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...
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 inability 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) (Evrson et al., 1997; Stetter et al., 2000). Evrson 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 order polynomials.
orders. Since the computation time for the calculation of an orientation map is very short and a huge workspace is not needed even for the 8th order polynomials, the proposed method is advantageous for prompt analysis of intrinsic optical imaging data. Finally, we propose the usage of the present model for better visualization of on-line maps during intrinsic signal optical recording.

2. Material and preparation

In the surgical operation and optical recording, the animals were cared for in accordance with the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Science" of the Japanese Physiological Society.

2.1. Surgical operation and optical recording

Seven cats were used for intrinsic optical imaging of orientation maps in the visual cortex. Surgical operations were done more than 1 week before the first optical recordings. In surgery and optical recordings, initial anesthesia was induced by ketamine hydrochloride (7.0 mg/kg, i.m.) and medetomidine hydrochloride (0.06–0.08 mg/kg, i.m.), and subsequently the animals were fixed on the stereotaxic frame and artificially ventilated with a 1:1 mixture of N2O and O2 containing 0.5–1.0% CO2 concentration and rectal temperature were, respectively, maintained within normal limits during surgery, 120–150 cycle, and rectal temperature were, respectively, maintained within normal limits during surgery, 120–150 cycle, 3.0–4.0% and 37–39°C. A chamber made of dental cement was illuminated with the light of 700 nm wavelength and the front of the animals using appropriate contact lenses. The corneal movement. The eyes were focused on a plane 30 cm in front of the animals using appropriate contact lenses. The corneal movement. The eyes were focused on a plane 30 cm in front of the animals using appropriate contact lenses. The corneal movement. The eyes were focused on a plane 30 cm in front of the animals using appropriate contact lenses. The corneal movement. The eyes were focused on a plane 30 cm in front of the animals using appropriate contact lenses. The corneal movement. The eyes were focused on a plane 30 cm in front of the animals using appropriate contact lenses. The corneal movement. The eyes were focused on a plane 30 cm in front of the animals using appropriate contact lenses.

The chamber was filled with 2% agar containing gentamycin and puncuronium bromide (0.1 mg/kg/h) to prevent eye movement. In the surgical operation and paralyzed by optical recording experiments, the animals were anesthetized over the rectangular region of interest (ROI) by the polynomial functions of x and y in the Cartesian coordinate system.

Let us define signal representing neural activity $s_{θ,t}(x,y)$ in response to stimulus θ at trial of data acquisition t as follows:

$$s_{θ,t}(x,y) = \frac{R_{θ,t}(x,y)}{R_{θ}(x,y)} - 1,$$  (1)

where $R_{θ}(x,y)$ and $R_{θ,t}(x,y)$ represent the recorded signals at trial t in response to visual stimulus θ and a blank stimulus, respectively. To determine the coefficients of the polynomial functions, we have only to minimize the mean square error $e_{θ,t}$ over the ROI for each stimulus and trial of data acquisition, which is defined by

$$e_{θ,t} = \int_{ROI} \left[ f_{θ,t}(x,y) - s_{θ,t}(x,y) \right]^2 dx dy. \tag{2}$$

To make it easier to carry out the fitting, it is convenient to expand the fitting functions by the orthonormal polynomials $ϕ_{μ}(x,y)$ defined inside the ROI:

$$f_{θ,t}(x,y) = \sum_{μ} c_{μ} ϕ_{μ}(x,y). \tag{3}$$

The orthonormal polynomial set can be generated by the Gramm–Schmidt procedure for constructing orthonormal bases. Values of the coefficients $c_{μ}$ should be determined such that the error $e_{θ,t}$ given by Eq. (2) takes the minimum. The minimization of $e_{θ,t}$ leads to the following equation:

$$c_{μ}(θ) = \int_{ROI} ϕ_{μ}(x,y)s_{θ,t}(x,y) dx dy. \tag{4}$$

Finally, we can obtain mapping signal $s_{θ,t}(x,y)$ that is closely related to neural activities in response to stimulus θ at trial t by subtracting the polynomial function $f_{θ,t}(x,y)$ from the recorded signal $s_{θ,t}(x,y)$:

$$s_{θ,t}(x,y) = s_{θ,t}(x,y) - f_{θ,t}(x,y). \tag{5}$$

Hereafter, we will call this method simply the polynomial subtraction method. This method follows the schematic rule depicted in Fig. 1.
The advantage of this method is the fact that smoothly varying noise components can be removed at each stimulus and each trial using the signal in response to the blank stimulus, while the other sophisticated methods use a whole set of recorded data. This indicates that we can adopt the present method to remove the noise from on-line maps during intrinsic signal optical recording.

2.3. Power spectrum analysis

In order to quantify the spatial arrangement of the orientation map, we calculated its power spectrum: first, the correlation function was obtained from the following equation:

$$C^{(i,j)}(x) = \frac{1}{S} \iint_{ROI} \bar{m}^{(i,j)}(\vec{x} + \vec{y}) \cdot \bar{m}^{(i,j)}(\vec{y}) d\vec{y},$$

(6)

where \(\bar{m}^{(i,j)}(\vec{x})\) represents the feature vector of the preferred orientation at cortical position \(\vec{x}\) in the \(i\)th recording session, that is, \(\bar{m}^{(i,j)}(\vec{x}) = (\cos \theta^{i,j}(\vec{x}), \sin \theta^{i,j}(\vec{x}))\). The integration on the right-hand side is taken over the ROI and \(S\) is its area. Then, we calculated the Fourier transform of the correlation function and summed up along the increasing waves.

3. Results

3.1. Illustration of the different methods

All orientation maps in the present paper were obtained by the application of the conventional vector sum method (Bonhoeffer and Grinvald, 1991). Since the raw data of recorded intrinsic signals often contain stimulus-unrelated stochastic components that vary smoothly over the ROI, the orientation maps exhibit skewed distribution of orientations.

To see the effectiveness of our method in visualizing orientation maps, in cat A, we compared the maps obtained from three different methods: (a) no filtering; (b–c) high-pass filtering (Fourier components of wavelengths larger than 1 and 0.6 mm were discarded); (d) polynomial subtraction method with polynomial functions up to the 8th order; and (e) generalized indicator function (GIF) method (Yokoo et al., 2001). In the orientation angle map obtained from the naive analysis without filtering (Fig. 2a1), preferred orientations were strongly biased to a particular orientation near the medial edge of the ROI. The orientation polar map exhibited low magnitude in the middle of the ROI (Fig. 2a2). Reflecting the artificial structure, no peak appeared at non-zero wave numbers in the power spectrum, and the orientation histogram showed a skewed distribution. When the cut-off frequency of the high-pass filtering is taken as 1 mm (typically chosen as the upper boundary for area 18), we could observe a slight improvement in the maps, but anomalous over-representation could not be eliminated around the medial edge of the ROI (Fig. 2b1 and b2). The orientation histogram still exhibited a biased distribution and a power spectrum whose peak was located at zero (Fig. 2b3). Although the power spectrum clearly peaked around 0.5 period per mm, by decreasing the cut-off frequency (Fig. 2c3), the power at the zero wave number still remained. Although the Fourier transformation, which provides a basis of the high-pass filtering method, assumes the periodic structure or infinite size of the system, our ROI is delimited by a finite free boundary. The residual DC component indicates that the application of the high-pass filtering method is not appropriate for the removal of the smoothly varying noise components particularly localized around the boundaries of the ROI. Consequently, signals around the edges of the ROI were still contaminated by the strong noise (Fig. 2c1 and c2), which affected the overall orientation distribution.

The polynomial subtraction method could successfully illustrate the orientation map all over the ROI (Fig. 2d1 and d2), and eliminate the skewed distribution (Fig. 2d3). We cannot distinguish the orientation maps from those obtained from the application of the GIF method (Fig. 2e1 and e2) in the sense that both power spectra have a peak located around 0.5 period per mm. The orientation histogram for the polynomial subtraction method is slightly less biased to the oblique orientations than that for the GIF method (Fig. 2d3 and e3). Thus, in this example, the polynomial subtraction method is as powerful as the GIF method despite it is a much simpler method.

3.2. Reproducibility

To examine whether the orientation maps are reconstructed from intrinsic signals recorded in different recording sessions conducted on the same day by changing illumination pattern on the visual cortex, we applied the polynomial subtraction method to recorded signals which exhibited low signal-to-noise ratio. Fig. 3a indicates the blood vessel pattern of the ROI in cat B, which was common with the two recording sessions. The absolute light reflectance patterns (the trial-averaged signals in response to a blank stimulus) in 1st and 2nd recording sessions are illustrated in Fig. 3b1 and b2, where red indicates bright and blue indicates dark. Fig. 3c shows difference maps of intrinsic signals in response to the orthogonal pairs of stimulus orientations; 3c1 and 3c2 for the 1st and 2nd recording sessions, respectively. In each difference map, some domains selectively activated by stimulus orientations (darker and brighter patches) were occluded by the smoothly varying background signal components. The profiles of the background signal components differed for different recording sessions. The quality of signal was low commonly for the two recording sessions, in that it is hard to recognize localized activities in response to stimulus orientations.

Due to the strong background noise, orientation angle maps for 1st and 2nd recording sessions (Fig. 3d1 and d2) were not only evidently dissimilar but also exhibited irregular arrangements of preferred orientations. Indeed, in the orientation map for the 1st recording session, reddish domains appeared in an antero-medial part while greenish domains in a postero-lateral part. The blue occupied larger territory in the middle of the ROI. The biased arrangements of preferred orientations were observed in the orientation angle map for the 2nd recording session, too; the map appears reddish all over the ROI. The irregularity in the orientation maps resulted in the orientation histograms showing skewed distribution.
The resulting power spectra show a peak centered on the DC component (Fig. 3e1 and e2). The evident dissimilarity in the orientation maps between the two recording sessions indicates that recorded signals were contaminated by stochastic components inherent in the signals, which do not depend on the stimulus orientation systematically.

Fig. 4a1 and a2 shows difference maps for the 1st and 2nd recording sessions of the same recordings shown in Fig. 3, which were obtained by the application of the polynomial subtraction method by taking into account polynomials up to the 8th order. It can be seen that the smoothly varying background component could be removed from each difference map. The orientation angle maps for the two recording sessions became quite similar (Fig. 4b1 and b2). The orientation histograms shown in Fig. 4c1 and c2 did not show spuriously biased distribution of preferred orientations, and the power spectra both present a peak located around 0.6 period per mm, which suggest a regular arrangement of the orientation domains.

To examine further the similarity of orientation maps between the different recording sessions, we calculated spatial correlation functions for the orientation maps obtained by applying the polynomial subtraction method between the two recording sessions.

The cross-correlation function of orientation maps between the two recording sessions is shown in Fig. 4d. The profile of the cross-correlation function indicates that similar preferred orien-
Fig. 3. Orientation maps in the two consecutive recordings from the same region of interest (ROI) of cat B, which were reconstructed by the naive method. (a) The image of the cortical surface in the ROI. (b) The cortical coordinate is shown to the right. The absolute light reflectance patterns in the 1st recording session (b1) and in the 2nd recording session (b2). (c) Difference maps for the 1st session (c1) and for the 2nd session (c2). The stimulus orientations are indicated by the tilted line segments and the pairs of orthogonal orientations are shown on the right side of the difference maps. (d) Orientation angle maps for the 1st session (d1) and for the 2nd session (d2). The preferred orientations are indicated by the color code shown below. (e) The power spectrum of the orientation map, and the orientation histograms representing the relative number of pixels against the preferred orientation in the 1st session (e1) and the 2nd session (e2). The two figures illustrate dissimilarly skewed distributions of preferred orientation over the cortical surface. All scale bars indicate 1 mm.
Fig. 4. Orientation maps in the two recording sessions of cat B, which were reconstructed by the polynomial subtraction method using up to the 8th order polynomials. (a) Difference maps for the 1st and 2nd sessions, shown in a1 and a2, respectively. (b) Orientation angle maps for 1st and 2nd sessions, shown in b1 and b2, respectively. (c) Power spectra and orientation histograms for the 1st and 2nd sessions, shown in c1 and c2, respectively. These orientation histograms indicate that the polynomial subtraction method eliminates spurious over-representation of particular orientations, and the power spectra show that preferred orientations are represented periodically. (d) Cross-correlation function of the orientation maps between the two recording sessions. The similarity between the orientation maps in the different sessions is apparent in these figures. The scale bars in (d) indicate 500 μm.

Correlations in the two recordings are likely to be spatially arranged closely to each other. The correlation strength at the center of the profiles gives the correlation coefficient. If the correlation coefficient takes unity, the two maps are identical; if it takes zero, the maps are completely independent. The correlation coefficient between the 1st and 2nd recording sessions was 0.55. This large value (compared to the values of correlation coefficient −0.011 for no filtering and 0.50 for the usual high-pass filtering) indi-
that both orientation maps are extremely similar. These results show that spatial arrangements of preferred orientations in the 2nd session reproduced those in the 1st session when the polynomial subtraction method was applied. The map similarity between the two recording sessions guarantees that the polynomial subtraction method works well in spite of low quality of signals and different cortical illumination conditions.

We also applied the high-pass filtering method to the same data used in Fig. 4 in order to reconstruct orientation maps and calculate the cross-correlation function (data not shown). As mentioned before, the high-pass filtering method did not improve the orientation maps around the edges of the ROI, and the reproducibility was somehow worse because the correlation coefficient of the orientation maps between the two imaging sessions was slightly lower than that for our method.

3.5. Noise properties

We examined the dependence of smoothly varying stochastic components of intrinsic signals upon cortical illumination patterns. First we show only the polynomial functions that fitted the smoothly varying components in Fig. 5a1 and a2 for the 1st and 2nd recording sessions, respectively. It is clearly confirmed that the profiles of the polynomial functions differed for different stimulus orientations and blocks of recordings (one block was a stack of five trials).

To see statistical properties of the smoothly varying components, we calculated patterns of the standard deviations over all the stimulus orientations and blocks at each pixel according to the following equations:

\[
\sigma(x, y) = \sqrt{\frac{1}{2\pi\Theta B} \sum_{\theta, t} [f_{\theta,t}(x, y) - \bar{f}(x, y)]^2}
\]

\[
\bar{f}(x, y) = \frac{1}{2\pi\Theta B} \sum_{\theta, t} f_{\theta,t}(x, y).
\]

Here \( B \) and \( \Theta \) represent the total numbers of bocks and stimuli, respectively. The averages of the smoothly varying components \( \bar{f}(x, y) \) were smaller by one order than the standard deviations \( \sigma(x, y) \). The patterns of the standard deviations for the 1st and 2nd recording sessions are shown in Fig. 5b1 and b2 where the color changes from blue to red when the standard deviation increases. The pattern for the 1st recording session did not resemble that for the 2nd recording session. Since the spatial pattern of cortical illumination changed between the two recording sessions with the same ROI, it is suspected that the different illuminations may be related to the difference in the standard deviation patterns. However, the source of the illumination-dependent noise is still unclear.

3.4. Order of polynomial functions

To see up to which order of polynomial functions we have to take into account to extract only stimulus-related signal components, we show changes of the orientation map recorded from cat G with the changes of the highest order of polynomials in Fig. 7. The power spectrum indicates the predominance of the DC component (Fig. 7a2) and the orientation map obtained without any filtering showed an artificial over-representation around the vertical orientation (Fig. 7a1 and a3). It turns out that the background noise was large enough to occlude mapping signal completely. By the subtraction of the 0th polynomial (DC components), all orientations emerged in the ROI (Fig. 7b1 and b3). The power spectrum still showed the predominance of low spatial frequency components (Fig. 7b2). The orientation map was drastically improved when we took into account polynomials up to the 3rd order (Fig. 7e1). The orientation histogram shows a much less biased distribution of preferred orientations (Fig. 7e3) and the power spectrum exhibits the localization of energy at a finite spatial frequency (Fig. 7e2). As the polynomial order increased, the localization of energy in the spatial frequency domain became sharper and sharper (Fig. 7d2-g2). This indicates that the arrangement of preferred orientations became more periodic. However, the orientation map and orientation histogram did not change so much.
Fig. 5. Analysis of noise fitted by the polynomial functions in the two recording sessions of cat B. (a) Noise subtracted from the raw data for each block and each stimulus in the 1st and 2nd sessions is shown in a1 and a2, respectively. (b) Patterns of standard deviations of the noise for the 1st and 2nd sessions are shown in b1 and b2, respectively. (c) Patterns of illumination-invariant noise in the 1st and 2nd sessions are shown in c1 and c2, respectively. The illumination-invariant noise components are quite similar, while the standard deviation patterns are dissimilar.

Fig. 6. Examples of orientation map reconstruction in four cats (C, D, E and F): from top to bottom, illustrations for cat C to cat F are shown. From left to right, the absolute light reflectance patterns, standard deviation patterns of noise, illumination-invariant noise patterns and reconstructed orientation angle maps are shown for each cat.
Fig. 7. Dependence of the reconstructed orientation maps, histograms and power spectra upon the highest order of the polynomials in cat G.
As a statistical criterion for determining the highest order $p$ of polynomial functions to fit the smoothly varying noise components best, we chose the Akaike information criterion (AIC), which tells us the best compromise between a better fit of a model to data (differences between intrinsic signals before and after the GIF method is applied in the present case) and the degree of freedom of the model (the number of polynomial functions for data fitting). Generally, the AIC is defined by $\text{AIC} = -2 \text{log-likelihood} + 2 \text{degree of freedom}$. The minimum description length (MDL) principle can also serve for determining the adequate order of polynomial functions, although the mathematical definition is slightly different in the second term representing the degree of freedom of the model, called the model complexity for the MDL principle. We could not find a quantitative difference in the determined value of $p$ between the two criteria.

Our data used for the estimation of AIC are defined by the differences in the stimulus-evoked intrinsic signals before and after the GIF is applied. From the definition of AIC, we cannot necessarily use all the data, because AIC requires only statistically independent data. Therefore, to sample statistically uncorrelated data, we firstly checked the noise correlation inherent in the original data, as shown in the left panel of Fig. 8. We found that the data were correlated over several micrometers in the cortical surface. Using statistically uncorrelated noise sampled at pixels mutually separated farther than 535 $\mu$m, the log-likelihood and AIC were estimated for different values of the highest order of polynomials $p$. The $p$ dependence of AIC is shown in black, while the $p$ dependence of the log-likelihood is plotted in grey. The minimum of AIC is given at $p \approx 2$. This indicates that the best model for fitting the smoothly varying noise components can be composed only from polynomial functions up to the 2nd order.

![Fig. 8. Correlation of noise inherent in the original data (left) and AIC for different values of $p$ (right). The noise defined by the differences between the recorded stimulus-evoked intrinsic signals and the signals obtained from the GIF application exhibited spatial correlation decreasing with the distance between the pixels on the cortical surface. Using statistically uncorrelated noise sampled at pixels mutually separated farther than 535 $\mu$m, the log-likelihood and AIC were estimated for different values of the highest order of polynomials $p$. The $p$ dependence of AIC is shown in black, while the $p$ dependence of the log-likelihood is plotted in grey. The minimum of AIC is given at $p \approx 2$. This indicates that the best model for fitting the smoothly varying noise components can be composed only from polynomial functions up to the 2nd order.](image)

4. Discussion

We have seen that the subtraction of the smoothly varying components fitted by the polynomial functions from the recorded intrinsic optical signals lead to clear orientation maps. In particular, orientation maps were quite similar between different recording sessions even when the naive or high-pass filtering methods resulted in dissimilar maps. This indicates that the polynomial subtraction method works well to visualize reliably functional maps in the visual cortex by subtracting the smoothly varying noise components characteristic of intrinsic optical recording.

The conception of this method is indeed not fundamentally different from the conventional high-pass filtering method, which uses a basis set of trigonometric functions for creating a high-pass filter. In the present method, polynomial functions are used for creating such a high-pass filter, as illustrated in Fig. 1. In Fig. 2, however, we can see the advantage of the filtering based on polynomial functions, compared to the conventional high-pass filtering method. Empirically, our method has two striking improvements: (1) The non-uniform responses to certain stimulus orientations localized near the edges of the ROI cannot
be well removed by the high-pass filtering because the spatial non-uniformity is often too steep. On the contrary, when we applied the polynomial subtraction method, the non-uniformity can be removed effectively by linear and/or quadratic functions and hence the resultant orientation maps are regularly organized even near the edges. (2) The periodic organization of orientation maps in area 17 has no anisotropy. In contrast, iso-orientation domains in area 18 are usually elongated perpendicularly to the 17/18 transition zone. Using the conventional high-pass filtering, by which Fourier components are removed irrespective of spatial anisotropy in the real space, the elongated structure can be disrupted. On the other hand, the polynomial subtraction method can retain anisotropic structure without any a priori information about the arrangement of the preferred orientations.

In other methods based on the principal component analysis (Everson et al., 1997; Stetter et al., 2000), the smoothly varying noise components (global signal) have been found to contaminate stimulus-related signal components (mapping signal). Therefore, the subtraction of the global signal from the recorded signal is very important to obtain mapping signal. Even if our method is not based on statistical theory, polynomial components changing smoothly in space can well represent the global signal and hence the subtraction of these components resulted in reliable orientation maps. The application of this method has successfully demonstrated that domains activated by temporally modulated plane stimuli appeared as patches inside area 18, which were aligned in parallel to the 17/18 transition zone (Tani et al., 2003).

The stimulus-independent noise stems from some fluctuation in the light reflectance but not in the shot noise in photons detected by the CCD camera because the noise of problem varies smoothly in space. The fluctuation in the light source is also ruled out because the noise was not spatially uniform. Based on our analysis, it is more likely that the smoothly varying noise is attributed to the intrinsic properties of electric circuits of the CCD camera and the changes of light reflectance due to the movement of the cortical surface. The two sources possibly generate the noise multiplicatively. When we conduct intrinsic optical recording, we need to remove a part of the skull and dura mater covering the cortex where we attempted to visualize functional maps. The removal of the skull and dura mater releases the suppression of cortical movement. To minimize the cortical movement, we filled the chamber with the agar. To minimize the suppression of cortical movement, we filled the chamber with the agar. Therefore, the polynomial subtraction method cannot be used for removing the blood vessel artifact, as seen in Fig. 6d4. In our optical imaging, to minimize such an artifact in the raw data, we usually use the cortical illumination light of the wave-length of 700 nm, which is in the range of light scattering regime but not hemodynamic regime.

On the applicability to variable spectral signals, it is impossible to evaluate whether or not our method works well based on the power spectrum. However, if we set the ROI which is sufficiently larger than the extent of stimulus-evoked intrinsic signals, the reconstructed activation patterns are not different from those obtained from the GPF method. This fact has been confirmed in the activation pattern in response to mechanical stimulation to one or two whiskers in rats and tonotopic maps in AI and AAF in the cat auditory field (data not shown). However, the signals after the smoothly varying noise components were removed by this method could not endure the precise quantitative analysis. Therefore, our method is better to be used in the on-line analysis for reconstructing tentative cortical activation patterns.

The sophisticated methods based on PCA (principal component analysis) can be used only in the off-line analysis. On the other hand, the polynomial subtraction method does not require a whole set of recorded data for removing a smoothly varying noise component from the recorded signal in response to each stimulus, as pointed out in Section 2.2. Therefore, so long as we record light reflectance in response to a blank stimulus first every trial of data acquisition and apply this method to the normalized light reflectance to each actual stimulus, we can decompose the recorded signal into mapping signal and global signal one by one during recording.

The applicability of the polynomial subtraction method for on-line monitoring of the quality of recorded signals provides a great advantage for successful optical recording of intrinsic signals.

References


