Video Article

High-speed Continuous-wave Stimulated Brillouin Scattering Spectrometer for Material Analysis

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Abstract

Recent years have witnessed a significant increase in the use of spontaneous Brillouin spectrometers for non-contact analysis of soft matter, such as aqueous solutions and biomaterials, with fast acquisition times. Here, we discuss the assembly and operation of a Brillouin spectrometer that uses stimulated Brillouin scattering (SBS) to measure stimulated Brillouin gain (SBG) spectra of water and lipid emulsion-based tissue-like samples in transmission mode with <10 MHz spectral-resolution and <35 MHz Brillouin-shift measurement precision at <100 ms. The spectrometer consists of two nearly counter-propagating continuous-wave (CW) narrow-linewidth lasers at 780 nm whose frequency detuning is scanned through the material Brillouin shift. By using an ultra-narrowband hot rubidium-85 vapor notch filter and a phase-sensitive detector, the signal-to-noise-ratio of the SBG signal is significantly enhanced compared to that obtained with existing CW-SBS spectrometers. This improvement enables measurement of SBG spectra with up to 100-fold faster acquisition times, thereby facilitating high spectral-resolution and high-precision Brillouin analysis of soft materials at high speed.

Video Link

The video component of this article can be found at https://www.jove.com/video/55527/

Introduction

Spontaneous Brillouin spectroscopy has been established, in recent years, as a valuable approach for the mechanical analysis of soft materials, such as liquids, real tissue, tissue phantoms and biological cells[1,2,3,4,5,6,7]. In this approach, a single laser illuminates the sample and light that is inelastically scattered from spontaneous thermal acoustic waves in the medium is collected by a spectrometer, providing useful information on the viscoelastic properties of the sample. The spontaneous Brillouin spectrum includes two Brillouin peaks at the acoustic Stokes and anti-Stokes resonances of the material, and a Rayleigh peak at the illuminating laser frequency (due to elastically scattered light). For a Brillouin backscattering geometry, the Brillouin frequencies are shifted by several GHz from the illuminating laser frequency and have spectral width of hundreds of MHz.

While scanning Fabry-Perot spectrometers have been the systems-of-choice for acquiring spontaneous Brillouin spectra in soft matter[1,2], recent technological advances in virtually imaged phase array (VIPA) spectrometers have enabled significantly faster (sub-second) Brillouin measurements with adequate spectral-resolution (sub-GHz)[3,4,5,6,7]. In this protocol, we present the construction of a different, high-speed, high spectral-resolution, accurate Brillouin spectrometer based on the detection of continuous-wave-stimulated-Brillouin-scattering (CW-SBS) light from non-turbid and turbid samples in a nearly back scattering geometry.

In CW-SBS spectroscopy, continuous-wave (CW) pump and probe lasers, slightly detuned in frequency, overlap in a sample to stimulate acoustic waves. When the frequency difference between the pump and probe beams matches a specific acoustic resonance of the material, amplification or deamplification of the probe signal is provided by stimulated Brillouin gain or loss (SBG/SBL) processes, respectively; otherwise, no SBS (de)amplification occurs[8,9,10,11]. Thus, an SBG (SBL) spectrum can be acquired by scanning the frequency difference between the lasers across the material Brillouin resonances and detecting the increase (decrease), or gain (loss), in the probe intensity due to SBS. Unlike in spontaneous Brillouin scattering, elastic scattering background is inherently absent in SBS, enabling excellent Brillouin contrast in both turbid and non-turbid samples without any need for Rayleigh rejection filters as required in VIPA spectrometers[10,11,13].

The main building blocks of a CW-SBS spectrometer are the pump and probe lasers and the stimulated Brillouin gain/loss detector. For high spectral-resolution, high speed CW-SBS spectroscopy, the lasers need to be single-frequency (< 10 MHz linewidth) with sufficiently wide wavelength tunability (20 - 30 GHz) and scanning rate (> 200 GHz/s), long-term frequency stability (<50 MHz/h) and low intensity noise. Furthermore, linearly polarized and diffraction-limited laser beams with powers of few hundreds (tens) of mW on the sample are required for the pump (probe) beam. Finally, the stimulated Brillouin gain/loss detector should be designed to reliably detect weak backward stimulated Brillouin gain/loss (SBG/SBL) levels (10^{-5} - 10^{-6}) in soft matter. To meet these needs, we selected distributed feedback (DFB) diode lasers coupled to

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polarization-maintaining fibers along with a stimulated Brillouin gain/loss detector combining an ultra-narrowband atomic vapor notch-filter and a high-frequency single-modulation lock-in amplifier as illustrated in **Figure 1**. This detection scheme doubles the intensity of the SBG signal while significantly reducing noise in the probe intensity, where the desired SBG signal is embedded[11]. Note that the role of the atomic vapor notch-filter used in our SBS spectrometer is to significantly reduce the detection of unwanted stray pump reflections rather than to decrease the elastic scattering background as in VIPA spectrometers that detect both spontaneous Rayleigh and Brillouin scattered light. Using the protocol detailed below, a CW-SBS spectrometer can be constructed with the capability of acquiring transmission spectra of water and tissue phantoms with SBG levels as low as 10^{-6} at <35 MHz Brillouin-shift measurement precision and within 100 ms or less.

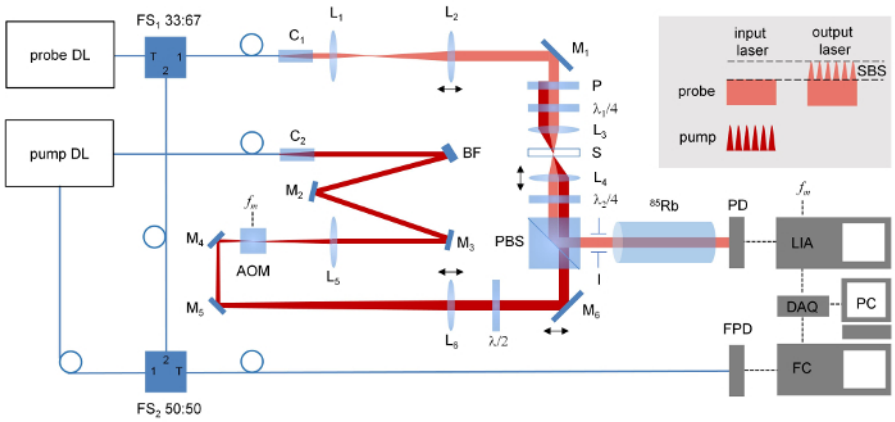

Figure 1: Continuous-wave Stimulated Brillouin Scattering (CW-SBS) Spectrometer. Two continuous-wave pump and probe diode lasers (DL), frequency detuned around the Brillouin shift of the sample, are coupled into polarization-maintaining single-mode fibers with collimators C_1 and C_2, respectively. The pump-probe frequency difference is measured by detecting the beat frequency between beams peeled from the pump and probe lasers using a set of fiber splitters (FS), a fast photodetector (FPD), and a frequency counter (FC). The S-polarized probe beam (light red), expanded using a Keplerian beam expander (L_1 and L_2), is right circularly polarized by a quarter-wave plate ($\lambda_1/4$) and focused on the sample (S) by an achromatic lens (L_3). For effective SBS interaction and optical isolation, the pump beam (deep red), expanded using a Keplerian beam expander (L_5 and L_6), is first P-polarized using a half-wave plate $\lambda_2/4$), then transmitted through a polarizing beam splitter (PBS), and is finally left circularly polarized by a quarter-wave plate ($\lambda_2/4$) and focused on the sample with an achromatic lens (L_4; same as L_3). Note that the pump and probe beams nearly counter-propagate in the sample and that an S-oriented polarizer (P) was used to prevent the P-polarized pump beam (coming out of $\lambda_1/4$) from entering the probe laser. For lock-in detection, the pump beam is sinusoidally modulated at f_m with an acousto-optical modulator (AOM). The SBG signal, manifested as intensity variations at frequency f_m (see inset), is demodulated with a lock-in amplifier (LIA) following detection by a large-area photodiode (PD). For significant elimination of stray pump reflections in the photodiode, a narrowband Bragg filter (BF) and an atomic notch filter (^{85}RB) around the pump wavelength are used alongside with a light-blocking iris (I). Data is recorded by a data acquisition card (DAQ) connected to a personal computer (PC) for further analysis of the Brillouin spectrum. All folding mirrors (M_1-M_6) are used to fit the spectrometer on a 18"×24" breadboard that is vertically mounted on the optical table for facilitating placement of watery samples. Please click here to view a larger version of this figure.

Protocol

Note: Unless stated otherwise, (i) connect all mounts to post holders and tighten the post bases with a clamping fork or mounting base to the optical table, and (ii) use output laser powers of 2 - 10 mW for all alignment procedures.

Note: Turn on all electrical/optoelectronic devices in the setup and allow 30 min of warmup time prior to use.

1. Prepare the Probe Beam Optical Path

1. **Mount and align the fiber collimator of the probe laser.**
    1. Connect the input fiber of a 33:67 FC/APC polarization-maintaining fiber splitter (port T of FS_1) to the fiber coupler of the probe laser. Connect the 67%-output fiber of the fiber splitter (port 1 of FS_1) to the fiber collimator (C_1). Attach the fiber collimator to a 6-axes kinematic mount ($Ø_x$, $Ø_y$,$Ø_z$, x, y, z). Place a power meter behind the fiber collimator and maximize the power from the laser by adjusting the x, y and z screws of the laser fiber coupler.
    2. Rotate the fiber collimator (or the optical element to be aligned) to adjust the laser polarization to the S-polarization direction, which here is perpendicular to the optical table plane. Confirm that the laser beam is S-polarized by measuring minimum (maximum) laser transmission (reflection) through an auxiliary polarizing beam splitter with a power meter.
    3. Mount two auxiliary alignment irises at an identical height from the optical table (3" in this setup). For beam propagation along the optical axis of the system and parallel to the optical table, this height should be maintained constant during the alignment of the entire system. Place one iris in a table mounting hole behind the fiber collimator (or the optical element to be aligned) at <50 mm distance. Place the second iris in a collinear table mounting hole sufficiently far from the first iris (>300 mm).
    4. Align the output beam of the fiber collimator (or the optical element to be aligned) along the optical axis of the system by adjusting the x, y, $Ø_x$ and $Ø_y$ screws of the kinematic mount until the laser beam is concentric to the center of both irises.

2. **Set up a Keplerian beam expander.**
    1. Mount a lens (L_1, f_1 = 25 mm) in a fixed optical mount.

2. Mount two auxiliary alignment irises by following the procedure in 1.1.3. Adjust finely the lateral position and pitch angle of the lens so that the transmitted beam is concentric to the center of both irises.
3. Mount a second lens (L_2, f_2 = 50 mm) in a fixed optical mount. Attach the mount post base to a linear translational stage aligned to the optical axis of the system. Place the stage such that the lens is at a distance of f_1+f_2 from the first lens. Align the lens as described in 1.2.2.
4. Place a shearing interferometer behind the second lens to confirm that the beam is collimated. Translate the second lens along the optical axis of the system until the interference fringes produced are parallel to the reference line ruled on the diffuser plate of the shearing interferometer.

3. **Fold the output beam of the beam expander.**
   1. Mount a mirror (M_1) in a kinematic mount with pitch ($Ø_x$) and yaw ($Ø_y$) adjustments. Orient the mirror to be 45° with respect to the optical axis along the elements C_1-L_1-L_2.
   2. Mount two auxiliary alignment irises by following the procedure in 1.1.3. Adjust the $Ø_x$ and $Ø_y$ screws of the mirror mount until the reflected beam is concentric to the center of both irises that defines the optical axis of the system.

4. **Set up the sample illumination optics.**
   1. Mount a zero-order quarter-wave plate ($\lambda_1/4$) in a 6-axes kinematic mount ($Ø_x$, $Ø_y$, $Ø_z$, x, y, z) at a distance of approximately 150 mm from the folding mirror (M_1), leaving sufficient space for placing a polarizer (P) before the waveplate as described in 2.7. Rotate the waveplate by 45° with respect to its fast axis to yield a circular polarization state.
   2. Mount a focusing lens (L_3, f_3 = 30 mm) in the same kinematic mount of the waveplate. Align the beam transmitted through the lens by following the procedure in 1.1.3-4.

5. **Set up the collection optics of the sample.**
   1. Mount a 6-axes kinematic mount ($Ø_x$, $Ø_y$, $Ø_z$, x, y, z) onto a differential linear translational stage at a distance of approximately 60 mm from the focusing lens (L_3). Mount a zero-order quarter-wave plate ($\lambda_2/4$) in the kinematic mount. Rotate the waveplate by 45° with respect to its fast axis and confirm that the laser beam is S-polarized by following the procedure in 1.1.2.
   2. Mount a collection lens (L_4, f_4 = 30 mm) in the same kinematic mount of the waveplate. Align the beam transmitted through the lens by following the procedure in 1.1.3-4. Confirm that the beam is collimated as described in 1.2.4.
   3. Mount a polarizing beam-splitter cube (PBS) onto a kinematic mount with pitch ($Ø_x$) and yaw ($Ø_y$) adjustments and place it behind the waveplate (as shown in Figure 1). Mount two auxiliary alignment irises by following the procedure in 1.1.3. Adjust the $Ø_x$ and $Ø_y$ screws of the beam-splitter mount until the reflected beam is concentric to the center of both irises that defines the optical axis of the system.

2. Prepare the Pump Beam Optical Path

1. **Mount and align the fiber collimator of the pump laser.**
   1. Connect the fiber of the amplified port of the pump laser to the fiber collimator (C_2). Mount and align the fiber collimator of the pump laser as described in 1.1.3 - 4.

2. **Tune the pump wavelength to the rubiduim-85 $D2_{Fg}$ = 3 absorption line.**
   1. Place a rubidium-85 vapor cell behind the fiber collimator of the pump laser (C_2).
   2. Situate an auxiliary photodetector behind the vapor cell to measure the transmission of the pump beam through the cell. Connect the photodetector to an oscilloscope. Press the 'Autoset' button on the oscilloscope to automatically set the amplitude and time trace of the readout signal from the photodetector.
   3. Set coarsely the laser wavelength to the rubidium D2 absorption line, 780.24 nm, by turning the temperature knob on the laser controller to a level where minimum light transmission is measured through the rubidium cell by the auxiliary photodetector (see step 2.2.2). Set the laser temperature to the identified level.
   4. Connect the output of a function generator to the current modulation input of the pump laser controller.
   5. Apply a triangle wave from a function generator to the current modulation input of the laser controller to slowly scan the laser wavelength across 60 pm (30 GHz). To this end, press the 'Channel Select' button on the function generator and select channel 1. Next, press the 'Ramp' button and then the 'Continuous' button to set the channel to produce a triangle waveform. Press the 'Amplitude' shortcut button to set the waveform amplitude to 2.25 Vpp (peak-to-peak voltage) and the 'Frequency/Period' shortcut button to set the waveform frequency to 5 mHz. Finally, press the 'On' button to turn on the channel of the function generator.
   6. Identify as precisely as possible the current level that brings the pump wavelength to the rubidium-85 $D2_{Fg}$ = 3 absorption line by measuring minimum light transmission through the rubidium cell using the auxiliary photodetector (see step 2.2.2). Set the laser current to the identified level by turning the current knob on the laser controller. Remove the rubidium cell and the auxiliary photodetector. Finally, disconnect the function generator from the current modulation input of the laser controller.

3. **Mount and align the laser-line clean-up filter.**
   1. Place the laser-line clean-up filter (a reflecting Bragg filter; BF) in a kinematic mount with pitch ($Ø_x$) and yaw ($Ø_y$) adjustments at a distance of 250 mm from the fiber collimator (C_2).
   2. Place a power meter in the transmission (reflection) optical path of the filter and minimize (maximize) the beam power by rotating the filter in the pitch axis to match the Bragg input angle (8° in this setup). Adjust finely the $Ø_x$ and $Ø_y$ screws of the kinematic mount to optimize the alignment.
   3. Fold the beam reflected off the filter back to a direction parallel to that of the beam at the filter input using two mirrors (M_2, M_3) mounted on kinematic mounts with pitch and yaw adjustments.
   4. Mount two auxiliary alignment irises by following the procedure in 1.1.3. Adjust the $Ø_x$ and $Ø_y$ screws of both mirror mounts until the beam reflected from the second mirror is concentric to the center of both irises that defines the optical axis of the system.

4. **Mount and align the acousto-optical modulator.**

1. Mount and align a lens (L_5, f_5 = 100 mm) to focus the pump beam into an acousto-optical modulator (AOM) as described in 1.2.2. After lens aliment, remove gently the lens L_5 from its mount prior to placing the AOM in order to avoid damage to the AOM.
2. Mount the AOM onto a 5-axes platform ($Ø_x$, $Ø_y$, x, y, z) at a distance of approximately 100 mm from the focusing lens (L_5). Ensure that the pump beam propagating through the entrance window of the modulator is S-polarized (see 2.1.2) to optimize the modulator performance.
3. Connect the RF output of the modulator driver to the RF input of the modulator using a 50-Ω coaxial cable. Turn on the driver and press the 'Mode' button on the driver so that the acousto-optical modulator operates in continuous-wave mode.
4. Place a power meter behind the modulator output to measure the power of the first-order diffracted beam only. Adjust the Bragg angle of the modulator to maximize the power of the first-order diffracted beam by rotating the modulator in the pitch axis ($Ø_x$).
5. Reposition finelythe focusing lens (L_5) in its mount to focus the pump beam into the modulator and achieve the desired fast rise/fall time (10 ns for ~50 µm beam diameter focus in this setup). Adjust the x, y, z, $Ø_x$ and $Ø_y$ screws of the mounting platform of the modulator to maximize the power of the first-order diffracted beam.
6. Fold the beam at the modulator output to a direction parallel to that of the beam at the modulator input using two mirrors (M_4, M_5) mounted on kinematic mounts with pitch ($Ø_x$) and yaw ($Ø_y$) adjustments as described in 2.3.3-4.
7. Mount and align a second lens (L_6, f_6 = 200 mm) at a distance of f_5+f_6 from the focusing lens at the modulator input to collimate the modulated pump beam as described in 1.2.3-4. This lens along with the focusing lens at the modulator input form a Keplerian beam expander for the pump beam, matching the pump and probe beam diameters prior to focusing on the sample (S).

5. Set up the pump P-polarization optics. Mount a zero-order half-wave plate (λ/2) in a rotation mount. Place the waveplate behind the second lens of the Keplerian beam expander of the pump beam (L_6). Rotate the waveplate to adjust the beam to the P-polarization direction, which here is parallel to the optical table plane. Confirm that the laser beam is P-polarized by measuring maximum (minimum) laser transmission (reflection) through an auxiliary polarizing beam splitter with a power meter.
6. **Fold and laterally shift the beam at the output of the waveplate.**
   1. Mount a mirror (M_6) in a kinematic mount with pitch ($Ø_x$) and yaw ($Ø_y$) adjustments at a distance of 50 mm from the half-wave plate (λ/2). Attach the post base of the kinematic mount to a linear translational stage aligned to the optical axis of the system. Orient the mirror to be 45^o with respect to the optical axis along the elements λ/2-PBS.
   2. Align the beam reflected from the mirror and the polarizing beam splitter as described in 1.3.1-2. Confirm that the pump beam transmitted through the polarizing beam splitter is collinear with the probe beam optical path using a laser viewing card.
   3. Translate the mirror by 3 mm in a direction perpendicular to the optical axis of the pump-probe focusing lenses (L_4 - L_3) to produce off-axis pump illumination of the sample (S) that minimizes stray pump reflections.

7. Set up the pump blocking optics in the probe optical path. Mount a linear polarizer (P) in a rotation mount. Place the polarizer between the folding mirror (M_1) and the first waveplate ($\lambda_1/4$) in the probe optical path, approximately 75 mm from each of these components. Rotate the polarizer to minimize (maximize) transmission of the pump (probe) beam.

3. Prepare the Scheme for Detecting the Frequency Detuning of the Pump and Probe Lasers

1. **Set up the fiber optics for the probe and pump lasers.**
   1. Connect the input fiber of a 50:50 FC/APC polarization-maintaining fiber splitter (port 1 of FS_2) to the fiber coupler of the non-amplified port of the pump laser. Connect the 33%-output fiber of the probe fiber splitter (port 2 of FS_1) to the 50%-input fiber of the pump fiber splitter (port 2 of FS_2) using a mating sleeve.
   2. Measure the optical power at the output fiber of the 50:50 pump fiber splitter (port T of FS_2) with a power meter and ensure that the total optical power is <10 mW to prevent saturation of the fiber-coupled photodetector (FPD). Connect the output fiber of the 50:50 pump fiber splitter (port T of FS_2) to the input of a high-speed fiber-coupled photodetector.

2. Connect the K male connector of the fast photodetector directly to the K female connector of the GHz band of a microwave frequency counter (FC).

4. Set Up the Stimulated Brillouin Gain/Loss Detector

1. **Prepare the rubidium-85 vapor cell.**
   1. Wrap the entire cell with a thermally conductive pad. Wrap a heat tape around the edges of the cell. Place a thermocouple at the center of the cell to monitor the heating temperature. Ensure that the thermocouple does not touch the heat tape. Connect the thermocouple to a thermometer to read out the cell temperature.
   2. Wrap the entire cell with a polytetrafluoroethylene tape to hold the heat tape and thermocouple in their places and to thermally isolate the cell from the environment. Leave the end of the heat tape unobstructed at both edges. Wire the two leads of the heat tape to a 0-30 V, 5 A DC power supply.
   3. Mount the cell in the reflection optical path of the polarizing beam splitter (PBS). Ensure that the probe beam hits the center of the cell.
   4. Mount an iris (I) before the cell. Open the iris so that the probe beam can completely pass through. This iris assists in minimizing stray pump reflections.

2. **Set up the photodetector.**
   1. Place the photodetector (PD) behind the rubidium cell. The photodetector, housed in an aluminum box, comprises a large-area photodiode and a homemade RC low-pass filter (R = 1 kΩ, C= 0.1 µF) that reduces noise of the reverse bias voltage. Ensure that the probe beam hits the center of the photodiode using a laser viewing card.

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2. Connect the photodiode cathode terminal to the 0-30 V, 5 A DC power supply using a 50 Ω coaxial cable. Apply a reverse bias of 25 V, by turning the voltage knob on the power supply, so that the photodiode is operated in photoconductive mode for high-frequency detection.

3. **Set up the lock-in amplifier.**
    1. Connect the photodetector to a 50Ω coaxial low-pass filter (LPF) of 1.9 MHz bandwidth using a 50 Ω coaxial cable. Connect the output of the coaxial LPF directly to the signal input of the lock-in amplifier (LIA). Press the 'Sig-Z In' button on the lock-in amplifier to set the signal input impedance of the lock-in amplifier to 50Ω.
    2. Connect channel 1 of a function generator to the reference input of the lock-in amplifier using a 50 Ω coaxial cable. Press the 'Channel Select' button on the function generator and select channel 1. Next, press the 'Sine' button and then the 'Continuous' button to set the channel to produce a sinusoidal waveform. Press the 'Amplitude' shortcut button to set the waveform amplitude to 0.7 Vpp and the 'Frequency/Period' shortcut button to set the waveform frequency to f_m=1.1 MHz.
    3. Connect channel 2 of the function generator to the external analog input of the acousto-optical modulator driver using a 50 Ω coaxial cable. Follow the procedure in 4.3.2 to set a 1 Vpp, f_m=1.1 MHz sinusoidal waveform on channel 2.
    4. Press the 'On' button on the function generator to turn on channels 1 and 2 and lock their phase relationship by pushing the 'Align Phase' bezel button on the function generator.
    5. Switch the 'Mode' button on the acousto-optical modulator driver to 'Normal' state. The pump beam is now optically modulated at f_m=1.1 MHz.

5. Final Preparations of the System and Performance Optimization

1. **Set up the data acquisition unit.**
    1. Connect the analog output of the microwave frequency counter (FC) to one analog input of the data acquisition unit (DAQ) using a coaxial cable. Press the 'DAC', '1' and '0' buttons on the frequency counter to set the frequency readout accuracy to 10 MHz. This channel monitors the pump-probe frequency detuning.
    2. Connect the 'X' output of the lock-in amplifier (LIA) to the second analog input of the data acquisition unit using a coaxial cable. Press the 'Output' button of the 'X' channel on the lock-in amplifier to activate the channel. Use this channel monitors the stimulated Brillouin gain (SBG) signal level.
    3. Split an output channel of a function generator into two separate channels using a BNC-tee connector. Connect one channel to the current modulation input of the probe laser controller and the second channel to the third analog input of the data acquisition unit using coaxial cables. Use this second channel to acquire the current modulation signal of the probe laser.
    4. Connect the USB output of the data acquisition unit to a computer. Write a program in a data acquisition software package to visualize and record the above-described signals from the data acquisition unit[14].

2. **Mount a water sample in the measurement chamber.**
    1. Fill a home-built 500 µm-thick glass chamber with distilled water. The chamber is comprised two round 25 mm diameter 0.17 mm thick glass coverslips spaced by a 500 µm-thick polytetrafluoroethylene tape.
    2. Mount a chamber holder on a 3-axis motorized translation stage. Place the measurement chamber in the holder and translate it to the joint focus point of the probe and pump focusing lenses (L_3 and L_4, respectively) using the motorized stage.

3. **Heat the rubidium cell.**
    1. Wear laser safety glasses for 780 nm laser use. Increase the power of the pump laser to obtain >250 mW on the sample by turning the current knob on the tapered-amplifier controller and measuring the power just before the sample with a power meter.
    2. Set the time constant of the lock-in amplifier (LIA) to 1 s by pressing the 'Settle Up/Down' buttons on the lock-in amplifier. Set the low pass filter of the lock-in amplifier to 24 dB/oct by pushing the 'Filter Slope Up/Down' buttons. Set the lock-in amplifier sensitivity to 1 mVrms by pressing the 'Sens Up/Down' buttons. Use the align phase function of the lock-in amplifier to adjust the phase shift between the reference and signal inputs of the amplifier to zero by pushing the 'Shift' and 'Phase' buttons.
    3. Monitor the stray pump reflections by observing the readouts on the 'X' channel of the lock-in amplifier.
    4. Retune the pump wavelength to the rubiduim-85 $D_2 F_g$ = 3 absorption line by gently turning the current knob on the laser controller to obtain a minimum stray pump reflection readout on the 'X' channel of the lock-in amplifier.
    5. Set 17 V DC on the power supply connected to the heat tape to warm up the rubidium cell to 90 ^{o}C. Wait a couple of minutes until the thermometer readout stabilizes on the desired cell temperature. Note: The signal readouts observed on the 'X' channel of the lock-in amplifier should rapidly drop during heating (due to the significant rise in the absorption of the cell).

4. **Measure and optimize the SBG signal in water.**
    1. Increase the power of the probe laser to obtain >10 mW on the sample by turning the current knob on the laser controller and measuring the power just before the sample with a power meter.
    2. Coarsely tune the probe wavelength to the rubiduim-85 $D2_{Fg}$ = 3 absorption line by turning the temperature knob on the probe laser controller and measuring a minimum laser power level behind the rubidium cell with a power meter.
    3. Finely tune the probe wavelength to be longer than the pump wavelength by turning the current knob on the probe laser controller until >10 mW, approximately constant, laser power levels are measured behind the rubidium cell with a power meter. Note: If the probe wavelength is shorter than that of the pump laser, then the additional absorption bands of the rubidium-85 cell significantly reduce the probe power at the cell output.
    4. Set the frequency detuning between the pump and probe lasers to match the Brillouin shift of water (~5 GHz) by turning the current knob on the probe laser controller and observing the frequency detuning readouts on the frequency counter (FC). Note: For the negative (positive) first-order diffracted beam, these readouts should be larger (smaller) than the Brillouin shift by the RF driving frequency of the acousto-optical modulator (210 MHz in this setup).
    5. Set the lock-in amplifier sensitivity to 100 µVrms and adjust the phase shift between the reference and signal inputs of the amplifier to zero by following the procedure in 5.3.3.

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6. Optimize the crossing efficiency of the pump and probe beams by (i) finely adjusting the $Ø_x$ and $Ø_y$ screws of the kinematic mount of the folding mirror of the pump beam (M_6), and (ii) slightly translating the pump focusing lens (L_4) along the optical axis of the system.
7. Ensure that higher signal readouts on the 'X' channel of the lock-in amplifier result predominantly from an increased SBG signal (rather than from stray pump reflections) by blocking the probe beam and measuring unchanged levels of stray pump reflections on the 'X' channel of the lock-in amplifier.
8. Repeat steps 5.4.6-7 until the SBG signal reaches a maximum (>2 µVrms), while keeping stray pump reflections at an unchanged minimum level.

6. Measure and Analyze an SBG Spectrum

1. **Create a calibration curve of probe modulation current vs pump-probe frequency detuning.**
    1. Set the frequency detuning between the pump and probe lasers to 5 GHz (around the Brillouin shift of water) by turning the current knob on the probe laser controller.
    2. Press the 'RES' and '5' buttons on the microwave frequency counter (FC) to set the gate time to 1 ms, providing a sampling interval of 100 ms between consecutive frequency detuning measurements. Apply a triangle wave to the current modulation input of the probe laser controller by following the procedure in 2.2.5 with waveform amplitude and frequency parameters of 150 mVpp and 50 mHz, respectively. This will allow to slowly scan the probe wavelength (and hence the pump-probe frequency detuning) across 2 GHz.
    3. Set the sampling rate of the data acquisition unit (DAQ) to 100 samples/s/channel and record the pump-probe frequency detuning and probe laser modulation current signals from the data acquisition unit for 20 s (over 4 - 6 GHz) using the home-written data acquisition program.
    4. Load the measurement data in a computational software program. Fit the pump-probe frequency detuning data with a linear model. Note that it is also possible to use a polynomial fit of higher order (due to the nonlinearity of the pump-probe frequency detuning measurements). Fit also the probe laser modulation current data with a linear model.
    5. Generate the calibration curve by storing in a computational software program the pump-probe frequency detuning fit samples as a function of the probe modulation current fit samples.

2. **Measure an SBG spectrum at high speed.**
    1. Mount the sample-under-test (S), for example, distilled water as used in the experiments, as described in 5.2.1 - 2. Repeat steps 5.4.1 - 8.
    2. Set the lock-in amplifier (LIA) time constant to ≥100 µs by pressing the 'Settle Up/Down' buttons on the lock-in amplifier. Apply a triangle wave to the current modulation input of the probe laser controller by following the procedure in 2.2.5 with waveform amplitude and frequency parameters of 150 mVpp and 50 Hz, respectively. This will allow to rapidly scan the probe wavelength (and hence the pump-probe frequency detuning) across 2 GHz.
    3. Set the sampling rate of the data acquisition unit (DAQ) to ≤100,000 samples/s/channel and record the SBG and the probe laser modulation current signals from the data acquisition unit for ≥10 ms (over 4 - 6 GHz) using the home-written data acquisition program.

3. **Visualize and analyze the SBG spectrum.**
    1. Load the measurement data recorded in 6.2.6 in a computational software program.
    2. Convert the measured probe laser modulation current values to pump-probe frequency detuning values by identifying these values in the calibration curve stored in 6.1.5.
    3. Subtract the average noise floor from the spectrum and visualize the SBG spectrum by plotting the SBG measurements against the pump-probe frequency detuning values.
    4. Fit the spectrum with a Lorentzian curve. For initial guess of the Lorentzian parameters, use the amplitude, frequency position and full-width at half of the highest point of the spectrum.
    5. Calculate the Brillouin shift and linewidth of the tested sample by retrieving the frequency position of the maximum and full-width at half-maximum of the Lorentzian fit, respectively.

Representative Results

Figures 2b and **3b** display typical point SBG spectra of distilled water and lipid-emulsion tissue phantom samples (with 2.25 scattering events and an attenuation coefficient of 45 cm^{-1}) measured within 10 ms and 100 ms, respectively. For comparison, we measured the SBG spectra in 10 s as shown in **Figures 2a** and **3a**. In these measurements, the rubidium-85 vapor cell was heated to 90 °C for attenuating stray pump reflections by ~10^4 and transmitting >95% of probe light; levels that were maintained stable for over an h[11]. Also, the spatial resolution, defined here as the lateral full-width at half-maximum of the SBS intensity detected from the focus, was estimated to be approximately 8 µm[10]. The mean Brillouin shifts obtained from the rapidly acquired spectra in water and tissue phantoms were 5.08 GHz and 5.11 GHz, respectively. These Brillouin shift estimates are comparable to those calculated from spectra recorded in 10 s and to previously published Brillouin data of aqueous samples[9,10,11]. The insets in the figures show histograms of the Brillouin shift estimates retrieved from 200 successive measurements of SBG spectra. The precision of the obtained Brillouin shift was evaluated in terms of the standard deviation of a Gaussian distribution fit to the observed Brillouin shift distribution. Standard deviations of 8.5 MHz and 33 MHz were obtained in the water and tissue phantom samples, representing a high measurement precision for detecting subtle changes in material mechanics. Although the pump power level used here was high (~250 - 270 mW), heating due to absorption of water at 780 nm was estimated to be <0.53 K, and thus can be neglected in the aqueous samples used in this work[10]. Moreover, no short-term instability of the SBG spectra of the water and lipid-emulsion samples was observed during 120 s of continuous exposure of the samples to these power levels.

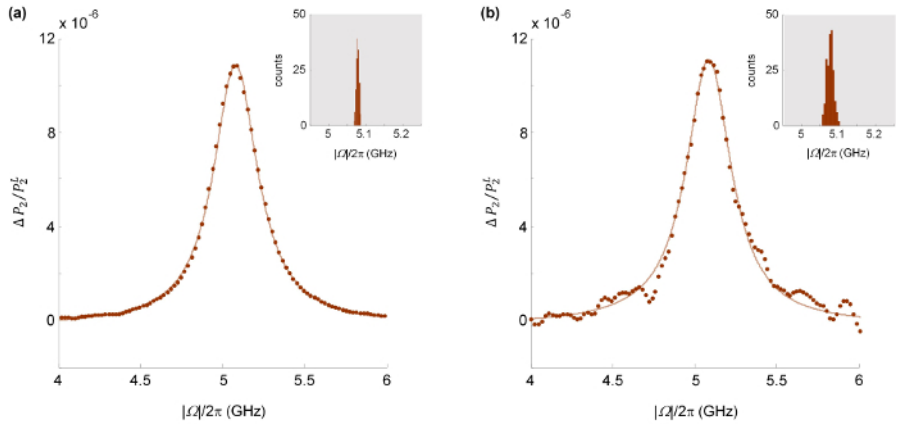

Figure 2: Stimulated Brillouin gain (SBG) Spectra of Water. Representative SBG spectra of water acquired in (**a**) 10 s and (**b**) 10 ms. Dots and solid lines stand for measurement values and Lorentzian fits, respectively. Insets show corresponding histograms of Brillouin shift estimates of water. Please click here to view a larger version of this figure.

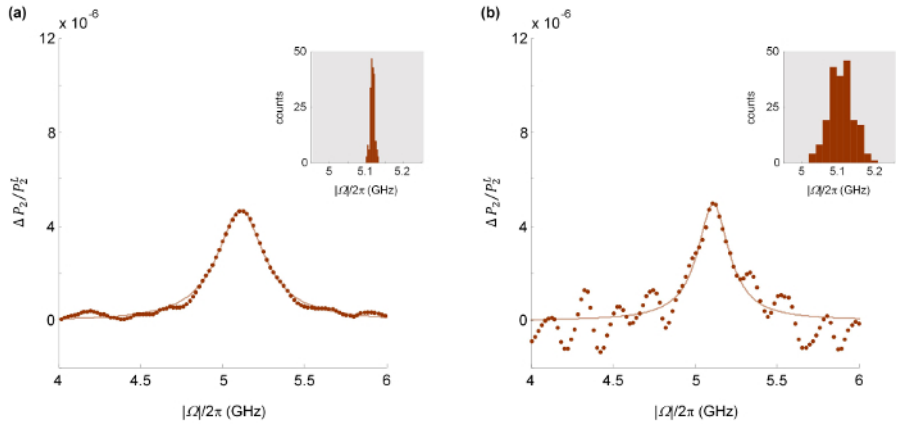

Figure 3: Stimulated Brillouin gain (SBG) Spectra of Tissue Phantoms. Representative SBG spectra of lipid-emulsion tissue phantoms (with 2.25 scattering events and an attenuation coefficient of 45 cm^{-1}) acquired in (**a**) 10 s and (**b**) 100 ms. Dots and solid lines denote measurement values and Lorentzian fits, respectively. Insets show corresponding histograms of Brillouin shift estimates of the tissue phantom. Please click here to view a larger version of this figure.

Discussion

The system, shown in **Figure 1**, was designed to be built on an 18" x 24" breadboard that can be mounted vertically on an optical table, facilitating placement of watery samples. As a result, it is important to strongly tighten all optical and mechanical elements and ensure that the pump and probe beams are collinear and concentric with the various elements prior to illuminating the sample in off-axis geometry.

Difficulties in observing the stimulated Brillouin gain signal may occur due to excessive stray pump reflections that mask the weak Brillouin gain of watery samples (~10^{-6}). To address these possible difficulties, ensure first that the chamber is positioned at the joint focus point of the probe and pump focusing lenses (L_3 and L_4, respectively). Then, close slightly the iris (I) placed before the rubidium cell and/or translate slightly the folding mirror of the pump beam (M_6) to further eliminate detection of stray pump reflections. Note that these procedures will also decrease the Brillouin signal, but may provide a better starting point for detecting the stimulated Brillouin gain signal in water. If the signal is still not detected, use methanol or carbon disulfide, which have a significantly stronger Brillouin gain than water[8,10]. Alternatively, for measurements of non-turbid samples, it is possible to use thicker glass chambers (ten times the confocal parameter of L_3/L_4) that significantly reduce detection of stray pump reflections.

In the protocol, we described high-speed measurements of stimulated Brillouin gain spectra over 2 GHz. To extend the measurements over a larger bandwidth (for example, in samples with multiple Brillouin frequency shifts separated by >1 GHz), it is essential to produce a calibration curve of the probe modulation current against the extended frequency detuning range of the pump and probe lasers. Desirably, this curve should be corrected for the small nonlinearity of the laser frequency sweep with modulation current. Alternatively, schemes for rapid monitoring of the pump-probe frequency detuning can be integrated to replace the microwave frequency counter (FC) in the spectrometer.

The Brillouin frequency shift and linewidth measured by the setup proposed here can be converted to the material complex longitudinal modulus at GHz frequencies for a known density and refractive index of the sample[4]. As in spontaneous Brillouin spectroscopy, other elements of the material stiffness tensor (*e.g.*, shear modulus) could be probed using SBS spectroscopy by detecting light scattered at different angels and polarization states from the pump light. The Brillouin spectrum would then exhibit lower signal-to-noise-ratio (due to the smaller crossing efficiency of the pump and probe beams in the sample[10,11,12]) and smaller Brillouin frequency shifts and linewidths (due to the reduced crossing

angle) than those obtained in the nearly backscattering geometry. Consequently, the use of longer measurement times and lasers with narrower linewidths would be required.

For measurements of Brillouin spectra in non-turbid samples, our current SBS spectrometer provides acquisition times that are comparable to those obtained by VIPA spectrometers[4] and that are 100-fold faster than those achieved by existing continuous-wave stimulated Brillouin scattering spectrometers (with similar Brillouin shift sensitivity)[9,10,11]. For Brillouin measurements in turbid media, our instrument is able to acquire Brillouin spectra of turbid samples with 2.25 scattering events in a time as short as 100 ms, which is 3-fold faster than that used by a VIPA spectrometer with a multipass Fabry-Perot-based Rayleigh rejection filter in turbid samples with 0.13 - 1.33 scattering events[13]. Unlike VIPA spectrometers, SBS spectrometers does not require any specialized Rayleigh rejection filters, and inherently provides excellent contrast, even in turbid samples with strong elastic scattering[10,11].

The current SBS spectrometer has not yet reached the shot-noise limit. The spectrometer noise is dominated by intensity noise in non-turbid samples and by electrical noise in turbid media[11]. As a result, the signal-to-noise-ratio (and hence the acquisition time) of the SBG signal is limited. To overcome this limitation, a low-noise electrical amplifier prior to lock-in detection could be used to further reduce the acquisition time of SBG spectra in scattering materials without decreasing the Brillouin shift sensitivity[11]. In addition, the use of shot-noise-limited laser sources with higher rejection of stray pump light in a true backscattering geometry would optimally increase the signal-to-noise-ratio of the spectrometer, allowing shorter times for recording SBG spectra with high Brillouin shift sensitivity[11].

Disclosures

The authors have nothing to disclose.

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