Multiple objects detection in biological images using a marked point process framework

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Abstract: The marked point process framework has been successfully developed in the field of image analysis to detect a configuration of predefined objects. The goal of this paper is to show how it can be particularly applied to biological imagery. We present a simple model that shows how some of the challenges specific to biological data are well addressed by the methodology. We further describe an extension to this first model to address other challenges due, for example, to the shape variability in biological material. We finally show results that illustrate the MPP framework using the "simcep" algorithm for simulating populations of cells.

Keywords : Marked Point Process, object detection, biological imagery

1 Introduction

Detecting multiple instances of a given object from images is a major issue in computer vision as it often represents the first step towards image understanding and interpretation. For example, in remote sensing, the description of land cover (especially when dealing with high resolution images) relies on a previous detection of objects in the scene such as buildings, trees or roads. In computational biology this problem also appears frequently in order to evaluate, characterize or classify a population of biological objects such as cells, vesicles within cells or RNA/protein complexes [1, 2]. A particular case can be the initialization of a tracking algorithm to study, for example, vesicles trajectories [3]. In addressing biological applications some specific issues have to be considered due to the variability of biological material within and between different classes of objects. For example, objects representing other biological material may be mixed with the actually targeted ones, thus the image cannot be simply modeled as a collection of objects of interest in a background. Besides, the size of these targeted objects is sometimes close to the voxel size, making the differentiation between objects and noise particularly complicated. In this paper, we present a methodological framework that provides tools to solve the different issues raised by multiple biological objects detection from microscopic images. We will particularly develop the following:

Issue 1: How to address the intensity heterogeneity that prevents from considering a global threshold on the intensity in order to separate to objects from background ?

Issue 2: How to deal with nuisance objects that do not belong to the targeted class of objects but cannot be considered as background neither ?

Issue 3: How to deal with a high density of objects that generates clusters of possibly overlapping objects ?

Issue 4: How to handle the shape variability between objects ?

Issue 5: How to detect objects that consist of a few pixels ?

Issue 6: How to deal with both 2D and 3D datasets ?

Throughout the literature that addresses this problem, we distinguish both global as well as local methods. Global methods usually consider a threshold to separate the background from pixels belonging to objects. Each n-connected group of pixels tagged as object is then analyzed. A watershed segmentation is then performed on the distance map inside each component to split it into individual objects. Each individual object is finally selected or rejected depending on its size and shape, considering for example a circularity parameter. This classical approach is usually the one proposed by common image analysis software such as Matlab, the particle analyzer of Fiji or Cell Profiler [4, 5]. Nevertheless, issue 1 is not addressed within this approach. In consequence, in order to remove background variation, a high pass filter has to be previously applied. Issues 2,3 and 4 are partially solved if the objects of interest have more or less a circular shape and can be bounded by particular minimum and maximum sizes that discriminate them from nuisance objects. The shape of the detected object is arbitrarily defined by the watershed algorithm, so issue 4 is not addressed. Finally, issue 5 is not addressed in case of noisy data. In local approaches, a first step usually consists of seeds detection. A growing process then extends each seed to define an object using, for example an active contour or marker controlled watershed. This process allows the object shape recovery only if they are initially properly localized by the seeds. Therefore, the seeds detection is crucial. Some strategies to obtain these seeds include local maxima after a global threshold or a template matching process [6]. Issue 1 can be partially solved by considering a low threshold when seeds are defined by local maxima. Issue 2 is not addressed whereas clusters are split arbitrarily when two growing objects intersect.

In this paper we present the marked point process modeling (MPP) as a framework to solve the different issues described above. These models derived from the application of point processes to spatial statistics. They have proven their efficiency and robustness in various fields of computer vision in order to evaluate populations of, for example, trees, buildings, roads, people in a crowd or flamingos. A survey of marked point processes applied to image analysis can be found in [7]. Herein we focus on biological images and show how to derive specific models to accurately address the different issues mentioned above.

2 Method

2.1 Marked Point Process

Let us consider an object space $\mathcal{O} \subset \mathbb{R}^m$ that contains the geometrical description of the object of interest. For example if we consider the set of disks with radius bounded by r_{min} and r_{max}, then $\mathcal{O} = [r_{min}, r_{max}] \subset \mathbb{R}$.

We consider the configuration set Ω as the union of all the possible finite sets of objects lying in a subspace $\mathcal{S}$ of $\mathbb{R}^n$ defined by the support of the image :

$$\Omega = \bigcup_{i=0}^{\infty} \Omega_i, \tag{1}$$

where

$$\Omega_i = \{\omega_1, \ldots, \omega_i\} \in (\mathcal{S} \times \mathcal{O})^i \tag{2}$$

is the set of configurations containing exactly i objects, $\omega_i = (p_i, m_i), p_i \in \mathcal{S}$ is the center of the object and $m_i \in \mathcal{O}$ are the marks. We define a marked point process [8] by athe Gibbs density as follows:

$$\forall \omega \in \Omega, d\pi(\omega) = \frac{1}{Z} \exp\left[-U(\omega)\right] d\pi_0(\omega), \tag{3}$$

where π_0 is the measure of the Poisson process and $U(\omega)$ is the energy function that evaluates each configuration of objects. The lower the energy function value the more probable is the particular object configuration. In the context of image analysis, the energy function embeds a data term, $U_D(\Omega|I)$, that evaluates the consistency of any object with respect to the data I as well as a prior, $U_P(\Omega)$, that reflects constraints on the objects geometry and repartition in the image plane.

Let us consider a first example, shown in figure 1, where the image $\{I(s), s \in L\}$ on the lattice L consists of circular cells on a dark background. We first define a data term that measures the contrast between a candidate object and its neighborhood as follows:

$$\begin{aligned} &P(I|\Omega = \{\omega_1, \ldots, \omega_i, \ldots, \omega_n\}) = \exp -U_D(\Omega|I) \quad \text{with} \\ &U_D(\Omega|I) = \sum_{i=1}^{n} u_d(\omega_i), \end{aligned} \tag{4}$$

where $u_d(\omega_i)$ is a contrast term we defined as:

$$u_d(\omega_i) = \begin{cases} 1 - \frac{d(\omega_i)}{d_0} & \text{if } d(\omega_i) < d_0 \\ exp\left(\frac{d_0 - d(\omega_i)}{3d_0}\right) - 1 & \text{otherwise.} \end{cases} \tag{5}$$

In equation(5), $d(\omega_i)$ is a distance between pixels in the object ω_i and pixels in the external boundary $\partial\omega_i$ (see figure 2). For example the Bhattacharrya distance is defined by:

$$d(\omega) = \frac{1}{4} \frac{(\mu_o - \mu_b)^2}{\sigma_o^2 + \sigma_b^2} + \frac{1}{2} \log\left[\frac{\sigma_o^2 + \sigma_b^2}{2\sigma_o\sigma_b}\right], \tag{6}$$

where μ_o (resp. μ_b) and σ_o^2 (resp. σ_b^2) are the mean and variance of pixels in ω (resp. $\partial\omega$.

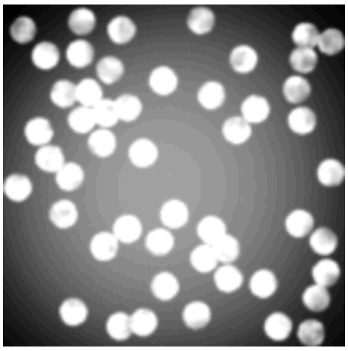

Figure 1: Example of an image containing a collection of objects on a background.

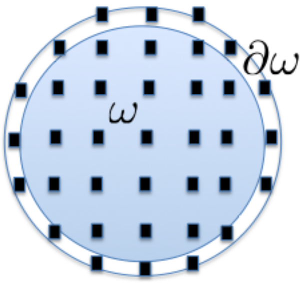

Figure 2: Discretization of a disk ω and its neighborhood $\partial\omega$

In order to prevent object overlap as much as possible, we add the following prior:

$$U_P(\Omega) = \sum_{i,j:\omega_i \cap \omega_j \neq \emptyset} f(\omega_i, \omega_j) \quad \text{with} \tag{7}$$

$$f(\omega_i, \omega_j) = \begin{cases} \infty & \text{if} \frac{|\omega_i \cap \omega_j|}{\min(|\omega_i|,|\omega_j|)} > o \\ 0 & \text{otherwise} \end{cases}$$

where $|\omega_i|$ refers to the size of object i and o is the maximum overlap ratio permitted. The solution is then defined as the minimizer of the global energy:

$$U(\Omega) = U_P(\Omega) + U_D(\Omega|I). \tag{8}$$

2.2 Optimization

Two practical issues arise when analyzing this problem. On the first place, the energy $U(\Omega)$ we want to minimize is not convex. Secondly, the normalizing constant (or partition function) Z defined in equation 3 is analytically and numerically intractable. Therefore, the optimization is classically performed using a simulated annealing coupled with a sampling algorithm. This last that can be, for example, jump and diffusion processes or MCMC approaches, particularly Reversible Jump MCMC (RJMCMC), as the number of objects is unknown. More recently, the multiple births and deaths (MBD) algorithm based on a discretization scheme of a stochastic differential equation has been proposed [9]. As an advantage, this algorithm permits to address a whole set of objects in the same iteration. Besides, there is no rejection in the birth step that allows any new objects introduction at every stage of the simulated annealing. Some faster suboptimal algorithms have also been proposed such as the multiple births and cut (MBC). In this paper we consider the MBD algorithm as the MBC algorithm is restricted to particular energy functions.

The MBC algorithm alternates births and deaths steps consisting in adding new objects and removing some of them with a certain probability that depends on the specific value of the energy function:

Algorithm 1 *Multiple Births and Deaths*

1 *Intialize the objects configuration with the empty set* $\Omega_0 = \emptyset$*, set* $T = T_0$*,* $i = 0$ *and* $\delta = \delta_0$

2 Births step : *Set* $i = i + 1$*, Generate randomly a set of objects* $B_i = \{b_i^j\}$ *and compute the data term for each object* $u_d(\omega_i^j)$*. The location and the marks of objects are drawn from a uniform distribution and the number of objects is drawn from a Poisson law of parameter* $\delta \times |L|$*,* $|L|$ *being the number of pixels. Set* $\Omega_i^b = \Omega_{i-1} \bigcup B_i$*.*

3 Sorting step : *Sort the objects in* Ω_i^b *by descending order of the data energy (from the "worst" to the "best").*

4 Death step : *For each object* $\{\omega_j \in \Omega_i^b\}$ *taken sequentially in the ordered list, remove* ω_j *from* Ω_i^b *with probability* $p = \frac{\delta a(\omega_j, \Omega_i^b)}{1 + \delta a(\omega_j, \Omega_i^b)}$ *such that*

$$a(\omega_j, \Omega_i^b) = \exp -\frac{1}{T}\Big(U(\Omega_i^b / \{\omega_j\}) - U(\Omega_i^b)\Big)$$

5 *If not converged decrease* T *(resp.* δ*) by a factor* α_T *(resp.* α_δ*) and go back to step 2.*

The convergence has been theoretically proved in [17], and is empirically obtained either after a fix number of iterations or when the configuration does not change during a couple of iterations.

2.3 Results

We validate this model on several synthetic images of cells simulated with the "Simcep" algorithm [10]. To do so, we compare the performance of the proposed MPP approach with the classical approach proposed by software such as Fiji or Matlab. This last one consists in binarizing the image and splitting the clusters using the watershed algorithm on the distance map. Resulting connected components are selected as objects depending on their size and a circularity coefficient. We first consider a noise free image of cells given on figure 3, and then we add noise on figure 4. Finally we increase the background heterogeneity due to the light source (see figures 5 and 6).

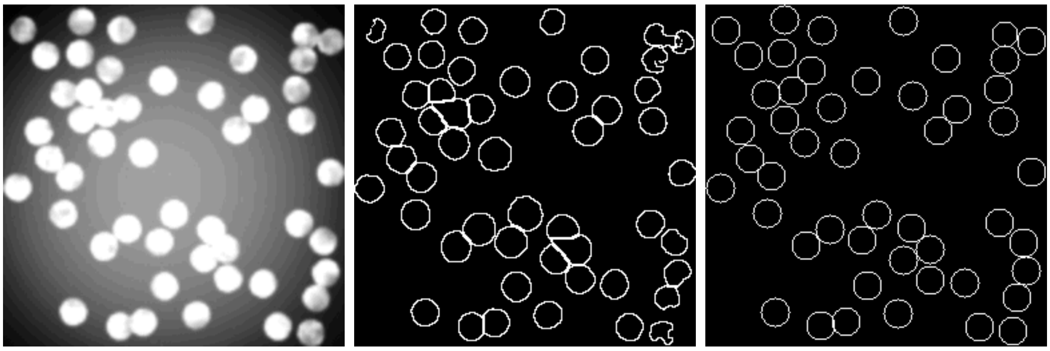

Figure 3: A noise free example of image cells (left) and the detection obtained using the fiji particle analyser (middle) and the MPP approach (right).

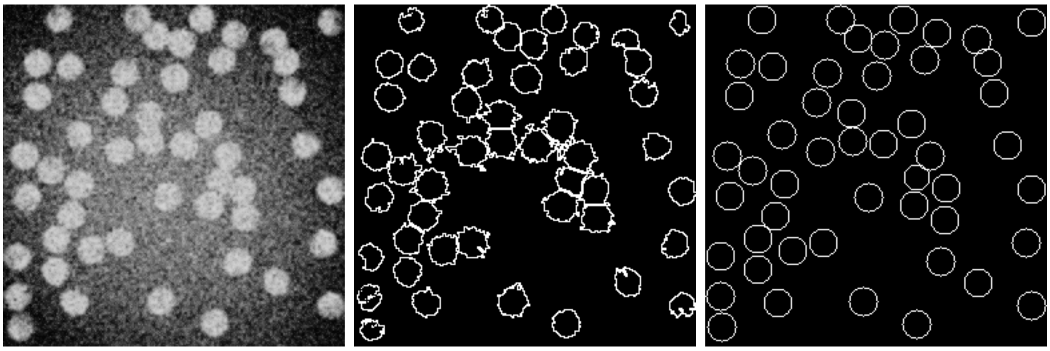

Figure 4: A noisy example of image cells (left) and the detection obtained using the fiji particle analyser (middle) and the MPP approach (right).

With this first model we partially address the different challenges (issues 1, 2 and 3). The main advantage of this approach is that the data is taken into account at the object level. In the data term, statistics of pixels contained in the whole object are considered providing a high robustness with respect to noise (see figure 4). Besides, we consider a local contrast term between the object and the surrounding pixels that gives robustness with respect to the background heterogeneity (see figures 5 and 6) compared to the classic approach. Finally, the shape model facilitates the discrimination between objects of interest and nuisance objects. Notice however that in the case of disk shaped objects the watershed algorithm (classic approach) performs quite well on the task of splitting clusters (see figure 3). However, this performance decreases rapidly with noise or background heterogeneity while the MPP approach remains robust. Finally, with this simple model, only circular cells are addressed. Several extensions have to be considered

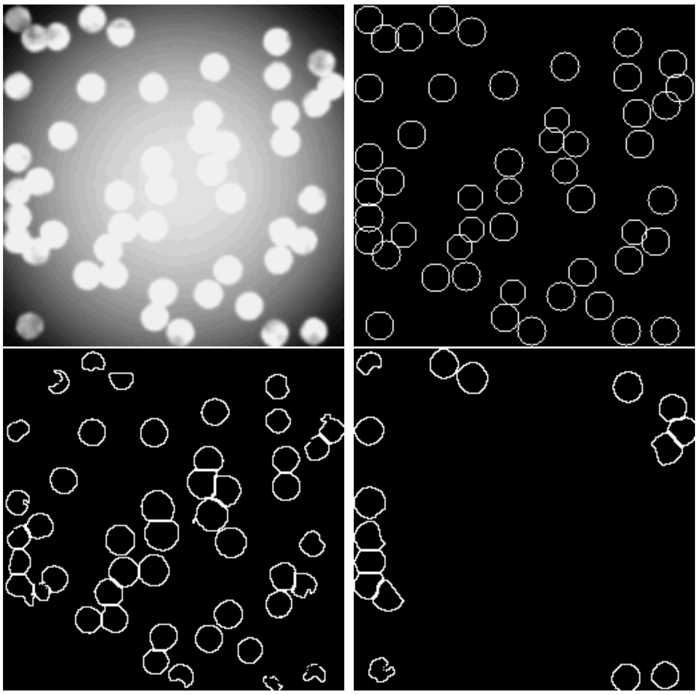

Figure 5: A first exemple of an heterogeneous background (top left), results with the MPP approach (top right) and with two parameter settings for the fiji particle analyser (bottom left and right)

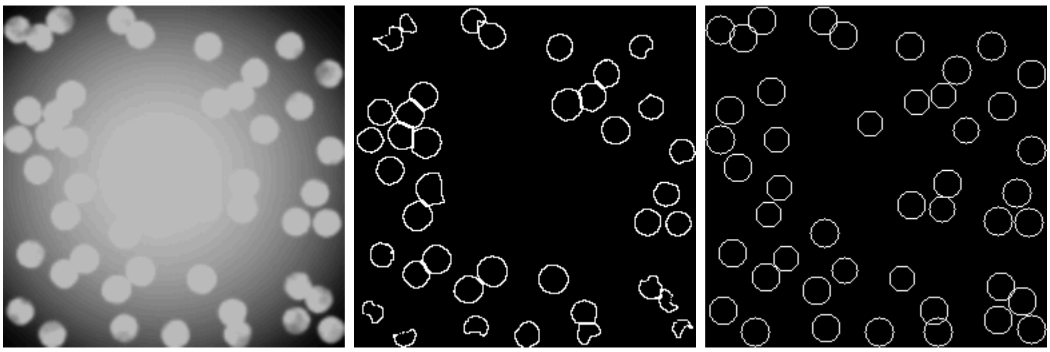

Figure 6: A stongly heterogeneous background (left), results obtained the fiji particle analyser (midlle) and with the MPP approach (right).

in order to generalize the approach.

3 Discussion

We have shown in section 2.3 that the MPP approach gives an answer to issues 1, 2 and, partially, 3. By considering objects instead of pixels as unknown variables we obtain a robust detection with respect to noise and to non homogeneous background. However, at this stage issues 4, 5 and 6 have not been yet addressed. In this section we discuss several extensions to the previous model that take into consideration the shape and size variability of the objects within the same MPP framework.

3.1 More general shapes

In section 2, we have considered an object space consisting of disks. However, it is straightforward to take into account more complex parametric spaces in order to define other geometries. For example, if we consider the image given on figure 7, it is clear that a model based on disks would fail to detect the cells. The particle analyzer based on the distance transform (Fiji) is also inadequate as shown on figure 7 (middle). This problem can be solved within the marked point process framework by considering ellipses as objects, such that the object space is defined by $\mathcal{O} = [a_{min}, a_{max}] \times [b_{min}, b_{max}] \times [0, \pi]$. We can see on the MPP result on figure 7 (right) that the objects have been correctly detected and delineated, contrary to the particle analyzer approach, that cannot correctly split clusters of ellipses. Several parametric shapes have been proposed in the literature including rectangles, segments or superquadrics. The mix of two shape spaces such as disks and ellipses is also possible. However, the parametric space that defines the shapes should have a low dimension, typically lower than 5, to avoid computational burden. This can be limiting in case of complex shapes such as the cells on figure 8, or when the objects are composed of a few pixels, causing the discretization to lead to a poor approximation of the parametric shapes. To overcome this limit, it has been proposed to define the object space as a dictionary of precomputed shapes. Such a dictionary can be obtained from previous segmentation maps as in [11] (see the result on figure 8) or by constructing an exhaustive description of shapes included in a small bounding box. On figure 8 we can see that the use of a dictionary combining shapes obtained by the particle analyzer algorithm and by an active contour approach allows to select the most relevant ones from each method and for different parameters, thus improving the global result. On figure 9, the dictionary is defined by the whole set of convex shapes bounded by 5×5 pixels square [12]. This last approach has been proven to overcome state of the art detection techniques, such as the one included in Icy which is based on wavelets (see figure 9 middle and right).

3.2 Energy function

The energy function is composed of a data term that fits the objects onto the image and a prior that favors or imposes properties on the whole configuration. In section 2

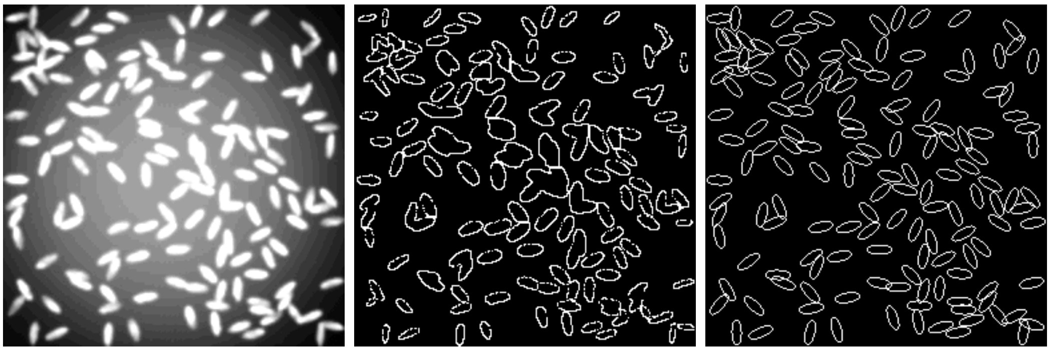

Figure 7: Example of elliptic cells (left), results obtained by the Fiji particle analyser (middle) and the MPP approach (right)

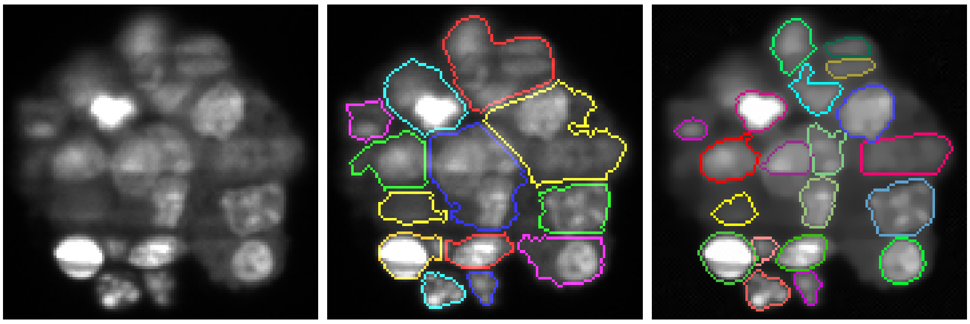

Figure 8: Spheroid containing cells of various shapes (left), results obtained with the Fiji particle analyser (middle) and the MPP approach based on a dictionary (right).

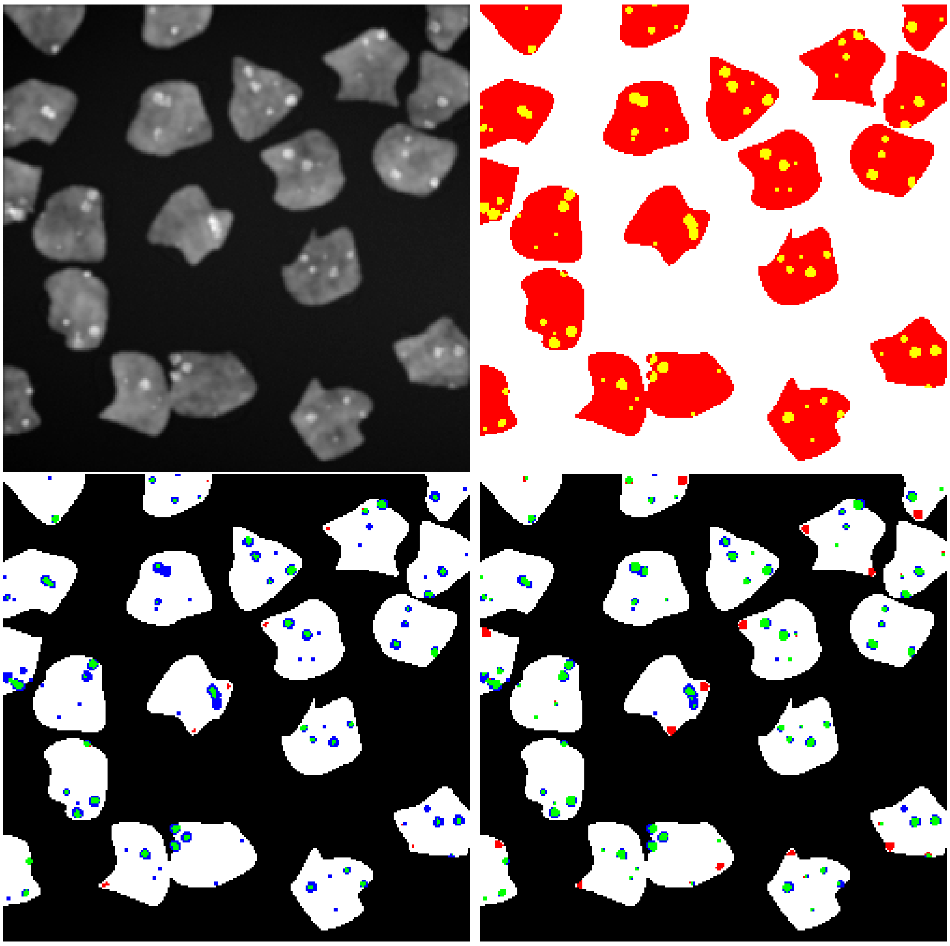

Figure 9: An example of small particles within cells (top left), ground truth (top right), results obtained by the wavelet approach proposed by Icy (bottom left) and by the MPP approach (bottom right)- Green : true positive - Blue : false negative - Red : false positve

we have considered a contrast term based on the Bhattacharrya distance between the pixel intensity inside the object and in a crown surrounding it. Several formulations to define the data term can be found that compare the mean, the median or the maximum value between the object and its neighborhood. These terms are based on the contrast norm of the object with respect to the surrounding pixels that are supposed to belong to the background. Another class of data terms is based on the scalar product of the normalized intensity gradient on the object frontier and the normal of the shape along this frontier. This notably leads to models that are invariant with respect to the image contrast [13]. In this setting the data term is entirely based on the object geometry (in the data) independently on the contrast. This is therefore fully adapted to cases when the contrast is not constant within the image due to heterogeneous illumination in the background or variability in the object intensity. The second part of the energy function consists of prior information. In this paper we have considered a repulsive term that prevents objects overlap. Some attractive properties can also be defined, for example, to favor clusters of objects or to align them by sharing similar angles.

3.3 Computational considerations

The Multiple Birth and Death algorithm has been proposed in [9] as an alternative to the classical RJMCMC scheme. As for the RJMCMC, the convergence to the configuration that minimizes the energy has been proven in a simulated annealing context [17]. Regarding the MBD algorithm, its main advantage lies in the birth step, where several objects are added simultaneously to the configuration independently of the temperature. Therefore, even at a low temperature, the system can investigate new objects that locally increase the energy. To improve the convergence speed, one can introduce a birth map to favor introducing new objects with a higher probability in relevant locations within the image. For example this birth map can be based on a precomputation of the data terms for each possible location of the objects. A tradeoff needs then to be found between the complexity of the birth map computation and the gain in terms of number of iterations before convergence. Some suboptimal algorithms have been proposed in order to speed up the convergence. For these algorithms there is no guarantee to reach the global optimizer, but they have proven to be efficient in practice. As for Markov Random Fields, the graph cut algorithm has been employed by replacing the death step in the MBD by a graph cut to select the most relevant objects. This has lead to the Multiple Birth and Cut (MBC) algorithm [18]. The main advantage of the MBC over RJMCMC and MBD schemes is that it prevents from embedding the algorithm into a simulating annealing scheme thus avoiding the calibration of the cooling parameters (initial value and decreasing coefficient of the temperature). To speed up the convergence speed one can consider a deterministic version of the MBC algorithm that can be compared to the ICM for Markov Random Fields. It simply consists of the removal of an object - during the death step - in the case that this change in the configuration induces an energy decreasing. A quantitative comparison between stochastic samplers, in terms of accuracy and computational time, is given in [19] on a particular application. However, the efficiency of these algorithms highly depends on their design (kernel choice in case of RJMCMC, birth map in case of MBD), thus each case needs to be specially studied.

3.4 Further MPP applications to biology imagery

In this paper we focus on object detection. However, other problems can be addressed within the marked point process framework as it has been proposed for remote sensing applications. In this particular context, a hierarchical model has been proposed by [14] to model groups of vehicles (i.e.: each vehicle is a first order object and a group of them is a second object order). Such a multi-level model can be applied to study populations of vesicles within cells. An extra dimension representing time can be added to MPP models in order to obtain an object tracking algorithm [15]. MPP can thus be employed to study vesicles trajectories. Finally, the transition between two states of a given object, for example a cell from alive to dead, can be addressed through change detection models [16].

4 Conclusion

The MPP approach, originally developed in the domain of spatial statistics for the modeling of populations, has been more recently successfully applied to solve image analysis problems and, more particularly, multiple object detection from images. In this paper we have shown that this framework is well suited to perform object detection in biological imagery. The different issues raised by these applications can be satisfactorily addressed with MPP modeling. This includes noise, shape variability and background heterogeneity. The non convexity of the functional to be minimized may lead to heavy computational time, especially when treating 3D datasets. However, some sub-optimal algorithms have been proposed that make the approach usable in practice. Some issues remain unsolved concerning MPP. Shapes are currently defined in a low dimensional parametric space or in a predefined dictionary. To consider general shapes defined in a shape space, as for example in [20], is a very challenging issue. Apart modeling issues we can also mention improvement in the optimization to speed up the convergence or to define parallel implementation [19]. Finally, estimating the parameters is still a largely open issue [21].

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