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Fiber optic probe for region of interest (ROI) selective time averaged multi-fluorescence imaging

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ABSTRACT

Time averaged imaging is one of the widely used methods to achieve improved image quality, used in different types of microscopic methods. Time averaged imaging refers to adjusting the exposure time of the imaging system to obtain optimal images. In state of the art microscopes, the region of interest (ROI) of illumination beam for time averaged imaging can be selected to be of regular shapes such as circle or rectangle. This forces smallest possible ROI to be larger than the actual sample's ROI which can be of a specific shape with complex contours. In this context, we present a flexible fiber bundle based illumination probe capable of illuminating samples of irregular shapes for time averaged imaging. Further, this probe is capable of multi-wavelength illumination, hence can be used for multi-fluorescence imaging. The fiber probe with features such as region selective and multi-wavelength illumination allows it to be used for optimal imaging of multi-fluorescence sample.

Keywords: Fiber optic imaging bundle, fiber probe, Selective illumination, microscopy

1 INTRODUCTION

Optical technology in particular biomedical imaging plays an important role in the disease diagnostics [1-8], biometric identification [4, 9] and therapeutic interventions [8, 10-12]. Biomedical imaging research has focused on the high resolution imaging, which has produced different imaging systems capable of imaging at micron and sub-micron scale resolutions [6, 13-18]. The state of the art high resolution imaging methods include but not limited to confocal microscope, structured illumination microscope, stimulated emission depletion (STED) microscopy, stochastic optical reconstruction microscopy (STORM) *etc*[15, 19-22]. Most of these high resolution imaging methods are table top microscopic systems. The use of flexible fiberprobe as imaging head enables the system to be portable.

Biomedical engineering and optics play a very vital role in the areas such as imaging [8, 10, 11] and translational medicine [1-8]. Over the years there has been a lot of advancement specifically in biomedical optics, lasers and imaging [12]. However, these techniques are limited in its potential when the selected region from the sample needs to be imaged without illuminating the non-selected regions. Such selective illumination is required for study of neuronal signaling. A comprehensive study of neuronal signaling demands the temporal precision of the order of milliseconds and illumination beam spot of diameter less than equal to 5 μm [23, 24].

In recent years, the selective illumination has served as an important aid in scientific advancement. However, these tools are capable of illuminating only one selected region at a time with the standard bounding box of circular or rectangular shape. These tools allow the improved localization of the light and are capable of illuminating multiple irregularly shaped regions in the sample. Most of these techniques use reflection mode imaging for the recording of the photo-activated processes or the effects of photonic stimulation. Furthermore, the use of the non-coherent or monochromatic light source prohibits them from using the samples having multiple photosensitive materials or reagents [18, 19]. The state of the art selective illumination methods use microscope objective for illumination which limits their use to the samples that are only suitable for benchtop microscope. Furthermore, there are instances when only a few cells or subcellular regions are of interest for imaging. However, the wide-field illumination generally reduces the image contrast. Additionally, wide-field imaging involves exposure to light over the entire FOV, which causes unintentional decay in the fluorescence signal in the non-selected sample region. In this context, this article describes the optical design and use of this method for selective time averaged multi-fluorescence imaging.

2.1 Optical setup

Figure 1. Optical schematic for spatiotemporally selective illumination and imaging fiber probe.

A schematic diagram representing the optical configuration of the developed targeted illumination and imaging fiber probe is shown in Figure 1. A laser light beam is directed towards the DMD to cover the complete pane of the DMD mirrors (14 mm X 10 mm). The DMD [0.7 XGA DDR, Texas Instruments] is a reflective SLM with 1024 X 768 micro-mirrors.

An array of the DMD mirrors acts as a reflective grating creating the higher ordered diffraction beams. The zeroth order beam reflected from the DMD is allowed to pass through a condenser lens assembly of the plano-convex lenses (focal length 35 mm and 75 mm) and a microscope objective lens (Magnification 20x; NA 0.4 air objective) reducing the beam size to 200 μm X 200 μm .

A FOIB [Sumitomo Electric, IGN-11/50] of 1 mm diameter is used as the probe arm of the developed system. A custom gradient index (GRIN) lens [GT-IFRL-100-cus-50-NC, Grintech GmbH] having a focal length of 0.3 mm is attached to the distal end face of the fiber probe. The FOIB-GRIN lens assembly is secured in a steel sheath (1.1 mm inner diameter and 1.2 mm outer diameter). A beam condenser assembly made up of the two plano-convex lenses is used along with the microscope objective to couple the light beam into the fiber bundle through the proximal end. An EMCCD [iXon 887, Andor] camera is used to capture the images from the proximal end of the fiber bundle. The bright field image collected through the fiber bundle is used as a reference input to select the regions in the sample to be illuminated. Using a commercial software [Pixel precision, 3DIcon], specific DMD mirrors are activated to perform selective illumination.

2.2 Proof of concept: selective illumination

The developed probe is capable of selective illumination as discussed in previous section. The developed probe was used to selectively illuminate parts of a mouse kidney cells such as cell boundaries. Figure 2 shows the images of cells from mouse kidney sections. The cells are imaged with widefield illumination, the image captured is used as a reference image. A spatial patterns were created on the DMD panel so as to illuminate the selected regions of the sample.

Figure 2. Selective illumination of mouse kidney cells. (a) Widefield image of mouse kidney cells. (b-d) Images of cells collected with selective illumination of cells and cell boundaries.

2.3 Selective time averaged multi-fluorescence imaging

In fluorescence imaging experiments, the sample can have an uneven concentration of fluorescent dyes. The uneven concentration of fluorescence in the sample always poses a hurdle in imaging with optimal quality. The exposure time of illumination system and the collection time of camera are controlled to improve image quality. However, the full FOV has to be illuminated for the longer time duration to capture a good quality image of the sample region with a low fluorescence concentration. This can cause either the pixel saturation in some parts of the image, bleach or even damage to the whole sample structure. On the contrary, for imaging the samples with high fluorescence concentration, the exposure time has to be reduced. It means, if the sample has multiple cells tagged with the different concentrations of the fluorescent dye then the multiple images have to be captured and stitched together for an optimal image. In another scenario, if the sample has multiple cells tagged with the fluorescent dyes which have different excitation wavelengths, then multiple images need to be captured separately at different times in order to

prevent unnecessary exposure to cells. In both the cases, multiple images have to be captured and stitched together which takes a longer image capture time and overexposes the samples to the irrelevant wavelengths of light. Also, it requires extra image processing to combine the images captured with different exposure times.

The developed probe enables a selective illumination of the multiple regions from FOV with the freedom to select each region with different exposure times (Figure 3). The potential advantage of the proposed probe comes from this perspective. The probe embodiment can illuminate multiple cells or cell organelles from the sample with the controlled exposure times. Figure 3 shows an image captured by illuminating three cellular regions from the test sample with two different wavelengths and different exposure times. The region 1 and 2 as shown in Figure 4.13 are two cells from mouse kidney section while the region 3 is the remaining part of the FOV. These three sample regions are illuminated for different time durations with a region specific monochromatic light and a single image is captured with long camera exposure time.

Figure 3. Multiple regions of mouse kidney section captured with different exposure times in a single frame. Region 1 was illuminated with 488 nm light for 0.2 s. Region 2 and 3 were illuminated with 561 nm light for 0.4 s and 0.1 s, respectively.

3 CONCLUSION

In this article, we have demonstrated the design and experimental evaluation of selective illumination optical fiber probe. The developed probe has been used to perform selective time averaged multi-spectral imaging. The developed probe eliminate the need of capturing multiple fluorescence images to get the optimal image as it illuminates selected regions for different exposure times. It has also improved the imaging speed for time averaged imaging of multi-fluorescence sample. It is envisaged that this novel optical configuration can offer a wide range of potential applications in a variety of fields such as diagnostic imaging, optogenetics, optical twizzers, to name a few.

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