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A method to obtain reference images for evaluation of ultrasonic tissue characterization techniques

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Abstract

A general problem when evaluating ultrasonic methods for tissue characterization is that "a golden standard" is seldom known. This paper describes a manual method to obtain a reference image, with the same geometry as the ultrasound image, indicating spatial location of the different tissue types present in the biological tissue scanned in vitro.

A $30 \times 10 \times 2$ mm^3 piece of formalin fixed porcine tissue was molded into an agar block, which on the top surface, contained a set of fiducial markers, spaced 2.5 mm. The block was submerged into 20 °C water and a set of parallel 7.5 MHz spatial compound ultrasound images of tissue and fiducial markers were recorded each 0.5 mm. Guided by the fiducial markers, the agar block was subsequently cut into slices 2.5 mm thick, photographed and finally analyzed histologically identifying these tissues: collagen rich, collagen poor, micro vessels and muscle fibres. Due to: (1) the cutting procedure, (2) the finite size of the ultrasound beam and (3) the spatial variation in propagation velocity, the macroscopic photographs did not align completely with the ultrasound images. Likewise, the histological image is a geometrically distorted version of the macroscopic photograph, due to the histological preparation process. The histological information was "mapped back" into the format of the ultrasound images the following way: On the macroscopic images, outlines were drawn manually which defined the border of the tissue. These outlines were superimposed on the corresponding ultrasound images (identified via the fiducial markers) and modified to encompass what appeared to be tissue regions on the ultrasound images and subsequently re-applied to the macroscopic image. This modified macroscopic outline was used as guideline when drawing outlines identifying regions of the various tissue types. Specifically, the macroscopic image revealed the borders between the different tissues, while the histological image identified the four tissue types.

A set of 12 reference images based on modified macroscopic outlines was created. The overlap between the ultrasound images and the macroscopic images—which are the geometrical basis for the final reference images—was between 77% and 93%.

A set of 12 reference images spaced 2.5 mm, identifying spatial location of four different tissue types in porcine muscle has been created. With the reference images, it is possible to quantitatively compare different ultrasound based tissue classification techniques.

Keywords: Ultrasound; Tissue characterization; Atherosclerosis; Histology

1. Introduction

The main cause of stroke is due to an atherosclerotic plaque deposit of lipid and collagen in the carotid artery. The only preventive treatment is surgery whereby the plaque is removed, but there is a 5% risk of complications during such a procedure. Not all plaques are dangerous [1], however, but with today's technology it is difficult to distinguish between the different kinds of plaque. It is believed that the contents of the plaque is related to how unstable and thereby how dangerous the plaque is [2]. It is therefore relevant to search for a method to characterize the tissue types in the plaque. Ultrasound is a non-invasive imaging modality that can be used to identify arteries with plaque deposit. Better

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methods with respect to the identification of the different tissue types are, however, needed. A general problem when evaluating ultrasonic methods for tissue characterization is that the exact reference is seldom known. This paper describes a manual method to obtain a reference image, with the same geometry as the ultrasound image, indicating spatial location of the tissue types present in the biological tissue scanned. This method is applied to a phantom made from porcine muscular tissue.

2. Materials and methods

2.1. Preparation and scanning

In order to have a phantom with distinct tissue types a 30 mm long, 10 mm wide and 2 mm thick piece of porcine muscular tissue was used. The phantom contains lipid, collagen and muscular tissue. For reference, an atherosclerotic plaque contains primarily lipids and collagen. The tissue was folded around a metal rod to somewhat mimic an artery. The tissue was then formalin fixed and subsequently molded into an agar block ($1\%_{\text{weight}}$ agar–agar) as described in [3]. On the top surface this block contained a set of fiducial markers made in the molding process by placing a lid with bars spaced 2.5 mm (in the x_t direction in Fig. 1) on top of the liquid agar. Subsequently the agar block was cooled down to obtain a solid form. As shown in Fig. 1 the experimental setup for ultrasound scanning consisted of: (1) a multi-angle compound imaging ultrasound scanner (the Xtra-system) developed at our laboratory [4], (2) a 192-element 7.5 MHz linear array transducer 40 mm wide [4], (3) a scanning tank with degassed demineralized water at 20 °C, (4) a 3D translation system capable of translating the transducer and (5) a control computer running Matlab (Mathworks, Inc., Natic, MA, USA).

The block was submerged into the scanning tank and parallel ultrasound images of the tissue and the fiducial markers were recorded, spaced 0.5 mm apart (in the x_t direction in Fig. 1) by mechanically translating the linear array transducer. Spatial compounding was used since this improves boundary definitions [4–6].

2.2. Cutting and histological staining

The agar block with the tissue specimen was cooled to 5 °C and placed in a stationary frame. Guided by the fiducial markers, the specimen was cut into slices 2.5 mm thick in order to have a set of images from which the contours of the tissue could be identified. The knife (a trimming blade) was lubricated in glycerol to minimize adherance between knife and tissue and was drawn through the agar block in one continuous movement. 12 slices were made. Each slice was photographed digitally

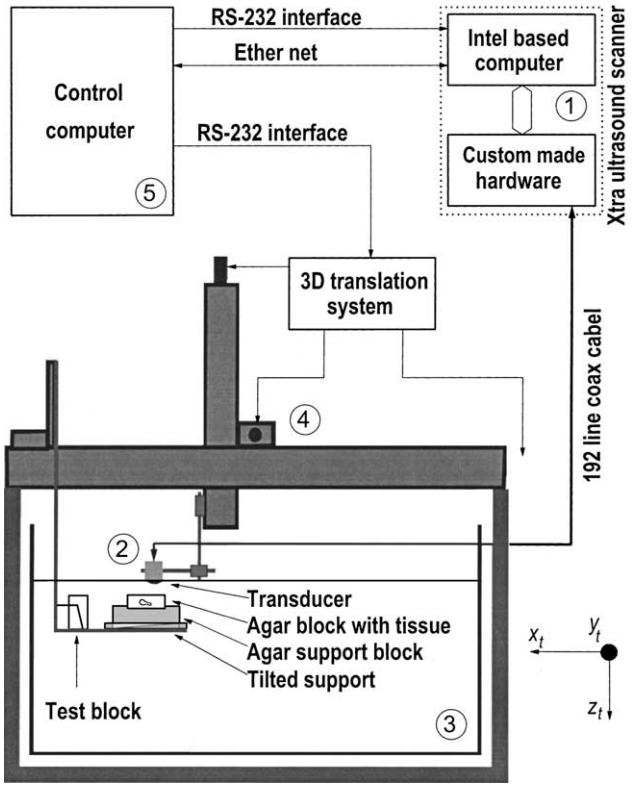

Fig. 1. Ultrasound recording setup showing the scanning tank and the *XYZ*-positioning system.

(macroscopic image). The markers on top of the agar block made it possible to identify approximately which ultrasound scan plane (x_t direction in Fig. 1) that corresponds to which tissue slice i.e. the scan planes nearest to the cutting planes through the markers were used. This procedure introduces a maximum error of 0.5 mm. The tissue slices were then analyzed histologically (stained with elastin trichrom). This analysis identified the following tissue types: collagen rich, collagen poor, muscle fibres and micro vessels of which the first two can be interpreted as collagen and lipids, respectively.

2.3. Creating the reference image

The basis for creation of the reference image is outlines on the three different sets of data, indicating the borders of the tissue and the different tissue types. The first step in the creation of the reference image was to outline the macroscopic images to obtain the contours of the tissue as shown in Fig. 2. Care was taken to ensure that only tissue in the cutting plane was encompassed by the outline since there is a small variation in shape over the 2.5 mm slice. This outline was then superimposed on the spatial compound ultrasound image that correspond to the associated macroscopic image. The outline was offset to best match the ultrasound

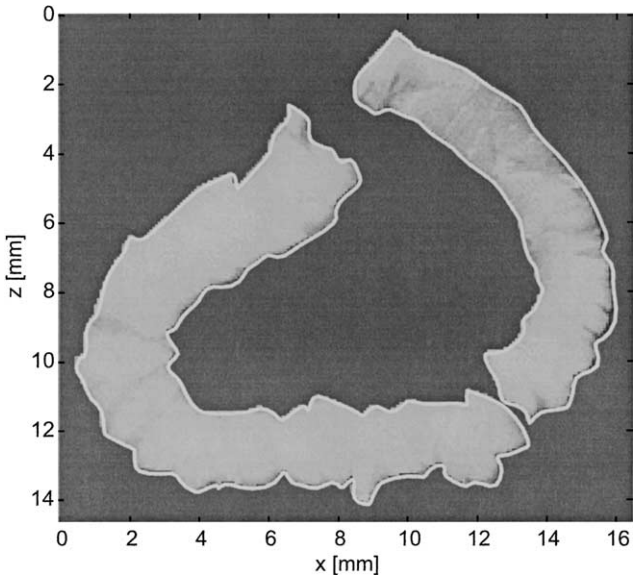

Fig. 2. Macroscopic image with outline defined by the boundaries of the specimen.

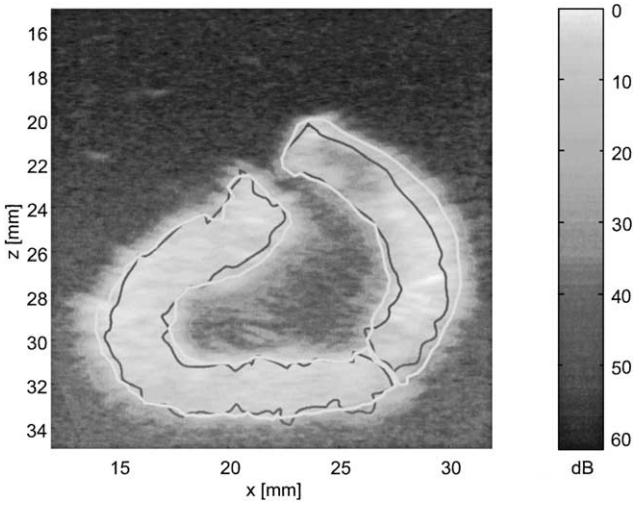

Fig. 3. Ultrasound image with the macroscopic outline (dark) and the modified macroscopic outline (bright).

image. An example can be seen in Fig. 3 (dark outline). From the dark outline it can be observed that the ultrasonic and macroscopic images are geometrically distorted relative to each other. The geometrical distortion is due to two fundamentally different sets of effects: The first is the cutting procedure, whereby the knife can displace (and in rare cases even twist) tissue segments resulting in a geometrical deformation. The second set is due to the ultrasound imaging method: The size of the point spread function is finite (finite width of main beam, side lobes and grating lobes), the propagation velocity is not constant (errors in re-mapping from time delay to distance as well as refraction). The latter set of effects are likely to make the ultrasound image a deformed and enlarged version of the tissue scanned. It is

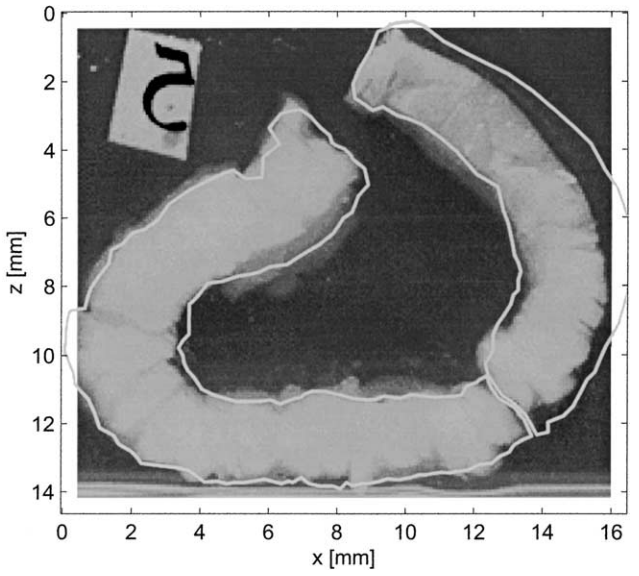

Fig. 4. Macroscopic image with the modified macroscopic outline.

furthermore noted that the histological images are geometrically distorted relative to the macroscopic images due to the histological preparation process, as can be seen from a comparison of Figs. 4 and 5, and thus cannot be used directly as reference images.

The information of the macroscopic and histologic images was "mapped back" into the format of the ultrasound images in the following way: The macroscopic outline superimposed on the ultrasound image (Fig. 3, dark outline) was modified to encompass areas defined approximately by the -10 dB demarcation in the ultrasound image (Fig. 3, bright outline) which covered what appeared to be the tissue region on the ultrasound image. This approach was chosen in order to resemble

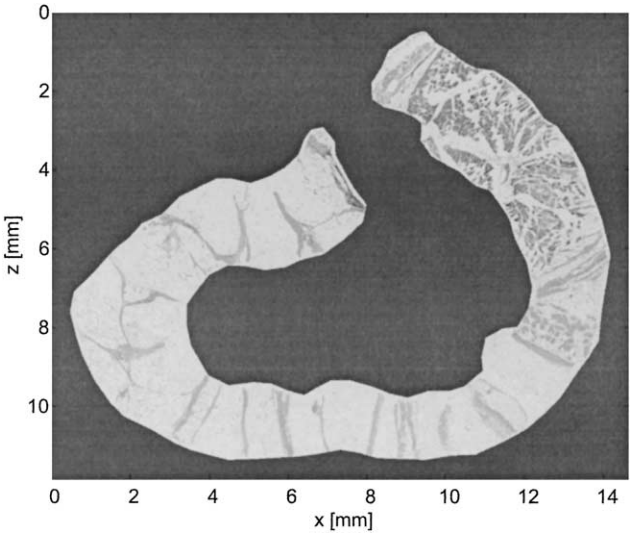

Fig. 5. Histology image corresponding to the macroscopic slice in Fig. 2.

as closely as possible the clinical situation, where plaque is identified and outlined on clinical images [7–9]. The modified macroscopic outline contains information from the macroscopic image about size and geometry and information from the ultrasound image about geometry and approximate size. Deformation effects due to e.g. the cutting procedure is then minimized in the final outline. The modified macroscopic outline was next applied on the macroscopic image as shown in Fig. 4 and used as guideline, when drawing the different areas indicating different tissue types. By combining the information of the histology image (Fig. 5) and the macroscopic image, the different tissue types, their location and the interfaces between these could be identified.

2.4. Evaluation of the reference image

The reference images were evaluated in two different ways: (1) A comparison of the area of the outline that encompasses the tissue region in the macroscopic image with the area of the outline modified to encompass the tissue region in the ultrasound image i.e. the two outlines in Fig. 3. (2) A calculation of the degree of overlap between the two above outlines. Specifically, if A_1 is the area of the macroscopic outline and A_2 is the area of the modified macroscopic outline and A_3 is the area of the joint region between the two, then the correspondence, γ, can then be calculated as follows:

$$\gamma = \frac{2A_3}{A_1 + A_2} \tag{1}$$

3. Results

A set of 12 reference images was created. Each different tissue type was assigned a specific number and encoded as different colors in the final reference image. An example is shown in Fig. 6. The areas of the macroscopic outlines and the modified macroscopic outlines are plotted in Fig. 7. As can be observed, with one exception, the area of the modified macroscopic outline is *consistently* larger than the macroscopic outline. The geometrical agreement between the ultrasound images and the macroscopic images, that are the basis for the final reference images is, $\gamma = 86.5 \pm 9.5\%$. An illustration of the overlap can be seen in Fig. 8.

4. Discussion

The fundamental problem of verifying this reference is that there does not exist a better one to compare against. Thus only the rationale behind the method and indirect measures can be used to assess the quality of the reference images.

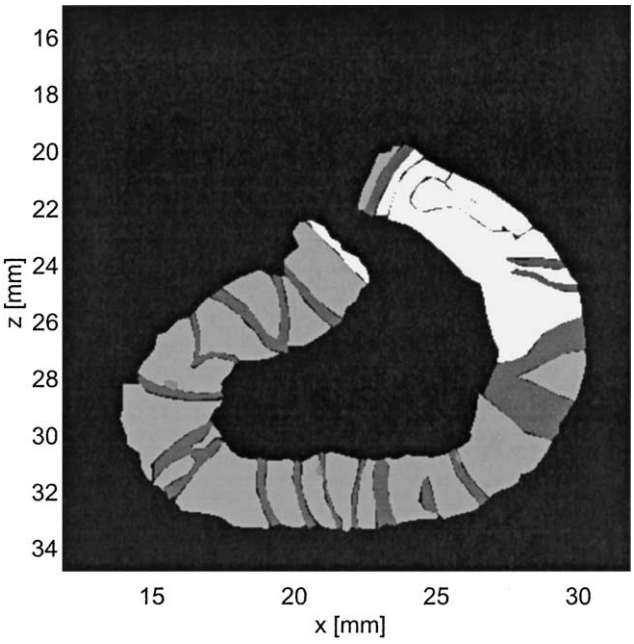

Fig. 6. Reference image with colors indicating tissue type. Dark gray = connective tissue, bright gray = lipids, white = muscle fibres.

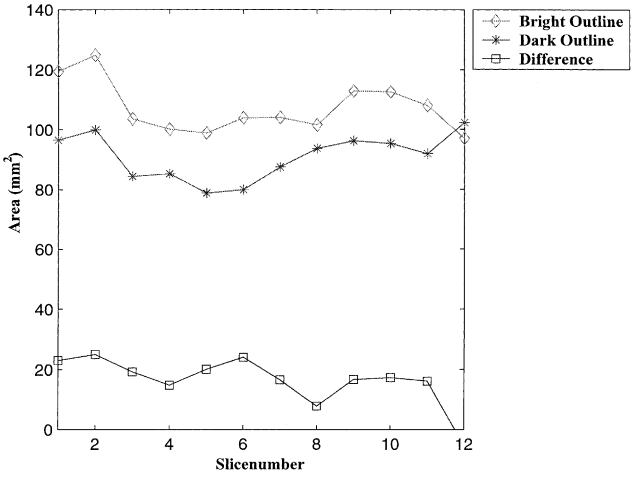

Fig. 7. The difference between the ultrasound and macroscopic areas in Fig. 3.

Two such measures have been investigated: The areas of the macroscopic outlines compared to the areas of the ultrasound outline indicates how much "larger" a given tissue area becomes on the ultrasound image, given the "clinical" outlining method used here. As can be observed from the results in Fig. 7, the area of the ultrasound outline is quite *consistently* 20 mm^2 larger than the macroscopic outline which corresponds to an approximate increase of 20%. The only exception is for slice 12, where the plaque has been squeezed during the cutting procedure and the lower part of this slice does not reflect the actual geometry. Thus this reference image should be discarded.

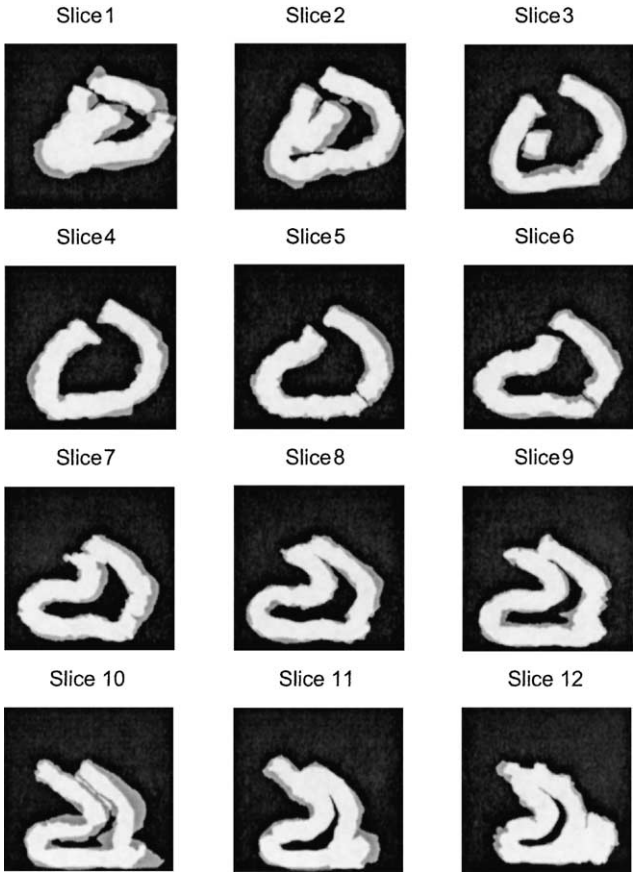

Fig. 8. Illustration of the overlap between the ultrasound data set and the macroscopic data set. White indicates an overlap and gray that only one of the data sets are present in this particular region.

The other measure indicates the degree of overlap between the ultrasound outline and the macroscopic outline. Because the ultrasonic outline is consistently larger than the macroscopic, this agreement is only about 86%. However, when observing the overlap in Fig. 8 it is clear that there is quite acceptable geometrical agreement between the geometrical shape of the ultrasonic outline and the macroscopic outlines.

With respect to identifying the outline on the ultrasound image an alternative method would be to modify (reshape) the macroscopic outline without changing the area. This would ensure that only the effect of displacement during the cutting procedure would be included. The degree of overlap would increase, but one would be faced with two problems: (1) some bright areas on the ultrasound image would have to be left out. To determine which could be a somewhat difficult task. (2) Even though a tissue region on an ultrasound image is a geometrically distorted (and enlarged) version of the corresponding tissue due to the imaging method, it might still contain relevant diagnostic information. As an example, an ultrasound image of a plaque contains valuable diagnostic information if it could reveal an atheromatous gruel encapsulated by a fibrous cap; whether this image is somewhat geometrically distorted relative to the actual tissue is less important (even though an assessment of the thickness of the cap would be the next goal). Thus this distortion should not necessarily be considered an error.

Another aspect of this method is how small tissue regions it is relevant to characterize. Plaque regions in specimens are seldom thicker than 4 mm measured radially in the intact artery [7]. The finite size of the ultrasound beam dictates the spatial resolution in the final ultrasound image and thus how small objects that can be detected. With the system used the point spread function has been measured to be 0.77 and 0.27 mm in the lateral and axis directions, respectively [5]. When tissue characterization is based on e.g. backscatterer, the speckle phenomena results in an unknown variation of the backscatterer level, requiring tissue regions larger than several speckle cells [11], in order to obtain a statistically stable estimate. Thus, part of the reference image with too small regions might need to be treated as one region, e.g. Fig. 6 for $x = [20; 24]$ mm and $y = [30; 32]$ mm where the dark and bright gray regions might have to be combined to a fourth material. Likewise for micro vessels, as these typically will be smaller than the point spread function.

An improvement to the method could be to fully automate the outline procedure and especially the modification part of this procedure. Instead of using the modified macroscopic outlines as guideline one could argue that the histology image should be used instead. However, since the effect of the histological staining is not exactly known, outlines from the histology images cannot be used directly. The staining might give rise to shrinking of the tissue due to the dehydration which is part of the histological analysis [12] and different tissue types might shrink different amounts. Furthermore tissue might be missing in cases where the surface from which the histological slices are cut are not completely smooth.

An alternative way to improve the presented method could contain these steps: The agar block is frozen and cut with a laser beam or water beam, so that no displacement takes place. Each slice should be spaced 0.5 mm or less and stained directly (e.g. Oil-red-O) and photographed. This would give a large series of images with very well-defined geometry and the histological images could then easier be used as reference images. Finally other imaging modalities, such as CT or MRI scanning prior to cutting, could be used to try to partly verify the reference.

Porcine muscular tissue has been used in this investigation which eventually aims at developing a method that could be applied to atherosclerotic plaque. The tissue types in an atherosclerotic plaque do to some degree resemble the tissue types in porcine muscular tissue. Both specimens contain a fibrous part, lipid rich regions and micro vessels. One major advantage of using

porcine muscular tissue is that the tissue regions are less complex. Tissue types were relatively easily detected in the porcine muscular tissue specimen whereas in atherosclerotic plaque some regions contain a mix of different tissue types. Furthermore the regions can be larger in porcine muscular tissue. Finally, porcine muscular tissue contains anisotropic tissue, which has also been shown to exist in plaque [10].

5. Conclusion

A method to create a reference image with the same geometry as the ultrasound image has been developed. The method is based on in vitro scannings of the tissue specimen and include manual outlining of the boundaries of the tissue. The reference images contain four different tissue types. Specifically, 12 reference images spaced 2.5 mm have been created. Based on geometrical considerations, it was found that 11 of these images can be used as a quantitative reference when comparing different ultrasound based tissue classification techniques.

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