Sensitivity estimation of spectroscopic optical coherence tomography

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ABSTRACT

This study aims to investigate the sensitivity of spectroscopic optical coherence tomography (OCT) to small changes in the absorption properties of the imaged object as well as to evaluate its ability to resolve spatial variations in the object's absorption coefficient. Spectroscopic OCT would have the advantage to provide spatially resolved spectroscopic information at multiple wavelengths across the available bandwidth of the light source in a single measurement. An ultrahigh resolution OCT system based on a Ti:sapphire source emitting in the range of 700 nm to 900 nm with an optical bandwidth of up to 165 nm was used to measure optical absorption of specially designed, non-scattering phantoms. High speed and high resolution digitization in combination with a Morlet wavelet transform was utilized to derive spectroscopic information from the full interference OCT data. Using a non scattering phantom, the preliminary results of the present work reveal the challenges that have to be overcome in order to extract spatially resolved quantitative spectroscopic information by OCT.

Keywords: Optical coherence tomography, Ti:Al_2O_3 laser, spatially resolved spectroscopy, absorption.

1. INTRODUCTION

Optical coherence tomography (OCT) is a promising optical imaging technique that enables non-invasive, high resolution *in vivo* imaging in transparent and non-transparent biological tissue. Utilization of broad-bandwidth light sources in OCT permits imaging of tissue morphology in detail at depths significantly greater than the penetration depth offered by conventional bright field and confocal microscopy[1-4]. Derivation of spectroscopic information with OCT in addition to the high-resolution tomographic imaging presents a very attractive possibility. Unfortunately, so far only a few studies have been performed in this direction mainly because sufficiently broad bandwidth light sources have not yet been available. Previous studies on non-biological samples demonstrated spectroscopic detection over a bandwidth of ~50 nm centered at 800 nm[5] as well as at 1.3 µm[6]. Other studies involved a combination of two separate light sources (centered at 1.3 and 1.5 µm respectively) designed to detect water content[7,8]. Recently an in vitro OCT study of blood oxygenation was reported employing a broad bandwidth (95 nm FWHM) Ti:Sapphire laser[9]. Utilization of femtosecond Ti:Sapphire lasers that emit broad bandwidth light centered at 800 nm not only enables sub-cellular level resolution OCT but may also provide spectroscopic information. This spectral region is important because it overlaps with the so-called therapeutic window, covering absorption features of several biological chromophores, like melanin, oxy- and deoxyhemoglobin[10]. Therefore, an extended version of OCT may permit extraction of spatially resolved spectroscopic information, and thus to allow for characterization of the functional and/or biochemical properties of the investigated tissue. Spectroscopic OCT could also function as a type of "spectroscopic staining," in analogy to staining in histopathology by enhancing the image contrast. Recently, a state of the art, broadband (260 nm FWHM) Ti:Al_2O_3 laser has been developed for ultrahigh, 1 µm axial resolution, that can permit spatially resolved spectroscopic OCT imaging at 800 nm center wavelength[11,12].

2. METHODS

An ultrahigh resolution OCT system (fig.1) was employed in the present spectroscopic studies. The system consists of a high speed (100 Hz, 400 mm/s) fiber-optic Michelson interferometer employing a state of the art, sub-10-femtosecond Ti:sapphire laser (Femtosource compact pro, FEMTOLASERS, 800 nm center wavelength, up to 165 nm optical bandwidth, 400 mW output power). The interferometer was interfaced with a microscope delivery system. Both the fiber-optic interferometer and the optical components of the microscope were designed to support the

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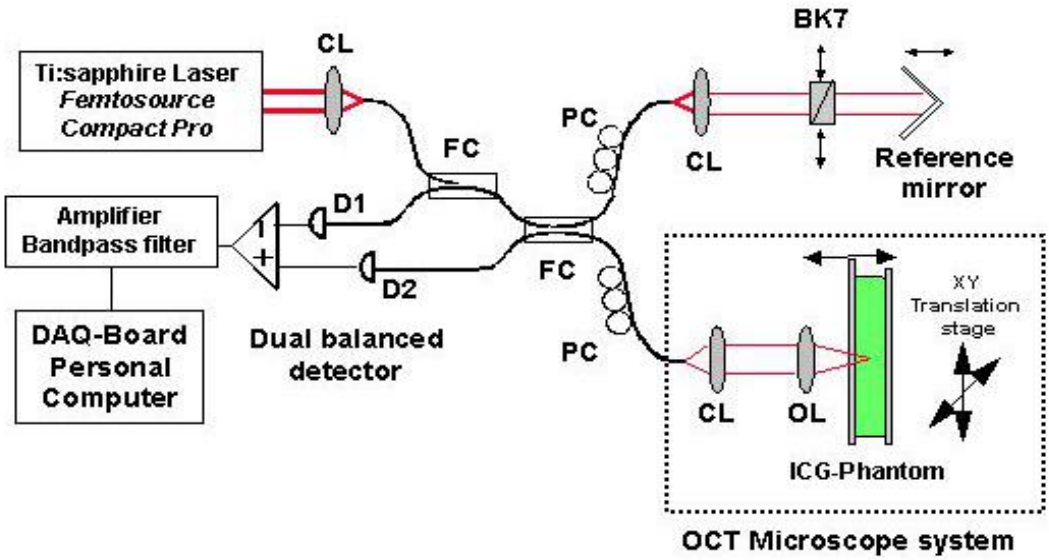
propagation of very broad band light throughout the OCT system and to compensate for any polarization and dispersion mismatch between the sample and reference arms of the interferometer. Axial resolution of 2.0 µm in air, corresponding to 1.4 µm in biological tissue has been previously demonstrated with this system[13]. A signal to noise ratio of ~ 90-95 dB was achieved at 1 MHz carrier frequency by using an incident power of 500 µW and employing dual balanced detection. Spectral information of the investigated sample can only be obtained by measuring the full interference signal and using proper digital signal post processing. In this case, full interference OCT data was digitized with a high speed (10Ms/s) and high resolution (16 bit) A/D converter. A Morlet wavelet transform was utilized to obtain spectral information from the fringe data. Since no interferometric triggering has been implemented yet into the current system, non-linearities in the scanning have been accounted for in the data processing software.

Figure 1. Ultrahigh resolution, spectroscopic OCT system: CL – collimating lens, FC – fiber coupler, PC – polarizations controller, OL – microscope objective lens, D1 and D2 – Si photodiodes, and BK7 – glass prisms for dispersion compensation.



The sample used for the spectroscopic OCT measurements was prepared by sandwiching a gel layer doped with ICG (Indocyanine Green) dye between two thin (~130 µm) glass cover slips. The reflections from the front and back surfaces of the top cover slip were used as a reference for the spectral response of the system (as well as to rule out any dispersion changes in the spectrum resulting from the presence of the glass). The reflection from the front surface of the bottom cover slip was used to determine the effect of the absorptive gel-ICG layer on the measured spectrum. In order to evaluate the sensitivity of the SOCT to small changes in the absorption properties of a medium, the thickness of the gel layer as well as the concentration of the ICG dye in it were varied. Reference measurements were performed with a sample containing undoped gel in order to register possible dispersion in the spectrum resulting from the presence of the gel. Since it is well known that the ICG absorption spectrum is both time and concentration dependent[14], the absorption spectrum of each sample used in the spectroscopic OCT experiments was measured with a spectrophotometer immediately before and after exposure to Ti:sapphire light in order to account for any time- or exposure to light related changes in the spectrum. Also, to minimize the number of parameters that can affect the magnitude of the measured OCT spectra, non-scattering phantoms were used in this study.

3. RESULTS AND DISCUSSION

The intereferometric fringes acquired with SOCT at the glass-gel and gel-glass interface of the ICG-gel sample are presented in fig.2a, while the corresponding spectra derived by a Morlet wavelet transform of the fringe data (average of 20 consecutive measurements) are shown on fig.2b. It is clear that the presence of a wavelength dependent absorber in the gel phantom causes alteration both in the magnitude and the shape of the measured OCT spectrum. The amount of absorption in the sample can be quantified at various wavelengths provided the absolute intensity of the incident and reflected light is measured and the sample thickness is known. Figure 3a shows a representative measurement of the OCT spectra from a 1 mm thick phantom. The thin black and the thick grey lines correspond to spectra measured at the glass-gel and gel-glass interfaces respectively. The thick black line demonstrates the expected change in the OCT spectrum after passing through the absorbing gel. It was determined using prior knowl-

edge of the phantom's thickness and absorption coefficient. The graph shows very good agreement between measured and expected spectra except for a small discrepancy in the wavelength region of 720 nm to 770 nm. The OCT spectral data was also used to determine

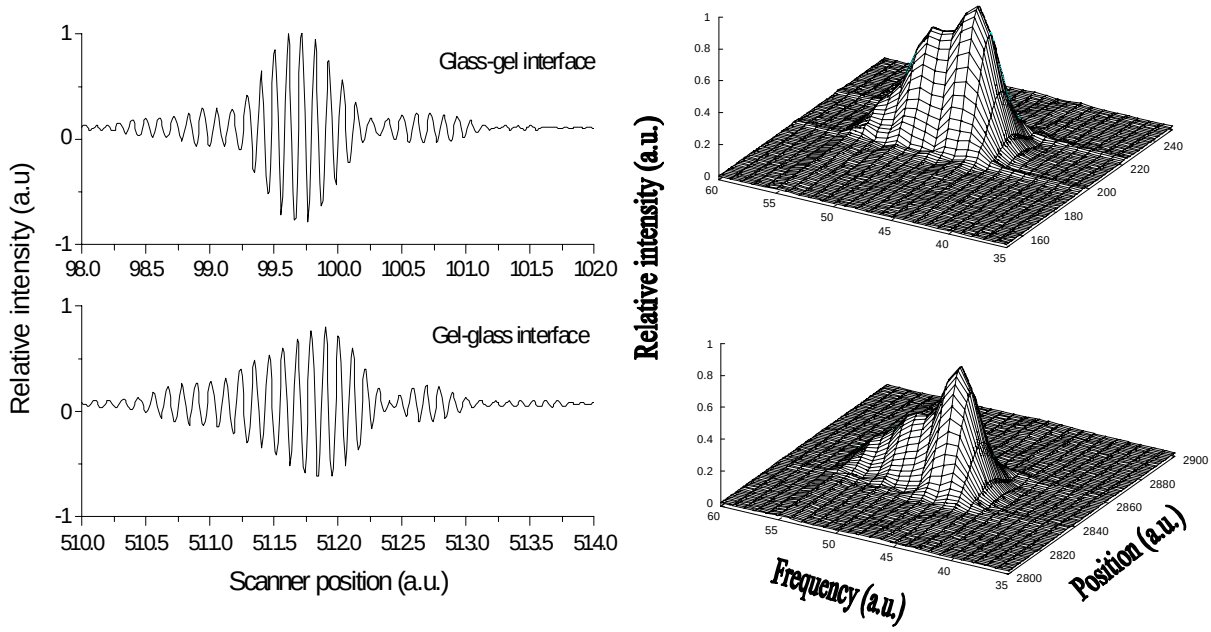


Figure 2. **a)** Interferograms acquired at the glass-gel and gel-glass interfaces of the ICG-gel layer; **b)** corresponding OCT spectra obtained through a Mortlet transform of the interferograms.

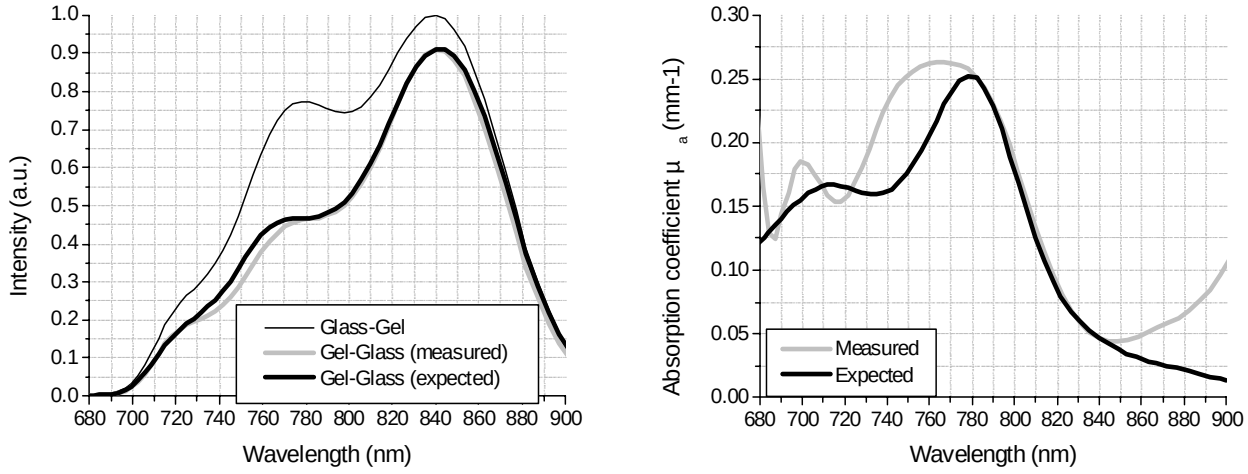


Figure 3. a) Measured and expected OCT spectra of ICG-gel phantom; b) Measured and expected absorption coefficient of the ICG-gel sample as a function of wavelength.

the absorption coefficient of the ICG-gel sample. Figure 3b presents a comparison between the phantom's absorption coefficient derived from the OCT measurements (gray line) and from transmission measurements performed with a commercial spectrophotometer (black line). It is obvious from the graph that OCT can be used for derivation of spectroscopic information, though care must be taken during processing and interpretation of the experimental

data. As fig. 3b clearly demonstrates, even small changes in the spectrum measured from an absorptive object caused for example by power fluctuations in the incident beam, can result in a very large error in the derived absorption coefficient. Considering the fact that the intensity of the back-reflected light in an OCT tomogram is sensitive to both spatial variations in the optical properties of the imaged object (index of refraction, scattering and absorption properties), and the imaging system related parameters (power fluctuations, non-linearities in the scanning and defocusing), quantitative spectroscopy in biological tissues can prove a very challenging task.

4. CONCLUSION

In this paper we have presented preliminary measurements aiming to evaluate the sensitivity of a broadband OCT system to detect small changes in the optical absorption of a non-scattering object. We have demonstrated that though in principle spectroscopic information can be derived from OCT fringe data, quantitative spectroscopic characterization of the imaged object's optical properties will require development of calibration and high sophisticated post-processing data algorithms. In the near future we will be implementing interferometric triggering in our OCT system that can remove the possibility for shifts in the spectral data from a scan to scan. We will also investigate the ability of spectroscopic OCT to resolve spatial variations in the sample absorption coefficient and will use specially designed phantoms to investigate the effect of scattering on the precision with which spectroscopic data is derived with spectroscopic OCT. Spectroscopic OCT would have the advantage to provide spatially resolved spectroscopic information at multiple wavelengths across the available bandwidth of the light source in a single measurement and would therefore have the potential to improve OCT image contrast and to obtain functional or biochemical properties of the investigated tissue.

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