A diamond voltage imaging microscope

D. J. McCloskey✉, N. Dontschuk✉, A. Stacey1,2, C. Pattinson3, A. Nadarajah1, L. T. Hall3, L. C. L. Hollenberg1,3, S. Prawer1 and D. A. Simpson1

Technologies that capture the complex electrical dynamics occurring in biological systems, across fluid membranes and at solid–liquid interfaces are important for furthering fundamental understanding and innovation in diverse fields from neuroscience to energy storage. However, the capabilities of existing voltage imaging techniques utilizing microelectrode arrays, scanning probes or optical fluorescence methods are limited by resolution, scan speed and photostability, respectively. Here we report an optoelectronic voltage imaging system that overcomes these limitations by using nitrogen-vacancy defects as charge-sensitive fluorescent reporters embedded within a transparent semiconducting diamond device. Electrochemical tuning of the diamond surface termination enables photostable optical voltage imaging with a quantitative linear response at biologically relevant voltages and timescales. This technology represents a major step towards label-free, large-scale and long-term voltage recording of physical and biological systems with sub-micrometre spatial resolution.

The development of fluorescent molecular sensors for imaging voltage changes in biological systems has revolutionized neuroscience, providing a tool to capture neuronal activity over large areas with sub-neuron resolution both in vitro and in vivo. However, the poor photostability of molecular voltage sensors limits recording times to a few minutes, posing problems for longitudinal studies of network evolution and disease processes. These limitations mean that lower-resolution techniques such as multielectrode arrays (MEAs) remain predominant tools in neuroscience, disease modelling, drug discovery and safety pharmacology. By embedding fluorescent, charge-sensitive defects within a transparent semiconducting substrate, solution voltage imaging can, in principle, be realized by the optical detection of local changes in the near-surface semiconductor space-charge layer. Changes to this space-charge layer are known to modulate the fluorescence of defects by altering the number of electrons bound to each, otherwise known as the charge state of the defect. Such a hybrid optoelectronic approach has been proposed and would occupy a voltage imaging regime unexplored to date, combining the spatial resolution of optical techniques with the long-term stability and minimal invasiveness of MEAs. The nitrogen-vacancy (NV) defect in diamond—a fluorescent atom-scale point defect—is bright, photostable and possesses three optically distinguishable charge states known to be responsive to voltage changes in solution, making it an ideal candidate system for developing this approach. As a substrate, a diamond is biocompatible and has well-developed nanofabrication pathways, meaning the proposed technique could potentially be applied to both intracellular and extracellular recording. In addition, the chemical inertness of diamond suggests that this approach to voltage imaging could enable time-resolved electrochemical microscopy, complementing the scanning probe techniques currently employed in the characterization of energy storage materials in a wide range of liquid electrolytes.

In this work, we realize optoelectronic voltage imaging with ensembles of NV centres by engineering the near-surface electrostatic environment of diamond for responsiveness to external potentials. We first establish precise electrochemical control of the diamond surface termination, which we use to tune the ensemble charge state population to an optimal composition for voltage sensing consisting exclusively of the fluorescent neutral (NV0) and non-fluorescent positive (NV+) states. This approach minimizes background fluorescence, avoids spectral overlap between NV0 and negative (NV-) state that would otherwise act to impede sensitivity, and eliminates any requirement for d.c. biasing. We then demonstrate the capabilities of our diamond voltage imaging microscope (DVIM) by performing the real-time imaging of capacitive charge injection by a microelectrode in solution. Finally, we show that this sensing mechanism can be replicated and enhanced in an array of diamond nanopillar optrodes, each possessing sub-millisecond fluorescence response times and two orders of magnitude greater voltage sensitivity than previously demonstrated using NV centres.

Results

Fabrication and operating principles. Our devices consist of high-density (HD) (of the order of 104–107 cm−2) ultrashallow (≤7 nm) NV ensembles formed by ion implantation into ultra-pure single-crystal diamond wafers (Methods). The diamonds are hydrogen terminated by indirect exposure to hydrogen plasma (Supplementary Fig. 1), which prevents the hydrogen passivation of shallow NV centres27–29 and renders the diamond surface electrically conductive in atmosphere via the formation of a two-dimensional hole gas30,31. The devices are mounted within custom-built fluid wells (Fig. 1a), which feature a planar platinum (Pt) electrode used to apply solution potentials for characterization while fluorescence excitation and collection are simultaneously performed from below. As indicated by the grey region in Fig. 1b, hydrogenation of the diamond surface results in the loss of virtually all detectable fluorescence, indicating the full conversion of NV ensemble to the dark NV+ state. The charge state of an NV centre is determined by the position of the Fermi level, EF, relative to the adiabatic charge state transition energies (Fig. 1c). The layer of negatively charged adsortates that forms on the hydrogenated surface creates a strong electrostatic field that shifts these transition energies upwards relative to EF (ref. 32), a phenomenon known as near-surface band bending.

1School of Physics, University of Melbourne, Parkville, Victoria, Australia. 2School of Science, RMIT University, Melbourne, Victoria, Australia. 3Centre for Quantum Computation and Communication Technology, School of Physics, University of Melbourne, Parkville, Victoria, Australia. ✉These authors contributed equally: D. J. McCloskey, N. Dontschuk. ✉✉e-mail: dan.mccloskey@unimelb.edu.au; simd@unimelb.edu.au

NATURE PHOTONICS | VOL 16 | OCTOBER 2022 | 730–736 | www.nature.com/nature photonics
The combination of an ultrashallow NV ensemble and the low bulk defect concentration of the diamond material used enables surface transfer doping to cause appreciable changes in the low bulk defect concentration of the diamond material used be intractable. The NV0 following hydrogenation (Supplementary Fig. 1), which NV ensembles with the same areal density show some population (Supplementary Note 1):

\[ \Delta \eta / \eta = \beta \Delta V, \Delta I = \text{the measured change in the fluorescence count rate, } \eta \text{ is the fluorescence at 0 V and } \Delta V \text{ is the change in solution potential.} \]

As shown in Fig. 1f, the initial surface oxidation pulses concurrently increase the fluorescence contrast and NV0 population until the voltage response plateaus in an optimal sensitivity region. At this point, the surface is partially oxidized (Fig. 1g, left) with \( E_r \) intersecting the NV0+ transition within the shallow implanted region (Fig. 1g, right). The partially oxidized diamond surface retains sufficient conductivity to allow for solvated charges to build up within an electrolytic double layer on the application of a solution voltage. As experimentally shown in Fig. 1h, positive solution voltages reduce the upwards band bending, increasing the NV0 population and fluorescence intensity, whereas negative solution voltages have the opposite effect. Integrating the raw spectra reveals a fluorescence response that is well approximated by a linear function across a range of gate voltages of around ±50 mV (Supplementary Fig. 1). For samples oxidized well beyond their maximum sensitivity, we begin to observe NV0– interconversion (Supplementary Fig. 1). However, the sensitivity of such a configuration is limited by low contrast resulting from the spectral overlap between NV0 and NV0 emissions.

**Imaging microelectrode charge injection in solution.** To verify the localized solution voltage imaging capabilities of the DVIM, we used it to image the spatiotemporal voltage transient resulting from the application of a voltage step to a proximal microelectrode. Figure 2a illustrates the experimental setup: an insulated Pt/Ir microelectrode with an exposed tip diameter of ≈8 μm (Supplementary Fig. 2)
was positioned as close to the diamond surface as possible without contact in a dilute buffered saline solution (Methods). Before the measurement of microelectrode signals, the voltage response of the imaged chip area was calibrated using d.c. voltages applied via the large Pt ring electrode (Methods). Figure 2b demonstrates the DVIM response within a dynamic range of ±70 mV, from which linear fits of the responses at each pixel can be used to construct a calibration map (Fig. 2c). A signal generator was used to apply 100 mV between the microelectrode and diamond surface while NV fluorescence was recorded using an inverted wide-field fluorescence microscope and a scientific complementary metal–oxide–semiconductor camera. This voltage is low enough to ensure the two surfaces behave as ideally polarizable electrodes (negligible Faradaic currents, Methods).  

**Characterization of a DVIM enhanced by photonic surface structuring.** Using equation (1), we determine a median per-area sensitivity of 2.0 µF cm⁻² and bulk solution resistivities of 25–200 kΩ cm, which we measured from similar solution preparations to that employed here.
As evidenced by Fig. 3b, these diamond optrodes with an array of 700 nm-diameter pillars produced by reactive ion etching (Methods) demonstrate a tenfold increase in photon collection efficiency. Figure 3c illustrates the characteristic fluorescence image of a diamond sensor surface, with a map of fluorescence responsivity values for each optrode shown in d. The extracted rise- and fall-time constants for the optrodesversus square-wave voltage pulse amplitude in solution. The values were obtained from stretched exponential fits (Methods) to the measured fluorescence transients, which were averaged over 500 trials. The error bars are 95% confidence intervals for the fit parameters obtained from the co-variance matrix of the fit residuals. The inset shows the representative response time trace for a 20 mV pulse averaged over 500 integrations. The solid line shows a stretched exponential fit to the data. f, Noise power spectral densities (NSD) from a single-diamond optrode (blue, top) and the square area highlighted in d (red, bottom). The calculated shot-noise-limited sensitivities are indicated by the dashed lines. Scale bars, 10 μm. These measurements were performed in highly conductive PBS (Methods).

sensitivity, we implement a straightforward approach for increasing light collection by patterning the DVIM surfaces with arrays of nanopillars to act as fluorescence waveguides\(^1\). Figure 3a shows a scanning electron microscopy image of a diamond sensor surface with an array of 700 nm-diameter pillars produced by reactive ion etching (Methods). As evidenced by Fig. 3b, these diamond optrodes provide a tenfold increase in photon collection efficiency from our shallow NV\(^\circ\) ensembles. Variations in fluorescence intensity from the optrodes shown in the image result from variations in wide-field laser excitation, as confirmed by the uniform brightness of the optrodes when observed with scanning confocal microscopy (Supplementary Fig. 3). Figure 3c demonstrates the photostability of the optrodes, with no degradation of the fluorescence signal amplitude detected over 40 s of continuous recording as a 20 mV square wave was applied to the sample in PBS using the Pt ring electrode. We also note that no degradation of sensing performance was observed over the period during which the measurements of this device were performed (more than four months; Supplementary Fig. 4). We confirmed that the linearity of the sensor response was preserved following nanofabrication both at the single-optrode level and over the aggregate field of view (Supplementary Fig. 3). We observed an increase in the overall fluorescence contrast of the optrodes (Fig. 3d) compared with the flat surface. This effect was confirmed by measuring the voltage responses of circular test structures spanning two orders of magnitude in diameter that we fabricated on the same diamond sample (Supplementary Fig. 3). As we employed an oil-immersion objective lens for sensitivity characterization, we primarily attribute this phenomenon to a reduction in the relative contribution of static background fluorescence originating from the immersion oil itself (Supplementary Fig. 1) to the fluorescence signals.

To evaluate the temporal response of the DVIM, an avalanche photodiode (APD) was used to capture the fluorescence dynamics from the illuminated area on the application of 3 ms square-wave voltage pulses with varying amplitudes from the large Pt counter-electrode. Unlike the microelectrode measurements described above, here we used a highly conductive buffered saline solution to minimize the RC time constant of the overall circuit. Figure 3e shows the extracted rise- and fall-time constants, whereas the inset shows a representative fluorescence response time trace. These responses are well fit by exponential functions and yield time constants consistent with capacitive charging of the diamond surface rather than NV charge state transition rates, which are expected to be <1 μs (ref. \(^1\)). As this charging time is limited by the diamond surface resistivity, it could be reduced in future devices by fabricating surface electrical contacts closer to the sensing region. The measured fluorescence response time of <300 μs corresponds to the maximum operating frequency of around 3 kHz. This knowledge allows us to reliably measure the noise spectral density of the DVIM as our camera is operated at its highest possible frame rate (over 128 × 128 pixels) of 1.6 kHz. Figure 3f displays the noise spectral density of a single optrode and a 9.25 μm × 9.25 μm area (Fig. 3d, white square). Above ≈10 Hz, the measured noise floors show no apparent dependence on frequency and are consistent with the photon shot-noise limits predicted by equation (1), which are denoted by dashed lines.

**DVIM sensitivity.** From the measured noise power spectra, we obtain a sensitivity of 55 μV Hz\(^{-1/2}\) per optrode or 77 μV Hz\(^{-1/2}\), accounting for the interpillar pitch of 1.4 μm. This value, more than six times the sensitivity of the unpatterned area of the same sample, could be further improved to 42 μV Hz\(^{-1/2}\) by reducing the pitch to 900 nm and utilizing hexagonal close-packed arrays in future devices. Diamond nanopillar structures are particularly attractive for electrophysiological applications due to their ability to facilitate close contact with cultured neurons\(^1\). With this use case in mind, Fig. 4a compares our measured optrode sensitivities with established technologies for the voltage imaging of neuronal cultures in vitro. Neuro-electrophysiological recordings can be broadly classified into three distinct modalities depending on the nature of the sensor–neuron interface. Planar sensors can be used to measure extracellular voltage signals with minimal perturbation to the cells in question. However, they provide poor confinement of transmembrane currents, which results in low signal powers that can only be detected by the most sensitive techniques, indicated by those occupying the region below the violet line in Fig. 4a. When protruding features can be engulfed by cultured cells, resulting in a well-sealed fluid cleft between the sensor and membrane, a class of higher-power ‘quasi-intracelluar’ signals
The optical readout mechanism is not subject to the same restrictions as imaging with sub-millisecond fluorescence response times. In addition, we measured a technologically feasible upper limit on the sensitivity of a single-diamond optrode using NV⁻⁺⁻ sensing (Fig. 4a, green square) based on the implementation of established material and apparatus optimizations (Supplementary Note 3). The limit of sensitivity using charge state interconversion between NV⁻ and NV⁺ of around 375 nV Hz⁻¹/² for a single optrode (Supplementary Fig. 5) is comparable to state-of-the-art HD MEAs, but offers more than one order of magnitude greater spatial resolution. In addition, the optical readout mechanism is not subject to the same restrictions on the overall number of active recording channels as MEAs⁶⁹, potentially allowing higher volumes of information to be extracted from dense neuronal cultures.

To model local bioelectrical charge injection and verify our calculated sensitivities, we developed a protocol to generate voltage signals with sub-millisecond response times (Supplementary Fig. 6). We also note that the applied signal was resolvable at the single-optrode level (Supplementary Fig. 6). To demonstrate that sub-millisecond detection is presently only limited by the photon shot noise, we gated our measurements to the applied signals and averaged the results over several trials, a practice for which a precedent exists for high-resolution mapping of extracellular signal propagation⁴⁰,⁴¹. Figure 4c shows the resulting detected voltage traces integrated over a flat region (1.7 μm × 1.7 μm), indicating a peak voltage of less than 800 μV at the sensor surface (5 mV applied to the microelectrode). The region of interest can also be broken into sixteen 425 nm sub-regions, thereby demonstrating voltage recording with sub-micrometre resolution. This represents a 20 times improvement over current HD MEA systems and is around twice the diffraction limit of our microscopy apparatus (≈215 nm). The averaged result of 2,000 trials is shown in Fig. 4c for clarity, but we note that the signal was resolvable after around 200 trials (Supplementary Fig. 6).

**Discussion**

We have demonstrated an optical voltage imaging sensor with a quantitative linear response utilizing the transitions of diamond NV centres between their neutral and positive charge states. Our method is made possible through tailored control over the NV ensemble charge state populations via electrochemical tuning of the diamond surface termination. This technology circumvents the need for on-chip readout circuitry, enabling resolutions more than twenty times higher than complementary metal–oxide–semiconductor HD MEAs as parallel readout is enabled from, in principle, as many channels as there are pixels available on modern scientific cameras (>1 million). Diamond voltage imaging microscopy can be immediately utilized in fundamental studies where the complex electrokinetic dynamics of diffuse liquids⁴² preclude or complicate the use of single-point probe measurements⁴³,⁴⁴, and may enable time-resolved imaging studies of battery systems.⁴⁵ Practical application of this technique to electrophysiology will likely require

---

**Fig. 4** Comparison with existing neuronal voltage imaging technology and demonstration of fast signal detection. a. Comparison of voltage sensitivities and signal detection thresholds at 1 kHz. The data points represent the best measured sensitivities of flat (blue) and nanopatterned (red) DVIMs and the theoretical best sensitivity of a DVIM (green). The solid lines show the scaling of sensitivity with the interrogated area (resolution) to assist with comparison with other technologies. The dashed horizontal lines show the estimated detection thresholds for intracellular (pink, top) and extracellular (violet, middle) mammalian neuronal action potentials. The shaded regions roughly indicate the sensitivity and resolution regimes available to current high-speed voltage imaging technologies (Supplementary Note 2 provides the justification and Supplementary Table 2 provides tabulated comparisons). b. Detection of 1 ms square-wave voltage pulses applied with a Pt microelectrode at a repetition rate of 10 Hz by a 4 × 4 array of optrodes (effective resolution, 5.2 μm). The red triangles indicate the rising edges of the applied pulses. c. Detection of 1 ms square-wave voltage pulses with sub-millivolt amplitude and 425 nm resolution by repeated integration on a 1.7 μm × 1.7 μm section of a flat DVIM chip. The shaded region indicates the duration of the applied pulse. All the displayed traces are unfiltered.
adhesion-promoting coatings to improve cell survival rates and enhance signal strength via biological seal resistance. The ionic nature of these coatings could conceivably cause changes to near-surface NV responses; however, our testing has shown no deleterious effects (Supplementary Note 4). Long-term photostability, rapid response of NV emissions and the transparency of diamond make DVIMs an attractive platform for the future studies of neuronal network formation and function, where the transparent substrate can be leveraged to enable multimodal voltage imaging with, for instance, transcriptional, structural or metabolic tags, as well as all-optical closed-loop systems utilizing optogenetic stimulation.

Recent advances in large-area single-crystal diamond wafer synthesis and in neuronal culturing on diamond surfaces and nanopillars provide optimism that DVIMs can be realized as a competitive in vitro electrophysiological platform. On the near-term horizon, nanowire optroteodes (<200 nm in diameter) may provide intracellular access, whereas newly developed diamond dry-etching techniques can be used to fabricate arrays of mushroom-like optroteodes for quasi-intracellular recordings. Looking ahead, although substantial (yet realistic) improvements in diamond materials engineering would be required (Supplementary Note 3 and Supplementary Table 4), we predict that voltage imaging using charge state transitions of fluorescent colour centres will enable label-free extracellular imaging of neuronal network dynamics with deep sub-cellular resolution.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41566-022-01064-1.

Received: 22 November 2021; Accepted: 20 July 2022.
Published online: 8 September 2022

References
1. Knöpfel, T. & Song, C. Optical voltage imaging in neurons: moving from technology development to practical tool. Nat. Rev. Neurosci. 20, 719–727 (2019).
2. Piatkevich, K. D. et al. Population imaging of neural activity in awake and fictive swimming zebrafish using a label-free extracellular imaging approach. Nature 574, 413–417 (2019).
3. Wang, W., Kim, C. K. & Ting, A. Y. Molecular tools for imaging and recording neuronal activity. Nat. Chem. Biol. 15, 101–110 (2019).
4. Abdelfatah, A. S. et al. Bright and photostable chromogenic indicators for extended in vivo voltage imaging. Science 365, 699–704 (2019).
5. Abbott, J. et al. Extracellular recording of direct synaptic signals with a CMOS-nanoelectrode array. Lab Chip 20, 3239–3248 (2020).
6. Emenegger, V., Obien, M. E. J., Franke, F. & Hierlemann, A. Technologies to study action potential propagation with a focus on HD-MEAs. Front. Cell. Neurosci. 13, 139 (2019).
7. Castelletto, S. & Boretti, A. Color centers in wide-bandgap semiconductors for subdiffusion imaging: a review. Adv. Photon. 3, 054001 (2021).
8. Lühmann, T. et al. Screening and engineering of colour centres in diamond. J. Phys. D: Appl. Phys. 51, 483002 (2018).
9. Petrikova, V. et al. Charge-sensitive fluorescent nanosensors created from nanodiamonds. Nano Lett. 17, 12307–12311 (2017).
10. Karaveli, S. et al. Modulation of nitrogen vacancy charge state and fluorescence in nanodiamonds using electrochemical potential. Proc. Natl Acad. Sci. USA 113, 3938–43 (2016).
11. Doherty, M. W. et al. The nitrogen-vacancy colour centre in diamond.
12. Schirhagl, R., Chang, K., Loretz, M. & Degen, C. L. Nitrogen-vacancy centers in diamond: nanoscale sensors for physics and biology. Annu. Rev. Phys. Chem. 65, 83–105 (2014).
13. Grotz, B. et al. Charge state manipulation of qubits in diamond. Nat. Commun. 3, 729 (2012).
14. Krečmarová, M. et al. A label-free diamond microfluidic DNA sensor based on active nitrogen-vacancy center charge state control. ACS Appl. Mater. Interfaces 13, 18500–18510 (2021).
15. Yang, K.-H. & Narayan, R. J. Biocompatibility and functionalization of diamond for neural applications. Curr. Opin. Biomed. Eng. 10, 60–68 (2019).
16. Hanlon, L. et al. Diamond nanopillar arrays for quantum microscopy of neuronal signals. Nanoscale 7, 03903 (2015).
17. Momenzadeh, S. A. et al. Nanoengineered diamond waveguide as a robust bright platform for nanomagnetometry using shallow nitrogen vacancy centers. Nano Lett. 15, 165–169 (2015).
18. Kehayas, P. et al. Solution nuclear magnetic resonance spectroscopy on a nanostructured diamond chip. Nat. Commun. 8, 188 (2017).
19. McCloskey, D. I. et al. Enhanced widefield quantum sensing with nitrogen-vacancy ensembles using diamond nanopillar arrays. ACS Appl. Mater. Interfaces 12, 13421–13427 (2020).
20. Abbott, J. et al. A nanoelectrode array for obtaining intracellular recordings from thousands of connected neurons. Nat. Biomed. Eng. 4, 232–241 (2020).
21. Liu, R. et al. High density individually addressable nanowire arrays record intracellular activity from primary rodent and human stem cell derived neurons. Nano Lett. 17, 2757–2764 (2017).
22. Spira, M. E. & Hai, A. Multi-electrode array technologies for neuroscience and cardiology. Nat. Nanotechnol. 8, 93–104 (2013).
23. Stötter, J., Show, Y., Wang, S. & Swain, G. Comparison of the electrical, optical, and electrochemical properties of diamond and indium tin oxide thin-film electrodes. Chem. Mater. 17, 4880–4888 (2005).
24. Kempaiah, R., Vasudevanmurthy, G. & Subramanian, A. Scanning probe microscopy based characterization of battery materials, interfaces, and processes. Nano Energy 65, 13931 (2020).
25. Liu, D. et al. Evolution of solid electrolyte interface on TiO2 electrodes in an aqueous Li-ion battery studied using scanning electrochemical microscopy. J. Phys. Chem. C 123, 30958–30971 (2019).
26. Schryen, C., Polakow, V., Wunderlich, R., Meijer, J. & Nebel, C. E. Active charge state control of single NV centres in diamond by in-plane Al-Schottky junctions. Sci. Rep. 5, 12160 (2015).
27. Stacey, A. et al. Depletion of nitrogen-vacancy color centers in diamond via hydrogen passivation. Appl. Phys. Lett. 100, 071902 (2012).
28. Findler, C., Lang, J., Osterkamp, C., Nesládek, M. & Jelezko, F. Indirect overgrowth as a synthesis route for superior diamond nano sensors. Sci. Rep. 10, 22404 (2020).
29. Ristein, J. Surface transfer doping of diamond. J. Phys. D: Appl. Phys. 39, R71–R81 (2006).
30. Maier, F., Riedel, M., Mantel, B., Ristein, J. & Ley, L. Origin of surface conductivity in diamond. Phys. Rev. Lett. 85, 3472–3475 (2000).
31. Broadway, D. A. et al. Spatial mapping of band bending in semiconductor devices using in situ quantum sensors. Nat. Electron. 4, 502–507 (2018).
32. Chaplin, B. P., Hubler, D. K. & Farrell, J. Understanding anodic wear at boron doped diamond film electrodes. Electrochim. Acta 89, 122–131 (2013).
33. Petrikova, V. et al. Luminescence of nanodiamond driven by atomic functionalization: towards novel detection principles. Adv. Funct. Mater. 22, 812–819 (2012).
34. Ristein, J., Zhang, W. & Ley, L. Hydrogen-terminated diamond electrodes. I. Charges, potentials, and energies. Phys. Rev. E 78, 041602 (2008).
35. Licht, S., Cammarata, V. & Wrighton, M. S. Direct measurements of the physical diffusion of redox active species: microelectrochemical experiments and their simulation. J. Phys. Chem. 94, 6133–6140 (1990).
36. Dunkerl, M. et al. Hydrophobic interaction and charge accumulation at the diamond-electrolyte interface. Phys. Rev. Lett. 106, 196103 (2011).
37. Spira, M. E., Shmoel, N., Huang, S.-H. M. & Erez, H. Multisite attenuated intracellular recordings by extracellular multielectrode arrays, a perspective. Front. Neurosci. 12, 218 (2018).
38. He, G. et al. Nanoneedle platforms: the many ways to pierce the cell membrane. Adv. Funct. Mater. 30, 1809890 (2020).
39. Ogi, J. et al. A 4.8-μm, noise CMOS-micro electrode array with density-scalable active readout pixels via disaggregated differential amplifier implementation. Front. Neurosci. 13, 234 (2019).
40. Viswam, V., Obien, M. E. J., Franke, F., Frey, U. & Hierlemann, A. Optimal electrode size for multi-scale extracellular-potential recording from neuronal assemblies. Front. Neurosci. 13, 385 (2019).
41. Müller, J. et al. High-resolution CMOS MEA platform to study neurons at subcellular, cellular, and network levels. Lab Chip 15, 2767–2780 (2015).
42. Bazant, M. Z., Thornton, K. & Adalja, A. Diffuse-charge dynamics in electrochemical systems. Phys. Rev. E 79, 021506 (2004).
43. Collins, L., Kilpatrick, J. J., Rep. Prog. Phys. 85, 066101 (2018).
44. Collins, L. et al. Full data acquisition in Kelvin probe force microscopy: mapping dynamic electric phenomena in real space. Sci. Rep. 6, 30557 (2016).
45. Salerno, M. & Dante, S. Scanning Kelvin probe microscopy: challenges and perspectives towards increased application on biomaterials and biological samples. Materials 11, 951 (2018).
46. Garrett, D. J., Tong, W., Simpson, D. A. & Meffin, H. Diamond for neural interfacing: a review. *Carbon* **102**, 437–454 (2016).

47. Petrakova, V. et al. Imaging of transfection and intracellular release of intact, non-labeled DNA using fluorescent nanodiamonds. *Nanoscale* **8**, 12002–12012 (2016).

48. Kitagawa, H. et al. Activity-dependent dynamics of the transcription factor of cAMP-response element binding protein in cortical neurons revealed by single-molecule imaging. *J. Neurosci.* **37**, 1–10 (2017).

49. Wu, B., Eliscovich, C., Yoon, Y. J. & Singer, R. H. Translation dynamics of single mRNAs in live cells and neurons. *Science* **352**, 1430–1435 (2016).

50. Lukinavičius, G. et al. Fluorogenic probes for live-cell imaging of the cytoskeleton. *Nat. Methods* **11**, 731–733 (2014).

51. Zhang, Z., Chen, W., Zhao, Y. & Yang, Y. Spatiotemporal imaging of cellular energy metabolism with genetically-encoded fluorescent sensors in brain. *Neurosci. Bull.* **34**, 875–886 (2018).

52. Barral, J. & D Reyes, A. Synaptic scaling rule preserves excitatory–inhibitory balance and salient neuronal network dynamics. *Nat. Neurosci.* **19**, 1690–1696 (2016).

53. Werley, C. A., Chien, M.-P. & Cohen, A. E. Ultrawidefield microscope for high-speed fluorescence imaging and targeted optogenetic stimulation. *Biomed. Opt. Express* **8**, 5794–5813 (2017).

54. Kim, S.-W., Takaya, R., Hirano, S. & Kasu, M. Two-inch high-quality (001) diamond heteroepitaxial growth on sapphire (1120) misoriented substrate by step-flow mode. *Appl. Phys. Express* **14**, 115501 (2021).

55. Chia, C., Machielse, B., Shams-Ansari, A. & Lončar, M. Development of hard masks for reactive ion beam angled etching of diamond. *Opt. Express* **30**, 14189–14201 (2022).

56. Smerek, M. & Dulęba, I. Circular object detection using a modified Hough transform. *Int. J. Appl. Math. Comput. Sci.* **18**, 85–91 (2008).

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

© The Author(s), under exclusive licence to Springer Nature Limited 2022
Methods

Sensor fabrication. HD near-surface NV ensembles were created via 2keV implants of 13C N ions at a dose of 10^15 cm^{-2} and an incidence angle of 7° (InnovIon) into commercial chemical-vapour-deposited electronic-grade <100> diamond wafers (Element Six), which were then annealed in a vacuum (∼10^{-4} torr) at 950 °C for 4h. The samples were initially oxygen terminated by boiling in a hot mixture of sulphuric acid and sodium nitrate for 30 min. Ti/Pt 15/50 nm electrical contacts were patterned on the diamond surface via photolithography using the T1 35E photoreist in image-reversal mode. Nanoporous arrays were fabricated via oxygen-plasma reactive ion etching patterns were exposed in a layer of PMMA A8 (Kayaku Advanced Materials) using electron-beam lithography, and etch masks composed of a 10/125 nm Cr/Au bilayer were deposited by electron-beam evaporator and lift-off. Reactive ion etching was performed using an Oxford Instruments PLASMALAB 100 ICP380 system. A two-step oxygen-plasma etching process was used, the first step being performed at 10 mtorr, 50 s.c.c.m. gas flow rate and 600 W of ICP power with a radio-frequency power for 10 min. In the second step, which was run for 30 min, the flow rate was increased to 60 s.c.c.m. and the radio-frequency power was reduced to 10 W. The samples were hydrogen terminated by indirect exposure to hydrogen plasma in a microwave plasma-enhanced chemical-vapour-deposition diamond growth reactor (Seki), where indirect exposure was achieved by shielding the samples from the plasma ball with a perforated molybdenum shell (Supplementary Fig. 1).

Sensitization and characterization. Custom-built fluidic devices were fabricated by patterning glass coverslips with a thickness of 50 µm with a Pt electrode and a Pt stripe for contacting the diamond samples. The coverslips were attached to custom printed circuit boards with circular cutouts using commercial two-part epoxy resin. Silicone fluid wells were made by pouring two-part silicone rubber into custom moulds. The bases of the moulds were flat enough that the resulting silicone wells would spontaneously form a watertight seal on contact with the glass coverslips. The samples were mounted to the coverslips with a thin layer of optically transparent, non-fluorescent silicone rubber (SYLGARD 184, Dow Corning) and electrical contact between the samples and Pt stripe was made using conductive silver epoxy (CircuitWorks). The cured silver epoxy was then encapsulated with silicone to prevent Faradic short-circuiting of the applied voltage signals through the solution.

All the voltage signals used in this work were applied using a Rigol DG4162 function/arbitrary waveform generator. The diamond devices were sensitized in PBS solution (137.0 mM NaCl, 10.0 mM phosphate, 2.7 mM KCl, pH 7.4; osmolality, 280–310 mOsm kg^{-1} (Gibco Thermo Fisher)) by the repeated application of oxidative voltage pulses (Fig. 1c) applied between a Pt counter-electrode and the diamond until the device sensitivity plateaued. The sensitivity was calculated from the measured fluorescence responses to 50 mV peak-to-peak square-wave voltage pulses applied through the counter-electrode using equation (1). For microelectrode experiments, commercial deionized water (Honeywell) was mixed with a small amount of PBS to create a dilute conductive solution in which the measurements were performed. The microelectrode (rounded tip Pt/Et electrode from Microprobes for Life Science) was positioned just above the diamond surface using a manual micromanipulator by first bringing the tip into contact with the surface as determined by a local change in diamond fluorescence at the tip location due to contact potential difference. The tip was then lifted off the surface until the local fluorescence change vanished and was allowed to settle for 20 min before measurement to ensure that no movement of the electrode occurred during recording. Through bright-field imaging of the microelectrode (Fig. 2a), we confirmed that the tip was situated within half the focal depth of our microscope objective from the surface (approximately 5 µm).

Fluorescence measurements. Fluorescence was excited with a 200 mW, 532 nm laser (Coherent Verdi) and collected through a layer of PMMA A8 (Kayaku Advanced Materials) using electron-beam lithography, and etch masks composed of a 10/125 nm Cr/Au bilayer were deposited by electron-beam evaporator and lift-off. Reactive ion etching was performed using an Oxford Instruments PLASMALAB 100 ICP380 system. A two-step oxygen-plasma etching process was used, the first step being performed at 10 mtorr, 50 s.c.c.m. gas flow rate and 600 W of ICP power with a radio-frequency power for 10 min. In the second step, which was run for 30 min, the flow rate was increased to 60 s.c.c.m. and the radio-frequency power was reduced to 10 W. The samples were hydrogen terminated by indirect exposure to hydrogen plasma in a microwave plasma-enhanced chemical-vapour-deposition diamond growth reactor (Seki), where indirect exposure was achieved by shielding the samples from the plasma ball with a perforated molybdenum shell (Supplementary Fig. 1).

Voltage response characterization. Calibration of the DVIM voltage response was performed with voltages applied between the large Pt counter electrode (Fig 1a) and the sensor. For microelectrode measurements, calibration was performed in the same dilute solution as the recordings, although similar responses to calibrations in PBS were observed. The voltage was stepped 15 times in increments of 10 mV, from 0 V to -70 mV, then to 70 mV and back to 0 V, with a step period of 25 s. The sensor fluorescence was recorded over a 768 x 768 pixel region at ten frames per second. Pixels in the calibration video were binned to match the binning of pixels in the processed recording data (32 x 32 pixels for the data displayed in Fig. 2). For each pixel, the average fluorescence for the last 15 s of each voltage step was fit as a linear function of the applied voltage using least-squares regression (polyfit from the NumPy library running in Python 3) to extract gradients m and offsets b of the linear functions as well as the co-variance matrix for the fit Γ. Per-pixel fluorescence contrasts f and uncertainties df were then calculated as

\[ f = -m/b, \]

\[ (df)^2 \approx \left( \frac{\sigma_m}{m} \right)^2 + \left( \frac{\sigma_b}{b} \right)^2 + 2 \left( \frac{\sigma_m}{m} \right) \left( \frac{\sigma_b}{b} \right) \left( \frac{\sigma_m}{m} \right), \]

where the co-variance matrix \( \Gamma = \begin{pmatrix} \sigma_{m,m} & \sigma_{m,b} \\ \sigma_{b,m} & \sigma_{b,b} \end{pmatrix} \).

Calibration was performed in PBS for optrode array measurements. Here 2 Hz square waves with one half-period at 0 V and the other half-period at ±2 mV, ±5 mV and from ±10 to ±50 mV (steps of 10 mV) were applied for 10 s. Fluorescence was recorded over 1,024 x 1,024 pixel regions at almost ten frames per second. The pixels belonging to each optrode were binned using a circular Hough transform algorithm\(^\text{38}\) before further processing. The fluorescence histogram of each optrode was fit with the sum of two Gaussian lineshapes, namely, \( g(x, \sigma_1, \sigma_2, \sigma_m, A_1, A_2) \), where \( \sigma \), and \( A \) denote the mean, standard deviation and integrated area of each Gaussian, respectively, obtained using the nonlinear least-squares curve-fitting function available from the scpy.optimize library. The per-optrode fluorescence contrasts and their uncertainty were then computed via

\[ f = \frac{2 \bar{x}_1 - \bar{x}_2}{\bar{x}_1 + \bar{x}_2}. \]

The fluorescence contrast sensitivities were then calculated by fitting straight-line functions to a zero intercept to \( f \) as a function of the excursion voltage from 0 V (Supplementary Fig. 3).

Radial averages (Fig. 2f) were weighted with the estimated standard deviation (quadrature sum of percentage contrast error \( df \) and estimated per-pixel noise). The radial axis was pixel valued as the integer floor of the true radius at the innermost corner of each pixel (with respect to the pixel chosen as the centre point). The error bars shown are 95% confidence intervals determined from the Student's t-test using a weighted radial standard deviation.

Fitting of APD measurements. Measurements of the DVIM response time in PBS performed with an APD used aggregate fluorescence collected from an ≈200 µm circular illuminated region of the sample. Due to the finite conductivity of the hydrogen-terminated diamond surface, we expect the fluorescence response of the DVIM (which results from the re-equilibration of the two-dimensional hole gas density in response to a change in density of the solvated charges at the diamond surface) to exhibit an RC-like time constant. However, the varying proximity of each point in the illuminated area to the Tu/Pt electrical contact on the sample surface means that we also expect a small amount of variation in the equilibration time constant between each point. To capture this effect on the aggregate fluorescence measured with the APD, we fit the measured response curves with stretched exponential functions that can account for a distribution of response times within the illuminated area\(^\text{39,40}\). These functions \( S(t) \) take the following form:

\[ S_{\text{fit}}(t) = a \left( 1 - \exp \left( -\frac{t}{\tau_{\text{fit}}} \right) \right), \]

where \( \Delta F = \langle F(t) - F_\text{b} \rangle \) is the baseline fluorescence measured with the sensor and any external electrodes grounded.

Raw fluorescence images \( F(t) \) with a solution gate voltage applied were converted to fluorescence contrast \( f \) images via
where $\alpha$ is the fluorescence contrast, $t$ is the time following the start (for $S_{\text{rise}}(t)$) or end (for $S_{\text{fall}}(t)$) of the voltage pulse, $\tau_{\text{rise}}/\tau_{\text{fall}}$ is the fluorescence rise-/fall-time constant and $\gamma$ is the stretch factor.

Data availability
Source data used in the production of this work is available via Zenodo at https://doi.org/10.5281/zenodo.6717484.

Code availability
Custom data analysis and simulation code used in the production of this work is available via Zenodo at https://doi.org/10.5281/zenodo.6717484.

References
57. Hall, L. T. et al. Detection of nanoscale electron spin resonance spectra demonstrated using nitrogen-vacancy centre probes in diamond. Nat. Commun. 7, 10211 (2016).

Acknowledgements
We thank W. Tong for providing poly-d-lysine and assisting with the coating process. We thank G. Berecki and S. Petrou for helpful discussions. D.A.S. and L.T.H. were supported by the Australian Research Council (ARC) through Discovery Project 200103712. L.T.H. was supported by the ARC through DECRA 2001011785. A.S. was supported by the ARC through DECRA 190100536. A.N. was supported by the ARC through Linkage 190100528. L.C.L.H. was supported by the ARC Centre of Excellence for Quantum Computation and Communication Technology. D.J.M. was supported by an Australian Government Graduate Research Training Scholarship. This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF).

Author contributions
D.J.M., N.D. and D.A.S. developed the technological concepts and designed the experiments with input from A.S., S.P. and L.C.L.H. The devices were designed and fabricated by D.J.M. and N.D. with input from A.N. Hydrogen termination was performed by A.S. with input from D.J.M. The electrochemical oxidation procedure was performed by C.P. with input from D.J.M. and N.D. Microelectrode measurements and corresponding data analysis were performed by D.J.M. and N.D. with input from L.T.H. The equivalent circuit model was developed by D.J.M. Optrode array measurements and data analysis were performed by D.J.M. with input from N.D. and L.T.H. The original manuscript draft was written by D.J.M., N.D. and D.A.S. All the authors contributed to reviewing and editing the manuscript. L.C.L.H., S.P. and D.A.S. supervised the work.

Competing interests
D.J.M., N.D., A.S. and D.A.S. are authors on a provisional patent granted to The University of Melbourne covering the fabrication of the NV$^+$ ensemble chip and its use in voltage sensing applications (IP Australia patent no. 2021901331). S.P. is a director and shareholder of Carbon Cybernetics, a company developing a diamond-based neural implant. The remaining authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41566-022-01064-1.
Correspondence and requests for materials should be addressed to D. J. McCloskey or D. A. Simpson.
Peer review information Nature Photonics thanks Fedor Jelezko and Milos Nesladek for their contribution to the peer review of this work.
Reprints and permissions information is available at www.nature.com/reprints.