

Impact of Digital Image Manipulation in Cytology

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• **Context.**—Digital images have become an important component of cytology practice. They are used in telecytology, automated screening, educational material, and Web sites and have potential for use in proficiency testing. However, there has been no formal evaluation to date to determine if digital image manipulation (intentional or unintentional) can affect their interpretation.

Objective.—To investigate whether alteration of digital cytology images affects diagnosis.

Design.—Acquired digital images of ThinPrep Papanicolaou test slides were manipulated (rotated 90° and brightness, contrast, red-green-blue color, and luminosity adjusted) using Photoshop. A test composed of these altered images, along with their original (unaltered) image and exact duplicates was given to 22 cytologists (13 cytotechnologists, 8 cytopathologists, and 1 fellow). All images were rated as negative, atypical (atypical squamous cells of undetermined significance), low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial le-

sion, or positive for cancer. Weighted κ and heterogeneity χ^2 statistics were used to measure levels of agreement and assess concordance between groups.

Results.—The level of agreement for identical duplicate images was excellent ($\kappa = 0.81$), compared with the poor agreement for manipulated image pairs ($\kappa = 0.21$), a statistically significant difference ($P < .001$). For all altered image types agreement was poor. There was no significant difference between cytotechnologists and cytopathologists in level of agreement ($P = .56$).

Conclusions.—Manipulation of a Papanicolaou test digital image, irrespective of the specific category of cytologic material photographed, significantly affects its interpretation by both cytotechnologists and cytopathologists. This suggests that care needs to be taken when digital cytology images are used, to specifically ensure that their alteration does not affect diagnosis.

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A digital image is an image that is captured and stored as an electronic data file. Digital images permit image quality and content to be evaluated at the time of capture, are easily duplicated and manipulated, and allow for better image storage, cataloging, retrieval, and sharing when compared with analog photography. As a result of these direct benefits, digital images are becoming an increasing component of modern cytopathology practice.^{1,2} They are being used for telecytology, educational materials including cytology Web sites, peer review, and automated screening of Papanicolaou (Pap) test slides and have the potential for use in future proficiency testing.^{3–5}

The necessary steps involved during the digital imaging process include image acquisition (eg, digital camera capture), storage (eg, disk medium or drive storage and retrieval), editing (eg, postcapture manipulation), and use (eg, viewing, displaying, printing, and sharing). Presently, there are no set standards regarding these various imaging steps in the field of pathology. Image file formats that use a lossy compression algorithm (eg, JPEG) reduce file

size and needed storage space. However, similar image compression and manipulation can inadvertently insert artifacts and/or remove elements from a digital image. In general, the greater the degree of compression the more information lost, which may ultimately reduce the image quality or detail.

As digital images have become more prevalent in the medical and scientific fields due to the widespread availability of sophisticated image manipulation software, the importance of fraudulent digital image manipulation has emerged as an issue of concern.^{6–15} Digital image manipulation refers to the use of computer program tools and software to perform image processing (altering of an image). Pixels that comprise a digital image can be changed as a group (global adjustment) or individually (focal adjustment) by means of image editors. A limited number of publications have addressed this contentious topic, proposing guidelines and simple rules on such issues as erasing and combining elements within a digital image.^{16,17}

However, there has been to date no formal evaluation in cytopathology to determine if digital image manipulation (intentional or unintended) can affect the respective interpretation. To specifically address this need, the aim of this study was to investigate whether global alteration of digital cytology images could significantly affect diagnosis.

MATERIALS AND METHODS

Image Acquisition and Manipulation

Digital images of ThinPrep Pap test slides, stained using the standard ThinPrep Imager stain, were acquired using a single Spot Diagnostic (Insight 4) digital camera at similar magnifica-

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No. of Images	Image Type	Image Manipulation
10	All original images	None altered
20	10 original + 10 identical duplicates	None altered
20	10 original + 10 altered duplicates	Half altered

tion. All images were stored in JPEG format. Using Adobe Photoshop 7.0 (Adobe Systems Inc, San Jose, Calif) on an Apple i-Book G4 (Apple Inc, Cupertino, Calif), 10 of the digital images were manipulated. This manipulation was restricted to global alterations (ie, all elements within the image were changed) and included changes in contrast, red-green-blue color balance, and/or brightness of the images. Contrast in certain manipulated images was only increased, in which case the nuclei were made to appear darker and more "hyperchromatic." With red-green-blue manipulation squamous cells in some of the images could be adjusted to appear less keratinized (ie, cytoplasm more blue than orange) along with darker nuclei. Brightness was only increased in select images, which resulted in darker nuclei. No focal changes were made. Once the images were manipulated, they were rotated 90° from the original acquired image in an attempt to disguise it from the original.

Test Administration

A test was compiled of 50 digital images of cervicovaginal cytology including altered, unaltered, and duplicate images (Table 1). The test was administered digitally (ie, viewed on a personal computer monitor) to 22 cytologists (13 cytotechnologists, 8 cytopathologists, and 1 cytopathology fellow). The same test was given to everyone. All 50 images were presented in the same order in a PowerPoint 2003 (Microsoft Corp, Redmond, Wash) presentation. Each image was advanced by the individual test taker, with no set time restriction. Test takers were instructed to rate each digital image as negative, atypical, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, or positive for carcinoma.

Statistical Analysis

An expert panel consisting of 3 cytopathologists was convened to evaluate each image to its perceived correct diagnosis. For each image, the diagnosis rendered by each respondent was compared with the expert panel. The weighted κ statistic was used to assess the level of agreement (concordance) on ratings for pairs of slides of the same image, which was either duplicated or enhanced, and whether agreement was greater than that expected by chance.¹⁸ A κ of 0.4 is considered fair, 0.75 good, and greater than 0.75 excellent. The heterogeneity χ^2 statistic was used to test for significant heterogeneity of concordance between groups, with Higgins and Thompson Heterogeneity Index (H) used as a measure of the amount of heterogeneity present.^{19,20} An index of H less than 1.2 indicates no heterogeneity (ie, no difference), H from 1.2 to 1.5 demonstrates a moderate difference, and H more than 1.5 indicates an extremely notable difference. To control for multiple comparisons, $P < .015$ was used to denote significance.

RESULTS

There was a 100% response rate with all questions answered. Table 2 illustrates the correlation of diagnoses for all pairs of altered images versus unaltered images. The κ measure of agreement ($\kappa = 0.21$) was poor for the altered images. Table 3 demonstrates the correlation of diagnoses for all pairs of unaltered duplicate images within the study. By comparison, the κ measure of agreement ($\kappa = 0.81$) was excellent for these duplicate unaltered images. This difference was moderately large ($H = 1.34$) and statistically significant ($\chi^2_1 = 134.5$, $P < .001$). When digital

Altered Images, Diagnosis	Unaltered Images, Diagnosis				
	Neg	ASC	LSIL	HSIL	Ca
Neg	23	19	5	1	0
ASC	27	26	11	1	0
LSIL	4	15	15	1	0
HSIL	24	23	3	19	0
Ca	0	2	1	0	0

* Numbers in boldface indicate identical interpretation of an image. Neg indicates negative; ASC, atypical squamous cells of unknown significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; and Ca, carcinoma.

Unaltered Images, Diagnosis	Unaltered Images, Diagnosis				
	Neg	ASC	LSIL	HSIL	Ca
Neg	98	7	0	2	0
ASC	7	42	6	2	0
LSIL	1	2	41	0	0
HSIL	1	2	1	8	0
Ca	0	0	0	0	0

* Numbers in boldface indicate identical interpretation of an image. Neg indicates negative; ASC, atypical squamous cells of unknown significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; and Ca, carcinoma.

images were altered, there was a tendency for raters to render a higher cytologic diagnosis (Figure 1, A and B). Altered images containing atypical squamous cells of undetermined significance were frequently overdiagnosed as dysplasia (38 answers) and even diagnosed as carcinoma in 2 instances. Furthermore, 1 digital image of low-grade squamous intraepithelial lesion was interpreted by an individual to also represent carcinoma after manipulation. Image alteration also resulted in cytologists undercalling the manipulated image as negative in 25 responses (Figure 2, A and B). By comparison, duplicate images that were not subject to any global adjustments were infrequently overdiagnosed as atypical or dysplasia and rarely resulted in underinterpretation.

No significant difference was identified in the degree of agreement in slide pair interpretation when comparing cytotechnologists and cytopathologists ($\chi^2_1 = 0.332$, $P = .56$; $H = 1.0$). κ for altered images was poor for both groups; 0.18 for cytotechnologists and 0.25 for cytopathologists. No individual altered image appeared to be significantly different ($\chi^2_1 = 7.349$, $P = .39$; $H = 1.0$). The agreement (κ) was poor for all images and ranged from 0.01 to 0.3. The weighted κ measure of agreement for each cytotechnologist or cytopathologist with the expert panel ranged from 0.04 (poor) to 0.62 (good). Agreement with the experts was generally better for altered digital images than those that were unaltered.

CONCLUSIONS

Digital images have become an integral component of contemporary pathology practice and education. Their application in the field of cytopathology includes telecytology, automated screening, education, training and certification, research, and publications. They represent an integral component of cytopathology Web pages that can be

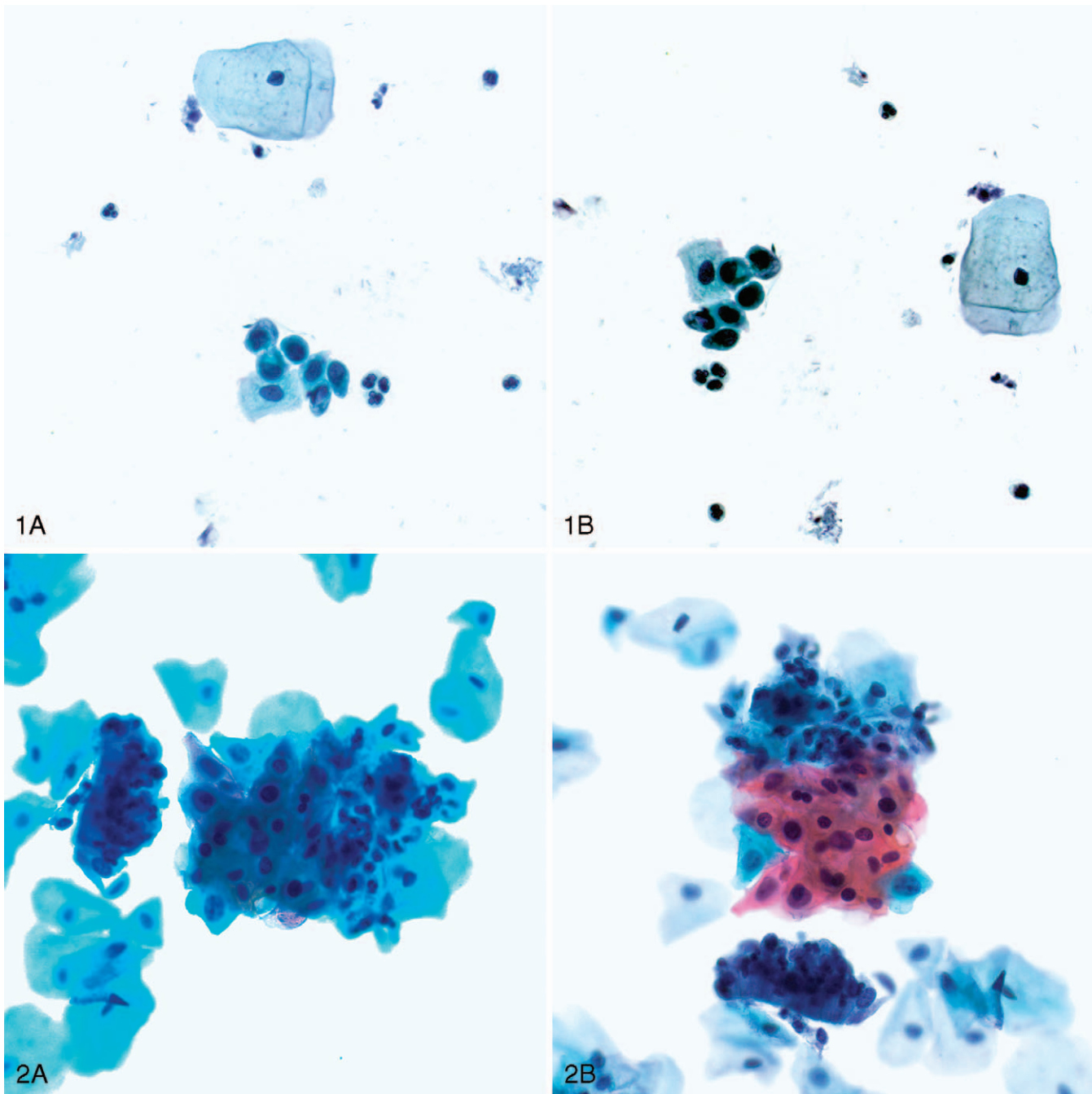


Figure 1. This pair of images demonstrates an example of an unaltered (original) captured digital image (A) and its altered (manipulated) version (B). Red-green-blue colors were all increased in the manipulated image with blue > green > red. Contrast and brightness were not changed. The image was then rotated 90° clockwise. This particular pair of images showed poor correlation in the study ($\kappa < 0.15$), with most raters (73%) overcalling the manipulated image high-grade squamous intraepithelial lesion compared with the original image, which was widely diagnosed as negative (Papanicolaou stain, original magnification $\times 600$).

Figure 2. This pair of images showed poor correlation in the study ($\kappa < 0.02$), with most raters (64%) undercalling the altered image (A) as negative, compared with the original image (B) showing atypical squamous cells of undetermined significance. The red-green-blue colors were manipulated with red completely removed and blue slightly increased. Contrast and brightness were unaltered and the image was rotated 90° clockwise (Papanicolaou stain, original magnification $\times 600$).

accessed through an Internet Web browser and hold promise for future proficiency testing. Specifically, telecytology is the practice of cytology at a distance by using telecommunication to transmit digital images. This may use static images (eg, captured fields of view), dynamic or real-time images (obtained with an interactive or re-

motely operated robotic microscope stage), or virtual (whole slide) images. Several studies have validated the use of telecytology for diagnostic, consultation, and education purposes.^{3,4,21,22} Following the adoption of liquid-based cytology, Pap test imaging systems were successfully introduced, resulting in improved diagnostic accu-

racy and efficiency in screening. These computer screening systems rely on high-resolution field of view scans of selected images in concert with imaging algorithms.

Virtual microscopy methods have further been shown to be effective for administration of standardized proficiency tests in cytology.⁵ In this case, the imaged (scanned or digitized) whole slide may contain more than 3000 high-power images that have been joined (tiled) together. As an additional example of their application in cytopathology, the National Cancer Institute Web-based atlas of the Bethesda System contains more than 300 digital images. A subset of these images was used for the Web-based Bethesda Interobserver Reproducibility Project, which involved more than 500 participants providing independent interpretations online.²³ Investigators involved with this online project demonstrated that Web-based studies may be useful in assessing interobserver agreement in classifying images.

This study demonstrated how global manipulations of a cytology digital image can significantly affect its morphologic interpretation. Such alterations may result in both diagnostic underinterpretation and overinterpretations, by both cytotechnologists and cytopathologists. This is most concerning because digital images are being increasingly used in the field of cytopathology. We were intrigued to find out that the performance of our altered digital images on expert review was generally better than those that were unaltered. We suspect that the manipulation of several cases may have eliminated the subjectivity often involved in cytologic interpretation. For example, when the nuclei in squamous cells that fall into the diagnostic category of atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion, are enhanced to make them appear more hyperchromatic, it may be easier for cytologists to agree that such cells appear dysplastic. It was not the focus of this study to define exactly what type of alteration or how much manipulation of a digital image is necessary to affect its interpretation. Nevertheless, it is interesting to examine the types of alterations we performed and the impact they had. All images underwent arbitrary adjustment of their red-green-blue levels (for red, the range was -100 to 85; for green, 0 to 95; for blue, -40 to 173). In addition, half of the images also had their brightness altered (range, -20 to 35) as well as the contrast adjusted (range, -30 to 38). Image manipulations were therefore largely multifactorial. The levels tool in Adobe Photoshop permitted several aspects of these digital images (tonal range and color balance) to be simultaneously adjusted. Standards regarding the use of digital images in cytopathology are required to avoid such potential misdiagnoses. This standardization needs to be broadly applied to the entire imaging process (capture, saving, editing, and sharing), as recommended by this study and others.²⁴

Digital images can potentially be altered at the time of saving (eg, compression) or when shared (eg, viewed or displayed on a monitor). Although in one previous study, no negative effect was seen with JPEG image compression in regard to the accuracy and confidence level of diagnosis on static telepathology,²⁵ monitor properties may vary, greatly affecting the display of digital images. Image editing software is readily available to allow for postcapture processing (manipulation) of digital images. Simple edits to a digital image may include zoom, resize, rotate, flip, crop, and sharpening, as well as adjustments made to

brightness, contrast, gamma, and color balance. Many of these adjustments can be carried out using software levels, curves, histograms, and tone maps. Rotating images may result in their distortion and require subsequent cropping. Cropping can also discard unwanted parts of an image. In so doing, image editors can be used to improve the composition of digital images. Images can also be altered by adding annotations (eg, text, arrows, measurements).

Digital image manipulation is routinely used in the field of digital radiography. In digital radiography, preprocessing (to correct for system irregularities such as dead pixels or dark noise), processing (manipulation of raw data such as unsharp mask, histogram sliding and stretching), and postprocessing (manipulation of the final appearance of the radiograph by the end user) of digital radiographs are used.²⁶ In one study, investigators showed that grading of diabetic retinopathy based on digitized retinal images was improved by software manipulation or processing of images.²⁷ Similarly, digital enhancement of subquality bite-mark photographs has been reported in the forensic literature.²⁸

In proposed digital imaging guidelines for pathology, global rather than focal adjustments of digital images are supported.¹⁷ Global adjustments involve changes across the entire image (eg, hue, contrast, brightness, and color changes). Focal adjustments are those made to only specific areas of an image (eg, deleting an object represented on an image). With regard to legal considerations, global enhancement of a digital image is considered to be far more acceptable than focal adjustments.²⁹ Although it is easy to appreciate how focal adjustments can misrepresent data, the present study indicates that global adjustments may also negatively alter digital data, causing misrepresentation of the original information. As examples, global changes to background color to highlight specific cells may suppress a diagnostic background diathesis on the slide. Alternatively, nuclear dysplasia with hyperchromasia can be adjusted by altering the hue, brightness, and contrast of a digital image. Further investigation into what specific features need to be most closely regulated is required.

In conclusion, this study demonstrates that manipulation of a digital image, irrespective of the cytologic material photographed, significantly affects its interpretation by both cytotechnologists and cytopathologists. This suggests that care needs to be taken when digital cytology images are used, to specifically ensure that their alteration does not affect diagnosis. Clearly, the entire imaging process needs to be standardized if digital images are to be fully adopted in the field of pathology.

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