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
Abstract

The objective of this study was to create and evaluate a routine (macro) using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, USA) for automatic counting of labeled nuclei by proliferating cell nuclear antigen (PCNA) immunohistochemistry. A total of 154 digital color images were obtained from eleven sections of reticular oral lichen planus stained by PCNA immunohistochemistry. Mean density (gray-level), red density, green density, blue density, area, minor axis, perimeter rate and roundness were parameters used for PCNA labeled nuclei discrimination, followed by their outlined presentation and counting in each image by the macro. Mean density and area thresholds were automatically defined based, respectively, on mean density and mean area of PCNA labeled nuclei in the assessed image. The reference method consisted in visual counting of manually outlined labeled nuclei. Statistical analysis of macro results *versus* reference countings showed a very significant correlation (r_s = 0.964, p < 0.001) for general results and a high level (89.8 ± 3.8%) of correctly counted labeled nuclei. We conclude that the main parameters associated with a high correlation between macro and reference results were mean density (gray-level) and area thresholds based on image profiles; and that Image-Pro Plus 4.5 using a routine with automatic definition of mean density and area thresholds can be considered a valid alternative to visual counting of PCNA labeled nuclei.

Keywords : Image processing, computer-assisted; Lichen planus; Proliferating cell nuclear antigen.

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