Peter Stilbs

Diffusion and Electrophoretic NMR

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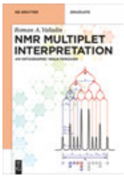

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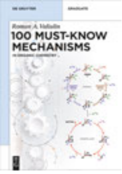

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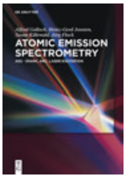

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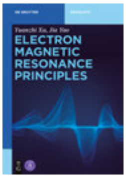

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Peter Stilbs

Diffusion and Electrophoretic NMR

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Preface

With great initial hesitation, I considered a surprising invitation in early 2017 by De Gruyter Publishers to write a book on NMR diffusometry in their series of graduate-oriented textbooks. A related one by Bernhard Blümich and coworkers on *Compact NMR* had already set a high standard indeed [1]. Three advanced monographs of excellent quality on translational motion and NMR also had appeared less than a decade ago: by William S. Price in 2009 [2], by Paul T. Callaghan in 2011 [3] and by Geir Humborstad Sørland in 2014 [4].

Being professor emeritus since 2012, I felt that there was not much more I could add to all this and to my own previous reviews on the subject and some families of applications. The first one (in *Progress in NMR Spectroscopy*) formally dates back to 1987 [5], and was widely influential in the past (it was actually authored in late 1985 and appeared in print in late 1986). At the time of writing this book, it had about 1,600 citations. However, in later years, it seems that much of the actual information in it is not noticed or actually read; especially numerous application studies listed and outlined there seem widely ignored or overlooked.

My most recent NMR diffusometry review dates back to 2011 [6]. It is similar in terms of outline to that in this book, although more compact. However, this text appears unnoticed by many potential NMR users too, most likely because it constitutes a chapter (among many other NMR-related ones with similar fate) in *Encyclopedia of Analytical Chemistry*. This would not be the first choice for looking for introductory information on NMR techniques and similar topics. Finally, an essay-like one (written in 2015, but finally published in paper form in 2017) describes personal historical recollections on the early evolution of multicomponent diffusometry techniques in high-resolution NMR [7].

Oleg Lebedev, series editor at De Gruyter, gradually convinced me that a book of primarily educational format and focus should indeed serve a purpose, especially for persons who want to get started with hands-on NMR diffusometry. That the intended book need and should not be “complete” with regard to choice of references and topics, and there was virtually no limit to the number of color illustrations that contributed a lot to my final positive decision. I just hope that colleagues around the globe understand the scope and format, and do not feel offended that links to their important contributions to the field might have somewhat arbitrarily been left out.

I want to clearly stress that this book is not intended to compete in any way with the detailed and quite complete texts mentioned earlier. It is simply meant to be an introductory and rather nonmathematical textbook for beginners, with some basic NMR experience. A main objective for a book of this kind is to introduce the subject to those who want to proceed quickly to a “hands-on” situation. It is easy to get misled by older literature and descriptions of numerous ingenious experimental procedures and subsequently get lost into unnecessarily complicated or awkward

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measurement procedures. Modern equipment is also intrinsically much more artifact-free than it was some decades ago, and there is less or no need for numerous "corrective" measurement procedures that were common in the past; some of which could even be quite contra productive today.

Nowadays, necessary equipment for NMR diffusometry is readily available in many labs around the globe, also because "gradient-enhanced" procedures became popular in high-resolution NMR. This has, in turn, promoted manufacture and sales of NMR probes with integral magnetic field gradient coils as a standard option. Such probes may also have multinuclear capability, through interchangeable inserts and generally have quite sufficient performance for many diffusion-related applications. Users who need higher specifications regarding gradient strengths and similar will find dedicated probes and gradient drivers available commercially. Some of that equipment may have also been designed for NMR microscopy (i.e., magnetic resonance imaging (MRI) on small objects). Here, the probe would have three gradient coils for the x-, y- and z-spatial dimensions and three independent gradient drivers. Coil design and gradient driver efforts in MRI have undoubtedly also promoted vastly superior instrumental performance in modern-day NMR diffusometry.

A companion method, electrophoretic NMR (eNMR), has been around for quite some time [8, 9]. It later found its way into high-resolution NMR, primarily through efforts by Charles S. Johnson, Jr. and coworkers around 1990 [10]. I personally spent some months in his lab in 1992 to learn some tricks. Later I realized that practical and reliable routine implementation of eNMR remained deceptive as a concept, at least in our hands – and over many years. In particular, we found that the competing and mostly irreproducible phenomenon of electro-osmosis was difficult to suppress or correct for. Various glass coatings (meant to suppress electro-osmosis) could work for a single sample, but were not stable enough to survive washing and sample changing during longer actual measurement series. In this context, I feel a need to apologize at this stage to my own past Ph.D. students who often had to suffer in despair with irreproducible experiments, especially Kim Paulsen, Erik Thyboll-Pettersson and Fredrik Hallberg.

Primarily through later-year innovative ideas by my local colleague István Furó and our common former Ph.D. student Pavel Yushmanov (nowadays electronics and fine mechanical wizard at the department), it appears that the eNMR technique has finally reached a reasonable state of maturity and stability. I have, therefore, included a final section on eNMR in this book. Despite a much more limited system applicability than NMR diffusometry, high-resolution eNMR is a technique with great and still largely unexploited potential in chemistry, even after three decades of existence.

Before finally accepting the offer to compose and write this book on my own, I contacted a few distinguished colleagues regarding possible coauthorship. Lack of time within the foreseeable future was the common reason to decline. Also, I remember very well a previous joint book project efforts by three colleagues – after more than a year of ambitious authoring, differing opinions on the content, level and style made it impossible to finalize the work. Well, there is also a saying "too many cooks spoil the soup." I do not really think that could have been the case with the coauthors I myself had in mind, but some practical, stylistic and logistic aspects of writing likely got simpler. However, I hope that a related saying, "cook a soup on a nail," will not characterize the final result (a stone soup version of this saying also circulates).

Before finally deciding to undertake this task, I also consulted Bernhard Blümich, who already contributed to this book series with the volume *Compact NMR* some years earlier. Bernhard advised me that authoring such a book requires a lot of devotion and daily discipline – of course, he was right. However, on many occasions during this intermittent two-year authoring, I rather got personal associations to the frantically writing Dr Mabuse in the 1933 movie classic *The Testament of Dr. Mabuse*, directed by Fritz Lang.

Anyway, I now want to thank all former collaborators in the topic areas of this book. In recent decades, contact and discussions with persons on the other side of the globe have been particularly valuable – special thanks go to William S. Price (University of Western Sydney), who has been a great support and a good friend for decades. He promptly assisted with various matters on numerous occasions while authoring this book. I have also had great "across the Globe" contact with Philip W. Kuchel and Paul T. Callaghan. Paul tragically passed away in 2012, and is sadly missed. Help and advice from local collaborators István Furó, Pavel Yushmanov and Sergey Dvinskikh have also been of immense importance for me during later years. István and Pavel also kindly provided a number of illustrations and related material for the eNMR sections of this text. In the past, I also enjoyed very fruitful and stimulating collaboration with Michael E. Moseley, Wyn Brown, Olle Söderman, Björn Lindman, Terence Cosgrove, Peter C. Griffiths, Lars Nordenskiöld, Aatto Laaksonen, Magnus Nydén and Harald Walderhaug, in particular. I also thank all former Ph.D. students, postdocs and visiting scientists over the years.

Thanks also go to Klaus Zick of Bruker Biospin and Gareth Morris and Mathias Nilsson for kindly providing information, documentation and figure material related to Bruker and Varian/Agilent PGSE instrumentation and the GNAT data processing package. Andrew Coy and Bertram Manz made available information, text and figure material related to PGSE operation on recent-year "bench-top" spectrometers manufactured by Magritek. Daniel Topgaard and Daniel Gallichan generously provided graphic tools and computer code for simulating echo formation, probably saving me weeks of time and effort.

Special thanks go to Oleg Lebedev who originally persuaded me to write this book, and to Lena Stoll of De Gruyter for innumerable consultations regarding technical typesetting and graphical matters. They all patiently and promptly dealt with. During the final months of writing and actual preproduction, their respective roles at De Gruyter were taken over by Mareen Pagel and Ria Fritz, with a continued pleasant, nonformal, constructive and very helpful atmosphere. I am also very grateful that they gladly accepted a considerably longer manuscript text than initially agreed on.

Finally, I want to thank my former Ph.D. supervisor Sture Forsén for directing me into the field of NMR diffusometry more than 40 years ago, when I moved from Lund to a different and indeed highly molecular transport-oriented scientific environment in Uppsala [7]. His encouragement and moral support for authoring this book is greatly appreciated as well.

Åkersberga, December 31, 2018 Peter Stilbs

References

[1] Blümich, B., Haber-Pohlmeier, S., & Zia, W. Compact NMR. Berlin/Boston: De Gruyter, 2014.

[2] Price, WS. NMR Studies of Translational Motion – Principles and Applications. Cambridge: Cambridge University Press, 2009.

[3] Callaghan, PT. Translational Dynamics & Magnetic Resonance, Principles of Pulsed Gradient Spin Echo NMR. Oxford: Oxford University Press, 2011.

[4] Sørland, GH. Dynamic Pulsed-Field-Gradient NMR. Berlin, Heidelberg: Springer, 2014.

[5] Stilbs, P. Fourier transform pulsed-gradient spin-echo studies of molecular diffusion. Prog Nucl Magn Reson Spectrosc. 1987; 19: 1–45.

[6] Stilbs, P. Diffusion and Electrophoretic Studies Using Nuclear Magnetic Resonance. In: RA Meyers, ed. (Nuclear Magnetic Resonance and Electron Spin Resonance Spectroscopy). Chichester, UK: John Wiley & Sons Ltd, 2011:1439–1461.

[7] Stilbs, P. Historical: Early multi-component FT-PGSE NMR self-diffusion measurements-some personal reflections. Magn Reson Chem. 2017; 55: 386–394.

[8] Holz, M., Lucas, O., & Müller, C. NMR in the presence of an electric current. Simultaneous measurements of ionic mobilities, transference numbers, and self-diffusion coefficients using an NMR pulsed gradient experiment. J Magn Reson. 1984; 58: 294–305.

[9] Holz, M. Electrophoretic NMR. Chem Soc Rev. 1994; 23: 165–174.

[10] Johnson, C., Jr, & He, Q. Electrophoretic Nuclear Magnetic Resonance. Adv Magn Reson. 1989; 13: 131–159.

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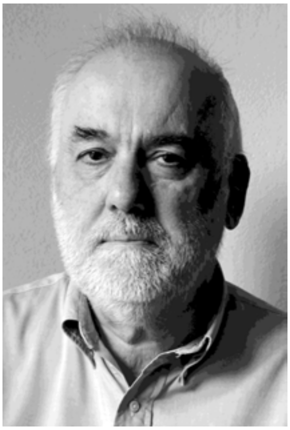

Peter Stilbs is Professor Emeritus of Physical Chemistry at the Royal Institute of Technology (KTH), Stockholm, Sweden. He received his doctorate degree in Physical Chemistry (TeknD) in 1974 at the Lund Institute of Technology under the guidance of second-generation nuclear magnetic resonance (NMR) pioneer Sture Forsén. After a postdoc year at Brock University (St. Catharines, Canada), he has held positions at Uppsala University (Sweden) and University of Bergen (Norway), before being appointed to the KTH chair in 1986.

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1 Historical perspectives and basic pulsed NMR concepts

The interest in studying transport processes such as self-diffusion, flow and electrophoresis via nuclear magnetic resonance (NMR) methods has grown rapidly in the past decades. There are several reasons involved, including recent availability of commercial high-performance instrumentation. The use of similar equipment and methodology for NMR microscopy (magnetic resonance imaging [MRI] on small objects) has also been utilized in these processes. Both techniques can also be combined for creating spatially resolved images that quantify transport processes in heterogeneous or confined systems.

Data available on lateral transport processes such as self-diffusion do not normally require further interpretation, unlike many other techniques that require elaborate theoretical frameworks and simplifying models for converting a measured quantity into something physically conceivable. Nowadays, the precision and accuracy of NMR-based diffusometry is good (1% is routinely achievable). The measuring range spans over five decades; however, it is often limited by other factors, such as rapid spin relaxation and convective overturning in the sample.

NMR techniques for self-diffusion studies originated in the year 1949 by the pioneering work of Erwin Hahn (1921–2016). At the time he was an unsupervised post-doc, who also invented, analyzed and experimentally implemented the concept of pulsed NMR spectroscopy on which the modern NMR rests upon [1, 2].

1.1 Early NMR work was field sweep based – Hahn conceived and developed pulsed NMR excitation and detection

Pulsed NMR is based on the concept of “free nuclear precession” of nuclear spins, originally put forward by Felix Bloch in a companion paper to one of the two independent first detections of NMR [3]. The original experimental study was done through a magnetic field sweep approach. His group included William Hansen and Martin Packard [4]. Edward Purcell made another pioneering study together with coworkers Henry Torrey and Robert Pound [5]. They independently detected NMR via a slightly different but still sweep-based set up. Purcell shared the 1952 Nobel Prize with Bloch, and their original announcement papers were published in the same issue of *Physical Review* in 1946 (see http://mriquestions.com/who-discovered-nmr.html for more historic information).

Several reviews in the earlier days of NMR detection have shown that this field was highly influenced by the recent personal experiences with radio and radar equipment in the military services during World War II, and by general electronics instrumental development at the time. In addition, there was abundance of surplus military electronics. My own speculation is that Erwin Hahn (Figure 1.1) was also

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Figure 1.1: The author standing proudly next to Erwin Hahn at a conference lunch break in Chamonix, France in 2005 (private photo).

militarily influenced in his pulsed NMR approach, and its subsequent evolution for generation and detection of spin echoes. He had previous service in the US Navy, teaching pulsed techniques such as sonar and radar for two years (see https://www.aip.org/history-programs/niels-bohr-library/oral-histories/4652). Hahn's 2006 Russell Varian Prize lecture also has historical information from that time [6]. In 1990 he also wrote a highly informative but often overlooked article on the development of NMR and magnetic resonance-based imaging (MRI and NMR microscopy) [7].

1.2 Hahn discovered spin echo formation in pulsed NMR

In a brilliant follow-up study to his less-cited single-pulse paper introducing the pulsed NMR concept experimentally, Hahn correctly described and interpreted spin echo formation effects he had noted when using two or more radiofrequency (rf) pulses [2]. He also showed that they provide a pathway to information for self-diffusion in a sample. The key concept is that echo amplitudes are affected quantitatively through molecular motion in a spatially varying magnetic field. Supported by another NMR pioneer, Charles P. Slichter, Hahn also derived a proper quantifying equation that linked echo attenuation to self-diffusion for a constant magnetic field gradient (i.e., a linearly changing magnetic field along a chosen direction).

1.3 The introduction of FT-NMR led to a transition from sweep to predominantly pulsed NMR detection

The basic signal response in pulsed NMR (the so-called free induction decay [FID]) is a composite signal received from induced transverse sample magnetization, which decays exponentially with time. The FID is thus an unresolved time-domain signal, originating from all pulse-excited nuclei in the sample. Hence, it was not originally very useful outside physics-related studies of single components. However, Fourier transformation of the FID results in a normal frequency-domain spectrum. Such procedures became realistic only through the rapid progress in the field of digital electronics, that is, through the introduction of laboratory computers from around 1970 and onwards, and a faster Fourier transform algorithm (FFT) developed by Cooley and Tukey in 1965 [8] (Figure 1.2).

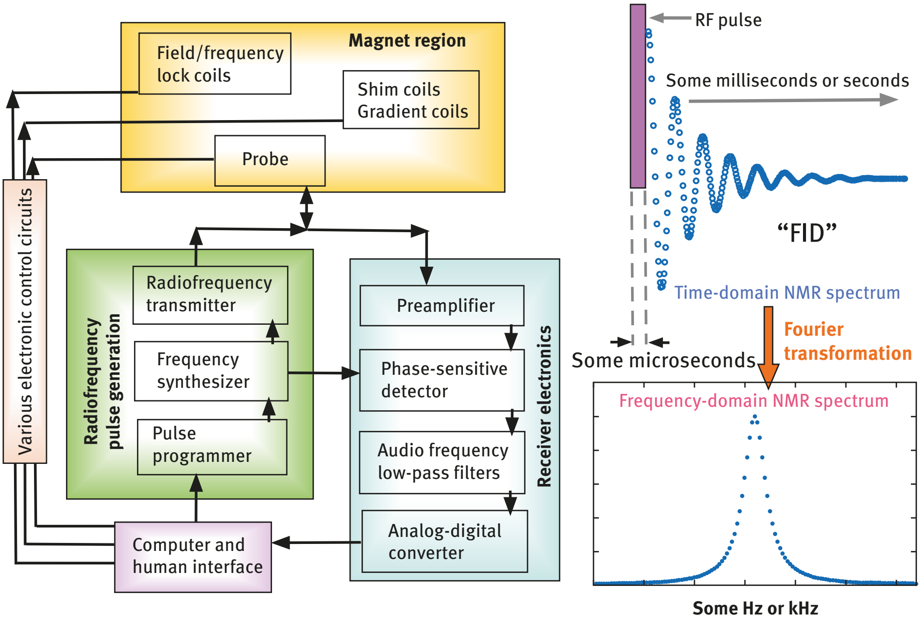

Figure 1.2: A simplified block diagram of a pulsed Fourier transform NMR spectrometer. The basic detection mode is a time-domain FID signal response from the sample, that follow a sequence of one or more relatively strong (e.g., 100 W) rf pulses. The FID is further digitized and Fourier transformed into a frequency domain spectrum. Normally one uses two detection channels rather than one, as further described in Section 1.11. Currently in use are permanent as well as superconducting magnet systems (as illustrated in Figure 4.1). Resistive electromagnets similar to those implied in Figure 1.3 and illustrated in Figure 6.33 were the standard electromagnets used in the 1950s to the late 1970s, but are quite rare nowadays. In a superconducting magnet geometry, the diffusion-detecting magnetic field gradients used in NMR diffusometry are normally applied along the sample axis and in the main magnetic field direction. In a permanent or resistive magnet system, this direction is normally transverse to the tubular-shaped sample axis.

The key step toward pulsed Fourier Transform NMR (FT-NMR) was taken in the late 1960s. Richard R. Ernst and Weston A. Anderson then demonstrated the feasibility of Fourier transformation of digitized time-domain FID's into frequency-domain NMR spectra [9]. They were both working for the NMR manufacturer Varian Associates at the time. Fourier transformation computations of punched paper-tape digitized data were done off-line on a mainframe computer. Ernst was later awarded the 1991 Nobel Prize for FT-NMR, other methodological development and subsequent creation of many new NMR techniques within his research group.

It should be noted that pulsed NMR detection and spin echo formation effects like those discovered by Hahn four decades earlier are a central component of most modern NMR techniques as well. These do include multidimensional NMR (the main tool used in the protein structure-related shared Nobel Prize of 2002), and also MRI, a field in which the Nobel Prize was awarded for physiology or medicine in 2003. Indeed, many feel that it is strange that Hahn did not even share any of these prizes. Apart from the already mentioned spin echo-related basic methodology Hahn and Maxwell also discovered and studied even smaller proton homonuclear and heteronuclear spin–spin couplings through pulsed spin-echo NMR [10, 11]. Similar studies through sweep NMR at the same time also observed this phenomenon [12, 13], although for quite large heteronuclear spin–spin couplings involving ^{31}P and ^{19}F. Along with Hartmann, Hahn also invented and experimentally tested the concept of the so-called Hartmann–Hahn condition of polarization transfer, which makes use of various types of solid-state NMR possible [14]. Many of Hahn's contributions are described in a multiauthor monograph titled "Pulsed Magnetic Resonance: NMR, ESR and Optics. A Recognition of E.L. Hahn" [15]. It appeared a year after the main modern NMR Nobel Prize in chemistry was awarded to Richard R. Ernst in 1991, and a decade before the shared MRI Nobel Prize in medicine or physiology was awarded to Paul Lauterbur and Peter Mansfield.

Sweep NMR remained the standard NMR approach for three early decades, and "high-resolution" ^{1}H NMR methodologies of various types grew hugely in popularity among chemists (Figure 1.3). Their applications were mostly routine analysis and determination of molecular structure in the context of synthetic work in organic chemistry. Other nuclei than protons were not normally accessible for typical commercial instruments. Pulsed NMR became a comparably peripheral technique for decades, which was mostly utilized in the realm of physics and nearby fields. Freeman and Morris described early development of commercially available NMR in a recent paper [16].

As a sideline, it is somewhat amusing to recall that both 1952 Nobel laureates are said to firmly have claimed that NMR was a technique for physicists, and was of no use whatsoever in chemistry. In the early 1950s, the Swedish biological/medical NMR pioneer Erik Odeblad approached Felix Bloch with a

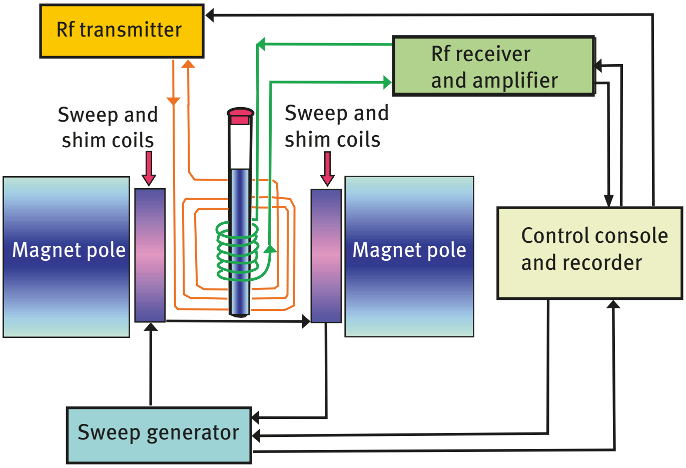

Figure 1.3: Schematic geometry and layout of the magnet/probe environment of a resistive iron magnet NMR system. Rf coils for excitation and detection could be applied transverse to the magnetic field in the hybrid variant drawn here, or both along the sample tube axis (the normal situation). Magnetic field gradients in such geometries were normally applied along the main magnetic field, but transverse to the sample axis.

request to also study biological materials by proton NMR on his equipment, but it was rejected. Later, Odeblad successfully started such studies on his own, building an existing equipment designed and built by Gunnar Lindström (who some years earlier was very close to make the first discovery of chemical shifts in NMR) [17].

1.4 The somewhat dormant technique of magnetic field gradient spin echo NMR diffusometry gradually achieves greater popularity in the 1970s

The number of pulsed NMR spectrometers gradually increased over the years, but they were relatively rare in comparison with the number of continuous wave (CW), magnetic field swept ones. One particular and unique application of pulsed NMR – self-diffusion studies, as pioneered by Hahn – stood out over the years to come and seemed to fascinate a small but diverse range of researchers in physics and chemistry. Such measurements were originally made in static (constant) magnetic field gradients similar to those present in Hahn’s “non-perfect” original instrument set up.

At the time, magnetic field gradients could be achieved either by using a “poor” magnet or by intentionally making the magnetic poles of a typical resistive iron magnet slightly nonparallel, using a thin metal plate shim spacer for alignment. It’s true that the concept of “shimming” in NMR indeed originates from early procedures for geometrical alignment of electromagnetic pole shoes that also used a huge wrench or Insex/Allen tool for tightening the supporting bolts and nuts.

A later development was the introduction of extra current-driven “(Golay) shim coils,” which was meant to be designed to minimize magnetic field gradients across the sample – or to create a correcting field gradient in a chosen direction [19]. Ideally, individual gradients generated in this approach are describable by one of a set of spherical harmonics, and the orthogonal properties of these render possible substantially independent adjustments (Figures 1.4 and 1.5). By proper design, real-world shim coils can be manufactured that also well-approximate such gradient sets over relatively large sample volumes, rather than an ideal point target. An innovative variant was suggested in 2010, in the form of many (originally 24) small, localized coils around the sample area [18]. Of course, modern numeric computational tools help immensely in such context, including design of gradient coils intended for self-diffusion measurement generation. Turner [20] and Hidalgo-Tobon [21] have described basic gradient coil design principles in considerable detail.

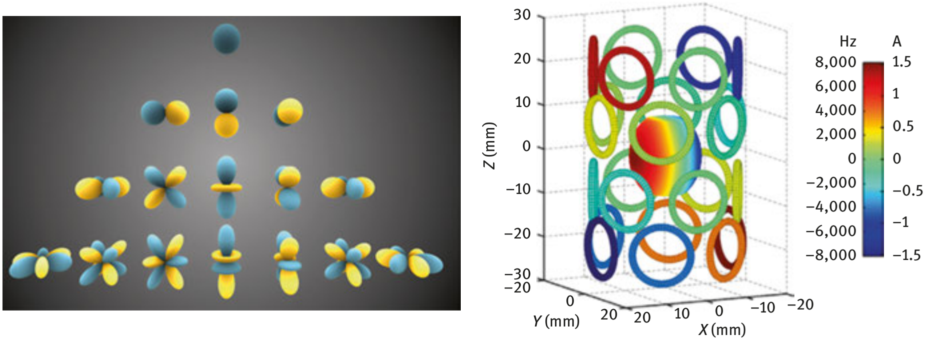

Figure 1.4: (left) Golay invented the concept of ideally orthogonal and independent shim coils in NMR in 1958 based on adding together magnetic field gradients from suitably shaped and oriented current-carrying coils. They should ideally be of the general shape of (real) spherical harmonics, illustrated in the picture. Blue regions are positive and yellow negative. The top one would correspond to the main magnetic field (B_0) and those in the second row to first-order *x, y* and *z* gradients, respectively. Those in the bottom rows illustrate the shape of second- and third-order gradients. (From Wikipedia. Figure courtesy of Inigo Quilez, Wikipedia Creative Commons Attribution Share-Alike 3.0 Unported license). (right) Juchem and coworkers [18] have presented an alternative gradient shaping concept that is based on many small, circular and individually controllable gradient coils around the sample. Reprinted with permission from Juchem et al., J Magn Reson. 2010; 204: 281–28; Copyright (2010) Elsevier.

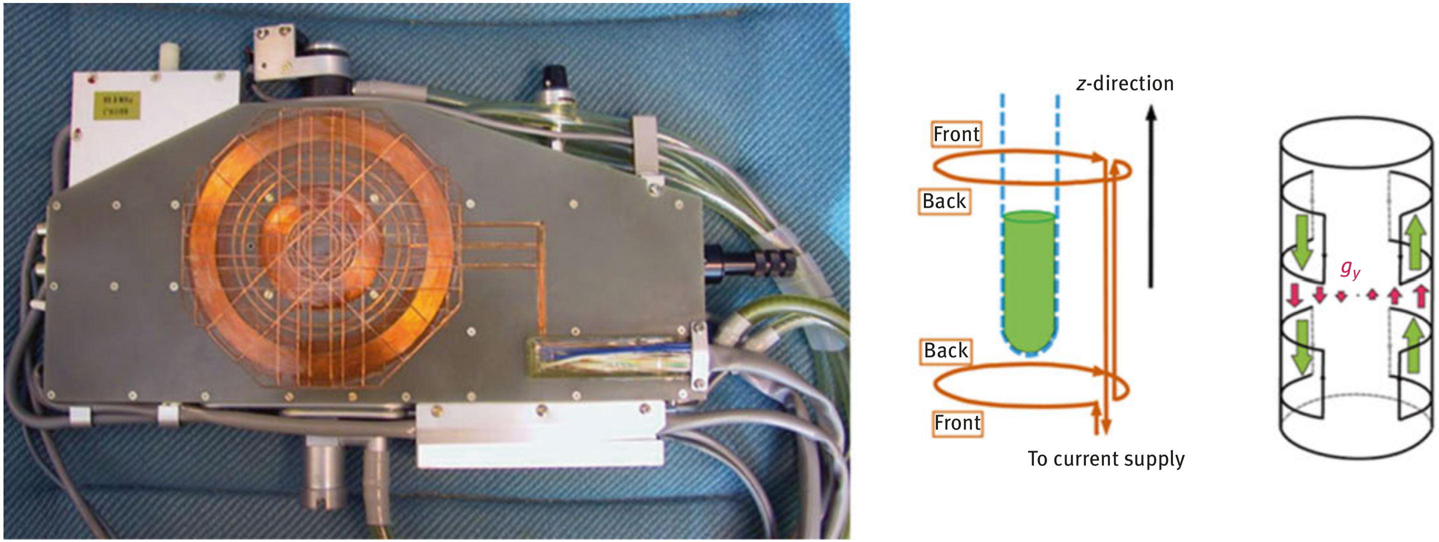

Figure 1.5: (left) Golay-type “shim coils” (one on each side) on the sidewalls of a late 1970s generation multinuclear iron magnet NMR probe (for the JEOL FX-100). Here, the sample axis is formally thought to be in the vertical magnet “*y*-direction” and the main magnetic field is in the *z*-direction. As seen, it was like a dozen individual coils on the probe sidewalls, of varying direction and “order” (i.e., first-, second- and third-order [cf. Figure 1.4]). The probe picture was previously published in Historical: early multi-component FT-PGSE NMR self-diffusion measurements-some personal reflections. *Magn Reson Chem*, 2017; 55: 386–394, and is reproduced here by permission; Copyright (2017) Wiley. (right) Schematic design of *z* and *y* Golay-type “gradient coils” for superconducting magnet geometry. The current leads are omitted for the g_y drawing for better clarity (see also Figures 4.2, 4.3 and 9.2–9.5 for actual coil designs). The sample axis is oriented along the main magnetic field direction (*z*), which on a normal NMR spectrometer type is vertically oriented. In MRI on larger objects, it is horizontal for practical reasons. *y*- and *x*-direction gradient arrangements are identical, although oriented at 90° differently along the cylinder axis. Note that in the context of NMR diffusometry there are normal “shim coil sets” (coarse superconducting ones [adjusted upon magnet installation] as well as finer adjustment user-accessible resistive ones for optimization of magnetic field homogeneity at the sample location) and also specialized ones (*z*-only, or *x*,*y*,*z*-type) with high current capability for actual generation of magnetic field gradients used in NMR diffusometry or imaging context.

1.5 Pulsed field gradient spin echo NMR opens doors for diffusometry and makes magnetic field gradient-based imaging possible

The next methodological step for NMR-based self-diffusion measurements was the idea of generating pulsed magnetic field gradients in the sample through dedicated gradient coils that were directed along the main magnetic field direction. The key concept seems to originate from a somewhat vague suggestion about using "alternating magnetic field gradients" by McCall, Douglass and Anderson in a remarkable 1963 contribution [22]. Here the authors also outline a large family of future application directions for NMR self-diffusion measurements. Stejskal (1932–2011) and his two years older PhD student Tanner achieved an instrumental, theoretical and methodological implementation of the so conceived pulsed magnetic field gradient spin echo (PGSE) NMR technique, as described in a seminal and widely cited 1965 paper [23]. It is part of the content in Tanner's 1966 thesis. Many pioneering application topics were covered here as well; among them were restricted diffusion using PGSE methodology [24–26] (Figure 1.6) and practical utilization of the three-pulse stimulated echo [27].

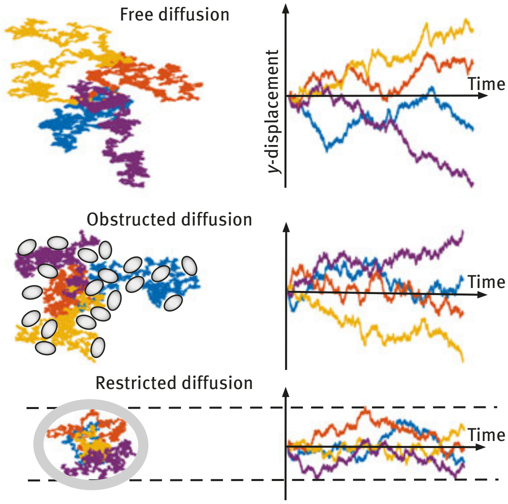

Figure 1.6: Illustrating translational diffusion in two dimensions, under three different conditions. Quantification of these through NMR diffusometry will be further discussed in later chapters.

Stejskal and Tanner did not use the acronym PGSE. Together with my former PhD student Michael E. Moseley, I introduced it in the late 1970s. It has subsequently been accepted and is used in main recent monographs on NMR diffusometry. Previously "PFG NMR" and similar were the most common abbreviations, and are still seen. Many feel that this and similar acronyms do not properly reflect that spin echo formation is at least as important a component of the experiment as the magnetic field gradient one.

Of course, main MRI or NMR microscopy techniques also would not be possible without the use of pulsed magnetic field gradients. Originally, computer control of these was not available as a methodological component in either PGSE diffusometry or NMR-based imaging. Today, it is the natural way – combined with computer-controlled digital data acquisition and data processing.

1.6 Frequency-resolved multicomponent NMR diffusometry emerged in the 1970s

A few years after the introduction of pulsed FT-NMR, Vold and coworkers in 1969 pointed out that multicomponent spin relaxation can be studied using Fourier transformation of signal responses in multi-pulse NMR [28]. They also mentioned that frequency-resolved FT-PGSE should be a feasible tool for quantifying self-diffusion of individual components of a mixture or solution. The first FT-PGSE demonstration experiment appeared almost five years later [29], and a number of chemically significant ones began to emerge in the late 1970s.

A mid-1980s review of FT-PGSE [30], already lists almost 50 applications of NMR-based multicomponent self-diffusion studies in diverse areas of chemistry. With the introduction of specialized commercial NMR instrumentation for this purpose around 1990, the popularity of FT-PGSE has further accelerated and established a valuable, multifaceted and widely available tool. Instrumental FT-PGSE performance is nowadays impressive indeed, and post-processing of data is also much facilitated through the availability of personal computer systems with easy data transfer and data interchange options. Such matters are discussed in greater detail in Chapter 6.

1.7 Computer and NMR spectrometer developments make FT-PGSE easier and a more powerful technique

Off-line data processing of FT-NMR data was initially very awkward and difficult, since spectrometer computers and spectrometer vendor software had no standard options for data transfer for off-line processing. One usually had to be content with basic data output like “peak height” or “peak integral” results for the experiment in question, until like the mid-1980s. Today, such operations are truly trivial, which has paved the way for more advanced data processing options, further described in Chapter 6.

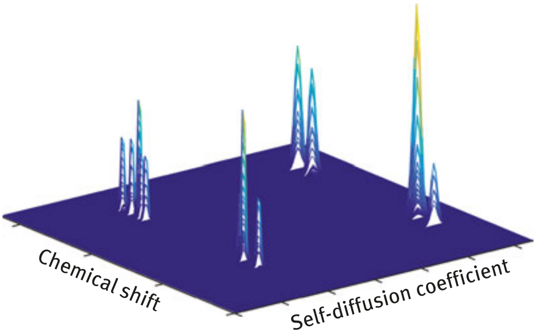

Figure 1.7: A 3D-like DOSY-mode FT-PGSE results display, summarizing *y*-axis self-diffusion and *x*-axis NMR frequency information through contour interpolation. A 2D-like vertical projection contour variant is normally preferred, since peaks then do not visually obscure each other.

In later decades, also a FT-PGSE 2D-type results display mode, generically named "DOSY" (Diffusion Ordered SpectroscopY), have significantly promoted and popularized the use of FT-PGSE for the purpose of quantifying molecular self-diffusion. Charles S. Johnson, Jr. and coworkers introduced it in the early 1990s [31, 32].

In their simplest form, DOSY displays are 2D frequency/self-diffusion contour interpolations over raw one-dimensional PGSE results for successive NMR frequency increments (Figure 1.7). The full spectral frequency dimension resolution (e.g., like 8k or 16k) is normally kept in such data displays, while that in the diffusion dimension is much more limited and is often presented on a logarithmic axis. More complex data processing procedures are discussed or listed in Chapter 6. It should be noted that only some more complex "DOSY variants" would rank as multidimensional NMR in the normal sense (i.e., COSY, NOESY). The introduction of a generic DOSY acronym has unfortunately caused considerable nomenclature and conceptual confusion in the context of NMR diffusometry. "DOSY" (instead of FT-PGSE, or PGSE, and similar) is nowadays absurdly common as a method-implying notation in the scientific literature, even in situations where no 2D- or 3D-type results display was ever generated.

1.8 NMR imaging (MRI and NMR microscopy) techniques emerged in the early 1970s

Followed by the pioneering work of Lauterbur [33], using a sweep spectrometer and magnetic field gradients in selected directions to coarsely produce a 2D-image slice of water-containing capillaries inside a normal NMR tube, magnetic-resonance–based imaging procedures later developed enormously in complexity and scope. By far the largest application volume is in the medical field and can sample whole body volumes. In such context it is named “MRI” – magnetic resonance imaging – to avoid the word “nuclear,” which was thought to be scary.

Detection in NMR imaging is almost exclusively based on proton NMR, and the signal-generating tissue components are primarily water and fat. Image contrast appears naturally through concentration effects, and the presence of less mobile (and more rapidly spin relaxing) bone structures. Further contrast is achievable by making excitation and detection spatially sensitive to nuclear spin relaxation effects. Those may be “naturally” occurring, or be generated through added “contrast agents,” which are normally based on complexes of paramagnetic ions such as Gd^{3+} that affect spin relaxation of water and fat in tissue. A special type of brain MRI is called functional MRI (fMRI), where water spin relaxation effects naturally occur as a result of localized metabolic processes in brain tissue that propagate via alteration of levels of oxygen-binding hemoglobin during “mind work” at a particular brain location.

When dealing with chemical or physical applications on small (centimeter or millimeter-like) objects, one normally uses the notation “NMR microscopy,” rather than “MRI.” A spatial resolution like 10 µm is achievable in NMR microscopy, while routine whole-body MRI spatial image resolution is limited to 1 mm or so. NMR-based imaging techniques are today based on computer-controlled pulsed magnetic field gradient generation (commonly in three dimensions) as well as pulsed rf excitation and detection similar to that of the NMR-based self-diffusion measurements described in this book. Much of the instrumentation components and detection procedures can thus be used interchangeably between PGSE and MRI/NMR microscopy. Further mutual benefit is achieved through shared use and development of measurement procedures and hardware components, such as gradient coil designs and controlling electronics for gradient generation.

Many basic NMR imaging operations and methodological building blocks were originally developed within basic methodological research on small-volume samples. Later it was found as a niche within larger-volume medical MRI. Examples include combining chemical shifts of normal spectroscopic NMR with spatial detection of NMR microscopy, thereby producing chemically separated images of multicomponent materials or tissues. Particularly useful concepts are to spatially detect diffusion and flow, and even to separate them detection-wise, also in heterogeneous materials. “Diffusion MRI” is nowadays a hugely important and advanced technique in medical radiology [34]. Its original medical application relates to (self-diffusion related) contrast in brain tissue after a stroke at the spatial location in question. Notably, corresponding contrast based on spin relaxation is rather poor or nonexistent. The composite experimental procedure is in essence to combine a PGSE sequence with the imaging capabilities of MRI. Flow patterns in blood vessels can similarly be spatially detected and quantified even in three dimensions.

Such methodology originates from a very seminal paper by Callaghan, Eccles and Xia [35], where flow and self-diffusion of a laminarly flowing liquid in a thin tube were separated experimentally and quantified spatially as well. At the time I personally found the implications of the image in Figure 1.8 quite stunning. As evident in many sections of this book, Paul Callaghan (Figure 1.9) has been a giant in its field of context.

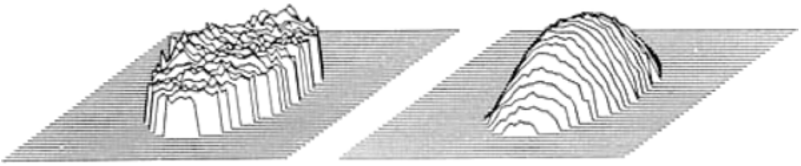

Figure 1.8: Simultaneous NMR microscopy imaging of self-diffusion (left) and laminar flow (right) of $CuSO_4$-doped water in a 0.7 mm inner diameter Teflon tube. For instrumental and data processing reasons at the time, the circular tube appears elliptical and there is a higher relative noise level in the diffusion profile. The flow profile fits almost perfectly to a Poiseuille distribution in space (From Callaghan et al, J Phys E: Sci Instrum. 1988;21:820–822 [35], reproduced with permission, Copyright (1988) IOP Publishing).

Figure 1.9: Sir Paul T. Callaghan (1947–2012), repeating Sir Isaac Newton's echo experiment that was aimed at quantifying the speed of sound. The study was made at the very same location, in Cambridge, September 2011 (private photo). Callaghan was a giant in both NMR microscopy and PGSE methodology, as clearly manifested in some 300 scientific publications and 2 monographs of huge significance [36, 37]. He also acted as an advisor to the New Zealand government in various matters, and was a great public, as well as scientific communicator. Callaghan founded the company Magritek, which presently makes bench-top, portable and educational-type NMR spectrometer systems, as mentioned elsewhere in this book. Apart from his NMR work, the titles of two of his other books illustrate to some extent his wide areas of influence; "Wool to Weta: Transforming New Zealand's Culture and Economy" and the educationally oriented "Are Angels OK?: The Parallel Universes of New Zealand Writers and Scientists." His educational lectures that relate to NMR diffusometry are highly recommended: http://www.magritek.com/support/videos/.

1.8.1 Further reading

Topgaard D. Multidimensional diffusion MRI. J Magn Reson. 2017; 275: 98–113.

1.9 Electrophoretic NMR emerges as a component-resolved technique

In contrast to the simplicity of basic PGSE studies, the closely related electrophoretic NMR (eNMR) technique still remains quite challenging and problematic one in comparison, even several decades after its conceptual introduction [38]. Saarinen and Johnson made the first high-resolution eNMR study in 1988 [39]. Suitable commercial eNMR equipment in the form of a third-party spectrometer add-ons are available and remedies for minimizing the influence of disturbing side effects such as electro-osmosis have also been developed. So, the eNMR feasibility situation is also now much brighter than it was a decade ago. The topic is described in greater detail in Chapter 8.

1.10 Outline, scope and required background for the present book

In the following sections, these families of methodologies will be described in more detail, together with sample applications from the literature and preferred experimental and methodological strategies for achieving optimal results. With some sidelines, the text coverage will focus on liquid samples. It is primarily written for newcomers who themselves consider using, or have already tried NMR diffusometry techniques. Some basic knowledge about NMR theoretical and practical experience with experimental NMR in solution will be assumed. Mathematical formalism and duplicate derivations of already existing key relations will be kept to a minimum.

Familiarity with the simple vector NMR model suffices for comprehension of the text in this book. Readers who feel unfamiliar with any NMR concepts such as 90° pulse, rotating frame, vector model, dephasing, transverse and longitudinal spin relaxation, phase correction, phase cycling and so on should first consult some updating textbook or web resource on basic NMR techniques in solution (see the list “Further reading”). A “pictorial vector model NMR crash course” is provided as well in Section 1.11 of this chapter.

There is also a huge and wide volume of literature in the general area of magnetic field gradient methodology in NMR in the context of MRI and microscopy. Unfortunately, medically MRI-oriented treatises may be misleading and unfair regarding certain aspects. After all, the main methodological MRI pioneers were indeed physicists and chemists, and most key concepts existed well before the widespread use of MRI. A quote I once saw in a popularized article in the newspaper *Newsweek* around 1990 is strikingly diagnostic regarding oddly differing perspectives here. It read something like *“Nuclear Magnetic Resonance used to be an obscure technique, utilized by chemists and physicists. But now the medical community has developed the field into . . ..”*

1.10.1 Further reading

The main recent NMR diffusometry texts were already listed in the Preface of this book.

For general updates on the NMR background, freely available lecture notes by Keeler at www.keeler.ch.cam.ac.uk/index.html look almost perfectly matched with the present chapter. The latest printed book version is more detailed: *Understanding NMR Spectroscopy*. *Nuclear Magnetic Resonance: Concepts and Methods* by Canet is also highly recommended. A well-received and relatively new nonmathematical textbook is by Jacobsen: *NMR Spectroscopy Explained: Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology*. Considerably more advanced NMR textbooks are *Spin Dynamics: Basics of Nuclear Magnetic Resonance* by Levitt and *Principles of Nuclear Magnetic Resonance in One and Two Dimensions* by Ernst, Bodenhausen and Wokaun. A rather unique pictorial textbook by Blümich; *Essential NMR*; is highly recommended as a companion book to the present text.

Other suitable textbooks, web resources and NMR-related software are listed as text or download links at www.ebyte.it/library/StansNmrLinks.html; “Stan’s NMR, MRI, NQR and ESR links.”

An excellent source of links for various NMR resources is www.spincore.com/nmrinfo/ and somewhat similar The Open NMR Project – NMR Wiki can be found at nmrwiki.org.

An excellent web-based and partly interactive introduction to basic NMR by Joseph P. Hornak has been available via this link: https://www.cis.rit.edu/htbooks/nmr/ for quite some time.

Paul T. Callaghan recorded a remarkable series of demonstration lectures on basic NMR and central concepts in NMR diffusometry available at http://www.magritek.com/support/videos/

In addition to these, there are various chapters in the multi-volume set *Encyclopedia of Nuclear Magnetic Resonance* (Wiley, New York, Grant, D.M. and Harris, R.K., original Eds.). It first appeared in 1996 and was later been supplemented with some additional contributions in 2002. If you are fortunate, this hardcover resource could be found at your local library. The original issues appear to be mostly out of print, and are hugely expensive nowadays. This Encyclopedia series morphed in 2007 into an electronic resource, eMagRes, which is still very active. It lists more than 700 articles at the time of writing, written by experts and pioneers in the respective areas (https://onlinelibrary.wiley.com/doi/book/10.1002/9780470034590).

The journal *Concepts in Magnetic Resonance*, Series A and B continuously publishes reviews on numerous specialized topics in NMR, and conceptually bridges a gap between an educational and a research level reading.

1.11 Basic pulsed NMR concepts – a pictorial vector model crash course

An attempt to provide a short summary of text is included here in the form of mainly visual illustrations and brief explaining text. It is meant to serve for basic understanding of the most central elements of NMR spectroscopy, as described within the framework of the semiclassical vector model. The content should be sufficient as background for those with at least basic knowledge of spectroscopy concepts in general and who have had at least some previous experience with NMR. The particular subject of spin relaxation is covered in some additional detail in Chapter 2. For more complete and deeper understanding of various NMR concepts, readers should consult the literature and electronic resources listed in Section 1.10.

1.11.1 Energy levels and frequencies in NMR spectroscopy

NMR spectroscopy is based on quantization of nuclear spin states for nuclei with spin quantum number (I) greater than 0, and corresponding energy differences of these in an applied magnetic field (B_0). The most favorable NMR nucleus is the proton, which has high natural abundance and occurrence and $I = 1/2$ as well as a high magnetogyric ratio (γ). Spin-1/2 nuclei have two spin states, denoted with red and blue spinning symbols in Figure 1.10. The resonance frequency (ν_0) that matches the energy gap between these (ΔE) is thus linearly dependent on the applied magnetic field. In the vector model, the “spins” are thought of as tiny magnets themselves, which precess along the applied magnetic field axis (z) at a rate equal to the NMR frequency, but at a random phase relation in the x–y plane. So, at equilibrium and for too many spins (there would be typically more than 10^{18} in a sample) there will just be a net nuclear magnetization component along the applied field direction, as illustrated in red in the “arrow bundle” part in the upper right section. Note that the vector model view has elements of quantization (energy levels), as well as from classical mechanics (rotation, precession).

1.11.2 The comparably very low frequency of NMR spectroscopy has several consequences

The basic NMR signal is quite attenuated in some respects relative to those of other spectroscopy forms. At thermal equilibrium, relative energy-level populations are given by the Boltzmann distribution. This difference is quite small in NMR – only some parts per million. Spectroscopic NMR frequencies are very low as well, and

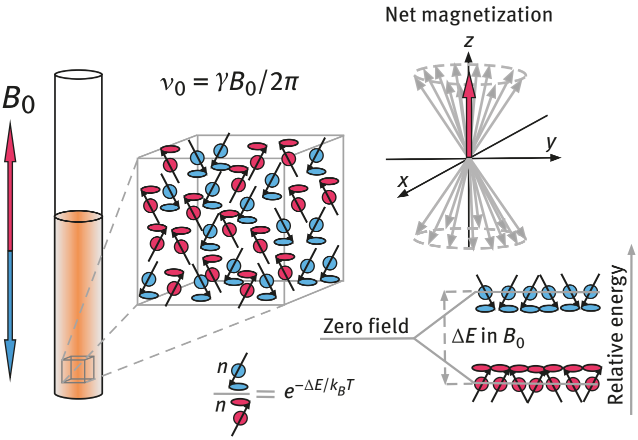

Figure 1.10: Illustrating quantized nuclear magnetization energy levels in an applied magnetic field (see text).

similar to other electromagnetic transitions such as Einstein coefficients for "stimulated absorption and emission," respectively describe the transition probabilities in general and are denoted as B_{12} and B_{21}. As a result of their equality, only the small excess fraction of spins indicated contributes to the actual net energy absorption process (Figure 1.11).

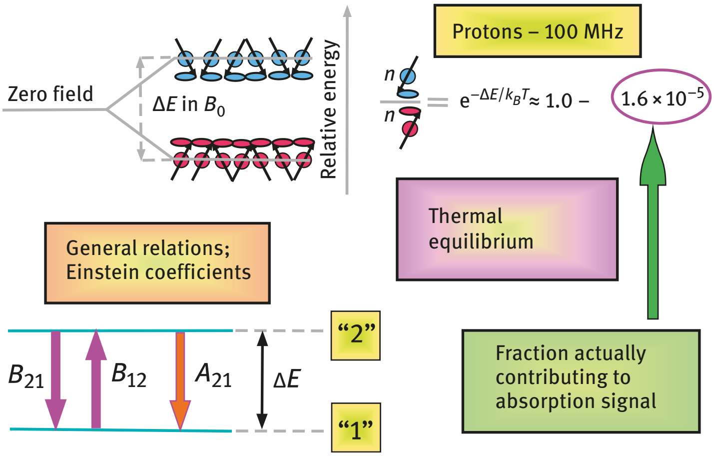

Figure 1.11: Illustrating basic energy-related factors that influence NMR spectroscopy very differently than higher-frequency spectroscopy forms (optical or other).

"Spontaneous emission," represented by A_{21} arrow in the figure is the normal mechanism for maintaining thermal equilibrium of energy level populations. This typically occurs at sub-nanosecond time scales for energy levels relevant to much higher frequency optical spectroscopy. However, the spontaneous emission probability and kinetic rate depend on the frequency or energy gap cubed, and spontaneous emission is totally negligible at NMR frequencies. Instead, various types of non-radiative spin relaxation mechanisms coupled to molecular motion operate, albeit at low rates. Typically re-equilibration after perturbation of the thermal Boltzmann equilibrium typically occurs at millisecond to second time spans. As discussed in the following section, such "spin relaxation" studies are an excellent pathway for measuring molecular reorientational rates rather than translational diffusion, which is the focus of this textbook.

1.11.3 The "rotating frame" description of excitation and emission in NMR

"Excitation" in the vector model NMR description is achieved through a rf electromagnetic signal perpendicular to the main magnetic field. It is considered to be taking place through the magnetic component of the electromagnetic field turning the magnetization along that perpendicular axis, *i.e.*, along some axis in the x–y plane in the coordinate system depicted in Figure 1.10. Visualization of its effect on the spin system here becomes very much simplified by introducing a rotating coordinate system – "the rotating frame." The z-axis is unaffected, but the x–y plane is now thought to rotate at the nominal NMR frequency (in the MHz range), which is set within some kHz of the signal location of interest. In such context one relabels the original "laboratory" axes (x and y) to x' and y', respectively (Figure 1.12).

Now the MHz oscillating/rotating rf field instead becomes stationary or low-frequency (typically Hz to kHz) and is denoted as B_1. It can be thought of as being applied in any direction in the x'–y'-plane by changing its phase. Note that one uses coherent excitation in NMR unlike many other types of spectroscopies (exceptions are many laser-based variants). Signal detection is made in a coherent manner as well, and corresponds to measuring spin magnetization in some selectable direction in the x'–y'-plane – also by selecting a "receiver phase" setting.

Tipping the magnetization from the equilibrium of z-direction can be done by applying a short rf pulse of chosen phase perpendicular to the z-direction. In the rotating frame this corresponds to applying a static B_1 magnetic field in the same direction rather than an MHz rf pulse. The net magnetization will be rotated around the same axis, and a receiver coil can pick it up. In the figure and according to convention, the B_1 field is thought to be oriented along the positive x'-axis and the receiver along the negative y'-axis. The angle rotated depends