tive CaM-KII was chronically expressed, appears to contradict this view of the role of CaM-KII in LTP. Such mice exhibit no increase in synaptic transmission, and normal LTP can still be generated (although there is a shift in the frequency dependence of LTP). These results led to the conclusion that CaM-KII is not part of the direct signal transduction cascade responsible for generating LTP but only modulates this machinery.

Can these conflicting data be reconciled? One possibility is that CaM-KII does directly mediate the generation of LTP but can also modulate the sensitivity of this transduction pathway. In particular, chronic CaM-KII activity (*12*) could decrease the sensitivity of this pathway (perhaps by phosphatase activation). This view is a special case of the "sliding threshold" model (*13*) in which a requisite component of the signal transduction pathway used to generate LTP (that is, CaM-KII) can itself also act to modulate the pathway. This modulation may occur primarily as a result of long-term genetic modifications and not of more acute perturbations. A homeostatic compensatory regulation of this kind may be an example of a general principle in biology that serves to establish a functionally relevant dynamic range for signal transduction. Simply put, if a pathway is continually activated it will tend to decrease its sensitivity; if it is not activated it will increase its sensitivity.

Clear examples of how difficult it is to interpret the results of genetic modification of transduction cascades comes from work on rod phototransduction (*14*). The advantage of this system is that the molecular basis of the cascade is known, so one can assess whether genetic modifications produce easily interpretable effects. Transgenic mice expressing a constitutively active transducin that mediates phototransduction would be expected to show saturation of the subsequent transduction cascade. However, a compensatory reduction in downstream enzymes prevents saturation. Another example comes from mice in which the inhibitory subunit of cyclic guanosine 3′,5′-monophosphate (cGMP)–phosphodiesterase is deleted. This would be expected to lead to a drop in cGMP concentration. Surprisingly, cGMP concentration is elevated as a result of an unanticipated disappearance of the catalytic subunits of the enzyme. In many other cases, modification of rod proteins leads to cell-specific degeneration. These examples indicate that extreme caution must be used in interpreting results obtained by genetic modification of signal transduction pathways, especially when genetic and anatomical controls are not feasible.

In theory, compensatory changes might be minimized by inducible genetic modification. For instance, heat shock promoters have been used in *Drosophila* to produce changes in gene expression within 30 min. The elegant new methods (*15*) for inducible gene expression in mice are comparatively slow (2 weeks) and may not eliminate the problems of compensatory change.

With the new results from Barria *et al.*, a strong case is emerging for the importance of CaM-KII in the direct control of synaptic strength and, by implication, in the storage of information (*16*). CaM-KII, as part of an important synaptic signal transduction pathway, may also control homeostatic pathways, which only become apparent during chronic manipulations, such as those that occur with current methods for genetic manipulations.

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IMAGING

Tissue Optics

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Our bodies are not impervious to light. When you step outside on a sunny day, the inside of your head is illuminated—in fact, sufficient light penetrates your skull to comfortably read this page (*1*). Shine a flashlight at your finger, and your finger will glow red, a demonstration that red and near-infrared light penetrate deeply into tissue. Just as farmers once candled eggs to check for chick embryos, transillumination has been used to screen for scrotal tumors, breast cancer, and blood oxygenation (*2*). Although the resolution and diagnostic accuracy of these early attempts at imaging with light were poor, this is changing. In this issue on page 2037, Tearney *et al.* (*3*) use light-based optical coherence tomography (OCT) to form tomographic images of human tissue in situ with a resolution exceeding that of magnetic resonance imaging (MRI), computerized tomography (CT), or ultrasound.

In tissue, light is both absorbed and scattered; scattering dominates in the red and near-infrared spectrum (see the figure). This native scattering renders us opaque and blurs transmission images. However, those photons surviving passage through the tissue emerge bearing clues about their voyage. Study of their behavior has spawned a field now known as tissue (or biomedical) optics (see related News story on page 1991). Optical approaches to tissue imaging have general advantages: They are inexpensive, noninvasive, transportable, and nontoxic. In addition, biological materials have native contrast that allows optical assessment of tissue chemistry and cellular structure.

OCT takes advantage of the scattering inherent in tissue to generate in vivo images. As photons meander through tissue, a small amount of light that has bounced off internal structures only once is coherently back-reflected from various boundaries within the tissue. Using interferometric techniques, this coherently backscattered light can be detected at intensities down to 1 part in 10^{11}, while simultaneously revealing the depth of the scattering event. At the heart of OCT is a Michelson interferometer, which detects interference between halves of a single light beam split along a sample arm, containing low-coherence light backscattered from multiple depths within the tissue; and a reference arm, containing light passing through an air or water gap of variable length (*4*). When sample and reference light are recombined, and the time delay through both paths matches within the coherence

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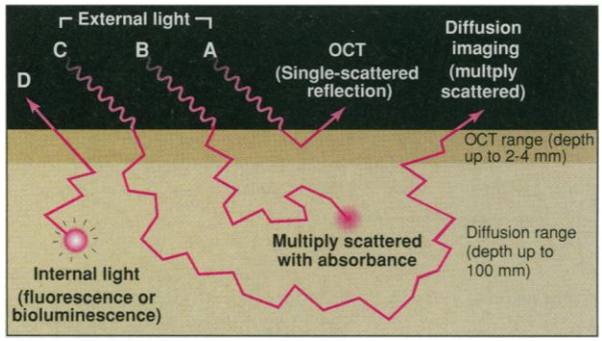
length of the source, they interfere. This interference indicates that a component of light backreflected by the tissue traversed a path with a time delay equal to the delay through the reference path. By varying the length of the reference path, multiple depths into the tissue can be sampled. As long as the speed of light in tissue (c divided by the refractive index) is constant, the length of the reference path is linearly related to tissue depth probed. This allows 10- to 20-μm spatial resolution into the tissue along the beam axis. Finer image resolution can be achieved by using short coherence-length sources. Images may be formed by scanning this depth-probing beam either in an x-y grid pattern or in a rotational circular scan.

OCT has been used before as an external or ex vivo imaging approach—for skin, cardiac vessels, embryos, eye, and other sites (*5*–*9*). Clinically, it has been the most useful in ophthalmology, where optical imaging of cross sections of the retina and other optic substructures has assisted diagnosis and therapy (*8*) of diseases such as macular degeneration and choroidal neovascularization.

In the present study, Tearney *et al.* have converted their device into a catheter-based system. The result is finely resolved images of esophageal tissue layers. This report is significant in several ways. First, the 10-μm spatial resolution of OCT outperforms that of MRI, ultrasound, and confocal microscopy, which image with 200-μm resolution. Even high-frequency (100 MHz) intraocular ultrasound produces only 20- to 40-μm resolution. Second, OCT can be used for biopsies in situations where excisional biopsies are difficult. Biopsy that requires tissue sampling is less than ideal for the eye, coronary vessels, brain, or nervous tissue, all of which can be irreversibly damaged by biopsy or are difficult to access. A catheter-based OCT system such as that reported by Tearney *et al.* lends itself to use during endoscopy, opening much of the body to view via minimally invasive approaches. (Although for this approach to have medical value, cross-validation with conventional pathology approaches will be needed.) Third, even if excisional biopsy is required, OCT may be useful in guiding the surgeon in selecting the optimal site for maximal biopsy yield. Last, extensions of the technique are possible, including multispectral analysis, optical Doppler tomography (ODT), and intravascular imaging. Each of these areas is under study.

OCT has several limitations. Excised tissue can be stained for the presence of antigenic or structural components, cultured for infectious agents, and viewed under a microscope with a resolution approaching 1 μm or better using near-field or electron microscopy. In contrast, OCT cannot identify the molecular components of tissue, normal or pathogenic. The spatial resolution of OCT is considerably less than that of an excision biopsy. Short-pulse OCT may increase axial resolution to 2 to 4 μm, but improvements in lateral resolution are more difficult. Scattering degrades beam coherence and limits the effective spot size inside tissue, while variations in refractive index over the sampled region further reduce image quality (*10*). Thus, the theoretical diffraction limit for resolution is never achieved in practice.



Path of light. Light moves randomly through tissue because of scattering, which dominates over absorbance for red and near-infrared light. At shallow depths, some photons are coherently reflected by a single scattering event (**A**), permitting detection by interferometric techniques. Images using this coherently reflected signal can be constructed, such as in OCT. At deeper depths, light is multiply scattered and can either be absorbed (**B**) or survive tissue transit to escape (**C**) and be detected. Images can be constructed using this diffuse light, as in optical diffusion tomography. Light can also be generated within the tissue (**D**) and then externally detected.

In addition, the depth of imaging for OCT is limited to 2 to 4 mm. At depths beyond several millimeters, essentially all photons have undergone multiple scattering and lost coherence. Here, diffusion theory, which treats photon migration through tissue as a diffusive process similar to the diffusion of heat or gases, works well (see News story on page 1991). Such an approach has allowed imaging of stroke, brain hemorrhages, and breast tumors in human subjects, although at a lower resolution than OCT (*11*), and has allowed chemical characterization of tissue.

Finally, illumination-based approaches work only if there is a distinctive and detectable optical signature. Subtle differences between tissues (such as between cancerous and normal cells) demand a high degree of spectral sensitivity and may not be detectable with OCT. Key molecules in many biological processes—those associated with gene activation or infection, for example—may possess no distinguishing spectral features, thus limiting assays of such events to in vitro tests removed from the relevant influences of the intact organism. Here, use of an internal light beacon, conceptually parallel to PET or nuclear medicine scanning, has allowed for real-time, noninvasive monitoring and imaging of infection and gene expression in vivo (*12*).

In the future, OCT and other optical techniques may usher in the use of light-assisted medical diagnosis, or "optical biopsy," leading to automated surgical tools or portable diagnostic devices that can identify tumors, provide feedback to surgical interventions such as tissue welding or electrocautery, and detect surgical errors in real time. Because each tissue has a unique spectral signature (for example, liver looks distinct from bowel because of differences in both absorbance and in the way the tissue scatters light), automated discrimination among tissue types is feasible. Biocompatible dyes, fluorescent markers, or other contrast agents will improve accuracy and resolution for many optical techniques, similar to the way such approaches improved CT and MRI. Such dye-based optical techniques would be the in vivo equivalent of the histologic stains available to the pathologist.

Of all of the light-based techniques, OCT provides the highest resolution structural images at shallow depths, but each approach is likely to find an application in which it excels. We are now leaving the dark ages of biology and medicine.

An enhanced version of this Perspective with links to additional resources is available for *Science* Online subscribers at http://www.sciencemag.org/

References and Notes

1. The intensity of transmitted light falls by about one decade of magnitude per centimeter traveled. Thus, the intensity of light under the skull is about 1% of the incident light. Although dark hair substantially reduces this number, enough light would likely be transmitted by the forehead to ensure good illumination.
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