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The promise of Brillouin microscopy in Medicine – a mini review

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Abstract

Brillouin scattering is inelastic scattering of light by thermally generated acoustic waves within a medium first reported in 1922. It allows measurement of mechanical properties and is widely used in Material science and Physics. After nine decades of development and refinement of its measuring equipment, especially the integration of VIPA Brillouin spectroscope with confocal microscope, the technique of Brillouin scattering now offers new promising applications in the field of cellular mechanics and pathophysiology of health and disease.

Keywords: Brillouin scattering; Brillouin spectroscopy; Brillouin microscopy; Elasticity; Biomechanics; Microscopy

Introduction

Brillouin scattering is named after Leon Brillouin for inelastic scattering of light (photons) by thermally generated acoustic waves (phonons) within a medium [1]. Mandelstam published similar prediction which is believed to be recognized as early as 1918 [2,3]. Gross in 1933 first experimentally verified this effect in water and various organic fluids using a mercury lamp with a scattering angle of 90°C [4].

Brillouin spectroscopy measures spectral changes upon scattering and provide direct information on the phonon properties that are closely related to the viscoelastic properties of the medium [5-7]. It is an empirical technique which allows for the determination of elastic moduli for a given material. This technique has been applied to measure numerous samples ranging from crystals, liquids, semiconductors to organic materials including animal and plant [2,8-14].

This paper reviews all currently available literature on Brillouin microscopy in the field of Medicine and biomedical research up to 2019. A summary table of currently available measurements of Brillouin shift in biologic tissue in medicine is tabulated.

The principle of Brillouin Scattering

Brillouin microscopy provides indirect means to measure the speed of sound in order to image the mechanical properties of a given sample. It is based on the inelastic scattering of light by high frequency acoustic waves spontaneously produced inside the specimen in a random way. When a photon is scattered by a phonon, it experiences a Doppler shift that depends on the velocity of the phonon. If the photons are detected as a function of frequency, the frequency shift (Brillouin frequency) can be measured. The shift can be seen downwards (Stokes) or upwards (Anti-Stokes) with the same magnitude. The measured shift depends on the speed of sound and the refractive index of the materials (See Figure 1).

The Brillouin frequency shift ν_B for an isotropic material can be expressed as [9]:

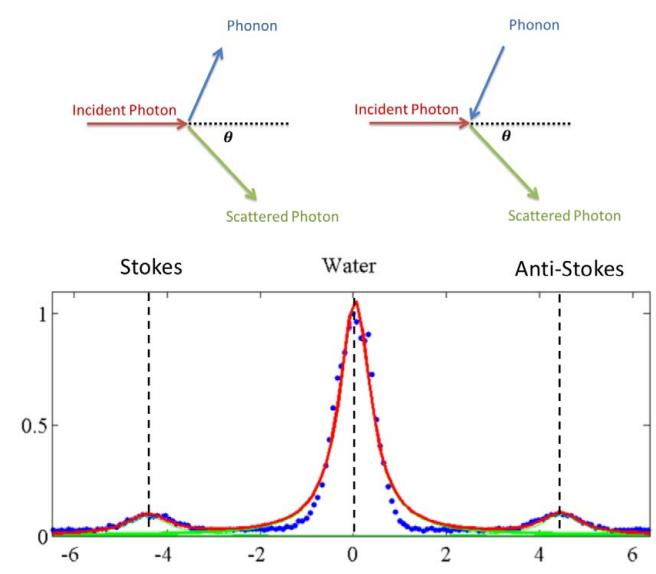

Figure 1: Depicting the principle of Brillouin scattering in samples.

$$\nu_B = \frac{2n}{\lambda} V \sin\left(\frac{\theta}{2}\right)$$

where $v = \sqrt{\frac{E}{\rho}}$ is the acoustic velocity, E the elastic modulus for solids and bulk modulus for liquids, ρ the density, n the refractive index, λ the optical wavelength and θ the scattering angle.

The line width of Brillouin spectrum Δ is related to the acoustic attenuation coefficient $\propto$ through $\Delta = \propto V/\pi$. These parameters are related to storage modulus M' and loss modulus M'' through $M' = \rho V^2$ and $M'' = \rho V^2 \Delta/\nu_B$. The measured complex modulus would be the same as the values obtained by hypothetical dynamic analysis at the GHz frequency range.

Brillouin Spectroscopy

The traditional Brillouin scattering system consists of a laser, optical components, a Fabry-Perot interferometer and a data acquisition system [5-7,14,15]. Recently Virtual imaged phased array spectrometer has been used in place of the Fabry-Perot interferometer [16,17]. Brillouin scattering is a weak inelastic scattering process arising from the interaction of light with inherent thermal density fluctuations propagating at the hypersound velocity inside the media. The frequency shift of the inelastic scattered light due to thermal density fluctuations in media is typically within the range of 50 GHz. Brillouin frequency shift in the order of GHz is too small to be resolved with conventional spectrometers. For resolution, Brillouin spectroscopy has relied on multiple-pass scanning Fabry-Perot interferometers or Virtual imaged phased array [16,17].

Interferometry is based on wave superposition. A Fabry-Perot interferometer in particular is made of two parallel mirrors where multiple light reflections between the two surfaces infer light wave interference. When the two waves with the same frequency combine, the resulting pattern is determined by the phase difference between the two waves. Depending on the wavelength of the light, the angle the light travels between the reflecting surfaces, the thickness of the mirrors and the refractive index of the material, waves that are in phase will experience a transmission peak maximum while waves that are out of phase will experience a transmission peak minimum. Multiple reflected rays are out of phase by a constant increment increase the sharpness of the inference maximum [11,16,17].

As the Brillouin signal is very weak, Brillouin spectroscopy based on multiple-pass scanning Fabry-Perot interferometer requires high illumination of the sample from the laser (typically a few tens to hundreds of milliWatts) and long acquisition time (minutes to hours) to perform the spectral analysis. Traditional Brillouin spectroscopes based on Fabry-Perot interferometer are suitable for Material science and Physics applications, but not for biomedical uses because the illumination and acquisition time exceed the safety limits of living biological tissue. To overcome this difficulty a virtual image phased array (VIPA) spectrometer has been used to replace the Fabry-Perot interferometer [11,16,17].

The VIPA is usually a solid etalon. The front surface is highly reflective with a narrow beam, entrance window. The back surface is partially reflective. High throughput is assured because nearly all the photons arriving at the VIPA are transmitted forward and can be detected. Consequently, spectral analysis can be performed with sub-second acquisition time and illumination power (sub-milliWatts) within the safety limits of living biological tissue. Measurement speed has been reported to be 1000 times faster than traditional scanning Fabry-Perot spectrometer [18,19,20,21].

Brillouin Microscopy

Recently Brillouin spectroscopy based on VIPA has been combined with confocal microscopy to yield a confocal Brillouin microscopy which is able to perform mechanical imaging in a non-invasive manner (See Figure 2 for simplified schematic setup) [16,17]. Such Brillouin microscopes extend the Brillouin scattering technique from point sampling spectroscopy to imaging modality and would open up many new possibilities for mechanical imaging of biomedical systems. Brillouin microscopy has been applied to measure the speed of sound in muscle, bone and eye tissue. It has been used to image eye in vivo, ex vivo biological cells, coronary vessels and many other biological tissues (See Table 1 for review) [11,20-23].

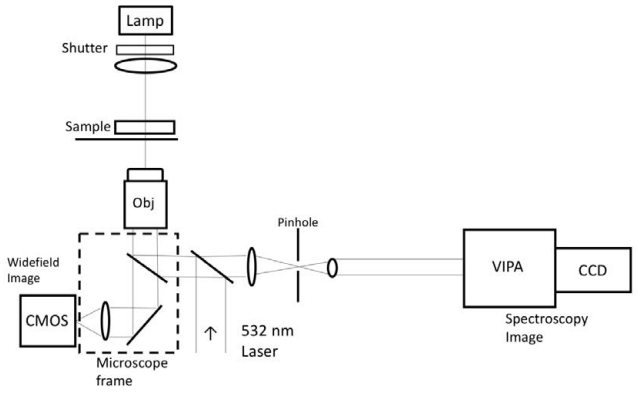

Figure 2: Depicting the schematic setup of a Brillouin scattering microscopy.

Name of Biological Tissue	GHz	Reference
Cornea	2.82-2.5	Vaughan JM 1980 [28] Scarcelli G 2012 [29]
Lens	2.38-3.1	Vaughan JM 1980 [28] Scarcelli G 2012 [29]
Single cell	2.5-3.5	Antonacci G 2016 [21]
Spinal cord	5.3	Schlußler R 2017 [17]
Water	7.4	Berghaus K 2015 [19]
CSF	7.6	Steelman ZA 2014 [30]
Red blood cell	7.8	Meng Z 2015 [31]
Skin	8	Troyanova-Wood M 2019 [25]
Blood vessel	10-14	Antonacci G 2015 [32]
Brain	14.5-16	Palombo F 2018 [34]
Cartilage	15.2	Palombo F 2014 [26]
Collagen	15.3	Palombo F 2014 [26]
Brain plaque	18.5	Palombo F 2018 [34]
Bone	19.8 23.0	Zhang D 2001 [35] Kawabe M 2012 [36] Cardinali MA 2019 [37]
Trabecular bone	7.8-8.4	Cardinali MA 2019 [37]

Table 1: Depicting Brillouin shift in measured biological tissue.

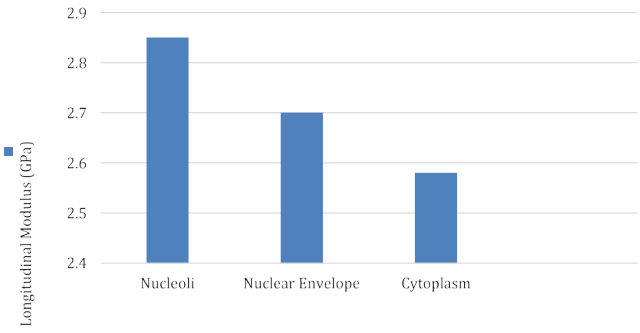

Figure 3: Depicting measured Longitudinal Modulus in subcellular organelles (After Antonacci G, 2016 [21]).

Brillouin microscopy has been shown to be able to allow the elasticity of the esophagus to be mapped for Barrett's esophagus or pre-cancer without the need for labelling or staining. This is a first application of Brillouin microscopy for cancer [24]. This was later applied to skin for melanoma [25]. Some physical scientists have been able to combine both Raman and Brillouin spectroscopy for microscopy [14,26].

More recently, at the time of this present manuscript, Brillouin scattering has been applied to flow cytometry. Brillouin spectroscopy probes the mechanical properties of material via light scattering. Brillouin spectroscopy inherently label free, non-contact and non-invasive, is able to classify cell populations based on their mechanical signatures with a high throughput. This shows the broad application of Brillouin spectroscopy [27].

The average diameter of the nucleus in a mammalian cell is approximately 6 micrometers while the spatial resolution of Brillouin microscopy is sub-micron. Brillouin microscopy has been used to study subcellular biomechanical properties of the cell. This development has been relatively recent. Currently, Brillouin microscopy has been able to resolve the differences between subnuclear organelles. This is a great advancement in the field of microscopy and bioimaging (See Figure 3) [11,16,20,21,26]. It is anticipated that Brillouin microscopy will continue to offer new promising applications in medicine.

Conclusion

Brillouin microscopy is a versatile and broadly applicable technique for microscopy. It can be used to study subcellular biomechanical properties of the cell. This development has been relatively recent. The high spatial resolution, three-dimensional imaging capabilities afforded by confocal imaging and together with the noninvasive nature of this technique offers promising new opportunities to further the field of cellular mechanics and pathophysiology of health and disease.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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