Contrast in NMR Imaging and Microscopy

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ABSTRACT

Nuclear magnetic resonance (NMR) imaging is increasingly being used for medical diagnosis and in scientific research and applications. In practice, it is often difficult to quantitatively relate the image intensity to the nuclear spin density, because other factors complicate a proportional interpretation. These factors include chemical shift, magnetic susceptibility variation, nuclear spin relaxation, and molecular motion (ordered flow and random Brownian motion). However, this kind of information is valuable in its own right and it can be quantitatively and accurately extracted from imaging experiments. This paper defines an image contrast as the image intensity change or modulation due to the contribution of molecular information other than the nuclear spin density. It is this kind of quantitative measurement that makes NMR imaging a useful tool for scientific research and practical applications. This article uses the concept of image contrast to summarize and describe various contrast mechanisms in NMR-imaging experiments.

INTRODUCTION

Nuclear magnetic resonance (NMR) imaging has proved exceptional in its ability to provide details of local molecular environments noninvasively. The first NMR image was published in 1973 (1), and its potential for medical diagnosis was documented immediately (2, 3). Whole-body scanners are now routinely used as indispensable tools for medical diagnosis (4–6). In addition to its widespread use in clinical medicine, NMR imaging is increasingly being used to measure such physical and biophysical phenomena as velocity and self-diffusion coefficients of fluid flows (7–15), in vivo vascular flow in botanical samples (16–19), and the spatial distribution of chemical species (20–26). With the scaling down of the receiver coil and the fine tuning of the instrument, resolution can be as fine as 10 μm³. Where the voxel resolution of the image is less than (100 μm)³, NMR imaging can be called NMR microscopy (27–30)—the human eye cannot resolve a volume element smaller than (100 μm)³. Although the proton (¹H) is still the most commonly used nucleus because of its sensitivity, imaging using other nuclei (¹⁷O, ¹⁹F, and ³¹P) has become possible.

In practice, it is often difficult to quantitatively relate NMR image intensity to nuclear spin density because other factors complicate a proportional interpretation. They include magnetic susceptibility variation due to the heterostructure of the sample, relaxation processes of the nuclei, chemical shift variations, and molecular motion (ordered flow and random Brownian motion). However, these kinds of information are valuable in their own right, and they can be quantitatively and accu-
ratey extracted from imaging experiments by incorporating appropriate image contrast schemes. This article defines an image contrast as the image intensity change or modulation due to the contribution of molecular information other than the nuclear spin density. Hence, an image contrast scheme can be referred to as a method to emphasize the contribution of molecular information through the intensity of the image. It is these quantitative measurements that make NMR imaging an exceptional tool in scientific research and practical applications.

This article summarizes the concepts of the image contrast and the techniques to extract contrasts in experiments. Rather than offering a comprehensive review of the literature and extensive derivation of equations, this article provides an overview of the topic, and interested readers can explore the concepts further by using the references given in the text and other literature. The article has three parts. The principles of NMR imaging and microscopy in the terminology of k-space are reviewed briefly. Then, various contrast factors in NMR-imaging experiments and contrast extraction techniques in NMR imaging are discussed. The last section is a further discussion of other contrast-based functions.

BASIC PRINCIPLES OF NMR IMAGING

The basic principles of NMR are well understood (31–37). At the heart of the technique is the linear proportionality between the nuclear Larmor frequency (ω) and the magnitude of the external magnetic field (Bo) in which the nuclei are immersed. In high-resolution NMR spectroscopy, specific efforts are made to improve the uniformity of the field over the sample space so that each chemically identical nucleus resonates at the same frequency. NMR Imaging

In NMR imaging, by contrast, a sample is placed in a magnetic field that has been deliberately made nonuniform. Thus, the Larmor frequency of the nuclear spins will differ from one location to another across the sample. The spatial position of a spin is, therefore, encoded by a precession frequency that has an additional term associated with the imaging gradient, as given by Eq. [1]:

\[ \omega(r) = \gamma B_0 + \gamma G \cdot r \]  

where \( \gamma \) is the gyromagnetic ratio of the nucleus, \( B_0 \) is the uniform polarizing field, and \( G \) is the applied linear field gradient vector.

To distinguish any spatial location in real space, three orthogonal gradients in the component of \( G \) parallel to \( B_0 \) are sufficient, namely \( G_x, G_y, G_z \). Conventionally, the direction of the polarizing field \( B_0 \) is defined as the z axis; hence, the gradient field is denoted as the variation of the polarizing field along three orthogonal axes, that is (Eqs. [2] and [3]),

\[ G = \nabla B_0 \]  

or

\[ G_x = \frac{\partial B_z}{\partial x} \]  

\[ G_y = \frac{\partial B_z}{\partial y} \]  

\[ G_z = \frac{\partial B_z}{\partial z} \]  

A one-dimensional (1D) example of such spatial frequency encoding is shown in Fig. 1; the magnetic field is varied linearly across the sample space. All the nuclei in the plane perpendicular to the field gradient will experience the same field strength and contribute to the signal amplitude at the same frequency. In other words, spatial displacements are turned into frequency displacements. The frequency spectrum therefore takes a form that represents the shape of the sample, known as the 1D image or projection profile.

The acquisition of the NMR signal occurs in the absence of the radio frequency (rf) field but in the presence of the applied mapping gradients. This signal is given by Eq. [4]:

\[ S(t) = \int_\rho(r) \exp[-i\omega(r)t]dr \]  

\( \rho(r) \) is the nuclear spin density, \( \omega(r) \) is given by Eq. [1], and the integral is over the defined three-dimensional (3D) space. In the heterodyne detection frame, where the reference frequency is \( \gamma B_0 \), Eq. [4] becomes Eq. [5]:

\[ S(t) = \int_\rho(r) \exp(-i\gamma G \cdot r)dr \]  

By defining a reciprocal space vector \( k \) (38) as
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Two dimensions by electronically selecting a two-dimensional (2D) slice through the sample. This strategy reduces the size of data array from $N^3$ to $N^2$ where $N$ is the number of discrete points to be sampled, and the result is consistent with the final 2D visual display on a computer screen. The means to selectively and noninvasively excite the nuclear spins within a thin slice is achieved by simultaneously using a narrow-band rf pulse and a gradient field in the direction orthogonal to the slice plane (imaging plane) (40), as illustrated in Fig. 2. Using linear response theory, in order to excite a rectangular slice in frequency domain, the time dependence of the rf pulse requires a sinc amplitude modulation because the rectangular function and the sinc function are a Fourier transform pair (41). However, it should be noted that the response of the nuclei spin system for large turn angles is highly nonlinear and can be described exactly only by solving the equation of motion of $M$ numerically (42, 43).

There are two basic parameters in slice selection: slice position and slice thickness. The slice position ($z$) is determined by the frequency ($\omega$) of the excitation field $B_x(t)$ with respect to the polarizing frequency ($\omega_0$) of $B_0$. If $\omega$ is identical
Figure 2  Means of slice selection in NMR imaging. The slice location is determined by the frequency offset of the rf field $B_1$ with respect to the Larmor frequency $\omega$ and the gradient strength. The slice profile is determined by the frequency profile of the rf excitation pulse. A rectangular slice profile can be obtained if the rf pulse is of the sinc form.

with $\omega$, the selected slice is at the center of the field. By changing the precession frequency of $B_1(t)$, the position of the imaging plane can be shifted along the gradient axis, as

$$z = (\omega - \gamma B_0)/\gamma G_z$$  \hspace{1cm} [9]

$G_z$ is the slice selection gradient along the z axis. The width of the selected slice is determined by the bandwidth of the $B_1$ field, $\Delta f$, and the magnitude of the gradient, as

$$\Delta z = \Delta \omega/\gamma G_z$$  \hspace{1cm} [10]

$\Delta \omega$ is equal to $2\pi \Delta f$.

In a 2D imaging experiment, a transverse plane is selected that is normal to the direction of the slice selection gradient. In this transverse plane, the direction of $k$-space is determined by the direction of the mapping gradient, but the magnitude of traverse in $k$-space can be determined by changing either the application time of the gradient or the gradient magnitude. The initial sampling point defines the origin of $k$-space. From then on, its position is determined by the time effect of the mapping gradients. There are several ways the entire $k$-space can be sampled. It is the method of $k$-space traverse that results in different NMR imaging techniques (44). Two common examples are given in Figs. 3 and 4: Cartesian raster sampling and polar raster sampling.

Two-dimensional Fourier imaging (45) and spin-warp imaging (3, 46) use Cartesian raster sampling. A pulse sequence of spin-warp imaging is shown in Fig. 5. The two transverse mapping gradients in the pulse sequence are commonly called the phase gradient and the read gradient. The phase gradient is applied using a fixed time, and its magnitude is stepped to provide different phase offsets in $k$-space. The read gradient is applied to advance the sampling point along the read direction in $k$-space during the signal sampling.

The filtered back projection-reconstruction (PR) sequence (1), shown in Fig. 6, uses a polar sampling raster (Fig. 4) and is based on the well-established algorithms used in X-ray tomography (47). In the spatial mapping period, the two transverse mapping gradients are applied simultaneously, following the cosine and sine relationships, respectively. The back-projected image is then interpolated into a Cartesian matrix to form the final 2D image.

The PR method is straightforward. It can be designed to achieve a short "dead time" before
sampling and therefore it is among the most sensitive of imaging methods (35, 48). There are, however, some disadvantages. One of them is due to the polar raster sampling, where the inner parts (which correspond to lower frequency components) are sampled more densely than is the outer region (which corresponds to higher frequency components). This implies that the fine details are more poorly represented than are the coarse features in the images using PR method. By comparison, the commonly used spin–warp sequence offers the same resolution for high and low frequencies, but it suffers some signal loss and unwanted $T_2$ contrast during the phase-encoding period. Interest in the PR method has increased in recent years because many materials, such as biological tissues and porous media, have short relaxation times (49, 50) so a shorter dead time is advantageous.

**S/N, Resolution, and Limits**

Nuclear magnetic resonance is an insensitive technique, the signal induced by the transverse magnetization in the receiver coil being on the order of several microvolts. This rather weak signal is superposed on the electronic noise in the instrument. It is the ratio of the available signal to noise ($S/N$) that is important in any experiment, and this ratio is a limiting factor in NMR microscopic imaging. At a given temperature, it can be shown (51) that the $S/N$ in an NMR experiment depends on $(\omega_0)^{7/4}$. Therefore, the signal can be maximized by using a large magnetic field and by using the nucleus with the largest possible magnetogyric ratio, the proton.

Sources of noise in NMR experiments can be numerous. In a well-designed instrument, the probe and the rf preamplifier obviously determine overall noise, and hence sensitivity. The fundamental thermal noise power for an rf coil arises from the random thermal motion of electrons in the wire of the receiver coil (Johnson noise) and cannot be avoided. By reducing the operating temperature, the overall thermal noise of the receiver coil–preamplifier can be reduced (52–54). The signal amplitude in NMR imaging, on the other hand,
(a) Pulse sequence to traverse in polar raster for the first quadrant

(b) Sampling the first quadrant

(c) 2-D Polar raster

**Figure 4** Polar raster sampling in 2D k-space imaging. The pulse sequence in (a) samples the first quadrant (b) in k-space. By changing the signs of the mapping gradients, the entire 2D k-space can be sampled in a polar raster (c).

**Figure 5** Pulse sequence of 2D spin-warp imaging. The leading gradient pulse in the read direction is used to delay the formation of the spin echo signal so that a full echo can be sampled. A gradient echo approach is used in the slice selection. The functions of the phase and read gradients are explained in Fig. 3.

is proportional to sample volume. As the image voxel size decreases, the number of spins contributing to the signal in the voxel also decreases, as does S/N. Thus, a minimum acceptable S/N corresponds to a minimum voxel resolution (51).

Sensitivity provides the first-order limitation to S/N and resolution in NMR microscopy. There are other factors that also limit S/N and resolution (55, 56). For example, $T_2$ relaxation gives a natural NMR linewidth in any sample, susceptibility of a heterogeneous sample degrades the linearity of the imaging gradient, and chemical shift perturbs the natural linewidth. For liquid or liquidlike samples that contain moving molecules, such as water solutions, polymer solutions, and biological samples, the self-diffusion of molecules sets an intrinsic lower limit to the resolution. This is because the 1D root mean square distance that molecules move under Brownian motion is $(2Dt)^{1/2}$ where $D$ is the self-diffusion coefficient of the molecules and $t$ is the observation time. Hence, Brownian motion leads to an irreversible loss of the echo signal (57).
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Figure 6 Pulse sequence of 2D back projection imaging where a half-echo signal is sampled (sampling starts at the center of the echo). A spin–echo approach is used in the slice selection. The functions of the two transverse mapping gradients are explained in Fig. 4.

Figure 7 shows a resolution scale in NMR imaging. The resolution in medical MR scanners is typically about 1 mm$^3$, limited largely by such practical factors as electronics, computer capability, and imaging time. NMR microscopy covers the resolution range from (100 μm)$^3$ down to about (1–10 μm)$^3$, limited in principle by the self-diffusion coefficient of the molecules. Between these two extremes, small-scale imaging has found itself in recent years increasingly important in scientific research and practical applications.

**IMAGE CONTRAST FACTORS AND THEIR EXTRACTION**

One conundrum in NMR imaging and microscopy concerns the relationship between the true nuclear spin density in the sample and the apparent density as indicated by the proton image intensity. It is well known that the relative water proton image amplitude can be highly inconsistent with known water content in different physical regions of a biological sample (18). This discrepancy could have several origins. The most common explanation is the inevitable $T_2$ contrast arising from the finite echo time $T_E$. This time delay can be reduced to some minimum value—a few milliseconds—but it is never possible to entirely eliminate it. In consequence it is impossible to measure $\rho(r)$ directly.

**Image Contrasts and Their Extraction**

Generally speaking, the time sequence of any imaging experiment consists of two phases, the preconditioning period and the imaging period (58). (This is true even when one cannot clearly divide a pulse sequence into the two phases.) The preconditioning period provides the desired image contrast, and the imaging period is responsible for the spatial resolution and differentiation of the local structure. If no specific contrast scheme is used, a “normal” spin density map will be obtained, although strictly speaking, all images are more or less weighted by the influences of several intrinsic contrast factors. In the previous discussions of imaging processes expressed in Eq. [7], the influences of contrast factors on the image intensity were ignored for the sake of simplicity. In practice, instead of Eq. [7], the relationship between the signal and the nuclear spin density in a NMR imaging experiment can be written as (Eq. [11])

$$S(k) = \int \rho(r)E_c(r)\exp[-i2\pi k \cdot r]dr \quad [11]$$

$E_c(r)$ is the normalized and combined contrast factor.

The usual image reconstruction returns not simply $\rho(r)$ but $\rho(r)E_c(r)$. The interest in the image contrast factors is twofold: First is the possible reduction of image artifact due to these contrasts; second is the potential information obtainable via the contrasts. This article discusses the latter interest. In general, $E_c(r)$ is a combined factor that could be a product of several contrasts, such as (Eq. [12]):

$$E_c(r) = \text{combined contrast factor}$$

![Figure 7 Resolution scale in NMR imaging.](image-url)
Table 1  Conventional vs. Contrast Imaging

| Conventional Contrast factor | FT[k] | S(k) ⇔ p(r)
|-------------------------------|-------|-------------------
| Contrast extraction E,(r) w contrast factor | FT[k] | S(k) ⇔ p(r)E,(r) |
| E,(r) = E,(T1)E,(T2)E,(δ)E,(q) [12] |       |                   |

where E,(T1) is the contrast factor representing the effect of the spin–lattice relaxation, E,(T2) is the contrast factor representing the effect of the spin–spin relaxation, E,(δ) is the contrast factor representing the effect of chemical shift, and E,(q) is the contrast factor representing the effect of molecular motion (velocity v and self-diffusion D). By choosing appropriate pulse sequences and parameters, one can emphasize the contribution of one particular contrast and find a way to extract it from experiments (Table 1).

There are two basic approaches by which the image contrast can be studied (Table 2). The first makes the pulse sequence sensitive to a particular contrast so that the image intensity bears or is weighted by the influence of that contrast. This approach is widely used in medical diagnosis, where, for example, a relaxation-weighted image is often used to study tissue abnormality. This approach offers the advantages of straightforwardness and time efficiency. However, it offers only a qualitative solution, and although it has been used extensively in medical research and diagnosis, its application at a microscopic resolution should be handled with more care. This is because, in comparison with the medical and small-scale applications, NMR microscopy requires a much stronger gradient G and often uses a much higher polarizing field Bo. The image intensity at high spatial resolution also can be strongly influenced by other factors, such as susceptibility variation due to structure inhomogeneity in the sample.

The second approach in the study of image contrast is to obtain the information quantitatively. There are three experimental schemes by which the contrast information can be extracted: the one-shot scheme, the two-image scheme, and the multiple-slicing scheme. In the one-shot scheme, the final image is directly proportional to the particular contrast studied. The pulse sequence must be made so that only the spins responsible for a particular contrast produce the final signal. For example, a time-of-flight imaging pulse sequence can be made to “sense” only the moving spins so that the resulted image is a map of velocity (59); a chemical-shift-imaging sequence can be designed to select just one resonant frequency.

The two-image scheme is probably the most commonly used approach. It requires two imaging experiments under two contrast conditions. The final image is computed from the two images, pixel by pixel, by means of some mathematical calculation, such as subtraction, division, or trigonometric operation. Examples of this scheme include imaging translational flows and Brownian motion (18, 60).

The multiple-slicing scheme involves acquiring multiple images under different contrast conditions. These conditions represent a progressive amount of particular contrast along a contrast direction, as illustrated in Fig. 8. Therefore, each 2D image merely represents a slice along that particular contrast direction (each image is contrast-weighted, as in the first approach). Subsequent analysis can be carried out, pixel by pixel, using that series of images. At each pixel location of the 2D image, a 1D data array can be formed with the array elements being the pixels along that contrast direction. By using the appropriate algorithm corresponding to that particular contrast, two-dimensional maps of the contrast information can be computed (7, 11, 61). Table 2 compares the four contrast extraction schemes.

Comparing the three different schemes, the one-shot scheme is obviously the most time-
Contrast-weighted 2-D 'raw' images

Appropriate Analysis on pixel-by-pixel basis

Contrast Image
\( (v, D, T_1, T_2) \)

Figure 8  Multiple-slicing approach in contrast extraction. The algorithms in the final calculation could be Fourier transform, least-squares fit, or moment method. Acquiring a complex data image enables the extraction of phase information (such as in a velocity measurement).

Relaxation Contrast

Two intrinsic time constants, \( T_1 \) and \( T_2 \), are used to describe the evolution of a nuclear spin system after being disturbed from its thermal equilibrium (a vector quantity \( M_0 \) along the \( z \) axis). \( T_1 \) is known as the spin–lattice (or longitudinal) relaxation time because the process involves an energy exchange between the spin system and its surrounding thermal reservoir. This time constant describes the return of the disturbed magnetization to its thermal equilibrium along \( B_0 \). The \( T_1 \) in liquids is dominated by high-frequency molecular tumbling motion, and it is usually several seconds long. \( T_2 \) is called the spin–spin (or transverse) relaxation time because it describes the decay in phase coherence between the individual spins in the transverse plane, resulting in signal loss. This decay in phase coherence can arise from external sources, such as a magnetic field inhomogeneity or a gradient, or from low-frequency molecular motion. \( T_2 \) in biological tissues is usually in the range of tens or hundreds of milliseconds.

\( T_1 \) and \( T_2 \) are among the contrasts that most often influence image intensity. The relaxation contrasts are present intrinsically in all NMR imaging experiments, and they are the primary factors that influence the image intensity. Although such contrasts are undesirable in the interpretation of nuclear spin density, they are useful in their own right. For example, \( T_1 \) varies greatly between different biological tissues or between normal and abnormal tissues, so that a knowledge of \( T_1 \) distribution is extremely useful in the differentiation of biological soft tissue.

\( T_1 \) contrast in an imaging experiment occurs when the interval between successive signal averages (\( T_R \), repetition time) is not long enough for the spin to fully recover its equilibrium. (If \( T_R \) is three times as long as \( T_1 \), the signal will recover to 95% of the signal intensity before the next pulse.) Hence, \( T_1 \) contrast can be incorporated into the
imaging experiment simply by repeating experiments at different $T_R$. The image contrast function is then given approximately by

$$E_c(T_1) = 1 - \exp(-T_R/T_1(r)) \quad [13]$$

where $T_1(r)$ is written merely to emphasize that $T_1$ is a function of the position in the sample.

During signal acquisition, the finite echo time between excitation and signal acquisition is often termed $T_E$. If $T_E$ is long compared with the $T_2$ of the sample, $T_2$ contrast will occur. By repeating the imaging experiment at a fixed $T_R$ but with different $T_E$, a $T_2$ relaxation map can be obtained with an image contrast function as

$$E_c(T_2) = \exp(-T_E/T_2(r)) \quad [14]$$

provided that $T_R$ is larger than $T_1$ to ensure that the $T_2$ contrast is dominant.

Subsequent calculation of $T_1$ and $T_2$ contrast can be carried out by employing either Eq. [13] or Eq. [14]. In these analyses, it also might be possible to obtain a corrected proton density image, $\rho(r)$, by extrapolating back to zero delay ($T_E = 0$, meaning no signal loss due to $T_2$) and infinite repetition time ($T_R = \infty$, meaning that the magnetization vector is fully restored along the $B_0$ direction) (62). This additional information could be important in NMR microscopy of biological samples because of the heterogeneity of the tissues. Because the physical boundaries between regions of differing susceptibility can strongly perturb the local magnetic field uniformity, severe relaxation contrast in submillimeter scale could alter the appearance of experimental proton maps.

Note that in the above discussion of $T_2$ extraction, we have simply repeated the imaging sequence at different $T_E$. This approach is generally valid as long as the diffusion attenuation due to the imaging gradients doesn’t play an important part in the signal attenuation. As pointed out recently by Haase et al. (63), an increase of $T_E$ normally increases the interval between the slice or mapping gradient pulses, as shown in Fig. 9. This gradient pair could act like a pulsed-gradient spin-echo (PGSE) gradient, resulting in an inaccurate $T_2$. (The next section details the effects of the PGSE sequence.) This situation is the worst in NMR microscopy because a large mapping gradient is required. Special care should be taken to ensure that the increment of $T_E$ doesn’t increase the spacing of gradient pulses.

**Flow and Diffusion Contrast**

Molecular translational motion occurs when liquid or liquidlike samples, such as water solutions, polymer solutions, and biological samples, contain moving molecules. Because self-diffusion of molecules leads to an irreversible loss of the echo signal (57), the random Brownian motion, self-diffusion ($D$), of molecules is important in NMR microscopy. Another type of motion of interest is the macroscopically ordered molecular motion, fluid flow ($v$). A molecule with a velocity $v$ will move to a new position over a specific time interval in the image pulse sequence. Hence it also leads to signal loss. Both motions can provide unique information about the sample, because velocity is associated with mass flow of the fluid and the self-diffusion coefficient reflects the microscopic structure of molecules and their surroundings.

To probe the motion of nuclear spins using MRI, the usual imaging sequence needs to be made motion sensitive (64, 65). The most precise and sensitive motion contrast algorithm is the narrow PGSE sequence (66, 67). Figure 10(a) shows the classical form of the PGSE sequence, where the dephasing and refocusing of the nuclear spins occur in two strong and identical gradient pulses. In the presence of the first gradient pulse, any phase shift that depends on the position of each nucleus is recorded. Between the pulses the molecules containing the nuclei keep changing their positions because of self-diffusion or flow. After
(a) Pulsed-Gradient Spin-Echo sequence

![Diagram of Pulsed-Gradient Spin-Echo sequence]

(b) Imaging pulse sequence in Dynamic NMR Microscopy

![Diagram of Imaging pulse sequence in Dynamic NMR Microscopy]

**Figure 10** PGSE pulse sequence in NMR imaging. (a) The PGSE sequence records the motion of the spins. (b) The pulse sequence used in dynamic NMR microscopy that combines the PGSE sequence with the 2D imaging sequence.

Following a similar approach used before in conventional MRI, a dynamic reciprocal space vector \( \mathbf{q} \), can be defined as

\[
\mathbf{q} = (2\pi)^{-1} \gamma g \delta
\]  

[15]
$g$ is the PGSE gradient (a vector quantity), and $\delta$ is the duration of the PGSE gradient pulses. When the gradient pulse width is narrow, and both translational flow and random Brownian motion occur, the result can be derived as a simple product of a phase shift term and an amplitude attenuation term $\left(7,11\right)$, as

$$E_x(q) = \exp(i2\pi q v \Delta) \exp(-4\pi^2 q^2 D \Delta) \quad \left[16\right]$$

$v$ is the velocity in the direction of $q$, $D$ is the self-diffusion coefficient, and $\Delta$ is the separation of the two PGSE pulses.

Figure 10(b) shows an imaging pulse sequence by which fluid flow and Brownian motion can be measured simultaneously. The first and the last of the three parts in the sequence correspond to the slice selection and the imaging segments of the pulse sequence shown in Fig. 6, providing the spatial resolution in three dimensions. The contrast period of the pulse sequence corresponds to the PGSE sequence shown in Fig. 10(a). Although filtered back projection is used as the imaging method in Fig. 10(b), the flow and diffusion contrast is equally applicable to reconstruction by the spin–warp imaging method. In the contrast period, a single dimension in $q$-space is chosen so that the overall imaging process is four dimensional. The method can be easily generalized to two or three dimensions in $q$-space, although this leads to excessively long experiments because of the low sensitivity associated with the NMR method and the consequent need for signal averaging.

The direction in which self-diffusion and flow is measured is determined by the direction in which $g$ is applied. The amplitude of $g$ determines the $q$-vector amplitude, $q$. If the dynamic dimension conjugate to $q$ is defined as $Z$ and the relevant components of the diffusion tensor and the velocity vector in the $Z$ direction are $D$ and $v$, $E(q)$ has the form of an oscillatory function of $q$ modulated by a gaussian decay, given by Eq. [16]. Imaging experiments are carried out with the $q$-contrast gradient being successively stepped in a number of discrete steps to some maximum value $g_m$. Each step corresponds to a slice in $q$-space, where one pair of complex images (one in phase and one quadrature phase) is reconstructed using the normal $k$-space reconstruction algorithm. Each image 'slice' in $q$-space is weighted progressively by $E_x(q)$ as the stepping up of $g$. Subsequent analysis along the particular $q$ direction using the multiple $q$-contrasted slices produces comprehensive information about molecular motion at each pixel of the image. There are several approaches $\left(68-70\right)$ by which such analysis can be carried out automatically. Figure 11 shows one example of this multiple-slicing technique in the measurement of velocity in an abrupt stepped tube. Computer simulation also has been used to predict the results of the experiment. The agreement is excellent $\left(14\right)$.

**Chemical Shift Contrast**

Chemical shift is the most common parameter used to identify the structure of molecules. For

![Figure 11](image)

**Figure 11** Velocity imaging of water flow through an abrupt expansion at constant speed. The stepped tube was made using two pieces of glass capillary; internal diameter 2.9 mm and 1.8 mm, respectively. Eighteen pairs of complex images were acquired at an array size of $64 \times 64$. Both the axial and the radial components of the velocity vector were imaged. Self-diffusion maps also were constructed simultaneously with the velocity maps. (a) Axial velocity map at the junction of the abrupt expansion. (b) Axial velocity profile through the center of the tube at the junction of the abrupt expansion. The solid line in (b) gives the theoretical velocity profile of Navier–Stokes equations obtained by finite difference numerical methods.
$^{1}$H NMR, the reference is commonly TMS, (CH$_3$)$_3$Si, and the proton in free water has a chemical shift around 4.7 ppm (33). In imaging experiments that use homogeneous water samples, however, this proton chemical shift is simply ignored because it represents zero displacement of the image object if the resonant frequency is exactly at the water frequency. If a sample contains more than one chemical species, however, the situation will be different. This is because, in our analysis so far, we have assumed that whenever there is no field gradient, the frequency domain signal of the sample is a single peak (only the first term in Eq. [1] exists). In case of two chemical environments, the direct relationship between the resonant frequency and the spatial position, given by Eq. [1], becomes

$$\omega(r) = -\gamma(B_0 + G \cdot r + \delta_i B_0 \times 10^{-6})k, \quad (i = 1, 2) \quad [17]$$

$\delta_i$ represents two different chemical shifts, in this case. Without the middle term, Eq. [17] is the precise equation in NMR spectroscopy used to investigate the chemical shift information. Without the last term, on the other hand, Eq. [17] becomes the standard relationship in imaging experiments governed by Eq. [1]. With all three terms, a simple imaging method will reconstruct two position-shifted images simultaneously. Provided the spectrometer has high enough resolution, the final image is a superposition of these two images and hence an image artifact. A well-known example is the artifact from fat, whose chemical shift is about 3 ppm away from that of water, as shown in Fig. 12.

Often, however, the chemical shift contains valuable information and techniques have been developed to obtain this information from the NMR image (71). The most comprehensive approach to chemical shift imaging is the chemical-shift-imaging (CSI) technique (20, 72), in which the frequency spread due to the chemical shifts becomes an extra dimension in the imaging experiment. Therefore, the ultimate form of the digital array in CSI is four-dimensional: three spatial (x,y,z) and one chemical ($\delta_i$). We can write the contrast factor in CSI experiments as

$$E_c(r, \nu) = \int g(r, \nu) \exp[i2\pi\nu t] d\nu \quad [18]$$

where $g(r, \nu)$ is the spectral profile function at r. The exact function of $g(r, \nu)$ can be written down only in very few cases. For example, for spectral peaks from a sample containing simple liquids, $g(r, \nu)$ bears a Lorentzian shape. In most cases, we use Fourier transformation to find out the line shape of $g(r, \nu)$.

A comprehensive chemical shift imaging (CSI) experiment can be very time consuming because the frequency spread due to the chemical shifts becomes an extra dimension in the imaging experiment. Although four-dimensional CSI experiments have been carried out successfully (73), one often must reduce the digital array size or the number of dimensions—or both—so that the experiment can be completed within reasonable limits of experimental time and computer capability. There are two extremes in the data size and dimension reduction in CSI experiments. One extreme is to reduce the spatial dimension to a single voxel (the region of interest, ROI) but to obtain the full chemical shift information from the selected volume. Examples of such an approach include ISIS (24), DRESS (74), and SPACE (75). This volume-selected spectroscopy approach, which is no longer a true imaging experiment, is useful for medical diagnosis and in vivo biological studies, but it requires a prior knowledge of the ROI. The alternative extreme is to reduce the observation window on the chemical shift spectrum to a single shift (the shift of interest, SOI) while retaining the spatial information of the image. The chemical-shift-selective (CHESS) imaging technique (21, 76, 77) is an example of an approach that produces an entire image of a preselected chemical shift component in the sample. CHESS imaging is much more straightforward in implementation and less time consuming than a full-feature CSI, if one knows a priori, which chemical shift to monitor.

**CONTRAST-DEPENDENT FUNCTIONS**

The contrast factors discussed above can be considered primary because each of them can be included directly into the Bloch equation. For example, the self-diffusion term can be included as the Bloch–Terry equation (78). However, image intensity also can be affected by other functions or parameters, such as magnetic susceptibility or temperature. These influences are secondary because they act via the effects of the primary contrast factors discussed previously. Spatially resolved images of these functions or parameters also can be constructed in a similar manner, as we have discussed previously, with the multiple-slicing technique, at a cost of longer experimental time.
Susceptibility

Although the external origin of the magnetization $M$ is the applied magnetic field, its intrinsic origin is magnetic susceptibility, $\chi_m$, because

$$M = \frac{\chi_m}{\mu_0(1 + \chi_m)} B$$  \hspace{1cm} [19]$$

$\mu_0$ is the absolute permeability ($4\pi \times 10^{-7} \text{ H m}^{-1}$). $\chi_m$ is a dimensionless number, positive for the paramagnetic materials and negative for diamagnetic materials (biological and organic materials).

Because the sample being imaged is heterogeneous, $\chi_m$ will depend on spatial position. At the boundaries of local internal structures among themselves or with air, a difference in two neighboring $\chi_m$—even just a few parts per million—will induce a local magnetic field gradient across the boundary. These induced internal magnetic field gradients will degrade the homogeneity of the applied field, not only at the boundaries but also in the space near the boundaries. This means that the susceptibility differences are local at the boundaries, their effects are distant into the nearby media. As a consequence, additional $T_2$ is induced in these regions, which results in image distortions and artifacts.

Because $\chi_m$ is about $-10^{-3}$ for most of the diamagnetic materials, it has a very small effect.
Nonetheless, the effect of magnetic susceptibility is field dependent. The higher the field, the bigger the effect. Simulations have shown that dramatic image distortions could be induced even if the susceptibility difference is only 3 ppm at a 20 kHz receiver bandwidth (29). Clearly, the image distortion is worst at microscopic resolution (79). An example is shown in Fig. 13, where the severe image distortion is caused by the interface between water and air.

**BOLD Contrast**

BOLD stands for blood oxygenation level dependent. The mechanism of the BOLD contrast is the following. By giving up oxygen, the hemoglobin iron in blood undergoes a change in spin state from diamagnetic low spin in the oxygenated state to paramagnetic high spin in the deoxygenated state. The increase in paramagnetic iron content in blood increases the difference in bulk magnetic susceptibilities among the blood and the surrounding tissue. Hence the coherence in NMR signals decays quickly.

Early studies (80) showed that the BOLD contrast in proton NMR depends strongly on the $T_2$ of the blood water, and the content of paramagnetic deoxyhemoglobin in red cells is responsible for the $T_2$ change. In NMR imaging experiments, however, blood water in microvessels is often difficult to image in soft tissue, such as in the brain. To enhance the effect of the BOLD contrast, some recent work has explored the effect of $T_2^*$ by using gradient echo imaging at high magnetic fields (up to 7 Tesla) (81-83). The work shows that, in addition to contributions from the blood water, the effect of BOLD contrast can be enhanced by including contributions from the perfused tissue water around the blood vessels in gradient echo experiments. In other words, whereas a spin echo signal is attenuated only by the effect of $T_2$, a gradient echo is additionally attenuated by dephasing because of microscopic static field inhomogeneities. Because of the extensive network of blood microcapillaries in brain tissue, the imaging signal from the brain reflects blood oxygenation via the enhanced BOLD contrast.

The blood oxygenation level varies when there is a change in the dynamic equilibrium between the normal supply and demand of oxygen to the brain tissue. A change in the demand can come from an increase of the neuronal activity in the brain, which could reflect some external visual or audio stimulation or some repetitive motion (83-86). Therefore, BOLD contrast can be used in functional brain imaging studies by MRI without administered contrast agents. Functional brain-imaging usually requires two imaging experiments with different stimulating conditions. A pixel-by-pixel image subtraction or division returns a net change of the brain activity. This type of image processing, as discussed previously, requires very high S/N with good and stable system hardware performance.

**Temperature**

Early development of imaging temperature distributions using NMR was motivated by the clinical application to hyperthermia cancer therapy during which cancerous tissue is targeted to be heated to just over 42 °C while the surrounding healthy tissue is kept to a temperature of less than 40 °C. Therefore a noninvasive temperature mapping technique could provide a monitoring tool for the efficacy and safety of the therapy. One MRI monitoring technique uses a fast-imaging technique to guide thermal surgery so that thermal profiles of the heated tissue can be imaged before the tissue is cooled (87). Recently, temperature-mapping studies have been extended to nonbiological materials, such as solids and polymers. In addition to temperature, other thermal phenomena also can be mapped via temperature dependency. For example, the mapping of the Rayleigh convection has been demonstrated using a flow-sensitive imaging sequence (88).

The most versatile NMR temperature-imaging scheme uses the self-diffusion of molecules to measure random thermal motion. Water self-diffusion measurements have been published (89) to show that every 1 °C temperature change will result in approximately a 2.4% change in the self-diffusion coefficient at room temperature. The theoretical relationship of diffusion vs. temperature can be derived from the Stokes–Einstein equation, which relates viscosity to translational self-diffusion. Assuming the activation energy $E_a$ is a constant, the Stokes–Einstein equation can be differentiated (90, 91) to give

$$\frac{D_T - D_0}{D_0} = \left(\frac{E_a}{kT_0}\right) \left(\frac{T - T_0}{T_0}\right)$$

$D_T$ is the measured self-diffusion coefficient at the measurement temperature $T$ (in Kelvin), $D_0$ is a reference diffusion coefficient at the reference
Figure 13  Effect of susceptibility artifact. The sample is water in 4 mm NMR tube. The length of the water is short compared with the height of the rf coil. The four images were acquired with the same sample location, same pulse sequence, and same parameters but different gradient directions, at 360 MHz proton frequency. The slice is selected across the middle of the NMR tube. (a) The phase gradient is $x$, pointing vertically upward; the read gradient is $z$, pointing to the right. (b) The same as (a), except the read gradient points to the left. (c) The phase gradient is $z$, pointing vertically upward; the read gradient is $x$, pointing to the right. (d) The same as (c) except the phase gradient points downward. The two profiles above (a) and (b) are drawn through the centers of (a) and (b), respectively. The artifacts result from the meniscus of water: The local gradients resulting from the susceptibility difference at the water–air interface distort the linearity of the imaging gradients.
CONTRAST IN IMAGING AND MICROSCOPY

Temperature $T_0$, $E_a$ is the activation energy that measures the amount of energy required to break hydrogen bonds, and $k$ is the Boltzmann constant. Note that $(kT)$ is about $4.41 \times 10^{-21}$ joule at room temperature, and $(E_a/kT)$ is a factor that determines the sensitivity of the temperature measurement using self-diffusion. For a series of multiple-imaging experiments, the factor $(E_a/kT)$ can be normalized so that the temperature can be mapped out. A phantom study using polyacrylamide gel and the two-image approach has shown that a 0.2 °C temperature difference can be determined over 0.3 cm$^3$ regions (92).

Two other NMR contrast factors, relaxation time and chemical shift, also show temperature dependence. Because nuclear spin relaxation is caused by the distributions of local interactions in their environment experienced by the nuclear spins, both $T_1$ and $T_2$ exhibit temperature dependence. For pure, degassed water, the $T_1$ value changes at a rate of about 100 ms/°C between 0 and 100 °C (90), and the $T_2$ value changes at a rate of about 74 ms/°C in the range of 30–50 °C (93). Temperature mapping using relaxation has been demonstrated in agar phantom, in polymers, and in biological samples in vivo (94–96).

The temperature dependence of chemical shift (97) results from the temperature dependence of the nuclear screening constant. An increase in screening decreases the effective external magnetic field. Early experiments demonstrated the temperature dependence of chemical shift for a variety of well-characterized compounds, such as water, methanol, and ethylene glycol (93, 98). For example, the OH peak of ethylene glycol shifts toward the methyl peak at a rate of about 2 Hz/°C between 300 K and 350 K at 300 MHz proton frequency (93). This temperature-dependent frequency shift can be detected either directly by a chemical-shift-selected imaging sequence (98) or through the additional phase shift in a gradient echo sequence (99).

Of the three schemes that monitor temperature, the self-diffusion scheme using the PGSE sequence is probably the most sensitive and reliable, with good linearity and simple interpretation. Furthermore, the temperature-sensitive phenomenon based on the self-diffusion measurement is almost universally applicable. In liquid or liquidlike systems at room temperature, the diffusion scheme can be modeled using the Stokes–Einstein equation. For solid or solidlike materials, such as some polymers, other theoretical models must be developed. In comparison, the chemical shift scheme and the relaxation scheme lack universal applicability. The relaxation scheme also suffers from several complexities. For example, the relaxation mechanism is relatively complex; the $T_1$ values are field dependent; the measurement of relaxation times is susceptible to instrumental imperfections, such as rf field inhomogeneity. The chemical shift scheme also is limited because it requires either a simple spectrum with narrow linewidth or distinct chemical shift peaks associated with the heated and unheated regions. Therefore, the chemical shift scheme has limited application in in vivo studies in which broad and complicated peaks are common.

SUMMARY

This article describes NMR imaging and microscopy and its applications with an emphasis on the image contrast extraction. Fig. 14 summarizes the relationships. Although the spatial resolution and the cost of the NMR imaging and microscopy might not be able to compete directly with other imaging techniques, such as optical microscopy, its value lies in its total noninvasive nature and in its rich contrasts.

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Figure 14 Summary of image contrasts. The lines relate some of the relationships we have discussed in this paper. In addition, one can also combine two different contrasts into one experiment, such as to image the velocity of a particular component that has been selected via the relaxation or chemical shift mechanism before the onset of the velocity-imaging sequence.
images shown in Fig. 11 were acquired at Paul T. Callaghan’s laboratory (Physics and Biophysics Department, Massey University, Palmerston North, New Zealand). The images shown in Fig. 12 and 13 were acquired at Lynn W. Jelinski’s laboratory (Center for Advanced Technologies in Biotechnology, Cornell University, Ithaca, NY).

REFERENCES


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