

One- and Two-Dimensional NMR Spectroscopy with a Magnetic-Resonance Force Microscope**

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Magnetic-resonance imaging (MRI) is a non-invasive method to generate three-dimensional images which have a high information content and is used in various fields, ranging from human medicine to material science. In microimaging, the spatial resolution of MRI can approach one micrometer in favorable systems.^[1] Magnetic-resonance force microscopy (MRFM)^[2–4] has opened an avenue for extending imaging to the nanometer range. Two-dimensional images mapping the spin density with 90 nm resolution have recently been obtained^[5] and single-spin resolution, as reported for electrons,^[6] can be envisioned.

As with MRI, the MRFM method is not limited to the three spatial dimensions. Spectroscopic dimensions can be added, providing detailed chemical and structural information at the atomic level. Such experiments are routinely performed in clinical MRI and are denoted as MR spectroscopic imaging (MRSI) or chemical-shift imaging (CSI).^[7] Spectral information, for example, from dipolar and quadrupolar interactions, has been used in MRFM experiments, in particular for generating new image contrast.^[8–10] The most important interaction—the chemical shift—however, has not been employed in MRFM, because of the difficulty of combining high spatial with high spectral resolution. Mechanical detection of chemical shifts, without spatial resolution, has been demonstrated on millimeter-sized samples^[11,12] with a setup where the field gradient vanishes at the sample position.

MRFM provides an image of the object's spin density by using the spatial variation of the resonance frequency in a magnetic field gradient, in full analogy to MRI. In contrast to MRI, the magnetization is detected mechanically with a micromechanical cantilever that measures the force on the spin magnetic moment in a magnetic field gradient. Spatial resolution and detection sensitivity can be significantly improved over inductively detected MRI,^[13] but the permanent presence of a gradient complicates spectroscopy. This problem is particularly true for chemical-shift spectroscopy,

because the interaction has the same symmetry properties as the interaction with the magnetic field (gradient). In principle, it is conceivable to extract chemical-shift information in a gradient by recording zero-quantum spectra.^[14] Other, related methods have also been discussed;^[15,16] all of them, however, have limitations and the full information content of a regular NMR spectrum is not reproduced.

An alternative approach, presented herein, is to temporarily move away the gradient source during the experiment (see Figure 1). The spectroscopic information can then be collected in a nearly homogeneous field. We shall demonstrate below that this method allows for chemical-shift imaging.

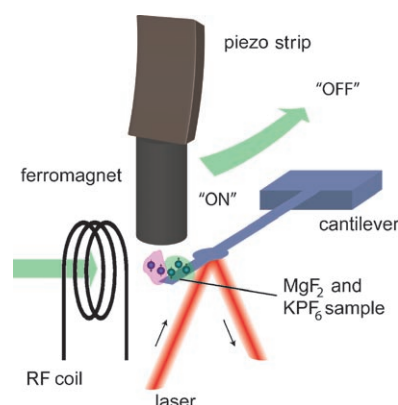


Figure 1. Schematic drawing of our magnetic-resonance force microscope. The sample is mounted on a mechanical cantilever and feels an attractive or repulsive force to the ferromagnetic gradient source, depending on the orientation of the spin magnetic moment. Radio frequency (RF) irradiation induces periodic spin inversions at the mechanical resonance frequency of the cantilever, resulting in an oscillation of the cantilever that is detected by a laser-beam-deflection sensor. Our setup also contains a piezo strip that can flip the gradient source away from the sample, allowing spectroscopy experiments to be performed in a homogeneous magnetic field.

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The experimental method applied follows the general principle of multidimensional NMR spectroscopy.^[17] The dimensions can be either spatial dimensions, corresponding to imaging dimensions, or spectral dimensions. In the examples discussed herein, there is always a single spatial dimension combined with either one or two spectral dimensions. The pulse sequence for the two-dimensional experiments, one spatial, one spectral dimension, is shown in Figure 2.

While acquiring a spectral dimension, the gradient source, in this case a small ferromagnet attached to a piezo actuator

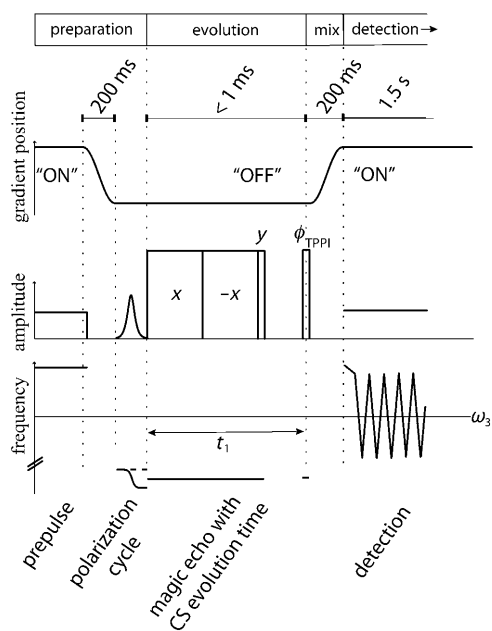


Figure 2. Pulse sequence for one-dimensional spectroscopy in the context of a two-dimensional experiment (one spectral and one spatial dimension). During the t_1 evolution period, the gradient is in the “off” position and the spectral information is encoded. Magic-echo nutation pulses are applied to remove the dipolar interaction. For read-out, the spins are periodically inverted so as to excite the cantilever and the signal is detected as the cantilever’s oscillation amplitude. The spatial resolution is given by the frequency range covered by the frequency sweeps during detection. The pulses in the preparation period are not essential for the principle of operation but serve for the suppression of artifacts (see the Supporting Information). For two spectral dimensions (to yield spatially resolved 2D NMR spectroscopy) a second evolution period can be added. (Further details are given in the text and the Supporting Information.)

(see Figure 1), is mechanically moved to a far-away “off” position, resulting in a relatively homogeneous field at the sample location (see the Supporting Information). Spin coherence is excited by a $\frac{\pi}{2}$ pulse and evolves, during t_1 , under the influence of the chemical-shift interaction and, if present, other interactions, such as J couplings, or dipolar and quadrupolar interactions (Figure 2). After t_1 , the magnetization is stored along the z direction and the gradient source is brought back to the “on” position directly over the sample. In the large field gradient, the signal can now be acquired with high spatial resolution. As usual, the indirect dimension is sampled by systematically incrementing the evolution time t_1 in a series of experiments.^[9] Finally, a chemical-shift-resolved spectrum is obtained by Fourier transform of t_1 . In the pulse sequence as shown in Figure 2, the imaging dimension is also sampled slice by slice, directly in frequency space, by systematically varying the center frequency ω_3 of the detection sweep. A simultaneous sampling of all the slices is, however, possible using a Hadamard encoding during (or just before) the detection period.^[18]

A one-dimensional MRFM image of our test sample, which consisted of two single crystals (KPF_6 and MgF_2), is given in Figure 3a. The spatial resolution, defined as the thickness of the resonant slice,^[19] is $2.0\ \mu\text{m}$. The signal intensity (shown by the black solid line in Figure 3a) represents the total ^{19}F spin density of the sample. The colored areas indicate the separate contributions of KPF_6 and MgF_2 . The two compounds were distinguished in a cross-depolarization (CDP) experiment that takes advantage of the fact that the fluorine magnetization in the KPF_6 crystal can be efficiently destroyed by pulses on the ^{31}P spins.^[10]

In Figure 3b and c localized chemical-shift spectra are shown. The spectra shown in blue correspond to the one-pulse experiment described above. The spectra shown in red are acquired under a magic-echo line-narrowing pulse scheme^[20,21] that selectively averages the dipolar interactions between spins, resulting in considerably sharper and better resolved signals. Figure 3b shows the spectrum at $d = 95\ \mu\text{m}$. At this spatial position only KPF_6 is probed and the spectrum consists of a single line at $\delta = -60\ \text{ppm}$. At $d = 135\ \mu\text{m}$ the sample is heterogeneous and a second peak, associated with MgF_2 , is observed at $\delta = -190\ \text{ppm}$. In addition, the intensity of the KPF_6 peak is reduced compared to Figure 3b, because there is less KPF_6 material at the new position.

To extend the above experiment to two spectral dimensions (in addition to a spatial dimension) an additional time-evolution period, t_2 , is added to the scheme shown in Figure 2 (see also the Supporting Information, Figure S2). As an example, we demonstrate a separated-local-field experiment^[22] that correlates the chemical shift with the dipole coupling. The resulting two-dimensional spectrum (shown in Figure 3d), is recorded at the same spatial position as Figure 3c. Along the first dimension, the chemical shift is used to resolve the two resonance signals from KPF_6 and MgF_2 , while the dipolar interaction is suppressed by the magic-echo sequence. Along the second dimension, the dipolar interaction is active while the chemical-shift interaction is, at least partially, removed by a Hahn-echo pulse.^[23–25] The KPF_6 shows a narrow line width along the

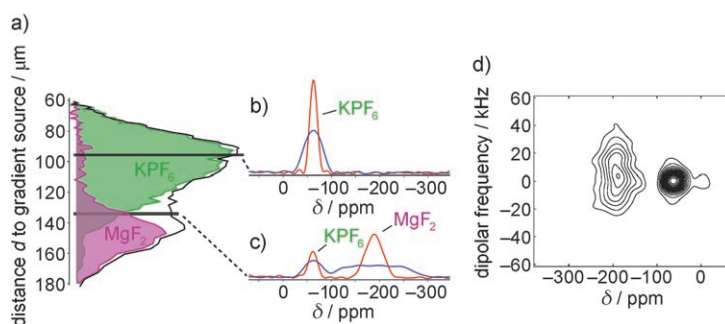


Figure 3. Spatially resolved chemical-shift spectra for KPF_6 and MgF_2 single crystals. a) The ^{19}F spin density as a function of z position. b) and c) Chemical-shift spectra at the two positions indicated in the sample. In (b) only KPF_6 is present, while in (c) both KPF_6 and MgF_2 are found. Red and blue solid lines correspond to experiments with and without line narrowing, respectively. d) 2D spectrum, correlating the dipolar and chemical-shift dimensions in a separated-local-field experiment. (Further details are given in the text and the Supporting Information.)

dipolar dimension ω_2 , as expected, because rapid rotational motion of the KPF_6 unit in the crystal partially quenches the dipolar couplings. The MgF_2 peak, in contrast, is considerably broader as no significant motion is present.

We estimate that the residual gradient in the gradient source in the “off” position is about $(23 \pm 3) \text{ T m}^{-1}$, roughly two orders of magnitude smaller than the 2.5 kT m^{-1} employed for signal detection and spatial encoding. For recording high-resolution chemical-shift spectra, this residual gradient could be reduced even further by moving the gradient source a greater distance away, or by decreasing its size, or by using suitable shim coils. Furthermore, it is conceivable to temporarily switch off the gradient by heating a ferromagnetic gradient source over the Curie temperature.

We have demonstrated chemical-shift imaging and its extension to two-dimensional spectroscopy using a magnetic-resonance force microscope. The spatial resolution, in this case about $2.0 \mu\text{m}$ in one dimension, can be significantly improved by reducing the size of the RF modulation during readout^[26] and by using higher field gradients.^[5] The experiments can be combined with Hadamard multiplexing schemes for the simultaneous acquisition of many slices in the spatial dimension, thus improving the signal-to-noise ratio.^[18] Two-dimensional imaging using Hadamard and Fourier encoding based on the field gradient in the static and RF field, respectively, has recently been demonstrated using the same apparatus^[27] and can be combined with one- or two-dimensional spectroscopy yielding detailed chemical information with high spatial resolution. We expect that further development of the method will lead to chemical-shift images of materials and living biological objects such as cells.

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