



Dispersive coherent Brillouin scattering spectroscopy

Ayumu Ishijima^{a,b,1,*}, Shinga Okabe^c, Ichiro Sakuma^{a,c,d}, Keiichi Nakagawa^{a,c}

^a Department of Precision Engineering, The University of Tokyo, Tokyo 113-8656, Japan

^b PRESTO, Japan Science and Technology Agency, Saitama 332-0012, Japan

^c Department of Bioengineering, The University of Tokyo, Tokyo 113-8656, Japan

^d Medical Device Development and Regulation Research Center, The University of Tokyo, Tokyo 113-8656, Japan

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ABSTRACT

Frequency- and time-domain Brillouin scattering spectroscopy are powerful tools to read out the mechanical properties of complex systems in material and life sciences. Indeed, coherent acoustic phonons in the time-domain method offer superior depth resolution and a stronger signal than incoherent acoustic phonons in the frequency-domain method. However, it requires scanning of delay time between laser pulses for pumping and probing coherent acoustic phonons. Here, we present Brillouin scattering spectroscopy that spans the time and frequency domains to allow the multichannel detection of Brillouin scattering light from coherent acoustic phonons. Our technique traces the time-evolve Brillouin oscillations at the instantaneous frequency of a chromatic-dispersed laser pulse. The spectroscopic heterodyning of Brillouin scattering light in the frequency domain allows a single-frame readout of gigahertz-frequency oscillations with a spectrometer. As a proof of concept, we imaged heterogeneous thin films and biological cells over a wide bandwidth with nanometer depth resolution.

1. Introduction

The characterization of the micro/nanoscale material's mechanical properties is critical for an understanding of their functionality [1,2]. Researchers have been looking for a means to measure on a tiny scale for several decades. However, very few tools are available for obtaining the mechanical properties of miniaturized samples including biological cells. Common mechanical tests (e.g., dynamic mechanical analysis and rheometer) used for bulk materials require contact mechanical forces on samples, and it is difficult to scale down their size to obtain small-scale spatial information. Although atomic force microscopy [3–5] enables two-dimensional (2D) measurements with a resolution up to nanometers, it is limited to surface measurements and requires the contact of the indenters with the specimens. Ultrasound elastography [6,7] provides access to mechanical properties in a non-invasive fashion but lack micrometer-level resolution. Thus, despite the advancements achieved in mechanical testing techniques, the realization of in-depth, noncontact microscopic measurements remains an open challenge.

An acousto-optical technique can meet this demand: Brillouin scattering spectroscopy interrogates material's mechanical properties from an inelastic light scattering by a phonon, referred to as Brillouin light

scattering [8]. It non-invasively reads out the viscoelastic properties of small-scale materials with high spatial resolution [9–11]. Brillouin scattering spectroscopy has various applications, including phonon physics studies [12–14] and characterization of solids [15–17], liquids [18–20], and biological samples [21–27]. In general, Brillouin scattering spectroscopy can be classified into two forms: (1) as frequency-domain Brillouin scattering spectroscopy, in which gigahertz-frequency-shifted Brillouin scattering light from incoherent acoustic phonons is recorded using a specific imaging spectrometer with a sub-gigahertz frequency resolution (e.g., virtually imaged phased array spectrometer [28]), and (2) as time-domain Brillouin scattering spectroscopy, in which the carrier frequency of Brillouin scattering light from coherent acoustic phonons, known as Brillouin oscillation, is recorded by coherent Brillouin scattering techniques (referred to as picosecond ultrasonics [19,29,30]). Although coherent acoustic phonons in the time-domain method offer superior depth resolution and a stronger signal when compared with incoherent acoustic phonons in the frequency-domain method, it does not allow multichannel detection. Moreover, it requires scanning the delay time between laser pulses for pumping and probing coherent acoustic phonons. Typically, the delay time is adjusted either by stepping a mechanical delay line or a repetition offset of two mode-locked

* Corresponding author at: Department of Precision Engineering, The University of Tokyo, Tokyo 113-8656, Japan.

E-mail address: ishijima@es.hokudai.ac.jp (A. Ishijima).

¹ Present Address: Research Institute for Electronic Science, Hokkaido University, Hokkaido, 001–0021, Japan.

pulsed laser sources with phase-controlling devices and stabilization electronics. Consequently, the conventional scanning method of delay time limits the signal acquisition speed or increases the electronic complexity. In recent years, various technological advancements have been underway to solve the above issues, such as the development of laser sources including free-running dual comb laser oscillators [31,32] and the simplification of the adjustment method for the repetition offset of two mode-locked pulsed laser sources [33].

In this article, we propose and demonstrate Brillouin scattering spectroscopy that straddles the time and frequency domains, which we refer to as dispersive coherent Brillouin scattering spectroscopy. Our technique harnesses coherent acoustic phonons and a chirped laser pulse to record time-evolving Brillouin oscillations in the frequency domain. Coherent acoustic phonons launched from a metallic thin film provide nanometer-scale depth resolution and intense Brillouin scattering light, as in time-domain Brillouin scattering spectroscopy. The chirped laser

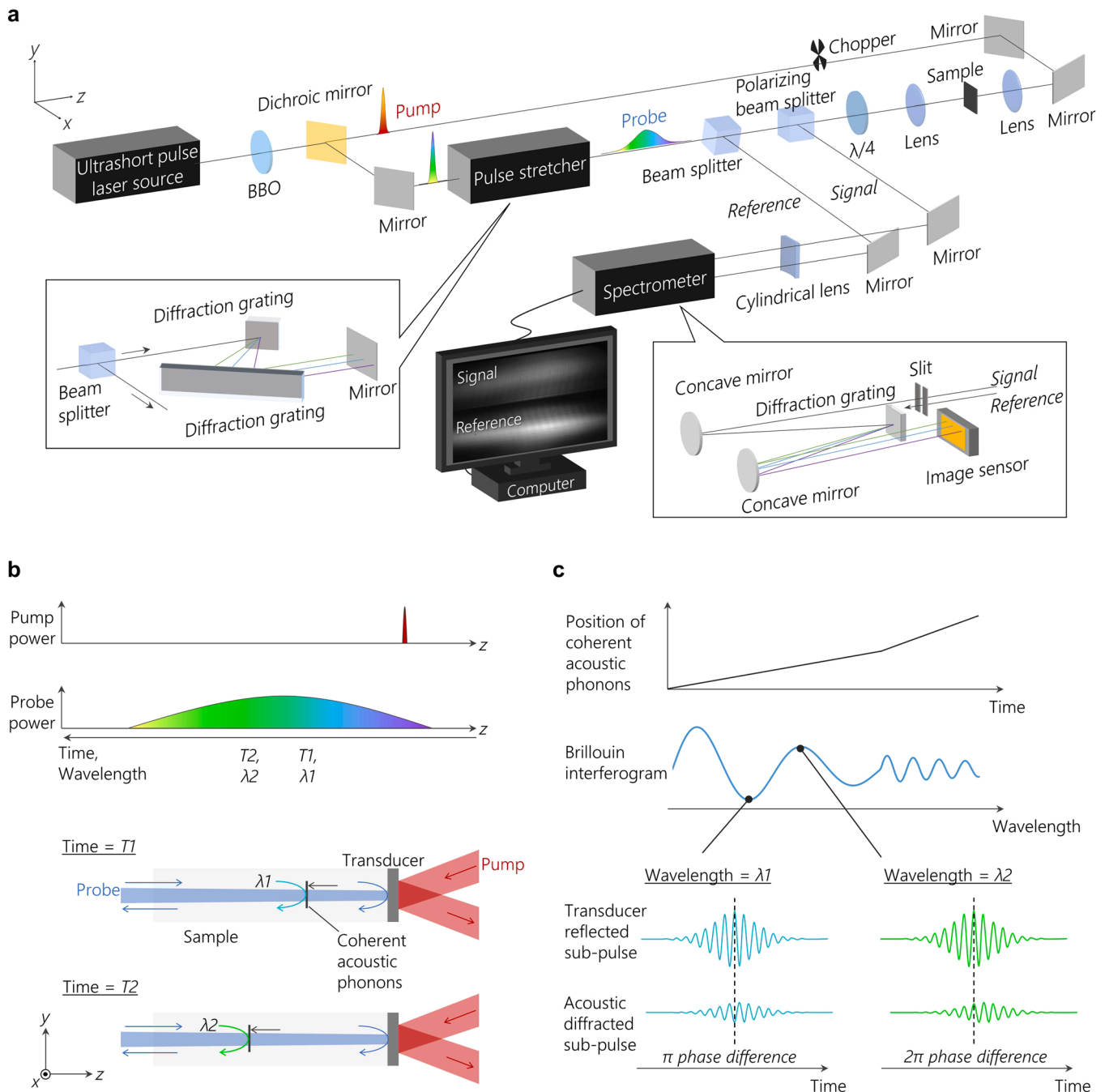


Fig. 1. Schematic and conceptual illustration of dispersive coherent Brillouin scattering spectroscopy. a) Schematic of the experimental setup. A near-infrared femtosecond laser pulse is loosely focused on a transducer surface to excite coherent acoustic phonons. A frequency-doubled laser pulse is stretched in the grating pair (left inset) and directed to a spectrometer (right inset) as a signal after the sample is illuminated. A fraction of the chirped laser pulse that bypasses the sample is also directed into the spectrometer as a reference (the light path is not shown in the right inset) for balanced detection. b) Principle of dispersive coherent Brillouin scattering spectroscopy. Each sub-pulse of the chirped laser pulse (of wavelengths λ_1 and λ_2) is diffracted by the coherent acoustic phonons at different timings (T_1 and T_2) and in different positions. c) The spectroscopic heterodyning of chirped laser pulse yields the interferogram in a frequency domain that beats at Brillouin frequency. This beat is caused by the phase difference between the diffracted chirped laser pulse from coherent acoustic phonons and the reflected chirped laser pulse from the transducer surface.

pulse with linear chromatic dispersion gives multichannel detection of Brillouin scattering light, as in frequency-domain Brillouin scattering spectroscopy. Hence, localized spectral encoding of the Brillouin oscillation to chirped pulse and decoding by a spectrometer eliminates the need for a spectrometer with a sub-gigahertz frequency resolution and mechanical or optical systems to achieve optical sampling. Our dispersive coherent Brillouin scattering spectroscopy is as versatile as conventional Brillouin scattering spectroscopy and holds great promise as a practical microscopic mechanical imaging technique.

2. Results

2.1. Working principle of dispersive coherent Brillouin scattering spectroscopy

In our technique of dispersive coherent Brillouin scattering spectroscopy, we use a single ultrafast short-pulse laser to pump and probe coherent acoustic phonons (Fig. 1a). Coherent acoustic phonons are launched into the sample by the absorption of an optical pump pulse and the subsequent rapid thermal expansion of a metallic thin film, which acts as a transducer (Fig. 1b). The pump pulse is chopped at half the laser repetition rate for compensating the laser fluctuation [34]. A femto-second laser pulse is temporally stretched by the pulse stretcher (left inset in Fig. 1a) and made incident on the sample as successive probes diffracted by propagating coherent acoustic phonons at different timing

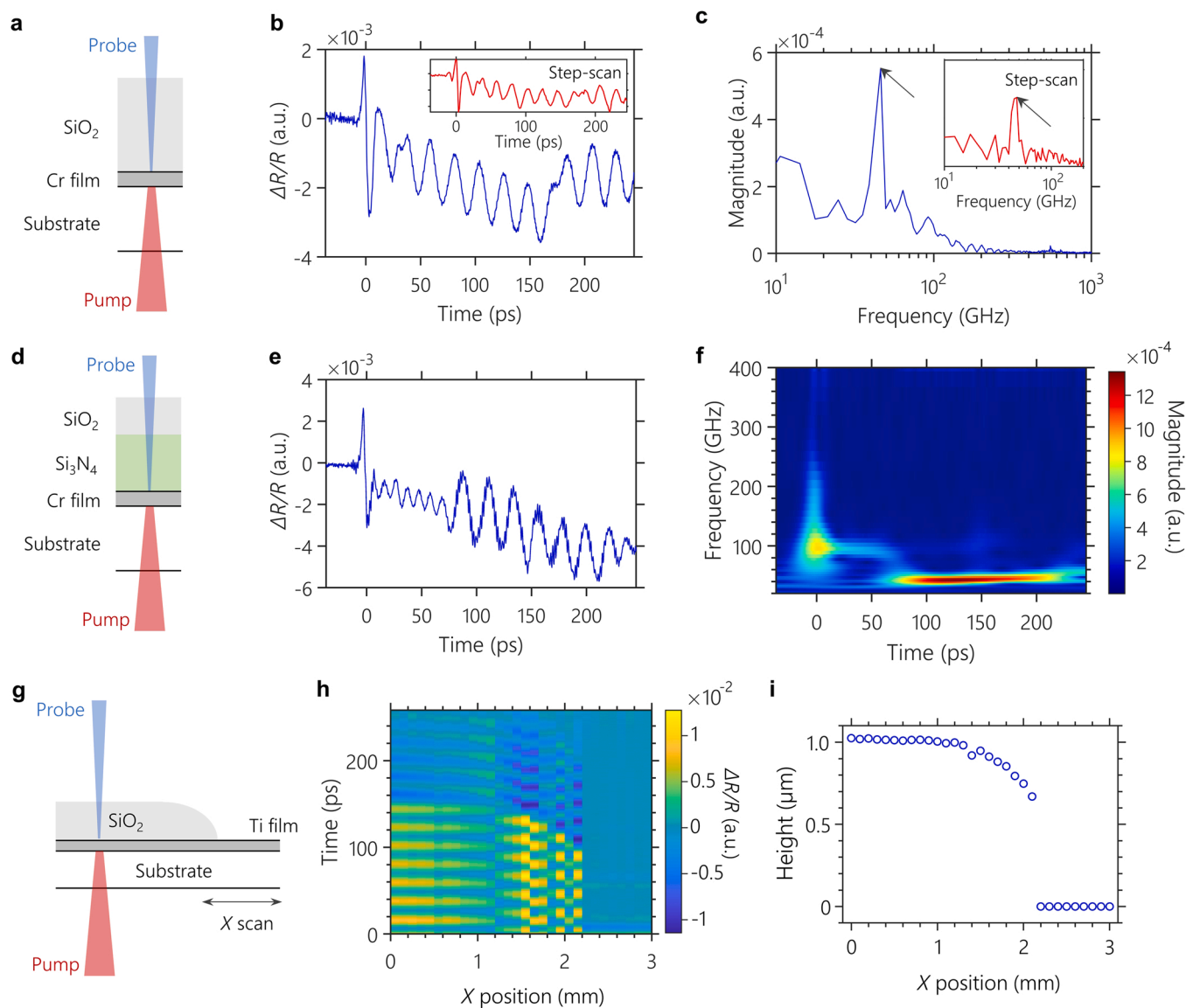


Fig. 2. Basic performance of dispersive coherent Brillouin scattering spectroscopy. (a) Schematic of the experiment with a 1 μm SiO₂ film sputtered on a 100 nm chromium film that launches coherent acoustic phonons through the transient absorption of an optical pump pulse (1.2 mJ/cm²). The pump pulse excites the coherent acoustic phonons from the glass substrate side, which plays the role of the transducer. A chirped probe pulse then detects acoustic propagation in SiO₂. (b) Brillouin oscillations acquired by dispersive heterodyne Brillouin spectroscopy (blue) and time-domain Brillouin spectroscopy with step-scan method (red). (c) The Fourier transform of the measured Brillouin oscillations indicates a peak at 44 GHz. (d) Schematic of the depth profiling experiment with the 700 nm Si₃N₄ film and 500 nm SiO₂ film sputtered on a 100 nm chromium film. (e) Recorded Brillouin oscillations and (f) the corresponding time evolution of the Brillouin frequency indicating peaks around 90 GHz and 44 GHz. (g) Schematic of the sample with 1 μm SiO₂ step created on a 300 nm titanium film. (h) Recorded Brillouin oscillations and (i) SiO₂ height at each scanning position.

and positions (Fig. 1b). In other words, the instantaneous frequency of the chirped laser pulse encodes the time delay with respect to the pump pulse. Heterodyning the diffracted chirped laser pulse from coherent acoustic phonons and the reflected chirped laser pulse from the surface of the transducer leads to the gigahertz-beating of the light intensity in the time domain. A spectrometer (right inset in Fig. 1a) disperses the chirped laser pulse in the frequency domain to create a Brillouin interferogram (Fig. 1c). The frequency f_B of these oscillations is related to acoustic velocity v , refractive index n , and probe wavelength λ through the relation $f_B = 2nv/\lambda$ for normal probe beam incidence. The Brillouin interferograms recorded by the image sensor are processed on a computer to construct a time trace of transient reflectivity with the time axis calibrated based on the optical settings (Fig. S1, Supporting information). Briefly, the time axis was calibrated from transient reflectivity waveforms obtained by varying the pump delay time by stepping an optical delay line set in the pump arm. For calculating the wavelength-to-time calibration coefficient, the wavelength at which the first transient reflectivity peak was detected against the delay time added to the pump pulse.

A high sampling frequency (4.2 THz) and an ample time window (282 ps) can be achieved with the large number of data points (1024 points). The temporal resolution of the system is determined by the sub-pulse duration [35] given by $(\tau_0\tau_c)^{1/2}$, where τ_0 is the Fourier transform-limited pulse duration and τ_c is the chirped pulse duration. The maximum time window is traded off with the temporal resolution, where increasing the chirp elongates the time window but degrades the temporal resolution (Fig. S2, Supporting information). We used a chirped pulse of a full-width at half-maximum (FWHM) temporal duration of 160 ps and 400 nm-centered wavelengths with an FWHM bandwidth of 7.8 nm corresponding to a 31 fs transform-limited pulse duration. Based on these parameters, our current system has a temporal measurement resolution of 2 ps. Furthermore, the probe bandwidth of 7.8 nm used in this study is narrow enough for probing photons to “see” the same phonon wavepacket in the linear range of dispersion [36].

2.2. Basic performance of dispersive coherent scattering Brillouin spectroscopy

To gauge the performance of dispersive coherent scattering Brillouin spectroscopy, we measured the Brillouin frequency of a silicon dioxide (SiO₂) thin film attached to a 100 nm chromium film (Fig. 2a). We used SiO₂ thin film as the sample for our proof-of-principle demonstration because the acoustic velocity ($v \sim 5.9$ km/s), refractive index ($n \sim 1.47$), and Brillouin frequency ($f_B \sim 44$ GHz) at $\lambda \sim 400$ nm were reported in earlier studies [37,38]. The time trace of the Brillouin oscillations (Fig. 2b and Fig. S3, Supporting information) and the Brillouin spectrum (Fig. 2c) obtained using our technique (averaged over 4000 shots, which corresponds to a data acquisition time of 40 s in our experimental setup) are in good agreement with those measured using time-domain Brillouin scattering technique (step-scan method). The thickness of the SiO₂ thin film calculated from a bump on the time trace around 180 ps originating from the SiO₂-air interface is 1 μ m, which matches the actual thickness of the SiO₂ film. Meanwhile, there were slight differences in the Brillouin oscillation amplitude and a non-oscillatory signal component owing to indispensable wavelength dependence on piezo-optical couplings [39]. It is also worth mentioning that the method falls short in measuring acoustic dispersion of the sample because time-evolving Brillouin oscillations are recorded to the wavelength of the probe pulse.

We show the nanometer-resolution depth profiling of a heterogeneous thin film consisting of silicon nitride (Si₃N₄) and SiO₂ (Fig. 2d); Si₃N₄ is widely used in optoelectrical devices, and its Young’s modulus is approximately three times higher than that of SiO₂ [38]. From the time trace presented in Fig. 2e, one can recognize the change in the phase-matching condition of the probe light and coherent acoustic phonons when the coherent acoustic phonons leave Si₃N₄ and enter SiO₂. High-frequency oscillations ($f_B = 90$ GHz) from the Si₃N₄ layer

appear first, and low-frequency oscillations ($f_B = 44$ GHz) start with a delay of 80 ps, which corresponds to the crossing of coherent acoustic phonons at the interface of the SiO₂ layer (Fig. 2f). Si₃N₄ has a higher Brillouin frequency than SiO₂, as expected, because the product of the material’s refractive index and acoustic velocity differs by a factor of 2.

We also performed profiling of a 1 μ m SiO₂ step created on a 300 nm titanium film (Fig. 2g). We obtained spatial profile by translating the sample over 3 mm with a 100 μ m pitch. From the transient reflectivity map shown in Fig. 2h, we can observe Brillouin oscillations from the SiO₂ layer up to a lateral position of 2.2 mm. The thickness of the SiO₂ thin film (Fig. S4, Supporting information) corresponds well to the bumps on the time trace around 150 ps originating from the SiO₂-air interface. In this context, the timing where coherent acoustic phonons reached the interface between titanium and SiO₂ (T_1) and SiO₂ and its surface (T_2) were defined as jumps in the transient reflectivity waveforms. We defined T_1 as the first peak of the oscillated transient reflectivity waveform. The spatial profile of the film thickness was calculated using the time integration of the acoustic velocity v between T_1 and T_2 at each measurement position. The lateral boundary of the film becomes apparent near 1.2 mm, and the thickness of the film decreases as it approaches the bare titanium region (Fig. 2i). Furthermore, we demonstrate a cross-sectional Brillouin frequency mapping capability. We used glycerol, a well-known and well-characterized prototypical glass-forming liquid [40], squeezed between two flat glass substrates; one of them was a 300 nm titanium film with a 1 μ m SiO₂ step. We can observe the interface between SiO₂ and glycerol from the contrast produced by the Brillouin frequency (Fig. S5, Supporting information), whereas their close refractive index values ($n \sim 1.47$ and 1.48 for SiO₂ and glycerol, respectively) make it difficult to see the interface with the naked eye.

2.3. Three-dimensional Brillouin imaging by dispersive coherent Brillouin scattering spectroscopy

To demonstrate the 3D Brillouin imaging capability, we imaged phosphate-buffered saline squeezed between two flat glass substrates; one of them was a 300 nm titanium film with a 1 μ m SiO₂ step (Fig. 3a). We tuned the time window from 282 ps to 844 ps for improving the low Brillouin frequency resolution and enlarging the depth direction field of view by simply adjusting the distance between the grating pairs (Fig. S6, Supporting information). The temporal measurement resolution was 3.8 ps in this configuration (Fig. S2, Supporting information). 3D transient reflectivity maps were obtained by raster scanning the sample in two dimensions (fixed z axis) with a 5 μ m step over 100 μ m, averaging over 2000 pairs of measurements without and with the pump (0.9 mJ/cm²) for each step. We can observe the interface between SiO₂ and phosphate-buffered saline from the contrast produced by the Brillouin frequency (Fig. 3b–3d). The spatial variation in the Brillouin frequency at the calculated depth positions is mapped, as depicted in Fig. 3e (Movie S1, Supporting information). The area occupied by SiO₂ in the cross-sectional image decreases with increasing height because of the slope set in the sample.

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The microscopic imaging capabilities were further illustrated using biological cell samples (Fig. 4a and Fig. S7, Supporting information). We used HeLa cells fixed on a 300 nm titanium film, and the sample was scanned in two dimensions with a 3 μ m step over 60 μ m to acquire 3D transient reflectivity maps. We averaged over 2000 pairs of measurements without and with the pump (0.04 mJ/cm²) for each step, resulting in an acquisition time of 40 s/step. The acoustic image clearly shows the spatial variations in the peak frequency of the internal structure, particularly the nucleus (yellow region) and cytoplasm of the cell (Fig. 4b–4d). The spatial variation in the peak frequency at the calculated depth positions is mapped in Fig. 4e (Movie S2, Supporting information). The high-frequency regions around the cells in the shallow area originate from the surface displacement of the Ti film and not the

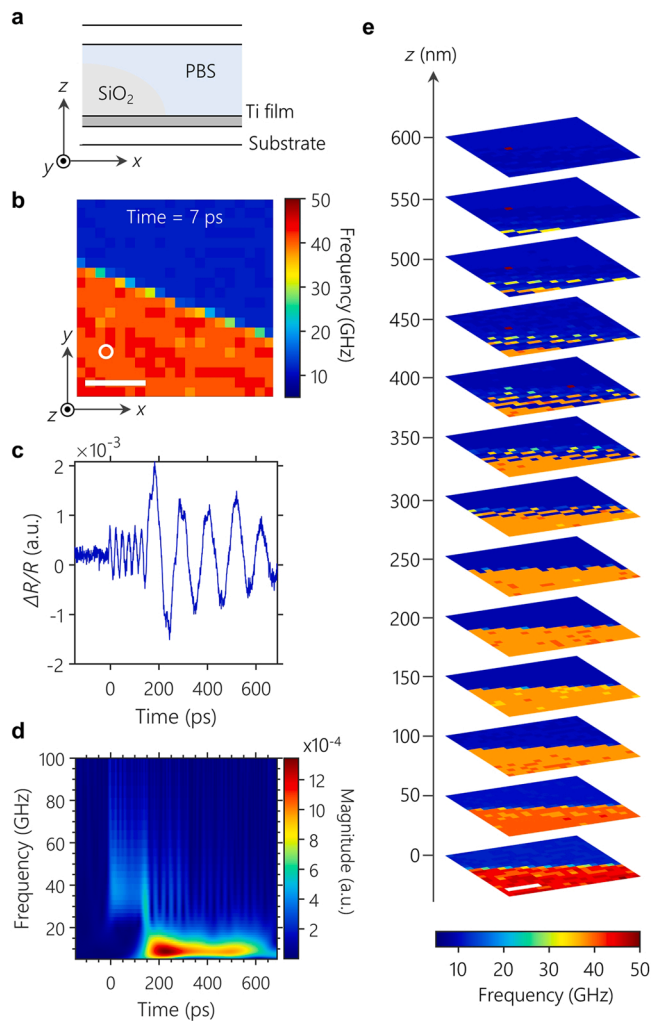


Fig. 3. Three-dimensional dispersive coherent Brillouin scattering microscopy of thin film sample. (a) The sample is phosphate-buffered saline (PBS) squeezed between two flat glass substrates; one of them is a 300 nm titanium film with a 1 μm SiO₂ step. (b) Representative cross-sectional Brillouin frequency image of the sample at 7 ps, showing a signal from SiO₂ (red) and phosphate-buffered saline (blue). The difference in the Brillouin frequencies of the two materials produces a high contrast in the Brillouin frequency image. (c) Recorded Brillouin oscillations and (d) corresponding time evolution of the Brillouin frequency at the position identified as a white circle in the Brillouin frequency image. (e) 3D Brillouin frequency images at different calculated depth position. Scale bars are 30 μm .

frequencies originating from the cells. Therefore, these regions were masked for improved visualization. Furthermore, we acquired the spectral images by plotting the spectral magnitude of a distinguishable frequency signature of the sample to clearly visualize the cellular structure in three dimensions (Fig. 4f and Movie S3, Supporting information). Although the image displayed in Fig. 4e and 4f are sectioned every 100 nm, the depth resolution is limited to ~ 400 nm from the spatial width of the wavelet used in the frequency analysis (see Materials and Methods). Note that the Brillouin frequency and the acoustic resonance frequency are hardly distinguishable for the sample thickness where the Brillouin frequency and acoustic resonance frequency or harmonics have close values [41], e.g., in the thin cytoplasm of the dehydrated HeLa cells. Therefore, we did not define the peak frequency as Brillouin frequency for the dehydrated HeLa cells sample.

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3. Discussion and conclusion

In this study, the feasibility of dispersive coherent Brillouin scattering spectroscopy is demonstrated. The technique combines two key mechanisms – localized spectral encoding of the Brillouin oscillation and decoding by a spectrometer – to perform the multichannel detection of Brillouin oscillations. Consequently, our technique can acquire Brillouin oscillations at least 100-times faster than the step-scan method used in conventional time-domain Brillouin scattering spectroscopy. The abovementioned comparisons were performed under the following conditions: identical laser repetition rate, measurement time window, and temporal resolution and an optical delay line with an infinitely short positioning time. In this case, the proposed method reduces the measurement time by the number of scanning points of the optical delay line. Therefore, we achieved a 126-fold (222-fold for imaging experiments) improvement in the signal acquisition speed, given that the temporal resolution was 2.2 ps (3.8 ps) and the measurement time window was 282 ps (844 ps).

However, as of the current proof-of-principle experimental setup, the acquisition speed does not surpass asynchronous optical sampling (ASOPS) systems [31,32,42–45], which improved the acquisition rate to a few seconds per point. The frame interval of the image sensor used as a multichannel detector limits the overall measurement time for our current setup. This limitation can be addressed through further system improvements from numerous directions, such as using a high-frame-rate camera with higher pulse repetition rate laser source. Furthermore, changing the operating wavelength to the near-infrared region to employ optical fiber-based dispersive Fourier-transform spectroscopic techniques [46,47] would further improve the acquisition rate of our method.

While the performance of our detection electronics was sufficiently high, the measurements were limited by the pointing and the shot-to-shot energy fluctuations of the Ti:sapphire regenerative amplifier. Despite significant reductions in laser noise through balancing and chopping (see Methods and Fig. S8, Supporting information), laser fluctuations remain the dominant noise source. Not performed in this study, but we can further reduce the noise by digital filtering (i.e., unweighted averaging) of the transient reflectivity waveform [34].

Moreover, while the measurement time window was limited to sub-nanosecond time scales by the size of the diffraction grating, it can be easily tuned to nanosecond time scales by the dispersion in the optical fiber. Enlarging the time window would potentially not worsen the signal-to-noise ratio because the pulse stretcher is consisted of passive optical elements. Note that the time window is traded with the temporal resolution at a given bandwidth of the probe pulse (Fig. S2, Supporting information). The optical fiber-based system can profoundly benefit from translating our technique toward a study on moving biological specimens and flow cytometric applications. Thus, its potential applications are versatile and introduce a new paradigm in Brillouin scattering spectroscopy to open a door for the prospect of unexploited fields in material and life sciences.

4. Materials and methods

4.1. Brillouin scattering spectroscopy system

The ultrashort laser source we used was a Ti:sapphire regenerative amplifier (Astrella-USP-1K, Coherent, US) producing 803 nm-centered wavelengths with a bandwidth of 70 nm, and a pulse duration of 35 fs amplified pulses in a maximum repetition rate of 1 kHz. Because the sensor readout used in the spectrometer led to slow data acquisition, the pulse repetition rate was down counted from 1 kHz to 100 Hz. The laser output was frequency-doubled (406 nm-centered wavelengths with a bandwidth of 7.8 nm) by a β -barium borate crystal (BBO-1001 H, Eksma Optics, LT) and separated from an 800 nm laser pulse by a harmonic separator (USB21, Thorlabs, US) to use as a probe pulse.

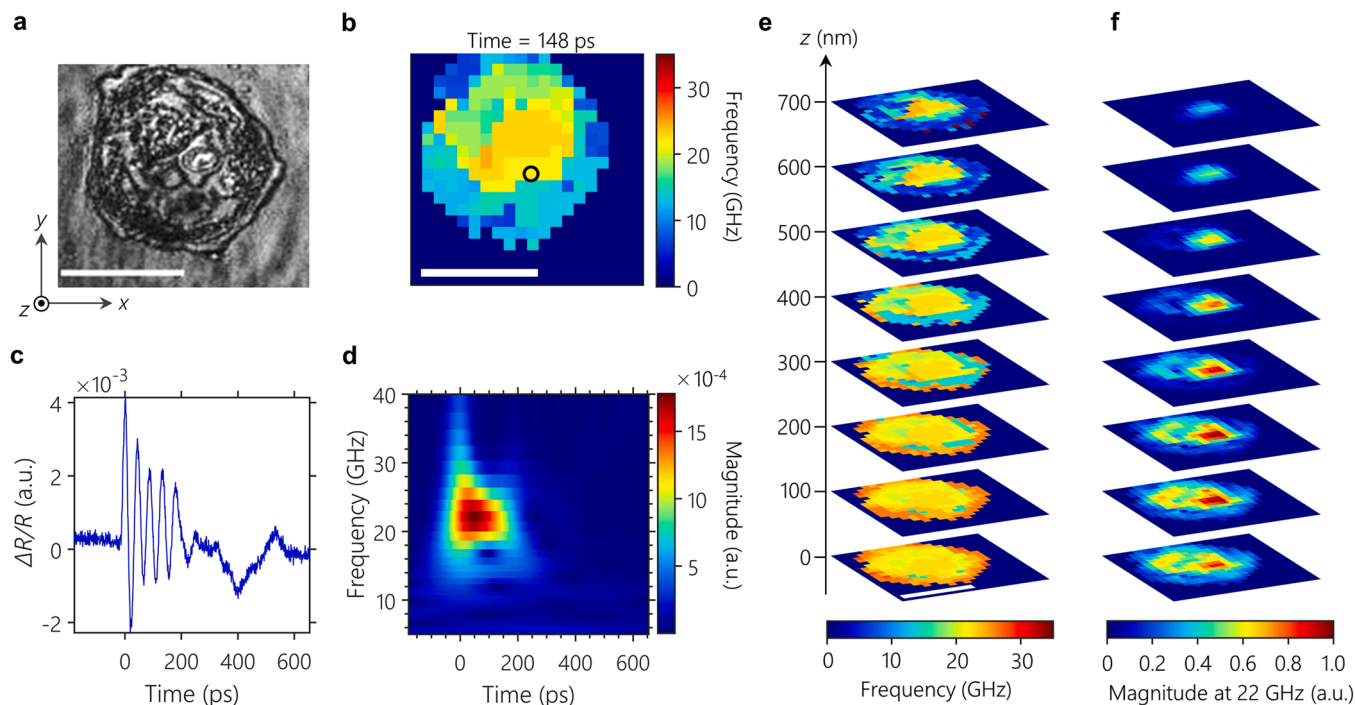


Fig. 4. Three-dimensional dispersive coherent Brillouin scattering microscopy of biological cell. (a) Brightfield image and (b) Representative cross-sectional acoustic image of the HeLa cell at 148 ps. (c) Recorded Brillouin oscillations and (d) corresponding time evolution of the Brillouin frequency at the position identified as a black circle in Fig. 4b. (e) 3D acoustic images and (f) 22 GHz spectral images at various calculated depth positions. The images are masked to remove the high frequency regions around the cells in the shallow area, which originate from the displacement of the Ti film surface. Scale bars are 30 μm .

An 800 nm fs laser pulse, modulated at 50 Hz by an optical chopper (MC2000B, Thorlabs), served as a pump pulse to excite coherent acoustic phonons. A probe pulse was time-stretched to by a pair of 36000 lines/mm gratings (PC3600, Spectrogon, SE). Subsequently, the probe pulse was split into a sample and a reference arm by a beam splitter (BS013, Thorlabs). After passing via a half-wave plate (WPH10M-405, Thorlabs), polarizing beam splitter (PBSW-405, Thorlabs), and a quarter-wave plate (SAQWP05M-700, Thorlabs) in the sample arm, a lens ($f = 40$ mm) focused a probe pulse on the sample. For microscopy experiments, we changed the lens to an objective lens with a numerical aperture of 0.42 (M-Plan Apo 20 \times , Mitsutoyo, JP). The probe pulse reflected from the sample, and a fraction of probe pulse that bypassed the sample was directed into a homebuilt Czerny–Turner spectrometer (spectral resolution of 0.037 nm, pixel resolution of 0.0169 nm/pixel, spectral bandwidth of 17.3 nm) coupled to a 16-bit sCMOS camera (Orca Flash4.0 V3, Hamamatsu Photonics, JP) by a cylindrical lens ($f = 200$ mm) for realizing balanced detection. Our spectrometer consisted of two spherical mirrors (CM508–500-F01, Thorlabs) and a 2400 lines/mm grating (GH50–24 V, Thorlabs). Using a digital delay generator (DG645, Stanford Research Systems, US), the camera and the optical chopper (MC2000B, Thorlabs) were synchronized to the laser’s repetition rate. The samples were mounted on motorized stages connected to a stage controller (SHOT304GS, Sigmakoki, JP).

4.2. Transient reflectivity calculation

Transient reflectivity waveforms were constructed from the captured images, yielding a sensitivity of approximately 10^{-5} with 10^3 laser shots (Fig. S8, Supporting information) by following procedures (Fig. S9a, Supporting information). First, we excluded pixels at or near the boundary of signal and reference regions. Second, each region corresponding to a specific delay time was vertically averaged over roughly 500 pixels. Finally, we normalized the value based on the signal (I_{sig}) and reference (I_{ref}) spectra recorded with (w) and without (w_0) the pump pulse:

$$\frac{\Delta R}{R} = \frac{I_{sig}^w}{I_{ref}^w} \bigg/ \frac{I_{sig}^{w_0}}{I_{ref}^{w_0}} - 1 \quad (1)$$

4.3. Time-domain Brillouin scattering spectroscopy with step-scan method

We used an optical setup similar to that described above without the pulse stretcher and spectrometer. Two photodiodes (SM1PD1A, Thorlabs) with current preamplifiers (PDA200C, Thorlabs) were used instead of the spectrometer. The peak voltage output from the current preamplifier was recorded using a data acquisition card (USB-6216, National Instruments, US) with an external sampling clock produced by a delay generator (DG535, Stanford Research Systems, US) to measure the pulse energy of each probe pulse [34]. The delay time between pump and probe pulses was changed by stepping the optical delay line set in the pump arm.

4.4. Brillouin imaging

Transient reflectivity waveforms at each measurement position of the sample were acquired by translating the sample mounted on a three-axis motorized stage connected to a stage controller. We produced a Brillouin frequency map by processing each waveform with continuous wavelet transformation using the generalized Morse wavelet to obtain the peaks of Brillouin frequency f_B at different timing (Fig. S9b and S9c, Supporting information). The frequency domain representation of the Morse wavelet [48] is

$$\Psi_{P,\gamma}(\omega) = U(\omega) a_{P,\gamma} \omega^{\frac{P^2}{2}} \exp(-\omega^\gamma) \quad (2)$$

where $U(\omega)$ is the unit step function, $a_{P,\gamma}$ is a normalizing factor, P^2 is the time-bandwidth product, and γ is the asymmetry parameter. We used the symmetry parameter γ of 3, and the time-bandwidth product P^2 of 5 and 60 for SiO_2 and the cell sample, respectively. The depth profile of the sample was constructed by calculating the acoustic velocity v from

the Brillouin frequency map through the relation of $\nu = f_B \lambda / 2n$. The refractive index n for each sub-pulse wavelength λ was calculated from the dispersion formula [49,50] for the SiO₂ sample. The refractive index n for phosphate-buffered saline and cell sample was assumed to be 1.33 and 1.36, respectively.

Note that the width of the wavelet mainly limits depth resolution. The wavelet duration in time is proportional to P ; therefore, the value of P^2 affects the depth resolution when the contrast mechanism in the final image is the Brillouin frequency. When sectioning the two-layered sample consisting of phosphate-buffered saline ($f_B = 10$ GHz) and SiO₂ ($f_B = 44$ GHz) as in Fig. 3, sampled phonon wavelength ($\lambda_{\text{phonon}} = \lambda/2n$) is 150 and 136 nm at $\lambda = 400$ nm, respectively. As for the wavelet size, the temporal width of 10 and 44 GHz wavelets are 91 and 21 ps for $P^2 = 5$ (Fig. S10a and S10b, Supporting information). From the calculated acoustic velocity of phosphate-buffered saline ($\nu = 1.5$ km/s) and SiO₂ ($\nu = 5.9$ km/s), the spatial width of those wavelets is 137 and 124 nm. For $P^2 = 60$, the temporal width of 10 and 44 GHz wavelets are 288 and 66 ps (Fig. S11c and S11d, Supporting information), respectively. The spatial width of those wavelets is 433 and 389 nm. These values are comparable to or larger than the sampled phonon wavelength. Therefore, depth resolution is limited by the width of the wavelet. Moreover, depth resolution is traded for frequency resolution, increasing depth resolution decreases frequency resolution (Fig. S11e and S11f, Supporting information).

4.5. Thin film preparation

The chromium, titanium, and silicon nitride thin layers were deposited on a 100 μm thick glass substrate (0111650, Paul Marienfeld, DE) by a sputtering system (iMiller CFS4EP, Shibaura Mechatronics Corp., JP). The silicon dioxide layer was deposited by a different sputtering system (SIH-450, Ulvac Inc., JP). We prepared the SiO₂ step on the titanium film by partially masking the titanium layer with 500 μm thick silicon before depositing SiO₂.

4.6. Biological cell sample preparation

HeLa cells were cultured on an EtOH-sterilized 300 nm Ti film deposited on a 100 μm thick glass substrate for 24 h. The cells were fixed with 4% (w/v) paraformaldehyde solution (161–20141, Fujifilm Wako, JP) for 15 min at room temperature. After washing with distilled water, the cells were exposed to air.

CRedit authorship contribution statement

A.I. conceptualized and designed the system. A.I. and S.O. constructed the systems and performed experiments and analyses. I.S. and K.N. assisted in constructing the system and conducting the experiments and analyses. A.I. supervised the study and wrote the manuscript. All authors have contributed to and approved the final manuscript.

Declaration of Competing Interest

The authors declare no competing financial interests.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.pacs.2022.100447](https://doi.org/10.1016/j.pacs.2022.100447).

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Ayumu Ishijima is an assistant professor at Hokkaido University, and a PRESTO researcher at Japan Science and Technology Agency. His research focuses specifically on the development of ultrafast photoacoustic technologies for visualizing biological cells using photoacoustic waves to enable studying biomechanical function of the cells. He received his Ph.D. in Engineering from The University of Tokyo and his Bachelor in Engineering from University of Electro-Communications, and conducted post-doctoral research at the University of Tokyo.



Okabe Shinga received his master's degree in Engineering from The University of Tokyo. His research focused on mechanical imaging of biological cells to elucidate biological phenomena. Currently, he is working as a medical device engineer.



Keiichi Nakagawa received a Ph.D. degree in engineering from The University of Tokyo, Tokyo, Japan, in 2014. He is currently a Lecturer at The University of Tokyo. His research interests are biomedical engineering, precision engineering, and optical engineering.