ESTIMATION OF 3-D NUCLEAR DENSITY FROM A 2-D IMAGE OF HEPATIC HISTOPATHOLOGICAL SPECIMEN

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Abstract: The nuclear density (the number of nuclei per unit area) is useful information for histopathological diagnosis, especially in diagnosing very early stages of hepatocellular carcinoma [1]. However, the observed nuclear density depends on the thickness of a specimen. This dependency originates from using two dimensional (2-D) nuclear density instead of 3-D density because nuclear density itself should originally be three dimensional (number of nuclei per unit *volume*). So, the purpose of this method is to estimate 3-D nuclear density. The method uses only a 2-D microscopic image, and features such as the average and variance of nuclear areas and the 2-D nuclear density are extracted from the 2-D image. The 3-D nuclear density is estimated by Bayes estimation using those as the observations. The 3-D nuclear density of a hepatic histopathologic specimen was experimentally estimated. The accuracy (90% confidence interval) was ±17% when a microscopic image taken with a x40 objective lens was used. The method uses only a 2-D microscopic image, which pathologists are usually observing, so the method is easy to use.

Introduction

Histopathological diagnosis is a technique for observing a histopathological specimen with a microscope. Histopathological diagnosis is done by pathologists at their discretion, so the differential diagnosis of borderline lesions is a serious problem. It is therefore important to develop a support system that analyzes histological images and provides important quantitative information for diagnosis.

We developed a support system for early well-differentiated hepatocelluar carcinoma (HCC) [1]. The system enables users to easily estimate the nuclear density (number of nuclei per unit area). Because the nuclear density of early well-differentiated HCC is usually higher than that of non-cancerous parts [1-2], pathologists can use the extracted nuclear density for diagnosis.

However, the observed nuclear density depends on the thickness of a specimen, as is shown in Figure 1 [3]. This dependency originates from using two dimensional (2-D) nuclear density instead of 3-D density, because nuclear density itself should be three dimensional (number of nuclei per unit *volume*).

To examine the dependency of 2-D nuclear density on the thickness of a specimen, we use a simulation. First, a virtual tissue was generated in which virtual nuclei were randomly distributed with the parameters shown in Table 1. The virtual tissue was cut as shown in Figure 2, and the 2-D nuclear density of the cut tissue was calculated. In the simulation, the depth of the focus was assumed to be deeper than the thickness of the virtual tissue, and the field of view was assumed to be large enough. The 2-D nuclear density as a function of the thickness is shown in Figure 3. The observed 2-D nuclear density linearly increased as the thickness increased.

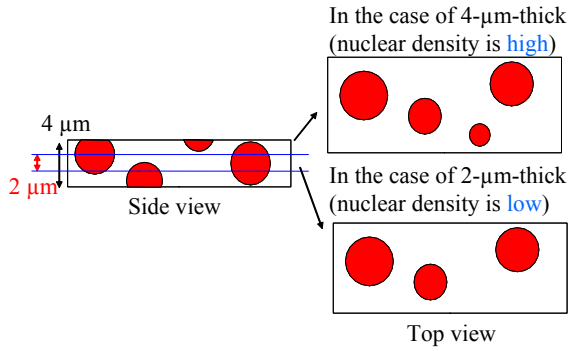

Figure 1: Observed nuclear density depends on thickness of specimens

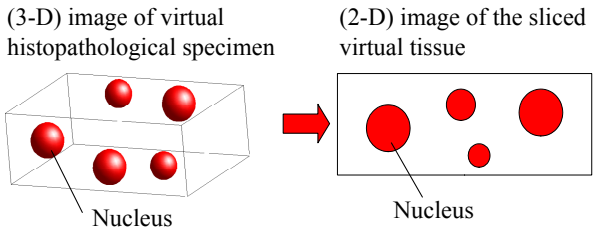

Figure 2: Slice of virtual tissue

Table 1: Parameters for generating distribution of nuclei in virtual tissue

3-D nuclear density	170×10^4 /mm^3
Average of nuclear radii	2.65 µm

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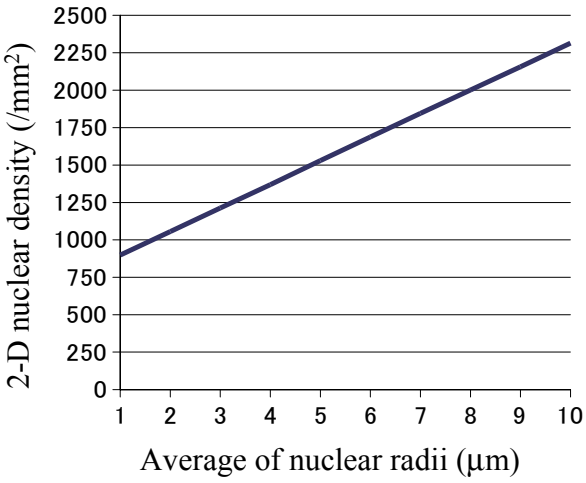

Figure 3: Dependency of 2-D nuclear density on thickness of specimen

This problem can be solved by calculating the 3-D nuclear density itself instead of using the 2-D density. However, as far as we know, there are almost no reports on measuring the 3-D nuclear density of histopathologic specimens.

So, we developed a method for estimating the 3-D nuclear density. The method uses only a 2-D microscopic image, which pathologists usually observe, so that they can easily use the method. Because particular facilities such as an automatic stage aren't needed besides a camera, it is advantageous that the method can be used in many institutions.

Materials and Methods

Pathologists usually observe microscopic 2-D images. However, determining the 3-D density from 2-D images is difficult. The 3-D nuclear density (d_{3D}) can be *estimated* using Bayes estimation as follows. The 3-D nuclear density is estimated using the feature values, which can be derived from 2-D images.

A Bayesian equation used for the estimation is shown in Equation 1.

$$P(\Theta \mid X_o) = \frac{P(\Theta)P(X_o \mid \Theta)}{P(X_o)} \qquad (1)$$

Here, X_o is an observed feature vector. The feature vector is composed of three features, including the 2-D nuclear density (d_{2D}), the average and the variance of the nuclear areas. Those features are calculated from a 2-D image used for the estimation. The vector to be estimated, Θ, is composed of three parameters: the 3-D nuclear density, the average and the variance of the nuclear radii. They were chosen as the parameters based on the assumption that the shapes of nuclei are almost spherical [4].

$P(\Theta)$ is a prior probability density, and was assumed to be uniform in the parameter space at the beginning.

$P(X_o|\Theta)$ for one Θ was calculated as follows.

(1) A virtual random distribution of nuclei was generated in a virtual tissue using the parameter Θ.
(2) The virtual tissue was sliced at a pre-determined thickness to make a virtual histopathological specimen, and a feature vector was calculated using the virtual specimen.
(3) By measuring how often the calculated feature vector coincides with the observed feature vector, X_o, $P(X_o|\Theta)$ for the Θ was calculated.

$P(X_o)$ can easily be calculated by integrating $P(X_o|\Theta)$ for all possible Θ. Then, $P(\Theta|X_o)$ is calculated using Equation 1.

Finally, the probability density for the 3-D nuclear density $P(d_{3D}|X_o)$ was derived by integrating $P(\Theta|X_o)$ for the other two parameters, the average and the variance of the nuclear radii.

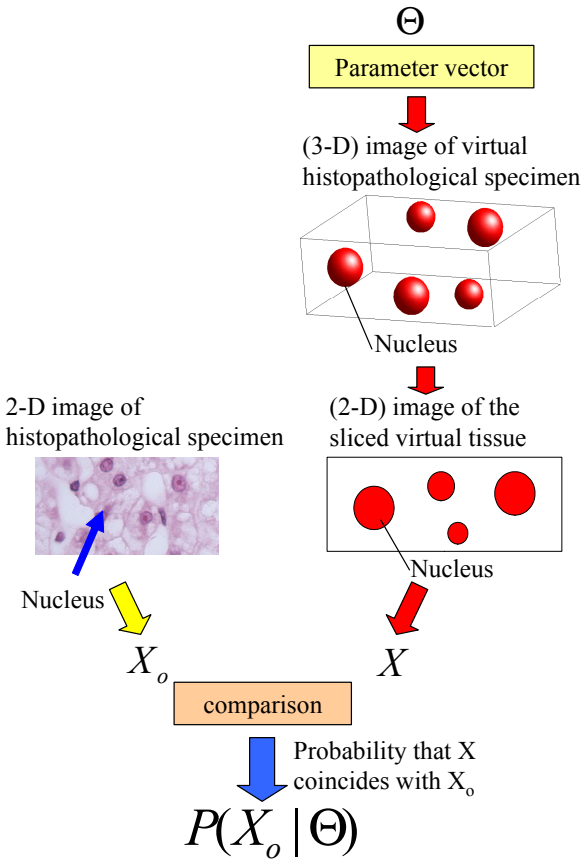

Figure 4: Calculation of $P(X_o|\Theta)$

Results

A microscopic image of a hepatic histopathological specimen (hematoxylin-eosin stained, 3-µm thick) was captured using a CCD camera with a x40 objective lens. The number of pixels was 1280 x 960, corresponding to an area 307 x 230 µm^2 on the specimen.

The captured image is shown in Figure 5. Nuclear areas of the same image are shown in Figure 6. This time, these nuclear areas were extracted by hand. This procedure will be semi-automated in the near future.

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From the extracted nuclear areas, feature values were automatically calculated. The 2-D nuclear density was 1.13×10^3 /mm^2, the average and the variance of the nuclear areas were 68.4 μm^2 and 283 μm^4, respectively. These feature values were used to estimate Θ, and the probability density of each parameter was calculated. The ranges and resolutions of each parameter used in the estimation are shown in Table 2.

Table 2: Range and resolution of each parameter

	Lower	Upper	Resolution
3-D nuclear density (/mm^3)	6×10^4	17×10^4	0.5×10^4
Average of nuclear radii (μm)	3.00	4.00	0.05
Variance of nuclear radii (μm^2)	0.00	0.50	0.05

The probability density of the 3-D nuclear density is shown in Figure 7. The probability density of the average of the nuclear radii is shown in Figure 8. The probability density of the variance of the nuclear radii is shown in Figure 9. The most probable d_{3D} was estimated to be 11.5×10^4 /mm^3. The most probable values for the other two parameters, the average and the variance of the nuclear radii, were 3.5 μm and 0.0 μm^2, respectively. The 90% confidence interval for the 3-D nuclear density was 9.4×10^4 /mm^3 $\leq d_{3D} \leq$ 13.3×10^4 /mm^3, which corresponds to the deviation of ±17%.

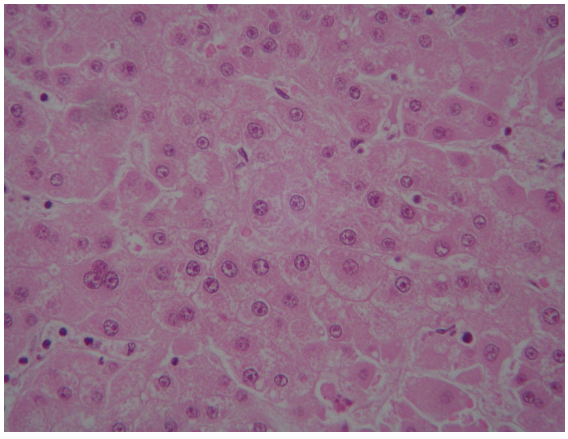

Figure 5: Microscopic image of hepatic histopathological specimen

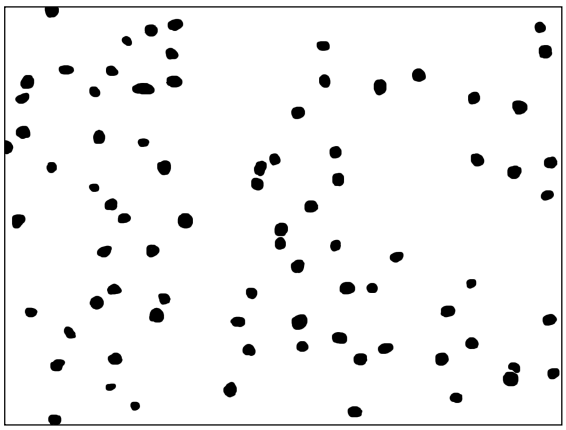

Figure 6: Nuclear areas extracted by hand from microscopic image

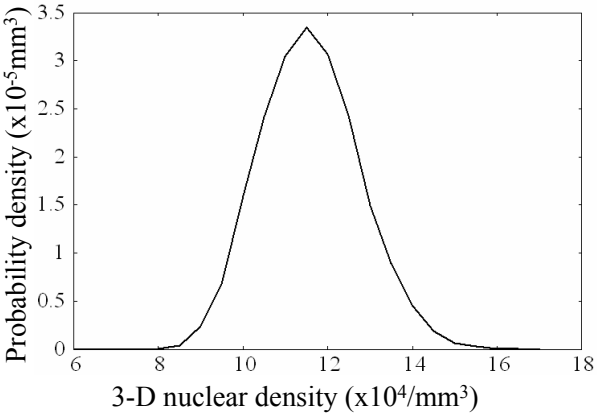

Figure 7: Probability density of 3-D nuclear density

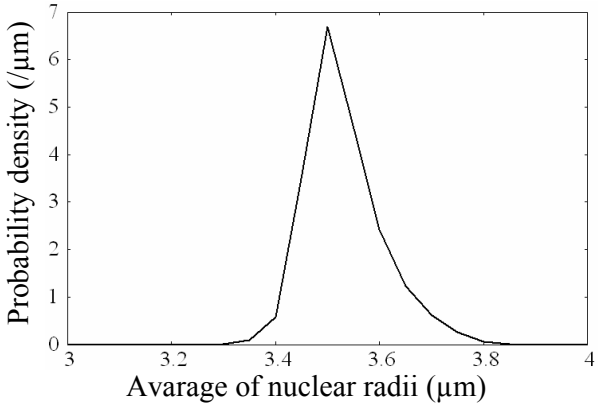

Figure 8: Probability density of average of nuclear radii

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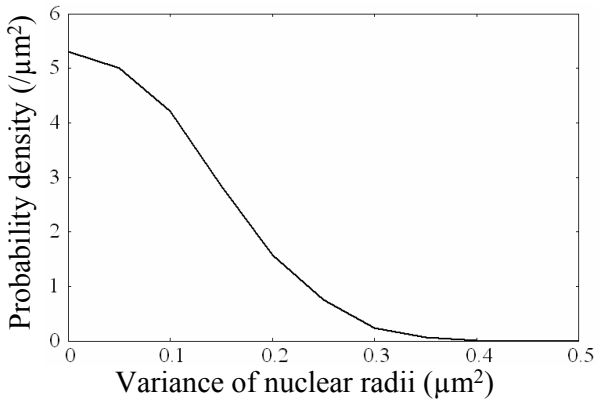

Figure 9: Probability density of variance of nuclear radii

Next, to confirm the validity of the method, d_{3D}'s for the virtual specimens, which were generated with the same d_{3D} using different thicknesses, were estimated. Feature vectors were calculated using the parameter vectors shown in Table 3, changing only the thickness from 2 μm to 4 μm. The ranges and resolutions of the parameters used in the estimation were the same as in Table 2.

Estimated d_{3D}'s are shown in Figure 10. Even if the thickness changed, the estimated d_{3D} changed little, showing the independency of d_{3D} on thickness.

Table 3: Parameter values

3-D nuclear density	11.5×10^4 /mm^3
Average of nuclear radii	3.50 μm
Variance of nuclear radii	0.00 μm^2

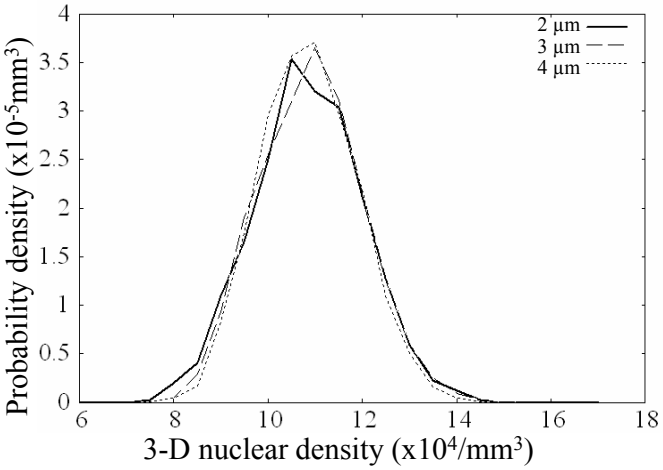

Figure 10: Effect of thickness on 3-D nuclear density

Conclusion

The 3-D nuclear density was estimated using only a 2-D histopathological image. The accuracy (90% confidence interval) was ±17% when a microscopic image taken with a x40 objective lens was used. We confirmed that d_{3D} is independent of the thickness by simulation. The method uses only a 2-D microscopic image, which pathologists are usually observing, so that they can easily use the method.

Future work will include improving the accuracy by improving the feature values. Improving the prior probability density ($P(\Theta)$), which was assumed to be uniform in the simulation, will also be useful for improving the accuracy.

In the simulation, the nuclear areas were extracted by hand. We are going to make a GUI system that enables pathologists to extract the nuclear areas semi-automatically, which will make a practical diagnosis support system.

Acknowledgements

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