SICE Annual Conference 2010
August 18-21, 2010, The Grand Hotel, Taipei, Taiwan

Detecting and Tracking Multiple Fluorescence Spot in Noisy DXT Image Sequence

Reo Sakakura , Yoshimitsu Aoki
Keio University Faculty of Science and Technology
Kanagawa , Japan
rsakakura@aoki-medialab.org

Keishi Shimizu , Shigetoshi Oiki
The University of Fukui
Fukui , Japan

Abstract— **Quantitative analysis of molecular structural change by means of Diffracted X-ray tracking (DXT) method requires tracking of dozens of bright spots in noisy image sequence. As the observation equipment upgrades, experimental data was cannot be analyzed manually with sufficient speed and accuracy. We propose automatic analysis method combining background estimation and frame-by-frame matching. We applied propose method to real DXT image data acquired for potassium ion channel structural changing analysis for evaluation. The result of experiments show that the method has high accuracy and be able to replace manual analysis.**

Keywords-component; background etimation;fluorescence image sequence;kernel density estimation;multiple object tracking;occurrence probability

I. Introduction

Recently, single molecular behavior measurement according to the function appearance of the functionality protein molecule is actively done in the study field like biophysics and molecular physiology. Especially, the membrane protein (ion channel, ion pump, transporter, etc.) that has the regulating function in the inside and outside of the cell is very important research object.

DXT method can obtain a minute change of molecular configuration in real time as a image sequence[1]. Structural change is indirectly analyzed by detecting and tracking the bright spot appears in the image sequence. However, DXT image sequence is very noisy and has a luminance gradient so that makes spot detection difficult. Existing approach to spot detection is background estimation using robust statistics[2]. Estimate background level for each pixels frame-by-frame after that subtract background image from input image to detecting pixels that spot exist. Though this method can detect spots that experts overlook , it cannot replace visual detection because percentage of over detection about 30% .

In the current state, existing automatic analysis method cannot demonstrate effectiveness, and will rely on specialist's watching detection and manual tracking for the DXT image sequence. However, the greater part of experimental data have been left without being analyzed enough because of huge amount of time and the labor are required for a manual analysis.

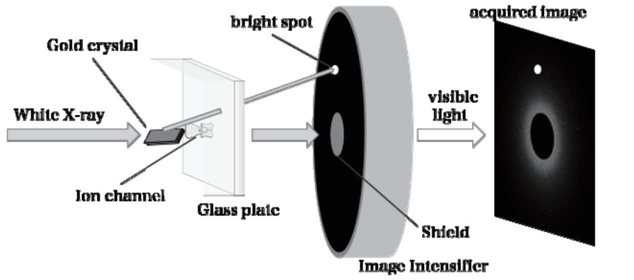

Fig1.DXT method experimental system(Actually fixed on a glass substrate to multiple ion channels.)

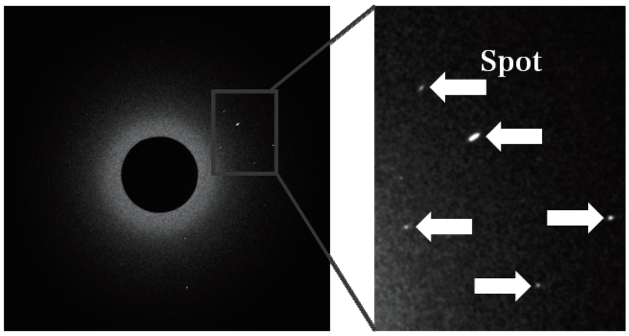

Fig2. DXT image and Spot

II. Proposed Method

Our proposed method is composed of spot detection based on occurrence probability of pixel value and point matching of frame-by-frame based on local search. It deals with the change of the background level by the background model's consecutive update. The flow of the proposal method is shown below.

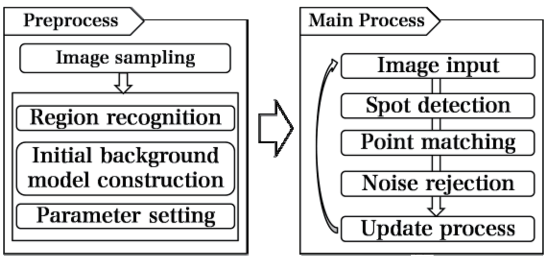

Fig3.The flow of proposed method

A. Kernel density estimation

DXT image sequence background has two problem. One is a background luminance gradient caused by the principle of DXT method. The other thing is flickering intensity value cause by noise. The fluctuation band by the noise is also different in each pixels because of the position of the image according to the above-mentioned luminance gradient. We do background subtraction based on background modeling to detect spots to deal with these issues. As background modeling technique, we used kernel density estimation.

Kernel density estimation is a particular nonparametric method that estimates the underlying density. In this method, the underlying probability density function (pdf) P(X) at X is calculated using sample Xi(i=1,...,N)

$$P(X) = \frac{1}{N}\sum_{i=1}^{N} K(X - X_i) \qquad (1)$$

where K is called window function it should satisfy $K(t) \geq 0$ and $\int K(t)dt = 1$. We chose K to be Gaussian, then the density estimated as

$$P(X) = \frac{1}{N}\sum_{i=1}^{N} \frac{1}{\sqrt{2\pi\sigma^2}} \exp(-\frac{1}{2}\frac{(X - X_i)^2}{\sigma^2}). \quad (2)$$

In our study, Xi is the set of observed brightness of each pixel in the past and we choose N = 300.

B. Spot detection

The initial set of background images, it is desirable that there are no spots. However DXT method cannot control the appearance and disappearance of spots during the video acquisition, background images were obtained for the ideal background will differ for each experimental condition is also difficult. So we build an initial background in the following way.

First, obtain 300 images at random from among all the images. Then remove what the value is zero and the high brightness derived from the spot from value of 300 in each pixels.

Kernel density estimation is to calculate the occurrence probability of the pixel intensities. If the pixel was background, the occurrence probability is higher because occurrence of value close in the past is high. Using this probability estimate, the pixel is considered to be a foreground pixel if P(X)<Pth and X>TH, where the threshold Pth is the global occurrence probability threshold over all images and TH is pixel value threshold set in each pixels.

Fig.4 is the result of spot detection changing Pth. This time, we use experimentally-determined Pth = 0.0003.

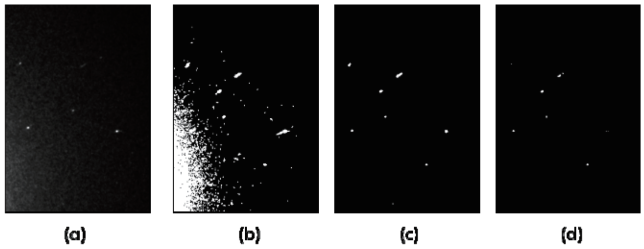

Fig.4 (a)original image (b) detection result of Pth = 0.001 (c) Pth = 0.0001 (d) Pth = 0.00001

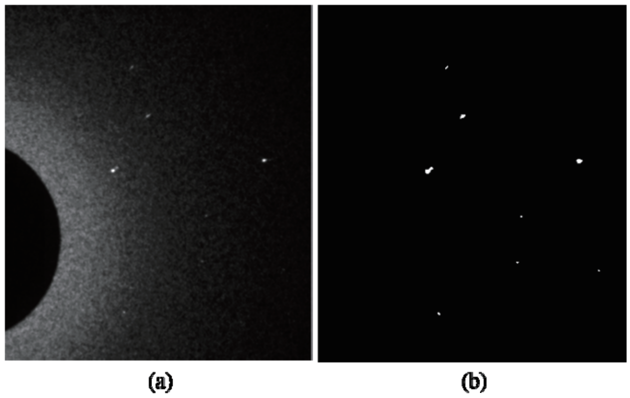

Fig.5 (a)Original image. (b) Result of Detection

The one that is adjacent in the detected pixel is treated as one spot candidate point. After that, the spot where the area is one pixel removed as a noise.

C. Spot Tracking

Tracked over time to spot potential obtained by the preceding point.

First, tracking process estimates the gravity point that spot which observed in the past using each velocity parameter (vx,vy) and acceleration parameter (ax,ay). Then, the candidate point which is nearest from estimated point was matched with past spot.

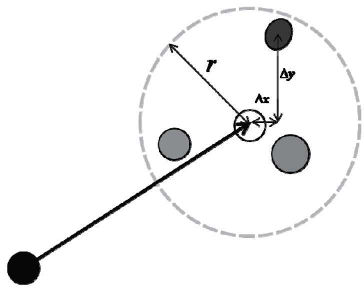

Fig.6 matching image r=20pixel

At present, the result of detection includes miss detection so we have to evaluate the case is miss detection or overlap when two spots matched same candidate point. If there were no slate point in search area, the spot was assumed per primam miss detection. In addition, if the spot was not detected in consecutive 10 frames , it was decided that was disappeared .

Candidate points for the first stage of the spot and finished the match appeared in the past or a new spot, a determination of noise. The other spots which did not matched are evaluated there are new spots if they were matched in frames t+1~t+5. Otherwise are noise.

D. Updating Background Model

The end of process for each frames, pixel value which pixel is determined the background is stored into the background model instead of oldest luminance data for each pixels.

III. EXPERIMENTAL RESULT

We experienced our method using real DXT image sequence. For Detection experiment, we use 2 data set (data1; 40images, data2; 30images) both was chosen suitably from different DXT image sequence. Tracking experiment , we verify 60 spots that seems exist in image sequence for a long time. Lacking ground truth for the real data, we evaluated the performance of our algorithm by visual comparison with visual detecting and manual tracking results.

The detection result of comparison are presented in Table I. Two performance measures are listed: detection rate, which is the ratio between the number of spot detected by visual and number of correct result of proposed method, and accuracy rate, which is the ratio between the number of false and correct. Ideally, the values for both ratios should be equal to 100%.

The tracking result of tracking are presented in Table II. The success judgment of the tracking was divided into two stages, success1 is tracking with same label number from beginning to the end, success2 is correct trajectory but label number was changed on the way.

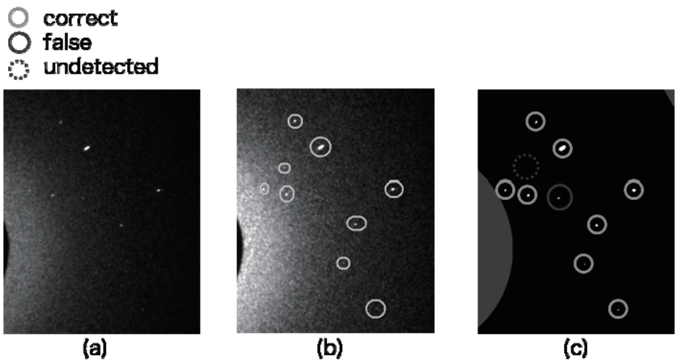

Fig.7 Example of the detection result determination (a)original image (b) visual detection (c) subtraction result

IV. CONCLUSION

We proposed automatic analysis method for DXT image sequence. From the outcome of an experiment, our method was shown high accuracy rate and it means method can replace manual tracking. The algorism running on a PC (Core2 (TM) Extreme 2.53GHz, 8GB of RAM), processing time per frame is about 2sec.We expect that faster execution times are still possible.

As future challenges, we are going to improve the detection rate while maintaining the accuracy rate. Currently, number of undetected has significant impact on the tracking so we expect that, improving the detection rate rise a success rate of tracking. In addition, it also kept to improve inadequate to address issues as they arise in a cross track.

REFERENCES

[1] Sasaki, Y.C., Okumura, Y., Adachi, S., Suda, H., Taniguchi, Y., and Yagi, N.,(2001),"Picometer-scale dynamical x-ray imaging of single DNA molecules",Phys. Rev. Lett. 87,248102

[2] H. Eda, K. Shimizu, S. Oiki and Y. Aoki, "Image Analysis for Potassium Ion Channel Dynamics. Spots Detection and Tracking on X-ray Diffraction Image." Pattern measurement symposium .Japan , vol.12th Page25-30(2007)

[3] Ahmed Elgammal, Ramani Duraiswami, David Harwood, Larry S.Davis, "Background and Foreground Modeling Using Nonparametric Kernel Density Estimation for Visual Surveillance",Proceedings of the IEEE,Vol.90,No.7,July2002.

[4] Hirofumi Shimizu, Masayuki Iwamoto, Takashi Konno, Amiko Nihei, Yuji C. Sasaki, Shigetoshi Oiki "Global Twisting Motion of Single Molecular KcsA Potassium Channel upon Gating",Cell 132,67-78,January 11,2008.

TABLE I. THE RESULT OF SPOT DETECTION

		data1	data2
Visual detection		1449	759
Proposed method	All	1036	624
	Correct	1024	576
	False	12	48
	Undetected	467	183
detection rate		**68.7%**	**75.9%**
accuracy rate		**98.8%**	**92.3%**

TABLE II. THE RESULT OF TRACKING

SUCCESS	1	62.3%	**83.1%**
	2	20.8%	
FALSE			**16.9%**