Sir,

The contribution of scientific technology in the field of histopathology and lab medicine is well recognized. Image analysis is one such division in which many researchers and practicing pathologists have used scientific technology to good advantage. Currently there are many systems available to an anatomic pathologist for the purpose of image analysis in immunohistochemistry (IHC) and for nuclear morphometry. Many of these systems, however, require expensive software and hardware attachments for image acquisition, analysis and storage. Consequently, a cost-effective alternative for image analysis would be a welcome tool for pathologists and researchers alike. In this connection, “ImageJ” is a freely available java-based public-domain image processing and analysis program developed at the National Institutes of Health (NIH). Currently, ImageJ’s Macintosh platform counterpart, “NIH Image” is widely used in biologic research.1-3

For the evaluation of IHC slides using ImageJ, images can be captured onto the hard drive of the workstation computer. Thereafter, captured images can be opened in NIH Image/ImageJ for evaluating indices of positivity on IHC slides as well as fluorescence images.4,5 Counting can be done in two ways off the image- the cell counter technique (analogous to the hematology differential counters) and the area method. In the ‘cell counter method’, cells can be counted off directly from the screen by placing marks of different colors onto positive and negative nuclei by mouse clicking. Finally, ImageJ will generate the IHC index automatically. An advantage of this technique over conventional counting across the microscope is its reproducibility and accuracy. With the ‘area method’, the total area occupied by positive (brown) nuclei and negative (blue) nuclei can be estimated by setting a “threshold” using ImageJ’s thresholding tool for selection of these nuclei separately. From this nuclear area data, the positive IHC index for that image can be calculated. One another useful feature of ImageJ is the ‘ROI Manager’ (Region Of Interest Manager) where the pathologist can specify and draw out areas to be evaluated, editing out the unwanted elements. All data can be imported into Microsoft Excel worksheets to give a summated set of results for a single slide.

Besides IHC analysis, NIH Image/ImageJ can do densitometry imaging to analyze intensity of western blot bands. In addition, a number of nuclear morphometric descriptors can be evaluated on Feulgen-stained sections after downloading specific plug-ins from the ImageJ website. The macros and plug-ins are available as source files and download to the ImageJ folder. The website gives step-by-step instructions on how to execute the various available functions. The latest PC (java) version, ImageJ.v1.31 can be downloaded from the web link http://rsb.info.nih.gov/ij/. Sample images of gels, fluorescent cells and cell colonies are included for learning the process of analysis. Accessibility of the aforementioned web link has been verified as of February 27, 2004.

In short, NIH Image/ImageJ delivers a lot despite being a freeware. Knowledge of its potential uses will go a long way in establishing objective, reproducible, cost-effective and timesaving methods of automated image-based IHC evaluation and morphometry for histopathologists in our country.

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Girish V,* Vijayalakshmi A
Deparment of Preventive Medicine and Division of Haematology/Oncology, Robert H.Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, 710 North Fairbanks, Room 8340, Chicago, IL60611.

Correspondence to : Vijayalakshmi Ananthanarayanan. 
E-mail: viju@northwestern.edu

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