A low cost Time-coded Confocal Microscope

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Abstract

Confocal microscopy is a high resolution microscope technique, which operates by scanning a diffraction limited focal spot over a sample. This focal point on the sample is then imaged through an objective lens and a pinhole to ensure that only the information coming from the focal plane is captured and the information from the other planes are blocked by the pinhole. Although this gives better lateral resolution, this method is inefficient in terms of the amount of light required and the scanning time. In this paper, we discuss an alternative solution using digital micromirror device (DMD) to perform multiple focal points scanning simultaneously. This is done by projecting a series of orthogonal codes onto the DMD. The corresponding images obtained from the orthogonal codes are then saved and processed offline in a computer to calculate a confocal image. We use incoherent illumination rather than a laser source as used previously. We also discuss a key issue in this confocal system, which is a suitable separation between each focal spots and a crosstalk between them. We demonstrate that the proposed confocal configuration with the DMD device improves the light efficiency, the scanning time through the parallel confocal spots and a laser source is not required.

Introduction

Confocal microscopy has had major impact in biological and biomedical research [1], since it provides users with (1) higher spatial resolution in comparison with widefield compound microscope [2] and, more importantly, (2) the optical sectioning capability allows one to perform microscopic imaging in 3D [3]. A typical conventional configuration for the confocal microscope is shown in Fig. 1. The differences between a widefield microscope and a typical confocal microscope are that :

(1) The sample illumination for the confocal system is a focused laser beam illuminating only a single point on the sample.
(2) Rather than using a CCD camera to capture the image the confocal system employs a pinhole and a photo detector (single point detection). The pinhole eliminates light that degrades resolution in both the lateral and the axial directions.
(3) The image is formed by scanning the focal point over the field of view.

The present system used an inexpensive LED light source removing the need for a laser. A drawback of the typical confocal system is the need of scanning system; there are three standard scanning approaches which are (i) sample scanning using a motorized sample stage, (ii) beam scanning using a set of galvo mirrors to scan the focus beam across the sample and (iii) lens scanning using Nipkow disk to provide multiple focal spots on the sample simultaneously. Although the lens scanning method can provide a parallel confocal scan which provides a faster image acquisition, it does, in fact, waste a lot of light.

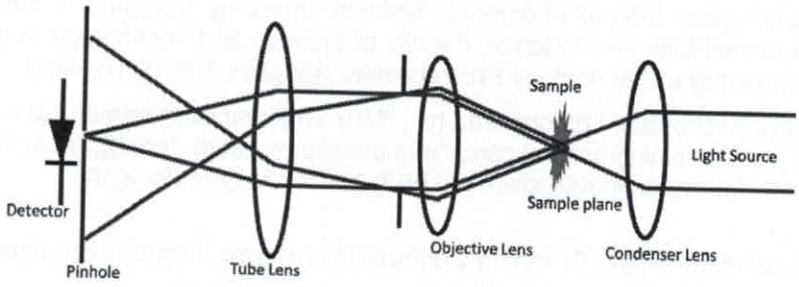

Fig. 1 shows a typical confocal microscope configuration

In this paper, we propose an alternative way to perform parallel focal spots scanning using a digital micromirror device (DMD) and a halogen lamp as the light source. This configuration enables us to use the light more efficiently and build a confocal microscope system at an economical cost.

System setup

The proposed confocal system as shown in Fig. 2 consists of a halogen light bulb illuminating a digital micromirror device (DMD) through Köhler illumination. The DMD (640×480 resolution and 10μm size) is aligned on the conjugate plane of the image plane of the objective lens. The DMD was extracted from a data projector. The DMD consists of micro size mirrors, which each of these micro-mirrors can be electronically controlled separately through MATLAB and act as an intensity spatial light modulator. Since each the pixel size of the mirrors is smaller the diffraction limit of Lens4, the reflected light from each pixel acts as a spatially coherent source. This coherent light is then projected onto the sample through Lens4 and the condenser lens. The focal point image on the sample is then imaged through a microscope system and a CCD. Since there is no physical pinhole in the system, the pinhole effect is then calculated offline in a computer by integrating only the intensity within 0.5λ/NA (diameter) of the Airy disc captured on the CCD [5,6].

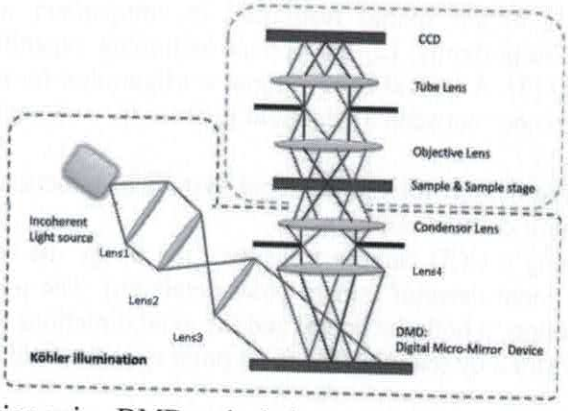

Fig. 2 confocal configuration using DMD and a halogen lamp; Lens1 (doublet f50), Lens2 (doublet f50), Lens3(doublet f150), Lens4(doublet f200), condenser lens (x10 NA0.25), objective lens (x10 NA0.25), Lens4(doublet f200)

Of course, a single point confocal scanning can be performed by switching on one pixel at a time, however, this is rather slow. One obvious choice to speed up the scanning speed is to illuminate the sample with multiple confocal spots simultaneously. Ideally the easiest way to scan the sample is to switch on the DMD pixels using patterns as shown in Fig.3. However, since the diffraction limited spot (Airy disk) has a finite size (Jinc function), if any two Airy disks were too close; there would be a crosstalk between the two Airy disks leading to loss of confocality. Therefore, in the experiment we separated each focal spot by 10 DMD pixels to ensure that the point spread function can be resolved as shown in Fig.4.

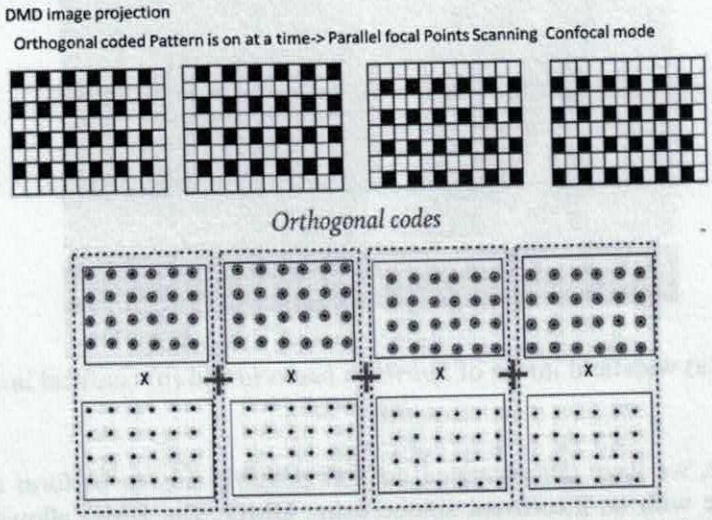

Fig. 3 Parallel confocal scanning using orthogonal codes to provide multiple diffraction limited spots on the sample. The top figure shows a series of orthogonal patterns sent to the DMD. The bottom figure shows the conceptual focal point images corresponding to the orthogonal codes in the top figure and also show the process to sum the images after multiplying by the virtual pinhole positions.

Results

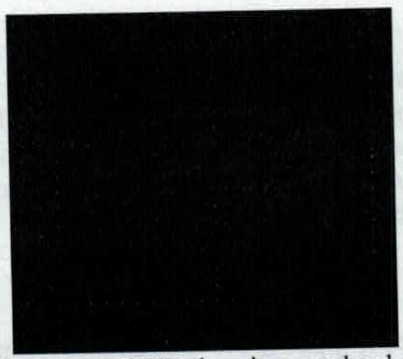

Fig. 4 shows a zoom-in image on CCD showing resolved diffraction limited spots.

As mentioned in the earlier section that each of the focal points has a separation of 10 pixels. We therefore captured 100 images using the othogonal codes to ensure that every pixel of the DMD were switched on once. The series of images were then processed to work out the confocal effect corrsponding to each of the sample positions for each image and then the full cofocal image was computed by summing all the confocal frames.

The system not only allows us to take confocal image, but it also enables us to take a widefield image by switching on all the DMD pixels; the DMD hence acts like a mirror illuminating every point on the sample simutaneously. Fig 5 shows a widefield image of *Spirillum bacteria* taken

by the CCD when all the DMD pixels were on and a confocal image of the same bacteria calculated by 100 frames captured by CCD corresponding to the series of the orthogonal codes.

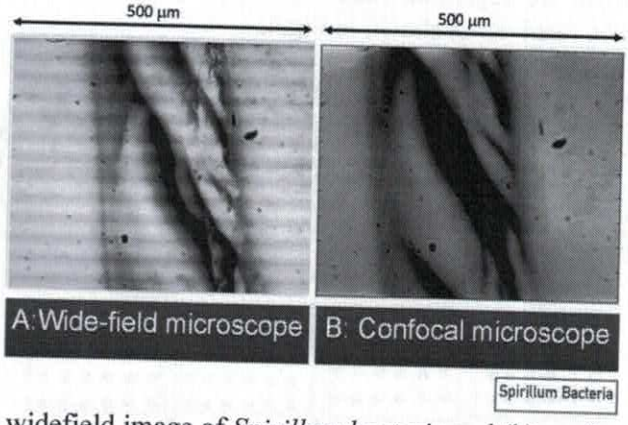

Fig. 5 shows (a) widefield image of *Spirillum bacteria* and (b) confocal image of *Spirillum bacteria*

Conclusion

In this paper, we have demonstrated another possible way to perform a parallel scanning confocal microscope with an incoherent source using DMD. The DMD allows us to convert the incoherent source to a spatially coherent source and perform the multiple focal spots scanning with no mechanical movement required. By projecting orthogonal codes on to the sample, the parallel confocal scanning can be achieved and the images corresponding to these codes are then captured by a CCD. The pinhole effect and confocal image are calculated offline in a computer by processing the images. We have also shown that it is possible to build a low-cost scanning confocal microscope and this microscope can also be used as a dual-mode microscope (widefield microscope and confocal microscope).

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