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Correspondence to:
Dipl. ing. Tomislav Takač
21000 Novi Sad
Narodnog Fronta 83/522

E-mail: tomatakac@gmail.com

FUSION OF MICROSCOPIC IMAGES

SPAJANJE MIKROSKOPSKIH SLIKA

Tomislav Takač[1], Vesna Spasić Jokić[1]

[1]Faculty of Technical Sciences, University of Novi Sad

Ključne reči
spajanje slike, površina, mikroskopski uzorak, korelacija, vremenski domen

Key words
image fusion, area, microscopic sample, correlation, time domain

Abstract

Digitalization of various data is trend in modern medicine. Numerous analog microscopes could be found in different kinds of laboratories in Serbia. Due to high prices of digital optical microscopes pragmatic solution was found- to enable acquisition of data and their digitalization using suitable analog microscope, camera and computer or working station.

1. INTRODUCTION

One possible system for improvement of analog optical microscope consists of three basic parts: (1) Sample platform controlled by step motor and computer; (2) commercially available digital camera fixed on microscope ocular and connected with the computer and (3) software application that enable connection of platform and camera with the computer image fusion. It is necessary to take two shots at some distance which will have some of the identical parts. Due to system imperfection it is almost impossible to get two identical snapshots as their brightness are not the same due to micrometer displacement of digital camera related to main optical axis of microscope. According to that it is necessary to find correlation between shots and develop software application which will enable overlapping of two images at the common regions and further their fusion in one image size up to GPixel.

The object to record is microscope sample with maximum size up to 1 cm. Microscope

· Field of view covers a very small part of the surface of prepared sample and full inspection could takes to much time. Well trained user needs between 30 s and 5 minute for scoping. Limitation of field of vision can be a problem in the cases in which the samples are bigger. Experienced observer can get a real picture by continuous moving of the sample but is not able to save the image. In some circumstances it is necessary to keep sample or to choose a less enlargement. Photographing in microscopy with conventional film as a medium is to slow and uneconomic. With the advent of high-quality photo-sensors and with cheapening of commercial digital cameras and accompanying hardware some necessary conditions are put in place to achieve the main goals: permanent archiving of records; processing and easy image manipulation; enabling of different measurement (dimensions, density, etc.). Microscopic images connecting starts with series of shots took at specific distance (depends of microscope enlargement, camera resolution and accuracy of positioning). Further, particular slides have to be conjugate in one big high resolution slide (mega-slide) during the few minutes.

2. MATERIALS AND METHODS

2.1. Mathematical baselines of image conjugation

Digital images contain information on the distribution of chromaticity (color) over the surface of preparation presented as rectangular matrix with pixels as its elements. Pixel represents information about the average value of color at the small part of object surface. As the color is described by three values (point in three - stimulus space) than image is considered as a matrix with three-dimensional vectors as its elements. Adjacent positions from which individual shots are taken have overlapping area. At this very area it is necessary to find relative shift expressed in pixels. Detail which exists at both images is a key point in shift determination. Procedure implies that the extract tablet (small part of image) from the first slide is the required pattern and the similar part has to be found at the second slide. If the dimensions of source image is 20 N *x* N pixels, and tablet size is 20 M*x*M than tablet will be compared with sub-matrixes. 2 D function which describes similarity of matrixes is so-called cross-correlation function with maximum which corresponds to the position of the most similar details.

2.2. FFT algorithm for determination of function of correlation

Discrete Fourier Transformation (DFT) is defined as

$$F(w)=\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{+\infty} f_{(x)} * e^{-j2\pi wx}\,dx \qquad (2.1)$$

and inverse transformation is given by

$$F(x)=\frac{1}{2\pi}\int_{-\infty}^{+\infty} f_{(w)} * e^{-j2\pi wx}\,dx \qquad (2.2)$$

Where are:

$f(x)$- signal over the spatial coordinate x

F(w) – spectra, complex image function f

w - frequency spatial coordinate over direction x

j- imaginary unit (j^2= -1)

These equations are not usable in computer application because mathematical processing of signal in digital system uses discrete values. According to Fourier functions the transformation of discrete signals is periodical, i.e. spectra of periodical signals is discrete. Further t in computer systems in which the signal and spectra are discrete it is reasonable to consider that signal and signal spectra are periodical. However, if we take only one period we can get signal in final range. Mathematically speaking, DFT of digital image presumes that signal is infinitely repeated as well as its spectra. Original signal (in spatial domain) and its spectra (image of signal in frequency domain) have the same number of discrete values. E.g. if the image dimension is (128 x 128) pixels, DFT of this image has 128 x 128 values.

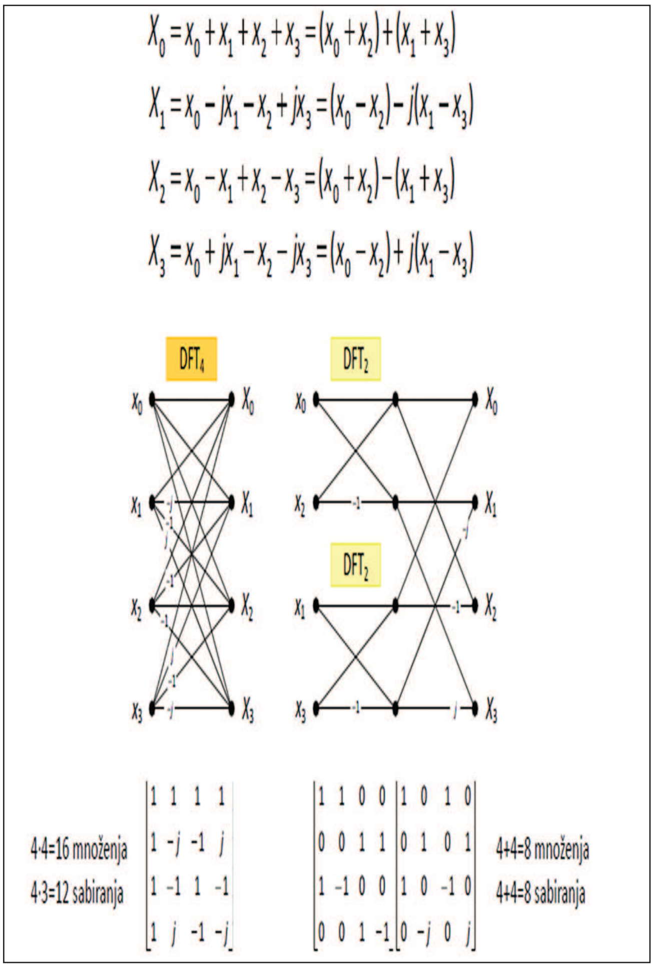

Figure 1. Decomposition of DFT in 4 points

Discrete Fourier Transformation represents transfer of periodical discrete signal x (n) in series of complex numbers x (k) and are defined as:

$$x(k)=\sum_{n=0}^{N-1} x(n)e^{-j2\pi \frac{n}{N} k} \qquad (2.3)$$

And further, inverse DFT is given as:

$$x(n)=\frac{1}{N}\sum_{k=0}^{N-1} x(k)e^{j2\pi \frac{n}{N} k} \qquad (2.4)$$

Calculations are performed using iterations for n and k, and exponents obtained by Euler formula:

$$e^{jz} = \cos z + j\sin z \qquad (2.5)$$

Direct calculation of above formula represents sophisticated algorithm which depends on N^2 and complexity degree of N2 and it is not suitable for practice.

For the purposes of this study, we used Convolution theorem which considers that convolution of function in spatial domain represents multiplication in complex domain. However, in this very case DFT algorithm is not so useful as it was necessary to perform direct transformation of two functions, then multiply their complex images and finally, results have to be inversely transformed which is unfavorable than to work in spatial domain.

Fast Fourier Transformation (FFT) is based upon successive partition of DFT in certain numbers of points. The main advantage of this solution is the decrease of complexity from N^2 to $N\log_2 N$. Also, the occupation of memory space is less due to calculating *in situ*. Efficiency is 99 % for N = 1024. Some disadvantages come from the requirement that radix 2 is possible to derive only for $N = 2^m$ (if it so not matched than it is necessary to perform *zero padding*). Over-range could be avoided by simple ½ entrance scaling

Decomposition of DFT in 4 points is given at Figure 1.

3. RESULTS

3.1. Conjugation (fusion) of two shots based on correlation function

Application of image correlation can be easily explained by two shots (a and b) taken from the points at the distance of 100 µm (100 steps of moving platform motor). Relative shift, expressed in pixels, should to be accurately measured. Figure 2 gives the method of obtaining the matrix (two-dimensional functions which correlations are required).

Source images have dimensions of 1100 x 960 pixels. Larger region (N x N) called target is inserted from one image. Smaller region (M x M) called patern is separated from another image. Basic condition that N + M = 960 has to be fulfiled.

Coordinate system of images a and b are $x_1O_1y_1$ and $x_2O_2y_2$. Area used for matrix creation are (704 x 704) pixels and (256 x 256) pixels. Upper left corners of the images are the origins of those images. Local coordinate systems of target and patern can be expresed as $x_1'O_1'y_1'$ and $x_2'O_2'y_2'$. Square values of target pattern dimensions are obtained by

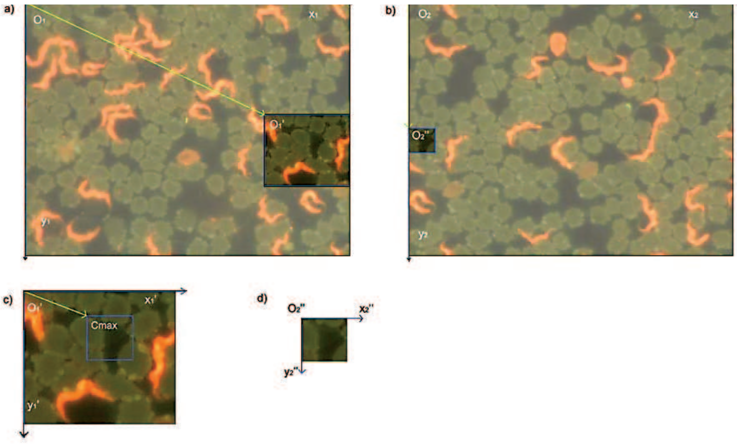

Figure 2. Starting points for the image fusion

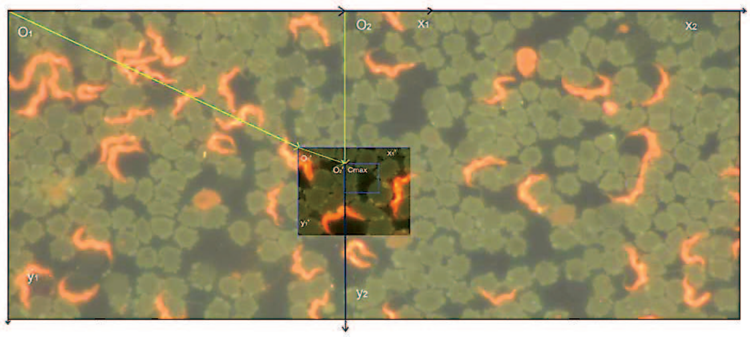

Figure 3. Conjugation (fusion) of the two images

special software. Just imagine one window which frame has the same dimension as pattern. This window can be placed somewhere at the target area for the purpose of finding the correlation between pattern and window pixels. Correlation result is a number stored in computer memory. The same procedure will be repeated until we sweep the whole area. The highest value of correlation function corresponds to the maximum match of pattern and corresponding area at the target. Fourier transformation of color image can be obtained by decomposition of the three matrixes of the same dimensions but with various chromatic component (red, blue, green). FFT algorithm is applied at each of these matrixes separately. During the analyze of pathological samples it was recognized that red component carries little number of information and is practically useless. During the program execution the user can make a choice about chromatic component, but the green color is recommended.

The next step is FFT of the two matrixes in aim to obtain two complex matrixes which elements can be complex multiplied. The result is Fourier image of correlation matrix. Using inverse Fourier transformation it is possible to obtain complex correlation matrix necessary for further derivation of correlation function maximum by finding: the highest real part; imaginary part near to zero; the highest moduo or the argument near to zero. .

Criteria which include imaginary part or argument are more reliable but periodical nature of tangens function can leads to some mistakes. Therefore the phase correlation is applied only in case of small shifts and is accompanied by amplitude monitoring. Only the real part of correlation matrix is considered in this paper, actually, element represented by index C_{max} at Figure a with maximum real part is required.

Measured value of relative shift represents the image of coordinate origin position (upper left corner) of the second image in coordinate system which origin is upper left corner of the first image. Point O_2 is coordinated by (Δx,Δy) in the space $x_1O_1y_1$. According to measured values of Δx and Δy it is possible to fuse source images so that common pixels only once can be inserted in the final image. Result of fusion is given at figure 3. Coordinate spaces of origin images as well as their square fragments used for determination of correlation matrix are left on the figure.

Image fusion can be performed on a few different ways. It is possible to take overlapping area from one image or it can be conjugated from three sub-images. If the last one is chosen than one sub-image belongs to the first image, one belongs to the second and the third represents overlapping area made up of pixels common to both images – gradient of transition or average pixel value. The other way enables less noticeable transition of image as there is a difference in average energies. The first way is better from the stand point of speed to get appropriate solution.

Apstrakt

Digitalizacija različitih podataka predstavlja trend u savremenoj medicini. Danas se u laboratorijama Srbije mogu naći brojni analogni optički mikroskopi. Zbog visoke cene digitalnih mikroskopa nadjeno je pragmatično rešenje koje omogućava akviziciju podataka i njihovu digitalizaciju primenom pogodnog analognog mikroskopa, kamere i računara ili radne stanice.

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