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44-MHz LiNbO₃ Transducers for UBM-Guided Doppler Ultrasound

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Abstract—In the post genome-sequencing era, physiological phenotyping of genetically engineered mice is critical to further our understanding of the functional consequences of specific genetic defects. We have developed a 40–50 MHz ultrasound biomicroscopy-(UBM) guided, pulsed Doppler system for the sensitive detection of in vivo blood velocity waveforms in the mouse embryonic cardiovascular system. Our approach uses separate transducers for simultaneous imaging and Doppler blood flow measurements. To this end, unfocused, air-backed lithium niobate (LiNbO₃) transducers provide sensitive Doppler detection and the flexibility of adjusting the axial position of the pulsed Doppler sample volume over many millimeters depth range of the collimated ultrasound beam. In this paper we describe the fabrication and characterization of the electromechanical and ultrasonic beam properties of 44-MHz LiNbO₃ Doppler transducers. We further demonstrate the utility of these Doppler transducers for interrogating blood vessels such as the dorsal aorta over a range of mouse embryonic stages and axial range-gate depths.

I. INTRODUCTION

The recent completion of the human and mouse genome sequences, together with the ready availability of many spontaneous and genetically engineered mutant strains, has led to widespread acceptance of the mouse as the preferred animal model for genetic studies of mammalian embryonic development and many human diseases. Ultrasound biomicroscopy (UBM) has emerged as an important in vivo imaging method for analyzing cardiovascular development and congenital heart disease in the mouse [1]. UBM uses high frequency ultrasound both for in utero imaging and for Doppler detection of blood velocity waveforms in developing mouse embryos. We have developed a 40–50 MHz UBM scanner with pulsed Doppler detection, designed specifically for mouse embryos [2]. In this scanner, UBM image and Doppler data are acquired with separate transducers (Fig. 1), enabling precise and accurate UBM-guided Doppler measurements in mouse embryonic blood vessels over a wide range of gestational stages [3]–[5]. This approach has the advantage of allowing the transducers to be optimized independently, either for imaging or Doppler detection, and enables the simultaneous acquisition of UBM images and Doppler blood velocity measurements.

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Fig. 1. Schematic of the multiple transducers and overlapping beams used in the UBM-Doppler scanner.

In this paper, we describe the fabrication and characterization of 44-MHz planar, lithium niobate (LiNbO₃) Doppler transducers currently used to make UBM-guided, pulsed-wave Doppler measurements in our laboratory. In contrast to lower frequency (<10 MHz) ultrasound transducers, in which piezoceramic materials such as lead zirconate titanate (PZT) and composites based on PZT are used predominantly, single crystalline LiNbO₃ has many material properties that are attractive for use in high frequency ultrasound transducers, as well as for its more traditional application in electro-optic and opto-acoustic devices [6]. The 36° Y-cut plate of LiNbO₃ is of particular interest for UBM applications; it has a high thickness-mode electromechanical coupling coefficient (kᵣ = 0.49), low relative dielectric constant (ɛ₃₃ = 30), and is commercially available in a thickness yielding a resonant frequency close to 45 MHz. Pairs of matched LiNbO₃ crystals have been used in 40–50 MHz continuous-wave Doppler transducers in the past [2], [7], but to our knowledge this is the first detailed characterization of LiNbO₃ transducers in this frequency range, and the first description of high-frequency LiNbO₃ transducers suitable for pulsed-wave Doppler applications.
II. Materials and Methods

A. Transducer Fabrication

Planar, air-backed LiNbO$_3$ transducers were fabricated in electrical subminiature version A (SMA) barrel connectors for Doppler measurements (Fig. 2). Circular 1.5-mm planar disks were cut from a chromium-gold (Cr-Au) electroded, (nominal) 76.2-µm thick LiNbO$_3$ plate (36° Y-cut LiNbO$_3$, Crystal Technology, Palo Alto, CA). Cutting was achieved using diamond slurry (Kay Industrial Diamond Corporation, Deerfield Beach, FL) and a homemade, thin-walled brass tube (ID = 1.5-mm) as a cutting tool mounted in a precision drill press (Model 7130, Servo Products Co., Pasadena, CA) set at the lowest speed (200-rpm). The 20-mm × 20-mm LiNbO$_3$ plate was mounted to a glass microscope slide with beeswax during the cutting process, and the wax was subsequently removed from the LiNbO$_3$ disks with solvents (submersion and gentle rubbing in 40°C trichloroethylene and acetone). The transducer housing was prepared by cutting off a hollow, 1.5-mm diameter socket pin connector (SIP socket pin, Mill-Max, Oyster Bay, NY), then cementing the socket pin into the female adaptor of the SMA barrel connector with silver (conductive) epoxy (Epotek EP110, Epoxy Technology, Billerica, MA). A LiNbO$_3$ disk then was mounted on the socket pin with silver epoxy, so that the disk surface was flush with the front face of the SMA connector. The space between the pin connector and the inner wall of the SMA was potted with an insulating epoxy (Stycast 1266, Emerson & Cuming, Woburn, MA), up to the level of, but not covering, the LiNbO$_3$ disk. A 3000-Å Cr-Au layer then was evaporated on the front face of the transducer, including the LiNbO$_3$ crystal and surrounding Stycast epoxy, to make electrical (ground) contact between the LiNbO$_3$ disk and the outer housing of the SMA barrel connector.

B. Transducer Characterization

1. Electrical Impedance: The complex electrical impedance of both the unmounted 1.5-mm LiNbO$_3$ disks and the completed transducers were measured using a Network-Spectrum-Impedance Analyzer (HP4396A + HP43961A RF Impedance Test Adapter, Hewlett Packard, Palo Alto, CA). A dielectric material test fixture (HP16453A, Hewlett Packard) was used to measure the impedance of the unmounted disks, while the completed transducers were connected directly to the impedance an-
alyer with a threaded Neill Concelman (TNC)-to-SMA adaptor.

2. Two-way Insertion Loss: The two-way insertion loss was measured between 30 and 55 MHz, in 1 MHz steps, for each transducer, comparing the power received by the transducer to the power available to a 50-Ω load, as described previously [8], [9]. Briefly, a 50-cycle tone burst at each test frequency was coupled through a directional coupler (Model ZFDC-10-1, Mini-Circuits, Brooklyn, NY) to the transducer. The front-end protection circuit included an expander (DEX-3, Matec, Northborough, MA) placed between the tone-burst generator and the coupler, and a limiter (1N50B, Wiltron, Morgan Hill, CA) placed at the output of the directional coupler. The transducer was aligned in a water bath, using a tilt stage to maximize the pulse-echo signal from a quartz optical reflector placed 6 mm from the transducer. Insertion loss was calculated by comparing the pulse-echo signal to the signal obtained after coupling the input pulse to an open circuit, ensuring 100% (electrical) reflection. The signals were captured with a digital oscilloscope (HP54510A, Hewlett Packard). The losses due to attenuation of ultrasound in water and reflection from the quartz surface were subtracted from the insertion loss measurements. The insertion loss measurement relates directly to transducer efficiency but does not take into account additional refraction/diffraction losses that can still occur. Nevertheless, the insertion loss measurement provides a standard and widely used quantitative method for comparing transducers.

3. Ultrasonic Beam Measurements: The near field of the transducer beam used for Doppler measurements was mapped over a 2-mm (X) × 2-mm (Y) × 4-mm (Z) volume, centered on-axis 7 mm from the transducer face, where Z is defined to be the axial (depth) direction. The beam-plotting system, controlled via a LabView virtual instrument (National Instruments, Austin, TX), was described previously [10]. Briefly, a 50-cycle tone burst at the manufacturer-specified speed-of-sound (6880 m/s) and crystal thickness (76.2 µm). The impedance data from 17 1.5-mm LiNbO3 disks in SMA connectors, effectively clamping the edges of the disks, the completed transducers did not exhibit these spurious peaks (Fig. 3; see Section IV). The six LiNbO3 disks showing the strongest resonance were mounted in SMA connectors, as described in Section II-A. Impedance data obtained before and after transducer fabrication were
similar, with the completed transducers showing smoother impedance curves (after removal of secondary peaks), a slight broadening in the width of the resonance and a mean decrease in resonance frequency to $43.7 \pm 0.12$ MHz ($N = 6$). This slight decrease in the resonance frequency is likely due to the damping caused by either a clamping effect from the socket pin connector in the transducer housing, or a mass-loading effect by the final Cr-Au electrode, or both effects.

Two-way insertion loss (IL) measurements from the air-backed, LiNbO$_3$ transducers showed a minimum IL of $-16$ dB at 44 MHz (Fig. 4), which is a considerable improvement over previous UBM transducers fabricated from piezo-polymers (IL $\approx -40$ dB) [9] and even piezo-ceramics (IL $\approx -20$ dB) [8], [11]. This increase in transducer sensitivity is obtained at the cost of decreased bandwidth ($-6$ dB bandwidth, BW $\approx 7$ MHz, i.e., $\sim 15\%$ fractional bandwidth) compared to alternative materials (piezo-polymers, BW $\approx 100\%$; piezo-ceramics, BW $\approx 40\%$), but this is not a problem for Doppler detection in which a precisely defined transmit frequency is used.

B. Ultrasonic Beam Measurements

Volumetric beam plots from the air-backed, LiNbO$_3$ transducers demonstrated the complicated pattern of maxima and minima expected in the near field of a planar transducer (Fig. 5). In the maps shown in Fig. 5, each contour plot was normalized to the maximum value in that plane and, therefore, shows relative pressure amplitude differences in each plane. The (interpolated) YZ contour plot illustrates the expected collimated nature of the ultrasound beam, and provides a qualitative measure of the decrease in acoustic pressure with distance from the transducer face.

A simple search routine was implemented to determine the maximum contiguous $-6$ dB contour in each XY plane, and the areas inside these contours equated to an equivalent circular area, to determine the mean ($\pm$ standard deviation) equivalent aperture diameter of $1.35 \pm 0.10$ mm. This indicates that the 0.125-mm thick wall of the socket pin connector likely clamps the outer edge of the 1.5-mm LiNbO$_3$ disk and effectively reduces the transducer aperture. Assuming our transducer acts similar to an ideal circular piston with diameter 1.35 mm, the far-field transition depth and divergence angle were calculated to be $13.1$ mm and $1.8^\circ$ [12] further confirming that the beam plots and Doppler measurements provided in this paper
were all made in the near field of the LiNbO$_3$ transducers (Fig. 5). The mean (± standard deviation) pressure amplitude was computed from the calibrated PVDF hydrophone measurements within each −6 dB XY contour, and showed a decrease in mean pressure from approximately 0.65 MPa at $Z = 5$ mm to 0.40 MPa at $Z = 9$ mm (Fig. 6).

C. Doppler Blood Velocity Waveforms

To demonstrate the utility of these LiNbO$_3$ transducers for acquiring in vivo Doppler blood flow waveforms, the dorsal aortas of mouse embryos staged between gestational days E10.5 and E14.5 were interrogated (Fig. 7). The Doppler waveforms show high signal-to-noise characteristics, resulting from the high sensitivity of the 44-MHz LiNbO$_3$ transducers. Furthermore, the collimated ultrasound beam was used to acquire the Doppler signals over more than 3 mm, moving the Doppler sample volume further from the transducer for progressively larger embryos. The heart rates and peak velocities derived from these measurements (between E10.5–14.5: heart rate ranged between 3 and 5 beats per second; peak velocity ranged between 30 and 150 mm/s) were consistent with previous analyses of dorsal aorta from our laboratory [3], [4].
Fig. 6. Pressure measured in −6 dB aperture as a function of axial distance (Z), for the same transducer as Fig. 2. The dashed line shows the linear regression data fit: \( P [\text{MPa}] = 0.968 - 0.0625 \ Z [\text{mm}] \) \((r = -0.90; p < 0.001)\).

**IV. Discussion and Conclusions**

UBM-guided Doppler ultrasound is an important *in vivo* approach for analyzing cardiovascular phenotypes of developing mouse embryos [1]. The use of separate transducers for UBM imaging and Doppler allows optimization of the transducers for their distinct functions, and enables simultaneous acquisition of UBM images and Doppler data [2]–[5]. In this paper we have described the fabrication and characterization of highly sensitive, 44-MHz, air-backed \( \text{LiNbO}_3 \) transducers for Doppler detection. The two-way insertion loss was measured to be −16 dB, which is a significant improvement over the −40 dB insertion loss typical of piezo-polymer transducers commonly used for UBM [9].

The use of a planar transducer introduces some limitations due to the lack of focusing and the complexities of the near-field beam patterns; but, at the high frequencies used in UBM and with current transducer fabrication approaches, the near field has the advantage of providing a reasonable lateral resolution (1–1.5 mm) as well as a collimated beam over a very wide depth of field, approximately 13 mm for the transducers described in this paper. This collimated beam allows the position and extent of the Doppler sample volume to be easily gated in the axial direction of the transducer. Furthermore, the complexities of the near field are largely averaged out over the Doppler sample volume, as demonstrated in our measurements of acoustic pressure along the collimated beam (Fig. 6), and reflected in the high-fidelity mouse embryonic aortic waveforms presented in this paper (Fig. 7).

Unexpected secondary peaks were evident in the impedance data from the unmounted 1.5-mm \( \text{LiNbO}_3 \) disks, which were eliminated after mounting the disks in the transducer housings. The nature of these spurious resonance peaks has not been studied in detail as they appeared to have no effect on the final Doppler transducers. It is interesting to note the qualitative similarity of these secondary resonant peaks to the anharmonic extensional/flexural modes identified in past studies of quartz crystals [13]. These complex trapped-energy modes are highly dependent on transducer and electrode dimensions, and they can be suppressed in quartz filters by confining the electrode to the central region of the crystal, essentially providing lateral clamping of the resonator due to the mass effect of the unelectroded quartz [14]. We spec-
ulate that similar trapped-energy modes may have produced the secondary peaks in our impedance data, which were eliminated by the lateral clamping of the LiNbO$_3$ disks in the transducer housings. However, a detailed theory for trapped-energy modes in LiNbO$_3$, similar to that developed for quartz resonators [14], does not seem to be available in the literature.

Other researchers recently reported pulsed-wave Doppler measurements in mouse embryos using the same piezopolymer transducer for both UBM imaging and Doppler [15], [16]. This approach has the advantage of higher spatial resolution than our measurements because the Doppler measurements are made with a focused transducer, and the need to align the two transducers is eliminated when both imaging and Doppler are performed with the same transducer [2]. The disadvantage of this alternative method is that UBM images and Doppler data cannot be acquired simultaneously, as in our system, which can be a significant impediment in the face of considerable movement often present due to maternal respiration and uterine contraction during embryonic imaging. In addition, the wider extent of the Doppler sample volume achieved with the planar transducers may be advantageous in providing quantitative data related to the complete range of velocities in a blood vessel (required, for example, to estimate blood flow), because the near-field Doppler beam provides reasonably uniform insonation over the diameter of even the largest mouse embryonic blood vessels (dorsal aorta diameter $\approx 300 \mu$m) [4]. It is likely that both types of transducers (focused and unfocused) will play important roles in future high-frequency Doppler studies of the developing mouse cardiovascular system.

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REFERENCES


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