A New Approach for Autofocusing in Microscopy

Elena Tsomko*, Hyoung Joong Kim* and Hyoung Seok Han

Abstract : In order to estimate cell images, high-performance electron microscopes are used nowadays. In this paper, we propose a new simple, fast and efficient method for real-time automatic focusing in electron microscopes. The proposed algorithm is based on the prediction-error variance, and demonstrates its feasibility by using extensive experiments. This method is fast, easy to implement, accurate, and not demanding on computation time.

Keywords: autofocusing, microscopy, prediction-error variance

I. Introduction

Autofocusing is one of the fundamental techniques in modern microscopy. Quality of cell images is very important for accurate study and diagnosis. However, adjusting microscope lenses manually is not so much sufficient for getting the best focus. Thus, in order to get cell image in the best focus automatically we need some measurements that would be computed precisely and very fast.

Various microscope focusing methods have been proposed before (e.g. [1-5], [7]). Evaluations of some of the autofocusing algorithms are given by [2], [6] and [8]. From these evaluations one can see that mostly calculations are computation-intensive and sometimes may be time-consuming. Some of the methods are not immune to noise in the original images.

In this paper, we propose a new method for autofocusing in microscopy. This method does not require computation-intensive operations, is very fast and accurate. Furthermore, it is immune to any kind of noise in the images (e.g. white, black, or both of them) and need not any transformations such as DCT or DWT.

II. Theoretical Background

Below, we describe the theoretical part of our method, i.e. we define the prediction-error variance value. The examples are given based on standard test images like Baboon, Lena, etc. However, the same features are peculiar to microscope images as well.

In general, images are highly correlated. When there is significant correlation between successive samples, it should be possible to predict the value of any given sample with a reasonably high degree of accuracy from some of the preceding samples.

The simplest predictor for an image is the one that uses the previous pixel, $u(x,y-1)$, in the image as the predicted value, (x,y), of the current pixel, $u(x,y)$. In this case, the prediction residue, $g(x,y)$ is nothing but the difference between the adjacent pixels. Hence, $g(x,y) = u(x,y) - u(x,y-1)$.

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Figure 1 shows the original Baboon image and its motion and Gaussian blurred counterparts, respectively. And Figure 2 shows the histogram of the differences between adjacent pixels for the original Baboon image (solid line) and, for example, its Gaussian blurred version (dashed line).

Fig. 1 Example of the original Baboon image (left), and its Gaussian (center) and motion blurred (right) versions, respectively

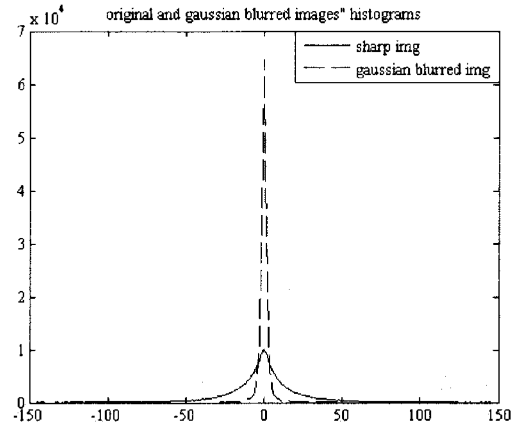

Fig. 2 Distribution of prediction residues of the original Baboon image (solid line) and its Gaussian blurred image (dashed line)

The standard deviations of these pictures are 184.4 and 1.60, respectively (see Table 2.1). In order to compute these values we took all the difference values of the neighboring pixels. However, to decrease the computational intensity for the proposed method only 300 samples were taken randomly, and experiments show that we can also rely on these variables' values as well (compare Tables 1 and 2). The variance values are computed by Equation 1 and Equation 2 that are given as follows:

$$\sigma_p^2 = \frac{1}{M(N-1)}\sum_{x=1}^{M}\sum_{y=1}^{N-1}[g(x,y)-\bar{g}]^2, \quad (1)$$

$$S_p^2 = \frac{1}{P-1}\sum_{k=1}^{P}[v(k)-\bar{v}]^2 , \quad (2)$$

where $\bar{g}$ represents the mean of $g(x,y)$'s.

The sample variance value in Equation (2) is computed using P sample values. We randomly select P difference values from the two-dimensional array $g(x,y)$ and allocate them to the one-dimensional array, $v(k), k = 1, \dots, P$.

Table 1. Variances of a set of standard test images: sharp ones and their blurred counterparts (for 300 random samples)

Images	S_p^2 of sharp image	S_p^2 of blurred image
Bicycle	634.34	3.51
Fish	152.86	2.28
Baboon	158.06	1.55
Mandrill	42.28	0.84
Birds	31.64	0.80
Lena	71.66	1.47

Table 2. Variances of a set of standard test images: sharp ones and their blurred counterparts (for all the difference values)

Images	S_p^2 of sharp image	S_p^2 of blurred image
Bicycle	587.7	3.49
Fish	155.6	2.48
Baboon	184.4	1.60
Mandrill	37.8	0.85
Birds	28.0	0.89
Lena	49.3	1.40

III. Algorithm

In order to automatically adjust the proper focus we suggest using prediction error variance of the images. While focusing, the variance value is computed for cell image in each step. Furthermore, since the tissue image's structure looks blurry itself, we add some noise to the captured image in order to increase its contrast. After several iterations, i.e. capturing the image from the smallest focus to the largest one, we compute the maximum variance value of the images. That one which has the largest one is focused best. The block-scheme of the proposed algorithm is given in Figure 3.

Note that no thresholds and transformations are used here. It makes the method faster and does not affect on its accuracy.

IV. Experiments

For our experiments we used various images downloaded from different sources [9-11]. They are the images of different cells, e.g. bacteria, fungus, micro-organisms, etc., and small insects (Fig. 4).

As one can see, mostly the images themselves look a little blurry. And if we take the variance values of the original image and the blurred versions of it, the difference will be not so significant. Thus, before starting experiments with autofocusing, we added some "salt and pepper" noise to them.

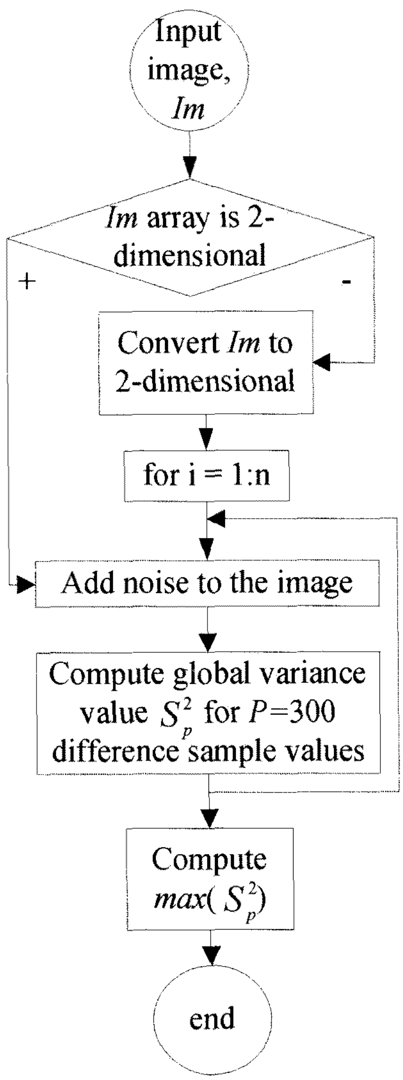

Fig. 3 Block-scheme of the algorithm for the proposed method

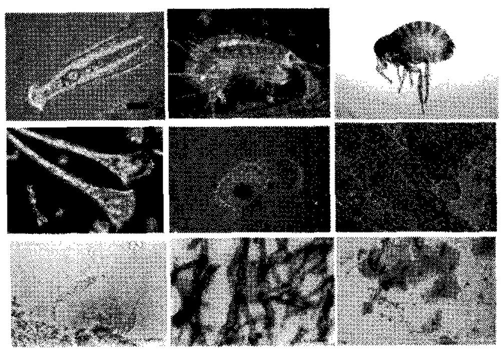

Fig. 4 Examples of the microscope images used in our experiments.

We implemented our experiments using Matlab. In order to simulate focusing process of microscope, we added Gaussian blurriness to the image more and more with each "focusing" step:

```
for i = 1:5 % make five steps for blurring the image
    hsize = 2*i+1;
    sigma = i*i+6;
    PSF = fspecial('gaussian',hsize,sigma);
    ...
end
```

In order to compute variance values of the captured images, we used neighbor pixels taken in different directions, i.e. horizontal, vertical and maximum magnitude value between the original pixel and its right and lower neighbors. Figure 5 shows that the variance of the original well-focused image (e.g. the first image in Fig.4) has the largest value, no matter in which direction the neighbor pixels had been taken.

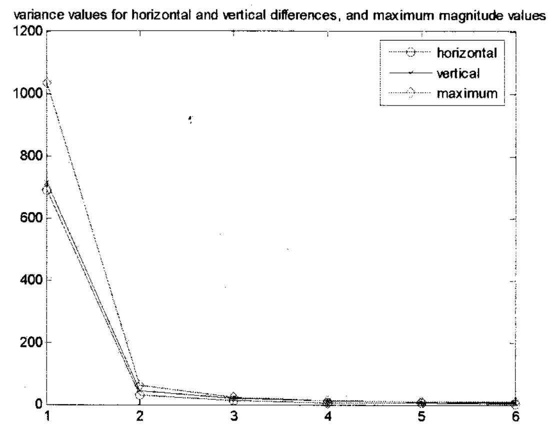

Fig. 5 Variance values for the first image of Fig.4

X-axis in Fig.5 are the steps of adding the blurriness. For the first step we just took original image, and for each of the next iterations we added more blurriness.

Totally in our experiments we used 250 microscope images and for all the images well focused original ones had the largest variance values.

V. Discussions and Conclusion

The proposed method for autofocusing in microscopy is fast, simple and efficient. Only P random sample pairs are used for computing the measure among $M \times (N-1)$ sample population. No thresholds and no transforms are required. Adjusting the focus can be done automatically based on the prediction-error variance value.

This work is started with simulating the focusing in Matlab. Further we plan to extend our work with real out-of-focused images taken by microscope, and also compare our method with other proposed ones. More images will be taken with different illuminations, angles of light, reflections, in dark fields and fluorescence mode and studied with performance results.

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