Specific acoustic impedance mapping of shrimp scale using scanning acoustic microscopy

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1. Introduction

Scanning acoustic microscopy (SAM) offers a significant advantage over traditional optical methods for imaging biological tissues. Unlike optical microscopy, which often requires sample preparation and can potentially damage delicate structures, SAM provides a non-destructive and rapid means of observation. By utilizing high-frequency acoustic waves, SAM creates detailed images of both industrial and biological tissues, offering valuable insights into their microstructure and properties [1-4)]. This technique is particularly well-suited for examining soft tissues, detecting anomalies, and conducting quality control in medical and biological research [5, 6)]. This paper introduces specific acoustic impedance mapping of shrimp scales as a non-invasive method, aiming to image local acoustic impedance distribution related to sound speed, which is crucial for tissue characterization. The proposed technique aims to image the local distribution of cross-sectional acoustic impedance in biological tissue, which is a parameter closely related to sound speed and potentially valuable for tissue characterization. By exploiting the relationship between acoustic impedance, sound speed, and density, this methodology enables micro-scale imaging through acoustic response.

The acoustic response contains a bunch of frequencies that evolve with time, giving rise to the need for decomposition using time-frequency analysis. The most basic methods for time-frequency analysis are time windowing and Fourier analysis, which focus on breaking down signals into respective temporal and frequency components. Both methods face difficulty in examining the signal components presented in multiple time scales. Time windowing requires careful selection of appropriate averaging time, while Fourier analysis involves pre-processing steps like data windowing.

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Advanced tools like short-term Fourier transform (STFT) and Wavelet transform have better signal decomposition in the multi-scale resolution [7)]. Within the domain of wavelet decomposition, Discrete Wavelet Transform (DWT) excels in decomposing signals into both time and frequency domains [8)]. However, the DWT imposes restrictions on the length of signals, which should be multiple powers of two. This limitation restricts the DWT application, and decomposition depends on whether the event span falls within a wavelet averaging window or not. The more advanced version of DWT, the maximal overlap discrete wavelet transform (moDWT), retains down-sampled values at each decomposition level and does not put restriction on signal length [9)]. Considering the benefits of the moDWT over other methods, it is suggested that combining the moDWT with soft computing models can offer a more effective and efficient approach for extracting the characteristic features through the acoustic response of shrimp scales.

2. Sample preparation and SAM imaging

Shrimp scales were obtained from healthy specimens at the Tromsø Aquaculture Station. Since the shrimp were not subjected to any treatments before euthanization, ethical approval was not required. The scales were freshly collected and carefully extracted from the edge using wide tweezers to avoid potential cracking.

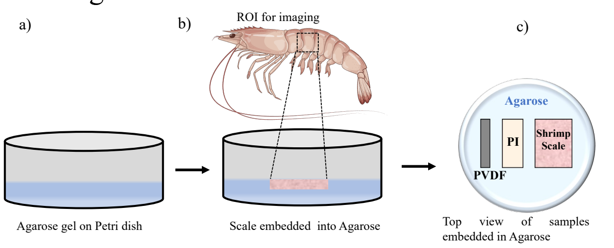

Fig. 1: A schematic representation of sample preparation provides a diagrammatic depiction of the process, and steps involved in preparing a sample for experimentation.

Fig. 1 (a) represents a thick agarose gel layer into a regular Petri dish, while in Fig. (b), a shrimp scale is carefully placed and pressed into the agarose layer.

Scanning acoustic microscopy was used to image the sample. For more information on how the SAM system operates, additional details can be found elsewhere [10]. The area of interest (ROI) was then imaged using SAM. Fig. (c) depicts a schematic representation featuring the reference samples polyvinylidene fluoride (PVDF) and Polyimide (PI), as well as the target sample shrimp scale. A 2 wt% agarose solution was prepared by dissolving agarose in 10 ml of distilled water, stirred at 100°C for 10 minutes. The gel was then poured into a Petri dish, and a shrimp scale was gently pressed into it. Cold distilled water was added, and SAM imaging was promptly initiated to preserve sample integrity[1]. Agarose helped maintain the sample's freshness and position, with its acoustic impedance like water. To measure the acoustic impedance of the shrimp scale, polyimide (PI) and polyvinylidene fluoride (PVDF) samples were also embedded in the agarose gel for comparative analysis.

3. Results and discussions

The proposed algorithm was validated using PVDF and polyimide samples. While the response was measured at multiple locations, only specific signals are presented for clarity. The time-varying frequency spectrum of the response signal required filtering to identify dominant frequencies. STFT and wavelet transform were used for this purpose[11]. Fig. 2 shows the frequency content of the filtered signal, illustrating the dynamic changes in the acoustic response over time.

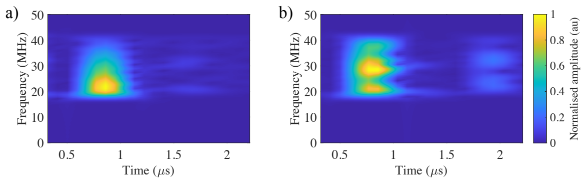

Fig 2: The figures display the STFT, responses for (a) Shrimp scale and (b) Polyimide, showing different evolving signal frequencies and their time-dependent characteristics.

The time domain responses are transformed into the frequency domain to facilitate interpretation. In this domain, the predominant peak frequency is identified and selected as the representative characteristic of the specimen. This approach allows for a clearer understanding of the specimen's properties, as the dominant frequency provides a concise summary of the acoustic response and acoustic impedance using the formulation of the reflection coefficient. The detailed analysis and visualization of these frequencies, highlighting the key peak, are presented in Fig. 3.

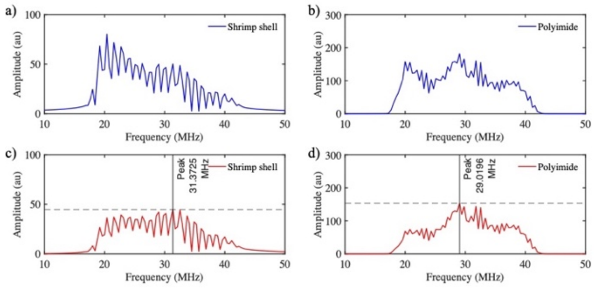

Fig. 3: The figures display the frequency spectra of the true responses for (a) shrimp scale and (b) polyimide, along with the wavelet-transformed signals in (c) and (d).

Using the reflection coefficient in the theory of acoustic wave, the mean impedance values of the considered shrimp scale are between 2.8 to 3.4 MRayl with an average standard deviation of 0.3 MRayl spatially.

4. Conclusion

SAM is a versatile, label-free imaging technology used in biomedical imaging, non-destructive testing, and material research. It reveals significant differences in reflected frequencies for materials like polyimide and shrimp scale, despite using the same transmitted signal. These differences arise from variations in material impedance, affecting wave propagation and reflection and thus evaluating impedance. This global analysis provides valuable insights into the functional aspects and complex biomechanical structure of various components within a shrimp's exoskeleton.

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