches to compute tumor mean DCE-MRI metrics, whether semiquantitative or quantitative, cannot capture the spatial heterogeneity of the tumor functions as measured by DCE-MRI, and therefore, valuable information could be missed during therapeutic response evaluation.

Texture analysis of semiquantitative or quantitative PK metrics from breast DCE-MRI has been shown to be effective in applications such as automatic lesion segmentation (37-39) and cancer diagnosis (40-46). Recently, Teruel et al. (47) presented a detailed analysis of 16 textural statistical features from T1-weighted DCE-MRI images that are capable of predicting early tumor response to NAC. However, this study used a 2-dimensional (2D) statistical texture description without taking advantage of the 3-dimensional (3D) information provided by the T1-weighted DCE-MRI. Moreover, these features were computed in a specific gray-level condition that reduced the number of potentially useful features. Golden et al. (48) used similar DCE-MRI texture features to predict therapeutic responses in terms of pCR, residual lymph node metastases, and residual tumor with lymph node metastases in patients with triple-negative breast cancer.

Here, we conducted a thorough analysis of hundreds of 3D statistical features extracted from parametric maps of semiquantitative and quantitative PK metrics that were obtained from a DCE-MRI study (25) of breast cancer response to NAC. We report our preliminary findings on the effectiveness of these features for early prediction of NAC response.

**MATERIALS AND METHODS**

**Patient Cohort and Study Schema**

In this institutional review board-approved and HIPAA (Health Insurance Portability and Accountability Act of 1996)-compliant study, 38 women (age range, 27–79 years) diagnosed with grades 2–3 invasive breast tumors and scheduled to undergo NAC consented to participate in a longitudinal research MRI study that includes DCE-MRI. In total, 31 of the 38 women were treated with standard-of-care therapy regimens that included 4 cycles of doxorubicin–cyclophosphamide administration every 2 weeks followed by 4 cycles of a taxane every 2 weeks, or 6 cycles of the combination of all 3 drugs every 3 weeks. The other 7 patients were enrolled in the NAC ISPY-2 trial, where patients were randomized to receive standard-of-care regimen or standard-of-care regimen plus experimental drugs. The ISPY-2 standard-of-care regimen started with a taxane administration followed by doxorubicin–cyclophosphamide administration. If used, the experimental drug was usually added to the taxane. In total, 4 of the 7 patients were placed in the treatment arm with experimental drug–3 received neratinib, a tyrosine kinase inhibitor, and 1 received ganitumab, a human monoclonal antibody against type 1 insulin-like growth factor receptor. The targeted agent, trastuzumab, was added to the NAC regimen for tumors with positive HER2 (human epidermal growth factor receptor 2) receptor status (n = 23).

MRI examinations for this research study were performed before NAC (visit 1, V1), after 1 cycle of NAC (V2), at midpoint of NAC (V3, usually after 3 or 4 NAC cycles, or before change of NAC agents), and after NAC completion but before surgery (V4). For the V2 – V1 studies, the MRI scan was undertaken at least 7 days after the administration of the previous NAC cycle to allow time for drug effects. This paper reports results for early prediction of NAC response, and thus, only the data from the V1 and V2 studies were used for texture analysis.

**DCE-MRI Data Acquisition and Analysis**

All breast MRI studies were performed using a 3T Siemens Tim Trio system with a body coil and a 4-channel bilateral phased-array breast coil as the radiofrequency transmitter and receiver, respectively. During each MRI session, following pilot scans and precontrast agent (CA) axial T2-weighted MRI with fat saturation and axial T1-weighted MRI without fat saturation, axial bilateral DCE-MRI images with fat saturation and full breast coverage were acquired with a 3D gradient echo-based TWIST (Time-resolved angiography With Stochastic Trajectories) sequence, which uses the strategy of k-space undersampling during acquisition and data sharing during reconstruction (49). DCE-MRI spatial resolution = 1.0 × 1.0 × 1.4 mm³ and temporal resolution = 14–20 seconds. Details of the acquisition parameters are described in Tudorica et al.’s study (25).

Breast tumor regions of interest (ROIs) were drawn by 2 experienced breast radiologists on postcontrast (~90–120 seconds after the gadolinium CA injection) multisection DCE images covering the entire contrast-enhanced tumor. For voxels within the tumor ROI, the DCE-MRI time-course data were separately fitted with the following 2 PK models: 1 with a 1-compartment–2-parameter standard Tofts model (SM) (50, 51) and another with a 2-compartment–3-parameter shutter-speed model (SSM) (52). Both model fittings returned parameters \( K^{\text{trans}} \) (rate constant for CA plasma-to-interstitium transfer) and \( V_e \) (volume fraction of extravascular and extracellular space), whereas the SSM also returned an additional parameter \( \tau_i \) (mean intracellular water molecule lifetime), which is used to account for the effect of cross cell membrane water exchange kinetics (in the extravascular space) in the SSM (52). Details of PK data analysis and mathematical formulations for the SM and SSM are described in Tudorica et al.’s study (25). CA intravasation rate constant, \( k_{ep} \), was calculated as \( k_{ep} = K^{\text{trans}} / V_e \). The \( dK^{\text{trans}} \)

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1 For more information, see: http://ispy2.org
parameter, defined as \( d \kappa^{\text{trans}} = \kappa^{\text{trans}(\text{SSM})} - \kappa^{\text{trans}(\text{SM})} \), provided a measure of the exchange effect on \( \kappa^{\text{trans}} \) estimation (53) and was also calculated. Voxel-based multisection parametric maps of the PK parameters (within the tumor ROI) were generated following the SM and SSM fittings.

In addition, the following 5 semiquantitative metrics were quantified from the DCE-MRI signal time-course data, and voxel-based parametric maps were generated (41, 54, 55):

1. Initial enhancement (WashIn) describes the initial signal increase from the precontrast value to the postcontrast value, as defined by the following equation:

\[
\text{WashIn} = \frac{s_{\text{max}} - s_0}{s_0}
\]

where \( s_0 \) is the average of the precontrast baseline signal intensity and \( s_{\text{max}} \) is the postcontrast signal intensity ~90 seconds after completion of CA injection.

2. Signal enhancement ratio (SER) characterizes the post-contrast signal curve shape, as defined by the following equation:

\[
\text{SER} = \frac{s_{\text{max}} - s_0}{s_{\text{last}} - s_0}
\]

where \( s_{\text{last}} \) is the last signal value.

3. After initial enhancement (WashOut), the signal curve behavior is described after the initial phase of contrast enhancement, as follows:

\[
\text{WashOut} = \frac{s_{\text{last}} - s_{\text{max}}}{s_{\text{max}}}
\]

4. Wash-in slope (SlopeIn) is a measure of the CA uptake rate, and is calculated using the following equation:

\[
\text{SlopeIn} = \frac{s_{\text{max}} - s_0}{t_{\text{max}}}
\]

where \( t_{\text{max}} \) is the time when \( s_{\text{max}} \) was measured.

5. Area under the curve (AUC), the signal integral value, is defined using the following equation:

\[
\text{AUC} = \int_{t_0}^{t_{\text{last}}} s(t)dt
\]

where \( t_0 \) and \( t_{\text{last}} \) are the first and last time points, respectively.

**Texture Feature Extraction**

This section presents the mathematical definition of the various texture features under study. We extracted several sets of features. The first 3 sets are based on the gray-level cooccurrence matrix (GLCM) (54, 56–60), the gray-level run length matrix (RLM) (59-63), and the size zone matrix (SZM) (60, 64, 65), and all belong to the family of statistical matrices. Once these matrices are constructed, texture features [such as Haralick features (56) and moments] can be derived. The fourth set of features is based on a local binary pattern (LBP) representation (66, 67), and the fifth set is based on the morphological operation called pattern spectrum (PS) (68, 69). In addition to these advanced features, we also include classic texture features that are based on moments of the image intensity pattern (70).

Statistical matrices have been extensively used in texture characterization, the best known of which is the GLCM, which leads to the definition of Haralick’s features (56). As a second-order statistic, and for an image \( f \), the \( \text{GLCM}_{\Delta}(i, j) \) represents the joint probability that a pixel with gray-level \( i \) occurs jointly with another pixel having a gray-level \( j \), for a given spatial offset \( \Delta \) between the pair of pixels. For an offset \( \Delta = (\Delta_x, \Delta_y) \), the GLCM is defined as follows:

\[
\text{GLCM}_{\Delta}(i, j) = \sum_{x} \sum_{y} \{ 1 \text{ if } f(x, y) = i \text{ and } f(x + \Delta_x, y + \Delta_y) = j \text{ otherwise} \}
\]

By design, the GLCM is dependent on the offset and is therefore not rotation-invariant (Figure 1A). When using 8-connexity, this is addressed by computing the GLCM in 4 directions, corresponding to offsets \( \Delta_x = (0, 1), \Delta_{25} = (1, 1), \Delta_{90} = (1, 0) \), and \( \Delta_{135} = (-1, 1) \), and the average matrix over all offsets can be used (57-59). In this study, we used the GLCM 3D formulation with 26-connexity, computed with 26 different offsets, and then we averaged the resulting matrices into a single matrix. On the basis of this matrix, we derived the following 16 second-order statistical features [known as the Haralick features (56)] for each image: average, contrast, correlation, energy (or second angular momentum), entropy, homogeneity, dissimilarity, inertia, variance, inverse difference momentum, sums average, sums
variance, sources entropy, differences variances, differences entropy, and maximum probability. The detailed mathematical definition of these Haralick features is listed in the Supplemental Appendix.

In addition to GLCM, another classical technique is a statistical matrix called the gray-level RLM (61), which has been extensively used for texture classification (60, 62, 63). Although the GLCM represents second-order statistical features, the RLM extracts higher-order statistical features. The matrix element $RLM_{f,g}(l, l)$ counts the total number of runs in the image $f$ with the gray-level $g$ and run length $l$ (ie, collinear pixels with the same intensity in the direction $\theta_i$, as shown in Figure 1C. This method is particularly effective for periodic textures, and it complements the information provided by the GLCM. From the RLM, we can extract features as the moments of order varying from $-2$ to $2$. Similar to the GLCM, this matrix requires computations using various directions to be rotation-invariant. In our study, we used the 3D RLM formulation computed in 26 directions.

Recently, Thibault et al. (60, 64, 65) introduced the gray-level SZM as an alternative to the joint RLM formulation. In this statistical matrix-based method, the value of the matrix element $SZM_f(s, g)$ is equal to the number of zones in the image $f$ with size $s$ and gray level $g$ (Figure 1D). The resulting matrix has a fixed number of rows equal to $t_s$ (the total number of gray levels), and a dynamic number of columns, determined by the largest zone size and size quantization. The structure of SZM reflects the image texture—the more homogeneous the texture (large, flat zones with similar gray levels), the wider and flatter the matrix. From this statistical matrix representation, we can calculate all the second-order moments as compact texture features (62), plus 2 more features, which are specific weighted variances (64).

Unlike GLCM and RLM, which depend on the offset $\Delta$ and the orientation $\theta$, respectively, the SZM matrix is invariant with respect to rotation and translation. In general, the RLM and GLCM are appropriate for periodic textures, whereas the SZM is more suitable for heterogeneous nonperiodic textures.

There are several SZM variants (65, 71). For example, the multiple gray-level SZMs incorporate the gray-level quantization. For an 8-bit image, it is computed from 8 SZMs for 8 different gray-levels ($N_k = 2^k, k = 1, 2, \ldots , 8$). The resulting matrices are combined by a weighted average using the following equation:

$$MSZM_f(s, g) = \sum_{k=1}^{8} w_k S_{SZM_{f,k}}(s, g)$$

where, $SZM_{f,k}$ is $SZM_{f}$ calculated from $T$ quantized in $N_k$ gray levels.

Remark. By design, all these statistical matrices are sensitive to image acquisition noise. To improve their robustness, the number of gray levels can be reduced before a matrix is constructed. Different methods exist to reduce the number of gray levels to $N$ possible values, for example, using a monotonically decreasing function or a cumulated histogram.

In addition to these statistical matrices-based features, we also extract a set of features known as the LBP (66). LBP is a simple yet very efficient texture operator, which labels the pixels of an image by thresholding the neighborhood of each pixel and produces a binary number. The LBP is widely used in pattern recognition because of its simplicity and computational speed and its efficacy in describing the local spatial structure of an image. The LBP provides a unique score for each pixel, and the scores’ distribution represents the texture features. Practically, for an image $f$ and a given pixel $p$ with 8 ordered neighbors $p_i$, $LBP(p) = \sum_{i=0}^{7} v_i 2^i$, with $v_i = 1$ if $p_i \geq p$, or 0 otherwise.

One of the most important properties of the LBP operator is its robustness to monotonic gray-scale changes caused, for example, by illumination variations. In Ojala et al.’s study (67), a rotation-invariant formulation is presented, making this feature even more versatile. However, no proper definition of LBP in 3D exists. The most effective solution remains to compute the 2D LBP score on the 3 plans (XY, XZ, and YZ) for each pixel, and then to use all these values to fill the same histogram.

The next texture-characterization technique we included in our study is the PS (68, 69). The PS features describe the shape and size of structures in an $n$-dimensional signal. Measurement of the PS is based on morphological operations, which use a variety of structuring elements to filter a signal at multiple spatial scales. The PS is the combination of a granulometry, and its dual operation is the antigranulometry. A granulometry is the distribution study of all the object sizes present in an image. Formally, a granulometry is a family of morphological openings $\Gamma = (\gamma_n)_{n \geq 0}$ that depend on a positive parameter $n$, which expresses a size factor for a fixed structuring element. The granulometric analysis of an image $f$ with respect to $\Gamma$ involves evaluating each opening of size $n$ with a measurement $f(\gamma_n(f))$. The PS curve $PS_n$ of $f$ with respect to $\Gamma$ and $\Phi$ [the antigranulometry, $\Phi = (\nu_n)_{n \geq 0}$, a family of closings] is defined by the following normalized mapping:

$$PS_n(f) = \frac{1}{f} \left( \int \gamma_n(f) - \int \gamma_{n+1}(f) \right) \text{ for } n \geq 0$$

$$PS_n(f) = \frac{1}{f} \left( \int \Phi_n(f) - \int \Phi_{n-1}(f) \right) \text{ for } n < 0$$

The PS value for each size $n$ is a probability density function (ie, a histogram), and it corresponds to a structure measurement: a peak in PS at a given scale $n$ indicates the presence of many image structures of this scale or size. PS size distribution is a powerful gray-level and rotational-invariant texture descriptor (72).

In addition to these advanced texture features, we also added a set of classical moment-based texture features that include volume, average, and standard deviation. The statistical matrices were computed using different gray-level reduction values and algorithms. Counting all the texture features (GLCM, RLM, SZM, LBP, PS, and moments features), the total number of extracted features for each parametric map was 1043, distributed as shown in Table 1.

Remark.

- All the features presented in this paper are deterministic mathematical functions. They do not use any random parameter and, consequently, are reproducible.
- The PS and LBP are powerful texture-characterization techniques. They describe a texture by providing distributions of patterns or sizes; therefore, their effectiveness comes from the combination of all the provided feature values. However, our small cohort size prevents simultaneous use of multiple features. In our study, we analyzed each feature individually (see section on Features’ Evaluation below), by
using one feature at a time for the subsequent RCB prediction.

Pathological Analysis
The status of pathologic response (to NAC) for each breast tumor was determined by pathological analysis of the post-NAC resection specimen. The following pathology parameters were measured from the resection specimen under light microscopy: cross-sectional tumor size in 2D, estimated invasive tumor cellular density, number of involved lymph nodes, and the greatest tumor dimension in the largest involved node. These measures were used to compute the RCB index value using an equation published by Symmans et al. (6). pCR is defined as the absence of residual invasive tumor with \( RCB = 0 \). A pathologic nonresponse (pNR) is defined as tumor cell density in a resection specimen that is either equal to or greater than the tumor cell density in a core biopsy specimen. Pathologic partial response is defined as findings intermediate between pCR and pNR. Non-pCR includes both pathologic partial response and pNR with \( RCB > 0 \).

Features’ Evaluation
This paper aims to determine the capability of each map–feature pair to early predict the RCB index value for patients with LABC undergoing NAC. To do so, we have extracted a total of 1043 texture features in 3D from each of the 13 parametric maps, as presented in the previous section. They include Haralick features (from GLCM), RLM, SZM, LBP, PS, and basic moments, computed with various parameters and with gray-level reduction values and techniques. The capability of each feature is then evaluated individually using the following pipeline (see Algorithm 1):

- For a given feature \( f \) and a given parametric map \( p \), we extracted the feature for each patient at visit \( V_1 \) and \( V_2 \).
- We computed the feature gradient, defined as the difference of feature values between \( V_1 \) and \( V_2 \).
- Gradient outliers were then removed to improve the algorithm robustness.

\begin{algorithm}
\begin{itemize}
  \item The remaining gradients\(^2\) were then centered (with an average of 0), whitened (with a normalized standard deviation of 1), and used as inputs in a ridge regression model (73) coupled with a leave-one-out cross validation to predict the corresponding normalized (to increase the computational precision) pathologically measured RCB index values.

  The ridge regression was preferred over the classical linear regression because it adds a penalty term to the coefficients as a way of regularization, leading to reduced risk of overfitting and improved generalization capability. These penalty advantages are particularly beneficial because of the small number of patients available in our cohort. Moreover, by coupling the ridge regression with the leave-one-out cross validation protocol, the risk of overfitting is further reduced.

  The predicted RCB index values using the feature were finally compared with the pathologically measured RCB values, and 4 correlations (using different paradigms) were computed for feature evaluation, namely, the Pearson product moment (linear), the Spearman rank–order (rank), the Kendall tau (rank), and the Goodman–Kruskal gamma (rank).
\end{itemize}
\end{algorithm}

RESULTS
According to the pathology analysis of the resection specimens, 9 patients were pCRs with \( RCB = 0 \), whereas the other 29 patients were non-pCRs, with RCB index values ranging from 0.43 to 4.1. These RCB index values were the inputs to the pipeline described by Algorithm 1 to evaluate the predictive

\(^2\) Practically, a maximum of 2 outliers were removed. Consequently, the smallest data set used contained 36 values.
ability for each of the 1043 3D texture features extracted from a total of 13 DCE-MRI parametric maps (both quantitative PK parameters and semiquantitative metrics). Consequently, a total of 13 559 map–feature pairs were investigated. To be considered as an effective predictor, a map–feature pair must have all 4 correlation coefficients $\geq 0.7$. This “effectiveness” threshold value was empirically determined by the visual analysis of “true RCB versus predicted RCB curves”.

Among the 13 559 map–feature pairs investigated, 1069 (7.9%) were found to meet the effectiveness conditions.

### Texture Features for Prediction of Therapy Response

The feature distribution among the 1069 best feature–map pairs with all 4 correlation coefficients $\geq 0.7$ is presented in Table 2. It is worth noting that the texture-extraction techniques (GLCM, RLM, SZM, etc.) provide an imbalanced number of features (Table 1). To fairly compare the effectiveness of these techniques, the distribution of the 1069 feature–map pairs was weighted according to the original distribution shown in Table 1—the higher the number of features provided by a technique, the lower the weight. For example, in Table 2, among the 1069 feature–map pairs, only 6 use moments, or 0.57%. The moments were also the technique generating the lowest number of features (6 features, representing 0.58% of all the generated features in Table 1). After correction through weighting, the 6 feature–map pairs using moments now represent 7.69% of the best pairs (Table 2). Because of this correction, the GLCM is the most frequently used feature-extraction technique, whereas the other techniques have comparable percent distribution.

### MRI Metrics for Prediction of Therapy Response

Figure 2 shows how often quantitative PK parameters and semiquantitative metrics provided good texture features for early prediction of therapy response under the effectiveness condition of all 4 correlation coefficients $\geq 0.7$. It appears that the SSM parametric maps ($K_{\text{trans}}(\text{SM})$, $dK_{\text{trans}}$, $k_p(\text{SSM})$, $K_{\text{trans}}(\text{SM})$, $T(\text{SSM})$, $v_5(\text{SSM})$, $\text{WashOut}$, $\text{AUC}$, $\text{SlopeIn}$, $\text{SER}$, $\text{Washin}$, $v_5(\text{SM})$, $k_p(\text{SM})$) are more likely to provide a good predictive feature than the SM PK parameters or the semiquantitative metrics.

After increasing the effectiveness condition threshold from 0.7 to 0.875, we find 9 feature–map pairs with all 4 correlation coefficients $\geq 0.875$, as shown in Table 3. The GLCM (coupled with Haralick features) and the SSM PK parameters are the most frequently used features that are effective for prediction of NAC response, accounting for 6 out of the 9 best pairs. This is consistent with the results presented in Table 2 and Figure 2.

Figure 3 shows examples of the correlations between the normalized pathologically measured RCB index values and the predicted RCB index values using $K_{\text{trans}}(\text{SM})$ + RLM + Gray-level nonuniformity feature (Figure 3A) and $v_5(\text{SSM})$ + Haralick + Contrast feature (Figure 3B) as predictive features, respectively. These correlations are nonlinear, even though the predictive models were built using the linear model of ridge regression. It is
not mathematically possible to model a nonlinear problem using a linear approach, which sets a mathematical limit to the performance prediction. It also explains why the predicted RCB index values are accurately ranked, but the range of predicted values is smaller than that of the pathologically measured RCB index values. Nonetheless, even with a ridge regression, we can observe in Figure 3 that the predictive models can separate the patients with pCR (green dots; n = 9) from those with non-pCR (red dots; n = 29) with high accuracy. At 100% sensitivity (ie, correctly classifying all pCRs), the specificities are 100% and 96.7% for $K_{\text{trans}}(SM) + \text{RLM}$ + Gray Level Non Uniformity feature and $v_e(SSM) + \text{Haralick} + \text{Contrast feature}$, respectively. Figure 4 shows the $v_e(SSM)$ map at visits $V_1$ and $V_2$ for a tumor with pCR (Figure 4A) and a tumor with non-pCR (Figure 4B). The Haralick contrast feature value increases by $\sim 450\%$ for the tumor with pCR and by $\sim 30\%$ for the tumor with non-pCR.

**DISCUSSION**

This preliminary study with a 38-patient cohort shows that changes in breast tumor heterogeneity as measured by changes in texture features of DCE-MRI voxel-based parametric maps can be useful markers for early prediction of breast cancer response to NAC, discriminating pCR versus non-pCR, as well as predicting low versus high post-NAC RCB index value. Although it clearly needs to be validated with larger patient populations, this noninvasive 3D imaging feature-extraction approach has the potential to become an important clinical tool in the emerging era of precision medicine to identify, in the early stages of treatment, nonresponding patients for alternative personalized therapy regimens, and stratify patients for better surgical decision-making, and after surgery care planning based on accurate prediction of RCB. For example, using the $\text{RLM} + \text{Gray-level nonuniformity feature}$ of the $K_{\text{trans}}(SM)$ map (Figure 3A), the 29 non-pCRs can be discriminated from the 9 pCRs with 100% sensitivity and specificity, and consequently, an AUC of receiver operating characteristic equal to 1, after only 1 out of 6–8 NAC cycles. In comparison, the mean parametric value previously suggested as an imaging marker for therapy response prediction (25) never reaches the effectiveness condition (all 4 correlations $>0.7$). When this feature is used to classify pCR versus non-pCR, the best AUC of the receiver operating charac-

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**Figure 3.** Fitted curves between the normalized pathologically measured residual cancer burden (RCB) index value and the predicted RCB: $K_{\text{trans}}(SM) + \text{RLM} + \text{Gray Level Non Uniformity feature}$ (A) and $v_e(SSM) + \text{Haralick} + \text{Contrast feature}$ (B). Patients with pathologic complete response (pCR) and non-pCR are represented with green and red dots, respectively.

**Figure 4.** Example that shows the changes in $v_e(SSM)$ maps change examples for the following 2 tumors: one tumor with pCR at $V_1$ (A) and $V_2$ (B), and the other tumor with non-pCR at $V_1$ (C) and $V_2$ (D). The Haralick contrast feature value (Figure 3B) increased by $\sim 450\%$ for the tumor with pCR and increased by $\sim 30\%$ for the tumor with non-pCR.
Efficient 3D DCE-MRI Texture Features

This study shows that texture features of DCE-MRI quantitative PK parameters are likely to be more useful than those of the semiquantitative metrics for early prediction of breast cancer NAC response, showing the benefit of performing PK modeling of the DCE time-course data. In addition, we found that the good predictive texture features are more likely to come from SSM than from SM parameters, although we observed comparable predictive capabilities between percent changes of tumor mean SM and SSM parameters after 1 NAC cycle in a separate study (25). This is possibly because of the fact that compared with SSM, the SM has a tendency to underestimate PK parameters (\(K_{\text{trans}}\) and \(v_e\)) in malignant breast tissue, but not in benign or normal breast tissue (53). The SSM PK parametric map of the same breast tumor is expected to provide a larger dynamic range of the voxel parameter values than its SM counterpart, and thus, provide a more robust characterization of heterogeneity and its changes induced by therapy.

The results presented in this study were obtained using a single 3D texture feature extracted from a single parametric map, when previous work used a set of 2D features (47, 48) for early prediction response. Features in 3D offer more robust and efficient characterizations of early breast tumor changes than classical 2D features. This study, conducted on 1043 features and 13 maps, provides better characterization and evaluation of feature and map capabilities compared with the study that used 2D features. If a larger cohort of patients becomes available in the future, such information will allow us to build more complex models combining multiple features and maps for a better early prediction of breast cancer response to NAC.

This study has several limitations. First, the sample size of the study is small, and thus, it is important to validate the initial findings from this study with a larger patient cohort in the future. Second, mean tumor DCE-MRI parameter values were used in this study to assess breast tumor response to preoperative therapy. The tumor heterogeneity that is reflected in the imaging metrics, for example, in the \(K_{\text{trans}}\) maps, was not captured in the mean DCE-MRI parameter values. The potential integration of mean values and texture features of DCE-MRI metrics may further improve the effectiveness of quantitative DCE-MRI for assessment of therapy response. The third limitation is the use of the ridge regression model (a linear technique) to predict a nonlinear phenomenon. A nonlinear regression technique trained with \(>1\) feature from different maps, and on a larger cohort of patients, should increase the early response prediction. The last limitation is that the statistical matrices and the PS are not robust to resolution variations, and consequently, their efficacy may be reduced if the resolution is degraded. However, resolution degradation does not automatically lead to performance degradation. Further study is needed to elucidate the relationship between the 2.

In conclusion, we have investigated the capabilities of thousands of 3D texture features derived from different quantitative DCE-MRI maps for early prediction of NAC, on a cohort of 38 patients with LABC. Tumor texture heterogeneity changes captured by 3D statistical features measured using quantitative DCE-MRI parameters such as \(K_{\text{trans}}(\text{SSM})\) are promising markers for early prediction of pathologic response outcome.

**Supplemental Materials**

Supplemental Appendix: http://dx.doi.org/10.18383/j.tom.2016.00241.sup.01

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REFERENCES


Intrathoracic Fat Measurements Using Multidetector Computed Tomography (MDCT): Feasibility and Reproducibility


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Abbreviations: Cardiovascular disease (CVD), body mass index (BMI), coronary artery disease (CAD), multidetector computed tomography (MDCT), computed tomography (CT), coronary CT angiography (CCTA), epicardial fat volume (EFV), region of interest (ROI), Advantage Workstation (AW), Hounsfield Unit (HU)

Intrathoracic fat volume, more specifically, epicardial fat volume, is an emerging imaging biomarker of adverse cardiovascular events. The purpose of this work is to show the feasibility and reproducibility of intrathoracic fat volume measurement applied to contrast-enhanced multidetector computed tomography images. A retrospective cohort study of 62 subjects free of cardiovascular disease (55% females, age \(49 \pm 11\) years) conducted from 2008 to 2011 formed the study group. Intrathoracic fat volume was defined as all fat voxels measuring \([-50, 250]\) Hounsfield Unit within the intrathoracic cavity from the level of the pulmonary artery bifurcation to the heart apex. The intrathoracic fat was separated into epicardial and extrapericardial fat by tracing the pericardium. The measurements were obtained by 2 readers and compared for interrater reproducibility. The fat volume measurements for the study group were \(141 \pm 72\) cm\(^3\) for intrathoracic fat, \(58 \pm 27\) cm\(^3\) for epicardial fat, and \(84 \pm 50\) cm\(^3\) for extrapericardial fat. There was no statistically significant difference in intrathoracic fat volume measurements between the 2 readers, with correlation coefficients of 0.88 \((P = .55)\) for intrathoracic fat volume and 0.12 \((P = .33)\) for epicardial fat volume. Voxel-based measurement of intrathoracic fat, including the separation into epicardial and extrapericardial fat, is feasible and highly reproducible from multidetector computed tomography scans.

INTRODUCTION

Cardiovascular disease (CVD) remains the number one cause of mortality among adult men and women in the USA despite recent decrease in the mortality rate (1). A major contributor to increased CVD events is central obesity, which has been implicated as a cardiovascular risk factor and a public health problem (2). Several studies have shown that increased abdominal visceral fat is a strong predictor of metabolic syndrome and CVD (3–5). Waist circumference and body mass index (BMI) are commonly used anthropometric measures for quantifying general and regional adiposity (3–8). However, both measures have been criticized for providing general measurements that do not directly correlate well with the underlying visceral fat component (6–8), such as abdominal and intrathoracic fat that are more highly correlated with cardiovascular risk compared with waist circumference and BMI alone (9, 10). An independent association between increased intrathoracic fat volume (ie, intrapericardial fat) and abdominal fat volume with atrial fibrillation and coronary artery disease (CAD) was shown (11–13). However, most of these studies do not explicitly describe the methodology used to quantify thoracic fat or define intrathoracic fat compartments. Multidetector computed tomography (MDCT) scans are often used in research protocols for the measurement of visceral or intrathoracic adiposity (14), but these have been limited in the clinical setting owing to cost and radiation exposure. Some centers have developed and validated in-house semi- and full-automated software as part of research tools to calculate the epicardial fat volume from noncontrast-enhanced computed tomography (CT) (15). Although the epicardial fat may be an important measurement for identifying individuals at increased CVD risk (11, 12), the feasibility of intrathoracic fat compartment measurements obtained from clinically acquired contrast-enhanced chest MDCT scans using commercially available software has not been assessed. In this paper, we discuss the definitions of intrathoracic fat, its clinical significance, and the methodology of quantifying intrathoracic fat volume.
The primary goal of this study is to show the feasibility of intrathoracic fat volume measurements from prior clinically acquired contrast-enhanced cardiac MDCT examinations using commercially available postprocessing software. The second goal of the study is to show the reproducibility of the intrathoracic fat volume measurements by testing the inter-reader variability using the Bland–Altman interobserver variability test.

**MATERIALS AND METHODS**

**Study Population**
The study was approved by the Institutional Review Board with a waiver of informed consent. In total, 62 normal subjects free of any CVD formed the study population. The normal subjects were included after retrospectively reviewing the medical charts and coronary CT angiography (CCTA) reports of 675 adult subjects with atypical chest pain presenting either in the emergency department or at an outpatient clinic, who underwent CCTA between January 2006 and December 2011. The 62 subjects (34 females, 55%, and 28 males, 45%; mean age, 49 ± 11 years; age range, 24–72 years) fulfilled the following inclusion criteria:

1. No evidence of CAD (normal electrocardiogram and normal retrospectively gated CCTA).
2. No CAD risk factors, such as hypertension, hypercholesterolemia, diabetes, or structural heart disease (normal medical history and physical examination).
3. Low pretest probability for CAD based on Framingham criteria (16).

No major adverse cardiac events were noted at the subsequent 6-month chart review in any subject. Height, weight, BMI, age, and gender were recorded for all subjects.

**CT Acquisition Technique**
All subjects underwent assessment of the vital signs (blood pressure and heart rate) at least 1 hour before the CCTA. Subjects with heart rate >65 beats/min underwent oral premedication with 50 mg of metoprolol at least 45 minutes before CT acquisition. Heart rate of ≤60 beats/min was achieved in all patients during the scan. All subjects underwent premedication with 1 puff of sublingual nitroglycerin 1–5 minutes before the CT scan. All scans were acquired with the patient in the supine position and with arms elevated above and behind the head on a 64-row MDCT (Lightspeed VCT; GE Healthcare, Milwaukee, WI). Image acquisition was performed with electrocardiogram gating in the craniocaudal direction at end-inspiration within a single breath-hold. The scan z-axis coverage ranged from 2 cm above the most cephalad coronary artery to 2 cm below the cardiac apex. The scan parameters were as follows: section thickness = 0.625 mm, tube voltage = 100–120 kVp, gantry rotation time = 0.35 seconds, and the current unit (mA) was adjusted for patient size based on a BMI look-up table.

Iso-osmolar contrast material (Visipaque 370; GE Healthcare) was administered through an 18-g intravenous cannula placed in the right antecubital vein. A test bolus of 15 mL of contrast material was injected at 5 mL/s with the region of interest (ROI) placed in the aortic root at the level of the left main coronary artery. For each patient, a Hounsfield Unit (HU) time graph was obtained, from which the scan delay was calculated as peak enhancement plus 6 seconds. The dedicated CCTA acquisition was then acquired using a triphasic contrast bolus with a total of 80 mL of contrast material. The first 50 mL of contrast material was followed by 30 mL of contrast material diluted with 30 mL of normal saline. A 50 mL of normal saline push comprised the final phase of the bolus injection. The entire volume was delivered at a rate of 5 mL/s. All examinations were performed using retrospective gating with tube current modulation (100% peak tube current during mid- to end-diastole and up to 80% reduction at end-systole) to reduce radiation exposure.

**Definition of Thoracic Fat Compartments**
The intrathoracic adipose tissue is defined as the adipose tissue surrounding the heart, enclosed by the inner aspect of the sternum, spine, and lungs, extending from the bifurcation of the pulmonary artery through the cardiac apex over the diaphragm. It includes both the extrapericardial and epicardial fat.

The epicardial adipose tissue is defined as the adipose tissue enclosed by the visceral pericardium and is concentrated in the atrioventricular and interventricular grooves, along the major branches of the coronary arteries, around the atria, over the free wall of the right ventricle, and over the apex of the left ventricle (17) (Figure 1). The extrapericardial fat is defined as the adipose tissue situated on the external surface of the parietal pericardium within the mediastinum, alternatively termed the mediastinal fat (17).

**CT Image Reconstruction, Fat Measurement Technique, and Postprocessing**
All reconstructed images were postprocessed on a GE Advantage Workstation (AW) (version 4.5, GE Healthcare) using the Refor-
mat software tool (GE Healthcare). Further 10–14, 10-mm-thick contiguous axial sections were obtained using the Reformat tool and batch lines off the previously obtained CCTA axial images, with coverage extending from the bifurcation of the pulmonary artery through the cardiac apex over the diaphragm (Figure 2). The fat volume of each thoracic compartment was obtained by tracing the mediastinal and epicardial areas in a systematic fashion as detailed below, and data were processed using a histogram-based statistical program based on the method described by Borkan et al. (18). The field of view encompassed all soft tissues of the chest at that level. All CT reformats were performed on the same AW workstation to reduce measurement error. Reformats were loaded into the Reformat software on the AW workstation to measure the thoracic fat compartments using semiautomatic segmentation.

The total fat represented the adipose tissue covering from the bifurcation of the pulmonary artery through the cardiac apex over the diaphragm (Figures 1 and 2). The resulting histogram displayed the computer-generated volume of all 10–14 10-mm-thick sections. The fat volume was then calculated in cubic centimeters by designating an attenuation threshold that would isolate and quantify fat. The threshold range was set from $-250$ to $-50$ HU to allow for the lower density of fat by bone artifact (18). Another ROI was manually traced at the interface between the mediastinal fat and lungs and adjacent vertebral bodies and paraspinal musculature on all sections. This tracing was placed at the inner edge of the interface to completely exclude the subcutaneous fat, osseous structures, and lungs. The area outside this tracing was deleted, leaving an internal area designated as “intrathoracic”. The intrathoracic volume comprised the extrapericardial and epicardial volumes (Figure 1). The intrathoracic and fat volumes were calculated as explained above. The last ROI was traced along the pericardium on all sections to completely include the epicardial volume/fat. The region outside this tracing was deleted, leaving an internal area designated as “epicardial” (Figure 3). Similar to the other regions, the epicardial volume and epicardial fat volume were calculated. Subtracting these volumes from the intrathoracic volume and fat volume resulted in the “extrapericardial” and fat volumes, respectively. This methodology of obtaining the fat volume measurements in the thorax is in agreement with the defined anatomy of the epicardium, pericardium (pericardial sac), and mediastinum (15, 17).

**Reproducibility**

All thoracic fat and volume measurements were performed by a single CT-certified technologist with >5 years of experience in advanced image processing. To test for inter-reader variability of the contouring, all 62 examinations were independently analyzed 6 months later by a second reader (a cardiothoracic fellowship-trained radiologist with >7 years of experience) using the same methodology used by the first reader, and blinded to the patient information and results from the first reading.

**Statistical Analysis**

Continuous data were presented as mean ± standard deviation or median (25th–75th interquartile range), as appropriate. Categorical data were presented as numbers and percentages. The univariate association between the tested variables was assessed with the Student $t$ test for continuous variables with normal distribution, Wilcoxon test for continuous variables without normal distribution, and $\chi^2$ test or Fisher test for categorical variables, as appropriate. A general linear model was used to evaluate the associations between the thoracic fat volumes and...
Women had 36% less extrapericardial fat than men (103 ± 57 cm$^3$; $P = .004$). Women also had less intrathoracic fat (difference of 40 ± 18 cm$^3$ from the men’s volume of 162 ± 79 cm$^3$; $P = .03$).

### Influence of BMI on Thoracic Fat Compartments

Our study population group is represented by 12/63 (19%) normal, 33/63 (52%) overweight, and 18/63 (28%) obese subjects (mean, 28.5 ± 4.7 kg/m$^2$; median, 28 kg/m$^2$; range, 16.6–43 kg/m$^2$). The mean BMI in women was 28.5 ± 5.3 kg/m$^2$ and ranged between 16.6 and 43 kg/m$^2$ (median, 27.7 kg/m$^2$), and in men, the BMI was 28.6 ± 4 kg/m$^2$, ranging between 24 and 42 kg/m$^2$ (median, 28.1 kg/m$^2$). There was no statistically significant difference in the BMI distribution according to gender ($P$-value of .65). The mean BMI distribution by the 3 groups was as follows:

- **group 1 (n = 12):** 23 ± 2 kg/m$^2$, range: between 16.6 and 24.8 kg/m$^2$ (median, 23.8 kg/m$^2$);
- **group 2 (n = 33):** 27.4 ± 1 kg/m$^2$, range: between 25 and 29.6 kg/m$^2$ (median, 27.7 kg/m$^2$); and
- **group 3 (n = 18):** 34.3 ± 4.1 kg/m$^2$, range: between 31 and 42.9 kg/m$^2$ (median, 32.5 kg/m$^2$).

There was statistically significant difference in the BMI distribution according to obese groups ($P$-value of .0001). There were 6/12 (50%) women in the normal group, 17/33 (49%) women in the overweight group, and 10/18 (60%) women in the obese group. No statistically significant difference was observed

### RESULTS

#### Baseline Characteristics

The mean systolic and diastolic blood pressures were 122 ± 9 and 73 ± 10 Hg, respectively. The median BMI was 27 kg/m$^2$, and the 25th–75th interquartile range (IQR) was 24–30 kg/m$^2$. The sample study characteristics for all subjects, stratified by gender, are presented in Table 1.

#### Thoracic CT Fat Compartments

Thoracic MDCT fat volume measurements in cubic centimeters in all patients and stratified by gender are presented in Table 1. The intrathoracic fat volume consisted of 59% extrapericardial fat volume (83 cm$^3$ out of the total of 140 cm$^3$) and 41% epicardial fat volume (57 cm$^3$ out of the total of 140 cm$^3$). Women had 36 ± 12 cm$^3$ less extrapericardial fat than men (103 ± 57 cm$^3$; $P = .004$). Women also had less intrathoracic fat (difference of 40 ± 18 cm$^3$ from the men’s volume of 162 ± 79 cm$^3$; $P = .03$).

### Table 1. Study Sample Characteristics

<table>
<thead>
<tr>
<th></th>
<th>All subjects (N = 62)</th>
<th>Women (N = 34/62)</th>
<th>Men (N = 28/62)</th>
<th>P-Value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48 ± 11</td>
<td>49 ± 11</td>
<td>47 ± 11</td>
<td>.51</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>27, 24–30</td>
<td>27, 24–30</td>
<td>27, 25–30</td>
<td>.73</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>79, 72–91</td>
<td>78, 70–88</td>
<td>81, 74–95</td>
<td>.25</td>
</tr>
<tr>
<td>Intrathoracic fat [cm$^3$]</td>
<td>141 ± 72</td>
<td>125 ± 62</td>
<td>162 ± 79</td>
<td>.04$^b$</td>
</tr>
<tr>
<td>Epicardial fat [cm$^3$]</td>
<td>58 ± 27</td>
<td>56 ± 28</td>
<td>59 ± 26</td>
<td>.62</td>
</tr>
<tr>
<td>Extrapericardial fat [cm$^3$]</td>
<td>84 ± 50</td>
<td>69 ± 38</td>
<td>103 ± 57</td>
<td>.02$^b$</td>
</tr>
</tbody>
</table>

The sample characteristics are presented as median, interquartile range (IQR) or mean ± standard deviation (SD), where appropriate, in all subjects and stratified by gender.

$^a$P is the statistical significance between the thoracic fat compartment measurements and gender using the Student $t$ test.

$^b$Significant at level <.05 using Wilcoxon test for continuous variables without normal distribution and Student $t$ test for continuous variables with normal distribution.

### Table 2. Thoracic Fat in Cubic Centimeters Stratified by Obesity

<table>
<thead>
<tr>
<th></th>
<th>Normal (N = 18)</th>
<th>Overweight (N = 33)</th>
<th>Obese (N = 18)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat, cm$^3$</td>
<td>736 ± 300</td>
<td>700 ± 235</td>
<td>783 ± 284</td>
<td>.50</td>
</tr>
<tr>
<td>Mediastinal fat, cm$^3$</td>
<td>134 ± 88</td>
<td>133 ± 53</td>
<td>146 ± 64</td>
<td>.72</td>
</tr>
<tr>
<td>Pericardial fat, cm$^3$</td>
<td>57 ± 37</td>
<td>55 ± 22</td>
<td>56 ± 21</td>
<td>.87</td>
</tr>
<tr>
<td>Epicardial fat, cm$^3$</td>
<td>76 ± 54</td>
<td>78 ± 37</td>
<td>91 ± 48</td>
<td>.59</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
with respect to gender and obesity groups (P-value of .94). Table 2 presents thoracic fat in cubic centimeters stratified by the 3 obesity groups. No statistically significant difference was seen between total, mediastinal, pericardial, and epicardial fat and BMI as a continuous variable. In addition, no statistically significant difference was found between the obese and overweight groups compared with the normal group. The statistically significant difference was not reached even with gender stratification.

Inter-Reader Agreement
There was no statistically significant difference in the intrathoracic or epicardial fat volumes between the readers by either Pearson correlation coefficient or Bland–Altman analysis (P-value = .50 for intrathoracic fat volume and 0.33 for epicardial fat volume) (Table 3). Figure 4 shows the Bland–Altman plots showing the inter-reader differences for the intrathoracic (A) and epicardial (B) fat volume measurements.

**DISCUSSION**
In this cross-sectional retrospective cohort study, we showed the feasibility and inter-reader reproducibility of intrathoracic fat volume measurements on prior clinically acquired contrast-enhanced cardiac MDCT examinations using commercially available postprocessing software.

The layers surrounding the heart are composed of intra- and extrapericardial fat (17). We used the pericardium as a landmark to divide the intrathoracic fat into intra- (epicardial) and extrapericardial fat compartments, as they have different embryological origins, blood supply, and functional properties such as the secretion of adipokines. The extrapericardial fat, also known as paracardial fat, is defined as the fat tissue external to the parietal pericardium. It originates from the primitive thoracic mesenchyme that also forms the outer thoracic wall and is supplied by a pericardiophrenic artery, which is a branch of the internal mammary artery (17).

In contrast, the epicardial fat, also known as intrapericardial fat, is defined as the fat tissue enclosed by the visceral pericardium that is composed of mesothelial cells and is supplied by the coronary arteries that also supply the myocardium (17, 20). The epicardial fat is in direct contact with the surface of the myocardiun and coronary arteries with no separation by a physical fascia, and it is virtually impossible to accurately dissect the epicardial fat from the myocardium ex vivo (21). Thus, molecules secreted by the epicardial fat may diffuse between the fat and these adjacent structures. The epicardial fat, for example, the omental and mesenteric fat, shares a common origin as arising from the splanchnopleuric mesoderm associated with the gut (17). A dichotomous role, both protective and detrimental, has been attributed to the epicardial fat. Under normal physiological conditions, the epicardial fat may serve as a buffer, absorbing fatty acids and protecting the heart against high fatty acids levels, and may release factors such as adiponectin that blunt the toxic effects of high fatty acid levels on the myocardium (22). Nevertheless, with fat accumulation in the epicardial fat depot, recognized as one of the ectopic sites in increased abdominal visceral adiposity, the epicardial fat may promote atherosclerotic changes in the coronary arteries and myocardium by triggering a cellular and molecular inflammatory cascade that leads to increased lipolysis, decreased adiponectin, and increased leptin levels (17). Adiponectin is known for its anti-inflammatory and antiatherogenic properties; thus, reductions in adiponectin may reduce its potential vasoprotective effects and play an important role in metabolic syndrome and CVD (17, 22–24). Excessive epicardial fat, but not extrapericardial fat, has been shown to be associated with CAD and decreased cardiac function (23, 24) and it is an emerging imaging biomarker for identifying patients at risk for CVD.

The term epicardial fat is variably used to describe the adipose tissue in the space either between the visceral and parietal pericardium, that is, pericardial sac, between the pericardium and the myocardium, just external to the pericardium, or in the intrathoracic space (11, 12, 21). All studied subjects had normal pericardium thickness, and the studied ROI was placed on the pericardium itself, which we used as a landmark to discern the intra- from extrapericardial fat compartments; therefore, the space between the visceral and parietal pericardium was included in the epicardial fat volume measurements. In reality, the layers of the pericardium are closely opposed in normal subjects and separated by a small amount of physiological fluid. In addition, the heart and coronary arteries are in-

<table>
<thead>
<tr>
<th>Table 3. Interrater Variability Between 2 Readers for Intrathoracic CT Fat and Volume Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pearson Correlation of the Means of the 2 Raters (N = 62)</strong></td>
</tr>
<tr>
<td><strong>Method Comparison Test Using Bland–Altman Procedure (N = 62)</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td><strong>Intrathoracic fat (cm³)</strong></td>
</tr>
<tr>
<td><strong>Intrathoracic volume (cm³)</strong></td>
</tr>
<tr>
<td><strong>Epicardial fat (cm³)</strong></td>
</tr>
<tr>
<td><strong>Epicardial volume (cm³)</strong></td>
</tr>
</tbody>
</table>

*P is the statistical significance using Pearson correlation or Bland–Altman test, where appropriate.

**Significant at level <.05 using Bland–Altman test.”**
cluded in the epicardial fat volume measurement, primarily because there is mutual vascular supply and no separate physical fascia between the 2; therefore, any attempt to exclude the heart would increase the postprocessing time and may lead to measurement errors. Because the arteries and the heart are primarily made of soft tissue and possess fluid attenuation characteristics, which are enhanced by the contrast agent in this study, there is very little fat within the heart itself that would be included. Lipomatous hypertrophy of the atrial septum is conceivable as a fair amount of fat in the interatrial septum, which would be included when present, and in abnormal subjects, small amounts of fatty myocardial replacement from old infarcts would be included.

The results of this study showed excellent inter-reader reproducibility for quantifying intrathoracic fat volumes from contrast-enhanced CT scans using commercially available software for which we used thoracic fat tissue voxels with HU between −250 and −50, as previously described (18). In the literature, various window widths, ranging from −250 to −30 HU (15, 23–26), have been described. Nevertheless, it should be noted that the original studies defining the CT fat range were performed using single-section CT scanners and different section thicknesses (18, 27). Whether the fat window width will change using the newer-generation CT scanners and whether there is any significant difference in the fat volume measurements between the 2 different fat tissue CT window widths (−250 to −50 HU and −190 to −30 HU, with the latter being most recently used) are open questions for future research using a larger sample size. Typically, voxel-based fat measurements are significantly different when different CT window widths are used. They also vary with section thickness used. Our reconstructed images had 10 mm of section thickness, which follows Borkan study where fat is identified with HU between −250 and −50. The section thickness described in the study where they used HU between −190 and −30 was 5 mm. If the CT fat window widths do not use the recommended section thicknesses, the quantitative data may be inaccurate, and their future application questionable. We correlated the 2 different fat tissue CT window widths that were assessed using the Bland–Altman analysis. Epicardial fat volume (EFV) using a CT window width of −190 and −30 HU was significantly greater than that using a CT window width of −250 and −50 HU (mean ± STD of 69 ± 34 cm³ versus 58 ± 27 cm³, P-value of <.0001). There was statistically significant difference in the EFV measurements between different

Figure 4. Inter-reader agreement (Bland–Altman plots) for intrathoracic and epicardial fat volumes. Dotted lines represent 95% limits of agreement. Mean difference and 95% confidence interval for intrathoracic fat volume after applying the threshold of minimum of −250 Hounsfield Unit (HU) and maximum of −50 HU (A). Mean difference and 95% confidence interval for epicardial fat volume after applying the threshold of minimum of −250 HU and maximum of −50 HU (B).
In conclusion, this study stresses the importance and shows the feasibility and reproducibility of intrathoracic fat volume quantification from previously performed chest MDCT as part of standard patient care among relatively healthy individuals free of CVD, using commercially available software. The feasibility and inter-reader reproducibility reported in this study will help in future quantification of epicardial fat volume, an emerging surrogate marker for cardiometabolic risks.

Conflict of Interest: None reported.


A Population-Based Digital Reference Object (DRO) for Optimizing Dynamic Susceptibility Contrast (DSC)-MRI Methods for Clinical Trials

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Key Words: dynamic susceptibility contrast MRI, digital reference object, brain tumor perfusion
Abbreviations: Dynamic susceptibility contrast (DSC), magnetic resonance imaging (MRI), digital reference object (DRO), cerebral blood flow (CBF), cerebral blood volume (CBV), contrast agent (CA), repetition time (TR), echo time (TE), The Cancer Imaging Archive (TCIA), flip angle (FA), 3-dimensional (3D), finite perturber finite difference method (FPFDM), extravascular extracellular space (EES), gadopentetate dimeglumine (Gd-DTPA), arterial input function (AIF), percent signal recovery (PSR)

The standardization and broad-scale integration of dynamic susceptibility contrast (DSC)-magnetic resonance imaging (MRI) have been confounded by a lack of consensus on DSC-MRI methodology for preventing potential relative cerebral blood volume inaccuracies, including the choice of acquisition protocols and postprocessing algorithms. Therefore, we developed a digital reference object (DRO), using physiological and kinetic parameters derived from in vivo data, unique voxel-wise 3-dimensional tissue structures, and a validated MRI signal computational approach, aimed at validating image acquisition and analysis methods for accurately measuring relative cerebral blood volume in glioblastomas. To achieve DSC-MRI signals representative of the temporal characteristics, magnitude, and distribution of contrast agent-induced T1 and T2* changes observed across multiple glioblastomas, the DRO’s input parameters were trained using DSC-MRI data from 23 glioblastomas (40,000 voxels). The DRO’s ability to produce reliable signals for combinations of pulse sequence parameters and contrast agent dosing schemes unlike those in the training data set was validated by comparison with in vivo dual-echo DSC-MRI data acquired in a separate cohort of patients with glioblastomas. Representative applications of the DRO are presented, including the selection of DSC-MRI acquisition and postprocessing methods that optimize CBV accuracy, determination of the impact of DSC-MRI methodology choices on sample size requirements, and the assessment of treatment response in clinical glioblastoma trials.

INTRODUCTION
Dynamic susceptibility contrast (DSC)-magnetic resonance imaging (MRI) noninvasively measures brain tumor cerebral blood flow (CBF) and cerebral blood volume (CBV), and it has found increasing clinical applications for patient management (1-18). To facilitate multi-institutional comparability and consistency, national initiatives, including National Cancer Institute’s Quantitative Imaging Network, Radiological Society of North America’s Quantitative Imaging Biomarkers Alliance, and the National Brain Tumor Society’s Jumpstarting Brain Tumor Drug Development Coalition, are underway to standardize acquisition and analysis protocols for DSC-MRI (19, 20). A challenge to such efforts is the relative paucity of studies systematically evaluating the influence of DSC-MRI methodology on CBV accuracy. In practice, such validation studies are difficult to perform in patients because of the need for multiple contrast agent (CA) injections and lack of a noninvasive gold standard CBV measure for reference. As an alternative to in vivo validation, in silico digital reference objects (DROs) provide a means for computing synthetic MRI signals and derived kinetic parameters for a range of clinically relevant input conditions. Such a DRO was recently developed for dynamic contrast-enhanced MRI to investigate the biases and variances of algorithms used for image analysis (21).

The goal of this report is to describe the development of a DSC-MRI DRO that recapitulates the heterogeneous signal characteristics measured in glioblastomas. In general, there are two underlying strategies that can be pursued for DROs emulating MRI data. When the primary objective is to establish multisite analysis consistency, synthetic signals can be computed using simple heuristic models approximating the underlying biophysics of signal formation, as the endpoint is to assess the agreement between software estimates of a parameter such as CBV.
that is explicitly defined by the “ground truth” time course. However, if the intention is to optimize acquisition protocols and CA dosing schemes, such as those used in DSC-MRI, or if the accuracy of the analysis is dependent upon certain physical or physiological assumptions, the synthetic signals should accurately reflect the biophysics of the MRI signal. For the DSC-MRI DRO, we pursued the latter strategy because it enables a more accurate and comprehensive investigation into the DSC-MRI methodology.

In brain tumor DSC-MRI, the acquired signals reflect a complex combination of $T_1$, $T_2$, and $T_2^*$ changes that depend upon numerous features including CA kinetic parameters (CBF, permeability, intra- and extravascular volume fractions), pre-contrast $T_1$ and $T_2$ vascular architecture, cellular microstructure (size, shape, spatial distribution), transvascular and transcellular water exchange, and CA $T_1$ and $T_2$ relaxivity. The sensitivity of the DSC-MRI signal to relaxation time variations is influenced by the acquisition parameters (repetition time [TR], echo time [TE], flip angle [FA], pulse sequence type) and CA dosing scheme (preload and bolus dose and timing). Accordingly, for the DRO to yield realistic signals, its design must reasonably approximate the magnitude and heterogeneity of these physical and physiological parameters in vivo. To that end, we developed a DSC-MRI DRO that is driven by a validated computational strategy to compute MRI signals for realistic 3-dimensional (3D) tissue structures (22); partially constrained by parameter inputs defined from in vivo data; and, for unknown parameters, trained using a public database of DSC-MRI data in glioblastomas.

**METHODOLOGY**

The computational approach used herein, termed the finite perturber finite difference method (FPFDM) (22, 23), models the effects of water protons diffusing in heterogeneous magnetic field medium based on a 3D tissue structure. The FPFDM computes magnetic field perturbations induced by susceptibility variations between the simulated tissue compartments, and it determines the resulting gradient echo transverse relaxation rates. In addition to a 3D matrix that defines the tissue structure (eg, blood vessels and cells), requisite FPFDM inputs include the static magnetic field strength, the CA concentration in each compartment for determining intercompartment susceptibility differences, the water proton diffusion coefficient, and the DSC-MRI pulse sequence parameters. To ensure clinical relevancy, the DRO derived from these input parameters should replicate the magnitude and heterogeneity of CA-induced $T_1$ and $T_2$ changes during bolus passage through vessels and into the extravascular extracellular space (EES).

**CA Kinetics**

The 2-compartment pharmacokinetic model described by Brix et al. (24) was used to simulate concentration–time profiles in plasma ($C_p$) and the EES ($C_e$). Inputs to the Brix model include vascular volume fraction, blood flow, CA transfer coefficient ($K^{trans}$), and volume fraction of the EES ($v_e$). Rather than use previously reported mean CBF and CBV values in glioblastoma, our simulated kinetic curves better represented clinical data if the DRO voxels matched the paired, voxel-wise distribution of these parameters across patients (as compared with randomly distributed unpaired parameters). Accordingly, we extracted DSC-MRI data from 23 patients with glioblastoma ($\geq$40 000 voxels) in The Cancer Imaging Archive (TCIA) database for characterizing the distribution of paired CBF and CBV values. For this patient cohort, DSC-MRI was acquired at 3T, consisting of General Electric (General Electric Healthcare, Waukesha, WI, USA) (n = 14) and Siemens (Siemens Medical Systems, Erlangen, Germany) (n = 9) scanners using single-echo gradient echo-planar imaging (TR = 1–1.25 seconds, TE = 30 milliseconds, $FA = 70–80^\circ$, field of view = 240 × 240 mm², section thickness = 4–5 mm, matrix = 96² or 128²) before, during, and after administration of 0.1 mmol/kg gadopentetate dimeglumine (Gd-DTPA) infusion at 4 ml/s followed by a saline flush. Five minutes before bolus injection, a 0.05 mmol/kg Gd-DTPA preload was administered to minimize $T_1$ leakage effects. Residual leakage effects were corrected using the Boxerman–Schmaida–Weisskoff approach (25). Voxel-wise relative CBV and CBF maps were calculated from the leakage-corrected DSC-MRI data and an automated measure of the arterial input function (AIF), using circular singular value decomposition–based deconvolution (26–29). The voxel-wise distributions of $K^{trans}$ and $v_e$ were characterized using a retrospective analysis of dynamic contrast-enhanced MRI signals extracted from a dual-echo DSC-MRI data set in 11 glioblastomas (30). Because DSC-MRI data yield relative tumor CBV and CBF measures, their values were scaled using data obtained from dynamic computed tomography perfusion imaging (31). In addition, the AIF used as input for the DRO’s kinetic modeling was computed as the average AIF among all patients in the TCIA data. Figure 1, A, B, and C shows the average AIF values, CBV and CBF paired distribution, and $K^{trans}$ distribution, respectively.

To define a computationally manageable number of tissue models in the DRO that still accurately reflected the in vivo voxel-wise heterogeneity, the 2-dimensional paired distribution of CBV and CBF was first binned into intervals of 5 ml/100 g/min and 1 ml/100 g, respectively. The resulting distribution was then scaled to yield 100 combinations of CBF and CBV pairs, which were then used to define the number and vascular properties of the tissue structures.

**Tissue Structures**

Although the component of DSC-MRI signal associated with CA-induced $T_1$ changes is easily calculated by assuming fast water exchange (32–34), the CA-induced $T_2$ changes depend on vascular and cellular microstructural geometry, precluding use of a simple analytical model. To reflect this complexity, we modeled tissue structures using ellipsoids (cells) (22, 23, 35) packed around randomly oriented cylinders (vessels) (36–45). Previously, we showed that modeling cells as ellipsoids rather than spheres provides a more accurate estimate of the magnitude of $T_2$ changes observed in clinical DSC-MRI studies (22, 23, 35), whereas modeling the vasculature structure as randomly oriented cylinders has been shown to accurately estimate the $T_2$ effects that occur when CA is distributed within blood vessels (36–45). The cylindrical vascular volume fraction was fixed using the in vivo extracted CBV values, and vessel sizes varied from 5 to 30 µm (46). Tumor cell volume fractions were allowed to vary within a physiologically relevant range (45%–65%) (47),
and the mean cellular axis radii for a given voxel varied between 4 and 15 μm (46). Figure 2 shows a representative 3D volume rendering of 2 tissue structures, one with homogeneous ellipsoids with a constant aspect ratio (Figure 2A) and one showing ellipsoids with heterogeneous shapes (Figure 2B).

**Computation of DSC-MRI Signal**

The susceptibility differences between the vascular and extravascular compartments were computed using \( \Delta \chi = \chi_m [CA] \), where \( [CA] \) is the compartmental CA concentration (\( C_p \) and \( C_e \)) and \( \chi_m \) is the CA molar susceptibility (0.027 \( \times 10^6 \) mM\(^{-1}\)) (48). In addition to all the aforementioned input parameters, the FPFDM calculates the DSC-MRI signal as described previously (22) using a water proton diffusion rate (\( D \)) of 1.3 \( \times 10^{-3} \) mm\(^2\)/s (49), relevant pulse parameters (TE, \( B_0 \), FA, TR), and precontrast T1 values ranging from 1 to 2.2 seconds. Figure 3 shows representative simulated \( C_p \) and \( C_e \) time curves (Figure 3A), and the corresponding gradient echo DSC-MRI signal ratio (\( S/S_0 \)) time curves (Figure 3B) for the 2 tissue voxels are shown in Figure 2.

**DRO Training**

Given the large number of input parameters and a wide range of potential permutations, it is critical to ensure that the DRO’s simulated DSC-MRI signals accurately represent the temporal characteristics, magnitude, and distribution of CA-induced T1 and T2* changes observed across typical glioblastomas. To achieve this, we used the voxel-wise TCIA data described above (>40 000 voxels) for identifying the appropriate combination of...
input parameters. In particular, all computed signals, for an equivalent preload dosing scheme and pulse sequence parameters to those in the TCIA data set, underwent a selection criteria process based on their percent signal recovery (PSR) and the mean and standard deviation of the signals across the DRO. The PSR is a useful metric for comparison because it reflects the magnitude of the signal drop during bolus passage and the postbolus signal recovery. The DRO’s input tissue structure (eg, cell size, shape), kinetic parameters (eg, CBF, $K_{\text{trans}}$), and physical parameters (precontrast T1) were systematically permuted until the distribution of PSR values and the mean and standard deviation of signals across the DRO agreed with those found in the voxel-wise TCIA data. The PSR agreement was evaluated using a 2-sample Kolmogorov–Smirnov test. In addition, a 95% agreement between the FWHM and the maximum signal drop was used to determine the agreement between the mean signals. To achieve this level of agreement, the iterative process required a DRO consisting of $\sim$10 000 unique voxels. The data training based on this selection criterion ensured the removal of computational signals from the DRO, because of an unrealistic combination of tissue parameters. Figure 4A–B shows the agreement between the in vivo and in silico data. The training phase ensured concordance of the percent signal recovery (PSR) distributions for in vivo and in silico data, supported by 2-sample Kolmogorov–Smirnov test (C).

**DRO Validation**

To validate the DRO’s ability to produce reliable signals for pulse sequences and that the CA dosing schemes are different from those in the training data set, we compared simulated dual-echo signals with those found in an in vivo dual-echo DSC-MRI “validation” data set. The validation data set was acquired in patients with glioblastoma ($n = 3$) at 3T using a dual gradient echo-planar imaging protocol with the following parameters: TR = 1.5 seconds, TE1/TE2 = 7.0/31.0 milliseconds, field of view = 240 × 240 mm², section thickness = 5 mm, matrix = 96². Measurements were taken before, during, and after administration of Gd-DTPA (0.1 mmol/kg Gd-DTPA, 4 ml/s infusion rate followed by 20 ml of saline flush). In the simulation, the structural and kinetic inputs derived during the training phase remained the same, but the acquisition parameters and dosing scheme were chosen to match those used in the patient data. The goal of this validation study was to determine whether the DRO fully captures the heterogeneity (eg, magnitude and temporal characteristics such as PSR) of the DSC-MRI signals acquired in this separate (and smaller) cohort of patients. To identify this subset of voxels within the DRO, a correlation analysis was performed between the signals in the in vivo and DRO data. The range of PSR values found in the in vivo and DRO data was

| Table 1. Input Parameters for Tumor and Normal Tissue |
|-----|-----|-----|-----|-----|-----|
|     | CBV (%) | CBF (ml/100 g/min) | $K_{\text{trans}}$ (min⁻¹) | $T_{1\text{O}}$ (s) | $v_e$ (%) | Cell Radii ($\mu$m) | Vessel Radii ($\mu$m) |
| WM  | 3.5 ± 0.6  | 38.4 ± 8.3  | 0.0 ± 0.0  | 1.8 ± 0.2  | 25.4 ± 0.3 | 8.4 ± 2.7  | 9.9 ± 1.0 |
|     | (2.5–4.6) | (26.6–55.2) | (0.0–0.0) | (1.3–2.5) | (24.8–26.0) | (4.4–12.6) | (6.0–13.6) |
| Tumor | 6.1 ± 2.8  | 150.3 ± 55.6 | 0.19 ± 0.08 | 1.8 ± 0.2  | 24.3 ± 1.5 | 8.4 ± 2.7  | 9.9 ± 2.5 |
|     | (1.5–13.8) | (43.9–268.2) | (0.03–0.47) | (1.0–2.6) | (22.3–26.5) | (4.4–12.6) | (1.2–19.7) |

Values are expressed as mean ± SD and (minimum–maximum).
compared to ensure that the DRO captured the signal heterogeneity measured in the validation set for both TEs. A parameter termed percent relaxation drop (PRD) was also formulated in a similar fashion as PSR using the derived dual-echo $\Delta R_2^*$ time courses and compared between the in vivo and DRO data.

All simulations were performed using Matlab (MathWorks, Natick, MA) running on a high-performance 32-core system with 2.3 GHz processors and 128 GB of RAM.

RESULTS

Validation

Figure 5 compares simulated and in vivo dual-echo DSC-MRI data. The DRO could accurately recapitulate the TE = 7 milliseconds and TE = 31 milliseconds signals and the derived dual-echo $\Delta R_2^*$ time courses, which remove T1 leakage effects but retain T2* leakage effects. The PSR and PRD heterogeneity of the in vivo data was also fully reflected in the DRO. This indicates that the trained DRO can accurately model the underlying CA-induced T1 and T2* effects and the associated DSC-MRI signals for different sets of pulse sequence parameters and CA dosing schemes.

Application 1: Influence of Acquisition and Postprocessing Methods on CBV Accuracy

It is well established that T1 and T2* CA leakage effects confound the reliable measurement of CBV (25, 50). DSC-MRI acquisition strategies have been proposed to reduce T1 leakage effects, including the use of preload CA administration, low FAs, long TEs and TRs, and dual-echo pulse sequences. In addition, postprocessing methods have been developed that eliminate residual T1 and/or T2* leakage effects (25, 51-59). However, validation of these acquisition and postprocessing strategies in vivo has been limited because of the lack of a reliable gold standard reference. A potential application of the population-based DRO is the systematic investigation of the acquisition and postprocessing methods that influence the reliability of CBV measurements.

To this end, we computed the percentage difference between tumor CBV simulated with and without ($K^{trans} = 0$) CA leakage effects for a single-dose bolus injection protocol (no preload), FA = 30° and 90°, TE = 30 milliseconds, and TR = 1 and 2 seconds. We also compared CBV accuracy with and without the application of postprocessing leakage correction using the Boxerman–Schmainda–Weisskoff approach. Results of this analysis are shown in Figure 6. For TR = 1 second, FA = 90° yielded more accurate CBV values than FA = 90°, with and without postprocessing leakage correction (Figure 6A). As expected, the uncorrected 90° FA data yielded substantially underestimated CBV across the DRO voxels, reflecting the strong sensitivity to T1 leakage effects. For TR = 2 seconds, a greater fraction of voxels overestimate CBV, indicating a shift toward T2*-dominated leakage effects due to reduced T1 sensitivity.
The population-based DRO can also be used to optimize DSC-MRI for assessment of treatment response in clinical trials. For example, the influence of acquisition and postprocessing methods on the sensitivity of DSC-MRI to a given CBV change can be used to determine protocols that minimize the sample size needed to power a clinical trial. In this context, the DRO serves as an atlas of possible tumor DSC-MRI signals. By using the correlation analysis discussed in the validation section, a virtual patient DSC-MRI data set can be generated by replacing voxel-wise in vivo tumor signals with an atlas-matched version. This analysis can be propagated across an existing clinical trial database to compute in silico pre- and post-treatment DSC-MRI data. Because the simulated signals for a given voxel originate from a unique set of input conditions, the DSC-MRI signals can be recomputed for any combination of acquisition parameters, such as a new FA or CA dosing scheme. This permits systematic investigation of how acquisition and postprocessing methods influence the inter- and intra-subject CBV heterogeneity, pre- and post-therapy. Alternatively, an assumed effect size distribution (eg, 20% ± 5% decrease in a tumor’s CBV) could be applied to the untreated cohort of virtual patients and can be used to identify, within the DRO, the “treated” DSC-MRI signals for each voxel.

Figure 7A–B illustrates a simulated pretreatment CBV map for a virtual patient computed using 2 different CA dosing schemes: a single-bolus dose with no preload (method 1) and a single-dose preload preceding a single-bolus dose (method 2). The corresponding treated CBV maps (modeled as a 20% mean reduction in tumor CBV) for both methods are shown in Figure 7C–D. The pre- and post-treatment CBV distributions across the entire tumor region of interest for both acquisition methods are shown in Figure 7E–F. In this example, CBV estimates derived from method 2 were more sensitive to treatment response compared with those derived from method 1, as indicated by the change in CBV. Similar analyses could be extended to cohorts of virtual patients to identify the most robust and sensitive DSC-MRI acquisition and postprocessing strategies for use in clinical trials.

DISCUSSION
We have described the development of a DRO that recapitulates the DSC-MRI signal characteristics observed in human glioblastomas. The DRO enables signals to be computed for ranges of physiological, physical, and acquisition parameters. Clinical relevance is ensured through the use of a training data set. Furthermore, we validated the DRO’s ability to produce reliable signals for different CA dosing schemes and acquisition parameters. Although in silico models may be limited by the accuracy of the biophysical model used, they provide a feasible and robust alternative to in vivo studies, which, in the case of DSC-MRI, may require multiple contrast injections and MRI scans and often lack a reliable “ground truth” for establishing accuracy.

Two key features of the proposed DRO are instrumental to its ability to provide signals that emulate clinical data. First, the DSC-MRI signals are derived using a validated computational approach that enables the incorporation of realistic tissue structures. Unlike heuristic models of DSC-MRI (34), this approach does not make assumptions regarding the voxel-wise CA T2* relaxivity, a parameter that is highly dependent upon vascular and cellular microstructure. In the proposed DRO, the voxel-wise microstructure determines the compartmental volume fractions and the associated CA relaxivity. Second, the training phase ensures that the range of simulated signals reflects the heterogeneity observed in vivo. Without training, there is the potential to introduce bias into the optimization of acquisition and postprocessing methods, as such methods may have not have uniform accuracy across the range of parameters.

Although we have presented two potential applications for the proposed DRO, there exist numerous opportunities for its use. Studies seeking to characterize and explore the biophysical basis of DSC-MRI data in brain tumors have yielded new biomarkers sensitive to the underlying tumor microstructure (eg, morphological features of vessels and cells) (23, 60-62) and hemodynamics (eg, vascular architectural imaging) (63). For these advanced methods, the DRO provides a tool with which to systematically investigate the sensitivity of DSC-MRI to such features and identify optimal acquisition protocols. Furthermore, the DRO can also be used to assess the accuracy of kinetic parameter estimates derived from newly developed pulse sequences, such as the recently proposed multiecho spin and gradient echo (SAGE) approach (64-69).

Although we trained the DRO with and validated it against in vivo data, any simulation approach that models complex biophysical phenomena has limitations. As described previously (22), the current computational approach does not consider the...
effects of arbitrary or heterogeneous CA distribution within a given tissue compartment such as the EES. The DRO could also be expanded to include the effects of transvascular water exchange rate, intravascular flow dynamics, atypical cellular geometries, and more heterogeneous vascular tree models. However, increasing the biological complexity of the input tissue structures also increases the number of unknown parameters that would need to be characterized.

The proposed DSC-MRI DRO provides a tool that can be leveraged by groups aiming to optimize and standardize acqui-

**Table 2. Summary of Pulse Sequence Parameter Values and CA Dosing Schemes**

<table>
<thead>
<tr>
<th>TR (ms)</th>
<th>FA (°)</th>
<th>TE (ms)</th>
<th>B0 (T)</th>
<th>Preload + Bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1000, 1500, 2000)</td>
<td>(30, 60, 90)</td>
<td>(20, 30, 40, 50)</td>
<td>(1.5, 3)</td>
<td>(0 + 1, 1/4 + 3/4, 1/2 + 1/2, 1/2 + 1, 1 + 1)</td>
</tr>
</tbody>
</table>

All possible combinations yielded 360 different acquisition methods. Dosing schemes are presented as a fraction of a standard 0.1 mmol/kg dose.
sition and analysis methods for prospective clinical studies. It also enables the evaluation of bias and variance introduced by multivariate data analysis. Such efforts are critical for establishing comparability of DSC-MRI data and interpreting multisite clinical trial data. To facilitate this effort, a range of DSC-MRI DROs is available for download from The Cancer Imaging Archive (www.cancerimagingarchive.net) under the collection name Barrow-DRO. The provided files contain multiple versions of the DRO, computed across a wide range of pulse sequence parameters and preload dosing schemes, all saved in Digital Imaging and Communications in Medicine (DICOM) and Matlab formats. Table 2 summarizes the range of pulse sequence parameters and CA dosing schemes that, when combined, yield 360 different acquisition methods. Each DRO file is a DSC-MRI time series data set similar to what would be acquired clinically and includes predefined regions of interest for the AIF, normal tissue and tumor voxels. Accordingly, these data may be processed using commercial or customized DSC-MRI analysis packages. The data set summary page details the organization of the files, the regions of interest, and the instructions for use.

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An MR-Based Viscosity-Type Regularization Method for Electrical Property Tomography

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discuss here, a method based on viscosity-type regularization is proposed for magnetic resonance electrical property tomography (MREPT) to mitigate persistent artifacts when it is used to reconstruct a map of electrical properties based on data from a magnetic resonance imaging scanner. The challenges for solving the corresponding partial differential equation (PDE) are discussed in detail. The existing artifacts in the numerical results are pointed out and classified. The methods in the literature for MREPT are mainly based on an assumption of local homogeneity, which makes the approach simple but leads to artifacts in the transition region where electrical properties vary rapidly. Recent work has focused on eliminating the assumption of local homogeneity, and one of the solutions is convection–reaction MREPT that is based on a first-order PDE. Numerical solutions of the PDE have persistent artifacts in certain regions and global spurious oscillations. Here, a method based on viscosity-type regularization is proposed to effectively mitigate the aforementioned problems. Finite difference method is used for discretizing the governing PDE. Numerical experiments are presented to analyze the problem in detail. Electrical properties of different phantoms are successfully retrieved. The efficiency, accuracy, and noise tolerance of the proposed method are illustrated with numerical results.

INTRODUCTION

Magnetic resonance imaging (MRI)-based magnetic resonance electrical property tomography (MREPT) (1, 2) is a strategy for noninvasively reconstructing electrical properties (EPs) (conductivity and permittivity) of the human body without using additional hardware to a magnetic resonance (MR) scanner. It has been intensively developed in recent years for reconstructing the EPs of human tissues based on the measurable data (radiofrequency [RF] field distribution called $B_1$-map) (3) from an MRI scanner because the $B_1$-field carries the information of EP distribution in the human body.

MREPT is an important technology in various areas in medicine and biology. The constructed EP map can show the anatomical structure of the human body by the high contrast of EPs between different tissues (4). Cancerous tissues, including those at the early stages, are highly distinguishable from healthy ones (5, 6). Therefore, the EP map provides a good tool for early cancer detection. It can also provide guidance to design body-centric communications (7) and electromagnetics-based therapies (8). EP maps can also help in understanding the activities of cells, in particular with exposure to either static or dynamic electromagnetic waves (9). Moreover, an EP map is crucial for accurate calculation of specific absorption rate, which is a main parameter for assessing the risk of RF and microwave radiation and that of ultrahigh-field MRI scanning (10, 11).

MREPT was originally proposed by Haacke et al. (12) and first implemented by Wen (13). The Helmholtz equation for homogeneous material was proposed for calculating both conductivity and permittivity with positively circularly polarized component of the magnetic field ($B_1^+$), which corresponds to the RF transmit field. Another MREPT method was developed and systematically studied by Katscher et al. (14) and Voigt et al. (15). The integral forms of Faraday’s law and Ampère’s law were applied to formulate the governing equation based on $B_1^+$. However, these methods are based on an assumption of local homogeneity for simplification. This assumption leads to artifacts in the region where EPs vary either quickly or abruptly (3, 16, 17). The reconstruction errors are rigorously analyzed by Seo et al. (18). A recent work was focused on removing the local homogeneity assumption (19).
One full generalization of these methods was performed by Sodickson et al., and the technique was named Local Maxwell Tomography (LMT) (20, 21). The governing equation of this method was derived from Faraday’s law and Ampère’s law. The unmeasurable magnetic field components are eliminated by using Gauss’s law. The method is physically feasible and free of assumptions. Magnetic fields measured using an MRI scanner with multiple channels are used to solve the unknown. Although the assumption of local homogeneity is removed in LMT, higher-order partial differentiation over the magnetic field is needed in the calculation, which significantly increases this method’s sensitivity to noise.

Another MREPT method (22) was proposed on the basis of the fact that the magnetic field $B_1^x$ near the center of a birdcage or a transverse electromagnetic coil is homogeneous along its central axis (23, 24), and it is assumed that the contribution of the along-axis component of the magnetic field is negligible when the region of interest is close to the center of the coil. A first-order PDE including a gradient term of the unknown EP was formulated via properly manipulating Maxwell equations, and the gradient term describes the inhomogeneity of the object under investigation. This method is more general than the MREPT proposed by Katscher et al. (14) and has a simpler form than LMT.

Hafalir et al. (25) then named this method as convection–reaction MREPT (cr-MREPT), as its governing equation is similar to the convection–reaction equation (26). The first-order PDE was then solved by Hafalir et al. (25) for 2-dimensional reconstruction using a strong form with finite element method (FEM) (27). An obvious artifact was observed in the region where the gradient of $|B_1^x|$ is close to zero. Although 2 methods, the double-excitation and the constraint MREPT, were further proposed by Hafalir et al. (25) to improve the results, artifacts reappear when noise exists. Meanwhile, cr-MREPT does not work with every phantom. Even with the phantoms used by the authors, the reconstructed EPs deviate seriously from the true values in a large region close to the central artifact. High sensitivity of noise was also observed.

In parallel with the work of Hafalir et al., Ammari et al. (19) also attempted to solve this PDE via an adjoint-based iterative reconstruction algorithm. The real and imaginary parts of the equation are separated to produce a coupled equation pair solved by the iterative scheme. This method can construct a blur image of the EPs without the aforementioned artifacts, but it is not applicable in the region where the gradient of $|B_1^x|$ field is close to zero, and the produced linear system is ill-conditioned. The method also converges slower than the FEM-based method (19).

The solution of the first-order PDE shows persistent artifacts where $\nabla |B_1^x|$ approaches zero. Other than that, global spurious oscillations exist in the solution of this first-order PDE. Therefore, directly solving it with numerical methods is difficult. Here, the existing challenges for solving the PDE are discussed in detail. The existing artifacts in the numerical results are pointed out and classified. An effective viscosity-type regularization (28) solution is applied to mitigate the aforementioned problems. A Laplacian term is introduced into the first-order PDE for stabilizing the entire system, which can be solved by either the finite difference (FD) method or FEM. The governing PDE is in the first order, so its solution will be a linear function that depends on the space coordinates, and it can present sharp variations in space. Because the PDE is not a singularly disturbed equation (29, 26, 30), the introduced second-order Laplacian term will modify the PDE to obtain a second-order solution, which is an approximation of its exact solution and presents a smoother property in space.

The derivation of the formulation is briefly presented for the convenience of discussion; behaviors of the equation are analyzed in detail before introducing the viscosity-type regularization. Numerical experiments are used to facilitate the analysis of the existing problems and to show the effectiveness of the proposed approach. Finite difference method is applied for discretization. The proposed method is applied for reconstructing the EPs of different phantoms. Accuracy, stability, and noise tolerance of the method are illustrated with numerical results.

**Theory and Methodology**

The governing PDE for cr-MREPT are given as follows:

$$\mathcal{L} + \mathcal{M} \cdot \nabla \gamma - \gamma \nabla^2 H_1^+ + i \omega H_1^+ = 0.$$  \hspace{1cm} (1)

This equation can be solved for reconstructing EPs of a 3-dimensional object (see online supplemental Appendix for the relating derivation of this equation). If $\gamma$ does not vary severely along the $z$ direction, $\frac{\partial \gamma}{\partial z}$ becomes negligible. Equation (1) is then simplified as follows for a single section located at the center of birdcage:

$$\mathcal{L}_{xy} + \mathcal{M}_{xy} \cdot \nabla_{xy} \gamma - \gamma \nabla^2 H_1^+ + i \omega H_1^+ = 0.$$  \hspace{1cm} (2)

$\mathcal{L}_{xy}$ and $\mathcal{M}_{xy}$ are vectors $\mathcal{L}$ and $\mathcal{M}$ without the $z$-component, respectively.

$$\nabla_{xy} = \frac{\partial}{\partial x} + \frac{\partial}{\partial y}.$$ Notice that $\nabla^2 H_1^+$ still needs to be evaluated in a 3-dimensional form. Magnetic field $H_1$ is not measurable using MRI, but its contribution is negligible compared with $H_1^+$, as $H_1$ is generally close to zero in the center of the birdcage coil. Therefore equation (2) could be further simplified as follows:

$$\mathcal{L}_{xy} \cdot \nabla_{xy} \gamma - \gamma \nabla^2 H_1^+ + i \omega H_1^+ = 0.$$  \hspace{1cm} (3)

For homogeneous regions, $\nabla_{xy} \gamma = 0$; therefore, equation (3) can be simplified as $\gamma = \frac{i \omega H_1^+}{\nabla^2 H_1^+}$, which is the governing equation proposed by Haacke et al. and Wen (12, 13), named, in this content, standard MREPT (stdMREPT).

Therefore, the original inverse problem equivalently becomes a problem of solving the first-order PDE (3). It has the form of a stationary convection–reaction equation, which is a mathematical model for fluid dynamics (31). Thus, the MREPT based on equation (3) is called cr-MREPT by Hafalir et al. (25).

The coefficients of PDE (3) are complex, making the PDE exhibit different mathematical and numerical behaviors from the stationary convection–reaction equations that have real coefficients. In particular, both equations show global spurious oscillations in their solutions (26). For convection–reaction equations (real coefficients), these global spurious oscillations are caused by the mesh density for discretization, and the results converge better when the mesh size decreases. However, this fact
does not hold for equation (3), where the coefficients are complex. Instead, the spurious oscillation in equation (3) is caused by a fundamental mathematical drawback of itself; this is further discussed as follows.

Equation (3) can be reformed as follows:

\[ F(r) \cdot \nabla_{xy} \gamma(r) = f(r, \gamma(r)) \quad (4) \]

with \( F(r) = L_{xy} \) and \( f(r, \gamma(r)) = \gamma \nabla^2 H_i^+ + i \omega \mu H_i^+ \). According to the Cauchy–Kowalevski theorem (32), a necessary condition for equation (4) to have a smooth solution is that \( f(r, \gamma(r)) \) is analytic in region \( \Omega \) (19). However, \( \nabla^2 H_i^+ \) is not smooth in the region where electrical parameters vary rapidly (as in Figure 3H for a 2-layer cylindrical phantom with difference \( \varepsilon_r \) and \( \sigma \) in different layers). Discontinuity of \( \nabla^2 H_i^+ \) exists at the boundary where electrical parameters vary rapidly. Therefore, a smooth solution of \( \sigma \) and \( \varepsilon_r \) cannot be obtained with equation (3), and a better strategy is needed for good numerical stability, accuracy, and efficiency.

Here, a viscosity-type regularization method (28) is applied to equation (3) to eliminate the global spurious oscillation in its solution. The governing equation becomes the following equation:

\[ \rho \nabla^2 \gamma + L_{xy} \cdot \nabla_{xy} \gamma - \gamma \nabla^2 H_i^+ + i \omega \mu H_i^+ = 0 \quad (5) \]

where \( \rho \nabla^2 \gamma \) is the additional Laplacian term, and \( \rho \) is a constant that is always < 1 but > 0. This approach does not lead to a singularly disturbed problem (29, 26, 30). The additional Laplacian term plays a regularization role. This stabilized MREPT method is named as stabMREPT. There is no good way for deciding the optimum value of \( \rho \) yet, but it can be chosen as several times or a fraction of the maximum of \( |L_{xy}| \), which is the \( x \)-component of \( L_{xy} \). There are 2 ways to add the Laplacian term. One is adding it to the whole calculation domain, as shown in equation (5), and the other is adding it to the region where it is necessary (33) to produce more accurate results, but the optimal coefficients of the added Laplacian term need to be determined in a complex way, rendering it complicated. Here, only equation (5) is implemented for showing the idea. Central difference formula of the FD method is used for discretization.

**Discretization Strategy**

A square domain denoted as \( \Omega \) and bounded by \( \partial \Omega \) is discretized by small squares with \( N_x + 2 \) and \( N_y + 2 \) grid points along the \( x \) and \( y \) directions, respectively, giving \((N_x + 2) \times (N_y + 2)\) data points in space. \( \Delta x \) and \( \Delta y \) are the step lengths along \( x \) and \( y \) directions, respectively. Use \( i \) and \( j \) to index the mesh points along \( x \) and \( y \) with \( i := \{ii \in \mathbb{Z}, 0 \leq i \leq N_x + 1\} \) and \( j := \{j \in \mathbb{Z}, 0 \leq j \leq N_y + 1\} \), respectively. The values of \( H_i^+ \) and \( \gamma \) at the point \((i, j)\) are represented as \( H_{(i,j)} \) and \( y_{(i,j)} \), respectively.

Allowing \( L_{xy} = [L_x, L_y] \) with \( L_x = -\partial H_i^+ / \partial x + i \omega \mu H_i^+ / \partial y \) and \( L_y = -i \omega \mu H_i^+ / \partial x - \partial H_i^+ / \partial y \). The values of \( L_x \) and \( L_y \), at point \((i, j)\) are represented with \( L_{x(i,j)} \) and \( L_{y(i,j)} \) respectively. Using first-order central difference formula, \( L_{x(i,j)} \) and \( L_{y(i,j)} \) are given as follows:

\[ L_{x(i,j)} = -\frac{H_{(i+1,j)} - H_{(i-1,j)}}{2\Delta x} + i \frac{H_{(i,j+1)} - H_{(i,j-1)}}{2\Delta y}, \quad (6) \]

\[ L_{y(i,j)} = -i \frac{H_{(i+1,j)} - H_{(i-1,j)}}{2\Delta x} - \frac{H_{(i,j+1)} - H_{(i,j-1)}}{2\Delta y}. \quad (7) \]

Here, the imaginary unit \( i \) can be easily distinguished from the index \( i \) on the basis of its location. Representing the data of \( \nabla^2 H_i^+ \) at the point \((i, j)\) as \( d^2H_{(i,j)} \), the second-order central difference formula is used to discretize \( \nabla^2 H_i^+ \), which gives the following equation:

\[ d^2H_{(i,j)} = \frac{H_{(i+1,j)} - 2H_{(i,j)} + H_{(i-1,j)}}{\Delta x^2} + \frac{H_{(i,j+1)} - 2H_{(i,j)} + H_{(i,j-1)}}{\Delta y^2} \]

\[ + \frac{H_{(i,j+k)} - 2H_{(i,j)} + H_{(i,j-k)}}{\Delta z^2} \quad (8) \]

\( k \) is the index for sections along the \( z \)-direction. Two extra sections at \(-\Delta z \) and \( \Delta z \) away from the central section are used to calculate the partial differentiation of \( H_i^+ \) over \( z \).

Another way for calculating \( L_{x(i,j)} \) and \( L_{y(i,j)} \) is by using the Savitzky–Golay (SG) filter (34), which has been widely used to smooth noisy data. Here, \( H_i^+ \) is approximated with the following equation:

\[ H_i^+ = a_1x^2 + a_2y^2 + a_3xy + a_4x + a_5y + a_6. \quad (9) \]
where \((x, y)\) is the corresponding coordinate of \(H^+_1\), \(a_p, p = 1, \ldots, 6\) are polynomial coefficients, which need to be decided with the measured data of \(H^+_1\). As an example, at mesh grid point \((i, j)\), \(H_{(i,j)}\) has 8 neighbors, as shown in Figure 1. These neighbors or \(H_{(i,j)}\) itself could be used for interpolation with Stanley et al. (9), which will produce an overfitting linear system about the unknown coefficients \(a_p, p = 1, \ldots, 6\). A least-square method could then be used for solving the unknown.

The derivatives over \(H^+_1\) at the point \((i, j)\) are then calculated using the following equations:

\[
\frac{\partial H^+_1}{\partial x} = 2a_3x_i + a_4y_j + a_1, \tag{10}
\]

\[
\frac{\partial H^+_1}{\partial y} = 2a_3y_j + a_4x_i + a_2, \tag{11}
\]

\[
\frac{\partial^2 H^+_1}{\partial x^2} + \frac{\partial^2 H^+_1}{\partial y^2} = 2a_6 + 2a_5. \tag{12}
\]

Here, \((x_i, y_j)\) is the coordinate of the grid point \((i, j)\). Notice that the Laplacian term over \(H^+_1\) includes the second-order partial differentiation of \(H^+_1\) over \(z\), which is calculated with a central difference formula, with the data at sections located at \(-\Delta z\) and \(\Delta z\). With equations (6)–(8), or (10)–(12), equation (5) can be easily discretized with the first- and second-order central difference formula, as follows:

\[
- \text{io} \mu_0 \partial_{ij} H^+_1 = \rho \left( \frac{\partial^2 H^+_1}{\partial x^2} \right) + \rho \left( \frac{\partial^2 H^+_1}{\partial y^2} \right) + L_{x} \left( \frac{\partial H^+_1}{\partial x} \right) + L_{y} \left( \frac{\partial H^+_1}{\partial y} \right) - \gamma_{ij} d^2 H^+_1 \partial_{ij}. \tag{13}
\]

A linear system is produced via assembling (13) for all points indexed by \((i, j)\). The linear system is denoted as \(\mathbf{Ag} = \mathbf{b}\). The sparsity of the tridiagonal matrix \(\mathbf{A}\) allows

**Figure 3.** \(\sigma\) (A) and \(\epsilon_r\) (B) of phantom shown in Figure 2 are reconstructed by applying convection–reaction magnetic resonance electrical property tomography (MREPT) (cr-MREPT) based on equation (3). \(\sigma\) (C) and \(\epsilon_r\) (D) reconstructed using equation (2). Real (E) and imaginary (F) parts of \(H^+_1\), absolute value of \(L_z\) (G) and \(\nabla^2 H^+_1\) (H), are also presented.

**Figure 4.** \(\sigma\) (A) and \(\epsilon_r\) (B) reconstructed via least-square minimization with \(\lambda = 0.01\max(\mathbf{A})\) for the phantom shown in Figure 2. Central difference is used for \(\nabla^2 H^+_1\).
efficient calculation with Gaussian elimination. The Dirichlet boundary condition is assumed here. Vector $g$ represents the unknowns $\gamma_{i,j}$. The relative permittivity and conductivity at $(i,j)$ can be calculated based on the real and imaginary parts of $\gamma_{i,j}$.

**Numerical Experiments**

In the calculation, $H_1^+$ as input is simulated with FEM-based software COMSOL (35). A 16-leg high-pass shielded birdcage coil with a radius of 14.5 cm and a leg-length of 24 cm (36) is built to work at 127.74 MHz (3 T MRI system) to produce a homogeneous $H_1^+$ within the coil, and it is placed with its axis along the $z$-axis. The coil is driven by 2 ports with 500 V for each. Both ports are geometrically 90° apart with a 90° phase difference.

The phantom of 2 coaxial cylinders used for simulation is shown in Figure 2A. The cylinders have the same height $h = 24$ cm. The radii of the exterior and interior ones are $r_1 = 5$ cm and $r_2 = 2.5$ cm, respectively. The exterior cylinder is filled by a material with $\varepsilon_{\mathrm{r1}} = 75$ and $\sigma_1 = 0.5$ S/m, whereas for the interior one, $\varepsilon_{\mathrm{r2}} = 50$ and $\sigma_2 = 1$ S/m.

The phantom is placed in the birdcage coil, with its axis and center coinciding with those of the coil. A Dell Precision workstation (Round Rock, Texas) with Intel Xeon CPU with 2 processors (4 cores for each) and 48 GB RAM is used for simulation. The phantom is meshed with tetrahedral elements. The maximum mesh size for $-0.5$ cm $< z < 0.5$ cm is 1 mm. The mesh size is then increased gradually inside out. This strategy guarantees the accuracy of the calculated $H_1^+$ at the sections of interest and minimizes the number of elements to reduce the required memory.

The domain $\Omega$ is defined as the square inscribed to the cross section of the exterior cylinder, indicated by the dashed lines in Figure 2B. It is then meshed with $N_x = N_y = 99$. The data of $H_1^+$ are directly extracted from COMSOL.

The mesh used here will lead to a space resolution of 0.75 mm. Because simulation data are used here, not much concerns are needed. But, in practice, this resolution will need a long MR scan and produce low signal-to-noise ratio (SNR). A coarse mesh can be used first to perform the MR scan. With the obtained $H_1^+$, one can easily apply the cyclic regularized Savitzky-Golay filter (37) to reconstruct $H_1^+$ of a finer mesh with the required resolution. This will also help in reducing noise.

Relative permittivity $\varepsilon_r$ and conductivity $\delta$ of phantom shown in Figure 2 are first reconstructed by equation (3), the cr-MREPT method. Differentiations of $H_1^+$ are calculated with the central difference formula. As shown in Figure 3, A and B, significant artifacts are observed around the center of the interior cylinder and in the regions where $\varepsilon_r$ and $\sigma$ are discontinuous. Furthermore, global spurious oscillations are also clearly seen. For checking the effect of neglecting the contribution of $H_z$, equation (2) is also applied for the reconstruction. Results are shown in Figure 3, C and D, and not much improvement is seen. The global spurious oscillations and the artifacts still exist.

Hafalir et al. (25) report that the central artifacts are caused by the fact that $\mathcal{L}_z$ and $\mathcal{L}_y$ are close to 0. Figure 3, E and F shows

**Figure 5.** $\sigma$ (A) and $\varepsilon_r$ (B) of the phantom in Figure 2 are reconstructed by cr-MREPT with $N_x = N_y = 199$.

**Figure 6.** Reconstructed results of $\sigma$ (A) and $\varepsilon_r$ (B) for the 2-cylinder phantom shown in Figure 2 with stabilized MREPT (stabMREPT). $\delta = 0.37$, $N_x = N_y = 199$. 

$z = 0, z = -0.25$ cm, and $z = 0.25$ cm is extracted as inputs for the reconstruction.

The domain $\Omega$ is defined as the square inscribed to the cross section of the exterior cylinder, indicated by the dashed lines in Figure 2B. It is then meshed with $N_x = N_y = 99$. The data of $H_1^+$ are directly extracted from COMSOL.

The mesh used here will lead to a space resolution of 0.75 mm. Because simulation data are used here, not much concerns are needed. But, in practice, this resolution will need a long MR scan and produce low signal-to-noise ratio (SNR). A coarse mesh can be used first to perform the MR scan. With the obtained $H_1^+$, one can easily apply the cyclic regularized Savitzky-Golay filter (37) to reconstruct $H_1^+$ of a finer mesh with the required resolution. This will also help in reducing noise.

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Hafalir et al. (25) report that the central artifacts are caused by the fact that $\mathcal{L}_z$ and $\mathcal{L}_y$ are close to 0. Figure 3, E and F shows
the real and imaginary parts of the simulated $H^r_1$ at the central section ($z = 0$), denoted as $\Re H^r_1$ and $\Im H^r_1$, achieve to their extrema in the vicinity of the center, as shown in the figures, indicating that $\partial H^r_1/\partial x$ and $\partial H^r_1/\partial y$ are close to zero. This results in $\mathbf{L}_x^{(i,j)} = 0$ and $\mathbf{L}_y^{(i,j)} = 0$ for the points in the center region. The absolute values of $\mathbf{L}_x$ are shown in Figure 3G ($|\mathbf{L}_x| = |\mathbf{L}_y|$).

A minimization method is also applied to mitigate the global spurious oscillation when solving equation (3). The original linear system produced by equation (3) is written as $\mathbf{A}g = \mathbf{b}$, which is then transformed into a least-square minimization problem $\min ||\mathbf{b} - \mathbf{A}g||^2 + \lambda ||g||^2$ with a penalty term $\lambda ||g||^2$. The damping parameter $\lambda > 0$. In this way, the solution for $g$ is regularized. It is then solved with the iterative algorithm LSQR, which is based on the Golub–Kahan bidiagonalization process (38). The condition number of $\mathbf{A}$ here is about $10^7$, so $(\mathbf{A}^T \mathbf{A} + \lambda \mathbf{I})g = \mathbf{A}^T \mathbf{b}$, $\mathbf{A}$ being not symmetric, is not suitable for the calculation (it is equivalent to the applied minimization problem), because the 2-norm condition number of $\mathbf{A}^T \mathbf{A}$ is exactly the square of the one of $\mathbf{A}$.

This minimization is conducted with $\lambda = 0.01 \max ||\mathbf{A}||^2$, which is a large value for $\lambda$. As shown in Figure 4, the results converge better to the true values, but an obvious “4-blade” artifact is observed. These global spurious oscillations remain.

Moreover, this global spurious oscillation is independent of grid sizes $\Delta x$ and $\Delta y$. Figure 5, A and B show the results calculated with $N_x = N_y = 199$, which is a much finer mesh in the region $\Omega$. Global spurious oscillation still exists. Many artifacts appear with very large values. The reconstructed values of $|\epsilon_r|$ and $|\sigma|$ achieve to 500 and 10.

Therefore, neither directly the solution of equations (2) and (3) nor that of the least-square minimization method produces accurate results. This is expected by the fundamental drawback existing in the governing PDE, which is explained in section Theory and Methodology. However, reconstruction with the proposed stabMREPT based on the new second-order PDE (5) shows much better performance, which is shown as follows.

The same phantom (shown in Figure 2) is used for the proposed stabMREPT. The region $\Omega$ is discretized with the same mesh density ($N_x = N_y = 199$). The parameter $\rho = 0.37$, which is 7 times of the maximum value of $|\mathbf{L}_x|$. Figure 6 shows the reconstructed conductivities and permittivities. The normalized root-mean-square error (NRMSE) is defined as $\%_{\text{error}} = 100|\epsilon_{\text{true}} - \epsilon_{\text{recon}}||/||\epsilon_{\text{true}}||$. The reconstructed results of $\sigma$ and $\epsilon_r$ are very close to the true values. Comparison of the reconstructed $\sigma$ and $\epsilon_r$ with their true values at the line $y = 0$ yields the plots shown in Figure 7. A very good match is observed. Relative errors are all $<1\%$ except the boundary where the electrical parameters are discontinuous. Moreover, including $H_z$ into equation (5) produces the same results.

To further check the performance of stabMREPT compared with stdMREPT at both the boundary and at the homogeneous regions (no abrupt change in EPs), the NRMSE is calculated here for the reconstructed $\sigma$ of the 2-cylinder phantom. The parameters for the reconstruction are the same as those used for Figure

![Figure 7](image_url)

**Figure 7.** Comparison between the true values and the reconstructed ones of $\sigma$ (A) and $\epsilon_r$ (B) along $y = 0$. stabMREPT is applied for the 2-cylinder phantom. The regularization parameter $\delta = 0.37$, $N_x = N_y = 199$.

![Figure 8](image_url)

**Figure 8.** The map of normalized root-mean-square error (NRMSE) for $\sigma$ reconstructed with stdMREPT (A) and stabMREPT (B). The 2-cylinder phantom is used. $\delta = 0.37$, $N_x = N_y = 199$. 

6. The map of NRMSE is shown in Figure 8. A reconstruction error of stdMREPT at the boundary of interior cylinder is \(>100\%\), whereas a large error is also observed in the region far from the boundary. A reconstruction error at the boundary of the cylinder is also observed in the results obtained with stab-MREPT, which is about 45\%, and this error decreases gradually along the radius. But the reconstruction error at the region far away from the boundary is much smaller than the one at stdMREPT, where the largest error is \(<3\%\). Therefore, stabMREPT performs better than stdMREPT in both the boundary and homogeneous regions.

To further test the proposed approach, a simplified human head phantom as shown in Figure 9 is simulated. The model has 5 different regions indicated by different colors. Each region relates to a tissue type of the human brain (4). These tissues are, from external to internal, scalp \((\epsilon_r = 62, \sigma = 0.54 \text{ S/m, gray})\), skull \((\epsilon_r = 21, \sigma = 0.12 \text{ S/m, deep blue})\), gray matter \((\epsilon_r = 73, \sigma = 0.59 \text{ S/m, yellow})\), white matter \((\epsilon_r = 52, \sigma = 0.34 \text{ S/m, light blue})\), and cerebrospinal fluid \((\epsilon_r = 84, \sigma = 2.14 \text{ S/m, red})\).

Figure 9. Simplified head model with 5 different regions characterized with electrical parameters of scalp \((\epsilon_r = 62, \sigma = 0.54 \text{ S/m, gray})\), skull \((\epsilon_r = 21, \sigma = 0.12 \text{ S/m, deep blue})\), gray matter \((\epsilon_r = 73, \sigma = 0.59 \text{ S/m, yellow})\), white matter \((\epsilon_r = 52, \sigma = 0.34 \text{ S/m, light blue})\), and cerebrospinal fluid \((\epsilon_r = 84, \sigma = 2.14 \text{ S/m, red})\).

Figure 10. True \(\sigma\) and \(\epsilon\), of the head model are given in (A) and (B). Results are shown in stabMREPT (C) and (D). Results of cr-MREPT are given in (E) and (F). Results of stdMREPT are given in (G) and (H). The first and second rows are results of \(\sigma\) and \(\epsilon\), respectively.

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in Figure 10. True values are shown in Figure 10, A and B. The reconstructed $\sigma$ with stabMREPT (Figure 10C) matches quite well to true values with NRMSE < 5%, although boundary artifacts are observed in the results of $\varepsilon_r$ (Figure 10D). These results are better than those computed with cr-MREPT (Figure 10E and F) and stdMREPT (Figure 10, G and H). Figure 11 shows a comparison of the variation of $\sigma$ and $\varepsilon_r$ along the line of $x = 0$ for stabMREPT and stdMREPT. A strong oscillation exists in the stdMREPT results.

In practical measurement, the data of $H_1$ are always polluted by noise at different levels. The noise tolerance of stabMREPT is tested using the simplified human brain model. The SNR is defined as $\text{SNR} = 20\log_{10}(|H_1^*|/|N|)$, where $H_1^*$ is the simulated data. $N$ is the added complex white Gaussian noise generated with a given noise level in decibel. SNR is set to be 60 dB and $\rho = 4.1 \times 10^{-3}$ in equation (6). The SG filter is used for calculating the derivatives of $H_1^*$ to smooth noisy data. Figure 12, A and B shows the results reconstructed by cr-MREPT. The accuracy of the results obtained is low. Results of stdMREPT are given in Figure 12, C and D. The results are severely degraded by the noise. Very large values and global oscillations are observed in the results. On the contrary, the noise has no strong effect on the results of $\sigma$ reconstructed with stabMREPT, although the results for $\varepsilon_r$ are slightly affected, as shown in Figure 12, E and F. Moreover, the reconstructed results are severely affected when the noise level is increased to be $\geq 50$ dB. However, the effect of noise on stabMREPT becomes rapidly insignificant with an increase in SNR. Examples are given in Figure 12, G and H for stabMREPT with SNR = 65 dB, a considerable improvement is
REFERENCES


DISCUSSION AND CONCLUSION

Here, a viscosity-type regularization method is used to effectively improve the performance of the newly proposed cr-MREPT, which is based on a first-order complex coefficient PDE. The proposed method is then named as stabMREPT. The regularization is implemented on the basis of the fact that adding the Laplacian term does not lead to a singularly disturbed problem. The PDE is discretized using FD method.

The reconstruction method based on the same PDE was named as cr-MREPT by Hafalir et al. (25). However, several drawbacks exist in this method, although it is rarely reported in the literature. Here, these drawbacks are analyzed in detail. Different numerical simulations are given for the purpose of illustration: 2 PDE properties are coupled. The governing equation of cr-MREPT is equation (1), which is in the form of pure convection–reaction PDE. It is well known in the literature that such an equation is difficult to solve numerically. Here, these drawbacks are analyzed in detail. Different numerical simulations are given for the purpose of illustration: 2 PDE properties are coupled. The governing equation of cr-MREPT is equation (1), which is in the form of pure convection–reaction PDE. It is well known in the literature that such an equation is difficult to solve numerically.

The viscosity-type regularization method is then suggested. As illustrated in the paper, this method can efficiently mitigate the persistent artifacts existing in the convection–reaction PDE. The regularization parameter \( \rho \) needs to be positive and smaller than 1. It can be chosen as several times or a fraction of the maximum of \( |\mathbf{L}_x| \), which is the \( x \)-component of \( \mathbf{L}_{cr} \). Blurring can be observed at the boundaries of different tissues, but the reconstruction accuracy can be considerably better. The accuracy of the proposed method is shown by numerical results obtained by using simple and complex phantoms. Good performances in several numerical experiments are observed by comparing them with other existing methods.

Although the noise tolerance is not yet good for practical use, it is already considerably better than stdMREPT and cr-MREPT. The noise tolerance of this method was compared with that of other methods. Another algorithm based on FEM will be implemented in the next step to add the regularization term only when it is needed. A regularization-based Sg method (37) can be used for reconstructing \( H = \mathbf{T} \) from the measured noisy data, which will further reduce the influence of noise and increase the noise tolerance of the method.

Supplemental Materials

Supplemental Appendix: http://dx.doi.org/10.18383/j.tom.2016.00283.sup.01


Low-Noise Active Decoupling Circuit and its Application to $^{13}$C Cryogenic RF Coils at 3 T

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Abstract: We analyze the loss contributions in a small, 50-mm-diameter receive-only coil for carbon-13 ($^{13}$C) magnetic resonance imaging at 3 T for 3 different circuits, which, including active decoupling, are compared in terms of their Q-factors and signal-to-noise ratio (SNR). The results show that a circuit using unsegmented tuning and split matching capacitors can provide >20% SNR enhancement at room temperature compared with that using more traditional designs. The performance of the proposed circuit was also measured when cryogenically cooled to 105 K, and an additional 1.6-fold SNR enhancement was achieved on a phantom. The enhanced circuit performance is based on the low capacitance needed to match to 50 Ω when coil losses are low, which significantly reduces the proportion of the current flowing through the matching network and therefore minimizes this loss contribution. This effect makes this circuit particularly suitable for receive-only cryogenic coils and/or small coils for low-gamma nuclei.

Introduction

The sensitivity of the magnetic resonance (MR) detection circuit is one of the most important aspects of a magnetic resonance imaging (MRI) experiment. Signal-to-noise ratio (SNR) improvement allows higher image resolution and/or reduced acquisition time. In an MR experiment, the noise mainly comes from thermal noise of the coil (and electronics), and the sample noise is due to the interaction of radiofrequency (RF) fields with the lossy sample. For proton imaging in humans, often, the sample losses are dominant because of the relatively high Larmor frequency of protons and the subject’s (patient) large size. However, increasing attention has been drawn toward imaging of other nuclei with lower Larmor frequencies, emphasizing the importance of coil losses. Carbon-13 ($^{13}$C) is of particular interest because it is used for hyperpolarized metabolic MR. This is an exciting new method with potential in early diagnosis of disease, staging, and therapy monitoring (1-5).

Following the trend already established for proton imaging, the SNR of $^{13}$C imaging can, in principle, also be improved using smaller surface coils in phased arrays or for acceleration with parallel imaging. However, the assumption is that sample-dominated noise can be achieved. Examples of receive-only double-tuned (4-6) and phased-array (7) coils using small surface elements for $^{13}$C have already been reported, showing improved performance over detection using bigger-volume coils. However, developing low-loss $^{13}$C small surface coils that work in a sample-dominated noise regime to replicate the enhancements provided by surface coils already seen for proton imaging remains a challenge.

At the lower Larmor frequency of $^{13}$C (4 times lower than that of hydrogen-1 [$^{1}$H]), the noise contribution of the coil becomes more significant. One can approximate the SNR of a nuclear magnetic resonance experiment, in terms of coil and sample losses, as it is done in Styles et al.’s study (8) and calculated using the following equation:

\[
\text{SNR} = \frac{\omega \cdot B_1}{\sqrt{R_S \cdot T_S + R_C \cdot T_C}}
\]

where $R_S$ and $R_C$ are the equivalent resistances of sample and coil, respectively, $T_S$ and $T_C$ their temperatures, $\omega$ the operating frequency, and $B_1$ the field per unit current.

In this context, the SNR of the experiment can be further increased by cooling the coil. The resistivity of copper decreases ca. 8 times when cooled to liquid nitrogen temperature (77 K), allowing a potential ~3-fold SNR increase for the case of no sample losses. The SNR gain obtained from cooling then depends on the balance between coil and sample losses, making it difficult to directly compare coils with different geometries and resonance frequencies. The following are some examples found in the literature of Q factors for cryogenic coils similar in size to the ones we will study here:

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(1) A 30-mm-diameter copper coil for $^{39}\text{K}$ at 9.4 T (18.68 MHz), with an unloaded quality factor improvement $Q_{77K}/Q_{290K} = 450/223$ (9).

(2) A 17-mm-diameter copper coil for $^1\text{H}$ at 1.5 T (64 MHz) with unloaded $Q_{77K}/Q_{290K} = 260/125$ (10).

(3) A 35-mm-diameter copper twin horseshoe resonator for $^1\text{H}$ at 3 T (128 MHz) with unloaded $Q_{77K}/Q_{290K} = 900/300$ (11).

Cryogenic coils, as those mentioned above, are more often used only as receiver coils, whereas the transmission is performed by a separate volume coil that provides better homogeneity. Therefore, an additional circuitry needs to be added to the coil to detune it during the transmission (active decoupling), preventing inhomogeneities created by the current flowing in the receive coil and also protecting the preamplifier. Decoupling between transmitter and receiver coils has traditionally been implemented by adding a PIN diode in series with an inductor, which is chosen to create high impedance together with one of the capacitors within the coil, therefore greatly reducing the current flow (12, 13).

Some thorough studies have been already conducted, quantifying coil losses for proton imaging at different field strengths (14), showing good agreement between predictions and measurements. Here, we focus on the noise contribution of the circuit used to tune, match, and actively decouple receive-only $^{13}\text{C}$ coils at 3 T (32.13 MHz), and show that, at this frequency, the loss contribution of the tuning capacitor(s) can easily be dominant. In this context, we propose a novel active decoupling circuit and compare its performance with that of traditional schemes. The results show that the proposed circuit significantly reduces the losses, particularly for coils with low resistance. Measured Q-factors of up to 793 at 105 K for an unloaded 50-mm copper loop are obtained, showing the potential of this circuit for cryogenic coils.

**Theory**

The equivalent circuit diagram of a nuclear magnetic resonance experiment can be simplified as shown in Figure 1. If the coil inductance is fixed, the SNR of the experiment is then dependent on the amount of noise present. The balance between coil, sample, and matching network losses depends mainly on the coil size and test frequency, with coil losses being dominant for lower frequencies and smaller sizes. However, it is frequently overlooked that noise source is the matching network, which, particularly for coils with active decoupling, can become very relevant.

In Figure 1 the matching network is shown as a “black box” that transforms the impedance of the coil to 50 $\Omega$ (adding a certain amount of losses to $R_M$). Let’s consider instead the circuit shown in Figure 2A (omitting $R_s$ for the moment), which uses a capacitor in parallel to tune the coil. In this case, we have included the losses added by the capacitors, using their equivalent series resistance (ESR) in series with the ideal capacitors. It can be shown (see Supplemental Appendix) that the losses in this circuit are equivalent to the ones shown in Figure 2B, as long as the resistances ($R_C$, $ESR_T$, and $ESR_M$) are much lower than the impedances of the components. In that case, the total losses in the resonator can be approximated using the following equation:

$$R_{eq}^{par} = R_C + ESR_T + ESR_M$$

Therefore, and to include the capacitor losses in our analysis, for the rest of this study, we will calculate the Q-factor of coils using the following equation:

$$Q_{eq}^{ser} = R_C + ESR_T + ESR_M$$

For the case of a series tuned coil (as shown in Figure 2C), the total resistance in the resonator can be approximated as that in Figure 2D (see Supplemental Appendix). Again, this approximation is only valid for coils with equivalent resistance that is much lower than its reactance. Then the total resistance of the series tuned coil ($R_{eq}^{ser}$) can be expressed using the following equation:

$$R_{eq}^{ser} = R_C + ESR_T + ESR_M$$

**Figure 1.** Circuit equivalent of the magnetic resonance imaging (MRI) experiment, including the noise contribution of coil ($R_C$), sample ($R_S$), matching network ($R_M$), and preamp (NF).

**Figure 2.** Parallel (A) vs. series (C) tuned coil circuits, and their respective simplified approximations (B)(D).
\[ Q = \frac{\omega \cdot L}{R_{eq}} \]  
(4)

where \( R_{eq} \) includes now not only the losses in the loop but also the losses in the capacitors used for tuning and matching [as derived in equations (2) and (3)].

Even though both approximations of \( R_{eq} \) are valid for only coils with low losses, their behavior is quite different for the extreme case, where losses tend to zero (\( Q \) tends to \( \infty \)). In the parallel tuned circuit, lower losses require a lower matching capacitor (higher impedance) to perform the impedance transformation to 50 \( \Omega \), which, in practice, reduces the current flowing through this capacitor, minimizing its contribution to the total losses.

For the case of the series tuned coil, lower losses require a higher capacitor for matching (lower impedance), which is problematic because capacitors with higher capacitances have inherently higher losses. In the extreme case where a capacitor with very low impedance (\( \approx \text{m} \Omega \)) is needed for matching, the analysis performed here is not valid anymore, and the losses in this capacitor would become dominant. This case is not very realistic though.

**A Novel Low-loss Active Decoupling Circuit Using Split Matching Capacitors**

There are numerous ways to implement the active decoupling via pole insertion, depending on the particular coil design. Here, the losses of 3 different circuit schemes are compared: the 2 classical (series and parallel tuned coils) and 1 novel, which significantly reduces the losses by using only 1 parallel tuning capacitor.

Figure 3 depicts the 3 receive-only circuit schemes under study. Coil 1 (\( C_m = 2000 \, \text{pF}, \, C_t = 470 \, \text{pF} \)) is a series tuned coil with segmented tuning capacitor, widely used because it allows preamp decoupling schemes (15). In this scheme, the segmenting capacitor is needed to prevent the loop from closing the Direct Current (DC) decoupling circuit. Coil 2 (\( C_m = 47 \, \text{pF}, \, C_t = 470 \, \text{pF} \)) is a parallel tuned coil with 2 balanced matching capacitors and an active decoupling pole formed with one of the tuning capacitors.

Coil 3 (\( C_m = 22 \, \text{pF}, \, C_t = 230 \, \text{pF} \)) is a newly proposed parallel tuned coil with a balanced matching network, where one of the matching capacitors is split and used to create a pole together with the inductor \( L_1 \) and the tuning capacitor (opening the loop at the test frequency). The nonsplit matching capacitor is also used to create an additional pole with \( L_2 \), which effectively breaks the ground path of the loop and improves the decoupling performance. The values of \( L_1 \) and \( L_2 \) to create the poles are given by the following equations:

\[ L_1 = \frac{1}{\omega^2 \left( \frac{1}{C_t} + \frac{1}{2 \cdot C_m} \right)} \]  
(5)

\[ L_2 = \frac{1}{\omega^2 \cdot C_m} \]  
(6)

As shown in the previous section, the equivalent resistance of a low-loss parallel tuned coil depends on only the losses in the loop and the tuning capacitor. This permits the addition of extra matching capacitors with a minimal effect over the total losses. Therefore, the main advantage of the design proposed for coil 3 is that only 1 tuning capacitor is used to close the loop, thus reducing the resistance added to the resonator. In this case, the loop does not short-circuit the DC decoupling path because of the added split matching capacitor.

**METHODS**

**Coil Geometry**

Selecting the right inductance of a receive coil (single loop) is not a trivial task, and it is mainly dependent on the frequency of the experiment and the imaging target depth. A higher inductance provides higher induced signal while reducing the current in the resonator. However, sample losses scale up with the inductance and can be easily dominant, particularly at higher frequencies.

For the case of cryogenic coils, a significant extra reduction of coil losses is made, which can provide an SNR enhancement as long as sample losses are low. Therefore, as sample losses scale with the inductance, there are also reasons to keep the inductance as low as possible. Mispelter et al. (16), in their study, propose a rule of thumb of keeping the coil impedance between 20 and 200 \( \Omega \), which seems appropriate for a general case.

In the rest of this study, we will perform our analysis using a (somewhat) arbitrary 50-mm-diameter loop coil, wound with 2.3-mm-thick copper wire. This provides an inductance of \( \sim 100 \, \text{nH} \) (\( \sim 20 \, \Omega \) at 32.13 MHz). The main purpose of using this geometry is to use a low-inductance coil, to explore the limits of SNR that can be gained by cooling down a copper coil while keeping sample losses minimal. This coil size is useful for small animal imaging, as it can cover the entire depth of a small rodent, and it also has the potential to be used as a building block for arrays.
The inductance of a 50-mm loop wound with 2.3-mm-thick copper wire can be approximated using the following equation:

$$L = \mu_0 a a \left[ \ln \left( \frac{16a}{d} \right) - 2 \right] = 99 \text{ nH} \quad (7)$$

Where \( a \) is the radius of the loop and \( d \) is the diameter of the wire. Its resistance is calculated using the following equation:

$$R_C = \frac{2a}{d\delta} \quad \text{with} \quad \delta = \sqrt{\frac{2\rho}{\omega \mu_0}} \rightarrow R_C = \frac{a}{2 \delta \rho \mu_0} = 30 \text{ m\Omega} \quad (8)$$

where \( \rho \) is the wire resistivity and \( \delta \) is the skin depth.

**Cryostat**

For the cryogenic measurements, a simple cryostat was fabricated. The cryostat has the following 2 main parts: a liquid nitrogen deposit enclosed in a Styrofoam isolation and a “cold finger,” made of 2 ceramic bars \((10 \times 10 \times 300 \text{ mm}^3)\) of aluminum nitride (Precision Ceramics, Birmingham, UK) partially immersed in a liquid nitrogen deposit. The coil is attached to the other end of the ceramic bars with nylon cable ties, and thermally insulated using a coil holder made of Rohacell (Evonik GmbH, Essen, Germany) and a layer of 5 mm of aerogel (Aspen Aerogels Inc., Northborough, MA, USA) placed between the coil and sample.

This setup allows to cool the coil to 105 K in a consistent way, with the 5-mm aerogel layer providing sufficient thermal insulation for \(>1\) hour of experiment without significant thermal transfer to the sample. Therefore, the coil-to-sample distance when the coil is mounted on the cryostat is about 5 mm. For this reason, the measurements at room temperature were done using a 5-mm-thick polytetrafluoroethylene (PTFE) spacer placed between the coil and phantom, to make them comparable. Although this spacing is not necessary at room temperature, it is not the purpose of this study to evaluate the loss of coupling to the sample when extra distance to the coil is included.

**Simulation Study**

The losses in the 3 actively decoupled coils proposed in Figure 3 were first evaluated via numerical simulations, using a commercial full-wave simulator with its circuit co-simulation (CST Computer Simulation Technology AG, Darmstadt, Germany) feature to add the losses in the lumped elements. The copper conductivity at room temperature and 105 K was taken from Matula et al.’s study (17), with values of 5.96 \( \times 10^7 \) and 2.87 \( \times 10^8 \) \([\text{S/m}]\), respectively. Capacitor losses are modeled using its ESR, which can be calculated from its datasheet, by dividing their reactance at the frequency of interest over the Q-factor. Ceramic capacitors from the CHB series (TEMEX Ceramics, Persac, France) were used. In this case, we found that capacitors that ranged between 22 and 470 pF have an ESR of about 20 m\( \Omega \), whereas capacitors of \(\approx\)1000 pF have an ESR of \(\sim\)40 m\( \Omega \). At the time of writing this paper, there were no available data about the ESR of these capacitors at low temperatures; therefore, based on our own characterization, we take the approximation of the ESR that reduces to half when cooled down to liquid nitrogen temperature.

The isolation provided by the diodes used for the active decoupling can potentially play an important role in the total circuit losses. Several nonmagnetic PIN diodes were tested, and finally, MMP7072-128-1 PIN diodes (MACOM, Lowell, MA, USA) were used. The isolation provided by these diodes was measured for 32.13 MHz at room temperature and 105 K, with obtained values of 60 and 120 k\( \Omega \), respectively. These values of equivalent resistance were then also included in the simulations to improve their accuracy. Simulations of the unloaded coils without including diode losses were also performed, to determine the importance of these losses relative to the losses in the capacitors.

Simulations were repeated with the coils unloaded (surrounded by free space) and loaded with a spherical phantom emulating the sample placed at a distance of 5 mm from the coil. The phantom is 38 mm in diameter and is filled with muscle simulating liquid as defined by Gabriel et al. (18), which, at 32.13 MHz, has electrical properties, \(\varepsilon_r = 87.92\) and \(\sigma = 0.686\) \([\text{S/m}]\). The boundary condition was a conductive wall cube 600 mm in length with the coil placed in the center.

**Bench Characterization**

The coils were then fabricated and characterized in the laboratory. The different active decoupling circuits were mounted on an FR4 printed circuit board etched in house with an appropriate pattern to mount the lumped elements. Because of the relatively high penetration depth at 32.13 MHz (11.7 \(\mu\text{m}\)), the printed circuit board with 3 oz (105 \(\mu\text{m}\)) copper clad was used. High Q variable capacitors (Johanson Technology Inc., Camarillo, CA, USA) of the 80H85 series where added to allow for fine tuning and matching.

The phantom used to emulate the sample loading is a 38-mm-diameter sphere containing a 1.0M solution of \(^{13}\text{C}\) sodium bicarbonate (85 mg/mL) in water. The electrical properties of this solution could not be measured, as the phantom was sealed, but because of the \(^{13}\text{C}\) enrichment, it is expected that the conductivity of such a solution will be higher than the nominal values for the muscle simulating liquid described in Gabriel et al.’s study (18).

The coils were first characterized in terms of the unloaded and loaded Q-factor by measuring the reflection coefficient \(S_{11}\) with a vector network analyzer (VNA). For a coil matched to 50 \(\Omega\), half the power is dissipated at the signal source, so that the Q-factor is given by the following equation:

$$Q = \frac{2 \cdot f_0}{\Delta f_{-3dB}} \quad (9)$$

where \(\Delta f_{-3dB}\) is the \(-3\) dB bandwidth measured from the \(S_{11}\) parameter.

The decoupling performance of the coils was also evaluated on the bench using a VNA. The following measurement method is used: a small pickup loop (15 mm in diameter) is placed close to the coil under test (eg, 5 mm). Both coils are then connected to the 2 ports of the VNA, and the transmission coefficient \(S_{12}\) is measured for the ON and OFF states of the PIN diodes. The isolation provided by the decoupling circuit is then calculated as the difference in dB between both states.

**Scanner Measurements**

Imaging experiments were performed using a 3 T clinical scanner (Signa HDx, GE Healthcare, Waukesha, WI, USA), using the phantom described in the previous section. The transmission coil
is a $^{13}$C commercially (GE Healthcare, Waukesha, WI, USA) available 60-cm-diameter volume coil, of the clamshell type, previously described in Nelson et al.’s (4) and Tropp et al.’s (19) studies. A single echo time spiral imaging sequence was used with a field of view $= 80 \times 80$ mm, section thickness $= 50$ mm, and pixel size $= 5$ mm, similar to the commonly used in vivo spiral imaging sequence used for hyperpolarized examinations (20). Measurements were repeated 32 times, and the obtained images were averaged in magnitude. The total scanning time for the entire sequence, including repetitions, was 32 seconds. The SNR profile, perpendicular to the receiving coil plane, was calculated from the magnitude image as described in Henkelman et al.’s study (21).

All the coils were first connected to a WMA32C low-noise preamplifier (WanTCom, Chanhassen, MN, USA), which has a nominal input impedance of $3 \Omega$ and a measured noise figure at room temperature of 0.7 dB at 32.13 MHz. This preamplifier is not rated for low temperatures, and it was kept at room temperature during the tests where the coils were cryogenically cooled.

## RESULTS

### Room Temperature

The results obtained from the simulations and bench measurements are summarized in Table 1. The coil resistance $R_c$ and inductance $L$ are taken from the electromagnetic simulation, with good agreement with the theoretical equations (7) and (8) for a wire loop. The losses added by the matching network $R_m$ are then calculated from the unloaded Q-factor obtained using the circuit co-simulation, as the difference between the total losses and $R_c$. The losses added by the sample are calculated as the difference between the losses obtained in the loaded and unloaded simulations. The SNR obtained with each of the coils is shown in Figure 4, averaged over 3 voxels and plotted against the phantom depth.

### Active Decoupling Performance

Figure 5 shows the measured active decoupling performance for coil 3, which, at 32.13 MHz, provides a decoupling of 41 dB at room temperature and 48 dB when cooled. The traditional circuits used in coils 1 and 2 provide a better decoupling (see last column of Table 1), and this is because of the Q-factor of the inserted pole. For coils 1 and 2, the pole is made using a tuning capacitor ($\sim 470$ pF) in parallel with an inductor of $\sim 52$ nH. For coil 3, the capacitance of the pole is dominated by the matching capacitors ($20 – 40$ pF), which then require much higher inductors ($620 – 1240$ nH) in parallel to resonate. These values of inductance are $> 10$ times higher than those used in coils 1 and 2, reducing the Q of these components. This effect is better seen when cooling, as the resistance of the inductors decreases, increasing the Q of the poles and the isolation. Moreover, $40 – 50$ dB of isolation is likely sufficient for most applications, and it is similar to published data from similar experiments (22, 23).

### SNR: Room Temperature vs. Cryogenic Temperature

Using the cryostat described before, the Q-factor of the coil was measured, with a ratio of 793/586 obtained between unloaded

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**Table 1. Coil Parameters Obtained from Simulations and Bench Measurements**

<table>
<thead>
<tr>
<th>Coil</th>
<th>$T$ [K]</th>
<th>$L$ [nH]</th>
<th>$R_c$ [mΩ]</th>
<th>$R_m$ [mΩ]</th>
<th>$R_s$ [mΩ]</th>
<th>$Q_u$ (only capacitors/full circuit)</th>
<th>$Q_L$</th>
<th>$Q_u$</th>
<th>$Q_L$</th>
<th>Active decoupling [dB]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coil 1</td>
<td>290</td>
<td>95.2</td>
<td>62</td>
<td>3</td>
<td>3</td>
<td>127/220</td>
<td>209</td>
<td>216</td>
<td>195</td>
<td>62</td>
</tr>
<tr>
<td>Coil 2</td>
<td>290</td>
<td>95.2</td>
<td>39</td>
<td>3</td>
<td>3</td>
<td>316/303</td>
<td>286</td>
<td>293</td>
<td>243</td>
<td>62</td>
</tr>
<tr>
<td>Coil 3</td>
<td>94.3</td>
<td>94.3</td>
<td>19</td>
<td>3</td>
<td>3</td>
<td>435/401</td>
<td>380</td>
<td>390</td>
<td>316</td>
<td>41</td>
</tr>
<tr>
<td>Coil 1</td>
<td>105</td>
<td>94.3</td>
<td>37</td>
<td>3</td>
<td>3</td>
<td>372/371</td>
<td>363</td>
<td>403</td>
<td>341</td>
<td>67</td>
</tr>
<tr>
<td>Coil 2</td>
<td>105</td>
<td>94.3</td>
<td>19</td>
<td>3</td>
<td>3</td>
<td>559/584</td>
<td>563</td>
<td>572</td>
<td>464</td>
<td>67</td>
</tr>
<tr>
<td>Coil 3</td>
<td>94.3</td>
<td>94.3</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>835/801</td>
<td>756</td>
<td>793</td>
<td>586</td>
<td>48</td>
</tr>
</tbody>
</table>

Abbreviations: $L =$ loop inductance, $R_c =$ loop resistance, $R_m =$ resistance added to the resonator by the circuit elements, $R_s =$ resistance added by the sample (for the loaded case), $Q =$ quality factor of the coil (unloaded and loaded), Active decoupling = difference between the signal transferred from Tx to Rx coil in the ON and OFF states of the circuits (in dB).
and loaded Q (at 105K). Using these values, we can calculate the expected SNR enhancement when cooling from 290 to 105 K using the following equation:

\[
\frac{SNR_{105K}}{SNR_{290K}} = \frac{Q_{\text{unloaded} \ 290K} + Q_{\text{loaded} \ 290K}}{Q_{\text{unloaded} \ 105K} + Q_{\text{loaded} \ 105K}} = 1.63
\]

To confirm this enhancement, imaging experiments were performed with coil 3 cooled to 105 K using the cryostat described before. Images were then acquired at room temperature and at 105 K, in an otherwise identical setup. The obtained SNR and images are shown in Figure 6, where the expected SNR enhancement is confirmed. This result shows that coil losses are indeed the primary source of noise for this combination of frequency, coil, and sample size.

**DISCUSSION**

The measured Q-factors for the 3 studied circuits agree well with the simulations for the unloaded case, which shows that the model used for the lumped element losses is reasonable and that the losses in the circuitry are ultimately dominated by the ESR of the capacitors. The extra losses added by the PIN diodes are not very significant in this case as long as PIN diodes with good isolation are used. However, it can be noted that their effect is higher for coil 3 than for the rest, which is explained because of the lower tuning capacitance (higher reactance) required in this circuit, which makes the equivalent parallel resistance of the PIN diode relatively more important. The unsegmented coil (coil 3) showed the largest measured Q-factor in the presence of the sample, confirming that segmentation is not beneficial for the small loop size used here.

The SNR results obtained are consistent and show proportionality to the Q-factor, with coil 3 providing the best SNR (about a 20% improvement compared with coil 2, and almost 40% more than coil 1). The effect of capacitor losses is even more relevant if the thermal losses of the coils are reduced by cryogenically cooling them, accounting for almost half of the total losses for coil 3 when unloaded. The measured Q-factor of the loaded coils is lower than the simulated values, but this can be explained because of the higher conductivity of the 13C-enriched solution compared with the muscle tissue properties described in Gabriel et al.’s study (18).

The results obtained in this study show that for some MRI experiments at lower frequencies, the circuit scheme chosen to drive the coil can have a very important effect on the total losses. When the coil resistance is very low, the losses of the resonator can be easily dominated by the ESR of the tuning capacitor(s). In this case, it is advantageous to use a parallel tuned scheme and close the loop with only 1 capacitor, such that the resistance added to the resonator is minimized. The reason, as shown in the Supplemental Appendix, is that for such a configuration, the losses in the matching capacitors can be almost neglected. To prevent the loop from shorting the DC decoupling circuit, an extra matching capacitor needs to be added and used to create the tuned trap activated by the decoupling DC signal. The result might be counterintuitive for RF coil designers at higher frequencies, as segmenting the tuning capacitor is one of the most common techniques used to minimize sample losses. The results obtained here show that, depending on the balance between loop, circuit, and sample losses, adding segmenting capacitors may be counterproductive in some cases.

The new coil circuit proposed is particularly useful for cryogenic coils, as the impedance transformation required for parallel tuned coils with very low losses causes dominance of the losses in the tuning capacitor on the losses of the tuning/matching/decoupling network. Here, we have also confirmed this result through bench characterization and imaging experiments on a phantom. An ~1.6-fold SNR improvement at the
phantom surface was obtained using a 50-mm-diameter loop. This enhancement was obtained even though the sample loss contribution introduced by the phantom was significantly higher than that of the physiological tissue (as shown with the discrepancy of loaded Q-factors in Table 1). Therefore, an even higher enhancement is expected for in vivo experiments.

One shortcoming of this circuit, which may make it unsuitable for some applications, is the lower decoupling due to the losses in the high inductors needed for the poles. We have shown a decoupling of ~40 dB at room temperature compared with that at 60 dB provided by the traditional circuits. For cryogenic coils, this value gets significantly better because of the loss reduction of the matching capacitors and the inductors’ resistance, which effectively increase the Q-factor of the poles. Moreover, an isolation over 40 dB may still be enough for most applications, where both transmitting and receiving coils are separated by at least a few centimeters.

CONCLUSION

Given the added complexity and technical difficulties introduced by cryogenic coils for in vivo experiments, it is important to reduce the coil losses to its minimum at room temperature before cryogenically cooling. In this study, an improved circuit scheme for receive-only RF coils is proposed, which significantly reduces the coil losses for coils with very low resistance. For $^{13}$C at 3 T, >20% SNR improvement was obtained at room temperature (compared with traditional schemes) using a simple 50-mm-diameter loop (~100 nH). The applicability of this circuit to cryogenic coils is also shown by cryogenically cooling it to 105 K, with an extra ~1.6-fold SNR improvement obtained compared with room temperature. The overall performance of this circuit is based on the assumption of a low-loss coil, which requires very low capacitances for impedance matching. This makes this circuit particularly suitable for cryogenic coils. The SNR improvements of the proposed circuit will be even more noticeable for coils with smaller inductance (lower frequency and/or size).

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REFERENCES


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