Morphological source separation for particle tracking in complex biological environments

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Abstract

Tracking nano-metric particles in a biological environment is a very difficult task because of the low signal intensity and the high mobility of these small objects. The task becomes nearly impossible for classical tracking procedures when the targets are labeled with a marker that is not strictly specific, because in this case dynamic structures in the cell are also visible. To address this limitation, we propose to use a source separation technique based on sparsity principles which allows the discrimination of objects with different morphologies. We prove in a real case that tracking in the source separated images allows to track particles that interact with other sources, something which was not feasible until now. This capability opens up new perspectives for the analysis documenting intricate interactions between cellular compartments.

1 Introduction

The classical paradigm of particle tracking is a detection step followed by an association procedure between the measurements and the active tracks sets. However, in practice the set of detections is generally corrupted by detections, called clutter, that do not correspond to a target. The performance of tracking algorithms quickly decreases with an increasing level of clutter because false tracks appear and associations of tracks with clutter occur. Clutter occurs mainly from two phenomena: (1) sensor's noise, and (2) objects that are labeled but are not of interest. The latter is common in biological images because targets such as ribonucleic acid (RNA) particles, vesicles or proteins, evolve in cells or intercellular spaces that are very crowded environments. Since in many fluorescent biological images the labeling is not strictly specific to the targets, structures such as membranes or filaments emit light which creates a dynamic structured background and generates a high density of clutter. It therefore degrades significantly tracking results.

In this paper, we propose a new method for tracking small targets in a complex environment. Our approach consists of a combination of a detection step using the results obtained by a Blind Source Separation (BSS) technique, followed by a Bayesian tracking procedure. We have adapted the BSS method to the characteristics of fluorescence biological images.

We show in Section 2 that for the BSS task the Morphological Component Analysis (MCA) [5] algorithm achieves very good performances in real fluorescence biological images when using our proposed set of dictionaries. In Section 3.1 a detection procedure using MCA is described. We compare its results with detections of a classical wavelet based approach in fluorescence images from plant cells. In Section 3.2 the combination of our detection procedure with a Bayesian tracking step is shown to outperform a classical tracking procedure. Also the capacity of the proposed approach to track targets that interact with the background is demonstrated.

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2 Blind Source Separation in biological images

2.1 Morphological Component Analysis

The aim of blind source separation techniques is to recover the set of sources $S = [s_1^T, ..., s_n^T]$ from the observation of their mixture: $Y = AS+N$, where A is the mixture coefficients matrix, Y is the measured signal and N is the matrix containing measurement noise and model error. While well known Independent Component Analysis methods [2] rely on statistical principles, very recently some methods based on morphological diversity and sparsity have emerged [5]. They require the definition of a dictionaries set such that each source can be represented in a very sparse way in a given waveform dictionary of the set and in a very non-sparse manner in every other. In the case of noisy measurements, a signal s_i is said to be sparse in a waveform dictionary D_i if it can be represented from a very few dictionary elements: $s_i = \alpha_i D_i$, where most of the coefficients α_i are nearly zero.

The MCA algorithm [5] was primarily designed for the analysis of natural images and astronomical data. It decomposes the signal Y in a union of over-complete dictionaries using a modified block coordinate relaxation method which minimizes iteratively the following score function:

$$\{\alpha_i\}_{1...n} = \underset{\{\alpha_i\}_{1...n}}{\operatorname{argmin}} \left[\sum_{i=1}^{n} ||\alpha_i||_0 + \lambda ||Y - \sum_{i=1}^{n} \alpha_i D_i||_2^2 \right]$$

The first term of the sum reflects a sparsity constraint while the second term is the reconstruction error and introduces a data-driven constraint.

2.2 Dictionaries set for biological images

In order to separate small particles, whose tracks are wanted, from background in biological images, we propose to use the MCA algorithm by considering the particles as one source and background structures as other sources. We therefore have to define at least two dictionaries that are mutually incoherent and model in a sparse way biological images.

In many fluorescence biological images small particles appear as nearly Gaussian spots because the point spread function of the acquisition system is very well approximated by a Gaussian function [7]. We therefore propose the Undecimated Discrete B3-Wavelet Transform (UDWT) [6] as the waveform dictionary D_1 for this source, because it is built from nearly Gaussian functions. The dictionary D_2, that represents the background must also be selected according to its morphology. Very often the non isotropic background in fluorescence images is composed of linear structures, such as membranes and filaments. The Discrete Curvelet Transform (DCvT) [1] is well adapted to these signals in 2d because it represents C^2 smooth curves optimally. For images in which the background looks like texture, the Discrete Cosine Transform (DCT) is an appropriate dictionary because it models locally periodic signals in a sparse way. These three dictionaries have very fast implementations of direct and inverse transforms in 2d, which makes MCA computationally feasible.

An example of MCA capabilities when it is applied with an appropriate set of dictionaries is presented now.

Figure 1 shows an image of epidermal root cells of

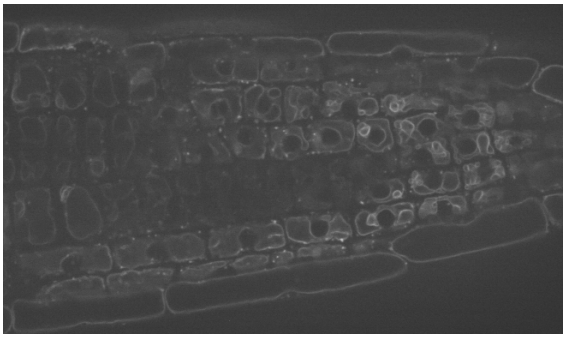

Figure 1. A GFP KOR1 protein labeled vacuoles membranes and small compartments.

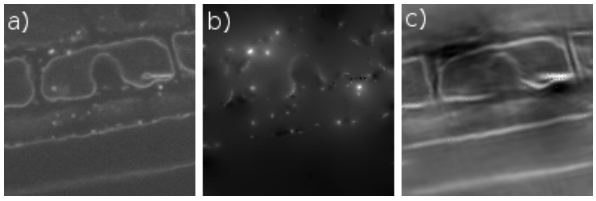

Figure 2. Results of MCA using the proposed dictionaries: (a) Original crop (b) Wavelet part $\alpha_1 D_1$, (c) Curvelet part $\alpha_2 D_2$.

Arabidopsis plants. The seedlings expressed the Green Fluorescent Protein (GFP) fused to the KORRIGAN1 (KOR1) protein. 20 stacks of 11 slices each were acquired with a disk scanning confocal microscope because tracks of KOR1 compartments are wanted. We apply MCA because signals of KOR1 proteins in compartments appear as small isotropic spots superposed to signals of membranes of vacuoles. There is no restriction on the dimensionality of sources in our framework, but a processing of MCA slice by slice was preferred because the cost of using 3d dictionaries representing surfaces is prohibitive. We therefore have chosen to apply MCA with two dictionaries: UDWT for spots and DCvT for membranes. Here the DCT is useless because of the lack of texture in these images. The separation of compartments and membranes signals is very well

achieved as shown in Figure 2.

3 Tracking small particles in MCA processed images

3.1 Spots detection

We propose to detect small particles by analyzing the wavelet coefficients resulting from the BSS technique proposed in Section 2.2 and then follow them through time with a kinetic Bayesian tracking algorithm (Section sec:res). In the following we call this combined BSS-detection method MCA-UDWT as opposed to the method UDWT which only uses an undecimated B3-wavelet transform without BSS.

One or few wavelet scale coefficients of the MCA-UWDT are selected according to the particles sizes. A binary mask of each slice of the original image is obtained from the binarization of these coefficients by thresholding. The mask is used to obtain 3d positions of individual particles by a 3d connected components extraction.

In the case of the biological images presented in Section 2.1 the second scale of the MCA-UDWT is analyzed because it corresponds to the size of KOR1 compartments. For the purpose of validation, a manual detection process to assess precise detection performance is highly time consuming in our case because the particles are very numerous (in the range of several thousands) and the third dimension considerably slows down the manual localization. This is why we consider only one slice of the stacks and crop it. Conclusions on performance improvement should remain valid in 3d because loosing the z information makes the automatic detection process even more difficult, placing us in a worst case scenario. We compare the new detection performance to those of a method that has been demonstrated to be efficient in many biological images [4]. When using a single scale the latter consists in thresholding the second scale of UDWT coefficients and extracting detections in a similar way as described for MCA-UDWT.

In Figure 3 the scales 2 of MCA-UDWT and UDWT are depicted. It shows that there no longer remains any linear structure in the second scale given by MCA-UDWT filtering, which makes the detection process much more easier than in the second scale of UDWT which contains many linear residues. The corresponding binarized images clearly highlight this fact.

An expert identified 2507 spots in the movie of 20 images corresponding to this crop. Figure 4 represents the number of recovered detections and the number of false detections for various strategies of thresholding second scales coefficients. It shows that MCA-UDWT always recovers a higher number of targets than UDWT for

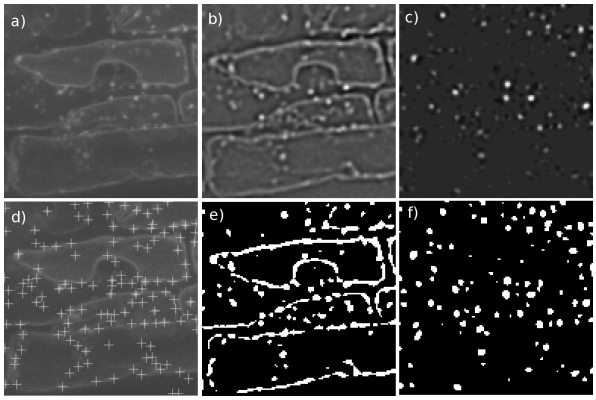

Figure 3. (a,d) Original crop and corresponding manual detections, (b,e) 2nd scale of UDWT and resulting binarized image by permissive thresholding, (c, f) 2nd scale of MCA-UDWT and resulting image by permissive thresholding.

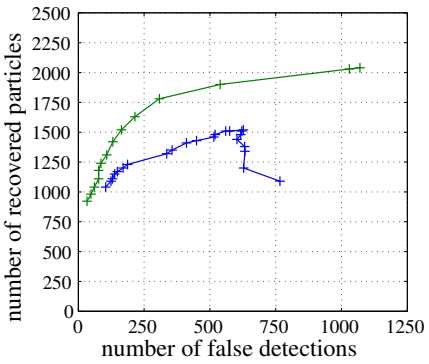

Figure 4. Detection performance based on MCA-UDWT (green) and UDWT (blue).

a given number of false detections. This is especially true when the threshold is permissive (right part of the curves): in this case MCA-UDWT is able to recover low intensity particles, while the UDWT cannot because it recovers also linear structures which aggregate with true detections, and therefore worsens results quality.

3.2 Tracking of particles

Once the detection step has been performed, a kinetic Bayesian tracking procedure with a mixture of predicting filters [3] is used to build the tracks. The mixture of three kinetic filters: Brownian, constant speed and constant acceleration filters, is able to model most common biological motions such as diffusion or motion along filaments or tubules.

In Table 1 we present the number of manually identified displacements of targets between two consecutive frames that are recovered by Bayesian tracking when combined with MCA-UDWT and UDWT detec-

tion procedures. The MCA-UDWT approach clearly improves the tracking result quality due to a better set of detections: 19% of displacements are additionally recovered, while at the same time the rate of false displacements is decreased by 4%.

	UDWT	MCA-UDWT
recovered displacements rate	1176/2005 = **0.59**	1572/2005 = **0.78**
false displacements rate	283/(1176+283) = **0.19**	277/(1572+277) = **0.15**

Table 1. Tracking results on a small 2d crop of the original images.

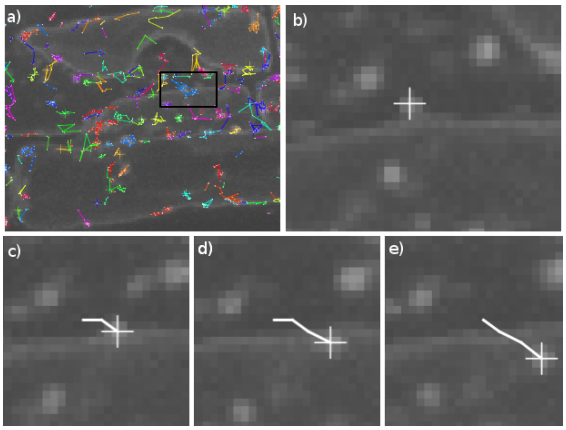

Figure 5. Results of tracking: (a) tracks in a small 2d crop, (b, c, d, e,f) tracking of a vesicle that is crossing a membrane.

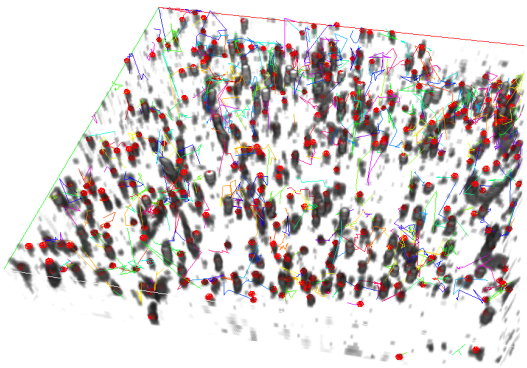

Figure 6. Detection and tracking result using MCA-UDWT detections in original 3d images.

A qualitative inspection of results obtained with the proposed approach reveals that 22% of displacements miss because of events, such as fusion of closely spaced targets, that are not related to BSS and for which we are currently developing other solutions. This confirms the efficiency of MCA-UDWT combined with Bayesian tracking to solve the issues described in Section 1. In Figure 5 we show that thanks to MCA-UDWT we are able to track particles that interact with membranes, or cross them. This capability of the approach is opening new perspectives for biological studies: we can quantify interactions with background structures because each separated image gives information about positions and dynamics of a source.
Results of detection and tracking in 3d of the proposed approach are presented in Figure 6. The same benefits that were proven in 2d are observed: the resulting tracks are not degraded by the presence of any remaining background structure. We are planning now to quantify the improvement as for the 2d case.

4 Conclusion

We have proposed the use of the MCA algorithm with representation dictionaries adapted to biological fluorescence images in order to separate small isotropic targets from other fluorescent sources coming from structured dynamic background. The benefits of the method was proven in an example of real and challenging biological case. The combination of this technique with a Bayesian tracking procedure allows following particles that were previously impossible to track because of their interactions with background structures. These results open up new perspectives in studies of interactions and relative motions between small particles and other structures in biological images.

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