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Color Enhancement and Edge Detection for Confocal Microscopy Fluorescent Images

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ABSTRACT

We propose image enhancement pre-processing techniques for the confocal fluorescent images captured from the release of cathepsin B-green fluorescent protein (GFP) during apoptosis. To this end, we improve the color quality of the images, through local neighborhood statistics, then color edge detection is performed to isolate the lysosomes allowing us to observe more accurately the changes that have occurred. The proposed methods aim to be the starting point for further automatic processing of the measurements, color and shape segmentations, and analysis. Of special interest to us will be to monitor localization of subcellullar particles and a leakage of proteins from these particles.

1. INTRODUCTION

We present a pre-processing method for an automatic system of tracking and analysis of micro particles in confocal microscopy fluorescence images, consisting of the combination of color contrast enhancement and gradient type edge detection.

The importance of accurate early vision algorithms, among them edge detection, for microscopy images, in general, can not be over emphasized, as further processing towards measurements and diagnosis will entirely depend on the "goodness" of the early estimates. As traditional smoothing (low-pass filtering) techniques will remove noise together with small detail and high-pass filtering will accentuate edges but also noise, there is a need to develop methods which balance the preservation of edges with the removal or discount of noise.

Programmed cell death or "apoptosis" is an important biological process regulating embryonic development and maintaining adult tissue homeostasis [1]. In order to understand the role of lysosomal protein, cathepsin B, in apoptosis, it is essential to follow its movements and subcellular localization inside the cell by live cell microscopy. In this context, one of the important measurement for the biologist, will be to quantify the approximated amount of protein released from the lysosomes in a interval of time.

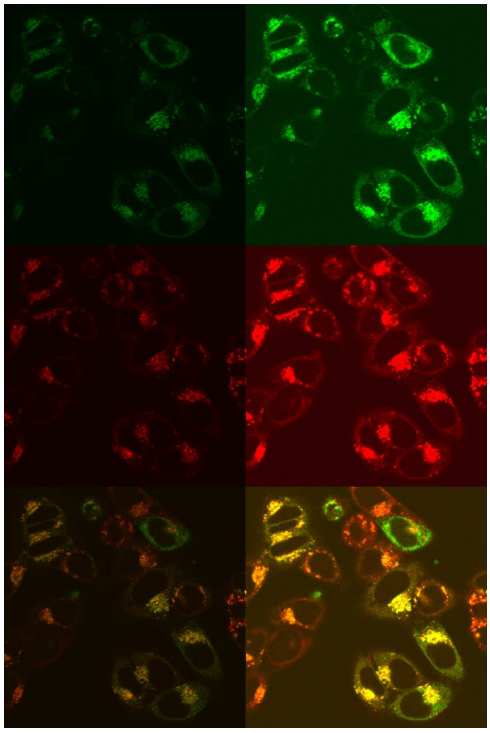

Fig. 1. Original (left) and enhanced (right) *FL* images at the beginning of measurement time.

In real time microscopy applications, such as in confocal fluorescence microscopy, the produced quality of fluorescent (FL) images is not always the best even for the human observer, and it is worse in the case of automatic computer analysis programs, in charge of diagnosis. To improve quality of images for further automatic processing stages, we make use of an enhancement method presented by Lee [2] for gray-level images. This method has been extended to color, allowing us to balance the overall contrast of the picture at both ends, preserving the edges of the micro particles for further detection, localization and analysis. Obviously, the assessment of the quality of the en-

hanced images will depend on the human observer and/or in the requirements that further processing techniques put into their input data.

Next, to well-localize the borders of the particles of interest to us in the low or high contrast images obtained from the local mean and variance color enhancement methods, we make use of a color gradient technique [3] of edge detection. The importance of edge detection for many practical fields, such as in Computer Vision, Robotics, Pattern Recognition and Satellite and Video Telecommunications, is a well known fact, which has influenced the research and development of a very large number of solutions to this problem. Marr [4] in his pioneering work of edge detection by approximating one of the most frequent response, to light excitation, observed in the retinal ganglion cells of mammalians, has put the basis for physiological vision understanding. Canny [5], proposed a two step response approximation consisting of smoothing and differentiation. As the retinal ganglion cells response is not unique, several types of more effective response approximations can be designed using parameter optimization techniques, e.g. window size selection by statistical methods [6]. In [7] a review of the early algorithms can be found and for a more modern review of edge detection algorithms, see e.g. [8].

2. LIVE CELL MICROSCOPY

Human cervical cancer cells, Hela cells, growing on glass-bottom petri dishes and expressing cathepsin B fused with a green fluorescent protein (GFP) [9] were incubated with 50 nM Lysotracker Red that accumulates into the lysosomes. To induce apoptosis the cells were incubated with 1 μM Staurosporine.

The images were collected using a inverted Zeiss LSM 510 META laser scanning confocal microscope with 63X/1.4 oil DIC objective. Lysotracker Red and cathepsin B-GFP were imaged by using 543 *n*m HeNe laser and 488 *n*m line from Argon laser for the excitation of fluorophores, respectively. A 545 *n*m dichroic mirror divided red and green fluorescence, and emissions were collected to photomultipliers through LP 560 *n*m and a 500-530 band pass filters, respectively.

3. COLOR LOCAL MEAN AND VARIANCE CONTRAST ENHANCEMENT

The importance of image enhancement is defined by the fact that the image acquisition systems are subjected to several types of aberration, which will damage the overall quality of the pictures. Furthermore, further processing stages will be impaired by the poor quality of the input data. Obviously, low contrast of images captured by using low excitation energy to minimize photodamage of live cells will obscure important cellular details, like the presence of relevant intracellular particles. On the other hand, photobleaching of fluorophore caused by an excessive amount of illumination, will increment a number of false negative particles and the high luminance of the particles "obscures" their internal microstructures.

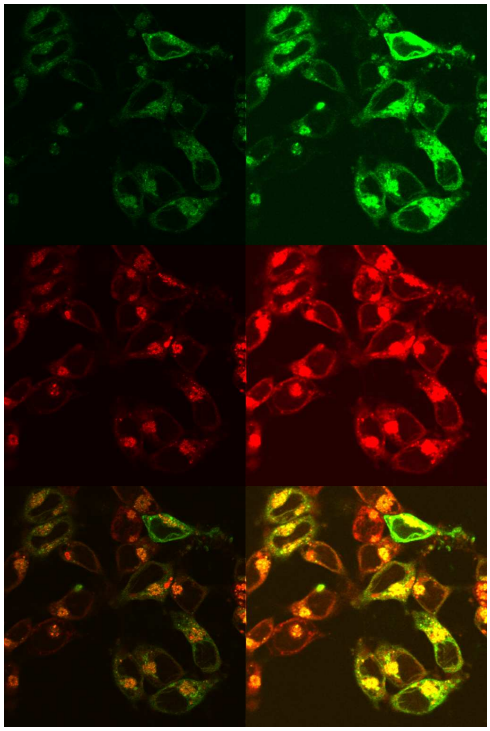

Fig. 2. Original (left) and enhanced (right) *FL* images at the middle of measurement time.

Grey-level enhancement methods like homomorphic filtering, histogram modeling operations, contrast stretching and unsharp masking [10], [11], have been used widely to improve some specific characteristics of the image for further human observation or machine computation and analysis. A particularly successful method is the one presented by Lee [2], which is suitable for real-time applications with superior performance over other more complicated methods. Its simplicity, in the positive sense, makes this method attractive. The original method was presented for correcting grey-level images at both ends of gray scale (0 and 255).

The main idea behind Lee's algorithm is to improve the image quality, by adding the local mean and its scaled local variance. This can be represented as follows:

$$\widehat{I}(x,y) = m(x,y) + \kappa\{I(x,y) - m(x,y)\}, \qquad (1)$$

where $I(x,y)$ represents the image brightness in coordinates (x,y), $m(x,y)$ is the local $[N \times N]$ neighborhood mean, κ is the variance scalable constant, and $\widehat{I}(x,y)$ is the enhanced image. For different values of κ different features of the image will be enhanced, ranging from edge sharpening ($\kappa > 1$) to smoothing ($\kappa = 0$) corresponding to high-pass and low-pass filtering, respectively. To deal effectively with narrow distributed histogram images, the first summand local mean $m(x,y)$ in (1), can be scaled and shifted to produce:

$$\widehat{I}(x,y) = \alpha\{m(x,y)\} + \beta + \kappa\{I(x,y) - m(x,y)\} \quad (2)$$

which enhances the image contrast at both ends $[0, 255]$ and between the whole grey-level range.

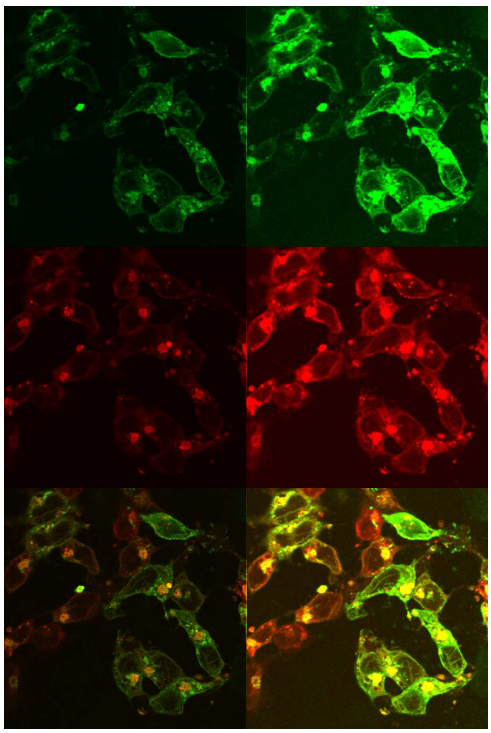

Fig. 3. Original (left) and enhanced (right) *FL* images at the end of measurement time.

The extension to RGB color system images can be performed using (2) for each color separately, obtaining the enhanced color image $\widehat{C}$ as:

$$\widehat{C} = [\widehat{R},\ \widehat{G},\ \widehat{B}], \tag{3}$$

which allows selective enhancing of color and/or image features by tuning the model coefficients α, β and κ.

In Figures (1), (2) and (3), are shown the original (left) and the color local statistics enhanced images (right) for different times of picture shooting. In all the figures, from top to bottom, the first picture shows the green fluorescent protein linked to the target protein, the second shows the red lysotracker, and the third shows the colocalization (merged image) of the two previous. The values of the model coefficients, for all sets of data, are: $\alpha = 1.5$, $\beta = 0.5$ and $\kappa = 0.5$.

4. COLOR GRADIENT EDGE DETECTION

The analytical treatment of multi-images as vector fields has been presented in [12], and extended in [3], to present a single output from the gradients of the image components. Several edge detection techniques [8] can be extended to detect edges in color type images, but at this stage, we retain the method given in [3], mainly due to its straightforward application to the RGB color system. The surface function in which local maxima (edges) will be contained is given by:

$$d\widehat{C}^2 = \partial\widehat{R}^2 + \partial\widehat{G}^2 + \partial\widehat{B}^2. \tag{4}$$

Representing by *r, g* and *b* the unitary vectors corresponding to the *R, G* and *B* coordinate axes, and by introducing vectors of partial color derivatives u and v as:

$$\begin{aligned} u &= \frac{\partial\widehat{R}}{\partial x}r + \frac{\partial\widehat{G}}{\partial x}g + \frac{\partial\widehat{B}}{\partial x}b \\ v &= \frac{\partial\widehat{R}}{\partial y}r + \frac{\partial\widehat{G}}{\partial y}g + \frac{\partial\widehat{B}}{\partial y}b, \end{aligned} \tag{5}$$

then (4) can be written as:

$$d\widehat{C}^2 = u'u\ dx^2 + 2u'v\ dx\ dy + v'v\ dy^2. \tag{6}$$

Using the polar coordinate system, it has been shown in [3] that the orientation θ of maxima values is given by:

$$\theta = \frac{1}{2}arc\ \tan(\frac{2u'v}{u'u - v'v}). \tag{7}$$

It is clear that the gradient is an operator of directional type, as such, different approximations of the gradient are obtained in dependence of the orientation in which the data image has been feeded to the operator. Thus, by flipping $\updownarrow$ and rotation $\curvearrowright$ along the coordinate axes of the input image data, we obtain in total four approximations of the gradient which will be used to calculate a more complete gradient map GM as:

$$GM = \sqrt{d\widehat{C}^2 + d\widehat{C}^2_{\updownarrow} + d\widehat{C}^2_{\curvearrowright} + d\widehat{C}^2_{\updownarrow\curvearrowright}}, \tag{8}$$

where, $d\widehat{C}^2_{\updownarrow}$, $d\widehat{C}^2_{\curvearrowright}$, and $d\widehat{C}^2_{\updownarrow\curvearrowright}$ are the gradients obtained from the flipped image from left to right, rotated by 180^0 and a combination of both, respectively. The maximum operation can also be used instead of the square root. Obviously, to apply (8) the inverse flipping and rotation operations have to be applied. The increase in computation load is justified by the obtained increase in localization of the edge pixels.

At this time, a simple threshold Th operation which will classify as edge candidates EC a certain percentage of a local maximum value, is applied $EC = Th(GM)$, finally to suppress some of the false negatives that have remained, morphological thinning $Thin$ is used to obtain edges $E = Thin\{EC\}$ that are well localized and have one pixel of width.

5. EXPERIMENTAL RESULTS

The purpose of our experiments is two-folded: first we are interested in identifying the clusters of lysosomes (detecting outer edges), and second, in looking at the internal structure of the clusters (lysosome edges).

For the first case, we need to increase the contrast of the original color image, to make the clusters more "visible" for the color gradient edge detector. By trial and error correction, we have estimated that the best quality of enhancement has been obtained for the following model coefficient values in (2): $\alpha = 1.2$, $\beta = 0.5$ and $\kappa = 0.5$. The size of the neighborhood is: $N = 5$. After obtaining GM by (8), the edge candidates EC will be obtained by taking 40 % of the values which are greater than the local

maximum, finally thinning is applied for refinement of the width of EC. The obtained results for a region, from the FL images shown in Figure (1), containing two clusters of lysosomes belonging to two different neighboring cells, are presented in Figure (4), from left to right, for the green, red and merged images, respectively. It is observed that the clusters outer edges are well localized and that very few spurious pixels have remained.

For the second case, the procedure described above will be followed, with changes in value of the model coefficients for (2), as to observe internal cluster information we need to decrease the contrast of FL images, namely: $\alpha = 0.4$, $\beta = 0.7$ and $\kappa = 0.5$. The threshold for the color gradient edge detector is increased to 60 %, as the tiny lysosome structures need to be preserved. The obtained results are presented in Figure (5), for the same time and region as in Figure (4), from left to right, for the green, red and merged images, correspondingly. It has been observed that, although the majority of the lysosome edges are present and the outer cluster edge is well defined, some of the contours are broken due mainly to failure in edge discrimination of overlapped lysosomes represented in FL images with a very close intensity value.

It is clear that the potential for automatic tracking of micro particles and measurements of leakage in a time interval during apoptosis, in FL images, will be improved by having an exact approximation of the localization of clusters of micro particles or even of individual lysosomes.

6. CONCLUSIONS AND DISCUSSIONS

A new pre-processing algorithm intended for being used in an automatic system for confocal fluorescence microscopy has been introduced. The method can be used for image tracking and analysis of movement and deformations of micro particles in a time interval. An effective grey-level enhancement technique has been extended to enhance color images and the color gradient edge detector has been transformed to obtain a more complete gradient map which increases the probability of correct classification and localization of the edge tokens. The obtained results justify the validity of the methods.

Further work will be devoted to the automatization of the presented method, namely: the selection of the model coefficients of the color local mean and variance enhancement operation, together with the size of the neighborhood in which we are operating. Also, an automatic process of selection of the thresholding operation of the color gradient edge detector has to be found. Finally, an analysis of the color coordinate systems will be carried out, to select the one which is most suitable for the confocal microscopy fluorescence type of images.

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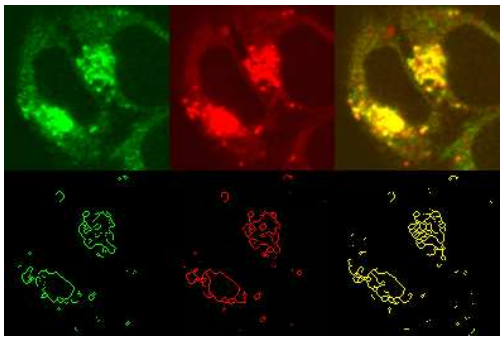

Fig. 4. Enhanced regions (upper row) and detected edges (lower row) in *FL* images.

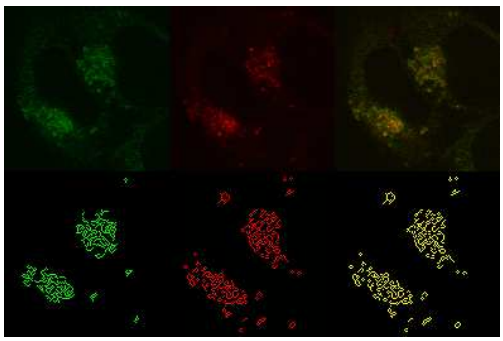

Fig. 5. Enhanced regions (upper row) and detected edges (lower row) in *FL* images.

REFERENCES

[1] J. F. Kerr, A. H. Wyllie, A. R. Currie, "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics.", *Br J Cancer.,* 26:239-257,.1972.

[2] J-S. Lee, "Digital Image Enhancement and Noise Filtering by Use of Local Statistics", *IEEE Trans. on Pattern Analysis and Mach. Intell.,* Vol. PAMI-2, no. 2, pp. 165-168, 1980.

[3] S. Di Zenzo, "A note on the gradient of a multi-image", *Computer Vision Graphics and Image Processing*, vol. 33, no. 1, pp. 116-125, Jan., 1986.

[4] D. Marr and E.C. Hildreth, "Theory of edge detection", *Proc. Royal Soc. London,* B, vol. 207, pp. 187-217, 1980.

[5] J. Canny, "A computational approach to edge detection", *IEEE Trans. Pattern Anal. Mach. Intell.,* vol. PAMI-8, no. 6, pp. 679-698, 1986.

[6] E. Albán, V. Katkovnik, K. Egiazarian, "Adaptive window size gradient estimation for image edge detection", *Proc. of SPIE Elect. Imaging, Image Processing: Algorithms and Systems II*, USA, pg. 54-65, January, 2003.

[7] T. Peli and D. Malah, "A study of edge detection algorithms", *Comp. Graphics and Img. Processing.,* vol. 20, pp. 1-21, 1982.

[8] D. Ziou, and S. Tabbone, "Edge detection techniques - an overview", *NEC Research Institute Scientific Literature*

[9] M. Linke, V. Herzog, K. Brix, "Trafficking of lysosomal cathepsin B-green fluorescent protein to the surface of thyroid epithelial cells involves the endosomal/lysosomal compartment.", *J Cell Sci.,* 115(Pt 24):4877-89, Dec 15, 2002.

[10] A. K. Jain, *Fundamentals of Digital Image Processing,* Prentice-Hall, Englewood Cliffs, NJ 1989.

[11] A. Rosenfeld and A. C. Kak, *Digital Picture Processing,* Vol. 1 and 2, New York, U.S.A., Academic Press Inc., 1982.

[12] R. Machuca and K. Phillips, "Applications of Vector Fields to Image Processing", *IEEE Trans. Patt. Anal. Mach. Int.,* vol. PAMI-5, no. 3, pp. 316-329, May 1983.