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Three-dimensional reconstruction and lateral views in optical microscopy

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ABSTRACT

The three-dimensional properties of an optical microscope are analyzed and a defocusing technique is proposed to recover the spatial distribution of the specimens under investigation. Limitations and real capabilities of the 3D reconstruction are pointed out. As a result, lateral views are obtained by means of operations in the spatial frequency domain.

In such a way, it is possible to represent side views of an object within the angular aperture range of the microscope. A theory concerning image formation is discussed and simulations of side view reconstructions are reported.

1. INTRODUCTION

Three-dimensional (3D) reconstruction of observed objects is one of major task of today's imaging science. In particular, in the microscope imaging of biological specimens, the goal of obtaining a spatial distribution of an intensive physical property of the objects should be achieved avoiding alterations to the structures.

By using an optical sectioning instead of a physical one it is possible to recover internal planes, not destroying or altering the specimens. The 3D properties of an optical microscope have been discussed in many articles (see for example [1,2]) and restored images have been obtained in particular situations [3]. However, the problem is not so simple as it may appear in the scientific literature, where the various methodologies often are based on a priori information which permits a satisfactory reconstruction.

We present a general technique based on defocusing, with no a priori information. In such a way, limits and possibilities of the reconstruction process are stressed, and we prove that a total 3D reconstruction is not possible, since in the defocusing operation a part of information is irreparably lost. This fact can be easily seen by a spectral analysis and a calculation of the Optical Transfer Function (OTF) of the system leads to define the limits of restoration. On the other hand, we analyze the possibilities of the defocusing method and to this end we used a simplified but effective model of the microscope. The model is obtained under the hypotheses of geometrical optics. This means to disregard diffraction, i.e., to state that the system is rigorously stigmatic. The assumption is not correct, but the error is a minor one: one must bear in mind that if a 3D reconstruction has some limits in a diffraction-free microscope, it can hardly be presumed that a better behaviour will be exhibited by any instrument with diffraction limitations.

In the next section the image formation theory is presented. In section 3, we analyze the restoration task. Simulation and results are discussed in the last section.

2. IMAGE FORMATION

The properties of out-of-focus images have been extensively studied in the last decades (see for example [4]). Assuming the microscope as a linear system, if the plane of acquisition (e.g. a TV camera target) is not properly focused, then the relationship between the actual image intensity $i(x,y)$ and the ideal (in focus) one $i_o(x,y)$ is simply a convolution by some function $s(x,y)$.

$$i(x,y) = i_o(x,y) * s(x,y) \qquad (1)$$

Equation (1) holds if the image plane is perpendicular to the optical z-axis.

Then, x and y are coordinates perpendicular to the z-axis; $s(x,y)$ is a function depending on the imaging system used and on the degree of defocusing. Once $s(x,y)$ and $i(x,y)$ are known, an inverse convolution yields the required in-focus image $i_o(x,y)$.

The 3D image formation of microscopic objects follows a similar approach. In this case $s(x,y,z)$ is the 3D Point Spread Function (described later on), $i_o(x,y,z)$ represents the 3D object, i.e., the 3D transmission coefficent while $i(x,y,z)$ is the image obtained; more precisely, it is a set of 2D images at fixed z, formed by the in-focus planes and by the out-of-focus planes under and over the in-focus ones. The result is a set of blurred images and the goal consists in reconstructing the in-focus planes, i.e., the object, starting from $i(x,y,z)$. In a computerized analysis, z is a discrete quantity, and every plane $i(x,y,z)$ is a digitized image.

We consider incoherent illumination, as one normally has in microscopy; introducing the 3D Fourier Transform I, I_o and S of i, i_o and s respectively, the well-known convolution property yields

$$I(f_x,f_y,f_z) = I_o(f_x,f_y,f_z)\, S(f_x,f_y,f_z) \qquad (2)$$

If we consider geometric optics, an expression for s is:

$$s(x,y,z) = \begin{cases} \dfrac{A}{z^2} & \text{if } \sqrt{x^2+y^2} < |z| \tan\theta \\ 0 & \text{otherwise} \end{cases} \qquad (3)$$

where A is a constant and θ is the angular aperture of the microscope.
The calculation of S leads to a very important result which has a considerable effect on the 3D reconstruction. The Fourier Transform of equation (3) gives the following condition:

$$S(f_x,f_y,f_z) = 0 \qquad \text{if } |f_z| > \tan\theta \sqrt{f_x^2+f_y^2} \qquad (4)$$

i.e., S is zero inside the double cone in the Fourier space, whose angle of aperture is formed by the f_z axis and $\pi/2 - \theta$.
In the Appendix eq.(4) is proven and the analytic expression of S in the in the region where it does not vanish is calculated.

The fact that $S(f_x,f_y,f_z)$ is zero in a wide region can be explained by some considerations in the frequency domain [5].

The wave coming from the object under investigation can be decomposed as a superposition of plane waves with different directions [6]. Only the plane waves entering the microscope at angles not higher than the input angular aperture θ of the microscope contribute to the image formation. In fact, high frequencies are lost.

A more accurate expression for $s(x,y,z)$ can be used [7] which does not neglet diffraction, but the conclusion we obtain is always valid, i.e., S has regions where it vanishes.

3. 3-D RECONSTRUCTION

We aim at obtaining internal planes of the specimens and a restoration has to be performed to eliminate the contribution of the out-of-focus planes. Starting from equation (1), an inverse convolution yields i_o, i.e., the set of in-focus planes. The procedure could be the following one : calculate I from the images, through a Fourier Transform; measure or calculate s and deduce its transform. Then (2) yields I_o, from which an inverse transform derives the desired i_o. This simple operation cannot be performed since S is equal to zero in entire regions of the frequency domain. This implies that a perfect 3-D reconstruction is not possible.

On the contrary, a correct information can be obtained about the projections of the object in all directions comprised within an angle relative to the optical axis. By using the projection theorem, if we imagine to superimpose the two frames : f_x, f_y, f_z, and x, y, z, then the projection of the object parallel to a direction z', is the inverse Fourier transform of the function obtained cutting the Fourier space by a plane through the origin perpendicular to z'.

Hence if zz' angle is $< \theta$ such projection is obtained. If not, the above said plane is in the region where S vanishes, and no information can be recovered. Analitically, let us consider a new reference frame x',y',z', obtained by adding two orthogonal axes x' and y' to z', leaving the origin unchanged, and let f_x, f_y, f_z be the spatial frequencies conjugate to x', y', z'. Then, if A is the orthonormal matrix that links the x y z and x' y' z' frames, one has :

$$dx\,dy\,dz = \det(A)\, dx'dy'dz' = dx'dy'dz'$$

and

$$x f_x + y f_y + z f_z = x'f'_x + y'f'_y + z'f'_z \qquad (5)$$

then

$$I_o(f'_x, f'_y, f'_z) = \iiint i_o(x',y',z') \exp(-2\pi j(x'f'_x + y'f'_y + z'f'_z)\, dx'dy'dz' \qquad (6)$$

If one considers the plane $f'_z = 0$, then (6) can be written in the following way :

$$I_o(f'_x, f'_y, 0) = \iiint i_o(x',y',z') \exp(-2\pi j(x'f'_x + y'f'_y) =$$

$$= \iint \exp(-2\pi j(x'f'_x + y'f'_y) \int i_o(x',y',z')\, dz'\, dx'dy' =$$

$$= F \left(\int i_o(x',y',z')\, dz' \right) \qquad (7)$$

Equation (7),which expresses the projection theorem, gives the Fourier transform of the projection of i_o along the z' direction, perpendicular to the plane $f'_z = 0$.

Once one has calculated I_o (f_x, f_y, f_z), it is sufficient to calculate the Fourier transform in the plane $f'_z = 0$, by means of interpolations of the values of I_o . An inverse transform of equation (7) gives the projection along the z' direction.

We have pointed out that I_o cannot be recovered; nevertheless,in the region where S does not vanishes, we can apply a Wiener filter on I, so obtaining a Fourier transform to be used in the projection theorem.

4. RESULTS AND DISCUSSION

Simulations and actual experiments have been performed.

A three dimensional image has been simulated, consisting in a pyramid with eight planes. The point spread function s is a double cone. A 3-D defocused image has been obtained by means of a convolution between the original image and the cone. Starting from this blurred image , a restoration has been performed and side views have been obtained.

The calculations have been performed on 32x32x16 matrices, in which some zero points have been added to avoid aliasing effects.

Fig. 1 shows a defocused set of planes. Fig. 2 shows the reconstructed images of the pyramid . From these images the different point of view are evident.

In the case of actual experiments difficulties arise when getting the set of images, since it is difficult to adjust with accuracy the defocusing. The software implemented needs a set of images where the in-focus planes are taken at a constant sz distance, and this task is not easy to get if an automatic controlled adjustment of the focus is not available.