LED illumination module for fluorescence and spectroscopic studies in microscopy

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Abstract. In this report we produce a microscopic illuminator consisting of four light emitting diodes (LEDs) with different wavelength. The radiation power of each LED could be controlled independently. The dichroic mirrors are used as a beam combiner. So, it is possible to create a highlight of the required spectral composition on the object under study. This illuminator is useful both for fluorescent analysis or spectral imaging.

1. Introduction
Nowadays, to study some types of micro-objects using a microscope, it is often not enough to obtain their image. There are various methods to increase the information content of research. In particular, for the study of biological objects using the technique of phase contrasting, fluorescent analysis, spectral imaging and other methods [1]. Spectral imaging requires either a wavelength-tunable light source [2, 3] or a wavelength-tunable optical filter, for example an acousto-optical [4], which allows us to take a series of images at required different wavelengths and thus analyze the spectrum of the light coming from each point of the object.

To excite the fluorescence in the object under study, narrow-band short-wave optical radiation should be directed at it. Typically, such light is generated using a filter that cuts out a portion of the spectrum of the radiation from a broadband source. Sometimes laser, LED of other sources are used for this technique [1].

There are a large number of different markers introduced into the object of study. They fluoresce when illuminated at different wavelengths. Recently, the technique of using multiple markers simultaneously, which are introduced into different parts of the object or mixed. This technique is applied first of all for living cells study. It allows different areas fluoresce at different spectral ranges.
For this technique it is more useful to implement a specialized light source that generates a radiation in several narrow spectral ranges. Such a source may also contain a wideband source and several light filters however it is more efficient if the lighter consists of several LEDs with different spectral ranges emitting simultaneously [5].

In our laboratory, tasks often arise when it is important not only to obtain luminescence, but also to measure the spectrum of luminescent radiation at each point of the object. This requires both a fluorescent illumination module and a spectral imager. Such systems for «mixed» fluorescent analysis and spectral imaging were previously created [6, 7], but their applicability is very limited in the number of spectral channels either in the lighting or in the registration branch.

To study of a large number of objects with minimal human involvement in this process it is required to automate the measurement process and synchronize the devices used: spectral imager, video camera, fluorescent module, microscope stage.

For spectral imaging we utilize a home-made acousto-optical spectral imaging module developed and fabricated specially for the microscope. It is described in detail in [8]. But there is no commercially available fluorescent illumination module which can be synchronized with our acousto-optical module. That is why we developed a LED illuminator for fluorescent microscopy of modular construction and with independent control of the radiation power of each LED source. In the near future we plan to synchronize it with other nodes of the microscope.

2. Designed LED illuminator

We used four sources LED1-LED4 of 3 W power with different spectra, as shown in figure 1 (a). Their radiation indicatrix are shown on figure 1 (b).

![Figure 1. LEDs spectra (a) and radiation indicatrix (b).](image)

The optical layout of the developed fluorescent illuminator is shown in figure 2. Lenses L1-L4 are used for beam collimation. We utilize equal collimating lenses, but their positions relative to the corresponding LEDs are adjustable to compensate the difference of LEDs indicatrix (figure 1 (b)) and
to ensure maximum uniformity of illumination of the object under study. Actually, lens positioning should be done once before the measurements. The combination of collimated beams is carried out by dichroic mirrors DM1-DM3.

![Optical scheme of the designed illuminator.](image)

**Figure 2.** Optical scheme of the designed illuminator.

The microscope attached with the designed fluorescent LED illumination module and acousto-optical spectral imager AOS is presented in the figure 3.

The designed fluorescent illumination module represents a beam combiner unit (1), to the housing of which two LED sources (2), (3) can be attached, or one LED source and a similarly designed beam combiner unit with a longer wavelength border. This allows us to apply simultaneously as many LED sources with different wavelengths as we need. In the case when only one LED source is utilized, it can be attached directly to the microscope. Thus, the modular principle of the developed fluorescent LED illuminator is implemented.

![Microscope with acousto-optic spectral imager (AOS) and designed illuminator.](image)

**Figure 3.** Microscope with acousto-optic spectral imager (AOS) and designed illuminator:
1 – beam combiner unit; 2, 3 – LED sources; 4 – control unit.

Each LED source is connected to a single control unit (4) that allows us to control the intensity of each LED source separately. By this time, we have manufactured a control unit in which manual
control of the power of each LED is implemented. In the future, software control and synchronization will be realized.

3. Conclusion
We developed a fluorescent LED illumination module which is convenient to apply with acousto-optical spectral imaging module to obtain a device for mixed fluorescent analysis and spectral imaging in microscopy of biological objects.

The modular design of the developed fluorescent illuminator allows the use any number of LEDs with spectral characteristics required for specific object studies. The power of each LED is graduated smoothly and independently of the others. Synchronization of all microscope devices will allow us to create an automated tool for the study of a large number of samples with minimal human involvement in the measurement process.

The designed illuminator could find application not only in microscopy. It can also be used in colorimetry tasks, for spectral visualization of objects with their non-destructive testing and in other applications.

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