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www.elsevier.com/locate/talanta

PII: S0039-9140(15)30550-6
DOI: http://dx.doi.org/10.1016/j.talanta.2015.12.018
Reference: TAL16187

To appear in: *Talanta*

Received date: 30 September 2015
Revised date: 2 December 2015
Accepted date: 10 December 2015

Cite this article as: Xiao-Xia Fang, Han-Yang Li, Pan Fang, Jian-Zhang Pan and Qun Fang, A Handheld Laser-Induced Fluorescence Detector for Multiple Applications, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2015.12.018

A Handheld Laser-Induced Fluorescence Detector for Multiple Applications

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ABSTRACT

In this paper, we present a compact handheld laser-induced fluorescence (LIF) detector based on a 450 nm laser diode and quasi-confocal optical configuration with a total size of 9.1 × 6.2 × 4.1 cm. Since there are few reports on the use of 450 nm laser diode in LIF detection, especially in miniaturized LIF detector, we systematically investigated various optical arrangements suitable for the requirements of 450 nm laser diode and system miniaturization, including focusing lens, filter combination, and pinhole, as well as Raman effect of water at 450 nm excitation wavelength. As the result, the handheld LIF detector integrates the light source (450 nm laser diode), optical circuit module (including a 450 nm band-pass filter, a dichroic mirror, a collimating lens, a 525 nm band-pass filter, and a 1.0 mm aperture), optical detector (miniaturized photomultiplier tube), as well as electronic module (including signal recording, processing and displaying units). This detector is capable of working independently with a cost of ca. $2000 for the whole instrument. The detection limit of the instrument for sodium fluorescein solution is 0.42 nM (S/N = 3). The broad applicability of the present system was demonstrated in capillary electrophoresis separation of fluorescein isothiocyanate (FITC) labeled amino acids and in flow cytometry of tumor cells as an on-line LIF detector, as well as in droplet array chip analysis as a LIF scanner. We expect such a compact LIF detector could be applied in flow analysis systems as an on-line detector, and in field analysis and biosensor analysis as a portable universal LIF detector.

Keywords: handheld instrument, laser induced fluorescence detection, capillary electrophoresis, flow cytometry, chip scanner

Abbreviation: laser-induced fluorescence (LIF); fluorescein isothiocyanate (FITC); capillary electrophoresis (CE); point of care testing (POCT); numerical aperture (NA); light-emitting diode (LED); photodiode (PD); avalanche photodiode (APD); analog-to-digital converter (ADC); signal to noise ratio (SNR); forward scattered (FSC).

Introduction

Laser-induce fluorescence (LIF) is one of the most sensitive detection techniques and widely used in capillary electrophoresis (CE) [1-2], microfluidic chip-based analysis [3] and microarray chip-based analysis [4]. Currently, there is a growing urgent requirement for miniaturized LIF detectors in many research fields, such as field analysis [5-6], point of care testing (POCT) [7], environmental analysis [8], and bioanalytical analysis [9-10].

Various miniaturized LIF detection systems have been developed. [11-13] Fruetel et al. built a miniaturized epifluorescent LIF module in a handheld microchip-based CE analyzer for protein analysis with a total size of 11.5 × 11.5 × 19.0 cm. [14] The LIF module integrated a 405-nm laser diode, two 0.60 numerical aperture (NA) aspheric lenses, a photomultiplier tube and other optical components, with an overall size of 7.5 × 5.5 × 3 cm. The detection limits for fluorescent dyes and fluorescamine-labeled proteins were in the picomolar and nanomolar range, respectively. Mathies' group developed a confocal LIF-based portable scanner for microchip capillary array electrophoresis. [4] The instrument, with a size of 30 × 30 × 20 cm with a solid-state 488 nm laser, contained a LIF system, a pneumatic system, four temperature control systems and four high voltage power supplies. The confocal LIF system with a rotating objective was used as a scanning detector for multichannel capillary array electrophoresis in this analyzer. The detection limit of the instrument was <20 pM for on-chip capillary electrophoresis of fluorescein dyes. Singh et al. reported a microfluidic platform for point-of-care testing of biological toxins in body fluids with a total size of 23 × 20 × 13 cm. [9] A miniaturized LIF system with two lasers (532 nm and 633 nm) and two detection channels was used in microfluidic electrophoretic immunoassays. The detection limits were 0.3 nM for SEB, 0.5 nM for Shiga toxin I and 20 nM for ricin before sample preconcentration. In most of these miniaturized LIF systems, 405-nm laser diodes were used as light source, but the laser wavelength is far from the maximum excitation wavelength (ca. 490 nm) of green fluorescent dyes frequently used in current LIF detection, such as fluorescein isothiocyanate (FITC) and calcein AM, resulting in the decrease of detection sensitivity.

Some research groups [15-18] used light-emitting diode (LED, 490 nm or 478 nm) instead of laser diode as light source and photodiode (PD) or avalanche photodiode (APD) instead of photomultiplier tube as optical detector, to further reduce the size and cost of LIF detection systems and provide more suitable excitation wavelengths for green fluorescent dyes. The authors' group also used a concave LED and a photodiode to build a LIF detector. [17] Although an optical module size of 5 × 12 × 6 cm and a detection limit of 0.92 μM for sodium fluorescein were obtained, the miniaturization and integration of the whole LIF system had not been realized.

In most of the researches in miniaturization of LIF systems, the miniaturized LIF detection systems were usually used as a detection module of a whole analysis system, rather than an integrated detector system capable of working independently. In this work, we developed a compact handheld LIF detector based on quasi-confocal optical configuration, which integrated a 450 nm laser diode light source, optical modules, a photomultiplier tube, as well as signal recording, processing and displaying unit with a total size of 9.1 × 6.2 × 4.1 cm. It could be used as an independent detector and flexibly coupled to various analysis systems. Since there is few report on the use of 450 nm laser diode in LIF detection, especially in miniaturized LIF detector, systematic investigations on optical modules matching the 450 nm laser diode were carried out to meet the needs of a miniaturized LIF detector. We applied the LIF detector in multiple applications including high-speed capillary electrophoresis of amino acids, flow cytometry of tumor cells and scanning detection of microfluidic droplet array chip.

Material and methods

2.1. Chemicals and Reagents

All chemicals of reagent grade were used and demineralized water was used throughout. Standards of arginine, phenylalanine, alanine, glycine, glutamic acid and aspartic acid were purchased from Kangda Amino Acid Works (Shanghai, China). Sodium fluorescein and FITC were purchased from Sigma-Aldrich (St. Louis, USA). A 5 mM borate buffer (pH 9.2, Sinopharm Chemical Reagent Co., Shanghai, China) was used as the working electrolyte for CE separation. A series of sodium fluorescein solutions with different concentrations for system performance testing were prepared by diluting 1 mM sodium fluorescein with 5 mM borate buffer. A mixture of 1 μM FITC-labeled amino acids solutions for capillary electrophoresis separation were prepared as previously described elsewhere [19]. In flow cytometry of tumor cells, 5 μL PBS solution with 10% (v/v) of calcein AM was added to 1 mL HepG2 cell suspension after incubate at 37°C for 30 min. After centrifuging for 2 min in 1000 rpm, the supernatant was removed. The HepG2 cell suspension was diluted by 2 mL PBS before analysis.

2.2. Building of the LIF detector

Images and schematic diagram of the LIF detector are shown in Fig. 1. A 450 nm laser diode (PL450B, Osram, Munich, Germany) was used as light source. After passing through a 2.0 mm aperture and a 450 nm band-pass filter (BF450, HB Optical Technology Co., Shenyang, China), the laser beam was reflected by a dichroic mirror (DM505, HB Optical Technology Co., Shenyang, China) with high reflectance below 505 nm and focused by a collimating lens (0.30 NA, 7.0-mm-long working distance, Leimai Electronics, Guangzhou, China) with a focusing spot size of 15 μm. The emitted fluorescent light was collected by the same collimating lens. After passing through the same dichroic mirror and a 525 nm band-pass filter (BF525, HB Optical Technology Co., Shenyang, China), reflecting by a mirror, and passing through a 1.0 mm aperture, the fluorescence beam was detected by a miniaturized photomultiplier tube (H10722-01, Hamamatsu, Shizuoka, Japan). The output signals from board and displayed on the screen of the detector.

2.3. Electronic circuit module of the handheld LIF detector

A block diagram of the LIF detector circuit is shown in Fig. 2, and more detailed information is provided in Fig. S1, Fig. S2, and Table S1. A 16-bit MSP430F1611 (Texas Instruments, Dallas, USA) was selected to serve as the embedded microcontroller. A build-in 12-bit analog-to-digital converter (ADC) was utilized to acquire the signal of fluorescence intensity and a 12-bit digital-to-analog converter (DAC12) was implemented to regulate the negative high voltage of integrated photomultiplier tube module. The data acquired were stored in a TF card which connected to the SPI port of MSP430F1611. The laser diode was supplied with a constant current source which was controlled by the MSP430F1611. A four-button keypad and a 320×240 pixels TFT LCD screen were incorporated to construct the user interface. The USB interface for communication also was provided by a UART to USB Chip FT232 (Future Tech., Wokingham, United Kingdom). The circuit was powered by a 3.6 V Li-ion battery. The main part was supplied by 3.3 V which was regulated by SP6203 (Sipex, Milpitas, USA) and the photomultiplier tube was supplied by ±12 V voltages, which were converted by power convert module E0312T (Mounsun, Guangzhou, China).

2.4. Application of the detector

For application in CE separation, a mini x-y-z translation stage with size of 6 × 1.4 × 4.6 cm (Daheng Group, Inc., Beijing, China) was fixed on the LIF detector for adjusting the position of the capillary to make the laser focusing point at the channel center of the capillary. In CE separation, a slotted-vial array device [20] for achieving automated sample injection and a high-voltage power supply (Tianjin Dongwen Co., Tianjin, China) for providing high voltage in the range of -6000-0 V were used for electrophoretic separation. High speed CE separation for FITC-labeled amino acids was carried out using the translational spontaneous injection method as previously described elsewhere. [19]

In the flow cytometry, a tapped tip capillary (500 μm i.d. 690 μm o.d.; tip diameter, 100 μm i.d.; Reafine Chromatography Co., Yongnian, China) inserted in a 750 μm i.d. square capillary (Wale Apparatus, Hellertown, USA) was used to generate hydrodynamic focusing flow with sheath flow. The flow rates were controlled by a syringe pump (PHD 2000, Harvard Apparatus, Holliston, USA) and a liquid-level difference driving device. A photodiode (OPT101, Texas Instruments, Dallas, USA) was used for forward scattered signal detection.

In the scanning detection for droplet array chip, a glass chip with 6 × 6 nanowell array fixed on an automated x-y-z translation stage (Zolix, Beijing, China) was used, with a well size of 100 μm depth and 400 μm diameter. The microchip fabrication and droplet generation methods are as described previously. [21]

Results and discussion

3.1. Quasi-confocal configuration

Miniaturization of a LIF detector usually includes the simplification of optical structure and the miniaturization of optical elements. Currently, confocal configuration [22-24] is one of the most frequently used formats in LIF detection systems. It is suitable for complex optical signal analysis, especially fluorescence imaging, tomography and high-sensitivity detection. Furthermore, compared with other types of LIF optical configurations such as orthogonal configuration, the LIF detectors with confocal configuration have more open top space for installing sample vessels, such as capillary or microchip, which significantly facilitate the experimental operations. However, they also have limitations such as sophisticated optical structure and the difficulty in miniaturization. In this work, in order to achieve the miniaturization of LIF system, we used the quasi-confocal configuration [25] and systematically optimized the main optical modules and Raman effect of water in this system.

Optical modules

The size of the optical modules is an important factor governing the whole size of the detector.

3.2.1. Light source

For reducing the size of light source, the authors' group had used a concave LED as the light source for LIF detector in a previous work [17]. Although the collimation property of the concave LEDs is much better than common LEDs, the loss of light intensity in light focusing process is still much larger than a laser light source with the same optical power. We also tried to use diode-pumped lasers [26-28], however their size and power consumption were still too large to be used in a handheld LIF detector. Laser diodes have small size, low price, stable optical output and multiple available wavelengths (405 nm, 450 nm, 520 nm, 635 nm, etc.), which are perfectly suitable for miniaturized portable devices. Compared with 405 nm laser diode, 450 nm laser diode (commercially available) has excitation wavelength more closed to green fluorescent dyes. It also has moderate cost compared with expensive 488 nm laser diode recently available in market. Therefore, we chose a 450 nm laser diode as the light source of the LIF detector and developed a set of miniaturized optical modules matching the light source.

3.2.2. Focusing lens

Most conventional LIF systems use normal microscope objective with large numerical aperture as the focusing lens to obtain small focusing spot and achieve high detection sensitivity and spatial resolution. [29-30] However, microscope objectives are usually too large for a handheld LIF detector. Therefore, we used a small collimating lens with a size of 10 × Φ9 mm to replace the 20 × microscope objective

(Dayueweijia Technology Co., Beijing, China) as the focusing lens of the handheld LIF detector, which was originally used in a 450 nm laser diode for laser beam collimation. The characteristics of both the normal microscope objective and the collimating lens are shown in Table 1. Compared with the microscope objective, the collimating lens has smaller size, lower price, better transmittance of 450 nm laser (14.9% better than microscope objective), and pretty good numerical aperture value (better than 10 × microscope objective with 0.25 NA). The objective lens is 30 times larger than the collimating lens with almost the same numerical aperture, focus spot size (as shown in Fig. 3) and collection efficiency of fluorescence signal. Thus, we used the collimating lens as the focusing lens in this LIF detector. The total size of the laser diode light source with the collimating lens was only 12 × 12 × 18 mm.

3.2.3. Pinhole

In a typical confocal optical configuration, usually a small pinhole [31] and a second focusing lens are used to filter the scattering light. However, such a configuration needs relatively long focusing distance between the pinhole and focusing lens. For reducing the detector size, we did not use the second focusing lens and the small pinhole as the formal confocal configuration, but only used an aperture with a relatively large diameter of 1.0 mm coupled with the filter to eliminate the scattering light as the quasi-confocal configuration. The 1.0 mm diameter of the aperture was chosen according to the size of the fluorescence spot of sodium fluorescein filled in the capillary imaged on the window of the photomultiplier. Such a simplification in optical configuration led to an increase of ca. four times in detection limit than conventional confocal fluorescence detection systems. [32] This reduce in detection performance could be accepted for a miniaturized LIF detector.

3.2.4. Filter

The effects of different filters were studied using different combinations of three 9-cm diameter excitation and emission filters, including a 450 nm band-pass filter for 450 nm laser diode, a 488 nm long-pass filter (BLP01-488R-9-D, Semrock, Rochester, USA) and a 525 nm band-pass filter for signal collection. A 20 nM sodium fluorescein solution was used as model sample. As shown in Table 2, the highest signal to noise ratio (SNR) result was obtained with the combination of the 450 nm band-pass filter, the 488 nm long-pass filter and the 525 nm band-pass filter (Fig. S3). For maintaining consistent conditions, the intensity of the laser beam after passing through the 450 nm band-pass filter was enhanced to 20 mW being equal to that of the original laser diode. However, when the 488 nm long-pass filter was not used, the SNR value only reduced by 1.78%, showing its insignificant effect in improving SNR. Therefore, we chose the 450 nm band-pass filter and the 525 nm band-pass filter as the excitation and emission filters, respectively.

3.3. Raman effect of water

The Raman signal of water with 450 nm excitation light is at 535 nm (Fig. 4a), which is close to the fluorescence wavelength of FITC (519 nm) and will disturb the fluorescence detection in this system. To measure the interference of the Raman effect of water in this detector, ethanol was used as control solution, because it has almost the same refractive index value as water (1.33299 and 1.36048 at 20°C, respectively [33]), while no obvious Raman signal in the range of 450 to 700 nm (as shown in Fig. 4a). The Raman effect was studied by injecting air, ethanol and water sequentially into a 50 μm i.d. capillary. The signal levels for ethanol and water were 256 and 262 (Fig. 4b), respectively, showing that the Raman signal was only 2.3% of the background signal, which could be omitted.

3.4. Performance of the LIF detector

To demonstrate the performance of the handheld LIF detector, we used sodium fluorescein as the model sample. As shown in Fig. 5a, a detection limit of 0.42 nM (S/N = 3) for sodium fluorescein was obtained with the highest sampling frequency of 5 kHz, which could meet most of the requirements of

routine biochemical detection and analysis. The whole detector was miniaturized into a total size of only 9.1 × 6.2 × 4.1 cm (width × length × height), weighing 350 g. Due to the simplification in optical modules and configuration, the cost of the LIF detector was reduced to ca. $2000. We also tested the working stability of the detector in a long-term (5 h) continuous detection of 100 nM sodium fluorescein, the relative standard deviation (RSD) was below 1%.

3.5. Application of the LIF detector

3.5.1. Capillary Electrophoresis

The detector was applied in the CE separation of 1 μM FITC-labeled amino acids after optimizing the effects of separation high voltage, sample concentration and high voltage of photomultiplier tube. As shown in Fig. 5b, almost baseline separation of six FITC-labeled amino acids (arginine, phenylalanine, alanine, glycine, glutamic acid and aspartic acid) and FITC was achieved within 7 s with an effective separation length of 20 mm and electrical field strengths of 1100 V/cm. High separation efficiencies of 0.24-0.45 μm plate heights were obtained, which are comparable to most of microchip-based CE systems. [34]

3.5.2. Flow cytometry

The detector was further used in flow cytometry of calcein AM labeled HepG2 cells. We optimized the position of laser focusing spot, shape of hydrodynamic focusing flow in the square capillary, as well as detection angle and position of the photodiode for forward scattered signal. Preliminary results (Fig. 5c) showed that the fluorescence signals of single cells could be detected with good correspondence to the forward scattered (FSC) signals with a detection throughput of 240 cells/min, which demonstrates the application potential of the present device in flow cytometer as a fluorescence signal detector.

3.5.3. Scanning detection

After optimizing the distance to the chip and the scanning speed, the detector was also used in the fluorescence scanning detection of a droplet array chip with 3 × 6, 100 nL droplets of 500 nM sodium fluorescein. A three-dimension surface plot (Fig. 5d) shows the fluorescence intensity profile of the droplet array chip with 4.59% (n = 18) RSD of the highest fluorescence intensity for each droplet. Droplets with different concentrations of sodium fluorescein of 0 M, 100 nM, 200 nM, 500 nM, 800 nM, and 1000 nM were also detected with a good linear regression equation of $y = 409.5x + 9.79$ ($r2 = 0.9986$).

Conclusions

In this work, we developed a compact handheld quasi-confocal LIF detector with 450 nm laser diode as light source. Actually this is the first report on using a 450 nm laser diode in a miniaturized LIF detector. After investigations to various modules and optical configuration of the detector, higher miniaturization and integration level of the detector over most of the previously-reported miniaturized LIF detection systems was achieved. Its detection performance is comparable to those previously reported systems using semiconductor pump laser as light source. [32]

The present detector has broad applicability due to its compact size and the ability of independently working. In addition to the CE separation and flow cytometry as demonstrated in this work, it can also be used as an on-line LIF detector for flow analysis systems, such as multiphase microfluidics and liquid chromatography. Furthermore, this detector could also be combined with microfluidic chips, microarray chips or biosensors and used in field analysis, point of care testing and environmental or industrial monitoring as a portable universal detector or a portable LIF scanner.

Acknowledgments

Financial supports from Natural Science Foundation of China (Grants 20825517, 21027008, 21227007, 21435004, and J20131203) and Natural Science Foundation of Zhejiang Province of China (Grants LY14B050002) are gratefully acknowledged.

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Figure Captions

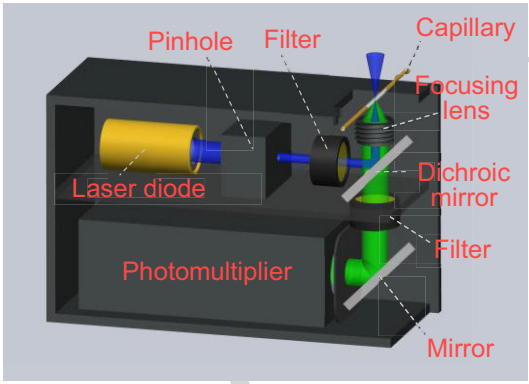
Fig. 1. (a, b) Images of the handheld LIF detector. (c) Schematic diagram of the LIF detector (not in scale).

Fig. 2. Electronic schematics of the specially developed circuit board.

Fig. 3. CCD images showing the laser spots in the capillary channels when focused by (a) a collimating lens for 450 nm laser and (b) a 20 × normal microscope objective.

Fig. 4. (a) Fluorescence spectra of water, borate buffer and ethanol when excited by a 450 nm laser diode. (b) Signal recordings of sequential injection of air, ethanol and water into a 50 μm i.d. capillary with a high voltage of -900 V for the photomultiplier tube, sampling frequency of data acquisition: 100 Hz.

Fig. 5. (a) Fluorescence signal recordings of sequential injections of 10 nM, 20 nM, 50 nM, and 100 nM sodium fluorescein solutions into a 50 μm i.d. capillary, sampling frequency of data acquisition: 100 Hz. (b) Electropherogram of a mixture of 1 μM FITC-labeled amino acids under optimized conditions: tapered-tip capillary; sample reservoir removing angle, 90°; effective separation length, 20 mm; electric field strength, 900 V/cm; working electrolyte, 5 mM borate buffer (pH 9.2); sampling frequency of data acquisition, 1000 Hz. (c) Signal recording of forward scattered light and fluorescence in flow cytometry of HepG2 cells labeled by calcein AM. Conditions: high voltage of photomultiplier tube, -600 V; sampling frequency of data acquisition, 5000 Hz. (d) Fluorescence surface plot of a droplet array chip with 3 × 6 100 nL droplets of 500 nM sodium fluorescein. Conditions: high voltage of photomultiplier tube, -600 V; sampling frequency of data acquisition, 1000 Hz.



Highlights[1]

A handheld laser-induced fluorescence detector with 450 nm laser as light source

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laser-induced fluorescence (LIF); fluorescein isothiocyanate (FITC); capillary electrophoresis (CE); point of care testing (POCT); numerical aperture (NA); light-emitting diode (LED); photodiode (PD); avalanche photodiode (APD); analog-to-digital converter (ADC); signal to noise ratio (SNR); forward scattered(FSC).

Investigation of optical module effects for achieving system miniaturization

High miniaturization and integration level over most previous LIF detection systems

Broad applications in capillary electrophoresis, flow cytometry and droplet analysis

Table 1. Performance of two focusing lenses.

Focusing lens	**Size (mm)**	**Diameter of laser spot (μm)**	**Numerical aperture**	**Transmittance of 450-nm laser (%)**	**Collection efficiency of fluorescence (%)**
20× normal microscope objective	43×Φ24	Φ14.5	0.40	83.53	77.27*
Collimating lens for 450 nm laser	10×Φ9	Φ14.6	0.30	95.97	80.77*

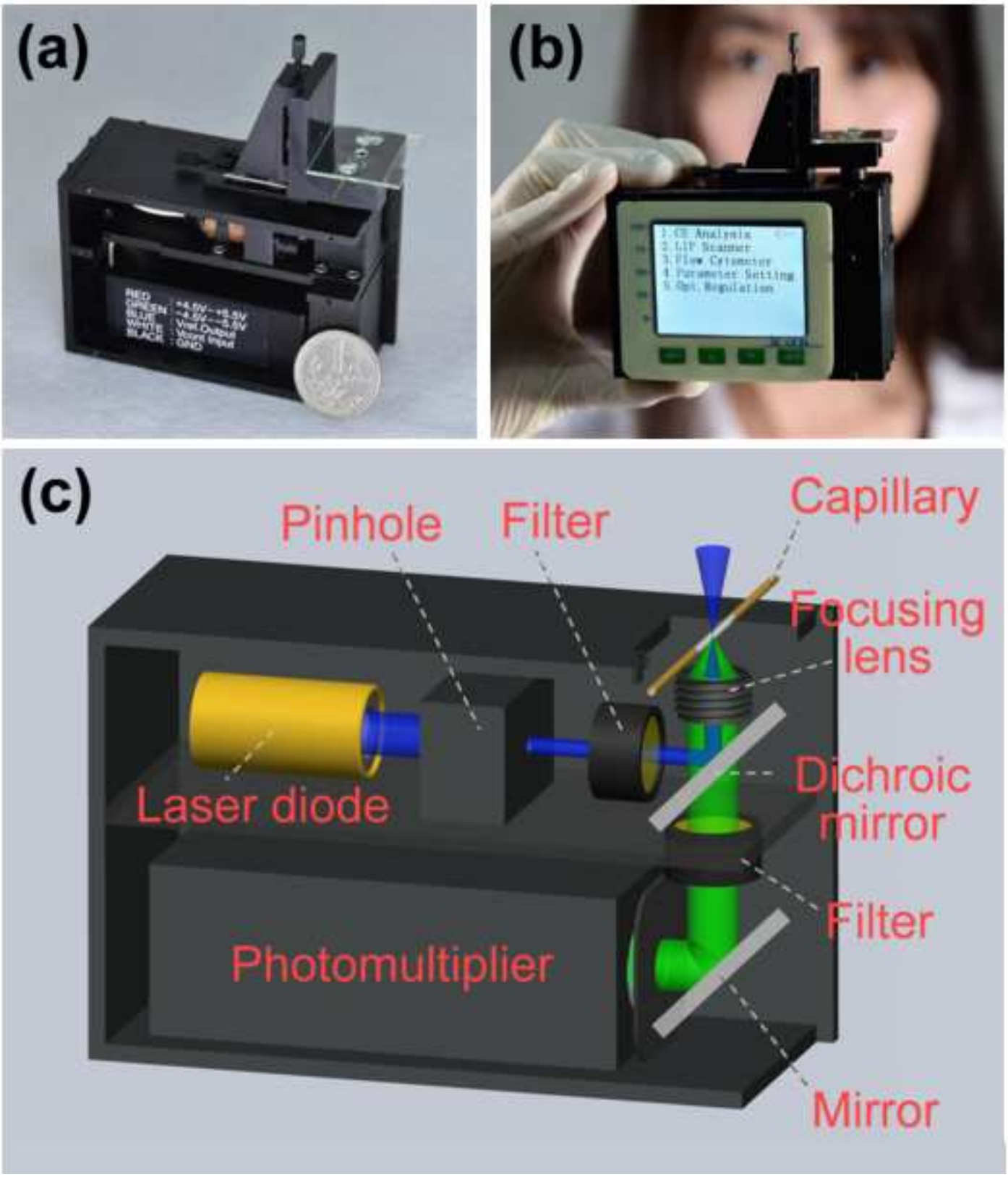
*The collection efficiencies of the both focusing lenses were tested using a point light source which has the same wavelength as the fluorescence light of FITC.

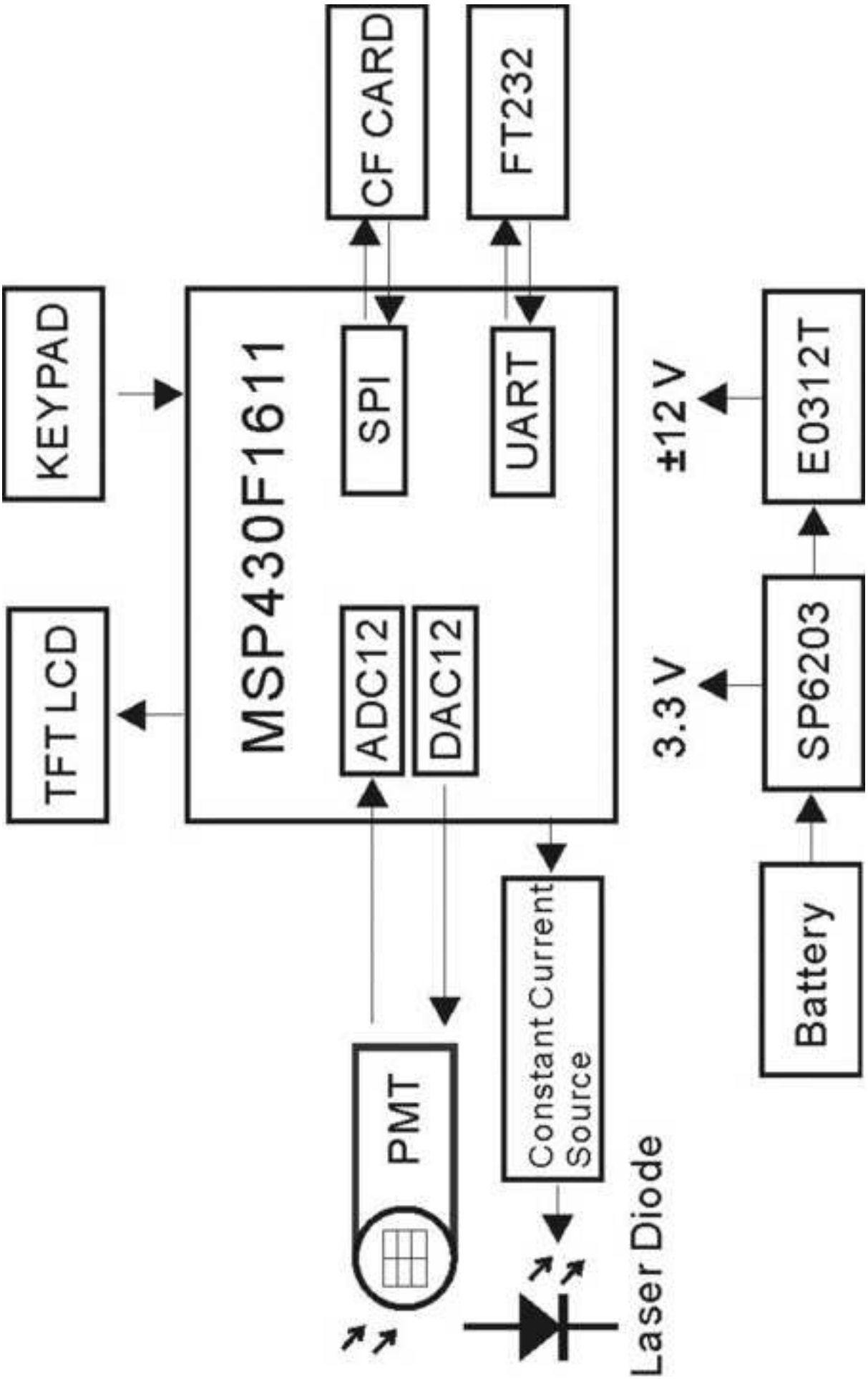
Table 2. Signal to noise ratios of the LIF detector when using different combinations of filters.

Combination No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
450 nm band-pass filter	N	Y	N	Y	N	Y	N	Y
488 nm long-pass filter	N	N	Y	Y	N	N	Y	Y
525 nm band-pass filter	N	N	N	N	Y	Y	Y	Y
Signal to noise ratio	*	*	*	*	5.89	11.09	5.39	11.29

*The signals are out of the detection range.

"Y" means the corresponding filter is used in this combination, while "N" has the opposite meaning.





Figure

Figure
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