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Localized Blood Flow Imaging Using Quantitative FENSI

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Abstract

FENSI was previously introduced as a novel functional imaging method for measuring changes in localized blood flow in response to a stimulus. However, FENSI was limited to a qualitative functional MRI tool, due to magnetization transfer (MT) effects and different tagging plane profiles between tag and control images. In this work, a revised FENSI acquisition is proposed to enable quantitative imaging, which is capable of providing absolute localized blood flow maps free from MT and slice profile errors. The feasibility and accuracy of measuring microvascular (arteriole, capillary, venule) blood flow by using quantitative FENSI was validated by our phantom studies. Additionally, localized cerebral blood flow, $366 \pm 45 \mu\text{L}/\text{min}/\text{cm}^2$ in gray matter and $153 \pm 23 \mu\text{L}/\text{min}/\text{cm}^2$ in white matter, was measured in healthy subjects during resting state, while a flow change of $73 \pm 13\%$ was detected during a visual task.

Keywords

FENSI; perfusion; localized blood flow; blood flux; magnetization transfer

INTRODUCTION

Cerebral blood flow is a critical determinant of brain health and cognitive performance. MRI-based blood flow measurement methods have been studied extensively as noninvasive approaches for examining brain physiology and function. For instance, by using water in blood as an endogenous contrast agent, arterial spin labeling (ASL) techniques are able to provide a measurement of classical perfusion (1-4), which is defined as the volume of blood delivered to a unit volume of tissue per unit time ($\text{mL}/100 \text{ g}/\text{min}$). Besides ASL, phase contrast (PC) MRI is another noninvasive flow imaging technique, in which flow-sensitizing gradients in the MR pulse sequence induce phase shifts proportional to the velocity of moving spins along the gradient direction. PC MRI is in widespread use for measuring high velocity ($\geq 10 \text{ cm/s}$) and volumetric flow rate in large vessels (5). However, PC MRI does not allow for interrogation of the localized and microvascular flow in small vessels (arteriole, capillary, venule). For ASL, it allows measurement of localized delivery of blood, but it can have a strong dependency on blood flow pathway and arterial transit time (ATT), as most of the ASL approaches aim to tag arterial spins in the large feeding arteries while imaging blood signal downstream in the brain tissue of interests. In this work, we propose a quantitative method to allow for interrogation of highly localized, slow blood flow in small vessels, a regime in which very few non-invasive methods are available.

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Recently, the technique of flow-enhanced signal intensity (FENSI), has been developed as a qualitative tool for the assessment of localized flow changes during functional tasks and in aging (6,7). Similar to ASL, FENSI is also a pairwise subtractive method, and the flow-weighted signal is obtained by subtracting a tag image from a control image. The imaging geometry of FENSI is shown in Figure 1a. The general setup includes a thin tagging plane that is placed in the middle of a thick imaging slice. A FENSI tag image is acquired after repeatedly saturating the thin tagging plane for some time using 90° radiofrequency (RF) pulses followed by gradient spoilers (Figure 1c), while the control image is acquired after a single or a few tagging pulses are used. After subtraction of tag from control, the obtained FENSI signal corresponds to imaging all of the MR spins that have passed through the thin tagging plane during the tagging period.

FENSI has many advantages as a noninvasive blood flow imaging method. Different from ASL, which tags flowing spins in feeding arteries proximal to the imaging volume, FENSI can have an arbitrary tagging plane and tag flowing spins very close to or inside the microvasculature (arterioles, capillaries and venules) with slow velocity ($\leq 1\text{cm/s}$). With the capability to provide a highly-localized flow signal by tagging a thin slice inside the imaging slice, FENSI is expected to be less vulnerable to arterial transit time effects and blood delivery pathways. The specific tagging and imaging geometry also enables FENSI to be sensitive to blood flow directions, that is, only flowing spins with a velocity component perpendicular to the tagging plane will contribute to the FENSI signal.

As originally proposed, FENSI was limited to a qualitative functional MRI tool due to two major systematic errors from static tissue in the imaging slice, both of which introduce apparent flow. The first one is the unbalanced magnetization transfer (MT) effects resulting from the use of an unequal number of RF pulses between tag and control images. The second source of error comes from differences in the tagging plane profiles between tag and control images. Repetitive implementations of tagging RF pulses produce a wider and sharper tagging plane profile in tag images than that by a single implementation of the RF pulse in control images. When subtracting the tag image from the control image, the tag profile difference between the two images results in an apparent flow from static tissue. In a qualitative functional imaging protocol, these effects do not behave differently between task and rest portions of the paradigm, so there is no noticeable effect for the creation of activation maps for functional imaging. However, in order to get quantitative flow images, these artifacts must be removed. In this work, we further developed FENSI from a qualitative functional MRI tool to a quantitative method, named quantitative FENSI (qFENSI), capable of providing a noninvasive, accurate, and absolute measurement of blood flux through the microvasculature.

MATERIALS AND METHODS

MR Pulse Sequence

In the original FENSI sequence, the tag image was acquired with a tagging module applied to a thin tagging plane of 0.4 mm for duration of 330 ms (consisting of thirty 90° RF pulses, each followed by a gradient spoiler). And the control image was acquired with one tagging pulse applied before the readout (Figure 1a and c).

In order to balance magnetization transfer effects between tag and control images, a RF concatenation scheme (8-10) is adapted for qFENSI tag and control pulses. RF concatenation was previously employed in the pulsed ASL technique of Transfer Insensitive Labeling Technique (TILT) (9,11-13). As shown in Figure 1c and d, the 90° tagging pulse is replaced by two consecutive 45° RF pulses. Following the recommendations of the RF concatenation scheme (8,9), the second 45° pulse is reversed in time and the second slice

selective gradient has opposite sign compared to the first one. For the control images (Figure 1d), the sequence has exactly the same RF pulse pair and gradients as that of the tag image, except that the second 45° RF pulse is applied with a 180° phase shift to achieve an effective 0° excitation from the pair of pulses. By applying concatenated RF pairs, the same number of RF pulses with the same timing and location are used in both control and tag sessions, and thus the MT effects from the static tissue in the image region are cancelled out after the subtraction procedure.

However, application of the RF concatenation scheme in FENSI does not eliminate the tagging plane profile artifact. Repetitive implementations of (+45°, +45°) in tag will still generate a wider and sharper tag profile than that by (+45°, -45°) in control. To further remove this tag profile artifact, three 90° saturation pulses with spoiling gradients are applied to a thicker post-tag saturation slice to suppress MR signal inside this wider region after the repeated tagging or control modules (Figure 1b and d). The slice thickness of the post-tag saturation RF pulses is chosen to be wide enough to cover the significant main lobe and side lobe differences in the tag and control tagging plane profiles.

By using the RF concatenation scheme to make qFENSI insensitive to MT effects and by applying post-tag saturation module to eliminate tagging plane profile artifacts, qFENSI is able to perform quantitative flow mapping. The updated qFENSI sequence and corresponding geometry is shown in Figure 1b and d. More detailed MR pulse sequence parameters can be found in Table 1.

Numerical Simulations

Numerical Bloch simulations were performed to determine the expected widening of the tagging plane from repeated tagging RF pairs and to set the post-tag saturation slice thickness to cover the widening region. The tagging plane profile was simulated as a function of number of RF pulse pairs (N) implemented in the qFENSI sequence. The simulation parameters were chosen to match the experimental settings as in Table 1, Tag Plane Prescription **B**: windowed-sinc 45° RF pulses with duration of 2560 μs, RF spacing in one concatenated pulse pair (τ) = 2960 μs, spoiler duration and amplitude = 3600 μs/[±10, ±12, ±14, ±16 mT/m], the preset thickness of the thin tagging plane = 3 mm, thickness of the imaging slice = 20 mm, number of RF pairs (N) = 1, 10, 50 and 100. T1 (1820 ms) and T2 (99 ms) relaxation effects of gray matter at 3 T were considered in the simulation (14). Several different amplitudes of spoiler gradients were used to avoid the formation of echoes in the string of saturation pulses.

Flow Quantification

The flux quantification of qFENSI can be derived by the following steps:

1. Flow-weighted images, ΔM , can be obtained by subtracting the tag image from the control image. This flow-weighted image contains microvascular blood flow information and will be converted to volumetric blood flux.
2. To determine the conversion factor from MR signal to water volume, we used a voxel with cerebrospinal fluid (CSF). The equilibrium magnetization signal of CSF of an imaging voxel volume is calculated by Equation [1],

$$CSF \text{ signal per mL} = \frac{M_{o,CSF}}{V_{img} - V_{posttag}} \quad [1]$$

$$M_{o,CSF} = \frac{M_{control,CSF}}{1 - \exp\left(-\frac{TR}{T_{1,CSF}}\right)}$$

where V_{img} (mL) is the imaging voxel volume and V_{posttag} is the post-tag saturation volume inside the imaging voxel, and the CSF equilibrium signal of $M_{0,\text{CSF}}$ is from a volume of $(V_{\text{img}} - V_{\text{posttag}})$. TR is the repetition time (5 s). $M_{0,\text{CSF}}$ is calculated from the mean of the control images ($M_{\text{control,CSF}}$) with consideration of CSF T1 relaxation ($T_{1,\text{CSF}} = 3.7$ s at 3 T) (15).

3. By Equation [2], We can obtain the volume of moving spins during the tagging interval, Δt , by normalizing the flow-weighted image (ΔM) by the CSF equilibrium signal per mL (Equation [1]),

$$\text{Volume of tagged flowing spins (mL)} = \Delta M / \left(\frac{M_{0,\text{CSF}}}{V_{\text{img}} - V_{\text{posttag}}} \right) \quad [2]$$

4. Assuming a single compartment model as in (16,17), the blood flux Q in $\mu\text{L}/\text{min}/\text{cm}^2$ can be obtained by dividing the volume of tagged flowing spins from Equation [2] by the cross-sectional area of a single voxel ($A_0 = (22 \text{ cm}/64)^2$) and the tagging duration Δt ,

$$Q = \frac{60 \times 1000}{A_0 \cdot \Delta t \cdot \lambda \cdot \alpha} \cdot (V_{\text{img}} - V_{\text{posttag}}) \cdot \frac{\Delta M}{M_{0,\text{CSF}}} \quad [3]$$

where the scalar 60 converts the unit from second to minute and 1000 converts from mL to μL . λ is the water content of blood, i.e., 0.76 (16,18), α is the tagging efficiency with qFENSI ($\alpha = 1$ was used in the calculation).

5. By accounting for blood transversal and longitudinal relaxation effects, the final calculation for volumetric flow rate of blood is shown in Equation [4],

$$Q = \frac{60 \times 1000}{A_0 \cdot \Delta t \cdot \lambda \cdot \alpha} \cdot (V_{\text{img}} - V_{\text{posttag}}) \cdot \frac{\Delta M}{M_{0,\text{CSF}}} \cdot \exp\left(\frac{w}{T_{1,\text{blood}}}\right) \cdot \exp\left(\frac{TE}{T_{2,\text{blood}}}\right) \quad [4]$$

where w is the post-tag time delay between tagging and image readout (500 ms in this study), and TE is the echo time (44 ms). $T_{1,\text{blood}}$ (1680 ms) and $T_{2,\text{blood}}$ (275 ms) denote the longitudinal and transversal relaxation times of blood at 3T respectively, which were obtained from the literature (14).

Phantom Validation

Phantom studies were performed in order to validate flow measures and the removal of MT and tagging plane profile errors from the updated qFENSI technique. First, a static phantom was constructed using a 2% agar gel to examine and compare the magnetization transfer and tagging plane profile errors between the qualitative and quantitative FENSI sequences.

The second phantom study was performed to investigate and validate the accuracy of the flow measurements with qFENSI on a flow phantom. The phantom was made with a 2% agar gel and a tubing of 1.02 mm inner diameter. The tubing was wrapped four times around a plastic frame (10 cm width) inside the agar phantom (Figure 2a and b). qFENSI acquisitions were carried out with a matrix size of 64×64 and a field of view of 220 mm as listed in Table 1, Tag Plane Prescription A. The imaging plane was placed perpendicular to the flow in the tubing (Figure 2a). With these imaging parameters, a 7% volume fraction of tubing flow was obtained in imaging voxels that contained tubing. A syringe pump was used to maintain the flow rates (Q_0) inside the tubing with preset values of 490.3, 343.2, 245.2, 147.1, and 49.0 $\mu\text{L}/\text{min}$ for each scan, corresponding to average flow velocities (v_0) in the

tubing of 10.0, 7.0, 5.0, 3.0, and 1.0 mm/s. The relationship between these two groups of flows is converted according to $Q_0 = 60 \cdot \pi r^2 \cdot v_0$, where r denotes the radius of the tubing.

Human Experiments

Ten healthy subjects (seven male and three female, 33-80 years old) were studied on a 3 T clinical scanner (Trio, Siemens, Erlangen, Germany) using standard body coil transmission and twelve-channel head array receive coil, following a protocol approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign. To reduce movement, padding was used to stabilize the subject's head. Resting blood flow maps were acquired with qFENSI in an axial slice at the level of the corpus callosum for each subject, averaged from 30 pairs of control/tag images with a repetition time (TR) of 5 s for each image. For all in vivo imaging experiments, the protocol from Table 1, Tag Plane Prescription **B** was used.

Functional acquisitions were performed on another four healthy subjects (three male and one female, 20-30 years old). The four subjects performed a visual functional task, in which they passively viewed a blocked visual presentation consisting of 30 seconds of display of a fixation cross followed by another 30 seconds of a red/black checkerboard reversing at 8 Hz. Five repeats of the task blocks were employed per functional scan with a total duration of five minutes. The visual presentation was displayed to the subjects through back projection (BrainLogics, Psychology Software Tools, Inc., Sharpsburg, USA). A coronal imaging slice passing through the primary visual cognitive area, V1, was acquired with qFENSI. 30 pairs of control/tag images were obtained with a TR of 5 s for each image. All the activation maps were formed using FSL (FMRIB Software Library, Oxford, UK) (19). The time series was high-pass filtered with a cutoff of 60 s. Spatial filtering was performed with a Gaussian kernel with a full width at half-max of 5 mm. The signal was modeled by multiplication of a tag-control regressor with a block model of the task that was convolved with a gamma-function hemodynamic response function. Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z \geq 2.3$ and a (corrected) cluster significance threshold of $P = 0.05$ (20). The activation results were overlaid on a high-resolution 2D turbo-spin echo acquisition taken with the imaging slice at the same location as the qFENSI acquisitions, at the thin tagging plane location.

A post-tag delay of 500 ms was used for both static and functional acquisitions to improve the assumption that only microvascular blood flow contributes to the qFENSI signal. This delay allows time for fast spins that were tagged in large vessels to leave the imaging volume before data acquisition.

Flux rate maps were quantified by Equation [4]. Mean flux rates and mean temporal signal-to-noise ratio (SNR) in different ROIs were calculated. For resting blood flow imaging, the ROIs were gray matter (GM) and white matter (WM) areas, which were obtained by projecting the GM and WM atlases (HarvardOxford) provided by FSL (21) to subject space, resulting in probabilistic maps of GM and WM distributions. Then, the GM and WM masks were generated by applying a threshold of 98% to avoid significant cross-contamination between GM and WM voxels (17). The temporal SNR is calculated by dividing the mean flow-weighted signal in each voxel by the standard deviation through time. By averaging the temporal SNR of voxels in a ROI, we obtain the mean temporal SNR of a single image for that ROI (22,23). For the data acquired during the visual functional tasks, the ROI was defined as the activated GM voxels (those that exceeded the cluster-level statistical threshold as explained above). This functional ROI was used to examine task-related changes in measured flow.

RESULTS

Numerical Simulations

Figure 3 depicts the simulated slice profiles by applying different numbers of $(+45^\circ, +45^\circ)$ RF pairs, with the same pulse sequence parameters that were used in the in vivo scans. As shown, a single implementation of a $(+45^\circ, +45^\circ)$ pair generates the narrowest tagging width, largest transition width and lowest amplitude of side lobes, while 100 $(+45^\circ, +45^\circ)$ pairs give the widest tagging band, sharpest transition width and highest side lobe amplitude. Therefore, in order to eliminate the tagging plane profile artifacts, the slice thickness of the post-tag saturation RF pulses need to be wide enough to cover the spatial extent of the repetitive tagging RF pairs. Our simulation and empirical conclusion is that the slice profile artifacts are effectively suppressed when using a post-tag saturation slice with a thickness approximately three times wider than the preset tag plane profile width for a single $(+45^\circ, +45^\circ)$ pair.

Phantom Validation

In the first phantom experiment, a static phantom was used and no flow was expected. With the original qualitative FENSI, the mean residual signal in agar ($\Delta M/M_0$) was around 10%, which is much higher than the signal level that would result from blood flow in brain (1-2%). In contrast, the residual error is reduced to noise level ($\leq 0.1\%$) when the proposed quantitative FENSI (qFENSI) is used. The lack of static tissue water contamination in the flow signal demonstrates that both magnetization transfer and slice profile artifacts were successfully eliminated. Two sets of protocol parameters (Tag Plane Prescription A: tagging plane, post-tag saturation slice, imaging slice and tagging duration = 1, 3, 20 mm and 1 s respectively; and Tag Plane Prescription B: 3, 8, 20 mm and 3 s) listed in Table 1 were tested in the agar phantom and validated that no significant residual signal was produced by static water with the updated qFENSI technique.

Next, the flow phantom was used to verify the accuracy of the flow measures. One example of a flow weighted image ($\Delta M/M_0$) of the flow phantom is shown in Figure 2c. The location of the tubing segments in the flow phantom can be easily determined by visual inspection, as no net signal from agar was left in the background when using qFENSI. The preset (Q_0), measured (Q) flow rates, and expected mean velocity (v_0) in the tubing are listed in Table 2 for several preset flow rates. With the geometry and timing parameters (Tag Plane Prescription A) shown in Table 1, qFENSI was designed to be sensitive to flowing spins that have velocities between 3 and 10 mm/s. These parameters are chosen to target the highly-localized microvascular flow rates. Spins moving faster than 10 mm/s will leave the imaging slice, and spins slower than 3 mm/s still stay inside the post-tag saturation slice, and have their signal spoiled by the three 90° post-tag saturation RF pulses. As shown in Table 2, the updated qFENSI acquisition provides an accurate and localized measure of volumetric flow from spins with average velocities between 3 mm/s and 10 mm/s, but underestimates volumetric flow with average velocities outside of this range. As expected, in the last column of Table 2, the measured flow rate was much smaller than the preset value in the tubing for an estimated mean velocity of 1.0 mm/s.

Human Experiments

Figure 4a shows the resting state qFENSI flux maps acquired in nine subjects (one subject's data was excluded due to excessive motion). The protocol parameters from Table 1, Tag Plane Prescription B were used for all in vivo data sets. The average flux in the GM ROI of the nine subjects is $366 \pm 45 \mu\text{L}/\text{min}/\text{cm}^2$ and $153 \pm 23 \mu\text{L}/\text{min}/\text{cm}^2$ in the WM ROI (Figure 4b). The mean temporal SNR of one acquisition with qFENSI is 0.8 ± 0.2 in GM and 0.5 ± 0.1

in WM. These SNR values will increase to 4.4 and 2.7, respectively, by averaging over the 30 measurements acquired in our experiments.

Figure 5a, shows the z-score visual activation maps by co-acquired blood oxygenation level dependent (BOLD) (1st row) and qFENSI (2nd row) acquisitions. The functional flux results are listed and compared in Figure 5b for an ROI defined by the voxels in the activated clusters from the visual task. The measured average flux in the ROI, i.e., the mean flux through the whole functional task including both rest state and activation conditions, of four subjects is 375 ± 33 $\mu\text{L}/\text{min}/\text{cm}^2$, and the flux change is 73 ± 13 %.

DISCUSSION

In this work, qFENSI was developed and applied to non-invasively measure highly-localized microvascular blood flow and flow changes in the human brain during resting state and a visual task. qFENSI is able to provide a measure of the blood flux in vessels very close to and including the microvasculature in localized tissue with units of $\mu\text{L}/\text{min}/\text{cm}^2$. This measure is expected to provide information on localized slow flowing blood, unlike other flow measures which have a strong dependence on flow delivery pathways and arterial transit times.

The qFENSI method is being proposed as a complement to ASL techniques. Some of the information provided by qFENSI might overlap with the perfusion information provided by ASL, however, the FENSI method provides additional information about the microstructure of local delivery in the tissue vascular beds. This information is not available with most of ASL approaches, although some of it may be in the signal of other ASL techniques which minimize transit time effects, such as Velocity-Selective ASL (22). We suggest that the FENSI method will provide interesting complementary information for examining things like: changes in microstructural organization of brain microvasculature with aging (24), information about the specific flow related to a neurovascular unit, and provide early information on vasculature reorganization at early stages of neurological diseases. In addition, the technique will provide a general way to measure volumetric flow in other tissues, besides brain, such as in the bone or muscle, without the need to identify major feeding vessels for tagging.

In qFENSI, the thin tagging plane is placed in the middle of the imaging volume. Note that this “imaging volume” is not actually what is being localized in the proposed method. It is simply the volume in which the flowing spins will accumulate after leaving the tagging plane. We choose to place the tagging plane in the middle of the imaging volume as we are trying to tag blood far down the arterial tree, where it may not be uniformly directed in the ascending direction (25,26). We desire to complement existing methods in the field of blood flow imaging by providing a technique that measures volumetric flow rate of slow flowing blood on a highly localized scale and propose that tagging in the middle of the imaging volume as in qFENSI provides the most straightforward implementation for this purpose. We could place the tagging plane below the imaging volume, however, this ASL-like geometry setup would impose additional transit constraints for flowing spins we are imaging beyond those required for elimination of the tagging slice profile artifacts, as post-tag saturation is still needed in order to place the tagging plane very close to the imaging slice of interest. By contrast, the required transit of spins from labeling to detection has been minimized in our current qFENSI setup.

Currently in the literature, there are two other noninvasive blood flow imaging techniques which are potentially able to provide a localized flow measure by tagging very close to imaging tissues of interest. The first one is Flow-sensitive Alternating Inversion Recovery

(FAIR), especially single slice FAIR (27), in which the arterial transit time value is assumed to be small. The second potentially localized measure is Velocity-Selective ASL (VSASL), whose labeling is velocity-selective instead of spatial selective (22). It should be noted that in VSASL, a post-tag delay of 1600 ms is often used to optimize its SNR efficiency (28,29). Both techniques are ASL methods that measure perfusion, i.e., how much blood is delivered to a unit volume of tissue per unit time and the flow is calculated as perfusion in the unit of mL/100 g tissue/min.

However, qFENSI measures a different aspect of blood flow, localized volumetric flow rates of blood as it passes across the thin tagging plane during the tagging time. qFENSI flow is quantified as flux in the units of $\mu\text{L}/\text{min}/\text{cm}^2$. Additionally, the tagging approach of qFENSI differs from the ASL methods. Both FAIR and VSASL apply instantaneous tagging RF pulses, either spatial selectively or non-selectively, and mainly tag arterial blood flow while it is moving fast. In contrast in qFENSI, repetitive tagging RF pulses are implemented to continuously tag slow-moving spins flowing across the thin tagging plane, making it possible to obtain blood flow signal from microvessels with a reasonable temporal SNR. For comparison, we can examine the SNR measures per image in the literature which are calculated similarly to our qFENSI SNR calculations with reported values of 2.1 ± 0.3 by the pulsed ASL method of PICORE/QUIPSS II and 1.1 ± 0.2 by Velocity-Selective ASL (VSASL with $V_c = 2 \text{ cm/s}$) (22). Some of the higher SNR of the pulsed ASL method is due to inversion labeling, whereas qFENSI uses saturation labeling. In addition, the ASL methods tag all inflowing blood, while qFENSI targets microvascular flow perpendicular to the tagging plane.

Although a direct comparison between perfusion and flux cannot be done due to the different flow mechanisms being examined, a thought experiment can be performed to check for relative agreement between the two measures. In steady state, perfusion delivered to a voxel would be balanced by flow leaving the voxel, using conservation laws within a voxel. For our qFENSI experiment, we have effective tag profiles of around 5 mm full width at half maximum (FWHM) after the repeated tagging module (see discussion to follow). Thus, we are measuring the flux through this 5 mm tag plane. If we convert our flux measure into a flow through the volume of the tagging plane, we get the same units as in perfusion. For our qFENSI measures, this gives a mean perfusion of 75 mL/min per 100 g tissue (activated GM) from the functional study, 31 mL/100 g/min (WM) and 73 mL/100 g/min (GM) from our static imaging study, assuming a density of tissue of $1 \text{ g}/\text{cm}^3$. These results agree closely with the expected perfusion values from the literature (17,22) and the average GM/WM flow ratio reported by (30-33). Additionally, the flow changes ($73 \pm 13\%$) measured with qFENSI match the reported values for similar functional tasks acquired with perfusion measurement methods (34,35). Although it is instructive to check order-of-magnitude agreement between the flow measures, we do not encourage this interpretation as the two approaches are measuring different aspects of flow which may be uncoupled in certain physiological situations. Further studies will provide normative measures for qFENSI across various populations.

Selection of experimental parameters

By employing the RF concatenation scheme, the magnetization transfer effects in qFENSI are well balanced between tag and control acquisitions. However, our preliminary results from numeric Bloch equation simulation and agar phantom experiments showed that a post-tag saturation slab must also be used to address differences in tagging plane profile between tag and control images. Our simulation and experimental results showed that these slice profile differences can be effectively suppressed by applying three saturation RF pulses after the tagging module with a thickness approximately three times wider than the preset slice profile width for a single ($+45^\circ$, $+45^\circ$) RF pair. Our phantom and in vivo results confirm that

both the MT effects and the tagging plane profile effects have been successfully eliminated in the flow images obtained with qFENSI.

Motion is another critical aspect to consider when designing the tagging and imaging geometry for the qFENSI sequence. The FENSI method is sensitive to small movements, and this is especially true for qFENSI as the tagging is realized by two concatenated 45° RF pulses. The spins in the slice excited by the first 45° pulse must significantly overlap with that by the second 45° pulse. Otherwise, if there is no overlap between these two pulses at all, a tag pair ($+45^\circ$, $+45^\circ$) will provide an identical tagging profile as a control RF pair ($+45^\circ$, -45°) and will lead to zero flow signal after subtraction. In human scans, it's more robust to use a wider tagging plane, since pulsations during the cardiac cycle can cause significant brain motion, with peak displacements on the order of 0.5 mm (36). Therefore, we chose a 3 mm tagging plane to increase the likelihood of significant overlap in the pulse profiles of the concatenated RF pulse pairs.

In human studies, a wider tagging plane (3 mm) requires a wider post-tag saturation slice (8 mm), which can decrease SNR. Meanwhile, a wider imaging slice (20 mm) and longer total tagging duration (3 s) were used to improve SNR. Although the imaging slice thickness is 20 mm, the flow weighted signal in qFENSI results from spins flowing through the thin tagging plane of 3 mm. This gives qFENSI approximately the same imaging resolution as other functional neuroimaging methods. The thick imaging slice helps to increase the SNR and to allow a build-up of signal that has passed through the tagging plane.

Challenges and future application

Currently, one drawback to the current implementation of qFENSI for human scans is the sensitivity to main magnetic field inhomogeneity, or off-resonance, effects on the tagging efficiency. The difference in time between the centers of the two concatenated RF pulses, i.e., τ in Figure 1d, makes qFENSI vulnerable to off-resonance influences which induce extra phase offset between these two pulses. The sensitivity to off-resonance artifacts can cause signal loss, variability in the effective flip angle for tag and control pulses, and result in underestimation of blood flow. A correction method proposed previously may be used to address some of this quantification error (37).

In addition to the sensitivity to off-resonance effects, a challenge of the current implementation is the loss of SNR from the use of the post-tag saturation slab. Improved design of the tagging plane profile of the tagging RF pulse pairs, using Shinnar-Le Roux (SLR) RF pulses, could save more slow flow signal by allowing for a narrower post-tag saturation slice (38). Another drawback of the current method is that qFENSI is a single-slice method, which makes the technique quite SNR inefficient compared to multi-slice flow imaging techniques. Future work will involve further development of qFENSI for multi-slice acquisition.

With the ability to noninvasively provide highly-localized, directional-sensitive measures of blood volumetric flux, qFENSI is of great potential in many research and clinical applications. qFENSI will enable the direct measurement of highly-localized cerebral blood flow and its spatial architecture in different areas of the brain. This multiparametric (amount and orientation) measure of flow may provide sensitive measures that may reflect early changes in the vitality of the brain with age, in circulatory disorders, in the development of tumors or neoplasms, and to investigate neurovascular coupling in gray matter relevant to functional MRI techniques.

CONCLUSION

In this work, we modified the FENSI volumetric flow imaging technique from a qualitative functional imaging tool to a quantitative flow measurement technique called quantitative FENSI or qFENSI. qFENSI is able to provide accurate absolute microvascular blood flow maps by eliminating magnetization transfer and tagging plane profile artifacts associated with the initial qualitative method. The feasibility and accuracy of quantitative FENSI to measure slow flow has been validated by our phantom studies. Also, cerebral blood flow maps of human brain quantified as volumetric flux were obtained successfully with qFENSI. Specifically, we found flow rates in gray matter ROIs during a visual task to have a mean volumetric flow rate of $375 \pm 33 \mu\text{L}/\text{min}/\text{cm}^2$ with a flow change of $73 \pm 13\%$ in visual areas during functional activation, and $366 \pm 45 \mu\text{L}/\text{min}/\text{cm}^2$ in gray matter and $153 \pm 23 \mu\text{L}/\text{min}/\text{cm}^2$ in white matter for a second set of subjects at rest.

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Figure 1.

Schematics of the tagging and imaging geometry for (a) qualitative and (b) quantitative FENSI: thin tagging plane (dash yellow), post-tag saturation slice (blue) and imaging slice (red). (c) and (d) are the corresponding pulse sequences for qualitative and quantitative FENSI, respectively: tagging pulses (yellow), post-tag saturation pulses (blue), SE-EPI readout (red) and spoil gradient (stripe) with varying amplitude. 90° saturation RF pulses were used for tag and 0° RF pulse for control in qualitative FENSI (c), while RF concatenation pairs were applied in quantitative FENSI (d): (+45°, +45°) for tag image and (+45°, -45°) for control image. τ denotes the RF spacing between two RF pulses.

Figure 2.

(a) Side view of the flow phantom for the phantom validation study. The imaging slice (red), tagging plane (yellow) and post-tag saturation slice (blue) are placed perpendicular to the flow in the tubing (green). The phantom is filled with 2% agar. (b) Top view of the flow phantom. (c) One example ($Q_0 = 343.2 \mu\text{L}/\text{min}$) of flow-weighted maps ($\Delta M/M_0$) of the flow phantom measured with qFENSI. The location of the tubing segments can be easily determined by visual inspection and the background signal of agar is well suppressed.

Figure 3.

Simulated tagging plane profile as a function of number of RF pulse pairs (N) implemented in the qFENSI sequence. The region between two cyan vertical lines denotes the preset thin tagging plane (3 mm). The region between two red vertical lines denotes the post-tag saturation slice (8 mm) and the imaging slice is 20 mm. T1 (1820 ms) and T2 (99 ms) of gray matter at 3 T were considered in the simulation.

Figure 4.

(a) Resting blood flux maps acquired with qFENSI in nine healthy subjects (color bar in $\mu\text{L}/\text{min}/\text{cm}^2$). (b) Resting blood flux ($\mu\text{L}/\text{min}/\text{cm}^2$) and SNR values analysis for each subject. The estimated mean flux over gray matter (GM) ROI is $366 \pm 45 \mu\text{L}/\text{min}/\text{cm}^2$ and $153 \pm 23 \mu\text{L}/\text{min}/\text{cm}^2$ over white matter (WM) ROI.

Figure 5.

(a) Coronal z-score activation maps by qFENSI-based BOLD (1st row) and qFENSI (2nd row) during visual stimuli for each subject. (b) Cerebral blood flux ($\mu\text{L}/\text{min}/\text{cm}^2$), flow change percentage and SNR values in activated voxels for visual task

Table 1

MR pulse sequence parameters for qFENSI. Tag plane prescriptions A and B represent different tagging plane prescriptions that were used in the acquired data.

Imaging parameters:

FOV = 220 mm, in-plane matrix size = 64×64, TR/TE = 5000/44ms, Spin echo (SE)-Echo planar imaging (EPI) readout.

qFENSI Module:

windowed-sinc 45° RF pulse with duration = 2560 μ s, RF spacing in one concatenated pulse pair (τ) = 2960 μ s, spoiler duration and amplitude = 3600 μ s/[± 10 , ± 12 , ± 14 , ± 16 mT/m].

Tag Plane Prescription A: tagging plane/post-tag saturation slice/imaging slice = 1/3/20 mm, RF pair spacing = 10 ms, tagging duration = 1 s.

Tag Plane Prescription B: tagging plane/post-tag saturation slice/imaging slice = 3/8/20 mm, RF pair spacing = 30 ms, tagging duration = 3 s*.

* Both parameter sets (**A** and **B**) were investigated and tested in phantom and set **B** was used in vivo.

Table 2

Preset and measured flow rates in flow phantom (μL/min)

Set Q_0	490.3	343.2	245.2	147.1	49.0
Measured $Q \pm SD$	470±54	385±51	297±41	110±24	14±38
Expected mean v_0 (mm/s)*	10.0	7.0	5.0	3.0	1.0

* v_0 is the expected mean velocity in the tubing for each set volumetric flow rate, i.e., $Q_0=60\cdot\pi r^2\cdot v_0$, where r denotes the radius of the tubing.