Discovery of Effective Spectrum for Classifying iPS Cells Taken with CARS Microscope

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Abstract: There is a technique using the CARS (Coherent Anti-Stokes Raman Scattering) microscope to identify iPS cells. CARS microscope can visualize the different molecular structures of iPS cells in each spectrum, so it is possible to identify iPS cells without destroying them. However, the information on molecules in the spectrum obtained by the CARS microscope is so diverse that it takes a great deal of time and effort to identify them. We propose a method to automatically identify the spectrum, which is effective for iPS cell identification, thereby reducing the time and effort required for identification using the CARS microscope. In this paper, we propose a network that handles multi-resolution information in parallel to learn both image classification and segmentation simultaneously. Moreover, the effective spectrum for classifying iPS cells are discovered by using the network gradients and the F-measure for cell segmentation. By the experiments on four kinds of iPS cells, we confirmed that the accuracy of the proposed method for classifying iPS cells achieved 99%. Furthermore, the effective spectrum for each iPS cell could be automatically identified.

1 INTRODUCTION

iPS cells (Takahashi et al., 2006) are capable of transforming into almost any types of cells, and regenerative medicine research (Hideyuki et al., 2019) using their characteristics is actively conducted. To use iPS cells for regenerative medicine, it is necessary to transform them into other cell types (called “differentiation”). However, because of the variability in the efficiency and direction of differentiation of iPS cells into the other cells, when iPS cells are differentiated from iPS cells, cell types other than the intended ones or cells that have not been fully differentiated may be mixed in. Furthermore, when cells differentiated from iPS cells are transplanted into an organism, it is known that tumors can be formed if undifferentiated iPS cells are mixed in.

There is a method using CARS (Coherent Anti-Stokes Raman Scattering) microscope (Cheng et al., 2004) to identify iPS cells and their differentiated cells. CARS (Begley et al., 1974) is a phenomenon in which two different spectra of light are irradiated on a material, and light with a spectrum different from both is generated. CARS microscope allows us to visualize the molecular structure of the cell in each spectrum. Therefore, CARS microscopy makes it possible to identify iPS cells without destroying (killing) cells. However, the information on molecules in the spectrum obtained by the CARS microscopy is so diverse that it takes a great deal of time and effort to identify them. Therefore, it reduces the time and effort required for identification using the CARS microscope by automatically identifying the spectra that are effective for iPS cell identification. We perform automatic classification using CNN from spectrum images obtained by the CARS microscope. Furthermore, from the results of the classification, we discover the effective spectrum for classifying each iPS cell.

There is a method using Grad-CAM (Selvaraju et al., 2017) to identify the spectrum that is effective for identification. By using Grad-CAM, it is possible to identify the effective spectrum as the one with a large importance value in the feature maps (Takeshi et al.,

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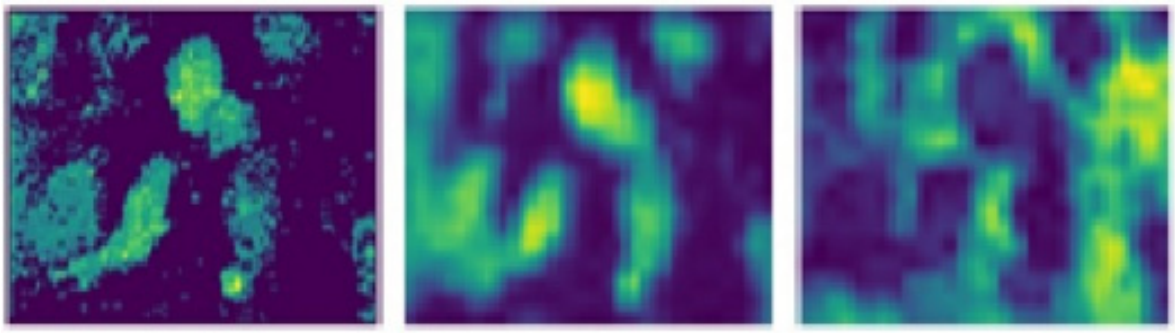

(a) (b) (c)

Figure 1: Example of visualization results for the portion of each spectrum that was identified as effective for classification. (a) Visualization of the location of cells in the input image. (b) Example of a visualization result in which cells are captured by the portion that is considered effective for classification. (c) Example of a visualization result in which cells are not captured by the portion that is considered effective for identification.

2019). However, as shown in Figure 1, this method judges an object to be effective even when the effective spectrum for classification does not capture the cells such as a culture medium.

We proposed the automatic detection method of the effective spectrum shown in Figure 2 to solve the shortcomings of the Grad-CAM based effective spectrum identification. The multi-scale network learns by handling feature maps with multiple resolutions in parallel. The effective spectrum calculation module uses the gradients like Grad-CAM to calculate the important feature map and then multiplies it by the F-measure for cell segmentation obtained from the multi-scale network. By multiplying F-measures, we can reduce the importance of the spectrum that does not capture cells.

In experiments, we classify four types of iPS cells using images captured by the CARS microscope. We also identify the effective spectrum from the classification results. As a result, we were able to successfully classify iPS cells with 99% accuracy and discover class-specific effective spectrum.

This paper is organized as follows. Section 2 describes the related works. The details of the proposed are presented in section 3. Section 4 shows experimental results. Finally, conclusion and future works are described in section 5.

2 RELATED WORKS

2.1 Identification of iPS Cells using CARS Microscope

Researches on the application of iPS cells to regenerative medicine are actively conducted. A method for identifying high-quality iPS cells is to use the CARS microscope (Michiel et al., 2007). CARS microscope utilizes the phenomenon of CARS which is the generation of light with a spectrum different from that of either light when two lights with different spectra are incident on a material. CARS microscope allows us to visualize the molecular structure of iPS cells in a non-destructive, non-invasive, non-staining, and non-labelling manner. Thus, we can identify the cells in their living state. However, the spectrum obtained by the CARS microscope contains a variety of molecular information, and it is very costly to obtain all the molecular information. Therefore, we reduce the cost by discovering the effective spectrum for classifying cells. In this paper, we propose a classification method of iPS cells using the CNN which has a structure like the HR-net (Saad et al., 2017), and an effective spectrum for classifying iPS cells is discovered automatically.

2.2 Effective Spectrum Discovery using Grad-CAM

There is a method for discovering the effective spectrum for classification by using Grad-CAM (Takeshi et al., 2019). This method identifies effective spectrum by comparing the average of the gradients of the convolutional layers computed in the same way as Grad-CAM for each feature map. However, in this method, the spectrum may be judged to be effective even when the result is shown in Figure 1(c). In other words, when the gradient of a convolutional layer is computed for the input cell image and the gradient of non-cell pixels shows a large value, this spectrum is erroneously judged to be effective.

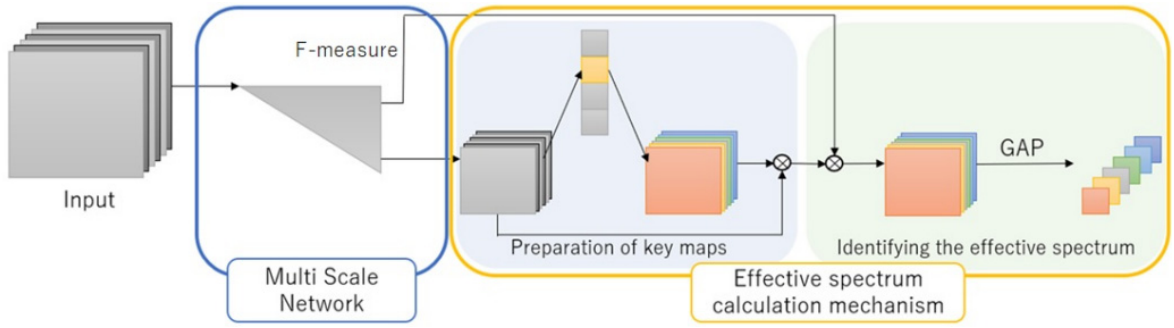

Figure 2: Overview of the proposed method. It consists of a multi-scale network and an effective spectrum calculation module, and it identifies the effective spectrum for classifying the input cell by the magnitude of the final output value.

In this paper, we use segmentation (Long et al., 2015) to solve this problem. Segmentation becomes more accurate as the per-pixel accuracy improves. Therefore, when the cell locations are recognized more accurately, the F-measure becomes large. When cell segmentation does not work well, the F-measure becomes small. By multiplying the F- measure for segmentation with the gradients of feature maps, it is possible to suppress the importance values when the gradients of non-cell pixels are large, and we discover effective spectrum correctly. Therefore, by using the result of cell segmentation, we can prevent the example as shown in Figure 1(c).

3 PROPOSED METHOD

The proposed effective spectrum identification network shown in Figure 2 consists of a multi-scale network shown in Figure 3 and an effective spectrum calculation module. Section 3.1 describes the multi-scale network. Section 3.2 describes the effective spectrum calculation module.

3.1 Multi-scale Network

The purpose of the multiscale network is to learn the features while focusing on the location of cells. Multi-scale networks have two characteristics. The first one is the network structure used to learn by handling the features of multiple resolutions in parallel. The second one is the skip connection to compensate for the information in the input spectrum.

The structure of the multiscale network is shown in Figure 3. The reason for training multiple resolutions in parallel (Sun et al., 2019) is to efficiently learn the classification and segmentation at the same time. Segmentation is the task that class labels are assigned to each pixel in an image, and it is possible to learn cell location information by incorporating segmentation learning. Therefore, we expected that the network would learn to use much information of cells during classification because it would understand the location of the cells better than the case without segmentation learning. The multiscale network used convolution with a kernel size of 3 with stride 2 to reduce the resolution, and bilinear interpolation to increase the resolution. In the multiscale network, depth wise convolution was applied to only the first layer, and normal convolution was applied to the remaining layers. To perform image classification, all feature maps of the input image are aggregated into a feature map with a reduced resolution of 1⁄4. To perform segmentation, all feature maps are aggregated into a feature map of the same size as the input image. We also introduce attention in the channel direction during training to make it easier to identify the spectrum that is effective for classification.

Multi-scale networks used convolution to extract features. The convolution calculates the output of one channel by multiplying all the input channels by their weights. Since the information of all spectra is mixed, it is impossible to identify effective spectrum from the feature maps. To solve this problem, we used the skip connection like ResNet (He et al., 2016), and added the input features to the output feature maps of the multi-scale network. By using the skip connection, it is possible to compensate the original spectrum for the output features and identify which spectrum is effective. To match the size of the input image and the output feature maps from the multi-scale network, we used average pooling with filter size 4 and stride 4.

3.2 Effective Spectrum Calculation Module

The effective spectrum calculation module identifies the effective spectrum for classification from the

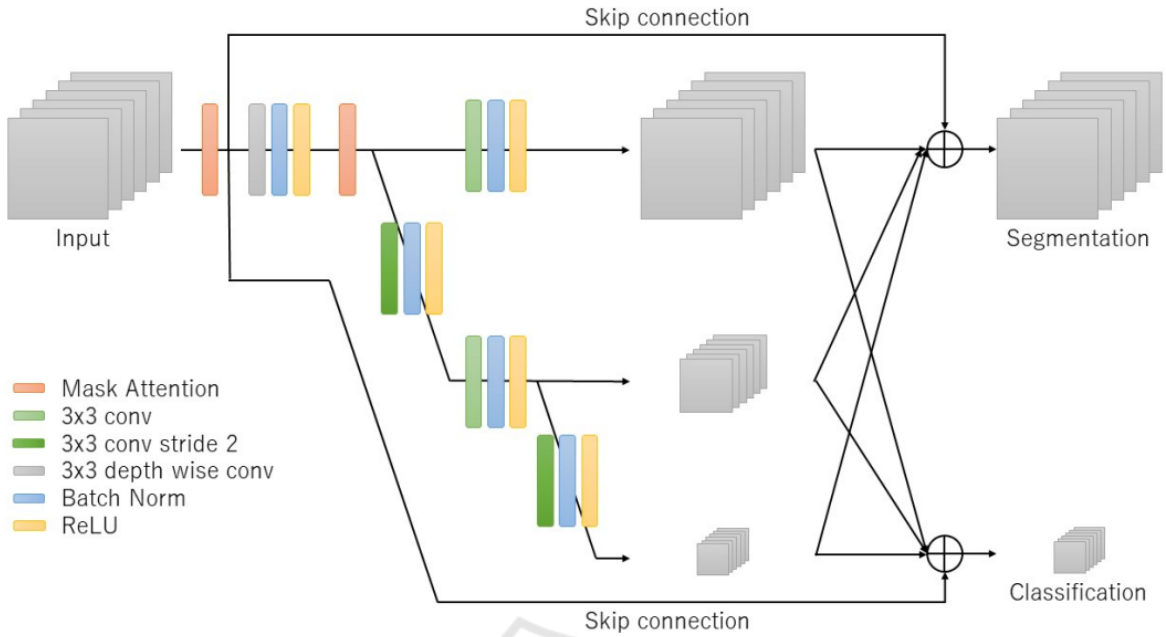

Figure 3: Overview of multi-scale networks.

feature maps in the multi-scale network. This module can identify the effective spectrum while suppressing the spectrum that does not capture cells as shown in Figure 1(c). The module consists of the preparation of keymaps and identification of the effective spectrum. The keymaps indicate the importance of each spectrum. The preparation of a keymap is performed in the following three steps. First, we obtain the final convolutional layer with the lowest resolution in the multi-scale network. Second, we calculate the gradient of the convolutional layer for the correct class. Third, by multiplying the calculated gradient value by the feature map, a keymap is created that identifies the important areas for identification. By multiplying the feature map by the gradient value, the keymap has larger values for important pixels for classification.

Identification of the effective spectrum is performed in the following three steps. First, we obtain the feature maps with the highest resolution in the multi-scale network and evaluate whether the cell locations are segmented well by using the F-measure or not. Second, the keymaps for each spectrum are multiplied by the F-measure for each spectrum. The effective spectrum is identified by the magnitude of the multiplied values. By multiplying the F-measure, the spectrum that does not capture cells is suppressed, and the shortcomings of the existing methods are improved.

The creation of the segmentation labels required to learn segmentation in section 3.1 and to obtain the F- measure in section 3.2 is described in section 4.2.

4 EXPERIMENTS

In this section, we show the experimental results. Section 4.1 describes the dataset and augmentation used in this study. Section 4.2 gives the overview of experiments. Section 4.3 presents the results of evaluation experiments.

4.1 Dataset and Experimental Setup

The experiments were conducted using multi-spectrum images of iPS cells captured by the CARS microscope. The classes in the dataset consist of four classes; ectoderm (ECT), mesoderm (MES), endoderm (END), and undifferentiated (UND). The total number of data for each class is 100; 25 for ECT, 25 for END, 25 for MES, and 25 for UND. These data were originally from the same cell line and were imaged after 1 week of incubation under different culture conditions. The number of spectra visualized per sample was 609, and the size of each image was 70x110 pixels. Due to the small number of images, we use 5-hold cross-validation. We use 80 images for training and 20 images for validation.

Data augmentation is proven to be an efficient technique to improve the overall model performance. In our experiments, data augmentation was used to improve the performance of the multiscale network.

Table 1: The overview of experiments.

	Processes
I	Create a simplified segmentation label
II	Learning Multiscale Networks
III	Back propagation of the final convolutional layer using a trained multiscale network
IV	Multiply the gradient by the convolutional layer of the final layer
V	Calculating F-measure from high-resolution feature maps of multiscale networks
VI	Multiply the results of IV and V
VII	Sum up the results for each class

In training, we performed random horizontal and vertical flipping after random cropping of the image to a size of 64 x 64 pixels. In validation, the image size was changed to 72 x 112 by zero padding to make the calculation easier.

We used the Pytorch library and trained the network with Adam for 100 epochs. The base learning rate (lr_base) was set to 0.01, and when there were 50 epochs, the learning rate was set to 0.001 and the network was trained. We use a batch size of 40 and a momentum of 0.9. The loss function is a combination of Cross entropy loss during training for discrimination and Dice loss during training for segmentation.

4.2 Overview of Experiments

In this section, we describe the flow of experiments. The overview is given in Table 1. A simplified label for segmentation is created by summing all 609 spectra and then binarizing Otsu's method (Otsu, 1979) because it is only necessary to distinguish cells from the background. The network is trained in Table 1, II to create a model to identify iPS cells. All operations below III in Table 1 are performed using the trained model. Table 1 VII sums up the results of the 5-fold cross-validation up to Table 1 VI. Cells that are misclassified by the multiscale network is excluded from the calculation.

4.3 Experimental Results

We conducted experiments on the dataset obtained by the CARS microscope, and the classification results of the four kinds of iPS cells are shown in Table 2. We compared the accuracy of the network with and without multiple resolutions. The network without multiple resolutions is a standard CNN that classifies the cells with only feature maps of low-resolution. Table 2 demonstrated the effectiveness of usage of a multi-scale network structure. This result suggests that it is more effective to learn the feature maps while retaining location information.

Table 2: Comparison results. "Single" shows the result without using multiple resolutions, and "Multi" shows the result with multiple resolutions.

Acc(%)	ECT	END	MES	UND	Mean
Single	92	100	96	96	96
Multi	96	100	100	100	99

Table 3: Confusion matrix of the multiscale network.

	Label			
	ECT	END	MES	UND
ECT	24	0	0	0
END	1	25	0	0
MES	0	0	25	0
UND	0	0	0	25

We compared the results of identifying effective spectrum by the proposed method and the conventional method using Grad-CAM. Figure 4 shows the importance of each class of iPS cells for the proposed method and Figure 5 shows the importance of each spectrum for the conventional method. Figure 4 demonstrated that the spectrum around $850cm^{-1}$, $1200\,cm^{-1}$, and $1750\,cm^{-1}$ for ectoderm (ECT), $1800cm^{-1}$ and later for mesoderm (MES), $1300cm^{-1}$ and $1500cm^{-1}$ for endoderm (END), and the first half of the spectrum around $750cm^{-1}$ for undifferentiated (UND) are effective for classification. When we compare Figure 4 with Figure 5, the proposed method makes it easier to identify the differences in the effective spectrum of each cell.

Figure 6 showed the feature maps that correspond to top five effective spectra discovered by the proposed method and the conventional method (Takeshi et al., 2019). As the result of the conventional method, the feature maps that non-cells have high value were ranked high. On the other hand, in the case of the proposed method, only the feature maps that captured cells were ranked high. This result demonstrated the effectiveness of our method using both the F-measure for segmentation and the gradients of feature maps. However, the results in Figure 4 showed that the ECT and END classes have similar values when the importance of the spectrum is not high. This may show that the features of ECT and END classes are more similar than those of the other two classes, and the ECT class is misclassified as END class in Table 3.

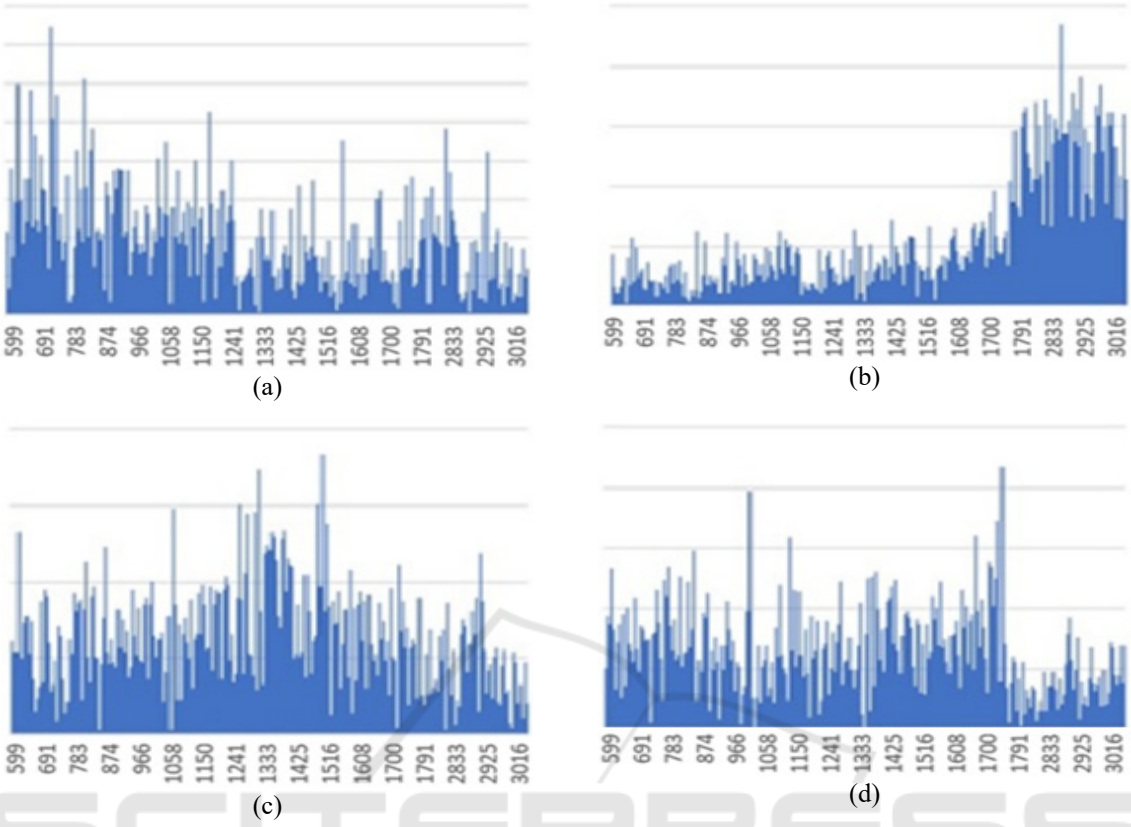

Figure 4: The importance of each class of iPS cells by the proposed method. The result is the sum of all values from samples in each class. The vertical axis of the figure shows the total importance value, and the horizontal axis shows the spectrum (cm^{-1}). (a) Results from of cells in the ectoderm (b) Results of cells in the mesoderm (c) Results of cells in the endoderm (d) Results of undifferentiated cells.

5 CONCLUSION

In this paper, we propose the multi-scale network for classifying iPS cells from the CARS microscopy images. Effective spectrum is identified by multiplying the F-measure based on cell segmentation and the importance based on gradients of feature maps. By using the proposed method, we were able to identify the effective spectrum for the classification of four kinds of iPS cells. This result means that it is possible to suggest the molecular information that characterizes each cell type from the imaging data of the CARS microscope without any prior information or prejudice. In the future, we would like to make it possible to obtain similar results when there are multiple iPS cells in an image.

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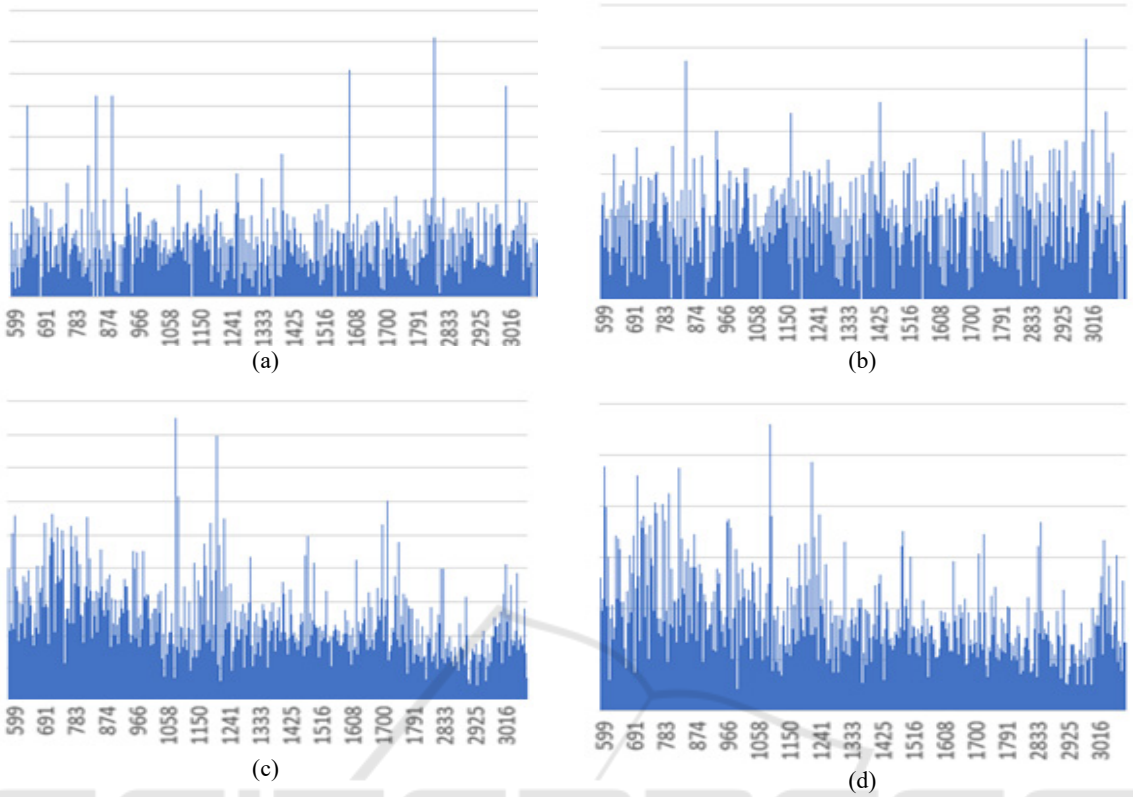

Figure 5: The importance of each class of iPS cells by the conventional method. The result is the sum of all values from samples in each class. The vertical axis of the figure shows the total importance value, and the horizontal axis shows the spectrum (cm^{-1}). (a) Results from of cells in the ectoderm (b) Results of cells in the mesoderm (c) Results of cells in the endoderm (d) Results of undifferentiated cells.

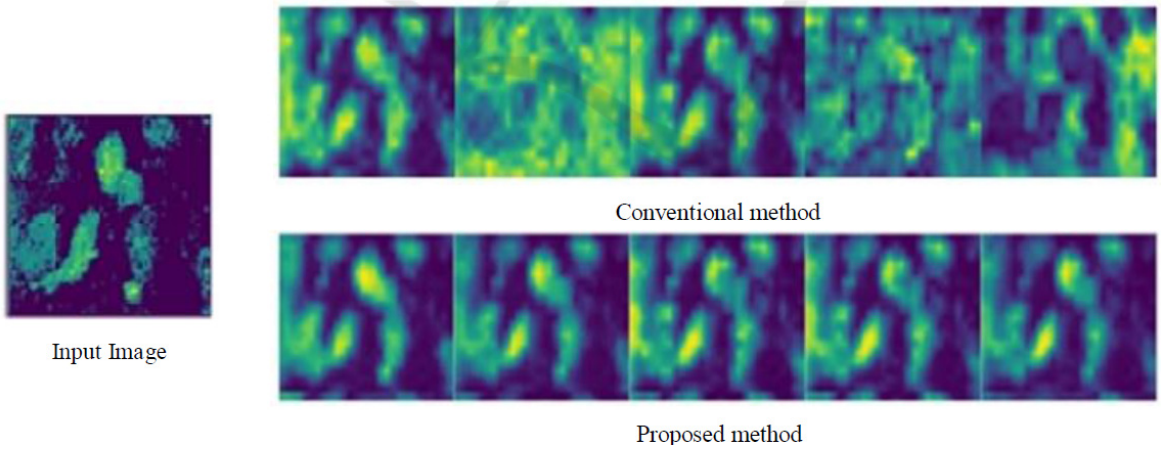

Figure 6: Visualization results of the top five important spectrum. The left column shows the input image, the top row shows the results by conventional method, and the bottom row shows the results by the proposed method. From left to right images shows the feature maps with the first to the fifth important spectrum.

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