Published in final edited form as:

Opt Lett. 2012 April 15; 37(8): 1388–1390.

Cerebral blood flow imaged with ultrahigh-resolution optical coherence angiography and Doppler tomography

Hugang Ren, Congwu Du, and Yingtian Pan*

Department of Biomedical Engineering, Stony Brook University, Stony Brook, New York 11794, USA

Abstract

Speckle contrast based optical coherence angiography (OCA) and optical coherence Doppler tomography (ODT) have been applied to image cerebral blood flow previously. However, the contrast mechanisms of these two methods are not fully studied. Here, we present both flow phantom and *in vivo* animal experiments using ultrahigh-resolution OCA (μ OCA) and ODT (μ ODT) to investigate the flow sensitivity differences between these two methods. Our results show that the high sensitivity of μ OCA for visualizing minute vasculature (e.g., slow capillary beds) is due to the enhancement by random Brownian motion of scatterers (e.g., red and white blood cells) within the vessels; whereas, μ ODT permits detection of directional flow below the Brownian motion regime (e.g., laser-induced microischemia) and is, therefore, more suitable for brain functional imaging.

Cerebral blood circulation is essential to preserve the function of a living brain. Visualization and quantification of cerebral blood flow (CBF) can greatly advance our understanding of cerebral microcirculation and hemodynamics. Recent technological advances in OCT-based flow imaging techniques have evolved to two different approaches, i.e., optical coherence angiography (OCA) and Doppler tomography (ODT) for vasculatural visualization and quantitative imaging of CBF *in vivo*. OCA extracts comprehensive vascular contrast based on image processing methods to separate apparent flows from the background phase noise, including phase-based approach such as optical micro angiography (OMAG) [1] by Hilbert analysis in the lateral direction, Doppler variance method [2] by characterizing the broadening of the Doppler spectrum, frame subtraction method [3] by high-pass filtering in the slow lateral direction, and speckle variance method [4] by analyzing the intensity variance across sequential cross-sections. Meanwhile, ODT measures the Doppler phase change induced by moving scatterers (e.g., red and white blood cells) to enable quantitative CBF imaging. In addition to phase subtraction method (PSM) [5], which measures the Doppler phase shift between adjacent A-scans, several new algorithms have been reported to enhance flow detection sensitivity, including joint spectral- and time-domain method [6] with 2D fast Fourier transform (FFT) analysis, digital frequency ramping method [7] to circumvent the need of hardware modification for flow quantification, volumetric flow imaging [8] by Hilbert analysis to remove background phase noise, and dual-beam approaches [9,10] to enable detection of both slow and fast flows. However, it is known that the vascular turnouts provided by speckle contrast OCA surpass those detected by ODT, especially for capillary beds. In this letter, we present experimental results on both flow phantom and *in vivo* animal (mouse brain following laser-induced micro ischemia)

studies to elucidate the mechanistic differences between these two methods for CBF detections.

The imaging platform used in this study—combining 3D ultrahigh-resolution OCA (μ OCA) and ODT (μ ODT)—involved modification of previously reported ultrahigh-resolution spectral-domain OCT (μ OCT) setup [11], in which a sub-8-fs laser was used for ultra-broadband illumination ($\lambda = 800$ nm, $\Delta\lambda \approx 128$ nm) to a 2×2 broadband fiber optic Michelson interferometer. Its reference arm was connected to a grating-lens-based optical delay line (together with a prism pair in the sample arm) to compensate the dispersion mismatch and maximize the bandwidth (e.g., $\Delta\lambda_{cs} = 154$ nm) of the cross-spectrum ($S_{cs}(\lambda) \equiv [S_s(\lambda) \cdot S_r(\lambda)]^{1/2}$) between the sample and reference arms ($S_s(\lambda)$, $S_r(\lambda)$: sample and reference power spectra), so that an axial resolution (i.e., coherence length $L_c = 2(\ln 2)/\pi \cdot \lambda^2/\Delta\lambda_{cs}$) of $1.8 \mu\text{m}$ in brain tissue was reached. Light from the sample arm was collimated, scanned transversely by a precision servo-mirror (x -, y -axes), and focused by an f16 mm/NA 0.25 microscopic objective (e.g., transverse resolution: $\sim 3 \mu\text{m}$) on the capillary beds of mouse cortex through a cranial window. The back scattered light from cortical brain at different depths (along z -axis) was collected back to the sample arm and recombined with the reference light to be detected by a spectral imager in which the collimated light ($\phi 10$ mm) was spectrally diffracted ($1200^{-1}/\text{mm}$) and focused ($f = 85$ mm) onto a linear CCD camera (2048 pixels, Atmel) running at 27 kHz. Synchronizing the CCD camera with sequential x -scans (e.g., 500 pixels), 2D μ OCT (z - x image) was acquired at ~ 54 fps and streamlined via camera link to the hard disks (300 MB/s, Raid 0) of a workstation for parallel 2D/3D image processing and display by inverse FFT. However, unlike μ OCT for architectural imaging, specific scanning schemes were implemented to facilitate simultaneous μ OCA and μ ODT for in vivo visualization of micro vascular networks and quantitative imaging of capillary CBF. For μ OCA imaging, the camera was configured at its highest rate (27 kHz) to acquire 4 consecutive $I(z, x)$ images per z - x plane (e.g., $I_j(z, x) = \text{FFT}^{-1}[I(k, x)|_k], j = 0 \rightarrow 3$); thus, μ OCA could be expressed as their relative standard deviation [$\sigma_{\text{OCA}}(z, x) = \Sigma |I_j(z, x) - I(z, x)| / (4I(z, x))$, where $I(z, x) = \Sigma I_j(z, x) / 4, j = 0 \rightarrow 3$] based on modified speckle contrast approach [4]. For μ ODT imaging, the camera was configured to acquire at 10 kHz (down binned to 5, 1 kHz) and the Doppler flow images were reconstructed by our newly developed phase-intensity-mapping algorithm (PIM) [12] for enhancing CBF detection sensitivity.

A flow-phantom study was performed to compare the differences between OCA and ODT for flow detection, in which 0.5% intralipid in a translucent capillary tubing ($\phi 280 \mu\text{m}$) was driven by a precision syringe pump (CMA400, Micro dialysis) for accurate flow rate control. The bidirectional capillary tubes were immersed in a solidified scattering scaffold by mixing 0.5% intralipid with 1% agarose to mimic brain tissue. The micro fluidic chamber was tilted $\theta \approx 85^\circ$ to the OCT beam to reduce apparent Doppler flow. Fig. 1 compares the results of μ OCA and μ ODT at the pump flow rate (v_p) of 0, 23.6, 47.2 $\mu\text{m/s}$. At $v_p = 0 \mu\text{m/s}$ (with pump stopped and exhaust curtailed), μ ODT showed no directional flow ($v_{\text{ODT}} \approx 0$) as expected. In contrast, μ OCA showed that the speckle contrast in the tubes ($\sigma_{\text{OCA}} = 16k$ counts)—representing their flows—was significantly higher than that of background noise level ($\sigma_b = 10k$ counts), resulting from Brownian motion of solidified scaffold (scatterers) and the system noise. These results indicate that unlike μ ODT that detected directional flow (i.e., random Brownian motion was canceled), μ OCA detected random motion within the tubes. This implies that the higher sensitivity of μ OCA for detecting microvasculature was likely contributed from the Brownian motion of scatterers within the blood vessels including capillaries rather than directional flow (CBF). For instance, when the pump rate was increased to $v_p = 23.6, 47.2 \mu\text{m/s}$ (~ 15 min was allowed to stabilize flow in each case), the results showed that μ ODT was able to identify directional flows and their flow rate increase;

whereas μ OCA based on speckle contrast exhibited minor increase, likely due to the high offset of Brownian motion.

For more quantitative analysis of the differences between these two approaches, we measured the changes of μ OCA and μ ODT with gradual increases of the pump rate v_p from 0 $\mu\text{m/s}$ to 35 $\mu\text{m/s}$. At each v_p , the μ ODT image was reconstructed and a small circular area around the tube center was selected as the region of interest (ROI) and its mean flow velocity was calculated to represent its flow rate (v_{ODT}). Fig. 2 illustrates the quantitative analysis, in which curve fitting showed a close linear correlation between μ ODT data v_{ODT} and the pump rate v_p ($R^2 = 0.98$). On contrary, the non-directional flow index (σ_{OCA}) behaved very differently as shown by the red triangles. For $v_p \leq 12 \mu\text{m/s}$, $\Delta\sigma_{\text{OCA}} \approx 5k$ counts due to predominant Brownian motion and remained unchanged; for $v_p > 12 \mu\text{m/s}$, it increased slightly with v_p . Quantitative analysis in Fig. 2 confirms that the high vasculatural detectability of μ OCA was mainly attributed to the Brownian-motion offset ($\Delta\sigma_{\text{OCA}} \approx 5k$ counts). More importantly, μ ODT provided better sensitivity for detecting directional flow and the minimal detectable flow rate ($v_{\text{ODT}} \approx 5 \mu\text{m/s}$, with no offset) was substantially lower than that of μ OCA which was under Brownian-motion limit with a large offset of $\Delta\sigma_{\text{OCA}} \approx 5k$ counts in the phantom study.

To further validate these phenomena *in vivo*, we applied a mouse cerebral microischemia model. In this study, CD-1 mice (8 weeks old) were anesthetized with inhalational 2% isoflurane and then mounted on a custom stereotaxic frame. A $\phi 5 \text{ mm}$ cranial window was created on the somatosensory motor cortex with the dura left intact. The exposed brain surface was covered by 1% agarose gel and sealed with a glass coverslip. After μ OCA pre-scan to accurately locate the coordinates of individual blood vessels, a pigtailed green laser (532 nm, 60 mW) was interconnected via the sample arm to disrupt the vessels (2 min exposure for capillary CBF and multiple exposures for intermediate CBF). The physiology of mice, including electrocardiography (ECG), respiration rate and body temperature, was continuously monitored.

Fig. 3 shows the comparative results of μ ODT (pseudo color) and μ OCA (grayscale) over a field of view of $2 \text{ mm} \times 2 \text{ mm}$ on mouse cortical brain. Because of ultrahigh resolution ($\sim 1.8 \mu\text{m} \times 3 \mu\text{m}$) of μ OCT, high vasculatural details including capillary beds (e.g., $\phi 3\text{--}8 \mu\text{m}$ capillaries) were readily visualized by μ OCA. Furthermore, the enhanced flow sensitivity by PIM allowed for detection of the quantitative CBF networks, especially minute slow CBFs (e.g., $10 \mu\text{m/s}$) in capillaries, with almost identical vascular densities as those by μ OCA. To compare with the baseline data (panels a, b), the upper half (c, d) and lower half (e, f) panels show laser disruptions of a $\phi 35 \mu\text{m}$ (1) and a $\phi 27 \mu\text{m}$ (2) branch arterioles whose locations are marked by two green dots. As shown in μ ODT images (d, f), the downstream CBFs including those in the interconnected capillary beds diminished in two dashed green circles (the margins were determined by segmentation of ratio image, i.e., $\Delta = (d - b)/b$ or $(f - b)/b$ using threshold $\Delta = -35\%$). In other words, the CBFs in those vessels were deactivated as a result of laser disruptions. On contrary, the vascular shutdown of capillary beds by two laser disruptions (e.g., dashed green areas segmented with $\Delta = (c - a)/a$ or $(e - a)/a$ using threshold $\Delta = -35\%$) was drastically smaller in μ OCA images. Interestingly, except those in close proximity to the disrupted spots (1, 2) where laser thermal coagulation might either solidify the blood or dramatically increase its viscosity that tranquilized Brownian motion, the speckle contrast signals within the arterioles (at $\sim 100 \mu\text{m}$ downstream) remained to be visualized, as highlighted by green arrows (decreased -28% for 1 and -24% for 2 by μ OCA versus over -95% by μ ODT). This was attributed to the contrast enhancement by the Brownian motion of blood inside the deactivated vessels. Therefore, the results of this *in vivo* study further validate that, while μ OCA may detect more vasculatures such as capillary beds due to Brownian motion enhancement, μ ODT provides more sensitive CBF

quantification (e.g., unidirectional flow) and is thus more suitable for brain functional studies.

In summary, we present new experiments to elucidate the differences between OCA (speckle contrast based) and ODT for imaging cerebral hemodynamic responses to functional brain activation and neurotoxicity. Although both μ OCA and μ ODT techniques are based on detecting Doppler phase shift of moving backscatterers, our tissue-flow phantom and *in vivo* mouse brain studies suggest that these two approaches operate under different regimes of flow-induced motion detections. In the low-flow domain, μ OCA provides high vascular turnouts resulting from the enhancement of Brownian motion of free-moving red and white blood cells within the blood vessels. This renders μ OCA to visualize more vasculature networks, including deactivated branch vessels as evidenced by the images of no-flow tubes (Fig. 1) and laser disrupted arteriolar branches [Figs. 3(c) and 3(e)]. On contrary, μ ODT detects directional flows and is thus more suitable than μ OCA for studying cerebral hemodynamic responses to brain functional changes. By combining ultrahigh resolution of μ ODT and high phase sensitivity of PIM, we are able to readily detect micro vascular CBF with capillary details (e.g., ϕ 3–8 μ m capillaries) and at ultrahigh sensitivity (10 μ m/s). It is noteworthy that more work is needed to compare the results of flow detectability by other OCA approaches (e.g., phase-based OMAG or DFR-OCT [7]) and the potential differences of Brownian motion offset value between intralipid ($\Delta\sigma_{OCA} \approx 5k$ counts) and blood (potentially lower, due to less Brownian motion) in capillary tubes of smaller sizes (e.g., ϕ 100, 30, 8 μ m).

Acknowledgments

The work was supported in part by National Institutes of Health grants K25-DA021200 (C. D.), 2R01-DK059265 (Y. P.), 1RC1DA028534 (C. D. and Y. P.), and 1R21-DA032228 (Y. P. and C. D.).

References

1. Wang RK, Jacques SL, Ma Z, Hurst S, Hanson SR, Gruber A. Opt Express. 2007; 15:4083. [PubMed: 19532651]
2. Yu L, Chen Z. J Biomed Opt. 2010; 15:016029. [PubMed: 20210473]
3. Srinivasan VJ, Jiang JY, Yaseen MA, Radhakrishnan H, Wu W, Barry S, Cable AE, Boas DA. Opt Lett. 2010; 35:43. [PubMed: 20664667]
4. Mariampillai A, Standish BA, Moriyama EH, Khurana M, Munce NR, Leung MKK, Jiang J, Cable A, Wilson BC, Vitkin IA. Opt Lett. 2008; 33:1530. [PubMed: 18594688]
5. Zhao Y, Chen Z, Saxer C, Xiang S, de Boer JF, Nelson JS. Opt Lett. 2000; 25:114. [PubMed: 18059800]
6. Szkulmowski M, Szkulmowska A, Bajraszewski T, Kowalczyk A, Wojtkowski M. Opt Express. 2008; 16:6008. [PubMed: 18545302]
7. Yuan Z, Luo Z, Ren H, Du C, Pan Y. Opt Express. 2009; 17:3951. [PubMed: 19259236]
8. Tao YK, Davis AM, Izatt JA. Opt Express. 2008; 16:12350. [PubMed: 18679512]
9. Makita S, Jaillon F, Yamanari M, Miura M, Yasuno Y. Opt Express. 2011; 19:1271. [PubMed: 21263668]
10. Zotter S, Pircher M, Torzicky T, Bonesi M, Götzinger E, Leitgeb RA, Hitzinger CK. Opt Express. 2011; 19:1217. [PubMed: 21263663]
11. Yuan Z, Chen B, Ren H, Pan Y. J Biomed Opt. 2009; 14:050502. [PubMed: 19895098]
12. Ren H, Du C, Yuan Z, Park K, Volkow N, Pan Y. Mol Psychiatry. 2011; 16:1038. [PubMed: 21110160]

Fig. 1.

(Color online) Comparative results of μ OCA and μ ODT for bi-directional flows in a translucent $\phi 280 \mu\text{m}$ capillary tube at the pump rate of $v_p = 0, 23.6$ and $47.2 \mu\text{m/s}$. While the non-directional flow imaged by μ OCA was offset by Brownian motion and only exhibited minor increase with v_p , the directional flow imaged by μ ODT varied linearly with v_p .

Fig. 2.

(Color online) Quantitative analyses of the flow-rate changes of directional flow by μ ODT and non-directional flow by μ OCA with the pump rate v_p . μ OCA provided high sensitivity for detecting vasculature of minute slow flows due to the enhancement by random Brownian motion ($\Delta\sigma_{OCA} \approx 5k$ counts), μ ODT was able to detect directional flow velocity at ultrahigh sensitivity of $v_{ODT} \approx 5 \mu\text{m/s}$ in flow-tissue phantom.

Fig. 3.

(Color online) Comparative μ OCA (upper panels) and μ ODT (lower panels) images of mouse cortical brain *in vivo* during (a), (b) baseline, (c), (d) after first and (e), (f) second laser coagulations that induced microischemia. The locations of laser disruptions are marked with green dots. The dashed green circles highlight the spreading of CBF shutdowns due to laser disruptions of two arterioles. Apparently, because of offset by Brownian motion, the areas of vasoconstriction (i.e., CBF shutdown) in μ OCA were smaller (underestimated) than those in μ ODT. Except at the coagulation spots, the disrupted vessels (both up- and down-streams) remain visible (although dimmer) due to Brownian motion.