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Real-time GHz Ultrasonic Imaging of Nematodes at Microscopic Resolution

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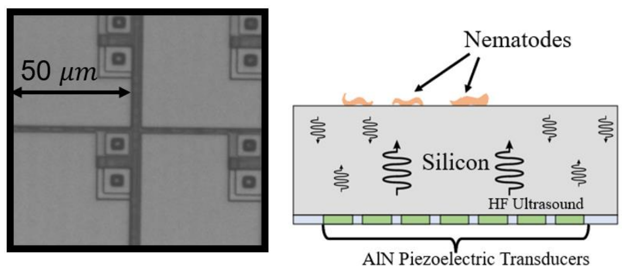
Nematodes are worm-like multi-cellular animals that are known to cause damage to the roots, stems, and foliage of plants. They are responsible for billions of dollars' worth of damage per year in the crop industry worldwide [1]. Plant-pest management should accurately detect and identify nematodes to carry out necessary action in the farm before any damages occur. Several identification methods of nematodes are based on biochemical, morphological, and molecular features [2]. Most of these require extraction and processing of soil samples, chemical analysis, Polymerase Chain Reaction (PCR), enzymes extraction, and other complex biochemical processing [3-6]. These processes can be time-consuming, laborious, and requires specialized facilities/laboratories to perform analysis. Similarly, several imaging techniques previously used to visualize and study nematodes include visible light, thermal, hyperspectral, and atomic force microscopes [7,8]. Most of these microscopy techniques are expensive and bulky. In addition, several limitations among these techniques arise due to the impermeability of light in soil. Ultrasound imaging has been frequently used in medical diagnostic processes where the frequency used ranges from 2 – 15 MHz. In this work, we propose high frequency (1.8 GHz) ultrasonic imaging as a potential tool to visualize and study microscopic pests such as nematodes. In previous work, we demonstrated the use of this GHz ultrasonic imager array to sense nematodes in air, water, and soil [9]. This work further analyzes the reflected echo data to extract spatial features of two different nematode species (*Steinernema carpocapsae* and *Steinernema feltiae)*. The ultrasonic imaging modality enables measurements of acoustic impedance and phase shifts allowing potential differentiation between various nematode species. Furthermore, the high frequency ultrasonic imaging approach can be a powerful tool to visualize, as well as detect microscopic pests at a very high sampling rate.

We use a compact 128 x 128-pixel array of Aluminum Nitride (AlN) transducers that allow imaging at a sampling rate of up to 12 fps enabling real-time visualization of nematodes motion with a spatial resolution of each pixel as 50 µm (Figure 1A). The necessary circuitry is integrated within the pixel using a 130 nm CMOS process that results in a low-power and low-cost chip. As the wavelength is inversely proportional to frequency, imaging at GHz frequency allows for high imaging resolution (~ 4.5 µm at 1.85 GHz). When voltage is applied to each transducer, the wave travels through the silicon, reflects on the backside, and is received back by the transducer (Figure 1 B). The intensity of wave reflecting depends on the acoustic impedance of material located on the silicon backside. The reflected echo amplitude for 128 x 128 pixels can be plotted to obtain an image.

The experimental setup has an optical microscope camera (Hayear HY-2307) mounted on top, and the ultrasonic imager placed right below it. The camera is synchronized with the imager's data acquisition to verify that the ultrasonic images show nematodes. Similarly, the optical images were used to measure nematode dimensions and moving velocity and compare them with the values obtained through the acoustic images. Nematodes *Steinernema carpocapsae* and *Steinernema feltiae* (obtained from BioBest Sustainable Crop Management) were washed thoroughly with water to separate any impurities/food particles. Few nematodes from both species were then dispensed on the imager surface and left to dry separately. As the water dried up, both ultrasonic and optical reading was taken until all the nematodes completely dried up. ImageJ [10] and Tracker[11] were used to measure the spatial dimensions of the nematodes and their moving speed, respectively.

The presence of nematode was verified by its shape and motion in the ultrasonic images. The nematodes can be clearly seen crawling on the imager's surface (Figure 2). For both the species, the measured length from ultrasonic images is in the same range as obtained from optical (two paired parametric t-test $p > 0.5$ for both species) as shown in Figure 3A. The measured velocities for both species was obtained to be in the range ~0.05 – 0.5 mm/s, as shown in Figure 3B. The measured moving velocities also correspond to values reported from literature [12] for nematode species of similar dimensions.



A B

Figure 1 A) Four pixels (50 μm * 50 μm each) with integrated CMOS T/R circuits. B) Schematic showing ultrasonic imaging of nematodes.

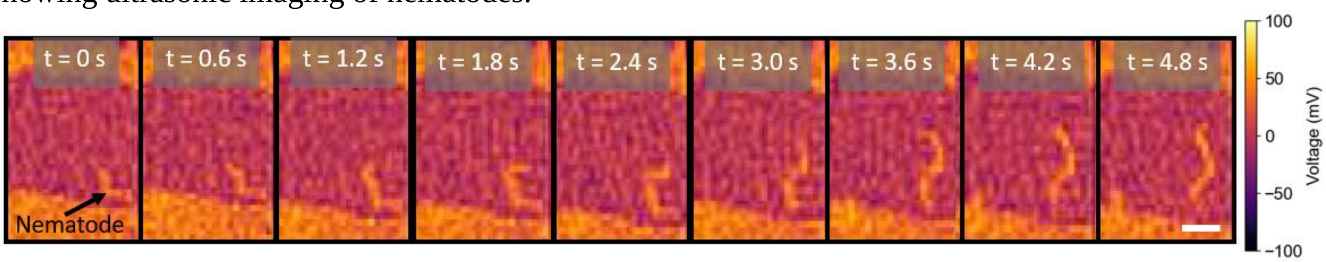
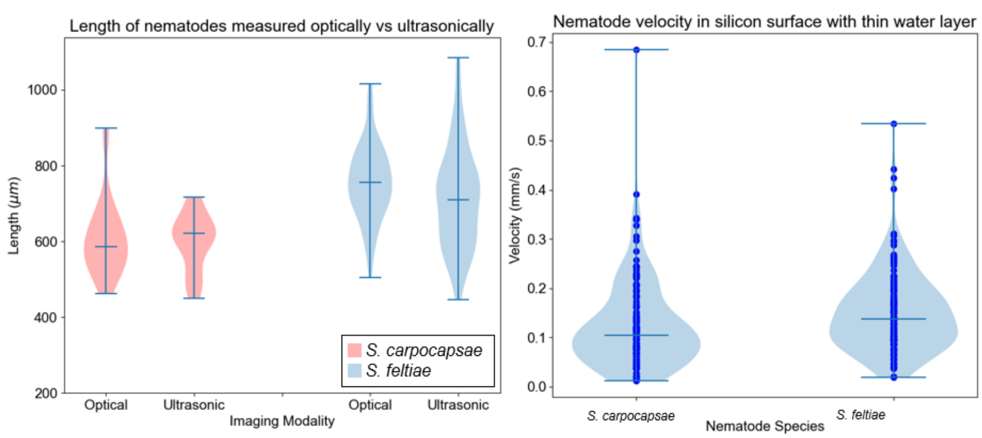


Figure 2. Series of ultrasonic images (reflected echo) shows nematode (*S. feltiae)* moving on the imager surface. The white scale bar corresponds to 300 μm.



A B

Figure 3 A) Length distribution of *S. carpocapsae* (n = 24) and *S. feltiae* (n = 86) measured optically and ultrasonically. B) Velocity distribution measured from ultrasonic images for *S. carpocapsae* and *S. feltiae* swimming in a thin water layer on silicon surface (n = 8 for both species).

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