TIME RESOLVED ACOUSTIC MICROSCOPY FOR MEASUREMENT OF ELASTIC PROPERTIES OF SINGLE CELLS

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Abstract

Quantitative measurement of the sound velocity inside cells can give information of the polymerization state of the cytoplasm and thus of its physiological status. Time resolved acoustic microscopy has been used to investigate elastic properties of biological samples with thickness greater than 15 μm [1]. To investigate single cells with a thickness of approx. 5 μm a combined instrument consisting of an inverted optical microscope and an acoustic microscope with direct digitization of the received ultrasound signal with a center frequency of 1 GHz was developed. Using defocusing techniques in conventional light microscopy the specimen thickness was estimated. The travel time of the ultrasonic pulse was determined by cross correlating the received signal with a reference signal. Interpolation techniques were necessary to achieve the needed time resolution. Using the thickness and travel time information for calculation the longitudinal sound velocity of several samples have been investigated and the accuracy of the technique was evaluated.

Introduction

Elastic properties of cells can give important information on processes such as division, migration or attachment and can give direct infromation of the polymerisation state of the cytosol or the kernel. Acoustic microscopy is a powerful tool for the examiniation of elastic properties of materials. But up to now the application of acoustic microscopy to examining the elastic properties of single cells on a subcellular level was somehow limited.

To overcome some limitations from conventional acoustic microscopes a new combined acoustic and optic microscope was developed to investigate the elastic properties of cells and their connection to chemical properties or structures of the cell found with syncrounous optical microscopy.

The new microcope is a time resolved 1 GHz acoustic microscope to employ time of flight measurements. From these it is possible to calculate the local bulk sound velocity of a cell. Since the microscope uses a very short burst of ultrasound with a length of 6 $nsec$ layers with a thickness greater than 2 μm can be resolved. If the top of the cell is visible in the acoustic signal, it is possible to determine the local heigth of the cell from the acoustic signal alone. If the signal from the top of the cell is not visible the optical microscope can be employed to find the height profile of the cell.

Theory

Using a simple solid state model for the cell the elastic bulk modulus K is connected to the longitudinal sound velocity c_L as the square root of the ratio of K and the density.

$$c_L = \sqrt{\frac{K}{\rho}} \quad \Leftrightarrow \quad \rho \cdot c_L^2 = K$$

If the the cell density can be estimated as constant in the cell the elastic modulus K can be derived by measuring the longitudinal sound velocity. Figure 1 shows a straight forward time of flight approach using time resolved acoustic microscopy.

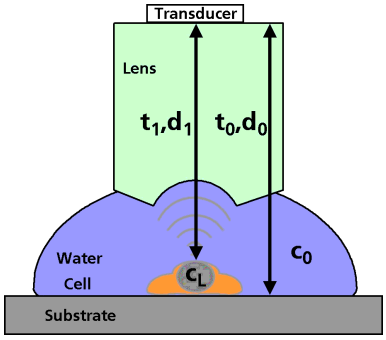

Figure 1: Measuring the longitudinal sound velocity of a biologigal cell through a time of flight measurment.

If the distance between the transducer and the substrate is d_0 and the distance between the lens and the cell is d_1 the thickness of the cell amounts to $d_2 = d_0 - d_1$. The measured travel time of the ultrasound pulse on its way from the lens to the substrate and back without passing through the cell will be t_0 and the measured time of flight on the same way but with passing through the cell will be $t_0 + dt$. Then the local sound speed c_L is:

$$c_L = \left(\frac{dtc_0}{2d_2} + 1 \right)^{-1} c_0$$

A combined acoustic and optic microscope

Figure 2 shows the combined microscope. Its base is an inverted optical microscope (Olympus Optical Eu-

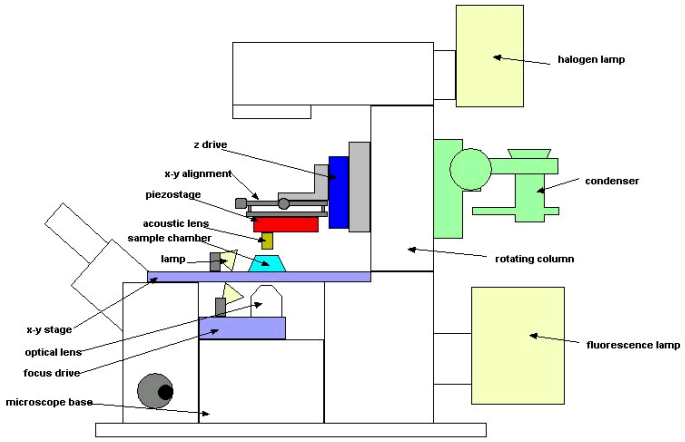

Figure 2: Schematics of the combined microscope in acoustic mode.

rope, Germany). It posseses two light pathes. The first uses a halogen lamp and a condensor for transilumination while the second uses a mercury burner for epiflourecence in a reflected light setup. Additional lamps where attachted to the microscope to illuminate the acoustic lens for the alignment.

The acoustical scanner consists of the acoustical lens with $1\ GHz$ center frequency and an apperture of $100°$ (KSI, Germany) mounted on a piezo stage which has a scanning range of $80 \cdot 80\ \mu m^2$ and a maximum scanning frequency of $100\ Hz$. The scanner is attachted to a mechanical aligment stage for x-y alignement and tilt correction of the lens. A motorized linear stage is used for the focusing of the acoustic lens with a resolution of $0.1\ \mu m$. The acoustic scanner is exchangeable with the transillumination condenser by means of a rotating column.

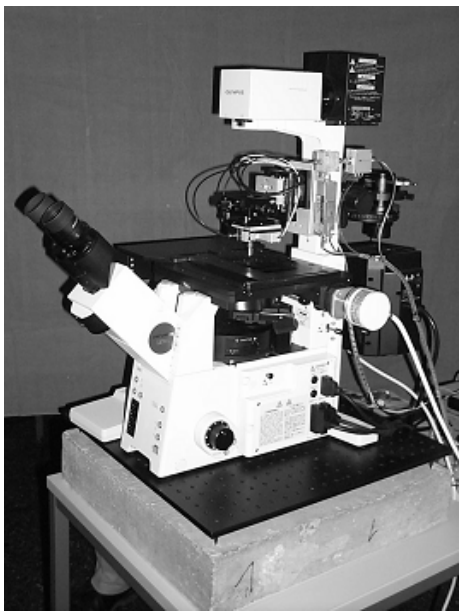

Figure 3: Photograph of the combined microscope in acoustic mode.

The radio frequency part of the microscope consists of a simple short pulse generator with a step recovery diode, a switch, an amplifier and a fast analogue to digital converter running at $4\ GS/sec$ as described in [2]. The switch connects either the pulse generator or the amplifier to the lens. The generated pulse has a width of approx. $0.5\ nsec$ and an amplitude of $5\ V$. The trigger signals and the signals for driving the piezo stage are generated by a microcontroller. A software running on a standart personal computer acquires the digital data which is then stored onto hard disk for later postprocessing and generates C-scan images from the echo ampitudes.

Alignment of the lens is done every time the acoustic scanner is rotated onto the sample. The mechanical alignment stage is adjusted until the center of the lens is in the center of field of view of the light microscope. After mechanical alignment the lens is moved into different positions with the piezo stage. Since the actual center of the lens is not easly found in the optical image an additional alignment correction term is calculated from the estimated center positions of the lens in different positions by making a least square fit.

Time resolved acoustic microcopy at 1GHz

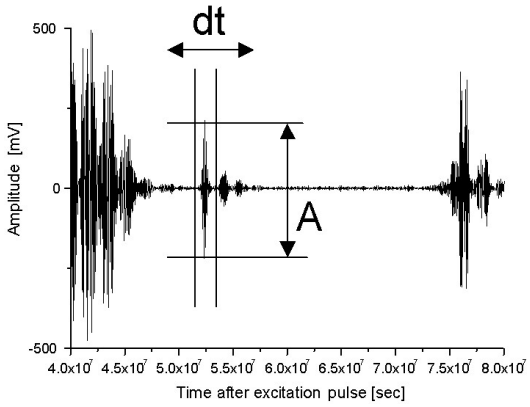

Figure 4: Digitized Signal from a glas substrate with a $1\ GHz$ $100°$ acoustic lens. The Echo from the substrate is located at approx. $520\ nsec$.

Figure 4 shows the received signal of the $1\ GHz$ lens on top of a glass substrate when driven by a short pulse. The signal shows three main echoes. The first echo is generated by the lens water interface while the second signal is the echo from the sample substrate interface. The last echo shows the ultrasound pulse after it has traveled two times through the lens. Small echoes in the signal are due to impedance mismatches in the radio frequency electronics and dependent on cable length.

There are two main parameters to measure in the signal. The first parameter is the amplitude A of the sample substrate interface which is dependent on the reflec-

tion coefficient of the interface and the damping in the travel path of the ultrasound pulse. The second parameter is the arrival time of the echo $t_0 + dt$. While the amplitude is used to generate C-Scan pictures to investigate the sample qualitatively the arrival time is used to calculate the local bulk sound velocity for quantitative measurements.

3D Reconstruction of single cells

Additionally to the echoes from the sample substrate interface it is possible to detect echoes from top or inside the cell. These are approx. $50 - 100$ times smaller than the echo from the substrate interface. Since the complete signals from every pixel are recorded it is possible to fill a 3D Volume from one acoustic x-y scan. Inside this volume structures of the recorded sample can be identified.

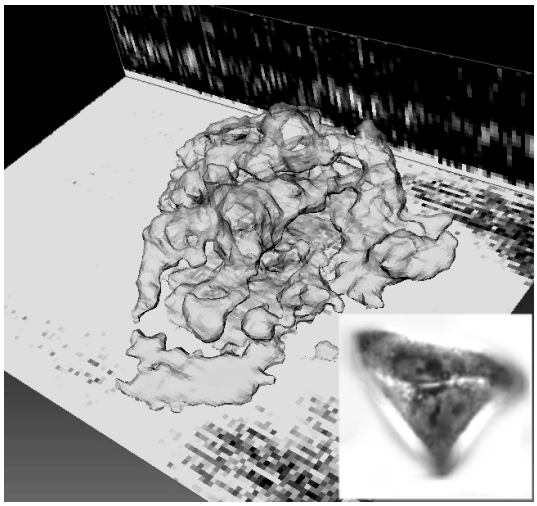

Figure 5: Manual 3D-reconstruction from a HaCat cell. The image in the corner shows the corresponding optic image.

Figure 5 shows a manual 3D reconstrucion from a HaCat cell. The segmentation was done by selecting areas of high signal amplitude in all B-Scans. The corner picture shows the optical image of the cell. There are two cells attachted to each other. Both kernels can be identifiyed in the 3D reconstruction and the seperation between the cells is visible. The cell has a width of approx. $40\ \mu m$ and a height of $7\ \mu m$.

Results

The sound velocitiy of samples from two different cell lines were investigated. The cell lines were C2C12 myoblast and L929 fibroblast. The height profile was either estimated from the recorded acoustic data for the C2C12 myoblast or estimated from an optical stack for the L929 fibroblast.

Figure 6 shows on the left side an optical phase constrast image of a C2C12 myoblast and on the right side

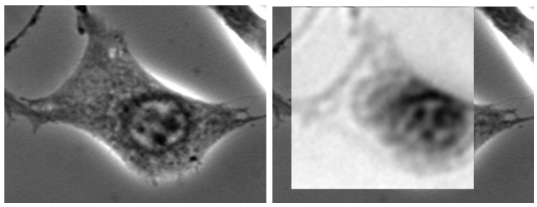

Figure 6: Optic phase contrast image from a C2C12 myoblast and superimposed with acoustic amplitude image.

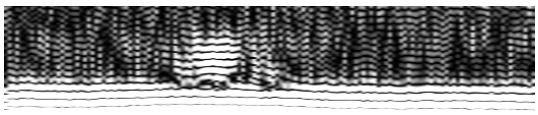

Figure 7: Radio Frequency B-Scan of a cross section from C2C12 Cell.

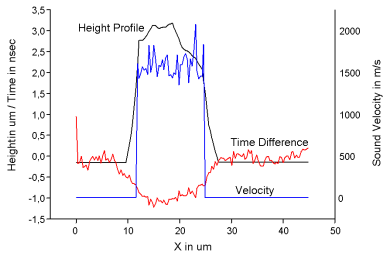

Figure 8: Sound speed of a cross section from C2C12 Cell.

the optical image superimposed with an acoustical amplitude image both in focus. Similar features are visible in the kernel structure of the cell, while different features visible in the cytosol of the cell.

From the acoustical data a cross section along the y-axis was taken as shown in Figure 7. The figure shows the echo from the top of the subtrate and in the middle the echos from the cell especially from the kernel of the cell. A Height profile of the cell cross section was estimated from the B-Scan and the arrival time of the sample subtrate interface dt was measured using a 20 times oversampling and a maximum peak detection. The height profile, the arrival time and the calculated velocity are shown in Figure 8. The mean value is near to the expected longitudinal sound velocity approx. $1600\ m/s$. Also there are regions in the cross section, were the local mean of the sound velocity seems higher than in other region indication the very highly condensed nucleole.

The optical and acoustic image from in focus positions of a L929 fibroblast are shown in Figure 9. To estimate the sample thickness of the cell, the following procedure was used. Optical images were taken at different focus positions as seen in Figure 10 on the left side. In each image the regions that were in focus were marked. The height profile seen in Figure 10 on the

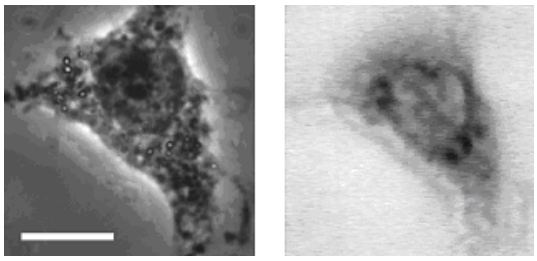

Figure 9: Optical phase contrast and acoustical amplitude image of a L929 fibroblast.

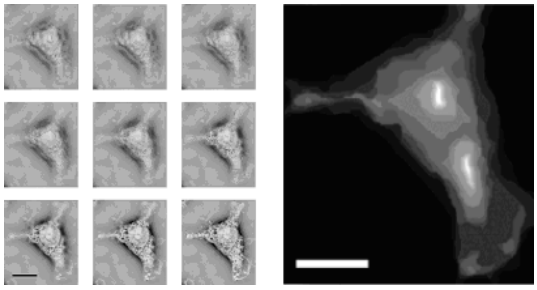

Figure 10: Optical Stack and height profile of a L929 fibroblast.

right side was then reconstructed from these marked areas and the corresponding focal position.

From the recorded data of the acoustic microscope the time shift information was extracted by correlation technique and second order polynominal interpolation. From this information and the height profile obtained by optical microscopy the velocity image in Figure 11 was calculated showing a very high sound velocity in the highly condensed nucleole.

Resolution

With a 1 GHz lens the lateral resolution of the acoustic microscope is approx. 1 μm. Since the pulse length is around 6 $nsec$ it is possible to resolve layers with thicknesses greater than 2 μm with the sound velocity of water. The alignment error beetween optical and acoustical images was found to be better than 3 μm in both axis.

Absolute resolution of the measurement of the bulk sound velocity is limited due to the height estimation error of ± 0.5 μm and the arrival time error ± 250 $psec$. The error is the approximatily the same for optical and acoustical microscope and due to the focal length of the optic objectiv or the signal to noise ratio in the acoustic signal.

Adding those estimated errors we can calculate that the measured sound velocity to be in the range of $c_L = 1700 \pm 270$ m/s for a 5 μm thick layer with a true sound velocity of 1650 m/s. To reduce the error averaging was employed with a tradeoff to the maximum frame rate. If we only take the height estimation error into account the measured values have a range of $c_L = 1670 \pm 170$ m/s for the same layer. Additionaly there is the mechanical vibration of the scanner and the substrate.

Figure 11: Velocity image of a L929 fibroblast with velocities from 1600m/s(black) to 1650m/s(white).

Conclusion

It was shown that with time resolved acoustic microcopy with short pulses it is possible to image single cells at 1 GHz. It was also possible to measure the bulk sound velocity either from the acoustic scans alone or with the help of the optic microscope. B-scans of cells indicated that it is possible to resolve structures in the cell with high frequency ultrasound.

Local variations in the sound velocity of the kernel of a cell can be seen in the data even though the absolute error can be greater than the variations. Nevertheless as the height of the cell does not vary much over a small area relative changes in sound velocity are detectable. There an error in the height estimation does only add an offset to the measured values.

To reduce the absolute error of the measured sound velocity the height estimation error has to be reduced. This can be done by either using optical objectives with very high numerical apperture for the optical procedure or by improving the signal to noise ratio in the acoustic signals.

References

[1] C. M. W. Daft and G. A. D. Briggs, "Wideband Acoustic Microscopy of Tissue", IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control Vol.36:258-263 (1989).

[2] A. Briggs, "Acoustic microscopy" . Oxford University Press, Oxford, 1992.