Simultaneous analysis of flow velocity and spectroscopic properties of scattering media with the use of joint Spectral and Time domain OCT

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Abstract

In this paper we describe how to simultaneously retrieve spectroscopic and flow velocity information of scattering media such as biological tissues with the use of Optical Coherence Tomography (OCT). The main problem which must be overcome during spectral information recovery is random speckle noise. It cannot be effectively removed if the medium is stationary. However, if during the measurement time there is a continuous change of locations of scattering particles causing the phase change, an efficient averaging of speckle-affected spectra takes place. In order to implement this idea we propose to make use of the measurement protocol of joined Spectral and Time domain OCT method which was originally designed for flow velocity estimation. As a result a multi-purpose method was tailored which makes possible structural imaging, flow velocity measurement and spatially resolved light attenuation determination.

Spectral optical coherence tomography (SOCT) technique offers significantly improved imaging sensitivity and faster practical performance when compared to Time domain OCT [1]. Here, the intensity and relative delay of backscattered or reflected light is coded in a pattern of channeled spectrum produced at the output of an interferometer system and collected by a spectrometer with a multipixel photodetector. This enables the acquisition of information of one line of a tomogram within few microseconds. This feature combined with an ability to perform high resolution imaging makes this technique suitable for *in-vivo* non-invasive comprehensive studies of tissue based on three-dimensional imaging. It has been already successfully applied to ophthalmic imaging of the anterior eye and retina [2]-[3]. Moreover, the OCT fringe signal can be successfully adopted to functional analysis of biomedical objects thereby enabling fundamental studies of the retina physiology and its diseases.

Flow velocity mapping is of great importance for he functional OCT studies [4]. Recently, we have proposed an efficient method of obtaining flow velocity information from he OCT data. This method is called Joint Spectral and Time domain OCT [6]. It enables measuring a relative Doppler shift from consecutively collected spectral fringe patterns, which allows calculating the value of an axial velocity component i.e. the velocity in the direction parallel to the incident beam.

Another important issue for functional studies is a depth resolved extinction analysis, which can provide information about relative variation of the chemical content in a measured tissue. This can be used, for example to determine the level of blood oxidization or blood perfusion. The preliminary results of extinction measurements by Spectral OCT have been already demonstrated [5]. As long as the objects under investigation are turbid media, a random modulation of light intensity appears due to the random phase distribution of all light rays within the light beam and manifests itself as a random modulation of an observed spectral envelope. In this case it is impossible to extract an undisturbed spectral envelope of the fringe signal. Therefore, effective averaging is needed to eliminate Fourier domain speckles. This can be, in principle, done by averaging spectral fringes obtained for the same lateral position of a probing beam providing that the time-dependent phase of scattered light in consecutive measurements is uncorrelated.

In this contribution we propose an efficient method of reducing unwanted signal modulations and retrieving the spectroscopic data including both scattering and absorption coefficients. The great advantage of the described method is that the same measurement protocol can be used to obtain data enabling the calculation of flow velocity as well as extinction coefficients.

Joined Spectral and Time domain OCT method has been described in details elsewhere [6]. In short, for every single lateral position OCT interference patterns are registered in a time increment to detect possible modulation due to the Doppler effect. Therefore signal intensity I_i is both wave number and time dependent:

$$I_i(k,t) \propto \cos(2z_i k + 2v_i kt) \qquad (1)$$

where index i indicates *i-th* layer, z_i its location and v_i its velocity. Because such a set of data can be Fourier transformed in both spectral and time spaces this is where Spectral OCT meets Time domain OCT. Using Fourier transform in the spectral domain a regular structural

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image of the object is created, whereas using it in the time domain the information about the Doppler frequency (and thereby velocity) is obtained. Performing both transforms, one after another, velocity v distribution in depth z is retrieved and the velocity map of an object can be generated.

The measurement protocol assumes the collection of a number of spectra before moving the probing beam to the adjacent position. We assume that the scanner driving the signal has the shape of a continuous ramp. But then we have to ensure that a set of channeled spectra taken for analysis correspond to the transverse region of dimension comparable to the size of a focused beam. Because one has to collect more spectra than in regular imaging, the method requires high speed to keep the total examination time at a reasonable level.

The Spectral OCT enables extracting information to construct a single line of tomogram of the measured object from the spectral fringe signal acquired in a single exposure of a CCD camera. The signal resulting from the interference of light I_R reflected from a stationary reference mirror (terminating one arm of an interferometer) and light returning from *i-th* interface of the sample I_i can be expressed as:

$$I(k) = I_R(k) + \sum_i I_i(k) + 2\sum_{i>j} \sqrt{I_i(k) I_j(k)} \cos\left(2 z_{ij} k\right) + 2\sum_i \sqrt{I_i(k) I_R(k)} \cos\left(2 z_i k\right) \quad (2)$$

Light propagating through scattering and absorptive media is being attenuated, which is qualitatively described by the Lambert-Beer law. Therefore within the distance between two surfaces (denoted 1 and i) light intensity decreases exponentially with the wavelength dependent ratio:

$$I_i(k) = I_1(k) \exp\left(-(\alpha(k) + \kappa(k))(z_i - z_1)\right), \quad (3)$$

where $\alpha(k)$ is the absorption coefficient and $\kappa(k)$ characterizes scattering. If we assume that both coefficients are constant within the analyzed volume of a sample, we can express the envelopes of fringe signal contributions (spectral envelopes) corresponding to depths z_1 and z_i, as:

$$S_1(k) = 2\sqrt{I_1(k) I_R(k)}, \quad S_i(k) = 2\sqrt{I_i(k) I_R(k)}. \quad (4)$$

Thereforewavelength dependent normalized light attenuation along the distance $Z=z_i-z_1$ can be calculated from equation (3):

$$\frac{I_i(k)}{I_1(k)} = \left(\frac{S_i(k)}{S_1(k)}\right)^2. \quad (5)$$

Since the desired information about selective (wavelength dependent) attenuation of probing light is coded in the ratio of spectral envelopes, one can use spectral OCT signals and process them to retrieve spectral envelopes for each depth position of the scattering sample. Although the quality of data is sufficient for a generation of structural images, it is not good enough to get spectral information because the envelopes are usually affected by additional speckle noise [7]. To reduce speckle noise the averaging is performed with an additionally randomized phase – which happens naturally in the case of moving particles such as blood in vessels. Consequently, to implement this procedure a set of consecutive OCT fringe patterns is collected for every single position of a probing beam. This is exactly the same measuring regime as the one used for the STdOCT method.

In the proposed procedure, firstly, we determine the flow regions on the tomograms and then carry corresponding spectral fringes for further processing. For each predetermined transverse position, the Fourier transformation along optical frequency is performed on each *M-th* spectral fringe signal. These complex-valued signals are later filtered by multiplication by a Gaussian function centered at z_i position which is an equivalent of convolution in the *k-space*. Typically we used the Gaussian filter with FWHM (Full Width Half Maximum) enabling to transmit 25 axial pixels corresponding approximately to 40 micrometers of thickness. The result corresponds to the spectral shape of light scattered back from a chosen layer $S_i(k)$. Typically M=20 is sufficient to determine flows but it is inadequate to obtain smooth reconstruction of the spectrum. Therefore we repeat the same procedure for L neighboring transverse points. Finally, we average *LxM* reconstructed spectral envelopes to obtain a smooth spectral shape.

The experimental data presented in this paper were obtained with a prototype Spectral OCT instrument constructed at the Nicolaus Copernicus University. The instrument enables collecting 25,000 axial lines (A-scans) per second preserving a high sensitivity of 95dB. The high axial resolution (3.5 μm) was delivered by the use of a broadband light source (Broadlighter, Superlum, Moscow) emitting light from three multiplexed superluminescent diodes, giving a total wavelength span of 100nm at FWHM, centered at 830 nm. Light enters the Michelson interferometer based on single mode fibers with a broadband optical fiber coupler (AC photonics). The sample arm is equipped with the optical scan head, which enables the probe beam to be directed onto the retina in two lateral directions with an electronically controlled pair of galvanometric scanners (CamTech). When imaging the human eye, two lenses arranged in a telescope configuration are used to form a collimated Gaussian beam which is incident on the cornea and then focused onto the retina by the natural optics of the eye.

Figure 1 shows a result of simultaneous spectroscopic and Doppler measurement of ethanol solution of latex microspheres mixed with a laser dye (IR140LP) flowing through a glass capillary (700um diameter) positioned at angle 85˚ to the probing beam. The dye has maximum

peak absorption at approximately 805 nm (blue curve in Figure 1c). Additional measurement was performed for aqueous solution of intralipid. Figures 1a and 1b show the structural image and velocity profiles calculated with the STdOCT method for both cases: of microspheres and intralipid. The data used to determine spectral envelopes and thus attenuation curves via equation (5) are taken from a region corresponding to the part of cross-sectional images delineated by the color rectangles visible in Fig. 1a. The obtained extinction spectrum was compared with the one produced by a commercial spectrophotometer for the pure ethanol solution of the dye. As it can be seen in Fig. 1c the result of OCT measurement correlates well with the data obtained from the spectrophotometer despite that in the case of OCT we measured a scattering medium while in the spectrometric experiment we used a clear solution. The profile obtained for an intralipid solution decreases with the wavelength due to λ-dependent scattering of the medium.

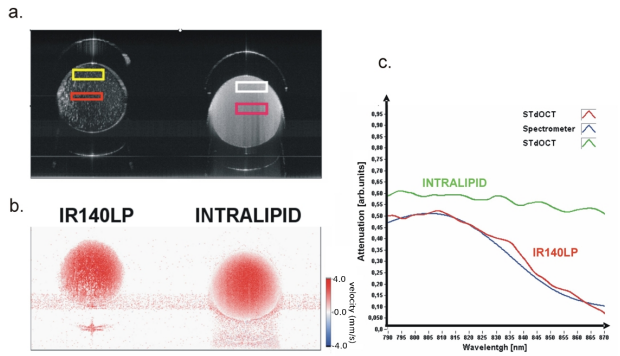

Fig. 1. Simultaneous Doppler and spectroscopic analysis of flowing micro-spheres mixed with IR140LP laser dye using (STdOCT): **a.** intensity plot showing cross-sections of two glass capillaries filled with latex microspheres mixed with the dye (left) and 2% solution of intralipid (right); **b.** map of the axial velocity distributed within the sample **c.** plot of the normalized extinction curve calculated using the STdOCT; a blue line corresponds to the normalized absorption curve of the ethanol solution of IR140LP laser dye measured by a commercial spectrophotometer.

In another experiment we tested our method in a real biological sample. In this case we measured the selected area of the human retina *in vivo* (Fig. 2a).We have chosen the region close to an optic disk where it is relatively easy to find large blood vessels. Calculated color coded velocity profiles are marked indicating the cross sections of blood vessels located in the retina. Again, the areas over which the data were averaged are marked with color rectangles of approximately the same area. The green one is located inside the vessel cross-section and the red one – in the static tissue. We expect better result of retrieval of spectral envelope for the vessel region due to additional averaging caused by the blood flow. In Figures 2b and 2c we compared the grey curve corresponding to the spectrum of the used light source with the averaged spectral envelopes from both areas. The blue curve in Fig. 2b corresponds to the spectral envelope obtained for green rectangle area – the profile is very similar to the original one. Such a correspondence is not seen in Fig. 3c where the spectral envelope obtained for the red rectangle area shows random modulation and therefore differs significantly from the red curve.

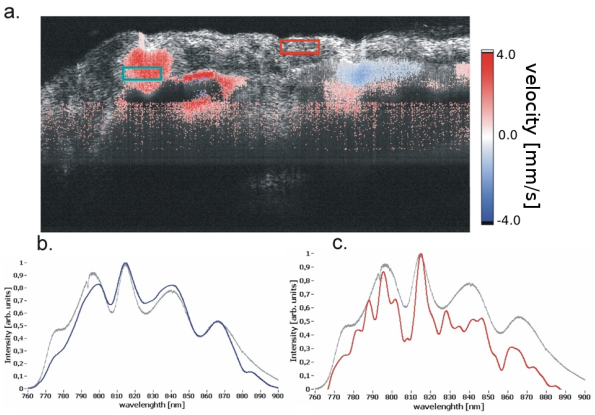

Fig. 2. Efficiency of spectral envelopes retrieval for stationary vs flowing medium of human retina imaged *in-vivo*.

In this paper we have demonstrated an efficient method of retrieving information on the depth dependent extinction in turbid media by combining Spectroscopic Fourier domain OCT method with Doppler techniques. We demonstrated that we are able to reduce efficiently speckles by averaging an OCT signal in case the measured medium is moving. We have shown preliminary results for phantom and retinal blood vasculature.

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