GATOR1 to repress mTORC1 signalling[11].

Together, these data trace out a model in which the regulatory factors modulating mTORC1 activity can sense the intracellular level of specific amino acids that limit the rate of protein synthesis. Using this strategy, there would be no need for the intracellular concentration of every amino acid to be monitored in a cell; instead, only those used the most and the least frequently in protein synthesis would need to be tracked. That would enable the evolution and operation of a relatively simple, robust and economical regulatory network for maintaining a balance between protein synthesis and degradation.

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Physical chemistry

Single reaction events imaged in total darkness

Frédéric Kanoufi & Neso Sojic

Single photons emitted from individual electrochemically excited molecules in solution can now be detected. The technique can be used to image cells at nanometre resolution, without using an external light source. **See p.244**

If you want to admire the Milky Way in all its splendour, you should choose a moonless (and possibly romantic) night, away from urban light pollution. Exactly the same principle applies in microscopy, at the other end of the observational size scale: it is much easier to observe faint objects in complete darkness. On page 244, Dong *et al.*[1] report their use of this simple but powerful idea in a direct optical method for imaging single photons generated by individual chemical-reaction events in solution. The authors' method is conceptually different from the fluorescence-based approaches used in conventional single-molecule microscopy, because the excitation process that leads to photon emission is controlled by electrochemistry and chemical reactivity, and does not require irradiation with light. The authors demonstrate that their imaging method can be used for super-resolution microscopy of living cells.

Chemical reactions typically involve the collisions of individual molecules in solution. However, because measurements of reactions usually record the average parameters of ensembles of these molecular events, the characteristics of individual events are usually obscured, and the precise location and time of each reacting molecule in the solution is unknown. This issue can be partly overcome by restricting the volume of the solution, or by separating events in space and/or time, allowing each one to be detected alone. For example, high-throughput single-molecule measurements have been developed in ultrasmall containers, to detect individual biomolecules[2]. Extraordinary achievements in single-molecule studies have also have been reported using scanning tunnelling microscopy[3], nano-electrochemistry[4] and an optical technique called super-resolution localization microscopy (which achieves a resolution higher than the diffraction limit — a fundamental restriction that usually limits the resolution of optical imaging techniques)[5].

After decades of development, methods in which samples are irradiated with light have arguably become the most useful means of 'seeing' individual molecules — the 2014 Nobel Prize in Chemistry was awarded for discoveries in this field (see go.nature.com/3zs4aph). Biologists, physicists and chemists quickly adopted these revolutionary microscopy techniques, all of which use clever arrangements of light and dark — similar to the chiaroscuro technique in art. However, because these methods require the use of short, high-power laser pulses, their performance is often limited by background scattered light and photobleaching (the extinguishing of fluorescence as a result of the continuous excitation of fluorescent molecules by light). In biological applications, samples can also be damaged by the intense laser pulses, or produce their own fluorescence, obscuring the image of the fluorescent molecule of interest.

Some organisms can produce light *in situ* using biochemical reactions, rather than relying on external light sources — such bioluminescence illuminates the darkest regions of the oceans, for example. Dong and colleagues' approach to single-molecule imaging takes a

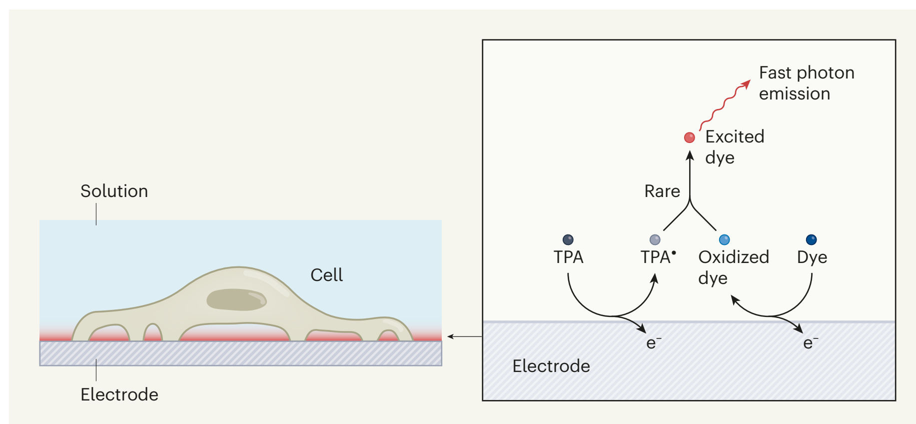

Figure 1 | A microscopy technique involving single-molecule imaging of electrochemical reactions. Dong *et al.*[1] have produced images of cells attached to the surface of electrodes using a phenomenon called electrochemiluminescence. The electrode is immersed in a solution of dye molecules (a ruthenium complex) and a co-reactant (tripropylamine, TPA). TPA is oxidized (loses an electron, e^-) at an electrode, and produces a radical ($TPA^•$). The radicals have an extremely short lifetime, and are therefore found only at very high dilution in a thin region above the electrode surface. The radicals thus encounter dye molecules only rarely. If dye molecules are oxidized at the electrode and encounter a radical, the dye enters its excited state. The excited dye molecules almost instantaneously emit a photon, revealing the location and time of the reaction event. By imaging photons over time, an image of the electrode surface is produced. Photons are not produced at regions where the cell is attached, thus creating a negative image of the cell.

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similar tack: the authors detect photons emitted by a dye molecule that has been excited using an electrochemically triggered phenomenon called electrochemiluminescence (ECL)[6,7]. Because this phenomenon does not require any optical excitation, the experiments can be performed in complete darkness.

Analytical methods based on ECL are powerful and have been widely adopted, because the use of an electrochemical trigger, rather than light, allows an ultrasensitive readout of the optical signal. This has been especially useful in clinical diagnostics[8]: about two billion ECL-based immunoassays are run worldwide each year[9]. In the past decade, ECL has also been adapted for use in imaging[10].

In principle, the near-zero background optical noise of ECL-based methods should enable them to reach the ultimate analytical limit: the detection of a single molecule. But although single-molecule detection is now common in fluorescence-based imaging techniques, methods for detecting single ECL events have been almost completely lacking. Just one approach was known, in which single photons are detected from individual ECL reactions under stringently controlled experimental conditions, but without any spatial resolution[11]. Many authors have reported ECL imaging of single nano- or micrometre-scale objects (see refs 12 and 13, for example), but not of single molecules.

Dong *et al.* have addressed this challenge by developing a method that images the positions of single photons emitted by individual excited dye molecules in ECL events, which are triggered near the surface of an electrode. The authors use a dilute solution of the dye to ensure that these molecules are spatially separated, a strategy used in many single-molecule measurements. However, the isolation of individual ECL events in space and time also arises from careful control of the reactivity of both the dye and the other reagent that takes part in each event (Fig. 1). Light emission occurs when a dye molecule reacts with a radical that is generated by the electrode. The extremely short lifetime of the radical ensures that it is present only at very high dilution, near the electrode surface. The diluted dye molecules therefore encounter the diluted radicals so rarely that each millisecond-duration snapshot of the experiment captures only one reaction, and thus only one photon.

By analysing multiple experiments, the authors convincingly show that the statistical distribution of the number of photons detected per exposure follows Poisson statistics at different exposure times and at different dye concentrations — thus demonstrating that the collisions of dye molecules with the electrode occur randomly. Furthermore, by analysing the relationship between the dye concentration and the time interval between detected events, the authors show that the rate of the process is controlled by the diffusion of the dye to the electrode surface, rather than by its adsorption at the electrode; by contrast, adsorption controls the rate of many single-molecule fluorescence studies.

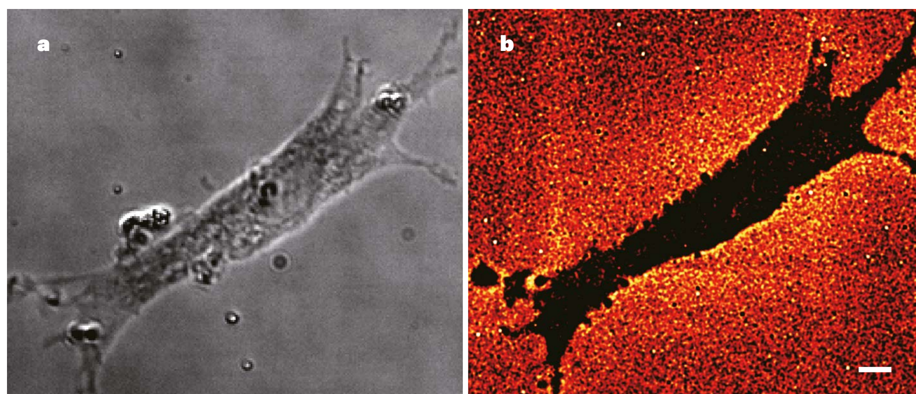

Figure 2 | Cell imaging using ECL-based microscopy. a, An image of a cell obtained using an optical microscopy technique. **b**, A negative image of the same cell produced using Dong and colleagues' microscopy technique[1]. The image combines 15,000 frames, each with an exposure time of 10 milliseconds. Scale bar, 5 micrometres. (Images from Supplementary Information Fig. 27 of ref. 1.)

The authors' findings open the way to a new concept in imaging: a chemistry-based approach to super-resolution microscopy. By imaging the photon emissions at the surface of a nanoelectrode over time, Dong *et al.* were able to visualize the surface with a spatial resolution down to 22 nanometres. They extended this approach to image cells attached to an electrode: the adhesive regions of the cells hinder the diffusion of ECL reactants to the electrode surface, blocking photon emission and thereby producing a negative image of those regions[14] (Fig. 1). The resulting images were remarkably sharp (Fig. 2), and correlated with images produced using super-resolution fluorescence microscopy, validating the effectiveness of the approach. Notably, the ECL technique does not require labels to be attached to the cells.

"The findings open the way to a new concept in imaging: a chemistry-based approach to super-resolution microscopy."

Although single photons are imaged, these are emitted by only a fraction of the excited dye molecules produced in solution; the excited molecules, in turn, are produced using just a small fraction of the electron flow through the electrode. In other words, it is not possible to correlate every electron transferred to the electrode from the reagents during an ECL reaction unequivocally to a detected photon. The detection and localization of single-electron events therefore remains a challenging objective in electrochemistry.

The long-term goal is to develop Dong and colleagues' method further, foster its adoption by diverse scientific communities, and design an ECL-based single-photon microscope suitable for commercialization. Moreover, the reported approach opens up exciting opportunities for the investigation of individual electrochemical reactions, or of any reaction involving electron transfer. It could also lead to the development of new strategies for bioassays and cell imaging, complementing well-established fluorescence-based single-molecule microscopy techniques.

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