Nuclei Extraction from Histopathological Images using a Marked Point Process Approach

Maria Kulikova\textsuperscript{a} and Antoine Veillard\textsuperscript{ab}, and Ludovic Roux\textsuperscript{ac}, and Daniel Racoceanu\textsuperscript{ab}

\textsuperscript{a}CNRS – IPAL UMI, 1 Fusionopolis Way, #21-01 Connexis, Singapore 138632; 
\textsuperscript{b}School of Computing, National University of Singapore, Singapore 117417; 
\textsuperscript{c}University Joseph Fourier, France 38041;

ABSTRACT

Morphology of cell nuclei is a central aspect in many histopathological studies, in particular in the histological grading of cancer. Therefore, the automatic detection and extraction of cell nuclei from microscopic images obtained from cancer tissue slides is one of the most important problem in digital histopathology.

We propose to tackle the problem using a model based on marked point processes (MPP), a methodology for extraction of multiple objects from images. The advantage of MPP based models is their ability to take into account the geometry of objects; and the information about their spatial repartition in the image. Previously, the MPP models have been applied for the extraction of objects of simple geometrical shapes. For histological grading, the pathologists mostly rely on the information about the nuclei pleomorphism. So that, the accurate nuclei delineation became an issue of even greatest importance than optimal nuclei detection. Recently, the MPP framework has been defined on the space of arbitrarily-shaped objects allowing more accurate extraction of complex-shaped objects. The nuclei often appear joint or even overlap in histopathological images. The model still allows to extract them as individual joint or overlapping objects without discarding the overlapping parts and therefore without the significant loss in delineation precision.

In this paper, we also aim to compare the MPP model with two state-of-the-art methods selected from a comprehensive review of the available methods.

The experiments are performed using a database of H&E stained breast cancer images covering a wide range of histological grades.

1. INTRODUCTION

Histopathology, the microscopic observation of biological tissues, became the gold standard in the diagnosis and prognosis of a number of common and critical pathologies such as breast cancer. The analysis of breast cancer surgical slides in order to rate the malignancy of breast tumors is a highly technical and tedious task. In most of the major pathology departments, the pathologist follows a protocol called the Nottingham Grading System (NGS)\textsuperscript{1} in which the analysis of size, shape and appearance of cell nuclei is required (criterion known as the nuclear pleomorphism in the NGS). More generally, the observation of cell nuclei is a major aspect of most histopathological studies. The computer vision methods developed in histopathology aim to facilitate the task of pathologists. Therefore, the accurate detection and extraction of nuclei by computer vision algorithms is a major challenge in digital histopathology.

In order to tackle this problem, we propose an application of a Marked Point Processes (MPP) based model developed for multiple complex-shaped object extraction from images;\textsuperscript{2} and a comparative study with two state-of-the-art algorithms: the Gradient in Polar Space (GiPS) model\textsuperscript{3} and a level set based model proposed by K. Mosaliganti \textit{et al.}\textsuperscript{4}

The ability to specify prior-knowledge about the objects sought, the independence on their initialization or moreover the parallelization capabilities of such a model represent an interesting potential for effective and efficient nuclei extraction from histopathological images.

\textsuperscript{1}maria.kulikova@cnrs.ipal.fr
2. RELATED WORK

Several works have tackled the problems of the reliable detection of cell nuclei from histopathological images, the accurate delineation of their boundaries and the management of cell occlusion or overlapping. For reference, Gurcan et al.\textsuperscript{5} proposed a review of existing histopathological image analysis methods.

Petushi et al.\textsuperscript{6,7} introduced a method for labeling of several histological and cytological micro structures in high resolution images of H\&E stained breast cancer slides of different grades. For nuclei detection, an adaptive optimal thresholding (Otsu thresholding) was used followed by opening and closing morphological operators for filling small gap and separating slightly connected nuclei. The objective was to identify different types of nuclei which belong to inflammatory cells, lymphocytes, epithelial cells, cancer cells; and high nuclei density regions, but not to accurately delineate the nuclei.

Yang et al.\textsuperscript{8} proposed a method to accurately extract cell nuclei from time-lapse fluorescence image sequence. In such images, nuclei are bright objects on a dark background, so they can be easily extracted from background by thresholding. Contiguous nuclei are separated by using a marker-controlled watershed. To reduce the effects of oversegmentation, they added context information to merge the different parts of extracted nuclei or to be able to extract a cluster of nuclei.

Jung and Kim\textsuperscript{9} also proposed to extract cell nuclei and to deal with clustered nuclei using a marker-controlled watershed method. Then, an ellipsoidal modeling of contours is used to adjust cell nuclei contours. The method was tested on two types of images: cervical images and immunohistochemistry images of breast.

Sertel et al.\textsuperscript{10} proposed a method to detect nuclei of centroblast cells (large malignant cells) on H\&E stained histology images of follicular lymphoma (lymphoid malignancy). From the input RGB image, the color band having the highest contrast is selected, resulting in a bimodal grey levels distribution corresponding to nuclei and non-nuclei. A locally adaptive thresholding provides the extraction of nuclei. A spatial voting approach based on fast radial symmetry transform is used to separate overlapping cells from each other. At last, centroblasts are identified by assessing size, eccentricity and texture of the nuclei.

The empirical studies proposed in the previous papers show that the above methods perform well at detecting nuclei. However, their ability at accurately delineating nuclei boundaries is somewhat limited. In addition, they do not handle efficiently nuclei overlapping. Recently, several authors have proposed the use of active contour models in combination with other methods for accurate cell or nuclei extraction and overlapping management.

In cytology, Yang et al.\textsuperscript{11} proposed accurate lymphocyte nuclei and cytoplasm extraction on blood smears using a robust color gradient vector flow active contour model. They assessed the ability of their method to accurately extract different types of lymphocytes by measuring a ratio between the area of extracted nuclei (or cytoplasm) and the area of the ground truth of nuclei (or cytoplasm). In histology, Ali and Madabhushi\textsuperscript{12} proposed nuclei and lymphocyte extraction from H\&E stained breast and prostate cancer histological images. Their model used shape prior information and region-based active contours. The initialisation of their model is performed by using watershed. A double assessment of the method was proposed: capacity to detect nuclei or lymphocytes and capacity to resolve the problem of overlapping nuclei or lymphocytes. Finally, Dalle et al.\textsuperscript{3} and K. Mosaliganti et al.\textsuperscript{4} developed methods of nuclei extraction from H\&E histopathological images and 3D microscopic images respectively. As we chose these methods for the comparative study, we give their brief description in section 3.

All in all, it appears that nuclei detection and extraction, and the ability to deal with overlapping nuclei is a key challenge for the prognosis of cancers both at the clinical level and at the algorithmic level. Active contour models improved the accuracy of nuclei boundaries delineation, and an additional information such as shape priors or post-processing such as Voronoi tessalation can solve the problem of nuclei overlapping.

However, active contours models present the disadvantage of being highly sensitive to initialization. Therefore, the quality of the extraction of the active contours based approaches presented above is highly dependent on an accurate prior detection of the amount and position of objects in the image. The MPP model presented in the following section avoids this issue as it does not rely on a prior detection phase before the extraction of the objects.
3. METHODS

In this section we give quite detailed description of an MPP approach for multiple arbitrarily-shaped object extraction; and an overview of the two other state-of-the-art methods for nucleus extraction used as a reference for the empirical study described in section 4.

3.1 Marked Point Processes based model (MPP)

Stochastic Marked Point Processes are a well known methodology developed for multiple object extraction from images. The advantage of MPP based models is their ability to take into account the geometry of objects; and the information about their spatial repartition in image by modeling inter-object relations in the scene showed by the image. In previous work, MPP models have been successfully applied for the extraction of objects of simple geometrical shapes from remote sensing images.\textsuperscript{13–15} As mentioned before, the pathologists rely on the information about the nuclear pleomorphism in images in order to establish a grade of a tumor. So that, the accurate nuclei delineation became an issue of great importance. More recently, the MPP framework has been defined on the space of potentially arbitrarily-shaped objects\textsuperscript{2} allowing thus more accurate extraction of complex-shaped objects. Unlike the active contour based methods presented in section 2 which require a prior detection of the objects, the MPP framework constructs the objects using a methodology known as “high order active contour” without requiring the initialisation of objects’ location or the number of objects to be known in advance.

In the image scene, nuclei often appear joint or even overlap. The model still allows to extract the objects of interest as individual joint or overlapping objects without discarding the overlapping parts and therefore without the resulting loss in delineation precision.

Here below, we describe the model and the algorithm.

In this model, an individual object is represented by its boundary in the image $I$ as a closed planar curve $\gamma: [0,2\pi] \rightarrow I \subset \mathbb{R}^2$ defined as $\gamma(t) = x_0 + (r_0 + \delta r(t)) (\cos(t), \sin(t))$ where $x_0 \in \mathbb{R}^2$ is the centre of mass and $\delta r: [0,2\pi] \rightarrow \mathbb{R}$ the radial variation around a circle of radius $r_0$ with centre at $x_0 \in I$. In practice, arbitrarily-shaped curves are obtained by adapting randomly generated configuration of circles of radius $r_0 \in [r_{\min}, r_{\max}]$ to the image. This is done via the minimization of the curve energy $E(\gamma)$ by gradient descent which helps in restricting the search space.

The energy $E(\gamma) = E_{\text{im}}(\gamma) + E_{\text{o}}(\gamma)$ is composed of an image term and an object shape term, where

$$E_{\text{im}}(\gamma) = \lambda_g \int_{[0,2\pi]} dt \ n(t) \cdot \nabla I(\gamma(t)) + \lambda_G \int_{R(\gamma)} dx \ (G(x) - \bar{G}(x)) ,$$

(1)

with $n(t)$ the unnormalized outward normal to the object boundary and $R(\gamma)$ the object interior; and $\lambda_g$ and $\lambda_G$ weighting parameters. The first term encourages objects with boundaries of high image gradient response. The image gradient field $\nabla I$ is computed using a Canny-Deriche filtering for edge detection. The second term places objects according to a Gaussian model of the interior and exterior object with $G(x) = (f(x) - \mu)^2$ modeling the interior and $\bar{G}(x) = (f(x) - \bar{\mu})^2$ the exterior (background). The mean and variance terms $\mu, \sigma, \bar{\mu},$ and $\bar{\sigma}$ are learned using object and background samples.

The object shape term $E_{\text{o}}(\gamma)$ is defined as

$$E_{\text{o}}(\gamma) = E_{\text{smth}}(\gamma) + E_{\text{sh}}(\gamma) ,$$

(2)

with $E_{\text{smth}}(\gamma) = \int_{[0,2\pi]} dt |\dot{\gamma}(t)|^2$, favouring the smoothness and a uniform parametrization of the object boundary and $E_{\text{sh}}(\gamma) = 2\pi \sum_{k \in \mathbb{Z}} f(k)|\hat{\delta r}(k)|^2$ with $\hat{\delta r}(k) = \frac{1}{2\pi} \int_{[0,2\pi]} dt \exp(-ikt)\delta r(t)$ representing the prior energy associated with the shape of the curve and defined by the variance of Fourier components $\sigma(k) = \frac{1}{2\pi} \int_{[0,2\pi]} dt |\hat{\delta r}(t)|$ at different frequencies $k$. It encourages or restricts perturbations of the circle at different frequencies $k$.

For a configuration $\omega$ of objects $\gamma_i$ in image, the associated configuration energy is defined by
\[ H(\omega) = c_0 \sum_i H_1(\gamma_i) + \sum_{i \neq j} H_2(\gamma_i, \gamma_j), \]

where the image data term is \( H_1(\gamma_i) = E(\hat{\gamma}_i) \) where \( \hat{\gamma}_i \) is the contour \( \gamma_i \) after gradient descent, and \( H_2 \) is the interaction term which controls the overlapping between the objects: \( H_2(\gamma_i, \gamma_j) = \frac{\exp(-|R(\gamma_i) \cap R(\gamma_j)|)}{\min(H(\gamma_i), H(\gamma_j))} + \delta(\gamma_i, \gamma_j), \)

where \( \delta(\gamma_i, \gamma_j) = \infty \) if \( |x_i^o - x_j^o| \leq \epsilon \); otherwise it is equal to zero. \( \delta \) is a hard-core repulsion term between two objects and \( c_0 \) is a weighting parameter.

Let \( \Omega_c \) be the set of all possible configurations \( \omega \). A Gibbs probability density is defined w.r.t. the Lebesgue-Poisson measure \( \lambda \) on \( \Omega_c \) and takes the form:

\[ p(\omega) = \frac{z^{||\omega||}}{Z_\beta} \exp\{-\beta H(\omega)\}, \quad (3) \]

with a normalizing factor \( Z_\beta = \int_{\Omega_c} d\lambda(\omega) z^{||\omega||} \exp\{-\beta H(\omega)\} \) and parameters \( \beta > 0, z > 0 \).

The optimal configuration of objects in the image is obtained by sampling from the Gibbs probability distribution using a Markov chain, which consists of a discrete-time multiple birth-and-death process converging within the logarithmic annealing scheme to a continuous-time process giving a global minimum of \( H \).

First, the ‘birth’ step adds an unknown number of circles to the current configuration (empty at the first step) sampled from the Lebesgue-Poisson distribution with intensity (independent of the temperature and configuration energy) \( z = \delta z_0 \), where \( z_0 \) is the Poisson mean. Then, every circle is evolved by gradient descent with gradient field of \( E \) adapting the objects in the configuration to the image. Finally, the ‘death’ step removes a number of components from the current configuration with some probability \( p_d \) dependent on the current temperature \( T \) and for every component, the configuration energy difference without and with the component: \( \Delta_i H(\omega) = H(\omega \setminus \gamma_i) - H(\omega) \). The optimal configuration is found if all the objects added in the birth step and only them are removed in the following death step. If not, the temperature \( T \) and time step \( \delta \) are decreased using a geometric annealing scheme, and the birth-and-death procedure is repeated.

### 3.2 Gradient in Polar Space model (GiPS)

The GiPS method\(^3\) has been specifically developed for H&E stained surgical histopathology images. First step of the algorithm consists in nuclei detection with thresholding. Then, a Gamma-correction is performed to enhance the input image. A binary image is then generated by applying thresholding on the enhanced image. Then, dilation and erosion morphological operators are applied on the binary image in order to separate the joint or overlapping cell nuclei. The centers of mass of the nuclei are identified by applying a distance transform on the eroded image. The second step consists in the extraction of nuclei. This step starts by segmenting the image into patches containing the cell nuclei. A polar transform of the coordinate system is then performed on every patch with the center of mass of the nucleus as the origin. Finally, a median filter is applied for noise removal, and a biquadratic filtering is used to produce a gradient image from which nuclei boundaries are obtained.

### 3.3 K. Mosaliganti’s Level Set based model (KMLS)

K. Mosaliganti et al.\(^4\) have proposed a method for nuclei extraction using level sets. They applied their method on three different types of 3D microscopic images: mouse mammary glands at 20X magnification, cell culture observed at 10X and confocal microscopy of zebra fish. First, a thresholding and then a convex shape of nuclear intensity with a Gaussian kernel are applied to the input image to separate roughly foreground (nuclei) from background. Then, a level set segmentation is performed in order to refine the nuclei boundaries. Finally, the overlapping or joint nuclei are separated using a Voronoi diagram of nuclei.
4. EMPIRICAL STUDY

4.1 Data
We established a database of cell nuclei images from breast cancer surgical slides of 6 patients in close collaboration with the pathology department of a university hospital. The slides have already been analysed and graded by pathologists following the NGS, a state-of-the-art protocol to rate the malignancy of breast cancers from the analysis of histopathological images. Since the size and aspect of the nuclei can vary greatly according to the malignancy of the cancer, the selection of images was made to span a large range of histological grades from the NGS. The images correspond to high power fields acquired at an optical magnification of 400 times and have a resolution of \(1024 \times 1024\) pixels with each pixel covering a surface of \(0.25 \mu m \times 0.25 \mu m\) and contain both clusters of nuclei and isolated ones. The 1104 cells in the database are delineated manually in order to provide the basis for the validation of the algorithms.

4.2 Protocol
We assess 2 different aspects of the algorithms: the detection of the cell nuclei and the accuracy of their extraction.

For every image, the first problem is to match every nucleus detected by the algorithms (called ‘candidate’) to the corresponding reference nucleus delineated manually (called ‘reference’). Note that any of the following odd situations can happen: a candidate may not overlap with any reference, a candidate may overlap with two or more references or a reference may overlap with two or more candidates. Therefore, this matching task is not straightforward.

The solution we adopted is to pair candidates with references in a 1-to-1 fashion in order to maximize the total overlapping area between candidates and references. Candidate-reference pairs without any overlapping are discarded. In general, leftover cells will be found among both the candidates and the references. This assignment problem (a particular type of combinatorial optimization problems) is solved using the Hungarian assignment method.

The detection of cell nuclei is assessed with the F-measure, which is a harmonic mean of 2 criteria: precision score \(\text{prec}\) and recall score \(\text{rec}\), and is defined as follows:

\[
\text{F-measure} = 2 \frac{\text{prec} \times \text{rec}}{\text{prec} + \text{rec}}
\]

Let \(p\) be the number of pairs established with the above method (i.e. the number of well-detected nuclei), \(r\) be the number of reference nuclei and \(c\) be the number of candidates. The precision score, defined by \(\frac{p}{c}\), measures the proportion of true positives among all the cells detected by the algorithm. The recall score defined by \(\frac{p}{r}\) measures the proportion of actual positives which were correctly recognised by the algorithm.

The accuracy of extraction (delineation of nuclei) is computed for every pair ‘candidate-reference’ using the Jaccard index. If \(A\) is the surface of a candidate nuclei in the image and \(B\) is the surface of the corresponding reference, the Jaccard index for this pair is defined as:

\[
\frac{|A \cap B|}{|A \cup B|}
\]

The score ranges from 0 (no overlapping) to 1 (perfect correspondence). A global extraction score is computed by taking the arithmetic mean of the individual scores.

4.3 Results and Discussion
The global detection and extraction scores for the 3 different methods on the 1104 nuclei from the image database are provided in table 1.

A number of 1204 nuclei were detected using the MPP approach, 1678 using the KMLS method, and 735 using the GiPS method as compared to 1104 actual ones. In terms of nuclei detection, the MPP model obtains thus the best F-measure of 0.7038 followed by KM with 0.6292 and GiPS with 0.5909.

At first glance, it may seem that the results are lower than what was achieved in some related works presented in section 2. This is in fact explained by differences in the modality of the histopathological images which are used. Better results are typically obtained with cytological images such as fine needle aspirate biopsies where a single type of objects (cells) are well differentiated from a uniform background. In comparison, histopathological images such as the H&E stained digitised surgical slides used in our work are very high content images showing a wide variety of objects and structures, which are often poorly differentiated from a heterogeneous background.
Table 1: For each method: number of nuclei detected, number of ‘candidate-reference’ pairs constructed, global detection score (F-measure) and global extraction score (average Jaccard index).

<table>
<thead>
<tr>
<th>Method</th>
<th>nb. of nuclei detected</th>
<th>nb. of pairs constructed</th>
<th>global detection score (F-measure)</th>
<th>global extraction score (average Jaccard index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPP</td>
<td>1204</td>
<td>827</td>
<td>0.7038</td>
<td>0.6489</td>
</tr>
<tr>
<td>KMLS</td>
<td>1678</td>
<td>942</td>
<td>0.6674</td>
<td>0.6292</td>
</tr>
<tr>
<td>GiPS</td>
<td>735</td>
<td>596</td>
<td>0.6337</td>
<td>0.5909</td>
</tr>
</tbody>
</table>

In terms of nuclei extraction (delineation of nuclei), the MPP model obtains the best average Jaccard index of 0.6489 followed by KM with 0.6292 and GiPS with 0.5909.

Histograms in figure 1 illustrate the distribution of the Jaccard indices for individual pairs of nuclei. It gives a more detailed insight into the quality of the delineation of nuclei. MPP and KMLS methods have relatively comparable distributions, which can be explained by the fact that they both use active contours. Comparing performances of MPP and GiPS is less evident: although MPP has more well-delineated nuclei than GiPS, it also provides a slightly greater number of poorly extracted nuclei (Jaccard index less or equal to 0.4). Therefore, GiPS performs in general a more stable quality extraction (although less good on average) than the MPP model. This tendency can be explained by the fact that the MPP model requires prior information on the size and shape of the objects which may fit some nuclei better than others.

Figure 2 shows an example of resulting nuclei delineation for each of the methods on the same sample of image.

Figure 1: Distribution of the pairwise extraction accuracy scores (individual Jaccard indices) for each of the 3 methods.

5. CONCLUSION

The MPP model proved a promising solution for the extraction of cell nuclei from breast cancer slide images able to overperform the two other state-of-the-art methods. This study also suggests a number of improvements to the method. First, a number of parameters corresponding to the initialization of the circles and the parameters arising from the image modeling were calibrated experimentally. Setting these parameters via machine learning would likely improve the overall results of the method. Moreover, the stability of the extraction also proved to be somewhat of a concern. A way to address this would be to define multiple classes of objects in order to better
capture the variety in terms of size, shape, and texture of nuclei. This is possible because of the generality of the MPP model with arbitrarily-shaped objects involved.

**Acknowledgments**

This work was performed within the project MICO COgnitive MIcroscope: a cognition-driven visual explorer for histopathology for application to breast cancer grading, a project supported by ANR the French National Research Agency, program TecSan 2010 ANR-10-TECS-015. We would like to express our gratitude to INRIA for providing the ASOE 0.1 software which was developed in EPI Ariana.

**REFERENCES**


