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ORIGINAL ARTICLE

AN AUTOMATED FOCUS FUSION ALGORITHM FOR INFORMATIVE CYTOLOGICAL IMAGE

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Abstract To date, digital pathology is based on single focal plane images, and with its rapid development, image quality and information are becoming critical concern in the field. This paper presents a new multiple focal-plane based focus image fusion algorithm to enhance the image quality. First of all, we will introduce a new focus image fusion algorithm; next, we will demonstrate some experimental data and results on cytopathology slide images; and finally, we will verify the effectiveness of this algorithm using computer vision techniques and cytopathology diagnosis, and draw the conclusions.

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Key words: Cytopathology; Focal Plane; Fused Image; Best Focal-plane Image; Entropy.

原 著

細胞診標本画像用自動フォーカス合成に関するアルゴリズム

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抄録 近年，デジタルパソロジーの急速な発展に伴い，標本画像の画質に対する関心と要求も高まってきている．いままでのデジタルパソロジーは全てシングル z-Plane の画像を使っているので，画質と画像情報不足の問題が浮上してきた．本論文では，まず新しい画像フォーカス合成のアルゴリズムを提案し，続いて，細胞診標本画像結果を示し，最後に，画像処理と病理診断の観点から画像結果に対して評価を行い，本アルゴリズムの有効性を検証した．

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キーワード：サイトパソロジー；フォーカスプレン；合成画像；ベストフォーカス画像；エントロピー．

Introduction

Pathologists have been using their tool of trade, the microscope, since the early 17th century, when it was first described by Antony van Leeuwenhoek and Robert Hook[1)]. Today, with the advent of the Internet and new technologies that have allowed cameras to digitalize images, digital pathology is being developed that will have an impact on the way the pathologist will practice.

Digital pathology is the use of computer technology to convert analog microscopic images into digital images that are similar to digital

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photography; there are many functions that digital pathology offers that are not possible with light microscope examinations. These include integrating the workflow of the pathologist with electronic medical records, retrieval of previous biopsy images using the computer, split screen comparison of 2 or more images from the same case or different cases, ability to use whole-slide-imaging (WSI) data for computer-assisted image analysis and manipulation, transmission of digital images to the patient's electronic chart, the internet and other links, and so forth[2~4].

Furthermore, developers are also looking at WSI as a front end process for computer aided diagnosis tools and anatomic pathology laboratory information systems in conjunction with other relatively new technologies. In this circumstance, the quality and accuracy of image data is becoming increasingly important.

In this manuscript, we will present a new multiple focal-plane based image fusion algorithm to improve the image quality and information. We will also perform a group of assessments on the fused cytology slide images using computer vision techniques and pathological diagnosis exams, in sequence, a promising conclusion, which is expected to expand the applications of digital pathology, will be drawn.

Materials & Methods

Cytology glass slides preparation

Material was obtained from a 74-year-old woman with a right mammary tumor, and the imprint was prepared by pressing the cut surface against a microscopic slide. The imprinted microscopic slide was Papanicolaou stained and used for obtaining the best focal-plane image (BFI) and Fused Image (FuI) analyses. The tumor was histologically diagnosed as solid tubular carcinoma.

Several other Papanicolaou stained slides were prepared for the purpose of assessment of image quality and information contents.

Multiple focal plane Image fusion method

Image fusion refers to a process that extracts redundant and complementary information from a set of input images and fuses it into a single and more informative complete image.

In our study, we selected Claro's high-end WSI device -Vassalo model as our experimental tool. The tool configuration is as follows:

a) Z-plane accuracy is 0.5μm
b) Carl Zeiss EC-plan 40x objective lens is utilized
c) Hitachi 3CCD camera (HV-F31) is employed
d) Computer model: DELL Vostro 220 mini-tower with Windows XP Pro, SP3
e) Application Software: Vassalo Ver1.3.5, iViewer Ver 5.1.9

First, we introduce a new figure of merit to evaluate and extract image information to be fused:

$$C(i,j)=\sqrt{(I(i,j-1)-I(i,j+1))^2+(I(i-1,j)-I(i+1,j))^2} \quad (1)$$

C(i, j) stands for the measurement clarity of pixel located at (i, j), I(i, j) is the gray-scale of pixel at location (i, j). We calculate all C(i, j) values in both images to be fused.

Next, suppose we have image a and image b to be fused. Image R is the fusion result, for each pixel in both images. The clarity values are computed utilizing the aforementioned formula respectively, based on the comparison of the corresponded clarity values pair. The gray-scale value is derived from the image with more measurement clarity.

$$I_R(i,j)=\begin{cases} I_a(i,j) & C_a(i,j)\geq C_b(i,j) \\ I_b(i,j) & C_a(i,j)<C_b(i,j) \end{cases} \quad (2)$$

Here, Ia(i, j) is the gray-scale value of pixel (i, j) in image a, Ib(i, j) is the gray-scale value of pixel (i, j) in image b, and IR(i, j) is the fused gray-scale value of pixel (i, j) in image R.

Ca(i, j) stands for clarity of pixel (i, j) in image a, and Cb(i, j) stands for clarity of pixel (i,j) in image b.

Finally, the artifact boundaries in the fused image must be removed or processed. As indicated above, the fused boundaries are usually composed of pixels from different images, which cause fusion artifacts on the boundaries. In order to suppress these artifacts, we introduced the following technique.

$$I_R(i_o, j_o) = I_a(i_o, j_o) * N_a + I_b(i_o, j_o) * N_b \qquad (3)$$

Suppose a pixel (io, jo) on the boundary, the corresponded gray-scale level in image a is Ia (io,jo), in image b is Ib(io,jo), and in fused image is IR(io,jo); Na and Nb are dynamically calculated coefficients.

For all pixels on the boundary, we select a MxM kernel. There are (MxM-1) pixels in the sum in the kernel except for the pixel (io, jo) itself, where all pixels surrounding the pixel (io, jo) will be checked if it comes from image a or image b. When ma and mb denote the counts from image a and image b respectively, then,

$$\begin{aligned} &Na = ma \,/\, (M \times M - 1), \text{ and} \\ &Nb = mb \,/\, (M \times M - 1) \end{aligned} \qquad (4)$$

In our study, we used a 3x3 kernel size.

All pixels on the boundaries must be re-calculated with equation(3, 4).

Image assessment method

In order to quantify the information of the image, here we employ the Entropy method. Entropy can measure the information content of images[5, 6].

In information theory, the definition of the information entropy is quite general, and is expressed in terms of a discrete set of probabilities pi.

$$H(X) = -\sum_{i=1}^{n} p(x_i) \log_b p(x_i) \qquad (5)$$

According to Shannon's assumption, one element of a large number of messages from an information source is just as likely as another, so the digital number of one pixel in an image is just as likely as another pixel. In any one image the number of pixels can be very large. In such cases, to quantify the information contents of an image one can just satisfy the Shannon's assumption. Hence, it is reasonable to use Shannon's entropy in image analysis; the formula can be modified as:

$$H = -\sum_{i-1}^{G} d(i) \ln_2 \{d(i)\} \qquad (6)$$

Where G is the number of grey level of the image histogram range, for a typical 8-bit image, it ranges between 0 to 255, and d(i) is the normalized frequency of occurrence of each grey level. To sum up the self-information of each grey level from the image, the average information content is estimated in the units of bit per pixel.

From the equation (6), it can be seen that entropy can directly reflect the average information content of an image. The maximum value of entropy is produced when each grey level of the whole range has the same frequency. Therefore, from the fused image, if its entropy value is higher than its corresponded single focal plane images, it may be deduced that the fused image contains more information that any of the single focal plane images. Although it is not clear if that "more information" is in the form of noise or useful information, which can be distinguished by other methods, like Image Noise Index, etc.

In this study, we assume all cytopathological images are basically noise-less.

Results

Image assessment using Entropy

So far, the best focal-plane image has been widely used in digital pathological diagnosis. In order to evaluate entropy values in the assessment of the best focal-plane images and fused images, several sets of images were used. Images are all acquired from different cytopathology slides using the same optical magnification. The number of images used to create the fused image are denoted along the images respectively (Fig. 1).

The entropy values of all image sets are shown in table 1.

Comparing the calculated entropy values from the table, we found that all fused images have bigger entropy values than their corresponding best focal-plane images ($P < 0.05$ by Mann-Whitney test), i.e., the fused images have more information than the best focal-plane images. Furthermore, we found that within the thickness ranges, the greater the number of layers fused provided proportionately more information from the fused images.

Image assessment using Pathological Diagnoses Exams

Fig. 2 shows both the best focal-plane images (BFI) and fused image (FuI) of a Papanicolaou stained slide, which was obtained from a 70-year old woman with a right mammary tumor. The top-left sub-figure (a) shows a focused image thumbnail of a cluster of epithelial cells; the top-right sub-figure (b) is the ROI (region of interest) image of (a) grabbed in optical magnification of 40x. However, although it is the best focal-plane image, due to the thickness of the cells, the image still looks blurred. In contrast, the bottom-left sub-figure (c) is the fused image thumbnail with identical area to proceeding sub-figure (a), and in perception, sub-figure (c) has much more clarity than sub-figure (a). Similarly, the bottom-right sub-figure (d) illustrates the 40x optically magnified original image of (c), and unlike the sub-figure (b), this image is acquired by fusing up to 50 focal-plane images together, in which a "bipolar" myoepithelial cell (↓) is clearly outlined.

The same patient's Histological tissue section (Hematoxylin & Eosin stained) images (Fig. 3) are observed, the left-handed image (a), acquired in magnification of 10x, sees poorly differentiated carcinoma growing as solid areas devoid of gland formation; the right-handed image (b), grabbed in magnification of 40x, reveals scattered myoepithelial cells among the carcinoma cells (↑) and a scattering of myoepithelial cells fringing the carcinoma cells (▲).

Discussion

A very critical goal achieved by an automated image fusion algorithm in this study was to evaluate the effectiveness and performance of the fused images applied for pathological diagnosis. It has been proven to be reliable that a focus fusion algorithm is a practical and informative approach for cytopathology slide digitalizing and diagnosis period. By quantifying the entropy, and comparing fused image with its best focal-plane image, the fused image, which has more information content, shows greater advantages than corresponded the best focal-plane image.

Although image fusion approaches and their applications have been widely investigated, few studies have been conducted for digital pathology application to date[7~12]. The theory of image fusion has advanced rapidly in the past several years. Image fusion approaches ranging from extreme simplicity to considerable complexity have been proposed, which can be

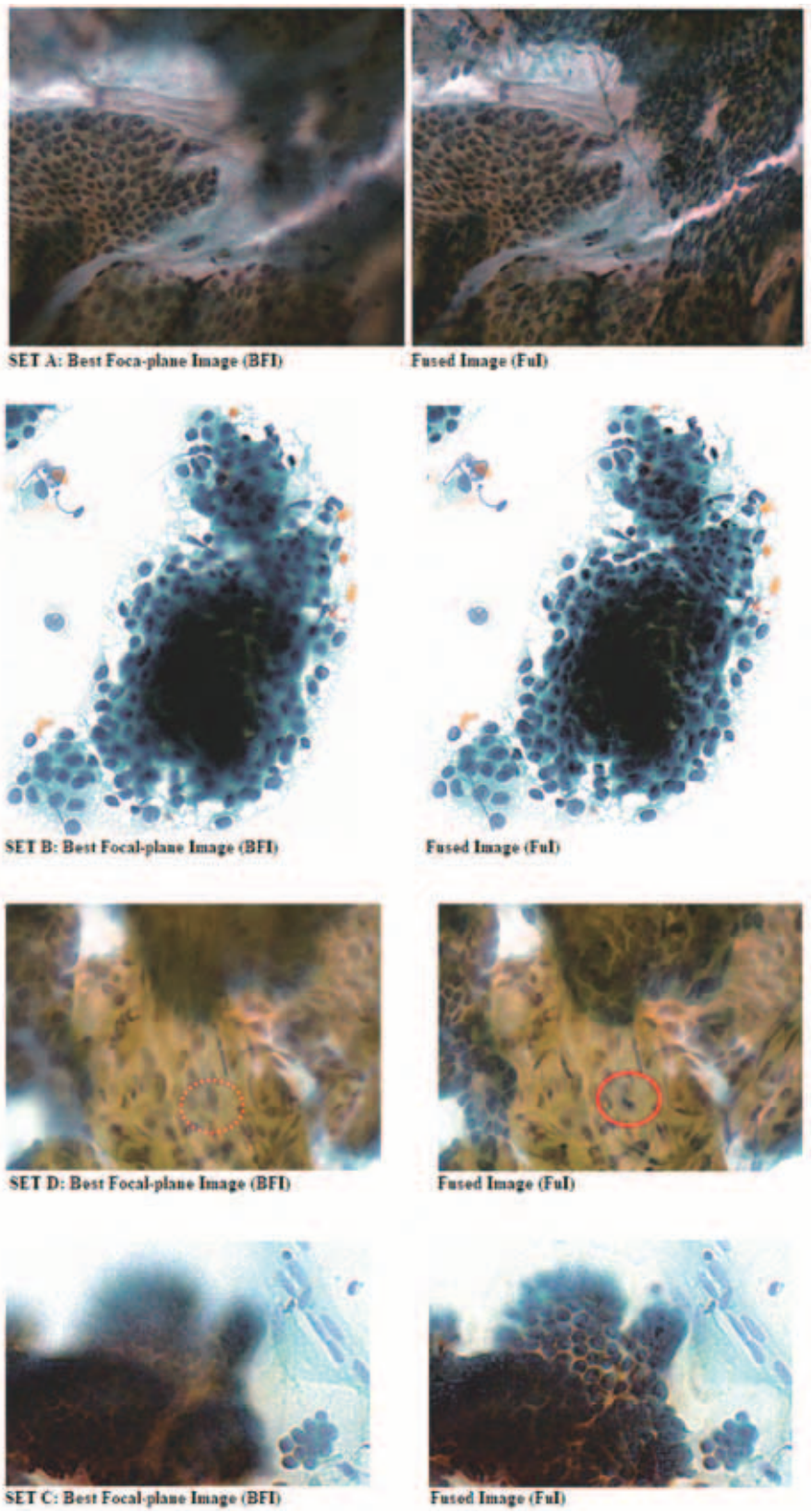

Fig. 1 Comparisons of information between the best focal-plane Images (BFI) and Fused Images (FuI) among SET A, SET B, SET C, and SET D. BFI: optical magnification 40x, single focal-plane; FuI: optical magnification 40x, multiple focal-planes.

Table 1 Comparison of entropy values of the best single focal-plane image (BFI) with an entropy values of Fused Images (FuI)

Image Set	BFI (bit/pixel)	FuI (bit/pixel)
Set A	6.8645	7.7082
Set B	6.5732	7.0411
Set C	6.8487	7.3806
Set D	6.8543	7.2089

$P < 0.05$ compared to entropy values by Mann-Whitney test

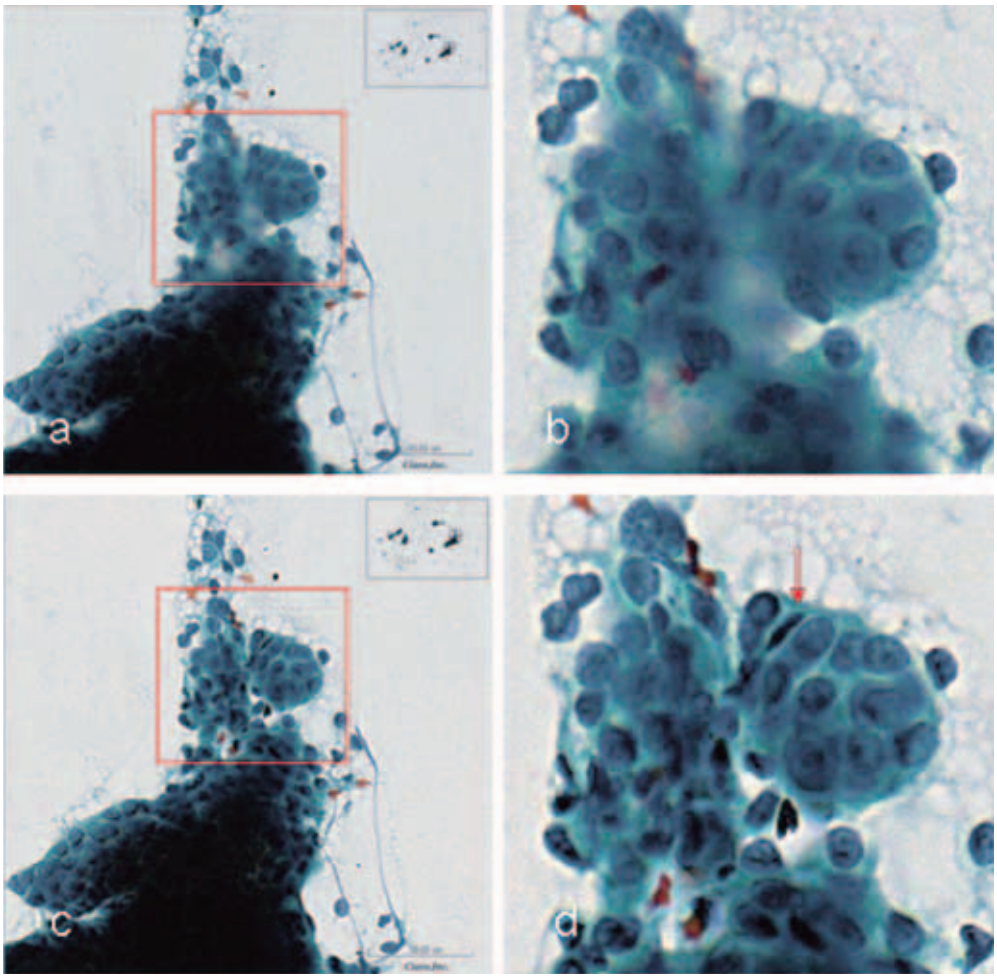

Fig. 2 Comparison of Pathological features between Best Focal-plane Image (BFI) and Fused Image: (FuI), Papanicolaou stained slide of a 74-year-old woman with a right mammary tumor, (a) thumbnail of the Best Focal-plane Image, (b) original best focal-plane image: optical magnification 40x, single focal-plane, (c) thumbnail of Fused Image, (d) original Fused image: optical magnification 40x, 50 focal-planes, distance between each focal-plane 50μm.

broadly classified into two areas: spatial domain fusion and transform domain fusion. The fusion methods such as averaging, Brovey method, principal component analysis (PCA) and IHS based methods fall under spatial domain approaches.

Another important spatial domain fusion method is the high pass filtering based technique.

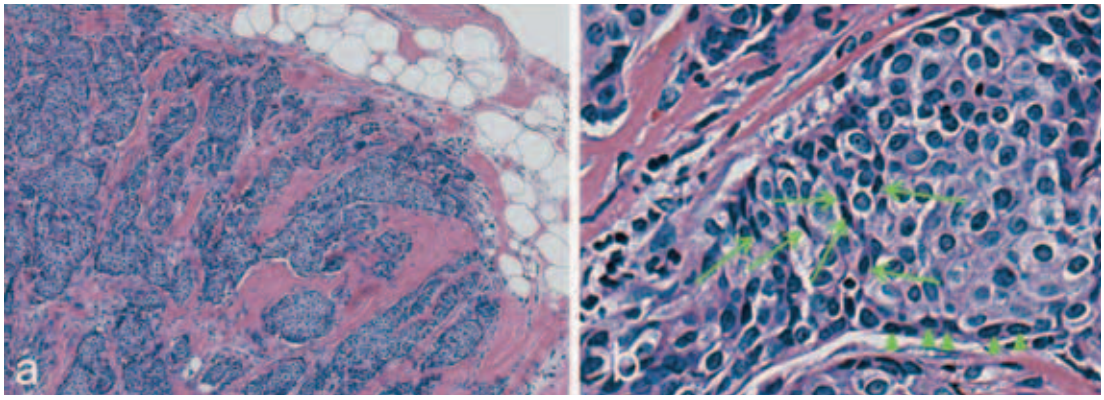

Fig. 3 Images of HE stained tissue section slide: Myoepithelial cells among solid tubular carcinoma cells: (a) Low magnification image shows poorly differentiated carcinoma growing as solid areas devoid of gland formation (10x, HE staining); (b) Partially, High resolution image shows scattered myoepithels among the carcinoma cells (↑) and a scattering of myoepithelial cells fringed the carcinoma cells(▲). (40x, HE staining)

The disadvantage of spatial domain approaches is that they produce spatial distortion in the fused image. The discrete wavelet transform has become a very useful tool for fusion. Some other fusion methods are also available, such as Lapalcian pyramid based, curvelet transform based etc. These methods show a better performance in spatial and spectral quality of the fused image compared to other spatial methods of fusion. Recently, a generic framework covering a large set of these approaches has been given by Zhang and Blum[13].

Since it is known that image fusion algorithm performance is application dependent, we focus on fusion algorithms especially for cytology imaging application. Taking the characteristics of cytological images into account, we have proposed a new image fusion algorithm particularly for Cytological imaging application. Although image-based focusing algorithms[14~18] and image registration algorithms[19~25] are usually required before fusing, we only address the image fusion algorithm in this paper.

Based on all experimental data and results, the following conclusions can be drawn:

- The fused image created by the proposed algorithm is more informative and of better quality than either the best focal plane image or any other single focal plane image, which can lead to a more reliable exam result.
- For cytopathology diagnosis, a lot of time can be saved to just review a single fused image rather than dozens of layered images
- Furthermore, many new features and evidence can be discovered in the fused image for cytopathology diagnosis. Those features so far rarely show up or are very weak in the best focal plane image or multi-layered images, and are barely visible in the microscopy image.

We have validated this new focus fusion approach for cytopathology diagnosis in our study and found the new approach could be one of the best methods to resolve pathologist's concerns about image quality. Furthermore, the application of new focus fusion algorithm in cytopathology image may allow us to make full advantage of entering 3D cell information for exams and diagnosis.

Considering the thickness of cytopathology slides, we have verified this novel approach using cytopathology slides. Our future research will validate the same technique in histopathology, immunohistochemistry (IHC), immunofluorescence, and fluorescence in situ

hybridization (FISH) in sequence.We expect this approach will significantly accelerate the speed of application and expanding of digital pathology where the image quality and image size are critical. Finally, we think this method lays a solid foundation for automatic pathology diagnosis.

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