

Online analysis method for intrinsic signal optical imaging

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Abstract

The intrinsic optical imaging technique has been widely applied for the visualization of functional maps in the sensory cortices of mammals. Many current studies refer this mapping in order to focus thereafter on particular features, at some particular locations: a fast and accurate mapping is therefore required. However, even during a successful experiment, the recorded raw data are usually contaminated by some kinds of noise that cannot necessarily be averaged out over the trials. An adequate image data analysis method has to be applied to extract signals closely related neural activities in response to presented stimuli. Thus far two different analysis methods could be adopted: the band-pass filtering and the GIF method [Yokoo T, Knight BW, Sirovich L. An optimization approach to signal extraction from noisy multivariate data. *NeuroImage* 2001;14:1309–26]. While the latter one is very efficient but requires the whole data in order to maximize the signal to noise ratio, the simple band-pass filtering technically reaches its limits very quickly. Here we propose another filtering method based on the polynomial subtraction of spatially smoothly modulated components. This simple method can visualize well-organized *iso*-orientation domains of the cat visual cortex with reliability similar to more sophisticated ones while allowing an online visualization of the clean data.

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1. Introduction

Intrinsic optical recording has been widely applied to visualization of various kinds of functional maps over the cortical surface. Owing to the technique, we can observe the tangential organization in the representation of sensory features in a wide area of the cortical surface. In particular, the layouts of orientation preferences and ocular dominance maps in the primary visual cortex of cats and monkeys have been studied in depth by the use of the intrinsic optical imaging (Bonhoeffer and Grinvald, 1991; Crair et al., 1997; Chapman and Bonhoeffer, 1998; Chapman et al., 1996; Coppola et al., 1998; Grinvald et al., 1986; Kim et al., 1999; Ohki et al., 2000). Furthermore, this technique has often been used in the combination with other conventional techniques not only for the observation of the spatial arrangement of optimal stimulus features but also for the elucidation of more detailed properties of cortical function, structure and development (Chapman and Bonhoeffer, 1998;

Chapman and Godecke, 2000; Chapman et al., 1999; Coppola et al., 1998; Crair et al., 1997, 1998; Godecke et al., 1997; Kim and Bonhoeffer, 1994; Sengpiel et al., 1999; Sharma et al., 2000; Tani et al., 2003; White et al., 2001). To this end, an efficient analysis method is required for removing the undesirable noise from the recorded data.

In addition to the powerfulness of the analysis method, we also focused on the fact that many experiments use the resulting functional maps as a preliminary localization of some particular features. Other techniques like tracer injections (Kisvarday et al., 2000), intracellular (Gibber et al., 2001) or more commonly extracellular recordings usually follow the mapping. It therefore becomes necessary to reconstruct functional maps as quickly as possible, stopping the recording as soon as an efficient mapping had been performed. To this end, a fast method that could be directly implemented in the acquisition software would be very much appreciated.

The fast, conventional data analysis of recorded optical signals consists of the filtering of a particular range of spatial frequencies. The cut-off of high spatial frequency components of recorded signals leads to smoothing of optical signals, resulting in functional maps exhibiting continuous representation of stimulus features. This filtering is not essential for the reconstruction of maps but it is useful for the observation of tiny structure in

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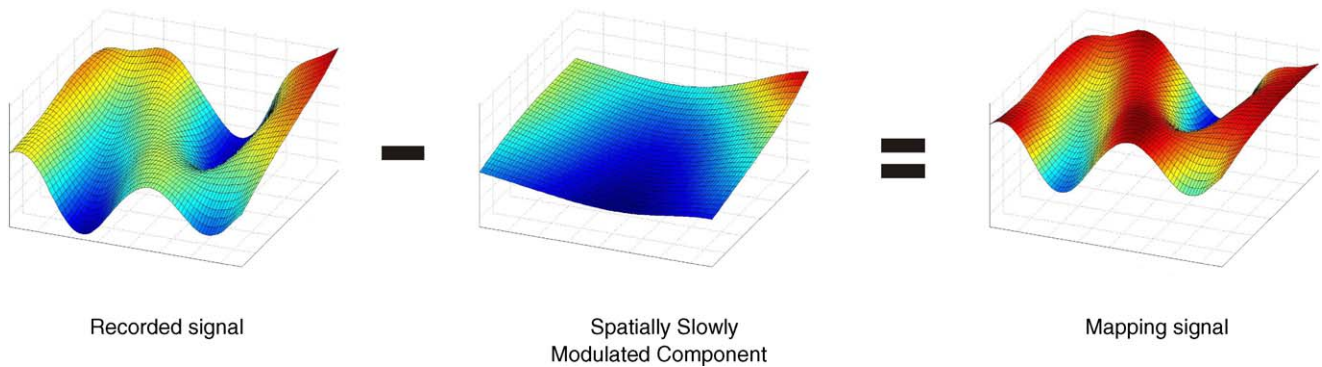


Fig. 1. A schematic picture of the subtraction method. Mapping signal (right) is obtained by the subtraction of a spatially smoothly modulated component (middle) from a recorded signal (left).

the maps such as the pinwheel structure in orientation maps (Bonhoeffer and Grinvald, 1991; Crair et al., 1997; Ohki et al., 2000) and direction discontinuity lines in direction maps (Kim et al., 1999; Weliky et al., 1996). On the other hand, the cut-off of low spatial frequency components eliminates global fluctuation in the recorded optical signals over the cortex, as depicted in Fig. 1. This filtering is more important to obtain the overall map structure, because such spatially smoothly modulated signals, which are often contained in the recorded optical signals, drastically change the spatial arrangement of optimal features in individual maps.

Thus far, the high-pass filtering has been used for extracting components of signals closely related to neural activities in response to presented stimuli. One disadvantage is the impossibility for the resulting signal to exceed the periodicity defined by the cut-off characteristic frequency. A classical example is found in cat area 18, where orientation map is periodic along a rostro-caudal axis, and elongated along the perpendicular axis. Then, when a narrow band filter is adopted, this method leads to the spurious spatial periodicity of feature representation even if the obtained maps appear to be nicely organized. Another disadvantage of the high-pass filtering is the distortion of maps near the edges of the region of interest (ROI): the appearance of a strong noise near the edge cannot be well fitted by low-frequency sine and cosine functions, and cannot therefore be eliminated. Furthermore, it cannot sufficiently improve images of maps when components of optical signals unrelated to presented stimuli are strong enough to occlude components of signals closely related to stimuli. Such disadvantages originate in the fact that the low-frequency cut-off procedure cannot eliminate effectively the components of smoothly modulated signals unrelated to presented stimuli.

In order to obtain biologically plausible maps from noisy recorded optical signals, sophisticated analysis methods have been proposed based on the principal component analysis (PCA) (Everson et al., 1997; Stetter et al., 2000). Everson et al. (1997) found that components with intermediate eigenvalues reflect columnar structure when they applied the principal component analysis to recorded signals. However, there are no objective criteria to judge which decomposed components represent mapping signals before visual inspection of all the patterns of decomposed signals. Stetter et al. (2000) also applied the PCA to decompose

optical signals into mutually uncorrelated different eigenvectors. They found that eigen-modes with some of the largest eigenvalues mainly contributed to stimulus-independent global signal and signal from blood vessels; the 4th–7th eigen-modes contained mapping signal, which closely related to a spatial pattern of ocular dominance columns. Recently, Yokoo et al. (2001) proposed an advanced method called the generalized indicator function method based on the PCA. This method is to project the recorded data onto the sub-space of data, in which the signal-to-noise ratio is maximized. The method is expected to be generally applicable to the image data analysis, without prior knowledge of noise properties. Although these sophisticated methods are powerful to extract mapping signal from noisy recorded signal, whole processes to get resultant functional maps from original recorded signal are somehow complicated and often require a huge amount of work space in computers because of processing high-dimensional matrices as in matrix diagonalization. Thus, a method that can be treated with ease and remove major noise components is desired.

In the present study, we devised a new method to subtract smoothly modulated components that contaminate and occlude components of optical signals closely related to neural activities in response to visual stimuli, as illustrated by Stetter et al. For the subtraction, we first calculate the components by fitting the recorded signals with polynomial functions, which are defined inside the ROI. Then, we subtract the polynomial functions from the recorded signals, and illustrate maps using the residual signal components. Owing to this method, we could visualize almost the same orientation maps from the data obtained in different recording sessions of the identical cats, though the conventional high-pass filtering method could not reconstruct the maps. The reproducibility of the maps obtained from different recording sessions indicates the efficiency and reliability of the proposed method for intrinsic optical recording. Also noise analysis suggests that smoothly modulated signal components were composed of fluctuation in absolute light reflectance and noise induced by movement of cortical surface due to the pulsation of large blood vessels. Furthermore, we examined up to which order of polynomial functions we should take into account to better reconstruct orientation maps. The orientation maps were drastically improved when we took into account the 3rd order polynomials, and the map will not change so much for higher

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