EasyChair Preprint

№ 982

High Speed Fluorescence Endomicroscopy with Structured Illumination for Robot Assisted Minimally Invasive Surgery

Haojie Zhang and Guang-Zhong Yang

May 9, 2019

High Speed Fluorescence Endomicroscopy with Structured Illumination for Robot Assisted Minimally Invasive Surgery

Haojie Zhang and Guang-Zhong Yang

The Hamlyn Centre for Robotic Surgery, Imperial College London, UK

hz1112@ic.ac.uk

INTRODUCTION

While confocal fluorescence microscopy provides an effective means of eliminating signals from out-of-focus light, its costs, complexity and low frame rate have motivated the development of alternative microscope techniques. Structured illumination microscopy (SIM) offers confocal-like out-of-focus background rejection without the need of beam scanning [1]. In this technique, a predefined illumination pattern is projected onto the sample, where only in-focus information is modulated due to rapid attenuation of high spatial frequency pattern of defocussed areas [2].

The imaging speed of SIM is related to the process of shifting the illumination pattern. In many early SIM systems, physical gratings are used to generate patterned illuminations via a mechanically moving stage. This is difficult to realize a very precise pattern shift, especially at a high shifting rate, which can cause artefacts in reconstructed images. The spatial light modulator (SLM) based SIM system enables precise and rapid control of the excitation pattern, allowing an accurate fast pattern shifting [3]. SLM has therefore been considered in many SIM designs [1,2,3]. However, its use with flexible fibres suitable for robot assisted minimally invasive surgery remains largely unexplored. An initial implementation of this kind of design can only realise an effective SIM frame rate of about 2 fps [1].

In this work, a fluorescence endomicroscopy with a flexible fibre bundle is demonstrated using a SLM-based SIM to produce optically sectioned images at acquisition speed as high as 40 fps. The modulation and optical sectioning of the endomicroscopy is also demonstrated.

MATERIALS AND METHODS

Imaging system

A schematic of the proposed imaging system is shown in Figure 1. The output beam from a CW solid-state laser (Vortran Stradus, λ = 488 nm, TEM_{00}) is expanded by a 6× telescope to a beam diameter of approximately 9 mm. Then, it is projected onto the SLM via a polarizing beam splitter. The SLM incorporates a phase-only ferroelectric liquid crystal on silicon device (Forth Dimension Displays QXGA-3DM) with 2048 ×1536 pixels and 8.2 µm pixel pitch. The grid pattern was generated with the SLM by displaying a grating which diffracts the light [4]. Since the SLM only changes the phase of the light, the contrast of the projected pattern is strongly affected by the polarization state of the light. For maximal contrast, a half-wave plate is placed between the SLM and the beamsplitter to rotate the polarization state of the light. A polariser is used to remove unwanted polarisation light. A spatial filter is applied at the focus of two achromatic doublet (f = 150 mm) to allow only the ±1 diffraction orders to pass. The spatial filter blocks the extra small peak between two high peaks and offers better contrast modulation at the specimen plane [5].

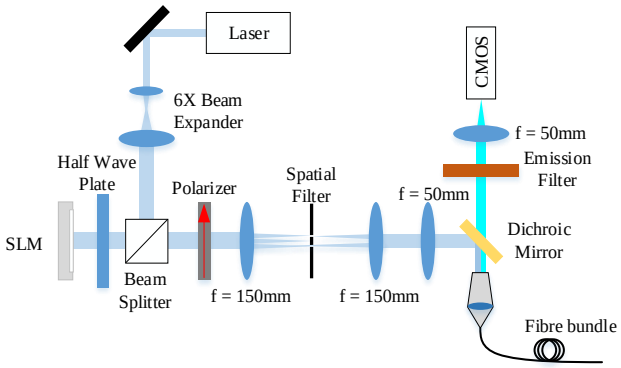

Fig. 1 Schematic of the proposed imaging system. A grid pattern is produced by a SLM and projected onto a sample via an imaging fibre bundle.

Image acquisition and processing

To construct the SIM endomicroscope, an imaging fibre bundle is used to guide both the grid patterned illumination to the specimen and the resulting fluorescence emission back to a CMOS camera. The imaging fibre bundle (Cellvizio Gastroflex UHD) has 30,000 cores with a 600 µm useful diameter. The distal end of the fibre bundle is equipped with a micro-objective (×2.5 magnification, working distance = 50 µm, field of view = 240 µm). The resultant fluorescence is relayed through the fibre bundle, isolated with a dichroic mirror and an emission filter, and imaged onto the CMOS camera (Point Grey FLEA 3) via an achromatic doublet (f = 50 mm). Typical image size is 512 × 512 pixels. Three fluorescence images (I_1, I_2, I_3) of the sample are taken at three different grid positions with a phase-shift of 90° between two adjacent grating [6]. A final optically-sectioned image (I_{SIM}) is then generated by using the following equation [6]:

$$I_{SIM} = \sqrt{(I_1 - I_2)^2 + (I_1 - I_3)^2 + (I_2 - I_3)^2}.$$

Since the maximum frame rate of the SLM is around 1 kHz, the speed of the endomicroscope depends on the

camera frame rate, which is around 120 fps, resulting a SIM sectioning speed of 40 fps.

RESULTS

In order to investigate the modulation ability of the system, a uniform fluorescent plane was first illuminated. Figure 2(a) is a computer-generated rectangular grid pattern to be displayed on the SLM. Figure 2(b) is a raw image of the uniform plane illuminated with the pattern in Figure 2(a). The obvious contrast between the ON and OFF pixels of the projected pattern demonstrate good modulation and perfect ON/OFF photo-switching of the fluorophores. The 2D Fourier transforms of Figure 2(b) is shown in Figure 2(c)&(d). The outer ring in Figure 2(c) is from the honeycomb structure of the fibre bundle, which determines the system resolution. The excellent contrast between the ON and OFF pixels can be future proved by the numerous sidebands in Figure 2(d).

A lens tissue paper stained with acriflavine was then illuminated with the same rectangular grating pattern to test the optical sectioning ability of the endomicroscopy. A raw image of the grating-illuminated tissue paper is illustrated in Figure 3(a), showing a clear distinguish between the ON/OFF region. Figure 3(b) is reconstructed from three raw images by using the algorithm showing above. The widefield image in Figure 3(c) is the average of the three raw images, corresponding to the non-sectioned fluorescence image [1]. Figure 3(d) shows profile along the lines in (b) and (c), demonstrating a clear reduction in fluorescence background when comparing the intensity distribution in both cases.

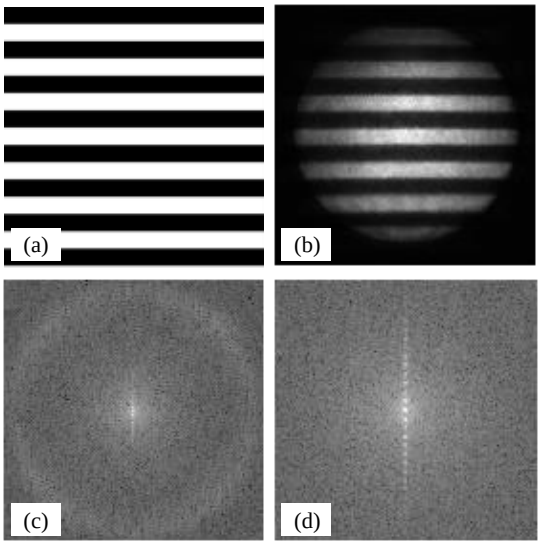

Fig. 2 (a) Pattern with a rectangular grating to be displayed on the SLM. (b) Image of a thin uniform fluorescent plane illuminated with the pattern in (a). (c) Corresponding Fourier transform of the image in (b). (d) Centre origin of the Fourier transform in (c).

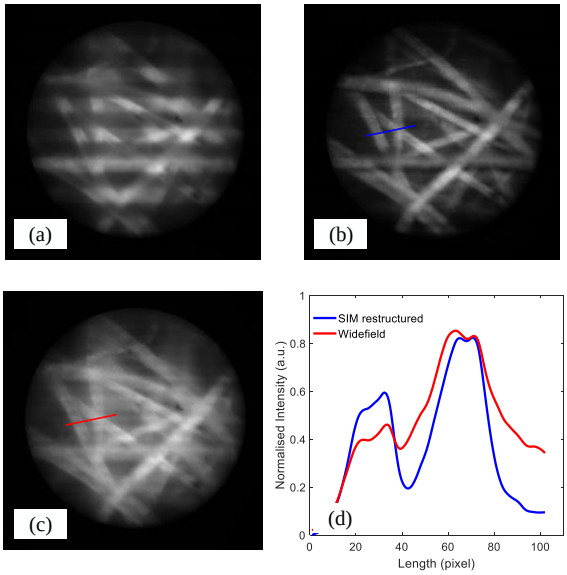

Fig. 3 (a, b and c) grating-illuminated, SIM and widefiled images of lens tissue paper stained with acriflavine. (d) SIM (blue) and wide-field (red) profile along the lines in (b) and (c).

DISCUSSION

A high-speed fluorescence endomicroscopy with a flexible fibre bundle is demonstrated using SLM-based SIM to produce optically sectioned images. The system can achieve camera frame rates up to 120 fps and a SIM image rates up to 40 fps, which is much higher than previous results with a similar design. Initial results show that the system has an excellent modulation and optical sectioning.

ACKNOWLEDGMENTS

This research is conducted with support from the EPSRC Grant EP/N019318/1 (REBOT: Robotic Endobronchial Optical Tomography).

REFERENCES

[1]. Bozinovic, Nenad, et al. “Fluorescence endomicroscopy with structured illumination.” Optics express 16.11 (2008): 8016-8025.

[2]. Xu, Dongli, et al. "Fast optical sectioning obtained by structured illumination microscopy using a digital mirror device." Journal of biomedical optics 18.6 (2013): 060503.

[3]. Kner, Peter, et al. "Super-resolution video microscopy of live cells by structured illumination." Nature methods 6.5 (2009): 339.

[4]. Martínez-García A, et al. “Operational modes of a ferroelectric LCoS modulator for displaying binary polarization, amplitude, and phase diffraction gratings.” Applied optics. 2009 May 20; 48(15):2903-14.

[5]. Fu HL, et al. “Optimization of a widefield structured illumination microscope for non-destructive assessment and quantification of nuclear features in tumor margins of a primary mouse model of sarcoma.” PloS one. 2013 Jul 23;8(7):e68868.

[6]. Neil, Mark AA, et al. “Method of obtaining optical sectioning by using structured light in a conventional microscope.” Optics letters 22.24 (1997): 1905-1907.