Design and optimization of line-field optical coherence tomography at visible wavebands

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Abstract: Parallel line-field Fourier-domain optical coherence tomography (LF-FDOCT) has emerged to enable relatively higher speeds than the conventional FDOCT system. In the LF-FDOCT, one B-scan is captured at a time instead of scanning the beam to acquire hundreds of A-scans. On the other hand, spectroscopic OCT using the visible waveband provides absorption information over multiple wavelengths at each voxel. This information of spectral absorption enables quantitative measurement of blood oxygenation, voxel by voxel. Here, we presented the design and optimization of a LF-FDOCT system at the visible waveband (520–620nm), especially using a generic Camera Link area sensor (2048×1088 pixels). To optimize the axial resolution and depth of imaging volume, we simulated various parameters and found that two Nyquist optima can exist, the origin and implication of which has been discussed. As a result, our system acquired 1088 A-scans in parallel at the camera’s frame rate of 281 frame per second, achieving an equivalent rate of over 300,000 A-scan/s, while minimizing sacrifice in the point spread function (2.8×3.1×3.2×3.2 μm3, × × y × z) and the field of view (750×750×750 μm3). As an example of application, we presented high-speed imaging of blood oxygenation in the rodent brain cortex.

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1. Introduction

As a non-invasive three-dimensional (3D) imaging modality, optical coherence tomography (OCT) is being used for a wide range of applications from clinical ones like ophthalmic inspection [1,2] and skin testing [3,4] to basic research including animal brain imaging [5,6]. The basic form of OCT is composed of a broadband incoherent light source and a Michelson interferometer. Time-domain OCT (TDOCT) was the form first demonstrated [7], but its speed was restricted to the speed of mechanical motion of the reference mirror. Based on the relationship between wavenumber and axial position as a Fourier-transform pair, Fourier-domain OCT (FDOCT) was emerged as a more prominent form. FDOCT captures a spectral fringe of the interference between the reference and sample beams, and A-scan can be obtained by a Fourier transformation of the interferogram without moving the reference mirror as in TDOCT [8–10]. This approach enables FDOCT to acquire the axial profile of a sample’s field reflectivity at once; thus, the imaging speed of FDOCT is generally 1–2 orders of magnitude larger than TDOCT while they operate at the same signal-to-noise ratio (SNR) [11]. Also, the SNR is N/2 times higher than that of TDOCT where N denotes the pixel number of the camera to record the spectral fringe while they operate at the same imaging speed [12].

Recent studies introduced another form that further enhances the A-scan rate, parallel line-field FDOCT (LF-FDOCT) [13–15]. Conventional FDOCT systems mostly scan a sample spot by spot (A-scan by A-scan) along the two orthogonal axes, where the time interval between consecutive A-scans is limited by either the speed of a scanning galvo-mirror or the maximal acquisition
rate of a line camera. In contrast, LF-FDOCT uses a two-dimensional (2D) area camera that captures spectral fringes simultaneously from multiple locations of a sample along the fast axis or x axis. This approach enables LF-FDOCT to acquire the cross-sectional profile or B-scan at once, leading to a higher equivalent A-scan rate than conventional FDOCT [16,17].

On the other hand, OCT has been also advanced in terms of what it can image beyond the tissue structure, so called functional OCT. For example, by analyzing a complex-signal correlation difference between two or more B-scans repeated on the same location of a sample, OCT enabled label-free angiography of vasculature as movement of red blood cells (RBCs) makes the correlation difference larger than static background tissue [18–21]. When analyzing phase differences between multiple consecutive A-scans, OCT enables measurements of the axial velocity of blood flow [22,23]. These functional OCT approaches to blood perfusion imaging have been applied for inspection of vascular abnormalities in human eye [24,25] and neuroscience research in rodent cerebral cortex [26,27], among others.

Related to this vascular functional OCT, another approach has been investigated to image blood oxygenation. While the near-infrared waveband is generally preferred in OCT because of its longer tissue penetration depth, the blood oxygenation imaging should use the visible waveband, over which the light absorption of hemoglobin is 1–2 orders of magnitude higher than tissue scattering [28,29]. By exploiting distinct molecular extinction spectra of oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb), and by analyzing spectral fringes in a wavelength-resolved manner, visible-waveband OCT enables us to measure the ratio of oxy- and deoxy-hemoglobin concentrations or the oxygen saturation while maintaining the 3D imaging capability of OCT [30,31]. In detail, a short-time Fourier transform segmenting the spectral fringe into several sub-bands, along with least-square fitting, have been mainly used [21,28,32] to measure the oxyhemoglobin concentration [HbO2], deoxyhemoglobin concentration [Hb], total hemoglobin concentration [HbT], and oxygen saturation (SO2).

In this paper, we combine these two advances: LF-FDOCT for speed enhancement and the visible waveband for blood oxygenation imaging. First, we designed and optimized a spectroscopic LF-FDOCT system at the visible waveband. For this system, a 100-nm bandwidth from 520 nm to 620 nm was chosen from a supercontinuum laser, and a 2D area camera with 281 fps frame rate and 2048 (horizontal) × 1088 (vertical) pixels was used, where spectral fringes were received in the horizontal direction and the vertical direction represented the fast axis or X axis. Especially, we optimized our design to make the imaging volume to be almost cubic as 750 μm (x) × 750 μm (y) × 750 μm (z; 20-dB depth of view in air) and the resolution volume to be almost isotropic as 2.8 μm (x) × 3.1 μm (y) × 3.0 μm (z), when using our 10× objective. Then, we implemented data processing software for the wavelength-resolved analysis of spectral fringes. Combined, the system enabled us to image blood oxygenation in the mouse cortex at an equivalent A-scan rate of 305 kHz.

2. System design and instrumentation

2.1. Overall design

A schematic of our custom-built LF-FDOCT system is presented in Fig. 1. We used a supercontinuum laser source (EXW-12, NKT), the whole waveband of which spans between 450 nm and 2400 nm. A long-pass filter and a short-pass filter were installed to pick the 100-nm bandwidth (520–620 nm). The laser beam emitted from a fiber collimator had a 1/e2 diameter of 2 mm. This Gaussian beam passed through a 4-f lens system that worked as an expander. We optimized this beam expander such that the diameter of the output beam became ~10 mm, close to the aperture of the objective.

After the expander, we used a cylindrical lens to converge the beam in the vertical direction while keeping the beam collimated in the horizontal direction. The beam was split into the sample and reference arms by a 50:50 beam splitter (BS004, Thorlabs). In the sample arm, the
beam was directed to a one-axis scanning galvo-mirror (GVS011, Thorlabs), at which the beam was deflected for the y-axis scanning. The beam was focused onto the sample plane by a 10× objective (378–823–5, Mitutoyo; focal length of 20 mm). We tested several focal lengths of the cylindrical lens and chose 10 mm (LJ1878L2-A, Thorlabs) to make the beam as a sharp line as possible at the sample plane (3.1 $\mu$m in width in full width half maximum (FWHM)).

In the reference arm, the beam passed through a neutral-density filter (NDC-50C-2-A, Thorlabs) to adjust the reference beam intensity as needed. The same 10× objective was used to shape the beam to a line on a mirror (PF05-03-G01, Thorlabs).

The beam splitter combined the back-scattered sample beam and the mirror-reflected reference beam. The combined beam was deflected by 90 degrees by a mirror and then compressed by another 4-\(f\) optical system. We used a diffraction grating to disperse the compressed beam in the horizontal direction. The beam width on the grating, controlled by the 4-\(f\) system, the beam angle on the grating, and the grating density served as key input parameters in and were optimized by the following simulation. This dispersed beam, which contains spectral fringes in the horizontal direction and lateral location information in the vertical direction, was focused on the area camera, a complementary metal-oxide-semiconductor (CMOS) sensor array (GZL-Cl-22C5M-C, Point Grey; pixel numbers, 2048 (horizontal) x 1088 (vertical); pixel size, 5.5 $\mu$m x 5.5 $\mu$m). The focal length of L3 was another key input parameter to the following simulation.

Compared to the line alignment in fiber-based FDOCT, free-space LF-FDOCT required careful beam alignment such that the pixels in each column represent the same wavelength and the pixels in each row represent the same location. Figure 2(a) shows the beam propagation path for the horizontal direction representing the wavelength axis, and Fig. 2(b) shows the beam propagation path for the vertical direction representing x axis of the sample plane. In the horizontal plane, the imaging mechanism is identical to that of the flying-spot FDOCT. The beam was kept collimated, and thus focused to a spot at the sample plane (i.e., the focal plane of the objective). This beam was dispersed by the diffraction grating and then focused by the camera lens (L3); as a result, a rainbow line appearing on the camera. In the vertical plane, the cylindrical lens worked as a focal lens, eventually making the beam divergent in front of the diffraction grating. Since the diffraction grating has no groove in this plane, the beam divergence remained the same in front of the camera lens. Therefore, the beam became a micron-width line on the camera, encoding x-axis locations onto the vertical-direction pixels. Combined, the beam was focused to be a thin

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**Fig. 1.** A schematic of our LF-FDOCT system. BS, beam splitter; OBJ, objective; ND, neutral density.
line on the sample plane and then was projected to be a continuous series of rainbow lines on the camera.

(a) Horizontal Plane

(b) Vertical Plane

**Fig. 2.** Beam propagation path of the LF-FDOCT system on the horizontal (a) and vertical (b) plane.

2.2. Simulation for optimization of spectrometer design

To determine the design parameters that optimize the important performance parameters, the axial resolution and the depth of volume (DOV), we simulated how the performance output parameters are affected by several input design parameters, including the grating density, the beam waist and angle on the grating, and the focal length of L3. In the horizontal direction, the diffraction grating worked as a 1-D spatial disperser. As the source light passed through the band-pass filter with the bandwidth of $\Delta \lambda$, the beam length $\Delta X$ of the projection of a spectrum along the horizontal direction on the camera sensor plane is determined by [33]:

$$\Delta X = \left(\frac{f}{d \cdot \cos \theta}\right) \cdot \Delta \lambda$$

(1)

where $f$ is the focal length of the lens between the grating and camera, $d$ is the grating period, and $\theta$ is the diffracted angle of the beam to the diffraction grating. However, when the beam length $\Delta X$ is longer than the camera sensor size ($M \cdot A$), the total bandwidth captured by the camera becomes narrower as $\Delta \lambda' = \left(\frac{d \cdot \cos \theta}{f}\right) \cdot M A$, where $M$ is the number of the horizontal pixels of the camera and $A$ is the camera’s pixel interval. Thus, the effective spectral bandwidth $\Delta \lambda'$ becomes:

$$\Delta \lambda' = \begin{cases} 
\Delta \lambda \\ 
\frac{\Delta \lambda}{MA \cdot d \cdot \cos \theta}
\end{cases} \quad \text{when } \Delta X \leq M \cdot A \text{; i.e., } f \leq M \cdot A \cdot d \cdot \cos \theta / \Delta \lambda$$

(2)

Figure 3(a) plots an example of this effective spectral bandwidth as a function of the focal length.

The axial point spread function (PSF) size in FD-OCT is determined by this spectral bandwidth. As a Gaussian spectrum is used to calculate the axial PSF size, we scaled the FWHM of our rectangular spectrum into that of Gaussian spectrum, using a transformation coefficient $T = \frac{2 \sqrt{\ln 2}}{2 \sqrt{3}}$. Our bandpass-filtered light had a rectangular spectrum with the width of 100 nm, so the effective FWHM under the Gaussian spectral shape assumption should be $2 \sqrt{\ln 2} \cdot \frac{100}{2 \sqrt{3}} = 68$ nm. Thus, our
Fig. 3. (a) An example of the effective bandwidth varying with the focal length of the lens; (b) Examples of the imaging depth and the axial resolution varying with the focal length. These examples were plotted using $\lambda_0 = 575$ nm, $\Delta \lambda = 100$ nm, $W = 4$ mm, $A = 5.5$ $\mu$m, $d = 1/1200$ mm, $\theta = 30^\circ$ and $M = 2048$.

The axial PSF size is expressed by:

$$\text{Axial PSF size} = 2\ln 2 \frac{\lambda_0^2}{\pi T \Delta \lambda'} = \begin{cases} 2\ln 2 \frac{\lambda_0^2}{\pi T \Delta \lambda} & (f \leq \frac{MAd \cos \theta}{\Delta \lambda}) \\ 2\ln 2 \frac{\lambda_0^2}{\pi TMAd \cos \theta} & (f > \frac{MAd \cos \theta}{\Delta \lambda}) \end{cases}$$

Figure 3(b) plots an example of the axial PSF size as a function of the focal length.

On the other hand, the DOV of FD-OCT is determined by the spectral sampling interval. First, the spectral resolution of the diffraction grating is given by [34]:

$$\delta \lambda = \frac{\lambda_0 \cdot d \cdot \cos \theta}{W}$$

where $W$ is the waist of the diffracted beam on the grating, which is determined by the grating equation and the waist of the incident beam. As the dispersed beam is focused on to the camera, this spectral resolution corresponds to the distance on the camera sensor plane as

$$\delta x \approx f \delta \theta = f \left( \frac{d \theta}{d \lambda} \right) = f \frac{d}{d \cos \theta} \delta \lambda$$

where the differential of the diffraction grating equation was used, and the 1st order diffraction was adopted.

To sufficiently sample such resolved spectrum (i.e., to meet the Nyquist sampling criterion), the camera’s pixel interval $A$ ($\mu$m) should satisfy

$$2A \leq \delta x$$

leading to a condition of $2A \leq \frac{f \Delta \lambda}{d \cos \theta} = \frac{\lambda_0 f}{W}$.

Second, the spectral sampling interval depends on this relation between the spectral resolution and the camera’s pixel interval, as well as the aforementioned relation between the beam length and the camera’s sensor size. When the spectral resolution is so high that the camera pixels sample effectively different wavelengths (i.e., the Nyquist sampling condition Eq. (6) is not met;
\( \delta x < 2A \), the spectral sampling interval \( \delta \lambda \) is given by:

\[
\delta \lambda = \begin{cases} \frac{\lambda}{M} = \frac{\lambda}{A} \frac{d \cos \theta}{f} & \text{when } \Delta X \leq MA; \ i.e., \ f \leq \frac{MA d \cos \theta}{\Delta X} \\
\frac{\delta x}{\Delta x} = \frac{\lambda}{A} \frac{d \cos \theta}{f} & \text{otherwise} \end{cases}
\]

(7)

where \( M' \) is the effective pixel numbers that cover the bandpass-filtered source spectrum \( \Delta \lambda \); i.e., \( M' = \frac{\Delta X}{\Delta \lambda} = \frac{f \Delta \lambda}{\lambda' A} \). Interestingly, this spectral sampling interval is independent of whether the beam length \( \Delta X \) is longer or shorter than the camera sensor size. However, when the spectral resolution is so low that adjacent camera pixels cannot distinctively sample a wavelength (i.e., the Nyquist sampling condition of Eq. (6) is satisfied; \( \delta x \geq 2A \)), the effective spectral sampling interval should equal to the half of the spectral resolution \( \left( \delta \lambda / 2 \right) \) derived in Eq. (4). Thus, the effective spectral sampling interval \( \delta \lambda' \) becomes:

\[
\delta \lambda' = \begin{cases} \frac{\delta x}{2} = \frac{\lambda_0 d \cos \theta}{2W} & \text{when } \delta x \geq 2A; \ i.e., \ f \geq \frac{2AW}{A_0} \\
\delta \lambda' = \frac{\lambda}{A} \frac{d \cos \theta}{f} & \text{when } f < \frac{2AW}{A_0} \end{cases}
\]

(8)

Third, the DOV is determined by this effective spectral sampling interval:

\[
DOV = \frac{\lambda^2}{4 \delta \lambda'} = \begin{cases} \frac{\lambda_0}{2} \frac{d \cos \theta}{W} \frac{f}{f - \frac{2AW}{A_0}} & \text{when } f \geq \frac{2AW}{A_0} \\
\frac{\lambda_0}{2} \frac{d \cos \theta}{W} & \text{when } f < \frac{2AW}{A_0} \end{cases}
\]

(9)

Figure 3(b) also plots an example of the DOV as a function of the focal length.

It should be noted that this result considers both effects of the camera pixel pitch and sensitivity roll-off. From the traditional perspective, the maximum ranging depth is solely determined by the sampling pitch of the spectrometer \( \delta \lambda \). While the sensitivity roll-off caused by the finite spectral resolution limits the DOV. When the optical resolution is much worse than the sampling resolution (e.g., when a single wavelength illuminates multiple pixels), the DOV is limited but the maximum retainable depth remains the same. In our result, the DOV was determined in a “hybrid” sense. When the focal length decreases below the cut-off [Fig. 3(b)], the wavelength sampling interval becomes larger leading to a decrease in DOV, as the traditional perspective explains. When the focal length increases above the cut-off, the projected spectrum is broader than the detector which leads to a shorter wavelength sampling interval and thus longer DOV, again as expected from the traditional perspective. However, the spot size increases, which means the sensitivity roll-off will be stronger, effectively limiting the DOV.

Finally, the axial sampling interval is given by \( \text{DOV} / (M/2) \). We added a plot of the double of this axial sampling interval into Fig. 3(b) to visualize the regions of under-sampling and over-sampling. As the effective axial resolution is determined by the maximum between the axial PSF size and the double of sampling interval, the axial resolution should be the higher points between the red and the red-dotted lines. Thus, within the indicated “under-sampling” region, the axial resolution is determined to be the double of sampling interval, not the PSF size.

Interestingly, in the example displayed in Fig. 3(b), there existed two optimal focal lengths for the axial Nyquist sampling (i.e., neither over- nor under-sampling) – those intersections between the red-solid and red-dashed lines. The second optimum with the longer focal length was not expected, and we interpret it as follows. When the focal length is short enough for the camera to capture the whole bandwidth of bandpass-filtered source light, the axial PSF size does not vary with the focal length [the initial plateau of the red-solid line in Fig. 3(b)]. But, if the focal length becomes so long that the camera captures only a part of the whole bandwidth (the decay in Fig. 3(a)), the axial PSF size increases accordingly (Eq. (3), bottom; the positive-slope part of the red-solid line in Fig. 3(b)). Meanwhile, even when the focal length was so short that the axial PSF...
size did not vary, the DOV increases with the focal length because the spectral sampling interval decreases [Eq. (9), bottom; the initial positive-slope part of the red-dashed line in Fig. 3(b)]. Thus, this increasing DOV (and thus the increasing axial sampling interval) can intersect with the half of the axial PSF size that did not vary, producing the first Nyquist optimum. However, as the focal length becomes longer, the increase in DOV stops [Eq. (9), top; the later plateau of the red-dashed line in Fig. 3(b)] and thereby this constant DOV can intersect with the half of the axial PSF size that increased with the focal length, which produces the second Nyquist optimum.

As Fig. 3(b) only shows an example with the specific set of parameters, we plotted the same for various grating densities, diffracted angles, and beam widths in Fig. 4. The number of the Nyquist optima varied with those input design parameters (e.g., no Nyquist optimum when \( W = 2 \) mm, \( \theta = 15 \) deg, grating density = 600 lines/mm).

![Fig. 4](image)

Fig. 4. The relationships between the axial PSF size (black) and the double of sampling interval (color), or the under- and over-sampling regions, for various focal lengths, grating diffracted angles, and grating beam widths.

A tradeoff exists between the two optima; the left optimum (the one with the shorter focal length) offers a better axial resolution but a smaller DOV, while the right optimum provides us with a longer DOV by sacrificing the axial resolution. When there exist two optima for a given set of the grating density and angle, we chose the right one (i.e., the one with the longer focal length). Whereas the left optimum did not vary with the beam width, the right one varied and thus provided us with another degree of freedom in design optimization. This additional degree of freedom enabled us to easily adjust the axial resolution and DOV by changing the focal length and matching the beam width.

To better understand this inter-relationship between design parameters, we plotted the second Nyquist optimal focal length as a function of the beam widths for various grating angles and densities (Fig. 5). The second Nyquist optimal focal length did not vary with the grating angle but
only the range of the beam width over which the optimum exists varies with the angle. Generally, a larger diffracted grating angle allowed a broader range of the beam widths with the optimum existing, especially in small beam widths. Note that a larger grating angle enlarges both the DOV and axial resolution at the optimum as can be seen in Fig. 4.

Fig. 5. The relationship between the diffracted beam width and the optimal focal length for the second Nyquist optimum where the half of the sampling interval equals the PSF size. When the optimum for an angle existed, the optimum for a smaller angle always existed and was identical to that. Thus, the lines with smaller angles are overlaid on those with larger angles in this figure.

2.3. Instrumentation and characterization

In our instrumentation, we chose the light source to be $\lambda_0 = 570$ nm and $\Delta \lambda = 100$ nm, and the camera with $A = 5.5$ $\mu$m and $M = 2048$. The center wavelength and bandwidth were chosen such that the captured spectral information is sufficient for distinguishing the differences in absorption between the oxygenated and deoxygenated hemoglobin. For these given parameters, we determined the variables to be $d = 1/1200$ mm, $W = 3$ mm, and $\theta = 30^\circ$ as the initial design. First, we chose the grating of 1200 lines/mm (GR25-1205, Thorlabs) because a larger grating density gives us a larger flexibility in aiming the DOV and axial resolution (compare the range of the second intersection in $y$ axis in Fig. 4) while the diffraction efficiency becomes more sensitive to the incident angle with a larger density (e.g., 1800 lines/mm). Second, we chose the beam width of 3 mm because it was the minimum beam width at which the second Nyquist optimum exists at every grating angle simulated above (i.e., greater design flexibility; Fig. 5) while a smaller beam width tends to provide a better axial resolution (Fig. 4). This selection of the variables as the initial design resulted in the optimal focal length of 88 mm. Since a smaller beam width tends to provide a better axial resolution while a shorter focal length was optimal for a smaller beam width, we used a lens with 75-mm focal length (AC254-075-A, Thorlabs) for our instrumentation. Finally, we chose the diffracted grating angle of $30^\circ$ as the theoretical design optimum because it was closest to that corresponding to the grating’s blazed angle ($23^\circ$ diffracted angle at the $17^\circ$ incident blazed angle). When applied these parameters to the above formulae, the theoretical size of the axial PSF was $2.1 \mu$m [Eq. (3)], and the theoretical DOV was $1185 \mu$m with the axial sampling interval being $1185/1024 = 1.16 \mu$m. Since this sampling interval was larger than a half of the PSF size, the effective axial resolution is determined by the sampling interval to be $2\times1.16 = 2.3 \mu$m, being slightly larger but close to the axial PSF of $2.1 \mu$m (i.e., the second Nyquist optimum). In this configuration, the camera over-samples the spectral information over the wavelength ranging from 520 to 620 nm as the spectral PSF size of 0.14 nm and the spectral sampling interval of 0.049 nm make the effective spectral resolution to become 0.14 nm.
After careful alignment, we measured the DOV and axial resolution by using a mirror as the sample. The widely used dispersion compensation algorithm [35] was applied in post-processing. When we obtained A-scans over 20 positions of the mirror sample in depth with a step of 50 µm, the DOV and axial sampling interval were measured to be 1.6 mm and 1.6 µm, respectively, which were 34% larger than the above theoretical values. This discrepancy might be attributed to a few factors, including the physical alignment that led to slight changes in the parameters (e.g., the final beam width was slightly changed after the alignment) and the assumptions made during the simulation (e.g., the resolution and DOV equations were derived from the Gaussian-shape spectrum). When we measured the axial resolution in FWHM from the A-scans, the axial resolution was 3.2 µm. As expected in the simulation, the axial information was under-sampled, and the axial resolution was determined by the sampling interval than the PSF. Referring to the previous research [14], three main noise sources dominate in the FD-OCT system, including the electronical noise $N_{el}$ of the detector, shot noise $N_{sh}$ and optical relative intensity noise $N_{RIN}$. The maximum SNR in a single pixel is achieved when $N_{RIN}$ equals to $N_{el}$. The number of electrons per pixel generated by the reference arm light required for the maximum SNR, however, was $N_{ref} = 1.13 \times 10^5$ in our system, which was beyond the full well depth of the camera (12900 electrons). Thus, the maximum SNR of our LF-FDOCT system was limited by the saturation level of the camera. Under this condition, each noise of the system was evaluated as $N_{sh} = 95$, $N_{el} = 18.8$, and $N_{RIN} = 1.5$ electrons, implying that the sensitivity of the system was dominated by the shot noise. Considering the spectrometer efficiency of 39.2% and the x-axis pixel number, the theoretical shot noise limited sensitivity was estimated to be 93.0 dB, where the total sample illumination power was 19.5 mW and the exposure time of the camera was 400 µs. Experimentally, the A-scan data with a moving mirror also enabled us to determine the sensitivity and the sensitivity drop-off to be 85 dB with $-45$ dB attenuation and $-22$ dB/mm, respectively.

Whereas the lateral FOV and resolution are independent of the axial DOV and resolution in FDOCT, they can be dependent in LF-FDOCT (e.g., the focal length of the camera lens affects both the lateral and axial resolutions). It is also known that the lateral resolution of this type of parallel illumination system is double of that of the flying spot system due to the crosstalk effect [13]. We used a 10x microscope objective (Mitutoyo, NA = 0.26, theoretical lateral PSF size = 1.1 µm at our center wavelength of 570 nm), with the expected lateral PSF size of our system being 2.2 µm when taking into account the crosstalk effect. We used the USAF resolution target to adjust and measure the lateral FOV and resolution. The lateral FOV is determined by the lateral sampling interval and the lateral pixel number (1088). The lateral sampling interval is determined by the objective lens (f = 20 mm), the 4-f system (the focal length ratio = 3.4), the camera lens (f = 75 mm), and the camera pixel’s lateral size (5.5 µm). Thus, the expected lateral sampling interval was 0.45 µm. Using the USAF resolution target, we determined that the actual lateral FOV and sampling interval in the x direction were 750 µm and 0.69 µm, respectively. Figure 6(a) displays an example of the en face image of the USAF resolution target, and an enlarged image from the yellow box shown in Fig. 6(a) is displayed in Fig. 6(b). From this image, we measured the lateral resolution to be 2.8 µm in the x direction [group 8–4; Fig. 6(c); 27% larger than the crosstalk considered, theoretical PSF size]. To match the lateral FOV in the y direction to that in the x direction while minimizing the waste of B-scans according to the Nyquist theorem, we adjusted the range and step of the galvanometer input voltage such that 512 B-scans are repeated with 1.5-µm step, which resulted in the lateral resolution of 3.1 µm in the y direction [group 8–3; Fig. 6(d)].

One of the disadvantages of LF-FDOCT is the presence of optical aberrations [36]. We observed aberrations during the alignment. For the alignment, we put a mirror on the sample plane and inserted a notch filter (532 nm, 1 nm FWHM) before the grating, generating a narrow line on the camera. We aligned the system such that the line on the camera becomes the narrowest (for spectral resolution), while a focus line on the sample mirror remained as narrow as possible.
**Fig. 6.** (a) The *en face* image of the USAF resolution target. (b) The magnified image of smaller groups obtained from the yellow box in (a). (c) The intensity distribution along the red line shown in (b). (d) The intensity distribution along the blue line shown in (b).

**Fig. 7.** A cross sectional image of a tape.
However, due to optical aberrations, the narrow line on the camera became slightly distorted and defocused when we move the line-field probe beam in y axis for 3D scanning [37,38]. This effect might be attributed to some artifacts observed in Fig. 6(a), as the artifact becomes more distinct near the edge of FOV.

Figure 7 displays an example of B-scan that can be acquired from a single frame of our camera. The B-scan captured a cross-sectional image of a tape and is displayed in logarithm.

3. Application to rodent cortex imaging

3.1. Animal preparation

For the further application, the LF-FDOCT system was validated by in vivo imaging of blood oxygenation in the murine brain. Craniotomy surgery was performed on the test mouse. In the surgical experiment, the mouse was initially anesthetized with 2.5% isoflurane, and ventilated with a mixture of oxygen and air. An area of the skull at the back of the head was shaved without damaging the somatosensory cortex. An approximately 10 mm midline incision was fabricated on the scalp and the skin situating the two sides was retracted. A circular hole on the skull of diameter 6 mm orientating the region of interest of the cortex was thinned using a dental burr until the cortex can be faintly seen. A patch of the thinned skull was carefully cut using scissors while keeping the dura intact. A small glass cover slip was adopted to cover the exposed area of the cortex, and the dental acrylic filled with a 1% agarose solution in artificial CSF was affixed around the exposed area, which helped protect the brain from contamination and dehydration. A custom metal head post was cemented to the skull, the performance of which was to hold the head onto the custom stereotaxic frame during the imaging measurements. After the surgery, the animal was placed into the LF-FDOCT system for our in vivo imaging. The animal was kept under isoflurane anesthesia during imaging. Temperature, oxygen saturation, and pulse rate were continuously monitored using a pulse oximetry and a rectal probe in the time of surgical procedure and experiment. The body temperature and oxygen saturation were maintained at 37 °C and 95%, respectively. The pulse rate remained within the normal range of 250-350 pulses/min. All animal-based experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Brown University, according to the guidelines and policies of office of laboratory animal welfare and public health service, National Institutes of Health.

3.2. Structural imaging

Through the cranial window, we conducted LF-FDOCT imaging of the rodent cerebral cortex in vivo. As can be seen in an en face image (Fig. 8), our LF-FDOCT effectively visualized blood vessels. Vessels appeared dark as they absorb visible light, which was opposite from general OCT angiograms where vessels appear bright as blood flow results in larger signal decorrelations [36,39]. As the mouse brain is highly scattering, the resolution in the resulting image was worse than that of the image of 1951-USAF resolution target (Fig. 6). Nevertheless, the brain image showed blood vessels with diameters down to 20 µm in Fig. 8.

3.3. Spectroscopic data processing

As an example of possible applications, we performed spectroscopic processing of our LF-FDOCT data. The visible waveband used in our LF-FDOCT system allows for mapping blood oxygenation. We adopted the published methods for spectroscopic processing of visible-band OCT data [40-42]. In brief, we split the acquired visible-band spectrum data into several segmentations in the wavenumber space, and each segment were Fourier transformed into the z-space, by which each of these resolution-sacrificed voxels owns the spectral information as being “sampled” at the center wavelengths of the segments. This processing based on the short
time Fourier transform (STFT) can be expressed by:

\[
STFT(k, z; w) = \int i_k(k') \cdot w(k - k'; \Delta k) \cdot e^{-ik'z} \cdot dk'
\]  

(10)

where \(i_k(k')\) denotes the OCT fringe data segment centered at \(k\), \(w\) is a Gaussian window function, \(\Delta k\) is the wavenumber width for the window function, and \(z\) is the axial pixel.

Based on the modified Beer-Lambert Law describing a parametric model of light propagation [43], the absorbance spectrum of a blood vessel was related to a B-scan of spectroscopic OCT:

\[
\mu_a(x, z, k) = \frac{1}{2D} \ln \left( \frac{I_{R2}(x, z, k)}{I_{R1}(z, k)} \right)
\]  

(11)

where \(I_{R2}(x, z, k)\) is the spectroscopic OCT signal at a voxel \((x, z)\) within the vessel, \(I_{R1}(z, k)\) is the spectroscopic OCT signal averaged over the other non-vessel voxels along the \(x\) axis at the same depth, and \(D\) indicates the diameter of the blood vessel. Since the oxy- and deoxy-hemoglobin extinction coefficients have distinct curves [29,44], the absorption spectrum was used determining blood oxygenation at each voxel, via a least square fitting of

\[
\mu_a(x, z, k) = [HbT]\left\{SO_2\mu_{a, HbO_2}(x, z, k) + (1 - SO_2)\mu_{a, Hb}(x, z, k)\right\}
\]  

(12)

where \([HbT]\) is the total hemoglobin concentration, \(SO_2\) is the oxygen saturation, \(\mu_{a, HbO_2}\) and \(\mu_{a, Hb}\) are the molar extinction coefficients of the oxy-hemoglobin and deoxy-hemoglobin, respectively. We used ten waveband segments for the STFT and fitting. Finally, we applied a median and Gaussian image filters to the \(SO_2\) map while excluding bad-fitting voxels (\(p > 0.05\)) from the median filtering. As a result, the spectroscopic analysis produced a map of \(SO_2\) as shown in Fig. 9.
4. Conclusion
We have presented the optimized design of LF-FDOCT at the visible waveband, where three considerations have been taken into account to optimize the design. First, the beam diameter was considered important throughout the whole system, especially the one on the diffraction grating. The relation of the beam diameter and angle to the aperture of the objective lens was also important in this line-scanning scheme. Second, the selection of the wavelength bandwidth out of the available range of 410–2400 nm (the continuum laser; EXW-12, NKT) required consideration of multiple factors. Among others, we carefully considered the tradeoff that a broader bandwidth improves the axial point spread function while it decreases the imaging depth. In this consideration, interestingly, we found that two Nyquist optima can exist and discussed its origin and implication (Fig. 4). The selection of waveband should also consider the hemoglobin absorption spectra when applied to mapping blood oxygenation. Third, on the area sensor, the spectral resolution and the line-scanning beam’s both length and width should be optimized. As a result, this study achieved the bandwidth of 100 nm, the 3D FOV of 750 μm (x) × 750 μm (y) × 750 μm (z), the 3D point spread function size of 2.8 μm (x) × 3.1 μm (y) × 3.2 μm (z), and the imaging speed of 281 B-scans/s (equivalently 305,000 A-scan/s). The feasibility of high-speed LF-FDOCT imaging of blood oxygenation was shown in the rodent cerebral cortex. Improvement and validation of blood oxygenation maps require further study. When further developed, LF-FDOCT may advance the OCT’s capability of tracking individual RBC passage in capillaries by adding the individual RBCs’ oxygenation information [45]. The one-shot acquisition of a B-scan will also increase the cross-sectional FOV and the temporal resolution of the RBC passage imaging, from 300 μm and 4 ms in our previous paper to 750 μm and 3.5 ms in the system built here, and up to 750 μm and 1 ms when built with a 1,000-fps camera.

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