

A reference for ultrasound spatial compound images of carotid plaque

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1 Introduction

When evaluating new medical imaging methods on biological tissue, a reference is seldom available. This paper deals with making a quantitative reference based on human pathological tissue *in vitro*. The reference is made from a destructive analysis of the tissue, so all relevant ultrasound recordings must be done prior to creating the reference. The specific aim of this research is evaluation of different implementations of spatial compound imaging.^[4, 6, 7, 9]

In this particular study, the ultrasound data from scanning of the tissue was saved as raw rf signals, allowing for the application of a range of different processing methods on the recorded ultrasound signals.

2 Materials and Methods

The pathological tissue consisted of ten atherosclerotic carotid plaques fixed in formalin. The plaques were removed by endarterectomy.

A. Weight, volume and ultrasound scanning

First, the weight and the volume of the ten plaques were found by use of Archimede's principle. The plaques were next molded into agar blocks, which had rows of fiducial markers on the top of the block. These markers could both be recognized by ultrasound and later by the operator, who sliced the agar block. A photo of such a block is shown in Figure 1.

The ultrasound scanning were done by placing the agar block with the tissue specimen in a scanning tank with demineralized, degassed water. Cross-sectional images inter-spaced 0.5 mm were recorded by used of the Xtra system.^[7] The approximately 65 scan planes per plaque



Figure 1 Photo of agar block with atherosclerotic tissue embedded in the agar. The fiducial markers are seen as the two columns at left and right.

covered the entire tissue specimen and were situated perpendicular to the long axis of the plaque (and the agar block).

B. Slicing of the agar block with plaque

The tissue specimens involved in this study contained calcified tissue, which can be very hard to cut. Thus, for the purpose of performing the slicing of the agar block with plaque and later the microtome slicing involved

with the histological analysis, the plaque had to be decalcified. This was done by placing the agar block with plaque in 0.33 M EDTA under stirring for a period of 1-2 months.

After decalcification, the agar block was cooled to 5°C and then placed in a custom-made 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 to avoid compressing or in other ways deforming the agar and plaque. However, for some slices, the tissue deformed slightly during cutting. Immediately after the knife had cut through the tissue, the agar slice was marked with a paper label containing the slice number. Next, the face of the 2.5 mm tissue slice was photographed. An example of such an anatomical photograph is shown in Figure 2. A calibration device 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 by a standard histological process.

C. Identifying tissue types

The 2.5 mm thick tissue slices were analyzed histologically (formalin-fixed paraffin sections). During this process, tissue shrinkage typically takes place.^[1] The deparaffinized tissue sections were stained with elastin-trichrome staining. Because the plaques were decalcified before processing, it was not possible to always identify and quantify calcification.

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.

Table 1 Overview of the tissue types identified histologically in the plaques analyzed in this study. Note that material 7 is not represented here.

ID	Description
1	Thrombus (old and new)
2	Collagen rich
3	Collagen poor with cavity
4	Collagen poor without cavity
5	Collagen poor with blood elements, without cavity
6	Media
8	No histological information available
9	Presumed calcification

D. Creating the reference maps

The reference maps were obtained by the following method. Figures 2 to 4, show the ultrasound image, the anatomical photograph and the histological image from exactly the same scan plane in the plaque.

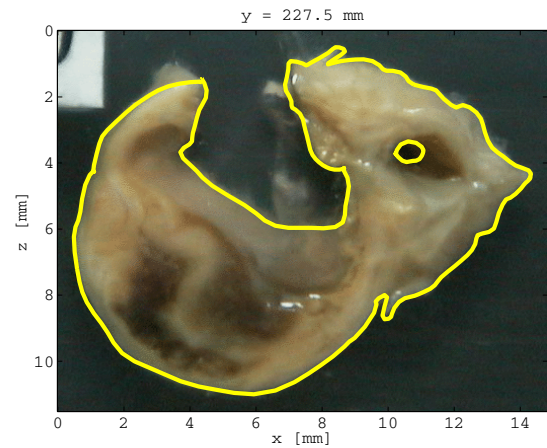


Figure 2 The anatomical photograph for a typical slice through one of the plaques.

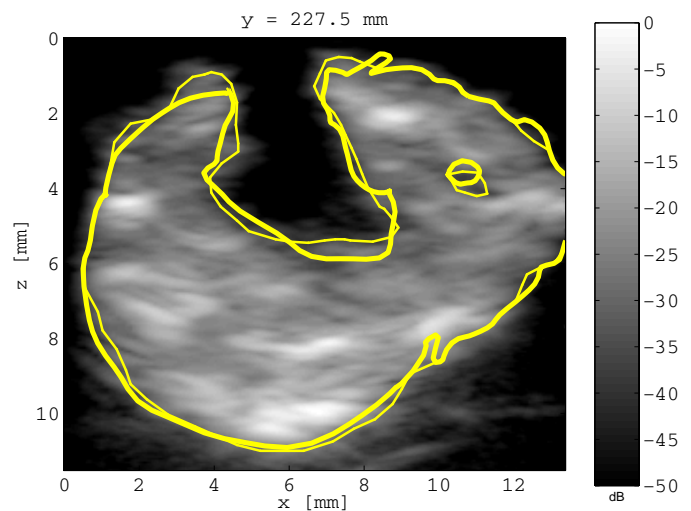


Figure 3 The multi-angle compound image for the same scan plane as in Figure 2. The thick outline corresponds to that in Figure 2.

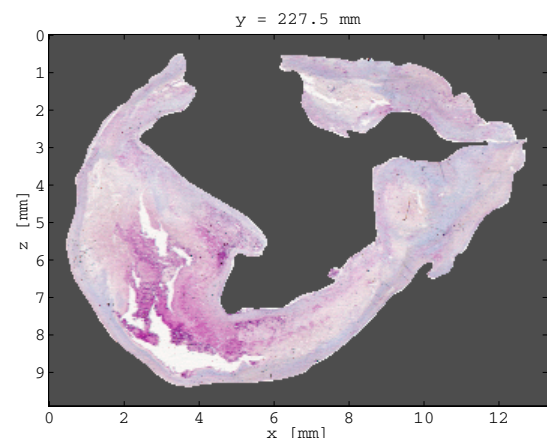


Figure 4 The histological image for the same scan plane as in Figure 2.

First, outlines of the tissue were drawn on the anatomical photographs, taking care that only tissue in the cutting plane was included. Figure 2 shows an example of this (thick outline).

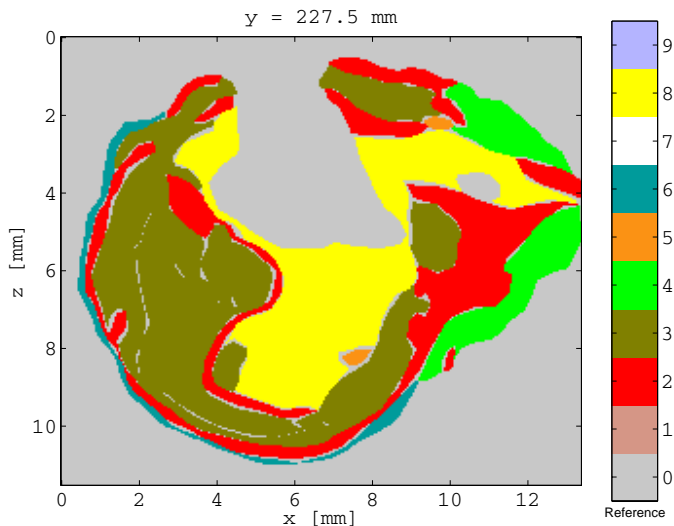


Figure 5 The reference map corresponding to the ultrasound image in Figure 3. The colors correspond to the definitions in Table 1. Note that material 7 is not used.

The anatomical outline was then copied to the associated 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). Often, this outline did not perfectly match the ultrasound image, mainly due to tissue displacement during slicing. This unavoidable 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.

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 location and shape of a given segment of a thick outline was only adjusted *drastically*, when it was very likely that the deviation was due to tissue displacements during slicing (as described above). During adjustment of the outline, care was taken to keep constant the area of the entire outline. The resulting outline (thin outline) can be seen in Figure 3.

The result of this, the modified (thin) outline, was finally re-imposed on the anatomical photograph. The reference map was then created by outlining the individual tissue types directly on the computer screen showing the content of the anatomical photograph with the thin outline superimposed. Tissue types and location were identified based on both the anatomical photograph (Figure 3) and the histological image (Figure 4). The regions were placed taking account of the displacement of the thin outline relative to the contour of the tissue. The reference map corresponding to this example is seen in Figure 5.

Note that material 8 ("No histological information available") in Figure 5 are present in regions where the lack of histological information resulted in inconclusive material identifications.

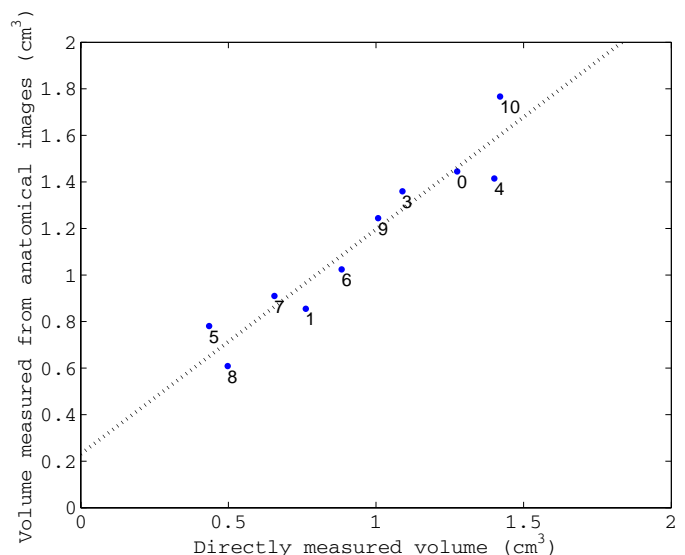


Figure 6 Volume measured with Archimede's principle versus volume derived from the outlines of tissue on the anatomical photographs. Slope of regression line: 0.97. $r = 0.95$.

3 Results

Ten plaques were processed giving a total of 123 slices. Each slice is represented by associated ultrasound data, anatomical photograph, histological image and thereby reference map.

The relative number of overlapping pixels for the outer outline of the reference map (Figure 5) and the outlines drawn on the anatomical photographs (Figure 2) varied between 30% and 97%. The average agreement was 85%.

In an attempt to verify the area of the anatomical outlines, Figure 6 compares the volume of the plaques obtained directly, $V(q)$, and the volume calculated based on the outlines of the tissue on the anatomical photograph. The latter is calculated from an estimate of the volume between the slice faces. As the slope of the regression line is close to unity, the volume of a plaque measured from the anatomical photographs is on average 0.23 cm^3 larger, than the volume measured directly.

4 Discussions

An average geometrical agreement of 85% for this kind of biological tissue - which sometimes can be very hard to slice - is considered satisfactory. Due to variations in speed of sound, there might be cases where the ultrasound image is geometrically distorted relative to the plaque. However, in most cases, the lack of geometrical agreement is due to displacement caused by the cutting.

Figure 6 shows a good correlation between the directly measured volume and the volume estimated from the anatomical outlines. The regression line features an off-set, however, which can be due to:

- If the tissue slices are not strictly parallel the area calculated from the outlines will be too high.
- Some tissue types shrink when fixed in formalin,^[10, 11, 12] even though the observations are not completely consistent.^[2] Differences might be due to tissue type and methodology. If the plaques underwent shrinkage due to the formalin fixation, it is plausible that this shrinkage was partially reversed by the permanent decrease in formalin concentration brought about when the plaques were encapsulated in agar.
- The plaques were molded into 45°C hot agar. This could have caused swelling, which was not reversed when the temperature subsequently fall to 21°C.
- The liquid agar could have entered small cracks in the surface of the plaque which are not found and outlined in the anatomical photograph.

One limitation of the present method is that the histological analysis cannot identify all calcification with a high degree of assurance. Therefore, regions of calcification should be identified by other means (*e.g.* X-ray based CT). Those regions found this way should then be merged with the histological data. Data from a planar 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

Based on ten formalin fixed atherosclerotic plaques, approximately seventy reference maps have been created. These can be quantitatively compared with associated ultrasound images recorded previously. The reference maps show tissue types such as *e.g.* adipose tissue and fibrous tissue. The limitations are mainly that most reference maps contains a small amount of unidentified tissues and that calcified tissue is difficult to identify with the present method. The latter can probably be solved by using CT.

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7 References

- [1] Baker JR: Principles of biological microtechnique. Methuen, London; pp. 75-86. 1958.
- [2] Dalager-Pedersen S, Falk E, Ringgaard S, Kristensen I and Pedersen E: Effects of Temperature and Histopa-

thologic Preparation on the Size and Morphology of Atherosclerotic Carotid Arteries as Imaged by MRI. Journal of magnetic resonance imaging 10: pp. 876-885. 1999.

[3] El-Barghouty NM, Levine T, Ladva S, Flanagan A, Nicolaides A: Histological verification of computerised carotid plaque characterisation. Eur. J. Vasc. Endovasc. Surg., Vol. 11, pp. 414-416, 1996.

[4] Entekin R, Jackson P, Jago JR, and Porter BA: Real time spatial compound imaging in breast ultrasound: technology and early clinical experience. Medicamundi, Vol. 43, Issue 3, pp. 35 - 43. September 1999.

[5] European Carotid Surgery Trialist' Collaborative Group (ECST). MRC European Carotid Surgery Trial: Interim results of symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis. The Lancet, Vol. 337, pp. 1235-1243, 1991.

[6] Hernandez A, Basset O, Chirossel P, Gimenez G: Spatial Compounding in Ultrasonic Imaging Using an Articulated Scan Arm. Ultrasound in Med. & Biol., Vol. 22, No. 2, pp. 229-238. 1996.

[7] Jespersen SK, Wilhjelm JE, Sillesen H: Multi-Angle Compound Imaging. Ultrasonic Imaging, Vol. 20, pp. 81-102, 1998.

[8] North American Symptomatic Carotid Endarterectomy Trial Collaborators (NASCET): Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. New Engl. J. of Med., Vol. 325, No. 7, pp. 445-453, 1991.

[9] Shattuck DP, Ramm OTv: Compound Scanning with a Phased Array. Ultrasonic Imaging, Vol. 4, pp. 93-107. 1982.

[10] Schned AR, Wheeler KJ, Hodorowski CA, Heaney JA, Ernstoff MS, Amdur RJ, Harris RD: Tissue-shrinkage correction factor in the calculation of prostate cancer volume. Am J Surg Pathol. 20(12). pp. 1501-6. 1996.

[11] Weind, KL, Ellis CG, and Boughner DR: Porcine aortic valve cusp dimensional changes with aldehyde fixation (Abstract). Can J Cardiol 16: 110F, 2000.

[12] Wisecarver JL: Allowing for shrinkage. (abstract) Am J. of Gastroenterology. Vol. 94, No. 7. p. 1743. 1999.

[13] Wilhjelm JE, Grønholdt M-LM, Wiebe B, Jespersen SK, Hansen LK and Sillesen H: Quantitative Analysis of Ultrasound B-mode Images of Carotid Atherosclerotic Plaque: Correlation with Visual Classification and Histological Examination. IEEE Transactions on Medical Imaging, Vol. 17. No. 6. pp 910 - 922. 1998.