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Composite Imaging Method for Histological Image Analysis

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Abstract— **A composite imaging method has been developed that enables the user to directly capture a composite image by one-image capturing. It was experimentally verified that the composite images of bright-field, dark-field, and phase-contrast images can be captured with an arbitrary composition ratio. The difference in pixel values between the captured composite image and the computer composite image was small. This imaging method is realized only by placing below the condenser a masking plate, which can easily be made using Neutral Density filters. Therefore, little additional time and cost are needed. The composite imaging method was applied for extracting *Helicobacter pylori* in microscopic images of HE-stained gastric histological sections. *H pylori* is difficult to extract because the colors in *H pylori* are similar to those in other areas. It is experimentally shown that a composite image of phase-contrast and dark-field images captured using the proposed method improves the accuracy for extracting *H pylori*.**

I. Introduction

HISTOPATHOLOGICAL diagnosis by pathologists is discretionary. So, 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.

We previously developed a support system for diagnosing early hepatocellular carcinoma that enables the user to easily estimate the nuclear density and the nuclear areas [1]. However, automatic extraction of cell membranes, which is necessary for estimating N/C ratio, was difficult because the color difference between the cell membrane and the cytoplasm was small in microscopic images of hematoxylin and eosin (HE)-stained hepatic histological sections.

We applied a multimodal method, which uses different kinds of imaging methods, to extract the cell membranes [2]. Three kinds of imaging methods (bright-field, dark-field, and phase-contrast imaging) were used because they are readily available for general pathologists. The correct rate of cell membrane extraction was improved by additional color information obtained by dark-field and phase-contrast images but not conventional bright-field images. The multimodal method using three kinds of imaging methods effectively improved the accuracy for nuclear position extraction and contours extraction [3].

The multimodal method, however, requires repeating image capturing two or three times while switching the imaging method. Because the imaging methods are switched by revolving a combined condenser, the images are sometimes slightly misaligned. An additional process to correct the misalignment is sometimes required.

To solve this problem, we have been investigated a method that enables the user to capture a composite image directly [4], [5]. The method, which we call a composite imaging method, uses a masking plate between a field lens and a condenser. The composition of three kinds of images is realized by adding the lights of those images with an appropriate composition ratio. The masking plate is used to attenuate and/or cut each of the three kinds of lights.

A similar method, which modifies a condenser's light mask, has been reported [6]. Processing a small condenser's light mask, however, is not easy for most users. Bright-field image is added by slightly turning the light annulus of the condenser into an off-centered position. Therefore, the composited bright-field image is modified from the original one. Using polarization techniques to regulate the light intensity might also modify the dark-field image because the reflection ratio depends on the polarization of the light.

The composite imaging method in this paper does not require processing of the condenser light mask. Instead, a masking plate, which is made using Neutral Density (ND) filters, is placed *outside* the condenser. ND filters are low cost, can easily be cut using a cutter, and can reduce the light intensity without modifying the light pattern. Polarization property of the light is also not affected. Thus, three kinds of images can be composited with very little modification. Experimental results show the effectiveness of the method. The computer composite image and the composite image captured by our method barely differ.

The composite imaging method was applied for extracting *Helicobacter pylori* in microscopic images of HE-stained gastric histological sections. *H pylori* is difficult to extract because the color difference between *H pylori* and other areas is small [7]. It is experimentally shown that the accuracy for extracting *H pylori* can be improved by using a composite image of phase-contrast and dark-field images captured using the proposed method.

II. Imaging Methods

A. Bright-field imaging

Fig. 1(a) shows the light path of bright-field imaging. Parallel light beam is concentrated into a cone of light using a condenser to illuminate the specimen. The light is, then,

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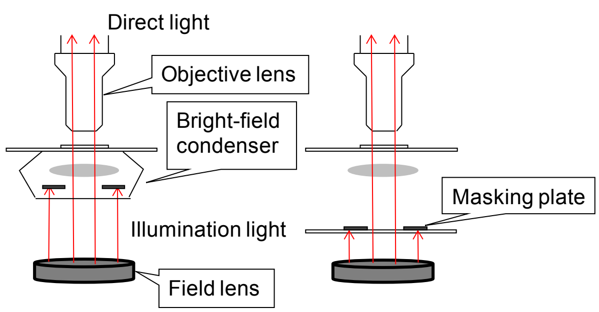
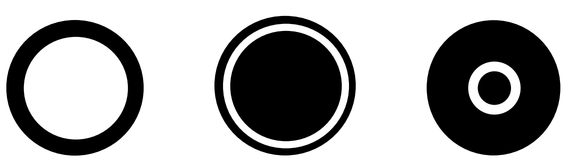
attenuated by the specimen. Therefore, a bright-field image reflects the distribution of absorption and is suited to observe stained histological sections. To improve the contrast of the image, the outer area of the parallel light beam is usually cut at the condenser as shown in Fig. 1(a). The same illumination pattern can be realized using a masking plate placed between the field lens and the condenser as shown in Fig. 1(b). In this case, which we call quasi-bright-field imaging, the aperture diaphragm of the condenser is fully opened, and the masking plate shown in Fig. 2(a) is used.

Microscopic images of a hepatic histological section for both bright-field and quasi-bright-field imaging are shown in Fig. 3(a) and (b). A phase-contrast objective lens (×40, NA0.75, Nikon) was used with a microscope (Eclipse 80i, Nikon). The masking plate was about 22 mm, and it was easily made by cutting thin films. As shown in Fig. 3, the difference between two imaging methods was very small. The average error of pixel values (R, G, B) between two images was 2.1.



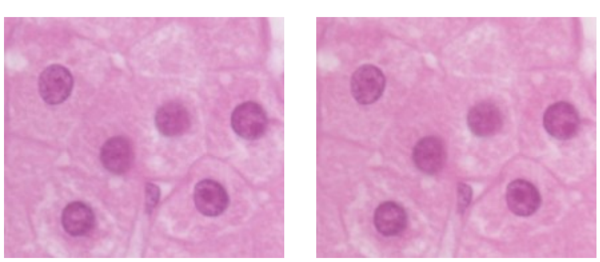
(a) Bright-field imaging (b) Quasi-bright-field imaging

Fig. 1. Light path in bright-field and quasi-bright-field imaging.



(a)Bright-field (b) Dark-field (c)Phase-contrast

Fig. 2. Masking plate.



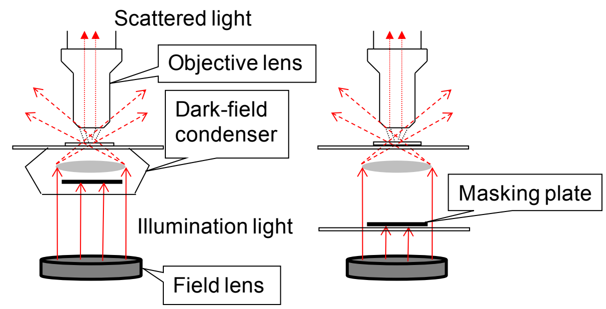
(a) Bright-field image (b) Quasi-bright-field image

Fig. 3. Examples of captured images.

B. Dark-field imaging

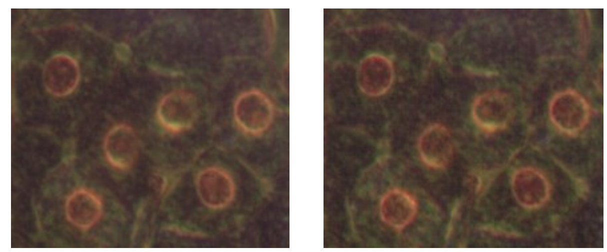
In dark-field imaging, the illumination light corresponding to bright-field imaging is cut as shown in Fig. 4(a), and scattered and reflected lights are observed. The same illumination pattern, which we call quasi-dark-field imaging, is realized using the masking plate that has an annular aperture shown in Fig. 2(b) with fully opening the aperture diaphragm of the condenser.

Fig. 5(a) and (b) show dark-field and quasi-dark-field images. The average error of pixel values was as low as 2.6 between two images.



(a) Dark-field imaging (b) Quasi-dark-field imaging

Fig. 4. Light path in dark-field and quasi-dark-field imaging.



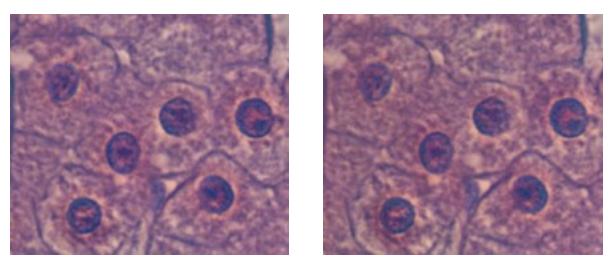
(a) Dark-field image (b) Quasi-dark-field image

Fig. 5. Examples of captured images.

C. Phase-contrast imaging

In phase-contrast imaging, phase differences in light caused by differences in refractive index are converted into brightness changes in the image. This imaging technique is usually used to observe transparent and/or unstained sections. Quasi-phase-contrast imaging is realized using the masking plate that has an annular aperture shown in Fig. 2(c).

Fig. 6(a) and (b) show phase-contrast and quasi-phase-contrast images. The average error of pixel values between two images was as low as 4.4.



(a) Phase-contrast image (b) Quasi-phase-contrast image

Fig. 6. Examples of captured images.

D. Composite imaging (Bright-field and Dark-field)

The bright-field masking plate (Fig. 2(a)) and the dark-field masking plate (Fig. 2(b)) have no common white region. Thus, the bright-field light and the dark-field light can be added using a composite masking plate of those masking plates as shown in Fig. 7(a). The ratio of the light intensities between two imaging methods is adjusted using an ND filter. The light intensity of bright-field imaging is usually larger than that of dark-field imaging. Therefore, the ND filter is used for the bright-field light. Fig. 8(a) shows an example of the captured composite image where an ND 2.1 filter was used for the bright-field light. A composite image is also generated using Eq. (1), which we call a computer composite image. In Eq. (1), f_{BF+DF}, f_{DF}, and f_{BF} are the pixel values of a composite image, a dark-field image, and a bright-field image, respectively. α_{DF} is the ratio of the dark-field image. β is a parameter to adjust the contrast.

$$f_{BF+DF} = (\alpha_{DF} f_{DF} + (1 - \alpha_{DF}) f_{BF}) \times \beta \quad (1)$$

α_{DF} in the composite image (Fig. 8(a)) was estimated by optimizing both α_{DF} and β so as to minimize the average error of pixel values between the composite image and the computer composite image. In the case of Fig. 8(a), α_{DF} was estimated to be 0.50. The average error of pixel values between the composite image and the computer composite image (Fig. 8(b)) was only 3.9. Almost the same composite image was obtained by the composite imaging method.

E. Composite imaging (Bright-field and Phase-contrast)

Fig. 7(b) shows a composite masking plate of bright-field and phase-contrast imaging. Bright-field light is attenuated by the ND filter also in this case because the light intensity is usually larger in bright-field imaging than in phase-contrast imaging. Fig. 9(a) and (b) are the captured composite image and the computer composite image that was made by minimizing the average error as in the case before. α_{PC} (the ratio of phase-contrast image) was estimated to be 0.45, and the average error of pixel values was 4.3. Although phase-contrast imaging utilizes interference of light, the error of pixel values was small.

F. Composite imaging (Phase-contrast and Dark-field)

Fig. 7(c) shows a composite masking plate of phase-contrast and dark-field imaging. Fig. 10(a) and (b) show the composite image and the computer composite image. α_{DF} was estimated to be 0.65 in this case. The average error of pixel values was 5.0. The error was also low in this case.

G. Composite imaging of three imaging methods

All three image methods are composited using the composite masking plate shown in Fig. 7(d). Fig. 11(a) and (b) show the results. The average error was 6.8 between two images. Three imaging methods were composited with small error in pixel values.

III. APPLICATION FOR EXTRACTING *H PYLORI*

Histological diagnosis is widely used to identify *H pylori* infection. *H pylori* in HE-stained sections is, however, difficult to identify because the color difference between the *H pylori* and other areas is small in bright-field images as shown in Fig. 12(a). Thus, additional staining methods such as modified Giemsa and Gimenez are used to help identify *H pylori* infection [8]. It will, therefore, be helpful if *H pylori* in

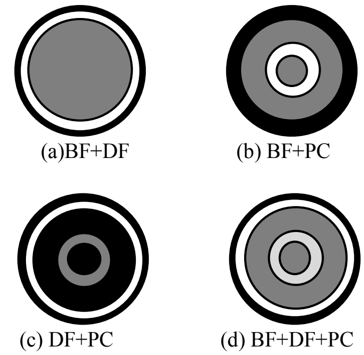
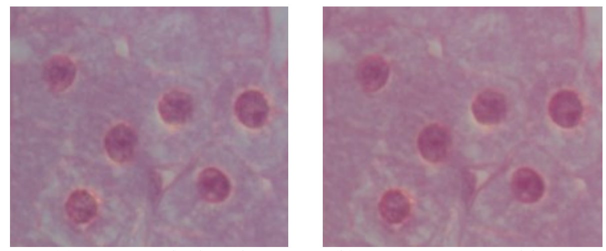
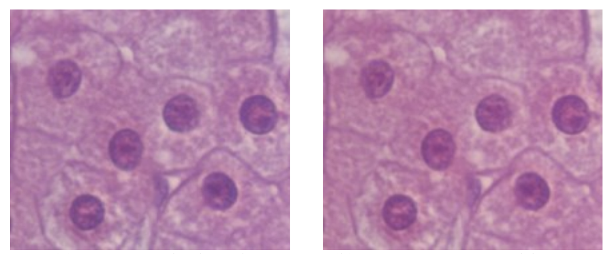
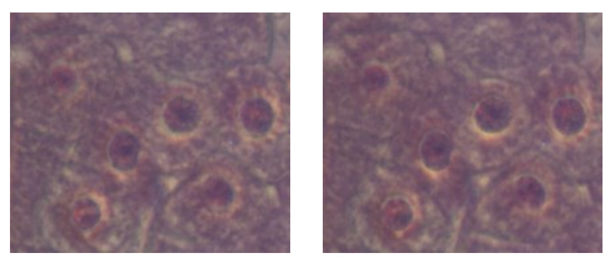
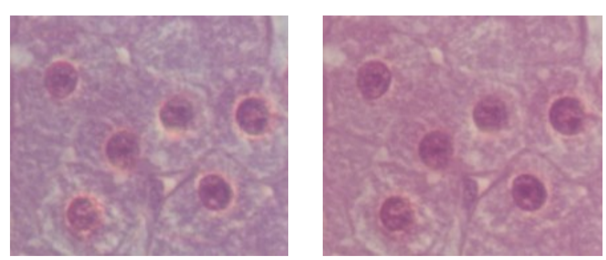


Fig. 7. Composite masking plate. BF: Bright-field, DF: Dark-field, PC: Phase-contrast.


(a) Composite imaging (b) Computer composition
Fig. 8. Composition of bright-field and dark-field imaging (α_{DF}=0.5).


(a) Composite imaging (b) Computer composition
Fig. 9. Composition of bright-field and phase-contrast imaging (α_{PC}=0.45).


(a) Composite imaging (b) Computer composition
Fig. 10. Composition of phase-contrast and dark-field imaging (α_{DF}=0.65).


(a) Composite imaging (b) Computer composition
Fig. 11. Composition of bright-field, phase-contrast, and dark-field imaging (α_{DF}=0.35, α_{PC}=0.28).

microscopic images of HE-stained sections become more detectable.

To validate the effectiveness of the composite imaging method, *H pylori* were extracted using only the color information of each pixel. Three kinds of images (bright-field, dark-field, and phase-contrast images) and their composite images were captured for three HE-stained gastric histological sections. *H pylori* were extracted using the classifier trained for the image. We used a feed-forward neural network as a classifier because it performed better than a SVM at extracting cell membranes [2]. Colors (R, G, B) and their chromaticities were used for the color information. Training data were made for each image using the GUI used for cell membrane extraction [2]. Each pixel in the image was classified into *H pylori* if the output of the classifier was larger than a threshold. After large areas were removed, the resultant small areas were extracted as *H pylori*.

The numbers of correctly extracted *H pylori* (N_{TP}) and wrongly extracted areas (*non-H pylori*) (N_{FP}) were used to evaluate the results. Both N_{TP} and N_{FP} depend on the threshold value of the classifier, so the results were evaluated to be better when N_{TP} was larger with smaller N_{FP}. The three kinds of images and their composite images were evaluated, and the best results were obtained for the composite image of phase-contrast and dark-field images in all the cases. Fig. 12(b) shows an example of the composite image. The colors of *H pylori* are basically greenish in the composite images of phase-contrast and dark-field images. Fig 13(a) and (b) show examples of areas extracted as *H pylori*. In the case of the bright-field image (Fig. 13(a)), N_{TP} and N_{FP} were 18 and 11, respectively. In case of the composite image (Fig. 13(b)), N_{FP} was reduced to only 2 with almost the same N_{TP} of 17, showing the effectiveness of using the composite imaging method.

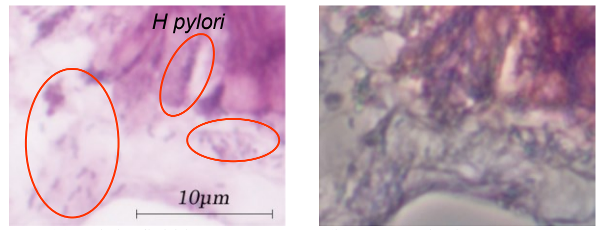
Although similar results might be obtained by using the computer composite image of phase-contrast and dark-field images, the composite imaging method has an advantage in that there is no misalignment that needs to be corrected. Such a misalignment can become a major problem when extracting small objects such as *H pylori*.

IV. Conclusion

A composite imaging method was developed that enables the user to capture a composite image by one-image capturing. It is experimentally shown that composite images of bright-field, dark-field, and phase-contrast images can be captured with little error in pixel values. This imaging method is realized only by placing below the condenser a masking plate, which can be easily made using ND filters. Therefore, little additional time and cost are needed.

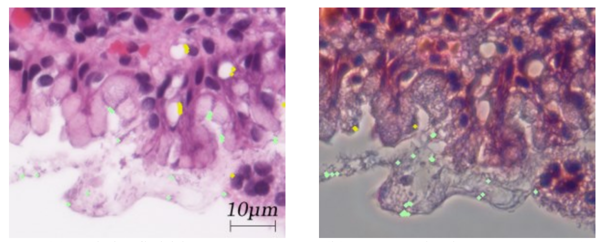
The composite imaging method was applied for extracting *Helicobacter pylori* in microscopic images of HE-stained gastric histological sections. It is experimentally shown that the accuracy for extracting *H pylori* is improved when using the composite image of phase-contrast and dark-field images.

Future work includes the expansion of this technique. For example, switching among various imaging methods and adjustment of the composition ratio should be automated by replacing the masking plate with a spatial light modulator (SLM). The SLM should also enable fast switching among imaging methods and capturing of plural images in short time. Those images should be processed instantly and exhibited on a display for supporting histological diagnosis.



(a) Bright-field image (b) Composite image (PH+DF)

Fig. 12. Example microscopic images of HE-stained gastric histological section. α_{DF} of composite image is 0.33.



(a) Bright-field image (b) Composite image (PH+DF)

Fig. 13. Results of *H pylori* extraction. Green: Correctly extracted *H pylori*, Yellow: Wrongly extracted areas (*non-H pylori*). α_{DF}=0.33.

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