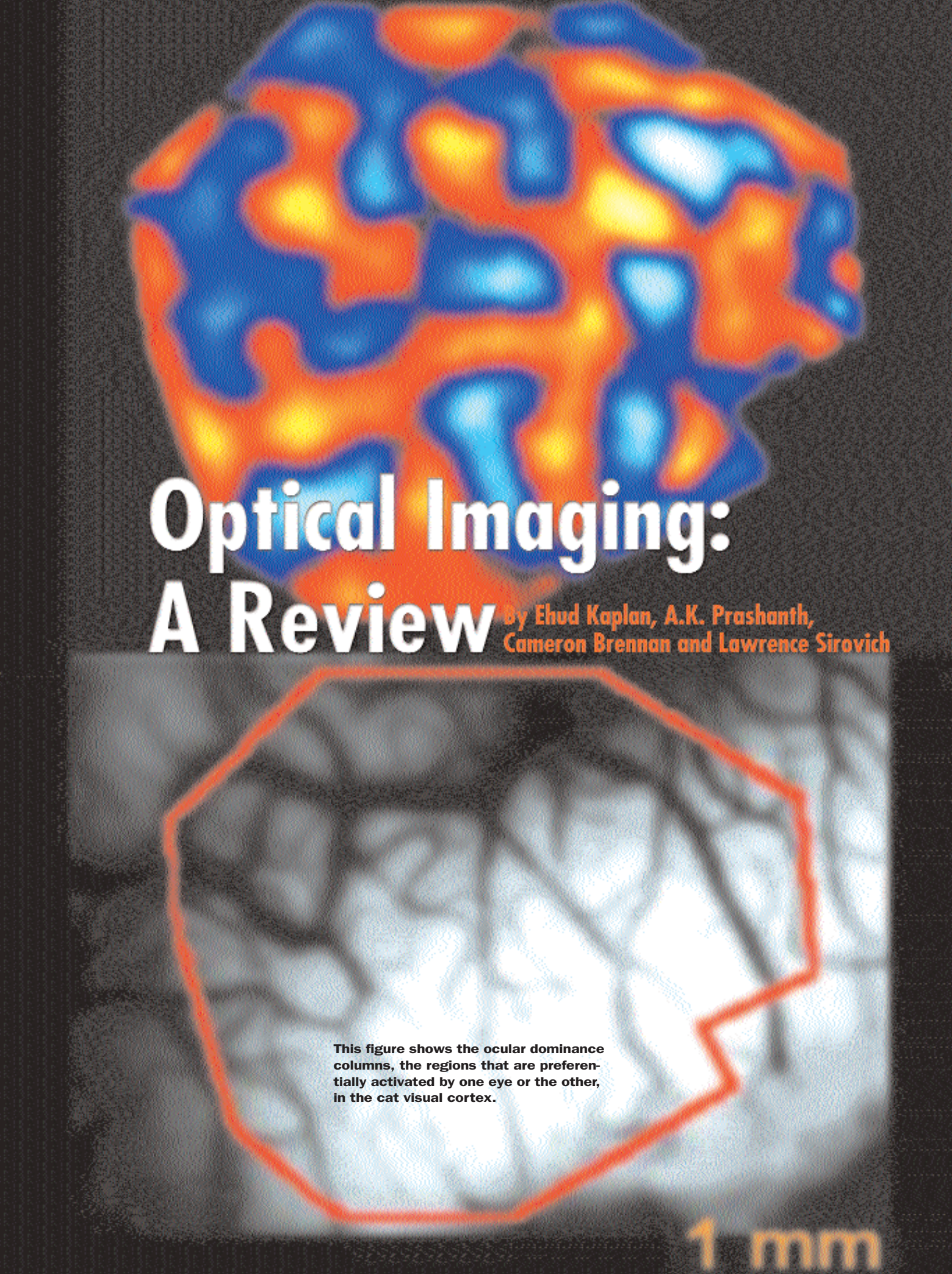

Optical Imaging: A Review

by Ehud Kaplan, A.K. Prashanth, Cameron Brennan and Lawrence Sirovich

This figure shows the ocular dominance columns, the regions that are preferentially activated by one eye or the other, in the cat visual cortex.

You can see a lot by ***looking*** – (*Yogi Bera*). This aphorism captures the power and appeal of imaging: through the visualization of the structure or function of the object of our interest, we can learn a great deal about it. Modern imaging technologies are advancing rapidly, and are becoming important in biology, medicine, and numerous other areas of science. There are many approaches to imaging, each with its own advantages and limitations of targets and of spatial and temporal resolutions. These approaches include magnetic resonance imaging (MRI), positron emission tomography (PET), magnetoencephalography (MEG), optical imaging, ultrasound imaging, and various types of microscopy, including both light and electron microscopy. In this review we shall briefly sketch one particular approach to imaging: optical imaging. We shall illustrate its use in the study of the functional organization of the mammalian brain. A more comprehensive review of optical imaging as applied to the brain can be found in Grinvald *et al.*, 1999.

The various types of optical imaging

Dyes, intrinsic signals, multi-photon

The term *optical imaging* is used to refer to imaging methods that employ (usually visible) light for imaging. It includes two major types of imaging. The first requires the use of some *extrinsic agent*, such as a dye, to create or enhance contrast. The optical properties of the dye, such as fluorescence or absorption, change and signal a change in the state of the material under study, such as a change in the electric field, concentration of some chemical, and so on. The second type of optical imaging requires no extrinsic agents, and takes advantage of optical changes that are *intrinsic* to the imaged material. These methods can be used, for example, to visualize the distribution of calcium in a cell or tissue, show us how a particular gene is expressed in the body or in an organ, the spatio-temporal distribution of electrical activity in neuronal tissue, etc. The ability of this type of imaging to provide information at high spatial and temporal resolution over a large area makes it a source of data that cannot be obtained with more conventional methods, which use localized and usually static sampling.

Optical imaging in biology and medicine

Visualizing both structure and localization of function ("space")

Static imaging (photography) informs us about structure and about the localization of function. For example, we can determine where the heart is, its shape, color, and so forth. We can also learn about functional properties: which heart muscle fibers contain a particular protein, or which cortical neurons receive input from the left eye. This type of data informs us about the functional organization of the target organ.

Dynamics, development, plasticity ("time")

One of the most important advantages of modern imaging over static photography is its ability to provide dynamical information, showing how the system under study changes with time. We can now see which heart muscle fibers contract first, the sequence of excitatory waves that sweep across the entire heart, and so forth. This can be done on several different scales: microseconds and milliseconds for fast events in neurobiology and cardiac function, minutes and hours for differentiation and plasticity, and days, months and years for developmental studies. Dynamical information is crucial to the development of complete mathematical models of the system under study, and the recent explosive increase in computer power makes it possible for scientists to use the vast amounts of information contained in a temporal sequence of images.

Imaging approaches

Contrast agents: dyes, "direct" imaging

Voltage sensitive dyes

These dyes change their fluorescence or absorption in response to changes in the voltage across the membrane of a neuron or muscle fiber. They bind to the membrane, and respond in picoseconds to the electrical events. They bleach easily, and often cause photodynamic damage. It is frequently difficult to get adequate staining of biological membranes *in vivo*. In addition, the signal-to-noise ratio (SNR) of these dyes is usually small. These factors limit their use *in vivo*, but they can often be used successfully *in vitro*.

Indicator dyes

Calcium dyes are an example of dyes that report the concentration of a substance. They produce relatively large signals, and are used widely in biology. Similar dyes are available for a wide range of substances: electrolytes, proteins, and so on. Of special interest are green fluorescing proteins (GFPs), which can be tailored to attach to particular organic molecules, making the distribution of these molecules visible.

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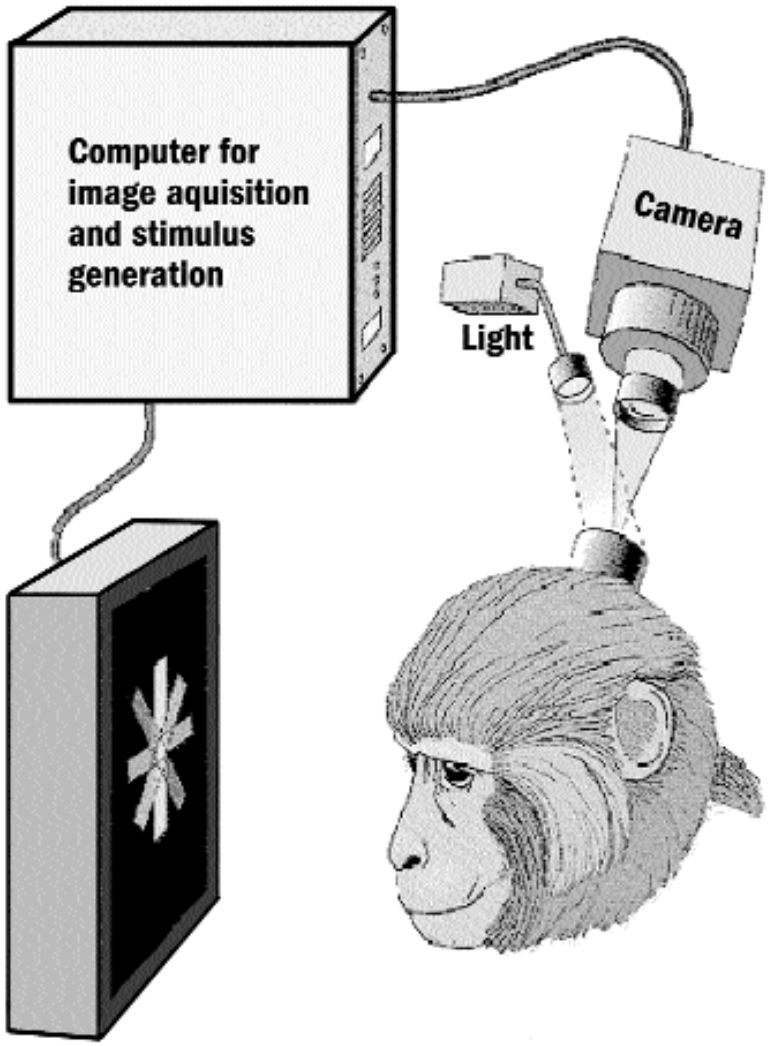

Figure 1. Experimental setup for (intrinsic) optical imaging of the brain. Similar methods can be applied in other areas.

Intrinsic optical signals ("indirect" imaging)

Biological activity often changes the optical properties of the tissue, such as the amount (intensity) or spectrum of the light being reflected. Changes in light scatter due to changes in the index of refraction of the tissue are another source of information. These changes can be used to visualize brain regions that are activated by stimulation, and provide detailed structural and functional information (see below).

The intrinsic optical signals from the brain depend (directly or indirectly), at least partly, on the blood and its level of oxygenation. Therefore, the spatial resolution of optical imaging of the brain is determined by the distance of the imaged neural tissue from the capillary bed. This limit is estimated at ~50 microns.

Multi-photon imaging

A relatively new approach to high-resolution imaging is multi-photon imaging, in which long wavelength laser light is used to create a thin layer in which the photon density is very high, within the imaged material. This high density of photons increases the probability of *simultaneous* absorption of two (or more) photons in a molecule of a fluorescent dye, and one can thus obtain an image that is restricted only to the thin layer. By changing the depth at which the photon density is high, one can obtain images from various depths, and these can be combined to obtain three-dimensional information. Because of the lower energy of long wavelength light, less photodynamic damage is caused to biological specimens, and this makes multiphoton imaging a preferred approach for *in vivo* studies. In addition, *all* the light is used in this imaging mode, in contrast to confocal microscopy, where light that is not confocal is excluded from the image but passes through the tissue, causing damage but adding no information. When this approach is coupled with the use of green fluorescing proteins (GFPs), which can be tailored by molecular biologists to tag substances of interest, one can obtain high resolution, three-dimensional information about certain cell types or parts of cells. Functional imaging (imaging of certain biological functions) can also be achieved this way.

Other optical imaging approaches

In most applications of optical imaging the focus is on the *intensity* of the reflected or fluorescing light. However, other aspects of the captured light, such as the *angle of polarization* or the *phase* of a modulated light, often contain valuable or unique information. In some cases these variables provide much more reliable signals than the amplitude signal (for example, Chance *et al.*, 1998).

Analysis methods

The analysis of imaging data presents special challenges to the scientist. This is because images contain an enormous amount of useful information, which needs to be extracted and arranged in a usable fashion. In addition, images often contain faint signals buried underneath irrelevant noise and artifacts: instrumentation noise, physiological artifacts such as movement, spontaneous (endogenous) activity, and so on. The separation of the relevant signal from the noise is at least as important as the acquisition of the images themselves, and analysis methods are under constant development and refinement.

Differential imaging

In many imaging situations, a widely used approach is to perform differential imaging: the images obtained under one experimental condition are averaged, and the average is then subtracted (sometimes divided) from the average of images obtained under another experimental condition. This approach focuses on the difference between the two experimental conditions, but it also discards important signals that are common to both. A popular approach, which is embedded in a widely used analysis package (Statistical Parametric Mapping, or SPM), couples differential imaging with statistical analysis of the region of interest. This is the approach used in most fMRI studies for data analysis.

Principal components analysis

An alternative to the usual method of differential imaging is the use of decomposition methods, such as principal components analysis (PCA) or independent components analysis (ICA), in which the original imaging data are recast into a new coordinate system, which may be more appropriate for the purposes of the analysis (Everson *et al.*, 1997; Bell & Sejnowski, 1995). These methods offer an advantage in terms of both sensitivity and robustness, advantages that are important when the SNR is low. PCA can be combined with differential imaging and statistical testing, as in the method described recently by Gabbay *et al.* (2000). In addition, these approaches provide information about the dynamics of the investigated process. This type of information is difficult to obtain by differential imaging.

Other methods of analysis

There are numerous other approaches to the analysis of images, and a detailed review of all of them is beyond the scope of this article. Wavelets, one popular approach, does deserve special mention, however, since in some cases it is the most natural path to follow, because of the inherent multi-scale nature of wavelets, and because of the similarity between wavelets and receptive fields (sensitivity profiles) of neurons found in the visual system (see, for example, Carmona *et al.*, 1995). For an in-depth review of a number of other analysis methods, including frequency domain methods, see Mitra & Pesaran, 1999.

Two examples

1. Imaging of cortical activity

Experimental setup

In our attempts to understand the function of the brain, a crucial step is the acquisition of information about the spatio-temporal distribution of neural activity. In other words, we wish to know which neuron or neuronal ensembles are active, and what the temporal pattern of their activity is.

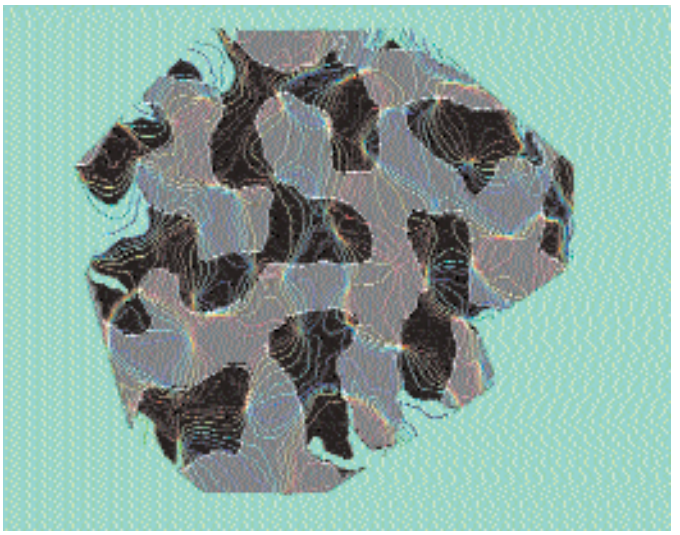

Figure 2. Ocular dominance columns (gray and black) and orientation selective domains in the cat visual cortex. Gray regions respond primarily to stimulation of the right eye. Black regions respond primarily to the left eye. Colored lines connect the locations of neurons that prefer particular orientation: red indicates preference for vertical, yellow indicates preference for horizontal, and so on around the clock. The cat was stimulated repeatedly with drifting gratings, oriented at various orientations, and the regions activated by each orientation were imaged. All the data were then combined to create the iso-selectivity contour map shown here. For more details on the analysis, see Everson *et al.*, 1998. The entire picture spans approximately 6 × 4 mm. (Brennan & Kaplan, Unpublished data).

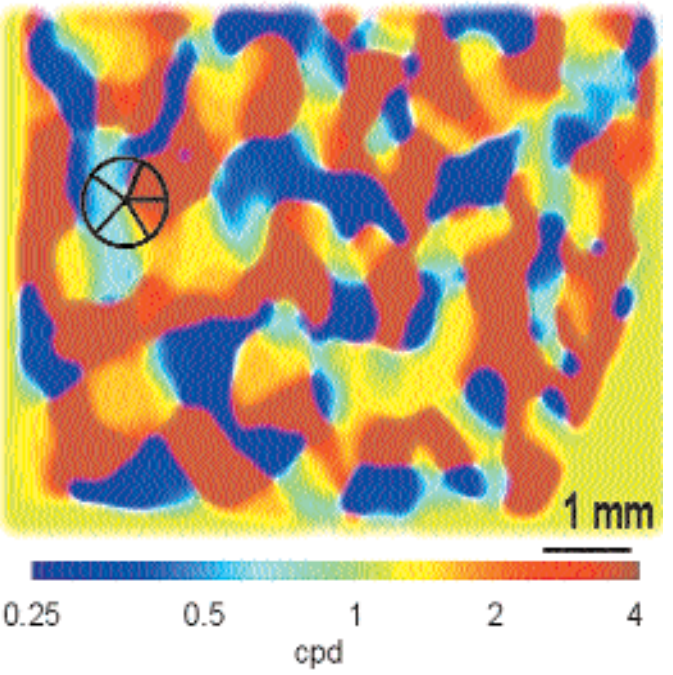

Figure 3. Size selective regions in the cat visual cortex. Each color represents a cortical region selective to a different size (or spatial frequency, in cycles/degree) of objects in the visual world. The pinwheel on the left shows one of many places where regions selective for various sizes meet at a singularity (Prashanth & Kaplan, unpublished data). The cat was stimulated repeatedly with drifting gratings of various spatial frequencies, and the regions activated by each spatial frequency were imaged. All the data were then combined to create the iso-selectivity contour map shown here. For more details on the analysis, see Everson *et al.*, 1998.

Optical imaging of intrinsic signals can provide detailed information about the spatial pattern of the activity. However, because the neural activity is made visible through changes in the 'hemo-dynamic filter,' the temporal resolution is limited to slow processes, on the order of a few seconds.

As mentioned above, there are three sources to the optical signal in such experiments: 1) an increase in the amount of deoxyhemoglobin, which changes the absorption spectrum of the tissue; 2) a large, subsequent increase in blood flow to the active brain region, and 3) a change in light scattering due to cell swelling.

The experimental setup is shown in Figure 1. Light (600-720 nm) illuminates the exposed cortex, and the reflected images are captured by a camera (usually a CCD camera). The frame rate and resolutions vary among experiments, but are usually in the range of 2-30 frames per second, with a resolution of 500 × 400 pixels, digitized at 12 bits/pixel.

Imaging of functional neuronal ensembles

In the past decade, optical imaging of intrinsic signals has provided new details about the functional organization of the cortex, the huge neuronal sheet that covers the older parts of the brain. It was known for some time that neurons in the visual cortex have certain preferences: some are driven primarily by one eye, some are tuned to the orientation of edges and lines in the visual world, and some are concerned with size, color, or movement. In addition, it was known that neurons of certain preferences tend to cluster together as columns (Mountcastle, 1957; Hubel & Wiesel, 1962), but the details of this clustering could not be easily determined from studies that recorded the activity and selectivity of individual neurons with microelectrodes.

Optical imaging has provided greatly improved views of the ocular dominance columns and orientation selective domain (Figure 2), or the size selective regions (Figure 3) of the visual cortex of cats and monkeys. The details and scope of these images provided crucial new information about the functional architecture of the cortex. Of special theoretical interest is the discovery (Blasdel & Salama, 1986; Bonhoeffer & Grinvald, 1991) of the "pinwheel" organization of orientation of selective domains in the visual cortex. Such structures might shed light on the computational strategies that the cortex must perform in analyzing the visual world.

2. Imaging of single cells and cellular components

In the previous section we focused on imaging of large groups of neurons. However, optical imaging can provide a wealth of new information about single cells and sub-cellular compartments. Thus we can visualize the spread of currents across the cell membrane as electrical activity takes place, trace the spread of various molecules such as calcium throughout the cell, and so on. Of particular recent interest have been explorations of the activity of dendritic spines, the tiny boutons through which nerve cells communicate with each other (Helmchen *et al.*, 1999). The activity in tiny parts of a cell, measuring just a few microns, can now be visualized with unparalleled spatio-temporal resolution in real time in the living brain.

Conclusions

Imaging can now provide a wealth of new information about a wide range of subjects. Optical imaging, as used in biology and other areas, can provide new views of the detailed structure and function of objects ranging from sub-cellular compartments to neuronal ensembles that span millions of cells. The continuing development of new dyes, imaging devices and analysis methods ensures that this field will continue to be in the forefront of scientific endeavor.

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References

Bell, A.J. & Sejnowski, T.J., "An information-maximization approach to blind separation and blind deconvolution," *Neural Computation,* **7** (6), 1129–59 (1995).

Blasdel, G.G. & Salama, G., "Voltage-sensitive dyes reveal a modular organization in monkey striate cortex," *Nature,* **321**, 579–85 (1986).

Bonhoeffer, T. & Grinvald, A., "Iso-orientation domains in cat visual cortex are arranged in pinwheel-like patterns," *Nature*, **353**, 429–31 (1991).

Carmona, R.A., *et al.*, "Wavelet Analysis for Brain Function Imaging," *IEEE Transactions on Medical Imaging*, **14** (3), 556–4 (1995).

Chance, B, *et al.*, "A novel method for fast imaging of brain function, non-invasively, with light," *Optics Express*, **2**, 411–23 (1998).

Everson, R., *et al.*, "Separating spatially distributed response to stimulation from background," *I. Optical Imaging. Biol. Cybern,* **77**, 407–17 (1997).

Everson, R.M., *et al.*, "Representation of Spatial Frequency and Orientation in the Visual Cortex," *Proc. Natl. Acad. Sci. USA,* **95**, 8334–8 (1998).

Gabbay, M., *et al.*, "A principal components-based method for the detection of neuronal activity maps: Application to Optical Imaging," *NeuroImage*, **11**, 313–25 (2000).

Grinvald, A., *et al.*, "*In-Vivo* Optical Imaging of Cortical Architecture and Dynamics," *Modern Techniques in Neuroscience Research*, Windhorst, U. & Johansson, H. (Eds.) Springer, Berlin, 893–969 (1999).

Helmchen, F., *et al.*, "*In vivo* dendritic calcium dynamics in deep-layer cortical pyramidal neurons," *Nature Neuroscience*, **2**, 989–96 (1999).

Hubel, D.H. & Wiesel, T.N., "Receptive fields, binocular interaction and functional architecture in the cat's visual cortex," *J. Physiol.* (Lond.), **160**, 106–54 (1962).

Mitra, P.P. & Pesaran, B., "Analysis of Dynamic Brain Imaging Data," *Biophysical Journal*, **76**, 691–708 (1999).

Mountcastle, V.B., "Modality and topographic properties of single neurons of cat's somatic sensory cortex," *J. Neurophysiol.*, **20**, 408–34 (1957).

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