A Detection Algorithm Based on Matrix Factorization for Live Mitochondria in Fluorescent Microscopic Images

[Extended Abstract]

***Abstract*--With the development of biomedicine, detection of mitochondria in fluorescence microscopic images is an important method to explore the nature of life phenomena. However, limited by fluorescence microscopy, two parts which are the shadow of cytoplasm and live mitochondria are contained in general microscopic images. For this reason, the signal-to-noise ratio (SNR) of the live mitochondria time sequence images drops rapidly and background complexity increases greatly, which can't satisfy the requirements of traditional particle detection algorithm. We present a new detection algorithm for live mitochondria in fluorescent microscopic images in this paper. In order to realize this method rapidly, we use augmented Lagrange multiplier algorithm. Shadow of cytoplasm is removed by this algorithm effectively, and mitochondria can be detected in fluorescent microscopic images accurately. Therefore, the proposed algorithm provides an efficient and accurate tool to detect mitochondria in live cells.**

Keywords--mitochondria; matrix factorization; optimization problem; augmented Lagrange multiplier algorithm

I. INTRODUCTION

With the development of fluorescence microscopic imaging technology, fluorescent microscopy becomes one of the most important tools in the biomedical science to study apoptosis of cells and explore the nature of life phenomena recently. In 2006, Conradt[1] published a paper which showed the mitochondria transfer had an influence on cell regulation mechanism. Thus detection of mitochondria in biological fluorescence microscopic image sequences is a research hotspot in the area of biomedical image processing.

Limited by fluorescence microscopy, the signal-to-noise ratio (SNR) of microscopic images can't satisfy requirements of the traditional detection algorithms. Fluorescent biological images often present background unevenly, and mitochondria particles are of small size and have no obvious boundaries. These particles are labeled by green fluorescent protein (GFP) and attached to axon, which have an inhomogeneous grey level distribution[2]. So it is difficult to develop an algorithm that can detect mitochondria in fluorescent microscopic images. The main difficulties are shown as below:

1) Lack of reliable features: Good features that we use to distinguish objects of interest from others are critical in designing object tracking algorithms. Common features in images include color, edges, intensity and so on. However, mitochondria are of small size and influenced by an uneven background in microscopic images. So, we are short of reliable features to detect mitochondria in images.

2) Influence of background interference: Because the fluorescence microscopic imaging technology is not consummate, the signal-to-noise ratio (SNR) of the live mitochondria time sequence images is generally low. Interfered by cytoplasm shadow, image background complexity is very high. Microscopic images can't meet the requirements of traditional particle detection algorithm.

Automatic detection of mitochondria in live cells is a very challenging task, a large number of methods have been proposed to complete this task over the past decades. Olivo[3] proposed a multi-resolution algorithm for the detection of particles, which is based on à trous wavelet transform. This algorithm can obtain accurate results when the SNR of the microscopic image is high. But with the decrease of the SNR and increase of background complexity, the performance of the algorithm drops rapidly. Jiang[4] used a machine learning method, which is based on Haar features, and it can detect a part of fast moving target particles in images.

Baraniuk[5] and Donoho[6] introduced a new technique called compressive sensing, which has been widely used in the field of signal processing and image processing. This theory which makes full use of the characteristic that most signals are sparse in reality and reduces the sampling rate greatly on the premise of reconstructing original signals accurately which goes beyond the classic Nyquist-Shannon sampling theorem. Matrix factorization theory which is based on the theory of compressed sensing is proposed, and we can use this theory to decompose a high dimensional signal into a low-rank component and a sparse component. This step provides great help to further processing of signals.

We present an effective automatic detection algorithm which is based on matrix factorization method for live mitochondria in fluorescent microscopic time sequence images. Because microscopic image sequence has many similarities between frame and frame, we put every frame of the image sequence into a matrix whose rank is 1. According to this property, the matrix can be decomposed by ℓ_1 optimization, and the inhomogeneous grey level distribution of background in every frame and mitochondria are viewed as the low-rank component and the sparse component respectively. In this way, we get images which contain nothing but live mitochondria and separated mitochondria from cytoplasm effectively. The new images have higher SNR and lower background complexity, which meet the requirements of general particle detection algorithm to detect live mitochondria accurately.

II. MITOCHONDRIAL DETECTION MODEL BASED ON MATRIX FACTORIZATION

The images that are used in this article are the mitochondrial fluorescence microscopic sequences with drosophila neuronal axons, in which mitochondria are labeled by GFP. Dynamic characteristics of mitochondria of drosophila neuronal axons were studied in exploring the

pathogenesis of amyotrophic lateral sclerosis (ALS) has achieved important results in article[7]. The SNR of these images declines and background complexity augments since axon cells contain cytoplasm shadow. All of these make it very difficult to detect mitochondria in images. However, when we observe the image sequence, we find that there are many similarities of cytoplasm shadow between frames. Moreover, according to Pilling's research[8], 57% of mitochondria are stationary, and only 43% of mitochondria are dynamic.

Therefore, we suppose each frame $\boldsymbol{D}_{\mathrm{i}}$ can be decomposed into cytoplasm shadow in neural axon cells and mitochondria labeled by GFP, which can be denoted by $\boldsymbol{A}$ and $\boldsymbol{E}$ respectively. Model can be formulated as:

$$\boldsymbol{D} = \boldsymbol{A} + \boldsymbol{E} \tag{1}$$

We stack each frame $\boldsymbol{D}_{\mathrm{i}}$ as a column of a matrix $\boldsymbol{D}$, whose rank is 1. After we decompose $\boldsymbol{D}$, mitochondria $\boldsymbol{E}$ has only a few elements which are not zero, which means that $\boldsymbol{E}$ is sparse. Cytoplasm shadow in one frame of image sequence is very similar to that in other frames, so cytoplasm shadow $\boldsymbol{A}$ can be taken a low–rank matrix. In order to decompose (1) exactly, the objective function can be set to $\mathrm{rank}(\boldsymbol{A}) + \lambda\|\boldsymbol{E}\|_0$, and the problem is transformed into an optimization problem. Candès[9] called the optimization problem was Robust PCA(RPCA). The matrix $\boldsymbol{D}$ can be decomposed into a low-rank matrix $\boldsymbol{A}$ and a sparse matrix $\boldsymbol{E}$ by solving the optimization problem, so we can achieve the goal that live mitochondria are separated from shadow of cytoplasm.

III. ALGORITHM

Matrix recovery[10] (MR) is dubbed as Robust PCA or sparse matrix with low-rank matrix factorization as well. This is a curious phenomenon that we can identify elements of a matrix which are "polluted" seriously and reconstruct original matrix at the same time. (In fact, we are interested in all of those "polluted" elements which are mitochondria in live cells.) We suppose the original matrix is a low-rank matrix and polluted elements are sparse. Hence matrix recovery can be expressed by the optimization problem:

$$\begin{aligned} &\min \quad \mathrm{rank}(\boldsymbol{X}) + \lambda\|\boldsymbol{E}\|_0 \\ &\text{subject to} \quad \boldsymbol{A} + \boldsymbol{E} = \boldsymbol{D} \end{aligned} \tag{2}$$

where $\|\cdot\|_0$ represents ℓ_0-norm of a matrix, and λ is a positive weighting parameter for noise. However, this formula is combinatorially complex and intractable to solve, so the complexity of computing is exponential. In order to reduce the complexity of computing, we relax it by replacing ℓ_0-norm with ℓ_1-norm, which means that ℓ_1-norm and nuclear norm have been shown to be surrogates for ℓ_0-norm and rank of the matrix. The original problem can be transformed as:

$$\begin{aligned} &\min \quad \|\boldsymbol{X}\|_* + \lambda\|\boldsymbol{E}\|_1 \\ &\text{subject to} \quad \boldsymbol{A} + \boldsymbol{E} = \boldsymbol{D} \end{aligned} \tag{3}$$

Here, $\|\cdot\|_*$ and $\|\cdot\|_1$ are nuclear norm and ℓ_1-norm of a matrix respectively. $\|\cdot\|_*$ is denoted as $\|\boldsymbol{X}\|_* = \sum_{i=1}^{r}\sigma_i(\boldsymbol{X})$, and $\sigma_i(\boldsymbol{X})$ is the ith singular value of the matrix $\boldsymbol{X}$. $\|\cdot\|_1$ is defined as $\|\boldsymbol{X}\|_1 = \sum_{i,j}^{n}\boldsymbol{E}_{i,j}$, which is the sum of the absolute values of matrix entries, and $\boldsymbol{E}_{i,j}$ is the element of the matrix $\boldsymbol{E}$. This is a convex optimization problem whose size of dimension is very large. It is critical to solve this problem quickly, so we propose augmented Lagrange multiplier algorithm to solve (3). Augmented Lagrange multiplier algorithm of matrix factorization method is described as:

$$L(\boldsymbol{A},\boldsymbol{E},\boldsymbol{Y},\mu) = \|\boldsymbol{A}\|_* + \lambda\|\boldsymbol{E}\|_1 + \langle \boldsymbol{Y}, \boldsymbol{D} - \boldsymbol{A} - \boldsymbol{E}\rangle + \frac{\mu}{2}\|\boldsymbol{D} - \boldsymbol{A} - \boldsymbol{E}\|_F^2 \tag{4}$$

where $\|\cdot\|_{\mathrm{F}}$ is Frobenius norm. In order to minimize (4), we update $\boldsymbol{A}$ and $\boldsymbol{E}$ alternately. First of all, we obtain $\boldsymbol{A}$ to minimize L(·) by fixing $\boldsymbol{E}$ and $\boldsymbol{Y}$. Then, we obtain $\boldsymbol{E}$ to minimize L(·) by fixing $\boldsymbol{A}$ and $\boldsymbol{Y}$ at the same way. In this way, the problem is solved to get the optimal solution.

When we update $\boldsymbol{A}$, the formula is:

$$\arg\min_{\boldsymbol{A}}\|\boldsymbol{A}\|_* + \frac{\mu}{2}\|\boldsymbol{D} - \boldsymbol{A} - \boldsymbol{E} + \mu^{-1}Y\|_F^2 = \boldsymbol{D}_{\mu^{-1}}(\boldsymbol{D} - \boldsymbol{E} + \mu^{-1}\boldsymbol{Y}) \tag{5}$$

When we update E, the formula is:

$$\arg\min_{\boldsymbol{A}}\|\boldsymbol{E}\|_1 + \frac{\mu}{2}\|\boldsymbol{D} - \boldsymbol{A} - \boldsymbol{E} + \mu^{-1}\boldsymbol{Y}\|_F^2 = S_{\frac{\lambda}{\mu}}(\boldsymbol{D} - \boldsymbol{E} + \mu^{-1}\boldsymbol{Y}) \tag{6}$$

According to (5) and (6), the solutions will be updated until the original problem is converged. Actually, there is no need to calculate exact solutions of sub-problem in each step. We update $\boldsymbol{A}$ and $\boldsymbol{E}$ to obtain an approximate solution of the sup-problem only once, and it is enough to make the algorithm converge quickly and acquire the optimal solution. This algorithm is called augmented Lagrange multiplier algorithm. Here, $\boldsymbol{D}(\cdot)$ represents singular value decomposition, which is expressed by $svd(\cdot)$ in algorithm. The soft-thresholding scalar operator $\boldsymbol{S}(\cdot)$ is defined as:

$$\boldsymbol{S}_{\varepsilon}(x) = \begin{cases} sign(x)(|x| - \varepsilon) & |x| > \varepsilon \\ 0 & \text{otherwise} \end{cases} \tag{7}$$

In the algorithm, λ is balance parameter determines the proportion of mitochondria separated from cytoplasm. We make λ=1600 according to resolution of the images. ρ is accelerating factor, the value range of which is from 0 to 1. When ρ=0.7, the algorithm gets the fastest convergence speed in the experiments. Initial value μ_0 can be set as any value greater than 1 which is set as 2 in experiments.

The detail of augmented Lagrange multiplier algorithm can be expressed as follows:

Algorithm 1: Augmented Lagrange multiplier algorithm

Input: $\boldsymbol{Y}_0$, $\boldsymbol{E}_0 = 0$, μ_0=2, $k = 0$, λ=1600
While not converged **do**
$(\boldsymbol{U},\boldsymbol{S},\boldsymbol{V}) = svd(\boldsymbol{D} - \boldsymbol{E}_k - \mu_k^{-1}\boldsymbol{Y}_k)$
$\boldsymbol{A}_{\mathrm{k+1}} = \boldsymbol{U}\boldsymbol{S}_{\boldsymbol{\mu_k}^{-1}}[\boldsymbol{S}]\boldsymbol{V}^{\boldsymbol{T}}$
$\boldsymbol{E}_{k+1} = \boldsymbol{S}_{\lambda\mu_k^{-1}}[\boldsymbol{D} - \boldsymbol{E}_k + \mu_k^{-1}\boldsymbol{Y}_k]$
$\boldsymbol{Y}_{k+1} = \boldsymbol{Y}_k + \mu_k(\boldsymbol{D} - \boldsymbol{A}_{k+1} - \boldsymbol{E}_{k+1})$
$\mu_k = \rho\mu_{k-1}$
k=k+1
end while

IV. EXPERIMENTAL RESULTS

A. Image acquisition

The images that are used in this paper are the mitochondrial fluorescence microscopic sequences with drosophila neuronal axons, which contain 60 frames. Each frame of this sequence has resolution 997*801. We stack each frame as a column of our matrix $\mathbf{D} \in \mathbf{R}^{60\times798597}$. This matrix can meet the matrix factorization requirement that rank of the matrix is 1.

B. Analysis in microscopic image

We adopt the method proposed in this paper to process 60 frames of fluorescence microscopic image sequences. Three frames are selected from processed frames randomly, and results are shown in Figure 1. Figure 1(a) shows three frames from original microscopic sequence; 1(b) and 1(c) show the corresponding low-rank matrix and sparse matrix respectively. Figure 1(b) includes cytoplasm shadow which causes interference on mitochondrial detection, and there is not any mitochondrion in the shadow. In another way, Figure 1(c) contains nothing but live mitochondria, which we are interested in and want to detect. Compared with Figure 1(a), shadow of cytoplasm has been removed basically, and only a few shadow which is the endpoint of drosophila neuronal axons remains in Figure 1(c). Moreover, the remaining part can't interfere with particles detection in images. In this way, we separate mitochondria from the cytoplasm shadow successfully, and obtain higher SNR and lower background complexity of each frame. The frame can satisfy the requirements of general particle detection algorithm. Selection of balance parameter λ is very important in the method since it determines the proportions of sparse component and low-rank component in results. Table I shows the parameters are used in the experiment.

In this paper, we use isotropic undecimated wavelet transform (IUWT) algorithm to detect mitochondria in original microscopic images and in processed microscopic images by matrix factorization respectively; and results are shown in Figure 2(a) and Figure 2(b) respectively. At the same time, mitochondria are detected by spot-enhancing filter algorithm[11] in original microscopic images which are marked in Figure 2(c) as well. The IUWT algorithm can obtain exact results when the SNR of images is very high, but the performance of this method drops quite rapidly when the SNR decreases and background complexity increases. Comparing yellow rectangular regions in 2(a) with 2(b), we notice that owning to the interference of cytoplasm shadow, a few mitochondria are detected in yellow rectangular region of 2(a), and a lot of mitochondria can't be detected exactly. In another way, when the same image is processed by the method which is proposed in this article, the number of mitochondria which are detected in yellow rectangular region of 2(b) increases significantly, and the positions of mitochondria are more accurate. We cannot fail to notice the face that the number of mitochondria which are detected by spot-enhancing filter algorithm in green rectangular regions of Figure 2(c) is less than in Figure 2(b). Some mitochondria which are get together in an area are difficult to be detected in 2(c). The number of mitochondria are detected in 2(a) is about 300, in 2(b) is about 430, in 2(c) is about 340. Therefore, it is more effective and accurate to detect mitochondria in microscopic images which are processed by matrix factorization method rather than in original microscopic images directly.

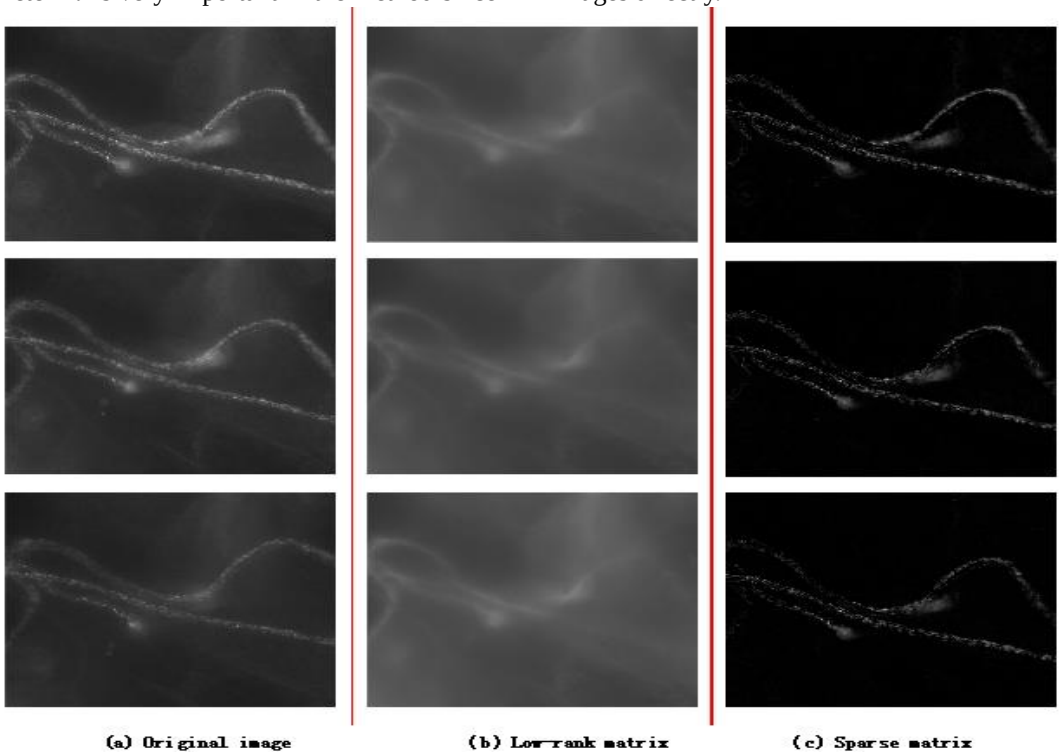
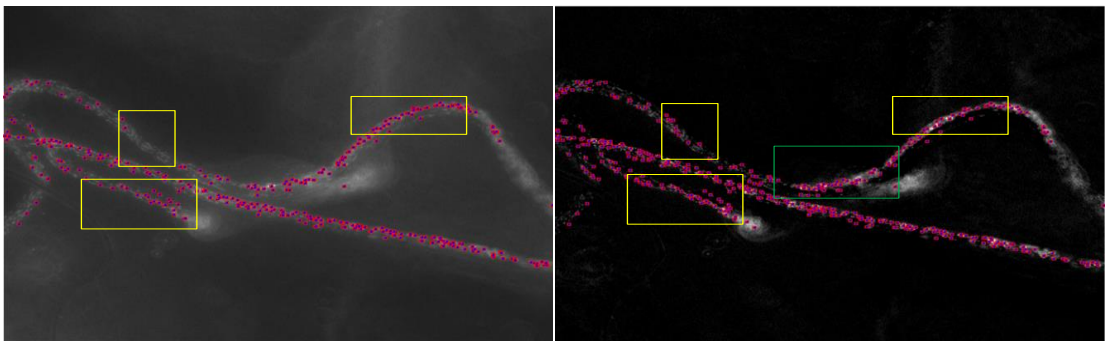
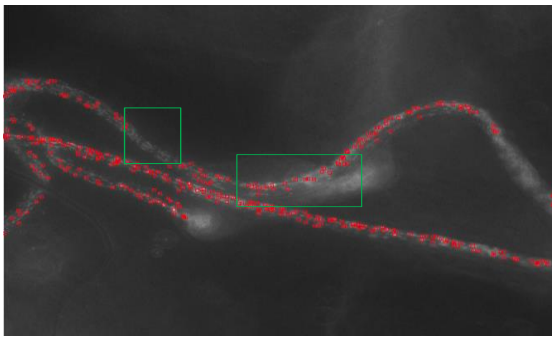


Fig.1 Results of matrix factorization method



(a) Detection results of the original images by IUWT algorithm

(b) Detection results of the processed images by IUWT algorithm



(c) Detection results of the original images by spot-enhancing filter algorithm

Fig.2 Comparison of mitochondria particle detection results (a) Detection results of the original images by IUWT algorithm. (b) Detection results of the processed images IUWT algorithm. (c) Detection results of the original images by spot enhancing filter algorithm.

TABLE I PARAMETERS OF AUGMENTED LAGRANGE MULTIPLIER ALGORITHM

Parameter	Parameter value
Balance parameter λ	1600
Accelerating factor ρ	0.7
Initial value μ_0	2

V. CONCLUSION AND OUTLOOK

We present matrix factorization method based on augmented Lagrange multiplier algorithm to separate mitochondria from the cytoplasm shadow in microscopic images, improve the SNR of the image, and reduce the background complexity. Then general particle detection algorithm can detect live mitochondria in processed fluorescent microscopic images rapidly and accurately. This method provides an efficient analysis tool for further studies on mitochondria in live cells.

Currently, automatic tracking of trajectory of live mitochondria in fluorescence microscopic images is a research hotspot and difficulty in the field of biomedicine as well. Tracking live mitochondria will lead to obtain some movement parameters such as movement velocity and acceleration to research their dynamic characteristic. Particle dynamics based on tracking fluorescent microscopic particles provides strong research method on some researches like exploring the mechanism of neural activity and the happening of cancer. However, automatic detection is the basis of automatic tracking of mitochondria. Therefore, our next goal is tracking mitochondria in live cells.

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