IHC Optical Density Score: A New Practical Method for Quantitative Immunohistochemistry Image Analysis

To the Editor:

Immunohistochemistry (IHC) is a powerful method for identifying the presence and the expression extent of specific antigens in formalin-fixed, paraffin-embedded tissues, which is widely used for diagnostics and research purposes because of its reliability, ease of use, and versatility.[1–3] Such qualitative and semiquantitative assessments have important diagnostic and prognostic implications, particularly for lymphoma, cutaneous tumors, that directly influences grading and classification of the disease, affecting patient management.[1,2,4] However, till today, pathologic analysis of tissue samples remained a subjective and time consuming procedure, wherein the antibody staining intensity is manually judged.[2] Therefore, despite development of practical scoring systems,[5,6] the scoring decision is directly influenced by visual bias.[2] However, introduction of advanced digital image processing systems such as Image J (NIH, Bethesda, MD), and their compatible open source plugins such as IHC Profiler highlight the possible hope to run the most precise and high volume IHC quantitative analysis using color deconvolution and computerized pixel profiling leading to the automated scoring of the respective image.[2] Although IHC Profiler generates a nice histogram profile of 3,3-diaminobenzidine (DAB) image—which corresponds to number counts of a pixel intensity—and computes the score automatically,[2] the final score is shown in a semiquantitative way (high positive, positive, low positive, or

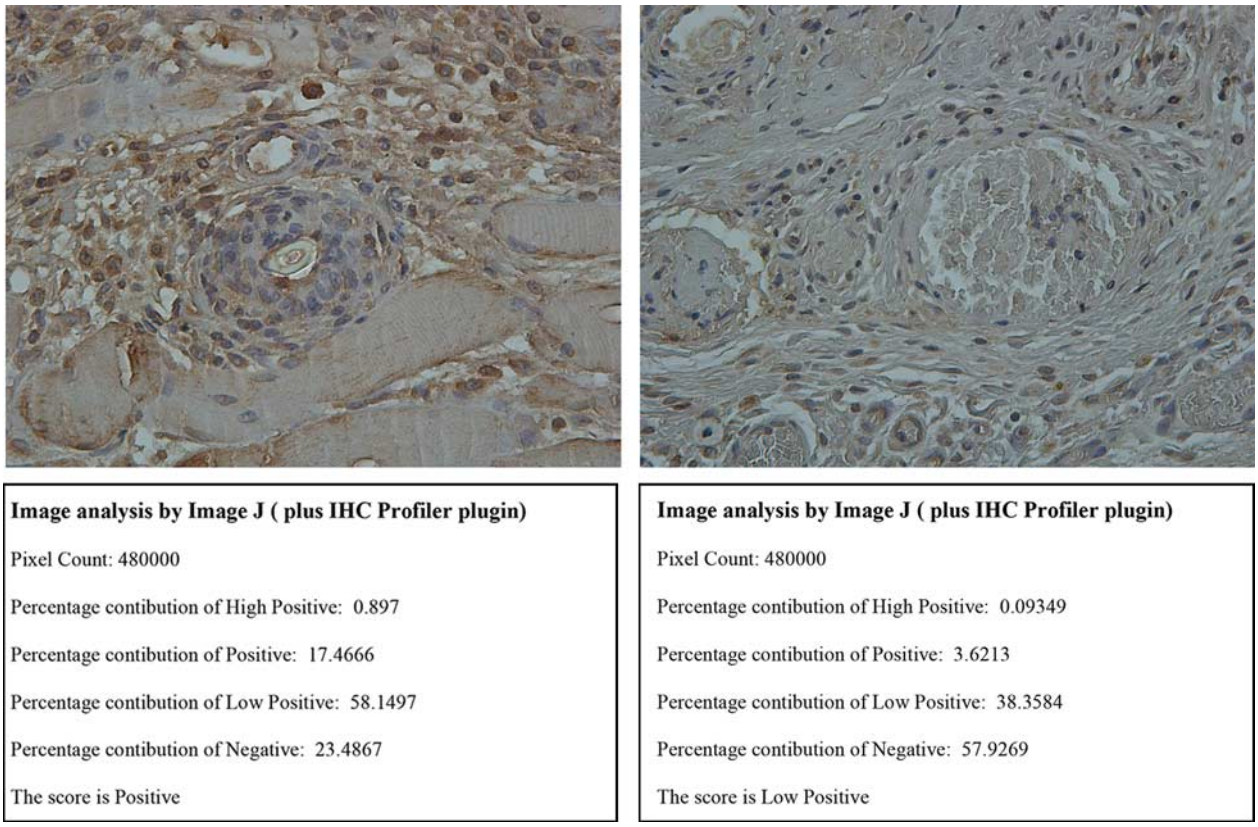

FIGURE 1. Image analysis and comparison by Image J, plus IHC Profiler plugin. IHC indicates immunohistochemistry. IHC Profiler counted the pixels, calculated the percentage contributions, and then declared the score in the left and right images as positive and low positive, respectively. To have the opportunity to perform quantitative comparisons IHC optical density score was calculated for the left and the right images as 2.54 and 1.84, respectively, based on IHC Profiler percentage contributions. Rat skin sections were incubated with the anti-hHGF antibody (1:100 dilution; R&D Systems, Abingdon, UK) followed by the second antibody-horseradish peroxidase conjugate (1:200; Dako). Staining was performed with DAB using a cell and tissue staining kit (BD Biosciences Pharmingen).

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negative). To solve this issue during working with such programs, and the need for the exact numbers in image analysis and comparisons, the following algebraic formula is recommended to calculate the IHC optical density score (from 1 to 4) for the IHC images.

$$\textit{IHC optical density score} = \left(\frac{\begin{array}{l}\text{Percentage contibution of high positive} \times 4 \\ +\text{Percentage contibution of positive} \times 3 \\ +\text{Percentage contibution of low positive} \times 2 \\ +\text{Percentage contibution of negative} \times 1\end{array}}{100} \right).$$

As an example, image analysis and comparison of 2 DAB stained skin sections were performed using IHC Profiler. This program counted the pixels, evaluated the percentage contributions, and then declared the semiquantitative scores for our sections (positive vs. low positive). In addition, IHC optical density score was calculated for these 2 stained sections. (2.54 vs. 1.84) to have the opportunity to perform exact quantitative analysis and comparison (Fig. 1).

In brief, the advanced digital image processing systems opened new doors to further progress in an unbiased, unsupervised, and automatic IHC image analysis by measurement of optical density, which is proportional to the expression extent of specific antigens. Furthermore, application of our new scoring method, *IHC Optical density score*, might help the scientists in quantitative comparisons.

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