Liver Fatty Change Classification Using 25MHz High Frequency Ultrasound

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Abstract - Liver fatty change (steatosis), the accumulation of fat within liver cells, is a common histological finding in liver biopsies. The histological changes of steatosis such as the distribution of fatty droplets cannot be resolved by conventional ultrasound. High frequency ultrasound (≥ 20 MHz), on the other hand, has the potential to reveal more detail of steatosis. In this study, B-mode images of high frequency ultrasound of 19 fresh human liver samples were obtained to evaluate ultrasound's ability in determining the steatosis grade. B-mode images were acquired by a mechanically controlled 25MHz single crystal probe. Image features derived from gray level concurrence and non-separable wavelet transform were extracted to classify steatosis grade using a classifier known as the support vector machine. Each liver sample subsequently underwent histological examination and liver steatosis was graded from 0 to 3 (0: no fatty change; 1: less than 33% of hepatocytes affected; 2: 33% to 66% of hepatocytes affected; and 3: more than 66% of hepatocytes affected). The four grades were then combined into 2 classes- grade 0 and grades 1-3 (non-steatosis and steatosis); 3 classes- grade 0, grade 1 and grades 2-3 (non-steatosis, mild steatosis, and prominent steatosis); and 4 classes (all different grades). Classification results were correlated with histology. It is shown that the best classification accuracy of 2, 3 and 4 classes were 90.5 %, 85.8 % and 82.6%, respectively. Compared with the results acquired from conventional ultrasound at 7 MHz (the best classification accuracy of 2, 3 and 4 classes were 81.6 %, 75.8 % and 74.2 %, respectively), the classification accuracy was clearly better at 25 MHz. Thus, liver steatosis can be more accurately characterized using high frequency B-mode ultrasound. Limitations and potential solutions of liver steatosis using high frequency ultrasound are also discussed.

Introduction

Liver fatty change (steatosis), the accumulation of fat within liver cells, is a common histological finding in liver biopsies. Steatosis in nonalcoholic patients is widely believed to be a benign condition with little or no risk of disease progression. However, marked steatosis may result in necroinflammation,

fibrosis and even cirrhosis [1]. B-mode ultrasound is usually recommended as a first step to evaluate the patient with mild elevated values of serum liver enzymes and it has been used for liver tissue classification. Steatosis change increases the echogenicity of liver relative to that of the kidneys and this characteristic has been adopted in clinical diagnosis. Steatosis can also be classified using image features. Mojsilovic et al. proposed a non-separable wavelet transform method to extract the B-mode image features for classification of healthy liver, cirrhosis and steatosis [2]. However, B-mode ultrasound at lower frequencies (≤ 10 MHz) generally does not have adequate resolution to reveal morphological changes of steatosis.

High frequency ultrasound (≥ 20 MHz) has superior resolution, thus allowing imaging finer structures of tissue. Nevertheless, high frequency ultrasound has not been widely applied to liver tissue characterization primary due to its limited penetration. The intra-vascular approach (through inferior vena cava to hepatic veins) provides a potential solution for the application of high frequency ultrasound to human liver. In this study, we evaluate the feasibility of tissue characterization of human liver using 25 MHz B-mode ultrasound *in Vitro*. Results of the feasibility study can be a foundation of intra-vascular high frequency ultrasound for liver disease classification.

Image Feature Extraction and Classification

In this study, the gray level concurrence (GLC) method and the non-separable wavelet transform (NSW) method were adopted for image features extraction. With these features, steatosis grades were classified using the support vector machine (SVM), a classification technique proposed by Cortes and Vapnik [3].

In the GLC method, a spatial gray-level dependence matrix (i.e. concurrence matrix) is first calculated. The matrix, $Co(i, j; d, \theta)$, represents the probability that the first pixel has a gray-level of i and the second pixel has a gray-level of j under the condition that the distance between two pixels is d in the direction of θ . For example, the following equation indicates the concurrence matrix at $\theta=0^\circ$. The concurrence matrix at other angles can be similarly defined [4, 5].

$$Co(i, j; d, 0^\circ) = \# \left\{ \begin{array}{l} ((k, l), (m, n)) \in (L_y \times L_x) \\ k = m, |l - n| = d \\ I(k, l) = i, I(m, n) = j \end{array} \right\} / T(d, 0^\circ) \quad (1)$$

where (k, l) and (m, n) are coordinates of two pixels in an image; the image has a size of $L_y \times L_x$, and I represents the intensity of a particular pixel (0 to 255). The symbol $\# \{ \cdot \}$ denotes the total number of occurrences and T is the total number of pixel pairs with the pre-specified d and θ . Figures 1a and 1b show the concurrence matrices ($d=6$, $\theta=0^\circ$) of 25MHz ultrasound images of a steatosis liver and a non-steatosis liver, respectively. Generally, the probability pattern of the concurrence matrix of a steatosis liver is more diversely distributed than that of a non-steatosis liver. Image features such as the angular second moment, contrast, correlation, entropy and sum entropy can be derived from the concurrence matrix [4, 5].

Fig. 1. B-mode sub-images of 25 MHz ultrasound with its corresponding concurrence matrix in a steatosis liver (grade 1) (a) and a non-steatosis liver (b).

In addition to GLC, NSW was also applied to extract the image features. The quincunx wavelet transform was applied [2, 5]. In this case, the original image was filtered by a 2-D diamond shaped high-pass filter and a low-pass filter of the same shape before it was down-sampled to a new image with half of the original area. Filtering and down-sampling were performed four times, thus producing high pass filtered sub-images H1-H4 and low pass filtered sub-images L1-L4. The variances of images H1-H4 and L4 were taken as the image features [2, 5]. Figure 2 compares the NSW output images of a steatosis liver (a) to those of a non-steatosis liver (b) (25 MHz ultrasound). In this particular case, the variances of the output

Fig. 2. Non-separable wavelet transform of a steatosis liver (grade 1) (a) and a non-steatosis liver (b). H1-4: images after high-pass filtering and down-sampling, L4: image after final low-pass filtering and down-sampling.

images (H1-H4 and L4) of a steatosis liver exceed those of a non-steatosis liver.

The features extracted by the above two methods were used by SVM for classification [5]. The SVM maps features of the training examples to a higher dimensional space and finds a hyper-plane to separate the two classes with the decision boundary set by the support vectors. The testing samples were then classified by the trained SVM. The three-class SVM classifier can be generated from a two-class classifier by further dividing one of the original classes into two classes. A classifier of higher classes can be produced similarly. A multi-class SVM classifier, developed by Junshui Ma (NIS-2, Los Alamos National Lab.) and Yi Zhao (EE department, Ohio State University), was used (<http://www.csie.ntu.edu.tw/~cjlin/libsvm>).

Image Acquisition and Processing

Nineteen fresh human liver samples obtained from surgical specimens were studied. These specimens were cut to bar shape and immersed in normal saline. After scanning by 25 MHz and 7 MHz ultrasound, these specimens received histological examination for steatosis grading.

A. High frequency ultrasound (25 MHz)

A lithium-niobate single crystal focused transducer (NIH Resource Center for Medical Ultrasonic Transducer Technology, Penn State University) was used. The transducer's center frequency is 44 MHz (−6 dB bandwidth: 34-59 MHz). A block diagram of the experimental setup is shown in Fig. 3. To extend the depth of focus of the fixed focus transducer, two images were acquired and combined (also known as depth scan) [6]. The two images corresponded to two different transducer positions which were 2 mm apart in the axial direction. For 25 MHz imaging, 3 cycles of a sine wave pulse with a center frequency of 25 MHz was transmitted. The spacing between two adjacent scan lines was 20 μ m. All images here are displayed with a 36-dB dynamic range.

Fig.3 A block diagram of the setup of 25MHz ultrasound.

B. Conventional ultrasound (7 MHz)

After the 25 MHz data was acquired, the same specimen was also scanned using a 7 MHz linear array transducer (10L, GE LOGIQ 500, Chalfont St. Giles, UK) in a water tank. The images were then acquired using a frame grabber (UPMOST UPG401B, Taipei, Taiwan) with 256 gray levels and 320 x 240 pixels resolution.

C. Image processing

A sub-image of 64 x 64 pixels (11.0 mm x 11.0 mm and 2.8 mm x 2.8 mm equivalent to $51.3 \lambda \times 51.3 \lambda$ and $46.7 \lambda \times 46.7 \lambda$ for 7MHz and 25 MHz, respectively, where λ is the ultrasound wave length) was selected from each image. Five to ten sub-images in each frequency of each specimen were selected; the cross-correlation coefficients among the images were all below 0.5. Image features were extracted using GLC and NSW from these sub-images.

After ultrasound scanning, the specimens were fixed in formalin and embedded in paraffin. Several sections with a thickness of 5 μ m were cut from each specimen and were stained with Masson's Trichrome stain for histological examination. Slices of all specimens were photographed to histological pictures (i.e., low-power magnifying) by a digital camera (Sony S85, Tokyo, Japan). The steatosis grade was classified by an experienced pathologist according the following criteria [1]: 0: no fatty change; 1: less than 33% of hepatocytes affected; 2: 33% to 66% of hepatocytes affected; and 3: more than 66% of hepatocytes affected. A semi-automatic method to calculate the percentage of affected hepatocytes was also adopted. Microscopic pictures (i.e. high-power magnifying) of the liver slices were photographed by a digital camera (Nikon Coolpix 4500, Tokyo, Japan) mounted on a microscope (Teng-Bo Roomkop XTL 300A, Taipei, Taiwan). The digitized images were then sent to a personal computer and were processed using a commercial image processing software (Adobe Photoshop, 4.0.1 TC, San Jose, CA).

Fig. 4 (a) A microscopic picture of a steatosis liver (grade 2) with Masson's Trichrome stain, image size is 2.1 mm x 1.6 mm. (b) The steatosis affected hepatocytes (black arrow in (a)) are marked in white, unaffected hepatocytes are in gray and the other tissues (white arrow in (a)) are in black.

The hepatocytes affected by steatosis (the black arrow in Fig. 4a) were marked by white color as shown in Fig. 4b, the unaffected hepatocytes were marked by gray color and the other tissues (the white arrow in Fig. 4a) were marked by black color in Fig. 4b. The percentage of steatosis can be calculated as the sum of affected hepatocytes (number of pixels of the white areas) divided by the sum of total hepatocytes (number of pixels of the white and the gray areas). Table 1 lists the mean steatosis percentage (counted from 5 images in each specimen) and the grading of the 10 steatosis specimens. It was further verified that the grading results were the same as

Table 1: Mean percentage of steatosis and grading of 10 specimens of steatosis liver

Specimen No.	1	2	3	4	5	6	7	8	9	10
Mean percentage	9.5	49.2	12.6	14.5	34.4	75.9	4.3	17.8	2.2	18.0
Steatosis grade	1	2	1	1	2	3	1	1	1	1

those done by the pathologist. The all four grades of steatosis were then combined into 2, 3 and 4 classes according to the following criteria: 2 classes- grade 0 and grades 1-3 (representing non-steatosis and steatosis); 3 classes- grade 0, grade 1 and grades 2-3 (representing non-steatosis, mild and obvious steatosis); and 4 classes (all different grades). The classification accuracy was tested using the support vector machine by the leave-one-out method. After testing, the classification results were recorded. The process was repeated until all images had been tested. The accuracy was then determined as

$$\text{Accuracy} = \frac{CI}{N} \quad (2)$$

where CI is the number of correct incidences in a particular class and N is the total number of samples in that class. Various combinations of input features (angles and distances of GLC; filter pairs of NSW) and the SVM parameters (penalty term and kernel function parameters) were tested to find the best performance achievable.

Results

Among the nineteen specimens, nine specimens were non-steatosis and ten specimens was steatosis. The combination of all features of the GLC ($d=6$, $\theta=0^\circ$) and all feature of the NSW yielded the best results among all the different combinations. For 25 MHz ultrasound images, the best accuracy of 2, 3 and 4 classes were 90.5 %, 85.5 % and 82.6%, respectively. For conventional ultrasound images at 7 MHz, the best classification accuracy of 2, 3 and 4 classes were 81.6 %, 75.8 % and 74.2 %, respectively. Compared with the results acquired from conventional ultrasound, the classification accuracy was markedly better with high frequency ultrasound. The comparison was graphically shown in Fig. 5.

Figure 6 compares the images between 25 and 7 MHz. The hepatocytes affected by steatosis in the microscopic picture (arrow in Fig. 6a) are shown as bright ovoid or strip shaped structures in the histological picture (arrow in

Fig. 5 Comparison of the steatosis classification results between the 25 MHz and 7 MHz.

Fig. 6 (a) A microscopic picture (i.e., high-power magnifying) of a steatosis liver (grade 2) with Masson's Trichrome stain, image size is 2.1 mm x 1.6 mm. (b) A histological picture (i.e., low-power magnifying) with Masson's Trichrome stain, image size is 19.6 mm x 16.0 mm. (c) The corresponding 25MHz ultrasound image, image size is 18.6 mm x 11.8 mm. (d) The corresponding 7 MHz ultrasound image, image size is 20.0 mm x 15.0 mm.

Fig. 6b). Furthermore, the pattern of steatosis distribution in Fig. 6b is more clearly demonstrated in the 25 MHz ultrasound image (arrow in Fig. 6c) than the 7 MHz ultrasound image (arrow in Fig. 6d). The higher spatial resolution at 25 MHz is believed to play a critical role in the resultant higher accuracy.

Discussion and Conclusions

The 25 MHz images had better accuracy for steatosis classification. Comparing the histological pictures of steatosis livers with the 25 MHz ultrasound images, some similarities were found. Figure 7a shows that the steatosis-affected hepatocytes (grade 1) locate mainly in the pericentral area of acinus and the clusters of affected hepatocytes appear as an ovoid shape (arrow). The 25 MHz ultrasound image shows a similar pattern to the clusters of affected hepatocytes shown in Fig. 7a (arrow in Fig. 7b). Figure 7c shows that the steatosis-affected hepatocytes (grade 1) in another liver locate mainly in the periportal area (arrow). Again, the 25 MHz B-mode image in Fig. 7d presents a similar pattern (arrow). Also, comparing the 25 MHz liver images of mild steatosis with severe steatosis, the brightened lesions occupy larger areas with higher grade of steatosis (Fig. 6c).

High frequency ultrasound has good spatial

Fig. 7 (a) and (c) show the histological pictures of 2 steatosis liver (grade 1) with Masson's Trichrome stain, image size is 21.0 mm x 16.0 mm; (b) and (d) The corresponding 25MHz ultrasound images, image size is 18.6 mm x 11.8 mm.

resolution and reveals more details of steatosis than conventional ultrasound at lower frequencies, but the shorter penetration depth and the smaller focal zone limit its clinical applications. Intra-abdominal organs are hard to be accessed by the high frequency ultrasound because high frequency sound waves cannot penetrate through the abdominal wall. The intra-vascular approach may be a potential solution. In addition to the limited penetration, the small depth of focus of the single crystal probe creates another limitation. For example, fibrosis patterns may not be adequately demonstrated using high frequency ultrasound. The degree of fibrosis ranges from fibrous expansion in the portal area to cirrhosis. The regenerative nodules of a cirrhotic liver are enclosed by fibrotic tissues and existence of regenerative nodules is one of the key features for pathological diagnosis of cirrhosis. The diameter of regenerative nodules is generally larger than 3 mm in patients with diseases such as late phase of hepatitis B cirrhosis and autoimmune cirrhosis, and primary biliary cirrhosis. Although in our previous study, we had successfully classified the fibrosis grade by SVM using 7 MHz ultrasound [5]. However, the increased spatial resolution of 25 MHz may not improve the classification of fibrosis due to the limited penetration and the small depth of focus.

In conclusion, the 25 MHz ultrasound images had better accuracy for steatosis classification. But the limitations of poor penetration depth and small focal zone need to be overcome for further clinical applications.

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