Principles of Pseudoscanning in Optical Microscopy

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ABSTRACT

We present in this paper the basic principles of a new technique in scanning optical microscopy (SOM) which we call pseudoscanning. A microscope employing the technique of pseudoscanning has the same capabilities as a confocal SOM in terms of resolution and depth of focus but features no mechanical moving parts. To achieve pseudoscanning, a 2-dimensional (2D) LED array was used as a light source and a 2-D Multichannel Detector (2D-MCD) was used to track the point image as it moves across the image plane. The 2D-MCD must be configured in a manner in which its various pixels act independently of each other. The pseudoscanning microscope features a higher temporal bandwidth than the conventional CSOM because object-point scanning and consequent image tracking are done electronically.

INTRODUCTION

Compared to a conventional microscope, a scanning optical microscope (SOM) has the advantages of superior resolution that is beyond the classical diffraction limit [1], and a very narrow depth of field that leads to images of high contrast [2]. Since there is a strong rejection of the contributions from the non-conjugate planes such as those from lenses and mirrors in the optical system, the resultant image will be free of attendant speckles even when a laser is employed as illumination source.

The basic operation of an SOM involves the imaging of a point light source into the object plane and then tracking the resultant

image point by a photodetector. The composite image is formed by scanning the object point across the sample. The size of the photodetector determines the degree of coherence of the optical system [3]. A confocal scanning optical microscope (CSOM) is achieved when the detector assumes the dimensions of a point [1].

Probing of the object is implemented by either scanning the point light source across the source plane or raster scanning the object plane across a fixed point source. In the former techique two moving mirrors are usually employed to effect two-dimensional motion of the probe point on the object plane. Precise control of these mirrors can be achieved through the use of a galvanoscanner [4], stepper motor, polygon mirrors, piezoelectric transducers, acousto-optic modulators [5]. Other methods of light source scanning involves the use of a Nipkow disk [6,7] or a CRT scanner [8]. Two-dimensional scanning of the sample across a point light probe can be accomplished using stepper motors or loudspeakers [9,10]. One drawback in the construction of an SOM is that high-resolution mechanical scanning inevitably requires precise mechanical parts, stability, and sophisticated feedback and timing electronics which makes the SOM difficult and expensive to construct.

This paper discusses the principle and implementation of pseudoscanning in optical microscopy. We established the equivalence of a pseudoscanning microscope (PSM) and a CSOM. Equivalence enables the PSM to yield the same superior resolution, and contrast as that of a CSOM but without its moving parts.

PRINCIPLES OF PSEUDOSCANNING MICROSCOPY

Shown in Fig. 1 is a simplified diagram of confocal scanning optical microscope. In a CSOM the image amplitude of a point object is given by [1].

$$i(x',y') = h_1(x,y)h_2(x,y) \qquad (1)$$

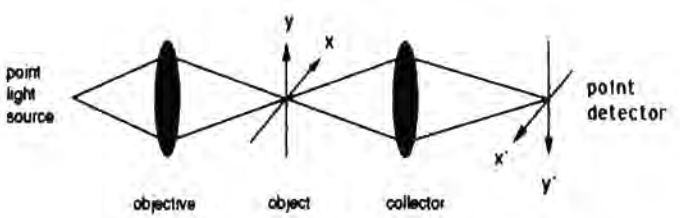

Fig. 3. Diagram of a pseudoscanning optical microscope.

where $h_1(x,y)$ $h_2(x,y)$ are amplitude point spread function (PSF) of the objective and collector lens respectively. Thus unlike in a conventional microscope where the PSF of the optical system is solely determined by the objective, both the objective and collector lenses in a CSOM contribute to the fidelity of the imaging process. For a circular aperture of diameter d, the impulse response is expressed as

$$h(r) = \text{const. } J(2\pi d r) / 2\pi r \qquad (2)$$

where $J(2\pi r)$ is the Bessel function of order 1. Substituting Eq. (2) into Eq. (1) yields

$$i(x',y') = \text{const. } J^2(2\pi d r) / 4\pi^2 d^2 r^2 \qquad (3)$$

where $r = (x^2 + y^2)^{1/2}$ is the radial distance from the optical axis and d is the diameter of the aperture. Figure 2 shows the impulse response of a CSOM and that of a conventional microscope. In the calculation it assumed that both optical systems have the same value of their objective numerical aperture. It is seen that the CSOM results in images of better fidelity as seen in a narrower impulse response that is devoid of sidelobes (Fig. 2b).

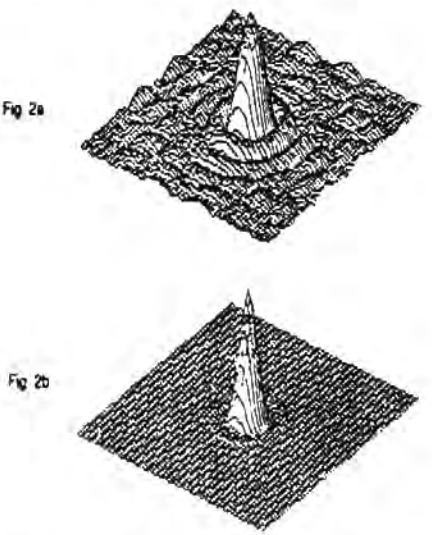

Fig. 2. Graphical behavior of 2D impulse response function for; a) conventional optical microscope, and b) scanning optical microscope for the same numerical aperture values. Note the narrower peak and the absence of sidelobes in Fig. 2b.

The measured composite image intensity is obtained by scanning across the entire object field,

$$I_c = \sum_{m,n=1}^{N} i_{mn}(x_m, y_n)\ i_{mn}^*(x_m, y_n) \qquad (4)$$

where the sampling distance between two successive points (m,n) and (m+1, n+1) satisfies the Rayleigh criterion limit. The constant in Equation (3) changes value from point to point across the object plane corresponding to the absorption characteristics (transmittance geometry) of the particular point. Note that Equation (4) does not contain cross terms involving mixed amplitudes since the image points are incoherently related to each other. Cross-terms can give rise to speckles, and other unwanted coherent effects such as the appearance of spurious peaks [11]. The entire image field is composed of N x N points. In the case when it is the point light source that moved across the object plane, a corresponding shift in the position of the point detector is required to detect the image point. This is in accordance with the lens equation.

In a pseudoscanning microscope, a two-dimensional array of point light sources and a two-dimensional multichannel detector are placed in the source and image plane respectively (Fig. 3). Scanning of probe point across the object plane is accomplished by sequential pulsing of the point light sources across the source plane. Tracking of the corresponding point image is done by activating that point detector whose location satisfies the lens equation from the object plane to the image plane. For the point sources to be incoherently related during detection, the pulse widths between successive excitation must not overlap in time. It is also required that every detector in the array functions independently of each other.

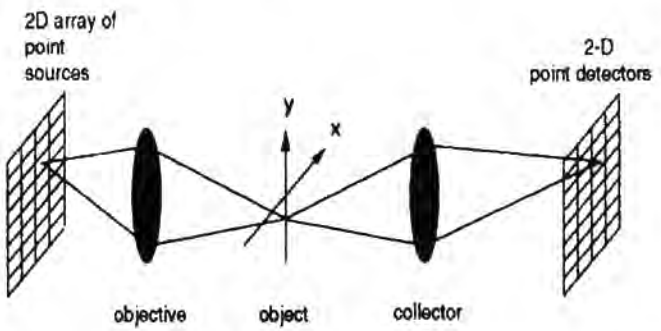

Fig. 3. Diagram of a pseudo-scanning optical microscope.

The imposed incoherence (both temporal and spatial) on light source pulsing and detection in the PSM leads to a composite image intensity (I_c) with no cross terms (interference effects),

$$I_c = I_{11} + I_{12} + \ldots + I_{mn} \; ; \; m,n = 1,2,\ldots, N \qquad (5)$$

where the subscripts signify the locations of the individual point detectors. When Equation (5) is written in terms of the image amplitudes, it reduces into an expression identical to Equation (4). Hence the PSM features the same imaging capabilities as that of a CSOM.

IMPLEMENTATION OF PSEUDOSCANNING

This section deals with the practical implementation of the PSM as a device. For device compactness, the dimensional compatibility with other commercially-available optical components, the size of the light source array must be made small without sacrificing its number of elements. While an array of laser diodes is the best choice, one is most likely to settle with an LED array because of their commercial availability. Strongly-beam guided GaAs LED arrays with 16 x 16 elements and a side dimension of 60.68 mm are available [12]. The size of the array's projection in the object plane can be controlled using a zoom lens or a variable-focus beam expander adapted in reverse from the source plane to the object plane.

A self-scanned solid state sensor (e.g. CCD camera or a photodiode array) can be employed as the 2D multichannel detector (2D-MCD) in the PSM. The size of a pixel is about 15 x 15 microns [13]. In the PSM application, only one pixel (or group of pixels) of the 2D-MCD is needed at any observation time and therefore there will be N^2 times of read-outs and blanking needed to form the composite image. Blanking is needed to prevent charge build-up due to thermally-generated dark current which compromises the dynamic range, and linearity of pixels during image tracking through blooming and existence non-zero bias level during detection by a particular pixel [14].

Temporal banwidth of the PSM is limited only by the response time of the GaAS LED and the detector. This could easily be in the microsecond regime with the current LED/Detector technology. Fast response is necessary for fluorescent applications, and real-time viewing.

It should be noted however, that the high number of read-outs required in the PSM application may lead to a considerable read

noise contributed by the output amplifier during read-out of the 2D-MCD [15]. This can be minimized by increasing the `effective' pixel size equivalent to a cluster of 2D-MCD pixel. This method, however compromises the image resolution.

The LED array mentioned above was not really commercially meant for this type of application and thus its inherent limitation in size and number of elements. However, it is expected that the alternative applications such as the PSOM will lead to a wider variety of electro-optical devices and components.

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