Ultrahigh-resolution full-field optical coherence microscopy using InGaAs camera

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Abstract: Full-field optical coherence microscopy (FFOCM) is an interferometric technique for obtaining wide-field microscopic images deep within scattering biological samples. FFOCM has primarily been implemented in the 0.8 μ m wavelength range with silicon-based cameras, which may limit penetration when imaging human tissue. In this paper, we demonstrate FFOCM at the wavelength range of 0.9 - 1.4 μ m, where optical penetration into tissue is presumably greater owing to decreased scattering. Our FFOCM system, comprising a broadband spatially incoherent light source, a Linnik interferometer, and an InGaAs area scan camera, provided a detection sensitivity of 86 dB for a 2 sec imaging time and an axial resolution of 1.9 μ m in water. Images of phantoms, tissue samples, and *Xenopus Laevis* embryos were obtained using InGaAs and silicon camera FFOCM systems, demonstrating enhanced imaging penetration at longer wavelengths.

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1. Introduction

Full-field optical coherence microscopy (FFOCM), also termed full-field optical coherence tomography (FFOCT), is an interferometric technique that utilizes spatially incoherent illumination and array detection to provide high-resolution transverse images of reflected light within biological specimens [1-10]. In FFOCM, broad bandwidth light illuminates an interferometer, splitting portions of the light to the sample and reference arm. The sample reflectance is imaged onto an array-based detector where it interferes with an image of the reference reflector. Images from within the tissue are typically reconstructed by arithmetic combination of the resulting interference patterns at different reference arm path lengths [1]. The advantages of FFOCM compared with other reflectance-based optical sectioning techniques, including parallel detection, high axial resolution, and the ability to use inexpensive spatially incoherent sources, are well documented [1,2]. Recently, thermal or arc light sources with broad spectral bandwidths such as tungsten-halogen lamps and xenon arc lamps were incorporated into FFOCM setups, demonstrating sub-micrometer axial resolutions [2-4,11]. Microstructural FFOCM images of various biological samples, have been demonstrated, showcasing the ultrahigh-resolution capabilities of this technology [2].

In most biological tissues, scattering of light inside the tissue is the dominant factor that limits the imaging penetration depth [12,13]. Since the scattering becomes weaker at longer wavelengths [12-14], the use of a light source with longer wavelengths should significantly improve the penetration depth. To date, silicon-based CCD (charge coupled device) or CMOS (complementary metal oxide semiconductor) cameras have been primarily used as the 2dimensional detector array for FFOCM, predominantly because reliable silicon (Si) arrays with high-speed imaging capabilities are readily available. Beyond 1.0 µm, however, the sensitivity of silicon-based detectors degrades significantly, making it difficult to utilize these devices at longer wavelengths. Recently developed Indium Gallium Arsenide (InGaAs) area cameras, with a good responsivity in the $0.9 - 1.7 \,\mu\text{m}$ wavelength range, may enable imaging at longer wavelengths, thereby increasing the penetration depth of FFOCM. Relatively low transverse resolution (~35 µm) wide-field OCT, which utilized collimated illumination of coherent light source (mode-locked Cr4+:forsterite laser), has showed the potential of InGaAs area cameras for full-field imaging [15]. In this manuscript, we report the use of InGaAs area scan camera in an ultrahigh transverse and axial resolution FFOCM system operating at a wavelength range of $0.9 - 1.4 \,\mu\text{m}$. A theoretical analysis of the detection sensitivity for the InGaAs FFOCM system is presented. Finally, the penetration depth of the InGaAs system is directly compared to that provided by a similar silicon array FFOCM system in a variety of samples, including an Intralipid phantom, tissues ex vivo, and Xenopus laevis embryos.

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2. Experimental setup



Fig. 1. Schematic of the experimental setup. Xe, xenon arc lamp; ND, neutral density filter; BS, beam splitter cube; OL, microscope objective lens, GP, glass plate; DAQ, data acquisition board in computer.

Like other previously published FFOCM designs [1-4], our system was based on the Linnik interference microscope configuration (Fig. 1) [1-3,16]. Spatially incoherent broadband light from a xenon arc lamp (Oriel 6263) provided bright illumination through a multi-mode fiber (1.0 mm diameter, Oriel 77519). In the reference arm of the interferometer, a neutral density (ND) filter was used to adjust the intensity of reference light. A glass plate was used in the sample arm for dispersion compensation of the neutral density filter. Two identical microscope objective lenses were utilized in both reference and sample arms. Aberration-free water-immersion objective lens availability was an issue in 0.9 - 1.4 µm wavelength range. Several inexpensive off-the-shelf microscope objectives were tested, and an objective lens from Optics for Research (OFR-LMO 20×, 0.45 NA in air, working distance 2.1 mm) provided good imaging performance in this wavelength range. With water-immersion, the round-trip loss of this objective was about 4.5 dB, which can be mainly attributed to the mismatch between our illuminating wavelength and the design wavelength of the stock dielectric anti-reflection coating $(0.8 \ \mu m)$. An estimate of the lateral resolution in water, measured from the full-width-half-maximum (FWHM) of the derivative of the edge response function [17,18] was 2.0 µm. Interference images were captured with an InGaAs area scan camera (SU320MSW-RS170, Sensors Unlimited Inc., 320×256 pixels, 12 bits, 60 Hz) in free-running mode. A synchronized sinusoidal signal was generated from the camera's pixel clock and used to drive a piezoelectric transducer (AE0505D16, Thorlabs) which changed the reference mirror position. Four images acquired over a modulation period with different phase quadratures were processed in order to obtain the coherent signal, which contained the optically sectioned image data [1,2,4].

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The optical spectrum at the detector was measured by an optical spectrum analyzer (HP 70950B, Hewlett Packard), using a water-immersed gold-coated mirror as the sample. Figure 2(a) shows the system's optical spectrum obtained by multiplying the spectral responsivity of the InGaAs camera with the measured optical spectrum. Although the spectral response of the InGaAs camera extends to $1.7 \,\mu m$, water absorption limits the effective spectral range above ~ 1.4 μ m, resulting in the system spectrum of 0.9 - 1.4 μ m range centered around 1.15 μ m. The axial point spread function (PSF) of the system was measured by translating the goldcoated mirror in the sample arm with 0.1 µm steps using a motorized translation stage (Picomotor 8302, New Focus). The PSF, measured as a plane response function, was in good agreement with the PSF calculated from the optical spectrum of the system (Fig. 2(b)). The spectral spikes of the xenon arc lamp source gave rise to the side lobes in PSF. The FWHM axial resolution was measured to be $1.9 \,\mu\text{m}$ (in water). Differences between the measured and calculated PSF can be attributed to two factors: The spectral responsivity of the camera used for this calculation (obtained from vendor specifications) may be slightly different from actual spectral responsivity, and the finer spectral structures of the source may not have been resolved by our optical spectrum analyzer (0.08 nm), As a result, it is likely that the spectrum we utilized for PSF calculation was only an estimate of the true spectral content of the source.



Fig. 2. (a) Measured optical spectrum at the detector and (b) point spread function of the FFOCM system.

3. Detection sensitivity

Signal to noise ratio (SNR) is a key system parameter that affects FFOCM image quality and penetration depth. The SNR analysis of shot noise limited silicon-based FFOCM systems using the four-quadrant phase shift method have been previously presented in Ref. 4. The electrical noise, including the read out noise and the dark noise, of commercial InGaAs cameras may not be negligible compared to the shot noise, and therefore should be considered in the SNR analysis. Assuming that the well of each InGaAs array element is full upon maximum illumination, and neglecting the relative intensity noise (RIN), it can be shown that the system SNR is given by

$$SNR = \frac{2NR_{s}R_{r}}{(R_{r} + R_{inc})^{2}} \frac{\xi_{max}^{2}}{\xi_{max} + \eta^{2}},$$
(1)

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where *N* is the number of image accumulations, R_r is the reflectance of the reference mirror, R_s is the sample reflectance, R_{inc} is the portion of incoherent light remitted from the sample, ξ_{max} is the full-well-depth (FWD) per camera pixel, and η is the noise-equivalent electrons (NEE) representing total electrical noise. From Eq. (1), it can be seen that increasing the FWD provides a higher SNR, since it enables illumination of the sample with higher power. The FWD of the camera used in our system can be increased by custom adjustment of the camera gain setting. However, increasing the gain also causes the electrical noise of the camera to increase proportionally. With $\xi_{max} \rightarrow M\xi_{max}$ and $\eta \rightarrow M\eta$, the sensitivity of the system defined by the minimum detectable reflectance (SNR = 2) can be expressed as

$$S[dB] = 10 \times log\left[\frac{(R_r + R_{inc})^2}{MNR_r\xi_{max}} \left(1 + \frac{M\eta^2}{\xi_{max}}\right)\right].$$
 (2)

With ξ_{max} =800,000 and η =400 for the SU320MSW camera, a sensitivity enhancement of 7.8 dB could theoretically be obtained when $M = \infty$. In practice, however, the FWD should not be indefinitely increased because the number of photons converted into electrons is limited by the brightness of the light source. Additionally, the sensitivity enhancement is not significant above a certain value of M, because of the increased electrical noise. Considering the above factors, we increased the FWD of the SU320MSW InGaAs camera to 6,000,000 (M=7.5) by increasing the gain (electron/count) of the camera through the adjustment of the current mirror gain between the detector and the integration capacitor, which corresponded to an expected sensitivity enhancement of 5.6 dB. Table 1 summarizes the theoretical and measured sensitivities for the two gain settings, demonstrating a sensitivity improvement of 5.6 dB for the higher gain setting, which agreed with the theoretical improvement.

Table 1. Sensitivity for different gain settings (M) of the InGaAs Camera. $R_r = 2.5$ %. Sample was a partial reflector with -57 dB reflectance (A gold-coated mirror with a neutral density filter).

	M=1, N=1	M=7.5,N=1	M=1, N=100	M=7.5, N=100
Theoretical sensitivity (dB)	74.2	79.8	94.2	99.8
Measured sensitivity (dB)	68.8	74.3	88.7	94.1

Our measured sensitivities were approximately 5.5 dB lower than the theoretical sensitivities, which may in part be attributed to the objective lens losses (~ 4.5 dB). Since the full-well of the InGaAs detector at M=7.5 is filled when the sum of sample and reference arm reflectances are ~2.5%, and the amount of incoherent light typically coming back from scattering samples, R_{inc} , is approximately 1% [2], the reference mirror reflectance was set to R_r =1.5% for biological tissue imaging. For these parameters, a ~ 72 dB sensitivity (~ 92 dB with N=100) is expected. These values are in concert with previous calculations and measurements obtained for silicon-based FFOCM systems and as a result, we would expect to achieve a similar SNR performance using the InGaAs camera.

4. Imaging penetration depth: InGaAs camera vs. Silicon camera





Fig. 3. FFOCM images acquired through 2.1 mm thick Intralipid solution. (a) and (b) are the images through 3 % Intralipid solution with the systems using Si camera and InGaAs camera, respectively. (c) and (d) are the images through 4 % Intralipid solution with Si camera system and InGaAs camera system, respectively.

To compare the imaging penetration depth of FFOCM systems with different wavelength ranges, we built another FFOCM system with a silicon CCD camera (DS-12-16K5H, $128 \times$ 128 pixels, 12 bits, 490 Hz, Dalsa) using the same objective lenses (OFR-LMO 20x). The shorter-wavelength, Si FFOCM system operated in the 0.65 - 0.95 µm wavelength range. A tungsten-halogen lamp (Oriel 6333) was used as the light source for Si system, providing a measured axial FWHM resolution of 1.1 µm. Since the primary spectral spikes of the xenon arc lamp were located in the wavelength range of $0.8 - 1.0 \,\mu\text{m}$ and the bright illumination of the xenon arc lamp was not necessary for the silicon CCD camera due to its much smaller FWD with respect to the InGaAs camera, the tungsten-halogen lamp was considered to be a proper light source for the Si FFOCM system. The sensitivities of both systems were set to 72 dB by adjusting the number of image accumulations (N). Images of a 1951 USAF resolution target were obtained from both systems through a 2.1 mm layer of Intralipid solution, which is a phantom commonly utilized to simulate scattering by biological tissues [19,20]. Figures 3(a) and (b) show the FFOCM images of the resolution target obtained through a 3 % Intralipid solution. At this Intralipid concentration, images from both Si and InGaAs FFOCM systems show the resolution target, but the smallest Group 7 element 6 bars were not clearly resolved with the Si system. The signal levels of the brightest bars were approximately 6 dB and 24 dB higher than the noise level with the Si and InGaAs systems, respectively. At 4%, the resolution chart was completely obscured by the Intralipid in the Si system and the entire signal was below the noise level. With the InGaAs system, however, many of even the smallest Group 7 bars could be visualized underneath the 4% layer; the signal level at the longer wavelength was approximately 8 dB above the noise level.

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5. Images of biological samples



Fig. 4. FFOCM images of head mesenchymal cells of the fixed *Xenopus laevis* embryo, *ex vivo*. (a) Movie of a series of en face images from ventral side (top) to dorsal side (bottom) (presented at 30 fps, smaller (compressed) version: 2.5 MB, larger (uncompressed) version: 15 MB). (b) Cross-sectional image acquired from 1640 *en face* tomographic images. Scale bar: 100 μm.

Various biological samples were imaged with our InGaAs FFOCM system. In order to demonstrate sub-cellular resolution, we imaged a *Xenopus laevis* embryo, *ex vivo*. For the sample preparation, a whole embryo at stage 49 (according to Nieuwkoop and Faber tables) was fixed in MEMFA (0.1M MOPS [pH7.4], 2mM EGTA, 1mM MgSO₄ and 3.7% formaldehyde) for about an hour. For this sample, fixation was required as we empirically found that the cellular structures of the unfixed *Xenopus* embryo degraded during the extended period of time required for imaging the entire three-dimensional volume. Prior to imaging, the embryo was transferred into a petri dish and positioned with the ventral side up. The embryo was covered with 1× PBS (phosphate-buffered saline), which served as the immersion medium for the microscope objective. A three-dimensional data set with a volume of 320 μ m × 260 μ m × 1620 μ m was acquired by moving the sample axially (z) in 1.0 μ m steps with a high-precision motorized translation stage. A series of *en face* images acquired from top (ventral side) to bottom (dorsal side) of the embryo with 1.0 μ m steps is presented as a movie (shown at 30 fps) in Fig. 4(a). 30 images were accumulated to construct a single *en face* image, resulting in a sensitivity of 86 dB. The acquisition time per image was 2 s. Figure

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4 shows *en face* and cross-sectional images of head mesenchymal cells of the embryo. The cell walls and the nuclei were clearly visualized at depths of up to 1.3 mm from the surface, demonstrating the high-resolution and deep penetration capabilities of the InGaAs FFOCM system. Even though the side lobes of the PSF were relatively high, they were not readily apparent in the cross-sectional reconstructions of the Xenopus (Fig. 5(b)). If problematic in future application of InGaAs FFOCM with the arc lamp source, spectral shaping and/or deconvolution techniques [21] can be utilized to improve cross-sectional imaging by reducing the PSF sidelobes.

Another embryo at stage 47 was prepared as described above (fixed), and the heart and the eye were imaged. A cross-sectional image, shown in Fig. 5(a), reveals the structure of the embryo heart, including the ventricle, the two chambers comprising the atrium, and the atrio-ventricular valve. From the movie of a series of *en face* images (1.5 μ m step) shown in Fig. 5(b), the microstructure of the embryo eye, including the epithelium, retinal pigmented epithelium, and neural retina can be visualized with the InGaAs FFOCM system.



Fig. 5. (a) Cross-sectional FFOCM image of *Xenopus laevis* heart, *ex vivo*. V: ventricle, A: atrium, S: atrial septum, AV: atrio-ventricular valve. (b) Movie of a series of *en face* images of *Xenopus* eye (presented at 20 fps, smaller (compressed) version: 2.5 MB, larger (uncompressed) version: 5.8 MB). E: epithelium, RPE: retinal pigmented epithelium, NR: neural retina. Scale bar: 100 μ m.

To compare penetration depth in highly scattering tissues, we imaged fresh swine small intestine *ex vivo* with both the InGaAs system and the Si system (Fig. 6). The sensitivity of both systems was 83 dB (1fps imaging with the InGaAs system and 0.6 fps with the Si system). Cross-sectional FFOCM images provided clear views of the characteristic villi of the small intestine. Small regions of bright reflectivity, consistent with nuclei, can also be observed in images obtained by both systems (Fig. 6). Image penetration was measured by determining the location within the image where the SNR was equal to two. The penetration in the swine intestine in the center of the villous projection was 620 μ m with the InGaAs FFOCM system (Fig. 6(a)) and 380 μ m with the Si system (Fig. 6(b)). A sequence of *en face* images acquired with the InGaAs system in 1 μ m steps is presented as a movie in Fig. 6(c).

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Fig. 6. Cross-sectional FFOCM images of swine small intestine, *ex vivo*, acquired (a) from InGaAs system and (b) from Si system. Detection sensitivity of both systems was set at the same value of 83 dB. (c) Movie of a series of *en face* images of swine small intestine from InGaAs



Fig. 7. Cross-sectional FFOCM image of human thyroid tissue, *ex vivo*. F: follicles. Scale bar: 100 μ m. system (presented at 20 fps, smaller (compressed) version: 2.6 MB, larger (uncompressed) version: 9.5 MB) . Scale bar: 100 μ m.

Figure 7 shows a cross-sectional image of fresh human thyroid tissue, obtained *ex vivo*. Follicles of different sizes were observed. Human tissues are typically much more scattering than *Xenopus* tissues, but we were able to visualize structures located deeper than 700 μ m from the surface. Enhanced imaging penetration may be advantageous for *in vivo* imaging,

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especially through highly scattering tissues. However, the current imaging speed of the FFOCM system (~1s/frame for ~85 dB sensitivity) may need to be increased for *in vivo* imaging in order to minimize artifacts caused by patient motion.

6. Conclusion

With the exception of ophthalmic applications, where the aqueous and vitreous humors are transparent, 1.2-1.4 μ m has become the standard wavelength range for coherence gating-based imaging due to enhanced imaging penetration at these wavelengths. Although the high-resolution capability of FFOCM has great promise for non-invasive diagnosis, previous embodiments have primarily relied upon Si-based detector arrays that cannot effectively be used beyond 1 μ m. In this paper, we have demonstrated an ultrahigh-resolution FFOCM system using an InGaAs area scan camera that makes use of the entire spectral range of 0.9 μ m to 1.4 μ m. We have experimentally demonstrated, in both phantoms and tissues *ex vivo*, that this longer wavelength system has a superior imaging penetration depth than a comparable system at 0.8 μ m. Furthermore, images of *Xenopus* embryos and swine intestinal epithelia obtained with the InGaAs system were comparable in quality to those obtained with silicon-based systems, demonstrating the capability of InGaAs FFOCM to provide similar architectural and subcellular information content. We therefore believe that the FFOCM system with InGaAs camera has significant potential for ultrahigh-resolution imaging in highly scattering samples and in applications where large image penetration is desirable.

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