Morphometric analysis of dermal collagen by color clusters segmentation

Análise morfométrica do colágeno dérmico a partir da segmentação por conglomerados (clusters) de cor*

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Abstract: Morphometric analysis of dermal collagen can provide quantitative support to dermatologic research. The authors of this article disclose a technique of digital image analysis which allows the identification of microscopic structures by color cluster segmentation regarding the estimate intensity and density of dermal collagen fibers.

Keywords: Image cytometry; Cluster analysis; Collagen

Resumo: Análise morfométrica do colágeno dérmico pode fornecer subsídio quantitativo para a pesquisa em dermatologia. Os autores demonstram uma técnica de análise de imagem digital que permite a identificação de estruturas microscópicas, a partir da segmentação por conglomerados (clusters), de cor aplicada à estimativa da intensidade e densidade das fibras colágenas da derme.

Palavras-chave: Análise por conglomerados; Citometria por imagem, Colágeno

Digital photography constitutes a pixels matrix and its intensities of colour, position, combinations and interrelations are definied and unchangeable for each image, fact that favours quantitative analysis and the counting of structures. This technique is called morphometry.[1]

Histological cuts of computational morphometry represent an important tool in biomedical research, integrating the objectiveness of the measurements, high level of reproducibility, low cost, independence of human subjectiveness and partiality, possibility of quantitative analysis of the variables and a great number of publications available.[2]

The estimate epidermic thickness, hyperkeratosis, parakeratosis, melanic pigmentation, depth of tumours, inflammatory infiltrate, volume of the glands, immunohistochemical marks, heterogeneity of chromatin, dermic elastosis and collagen alterations are some direct applications of morphometry to microscopic skin cuts.[3-5]

In spite of the availability of specific commercial systems of morphometry, structures can be quantified using a simple microscope of light, attached to digital cameras and analyzed by free softwares such as the ImageJ, making it possible to promote the diffusion of quantitative research in dermatology.[6,7]

In this study we present a strategy to estimate the density and intensity of collagenous fibers on the skin, which is an important variable in studies about ageing, genetic syndromes, fibromatosis and colagen diseases, besides therapeutic comparissons.

There are various systems of color to operate morphometry, outstanding the HSB, the LAB, the XYZ and the RGB, the most commonly used. This means that the pixels of an image can be interpreted as shinning points with intensities of color that can be decomposed into channels such as: red (R), green (G) and blue (B). If each pixel projects its composition of color into a tridimensional orthogonal system RXGXB, it is possible to identify in this virtual space groups of points which are related to the shades of color of the image. Cluster analysis is a computational tool that can identify such groupings of points and substitute them by its median value (centroid), creat-

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ing a segmentation of the image according to the intensity of color.[7,8]

It is possible to use the *plugin* called *k-means clustering* in the ImageJ software which allows segmentation by conglomerates represented spatially by different systems of color.[6-8]

Initially, for a trustworthy evaluation, it is necessary strict standardization of the body area to be studied, of the technic to obtain material and to process the surgical section, microscopic cuts with the same thickness, staining of the plates with the same solution, choice of stainings that best stands out the structure to be studied (e.g. Masson trichrome), photographies captured in the same microscopic system and with the same optical enlargement, illumination, resolution, ISO, opening and speed of the camera obturator preferentially captured by the same individual(blinded concerning the groups of analysis) at the same time, sufficient sample of the numbers of images per plate and per patient in standardized skin sites (e.g, papillary dermis).

After these carefull procedures photographies of histologic cuts of skin stained by the Masson trichrome should be prepared for analysis starting from the standardized enlargement of the contrast between the shades and the cutting out of areas of interest (Pictures 1A, 1B e 1C). The resulting image can be divided into five or six different shades of *pixels*, as from the segmentation by color clusters (Picture 1D).

The analysis of the histogram of the new image allows us to evaluate the frequency and the intensity of each group of color (varies from 0 to 255) informing the density of the collagen and its estimate density of shade in relation to the background color (Picture 2).

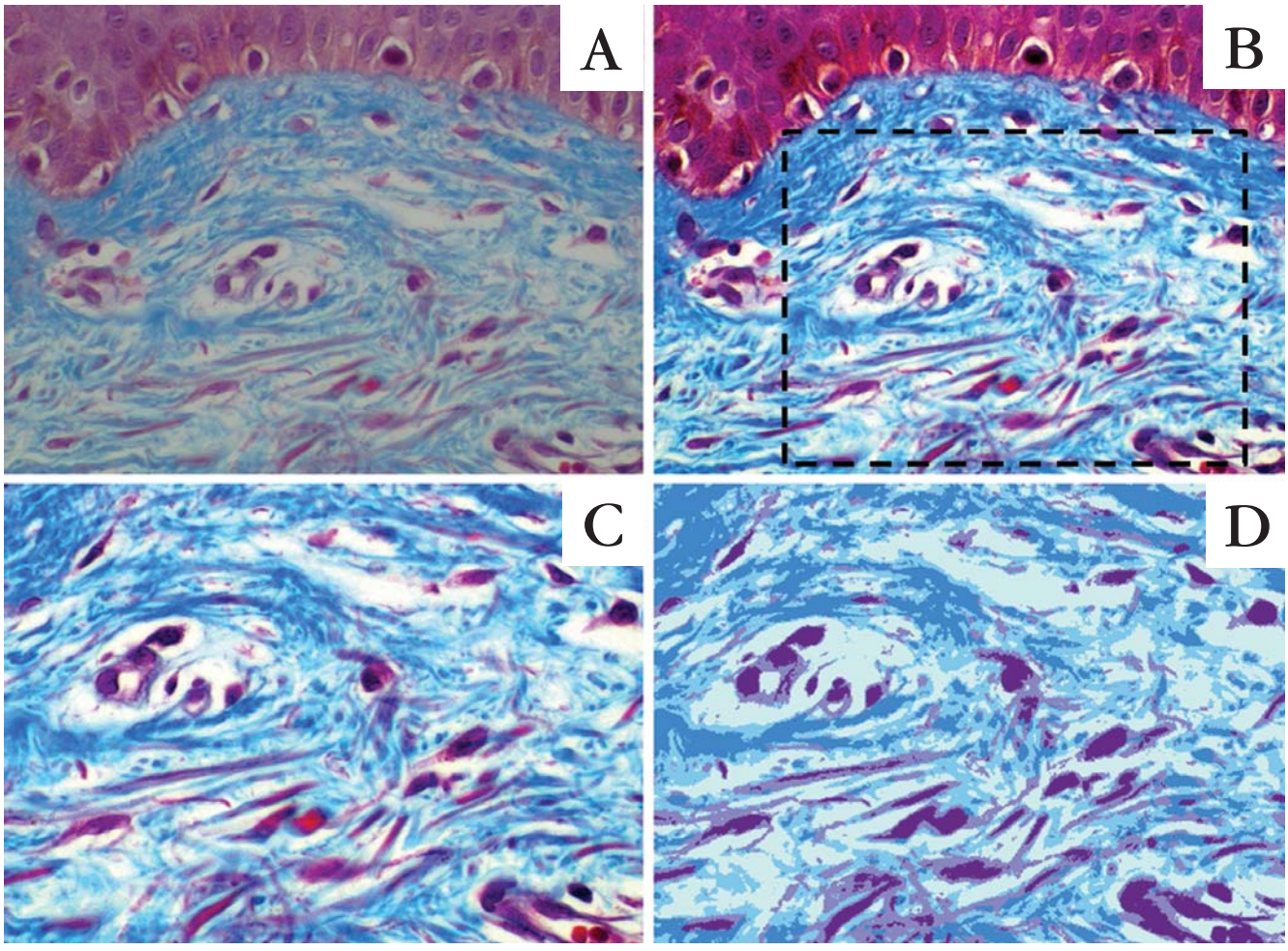

Figure 1. A. Photography of superficial and papillary dermis of the anterior chest of an adult, stained by Masson trichrome ; enlarged 400x; **B.** Increase in the contrast and detemination of the areas of interest for analysis; **C.** Cutting out of the image for segmentation (five groups), showing two tonalities of collagen (dark and light blue), inflammatory cells, red blood cells, endothelium e and fibroblasts (purple and redish), elastic fibers (pale red) besides the background (white); **D.** Segmented image represented by the five median colors (centroids) of each group.

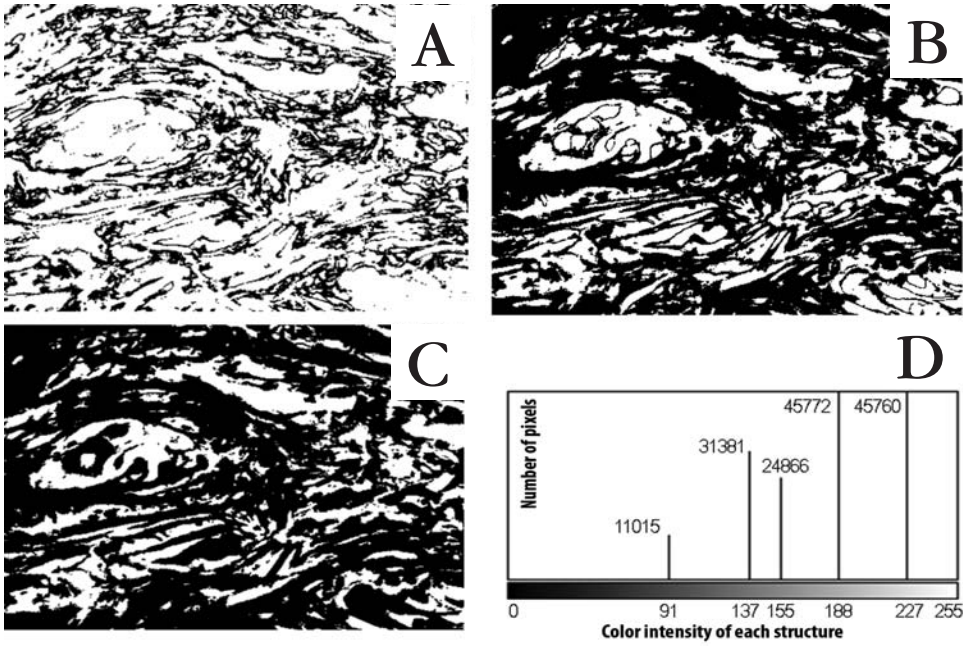

Figure 2. A. Individual binary segmentation of thin collagen fibers (28,8%); **B.** Thin fibers added to the thick and elastic ones (64,2%); **C.** Segmentation of all dermal structures,including nuclei (71,2%); **D.** Histogram showing the intensity of color of the centroid of each structure. Thick collagen is darker (137) than the background (227), resulting in a difference of 90 tons of color.

Alternatively, the direct segmentation of the dermis from binary images can be used to estimate the density and the intensity of dermal collagen without categorizing the different groups of color. However, this fact presents some disadvantages as there is no individualization of the collagen of the elastic fibers, endothelium, red blood cells, fibroblasts and inflammatory cells; which can lead to overestimated values (Picture 2). The method of segmentation by color clusters allows the control of this obliquity and also the joint estimation of these other structures.

Picture 3 demonstrates the quantitative estimate of dermal collagen in two different skin cuts with different densities and intensities of fibers showing the important morphometrical subsidy that the technic offers to dermatologic research.

The method also proved to be flexible enough for the use of different numbers of *cluster* selected to individualize groups of different stainings. The same described technic can be used to evaluate skin cuts stained by Hematoxylin and Eosin. However, the greatest colorimetrical distinction of the Masson trichrome for collagen also favours the segmentation of different fibers and other dermal structures.

As the technic identifies the groups of *pixels* that represent independent structures, this technic of analysis using clusters is less sensitive to differences in perception inherent to the technic of plate staining and illumination of the photography, effects that generate the false impression of alterations in the intensity of color.

Morphometric computational systems should be used for quantitative analysis in dermatologic researches and preferentially validated by qualitative technics (estimated visual cross evaluation), biomechanic technics (tests on rugosity, texture, hydration, elasticity), biochemical technics (expression of cytokine, proteins, enzymatic degradation) or functional tests (improvement on the disease, reduction of

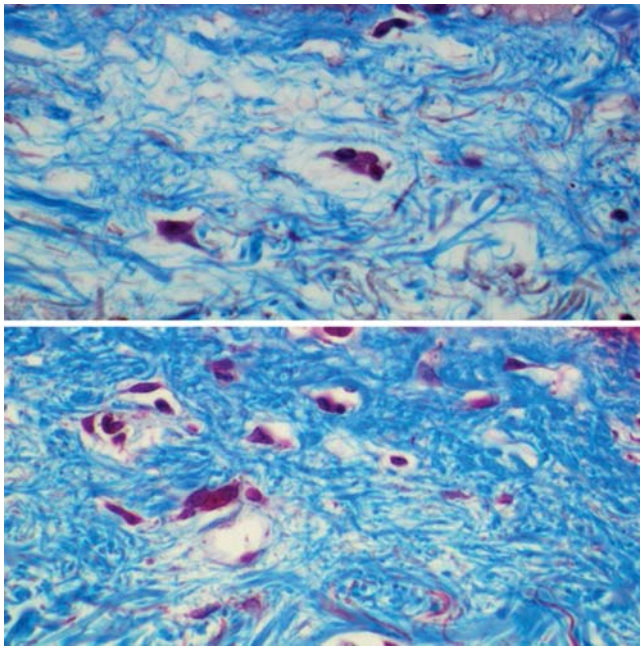

Figure 3. Areas of interest for the analysis by segmentation of conglomerates of color (dermis stained by the Masson trichrome; enlarged 400x), concerning the density and intensity of the collagen **A.** Less dense collagen (62,8%) and less intense (76) in relation to the background; **B.** Denser collagen (74,5%) and intenser (80) in relation to the background.

symptoms).

The study of dermal collagen can still be supplemented by the analysis of fractions of collagen I and III (imuno-histochemistry), apart from the evaluation of neocollagen-genesis starting from picrossirius staining (microscopy of polarized light), thickness and orientation of fibers. Such evaluations can be quantified using morphometry technics.[9,10] ❑

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