High-voltage power supply for capillary electrophoresis microchip based on ARM

Xiaobo Yang, Weiping Yan#, Hongfeng Lv, Zhihuan Liu and Lubing Xie

School of Electronic Science and Technology, Dalian University of Technology, Dalian 116024, China
Corresponding Author / E-mail: yanwp@dlut.edu.cn, TEL: +86-411-8470-7428, FAX: +86-411-8470-6706

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A high-voltage power supply system with the core STM32F103 microprocessor and three high-voltage modules was presented. It mainly included microprocessor, amplifier-filter circuit, ADC, DAC, keyboard, relay array, LCD (240× 128) and RS232 interface. The stability of high-voltage output had been improved by adopting closed-loop feedback control. Electrodes switch was achieved by relay array which satisfied the voltage switch in the process of sample injection and separation, shortened the switch time, improved the detection accuracy, and greatly reduced the human disturbance. The injection voltage and time could be set by keyboard as well as the separation voltage and time. LCD could not only show parameter setting, but also monitor the current detecting state. The analysis of system parameters were conducted with the function of MFC for graphics drawing by upper computer software based on Visual C++. Three continuously tunable output voltages were obtained from 0V to 5000V. The relative error of output voltages was less than 0.3% and the maximum output current was 0.25mA. System tests of main performance and its application had been conducted and ideal results were obtained.

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NOMENCLATURE

ADC = analog-to-digital converter
DAC = digital-to-analog converter
MFC = microsoft foundation class
DMA= direct memory access

1. Introduction

Microfluidic studies the behavior of fluids at the microscale and the design of systems to take advantage of such behavior in multidisciplinary field encompassing physics, chemistry, engineering, and biotechnology. Many new applications are created by virtue of microfluidic integrating sensors, actuators, and other electronics recently [1, 2]. Compared with conventional separation and analytical methods, microfluidic exhibits special potential advantages including shorter analysis time, lower sample and reagent volume requirement, lower applied voltage and detection limits, easy to integrate and so on [3-5]. Importantly, new principles of fluid manipulation have enabled detection and handling of nanoliter fluid samples. In recent years, these principles have been applied to the development of capillary electrophoresis microchip system.

Capillary electrophoresis microchip has been widely used in biomedical measurement, protein separation, medical research and other areas of analytical chemistry as an effective way of microfluidic separation and analysis. In the detection of capillary electrophoresis microchip, high voltages must be applied in the reservoirs of microchip to perform the functions of sample injection and separation by switching the electrodes, the accuracy of results will be greatly depend on the stability and reliability of the high-voltage output, so high-voltage power supply plays a very important role in the detection and analysis of capillary electrophoresis microchip system [6].

Bao and coworkers [7] reported a special high-voltage power supply for capillary electrophoresis microchip controlled by singlechip. But the data processing must be done by PC and thus it couldn't meet the demand of miniaturization and integration. Cai [8] presented a structure whose adjustable and output voltages were 0~10V and 0~3000V respectively. The adjustable voltages were linear with output voltages. However it couldn't be applied in the multichannel detection. So a high-voltage power supply system utilizing the STM32F103 microprocessor and three integrated high-voltage modules was developed with the purpose of improving the stability and accuracy of high-voltage output. In this work, main control board, relay array circuit and software with user interface were designed. In addition, the performance, stability and application of system had also been investigated.

2. Experimental details

2.1 Experimental principle

The typical working principle of microfluidic chips is made up of sample injection and sample separation as shown in Fig. 1. In the process of sample injection, high voltage is applied between U_1 and U_2 (injection channel), the other voltage is applied between U_3 and U_4 (separation channel) to ensure that the sample can be driven from U_1 to U_2 and not spread. In the separation process, the voltage between U_3 and U_4 is changed and the sample is driven into the separation channel. Due to the differences of charge-mass ratios and dielectric constant of the particles included in sample, the speed of particles produced under the electric field is different too. Thus the sample is separated finally. The formula of electrokinetic injection volume is defined as follows:

$$Q = \frac{(\mu_{ep} + \mu_{eo})V_i \pi r^2 C t_i}{L_t}$$

where V_i is the injection voltage, r is the capillary diameter, C is the sample concentration, t_i is the injection time, L_t is the length of injection channel, μ_{ep} is the electrophoretic mobility, and μ_{eo} is the electroosmotic mobility.

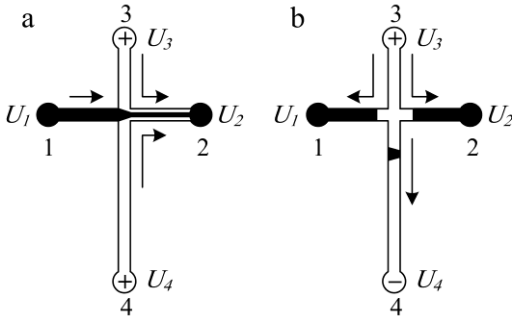


Fig. 1 Typical electrophoresis principle in the microfluidic chip composed by (a) sample injection and (b) sample separation.

2.2 System structure

As illustrated in Fig. 2, the STM32F103 microprocessor [9, 10] was the core of high-voltage power supply system and integrated high-voltage modules were adopted as the body of system based on the consideration of reliability, security and portability. High-voltage modules were controlled by closed-loop feedback and were supplied with 24V DC. The range of monitoring voltages, output voltages and currents were 0~5V, 0~5000V and 0~0.25mA respectively. The monitoring voltages were linear with output voltages, namely output voltages from 0 to 5000V were controlled by monitoring voltages from 0 to 5V. The monitoring voltages of high-voltage modules were firstly processed by amplifier-filter circuit [11] and then were sent to built-in ADC in STM32F103. The microprocessor compared preset voltages with voltages sent to ADC and then output voltages of high-voltage modules were controlled by DAC (TLC5618A, Texas Instruments). At the same time, relay array (GRL2412) driven by ULN2803 were used to realize electrodes switch as well as to improve the speed and automatic degree of electrodes switch. LCD (240128A series) could not only show parameter setting, but also monitor the current detecting state. Users could set the output voltages and time in the process of sample injection and separation by matrix keyboard (4×4). System would execute the set voltages, injection and separation time automatically as soon as the scanning program of board scanned the press of execution button which could avoid unnecessary manual intervention.

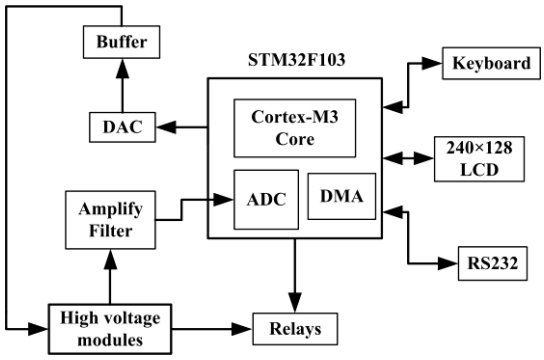


Fig. 2 Block diagram of overall system structure showing the components and their correlation.

The main peripheral circuits of STM32F103 consisted of power management, boot configuration, clock circuit, JTAG and hardware reset. In order to filter high-frequency ripple and perform the function of level transformation, a second-order Bathurst low-pass filter was designed. In addition, to obtain fast and exact speed of electrodes switch during the injection and separation process, six high-voltage relays were adopted. As depicted in Fig. 3, ULN2803 was used to enhance the driving capacity of GPIO. Diodes D_1~D_9 (IN5819) served as protection diodes to avoid damaging contacts and breakdown of collector junction of Darlington transistor when the relay coils were disconnected. C_1 and C_2 were parallel capacitance to filter for 24V power supply.

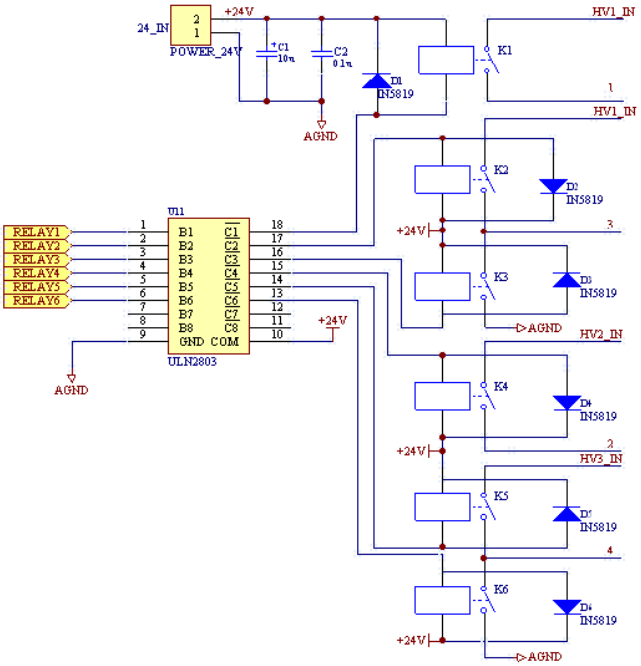


Fig. 3 Circuit diagram of the relay array.

Software design had a large proportion in the system development. Data processing, keyboard, display, serial communication and relay switching were realized based on embedded operating system. Test of switching time for high-voltage relays and stability parameters for high-voltage modules were implemented with the function of MFC for graph display by upper computer software based on Visual C++ 6.0 programming environment. The flow chart of upper computer software was shown in Fig. 4.

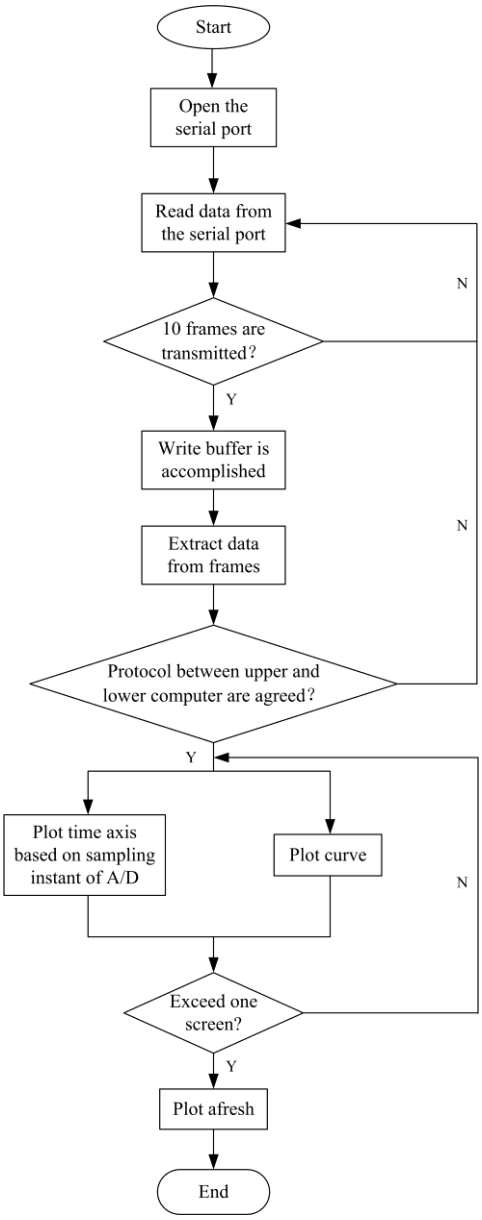


Fig. 4 Flow chart of upper computer software.

2.3 Microchip fabrication

Up to now, several materials such as silicon, glass, plastic, poly-(dimethyl-siloxane) (PDMS) have been used in the microchip fabrication [12-14]. In this work, microchip was fabricated with glass for its advantages of excellent optical properties, good insulation, upstanding heat-dissipating property and relatively easy surface modification. The photomask pattern was designed by Auto CAD software and then was printed on a transparency file using a high-resolution printer. The injection and separation channels were designed as 10 mm and 40 mm long, respectively.

With standard photolithography and wet chemical etching techniques [15], the microchip was fabricated on the 50 mm×35 mm soda lime glass substrate precoated with 145 nm Cr layer and 570 nm positive Az-1805 photoresist. The microfluidic channels (including injection and separation channels) were etched in a stirred HNO_3/HF etchant to the depth of 60 μm and width of 100 μm. The thermal bonding was performed with a change temperature control program in a muffle. Prior to bonding, access holes were drilled in the top plate using an ultrasonic drill. Layout of the microfabricated chip is shown schematically in Fig. 5. This kind of chip proved to be strong under normal pressure as well as rinsing the microchannels and could be used for several weeks.

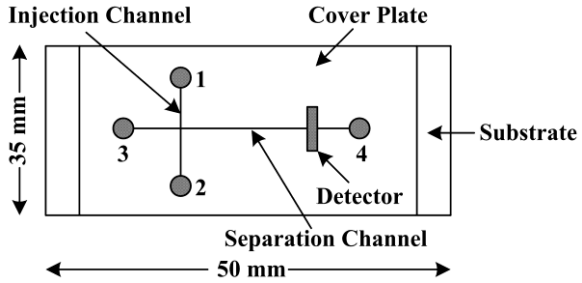


Fig. 5 Layout of the cross-shaped capillary electrophoresis microchip, comprising an injection channel and a variable length separation channel: 1 sample inlet, 2 sample outlet, 3 buffer inlet, and 4 buffer outlet.

2.4 Electrophoretic procedure

The electrophoretic separation was performed in the cross of single and multichannel capillary electrophoresis microchip using the home-made high-voltage power supply system. The microchannels of chip were pretreated with 0.1 mol/L NaOH for 1 hour firstly and then rinsed with deionized water and $Na_2B_4O_7$ buffer. Buffer solution was filled into the buffer inlet 3 and outlet 4, and subsequently Rhodamine B (1.0×10^{-3}mol/L) as the sample was filled into the sample inlet 1. Platinum wire electrodes were inserted into the reservoirs. In the sample loading procedure, 600V was applied between the sample inlet 1 and outlet 2 and the sample separation occurred when applying a potential of 800V between reservoirs 3 and 4. After separation the microchannels were immediately rinsed with deionized water to prevent it from blocking.

3. Results and discussion

3.1 High-voltage output characteristic

The high-voltage outputs of each route were measured time after time in the test. The set value was defined as the value entered from keyboard; the adjustable value was the output value of DAC after filtering; the output value was the actual high-voltage output and the ideal proportional relationship of them was 1000:1:1000. The comparison between the set value and deviation value of output voltage is shown in Fig. 6. The deviation value of the second route is 6V which is minimal and the third route is 11V which is maximal. The major reason is inconsistent performance of high-voltage modules.

To get the best result in the detection of capillary electrophoresis microchip, the second route was applied in the two ends of separation channel which need the highest accuracy and was connected with electrode 3. The first route whose maximal deviation value was only 6V under 600V was applied in the two ends of injection channel which performed in the output range of 0~600V to ensure that the injection channel was full of sample in the injection process and to avoid the background noise caused by the flood of sample into separation channel in the separation process. The third route was connected with electrode 4 and only performed in the injection process cooperated with electrode 3 to avoid flow of sample into the separation channel in the cross because of its maximal deviation value and the maximal deviation value was only 6V in the range of 0

~400V.

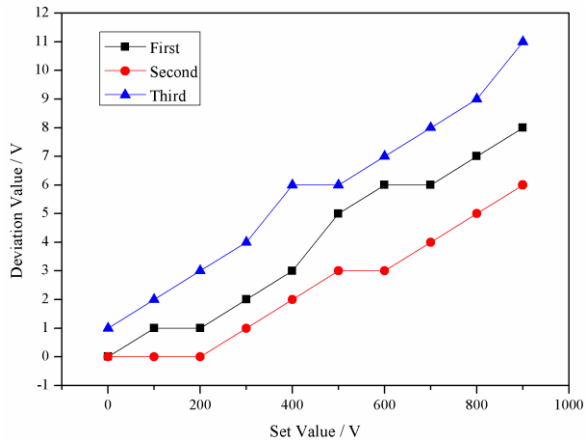
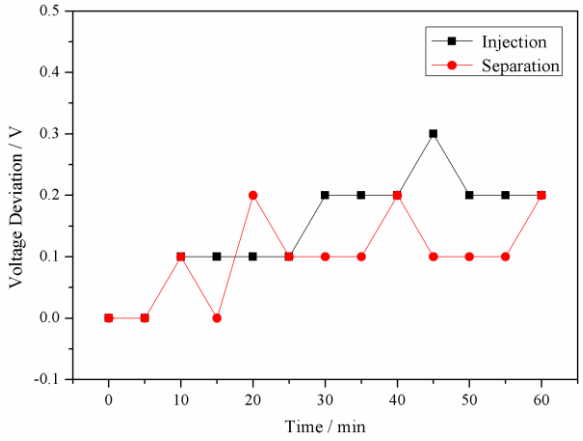


Fig. 6 Comparison between set value and deviation value of each route of high-voltage power supply.

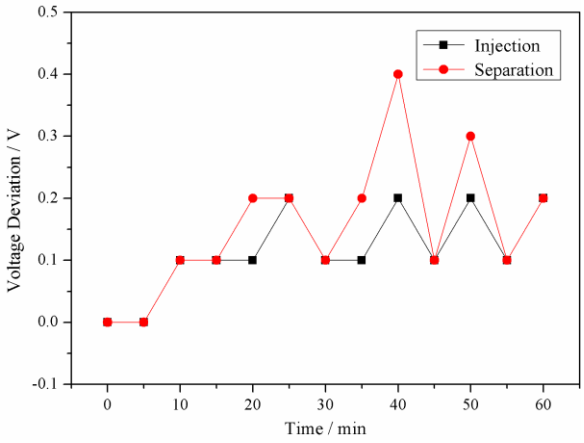
3.2 Time shift characteristic

Time drift characteristic is a significant parameter measuring the performance of power supply which is defined as the relationship between voltage output and time. Several tests need to be made in the detection procedure of capillary electrophoresis microchip. In order to avoid voltage deviation caused by march of time in the injection and separation process which would induce inaccurate test results, 10 times test of time drift characteristic for each route of high-voltage output were made in the range of voltages required for injection and separation and average value was adopted. Each test time was 1 hour. Further, test began to make after startup in 30 minutes to ensure the steady voltage output because of preheating needed for high-voltage power supply after electrifying.

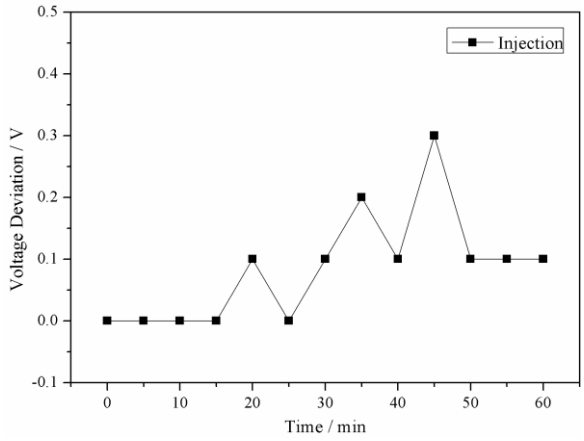
The output of first route was connected with electrode 1 of capillary electrophoresis microchip (identical with Fig. 5) in the injection process and with electrodes 1 and 2 in the separation process. The second route was connected with electrode 3 in the injection and separation process. The third route was connected with electrode 4 which only performed in the injection process and was switched by relay array to ground in the separation process. The time drift characteristic of high-voltage power supply is shown in Fig. 7 in which the first and second routes performed in the injection and separation process, the third route only performed in the injection process. As depicted in Fig. 7, the time drift characteristic of system is less than 1V and with accuracy less than and equal to 0.1%/hour which can meet the demand of experiment.



(a)



(b)



(c)

Fig. 7 Time shift characteristic of high-voltage power supply including (a) the first route, (b) the second route and (c) the third route.

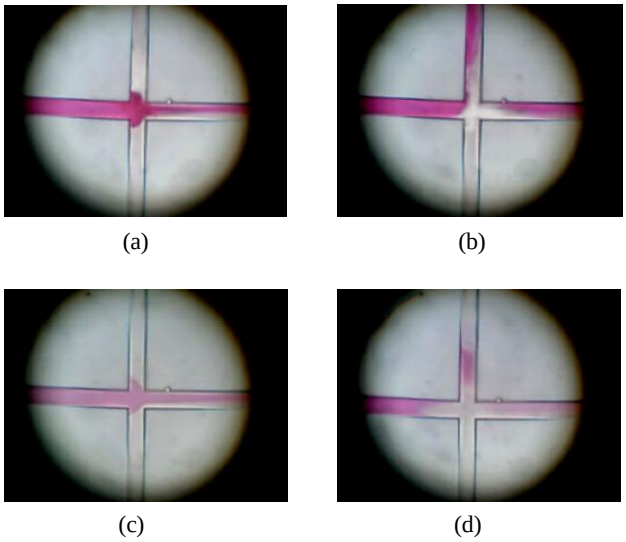
3.3 Sample injection and separation

Accurate voltage output applied in the reservoirs and appropriate voltage ratios between the reservoirs are crucial in the injection process of cross-shaped capillary electrophoresis microchip, which not only make the injection channel filled with sample, but also make the sample not spread to the separation channel. Different results could be obtained by changing the voltages applied in the two ends of channels. As depicted in Fig. 8(a) and (b), improper voltage ratios resulted in conditions of streaking in which the signal intensity was very weak in the detection area and sample composition couldn't be determined. So they are the key factor to realize precise analyses for capillary electrophoresis microchip. Fig. 8(c) showed the effect under the circumstance of proper voltage ratios in the injection process, which the voltages applied in the reservoirs 1, 2, 3, 4 were 600V, 0V, 400V, and 600V respectively. As demonstrated in Fig. 8(c), the sample was extruded into a wedge of good symmetry, which ensured the sample shape in the separation channel after switching the voltages.

Voltages of each reservoir should be changed quickly to ensure that only the sample in the cross can be driven to the separation channel and the sample in the injection channel return to the corresponding reservoirs in the separation process of cross-shaped capillary electrophoresis microchip. The sample in the cross is driven to the separation channel and there are no conditions of streaking under this circumstance. The separation result in the cross

corresponded to the foresaid injection condition is shown in Fig. 8(d), which the voltages applied in the reservoirs 1, 2, 3, 4 were 600V, 600V, 800V and 0V respectively. As illustrated in Fig. 8(d), the sample length driven to the separation channel in the separation process and the sample length extruded into the cross in the injection process were almost same. The sample of this shape could form narrow peak in the detection area and ideal results could be obtained.

As indicated by the above experiments, the voltage output of designed high-voltage power supply was steady, accurate and the speed of electrodes switch was relatively rapid which could be used for the analyses of capillary electrophoresis microchip.



(a) (b)

(c) (d)

Fig. 8 Effect of injection process (a, c) and separation process (b, d) in different conditions (a, b is under the circumstance of improper voltage ratios and c, d is in the condition of proper voltage ratios) for the cross-shaped capillary electrophoresis microchip.

3.4 Multichannel detection

The schematic diagram of detection system is shown in Fig. 9. It mainly consists of laser induced fluorescence detection subsystem, channel identification unit and fluorescent signal processing subsystem. The laser induced fluorescence detection subsystem is composed by a green laser diode double-pumped solid-state laser of 532 nm (peak wavelength), two pieces of optical fiber arranged V-shaped for incidence laser and receiving fluorescence, micro lens and mechanical scanning platform. The channel identification unit is composed by a red LED, optical modulator board and optoelectronic receiver. The signal processing subsystem is made up of filters, avalanche diode (APD), signal preamplifier and embedded operating system. The turntable was drove by electric motor to conduct uniform circular motion, and the scan axle conducted sector scan with certain angle at the same time. So the incidence and emission optical fibers fixed on the scan axle scanned back and forth above the capillary array electrophoresis microchip to perform the multi-channel scan.

The beam of red LED irradiated on the opaque areas of optical modulator board, the optoelectronic receiver couldn't receive optical signals, thus the detector outputted low potentials; when the beam irradiated on the transparent zones, the optoelectronic receiver could receive optical signals, and the detector outputted high potentials. Because the widths of transparent and opaque areas corresponded to the width of capillary channels and the distances between capillary channels, the scan axis drove optical modulator board to move around and the channel identification pulse sequence was formed. The detection system fixed on the scan axis scanned an arc-shaped trajectory between the two pulses.

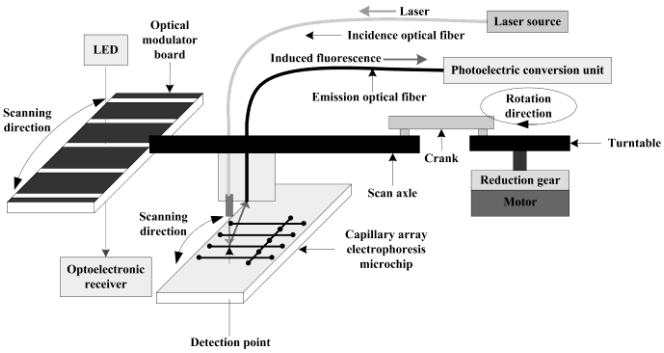


Fig. 9 Schematic diagram of multichannel detection structure comprising laser induced fluorescence detection subsystem, channel identification unit and fluorescent signal processing subsystem.

Firstly, purified water and Rhodamine B (1.0×10^{-4} mol/L) were introduced into the channels 1, 2 and 3, 4, respectively. The volume of Rhodamine B in channel 4 was larger than its in channel 3. The result is shown in Fig. 10, the straight lines represent the signals of high potentials received by optoelectronic receiver and the gaps between it correspond to the opaque areas of optical modulator board. Because the widths of transparent and opaque areas correspond to the width of capillary channels and the distances between capillary channels proportionately, the optoelectronic receiver acquires the signals of high potentials when the scan axle scans above the microchannels. Therefore, the fluorescence peaks detected by APD appear in the gaps between straight lines 1 to 5 synchronously and the channel identification is achieved.

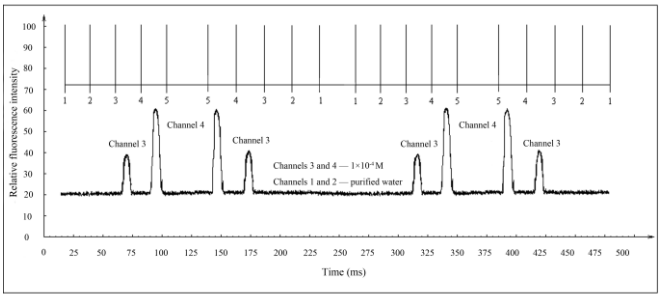


Fig. 10 Typical electropherograms of separation of samples. Channels 1, 2 and 3, 4 were filled with purified water and Rhodamine B sample (1.0×10^{-4} mol/L). The volume of sample in channel 4 was larger than its in channel 3. Analytical conditions: 10m mol/L $Na_2B_4O_7$ running buffer; the sample loading operation of 600 V for 20 s; the separation voltage of 800 V.

As seen in Fig. 10, there is no fluorescence peak in the gaps between straight lines 1 to 3 because of the injection of purified water in channels 1 and 2. The other two peaks in the gaps between straight lines 3 to 5 are the fluorescence signals of Rhodamine B detected in channels 3 and 4. Since the volume of Rhodamine B in channel 4 is larger, the fluorescence peak is higher than its in channel 3. It can also be observed that the locations of fluorescence peaks detected in channels 3 and 4 are different to some extent when the scan conducts

in the direction from 1 to 5 and 5 to 1 in system. It is proved from repeated experiments that it exists mainly because of the detection error brought by the mechanical path difference of scanning structure, which need to be eradicated in the near future.

4. Conclusions

In summary, this paper demonstrates a home-made high-voltage power supply system for capillary electrophoresis microchip based on the STM32F103 microprocessor and three integrated high-voltage modules. The stability of high-voltage output had been improved by closed-loop feedback control and electrodes switch was achieved by relay array. Three continuous tunable output voltages from 0~5000V were obtained by controlling from 0~5V using the designed system. High-voltage output, time drift characteristic and electrodes switch time, relationship between load and output voltage had been performed and the results showed that the system had come up to the design requirements. In addition, the system was applied to single and multichannel injection and separation detection of capillary electrophoresis microchip with Rhodamine B sample and the results showed that the system had advantages of small size, steady output voltages, higher automation, and could completely meet the need of capillary electrophoresis analysis.

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