Design of POCT Testing Device for Fluorescence Immunochromatography

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Abstract. This study is based on fluorescence immunochromatography technology, using antigen-antibody immunological reaction and chromatographic reaction, and in the form of fluorescence immunochromatographic test paper, to achieve the purpose of rapid and accurate quantitative detection of objects. Develop a portable fluorescent immunochromatography POCT detection device for all levels of medical units, clinics and families. The device uses a V-type non-confocal optical path and mainly includes fluorescence signal reception, signal processing, motor drive and other modules. By detecting the fluorescence intensity released by fluorescent markers and combining the quantitative model of light intensity ratio and concentration, Establish the relevant algorithm to realize the qualitative and quantitative detection of the target.

1. Introduction

After undergoing four stages of development, including qualitative detection using the gold chromatographic method, manual semi-quantitative detection using a colorimetric card, manual quantitative detection with a readout meter, and semi-automatic quantitative detection, Point-of-Care Testing (POCT) has gained widespread usage in various areas such as infectious disease detection, blood gas analysis, blood glucose monitoring, immune diseases assessment, food safety testing and more. This is due to its accuracy, convenience and speed [1]. Immuno-chromatography (ICA), which combines antigen-antibody specific reactions with chromatography technology, is commonly employed for qualitative substance testing in the POCT field. However, practical applications often require the incorporation of colloidal gold particles quantum dots or fluorescent microspheres as markers within immunochromatography to enable the transition from qualitative to quantitative detection. This enhances sensitivity and accuracy of measurement. Consequently Fluorescence Immunoassay (FIA), an innovative technique that merges specific immunological reactions with sensitive fluorescence methods has been rapidly developed [2].

FIA employs fluorescent markers such as colloidal gold particles quantum dots or lanthanides to quantitatively measure target concentrations by detecting fluorescence signal intensity. With its high sensitivity strong specificity and robust anti-interference capabilities this technology finds extensive use in rapid virus detection enabling it to overcome limitations associated with traditional methods like time-consuming procedures low sensitivity cumbersome processes etc.. Due to its advantages of strong specificity high sensitivity and practicality fluorescent immunoassay technology is widely applied in virus identification tasks along with measuring bioactive compounds like hormones proteins microorganisms.

2. Principle and structure of the system

2.1. Principles of immunochromatography

Immunochromatography takes strip fiber chromatographic material fixed with detection line and control line as the fixed phase, and the test is also a mobile phase. The measured substance moves on the chromatographic strip through capillary action. The specific immune reaction of the object to be measured occurs at the T-line. The immune response of the free substance occurs at line C. This method has the characteristics of specificity, simple operation and rapid operation, and is widely used in clinical diagnosis, environmental monitoring, food safety and other important fields [3]. As shown in Figure 1.

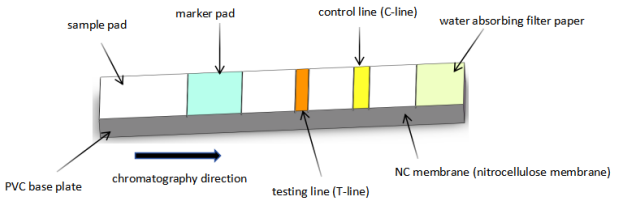

Fig. 1. Schematic diagram of immunochromatogram.

2.2. Optical structure design

The system adopts the fluorescence detection principle of laser induced fluorescence technology, and based on

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this principle, the optical detection mechanism is designed.

2.2.1 Optical path design

The commonly used optical path design has V-type non-confocal optical path and confocal optical path, confocal optical path has high cost and good stability, V-type optical path design has simple structure and low cost, and the V-type optical path design is selected to compare the above two design performances. In the design, the traditional optical module + mechanical scanning structure method is adopted [4]. The excited light emitted by the light source is first focused on the test strip, and then focused on the photodetector through the collected light path. Since each optical module can only collect a little fluorescence signal on the test strip, an additional mechanical transmission device is required to realize the scanning of the entire detection area [5]. In this scheme, the optical module is small, making the whole system more portable. The photodetector uses Hamamatsu S1087, and the LED uses environmentally friendly, mercury-free, immediate on/off, narrow band width, low power consumption, good continuity, small volume and low voltage UV-LED.

2.3. Mechanical structure design

The optical module consists of a light source and photodiode and is sealed in an optical camera obscura. The stepper motor drives the optical camera to scan and detect along the guide rail. This closed environment design can ensure that the light emitted by the light source can be directed to the test strip with maximum intensity, and the light reflected by the test strip can be received by the photodetector with maximum intensity. This design can not only avoid the noise interference caused by the light interference in the external environment, but also improve the detection accuracy and sensitivity of the system. The mechanical structure design is shown in the figure 2.

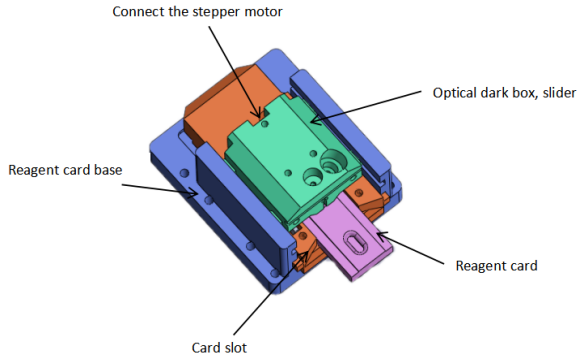


Fig. 2. Optical module mechanical structure design.

In order to ensure the stability of fluorescence intensity, the mechanical properties of the base of the moving parts were analyzed, the corresponding materials were set, and the solution was carried out after the definition and mesh division. The data show that the maximum load of the base is 2N, while the maximum stress experienced by the base is 2.5N/m2 and the maximum displacement is 0.028mm. The flexural yield strength of the base is 2.75N/m2. Based on these evaluation data, it can be determined that the base meets the design requirements. As shown in Figure 3.

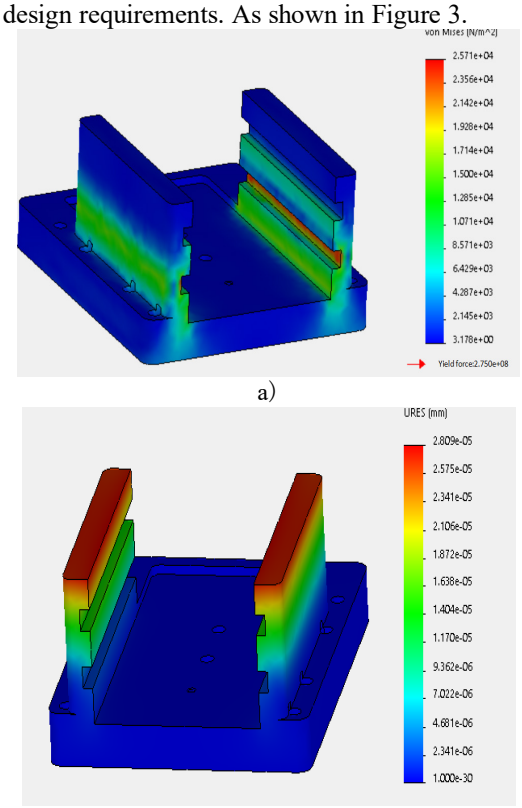


Fig. 3. Finite element analysis.

2.4. System hardware design

According to the functional requirements, a series of modular design concepts are adopted in the design of an embedded system based on STM32F103VET6 microcontrol unit (MCU), aiming to achieve efficient signal processing, accurate drive control, stable power management, intuitive human-computer interaction interface and reliable data storage functions. This design mainly includes the following key modules: microprocessing unit and its basic support system, signal acquisition and processing module, drive module, power supply module, user interaction interface module and data storage module. These modules together constitute a complete system, which can meet the needs of complex embedded applications. The following is the overall hardware control scheme. As shown in Figure 4.

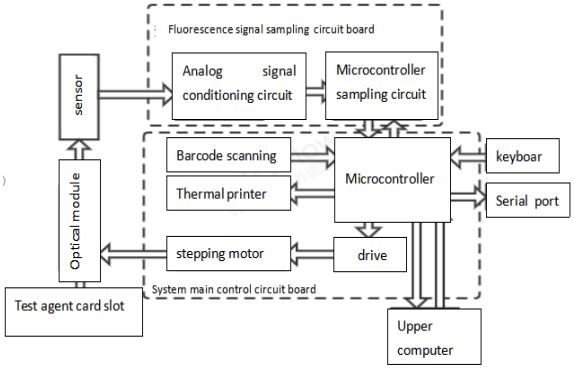


Fig. 4. Hardware control overall design scheme

2.4.1 Signal amplification

After I/V is converted into voltage signal, the voltage amplitude can reach mV, and the signal detection amplitude is too small, so the signal needs to be further amplified. This study uses the transresistance effect to amplify the signal. When the input signal of a diode changes, its trans-resistance (that is, the ratio of the input voltage to the output current) will also change accordingly. A transresistance amplifier with a T-network feedback configuration can convert an input current source into an output voltage. The current-to-voltage gain is based on the T-network equivalent resistance, which is larger than any resistance used in the circuit, allowing for very high gains that reduce noise, stability issues, and errors in the system.

2.4.2 Motor drive

The mechanical structure of the detector includes a stepper motor and a sliding track. The stepper motor rotates, the detection light chamber moves along the sliding track, and the test strip below the track is scanned. The tester selected model TMC5130A as the motor drive IC. TMC5130A is a two-phase stepper motor driving IC, the advantages are low noise, low energy consumption, stable operation, strong output power. Its operating voltage range is 8-60V; The maximum drive motor current can reach 3A or 6.5A. The detector uses a four-wire bipolar stepping motor, the working voltage is +5V, the maximum working current is 800mA. TMC5130A composite design requirements.

3. Algorithm Design

In the process of signal acquisition, the internal noise of the instrument, the influence of scattered light, and the interference of A/D quantization noise will cause errors when the same sample is measured under the same conditions. In order to reduce the impact of the interference brought by the error, the need to collect the signal filtering processing, hardware design often adopts the method of simulator filtering, if you can cooperate with the software filtering will achieve better filtering effect. Therefore, the digital filtering algorithm is added.

A Gaussian signal is a mathematical function whose shape has a Gaussian or normal distribution characteristic. In signal processing and statistics, Gaussian signals are often used to represent the output of a system with Gaussian noise or some random process with a specific property. The graph of a Gaussian function is presented as a bell curve with a symmetric center, with the maximum value at the center and gradually decreasing to both sides, never touching the horizontal axis. The one-dimensional mathematical expression of the Gaussian signal (the Gaussian function) is:

$$f(x) = A * e^{-(x-\mu)^2} / (2\sigma^2) \quad (1)$$

Where is the amplitude of the Gaussian function. is the independent variable. is the position of the mean or peak, which determines the center of the waveform. is the standard deviation, determines the width of the wave, the wave dispersion degree. The square of A is called the variance. represents the natural exponential function.

The following article will filter and contrast the double-peak Gaussian signal with Gaussian white noise. The visualization of the Gaussian signal with the addition of noise is as follows, Figure 5 is the original signal and Figure 6 is the noise signal.

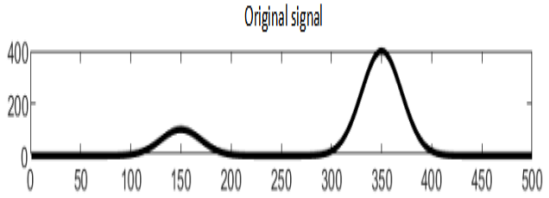


Fig. 5. Raw signal.

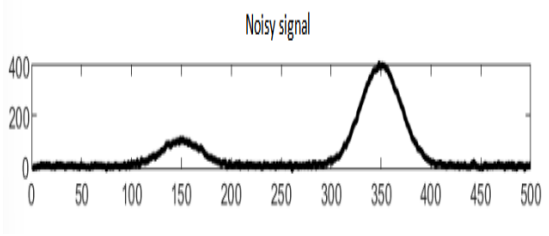


Fig. 6. Noisy signals.

3.1. Compound filtering algorithm

In this design, the method of combining the moving average filter and wavelet transform is proposed to denoise the photoelectric signal collected by the instrument, which can effectively remove the low frequency interference and high frequency noise, and make the sampling curve smoother. The detection accuracy is further improved. The moving average algorithm is used to remove high frequency noise and retain curve edge details. As can be seen from equation (1), the smoothness of moving filter denoising is determined by N, and the queue length N needs to be taken as a value. The value of N can be obtained by the following formula (2) :

$$WC = \frac{FC}{FX} = \frac{1}{2.21N + 0.32} N \geq 2 \quad (2)$$

(1) Wavelet decomposition

First, the processed signal should be decomposed by wavelet, and the wavelet decomposition needs to select the appropriate wavelet basis and the number of decomposition layers. The selection of wavelet basis needs to be comprehensively considered from the three evaluation parameters of root-mean-square error, signal-to-noise ratio and smoothness. Different wavelet base processing signals show different characteristics, and no wavelet base can perfectly cope with all signal types. According to experience, Sym6 wavelet base is chosen. Its mean square error is small, the smoothness is

moderate, comprehensive consideration is chosen as the wavelet base of this algorithm.

Then it is necessary to select the appropriate number of decomposition layers. The higher the value of decomposition layers, the higher the separation degree of signal and noise, but the greater the distortion degree of the reconstructed signal. Therefore, it is necessary to comprehensively consider to obtain the appropriate number of decomposition layers. In the design of biochemical instrument, photoelectric signal denoising often adopts 5-layer decomposition, so the reconstruction scale of wavelet decomposition is chosen to be 5.

(2) thresholding processing

Since noise is a random signal, the threshold of noise must be estimated first, the commonly used methods are: maximum minimum threshold estimation, fixed threshold estimation, unbiased risk estimation and heuristic estimation. The threshold selection rule is based on the model:

$$y = f(t) + e \tag{3}$$

e Is Gaussian white noise and t is the raw signal. Therefore, the threshold of noise in the wavelet domain can be judged by the original signal. Maximum minimum value Threshold estimation has a very good effect on weak signal extraction when there is less noise in high frequency band.

$$\lambda = \begin{cases} 0.3926+0.1829(\dfrac{\ln N}{\ln 2}) , & N>32 \\ 0, & N\leq 32 \end{cases} \tag{4}$$

Where N is the signal size.

After getting the threshold value λ , it is also necessary to choose the appropriate threshold function, the commonly used threshold function has hard threshold function and soft threshold function. The wavelet coefficient obtained through the processing of the soft threshold function makes the signal continuous signal without excess oscillation, which meets the requirements. Using the formula:

$$Z = \begin{cases} \text{sign}(x) & (|x|-\lambda) \\ 0, & |x|\leq\lambda \end{cases} \tag{5}$$

When the absolute value of the coefficient is less than the threshold value, the function value is zero; When the absolute value of the coefficient is greater than the threshold value, the result of subtracting the threshold value from the absolute value of the coefficient is taken. Where sign is the sign function and x is the input wavelet coefficient matrix. When the value of x is greater than 1, the sign function value is 1; x value equals 0, the function value is 0; If the x value is less than 1, the function value is -1.The obtained wavelet coefficient matrix is reconstructed by using Q-shift inverse transformation.

The following figure 7 shows the result of filtering the Gaussian signal:

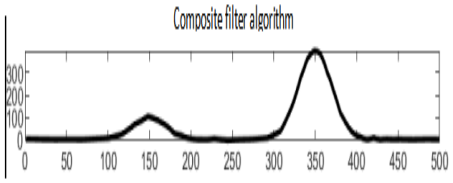


Fig. 7. The result of composite filter processing.

3.2. Concentration calculation

After the test substance is added to the test strip, the antigen will react with the antibody, and the antibody label and fluorescent microsphere will bind to it. At this time, under the irradiation of the excitation light, the fluorescence reaction in the T-line region and the C-line region will be obvious, while the fluorescence reaction in other regions will not or rarely occur. It is reflected in the absorption spectrum as two peaks, and the rest of the spectrum is ideally the same as the baseline. The greater the concentration of the object to be measured, the more fluorescent markers will be bound and the greater the light intensity value will be emitted, so there is a mathematical model to describe this relationship between the ratio of the concentration of the object to the detected light intensity.

3.2.1 Algorithm of light intensity ratio

In an ideal case, the baseline is a horizontal line, and there will be a crest where the T and C lines are located, but not in other areas. At this point, calculate the area of the T-line crest and the C-line crest, listed as ST and SC, assuming the concentration of the object to be measured is C, you can get an area and concentration relationship:

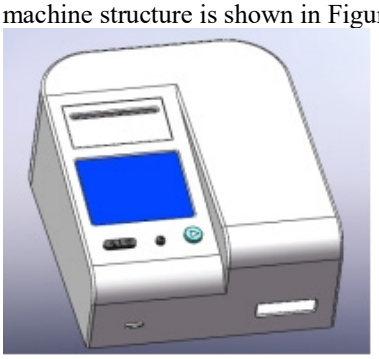
$$C = f(S_T / S_C) \tag{6}$$

To calculate the area ratio of line T to line C. First you need to find the area of the crest of both. But the starting point and the end point of the wave crest are difficult to judge intuitively. This is because in the production process of the test strip, the specific size is not consistent, that is, the strip width of the T line and the C line is not a fixed value on different test strips. Therefore, it is obviously unreasonable to simply judge the starting point and end point of the wave crest from the fixed position point. Therefore, in the program design, it is necessary to find out the starting point and endpoint of each wave crest. Scan the strip of travel in 50mm, walk 600steps, each step to collect a signal value, and finally can get 600 sampling values. These 600 points are denoting M1, M2..... M600[6]. Start with M1 and calculate M2-M1, then M3-M2, and so on until the difference is greater than the preset value (according to experimental calibration), at which point M3 is the starting point: then continue to iterate until the difference is greater than the negative default value, at which point it is the endpoint. Knowing the starting point and the end point, we can find the area of the crest. Finally, the detection ratio is obtained. Put into the curve equation to find the concentration of the substance to be measured.

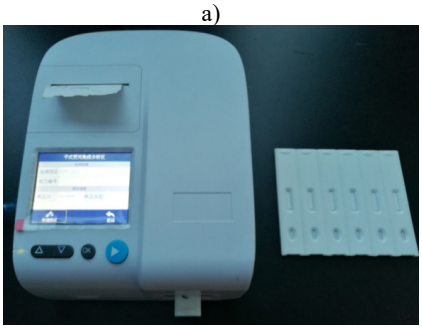
4. The whole machine installation and test

4.1. Complete machine installation

The whole machine structure is shown in Figure 8.



a)



b)

Fig. 8. Structure of the whole machine.

4.2. Instrument performance test

Before detection, make sure the immunochromatography card reader and supporting software can be connected successfully, and prepare the recombinant protein of PCT into solutions of 100, 50, 25, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05 and 0ng/ml. Repeat the detection 3 times respectively, and conduct the detection at 15 minutes (when the chromatography lasts for 15min, the reading value can reach stability, and the relevant indicators are also excellent), and make the standard curve. R2>0.99, which can be seen that the fluorescence quantitative detection system has good linear characteristics. The fitting curve determines the concentration of the solution at the test size, and realizes the quantitative detection. The log-log curve of PCT protein concentration and T/C value is shown in Figure 9.

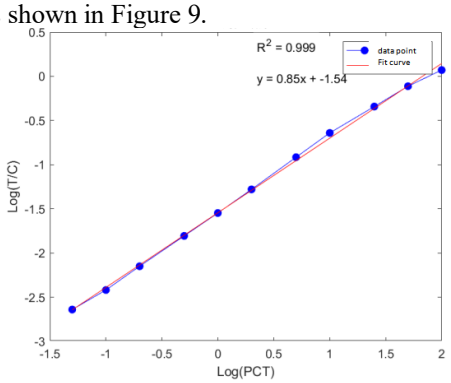


Fig. 9. Logarithmic curve of PCT protein concentration and T/C value.

5. Conclusion

According to the fluorescence detection principle of laser induced fluorescence technology, and based on this principle, the optical detection mechanism is designed. Through the whole system design and finite element analysis, the rationality of the device is verified by experiment. On this basis, a variety of algorithms are designed for the internal noise generated in the process of signal acquisition. Finally, a mathematical model is proposed which combines the moving average filter and wavelet transform to achieve the denoising effect of the electrical signal. At the same time, the mathematical model of light intensity and concentration based on the least square method is curve fitting. At the same time, the feasibility of the algorithm is verified. Compared with the existing fluorescent immunochromatography POCT device, it has small size, high sensitivity, and can realize qualitative and quantitative detection at the same time. These studies provide important value for the realization of precision medicine, early diagnosis of diseases and epidemic detection.

6. Outlook

Fluorescence immunochromatography technology can be applied to the analysis of a variety of biological samples, including serum, urine, saliva and other body fluid samples, as well as food and environmental water samples monitoring field, the detection range covers biomolecules, cytokines, hormones, drug residues and other target substances, has a wide application prospect and can be combined with other technologies, such as microfluidic technology, electrochemical detection technology, To achieve multi-parameter and multi-dimensional biological analysis, and improve the accuracy of analysis. The trend of multi-technology integration will further promote the application of fluorescence immunochromatography technology in medicine, biology and other fields, and provide more powerful tools and support for scientific research and clinical diagnosis.

References

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