and undertake a systematic and quantitative study of the accuracy and resolution capability of the technique[5]. In particular, they focus on three main topics: impact and optimization of the signal-to-noise ratio, suppression of false localizations due to unspecific or multiple-site binding, and sophisticated mechanical drift correction. For the latter, they introduce new concepts based on the imaging of complex *a priori* known DNA origami structures, which they term templated and geometry-templated drift correction. These corrections achieve <1 nm residual drift over the imaging timescale.

To demonstrate the imaging resolution of DNA-PAINT, a DNA-origami grid with a dense pattern of pixels, 5 nm apart, was prepared, mimicking the monomer arrangement in a microtubule. DNA-PAINT allows the visualization of each individual target, and can also be used for multicolour grids. This work is an important step forward in the optical microscopy of molecular structures. The demonstrated resolution power of DNA-PAINT rivals that of electron microscopes, but with the added specificity of fluorescent labelling. The next important steps are (1) to extend the nanometre resolution to the third dimension, which would allow for obtaining 3D structural information of single biomolecules or biomolecular complexes that would come close to resolutions achieved with cryoelectron microscopy or X-ray scattering methods; and (2) to develop new toolboxes for applying the concept of PAINT to proteins, not just DNA, for example by introducing specific binding sequences into a protein of interest to which a suitable dye-labelled ligand can reversibly bind. The promise is that far-field optical imaging can provide structural (and potentially dynamic) information on single molecules or molecular complexes with close to atomistic spatial resolution, which could open a new world to optical microscopy. ❐

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References

1. Hell, S. W. & Wichmann, J. *Opt. Lett.* **19,** 780–782 (1994).
2. Betzig, E. *et al. Science* **313,** 1642–1645 (2006).
3. Rust, M. J., Bates, M. & Zhuang, X. *Nature Methods* **3,** 793–795 (2006).
4. Sharonov, A. & Hochstrasser, R. *Proc. Natl Acad. Sci. USA* **50,** 18911–18916 (2006).
5. Dai, M., Jungmann, R. & Yin, P. *Nature Nanotech.* **11,** 798–807 (2016).
6. Iinuma, R. *et al. Science* **344,** 65–69 (2014).
7. Jungmann, R. *et al. Nature Methods* **11,** 313–318 (2014).
8. Jungmann, R. *et al. Nature Methods* **13,** 439–442 (2016).

TISSUE ENGINEERING

Nanoelectronics for the heart

Real-time three-dimensional mapping and control of *in vitro* cardiomyocytes opens new paths for post-surgery heart monitoring and stimulation.

Vladimir Parpura

Myocardial resection is an open-heart surgery removing damaged or diseased areas of the cardiac muscle to improve its rhythm and/or function. Biomedical engineering and synthetic biology have focused on the fabrication of cardiac patches that could be used to fill the resulting resection void. Various 3D scaffolds have been developed for the regeneration of heart muscle[1], and postnatal dermal fibroblasts have been reprogrammed to become cardiomyocyte-like cells endowed with contractility[2]. Together, these results imply that a culture of the patient's own skin cells within a 3D scaffold could be used to populate the resected heart areas. However, this approach requires devices that are able to continuously monitor the functional status throughout the implanted patches and, simultaneously, control their electrical activity. Writing in *Nature Nanotechnology*, Charles Lieber and co-workers from Harvard University now report the development of a flexible scaffold that can house cardiomyocytes as well as map and modulate their electrical activity in three dimensions[3].

In their bottom-up approach, the researchers first produced doped p-type silicon nanowires contact-printed onto an SU-8 polymer film surface. They arranged the nanowires in a field-effect transistor (FET) configuration rather than as simple electrodes, minimizing the spurious increase of the impedance as a result of decreasing the device's physical dimensions[4,5]. Metal source–drain interconnects were inserted to address each FET individually, and the resulting rectangular pad (20 μm × 4 μm × 350 nm) corresponded to a single recording device. The electrical properties of individual sensor pads were characterized using phosphate-buffered saline. The quantified time resolution on the order of 0.01 ms, together with favourable sensitivity and high signal-to-noise ratio, made the pads suitable for the detection of action potentials.

The considered scaffold was made of four superimposed layers, each one containing a 4 × 4 array of pads; and four circular palladium–platinum microelectrodes were incorporated for stimulation. Passive poly(lactic-co-glycolic acid) electrospun fibre films were inserted between the four layers, and the resulting final 3D scaffold (5 mm × 5 mm × 200 μm) had a bending stiffness similar to that of conventional scaffolds used for cardiac tissue growth. The scaffold attached to a modified Petri dish (Fig. 1) housed rat ventricular cardiomyocytes in culture. Their electrical activity was evident throughout the entire scaffold after 8 days *in vitro*, showing sarcomere length and conduction velocity similar to that found in *in vivo* rat heart tissue. Over the course of culture, there was an order-of-magnitude reduction in the beating frequency. This frequency could be acutely up- or down-modulated by the global application of norepinephrine or heptanol, respectively, the latter being a blocker of gap junctions, which connect cardiomyocytes. The focal application of norepinephrine caused arrhythmia, indicating that the original pacemaker activity could be modulated, as further explored by using the stimulation electrodes. The authors were able to lock the pacemaker activity to a given stimulation electrode and shift the directionality of the action potential propagation by changing the stimulus input between different electrodes, showing spatiotemporal control over the tissue excitability.

These proof-of-principle experiments indicate that the constructed 3D scaffold would make it feasible to monitor and stimulate the cardiac activity post-surgery.

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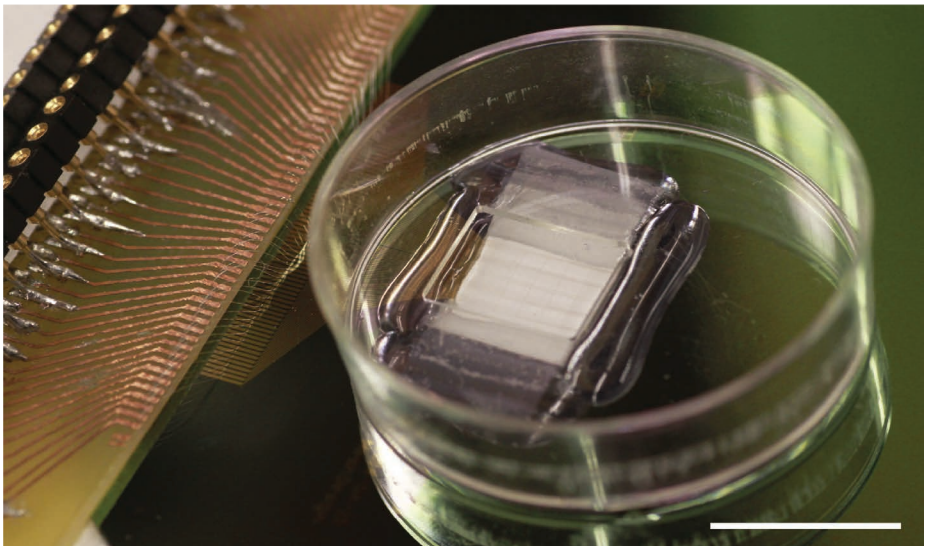

Figure 1 | Photograph of the 3D scaffold attached to a modified Petri dish. The input–output connections of the chip are visible on the left. Scale bar, 1 cm. Reproduced from the Supplementary Information for ref. 3, Nature Publishing Group.

Using the above-mentioned fabrication strategy, the scaffold layout could be readily altered to generate different sizes and shapes, as well as the number and distribution of FET arrays. Remarkably, less than 10% of the FET sensors failed within 2 weeks of usage. However, these silicon nanowire devices would eventually fail. When longer-term recordings are desirable, metal passivation of silicon nanowires could be utilized to achieve stable recordings over the course of several months[6]. Furthermore, additional sensing (for example by using a strain gauge transducer) and/or stimulation (for example with light-emitting diodes combined with channelrhodopsin expression in cardiomyocytes[7]) modalities could be added to the present scaffold design. Further biocompatibility testing by chronic implantation in animal models *in vivo* seems warranted given the potential translational application of this scaffold. Although it has been shown that silicon nanowires do not cause significant production of free radicals within mouse peritoneal monocytes[8], there are some concerns regarding their pulmonary toxicity in rats *in vivo*[9]. Consequently, it will be important to establish safe exposure limits, both general and muscle specific, in humans. ❐

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References
1. Blan, N. R. & Birla, R. K. *J. Biomed. Mater. Res. A* **86,** 195–208 (2008).
2. Ieda, M. *et al. Cell* **142,** 375–386 (2010).
3. Dai, X., Zhou, W., Gao, T., Liu, J. & Lieber, C. M. *Nature Nanotech.* **11,** 776–782 (2016).
4. Cohen-Karni, T., Timko, B. P., Weiss, L. E. & Lieber, C. M. *Proc. Natl Acad. Sci. USA* **106,** 7309–7313 (2009).
5. Duan, X. *et al. Nature Nanotech.* **7,** 174–179 (2012).
6. Zhou, W. *et al. Nano Lett.* **14,** 1614–1619 (2014).
7. Bruegmann, T. *et al. Nature Methods* **7,** 897–900 (2010).
8. Leonard, S. S. *et al. Environ. Health Insights* **8,** 21–29 (2014).
9. Roberts, J. R. *et al. J. Nanomater.* **2012,** 398302 (2012).

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NANOBIOTECHNOLOGY

Net charge of trace proteins

Isoelectric points of small samples of proteins can now be measured using atomic force microscopy and substrates with a known charge.

Gilbert C. Walker

For successful integration of proteins into biosensors or composite materials, it is essential to understand the properties of proteins, for example their solubility in different environments. One of the most discouraging moments during the manipulation of protein solutions and nanoparticles coated with proteins is when they irreversibly precipitate out of solution, wasting painstaking efforts at sample preparation. Most often, such precipitation occurs at the protein's isoelectric point (pI) — the pH value at which the net charge on a protein is about zero. In general, knowing the pI of a protein is important because it tells us the range of solution acidities where the protein will remain soluble, and it allows us to predict how the protein will interact with other charged materials. Current methods to determine the pI, such as isoelectric focusing, require relatively large amounts of protein, which is not suitable when only trace amounts are available. Now, writing in *Nature Nanotechnology*, Julius Vancso and colleagues at the University of Twente, A*STAR institutes in Singapore, Warsaw University of Technology, and the National University of Singapore report a method to measure the pI of minute quantities of protein using atomic force microscopy[1].

Proteins contain chemical groups that ionize (usually by either losing or gaining protons to become negatively or positively charged, respectively) at different solution acidities. In highly acidic solutions (where the pH is low), proteins tend to have positive charges, whereas in lowly acidic solutions (where pH is high), proteins tend to be negatively charged. In each case, similarly charged proteins repel each other. However, when a protein is charge neutral (that is, at its pI), it experiences the smallest repulsion from another protein, thereby causing precipitation. An everyday example of such precipitation is curdled milk. When milk pH

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