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EVALDAS SAPELIAUSKAS

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EVALDAS SAPELIAUSKAS

APPLICATION OF CAPACITIVE MICROMACHINED ULTRASOUND TRANSDUCERS FOR BIOSENSORS

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Scientific Supervisor:
Prof. Dr. Darius Viržonis (Kaunas University of Technology, Technological sciences, Electronic Engineering – 01 T).

Editor: Gavin James Grant Stewart (KTU).

Dissertation Defence Board of Electrical and Electronic Engineering Science Field:
Assoc. Prof. Dr. Elena Jasiūnienė (Kaunas University of Technology, Technological sciences, Electrical and Electronic Engineering, 01T) – **chairwoman**;
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Prof. Dr. Rimantas Kodžius (Shanghai University, Biomedical Science, Biology – 01B).

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Address: K. Donelaičio St. 73-402, 44249 Kaunas, Lithuania.
Tel. no. (+370) 37 300 042; fax. (+370) 37 324 144; e-mail doktorantura@ktu.lt.

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The doctoral dissertation is available on the internet http://ktu.edu and at the library of Kaunas University of Technology (K. Donelaičio St. 20, 44239 Kaunas, Lithuania).

INTRODUCTION

Currently, Lithuania's research funding policy is organized for smart specialization priorities. One of the priorities – early diagnosis of chronic, neurodegenerative and lifestyle-related diseases. The most effective way of diagnosis of such diseases is biomolecular diagnostics, allowing the detection of pathogenic molecules in the early stage of the disease. In clinical practice, many different methods of molecular diagnostics using various analytical biochemistry are used, of which the best known is the ELISA (Enzyme-linked Immuno Sorbent Assay), the most common one in immunology. This method consists of several processes: preparation of the analyte solution with antigens, specific interaction with the enzyme-labeled antibodies and activation of enzymes. Activated enzymes change the color of the biochemical substrate solution, whose intensity is determined by the number of interactions. This is a long-lasting (from several hours to several days) method that requires specific staff expertise and specialized laboratories [1]. To speed up ELISA-like and other similar molecular diagnostic methods and to make them more accessible, biosensors with high potential for automated measurements are being developed [2]. When biosensors become a key tool in molecular diagnostics, it would change the molecular diagnostic paradigm - from lengthy and costly research laboratory operations it would be moved to a quick and informative results for different conditions: primary health care, household, military field, space and others. Such paradigmatic play has already begun in some diagnostic areas, such as the measurement of blood sugar levels, where compact and affordable biosensors, to a large extent, replaced laboratory tests. Therefore, the international market for biosensors, especially for detection of specific interactions of biomolecules, is currently increasing [3].

Research relevance

The research presented in this paper was carried out to obtain the essential engineering and basic knowledge for using capacitive micromachined ultrasound transducer structures for biodetection [4-7]. The main results achieved were CMUT's structure for surface modification and biofunctionalization. Important information about signal transduction during specific interactions of materials into electrical signals principles was deducted both experimentally and by numerical simulations. The principles that served to determine the quantitative specific interaction parameters analytically and experimentally were derived. In terms of engineering, the research led to the development of prototype biosensors based on CMUT structure to reveal their potential for miniaturization, integration with electronics and reduction of molecular diagnostic costs.

The aim of the research was to design and demonstrate a prototype sensor fabricated using CMUT structures for detection of specific interactions of biomolecules.

The problem

Earlier published researches confirm the principle of the CMUT structure for biosensor function, using specific biochemical elements interactions.

This biochemical interaction can be detected by measuring the resonance frequency of the sensor's structures in gaseous environment. During previous researches it was also shown that the CMUT structure can provide more information than the competing bio sensing platforms available on the market. The main problem that requires research and newly created scientific knowledge is that the vast majority of biochemical interactions occur mostly in liquid medium. This liquid medium is not suitable for resonant measurements due to very high energy losses. The previously used methods when the resonant measurement is done after drying out the sample have high uncertainties. Therefore, this research seeks to answer the question of what methods and tools are possible using capacitive micro machined ultrasound transducers in order to obtain real time information about occurrences or absence of specific interactions between biochemical materials in liquid media.

Work hypotheses

The structural resonance that was used in previous works can be change to the transverse wave resonance which will propagate between solid-liquid interfaces, such as Scholte type acoustic waves, using a comb type CMUT structure. When specific interactions occur, a layer of biomolecules is formed on the surface of the sensor, which can induce changes of velocity to the Scholte type wave and the resonance frequency of the comb type structure. When we track the wave velocity and the resonance frequency of the structure, in real-time we can detect the formed layer of biomolecules that was formed by specific interactions, and also we can measure some of the dynamic properties of this interaction.

The objectives were as follows:

1. CMUT structure analysis for biodetection methods and its potential for real time operation in liquid analyte.
2. CMUT biosensor structure prototype creation with integrated microchannels for the detection of specific biological materials. Research of acoustic and mechanical transient processes appearing in a CMUT with integrated microchannel.
3. Justify the suitability of a CMUT with an integrated microchannel for the detection of specific biological interactions.
4. Research the possibility of the CMUT structure to be used as a sensor and as a liquid mixing device.

Scientific novelty

1. Scientific knowledge will be obtained about the possibilities of using capacitive micromachined ultrasound transducer structure together with integrated microchannels for bio-sensing in liquid media when specific interaction takes place. By fabricating, experimentally and using empirical models testing the prototypes of the CMUT chips with interdigital type structures, we have determined that this structure can be used to transmit and receive Scholte type acoustic waves.
2. The informativity of Scholte wave velocity, when detecting specific forming of biomolecular layer in real time, was justified.
3. Using the CMUT prototype with integrated microchannels, liquid manipulation methodology was created, which justified the structure's possibilities to control liquid diffusion in the microchannel; this increases the sensor's application possibilities.

Practical value of research results

The main engineering and fundamental knowledge was acquired for the fabrication and usage of biosensors based on CMUT structures.

Functionalization methods based on the measured signal information and usefulness for analyte detection were designed.

The technological concept was justified by demonstration of operation of the prototype sensor in the real time.

Hypotheses

1. Biosensor, with integrated microchanels and CMUT structures, can detect biochemical analyte in liquid in real-time by transmitting and receiving *Scholte* type waves through the specific biochemical interaction zone.

2. The phase velocity of *Scholte* waves, transmitted through the specific interaction zone, depend on biomolecular interactions in the mentioned zone, the elastic modulus of analyte adsorbing materials and viscosity.

3. The sensitivity of the biosensor is enough to detect the changes of elastic properties of the phospholipid bilayer in the real time.

4. CMUT structures, functioning as biodetectors, can also be used to control liquid diffusion inside the microchannel and change conditions of the specific interaction.

Thesis approval

The main results of the doctoral research have been published as 2 publications in the journals listed in ISI Web of Science with impact factor, 5 publications in conference proceedings and 7 presentations at international scientific conferences.

Structure of the thesis

The thesis consists of an introduction, five parts, conclusions, a list of references and a list of the author's publications. The total scope of the thesis is 85 pages including 46 figures, 1 table and 133 bibliographic references.

1. STRUCTURE OF CAPACITIVE MICROMACHINED ULTRASOUND TRANSDUCER BIOSENSOR AND ITS USE FOR EXPERIMENTS

Immunology is one of the biomedical sciences that forms the important part of the biosensors market. Genetic diagnostics and genome research provides another market opportunity for biosensing since large numbers of specific biomolecular interactions (such as DNA hybridization) are to be registered as quickly and reliably as possible. The biosensor is an analysis system, connected to the signal converters or detectors, consisting of specific analyte molecules, that can register the aforementioned physico-chemical changes occurring during the interactions with the analyte and then it changes these measurable signals to electrical signals which are proportional to the concentration of the analyte. The advantages of biosensors compared to other currently widely used analysis systems are their high sensitivity, specificity, and real-time measurements that do not require complex sample processing and labeling [8, 9]. A generalized structure of biosensor is shown in Fig.1.
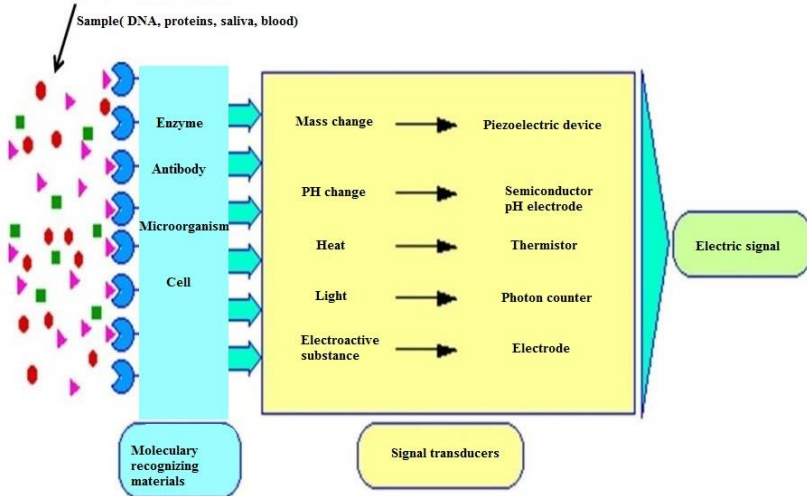

Fig.1. Generalized structure of the biosensor.

This biosensor consists of two functional parts: the bioactive component, providing a specific biochemical interactions and the detector of those interactions which changes information, resulting from the physical nature of biochemical interactions into electrical signals. A large number of biosensors for

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molecular diagnostics are being developed in Lithuania and other countries, however the complexity of biomolecular detection, fabrication cost of analytical chips, and specific requirements for analyte preparation leave other methods with limited capabilities of miniaturization and integration with compact electronics [10, 11].

The main structure of the CMUT element is its capacitive cell that has one moveable and one fixed electrode. Between these electrodes (the movable one is usually structurally aligned with the membrane) there is a vacuum gap, which creates a cavity for the displacement of the membrane. When the electrical field is applied between these electrodes, the membrane is attracted towards the substrate of the structure by a Coulomb force and the induced stress within the membrane balances the attraction. The balance between these forces determines the membrane and electrode placement, speed, acceleration and electrical properties. The cross-section of CMUT cell structure is shown in Fig.2. In this case, the top flexible electrode is aligned with the membrane and together with protective layer creates the critical thickness [12, 13]. This layer determines the electromechanical properties of the cell and the passivation layer is used to protect the top electrode from external mechanical, chemical and electrical sources.
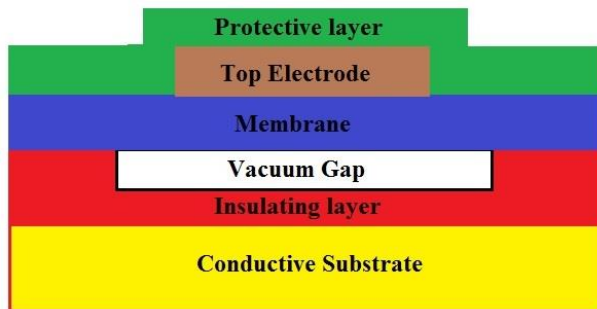

Fig.2. Cross section of single CMUT capacitive cell

The vacuum gap for the cell is formed in the insulation layer, which protects the device from short circuit if the membrane touches the bottom of the gap. The operation of CMUT is based not on the piezoelectrical effect as with the majority of known ultrasonic transducers, but on electrostatic forces between the electrodes explained by the Coulomb law. This working principle leaves us with a wide variety of materials to choose from in making CMUT structures. The vacuum gap, separating the membrane from the bulk, is formed using micromachining processes. It is an additional advantage when compared with similar microelectromechanical devices – when the membrane moves inside the vacuum, energy losses are reduced [14]. This is why CMUT structures have better resonant characteristics at high frequencies and in dampening

environments such as liquids [15, 16]. For the first stage of the research we have designed and fabricated two types of CMUTs: ones that operate at 7 MHz resonant frequency in air (the "low frequency CMUT") and ones that operate at 40 MHz resonant frequency (the "High frequency CMUT"). Moreover, low frequency CMUT working surface was coated with gold and the high frequency CMUT's surface was silicon nitride. These two different CMUT structures were used to compare the bimolecular interactions taking place on different surfaces. Also, we wanted to check if the CMUT surface, passivated with silicon nitride, can be used for registering bimolecular interactions. Both CMUT type structures are shown in Fig.3.
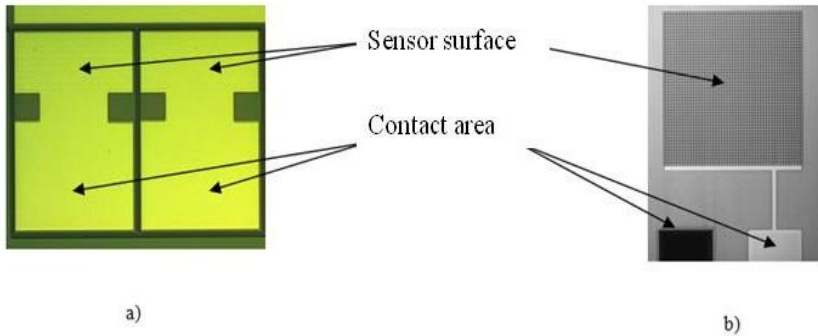

Fig.3. Micrographs of experimental sensors: a – two 7 MHz elements of the sensor; b – 40 MHz sensor

The third type of CMUT structures that are discussed in this study can transmit and receive *Scholte* waves (propagating at the boundaries of the surface and liquid) [17]. It uses a special planar periodic comb-like or interdigital structure that maintains the resonance of the wave. Interdigital structures' ability of transferring the energy by transverse waves depends on the CMUT membranes design and on the number of "finger" pairs that are in the direction of the propagating wave. Therefore, we can obtain high sensitivities in liquid environments by optimizing the design of the CMUT cell structures and the secondary interdigital structure. Since biomolecular interactions take place in liquids, optimizing the structure will have additional advantages over CMUT sensors that employ the membrane mechanical resonance principle. The idea of the interdigital transducer is illustrated in Fig.4. The transducer "fingers" are placed on the surface in such a way that it could be actuated using signals Tx1 (t) and Tx2(t). These signals have a narrow spectrum and differ in phase by:

$$Tx1(t) = A\sin(\omega t)\,; Tx2(t) = A\sin(\omega t + \varphi), \qquad (1)$$

here: ω and φ is angular frequency and phase shift accordingly.
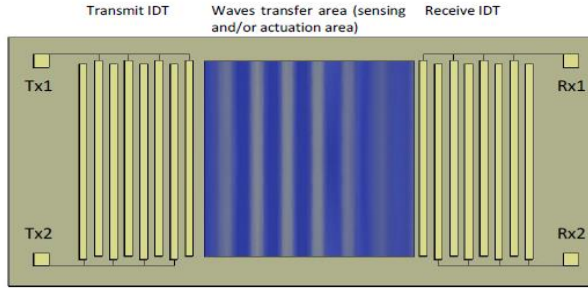
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Fig.4. Schematic picture of a surface acoustic wave design

Applying signals to Tx1 and Tx2, CMUT interdigitated membranes are deflected towards the substrate (see Fig.2). The material in the transducers vicinity is also deformed according to the deflection. The transverse wave is generated on the surface of the device and then part of the energy is converted to the *Scholte* type wave if the device surface is in contact with liquid.
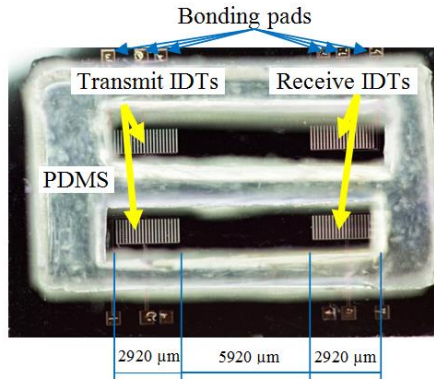

Fig.5. CMUT device with two open microchannels

Since phase velocity of the *Scholte* wave is a function of surface material of the device and the liquid properties, we assume that the measured *Scholte* wave properties can be directly related to the parameters of the biomolecule interactions (for example, natural or artificial phospholipid bilayers anchored to the sensing area) (Fig.5). In order to investigate the various physical properties of *Scholte* waves and prepare for experimental work with phospholipids, we designed a series of interdigital CMUT sensing structures. All of the designed structures have acoustic delay lines, structures that can transmit and receive *Scholte* type of waves and a gap between which it is functionalized to measure analyte parameters [18, 19].

2. EXPERIMENTAL METHODS

2.1. Measurement of resonant frequency using CMUT with oscillator circuit

Several experiments were carried out using oscillator circuits with high gain feedback loops that were implemented with CMUT as a nonlinear element, which changes the autogenerating frequency. The principle schematic of the circuit is shown in Fig.6. Resistor R1 and capacitor C2 is protective circuit from bias voltage Vdc. TV1 VT2 transistors are connected in an auto generator circuit from which the operating resonance frequency can be determined by capacitors C3, C4 and C5. L1 is parasitic inductance that has some influence for the oscillator circuit. The CMUT electromechanical resonance also additionally regulates the oscillator operating frequency. The capacitor C1 is voltage source filter, C6 – protective output capacitor. This kind of oscillator circuit has great potential to work with low Q resonators, since it has greater self-generation sensitivity than circuits used by Lee et. al. [20, 21].
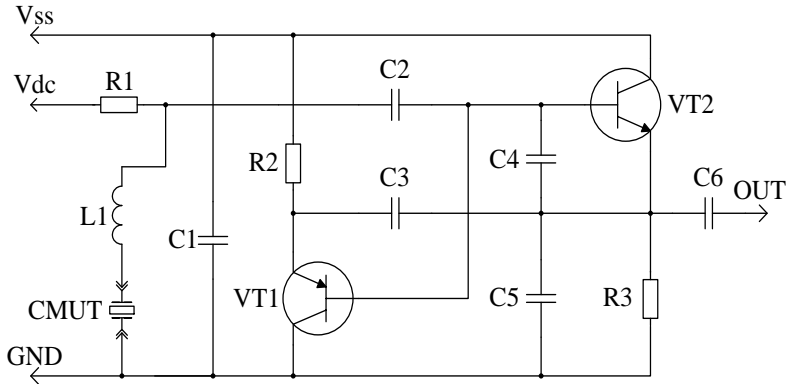

Fig.6. Principle schematic of oscillator circuit with high gain feedback loop

A digital oscilloscope FLUKE 199C with integrated real time fast Fourier transformation (FFT) algorithm was used to register the output of oscillator's resonance frequency.
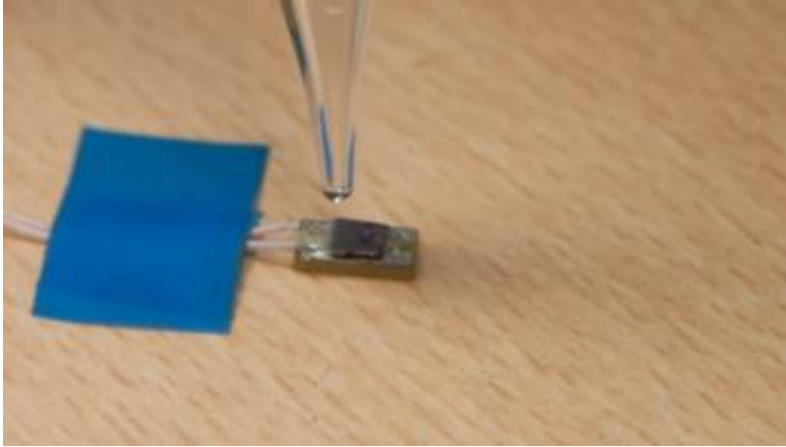
Fig.7. Picture of sensor surface modification with liquid in air

The frequency spectra of the real part of the impedance were measured using a network analyzer Agilent 4395A equipped with the impedance measurement kit. During the experiments, an additional voltage source was used to maintain the necessary bias voltage. The results were interpreted in respect of the equivalent circuit model and results from finite element analysis [22].

4.2. Measurements of biomolecular interactions inside the microchannel

The scheme of the experimental system for biomolecular interaction structure is shown in Fig.8. CMUT structures 2a and 2b are inside of a microchannel 1. In this scheme one of the structures, acting as a transmitter, is connected to generator 10 (Agilent 33522A). The second CMUT structure, acting as the receiver, is connected to two primary amplifiers 7. The outputs of the amplifiers are connected to oscilloscope 8 (Fluke 190-502/AM). The bias voltage source 9 (Agilent N5752A) is common for all of the CMUT structures. There is also a synchronization cable connecting transmitter and receiver, which is used for synchronizing the excitation pulses. This measurement system is usual for delay line type measurements.
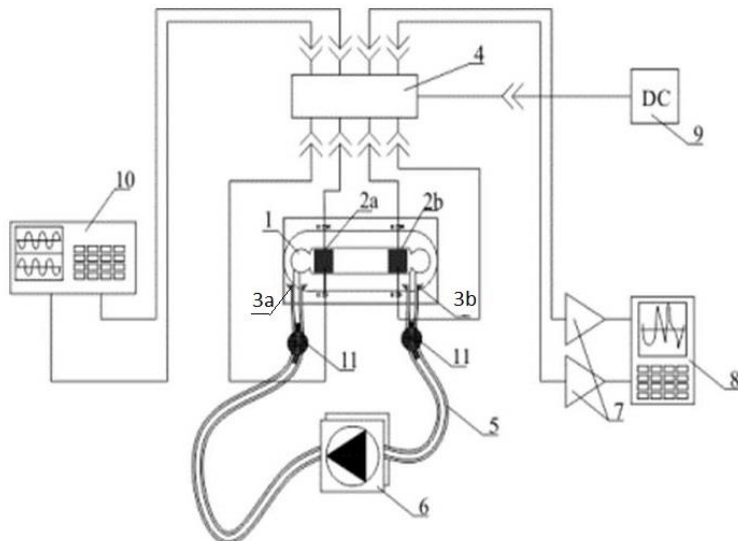
Fig.8. Experimental measurements structure: 1 – microchannel; 2a and 2b – two CMUT structures on a single chip; 3a,3b – connections for the input and output of analyte; 4 – T hub for connecting bias voltage; 5 – connection tubes; 6 – peristaltic pump; 7 – receivers; 8 – digital scope; 9 – DC source; 10 – signal generator with two outputs; 11 – liquid valves

By additional processing of the received time signals, analysis of resonant frequency and phase velocity of the wave was performed. This analysis allowed us to relate changes in wave propagation parameters to the changes of wave phase velocity. Transient processes in the microchannel were excited by a sinus form impulse with the amplitude of 10 V and using the generator's burst function. The duration of the impulse was selected from one to twenty periods, according to the measurement protocol. For additional processing, the oscillographs, with more than 1200 discrete values and not worse than 4.2 ns time resolution, were also recorded. This resolution allows us to identify the signal's resonant frequency up to 10 MHz with high certainty. Network analyzer Agilent 4395A was used as a reference measurement channel.

4.3. CMUT surface functionalization with antibodies

The immune pair elements were immobilized on the surface of the sensor by adsorbing them from liquid solution and binding them with aldehyde gases. The device prototype was functionalized with bovine serum albumine, which exhibit biomolecular interactions with the antibody of the same virus [1].

A cross-section of CMUT sensor's structure when the membrane is modified and prepared for experiments is shown in Fig.9.
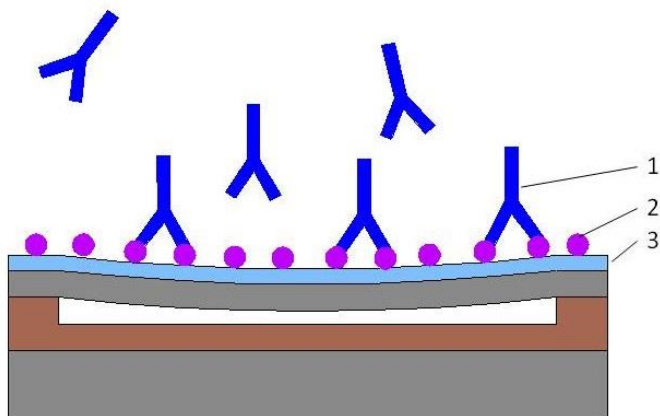

Fig.9. Cross-section of CMUT cell with immobilized antigen. 1-virus antibody, 2-antigens immobilized on the surface of the device, 3-electrode

Using network analyzer Agilent 4395, resonance measurements were carried out for non-functionalized CMUT structures. After registration of CMUT resonance values, the sensor's surface was functionalized by drop coating with 1.5 µl water solution of Bovine Leukemia Virus (BLV) antigen gp51 (1 mg/ml). Then CMUT elements were kept for 20 minutes above the 5% glutar aldehyde solution. The proteins that were not immobilized were washed away with deionized (DI) water. After washing with DI water, the devices were dried out and the primary resonance frequency and impedance measurements were carried out. These steps were repeated until there was no more shift in the frequency and impedance measurement values. To eliminate the signal from nonspecific biomolecular interactions with other proteins, which are present inside the blood, one of the modified devices was made to work as a reference by exposing its surface to non-infected and 3 times diluted blood serum for 20 minutes. After each of the washing steps, CMUT chips were dried out to avoid any short circuits due to moisture over open contacts and distortion of the readings by the excess moisture in the bioelements.

Several experiments were carried out using 3 times diluted BLV infected cattle blood serum, 10 times diluted BLV infected cattle blood serum and 3 times diluted not infected cattle blood serum [23]. The overall algorithm for the biosensor functionalization and measurement is given in Fig.10.

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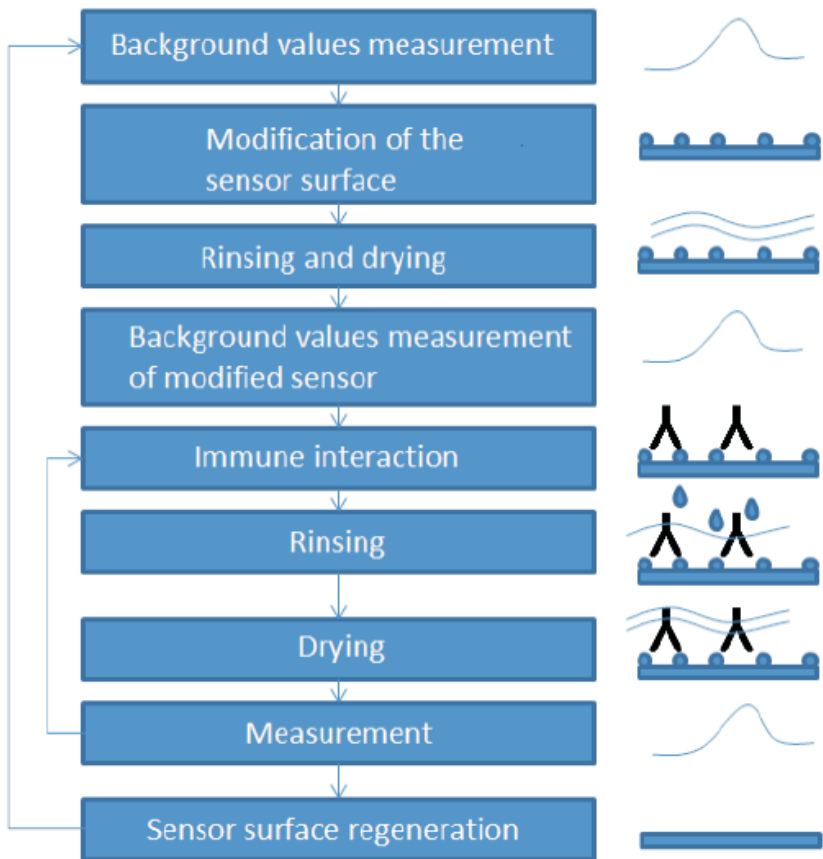
Fig.10. Algorithm for functionalization and immobilization of antibodies

4.4. Research of liquid mixing and manipulation

Research was done to explore the speed of dynamic diffusion of liquid going through the microchannel. These experiments were carried out using CMUT chips with four channels - two phases IDT transmitters with 20 fingers and two IDT receivers CMUT chips were assembled together with a PDMS microchannel with input and output ports for liquids. A picture of the assembled sensor is shown in Fig.11.

Fig.11. IDT CMUT sensor with microchannel for manipulation's experiments

By changing the input of the peristaltic pump from clear liquid to a colored one, it was determined how the liquid diffuses in the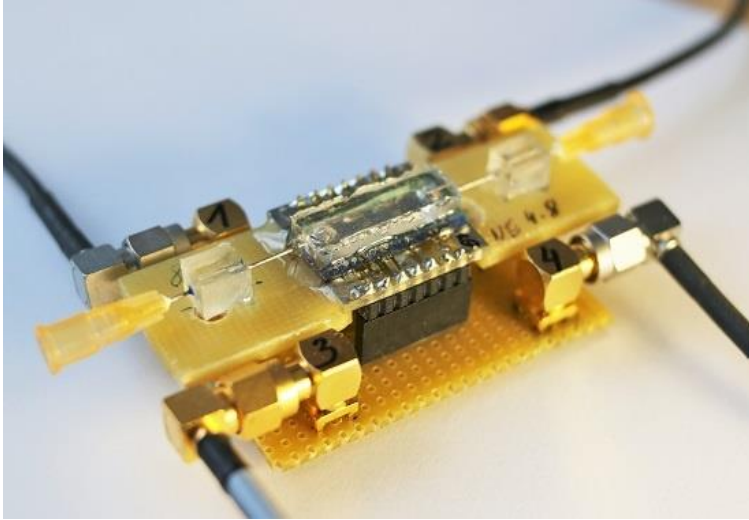 microchannel with help of the acoustic streaming or without it. To create a vivid purple color for IPA alcohol solution, 0.1% calcium permanganate was used. An image processing algorithm was created for automatic color intensity measurement inside the microchannel.

One of the experimental phase is shown in Fig.12.
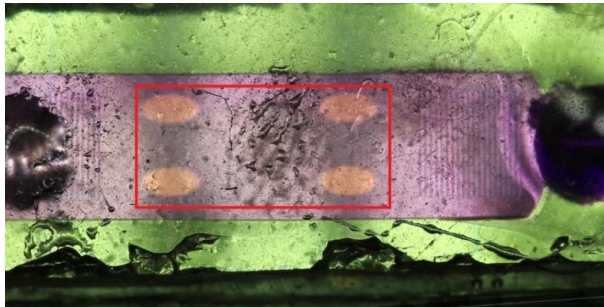

Fig.12. A photograph of experimental device. Inside the microchannel is IPA solution with 0.1% calcium permanganate. Red zone was used for brightness index analysis

The figure contains a photograph of the microchannel when the color intensity reached maximum value; the red framed zone is the region of interest, which was used for automatic detection and color intensity measurements.

In the first phase of the experiment, we wanted to measure the diffusion dynamics of colored liquid when going through the microchannel. In the second phase, we wanted to find out if interditgital CMUT structures can work as a micropump and create a directional pressure.
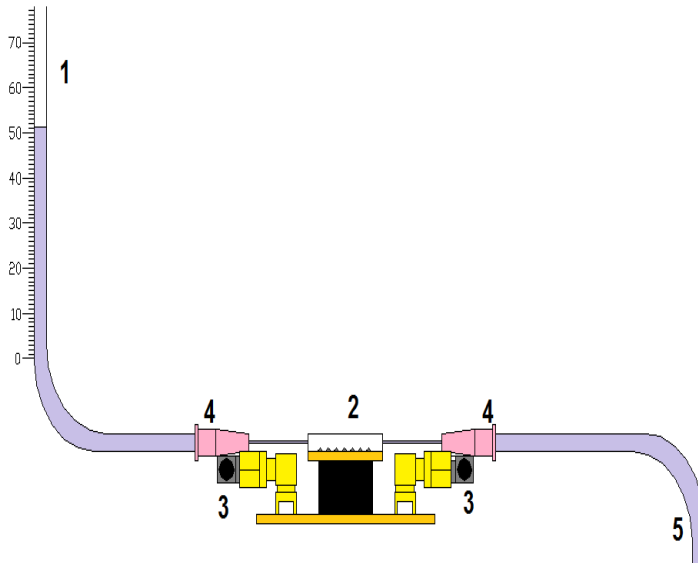
Fig.13. Schematic of acoustic pumping experiment: 1 – liquid column, 2 - sensor, 3 – electric connections, 4 – liquid connection, 5 – test tube

3. RESULTS OF CMUT RESEARCH

3.1. Resonant frequency measurements of structural CMUT in air

Results from testing low frequency CMUTs working with the oscillator circuit are shown in Fig.14 and 15. The input of the oscillator, shown in Fig.14, shows how bias voltage influence CMUT's resonance frequency. When bias voltage increases, the resonance frequency of the CMUT decreases due to the so-called "equivalent spring dampening" effect, which is a characteristic nonlinear parameter of CMUT [24]. According to Fig.15, the oscillator output frequency is almost linear. It is a property of the resonator circuit: in this circuit CMUT is just a component that changes the autogeneration frequency; this is why the resonance frequency between CMUT and oscillator's output is nonlinear.

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Fig.14. CMUT characteristics with various bias voltages starting from 40 V and ending at 70V

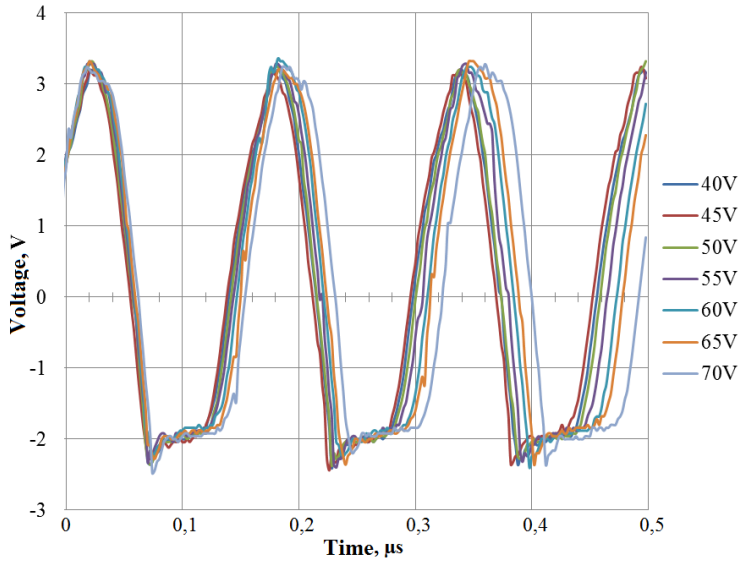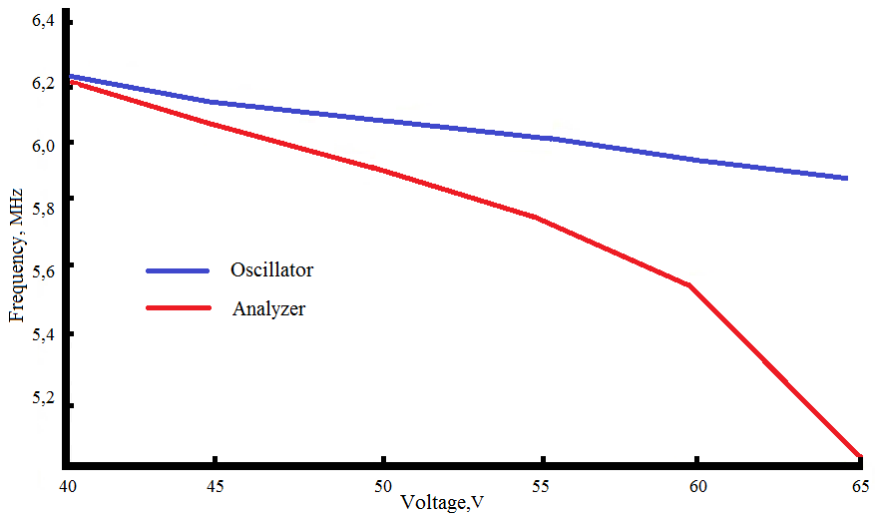**Fig.15.** Resonance frequency of oscillator and CMUT at various bias voltages

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3.2. Resonant frequency measurements of CMUT membrane in liquid

The informativity of the CMUT resonator, connected to the oscillator circuit (Fig. 6) as a sensor, was confirmed by performing experiments while submerging CMUT into different liquids. The real-time oscillator output of the resonance frequency shift is shown in Fig.16. Here the CMUT is submerged into a transformer oil (Neste TRAFO 10X, 895 kg/m^3 blue line) for 50 seconds and then submerged into isopropyl alcohol (786 kg/m^3, red line). The most important fact is that the transformer oil shows a larger resonance shift than the isopropyl alcohol, and this can be explained by the higher viscosity of the oil. These results show that the created measurement channel can adequately measure the composition of liquid mixture. However, the informativity is limited due to the low resonance quality of the immersed CMUT chip and a high noise level (about 20% of signal amplitude). Results show that, in liquids, high sensitivity resonant measurements are not promising.

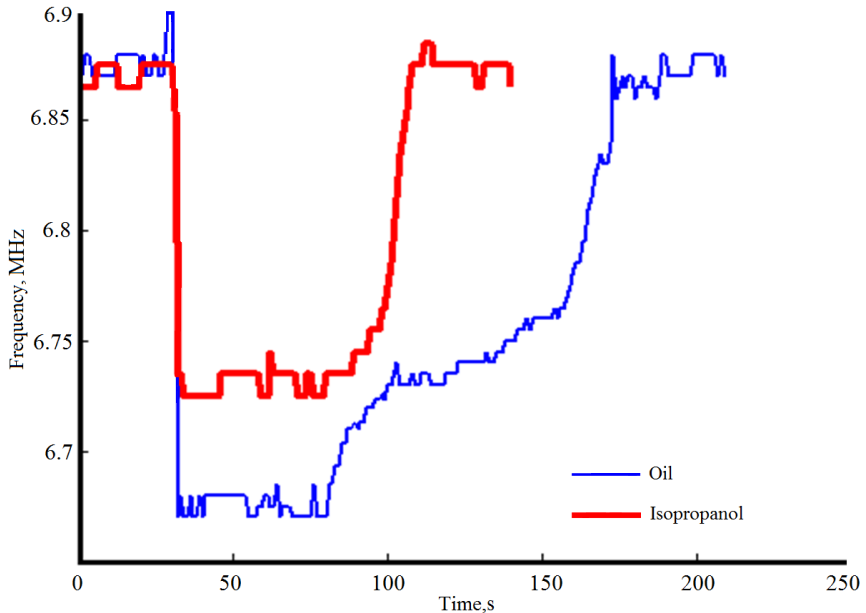
Fig.16. Real time resonance frequency shifts of the CMUT submerged into transformer oil (blue line) and isopropyl alcohol (red line)

3.3. Testing of interdigital CMUT

For functionalization tests, CMUT devices were assembled on printed circuit boards together with a microchannel. After assembly, the devices were tested with network analyzer (Agilent 4395A). A real part of the impedance

spectrum, acquired with the analyzer, while CMUT was working in air and in liquids, is shown in Fig.17. As we can see, the CMUT in air exhibits higher values of the real part of the complex electromechanical impedance (resistance, 98 ohm) and resonance frequency (21 MHz, with bias voltage of 40 V). Due to various losses in liquid (DI water), the resonance frequency of the CMUT and the max complex electromechanical impedance values are much lower (9.5 MHz and 60 ohms respectively). We interpret these readings as specific for correctly functioning CMUT structure. The secondary resonance peak, appearing at 19 MHz, is associated with different working conditions of individual CMUT cells when the device is working in air. These side resonances are almost completely damped when in immersion.
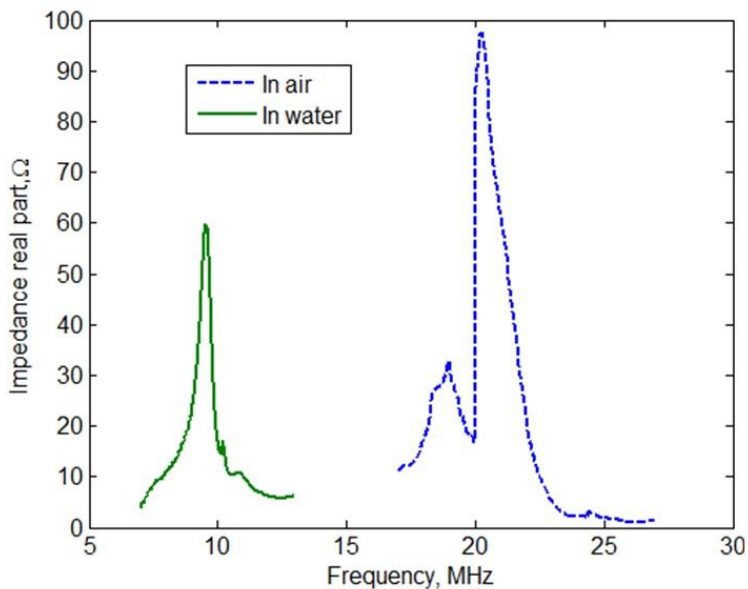
Fig.17. Real part of the impedance for interdigital type CMUTs working in air (blue) and in deionized water (green)

At the second functionalization test phase, the ability of the fabricated structure to send and receive *Scholte* type waves was investigated. One of the IDT CMUT structure was connected to two phase generator Agilent 33522A, and the other structure, analogous to the previous one, was connected to the primary signal amplifier and oscilloscope (Fluke 190-502/AM).
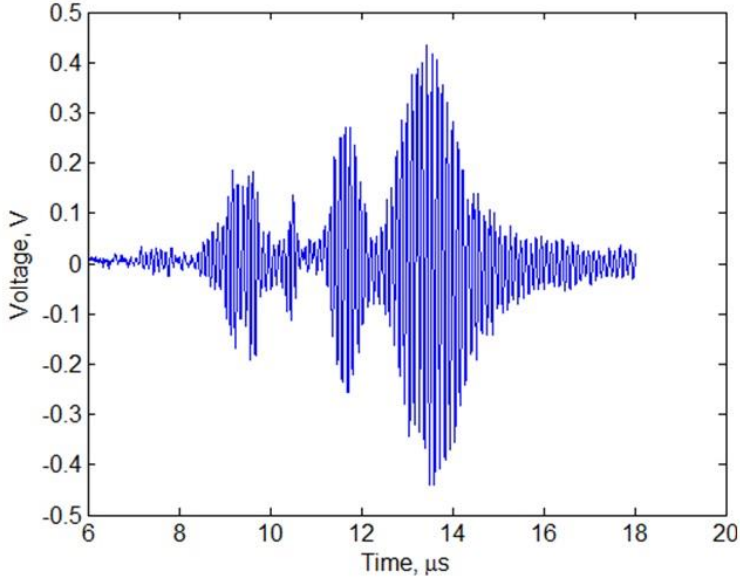
Fig.18. Transverse waves received using CMUT structure when the device is excited using harmonic 10 MHz, 10 periods, 10 Vpp pulses

For deionized water, *Scholte* waves were excited with 10 MHz harmonic impulse with peak-to-peak amplitude of 10 V. The received signal is shown in Fig.18. Analytical and finite element models were created [25] to identify and analyze the transmitted and received *Scholte* waves. Several delta impulses can be seen from the results that can be correlated to the received transverse waves. Impulse with the maximum amplitude is associated with received *Scholte* wave. Other received impulses are associated with the other type waves reflected from the edges of the CMUT chip.

The third phase of the functional tests involved characterizing how the received *Scholte* waves depend on small changes in the liquid mixture. A 1 ml/min liquid flow was induced through the microchannel. Isopropyl alcohol was used as the liquid substrate, which was periodically changed to deionized water and back in different ratios of IPA and DI water: 10:1, 20:1, 50:1 (IPA: Water). A peristaltic pump ensured constant flow and its input was switched to different liquid containers. The received signal amplitude was tracked and registered in real time using a personal computer. The results of these tests are shown in Fig.19.
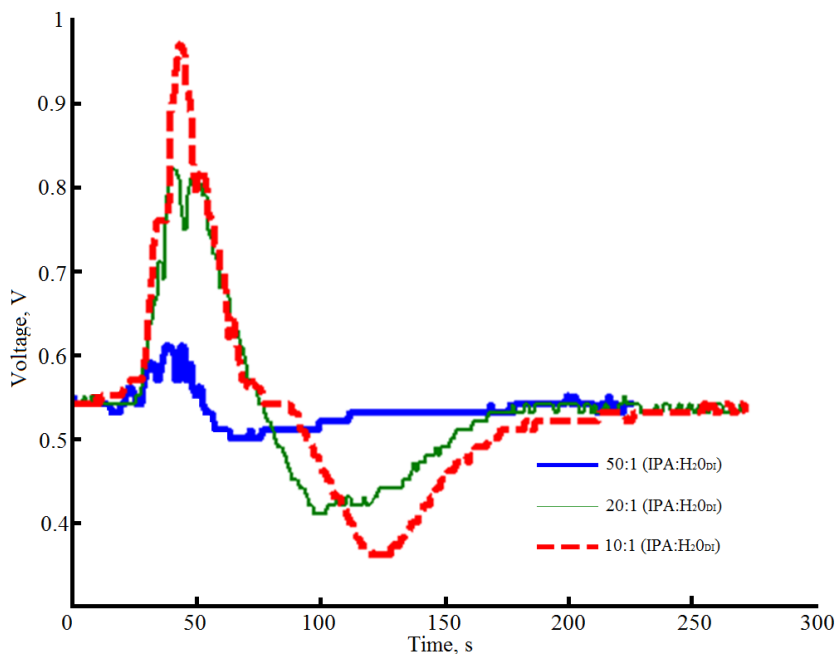
Fig.19. Testing of designed device in the real time with varying liquid contents

From the results, we can see that the received signal amplitude is sensitive to the analyte mixture inside the testing zone. The results of these tests allowed us to prepare for more complex experiments, leading us to the concept of miniaturized phospholipid biosensor with microelectromechanical elements.

3.4. Verification of analytic model and finite element model by experimental data

For interpretation of CMUT readings, we created two models (analytical and finite element), that let us explore acoustical transient processes on a physical level that exist in solid bodies, which are in contact with liquid. Both models were verified between each other and by using results from the experiments. The analytical model allowed us to calculate the transient acoustic processes appearing in the microchannel and wave dispersion dependencies. Both models were used to consider two cases in a 200 µm height microchannel. In the first case, the microchannel was filled with deionized water (c_L=1500 m/s, ρ=1000 kg/m^3). Second case – isopropyl alcohol (c_L=1170 m/s, ρ=785 kg/m^3). The dispersion curves are shown in Fig. 20.
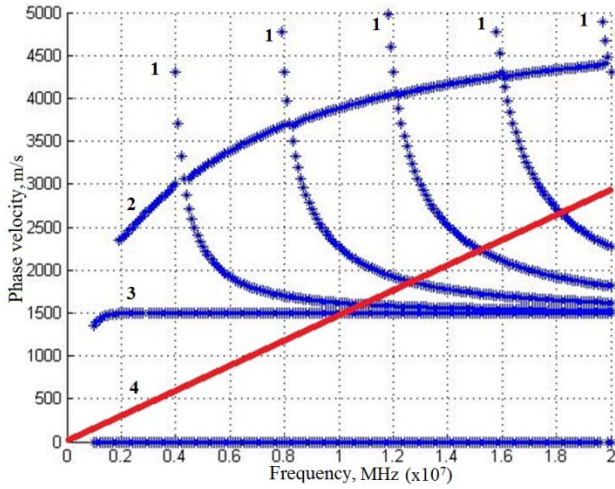
a)
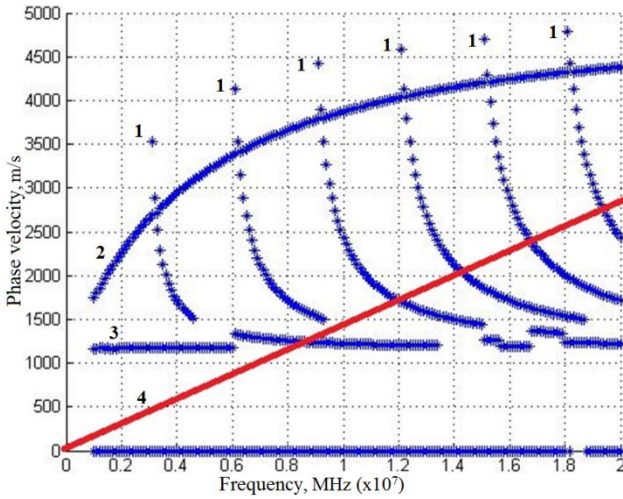
b)

Fig.20. Dispersion transient process curves in a 200 μm height microchannel: a – microchannel filled with deionized water; b – microchannel filled with IPA. Dots show the recalculation of frequency to transverse wave phase velocity functions, determining IDT CMUT with a period between elements of 146 μm. 1 – dispersive waveguide components, 2 – component reflected from microchannel wall, 3 – Scholte wave, 4 – transverse wave resonance with comb type CMUT structure recalculated to the phase velocity function.

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From these curves, we can identify at least two types of waves – dispersive guided waves with high dependency of the phase velocity in respect of the frequency and low dispersion *Scholte* wave with phase velocity of 1450 m/s in water at 10 MHz and in isopropyl alcohol 1153 m/s at 8.3 MHz. The red lines in the diagram show the function of the transverse wave resonance with comb type CMUT structures recalculated into phase velocity, when the distance between each of the CMUT structure "fingers" is 146 μm. A finite element model output was compared with experimental data from send-receive experiments using IDT CMUT structures. During the experiments, the microchannel was filled with isopropyl alcohol. The transmitting IDT was exited with a single 100 ns sinusoid cycle with the amplitude of 10 Vpp (Vpp – the amplitude between positive and negative peaks, peak to peak value). Bias voltage of 60 V was used. Transverse wave phase velocity was found from the frequency spectrum of transmitted and received signal. In these calculations, a distance of $p_1 = 146$ μm, fig. 21, distance between CMUT "fingers" was used.

$$v_{p_1} = f \cdot p_1. \tag{2}$$

here: p_1 – distance between CMUT „fingers "

Fig.21. Interdigital type CMUT structure schematic
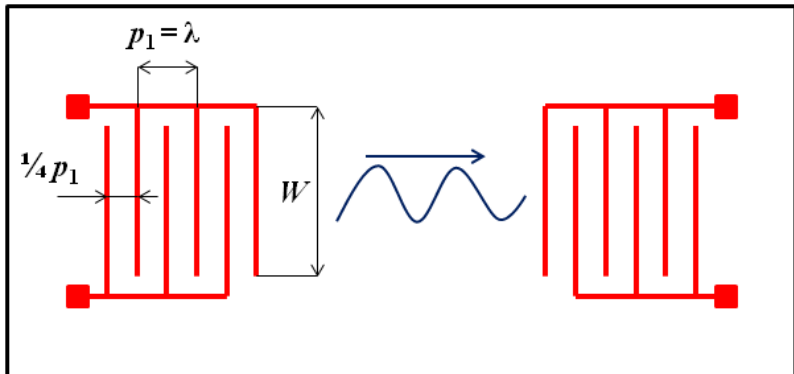
The phase velocity spectrums of experiments and from the simulated models are shown in Fig.22. The vertical lines show calculated CMUT structural resonance frequency using an analytical model that was equal to 9.3 MHz (or 1538 m/s when changed to phase velocity) and phase velocity of *Scholte* wave was equal to 1153 m/s (or 7.9 MHz, resonance frequency of the CMUT structure).
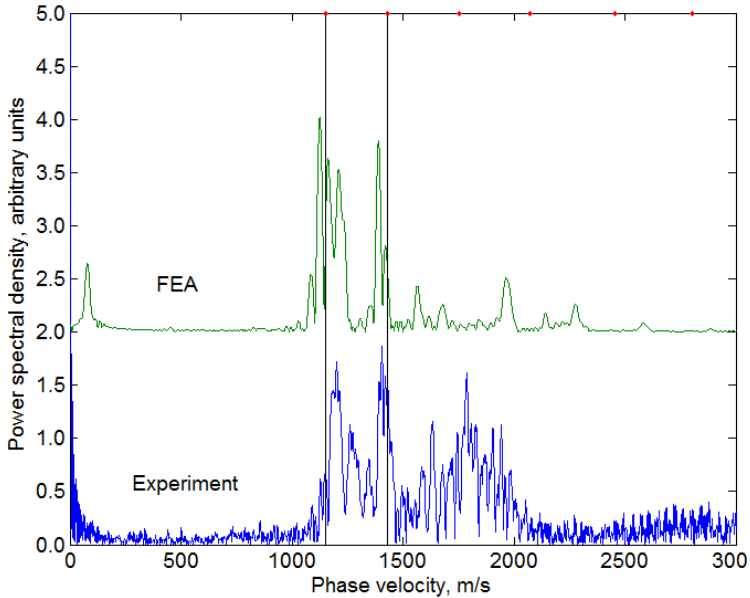
Fig.22. Simulated model spectrum of the received signal, when microchannel is filled with IPA, using the finite element method and data from the experiments. The vertical lines show CMUT structure's resonance values from the analytical model (at 9.3 MHz or 1538 m/s) and *Scholte* waves (at 7,9 MHz or 1153 m/s)

From these figures, we can clearly see two distinct maximums depicting the structural resonance and *Scholte* wave resonance. We can observe that the phase velocity from the analytical model is close to the values of the finite element model and close to the experimentally obtained readings. This ensures the adequacy of both of these models. These models can be used in different ways, also for the interpretation of experimental data. The main conclusion of this section is that the *Scholte* wave phase velocity parameter was identified as very informative, which potentially can be used as a sensing function in liquids. Also in this section, we showed that the designed finite element model is adequate in registering important transient processes in the sensor measurement channel. That is why this model was used for the interpretation of experimental data and also for preparation of experiments and for forecasting the potential results.

3.5. Measurement experiments of dynamic liquid mixture contents

The main objective of these experiments was to continuously register *Scholte* wave phase velocity. The microchannel was filled with different

isopropyl alcohol (IPA) and water solutions. These solutions were prepared with IPA as the main ingredient, adding 10 % and 20% of deionized water by volume.

a)

b)

Fig.23. Signal of *Scholte* wave when CMUT structure is working in isopropyl alcohol and two different isopropyl alcohol and water solutions: a – signal in time domain; b – received wave phase velocity
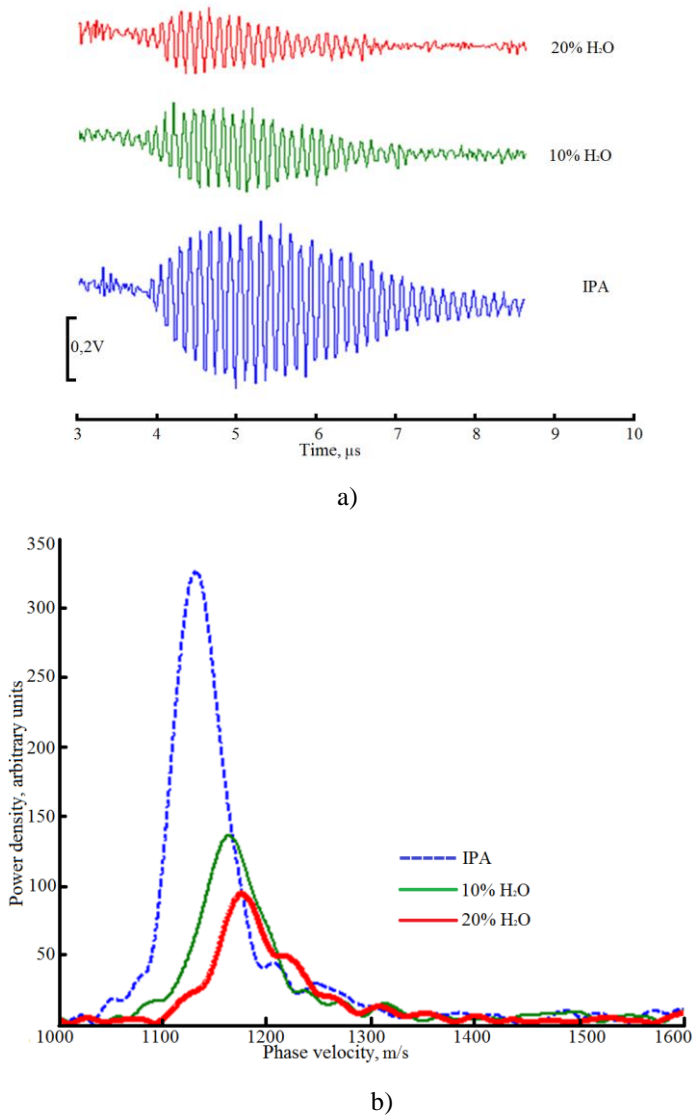
To detect the characteristics of received waves, *Scholte* waves were excited with a broadband one period signal. One period of 100 ns bipolar impulse with an amplitude of 10V was used for one of the CMUT structures. The second CMUT structure was used only as a reference. The received signal of three different liquid solutions is shown in Fig.23a. We can observe that in different liquids, the *Scholte* wave is received at different times. The shorter the time of the wave propagation, the higher the wave phase velocity. However, the measurement of the liquid properties using the signal propagation time approach is limited because of the limited resolution of the oscilloscope. A rough estimation of *Scholte* wave propagation time shows that the shortest delay time of impulse (the highest wave propagation speed), according to the envelope of impulse maximum, is 4.9 µs, and longest – 5.2 µs. Calculation to the phase velocity gives us 1208.2 m/s and 1138.5 m/s (when distance between centers of transmitting and receiving CMUT structures are 5920 µm).

The phase velocity spectrum from the received signal is shown in Fig.23b. The analysis of phase velocity spectrum shows 1134 m/s in the case of IPA, 1182 m/s in the case of 10 % solution of water and 1195 m/s in the case of 20 % water solution. These values are more reliable than the ones measured using a propagation delay line. At the same time, we can observe that the difference between values using both methods is very small. The measured *Scholte* wave phase velocity is close to the ones published earlier, while using analytical model [26, 27]. Sensitivity evaluation of the measurement channel showed the *Scholte* wave phase velocity changes up to 2% to each 10% change of the liquid mixture. It is also probable that in higher concentrations, the sensitivity curve will become nonlinear due to many different other physical parameters (for example changes of the irrigation rate of the sensor's surface).

3.6. Detailed finite element model verification for *Scholte* wave velocity measurements

CMUT experimental data was used to verify the finite element model. Assumptions of the analysis: acoustic waves in IPA are traveling at a speed of 1170 ms/ and in water at 1500 m/s. Also, we have assumed the viscosities of these liquids: IPA 785 kg/m3 and water 1000 kg/m3. The transient responses acquired during the analysis is shown in Fig. 23 a and b. To visualize the data, the results were modified by adding constants to the data values so the curves would not overlap over each other. Comparison of the results acquired during simulation are shown in Fig.24 a.
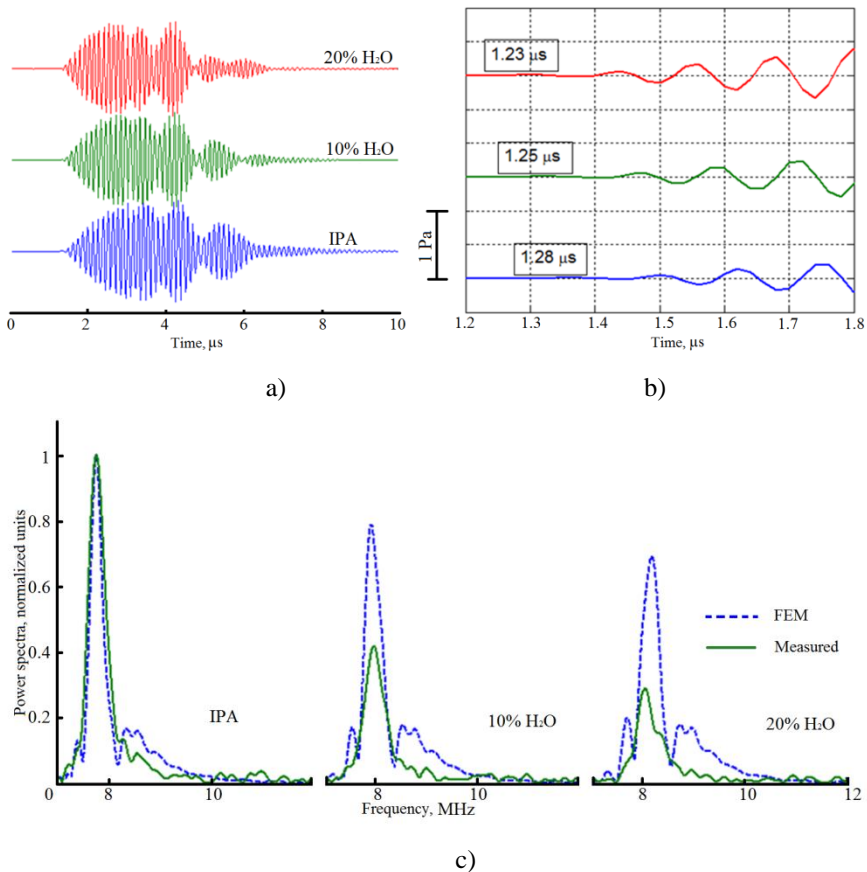
Fig.24. Finite element model comparison with experimental data: a – realization of simulated data in time domain; b – zoomed in time domain from 1.2 to 1.8 µs, showing *Scholte* wave propagation time; c – comparison of simulated and received signal frequency spectra

From these results, we can observe that when water content increases, the amplitude of the signal decreases and damping increases. Also, when water content is increased, the *Scholte* wave propagation time decreases. The differences of the simulated results from experiments (Fig. 23) can be explained by experimental conditions that differ from simulation. For example, simulated results show the ringing of the silicon bulk, which additionally modulated pressure values at the receive points. This effect was not observed during the experiments because the simulated CMUT chip was thinner than the one used in experiments (200 µm instead of 500 µm) and the physical chip was glued to a

printed circuit board, which additionally damped the bulk waves. Also during the experiments, the distance between the centers of the CMUT structures were 5920 µm, while due to memory limitations, this distance was reduced to 4380 µm in the simulation. This is why we have different dampening effects: difference of maximum amplitude of the simulated signals is substantially lower.

The time resolution of simulated data and amplitude was enough to identify the *Scholte* wave propagation time on the first increase in pressure. Zoomed in time axis is shown in 24 b), showing wave propagation times. These delay times correspond to the *Scholte* wave propagation through the analyte zone of 1460 µm. From this data, we can recalculate the phase velocity of *Scholte* wave for all cases: case of IPA it is 1141 m/s, 1168 m/s in 10% water solution and 1195 m/s in 20 % water solution. These values closely correspond to the values obtained from the experimentally received signal spectrum: 1135 m/s, 1182 m/s and 1195 m/s respectively. The slight discrepancy in values could be explained by the inadequate time axis resolution of the simulated data. Comparison between simulated and experimentally received data frequency spectra is shown in Fig.24 c). Results of the resonance frequency are clearly intact. Only in a 20 % water solution case do we have higher discrepancies between the simulated and experimental data, because of the higher signal to noise ratio and non-controlled experimental conditions that were mentioned previously.

This analysis and results comparison show that the finite element model is adequate for representing transient processes based on the *Scholte* wave phase velocity measurements. This model was used to verify the measurement principle.

3.5. Modeling of phospholipide membrane elasticity

While changing the elasticity modulus of phospholipid membrane layers in the simulation model, we found a series of *Scholte* wave time domain realizations that are shown in Fig.25 a. The elasticity modulus of the phospholipid membrane was changed from 1 to 5 GPa [28, 29]. Maximum amplitude and *Scholte* wave resonance, calculated at receive points (see Fig.18), were simulated as the sensor readings. These simulated readings, shown in Fig.25, were interpreted as a function of phospholipid membrane elasticity.

We can observe that the increase in the phospholipid membrane elasticity the resonance of *Scholte* wave with CMUT structure increases and the wave amplitude decreases.
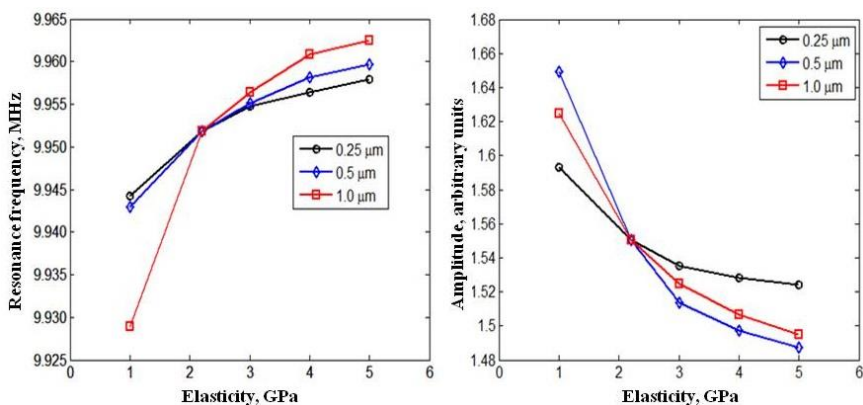
Fig.25. Results of finite element analysis showing the phospholipid membrane elasticity effect on the *Scholte* wave: a) resonance frequency; b) amplitude; different line types show different simulated layer thicknesses

Different line colors and markers show the simulation results for different sizes of finite elements representing phospholipid membranes. All of these lines coincide into one point equal to 2.2 GPa, a characteristic value of water. Above this point, the average function slope (the potential device sensitivity) is 3.8 kHz/GPa when simulating 1 µm size elements, 2.8 kHz/Gpa when model includes 0.5 µm size elements and 2.2 kHz/GPa when simulating 0.25 µm size elements. In particular, it was shown experimentally that Scholte wave phase velocity, as measured by the proposed sensing platform, is sensitive to quite low changes of the IPA and deionised water proportions. Our experiments show the average sensitivity of 2.9 m/s per one percent of the IPA and deionised water solution within 0% and 20% water content range. Experimental data obtained during sensing of the liquid properties were used to verify the finite element model, which reproduces the excitation and propagation of the Scholte waves and also simulates the tethered phospholipid bilayer. A very good fit between the measured and simulated frequency spectra of the received signals was shown. The verified model allowed us to do the theoretical proof of the principle for tethered phospholipid elasticity sensing, which can be used for phospholipid-incorporated toxins detection.

3.6. Real-time monitoring of experimental deposition of biochemical elements

To illustrate the adequacy of the created measurement channel, to measure the biomolecular interactions with the analyte zone in the sensor area, a real-time experiment was carried out by introducing phospholipid vesicle solution into the microchannel after the analyte sensor zone was modified with anchor molecules

that specifically interacts with phospholipids [29]. The data of the three experiments are shown in Fig.27. The primary increase in resonance frequency just after introduction of vesicles serum (II) could be related to an increase in equivalent mass density of the liquid and respectively the increase of *Scholte* wave velocity. Further incubation of the solution shows the decrease of the frequency of *Scholte* wave resonance with CMUT structure (III). This decrease could be linked to lower phase velocity of the *Scholte* waves. This effect can be explained by a process of phospholipid interaction with anchored molecules. When a thin, nanometer-sized phospholipid bilayer is formed on the analyte zone surface, the *Scholte* wave velocity changes (IV). This change is linked to the mechanical properties of anchored molecules attached to the phospholipid membranes, mainly: viscosity, elasticity modulus and mechanical stress. From the experimental data, we can observe that all three experiments had (despite the fact that measurement conditions were slightly different) a recurring experimental phases. These results were considered as the main justification for the concept of measuring *Scholte* wave phase velocity for detection of biomolecular interactions.
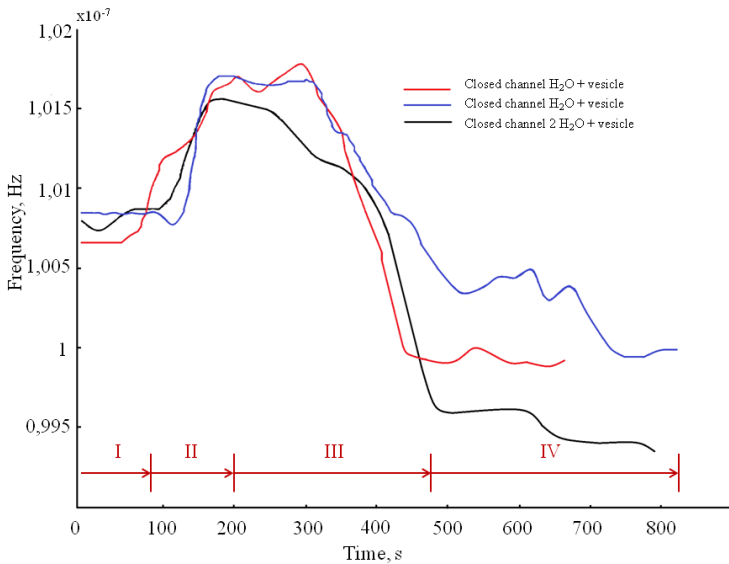

Fig. 27. Registering of phospholipid membrane deposition in real time

3.8. Using the CMUT structure for liquid manipulation

The objective of these experiments was to evaluate acoustical streaming influence to the diffusion of liquids that are pumped through the microchannel, when the sensor is operating in different operating modes.

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The diffusion measurements were carried out with three different conditions: without phase shift of the *Scholte* wave excitation, exiting the wave with + 90^0 shift of phase, aligning acoustic streaming direction with the pumping direction of peristaltic pump and exciting the waves with -90^0 phase shift, when the acoustic streaming direction is opposite to the peristaltic pump pumping direction. In all cases, the sensor structure was excited with continuous harmonic two channel 10 MHz signal with amplitude of 10 Vpp. The bias voltage was set at 70 in all cases.
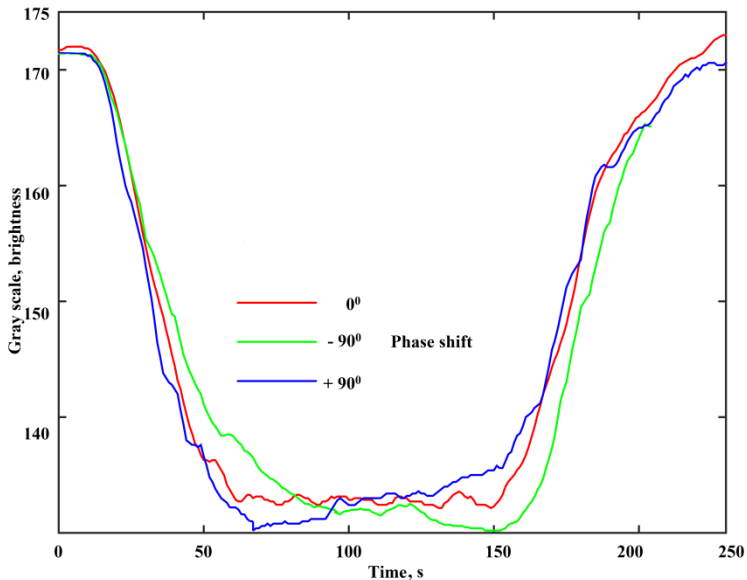
Fig. 28. The dynamics of color intensity, when acoustic streaming is in effect. Positive acoustic streaming matches the direction of peristaltic pump pumping direction

The data of colored liquid diffusion through the microchannel (see section 4.4) is shown in Fig.28. When the phase shift is zero, the maximum stabilization value was reached after 100 seconds (red line). We have assumed that this is the base line of liquid diffusion that will be used for interpretation of results. Due to the acoustic streaming effect, the maximum intensity values are reached at different times with positive and with negative phase shift values when in comparison to the base line. It clearly shows that with positive phase shift liquid diffusion increases and reaches the maximum index value at the 75-th second, while negative phase shift slows the diffusion down and the maximum color index value is reached only at the 150-th second.

3.9. Using interdigital type CMUT for micro pumping

The results of the experiment using interdigital type CMUT structure for acoustic liquid manipulation in microchannel and acoustic pumping efficiency are shown in Fig. 29. In the figure, Q is the flow rate values calculated according to the column height and was calculated from liquid volume that was pumped through the microchannel divided by the time:

$$Q = \frac{\Delta h \pi r^2}{\Delta t}. \tag{3}$$

Here Δh change in column height, r – inner radius of the tube, Δt – change in time.

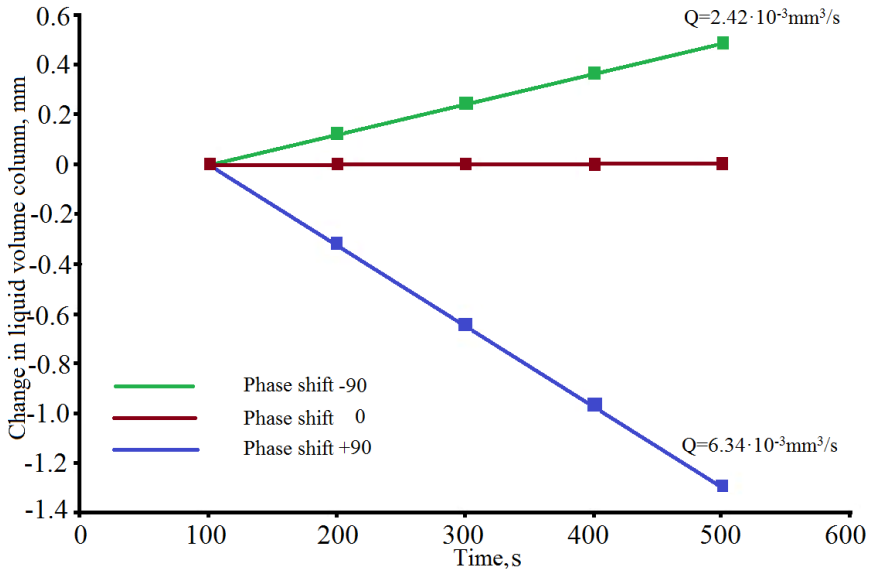**Fig.29.** Acoustic streaming manipulation in the microchannel when phase shift is 0, when phase shift is 90^0 and when phase shift is - 90^0

From these results, the hydrostatic component is already eliminated because acoustic pressure direction is the same as the hydrostatic pressure direction. When phase shift is negative 90^0, the flow is lower due to energy losses.

CONCLUSIONS

1. Analysis of CMUT's structure for biodetection showed:

    1.1. The CMUT structure is capable of transmitting and receiving transverse acoustic waves propagating at the solid-liquid interface also known as Scholte type waves;

    1.2. CMUT interdigital type chip integrated together with microchannel, electronics with Scholte wave phase velocity measurement and specialized software is suitable as a biodetection measurement channel;

    1.3. Scholte wave phase velocity, measured using comb type CMUT structure, is sensitive to small changes of liquid media. Experimental results show that the average sensitivity of Scholte wave phase velocity is 2,9 m/s for 1 percent of isopropyl alcohol and deionized water solution in the range of 0 to 20 %.

2. After the research of acoustic and mechanical transient processes appearing in a CMUT with a comb type structure, it was determined that:

    2.1. Sensitivity of the sensor, measuring the Scholte wave resonance frequency, when measuring the deposited phospholipid membrane elasticity is not less than 2 kHz/GPa;

    2.2. It is possible to detect realistic phospholipid membrane elasticity changes, which can in practice be less than 1 GPa, when Scholte wave resonance frequency measurement resolution is not less than tens of herz, which is possible using the available equipment.

3. Real-time experiments of phospholipid membrane depositions from vesicle solution showed that the created measurement channel is sensitive enough and the signal of resonance frequency repeats even with small changes of the experiment conditions. These experiments justify the principle of sensor operation and effectiveness. However, in the future, the influence of temperature and other environmental factors on the measured signal should be investigated.

4. The same CMUT structure, which was used for biosensing experiments was used as a micropump with the debit of up to $6.34 \cdot 10^{-3} mm^3/s$.

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INFORMATION ABOUT THE AUTHOR OF THE THESIS

Date and place of birth: January 8, 1975, Panevėžys.

Education: 1982–1993 – Panevėžys 10rd secondary school;

1993–1997 – bachelor's degree in Mechanical Engineering at Kaunas university of Technology;

1997-1998 - in Mechanical Engineering at Kaunas university of Technology;

1999-2001 – master's degree in Mechanical Engineering at Kaunas University of Technology Panevėžys Institute.

Contacts: e.sapeliauskas@gmail.com, tel.: +370 698 80721.

REZIUMĖ

Darbo aktualumas

Vienas iš šiuolaikinės medicinines diagnostikos prioritėtų – lėtinių, neurodegeneracinių ir su gyvenimo būdu susijusių ligų ankstyvoji diagnostika. Efektyviausias tokios diagnostikos būdas yra biomolekulinė diagnostika, leidžianti aptikti patogenines molekules ankstyvojoje ligos stadijoje. Klinikinėje praktikoje molekulinei diagnostikai naudojami įvairūs analitinės biochemijos metodai, iš kurių žinomiausias yra ELISA, labiausiai paplitęs imunologijoje. Šis metodas susideda iš keleto procesų: analitėje esančių nežinomų antigenų imobilizavimo, specifinės jų sąveikos su fermentais žymėtais antikūnais sukėlimo ir fermentų aktyvavimo. Aktyvuoti fermentai nudažo biocheminio tirpalo substratą spalva, pagal kurios intensyvumą nustatomas įvykusios sąveikos intensyvumas. Tai ilgai trunkantis (nuo keleto valandų iki keleto dienų) metodas, reikalaujantis specifinės personalo kompetencijos ir specialios laboratorijos. Siekiant ELISA ir kitus panašius molekulinės diagnostikos metodus paspartinti, atpiginti ir padaryti labiau prieinamus, yra kuriami biojutikliai, turintys didelį automatizuoto matavimo potencialą. Būtent biojutiklių naudojimas leido iš esmės paspartinti ir atpiginti genų sekvenavimo procesą. Biojutikliams tapus pagrindiniu molekulinės diagnostikos įrankiu plačiame medicininės diagnostikos

metodų spektre, iš esmės keistųsi molekulinės diagnostikos paradigma – nuo ilgai trunkančių brangių tyrimų laboratorijoje būtų pereita prie greitų ir informatyvių tyrimų įvairiose sąlygose: pirminės sveikatos priežiūros punktuose, buityje, karo lauke, kosmose ir kt. Tokia paradigminė slinktis jau yra prasidėjusi, ir tam tikrose diagnostikos srityse, pavyzdžiui, matuojant cukraus kiekį kraujyje, kompaktiški, kiekvienam diabetikui prieinami biojutikliai yra didele dalimi pakeitę laboratorinius tyrimus ir sukūrę atitinkamą rinkos nišą. Todėl tarptautinėje rinkoje identifikuojamas didelis biojutiklių, ypač skirtų specifinei medžiagų sąveikai nustatyti, sukūrimo poreikis. Apibendrinta biojutiklio struktūra parodyta 1 pav. Biojutiklį sudaro dvi funkcinės dalys: bioaktyvus elementas, užtikrinantis specifinę biocheminę sąveiką ir sąveikos detektorius, keičiantis dėl biocheminės sąveikos atsiradusią fizikinio pobūdžio informaciją į elektrinį signalą. Pasaulyje yra kuriama nemažai biojutiklių, skirtų molekulinei diagnostikai, tačiau dėl naudojamų specifinės sąveikos atpažinimo metodų sudėtingumo, analitinių lustų gamybos kaštų ir dėl specifinių reikalavimų analitės parengimui daugelis šių priemonių vis dar turi ribotas miniatiūrizavimo ir integravimo su kompaktiška elektronika galimybes [10].

Šiame darbe atlikti tyrimai buvo skirti gauti esmines inžinerines ir fundamentines žinias, kurių reikia siekiant panaudoti talpinių mikromontuojamų ultragarso keitiklių struktūrą biodetekcijai. . Gautos žinios apie CMUT struktūros funkcionalizavimo būdų tinkamumą biojutiklio funkcionalizavimui, svarbi informacija apie specifinės medžiagų sąveikos keitimo į elektrinį signalą principus, leidusi nustatyti kiekybinius specifinės sąveikos parametrus, analitiškai ir eksperimentiškai pagrįstas su specifine medžiagų sąveika siejamų elektrinių signalų informatyvumas. Inžinerine prasme, atlikti tyrimai leido pagrįsti CMUT struktūra grindžiamų biojutiklių technologiškumą, atskleisti jų miniatiūrizavimo, integravimo su elektronika ir molekulinės diagnostikos savikainos mažinimo potencialą.

Darbo tikslas - sukurti ir pademonstruoti specifinės biologinės kilmės medžiagų sąveikos jutiklį, grindžiamą CMUT struktūros dinaminėmis elektromechaninėmis savybėmis.

Nagrinėjama problema

Ankstesniuose paskelbtuose moksliniuose darbuose yra patvirtintas principinis CMUT struktūros tinkamumas biojutiklio funkcijai atlikti, panaudojant specifinę biocheminių elementų sąveiką. Biocheminė sąveika aptinkama matuojant jutiklio biodetektoriaus struktūrinį rezonansą dujų aplinkoje. Taip pat ankstesniuose darbuose parodyta, kad CMUT struktūra pasižymi didesniu informatyvumu, nei konkuruojančios biodetektorių platformos. Neišspręsta problema, reikalaujanti naujų mokslo žinių, yra tai, kad didžioji dauguma diagnostinę prasmę turinčių biocheminių sąveikų vyksta skystoje terpėje, o ne dujų aplinkoje. Skystoje terpėje rezonansinis matavimas nėra parankus dėl didelių energijos nuostolių. Anksčiau naudotas matavimo

būdas, kai jutiklis sąveikos metu yra patalpinamas į skysčio aplinką, o rezonanso matavimui yra išdžiovinamas, yra susijęs su nepateisinamai dideliais neapibrėžtumais. Todėl šiame darbe yra siekiama atsakyti į klausimą, kokiomis priemonėmis ir metodais įmanoma, panaudojant talpinių mikromontuojamų ultragarsinių keitiklių struktūrą, gauti informaciją apie įvykusią arba neįvykusią specifinę biocheminių medžiagų sąveiką skystyje realiame laike.

Darbinė hipotezė

Struktūrinį rezonansą, naudotą ankstesniuose darbuose, galima pakeisti skersinių bangų, sklindančių jutiklio paviršiaus ir skysčio sąlytyje, pavyzdžiui *Scholte* tipo akustinių bangų rezonansu su šukų tipo struktūrą turinčiu CMUT. Vykstant specifinei biocheminių medžiagų sąveikai, jutiklio paviršuje formuojasi biomolekulių sluoksnis, dėl kurio keičiasi *Scholte* tipo bangų sklidimo greitis, atitinkamai – ir rezonanso su šukų tipo struktūra dažnis. Matuojant bangų sklidimo greitį ir (arba) rezonanso su CMUT struktūra dažnį galima realiame laike aptikti specifinės medžiagų sąveikos dėka besiformuojantį biomolekulių sluoksnį bei išmatuoti dinamines specifinės sąveikos savybes.

Darbo uždaviniai

1. Išanalizuoti CMUT struktūros taikymo biodetekcijai realiame laike būdus ir atskleisti potencialias darbo su skysta analite galimybes.

2. Ištirti akustinius ir mechaninius pereinamuosius procesus vykstančius CMUT su integruotu mikrosrautų kanalu.

3. Pagrįsti CMUT su integruotu mikrokanalu tinkamumą specifinei biologinės kilmės medžiagų sąveikai registruoti.

4. Ištirti galimybę tą pačią CMUT struktūrą panaudoti jutiklio funkcijai ir skysčio mikrosrauto manipuliavimo funkcijoms atlikti.

Mokslinė vertė

1. Gautos mokslinės žinios apie galimybę talpinių mikromontuojamų ultragarsinių jutiklių struktūrą su integruotų mikrosrautų kanalu panaudoti biodetekcijai, skysčio terpėje specifiškai sąveikaujant biomolekulėms. Laboratorijoje pagaminti ir eksperimentiškai bei skaitmeniniu modeliavimu ištirti prototipiniai CMUT lustai, turintys šukų tipo struktūrą; nustatėme, kad tokia struktūra yra tinkama žadinti ir priimti *Scholte* tipo akustines bangas.

2. Moksliškai pagrįstas *Scholte* tipo akustinių bangų fazinio greičio informatyvumas realiuoju laiku aptinkant specifinį biomolekulių sluoksnio sėdimą iš skysčio ant jutiklio paviršiaus .

3. Panaudojus prototipinį CMUT jutiklį su integruotu mikrokanalu, sukurta skysčio mikrosrautų manipuliavimo matavimo metodika, kuria pagrįstas sukurtos struktūros tinkamumas valdyti skysčio difuziją per mikrokanalą; šis taikymas praplečia jutiklio panaudojimo ribas.

Ginamieji teiginiai

1. Biojutiklis, kurį sudaro su mikrokanalu integruotos CMUT struktūros, siunčiančios ir priimančios *Scholte* tipo bangas per specifinės biocheminės sąveikos zoną, gali aptikti biocheminę analitę skystoje terpėje realiuoju laiku.

2. *Scholte* tipo bangos, sklindančios per specifinės biocheminės sąveikos zoną, fazinis greitis priklauso nuo minimoje zonoje vykstančių fizikinių mechaninių procesų, tame tarpe – nuo iš analitės adsorbuojamų medžiagų elastingumo modulio ir tankio.

3. Biojutiklio prototipo jautris yra pakankamas tam, kad realiuoju laiku būtų galima matuoti fosfolipidinių bisluoksnių elastingumo pokyčius.

4. CMUT struktūros, funkcionuojančios kaip biodetektoriai, taip pat gali būti panaudotos ir kaip priemonė valdyti skysčio difuziją mikrokanale, keisti medžiagų specifinės sąveikos sąlygas.

IŠVADOS

1. Talpinių mikromontuojamų ultragarsinių keitiklių struktūros taikymo biodetekcijai analizė parodė, kad:

    2.1. Sukurta CMUT struktūra gali sukelti ir priimti skysčio ir kieto kūno sąlytyje sklindančias skersines akustines bangas, apibendrintai vadinamas *Scholte* tipo bangomis;

    2.2. CMUT lustas, turintis šukų tipo keitiklio struktūrą, integruotą mikrosrautų kanalą, *Scholte* tipo bangų fazinio greičio matavimo elektroniką bei specializuotą programinę įrangą yra biodetekcijai tinkamas matavimo kanalas;

    2.3. *Scholte* bangos fazinis greitis, išmatuotas naudojant šukų tipo CMUT struktūrą, yra jautrus nedideliems skysčio sudėties pokyčiams. Eksperimentų rezultatai rodo, kad sukurtas *Scholte* bangos fazinio greičio matavimo kanalas turi vidutinį 2,9 m/s jautrį vienam izopropilo ir dejonizuoto vandens tirpalo procentui 0 – 20 % ruože.

2. Ištyrus akustinius ir mechaninius pereinamuosius procesus CMUT „šukų tipo" struktūroje, nustatyta:

    2.1. tikėtinas jutiklio, matuojančio *Scholte* bangos rezonansinį dažnį, jautris jutiklio paviršiuje pritvirtintos fosfolipidinės membranos tamprumui yra ne blogesnis kaip 2 kHz/GPa;

    2.2. kad būtų įmanoma aptikti realistinius fosfolipidinių membranų tamprumo pokyčius, kurie praktiniu atveju gali būti mažesni už 1 GPa, *Scholte* bangos rezonansinio dažnio matavimo skyra turi būti ne mažesnė kaip dešimtys hercų, kas yra pasiekiama naudojant darbe naudotą matavimo sistemą.

3. Atlikti fosfolipidinių membranų nusodinimo iš vezikulių suspensijos stebėsenos realiame laike eksperimentai, kurie parodė, kad sukurtas

matavimo kanalas yra pakankamai jautrus šio biocheminio elemento fizinėms savybėms: gautas rezonansinio dažnio kitimo signalas kokybine prasme atsikartoja netgi nežymiai keičiant eksperimento sąlygas. Galima teigti, kad šie eksperimentai pagrindžia jutiklio veikimo principo tinkamumą. Vis dėlto, ateityje reikia ištirti temperatūros ir kitų aplinkos įtakos veiksnių poveikį matuojamam signalui.

4. CMUT struktūra naudota biojutiklių eksperimentuose, panaudota kaip akustinis mikrosiurblys, tinkantis skysčio debitui iki $6.34 \cdot 10^{-3} mm^3/s$.