Quantitative Magnetization Transfer Acquisition Protocol Optimization for B1-Insensitivity through Sensitivity-Regularization of the Cramér-Rao Lower Bound

**Mathieu Boudreau MSc1 and G. Bruce Pike PhD1,2**

1Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada

2Hotchkiss Brain Institute and Department of Radiology, University of Calgary, Calgary, Alberta, Canada

**In submission to: Magnetic Resonance in Medicine**

**Corresponding author**

Mathieu Boudreau

Room WB-325, McConnell Brain Imaging Centre

Montreal Neurological Institute

McGill University, Montreal

Quebec, Canada

H3A 2B4

E-mail: mathieu.boudreau2@mail.mcgill.ca

Phone: (438) 822-8747

**Grant support:** Funding for M.B. was provided by the National Sciences and Engineering Research Council of Canada with the Alexander Graham Bell Canada Graduate Scholarships-Doctoral program. Funding to GBP from the Canadian Institutes of Health Research also supported this research.

**Running title:** qMT Optimization through Sensitivity-Regularized CRLB

**Word count: 4772**

**Title: Quantitative Magnetization Transfer Acquisition Protocol Optimization for B1-Insensitivity through Sensitivity-Regularization of the Cramér-Rao Lower Bound**

**Abstract**

**Purpose:** To develop and validate a regularization approach of optimizing B1-insensitivity of the quantitative magnetization transfer (qMT) pool-size ratio (F) parameter.

**Theory and Methods:** A simple expression describing the impact of B1 inaccuracies on qMT fitting parameters was theoretically derived using a sensitivity analysis. To simultaneously optimize for robustness against noise and B1-inaccuracies, the optimization condition was defined as the Cramér-Rao lower bound (CRLB) regularized by the B1-sensitivity expression for the parameter-of-interest (F). The optimal regularization parameter was determined for B1-sensitivity robustness while minimizing the effect on the CRLB. qMT protocols were iteratively optimized from an initial search space (12 MT flip-angles, 26 off-resonance frequencies, variable flip angle T1 mapping), with and without B1-regularization. Three 10-point qMT protocols (uniform, CRLB, CRLB+B1-regulatization) were studied using Monte Carlo simulations considering a wide range of conditions (SNR, B1-inaccuracies, tissues).

**Results:** The B1-regularized CRLB optimization protocol had the best robustness of F against B1 errors, for a wide range of SNR for both white matter and grey matter tissue types. For an SNR of 100, this protocol resulted in errors in mean F<1% for B1 errors ranging between -10 to 20%, the range typically observed in vivo in the human head. Both CRLB-optimized protocols resulted in the lowest σF values for all SNRs, and were not impacted by B1 inaccuracies.

**Conclusion:** This work demonstrated the effectiveness of a regularized optimization approach for improving the robustness of auxiliary measurements sensitivity of qMT parameters, and that it could be possible to omit B1 mapping altogether with minor impact on the fitted pool-size ratio values.

**Keywords:** Magnetization Transfer, pool-size ratio, B1 mapping, Cramér-Rao lower bound, optimization, sensitivity analysis.

# INTRODUCTION

Quantitative magnetization transfer (qMT) imaging is a class of techniques that indirectly probe macromolecular content undetectable using conventional MRI due to their inherently small T2\*. Most qMT techniques quantify properties of macromolecular hydrogen (“restricted pool”) relative to hydrogen in nearby liquid water molecules (“free pool”) by solving the Bloch-McConnell equations describing magnetization exchange between these two interacting pools ([1](#_ENREF_1_1)). Particularly, the pool-size ratio F (ratio of equilibrium magnetization between both pools) is a qMT parameter that correlates strongly with myelin content ([2](#_ENREF_1_2),[3](#_ENREF_1_3)). As such, the pool-size ratio is a potential biomarker for lesion monitoring in multiple sclerosis (MS) patients ([4](#_ENREF_1_4),[5](#_ENREF_1_5)), and has been shown to correlate with de- and remyelination in a mice model of MS ([6](#_ENREF_1_6)).

Several techniques have been developed to acquire and model qMT data. qMT data is most commonly acquired using pulsed off-resonance MT-prepared spoiled gradient echo (SPGR) sequences ([7](#_ENREF_1_7)), however techniques using inversion recovery ([8](#_ENREF_1_8)) and balanced steady-state free precession have also been proposed ([9](#_ENREF_1_9)). Analytically solving the Bloch-McConnell equations is challenging unless a long continuous-wave MT pulse is used ([10](#_ENREF_1_10)), which is impractical for in vivo measurements. Several fitting models have been developed to estimate quantitative parameters from pulsed SPGR qMT data ([7](#_ENREF_1_7),[11](#_ENREF_1_11),[12](#_ENREF_1_12)), each with unique sets of experimental assumptions and approximations. In addition, SPGR qMT techniques require several additional quantitative measurements, such as main field (B0) mapping, transmit radiofrequency (RF) field (B1) mapping, and longitudinal relaxation time (T1) mapping. In this context, B0 mapping is used to calibrate the off-resonance frequency values in the presence of main field inhomogeneity ([13](#_ENREF_1_13)), B1 mapping to correct the RF field amplitude variations due to coil loading ([14](#_ENREF_1_14),[15](#_ENREF_1_15)), and T1 mapping to constrain the magnetization transfer fitting parameters ([7](#_ENREF_1_7),[10](#_ENREF_1_10),[16](#_ENREF_1_16)). These three measurements, in addition to the 10+ qMT measurements needed to fit the full set of fitting parameters ([17](#_ENREF_1_17)), makes it a challenge to acquire qMT data in a clinically feasible acquisition time.

Several strategies have been developped to improve the SPGR qMT acquisition time which originally consisted of over 60 qMT measurements ([7](#_ENREF_1_7)), limiting it to a single slice. The first three-dimensional qMT brain scan was achieved using a “uniform” acquisition protocol by reducing the number of off-resonance frequencies (Δ) to 5 (uniformly ranging between 400 Hz and 20 kHz) and MT flip angles (FAMT) to 2 (high and low values), for a total of MT-weighted 10 measurements ([18](#_ENREF_1_18)). Other studies went further, optimizing the protocol Δ and FAMT values using the Cramér-Rao lower bound (CRLB) as an optimization condition to minimize parameter variances, using simulated annealing ([19](#_ENREF_1_19)) or an iterative reduction procedure from an initial search space ([17](#_ENREF_1_17)). Rapid k-space readout techniques such as echo planar imaging have also been proposed to improve acquisition times ([20](#_ENREF_1_20)). The choice of B0/B1/T1 mapping techniques have also evolved over time, with researchers typically choosing the most rapid and reliable technique available at their disposal. For instance, the evolution from single-slice qMT imaging to whole-brain imaging required a switch from single-slice T1 mapping techniques (e.g. inversion recovery – IR, Look-Locker – LL) to 3D techniques (e.g. Variable Flip Angle – VFA). However, recent work has shown that this transition may have an important impact on the fitting parameters, since IR is a B1-insensitive technique ([21](#_ENREF_1_21)), whereas VFA is a B1-sensitive technique ([22](#_ENREF_1_22)). For a uniform 10-pt SPGR qMT protocol, it was demonstrated that the pool-size ratio F is much less sensitive to B1 errors if the qMT protocol uses VFA T1 mapping, relative to B1-insensitive T1 mapping techniques ([23](#_ENREF_1_23)). Since that work used a fixed qMT acquisition protocol to demonstrate the benefit of using VFA T1 mapping for F, it raised an interesting question: is it possible to further improve the robustness of F against B1 inaccuracies by optimizing the qMT acquisition protocol for B1-insensitivity?

The aim of this work is to develop a method to optimize the B1-sensitivity of qMT parameters (particularly F) for a fixed T1 mapping method, by regularizing the CRLB optimization condition with a B1-sensitivity term. We first developed a B1-sensitivity expression which was used to regularize the CRLB condition. Using simulations, we then explored the B1-sensitivity of qMT for different uniform protocol configurations. The optimal regularization term for the pool-size ratio was determined, and a sample qMT protocol was iteratively optimized using the CRLB condition both with and without the regularization term. The robustness of three protocols (uniform, CRLB, CRLB + B1 regularization) were then investigated using Monte Carlo simulations for a range of signal-to-noise (SNR), B1-inaccuracies, and tissue values.

# THEORY

In the presence of a small inaccuracy of a measurement parameter, such as B1 in qMT, this error will propagate to the fitting parameters of the model. The behavior of how this propagate error will impact each fitting parameter can be explored through a sensitivity analysis, by expanding the fitted signal in the presence of a ΔB1 with a Taylor expansion ([24](#_ENREF_1_24)). Assuming a small ΔB1 and a good fit (*M*(*B1*+ ΔB1) ≈ *M*(*B1*) ≈ *M*meas, where *M* is the signal generated by the fit), a first-order approximation of the Taylor expansion of the fitted signal results in the following matrix equation ([23](#_ENREF_1_23)):

|  |  |  |
| --- | --- | --- |
|  |  | **[1]** |

where *p* are the model fitting parameters (e.g. for the Sled and Pike([1](#_ENREF_1_1)) model of qMT: F, kf, T2,f, T2,r), is the column vector of errors in fitted parameters [ΔF, Δkf, ΔT2,f, ΔT2,r]ʹ, and are matrices with sensitivities values elements relative to *pi* or *B*1 (columns) for each measurement *n* (rows). can also be described as being the Jacobian of the measurement for the fitting parameters, which we’ll call the Jacobian sensitivity matrix moving forward.

Given a known ΔB1 value and Jacobian sensitivity matrices for *p* and *B*1, Eq. 1 can be solved for . However, since Eq. 1 is typically an overdetermined system of linear equations (), the optimal solution is found by minimizing the 2-norm for :

|  |  |  |
| --- | --- | --- |
|  |  | **[2]** |

Although Eq. 2 provides an estimate of the error propagated to the fitting parameters by an error in B1 got a given measurement protocol, it is insufficient in itself for optimal protocol design. qMT protocols must also be designed for robustness again noise naturally occurring in measured signals. For this purpose, the Cramér-Rao lower bound (CRLB) has been shown to be an adequate and sufficient estimate to minimize the variance in fitted qMT parameters due to experimental noise ([19](#_ENREF_1_19)).

Consider the Fisher information matrix (FIM) **J**, which has elements:

|  |  |  |
| --- | --- | --- |
|  |  | **[3]** |

where σ is the standard deviation of the noise, and **x***n* is the acquisition protocol for the *n*th measurement out of N unique measurements. The CRLB is defined as the diagonal elements of **J**-1, and the trace of this matrix provides an overall estimate of the minimum variance of a model. However, because the qMT fitting parameters differ largely in their order of magnitudes, the parameter-normalized CRLB (*V*) is defined ([19](#_ENREF_1_19)):

|  |  |  |
| --- | --- | --- |
|  |  | **[4]** |

In this work, we propose a regularization approach to minimizing both for noise (Eq. 4) and B1-error propagation (Eq. 2), using an iterative optimization approach for the acquisition protocol design ([17](#_ENREF_1_17)). Particularly, we are interested in minimizing the propagation of B1-error to the pool-size ratio *F* (Δ*F*) because of its demonstrated potential as a biomarker for myelin content. Thus, to optimally reduce an acquisition protocol of N unique measurements to N-1 measurements, at each iteration we solve for:

|  |  |  |
| --- | --- | --- |
|  |  | **[5]** |

where λ is the regularization parameter constant, and **x**N-1 is the N-1 optimal qMT subset protocol of **x**N for a given iteration. The regularization parameter λ value controls the tradeoff between CRLB (noise) and *F* sensitivity to B1-inaccuracies during the optimization.

# METHODS

The core qMT functions and routines used in the simulations and fitting of this work are from qMRLab (http://github.com/neuropoly/qMRLab), an open-sourced quantitative MRI software packaged that evolved from qMTLab ([25](#_ENREF_1_25)) and is written in MATLAB (MATLAB 2017a; MathWorks Inc., Natick, MA). The additional source code developed in this work, particularly for numerically estimating the Jacobians matrices of the system, the protocol optimization algorithms, and the Monte Carlo simulations, is released as its own open-source package (http://github.com/mathieuboudreau/qMTLab\_Tabs). The code was developed to wrap around the qMRLab code, so that it may be easily adaptable with other qMT software packages or in-house code.

## Uniform Protocols

The regularization term in Eq. 5 proposed for optimization against B1 was derived as a result of a first-order approximation of a Taylor series. To test this approximation, **Δ*p*** values (ΔF, Δkf, ΔT2,f, ΔT2,r) were calculated from Eq. 2 for a range of ΔB1 typically observed in vivo (±30%, assuming B1 = 1.0 n.u.), and were compared to values estimated by fitting the signal to the Bloch-McConnell equations ([7](#_ENREF_1_7)). A “uniform” qMT measurement protocols was used, meaning a protocol with logarithmically uniform off-resonance frequencies for each MT flip-angle (αMT) preparation pulse (see Table 2). Jacobian sensitivity matrices calculations for Eq. 2 (and) were estimated from numerical partial derivatives (10-2 % relative increase in parameter values). Two different qMT cases were considered for : B1-independent T1 measurements (IR) and B1-dependent T1 measurements (VFA). Signal simulation details (protocol and tissue parameters) are described in full detail in a recent study ([17](#_ENREF_1_17)).

Prior to protocol optimization, we were also interested in investigating values (from Eq. 2) on the number of MT flip angles and off-resonance values for other uniform qMT protocols. MT-prepared SPGR (TR = 25 ms, α = 7°) pulse sequence protocols using every combination of three αMT values (150°, 400°, 650°) were used (each unique αMT, each combination of two αMT values, and all three). Logarithmically-uniform offset frequencies for each αMT values ranged between 300 Hz and 20 kHz. To fairly assess all “uniform” protocols, the total number of acquisitions were limited between 8 and 30 by varying the number of offset values per αMT sets. For example, a single-αMT 10-point protocol would have 10 off-resonance frequencies, and a two-αMT 10-point protocol would have the same 5 off-resonance frequencies for both αMT. qMT signals were generated for tissue values within the typical white matter range (Table 1). A 5% overestimation in B1 value (ΔB1 = +0.05 n.u.) relative to the expected value (B1 = 1 n.u.) was used for all protocols to solve Eq. 2 for , and a VFA T1 mapping method was assumed (TR = 15 ms, α = 3° and 20°).

## Protocol Optimization

qMT protocols were iteratively optimized ([17](#_ENREF_1_17)) from a large initial search-space set of potential protocol values for αMT and Δ (for fixed TR and α, 25 ms and 7°). The most time-intensive component of the optimization algorithm is computing the Jacobian sensitivities ( and ). The Jacobian sensitivities were precomputed using parallel processing and cached for rapid access during the optimization algorithm. Note that both terms in Eq. 5 require element values from the Jacobian sensitivity matrices (through Eq. 4 and 3). The optimization search-space consisted of 312 points; each combination of 12 αMT values (ranging between 150° to 700°, in 50° increments) and 26 Δ values (ranging between 300 Hz and 20 kHz, with logarithmically uniform steps). A few (<5%) protocol points resulted in outlier numerical partial derivative values (non-smooth Jacobian sensitivity curve at those points), which may be due to signal simulation rounding errors or imprecise free-pool saturation fraction interpolations for derivatives of the open-source software used. Those protocol points were replaced with the nearest-neighbor points calculated from a higher-resolution 2929-point search-space (29 αMT and 101 Δ values). The Jacobian sensitivity matrices were calculated for white matter tissue values (Table 1).

Prior to protocol optimization, an optimal value for the regularization parameter λ was determined. The iterative optimization algorithm evaluating Eq. 5 was executed for a range of λ values (λ = 0, 0.01, 0.1, 0.5, 1, 2, 5), assuming ΔB1 = 0.05 and VFA T1 mapping (TR = 15ms, α = 3° and 15°). Because TR, TE, and α were fixed for all protocol points, the standard deviation of the noise in Eq. 3 (σ) was arbitrarily set to 1 for all calculations. The ΔF values and variance-efficiency ([variance × # acq. points]-1/2, where the variance is interpreted to be the parameter-normalized CRLB *V*) curves were compared for each N during the iterative optimization procedure. λ = 0.5 was determined to sufficiently minimize ΔF without substantially reducing the variance-efficiency. Two sets of 10-point protocols were optimized by iteratively finding the N-1 protocol subset which minimized Eq. 5 for ΔB1 = 0.05 (and assuming the VFA as above) and two cases of λ (λ = 0, noted CRLB, and λ = 0.5, noted CRLBλ=0.5).

## Monte Carlo Simulations

Ideal (noiseless) MT-prepared SPGR signals were simulated for three 10-point protocols (Table 2: Uniform, CRLB, and CRLBλ=0.5) and two tissue types (Table 1: white matter, grey matter). Rician noise was added to each simulated MT signal and an MT-off signal, for normalization (*M*MT/*M*MT-off). Six different SNR levels were considered (SNR = 25, 50, 75, 100, 150, 200). Sets of 10,000 noisy MT signals were independently generated and compared for each combination of qMT protocols, tissues, and SNR. Each dataset were subsequently fitted for qMT parameters (F, kf , T2,f, and T2,r) for a range of B1 errors (±30% in increments of 5%), and considering a two-FA VFA T1 mapping method (TR = 15ms, α = 3° and 15°).

# RESULTS

## Uniform Protocols

Figure 1 shows the simulated errors in each fitting parameter (ΔF, Δkf, ΔT2,f, ΔT2,r) estimated from the first-order approximation of the Taylor expansion in Eq. 2 (solid lines) and from the relative error in fit using the Sled and Pike model (dash line) in the presence of B1 errors (±30%). Data was simulated for a B1-independent T1 measure (IR, red) and a B1-dependent T1 measure (VFA, blue) separately. The overall trends in the error curves produced by model fits reproduced well similar simulations which were reported recently ([23](#_ENREF_1_23)) (Boudreau et al 2017, Figure 3), but differed in core qMT simulation and fitting software, thus establishing confidence in the use of this open-source qMTLab software ([25](#_ENREF_1_25)) for this work.

The parameter errors estimated from Eq. 2 approximated well the ones resulting from fitting for all parameters for B1 errors within ±5%. For VFA T1 and ΔB1 = 0.05 n.u. (+5 %), the Δ*p* values (Eq. 2, Fit) are: ΔF = (-0.94 %, -1.06 %), Δkf = (14.77 %, 16.88 %), ΔT2,f = (-2.56 %, -1.97 %), and ΔT2,r = (-0.51 %, -0.65%). Both ΔF (for VFA) and ΔT2,r showed linear trends for the “Fit” case, which resulted in better agreement with Eq. 2 overall. A ΔB1 of 0.05 n.u. was selected for the iterative optimization calculation (Eq. 5) later on in this work.

Figure 2 shows the simulated errors fitting parameters for a 5% ΔB1 using a wide range of uniform qMT acquisition protocols (assuming VFA T1) which varied in number of FAMT, number of off-resonance frequencies per FAMT, and total number of acquisitions points. Most curves (sets of FAMT combinations) trend asymptotically with increasing number of acquisition points, but generally not towards 0% parameter error values (except for a few ΔT2,r cases, # FA > 1 protocols that contain 650°). For ΔF, the three # FA = 1 curves (dark blue, orange, yellow) resulted in the largest ΔF values overall, suggesting it’s beneficial to include at least two flip angles in your qMT protocol to give it lower B1-sensitivity. The three protocols that had # FA > 1 that included FA=650° (green, light blue, red) resulted in ΔF curves that overlapped and intercepted ΔF = 0 % values near 10 and 15 acquisition points.

## Protocol Optimization

Figure 3 displays the values of the Jacobian sensitivity matrices (**a-d** are the columns of , **e** is , and f is ). Each plot may also be interpreted as the sensitivity of the Z-spectrum relative to each parameter-of-interest (i.e. the change in Z-spectrum signal value due to a small increase in a given parameter). The peak in the sensitivity curve for F occurs at an order of magnitude higher off-resonance frequencies for high FAMT (>500°) relative to the low values (~150-300°), whereas the peak sensitivity for kf remained constant near Δ = 1-2 kHz. The peak in sensitivity curve for also remained constant near Δ = 1-2 kHz, which may explain why kf has the largest errors out of all other fitting parameters due to ΔB1 (Eq. 1) for the VFA case in Figure 1. The higher sensitivity of F at high off-resonance (>10kHz) values relative to might be a contributing factor in its higher robustness against B1, as was mentioned in the previous section for Figure 2 for the uniform protocols (# FA > 1) that contained the high FAMT value (650°).

The variance-efficiency and ΔF values (for ΔB1 = 0.05) over the course of the iterative optimization of the 312-point protocol search space are shown in Figure 4 for a wide range of regularization parameter λ values. The highest variance-efficiency curve occurs for the λ=0 case (i.e. unregularized parameter-normalized CRLB, Eq. 5), as well as for the λ = 0.01 case. The magnitude of ΔF steadily increases to 1% as the protocol is iteratively reduced to ~150 acquisition points, and then proceeds to decrease to ~0.5% for N < 25. Increasing the regularization parameter by an order of magnitude (λ = 0.1) substantially reduces ΔF values for # acq. points > 25 by up to a factor of two while keeping the variance-efficiency relatively unaffected, however ΔF reconverges to ~-0.5% abruptly when the # acq. points are lower than 25. A regularization parameter of 0.5 was the lowest value tested which succeeded in ΔF converging to values near 0 for a low N; for N = 10, λ = 0.5 resulted in ΔF = -0.04% compared to -0.53 % for λ = 0, a relative improvement by a factor of 13. A slight overall reduction in variance-efficiency occurs for λ = 0.5; for N=10, the variance-efficiency reduced by 6.3% for λ = 0.5 relative to λ = 0. For higher λ values, the regularization term in Eq. 5 dominates early in the iterative optimization at the cost of lower variance-efficiency, which never recovers to its unregularized values. For intermediately-high λ values (λ = 1, 2), a second region where the regularization term in Eq. 5 dominates the iterative optimization is observed near N = 60 and 120 respectively, also at the expense of variance-efficiency. Overall, a λ value 0.5 appears to show the best compromise between ΔF (insensitivity of F against B1) and variance-efficiency out of the parameters evaluated.

The 10-point optimized protocols for λ = 0 (CRLB) and λ = 0.5 (CRLBλ=0.5) are shown in Figure 5, overlayed on the 312-point protocol search-space (displayed as line plots for better visibility of the optimized protocols). The details of these optimized protocols are also listed in Table 2. Overall, both optimized protocols share 7 out of 10 (Δ, FAMT) pairs, with only three acquisition points changing when the regularization term in Eq. 5 (λ = 0.5) is added. Both protocols have coverage of low, medium, and high off-resonance values, as well as low and high FAMT values.

## Monte Carlo Simulations

Distributions statistics (mean, σ) of the Monte Carlo simulations are shown for the fitted parameter-of-interest F as a function of ΔB1 (SNR = 100) in Figure 6, and as a function of SNR (ΔB1 = 0 and 15 %) in Figure 7, for the three protocols listed in Table 2. Figure 6 a and b displays the difference (%) in mean F relative to the mean F value for the (ΔB1 = 0, SNR = 100) case. Figure 7 a and b displays the difference (%) in mean F relative to the idFor eal (noiseless) fitted F value.

For CRLBλ=0.5, values were less than 1% (grey zone) for ΔB1 ranging between -10% and 20% (Figure 6, for both WM and GM), yet the same was only true between -5% and 10% for the CRLB-optimized protocol and between -5% and 5% for the Uniform protocol. The standard deviations of the F distribution were substantially lower (by a factor of ~1.75) for both CRLB and CRLBλ=0.5 protocols relative to the Uniform protocol. CRLBλ=0.5 σ values were slightly higher than CRLB values (6.7% higher), but both curves nearly overlapped for all ΔB1 values.

In the absence of B1 errors (ΔB1 = 0), values for both optimized protocols (CRLB and CRLBλ=0.5) were below 1% for all datasets with SNR greater than 75 (Figure 7, for both WM and GM). A minimum SNR of 100 was necessary for the Uniform protocol for to be below 1%. In the presence of a 15% overestimation of B1, the vs SNR curve for CRLBλ=0.5 remained largely unchanged for WM. For GM, the vs SNR curve for CRLBλ=0.5 saw a slight increase, however remained within 1% for SNR > 100. Both the CRLB and Uniform protocols saw larger deviations in values for the ΔB1 = 15% case, even at high SNR values (>100). The σ curves as a function SNR increased rapidly for SNR values below 75 for all curves. For all protocols, σ did not vary substantially between both ΔB1 cases (0% and 15%), The difference between the protocols behaved similarly as discussed above for Figure 6, with no substantial difference between the CRLB and CRLBλ=0.5 cases but an overall improvement for both relative to the Uniform protocol.

# DISCUSSION

This paper describes a methodology to design an optimized qMT protocol for reduced B1-sensitivity of the pool-size ratio F by regularizing the CRLB with a first-order sensitivity analysis. We found using Monte Carlo simulations that, for a regularized CRLBλ=0.5, errors propagated to fitted F were limited to below 1% in the presence of B1 errors ranging between -10 and 20%, consistent with the range of B1 values typically observed in the human brain at 3T ([26](#_ENREF_1_26)). Both regularized and conventional CRLB optimization resulted in an improvement of B1-insensitivity for F relative to a two-FAMT uniform protocol. Sensitivity analyses of uniform protocols suggested that acquiring data at a high-FAMT/high-off-resonance frequency value-pair may be an important contributing factor in designing a B1-insensitive acquisition protocol, where F likely has a higher robustness against B1 when VFA T1 mapping is used (as noted in the Jacobian sensitivity plots). This simulation work demonstrates the effectiveness of a regularized approach in optimizing qMT protocols for B1-insensitivity of F, and the work presented here can serve as a methodology in designing qMT protocols which could omit B1 measurements in the qMT protocols altogether if F is the parameter-of-interest for the study.

The work presented here considered a specific qMT fitting model (Sled and Pike([7](#_ENREF_1_7))) which fitted quantitative MT data for four parameters of the Bloch-McConnell equations (F, kf, T2,f, T2,r). Several other qMT fitting models for MT-prepared SPGR data exist, such as Yarnykh’s model that neglects direct saturation effects ([11](#_ENREF_1_11)) and Ramani’s continuous wave power equivalent model ([12](#_ENREF_1_12)). Each qMT fitting model depend on different approximations or assumptions, and some fitting parameters are defined slightly differently. For example, Yarnykh’s model suggests acquiring data only at off-resonance frequencies greater than 1 kHz, and uses a different fitting parameter set (e.g. T2,f is neglected, and their pool-size ratio parameter is defined as *f* = M0,r / (1+ M0,r) instead of Sled and Pike’s F = M0,r / M0,f parameter). The more limited range in off-resonance frequencies reduces the range available values in the Jacobian sensitivity matrix available during optimization, so optimizing against auxiliary measurements error propagation could be more challenging. Different fitting parameters sets will likely also change how the measurement errors are propagated to these parameters, which would likely change the B1-sensitivity between different fitting models for identical SPGR qMT acquisition protocols. Single-point qMT fitting models ([27](#_ENREF_1_27),[28](#_ENREF_1_28)) may provide additional challenges for optimizing against auxiliary measurement error sensitivity, considering that several parameter restraints are built-in within the models, which would provide additional limitations when solving Eq. 2. Our analysis of uniform protocols and Jacobian sensitivity matrices also suggests that B1-insensitivy of F may be a result having both small and large MT flip angle acquisition in your protocol, particularly at mid and high off-resonance frequencies, and such a configuration is not possible with a single-point measurement protocol.

We proposed a regularization approach to add an auxiliary measurement (e.g. B1) sensitivity component to the CRLB condition in the optimization algorithm. An alternative approach could have been to do a formal analysis of the error propagation of auxiliary measurement with the CRLB equations to be used as a condition in the optimization algorithm. Lankford and Does ([29](#_ENREF_1_29)) recently presented such a treatment in the context of T2 mapping. Their statistical analysis of propagation of error from parameter constraints demonstrated, under certain circumstances, it can be beneficial to measure B1 when doing multi-echo T2 mapping. Although not designed as a protocol optimization condition, it may be possible to adapt their theory in order to do so. Their framework was presented in a general manner to be applicable to other quantitative measures using auxiliary measurement, however it was only derived for a single level of parameter constraints. While this could be applicable for a B1-error propagation analysis of qMT if using a B1-independent T1 mapping method such as inversion recovery, a B1-dependent T1 mapping method such as Variable Flip Angle complicates the error propagation analysis beyond what is presented in Lankford and Does, as there are two interacting constraints on the qMT model (B1 and T1(B1)). One benefit of our regularized approach for optimizing acquisitions is in its conceptual simplicity and ease in implementation. Nonetheless, a formal propagation of error analysis would be a great candidate to compare the sensitivity to errors in constraints between different qMT models as discussed above, and should be explored in future work.

Several limitations should be considered when interpreting this work. An iterative optimization approach was chosen to estimate an optimal acquisition protocol from a larger initial search space. Iterative reduction of the protocol search space is not guaranteed to result in the global minima for the optimization condition. Global optimization using simulated annealing ([19](#_ENREF_1_19)) could be another valid approach to optimize an experimental design using Eq. 5. However, iterative optimization approaches benefit from an ease in implementation, rapid computation, and from the flexibility to choose the number of measurements of the experimental design after the optimization is complete, while a simulated annealing approach typically optimizes for a fixed pre-determined number of acquisitions. We opted for Monte Carlo simulations in lieu of an in vivo study to validate the regularized approach to B1-sensitivity protocol optimization. This provided us with accurate knowledge and greater control of the system conditions (e.g. tissue values, B1 error values, and noise level) we wished to investigate. In vivo evidence of the benefits of qMT protocol optimization using CRLB has already been reported in several different studies ([17](#_ENREF_1_17),[19](#_ENREF_1_19),[20](#_ENREF_1_20)), and Eqs. 1 and 2 were developed as a result of a recent comprehensive B1-sensitivity analysis of qMT study ([23](#_ENREF_1_23)) that compared predictions from simulation with in vivo measurements of F in the absence of B1 maps. Lastly, the optimization algorithm investigated here only considered a single tissue type (WM) during the protocol design. Although the resulting protocol was also evaluated for another tissue type in the Monte Carlo simulations (GM), the B1-sensitivity of F was slightly in GM more variable than for WM, even though both were restricted to below 1% for the B1 errors typically experience in vivo. If desired, the optimization condition (Eq. 5) could be adapted to consider multiple tissue types in a manner proposed by Cercignani et al ([19](#_ENREF_1_19)), by minimizing for the tissue which results in the maximum value of Eq. 5, which would require the precomputation of the Jacobian sensitivity matrices of all tissues-of-interest.

Overall, this work presents a framework for designing optimal qMT acquisition protocols for improved robustness against inaccuracies of auxiliary measurements (e.g. B1) by regularizing the Cramér-Rao lower bound with fitting parameter sensitivity information. We demonstrated this methodology by optimizing a qMT protocol for pool-size ratio robustness against B1-inaccuracies, and then evaluated the protocol for a range of signal-to-noise ratios, B1-inaccuracies, and tissue types. These findings imply that B1 mapping could potentially be omitted from such a qMT optimized acquisition protocol with minimal impact on the pool-size ratio estimations (< 1% error). Future work may include optimizing protocols for reduced sensitivity of multiple auxiliary measurements, and comparing the optimization of auxiliary measurement sensitivity between different qMT fitting models. Another interesting way forward could be combining Z-spectrum compressed sensing ([30](#_ENREF_1_30)) with this optimization approach, in order to increase the number of measurements in the protocol to further maximize the auxiliary measurement sensitivity in a time-efficient manner.

# REFERENCES

1. Sled JG, Pike GB. Quantitative interpretation of magnetization transfer in spoiled gradient echo MRI sequences. Journal of Magnetic Resonance 2000;145(1):24-36.

2. Schmierer K, Tozer DJ, Scaravilli F, Altmann DR, Barker GJ, Tofts PS, Miller DH. Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. J Magn Reson Imaging 2007;26(1):41-51.

3. Schmierer K, Wheeler-Kingshott CAM, Tozer DJ, Boulby PA, Parkes HG, Yousry TA, Scaravilli F, Barker GJ, Tofts PS, Miller DH. Quantitative magnetic resonance of postmortem multiple sclerosis brain before and after fixation. Magnetic Resonance in Medicine 2008;59(2):268-277.

4. Levesque IR, Giacomini PS, Narayanan S, Ribeiro LT, Sled JG, Arnold DL, Pike GB. Quantitative magnetization transfer and myelin water imaging of the evolution of acute multiple sclerosis lesions. Magn Reson Med 2010;63(3):633-640.

5. Davies GR, Tozer DJ, Cercignani M, Ramani A, Dalton CM, Thompson AJ, Barker GJ, Tofts PS, Miller DH. Estimation of the macromolecular proton fraction and bound pool T2 in multiple sclerosis. Mult Scler 2004;10(6):607-613.

6. Turati L, Moscatelli M, Mastropietro A, Dowell NG, Zucca I, Erbetta A, Cordiglieri C, Brenna G, Bianchi B, Mantegazza R, Cercignani M, Baggi F, Minati L. In vivo quantitative magnetization transfer imaging correlates with histology during de- and remyelination in cuprizone-treated mice. NMR Biomed 2015;28(3):327-337.

7. Sled JG, Pike GB. Quantitative imaging of magnetization transfer exchange and relaxation properties in vivo using MRI. Magn Reson Med 2001;46(5):923-931.

8. Dortch RD, Li K, Gochberg DF, Welch EB, Dula AN, Tamhane AA, Gore JC, Smith SA. Quantitative magnetization transfer imaging in human brain at 3 T via selective inversion recovery. Magn Reson Med 2011;66(5):1346-1352.

9. Gloor M, Scheffler K, Bieri O. Quantitative magnetization transfer imaging using balanced SSFP. Magn Reson Med 2008;60(3):691-700.

10. Henkelman RM, Huang X, Xiang QS, Stanisz GJ, Swanson SD, Bronskill MJ. Quantitative interpretation of magnetization transfer. Magn Reson Med 1993;29(6):759-766.

11. Yarnykh VL. Pulsed Z-spectroscopic imaging of cross-relaxation parameters in tissues for human MRI: theory and clinical applications. Magn Reson Med 2002;47(5):929-939.

12. Ramani A, Dalton C, Miller DH, Tofts PS, Barker GJ. Precise estimate of fundamental in-vivo MT parameters in human brain in clinically feasible times. Magn Reson Imaging 2002;20(10):721-731.

13. Skinner TE, Glover GH. An extended two-point Dixon algorithm for calculating separate water, fat, and B0 images. Magn Reson Med 1997;37(4):628-630.

14. Jin J, Chen J. On the SAR and field inhomogeneity of birdcage coils loaded with the human head. Magn Reson Med 1997;38(6):953-963.

15. Wiggins GC, Triantafyllou C, Potthast A, Reykowski A, Nittka M, Wald LL. 32-channel 3 Tesla receive-only phased-array head coil with soccer-ball element geometry. Magn Reson Med 2006;56(1):216-223.

16. Caines GH, Schleich T, Rydzewski JM. Incorporation of magnetization transfer into the formalism for rotating-frame spin-lattice proton NMR relaxation in the presence of an off-resonance-irradiation field. Journal of Magnetic Resonance (1969) 1991;95(3):558-566.

17. Levesque IR, Sled JG, Pike GB. Iterative optimization method for design of quantitative magnetization transfer imaging experiments. Magn Reson Med 2011;66(3):635-643.

18. Cercignani M, Symms MR, Schmierer K, Boulby PA, Tozer DJ, Ron M, Tofts PS, Barker GJ. Three-dimensional quantitative magnetisation transfer imaging of the human brain. NeuroImage 2005;27(2):436-441.

19. Cercignani M, Alexander DC. Optimal acquisition schemes for in vivo quantitative magnetization transfer MRI. Magn Reson Med 2006;56(4):803-810.

20. Battiston M, Grussu F, Ianus A, Schneider T, Prados F, Fairney J, Ourselin S, Alexander DC, Cercignani M, Gandini Wheeler-Kingshott CAM, Samson RS. An optimized framework for quantitative magnetization transfer imaging of the cervical spinal cord in vivo. Magn Reson Med 2017.

21. Barral JK, Gudmundson E, Stikov N, Etezadi-Amoli M, Stoica P, Nishimura DG. A robust methodology for in vivo T1 mapping. Magn Reson Med 2010;64(4):1057-1067.

22. Liberman G, Louzoun Y, Ben Bashat D. T(1) mapping using variable flip angle SPGR data with flip angle correction. J Magn Reson Imaging 2014;40(1):171-180.

23. Boudreau M, Stikov N, Pike GB. B1 -sensitivity analysis of quantitative magnetization transfer imaging. Magn Reson Med 2017.

24. Cruz JB. System sensitivity analysis: Dowden, Hutchinson & Ross; 1973.

25. Cabana J-F, Gu Y, Boudreau M, Levesque IR, Atchia Y, Sled JG, Narayanan S, Arnold DL, Pike GB, Cohen-Adad J, Duval T, Vuong M-T, Stikov N. Quantitative magnetization transfer imaging made easy with qMTLab: Software for data simulation, analysis, and visualization. Concepts in Magnetic Resonance Part A 2016:n/a-n/a.

26. Boudreau M, Tardif CL, Stikov N, Sled JG, Lee W, Pike GB. B1 mapping for bias-correction in quantitative T1 imaging of the brain at 3T using standard pulse sequences. J Magn Reson Imaging 2017.

27. Underhill HR, Rostomily RC, Mikheev AM, Yuan C, Yarnykh VL. Fast bound pool fraction imaging of the in vivo rat brain: association with myelin content and validation in the C6 glioma model. NeuroImage 2011;54(3):2052-2065.

28. Yarnykh VL. Fast macromolecular proton fraction mapping from a single off-resonance magnetization transfer measurement. Magn Reson Med 2012;68(1):166-178.

29. Lankford CL, Does MD. Propagation of error from parameter constraints in quantitative MRI: Example application of multiple spin echo T2 mapping. Magn Reson Med 2017.

30. Mclean M, MacDonald ME, Lebel RM, Boudreau M, Pike B. Accelerated z-Spectrum Imaging. 2017; Hawaii.

Table 1. qMT tissue parameters used to simulate white matter and grey matter tissue values in the Monte Carlo simulations. The parameter definitions are: F ­ pool-size ratio, kf  – exchange rate constant, T1,f – longitudinal relaxation time of the free pool, T1,r – longitudinal relaxation time of the restricted pool, T2,f – transverse relaxation time of the free pool, T2,r – transverse relaxation time of the restricted pool. The fitting parameters for qMT are F, kf, T2,f, and T2,r; T1,f is calculated from the observed T1 and the fitting parameters, and T1,r is conventionally fixed to 1 s.

|  |  |  |
| --- | --- | --- |
| Parameter | White Matter | Grey Matter |
| F | 0.15 n.u. | 0.075 n.u. |
| kf | 4.0 s-1 | 2.5 s-1 |
| T1,f | 0.9 s | 1.3 s |
| T1,r | 1.0 s | 1.0 s |
| T2,f | 30 ms | 55 ms |
| T2,r | 12 μs | 11 μs |

Table 2. qMT protocols used in the Monte Carlo simulations. The repetition times, excitation flip angles, and number of acquisitions were matched for all protocols. The Uniform protocol is a two MT flip-angle with logarithmically uniform off-resonance frequencies. The CRLB protocol was optimized using Eq. 5 with the regularization parameter set to 0, and CRLBλ=0.5 was optimized using a regularization parameter of 0.5.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Uniform | | | CRLB | | | CRLBλ=0.5 | | |
| Acq. # | TR/α | αMT | Δ (Hz) | TR/α | αMT | Δ (Hz) | TR/α | αMT | Δ (Hz) |
| 1 | 25ms/7° | 142° | 432.9 | 25ms/7° | 200.0 | 300.0 | 25ms/7° | 200.0 | 300.0 |
| 2 | 1 087.5 | 250.0 | 1 903.9 | 250.0 | 1609.5 |
| 3 | 2 731.6 | 700.0 | 1 609.5 | 700.0 | 1609.5 |
| 4 | 6 861.6 | 700.0 | 12 083.6 | 700.0 | 12 083.6 |
| 5 | 17 235.5 | 700.0 | 1 903.9 | 700.0 | 2 252.2 |
| 6 | 426° | 432.9 | 250.0 | 2 252.2 | 200.0 | 1 903.9 |
| 7 | 1 087.5 | 150.0 | 300.0 | 650.0 | 300.0 |
| 8 | 2 731.6 | 700.0 | 1 360.6 | 200.0 | 1 360.6 |
| 9 | 6 861.6 | 200.0 | 1 609.5 | 700.0 | 1 903.9 |
| 10 | 17 235.5 | 700.0 | 2 252.2 | 150.0 | 300.0 |

# FIGURE LEGEND

Figure 1. Simulated qMT parameter errors due to B1-inaccuracies (-30% < ΔB1 < 30%) considering a B1-independent T1 measurement (red: IR – inversion recovery) and a B1-dependent T1-measurement (blue: VFA – variable flip angle). Solid lines are parameter errors calculated from minimizing Eq. 2 (first-order approximation of the Taylor expansion), and dashed lines are parameter errors calculated from fitting the qMT signal according to the Sled & Pike model. The tissue parameters (white matter) and qMT protocol (uniform) were matched to those presented in Boudreau et al. 2017 (see Fig. 3 of the paper).

Figure 2. Simulated qMT parameter errors estimated from Eq. [2] for ΔB1=0.05 for a wide range of logarithmically-uniform (offsets) qMT protocols. Single (blue, orange, yellow), dual (purple, green, light blue), and triple (red) flip angle combinations of 150°, 400°, and 600° were compared. The number of offset frequencies were uniformly distributed between 300 Hz and 20 kHz, and matched for the total number of acquisition points (# offsets × # flip angles).

Figure 3. Sensitivity values for each qMT fitting parameters (F, kf, T2,f, T2,r) and B1 measurement values considering a B1-independent T1 measure (IR – inversion recovery) and a B1-dependent T1 measure (VFA – variable flip angle). The 312-point protocol shown (12 flip angles x 26 offset frequencies) represents the initial search-space used for protocol optimization. The sets of sensitivity values for each fitting parameter (a–d) consists of the matrix columns of the Jacobian sensitivity matrix (S*p* in Eq. 2 and 5).

Figure 4. Variance-efficiency (a) and ΔF (b) (Eq. 2, ΔB1 = 5%) values during the iterative optimization of the sensitivity-regularized Cramer-Rao Lower-Bound equation (Eq. 5). Variance-efficiency is defined here as (variance × # acq. points)-1/2, where the variance is interpreted to be the parameter-normalized Cramer-Rao Lower Bound (Eq. 3).

Figure 5. Comparison between the 10-point protocols iteratively optimized from a 312-point search space using solely the parameter-normalized CRLB (λ = 0) and regularized CRLBλ=0.5. The different flip angle z-spectrums of the initial optimization search-space are displayed in blue to emphasize the 10-point protocols. The flip angle z-spectrums (150° to 700°, in 50° increments) range from the highest MT-signal values curve (150°) to lowest (700°).

Figure 6. Means (a, b) and standard deviations (c, d) of the distribution of pool-size ratios (F) for sets of Monte Carlo simulations (10,000 runs, SNR = 100) fitted using a range of B1 errors (ΔB1 = ±30%, B1 = 1 n.u.) and for two sets of qMT parameters (white matter – a,c; grey matter – b, d). Mean F values (% error) shown here were compared relative to the accurate B1 value case (ΔB1 = 0), and the grey region represents the region of ±1% relative error. Simulated signal values were generated and fitted for three different 10-point qMT protocols: Uniform (blue) – two-FA protocol with logarithmically-uniform off-resonance frequency values, CRLB (red) – protocol optimized by iteratively minimizing the increase in the parameter-normalized Cramer-Rao Lower-Bound of the system, and CRLBλ=0.5 (yellow) – protocol optimized similar to CRLB, regularized by the estimated error of F (ΔF) in the presence of a B1 error (Eq. 5).

Figure 7. Means (a, b) and standard deviations (c, d) of the distribution of pool-size ratio values (F) for sets of Monte Carlo simulations (10,000 runs) fitted using a range of SNR values (25, 50, 75, 100, 150, and 200) and for two sets of qMT parameters (white matter – a,c; grey matter – b, d). Mean F values (% error) shown here were compared relative to data fitted for an ideal SNR case (noiseless), and the grey region represents the region of ±1% relative error. Data was fitted assuming ideal B1 values (B1 = 1 n.u., solid lines) and a 15% overestimation in B1 (B1 = 1.15 n.u., dotted lines). Simulated signal values were generated and fitted for three different 10-point qMT protocols: Uniform (blue) – two-FA protocol with logarithmically-uniform off-resonance frequency values, CRLB (red) – protocol optimized by iteratively minimizing the increase in the parameter-normalized Cramer-Rao Lower-Bound of the system, CRLBλ=0.5 (yellow) – protocol optimized similar to CRLB, regularized by the estimated error of F (ΔF) in the presence of a B1 error (Eq. 5).



Figure 1. Simulated qMT parameter errors due to B1-inaccuracies (-30% < ΔB1 < 30%) considering a B1-independent T1 measurement (red: IR – inversion recovery) and a B1-dependent T1-measurement (blue: VFA – variable flip angle). Solid lines are parameter errors calculated from minimizing Eq. 2 (first-order approximation of the Taylor expansion), and dashed lines are parameter errors calculated from fitting the qMT signal according to the Sled & Pike model. The tissue parameters (white matter) and qMT protocol (uniform) were matched to those presented in Boudreau et al. 2017 (see Fig. 3 of the paper).



Figure 2. Simulated qMT parameter errors estimated from Eq. [2] for ΔB1=0.05 for a wide range of logarithmically-uniform (offsets) qMT protocols. Single (blue, orange, yellow), dual (purple, green, light blue), and triple (red) flip angle combinations of 150°, 400°, and 600° were compared. The number of offset frequencies were uniformly distributed between 300 Hz and 20 kHz, and matched for the total number of acquisition points (# offsets × # flip angles).



Figure 3. Sensitivity values for each qMT fitting parameters (F, kf, T2,f, T2,r) and B1 measurement values considering a B1-independent T1 measure (IR – inversion recovery) and a B1-dependent T1 measure (VFA – variable flip angle). The 312-point protocol shown (12 flip angles x 26 offset frequencies) represents the initial search-space used for protocol optimization. The sets of sensitivity values for each fitting parameter (a–d) consists of the matrix columns of the Jacobian sensitivity matrix (S*p* in Eq. 2 and 5).



Figure 4. Variance-efficiency (a) and ΔF (b) (Eq. 2, ΔB1 = 5%) values during the iterative optimization of the sensitivity-regularized Cramer-Rao Lower-Bound equation (Eq. 5). Variance-efficiency is defined here as (variance × # acq. points)-1/2, where the variance is interpreted to be the parameter-normalized Cramer-Rao Lower Bound (Eq. 3).



Figure 5. Comparison between the 10-point protocols iteratively optimized from a 312-point search space using solely the parameter-normalized CRLB (λ = 0) and regularized CRLBλ=0.5. The different flip angle z-spectrums of the initial optimization search-space are displayed in blue to emphasize the 10-point protocols. The flip angle z-spectrums (150° to 700°, in 50° increments) range from the highest MT-signal values curve (150°) to lowest (700°).



Figure 6. Means (a, b) and standard deviations (c, d) of the distribution of pool-size ratios (F) for sets of Monte Carlo simulations (10,000 runs, SNR = 100) fitted using a range of B1 errors (ΔB1 = ±30%, B1 = 1 n.u.) and for two sets of qMT parameters (white matter – a,c; grey matter – b, d). Mean F values (% error) shown here were compared relative to the accurate B1 value case (ΔB1 = 0), and the grey region represents the region of ±1% relative error. Simulated signal values were generated and fitted for three different 10-point qMT protocols: Uniform (blue) – two-FA protocol with logarithmically-uniform off-resonance frequency values, CRLB (red) – protocol optimized by iteratively minimizing the increase in the parameter-normalized Cramer-Rao Lower-Bound of the system, and CRLBλ=0.5 (yellow) – protocol optimized similar to CRLB, regularized by the estimated error of F (ΔF) in the presence of a B1 error (Eq. 5).



Figure 7. Means (a, b) and standard deviations (c, d) of the distribution of pool-size ratio values (F) for sets of Monte Carlo simulations (10,000 runs) fitted using a range of SNR values (25, 50, 75, 100, 150, and 200) and for two sets of qMT parameters (white matter – a,c; grey matter – b, d). Mean F values (% error) shown here were compared relative to data fitted for an ideal SNR case (noiseless), and the grey region represents the region of ±1% relative error. Data was fitted assuming ideal B1 values (B1 = 1 n.u., solid lines) and a 15% overestimation in B1 (B1 = 1.15 n.u., dotted lines). Simulated signal values were generated and fitted for three different 10-point qMT protocols: Uniform (blue) – two-FA protocol with logarithmically-uniform off-resonance frequency values, CRLB (red) – protocol optimized by iteratively minimizing the increase in the parameter-normalized Cramer-Rao Lower-Bound of the system, CRLBλ=0.5 (yellow) – protocol optimized similar to CRLB, regularized by the estimated error of F (ΔF) in the presence of a B1 error (Eq. 5).