Research documentation of DI Martin Nausner
1st February - 31st July 2009, at New York University

Research Proposal:
Adapting Spin Noise Magnetic Resonance Techniques To Magnetic Resonance Imaging

Suggested New Research Title:
Acquisition and Data Processing of Nuclear Magnetic Spin Noise
Introduction

The phenomenon of spin-noise was predicted by Bloch in 1946 [1], but at the time could not be detected. Sleator and Hahn were able to measure spin-noise for the first time using a solid sample at liquid helium temperature [2,3]. Later, Ernst and McCoy demonstrated that spin-noise was also observable at ambient temperature with a commercial liquid-state NMR spectrometer [4].

Recently, nuclear spin-noise was detected in the presence of magnetic field gradients along different directions and exploited to reconstruct a two-dimensional image of the cross section of a phantom without the use of radio-frequency irradiation [5]. Due to the weakness of the spin-noise signal, which originates from a system in equilibrium, as opposed to the signal obtainable from pulsed NMR, where manipulated states are generally not in equilibrium, the applications of this phenomenon are currently limited.

Earlier we have systematically investigated the line shapes observed in proton nuclear spin-noise spectra which depend in a complex way on the properties of the resonance circuit, the number of spins present, transverse relaxation, inhomogeneous broadening, and radiation damping [6]. We also introduced the Spin-Noise Tuning Optimum (SNTO), where the spin-noise signal is indicates tuning conditions of the NMR probe, that may increase the intensity of the observed signal.

As we were able to explain the observed trends qualitatively, the original motivation for my research documented here, was to adapt these spin-noise magnetic resonance techniques to magnetic resonance imaging. However it became clear, that fundamental aspects of these techniques had to be studied in more detail, as some deviations, currently cannot be explained in a quantitative way. The discrepancies observed between the theory and our experiments are most probably due to the non-ideality of real NMR-probe rf-circuits, which include more than the necessary minimum number of electronic components to meet the requirements of high resolution, high sensitivity multi-nuclear NMR spectroscopy and magnetic resonance imaging (MRI). In particular, multiply tuned coils and rf-traps usually exhibit very irregularly shaped tuning curves like the one shown in Fig. 1.

One of the most obvious consequences of this situation seems to be the large offset between the conventional tuning optimum and the SNTO. With such tuning curves no single quality factor Q will be sufficient to describe the circuit near resonance, and especially at larger offsets. Modifications to the Nyquist equations [7] will have to be made to account for this non-ideality.

I was given the possibility to investigate in these important parameters and their consequences on a number of NMR spectrometers at New York University (NYU), at New York Structural Biology Center (NYSBC) and at Havard Medical School (HMS) in Boston, Massachusetts. I was also invited to give a talk at NYSBC and discuss the relevance of spin-noise for future spectroscopic applications.
Theoretical Background

The total power $P$ of the spin-noise signal at Larmor frequency $\omega_s/2\pi$ is given by Sleator et al [3] as

$$P = \frac{\pi Q \omega_s N \gamma^2 \hbar^2}{V_c}$$

(1)

where $Q$ is the quality factor of the rf-circuit, $N$ is the number of spins of gyromagnetic ratio $\gamma$ in a coil of volume $V_c$. The remarkable coincidence of this power with the power expected from spontaneous emission enhanced by radiation damping in a tuned cavity led to the conclusion spin-noise and spontaneous emission were part of the same controversial issue [3].

While this equation describes the total spin-noise power, one needs to resort the equations based on the Nyquist noise [7] relationship modified for the presence of resonating spins in order to describe the line shapes.

In the formulation of McCoy and Ernst [4] the line shape of the spin-noise power signal is described by

$$W(\omega) = q \frac{1 + a(\Delta\omega)\lambda_r^0}{\left[1 + a(\Delta\omega)\lambda_r\right]^2 \left[1 + d(\Delta\omega)\lambda_r + 2Q(\Delta\omega_c/\omega_c)\right]^2}$$

(2)

where $\Delta\omega$ is the resonance offset, $\Delta\omega_c$ is the offset from the rf-circuit's tuning center frequency $\Delta\omega$, $q$ is a frequency independent factor depending on the circuit resistance and temperature and the radiation damping rate

$$\lambda_r = 1/T_{rd} = \frac{1}{2} \eta Q \gamma \mu_0 M_z$$

(3)

with $M_z$ being the longitudinal magnetization, $\eta$ the filling factor, and $\mu_0$ the permeability of free space. The difference between the sample temperature $T_s$ and circuit temperature $T_c$ is taken into account via

$$\lambda_r^0 = \lambda_r \frac{T_c}{T_s} = \lambda_r \theta$$

(4)

The absorptive $a(\Delta\omega)$ and dispersive $d(\Delta\omega)$ components of the line shapes are responsible for the different appearances of the spectra under the influence of various homogeneous and inhomogeneous interactions.
Collecting data of fundamental spin-noise behavior

**Tuning dependence and Spin-Noise Tuning Optimum**

According to Eq. 2 the spin-noise signal can be a negative deviation from the otherwise flat baseline, if the tuning frequency of the circuit is equal or close to the Larmor frequency. As can be seen from the experimentally observed tuning dependence of spin-noise illustrated in Fig. 1, this is generally not found to be true in practice.

![Figure 1](image)

**Fig. 1:**
Tuning dependence of the spin-noise signal of H$_2$O (10% $^2$H$_2$O) on a 500 MHz cryogenically cooled TXI probe. The large red curve is the "wobble" curve as displayed by the tuning routine of the Bruker TopSpin software. The small inset spectra display the change of line shapes of the spin noise power spectra connected to the tuning offset. The baselines in these spin-noise power spectra represent the thermal noise of the system.

The "dip" line shape in the red inset spectrum is not found as predicted by Eq. 2 at the minimum position of the tuning curve, but at an offset of -464 kHz.

We call this special tuning setting, where the spin-noise spectrum shows the symmetrical dip, the spin-noise tuning optimum (SNTO). It exhibits only minor dependence on the sample properties but varies considerably between different probes thus has to be determined for each probe. Interestingly it could be shown, that this setting is optimized for detecting conditions, and an increase of more than 30 % of the signal-to-noise ratio (SNR) on two different spectrometers with different probes could be reported [6,8].
$^1$H pulse spectra with water presaturation of 2 mM sucrose in aqueous solution were acquired at a number of different tuning offsets. In Fig. 2 it can be seen, that while the detuning does not affect the RMS-noise, the signal itself increases to a maximum at the SNTO offset, which results in an increase of the signal-to-noise ratio, in this case of almost 50%.

**Fig. 2:**
Tuning dependence of the signal amplitude (a), RMS noise levels (b), and signal-to-noise ratio (c) of the anomeric doublet (d) in phase sensitive (absorptive) pulsed $^1$H NMR spectra of 2 mM sucrose in H$_2$O (10% $^2$H$_2$O), acquired under the following conditions: pre-saturation of the water signal for 1.5 s with an rf-power of $B_1\gamma/(2\pi) \sim 150$ Hz.

The position of the tuning optimum (as determined by the spectrometer's "wobb" routine) is at zero offset frequency emphasized by the color in the corresponding graphs, while the SNTO is in red.

This method could be verified and applied in the course of this project to optimize the tuning for detection on a number of different spectrometers and probes:

- 500 MHz Bruker spectrometer with TXI probe at NYU
- 500 MHz Bruker spectrometer with TXI cryoprobe at NYSBC
- 600 MHz Oxford spectrometer with TCO probe at HMS ($^{13}$C)
- 800 MHz Bruker spectrometer with TXI cryoprobe at NYSBC
- 800 MHz Bruker spectrometer with TXI cryoprobe at NYSBC
One important question to answer was, how a signal, that is believed to be caused by the interaction of the random fluctuations with the electric current of the receiving circuit, would grow relative to the thermal noise itself.

In datatrocessing it is known from statistics, that as a direct result of the Central Limit Theorem (CLT) [9], the signal to noise ratio grows with the square root of number of scans. This is known to be true for pulsed NMR spectroscopy, but has not been shown for spin-noise NMR spectroscopy yet.

**Fig. 3:**
The spin-noise power spectrum of H$_2$O was acquired on a 500 MHz spectrometer, equipped with a TXI cryoprobe, at an offset of $+ 408$ kHz, where the water spin-noise signal represents a "bump" and at an offset of $- 464$ kHz where it represents a "dip". The signal-to-noise ratio grows to the power of 0.44 respectively to the power of 0.46 of number of scans.

(Signal 4.7 ppm, Noise range 6 - 7.5 ppm, 8 - 16k scans).
Developing and testing of data processing methods

**Real and artificial model Free Induction Decays (FIDs)**

Acquisition of real spin-noise data for one dimensional FIDs is limited by the Bruker spectrometer hardware to a maximum of 500 k complex datapoints. As these timeseries are insufficiently long to test continuous data processing methods, a way to generate artificial data of similar type had to be found.

As illustrated in Fig. 5, fluctuating signals that decay over time were simulated by the convolution of an array of random complex numbers, which reproduces the various directions of spin magnetization at a certain time, with an exponential function, which represents the relaxation of the spins through reorientating themselves along the external magnetic field. Thermal noise was simulated by an array of larger random complex numbers, added to the generated fluctuating random decays.

![Fig. 5: Schematic illustration of the generation of an artificial free induction decay for data processing.](image)

This way arbitrarily long superpositions of equidistant (randomly starting) FIDs of random phase and amplitude, but at a given frequency were computed with one or more signals covered in random noise.
Autocorrelation

The autocorrelation function, which can be described as the cross-correlation of a signal with itself, is a well known tool in signal processing to detect repeating patterns in a time series, such as a periodic signal that is covered in random noise.

According to the Wiener-Khinchin theorem [10] the power spectral density is the Fourier transform of the corresponding autocorrelation function. Thus the power spectra of the hidden signal in the earlier artificially generated FID can be recovered by Fourier transform of the autocorrelated time series.

For a complex function $f$, the autocorrelation is defined by

$$f \ast f = \int_{-\infty}^{\infty} f(\tau) \bar{f}(\tau - t) \, d\tau \quad (5)$$

where $\ast$ denotes the cross-correlation and $\bar{f}$ is the complex conjugate.

Blockwise processing

Another method to process spin-noise data is to cut the one dimensional contiguous FID into small data blocks, Fourier transform each block and sum them up afterwards, which can be seen as an equivalent approach to random noise averaging in pulsed NMR.

**Fig. 6:**
Schematic illustration for blockwise processing of spin-noise data
Autocorrelation and blockwise processing of a real pulse spectra

Two different methods, autocorrelation and blockwise processing, for data processing were studied in detail and compared with each other. To test these two methods a one dimensional spin-noise FIDs of H$_2$O in $^2$H$_2$O was acquired, and by observing the linewidth of the resulting spectra the size of the datablocks were optimized to 550 datapoints per block Fig. 7a., respectively 550 datapoints for the autocorrelation time-lag.

In a direct comparison the blockwise processing performs better than the autocorrelation as can be seen by the overlay in Fig. 7b. This is in contrast to theory, which claims that the two methods are equivalent.

Fig. 7:
Size optimization of the datablocks (a) by observing the signals full width at half maximum (FWHM). Direct comparison of a blockwise processed spectra with an autocorrelated spectra with a blocksize and time-lag of 550 datapoints (b).
Spin-noise tuning optimum applied to biological NMR

The SNTO setup was applied to protein NMR samples, using an ubiquitin standard NMR sample in 50 mM Na$_3$PO$_4$, 9:1 H$_2$O : $^2$H$_2$O, and tested on several 2D and 3D routine pulse programs such as HSQC, HNCO, HNCA, C$_\beta$C$_\alpha$(CO)NH.

The experiments were implemented on a 500 MHz Bruker Avance spectrometer with a TXI cryoprobe at NYSBC. The SNTO was determined with the 2 mM sucrose NMR standard sample at an offset of -165 kHz at 500.298 MHz tuning frequency and verified with the ubiquitin standard sample.

The overlay of the 1D projections of the HSQC spectra under SNTO and "wobble" optimum conditions indicated a increase of 20% of SNR at the SNTO. Thus the more sensitive HNCO spectra were acquired and the overlay of the 1D projections indicated about the same increase of SNR. Two isolated peaks in the 23 raw plane were selected (row 91 and row 110) and the signal-to-noise ratios with a defined noise region of 6.5 - 8.5 ppm clearly confirmed the 20% increase of SNR at the spin-noise tuning optimum.

The acquired HNCA and C$_\beta$C$_\alpha$(CO)NH data could not yet be analyzes at the time of the report.

Fig. 7: 1D data block of an ubiquitin HSQC spectra at the "wobble" optimum, at 0 kHz offset, in blue, and at the SNTO, at -165 kHz offset, in red.
Fig. 8:
1D data block of an ubiquitin HNCO spectra at the “wobble” optimum, at 0 kHz offset, in blue, and at the SNTO, at -165 kHz offset, in red.

Fig. 9:
2D plot of the 23 raw plane of the ubiquitin HNCO spectra. The isolated peaks of row 91 and 110 are marked with a black line, and further discussed in Fig. 10.
Fig. 10:  
1D data blocks of an ubiquitin HNCO spectra at the "wobble" optimum, at 0 kHz offset, in black, and at the SNTO, at -165 kHz offset, in red.  
(a) the isolated peak of row 91 shows an signal-to-noise increase of 20 % at the SNTO compared to the "wobble" setup  
(b) the signal of row 110 verifies the increase at the SNTO with 19.7 %  
For the SNR calculations the noise region was defined from 6.5 to 8.5 ppm.
Conclusion

The main result of this research stay, which is embedded in the larger context of research efforts at NYU and JKU are:

1) SNTO tuning is generally applicable on different instruments yielding an increase from 10% to 50%.

2) The spin-noise to random noise ratio behaves like the normal SNR with respect to accumulations (i.e. $\sqrt{n}$)

3) A simulation model for spin-noise data was developed.
Publications and Presentations

Part of this work was presented on posters

"Nuclear magnetic spin noise: non-linearity, frequency shifts, spin noise tuning optimum"
M. Nausner, J. Schlagnitweit, V. Smrečki, A. Jerschow, N. Müller

"Non-linearity, frequency shifts and other surprises with nuclear magnetic spin noise"
N. Müller, M. Nausner, J. Schlagnitweit, A. Jerschow, V. Smrečki
EUROMAR 2009 (July 5 - 9, 2009 Göteborg, Sweden, poster Re17, Program and Abstract Book p. 89).

and in an invited talk

"Non-linearity and frequency shifts of nuclear magnetic spin-noise"
Martin Nausner
NYSCBC meta-group meetings (April 29, 2009, special lectures,
http://www.nysbc.org/metagroup/)

A publication with recent results, which were found during my research at NYU, is in preparation.
References

Abbreviations

$C_B C_A (CO) NH$ 3D triple resonance pulse program for protein NMR, designed to correlate the $^1H$ and $^{15}N$ amide resonances of one residue with both $^{13}CA$ and $^{13}CB$ resonances of its preceding residue via the intervening $^{13}CO$ spin

$CLT$ Central Limit Theorem

$FID$ Free Induction Decay

$HNCA$ Common 3D triple resonance pulse program for protein NMR, designed to correlate $^{15}N$ and NH chemical shifts with the intra- and interresidue $^{13}CA$ carbon shifts

$HNCO$ Common 3D triple resonance pulse program for protein NMR, designed to correlate connectivities between the $^{15}N - ^1H$ pair of one residue to the carbonyl $^{13}CO$ resonance of the preceding residue

$HSQC$ Heteronuclear Single Quantum Correlation 2D pulse program

$MRI$ Magnetic Resonance Imaging

$NMR$ Nuclear Magnetic Resonance

$RMS$ Random Mean Square

$SNTO$ Spin Noise Tuning Optimum

$SNR$ Signal-to-Noise Ratio

$TCI$ Triple Resonance NMR Probe with cryogenically cooled $^1H$ and $^{13}C$ coil

$TCO$ Triple Resonance NMR Probe with cryogenically cooled $^1H$ outside and cryogenically cooled $^{13}C$ coil inside

$TXI$ Triple Resonance NMR Probe with cryogenically cooled $^1H$ coil

$HMS$ Havard Medical School

$NYSBC$ New York Structural Biology Center

$NYU$ New York University