Fractional Entropy Based Active Contour Segmentation of Cell Nuclei in Actin-Tagged Confocal Microscopy Images

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Abstract

In the framework of cell structure characterization for predictive oncology, we propose in this paper an unsupervised statistical region based active contour approach integrating an original fractional entropy measure for single channel actin tagged fluorescence confocal microscopy image segmentation. Following description of statistical based active contour segmentation and the mathematical definition of the proposed fractional entropy descriptor, we demonstrate comparative segmentation results between the proposed approach and standard Shannon's entropy obtained for nuclei segmentation. We show that the unsupervised proposed statistical based approach integrating the fractional entropy measure leads to very satisfactory segmentation of the cell nuclei from which shape characterization can be subsequently used for the therapy progress assessment.

1 Introduction

Segmentation of cellular structures is an essential tool in cell microscopic imaging as it enables measurements which can be used to track cell divisions or help to reconstruct corresponding cell lineage trees providing data for calculation of different parameters like cell proliferation rate for instance. More specifically, the work presented in this paper has been carried out in a context of analyzing changes of cell cytoskeleton properties in a response to ionizing radiation insult. The final goal of this research effort is to better understand cell bio-mechanical responses during cancer radiation therapy. In this context, we propose an unsupervised segmentation approach of fluorescence confocal microscopy images which

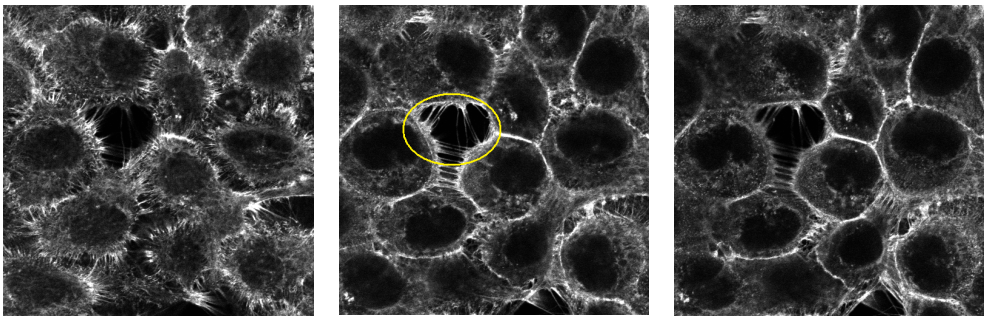
represents practical computational problem when considering many monolayer acquisitions – in order to effectively extract nuclei as a first step for providing spatial reference frame for analyzing cytoskeleton changes.

To date, only few methods have been proposed to address direct segmentation (without any denoising preprocessing of acquired images) of cell structures in fluorescence confocal microscopy images. In former approaches proposed in [8] and [12], authors focused on nuclei segmentations. In [14], authors proposed cell segmentation in 2D-fluorescent images with two channels (actin and nucleus tagging) using a multiphase level-set combining Chan-Vese [2] and geodesic active contour models, together with repulsive force introduced to prevent segmented cells from overlapping. In [7, 15] automated 3D cell segmentation from a 3D confocal acquisition of early Zebrafish embriogenesis is proposed; Two different fluorescent markers (red for nuclei and green for membrane) are used to easily discriminate nuclei from cell membranes. In [15], authors introduced an adapted version of the subjective surface technique [13] for surface reconstruction from missing boundary information whereas [7] use a multiphase level-set based on probability correlation functions.

Within a level set framework as in [7, 14], our method aims at a different objective: segmentation of microscopic 2D images extracted from a full single channel confocal acquisition with only one fluorescent marker used for actin tagging. The filament actin (F-actin) is believed to play a vital role in cell structure [3]. As Actin is one of the three most common proteins in human cytoskeleton, analysing its changes and properties could be instrumental in analysis of cell properties. For example this can be associated with cancer evolution. Nevertheless, due to a highly complex actin appearance, a high level of noise and a strong non-homogeneity of intensity and gradient information, the segmentation of cell structures in such imaging data, is a very challenging task. Moreover, a particular attention is given to completely avoid any enhancement preprocessing [10] and to reduce to its minimum, manual interventions during the whole segmentation process.

The data used in this paper were obtained from human prostate cells (PNT2). Actin were labelled with phalloidin-FITC and all imaging was carried out using a Zeiss LSM510 confocal microscope. Fig. 1 shows different slices from the microconfocal acquisition of the monolayer PNT2 cell culture. The stack volume is defined on the 512 512 98 grid of pixels each 0.21 m 0.21 m 0.11 in size. Actin is mostly present at the periphery of the cells and within the cytoplasm. We can then notice that high intensities of actin tagged confocal images allows biologists to roughly delineate cell membranes whereas darkest areas are identified as nuclei. Owing to the high level of Poisson noise corrupting these images and the particular texture of actin, classic region based active contour approach, like the Chan and Vese one [2], fails even in segmenting properly the boundaries of nuclei corresponding to each cell [6]: We then propose to tackle this segmentation using statistical based active contour (see [5] for an overview on the work on this area) more adapted to this particular context than classic region based ones.

The remaining of this article is organized as follows: in Section 2, the framework of statistical based active contour using entropy estimation is first presented, subsequently the corresponding Partial Differential Equation (PDE) which steers the evolution of the contour is described and finally attention is focused on the particular proposed fractional entropy descriptor; Section 3 focuses on experiments on microscopic images followed by conclusions and perspectives drawn in Section 4.



(a) (b) (c)

Figure 1: Examples of actin tagged fluorescence confocal microscopy images extracted from a 3D microconfocal acquisition of the monolayer PNT2 cell culture. (a) Lower slice (with low z-stack index), (b) Mid-slice with the lowest level of structural noise (a "hole" is highlight in yellow which should not to be confused with a nucleus), (c) Upper slice with non-homogeneity of the fluorescent marker on the left hand side.

2 Active contour segmentation using a fractional entropy descriptor

Formerly introduced in [1], integral based features active contour methods are derived from traditional region based approaches by utilizing integral statistics as descriptors of the inner ($_{in}$) and outer ($_{out}$) regions delimited by the active curve at a given iteration of the segmentation process.

First, let H_i denotes an integral entropy estimation associated to a particular region $_i$ within image such as

$$H_i = \int_i \hat{p}(\mathbf{I}(\mathbf{x})_i)\, d\mathbf{x} \tag{1}$$

with a monotonic increasing function, $\mathbf{I}(\mathbf{x})$ the luminance of pixel $\mathbf{x}$ x y and $\hat{p}$ the non-parametrically estimated Probability Density Function (PDF) of region $_i$. More precisely, usually PDF $\hat{p}$ is estimated using Parzen window technique such as:

$$\hat{p}(\mathbf{I}(\mathbf{x})_i) = \frac{1}{i} \int_i G(\mathbf{I}(\mathbf{x}))\, d\mathbf{x} \tag{2}$$

where 0 2^n 1 , n is the quantization level of image intensity function, and G is the Gaussian kernel of standard deviation . In the framework of statistical region based active contour segmentation, corresponding functional to minimize H_T is defined as a competition between inner and outer regions characterized by the considered entropy descriptor H of Eq. (1) such as:

$$H_T = H_{in} + H_{out} \int g\, ds \tag{3}$$

where g is a positive real value and s standard arclength of the curve. This functional combines measures of the considered entropy descriptor of inner $_{in}$ and outer $_{out}$ regions of the curve for a given iteration of the segmentation with an additional regularization term minimizing the curve length. The Euler derivative of Eq. (3) and usual minimization scheme leads to the Partial Differential Equation (PDE) steering the evolution in the orthogonal direction $\mathbf{N}$ of the active curve :

$$\frac{\partial}{\partial} = A(s)_{in} p(\mathbf{I}(s)_{in}) - A(s)_{out} p(\mathbf{I}(s)_{out}) g \mathbf{N} \tag{4}$$

where A is related to the proposed descriptor and is defined by:

$$A\ s\ _i \quad \frac{1}{i}\ _i \quad \hat{p}\ \mathbf{I}\ \mathbf{x}\ _i\ \hat{p}\ \mathbf{I}\ \mathbf{x}\ _i\ G\ \mathbf{I}\ \mathbf{x}\ \mathbf{I}\ s\ d\mathbf{x} \tag{5}$$

For illustration, let's consider the particular case of Shannon's entropy: function is given by $r \quad r \log r$ and then $H\ _i \quad {}_i \hat{p}\ \mathbf{I}\ \mathbf{x}\ _i \log \hat{p}\ \mathbf{I}\ \mathbf{x}\ _i\ d\mathbf{x}$. As it will be shown in the Experiment section, standard Shannon's entropy have some limitations in terms of segmentation performance: more specifically, this measure makes segmentation of corrupted (Gaussian noise) textured images challenging [4], in the case of high level of structural noise, the segmentation results are not that satisfactory. This can be explained by the fact that Shannon's entropy tassumes that the corrupting noise (and then the corresponding PDF $\hat{p}$) can be parametrically modeled within the exponential family [5] which is not true when considering confocal microscopy data. In this particular context, fractional entropy like the Rényi, given by:

$$H_R\ _i \quad \frac{1}{1} \log \quad {}_i \hat{p}\ \mathbf{I}\ \mathbf{x}\ _i\ d\mathbf{x} \tag{6}$$

is of primary interest. Rényi's entropy shows some relaxation possibilities regarding the shape of the PDF $\hat{p}$ which can be used by a judicious setting of (considered as strictly positive and lower than one in this study [11]). Unfortunately, Rényi's entropy as expressed in Eq. (6) is part of the non-integral entropy family that can not be easily associated to a region-based criterion in a classic active contour based segmentation. Nevertheless, taking benefits of the properties of Rényi's entropy, we propose to define a fractional entropy measure adapted to the framework of statistical region-based active contour segmentation. For this, let consider Eq. (1) with function and its derivative given by:

$$r \quad \frac{1}{1} \log\ r \quad \text{and} \quad r \quad \frac{}{1 \quad r} \tag{7}$$

Considering function of Eq. (7), we obtain an integral entropy measure integrating a fractional parameter.

3 Experiments and results on nuclei segmentation

In this section, comparative segmentation results obtained are first described for the unsupervised nuclei segmentation within the mid-slice of the considered single channel confocal microscopic acquisition (Fig. 1(b)).

The PDE from Eq. (4) is implemented in the level-set framework in order to be able to automatically handle topological changes [9]. The initialization of the active contour is a set of small circles uniformly distributed all over image which allows an easy initialization of the algorithm. Classic AOS scheme is used for implementation in order to obtain a reasonably fast convergence segmentation.

Fig. 2 shows results obtained with the standard Shannon's entropy criterion and the proposed fractional entropy descriptor. Considering experiments based on Shannon's entropy (Fig. 2 (left)), as one can notice, the method does not lead to satisfying results . Figs. 2 (middle and right) shows results of nuclei segmentation on the same slice, but with the proposed fractional entropy criterion: the nuclei segmentation is definitely improved. As one can notice, as actin is a complex structure, some artifacts could appear. It is possible to

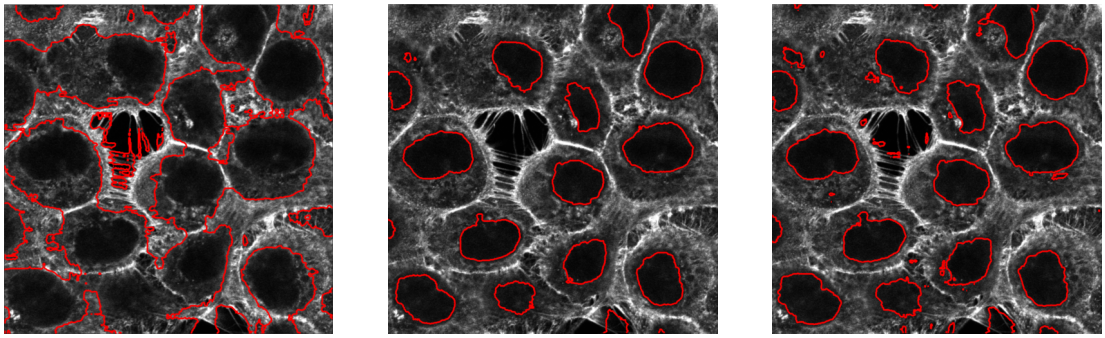


Figure 2: Comparative results of nuclei segmentation. Left: Shannon's entropy ; Middle and Right: Fractional entropy descriptor with 0 5 (middle) and 0 7 (right) ; for all experiments g 10.

overcome this drawback with an adapted choice of parameter. As one can see in Fig. 2, for 0 5, smaller number of artifacts related to value and those results show that this parameter plays an important role in the sensitivity of the criterion to the level of corrupting noise. Moreover, it is important to notice that the proposed fractional entropy measure can also distinguish a hole from a nucleus (which method based on Shannon's criterion was not able to achieve), whereas the associate PDFs are statistically very similar.

Finally, Fig. 3 shows some segmentation results obtained on the whole stack of acquired images. Results shown are obtained with 0 5, and g 10. To obtain these results, a propagation initialization strategy, starting on middle slice is used which makes integration of some spatial coherence within the segmentation scheme to avoid propagation of false detection due to complex appearance of actin.

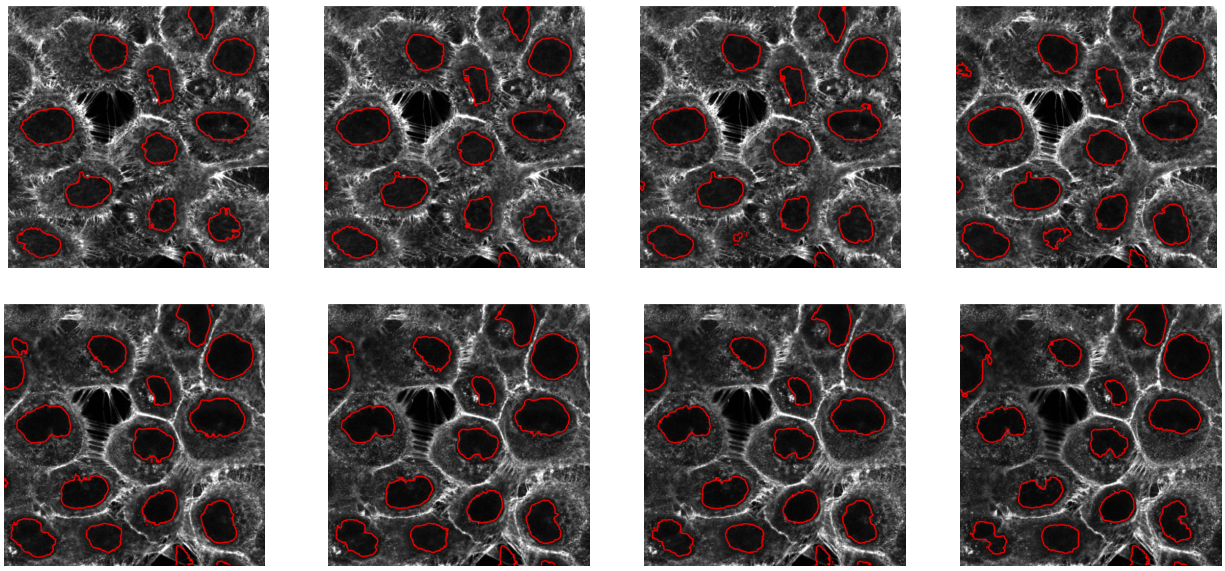


Figure 3: Segmentation of nuclei made on upper (upper row) and lower (bottom row) slices of the all stack, mid-slice of Fig. 2 being the initialization level). 0 5 and g 10.

These results have been qualitatively considered as very satisfactory from an expert point of view and a very good start for further investigations on that particular data.

4 Conclusion

The contribution of the segmentation approach presented in this article is twofold: (i) An unsupervised cell nuclei segmentation method is proposed for single channel actin tagged acquisitions without any enhancement or denoising preprocessing of the considered images. (ii) Whereas in the framework of statistical based active contour methods standard Shannon's entropy is most often considered as the region descriptor, we proposed an original fractional entropy measure inspired from Rényi's entropy making possible a relaxation of the sensibility of the descriptors to strong variations of the shapes of the non parametrically estimated related PDF. Main motivation was to overcome the limitations of Shannon's entropy which appeared not adapted to our segmentation problem. We are currently working on locally relating the optimal choice of parameter with the level of noise and/or the type of texture characterizing the image to segment. From an application point of view, we are finalizing a 3D version of our slice-by-slice segmentation approach to have a direct visualization of the 3D shape of the nuclei. Membrane segmentations will be the next step.

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