

# DEPARTMENT OF BIOTECHNOLOGY B.D.COLLEGE, PATNA – 1

## B.SC PART II BIOPHYSICS

BY

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### Nuclear magnetic resonance

NMR is a method of physical observation in which nuclei in a strong constant magnetic field are unsettled by a weak oscillating magnetic field (in the near field and therefore not involving electromagnetic waves) and respond by producing an electromagnetic signal with a frequency characteristic of the magnetic field at the nucleus. This process occurs near resonance, when the oscillation frequency matches the intrinsic frequency of the nuclei, which depends on the strength of the static magnetic field, the chemical environment, and the magnetic properties of the isotope involved; in practical applications with static magnetic fields.

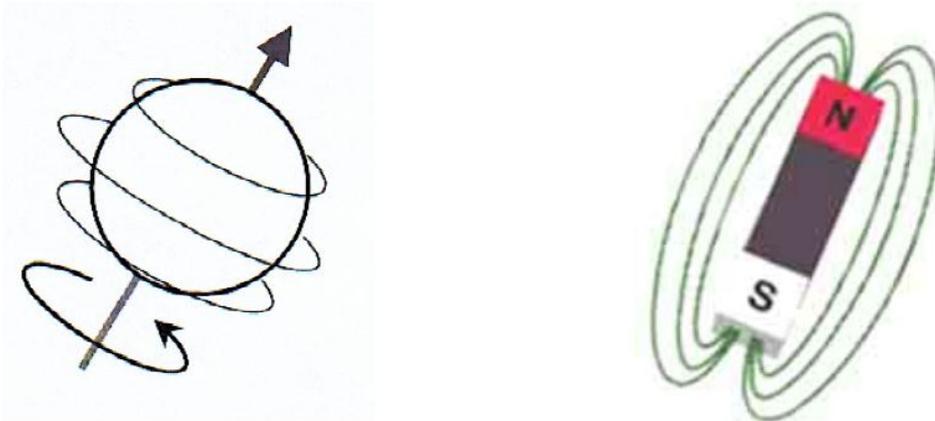
It has made a tremendous impact in many areas of chemistry, biology and medicine. In this report a student-oriented approach is presented, which enhances the ability of students to comprehend the basic concepts of NMR spectroscopy and the NMR spectra of various nuclei. The origin of chemical shifts, coupling constants, spin relaxation and the Nuclear Overhauser Effect (NOE) will be discussed and their relation to molecular structure will be provided. A wide range of applications of NMR spectroscopy is presented, including exchange phenomena, the identification and structural studies of complex biomolecules, such as proteins, applications to food analysis, clinical studies, NMR as a microscope and magnetic tomography.

Nuclear magnetic resonance (NMR) spectroscopy was discovered shortly after the Second World War, and since then its applications to chemistry have been continuously expanding. It was natural then that NMR took an important part in undergraduate chemistry education, being taught within various courses: physical chemistry, organic, inorganic and analytical chemistry. In recent years, the applications of NMR have been extended to biology and medicine, so they have also become an integral part of courses in these subjects.

#### The nuclear magnetic moment

Any motion of a charged particle has an associated magnetic field. This means, that a magnetic dipole is created, just like an electrical current in a loop creates a magnetic dipole, which in a magnetic field corresponds to a magnetic moment  $\mu$  (Figure 1). The magnetic moment  $\mu$  of a nucleus is intimately connected with its spin angular momentum. To be more precise  $\mu$  is proportional to  $I$ , which is the angular momentum quantum number

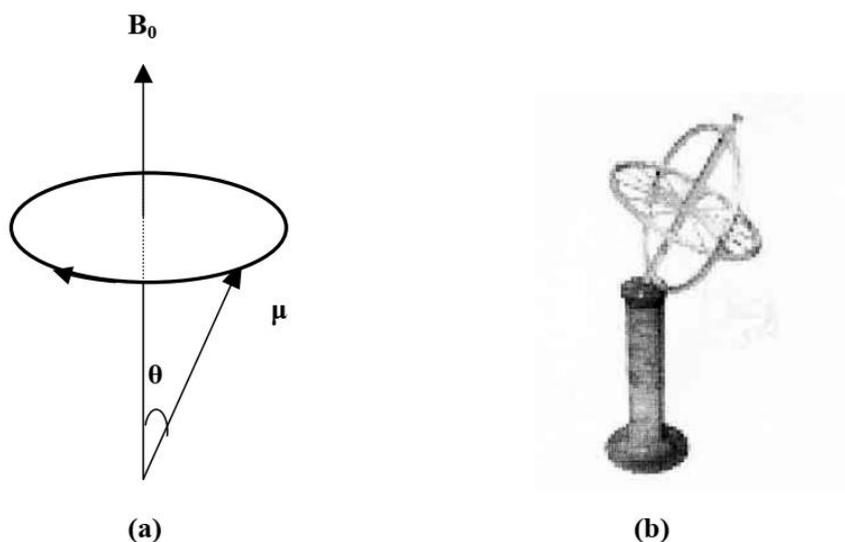
usually called the nuclear spin, with a proportionality constant  $\gamma$  known as gyromagnetic ratio:  $\boldsymbol{\mu} = \gamma \mathbf{I}$ .



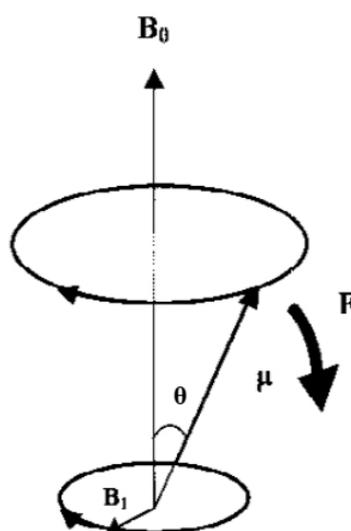
**FIGURE 1.** *A spinning nucleus can be regarded as a microscopic magnet.*

### Larmor precession

Let us consider the classical motion of a magnetic moment,  $\boldsymbol{\mu}$ , in a uniform externally applied strong magnetic field  $\mathbf{B}_0$  under the condition of constant total energy. The movement of  $\boldsymbol{\mu}$  traces out a cone about  $\mathbf{B}_0$ , which is analogous to the motion of a gyroscope running in friction-free bearings under the influence of the Earth's field (Figure 2). Such motion is referred to in general as Larmor precession. The precession frequency,  $\nu_0$ , is given by:  $\nu_0 = |\gamma| B_0 / 2\pi$  (2) Suppose there is an additional externally applied, but weak magnetic field,  $\mathbf{B}_1$ , perpendicular to  $\mathbf{B}_0$ . Such a field will also exert a torque on  $\boldsymbol{\mu}$ , tending to change the angle  $\theta$  between  $\boldsymbol{\mu}$  and  $\mathbf{B}_0$ . However, if  $\mathbf{B}_1$  is fixed in direction and magnitude, it will alternately try to increase and decrease  $\theta$  as  $\boldsymbol{\mu}$  precesses. Since  $\mathbf{B}_1$  is stated to be weak, the net effect will be a slight wobbling in the precession of  $\boldsymbol{\mu}$ . Alternatively, the motion of  $\boldsymbol{\mu}$  can be described as caused by a resultant field  $\mathbf{B}_0 + \mathbf{B}_1$ . If, on the other hand,  $\mathbf{B}_1$  is not fixed, but is rotating about  $\mathbf{B}_0$  with the same frequency as the precession of  $\boldsymbol{\mu}$ , its orientation with respect to  $\boldsymbol{\mu}$  will be constant. Suppose this orientation is such, that  $\mathbf{B}_1$  is always perpendicular to the plane containing  $\mathbf{B}_0$  and  $\boldsymbol{\mu}$  as in Figure 3; then the torque exerted on  $\boldsymbol{\mu}$  by  $\mathbf{B}_1$  will always be away from  $\mathbf{B}_0$ .



**FIGURE 2.** (a) Precession of a magnetic moment  $\mu$  about an applied magnetic field  $B_0$ . (b) The nuclear precession is analogous to the motion of a gyroscope under the influence of the Earth's field.

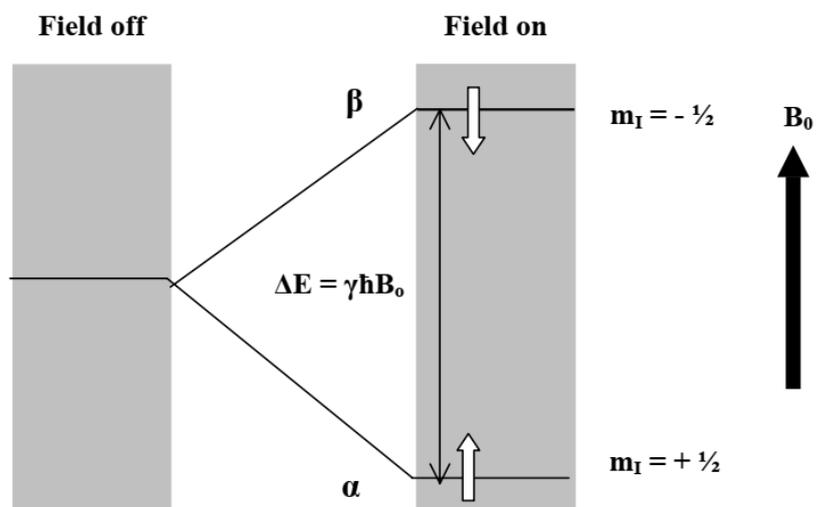


**FIGURE 3.** The effect of a rotating magnetic field,  $B_1$ , on a precessing magnetic moment,  $\mu$ . When  $B_1$  is perpendicular to the  $B_1 - \mu$  plane, there is a force  $F$  acting to increase the angle between  $B_0$  and  $\mu$ . If  $\mu$  and  $B_1$  are rotating at the same rate, this force acts always away from  $B_0$  and therefore has a consistent effect (Harris, 1983).

Consequently, an accumulated effect on  $\mu$  is possible. Since changing  $\theta$  corresponds to changing the energy of  $\mu$  in  $B_0$ , this condition is described as resonance – the frequency,  $\nu$ , of the field  $B_1$  required must equal the Larmor precession frequency of equation. (2) (Harris, 1983). The energy for the change of  $\theta$  is, of course, derived from the rotating field  $B_1$ , which is supplied by radio frequency electromagnetic radiation.

## Quantum mechanical description

According to the classical picture the atomic nucleus, assumed to be spherical, rotates about an axis and thus, possess a nuclear or intrinsic angular momentum  $\mathbf{P}$ . Quantum mechanical considerations show that, like many other atomic properties, this angular momentum is quantized: where  $\hbar = h/2\pi$ , ( $h$  is Planck's constant). The nuclear spin can have values  $I = 0, 1/2, 1, 3/2, 2, \dots$  up to 7. As it will be explained below, neither the values of **neither**  $I$  nor those of  $\mathbf{P}$  can yet be predicted from theory.



**FIGURE 4.** The nuclear spin energy levels of a spin- $1/2$  nucleus in a magnetic field (Atkins, 1998).

To observe a nuclear magnetic absorption, we have to adjust either the frequency  $\nu_0$  of the radiation or the strength of the magnetic field at the nucleus,  $\mathbf{B}_0$  until equation (2) holds, i.e. until the point where resonance (energy absorption) occurs.

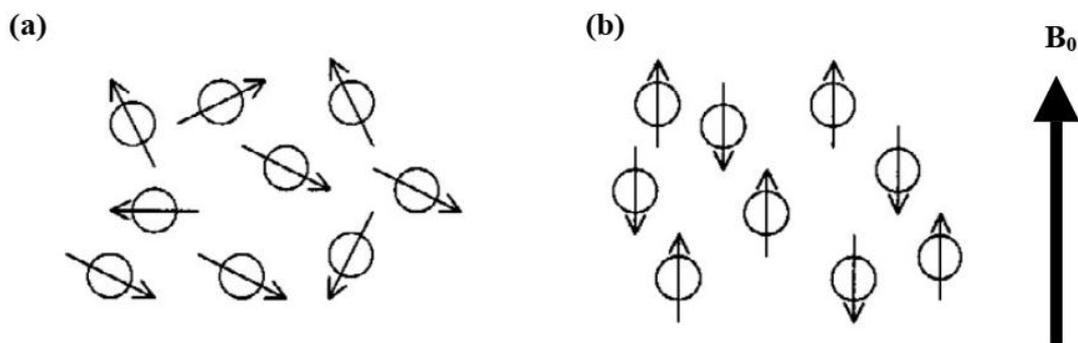


Fig: The effect of the magnetic field  $\mathbf{B}_0$  on the orientation of the spin magnetic moments.

## THE NMR SPECTRA OF VARIOUS NUCLEI

### The case of $^1\text{H}$

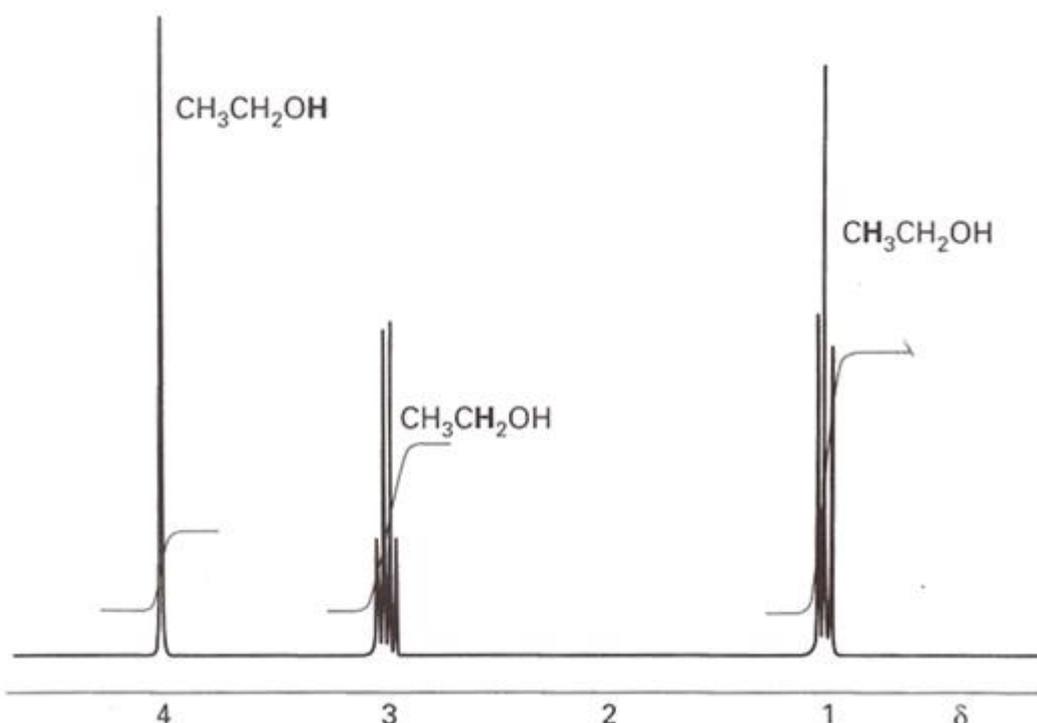
The vast majority of molecules of interest to chemists contain hydrogen atoms and, as this nucleus has one of the strongest resonances, it is not surprising that  $^1\text{H}$  NMR has found the widest application. Protons in different chemical groups have different shielding constants, and the resonance condition is satisfied at different  $\nu_0$ . Let us understand the spectrum of ethanol shown in Figure 7 (Atkins, 1998).

Thus, for a given applied field, the CH hydrogen nucleus will precess with a smaller Larmor frequency than that of OH (Banwell, 1994; Atkins, 1998).

Two very important facets of NMR spectroscopy appear in Figure 7:

(i) Identical nuclei, i.e.  $^1\text{H}$  nuclei, give rise to different absorption positions when in different chemical surroundings. For this reason the separation between absorption peaks is usually referred to as chemical shift.

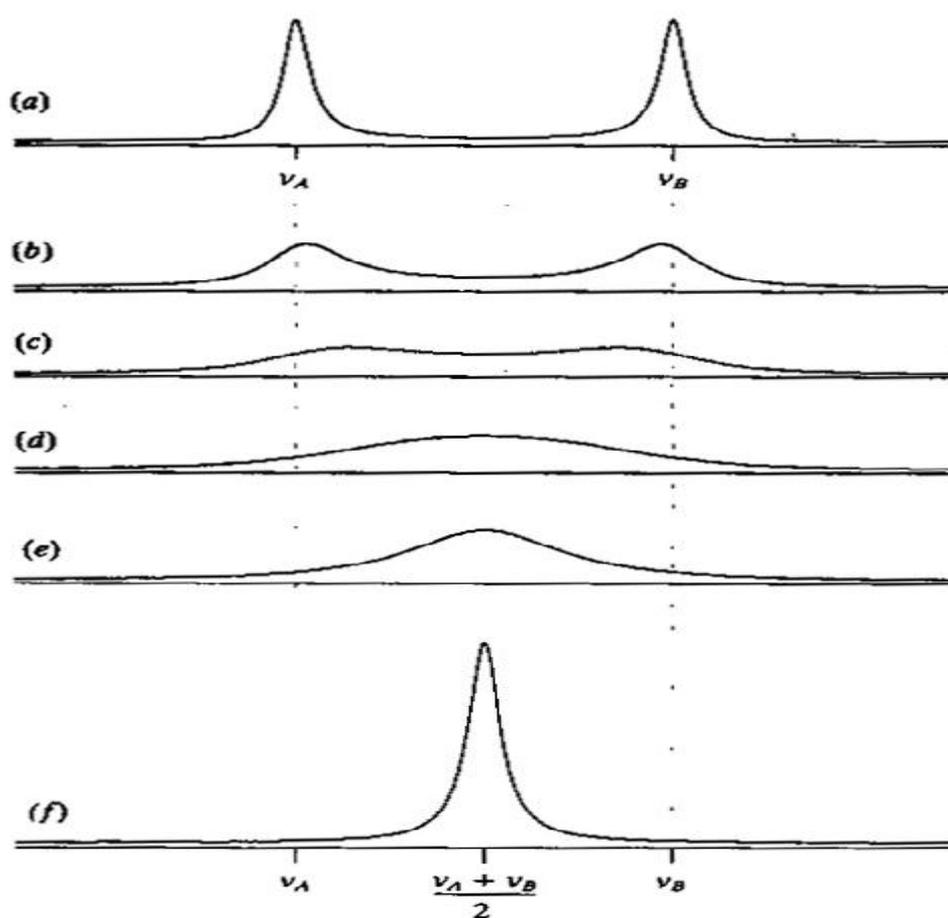
(ii) The area of an absorption peak is proportional to the number of equivalent nuclei (i.e. nuclei with the same chemical shift). Note that in the case of ethanol, we can distinguish which group of lines corresponds to which group of protons by their relative intensities.



The  $^1\text{H}$ -NMR spectrum of ethanol. The bold letters denote the protons giving rise to the resonance peak, and the step-like curve is the integrated signal (Atkins, 1998).

## Use of NMR to monitor rate processes

The student may have been puzzled by one aspect of the ethanol spectrum shown in Figure 7. The resonance of the  $-OH$  hydrogen is shown as a single line whereas we might expect it to be coupled with the neighboring  $CH_2$  nuclei and hence have multiplet structure. The reason it does not couple is attributed to exchange phenomena. How does it happen? Let us consider a more general situation in which a nucleus exchanges between two distinct sites, which are designated A and B. An example would be a particular proton on a ligand where the ligand binds to a macromolecule. In this case, the free and bound ligands are apt to have very different chemical shifts. In the limit where no exchange occurs, two distinct resonance lines will be present, one corresponding to each molecular environment. If exchange is extremely rapid, only one line will be evident. Its position will be between the two originally separated lines.

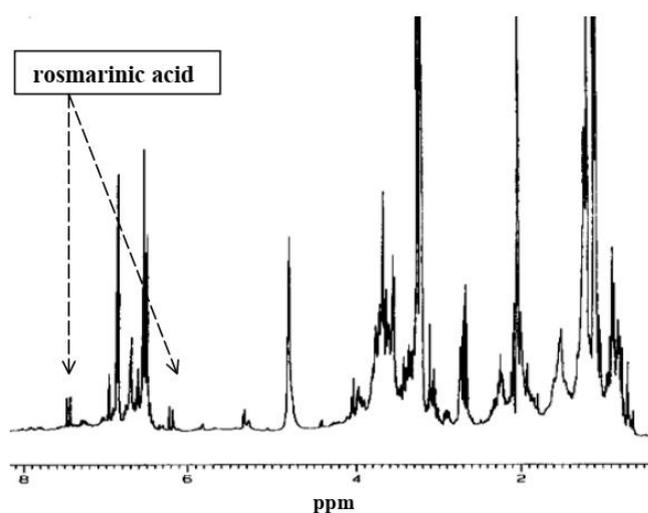


The effect of the chemical exchange between two sites A and B on the NMR spectra (Banwell, 1994).

## Materials science

NMR spectroscopy, in solid state, can be used to investigate new materials of great technological importance such as glasses, ceramics, polymers, synthetic membranes and superconductors. Furthermore, it can be used to investigate reactions taking place in catalytical surfaces.

## Food chemistry

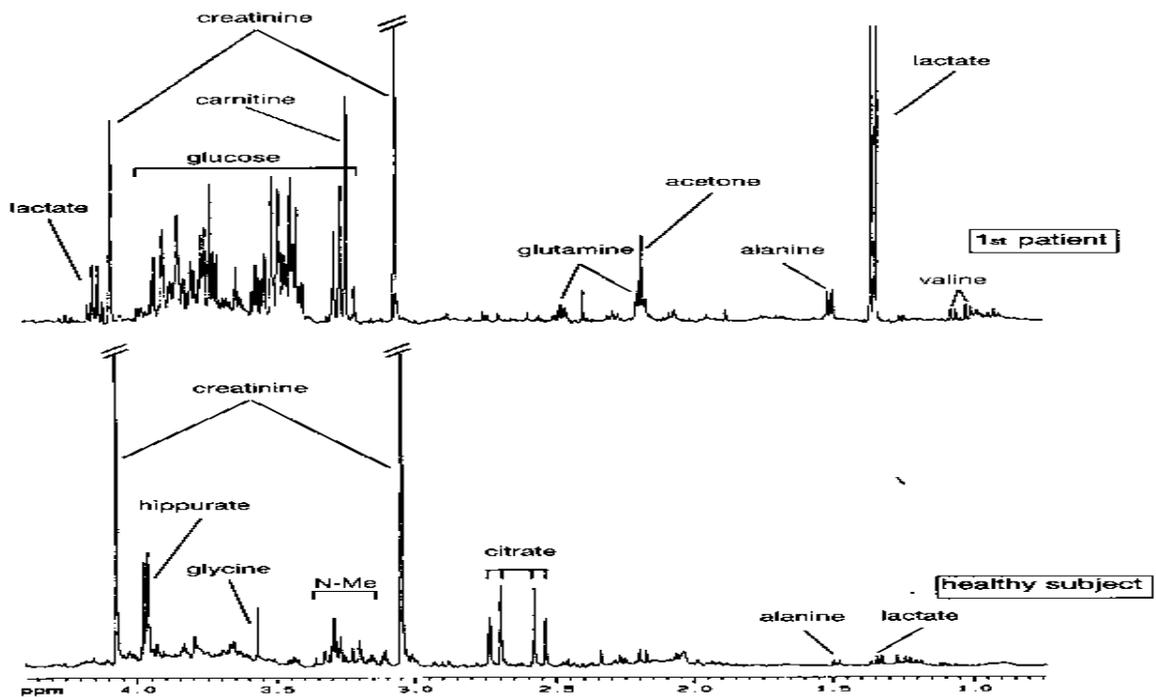


$H^1$  NMR spectra of the ethanolic extract of the plant *Origanum vulgare* (Greek oregano). The arrows denote the presence of the antioxidant compound rosmarinic acid

NMR spectroscopy can be used in the verification of the wine aging and authenticity as well as in the identification of the oil's fatty constituents (Belton, 1995). It can also contribute in the investigation of the mechanisms that are responsible for food decomposition without the need of sample destruction as it is common with the classical chemical analysis techniques. High resolution NMR techniques have found interesting applications in the analysis of complex mixtures of various extracts of natural products.

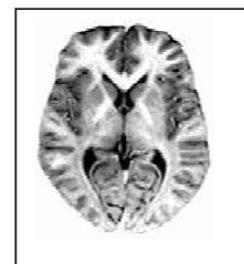
## Clinical applications

NMR has found numerous applications in the localization and characterization of metabolites in biological fluids *in vivo* and *in vitro* and, thus, it can be utilized in the diagnosis of many kinds of diseases.



$H^1$ -NMR spectra (400 MHz) of urine from a healthy subject and from one patient suffering from Paraquat intoxication (Bairaktari et al.,1998).

**Medical community** has been very enthusiastic with these new techniques due to many advantages compared to X-rays or to other imaging methods that use radioactive compounds. For instance, the blood volume that is conveyed by the heart in one pulse can be measured, allowing the study of the heart in action. Moreover, soft tissues that it is impossible to be detected using X-rays are clearly shown with the MRI technique, allowing the diagnosis of tumors and of other diseases.



Magnetic Resonance Imaging (MRI): Anatomical view of the brain.