Thrombus formation in a rat experimental model

Introduction

Compared to other scanning techniques in microscopy, the scanning acoustic microscopy and tomography provide an unique combination of three features, which makes it useful in a wide range of applications:

1.) It has a pronounced „subsurface capability“ that makes layer and subsurface reconstruction possible.
2.) The image generated by the Scanning Acoustic Microscope (SAM) reflects the stiffness, viscosity, adherence, density and topography of the sample.
3.) Due to a pulsed operation and a high frequency, the SAM is non-destructive to most samples. This is of special importance in medical diagnostics and research involving living cells and tissues.

Although the technique was introduced more than a decade ago, this technique of imaging and measuring acoustic properties is still not widespread in the medical and biological research fields, primarily due to the limited number of instruments available.

This report presents briefly some of the preliminary images obtained in rats in- and ex-vivo at the Leica Scanning Acoustic Microscope Application Laboratory. Our study concerned the imaging of the thrombus in a 0,7 mm diameter femoral artery in a living anaesthetised rat and imaging of the same artery after its removal from the animal.

Thrombus formation in a rat experimental model

Resent advances in microsurgical techniques have significantly enhanced the success rate of surgical procedures in small blood vessels. However, even in optimal situations with the most skiful microsurgeons there is a certain failure rate due primarily to thrombus formation in the arteries at the site of surgical intervention.

Two male anaesthetised Wistar rats were used in the study. The rats were placed under a stereo microscope and a skin incision was made across the right thigh in the line between the thigh and the groin. Using a microsurgical technique (the surgeon uses a stereo microscope and delicate special instruments) the femoral vessels were laid bare and the femoral artery (0,7 mm diameter) was carefully dissected free in a length of 1-1,5 cm.

A side vessel was ligated and the cut.
After surgery the rat was placed in a glass container filled with warm saline that acted as coupling liquid. The head was kept above the saline. The rat was then placed in the KSI SAM 50 and the femoral vessels were scanned at 25 MHz (Fig. 1)

Fig. 1: The image obtained in-vivo at a frequency of 25MHz with a field view of 8x8mm. The femoral atery (marke by circle) is seen running parallel to the femoral vein. Due to the fact that the animal is alive, blood is passing continously within the artery at a pulse rate of approximately 400 per minute during the ultrasonic scanning. Contrast changes in the artery and vein are apparent in the SAM-image due to the different acoustic properties of the vessel wall and the blood.

Following this 25 MHz scanning a thrombogenic injury was performed on the femoral artery. The artery was incised transversely over one-third of the circumference and the defect closed with special sutures that intentionally inverted a full thickness section of the arterial wall into the centre of the artery. Within a few minutes a thrombus formed at this site and the rat was again placed in the container with coupling liquid, placed in the KSI SAM 50 and scanned 25 MHz (Fig. 2)

Fig. 2: The image obtained in-vivo at a frequency of 25MHz with a field view of 8x8mm. Again the femoral artery is seen running parallel to the femoral vein. The arterial wall is clearly distinguished from the lumen (centre) of the artery. The circle indicates a narrowing of the arterial lumen that may correspond to the site of injury. The thrombus could be seen in the centre of the marked area.

For ex-vivo scanning of the artery with the thrombus, a section of the artery comprising the site of injury and approximately 2-3 mm of the artery on each side of this was ligated and excised. In this way any thrombus material and blood were captured inside the artery. The artery was then placed in the KSI SAM 50 and the SAM 2000 and scanned at 25 and 200 MHz, respectively (Fig. 3 and 4).

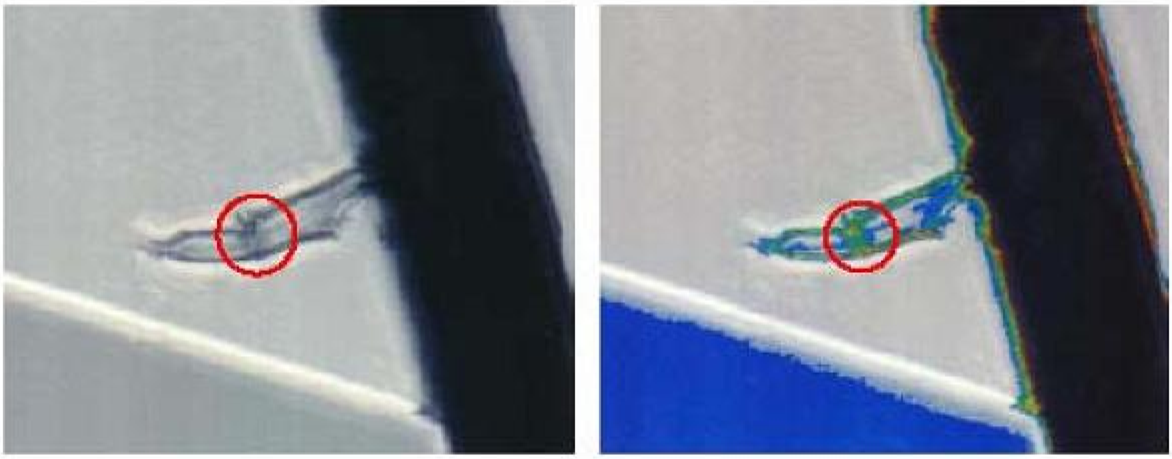

Fig. 3: The image obtained ex-vivo at a frequency of 25MHz with a field view of 8x8mm. The femoral artery is seen with the legated ends and side vessel. A dark area that may depict a thrombus is seen in the lumen below the sutures

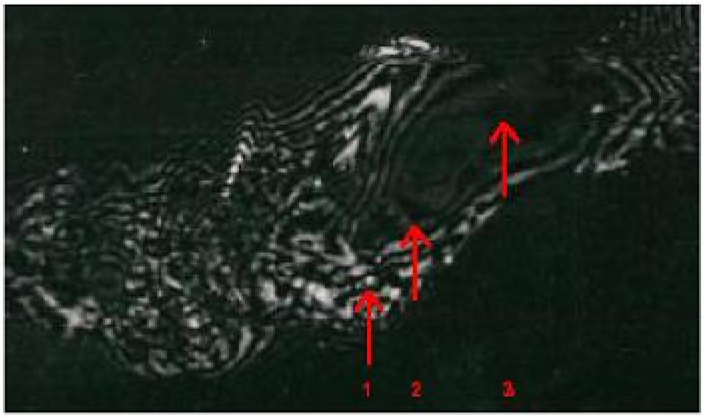

Fig. 4: The image obtained ex-vivo at a frequency of 200MHz with a field view of 1x1 mm using a gate width setting that included the top of the artery and the thrombus. The first arrow indicates the position of the structure, the second one the wall of the artery and the third one the thrombus. Different acoustic properties (layers) are reflected by the varying contrasts.

State of the art and future aspects

The use of scanning acoustic microscope offers the possibilities of ultrasonic imaging at low and high resolutions and quantitative analysis of acoustic parameters such as the speed of sound, the density of the tissue and the impedance of the tissue, all of which may be of great importance to medical research and diagnositcs. Especially the possibility of imaging the layered structure of organs and possibly the emerging of a new ultrasonically based description of the layer configuration, may significantly enhance our understanding of physiological and pathophysiological phenomena. Further development of this technique could result in new techniques for in-vivo diagnostics such as real-time imaging of ongoing processes such as the formation of vascular thrombi and direct microscopy of more or less stationary processes such as tumours.

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