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Optical coherence tomography (OCT) reveals depth-resolved dynamics during functional brain activation

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Abstract

Optical intrinsic signal imaging (OISI) provides two-dimensional, depth-integrated activation maps of brain activity. Optical coherence tomography (OCT) provides depth-resolved, cross-sectional images of functional brain activation. Co-registered OCT and OISI imaging was performed simultaneously on the rat somatosensory cortex through a thinned skull during forepaw electrical stimulation. Fractional signal change measurements made by OCT revealed a functional signal that correlates well with that of the intrinsic hemodynamic signals and provides depth-resolved, layer-specific dynamics in the functional activation patterns indicating retrograde vessel dilation. OCT is a promising new technology which provides complementary information to OISI for functional neuroimaging.

Keywords

Optical coherence tomography (OCT); Functional optical coherence tomography; Optical intrinsic signal imaging (OISI); Depth-resolved functional dynamics; Somatosensory cortex; Scattering change; Retrograde vessel dilation

1. Introduction

Optical imaging has become an important technique to investigate the neuronal and vascular responses to brain activation (Orbach et al., 1985; Grinvald et al., 1988; Villringer and Chance, 1997; Gratton and Fabiani, 2001; Franceschini et al., 2006; Hillman, 2007). Optical methods can offer both high spatial and temporal resolutions and are therefore particularly promising for measuring the hemodynamic, metabolic, and neuronal activity *in vivo*. Optical

intrinsic signal imaging (OISI) is a well-established optical imaging method which utilizes a CCD camera to map the clustered activation of neurons. OISI can provide highly sensitive measures of neuronal (Rector et al., 2001, 2005) and vascular (Frostig et al., 1990; Tso et al., 1990) responses to brain activation, and is currently being used extensively for study of the neuro-vascular relationship (Devor et al., 2003, 2005; Sheth et al., 2004, 2005). However, since CCD cameras integrate the back-scattered light from various depths, OISI cannot detect depth-resolved functional activation. To overcome this limitation, multiphoton microscopy has been used for functional neuronal imaging (Kleinfeld et al., 1998; Bacsikai et al., 2003; Chaigneau et al., 2003; Svoboda and Yasuda, 2006). In addition, new methods such as laminar optical tomography (LOT) have been developed for depth-resolved tomographic imaging (Hillman et al., 2004, 2007). Optical coherence tomography (OCT) is another promising method for depth-resolved imaging in highly scattering tissues such as the cerebral cortex. OCT is an emerging biomedical imaging method which can provide high-resolution, cross-sectional images in vivo and in real time (Huang et al., 1991; Schmitt, 1999). OCT enables the measurement of small, scattered signals over several orders of magnitude in dynamic range and can image deeper than multiphoton microscopy. Several groups have used OCT to study functional activation in neuronal tissues. Maheswari et al. demonstrated depth-resolved, stimulus-specific profiles during functional activation in the cat visual cortex (Maheswari et al., 2002, 2003), and showed a good agreement between the depth-integrated functional OCT signals and the OISI profiles (Rajagopalan and Tanifuji, 2007). They attributed these signals to variations in scattering due to localized structural changes such as capillary dilation and cell swelling. Lazebnik et al. (2003) demonstrated scattering changes corresponding to fast and slow signals triggered by action potential propagation in the sea slug abdominal ganglion. Satomura et al. (2004) observed the delayed swelling of the cortical surface in the somatosensory cortex following the electrical stimulation of the rat hind paw. Bizheva et al. (2006) and Srinivasan et al. (2006) reported depth-resolved functional OCT signals in the retina in response to visual stimulation. Recently, Wang et al. (2007) and Wang and Hurst (2007) demonstrated a new Fourier Domain OCT method termed optical angiography (OAG) to visualize the three-dimensional cerebral microcirculation of adult living mice through the intact cranium. Other groups have also explored low coherence interferometry methods for measurement of functional retinal activation (Yao et al., 2005) as well as nerve axon displacement (Akkin et al., 2004; Fang-Yen et al., 2004). In a previous study, we presented preliminary results using OCT to measure subsurface scattering changes due to functional activation in the rat somatosensory cortex (Aguirre et al., 2006). The rat somatosensory cortex is a well-established model system for neurophysiology, and these results demonstrated that OCT can provide high-resolution, cross-sectional measurement of functional hemodynamic response to electrical stimulation in the rat cortex. OCT data correlated well with simultaneously acquired OISI data. This allowed comparison of OCT results with extensively studied intrinsic optical signals. Our previous results suggested that OCT could have an important role in future studies of the functional neurovascular response. In this paper, we perform spatial and temporal correlations of OCT and OISI signals in order to gain further insights for interpretation of OCT results.

2. Materials and methods

2.1. Experimental system

Simultaneous OCT and OISI were performed on the rat somatosensory cortex. Fig. 1(A) shows the schematic of the co-registered OCT and OISI system. The OCT system used time domain detection and consisted of a balanced interferometer configuration, a rapid linearly scanning reference delay line operating at 1140 Hz, and logarithmic demodulation. The light source was a Nd:Glass femtosecond laser generating ~100 femtosecond pulses at 1060 nm

center wavelength. The laser spectrum was broadened by a single mode fiber (HI-1060) to generate a bandwidth of 40 nm, which yields an axial resolution of 18 μm in air (or 13 μm in tissue). The optical bandwidth was deliberately reduced compared with previous studies using this light source (Bourquin et al., 2003) in order to provide a larger resolution voxel for enhanced integration of small signals. The OCT sample arm included a collimating lens followed by a pair of galvanometer scanners to allow precise two-dimensional control of the OCT scan orientation. The image acquisition rate was 3 Hz, and each frame had 380×1000 pixels over a $3 \text{ mm} \times 2.8 \text{ mm}$ (transverse $\times$ depth) cross-section. The scanned OCT beam was focused by a near-infrared achromat lens ($f = 60 \text{ mm}$) and then redirected onto the specimen by a dichroic hot mirror which reflected the near-infrared while passing visible light as shown in Fig. 1(B). The OCT focal spot had a $1/e^2$ diameter of $\sim 36 \mu\text{m}$. The measured system sensitivity from a single high-reflector was $>95 \text{ dB}$ with 14 mW incident power on the sample. The role of the dual-balanced detector is to add the interference signals and to subtract the excess noise from the light source in order to enhance the signal-to-noise ratio. The demodulated OCT signal was recorded by the computer (computer #1) with a 12-bit, 5 MHz analog to digital (A/D) converter.

OISI was performed using a single wavelength (570 nm using Hg:Xenon illumination and a band-pass filter) which corresponds to an isosbestic point for oxygenated and de-oxygenated haemoglobin (Malonek et al., 1997). Therefore, cortical reflectivity at 570 nm provided a measurement of changes in total haemoglobin concentration, which is proportional to total blood volume if hematocrit is assumed constant. Reflected visible light from the cortex was collected with a camera lens and imaged onto a high sensitivity CCD (Coolsnap fx, Roper Scientific, 1300×1030 pixels, 12 bit), which was read by another computer (computer #2 in Fig. 1). During the stimulation experiments, an additional 570 nm band-pass filter (10 nm bandwidth) was placed in front of the CCD lens to reject near-infrared light transmitted by the dichroic. The CCD field of view (FOV) and the OCT scanning region were co-registered prior to each experiment by removing this band-pass filter and observing the OCT scanning beam on the CCD camera. The depth of focus of the OISI system is approximately 0.4 mm.

Fig. 2 shows the co-registered OCT and OISI images. OISI is used to localize and characterize the functional activation region for placement of the OCT beam. OCT cross-sectional images clearly delineate the thinned skull and the meningeal layers separating the skull from the underlying cortex. Cortical surface vessels can also be identified. Three-dimensional OCT volume data, as shown in Fig. 2C and D, reveals the blood vessel networks and facilitates co-registration of the OCT imaging regions with the OISI images.

Temporal synchronization of the OCT and OISI recordings was achieved using a third computer (computer #3 in Fig. 1). The digital to analog converter card on this computer drove the electrical stimulus generator and served as the master clock for the experiment by simultaneously acquiring relevant synchronization signals on a timebase locked to the stimulus drive. The signals acquired included the stimulus drive itself, a separate trial initiation pulse which denotes the beginning of a stimulus sequence, the CCD camera exposure, the OCT image frame synchronization, and a blood pressure trace used for monitoring animal physiology.

2.2. Animal preparation

Five (5) male Sprague–Dawley rats (250–350 g) were initially anesthetized with 2–2.5% isoflurane and immobilized in a stereotactic frame before beginning the cranial preparation. A tracheotomy was performed to allow artificial ventilation, and cannulas were inserted in the femoral artery and vein. The animals were artificially ventilated with 1.5–2.0% isoflurane, 70% N_2O and 30% O_2 . Body temperature was maintained near 37 $^\circ\text{C}$ with a heating blanket. An area of skull overlying the primary somatosensory cortex on the

contralateral side to the stimulated forepaw was thinned with a dental burr until transparent. The skull thickness was measured by OCT to be 100–200 μm . A barrier of petroleum jelly was built around the thinned skull and filled with mineral oil to reduce surface reflection from the skull. Subsequently, isoflurane was discontinued, and anesthesia was maintained with a 50 mg/kg bolus of α -chloralose, followed by continuous intravenous infusion at 40 mg/kg/h. Rectal temperature, ECG and expired CO_2 were continuously monitored during both surgery and OISI/OCT experiments. All experiments were conducted according to protocols approved by the institutional animal care committees at the Massachusetts Institute of Technology and the Massachusetts General Hospital.

2.3. Stimuli and data acquisition

Forepaw stimulation was performed using 20 s trials. Each trial consisted of a 1 s pre-stimulus period, followed by 4 s of stimulation with ~ 1.8 mA, 300 μs pulses at 3 Hz using a stimulus isolation unit. The stimulus amplitude was chosen to be just below the animal's twitch threshold as determined by palpation. A 15 s post-stimulus period was then used to allow full recovery to baseline. The trial was repeated 60 times during data acquisition over a 20 min period. Prior to data processing, digitized OCT signals were converted from a log scale to a linear scale.

2.4. Data analysis

Each OCT cross-sectional image was first spatially averaged by a 3×3 window to reduce the speckle. The purpose of pixel averaging is to reduce the noise while maintaining the image voxel resolution. The OCT image size in our study is $3 \text{ mm} \times 2.8 \text{ mm}$ with 380×1000 pixels. So the individual pixel size is $8 \mu\text{m} \times 2.8 \mu\text{m}$. A 3×3 pixel window will give $24 \mu\text{m} \times 8.4 \mu\text{m}$. This size is less than the focus volume $36 \mu\text{m} \times 13 \mu\text{m}$. If we choose a larger window, although the noise will be reduced further, the image resolution will also deteriorate. Fig. 3 shows the effect of different window sizes (1×1 , 3×3 , and 7×7 , respectively). As shown in (A–C), increasing the window size smoothes the image. As expected, averaging attenuates the overall signal intensity, however the patterns of the response (positive and negative) remain. This is also revealed by the histogram in (D).

According to previously published literature (Maheswari et al., 2003), the functional OCT signals were calculated as follows:

1. The ratio of the post-stimulus scans over pre-stimulus baselines was computed for each individual trial:

$$\gamma(x, z, t) = \frac{R_{OCT}^{post}(x, z, t)}{\left| \sum_{t=1}^N R_{OCT}^{pre}(x, z, t) \right| / N} \quad (1)$$

where R_{OCT} is the reflectivity at position (x, z) at frame t of each trial. The superscript *post* and *pre* indicate the post-stimulus and pre-stimulus scans, respectively. N denotes the number of frames acquired during the pre-stimulus period. The division operation removes the unchanging common variations due to morphological structures and extracts only the changes due to forepaw stimulation.

2. Fig. 3(E) shows the non-averaged OCT signal $\gamma(x, z, t)$. The signal variations arising from the stimulation are clearly visualized. The Fourier transform of the OCT signal (F) reveals the stimulation frequency (0.05 Hz) and its harmonics. Artifacts from respiration are attenuated because the skull was not opened and the animal is in a stereotactic frame. OCT signals were then averaged across trials ($\langle \gamma(x, z, t) \rangle$) to

further reduce the effects of physiologic noise in the measurements. The final differential OCT signals $\langle \gamma_{OCT_diff}(x, z, t) \rangle$ were obtained by:

$$\langle \gamma_{OCT_diff}(x, z, t) \rangle = \langle \gamma(x, z, t) \rangle - 1 \quad (2)$$

Each time point of the final resulting 20 s response to stimulation consisted of an average of 60 independent samples.

The functional OISI signals were calculated in a similar way. The reflectivity recorded by CCD camera $R_{OISI}(x, y, t)$ was plugged into the previous equations to calculate the final trial-averaged differential OISI signal time-courses $\langle \gamma_{OISI_diff}(x, y, t) \rangle$.

3. Results

3.1. Co-registration of functional OCT and OISI

Fig. 4 illustrates the co-registered OISI and OCT imaging in the region of functional activation. The functional signal was computed as a ratio of the reflectance at each time point to the mean reflectance in the pre-stimulus period and is therefore representative of a percent signal change from the baseline. Fig. 4(A) shows a representative ratio OISI image from the time window around the peak of maximal activation ($t = 4-6$ s). The region of functional activation corresponding to forepaw stimulation is clearly identified. A decrease in the reflectance at 570 nm (denoted by the blue region) corresponds to increased concentration of haemoglobin. Fig. 4(B) plots the time-course of the averaged fractional reflectivity changes in the region denoted by the dashed box in Fig. 4(A). The decreased signal intensity during the stimulation period arises from the stimulus-induced increase in blood volume absorbing more light. The time-course and maximum fractional signal changes (~ 0.03) agree with previously published results (Malonek et al., 1997; Devor et al., 2005; Dunn et al., 2005).

Fig. 4(C) shows the functional OCT image from the time window around the peak of maximal activation ($t = 4-6$ s). The OCT scan location is shown over the region of activation indicated by the red arrow in Fig. 4(A). Similar to OISI fractional change map, the OCT fractional change map was computed by normalizing all time points to the baseline pre-stimulus period. The OCT functional map reveals a localized area of activation with both positive and negative changes in the cortex. The temporal sequences of activation for the representative regions of interest (ROI) in Fig. 4(C) are plotted in Fig. 4(D). Size and shape of the boxed areas for time-course analysis were selected based upon visual perception of the boundaries of the localized change. The OCT functional signal time-courses reveal clear increases and decreases that deviate from baseline, reach a peak near the cessation of the stimulus, and then gradually return to baseline. The time-course of OCT signal changes correlates well with the co-registered OISI time-course as shown in Fig. 4(B). It is noted that the typical fractional changes in functional OCT (0.1–0.5) are larger than OISI, and the noise is also larger due to the enhanced sensitivity using OCT coherence detection. Nevertheless, the observed changes are statistically significant ($p < 10^{-7}$). Fig. 4(E) illustrates the map of p values showing the statistical significance of the fractional OCT changes from the baseline. In addition, the lateral line profiles of OISI signal changes and the depth-integrated OCT signal changes are plotted in Fig. 4(F), showing the spatial correspondence of OCT and OISI signals.

All five animals showed similar responses. Representative responses from different ROIs (one positive and one negative) for each animal are shown in Fig. 5.

3.2. Spatial-temporal evolution of functional activation

Fig. 6 shows the temporal sequence for the functional OCT images. The spatially distinct activation regions evolve during the stimulation period, reach the peak around the end of stimulation, and gradually fade back towards baseline during the recovery period.

Fig. 7 presents the results of functional OCT activation and control experiments. Fig. 7(A) shows the localization of the activation area from OISI during the contralateral forepaw stimulation. Two OCT scans were denoted by arrows “1” and “2” with scan “1” imaging across the activation area and scan “2” imaging outside the region of activation. Fig. 7(B) shows the functional OCT image at time window 4–6 s over the activation region denoted by arrow “1”. Localized regions of activation are visualized. Both positive and negative changes corresponding to the stimulation pattern are prominent. In contrast, as shown in Fig. 7(C), the functional OCT image at time window 4–6 s over the scan denoted by arrow “2” does not reveal any significant activation region. Small signal changes are observed which agree with the small changes in Fig. 7(A). Fig. 7(D) shows the functional OCT image at a time window 4–6 s over the scan denoted by arrow “1”, but with the stimulation was performed on the ipsi-lateral forepaw. There is no distinct activation region observed in the OCT image. Paired *t*-test between (B and C), and (B and D) are performed. In both cases, the difference is statistically significant ($p < 10^{-7}$).

3.3. Profile analysis for functional OCT signals

To further understand the characteristics of the positive and negative signals, detailed profile analyses were performed on the functional OCT images. Fig. 8(A) illustrates the axial (x1 and x2) and transverse (z1 and z2) lines on OCT structural and functional images used to perform profile analysis. The functional OCT image is taken from the time window around the peak of maximal activation ($t = 4–6$ s). Fig. 8(B–E) plot both differential functional signal (left panels) and the corresponding profiles at both baseline (pre-stimulation) and during the stimulation period (right panels) along the lines indicated by x1, x2, z1, and z2, respectively. First, distinct anatomical features such as the edges of the skull and the cortical surface can be appreciated in Fig. 8(B–D) (denoted by the black arrows). The pre- and post-stimulation plots indicate that the signals from skull edge and cortical surface remain stable during the stimulation process. This confirms that the OCT signal changes are not from global motion artefacts. Second, there exist two distinct patterns for OCT signal changes. For scenario “1”, the identified peaks in the scattering intensities are either increased or decreased. This scenario is illustrated in the enlarged inset on the right panel of Fig. 8(B). For scenario “2”, the center of a scattering peak is shifted, as indicated by the enlarged inset on the right panel of Fig. 8(C). Both scenarios can result in positive and negative changes in functional OCT signals. A typical OCT A-scan profile might contain the combination of both patterns.

3.4. Integrated OCT signals

In order to compare with OISI results, we analyzed the integrated OCT signals over the imaging region. In the OISI images (see Fig. 4A and B), the response curve is averaged over the region of interest (ROI) denoted by the dotted black box. This ROI is selected by the visual inspection of activation areas (blue color), in a way similar to the intensity threshold. In contrast, in the OCT cross-sectional images (see Fig. 4C), there are two prominent differences. First, both positive and negative signals are present. Second, compared to the single-location, wide-spread OISI activation region, the OCT activation regions are discrete and non-continuous local foci. Therefore, we applied a statistical significance threshold to select the activation ROI. Basically, for each pixel, the measurements in the baseline (0–1 s) and activation window (4–5 s) are collected as two groups (baseline and activation). A Student's *t*-test is performed on these two groups to test the null hypothesis that the

activation signals and baseline signals are the same. Then the pixels with a significance level $\alpha < 0.05$ are chosen since the null hypothesis is rejected for those pixels. Fig. 9(A) shows an activation image where only the ROIs with significance level $\alpha < 0.001$ are selected. In Fig. 9(B), the time course of the averaged positive signals, negative signals, and the summed signals are plotted. From the plot, both the positive and negative signals increase in magnitude during the activation period, and recover after the cessation of the stimulation. The temporal trend of signal variation agrees well with the stimulation pattern. The summed signal shows a net positive change during the activation, which indicates the increasing of overall OCT back-scattering signals. Fig. 9(B) also shows the corresponding OISI time course indicating well-matched temporal responses.

3.5. Depth-dependent activation pattern in OCT

Compared to depth-integrated OISI, OCT can resolve functional activation at different depths. The temporal properties of functional OCT time-courses at different depths (z) were analyzed. Fig. 10(A) shows a structural OCT image with two regions indicated by the red (a large vessel on the surface of cortex) and blue (in the cortex) boxes. The time-courses of functional OCT for these two regions are plotted in Fig. 10(B). The blue curve shows a time-course that corresponds well with the stimulation pattern. In contrast, the red curve indicated a time delay of approximately 2 s in both the onset and the peak of the response with respect to the stimulation pattern. Since the averaged OISI time-course matches well with the stimulation as indicated by Fig. 4(B), we choose the averaged OISI time-course as the reference and cross-correlated it with the functional OCT time-courses at each individual pixel:

$$C(d) = \frac{\sum_i \left[\left(\gamma_{OCT_diff}(i) - \bar{\gamma}_{OCT_diff} \right) \times \left(\gamma_{OISI_diff}(i-d) - \bar{\gamma}_{OISI_diff} \right) \right]}{\sqrt{\sum_i \left(\gamma_{OCT_diff}(i) - \bar{\gamma}_{OCT_diff} \right)^2} \sqrt{\sum_i \left(\gamma_{OISI_diff}(i) - \bar{\gamma}_{OCT_diff} \right)^2}} \quad (3)$$

where $OCT_diff(i)$ and $OISI_diff(i)$ ($i = 0, 1, 2, \dots, N-1$) are series of functional OCT and OISI signals at each time point, respectively. $\bar{\gamma}_{OCT_diff}$ and $\bar{\gamma}_{OISI_diff}$ are the means of the corresponding series and d represents the delays ($d = 0, 1, 2, \dots, N-1$). To account for the change in magnitude of OISI signals and OCT signals for individual pixels, both data are first normalized for calculating the cross-correlation (i.e. the auto-correlation of the data series at delay 0 is equal to 1).

The positive functional OCT signal trend will result in a negative correlation coefficient with OISI signal, and the negative functional OCT signal trend will give positive correlation coefficient. In the analysis, we take the absolute value of the correlation coefficient, since it indicates the degree of match between those two curves. We selected the pixels with correlation coefficients > 0.7 (which have good correlation with the OISI signals) and calculated the shift of the correlation peak, which is an indication of time lag between the functional OCT and OISI time-courses. Fig. 10(C) shows the spatially resolved time lags overlaid with the structural OCT image. Different colors indicate different time lags with respect to the averaged OISI time-course. Positive values indicate the OCT time courses are delayed compared to the OISI time course, while negative values indicate the OCT time courses precede the OISI time course. Fig. 10(D) displays the histogram of the time lags. The majority of the cortex region shows the functional OCT response closely follows the OISI response (with time lag within ± 1 s), while there are some regions near the surface of the cortex exhibiting delayed responses (with time lag > 1 s). Fig. 10(E) plots the averaged

temporal response of all the pixels with different time lags, including $[-1.5 \text{ s}, -0.5 \text{ s}]$, $[-0.5 \text{ s}, 0.5 \text{ s}]$, $[0.5 \text{ s}, 1.5 \text{ s}]$, $[1.5 \text{ s}, 2.5 \text{ s}]$, and $[2.5 \text{ s}, 3.5 \text{ s}]$. The temporal positions of their peak response from these time courses are different, indicating different dynamics.

Fig. 11(A) shows another example of the time lag image of the functional OCT activation with respect to the OISI image. The averaged time lag for a given depth from the skull surface is plotted versus depth in Fig. 11(B). There exists a two-phase behaviour for the averaged time lag. In the first 200 μm of the cortex (corresponding to the region 100–300 μm from the skull surface), the averaged time lag decreases as the depth increases (characterized by slope S1). From 200 μm and deeper into the cortex (corresponding to $>300 \mu\text{m}$ from the skull surface), the averaged time lag does not change significantly with the depth (characterized by slope S2). We analyzed the spatial-temporal correlation between OCT and OISI on 9 independent runs (each run include 60 stimulation trials) from 5 rats, and the distribution of S1 and S2 are indicated in Fig. 11(C). The averaged $S1 = -4.1 \pm 1.3 \text{ ms}/\mu\text{m}$, while the averaged $S2 = -0.18 \pm 0.35 \text{ ms}/\mu\text{m}$. There is statistically significant difference between S1 and S2 ($p < 0.0001$).

4. Discussion

4.1. OCT signal characteristics

In our experiments, OCT measures the depth-dependent scattering changes during the functional brain activation. The scattering signals measured here are different from the absorption changes measured by OISI, and also do not directly indicate blood flow values (however, Doppler OCT (Chen et al., 1997), an extension of OCT, is sensitive to blood flow). During the stimulation, a robust and highly localized OCT response is observed which is well-correlated with the stimulus and the hemodynamic response measured by OISI. The response is weak outside the region of hemodynamic activation identified by OISI, and is absent when the ipsi-lateral forepaw is stimulated. Furthermore, the blood pressure traces reveal no signs of sympathetic nervous system response, which could suggest shock-induced motion artefacts. The line-profile analysis in Fig. 8 confirmed that there is no shift in prominent morphological landmarks, such as the skull surface, which further excludes the possibility of artefacts from global motion. The data presented here is analyzed without requiring any spatial registration. The stereotaxis reduces the movement of skull dramatically, as seen by the unchanged edge of skull shown in the original Fig. 8(B–C). However, a spatial registration using cross-correlation is expected to further improve the result.

In the structural images of the cortex, there are apparent “shadows” which run perpendicularly to the cortical surface (e.g. Figs. 2B, 9A and 10A). This “shadowing” effect is primarily due to the strong forward scattering of the red blood cells in the large vessels. This effect results in the loss of OCT signal under the vessels and therefore will also affect the functional results. And since the OCT signal is acquired in the axial direction, the deeper activity could be influenced by the shallower regions. Therefore, we should be careful in data interpretation. One possible approach is to analyze the temporal response. If the temporal responses from shallower and deeper regions are different, it might suggest that the deeper activations are not due to the influence from the more superficial activation.

To understand the exact etiology of the OCT functional signal will require further investigation. The presence of highly localized regions of activation in the cortex suggests that localized swelling or vascular dilation, rather than simply bulk brain swelling, is a potential contributor. For example, in the case of Fig. 8(C), the loci of the scattering peak shifted about 3 pixels in the z direction, which corresponds to approximately 8.5 μm . This is consistent with the observation by previous researchers regarding pial arteriole dilation

during somatosensory stimulation (Ngai and Winn, 2002). There is a pial vessel at axial pixel position ~365 from the OCT structural image Fig. 8A (left), indicated by the black arrow. Furthermore, the possibility exists that localized scattering increases and decreases reflect local changes in red blood cell density or hematocrit, an observation that would have some support in previous literature (Kleinfeld et al., 1998). Future work will focus on more comprehensive characterization of the response, including the use of techniques such as high-speed OCT to provide three-dimensional activation patterns, and Doppler OCT or optical angiography to measure the blood flow changes during the activation (Wang et al., 2007). Recent advances in Fourier-domain OCT enables 10–100-fold increase in imaging speed (Choma et al., 2003; de Boer et al., 2003; Leitgeb et al., 2003). However, the basic nature of OCT signals and the information content are essentially the same. Fig. 12 shows a representative results obtained using Fourier-domain OCT imaging on the rat cortex. The details of the imaging system are described elsewhere (Andrews et al., 2008). Larger imaging field of view is achieved by the Fourier-domain OCT due to the enhanced imaging speed, while the activation patterns are consistent with the results obtained with time-domain OCT.

The presence of positive and negative functional OCT signals and the speckled appearance of the response under differential signal analysis implicate swelling mechanisms as an important contributor. Compared to OISI signals, which are integrated over depth, OCT signals show a larger magnitude of fractional changes, but are more likely to be subject to speckle shifts. The depth-integrated OCT signals showed in Fig. 7 reveal net positive changes during the stimulation. This might be related to the changes in density of red blood cell (RBC) as previously reported by Kleinfeld et al. (1998).

4.2. Depth-dependent signal dynamics

Compared to depth-integrated OISI, OCT has the unique advantage of resolving the functional response at different depths. This capability is important when investigating the dynamic responses at different cortical layers. In our study, we observed that the functional response varied with depth (Figs. 10 and 11). The OCT response from deeper (>200 μm) cortical layers synchronizes with the OISI response with an average time lag of approximately 0 s. In contrast, for more superficial cortical regions (<200 μm), we observed a delayed response with respect to the stimulation pattern, and there is a gradual increase in time lag when the depth decreases towards the cortical surface. We hypothesize that this phenomenon is consistent with retrograde (upstream) vessel dilation. The first 200 μm in the cortex corresponds to layer 1, where neurons are scarce, and is verified by the representative histology of rat cortex shown in Fig. 11(D). The functional response from the deeper layers is transmitted upstream to the surface vessels through retrograde vessel dilation (Girouard and Iadecola, 2006), therefore there exists a depth-dependent time delay in the response. In our experiment, we observed a consistent response in those 5 animals and the statistics is summarized in Fig. 11(C). The averaged propagation speed (1/S1) of 0.27 ± 0.11 mm/s, which agrees with previously reported retrograde dilation speed of ~0.3 mm/s in cerebellar cortex (Iadecola et al., 1997). Fig. 10(B) indicates there is a ~2 s time delay between the responses from the capillary (blue box) and the arterioles (red box). This time lag is consistent with an earlier observation by Maloney et al. (1997) who report a 1–2 s time lag between the blood volume increase (measured by OISI) and the cerebral blood flow increase (measured by laser Doppler flowmetry). Our current study does not implement Doppler OCT to measure the blood flow, while further studies using Doppler OCT methods promise to provide a quantitative assessment of changes in vascular flow with respect to depth and better understanding of the physiology of neurovascular coupling.

5. Conclusion

We presented depth-resolved functional OCT imaging of the neurovascular response to somatosensory stimulation in the rat. The time-course of the OCT response correlates well with OISI, suggesting that the OCT signal may reflect changes in hemodynamics. OCT signals include both positive and negative OCT functional changes. We also observe layer specific dynamic responses in the OCT signals which might indicate retrograde vessel dilation. Understanding the exact etiology of the OCT functional signal will require additional investigation. With further development, OCT has the potential to become a new method complements OISI for basic and applied neuroscience research.

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Fig. 1.
 (A) Schematic of co-registered optical coherence tomography (OCT) and optical intrinsic signal imaging (OISI) system. TIA: Transimpedance amplifier (TIA). (B) Photograph showing the OCT sample arm combined with CCD field of view through the dichroic hot mirror.

Fig. 2.

Precise co-registration of OCT imaging to the region of functional activation. (A) The OCT scan is directed across the region of interest (indicated by blue double-head-arrow) as measured with OISI. Anatomical features such as blood vessels (V) can be visualized in OISI and can be used to co-register with OCT image. (B) Structural OCT image reveals high-resolution visualization of the skull (S), surface vasculature (V), and meningeal layers, including the dura mater (D) and arachnoids (A). Cortex region (C) can also be visualized. (C) Three-dimensional rendering of the volume acquired by OCT. (D) En face OCT image reveals the characteristic blood vessel network which can be used to register the OCT imaging volume with OISI.

Fig. 3.

Effects of different averaging window sizes. (A) 1×1 ; (B) 3×3 ; (C) 7×7 . The histograms of the signals resulting from these three averaging windows are shown in (D). Bar: $500 \mu\text{m}$. (E) Non-averaged OCT raw signal (normalized to baseline) trace showing functional responses (blue curve) corresponding to the stimulation trial (beginning of each trial is indicated by the red curve). (F) The Fourier transform of the signal trace revealing the stimulation frequency (0.05 Hz, indicated by the red arrow) and its harmonics. The inset shows an enlarged view (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 4.

Representative results of OISI and OCT imaging of functional activation. (A) En face functional activation map from OISI with co-registered OCT scan indicated by the red arrow. (B) The averaged time-course for OISI over the region denoted by dashed box in (A) with stimulation period shaded grey. (C) Functional OCT image superimposed with the structural image. In order to simultaneously display these two images, we threshold the functional OCT signals to those with responses $> 5\%$ (or $< -0.5\%$). (D) Representative functional OCT time-courses for regions of interest specified by solid arrow (positive changes) and hollow arrow (negative changes). (E) Map of p values showing the statistical significance of the fractional OCT changes from the baseline. (F) The lateral line profile of OISI signal changes and the depth-integrated OCT signal changes showing the spatial correspondence of OCT and OISI signals. The OISI and OCT signal changes are scaled to overlap in order to provide better comparison.

Fig. 5.

Representative responses from ROIs on five different animals. Each color represents the results from one animal. Both positive (solid line) and negative (dashed line) responses are presented. Although temporal location and intensity of the peak varied slightly from animal to animal, in general, the response corresponded well with the stimulation pattern.

Fig. 6. Spatial-temporal evolution of functional OCT signals in the rat cortex. A fractional change map demonstrates the presence of positive (warm colors) and negative (cool colors) changes in OCT signals during stimulation. Functional OCT images at each individual temporal point ($t = 1\text{--}15\text{ s}$) reveal the presence of highly localized regions of activation in the cortex that persist throughout stimulation. Bar: $500\text{ }\mu\text{m}$.

Fig. 7.

(A) Localization of activation area in OISI. Two OCT scans are denoted by arrows “1” and “2”. (B) Functional OCT image at time window 4–6 s over the activation region denoted by arrow “1” during the stimulation of the contra-lateral forepaw. (C) Functional OCT image at time window 4–6 s over the scan denoted by arrow “2” during the stimulation of the contra-lateral forepaw. The activation signal is significantly less than in region “1”, which agrees with the OISI signals in (A). (D) Functional OCT image at time window 4–6 s over the scan denoted by arrow “1” during the stimulation of the ipsi-lateral forepaw. Note that no activation signal is detected.

Fig. 8.

Axial and transverse line profiles of functional OCT signals. (A) Position of the axial lines (x1 and x2) and transverse lines (z1 and z2) on OCT structural (left) and functional (right) images. (B) Left: Plot of differential functional signal along line x1. Right: Plot of raw linear signals at both baseline (pre-stimulation) and during stimulation period along line x1. Inset shows enlarged ($\times 1.5$) view of region denoted by "1". Black arrow indicates the edge of skull. (C) Left: Plot of differential functional signal along line x2. Right: Plot of raw linear signals at both baseline (pre-stimulation) and during stimulation period along line x2. Inset shows enlarged ($\times 2$) view of region denoted by "2". Black arrow indicates the edge of skull. (D) Left: Plot of differential functional signal along line z1. Right: Plot of raw linear signals

at both baseline (pre-stimulation) and during stimulation period along line z1. Black arrow indicates the edge of skull. (E) Left: Plot of differential functional signal along line z2. Right: Plot of raw linear signals at both baseline (pre-stimulation) and during stimulation period along line z2.

Fig. 9.

(A) The functional OCT image (red-blue color scale) at time window 4–6 s with significance level $\alpha < 0.001$ superimposed with the OCT anatomical image (grey scale); (B) plots of positive, negative, and summation OCT signals at regions with significance level $\alpha < 0.001$. Note that a net positive change occurs during the activation period. The time course of OISI signal ($\times 10$) is also included to show the temporal correspondence (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 10.

(A) Structural OCT image with two regions indicated by red and blue boxes. (B) The time-courses of the functional OCT for these two regions. (C) Spatially-resolved time-lags overlaid with structural OCT image. Different colors indicate different time-lags with respect to the averaged OISI time-course. (D) The histogram of the time-lags. (E) Temporal plots of averaged signal responses with time lags at different windows (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 11.

(A) Structural OCT image overlaid with the time-lags with respect to the averaged OISI time-course at different activation regions. The time-lags are color-coded (unit: second). (B) Plot of the averaged time-lags at the same depth (from the skull surface) versus the depth. Two distinct regions are indicated by different slopes (S1 and S2). (C) Box plot of the S1 and S2 from 9 independent runs on 5 rats showing statistically significant difference ($*p < 0.0001$). (D) Histology of rat somatosensory cortex indicating layered structures (layer I, II, III are specified).

Fig. 12.

Swept-source OCT image of functional activation. (A) Corresponding OISI image showing activation area (denoted by blue color); (B) En face OCT image; (C) Cross-sectional OCT imaging showing regions of functional activations. OCT image in (C) is acquired along the plane indicated by the red lines in both (A) and (B) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).