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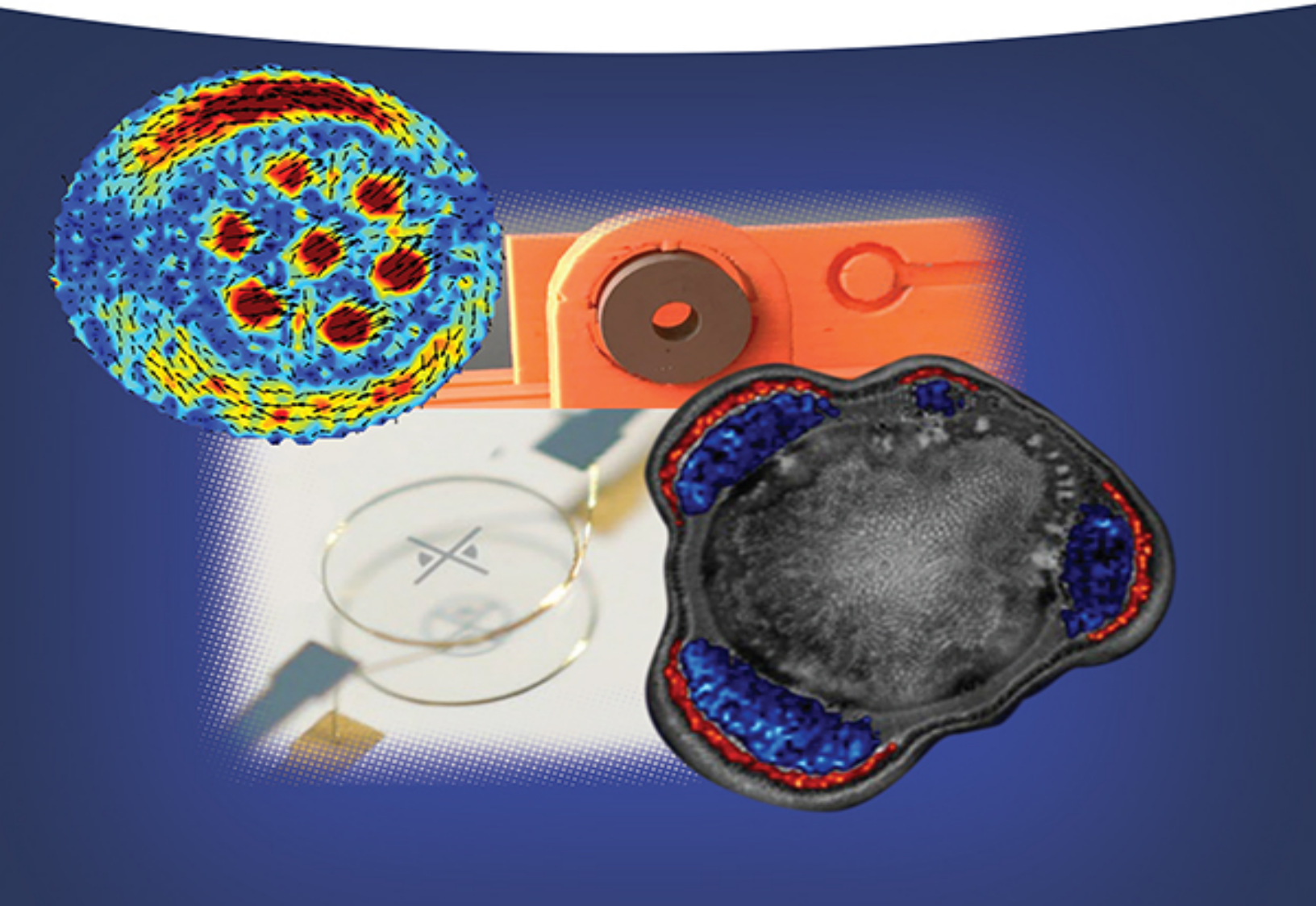
Edited by Sabina Haber-Pohlmeier, Bernhard Blümich, and Luisa Ciobanu

Magnetic Resonance Microscopy

Instrumentation and Applications in Engineering, Life Science, and Energy Research



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Foreword

This book is the fourth in the Wiley-VCH series on *Magnetic Resonance Microscopy*, a series linked in spirit to the *International Conference on Magnetic Resonance Microscopy* (ICMRM). Winfried Kuhn and Bernhard Blümich organized the first meeting of this biannual conference in 1991 in Heidelberg, which led to the first book in this series. That Heidelberg meeting is also when Paul Callaghan burst upon the scene (with his student Yang Xia, who remains active in these meetings) with his new, but now classic, book *Principles of Nuclear Magnetic Resonance Microscopy* (Oxford University Press) – what timing! The wider appearance of magnetic gradient fields in the portfolio of magnetic resonance methods for imaging and studies of molecular transport phenomena was an exciting prospect. It motivated the still ongoing ICMRM conference series and the associated books, which summarize the progress in this field with chapters written by leading experts, among them Nobel Prize awardees Paul Lauterbur and Sir Peter Mansfield as well as Sir Paul Callaghan, who shaped that community like a force of nature from then on until his untimely death in 2012. Also, our brilliant colleague Robert Blinc from Slovenia attended the first ICMRM but had to leave early following an announcement during one of the sessions effectively saying “Professor Blinc, you are needed back in your country,” at which Robert Blinc stood up and left to facilitate the independence of Slovenia from Socialist Federal Republic of Yugoslavia. We were witnessing the birth of a country, a unique experience for most of us. At the third meeting in Würzburg, the brave suggestion to hold a meeting in North America was accepted. Thus, the fourth meeting was in Albuquerque and ICMRM has now a truly international

presence, having ventured as far away as Utsunomiya and Beijing. These meetings, originally dubbed the Heidelberg Meetings, have been at the forefront of amazing developments and accompanying applications of magnetic resonance. Despite the inclusion of the word *microscopy* in their name, they represent the much broader area of magnetic resonance with spatial resolution, which is expressed by the title of the second book from the Albuquerque meeting, *Spatially Resolved Magnetic Resonance*, as well as the organizing *Division of Spatially Resolved Magnetic Resonance* of the *AMPERE Society*. Thanks to the advances over three decades, we have micrometer spatial resolution in magnetic resonance imaging today, while in the early days the word microscopy was understood as a tool to see things hard to visualize just by eye. We believe the broad range of the science and applications of magnetic resonance represented in these meetings is unique to all science and the field displays no hint of imminent stagnation – welcome news to all of us. I would like to close this Foreword with the observation that Bernhard Blümich, who with Winfried Kuhn founded these meetings 30 years ago, is still actively involved here as one of the editors of this book. I salute him for his continued contributions to the field and support of this conference.

Eiichi Fukushima
Albuquerque, 2021

Preface

Magnetic resonance microscopy (MRM) has focused on magnetic resonance imaging (MRI) applied to objects of smaller scale and higher spatial resolution for more than three decades. After the pioneering work by Eccles, Callaghan, Aguayo, Blackband, Johnson et al. in 1986, MRM quickly spread to, among other fields, chemistry, histology, and materials research. Since 1992, the edited book series *Magnetic Resonance Microscopy* has provided an important voice describing the latest developments in spatially resolved magnetic resonance methods and their applications far beyond the scope of medical diagnostics. An excellent introduction to MRM, focusing on the practical aspects of high magnetic fields and on the study of biological systems, was authored in 2017 by Luisa Ciobanu: *Microscopic Magnetic Resonance Imaging: A Practical Perspective* (Pan Stanford, Singapore, 2017). Our book complements this monograph by showing the use of MRM and related techniques in a much broader area and on a wider scale, which extends from chemical engineering to plant research and battery applications, highlighting the interdisciplinary nature of MRM.

The book opens with a section on hardware and methodology, covering aspects of micro-engineering, magnet technology, coil performance, and hyperpolarization to improve signal-to-noise ratio, a major bottleneck of MRM. Specific pulse sequences and developments in the field of mobile nuclear magnetic resonance are further topics of this first chapter. The following parts, 2 and 3, review essential processes such as filtration, multi-phase flows and transport, and a wide range of systems from biomarkers via single cells to plants

and biofilms. Part 4 focuses on energy research, which is becoming increasingly important due to the globally growing environmental problems. It reports on battery types and their developments and how battery states can be recorded and characterized with MRM. However, we would like to point out that only a small number of applications could be addressed in this book. Finally, the last chapter advocates that theory and applications should not be treated separately, because much can be gained from their complementarity.

The main aim of this book is to convince aspiring and established scientists from all fields that MRM is a versatile nuclear magnetic resonance (NMR) method that is capable of answering many questions from both the laboratory and everyday life. The book seeks to inspire a new readership from industries and innovative research directions to create synergies by adding MRM to their expertise.

The editors thank all the authors for contributing their invaluable knowledge to this book during a time challenged by COVID-19. Our thanks also go to the kind staff of the Wiley books department, who helped us with advice and support throughout the whole editing process.

Sabina Haber-Pohlmeier
Luisa Ciobanu
Bernhard Blümich
Summer 2021

Part I
Developments in Hardware and Methods

1
Microengineering Improves MR Sensitivity

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1.1 Introduction

Thirty years have passed since 1991 when Paul Callaghan published his book on magnetic resonance microscopy [1], and many works have subsequently appeared that have made numerous advances in this exciting field possible. Our goal for this chapter is to (informally) revisit some of Callaghan's analysis, to reflect on it, and then take account of some of the advances and insights that have been reported since then.

1.1.1 Comparative Electromagnetic Radiation Imaging

Paul Callaghan's book [1] is perhaps the first publication to consider magnetic resonance imaging (MRI) in the same light as optical microscopy. This will also be our starting point.

Until the advent of super-resolution microscopy, refractive optical microscopy was essentially a radiation scattering method, in which a beam of photons from an independent light source was sent on its way to scatter off objects, followed by traversal of the beam through a focusing objective on its way back to a detector, to thereby reveal the structure and composition of the scattering object. The

limitations of this approach, in terms of resolution, is known as the Abbe limit $\delta = \lambda/(2\ n \sin\theta)$, where n is the refractive index, θ the half-angle of the spot subtended by the lens, and λ the radiation wavelength.

Using radio waves taken for convenience at 300 MHz, a thus interpreted refractive MRI system would have a resolution of ~500 mm, which is a dire prospect for applications of MRI. In a seminal paper, Mansfield et al. [2] reported on a form of nuclear magnetic resonance (NMR) diffraction, in which they considered a solid-state periodic lattice of spins in a macroscopically sized lattice, revealing diffraction patterns on the order of the lattice. As a follow-up to this idea, Blümler et al. [3] and Bernhard Blümich [4] reported (the latter in a paper dedicated to Paul Callaghan) on an interesting intertwining of concepts of the $\boldsymbol{k}$-space vector of refractive MRI and the spatial periodicity of a lattice-like diffractive structure, further exploring diffractive imaging. Blümich's paper contains a few more gems worth discussing, but would distract us too far from the optical viewpoint we are considering here.

Near-field effects can be further exploited to increase the resolution of an imaging system. At optical wavelengths, one is hardly able to extend beyond 200 nm of resolution with currently available light sources. "The diffraction limit of light is 100 times the size of structures that cell biologists study as they characterise events in organelles or membranes," Hari Schroff (NIH/NIBIB) is quoted to say in [5], yet below 200 nm "is where most cellular action is," the author notes. The alternative is to avoid scattering as an imaging paradigm, instead, to image photon sources (also known as quantum emitters).

Interestingly, deep space astronomy always worked this way around by observing photon emitters, so that astronomers only consider objects that were once

themselves sources of radiation, such as stars and their predecessors and descendants. In astronomy, the limit of resolution is therefore not dominated by the wavelength of the radiation, which can be very small when compared to the size and distance of the astronomical objects, but rather by the measuring instrument's principle of operation, its detection sensitivity, and in particular, its effective aperture.

When imaging radiation sources, such as single photon emitters in molecules, we now know that we can greatly improve on the Abbe limit, by about a factor of 10, especially when combined with techniques of stimulated emission and depletion, and one of the numerous variations based on fluorophore emission dynamics. These techniques, which have revolutionized cellular biology and won its inventor Stefan Hell the Nobel Prize in 2014, are of course not accessible to astronomers, who would have to wait too long for excitation signals to pass from observer to object and back again. But for cell biology this is not problematic. Although at currently ~30 nm, the resolution is still far from the desired 1 nm limit, advances in image processing present a feasible route to achieve further improvements. But the technique also raises some questions. Sample preparation is very difficult, and imaging is indirect as fluorophores have to be invasively attached to interesting molecules, almost certainly modifying their behavior.

Magnetic resonance microimaging is a noninvasive technique that is clearly more closely related to stimulated emission depletion (STED) microscopy than to conventional scattering light microscopy. A localized atomic nucleus' spin is a quantum absorber/emitter. By localizing the excitation field spatially or by frequency, a sub-selection of the spins in a sample can be prepared to absorb radiation. Further localization can ensure that emission of radiation energy is again sub-selected, for example along the

geometrical intersection of two orthogonal manifold slices, the one for excitation, the other for emission. A range of further techniques, such as available through relaxation contrast, or phase accumulation, can again further sub-select spins before readout, thereby improving resolution in direct analogy to the techniques of fluorophore emission dynamics. Noninvasive Faraday-detected MRI has been reported down to ~3 µm resolution [6], which is already five orders of magnitude below the radiation wavelength. Nevertheless, even though MRI records radio frequency emissions, this is done almost exclusively through near-field interactions, i.e. by Faraday induction, and not from a beam or ray that requires a lens for focusing.

One of the limitations in NMR is certainly that a single quantum emission event is not yet readily observable as it would be in photonics, even though Dan Rugar showed that a single spin can be observed [7]. Thus all Faraday induction-acquired MRI images have to resort to averaging of a vast number of emission events, and over extended time, to yield useful information. If detection sensitivity were to be increased, fewer emitters could be used, and could perhaps be averaged over shorter times.

1.1.2 Limit of Detection

The statistical polarization level, i.e. that proportion of the total spin population that is available for quantum emission, is an additional cause of lack of signal. Proton spins for example are indistinguishable fermions, with a level occupation that follows

$$\langle N_i \rangle = \frac{1}{\mathrm{e}^{(\varepsilon_i - \mu)/kT} \pm 1} \qquad (1.1)$$

and which collapses to Maxwell–Boltzmann statistics when $e^{(\varepsilon i - \mu)/kT} \gg 1$, because the energy of a proton flip $\gamma\hbar B_0 =$

3.3×10^{-25} J is tiny compared with the thermal energy kT $= 4.11 \times 10^{-21}$ J. Thus at typical equilibrium polarization levels at 11.7 T, a factor of only 10^{-4} in excess in population difference with respect to the Fermi level μ can contribute to the signal. An imaging voxel size is therefore principally limited by polarization, because we find – for microcoils at their limit of detection – a sample containing around 10^{13} spins is needed to form an observable signal. Clearly, this sets a lower concentration limit once the voxel size is specified. For example, at the average size of a single eukaryotic cell of $(10\ \mu m)^3$, containing the required nuclei, implies a concentration of at least 1.66 µM. By increasing polarization, the voxel size is thus principally reduced, or the lower concentration limit is reduced, which could be achieved by resorting to out-of-equilibrium polarization techniques such as parahydrogen-induced polarization (PHiP), signal amplification by reversible exchange (SABRE), or dynamic nuclear polarization (DNP), all of which are rather hard to perform noninvasively, and hard to selectively localize too. We will return to this point shortly. One of the key advantages of MR-based microscopy is the ability to noninvasively reveal molecular composition, correlated with morphology. From the perspective of biological systems, this can be leveraged to monitor, for example, spatially resolved metabolism. To estimate the best achievable spatial resolution (voxel size), signal-to-noise ratio (SNR) should be considered in the context of the metabolically active system. Key parameters are the molecule abundances (concentrations) and timescale that are targeted. Consider a spatially resolved fluxomic investigation: can one estimate a realistic MRI spatial resolution taking into consideration the expected biological dynamics? Alternatively, what is the smallest biological structure with which metabolic flux can be measured –

thus, is it possible to monitor flux at the level of an organelle, single cell, cell cluster, or tissue?

Water is the most abundant molecule in biosystems and can be used as a useful reference from which scaling based on metabolite concentrations can be made. Using only the physical volume of a water molecule (0.03 nm^3), and an optimistic limit of detection (LOD) of 10^{13} spins, then an order of magnitude estimate of the smallest voxel is 0.1 pl (approximately 4.5 µm isotropic resolution). This is approximately the volume of a single red blood cell. Intracellular metabolite concentrations vary over several orders of magnitude, with the most abundant molecules typically in the tens of millimolar regime. The best-case scenario scaling factor is then 10^4 relative to water (assuming [water] = 55 M), and thus the smallest voxel volume increases to 1000 pl (100 µm isotropic resolution). For reference, this would correspond to 10 000 red blood cells or 2 fat cells (volume 600 pl per cell). Can the resolution be improved by signal averaging as a means to enhance SNR? Assuming metabolism is active during the measurement then one must consider the turnover rate of the target metabolite(s) relative to the time over which signal averaging is performed. Enzyme catalytic (second-order) rate constants span several orders of magnitude (k_{cat}/K_M ~10^1–10^9 s^{-1} M^{-1}), with a median of ~10^5 s^{-1} M^{-1} [8]. If the metabolite concentration is 100 mM, then the metabolite will encounter the “median enzyme” with a rate of 10^4 s^{-1}. At this concentration and a volume of 1000 pl, the metabolite concentration would drop below the LOD (~10 mM) in 6000 days giving more than sufficient time for signal averaging. At the diffusion limit 10^9 s^{-1} M^{-1} then, 6 days are required before the signal is not observable. This rough estimate takes many liberties in the assumptions (catabolic and anabolic reactions, multiple pathways,