Identification of melanoma cells: a method based in mean variance of signatures via spectral densities

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Abstract: In this paper a new methodology to detect and differentiate melanoma cells from normal cells through 1D-signatures averaged variances calculated with a binary mask is presented. The sample images were obtained from histological sections of mice melanoma tumor of $4 \, \mu m$ in thickness and contrasted with normal cells. The results show that melanoma cells present a well-defined range of averaged variances values obtained from the signatures in the four conditions used.

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References and links
1. Introduction

Malignant melanoma is an aggressive and deadly form type of skin cancer that begins in the melanocytes, the cells that make melanin and gives skin its color [1,2]. Melanoma is the ninth most common cancer in Europe [3] and the fifth and seventh most common cancer in the United States for men and women, respectively [4]. According of American Cancer Society 76,380 persons were diagnosed with invasive melanoma in 2016 with an estimated of approximately 10,130 deaths [5]. The most dangerous characteristic of melanoma is the capability of deep invasion, as it can spread over the body through lymphatic and blood vessels and although accounts for less than 5% of skin cancer cases in USA, is responsible of the majority of skin cancer deaths [6–8].

The major etiologic risk factor is the artificial UV light exposure due to sun tanning affecting the ADN in melanocytes [1,9]. The incidence has been dramatically increasing for at least 30 years and continues to rise at worldwide despite efforts to increase sun safety awareness. Although is generally understands that excessive skin sun exposure can be harmful, people have not changed behaviors even knowing the risk [4,6–9].

Early detection with surgical excision at an earlier stage of the tumor is essential for saving lives. In fact, surgery is still the treatment for people with early stage melanoma. Generally, in cancer, early detection alone could not necessarily lead to good prognostic or increase survival rates; however, in melanoma early detection is associated with higher cure rates.

One of the screening methods to detect early lesions include physical skin self-examinations to assess moles with the Stolz method or ABCDE rule (asymmetry, border, color, diameter, and evolution of pigmented lesions) [8,10]. There are other methods like serial dermoscopic photographs, confocal scanning microscopy (CSM), optical coherent tomography (OCT), pump-probe microscopy (PPM) and photoacoustic microscopy (PAM) to evaluate individual lesions over time to identify potentially suspicious changes [4]. The main disadvantages of dermoscopy is a low sensitivity. CSM gives poor resolution of chromatin patterns, nuclear contours and nucleoli; OCT can give higher detection depth and resolution, but special materials are needed to reduce the scattering; PPM has a high cost and large setup and PAM can detect deep tumor with relative high resolution, although it typically needs the staining of metal nanoparticle to enhance the imaging contrast [4,11,12]. Different methodologies based on image processing have been developed for the diagnosis of melanoma, such as the new methodology for diagnosis of skin cancer on image of dermatologic spot by spectral analysis, this methodology has a confidence level of 95.4%, and it is also a non-invasive tool for the patient [13].

Nowadays histologic examination continues to be the criterion standard for melanoma diagnosing [11,12]. To confirm a diagnosis of cutaneous melanoma requires obtaining a full thickness excisional biopsy with small side margin [14]. In patients with increased melanoma risk is very often necessary to obtain numerous biopsy specimens with a significant cost and morbidity for the patient. By other hand, while some melanomas are easily recognized by the naked eye, many can be difficult to distinguish [11]. The histologic appearance of melanoma is highly heterogeneous with numerous morphological variants. A typical melanomas shown pigmented epithelioid tumor cells and the disease progression and metastatic effect is characterized by morphological heterogeneity indicating that phenotypic changes evolution
accompanies disease progression [1]. Additionally, classifying some melanocytic lesions by conventional microscopy can be problematic if they exhibit some architectural or morphologic characteristics for not well trained pathologist [11].

There is therefore an emerging need to develop innovative technologies that can facilitate the early histologic diagnosis of melanoma for saving lives and potentially help to distinguish lesions with risk of metastasis.

2. Materials and methods

2.1 Murine melanoma model

In order to establish the melanoma model, adherent B16F0 cells (ATCC Manassas, VA) were detached with 1 min trypsin-EDTA (SIGMA-ALDRICH, St. Louis MO) and harvested by centrifugation and washed once with PBS. The tumorigenicity assay with B16F0 cells was performed by subcutaneous injection of 8 week-old BALB/C nu/nu male SPF mice with $1 \times 10^5$ cells that were resuspended in 0.2 ml of PBS. The mice were purchased from the “Salvador Zubiran” National Institute of Medical Science and Nutrition (México, DF) and were kept in microisolation boxes, fed with NubLab medium, and allowed filtered and sterilized water ad libitum. The injection sites were observed regularly for development and progression of tumors. All animals used in this study were maintained under standard conditions established by the guidelines for animal care and use according with Mexican Official Norm (NOM) NOM-039 and NOM-062-ZOO-1999. Tumor growth was monitored by measuring the tumor length (L) and width (W) using a diagnostic X-ray apparatus (General Electric Medical Systems Monitrol/15) operating at 30 kV and 100 mA, and an Ultrasound Transducer (General Electric Logiq 400 Pro) operating at 11 MHz.

Two weeks later of the tumour cells inoculation, the mice were euthanized and each developed tumor was dissected from surrounding tissues and cut into several pieces (approximately 5 x 5 x 5 mm). The pieces were fixed in 10% formaldehyde/phosphate-buffered saline (PBS) at pH 7, and paraffin embedded. Tissue sections (4 µm in thickness) were stained with hematoxylin and eosine (H&E).

2.2 Image acquisition and construction of image bank

The imagen bank was created with images of the stained tissues slides captured with an Infinity I Camera on a Nicon Eclipse T5100 light microscope adapted to imaging software Infinity Analyze 6.0 (Lumenera, Corporation Ottawa ON Canada, 2011).The images obtained were classified as a melanoma tumor. The tumor showed a moderate cellular pleomorphic form, characterized by rounded or polygonal cells with oval and hyperchromatic nucleus. Cells were disposed in acinus and mitotic activity was moderate. An image is shown in Fig. 1.
2.3 Mathematical analysis

First, the original image is converted to grayscale, then the image was divided into twenty five sub-images, where twenty five sub-images is the optimal number after a numerical analysis, Fig. 2.

Real and imaginary Fourier transform are obtained from each sub-image, $I_i(x,y)$, $x = 1, \ldots, N$, $y = 1, \ldots, M$, and $i = 1, \ldots, 25$, Fig. 3.
The image of \( \text{Re}\{\text{FT}(I(x,y))\} \) and \( \text{Im}\{\text{FT}(I(x,y))\} \) are filtered by a binary disk mask \( DM(x,y) \), in order to obtain the same length for each profile, defined like,

\[
DM(x,y) = \begin{cases} 
1, & \text{if } d\left((c_x,c_y),(x,y)\right) \leq n \\
0, & \text{otherwise},
\end{cases}
\]

(1)

Where \((c_x,c_y)\) is the center pixel of the image, \( n = \min\{c_x,c_y\} \) and \( d(t,s) \) is the Euclidian distance between \( t \) and \( s \) points, thus the \( DM(x,y) \) image is centered in the \((c_x,c_y)\)-pixel. An example of that binary filter \( DM(x,y) \) and the results of the filter process are shown in Figs. 4(a), (b) and (c). Mathematically these operations are given by

\[
f_R(x,y) = DM(x,y)\text{Re}\{\text{FT}(I(x,y))\},
\]

(2)

\[
f_I(x,y) = DM(x,y)\text{Im}\{\text{FT}(I(x,y))\}.
\]

(3)

For the images \( f_R(x,y) \) and \( f_I(x,y) \), were obtained 180 profiles of 2n pixels length that passes for \((c_x,c_y)\). They are separated by \( \Delta \theta = 1^\circ \) sampling in this manner the entire disk. Figures 4(b) and (c) show (in black solid line) the profile called the zero-degree profiles and denoted by \( P^\theta_R(x) \) and \( P^\theta_I(x) \), respectively. In general, the profile equations are expressed like, in accord with Barajas-García [15]

\[
P^\theta_R = f_R(x,y(x)),
\]

(4)

\[
P^\theta_I = f_I(x,y(x)),
\]

(5)

where \( x = 1,...,n, y(x) = m(x-x_i) + y_i, m = (y_2 - y_1)/(x_2 - x_1) \) is the slope of \( y \), \((x_i,y_i) = (c_x + r \cos \theta , c_y - r \sin \theta) \) and \((x_2,y_2) = (c_x + r \cos (\theta + \pi) , r \sin (\theta + \pi)) \) are the two distinct end points of that line segment, \( r = \min\{c_x,c_y\} \) and \( \theta \) is the angle that \( y \) has according to the horizontal axis in the Cartesian plane (considering that the origin (0,0) of the Cartesian plane is set at the center pixel of the image \((c_x,c_y)\)). Now, the profile whose sum has the maximum value will be selected, that is

\[
\lambda_{\theta} = \max_{0 \leq \theta \leq \pi} \left\{ \sum_{x=1}^{n} P^\theta_R(x), T^\theta_R(x) = P^\theta_R(x) \right\},
\]

(6)
Fig. 4. (a) Binary disk $DM(x,y)$, (b) $f_\alpha(x,y) = DM(x,y) \cdot \text{Re}\{FT(I(x,y))\}$. The solid line shows the profile $P_\alpha^R(x)$ and the dashed line the profile $T_\alpha(x)$. (c) $f_\beta(x,y) = DM(x,y) \cdot \text{Im}\{FT(I(x,y))\}$. The solid line shows the profile $P_\beta^R(x)$ and the dashed line the profile $T_\beta(x)$.

$$\lambda_\beta = \max_{0 < \theta < 2\pi} \left\{ \zeta_\beta^\theta = \sum_{x=1}^n P_\beta^\theta(x) \right\}, T_\beta(x) = P_\beta^\theta(x),$$

(7)

where the scalars $\zeta_\alpha^\theta$ and $\zeta_\beta^\theta$ represent the addition of the intensity values in each profile, $\alpha$ and $\beta$ are the angle of the profile in $f_\alpha(x,y)$ and $f_\beta(x,y)$ whose sum has the maximum value, respectively. Those profiles are called the maximum energy profiles [15,16]. Figures 4(b) and (c) show the maximum energy profiles (in black-dashed line) for the real and imaginary parts of the Fourier transform of the sub-image $I_i(x,y)$ Fig. 3(a). These profiles are given in the Cartesian plane in Figs. 5(a) and (b). These figures show the symmetry of $T_\alpha(x)$ and the antisymmetry of $T_\beta(x)$ in the vertical axis $x = c_x$.

Fig. 5. (a) The maximum energy profile $T_\alpha(x)$, (b) The maximum energy profile $T_\beta(x)$. 
Next, based on the maximum energy profile obtained by Eq. (6), two binary functions \( \zeta_{\text{Re}P}(x) \) and \( \zeta_{\text{Re}N}(x) \) are built by

\[
\zeta_{\text{Re}P}(x) = \begin{cases} 
1, & \text{if } T_R(x) > 0, \\
0, & \text{if } T_R(x) \leq 0,
\end{cases}
\]

\[
\zeta_{\text{Re}N}(x) = \begin{cases} 
0, & \text{if } T_R(x) > 0, \\
1, & \text{if } T_R(x) \leq 0,
\end{cases}
\]

(8)

(9)

where \( x = 1, \ldots, n \). Similarly, based on the maximum energy profile obtained by Eq. (7), the \( \zeta_{\text{Im}P}(x) \) and \( \zeta_{\text{Im}N}(x) \) binary functions are given by

\[
\zeta_{\text{Im}P}(x) = \begin{cases} 
1, & \text{if } T_I(x) > 0, \\
0, & \text{if } T_I(x) \leq 0,
\end{cases}
\]

\[
\zeta_{\text{Im}N}(x) = \begin{cases} 
0, & \text{if } T_I(x) > 0, \\
1, & \text{if } T_I(x) \leq 0,
\end{cases}
\]

(10)
\[ \zeta_{\text{tm,}n}(x) = \begin{cases} 0, & \text{if } T_i(x) > 0, \\ 1, & \text{if } T_i(x) \leq 0. \end{cases} \] (11)

the first two sub-index in Eqs. (8)-(11) indicate if the profiles comes from the real part (Re) or the imaginary part (Im) of the Fourier transform of the sub-image. The third sub-index means that the positive values (P) or negative values (N) of the profile were taken. Finally, taking the vertical axis \( x = c_x \) as the rotation axis, the \( \zeta_{\text{Re,P}}(x) \), \( \zeta_{\text{Re,N}}(x) \), \( \zeta_{\text{Im,P}}(x) \) and \( \zeta_{\text{Im,N}}(x) \) functions are rotated 360° to obtain concentric cylinders of height one, different widths and centered in \( (c_x, c_y) \) pixel [15,16]. Taking a cross-section of those concentric cylinders, the binary rings masks associated to the given sub-image are built. Figure 6 shows the binary rings masks corresponding to the image in Fig. 2(b) or 3(a) when \( i = 1 \). The sub-index notation is according with the Eqs. (8)-(11), the binary rings masks are named \( M_{\text{Re,P}}(x,y) \), \( M_{\text{Re,N}}(x,y) \), \( M_{\text{Im,P}}(x,y) \) and \( M_{\text{Im,N}}(x,y) \).

Fig. 8. Two different kind of tissues analyzed. (a) Variance of the images is compared. (b) Mean variance of the images is compared.
Fig. 9. Comparison of two kind of tissue. (a) Using condition real positive. (b) Using condition real negative. (c) Using condition imaginary positive. (d) Using condition imaginary negative.
Each mask is applied in the modulus of the Fourier transform of each sub-image. Before the modulus is obtained, the Fourier transform of each sub-image is transformed to an inverse filter. Thus, the modulus of this inverse filter for each ring is summed and then assigned to the corresponding ring index to obtain the signature for each sub-image [17], Fig. 7. Therefore, there will be four different signatures (one for each mask) for each sub-image. Thus the variance of each signature is calculated and for each sub-image there will be four variances corresponding to the four conditions of the four masks involved. Four average variances are obtained taking in account the 25 sub-images.

3. Results

In this work, 166 images of normal skin sections of nude mice and 166 images of sections of mice melanoma tumor were analyzed. If the variance of each image of tissue is obtained and graphed, Fig. 8(a), we can see several overlaps among these two kind of tissues. However, if for each image, we have 25 sub-images and a mean variance is calculated, Fig. 8(b), it is possible to see overlaps in the results. In another words, it is possible to find some confusions in the results. But, when we applied the methodology developed in this work, it is possible to see a good result when the different tissues are compared. No overlaps can be seen, Fig. 9.

Several filters were used, but the inverse filter had the best results in the comparison of these two kind of tissue (melanoma and normal cells). An inherent property of this method is that it is possible to obtain good results does not matter if the tissue has different color staining or the image has different format (bmp, tif or jpg). The variation observed in benign tissue curve (Fig. 9) is due to the different color staining in the samples.

4. Conclusions

This paper presents a new methodology to characterize melanoma tissue versus benign tissue through 1D-signatures averaged variance calculated with a binary mask. The modulus used in this performance is calculated from an inverse filter. The results show that melanoma cells presents a well-defined fringe of averaged variance of the signatures. The different tissues can have different color staining or the images can have different format.

This method can be helpful for no-expert people to melanoma cells recognition.

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