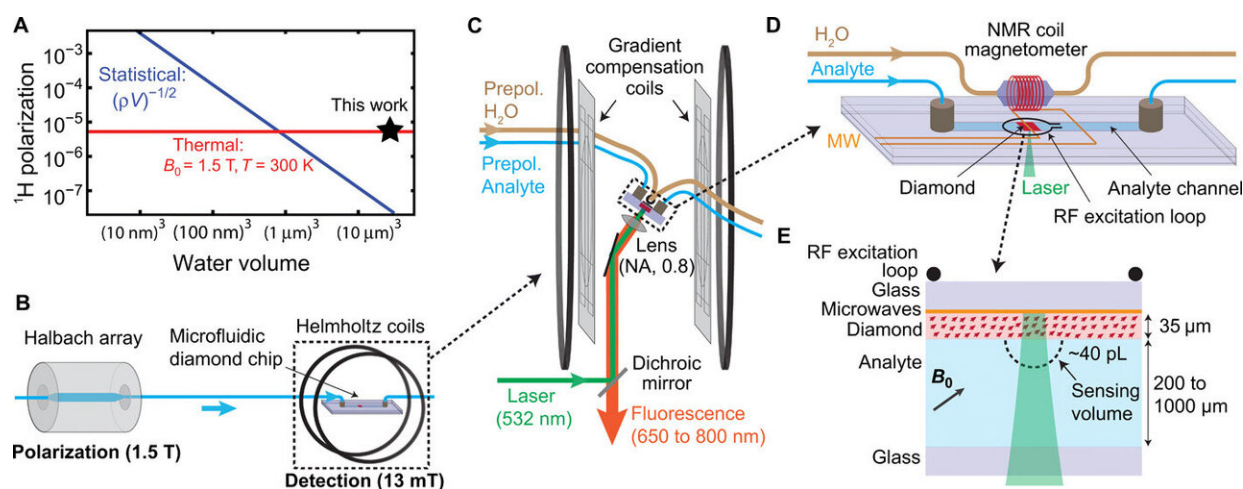


Two-dimensional (2-D) nuclear magnetic resonance (NMR) spectroscopy with a microfluidic diamond quantum sensor

August 2 2019, by Thamarasee Jeewandara



Microfluidic pre-polarization NMR setup. (A) Comparison of statistical and thermal polarization of protons in water as a function of detection volume. The room temperature water proton density is $\rho = 6.7 \times 10^{28}\text{ m}^{-3}$. (B) Pre-polarization concept. Analyte is pre-polarized by flowing it through a permanent magnet (1.5-T Halbach array). It is subsequently shuttled to a microfluidic chip housed in a stabilized, lower magnetic field ($B_0 = 13\text{ mT}$, Helmholtz coils) where it is detected by NV NMR. (C) Detection setup. Pre-polarized analyte flows to a microfluidic chip where it is stopped via fluidic switches (not shown), and the NV NMR signal is detected using a custom-built epifluorescence microscope with a numerical aperture (NA) of ~ 0.8 . A set of eight gradient compensation coils is used to eliminate first- and second-order magnetic field gradients along the field direction. The field is stabilized temporally using a coil-based NMR magnetometer in combination with low-inductance feedback coils wound around the main Helmholtz coils. (D)

Microfluidic chip setup. The chip is constructed using glass and adhesives. Two fluidic lines pass to the detection region, one consisting of water (for NMR coil magnetometer) and the other with analyte (for NV NMR). A radio frequency (RF) excitation loop, placed in between the NMR coil magnetometer and the NV NMR sensor, excites nuclear spin coherence in both channels. The NMR coil magnetometer consists of a 3-mm-diameter coil wound around a $\sim 10\text{-}\mu\text{l}$ water volume. The RF excitation loop and NMR coil magnetometer were placed orthogonal to one another to minimize cross-talk. Copper microwave (MW) lines, printed on the interior of the glass chip, provide spin control over NV electron spins. (E) NV NMR geometry. An NV-doped diamond membrane (1 mm by 1 mm by 0.035 mm) is located on the surface of a microfluidic channel (width: 2 mm, height: between 0.2 mm and 1 mm) in contact with the analyte. Laser illumination (532 nm) bounces off the printed microwave line, and fluorescence (650 to 800 nm) is detected. The effective analyte detection volume is ~ 40 pL. Credit: *Science Advances*, doi: 10.1126/sciadv.aaw7895

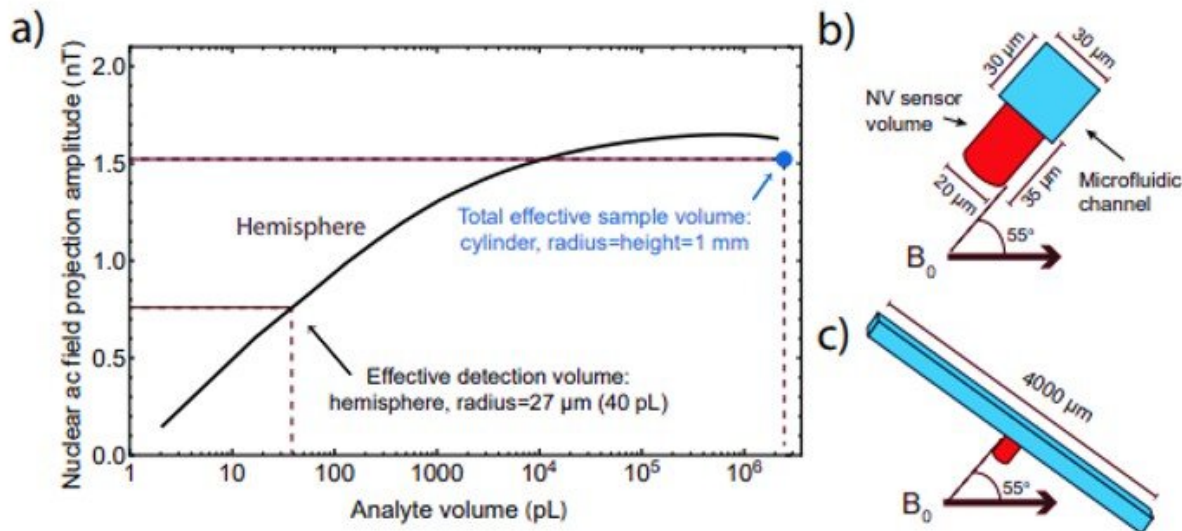
Quantum sensors based on nitrogen-vacancy (NV) centers in diamond are a promising detection mode for nuclear magnetic resonance spectroscopy due to their micron-scale detection volume and noninductive-based sample detection requirements. A challenge that exists is to sufficiently realize high spectral resolution coupled with concentration sensitivity for multidimensional NMR analysis of [picolitre](#) sample volumes. In a new report now on *Science Advances*, Janis Smits and an interdisciplinary research team in the departments of High Technology Materials, Physics and Astronomy in the U.S. and Latvia addressed the challenge by spatially separating the polarization and detection phases of the experiment in a microfluidic platform.

They realized a [spectral resolution](#) of 0.65 ± 0.05 Hz, an order-of-magnitude improvement compared with previous diamond NMR studies. Using the platform, they performed 2-D correlation spectroscopy of liquid analytes with an effective detection volume of ~ 40 picoliters. The

research team used diamond [quantum sensors](#) as in-line microfluidic NMR detectors in a major step forward for applications in mass-limited chemical analysis and single-cell biology.

Nuclear magnetic resonance (NMR) spectroscopy is a powerful and well-established technique for compositional, structural and functional analysis in a variety of scientific disciplines. In [conventional NMR spectrometry](#) the signal-to-noise ratio (SNR) is strongly dependent on the external field strength (B_0). As the spectral resolution increased, the B_0 increased as well, motivating the development of increasingly large and expensive superconducting magnets for improved resolution and SNR, resulting in a [two-fold increase in field strength](#) within the past 25 years.

However, even with large B_0 values, the detection of microscale volumes often required isotopic labeling, concentrated samples and long experimental timelines. To improve the sensitivity for small sample volumes, researchers developed [miniature inductive coils](#), which allowed several advances, including [spectroscopy of egg cells](#) and [in vitro diagnostics](#). The existing sensitivity and detection limits are yet suboptimal for metabolic analysis of [single mammalian cells](#) or for inclusion into [in-line microfluidic assays](#). As an alternative NMR detection strategy, quantum-sensors based on nitrogen-vacancy (NV) centers in diamond have emerged due to their sub-micrometer spatial resolution and noninductive-based detection.



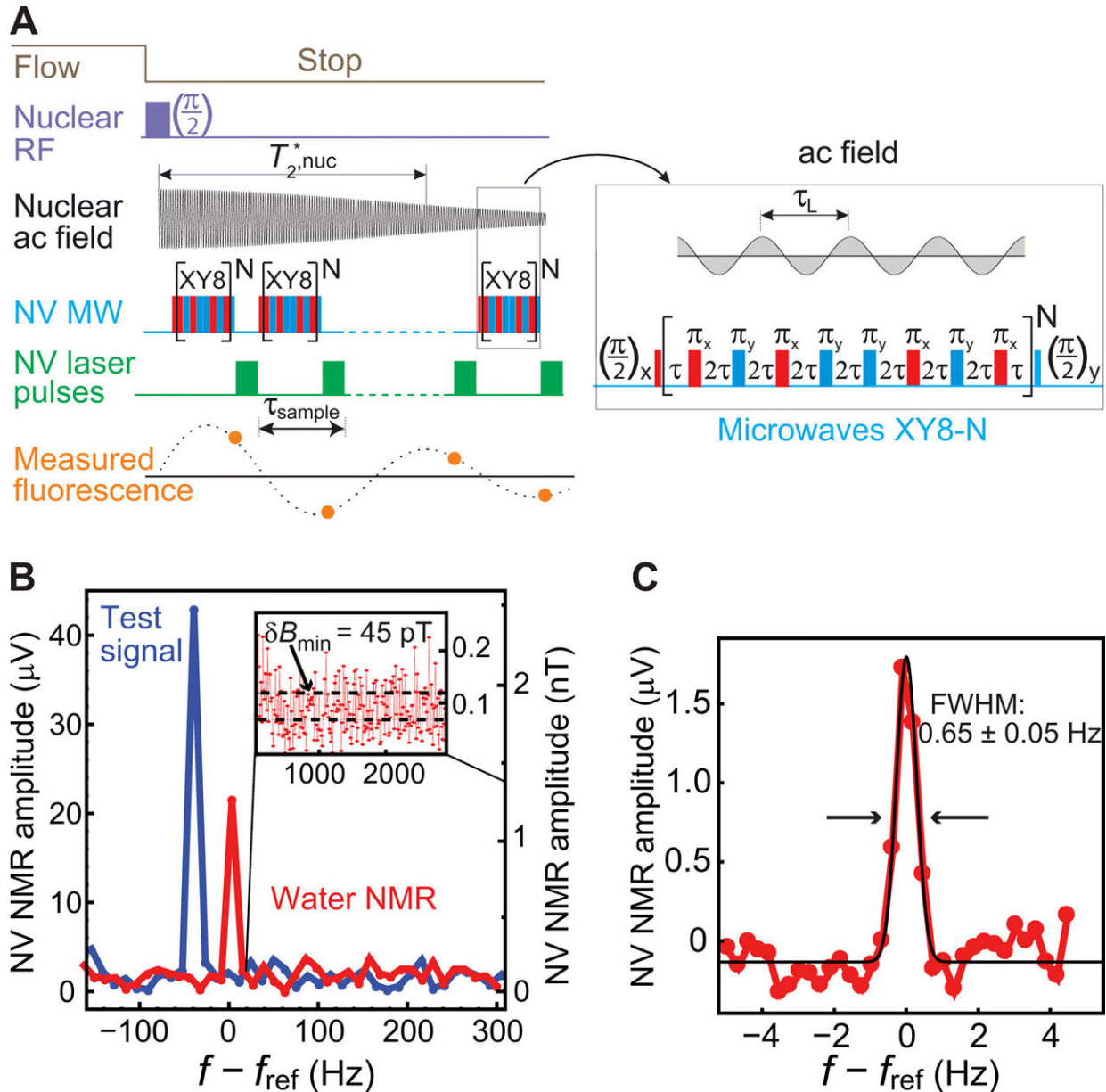
Nuclear ac magnetic field projection amplitude (integrated across the sensor volume) as a function of water volume. The effective detection volume (~40 pL) is defined as the volume where the nuclear ac field projection amplitude is equal to half of that from the total effective sample volume. Credit: Science Advances, doi: 10.1126/sciadv.aaw7895

Scientists previously implemented the method to detect early [nanoscale fluctuations](#) of nuclear magnetization to enhance polarization. They used [viscous solvents](#) to improve the frequency resolution to ~ 100 Hz to obtain the resolution of large chemical shifts at B₀ = 3 T (Tesla). Although further improvements in resolution can be made by increasing the detection volume (V), they were at a cost to SNR. In the present work, Smits et al. report an order-of-magnitude improvement in spectral resolution to realize a concentration sensitivity of ~ 27 M s^{1/2}. To achieve this, they spatially separated the polarization and detection phases of the experiment in [a microfluidic setup](#).

The research team used strong permanent magnets (1.5 Tesla) to generate nuclear spin polarization and performed the detection at 13 mT

using [Helmholtz coils](#) to simplify the task of stabilizing NMR linewidths to sub-hertz levels. They facilitated the use of diamond quantum sensors as in-line microfluidic NMR detectors at low [microwave frequencies](#). The improvements allowed Smits et al. to perform two-dimensional (2-D) correlation spectroscopy (COSY) of liquid analytes within an effective detection volume of ~ 40 pL (pico-liters). The researchers intend to combine this platform with advances in dynamic nuclear polarization using [external polarizing agents](#) and potentially optical hyperpolarization with NV centers to allow NMR spectroscopy of metabolites at physiological concentrations at single-cell spatial resolution.

In the experimental setup, Smits et al. housed the fluid analytes in a helium-pressurized container with variable flow rates of up to $50 \mu\text{l/s}$. The dwelling time for the analytes approximated 6s, longer than the spin relaxation time of the analytes studied (for example T_1 for water ≈ 3 s) leading to an equilibrium polarization of $\sim 5 \times 10^{-6}$. The analyte then flowed to a detection region for identification by NV NMR. To conduct NV NMR detection, the scientists used a custom-built epifluorescence microscope and oriented diamond membranes fabricated in the study, on four possible NV axes to align with the magnetic field in the setup.



Characterization of pre-polarized NV NMR. (A) The synchronized readout pulse sequence. It consists of a train of XY8-N pulses that perform successive phase measurements of the ac magnetic field produced by processing nuclei. The measured fluorescence reflects an aliased version of the nuclear ac field projection. The entire sequence is repeated every 2.5 to 4.25 s (1.25 s for flow and the remainder for detection). (B) NV NMR spectra (absolute value of Fourier transform) of water (red) and an applied 2.5-nT amplitude test field (blue) for an effective acquisition time of 5.2 s (average of 60 traces; total measurement time, 150 s). The NMR signal amplitude obtained from the

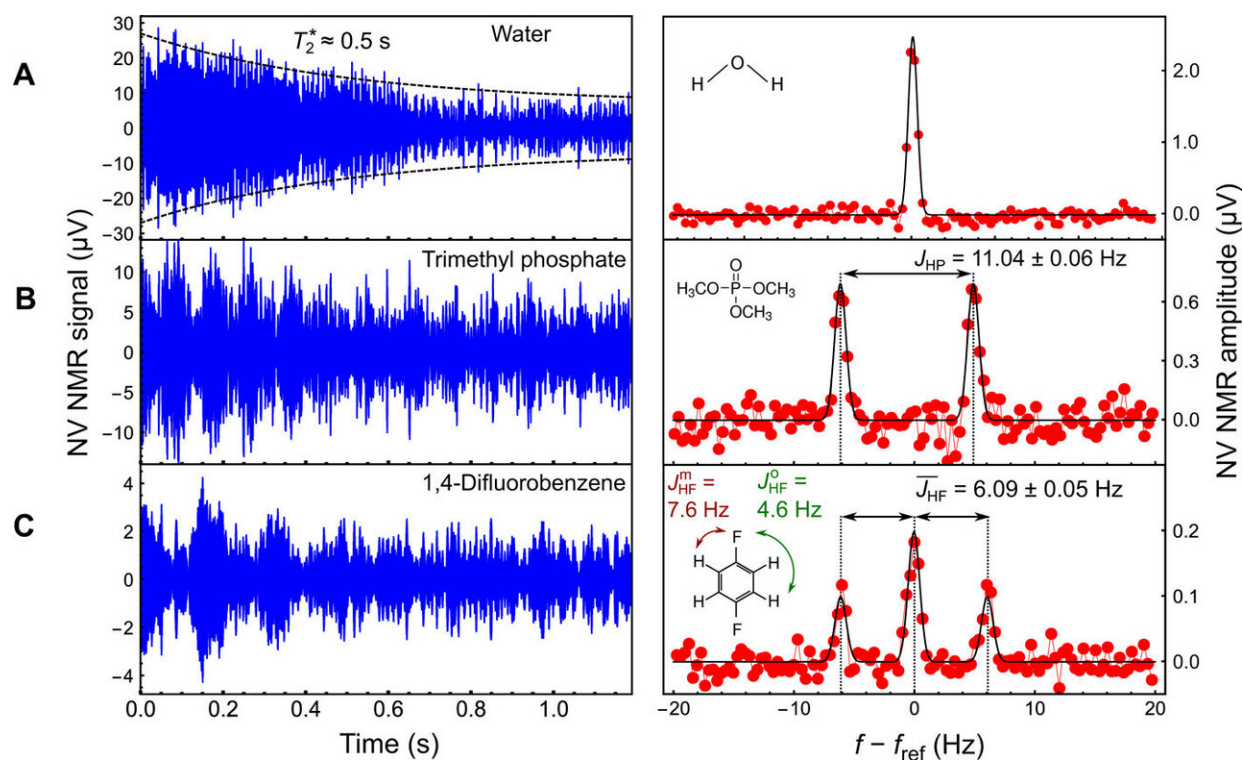
processed photodetector signal is recorded in μV . The conversion to magnetic field amplitude (in nT) is derived from the calibrated test field. Inset: The SD of the noise floor reveals $a_{\text{Bmin}} = 45 \text{ pT}$. From these data, we infer a minimum detectable concentration of $27 \text{ M s}^{1/2}$ ($\text{SNR} = 3$). Incorporating all experimental dead time, the concentration sensitivity is $\sim 45 \text{ M s}^{1/2}$. (C) A high-resolution NV NMR spectrum of water (imaginary part of Fourier transform) reveals a full width at half maximum (FWHM) linewidth of $0.65 \pm 0.05 \text{ Hz}$. Data were obtained by averaging 60 traces, each 3 s long. Credit: Science Advances, doi: 10.1126/sciadv.aaw7895

Smits et al. fabricated the microfluidic chip to house the diamond sensor, the device contained a copper chip on a glass slide to deliver the microwaves. The scientists also included a radio frequency (RF) excitation loop between the diamond and the feedback NMR coil, and a microfluidic channel enclosing the diamond sensor and the contacting analyte. They engineered microfluidic ports to combine external analyte tubing within the chip and used a $20 \mu\text{m}$ diameter laser beam to excite the NV centers through a $35 \mu\text{m}$ -thick diamond membrane.

The research team subsequently applied a series of XY8-5 microwave pulse sequences to the NV center to detect the nuclear ac field. They used deionized water to determine the sensitivity and spectral resolution limits of the apparatus. To optimize the spectral resolution, they adjusted the gradient compensation coils and demonstrated the capabilities of the NV NMR spectrometer by obtaining proton NMR spectra of diverse fluid analytes.

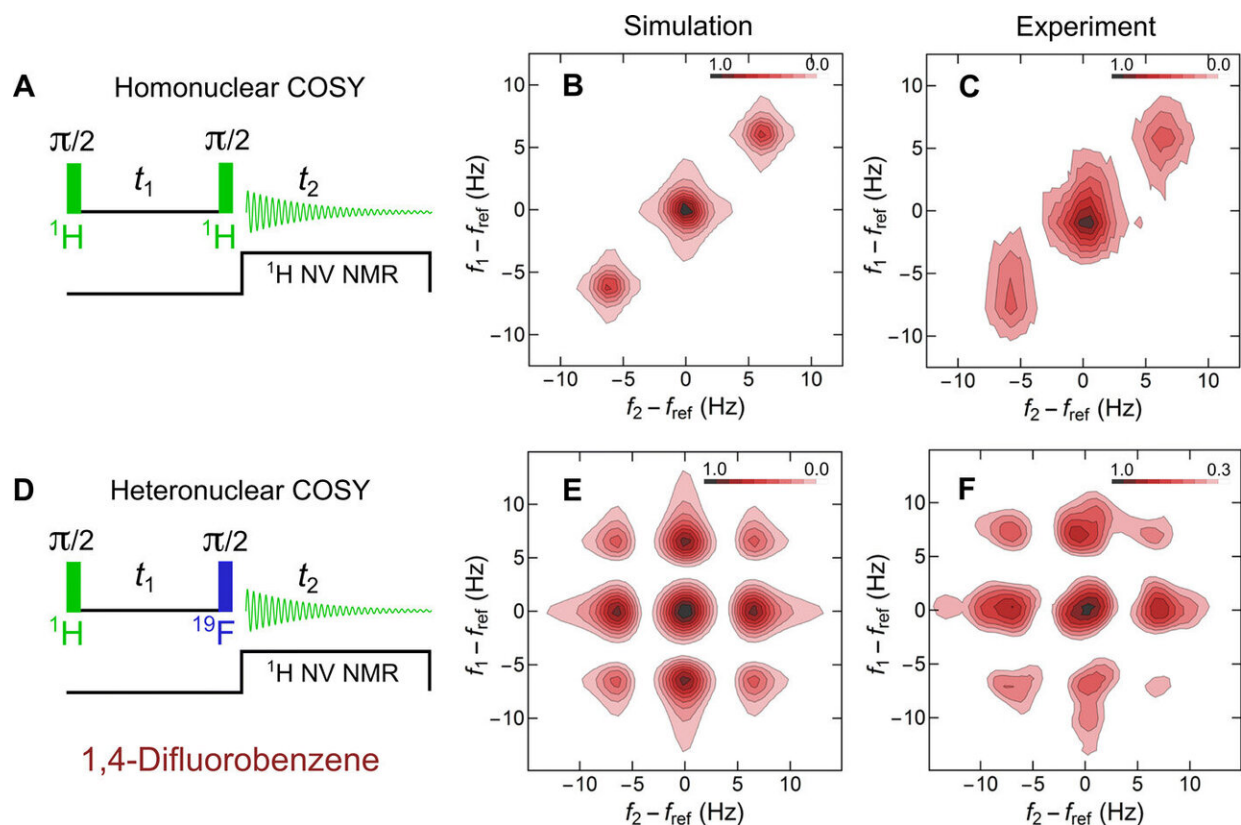
For example, the scientists obtained characteristic NV NMR spectra for trimethyl phosphate (TMP) and 1,4-difluorobenzene (DFB) compounds in the study. After establishing the potential to detect NMR spectra with sub-hertz resolution and high signal-to-noise ratios (SNRs) for the two compounds, they used the platform to perform 2-D [COSY NMR](#)

[spectroscopy](#). For this, Smits et al. performed two variants of the 2-D COSY analysis to probe nuclear interactions within DFB (1,4-difluorobenzene) and performed all simulations using the [SPINACH software package](#) for 2-D NMR.



1D NMR. Time-domain (left) and frequency-domain (right) NV NMR signals for (A) water, (B) trimethyl phosphate (TMP), and (C) 1,4-difluorobenzene (DFB). Signals were averaged over ~ 103 traces for a total acquisition of ~ 1 hour. A ~ 1 -kHz-bandwidth bandpass filter is applied to the time-domain data for better visualization. The frequency-domain spectra show the imaginary component of the Fourier transform. Each spectrum is fit with Gaussian functions (black lines). For TMP, we constrain the widths of both lines to be equal with a 1:1 amplitude ratio and find $J_{HP} = 11.04 \pm 0.06$ Hz. For DFB, we constrain the widths of all three lines to be equal with a 1:2:1 amplitude ratio and find $J_{HF}^- = 6.09 \pm 0.05$ Hz. Credit: Science Advances, doi: 10.1126/sciadv.aaw7895.

The demonstrated sub-hertz resolution and multidimensional NMR techniques can pave the way for the use of diamond quantum sensors within [in-line hyphenated analysis](#), [single-cell metabolomics](#) and [mass-limited pharmacodynamics](#). Smits et al. aim for the resulting high spatial resolution and epifluorescence format to facilitate high-throughput chemical analysis and [NMR imaging of cell cultures](#) with single-cell resolution. Limitations of the present device include the substantial averaging times required at physiological concentrations ranging from the micromolar to the millimolar volume. The researchers propose to use [higher magnetic fields](#) with longer and more sensitive XY8-N microwave pulse sequences to improve NMR sensitivity and photon collection efficiency in contrast to the existing methods. In the long-term, they expect the largest gains in sensitivity to occur via [non-invasive optical hyperpolarization](#) methods.



2D COSY NMR of DFB. (A) Homonuclear COSY pulse sequence, (B) simulated spectrum, and (C) experimental NV NMR spectrum of DFB. (D) A modified heteronuclear COSY sequence reveals off-diagonal peaks in both (E) simulation and (F) experiment. Color scales correspond to the normalized absolute value of the 2D Fourier transform. Vertical axes ($f_1 - f_{ref}$) correspond to the frequencies of the t_1 dimension, and horizontal axes ($f_2 - f_{ref}$) correspond to the frequencies of the t_2 dimension. In (C), 14 values of t_1 in 0.021-s increments up to 0.294 s were used. Total acquisition time was 22 hours. In (F), 16 values of t_1 in 0.021-s increments up to 0.336 s were used. Total acquisition time was 25 hours. In both cases, the t_2 acquisition spanned from 0 to 1.25 s. All simulations were performed using the SPINACH package. Simulation and experimental data use the same windowing functions. Credit: Science Advances, doi: 10.1126/sciadv.aaw7895.

The use of low external field strength (B_0) of 13 mT was another limitation in the study since it restricted the ability to resolve spectral splitting due to chemical shifts. The team aims to improve the chemical shift resolution by increasing B_0 to ~ 0.25 T, using the present detection scheme. In addition, although the NMR microfluidic sensor had an effective detection volume of ~ 40 pL, the scientists required several milliliters of analyte to fill the overall flow of the apparatus. Future microfluidic chips can therefore either miniaturize or omit pre-polarization steps or use smaller microfluidic channels for detection in a larger fluidic system.

In this way, Janis Smits and co-workers demonstrated the use of diamond quantum sensors for microfluidic NMR applications. They showed that separating the polarization and detection steps allowed an order-of-magnitude improvement in spectral resolution compared with existing diamond NMR studies. The scientists validated the platform by performing 2-D NMR on fluid analytes and propose its future applications in multidisciplinary research fields.

- More information:** 1. Janis Smits et al. Two-dimensional nuclear magnetic resonance spectroscopy with a microfluidic diamond quantum sensor, *Science Advances* (2019). [DOI: 10.1126/sciadv.aaw7895](https://doi.org/10.1126/sciadv.aaw7895)
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