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Brillouin Light Scattering Microspectroscopy for Biomedical Research and Applications: introduction to feature issue

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Abstract: There has been a marked revival of interest in brillouin light scattering spectroscopy/microscopy over the last decade in regards to applications related to all optically studying the mechanical problems associated with systems of biological and medical interest. This revival has been driven by advancements in spectrometer design, together with mounting evidence of the critical role that mechanical properties can play in biological processes as well as the onset of diverse diseases. This feature issue contains a series of papers spanning some of the latest developments in the field of Brillouin light scattering spectroscopy and microscopy as applied to systems of biomedical interest.

1. Introduction

Optical spectroscopy that are currently routinely used in biomedical research and applications can give information on chemical and structural properties of the probed samples. In contrast Brillouin Light Scattering (BLS) spectroscopy can yield information on the high-frequency mechanical properties. BLS has been a routine tool in condensed matter physics and material science to extract the acoustic velocities and elastic moduli. It's principles unlike with other optical spectroscopy techniques, such as Raman or IR absorption spectroscopy, involves the scattering of light from collective excitations associated with (either inherent or induced) acoustic vibrations of a sample. Recent advances in spectrometer design, together with growing interest in measuring the mechanical properties of biological and medical samples has seen renewed interest in Brillouin spectroscopy as applied to studying biological systems as well as for medical diagnostics applications. When combined with confocal microscopy, BLS spectroscopy becomes a very powerful tool for mapping mechanical properties of biological systems in three dimensions.

Though much progress has been made, many challenges lie ahead, that are related to the difficulty of measuring the Brillouin signals (which are inherently weak and occurs in the immediate vicinity of the elastic scattering peak) as well as the interpretation and the significance of the measured high-frequency elastic moduli in complex biological systems. This feature issue compiles a collection of articles by various researchers in the field spanning experimental applications of BLS to systems of biomedical interest, the interpretation of the measured BLS-spectra in such systems, variations on BLS spectroscopy methods, and some relevant developments in instrument design and data analysis.

Most current BLS imaging spectrometers rely on a set of angular dispersive optical elements called Virtually Imaged Phased Arrays (VIPAs). To achieve sufficient finesse to resolve the BLS spectra in non-transparent samples, two such elements are often placed in a cross-dispersion configuration, such that the spectrometer as a whole is spatially quite

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Biomedical Optics EXPRESS

expansive. Here *Fiore et al.* [1] present a novel method of using only a single VIPA, namely by passing the light through it twice (with the second time the beam having been rotated by 90°). The result is that they effectively achieve a cross-dispersion setup using only a single such element, which holds potential for saving costs and compaction of BLS spectrometers.

A major limitation for all forms of optical microscopy and spectroscopy is that many samples of biological interest are not transparent, resulting in significant stray light scattering and the inability to peer within their interior (which is usually the region of interest). This is even more pronounced for BLS where one is attempting to measure a signal that is very close to the probing laser line. One approach for overcoming this in optical microscopy is so-called "clearing" of the sample, which involves replacing the natural cellular content with a medium that has an equal refractive index to the supporting structure. Here *Gomez et al*. [2] take a look at the effect of "optical clearing" of biological samples on the BLS spectra. Despite the significant expected changes that clearing can be expected to cause on the mechanical properties they observe only subtle changes in the BLS spectra between cleared and non-cleared samples.

In many biological samples the variation in the BLS signals from position to position is only very subtle, and it can thus be challenging to obtain satisfactory contrast in BLS mapping. Furthermore, standard fitting procedures can be derailed by the presence of multiple spectral peaks or unaccounted for perturbations in the peaks shape brought about by e.g. scattering in the sample. Here *Fioretto at al* [3]. introduce the concept of spectral moment analysis for studying both BLS and Raman spectra. They show that it can provide a reliable method for a fast access to intensity and frequency position of spectral peaks. Moreover, the potential of joint Brillouin and Raman micro-spectroscopy is shown for the case of natural fibers.

Extracting the BLS spectra from a measurement performed using a VIPA cross-dispersion imaging spectrometer is not trivial and requires suitable calibrations with control samples and correct scaling function of the spectral dispersion axis. Here *Correa et al.* [4] present a detailed workflow starting from the raw output of an sCMOS camera, through to extracting the measured spectra and fitting a corrected and filtered spectral projection to obtain the BLS frequency shift and peak width. This is demonstrated in the form of studies on tissue-mimicking collagen gels.

The mechanical properties of materials for biomedical applications are usually affected by relaxation processes, *i.e.* by diffusion processes occurring at the molecular level, which are responsible for viscoelastic behavior. Relaxation processes are responsible for orders of magnitude increase of the elastic moduli from the quasi-static condition to the GHz regime probed by Brillouin scattering. Here *Pochylski* [5] shows the effect of the structural relaxation on the longitudinal modulus measured by Brillouin spectroscopy at different scattering geometries in a model aqueous polymer (PEG300) mixture.

A viscoelastic response is also typical of elastomeric scaffolds. Here *Rohman et al.* [6] report the results of a combined quasi-static, ultrasonic and Brillouin scattering characterization of poly(ester-urethane)-based scaffolds, suggesting a fractional derivative viscoelastic model for this material.

BLS provides a contrast mechanism for detection of normal tissue structure and changes in viscoelastic properties measured at GHz frequencies. As such it has shown potential for diagnosis of pathology. *Troyanova-Wood et al.* [7] applied BLS microscopy to *ex vivo* samples from a porcine model of malignant melanoma and found a marked difference in Brillouin frequency shift between melanomas (8.55 ± 0.18 GHz) and healthy tissues (7.97 ± 0.02 GHz), with regressing melanomas lying in between (8.11 ± 0.07 GHz). These results demonstrate that BLS spectroscopy is a sensitive probe for discrimination of skin cancer in tissues, with potential for tumor margin detection.

The risk of photodamage due to laser irradiation of cells and tissues can be a matter of concern in biomedical applications of BLS and other laser techniques. It is well documented

Biomedical Optics EXPRESS

that the use of longer wavelength incident light reduces the occurrence of absorption-mediated photodamage with respect to short visible wavelengths. However, it also decreases the signal intensity due to the inverse relationship with the forth power of incident wavelength (as well as reducing the frequency shift). *Nikolic and Scarcelli* [8] showed that the use of 660 nm wavelength enables live cells to be irradiated with 82 times more energy than at 532 nm, thus shortening the acquisition time (by ca. 34 times). This has the advantage that measurements can be performed over longer periods of time, larger fields of view, with shorter point-to-point steps and improved precision.

As well as probing soft tissues, BLS can also prove useful to analyse mineralized tissues such as bones. In this case BLS can assess bone strength, or distinguish between healthy and pathological condition of bones. Here, *Cardinali et al*. [9] demonstrate the ability to probe the femoral head with BLS. In addition to the characteristic signatures of collagen-rich areas, they also find the existence of a low-frequency component which they attribute to softer tissues.

BLS microscopy is a promising tool for the investigation of extracellular matrix (ECM) biomechanics, with spectra that reflect the structure and heterogeneity of the sample. *Bevilacqua et al.* [10] presented a study whereby Brillouin microscopy is applied to the ECM in live zebrafish and showed that selection of optimal mapping conditions enables contributions from different components within the probed volume to be resolved. This result stresses the importance of careful consideration of the spatial scales in Brillouin microscopy measurements with respect to the biological structures of interest and shows potential for elucidating the role of ECM biomechanics in vertebrate morphogenesis and axis elongation.

In the context of ECM biomechanics, *Wu et al.* [11] also applied BLS microscopy to investigate the effect of trypsin digestion on *ex vivo* sections of porcine articular cartilage to mimic early abnormalities typical of osteoarthritis (OA). Enzymatic digestion (for 4 hours with 1 mg/ml trypsin) accounts for a decrease (150 MHz) in Brillouin frequency shift of cartilage that is interpreted in terms of a small (4%) increase in water content based on a biphasic model. Early diagnosis of OA is the key to effective treatment; hence BLS microscopy, which is capable to detect such small changes in hydration levels, may provide a new diagnostic tool for early-stage OA.

Accumulation of advanced glycation end-products (AGEs) in biological tissues has been associated with ageing and diseases such as diabetes, Alzheimer's disease, atherosclerosis and osteoporosis. Collagen is the most abundant structural protein of the ECM, and collagen fibrils are the smallest structural elements of load-bearing tissues. *Andriotis et al.* [12] treated collagen fibrils with ribose and reported an increased hydration and decreased transverse stiffness for AGEs-bearing collagen fibrils compared to control samples measured using AFM and BLS microscopy. This finding can help elucidate the interplay between AGE adducts cross-links, the hydration and nanoscale mechanics.

Comparison of BLS with well-established techniques has helped established its relevance, yet its full potential remains to be discovered. Here *Coppola et al.* [13] compare BLS and traction force maps obtained with micropillar substrates and discern to what extent the extracted mechanical parameters are correlated.

Though the majority of BLS studies on biological systems over the last decade employ so-called spontaneous BLS, an alternative method involves optically exciting a transient density grating in the sample with two pulsed lasers and measuring the scattering from this with a continuous wave probing laser. This so-called Impulsive Stimulated Brillouin Scattering (ISBS) has previously been shown to significantly enhance the BLS signal and thereby also increasing the imaging speed for BLS spatial mapping, however typically at the expense of increasing the light dosage on the sample. Here *Ballman et al.* [14] assess the accuracy of ISBS and show that it in practice can also achieve a higher spectral resolution than is achieved with spontaneous BLS.

Biomedical Optics EXPRESS

Another alternative approach involves generating phonons with a femtosecond (fs) laser, and following the propagation of the phonon wavepackets in time with a second femtosecond laser pulse. Such an approach for measuring BLS can both increase the signal-to-noise ratio as well as provide additional information in the time domain. Here, *Perez-Cota et al.* [15] use this approach to monitor the encystation of Acanthamoeba castellanii. They demonstrate that the GHz-regime mechanical properties change in time and in a manner related to the different cycles of encystation.

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