

Exploring K-means, the Kirsch Compass kernel and Fourier-transform filtering for texture-based image segmentation.

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Abstract—This paper provides an analysis of different image processing techniques to segmentate regions with almost no pixel value variations. The aim of this study is to analyze problematic images which cause the state of the art algorithms to experience difficulties in solving the segmentation problem. I apply various image processing techniques that substantially improve the results on this specific subset of images and provide a comparison of my results with those obtained using the best algorithms which include MultiCellSeg, Topman and TScratch.

Index Terms—Wound healing assay, image segmentation, K-means, Kirsch Compass kernel, high/low pass filters, Otsu's method.

I. INTRODUCTION

IMAGE segmentation is one of the most important challenges in Computer Vision. This technique has a wide variety of applications in object recognition, object tracking, medical imaging and many other fields. While humans have no problem in clearly defining the boundaries of an object when we see it, Artificial Intelligence can sometimes experience problems in doing such task. This paper reviews some limitations of the state of the art algorithms for wound healing image segmentation. The algorithms include MultiCellSeg, Topman and TScratch. I also analyze two challenging images (plus one for reference) and give a better solution based on different methods chosen specifically for each image. This paper does not intend to undervalue AI capabilities, it rather focuses on problematic images and image processing techniques that can improve the results.

The point of interest of this paper are a subset of 3 images [Figure 1] from a set of 54 DIC images (pixel size $1.24 \times 1.24 \mu\text{m}$), denoted SN15. This images were acquired using a LSM-510 microscope (Zeiss, Germany) in non-confocal mode, from 27 different wells of DA-3 cells. The experiment was performed in Ilan Tsarfay's laboratory at Tel Aviv University. A cut (or scratch) was performed to the tissue and the main objective is to segmentate the scratch from this tissue.

II. STATE OF THE ART ALGORITHMS

A. Topman

Topman [1] is an algorithm developed in Matlab that performs segmentation based on the standard deviation (SD) of the image. It uses two windows of different size to calculate SD and combines the information to find regions of

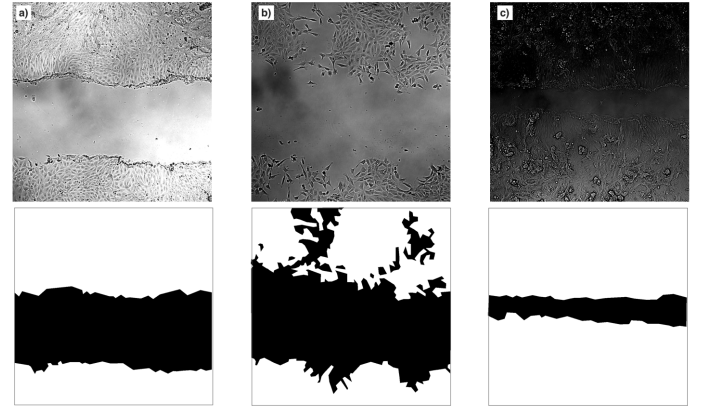


Fig. 1. Problematic images of interest: a) Good illuminated and non-complicated edges for reference. b) The high complexity of the patterns in the tissue is a challenge. c) Very poor lighting conditions will result in bad segmentation by the algorithms. The bottom images correspond to the manually segmented images provided with the dataset.

high texture. It has been used successfully to calculate cell migration rates and achieves a very high correlation between its results and the manually segmented images. I show the result of applying this algorithm to images a), b) and c) in the Figure below.

Looking at the results by Topman (Figure 2) for the specially challenging images selected, we can see how the Topman algorithm results in a high amount of noise. In order to eliminate this noise we will have to be willing to give up definition and structure of the edges.

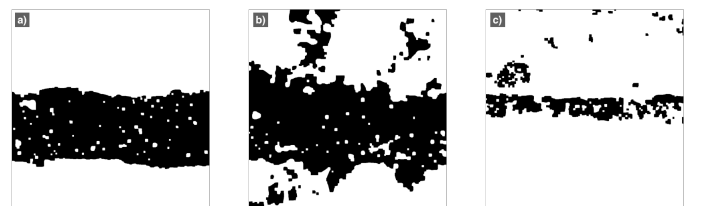


Fig. 2. Results of Topman algorithm applied to images a), b) and c) of Figure 1.

B. MultiCellSeg

MultiCellSeg is a segmentation algorithm developed by a group of scientist [2] based on Support Vector Machines

(SVMs). Using image features, a large number of SVMs is applied followed by some extra final classification and graph-cut segmentation to improve results. The most important advantage of this algorithm is that it is parameter-free and can adapt well to a wide variety of images.

Regarding the results in Figure 3, the MultiCellSeg algorithm can experience problems when working with poor lighting conditions such as image c). Also, the complex patterns from image b) are not so well captured using this algorithm as compared to using Topman.



Fig. 3. Results of MultiCellSeg algorithm applied to images a), b) and c) of Figure 1.

C. TScratch

TScratch is a software tool specially designed for automated analysis of this kind of segmentations [3]. It uses an edge-detection algorithm (10) based on the discrete curvelet transform (9). This provides a measure of the amount of detail in an image. Focusing on small windows of the original image, the algorithm is capable of detecting and comparing the textures between the scratch and the cell populated regions. TScratch is not only an algorithm but a whole software with its own graphical user interface which allows the user to vary the parameters used.

Looking at the results obtained by this algorithm we can see that the patterns formed in image b) are too complex for it to perform a good segmentation. Also dark images like c) can result in unwanted results.



Fig. 4. Results of TScratch algorithm applied to images a), b) and c) of Figure 1.

III. DEVELOPING SECTIONS

Having seen the results of the top three algorithms I will now address the specific problems of each image (a), b) and c)) and will propose a solution to each problem.

A first step common to all procedures consists of image equalization. This is a very important step consisting of exploiting the whole range of values of the gray-scale. This will not only improve contrast but also enhance structure and texture properties of the cell populated areas. If not performed,

some tissue areas will end up being classified as "cut" only because the detail does not show.

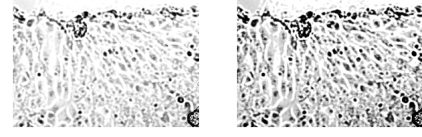


Fig. 5. Equalizing the image enhances texture in cell-populated regions.

A. High Pass Filter Approach

Looking at image Figure 1 a), a clear difference between the two regions is that the scratch is formed by a smooth, detail-free surface. With this in mind, I perform a Fast Fourier Transform and apply a High-pass filter to the transformation. When applying the inverse transformation I obtain, as expected, the edges and details which are mainly on the cell-populated areas. After that, two median filters are applied, one after the other, of size 11px along the vertical dimension and 41px along the horizontal dimension. This asymmetry allows to maintain some of the structure of the edges. Having reached the image in Figure 6 II, I tried to apply a binarization following Otsu's method but the result was very far from what I expected. That is why I performed a K-means clustering using $k=7$ different labels. Using 7 clusters allowed for a more fine segmentation. I developed an algorithm that automatically performs K-means segmentation and then iterates through all the resulting labels and selects the largest cluster, which can be seen in Figure 6 IV.

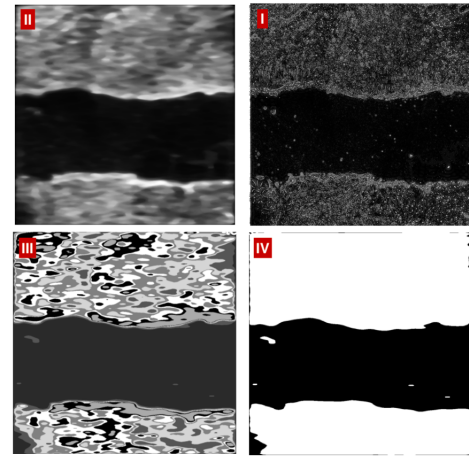


Fig. 6. I: Result of applying a high-pass filter to the Fourier Transform. II: two asymmetrical median filters are applied. III: K-means segmentation. IV: Keeps the largest cluster.

B. Kirsch Compass kernel

Image Figure 1 b) is characterized by a high level of detail and structure along the edges that separate the tissue from the scratch. The edges are oriented in all directions and the Kirsch Compass kernel (KCK) [4] provides a tool for obtaining the brightest edges out of 8 different directions (N, NW, W, SW, S,

SE, E and NE). This technique consists on applying different convolutions that result in enhanced edges along the eight different directions. After that, the brightest pixel is selected from the eight convolutions performed resulting in an image with the maximum edge strength.

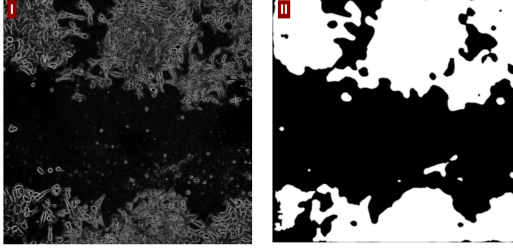


Fig. 7. I: Result of applying KCK. II: Final segmentation result

Using the resulting image (Figure 7 I) I proceed to apply two median filters using kernels of sizes (11x11)px and (31x31)px. These are small enough to preserve the detail of the edges which is the main objective of this section. Finally, the last binarization is applied using a threshold of 0.75 times the one obtained by Otsu's method.

C. Solution for poor lighting conditions

The last image (Figure 1 c) is the one with which the algorithms perform the worst. Even taking noise into account, Topman achieves a SSIM of 0.902 but the other two MCS and TScratch are not able to properly segmentate the image result in SSIMs of 0.759 and 0.677 respectively.

The approach to this problem has been to perform the image equalization and then test different high-pass filters to find the optimal result. Using a sharp cut-off high-pass filter with a radius of 0.005 has proven to give the best result which can be seen in Figure 8 I. This is followed by a median filter using a kernel size of 3px along the vertical direction and 201px along the horizontal direction (Figure 8 II). Lastly, I tested different thresholds to binarize the result and applied a low-pass filter to eliminate noise (Figure 8 III).

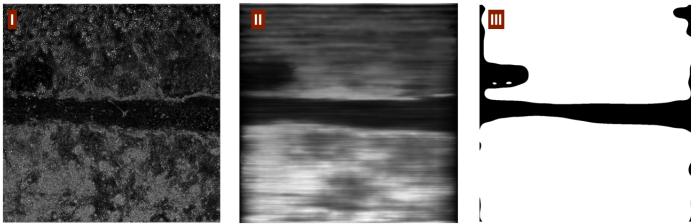


Fig. 8. I: Result of applying the high-pass filter. II: Asymmetrical median filter. III: Binarization and low-pass filter, final result.

IV. RESULTS AND CONCLUSIONS

The metric used to compare the results of the reviewed algorithms and of this paper is the SSIM which has been proven to be successful in quantifying image similarities [5]. The correlation matrices below show the SSIMs achieved by

each algorithm and the SSIMs achieved in this study. Note that this study focused on solving the segmentation for these three specific cases while the other algorithms aim at providing a general solution to the problem. Each correlation matrix corresponds to one of the images of interest (a,b,c). The first row of each matrix corresponds to the SSIM between the manually segmented target and the results obtained.

Image a), used as reference, is well segmented by all three algorithms. Topman results in a high level of sound and that is why it's $SSIM_{Topman-Manual}=0.933$ is the lowest. MultiCellSeg does slightly better than TScratch probably because it manages to completely whiten the cell populated areas without patches on the edges of the image. Lastly, my result has a SSIM of 0.959 when compared to the manually segmented image achieving the highest similarity.

Manual	1	0.933	0.942	0.951	0.959
Topman	0.933	1	0.923	0.941	0.944
TScratch	0.942	0.923	1	0.955	0.949
MultiCellSeg	0.951	0.941	0.955	1	0.959
My result	0.959	0.944	0.949	0.959	1
	Manual	Topman	TScratch	MultiCellSeg	My result

Fig. 9. SSIMs between the results and the manually segmented images for image a). The matrix also includes SSIMs between results.

Image b) offers more of a challenge to both the referenced algorithms and my image processing capabilities. The Kirsch Compass kernel is proven successful in extracting the complex edges of the tissue-cut frontier. This method manages to improve the result achieving a SSIM of 0.866.

Manual	1	0.852	0.855	0.83	0.866
Topman	0.852	1	0.838	0.81	0.857
TScratch	0.855	0.838	1	0.843	0.83
MultiCellSeg	0.83	0.81	0.843	1	0.811
My result	0.866	0.857	0.83	0.811	1
	Manual	Topman	TScratch	MultiCellSeg	My result

Fig. 10. SSIMs between the results and the manually segmented images for image b). The matrix also includes SSIMs between results.

Finally, image c) is clearly the most challenging. The bad lighting conditions make it really hard for the algorithms to properly extract texture features from the tissue. Topman however does a great job as compared to MCS and TScratch. The combination of high and low-pass filters applied in this study provides a slightly better result with an SSIM of 0.909 when compared to the manually segmented image.

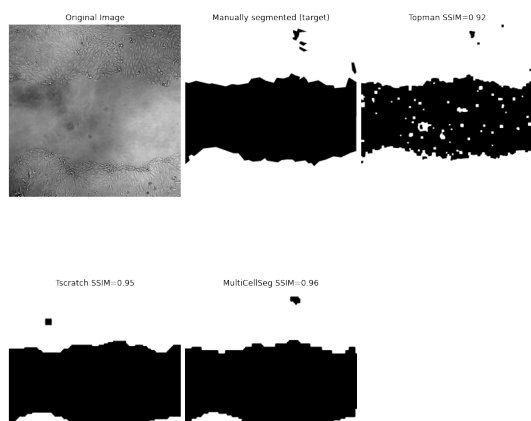


Fig. 11. SSIMs between the results and the manually segmented images for image c). The matrix also includes SSIMs between results.

In order to improve results the most critical thing is with no doubt the image capturing process. Taking microscopic images with uniform brightness and good lighting conditions will solve one of the final problems remaining of these algorithms. The results accomplished by Topman, MultiCellSeg and TScratch (see Appendix) are unprecedented and offer great value to anyone performing wound healing assays. However, in order to completely solve the general problem either a higher quality dataset must be collected or new capabilities should be implemented in these procedures. This study hopes to shed some light into possible approaches or techniques to obtain better results when dealing with problematic images.

V. APPENDIX

You can find the code used in this project in <https://github.com/ignaciocordova/image-segmentation-project> containing the "impavi" module and three notebooks to visualize all the process.



REFERENCES

[1] Topman G, Sharabani-Yosef O, Gefen A. A standardized objective method for continuously measuring the kinematics of cultures covering a mechanically damaged site. *Med Eng Phys.* 2012 Mar;34(2):225-32. doi: 10.1016/j.medengphys.2011.07.014. Epub 2011 Aug 5. PMID: 21820939.



[2] Zaritsky A, Natan S, Horev J, Hecht I, Wolf L, Ben-Jacob E, Tsarfaty I. Cell motility dynamics: a novel segmentation algorithm to quantify multicellular bright field microscopy images. *PLoS One.* 2011;6(11):e27593. doi: 10.1371/journal.pone.0027593. Epub 2011 Nov 9. PMID: 22096600; PMCID: PMC3212570.

[3] Gebäck T, Schulz MM, Koumoutsakos P, Detmar M. TScratch: a novel and simple software tool for automated analysis of monolayer wound healing assays. *Biotechniques.* 2009 Apr;46(4):265-74. doi: 10.2144/000113083. PMID: 19450233.

[4] Kirsch, R. (1971). "Computer determination of the constituent structure of biological images". *Computers and Biomedical Research.* 4 (3): 315–328.

[5] Jim Nilsson, Tomas Akenine-Möller (2020). "Understanding SSIM". Cited as: arXiv:2006.13846 [eess.IV].