Use of EMCCD camera for Brillouin spectroscopy

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Application Note

Abstract

The Brillouin spectroscopy is a measurement method for the non-invasive determination of rheological and biophysical tissue properties. Its application in ophthalmology can offer the possibility to determine the deformation properties of eye lens with spatial resolution. Based on spectral data of the Brillouin signals the bulk modulus within the lens tissue can be derived. To perform in vivo measurements a fast and sensitive detector is stringently necessary. A measurement set-up for confocal Brillouin microscopy based on a high-resolution dispersive device is presented. In vivo measurements on rabbit eyes are only possible due to use of a highly sensitive EMCCD camera.

Introduction

Brillouin-scattering occurs when coherent light is inelastically scattered by thermal density fluctuations inside a material. These fluctuations can be regarded as acoustic waves, which are propagating in all directions through the medium. The incident coherent light (laser light) undergoes a frequency shift which is equal to the frequency of the scattering sound wave. The Brillouin-signal is very weak of intensity. Brillouin spectroscopy measures the spectral change ($\Delta\nu_B$) in the scattered light relative to the incident monochromatic light. Because the atoms oscillate around their equilibrium position, the scattered light is shifted according to the Doppler principle by the same amount to higher and lower frequencies relative to the incident light frequency. These phenomena are referred as Anti-Stokes and Stokes Brillouin scattering (Fig. 1).

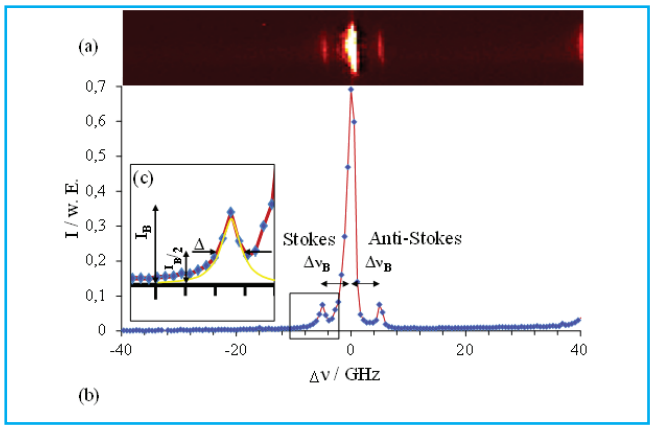

Figure 1: Example of a Brillouin-spectrum: characteristic EMCCD image of water (a) and corresponding normalized Brillouin-spectrum (b). The Stoke- and Anti-Stoke-frequency shift of the Brillouin-signals from the central elastically signal is 4.96 GHz. Inset (c) shows magnification of the Stokes-Brillouin-signal. The yellow curve describes the fitted Lorenz-function. Δ refers to full width at half maximum of the spectral linewidth and is 1.68 GHz. I_B shows the maximal Intensity of the Brillouin-signal. The linewidth Δ can also deliver further important information on tissues viscous characteristics.

The measured Brillouin shift $\Delta\nu_B$ is related to the refractive index n and the speed of sound V at the measured point in the following way:

$$\Delta\nu_B = \pm\frac{2n\nu_L}{c}V\cos\left(\frac{\theta}{2}\right) \qquad (1)$$

θ describes the angle between back-scattered detected light and incident laser light with the frequency ν_L.
c denotes the speed of light in vacuum.

Setup and Methods

Two identical Vitually Imaged Phase Arrays (VIPA) were used in a multi-pass configuration for spectral resolution of the low Brillouin shift $\Delta\nu_B$. (Fig. 2).

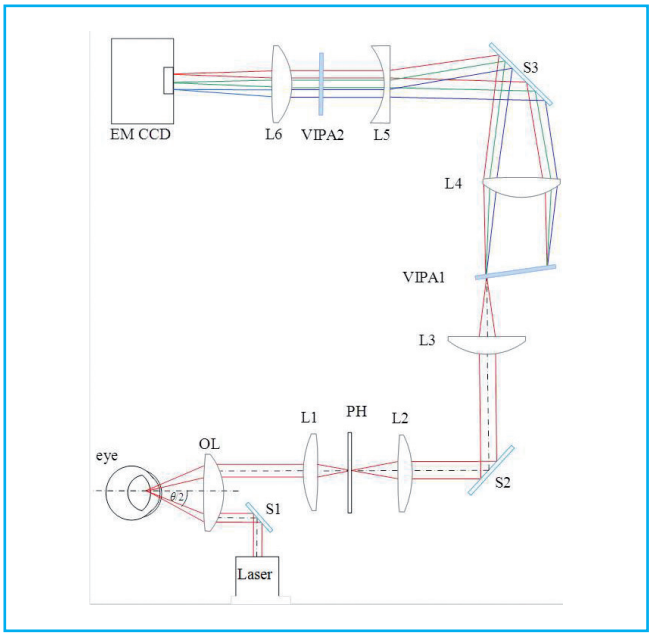

Fig. 2: Schematic diagram illustrating the experimental configuration (OL = objective lens, L1/L2/L4/L6 = collector lenses, L3 = positive cylindrical lens, L5 = negative cylindrical lens, S1/S2/S3 =mirrors, PH = pinhole).

A semiconductor laser specially designed for this measurement delivers a coherent beam source with a wavelength of 780 nm. This wavelength represents an acceptable compromise between beam exposure of the biological tissue to be examined and the achievable spectral separation of the inelastic and elastic scattered light components. Via an objective lens (OL) with a focal length of 40 mm the laser beam is focused into the eye to be examined. Due to the decentralized beam path through the objective lens, the incident beam at the eye lens is at an angle of θ/2 = 12°.

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The back-scattered light portion is collected again through the same lens and imaged via a collector lens (L1) onto a pinhole (PH). The configuration therefore utilizes the advantages offered by confocal microscopy namely that aberration demands on the objective and collector lenses are low and optical sections can be performed. In other words, only that region in a three-dimensional object is represented that is located close to or in the focal plane of the OL. With a pinhole diameter of 100 µm, scattered light from a sample volume of about 0.013 mm (x) × 0.013 mm (y) × 0.062 mm (z) is line-focused onward through the positive cylindrical lens (L3) into the first VIPA. This customized VIPA is tilted to the beam path at an angle of 2°, thus achieving a high angular dispersion. Via another lens (L4) and a negative cylindrical lens (L5) the light that is already dispersed into its frequencies is line-focused into a second, identical VIPA (V2) arranged perpendicularly to the first. This tandem set-up is a new performance in order to minimize any loss of intensity due to reflection at the optic components. It was only the multi-pass set-up that enabled us to perform measurements in biological tissue because the elastic scattering there is markedly more pronounced than in synthetic materials (e.g. acrylic) or water (Fig. 1). The detector of the measured signals, the EMCCD camera, is the key component in the configuration for achieving in vivo measurements. With a -80 °C Peltier-cooled iXon3 DU888 DC-BV EMCCD camera (Andor Technology) at the laser power of 12 mW, it was possible to reduce the exposure time to 0.3 seconds. Thus, our experimental setup fulfills the requirements of the laser class 1.

Measurements and results

In vivo measurements were performed unilateral on "White New Zealand" rabbits (Fig. 3).

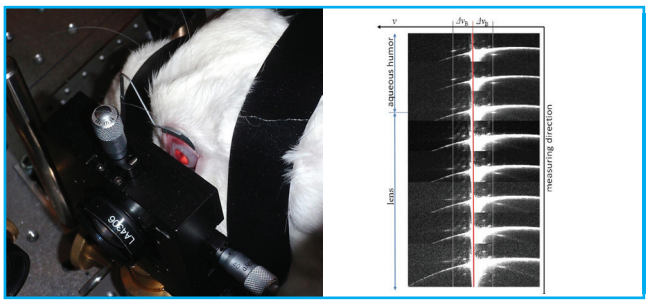

Figure 3a) In vivo measurement of a rabbit

Figure 3b) Brillouin spectra axially through the front portion of the rabbit's eye (EMCCD image)

During the measurement, the animal was fixed on a stage, which could be moved by linear motors in the x, y and z direction in relation to the measurement beam path. Using this setup an axial scan of the eye was performed in steps of 500 µm. The maximum measurements depth, which was reached in the rabbit eye, was 10.5 mm.

Figure 4 shows a measured axial profile of the eye starting from the corneal surface to the vitreous body. The graph clearly distinguishes the elements of the anterior segment of the eye based on their Brillouin scattering properties. The aqueous humor has a nearly constant Brillouin shift. For the crystalline lens, the graph shows the increase of Brillouin shift in anterior of the cortex from 5.3 GHz to 7.0 GHz - the nucleus of the lens. After this measurement point the Brillouin shift decreases up to a point where a fluctuation occurs. On this measuring depth of circa 9.5 mm it seems that here is the transition to the vitreous humor.

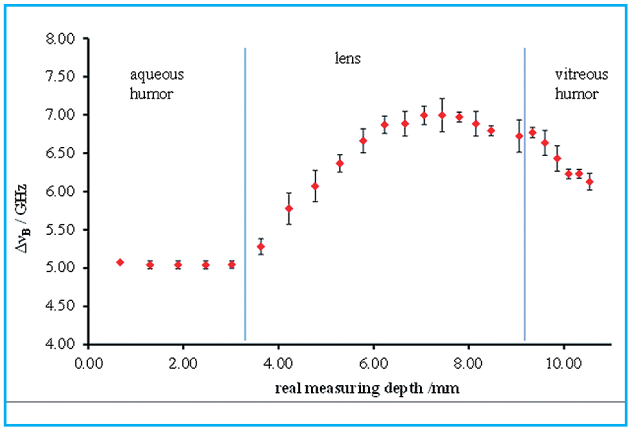

Figure 4: In vivo Brillouin depth profile of a rabbit anterior chamber of the eye.

The Brillouin shift $\Delta\nu_B$ is related to the bulk modulus K along the following equation:

$$K = \left(\frac{\frac{c}{\nu_L}\Delta\nu_B}{2\,n\,\cos\left(\frac{\theta}{2}\right)}\right)^2 \rho \qquad (2)$$

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To calculate the bulk modulus you need the refractive index n and the density ρ. They were referred from literature values. Figure 5 shows the calculated bulk modulus of anterior chamber of the rabbit eye.

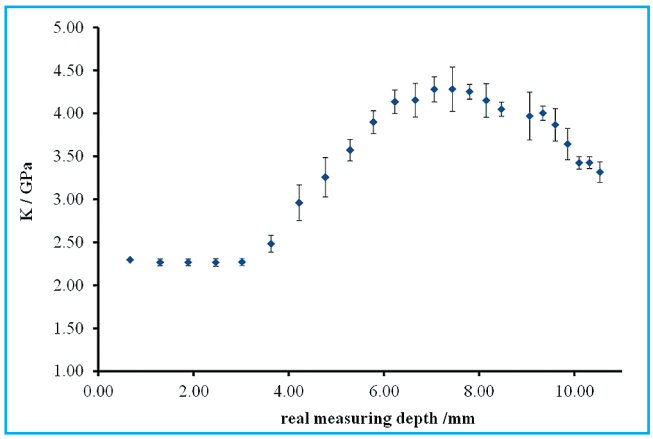

Figure 5: Bulk modulus of the anterior chamber of the rabbit eye.

Conclusion

The presented method shows the high potential of the Brillouin spectroscopy in the ophthalmology. It is possible to measure spatially resolved information in vivo regarding the rheological properties. Only by using an extrem sensitive iXon3 EMCCD camera measurement times of 0.3 s can be achieved. In combination with the laser power of 12 mW and a wavelength of 780 nm the method is in compliance with laser safety class 1. It seems to be realistic that this method can be useful in a wide field of in vivo examination in ophthalmology in the near future.

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