NMR IMAGING OF FLOW: MAPPING VELOCITIES INSIDE MICROFLUIDIC DEVICES AND SEQUENCE DEVELOPMENT

SUSANNA AHOLA

Department of Physical Sciences
University of Oulu
Finland

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The subject of this thesis is flow imaging by methods based on the nuclear magnetic resonance (NMR) phenomenon. The thesis consists of three related topics: In the first one the feasibility of measuring velocity maps and distributions inside a microfluidic device by pulsed field gradient (PFG) NMR has been demonstrated. The second topic was to investigate microfluidic gas flow using a combination of a special detection technique and a powerful signal enhancement method. The third topic is related to the unambiguous determination of velocities under challenging experimental conditions and introduces a new, improved velocity imaging sequence.

In the first part, well established imaging methods have been used to study water flow inside a micromixer. A surface coil matching the region of interest of the mixer was home built and used in the measurements in order to gain a better signal-to-noise ratio. Velocities inside the mixer have been measured by phase-encoding velocity, with unprecedented spatial resolution. Two dimensional NMR imaging and velocity maps revealed clogging and different manufacturing qualities of the mixers. In addition to the velocity maps, which display an average velocity for spins within one pixel, complete velocity distributions (so called average propagators) were measured. It was found that in the absence of spatial resolution in the third dimension, the propagator data can provide valuable insight to the flow system by revealing overlapping flow passages.

The next topic was gas flow inside a microfluidic device. It was investigated by time-of-flight flow imaging. The measurement of the weak gas signal was enabled by the use of two signal enhancement techniques: remote detection NMR and parahydrogen induced polarization (PHIP). The results demonstrate that a very significant signal enhancement can be achieved by this technique. In the future it may enable the investigation of interesting chemical reactions inside microrreactors.

The third and last topic of the thesis deals with measuring flow by the so called multiecho sequences. When multiecho sequences are used in combination with phase encoding velocity, an error may be introduced: the multiecho sequence may produce a cumulative error to the phase of the magnetization, if it is sensitive to RF pulse imperfections. The problem has been elaborately explained and various solutions discussed, among the newly proposed one. Experimental results demonstrate the performance of the new velocity imaging sequence and show that the new sequence enables the unambiguous determination of velocities even in challenging experimental conditions resulting from inhomogeneous radio frequency fields of the measurement coils.

**Keywords:** nuclear magnetic resonance, NMR imaging, flow, velocity, PHIP, polarization, remote detection, microfluidic devices, velocity mapping, propagators
This work has been carried out in the NMR research group at the Department of Physics of the University of Oulu. I would like to thank the heads of the department, both the present and his predecessor, professors Matti Weckström and Jukka Jokisaari, for placing the facilities at my disposal. I also thank the technical staff and the secretariat on the Department for all their help during production of this thesis.

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Oulu, November 2011

Susanna Ahola


### List of Original Papers

The present thesis consists of an introductory part and four original research papers, which are referred to in the thesis by their Roman numerals:

<table>
<thead>
<tr>
<th>Roman Numeral</th>
<th>Paper Title</th>
<th>Authors</th>
<th>Journal/Volume/Issue</th>
</tr>
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<tbody>
<tr>
<td>II</td>
<td>Velocity distributions in a micromixer measured by NMR imaging.</td>
<td>S. Ahola, V. Telkki and S. Stapf</td>
<td>Manuscript submitted for publication.</td>
</tr>
</tbody>
</table>

The author of this thesis is responsible for the experimental work, data analysis and image processing of Papers I and II. In paper II, the author has also performed the flow simulations. In Paper III, the author performed the experiments together with V. -V. Telkki and V. V. Zhivonitko. The polarization system of Paper III has been designed and built by the other authors. In paper IV the author has carried out the experiments together with Juan Perlo. Data analysis and image processing has been done by the author. The simulations comparing the performance of various multiecho sequences were carried out by Juan Perlo.
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Definitions</th>
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</thead>
<tbody>
<tr>
<td>ALTADENA</td>
<td>Adiabatic longitudinal transport after dissociation engenders net alignment</td>
</tr>
<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
</tr>
<tr>
<td>CP</td>
<td>Carr-Purcell</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EPI</td>
<td>echo planar imaging</td>
</tr>
<tr>
<td>HP</td>
<td>hyper polarization</td>
</tr>
<tr>
<td>MLEV</td>
<td>Malcolm Levitt’s composite pulse decoupling sequence</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>µTAS</td>
<td>miniaturized total chemical analysis system</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMRI</td>
<td>nuclear magnetic resonance imaging</td>
</tr>
<tr>
<td>PASADENA</td>
<td>Para-hydrogen and synthesis allow dramatic enhancement of nuclear alignment</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PEPI</td>
<td>π echo planar imaging</td>
</tr>
<tr>
<td>PHP</td>
<td>parahydrogen-induced polarization</td>
</tr>
<tr>
<td>RARE</td>
<td>rapid acquisition with relaxation enhancement</td>
</tr>
<tr>
<td>RD</td>
<td>remote detection</td>
</tr>
<tr>
<td>RF</td>
<td>radio frequency</td>
</tr>
<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
</tr>
<tr>
<td>TOF</td>
<td>time-of-flight</td>
</tr>
<tr>
<td>UV</td>
<td>ultra violet</td>
</tr>
<tr>
<td>XY</td>
<td>compensated CP sequence based on x and y phase alternation of the refocusing pulses</td>
</tr>
</tbody>
</table>
Contents

Abstract Acknowledgements List of Original Papers Abbreviations
Contents

1 Introduction .................................................................................. 1
1.1 NMR imaging ........................................................................... 1
1.2 Microfluidics and flow ............................................................... 1
1.3 Outline of the thesis ................................................................. 2

2 NMR imaging of flow ................................................................. 3
2.1 The source of the NMR signal ................................................... 3
2.2 Encoding of position and motion ................................................ 4
2.2.1 The k-space and the q-space .................................................. 4
2.2.2 The imaging equation – Fourier transform pairs .................... 6
2.2.3 NMR imaging of flow by phase encoding velocity .................. 6
2.3 Radio frequency surface coils used in NMR ............................... 6

3 Fluid mechanics ............................................................................ 11
3.1 Reynolds number ...................................................................... 11
3.2 Laminar flow ........................................................................... 11

4 Monitoring of fluid motion in a micromixer by dynamic NMR microscopy ........................................... 14
4.1 Introduction .............................................................................. 14
4.2 Average velocity maps inside a chemical micromixer .................. 16
4.3 Velocity distributions inside a chemical micromixer .................... 19

5 Time-of-flight microfluidic gas flow imaging using PHIP and RD NMR .................................................... 22
5.1 Remote detection NMR .............................................................. 22
5.2 Parahydrogen-induced polarization ............................................ 23
5.3 Microfluidic gas flow imaging utilizing PHIP and RD NMR ........ 24

6 Multiecho sequence for velocity imaging in inhomogeneous RF fields ................................................... 26
6.1 Multiecho sequences ............................................................... 28
6.2 The problem ............................................................................ 29
6.3 Solutions .................................................................................. 30
6.4 Velocity imaging sequence ....................................................... 31
6.5 Proof of principle measurements .............................................. 32

7 Summary and conclusions ........................................................... 35

References ....................................................................................... 36
**1 INTRODUCTION**

### 1.1 NMR imaging

Research methods based on the nuclear magnetic resonance (NMR) phenomenon, discovered more than 60 years ago [1, 2], have established an indispensable status in chemical analytics (NMR spectroscopy) and in medical diagnostics (magnetic resonance imaging, MRI). The success of the NMR methods lies in their non-invasiveness and versatility: the available information ranges from spectroscopic to spatial and dynamic information, including their combinations. NMR signal can be detected from a matter in any form; from gas, liquid or solid state. NMR methods are used by physicists, chemists and biochemists in their study of the structure of matter. Today, NMR methods are also widely used in the field of chemical engineering [3]: NMR methods have been applied, for example, to the study of porous media, polymers, fluids and flow of increasing complexity, reactors and reactions. Yet, the number of applications is growing. There are numerous contrast parameters available in NMR imaging [4, 5] - both spectroscopic (chemical shifts, relaxation rates etc.), spatial (spin density) and dynamic (velocity, acceleration, diffusion) with microscopic spatial resolution - making it the most information rich spectroscopic technique. The versatility of NMR is perhaps best highlighted in its history of Nobel prizes: physics (Rabi 1944, Bloch and Purcell 1952), chemistry (Ernst 1991, Wüthrich 2002) and medicine (Lauterbur and Mansfield 2003).

### 1.2 Microfluidics and flow

Microfluidics [6, 7] is a field of science and technology, which deals with manipulation of fluids in a micrometer scale. Microfluidic devices comprise for example micromixers, micro heat exchangers, micro adsorbers, and micro reactors and separators. The first miniaturized analytical devices were presented already in the 1970’s [8, 9] but not until a couple of decades later did the topic start to draw more attention after the miniaturized ‘total chemical analysis system’, µTAS, was proposed by Manz et al[10]. According to Manz, the basic theory of hydrodynamics and diffusion indicates faster and more efficient separations and shorter transport times for µTAS. At the same time the consumption of carrier, reagent, or mobile phase should be much smaller than in macroscopic systems. It is thus expected that the miniaturization combined with integration of multiple functionalities, could result in structures that exceed the performance of traditional large systems and could enable reactions which were not possible before. Furthermore, the possibility of simply duplicating the chip offers the possibility of low cost mass production.

Currently, the most developed application of microfluidic systems is the screening of conditions for protein crystalization [11-13]. In addition, microfluidic devices have also been applied to various bioanalyses[14]: DNA sequencing and separation, polymerase chain reaction (PCR), electrophoresis, enzymatic and immunoassays, cell counting, sorting and culture [15-17]. Microfluidic devices have also been used for examination and manipulation of samples consisting of a single cell [18,19] or a single molecule[20,21]. The key areas of drug discovery, such as chemical synthesis and screening of compounds, may also be improved by microfluidic tools and enable high-throughput screening in drug development [22,23]. In addition, a number of potential high impact applications have been suggested: For example tests for public healthcare [24], implants for therapeutic delivery, tissue engineering, and biosensing [25], and even forensic DNA analysis [26].
It is said, that flow in a microfluidic device is (almost) always laminar [27]. In laminar flow, the molecules flow along direct streamlines, and their position can be predicted quite accurately. There can be some mixing due to diffusion between the streamlines though. The dimensionless Reynolds number is used to characterize flow. Among other variables, it depends on the characteristic length of the system. In microfluidics, already by definition, the characteristic length is quite small, and most often the flow inside a microfluidic device is laminar.

To design a chip for a certain purpose, one must be able to model and simulate the performance of the design. However, because of factors such as surface tension, energy dissipation and fluidic resistance dominates the system, the simulation is non-trivial and reliable experimental methods are needed to verify the results of the simulations. In addition, the microfluidic chips themselves are not giving any information as such, but require a signal detection method. Various detection methods have been reported: chemiluminescence, electrochemiluminescence, electrochemical detection, UV – visible absorption detection, fluorescence, mass spectrometry and NMR methods [28-33]. Optical methods are most frequently used. However, they have certain limitations: The manufacturing material of the chip has to be optically transparent. In addition, most optical methods are of the on face – type. They also require the addition of tracers to the flow, which may alter the flow properties, especially at microfluidic dimensions. Ideally, non-invasive sensors, which can monitor flow in situ, would be preferred. NMR imaging holds great potential in the study of microfluidic devices because of the non-invasiveness and the versatility of the methods. However, because the NMR signal is inherently rather weak, the study of microfluidic devices is quite challenging because of the small amount of sample fluid and demand for very high resolution.

1.3 Outline of the thesis

The thesis is divided into two main parts; the first contains introduction and theoretical background to the topic, as well as summaries of the original journal articles, which are included in the second main part. Chapter 2 contains a brief review on theory of NMR, strictly from the perspective of focusing on NMR imaging of flow. The theoretical part is based on the books of Callaghan [4], Blümich [5] and Haacke et al. [34]. Chapter 3 introduces a couple of relevant concepts from the field of fluid mechanics. Original Papers I and II are summarized in Chapter 4, as they are closely related and both focusing on demonstrating the applicability of NMR imaging to the study of flow inside a chemical micromixer. Papers III and IV are a little bit more independent pieces of work, and they are summarized in Chapters 5 and 6, respectively. Paper III presents a remote detection NMR study of gas flow inside a microfluidic device combined with an elaborate spin polarization technique. Paper IV analyses the use of certain type of imaging sequences in the context of velocity imaging, and presents an amendment which enables the unambiguous determination of velocities by such sequences. Chapter 7 contains a brief overall summary on the thesis.
2 NMR IMAGING OF FLOW

Such nuclei that do not have an even number of both protons and neutrons possess an intrinsic feature called the nuclear spin. The nuclear spin gives rise to a magnetic dipolar moment, which interacts with a magnetic field. In an NMR experiment, the magnetic field at the nuclear site has contributions from the external static magnetic field of the spectrometer and from the switchable, constant field gradients. Also the molecular environment does affect the magnetic field experienced at the nuclear site: For example, nuclei in different chemical positions experience slightly different external magnetic fields, because the electron clouds shielding the nuclei are dissimilar. The magnetic dipole moment of one nucleus has an effect to the field experienced at another nuclear site, hence, their energy levels are coupled. These types of interactions cause fine structure to the NMR spectrum. In this Chapter the focus is only on the behaviour of nuclear magnetization in an external magnetic field, which is space and time dependent by use of pulsed field gradients.

2.1 The source of the NMR signal
The origin of the NMR signal lies within the nuclei of the material under investigation. Nuclei that possess spin will precess once placed in a magnetic field. The spins are precessing with a certain frequency, the Larmor frequency:

\[ \omega = -\gamma B. \]  

(1)

which depends on the strength of the applied magnetic field \( B \) at the nuclear site, and on the property of the nucleus called the gyromagnetic ratio \( \gamma \). The NMR signal is a sum of signals arising from all contributing spins, which precess with the Larmor frequency but each with a random phase (in thermal equilibrium). Therefore, with magnetic field pointing towards one axis, say \( Z \) there is only one component of magnetization, the \( Z \)-component, which is stationary and different from zero due to population differences of energy states. The macroscopic magnetization, which is observed in an NMR or NMR imaging (NMRI) experiment, is used to encode position and motion. In thermal equilibrium, and in temperatures which are not very low, the magnitude of the macroscopic magnetization per unit volume is:

\[ M_z(t) = \rho_s(t) \frac{2\gamma^2 h^2 I(I+1)}{3kT} B_0, \]  

(2)

where \( \rho_s = N/V \) is the number of spins per unit volume, \( B_0 \) is the magnitude of the magnetic field, \( I \) is the spin quantum number, \( T \) is the temperature and \( k \) and \( h \) are the Boltzmann and Dirac constants, respectively. At room temperature the energy levels corresponding to spin ‘up’ and spin ‘down’ state are very close, and thus there is no big difference in the population of the different states. Therefore the magnetization \( M_0 \) is rather small, making NMR an inherently insensitive method. There is a lot of work, also on-going, on enhancing the signal strength by increasing the population difference of the spin states involved in the transition that gives rise to the signal, from that of the thermal equilibrium state. This type of signal enhancement is referred to as hyperpolarization.

For imaging purposes it is reasonable to note that the macroscopic magnetization is a vector quantity, and both its phase and magnitude have to be measured in order to obtain an NMR image. In thermal equilibrium, in a static and homogeneous magnetic field, the magnetization is a vector pointing in the direction of the magnetic field, in this case the \( z \)-

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direction, and the transverse x- and y- components average to zero. Before any signal can be acquired, the magnetization needs to be disturbed from equilibrium. This is typically done with a radio frequency (RF) pulse, which has the effect of flipping the magnetization away from the z-axis. Given the RF pulse has the correct power and duration, the magnetization is tipped to the transverse plane. Now signal can be detected. Also by suitable RF pulse and magnetic field gradient manipulation this magnetization vector can be manipulated to contain information on spin position and motion in addition to the spectroscopic information which the spins alreadypossess. The behaviour of the magnetization vector in magnetic field $B_{stat}$ can be solved from the empirical Bloch equation:

$$\frac{dM}{dt} = \gamma M \times B_{stat} + \frac{1}{T_1}(M_0 - M) + \frac{1}{T_2} M_z \tag{3}$$

in which the relaxation times $T_1$ and $T_2$, longitudinal and transverse, respectively, are also included. When no RF pulse is applied, the magnetic field $B_{stat}$ is the same as $B_0$, the static and homogeneous field of the spectrometer or scanner. During an RF pulse the field $B_{stat} = B_0 + B_{RF}$, the latter being the field of the RF pulse. During a gradient pulse a magnetic field gradient $G$, produced by currents in gradient coils, is also present. Once diverted from equilibrium, by an RF pulse for example, the magnetization vector tends to return back to its equilibrium position, as is described by the relaxation terms.

2.2 Encoding of position and motion

2.2.1 The k-space and the q-space

The signal measured in an NMR experiment is proportional to the transverse magnetization $M_z$. Considering a situation, where the longitudinal magnetization $M_{long}$ is tipped to the transverse plane by a $\pi/2$ RF pulse the magnetization evolves as

$$M_z(t, t) = M_{long} \exp \left(-\frac{t}{T_1} + \frac{i}{2} \omega(t, t) dt \right) \tag{4}$$

in a magnetic field, which is both space- and time-dependent. The Larmor frequency $\omega$ is a function of time as the spins are moving in the space- and time-dependent magnetic field $B(t, t)$, where $t$ is the position vector and $t'$ denotes time. During the time $t$ the spins gain a phase $\phi$ as they are precessing with the Larmor frequency

$$\phi(t, t') = \int_0^t \omega(t, t') dt' = -\frac{i}{2} B(t, t') \tag{5}$$

The expression for the magnetic field can be expanded into a Taylor series:

$$B(x) = B\left|_{x=0} + \frac{\partial B}{\partial x} x + \frac{1}{2} \frac{\partial^2 B}{\partial x^2} x^2 + \ldots \right.$$  \hspace{1cm} \tag{6}

(where it is assumed that the field gradient is along x-axis). In equation (6) the first term refers to a spatially independent magnetic field; this is the 'spectroscopic term'; in NMR spectroscopy the applied magnetic field is homogeneous. The second term refers to a magnetic field which is linearly dependent on position – in other words, a constant field gradient is applied as is done in imaging experiments. The third term refers to non-linear space dependency of the magnetic field. Such situations are possible for example with portable NMR devices, which use single sided permanent magnets, but in this work high field
magnets with homogeneous magnetic fields and constant field gradients throughout the sample volumes have been used.

Thus, the phase becomes

$$\phi(t) = -\gamma \int_0^t \left[ \frac{\partial B_z(t)}{\partial t} + \frac{\partial B_y(t)}{\partial x} \right] \frac{\partial \chi(t)}{\partial x} dt$$

in a magnetic field which depends only linearly on space (thus, the second derivative with respect to x equals zero). The position coordinate of the spin, $\chi(t)$, can be expanded to a MacLaurin series

$$\chi(t) = \chi_0 + \frac{1}{2} a_{m1} t^2 + \ldots ,$$

where $\chi_0$ is the spin’s velocity. Limiting to systems of steady flow, in which the spins move with constant velocities, and thus $a_m = 0$, the phase of the magnetization becomes:

$$\phi(t) = -\gamma \int_0^t \left[ \frac{\partial B_z(t)}{\partial t} + \chi_0 \frac{\partial B_y(t)}{\partial x} \right] dt + \frac{1}{2} a_{m2} t^2$$

Now, in equation (9) the first term refers to NMR spectroscopy: the phase of the magnetization depends on the magnetic field at the nuclear site. That means that it depends on the external field but also on the molecular environment of the nuclei. The second term refers to MRI, in which the information of the position is encoded into the phase of the magnetization by applying a constant field gradient to the magnetic field. The last term refers to measurement of translational motion by diffusion and flow using variable magnetic field gradients. By denoting that

$$G = \left( \frac{\partial B_z}{\partial x}, \frac{\partial B_y}{\partial y}, \frac{\partial B_z}{\partial z} \right)$$

$$m_j = \int_0^t G(t)^i dt$$

the expression for the transverse magnetization becomes:

$$M_j = M_0 e^{i \omega t} e^{-i \omega \xi},$$

the effects of relaxation and spectroscopic terms are ignored. The variable $m_j$ is the $j^{th}$ moment of gradient modulation. The Fourier conjugate variables for position and velocity, i.e. the $k$- and $q$-space variables are defined as

$$k = \frac{\gamma}{2 \pi} \int_0^t G(t)^k dt$$

$$q = \frac{\gamma}{2 \pi} \int_0^t G(t)^q dt$$

differing from the gradient moment $m_j$ and $m_i$ by a factor of $\gamma$. The NMR signal of spins moving with constant velocities in a space- and time dependent magnetic field can be expressed in terms of the variables $k$ and $q$. NMRI is an indirect method: In an imaging
experiment, the NMR signal is always measured in the reciprocal \(k\)-space and NMR flow imaging is often referred to as \(q\)-space imaging. The reciprocal \(k\) and \(q\) spaces are sampled by varying the magnetic field gradients.

2.2.1 Imaging equation—Fourier transform pairs
In a case of stationary spins, \(\nu = 0\) and thus \(M = M_{eq} e^{-\gamma B_0 t}\) (equation (12)). The signal measured in an NMR experiment is directly proportional to the transverse magnetization. The strength of the signal also depends on other factors, such as the gain factors from electronic detection system. These other factors are assumed to be constant throughout the sample volume, and can be absorbed in the effective spin density \(\rho(t)\) which is what we indirectly observe in NMR imaging. The signal measured in \(k\)-space is thus

\[
g(k) = \int \rho(t) e^{i2\pi k \cdot r} dt.
\]

As was already noted before, \(k\) and \(t\) are Fourier conjugate variables. The effective spin density can be resolved from the measured \(k\)-space signal by performing an inverse Fourier transformation:

\[
\rho(t) = \int g(k) e^{i2\pi k \cdot r} dk.
\]

In the equation above, the relaxation effects are ignored, i.e. it is valid for the case where the repetition time of the imaging experiment \(T_R \gg T_1\) and the echo time \(T_E \ll T_2\). Such a case is not practically possible, instead the effective spin density typically contains relaxation contrast and thus \(\rho = \rho(T, T_1, T_2)\). This fact is actually utilized in imaging to resolve materials with different relaxation properties. For example, in medical imaging different tissues are imaged with different imaging parameters such as the repetition rate \(T_R\) and echo time \(T_E\).

2.2.3 NMR imaging of flow by phase encoding velocity
There are different ways to visualize flow by NMR imaging. The methods can be categorized as being either ‘steady state’, ‘time-of-flight’ or ‘phase encoding’. The first two rely on basic imaging techniques: in the steady state method, imaging is performed with such short repetition rate that signal from stationary spins gets saturated, whereas the flowing spins are being refreshed and continue to produce signal. Such methods are used when contrast in flow vessels is needed, like in angiography. In time-of-flight sequences the evolution of a spin package is followed at controlled time intervals. There are numerous ways to tag the spin package, the ‘bolus’, it can involve injecting a contrast medium or it can be simply selective excitation. Following tagging, regular imaging takes place at known time intervals. All of these approaches require that changes in spin position are small over the time required to record an image.

Here, the ‘phase shift’ methods are discussed. The phase encoding of velocity is typically realized by using bipolar pair of gradients, consisting of two gradient pulses of duration \(\Delta\) and separation \(\Delta\). The bipolar pair of gradients can be realized in different ways, for example by a pair of gradients of opposite sign, or by pair of gradients of the same sign but with an RF inversion pulse in between. These examples are shown in Figure 1 below. The effect of the two alternative velocity encoding schemes are the same, what comes to measuring velocity. However, in the spin echo sequence the echo amplitude decays with \(T_2\) as the 180° refocusing pulse has the effect of refocusing the phase differences caused by magnetic field inhomogeneities.

experiment, the NMR signal is always measured in the reciprocal \(k\)-space and NMR flow imaging is often referred to as \(q\)-space imaging. The reciprocal \(k\) and \(q\) spaces are sampled by varying the magnetic field gradients.

2.2.2 The imaging equation—Fourier transform pairs
In a case of stationary spins, \(\nu = 0\) and thus \(M = M_{eq} e^{-\gamma B_0 t}\) (equation (12)). The signal measured in an NMR experiment is directly proportional to the transverse magnetization. The strength of the signal also depends on other factors, such as the gain factors from electronic detection system. These other factors are assumed to be constant throughout the sample volume, and can be absorbed in the effective spin density \(\rho(t)\) which is what we indirectly observe in NMR imaging. The signal measured in \(k\)-space is thus

\[
g(k) = \int \rho(t) e^{i2\pi k \cdot r} dt.
\]

As was already noted before, \(k\) and \(t\) are Fourier conjugate variables. The effective spin density can be resolved from the measured \(k\)-space signal by performing an inverse Fourier transformation:

\[
\rho(t) = \int g(k) e^{i2\pi k \cdot r} dk.
\]

In the equation above, the relaxation effects are ignored, i.e. it is valid for the case where the repetition time of the imaging experiment \(T_R \gg T_1\) and the echo time \(T_E \ll T_2\). Such a case is not practically possible, instead the effective spin density typically contains relaxation contrast and thus \(\rho = \rho(T, T_1, T_2)\). This fact is actually utilized in imaging to resolve materials with different relaxation properties. For example, in medical imaging different tissues are imaged with different imaging parameters such as the repetition rate \(T_R\) and echo time \(T_E\).

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When the first gradient pulse is applied a spin isochromat experiences a magnetic field \( \mathbf{B}(t) \) during the time \( \delta \). Within that time the phase of the spins is “wound” to a certain value \( \phi(t) \).

The gradient is switched off and during the time \( \Delta \) the spins move to a different location \( r_2 \) where they will experience a different magnetic field \( \mathbf{B}(t) \) when the gradient is switched back on again. During the time \( \delta \) the phase of the spins is “unwound” by the amount \( \phi(t) \).

The phase of spins moving with a constant velocity after the bipolar gradient pulses is directly proportional to the velocity of the spins:

\[
\phi = -\gamma \mathbf{a} \cdot \mathbf{G} \mathbf{v} = -2\pi \mathbf{q} \mathbf{a} \mathbf{v}.
\]  

The fact that the phase angle \( \phi \) is only defined to a periodicity of 2\( \pi \), determines the choice of \( \mathbf{q} \) for a given maximum range of velocities.

**The Average Propagator**

In general, a group of spins in a certain volume element can posses a distribution of velocities. Then, by simply measuring the phase, results in an average velocity for spins within that volume element. Another approach is to measure the whole velocity distribution by applying the velocity encoding gradients in a stepwise manner. and, if followed by an imaging module, with one-, two- or three-dimensional spatial resolution. The average propagator \( \mathcal{P}(t',\Delta) \) [36] represents the distribution of displacements from \( t' \) to \( t \) during a time interval \( \Delta \). In pulsed field gradient (PFG) NMR experiment, the signal is measured in the inverse \( q \)-space:

\[
\mathcal{S}(q) = \int \mathcal{P}(t',\Delta) e^{-2\pi i q(t-t')} dt'.
\]  

By defining the average propagator \( \mathcal{P}(R,\Delta) \), which represents the distribution of displacements from \( t' \) to \( t \) during a time interval \( \Delta \), as

\[
\mathcal{P}(R,\Delta) = \int \mathcal{P}(t',\Delta) \delta(t-t') dt'
\]

and noting that the signal depends only on the net displacement \( R = t' - t \) and not on the starting point, the equation (18) of the signal becomes

\[
\mathcal{S}(q) = \int \mathcal{P}(R,\Delta) e^{-2\pi i qR} dR.
\]

The propagator is obtained from the \( q \)-space signal \( \mathcal{S}(q) \) by inverse Fourier transformation. In an NMR experiment the velocity always results from the measured displacement \( R \) during
the time $\Delta$, that is to say $\nu = |R|/\Delta$. Hence the propagator can be also denoted as $\mathcal{P}(R)$, where $\nu$ is the velocity.

### Propagator for self-diffusion and flow

In fluids, there is always some self-diffusion present when the absolute temperature is above absolute zero. Diffusion arises from the random movement of molecules propelled by thermal energy. This means that in practice, when we measure propagators for flow, we need to take into account, that also diffusion is present and has an effect on the results. The propagator for self-diffusion is

$$ \mathcal{P}(R, t) = (4\pi D t)^{-1/2} e^{-|R|^2/4Dt} \quad \text{(21)} $$

where $\mathcal{D}$ is the self-diffusion coefficient of the fluid and $R$ is the displacement vector. Superimposing the diffusion with flow propagator yields

$$ \mathcal{P}(R, t) = (4\pi D t)^{-1/2} e^{-|R-x t|^2/4Dt} \quad \text{(22)} $$

for constant velocities. This propagator is a normalized Gaussian function of dynamic displacement $R$, with a width which increases in time. The combined effect of diffusion and flow to the $q$-space signal is a phase shift due to flow and an attenuation of the echo amplitude due to diffusion.

### 2.3 Radio frequency surface coils used in NMR

In many of the experiments of this thesis radio frequency surface coils have been used both for RF pulsing and signal detection. In Papers I and II a surface coil was used to enhance the signal strength from fluid inside a chemical micromixer. Paper IV introduces a new pulse sequence for unambiguous determination of velocities when using a multiecho sequence in combination with an inhomogeneous RF field. It is thus important to understand how a surface coil is ‘special’: The surface coils produce a very inhomogeneous $B_1$ field as compared with other types of coils commonly used in NMRI, which of course has an effect on the images acquired. The receptivity pattern of a surface coil is described by its magnetic field lines. A magnetic dipole oscillating at a frequency $\omega_0$ at position $R$ from a loop of wire induces a time-varying voltage in the loop; this is how signal is detected in an NMR experiment. According to the principle of reciprocity, the voltage amplitude is directly related to the strength of the magnetic field $B_0$ at the point at the oscillating dipole by a unit current in the loop. The magnetic field produced by a unit current through the receiver coil, $B_0$, can be obtained for any coil geometry from the Biot-Savart law:

$$ B_0 = \mu_0 I d l \times r / r^2 \quad \text{(23)} $$

in which $l$ is the steady current in the coil, $dl$ is a differential element of the wire, $\mu_0$ is the vacuum permeability, $r$ is the displacement vector from the wire element to the point at which the field is being computed. The $B_1$ magnetic field lines for a simple surface coil, consisting of a single, flat, circular loop of wire, are presented in Figure 2 (for a plane perpendicular to the plane of the coil and positioned at the center of the coil). The magnetic field lines appear strongest in the direction of the coil axis, thus the best efficiency is achieved, when the coil is oriented with its axis perpendicular to the external magnetic field $B_0$ of the spectrometer, as the purpose of the coil is to create and measure transverse magnetization.
The sensitive volume of the surface coil is determined by the inhomogeneous distribution of $B_{1\perp}$ (reception) and by nonlinear relationship between $B_{1\perp}$ and the induced transverse magnetization (excitation). Furthermore, the experimental parameters, such as resonance offset, relaxation times and the pulse sequence used, affect the sensitive volume. For example, when pulsing repetitively, the transverse magnetization in the steady state is given by

$$\mathbf{m}(t) = \mathbf{m}(0) \exp(-t/T_2^*)$$

where $T_2^*$ is the transverse relaxation time. The sensitive volume of the surface coil is determined by the inhomogeneous distribution of $B_{1\perp}$ (reception) and by nonlinear relationship between $B_{1\perp}$ and the induced transverse magnetization (excitation). Furthermore, the experimental parameters, such as resonance offset, relaxation times and the pulse sequence used, affect the sensitive volume. For example, when pulsing repetitively, the transverse magnetization in the steady state is given by

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where $T_2^*$ is the transverse relaxation time.
\[ M_s = M_0 \sin \alpha = M_0 \frac{1 - \exp \left\{ -T_p / T_1 \right\}}{1 - \cos \alpha \exp \left\{ -T_p / T_1 \right\}} \sin \alpha \]  

The flip angle \( \alpha \) depends on spatial coordinates, and thus also the transverse magnetization. When the repetition rate is fast, also \( M_s \) is a function of the flip angle. (In addition to this, the spin density (and thus \( M_0 \)) and relaxation rates may appear to depend on the spatial coordinate.) In rapid pulsing, for example, the sensitive volume of the coil is increased as the spins farther away from the coil experience smaller flip angles and return to equilibrium position in less time than spins near the coil. [5]

**Paper I and Paper II: Surface coils mounted on a micromixer**

The surface coil used in the study of a chemical micromixer (Paper I of this work) was made from a single winding of 1.0 mm copper wire, and the shape was roughly rectangular. The size of the coil was chosen to match the region of interest of the micromixer. Thus, the coil was roughly 15 mm x 22 mm. The thickness of the fluid volume in the planar micromixer is 0.8 mm, significantly smaller than the dimensions of the coil and it can be estimated that the field strength is nearly constant in this volume. Thus the inhomogeneous RF-field of the coil did not play a significant role in this study and the experiments were performed in a standard way. The filling factor, however, was significantly improved by using the surface coil; as a careful estimate the filling factor was almost six times better with the surface coil than with a birdcage coil (calculating with the following parameters: birdcage coil i.d. 30mm, length 30mm, thickness 3.8 mm, surface coil sensitive volume 15 mm x 22 mm x 11mm and micromixer fluid volume 66 \( \mu l \)).

**Paper IV: Surface coil with a phantom system of flow in a pipe**

The coil used in the multiecho sequence study (Paper IV of this work) was a circular coil of 15 mm diameter wound from 1.5 mm copper wire. The flow system consisted of a cylindrical tube with inner diameter 3.8 mm. The sample tubes fit well into the sensitive volume of the coil (in depth), but the inhomogeneity of the rf field is significant and non-trivial, because a multiecho sequence is used. Nevertheless, because the effects from the RF field inhomogeneity are the same for each velocity encoding step, it has no effect in the velocity maps measured as long as the signal-to-noise ratio (SNR) is sufficient even for those regions which are furthest away from the surface coil.

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3 FLUID MECHANICS

In Paper IV “Multiecho sequence for velocity imaging in inhomogeneous rf fields” the performance of the new pulse sequence was demonstrated by imaging laminar pipe flow, which is a typical test system for NMR flow experiments. In Papers I, II and III fluid flow in a microfluidic device was studied. Because of the small dimensions, flow of liquids in microfluidic devices is practically always laminar [27]. Hence the concept of laminar flow is essential also for microfluidic devices. This chapter briefly discusses a couple of relevant concepts from the field of fluid mechanics.

3.1 Reynolds number

The Reynolds number [38] is a dimensionless number characterizing flow. It is given by the ratio of the inertial forces to viscous forces. If fluid flows through a pipe and the pressure difference across the ends of the pipe is increased, the fluid will start to flow faster. If the pressure continues to increase, at some point the parabolic velocity distribution is lost and the fluid will start to flow along random paths instead of direct stream lines. It is not only the velocity of the fluid alone which determines this point of transition; it depends also on the fluid properties and on the dimensions of the flow system. At low Reynolds numbers the viscous forces dominate and fluid flows smoothly along streamlines. Such flow is laminar. At higher Reynolds numbers the inertial forces start to dominate and produce random eddies, vortices and other flow fluctuations. This type of flow is turbulent. The different flow regimes can be resolved in terms of the Reynolds number: In pipe flow, experiments show that fully developed laminar flow occurs when Re < 2300 and turbulent flow when Re > 4000. Between 2300 and 4000 both laminar and turbulent flows are possible, depending on other factors such as pipe roughness and flow uniformity. These number are also called transition (or critical) Reynolds numbers. The definition of Reynolds number depends on the case – on the fluid properties such as density, viscosity and velocity and on dimensions of the flow system. For flow in a pipe the Reynolds number is defined as

\[ Re = \frac{\rho v L}{\mu} \]  
(26)

where \( \rho \) is the density of the fluid, \( v \) is the mean velocity of the flow, \( L \) is the characteristic length of the pipe and \( \mu \) is the dynamic viscosity of the fluid.

3.2 Laminar flow

Water, for instance, is a Newtonian fluid - its stress versus rate of strain curve is linear and passes through the origin. The constant of proportionality is known as the viscosity

\[ \tau = \mu \frac{dv}{dr} \]  
(27)

where \( \tau \) is the shear stress exerted by the fluid (‘drag’), \( \mu \) is the fluid viscosity and \( dv/dr \) is the velocity gradient perpendicular to the direction of shear.
**Cylindrical pipe**

A Newtonian, incompressible fluid is flowing in a pipe of length $L$ and radius $r_o (L >> r_o)$ positioned along the $z$-axis. According to the ‘no-slip’ boundary condition, the liquid touching the walls is stationary due to friction. The liquid in the middle of the pipe is moving fastest. It can be imagined that the fluid consists of infinitely thin cylindrical elements of fluid, lamina. A lamina, which is not in the middle or next to the pipe wall experiences a pull from the faster lamina closer to the middle of the pipe to the lamina next to it and a drag force from the slower lamina closer to the walls of the tube. A pressure difference $\Delta p$ is applied across the ends of the pipe to keep the fluid moving. In steady flow, the drag and pull forces and the force due to the applied pressure are balanced as the flow is constant in time. This yields a parabolic velocity profile [39]:

$$v_A(r) = \frac{(r^2 - r_0^2) \Delta p}{4 \mu L} = v_f(0) \left[ 1 - \left( \frac{r}{r_f} \right)^2 \right]$$

(28)

which is illustrated in Figure 4. Equation (28) is also known as the Hagen-Poiseuille law. At the wall $r = r_w$ and velocity becomes zero, and in the middle of the pipe $r = \theta$ and velocity has its maximum value, $v_f(\theta)$.

Figure 4. Parabolic velocity profile of flow in a cylindrical pipe and the propagator presentation of such flow obtained by integration over the position.

The volume flow rate through the pipe is given by integrating over the pipe radius:

$$V = \int_0^L 2\pi r v(r) dr = \frac{\Delta p r_c^4}{8 \mu L}$$

(29)

which is also known as Poiseuille’s law. The mean velocity of the flow is given by the volume flow rate divided by the cross sectional area of the pipe:

$$\bar{v} = \frac{\Delta p r_c^2}{8 \mu L}$$

(30)

This is exactly half of the maximum velocity.

---

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(30)

This is exactly half of the maximum velocity.
\[ v_0(0) = \frac{\Delta p e}{4 \mu L} . \] (31)

As indicated in Figure 4, the propagator for laminar flow in a cylindrical pipe is a rectangle, which means that all velocities in the range from zero to maximum appear with equal weights.

**Rectangular Duct**

The velocity profile for laminar flow in a rectangular duct \[ \text{[40]} \] is dependent on the aspect ratio of the duct: Let us assume a fluid of dynamic viscosity \( \mu \) is flowing steadily under the pressure gradient \( \Delta p \) in a rectangular duct of large aspect ratio \( h/e \). The duct is parallel to \( x \)-axis, and the cross section of the pipe is in the \( y,z \)-plane. The velocity \( u(y,z) \) along the flow direction \( x \) satisfies Poisson equation, \( \nabla^2 u(y,z) = -\frac{\Delta p}{\mu} \), with no-slip boundary conditions at the walls \( u(\pm e/2, z) = 0 \) and \( u(y, \pm h/2) = 0 \). Hence, the velocity profile \( u(y,z) \) is \[ \text{[40]} \]

\[ u(y,z) = u_0 \left[ 1 - \frac{2y^2}{e^2} \right] + \sum_{n=1}^{\infty} \left( -1 \right)^n \frac{32}{(2n-1)^2 \pi^2} \frac{\cosh((2n-1)\pi(z/h))}{\cosh((2n-1)\pi)(z/h))} \times \cos((2n-1)\pi(y/h)) \] (32)

where \( u_0 = \frac{\Delta p e}{\mu} \) is the maximum velocity. The terms of the sum decrease rapidly, roughly as \( 1/n^2 \). The velocity varies over a length scale determined by the thickness \( e \), the height \( h \) being involved through the aspect ratio \( h/e \). Considering now the velocity profile along the longer side of the rectangular pipe, that is the velocity values as a function of \( z \) with fixed values of \( y \). The shape of the velocity profile depends on the aspect ratio \( h/e \). When the aspect ratio \( h/e > 2 \), as in the case of our micromixer \( (h/e = 200 \mu m/65 \mu m = 3) \), the profile is clearly non-parabolic but flat except near the walls.

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4 Monitoring of Fluid Motion in a Micromixer by Dynamic NMR Microscopy

4.1 Introduction

As discussed in Chapter 1, the microfluidics deals with the behaviour, precise control and manipulation of microliter and nanoliter volumes of fluids. To NMR imaging, these are very small amounts of sample, and in quite difficult environment. The latter because typically microfluidic devices are plate-like chips, and result in susceptibility differences inside the sample volume of the NMR spectrometer as many interfaces between different media are present. What is common to all NMR studies on microfluidic devices is, that they all require some sort of “tricks” to enhance signal. These “tricks” may relate to hardware (dedicated coils), remote detection NMR or hyperpolarization of the spins, or combination of these.

Prior to Paper I, NMR spectroscopy on microfluidic devices had first been realized by building miniature coils which matched the volume of interest on or off the chip. This enhanced the signal strength because the filling factor of the coil was better. In ref. [41] a chip-based capillary electrophoresis (CE) device was integrated with NMR spectroscopy, using a dedicated RF coil. In ref. [42] time resolved NMR spectroscopy was used to study changes in protein conformation. The spectra were measured with a commercial solenoidal microcoil. In ref. [43], an integrated NMR planar microcoil was developed for on-chip-NMR spectroscopy. In Paper I, the feasibility of creating velocity maps inside a chemical micromixer was demonstrated for the first time. After the first preliminary measurements, Paper II presents a more thorough quantitative study of the entire velocity distributions inside a similar mixer. Both in Paper I and II, a dedicated coil was built to increase the SNR of the experiments. Mixer and coil mounted at the probe body are shown in the photograph of Figure 5:

![Figure 5](image)

Micromixer used in Paper I and II:

In Paper I and Paper II of this work flow imaging inside a chemical micromixer was done. A micromixer (type MOH 030) was obtained from Mikroglas chemtech GmbH, Mainz, Germany (www.mikroglas.de). The mixer designed to mix two miscible or immiscible fluids -liquids or gases- together by “multilamination”. A schematic presentation of the micromixer

![Micromixer](image)
is shown in Figure 6. The micromixer consists of a three layer design, with a cover plate on each side. The cross sections of the channels are rectangular. The two fluids that are mixed flow into the chambers on the first level of thickness 0.3 mm and a volume of 28 µl per fluid. On the next level, the fluid flow is split into 15 different vertical channels (per fluid), which are then combined in the third level in an alternating fashion to enhance the mixing which occurs by diffusion. The channel dimensions on the third level are 65 µm width, 200 µm height and 45 µm separation at the end of the triangular section. Following this section is a 25 mm long mixing chamber, the width of which decreases towards the outlet of the mixer.

![Figure 6. A schematic presentation of the micromixer used in the study. A) Layer structure of the micromixer: the micromixer consists of three plates and a cover plate on each side. The lowest mixer plate contains two large inlets. The next plate has 30 channels perpendicular to the xz-plane, connecting the inlets to the interlaced thin channels. The last plate contains the thin channels, and the mixing chamber, where all the 30 channels combine into one volume. B) Labelling of the inlets and channels, and coordinate system used.](image)

The micromixer is manufactured of a photosensitive Foturan glass. According to the manufacturer, material thicknesses of up to 2 mm can be illuminated. Smallest structures of 25 µm are possible, with a roughness of 1 µm. The manufacturing process of the chip is the following [44]: First the desired areas of the Foturan glass plate are exposed to ultraviolet (UV) light by using a mask. Silver atoms are formed in the illuminated areas. Then the plate goes through heat treatment, during which the glass crystallizes around the silver atoms. Etching is then done with acid. At room temperature, the crystalline regions have 20 times higher etching rate than the vitreous regions. This is how one plate of the micromixer is made. After all the plates are done, they are joined together by diffusion bonding, glass soldering or gluing, depending on the materials or requirements of the chip.

In the two studies, two similar but physically different micromixers were used. We were able to resolve notable differences in the two mixers, as can be seen in the static spin echo images of the two distinct micromixers in Figure 7.

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There are dark regions in the older mixer, which are not present in the newer piece. Likely these regions result from some interfaces, of glue or air for example, between the glass plates. There can be differences in the manufacturing process of the two chips; here probably in the method which is used in joining the etched glass plates together.

4.2 Average velocity maps inside a chemical micromixer

Paper I reports our preliminary study on performing velocity imaging on microfluidic devices. We used a standard, velocity encoded spinecho sequence, presented in Figure 8, to create two dimensional maps of average velocity inside the chemical micromixer. The sequence required two scans, one with the velocity encoding gradient switched on, and a reference scan with zero value of $G_{enc}$.

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mixing chamber, coherent flow structures are immediately lost.

In Figure 9, velocity maps displaying velocity component $v_y$, which is perpendicular to the main flow axis, are shown. In Figure 9A, both pumps were running at 5 ml/h to provide identical volume flow rates of both liquids (resulting in identical velocities inside the chambers since both total fluid volume and channel dimensions are identical). In Figure 9B one of the pumps was stopped. In the bottom reservoir, a rather regular flow pattern was identified (blue corresponds to leftward motion, red to rightward motion). In the upper chamber larger velocities toward the left are found, and no velocity was detected when the pump is switched off (Figure 9B). In the channels within the triangular section, the left- and rightward velocity components tend to grow toward the outer edge where the inclination angle relative to the main flow axis becomes larger. Due to the choice of velocity ranges, the outermost velocities are not depicted correctly (identifiable by an apparent change in sign where a phase wrap of $2\pi$ occurs in the velocity encoding angle). At the beginning of the mixing chamber, coherent flow structures are immediately lost.
The results for the "upward" velocity component $v_z$ are shown in Figure 10A and B. The only difference to the experiments in Figure 9 was the direction of the velocity encoding gradients, and their strength which have been adjusted to a somewhat wider range of velocities. The measurements were carried out under identical conditions with those of Figure 9.

Figure 9. Velocity images for the $v_z$-component. A) Both pumps on at 5 ml/h. B) One of the pumps stopped. [Paper I]

Figure 10. Velocity images for the $v_z$-component. A) Both pumps on at 5 ml/h. B) One of the pumps stopped. [Paper I]
From these velocity maps, some defects of the micromixer could be identified. Clogging is quite a typical problem with microfluidic devices and it is also visible in this measurement data. The leftmost channel of the triangular section does not contribute to flow as the velocity values oscillate randomly about zero. This can be seen from Figure 9A. The channel is indeed filled with liquid, as it is clearly visible in the still image of Figure 7, right. Note that this clogging could not be detected from the static NMR image or by visual inspection. It also appears that not all the channels contribute evenly to the flow. Some of the channels feature lower-than-average velocities throughout their length, see for example the channels with numbers 7, 9 and 14 in Figure 10 A and B. This may be due to variations in effective channel diameter; a constriction would lead to an increase of the pressure drop and thus to a lower velocity and smaller contribution to the net fluid transport. In the still image (Figure 7, right), two of these three channels were indeed identified as having smaller signal intensity, i.e., possibly a smaller effective diameter. The contrast is enhanced when velocities are determined directly.

These velocity maps display average velocities per pixel. Bearing in mind that the mixer is a three dimensional object, and our images have two dimensional spatial resolution it must be noted that the spins inside most pixels actually contain a rather wide range of velocities because the walls of the channels are always present. In addition, there are areas where two different channels overlap. Unfortunately spatial resolution in the third dimension was not practically possible.

### 4.3 Velocity distributions inside a chemical micromixer

In the measurements of Paper II, the velocity imaging sequence was improved by modifying the velocity encoding gradients. The velocity encoding gradient $\mathbf{G}_c$ is now applied in a step wise manner to scan the whole velocity distribution inside the chemical micromixer. The sequence used in the measurements of Paper II is presented in Figure 11.

![Figure 11](image1.png)

**Figure 11.** The pulse sequence used in Paper II. The sequence is almost identical to the sequence used in Paper I, but this time the value of the velocity encoding gradient changed in a step-wise manner ranging from $G_{\text{mic}}$ to $-G_{\text{mic}}$, collecting multiple datapoints from the $\phi$-space and thus measuring the whole distribution of velocities, that is, the propagator.

The measurement produces velocity distribution inside a chemical micromixer with 2D spatial resolution. This data can be used for creating a 2D velocity map of the mixer, for example, as presented in Figure 12. There are alternative ways for creating the map; the problem is, which value of velocity to choose from a distribution of values, to represent the spins within that pixel. In Figure 12A the most probable value of velocity per pixel is

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![Figure 11](image2.png)

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Ideally, the image encoding would be done in all three dimensions, with spatial resolution high enough to assume a single value of velocity per pixel, to avoid overlapping different channels; the slow spins of the large inlet and the fast spins of the thin channel.

Figure 12. Flowmaps inside the micromixer. (A) A flowmap inside the micromixer displaying the most probable velocity. (B) A flowmap inside the micromixer displaying average velocity per pixel, calculated from the propagator data. (C) Insert to figure A (to the area denoted with the dashed line rectangle). (D) Insert to figure B (to the area denoted with the dashed line rectangular area). (E) Propagator data from a pixel/voxel that contains both slow and fast spins, from large and thin channels. The dashed line cross in figure D indicates roughly the position where the propagator has been selected from. Note that the scale in Figures C and D is different from Figures A and B.

displayed. This is usually the method of choice when using this so called “propagator” formalism in creating velocity maps. Figure 12B displays the average velocity per pixel, obtained from the velocity distribution. Basically the same could be obtained from measuring only with one value of velocity encoding gradient, as is done in Paper I. Figure 12C is an insert to Figure 12A. It can be seen, that in the areas where the large inlet overlaps with the thin channels, the most probable velocity of course corresponds to that of the slow spins in the large inlet and it appears that the thin channels are “missing” in these areas. Figure 12D is an insert to Figure 12B. In the case of average velocity map, the thin channels are not missing, but display a lower velocity than elsewhere in the same channels. This is because in the 2D measurement the slow spins from the large inlet have a major impact on the average. Figure 12E shows the velocity distribution inside a pixel, which contains the two different channels; the slow spins of the large inlet and the fast spins of the thin channel.

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flow channels. However, by adding another indirect dimension to the experiment the duration would be further increased: not only multiplied by the number of pixels in the third dimension, but more signal accumulations would be required to gain an acceptable signal-to-noise ratio. In many cases this is not practically possible. The propagator data can compensate for the lack of the third dimension imaging data. In some instances, the propagator data reveals the two distinct velocity distributions within a pixel, as in Figure 12E.

We did also investigate how well the experimental velocity distributions correspond to theoretical distributions. Figure 13 presents some exemplary propagators together with a theoretical curve calculated for flow in a rectangular duct of similar size and shape.

![Figure 13](image)

**Figure 13.** Exemplary propagators measured from the thin channels parallel to z-axis. (A) A good match between the theoretical and experimental propagator, here for channel 1. (B) Agreement in the velocity range, but some difference in the shape, can be seen in the propagator of channel 10. Different velocity ranges: The velocities are (C) slower or (D) faster for some channels. The black and white outline graph (E) displays the area from which the propagators were obtained.

In Figure 13 the theoretical propagator is the same in each graph. We found that overall we have quite a good agreement between the theoretical propagators and measured ones. Some deviations were found, indicating that not all the channels are the same in size and shape.

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Remote detection (RD) NMR technique enables tremendous signal enhancement by separating the signal encoding and detection stages. The technique is ideal for flow systems with poor filling factor and probe fluids with sufficiently long $T_1$, for example, for a fluid flowing through a microfluidic chip. In the case of gas flow, the poor filling factor is even bigger issue, because, as compared to liquids, the density of gas is about three orders of magnitude lower. Thus even with the sensitivity enhancement given by the RD method, NMR imaging of gas flow at microscale dimensions is practically impossible and requires a further signal boost from hyperpolarization. In the study of Paper III, parahydrogen-induced polarization (PHIP) technique [47] was used to increase the signal from that of thermal equilibrium. This enabled the NMR imaging of microfluidic gas flow inside a microfluidic device by RD NMR. Paper III is the first report of PHIP gas flow imaging in a microfluidic device by RD NMR.

5.1 Remote detection NMR

Traditionally in NMR the same hardware, essentially the same RF coil, is used both for encoding and detecting the signal. To emphasize this, the traditional way can also be called direct detection. In remote detection NMR [45, 46] these steps are performed with separate RF hardware, as depicted in Figure 14.

The RD pulse sequence for measuring an indirectly detected NMR spectrum consists of three main parts: encoding step, travel delay and detect. First, an excitation pulse is applied. Then the time domain signal is stored after incremented evolution times $t$ as long-living longitudinal magnetization. The magnetization will remain intact as long as the longitudinal relaxation time $T_1$ of the spin system. Following encoding, the spins flow into the detection system, where the signal is detected after the magnetization is flipped back to the transverse plane by a readout RF pulse. The readout pulse and acquisition is repeated many times to cover all the sample fluid which was encoded in the encoding coil, as the residence time in the detection solenoid is typically shorter than in the encoding coil. In other words, the signal is measured with different travel times, and thus time-of-flight (TOF) flow information is naturally present in a remote detection experiment. The spectrum has to be acquired in an indirect fashion – point by point, by incrementing the evolution time of the magnetization, and adding another dimension to the NMR experiment. The acquisition of complex NMR signal requires repeating each reconstruction twice in a phase sensitive fashion. Time-of-flight images can be acquired by applying spatial phase encoding gradient pulses after the
excitation pulse in the encoding coil volume. These TOF images give insight to the flow and can be used to determine the flow velocities, for example. Similarly, also velocity encoding gradients can be included in the RD pulse sequence [48]. It is possible to obtain data both in the spectral and spatial dimensions [49] but as all the data is collected in an indirect fashion the experimental times then become exceedingly long.

### 5.2 Parahydrogen-induced polarization

Significant signal enhancement in NMR can be achieved by parahydrogen induced polarization [47]. The origin of this signal gain is in molecular hydrogen $H_2$, which has both symmetric and antisymmetric nuclear spin states. Molecules in the different states are referred to as ortho-$H_2$ and parahydrogen ($\rho-H_2$), respectively. The spin state behavior of $\rho-H_2$ is interesting for NMR, the application of which is often limited because of the low sensitivity resulting from the small population differences between different spin states in thermal equilibrium. All of the $\rho-H_2$ population is in a singlet state, and it is fairly easy to enrich $H_2$ with $\rho-H_2$. At room temperature, the ratio between ortho and para populations is 3:1. At low temperatures, the para-state becomes more popular, and for example in liquid nitrogen temperature (77 K) the population ratio is 1:1. The $\rho-H_2$ enrichment is quasi stable, and can be maintained for days, but with a catalyst the transition can happen much faster. This implies that a very high NMR signal enhancement could be available, and that the polarization of the spins is easily achieved and manipulated.

However, in the $H_2$ molecule the hydrogen nuclei are in magnetically equivalent positions. That results in a total spin angular momentum of zero for the para isomer. This means that the parahydrogen $H_2$ molecule cannot be detected by NMR. The high polarization of the parahydrogen molecule can benefit an NMR experiment only if the magnetic equivalence of the two hydrogen nuclei is broken. That can be achieved by a hydrogenation reaction, and in particular by such a hydrogenation reaction, where the two hydrogen nuclei end up in magnetically inequivalent positions in the product. One such reaction is the hydrogenation of propene into propylene. The art is to perform the hydrogenation reaction in such conditions, that the polarization is preserved. The success of polarization transfer depends much on the choice of catalyst. The catalyst must be such that it enables the pairwise addition of $\rho-H_2$ nuclei to the product molecule. This is best achieved by a homogeneous catalyst. However the catalyst is typically toxic, and thus one would prefer to use a heterogeneous catalysis, which can yield a catalyst free product. An immobilized homogeneous catalyst achieves the benefits from both: it enables the pairwise addition of hydrogen to the product, but also allows the separation of the product from the catalyst, extending the applicability of PHIP to systems that do not tolerate the toxic catalyst.

Two different reaction scenarios for transferring the polarization are known: “Para-hydrogen and synthesis allow dramatic enhancement of nuclear alignment” (PASADENA) [47] and “Adiabatic longitudinal transport after dissociation engenders net alignment” (ALTADENA) [50]. PASADENA involves the sudden breaking of magnetic equivalence when hydrogenation is carried out in a high magnetic field. ALTADENA involves hydrogenation in a low magnetic field followed by adiabatic transfer of the product to high magnetic field and also results in non-equilibrium population distribution of the spin states. PASADENA and ALTADENA produce different NMR spectral patterns, because the population of spin states in the hydrogenation reaction products is different in the schemes.

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5.3 Microfluidic gas flow imaging utilizing PHIP and RD NMR

In original Paper III microfluidic gas flow was studied. NMR imaging of gas flow at a microscopic scale was enabled by PHIP and RD NMR. This was the first demonstration of PHIP gas flow imaging in a microfluidic device by RD NMR. Earlier, gas flow in a microfluidic device has been studied by using hyperpolarized xenon [51, 52]. However, using PHIP instead of Xenon has some advantages: PHIP does not require expensive noble gases or instrumentation, and (even) better enhancement factors can be achieved (in natural abundance). In addition, unlike Xe, the noble gas, parahydrogen is not inert, and it can take part in scientifically and technologically more interesting chemical reactions. For this reason it is very interesting to apply PHIP technique to microfluidic devices. With the same technique, RD NMR and PHIP, a reaction inside a microreactor could be monitored.

The experimental scheme is summarized in Figure 15. The parahydrogen enriched gas is mixed with propene and then taken to reaction chamber. In the chamber the mixture is heated and flown through immobilized homogeneous Wilkinson’s catalyst [53]. Hydrogenation reaction takes place in Earth’s magnetic field, and the gas mixture is then adiabatically transferred to the NMR spectrometer. There it first enters the microreactor inside an encoding coil and a gradient system. Thereafter the gas flows through the detection coil and finally out from the spectrometer. Inside the NMR RF coils (Figure 15b), the gas experiences the NMR pulse sequence (Figure 15d) used to encode and detect the signal. Signal is measured from the detection coil, repeatedly at different travel times to cover all the encoded fluid. More details are disclosed in the original publication and its supporting material.

First the overall sensitivity enhancement of the method was demonstrated by measuring a spectrum of continuously flowing polarized gas mixture. A 600-fold enhancement comes from the RD method. The pulse length in the encoding coil is 600 times longer than in the detection coil, and by the principle of reciprocity this means that the detection coil is also 600 times more sensitive. By comparing the signal from polarized gas mixture to that of thermally polarized gas, an enhancement of 80 was obtained by PHIP. Together these yield 48000-fold signal enhancement, present in the spectrum of (Figure 15c).
Next, PHIP propane flow through a capillary was measured (Figure 16b). The TOF panels show very little dispersion and the flow appears as “plug flow”. This is because, although the gas flow here is laminar (the Reynolds number is very small), the diffusional mixing is very fast compared to the travel time. This means that the diffusion in the transverse direction mixes the different flow lamellas many times before the signal is detected. For comparison, laminar water flow in the same capillary was measured (Figure 16a), and here the TOF panels show a lot of dispersion due to laminar flow. In water, the diffusion happens much slower than the travel times. These different types of flow and their RD travel time curves were more comprehensively analyzed by Telkkki et al. [54].

Whilst the results from the water and gas flow in a capillary were exactly as expected, the results from the microchips (Figure 16c and d) were somewhat surprising. In chip 1 the gas appeared to flow along the edges of the chamber (Figure 16c). The geometry of the chip was found to be different from that designed, the center of the chamber being much thinner than the edges. In chip 2, the left side channel seemed to contain less gas than the right one, although designed to be equally thin. Evidently, the TOF images revealed flaws in the chips.
These flaws were also confirmed by water NMR images and by using a dye and optical detection.

Figure 16. TOF RD MRI images measured from microfluidic systems. a) Water and b-d) hyperpolarized propane flowing in the capillary tubing leading through the encoding coil, a) and b), and microfluidic chips, c) and d). The flow channels are outlined with white lines. The first images on the left are the result of summation of subsequent images measured at different travel time instances (time projection). The travel time instances are indicated in milliseconds. The spatial resolution in the y and z directions is 0.5x1.6 mm and 1.5x2.5 mm, respectively, depending on the experiment. Paper [11].

These measurement results show, that by combining PHIP with RD NMR techniques a $10^4$–$10^5$-fold sensitivity enhancement can be achieved, which enables noninvasive, traceless gas flow profiling in microfluidic devices. The TOF images of flow reveal differences between different types of flow (here gas and liquid flow) and expose manufacturing imperfections. In theory, PHIP technique can yield 11 times better signal enhancement as compared with HP.
Xe (Paper III supplementary information). This time, in practice 88 times better enhancement was obtained. (It should be noted that neither the HP Xe experiment nor our parahydrogen experiment used in this comparison was performed under optimized conditions, and that explain why the theoretical and experimental numbers do not match.) Bearing in mind the other benefits of PHIP technique, the cost effectiveness and reactivity, PHIP combined with RD NMR indeed is a very powerful technique for the study of microfluidic devices!
In Paper IV a new multiecho sequence called CP/CPMG-RARE is presented for measuring velocity distributions with inhomogeneous RF fields. In addition, as demonstrated by the experiments of the study, the inhomogeneities may distort the measurement of velocity and propagators even when using birdcage coils which produce a highly homogeneous RF field. In the context of phase encoding velocity, correlation between displacement and relaxation time can be measured with multiecho sequences. It would thus be of interest to apply these sequences also in the study of micromixers. However, the combination of phase encoding of velocity and a multiecho sequence is not straightforward, in particular when a sequence using refocusing pulses is chosen. A refinement of the technique was needed before it could be used in the study of flow, and especially under challenging experimental conditions such as with radio frequency surface coils.

6.1 Multiecho sequences

Multiecho sequences offer several advantages compared to single-echo methods. They can reduce the experimental time to a fraction of the time needed for a comparable single-echo sequence. This does not only enable faster acquisition of data, but can improve the sensitivity by allowing more signal accumulations per given time. It is also possible to acquire snapshot images of transient (flow) phenomena by using multiecho sequences. However, with some sequences there may be some practical limitations to this, relating to phase cycling scheme and number of scans required.

Traditionally, MRI is not a particularly fast imaging technique. The total experimental time $T_f$ for a 2D imaging sequence using read encoding is given by [34]

$$T_f = N_{pe} N_t T_o$$

(33)

in which $N_{pe}$ is the number of acquisitions, $N_t$ is the number of phase encoding steps in $j$-direction and $T_o$ is the repetition rate, the time it takes to acquire one echo (and thus one line of data in $k$-space) including relaxation delay. Mansfield et al. [55] discovered that instead of acquiring only one line of data in $k$-space at a time, one can sample the whole $k$-space, or a segment, after a single excitation. In principle, this would speed up the experiment by a factor of $N_t$, if using the notations above. The sequence of Mansfield et al. was named echo planar imaging (EPI). In an EPI sequence the time signal decays with the rate $1/T_1$, as the magnetic field inhomogeneities are not refocused. Such a multiecho sequence can of course also be realized as a spinecho sequence by including refocusing 180° RF pulses within the pulse sequence, also known as the PEPI [56] ($r$ echo planar imaging) sequence. The RARE (rapid acquisition with relaxation enhancement) sequence [57] is much alike, but the phase encoding is not done in a cumulative way as in PEPI. In these multispinecho sequences, the signal decays with the rate $1/T_1$ assuming perfect quality of RF pulses. Unfortunately, this is rarely the case in practice.
6.2 The problem

The core of the imaging sequence is a multispinecho sequence producing a train of spinechoes (Figure 17).

![Figure 17. A multiple spinecho sequence.](image)

The behaviour of the signal depends on the phases of the applied excitation and refocusing pulses. If the pulses are applied with a 90° phase difference the signal behaves as in a CPMG sequence (ref. [58]), shown in Figure 18a. In this figure the echo amplitude is plotted as a function of echo number. The echo amplitude maintains the same sign from echo to echo and decays according to $T_2$ relaxation. In the second case the excitation and refocusing pulses are applied with the same phase. The signal behaviour is very different from the previous and is shown in Figure 18b. The magnetization amplitude oscillates from echo to echo, and also vanishes after a transient period much shorter than $T_2$, unless the refocusing pulses are perfect $\pi$ pulses. This behaviour is as expected for a CP sequence.

![Figure 18. a) Echo amplitude decay for the CPMG sequence. The excitation pulse is applied with phase $\phi = 0°$, and the refocusing pulses with phase 90°. b) Echo amplitude decay for the CP sequence where the phase of the excitation pulse is also $\phi = 90°$.](image)

Once such a multispinecho sequence is combined with phase encoding of velocity a problem arises; as the velocity information is encoded in the phase of the magnetization, it cannot be adjusted to either 90° or 0°, but it can be anything. Thus, when a CPMG sequence is applied after phase encoding of velocity, two components are present – one for which the CPMG condition is valid, and another which behaves as signal in a CP sequence. In this case the transverse magnetization can be understood as a sum of these two components, denoted as $\Delta M_r$ and $M_r$, and they both need to be measured accurately in order to measure the phase correctly. The phase encoding of velocity is based on the fact that the velocity is directly proportional to the phase measured for a spin isochromat with a PGSE sequence (equation (17)). If the phase of the magnetization is not preserved correctly during the echo train, the velocities measured will be incorrect. The problem was illustrated by measuring the rectangular propagator of Poiseuille flow through a cylindrical tube as a function of the echo number, the results are shown in Figure 19.

![Figure 19.](image)
At small echo numbers both of the components are still present, and an undistorted propagator is measured. However, the CP-component starts to vanish much faster than the CPMG-component, due to RF pulse imperfections. As a consequence, information about the velocity sign gets lost.

6.3 Solutions

Various multiecho sequences, such as MLEV [59] and XY [60,61], have been presented already in the literature to preserve the phase of the magnetization during a multiecho train. The CP-CPMG approach presented in this study is much more tolerant to RF imperfections than the XY- and MLEV sequences, but requires the two components to be measured in two separate experiments.

We evaluated the performance of various sequences by the following criterion: Amplitude of the echo as a function of the echo number was calculated for different sequences and with variable refocusing pulse flip angle. From this data, the echo number corresponding to 1/\#amplitude was chosen to the graph comparing the performance of different sequences as a function of the flip angle. The results obtained by using this criterion are summarized in Figure 20.
The results of Figure 20 show that CPMG scheme is the most sensitive to RF pulse inhomogeneities. This is because both of the components, CP- and CPMG-, must be considered, and the limit is fully determined by the decay of the CP-component. In addition, because of the oscillation of the CP-component only every other echo was considered. MLEV-4 has an almost flat response in the range of ±5°, but beyond this the performance degrades rapidly. The higher (θ = 8 or 16) order MLEV loops are much more tolerant to RF pulse inhomogeneities, as indicated by their flat response, but the performance suffers from the fact that the phases of only (θ/2)th echo are coherent. The XY- 4, 8, and 16 sequences are not shown in the plot of Figure 20, but they were also studied obtaining a similar behavior as for their respective MLEV loops. The case of composite refocusing pulses [62] was also analyzed, but the performance was slightly inferior to that of the order 4 loops.

We have presented an approach that preserves only one component per experiment. Thus, two experiments switching the phase of the refocusing pulses by 90° are used to sample the CP- and CPMG-components one by one [63,64,65]. A similar method, making use of soft and slice-selective rf pulses, has also been presented [66] during Paper III has been in process. From Figure 20, it can be seen that the CP-CPMG method is highly tolerant to pulse imperfections. It is also worth noting that all echoes can be used, unlike in MLEV(θ) or XY(θ) sequences in which only the phases of (θ/2)th echo are coherent. This fact improves already the performance of the CP-CPMG approach for the case of perfect 180° pulses by a factor of 2 compared to others. Furthermore, in cases of a flip angle 180° ± 40° and beyond, the performance of the CP-CPMG method is more than one order of magnitude better than the performance of the other approaches. In the CP-CPMG scheme, a slight increase in the number of echoes available when moving away from the perfect 180° condition is a consequence of small admixture of $T_1$ to $T_2$.

### 6.4 Velocity imaging sequence

A new velocity imaging sequence, called CP/CPMG-RARE, including the solution discussed above, was introduced and is presented in Figure 21. The sequence consists of a velocity encoding part, a filter and a multiecho imaging module. Velocity encoding is realized by a conventional PGSE method following the slice selective excitation pulse. Instead of the spinecho sequence chosen for velocity encoding, any other scheme for phase encoding velocity could have been chosen. The output of the velocity encoding is an echo phase encoded by displacement. A filter is applied to preserve one, and only one, component of magnetization per experiment. The filter must be applied in order to measure undistorted

Figure 20. Performance of several sequences as a function of the flip angle. The maximum number of echoes, which can be used, is defined as the decay to 1/e of the initial amplitude (see text). [Paper IV]
propagators. This is because the CP-component of magnetization does indeed vanish much faster than the CPMG-component, but it might disturb the first few propagators that are measured. After filtering the magnetization a multiecho RARE [57] like imaging module is applied to cover the $k$-space. A minimum of four signal acquisition shots are required for generating a velocity encoded image; two shots are required by the CP-CPMG scheme and in addition, two shots as in velocity imaging in general (to acquire a phase reference). In the imaging part, the $k$-space trajectory can be freely chosen to enhance contrast by relaxation as done in conventional RARE sequence.

6.5 Proof of principle measurements

To prove that our approach is working, we chose to measure the same setup as in Figure 19. The hardware is the same, but this time the new velocity imaging sequence was used with imaging phase and read gradients set to zero. A series of propagators as a function of the echo number was measured using a standard birdcage coil. The results are presented in Figure 22 and it can be seen that the method is able to determine the velocities unambiguously. The results of Figure 19 and Figure 22 are obtained by using a birdcage coil, the RF field of which is generally considered highly homogeneous. As can be seen by comparing the two figures, the measured propagators are severely distorted unless the more robust method is used even in such experimental conditions.
Velocity imaging experiments were carried out by using a surface coil which produces a highly inhomogeneous RF field. A model system consisted of laminar flow in two pipes in opposite directions. The results are shown in Figure 23. This experiment does not only prove the validity of the method, but also shows typical distortions and how they might lead to wrong interpretations in situations of more complicated flow patterns. The spatial resolution in the image is fine enough to assure a constant velocity per pixel. In this limit the velocity can be measured either by sampling the full propagator or by using a single step in the velocity encoding gradients. Velocity is then measured from the propagator or by calculating the phase difference between the velocity encoded and the reference measurement, respectively.

Figure 23. The same as Figure 19, except for implementing the CPMG+CP approach. The experiment was obtained by implementing the sequence of Figure 21 without imaging gradients. The following experimental parameters were used: \( \Delta = 90 \) ms, \( \delta = 1 \) ms, maximum gradient 0.18 T/m, 32 steps to sample the \( \beta \)-space from negative to positive values. To avoid flow out a 5 mm thick slice was selected perpendicular to the flow direction. (Paper IV)
In Figure 23a a velocity map of the two pipe system obtained by implementing the new sequence of Figure 21 is shown. The two axially symmetric paraboloids with opposite signs are as expected for this model system of laminar flow in opposite directions and thus velocity was measured correctly. In Figure 23b a CPMG phase cycling scheme is used resulting in an obviously erroneous velocity image; the propagator per each pixel becomes mirrored as shown in Figure 19, because the odd component is lost during the echo train. Velocities in both directions are equally probable and due to noise the algorithm picks sometimes positive and sometimes negative velocities to be presented in the final velocity image. A condition for a CPMG phase cycling scheme is used to be introduced, and two axially symmetric paraboloids obtained (Figure 23c)– but the velocity is still not depicted correctly. If the single step approach is used, the loss of the CP component is critical and the velocity cannot be calculated; it is undetermined and fluctuates about zero (Figure 23d).
7 SUMMARY AND CONCLUSIONS

This thesis consists of four separate journal articles, Paper I - IV. Although most of the Papers are quite separate pieces of research work, there are some common denominators: All of the Papers deal with NMR imaging of flow and address the issue of NMR’s low sensitivity in some way.

In Paper III, this issue was tackled in a “fundamental” level by using a hyperpolarization technique to affect the population of spin states to strengthen the macroscopic magnetization from its “natural” thermal equilibrium value. In addition, further signal gain was attained by using RD NMR as detection method. The measurements of Paper III demonstrate that a significant sensitivity enhancement can be achieved by combining the PHIP and RD NMR techniques, and that by using such a technique it is even possible to study microfluidic gas flow.

Paper IV represents the other extreme when it comes to addressing sensitivity enhancement: in principle multiecho sequences can be used to gain a better signal-to-noise ratio in a given time, but this was not the main point of the study. Paper IV presents a new pulse sequence for measuring flow distributions under inhomogeneous RF conditions. The sequence enables unambiguous determination of velocities by a multiecho imaging sequence, even when the RF field is very inhomogeneous. By using a multiecho sequence the experimental time needed to produce the velocity maps of the micromixer could be reduced by a fraction of its original value. For very slow flow the sequence presented in Paper IV of this thesis could be used. The new multiecho sequence is naturally also applicable to other systems of steady flow. Even a small inhomogeneity of the RF field results in a position-dependent deviation of the pulse flip angle from the ideal. The new CP/CPMG-RARE provides correct and unambiguous results even in conditions of severe RF inhomogeneities.

In Papers I and II the sensitivity issue was tackled “lightly” by improving the signal by optimizing the RF coil to the planar chip geometry. This simple procedure enabled the velocity mapping inside a chemical micromixer, which was demonstrated in Paper I for the very first time. Paper I, together with Paper II, show that NMR imaging and velocity maps can reveal clogging inside a chemical micromixer, and can differentiate between different manufacturing methods and qualities of the chips. The study of Paper I was a rather preliminary report, and we wanted to extend the method and see what kind of insight could be gained from measuring propagators, i.e. velocity distribution functions, inside each pixel instead of settling for an average value for a range which is known to be quite wide. It was found that the propagators are a very sensitive tool in revealing differences between different channels, which should be similar in theory. Also, it could be concluded that in this type of case, where 3D spatial resolution is not practically possible, the propagator data can still provide useful insight to the flow.
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Original Papers

I S. Ahola, F. Casanova, J. Perlo, K. Mümennann, B. Blümich and S. Stapf
Monitoring of fluid motion in a micromixer by dynamic NMR microscopy.

II S. Ahola, V. Telkki and S. Stapf
Velocity distributions in a micromixer measured by NMR imaging.
Manuscript submitted for publication.

III V.-V. Telkki, V. V. Zhivonitko, S. Ahola, K. V. Kovtunov, J. Jokisaari, and I. V. Koptyug
Microfluidic Gas-Flow Imaging Utilizing Parahydrogen-Induced Polarization and Remote-Detection NMR

IV S. Ahola, J. Perlo, F. Casanova, S. Stapf and B. Blümich
Multiecho sequence for velocity imaging in inhomogeneous rf fields.

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