Diatom autofocusing in brightfield microscopy: a comparative study

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Abstract

We present a number of autofocusing methods in lighting microscopy for its use in diatom identification. Among these, the Tenengrad method has been considered one of the best. The basic requirements for a practical autofocusing system are speed, sharpness and robustness to noise. Recently other focus measures based on a modified Laplacian method are said to perform better than Tenengrad. We investigate two sound methods based on a modified Tenengrad and a modified Laplacian. Measurements show that they provide a reliable and suitable focus measure that outperform similar methods. We investigate the window size analysis dependency and perform an univariate analysis on the focus measures. The focusing techniques are implemented in an automatic slide scanning system for diatom detection and identification for its use in the ADIAC project. [1]

1. Introduction

Diatoms are unicelular algae related with brown algae that grow anywhere where there exists enough light and moisture. They are ecologically very important because they contribute around the 20% of the world's carbon fixation [3]. Besides the ecological interest, there exists a number of other fields where diatom analysis is useful: geological, climatological, geographical,archeological or forensic research. In many applications diatom analysis require both the presence of experts and the identification of a large range of diatom species and therefore it is a tedious and time consuming task. However, most of the existent computer-based diatom analysis methods do not tackle the whole automatization process [2, 7]. One key aspect in the automatization process is to determine reliable and fast autofocusing methods. Groen et al. has identified eight different criteria for comparing autofocus algorithms [4]. Many focusing techniques have been proposed in the literature [5, 9, 10, 6, 1]. Most of them extract a focus measure that gives a maximum for the best focused image. Defocus algorithms can be classified into two categories: those based on the statistical variance of pixel values and those based on spatial-frequency content of the image. In this paper, we propose two new algorithms based on the computation of the variance of the image gradient or image Laplacian that outperform existing methods according with some novel feature focus metrics.

2. Materials and dataset

Different diatom samples from fresh water and human tissue [2] were analyzed with a Zeiss Axiophot photomicroscope illuminated with a 100W halogen light with 40X lenses. For image acquisition, we used a Scion frame grabber that includes the NIH image processing shareware connected to a CCD analog camera from Pulnix. Two PC image analysis systems (Pentium II &III) were used one for image acquisition and the other one for algorithm computation. Furthermore, for computer intensive calculations a SUN Enterprise 450 with four processors was used. Images have been digitized with 8 bit/pixel and 256x256 pixel image format. The microscope slide was moved with a X-Y-Z motorized stage from Prior Instruments, with a step size of 0.1 μm for the X-Y axis and 1 μm for the Z-axis.

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3. Methods

Several methods have been proposed in the literature to solve the problem of autofocus [4, 5, 9, 10, 1]. These methods define a focus function which measure the relative sharpness of images at different object distances. The distance at which the function returns the largest value will be the object distance at which the image is best focused.

In the next sections we describe a number of diferent focus functions studied in this paper. Let $I(m,n)$ be the image intensity function of size $N \times M$ and let us assume a stack of k images taken by changing microscopy focus in steps of $1\mu m$. Figure 1 shows some examples of the image stack above an below the best focused image located at the center of the panel.

3.1. Grey level local variance methods

A well focused image is expected to have a high variation in grey levels. The local variance at point (m,n), with $m = 1, ..., M$ and $n = 1, ..., N$, is given by

$$lv(m,n) = \frac{1}{w_x w_y} \sum_{i}^{w_x} \sum_{j}^{w_y} \left[I(m+i, n+j) - \overline{I}\right]^2 \quad (1)$$

where $\overline{I}$ is the mean grey level value

$$\overline{I} = \frac{1}{w_x w_y} \sum_{i}^{w_x} \sum_{j}^{w_y} I(m+i, n+j) \quad (2)$$

and $w_x \times w_y$ is the size of a window centered on the point (m,n)

The focus measure based on the local variance will be given by the global variance

$$VAR(I) = \frac{1}{NM} \sum_{m}^{M} \sum_{n}^{N} \left[lv(m,n) - \overline{lv}\right]^2 \quad (3)$$

where $\overline{lv}$ is given by

$$\overline{lv} = \frac{1}{NM} \sum_{m}^{M} \sum_{n}^{N} lv(m,n) \quad (4)$$

3.2. Gradient magnitude based methods

A well focused image is expected to have sharper edges, so the use of image gradients are instrumental in order to determinate a reliable focus measure. Given an image gradient, the focus measure have to pool the data at each point as an unique value. Tenenbaum and Schalag et al. investigated a method, called Tenengrad, that is considered as a benchmark in this field [5]. This method estimate the gradient magnitude at each image point and sum all the magnitudes greater than a threshold T. The Sobel operator is used with the convolution masks

$$S_x = \begin{pmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{pmatrix} \qquad S_y = \begin{pmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{pmatrix} \quad (5)$$

and the gradient magnitude is calculated as

$$S(m,n) = \sqrt{[G_x(m,n)]^2 + [G_y(m,n)]^2} \quad (6)$$

where $G_x(m,n)$ and $G_y(m,n)$ are, respectively, the convolution of the input image $I(m,n)$ with the masks S_x and S_y.

The Tenengrad focus measure is given by

$$TEN(I) = \sum_{m}^{M} \sum_{n}^{N} [S(m,n)]^2 \quad for\ S(m,n) > T \quad (7)$$

Another alternative for pooling the gradient information is to calculate the variance of gradient magnitudes. Based on this idea, a new focus measure will be given by

$$SOB\_VAR(I) = \sum_{m}^{M} \sum_{n}^{N} \left[S(m,n) - \overline{S}\right]^2 \quad for\ S(m,n) > T \quad (8)$$

where $\overline{S}$ is the mean of magnitudes given by

$$\overline{S} = \frac{1}{NM} \sum_{m}^{M} \sum_{n}^{N} S(m,n) \quad (9)$$

3.3. Second derivative based methods

The use of a second derivative operator is one technique for passing the high spatial frecuencies, which are associated with sharp edges. As a second derivative operator we use the Laplacian operator, that can be approximate using the mask

$$L = \frac{1}{6} \begin{pmatrix} 0 & -1 & 0 \\ -1 & 4 & -1 \\ 0 & -1 & 0 \end{pmatrix} \quad (10)$$

For pooling the data at each point, we use two methods. The first one is the sum of all the absolute values, driving to the following focus measure

$$LAP(I) = \sum_{m}^{M} \sum_{n}^{N} |L(m,n)| \quad (11)$$

where $L(m,n)$ is the convolution of the input image $I(m,n)$ with the mask L.

The second method calculates the variance of the absolute values, providing a new focus measure given by

$$LAP\_VAR(I) = \sum_{m}^{M}\sum_{n}^{N}\left[|L(m,n)| - \overline{L}\right]^2 \qquad (12)$$

where $\overline{L}$ is the mean of absolute values given by

$$\overline{L} = \frac{1}{NM}\sum_{m}^{M}\sum_{n}^{N}|L(m,n)| \qquad (13)$$

4. Results

Here we propose a variant of this method based on use the variance of the magnitude of a Sobel gradient. The rationale of this approach is to define a more discriminative measure similar to a second derivative (Laplacian) but increasing the robustness to noise. Fig. 1-2 show a comparison of focus measurements without and with noise respectively [3].

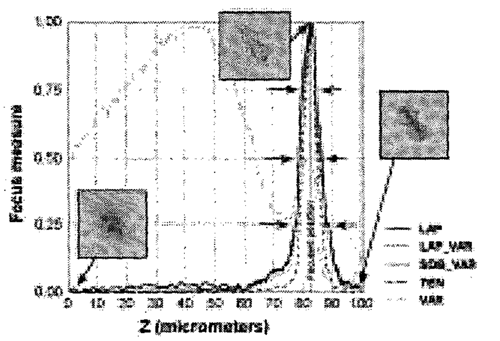

Figure 1. Comparative focus measurements

4.1. Window size selection

Another criteria to take into account is the window size selection for variance and gradient calculation. Fig. 3a shows as a box plot that the variance method provides a better focus estimate for small windows, but with the tradeoff of a higher computational cost than for large windows. However, Fig. 3b shows that the Sobel variance provides better results for large windows that leads to a reduction in the computational complexity. Next, we analyze the shape of the focus measures.

[3] For a subjective focus and image fusion assessment see: http://www.iv.optica.csic.es/projects/autof.html

4.2. Focus assessment

- Sharpness. Sharpness is not the only criteria for selecting a good focus measure. In fact, as Subbarao et al [9] has pointed out any focus measure can be artificially sharpened by simply squaring the focus measure. Kurtosis is a candidate for peakedness. However it suffers of a high noise sensitivity. We define an progresive sharpness measure based on the absolute sum of differences of three equidistant focus levels from the maximum value:

$$F_1 = k_1\sum_{i\neq j}|d_i - d_j| \qquad (14)$$

where d_i and d_j represent the level intersections in the central lobe of the focus measure (represented by the horizontal arrows in Figs. 1&2) and k_1 is a normalized constant. Fig. 4 shows that both Sobel+variance and Tenengrad methods provide the sharper focus response (i.e. a smaller F_1 value).

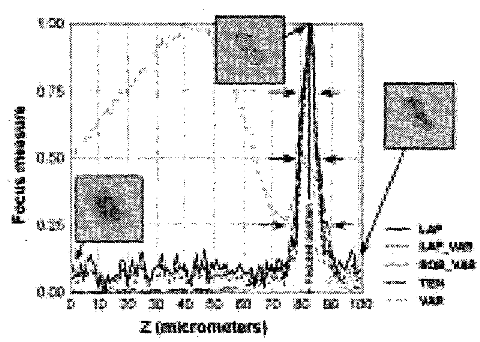

Figure 2. Same as Fig.1 but for noisy data

- Smoothness and noise sensitivity

We define a relative measure based on an accumulative sum of the absolute gradient value of the focus measure (Fig. 2) with respect to a fitting Gaussian with ($\sigma = 1$).

$$F_2 = \frac{\sum_k |AF'_k|}{\sum_k |AF_k|_G} \qquad (15)$$

where AF'_k represents the first derivative of the focus measure. Next table shows that both Sobel+variance and Tenengrad methods provide the smaller values of F_2 that correspond to a better noise tolerance.

Sobel+var	Tenengrad	Laplacian	Laplacian+var
1.0411	1.0646	2.507	2.101

4.3. Multi-focus fusion techniques

Since diatoms have a 3-D valve we are investigating fusion-based methods for combining different focal planes. We tested several fusion methods and asked to a group of diatom experts about the best result. We used a Laplacian pyramid-based method for its calculation [8]. The recombination process produces a contrast enhanced image and the fine diatom striae becomes more visible.

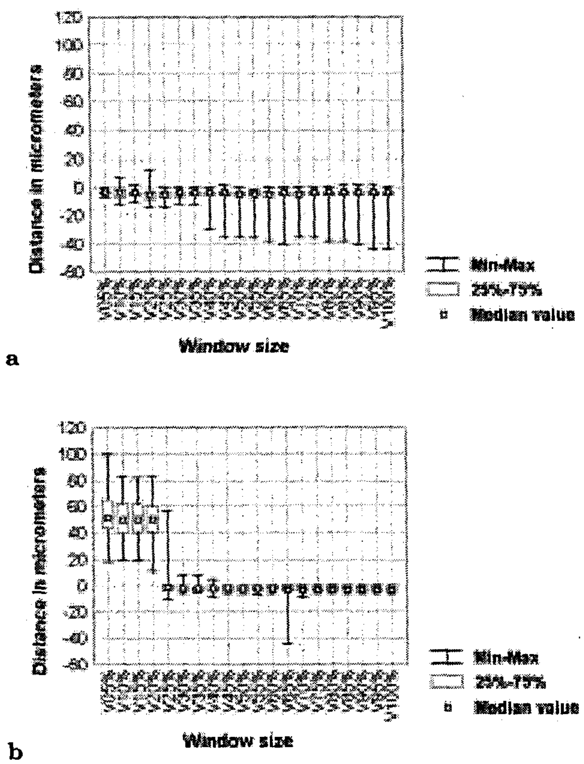

Figure 3. Window size evaluation. (a) Variance measure. (b) Variance of Sobel measure. Horizontal axis gives window size as percentage of the full image size. The zero level corresponds to best focus.

5. Conclusions

Two novel approaches in autofocusing based on a combination of gradient and variance processing are presented. An extensive work has been accomplished for a large number of different specimens. The proposed focus measures were compared with a number of techniques in the literature. Significant performance enhancement can be observed by the proposed method through new focus metrics.

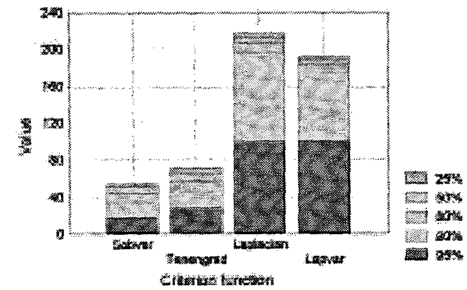

Figure 4. Focus noisy measures peakedness results for four selected methods

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