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題目(和文)	H&E染色ハイパースペクトル画像を用いた弾性繊維及び膠原繊維の分別に関する研究
Title(English)	Elastic and Collagen Fibers Discrimination Methods using H&E Stained Hyperspectral Images
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Dissertation Outline

Specimen staining is an important process in pathology diagnosis. The color information from tissue structure shows the tissue's condition. Currently, the standard staining in pathology diagnosis is Hematoxylin and Eosin (H&E) stain which shows the general information of the morphological structure of the tissue. In the particular diagnosis, pathologist needs to observe the growing progress of specific component i.e. elastic fibers over time. However, it is difficult to recognize the elastic fibers using H&E stained image, because it has a similar color and pattern with the collagen fiber. To recognize elastic fibers, Verhoeff's Van Gieson (EVG) stain is commonly used. However, EVG stain is an additional staining method after H&E stain has been done, which needs an additional effort and cost.

This study proposes a new method to classify elastic fibers from collagen ones with H&E stain using hyperspectral images (HSI). The proposed method performs pixel wise classification, which makes it possible for pathologists to observe the growing progression of the elastic fibers precisely. Consequently, it would improve the efficiency and accuracy of the pathology diagnosis process. The proposed method analyzes the spectral and spectral-spatial information of hyperspectral images in segmenting elastic and collagen fibers from the H&E stained images. The spectral features of the H&E hyperspectral image are investigated using Linear Discriminant Analysis (LDA) in segmenting elastic and collagen fibers. The proposed method adobts U-net based architecture deep learning to improve the discrimination accuracy using a combination of spectral-spatial features of the hyperspectral image. The experimental results were evaluated visually by pathologists and statistically by comparing to the segmentation of EVG stained image as ground truth image. The dissertation consists of five chapters as follows.

Chapter 1 introduces the pathology workflow process and background including problems as well as the objective of this study. It also explains the overview of hyperspectral images and reviews previous related studies.

In pathology diagnosis, specimen staining is an important process, in which the color information obtained by tissue components is useful for further pathological analysis. Hematoxylin and Eosin (H&E) stain is a standard staining method in pathological diagnosis to observe the morphological structures of tissues. In H&E stained images, fibers and cytoplasm are stained pink, and nuclei is stained blue. On the other hand, previous studies about pathological analysis showed an obvious correlation between the abnormality of elastic fibers ad diseases. Specifically, it is well known that pancreatic cancer tissues have a specific density and distribution of elastic fibers in the walls of vessels and ducts associated with the tumor phenomenon in pancreatic ductal carcinoma. However, the appearance of elastic fibers is not easy to be recognized from H&E stained images, due to the similar color and pattern with collagen fibers. After H&E stained tissue specimen has been received by pathologist, the pathologist will request additional Verhoeff's Van Gieson (EVG) stained image to distinguish elastic from the collagen component. This EVG stained image is commonly used because EVG stain is able to dye elastic and collagen fibers in different colors, deep blue and orchid, respectively. Nevertheless, EVG stain is an additional stain that is done while the H&E stain image has been done. It means that the procedures of EVG stain need more effort in chemical process and cost than H&E stain.

In practical diagnosis, pathologists need to know how elastic fiber region has been growing over time, compared to the previous condition. Hence, this study purposes to perform pixel wise classification of elastic and collagen fibers in H&E stained image using spectral features and the combination of spectral-spatial features of hyperspectral image. Thus make it possible for pathologists to observe the growing progression of the elastic fibers precisely.

Hyperspectral image (HSI) contains information in an almost continuous spectrum compared to the intensity curve from RGB imaging that provides data centered at only three prominent wavelengths. The spectral information contained with the continuous hyperspectral image can be utilized to more accurately analyze and understand microscale features that are not feasible using the discrete RGB imaging dataset.

Hence the advantage of the HSI is providing more comprehensive information and increase the potential of making new discoveries compared to wider wavelength band images. Instead of advantage, HSI also has a disadvantage; that is while the potential is larger, data and related parameters also more numerous, making it potentially more difficult and time consuming to use.

This study will answer the question about what information and phenomenon can be obtained from these images. How the performance of RGB image, spectral features based hyperspectral image, spectral-spatial based hyperspectral based can be used to segmented elastic and collagen fibers in H&E stained image can be obtained.

Hyperspectral image analysis is being done used for medical diagnosis because of its ability to provide a tangible image of biomarker information time and tissue spectral information. Some studies related to the usage of multispectral and hyperspectral imaging have been done for medical diagnosis purposes. Pinky A. Bautista et, al., introduced a digital staining methodology for pathological tissue samples by utilizing the transmittance spectra of the unstained and Hematoxylin and Eosin (H&E) stained multispectral images (16 bands) of specific tissue components and classified in to four tissue components, e.g. nucleus, cytoplasm, red blood cells, and the white region. The originality of this previous study was invented an H&E digital stain method using a multispectral image that potentially used to skip the chemical staining process to increase the pathology diagnosis efficiency. The limitation of this previous study is only for visualization of general morphological structure purposes. The improvement of this study is that the purpose of this study is for pixel wise classification elastic fibers with quantitative evaluation. Ishikawa et.al., developed a pattern recognition method based on HSI, called hyperspectral analysis of pathological slides based on stain spectrum (HAPSS), to detect cancers in hematoxylin and eosin-stained pathological slides of pancreatic tumors. The samples, comprising hyperspectral cubes of 420–720 nm, were harvested for HSI and tissue microarray (TMA) analysis. As a result of conducting HAPSS experiments with a support vector machine (SVM) classifier, it obtained a maximal accuracy of 94%, a 14% improvement over the widely used RGB images. Thus, HAPSS is a suitable method to detect tumors in pathological slides of the pancreas automatically. The originality of this previous study by Ishikawa et.al. is by inventing a pattern recognition to detect cancer in H&E stained hyperspectral image. The limitation is that the object target is the nucleus, which is easy to be recognized visually using H&E stained image. The improvement in this study is the object target is elastic fibers, which have a similar pattern and color with collagen fibers in H&E stained image and difficult to be recognized visually. Abe et al. did the study related to elastic quantification. They segmented the elastic and collagen fiber in EVG stained image using Quadratic Discriminant Analysis (QDA). It shows the good quantification study for elastic and collagen in EVG stained image. However, EVG stain is an addition stain in histopathology diagnosis, which is done after the H&E stain image has been generated and it only specifically used to distinguish elastic and collagen fibers. In this proposed study, elastic fibers segmentation is done using H&E stained image as a standard image.

Chapter 2 explains the broad outline of the experimental procedure relevant to this study, including material and apparatus such as the tissue samples, hyperspectral camera specification and some preprocessing methods.

This experiment was performed using Hyperspectral Image (HSI) of human pathological pancreatic Tissue MicoArrays (TMAs), using a combination of microscopy and a Hyperspectral (HS) camera. H&E staining was performed to generate images with differences in light absorption which depend on various factors such as physical characteristics of elastic and collagen components. After that, spectral signatures obtained from absorbance images were analyzed to classify and identify the tissue samples. In particular, the proposed method used for distinguishing (61 bands) between waveforms of absorbance of pigments by classifying elastic and collagen fibers components. Procedures in this study consist of several steps, i.e., tissue sample, image acquisition, preprocessing, training, segmentation, and verification. H&E stained images are used as the target of object analysis, and EVG stained images are used for label and ground truth images. To get a reliable label and ground truth, two types of stained images i.e., H&E stained and EVG stained images, which have almost the exactly same biological structures are prepared. EVG stained specimens are obtained by dying unstained specimens that are obtained by bleaching the H&E stained specimens. EVG stained tissues are captured by using the Hamamatsu Whole Slide Image (WSI) scanner as an RGB image. H&E hyperspectral images are captured by an optical microscope (Olympus BX-53) with a white LED light source which has almost similar characteristics to halogen light and a 61-wavelength bands hyperspectral camera from EBA Japan NH-3. Intensity calibration is done to account for the difference in the spectrum of light sources (LED). A high dynamic range image is generated by replacing the overexposure part of the image with the lower one. To make the object recognition on H&E stained specimen easier to be analyzed, absorbance spectral image is used, it is converted from captured transmittance hyperspectral images using the Beer-Lambert law equation.

As a pre-process to classify elastic and collagen fiber using the H&E stained image, the image registration method based on Speed up robust features (SURF) is employed to trace the fiber region of the EVG image to corresponding one of an H&E stained image. To obtain the ground truth images and label for training, EVG images are segmented into elastic, collagen, and other components using Linear Discriminant Analysis as Abe's method. Those ground truth images and labels have been corrected and verified by pathologists from Saitama Medical University.

Chapter 3 There are two possible factors to classify elastic and collagen fibers components in the HSI; they might be from the ratio of eosin and hematoxylin stain in the whole 61 wavelength bands from 420 to 720 nm and peak shift differences between elastic and collagen components. Therefore, a discriminant filter obtained from this spectral information is possible to separate elastic and collagen more accurately, which might not be found in RGB with three wavelength bands. The segmentation result showed that the hyperspectral image is superior to RGB imaging not only confirmed visually by pathologists but also quantitatively by T-statistic based on the dice coefficient. The paired T-Test statistic of elastic and collagen segmentation results using spectral information for the RGB image and the HSIwas 3.5082, with a p-value less than 0.05. It indicates that elastic and collagen segmentation based on spectral features in the HSI performs significantly better than RGB image.

Chapter 4 explains elastic and collagen fibers segmentation based on spectral-spatial features using U-net based architecture deep learning. The conventional machine learning needs lots of calculations for getting many spatial features in hyperspectral images corresponding to each wavelength to map and to fuse spatial and spectral features. On the other hand, U-net enables to train of both features automatically without redundancy from many calculations. The optimum U-net parameter set was obtained by trial experiments. The segmentation result showed that the HSI is superior to RGB image not only visually by pathologists but also in quantitative evaluation using a T-Test analysis based on the dice coefficient again. The paired T-Test statistic of elastic and collagen segmentation results using spectral-spatial information for the RGB image and the HSI indicates that elastic and collagen fibers segmentation based on spectral-spatial features in HSI performs significantly better than RGB images.

Chapter 5 contains discussion and conclusion, which explains elastic fibers segmentation performance comparison between spectral features and spectral-spatial features from pathologist and statistic perspective. The paired T statistic between spectral features and spectral-spatial features of the hyperspectral image indicates that elastic and collagen segmentation based on spectral-spatial features of HSI performs significantly better than spectral features only.

This study observes the potential method to segment elastic and collagen fibers using the H&E stained image as a standard stain in pathology diagnosis based on spectral and spectral-spatial features of HSI. The method is not only for visualization but also for pixel wise classification purposes. It would increase diagnostic accuracy and has the potential towards substituting the usage of the EVG staining method which is only used to recognize elastic fibers. Hyperspectral imaging systems are more expensive than RGB camera systems in general. However, it provides an accurate analysis result and increases the diagnosis efficiency. It also can be used not only for elastic fibers analysis but also for other tissue structures analysis purposes. The wider use of the HSI system stimulates increasing HSI system production which effects in decreasing the price.