

METABOLOMIC NMR ON A CHIP VIA INDUCTIVE COUPLING

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ABSTRACT

Inductively coupled resonators are explored as a tool to efficiently integrate detection by high-resolution NMR spectroscopy with microfluidic devices. Resonators are fabricated using lithographic techniques, and combined with standard microfluidic networks. The coupling efficiency, sensitivity, and spectral resolution of such devices is explored.

KEYWORDS: Nuclear magnetic resonance, Spectroscopy, Inductive Coupling, Metabolomics

INTRODUCTION

The goal of this work is to integrate high-resolution NMR spectroscopy with microfluidic separation and sample preparation. Nuclear magnetic resonance spectroscopy is widely applied to the study of biological molecules in a variety of contexts. Of particular interest here is its capability to resolve the constituents of complex metabolic mixtures containing dozens to hundreds of compounds at mM levels. Due to the linearity of the NMR response, such analysis is quantitative and general. Medical diagnostics based on NMR metabolomic analysis (so far, of macroscopic samples) is a rapidly developing field, with a large number of diagnostic assays emerging [1]. At the same time, microfluidic devices offer convenient and efficient possibilities for sample separation and preparation. Combining the two approaches would empower microfluidic assays with the resolution and specificity of NMR spectroscopy.

There are two main challenges in the design of any NMR probe hardware: sensitivity and resolution. Particularly when the available sample volume is limited, optimum sensitivity of the detector system becomes crucial. The signal/noise ratio of inductive NMR detectors depends on the quality factor of the resonator, as well as its filling factor. In microfluidic systems, the NMR pickup coil must be of similar dimensions as the sample.

Miniaturized NMR systems have been described by several authors [2–5]. In most cases, these devices are operated as flow probes, i.e., the sample is brought into the active volume of the probe coil through a microfluidic capillary. MicroNMR

systems based on solenoid coils wound around the capillary, and, more recently, stripline resonators have been demonstrated.

In the present contribution, we pursue a somewhat different approach to microscale NMR. The idea is to integrate it directly with Lab-on-a-chip devices, without the need to extract the NMR sample from the chip and inject into a dedicated capillary NMR probe. Instead, we aim to directly insert the entire microfluidic chip into the NMR spectrometer. This principle of operation is illustrated in Figure 1.

To this effect, the probe coil must be integrated into the chip. Inductive coupling [6,7] allows for wireless interaction between the chip and the transmitter/receiver system of the NMR spectrometer. In the present contribution, we demonstrate the fabrication of microfluidic devices with integrated planar resonators, and their inductive coupling to a standard 600 MHz NMR spectrometer. Their performance in terms of rf field strength, resolution, and sensitivity is explored. An example of such a chip is shown in Figure 2.

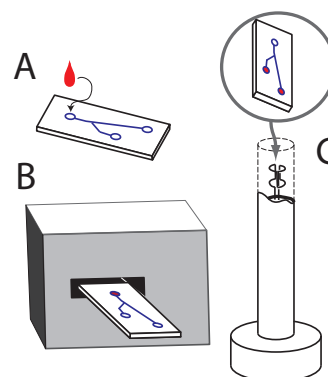


Figure 1: Basic concept of microfluidic NMR: A: Sample is loaded onto Lab-on-a-chip device; B: development box actuates microfluidic sample preparation/separation; C: chip is inserted into NMR probe assembly for spectroscopy

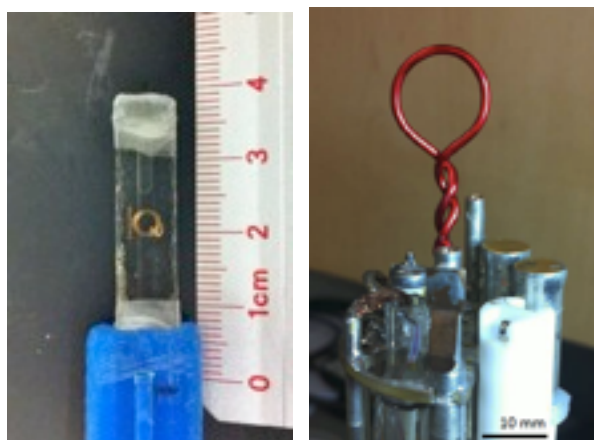


Figure 2: (left) Microfluidic device with integrated RF resonator: a coil is fabricated to tight specifications, such that its self-resonance lies precisely at the Larmor frequency. (right) Exposed probe coil to be inductively coupled.

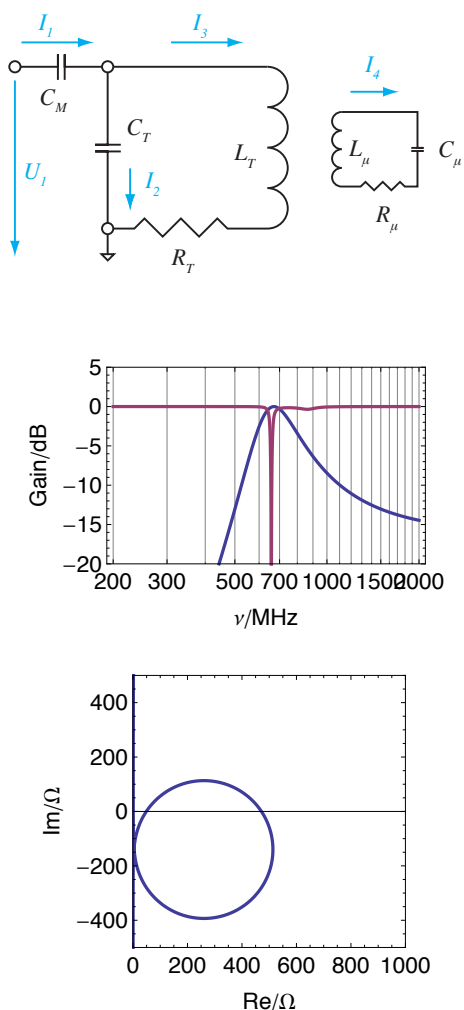


Figure 3: (top) Schematic circuit diagram of an inductively coupled resonator and probe system. (center) reflection ratio (red line) and fraction of power dissipated in the microcoil (blue line) as a function of frequency. (bottom) Smith chart of the apparent impedance observed at the connector of the probe circuit

SO₄. Spectral resolution was determined using samples of 5% H₂O in D₂O.

RESULTS AND DISCUSSION

Figure 4 shows a microfabricated resonator along with the reflection curve, from which the overall quality factor can be determined; the value of Q is around 70 in this case.

The radiofrequency performance of this resonator is shown in Figure 5 in the form of a nutation diagram. In this experi-

THEORY

Inductive coupling of a resonator to a probe circuit can be schematically represented by the circuit diagram shown in Figure 3. The coupling between the probe coil (right panel in Figure 2) and the resonator coil (left panel in Fig. 2) is mediated through the mutual inductance of the two coils. Figure 3 shows the calculated response of this arrangement, assuming typical geometries and component values consistent with the device shown in Figure 2. Given a high enough coupling constant between the two inductances, it is possible to find an arrangement where most of the incident rf power is dissipated in the resonator, as opposed the probe coil (blue line). This means that there is essentially no penalty in rf efficiency or sensitivity to the wireless coupling arrangement over a direct connection of the microprobe through a coaxial waveguide.

EXPERIMENTAL

Microfluidic devices were fabricated using standard lithographic techniques from Pyrex glass. The NMR resonators were made by two different routes. In the first, a glass substrate was seeded with a few 100 nm of Au. This seed layer was patterned using a positive photoresist, and the resonator structure was then created by electroplating. Finally, the seed layer was removed by flash etching after stripping of the photoresist.

The second approach departed from pre-fabricated polyimide foil covered with 28 μm of Cu (Upisel, UBE Industries, Inc.). In either case, the final resonator structure was covered with 20 μm of SU-8. This ensured that the structure is exposed to a well-defined dielectric, therefore improving the stability of the self-resonance frequency.

Resonator performance was tested using a network analyzer, by measuring the reflection ratio from a coaxial cable terminated by a small probe coil of a few turns. Resonators with varying geometry (number of coil turns, shape and size of capacitive structures) were fabricated, and the ones with self-resonance at 600 MHz were selected for NMR testing.

NMR measurements were conducted on a 600 MHz Varian VNMR system, using a dedicated probe head with a single loop inductor of 15mm diameter (Figure 2). Resonators were fixed to a microfluidic chip containing a rectangular sample chamber of 15 μl total volume, with a volume of 4 μl exposed to the rf field of the microcoil. The probe efficiency and rf homogeneity were tested by recording nutation sequences of water doped with 2mM of Cu-

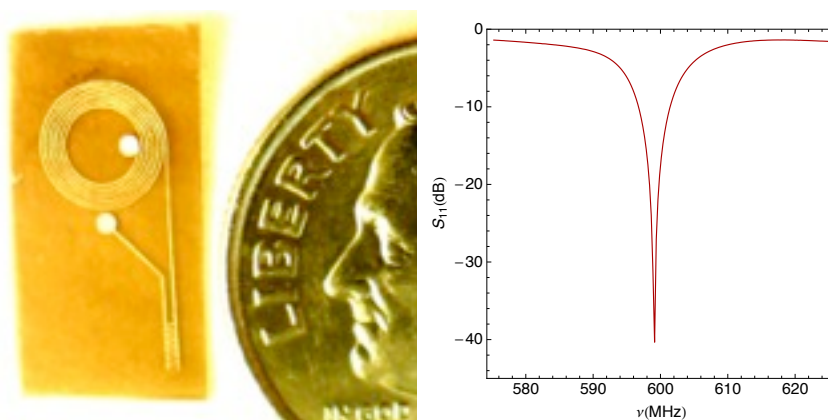


Figure 4: (left) Cu resonator consisting of planar coil and capacitive tuning. (right) reflection ratio of this coil coupled to the NMR probe. The Q factor is around 70.

ment, a single pulse NMR spectrum is recorded repeatedly, while systematically increasing the duration of the excitation pulse. At fixed excitation power, the radiofrequency field in the probe is given by the nutation frequency of the protons in the sample. The magnitude of the rf field per square root of power is an important characteristic of probe performance. At the same time, the gradual decay of the signal amplitude over several nutation cycles provides a measure of the homogeneity of the radiofrequency field. The nutation diagram shows an line intensity ratio of the 450 over a 90 degree pulse of 66%. This is an acceptable value for many simple NMR experiments. At 60 rf power, the 90 degree pulse length is 5 μ s, corresponding to 50kHz nutation frequency. The probe efficiency therefore amounts to 6.5 kHz/ \sqrt{W} .

The best spectral resolution that could so far be achieved with this device was 3.5 Hz, with a typical value of about 7 Hz. Since this is insufficient to resolve most three-bond J couplings, it is difficult with the present setup to resolve complex metabolomic

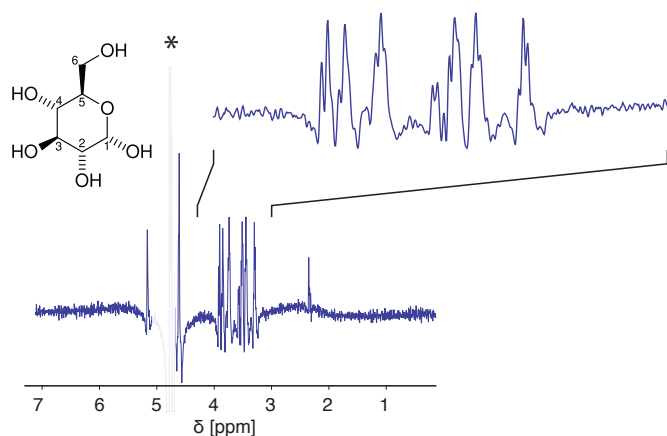


Figure 6: Single acquisition spectrum of a 5ul sample of 500mM D-glucose in D₂O. The baseline distortion is due to removal of a broad background signal (cf text). Spectral resolution is around 7 Hz.

design is adequate for NMR spectroscopy, the spectral resolution is not yet sufficient due to susceptibility broadening.

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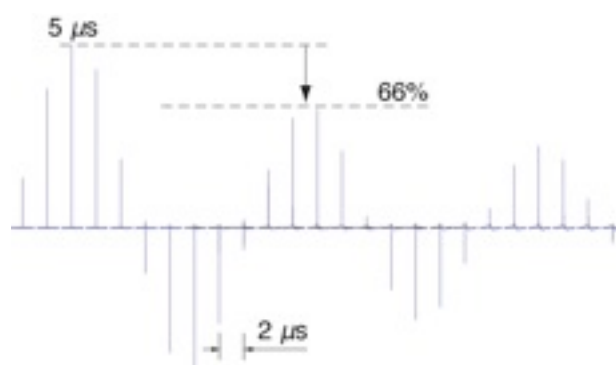


Figure 5: Nutation spectra acquired at 60W excitation power. 90 degrees flip angle is achieved with a 5 us pulse, corresponding to 50 kHz nutation frequency.

mixtures. The resolution is clearly limited by the static field homogeneity.

Figure 6 shows a single-scan spectrum of a sample of 500mM D-glucose in D₂O. The baseline distortion in the figure is an artifact of the spectral processing, which was optimized to suppress a broad background signal from polymer materials used in the construction of a prototype NMR probe. While the basic structure of the glucose proton spectrum is conserved, the individual resonances are not completely resolved. The residual line broadening is probably due to differences in magnetic susceptibility between the chip material (pyrex glass) and the surrounding medium (air). Changes in the design of the chip and the probe are currently investigated to alleviate this problem.

CONCLUSION

We have successfully demonstrated inductively coupled Lab-on-a-chip devices for micro NMR spectroscopy. While the radiofrequency performance of the current