International Journal of Electrical, Electronic and Communication Sciences
ISSN: 2517-9438
Vol:4, No:11, 2010

Semi-automatic background detection in microscopic images

Alessandro Bevilacqua[1,2], Alessandro Gherardi[2], Ludovico Carozza[2], Filippo Piccinini[2]

***Abstract*—The last years have seen an increasing use of image analysis techniques in the field of biomedical imaging, in particular in microscopic imaging. The basic step for most of the image analysis techniques relies on a background image free of objects of interest, whether they are cells or histological samples, to perform further analysis, such as segmentation or mosaicing. Commonly, this image consists of an empty field acquired in advance. However, many times achieving an empty field could not be feasible. Or else, this could be different from the background region of the sample really being studied, because of the interaction with the organic matter. At last, it could be expensive, for instance in case of live cell analyses.**

We propose a non parametric and general purpose approach where the background is built automatically stemming from a sequence of images containing even objects of interest. The amount of area, in each image, free of objects just affects the overall speed to obtain the background. Experiments with different kinds of microscopic images prove the effectiveness of our approach.

***Index Terms*—microscopy, flat field correction, background estimation, image segmentation**

I. Introduction

IMAGE analysis techniques are crucial for several application in biomedical imaging, to analyze cells and structures in histological samples. The first step for most of the image analysis techniques is the separation of foreground and background [1], in order to characterize with the highest accuracy the objects being studied. The background can often be considered as a "virtual" object composed of random noise [2] or illumination variations. Or alternatively, in the presence of culture medium, the background can be a "real" object, made of sparse (small) structures, such as impurities [3].

There are mainly two approaches. The first one provides that an empty field is acquired and often temporally averaged [4] [2]. However, an empty field could not be at one's disposal. The second class of methods detects the background also in the presence of foreground objects. However, in those cases the background is often detected by difference, after foreground detection and removal. To this purpose, prior information are used regarding the foreground objects [5], such as intensity brightness levels [6] or shape [7] of cells. All these methods could work fine, but they rely on object-dependent segmentation methods that even have already been built.

[1] DEIS (Department of Electronics, Computer Science and Systems), University of Bologna; alessandro.bevilacqua@unibo.it
URL:http://cvg.deis.unibo.it
[2] ARCES (Advanced Research Centre on Electronic Systems), University of Bologna; {abevilacqua, agherardi, lcarozza, fpiccinini}@arces.unibo.it

The non-parametric approach we propose is of general purpose and compliant with the presence of objects in the microscope field of view. It incrementally gathers information regarding background regions while the user moves the holder. Of course, in case there is no background in any part of the images, then our method fails. On the other side, in this case the background difference to detect the foreground could not be necessary, since the whole image is foreground. This work is organized as follows. In Sect. II, some relevant studies are discussed. Sect. III describes in detail the acquisition and the background detection stages of the algorithm we implemented. Extensive experimental results are discussed in Sect. IV, where two sequences of images of stem cells and a histological sample are considered. Some conclusions are drawn in Sect. V and some hints for future directions are given.

II. Previous works

To segment white blood cells in microscopic blood images, the algorithm proposed in [6] uses prior assumptions about the intensity levels of white and red blood cells, nucleus and cytoplasm. In addition, white blood cell's nuclei are segmented by using a well known gradient based approach to detect nuclei's contours. However, the author does not propose any background estimation and subtraction algorithm, although realizing that, since illumination is imbalanced, the image contrast between cell boundaries and the background varies depending on the condition during the capturing process.

The purpose of the work described in [7] is to achieve a robust background model for stem cell tracking. Here, the first step of background detection is to detect foreground objects. Then, the outcome is removed from the image and what remains is a background region. The final step exploits the background regions of each sequence's image to perform an effective spatio-temporal background estimation. Although being promising, this method works for those specific images only, since it is based on an explicit model of the foreground to detect and remove cell objects.

The approach described in [3] aims at detecting the cell boundaries to separate cells (a very small part of the image) from background. Here, the background is detected simply as being the largest part of the image and used in a preprocessing step. In practice, here the background does not work either to compensate for light or to detect the regions of interest by subtraction.

The authors in [4], before performing confocal mosaicing, use a reference background image to normalize each input image and to correct for illumination curvature and vignetting.

No details are given on how this reference background is achieved, thus supposing it is simply an empty field.

In [2], the background image is used just to remove camera noise in confocal microscopy: the program carries out automatic image subtractions using a *dark* background image representing the noise that the camera detects with closed illumination shutters, during one total exposure time. Besides, to face the effects of the uneven illumination, the authors carry out flat-fielding by using a light pattern template that is multiplied with each raw image.

In [5], the authors do not look for a background image, rather for a background histogram, to be used successively for segmentation purposes. To detect background regions in a first image, a complex cell detector is used to label cells. The background histogram is built considering the pixels of the complementary region. The authors then exploit the bimodality of the images histogram to roughly identify cell regions by thresholding.

In [8], each image is recovered through resolving, *via* convex optimization, the typical inverse problem describing the image formation process. Besides the system noise, here the authors explicit an *additive* bias component, that is the light field that is modeled as a $K-th$ order polynomial surface. However, the method has been conceived to strongly exploit the way the image is built using the differential interference contrast microscopy, where the bias is added to well defined cells.

III. Background estimation

The knowledge of a highly accurate background is necessary in order to estimate the light field distribution and to perform subsequent image processing algorithms. The estimation of the background of the specimen under analysis on the microscope can be performed incrementally by acquiring and analyzing a sequence of images, captured in real-time, until a sufficient amount of information is retrieved from the input images.

A. Image acquisition

The background is built incrementally using several images acquired randomly or in time sequence. The algorithm performs this acquisition automatically, without the need of any user intervention except for moving the specimen holder in case of non-automated microscopes. However, the system can also be configured to guide the user to select a number of images acquired randomly on the specimen. This can be used to target particular cell cultures with non uniform cell distributions, or in case of histological examinations where most of the biological content is unevenly distributed and the background is present in limited regions only.

Both these cases cover the most relevant part of the biological routine examinations.

B. Background detection

The image is conceptually subdivided into two complementary regions: foreground and background. We aim to detect the background by discarding the foreground objects. Here, we *do not consider* the background as being the most uniform region in terms of absolute pixel value. Rather, we relax this constraint by considering that by its nature the background represents the region of the image retaining the most uniform derivatives. In fact, organic matters can show a low variation that however can be perceived at numerical level. Practically speaking, we have conceived our algorithm not to detect the whole background, but to prevent it could detect any foreground pixel. As the reckoning to pay for this safe behavior, it just requires to analyze a little more images. The algorithm can be outlined as follows: where the central derivatives D along rows and columns of the images are computed using fast convolution masks $M_x = [-1/2 \;\; 0 \;\; +1/2]$ and $M_y = M_x^T$. P represents the minimum amount of background area (in percentage with respect to the whole image) to be collected in the sequence of images in order to estimate the final background. Although being important, this parameter is not too sensitive: the lower the value, the faster the algorithm. Th_B represents the threshold of the gradient magnitude under which the image region is considered to be *uniform*.

Algorithm 1 background estimation algorithm

1. *while* background region < P
2. $D =$ derivatives along x and y direction of image I_i
3. *foreach* (x, y) *in* I_i
4. *if* $D(x, y) < Th_B$
   $D_{back}(x, y) = 1$
   else
   $D_{back}(x, y)$ is void
5. $B_i =$morphological opening of D_{back}
6. $i = i + 1$
7. $B =$ smooth_fit (median B_i)

A morphological opening is then performed on the "noisy" estimation of background pixels D_{back}, thus reducing the number of "holes" in the background image B_i being built incrementally. The background is then computed by performing a temporal median filter of each estimated background image over the sequence acquired. Some pixel positions may present void values since no common background regions may have been detected. Therefore, to remove these possible empty regions a smooth 2-D fitting is performed to estimate the final background B. Despite its simplicity, the estimated background can be very accurate, as it can be seen in Sect. IV.

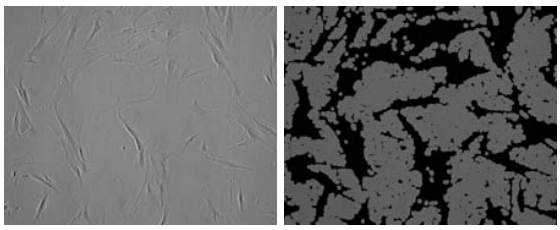
IV. Experimental results

The experimental results have been performed on two test sequences of 1280×1024 resolution images, having very different features as far as the background detection is concerned. The first sequence, hereinafter "stem-cells" is composed of 9 images representing stem cells with a confluence of 35%, where the content of the cells is uniform at human eyes and the contrast is nearly absent. The second sequence, hereinafter "histological sample", is a set of 77 images acquired from an histological specimen of bone tissue, showing varying values and well defined contrast and object's contours. Both sequences have been acquired by live acquisitions.

In our experiments, $Th_B = 4$, which has been determined experimentally by analyzing the cells. This value is very low and in normal cases (i.e., dealing with matters not having "same" pixel values as their background!) it could be increased without any damage for the algorithm. As for the other threshold, P, it has been set to 80%. Recalling that this parameter is intrinsically related to the amount of background pixels detected, this value can be considered sufficient to capture the shape of the background from a statistical point of view.

The number of images required to properly estimate the background depends on the content of the images. For the kind of image content considered in our experiments, we have seen that an accurate background estimation would require typically up to 10 images for medium density cells. However, depending on the biological content under examination, a different amount of images could be necessary.

An intermediate step of the algorithm is shown in Figure 1. In Figure 1(a), the input image belonging to the "stem-cells" set is given in input to the background extraction algorithm and the respective output is shown in Figure 1(b). Here we can see all the background regions detected by the segmentation procedure, where the cells' regions are depicted in black.



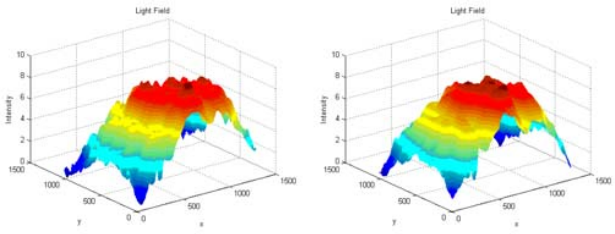
(a) (b)

Fig. 1: One image of the "stem-cells" sequence (a) and the mask of the detected background pixels (b).

In Figure 2(a), the background estimated from the first set of stem-cells images and normalized to its minimum value is shown together with the "ground-truth" (b), represented by the background built by averaging a sequence of four empty field images. As it can be seen, the estimation algorithm performs well to depict the real background even for a small number of input images.

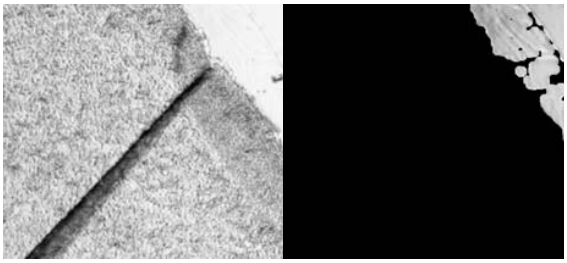
As for the second test sequence, Figure 3(a) shows one histological image acquired on the specimen and its normalized background segmentation (Figure 3(b)) as detected by our algorithm. It is worth noticing how the background in histological images is quite limited and it could not be present at all. Therefore, a higher number of images have to be analyzed by the algorithm while performing continuous live acquisitions.

The final background, after having analyzed 75 images to reach the coverage of 80% is shown in Figure 4(a). In Figure 4(b), the "ground-truth" background estimated from empty "histological sample" images. Also for this sequence the estimation algorithm performs well to depict the real background.



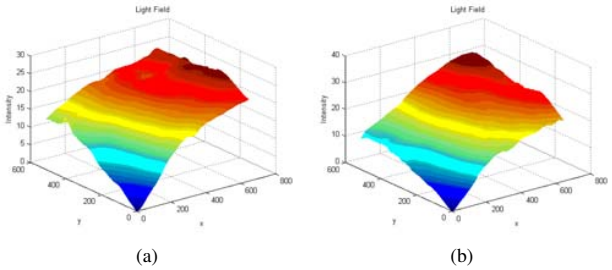
(a) (b)

Fig. 2: Estimated background (normalized) for the first set of images referring to the "stem-cells" culture (a) and the corresponding ground-truth (b).



(a) (b)

Fig. 3: Regions of normalized background (b) detected in one image (a) of the "histological sample" sequence.



(a) (b)

Fig. 4: Estimated background (light-field) for the second set of images referring to the "histological sample" sequence (a) and the corresponding ground-truth (b).

In order to quantify numerically the performances of the proposed estimation algorithm, an error metric is considered to assess the final estimated background image. In particular, we have used the Relative Percentage Mean Error (RPME), that is defined as the average of the absolute difference between the estimated background and the reference ground-truth, divided by the maximum signal of the reference. Table I shows the results on the two set of test sequences.

A mean 5.1% RPME error is attained on the first sequence of stem cells and 7.1% on the second histologic sequence. These good results on both the sequences confirm that our method is able to estimate the specimen background with a

Table I: Relative percentage mean error of estimated background

Set	RPME
stem-cells	5.1%
histological sample	7.1%

good accuracy even under very different conditions without the need of changing the parameters according to the biological matter under examination.

V. Conclusions

This work presents a non parametric illumination field estimation and compensation method for optical microscopy images. The method developed recovers the illumination field by analysing the background of an image, that is detected automatically even in the presence of objects of interest. To this purpose, the approach rely on the fact the background in microscopy is usually uniform and it can be considered as a single virtual object. No other assumptions or prior knowledge is exploited to detect the background, even in the presence of objects of interests. The experiments carried out on two sequences showing different biologic matters (cell cultures and histologic sample) prove the effectiveness of our algorithm.

Acknowledgments

We would like to thank Dr. Enrico Lucarelli (biologist) and his staff of the Bone Regeneration Laboratory (BRL) of the Rizzoli Orthopedic Institute (Bologna, Italy) for providing the data used in this work.

References

[1] A. Sacan, H. Ferhatosmanoglu, and H. Coskun, "Celltrack: an open-source software for cell tracking and motility analysis," *Bioinformatics*, vol. 24, no. 14, pp. 1647–1649, 2008.

[2] Emilie Flaberg, Per Sabelstrnaöm, Christer Strandh, and LaszloSzekely, "Extended field laser confocal microscopy (eflcm): Combining automated gigapixel image capture with in silico virtual microscopy," *BMC Med Imaging*, vol. 8, pp. 1–13, 2008.

[3] E. D. Cheng, S. Challa, R. Chakravorty, and J. Markham, "Microscopic cell segmentation by parallel detection and fusion algorithm," in *Proc. of the* 10^{th} *IEEE International Workshop on Multimedia Signal Processing*, 2008, pp. 94–100.

[4] Y. G. Patel, K. S. Nehal, I. Aranda, Y. Li, A. C. Halpern, and M. Rajadhyaksha, "Confocal reflectance mosaicing of basal cell carcinomas in mohs surgical skin excisions," *Journal of Biomedical Optics*, vol. 12, no. 3, pp. 034027–1–034027–10, May/June 2007.

[5] Kang Li, Mei Chen, Takeo Kanade, Eric Miller, Lee Weiss, and Phil Campbell, "Cell population tracking and lineage construction with spatiotemporal context," *Medical Image Analysis*, vol. 12, no. 5, pp. 546–566, Oct. 2008.

[6] F. Sadeghian, Z. Seman, A. R. Ramli, B. H. Abdul Kahar, and M. Saripan, "A framework for white blood cell segmentation in microscopic blood images using digital image processing," *Biological Procedures Online*, vol. 11, pp. 196–206, December 2009.

[7] N. N. Kachouie and P. W. Fieguth, "Background estimation for microscopic cellular images," in *IEEE International Conference on Image Processing, San Diego, CA, USA, October 12-15*, 2008, pp. 3040–3043.

[8] Kang Li and Takeo Kanade, "Nonnegative mixed-norm preconditioning for microscopy image segmentation," *Lecture Notes in Computer Science*, vol. 5636, pp. 362–373, 2009.