Three-band, 1.9-μm axial resolution full-field optical coherence microscopy over a 530-1700 nm wavelength range using a single camera

Antoine Federici, Arnaud Dubois

To cite this version:
Antoine Federici, Arnaud Dubois. Three-band, 1.9-μm axial resolution full-field optical coherence microscopy over a 530-1700 nm wavelength range using a single camera. Optics Letters, Optical Society of America, 2014, 39 (6), pp.1374-1377. 10.1364/OL.39.001374. hal-00956323

HAL Id: hal-00956323
https://hal-iogs.archives-ouvertes.fr/hal-00956323

Submitted on 7 Mar 2014
Full-field optical coherence microscopy (FF-OCM), also often referred to as full-field optical coherence tomography (OCT), was introduced a few years ago as an alternative to conventional OCT. FF-OCM uses an interference microscope and an area camera combined with a low-coherence illumination source for acquisition of en face tomographic images without transverse scanning [1,2]. The technique was applied to high-resolution (reaching $\sim$0.9 $\mu$m at $\sim$700 nm center wavelength [3]) noninvasive three-dimensional imaging of various semitransparent samples.

Several extensions of conventional FF-OCM have been developed, including the ability to exploit the spectroscopic response of the imaged sample. The purpose of this complementary imaging modality is to enhance image contrast, permitting better differentiation of the sample structures through their spectroscopic properties and providing additional information on the sample composition. A first technological approach involves detecting the whole interferometric signal and analyzing it using Fourier mathematics to provide spatially resolved spectroscopic information [4]. However, this method requires intensive computation and high mechanical stability to avoid degradation of phase-sensitive information. When implemented to conventional scanning OCT [5], this method is much faster, but implies a trade-off between spectral resolution and spatial resolution. This concern can be overcome in en face imaging by using high NA objectives for confocal sectioning as demonstrated in spectral-domain OCT (SD-OCT) [6]. Nevertheless, the working distance may then be a limitation of the accessible imaging depth.

An alternative to take advantage of the spectroscopic response of the sample is to image it in several distinct bands. High-resolution FF-OCM [2] and conventional OCT [3] were demonstrated in two bands, at $\sim$800 nm and $\sim$1200 nm center wavelengths, using, respectively, a halogen lamp and a supercontinuum laser as broadband light sources and two different detectors. Even though imaging can be performed simultaneously in the two bands, these approaches are not technologically convenient for multiband imaging since each band requires a dedicated detector. Three-band FF-OCM, using a single camera and three distinct light-emitting diodes (LEDs) in the red (R), green (G) and blue (B) bands, was reported to produce true-color images [9]. However, this system has a moderate axial resolution of $\sim$10 $\mu$m due to the relatively narrow power spectrum of LEDs. Besides, the spectral range is limited to the visible, which is not optimal for deep imaging. Three-band imaging was also implemented in SD-OCT, at high speed and high spatial resolution, but over a spectral range limited to $\sim$40 nm [6].

Three-band imaging is particularly interesting to produce color images using the conventional RGB three color channels. For high-resolution three-band FF-OCM, the bands have to be broad and possibly in the infrared to enable deep imaging, which constitutes a technological challenge. In this Letter, we report on a high-resolution high-sensitivity three-band FF-OCM system using a single camera and a single light source, covering a spectral range from 530 to 1700 nm.

The layout of our three-band FF-OCM imaging system is depicted in Fig. 1. A halogen lamp is used as the low-coherence light source. It is set up in a Köhler illumination arrangement to achieve uniform illumination of the sample. A broadband beam splitter separates the light beam into a reference arm and a sample arm of a Linnik-type interferometer with a microscope objective placed in each arm [10]. One surface of a wedge prism made of BK7 glass is used to provide a plane reference surface with 4% reflectivity, which is placed in the focal plane of the microscope objective in the reference arm. The angle of the wedge prism prevents the light reflected...
of four phase-shifted interferometric images [10]. The phase-shift is generated by making the reference surface oscillate using a piezoelectric transducer synchronized with the camera triggering signal.

Two different 400-mm focal length doublet lenses, one optimized for the 600–1000 nm wavelength range and the other one for 1000–1600 nm, are used depending on the filter combination, to project the interferometric images onto the camera sensor. Kinematic bases are employed for interchanging the lenses quickly and ensuring that still exactly the same part of the sample is imaged.

Three illumination bands [see Fig. 2(b)] within the range of spectral sensitivity of the camera are selected by using three filter sets held in filter wheels (see Table 1). The three bands, centered at 635, 870 and 1170 nm, spread out over the 530–1700 nm spectral range. Changing the filter set and the tube lens takes only a few seconds. Nevertheless, this requires the sample to be stable during this time, which may prevent from in vivo biomedical imaging.

Water-immersion microscope objectives typically cannot be used to image at wavelengths longer than ~1.3 μm, because of the absorption of light by water. Besides, refractive objectives cannot function efficiently over the broad spectral range considered here (530–1700 nm) because of optical aberrations and limited transmittance. Instead, reflective microscope objectives are used here (15×, 0.28NA), which are reverse Cassegrains following the Schwarzschild design. The primary mirror has a spherical concave surface with a center hole. The secondary mirror is a small convex spherical mirror that is machined into a spider assembly. The mirrors are broadband coated with gold for optimal reflectivity from visible to infrared. Accordingly, they have zero chromatic aberration, and negligible coma, spherical, and astigmatic aberrations.

Due to the broad spectra considered here, dispersion mismatch between the two arms of the interferometer may lead to a significant decrease of signal contrast and axial resolution. This phenomenon is partially avoided thanks to the rotating glass plate placed in the reference arm, whose role is to adjust the zero-delay position as well as to keep the dispersion mismatch minimized as the imaging depth in the sample is changed. Theoretically, a perfect compensation is achieved if the group velocity dispersion of the glass plate is equal to the one of the sample. By assuming that biological samples are mainly made of water, fused silica appears well suited with regard to its dispersion properties [12]. Both dispersion compensation plates (DCPs) are initially oriented at the same angle θ₀ (see Fig. 1). According to numerical simulations, θ₀ = 30° appears to be a good trade-off for minimizing both the deviation of the light beam and the losses by reflection on the DCP surfaces when the plate is rotated by an additional angle δθ. This value is then chosen as fixed angle for the DCP in the sample arm and as starting angle for the reference DCP rotation. For the three bands, we measured the variation of the optical path length difference, called d, with respect to δθ and fitted it with a polynomial function of order 3 (Fig. 3) in order to set the rotating movement parameters of the reference DCP during the acquisition.

The experimental data differ by less than 1% with
that one of the scope objectives and the mean wavelength transverse resolutions in the three bands. Like the axial resolution, it is desirable to have the same transverse resolution in the three imaging bands for superposition of the images. The acquired data are then processed by convolving every en face image with a suited two-dimensional Gaussian function, so that the transverse resolution is constant over the three bands.

The FF-OCM signal is proportional to the square root of the sample reflectivity and to the number $N$ of interferometric images that are accumulated. In order to achieve identical signals in the three bands for a sample with spectrally constant reflectivity, we first adjust the exposure time of the camera depending on the band so that the number of photoelectrons is always close to the full-well capacity, and then set $N$ depending on the band to compensate for variation of the fringe contrast. This provides a detection of reflectivity variations with a precision of $\sim 10\%$. By using a BK7 glass plate whose reflectivity varies by less than $5\%$ between the bands, we choose $N$ equals to 30 (band 1), 26 (band 2), and 24 (band 3) to obtain a quasi-constant FF-OCM signal over the three bands given the precision. In these conditions, the acquisition frame rate of en face tomographic images varies from 1 Hz in band 1 to 4 Hz in band 3, while the detection sensitivity defined as the lowest detectable sample reflectivity $\sim 10\%$ is close to 85 dB for the three bands. Thus, for each band, the performances are comparable to the ones of conventional FF-OCM.

Three-band imaging can reveal the presence of details in a sample, owing to their wavelength-dependent scattering and absorption properties, which are imperceptible otherwise. As an illustration of the system ability, a wood sample with two drops of different pigment colors deposited at its surface was imaged (Fig. 5). Since the reflectivity of wood structures does not vary significantly in the three bands, the surface of the sample appears white. Red stains are visible on the surface in the RGB representation due to side lobes in the interferogram envelope of band 3 [Fig. 4(c)]. A slight red hue can also be observed on the wood layers. This tendency is consistent with the fact that longer wavelengths penetrate deeper than shorter wavelengths due to reduced scattering.

Table 1. Features and Experimental Performances in the Three Bands

<table>
<thead>
<tr>
<th>Filter Combination</th>
<th>Band 1</th>
<th>Band 2</th>
<th>Band 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_0$ (nm)</td>
<td>635</td>
<td>870</td>
<td>1170</td>
</tr>
<tr>
<td>$\delta \lambda$ (nm)</td>
<td>120</td>
<td>220</td>
<td>390</td>
</tr>
<tr>
<td>$\delta z$ ((\mu m))</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>$\delta(x, y)$ ((\mu m))</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>$\delta(x, y)$ ((\mu m))</td>
<td>1.4</td>
<td>1.9</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*After post-acquisition image convolution.

One can notice that while it is almost impossible to simultaneously detect and distinguish the two painted regions with only one or two bands, three-band imaging with RGB representation clearly reveals them. Intensity profiles of the RGB image are shown to highlight the presence of these two pigments and provide a measurement of their size. One can deduce from Fig. 5 that one of the pigment absorbs light between 600 and 1000 nm.
An example of how three-band FF-OCM can be used for biological sample characterization is shown in Fig. 6. Two distinct human hairs, a light one and a dark one, were imaged.

Hair darkness is relative to the quantity of eumelanin pigments. Since eumelanin absorption decreases as wavelength increases [14], dark hair absorbs visible light, but not infrared light, much more readily than light hair. This is why a difference of imaging penetration depth can be observed between the hairs in band 1 but not in band 3. Scattering reinforces this difference since it is weaker in the infrared and thus the imaging penetration depth is less limited. However, signal penetration here is dominated by absorption since the difference between absorption coefficients is much more important than between scattering coefficients for distinct eumelanin in concentrations [14]. Three-band imaging can thus reveal spectral absorption properties of samples. One can notice that the sections of the hairs do not appear circular in the images. This artifact comes from a distortion of the optical wavefront due to the curved shape of the upper interface between the sample and air, and to the inhomogeneity of the refractive index within the sample.

This Letter presents an achromatic FF-OCM setup capable of high-resolution three-band imaging over a wavelength range from 530 to 1700 nm. Great care was taken to reach similar performances over the three bands. The system has a constant axial resolution of ~1.9 μm and a transverse resolution of 2.4 μm. Detection sensitivity close to 85 dB is obtained in the three bands. A dispersion compensation system is set to preserve these performances as the imaging depth is varied. Compared to conventional one-band imaging and even dual-band imaging, the technique provides image contrast enhancement and improved sample differentiation capability without significant loss of spatial resolution. Although three successive image acquisitions are required to produce a RGB image, unlike [7] and [9] one single camera and one single light source are employed, which make the system handler to use. Besides, this technological approach could be easily extended for imaging in more than three bands. This would provide real spectroscopic analysis of the imaged sample at the price of longer acquisition time and trade-off between spectral resolution and spatial resolution.

The authors wish to acknowledge the Agence Nationale de la Recherche and the European network for biophotonics “Photonics4Life.” They are grateful to Guillaume Dupuis at “Centre de Photonique Biomédicale” for providing gold beads.

References