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A High-throughput Active Contour Scheme for Segmentation of Histopathological Imagery

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Abstract

In this paper a minimally interactive high-throughput system which employs a color gradient based active contour model for rapid and accurate segmentation of multiple target objects on very large images is presented. While geodesic active contours (GAC) have become very popular tools for image segmentation, they tend to be sensitive to model initialization. A second limitation of GAC models is that the edge detector function typically involves use of gray scale gradients; color images usually being converted to gray scale, prior to gradient computation. For color images, however, the gray scale gradient image results in broken edges and weak boundaries, since the other channels are not exploited in the gradient computation. To cope with these limitations, we present a new GAC model that is driven by an accurate and rapid object initialization scheme; hierarchical normalized cuts (HNCut). HNCut draws its strength from the integration of two powerful segmentation strategies-mean shift clustering and normalized cuts. HNCut involves first defining a color swatch (typically a few pixels) from the object of interest. A multi-scale, mean shift coupled normalized cuts algorithm then rapidly yields an initial accurate detection of all objects in the scene corresponding to the colors in the swatch. This detection result provides the initial contour for a GAC model. The edge-detector function of the GAC model employs a local structure tensor based color gradient, obtained by calculating the local min/max variations contributed from each color channel. We show that the color gradient based edge-detector function results in more prominent boundaries compared to the classical gray scale gradient based function. By integrating the HNCut initialization scheme with color gradient based GAC (CGAC), HNCut-CGAC embodies five unique and novel attributes: 1) Efficiency in segmenting multiple target structures; 2) The ability to segment multiple objects from very large images; 3) Minimal human interaction; 4) Accuracy; and 5) Reproducibility. A quantitative and qualitative comparison of the HNCut-CGAC model against other state of the art active contour schemes (including a Hybrid Active Contour model (Paragios-Deriche) and a region based AC model (Rousson-Deriche)), across 196 digitized prostate histopathology images, suggests that HNCut-CGAC is able to outperform state of the art hybrid and region based AC techniques. Our results show that HNCut-CGAC is computationally efficient and may be easily applied to a variety of different problems and applications.

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High-throughput System; Active Contour Model; Segmentation; Prostate Cancer; Digital Pathology

1. Introduction

With the recent advent and cost-effectiveness of whole-slide digital scanners, histopathology glass slides can be easily converted into digital slides and stored as high resolution digital images (May, 2010). Pathologists can now analyze the digitized slides, thereby making their diagnoses on the computer monitor instead of the traditional microscope. More importantly, this technology makes the computerized quantitative image analysis of digitized histopathology possible (Gurcan et al., 2009). In histopathology specimens, the morphological appearance of different structures, such as as glands or nuclei, are often highly reflective of disease outcome. For example, each gland in normal prostate histopathology is comprised of a central lumen area, surrounding epithelial cytoplasm, and a ring of epithelial nuclei defining the outer boundary of the gland (Doyle et al., In Press). However, in low grade prostate cancer (CaP) the central lumen area shrinks and is almost completely absent in high grade CaP. Additionally, gland morphology is known to progressively change from low (less aggressive) to high grade (poor outcome) prostate cancer (Montironi et al., 2005). Additional visual changes include changes in the area of the epithelium or lumen, the shape, the size, the number, and differentiation of the glands (Gleason, 1992). In the case of several diseases, such as prostate, breast, and ovarian cancer, shape and morphological attributes of glands, tubules, and nuclei on the tissue specimens correlate with disease aggressiveness (Venkataraman et al., 2009; Karpinska-Kaczmarczyk et al., 2009; Farjam et al., 2007; Haba et al., 1993). Hence, an important pre-requisite to predicting disease outcome is the ability to accurately and efficiently detect the location of glands and segment them so that important morphological features pertaining to disease outcome may be obtained. Therefore, there is a clear need to develop high-throughput computer-assisted analytical tools for segmenting histological structures on digital pathology slides, which can be as large as several thousand by several thousand pixels.

Active contour (AC) models have emerged as popular segmentation tools for separating the objects/structures of interest from the background via continuously deformable curves. Most AC models deform in order to delineate the boundaries of the desired objects in the image through minimizing an energy functional (Caselles et al., 1997). While AC models are able to accurately capture the shape of the object, thereby enabling extraction of higher-level shape and morphological features, most AC models are not able to simultaneously segment multiple structures on very large images. This is primarily due to the fact that most boundary-based AC models require accurate model initialization in order to be able to handle very large images (Fatakdawala et al., 2010). Though region-based models do not require accurate initialization (as we will discuss later), simultaneous and concurrent segmentation of multiple structures on very large images is still a challenge for region based models, especially in the presence of a complicated background. For example, a prostate needle core biopsy digitized at 40x magnification results in an image that is greater than 2GB in size. A single prostate biopsy core could comprise several thousand glands. If the objective is to simultaneously segment boundaries of all glands in such an image, most AC schemes would require careful model initialization in the proximity of each object of interest. If the objective were to segment all glands in the image, this could involve multiple, careful initializations of the AC model to segment the thousands of glands that might be present on a single prostate biopsy core image. Since most AC models are unable to handle the simultaneous segmentation of so many structures of interest from such large images,

there is a need for rapid identification of the objects of interests in order to initialize the AC model. Manual initialization of thousands of objects simultaneously is clearly not feasible. Consequently, a deformable AC model would ideally require automated initialization of the objects of interest. Additionally, for most boundary based AC models, the evolution function is dependent on the gray scale intensity gradient. Most AC models convert color images into an equivalent gray scale representation and hence do not exploit the color tensor information present in these images (Caselles et al., 1997).

In this paper we present a new high-throughput segmentation tool for accurate, efficient and automated extraction of contours of histological structures (e.g. glands) so that the morphological information from histological images can be employed for building diagnostic and prognostic classifiers. We will show the application of a new color gradient based AC model with minimal user interaction for rapid, accurate model initialization in the context of gland segmentation on digitized prostate histopathology. The scheme, as we will show, is readily extensible to a variety of domains and applications both within and outside digital pathology.

The rest of this paper is organized as follows: In Section 2, we discuss previous related work. In Section 3, a brief overview of the new color gradient based AC scheme with minimally interactive object initialization, along with the novel contributions of this work are presented. In Section 4, we present the methodological details of our AC model. In Section 5, we describe the data sets and experimental design. In Section 6, we present the results of qualitative and quantitative evaluation of our AC mode. Concluding remarks are presented in Section 7.

2. Previous Related Work

Based on the type of image information used to drive the model, AC schemes may be categorized as either (a) boundary- (Caselles et al., 1997) or (b) region-based (Chan and Vese, 2001). The geodesic active contour (GAC) model proposed by Caselles et. al. (Caselles et al., 1997) is an important boundary-based AC model. Beginning with a user specified initial boundary, GAC models utilize a positive-decreasing gradient function as the stopping criterion. This attracts the contour towards the edges of the target objects. The

edge-detector function is a positive-decreasing function, defined as $g(f(c)) = \frac{1}{1+g(f(c))}$ where $g(f(c)) = \frac{1}{1+g(f(c))}$ (c)) is the magnitude of the gradient at every pixel c in the image. The minima of the function g(f(c)) is achieved as the gradient magnitude, s(f(c)), approaches the maximal value at the object boundaries. When this happens, the curve stops its evolution, right at the edge of the desired object. One limitation of boundary-based GAC models is that they are highly dependent on the edge-detector function. Most boundary based AC models (Cohen, 1991; Caselles et al., 1997; Malladi et al., 1995) define the function g(f(c)) as the gradient of the gray scale image. For color images, the most common approach in computing the image gradient involves first converting the vector image to a scalar (gray-scale) image by eliminating 2 of the 3 color channels (e.g. removing the hue and saturation channels while retaining the luminance channel) (Sapiro, 1997). The directional gradient is then calculated from this single channel image. However, this color conversion procedure results in broken edges and weak boundaries due to the loss of information from the other channels. This limitation of GAC models in exploiting gray scale gradients can be appreciated in Figure 2(c). Broken edges and weak boundaries adversely affect the curves' evolvement, such as causing it to miss the boundaries of objects whose gradients are not large enough (see Figures 5(e), (f) and Figures 6(e), (f)). Consequently, there is a need for the computation of color gradients directly from the color image.

As mentioned in Section 1, a major limitation of boundary-based AC models is the need for explicit initialization in the vicinity of the target object of interest (Cohen and Kimmel, 1997). Recently, region-based AC (RAC) models have been proposed to address some of the limitations of GAC models. The region-based model essentially employs statistical information derived from different regions (foreground and background) to drive the AC model, which is independent of the edge-detector function and does not require precise initialization. One important RAC model is the Rousson-Deriche (RD) model (Rousson and Deriche, 2002). The RD model assumes that the image plane comprises two regions and the intensities of pixels within each region satisfy a Gaussian distribution. The contour evolves as a result of competition between the log probability of current pixels c belonging to the foreground and background regions. However, RD and other RAC models have their own limitations. For instance, the model may lead to inaccurate boundaries if the boundary information is ignored. RAC models may also require more computations for the randomly initialized contour to converge to the boundaries of the objects. Moreover, most of the models make a strong assumption that the number of distinct regions in the scene is known. Further, if the background of the image is too complicated, such as in digitized histopathology, the RAC model may not be able to segment the regions of interest (see Figures 5(i), (j) and Figures 6(i), (j)). In fact, even in scenarios where the background is not very complicated, the RAC model may latch onto the incorrect object boundary.

While hybrid AC models (Paragios and Deriche, 2002a,b) have been proposed to combine the strengths of boundary-based and region-based models, like RAC models, they too might sometimes fail without accurate initialization. Even though the edge detection function is incorporated into the regularization term, the hybrid model is actually a variant of the region-based model. Since the region term in the hybrid model tends to dominate the driving forces during curve evolution, the hybrid model shares most of the limitations of regionbased models (see Figures 5(k), (1) and Figures 6(k), (1)). Therefore, the hybrid AC model is plagued by many of the limitations that afflict region-based models. Hybrid AC models are also constrained, like most boundary and region based models, in their inability to simultaneously segment multiple objects in very large images. This may explain why, up until now, relatively few shape-based segmentation tools have been proposed for the automated analysis of digitized histopathology imagery (Gurcan et al., 2009). In Fatakdawala et al. (2010), an expectation-maximization (EM) algorithm based method was utilized for automatically detecting the centers of lymphocytes on breast cancer histopathology images. The initial contours for the curve evolution function were defined with these detected centers. However, only image patches of size 200×200 pixels were considered in this study. In Hafiane et al. (2008), the results from fuzzy c-means clustering were employed to initialize the active contour model for segmenting the nuclei on prostate histopathology.

None of the initialization schemes proposed above, however, are able to address the demands of on the fly, rapid and efficient segmentation of a specific target of interest on very large images. In Janowczyk et al. (2009), Janowczyk et. al. introduced hierarchical normalized cut (HNCut), an object detection scheme that integrated the mean-shift clustering (Comaniciu and Meer, 2002) scheme with the normalized cuts algorithm (Shi and Malik, 2000) within a multi-resolution framework. The HNCut scheme used a hierarchically represented data structure to bridge the mean shift clustering and normalized cuts algorithms. This allows HNCut to efficiently traverse a pyramid of the input image at various color resolutions, efficiently and accurately pre-segmenting the object class of interest. By simply specifying a few pixels from the object of interest, the HNCut scheme can be used to rapidly identify all related and similar objects within the image. By specifying representative pixels from a different object, the HNCut scheme can be used to rapidly identify all image pixels corresponding to the target of interest.

3. Overview and Novel Contribution

Figure 1 illustrates the flowchart showing the working of the HNCut-CGAC model in the context of gland segmentation on prostate histopathological imagery. As the flowchart shows, the model includes two modules. In the first module, a HNCut initialization scheme (Janowczyk et al., 2009) is employed for rapid, minimally supervised, specification of the target object of interest. Based on the pre-segmentation results from the first module, a level set functional is initialized in the second module. The AC model employs a novel color gradient based function as its edge detection function. The color gradient is based on the local structure tensor, which is obtained by calculating the local min/max variations contributed from each color channel (e.g. R, G, B or H, S, V). This results in significantly stronger object boundaries compared to those obtainable via the gray scale gradient alone. By integrating the HNCut initialization scheme with the color gradient based GAC model, our high-throughput system has five unique and novel attributes:

- 1. Efficiency in segmenting multiple target structures. The HN-Cut initialization scheme allows for rapid detection of the locations of the target structures, thereby providing an initialization for the AC model. The level set representation of color gradient based AC model evolves the embedded level set functional, which is able to automatically handle changes in contour topology. The scheme can thus handle the simultaneous segmentation of multiple objects in parallel.
- 2. The ability to handle large images. The integration of the HNCut initialization within the GAC framework allows our scheme to segment multiple instances of the target object on arbitrarily large images.
- **3. Minimal human interaction**. The system requires minimal human intervention during the HNCut stage. This intervention is in the form of a user selected swatch that reflects the color contained in the target object of interest. The subsequent steps are completely free of any human intervention.
- **4. Accuracy**. The system is able to segment structures in the image with an accuracy comparable to that of a human expert. This is particularly relevant in histopathology imagery where a human expert may simply be unable to manually segment thousands of instances of the target object on a very large digital slide.
- **5. Reproducibility**. The model comprises very few free parameters and, except for the user selected swatch for the HNCut module, requires no additional user intervention. This makes the scheme robust and highly reproducible.

4. HNCut initialized active contour scheme

4.1. Notation

Let $C = (C, \mathbf{f})$ (or C = (C, f)) define a color (or gray scale) image, where *C* is a 2D Cartesian grid of pixels c = (x, y) and $\mathbf{f}(c) \in \mathbb{R}^3$ (or $f(c) \in \mathbb{R}^1$) is a function that assigns intensity values (or an intensity value) to pixel $c \in C$. A list of commonly used notations and symbols in this paper is illustrated in Table 1.

4.2. Hierarchical Mean Shift based Normalized Cuts initialization scheme

The HNCut scheme draws its strength from the integration of two powerful segmentation strategies—frequency weighted mean shift clustering and normalized cuts. The scheme is outlined in the following three steps:

1. User selects the domain swatch

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A user, via manual selection, defines a color swatch *S* from the color function **f** such that $S_1 = {\mathbf{f}_{1,\alpha} | \alpha \in {1, ..., N}}$ creates a selection of color values that are representative of the object of interest from *C*.

2. Frequency weighted mean-shift clustering for generating a multi-resolution color pyramid.

The mean shift algorithm is a non-parametric clustering technique (Co-maniciu and Meer, 2002). It can be employed to identify the local maxima of a density function and detect modes of clusters by using a density gradient estimator. In this step, an improved version of the mean-shift algorithm called frequency weighted mean-shift (FWMS) algorithm is employed to generate multiple levels of a pyramidal scene representation $C_{\kappa} = (C, \mathbf{f}_k)$, where $k \in \{1, ..., K\}$ represent the *k*-th levels of a color pyramid produced at each iteration of the FWMS algorithm. At each level *k*, the unique values in the color vector $\mathbf{F}_k = \{f_{k,1}, f_{k,2}, ..., f_{k,/\mathbf{F}_k/}\}$ are determined under the constraint that any two values are equivalent if $||f_{k,i} - f_{k,j}|| \le \varepsilon$, where ε is a predefined similarity constraint. As a result, the vector \mathbf{F}_k can be constructed from \mathbf{F}_k , where $\mathbf{\hat{F}}_k \subset \mathbf{F}_k$ and $\mathbf{\hat{F}}_k$ is a set of only the unique values present in \mathbf{F}_k , where the cardinality of set $\mathbf{\hat{F}}_k$ is defined as

$$M_k = |\widehat{\mathbf{F}}_k|. \tag{1}$$

For all $f_{k,i} = \hat{F}_{k,j}$, the element of the weight vector $\mathbf{w}_k = \{w_{k,1}, \dots, w_{k,M_k}\}$ associated with $\hat{\mathbf{f}}_k$ is computed as

$$w_{k,j} = \sum_{i=1}^{M_k} w_{k-1,i}$$
(2)

where $i, j \in \{1, ..., M_k\}$. Intuitively, $w_{k,j}$ in (2) is summing the weights from the previous level into the new unique values. Additionally, the weights satisfy the equation

$$\sum_{i=1}^{M_k} w_{k,i} = N.$$
(3)

As a result, $w_{k,j}$ is a count of the number of original colors that have migrated to $\mathbf{\hat{F}}_k$ through mean shifting (Comaniciu and Meer, 2002). Then, based on the weight vector \mathbf{w}_k , the fixed point iteration update becomes

$$f_{k+1,j} \leftarrow \frac{\sum_{i=1}^{M_k} w_{k,i} \widehat{f}_{k,j} G(\widehat{f}_{k,j} - \widehat{f}_{k,i})}{\sum_{i=1}^{M_k} G(\widehat{f}_{k,j} - \widehat{f}_{k,i})},$$
(4)

where the Gaussian function G, with a bandwidth parameter σ , is defined as

$$G(\widehat{f}_{k,j} - \widehat{f}_{k,i}) = \exp(-\frac{\left\|\widehat{f}_{k,j} - \widehat{f}_{k,i}\right\|^2}{\sigma^2}).$$
(5)

The function $G(\cdot)$ is used to estimate the kernel density at color data point $\mathbf{f}_{k,i}$.

3. Normalized cuts segmentation on frequency weight mean shift reduced color space.

Normalized cuts (NCuts) is a graph partitioning method (Wu and Leahy, 1993). The hierarchical pyramid created by mean shift and corresponding to various levels of color resolution serves as the initial input to the NCuts algorithm. NCuts takes a connected graph with vertices and edges and partitions the vertices into disjoint groups. By setting vertices to the set of color values and having the edges represent the similarity (or affinity) between the color values, the vertices can be separated into distinct groups, each of which is comprised of similar colors. By operating in the color space, as opposed to the spatial domain (on pixels), the scheme is very fast. Normalized Cuts (Shi and Malik, 2000) is employed on the small number of unique values in the bottom color level $\mathbf{\hat{F}}_{K}$ to remove those colors that are not contained within the object specific color swatch. Let $G_K = \{V_K, W_K\}$ be an undirected weighted graph with vertex set V_K and similarity matrix W_K constructed on C_{κ} at the lowest level of the color pyramid. V_K is comprised of unique color values in $\mathbf{\hat{F}}_{K}$. Assuming there are N unique color values in $\mathbf{\hat{F}}_{K}$, $W_{K} \in \mathbb{R}^{N \times N}$ is a similarity/adjacency matrix of the graph that measures the similarity of color value among any two vertices/points, whose elements are defined as (Shi and Malik, 2000)

$$w_{ij} = \exp\left(\frac{-\left\|f_{K,i} - f_{K,j}\right\|^2}{\sigma_1^2}\right) \times \begin{cases} \exp\left(\frac{-\left\|c_i - c_j\right\|^2}{\sigma^2}\right), & \text{if } \|c_i - c_j\| < \theta; \\ 0, & \text{otherwise.} \end{cases}$$
(6)

where c_i, c_j are in \mathcal{C}_{κ} , and θ is a pre-defined spatial radius threshold. In (6), the first Gaussian function measures intensity similarity between vertices c_i and c_j and σ_1 is a bandwidth parameter. The second Gaussian function measures spatial distance between c_i and c_j , and σ_2 controls the width of the neighborhoods. The segmentation problem is transformed into finding a vector that can optimally bipartition graph G_K , which in turn is equivalent to solving the following generalized eigenvalue system (Shi and Malik, 2000):

$$(D_{K} - W_{K})\mathbf{v} = \lambda D_{K}\mathbf{v},\tag{7}$$

where D_K is called the degree matrix. The entries of diagonal matrix D_K are column (or row, since W_K is symmetric) sums of W_K . The optimal bipartition of the graph is the eigenvector with the second smallest eigenvalue (7). The NCut algorithm

partitions $\mathbf{\hat{F}}_{K}$ into two color sets $\mathbf{\hat{F}}_{K}^{A}$ and $\mathbf{\hat{F}}_{K}^{B}$. After the partition, $\mathbf{\hat{F}}_{K}^{A}$ or $\mathbf{\hat{F}}_{K}^{B}$ are matched against the colors in swatch S_{1} (selected in Step 1). After subsequent

iterations, the segmentation results are obtained from one of \mathbf{F}_{κ}^{A} and \mathbf{F}_{κ}^{B} that uniquely contains all colors in color swatch *S*1, the other set of colors is discarded. The resulting detection results make for an excellent initialization for the subsequent application of the color gradient based AC model.

4.3. Local Structure Tensor Based Color Gradient

Color gradient based AC models have been proposed previously in Sapiro (1997) and Yang et al. (2005). A major difference between the HNCut-CGAC model and the color gradient vector flow snake in Yang et al. (2005) (where the color gradient serves as an external force to drive the snake) is that in HNCut-CGAC, the color gradient serves as the edge detector function. The color gradient function employed in HNCut-CGAC is inspired by the Cumani

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operator (Aldo, 1991), a second-order differential operator for vectorial images. The Cumani operator is based on Di Zenzo multi-valued geometry (Di Zenzo, 1986). For a color image $C = (C, \mathbf{f})$, the L_2 norm of \mathbf{f} can be written in matrix form as

$$d\mathbf{f}^2 = \begin{bmatrix} dx \\ dy \end{bmatrix}^T \begin{bmatrix} g_{11} & g_{12} \\ g_{21} & g_{22} \end{bmatrix} \begin{bmatrix} dx \\ dy \end{bmatrix},\tag{8}$$

where

$$g_{11} = \left(\frac{\partial \mathbf{f}}{\partial x}\right)^{T} \left(\frac{\partial \mathbf{f}}{\partial x}\right) = \left(\frac{\partial f_{1}}{\partial x}\right)^{2} + \left(\frac{\partial f_{2}}{\partial x}\right)^{2} + \left(\frac{\partial f_{3}}{\partial x}\right)^{2},$$

$$g_{12} = g_{21} = \left(\frac{\partial \mathbf{f}}{\partial x}\right)^{T} \left(\frac{\partial f_{1}}{\partial y}\right) = \frac{\partial f_{1}}{\partial x} \cdot \frac{\partial f_{1}}{\partial y} + \frac{\partial f_{2}}{\partial x} \cdot \frac{\partial f_{2}}{\partial y} + \frac{\partial f_{3}}{\partial x} \cdot \frac{\partial f_{3}}{\partial y}, \text{ and}$$

$$g_{22} = \left(\frac{\partial \mathbf{f}}{\partial y}\right)^{T} \left(\frac{\partial \mathbf{f}}{\partial y}\right) = \left(\frac{\partial f_{1}}{\partial y}\right)^{2} + \left(\frac{\partial f_{2}}{\partial y}\right)^{2} + \left(\frac{\partial f_{3}}{\partial y}\right)^{2}.$$
(9)

The matrix $\begin{bmatrix} g_{ij} \end{bmatrix} = \begin{bmatrix} g_{11} & g_{12} \\ g_{21} & g_{22} \end{bmatrix}$ contains the coefficients of the first fundamental form in the color space and is also referred to as the local structure tensor. It locally sums the gradient contributions from each image channel. Here f_1, f_2 and f_3 are intensities of each channel for any pixel *c* in *C*. For the matrix $[g_{ij}]$, the maximum and minimum eigenvalues of the matrix $(\lambda_+ \text{ and } \lambda_-)$ represent the extreme rates of change in the direction of their corresponding eigenvectors. λ_+ and λ_- may be formally expressed by

$$\lambda_{\pm} = (g_{11} + g_{22} \pm \sqrt{\Delta})/2, \tag{10}$$

where

$$\Delta = (g_{11} - g_{22})^2 + 4g_{12}^2. \tag{11}$$

The color gradient at any $c \in C$ may hence be expressed as (Sapiro, 1997)

$$s(\mathbf{f}(c)) = \sqrt{\lambda_{+} - \lambda_{-}}.$$
(12)

From equations (8)-(12), it is easy to show that the gray scale gradient $\sqrt{\frac{\partial^2 f_i}{\partial x^2} + \frac{\partial^2 f_i}{\partial y^2}}$, where $i \in \{1, 2, 3\}$, (widely employed for edge detection (Caselles et al., 1997)) is a special case of the color gradient $s(\cdot)$. Note that the methodology for computing the color gradient described above could be easily applied to different vectorial color representations such as RGB, HSV, and Luv (Gonzalez and Woods, 2008). Figure 2 illustrates the role and importance of the color gradient function (12) in driving the curve evolution function for an AC model. The color gradient representation (Figure 2(b)) for the digitized prostate histopathology image (Figure 2(a)) results in more prominent boundaries compared to the corresponding gray scale gradient (Figure 2(c)). Figure 2(c) was generated with the MATLAB function rgb2gray, where the RGB color image is first transformed into HSV color space. The hue

and saturation channels are then eliminated to yield a scalar luminance, gray-scale image (Caselles et al., 1997).

4.4. Geodesic Active Contour Model

4.4.1. Energy functional—We assume that the image plane $\Omega \in \mathbb{R}^2$ is partitioned into 2 non-overlapping regions by a curve \mathbb{C} . The foreground region Ω_f , background region Ω_b and the curve \mathbb{C} have been defined in Table 1. The relationship among them are

$$\Omega = \Omega_f \cup \Omega_b \cup \mathbb{C},\tag{13}$$

and

$$\Omega_f \cap \Omega_b = \emptyset, \tag{14}$$

where Ω_f and Ω_b represent the set of image locations corresponding to the target regions of interest (or foreground) and the other non-target regions (or background), respectively. The optimal partition of the image plane Ω by a curve \mathbb{C} can be obtained through minimizing the energy functional

$$E_1(\varphi) + E_2(\varphi) = \alpha \int_{\mathbb{C}} g(\mathbf{f}(c)) dc + \beta \int_{\Omega_f} g(\mathbf{f}(c)) dc.$$
(15)

In Equation (15), the first term $E_1(\varphi)$ is the energy functional of a traditional GAC model, obtained as the integral of an edge detector function $g(\mathbf{f}(c))$, for each pixel c over the curve \mathbb{C} . This external image force pushes or attracts the curve \mathbb{C} to the high gradient regions. Minimization of this energy term is equivalent to minimizing the weighted Euclidean length of the curve \mathbb{C} . The second term $E_2(\varphi)$ which is an area minimization term is inspired by the balloon force proposed in Cohen (1991). The inflation force, like a balloon, stops the curve \mathbb{C} when the object edges are strong. Alternatively, the curve may pass through the object border if the edge is too weak with respect to the inflation force (Cohen, 1991). Minimization of this term is equivalent to minimizing the weighted foreground areas enclosed by the curve \mathbb{C} . Note that the edge detector function in the traditional GAC model and the balloon force are based on the calculation of the gray level gradient of the image, such as the Canny-Deriche edge in Cohen (1991). In this paper, the edge-detector function $g(\mathbf{f}(c))$ is based on the color gradient, which is defined as

$$g(\mathbf{f}(c)) = \frac{1}{1 + s(\mathbf{f}(c))},\tag{16}$$

where $s(\mathbf{f}(c))$ is the local structure tensor based color gradient, previously defined in Section 4.3.

In traditional level set methods, a re-initialization phase is required as a numerical remedy for maintaining stable curve evolution (Li et al., 2010). To overcome this drawback, an additional energy term (Li et al., 2010) is added to remove the re-initialization phase

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$$E_3(\varphi) = \int_{\Omega} \frac{1}{2} \left(\left\| \nabla \varphi \right\| - 1 \right)^2 dc.$$
⁽¹⁷⁾

The combined energy functional in this paper is hence defined as

$$E(\varphi) = \alpha E_1(\varphi) + \beta E_2(\varphi) + \gamma E_3(\varphi),$$

= $\alpha \int_{\mathbb{C}} g(\mathbf{f}(c)) dc + \beta \int_{\Omega_f} g(\mathbf{f}(c)) dc + \gamma \int_{\Omega_0^2} \frac{1}{2} (\left\| \nabla \varphi \right\| - 1)^2 dc.$ (18)

By employing the Heavside function $H(\varphi)$, we can unify integrals in Equation (18) as (Chan and Vese, 2001; Zhao et al., 1996)

$$E(\varphi) = \alpha \int_{\Omega} g(\mathbf{f}(c)) \left\| \nabla H(\varphi) \right\| dc + \beta \int_{\Omega} g(\mathbf{f}(c)) H(\varphi) dc + \gamma \int_{\Omega} \frac{1}{2} (\left\| \nabla \varphi \right\| - 1)^2 dc,$$
(19)

where $c \in \Omega$. Using the fact that $\|\nabla H(\varphi)\| = \delta(\varphi(\mathbf{f}(c)))\|\nabla \varphi\|$ (Chan and Vese, 2001; Vese and Chan, 2002), the energy functional reduces to

$$E(\varphi) = \alpha \int_{\Omega} g(\mathbf{f}(c)) \delta(\varphi(\mathbf{f}(c))) \left\| \nabla \varphi \right\| dc + \beta \int_{\Omega} g(\mathbf{f}(c)) H(\varphi) dc + \gamma \int_{\Omega} \frac{1}{2} (\left\| \nabla \varphi \right\| - 1)^2 dc.$$
(20)

4.4.2. Curve evolution function of GAC model—Based on the theory of the calculus of variations (Gelfand and Fomin, 2000), the curve evolution function can be derived from the level set framework by minimizing the energy functional in Equation (20). The curve evolution function is now defined by the following partial differential equation (PDE):

$$\begin{cases} \frac{\partial \varphi}{\partial t} = \delta(\varphi) \left\{ \alpha \operatorname{div} \left[g(\mathbf{f}(c)) \frac{\nabla \varphi}{\left\| \nabla \varphi \right\|} \right] + \beta g(\mathbf{f}(c)) \right\} + \gamma \left[\Delta \varphi - \operatorname{div}(\frac{\nabla \varphi}{\left\| \nabla \varphi \right\|}) \right], \\ \varphi(0, c) = \varphi_0(c), \quad \forall c \in C. \end{cases}$$
(21)

where α , β , and γ are positive constant parameters defined empirically as $\alpha = 4$, $\beta = 0.5$, and $\gamma = 0.04$, respectively. $\delta(\varphi)$ is the Delta function (see Table 1), **div**(·) is the divergence operator, and $\varphi_0(c)$ is the initial evolution functional that is obtained from the HNCut segmentation result (see Section 4.2). φ_0 is defined as a piecewise linear function:

$$\varphi_0(c) = \begin{cases} -\xi, & c \in \Omega_b, \\ 0, & c \in \mathbb{C}, \\ \xi, & c \in \Omega_f. \end{cases}$$
(22)

where Ω_{f} , \mathbb{C} and Ω_{b} represent the target regions of interest, the boundaries of the target regions, and the other non-target regions, respectively. In Equation (22), Ω_{f} , \mathbb{C} , and Ω_{b} are all obtained via the application of the HNCut scheme. ξ is a positive constant and set empirically to $\xi = 4$.

5. Experimental Design

5.1. Datasets

We quantitatively and qualitatively compared the performance of the HNCut-CGAC model against other AC schemes (see Table 3) on a total of 196 images obtained from two different patient cohorts from the Hospital at the University of Pennsylvania (UPENN). In Table 2, the first cohort comprised 126 Hematoxylin & Eosin (H&E) stained and digitized prostate needle core biopsy specimens. Each of the 126 images was obtained by digitizing the corresponding glass slide at 20x optical magnification using an Aperio whole-slide digital scanner. The second data set is comprised of 70 H&E stained images of quadrant histological sections obtained from radical prostatectomy studies.

5.2. Ground truth generation

For all 196 images considered in this study, the objective was to segment the boundaries of the glandular regions. Since it was impossible to have an expert pathologist manually segment each and every gland in each of the 196 images (to provide ground truth for quantitative evaluation), the expert was asked to randomly pick region of interests on the digitized image where clusters of glands were visible. The expert then proceeded to meticulously segment gland boundaries from within the randomly chosen ROI on each of the 196 digitized images considered in this study. Consequently, quantitative evaluation of the different AC models was limited to these ROI's across the 196 images.

5.3. Comparative strategies

Table 3 lists four AC models that we implemented, solely for the purpose of quantitative comparison with the HNCut-CGAC model.

5.3.1. Rousson-Deriche model (Rousson and Deriche, 2002)—Most region-based AC models are inspired by the Mumford-Shah functional (Mumford and Shah, 1989). Mumford-Shah functionals approximate image intensities via a piecewise smooth (or constant) function. In Zhu and Yuille (1996), the authors established relations between statistical methods and the piecewise constant model by a more general energy functional, where the image intensities within each region are approximated by a statistical distribution. The Rousson-Deriche model assumes that the image plane Ω is partitioned into two regions Ω_f and Ω_b by contour C. If we further assume that image intensities in each region are statistically homogeneous and the intensities of each region are approximated by a Gaussian distribution, the energy functional can be derived as follows:

$$E(\varphi) = -\alpha_1 \int_{\Omega} [H(\varphi) \log p(\mathbf{f}(c)|\theta_f) + (1 - H(\varphi)) \log p(\mathbf{f}(c)|\theta_b)] dc + \beta_1 \int_{\Omega} |\nabla H(\varphi)| dc$$
(23)

where $H(\varphi)$ is the Heaviside function and $p(\mathbf{f}(c)|\theta_i)$ ($i \in \{f, b\}$) are the multivariate Gaussian distribution function with parameter $\theta_i = \{\mu_i, \Sigma_i\}$, where μ_i and Σ_i are the mean and covariance of the intensity in the region $i(i \in \{f, b\})$ and are estimated by

$$\mu_i = \frac{1}{|\Omega_i|} \int_{\Omega_i} \mathbf{f}(c) dc,$$

$$\sum_i = \frac{1}{|\Omega_i|} \int_{\Omega_i} (\mathbf{f}(c) - \mu_i) (\mathbf{f}(c) - \mu_i)^T dc.$$
(24)

By minimizing the energy functional (23) via the variational principle, the optimal bipartitioning of the image plane Ω can be obtained by evolving the curve evolution functional as follows

 $\begin{cases} \frac{\partial \varphi}{\partial t} = \alpha_1 \delta(\varphi) \left[\log p(\mathbf{f}(c)|\theta_1) - \log p(\mathbf{f}(c)|\theta_2) \right] + \beta_1 \delta(\varphi) div \left[\frac{\nabla \varphi}{|\nabla \varphi|} \right], \\ \varphi(0,c) = \varphi_0(c), \quad \forall c \in C. \end{cases}$

(25)

where $\varphi_0(c)$ is the initial contour, which is generated randomly. From Equation (25), the contour evolves as a result of competition between the log probability of current pixel *c* belonging to foreground Ω_f and background region Ω_b .

5.3.2. Hybrid AC (HAC) model (Paragios and Deriche)—Since region-based AC models do not typically include boundary information, Paragios and Deriche presented a hybrid AC model in Paragios and Deriche (2002a,b) by incorporating a gradient-based edge detection function into the regularization term of region-based model. By incorporating $g(\mathbf{f}(c))$ into the second term of Equation (25), the RD model reduces to the HAC model as follows:

$$E(\varphi) = -\alpha_2 \int_{\Omega} [H(\varphi) \log p(\mathbf{f}(c)|\theta_f) + (1 - H(\varphi)) \log p(\mathbf{f}(c)|\theta_b)] dc + \beta_2 \int_{\Omega} g(\mathbf{f}(c)) |\nabla H(\varphi)| dc.$$
(26)

The major difference between (26) and the HAC model presented in Paragios and Deriche (2002a,b) is that $g(\mathbf{f}(c))$ is based on the color gradient whereas the edge detection function employed in Paragios and Deriche (2002a,b) are based on the gray scale image gradient. The corresponding curve evolution function can be derived as

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\begin{cases} \frac{\partial \varphi}{\partial t} = \alpha_2 \delta(\varphi) \left[ \log p(\mathbf{f}(c)|\theta_f) - \log p(\mathbf{f}(c)|\theta_b) \right] + \beta_2 \delta(\varphi) div \left[ g(\mathbf{f}(c)) \frac{\nabla \varphi}{|\nabla \varphi|} \right], \\ \varphi(0, c) = \varphi_0(c), \quad \forall c \in C. \end{cases} 
(27)
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5.4. Experiments performed

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A total of five experiments were designed to showcase the different attributes of the HNCut-CGAC scheme. A total of 5 AC models (HNCut-CGAC, HNCut-GAC, CGAC, HAC, and RD) were evaluated in terms of their gland segmentation ability across 196 images.

5.4.1. Experiment 1: Robustness of HNCut to choice of swatch—The aim of this experiment was to demonstrate that the HNCut-CGAC model requires minimal human interaction and is robust to the choice of the color swatch. In our experiments we employed six different color swatch selection methods.

- A. In the first experiment, color swatch S_0 is selected from a single randomly chosen gland from a randomly selected image. S_0 is then employed across all of the images in the two data sets.
- **B.** In the second experiment, 10 images were randomly selected across the two data sets. For each of the 10 randomly selected images, color swatches S_1 – S_4 are randomly selected from multiple glands. Then HNCut-CGAC model with color swatches S_1 – S_4 is applied to segment the gland regions across 196 images.
- C. In the third experiment, the color swatch S_5 is selected from multiple glands from a randomly selected image in the data sets.

5.4.2. Experiment 2: Comparison of HNCut-CGAC against CGAC model—The aim of this experiment was to show the efficiency and accuracy of HNCut-CGAC over the CGAC model with random initialization. Here CGAC refers to the color gradient based

geodesic active contour model. The CGAC model is randomly initialized with circles that are evenly distributed across the image. The model is then applied for gland segmentation across all 196 images.

5.4.3. Experiment 3: Comparison of HNCut-CGAC against Rousson-Deriche (RD) model (Rousson and Deriche, 2002)—The aim of this experiment was to compare the accuracy of the HNCut-CGAC model with respect to a state-of-the-art region based AC model (RD). The RD model is a popular region-based AC model where the model is driven by the Gaussian distributions of both foreground and background (Rousson and Deriche, 2002). Though region-based models have the advantage of being an initialization-free scheme, the GAC model with accurate initialization and efficient edge detection function is able to outperform the RD model in segmenting glands structures from histological images. In this experiment, the RD model is initialized via multiple random circles evenly distributed across whole-slide images.

5.4.4. Experiment 4: Comparison of HNCut-CGAC against Hybrid Active

Contour (HAC) model (Paragios and Deriche)—The aim of this experiment was to compare the accuracy of the HNCut-CGAC model with respect to a state-of-the-art hybrid AC model (HAC). In this experiment, the HAC model is initialized via multiple random circles evenly distributed across the whole-slide images.

5.4.5. Experiment 5: Evaluating GAC performance with color and gray scale gradients—The aim of this experiment was to show the accuracy of the HNCut-CGAC model over the HNCut-GAC model. HNCut-GAC refers to a gray scale gradient based geodesic AC model with HNCut initialization. Since our aim is to demonstrate the advantages in using color gradient based edge-detection function for GAC model, we replace the color gradient with gray-scale gradient in the edge-detection function for the HNCut-CGAC model. In order to make a fair comparison, the gray scale gradient based GAC (HNCut-GAC) model is initialized by the HNCut scheme as well.

5.5. Performance measures

The performance of each model is evaluated based on the boundary-based measurements and region-based overlapping measurements:

5.5.1. Boundary-based measurement—The gland segmentation results of the HNCut-CGAC, HNCut-GAC, CGAC, RD, and HAC models were evaluated in terms of mean absolute distance (MAD). We define $\mathbb{G} = \{ c_x | x \in \{1, ..., N\} \}$ and $\mathbb{S} = \{c_w/w \in \{1, ..., M\} \}$ as closed boundaries of manual and automated segmentation, respectively. *N* and *M* are numbers of pixels on the boundaries of the manual and automated segmentations, respectively. MAD may then be defined as

$$MAD = \frac{1}{M} \sum_{w=1}^{M} \{ \min_{\chi} ||c_w - c_{\chi}| |\}, \quad \forall c_w \in \mathbb{S}, \ \forall c_{\chi} \in \mathbb{G}.$$

In this paper, the boundaries of the automated segmentation result are defined as the contours of zero level set function of AC models after convergence. An MAD value of 0 reflects perfect segmentation.

5.5.2. Region-based overlapping measurements—Gland segmentation results of the HNCut-CGAC, HNCut-GAC, CGAC, RD, and HAC models were evaluated in terms of

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overlap (OL), sensitivity (SN), specificity (SP) and positive predictive value (PPV). For each image, the set of pixels lying within the manual delineations of the glands is denoted as $\mathcal{A}(G)$. The set of pixels lying within any boundary resulting from the HNCut-GAC, CGAC, RD, and HAC models are denoted as $\mathcal{A}(S)$. $\mathcal{A}(S)$ is comprised of those pixels whose level set functions are positive after convergence of AC models. $|\cdot|$ represents the number of pixels in a region. For example, |C| represents the total number of pixels in the image \mathcal{C} . OL, SN, SP, and PPV are then defined as

- 1. Overlap (OL) = $\frac{|\mathcal{A}(S) \cap \mathcal{A}(G)|}{|\mathcal{A}(S) \cup \mathcal{A}(G)|}$
- 2. Sensitivity (SN) = $\frac{|\mathcal{A}(S) \cap \mathcal{A}(G)|}{|\mathcal{A}(G)|}$,
- 3. Specificity (SP)= $\frac{|C|-|\mathcal{A}(S)\cup\mathcal{A}(G)|}{|C|-|\mathcal{A}(G)|}$, and
- 4. Positive Predictive Value (PPV) = $\frac{|\mathcal{A}(S) \cap \mathcal{A}(G)|}{|\mathcal{A}(S)|}$

An OL = SN = SP = PPV = 1 is indicative of perfect segmentation.

5.5.3. Computational Time—We measure the execution time of the three major components of the HNCut-CGAC model: the HNCut initialization scheme, local tensorbased color gradient algorithm, and active contour model for segmentation. The software implementation for each component was performed using MATLAB (Mathworks, Inc.). The execution time in seconds for each component of HNCut-CGAC, as well as the total execution time for the model, for digitized prostate histopathology images with different resolutions, is reported in Table 4. All operations were performed on a 2.6GHz Intel Core 8 processor with 72GB of RAM. Note that even for images with over 25 million pixels, the total run time is only in the order of 10–12 minutes.

6. Results and Discussion

Qualitative results of HNCut-CGAC, HNCut-GAC, CGAC, RD, and HAC models on two different studies are illustrated in Figures 5(b)-(1) and Figures 6(b)-(1). In order to better compare the segmentation results, two magnified regions in each whole-slide image have been shown. The magnified regions in Figures 5(e), (f) and Figures 6(e), (f) reveal the inability of the HNCut-GAC model in accurately segmenting the glands. The reason is that the gray scale gradient based edge-detection function results in inaccurate and spurious boundaries. The magnified regions in Figures 5(g)-(1) and Figures 6(g)-(1) illustrate that the CGAC model, RD model, and HAC model with random initialization are unable to accurately segment all the glands in the image. On account of the accurate HNCut based initialization and the improved robustness due to the color gradient based edge-detection function, the HNCut-CGAC model outperforms the other 4 AC models.

Table 5 shows the results of quantitative evaluation of segmentation by HNCut-CGAC, HNCut-GAC, CGAC, RD, and HAC models in terms of MAD, OL, SN, SP and PPV across 196 whole-slide images. For HNCut-CGAC and HNCut-GAC models, color swatch S_0 is used. The mean and standard deviation values for MAD, OL, SN, SP, and PPV in Table 5 show that the HNCut-CGAC outperforms the HNCut-GAC, CGAC, RD, and HAC models. While the HNCut-GAC yielded a higher SN value compared to the HNCut-CGAC model, the improvement came at the cost of a lower OL, SP, and PPV. Figures 3(a)–(d) show the distribution of the region based performance measures (OL, SN, SP, PPV) for the HNCut-CGAC (using swatch S_0) model across all 196 images.

The comparison of segmentation on HNCut-CGAC model with different color swatch selection methods in Experiment 1 are shown in Table 6. As evidenced by the results in Table 6, no significant differences in either the region or boundary based performance

measures were observed across the different color swatches (S1-S5). There are no significant differences in segmentation results of HNCut-CGAC model over 196 images for S_0 and S_5 as well. The MAD results of two color swatch selection methods S_0 and S_5 in Experiment 1 are illustrated using frequency histogram plots (see Figures 4) which shows no significant difference in the segmentation of HNCut-CGAC model for S_0 and S_5 across all 196 images.

7. Concluding remarks

In this paper we presented a high-throughput geodesic active contour model with minimal human intervention for rapid and accurate segmentation of multiple objects on very large imagery. An accurate and efficient initialization scheme is employed for detecting the locations of the objects, which allows a color gradient based geodesic active contour model segment the object boundaries. While hybrid and region based AC models are typically initialization free, in the case of very large images and where multiple objects have to be segmented concurrently, they may under-perform since both the Rousson-Deriche and the hybrid AC models make strong assumptions regarding a priori knowledge about the number of target objects in the scene to be segmented. This is evidenced by the poor performance of the Rousson-Deriche model and the hybrid active contour model on very large digitized prostate histological images. An additional novel aspect of our new AC scheme is the use of a local structure tensor based color gradient in the edge-detector function for GAC model, which allows for more prominent boundaries compared to the traditional gray scale gradient. A quantitative and qualitative comparison between the HNCut-CGAC, HNCut-GAC, CGAC, RD, and HAC models for the task of gland segmentation across 196 prostate histopathology images revealed that the HNCut-CGAC model easily outperformed other AC schemes. The HNCut-CGAC model presented in this paper offers an easy, accurate, minimally interactive, reproducible and efficient scheme for general object segmentation, especially on very large images.

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	Color swatch	Ð	-
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Figure 1.

The flowchart of HNCut-CGAC model shown in the context of gland segmentation on prostate histopathology imagery.



Figure 2.

(a) Original color image of needle core biopsy histopathology image, and corresponding (b) color gradient and (c) gray scale gradient obtained after converting the color image in (a) to its gray scale representation with the MATLAB function rgb2gray.



Figure 3.

The histogram for segmentation accuracy evaluation of HNCut-CGAC model with color swatch S_0 over 196 images are plotted. The plots reflect the number of studies (y-axis) for which (a) Overlap, (b) Sensitivity, (c) Specificity, and (d) Positive Predictive Value (PPV) values were below certain number(x-axis).



Figure 4.

A histogram plot showing the distribution in the values of MAD, for the HNCut-CGAC model using swatches (a) S_0 and (b) S_5 across 196 images. Note that there are no significant differences in MAD values for the two different swatches.



Figure 5.

The gland segmentation results (boundaries in green) of HNCut-CGAC, HNCut-GAC, CGAC, RD, and HAC models for a whole-slide needle core biopsy (a). (c) and (d) are two different patches (I) and (II) from the segmentation result (b) of the HNCut-CGAC model which have been magnified to show gland details. (e) and (f) are two magnified patches selected from the same location (I, II) (b) and showing the segmentation result of the HNCut-GAC model. (g) and (h) show corresponding results for the CGAC model. (i) and (j) show corresponding results for the RD model. (k) and (l) show corresponding results for the HAC model.



Figure 6.

The gland segmentation results (boundaries in green) of HNCut-CGAC, HNCut-GAC, CGAC, RD, and HAC models from a whole-slide needle core biopsy (a) in study 2. (c) and (d) are two different patches (I) and (II) from the segmentation result (b) of the HNCut-CGAC model which have been magnified to show gland details. (e) and (f) are two magnified patches selected from the same location (I, II) (b) and showing the segmentation result of the HNCut-GAC model. (g) and (h) show corresponding results for the CGAC model. (i) and (j) show corresponding results for the RD model. (k) and (l) show corresponding results for the HAC model.

Description of Notation

Symbol	Description
С	2D image scene
С	2D Cartesian grid of pixels $c = (x, y)$
f (<i>c</i>)	function that assigns intensity values to pixel c
w _{k,j}	the <i>j</i> -th element of weight vector \mathbf{w}_k at level k
$\varphi(t, c)$	the level set function
C	the zero level set $\mathbb{C} = \{c \in \Omega: \varphi(c) = 0\}$
Ω	bounded open set in \mathbb{R}^2
$H(\phi)$	Heavside function $H(\varphi) = \begin{cases} 1, & \varphi(c) \ge 0; \\ 0, & \varphi(c) < 0. \end{cases}$
δ(φ)	Delta function $\delta(\varphi) = \begin{cases} +\infty, & \varphi(c) = 0; \\ 0, & \varphi(c) \neq 0. \end{cases}$
Ω_f	foreground region $\Omega_f = \{c \in \Omega: \varphi(c) > 0\}$
Ω_b	background region $\Omega_b = \{c \in \mathcal{M} \ \Omega: \varphi(c) < 0\}$
$\mathcal{A}\left(\cdot ight)$	the set of pixels contained within the boundary of the object
•	the L_2 norm
\mathbf{F}_k (or \mathbf{f}_k)	the set of colors at level k

Description of the different data sets considered in this study.

	Name	Number
Dataset 1	Hematoxylin & Eosin (H&E) stained prostate needle core biopsy images	126
Dataset 2	H&E stained images of quadrant histological sections of prostate obtained from radical prostatectomy studies	70

The AC models considered in this work for comparison with the HNCut-CGAC model.

HNCut-CGAC	Color gradient based GAC model with HNCut initialization
HNCut-GAC	Gray scale gradient based GAC model with HNCut initialization
CGAC	Color gradient based GAC model with random initialization
RD	Rousson & Deriche's (RD) model with random initialization
HAC	Hybrid AC model with random initialization

The execution time in seconds for each component of HNCut-CGAC model as well as the total execution time for digitized prostate histopathology images corresponding to different image resolutions. The values in the parentheses reflect the average computation times. All operations were performed using a 2.6GHz Intel Core 8 processor with 72GB of RAM. Here *X* and *Y* represent the number of pixel columns (width) and rows (height) in the image, respectively.

Image Resolution (10 ⁶ pixels)	HNCut (seconds)	Color Gradient (seconds)	Active Contour (seconds)	Total Time (seconds)
$5 \le X \times Y \le 9$	9–15 (12)	1–2 (1.5)	100–200 (150)	110–217 (163.5)
$9 \le X \times Y \le 16$	15–24 (19.5)	2-3 (2.5)	200–400 (300)	217–427 (322)
$16 \le X \times Y \le 27$	24-60 (42)	3-6 (4.5)	400-800 (600)	427-866 (646.5)

The average and standard deviation of the MAD, OL, SN, SP and PPV for the RD, HAC, HNCut-GAC (S₀), CGAC, and HNCut-CGAC (S₀) models over 196 whole-slide images.

	RD	HAC	HNCut-GAC (S ₀)	CGAC	HNCut-CGAC (S ₀)
MAD	5.9 ± 1.94	5.4 ± 2.0	7.2 ± 0.80	9.99 ± 5.06	$\textbf{2.07}\pm0.20$
OL	0.25 ± 0.31	0.27 ± 0.22	0.47 ± 0.22	0.27 ± 0.51	$\textbf{0.69}\pm0.12$
SN	0.56 ± 0.34	0.60 ± 0.22	$\textbf{0.98}\pm0.12$	0.85 ± 0.21	0.89 ± 0.09
SP	0.81 ± 0.41	0.83 ± 0.11	0.84 ± 0.11	0.80 ± 0.11	$\textbf{0.96} \pm 0.02$
Λdd	0.42 ± 0.25	0.45 ± 0.23	0.46 ± 0.24	0.44 ± 0.21	0.75 ± 0.11

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Quantitative evaluation of segmentation results for HNCut-CGAC models with different color swatch selection methods (S₁₋S₅). The average and standard deviation of the MAD, OL, SN, SP and PPV values over 196 whole-slide images are reported.

Color swatch	MAD	10	NS	SP	Λdd
S_1	2.07 ± 0.22	0.69 ± 0.11	0.89 ± 0.10	0.96 ± 0.01	0.75 ± 0.11
S_2	2.07 ± 0.21	0.69 ± 0.09	0.0 ± 0.09	0.96 ± 0.02	0.75 ± 0.13
S_3	2.07 ± 0.20	0.69 ± 0.10	0.0 ± 0.09	0.96 ± 0.01	0.75 ± 0.11
S_4	2.07 ± 0.23	0.69 ± 0.12	80.0 ± 0.08	0.96 ± 0.03	0.75 ± 0.11
S_5	2.07 ± 0.21	0.69 ± 0.11	0.0 ± 0.09	0.96 ± 0.01	0.75 ± 0.12