Scientific contribution

Electronic Blood Sedimentation Monitoring with Microcontroller and Linear CCD Sensor

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Abstract

Modern electronics can be utilized to provide automated means of monitoring spontaneous blood sedimentation and by implementing wireless power and data transfer can be used in centrifugation systems as well. Here, we present the method of building the optical linear charge coupled device (CCD) sensor with microcontroller circuit for recording and monitoring the sedimentation process. Brief overview of the engineering behind the communication protocol is given together with key microcontroller techniques that are required for fast analogue voltage signal sampling. Proposed system has adaptable light integration protocol and shows exceptional durability under centrifugal force loads that are commonly used in platelet-rich plasma. The parts for building the system are sourced at common electronics stores and provides easy means for observing optical changes in blood samples with emphasis on sedimentation and forming layers with different optical properties.

Keywords: Blood sedimentation; Plasma; Platelet-rich plasma; Microcontroller; Optical sensor

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1. Introduction

For faster wound healing, the application of blood without erythrocytes (plasma) to the injured tissue is used as an effective method (Daif 2013, Božič et al. 2022). Appropriately prepared plasma is used, which has an increased concentration of platelets relative to the initial concentration in the blood before the preparation (Vozel et al. 2021a).

When treating patients with platelet-rich plasma, a sufficient volume of blood must be drawn from the patient. Using laboratory centrifuges, which allow relatively low centrifugal accelerations, we increase the platelet concentration in the isolate compared with the total blood sample (Božič et al., 2022). During centrifugation, the erythrocytes settle at the bottom of the vessel, leaving above platelet-rich plasma (supernatant), however, the profile of the platelets in plasma depends on the dynamics of the system. Sedimentation of erythrocytes concentrates them towards the bottom of the tube which induces formation of rolleaux and therefrom channels that enable movement of plasma with smaller particles (including platelets) in the opposite direction. In this way the compartment above the erythrocyte boundary gets enriched with platelets and extracellular vesicles. Eventually, the centrifugal force causes sedimentation of platelets and concentrates them in the layer at the boundary between erythrocytes and plasma. During this process the distribution of platelets along the length of the plasma column is changing. On the other hand, leukocytes are accumulated at the boundary between erythrocytes and plasma (**Figure 1**).

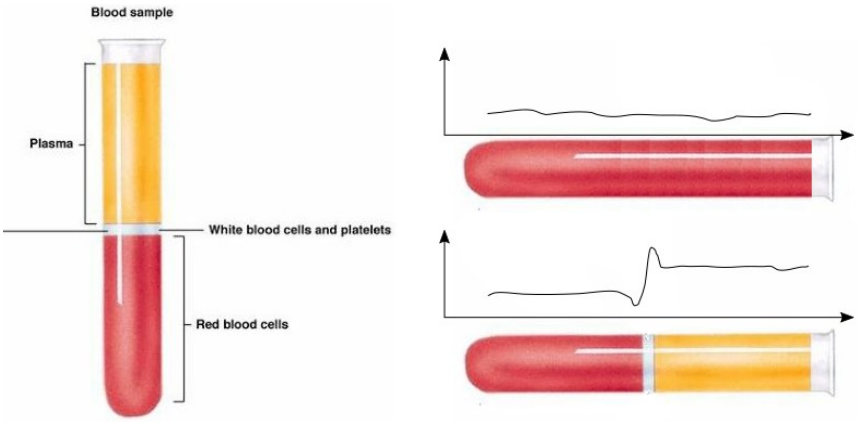

Figure 1. Blood sedimentation. At the bottom, there is a sediment of red blood cells, the platelet-rich layer and plasma (left). Different layers have different opacities; thus we can detect the layers with optical sensors (right). Above the epruvettes, a hypothetical signal is depicted.

Centrifuge parameters such as acceleration and centrifugation time strongly influence how the different blood particles in the sample will move under the influence of the centrifugal force. The blood of different patients may differ in composition and thus in its physical and chemical properties resulting in different sedimentation times. Parameters can be experimentally determined and mathematical models based on previous isolation protocols which could predict the settings of the centrifugation process have been developed (Chahla et al. 2017, Božič et al., 2022). The erythrocyte sedimentation time is crucial in the separation of erythrocytes from plasma as it provides a source of counterflow of platelets which is at the same time diminished when platelets are found in plasma devoid of erythrocytes. Mathematical modelling indicated that there is an opzimal time to enrich plasma with platelets and extracellular vesicles and that this takes place when erythrocyte boundary that moves towards the bottom of the tube coincides with boundary of platelets and extracellular vesicles that moves in the opposite direction (Božič et al., 2022). Further centrifugation may deplete plasma of platelets and extracellular vesicles. To test the model and enable optimization of the procedure, it would be of interest to follow the boundary of erythrocytes during preparation of plasma.

Within this work, we will present the development of an automated centrifuge module and tool for measuring spontaneous sedimentation, based on red blood cell settlement monitoring with the S11108 complementary metal-oxide-semiconductor CMOS linear CMOS sensor from Hamamatsu **(Figure 2**). Most of the development consists in the con-

struction of an electronic module for communication between microcontroller and the optical sensor. The microcontroller system is based around the ST Microsystems STM32F103 family microcontroller and, in addition to processing the sensor data, it takes care of deciding on a sufficient centrifugation time. It records the light transmission through the sample and in this way gives valuable insights to the researcher about the sedimentation process.

2. Methods

For prototyping, the linear CCD sensor Hamamatsu S11108 was chosen. Test tube (Vacutainer) containing the blood sample is inserted between the sensor and light emitting diode (LED) light source. The blood is illuminated with light source and the intensity of the transmitted light is recorded with an array of optical receptors. The Hamamatsu S11108 sensor has an array of 2048 photodiodes of size 14x14 μm on an active sensor length of 28.672 mm. This length is comparable to the length of Vacutainer tube. The signal generated by individual photodiodes/photoreceptors is relative to the light intensity and can be read at a frequency of 200 kHz to 10 MHz per pixel. The supply voltage is 5 V and the current consumption during operation at the lowest frequency is only 10 mA. The sensor is sensitive to light in the visible part of the spectrum between 400-1000 nm, with peak sensitivity at 700 nm. The video signal is linear between 0,3 V for dark point and 1,2-1,7 V for fully light saturated point.

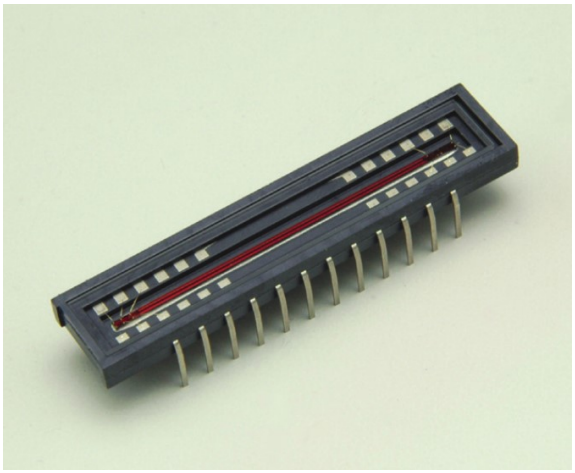

Figure 2. Hamamatsu S11108 linear CCD optical sensor with 2048 photoreceptors that form a single image of the light intensity in the test tube.

The communication protocol that sends data about pixel intensities is simple and is shown in time diagram in **Figure 3**. The sensor expects a digital clock signal which defines the temporal information to its electronics. Depending on the clock frequency, the light integration time during which the photoreceptors receive the light signal can be set by adjusting number of clock cycles. The frequency of image acquisition can be altered by changing the clock frequency. In addition to the clock signal, the sensor needs information when to start integration and send the data to the outside world. This is achieved by a start signal (denoted as ST in time diagram). Its length is important for setting the integration time, but it also triggers the shifting of the video signal at the sensor output. The sensor also generates a trigger signal (Trig) which can be used to synchronise with an analogue-to-digital translator. The end of the data transmission is indicated by an Electro – Optics – System (EOS) signal.

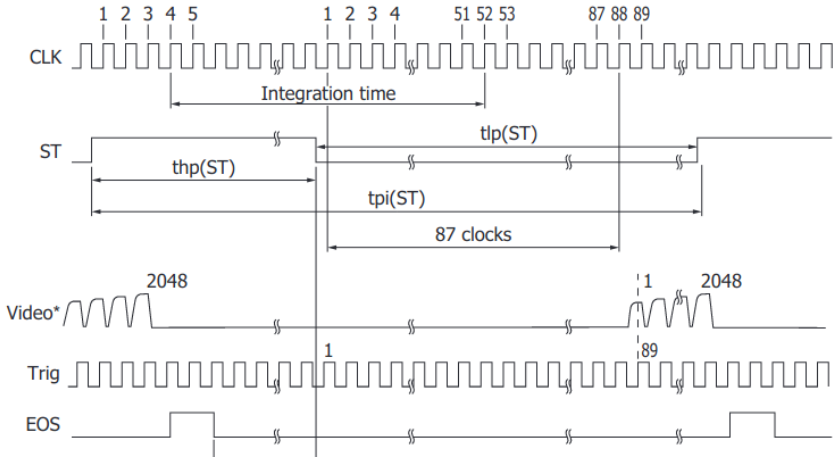

Figure 3. Timings of the communication protocol between CCD sensor and controlling circuit with all the required signals and their time durations in units of clock cycles. The actual time to read the whole image depends on the cock frequency and can vary substantially on this value.

The STM32F103 microcontroller on the well-known "Blue pill" (see **Figure 4**) development board was used to implement the communication protocol with the sensor and communication with the computer. The microcontroller has a universal set of timers which can be used to implement a clock signal for the sensor and a properly synchronised signal to start sending video data. Two timers are used which are connected in series; the first, TIM1, performs the task of a clock generator for the sensor, divides the processor clock and generates a 200 kHz digital signal at the output of the microcontroller by activating the Output compare function. At the same time, it triggers an internal event for the second timer TIM2, which uses the frequency of the first one to generate the ST signal. The output of the second timer generates a pulse width modulated digital signal at the output. The pulse length determines the integration time of the sensor.

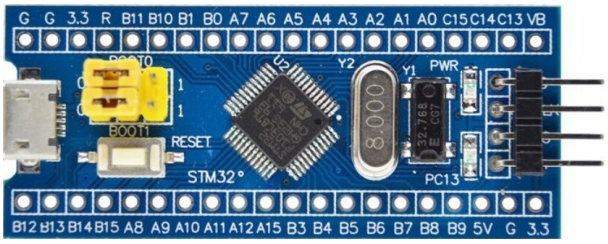

Figure 4. "Blue pill" development board with STM32F103 microcontroller on the board. The small microcontroller is fast enough to process the data output by the CCD sensor.

The analogue output signal of the sensor is fed to the input of an analogue-to-digital converter (ADC). The ADC can be operated at a maximum sampling rate of 500 kHz and can write the conversion results directly to microcontroller memory using a direct memory access (DMA) that requires no computational power from the microprocessor part. To run the analogue-to-digital conversions of the ADC, a trigger signal from the sensor was used, which tells when the video signal at the sensor output is ready for capture and is slightly lagging the sensor clock.

The timers and the ADC are configured to do their work independently of the processing unit and thus do not consume processor cycles. When the ADC finishes filling the memory and captures the entire image of the line scan sensor, an interrupt is triggered to tell the main processor that new data is available. These are processed and sent via USB to the computer.

3. Results

The system was developed in ST Microelectronics integrated development environment called STM Cube. The resulting communication between the sensor and microcontroller was observed by means of digital oscilloscope, ensuring all the timings and frequencies are correct and within the sensor specifications. The test of the sensor was done by illuminating Vacutainer tube containing different fluids and observing the voltage responses by oscilloscope and by examining data sent by the system to the computer. We have defined a threshold below which the measurement is considered to be opaque, giving us discrete information about how translucent is the material.

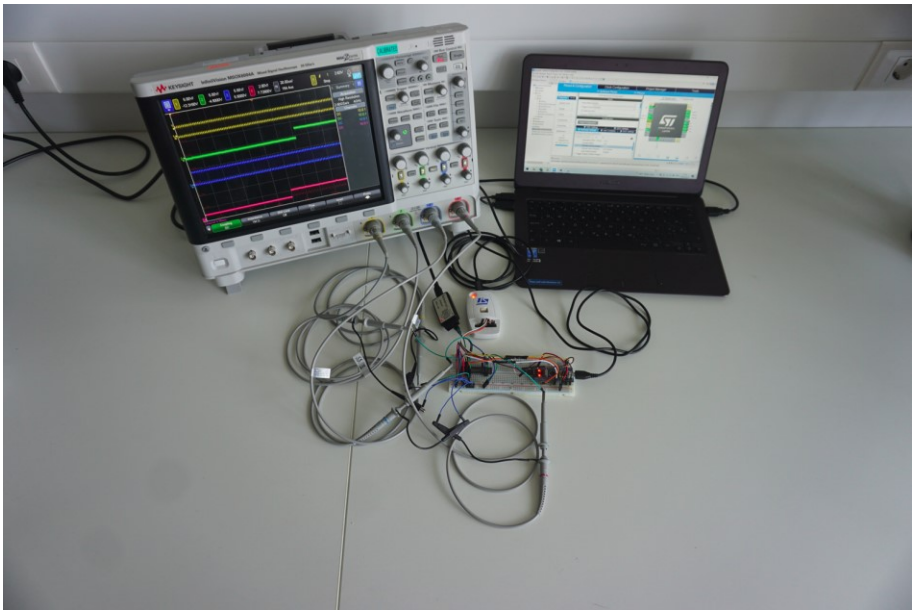

Figure 5. Testing the CCD sensor communication with microcontrollers with oscilloscope and computer. The development software running on the computer is used to reprogram the microcontroller board on the prototype board.

An important test was a durability test of the sensor in strong centrifugal force environment to determine the possibility of the sensor integration with centrifuge and observing sedimentation during the process of centrifugation. In **Figure 6**, the microscope image of the sensor endpoints, where the bonding wires are attached to the silicon dye, connecting the silicon with the outside world is shown. Thin wires are prone to breakage, therefore, we tested how the react under force. We exposed the sensor to gradually increasing centrifugal force of 10g, 50g, 100g, 400g and 600g and noted no deformation on the wires or any other part of the sensor. After the stress test, the sensor continued normal operation and showed no degradation of the performance.

4. Discussion

We have developed and tested a conceptual design for the construction of an optical detector for observing the spontaneous blood sedimentation with possibility to integrate it into centrifuge to observe the sedimentation while the sample is exposed to high centrifugal forces. The key to the final application is the detection of the time dependence of the movement of the erythrocyte boundary. If the centrifugation process is too long, the platelets are depleted from plasma and the efficiency of the platelet-rich plasma protocol is lost. The centrifugation time is strongly dependent on the physical and chemical parameters of the blood and can be determined in advance based on the information on the movement of the boundary.

Several open problems remain in the development process of centrifuge integration. A wireless communication with the centrifuge is supposed to be used, since the sensor is mounted on a rotor that rotates during operation, relative to the other electronics of the centrifuge. At the same time, power supply power to the sensor during operation will need

to be provided, which in the first version will be done using a rechargeable battery. In a practical implementation, it would be reasonable to take advantage of the fast motion of the sensor on the rotor and try to power the sensor by means of electromagnetic induction. However, the sensor will be valuable to estimate the sedimentation properties of erythrocytes also outside the centrifuge, to be inserted into mathematical model for estimation of the optimal centrifuge setting.

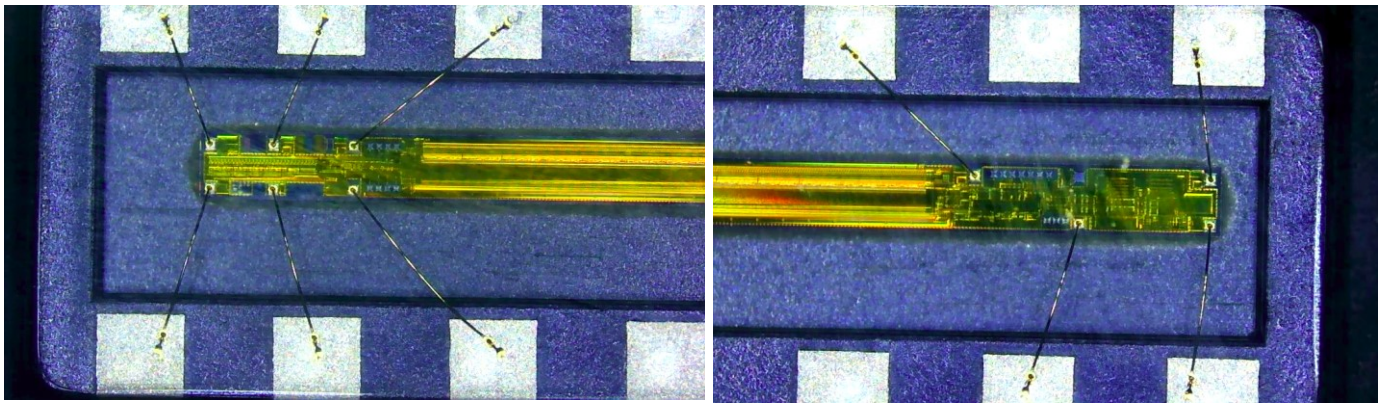

Figure 6. Microscope image of the sensor parts, where it interconnects with the outside world. After stress test, no deformation of the sensor was noted.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Božič D, Vozel D, Hočevar M, et al. Enrichment of plasma in platelets and extracellular vesicles by the counterflow to erythrocyte settling. Platelets. 2022; 33: 592–602. DOI: 10.1080/09537104.2021.1961716.
2. Chahla J, Cinque ME, Piuzzi NS, et al. A call for standardization in platelet-rich plasma preparation protocols and composition reporting: a systematic review of the clinical orthopaedic literature. J Bone Joint Surg Am. 2017; 99: 1769–1779. DOI: 10.2106/JBJS.16.01374
3. Daif ET. Effect of autologous platelet-rich plasma on bone regeneration in mandibular fractures. Dental traumatology. 2013; 29: 399–403. DOI: 10.1111/edt.12021
4. Jan Z, Drab M, Drobne D, et al. Decrease in Cellular Nanovesicles Concentration in Blood of Athletes More Than 15 Hours After Marathon. IJN. 2021; Volume 16: 443–456. DOI: 10.2147/IJN.S282200.
5. Vozel D, Božič D, Jeran M, et al. Autologous Platelet-and Extracellular Vesicle-Rich Plasma Is an Effective Treatment Modality for Chronic Postoperative Temporal Bone Cavity Inflammation: Randomized Controlled Clinical Trial. Frontiers in bioengineering and biotechnology. 2021a; 573. DOI: 10.3389/fbioe.2021.677541
6. Vozel D, Božič D, Jeran M, et al. Treatment with platelet- and extracellular vesicle-rich plasma in otorhinolaryngology-a review and future perspectives. Advances in Biomembranes and Lipid Self-Assembly, vol. 33. Elsevier. 2021b; pp. 119–153. DOI:10.1016/bs.abl.2020.05.003