Intravascular BOLD signal characterization of balanced SSFP experiments in human blood at high to ultrahigh fields

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Purpose: To investigate the intravascular contribution to the overall balanced SSFP (bSSFP) BOLD effect in human blood at high to ultrahigh field strengths (3 T, 9.4 T, and 14.1 T).

Methods: Venous blood prepared at two different oxygenation levels (deoxygenated: Y ≈ 71%, oxygenated: Y ≈ 94%) was measured with phase-cycled bSSFP for varying TRs/flip angles at 3 T, 9.4 T, and 14.1 T. The oxygen sensitivity was analyzed by intrinsic MIRACLE (motion-insensitive rapid configuration relaxometry)-R2 estimation and passband signal differences. The intravascular BOLD-related signal change was extracted from the measured data for microvasculature and macrovasculature, and compared with the extravascular contribution obtained by Monte Carlo simulations.

Results: The MIRACLE-R2 values showed a characteristic increase with longer TRs in deoxygenated blood, corroborating that SE-R2 data cannot be used to assess the intravascular bSSFP BOLD effect. Passband bSSFP signal differences measured at optimal flip angles of 30° at 3 T and 20° at 9.4 T/14.1 T revealed considerable relative intravascular contributions of 95%/70% at 3 T, 74%/43% at 9.4 T, 66%/46% at 14.1 T for TR = 5 ms, and 90%/65% at 3 T, 36%/27% at 9.4 T, 13%/15% at 14.1 T for TR = 10 ms in macrovascular/microvascular regimes.

Conclusion: The results indicate that intravascular effects have to be considered to better understand the origin of bSSFP BOLD contrast in functional MRI experiments, especially at short TRs. The MIRACLE-R2 method demonstrated the ability to quantify the apparent decrease in R2 due to rapid RF refocusing.

KEYWORDS
BOLD, bSSFP, fMRI, high to ultrahigh fields, human blood, intravascular contribution
1 | INTRODUCTION

Functional magnetic resonance imaging (fMRI) is an established tool to obtain insights into brain function in a noninvasive manner by using the BOLD contrast mechanism. The BOLD effect is primarily created by microscopic magnetic field gradients within and around blood vessels due to the presence of paramagnetic deoxyhemoglobin, reducing the detectable MR signal. Despite the widespread use of fMRI in research and clinical studies, a complete quantitative description of the overall observable BOLD signal change is still missing. The sensitivity to detect neuronal activity depends on the chosen sequence type, which further complicates the quantitative interpretation of activation patterns and often limits it to localization of the activated site.

Currently, high-resolution layer-resolved fMRI is receiving increased interest, implicating the need for dedicated acquisition techniques that are able to provide images with high SNR, no spatial distortions, and reduced sensitivity to large veins.

Balanced SSFP (bSSFP) is an interesting alternative sequence for fMRI, especially for laminar BOLD experiments, as it enables distortion-free imaging with high SNR efficiency and is in this respect superior to standard EPI. Using optimized TR and flip angle (α) settings, the oxygen sensitivity of bSSFP may be comparable or even exceed the one achievable with gradient-echo or T2-prepared imaging, as suggested by a previous study of the BOLD contrast in skeletal muscle. Moreover, Monte Carlo simulations have revealed that BOLD-related signal changes measured with bSSFP are selective to microvessels, similar to the spin echo (SE), and are therefore believed to be closer to the neuronal event than gradient echo.

To better understand the neurovascular bSSFP fingerprint observed in BOLD experiments, extravascular and intravascular contributions have to be identified separately. Recently, the extravascular BOLD component of bSSFP has been analyzed extensively, depending on vessel size, static magnetic field strength (B0), and sequence parameters such as TR or flip angle (α) by means of Monte Carlo methods. The intravascular contribution of bSSFP, on the other hand, has not yet been entirely explored in detail, as it requires knowledge about the apparent longitudinal (R1) and transverse (R2) relaxation rates of blood.

In the case of rapidly refocused bSSFP, the intravascular contribution is controlled by R2, which not only strongly depends on the oxygenation level (Y) but also on the refocusing interval (ie, the TR) due to diffusion-narrowing effects around red blood cells that contain paramagnetic deoxyhemoglobin. The resulting decrease in the apparent R2 with shorter TR values is similar to the echo-spacing dependence of R2 observed with the Carr-Purcell-Meiboom-Gill (CPMG) sequence. The bSSFP signal in blood can therefore not simply be estimated using literature R2 values, which are commonly derived based on either CPMG or single-echo SE techniques.

In this work, intravascular bSSFP BOLD signal changes are investigated in human blood samples, which are prepared at different oxygenation levels and measured at high to ultrahigh fields (3 T, 9.4 T, and 14.1 T). The oxygen sensitivity of bSSFP, depending on TR and flip angle, is quantified by (1) intrinsic R2 estimation from a series of phase-cycled bSSFP scans using motion-insensitive rapid configuration relaxometry (MIRACLE) as well as (2) direct calculation of passband bSSFP signal differences between oxygenated and deoxygenated blood samples. In addition, R1 and R2 values are derived based on standard SE techniques (ie, inversion-recovery spin echo with variable TIs for R1 estimation and single-echo SE with variable TEs for R2 estimation). Results from Monte Carlo simulations are used to quantify the extravascular BOLD contribution of bSSFP and SE. The relative intravascular and extravascular contributions to the BOLD effect are derived separately for microvascular and macrovascular regimes for both bSSFP and SE versus field strength and sequence parameters.

2 | METHODS

2.1 | Blood sample preparation

Fresh venous blood was collected from healthy adult volunteers at the local blood bank (20-30 mL/volunteer) under approval of the local ethics committee and stored in vials containing an anticoagulant (capacity: 10 mL/tube) for immediate transportation to the laboratory facilities. The oxygenation levels were then adjusted by bubbling air (21% oxygen) through the blood and mixing it until the desired Y level was reached (approximately 10 minutes was needed to achieve full oxygenation). The physiological hematocrit (Hct) of the blood was not altered. The Y and Hct values were assessed quantitatively using a radiometer (ABL80 FLEX CO-OX; Radiometer, Westlake, OH) before and after the MR measurement session.

For each session of MR experiments, four main blood samples were prepared in small 3-mL cylindrical tubes: two of them with an average oxygenation level of Y = 71 ± 5% (referred to as deoxygenated) and the other two with an average Y = 94 ± 4% (referred to as oxygenated). Average Hct level of the measured blood samples was approximately 42 ± 3% (range = 34%-47%, depending on the donor). Each MR session was conducted with blood samples from the same volunteer to avoid cross-contamination from different donors and possible resulting sample agglutination due to blood-type incompatibilities.

The blood samples were prone to a certain decrease in the oxygenation levels during the MR experiments. Therefore,
each MR session was limited to a maximal duration of about 6 hours, leading to an overall drop in the oxygenation levels of an average 7% at the end of the session. The Y and Hct values reported in this study refer to the mean of the values before and after the MR scanning.

### 2.2 Magnetic resonance experiments

The MR imaging of the prepared blood samples was performed at three different field strengths: (1) on a 3T whole-body scanner (Magnetom Prisma; Siemens Healthineers, Erlangen, Germany) using the standard 20-channel receive head coil of the manufacturer, (2) on a 9.4T whole-body scanner (Siemens Healthineers, Erlangen, Germany) using a custom-built 16-channel transceiver head coil (8 transmit/receive and 8 receive-only channels), and (3) on a 14.1T small-animal scanner (Magnex Scientific, Abingdon, United Kingdom/Bruker Biospin, Karlsruhe, Germany) using a custom-built linearly polarized transceiver birdcage coil.

For the purpose of MRI, the blood samples and a reference tube containing manganese-doped water (0.2 mM MnCl₂ in H₂O) were placed into a cylindrical phantom. The whole experimental setup to maintain a temperature of 37°C inside the blood samples and avoid red blood cell sedimentation is described in the Supporting Information (subsection 1) and illustrated in Figure 1.

A series of 3D phase-cycled bSSFP experiments was performed at 20 RF phase increments φ in the range [0°, 360°] to sample the bSSFP frequency profile for varying TR values as follows:

- **3T**: TR = [4, 5, 6, 8, 10] ms; resolution: 1.2 × 1.2 × 2 mm³; matrix size: 128 × 64 × 18
- **9.4 T**: TR = [3, 4, 5, 6, 8, 10] ms; resolution: 1.2 × 1.2 × 2 mm³; matrix size: 128 × 64 × 18
- **14.1 T**: TR = [2.5, 3, 4, 5, 6, 8, 10] ms; Resolution: 0.7 × 0.7 × 1.0 mm³ (matrix size: 85 × 85 × 26).

These measurements were repeated for bSSFP flip angles of α = [5, 10, 20, 30]° at 3 T and α = [5, 10, 20]° at 9.4 T/14.1 T, resulting in 10 scan sessions: four at 3 T and three at 9.4 T/14.1 T. The MR protocol of each session was completed by a B¹+ mapping sequence and the acquisition of standard SE-based reference R₁ and R₂ data. The B¹+ scaling factor cB¹⁺ = αact/αnom (≡ actual/nominal flip angle) was obtained using the vendor’s default implementation at 3 T/9.4 T (ie, TurboFLASH imaging with and without a preconditioning RF pulse²⁵,²⁶) and actual flip angle imaging²⁷ at 14.1 T. Standard reference R₁ and R₂ values were calculated based on single-slice inversion-recovery SE scans with variable TIs and single-slice single-echo SE scans with variable TEs, respectively. Different sets of TI/TR and TE values were used at each field strength, as summarized in Table 1, to account for the B₀ dependence of R₁ and R₂, respectively.

### 2.3 Data analysis

Data analysis and visualization of the results were performed using MATLAB R2019b (The MathWorks, Natick, MA).

Reference R₁ and R₂ values were determined voxel-wise through nonlinear least-squares fitting of the real part spin-echo data sets (Sn, n = 1, 2, … 5) acquired with variable inversion (TI,ₙ) and echo times (TE,ₙ), respectively, according to

![FIGURE 1](image-url)
the following exponential equations for $R_1$ (Equation (1)) and $R_2$ (Equation (2)):

$$S_n = S_0 \cdot (1 - C) \cdot e^{-T_in} \cdot R_1$$

$$S_n = S_0 \cdot e^{-T_{en}} \cdot R_2$$

$$R_1, IV = p_1 \cdot B_{20}^2 + p_2 \cdot B_{00} + p_3$$

$$R_2, IV = p_4 \cdot B_{20}^2 \cdot (1 - Y) + p_5 \cdot B_{00} + p_6$$

**Table 1** Sequence parameters (TI, TE, TR) used for the acquisition of standard SE reference $R_1$ and $R_2$ data depending on the field strength, at which the measurements were performed.

| $B_0$ | $R_1$ (TI (ms)) | $R_1$ (TR (s)) | $R_2$ (TE (ms)) | $R_2$ (TR (s)) |
|-------|-----------------|-----------------|-----------------|-----------------|
| 3 T   | [25 200 1000]   | 10              | [10 30 75]      | 3               |
|       | [2000 4000]     |                 | [150 300]       |                 |
| 9.4 T | [25 500 1500]   | 12              | [5 12.5 25]     | 3               |
|       | [3000 6000]     |                 | [50 100]        |                 |
| 14.1 T| [63 1000 2500]  | 15              | [2.6 10 20]     | 3               |
|       | [5000 10 000]   |                 | [40 80]         |                 |

**Table 2** Mean oxygenation (Y) and Hct levels along with mean SE-based reference measurements of relaxation rates ($R_1$, $R_2$) and mean $B_1$ scaling factor ($c_{B1}$) assessed in oxygenated (rows with white background) and deoxygenated (rows with gray background) blood samples for four scan sessions ($\alpha_{SSFP} = [5, 10, 20, 30]°$) at 3 T and three scan sessions ($\alpha_{SSFP} = [5, 10, 20]°$) at 9.4 T/14.1 T.

- The proportionality factor $S_0$ reflects the proton density and coil sensitivity, while $C$ is a parameter related to the inversion efficiency. On the obtained single-slice relaxation maps, circular regions of interest were drawn in all measured blood samples as well as in the MnCl$_2$-doped aqueous probe to assess mean reference $R_1$ and $R_2$ values (cf. Table 2 and Supporting Information Table S1). The blood $R_1$ and $R_2$ data obtained with reference SE techniques were then compared with the following intravascular (IV) $R_1$ and $R_2$ models derived by Khajehim et al$^{28}$ and Uludag et al,$^{29}$ respectively:

$$R_{1,IV} = p_1 \cdot B_{00}^2 + p_2 \cdot B_{00} + p_3$$

$$R_{2,IV} = p_4 \cdot B_{20}^2 \cdot (1 - Y) + p_5 \cdot B_{00} + p_6$$

A quadratic dependence on deoxyhemoglobin $(1 - Y)$ is assumed for $R_2$, while the weak dependence of $R_1$ on the oxygenation level is neglected (Equation (3)). The experimental single-echo SE blood $R_2$ values were fitted to a new model using Equation (4) with an additional linear $(1 - Y)$ term as

Note: Reference $R_1$ and $R_2$ estimation was based on inversion-recovery SE with five different TI values and single-echo SE with five different TE values, respectively.

Note: The displayed $Y$ and Hct values are the average between measured levels at the beginning and end of each session.
to derive the fitting parameters \( p_7, p_8, p_9, \) and \( p_{10} \). A linear \((1-Y)\) component was introduced, as this adjusted model (Equation 5) better reflected the SE-\(R_2\) data acquired in this work than Equation (4).

From the acquired complex bSSFP frequency profiles, \( R_2 \) was intrinsically estimated using the MIRACLE method\(^{23}\) as described in detail in the Supporting Information (subsection 2). For the analysis of differences in the bSSFP magnitude between oxygenated and deoxygenated blood samples, the acquired bSSFP profiles were centered voxel-wise (ie, \( B_0^{\text{corr}} \) corrected) after elimination of receiver-related phase offsets. The \( B_0^{\text{corr}} \) correction was based on shifting the magnitude profile according to the minimum in the first derivative of the phase, which is expected to correspond to the dip in the center of the bSSFP transition band. The bSSFP signal in the blood and reference probes was averaged for each phase cycle using the same central slices and regions of interest as defined for the MIRACLE \( R_2 \) analysis. In the assessed regions of interest, the mean \( c_{B1+} \) values were relatively close to one at 3 T and 9.4 T, but slightly lower at 14.1 T (Table 2 and Supporting Information Table S1).

In a last step, the average measured bSSFP signal amplitudes were normalized (Supporting Information subsection 3) to ensure comparability across field strengths and with the simulated extravascular bSSFP BOLD signal change, which was defined in units of the equilibrium magnetization \( M_0 \). The bSSFP signal differences between oxygenated and deoxygenated blood reflecting BOLD activation were then assessed as \( \Delta S_{\text{ox-deox}} = S_{\text{SSFP,ox}} - S_{\text{SSFP,deox}} \), where \( S_{\text{SSFP,ox}} \) and \( S_{\text{SSFP,deox}} \) are the normalized mean bSSFP signal levels of oxygenated and deoxygenated blood samples, respectively. Mean passband \( \Delta S_{\text{ox-deox}} \) values were obtained as the average of the four most central points in the bSSFP profile.

All values (SE \( R_1/R_2 \), MIRACLE \( R_2 \), bSSFP signal level) reported for deoxygenated/oxygenated blood refer to the average assessed in the two measured deoxygenated/oxygenated blood samples.

### Microvascular and macrovascular regimes

To assess the intravascular bSSFP BOLD signal contribution more specifically in relation to the extravascular component, microvascular and macrovascular regimes were defined as outlined in detail in the Supporting Information (subsection 4). For two different voxel types representing microvasculature and macrovasculature, the extravascular bSSFP BOLD contribution was quantified at the passband in units of \( M_0 \) by means of a Monte Carlo approach in complete analogy to Scheffler et al.\(^{13}\) The vessels of the vascular network were modeled as artificial cylinders with different diameters (ie, vessel sizes) and random orientations with respect to \( B_0^{\text{corr}} \).\(^{12,14,30}\) The relaxation rates of the extravascular (EV) water protons were set to \( R_{1,\text{EV}} = 0.003 \cdot B_0^{\text{corr}} - 0.0791 \cdot B_0^{\text{corr}} + 0.9247 \) and \( R_{2,\text{EV}} = 1.74 \cdot B_0^{\text{corr}} + 7.77 \) according to Khajehim et al.\(^{28}\) and Uludag et al.\(^{29}\) respectively.

The intravascular bSSFP BOLD contribution was calculated separately for microvasculature and macrovasculature by linearly interpolating the normalized measured signal levels at the passband in deoxygenated and oxygenated blood samples to the respective \( Y \) levels assumed for microvessels and macrovessels. The total BOLD-related passband bSSFP signal change consisting of a weighted sum of extravascular and intravascular contributions was then obtained by\(^{29}\)

\[
\Delta S = (1 - BV) \cdot \Delta S_{\text{EV}} + PD_{\text{rel}} \cdot \sum_i BV_i \cdot \Delta S_{\text{IV,i}}
\]

where \( i \) is the index denoting the different vascular compartments in a voxel as described in the Supporting Information (subsection 4) (ie, \( i = \{\text{arterioles, capillaries, venules}\} \) for microvasculature and \( i = \{\text{intracortical veins}\} \) for macrovasculature; \( BV \) is the total fractional blood volume relative to whole tissue volume (= \( \sum BV_i \)) (ie, 2.3% for microvasculature \[BV_{\text{arterioles}} = 0.5\%, BV_{\text{capillaries}} = 0.8\%, BV_{\text{venules}} = 1\%\] and 0.6% for macrovasculature); \( PD_{\text{rel}} \) is the relative proton spin density (= \( PD_{\text{rel}} / PD_{\text{EV}} \)); \( \Delta S_{\text{EV}} \) is the mean extravascular BOLD signal change calculated by Monte Carlo simulations separately for microvascular and macrovascular regimes; and \( \Delta S_{\text{IV,i}} \) is the mean intravascular BOLD signal change due to compartment \( i \), as extracted from the blood measurements.

In Equation (6), changes in \( BV \) between resting and activated states were not taken into account. A relative scaling was used for the spin density \( PD_{\text{rel}} = PD_{\text{rel}} / PD_{\text{EV}} \), as the relative (not absolute) contribution of extravascular and intravascular effects to the total BOLD signal change is of interest here. For gray matter, \( PD_{\text{rel}} \) was reported to be close to one\(^{31-33}\); therefore, \( PD_{\text{rel}} = 1 \) was assumed in this work. All BOLD-related signal differences were calculated in units of \( M_0 \) and therefore did not consider the increase of \( M_0 \) with the power of 1.5-1.7 as a function of \( B_0^{\text{corr}} \).\(^{34,35}\) The relative extravascular and intravascular bSSFP BOLD contributions of microvasculature and macrovasculature were analyzed for optimal flip angles according to Figure 4 in Scheffler et al.\(^{13}\) (ie, 30° at 3 T and 20° at 9.4 T/14.1 T).

Additionally, a similar analysis was performed for the SE case. To this end, the extravascular BOLD signal change was simulated using an initial full magnetization \( M_0 \), infinite TR, and an excitation flip angle of 90° based...
on a Monte Carlo method as described previously for bSSFP. The intravascular SE BOLD signal change was calculated for microvascular and macrovascular regimes according to Equation (6) using the derived SE $R^2$, $IV$ model (Equation (5)) combined with the SE signal expression $S_{SE} = M_0 \cdot e^{-TE \cdot R^2}$ (with $M_0 = 1$) and compared with the literature SE $R^2$, model (Equation (4)). Relative intravascular and extravascular SE BOLD contributions were analyzed for a range of TEs as well as for two sets of dedicated TEs: (1) TEs corresponding to the $B_0$-dependent tissue transverse relaxation times ($\equiv 1/R^2_{2, EV}$), as typically used for SE BOLD imaging, and (2) TEs maximizing $\Delta S$ (Equation (6)) for microvasculature.

3 | RESULTS

The characteristics of the measured deoxygenated and oxygenated blood at 3 T, 9.4 T, and 14.1 T, including $Y$ and Hct levels, reference $R_1$ and $R_2$ relaxation rates, and $B_1$ values, are summarized in Table 2. In Figure 2, the reference $R_1$ and $R_2$ measurements in human blood are compared with the literature models derived by Khajehim et al. (Equation (3)) and Uludag et al. (Equation (4)), respectively (dotted curves in Figure 2). The reference $R_1$ and $R_2$ data acquired in this work with standard SE techniques follow the same trend as predicted by these models. However, $R_1$ is overestimated in both oxygenated as well as deoxygenated blood, and $R_2$ is underestimated in deoxygenated blood at 9.4 T and 14.1 T with respect to the literature model values, whereas the 3T data are in very good agreement. The fit of the experimental SE-$R_2$ data to Equation (5) yielded the following model parameters: $p_7 = 5.3, p_8 = 64.8, p_9 = 3.5$, and $p_{10} = -4.0$, with an adjusted $R$-square of 0.993 (solid curves in Figure 2B).

The quantitative results for intrinsic blood $R_2$ estimation based on the complex bSSFP frequency profiles using MIRACLE are displayed in Figure 3. The MIRACLE-$R_2$ relaxation rates in deoxygenated blood show a pronounced increase with longer TRs, similar to the reported increase of $R_2$ based on CPMG with longer echo spacing due to diffusion-narrowing effects. The MIRACLE-$R_2$ of the oxygenated blood, on the other hand, remains largely independent of TR and in very good agreement with the reference SE-$R_2$ data. For TRs $\geq 8$ ms at 14.1 T, MIRACLE-$R_2$ values in deoxygenated blood are dropping and appear to be more affected by noise, as reflected by increased SDs (cf. Figure 3, bottom row). Generally, MIRACLE-$R_2$ and reference single-echo SE-$R_2$ agree better in deoxygenated blood at longer TR and higher flip angle when bSSFP is approaching a single-echo SE (long TR, 90° excitation flip angle) with similar diffusion sensitivity. The MIRACLE-$R_2$ values assessed in the MnCl$_2$-doped aqueous probe appear highly TR-insensitive, as
expected, and are close to the measured reference SE-R$_2$ values. The observed evident TR-dependence of MIRACLE-R$_2$ in deoxygenated blood corroborates that literature SE-based data cannot be used to quantify the intravascular BOLD contribution for bSSFP.

Normalized average bSSFP frequency profiles obtained for a range of TRs in deoxygenated and oxygenated blood at 3 T, 9.4 T, and 14.1 T are shown in Figure 4 (first and second column), representatively for a flip angle of 20°. Interestingly, the bSSFP frequency response in oxygenated blood is clearly asymmetric (Figure 4, first column). The corresponding signal differences $\Delta S_{\text{oxygenated}}$ (Figure 4, third column) increase with longer TRs in the bSSFP passband. Mean passband signal differences are quantified in Figure 5 versus the TR and flip angle, manifesting a consistent increase in $\Delta S$ versus TR, especially at higher field strength, in line with the MIRACLE-R$_2$ increase (Figure 3). The oxygen sensitivity of passband-bSSFP is further enhanced with higher flip angles (at all investigated field strengths) and with higher B$_0$ (Figure 5).

The overall bSSFP BOLD effect ($\Delta S$), differentiated between extravascular ($\Delta S_{\text{EV, tot}}$) and intravascular ($\Delta S_{\text{IV, tot}}$) contributions, is analyzed in Figure 6 for microvascular and macrovascular regimes (Equation (6)) and optimal B$_0$-dependent flip angles according to Scheffler et al.$^{13}$ As shown in Figure 6A, both extravascular and intravascular signal changes in units of M$_0$ increase with TR, except the intravascular component at 14.1 T. At such high field strength, the blood bSSFP signal reaches the noise level with increasing TRs due to the rapid R$_2$ relaxation at the oxygenation levels assumed for resting/activated states of intracortical veins and venules (Supporting Information, subsection 4), as shown in Figure 4 (third row, deoxygenated blood), leading to a smaller intravascular BOLD effect. The sensitivity to microvessels of bSSFP BOLD is

![FIGURE 3](image-url) Dependence of motion-insensitive rapid configuration relaxometry (MIRACLE)-based R$_2$ estimation in oxygenated (red circles) and deoxygenated (blue circles) blood on TR at 3 T (top), 9.4 T (middle), and 14.1 T (bottom) for three different balanced SSFP (bSSFP) flip angles (5°, 10°, and 20° from left to right). Using the concept of MIRACLE relaxometry, R$_2$ is intrinsically calculated based on the measured complex bSSFP frequency profiles. The MIRACLE-R$_2$ values assessed in the reference MnCl$_2$-doped water probe are plotted for comparison (yellow circles). For improved visualization, the calculated R$_2$ values are connected by dashed lines versus TR, and different scales are used per field strength. The reference SE-R$_2$ values reported in Table 2 and Supporting Information Table S1 are indicated for comparison next to each MIRACLE-R$_2$ plot as horizontal lines in the corresponding color (red/blue, oxygenated/deoxygenated blood; yellow, reference probe)
evident, yielding a 4 to 5 times higher $\Delta S$ for microvasculature as compared with macrovasculature across all investigated TRs and field strengths. The increase of both $\Delta S_{EY,tot}$ and $\Delta S_{EV,tot}$ with $B_0$ adds up to a 2.0/2.6 and 3.9/4.6 times higher microvascular $\Delta S$ at 9.4 T and 14.1 T, respectively, relative to 3 T for TR values of 5 ms/10 ms.

**Figure 4** Normalized average bSSFP frequency profiles in units of $M_0$ obtained with a flip angle of $\alpha = 20^\circ$ for different TRs in oxygenated (first column) and deoxygenated (second column) blood at 3 T (top row), 9.4 T (middle row), and 14.1 T (bottom row). The BOLD-related signal difference $\Delta S_{ox-deox}$ is displayed in the third column as the subtraction of the second from the first column versus the RF phase increment $\phi$.

**Figure 5** Normalized passband bSSFP signal change $\Delta S_{ox-deox}$ between oxygenated and deoxygenated blood versus TR for field strengths of 3 T (left), 9.4 T (middle), and 14.1 T (right) at flip angles of 5° (black), 10° (red), 20° (blue), and 30° (green, only acquired at 3 T).
Because the extravascular signal change increases more rapidly with TR than the intravascular one, the relative intravascular contribution in units of percentage decreases for longer repetition times. This TR dependence is especially prominent at ultrahigh field strength (Figure 6B). For the optimal flip angles analyzed in Figure 6 and short TR values, the intravascular contribution decreases moderately with field strength, concretely for TR = 5 ms from 95%/70% at 3 T to 74%/43% at 9.4 T and 66%/46% at 14.1 T in the macrovascular/microvascular regimes (Figure 6B). For TR = 10 ms, a more enhanced decrease from 90%/65% at 3 T to 36%/27% at 9.4 T and 13%/15% at 14.1 T, can be observed in microvasculature/macrovasculature. Generally, Figure 6B demonstrates that the intravascular component has a considerable impact on the overall bSSFP BOLD effect, particularly at short TRs.

For reference, intravascular and extravascular BOLD signal changes are analyzed in Figure 7 for SE in macrovasculature and microvasculature. Both $\Delta S_{EV,\text{tot}}$ and $\Delta S_{IV,\text{tot}}$ show a characteristic behavior versus TE with distinct maxima (Figure 7A). The SE $R_{2,\text{IV}}$ model derived in this work (solid red and yellow curves in Figure 7A) yields intravascular signal changes of the same order as the literature SE $R_{2,\text{IV}}$ model proposed by Uludag et al.29 (dashed red and yellow curves in Figure 7A). Clearly higher signal changes are observed for the microvascular regime as compared with macrovasculature (Figure 7B), similar to bSSFP. The intravascular SE-BOLD contribution is relatively high at 3 T (35%/37% for macrovasculature/microvasculature) but decreases rapidly for higher field strengths (11%/12% at 9.4 T and 4%/8% at 14.1 T in macrovasculature/microvasculature) for TEs set to tissue $T_2 = 1/R_{2,\text{EV}}$,29 (Figure 7B), in agreement with literature.20,36-38 Optimal TEs maximizing BOLD contrast in microvasculature are larger than tissue $T_2$ and yield reduced intravascular contributions of 19%/29% at 3 T, 4%/7% at 9.4 T, and 1%/5% at 14.1 T for macrovessels/microvessels.

**FIGURE 6** A, Total BOLD-related bSSFP signal change ($\Delta S$) in units of $M_0$, visualized as the sum of intravascular ($\Delta S_{IV,\text{tot}}$, red/yellow bars) and extravascular ($\Delta S_{EV,\text{tot}}$, blue/violet bars) contributions according to Equation (6) versus TR at 3 T (left), 9.4 T (middle), and 14.1 T (right). For the calculation of $\Delta S$, optimal bSSFP flip angles of 30° (3 T) and 20° (9.4 T/14.1 T) are used following Scheffler et al.13 The BOLD signal change $\Delta S$ is displayed separately for macrovasculature (red/blue bars) and microvasculature (yellow/violet bars). Note that different scales are used for $\Delta S$, depending on the field strength. B, Corresponding intravascular and extravascular BOLD bSSFP contributions in units of percentage, calculated as $\Delta S[\%] = (\Delta S_{EV,\text{tot}}/\Delta S) \cdot 100 + (\Delta S_{IV,\text{tot}}/\Delta S) \cdot 100 \equiv 100\%$.
A certain discrepancy was evident between the acquired SE-based blood $R_1$ and $R_2$ data and literature models (Figure 2). The literature data used for the fitting of $R_1$ (Equation (3)) were somewhat heterogeneous, mixing in vivo and ex vivo measurements as well as different $R_1$ acquisition strategies (e.g., Look-Locker EPI, IR-LASER, RAREVTR). Higher respective blood $R_1$ values as compared with the literature $R_1$ model were also found in another study based on inversion-recovery SE, including a weak linear dependence of $R_1$ on the oxygenation, which is not taken into account in Equation (3). Furthermore, Hct, which is proportional to the hemoglobin concentration, alters blood $R_1$ considerably, as reported in several studies. This dependence is not accounted for in the literature model plotted in Figure 2A. In this work, all measurements were performed with physiological Hct values. However, Hct varies depending on gender, with typically lower values in females. This may explain the variability in Hct, which we encountered especially for the different MR sessions at 3 T, and may contribute to the observed deviations between our SE-$R_1$ data and literature (Figure 2A).

The literature $R_2$ model (Equation (4)) may be biased at higher $B_0$ due to insufficient or lack of data at ultrahigh fields, as no data at $B_0 > 9.4$ T were included in the modeling. Additionally, a quadratic dependence on $Y$ was forced for the $R_2$ fit of the $9.4$ T data, even though the oxygenation dependence of $R_2$ was observed to be linear in the underlying 9.4 T data. This would explain the good agreement of our blood $R_2$ data with this model at 3 T, but increasing deviations at 9.4 T and 14.1 T (Figure 2B). The SE-$R_2$ error due to Hct variation ($\approx 10\%$) (Table 2) and drop in oxygenation ($\approx 7\%$) (see section 2) can be estimated, for example, for 3 T, using the blood $R_2$ data measured with single-echo SE at systematically varied Hct and Y levels reported in Zhoa et al (Table 1). Assuming a linear dependence of $R_2$ on Hct and a quadratic dependence of $R_2$ on Y, the variation in SE $R_2$ amounts to about 5% and 15% for 10% Hct and 7% Y.
variation, respectively, reflecting the strong dependence of $R_2$ on oxygenation.

The theoretical description of the bSSFP signal underlying the MIRACLE method does not take into account any diffusion and refocusing effects around microscopic field inhomogeneities (in this study, around deoxygenated blood cells). As a consequence, intrinsic $R_2$ estimation based on the acquired bSSFP frequency profile using MIRACLE revealed a clear apparent increase of the transverse relaxation rate for longer refocusing intervals (TRs) in deoxygenated blood (Figure 3), due to diffusion-related motional narrowing, similar to the well-known dependence of CPMG-$R_2$ in blood on the echo spacing. The apparent MIRACLE-$R_2$ values in deoxygenated blood showed a further tendency to decrease at higher flip angles, especially evident at ultrahigh fields (cf. Figure 3), possibly due to increased refocusing and thus reduced diffusion sensitivity while approaching the signal characteristics of a fully refocused CPMG ($180^\circ$ refocusing flip angle). This effect can be observed in Figure 4 in Scheffler et al., where signal differences decrease with increasing flip angle. Similarly, CPMG $R_2$ increases with reduced refocusing flip angles, as more and more transverse magnetization is refocused along the echo train, leading to an increase of the relevant TE for refocusing of diffusion-related dephasing. These results confirm that SE-based blood $R_2$ values reported in literature (eg, Uludag et al.) cannot simply be plugged into the analytical bSSFP signal equation to estimate the intravascular BOLD signal change.

As shown in Figure 4 (bottom row, middle column), the bSSFP signal in deoxygenated blood at 14.1 T is approaching the noise level at longer TRs due to the rapid $R_2$ relaxation at this field strength combined with relatively long TEs (ie, $1/R_2 < TE$). The observed drop in $R_2$ of deoxygenated blood for TRs ≥ 8 ms at 14.1 T (Figure 3, bottom row) can likely be attributed to a systematic bias due to the higher impact of noise at such low signal levels. Measuring with TE > $1/R_2$ leads to signal nulling and consequently erroneous MIRACLE-$R_2$ results.

The range of bSSFP acquisition parameters investigated in this work (ie, rather short repetition times and low flip angles) covers realistic settings. Although bSSFP fMRI contrast is generally expected to increase with longer TRs, bSSFP imaging often has to be performed with short TRs of only a few milliseconds to mitigate banding artifacts at high field strengths and to minimize scan times. Monte Carlo simulations of the extravascular bSSFP BOLD effect reported in previous work indicate that signal changes are highest for relatively low flip angles of about 30° at high field (<7 T) and 20° at ultrahigh field (≥7 T), which is beneficial in practice, as specific absorption rate constraints limit the achievable flip angle. Furthermore, in this study, the analysis of the acquired phase-cycled bSSFP signal focused on the passband. Transition-band bSSFP may give higher intravascular and extravascular signal changes (Figure 4 and Scheffler et al., Figure 1), but is highly sensitive to off-resonance effects and therefore is unstable, especially at high field strengths.

As visible from Figure 5, intravascular BOLD-related signal changes ($\Delta S_{\text{ox-deox}}$) of passband bSSFP increase with TR as expected, but are also amplified with higher flip angles and field strengths, in line with results reported by Dharmakumar et al. and Arumana et al. on the oxygen sensitivity of bSSFP in blood samples and in an ischemic leg cuff model, respectively. Intravascular effects appear to have a considerable impact on the bSSFP-BOLD contrast in short-TR acquisitions, contributing approximately 70% of the overall microvascular signal change at 3 T and more than 40% at 9.4 T/14.1 T for a TR of 5 ms (Figure 6B). For a longer TR of 10 ms, such as in Ehses and Scheffler, the intravascular bSSFP contribution decreases to about 65%, 27%, and 15% at 3 T, 9.4 T, and 14.1 T, respectively, for the microvascular regime. These findings agree well with simulations performed in Kim et al. at 3 T for similar TR and flip-angle settings, which suggest that the intravascular bSSFP contribution is over 60% of the total signal change at 3 T and therefore accounts for a large fraction of the bSSFP-fMRI contrast.

This relatively large contribution of intravascular signals compared with gradient-echo EPI has several implications for bSSFP fMRI. First, regions with large BV, such as cortical veins, will produce a significant BOLD contrast, as reported experimentally in Scheffler and Ehses. This might considerably bias signals from deeper cortical structures, similar to gradient-echo EPI. However, the imaging point spread function of bSSFP is much narrower compared with EPI, due to the absence of $T_2^*$ decay along the phase-encoding direction. Therefore, with a sufficiently high resolution and without spatial distortions, the large signal from cortical vessels might be effectively suppressed in deeper regions with bSSFP. Second, in microvascular regimes with smaller BV of about 2%, the intravascular contribution still amounts to about 20%-50%, as shown in Figure 6. This is probably not an inherent disadvantage, but rather demonstrates that the BOLD signal of bSSFP has a significant component related to blood volume changes similar to the VASO technique. As a consequence, our presented modeling of intravascular and extravascular contributions in bSSFP is still not fully correct, as we did not take into account changes in BV between resting and activated conditions. However, the strong dependence of intravascular over extravascular weighting on TR and flip angle (and field strength) might be used to adjust the desired ratio, or even allow disentangling BV and extravascular effects.

The relative intravascular contribution of SE, on the other hand, is higher than 30% at 3 T, but decreases relatively rapidly with $B_0$ to below 10% at 14.1 T for TEs set to tissue $T_2$ (≈ $1/R_{2,\text{EV}}$), because $R_2$ in venous blood increases faster with
B₀ than the corresponding tissue R₂ (Figure 7B). Absolute total BOLD-related SE signal changes are about 30, 20, and 10 times higher at 3 T, 9.4 T, and 14.1 T in comparison to bSSFP (Figures 6A and 7B). However, saturation effects resulting from finite TR as well as T₁*-related signal decay along the SE-EPI readout will lead to a reduction of the SE-BOLD signal change in actual fMRI experiments. At ultrahigh field strength, the BOLD efficiency of bSSFP has the potential to become comparable to SE,¹⁰,¹³ as BOLD-related signal changes increase more rapidly with B₀ in the case of bSSFP as compared with SE (Figures 6A and 7B). The BOLD efficiency at higher field strengths is further boosted by taking into account the supralinear increase of SNR with B₀ with the power of 1.5-1.7.³⁴,³⁵ Note that this effect was not considered for the calculation of BOLD-related signal changes in Figures 5-7, but a field-independent M₀ = 1 was assumed.

In this work, intravascular bSSFP signal changes for microvascular and macrovascular regimes were calculated based on measured signal differences, necessitating normalization to units of M₀. On the other hand, MIRACLE-R₂ fitting demonstrated its potential to quantitatively describe diffusion-narrowing effects for rapidly refocused bSSFP acquisitions. Future studies may focus on analyzing the dependence of MIRACLE-R₂ on TR and flip angle in more detail, to ultimately establish a quantitative model for given blood oxygenation levels and field strengths. This may include simulations and experiments to gain more insights into the possible mechanisms driving the bSSFP profile asymmetry observed in oxygenated blood samples (Figure 4), such as diffusion¹⁴ or chemical exchange effects.⁵³,⁵⁴

5 | CONCLUSIONS

The results from bSSFP imaging in blood samples with different oxygenation levels indicate that intravascular effects have a nonnegligible effect on BOLD-related signal changes at high to ultrahigh fields, particularly if imaging is performed at short TRs. Overall bSSFP-BOLD efficiency was clearly enhanced with higher B₀, corroborating the potential of bSSFP for fMRI experiments at ultrahigh field strength. Future studies may focus on using intrinsic MIRACLE-R₂ estimation based on phase-cycled bSSFP acquisitions to derive a quantitative model for the apparent diffusion-related TR and flip-angle dependence of the transverse relaxation rate.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

TABLE S1 Characteristics of the reference probe. Note: The mean spin echo–based relaxation rates ($R_1$, $R_2$) and mean $B_1$ scaling factor ($c_{B1}$) values of the reference MnCl$_2$-doped water probe (0.2 mM MnCl$_2$ in H$_2$O) are displayed for each field strength averaged across the different sessions.

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