An Achromatic Background Filter for Cytological Image Analysis

Sadao Isotani[1] and Waldemar Bonventi Jr.[2]

[1]Institute of Physics, University of São Paulo, Caixa Postal 66318, CEP 05315-970 São Paulo, Brazil.

[2]Faculty of Technology, 18080-000 Sorocaba, Brazil. www.fatecsorocaba.edu.br

sisotani@if.usp.br, bonventi@terra.com.br

Abstract*. In this work we evaluated an algorithm for filtering the achromatic background of colored cytological images. This algorithm makes use of an Isotropic White Locus Chromaticity Diagram, where the white region is selected by polar coordinates in the 1931 Chromaticity Diagram. The potential for application as a background filter is analyzed through applications to cytological images stained with the Papanicolaou method.*

1. Introduction

The processing of images is bringing the quantification and objective assessment for diagnoses in histopathology and cytopathology [Wied et al. 1989]. But massive processing is required for the use of standard methods [Cheng et al. 2001], [Dong and Xie 2005].

There exist at least two dozens of techniques of staining histological and cytological slides being the more used the Masson Trichromic and Papanicolaou methods. Image analysis methods were applied for the analysis in the hepatic cirrhosis [Matalka et al. 2006], [Tanano et al. 2003] and for the analysis of cervical intraepithelial neoplasia [Sakai et al. 2001].

The computerized nuclear morphometry and ploidy applied to the characterization of populations of human cancer cells was object of several works [Stenkvist et al. 1978], [Bibbo 1997], [Sakai et al. 2001], [Isotani et al. 2007]. In cytological analysis, colorimetric methods have been used [Wyszecki and Stiles 1982], which take into account human eye sensibility for color and illumination.

The segmentation is the first critical step to extract information of computer processed images [Munoz et al. 2003]. Although the identification of object and surface boundaries comes naturally to a human observer, accurate image segmentation has proved difficult and complex. Several techniques were presented to detect changes among chromatic values of image points, as edge detection, dilation/erosion, histogram cutting or thresholding, and clustering [Cheng et al. 2001]. The last two have been extensively used in colored segmentation, mainly in multidimensional histogram cutting. Bidimensional projections of color space were employed [Cheng and Yang 2001], chromatic filtering methods [Lucchese and Mitra 2004], color subtractive [Underwood et al. 2001] and clustering aided by tridimensional color histograms [Chen and Lu 2002], [Han and Ma 2002]. This kind of processing is computationally expensive due to histogram tridimensionality. Bi- and tridimensional histograms and several color representations involve the employment of pseudo-vectorial chromaticity coordinates and diagrams [Wyszecki and Stiles 1982], [Vandenbroucke et al. 2003].

In cells measurements the cytoplasm are quite transparent, transmitting almost all-incident light, while nuclei are colored. Since great part of the cell image is from interstices a step to facilitate the segmentation of images is the removal of background constituted by the pixels of these interstices. The form most common of removal of background is the use of threshold of intensity. However a value of threshold that optimizes the segmentation of nuclei of cells will bring difficulties when the objective will be the study of the cytoplasm because of the proximity of the values of intensity of background and of the cytoplasm. On the other hand a generic program where threshold is searched manually increases the complexity of the problem.

Therefore the use of the color properly said for the removal of background is an option to be considered. With this purpose we evaluate an achromatic method for removal of background which makes use of an Isotropic White Locus Chromaticity Diagram [Bonventi et al. 1994].

2. The Algorithm

The color is described by three values of colored stimuli RGB. To convert to the 1931 CIE system, we use the basis transformation and projections to (x,y) plane:

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = \begin{pmatrix} 2.7690 & 1.7518 & 1.1300 \\ 1.0000 & 4.5907 & 0.0601 \\ 0.0000 & 0.0565 & 5.5943 \end{pmatrix} \times \begin{pmatrix} R \\ G \\ B \end{pmatrix}, \quad x = \frac{X}{X+Y+Z} \text{ and } y = \frac{Y}{X+Y+Z} \quad (1)$$

Pairs (x,y) corresponding to white (grays) colors are in the elliptical region of the CIE Diagram. Our problem is the choise of RGB values that cover all white colors. Here we evaluate with this purpose the Isotropic White Locus Chromaticity Diagram [Bonventi et al. 1994]. The transformation from the 1931 CIE coordinates (x,y) to this new diagram is given by:

$$\begin{pmatrix} r \\ \theta \end{pmatrix} = P \cdot M \cdot R \cdot \left[\begin{pmatrix} x \\ y \end{pmatrix} - S \right] \quad (2)$$

where **P** is the transformation from the Cartesian to polar coordinates, **R** is a rotation to align the ellipsis, **M** takes into account the eccentricity and **S** places the origin at ellipsis' center.

$$M = \begin{pmatrix} 1/a & 0 \\ 0 & 1/b \end{pmatrix} \qquad R = \begin{pmatrix} \cos\theta & \sin\theta \\ -\sin\theta & \cos\theta \end{pmatrix} \qquad S = \begin{pmatrix} x_0 \\ y_0 \end{pmatrix} \quad (3)$$

The parameters corresponding to this transformation are: θ=40° (inclination of the elliptical region relative to the 1931 CIE axis), a=0.167 and b=0.083 (semi-axis), and x_0=y_0=1/3 (ellipsoid center coordinates), which were obtained by fitting the white elliptical boundary region in the 1931 CIE Diagram. The diagram of colors in this polar coordinate system shows that the white region is described by a circle of radius $0 \leq r \leq b \ \forall \theta$, minus a region between 235° and 336° that describes pink tones [Bonventi et al. 1994]. The border of the pink region fits with good accuracy a quadratic polynomial. Then, the exclusion of white region can be obtained using:

$$\textbf{WHITE LOCUS} = \{ 0 \leq r \leq b, \forall\theta \} - \{ r \leq 3.064 \cdot 10^{-6} \theta^2 - 0.00157 \cdot \theta + 0.2401, 235° \leq \theta \leq 336° \} \ (4)$$

3. Results

Figure 1a show an image of cervical intraepithelial cells stained with the Papanicolaou method. Figure 1b show the image resulting after the application of the present algorithm. We see that the background is almost filtered, except by small points in the cytoplasm region, probably due to the excess of coloration caused by residual dye. Application to images of malignant nuclei [Isotani et al. 2007] showed similar results.
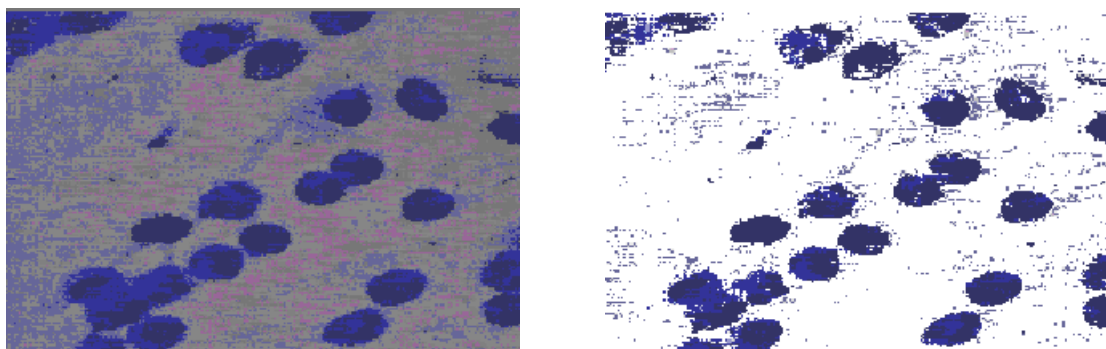

Figure 1 - a) Original cellular image with colored nuclei; b) cytoplasm filtered by the algorithm.

A preliminary comparison of the present algorithm with an algorithm of erosion used for the same purpose [Isotani et al. 2007], showed similar precision for visual requirements. Measurements of color of slides stained using the Papanicolaou method showed that the colors have small dispersion [Isotani et al. 2008], which allows parameterization. On the other hand the erosion filter requires the calculation of a threshold. Then, considering the computational advantage of the achromatic algorithm, we are examining the replacement of the erosion filter by the achromatic one [Isotani et al. 2007].

References

Bibbo, M. (1997) Comprehensive Cytophatology, Elsevier.

Bonventi Juniorr., W., Isotani, S. and Albuquerque, A. R. P. L. (1994) "A method for image background filtering of colored images using elliptical coordinates". In *Optical Methods in Biomedical and Environmental Sciences*, 3, pages 75-78, Elsevier.

Bonventi Junior., W. and Isotani, S. (2007) "Segmentação de imagens histológicas hepáticas por agrupamentos nebulosos de cores". In *Proc. of III Simpósio de Instrumentação e Imagens Médicas*, pages 1-4, São Carlos, Brasil.

Chen, T. K., Lu, Y. (2002) Color image segmentation-an innovative approach. In *Pattern Recognition*, 35(2), 395-405.

Cheng, H. D., Jiang, X. H., Sun Y., Wang, J. (2001) Color Image Segmentation. In *Advances and Prospects, Pattern Recognition*, 34(12), 2259-2281.

Cheng, S. -C., Yang, C. -K. (2001) "A fast and novel technique for color quantization using reduction of color space dimensionality". In *Pattern Recognition Letters*, 22(8), 845-856.

Dong, G. and Xie, M. (2005) "Color Clustering and Learning for Image Segmentation Based on Neural Networks". In *IEEE Transactions on Neural Networks*, 16 (4), 925-936.

Han, J., Ma, K. -K. (2002) "Fuzzy color histogram and its use in color image retrieval". In *IEEE Transactions on Image Processing*, 11(8), 944- 952.

Isotani, S., Brasil Filho, N., Sakai, Y. I., Albuquerque, A. R. P. L. (2007) "A system for the determination of image density and morphometry developed for the analysis of malignant nuclei". In *Proc. Twentieth IEEE International Symposium on Computer-Based Medical Systems*, pages 263-267, Maribor, Eslovenia.

Isotani, S., Sakai, Y. I., Capeli, O. M., Sakai, A. T., Pereira, S. M. M., Namiyama, G. M., Maeda, M. Y. S., Bonventi-Junior., W., Albuquerque, A. R. P. L. (2008) "A new toll for the analysis of color in Spcim applied to the exam of Papanicolaou". In *Proc. Simpósio Intercâmbio Brasil-Japão*, pages 1-3, São Paulo, Brasil.

Lucchese, L., Mitra, S.K. (2004) "A new class of chromatic filters for color image processing. theory and applications". In *IEEE Transactions on Image Processing*, 13(4), 534-548.

Matalka, I. I., Al-Jarrah, O. M., Manasrah, T. M. (2006) "Quantitative assessment of liver fibrosis: a novel automated image analysis method". In *Liver International*, 26, 1054-1064.

Munoz, X., Freixenet, J., Cufi, X., Marti, J. (2003) "Strategies for image segmentation combining region and boundary information". In *Pattern Recognition Letters*, 24 (1), 375-392.

Sakai, Y. I., Sakai, A. T., Isotani, S., Cavaliere, M. J., de Almeida, L. V. and Calore, E. E. (2001) "Morphometric Evaluation of Nucleolar Organizer Regions in Cervical Intraepithelial Neoplasia". In *Pathology Research and Practice*, 197, 189-192.

Stenkvist, B., Westman-Naeser, S., Holmquist, J., Nordim, B., Bengtsson, E. and Vegelius, J. (1978) "Computerized nuclear morphometry as an objective method for characterizing human cancer cell populations". In *Cancer Research*, 38, 4688-4697.

Tanano, H., Hasegawa, T., Kimura, T., Sasaki, T., Kawahara, H., Kubota, A. and Okada, A. (2003) "Proposal of fibrosis index using image analyzer as a quantitative histological evaluation of liver fibrosis in biliary atresia". In *Pediatric Surgery International*, 19, 52-56.

Underwood, R. A., Gibran, N. S., Muffley, L. A., Usui, M. L. and Olerud, J. E. (2001) "Color subtractive-Computer assisted image analysis for quantification of cutaneous nerves". In *Journal of Histochemistry and Cytochemistry*, 49(10), 1285-1291.

Vandenbroucke, N., Macaire, L., Postaire. J. G. (2003) "Color image segmentation by pixel classification in an adapted hybrid color space". In *Application to soccer image analysis. Computer Vision and Image Understanding,* 90(2), 190-216

Wied, G L, Bartels, P H, Bibbo, M. and Dytch, H. E. (1989). In *Human Pathology*, 20, 549-571.

Wyszecki, G. and Stiles, W. S. (1982) Color Science: Concepts and Methods, Quantitative Data and Formulae, 2nd ed., John Wiley and Sons.