Design of flow cytometer liquid circuit control system based on incremental PID algorithm

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Abstract. In the application and design of the flow cytometer, the problem of controlling the flow rate of liquid sample should be considered, which is directly related to the test performance of the instrument. In this paper, a flow cytometer liquid path controlling system based on incremental PID algorithm is designed. The STM32F429 is used as the core control chip, and a negative feedback correction network is introduced. At the same time, the incremental PID algorithm is used to control the speed of electronic motor. Combining with the hardware circuit, the automatic regulation and control of the flow rate of liquid path sample are realized. The experimental results show that the real-time sample flow can approach to the set value quickly and stably after the sampling started in this system. When different target values are set, the error between the measured value and the set value is less than 3%. The system is simple in design, high accuracy and stability, and can meet the requirements of intelligent control and high efficiency and precision in modern industrial instruments.

1. Introduction

Flow cytometer is a new high-tech instrument integrating laser technology, electronic measurement technology, chemical fluorescence technology and computer technology. The fluid path part is one of the important parts of the flow cytometer, and the control of the sample flow is the key factor that determines the working state of the fluid path. Therefore, it is of great significance to realize precise and efficient automatic control of sample aspiration. The negative pressure of the waste liquid tank is constant during the operation of the entire liquid path, and the sample flow interacts with the sheath flow. The faster the sheath flow, the slower the sample flow, and the speed of the sheath flow can be controlled by the motor. In order to meet the demand for efficient and accurate sample flow of the flow cytometer, it is necessary to optimize the control of the motor speed to indirectly ensure the accuracy and stability of the sample flow.

In Literature [1], pressure sensor was used for sampling, and the collected hydraulic signals were converted into voltage signals to judge the flow. This method cannot realize the real-time adjustment of sampling accuracy and sampling flow. Literature [2] adopted large-range flow sensor, measuring flow range was 4~30lu/min, and it adopted incremental PID algorithm to adjust the scraper pump, which was suitable for application scenarios with large flow and low precision requirements. Compared with other instruments, the flow rate of flow cytometry was relatively small, generally 10~80ul/min, which required the use of a small range flow sensor for measurement. In Literature [3], a turbo-type flow sensor and an inverse trigger were used to collect and shape the signal, and the flow control was mainly realized by the switching frequency of the solenoid valve in the balance cavity,
and the instantaneous flow value was relatively large. This method cannot be adjusted in real time and the flow is unstable. The flow cytometer in Literature [4] used a pressure sensor to collect the flow, and the sheath fluid was pressed into the flow chamber under the effect of gas pressure. The above methods cannot meet the special needs of small flow, high precision, and real-time sampling of flow cytometers. In this paper, a flow cytometer liquid path control system based on incremental PID algorithm is designed. Incremental PID has the advantages of simple principle and parameters, and it is easy to implement, so it is widely used in all fields [5]. In this paper, as the main control chip, the STM32 microcontroller is measured the sampling flow by using the flow sensor. The incremental PID algorithm, hardware design and system software design are also introduced. The practical application proves that the system adopts the incremental PID algorithm to control the motor speed, so as to indirectly control the sample flow, achieving the effect of rapid stability of the liquid circuit and high speed and accurate sample flow.

2. Working principle of liquid circuit

The flow cytometer fluid system includes a flow chamber, a sampling needle, and a series of valves, pumps, and tubing. The schematic diagram of the flow chamber is shown in Figure 1. The flow chamber includes a sample tube, a sheath liquid tube, and a nozzle. When the sample is collected, the sheath flow in the flow chamber surrounds the sample flow, and the sheath fluid flows in the same direction as the sample fluid. Under the constraint of the sheath fluid, the sample particles are arranged in a single stream and the particles are excited by the laser to obtain scattered light signals and fluorescent signals. The light signals are photoelectrically converted and collected and transmitted to a host computer for further processing and analysis [6]. During the counting process, the sample is collected using the negative pressure sampling principle, and the negative pressure in the waste liquid tank is kept constant. At the same time, the sample real-time flow from the flow sensor is read by the STM32 continuously, and the motor speed can be adjusted according to the real-time flow to make the sample flow stable near the target value. It is helpful to ensure the accuracy of sample data collection.

3. Control system design scheme

3.1. Overall block diagram of fluid control

As can be seen from Figure 2, the overall block diagram of the system introduces a negative feedback nonlinear correction network [7], where the controlled object is the real-time sample flow. The target sample flow is set on the PC. The PC transmits the target sample flow parameter to the STM32 through the network port. The STM32 controls the sheath fluid flow by controlling the motor speed,
thereby indirectly controlling the sample flow. During the counting process, the flow meter monitors and collects the sample flow in real time, and converts the analog signal into a digital signal. The digital sample flow rate information is transmitted to the STM32 through the IIC bus. In addition, the STM32 constantly compares the latest sample flow value with the target flow value to further adjust the motor speed.

3.2. Control system solution
The negative feedback correction network is used in this system to feed the output of the controlled object to the input for comparison processing, forming a closed-loop network [8]. The block diagram of the PID control system is shown in Figure 3. The closed-loop structure of the PID algorithm is adopted, and the target sample flow set by the PC is compared with the flow value fed back by the flowmeter to obtain the deviation value $e$. PID algorithm processing is performed on the deviation value $e$ to obtain the control amount $U$. The control amount $U$ is used to control the motor speed, so as to better control the sample flow rate and make it more stable and accurate.

![Figure 2. System control principle overall block diagram.](image)

![Figure 3. PID control system block diagram.](image)

3.3. Introduction to incremental PID algorithm
Incremental PID is a control algorithm with a wide range of applications in process control. It is suitable for actuators with integral memory elements. The end position of this type of actuator is related to the accumulated value of each previous input signal, each input signal determines the increment of the end position of two adjacent actuators. This is $\Delta U(k)$, not the actual position of the actuator [9]. Incremental PID algorithm is widely used in stepper motor control and stepper motor driven valve [10]. The expression of the incremental PID control algorithm is:

$$\Delta U(k) = U(k) - U(k-1) = K_p \Delta e(k) + K_i e(k) + K_d \Delta e(k)$$

As the delta $\Delta e(k) = e(k) - e(k-1)$.

$$\Delta U(k) = K_p [e(k) - e(k-1)] + K_i e(k) + K_d [e(k) - 2e(k-1) + e(k-2)]$$

Where $K_p$ is the proportional coefficient, $K_i$ is the integral time constant, $K_d$ is the differential time constant.

It is known from the expression that after determining the three parameters of $K_p$, $K_i$, and $K_d$, the control delta $\Delta U(k)$ is only related to the last 3 times of sampling values and no accumulation required. The advantage of the incremental PID algorithm is that it uses weighted processing. The computer will only output the control increment $\Delta U(k)$ each time, which is the amount of change in the position of the actuator, so the probability of the computer failure is small [11-13].
4. System hardware circuit design

4.1. Overall design of system hardware
The overall hardware design of flow cytometer liquid circuit control system includes power module, microcontroller minimum system module, liquid circuit drive module, flow needle and PC, etc. The overall hardware design diagram of the system is shown in Figure 4. The target sample flow is input on the PC and sent to the STM32 microcontroller. The STM32 outputs a control signal to the liquid drive module to control the motor speed, so as to continuously adjust the sample flow. The flowmeter with measuring range of 5~120ul/min is adopted for real-time sampling, and the actual sample flow is transferred to STM32 microcontroller. The STM32 upload the data to the PC for display. The PC is used to input commands and display data. The power module supplies power to each module so that the whole system can work properly.

4.2. Motor drive circuit module
A4984 chip is used in the motor drive module of the system. A4984 is a motor driver produced by Allegro company, which is easy to operate and has a built-in converter. The fixed-downtime current regulator in the motor driver can operate in slow or mixed attenuation modes with simple control and input drive capabilities up to 35V and ±2A. The motor drive circuit is shown in Figure 5.

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Figure 4. Overall hardware design.

Figure 5. Motor drive circuit.
The four pins of ENABLE, DIR, STEP and REF on the motor drive chip A4984 are connected to the single chip microcomputer, which means that the four pins of the single chip microcomputer can completely control the stepper motor. ENABLE is the enable end of the stepper motor controller. The microcontroller sends a command to ENABLE to determine whether the motor is in the enabled state or the motor is locked. DIR is the direction control terminal of the motor. It controls the forward or reverse rotation of the motor by receiving the level given by the microcontroller. STEP is the pulse control terminal of the motor. It controls the number of rotations and the speed of the motor by receiving the number of pulses and the pulse frequency sent by the microcontroller. REF is the reference voltage terminal of the motor, which is related to the driving current [14-15].

5. System software design

5.1. Main program design
The main program control flow chart is shown in Figure 6. After the system is powered on, the system is initialized and parameters are set, and then start to count. The current flow is collected by the flow meter and handed to the MCU for processing. The incremental PID algorithm is used in the program to compare the target value with the current flow collected by the STM32 microcontroller. Make the corresponding control to adjust the motor speed, and display the real-time sample flow on the PC.

5.2. Realization of incremental PID algorithm control
The system compares the actual value $y(k)$ collected by the flowmeter with the target value $r(k)$ set by the PC to obtain the difference value $e(k)$. As the input of PID controller, $e(k)$ can calculate the increment $\Delta U(k)$ to get the control variable and update the parameters. This can solve the lag of sample flow control and improve the response speed [16]. The PID algorithm is shown in Figure 7.

![Figure 6. Main program control flow chart.](image1)

![Figure 7. Incremental PID algorithm subroutine flow.](image2)
5.3. Implementation of flow control PID algorithm
By analyzing the PID control, the algorithm first compares the actual sample flow rate collected by the flow meter with the set value, calculates the deviation value, and then performs PID calculation to obtain the output result, which is used to control the sample flow rate by adjusting the motor speed. In order to determine the PID parameters with good control effect, it can achieve the purpose of quickly stabilizing the liquid circuit and adjusting the motor speed, thereby ensuring that the sample flow can reach the target value. This article uses the "trial and error" method in the empirical method. According to the influence of the P, I, and D parameters of the PID controller on the system performance as a theoretical basis, the controller parameters are repeatedly "tried" one by one in the order of proportional, then integrated, and finally differentiated until a satisfactory control effect is obtained. After several calculations and debugging, the final PID coefficients are $K_p=5$, $K_i=0.5$, $K_d=0$.

6. Experimental result
In order to verify that the system design can quickly stabilize the liquid circuit and adjust the motor speed in real time to ensure that the sample flow can reach the target value, the physical object is built and the experiment is carried out. The physical diagram of the flow cytometer liquid control system is shown in Figure 8. After system sampling is started, the background serial port monitors the real-time sample flow and takes the flow value every 1 second for data analysis. The diagram of real-time sample flow is shown in Figure 9. The target sample flow is set as 20ul/min, 50ul/min and 80ul/min respectively on the upper computer, and the PID parameters are $K_p=5$, $K_i=0.5$, $K_d=0$. The sample flow is collected by the flow meter in real time and displayed on the host computer. The flowmeter transmits the collected information to the single-chip microcomputer for processing. After the PID is turned on, the motor speed is adjusted according to the deviation between the actual sample flow and the target sample flow, so as to achieve the purpose of adjusting the sample flow. As can be seen from Figure 9, after the sampling is started, the sample flow oscillates several times and tends to stabilize rapidly to reach the target value as soon as about 10s. Moreover, the stability effect is good, which can meet the control requirements of the system.

Figure 8. System physical map.  
Figure 9. Sample real-time flow rate map.

To verify the stability of system control, target values are set as 20ul/min, 50ul/min and 80ul/min, respectively. The change of sampling flow is monitored through the serial port in real time. After PID is turned on and the sheath flow is stable, 10 groups of data are randomly selected to calculate the error between the target flow and the actual flow. The experimental data is shown in Table 1. It can be known from the test results in the table that under different target flows, the variation range of the real-
time sample flow after stabilization is within 1.1ul/min, and the stability error between the target flow rate and the actual flow rate is less than 3%. The higher the target flow, the more stable the sample flow, and the smaller the real-time flow stability error. The system has high stability in controlling sample flow, small error and good performance, and can meet the application technical standards of flow cytometry.

### Table 1. Errors at different target flows.

| Target the flow/ul/min | Actual flow/ul/min | Error/% | Target the flow/ul/min | Actual flow/ul/min | Error/% | Target the flow/ul/min | Actual flow/ul/min | Error/% |
|-------------------------|--------------------|---------|------------------------|--------------------|---------|------------------------|--------------------|---------|
| 1                       | 20                 | 2.815   | 50                     | 50.703             | 1.406   | 80                     | 80.138             | 0.173   |
| 2                       | 20                 | 2.585   | 50                     | 51.068             | 2.136   | 80                     | 80.083             | 1.038   |
| 3                       | 20                 | 2.000   | 50                     | 50.517             | 2.000   | 80                     | 79.926             | 0.093   |
| 4                       | 20                 | 1.835   | 50                     | 50.252             | 0.504   | 80                     | 80.283             | 0.354   |
| 5                       | 20                 | 2.960   | 50                     | 50.570             | 1.140   | 80                     | 79.850             | 0.188   |
| 6                       | 20                 | 2.455   | 50                     | 51.012             | 2.024   | 80                     | 80.608             | 0.760   |
| 7                       | 20                 | 2.770   | 50                     | 50.917             | 1.834   | 80                     | 79.776             | 0.280   |
| 8                       | 20                 | 1.520   | 50                     | 50.589             | 1.178   | 80                     | 79.317             | 0.854   |
| 9                       | 20                 | 1.465   | 50                     | 50.854             | 1.708   | 80                     | 80.065             | 0.081   |
| 10                      | 20                 | 2.245   | 50                     | 50.392             | 0.784   | 80                     | 80.642             | 0.802   |

In order to verify the effectiveness of this control system. In order to verify the effectiveness of this control system, this design is compared with a flow cytometer using a PK80167 pressure sensor. The volume consumption error data is shown in Table 2. Start the instrument and aspiration, the program will stop counting when the amount of aspiration is around 300ul. Calculate the actual consumption of the sample and compare it with the sample consumption displayed by the PC to obtain the sample consumption volume error.

It can be seen from Table 2 that the small-range flow sensor and the incremental PID algorithm are used in this system to adjust the sample flow in real time, which can make the PC display the sample volume consumption close to the actual consumption, and the error between the two is less than 2.5%. However, in the flow cytometer which uses pressure sensor to detect samples. The error between sample volume consumption and actual consumption is large. Experiments show that the control method used in this design has good effectiveness, and can better control the stability of the liquid circuit and adjust the flow error in real time. And it can save samples and improve detection accuracy.

### Table 2. Sampling consumption volume error comparison.

|                  | Traffic Setting/ul/min | Pre-test weight/g | Post-test weight/g | The weight difference/g | Actual consumption/ul | Display sample absorption/ul | Error/% |
|------------------|-------------------------|-------------------|-------------------|------------------------|-----------------------|----------------------------|---------|
| This design      | 10                      | 5.084             | 4.791             | 0.293                  | 293                   | 300.2                      | 2.46    |
| This design      | 30                      | 4.775             | 4.479             | 0.296                  | 296                   | 300.1                      | 1.39    |
| This design      | 50                      | 5.671             | 5.371             | 0.300                  | 300                   | 301.8                      | 0.60    |
| This design      | 80                      | 5.972             | 5.671             | 0.301                  | 301                   | 301.3                      | 0.10    |
| Use of pressure sensor | 10                     | 5.611             | 5.233             | 0.378                  | 378                   | 300.0                      | 20.63   |
| Use of pressure sensor | 30                     | 4.783             | 4.428             | 0.355                  | 355                   | 300.0                      | 15.49   |
| Use of pressure sensor | 50                     | 5.320             | 4.985             | 0.335                  | 335                   | 300.0                      | 10.45   |
| Use of pressure sensor | 80                     | 4.766             | 4.447             | 0.319                  | 319                   | 301.0                      | 5.96    |

7. Conclusions
In this paper, a flow cytometer liquid path control system based on incremental PID algorithm is designed, which can detect sample flow in real time and adjust in time. The sample flow error can be effectively reduced, and the accuracy of sample flow detection is improved. The STM32 single-chip
microcomputer is adopted as the system main control chip. The overall hardware design and system software design are introduced, and the liquid circuit motor drive circuit is designed to control the motor. The system is controlled by the incremental PID algorithm, so that the real-time flow rate of the flow cytometer fluid sample is stable within 1.1ul/min. The error between the real-time sample flow and the target value is less than 3%, and the sample consumption volume error is less than 2.5%. The flow can quickly approach the set value, and the liquid channel can quickly become stable. At the same time, the experimental results show that the system design is simple, and has high precision and strong stability, it is suitable for industrial control field.

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