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Image Processing in the Optical Microscopy: Photometry Correction-Decoding-Mosaicking

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Abstract: This article presents an application of the digital imaging in the reading of a slide in optical microscopy, by applying a photometric correction of images issued out of the microscope, an interpolation of images captured by a CDD sensor (decoding) and the implementation of a mosaicking in order to obtain a virtual slide of big size. This study is particularly decisive in a context of telediagnosis applications in optical microscopy, using this type of sensor and manipulating images of large dimensions. We also propose some solutions which improve the efficiency of the telediagnosis chain and the quality of the visualized images.

Key words: Image processing, optical microscopy, interpolation, decoding, mosaicking, virtual microscopy, telediagnosis

INTRODUCTION

The observation of the large field image, obtained by the mosaicking process which is used in the telediagnosis chain, shows a grid effect visible at the levels of join borders of the microscope issued images (Fig. 1 and 2). This effect is due to the inhomogeneous illumination of the slide scene.

An histogram stretching of the image allows to show this default.

This technique consists on applying a linear transformation on the original image (Fig. 3). This transformation is called «« histogram stretching »» (Fig. 4 and Fig .6).

Let g_{min} and g_{max} be the minimum and the maximum grey levels of the image pixels.

The stretching consists on the application of a linear function f to the image grey levels, such that:

$$f(g_{min}) = 0 \text{ and } f(g_{max}) = 255.$$

We have then:

$$f(x) = 255\,(x - g_{min})/(g_{max} - g_{min})$$

The illumination default, well visible now (Fig. 5), introduces the grid effect on the final mosaicked image.

PHOTOMETRY CORRECTION

To correct this default, we propose

- The application of an approximation of the illumination function on each image, each image to mosaick will be divided by this function (Fig. 7 and Fig. 8).
- The point by point correction. (Divide each image on its shading image).

Nevertheless, the scene black marks which represent the dust on the camera objective are complex to model analytically. Then we have chosen to make a point per point correction:

```
for (i = 0 ; i < 3 ; i++)
for (j = 0 ;  j< Image.height ; j++)
for (k = 0 ; k < Image.width ; k++)
{
      if (Shad.pixel[i][j][k] = = 0) Shad.pixel[i][j][k] = 1 ;
        Out.pixel[i][j][k] = (Average[i]*(Image.pixel[i][j][k])
          /Shad.pixel[i][j][k]) >>8 ;
}
```

The shading correction can be applied on the camera issued images, this reduces the execution time of three times (division over one channel instead of three channels for colour images).

Then, a decoding will be applied on the correction results in order to obtain the colour images.

INTERPOLATION AND DECODING

The microscope is equipped with a CCD sensor based camera. To acquire a coloured image, these sensors use a network of filters, introduced by BAYER in 1976[1].

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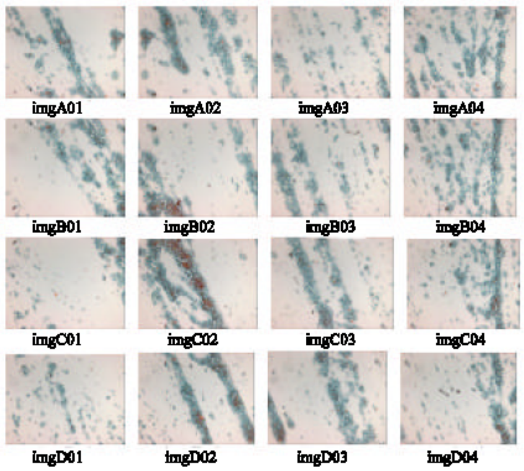


Fig. 1: The set of images to mosaick

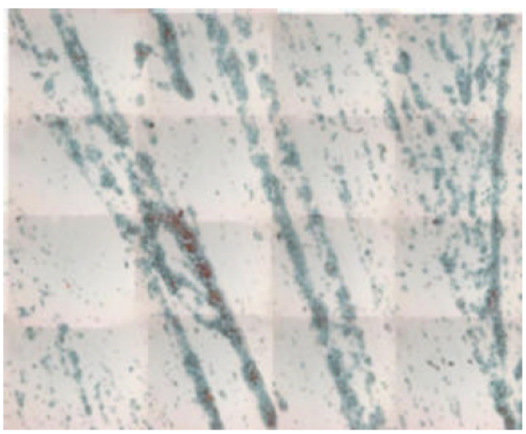


Fig. 2: Result of the mosaicking before the photometry correction

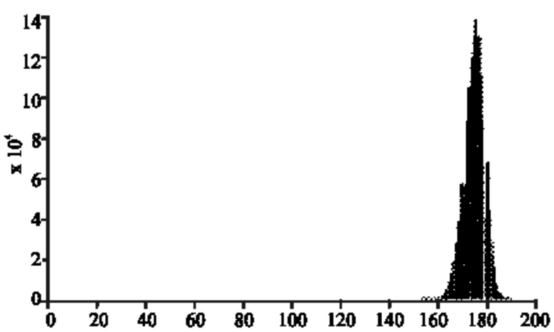


Fig. 3: Initial image of the 'shading' empty scene

The pixels partition of the camera issued image is given at Fig. 9.

This format is called 'BAYER' (Fig. 10).

As we can see, the green channel is sampled on the half of the total number of the image pixels, but every one

Fig. 4: Histogram of the original image of the shading

Fig. 5: Image of shading after histogram stretching

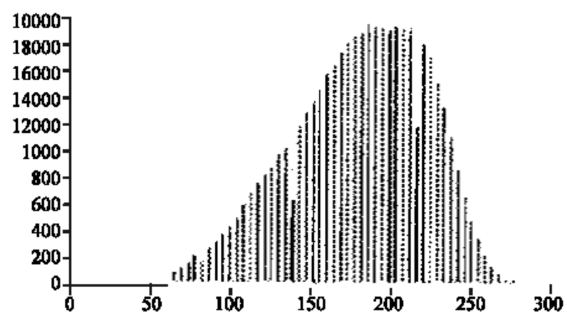


Fig. 6: Image histogram after histogram stretching

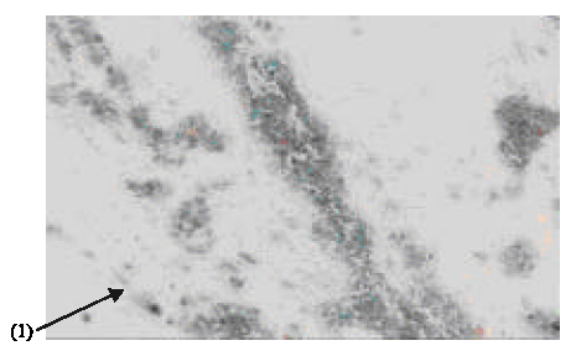


Fig. 7: Original image to mosaick (1) A redness appears at the image borders due to non homogenous illumination of the slide scene

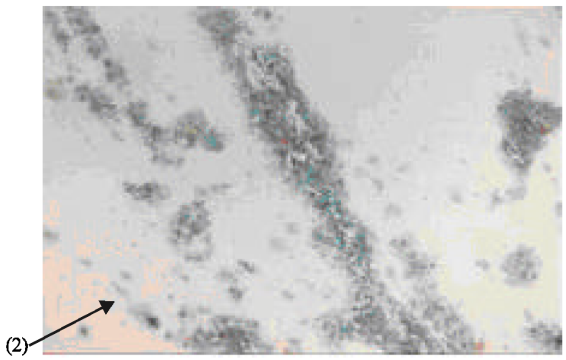


Fig. 8: Image after shading correction (2) Disappearance of the redness after photometry correction

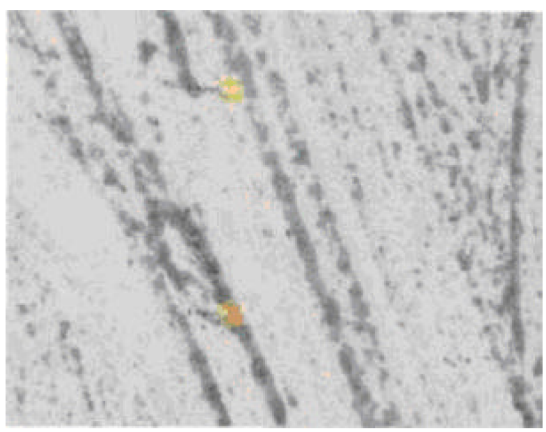


Fig. 9: Image resulting from the mosaicking after shading correction

G	B	G	B	G	B	G	B
R	G	R	G	R	G	R	G
G	B	G	B	G	B	G	B
R	G	R	G	R	G	R	G
G	B	G	B	G	B	G	B
R	G	R	G	R	G	R	G
G	B	G	B	G	B	G	B
R	G	R	G	R	G	R	G
G	B	G	B	G	B	G	B
R	G	R	G	R	G	R	G

Fig. 10: The pixels partition in 'BAYER' format.

G	B_1	G
R_1	G	R_2
G	B_2	G

$$R = (R_1+R_2) / 2$$
$$B = (B_1+B_2) / 2$$

G_1	R_1	G_2
B_1	G	B_2
G_3	R_2	G_4

Fig. 11: Interpolation of the two missing colours when we are in green pixel

B_1	G_1	B_2
G_4	R	G_2
B_3	G_3	B_4

$$R = (R_1+R_2+R_3+R_4) / 4$$
$$B = (B_1+B_2+B_3+B_4) / 4$$

R_1	G_1	R_2
G_4	B	G_2
R_3	G_3	R_4

Fig. 12: Interpolation of the two colours R and B

of the blue and the red channels occupies the quarter of the number of pixels. We denote this format by Y8.

In order to go from 'BAYER' format to 'RGB' format, the camera makes an interpolation to retrieve the two missing colours in every image pixel (Fig. 13 and Fig. 15).

This problem is the object of several recent publication[2,3].

To improve the efficiency of the telediagnosis chain, we will use the adjusted interpolation method of correlation of the linear interpolation version proposed in[4], which is fast and easy to implement (Fig. 14 and Fig. 16).

The two colours R and B are linearly interpolated with the nearest neighbours of the same colour.

There are four possible cases:

- When we are in a green pixel 'G' (Fig. 11), two cases are discussed:

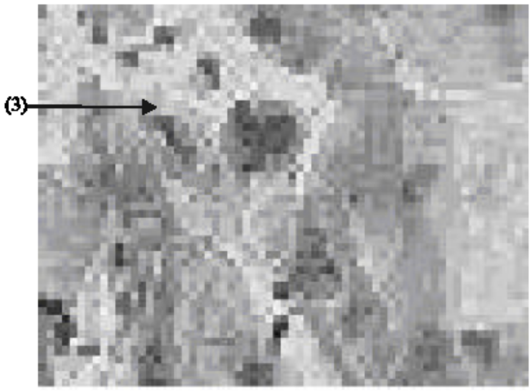


Fig. 13: Blue component interpolated by the camera software (zoom 2X) (3) Crystallization effect

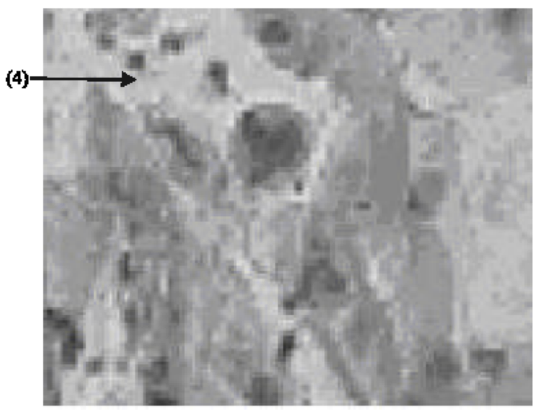


Fig. 14: Blue component interpolated by the developed software (zoom 2X) (4) Correction of the crystallization default

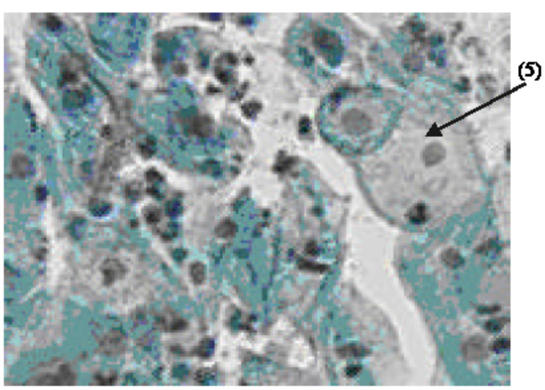


Fig. 15: Y8 image interpolated by the camera software (zoom 2X) (5) Presence of redness

- Interpolate the two missing colours 'R' and 'B',
- Take the average of the two nearest neighbours of the same colour.

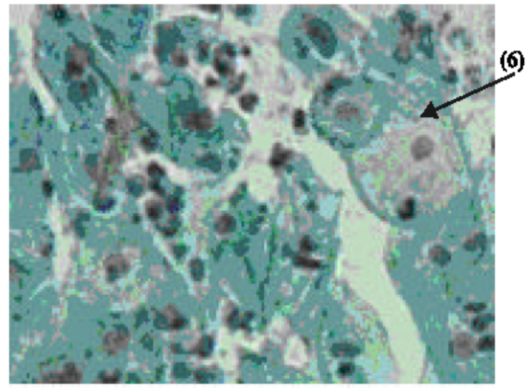


Fig. 16: Y8 image interpolated by the developed software (zoom 2X) (6) disappearance of the redness

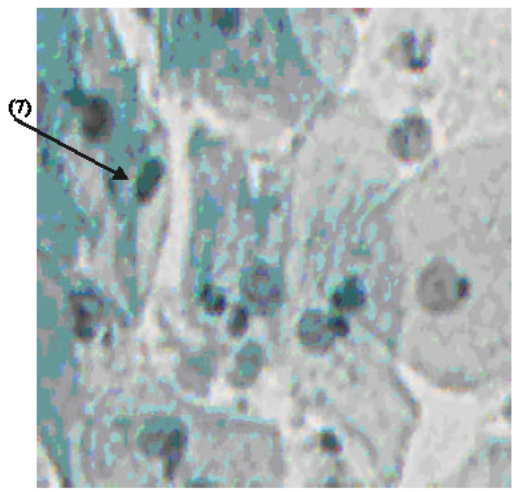


Fig. 17: Y8 image interpolated by the camera software (zoom 3X) (7) Presence of crystallization

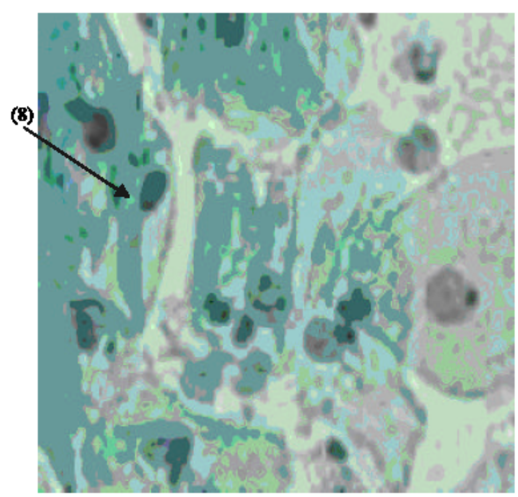


Fig. 18: Y8 image interpolated by the developed software (zoom 3X) (8) Disappearance of the crystallization

- When we are in an 'R' or 'B' pixel Fig. 12), in these two cases we take the average of the four nearest neighbours around the 'R' or 'B' pixel.

IMAGES MOSAICKING

The images obtained by microscopy are naturally very local. It is though necessary to replace them in a more global context, which is generally done using a diminution of the magnification.

The proposed project suggests to develop an alternative method which consists, on the contrary, to take several shifted images at a unique resolution. In order to obtain one image, you have to align all these images the most finely possible. Of course, this alignment has to be done automatically.

Determination of the joins borders: After the photometric correction, the elementary images are readjusted:

For two images we look for the superposition and the correspondence of each pixel of the recovering zone.

To obtain an image of large field, the fact to piece elementary images together creates artifacts due to the apparition of an artificial border at the level of the joins. That's why we are looking for an optimal join border, following certain criteria:

- The join border must follow the common edges of the two images, so that the possible visible transition already exists and therefore be natural.
- We must not create new contours in the mosaic.
- The residual geometric disparity must be minimal.

The correlation method is used to put in correspondence pixels in a recovering zone. The idea is to define a similarity measurement between the pixels of two images[5].

Looking for the correspondence between two images f and g $\rightarrow$ Looking for max of $|C_{fg}|$, where $|C_{fg}|$ is the correlation coefficient.

We obtain, then, a sequence of correlation values and the pixel corresponding to the best score will be chosen as matching the pixel of the centre of the fixed window in the other image.

The correspondence per correlation is a very efficient method in the case where the couple of images presents regions allowing to ease the matching[6].

Mosaicking of two images: The microscope issued images whose recovering zone is 85% wide, meaning a recovering zone of 15% (Fig. 19 and Fig. 20).

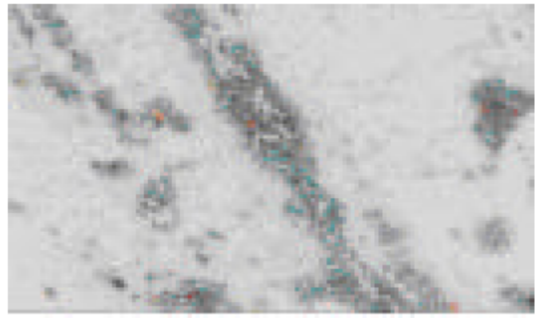


Fig. 19 : Image 01

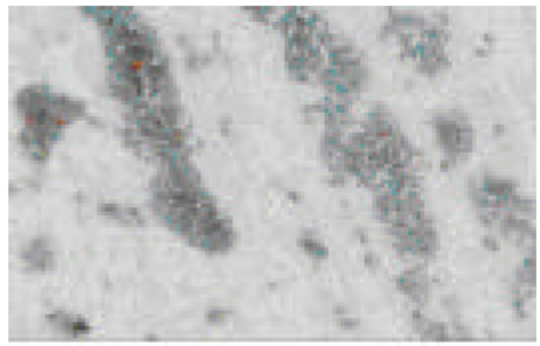


Fig. 20: Image 02

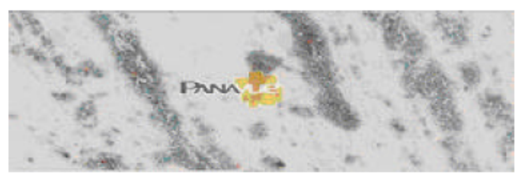


Fig. 21: Mosaicking result of the two images 'Fig. 17' and 'Fig. 18' by the 'Panavue' software

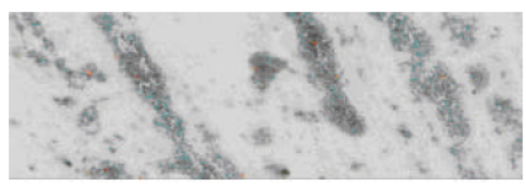
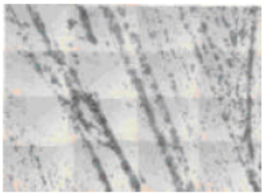


Fig. 22: Mosaicking result of the two images 'Fig. 17' and Fig. 18' by the developed software

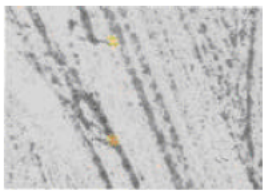
Making the mosaicking using the mosaicking package Panavue[7] (Fig. 21), then using the developed program, (Fig. 22), with a binary weighing of (0,1) and (1,0) of the photometry in the recovering zone and finally a progressive weighing from [0 to 1] always in the recovering zone, we note that the mosaicking time of two images by 'PANAVUE' takes 40s whereas the mosaicking by the developed program is about only 12s.

RESULTS AND DISCUSSION

We remind that the photometry correction is very efficient when using the 'shading' images of each elementary image of the slide.

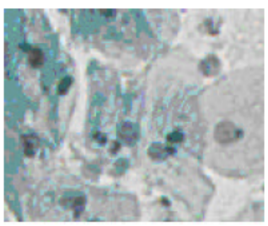


Before photometry correction

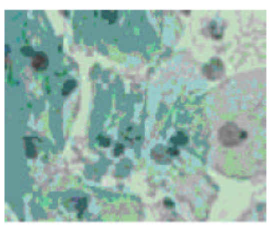


After photometry correction

The decoding at the output of the camera allows to improve the efficiency of the telediagnosis chain and to correct the crystallization defaults.



Camera interpolation



Interpolation by linear correlation

The implemented mosaicking allows us to optimize the computation time and to improve then the efficiency of the telediagnosis chain.

CONCLUSION

In this article, we have solved the problem of the grid which appears in the mosaicked images. We have discussed the different processings of the camera issued image before and after interpolation. The 'Bayer' image processing is very competitive, allowing to reduce the computation time. The shading correction can be applied on the camera issued images; this speeds up the execution time three times, (division over one channel instead of three channels in the coloured images). After that, a decoding will be applied on the correction results to obtain the coloured images. The mosaicking allows the reading of a big size and strong magnification slide in network, in the frame of a diagnosis protocol which develops, among others, a remote consultation system of a big size virtual slide for the medical diagnosis in haematology.

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