PART ONE | Native MRI contrast

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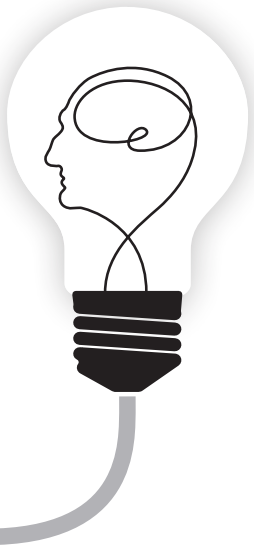
Chapter 2

Introduction to MRI

Extracted from Essential Bioimaging Methods (2009); Ch.13, 263-293



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Abstract

Over recent years, magnetic resonance imaging (MRI) has become an essential tool for the investigation of human brain disease. This chapter describes some of the principal MRI methods that are currently used for neuroimaging: T_1, T_2, susceptibility-contrast, diffusion and magnetisation transfer imaging. The mechanisms underlying the sensitivity of these techniques to pathophysiological state are explained.

Introduction

The present review will be restricted to the most common and for this thesis relevant applications of magnetic resonance imaging (MRI) in the brain, namely the depiction of hydrogen nuclei (protons) of mobile water molecules. The utility of MRI with respect to disease lies in the sensitivity of the technique to both micro- and macroscopic molecular motions. Nervous tissue consists of 70-80% water by weight, these water molecules being distributed through a variety of microscopic environments and physiological compartments. It is possible to generate MR images whose contrast reflects, amongst other factors, random molecular rotational motions (T_1 and T_2), random translational motion (diffusion), exchange with macromolecular protons (MTC) and blood flow (perfusion). The concentration and mobility of water molecules is modified in many pathologies and this is the basis of the high sensitivity of MRI to cerebral disease processes.
Furthermore, MRI contrast agents can also change these parameters allowing detection. In recent years this led to the emerging field of molecular and cellular imaging, where contrast agents targeted against disease-specific hallmarks or cells labelled with such agents are explored for early diagnostics and therapy follow-up. Also within the field of experimental neuroimaging this has taken a leap. A more extensive review is given in **Chapter 7**.

Biophysical background and methods

Some important principles are discussed here to provide a background for the rest of this article. For a more detailed introduction to MRI the reader is refer to various excellent textbooks.[1,2] Protons possess a nuclear magnetic moment (or 'spin'). In the absence of an external magnetic field these magnetic moments are randomly distributed in every direction. In the presence of a magnetic field however, a thermal equilibrium is achieved between spins oriented parallel and antiparallel to the magnetic field. The result is a net macroscopic magnetic moment, the bulk magnetisation (M_0), orientated in the direction of the external field (conventionally taken to be the *z*-axis). The individual spins precess around the *z*-axis at the Larmor frequency (ω, rad s^{-1}), which is proportional to the external magnetic field (B_0, Tesla):

$$\omega = \gamma B_0 \qquad [2.1]$$

where γ is a constant called the gyromagnetic ratio (26.751 10^7 rad T^{-1} s^{-1} for protons).

Relaxation

M_0 is proportional to the total number of protons present in the sample, and hence is also called the proton density. To be able to distinguish the magnetisation M_0 from the external magnetic field, M_0 is rotated by 90° into the transverse (*xy*) plane using a radiofrequency (RF) pulse at the Larmor frequency. Immediately following this 90° pulse, the initial magnetisation level can be detected. In time, the thermal equilibrium is restored, and the magnetisation vector returns to the *z*-axis. The characteristic times involved in this process are the spin relaxation

times: longitudinal relaxation time (T_1) for the restoration of the magnetisation along the *z*-axis, and the transverse relaxation time (T_2) for the decay in the *xy*-plane. (**Figure 2.1**)

T_2 and T_2*

The transverse relaxation time T_2 is also called the spin-spin relaxation time, referring to the molecular interactions behind the transverse relaxation process. The protons experience intramolecular dipolar interactions between two protons within the same molecule, as well as intermolecular interactions with protons of neighbouring molecules. This interaction becomes more efficient when the contact time between protons is relatively long, *e.g.* in viscous media. When the rotational correlation time of the molecules is short, as is the case for free water molecules, T_2 is relatively long (~ 3 sec). Water molecules interacting with macromolecules or solid surfaces generally have slower tumbling rates, which leads to a reduction in the relaxation time. Because water mobility often varies substantially between tissue types, and changes in situations of cellular stress, T_2-dependent contrast is very commonly used in MRI studies.

In addition to these spin-spin interactions, the transverse magnetisation is also perturbed by small local magnetic field differences. This results in different (local) Larmor precession frequencies of the spins under observation and thus in a loss of phase coherence causing a faster decay of the magnetisation in the *xy*-plane. The corresponding apparent relaxation time is called T_2* to distinguish it from the intrinsic transverse relaxation time T_2.

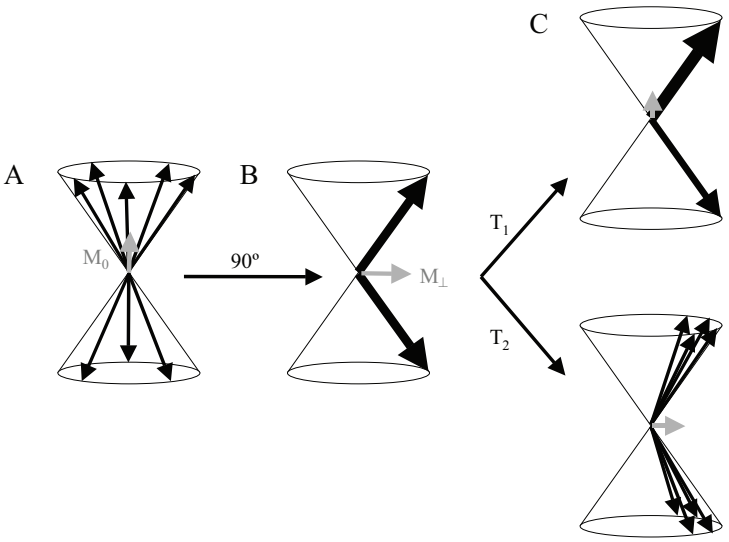

Figure 2.1 Schematic representation of the nuclear magnetic resonance principle
(**A**) The sample magnetisation M_0 arises from the uneven distribution of the spins (black arrows) between two different states, either parallel or anti-parallel to the main magnetic field B_0. The spins precess around the main magnetic field direction with the Larmor frequency ω. (**B**) After the application of a 90° pulse, the original distribution is shifted into the horizontal plane and phase coherence is established (the spins are all aligned along the same axis). The result is a sample magnetisation M⊥. (**C**) The spins return to the original distribution through T_1 relaxation. The loss of phase coherence is called T_2 relaxation. Both processes occur simultaneously but are depicted separately in the picture.

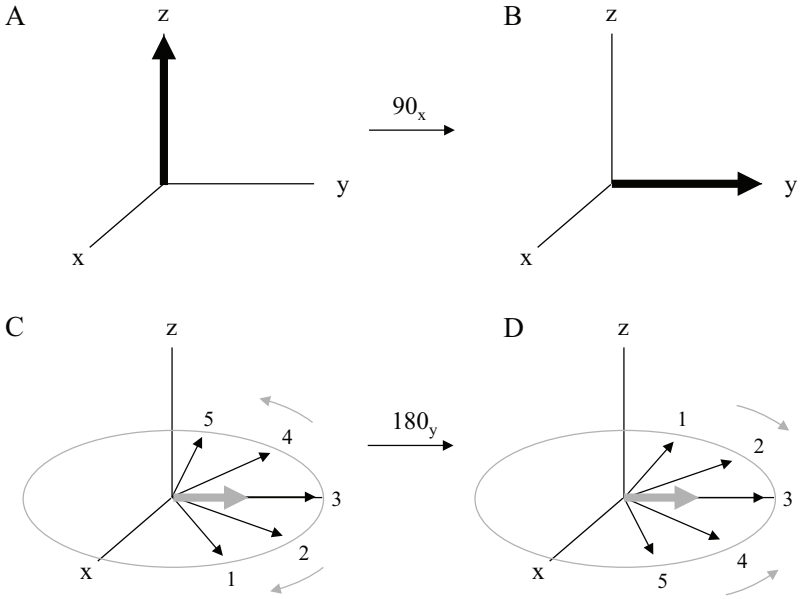

Figure 2.2 Spin echo MRI sequence
(**A**) Diagram showing the fanning out and refocussing of magnetisation in the course of a spin-echo sequence. (**B**) After the application of a 90° pulse, the original distribution is shifted into the horizontal plane. (**C**) The loss of phase coherence is primarily due to T_2* effects. (**D**) The 180° pulse flips the spins in the *xy*-plane, and the magnetisation refocusses along the y-axis. (**E**)The attenuation of the net magnetisation vector is due to T_2 relaxation.

This field-disturbing effect can be exploited as a source of contrast in tissue, since such magnetic field inhomogeneities typically occur at interfaces of structures with differing magnetic susceptibilities, like soft tissue and bone, or tissue and blood. T_2* contrast is of specific importance in blood oxygenation level dependent (BOLD) imaging, which is widely used in functional MRI investigations.[3] The paramagnetic nature of deoxygenated blood generates magnetic field gradients in blood vessels and surrounding tissues, leading to signal loss in T_2* weighted images. Fast T_2* weighted imaging is performed continuously to track transient changes in the magnetic field disturbances associated with the balance between oxyhemoglobin and deoxyhemoglobin in the blood, thus providing information on local neuronal activity. The second important application of T_2* contrast is its use in contrast enhanced MRI.[4] Specific exogenous (super)paramagnetic contrast agents, analogous to the tracers used in nuclear medicine, are being developed continuously in order to target specific areas or molecules, thus providing a means to map molecular events *in vivo*. Most commonly used T_2/T_2* contrast agents are iron oxide particles, causing hypo-intensities on the corresponding images by lowering T_2/T_2*, hence the name 'negative' contrast agents.

T_2 and T_2* measurements

Measuring relaxation times rather than making weighted images allows the quantification of observed changes. As already described, for detection the bulk magnetisation M_0 is rotated by

90° into the *xy*-plane. The ensuing loss of phase coherence due to T_2* effects can be reversed by the application of a series of 180° RF pulses following the initial 90° RF pulse, forming the so-called Spin-Echo (SE) sequence. The restoration of coherent magnetisation between the 180° pulses is called an echo. The amplitude of this echo is only attenuated by T_2 relaxation. (**Figure 2.2**)

T_2* can be measured by means of gradient echoes. In the spin-echo sequence the 180° pulse reverses the effects of local field inhomogeneities, whereas in a gradient echo sequence the echo is generated by reversing a magnetic field gradient. The main difference between a spin echo and a gradient is that the gradient echo does not refocus the dephasing due to field inhomogeneities, and therefore the echo is weighted according to T_2* rather than T_2. In addition to its use in T_2* imaging, gradient echoes are commonly used in rapid imaging sequences, as the echo time can be made much shorter than in spin echo sequences.

T_1

The other relaxation time must be considered is the longitudinal relaxation time T_1, also referred to as the spin-lattice relaxation time. The mechanisms behind this relaxation are complex, but it is facilitated by the presence of microstructures (macromolecules, membranes etc.), also called the lattice, that via dipolar interactions can absorb the energy of the excited protons. This energy transfer is most efficient when the rotational correlation rate of the molecules is in the same range as the Larmor frequency. In practice this means that T_1 becomes shorter as the molecular mobility decreases, but increases again for very slow molecular motion, as in solids. Both T_1 and T_2 reflect the properties of the physical micro-environment of water in tissue, albeit not in exactly the same way. Commonly used paramagnetic contrast agents (*e.g.* gadolinium or manganese-containing particles) decrease the longitudinal relaxation time T_1 leading to positive contrast.

T_1 measurements

The most well-known sequence to measure T_1 is the inversion recovery sequence. This sequence starts with a 180° RF pulse causing inversion of M_0, which then gradually recovers to its equilibrium. To detect the amount of magnetisation left, a 90° pulse is applied after a range of delay times. This rotates the magnetisation into the *xy*-plane, where it can be detected. (**Figure 2.3**) From the different delay times and the corresponding residual magnetisation levels, T_1 can be calculated.

Diffusion

Up to now, the translational motion of individual water molecules has not been considered. However, all molecules in a fluid are subject to Brownian movements, the extent of this motion depending on the temperature and the viscosity of the fluid. When an ensemble of molecules is followed in time, the root mean square displacement (x, m) shows a √t dependence:

$x=\sqrt{2dDt}$ [2.2]

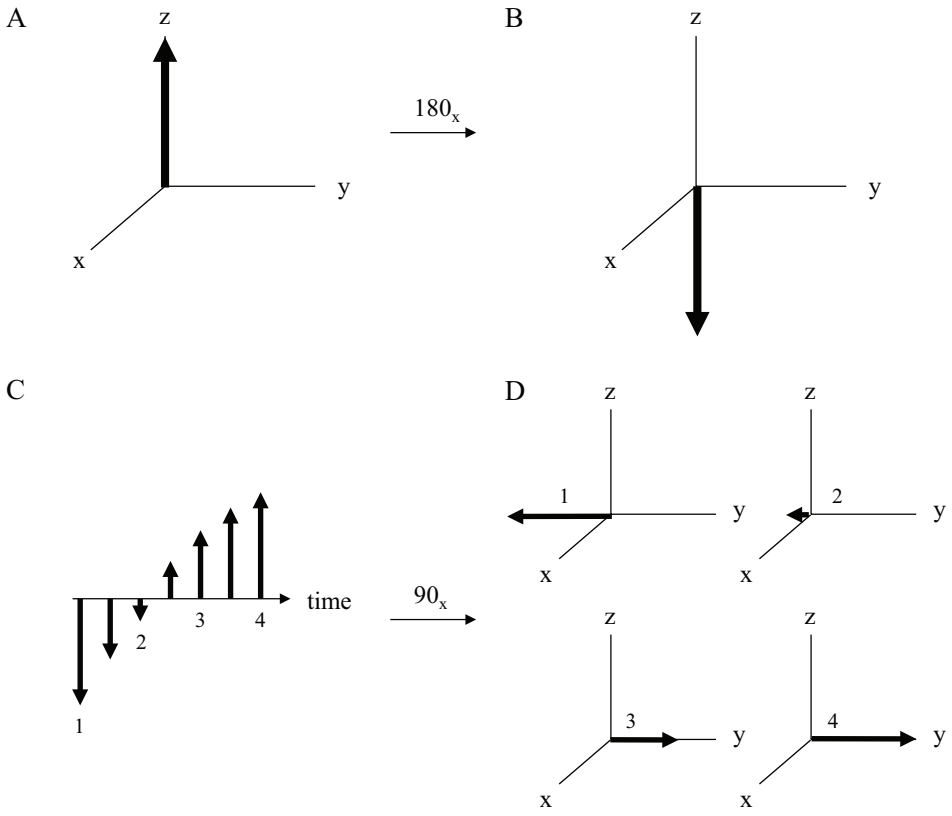

Figure 2.3 Inversion recovery MRI sequence
(**A**) Diagram showing the magnetisation changes during an inversion recovery sequence. (**B**) The 180° pulse inverts the magnetisation M_0. (**C**) In time, the magnetisation returns to equilibrium due to T_1 relaxation. (**D**) 90° pulses are applied to detect the residual magnetisation at a number of time points. After each 90° pulse, a waiting time is introduced to let the magnetisation return to equilibrium, after which the next cycle of 180° - 90° pulses is performed.

where D is the bulk diffusion coefficient of the fluid ($m^2\ s^{-1}$), t is the displacement time (s) and d (= 1, 2, or 3) is the dimensionality of the diffusion displacement. Normally, the displacement distribution of all molecules is Gaussian, where the mean displacement distance increases with increasing displacement times. However, if the molecules encounter barriers to diffusion, *e.g.* cell membranes, these determine the maximum displacement. These boundary restrictions imply that the displacement distribution is no longer Gaussian and is going to depend on the diffusion time. As a result, the measured apparent diffusion coefficient (ADC) is smaller than the intrinsic D. This ADC value is sensitive to the number of barriers, their geometry and their permeability: in other words to the tissue microstructure.
The above is true if isotropic diffusion can be assumed, *i.e.* diffusion that exhibits no directionality. Many biological tissues have a microstructure that favors molecular motion in a certain direction. In the brain, diffusional anisotropy occurs primarily in white matter tracts, caused by the myelin sheaths and other structures surrounding the nerve fibers, which restrict diffusion perpendicular to the axonal length. The anisotropic diffusion that arises when displacement along one direction

occurs more readily than along another is defined by a diffusion tensor, and diffusion tensor imaging (DTI) is the term used for measurements of the full diffusional properties of the sample in all three dimensions.[5]

Diffusion measurements

The ADC can be measured using a pulsed field gradient (PFG) experiment. In this experiment a sequence of two pulsed magnetic field gradients of equal magnitude but opposite sign and separated by an interval Δ temporarily change the resonance frequency of the observed spins (Eqn. 2.1) as a function of their position. If the spins remain at exactly the same position, the effects of the opposing gradient pulses compensate each other. However, as soon as translational motion occurs, the gradients induced frequency shifts do not exactly compensate each other anymore and a phase shift occurs. Because diffusion is random in all directions, no net phase shift results, but phase coherence is partially lost, resulting in attenuation of the echo amplitude. (**Figure 2.4**) The amount of this attenuation is determined by the length, amplitude and separation of the gradient pulses, summarised in the so-called b factor, and by the mean translational distance travelled during the interval Δ, which depends on the ADC. The signal intensity in a diffusion-weighted image (DWI) can therefore be described as:

$S_b = S_0 e^{-b.ADC}$ [2.3]

where S_b is the DWI signal intensity and S_0 is the signal without any diffusion gradients applied.

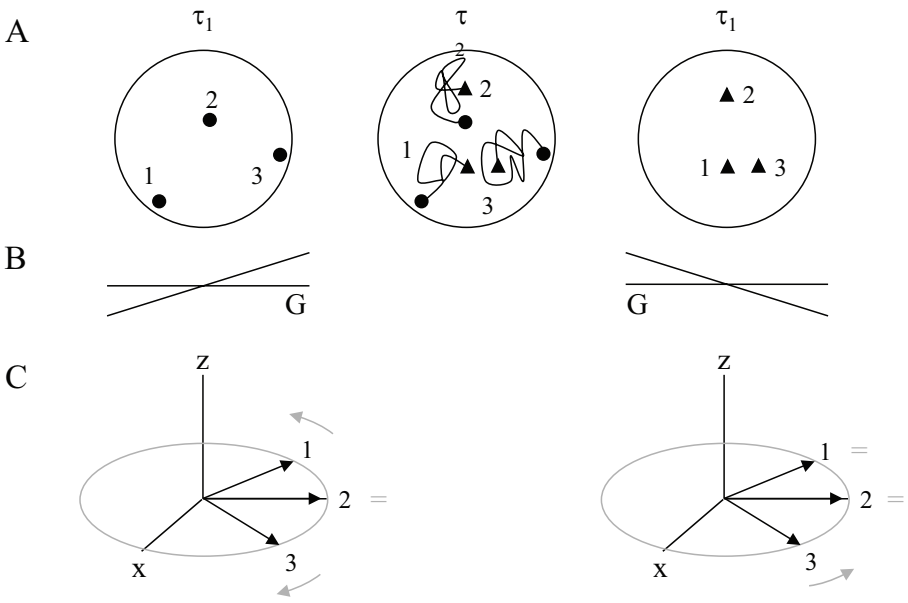

Figure 2.4 The principle of diffusion weighted MRI

(**A**) Water diffusion for three different spins within a sample. (**B**) Two diffusion gradients are applied with opposite sign and a delay time τ between them. (**C**) Diffusing spins experience different phase shifts (dependent on their position in the direction of the applied diffusion gradient) and are incompletely refocused, leading to a net loss in signal intensity

Magnetisation Transfer Contrast

A certain fraction of protons in tissue exist in a so-called 'bound' state, *i.e.* their motion is restricted because they are part either of macromolecules, or of water molecules within the hydration layers around macromolecules. These protons posses very short T_2 relaxation times (<100 μs) and hence are not accessible by standard spin-echo MRI methods. With currently available small-bore technology, minimum echo times are of the order of several ms, and hence the signal from these protons has already decayed before signal acquisition. While simple models for proton relaxation behaviour predict that such a proton population will have some influence upon T_1 and T_2, Wolff and Balaban[6] proposed the method of magnetisation transfer contrast (MTC) imaging[7], as a more direct means of probing these 'bound' protons.

MTC imaging is based on an inverse Fourier relationship between T_2 and the range of frequencies over which protons respond to RF excitation: protons in the 'bound' fraction possess a very short T_2 and hence exhibit a broad resonance width (~ 20 kHz) in the frequency domain. Conversely, bulk water protons have a long T_2 with a correspondingly narrow (~10 Hz) frequency domain resonance. (**Figure 2.5A**) This difference can be used to excite the 'bound' fraction independently from the bulk protons. If RF energy is supplied at a frequency offset from the central Larmor frequency (typically 1-5 kHz) the magnetisation in the 'bound' fraction will be reduced by saturation, while, in the absence of exchange, the bulk water pool would remain unaffected. (**Figure 2.5B**) However, on the time-scale of a typical MRI experiment there is a significant exchange of magnetisation between the two proton fractions, either by chemical exchange or by magnetic interactions. Since the 'bound' proton magnetisation has been reduced by the off-resonance irradiation, such exchange also leads to a reduction in both the magnitude of the observable bulk water magnetisation and its associated T1. (**Figure 2.5C**) The degree of reduction depends upon both the relative sizes of the two fractions and upon the rate of magnetisation exchange between them: both of these factors may be influenced by tissue pathology.

MTC measurements

In its most simple form, the MTC imaging experiment involves collecting an image (S_s) which is preceded by a long (~ 3 s) saturating off-resonance RF pulse, followed by a second image (S_0) without a pre-saturation pulse.[8] The Magnetisation Transfer Ratio (MTR) is then quantified as:

$$MTR = (S_0 - S_s)/ S_0 \quad [2.4]$$

A high MTR signifies the presence of a significant proton pool associated with macromolecules or cellular microstructure *and* a significant exchange of magnetisation between these protons and those of the bulk water. Reduction of the MTR suggests a disruption of tissue microstructure, the most successful application of MTC in experimental neuroscience being the investigation of pathological disruption of white matter due to demyelination.

In order to reduce imaging time, selective saturation of the 'bound' fraction may also be achieved by pulsed methods whereby gradient-echo images with short TR are obtained with a low angle excitation pulse preceded by a short (~10 ms) off-resonance pulse.[9] If the repetition time is sufficiently short (~100 ms) compared to the T_1 relaxation time of the 'bound' pool, an equilibrium is established after a number of cycles, resulting in substantial saturation of the 'bound' fraction.

It should be noted that unless total selective saturation of the bound pool is achieved, a situation impossible to achieve in practice, the magnitude of the MTC effect is dependent upon the duration, intensity and frequency offset of the off-resonance pulses. Caution is therefore required in the quantitative comparison of MTC imaging results obtained using differing experimental schemes.

Concluding remarks

The combination of appropriate imaging techniques can greatly elucidate our understanding of human brain pathologies. Prior clinical application the use of suitable animal models of disease or post-mortem human brain tissue can greatly aid the development of improved MRI techniques. This experimental imaging partnership has contributed to the development of novel imaging techniques, to the promotion of better diagnostic and prognostic measures, and to elucidation of the basic mechanisms of cellular injury leading to improved therapies.

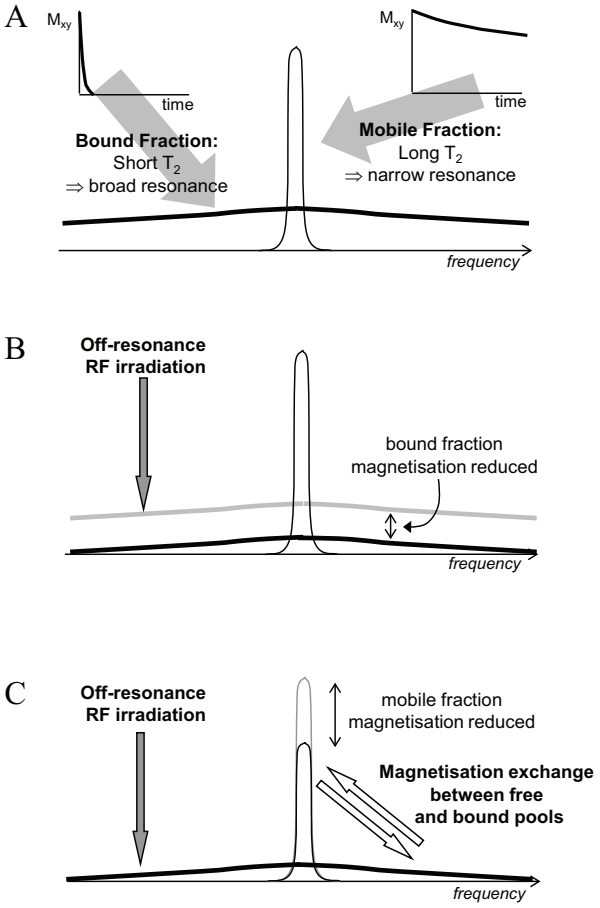

Figure 2.5 The principles of magnetisation transfer imaging
(**A**) Macromolecular-bound protons possess a very short T_2 and hence a broad resonance response in the frequency domain. Mobile water protons conversely exhibit a narrow frequency domain line-width. (**B**) In the absence of exchange, the application of RF energy at a frequency away from resonance perturbs only the bound proton fraction, causing the magnetisation of this pool to reduce towards zero. (**C**) Exchange of magnetisation between free and bound protons causes a reduction in the magnetisation of the mobile pool, and hence an observable reduction in MR image intensity.

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