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Miniaturized LED light source with a hybrid filter for fluorescent imaging

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Abstract

In this study, we developed a miniaturized needle-type fluorescent imaging device. This device allows deep brain function mapping of a rodent to be carried out with minimal invasiveness, simultaneously as the subject moves freely over a prolonged period. Further, we report on the design and fabrication process of our device. As such, micro-LED, which was employed as a fluorescent source, has been loaded with hybrid filters onto a CMOS chip. The LED was initially made further thinned to facilitate device miniaturization. The application of a hybrid filter removes green components from the blue LED and further contribute towards improved detection of fluorescence image for in-vivo applications.

1. Introduction

Conventionally, lens-based fluorescent microscopy is used for microscopic scale bio-imaging. On the other hand, a lens-free method has been gaining popularity over the years. Owing to favorable characteristics of exceptional small dimensions and lightweight, as well as being cost-efficient, has made the latter technique more appealing for implantable in-vivo imaging [1].

InGaN based blue micro-LED (μLED) has commonly been used as an excitation source targeted for green fluorescent proteins (GFPs) by virtue of its small dimension. The GFPs whereas has been widely employed as fluorophore markers in bioimaging application owing to its decent photo-stability.

We previously explored the use of fiber-coupled laser as GFPs excitation source [2]. While laser-based excitation source conveniently offers a narrower band spectrum and better control of irradiation spot, it requires the use of fiber optics to carry light signals from the laser. This factor compromised the capacity for brain imaging of freely moving conditions. Particularly, the stiff nature of the fiber material may lead to fracture from excessive bending during active brain scanning. In such an application, thinned μLED remains the superior option. Through laser lift-off (LLO), the sapphire substrate, which comprises of more than 90% of the original μLED thickness, can be effectively removed. Thus our research work conducts the fabrication and characterization of implantable fluorescence imaging devices attached to thinned blue μLED.

2. Micro LED Light Source with a Hybrid Filter

The broad emission band of the LED has proven to be one major hindrance in this application. This is due to the overlapping band in between the green emission component of the blue LED with the relatively weak fluorescent signal coming from the fluorophores. The intense green emission from the LED leads to poor signal detection sensitivity. Therefore, it is crucial to introduce an effective mean of filtering the overlapping component. Herewith, a hybrid filter composed of an interference filter, low-NA FOP, and blue absorption filter, has been developed with the aim to tackle the shortcoming mentioned above. Schematic diagram of the proposed light source is depicted in Fig. 1.

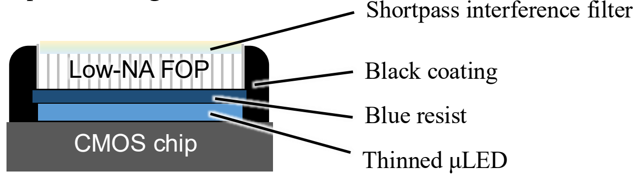

Fig. 1. Structure of the proposed light source

An interference filter effectively blocks photons at a specific wavelength. In our case, a short-pass band interference filter has been employed to attenuate the unfavorable longer wavelength band irradiated from the LED. The filter, whereas indiscriminately transmit all incoming photons from extremely tilted angles. This angle-dependent nature of interference filter still inevitably leads to poor SNR detection.

To overcome this, our interference filter has been coupled with a thinned low-NA fiber optic plate (FOP), which is made from bundled micron-diameters optical fibers. The FOP limits the angle of transmitted light. The NA characteristics are determined by the refractive indices of the core and cladding element of FOP. In our case, a low NA FOP was chosen as it absorbs relatively low angled incident light by virtue of the cladding material. Whereas high angle light gets transmitted through its core part. To maintain implant-ability feature of the device, the FOP thickness was reduced down to 120 μm using grinding and polishing apparatus. Furthermore, a drop-casted film of a blue absorption filter has been added at the bottom of the FOP to reduce the fluorescence of the μLED.

3. Fabrication process

A) Laser lift-off

LLO technique has been employed in the thinning process of the commercially available μLED (ES-CEBHM12A, epistar). The peak emission wavelength of this LED chip was approximately 460 nm. In this method, high-intensity UV laser pulses have been irradiated from the backside of the LED substrate. This caused the separation of InGaN LED crystal from its sapphire substrate. The release of intense energy from 266nm Nd:YAG laser-induced thermal decomposition on the surface boundary of the GaN layer, thus effectively break its interfacial bonding [3]. As such, the device prior thickness of 90μm has been reduced down to a single light-emitting layer of 8μm thick, thus realizing its miniaturization potential.

B) Device assembly.
The thinned 280 μm × 300 μm LED was mounted onto the irradiated area of a Si-CMOS chip. This CMOS part contains two aluminum pads; one for the cathode, another for the anode. First, Au bumps were bonded on the two electrodes by ultrasonic. The process then proceeded with the drop-casting of silver paste on top of the Au bonded pads. Next, the LED was lifted and placed. Both of the active contacts were connected to the pads of the chip. The alignment was done manually through the microscope. The sample then thermally cured at 120ºC for 30 minutes. The fabrication process was continued with the addition of a blue photoresist layer onto the back surface of the mounted μLED. The prior prepared layer of FOP and interference film was then added into the assembled devices (Fig. 2). Further, the sides of the device have been coated with black paint to reduce leakage.

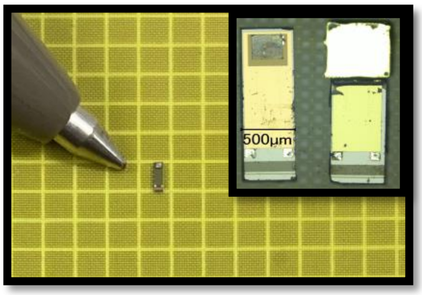

Fig. 2. Photograph of the imaging device. Inset picture shows CMOS chip with μLED mounted and another with filter loaded.

To complete with the fabrication process, the entire surface of the assemble device was covered with parylene coating. This step simultaneously provides biocompatible and hydrophobic layering for the assembled device. The process was carried out using PDS 2010 parylene coating equipment.

4. Evaluations

A) Spectrum comparisons
Short-pass interference filter has been added to cut-off emitted wavelength longer than 475 nm, spectral region at which GFP emission coincide with the higher end emission of the μLED. As shown in Fig. 3, our interference filter only effectively rejects incoming photons of low incident angle. As such, at normal angle of 0°, a sharp transmittance cut-off can be observed above 475 nm. Light rejection performance of the interference filter however diminished as the incident angle increased. For highly tilted light, the transmission peaks

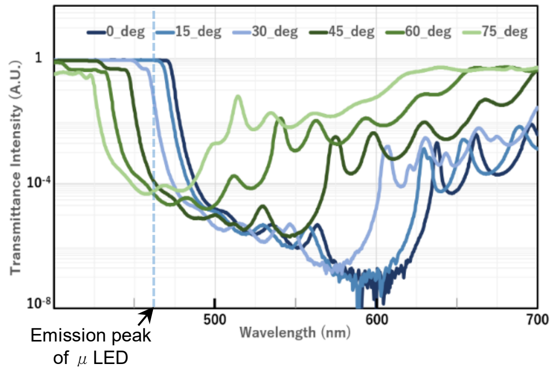

Fig. 3. Transmission spectra of the interference filter measured at different angles.

shift to shorter wavelength region. Hence the emergence of green components through the interference filter is not negligible. To address this, low NA FOP will be used as it enables the absorption of high angled incident photons.

B) Light emission profile,
In this measurement, the device was first submerged in 1mM fluorescein solution. Fig. 4 show the images obtained as the light beam from the μLED penetrating through the emission filter. Wide angled dispersion of light was observed as in Fig. 4(a) in the solution. This confirm the long wavelength passband of our interference filter that includes green components. By adding low-NA FOP as in Fig. 4(b), this undesirable component has successfully been blocked.

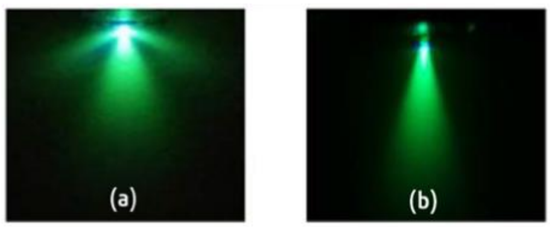

Fig. 4. Beam profile of μLED light sources with (a) Interference filter, (b) proposed excitation filter.

5. Conclusion

Here we successfully demonstrated a hybrid filter element, made from a composite layers of interference filter deposited onto a thinned low numerical aperture (low-NA) fiber optic plate (FOP) and a spin coated blue resist absorption film for the implantable fluorescence imaging device.

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