Optical Coherence-domain Imaging of Subcutaneous Human Blood Vessels in vivo

Sergey G. Proskurin

Biomedical Engineering, Tambov State Technical University, Tambov, 392000, Russia

Abstract Experimental methods of Optical Coherence Tomography (OCT) are applied for two-dimensional mapping of subcutaneous human blood vessels. Structural images of in vivo human finger and human palm macro vessels (0.2-1.0 mm) before and after optical clearing using the modified low power rapid scanning optical delay line are presented. Images are scanned with 12 µm minimum spatial resolution. The described modifications enable to apply low power (0.4-0.5 mW), low noise broadband near infrared light source and to obtain structural images with detection of not only reflected but also multiply scattered coherence-gated photons. The achieved transcutaneous probing depth is about 1.6-1.8 mm.

Keywords Optical Coherence Tomography (OCT), Low Power Rapid Scanning Optical Delay Line, Optical Clearing, Differential Imaging, Blood Vessels Visualization, Coherence Probing Depth (CPD)

1. Introduction

Optical Coherence Tomography (OCT)[1] is the new, important and fast developing modality which is based on the principles of scanning low coherence interferometry (LCI) and optical coherence-domain reflectometry[2]. Depth discrimination of multiply scattered light is performed due to application of a broadband light source. It could be a superluminescent diode (SLD), femtosecond pulsed laser or a swept source. Transversal resolution of OCT images is chosen by selecting focusing optics with different numerical apertures in the sample arm. Confocal microscopy principle is utilised in this case when a fibre tip works as a pinhole. Rapid low-coherence devices can be made on the basis of fibre optic Michelson interferometer with the rapid scanning optical delay line (RSOD)[3-7] in the reference arm (RA). By detection the phase shift of the interference fringes of consecutive A-scans it is possible to detect blood flow in capillaries, of about 20 – 70 µm in diameter, in human skin in vivo using 5 mW broadband source[6]. Simultaneous intensity, birefringence and flow measurements into the depth of about 1.0 - 1.2 mm was demonstrated in the upper part of a large blood vessel and in capillaries of a human finger[7,8]. At the same time very important problem of visualisation of subcutaneous human macro blood vessels, of about 1 mm in diameter, has not been solved till recently[9].

Note that further increase of the coherence probing depth (CPD) was investigated using the transitional mode between single backreflection and diffuse wave spectrescopy by LCI only. Particle dynamics study of highly scattering media was used utilising singly scattered, multiply scattered and diffuse light detection[10]. The probing depth of the coherence-gated detection of multiply scattered light in this study has reached 1.5 – 2 mm. Scattering properties of the suspension were similar to those of biological tissue.

In this paper we describe several modifications of the rapid scanning optical delay (RSOD) to apply a low power (0.5 – 0.2 mW) irradiation of a broadband infrared light source, superluminescent diode in time domain OCT system [9]. These modifications enable to utilise coherence-gated transitional multiply scattered mode to visualise human finger and human palm macro blood vessels with diameter of about 0.2 - 1 mm. Topical application of the optical clearing material[11] have been used to visualise structural signal from the blood, which appears inside the vessel after hand exercise only.

2. Materials and Methods

Figure 1 shows the schematic of the experimental set-up based on the scanning fibre optic Michelson interferometer with two 1x2 and one 2x2 fibre couplers.

Light from the sample arm (SA) goes through the system of lenses and is focused on the sample at the distance of about 5 cm from the SA scanning mirror (SM). The transversal resolution becomes respectively low, $L_o=70 \, \mu m$, to compare with other systems utilising focusing optics with high numerical aperture[6,7]. Confocal parameter in this case is about 3.5 mm. Choice of low numerical aperture of the sample arm optics is a compromise between lateral resolution and the probing depth[10]. Despite the loss of the lateral resolution this approach enables additionally discriminate backscattered and backreflected photons from
multiply and diffusely scattered ones.

The described approach increases CPD, but compromises spatial resolution. Yet, it is still enough to study nails and skin layered structures. Imaging of the structures like skin and nails is important in dermatology to study healthy, diseased, burned tissue. Imaging of subcutaneous blood vessels into the full depth of the lumen is necessary to study vessel abnormalities like embolism and aneurysm.

Figure 2 shows the modified implementation of the Fourier-domain scanning grating based double pass optical delay line (RSOD). This implementation is different from the similar ones described before[4,5]. After the collimator near infrared light (λ = 1.3, 1.5 μm) goes to the edge of the diode laser; CL, OL, optical lenses; SM, scanning mirror; ODL, optical delay line

Transversal sample arm scanning is performed by a Galvano scanner what is essential for in vivo application to compare with application linear scanning stages. The sample arm design also gives possibility to collimate the light and put an additional lens close to the sample. This increases transversal resolution at the upper layers, but does not give possibility to discriminate multiply scattered coherence-gated photons and to visualise transcutaneous structures deeper than 1.0 - 1.2 mm[7].

The described modifications of the Fourier-domain rapid scanning optical delay line make it easy to compensate dispersion and chirp of the coherence envelope, increase scanning depth without compromising the carrier frequency, f₀, stability, and to obtain more narrow spectrum of it[5],

\[ f_0 = \frac{4\Delta x}{\lambda} \frac{\partial \alpha}{\partial t}. \]  

Here a is the angle of the scanning RA mirror, Δx – the scanning mirror offset.

Attenuating reference arm power in LCI with a stable SLD source gives considerable, up to 34 dB, S/N ratio improvement[14]. In addition, since the described RSOD enables to recombine broadband light more efficiently, it is possible to use low power, low noise SLD which itself reduces source noise and incoherent broadband light[2,15], Iᵣᵣ, in the detected interference signal intensity, Iᵢ,

\[ I = I_{in} + I_{ref} + 2\sqrt{I_{in}I_{ref}} \cos(2\pi f_0 t), \]  

Here Iᵢ and Iᵣᵣ reference and sample arm light intensity, respectively. The incoherent component, Iᵣᵣ, appears as the phase noise[2] in coherence-gated systems in contrast to the heterodyne detection in quasielastic light scattering systems with application of a narrowband source with Δλ ~ 0.1 - 1.0 nm.

The employed pivoting point offset, Δx, also corresponds to the width, Δfₒ, of the carrier spectrum in the Fourier transform of the autocorrelation function which should be similar to the width of the bandpass filter[5],
\[
\Delta f = \frac{4\Delta \lambda}{\lambda^2} \left( \Delta x - \frac{L_r \lambda}{d} \right) \frac{d\lambda}{dt}.
\]

(3)

Here \(d\) is the pitch of the grating.

Introduction of the carrier in the LCI[2] gave possibility to tune off low frequency \(1/f\) noise and at the same time keep the carrier within the range of hundreds of kilohertz. Keeping the carrier \(f_0 < 100\) kHz gives the possibility to avoid increasing at higher frequencies white noise and to use narrow bandpass filters of about 5 - 15 kHz. Also, there is no need to use high frequency electronics. Special care should be taken setting the scanning mirror offset, \(\Delta x\). Bigger offset gives bigger values of the carrier, more than 100 kHz. Small offset will lead to partial overlapping of the broadband spectrum in the Fourier plane (1, 3).

Signal processing has been performed digitally offline with a standard demodulation algorithm, which was applied earlier[9,16]. This algorithm utilises short-time Fourier transform (STFT) with the sliding Hanning window. The processing takes from one to two seconds using 2.4 GHz, Intel Pentium IV processor. Using C++ language it is possible to design dedicated software and perform real time signal processing with up to 1 kHz of RA scanning frequency[8]. Using RA scanning frequency of about 80 - 100 Hz it is obviously possible to perform such processing with one to two frames per second, 300 - 500 lines per frame, what is enough for real time imaging and visual feedback.

3. Results and Discussion

The described modifications of the time domain OCT system, for the first time, gave the possibility to obtain images of a macro blood vessel (~1 mm in diameter) underneath the native human skin with transcutaneous depth of about 1.5 – 1.6 mm[9]. The images have been taken close to a Y-junction of blood vessels considering possibility of further investigation of blood flow with complex geometry [16-18] in vivo.

The described system also gives possibility to use two wavelengths close to 1.3 \(\mu m\) and 1.5 \(\mu m\) simultaneously. Separation of the images could be performed in the processing algorithm, since the two wavelengths will give the two different carriers (1, 3). Figure 3 shows the images of a human finger tip using 1.3 \(\mu m\) SLD (\(\lambda=1298\) nm, \(\Delta\lambda=52\) nm) and 1.5 \(\mu m\) SLD (\(\lambda=1482\) nm, \(\Delta\lambda=60\) nm). Note, the OCT image using 1.5 \(\mu m\) SLD and RSOD was obtained by us for the first time[19].

Transcutaneous probing depth of the light at \(\lambda = 1.5\) \(\mu m\) is approximately twice less to compare with more commonly used for the skin study wavelength 1.3 \(\mu m\) [6]. Therefore we choose standard for this cases 1.3 \(\mu m\) source. All further results are obtained using SLD with \(\lambda=1298\) nm, \(\Delta\lambda=52\) nm.

Figure 4 shows two blood vessels of a human subcutaneous Y-junction close to the junction with considerably low resolution but with the increased probing depth up to ~1.5 mm. Refractive index of the tissue, \(n=1.4\), is used in all cases.

Figure 5a) shows another blood vessel underneath the native skin of a human finger. Frequency of the reference arm scanner is set to the value of 80 Hz. The quality at the deeper layers starts to degrade after increasing the scanning frequency and only upper layers of the skin could be observed after 100 Hz even if the carrier is kept within the range of the bandpass filter.

Figure 5a) shows another blood vessel underneath the native skin of a human knuckle joint in vivo. (a) fist image; (b) 15 min after application of the optical clearing liquid; (c) after raster scan and averaging over four consecutive A-scans; (d) 20 min after application of the optical clearing liquid. Stratum corneum, epidermis, dermis, fascia and blood vessel are clearly seen in all images. Reticular dermis is not clearly distinguishable. Size of the images is 2x2 mm².

To increase the resolution and the penetration depth,
glycerol was utilised as an optical clearing liquid[11]. It was
topically applied onto the skin where the blood vessels (veins)
have been barely seen by the naked eye. Figure 5b) shows the
image of the same blood vessel after 15 min from the
beginning of the optical clearing. To reduce shot noise, phase
noise from the incoherent detection and speckle noise which
appears due to high spatial coherence of the source we per-
form averaging over 2 to 4 A-scans while changing the angle
of the scanner in the sample arm. The scanner moves 2 to 4
times slower in this case ensuring raster scanning and cor-
responding raster averaging over 2 to 4 lines within one pixel
size. In addition to that 0.1 - 0.3 mm pivoting point offset of
the sample arm scanning mirror was introduced to perform
movement of the beam across each pixel of the image. The
raster averaging elongates acquisition time proportionally up
to 2 to 3 sec but additionally increases signal-to-noise ratio
by 4 - 8 dB. This approach enables to reduce speckle noise,
increase contrast and better visualise deeper layers of the
skin, Figure 5c). A distinct structural signal backscattered
from blood was possible to detect as well. It appears inside
the blood vessel after one minute of hand exercise only,
Figure 5b,c). The achieved RA phase stability between
consecutive A-scans was about 10 - 20 %. Therefore it was
not possible to detect reliable phase shift between consecu-
tive interferograms and extract velocity information of the
blood flow in the shown vessels. Nevertheless, intensity
signal of the structural image from faster moving blood
corpuscles becomes stronger due to more scattering events
over probing volume for the chosen detection time.

Figure 5d) shows the same vessel after the 20 min from the
beginning of the process of optical clearing. The upper sur-
face structures are less distinguishable, although the struc-
tures at the depth of about 1.5 - 1.6 mm are better resolved.
The shape of the vessel appears to be more rounded. This
phenomenon is important and could be explained by the
change of average refractive index of the tissues around the
lumen of the vessel.

Figure 6 shows images of human palm with much more
dense skin than one of a finger. These images were obtained
before (a) and after (b) 15 min application of the optical
clearing liquid. No averaging has been performed in this case,
although the circled macro structures appear after the
cleaning only and surely are the blood vessels.

![Image](Image)

**Figure 6.** OCT images of a human palm in vivo, (a) before optical
clearing; (b) 15 min after the optical clearing. The circled structures are the
blood vessels. Size of the images is 2x2 mm²

Separate A-scan calculations reviled that the upper layers
are resolved with the accuracy of about 12 - 15 micron,
which corresponds to the axial resolution \( L_{ax} \). The lower
layers are resolved with the resolution of 30 - 50 microns
only. This strongly suggests that the multiply scattered
photons which are still coherent to the reference signal are
detected from the structures deeper than 0.8 -1.0 mm[10].
Since the spatial resolution gradually decays the bandwidth
of the source could be correspondingly chosen. This we
presume will additionally increase the coherence probing
depth while still compromising the lateral and depth resolu-

4. Conclusions

The modified low power RSOD in the reference arm of the
scanning interferometer enables to apply 1.3 μm wavelength
simultaneously with 1.5 μm. Application of any other
wavelength in between or close to these two is also possible.
This enables to make rapid differential and spectral OCT
imaging for two or several wavelengths. In addition the
modified RSOD is quite small ~ 11x7x5 cm³, what is im-
portant from practical point of view. Using this experimental
approach for the first time we have demonstrated subcuta-
neous macro (~0.2 - 1 mm) blood vessels underneath the
native human skin by detection of singly and multiply sca-
tered photons. The described set-up enables to increase
transcutaneous coherence probing depth up to 1.5 - 1.6 mm
and to resolve deeper tissue structures while compromising
spatial resolution. Coherent detection of least scattered
photons demonstrates transitional coherence-gating mode
between single scattered and diffuse photons[20]. Since
ballistic photons could be discriminated from the diffuse
ones at the depth of about 20 - 25 MFP[10,21] and having in
mind the reduced scattering coefficient of biological tissue ~
1 mm⁻¹, it seems to be reasonable to assume further increase
of transcutaneous coherence probing depth to the values of
about 2 - 2.5 mm.

Doppler OCT technique[6,7] was not applicable in this
case due to low phase stability of the consecutive A-scans.
In addition, all experiments have been performed when incident
light was perpendicular to the surface of the skin. Will it be
possible to extract valuable velocity information in such
transitional coherence-gated mode to study flows with
complex geometry[16-18] in human subcutaneous junction
of blood vessels is still a question to solve. Applying optical
circulator (Faraday rotator) instead of the first 1x2 fibre
splitter it is possible additionally reduce the source power by
the factor of two and eliminate the signal reflected back into
the source[15].

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