NONDESTRUCTIVE MEASUREMENT OF THE THICKNESS OF A PATHOLOGIC SPECIMEN USING AN OPTICAL MICROSCOPE

M. Takahashi*, Y. Yoneyama* and M. Nakano**

*Shibaura Institute of Technology, Saitama, 337-8570, Japan
**Chiba Medical Center, Chiba, 260-8606, Japan

mtaka@sic.shibaura-it.ac.jp

Abstract: A method for non-destructively estimating the thickness of a histopathologic specimen is presented. This estimation is necessary because the thickness sometimes differs from that set on the microtome, and a difference can lead to misdiagnosis. The method does not use a confocal laser microscope, which pathologists rarely use in actual diagnosis, but an optical microscope, which pathologists commonly use. It is thus suitable for practical use. The thicknesses estimated using specimens with two different thicknesses were reasonable compared to measurements using a destructive method. The precision was estimated to be about ±0.5 µm. The total estimation time including image capturing was a few minutes, sufficiently short for practical application.

Introduction

The specimens used in histopathologic diagnosis, a technique for observing histopathologic specimens (Figure 1) with a microscope, are usually made by fixing, sectioning, exsiccating, imbedding, slicing and staining. The tissue is sliced to a thickness of 2 to 6 µm using a microtome. However, the thickness sometimes differs from that set on the microtome.

A difference in the thickness is a serious problem because it might lead to misdiagnosis. For example, the observed nuclear density (number of nuclei per unit area) is an important element in histopathologic diagnosis, especially in diagnosing hepatocellular carcinoma in the early stages[3]. It depends on the thickness of the specimen[1][2], and a difference in the thickness of 1 µm would change the observed nuclear density by about 10%[1]. A difference in thickness would also cause an error in the 3-D nuclear density (number of nuclei per unit volume) estimated using 2-D microscopic images[2]. Therefore, it is important to know the true thickness of a specimen.

While the thickness can be measured using a microscopic image of a cross-section of the specimen, such a destructive method cannot be used for diagnosis when the specimen must be preserved. A confocal laser microscope can be used to non-destructively measure the thickness of a specimen[4], but such microscopes are very expensive and thus rarely used for routine diagnosis. Most pathologists do not have access to them.

We have developed a method for non-destructively estimating the thickness. It uses images captured using an optical microscope, so it is a practical method for pathologists.

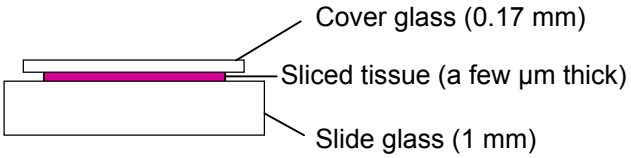

Figure 1: Cross-section of histopathologic specimen.

Materials and Methods

Hepatic histopathologic specimens (hematoxylin-eosin stained) were used.

The measurement system was composed of a PC with Athlon™ XP 2100 running Windows® 2000 and a microscope (E-400, Nikon) in which a CCD camera (Spot Insight IN1120, Diagnostic Instruments, 1600x1200 pixels) and a stage (OptiScan™ ES103, Prior) were installed.

Microscopic images of each specimen were captured while moving the computer-controlled stage upward (+Z direction) by 0.1 µm. The magnification factor of the objective lens was 100 (NA: 1.3).

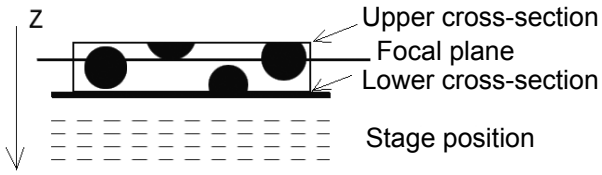

Figure 2: Method for capturing images.

As described below, areas were taken from each resultant 3-D image and used to estimate the thickness.

(a) An XZ-section MinIP (minimum intensity projection) image was generated by calculating the minimum value for all the y coordinates at the same XZ coordinates. This image was used to improve the contrast of the 3-D image, as described below, so that the accuracy was improved.
(b) Edge intensity I_{edge} was calculated for the MinIP image using a Sobel filter. The edge intensities

ISSN: 1727-1983

should be higher between the upper and lower cross-sections where there is tissue.

(c) The contrast of the edge intensity image was emphasized and normalized using histogram equalization. This normalization reduced the undesirable effect caused by the contrast difference, which was mainly caused by differences in the staining conditions, image capturing conditions, and the tissue itself.

(d) The number of pixels having an edge intensity greater than a threshold value was counted for each Z position. The position where the number of pixels rapidly increased (decreased) was extracted as the Z position of the upper (lower) cross-section. The distance between those two sections was taken as the estimated thickness.

Results

Hepatic histopathologic specimens which were made with setting the thickness on the microtome 2μm and 6μm were used in the experiment. First, we will give some of the results for the 6-μm-thick specimen.

We captured 180 images while changing the position of the focal plane by 0.1 μm. The resultant 3-D image was composed of 1600(X) x 1200(Y) x 180(Z) voxels. The size of one voxel was 0.073 x 0.073 x 0.1 μm.

The areas taken from the 3-D image were 22 (X) x 4 μm(Y). Figure 3 shows an example of a captured image at a certain Z position. The areas marked A to D were used for the measurement. They were selected because they did not include components generating strong edges such as nuclei and lymphocytes because strong edges cause many false weak edges outside the tissue and degrade the precision.

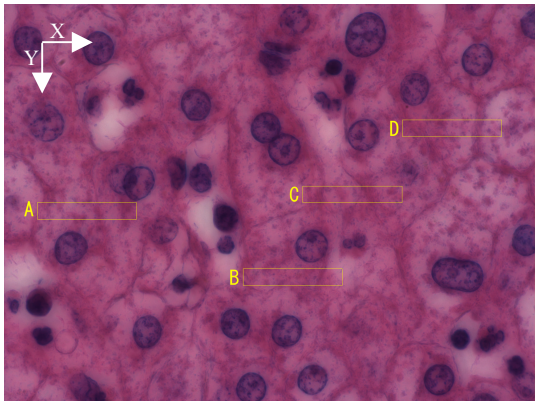

Figure 3: Example of image captured using 6-μm-thick specimen. Areas A to D were used for estimating thickness.

Figure 4 shows the XZ-section image of area A at a certain Y position. Figures 5, 6, and 7 show XZ-section MinIP images of area A using the R, G, and B channel, respectively. Inspection of these four images shows that the upper and lower cross-sections are clearer in the MinIP image using the R channel (Figure 5) than in those using the other channels. We therefore used the MinIP image using the R channel in the following process.

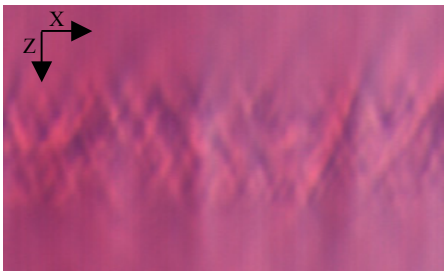

Figure 4: XZ-section image of area A at a certain Y position.

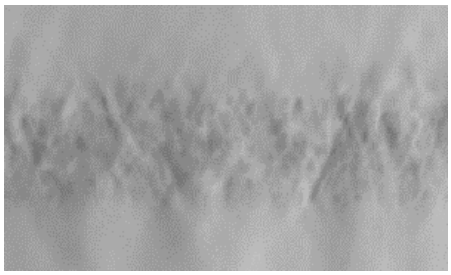

Figure 5: XZ-section MinIP image of area A using R channel.

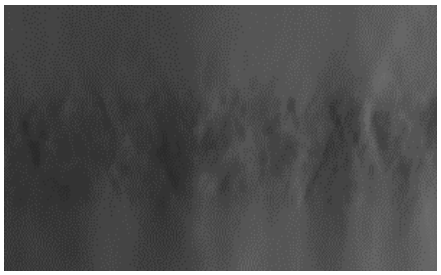

Figure 6: XZ-section MinIP image of area A using G channel.

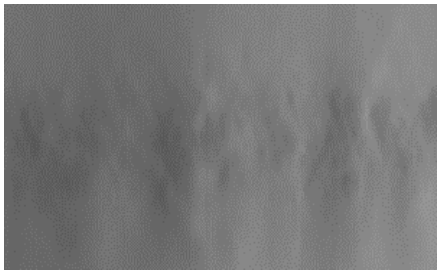

Figure 7: XZ-section MinIP image of area A using B channel

ISSN: 1727-1983

Figure 8 shows the edge intensity of the MinIP image using the R channel (Figure 5), and Figure 9 shows the result of histogram equalization. In both images, pseudo colors are used to show the intensity.

The histogram-equalized image was binarized as shown in Figure 10. In the figure, the black pixels have an edge intensity of more than the threshold value (230).

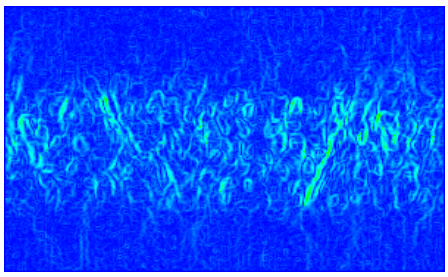

Figure 8: Edge intensity of MinIP image (Figure 5).

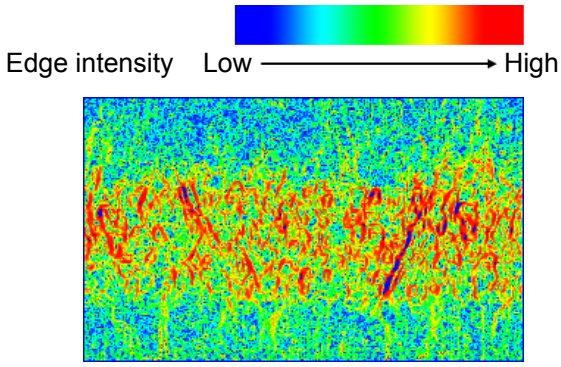

Figure 9: Image after histogram equalization.

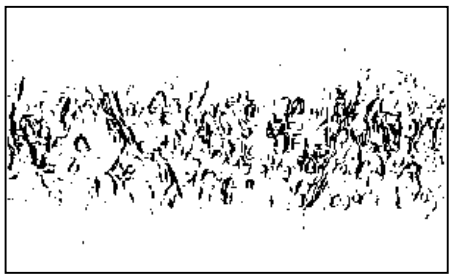

Figure 10: Binarized image (I_{edge}>230).

Figure 11 shows the number of black pixels counted for each Z position in Figure 10. In Figure 11, the results for the other three areas (B, C, and D) in the same image are also shown. The red broken lines show the positions estimated as the upper cross-section because the numbers of pixels rapidly increase at the positions for all four areas. Similarly, the blue broken lines show the positions estimated as the lower cross-section. These estimated positions are also shown in the binarized image in Figure 12. From these results, the thickness was estimated to be between 6.1 and 7.0 µm.

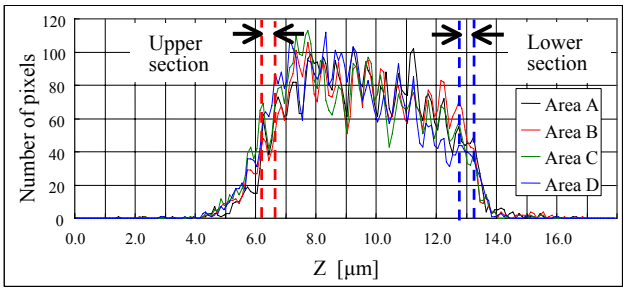

Figure 11: Number of pixels with I_{edge} > 230 for each Z for 6-µm-thick specimen.

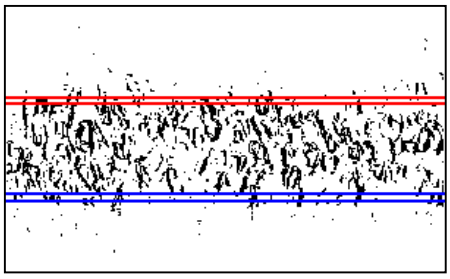

Figure 12: Positions of estimated upper and lower cross-sections for 6-µm-thick specimen.

Figures 13, 14, and 15 show the results for the 2-µm-thick specimen. We captured 150 images, and the areas taken from the image were 22(X) x 2 µm(Y). Following the steps described above, we estimated the thickness to be between 3.0 and 4.0 µm

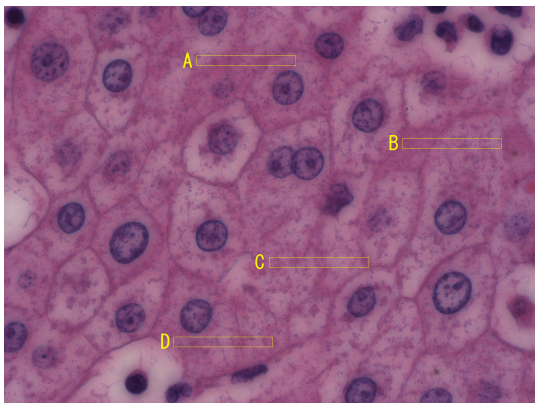

Figure 13: Example of image captured using 2-µm-thick specimen. Areas A to D were used for estimating thickness.

ISSN: 1727-1983

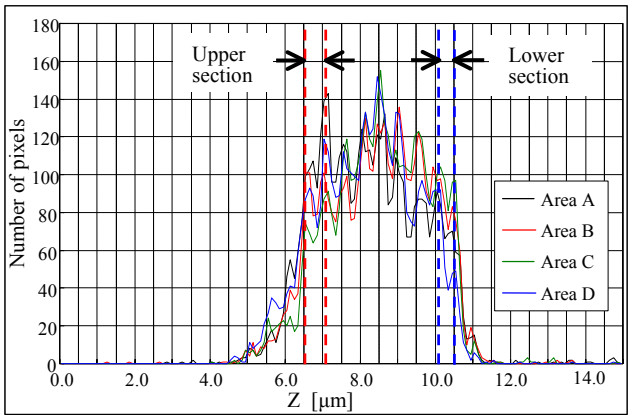

Figure 14: Number of pixels with $I_{edge} > 230$ for each Z for 2-μm-thick specimen.

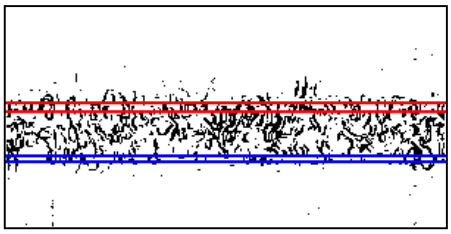

Figure 15: Positions of estimated upper and lower cross-sections for 2-μm-thick specimen.

To evaluate our results, we also measured the thickness using a destructive method. The specimen was cut using a glass cutter, and the thickness was measured using a microscopic image of the cross-section. The error was assumed to be one pixel of the image, which corresponds to 0.4 μm.

Table 1 shows the results.

Table 1: Estimated and measured specimen thickness (μm).

Microtome	6.0	2.0
Estimated thickness	6.1–7.0	3.0–4.0
Measured thickness	6.6±0.4	2.9±0.4

Discussion

The thickness estimated using our method matches the measured one quite well for the 6-μm-thick specimen, and both were a little bit larger than the thickness set on the microtome. Both were much larger than the thickness on the microtome for the 2-μm-thick specimen. Since making a thin specimen such as 2-μm-thick sometimes results in a large error in the thickness[4], the large difference between the estimated and measured thicknesses and the thickness on the microtome is not surprising. An error of about 1 μm for a 2-μm-thick specimen clearly shows the importance of estimating the actual thickness.

Conclusion

We have developed a method for non-destructively measuring the thickness of a histopathologic specimen. Since it uses images captured using an optical microscope, it should be widely applicable. The total time including image capture is only a few minutes, which is short enough for practical application.

Future work includes improving the accuracy by using more samples and automating the processes so that pathologists can easily use the system for actual diagnosis.

Acknowledgements

This research was partially supported by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research (C), 16500302, 2005.

References

[1] TAKAHASHI M., NAKANO M. (2005): 'Observed nuclear density is effected by the thickness of a specimen and the diameter of nuclei', Proc. of IAP2005 – The 4th Asia-Pacific IAP Cong., China, 2005, pp.325-326

[2] YOSHIMI T., TAKAHASHI M., NAKANO M. (2005): 'Estimation of 3-D Nuclear Density from a 2-D Image of Hepatic Histopathological Specimen', Proc. of EMBEC'05 – 3rd European Med. & Biol. Eng. Conf., Prague, 2005, Id1587

[3] NAKANO M., SAITO A., DOI M., TAKASAKI K. (1997): 'Stromal and blood vessel wall invasion in well-differentiated hepatocellular carcinoma', *Liver*, **7**, pp.41-46

[4] Sanitarian Society of Kanagawa Prefecture, Pathology Examination Study Group: 'A 2003 quality control report', Internet site address: http://www.jinringi.or.jp/kenkyuhan/byori/kenshyukai/200305/himitsu03.htm (in Japanese)

ISSN: 1727-1983