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Application of Improved Wavelet Transform in Biological Particle Detection*

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Abstract Particle detection of fluorescent images has become an indispensable tool in biological research. Here a simple and fast method for biological particles detection with high efficiency and accuracy, improved wavelet transform (IWT) was introduced. IWT originates from wavelet multiscale products (WMP). However, it resolves the problems in WMP and is more adaptive in dealing with different types of images. The performance of IWT, WMP and MSVST (multiscale variance stabilizing transform) was quantitatively evaluate by using both synthetic and real fluorescence images. Experimental results show that IWT performs much better than WMP in most cases, and has comparable results with the much complicated algorithm, MSVST. Besides, IWT is 20% faster than MSVST when processing the same images. Therefore, it was concluded that IWT can be generally used for the automatic detection of different kinds of biological particles, and the simplicity and accuracy make it a better choice for fluorescent image analysis.

Key words particle detection, IWT, WMP, MSVST
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With the widely use of bright fluorescent proteins and the development of live-cell imaging, it becomes possible to study subcelluar dynamics and obtain a better understanding of molecular mechanism underlying biological phenomenon. Quantitative analysis of these fluorescent imaging data involves the detection of biological particles (vesicles, viruses, bacteria, or single molecules) to gain useful information (number, position, intensity, etc.). Particle detection is fundamental for the single particle tracking techniques(SPT), which is to extract more complicated kinetic information (trajectory, velocity, life time, mean square displacement and so on) for further analysis. As we know, the signal to noise ratios (SNRs) of fluorescent images can be very low because they are contaminated by both photon noise (Poisson) and camera readout noise (Gaussian). Although manual detection can yield the most accurate results, it is very time consuming and impractical to be used on large set of image data. Therefore, it is essential to use a computerized algorithm to automate this process. Over past decades, a number of automatic detection methods have been proposed to address this difficult task, such as adaptive thresholding [1], top-hat filter [2], and morphological grayscale opening filter [3]. These methods do not give satisfactory results with biological images due to two reasons: first, biological images most often have low global SNRs and various local SNRs; second, particles have an inhomogeneous gray level distribution over the image while, at the same time, the image may present uneven background [4]. Recently, some wavelet-based techniques [4-5] were proposed to solve this problem. In this paper, we introduce an improved wavelet transform algorithm (IWT), based on wavelet multiscale products(WMP)[4], for particle detection and compare it with multiscale

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variance stabilizing transform (MSVST) [5], one of the best detection methods[6].

1 Detection method

1.1 WMP

The wavelet transform is a multiresolution analysis tool characterized by global localization properties in time/frequency or scale/space representations [4]. The isotropic undecimated wavelet transform (IUWT) [7] was first generally used in astronomical applications to detect isotropic objects [8] and then introduced to processing biomedical images. Based on an undecimated wavelet representation of the image and on the selective filtering of wavelet coefficients, Jean-Christophe Olivo-Marin approached the problem of feature detection as a process of extracting and combining multilevel elements of response, with each element coming from the analysis of an image at successive resolution levels[4].

Here we simply describe the algorithm. WMP uses a symmetric low-pass filter h, B_3-spline filter, that is [1 4 6 4 1]/16. Then we computed a convolution of the image with the filter through row by row followed by column by column, or we could just perform a 2D convolution with 2D B_3-spline filter $h_{B_3} \otimes h_{B_3}$. Here "$\otimes$" denotes tensor product. During the convolution, the image was extended by symmetric mirroring to avoid discontinuity problems at the borders. The same process was repeated recursively with a filter augmented at each scale i by inserting $2^{i-1}-1$ zeroes between two taps. Here "*" denotes convolution.

$$\begin{cases} A_i(x, y) = h^{i-1} * A_{i-1}(x, y) \\ W_i(x, y) = A_{i-1}(x, y) - A_i(x, y) \end{cases} \quad 0 < i \leqslant J \tag{1}$$

Then a hard threshold method was used to reduce the influence of noisy wavelet coefficients.

$$t_{\text{hard}}(W_i, t_i) = \begin{cases} W_i(x, y) & W_i(x, y) \geqslant t_i \\ 0 & W_i(x, y) < t_i \end{cases} \tag{2}$$

After that, a direct spatial multiscale product of each wavelet coefficients was performed to get a correlation image.

$$P_J(x, y) = \prod_{i=1}^{J} W_i(x, y) \tag{3}$$

Finally, the values in the correlation image were compared to a predetermined detection level to discriminate between particles and background and get a binary image of particles.

$$P_J(x, y) = \begin{cases} 255 & |P_J(x, y)| \geqslant l_d \\ 0 & \text{Otherwise} \end{cases} \tag{4}$$

By combining multiscale information elements by coefficient correlation, WMP can effectively detect particles in a simple way. However, when dealing with images with large Poisson noise, WMP fails to produce satisfactory results. Moreover, a hard threshold method[9] makes it difficult to be used to process different kind of images. Besides, the direct spatial multiscale product of each wavelet coefficients leads to discretization when images with low SNRs or particles of large size are processed.

1.2 MSVST

In order to better denoise Poisson noise, Zhang *et al.*[10] introduced a variance stabilizing transform (VST), which was applied to transform a discrete Poisson process into a Gaussian-like process with asymptotic constant variance. After that, he combined this VST with the filter banks of wavelets, leading to multiscale variance stabilizing transform. For MSVST, we applied VST on the approximation coefficients at each scale.

$$\begin{cases} A_i(x, y) = h^{i-1} * A_{i-1}(x, y) \\ W_i(x, y) = T_{i-1}(A_{i-1}(x, y)) - T_i(A_i(x, y)) \end{cases} \quad 0 < i \leqslant J \tag{5}$$

Where

$$T_i(A_i(x, y)) = b^{(i)} \operatorname{Sgn}(A_i(x, y) + c^{(i)}) \sqrt{|A_i(x, y) + c^{(i)}|} \tag{6}$$

$$\begin{cases} c^{(i)} = \dfrac{7\tau_2^{(i)}}{8\tau_1^{(i)}} - \dfrac{\tau_3^{(i)}}{2\tau_2^{(i)}} \\ b^{(i)} = 2\sqrt{\dfrac{\tau_1^{(i)}}{\tau_2^{(i)}}} \end{cases} \tag{7}$$

$$\tau_k^{(i)} = \sum (h^{(i)}[i])^k \tag{8}$$

The significant coefficients were determined by carrying out a false discovery rate (FDR) [11] control. After that, the hybrid steepest descent (HSD) [12] iterations were used for the reconstruction of image. Simply zeroing the approximation band at the last iteration to suppress the background, detail (particle) structures were picked up[5].

The MSVST method performs much better than WMP in both accuracy and adaptiveness. However, it is limited by two main reasons: First, the algorithm is quite complicated and requires high power computer to be used if large datasets are to be processed. Second, during the process, the Gaussian and Poisson components of noise need to be accurately estimated, which is very difficult and sometimes impossible. In fact, there are about three methods for the estimation of noise directly from real images[5, 13-14], but all of them break down when dealing with images with high numbers of photons. In this situation, we can only make a rough estimation and modify manually.

1.3 IWT

Both WMP and MSVST are based on the convolution of the image with the filter. The difference is that WMP performs a direct spatial product each wavelet coefficients while MSVST uses HSD iterations, which in fact is a spatial summation. Since features are embedded in different coefficients, product will reduce the noise but sometimes lead to discretization while summation keeps both signals and noise. Thus, we introduce IWT to find a balance between WMP and MSVST. IWT combines VST with wavelet coefficients just as MSVST (5). Then we changed the hard threshold of WMP t_i to:

$$\begin{cases} t_i = k\sigma_i \\ \sigma_i = \bar{\sigma}_i / 0.67 \end{cases} \tag{9}$$

Here $\bar{\sigma}_i$ is the median absolute deviation (MAD)[15] of wavelet coefficient at scale i, and k ranging from 1 to 10 can be easily adjusted. Next we select either one or more wavelet coefficients to compute a product or summation according to the actual data.

$$P_J(x, y) = \prod_{i=1}^{J} W_i(x, y) \text{ or } P_J(x, y) = \sum_{i=1}^{J} W_i(x, y) \tag{10}$$

Finally, we maintained the values above l_d to eliminate noise and dark particles, which is subjected to manual adjust to obtain particles with different shape and intensity.

$$P_J(x, y) = \begin{cases} P_J(x, y) & |P_J(x, y)| \geqslant l_d \\ 0 & \text{Otherwise} \end{cases} \tag{11}$$

IWT applies a VST to Gaussianize the data so that each sample is near-normally distributed with an asymptotically constant variance, making it suitable for denoising images containing both Gaussian and Poisson noise. By increasing the filters size during convolution, the analysis is inherent adaptive to different particle size [4]. In our actual experiment, we found that using 3 scales of filters could handle most images. Therefore, we obtained W_1, W_2, W_3 and $A3$. Noting that the first wavelet coefficient W_1 represented mostly high frequency noises and $A3$ represented background information, which leaved the real signal to wavelet coefficients W_2 and W_3. Thus, the FDR control is unnecessary for IWT. Since the HSD iterations method in MSVST is used mainly for the reconstruction of image, we can just zero $A3$ to suppress the background and to detect particles. Consequently, we were able to achieve better detection accuracy in a much simple way. The IWT program is written in Matlab R2008a (MathWorks, USA) and interactive. In next section, we fully evaluated these detection methods using synthetic and real data.

2 Experimental results

2.1 Experiments with synthetic data

Three types of images(Type A, B and C, Figure 1) filled with particles of different SNR and size were generated using an ImageJ (NIH, USA) plugin[6]. In this paper, SNR is defined as the difference in intensity between particle and background, divided by the standard deviation of the particle noise [16]. The size of

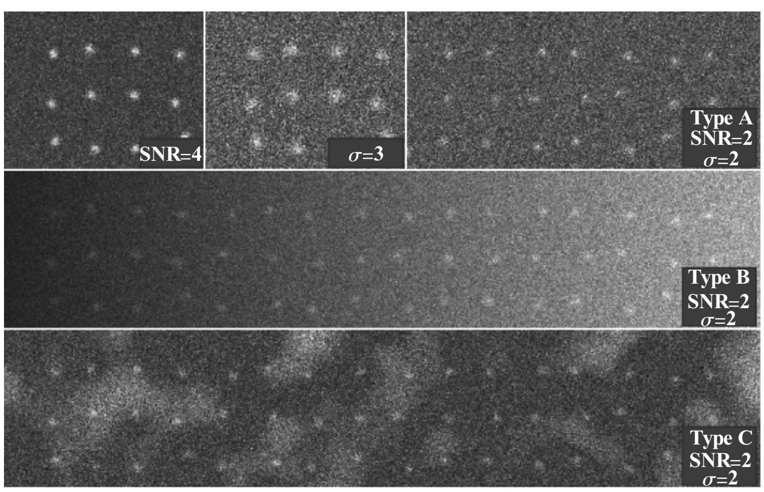

Fig. 1 Examples of synthetic images used in the experiments

The symmetrical GIPs are embedded into uniform (Type A), gradient (Type B) and non-uniform (Type C) backgrounds.

the particle is defined as sigma (σ), the standard deviation of the 2D Gaussian intensity profiles (GIPs). Type A images are constructed by adding a background level 10 to GIPs and applying a Poisson noise generator independently to every pixel of the noise-free image. For Type B images, the background level increased linearly in the horizontal direction from a value of 10 at the left image border to 50 at the right border. Type C images mimic the intensity distribution in the presence of large background structures, leading to a non-uniform background. In order to test the adaptiveness of each algorithm, we generated 10 frames of each Type of images (each contains 256 particles) with SNR ranging from 1 to 5 and sigma ranging from 1 to 6.

For quantitative comparison, we evaluated each performance of three methods by computing true positive rate (TPR) and false positive rate (FPR). Firstly, we defined N_{TP} as the number of true positives and N_{FP} as the number of false positives. Then $TPR = N_{TP}/(N_{TP}+N_{FN})$, and $FPR = N_{FP}/(N_{TP}+N_{TN})$. Because the number of true negatives (N_{TN}) is not defined, the modified $FPR^* = N_{FP}/(N_{TP}+N_{FN})$, where the number of false negatives, $N_{FN}=N_0-N_{FP}$ and N_0 is the total number of particles in the ground truth.

The performance of three detection methods was compared at the level of FPR^* =0.01. In the test of images with different SNR (Figure 2a, b, c), we found that IWT give almost the same performance as MSVST and better than WMP at SNR 1 to 2. But the difference of the performance decreased with SNR increased, and for SNR > 3, three methods performed equally well (TPR=1). The improvement of IWT over WMP is much more obvious in the test of different σ (Figure 2d, e, f). The low TPR of WMP was the result of discretization, especially when σ >2. Thus, we conclude that WMP is much more sensitivity to parameter change than MSVST and IWT.

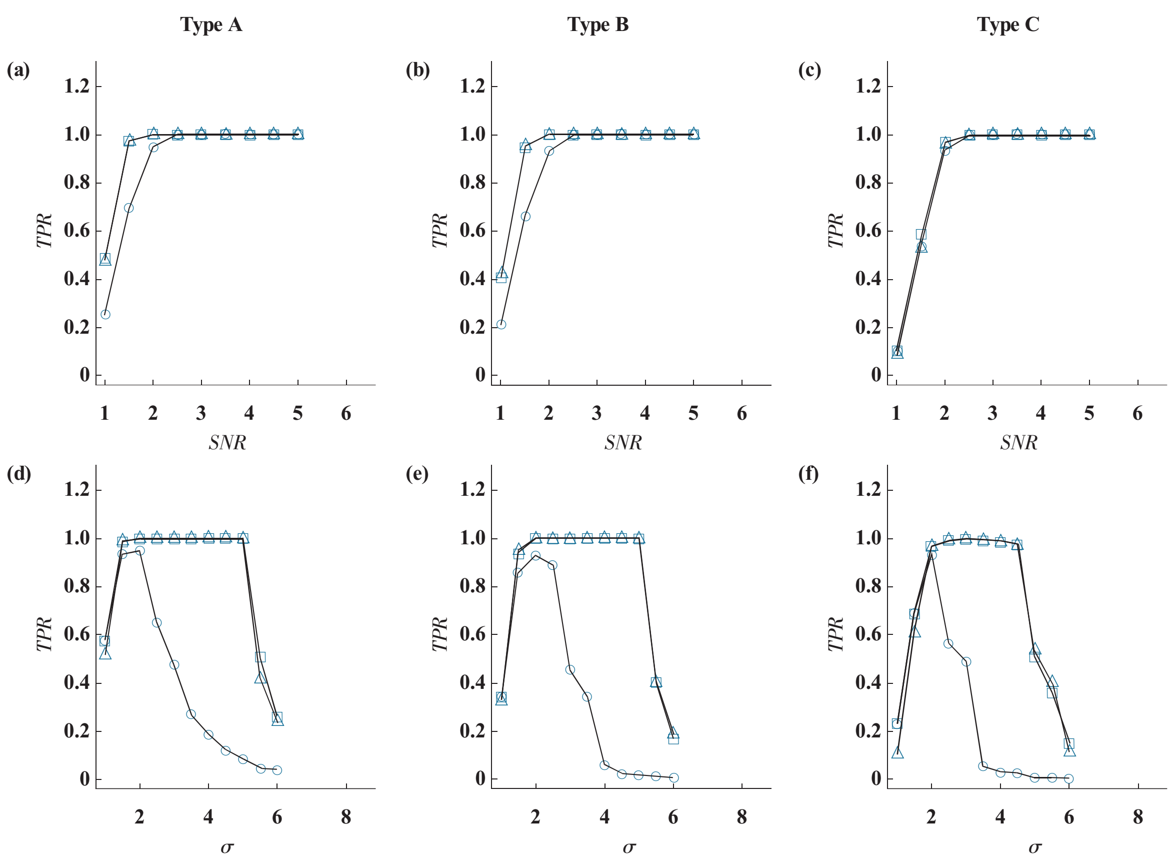

Fig. 2 Detection result of three type of images with different SNR or σ at the level of FDR^*=0.01

For (a, b, c), σ is 2 and SNR is 2 for (d, e, f). ○—○ : WMP; △—△ : MSVST; □—□ : IWT.

2.2 Experiments with real data

We also tested the detection methods using three real images. The first one is a TIRFM (total internal reflection fluorescence microscopy) movie of LβT2 cell labeled with pHluorin. The movie consists 50 frames and each containing 70 to 80 particles at *SNR* about 2～5. Figure 3b shows a single molecular PALM (photoactivated localization microscopy) movie of HEK293 cell labeled with mEosFP. The movie consists 50 frames and each containing 70 to 90 particles at *SNR* about 2～5. The third one is a TIRFM movie of PC12 cell labeled with GFP. The movie consists 10 frames and each containing 120 to 140 particles at *SNR* about 3～6. These movies contain particles of different *SNR* and varying size with non-uniform background, so they were more difficult for detection test than synthetic images.

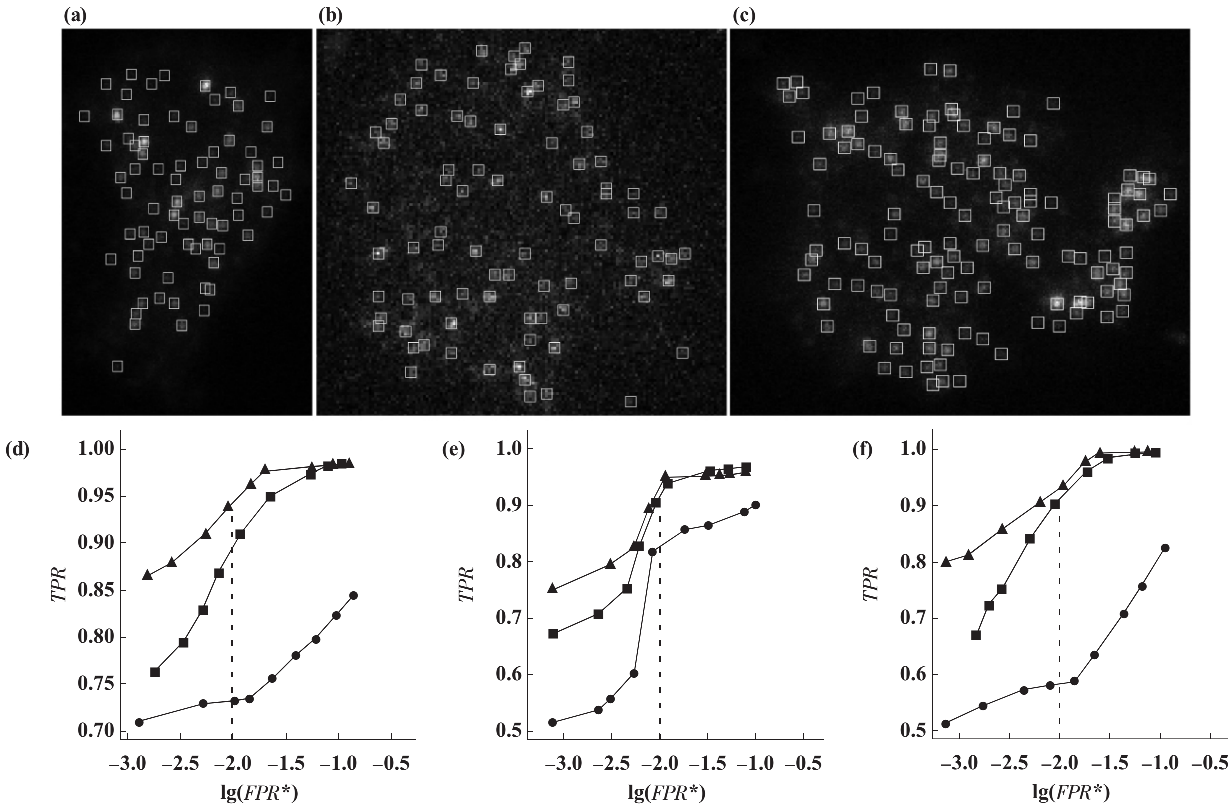

Fig. 3 Examples of three movies (a, b c) with manual particle detection (white squares) serving as ground truth

The corresponding results (d, e, f) are shown below the images. Dash line denotes *FPR**=0.01. ●—●: WMP; ▲—▲: MSVST; ■—■: IWT.

Since the ground truth of these real images was not available, the detection results were compared with manual inspection. From these results, we showed that the performance of the detection methods depend on the application types. MSVST showed the best performance and WMP was the worst. The IWT algorithm out-performed WMP in all aspects, and yielded comparable results to MSVST, especially when *FPR** >0.01 (Dash line in Figure 3). This agrees with the detection results of synthetic data. We could also see that when *FPR**=0.1, IWT obtained the best TPR of three. Moreover, IWT was 20 percent faster as compared with MSVST when denoising the same images (from Table 1).

Table 1 Time cost (second) of the detection methods with the three movies

Movie	WMP	MSVST	IWT
LβT2	6.31	8.30	6.43
HEK293	5.25	7.03	5.34
PC12	1.60	2.08	1.66

3 Discussion

In this paper we introduce a new method for particle detection and compare it with the other two wavelet methods, WMP and MSVST. The results from experiments on both synthetic and real images indicated that IWT has a great improvement over WMP. It increases the adaptiveness as well as accuracy. MSVST gives the best result but is too complicated to be implemented. The estimation of Poisson and Gaussian noise of images is very difficult and sometimes impossible when processing images with lots of photons. This is the very reason why MSVST fails to out-perform IWT in synthetic data (Figure 2) (The synthetic data is 16 bit and has a gray value above 60 000 while the real data has a much smaller value). Besides, MSVST consumes more time to compute than IWT (Table 1). Consequently, in biological particle detection, IWT can replace WMP that perform relatively similar to MSVST without complicated estimation and parameter setting. In our future work, we will try to make IWT more automated and intelligent for detection and recognition of biological particles while keeping the simplicity and convenience at the same time.

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改良的小波变换算法在生物微粒检测中的应用 *

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摘要 荧光图像的微粒检测已经成为了生物学研究中不可或缺的工具之一．介绍了一种改良的小波变换算法(improved wavelet transform，IWT)，该方法实现简单，能够以很高的速度和精度来进行生物微粒的检测．IWT 源自多尺度小波乘积算法(wavelet multiscale products，WMP)，但它不仅解决了 WMP 算法遇到的问题，而且在处理各类图像的时候具有更强的适应性．使用人工合成的图像和真实的图像来定量地分析 IWT、WMP 以及多尺度方差稳定变换算法(multiscale variance stabilizing transform，MSVST)的检测效果．实验结果表明，IWT 在大多数情况下的检测效果比 WMP 好很多，且与更为复杂的 MSVST 算法相当．此外，在处理相同图像时，IWT 的速度比 MSVST 快 20%．因此，IWT 算法能够普遍适用于各种生物微粒的自动化检测，其简单准确的特点使之成为荧光图像分析更好的选择．

关键词 微粒检测，IWT，WMP，MSVST

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