A DNP-NMR setup for sub-nanoliter samples

Pedro Freire da Silva. Supervisor(s): Prof. Giovanni Boero, Prof. Pedro Sebastião

The electrical and mechanical design for a non-magnetic, 2.5x2.5 cm² setup with µm-precision, along with 1.0x10 cm² optimized probes, was simulated and implemented to perform simultaneously EPR and DNP-NMR studies. The interaction coils were EM-FEM modelled and verified against optimized EPR and NMR experimental results for sub-nL samples of water, PDMS and TEMPO. DNP state-of-the-art, theory and future prospects were also presented.

I Introduction

Nuclear magnetic resonance (NMR) detection is a widespread technique use in a variety of fields both in spectroscopy and imaging that primes for its chemical insight, selectiveness and non-invasive behaviour. Its main downside is the sharp dependence on magnetic field strength and sample size, meaning sensitivity has proven to be the limiting factor to the wider use of this technique. Another common limitation is the difficult use of the technique for low gyromagnetic ratio nuclei, namely the biological cornerstone that is 13C, which shows a sensitivity 170 times smaller than 1H. This led to the development of complimentary techniques to achieve better resolution, in less time and with cheaper medium-field magnets, and that would allow for the study of more isotopes. These ranged from pulsed techniques to remove or insert interactions with other species (such as cross-polarizing 13C to match the polarization of 1H, CP-NMR, or the dipolar-dipolar decoupling that highly sharpens lines, through TROSY), to hyperpolarization techniques, such as the one implemented here, Dynamic Nuclear Polarization (DNP). Much like other cross-polarization techniques, DNP hyperpolarizes the nuclei up to the thermal polarization of the electrons, which means an increase of approximately 660 in signal or over 400 000 less time averaging out the noise. This technique was implemented, at a field of 0.7 T, to mass-limited samples in the sub-nL range, which mean a signal over a 10⁸ times smaller than regular samples and medium fields, using a versatile and low cost 3D-printed setup.

II Theory and simulations

The spectroscopic analysis of such a small sample requires a far higher spin sensitivity as that of regular systems, achieved with detection coils for a high filling factor of the volume used, and low noise designs. These were used from published designs with 130 CMOS printed coils and integrated electronics to achieve the highest possible SNR. The following equation states the dependences taken into consideration when the system was developed:

\[ \text{SNR} \propto \epsilon \eta B_0^{7/4} \gamma^{11/4} \frac{B_{1\perp}}{T_S} \sin(\gamma T B_{1\perp}) \]  

for a filling factor \( \eta \), unitary field produced by the detection coil \( B_{1\perp} \) perpendicularly to the fixed magnetic field \( B_0 \), sample temperature \( T_S \) and gyromagnetic ratio \( \gamma \) and all for a DNP enhancement of \( \epsilon \), proportional to \( B_{1\perp}^2 \).

It was thus necessary to analyze all the magnetic fields at play, the one created by the NMR coil, the unitary field, and the DNP-generating field created by the EPR coil repurposed for this experiment. This was done through FEM in COMSOL Multiphysics, solving the Maxwell equations for the geometry designed in Solidworks, exactly as used in the real setup and the working NMR/EPR frequencies at ~1 T. This allowed for an understanding of the geometrical restrictions created by the small sensitive volume of each coil and therefore of the necessity for a precise system, with losses per deviation, in the sensitive volume, of around 50%/20 µm. The same simulation allowed for the study and future optimization of the dielectric heating of the sample.
The desired DNP enhancement is based on relaxations effects of the excited polarization after microwave irradiation, which depend on cross-relaxation and auto-relaxations factors $\sigma$ and $\rho$, respectively, as:

$$\frac{d(I_z)}{dt} = -\rho(I_z - I_0) - \sigma(S_z - S_0)$$  \hspace{1cm} (2)

This equation can be rewritten to fit experimentally verifiable values such as the saturation factor $s$, the leakage factor $f$ and the coupling factor $\xi$:

$$\varepsilon = \frac{(I_z)}{I_0} = 1 - s \xi f \frac{\|y_{el}\|}{y_{n}}$$  \hspace{1cm} (3)

From these, the saturation factor is the only one that is dependent on the setup and not the sample used:

$$s = \frac{S_0 - \langle S_z \rangle}{S_0}, \hspace{0.5cm} 1 - 660 \text{ s} \leq \varepsilon_{exp} \leq 1 + 330 \text{ s}$$  \hspace{1cm} (4)

And is thus proportional to the EPR excitation field squared and its proximity to the sample. Considering the SNR is proportional to the enhanced magnetization, that the signal is geometrically correlated to the unitary field in a point of space and that, for high enhancements, $\varepsilon$ is proportional to the saturation factor created by the EPR coil’s excitation ($660s \gg 1$):

$$\frac{\varepsilon}{\varepsilon_0} = \frac{T_{EPR} B_{EPR}}{T_{NMR} B_{NMR}} \prod e^{-t_i/T_i}$$  \hspace{1cm} (5)

In which the small nuclear polarization times, usually in the 10-100 ms range, can be comparable to the shuttling times.
III Mechanical implementation

The mechanical design was fully developed in Solidworks and dimensioned through finite element simulations (FEM) targeting critical zones. A hard plastic with low thermal expansion coefficient was used, EX200, throughout the system with a printing tolerance of 50-100 µm.

![Solidworks model of the system. Table system for the EPR (a) and NMR (c) PCB's (in green), the microfluidic system (c) and detail of its attachment (d). Arrows indicate the correct placement of each part.](image)

The precision requirements were achieved using locking pieces on all moving parts, as above in (d), modelled to act as springs, thus reducing the possible moving room from up to 100 µm to zero. The control along an axis is done with an attachable piece (through an M6 nut/thread system) containing a magnetic micrometric screw.

![Final prototype seen from the back (a) and the front (b). M1.6 screws used to control friction and to lock movement are shown as well as the adjustment system and capillary interface. Image of the precision achieved when aligning the capillary with the NMR coil (c).](image)

IV Electrical implementation

To interface the NMR and EPR microchips, two compact PCB’s were designed in a configuration that would allow for contact of the two despite the space introduced by the Al wedge-wedge and Au ball-wedge wirebonding as subsequent epoxy cover layer.

![Schematic of the assembly of the PCB’s when aligned. Bottom PCB’s are 10x1 cm² and top ones are 2x1 (EPR) and 3x1 (NMR) cm².](image)

The EPR PCB required only DC inputs which were filtered or generated by an LDO on the PCB. The two symmetric outputs, in the 500 MHz range, were subtracted, band-pass filtered between 100-1000 MHz and outputted through an SMA cable soldered directly on the PCB. The top PCB connected contained only DC filtering.

![Schematic of the EPR PCB up to the top/bottom pins.](image)

The NMR PCB was fed by 5V with Tx and Rx voltages being generated by LDO’s, filtered and used to feed the TTL inverter to control the digital operation toggling pulse. It also required an RF excitation biased using a Bias Tee design and, when the same frequency is used for downconversion and RF excitation, an R to 2R network. Otherwise the lines would simply be separated and ESD protected by back-to-back diodes. The output was in the kHz range and biased at 1V. A protection voltage follower was used before it being amplified with a gain of 10. The design was a non-inverting OPAMP working at a 1 V virtual ground, made through a voltage divider from the highly capacitor-filtered VCC line feeding a higher impedance OPAMP feedback loop.
V Results and conclusions

After assembly and testing samples of water, PDMS and 50/200 mM TEMPO were used to optimize experimental parameters, such as NMR downconversion frequency and the exponential filter’s characteristic time or the maximum EPR modulation past which one would saturate the lock-in detector or broaden the linewidth. Selected results are shown in figure 10. The simulation results were then double checked experimentally and the optimal pulse length was found and used henceforth.

Having assembled the circuit, it was aligned using a modulation of the NMR pulse control line, thus inducing the same modulation frequency on the EPR coil, which was adjusted until the maximum signal was achieved.

After this easy alignment procedure, the setup was introduced into the magnet and the EPR excitation line to be excited and DNP-studied was searched for. This was done through a field sweep and a return to the discovered excitation matching field. Due to the hysteresis of the electromagnet and to have control over the excited frequency, a second smaller sweep using the control voltages of the varactors was done, setting the resonance frequency.

Due to magnet malfunction, the DNP results were not yet obtained, but will serve a purpose on future developments of the technique be it coil integration, parallelization, post-CMOS microfabrication or as a complementary technique in a micro total analysis system, μTAS.

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VII Bibliography
