A Portable Fluorescent Microfluidic Detection Device Based on FPGA

Gangshan Wu¹, Chiyuan Chen², Ning Yang*² and Peifeng Xu¹

¹Jiangsu Polytechnic College of Agriculture and Forestry, Jurong 211121, China
²Jiangsu University, Zhenjiang 212013, China

*yangning7410@163.com

Abstract. Fluorescence analysis method has high sensitivity, but the difference between effective and unwanted light in the process of fluorescence conversion and the noise of small-scale variable amplification. This paper designs a method of modulating the light source makes the fluorescent light be a kind of differential signal that is easily extracted by the photoelectric conversion circuit and amplified, and then uses digital devices FPGA and ADC to achieve signal acquisition and processing, and finally it is presented in the form of a dark room box. In this paper, a novel detection structure is used as a discussion point, and a low-power, high-precision, low-cost signal processing system has been introduced in detail, and mainly used in 500 The fluorescence in the wavelength range of -600 nm was tested. The mathematical relationship between different fluorescence intensities and the post-conversion variables was established. In the subsequent product development, the detection of different wavelengths of light can be achieved by replacing the excitation light source and the corresponding filter, providing a simple circuit processing method for designing photoelectric probes, biomedical detection, and chemical applications.

1. Introduction

There are many electron energy levels in the molecule that are close to each other. When the detection sample is irradiated with light of a specific wavelength, it will cause the electrons in the sample molecule to transition to a high energy level, become extremely unstable, and spontaneously lose energy and return to the original. The level of electrons in which fluorescence or phosphorescence occurs. Sensitivity is an important parameter of fluorescence analysis. Optical experiments often use spectrophotometry and colorimetry, while fluorescence analysis is 2 to 3 orders of magnitude higher [1]. FPGA (field programmable gate array) is very different from traditional PC and microcontroller development. It mainly focuses on parallel computing. The driver uses hardware description language (HDL), which is fast and parallel in the field of signal processing and communication. The advantage is ahead of other processor chips [2]. FPGA design covers communications, automotive electronics, avionics, defense, consumer electronics, military and medical electronics. The scale of contemporary FPGAs has also reached tens of millions of gates, which is suitable for implementing system-on-chip SOC. The design is flexible, the development investment is small, and the degree of fault tolerance is large. The FPGA is less risky than the ASIC, and the stealth cost is lower [3].

In recent years, there has been a unique favor for fluorescence in testing at home and abroad, and many talents have made relevant attempts, and many innovative methods have been proposed. Kulp et
al. designed a multi-color fluorescence detection system based on high-power white LED module and light rod homogenizing structure for orthogonal microfluidic PCR chip [4], for simultaneous detection of multiple gene targets of unknown pathogens. Immunosorbent assay for fluorescent ligation using an automated microfluidic photonic crystal enhanced fluorescence analysis platform for cancer biomarker detection in human serum [5]. A portable, low-cost, user-friendly and ultra-sensitive microcapsule array based on functional nucleic acid strategy was designed for the quantitative detection of copper ions [6].

The main research of this paper is the signal processing based on FPGA fluorescence detection, which is used for the collection of fluorescent signals in microfluidic chips. The FPGA uses a 12-bit parallel ADC with a sampling frequency of 65 MHz to acquire the fluorescent signal. Photoelectric conversion uses square wave modulation filtering to simplify the signal conditioning circuit.

2. Materials and Methods

2.1 Principle of Fluorescence Quantitative Detection

The reason why the fluorescence method can analyze and measure the sample is because there is a certain quantitative relationship between the fluorescence intensity of the substance and the solubility of the sample solution. According to the white law, the relationship between the transmitted light intensity and the solution concentration is:

$$\frac{I}{I_0} = 10^{\epsilon bc}$$

The fluorescence intensity F is proportional to the difference between the incident light intensity and the transmitted light intensity ($I - I_0$) and the fluorescence quantum yield $\phi$. When the influence of other factors is small, the relationship between the fluorescence intensity and the incident light intensity is:

$$F = k\phi I_0 2.303\epsilon bc \approx KI_0$$

Where $\epsilon$ is the molar coefficient, $b$ is the thickness of the liquid layer, $c$ is the concentration of the fluorescent substance in the solution, and $k$ and $K$ are different proportional constants. It can be seen from equation (2) that there is a linear relationship between the fluorescence intensity of the sample and the concentration of the fluorescent substance, and the basis of fluorescence quantification is this linear relationship. Therefore, increasing the detection sensitivity can be improved by photoelectric conversion and signal amplification. Electronic equipment is all right.

2.2 Theoretical analysis of photoelectric signal conversion

The modulation circuit is used to realize the regular illumination of the excitation light source, and the AC change of the fluorescence signal is indirectly controlled. After the conversion of the silicon photodiode, in the actual test process, the obtained electrical signal is a current signal of several hundred nanoamperes, which is presented. The waveform of a distorted sine wave. The main error of fluorescence measurement lies in the stray light and high-intensity excitation light in the photoelectric conversion environment. These error sources can be eliminated by using a monochromator or a filter. The filter has the advantages of being cheap and simple, and is divided into glass, film and interference. Three kinds of filters; secondly, the influence of various signals on the analog circuit after the conversion, such as the parameters and PCB topology of the op amp itself, etc. Selection, schematic optimal design, signal integrity analysis, and optimized PCB design. What the paper measures is not the precise amount of fluorescence produced, but the number of samples to be measured that is proportional to the amount of fluorescence. Therefore, in the obtained AC waveform, the required amount is the peak-to-peak value or effective value of the AC signal. It represents the difference information of the fluorescence that occurs after modulation. Fluorescence distance is different from that of the photoelectric converter, and the required amplification gain will be different.
Therefore, the INA128 is used to adjust the gain of the adjustable gain, so that the signal obtained by amplifying and filtering the AC signal has a level of one hundred millivolts, reaching the ADC sampling. The magnitude requirement.

2.3 Hardware Design

The hardware uses 1W blue LED chip (withstand voltage of 3.0-3.2V, maximum current is 300mA, emission wavelength is 460nm, luminous flux is 20-30LM), EP4ce5e22c8 main control chip, SG-S1223G silicon photodiode, OPA380, INA128, OPA227, LTC1966, MAX660, AD9226 and display. The fluorescence reagent used in the paper produces a fluorescence wavelength of about 500-600 nm in the photoluminescence phenomenon. The 530 nm narrow-band filter can be used to filter out excitation light and retain fluorescence.

The system adopts a projection optical path, and the modulation light source is embedded in a conical cavity, leaving a hole to illuminate the microfluidic chip bearing area. Below the carrying area is a signal conditioning circuit board, the circuit board is parallel to the carrying area, and the photodiode and the light emitting The holes are on the same vertical line. The specific 3D structure is shown in Figure 1(a)(b). The box casing has a gap in the microfluidic chip bearing area for replacing the microfluidic chip. In order to avoid the strong excitation light in the transmission process and the stray light around the photodetector affecting the fluorescence detection, a small black cap is made by the filter to cover the photodetector, so that only the light from the vertical direction can enter the detector, and the incident light path is The microfluidic chip is 45 degrees, making the possibility of strong excitation light entering the detector being reduced.

![Device Structural Chart](image)

**Figure 1. Device Structural Chart**

Considering that the photocurrent generated by the photodiode is only 2.6~3.6uA, the transimpedance amplifier formed by the general operational amplifier is affected by the parameters of the operational amplifier itself, such as offset voltage and bias current. 100,000 times magnification can be achieved with the OPA380 and photodiode. The schematic is shown in Figure 2(a).

When designing the intermediate stage amplifying circuit, considering that the photodiode output current is affected by the fluorescence intensity and the distance between the photodiode and the sample to be tested, the size difference is very large, so the INA128 design gain adjustable low noise amplifier circuit is adopted. The schematic diagram is shown in Figure 2(b). The magnification is controlled by a sliding rheostat with a resistance of 50KΩ, which can achieve 2~1000 times magnification.

In a circuit with two-stage amplification and a magnification of 100000 times or more, there must be noise interference. Gaussian white noise exists in the theoretical calculation. Therefore, a low-pass center frequency 1000 Hz, 500 Hz bandwidth band-pass filter is designed to preserve the 1 kHz signal
waveform. After testing, the waveform has no other interference. The filter is designed using OPA227, and the circuit schematic is shown in Figure 2(c).

Considering that there is error in the signal processing of a digital device and the external device can be used for calibration or measurement when the digital device program has problems, the paper uses LTC1966 to design the RMS detection circuit to realize the RMS detection of the AC signal and the AC signal. The effective value is converted to a voltage value for easy detection. Since the output voltage of the chip is less than 500mV, the voltage amplification is realized by the OPA227, and the sampling accuracy is improved. The schematic diagram is shown in Figure 2(d).

When designing the PCB, considering the problem of weak signal amplification processing, there is theoretically a phenomenon of noise drowning signals. The copper photodiode output pin and the OPA380 input pin are connected as short as possible, and the feedback resistor must be connected between the photodiode and the chip pin near the input to prevent signal feedback current from entering the chip. Copper is placed around the input copper wire and the feedback copper wire and the hole is grounded to the ground to form a low-impedance environment, which avoids parasitic parameters and leakage current of the circuit board and reduces the possibility of signal attenuation. The position of the filter capacitor resistor is placed as much as possible in the schematic topology to reduce the chip workload and result errors. The power supply pins of all chips must be connected to the 100nf capacitor to ground, and this capacitor is as close as possible to the chip to filter out high frequency interference in the supply voltage. The main component of the negative power supply module is copper-cutted, leaving the input and output ports, because the MAX660 is a switching power supply chip to prevent interference with the signal chain. Ensure that the signal chain of the entire signal conditioning circuit is in a straight line, eliminating crosstalk and attenuation. The specific circuit PCB is shown in Figure 2(e).

The AD9226 chip is used as the main chip of the system ADC, and the connection between the pin of the AD9226 and the FPGA is shown in Table 1. The overall circuit schematic is shown in Figure 3.

![Figure 2](image-url)  
*Figure 2. (a) OPA380 transimpedance amplifier circuit; (b) INA128 low noise amplifier circuit; (c) OPA227 bandpass filter; (d) rms detection circuit; (e) signal conditioning circuit PCB.*

| PIN  | Description   | FPGA PIN | PIN  | Description   | FPGA PIN |
|------|---------------|----------|------|---------------|----------|
| PIN_1 | Sample CLK    | PIN_72   | PIN_8 | Data 6        | PIN_65   |
| PIN_2 | Data12(LSB)   | PIN_71   | PIN_9 | Data 5        | PIN_64   |
| PIN_3 | Data 11       | PIN_70   | PIN_10| Data 4        | PIN_60   |
| PIN_4 | Data 10       | PIN_69   | PIN_11| Data 3        | PIN_59   |
| PIN_5 | Data 9        | PIN_68   | PIN_12| Data 2        | PIN_58   |
| PIN_6 | Data 8        | PIN_67   | PIN_13| Data 1(MSB)   | PIN_55   |
| PIN_7 | Data 7        | PIN_66   |      |               |          |
2.4. Internal signal processing

The signal processing module mainly stores DATA_in into a RAM. The RAM is a dual-port RAM with a bit width of 12 and a depth of 4096 of the M9K of the FPGA, which is convenient for the display module to call display. Because the known signal frequency is 1KHz, only the peak-to-peak information of the signal needs to be known. Since the signal is not a standard sine wave, the system also performs the extraction of the RMS detection when setting the peak-to-peak value, setting two variables. Complement each other and eliminate errors.

The peak-to-peak detection mainly finds the maximum and minimum values of a set of DATA_in in 1 millisecond, finds the average value of the upper and lower values of the previous set, and stores the result as the maximum and minimum registers. The added number is averaged as the maximum and minimum values of the next millisecond.

The RMS detection is mainly obtained by squaring all the DATA_ins in one group and averaging them in 1 millisecond. By using the multiplier core embedded in the FPGA, the maximum 18-bit multiplication can be realized, because of the multiplication in timing. The time required is relatively long, and enough time is reserved in this process to ensure that there is no error, but the structure of the FPGA itself is difficult to perform logarithmic operations, so the data is directly output to the display.

3. Results and Discussions

Signal conditioning circuit when no filter is directly modulated light source brachytherapy photodiode, the distance between 1 to 3 cm, acquired through the use of an oscilloscope waveform of the band pass filter shown in Figure 4. It can be seen that the waveform frequency is about 1KHz, the amplitude is about 3.96V, and the waveform is slightly distorted. From the oscilloscope, the peak-to-peak value is 3.96V, which is the waveform after the magnification is up to 600000 times.
The solution containing the fluorescent reagent powder is arranged on the microfluidic chip, and the test results of the system are turned on as shown in Table 2. It can be found that the concentration of the fluorescent substance is linear with the measured peak-to-peak value and the effective value, and the waveform is complete without distortion.

Table 2. Fluorescence test results.

| Fluorescent concentration | 10g   | 20g   | 30g   |
|---------------------------|-------|-------|-------|
| Waveform integrity        | Complete without distortion | Complete without distortion | Complete without distortion |
| Peak to peak (mV)         | 488   | 1034  | 1334  |
| Valid value (mV)          | 172   | 363   | 468   |

4. Conclusions
In this paper, a method of modulating the light source is designed to make the fluorescence become a differential signal that is easily extracted by the photoelectric conversion circuit and amplify it, and then use FPGA and ADC to realize signal acquisition and processing. The paper uses silicon photodiodes to extract optical signals into electrical signals, then performs signal amplification and filtering on the electrical signals, and finally inputs them into the FPGA for digital signal processing to achieve concentration detection of fluorescent reagents, including excitation light source driving design, optical path structure, Photodetector design, signal conditioning circuit design, and FPGA signal acquisition and processing design. In this paper, a low-power, high-precision, low-cost signal fast processing system is introduced in detail. Experiments are carried out mainly on fluorescence in the wavelength range of 500-600 nm, and the mathematical relationship between different fluorescence intensity and post-conversion variables is established. And designed a simple embedded system based on FPGA, using TFT screen display results. In the subsequent product development, the detection of different light wavelength requirements can be realized by replacing the excitation light source and the corresponding filter, which provides a simple circuit processing method for designing photoelectric probes, biomedical detection, and chemical applications.

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