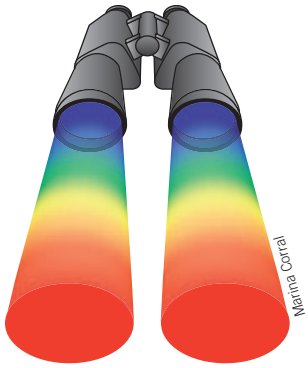
Near-infrared probes

Development of genetically encoded tools with absorption and emission spectra in the near infrared is worth the trouble.

Nearly 20 years since the introduction of GFP as the first genetically encoded fluorescent marker, biologists have had at their disposal an ever-growing palette of fluorescent proteins with colors that extend across the blue, green, orange and, increasingly, red and near-infrared parts of the electromagnetic spectrum. Tools that absorb and emit in the near infrared (NIR) are particularly promising for probing deep into living tissue.

A guiding theme for delivering light deep into living tissue is that, up to a point, redder is better. The near-infrared window that extends from 650 to 900 nm is ideal: in this region, the combined absorption from hemoglobin and water are minimal and tissue is maximally transparent to light.

In recent years we have seen notable advancements in the development of GFP-like fluorescent proteins that absorb and emit in the low 600-nm range, but further red-shifting of the fluorescent proteins' chromophores has proven hard thus far. Similarly, the activation wavelengths of currently available rhodopsin-based optogenetic tools are limited to 600–630 nm, which is below the NIR tissue transparency window.



Near-infrared probes could help biologists see deeper into living tissue.

For the design of NIR optical tools, researchers have started to explore the use of alternative proteins known as phytochromes that plants and bacteria use for sensing light. Bacterial phytochromes are particularly promising because they naturally absorb red and near-infrared light and because they use biliverdin as a chromophore, which is endogenously produced in mammalian cells. Newly engineered phytochrome-based NIR fluorescent proteins—IFP1.4, iRFP and Wi-Phy—have excitation and emission maxima in the low 700s (nm) and hold promise for deep *in vivo* imaging.

Bacterial phytochromes could also serve as templates for building NIR biosensors, extending the possibilities of existing tools, such as for deep calcium detection or multicolor imaging. One day, we may also see these proteins develop into optogenetic modulators of biological functions.

Much work will be needed before the full potential of bacterial phytochromes is really demonstrated. Surely matching the qualifications of monomeric, non-perturbative GFP will be hard. But the potential for highly penetrating non-toxic biological exploration is worth the effort. **Erika Pastrana**

Defective (but useful) diamonds

Nitrogen vacancy center defects in diamonds confer remarkably useful properties.

Diamonds may be a girl's best friend, but they will also become increasingly desirable to biological researchers. Diamonds have long served as useful tools in the physical sciences, where, among other applications, they have been used to subject samples to immense pressures. But their value to biological research lies in the remarkable properties of a particular defect in the highly regular carbon lattice that gives diamonds their conventional desirable properties. This defect consists of a single nitrogen atom adjacent to a missing carbon atom. The resulting nitrogen vacancy (NV) center exhibits fluorescence that is insensitive to photobleaching, retains its properties in nanodiamonds as small as 4 nanometers and, being carbon-based, has excellent biocompatibility.

Nanodiamonds are beginning to be used as probes for *in vivo* imaging and have been demonstrated to allow extraordinarily high-precision localization using super-resolution microscopy. Their use as cellular probes has been limited so far because they are not as easy for biologists to use as genetically encoded probes, but development of functionalization and targeting methods should start to change this. NV centers have been shown to function as highly efficient donors to dye-acceptor molecules during fluorescence resonance energy transfer and could be used to create a new class of sensors.

The most intriguing potential for NV centers, though, results from the properties of the unpaired electronic spin state of the center. This spin state is sensitive to magnetic fields, and the state has a fluorescence signature that can be read out using fluorescence microscopy. Use of a single NV center at the tip of a scanning atomic force microscope allows fine mapping of magnetic fields. Further development could result in a true benchtop miniature nuclear magnetic resonance (NMR) instrument and allow structure determination of a single protein. Alternatively, arrays of these centers could bridge the imaging gap between NMR instruments for molecular structure determination and the kind of magnetic resonance imaging machine used in hospitals for whole-body imaging.

Fluorescence signals from flawed diamonds open the door to their use in multiple biological applications.

It is too early to say where these bits of flawed diamond will have the greatest impact, but their potential makes them as fascinating to many scientists as ornamental diamonds are to their admirers. **Daniel Evanko**