Feasibility of Picosecond Laser-Doppler Flowmetry Provides Basis for Time-Resolved Doppler Tomography of Biological Tissues

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Abstract

A detectable signal is obtained from a laser Doppler flowmeter operating in the heterodyne mode with nano- and pico-second pulse laser sources. The ultrashort pulse probing *may be useful* for depth-dependent time-resolved laser Doppler velocity measurements of blood perfusion in biological tissues. © *1998 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(98)00402-X]

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1 Introduction

Time resolved laser spectroscopy (TRS) of stationary biological tissues is a rapidly developing method for biomedical tomography and diagnostics. With the time gating technique it is possible to filter away the photons traveling over a relatively long time in strongly randomized trajectories and to detect only the early arriving photons whose paths just slightly decline from an average trajectory, connecting the point of incidence of laser light with the point of detection. It has been reported in a number of publications that with TRS of early arriving photons, a spatial resolution of optical inhomogeneities 100 μm in size is possible.[1,2]

Laser Doppler flowmetry is an experimental technique used in biomedical monitoring and diagnostics of blood perfusion and transport. Doppler frequency shifts of light arising in the act of scattering of photons by moving erythrocytes are proportional to the velocity magnitude of the blood cells and can be measured experimentally. The resulting Doppler shift spectra are processed in order to retrieve information about the blood flow characteristics.

Continuous laser sources are commonly used in laser Doppler flowmeters (LDF). When applied to biotissues, the spatial resolution of the LDF is low, as a consequence of intense light scattering. On the basis of the data obtained experimentally with stationary TRS and the results of Monte-Carlo simulations[3] it would be expected that a better spatial and spectral resolution of the LDF could be attained, by combining a conventional laser Doppler with TRS techniques.

In our study we demonstrate the feasibility of measuring a heterodyne Doppler shift spectra for three pulsing laser sources: a nanosecond laser diode, a 35 ps duration of pulses Nd:YLF laser, and a 3–8 ps pulse duration dye laser. We consider the results obtained as a preliminary step towards a time-gated LDF for blood perfusion tomography. The results of the present study confirm the feasibility of obtaining a detectable Doppler signal on a noticeably shorter pulse duration scale than has been reported in earlier laser Doppler lidar systems.[4]

2 Theoretical Considerations

A heterodyne time-resolved LDF operates by mixing two successions of ultrashort laser pulses from the same source. The pulses in either succession are mutually incoherent, though each pulse in one succession has a coherent counterpart in the other. The pulses from the first succession, referred to hereafter as the reference beam, propagate in the optical system of the LDF without scattering and keep their original characteristics, e.g., shape, duration, and phase. Each pulse from the second succession (a signal beam) which is transmitted or reflected by

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the light scattering media, changes its properties as a result of multiple scattering and thereby carries information about the media properties. In tissues containing moving blood cells, extra linear in-time variations in phase of the light waves arise due to Doppler frequency shift ω_D. If a wave traveling in the direction given by a unity vector $\mathbf{n}_i$ strikes a moving particle with a velocity $\mathbf{V}$ and after scattering proceeds in the directions given by another unity vector $\mathbf{n}_s$, the Doppler shift is found from the equation:

$$\omega_D = \omega \cdot (\mathbf{n}_S - \mathbf{n}_i \cdot \mathbf{V}/c), \tag{1}$$

where ω is the frequency of the incident wave and c is the speed of light. The expression in brackets denotes the scalar product of the vectors. Given ω_D, ω, and the geometry of the scattering event, the magnitude $\mathbf{V}$ of the velocity of the particle can be found from Eq. (1).

Upon mixing at a photodetector (PD) the reference and signal pulse successions produce a photocurrent modulated with Doppler shift ω_D. The reference pulse is much shorter than its signal counterpart, which is broadened by multiple scattering, and if the two are mixed an interference component in the intensity of light will arise on the time interval where the pulses overlap. This opportunity may be used for time resolved laser Doppler tomography of biotissues.

Let us calculate a PD signal of the heterodyne LDF with an ultrashort pulsing laser source. In our analysis we follow a scalar wave approach. Each laser pulse is regarded as a wave packet with frequencies in the range of $\omega_o - \Delta\omega$ to $\omega_o + \Delta\omega$, where ω_o is the mean frequency and $2\Delta\omega$ is the frequency band of laser emission. A complex wave amplitude $U_r(t - t_p)$ of the reference beam pulse and the amplitude of the signal pulse $U_s(t - t_p)$, Doppler shifted and delayed by a time τ can be represented in the form

$$U_r(t - t_p) = \int_{\omega_o - \Delta\omega}^{\omega_o + \Delta\omega} S_r(\omega) \exp\{j\omega(t - t_p)\} d\omega, \tag{2}$$

$$U_s(t - t_p + \tau) = \int_{\omega_o - \Delta\omega}^{\omega_o + \Delta\omega} S_S(\omega) \exp\{j(\omega + \omega_D)$$
$$\times (t - t_p + \tau)\} d\omega, \tag{3}$$

where $S_r(\omega)$ and $S_s(\omega)$ are complex spectral amplitudes of the light waves, accounting for a spectral mode structure of laser emission, with j as the imaginary unity. Local time t is introduced with respect to the pulse arrival time t_p corresponding to the maximum of each reference pulse with a certain index p. A photocurrent $i(t - t_p)$ produced by interference of each coherent reference pulse and its coherent counterpart is proportional to the intensity of the detected light:

$$i(t - t_p + \tau) = i_{inc} + i_c, \tag{4}$$

where $i_{inc} = \alpha \cdot (U_r U_r^* + U_s U_s^*)$ is an incoherent term describing self-mixing of the reference and signal pulses and $i_c = \alpha \cdot 2\,\text{Re}\,(U_r U_s^*)$ is the heterodyne component due to coherent cross mixing of the pulses. Here α designates the sensitivity of the PD to the light in the given frequency band and the asterisk denotes complex conjugation. The cross component is of major interest for time-gated signal detection and we shall focus on this component. With expressions (2) and (3) we obtain

$$U_r U_S^* = \int_{\omega_o - \Delta\omega}^{\omega_o + \Delta\omega} d\omega \int_{\omega_o - \Delta\omega}^{\omega_o + \Delta\omega} d\omega_1 S_r(\omega) S_S^*(\omega_1)$$
$$\times \exp\{j(\omega - \omega_1 - \omega_D)(t - t_p) - j(\omega_1 + \omega_D)\tau\}.$$

The Doppler shifts due to blood transport in the tissues are much smaller than intermode frequency differences of laser emission, thus on passing through a low-frequency filter the PD signal will only contain the beatings between like spectral modes. This fact can be taken into account by the formal relation

$$S_r(\omega) S_s(\omega_1) = S_r(\omega) S_s^*(\omega_1) \delta(\omega - \omega_1),$$

where $\delta(\omega - \omega_1)$ is the Dirac delta function. On integration by ω_1 variable the expression for the coherent component of the photocurrent takes the form

$$i_c(t - t_p, \tau) = \alpha \cdot 2\,\text{Re}\left[\int_{\omega_o - \Delta\omega}^{\omega_o + \Delta\omega} S_r(\omega) S_S^*(\omega)\right.$$
$$\left. \times \exp\{-j\omega_D(t - t_p) - j(\omega + \omega_D)\tau\} d\omega\right]. \tag{5}$$

Equation (5) describes a pulse whose duration time Δt is of order $\pi/\Delta\omega$. In TRS and LDF applications the conditions $\omega_D \ll \Delta\omega \ll \omega_o$, $|t - t_p| \leq \Delta t$ and $\tau \leq \Delta t$ are valid. Under the conditions the phase terms in the exponential in formula (5) remain practically constant over duration time of both the reference and the signal pulses, so that Eq. (5) can be simplified

$$i_c(t_p, \tau) \approx \alpha \cdot 2\,\text{Re}\left[\exp(-j\omega_D t_p)\right.$$
$$\left. \times \int_{\omega_o - \Delta\omega}^{\omega_o + \Delta\omega} S_r(\omega) S_S^*(\omega) \exp\{-j\omega\tau\} d\omega\right]. \tag{6}$$

The integral in formula (6) represents a crosscorrelation function $B_{rs}(\tau)$ of the pulses. The function determines the amplitude $|B_{rs}(\tau)|$ and a phase shift φ_{rs} of the coherent component of the photocurrent. The result (6) can be rewritten in the form
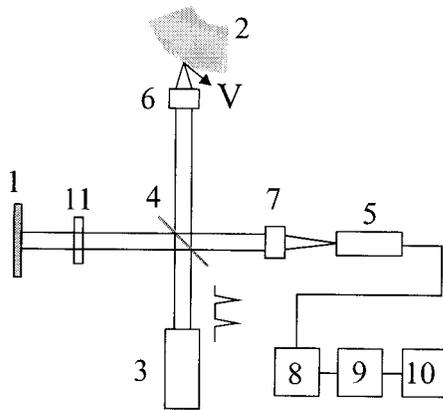
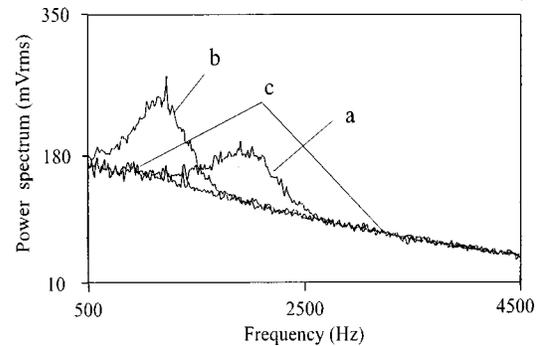**Fig. 1** The scheme of the laser Doppler heterodyne flowmeter with an ultrashort pulsing laser source.

Fig. 2 Doppler frequency shift spectra from a rotating disc with a pulsing diode laser (source S1). Laser pulse duration is 300 ns, repetition rate 700 kHz. Plot (a) corresponds to the velocity of the scattering surface of the disc $V = 4.8$ mm/s; plot (b) **V** $= 3.2$ mm/s; plot (c) unmoving disc.

$$i_c(t_p, \tau) \approx \alpha \cdot |B_{rs}(\tau)| \cos(\omega_D t_p + \varphi_{rs}). \qquad (7)$$

The conclusion is that the photocurrent pulsing with the repetition rate of a particular laser source contains a component modulated with the Doppler shift frequency. By demodulating the photocurrent, a harmonical signal at the Doppler frequency can be extracted, the value $\Delta\omega_D$ of the shift can be found, and a corresponding velocity magnitude **V** estimated on the basis of formula (1). In order to estimate the magnitude of the Doppler signal, the cross correlation function $B_{rs}(\tau)$ of the laser pulses incident on and scattered from the tissue should be measured experimentally.

3 EXPERIMENTAL SETUP

The experimental setup used in our experiment consists of a conventional interferometer which mixed light from the reference beam reflected by a stationary mirror (1) with the light of the signal wave reflected back by a rotating disc (2) (Figure 1). Both waves are obtained from the same laser (3) by splitting the laser beam at a glass plate (4) with a 1:10 intensity ratio. A photomultiplier tube (PMT) sensitive in the visible region is used as a photodetector (5). Three sources of ultrashort light pulses were used in the experiment:

- S1: a CQL800 (Philips) diode laser fed by a rectangular pulsing current with minimum value below lasing threshold (characteristics of the source: wavelength $\lambda = 675$ nm, repetition rate $f = 700$ kHz, pulse duration $\tau = 300$ ns, average power at the entrance in the interferometer $P = 0.5$ mW);

- S2: a second harmonic of CW mode-locked "Atares" Nd:YLF laser ($\lambda = 527$ nm, $f = 76$ MHz, $\tau = 35$ ps, $P = 1-3$ mW);

- S3: an R6G Coherent-700 dye laser synchronously pumped by the second harmonic of

Nd:YLF laser with cavity dumper ($\lambda = 578$ nm, $f = 3.8$ MHz, $\tau = 5-8$ ps, $P = 2-5$ mW).

The signal beam is focused on paper tape attached to the rotating disc. The reflected light is returned into the interferometer by an objective (6). The paper produced diffuse backscattered light without any noticeable specular component; a situation typical for biological tissues. The reference and the signal waves are focused onto the PMT by an objective (7) and the electrical signal from the PMT is passed through an integrating filter (8) to an amplifier (9) and then its spectrum is displayed by the HP3561A spectrum analyzer (10). The integrating filter with integrating time 200 μs effectively suppressed the first and the higher harmonics in the PMT signal at the frequency of the light source pulsation as well as intermode beatings. In experiments with different sources the neutral filters (11) have been set in the way of the reference beam in order to maintain the intensity ratio of the reference/signal waves at the PMT of order 50.

## 4 LASER DOPPLER SPECTRA WITH NANO- AND PICO-SECOND PULSE LASER SOURCES

The signal at the output of the integrating filter reproduces low-frequency beating in the intensity of the detected light from interference of the reference and the signal waves. The spectral composition of the signal represents the distribution in the Doppler shifts of light reflected from the rotating disc.

The experimental spectra with different sources are shown in Figure 2 (source S1), Figure 3 (source S2) and Figure 4 (source S3) for two different linear velocities (curves a and b) of the reflecting surface of the disc. The noise level in case of zero velocity of the disc is given by curve (c). Linear scale is used on both axes.
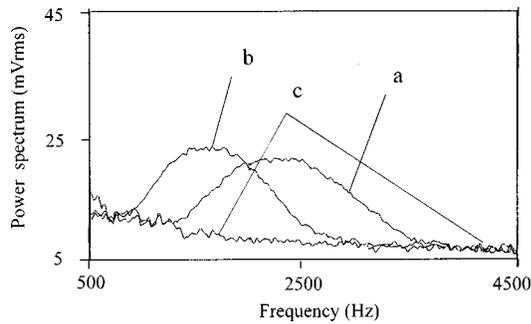
Fig. 3 Doppler frequency shift spectra from a rotating disc with a pulsing Nd:YLF laser (source S2). Laser pulse duration is 35 ps, repetition rate 76 MHz. Plot (a) corresponds to the velocity of the scattering surface of the disc **V**=7.2 mm/s; plot (b) **V**=4 mm/s; plot (c) unmoving disc.

All three figures display a characteristic broadened peak in the spectra at the Doppler frequency corresponding to the average velocity of the reflecting surface of the disc. The position of the maximum of the peak shifts towards lower frequencies as the velocity of the surface decreases (curve *a* to *b*). The values of the frequency corresponding to the location of the peak in the spectra and measured linear velocities of the scattering surface of the disc were found to be in good correspondence with formula (1). A mismatch in position of the peaks in spectra for identical wheel speeds, noticeable in Figure 2 and Figure 4, is a result of slightly different geometry for the two experiments. The width of the peak is determined predominantly by fluctuations in the speed of the disc driving motor and disc wobbling on the axis during rotation cycle.
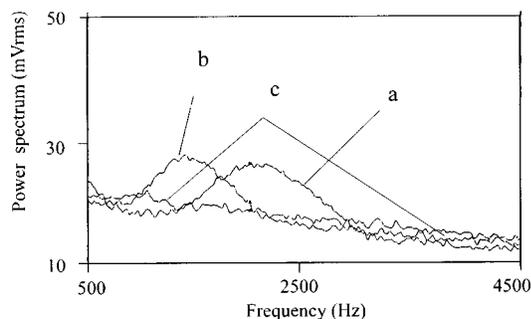
Fig. 4 Doppler frequency shift spectra from a rotating disc with a pulsing R6G dye Nd:YLF pumped laser (source S3). Laser pulse duration is 5–8 ps, repetition rate 3.8 MHz. Plot (a) corresponds to the velocity of the scattering surface of the disc **V**=4.8 mm/s; plot (b) **V**=3.2 mm/s; plot (c) unmoving disc.

In order for the peak of the spectra to be in the low-frequency range, the angle between the vector difference $n_s - n_i$ and the velocity vector **V** [see Eq. (1)] was close to 90° which significantly added to the broadening of the spectra.

5 CONCLUSIONS

It is experimentally demonstrated with the nano- and pico-second pulse lasers that the heterodyne Doppler signal can be detected with ultrashort pulses as well as with continuous laser light sources. A theoretical backup of the experimental results is provided.

The feasibility to detect a Doppler signal with ultrashort laser pulses provides a basis for time resolved laser Doppler tomographic experiments on biotissues. In the experiments, each narrow picosecond reference pulse is to be mixed with a certain part of corresponding coherent signal pulse broadened by multiple light scattering. By varying the time delay of the reference pulse with respect to the signal pulse it should be possible to obtain a heterodyne Doppler signal produced by the photons having a certain transient time. In the case of backscattering geometry of experiments, the shorter transient times of the photons correspond to a smaller penetration depth of light into the tissue. Thus scanning in the time delay is to a certain extent equivalent to scanning in depth of the tissue. The spectra obtained in this kind of experiment may potentially carry useful information about the spatial distribution of the velocity of blood in biotissues.

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