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A Low-Voltage Droplet Charging Circuit With Simulative Cell-Sorting Function for Flow Cytometer-Cell Sorter

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Background: Flow cytometer cell sorters have become important tools in many biological laboratories. Commercial electrically-deflected cell sorters that deflect wanted cells in electrically charged droplets need high-voltage amplifiers which are expensive and difficult to obtain. Effort was made to build an alternative droplet charging circuit with low-voltage amplifiers that are much easier to get and have more reasonable price.
Methods: A low-voltage charging circuit was designed. Every time a cell was to be separated, a pair of complementary charging pulses were produced: one was positive and the other was negative with equal amplitude. These were enlarged by two low-voltage charging amplifiers to drive two charging electrodes respectively.
Results: Due to the effect of addition, the voltage between the two electrodes was double as high as the output of either amplifier. The result of test experiment proved that the cell sorter with low-voltage amplifiers, which was cheaper and easier to obtain, could separate cells as efficiently as the instrument with high-voltage ones that were more expensive and more difficult to make. In addition, a simulative cell-sorting function was provided.
Conclusions: This low-voltage, easily-built and low-price charging circuit for flow cytometer cell sorter is a good alternative to the commonly used high-voltage one, especially to researcher who hopes to build his own personal instrument. Cytometry 39:306–309, 2000.

Key terms: low-voltage charging circuit; flow cytometer; cell sorter

As an important feature of flow cytometers (FCM), the cell-sorting technique has been greatly developed in the past decade (1,2,3), and has been applied in many fields (e.g. 4,5,6). Currently, most FCM cell sorters of research type use the electrical drop deflecting mode, which theory can be found elsewhere (1,7). In these cell sorters, charging voltage is very high. It was 150V on LLNL (1), and 100V on widely applied FACStar and the more recent FACSVantage SE. This is always achieved with a high-voltage amplifier, which is usually more expensive and more difficult to obtain than a common low-voltage one.

We designed a low-voltage charging system on the home-made flow cytometer cell sorter (8). In this system, two low-voltage amplifiers are applied to drive the two charging electrodes. When a cell is to be separated, one amplifier produces a positive pulse of +30V, at the same time the other one gives out a negative pulse of −30V. When no cell is sorted, both outputs of the amplifiers are neutral, 0V. Thus, the voltage between the two electrodes is ±60V or 0V, just as a high-voltage amplifier of 60V will do.

MATERIALS AND METHODS

Our cell sorter has the typical configuration of electronically deflecting mode which is now widely adopted (1). Figure 1 shows the overall diagram of the signal-processing circuit. A cell's scattering and fluorescence signals are routed into window discriminators that will determine if it is a desired one. The sorting logic circuit checks if the cells in adjacent drops are of the same kind, or if the space between different kinds of cells is large enough. The delay time and the number of drops to be charged are also set in this part. If a cell's signals satisfy a window's criteria and the sorting logic requirement, one channel will be in logic state "1", and the other will be "0". If no cell is detected, or, no cell fulfills either the window criteria or sorting

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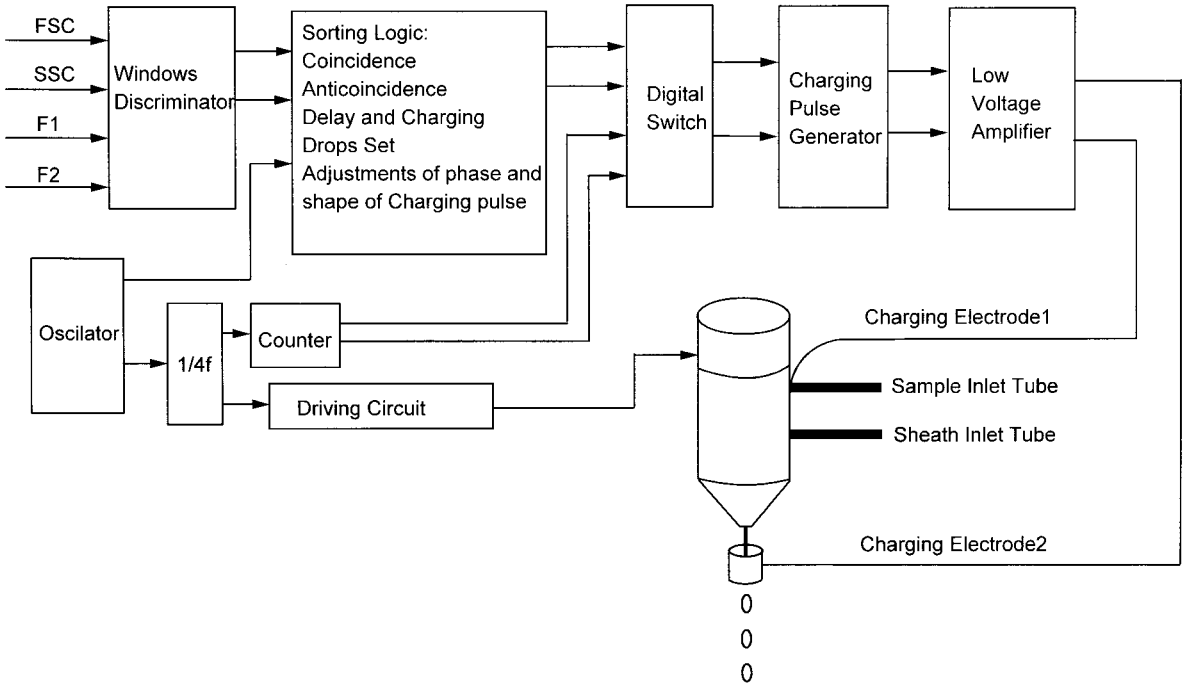

FIG. 1. Schematic diagram of signal-processing circuit.

logic requirement, both channels will be "0". At any time, the sorting logic circuit is in one of the three states: 00,01,10. Since no cell can activate both windows simultaneously, and the sorting logic circuit inhibits drops that contain different kinds of cells and are too close to each other, to be charged, the state of "11" is impossible to appear. If multiple neighboring drops all contained desired cells of the same kind, the "1" state will be longer and the charging pulse will be so too. Adjustments of the shape and phase of the charging pulse are also included in the sorting logic unit to achieve distinct separation streams. According to the different outputs of the left and right channels, the charging pulse generator produces different charging signals, positive or negative or zero. Then after amplification, the signals drive one electrode in the flowing chamber and the other around the stream in the air.

Figure 2(I) gives the detailed circuit of the charging pulse generator and low-voltage amplifiers. A counter 74LS161 is used for simulating cell sorting. The counter and the sort logic circuit are connected to a programmable digital selector 74LS157. It determines which output is selected to initiate the charging pulse generator that mainly includes a decoder 74LS139 and two analog switches DG211. The four logic states, 00,01,10, and 11, trigger the DG211 to produce three kinds of electrical signals: 0V, +5V, and −5V. They have relationship as indicated in Table 1. DG1 has complementary output to DG2 for the same logic input.

The signals are connected to voltage followers LF347 that serve as impedance buffers, then are amplified by A1 and A2 (LM343). Another two amplifiers (LM343) are used as voltage followers to strengthen the driving ability. Although either A1 or A2 can produce a voltage of 30V at most, the potential difference between their outputs, and so between the two charging electrodes, can be high up to 60V. This is illustrated in Figure 2(II).

Table 1
The Relationship Between Logic States and Produced Charging Signals

Output of channel 1, 2	Output of DG1	Output of DG2
00, 11	0V	0V
01	+5V	−5V
10	−5V	+5V

Since counter 74LS161 and the drop-forming circuit share a driving signal which has one fourth oscillator frequency, they synchronize and the counter encodes every drop cyclically. If its outputs are selected to trigger charging signals, all drops encoded as 01x (x=0 or 1) will be positively charged, and all encoded as 10x will be negatively charged, and the rest-00x and 11x-will remain uncharged. After flying through the electrical field, the stream is separated into three strands, which can be clearly viewed by eyes. This is useful to demonstrate the cell-sorting function and check the flowing status of stream.

BIOLOGICAL APPLICATION AND DISCUSSION

To evaluate the cell sorter with low-voltage amplifiers, a mixture of fluorescent microspheres (2μm, Poly-

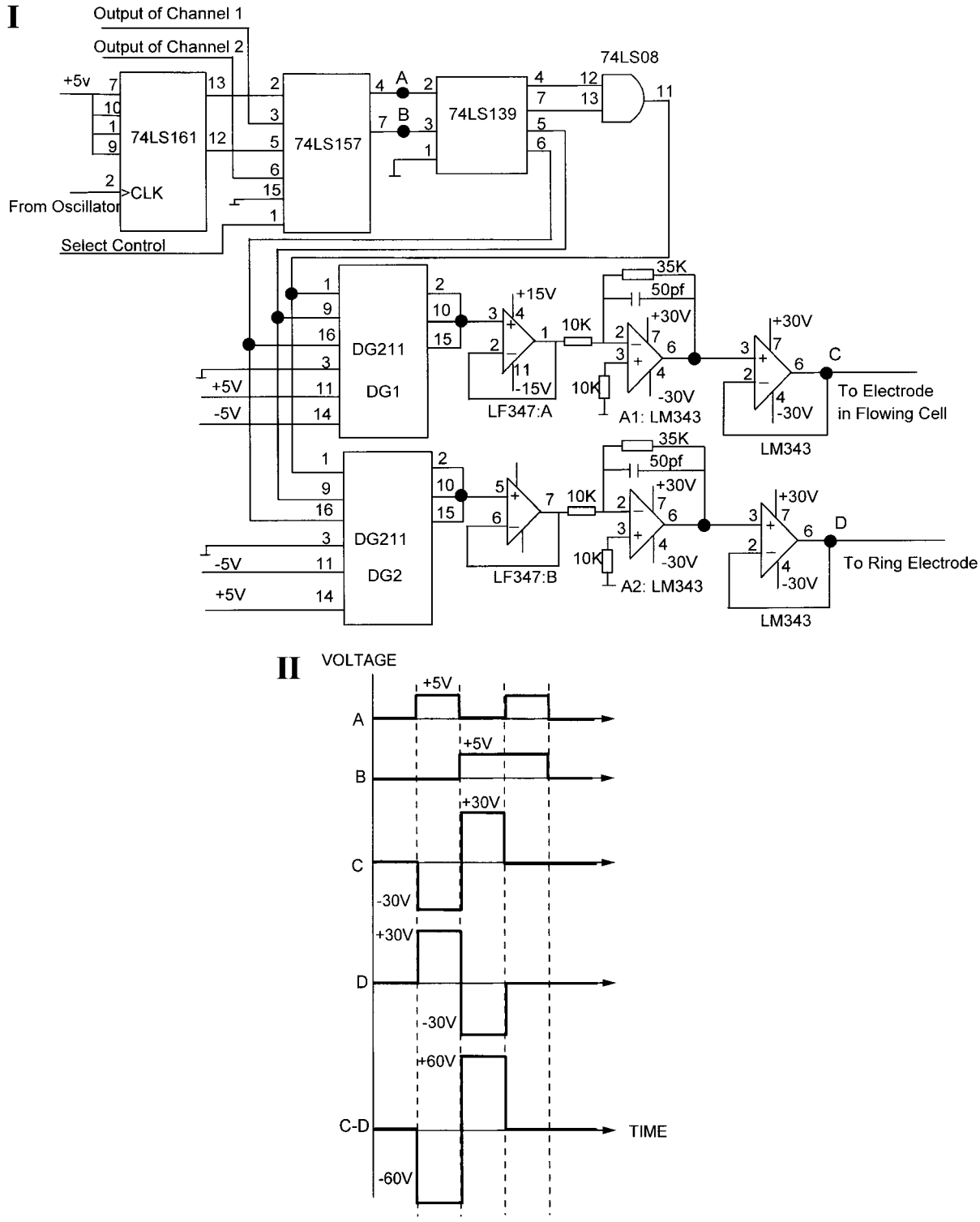

FIG. 2. Electrical diagram of droplet charging circuit with low-voltage amplifiers. **I**: Counter (74LS161) synchronizes with droplet formation, and it encodes every drop. The outputs of the counter or sorting logic circuit are first connected to a selector (74LS157) then to a decoder (74LS139). The two analog switches (DG211) work as charging signal generators. Different logical states trigger different signals on the switches. The small charging signals are connected to two linear amplifiers (LF347) which are used as impedance buffers, then amplified by another two linear amplifiers (LM343) which output amplitude can be as high as +30V or −30V. The last two amplifiers serve also as impedance buffers. **II**: Time diagram of the circuit in four different logical states. If the sorting logic outputs are 00 or 11, it means that there are no cells to be separated in both channels, or that a logical error occurs. In both cases, the signals at the ends of amplifiers are 0V. 01 means that there is a cell to be separated in channel 1, and the voltage at point C will be +30V while the voltage at point D is −30V. The potential difference between C and D will be −60V. In the case of logical state 10, the cell in channel 2 should be charged. The voltages at point C and D will be +30V and −30V respectively, and their difference will be +60V.

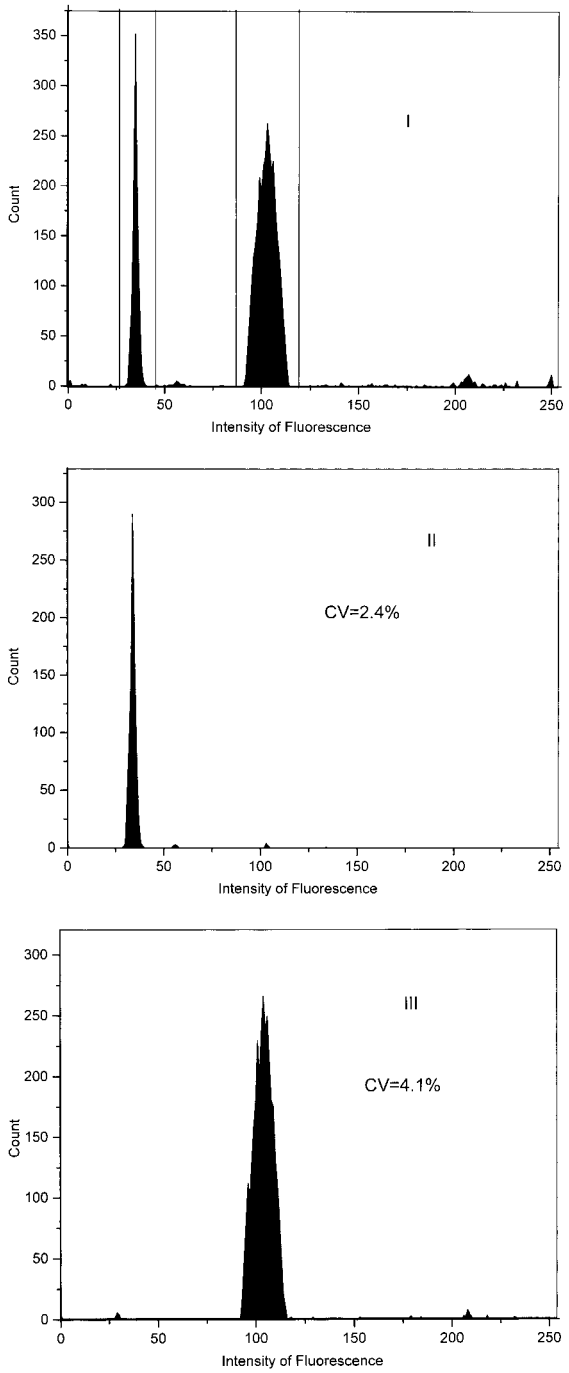

FIG. 3. Fluorescence distribution histograms. **I**: Mixture of plastic microspheres and chicken red blood cells. **II**: Separated fluorescent microspheres with purity of 99.2%. **III**: Separated chicken red blood cells with purity of 98.6%.

sciences, Inc., Warrington, PA) and chicken red blood cells fixed with ethanol (70%) (about 8μm) was measured and separated. The chicken blood cells and plastic microspheres are all excited at 488nm with an argon ion laser. The fluorescence light is collected at 585nm. Light can also be measured at 530nm. Chicken red blood cells emit fluorescence light when illuminated with laser. Part of the mixture was first measured with FCM, then two sorting windows were set via computer. Plastic microspheres and chicken red blood cells were separated when the sorting function was on. The drop formation rate was 30,000/sec, and the cell processing speed was about 1,500/sec. Every time a cell had to be separated, three neighboring drops were charged. The minimum space between two different kinds of cells was also three drops. The result is given in Figure 3. The purity of the separated microspheres and chicken red blood cells was 99.2% and 98.6% respectively. The rule that three drops are charged and at least three drops are spaced may be arbitrary. If the space is larger, for example, five drops or more, the purity can be higher. And if the additional doublet-discriminating function is added, the adhered cells can be prevented from separating more easily, and the purity will be higher too. This paper mainly concerns the characteristic of low-voltage circuit.

The result shows that the cell sorter using low-voltage amplifiers can separate cells as efficiently as those applying high-voltage amplifiers. If two or more pairs of low-voltage amplifiers are applied, more than two kinds of cells can be sorted in one experiment. Though commercial flow cytometer cell sorters can be easily purchased, it is also usual and important for researchers to develop their experimental instruments with novel structure and function. Low-voltage amplifiers are cheaper and easier to purchase, so it is more convenient to build a personal cell sorter.

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