

Functional optical coherence tomography reveals localized layer-specific activations in cat primary visual cortex in vivo

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Surface neural activity has been widely visualized using optical intrinsic signal imaging (OISI) from various cortical sensory areas. OISI of the cortical surface with a CCD camera gives integrated information across a depth of a few hundred micrometers. We visualize depth-resolved activation patterns of cat primary visual cortex by functional optical coherence tomography (fOCT). A comparison of the depth-integrated results of fOCT maps with the optical intrinsic signal profiles shows fairly good agreement. Our results reveal layer-specific activation patterns and indicate that the activation was not homogeneous. © 2007 Optical Society of America

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Optical intrinsic signal imaging (OISI) is a popular functional mapping technique to obtain high-resolution brain activation maps in animal models [1]. OISI in principle measures the reflectance changes from the cortex of the brain and has revealed organization of clustering of neurons having similar response properties called columns with dimensions around a few hundred micrometers [2,3].

In OISI, the cortical surface under activation by stimulation is illuminated with visible light and is imaged onto a CCD camera. Through this approach, OISI can monitor the concentration of oxy-deoxyhemoglobin of blood as changes in absorption and structural changes as changes in scattering. As the detection process is done with a CCD camera, the measured reflected light is actually integration of reflections over the depth determined by the collection optics. In other words OISI is suitable for detection of clustered activation of neurons. In contrast, anatomically most of the cortex is a layered organization consisting of six layers distinguished by cell types and density of cells [4]. As OISI detects the clustered activation, the potential difference in the layered organization across depth may go undetected.

Utilizing the potential of optical coherence tomography (OCT) [5] in resolving depth structures and taking advantage of the scattering changes during neural activity, we proposed the use of OCT in depth-resolved functional imaging and successfully demonstrated it in cat visual cortex [6,7]. Recently, supporting evidence for the potential of OCT in functional studies of squid axon [8] and of retina [9–11] and in the rat [12] have been reported. Here, we have obtained depth-resolved functional maps of cat visual cortex by functional OCT (fOCT).

To correlate the fOCT maps with the neural activity maps, we compared the results with the OISI results. The OISI technique in conjunction with the direct measurement of electrical activity has revealed that OISI indeed detects population activity of neurons [13]. This fact justifies our comparison of fOCT

maps with OISI. Indeed, a comparison of the integrated profiles obtained from fOCT maps correlates fairly well with the intensity profiles of the intrinsic maps.

We used a fiber based OCT imaging system consisting of a Mach–Zehnder type heterodyne interferometer (Fig. 1A). A broadband source with output power of 30 mW, central mean wavelength of 1.31 μm , and a spectral width of 50 nm was used. The sample arm viewing the animal side consists of an objective lens of numerical aperture 0.08 and was also fitted with a CCD camera. This allowed simultaneous viewing of the cortical surface with the introduction of visible light from an auxiliary laser source. The system has a depth resolution of 34 μm in free space and a spot size of 20 μm . The image acquisition rate was 2 Hz,

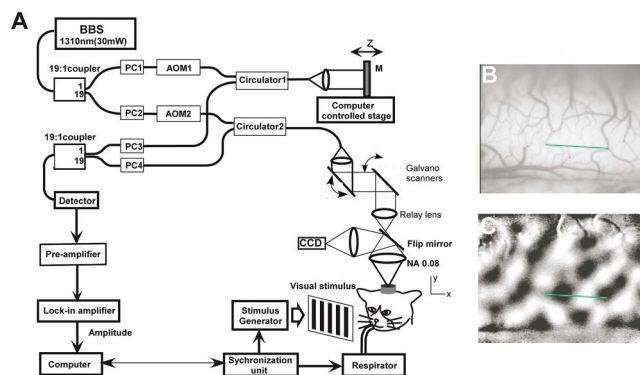


Fig. 1. (Color online) A, Schematic of the experimental system used. B, Exposed cortical surface of cat visual cortex and with C, an overlay of binarized activation map. BBS, broadband source; AOM, acousto-optic modulator; PC, polarization controller; M, mirror; O, objective lens. In C the dark and light patches represent, respectively, the activated regions for horizontal and vertical gratings, and the green lines indicate the region of the OCT scan. To reduce the respiratory fluctuation, data acquisition was done in synchronization with the heartbeat and respiration. The animal experimental protocol was approved by the Experimental Animal Committee of the RIKEN Institute, which follows the guidelines of the National Institutes of Health.

and each scan frame had 128×100 pixels over 1 mm transverse by 1 mm depth.

The fOCT experiments were conducted with anesthetized cats under artificial ventilation. The head of the animal was held tightly by attaching it to a metal rod. A stainless steel chamber was fixed onto the skull, and the inside of the sealed chamber was filled with agarose to keep the brain surface immobile. Figure 1B shows the exposed cortical surface with the clear blood vessels. The pupils of the eyes were dilated and fitted with contact lenses. The visual stimuli consisted of square-wave gratings presented in random on a CRT monitor. The stimuli consisted of five patterns with control or blank, horizontal (0°), vertical (90°), and oblique gratings (45° , 135°). A total of 40 trials were obtained for each stimulus. In a single trial, data acquisition was done for 8 s followed by a resting period of 5 s. Each trial period was 8 s with 2 s prestimulus and 6 s poststimulus durations. Stimulus duration was 2 s.

Prior to doing functional imaging with OCT, we performed OISI of the exposed cortical surface at a wavelength of 607 nm under identical stimulation conditions. OISI revealed that neurons responding to the same orientation are clustered and form an orientation map across the cortical surface. Figure 1C shows the binarized difference maps obtained when horizontal and vertical grating visual stimuli were presented to the cat. Dark and bright regions indicate the activated regions for horizontal and vertical gratings, respectively. An OCT x - z scan with an example as shown in Fig. 2A was done across the line indicated in the OISI map. The cortical surface border has been drawn manually, and the warm-colored regions indicate the scattering centers within the cortex. Following procedure had been applied to the OCT structural images before calculating the functional maps: (1) The scans were corrected for the misalignment of the surface position by use of a correlation-based procedure used previously [6]. (2) Pixel noise was removed with a smoothing filter of

window size $27 \mu\text{m} \times 21 \mu\text{m}$. Next, the ratio of the poststimulus over prestimulus scans for all four grating stimuli and the control condition where the control corresponds to blank screen condition was calculated as follows:

$$\gamma_s(x, d, t) = \frac{R_s^{\text{post}}(x, d, t)}{\sum_{\text{prescans}} R_s^{\text{pre}}(x, d, t)}. \quad (1)$$

Here R_s is the reflectivity at position (x, d) at time t . The superscripts *post* and *pre* indicate the poststimulus and prestimulus scans, respectively. The division operation removes the unchanging common variation and extracts only changes due to visual stimulation. Next, the ratio was averaged for all the scans obtained for each stimulus $\langle \gamma_s(x, d) \rangle$. Finally, the differential OCT signal $\langle \gamma_{\text{diff}}(x, d) \rangle$ was calculated as

$$\langle \gamma_{\text{diff}}(x, d) \rangle = \langle \gamma_{\text{grating}}(x, d) \rangle - \langle \gamma_{\text{control}}(x, d) \rangle. \quad (2)$$

With Eq. (2), by subtracting the differential OCT signal of the control, noise fluctuations such as respiration artifacts that were locked to the recording but not to the grating stimulus were removed. In this discussion, to make the comparison with OISI clearer and easier, we restrict ourselves mainly to the results obtained by calculating the difference $\langle \gamma_{\text{diff}}(x, d) \rangle$ obtained between two orthogonal gratings. The spatial map has been smoothed with a moving average filter of size around $100 \mu\text{m} \times 115 \mu\text{m}$.

Figure 2B shows the calculated fOCT maps obtained as a difference of the fOCT maps obtained for horizontal and vertical grating stimuli. Here, red and blue patches indicate the activation for horizontal and vertical grating stimuli, respectively. In this pseudo-colored map, red means more scattering change for horizontal than for the vertical. In the corresponding intrinsic map of Fig. 1C, black indicates more absorption change for horizontal than for the vertical grating stimuli. Hence the trends of increased absorption change and increased scattering change coincide. Here, in fOCT we assume there are only scattering changes. A pixel by pixel comparative t -test of the ratio calculated by Eq. (1) was done for the horizontal against the vertical grating stimuli. The tests revealed that the results obtained were statistically significant to within a 5% tolerance limit.

From the fOCT map shown, we can infer the following facts: (1) There is a discrete distribution of activation patches across depth and is stimulus specific. (2) In the very superficial region of less than 100 – $200 \mu\text{m}$, there are no activation patches indicating this superficial region may correspond to layer 1 where neurons are scarce. (3) In the regions deeper than 100 – $200 \mu\text{m}$, there exist several localized patches across depth showing no regular structure. (4) The localized patches extend up to the measured depth of around 1 mm.

To address whether the signals represent the regional difference of neural activity, we compared the intensity variation across the scanned line on the surface of OISI map with the integrated profile of fOCT. The integrated profile was calculated by inte-

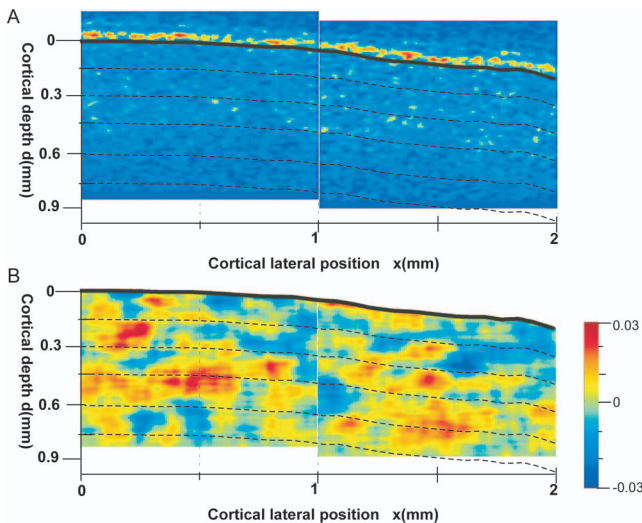


Fig. 2. Results of A, an OCT scan and B, a functional OCT map obtained across the line indicated in Figs. 1B and C. In B, the red and blue patches, respectively, represent the activated regions for horizontal and vertical gratings.

Table 1. Correlation Coefficients Between the Profiles Obtained by the OISI and fOCT Intensity Profiles Obtained from Different Scan Positions of a Single Cat under Two Different Stimulus Conditions

Position	0° −90°	45° −135°
1	0.58	0.65
2	0.32	0.29
3	0.24	0.39
4	0.89	0.77
5	0.407	0.539
6	0.566	0.559

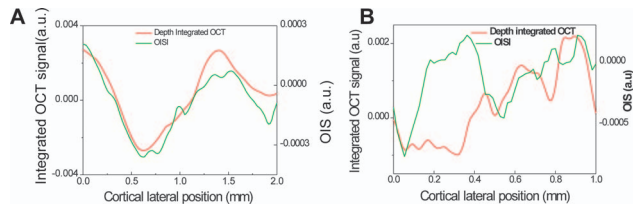


Fig. 3. Consistency of the OISI result with the integrated result of fOCT corresponding to correlation coefficients of A, 0.68 and B, 0.3. In A, the green line indicates the variation of OISI across the line indicated in Fig. 1C, and the red line is obtained by calculating the functional signal from integrating the OCT scans across the full scanned depth range of Fig. 2A. B is another example obtained from a different cat.

grating the OCT scans across the whole of the scanned depth range, followed by calculation of the differential OCT signal as described earlier in 1-D as in [6]. Here, the integration of the signal was done by summing up all the pixels across the direction of z to obtain a profile that varies across x . Figure 3A shows the result of such a comparison, with the red line indicating the integrated result and the green line indicating the OISI intensity variation across the respective lines for the example shown in Fig. 1C. A clear and remarkable agreement between the profiles could be seen corresponding to a correlation coefficient of 0.68. This indicates that the fOCT signal is indeed correlated with OISI and thus correlated with the neural-activity-evoked changes.

Experiments were done in 5 cats and found that a good correlation exists between the integrated fOCT signal and the intensity profiles obtained from intrinsic maps. Correlation coefficients vary in the range of 0.3 to 0.9. Table 1 gives the results of correlation coefficients for two different cases, namely 0°–90° and 45°–135° stimuli obtained from a cat under different scan positions. Correlation coefficients vary largely across both scan positions and stimulus conditions. To make the correlation relation clear, we have also conducted a comparative study between fOCT and electrophysiology, by which the neural activity is directly measured. We found a good correlation exists

between the two sets of results, which will be published elsewhere.

As an indirect way of monitoring the activity of neurons, OISI is one popular technique, and it can provide an indirect measure of the population activity. However, by its arrangement, the information obtained by the technique is integrated, and there is no way to discriminate the layer-specific information. By definition OCT could obtain structural information that is depth resolved. In this work, we have demonstrated that fOCT can become a potential tool in neuroimaging through monitoring dynamic changes in the neural system under stimulation.

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