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On Creating Reference Maps for Evaluation of Ultrasound Images of Carotid Plaque

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Abstract For the purpose of developing ultrasonic tissue classification methods, this work investigates a method to create reference maps of biological tissue. Specifically, formalin fixed carotid atherosclerotic plaque was molded into an agar block containing at set of fiducial markers. The block was scanned with spatial compound ultrasound at 7.5 MHz. Both tissue and fiducial markers were imaged. The block was afterwards sliced at the location of the fiducial markers. The slices were then photographed and analyzed histologically. From this data, reference maps with the same geometry as the ultrasound images were created. For each pixel in the ultrasound image, these reference maps indicate tissue type, such as collagen poor tissue, collagen rich tissue, etc. Because the histological method could not identify calcification with certainty, alternative methods (*e.g.* X-ray) had to be included to ensure proper identification of calcification.

Keywords Spatial compounding, histology, formalin fixation, tissue classification

1 Introduction

In most industrialized countries, the annual incidence of stroke is equal to approximately 0.2 % of the total population. In the European Union, this amounts to approximately 666 000 new incidences of stroke per year. Most strokes are believed caused by emboli released by atherosclerotic plaque located at the carotid bifurcation. Fortunately, these plaques can be removed by surgery and the procedure - carotid endarterectomy - has proven advantageous in reducing the risk of stroke.[4, 7]

Not all plaques are dangerous and recent research has indicated that composition in addition to size (*e.g.* degree of stenosis) is a predominant factor in the risk of a plaque causing stroke. Composition, however, is difficult to assess from ultrasound images. Several studies have found a statistical significant relation between relative volume of soft material (*e.g.* lipid and blood) contents of the plaque and the echogenicity of the plaque on ultrasound images *in vivo*.[2, 9] However, assessment of this for *individual patients* still seems premature and a number of reasons for this is given in [9]. Spatial compound imaging[3, 5, 6, 8] might be able to improve this, but this remains to be investigated.

To make the investigation of the potentials of spatial compounding more quantitative, this paper investigates a method to create reference maps revealing the tissue types throughout the ultrasound image. This map can then be used for automated quantitative evaluation of tissue classification techniques.

2 Materials and Methods

Ten atherosclerotic carotid plaques obtained at carotid endarterectomy were fixed in formalin. Each plaque (or slice of a plaque) underwent exactly the same procedure.

The plaque was placed - on a support made by sutures - in an acrylic molding frame. Forty-five degrees Celsius hot degassed liquid agar was slowly poured into the frame. The lid of the frame contained rows of rectangular openings that served as molds for a set of fiducial markers. These could both be recognized by ultrasound and later by the operator, who sliced the agar block. The fiducial markers were 1.5 mm wide and inter spaced 2.5 mm (both these measures are perpendicular to the scan planes).

2.1 Ultrasound scanning

The agar block with the plaque specimen was placed in a scanning tank with demineralized, degassed water and cross-sectional images inter-spaced 0.5 mm were recorded by used of the Ørsted·DTU experimental compound ultrasound scanner (the Xtra system[6]). The distance from the transducer sole to the center of the plaque was 20 mm. The scan planes covered the entire plaque and were located perpendicular to the long axis of the plaque (and the agar block). The number of planes ranged from 50 to 80.

2.2 Slicing of the agar block with plaque

In order to conduct both the slicing of the agar block with plaque and later the microtome slicing involved with the histological analysis, the plaque had to be decalcified. The agar block with plaque was therefore placed in 0.33 M EDTA under stirring for a period of 1-2 months.

Subsequently, the agar block was cooled to 5˚C and then placed in a special slicing frame, in which the block could be sliced from the end with a slice thickness of 2.5 mm, so that slicing planes were either through a fiducial marker or between two such markers. Care was taken not to compress or in other ways deform the agar and plaque. Nevertheless, for some slices, the tissue deformed slightly during cutting. Immediately after the knife had cut through the tissue, the agar slice was marked with slice number. Next, the face of the 2.5 mm tissue slice was photographed, as illustrated in Figure 1. An example of the resulting anatomical photograph is shown in Figure 2. A calibration cross was later placed at the same distance from the camera in order to allow metric calibration of the anatomical photographs. The plaque slice was subsequently analyzed histologically.

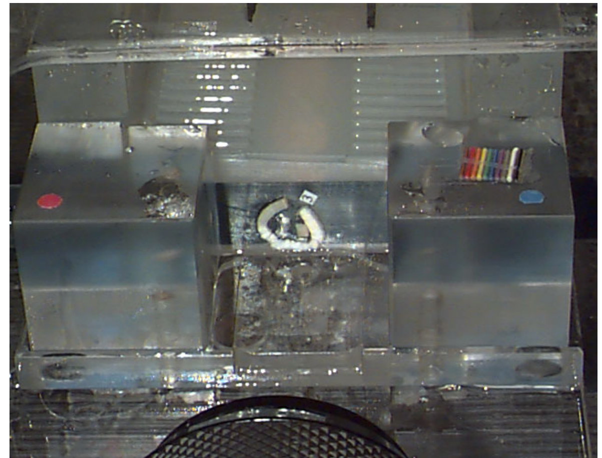

Figure 1 Model photo of an agar/tissue slice cut off from the end of the agar block, with the black knife still sitting behind the tissue slice. The lower part of the image shows the lens of the camera used to photograph the slice.

2.3 Histological analysis

The 2.5 mm thick tissue slices were processed for high-quality micro-examination (formalin-fixed par-affin sections). During this procedure all neutral lipids (free cholesterol, cholesterol esters, and triglycerides) were extracted. Five-micron thick paraffin sections were cut on a microtome (from the surface that was photographed as shown in Figure 1), sampled on glass slides, deparaffinized, stained with elastin-trichrome, mounted and coverslipped. Tissues shrink during dehydration and paraffin embedding; it remain shrunken during the subsequent procedures (degree of shrinkage was ~30% area reduction).[1]

Table 1 Overview of the tissue types identified histolog-ically in the plaques analyzed in this study. Note that material 7 does not exist in this study.

Tissue type
9. Presumed calcification
8. No histological info available
6. Media
5. Coll. poor wo cavity, w blood elem.
4. Collagen poor without cavity
3. Collagen poor with cavity
2. Collagen rich
1. thrombus (old and new)
0. Background

2.4 Tissue type identification

The deparaffinized tissue sections were stained with elastin-trichrome staining. Lipids were extracted during the histological processing and left behind unstained areas such as cavities, foam cells, and so-called cholesterol clefts.

Because the plaques were decalcified before processing, it was not possible to identify and quantify calcification by staining specifically for calcium. Nevertheless, the prior presence of calcification (in vivo) may be inferred by the presence of a characteristic connective tissue matrix in the processed tissue. As a consequence, the presence of calcification cannot always be identified.

The stained tissue sections were then analyzed visually by an experienced pathologist and the tissue types identified are shown in Table 1. Material 8 is used to denote tissue that could not be classified.

2.5 Creating the reference maps

The following method is explained by referring to Figures 2 to 5, which show the ultrasound image, the anatomical photograph and the histological image from exactly the same scan plane in the plaque.

Outlines of the tissue were drawn on the anatomically photographs, ensuring that only tissue in the cutting plane was included. An example is seen in Figure 2 (thick outline).

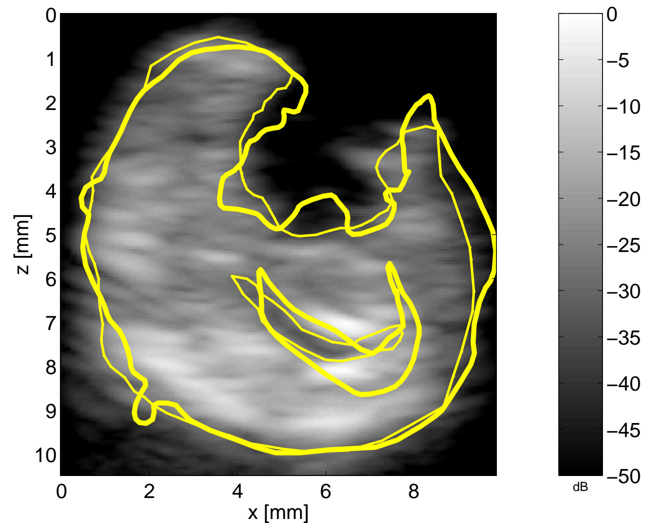

Figure 3 The multi-angle compound image for the same scan plane as in Figure 2. The thick and thin outlines corresponds to those in Figures 2 and 4, respectively.

The anatomical outline was then copied to the associated spatial compound ultrasound image and offset so as to match the image as well as possible, subjectively judged by visual inspection, as illustrated in Figure 3 (thick outline). Normally, this outline did not completely match the ultrasound image, mainly due to tissue displacement during slicing. The lack of geometrical match can be seen in the example in Figure 3 when the thick outline is compared to the contours of the ultrasound image.

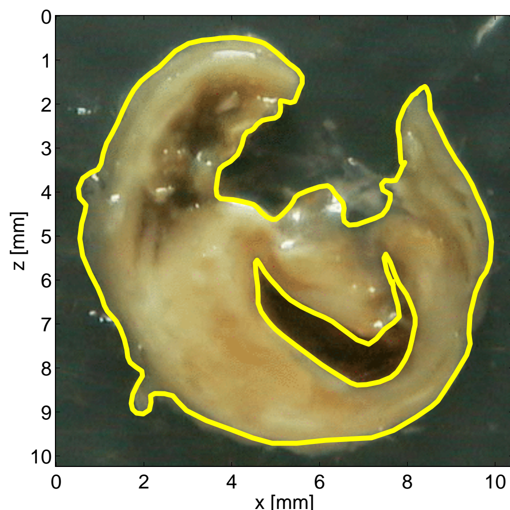

Figure 2 The anatomical photograph for a typical slice through a plaque.

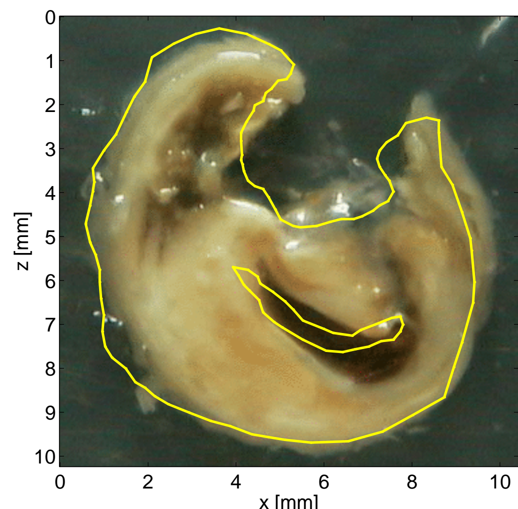

Figure 4 The anatomical photograph from Figure 2, with the ultrasound (thin) outline from Figure 3 superimposed.

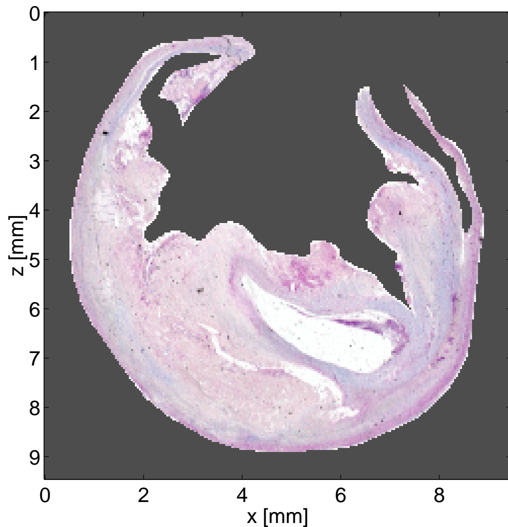

Figure 5 The histological image for the same scan plane as in Figure 4.

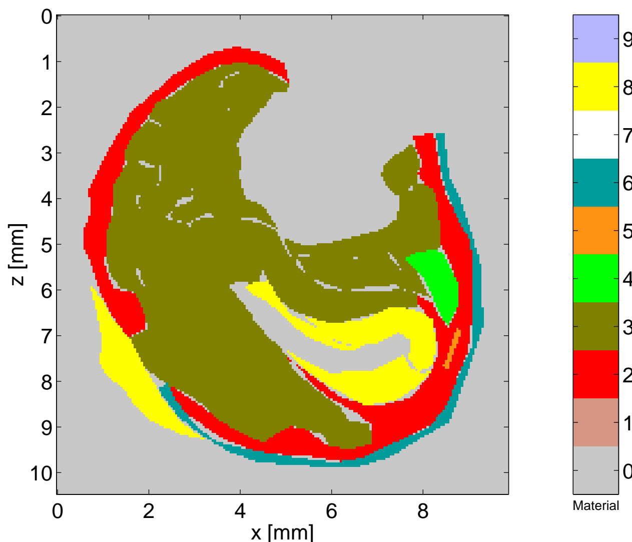

Figure 6 The reference map corresponding to the ultrasound image in Figure 3. The colors correspond to the definitions in Table 1. Note that material 7 is not used.

Then, when needed, segments of the (thick) outline were moved so that the entire outline matched the contours of the ultrasound image as well as possible. The position and shape of a segment of a thick outline was only adjusted *radically,* when it was obvious that the deviation was due to tissue displacements during slicing (as described in Subsection 2.2). Care was taken to keep constant the area of the entire outline. The resulting outline (thin outline) can be seen in Figure 3.

This modified (thin) outline was finally re-imposed on the anatomical photograph, as show in Figure 4. The reference map was then created by outlining the individual tissue types directly on the computer screen showing the content of Figure 4. Tissue types and location were identified based on both the anatomical photograph (Figure 4) and the histological image (Figure 5). The regions were placed taking account of the displacement of the thin outline in Figure 4 relative to the contour of the tissue. The final reference map corresponding to this example is seen in Figure 6.

Note that material 3 (collagen poor with cavity) in Figure 6 covers what appear to be different materials in Figure 4. In the latter figure, some of the tissue contains bleeding. However, material 3, "collagen poor with cavity", was not subdivided into tissues with and without blood components.

3 Results

Ten plaques were processed yielding a total of 123 slices each with associated ultrasound data, anatomical photograph, histological image and thereby reference map.

Figure 7 shows how well the anatomical photographs agree with the ultrasound images, for all plaques pooled together. Specifically, the figure shows the relative number of overlapping pixels for the outer outline of the reference map (Figure 6) and the outlines drawn on the anatomical photographs (Figure 2). This is the same as the relative overlap between the thin and thick outline in Figure 3. From Figure 7, 41 of the 123 slices agree more than 90%.

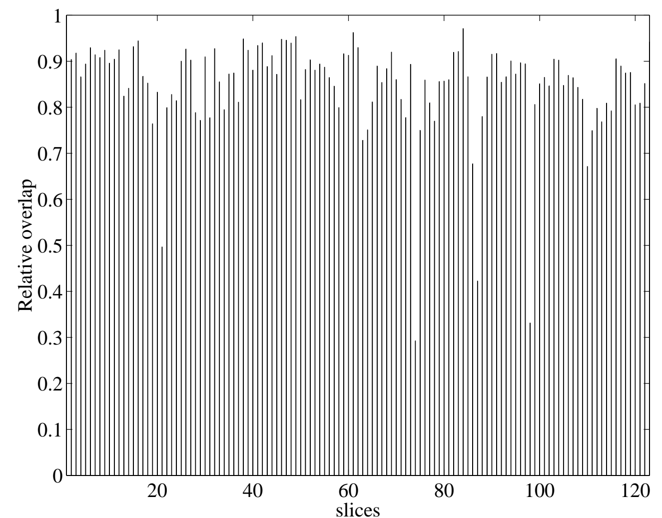

Figure 7 Geometrical agreement (relative overlap) between reference maps and anatomical photographs.

4 Discussions

The fiducial markers were clearly identifiable on the ultrasound image, thus the identification of the location of the ultrasound scan planes at fiducial markers and between fiducial markers, were associated with a maximum error of 0.25 mm. The precision of the slicing afterwards is more difficult to assess, but it is estimated that any part of the anatomical photograph plane is *maximally* 1 mm from the correct plane.

Figure 7 show an average agreement of 85% with just four scan planes exhibiting agreement below 50%. In general, the better the agreement, the more precise the cutting of the plaque. However, due to variations in speed of sound, there might be cases where the ultrasound image is geometrically distorted relative to the plaque.

Because the histological analysis cannot identify all calcification with a high degree of assurance, regions of calcification should be identified by other means (*e.g.* X-ray) and such regions excluded. Data from an X-ray investigation (not presented here) showed that about a third of the images had to be excluded due to calcification identified on X-ray but not on histology.

5 Conclusions

The results of the present study show that it is possible to create reference maps for formalin fixed plaque specimens, to which previously recorded ultrasound data can be compared quantitatively. The maps show the main constituents of the plaque, such as *e.g.* adipose tissue and fibrous tissue. However, as the histological analysis cannot guarantee identification of all calcification, scan planes with calcification must be identified by other means, *e.g.* X-ray.

These reference maps allow automated and quantitative comparison of different tissue classification techniques that can be applied to the recorded ultrasound data.

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