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# A compact and low-cost laser induced fluorescence detector with silicon based photodetector assembly for capillary flow systems

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ABSTRACT

A compact and low-cost laser induced fluorescence (LIF) detector based on confocal structure for capillary flow systems was developed and applied for analysis of Her2 protein on single Hela cells. A low-power and low-cost 450 nm laser diode (LD) instead of a high quality laser was used as excitation light source. A compact optical design together with shortened optical path length improved the optical efficiency and detection sensitivity. A superior silicon based photodetector assembly was used for fluorescence detection instead of a photomultiplier (PMT). The limit of detection (LOD) for fluorescein sodium was $3 \times 10^{-12}$ M or 165 fluorescein molecules in detection volume measured on a homemade capillary electroosmotic driven (EOD)-LIF system, which was similar to commercial LIFs. Compared to commercial LIFs, the whole volume of our LIF was reduced to 1/2–1/3, and the cost was less than 1/3 of them.

## 1. Introduction

Fluorescence detection has been one of the most sensitive and selective detection schemes available for fluid flow systems, such as flow injection analysis (FIA), capillary electrophoresis (CE), and liquid chromatography (LC) [1–3]. It is easy to focus laser beam to a spot of tens of micrometers to match the size of capillary inner diameter (i.d.), because of the coherent characteristic nature of laser. Therefore, laser induced fluorescence (LIF) detector is widely used in micro flow systems such as CE [4,5] and microfluidic chips [6,7], with limit of detection (LOD) ranged from nM to pM [8,9].

The most used optical structures in LIFs were orthogonal and confocal optical arrangements. Fang and coworkers built LIF system for microfluidic chip based on orthogonal optical arrangement. They deeply investigated various optical arrangements and demonstrated that the fluorescence collection angle of 45° in the chip plane gave the best result, with a scattered light intensity only 1/38 of that obtained at an angle of 90°. An LOD of 1.1 p.M. fluorescein was obtained, which was comparable to that of optimized confocal LIFs [10]. Sweedler and coworkers developed a LIF detector in orthogonal arrangement coupled CE system. The use of multiple emission wavelengths improved analyte identification based on differences in analyte fluorescence emission profiles and migration time. The amounts detected in a single pinealocyte incubated in 5-hydroxytryptophan ranged from $10^{-14}$ mol to $10^{-16}$ mol [11]. Generally speaking, the confocal structure in LIF systems was preferred more by researchers to achieve higher signal-to-noise ratio (SNR) because of the coherence and focusing performance of the laser [8]. Zare group developed advanced laser confocal fluorescence microscopy coupled with microfluidic chip. To improve the detection sensitivity, they widened the excitation laser focus in one direction from 1.8 μm to 40 μm by cylindrical optics. And by theoretical calculation, the LOD for trace proteins in a single cell could reach 7 molecules [12]. Recently, Pu and coworkers developed a confocal LIF detector for narrow capillary system. They presented a simple protocol to align the capillary with the optical system and used the position-lock capability of a translation stage to fix the capillary in position. A very low LOD of 70 fluorescein molecules was achieved [13]. Fang group developed a handheld LIF detector based on a 450 nm laser diode (LD) and confocal optical configuration with a total size of $9.1 \times 6.2 \times 4.1$ cm$^3$. A miniaturized photomultiplier tube (PMT) was employed for fluorescence detection. The hardware cost of the detector was $ 2000 with LOD of 0.42 nM for sodium fluorescein [14]. Besides, commercial LIFs, such as Picometrics ZETALIF [15] and Unimicro TriSep™ − 2010 LIF, are also based on confocal optical arrangements.

*Abbreviations:* LIF, laser induced fluorescence; EOD, electroosmotic driven; FITC, fluorescein isothiocyanate; PMT, photomultiplier tube; LD, laser diode; PD, photodiode; SNR, signal-to-noise ratio; LOD, limit of detection

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However, commercial LIFs suffer from some disadvantages, such as big volume (34 × 17 × 63 $cm^3$ to 25 × 40 × 22 $cm^3$) and expensive. In CE-LIF-MS analysis, for example, the connection capillary tubing between LIF and MS is rather long owing to the big size of the LIF, resulting loss of separation efficiency and peak broadening. The cost of a commercial LIF of Picometrics (France) is about 50,000 to 100,000 US dollars (USD)/unit, depending on the wavelength of laser. In this study, we developed a compact and low-cost LIF by employing a small LD as light source and a new photodetector *AccuOpt2000* assembly as fluorescence detector. The LOD and long term stability was similar to that of commercial LIF but the volume and cost were drastically cut. The developed LIF was applied for detection of Her2 proteins on a single Hela cell.

## 2. Experimental

### 2.1. Apparatus

A 0–20 kV DC power supply unit (DW-P303-1ACF0, Tianjin, China) was used for electroosmotic driven (EOD). Cell selection and transfer were performed under an inverted microscope (Nikon, TS100, Japan). Andor live cell imaging system (Revolution WD, Andor Technology) was used to observe the cells.

### 2.2. LIF systems

A 20 mW laser diode (LD) with wavelength of 450 nm (Lanji Photoelectricity Co., Ltd., China) was used as light source. The optical emission filter and dichroic mirror were purchased from Beijing Bodian Optical Tech (Beijing, China). After reflected by the dichroic mirror, the laser beam was focused by a microscope objective (Numerical Aperture 0.25, Yingxing Optical Instruments Co., Ltd., China) to illuminate the capillary flow cell. The emitting fluorescence was collected by the microscope objective, passing through the dichroic mirror (high reflectivity at wavelength shorter than 470 nm, high transmissivity at wavelength longer than 500 nm), collection lens, pinhole and emission filter (BP 524 nm, FWHM 30 nm), then detected by the new photodetector assembly *AccuOpt2000* (Dalian Scien & Tech Instrument Inc., China). The signal was acquired by chromatographic workstation Sepu3010 (Puhui Science and Technology Ltd., China). The whole volume of the LIF was 29 × 25 × 15.5 $cm^3$ which was 1/2–1/3 of a commercial LIF. The schematic diagram of optical path was shown in Fig. 1**(a)**, and the practical diagrams of the LIF were shown in Fig. 1**(b)** and **(c)**.

### 2.3. Materials and chemicals

Unless otherwise specified, all chemicals were analytical grade. Fluorescein sodium, fluorescein isothiocyanate isomer I (FITC) and rhodamine 123 were purchased from aladdin (Dalian, China). The recombinant protein Anti-ErbB2 (Her2) Affibody Molecule (conjugated with FITC) (0.38 mg/ml) which were produced in E.coli were purchased from the MultiSciences (Lianke) Biotech Co., Ltd. (Hangzhou, China) and Stored at − 20 °C. This product binds to the extracellular domain of human ErbB2 (Her2). The phosphate buffered saline (PBS pH = 7.2–7.4) was purchased from Dalian Meilun Biotech Co., Ltd. The 1% BSA in PBS was stored at 4 °C. Ethanol (purity ≥ 99.9%, chromatographic grade) was purchased from Merck. Fused-silica capillary tubing with 50 μm inner diameter (i.d.) and 365 μm outer diameter (o.d.) was purchased from XinNuo Optical Fiber & Chromatography Inc. (Handan, Hebei, China). Water used in the experiments was ultrapure water from Milli-Q system (Millipore Inc., Milford, MA, U.S.A.).

### 2.4. Cell culture and injection experiment

The Hela cells were obtained from the tumor cell bank of the Chinese Academy of Medical Science. Cells were cultured in RPMI-1640 medium (Gibco, USA) with 10% foetal bovine serum (Gibco, USA), 100 μg/ml penicillin and 100 μg/ml streptomycin (Invitrogen; Life Technologies, USA) at 37 °C in 5% $CO_2$ atmosphere. Cells were harvested by following treatment with trypsin to obtain single cell suspensions and washed three times with PBS. Each batch of cells (containing about 1 × $10^6$ cells) was blocked 15 min by 1% BSA to prevent the non-specific adsorption and then the supernatant was removed. And then, 1 μL conjugated affibody molecules (38 μg/ml) were added into the 100 μL cell suspension and incubated at 4 °C for 30 min. Then, the cells were washed with 1 ml PBS, centrifuged at 1000 rpm for 5 min and repeated thrice. The cells were resuspended in 100 μL PBS and ready for analysis. The results of stain were verified by immunofluorescence technique in each batch cells. Finally, the cells were sampled into the capillary EOD-LIF system by electric field driven.

### 2.5. Electroosmotic driven (EOD) conditions and analysis procedure

The capillary EOD-LIF system was employed for all assays. The total length of capillary was 30 cm and an effective length was 18 cm. The capillary was treated 1 h at room temperature by 0.2 M NaOH before use. The EOD running buffer was consisted of 2.0 × $10^{-2}$ M $Na_2B_4O_7$ − 9.3 × $10^{-2}$ M $H_3BO_3$ (pH = 8.3). The EOD voltage was 10 kV and injection time was 20 s. Fluorescein sodium, FITC, and rhodamine 123 stock solutions were prepared in alcohol (HPLC grade). The standard solution of fluorescein sodium and FITC were prepared by

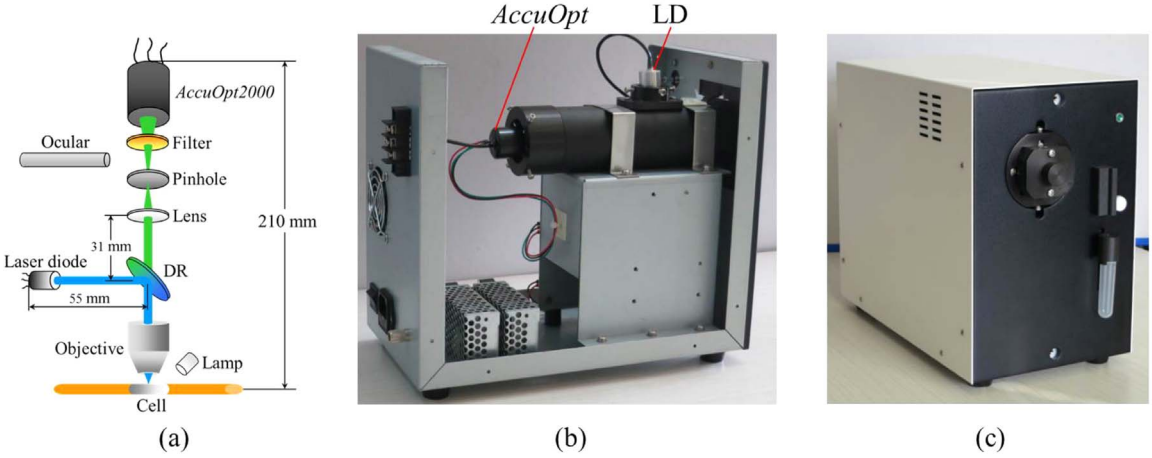


**Fig. 1.** Optical schematic diagram (a), internal configure diagram (b) and external graph of the LIF (c).