Unsupervised Light Spot Detection using Background Subtraction

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Abstract: Live cell imaging has been developing rapidly by the development of the microscope and fluorescence technique. Light spot detection in intracellular image is important for elucidation of form of morphology of animal. However, light spots are detected manually now, and human can not treat a large number of images. If automatic detection by computer is realized, we can obtain many objective data, and it will be useful for the biology development. In general, supervised learning is useful to develop a good detector. However, many particles are included in an intracellular image, and it is difficult to make a lot of supervised samples. Therefore, in this paper, we propose a light spot detection method based on unsupervised learning. Concretely, we use background subtraction and robust statistics to detect the light spots. In experiments using Wnt-3a images, the proposed method outperforms ImageJ which is usually used in cell biology.

1 INTRODUCTION

According to the development of fluorescence technique as GFP and microscope, we can get large number of intracellular images (Sakaushi et al., 2007; Sugimoto et al., 2010). It is expected to elucidate the form of morphology of animal. However, light spot detection in intracellular images by computer is a new research field, and the automatic detection methods are still little. Thus, light spots are detected manually now, and we need a lot of effort to obtain reliable data. This task wastes a lot of time and heavy burden of physically and mentally. In addition, the data becomes subjective. To solve these problems, we develop automatic detection based on robust statistics.

Wnt family of secreted signaling proteins has an important role in situation of embryogenesis (Takada et al., 2006). Wnt is one of signaling proteins. It involves cell's life and development. When the cell composed of multi-cellular organisms transfers the information from cell to cell, it secretes Wnt. The information transferred by Wnt is called Wnt signaling. It is essential signal made in various tissues of animals. However, if it is much transferable, cells are canceration. Therefore, properly controlling the secretional capacity of Wnt is important for treatment of cancer. However, we have not understood it yet. We require the detection and tracking method of signaling proteins from large number of intracellular images. We also need statistical analysis which is independent of subjectivity of observer. Thus, we propose light spot detection in Wnt-3a (Shibamoto et al., 1998) images by computer.

The sizes and shape of the Wnt-3a in images are inconsistent. In general, supervised learning is used to develop a good classifier (Kumagai, 2012). However, it is not easy to make a lot of supervised data by specialists. It wastes a lot of effort and time. In addition, intracellular image includes a lot of noises as shown in Figure 2(a), and there are also light spots as small as 1 pixel. Therefore it is difficult for specialist to distinguish between noises and small right spots. In fact, we could get only 3 images with ground truth. Thus, we propose the method for detecting Wnt-3a by background subtraction in which supervised information is not required. Since the images used in experiments were captured at long interval, we can not use sequential information to estimate the background (Shimai et al., 2007). To estimate the background from only one test image, we use median of a local region. We compute the difference between the estimated background and test image, and light spots are emphasized. To detect the light spots from the difference image, LMedS and binarization are used.

In experiments, Wnt-3a images obtained by National Institute for Basic Biology are used. The accuracy is evaluated by using 3 images with ground

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truth by a specialist. In general, evaluation with only 3 images is not sufficient. However, in a Wnt-3a image, many light spots are included as shown in Figure 4. Thus, we consider that the evaluation is sufficient. The accuracy of our method achieves 85.28%. This is much better than ImageJ (http://rsbweb.nih.gov/ij/) which is used in cell biology.

Section 2 explains the light spots detection by robust statistics (Huber, 1981). In section 3, experimental results one shown. Conclusions and future works are described in section 4.

2 LIGHT SPOT DETECTION AND NOISE REDUCTION

First, we estimate a background image from only an input image, and we perform background subtraction to emphasize the Wnt-3a. The Wnt-3a is detected from the difference image by using the robust statistics. However, since there are many noises which are similar to Wnt-3a, noises are also detected as Wnt-3a. Thus, it is difficult to detect the light spots by only one step and by Otsu's binarized method after candidates of light spots are detected by background subtraction.

In the following sections, we explain the details of our method.

2.1 Background Estimation

It is the best that we prepare the background image without foreground in advance. However, in Wnt-3a images, the background regions are also changed and we can not prepare the background image in advance. We can not use the sequential information too. Therefore, we make a background image from only one test image by median filter. In experiments, we apply a median filter with the size of 9 x 9 to the test image to estimate the background.

Foreground such as light spots are emphasized by subtracting the estimated background image from the test image. To classify the foreground and background, good threshold value is required. However, adequate threshold value is changed for every image. Therefore, the threshold is determined by Least Median of Squares (LMedS) which is one of robust statistics. Since the area of background is larger than that of foreground, the inlier becomes background and outlier becomes foreground.

2.2 Light Spot Detection using Robust Statistics

We use LMedS (Rousseeuw, 1984) to classify foreground and background. LMedS is more robust to outlier than least squares. In LMedS, the estimation result does not so change if the ratio of outlier is below 50 percents.

Next, we describe how to use LMedS criteria. We calculate difference image between test image and the estimated background image. Next, we calculate the median $d_{med}^2 = \text{med}\, d_i^2$ in the difference image. The standard deviation of error distribution is computed by using the median as

$$\overline{\sigma} = 1.4826\left(1 + \frac{5}{M-1}\right)\sqrt{d_{med}^2}, \quad (1)$$

where M is number of pixels in the image, and 1.4826 is a coefficient which error distribution normal distribution is in accordance with normal distribution. 5/(M-1) is a correction term for small number samples. We determined outlier (light spot candidates) as $\sqrt{d_i^2} \geq 2.5\overline{\sigma}$.

2.3 Noise Reduction

There are a lot of noises in images of Wnt-3a. Since only background subtraction can not classify noises and light spots, we distinguish the noises and light spots by Otsu’s binarized method (Otsu, 1979) after LMedS. However, there are noises with higher intensity than light spot. Therefore, if we use Otsu’s binarized method instead of LMedS, light spots are not detected well. Since there are small light spots with 1 pixel. We can not use the morphological operation. To classify noise and Wnt-3a, we pay attention to the neighboring intensities because noise is always the spike and the intensities of neighboring region is not large. Figure 1 shows the noise and light spot. Figure shows the neighboring pixels of noise have low intensity. Therefore, we compute average intensity of a local 3 x 3 region whose center is the point detected by background subtraction. The average intensities in 3 x 3 pixels are fed into Otsu's binarized method, and we detect light spots with unsupervised manner.

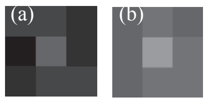

Figure 1: Comparison between around noise and light spot. (a) noise. (b) light spot.

Figure 2 shows the overview of our approach. Figure 2 (a) and (b) are an input image and estimated background image. Figure (c) shows the difference image between (a) and (b). Figure (d) and (e) show the result of LMedS and final result.

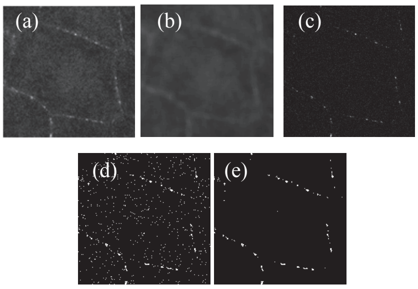

Figure 2: The flow of light spot detection. (a) Input image. (b) Estimated background image. (c) Difference image. (d) Result of LMedS. (e) Final result by Otsu binalization.

3 EXPERIMENTS

Effectiveness of our method is shown by experiments. In section 3.1, we describe image dataset. Section 3.2 shows experimental setting and results.

3.1 Wnt-3a Image Dataset

We use 3 intracellular images with Wnt-3a obtained by Takada laboratory in National Institute for Basic Biology. The images with ground truth are only 3, and we used given by a specialist 3 images for evaluation. In general, the evaluation using 3 images is not sufficient. However, the number of light spots in an image is large, and we consider that the evaluation is sufficient to evaluate the effectiveness. The size of images is 696 x 525 pixels. Example of image is shown in Figure 2 and 4. The image is the Wnt-3a which is generated by entering the mRNA in egg. The size and shape of light spots are different. About 200 light spots are included in an image.

3.2 Result

We compare the proposed method with ground truth position of Wnt-3a obtained by specialist. We judge that it is correct if the detected point is included in 5 x 5 pixels around ground truth. Table 1 shows evaluation results. Accuracy rate of proposed method achieves 85.28%. Even though the number of false positive is not so small. Figure 4 shows the example of detection result. Figure 4(a) shows ground truth with red square. Figure 4(b) shows our result in which almost light spots are detected.

Next, our method is compared with ImageJ which is frequently used in cell biology. The result by ImageJ is shown in Table 2. Accuracy rate of ImageJ achieves only 66.85% when the average number of false positive same as our method and the parameter "Noise tolerance" was set to 45. ROC curve is shown in Figure 3. Our method obviously better than ImageJ. Figure 4(c) shows detection result by ImageJ in which the detected light spots are indicated by yellow crosses. ImageJ failed to detect many light spots.

Table 1: Result of proposed method.

	Accuracy rate[%]	Number of false positive
Image 1	87.91	141
Image 2	85.06	72
Image 3	82.86	72
Average	85.28	95

Table 2: Result of ImageJ.

	Accuracy rate[%]	Number of false positive
Image 1	68.13	182
Image 2	71.84	76
Image 3	60.57	27
Average	66.85	95

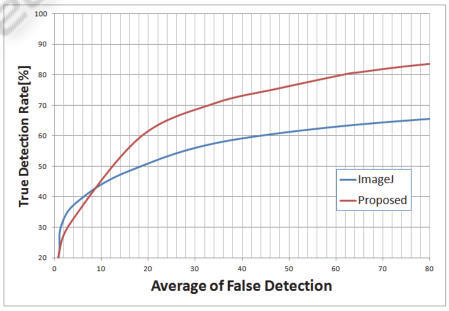

Figure 3: ROC curve of proposed and ImageJ.

As shown in Figure 4, the accuracy of our method is high and the problem is the false positive. When the intensity of background is high as shown in Figure 5, noises are detected as light spots because we assume that neighboring region of noise is low intensity. When intensities of neighboring region in background are large, average intensity value becomes large. Almost false positive are those kinds of errors. Figure 6 shows the ground truth and the result by our method. Our method detected 3 light spots in 5 correct light spots. We consider that difference of intensity between light spot and background is very small. Almost of all false negative are those kinds of errors.

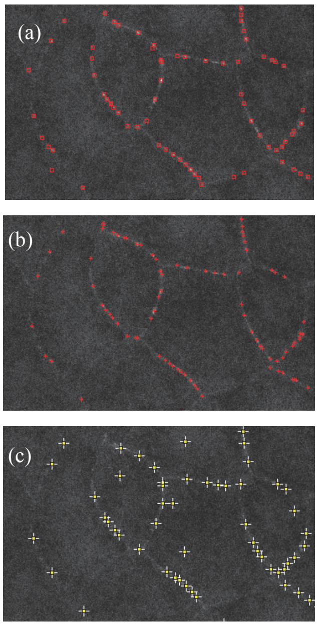

Figure 4: Example of light spot detection. (a) Ground truth by a specialist. (b) Detection result of (a) by the proposed method. (c) Detection result of (a) by the ImageJ.

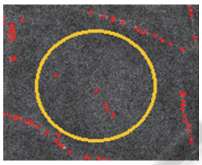

Figure 5: Example of high intensity background. There are no light spots in circle in fact.

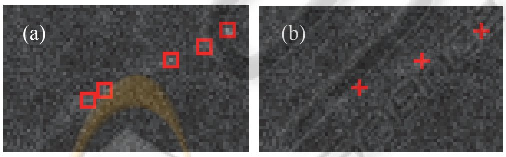

Figure 6: Example of false negative. (a) Ground truth. (b) Result of (a).

4 CONCLUSIONS

In this paper, we proposed unsupervised light spot detection based on background subtraction because development of supervised data is time-consuming. The accuracy of proposed method is high while the number of false positive is not small. The research field of intracellular image processing has started in recent years, there is little conventional method. Therefore, we can not compare except for ImageJ. Our method outperformed ImageJ and can detect light spots with various shape and size. However there are still some problems as

1. When the intensity of background is high, noises are detected as light spots.
2. When intensity of background and light spot are similar, detection is difficult.

The current method used only local region with 3 x 3 pixels. Namely, we pay attention to only a light spot now. One way is to use the neighboring contextual information around a light spot. If we use the neighboring information around a light spot, the computer may train the context information automatically. This is a subject for future works.

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