



Short Course II

Visualizing Large-Scale Patterns of Activity in the Brain: Optical and Electrical Signals

Organized by György Buzsáki, MD, PhD



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Table of Contents

Introduction	
Large-Scale Recording of Unit and Field Activity with Silicon Probes	1
<i>György Buzsáki, MD, PhD</i>	
Imaging the Spontaneous and Evoked Cortical Dynamics: Large-Scale Analysis of Temporal Structures of Neuronal Ensemble Activity	9
<i>Yuji Ikegaya, PhD, Gloster B. Aaron, PhD, and Rafael Yuste, MD, PhD</i>	
Rapid Visualization of Cortical Activity Using Intrinsic Signal Optical Imaging	19
<i>Michael P. Stryker, PhD</i>	
VSDI Explorations of Neocortical Dynamics	27
<i>Amiram Grinvald, PhD</i>	
Long-Term Optical Imaging of Intrinsic Signals in Anesthetized and Awake Monkeys	33
<i>Anna W. Roe, PhD</i>	
High Resolution [Ca ²⁺] Imaging Using 2-Photon Microscopy	43
<i>Karel Svoboda, PhD</i>	
In Vivo Imaging of Neuronal Structure and Function Using Multiphoton and Intrinsic Signal Imaging	51
<i>Lawrence C. Katz, PhD</i>	
Multiple Electrode Recording of Neuronal Ensemble Activity in the Rodent Hippocampus and Neocortex	59
<i>Matthew A. Wilson, PhD</i>	

Rapid Visualization of Cortical Activity Using Intrinsic Signal Optical Imaging

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Intrinsic signal optical imaging has been widely useful for revealing the organization of many cortical systems, from olfactory bulb to optic tectum, and it has found its widest use in the neocortex, where it has answered questions that had been outstanding for many years (1, 2). Nevertheless, intrinsic signal imaging has significant limitations beyond the obvious one of relying on a hemodynamic surrogate of neuronal activity, and microelectrodes are unlikely to fade into obsolescence in the foreseeable future. In particular, conventional methods of intrinsic signal imaging are slow and reveal the responses to only a very limited number of stimuli, and conventional analytic procedures work well only for some stimulus sets and fail with others. A new experimental and analytic approach to intrinsic signal imaging has improved its accuracy and acquisition time many-fold for some applications (3). This lecture focuses on the factors that limit the speed and precision of optical imaging. It concludes with some points of advice on the use of this technique for mapping sensory representations.

Intrinsic signal optical imaging takes advantage of a constellation of changes in optical properties that take place throughout the depth of the cortex in response to neural activity. These responses include changes in volume, changes in refractive properties, and changes in absorption of particular wavelengths of light (4). The most important of these changes for high resolution maps of neuronal activity is the change in absorption of red light by hemoglobin with blood oxygenation (5).

These intrinsic signal changes are small, with the stimulus-specific changes in absorption typically a few times 10 to 4 (2 or 3 parts per 10000). The small size of these signals relative to the signal-to-noise of CCD area detectors (or the even worse noise properties of video cameras) poses a problem. For example, consider a CCD array with a well depth of 10^6 photoelectrons, somewhat greater than any commercial high resolution device. Shot noise or photon counting statistics would allow a signal-to-noise ratio of 10 to 3 at best (1 part per 1000). Therefore, one must have some sort of averaging to capture a signal much smaller than the noise.

Issues in conventional episodic intrinsic signal imaging

Conventional methods of intrinsic signal optical imaging work by comparing the averages of many responses to different stimuli with one another or with the background response to a “blank” (absent) stimulus. The conventional stimulus-response protocol is essentially episodic: presentation of stimulus 1 for several seconds during which the reflected light across a region of cortex is collected in a series of

frames, followed by a relaxation period for the optical properties to return to a baseline state, followed by the presentation of stimulus 2, etc. The pace of stimulus presentation is set by the settling time of the optical response for return to baseline state before presentation of the next stimulus, and is typically on the order of 10 to 12 sec. This pace, the necessity of acquiring an average response to many stimulus presentations, and the limited period of time over which stable hemodynamic responses can be elicited, combine to limit the number of distinct stimuli that can be presented and compared, typically to 20 or fewer.

One difficulty with the conventional approach is that there are large fluctuations in reflectance that are not related to the stimuli that are presented. These fluctuations can obscure the activity-related responses. Since only the average responses are stored, it is in principle not possible to separate the “noise” fluctuations in reflectance from the signal of neural activity, and various post-hoc approaches are taken. Most commonly, the reflectance images from each stimulus condition are high-pass filtered to make their average levels equal both across stimulus conditions and across the spatial extent of each reflectance image. This is effective in many cases because the largest components of the reflectance fluctuations that are not related to the stimulus occur at low spatial and temporal frequencies. For many structures of interest, such high-pass filtering is innocuous when carried out with a cutoff two octaves lower in spatial frequency than the largest structure of interest, but it makes it impossible to draw conclusions about different levels of response elicited by different stimuli or over different large areas of the image. It is crucial for the experimenter to pay close attention to the filtering of the data in relation to possible structures of interest and the time course of their activation.

A major issue in episodic intrinsic signal optical imaging is normalization. The question is what should be the baseline or standard for assessing responses to different stimuli. It is important that this standard have low noise because the resulting maps will all have added to them any noise in this standard. The normal choices are the “cocktail blank,” which is the average of all the stimuli presented, or the real “blank,” that average image when no stimulus is presented. Collecting a sufficient number of absent-stimulus responses to make the noise low in the real blank significantly extends the duration of optical imaging experiments and reduces the number of real stimuli that one can use for mapping.

The cocktail blank has two great advantages: It is low noise, because it is the average of all of the other

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trials, and it incorporates any “nonspecific” activation that is common to all stimuli, thereby emphasizing the differences between responses to different stimuli in the resulting maps. But it has the terrible problem that the measurement of the responses to different stimuli depends on which other responses are incorporated in the cocktail blank for analysis. In practice, this often is a minor issue for qualities, like stimulus orientation in the visual cortex, which are conventionally mapped using a stimulus set distributed evenly around the possible orientations, but it is particularly an issue for maps of noncyclic qualities like spatial frequency. The problem is evident in Figure 1 [data from (6)], where the apparent responses to the intermediate frequency stimuli are very small when responses to only 6 low-to-mid frequency stimuli are included in the cocktail blank (filled blue triangles), but are much larger when the cocktail blank includes responses to the same 6 stimuli plus 2 additional high-frequency stimuli (open red triangles).

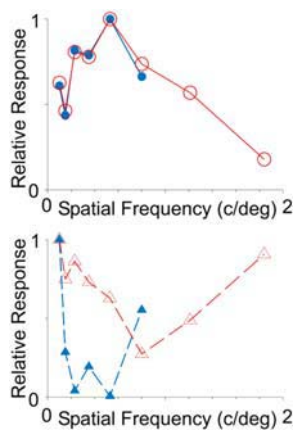


Fig. 1 Response amplitude as a function of preferred spatial frequency (SF) [data from (6)]. Above, blank normalization. Below, cocktail blank normalization. The dashed red line with open triangles shows the distribution of average response amplitudes as a function of spatial frequency. Response amplitudes are weakest for intermediate SFs, unlike the profile shown above. The dashed blue line is calculated from a subset of the data containing all but the highest 2 SFs (1.2 and 1.84 c°) and shows a peak at 0.8 c° . This profile is completely different from the one shown in red, calculated from the entire data set. Cocktail-blank normalization therefore makes the response profile dependent on the particular stimulus set used for analysis.

Analysis of the same data using real blank normalization (filled blue and open red circles, above) shows that the measurement of responses to the 6 lower-frequency stimuli are not greatly affected by the inclusion or omission of the data from the 2 additional high-frequency stimuli.

The fact that cocktail blank normalization also acts to obscure responses that are common to the stimulus set poses a problem when those common responses

are the responses of interest. This issue was evident in the measurement of the effects of monocular visual deprivation in young cats, where the deprived eye's responses largely lost orientation selectivity and collapsed onto an array of patches (7). These deprived eye patches simply had disappeared in earlier experiments because of the use of cocktail blank normalization, making it appear as if intrinsic signal imaging failed to detect the remnant responses to the deprived eye. With true blank normalization, the deprived eye patches stood out as the major feature of interest.

Continuous periodic Fourier imaging

The comparison of average responses in the conventional method of intrinsic signal optical imaging is analogous to the analysis of “transient” evoked potentials. The evoked potential literature long ago began to use “steady-state” evoked potentials as an alternative for making measurements that were much less susceptible to many kinds of noise. Recordings of steady-state evoked potentials use periodic stimulus presentation and measure only the component of the scalp electrical response at the temporal frequency of the stimulus, minimizing the effect of noise at other frequencies on the measurement. Norcia and co-workers have shown that responses to multiple stimulus frequencies in the steady-state evoked potential can be measured with little mutual interference through the use of appropriate processing techniques (8–10). A number of groups have applied a steady-state approach to brain mapping with fMRI, beginning with Engel, *et al.* (11).

Kalatsky and Stryker (3) took advantage of a parallel approach for measuring intrinsic optical signals evoked by sensory stimulation. While I will illustrate this method with examples from the visual system, the same approach is effective with other sensory modalities (12). They presented a periodic stimulus designed to excite all neurons of interest at successive phases of the stimulus and recorded the entire time series of reflectance images. For example, topographic mapping of the representation of azimuth used a period sweep of a vertical bar across the visual field. Maps of orientation columns in the visual cortex used drifting full-field grating that slowly rotated over many cycles of 360°. Typically, 5 to 15 min of response to continuous stimulation are compiled for analysis. The phase of the stimulus corresponds to a particular azimuthal position or orientation in these two examples, and the goal of the analysis is to measure for each position on the brain the phase of the stimulus that gave rise to the largest response.

The image data rates that must be stored for this approach are substantial. Imaging at full resolution of 1024 x 1024 12-bit pixels at 30 Hz creates over 60

Mbytes/sec, close to saturation of the conventional PCI bus on computers, and requiring Gigabit Ethernet and motherboards with the fast and wide PCI bus for storage and transmission of the data to a separate workstation for real-time analysis. Binning of the acquired images in the acquisition computer spatially at 2×2 pixels and temporally by 3 frames to make 16-bit data results in 512×512 pixel images at 10 Hz, or 5 Mbyte/sec, to be stored and transmitted to the analysis station. Even these files are large—1.6 Gbytes for a 5 min map.

The response at the frequency of stimulation is extracted from a complete time series of image intensities acquired at each pixel. The first step in this analysis is the removal of slow changes in the image intensities, which, because they are so large compared to the changes evoked by the stimulus, can affect the phase measured at higher frequencies (see Figure 2).

The necessity for this removal is initially counterintuitive, since one normally imagines that Fourier analysis can isolate the response components corresponding to the various stimulus frequencies. This intuition is not true, as demonstrated by the following thought experiment. Consider a model response function $R(t)$ consisting of a pure sinusoidal response contaminated by a linear slow change: $R(t) = A \cos(2\pi ft) - Bt$. This function has the following Fourier harmonic at frequency f : $Re = A/2$ and $Im = B/2\pi f$ for a period of time TN ($T = 1/f$), where Re and Im stand for the real and imaginary components, respectively, and N is the number of cycles of the test frequency. For reasonable values like those observed experimentally of $A = 2$, $B = 100\pi f/N$, and $N = 50$, one obtains $Re = 1$ and $Im = 1$. Hence, despite our having isolated the component of the response at the stimulus frequency using Fourier analysis, failing to remove the linear slow change component from the response would cause a huge artifactual shift in its measured phase (45° in this example), as well as a change of its strength.

As with the comparison between transient and steady-state evoked potentials, conventional episodic intrinsic signal imaging is contaminated by noise at all frequencies, but the relevant noise for periodic Fourier imaging is only that at the immediately adjacent frequencies. This property allows us to measure a signal, as shown in Figure 1, with an amplitude that is 10-fold above the relevant noise, despite the presence of noise that is 2 to 3 orders of magnitude greater at other frequencies.

The hemodynamic events that are set in motion by the stimulus have a certain time course that is of little interest in most studies that make use of intrinsic signal imaging. What is of greatest interest is the

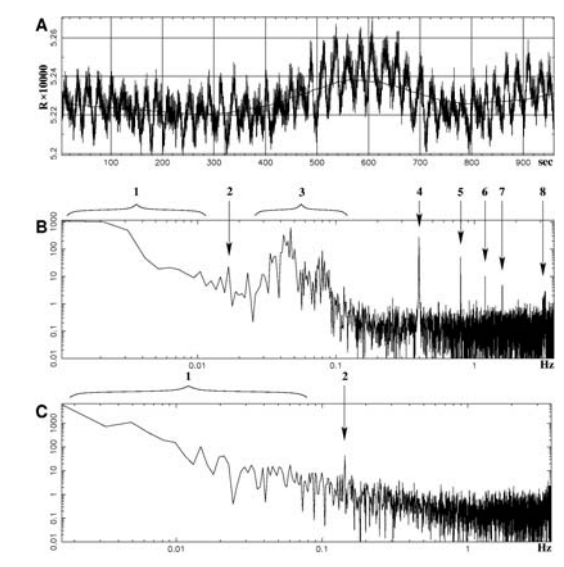


Fig. 2 Time course and power spectrum of the cortical light reflectance. (A) Time course of light reflectance at a single pixel from a set of 7,160 images of the cat primary visual cortex. The response is dominated by the vasomotor oscillations and slow variation of the baseline. To remove the slow variation, the response is time averaged over a window of integer number of stimulus cycles (2 cycles here), and the resulting curve (the smooth line) is subtracted from the response. (B) The power spectrum of the reflectance signal plotted in (A). Components of the time series are indicated: (1) slow variation, (2) response at the frequency of stimulation (1 min period), the amplitude and phase of which are used to construct cortical maps, (3) vasomotor signal, (4) ventilation artifact (fundamental frequency); (5), (6), and (7) second, third, and fourth harmonics of the ventilation artifact, and (8) heart beat artifact. The subject was artificially ventilated; as a result, the ventilation component is strongly periodic and sharp. The high-frequency part of the spectrum (above 0.2 Hz) shows presence of the so-called shot noise due to the Poisson distributed statistics of photon counting. The mapping signal (2) is small in this case, about 3 times the noise at that frequency, interpolated from neighboring frequencies. (C) The power spectrum of light reflectance signal at a single pixel from a set of 4,593 images of the mouse primary visual cortex. Mice usually do not have vasomotor oscillations, and their heart and respiration rates are much higher than those of the cat, so that these three major sources of contamination of the evoked response are not present in the portion of the spectrum illustrated. The sharp spike at 0.125 Hz is the response due to visual stimulation (8-sec period), which in this case is about 10 times the noise at that frequency.

instantaneous state of the stimulus that gave rise to the maximal response at each pixel of the image, not the state of the stimulus many milliseconds later when the response has grown maximally. Measurements of spike responses and other brief, short-latency responses generally do not have to worry about this issue. How are we to measure the long hemodynamic response lag with sufficient

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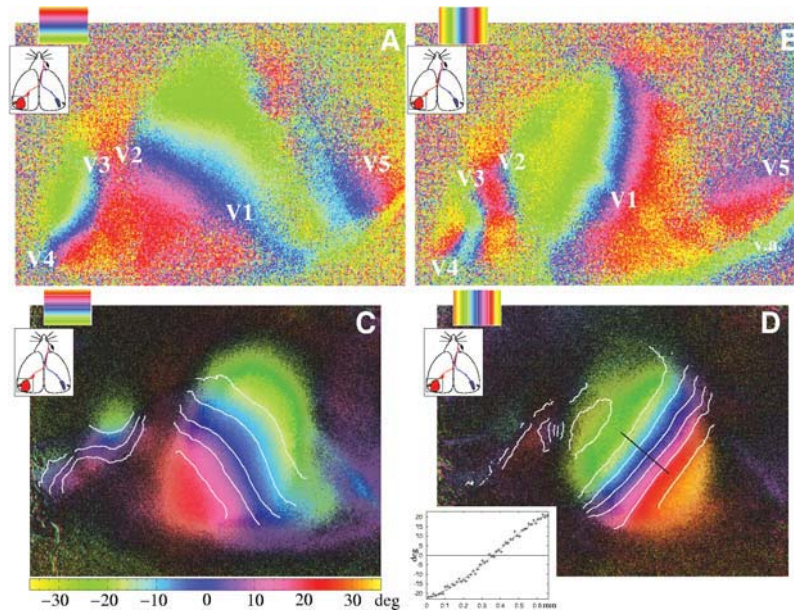


Fig. 3 Maps of multiple visual areas and precision of mapping [data from (3)]. (A and B) Phase maps of absolute retinotopy with isoelevation and isoazimuth lines, respectively, from a hybrid mouse. Imaging time was 12 + 12 min for these maps; the delay removed by the method of the stimulus reversal. Labels V1–V5 mark topographically organized areas. v.a., vascular artifact. (C and D) Polar maps (in which response magnitude is encoded by lightness) showing absolute retinotopy in another mouse together with isoelevation and isoazimuth lines drawn at 10° intervals. Imaging time was 10 + 10 min for these maps. Bottom inset of (D) shows the scatter of visual field azimuths for individual 8 × 8 micron pixels as a function of cortical position along the line segment shown in black.

accuracy to infer the responsible stimulus, when the stimuli are present continuously and periodically? If one conceives of the response as an instantaneous but variable neural response that is always followed by a constant slow hemodynamic event, then a solution is at hand (3). A time-reversed version of the stimulus will give rise at each point to an identical neural response followed by the hemodynamic event. If one subtracts the time-reversed from the original map, the constant hemodynamic event disappears, and one is left with a map of the instantaneous responses. If, on the other hand, one adds the two maps, then the instantaneous responses disappear (since they are in the opposite direction) and the resulting image contains only the constant hemodynamic delay.

The overall system used for continuous periodic Fourier imaging has the capacity for rapid and precise measurement. Figure 3 shows a plot of the precision of topographic mapping in the mouse from 10 min of mapping data. The plot of azimuthal position of receptive fields versus position on the brain in successive independent 8 × 8 micron pixels has a scatter of less than $\pm 3^\circ$.

The time needed to make high-quality maps in some circumstances can greatly be reduced using the new approach, by a factor of approximately 50. Reference (3) shows that orientation maps of cat visual cortex made from 1 min of data were comparable in quality to those from 1 h of data using the conventional, episodic approach. This comparison was done using identical imaging hardware for the two experimental protocols.

Five factors account for the greater speed and precision of the new approach using continuous recording and periodic stimulation over conventional episodic imaging: 1) Continuous recording of image data allows removal of slow changes from signal before analysis. 2) Continuous stimulation effectively drives each point on the cortex with a near-optimal stimulus; whereas the limited number of stimuli used with episodic approach requires that many regions never receive a stimulus that produces an optimal response. 3) Periodic stimulation and Fourier analysis decrease sensitivity to noise other than at the stimulus frequency. 4) Analysis of the time series of image data permits selection of the stimulus frequency to lie within a quiet portion of the reflectance spectrum. 5) The time needed with episodic imaging for relaxation of the hemodynamic response to baseline before presentation of the next stimulus (typically 50 percent of imaging time) and for presentation of blank stimuli is saved using the new approach. The importance of these factors depends on the circumstances of the individual experiment.

Final words of advice

In many publications, the optical maps are represented in smoothed form. For most stimulus qualities for which optical imaging has been useful, the similarity of neighboring pixels on the final, computed map is the best indicator of the signal-to-noise of the underlying data and thereby of the adequacy of the data for the question that is under investigation. Smoothing completely obscures this valuable source of information about the adequacy of experiments. It can fool both authors and readers, and can create

features in noise resembling those of genuine maps (13). Few other areas of biology would allow such concealment of the quality of the data. Indeed, imagine a biochemist reviewing a paper in which the only gel images presented were reconstructions from averages of scans from multiple gels. If it is necessary to smooth to see some feature of the findings, then one should have much less confidence in that feature, and the only fair way to represent the resulting map is with pixels the size of the low-pass filter kernel.

The new approach to intrinsic signal imaging requires periodic continuous stimulation. How is this possible with a stimulus dimension that is not actually periodic? In general, it appears adequate to use a continuous stimulus that increases or decreases monotonically as a function of time along the dimension to be mapped, and to make this stimulus periodic by following the one extreme stimulus value with the other, after a short interval needed to make the transition less prominent than it might otherwise be.

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