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Communication

Signal enhancement by diffusion: experimental observation of the "DESIRE" effect

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Abstract

Inadequate signal-to-noise ratio is a major factor limiting applications of magnetic resonance microscopy. The "Diffusion Enhancement of Signal and Resolution" (DESIRE) scheme promises potential sensitivity enhancements of between one and three orders of magnitude, but images using this mechanism have not been shown to date. Here, we report the first images obtained using the DESIRE method, and obtain excellent agreement between numerical simulations and experimental data with signal-to-noise enhancements of close to one order of magnitude.

Keywords: MR microscopy; Diffusion-enhanced MRI; Resolution; Magnetic resonance histology

1. Introduction

Despite advances made in the last several years in terms of its spatial resolution [1,2] the application of MR microscopy to the study of very small samples such as single biological cells [3–6] is still limited by a low signal-to-noise ratio (SNR) and long data acquisition times. Molecular diffusion has a strong influence on both the SNR and spatial resolution in magnetic resonance microscopy due to the fact that, on the time scale (ms) of the acquisition of individual $\vec{k}$-space lines, water molecules diffuse a distance on the order of the desired spatial resolution. Although diffusion limits the spatial resolution [7,8], it also has potential advantageous properties in terms of generating image contrast on a microscopic length scale through edge enhancement due to motional narrowing [9–11] and diffusive relaxation [12]. Moreover, it has been speculated that diffusion can possibly be used to increase significantly the sensitivity in magnetic resonance microscopy. Lauterbur and co-workers [13–15] have proposed a scheme termed diffusion enhancement of signal and resolution (DESIRE) to enhance the sensitivity by one to three orders of magnitude [5,16], although to date minimal experimental results have been reported.

The concept of diffusional enhancement is to saturate the nuclear spins within a small volume for an extended period of time. The molecules initially located in the saturated volume diffuse out and are replaced by new molecules which become saturated. As a result, after a certain saturation period the magnetization will be suppressed in a larger volume outside the directly saturated volume. The "missing" magnetization corresponds to all the spins that have been, at some point during the saturation period, within the saturated volume, even though these spins are now distributed over a larger volume. MR images are produced by detecting this missing magnetization via subtraction of images with and without saturation [16]. The expected enhancements, as well as the experimental requirements for the "DESIRE" method have been described in detail elsewhere [16]. Similar

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"hole-burning" techniques have been used in spectroscopic measurements of diffusion in various media [17–20]. In this paper, we report the first MR images obtained using the DESIRE technique, attaining signal enhancements of about one order of magnitude.

2. Experimental

All experiments were performed using a Varian Unity/Inova 600 MHz NMR spectrometer equipped with gradients with a maximum strength of 90 Gauss/cm. All experiments were performed at room temperature using a 3 mm diameter solenoidal radiofrequency (RF) coil.

The imaging sequence used is shown in Fig. 1; pulse and delay timings are defined in this schematic. The pulse sequence is based on a standard spin-echo sequence preceded by a saturation module. The role of this prepulse is to saturate the magnetization in a certain plane, in this case in the y direction. A constant slice-select gradient G_y is applied during a long, low-power RF pulse. The duration t_y and power of the pulse were chosen such that they correspond to a $\pi/2$ tip angle. The nominal thickness ($2a$) of the saturated plane, in the absence of significant diffusion during the saturation pulse, is defined to be the full-width-half-maximum (FWHM) of the frequency-domain sinc function:

$$2a = 1.21\frac{2\pi}{\gamma G_y t_y}. \quad (1)$$

After the pulse, any transverse magnetization is dephased using a spoiler gradient G_s. The prepulse is then repeated in order to keep saturating the magnetization in the selected plane for a time period on the order of T_1. After saturation, a conventional spin-echo imaging readout is used. Two images are acquired: one with saturation and a second with no saturation, and the images are subtracted on a pixel-by-pixel basis: the result is the diffusion enhanced image.

3. Results and discussion

Using the pulse sequence described in the previous section we obtained DESIRE enhanced images of a plant stem with a diameter of 1.5 mm. The images were acquired with a prepulse of total saturation time $t_{sat} = 900$ ms (consisting of 30 $\pi/2$ pulses) and a gradient $G_y = 60$ Gauss/cm. After each pulse a gradient G_s is applied to spoil any remaining transverse magnetization. In the standard spin-echo imaging module, the slice thickness defined by the gradient G_{ss} is chosen so that it encloses the entire area affected by the saturation pulse. From Eq. (1), the nominal saturation thickness is $2a = 8.16\,\mu$m. The actual saturation thickness is very close to the nominal thickness if the duration of the saturation pulse is sufficiently short so that the typical molecular distance $(Dt_y)^{0.5}$ is much less than the value of a. In this particular case $t_y = 5.8$ ms and $D = 1.4 \times 10^{-9}$ m^2/s (measured using a standard Stejskal–Tanner pulsed field gradient sequence), corresponding to $(Dt_y)^{0.5} \sim 2\,\mu$m.

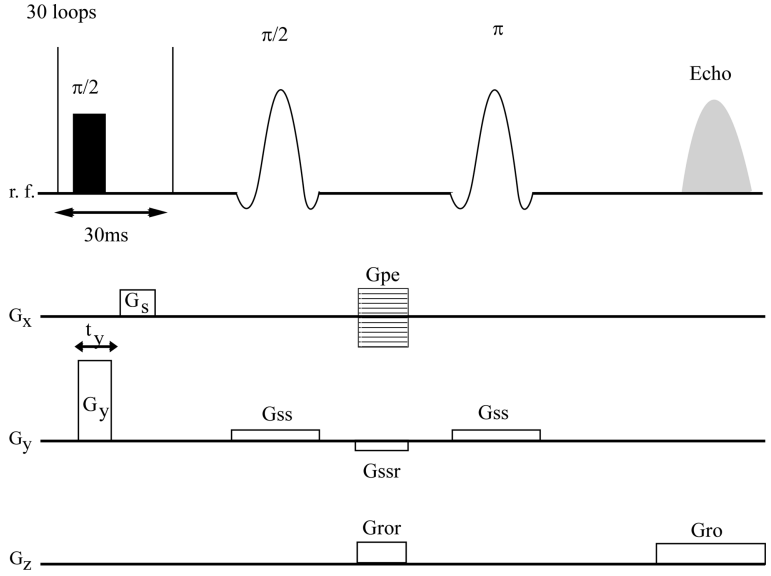

Fig. 1. Planar DESIRE pulse sequence. The first module consists of a long rectangular pulse applied simultaneously with G_y to saturate a thin slice through the sample. A short spoiler gradient pulse, G_s, is applied to destroy any transverse magnetization. Following saturation a regular spin-echo imaging sequence is run. The strength of the slice selection gradient, G_{ss}, is chosen so that the slice thickness is slightly greater than the dimension of the saturated volume.

Experimental verification of the saturation thickness is challenging due to its very small value. One can acquire a 1D spatially resolved projection along the axis from which the plane was saturated, but in order to be able to measure the saturation thickness accurately a spatial resolution much finer than the thickness is clearly necessary. Alternatively, one can compare results from thicker, more easily measurable saturation planes, and then use these values to extrapolate to the 1–10 μm range. We followed this latter approach. By keeping the gradient duration constant ($t_y = 5.8$ ms) and increasing its strength we produced saturation planes with thicknesses varying from 405 to 27 μm. A typical slice profile is shown in Fig. 2. In accordance with previous publications [17] the measured saturation width ($2a_{exp}$) can be defined by the FWHM of a Gaussian fit to the corresponding profile. The calculated and experimental slice thickness values, $2a_{calc}$ and $2a_{exp}$, are plotted in Fig. 3A. The fit shown is a straight line demonstrating a high degree of correlation between experimental and calculated values. A systematic overestimation is apparent from the 5% deviation of the slope with respect to unity. By extrapolating the linear fit (Fig. 3B) to the nominal saturation thickness ($2a_{calc} = 8.16$ μm) we extract $2a_{exp} = 7.83$ μm.

Having established the saturation thickness, we can now proceed to estimate the corresponding signal enhancement. According to Eq. (3) in [16], the enhancement $E(t)$ of the signal as a function of the saturation time is given by:

$$E(t) = \frac{1}{\varepsilon}\int_{|\vec{r}|>a} [1 - M(\vec{r},t)/M_0]\, \mathrm{d}V, \qquad (2)$$

where $\mathrm{d}V$ is the volume element, ε is the volume of the directly saturated region, $M(\vec{r},t)$ is the z component of the magnetization, and M_0 is the magnetization at thermal equilibrium. In Eq. (2) the time dependence of the magnetization is governed by the diffusion equation:

$$\left(D\nabla^2 - \frac{\partial}{\partial t}\right) M(\vec{r},t) = 0, \qquad (3)$$

where D is the diffusion coefficient. The initial condition, before the RF is turned on, is thermal equilibrium, with $M = M_0$ for all $|\vec{r}| > a$. Beginning at $t = 0$, we impose the boundary conditions $M = 0$ at $|\vec{r}| = a$ and $M = M_0$ for $r \to \infty$. For the 1D case considered here the solution of Eq. (3) is given by

$$M(y,t) = M_0 \,\mathrm{erf}\left[\left(\frac{y}{a} - 1\right) \Big/ 2\sqrt{Dt/a^2}\right]. \qquad (4)$$

From the experimental parameters used we calculate a theoretical signal-to-noise enhancement of ~10. This result, however, neglects T_1 effects. Inclusion of T_1 effects limits the expected enhancement [21]. For $T_1 = 1.17$ s (the measured value for our sample) the asymptotic limit is found to be $E \sim 8.64$.

The 2D diffusion enhanced MR images are shown in Figs. 4A and B. For comparison Fig. 4C shows a 2D image obtained using a regular spin-echo pulse sequence (the same data acquisition parameters TE and TR have been used and the slice thickness in this case is 1 mm). The displayed images are ZX plane slices with the Z-direction parallel to the applied magnetic filed. The Y direction is parallel to the axis of the plant stem. The image in Fig. 4A is obtained with a module consisting

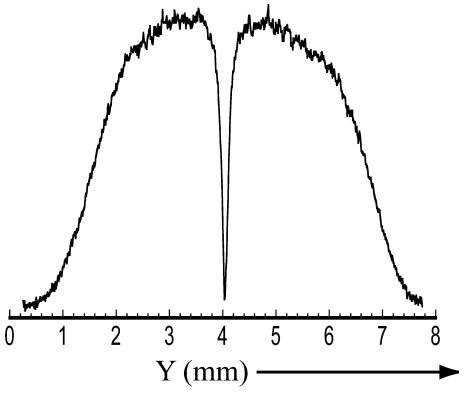

Fig. 2. 1D MR image of a plant stem showing the plane in which the magnetization was saturated. Data acquisition parameters: field-of-view 7.5 mm, number of complex data points 800, eight signal averages, TR = 2 s, TE = 14 ms, $G_{ss} = 4$ Gauss/cm, $t_y = 5.8$ ms, the total duration of the DESIRE prepulse was $t = 30 \times 30$ ms = 900 ms, saturation thickness ~100 μm.

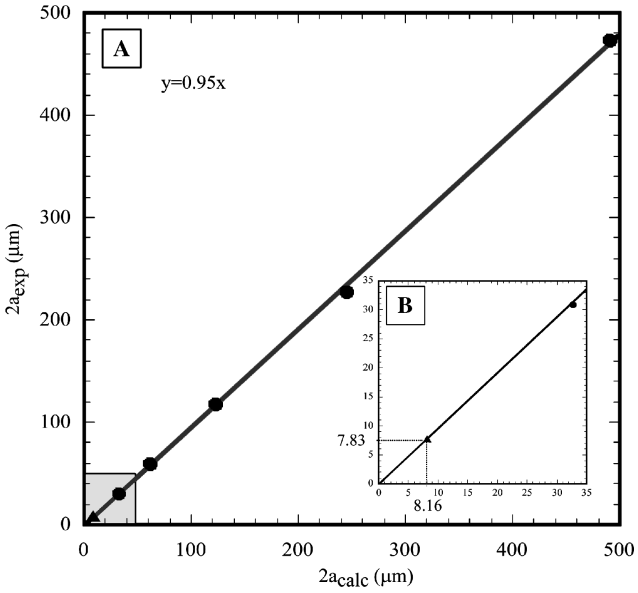

Fig. 3. (A) Comparison between the calculated slice thickness ($2a_{calc}$) and the experimentally measured slice thickness ($2a_{exp}$). The experimental values, are fitted by a straight line passing through the origin with slope = 0.95, and show a high correlation with the calculated values ($r^2 = 0.999$). ●, experimental points; ▲, extrapolated point. (B) An enlarged view of the region which contains the slice thickness of interest ($2a_{calc} = 8.16$ μm). By extrapolating we obtain $2a_{exp} = 7.83$ μm.

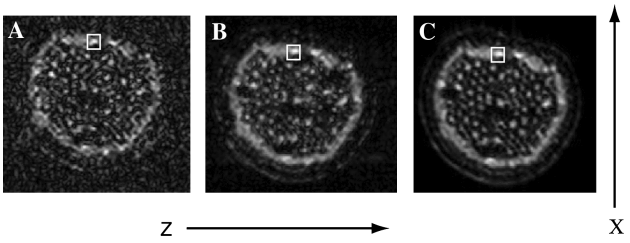

Fig. 4. DESIRE MR images of a plant stem. (A) 30 ms diffusion time (one localized $\pi/2$ pulse). Other data acquisition parameters: field-of-view 4×2 mm, data matrix 256×64, two signal averages, $G_{ss} = 4$ Gauss/cm TE = 11 ms, TR = 4.5 s, $G_y = 60$ Gauss/cm, $t_y = 5.8$ ms, resulting in a saturation thickness of ~ 8 μm. The SNR of the image, measured using the average signal in the region indicated by the box, was 2.06. (B) 900 ms diffusion time (30 $\pi/2$ pulses, 30 ms apart by). The SNR of the image was 16.3, giving an enhancement of 7.9 over the image in (A). (C) Conventional spin-echo image (1 mm thick slice). The SNR of the image was 210.

of only one $\pi/2$ pulse, while the image in Fig. 4B is obtained with a 900 ms long module, consisting of thirty pulses. The repetition time was 4.5 s, and the number of averages was two, resulting in a total experiment time of 10 min. The long repetition time was necessary only to avoid heating of the gradient coils. The in-plane resolution for all images is 31×31 μm. The SNRs of the images in Figs. 4A and B were 2.06 and 16.30, respectively, giving an enhancement of 7.9, significantly less than the value ~ 10 predicted in the absence of T_1 effects, and close to the T_1 asymptotic limit of E of 8.64. We notice that the theoretical value obtained for the enhancement ($E \sim 8.64$) is an excellent estimate. The slightly lower value obtained in practice could be due to the imperfection of the RF pulses and the inaccuracy in the estimation of the slice thickness. The SNR of the image in Fig. 4C, 1 mm thick slice, is 210. As expected, the SNR for the unenhanced, 8 μm saturated slice (Fig. 4C), is two orders of magnitude smaller. However, for the diffusion enhanced image (Fig. 4B) the SNR is only 12 times smaller.

4. Conclusion

This work demonstrates the feasibility of enhancing the signal-to-noise ratio and resolution in MR microscopy and localized NMR spectroscopy using a gradient–localized approach to DESIRE. We have produced MR images with a very small nominal saturation thickness (~ 8 μm) obtaining signal enhancements greater than a factor of eight. Out of the many possible DESIRE modalities, the particular one presented here might be quite useful in magnetic resonance histology (MRH) where MR images often need to be compared with very thin histological slides.

The image intensity obtained with the DESIRE sequence presented here is determined both by the proton density distribution in the saturated plane and the diffusion properties of spins within the image detection slice. In fact this unusual contrast mechanism is one of the potentially most interesting aspects of DESIRE. In conventional imaging sequences, diffusion contrast is represented by different degrees of *signal loss* within the imaging slice, whereas in DESIRE these are manifested by different degrees of *signal enhancement*. This should allow, in principle, extremely sensitive detection of regions of non-isotropic or restricted diffusion. In this paper, we used a simple plant stem sample which has extensive longitudinal symmetry, and unrestricted diffusion along the axis. Since the results obtained represent the first DESIRE images produced, the focus of the paper was to compare theoretical and experimental signal enhancements: in future publications we intend to explore image contrast in more challenging, heterogeneous systems.

A potentially mitigating factor in generating DESIRE images is that of magnetization transfer (MT). It is well known that MT effects are present in many standard imaging sequences in which slice-selective excitation and refocusing pulses can be considered as off-resonance pulses for neighboring slices. Since water associated with macromolecules has a very broad frequency spectrum, these effective off-resonance pulses saturate the macromolecular pool, which in turn exchange magnetization with the free water via dipole–dipole interactions, thus reducing the image intensity. In the DESIRE sequence the presaturation pulses are off-resonance for spins located outside the directly saturated plane but within the 1 mm thick detection slice. The degree of MT is a complicated function of pulse length and power, resonance offset, macromolecular content and rate of magnetization exchange, and is likely to be quite different for different samples. The literature places an upper bound on MT effects of $\sim 20\%$ for multi-slice imaging of biological samples, and $\sim 2\%$ for gels [22,23]. It should be noted that, whereas in multi-slice imaging MT reduces the signal intensity, in the DESIRE sequence any MT effect will actually cause greater enhancement. Since the enhancement achieved in our experiments was roughly 10% less than the theoretical value, we can conclude that MT effects for our particular data acquisition scheme and sample can probably be neglected. One possible experimental method to determine the degree of MT is to vary the thickness of the detection slice: the degree to which the enhancement factor decreases with slice thickness would potentially be an indication of the importance of MT.

Enhancements can be dramatically increased by saturating smaller regions and extending the method to locally saturating 2D and 3D regions. The task of generating an excitation locally in 2D or 3D can be accomplished using the $\vec{k}$-space approach to excitation first given by Pauly et al. [24] and more recently developed by

Stenger et al. [25,26]. Obtaining higher enhancements will require larger gradient strengths of 10's to 100's of T/m from systems which have been developed in the last few years [1,27].

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References

[1] S.-C. Lee, K. Kim, J. Kim, S. Lee, J. Yi, S. Kim, K. Ha, C. Cheong, One micrometer resolution NMR microscopy, J. Magn. Reson. 150 (2001) 207–213.
[2] L. Ciobanu, D.A. Seeber, C.H. Pennington, 3D MR Microscopy with resolution 3.7 μm by 3.3 μm by 3.3 μm, J. Magn. Reson. 158 (2002) 178–182.
[3] S.C. Grant, D.L. Buckley, S. Gibbs, A.G. Webb, S.J. Blackband, NMR spectroscopy of single neurons, Magn. Reson. Med. 44 (2000) 19–22.
[4] S.C. Grant, D.L. Buckley, S. Gibbs, A.G. Webb, S.J. Blackband, MR microscopy of multicomponent diffusion in single neurons, Magn. Reson. Med. 46 (2001) 1107–1112.
[5] L. Ciobanu, A.G. Webb, C.H. Pennington, Magnetic resonance imaging of biological cells, Prog. Nucl. Magn. Reson. Spectrosc. 42 (2003) 69–93.
[6] L. Ciobanu, C.H. Pennington, 3D micron-scale MRI of biological cells, Solid State Nucl. Magn. Reson. 25 (2004) 138–141.
[7] C.B. Ahn, Z.H. Cho, A generalized formulation of diffusion effects in μm resolution nuclear magnetic resonance imaging, Med. Phys. 16 (1989) 22.
[8] P.T. Callaghan, C.D. Eccles, Diffusion-limited resolution in nuclear magnetic resonance microscopy, J. Magn. Reson. 78 (1988) 1–8.
[9] B. Putz, D. Barsky, K. Schulten, Edge enhancement by diffusion in microscopic magnetic resonance imaging, J. Magn. Reson. 97 (1992) 27–53.
[10] B. Putz, D. Barsky, K. Schulten, Edge enhancement by diffusion-microscopic magnetic-resonance-imaging of an ultrathin glass-capillary, Chem. Phys. Lett. 183 (5) (1991) 391–396.
[11] W.B. Hyslop, P.C. Lauterbur, Effect of restricted diffusion on microscopic NMR imaging, J. Magn. Reson. 94 (1991) 501–510.
[12] P.T. Callaghan, C. Andrew, L.C. Forde, C.J. Rofe, Diffusive relaxation and edge enhancement in NMR microscopy, J. Magn. Reson. 101 (1993) 347–350.
[13] P.C. Lauterbur, W.B. Hyslop, H.D. Morris. NMR microscopy: old resolutions and new desires, in: XI International Society of Magnetic Resonance Conference, 1992, Vancouver, BC.
[14] H.D. Morris, P. Gorkov, F. Khan, J. Lyding, I. Adesida, P.C. Lauterbur, Diffusion mediated MRI signal amplification using microscopic rf coil, in: 37th Experimental NMR Conference (ENC), 1996, Pacific Grove, CA.
[15] H.D. Morris, P.C. Lauterbur. DEFT-Desire: an experiment to amplify small signal changes due to highly localized magnetic field gradients, in: 38th Experimental NMR Conference (ENC), 1997, Orlando, FL.
[16] C.H. Pennington, Prospects for diffusion enhancement of signal and resolution in magnetic resonance microscopy, Concept. Magn. Res. 19A (2003) 71–79.
[17] E.E. Sigmund, W.P. Halperin, Hole-burning diffusion measurements in high magnetic fields, J. Magn. Reson. 163 (2003) 99–104.
[18] Y.-Q. Song, Detection of the high eigenmodes of spin diffusion in porous media, Phys. Rev. Lett. 85 (2000) 3878–3880.
[19] M. Bendayan, J. Roth, A. Perrelet, L.J. Orci, Quantitative immunocytochemical localization of pancreatic secretory proteins in subcellular compartments of the rat acinar cell, Histochem. Cytochem. 28 (1980) 149–160.
[20] I.E. Dimitrov, S.R. Charagundla, R. Rizi, R. Reddy, J.S. Leigh, An MR imaging method for simultaneous measurement of gaseous diffusion constant and longitudinal relaxation time, Magn. Reson. Imag. 17 (1999) 267–273.
[21] W.B. Hyslop, Ph.D. Thesis in Department of Physics, 1998, University of Illinois at Urbana-Champaign.
[22] W.T. Dixon, H. Engels, M. Castillo, M. Sardashti, Incidental magnetization transfer contrast in standard multislice imaging, Magn. Reson. Imaging 8 (1990) 417–422.
[23] G.E. Santyr, Magnetization transfer effects in multislice MR imaging, Magn. Reson. Imaging 11 (1993) 521–532.
[24] J. Pauly, D. Nishimura, A. Macovski, A *k*-space analysis of small tip angle excitation, J. Magn. Reson. 81 (1989) 43–56.
[25] V.A. Stenger, F.E. Boada, D.C. Noll, Three-dimensional tailored RF pulses for the reduction of susceptibility artifacts in T*2-weighted functional MRI, Magn. Reson. Med. 44 (2000) 525–531.
[26] V.A. Stenger, F.E. Boada, D.C. Noll, Multishot 3D slice-select tailored RF pulses for MRI, Magn. Reson. Med. 48 (2002) 157–165.
[27] D.A. Seeber, J.H. Hoftiezer, W.B. Daniel, M.A. Rutgers, C.H. Pennington, Tri-axial magnetic field gradient system for microcoil magnetic resonance imaging, Rev. Sci. Instrum. 71 (2000) 4263–4272.