Autofocus algorithm using one-dimensional Fourier transform and Pearson correlation

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ABSTRACT

A new autofocus algorithm based on one-dimensional Fourier transform and Pearson correlation for Z automatized microscope is proposed. Our goal is to determine in fast response time and accuracy, the best focused plane through an algorithm. We capture in bright and dark field several images set at different Z distances from biological organism sample. The algorithm uses the one-dimensional Fourier transform to obtain the image frequency content of a vectors pattern previously defined comparing the Pearson correlation of these frequency vectors versus the reference image frequency vector, the most out of focus image, we find the best focusing. Experimental results showed the algorithm has fast response time and accuracy in getting the best focus plane from captured images. In conclusions, the algorithm can be implemented in real time systems due fast response time, accuracy and robustness. The algorithm can be used to get focused images in bright and dark field and it can be extended to include fusion techniques to construct multifocus final images beyond of this paper.

Keywords: Automated Microscopy, Autofocus, Fourier Transform, Pearson Correlation.

1. INTRODUCTION

Every day researchers in biological areas analyze a considered amount of microbiological samples. Automated systems more powerful are needed. In literature some developments have been cited, i.e an automatical system for phytoplanktonic algae identification [1]. One step involved in an automatical system is to capture microbiological images to get the best focused image from biological sample, it is a challenger task due nature problem. In some papers we can find autofocusing algorithms previously developed like Tenengrad algorithm [2], the gradient magnitude calculation of the Sobel operator [3] or other methods proposed based on different techniques [4] [5] [6].
We propose in this paper a new algorithm to get best focus image from microbiological sample based on usage of the Fourier transform to obtain the frequency content of several vector patterns in each captured image field at different Z distances and the Pearson correlation to construct a normalized focus measure. The spatial unidimensional Fourier transform pair can be defined like

$$H(f) = \int_{-\infty}^{\infty} h(x)\, e^{-j2\pi fx} dx \ , \tag{1}$$

and

$$h(x) = \int_{-\infty}^{\infty} H(f)\, e^{j2\pi fx} df \ . \tag{2}$$

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In eq. (1), $H(f)$ defines the spatial Fourier transform of $h(x)$ in one dimension. In eq. (2), $h(x)$ defines the spatial inverse Fourier transform of $H(f)$.

The linear correlation coefficient is sometimes referred to as simple correlation coefficient, Pearson product moment correlation coefficient or just Pearson correlation. The Pearson correlation coefficient r denotes a measure of intensity of association between two variables X and Y [9], the coefficient r can be obtained by the expression

$$r = \frac{\sum XY - \frac{\sum X \sum Y}{n}}{\sqrt{\left(\sum X^2 - \frac{(\sum X)^2}{n}\right)\left(\sum Y^2 - \frac{(\sum Y)^2}{n}\right)}} \tag{3}$$

where n represents the number of pairs of data present.

The Pearson coefficient r can never be greater than 1.0 nor less than –1.0, therefore we use $|r|$ to obtain the intensity of association between the two variables X and Y or the correlation. Obtaining a value of r close to 0.0 means that not correlation exists between the variables, in the same way obtaining a value of r close to 1.0 implicate that exists a strong correlation between the variables. The paper is written of the following way. Section 2 explains the materials and methodology, the results and discussion are explained in section 3 and section 4 contains the conclusions of this work.

2. MATERIALS AND METHODOLOGY

We obtained a stack of K captured images $f(x,y)_k$ of size NxM pixels from biological sample taken by stepping the microscope in Z axis, where $x = 1,..,N$, $y = 1,..,M$ and $k = 1,..,K$. In order to reduce the processing time in each image, the algorithm process a group of Q vectors V_q where $q = 1,..,Q$. These vectors are spatially equidistant to build a scan process pattern to each captured images $f(x,y)_k$ (Fig. 1). Δ defines the distance between V_q vectors, so the algorithm does not process the entire image, only the pattern defined. The number of vectors Q can be calculated by the expression $Q = int\left(\frac{N}{\Delta}\right) + 1$, where int means the integer part of the ratio in parenthesis. Vectors V_q can be computed by the expressions

$$V_1 = f(1, y_0,.., y_M)_k,\ V_2 = f(1+\Delta, y_0,.., y_M)_k,\ ...,\ V_q = f((q-1)\cdot\Delta+1, y_0,.., y_M)_k \tag{4}$$

We obtain the Fourier spectrum of V_q vectors, $|H_1(f)|$, $|H_2(f)|$,.., $|H_q(f)|$. We build a unique concatenated Fourier spectrum vector FSV_k for the captured image $f(x,y)_k$. Computing FSV_k from each captured image and comparing by Pearson correlation with the FSV_1 of the first captured image $f(x,y)_1$ called reference, defined to be the most out of focus image, we can obtain the best focused image finding the image with Pearson correlation coefficient r with the lower correlation value, the assertion can be explained because the image with most out of focus will have a lower correlation value when is compared with the best focus image. In Eq. (3) the $X = FSV_1$ and $Y = FSV_k$ for each computing of Pearson coefficient r, n will be the length of vectors X or Y. The Fig. 1 shows the scan process pattern defined by V_q vectors according to Eq. (4). We can control the V_q vectors spacing changing the value of variable Δ,

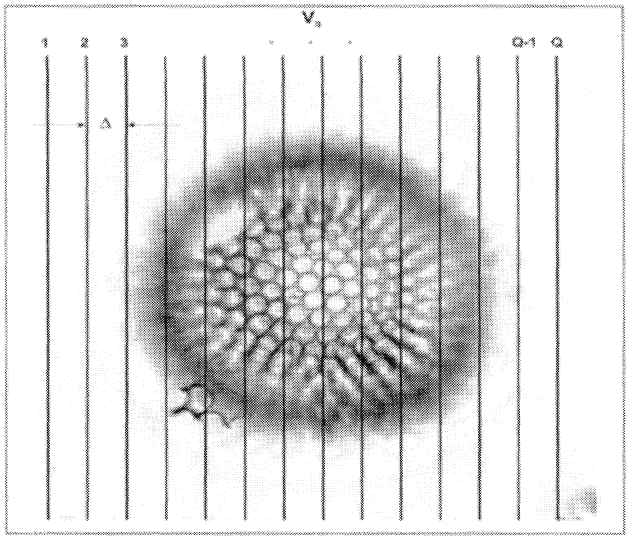

Fig. 1. Scan process pattern defined by V_q vectors across one captured image.

doing $\Delta \to 1$ will have more V_q vectors to compute, therefore doing $\Delta \to N$ will have less V_q vectors to compute and the algorithm will be less sensible to details on sample, in experiments we use different Δ values to obtain their respective graphics of algorithm sensibility to get the final focused image. Finally, Fig. 2 shows a general diagram of the algorithm proposed.

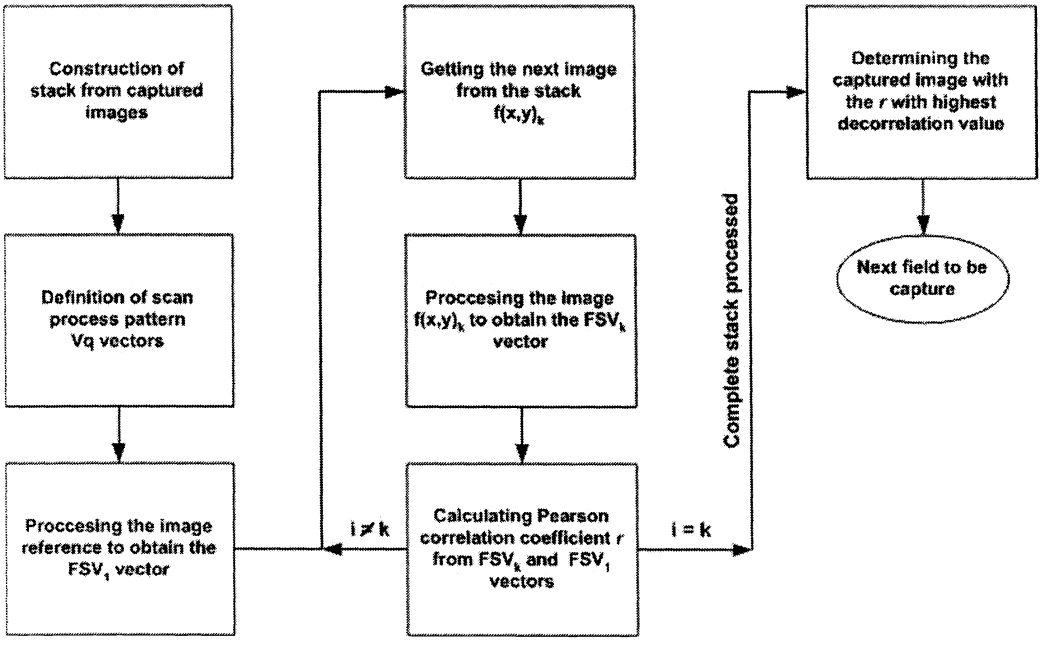

Fig. 2. General algorithm diagram.

3. RESULTS AND DISCUSSION

The Fig. 3 shows the curves of r Pearson coefficient when we change Δ value in range of 40 to 60 pixels with increments of 5 pixels, it is important to say that a $\Delta = 60$ means that just nine V_q vectors were processed. Observing the graphics all peaks are inside of the best focused image region, when $\Delta \to N$ the algorithm runs faster but it loss sensibility implicating that can't get the best focus image. The graphics from the experiments shows that 36 index image has the lower correlation value compared with the first captured image, so image 36 has the best focus.

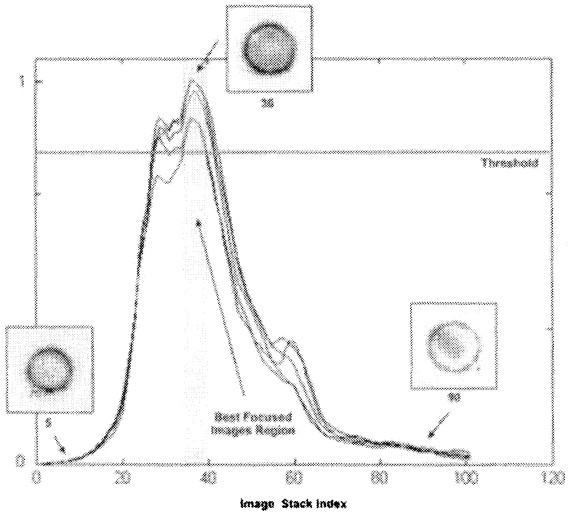

Fig. 3. Bright field Δ curves with values 40 to 60 pixels. Showing the best focused image and its index from captured image stack.

However, inside the best focused image region the difference in focus among them is so insignificant. The reader can observe that the diference between the images shown in Fig 4 it is not noticeable, these images are inside of the region where we can find the best focused image.

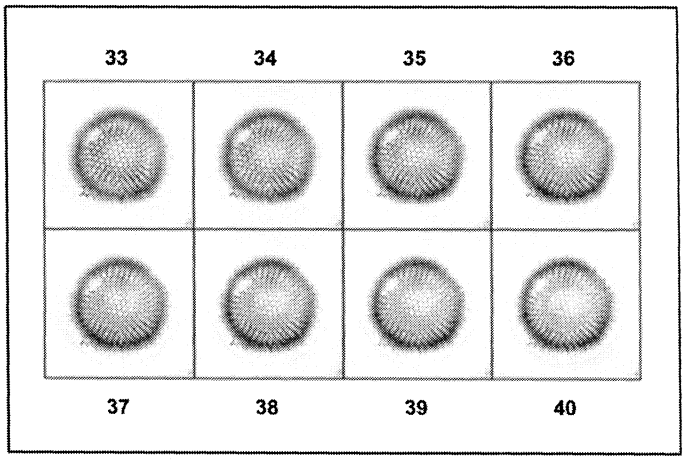

Fig. 4. Bright field images with r Pearson coefficient value to close.

The Fig. 5 and Fig. 6 show similar results than we obtained before, the main differences are that we work with dark field images and we can observe that the algorithm with the same variables values can get the best focused image. This is we can work automatically in both type of fields without change anything.

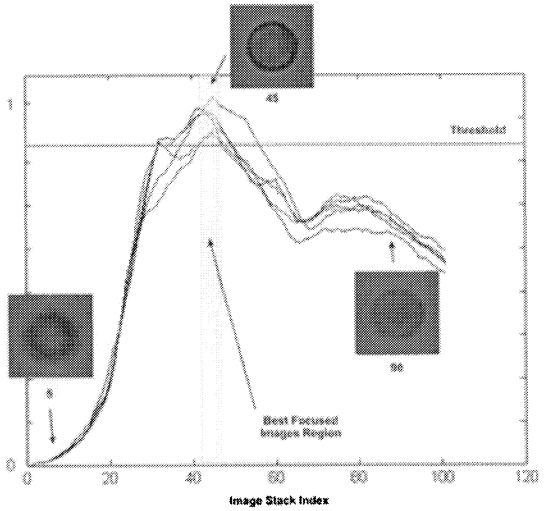

Fig. 5. Dark field Δ curves with values 40 to 60 pixels. Showing the best focused image and its index from captured image stack.

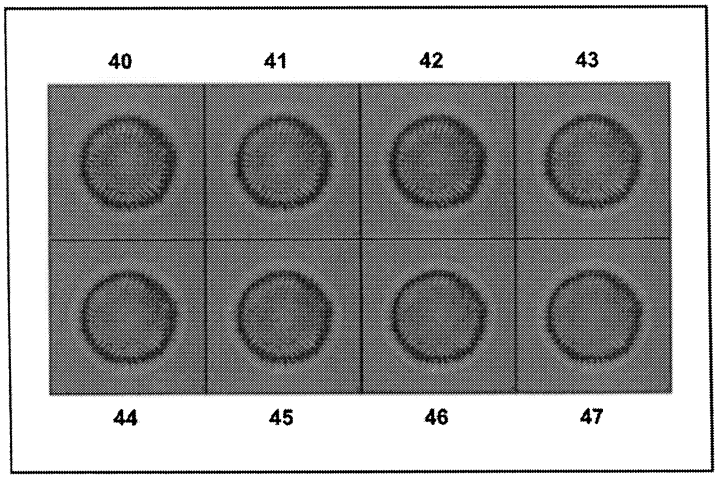

Fig. 6. Dark field images with r Pearson coefficient value to close.

4. CONCLUSION AND FUTURE WORK

The proposed focusing method offers a significant accuracy and robustness and performance suitable to be implemented in real time processing, besides it can process severals types of environments due the ilumination, like bright and dark

fields. Further work can be done including fusion techniques in the proposed algorithm to improve the final image quality, this is find the optimun threshold value where we can combine up to this value several images, observe the Fig. 3 and Fig. 5. More studies needs to be made to design and test a new scan process patterns and design more experiments to test different faces of Fourier transform.

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