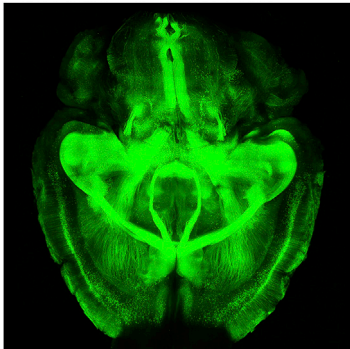


Through a Glass, Darkly

Advances in biology often derive from the development of new techniques that let us examine organisms in ways that were not previously possible. This Select presents several recent papers describing new methods for looking inside cells and tissues and visualizing biological processes.



Clarified Thy1-EYFP mouse brain imaged using one-photon microscopy. Image courtesy of Kwanghun Chung and Karl Deisseroth.

Cutting the Fat to See Clearly

The examination of tissues by light microscopy is impeded by lipid bilayers, which scatter light, restricting the depth to which imaging is possible, and limit accessibility to probes such as antibodies. A technique called CLARITY for fixing and permeabilizing intact tissues while removing lipids and leaving in place features such as membrane proteins and fine cellular structures, such as the synapses and spines of neurons, is now described by Chung et al. (2013). The tissue is infiltrated with hydrogel monomers, such as acrylamide, and formaldehyde. Upon activation of polymerization, a mesh is formed that supports the tissue and holds crosslinked molecules such as proteins in place with greater retention compared to other methods. Lipids and other molecules that cannot be crosslinked to the mesh can then be rapidly removed by electrophoresis. The technique allows a nearly constant refractive index to be maintained, permitting imaging at high resolution to a depth of at least several millimeters even by single-photon light microscopy. Fluorescence is maintained, and even multiple rounds of immunohistochemistry are possible in intact tissue. The accessibility to immunostaining will be particularly important for human tissues, which cannot be labeled in vivo. The authors examine tissue from the brain of an autistic patient, revealing ladder-like dendritic bridges between interneurons,

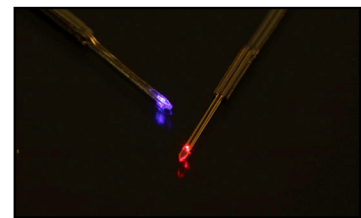
connections not typical of a normal brain but associated with mutations in cell adhesion molecules. CLARITY thus allows the visualization of fine structures in intact tissues, which previously required laborious sectioning and reconstruction after image acquisition.

Chung, K., et al. (2013). *Nature* 497, 332–337.

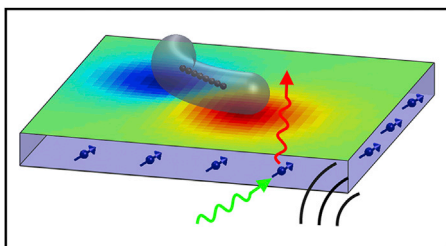
Journey to the Center of the Brain

Optogenetics is a powerful tool for understanding how neural circuits function to control behavior, enabling researchers to alter the function of light-sensitive receptors or ion channels through pulses of light. But tissues such as the mammalian brain pose particular challenges for this technique because they are difficult to access without causing damage, and the relatively bulky devices normally used to deliver light may affect the behavior of the animals studied. Kim et al. (2013) devise tiny optoelectronic devices that can be implanted in the mammalian brain and controlled wirelessly, allowing the stimulation of light-sensitive proteins with subcellular precision. The miniature light-emitting diodes (LEDs) are more than 1,000-fold smaller than conventional LEDs and can be designed to emit light in several wavelengths compatible with existing light-sensitive proteins. Activation at frequencies typically used in optogenetic experiments generates heat at a rate that is efficiently dissipated by the surrounding tissue. The devices may include a temperature sensor also capable of generating heat and an electrode able to distinguish individual action potentials, enabling use in electrophysiology. These devices can be attached to a needle using a silk-based adhesive, allowing implantation in a mammalian brain by injection followed by dissolution of the adhesive using artificial cerebrospinal fluid, leaving the device in place when the needle is withdrawn. Their tiny size and flexible construction allows the devices to be well tolerated over months, and they are powered by light-weight power scavenger devices, which can be attached to mice for use in behavioral assays. The authors use these devices to have the mice wirelessly self-stimulate their dopamine neurons in various behavioral assays, similar to what has been done with conventional optogenetic methods, and they suggest broad biomedical applications for similar devices.

Kim, T.-i., et al. (2013). *Science* 340, 211–216.



Powered blue and red μ -LED devices attached to a releasable injection substrate. Image courtesy of Michael Bruchas (Washington University) and John Rogers (University of Illinois).



Microscopic magnetic field patterns from living magnetotactic bacteria are imaged using red fluorescence from nitrogen-vacancy color centers in a diamond chip while probing the color centers with green light and resonant microwave fields. Image courtesy of David Le Sage.

Reading a Tiny Compass

Magnetic imaging has applications in biological research and in medicine, but existing methods have limited spatial resolution and thus cannot visualize subcellular structures, or their operating conditions preclude their use with living biological samples. Le Sage et al. (2013) present a method for the imaging of biomagnetic structures in living cells that has both subcellular resolution (400 nanometers) and wide field of view (100 microns), can be carried out within minutes, and can be combined with optical imaging. The authors apply the method to magnetotactic bacteria, which form membrane-bound organelles containing tiny particles of iron-containing compounds ordered in chains with a net dipole moment. The magnetosomes cause the bacteria to orient along geomagnetic field lines, which is believed to allow them to move to regions of optimal oxygen concentration. It was previously shown that fluorescence from nitrogen-vacancy (NV) color centers in diamond (nitrogen atoms adjacent to vacancies in the diamond lattice) allow magnetic sensing and imaging at nanometer scale, but high-resolution imaging of targets outside the diamond

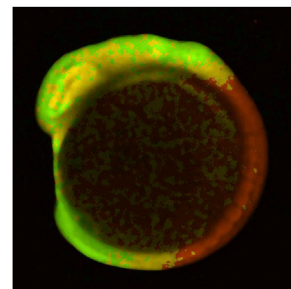
crystal has been a challenge. Living cells are placed in liquid on a diamond crystal containing a thin surface layer enhanced with NV centers. When the high-intensity green light used for NV excitation is applied at the proper angle, this light is reflected within the diamond and thus prevented from harming the cells, while low-intensity red NV fluorescence passes out of the diamond and is collected on a camera for magnetic imaging. Magnetosomes can thus be imaged in single cells, enabling study of the requirements for nanoparticle chain formation and visualization of chain formation in real time. The method could also be used to search for magnetoreceptor cells believed to exist in vertebrates, and it might be adapted to the study of firing neuronal networks in culture and the detection of free radicals generated in cells.

Le Sage, D., et al. (2013). *Nature* 496, 486–489.

FRETting Over a Small-Molecule Gradient

Patterning of the vertebrate body during early development is controlled by gradients of morphogens, which specify tissue identity. Retinoic acid (RA) is one such morphogen that promotes proper patterning of structures such as the hindbrain and the paraxial mesoderm. However, given the small-molecule nature of RA, it has not been possible to directly visualize the gradient of RA, complicating efforts to understand how this gradient is established. Shimozono et al. (2013) show that chimeric proteins combining the ligand-binding domains of RA receptors with fluorescent proteins enabling fluorescence energy resonance transfer (FRET) can be used to directly detect RA. The authors fuse cyan and yellow fluorescent proteins to each of two different versions of the RA receptor, and mutations introduced to one of these receptors yield a third construct; the three versions of the detector have distinct affinities for RA ranging from K'_d of 2 to 50 nM. Each of these specifically binds to RA (and not its metabolites), which causes a conformational change yielding FRET and enabling determination of the concentration of RA across a gradient in the early embryo of zebrafish. The authors show that the concentration of RA corresponds to the expression pattern of the enzyme that promotes the biogenesis of RA (high RA concentration) and that which promotes its degradation (low RA concentration). The results support a model of diffusion of RA from its source to a sink where it is degraded, thus generating a linear gradient. This tool for the direct visualization of a small-molecule gradient will enable further discovery in developmental biology and tissue engineering.

Shimozono, S., et al. (2013). *Nature* 496, 363–366.



Visualization of a two-tailed linear gradient of retinoic acid (RA) in a zebrafish expressing a genetically-encoded probe for RA. Image courtesy of S. Shimozono and A. Miyawaki.

Gabriel Hayes