

Low-cost modular design microscope for measuring fluorescent diamonds

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Received March 11, 2024; accepted March 27, 2024; published April 2, 2024

Abstract—Nowadays, microscopy is a widely used method for analyzing various materials. In our research, we use a developed two-channel fluorescence microscope to measure objects excited with two different wavelengths, e.g., fluorescent diamond powders with nitrogen vacancies. The results were compared with images illuminated with white LEDs observed using a microscope in transmission mode. The measurements yielded results with satisfactory resolution and image quality. Considering that the microscope is built with a budget design, it can be a cheaper alternative to commercial devices.

Since its invention, microscopy has been a valuable technique for observing many material details invisible to the human eye. The technique developed and described in this paper is fluorescence microscopy, which offers new possibilities, such as observing the response of molecules excited by radiation. The ability to observe the fluorescence of test substances is essential for studies of diamond small particles, which are widely used in biological diagnosis and labeling [1–4], including cytotoxicity studies on human pulmonary epithelial cells, cancer diagnosis [5], imaging living cells, long-term imaging, and tracking in complex cellular environments or biosensing and bioimaging due to the DNA-based coupling mechanism [6].

However, the limited number of substances subjected to fluorescence microscopy testing can be problematic. Many substances, especially biological ones that are of interest to scientists, do not show natural fluorescence. It is possible to partially solve this problem by synthesizing compounds that exhibit fluorescence. In such a case, a functional group (fluorescence center) is attached to the substance to be tested [7]. In this way, fluorescence centers become indicators of the presence of the examined substance [8]. Special requirements for fluorescence centers are needed for biological research as these fluorescence centers should be neutral to the biological tissue under study. Promising materials seem to be nanodiamonds [9]. The advantages of nanodiamonds have been indicated by their biocompatibility, non-toxicity, ease of detection of Raman signal, and intrinsic

fluorescence from their natural defects without complex pretreatment [10–11].

In nanodiamonds, as in other crystalline materials, there are defects in the crystal lattice. One of them is nitrogen-vacancy (NV) centers, point defects consisting of a pair of a nitrogen atom (N), which replaces a carbon atom, and a nearest-neighbor vacancy (V) in a crystal lattice. Such pairs may have a negative or neutral charge state and can act as fluorescence centers. They are also named color centers because they can absorb and emit radiation in the visible range [12].

Manufacturing nanodiamonds with a satisfactory number of NV centers requires irradiation with energetic neutrons, electrons, or ions and heating at a temperature of 600–1050°C to allow the migration of vacancies [12–13]. The substitutional nitrogen then captures the migrating vacancies to form NV centers. Additionally, surface functionalization can be used to conjugate proteins, metals, drugs, polymers, and lipids [3]. It has been shown that low toxicity, chemical stability, photostability, and excellent biocompatibility of such material have been achieved [6], [13–15]. Finally, nanodiamonds with negatively charged NV centers can be used as a fluorescence nanosensor based on a change in state of charge during optical excitation [13], [16].

A fluorescence spectrum is obtained by performing measurements of fluorescent diamonds with NV centers using a spectrometer. The spectrum shows a zero-phonon line falling at a wavelength of 637 nm. The transition of this line is due to electrons and has an energy of 1.945 eV. The transition occurs on the path between the ground energy state of the crystal lattice and the excited state [17].

Currently, several high-end microscopes are available on the market, ensuring high image quality. They are usually associated with the high cost of such devices. Additionally, these instruments are focused on one observation technique (e.g., standard, confocal, fluorescent), although constructions combining different techniques also appear [18]. That's why developing a

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modular, low-cost fluorescence microscope is very important.

In the first step, a single-channel system was developed. This system allows for observation of the sample in three ways. It is possible to obtain a fluorescent image, a reflected image (standard), and a mixed image (fluorescent + standard) showing fluoridation and other components of the examined sample simultaneously. Due to its modular design, the system can be modified easily. Modifications can include the replacement of the filter and light source depending on the examined sample and the expected wavelength of fluorescence excitation and emission.

Despite its simplicity, such a configuration change would be time-consuming and require re-aligning the entire system after each change. Thus, the final version of the developed system has a modular design. It was equipped with a channel that can excite biologicals at 430 nm and record results independently of those obtained from a channel that excites the sample with a 530 nm wavelength. In addition, the final version of the system has been supplemented (expanded) with a spectrometer module, allowing for the simultaneous spectral analysis of the tested sample.

A block diagram of the final version of the developed fluorescence microscope (two-channel one with the spectrometer) is shown in Fig. 1. The diagram shows two excitation sources, two detection channels, and a spectrometer. The optical path of the individual channels is also marked in the diagram. Selection of observations between the channels is done by switching on the corresponding light sources. This causes a change in the path of the laser beam, as indicated by the solid and dashed lines.

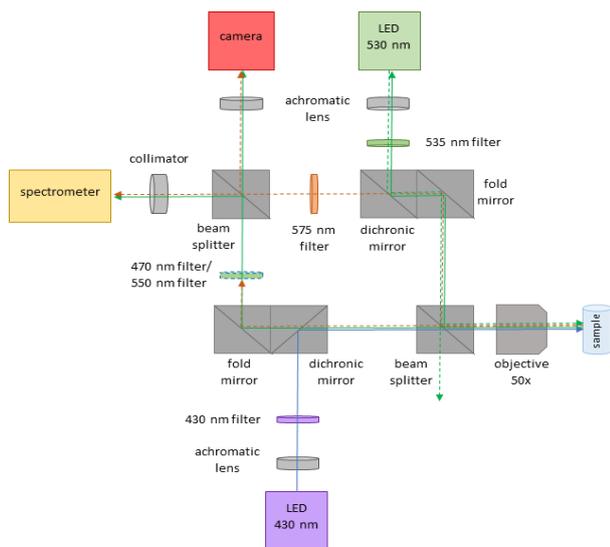


Fig. 1. Schematic of the two-channel fluorescence microscope with the marked laser beam path with a wavelength of 430 nm (solid line) and 530 nm (dashed line).

Several experiments were carried out to verify the developed system. They were mainly related to the fluorescence of diamond powders, so the 530 nm channel and a spectrometer were used. Two types of fluorescent diamond powders differing in the size of particles and NV center subtypes were used for the study: MD-NV-1 μ m-Hi and MD-NV-15 μ m-Hi (AdamasNano, USA). Both diamond powders contain nitrogen centers at 3000 ppb. The average particle size is 1 μ m and 15 μ m, respectively. All preparations were made as a mixture of diamond powder with distilled water. The mixture was spread in a small amount onto a microscope slide and placed directly over the objective. A 530 nm excitation source and a camera readout program were switched on.

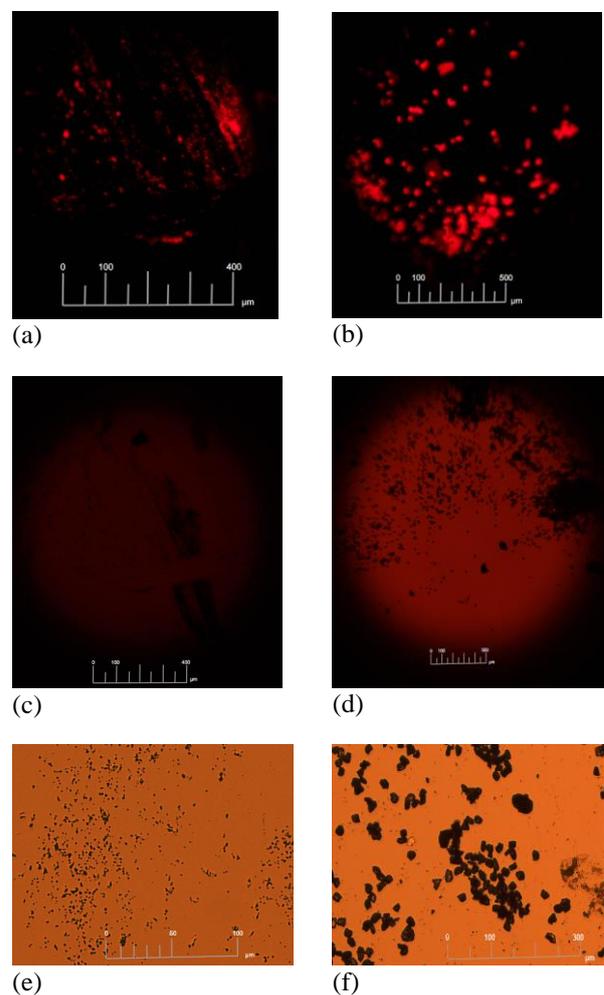


Fig. 2. Captured microscope images: fluorescence images of diamond powders with nitrogen vacancies MD-NV-1 μ m-Hi (first column) and MD-NV-15 μ m-Hi (second column) obtained using developed microscope (a)–(b), images obtained using developed microscope in transmission mode (c)–(d), and reference images obtained using microscope LAB 40 in transmission mode (e)–(f)

A view of the fluorescence image of the test slides was captured, as shown in Figs. 2 (a)–(b). The same samples

were also observed through a constructed microscope in a transmission mode (illuminated using white LED), as presented in Fig. 2 (c)–(d).

As can be seen in the mentioned Figs, experiments using samples utilizing diamond powders with NV centers confirm satisfactory accuracy and image quality of the developed system, especially about diamond powders with a particle size of several μm .

To compare the results presented by the constructed microscope, images of the same samples observed in transmitted white LED observed on a commercial microscope LAB 40 (Opta-Tech, Poland) were compared (Fig. 2. (e)–(f)).

In addition, a spectrometer was used to analyze the sample in more detail and to supplement the data with spectra of the samples studied. The obtained fluorescence spectra, created using the optical spectrometer Flame-S-XR1 (Ocean Optics, USA), with which the constructed system was equipped, are shown in Fig. 3.

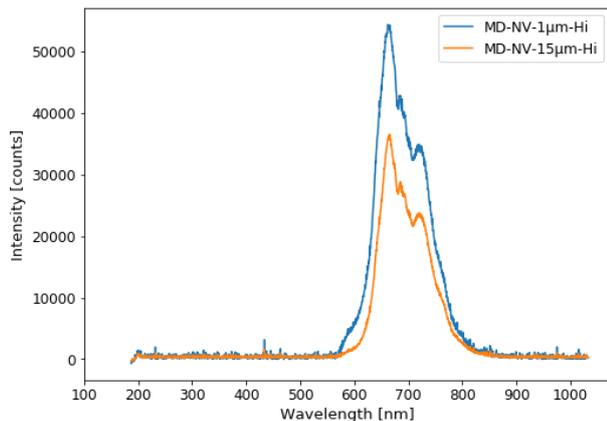


Fig. 3. Fluorescence spectra of preparations MD-NV-1 μm -Hi, MD-NVN-1 μm -Hi, MD-NV-15 μm -Hi (post-processing data).

The obtained spectra of the tested samples are NV-specific fluorescence spectra, consistent with expectations and comparable to data reported in the literature [19].

The main advantage of the developed microscope compared to other devices is its modular structure, which allows it to be easily adapted to the user's guidelines.

Additionally, low cost, simplicity of construction with satisfactory quality, and resolution of fluorescent images mean that the developed microscope can be considered a cheaper alternative to commercial devices.

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