The use of photoacoustics (PA) being a convenient non-invasive analysis tool is widespread in various biomedical fields. Despite significant advances in traditional PA cell systems, detection platforms capable of providing high signal-to-noise ratios and steady operation are yet to be developed for practical micro/nano biosensing applications. Microfabricated transducers offer orders of magnitude higher quality factors and greatly enhanced performance in extremely miniature dimensions that is unattainable with large-scale PA cells. In this work we exploit these attractive attributes of microfabrication technology and describe the first implementation of a vacuum-packaged microscale resonator in photoacoustic biosensing. Steady operation of this functional approach is demonstrated by detecting the minuscule PA signals from the variations of trace amounts of glucose in gelatin-based synthetic tissues. These results demonstrate the potential of the novel approach to broad photoacoustic applications, spanning from micro-biosensing modules to the analysis of solid and liquid analytes of interest in condense mediums.
In a relatively variant configuration, PA pulses are excited precisely between the prongs of a quartz tuning fork (QTF) immersed within the gas chamber [19,20]. The PA signal, in which case, is amplified by the resonant QTF \( f_0 = 2^{15} \) Hz rather than the cell resonance. While this scheme partly counteract the large mismatch of the acoustic impedances \( Z \) and the resulting signal loss \( \approx 10 \log_{10}(\text{abs}(-42g_z/Z_{\text{gas}}^{2})) \sim 65 \text{ dB} \) (section 2.2), the major challenges to signal stability and the physical constraints to diverse practical applications remain unchanged (see supplementary section 1 for further discussion).

In practice, design optimization of a cell resonator for a particular mode is extremely challenging. This is principally due to the limitations of approximate analytical models and the commonly employed top-down design approach; acoustic cell is fabricated to some rudimentary selected dimensions first, and the exact frequency response is measured later experimentally. In the resonant operation, the optically generated acoustic signals usually act as a broadband excitation source, bringing all the eigenmodes of the cell resonator into effect. Even in the case of simplest cylindrical geometry, frequency spectrum is generally crowded with multiple resonance peaks [14,21,22]. The nature and the degree of interference among different modes at the microphone detection region results in distorted or asymmetric peaks of comparable intensity [17,23]. This greatly undermines the design efforts and the benefits of selecting any specific resonance for measurement. Furthermore, the contribution of a particular mode to the signal amplitude becomes impossible in the time domain. The second consequence of this un-selective multimode excitation is the positive contribution to the thermo-viscous losses that ultimately determine the extent of acoustic amplification obtained at the cell resonance [13]. As a result, in most cases, available resonant Q-factors remain limited to just few hundreds.

While the cell resonators are widely applied to trace gas studies, relatively a small subset of efforts has been directed towards applications concerning condensed mediums. The progress in this dimension has largely been restricted due to the difficulties in achieving sufficient degree of sensitivity and steady operation with PA cells. Whether the sample being studied is gas, liquid or solid, beyond the fundamental design challenges, the principle restraints to the practical applications are essentially similar. The signal stability is the single most critical issue, as the resonant operation is affected by any change in the pressure, temperature and humidity/composition of the gas inside the cell. Interestingly the performance of QTF transducers too, both the \( f_0 \) and the \( Q \), subject to same consequences. The temperature-related fluctuations, however, usually play the dominant role in either case. For instance, theoretical studies [24] for the former show a net \( T \) dependence of PA signal on the temperature, while in actual measurements a much more profound impact \( T^{-1.7} \) has been reported [17,25,26].

This is easy to comprehend from the strong affiliation of the acoustic speed with the thermodynamic properties of the gas, \( c = c_0/\sqrt{T} \). Here \( c \) is the specific heat ratio, \( T \) the gas constant and \( T \) the thermodynamic temperature. Any variation of gas temperature is directly reflected as the drifts of the cell resonance and the Q-factor. This further discourages the use of very high \( Q \) resonators which are more liable to temperature fluctuations. Any potential solution to these challenges must provide ease of design and fabrication, high Q-factor performance as well as stable operation against any external instabilities.

Devices based on nano/microelectromechanical systems are capable of levels of performance that greatly surpass those of larger sensing systems. The unprecedented sensitivity in the detection of displacement [27], mass [28] and force [29] etc., has been achieved through the advances both in the microfabrication techniques that can mass produce devices with single-digit-nanometer precision as well as by the ingenious exploitation of these microsystems with various physical phenomenon. Here, we employ a microscale vacuum-packaged Si resonator with an off-chip optical readout to transduce PA elastic waves. This method offers a remarkably high Q-factor detection of miniscule PA signals while maintaining a steady and stable performance for prolonged measurements. The complete isolation of the micro-mechanical resonator from the ambient air effectively minimizes the influence of environmentally induced fluctuations. The employed coupling layout also benefits from the efficient solid-to-solid phase acoustic transmittance to the packaged resonator, in contrast to weak solid-to-gas coupling of the microphone diaphragm in the conventional setup. We demonstrate the potential of this method by correlating the vibration amplitudes of the micromechanical resonator with the intensity of PA signals from physiological range glucose concentrations, embedded in lab-synthesized gelatin tissues.

2. Functional principle

2.1. Elastic wave transduction at mechanical resonance

Like the harmonic oscillators in atomic force microscopy (AFM) that absorb energy via physical interaction to the sample surface, the sensing element can also be driven to motion through mechanical coupling with the sample by optically generated thermoelastic waves. The modulation rate of the optical excitation is adjusted to tune the acoustic coupling at the mechanical resonance where the substantial benefits of high SNR are present. In this case instead of sensing the frequency shift or static deflection, the change in the resonant amplitude is related to the signal intensity. Regardless of the source geometry [30,31], the PA signal due to pulsed excitation is given by \( p \propto c_0 \beta \omega \gamma Z_{\text{p}}/C_{\text{p}} \) where \( c, \beta, \mu, C_p \) and \( E_p \) represent the acoustic speed, expansion and absorption coefficients, specific heat of the medium and the intensity of optical excitation, respectively. It can be seen that the physical properties of the medium and the employed laser power both contribute to the generated PA intensity. For a normal force equivalent to smallest detectable force derivative \( F_{\text{min}} \) acting over \( l \times w \) (assuming a simple rectangular resonator), the ultimate pressure sensitivity can be expressed as, \( p_{\text{min}} = \sqrt{4k_bT\Delta T/(w^4 Z_{\text{gas}}^2)} \). Here, \( k \) the spring constant, \( k_B \) the Boltzmann’s constant, \( T \) the absolute temperature, \( \Delta T \) the band-width, \( w \) the width, \( l \) the length, \( Q \) the quality factor and \( \omega_n \) is the \( n \)th mode angular resonance frequency of the resonator. This relation implies that \( p_{\text{min}} \) at a specific \( \omega_n \) and \( T \) is a function of only \((1/Q)^{1/2}\). Further, it appears that using thinner, and therefore less-stiff resonant structures would yield higher sensitivities; however, this is only useful if the high Q-factor is maintained at the same time which unfortunately scales downward with increased surface-volume ratio [32]. The Q-factor depends on a number of parameters including the dimensions, geometry, materials as well as the dissipation by extrinsic mechanisms which will be further discussed in the later sections.

2.2. Matching the acoustic impedance

It is commonly assumed that for a given optical power, only a sensitive transducer and a large acoustic gain will serve to maximize the detection sensitivity. However, these elements constitute only one part in optimizing the measurement efficiency. Two distinct factors determine the sensitivity in any photoacoustic measurement, the overall signal-to-noise ratio (SNR) and to what extent the signal intensity is retained during the transmittance from the sample to the detector, namely the coupling efficiency. The latter is quantified by the transmission coefficient, \( T^i = 4Z_i(Z_i + Z_s) \) or alternatively the reflection coefficient, \( T^r = 1 - T^i \) [30,32,33]. Here \( Z_i \) and \( Z_s \) are the mechanical impedances of the interface materials given by the product of density \( \rho \) and the acoustic speed \( c \) of the medium, \( Z = \rho c \). The figure \( f_i \) characterizes the impedance losses faced by the signal as it transits across the sample-coupling medium and the coupling medium-transducer boundaries. These losses particularly become significant when the difference of characteristic acoustic impedances \( Z \) of the interface materials is very large (i.e. \( Z_1 < Z_2 < Z_3 \)). For instance, the transmission across gas-solid/solid-gas interface where \( Z_{\text{solid}} \gg Z_{\text{gas}} \)
crystal (QC) micromechanical transducers and quartz tuning forks (QTF) occupy a relatively middle place on the map. Nano/microelectromechanical systems (N/MEMS) offer enhanced performance in fractional dimensions, from very high frequency (VHF) ambient applications $Q \sim 10^3$ to surface-optimized ultra-high vacuum operation $Q \gg 10^5$.

These losses can be greatly circumvented in a ZPlatform substantial part of the signal is lost by reoperation. These losses set the ultimate upper bounds for the surface losses. These losses might be mitigated by careful selection of materials, fabrication processes and surface treatments [34–37], which is beyond the scope of this work.

The extrinsic loss mechanisms largely depend on the resonator dimensions, geometry and operating conditions. In order to determine the appropriate values for these parameters, we briefly refer to their contribution with regards to relevant loss mechanism. The vibrational energy of a mechanical resonator operating under atmospheric conditions is mainly dissipated by acoustic emission to support [38], squeeze-film damping [39] due to the presence of proximity stationary surface and by air drag [40] arising from the motion in viscous environment. The support loss accounts significantly when length $l$ of the resonator become comparable to its thickness $t$ or width $w$ (usually scale with $w/l$ and $t/l$). For this reason, the negative impact of the support loss is more pronounced when the dimensions lie in the sub-micrometer range as for the nanoelectromechanical resonators [41]. Further, for fundamental flexural mode, a clamp-clamp boundary condition might result ~15 orders of magnitude larger dissipation than a cantilever geometry of identical dimensions when the support and device thicknesses differ greatly i.e. $\text{support} > \approx t$ [38], which is the normal case for most micro/nano resonant structures. Squeeze-film damping effects can become serious if the distance between the resonator and the proximity stationary surface become smaller than $w/3$ [42]. These losses might be mitigated by the air damping which occurs in the most dominant fashion in the molecular ($K_n > 10$) and viscous flow regions ($K_n < 0.01$). Where $K_n$ is the Knudsen number describing the interaction of the moving object with the surrounding fluid environment, $K_n = l_{app}/w$, with $l_{app}$ being the mean free path of the air molecules and $w$ the width of the resonator. It is known that beams having larger cross-section $(w \times t)$, relatively suffer more seriously from drag losses at moderately low pressures (molecular regime) and ambient conditions than their identical $f_\text{c}-$counterparts with reduced $w$, $t$ dimensions. However, this role completely reverses at low operating pressures, as the air damping approaches to minimum and surface losses become more severe for the latter [34,40]. A substantial improvement in the resonator performance $Q_\text{ext} = Q_\text{int}$ is possible by isolating the resonator from the ambient environment in a miniature vacuum envelop. This configuration also effectively solves the problem of squeeze-film damping effects induced by the proximity housing walls, as $P < < P_{\text{amb}}$.

The choice of operating frequencies, on the other hand, is greatly influenced by the properties of optical excitation source. The typical duty factors available with the commercial pulsed laser diodes normally lie in the range of 0.01% – 0.1%, limiting the operating frequencies to under few kHz. The dimensional parameters can be selected for a desired resonance frequency based on the empirical relation, $f_0 = 0.162/\sqrt{E/P}$, where $E$ and $P$ are the material properties of the structural material. The resonance frequency $f_0$ varies only with the aspect ratio ($l/t$) and is independent of width $w$; it is possible to change the beam cross-section while holding the resonance frequency constant. Considering these guidelines for lower dissipation, compact size and limited operating frequencies offered by the pulsed laser diodes, a large cross-section (intended for higher $Q_{\text{ext}}$) cantilevered beam geometry was selected with $f_0 = 2.5\text{kHz}$; thickness, 7 $\mu\text{m}$; width, 150 $\mu\text{m}$; and length, 1940 $\mu\text{m}$.

### 3.2. Fabrication and hermetic encapsulation

Single crystal silicon was selected as the structural material for the resonator. The high purity with extremely low lattice defects make it a suitable choice for achieving large $Q_{\text{ext}}$. The device fabrication relies on photolithography and standard Si micromachining processes (see the supplementary section 2 for details on the fabrication process). Briefly, cantilever beam structures were realized using Bosch DRIE (Deep Reactive Ion Etching) on the device and handling sides of a silicon-on-insulator (SOI) substrate, respectively. The oxide layer was etched in a buffered HF solution (10%HF: NH₄F = 9:100).

A miniature vacuum enclosure is provided by micro recesses (depth: 3
Metal spacers were removed and 22 N force was applied to the activation of NEG strip at 525 K for 35 min. In the next step, the resonator compartment.

The substrate is also metalized with thin ITO (ITO), colored green. 2. Second step involves the hermetic bonding process with the getter placed in the respective chamber at 5 × 10^{-3} Pa, 675 K. The bottom chamber was sputtered with 100 nm-thin cavities for getter and resonator compartments. The basin of the resonator chamber was sputtered with 100 nm-thick Indium Tin Oxide (ITO), colored green. 2. Second step involves the hermetic bonding process with the getter placed in the respective chamber at 5 × 10^{-3} Pa, 675 K. The bottom substrate is also metalized with thin ITO film (100 nm) on areas facing the resonator compartment.

~200 μm) formed on a glass substrate with high optical transparency. Hermetic packaging of the resonator was implemented by wafer scale anodic bonding process on both sides of the SOI substrate. Fig. 2 shows the main steps involved in the process. For first wafer-level packaging, the glass substrate containing the recesses for resonator and getter chambers was bonded to the device layer. The cavities in the glass substrate were formed using pressurized sandblasting approach. A thin layer of 100 nm-thick ITO (Indium Tin Oxide) was sputtered using Ar +10%O₂ plasma (0.5 Pa, RF 100 W) on the surfaces of the etched cavities by lift off process to prevent the electrostatic pull-in collapse of the resonator by high voltage in the anodic bonding process. The glass substrate was precisely aligned with the processed SOI wafer carrying the resonator and the bonding process was performed in a custom-built setup at 600 V, 675 K, 1 atm pressure and t = 45 min.

For achieving optimum hermetic encapsulation, the final wafer-level anodic bonding process was implemented with an activated getter under high vacuum conditions. The preparation of the second glass substrate also required the surface metallization with 100 nm-thick layer of ITO on the areas facing the resonator chamber to balance out the electrostatic forces incurred during the bonding process. A non-evaporable getter NEG strip (St787/CTS/NI/8D, SAES Getters S.p.A) was cut to appropriate dimensions and placed in the respective chamber. Final bonding was implemented on a semi-automated platform. The two bonding parts were aligned to position the metallized areas with the resonator chamber along with the metal spacers to provide a route for vacuuming the cavities. The multi-step bonding process involved the chamber degasification to 5 × 10^{-3} Pa followed by the activation of NEG strip at 525 K for 35 min. In the next step, the metal spacers were removed and 22 N force was applied to the substrates. Finally, a voltage of 400 V was applied and maintained for about 1 h under 5 × 10^{-3} Pa external pressure at 675 K. The excess bonded areas on the edges of the completed device were trimmed off by mechanical dicing. Fig. 3 shows the optical micrographs of the fabricated chip, 5 × 2 mm².

3.3. Application to photoacoustic biosensing

Glucose is regarded as one of the most vital products of carbohydrate-metabolism in the digestion process. Proper control of blood stream glucose is crucial for the treatment of metabolic disorders like diabetes. In view of profound clinical significance, glucose was selected as the test analyte in the photoacoustic experiments. The PA biosensing operation of the high Q-factor micromechanical resonator was analyzed by probing the analyte induced variations in the properties of laboratory synthesized biological specimens. We first demonstrate the ability of the packaged microsensor to transduce the photoacoustic signals from gelatin-based tissue phantoms containing trace amounts of glucose. The temporal stability of the output response was evaluated for an extended measurement period and it is shown that the fluctuations of operating conditions produce only negligible impact on the device output. In the next step, it is demonstrated that the amplitude of the output signal varies in linear proportion with the PA intensity induced by the variations of glucose concentrations in the tissue matrix. The viability of this novel approach was validated with two distinct spectral wavelengths of excitation.

3.4. Selection of excitation wavelengths

The key considerations while choosing an appropriate wavelength are the minimum interferences from the non-specific compounds [43] and the ability to reach deep-tissue target sites for in-vivo applications [44]. The spectral features of metabolic products like glucose are generally prevailed by the strong vibration modes (O–H stretching and
bending) of the water rich background in biological tissues and body fluids (for instance the typical water content of glucose rich sites; skin stratum spinosum: 70%, blood plasma: 83%). Lower water absorption in the NIR spectrum offers three spectral regions: the short wavelength near-infrared (SW-NIR) 0.82–1.4 μm, the first overtone 1.48–1.85 μm and the combination 2–2.4 μm (see the supplementary section 5), which also coincide with some of glucose specific oscillations (mostly combination and higher overtones of νO-H, νC-H, νC-O, OCH bonds, n is the mode order) [45,46]. On the other hand, the optical penetration depth depends on the total attenuation coefficient μt, (μs = μw + μs, where μs and μw are the absorption and scattering coefficients of the media, respectively) [44]. As both the Rayleigh (∼1/λ3) and Mie scattering (∼1/λn with n≥1) decrease at longer wavelengths, moving further into NIR range becomes more favorable [47]. Taking into account these factors and the available spectral lines with commercial laser diodes, 905 nm and 1550 nm wavelengths were selected in the so-called NIR-I and NIR-III biological windows, respectively [48,49].

3.5. Synthesis of tissue phantoms

Tissue-mimicking synthetic phantoms efficiently recapitulate the physical properties and the extracellular matrix of native biological tissues, providing a clinically realistic in-vivo milieu [50]. Techniques for constructing the biological assays with tissue-like properties from water-based gels (hydrogels) are well developed [51,52]. Several tissue synthesis experiments were undertaken with various polymers including organosilicon compounds (dimethylpolysiloxane) and gelatin, in order to find the suitable gel material, optimal procedure and proportions of the constituents. The tissue assays used in the following PA studies were synthesized from the 1st grade experimental gelatin powder (FUJIFILM Wako PC#077-03155, Osaka, Japan) containing 15%, 2% of moisture and ash contents, respectively. Briefly, glucose solutions covering the physiological range (0 ~ 500 mg/dL) were prepared using D(+)-Glucose (Dextrose, Anhydrous, FUJIFILM Wako PC#047-31161, Osaka, Japan) and deionized water. Powdered gelatin was then heated above the gel point at 350 K and mechanically stirred for 10 minutes to form a homogeneous composition. The entrapped gases were removed in a 40 kPa vacuum chamber. The homogenous gel is congealed in lab-made glass phantom molds, V = 16(l)×16(w)×8(h) mm3 at 300 K and placed into a 278 K refrigerator in an air-sealed package for 48 h to allow the phantoms to fully cross-link. The synthesized tissues, after removing from the phantom-molds, were stored in an air-tight storage, 278 K temperature. Average tissue life was set to be 48 h after the cross-link period for the experimental studies.

4. Results and discussion

4.1. Ultra-sensitive operation: Q-factor and limit-of-detection (LoD)

The Q-factor was measured by driving the fabricated resonator to oscillation by a pulse actuation and acquiring the resulting response with laser vibrometry. For comparison, an identical measurement was performed in the ambient and the resonant quality factor Q was obtained from the time domain spectra (supplementary section 3). The resonance response matched well with the analytical estimations of fundamental f0. The measured quality factor of packaged resonator is ~ 150 times higher that of unsealed resonator (11750 and 80, respectively). The device is adequately sensitive to detect its own thermal noise fluctuations. The spectral density of thermoelastic displacement S1/2 = √(4kBT/πω0)k quantifies the detection limit of the resonator based on equipartition theorem for Q > 1, and generally referred to as thermoelastic calibration. The predicted S1/2 at the fundamental mode f0 is about 125.4 pm Hz-1/2 at 300 K and 1 atm pressure which closely relates to the experimental findings of Fig. 4a. As an expected consequence of high Q-factor, the inherent thermal noise also gets amplified at resonance. Fig. 4b and c highlight this effect. The high Q-performance of the packaged device translates the on-resonance displacement noise at the ambient to over 10-fold higher value which is in accordance with the corresponding ratio of minimum detectable pressures waves Pmin/Fpackaged/Fambient (section 2.1).

Analytical estimations of the contributions of clamping dissipation confirmed that the quality factor of the resonator is primarily determined by the air damping (supplementary section 4). This fact is further demonstrated experimentally in Fig. 5, where the Q-factor of the unpackaged resonator, held in a vacuum chamber, is plotted at various air pressures.

The experimental data are fit to a gas damping model [42], given below by eq.1, for an oscillating cantilever in the molecular flow region. Note that the clamping losses have been neglected due to insignificant contribution. Here l, t, ρ and E are the thickness, length, density and the Youngs modulus of the cantilever, respectively. P, T and M represent the air pressures, temperature and mass. The displacement noise is approximated by the following equation:

\[ Q_s \approx \frac{13}{P} \sqrt{\frac{RT}{M}} \left( \frac{l}{t} \right)^2 \sqrt{E} \]
marks the unsteady apparent deviation of the experimental data from the predicted model scheme is shown in Fig. 6. The optical excitation sources consist of near vacuum region. Based on this measurement, we expect a sealing pressure by the viscous losses, showing a linear decline with pressure in the free air, the general gas constant pressure, absolute temperature and the molar mass, respectively. For air, the general gas constant $R = 8.317$ kJ K$^{-1}$ and $M = 29$ kg mol$^{-1}$. The apparent deviation of the experimental data from the predicted model marks the unsteady effects near the crossover to viscous flow region. Compared to $Q_{\text{int}} \approx 12,000$ in the high vacuum region, the quality factor of the resonator is adversely affected by the viscous losses, showing a linear decline with pressure in the free molecular regime to a nearly sustained value $Q \approx 80$, in the continuum region. Based on this measurement, we expect a sealing pressure $\approx 5$ mPa in the intrinsic regime at $Q \approx 11750$ for the packaged device.

4.2. Integration of the packaged chip in photoacoustic measurement system

The schematic of optical excitation system and the signal acquisition scheme is shown in Fig. 6. The optical excitation sources consist of near infrared (NIR) pulsed-mode laser diodes, PLD (Stacked Arrays 155GS14S and 905D3S3J09S, Laser Components), providing temperature dependent spectral range and bandwidths ($\Delta \lambda_{P/Emitting Area}$) of $1520 \sim 1580$ nm, 20 nm; and $895 \sim 915$ nm, 8 nm, respectively. The interface platform comprises of 1 mm-thick transparent glass slab supported on an aluminum frame. The pulsed optical beam from the laser diode is directed by NIR-AR achromatic collimation and focusing lenses (Edmund Optics), and a 45° M (custom ordered for coefficient of reflection, $R \approx 97\%$ for the employed NIR wavelength) to the tissue phantom. The mounts for laser diodes, optical lens system and the fixture of the interface platform are mechanically machined into a single compact stainless-steel structure. The NIR-PLD pulse properties at $T = 26$ °C (mentioned as 300 K hereafter) correspond to peak power densities ($E_p$/Emitting Area) of $33.6$ kW/cm$^2$ at 1552 nm and $212.76$ kW/cm$^2$ at 906 nm with the pulse width $\tau_p = 150$ ns.

The microsensor chip is securely bonded on one end of the interface platform with a solidified adhesive glue, facilitating the acoustic coupling at the contact boundaries. The PA pulses produced by the optical excitation in the tissue phantoms are transmitted to the packaged resonator via interface platform. A function synthesizer (WF 1944B, NF Corp.) provides a trigger signal to the laser diode through a nanosecond pulse generator (supplementary Figure S 10). The frequency of the trigger signal is closely matched to the first resonance $2.48$ kHz of the microsensor for high SNR detection. The intensity of the photoacoustic signal is measured by monitoring the oscillation response of the resonator with a laser vibrometer microscopy system (AT 3400 LTS501 Graphtec Corp.). The electrical signal from the vibrometer is filtered by a lock-in amplifier (LI 5660, NF Corp.) using a time constant of 500 ms. A trigger signal-synchronized TTL logic output from the function synthesizer is used as a reference signal for lock-in detection. The current output of NIR-PLD is precisely monitored by a high-speed digital oscilloscope (DPO2024 200 MHz 1GS/s, Tektronix Corp.).

4.3. Characterization of the microsensor output response

In this section the characteristic response of the microsensor and the thorough performance of the PA measurement system are presented. Preliminary characterization experiments were carried out using tissue specimens with glucose magnitude equivalent to a normal physiological measure, 150 mg/dL. An optical beam generated from the pulsed NIR laser diode source ($\lambda = 1552$ nm, $\tau_p = 150$ ns) was used to excite the tissue phantom at a modulation rate in the proximity of fundamental flexural mode of the resonator. A single measurement spectrum contained vibration amplitudes of the resonator, acquired by sweeping the pulse repetition rate with a sampling frequency of 5 MHz. The intensity of the photoacoustic signal measured over the scan time, corresponds to respective vibration amplitudes of the resonator in the frequency domain. The acoustic response to optical irradiation depends on the interface platform comprises of PLD pulsed laser diode, CL collimating lens, FL focusing lens, M 45° reflecting mirror. Near infrared pulsed beam is used to illuminate the tissue phantom on an interface platform at $\tau = 150$ ns. The condensed assay contains D(+) -glucose (both $\alpha$- and $\beta$-Types) in the gelatin matrix (represented symbolically by the main structural alkyl group and the peptide chain with $A_\alpha$ denoting the amino acid groups). The generated PA waves are transmitted to the microsensor chip by mechanical coupling on the interface platform. A laser doppler vibrometer LDV measures the signal intensity $I_{PA}$ from the oscillation response of the resonator for different concentrations $C_g$ of the analyte in the irradiated assay. (see the supplementary section 6 for detailed electrical circuit and signal processing scheme).

![Fig. 5. Resonant Q-factor of the micromechanical resonator when operated at various air pressures. The experimental values of $Q$ (red circles) fit well to the predictions up to about 50 Pa. Whereas the measurements become less accurate near the crossover to viscous flow region. $Q = 11750$ indicates that the hermetic packaging has been able to achieve $\sim 5$ mPa cavity pressure in the intrinsic region.](image1)

![Fig. 6. Integration of the vacuum packaged sensor chip in photoacoustic measurement system.](image2)
physical properties of the specimen and the resulting frequency content of the PA transients span over a wide range [53]. Depending on the resonance linewidth $Q/f_0$, a resonator can only transduce a limited part of this spectrum as illustrated in Fig. 7a. The collected signal gradually rises to maximum at resonance before a steep decline, manifesting a quasi-linear response, and finally depletes away completely outside the available linewidth. The observed dynamical nonlinearity (peak tilting towards higher frequencies) may arise from a multitude of origins such as nonideal boundary conditions [54] and, the material [55], geometric and inertial effects [56]. Particularly, in the present case of the cantilever beam geometry, there is no single dominant effect and all of the aforementioned processes might contribute to overall nonlinear response [57]. While this peak scatter might lead to a significant phase flicker but as far as the amplitude measurements are concerned, the resonant amplitude of the device remained well below the bifurcation point, $A_r = 0.88(Qh)^{1/2}$, in all experiments [55]. This figure determines the upper bounds of the functional vibration amplitudes at the frequency hysteresis limit of the nonlinear response. Here, $A_r$ and $Q$ denote the critical vibration amplitude and the $Q$-factor of the resonator, respectively, and the coefficient $h$ is a function of nonlinear spring constant. It can be seen that a high $Q$ value makes the device more susceptible to nonlinear effects.

4.4. Evaluation of long-term stability of PA measurements

4.4.1. Amplitude stability

The fluctuations of the peak amplitude $A_0$ and the resonance frequency $f_0$ were observed in the PA measurements taken under identical experimental conditions, as shown in Fig. 7a for three recorded PA spectra. This prompted a careful examination of the temporal stability of the device output. To this effect, vibration response of the resonator was monitored continuously for over $\sim 2000$s while the PA measurements made on the tissue phantom. Care was taken to prevent any moisture formation at the tissue and supporting platform boundary to ensure the identical conditions throughout the measurements. Moisture condensation has been reported to influence the photoacoustic spectral measurements for in-vivo specimens [58]. The fractional fluctuations of the resonant amplitude, $\delta A/A_0 = (A - A_0)/A_0$ were observed to be $\sim 3.58 \times 10^{-2}$ over the entire measurement period, $A_0$ denotes the mean value. Fig. 7c (upper trace) clearly indicates that the device output follows a fairly stable response for extended period with no significant anomalies.

4.4.2. Frequency stability

The frequency stability was evaluated by measuring the fluctuations of the resonance response in the frequency domain. A continuous decline in the $f_0$ with the time was observed as the PA measurements proceeded (lower trace Fig. 7c). The most probable reason for this observation can be associated with the temperature variations of the resonant structure since the damping related interfering effects are not present in vacuum operation. Assuming the adiabatic expansion of the irradiated volume that leads to generating PA elastic waves, the conduction heat flux due to excitation pulse can be completely disregarded. The experimental conditions fulfill the criterion for adiabatic assumption; the thermal diffusion length $z = (4Dp)/D^{1/2}$ is much smaller than the size of optical beam $r$ [59]. The beam size $r$ is normally defined by the radius of the circular beam. For a rectangular ($l \times w$) cross section, we can reasonably assume $r \approx l/2 \sim 1 \times 10^{-7}$ m. The pulse width $\tau_p = 150$ ns and the thermal diffusivity of the tissue specimen $D = \sim 1 \times 10^{-7}$ m$^2$/s [60] yield $z = 2.45 \times 10^{-7}$ m and thus, conforming $z < < r$. Moreover, based on the low cavity pressure of the packaged resonator, $\sim 5$ mPa $< < P_{atm}$, the direct convection heat flow from the ambient may easily be considered as insignificant. This dismissal of thermal intervention from the ambient and the excitation system leaves the 632.8 nm measurement laser focused at the free end of the resonator as the potential contributor to the resonance frequency shifts. The effect of non-uniform temperature profile on the resonance performance might become much significant at very low operating pressures, even by a $\sim 800$ µW measurement laser in the present case [61,62]. These variations of the resonator temperature lead to the alteration of linear dimensions $l, w$ and the Young’s modulus $E$, by $(1 + \alpha \Delta T)$ and $E_0 + B(T - T_0)^{1/2}$, and consequently the $f_0$, with a typical temperature coefficient of $\sim -50$ ppm K$^{-1}$ [63]. Here $\alpha$ is the coefficient of thermal expansion, $E_0 = E(T_0) = \alpha K$ and the constants $B, T_0 > 0$. The observed frequency fluctuations are extremely small, $df/f_0 = (f - f_0)/f_0 \sim 1.04 \times 10^{-5} \approx \sim 10$ ppm, implying a variation.

Fig. 7. Typical response of the vibration packaged micro-resonator to photoacoustic elastic waves and the evaluation of long-term stability of the output response. a, The scan of the vibration amplitudes in response to PA waves from a test phantom. The inset marks the variations of peak response on both scales for three measurements. Blue line is the fit of the experimental data to the frequency response of a damped harmonic oscillator, $A(s) = A_0w_0^2[(\omega - \omega_0)^2 - \omega^2Q^2/4]^{1/2}$, with $A_0, \omega_0 = 2\pi f_0$ and $Q$ being the peak amplitude, the angular resonance frequency and the $Q$-factor, respectively. The deviation of the experimental data from the harmonic fit expresses a quasi non-linear response at the resonance. b, depicts the pulse profile of 1552 nm NIR-PLD at $\tau = 150$ ns with a delay of $\sim 75$ ns from the trigger (blue arrow). c, (upper trace) the symbols follow the variations of peak amplitudes for over 2000s, $\delta A/A_0 = 3.58 \times 10^{-2}$. The lower plot traces the corresponding frequency fluctuations, $df/f_0 \approx 1.04 \times 10^{-5}$. 

I. Latif, et al. 

Photoacoustics 18 (2020) 100189
ΔT ∼ 0.2 K. Notably, from operational perspective, the measurement accuracy is not affected by these marginal shifts since a single measurement captures the impact of temperature fluctuations on the entire frequency response. Furthermore, the PA intensity is related only to the peak oscillation amplitude, $A_0$. The intrinsic Q-factors of the micromechanical resonators have been shown to display a very weak correlation with the temperature, e.g. roughly following $Q_\text{int}^{-1} \propto T^{0.25}$ over $4 \sim 10 \text{ K}$ for high frequency Si resonators [64]. Consistently, in the present case $Q^{-1} \sim Q_\text{int}^{-1}$ (verified in section 4.1), the variation of ∼ 0.2 K is not expected to produce any considerable impact on the measured signal. This is evident in the upper trace of Fig. 7c. Thus, a stable and reliable estimate of the PA intensity can be acquired from the resonant response of the micromechanical resonator.

4.5. PA experiments at 1552 nm with different analyte concentrations

The experimental studies in this section involved the specimens with varying amounts of glucose to analyze the PA intensity variations. Using the identical experimental configuration, as discussed in the preceding section, all the test phantoms underwent 1552 nm, $t_p = 150 \text{ ns}$ pulsed irradiation while the PA spectra captured (a raw sample of the measurements is given in the supplementary section 7). An upward trend in the signal intensity was observed with increasing analyte concentrations in the tissue matrix as shown in Fig. 8a. This observation is consistent with the theoretical implications, $p \propto \epsilon_\mu / C_p$, wherein the variations of physical parameters $c$, $\beta$, $C_p$ and $\mu_\epsilon$ with increased analyte levels result in stronger PA signal [65]. Eliminating the instrumental drifts is crucial to obtain the accurate information on analyte specific PA intensity variations. The emitted radiant intensity and the wavelength of the PLD source both are temperature dependent (e.g. $\Delta T \sim 0.5 \text{ nm/K}$). While no separate temperature stabilization of NIR-PLD was implemented, measurements were performed when the diode temperature reached an equilibrium state following the output pulse profile (on average; pulse-pulse timing jitter $1 \sim 2 \times 10^{-9} \text{ s}$, pulse delay $7 \sim 8 \times 10^{-9} \text{ s}$, $\% \Delta f / f \sim 0.2\%$). The chemical invariance of the tissue composition can be soundly established; for the low infusing temperature of the tissue-synthesis process ($T=350 \text{ K max}$.) and the trace amounts of additive sugars (glucose), this is a fairly reasonable presumption [66,67]. Moreover, maintaining the identical conditions for the cross linking and storage processes, experimental studies were conducted within first $3 \sim 24 \text{ hours}$ of the set tissue life. Therefore, considering a stagnant and identical background based upon these careful practices, positive contribution to the signal intensity can be conceivably inferred from the analyte specific variations of the tissue matrix. This is further validated in a different set of experiments described in the following section.

4.6. PA experiments at 906 nm and higher irradiation intensity

Next set of control experiments were undertaken at higher radiant intensity of 906 nm NIR-PLD source aiming to validate the earlier experimental observations with 1552 nm excitation and to study, the effect of increased irradiation power on the output response and the operation at less analyte specific excitations. Experimental analysis on the long-term frequency and amplitude stability of the output signal along with a sample of raw measurements are provided in the supplementary section 7 and 8. A positive correlation was again noticed (Fig. 8b), but this time an overall higher PA intensity was observed for

---

Fig. 8. PA measurements at different excitation wavelengths and optical powers. Tissue phantoms containing various amounts of aqueous glucose (0 ∼ 500 mg/dL) were analyzed in the PA measurement system. a & b depict the PA intensity measurements carried out with 1552 nm and 906 nm PLD’s, respectively, at 1 atm and 300 K. c, shows the peak signal amplitudes for both set of measurements normalized by the mean optical power densities. Both linear fits show a good proportional dependence of the amplitude response on the PA intensity, yielding $R^2_{1552} = 0.960$ and $R^2_{906} = 0.971$. The responsivities of 87.8 pm/W cm$^{-2}$ mg/dL$^{-1}$ for 1552 nm and 29.4 pm/W cm$^{-2}$ mg/dL$^{-1}$ for 906 nm excitations were observed, as represented by the gradients of the linear fits (inset Fig. 8c). $n = 6$, performed on the same tissue phantom for each individual measurement subset in the pair. Error bars have been drawn considering the largest standard error used to calculate the maximum resolvable analyte concentration (see the main text, section 4.7).
the same test phantoms compared to the 1552 nm laser measurements. The largest optical throughput in SW-NIR region, about 2.5 and 9-fold higher peak transmittance of water compared to the first overtone and the combination regions, respectively (supplementary Fig. S7), offers the most optimal environment for analytical measurements in the entire NIR region. Unfortunately, this favor is offset by lower molecular absorptivities of glucose [48]. Larger gains in the present case result from ~6.5 times higher optical intensities used in comparison to that 1552 nm excitations. Nonetheless, the PA intensity variations were adequately resolved supporting the previous observations of 1552 nm experiments and, the assumptions of stable instrumentation and chemically consistent tissue specimens. It is also worth pointing out that while the generated photoacoustic pressure is proportional to incident optical intensity $P_p$, the PA intensity is given by $I_p = P^2/Z$, where $Z$ is the acoustic impedance of the medium. This implies that in terms of energy conversion, PA effect is nonlinear. The use of higher optical powers markedly extends the overall responsivity even at wavelengths that are less specific to the analyte.

4.7. Extended discussion on PA responsivity and resolution

The device response showed a linear dependence on the glucose content of tissue specimens as plotted in Fig. 8c (normalized by the mean power densities, $E_p \times r_i \times f / A_p$, $A_p$ is the beam cross-section). Experiments with both excitation wavelengths resulted in high correlation coefficients $R^2_{1552} = 0.960$ and $R^2_{906} = 0.971$. The PA responsivity, can be directly extracted from the gradients of linear fits. More precisely, we find, $(\partial A/ \partial C_g)_{1552} \approx 87.8$ pm/Wcm$^{-2}$ mgdL$^{-1}$ and $(\partial A/ \partial C_g)_{906} \approx 29.4$ pm/Wcm$^{-2}$ mgdL$^{-1}$ over 0 ~ 500 mg/dL. The higher responsivity at 1552 nm in comparison to 906 nm SW-NIR excitation, is in agreement with the glucose absorptivity in the respective spectral regions as reported by various spectroscopic studies [48,68]. The maximum resolvable variation of the PA signal i.e. the analyte concentration change $\delta C_g$, is set by the standard error of the amplitude fluctuations $\delta A$ and given by

$$\delta C_g = \delta A / \mathcal{R} = \frac{(A - A_0)}{\delta A / \mathcal{R}}$$

(2)

The data of Fig. 8c demonstrate the attainment of $\delta C_g \sim 39$ mg/dL and ~ 47 mg/dL for 1552 nm and 906 nm excitations, respectively.

Relatively deviant values in the upper linear fit can be accounted in part by the uncertainties in tissue homogeneity. This is plausible due to the largely differing molecular structures of the focal constituents i.e. glucose and water. In which case the optical absorption changes induced by the analyte do not follow a simple solution dynamics, $\mu_a^g + \mu_a^w$. Here, $\mu_a^g$ and $\mu_a^w$ quantify the glucose and water absorptions respectively, and more accurately related as [69]

$$\mu_a^g = \mu_{a, w}^g + \mu_{a, w}^w \left(1 - \frac{C_w}{C_w^0}\right)$$

(3)

$\mu_{a, w}^g$ represents the intrinsic glucose absorption from the water displacement effect and $C_w/C_w^0$ is the ratio of water molarities; in the presence of glucose to that of pure water. It is known that the addition of glucose reduces the water molarity by ~ 0.011% mM$^{-1}$ and the corresponding increment in $\mu_{a, w}^g$ results in lowering the $\mu_a^w$ of water molecules. This implies that the uniform distribution and the retention of moisture content in the tissue-simulating phantoms are the essential conditions for the invariant background assumption to hold. In practice, the changes of skin hydration levels, stratum corneum lipid configuration and the transdermal water loss lead to considerable interfering contributions in the reported in-vivo PA studies [58,70,71]. Another possible explanation might be based upon the fluctuations of temperature dependent water absorption $\mu_a^w(T, \lambda)$, where $T, \lambda$ are the temperature and the spectral wavelength, respectively. This effect is more pronounced in the first overtone region [72] compared to SW-NIR spectrum. For instance, $\Delta \mu_a^w \sim 1.1\% C^{-1}$ at 1552 nm, calculated from the interpolation of difference spectra 37-38 °C of the pure water (ref. 72). Maintaining an absolute constancy of physical parameters such as the temperature, is quite challenging even for in-vitro measurements. Nonetheless, for the experiments described in the above sections, the room temperature was maintained at $T=300$ K for more than 24 h prior to PA studies and the test phantoms were allowed to completely thermally equilibrate with the nominal laboratory temperature before the measurements. A potential concern might relate to the deviation of device response from linear dynamical regime. Nonlinear effects are not particularly surprising for high Q-factor micromechanical resonators; however, for very large discrepancies, the dynamic response cannot be directly translated to the intensity of the transduced signal without the complete knowledge of nonlinear dynamics in play.

5. Conclusion

The lack of efficient development techniques for traditional PA cell systems along with operational instabilities and low achievable SNR’s, present formidable challenges for practical applications. The novel approach, described herein, can provide extremely high quality factors and circumvent the substantial signal transmission losses. The primary aspect that sets this research apart from the conventional approaches is that the resonance mode of a microscale Si resonator has been employed for PA detection in order to address the critical problems in question. A high Q-factor ~ 11,750 resonator was realized through an optimal design process and wafer-scale hermetic packaging. The room temperature detection limit was measured to be ~ 100 pmHz$^{-1/2}$ from the thermomechanical noise spectra. The packaged device was interfaced in a photoacoustic system built from NIR pulsed laser diodes. To mimic the realistic in-vivo environment gelatin-based tissue phantoms were synthesized with varying amounts of embedded glucose in the physiological range. The experiments showed a stable amplitude and frequency response of the microsensor in the PA measurements. The current PA measurement system with integrated micromechanical resonator demonstrate a maximum resolution of ~ 40 mg/dL over 0 ~ 500 mg/dL range variations of glucose content in synthetic tissue phantoms. On the limiting side, this value is still lower than that specified by the regulatory standards for in-vivo clinical applications. However, employing more analyte-specific excitations might potentially lead to better resolutions and improved biosensing performance of the microsensor. A unique amenity of using micromechanical resonators is the ease and simplicity of the microfabrication process. Devices with desired operational characteristics can be precisely designed, fabricated and readily integrated into PA systems. We anticipate that the use of on-chip electronic readout would further simplify the measurement setup. The high Q-factor performance along with the functional operation with compact excitation sources make the packaged micromechanical resonators a versatile and promising candidate for micro-PA-sensing applications.

Declaration of Competing Interest

None.

Acknowledgements

Authors gratefully acknowledge the kind support of the Micro/Nanomachining Center (MNC) and the Micro System Integration Center (μSIC), Tohoku University, in the microfabrication, packaging and experimental studies.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pac.2020.100189.
I. Latif, et al.

Nonlinear limits for single-crystal silicon microresonators, J. Microelectromechanical Syst. 13 (2004) 715–724, https://doi.org/10.1109/JMEMS.2004.835771.

M.R.M.C. Da Silva, Non-linear flexural-flexural-torsional-extensional dynamics of beams—I, Formulation, Int. J. Solids Struct. 24 (1988) 1225–1234, https://doi.org/10.1016/0020-7683(88)90087-X.

P. Malatkar, Nonlinear Vibrations of Cantilever Beams and Plates, PhD Dissertation, Virginia Polytechnic Institute and State University, 1996 n.d.

M.A. Pleitez, T. Lieblein, A. Bauer, O. Hertzberg, H. von Lilienfeld-Toal, W. Mäntele, In vivo noninvasive monitoring of glucose concentration in human epidermis by mid-infrared pulsed photoacoustic spectroscopy, Anal. Chem. 85 (2013) 1013–1020, https://doi.org/10.1021/ac302841f.

R.I. Swieford, J.A. Morrell, Analysis of the repetitively pulsed dual-beam thermo-optical absorption spectrometer, J. Appl. Phys. 49 (1978) 3667–3674, https://doi.org/10.1063/1.325418.

J.-Y. Kong, O. Miyawaki, T. Yano, E. Meyer, D.W. Lee, P. Vettiger, C. Gerber, Temperature dependence of the force sensitivity of silicon cantilevers, Phys. Rev. B. 69 (2004) 045403, https://doi.org/10.1103/PhysRevB.69.045403.

P. Mohanty, D.A. Harrington, K.L. Ekinci, Y.T. Yang, M.J. Murphy, M.L. Roukes, Intrinsic dissipation in high-frequency micromechanical resonators, Phys. Rev. B. 66 (2002) 085416, https://doi.org/10.1103/PhysRevB.66.085416.

K.M. Quan, G.B. Christison, H.A. Mackenzie, P. Hodgson, Glucose determination by a pulsed photoacoustic technique: an experimental study using a gelatin-based tissue phantom, Phys. Med. Biol. 38 (1993) 1911–1922.

N. Riquelme, P. Díaz-Calderón, J. Enirone, S. Mataicaevich, Effect of physical state of gelatin-plasticizer based films on to the occurrence of Maillard reactions, Food Chem. 175 (2015) 478–484, https://doi.org/10.1016/j.foodchem.2014.12.008.

K. Simion, P. Reemann, A. Pöder, M. Pook, T. Kangur, K. Kingo, V. Jaks, U. Mäegor, M. Járvekülg, Effect of glucose content on thermally cross-linked fibrous gelatin scaffolds for tissue engineering, Mater. Sci. Eng. C. 42 (2014) 538–545, https://doi.org/10.1016/j.msec.2014.05.075.

K. Maruo, M. Tsurugi, M. Tamura, Y. Ozaki, In Vivo noninvasive measurement of blood glucose by near-infrared diffuse-refractance spectroscopy, Appl. Spectrosc. 57 (2003) 1236–1244, https://doi.org/10.1366/000370203664999090.

M. Kohl, M. Eisenpreis, M. Cope, The influence of glucose concentration upon the transport of light in tissue-simulating phantoms, Phys. Med. Biol. 40 (1995) 1267–1287, https://doi.org/10.1088/0031-9155/40/7/009.

J. Yao, L.V. Wang, Sensitivity of photoacoustic microscopy, Photoacoustics 2 (2014) 87–101, https://doi.org/10.1016/j.pacs.2014.04.002.

A.J. Welch, M.J.C. van Gemert, Optical-Thermal Response of Laser-Irradiated Tissue, Springer, Netherlands, Dordrecht, 2011 https://doi.org/10.1007/978-90-481-8831-4.

P.S. Jensen, J. Bak, S. Andersen-Engels, Influence of temperature on water and aqueous glucose absorption spectra in the near- and mid-infrared regions at physiologically relevant temperatures, Appl. Spectrosc. 57 (2003) 28–36 https://doi.org/10.1366/0003702032051165179.

Imran Latif received his M.S. degree in Mechanical Systems and Design from Tohoku University in 2016. Where his research focused on the development of micro/nanomechanical systems with various technologies such as photoacoustics and optical spectroscopy for practical applications.

Masaya Toda received the B.S. degree in physics from the Kobe University in 2002, and the M.S. degree in engineering science from the Osaka University in 2004. He received the D.Eng. degree from Osaka University in 2007, where his research focused on micromechanical cantilever sensing techniques. He was a Research Student with National Institute for Materials Science, Tsukuba, and the Max Planck Institute for Polymer Research, Mainz, Germany. He was a Post-Doctoral Researcher with MPIP in 2007 and with Tohoku University in 2008. From 2008 to 2013, he was an Assistant Professor at the Micromechanical Engineering, Tohoku University. He has been an Associate Professor in the Department of Micromechanical Engineering, Tohoku University, since 2013. His research interests include micromechanical cantilever sensors, heat measurement, polymeric multifilayer films, chemical sensors, and magnetic resonance force measurements.

Takahito Ono received his D.E. degree in mechatronics and precision engineering from Tohoku University in 1996. From 1996–1999, he was a Research Associate in the Department of Mechatronics and Precision Engineering, Tohoku University. He was an Associate Professor from 1999 to 2009. He is currently a Professor at the Department of Mechanical Systems Engineering, Tohoku University and the Director of Microsystem Integration Center, Tohoku University. He was a professor at the Department of Mechanical Engineering, Graduate School of Engineering at the University of Tokyo from 2013 to 2016. He is the Director at the Micro/Nanomachining research and education center, Tohoku University from 2018. His expertise are in the areas of microelectromechanical systems (MEMS), nanoelectromechanical systems (NEMS), silicon-based nanofabrication, ultra-sensitive sensing based on resonant devices, scanning probe technologies, and nanoprobe sensing for nanoscale science and engineering. His recent interests cover nanomaterials and their integration into microsystems for applications of IoT sensors, environmental monitoring, biomedical sensors, nano-energy, and scientific instrumentation.