Fluorescent and plasmonic labels and sensors have revolutionized molecular biology, helping visualize cellular and biomolecular processes. Increasingly, such probes are now being designed to respond to wavelengths in the near-infrared region, where reduced tissue autofluorescence and photon attenuation enable subsurface in vivo sensing. But even in the near-infrared region, optical resolution and sensitivity decrease rapidly with increasing depth. Here we present a sensor design that obviates the need for optical addressability by operating in the nuclear magnetic resonance (NMR) radio-frequency spectrum, where signal attenuation and distortion by tissue and biological media are negligible, where background interferences vanish, and where sensors can be spatially located using standard magnetic resonance imaging (MRI) equipment. The radio-frequency-addressable sensor assemblies presented here comprise pairs of magnetic disks spaced by swellable hydrogel material; they reversibly reconfigure in rapid response to chosen stimuli, to give geometry-dependent, dynamic NMR spectral signatures. The sensors can be made from biocompatible materials, are themselves detectable down to low concentrations, and offer potential responsive NMR spectral shifts that are close to a million times greater than those of traditional magnetic resonance spectroscopies. Inherent adaptability should allow such shape-changing systems to measure numerous different environmental and physiological indicators, thus providing broadly generalizable, MRI-compatible, radio-frequency analogues to optically based probes for use in basic chemical, biological, medical and engineering research.

Despite growing interest, MRI-based biosensing remains comparatively limited. Magnetic resonance spectroscopy can detect certain commonly occurring biomolecules but low sensitivity precludes high-resolution imaging of these and of many other potential biomarkers. Responsive MRI contrast agents offer alternatives but their reliance on changes in occurring biomolecules but low sensitivity precludes high-resolution imaging of these and of many other potential biomarkers. Responsive MRI contrast agents offer alternatives but their reliance on changes in environmental and physiological indicators, thus providing broadly generalizable, MRI-compatible, radio-frequency analogues to optically based probes for use in basic chemical, biological, medical and engineering research.

A first step towards multiplexable, high-sensitivity radio-frequency (RF) sensors can be made using recently developed microengineered multispectral MRI contrast agents. Whereas conventional T₁ and T₂ contrast agents modify NMR relaxivities, microengineered multispectral agents employ specially shaped magnetizable micro- or nanostructures to controllably shift NMR frequencies. Different structures shapes generate different local magnetic fields and associated NMR frequency shifts, enabling differently 'coloured' RF tags to be used for multiplexed labelling, analogous to optical tags. With their NMR frequencies geometrically determined, such multispectral tags can be transformed into RF 'colorimetric' sensors by incorporating flexible sensor elements that modify tag geometries in response to the environment. Stimuli-responsive hydrogels are one possibility. They offer reversible, tunable swelling that can be specifically sensitized to numerous biomolecules and environmental conditions. Redesigned around such gels, the dynamically reconfiguring magnetic elements of the resulting tags can transduce responsive hydrogel swellings into quantitative, NMR-readable, spectral shifts.

Here we introduce these geometrically encoded magnetic (GEM) sensors by demonstrating: (1) pH measurement, (2) spatiotemporal mapping of ion concentration gradients, (3) real-time tracking of cell metabolism, and (4) co-localized sensing through spectrally separable sensors that would otherwise be unresolvable. Although this reflects a limited set of examples, the ability to tailor gel responsiveness to different targets suggests that the same sensor modality could support RF monitoring of many different biomarkers and physiological or environmental processes. Localized pH sensing, in particular, can help indicate various pathologies including inflammation, ischaemia and cancer. Although not yet realized for clinical MRI, the biomedical importance of pH monitoring already motivates considerable research, including MRI spectroscopies (1H, 19F, and 31P) [16–18] and CEST agents [9,19], hyperpolarized substrates [20,21], and pH-dependent relaxation. All show promise, but can suffer from limited sensitivity, short agent lifetimes, or a need for multi-agent ratio-metric concentration normalization, respectively. Shape-changing GEM sensors, on the other hand, are not fundamentally lifetime-limited, offer high sensitivities (detailed below) and, unlike many MRI agents, do not rely on signal amplitude variations, providing instead concentration-independent frequency readouts for more precise, unambiguous quantitation.

The sensor design builds on a multispectral MRI agent geometry comprising spaced, magnetizable disk pairs that, owing to their magnetic shape anisotropy, automatically align themselves with applied magnetic fields [19] (see Fig. 1a and b). When magnetically saturated in the field of an NMR/MRI, such self-aligning assemblies generate tailorable, homogeneous, offset magnetic fields between the disks. The NMR frequencies of water self-diffusing through these homogeneous field regions are then shifted proportionally to the offset field magnitude. Examples of such field-shifted, or spectrally offset, NMR signals are shown in Fig. 1c through histograms of calculated magnetic fields, which closely mimic NMR spectra, in the vicinity of such magnetic structures. The spectral offsets, \( \omega_{\text{offset}} \), define the structures' NMR frequencies, or effective RF colours, and are tunable by changing structure shapes and materials according to:

\[
\omega_{\text{offset}} \approx -4Jf_{\beta} \left[ \frac{Lr^2}{(4r^2 + d^2)^{3/2}} \right]
\]
Here $h$, $r$, and $d$ are disk thickness, radii, and separation, respectively, $\gamma$ is the proton gyromagnetic ratio, and $J_S$ is the saturation magnetic polarization density of the disk material, which may reach 2 T (for iron), enabling large spectral offsets. These offsets are scale invariant, permitting a broad range of sensor sizes. Offsets do vary, however, if aspect ratios change. For disks separated by a responsive hydrogel spacer, differentiation predicts the additional NMR frequency shift, $\Delta \omega_{\text{offset}}$, as the gel changes size:

$$\Delta \omega_{\text{offset}} = \frac{3\gamma d^2}{4r^2 + d^2} \frac{\Delta d}{d} = \frac{1}{2} \frac{\Delta d}{d}$$

That is, the fractional change in NMR frequency shift scales roughly linearly with the fractional length change of the spacer (see also the inset to Fig. 1c).

Sensors described here are microfabricated (see Methods). They comprise 800–1,000-nm-tall, 300–400-nm-wide posts of biocompatible, antifouling, poly(ethylene glycol)-based hydrogel sandwiched between two 10–60-nm-thick, 900–1,000-nm-radius disks of nickel, or for biocompatibility, iron. Gel pH sensitivity arises from deprotonation of incorporated methacrylic acid side groups in relatively basic conditions. The resulting charged gel swells, increasing the disk separation, $d$, and thereby reducing the internal field magnitude and the resulting $\omega_{\text{offset}}$ value. Conversely, in relatively acidic conditions, protonation allows elastic recovery, decreasing $d$ and increasing the magnitude of $\omega_{\text{offset}}$.

As examples, the resonance shifts of nickel and of iron sensors with compressed and expanded hydrogel spacers are compared in Fig. 1d through NMR-z-spectra (Methods), which show the frequency-dependent water magnetization saturated out, $M_w$, as a fraction of initial water magnetization $M_0$. In Fig. 1e, a pH curve records resonance shifts from sensors submerged in a series of different pH buffers across the physiological pH range. Sensor response times are limited by gel shrinking and swelling times, but with submicrometre-scale to nanometre-scale hydrogel elements these times are easily sub-second (see Methods and Supplementary Video 1), allowing for real-time reporting and spatio-temporally well-localized measurements.
In buffered pH conditions, ionic concentrations can also be measured. Local ion concentrations influence much cellular activity, guiding intracellular function, intercellular communication, and, through chemotactic gradients, extracellular migration. In sensor terms, higher ion concentrations increase electrostatic shielding of the gel, reducing chemotactic gradients, extracellular migration. In sensor terms, higher ion concentrations increase electrostatic shielding of the gel, reducing chemotactic gradients, extracellular migration. In sensor terms, higher ion concentrations increase electrostatic shielding of the gel, reducing chemotactic gradients, extracellular migration. In sensor terms, higher ion concentrations increase electrostatic shielding of the gel, reducing chemotactic gradients, extracellular migration.

To verify extended operation in biological fluids, we also tracked the metabolic rates of Madin-Darby canine kidney (MDCK) cells placed with GEM sensors in an enclosed volume of Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum. With no circulating air, the sensors measure the cell growth medium’s acidification due to metabolic CO₂ production and cell necrosis in the increasingly hypoxic surroundings. Experiments were performed at 37 °C and at 32 °C with half the cell density used at 37 °C (Methods). Total oxygen consumption rates should decrease at lower cell numbers and at lower temperatures, which slow cell metabolism. As expected, recorded acidification and time to cell death (inferred by cessation of acidification) were faster at 37 °C than at 32 °C (Fig. 3). Comparing Figs 3b and 1e, spectral shifts also indicate decreases of roughly 1 and 0.7 (or log(10) and log(5)) pH units, confirming that at half the cell density the sensors register half the acidification.

While spacer gel expansions determine sensor frequency shifts, Δω_{offset}, initial ω_{offset} values are independently tunable over large frequency ranges by changing disk shapes and materials (see equation (1)). Thus different sensors can be spectrally isolated from one another, providing selective addressing and spatially co-localized, multiplexed sensing. As an example, we interleave two hierarchically patterned arrays of sensors of different geometries. Selective detection is demonstrated by decoupling the overlaid sensor images (Fig. 4).

Any biomarker detection is ultimately limited by reporter sensitivity, including absolute probe detectability and the relative biomarker-induced changes therein. Several factors combine to amplify GEM reporter sensitivity. First, the sensors’ spatially extended homogeneous field regions allow sensor states to be simultaneously sampled by many water molecules. Second, because water continually diffuses in and out of these regions, magnetization transfer imaging can greatly multiply the effective water signal volume (Methods). Third, large ω_{offset} values shift sensor signals far from any natural background and satisfy slow-exchange limitations even while facilitating more rapid water transfer, which further boosts signal. As an example, Extended Data Fig. 5 shows nearly 2.5% saturation transfer arising from approximately 75,000 GEM sensors submerged in a 15 mm × 15 mm × 0.1 mm water volume. This extrapolates to 5% signal change (the standard for reliable detection) at

Figure 2 | Spatiotemporal mapping of ion concentrations. a, Schematic of array of sensors (not to scale) submerged in a water tray filled with a 0.1 M, pH 7.5 buffer that contacts one end of a short tube filled with water-based agarose gel. As ions diffuse from the buffer solution into the agarose, locally depleted ion concentrations within the buffer are detected through induced shifts in sensor resonances. With an agarose volume one-tenth that of the buffer, complete mixing corresponds to a 10 mM ion reduction, but smaller dilution variations are also resolvable. b, Time evolution of sensor readings in zones I, II, III, and IV, corresponding to distances from the agar inlet of approximately 1–3 mm, 3–5 mm, 5–7 mm, and 7–9 mm, respectively. c, Spatial variation in sensor readings as a function of distance from agarose at various time points. Insets in b and c show numerical simulations of expected spatiotemporal variations in ionic strength, I, normalized to initial ionic strength, I_0. Inset x axes are in the same units as the main graph axes.

Figure 3 | Tracking cell metabolism. a, Schematic of experiment showing sensors suspended above MDCK cells in sealed volume of cell growth medium. b, Shifts in sensor resonance frequencies over time as cells acidify the surrounding medium through metabolic CO₂ production and cell necrosis. Different curve slopes and amplitudes confirm different metabolic rates and levels of acidification for experiments run at 37 °C and at 32 °C, with higher and lower cell densities, respectively.
a sensor concentration of approximately 10 femtomoles per litre, a promising level for targeted molecular imaging and potential monitoring of biomarkers that occur at concentrations undetectable with any other responsive MRI agent.

Converted to metal ion concentrations, the above example, which used iron-based sensors with 10-nm-thick, 900-nm-radius disks, corresponds to about 50 μM iron, below clinical Gd³⁺ and conventional PARACEST lanthanide ion concentrations, and comparable to those of specialized high-sensitivity polymeric and supramolecular PARACEST22,23. However, while GEM agents may consist predominantly of iron, Gd or PARACEST macromolecules are commonly tenfold more massive than their lanthanide ions alone. That is, despite each sensor being far larger than each lanthanide agent, for equivalent signal contrast the total mass of exogenous material required may be an order of magnitude less for GEM agents. At less than a picogram per sensor, this total mass amounts to a few micrograms per gram, or millilitre, of water. Notably, the above numbers derive from unoptimized first-generation sensors. Detection limits should improve with more accurate microfabrication34, and with optimized pulse sequence and sensor size and shape design.

Being ferromagnetic, GEM sensor disks are magnetically saturated in most MRI fields. This yields large, field-independent frequency offsets and, according to equation (2), proportionally large, responsive frequency shifts that further augment sensitivity by magnifying biomarker changes. Large frequency shifts are also particularly advantageous at lower, clinical MRI field strengths, where natural background can overwhelm the smaller shifts of traditional spectroscopic and chemical-exchange-based approaches. At clinical fields of 1.5 T, for example, the sensors’ 32-kHz spectral splitting per pH unit (Fig. 1e) represents approximately 500 parts per million (p.p.m.) separation. However, this results from thin nickel disks and a full-range hydrogel expansion of just 20% (see Methods). Switching to iron more than triples this splitting. Thicker disks can also increase spectral shifting several-fold, as can smaller disk diameters (see equation (1)). And hydrogel expansions can be increased substantially. Many responsive gels readily double in size; some hydrogels can even reversibly lengthen 20-fold25. Over narrow pH ranges, such expansions could improve sensitivity another one to two orders of magnitude. Combined, these modifications suggest potential spectral shifts approaching a million times those of conventional ¹H, ¹²F, or ³¹P NMR, which yield the order of 1 p.p.m. per pH unit16–18.

The inherent adaptability of the GEM reporter platform should also allow measurement of many variables besides pH. Different hydrogels, responsive to other environmental parameters, such as temperature, can be substituted. Additionally, through molecular imprinting28, or by incorporating catalytic enzymes27, enzyme cleavable substrates28, or specific receptor-ligand type bondings29 into the hydrogel, sensors can be reconfigured to measure (in a continuously reversible or irreversible manner) a broad array of analytes including numerous metabolites, antigens, and proteins. Specific protein recognition may in turn also enable sensitive RF mapping of reporter gene expression. Moreover, hydrogel responses can be tuned for optimal sensor range and linearity. For example, for pH sensing, expansions can be tailored through gel composition, crosslink density, and fractional acid content, with active ranges independently selected through acid pKₐ values.

While GEM sensors are already far smaller than biological cells, at present they still suffer from biological delivery issues associated with nano- to microscale materials. There is, however, growing interest in biomedical applications of materials in these size ranges30. Nor should nano- to microscale materials. There is, however, growing interest in present they still suffer from biological delivery issues associated with catalytic enzymes27, enzyme cleavable substrates28, or specific receptor-ligand type bondings into the hydrogel, sensors can be reconfigured to measure (in a continuously reversible or irreversible manner) a broad array of analytes including numerous metabolites, antigens, and proteins. Specific protein recognition may in turn also enable sensitive RF mapping of reporter gene expression. Moreover, hydrogel responses can be tuned for optimal sensor range and linearity. For example, for pH sensing, expansions can be tailored through gel composition, crosslink density, and fractional acid content, with active ranges independently selected through acid pKₐ values.

Figure 4 | Sensor multiplexing. a, Scanning electron micrograph showing array of interleaved sensors with alternately larger and smaller disk sizes. (Interspersed features are non-magnetic residual topography from the microfabrication process.) b, Schematic of hierarchical, interleaved sensor patterning. The words ‘ACID’ and ‘BASE’ are patterned out of two interleaved arrays of sensors. ‘ACID’ characters are spatially patterned from sensors with larger disks; ‘BASE’ characters are from sensors with smaller disks. At locations where characters overlap, both sensor variants are present. c, NMR z-spectrum showing two spectrally separated resonances from sensors comprising larger (‘ACID’) and smaller (‘BASE’) disks. The inset shows two MRI difference images of the same sample region acquired at different offset frequencies, showing selective addressing of different sensor populations. With alternate sensors interleaved every 6.4 μm, but an image resolution of 250 μm, images also show that different sensor signals can be simultaneously resolved even if coincident in the same MRI voxel, allowing co-localized multiplexed sensing. Images are created from signal intensity differences between magnetization transfer images acquired at an offset of ~1 MHz (at which water is unaffected by sensors) and at frequencies corresponding to the two different sensor resonances seen in the z-spectrum. Sensors, however, a nanometre change in disk separation can provide resolvable kilohertz shifts, raising the prospects of subnanometre displacement detection and potential NMR-accessible RF analogues to optical plasmonic and fluorescent molecular rulers.

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Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.
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Author Contributions G.Z. conceived of the project, designed the experiments, fabricated the sensors, analysed the data, and wrote the manuscript. S.J.D. designed all experiments and carried out experiments, Y. Chen for providing the MDCK cells, and J. Moreland for discussion and NIST Boulder cleanroom access. G.Z. (gary.zabow@nist.gov) oversaw the work, provided critical feedback, and helped write the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to G.Z.
Hydrogel composition. Hydrogel precursor solutions were mixed as a 1:1 ratio (w/w%) of poly(ethylene glycol) (n) dimethacrylate (with a poly(ethylene glycol) block molecular weight of approximately 200, Polysciences) and methacrylic acid (Sigma-Aldrich). (Chemical supplier names are included solely to specify experimental details; they do not indicate National Institute of Standards and Technology (NIST) or National Institutes of Health (NIH) endorsement of any particular company.) To this was added 5% (w/w%) of 2,2-dimethoxy-2-phenylacetophenone (also from Sigma-Aldrich), used as a photoinitiator. Cross-linking was performed using an i-line ultraviolet source with 5 cm² flood exposure through half-millimetre-thick borosilicate glass substrates. Before sensors were used, they were soaked in water or pH buffer (typically in pH 7 to pH 7.5) to wash out unreacted initiator and/or monomer, which might otherwise impede water access and hydrogel swelling or shrinking.

Hydrogel expansion. The 20% expansion quoted in the main text can be derived from equation (2), which shows that the frequency shift (about 10% between compressed and expanded gel spacer) is approximately half that of the gel expansion percentage. As a cross-check, however, we also tested a macroscopic sample of the hydrogel, formulated similarly to that used in the nanoscale hydrogel spacers. The test sample was polymerized against a substrate with surface relief patterns pre-etched at two different length scales: a series of etched lines (1 mm wide with 2-μm centre-to-centre spacing), which formed an optical diffraction grating, together with wider grids lines side up the diffraction grating into millimetre-scale squares. This pattern is transferred into the polymerized hydrogel surface and is visible when the hydrogel is removed from the substrate, simplifying measurement of gel expansion or contraction. Extended Data Fig. 2 shows such a hydrogel sample in the compressed and expanded state (immediately after being removed and lightly wiped dry after submerging in a pH 8 buffer). Images agree with an isotropic linear expansion of about 20%. Admittedly, in the sensor configuration, expansion may not be completely isotropic. The cylindrical gel spacers are laterally constrained at their top and bottom end surfaces through contact to the magnetic discs. During expansion this manifests as radial (transverse) compressive strain near the two ends of the spacer that may slightly increase the axial expansion (by an amount that depends on the Poisson’s ratio of the gel and on the extent of the radial strain towards the cylinder ends). Such additional expansion is likely to be small, however, because the transverse squeezing exists over only a fraction of the gel cylinder. Total expansion therefore seems in good agreement with sensor NMR field shift measurements.

pH buffers. All buffers were 0.1 M phosphate buffers mixed from monosodium phosphate and its conjugate base, disodium phosphate.

Curve fitting. To avoid any bias in extracting sensor resonant frequencies, each z-spectrum was automatically curve fitted to a linear background-subtracted Gaussian curve with the central frequency of the fitting Gaussian being taken as the sensor’s resonant spectral offset. (In the case of the agarose-buffer ion mapping experiment, where separate z-spectra were collected for each imaging voxel, signal-to-noise levels were sometimes insufficient to guarantee convergence of the curve-fitting algorithm. Therefore, to ensure consistency, those z-spectra were first averaged over several voxels in space and/or time).

Agarose-buffer interdiffusion experiment, simulation, and animation. Diffusion mapping was based on a 0.1 M, 7.5 pH phosphate buffer solution filling an approximately 15 mm × 15 mm × 0.1 mm water tray with one surface covered in a 14 mm × 14 mm array of 1,000-nm-radius sensors (lattice spacing 6.4 μm). A hole drilled into the tray material from underneath provided access to the water in the tray at a position 1–2 mm from one edge of the square water tray. A 1-mm-inner-diameter tube, filled with an ~3-mm length of agarose, was inserted into this hole such that one end of the agarose contacted the water in the tray.

To confirm that shifts in sensor resonant frequencies were due to changes in ionic strength, and not inadvertent changes in pH as the buffer stretch was diluted by the water in the agarose gel, a separate experiment was performed with pH 7.5 buffer solution poured directly over a volume of agarose gel twice as large as the buffer volume (to amplify any effect). After sitting for a day, the pH, as measured by conventional pH meter, had shifted by no more than 0.1 pH units, and did not shift any further thereafter. Since the ratio of agarose to pH buffer volume in the actual diffusion experiment was 20 times smaller than this, any pH changes in the actual experiment were assumed to be negligible.

Because of the finite dimensions of the agarose source/sink and the water tray, diffusion dynamics are not easily analytically approximated. Therefore, a numerical simulation of the ion diffusion between buffer and agarose was performed. Extended Data Fig. 3 shows sample simulation-derived in-plane ion concentration variations at various times. Theory insets to Fig. 2b and c were derived from these simulations.

The animation (Supplementary Video 2) derived from the sensor-recorded ion concentration data was generated by automatically curve-fitting all spectra collected,
in space and time. The animation comprises a sequential time series of spatial maps of the measured sensor resonant frequencies.

Hierarchically patterned ‘ACID’/‘BASE’ sample. The ‘ACID’/‘BASE’ sample was microfabricated using the protocol described above except that the patterning steps were represented in Extended Data Fig. 1b and f were modified. Arrays with interleaved rows of large and small disks were first optically exposed and then, before photore sist development, re-exposed with a pattern that removed disks everywhere except for locations corresponding to the ‘ACID’/‘BASE’ patterns. Since both sensor sizes were processed simultaneously, remaining hydrogel posts are wider for larger disk sensors than for smaller ones.

Z-spectra and magnetization transfer imaging. Sensor signals come predominantly from water whose NMR precession frequency is shifted by the homogeneous field between the sensor’s disks. Because self-diffusion continually replenishes the water within this homogeneous field region, however, the volume of water from which signal is acquired can be orders of magnitude larger than the volume between the disks. We exploit this diffusion-driven signal amplification by using a magnetization transfer imaging method. As shown schematically in Extended Data Fig. 4, trains of preparatory RF-pulses (generally n/2 pulses) are applied at an off-resonant frequency to ‘saturate out’ the magnetization of any water that is shifted to that particular frequency as it diffuses through the magnetic fields surrounding the sensor structure. This is followed by an on-resonance pulse and free-induction-decay acquisition that measures the remaining bulk water magnetization not yet saturated out. Noting the saturation as a function of the offset frequency of the applied RF-pulse train builds up the z-spectra used to measure sensor spectral shifts. Since the fields external to the sensor structures are inhomogeneous and decay rapidly in space, relatively little saturation occurs at most offset frequencies; conversely, when the frequency of the off-resonant preparatory pulses matches the frequency shift due to the extended internal homogeneous field regions of the sensors, a substantial saturation signal can appear from water as it diffuses in and out between the sensor disks. Exact signal amplification depends on how often water between the sensor disks is replenished before the accumulated magnetization deficit decays appreciably due to longitudinal relaxation. Since the time to diffuse a given distance scales quadratically with that distance, refresh rates are higher for smaller sensor structures allowing higher signal gains. A caveat is that the water exchange should not be so fast that it frequency–broadens the shifted sensor lines to such a degree that they overlap the unshifted background water line. Sensors with larger spectral shifts therefore allow faster water exchange rates and, accordingly, greater signal amplifications.

Sensor detection concentration limit. Detection concentration limit experiments (see Extended Data Fig. 5) were performed using an array of sensors made from 900-nm radius, 10-nm-thick iron disks. The sensor array was approximately 14 mm × 14 mm with square lattice spacing of 51.2 μm, equaling about 75,000 sensors, and was submerged in a 15 mm × 15 mm × 0.1 mm volume of water. Imperfect microfabrication did unnecessarily broaden sensor linewidths somewhat and lead to some fraction of these sensors being malformed, implying a true count of operable sensors less than 75,000. For calculations, however, we have assumed 75,000; that is, real sensor detection limits are probably better than those quoted here.

Cell metabolism experiments. All cells were incubated in DMEM cell growth medium with 10% fetal bovine serum at 37°C in a custom-designed 15 mm × 15 mm × 0.1 mm hydrogel sensor array with initial cell number and incubation time for each sample, to have the high-density experiment, a monolayer coverage of cells adherent to the sample holder base. Before MRI scanning, the medium was replaced with fresh growth medium and the sample holder sealed with a flat lid containing the array of sensors on its underside. To allow immediate recording, the MRI bore was prewarmed to 37°C or 32°C (to slow cell metabolism) by setting the gradient coil chiller instead to heat appropriately. Following experiments, it was noted that the phenol red pH indicator dye present in the DMEM cell medium had turned from an initially pinkish colour to a yellowish one, indicating that the medium pH had fallen at least below 6.8. Also, pipetting the cell medium liquid from a separate control experiment onto a piece of filter paper revealed a final pH of 6.5 ± 0.3, in agreement with the GEM sensor readings.

250-nm-radius double-disk structures. Paper results are based on 900–1,000-nm-radius sensors, but we are currently exploring how to shrink the sensors further. A sample z-spectrum is shown in Extended Data Fig. 6 from preliminary 250-nm-radius, 20-nm-thick, double-disk structures. As yet, our microfabrication is not optimized for these smaller structures. Signals are therefore lower than possible but still easily resolvable, suggesting the feasibility of considerably smaller structures.

Sensor response rates. GEM sensor response times are limited by the response rates of their hydrogel spacers. Macroscopic hydrogels often respond slowly because they are limited by hydrodynamic factors such as solution time for solute (or solvent) to penetrate through the gel. Being a primarily diffusively driven system, this is slow for large gels but speeds up roughly quadratically as gel sizes shrink. Sensors with nanoscale spacing posts, diffusion times through the gels are predicted to be in the millisecond range or below, enabling sensor responses that are fast on NMR timescales. This aids real-time tracking of local changes in the environment but can complicate measurement of the exact sensor response speed itself, since sensor transient dynamics may be too fast to be recorded by NMR.

In control experiments, however, we find that it is also possible to optically observe sensor action. We find that light reflecting off an array of the top disks of sensor structures can interfere with light reflecting off the sensor’s bottom disks or substrate, producing different interference colours that depend on the top-to-bottom disk separation in a manner akin to ‘thin-film interference’ phenomena more commonly observed in oil films, and so on. For appropriate viewing angles, the hydrogels change size the reflected light oscillates between being biased towards the red or blue end of the spectrum. Specifically, for close to normal incidence, the 20% length change in the hydrogel spacer used in the paper translates into a total optical path length change of 400 nm to 500 nm (assuming a water refraction index of ~1.33). This corresponds to almost a full oscillation through the reflected colours, implying that as the hydrogel spacers shrink, an initially reddish reflection, say, would be expected to turn green/blue and then partially return to red again (and vice versa). An example of this is shown in Supplementary Video 1, which shows an array of sensors covering a 15 mm × 15 mm substrate submerged in phosphate-buffered saline and subjected to a short pulse of a few drops of dilute hydrochloric acid, which lowers the local pH as it sweeps through the solution. The video (in real-time at 30 frames per second) shows rapid colour change, which implies rapid sensor response, following the acid front. (Note that to owing surface and resulting microfabrication errors, sensors at different points on the substrate have slightly different initial hydrogel spacer lengths, which impart different initial reflected colours at various points across the substrate).

To better quantify the rate of change, Extended Data Fig. 7 shows the colour change observed as a function of time at different points on the substrate as the acid front moves over. Also shown is a sample sequence of still frames from the same video. Once the acid front reaches any given point on the substrate, the reflected colour can be seen to change rapidly, with virtually all the colour change occurring within 100–200 ms. Unfortunately, we currently cannot discern what fraction of this time period is actually consumed by sensor response versus simply the time it takes for the acid first to locally mix with and acidify the solution surrounding the sensors. The data do, however, establish a ceiling for sensor response time of 0.2 s or less.

MRI pulse sequence parameters. All data were acquired on either a 14 T scanner with commercial birdcage transmit/receive coil or on an 11.7 T scanner with home-built solenoid transmit/receive coil. Field strength and coil type were chosen based on scanner and coil availability and are not necessarily optimal. Similarly, pulse sequence parameters are not necessarily optimized.

Compressed and expanded Ni and Fe sensor z-spectra. For each frequency point in each of the z-spectra (shown in Fig. 1d), a series of 15,000 off-resonant π/2 Gaussian-shaped pulses of length 0.1 ms with centre-to-centre spacings of 0.35 ms were first applied. Following these preparatory off-resonance pulses, a single on-resonance π/2 hard pulse was applied followed by free-induction-decay acquisition of the on-resonance water (see pulse schematic in Extended Data Fig. 4). A 6-s delay was added after the acquisition before the next series of off-resonance pulses were applied. Data presented are based on two averages.

Agarose-buffer ion diffusion. The same preparatory off-resonance pulse sequences were used in Fig. 2b and c as for the pH-curve z-spectra above. However, each train of off-resonance pulses was followed by a gradient-echo imaging sequence with repeat/echo times (TR/TE) = 20/2.2 ms, matrix size = 32 × 32 × 30 flip angle, total acquisition time for each image at each offset frequency = 640 ms). The same pulse sequence as for the pH-curve z-spectra was used for Fig. 3b with the experiment repeated continually until no further frequency change was observed.

Multiplexed ‘ACID’/‘BASE’ z-spectra. The same pulse sequence as for the pH-curve z-spectra was used in Fig. 4c.

Multiplexed ‘ACID’/‘BASE’ image. The same pulse sequence as for the pH-curve z-spectra was used for the inset to Fig. 4c. However, each train of off-resonance pulses was followed by a gradient-echo imaging sequence with TR/TE = 25/2.3 ms, matrix size = 64 × 32 × 30 flip angle, total FOV = 15 mm × 7.5 mm, and with centre-out phase encoding (total acquisition time for each image at each offset frequency = 800 ms). This acquisition was repeated 100 times allowing for signal averaging. Detection concentration limit z-spectrum. A similar acquisition sequence as to the pH-curve z-spectra was used in Extended Data Fig. 5 except this time applying 25,000 off-resonance π pulses.
250-nm-radius double-disk spectrum. A similar acquisition sequence as to the pH-curve z-spectra was used in Extended Data Fig. 6 except this time applying 15,000 pulses and an 8-s delay between acquisition of each point in the spectrum.

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Extended Data Figure 1 | Schematic of microfabrication protocol. See Methods for explanation of panels a–o.
Extended Data Figure 2 | Hydrogel expansion. Hydrogel sample in compressed (top) and expanded (bottom) state, showing ~20% linear expansion.
Extended Data Figure 3 | Diffusion simulations. Logarithmically shaded (greyscale bar) plots of numerically simulated ion-concentrations (normalized to initial concentration of unity) due to diffusion between pH buffer solution and water-based agarose gel.
Extended Data Figure 4 | Pulse protocol. Schematic showing off-resonant preparatory pulse train, followed by on-resonant excitation pulse and free-induction-decay (FID) acquisition. The sequence is repeated, each time with different offset frequency, to acquire each point in the z-spectrum.
Extended Data Figure 5 | Sensor sensitivity. $z$-spectrum, showing magnetization saturated out $M_s$ normalized to the initial water magnetization $M_0$ from sensors with Fe disks of radii 900 nm and thickness 10 nm with resonance around ~325 kHz.
Extended Data Figure 6 | Sensor miniaturization. z-spectrum, showing magnetization saturated out $M_s$, normalized to the initial water magnetization $M_0$, for double-disk structures of radii 250 nm with resonance around ~525 kHz.
Extended Data Figure 7 | Optically probed sensor response rates. Top panel, a series of consecutive still frames from Supplementary Video 1, showing the propagation of an acid front over an array of sensors. The resulting changes in reflected colours are due to changes in spacing between the top and bottom disks of the sensor, indicating rapid sensor response to introduced acid. Bottom panels, reflected light intensity in green and red channels (normalized to average light intensity across all colour channels) recorded frame-by-frame at the substrate points a and b indicated in leftmost top panel. Slightly different starting and end points within colour oscillation are due to unintentional sensor microfabrication variation across the substrate.