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## Ultrasound for tissue microscopy

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Application of ultrasonic methods for medical diagnostics is widely known due to the delicate effect on living tissues. The methods are less labor-intensiveness compared to X-ray tomography that makes it easier to observe dynamics of biological processes. A distinctive feature of focused ultrasound is the local nature of its interaction with an object. The probing beam has a focal zone with diameter and length determined by the angular aperture and operating frequency of the ultrasound emitter. The frequencies of 1-10 MHz are used in medical diagnostics to observe the structure of human organs. Radiation of higher frequency - 10-200 MHz, is used to study miniature samples, slices and to work with the laboratory animals.

Scanning acoustic microscopy (SAM) provides high-quality images of tissues and cells, comparable to the light microscopy, without any staining and with a short time of scanning. Ultrasonic waves pass through various tissues of the human body with certain speed values. The speed and attenuation of sound waves correlates with elasticity of tissues, thereby indicating their biomechanical properties. Elasticity varies depending on contents, such as: collagen fibers, blood, colloids, mucin, fat, crushed substances, cytoskeleton and so on. Due to the dependence, SAM allows to display changes in the tissues composition. In addition, chemical modifications such as fixation can affect acoustic properties and SAM can tracks these changes over time on the same sample to simplify statistical comparison of the measured data.

SAM was developed in the 70s of the last century at Stanford University [1]. Today, the SAM is modified: the shortest probe pulses and precision mechanical systems for the sensor moving provide a high resolution – lateral and in depth. Wave front of ultrasonic radiation is considered to be flat inside the focal zone of acoustic lens. Due to this, SAM can be used to calculate sound speed and attenuation, tissue thickness, and to image their distribution. The Imaging quality depends on the SAM frequency –the higher frequency corresponds to the smaller wavelength and diameter of focal zone, and a smaller pixel size as a result. Contrast of ultrasonic images depends on the biomechanical properties of tissues. Tissue sections that contain little structural protein possess sound speed and attenuation values that are similar to water in the coupling medium. In contrast, tissue sections containing structural proteins such as collagens have significantly higher the values than water. In general, the resolution of SAM is comparable to the resolution of optical microscopy. But since the principles of contrast formation of these two methods differ, their combination helps to clarify the structural and functional characteristics of tissues and cells as effectively as possible.

In this review, principle of SAM, samples preparation, examples of SAM application will be presented. To illustrate the method potential, the report uses both own original images and the results of other authors published with open access.

## References

[1] R. A. Lemons, and C. F. Quate, Acoustic Microscopy, in Physical Acoustics, edited W. P. Mason, and R. N. Thurston, (Academic Press., London, 1979)

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