

Simultaneous laser-induced fluorescence and contactless-conductivity detection for microfluidic chip

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Abstract

A combined detection system involving simultaneous LIF and contactless-conductometric measurements at the same place of the microfluidic chip was described. The LIF measurement was designed according to the confocal principle and a moveable contactless-conductivity detector was used in C⁴D. Both measurements were mutually independent and advantageous in analyses of mixtures. Various experimental parameters affecting the response were examined and optimized. The performances were demonstrated by simultaneous detection of Rhodamine B. And the results showed that the combined detection system could be used sensitively and reliably.

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In recent years, microfluidic chip or lab on a chip performing chemical and biochemical analysis procedures has received much attention owing to its advantages, such as high degree of integration, remarkable sensitivity, low reagent consumption and short separation time [1,2]. LIF, especially laser-induced confocal fluorescence method [3,4], is a widely used highly sensitive detection method. C⁴D is a versatile detection method and increasingly being used for microfluidic chip [5]. The contactless mode has several advantages over the contact mode, including the absence of problems (e.g., fouling, unwanted reactions) associated with the electrode-solution contact, a simple construction of the detector, and effective isolation from high separation voltages. A moveable contactless-conductivity detector offers distinct improvements compared to fixed-location detector in C⁴D according to Wang and his co-workers' report [6]. Moreover, the diversity of the analytes often requires a combination of various detection techniques in order to be able to reliably determine all the interesting components of sample. For accomplishing this mission, different detection techniques should be associated. Previously, combination of detection techniques coupled with microfluidic chip capillary electrophoresis have been reported by a few groups [7–9]. For example, dual conductivity/amperometric microchip detection system [6], dual fluorescence combined with amperometric microchip detection system [8], and simultaneous electrochemical and electrochemiluminescence detection for microchip [9] were described. It is a pity that none has combined the highly sensitive confocal LIF with moveable C⁴D system for microchip.

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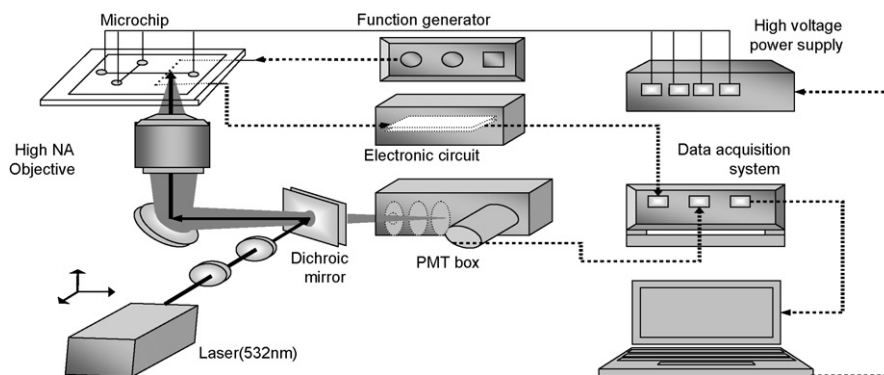


Fig. 1. Schematic setup of confocal LIF-C⁴D system.

In this letter, a novel combined detection system of simultaneous confocal LIF and moveable C⁴D for microfluidic chip was described and the performances were tested.

1. Experimental

Rhodamine B was obtained from Sigma–Aldrich Co. (St. Louis, MO). The Borax buffer solution (pH 9.0) was used, and Borax was purchased from Beijing Reagents Co. (Beijing, China). Ultra-pure water (18.2 MΩ) was purified by a Millipore Simplicity for preparation of all aqueous solutions. All chemicals were of analytical grade. Analyte solution and run buffer were filtered using 0.2 μm syringe filter. The PMMA chips were obtained from Dalian Institute of Chemical Physics, CAS. The shape of channel was trapezium, and one side of the channel was covered by a thin PMMA membrane of which the thickness was 50 μm. The widths of channel were 125 and 75 μm, respectively, and the depth was 25 μm.

The combined LIF-C⁴D system for microfluidic chip was built in our laboratory and displayed in Fig. 1. The emitted fluorescence passed through a high NA objective (40×), a dichroic mirror, a spatial filter (diameter 500 μm) and two pieces of interference filters (LF580, Huibo, China), to a photomultiplier tube (PMT) (CR114, Hamamatsu, Japan), which transferred fluorescence signal into electric signal. With reference to a reported scheme [10], the electronic circuit of the C⁴D was improved by adding a low-pass filter followed a changeable gain amplifier. There're several options for amplificatory multiple of inductive current: 300×, 600× and 1500×. The electronic circuit board was placed in a shielding box to protect the electronics from being interfered by external electric fields. A HP33120A function generator (Hewlett-Packard, Palo Alto, CA) was used for generating the sinusoidal signal (usually with a frequency of 289 kHz with peak-to-peak amplitude of 5 V). A HP34970A data acquisition system (Agilent

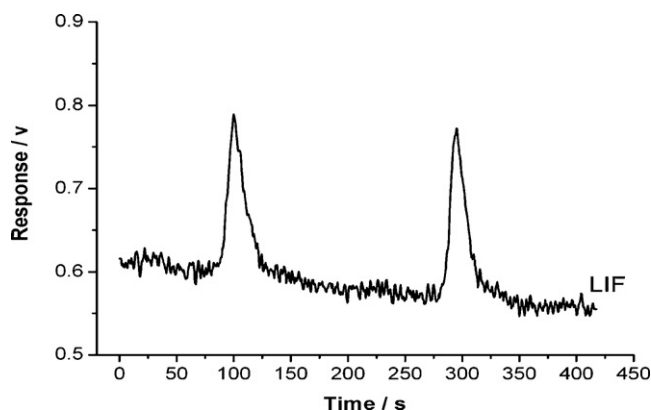


Fig. 2. Electropherogram of 5×10^{-9} mol/L Rhodamine B with LIF detector. Conditions: running buffer, borax (10 mmol/L, pH 9.0); injection voltage, +600 V, 8 mm for 30 s; separation voltage +1600 V, 50.2 mm for 170 s; laser power 11.5 mw; time R.S.D., 2.3%, peak value R.S.D., 7.4%, $n = 10$.

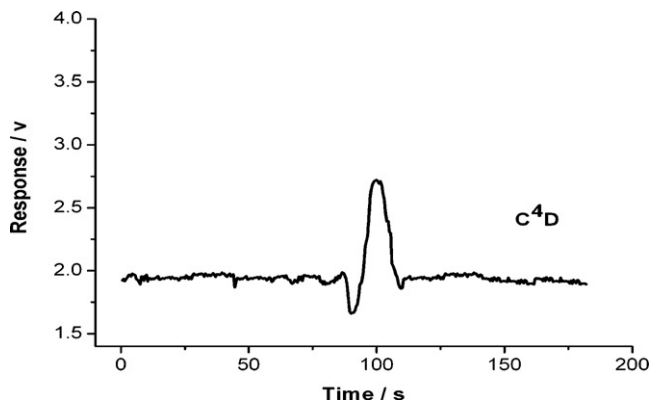


Fig. 3. Electropherogram of 1×10^{-7} mol/L Rhodamine B with C^4D detector. Conditions: frequency, 289 kHz; peak-to-peak amplitude, 5 V; amplifier multiple, 600 \times ; other conditions were the same as Fig. 1.

Technologies, Palo Alto, CA) was used for collecting both fluorescence signal and conductometric signal simultaneously. Data treatment was performed in HP BenchLink DataLogger.

The moveable contactless-conductivity detector was made on a PMMA plate by vacuum vapor deposition method. The electrodes were two rectangular-shaped, 10 μ m-thick copper films (0.8 mm \times 28.2 mm) with a distance of 800 μ m between them, and the end of the electrode was widened to 4 mm to facilitate the electrical connection.

2. Results and discussion

Both the measurements could be used independently or simultaneously. Various experimental parameters affecting the response were examined and optimized. A limit of detection (LOD) for LIF was less than 5×10^{-9} mol/L ($S/N = 8$), which sensitivity was lower than that of photo counting model detection (8.5 pM) [11] and much higher than absorbance detection (9.5×10^{-7} mol/L) [12] and LIF optic fiber detection (1×10^{-7} mol/L) [13] for Rhodamine B. Fig. 2 shows an electropherogram of Rhodamine B for two times and demonstrates high reproducibility and sensitivity.

The principal parameters affecting the response of C^4D were frequency and amplitude of the applied voltage. A sinusoidal excitation signal of 5 Vp-p and frequency of 289 kHz were applied. The intensity of the response and the relative peak value would be remarkably increased with increasing the amplificatory multiple. The amplificatory multiple of 600 \times was chosen because the most favorable response characteristics could be achieved at this amplificatory multiple. As shown in Fig. 3, a limit of detection of conductivity detection was 1×10^{-7} mol/L ($S/N = 6$) for Rhodamine B, which sensitivity was higher than that of inorganic ions detection (about 10^{-6} mol/L) reported using conductivity detection [14]. If this C^4D is used to detect inorganic ions, the limit of detection would be much lower.

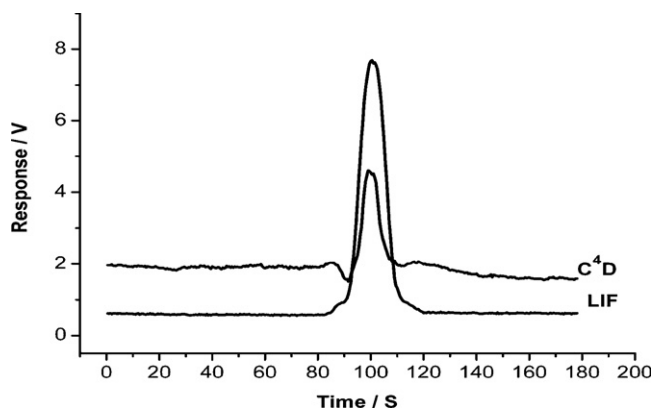


Fig. 4. Electropherogram of 2×10^{-6} mol/L Rhodamine B with simultaneous LIF and C^4D detection. Conditions were the same as Figs. 2 and 3.

The two measurements shared a common detection cell and were mutually independent. Fig. 4 showed that the responses were obtained at the same time (99 s, R.S.D. = 5.3%, $n = 9$). The combined detection system could simultaneously respond to ionic and fluorescent compounds, and thus would be applied more effectively in environmental and bio/chemical analysis.

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