MICROSCOPY

Motionless fast 3D scanning

A 3D laser scanning microscopy method requiring no moving parts promises to expand the *in vivo* study of fast neuronal signaling at cellular and subcellular levels.

The high speed of neuronal signaling and the complex three-dimensional (3D) structure of individual neurons and their networks complicate the study of *in vivo* neuronal functionality. Conventional laser scanning microscopy is too slow to image signaling in a 3D volume. Although clever schemes have been designed to greatly increase this speed, most of these methods rely on physical movement of some combination of beam-scanning mirrors, microscope objective or biological sample.

There are practical limits on the speed with which physical objects can be moved, which limit the imaging speeds of these physical inertia-based systems to below 100 Hz. Although such speeds allow many valuable biological experiments to be performed, there is a strong desire for speeds in the kilohertz range that would allow effectively simultaneous measurements at multiple points.

Recent years have witnessed the introduction of inertia-free acousto-optic deflectors (AODs). These devices use sound waves of a fixed frequency to control the deflection of the excitation laser in a laser-scanning microscope. Inertia-based systems are limited to steering a beam along a continuous path through the sample. But AOD-based scanning is not restricted to continuous path scans. Because sound frequency is used to control beam deflection, the beam can be moved almost instantly by a desired distance. The user can choose to either emulate a continuous path scan or operate in a 'random access' mode by jumping to specific points and staying at each one as long as desired to take a measurement before moving to the next point.

Peter Saggau's lab at Baylor College of Medicine is one of the pioneers in the use of AODs for microscopy. Most previous work has been limited to using a pair of AODs to position a beam in a lateral plane, but in 2005 Saggau demonstrated that AODs were not limited to lateral scanning (Reddy & Saggau, 2005). By applying counter-propagating sound waves that constantly varied in frequency to a pair of AODs, they effectively created a cylindrical lens of variable focal length and thus could control the axial focal position of the scanning beam. It turns out that it is possible to use two such pairs of AODs laterally and axially without interference between the two.

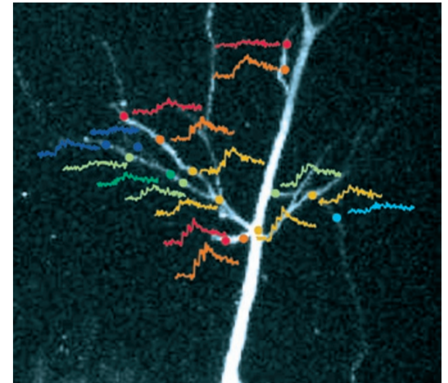

Figure 1 | Fast 3D imaging of dendritic calcium signals by random-access multiphoton microscopy. Image of a pyramidal neuron visualized in three dimensions with superimposed calcium transient graphs color-coded to indicate the axial depth of the recording site over a 30-µm range. Reprinted from *Nature Neuroscience*.

To demonstrate the power of this scanning method they built a random-access

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SYSTEMS BIOLOGY

REWIRING *E. COLI*

By adding new connections between unrelated genes to a gene network, researchers can investigate network robustness and evolvability.

When researchers want to understand how a biological network functions, typically they overexpress or knock out the gene of interest and watch the effects unfold. Such manipulations can be enlightening for understanding network robustness, which is essential for evolution.

Mark Isalan and his colleagues at the Center for Genomic Regulation in Barcelona, Spain have come up with a new approach to test the robustness and evolvability of gene networks by systematically examining the effects of adding new links between unrelated genes in *Escherichia coli*. As gene duplication is a major driving force for evolution, the researchers tested to see what would happen if open reading frames (ORF) were duplicated and linked to new promoters.

Isalan and colleagues generated 598 such 'rewired' gene networks from the genes for seven master transcription factors, seven sigma factors and eight downstream transcription factors. They created new promoter–ORF fusions, introduced them to the bacteria one at a time, either on plasmids or as *E. coli* chromosome integrations, and monitored the rewired networks' effects on cell growth. The bacteria tolerated about 95% of the rewired networks, which was initially surprising to the researchers. "I thought it would be like a cloning exercise when you make a gene in bacteria, and when the bacteria are unhappy, they usually get rid of it—they chew it up or they quickly select it out. That's not what happened here," recounts Isalan.

What was particularly interesting was that rewiring genes with many network connections (hub genes) had few detrimental effects on cell growth. Isalan speculates that *E. coli* "must have something like firewalls or some kind of buffering response to prevent things from going horribly wrong;... it would seem to imply that evolution can shuffle things around and get away with it without any immediate barriers to really making quite major perturbations to the network."

To test the evolvability of the rewired networks, the researchers applied environmental pressures to identify clones with fitness advantages under different conditions, which included serial passaging in liquid culture, extended longevity at 37 °C or survival after heat shock at 50 °C. With each experiment, they found that certain specific promoter-ORF combinations conferred distinct fitness advantages relative to the wild type. In particular, rewired *rpoS-ompR* promoter-ORF combinations increased survivability

multiphoton microscope with a four-AOD 3D scanning system and used it for high-speed 3D imaging of dye-filled pyramidal neurons in hippocampal brain slices (Reddy *et al*., 2008). They compared the imaging performance of AOD-based lateral scanning coupled with axial movement of the objective to fully AOD-based 3D scanning and showed there was a minimal performance difference between them.

Of course, you do not need high speeds to image static neurons in three dimensions. The value of the method is in its ability to sample multiple user-defined points on a neuron fast enough such that the sampling is effectively simultaneous. To demonstrate this capability, the researchers filled neurons with a calcium dye by patch pipette and acquired a full 3D image. This allowed them to select points of interest along neuronal dendrites. They then induced a short train of action potentials in the cell soma and measured the calcium response at the selected sites at speeds of up to 10 kHz. This showed clear reductions in the calcium response at points farther from the cell soma (**Fig. 1**).

Presently the effective axial range of the method is limited to 50 μm when using a high-magnification objective, so the system is not exploiting the full capabilities of multiphoton lasers. According to Saggau, some design changes already underway should substantially increase this range. While this system is already useful, when this limitation is overcome, the resulting system will offer a powerful tool for examining neuronal signaling in the intact brain.

Daniel Evanko

RESEARCH PAPERS

Reddy, G.D. & Saggau, P. Fast three-dimensional laser scanning scheme using acousto-optic deflectors. *J. Biomed. Opt*. **10**, 064038 (2005).

Reddy, G.D. *et al*. Three-dimensional random access multiphoton microscopy for functional imaging of neuronal activity. *Nat. Neurosci*., **11**, 713–720 (2008).

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in both longevity and heat shock experiments. Using a DNA microarray, they obtained a quantitative view of the *rpoS-ompR* network response to heat-shock selection. They found that although permeases were generally downregulated, chaperones and heat shock genes were upregulated. Overall, however, there were no major changes in expression across the network, again indicating its robustness.

The next obvious step is to try out the approach in higher organisms. Isalan's group is working on this, though it will not be exactly straightforward, as he explains: "The problem with eukaryotic systems is that they can have enhancers many, many kilobases away, and you can't simply take a small region and rewire it in exactly the same way we've done here."

And what of the survivability of higher organisms in response to gene network rewiring? Will they be as tolerant as the ever-resilient *E. coli*? "I suspect that in terms of a cancer cell, it might well survive," says Isalan. "If you want an embryonic cell to develop into an embryo—a mouse, say—I doubt it would be happy. But then again, my intuition was wrong about *E. coli*: I didn't expect 95% of the clones to work, so we don't know. I think this is not a case where intuition helps; really, we have to do the experiment."

Allison Doerr

RESEARCH PAPERS

Isalan, M. *et al.* Evolvability and hierarchy in rewired bacterial gene networks. *Nature* **452**, 840–845 (2008).

NEWS IN BRIEF

GENOMICS

Single-molecule DNA sequencing

Harris *et al.* have now published the long-anticipated first report of single-molecule DNA sequencing of a whole genome using the Helicos Biosciences technology. This 'next-next' generation sequencing method, which relies on imaging the incorporation of fluorescently labeled nucleotides to a template strand (called 'sequencing by synthesis'), is very fast, relatively inexpensive and does not require any amplification. The authors resequenced the M13 phage genome with 100% coverage and demonstrated high sensitivity for detecting mutations.

Harris, T.D. *et al. Science* **320**, 106–109 (2008).

CELL BIOLOGY

Red-shifted channelrhodopsin

The microbial light-activated proteins channelrhodopsin-2 (ChR2) and halorhodopsin are powerful tools for stimulating mammalian neurons. There has been great interest in generating a red-shifted variant of the blue light–driven ChR2 to develop new applications of the technology. Zhang *et al.* now describe such a tool, the VChR1 channelrhodopsin from *Volvox carteri*, which they discovered by genome database searching. ChR2 and the yellow light–stimulated VChR1 can be activated separately, which will facilitate studies of circuit behavior.

Zhang, F. *et al. Nat. Neurosci.*, **11**, 631–633 (2008).

PROTEOMICS

Proteome reanalysis with RePlay

Waanders *et al.* describe a new liquid-chromatographic technology that allows reanalysis of a proteomic sample via liquid chromatography–mass spectrometry, by splitting the flow and diverting a portion to a storage capillary for a later 'RePlay' run. This facilitates duplicate analysis (which can increase the number of protein identifications), reduces instrumental 'dead' time (compared to simply analyzing the sample twice) and allows targeted proteomics studies.

Waanders, L.F. *et al. Mol. Cell. Proteomics*, published online 29 April 2008.

GENOMICS

SNP mapping the lab rat

The lab rat is widely used as a model for various human diseases. The genome sequence for a single inbred rat strain is available, but it provides little insight into the huge genetic variation in different laboratory strains. The STAR Consortium now presents a detailed single-nucleotide polymorphism (SNP) map for the rat genome, containing ~3 million SNPs. They also obtained genotypes for a subset of 20,283 selected SNPs across 167 distinct strains. This resource will surely be welcomed for rat genetics studies.

The STAR Consortium, *Nat. Genet.* **40**, 560–566 (2008).

IMMUNOCHEMISTRY

A monoclonal antibody to Ago2

Members of the Argonaute (Ago) protein family serve as binding partners of small RNAs and are important in regulating gene expression. Biochemical analyses of Ago complexes thus far, however, have required overexpression of tagged Ago proteins. Rüdel *et al.* now report the development of a highly specific monoclonal antibody to Ago2 and demonstrate its application in western blotting, immunoprecipitation and immunofluorescence.

Rüdel, S. *et al. RNA*, published online 22 April 2008.