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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 $9.1 \times 6.2 \times 4.1$ cm^3. 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.

1. 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 $11.5 \times 11.5 \times 19.0$ cm^3 [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 $7.5 \times 5.5 \times 3$ cm^3. 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 $30 \times 30 \times 20$ cm^3 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 $23 \times 20 \times 13$ cm^3 [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

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

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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 $5 \times 12 \times 6$ cm^3 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 $9.1 \times 6.2 \times 4.1$ cm^3. 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.

2. 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.

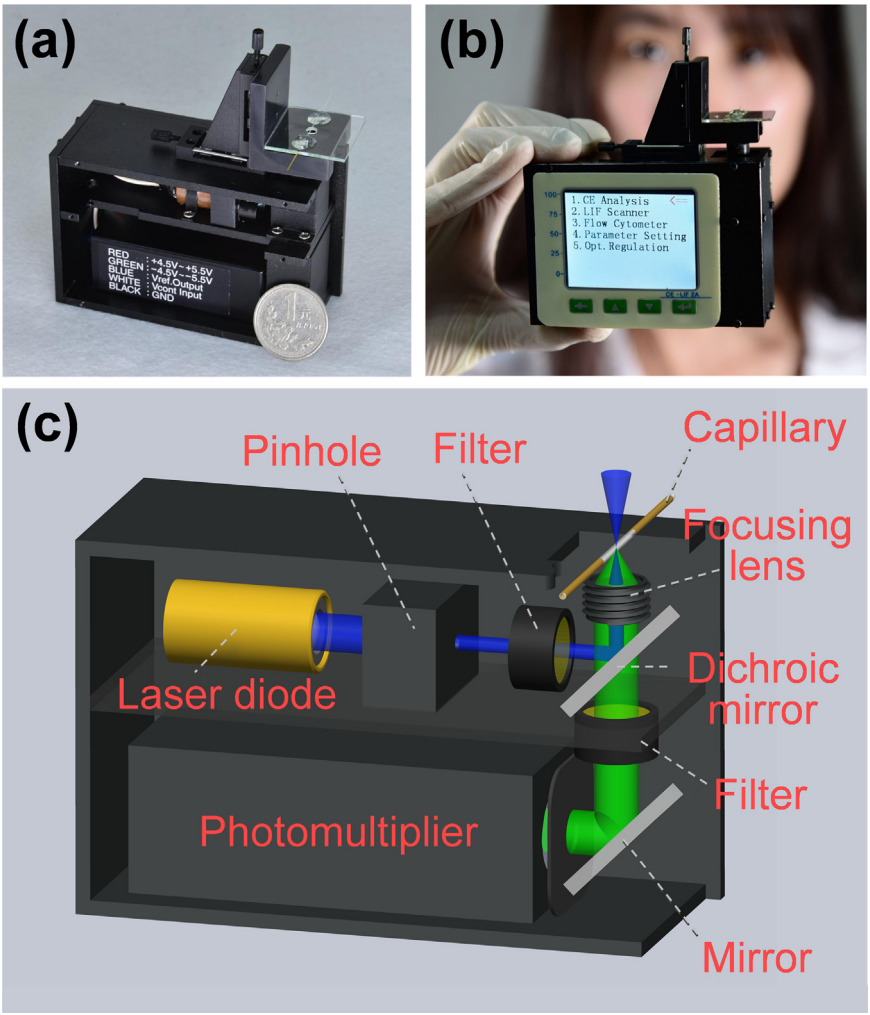

Fig. 1. (a and b) Images of the handheld LIF detector. (c) Schematic diagram of the LIF detector (not in scale).