Ultrasonic Thickness Evaluation of Seminiferous Tubule by Fuzzy Inference

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Abstract—**This paper describes a seminiferous tubules evaluation using an ultrasonic probe. In this system, we evaluate a diameter of seminiferous tubules for azoospermia patients. We employ a 5.0MHz ultrasonic single probe. In the experiment, we employ large and small nylon lines as the healthy and unhealthy seminiferous tubules. We made ball shape phantom from small and large lines in total 24. We acquire the waveforms by the ultrasonic probe and calculate amplitude values from the data that band pass filters applied. We then calculate cumulative relative frequency of amplitude values. Fuzzy if-then rules are made for the cumulative relative frequency of large and small lines. We evaluate a rate of large lines among all lines by using the fuzzy MIN-MAX center-of-gravity method. In the result, the mean absolute error was 5.98 %. The correlation coefficient was 0.98. The proposed method thus successfully evaluated the rate of the large lines.**

Keywords- medical system; ultrasonic; azoospermia; seminiferous tubule; fuzzy logic

I. INTRODUCTION

Azoospermia is one of the causes of male infertility. Azoospermia is the symptom that there is a complete absence of sperm in the ejaculate. Fifteen-20% of infertile men turn out to be azoospermia. Azoospermia is caused by two problems; a production problem which is called non-obstructive azoospermia (NOA) and a deliver problem which is called obstructive azoospermia (OA). The development of intracytoplasmic sperm injection (ICSI) opened a new era in the field of assisted reproduction and revolutionized the assisted reproductive technique protocols of couples with male factor infertility [1]. Fertilization and pregnancies can be obtained with spermatozoa recovered not only from the ejaculate, but seminiferous tubules.

The ideal technique of sperm extraction from testicle for NOA would be minimally invasive and avoid destruction of testicular function without compromising the chance of retrieval adequate numbers of spermatozoa to perform ISCI. Schlegel et al. have successfully developed the techniques with the assistance of an operating microscope as Micro-TESE [2]. TESE is a method of remove the seminiferous tubules in the testicle, and looking for the sperm in the seminiferous tubules. The possibility to including the sperm of larger seminiferous tubules is bigger than sclerotic seminiferous tubules. Some azoospermia patients have normal seminiferous tubules (Diameter: 0.250mm~0.300mm) and sclerotic seminiferous tubules (Diameter: 0.150mm). But there are the patients whose all tubules are small (Diameter: <0.150mm). The sperm cannot be collected if there are no large tubules in the testicle [3], [4]. Whether the sperm is able to be collected or impossibility is not understood if not operating. Additionally, the operation is costly and physically large burden for patients. Therefore, we need a system that noninvasively measures the thickness of the tubule testicular in the testicle before micro-TESE procedure. [5], [6].

Ultrasonic is known as a kind of the medical device for diagnosing human body. By using ultrasonic devices, several methods have been proposed to diagnose non-invasively the inside of the abdomen and blood [7]. Moreover, the ultrasonic quantitatively diagnoses inside of body with low cost and short time.

In our previous study [8], we proposed a method to evaluate a rate of large line of a phantom by a 1.0MHz ultrasonic array probe. The phantom lined up large and small lines in total 24 in parallelisms. The previous method evaluates the rate by only using amplitude value of ultrasonic wave forms. Because, a space resolution of the 1.0MHz probes is inadequacy for the small line, the 1.0MHz ultrasonic was not able to receive waves from small lines. To improve space resolution, we use higher frequency ultrasonic probe. In addition, this study evaluates the rate of large line by amplitude value and frequency analysis.

In this study, we propose a seminiferous tubule evaluation system with an ultrasonic single probe i.e., low cost device. We can non-invasively evaluate it by fuzzy inference evaluation procedure. We employ two different diameter nylon fishing lines as a small tubule and large tubule. We calculate the cumulative relative frequency of amplitude of acquisition data that passed two types of band pass filters. From the difference of frequency spectrum, we derive fuzzy IF-THEN rules and make a fuzzy MIN-MAX center-of-gravity method. The rate of large line was evaluated by the fuzzy MIN-MAX center of gravity method. As the result, the evaluated rate of large line increases according to the increment of the number of large line. Then the correlation coefficient was 0.98. This experimental result suggested the high possibility to determine the larger diameter testicular tubule under the assumption that the characteristics of human testicular tubule are similar to that of the fishing line.

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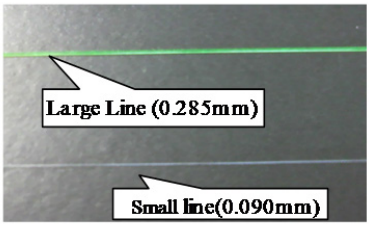


Figure 1. Large fishing nylon line and small fishing nylon line.


Figure 2. Phantom that put in lines and 15cm^3 water.

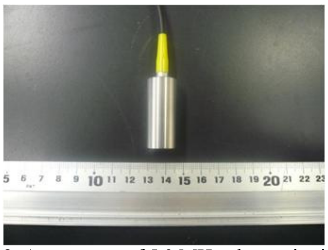


Figure 3. Appearance of 5.0 MHz ultrasonic single probe.

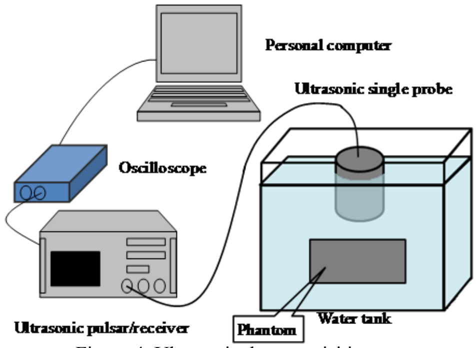


Figure 4. Ultrasonic data acquisition system.

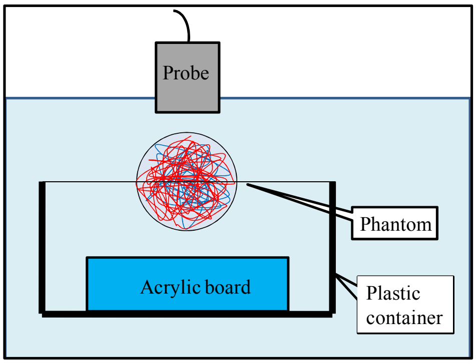


Figure 5. Acquisition method.

II. PRELIMINARIES

A. Phantom

In our experiment, we employ two different diameter nylon fishing lines as shown in Fig. 1. We employ these lines instead of seminiferous tubules. The diameter of small line is 0.090 mm and that of large line is 0.285 mm. Here, it is noted that diameter of healthy seminiferous tubule ranged from 0.25 to 0.30 mm and that of unhealthy tubule is smaller than 0.15 mm. Fig. 2 shows an appearance of these phantoms. It is put in 24 rolled lines (25cm) and 15cm^3 water (NOA patient's testicular capacity) in the rubber tube.

B. Ultrasonic Data Acquisition System

Fig. 3 shows an ultrasonic single probe. In this study, we employ 5.0MHz ultrasonic probe.

An ultrasonic data acquisition system is shown in Fig. 4. Fig. 5 shows an example of data acquisition situation. Sampling interval of the system is 20 ns. The ultrasonic waveform data are provided to a personal computer through the oscilloscope.

III. EVALUATION OF RATE OF LARGE LINE

Our method consists of six steps. Firstly, we acquire the waveforms by the ultrasonic probe. We extract echo of the nylon lines. Secondly, we apply band pass filters to the data. Thirdly, we calculate amplitude values from the data. Fourthly, we calculate cumulative relative frequency of amplitude values. Fifthly, we made fuzzy-if then rules to the cumulative relative frequencies of the particular amplitude. Finally, we calculate the rate of large line among all lines by the fuzzy MIN-MAX center-of-gravity method.

A. Frequency Analysis

The frequency of vibrating lines is determined by (1).

$$f = \frac{1}{2l}\sqrt{\frac{T}{\sigma}} \quad (1)$$

Here, the notation f denotes frequency, l denotes length of line, T denotes tension of line, and σ denotes linear density. In this research, assume that l and T are constant. Relation (2) means (1).

$$f \propto \frac{1}{\sqrt{\sigma}} \quad (2)$$

The density σ is represented with a diameter, Φ, of line, as shown in (3).

$$\sigma = \pi\frac{\phi^2}{4}M \quad (3)$$

Here, the notation M denotes degree of density. In this research we employ nylon line instead of seminiferous tubule for all phantoms. Therefore, M can be assumed as constant for all lines. By the same reason, M of seminiferous tubules are assumed as constant, Thus, relation (4) can be derived from (3).

$$\sigma \propto \phi^2 \quad (4)$$

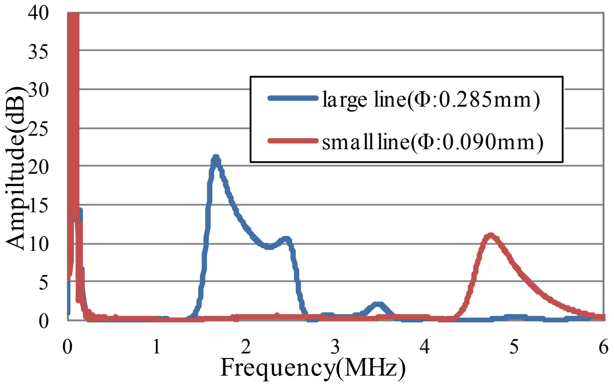


Figure 6. Frequency spectrum.

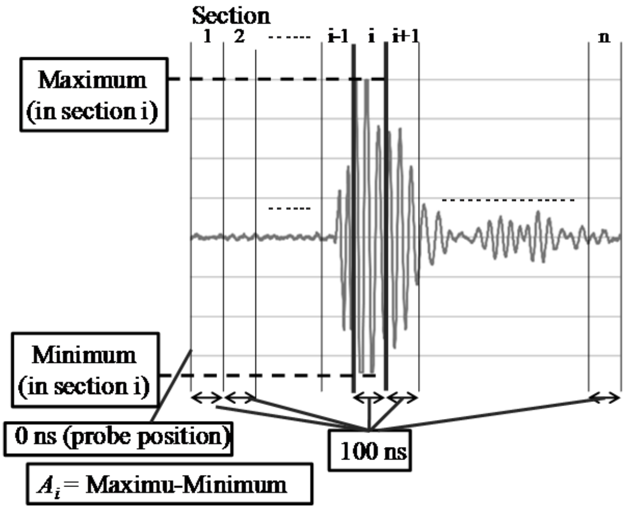


Figure 7. Calculation of Amplitude.

From (2) and (4), frequency of a vibrating line depends on diameter of line (5).

$$f \propto \frac{1}{\phi} \tag{5}$$

From this relation, we identify large line from small line by difference of the vibration frequency. Therefore, we examine frequency spectrum of large line and small line. Fig. 6 shows the result. As shown in Fig. 6, frequency of the large line is smaller than that of the small line. Therefore we use 1.5-2.5MHz band pass filters for large line, and use 4.5-5.5MHz band pass filter for small line to classify them.

B. Cumulative Relative Frequency of Amplitude

We use the difference of the distribution of the frequency spectrum for evaluating the rate of large line. We employ the cumulative relative frequency of amplitude of data obtained by applying the band pass filter to compare the rate of the big amplitudes.

We extracted the waves of the interval, 100 ns, as shown in Fig. 7. We then calculated peak to peak value in amplitude for the interval. In Fig. 7, the notation A denotes the peak to peak value, i.e., Maximum - Minimum. We calculated cumulative relative frequency from relative frequency from a (V) to

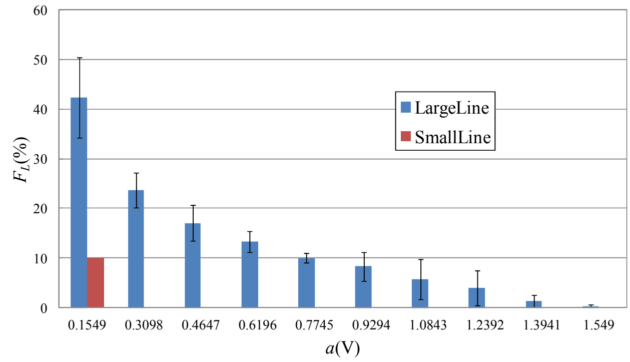


Figure 8. Average of F_L.

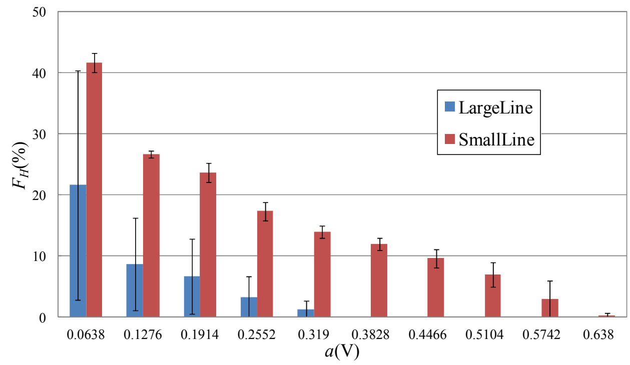


Figure 9. Average of F_H.

maximum amplitude MA. The maximum amplitude, MA_L obtained by applying 1.5-2.5MHz (=L) band pass filter was 1.549. The maximum amplitude, MA_H, obtained by applying 4.5-5.5MHz (=H) band pass filter was 0.638.The cumulative relative frequency was calculated by (6).

$$F_X(a) = \frac{\sum_{i=1}^{n} x_i}{n}, \quad x = \begin{cases} 1 & if\ a < A_i \le MA_X \\ 0 & otherwise \end{cases}, \quad for\ every\ i \tag{6}$$

Here, the notation $F_X(a)$ denotes cumulative relative frequency of amplitude of a. The notation X does the label of band pass filter. (X=L:1.5-2.5MHz, H:4.5-5.5MHz). Fig. 8 shows the average of $F_L(a)$ of learning data, on 24 large lines 16 times. Fig. 9 shows the average of $F_H(a)$ of learning data, on 24 small lines 16 times. As shown in these figures, $F_L(a)$ of large line is larger than that of small line, and $F_H(a)$ of small line is larger than that of large line.

C. Evaluation of Rate of Large Line

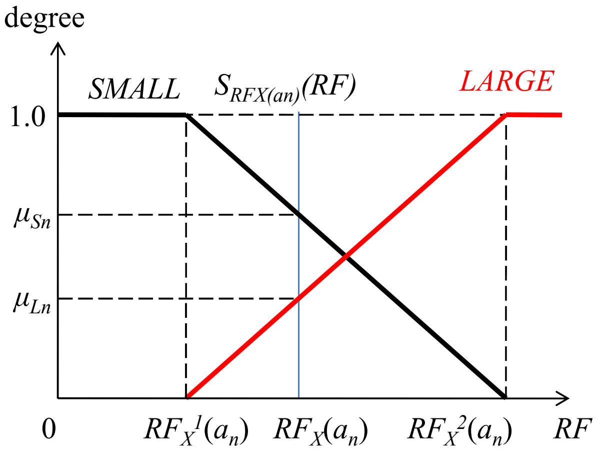
We evaluate a rate of large line in mixed lines. $RF_L(a)$ and $RF_H(a)$ is defined as the ratio of the cumulative relative frequency of low frequency domain and high frequency domain, respectively. These are calculated by (7) and (8).

$$RF_L(a) = \frac{F_L(a)}{F_L(a) + F_H(a)} \tag{7}$$

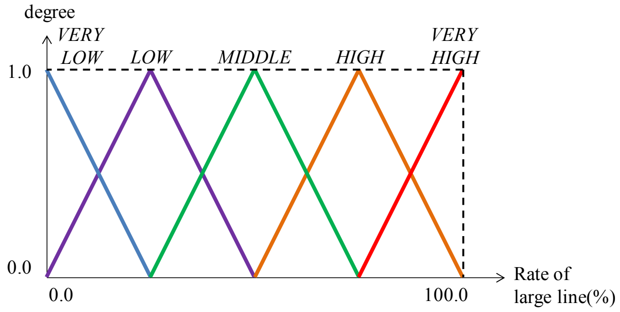
$$RF_H(a) = \frac{F_H(a)}{F_L(a) + F_H(a)} \tag{8}$$

TABLE I. Fuzzy IF-THEN Rules.

INPUT				OUTPUT
RF_L(a_1)	***RF_L(a_2)***	***RF_H(a_3)***	***RF_H(a_4)***	***Rate of large line***
SMALL	*SMALL*	*SMALL*	*SMALL*	*MIDDLE*
SMALL	*SMALL*	*SMALL*	*LARGE*	*LOW*
SMALL	*SMALL*	*LARGE*	*SMALL*	*LOW*
SMALL	*SMALL*	*LARGE*	*LARGE*	*VERY LOW*
SMALL	*LARGE*	*SMALL*	*SMALL*	*HIGH*
SMALL	*LARGE*	*SMALL*	*LARGE*	*MIDDLE*
SMALL	*LARGE*	*LARGE*	*SMALL*	*MIDDLE*
SMALL	*LARGE*	*LARGE*	*LARGE*	*LOW*
LARGE	*SMALL*	*SMALL*	*SMALL*	*HIGH*
LARGE	*SMALL*	*SMALL*	*LARGE*	*MIDDLE*
LARGE	*SMALL*	*LARGE*	*SMALL*	*MIDDLE*
LARGE	*SMALL*	*LARGE*	*LARGE*	*LOW*
LARGE	*LARGE*	*SMALL*	*SMALL*	*VERY HIGH*
LARGE	*LARGE*	*SMALL*	*LARGE*	*HIGH*
LARGE	*LARGE*	*LARGE*	*SMALL*	*HIGH*
LARGE	*LARGE*	*LARGE*	*LARGE*	*MIDDLE*



(a) Input membership functions.



(b) Output membership functions.

Figure 10. Fuzzy membership functions.

From the learning data, we select most suitable $RF_L(a)$ and $RF_H(a)$ to evaluate large line rate from the calculated absolute correlation coefficients between true rate of large line and $RF_L(a)$ or $RF_H(a)$ for all a. We denote the selected $RF_L(a)$ and $RF_H(a)$ as $a_1 \sim a_4$, as shown in Table II.

We evaluate a rate of large line in mixed lines using fuzzy inference. From Fig. 8 and Fig. 9, we obtain the following knowledge.

Knowledge 1: $RF_L(a_n)$ of large line is larger than that of small lines at same amplitude value.

TABLE II. Values of $RF_X^1(a)$ and $RF_X^2(a)$ in Figure 10(a).

	X	***a_n*(V)**	**$RF_X^1(a_n)$**	**$RF_X^2(a_n)$**
a_1	L	1.519	0.526	0.752
a_2	L	1.457	0.441	0.729
a_3	H	0.625	0.244	0.472
a_4	H	0.612	0.274	0.497

TABLE III. Experimental results.

Object		**Large line rate(%)**			
N_S	N_L	***True value***	***Proposed method***	***Previous method***	***MRA***
24	0	0.00	2.77	1.77	-3.58
20	4	16.67	26.76	27.65	73.67
16	8	33.33	35.85	49.98	28.64
12	12	50.00	49.74	53.83	45.63
8	16	66.67	70.17	64.55	71.94
4	20	83.33	79.04	79.01	77.88
0	24	100.00	81.58	96.57	108.32
Error(%)		-	5.98	8.62	12.67
Correlation Coefficient		-	0.98	0.92	0.81

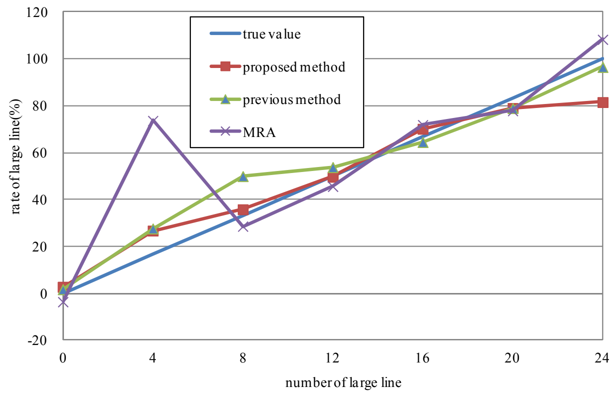


Figure 11. Result of large line rate.

Knowledge 2: $RF_H(a_n)$ of small line is larger than that of large lines at same amplitude value.

These knowledge are converted into sixteen fuzzy IF-THEN rules. The input and output fuzzy linguistic variables of fuzzy IF-THEN rules are shown in Table I. In it, "*VERY LOW*", "*LOW*", "*MIDDLE*", "*HIGH*" "*VERY HIGH*", "*SMALL*" and "*LARGE*" are fuzzy linguistic values, and these are defined by fuzzy membership functions as shown in Fig. 10. The notations $RF_L^1(a_n)$ and $RF_L^2(a_n)$ denote $F(a_n)$ of 24 small lines and $F(a_n)$ of 24 large lines, respectively. The notations $RF_H^1(a_n)$ and $RF_H^2(a_n)$ denote $F(a_n)$ of 24 large lines and $F(a_n)$ of 24 small lines, respectively. To determine these values, we acquire ultrasonic data 16 times for each line set, and we calculate the average values. We show the values of a_1, a_2, a_3, a_4, $RF_L^1(a_n)$, $RF_L^2(a_n)$, $RF_H^1(a_n)$ and $RF_H^2(a_n)$ in Table II. The fuzzy degree, μ_{Sn} and μ_{Ln} are calculated by (9) and (10).

$$\mu_{Sn}(RF_H(a_n)) = \min(SMALL, S_{RFH(an)}(RF)) \quad (9)$$

$$\mu_{Ln}(RF_L(a_n)) = \min(LARGE, S_{RFL(an)}(RF)) \quad (10)$$

Here, we define fuzzy singleton function $S_b(c)$ by (11).

$$S_c(d) = \begin{cases} 1 & if\ d = c \\ 0 & otherwise \end{cases} \quad (11)$$

The fuzzy IF-THEN rules are expressed by fuzzy MIN-MAX center-of-gravity method [9] as shown in Table I. For example, first row means:

If $RF_L(a_1)$ is *SMALL*, $RF_L(a_2)$ is *SMALL*, $RF_H(a_3)$ is *SMALL*, $RF_H(a_4)$ is *SMALL* THEN Rate of large line is *MIDDLE*

The center of gravity is decided as the rate of large line among all lines.

IV. EXPERIMENTAL RESULTS

We employed eight phantoms. They made from large and small lines in total 24 rolled lines and 15cm^3 water in the rubber tube. We acquired ultrasonic waveforms for the each 16 times. To compare our result with the other results, we show the results obtained by the method in [8] and the results by multiple regression analysis (MRA). Equation (12) shows the multiple regression analysis equation.

$$\begin{aligned} rate &= 8431.251 \\ &-8496.62 \times RF_H(1.519) - 212.44 \times RF_H(1.457) \\ &-8096.36 \times RF_L(0.625) - 139.167 \times RF_L(0.612) \end{aligned} \quad (12)$$

Table III and Fig. 11 show the means of the calculated large line rate and truth large line rate ($truth = N_L / (N_S + N_L) \times 100$ %). The notation N_S denotes number of small lines, and N_L denotes number of large lines. In Fig.11, the red line shows our evaluation result, the green line shows evaluation method in [8], the purple line shows MRA result and the blue line shows the true value. As shown in this figure, the rate of large line increased with the increment of the number of large line. The proposed method accorded with the truth value. The mean absolute error was 5.98 %, and the correlation coefficient was 0.98 in the proposed method. The proposed method obtained highest correlation coefficient among three methods.

V. CONCLUSION

In our study, we proposed a testicular tubules evaluation system with a 5.0MHz ultrasonic single probe. The fuzzy inference method used here works more exactly than the multi-regression analysis. As the experimental results, the proposed method overheads best accuracy in correlation coefficient and error ratio.

The single 5.0 MHz probe used here is lower cost than array probe used in [8]. Thus this method has advantage of cost and tractability as well as accuracy. In clinical practice, a single probe is easy handing and generally low invasive than array probe in the point of power.

In the future, we will evaluate diameter of seminiferous tubule phantom from difference of the distribution of the frequency spectrum.

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