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One-lens fluorescence microscope using CMOS image sensor

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A fluorescence imaging module includes a fluorescence sample, a lens, an emission filter, an image sensor, and an illuminating device disposed between the sample and the lens. The illuminating device comprises a truncated pyramid for bending light emitted by the excitation light source toward the central area of the fluorescence sample. Light from the excitation light source enters from a slanted side surface of the truncated pyramid and emerges at the top of the truncated pyramid to illuminate the sample. The fluorescence image of the sample is formed by the lens through the emission filter on the image sensor.

Keywords: One-lens fluorescence microscope, compact fluorescence microscope, fluorescence microscope module, CMOS image sensor, truncated pyramid illuminating device.

1 Introduction

An optical microscope is originally designed so that the eye can directly see the object under the microscope. A microscope typically consists of at least an objective lens and an eyepiece lens [1]. The distance between two lenses is typically the sum of the focal length of the objective, the focal length of the eyepiece, and a standard distance known as the tube length, which is 160 mm. The objective forms a real image at an intermediate plane. The eyepiece functions like a magnifier so that the eye can see a magnified image of the real image formed by the objective. A camera can be attached to the microscope to photograph the object under the microscope. Traditionally, a photo eyepiece replaces an eyepiece to project the image formed by the objective onto the film or image sensor of the camera. The camera may be inserted in the center tube of a trinocular microscope as illustrated in Fig 1.

Fig. 1. A camera is inserted in the center tube of a tribocular microscope.

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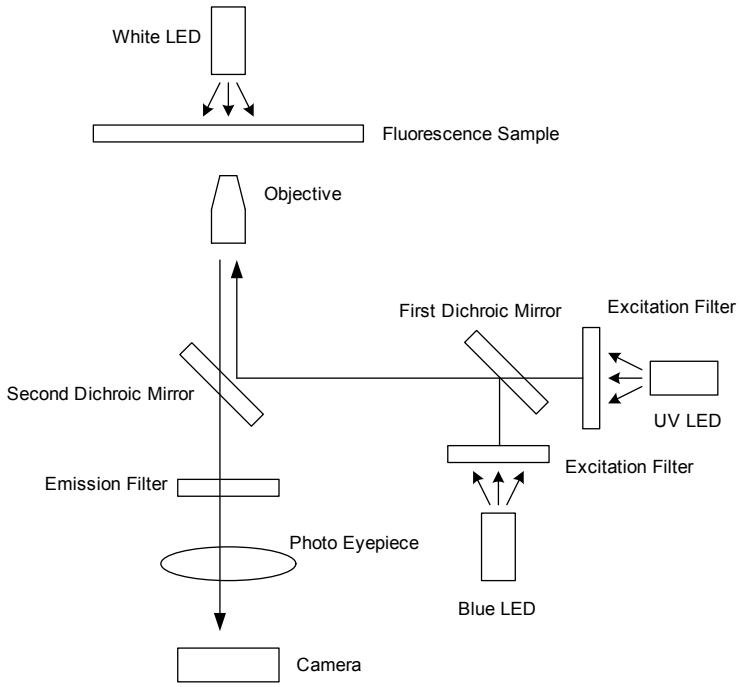
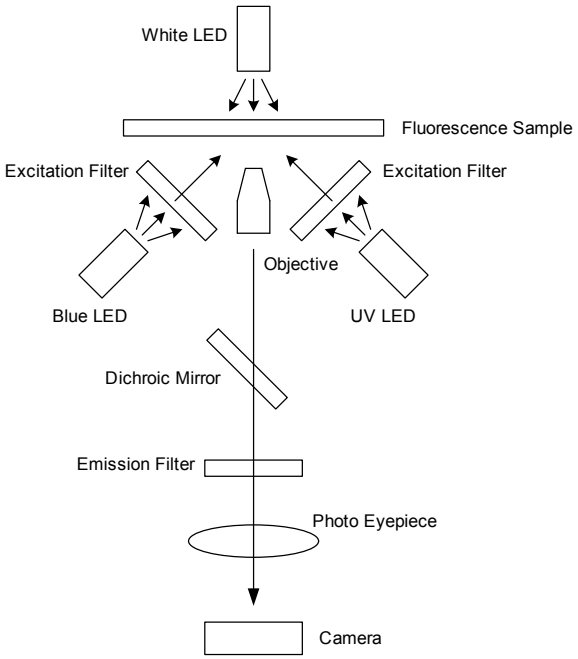
2 Traditional fluorescence microscope

The technique of fluorescence microscopy has become an essential tool in biology and biomedicine due to attributes that are not readily available in traditional optical microscopy. A fluorescence object emits fluorescence emission light after the absorption of excitation light. A variety of specimens exhibit autofluorescence when they are illuminated by excitation light. For non-autoflourescence speciments, the application of fluorophores has made it possible to identify cells and sub-microscopic cellular components with a high degree of specificity. The added fluorophores are excited by illuminating light of excitation wavelength and emit light having emission wavelength that is longer than the excitation wavelength. Fluorophores are stains that attach themselves to target structures. The widespread growth in the utilization of fluorescence microscopy is closely linked to the development of new synthetic and naturally occurring fluorophores with known profiles of excitation and emission, along with well-understood biological targets.

An example of traditional fluorescence microscope is depicted in Fig 2A [2,3]. A white LED illuminates a fluorescence sample on a microscope slide. A microscope objective in combination with an eyepiece images the sample on a camera. A UV LED and a blue LED are used to illuminate the sample. The illuminating light with UV and blue wavelengths excites the sample such that the sample emits fluorescence light. The sample may be autofluorescence or may be added with fluorophores. Excitation filters filter the excitation light emitted by the UV LED and the blue LED, respectively. The light beams after passing the excitation filters are combined by a first dichroic mirror. The combined excitation light beams are reflected by a second dichroic mirror to illuminate the sample. The emitted fluorescence light from the sample is transmitted through the second dichroic mirror, passes through an emission filter, and forms the image of the fluorescence sample on a camera. Another example of traditional fluorescence microscope is illustrated in Fig 2B [4]. The UV LED and the blue LED directly illuminate the fluorescence sample. No dichroic filters are required.



(A)



(B)

Fig. 2. (A) Traditional fluorescence microscope including a camera. (B) An alternative fluorescence microscope.

2 One-lens fluorescence microscope

In the construction of a fluorescence imaging module, first the required magnification is reviewed. Assume that the image sensor has a pixel size 1.4 μm and the desired resolution or the smallest feature of the sample is 0.2 μm. The required magnification is the pixel size divided by the smallest feature size, which is 1.4:0.2 = 7, provided that the lens has a resolution of 0.2 μm. Note that 0.2 μm resolution is commonly considered the best resolution that can be achieved by a lens.

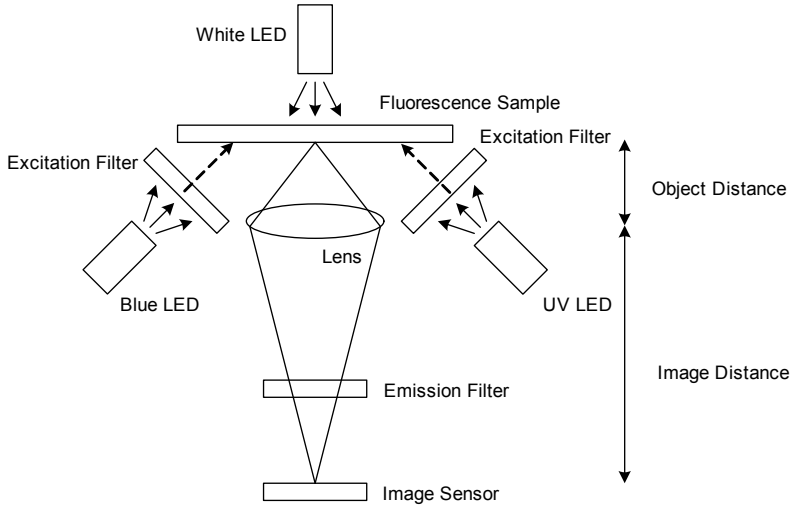


Fig. 3. Compact one-lens fluorescence microscope [5].

Figure 3 illustrates a compact one-lens fluorescence microscope proposed by Jutamulia *et al* [5]. A white LED illuminates a fluorescence sample on a microscope slide. A lens or lens system images the sample on a CMOS or CCD image sensor. CMOS image sensor is preferred since it is less expensive as compared with CCD image sensor. A UV LED and a blue LED are used to directly illuminate the sample. The illuminating light with UV and blue wavelengths excites the sample such that the sample emits fluorescence light. The fluorescence sample may be autofluorescence or may be added with fluorophores. Excitation filters are optionally provided to filter the excitation light emitted by the UV LED and the blue LED, respectively. The emitted fluorescence light from the sample is transmitted through an emission filter, and forms the fluorescence image of the fluorescence sample on the CMOS image sensor. No dichroic mirror is used in the proposed compact one-lens fluorescence microscope.

To provide the required magnification 7 (or M), the ratio of the image distance to the object distance must be 7 (or M). Using the lens equation $1/o + 1/i = 1/f$, where o is the object distance, i is the image distance, and f is the focal length of lens, we can calculate the image distance, the focal length of lens, and the total length, which is the sum of the object distance and the image distance, as functions of the object distance that meet the required magnification, e.g., 7, as shown in Table 1.

Table 1.

Magnification	Object Distance	Image Distance	Focal Length	Total Length (Object Distance + Image Distance)
7	5 mm	35 mm	4.375 mm	40 mm
7	10 mm	70 mm	8.75 mm	80 mm
7	15 mm	105 mm	13.125 mm	120 mm
7	20 mm	140 mm	17.5 mm	160 mm
7	25 mm	175 mm	21.875 mm	200 mm
7	30 mm	210 mm	26.25 mm	240 mm

3 Truncated pyramid illuminating device

From Table 1, it is seen that the compact one-lens fluorescence microscope of Fig 3 may be made shorter that the traditional fluorescence microscopes of Figures 2A and 2B. However, it would be difficult if not impossible to arrange the UV LED and the blue LED to illuminate the fluorescence sample for small object distance, e.g., 5 mm or 10 mm.

To solve the problem, a truncated pyramid having two parallel planes can be used for bending the illuminating light from the UV LED and the blue LED. For example, a truncated pyramid is illustrated in Figure 4A. The truncated pyramid bends the blue light emitted by the blue LED and the UV light emitted by the UV LED toward the central area of the fluorescence sample. Excitation filters are optionally provided to filter the excitation UV and blue light emitted by the UV LED and the blue LED, respectively. The truncated pyramid may be made from glass or other transparent materials. Light emitted from the UV LED and the blue LED may be collimated, and may be diverging. The UV LED and the blue LED may be replaced by other light sources including UV laser diode, blue laser diode, green and other laser diodes, and green and other LEDs.

On the other hand, the fluorescence light emitted from the sample to the lens may not be affected by the truncated pyramid, since the fluorescence light passes through the two parallel planes of the truncated pyramid. The sample will appear closer to the lens as illustrated in detail in Fig 4B. Figure 4C illustrates in detail the blue light from the blue LED is bent toward the central area of the sample.

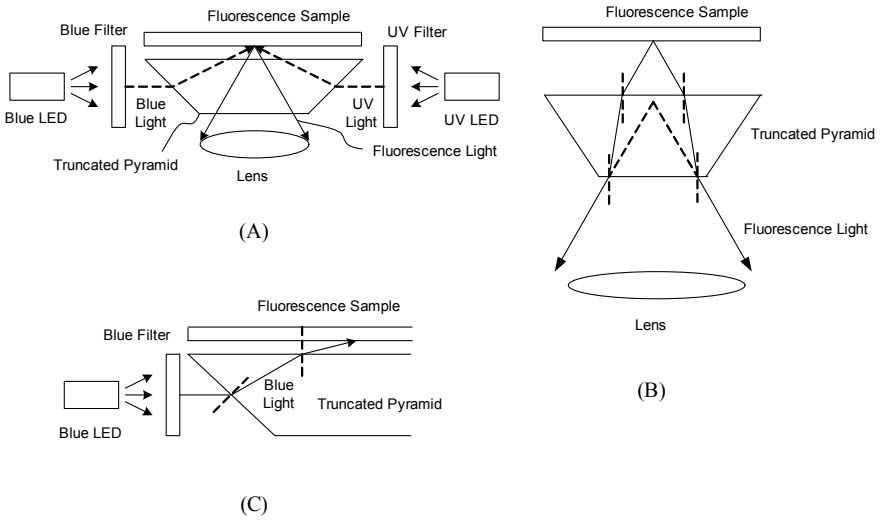


Fig 4. (A) Truncated pyramid illuminating device. (B) Fluorescence light passes through truncated pyramid illuminating device. (C) Blue light is bent by truncated pyramid illuminating device.

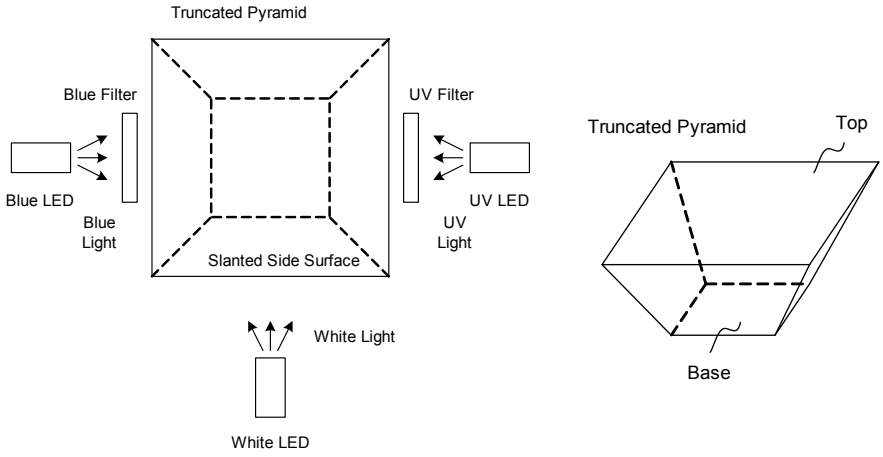


Fig. 5. (A) Truncated pyramid illuminating device having square base and square top. (B) Truncated pyramid illuminating device in perspective view.

Figure 5A shows a truncated pyramid having a square base and a square top. The slanted side surfaces connect the square base and the square top. Blue light emitted from the blue LED enters from the slanted side surface and emerges at the top square, and illuminates the central region of the sample. UV light emitted from the UV LED enters from the slanted side surface and emerges at the top square, and illuminates the central region of the sample. Excitation filters are optionally provided to filter the excitation light emitted by the UV LED and the blue LED, respectively. The white LED may be disposed on the side of the truncated pyramid. For example, light emitted from the white LED enters from the slanted side surface and emerges at the top square, and illuminates the central region of the sample. Another slanted side surface may be illuminated by a second white LED or light source having other excitation wavelength, e.g., green LED or green laser diode. The white LED may be replaced by other white light sources. In this manner, the white LED in Figure 3 is moved to the position under the sample, thus the total length of the

fluorescence microscope can be further shortened. Figure 5B illustrates the truncated pyramid in perspective view. The truncated pyramid may have polygonal base and polygonal top to accommodate a plurality of LEDs or light sources.

4 One-lens fluorescence microscope using truncated pyramid illuminating device

Figure 6 illustrates a compact one-lens fluorescence microscope using the truncated pyramid illuminating device. The illuminating device may comprise a truncated pyramid, a blue LED, a UV LED, and a white LED. The illuminating device may include a plurality of LEDs or light sources. The illuminating device includes at least one LED or any light source including diode laser that emits excitation light. The fluorescence microscope further includes a fluorescence sample, a lens, an emission filter, and an image sensor. The illuminating device is disposed between the fluorescence sample and the lens. In this manner, the emitted fluorescence light from the sample is transmitted through the emission filter. The lens forms the fluorescence image of the fluorescence sample on the image sensor.

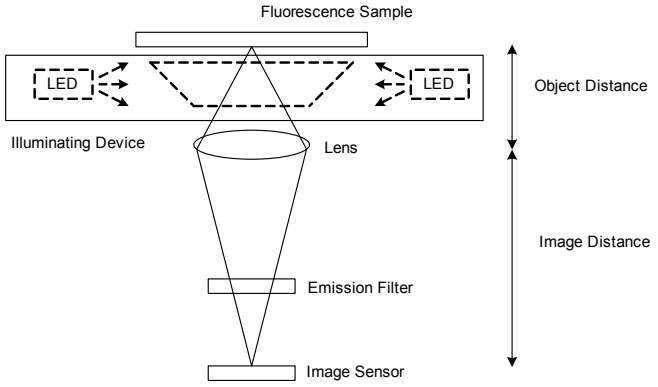


Fig. 6. Compact one-lens fluorescence microscope using the truncated pyramid illuminating device.

6 Concluding Remarks

It is possible to construct an illuminating device with a thickness approximately 5 mm including a truncated pyramid and the illuminating LEDs. Accordingly, a compact one-lens fluorescence microscope having a total length of approximately 40 mm, capable of magnifying a feature as small as 0.2 μm is possible. The magnified image detected by a CMOS image sensor is displayed on a large display monitor. Suitable and necessary image processing may also be performed.

For example, OmniVision's [6] OV16820 and OV16825CMOS image sensors enable 16-megapixel burst photography and can capture 4K2K, or Quad Full High Definition (QFHD), video at 60 frames per second (FPS). The pixel size is 1.34 μm.

References

1. Hecht E, Optics, Fourth Edition, (Addison Wesley, San Francisco), 2002.
2. Herman B, Fluorescence Microscopy, Second Edition, (Springer-Verlag, New York), 1998.
3. Lichtman J W, Conchello J-A, Fluorescence microscopy,Nature Methods, 2(2005)910-919.
4. Themelis G, Yoo J S, Soh K-S, Ntziachristos R S V, Real-time interoperative fluorescence imaging system usinglight-absorption correction, J Biomedical Opt, 14(2009)064012.
5. Jutamulia S, Gadjali H, Lei J, Fluorescence imaging module, US Patent Number8,785,885 (2014).
6. www.ovt.com (as of 26 August 2014).

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