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Citation: Review of Scientific Instruments **56**, 462 (1985); doi: 10.1063/1.1138323
View online: http://dx.doi.org/10.1063/1.1138323
View Table of Contents: http://scitation.aip.org/content/aip/journal/rsi/56/3?ver=pdfcov
Published by the AIP Publishing

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(Received 9 June 1984; accepted for publication 5 December 1984)

The automation of a microelectrophoresis apparatus is described. It relies on digital techniques for the electric field generation, the conductance measurement, the cuvette positioning, and the temperature rise estimation deduced from the conductance. A current source is used with programmable shape, frequency, duty cycle, and duration. All the measurement steps are successively controlled by the microprocessor. As described herein, the particles motion is recorded with a camera. The apparatus is built from independent (software and hardware) modules and other detecting devices could easily be adapted.

INTRODUCTION

Electrophoresis, the motion of charged particles (or molecules) in an electric field, is the basis of a variety of analytical and preparative techniques which are widely used in biology, medicine, and colloid industry. The electrophoretic mobility of a particle is a physical parameter which reflects its surface charge and thus its surface composition; it is particularly useful in the study of living cells, and especially the blood cells, the membrane of which is the site of many processes of relevance in enzymology, immunology, pathology, or virology. For example,[1] this technique has been used to characterize electrophoretic distributions of blood cells and to study the reactions of these cells with mitogens and antigens. Also, the differences in the mobility distributions of white blood cells from normal individuals and from patients with acute lymphocytic leukemia have been observed, correlations between charge and cell surface marker characteristics, and relationship between charge and cell aggregation have been reported. Most of the work in cell electrophoresis was done on human lymphocyte analysis, because different subpopulations can be distinguished (T and B lymphocytes types), the mobility profiles being relevant to the status of the immune system, and the presence or absence of disease. All these applications and others on proteins, viruses, nucleic acids, bioparticles, bacteria, and vesicles are reported in recent reviews.[2,3] Another field of interest for electrophoretic measurements is hemorheology: electrophoretic mobility must be taken into account when studying the flow properties of blood, the deformability of cells, and the interaction with other cells or with vascular walls (rouleaux formations, aggregation, and adhesion phenomena); this parameter is thus particularly important in microcirculation.[4]

For a long period the electric charge of particles in suspension has been determined by the microscopic measurement of their displacement under a given constant electric field (see Ref. 5 for a general review). Since the first paper by Flygare and Ware[6] different laboratories have developed the laser-Doppler anemometric method in order to measure the particles electric mobility.[7] Recently, commercial apparatus based on this principle have become available. Their main advantage is the apparent ease with which a velocity spectrum is obtained in a relatively short time (1–3 min). However, some complicating factors such as the electro-osmosis and the temperature rise during the measurement may not yet be completely solved, so that the microscopic method is still useful for precise electric mobility determinations.

We have previously described[8,9] a microelectrophoresis apparatus with a lateral orientation rectangular cell, vertically moved at a constant speed, while a sinewave field burst is applied. The resulting particles motion is photographed through a dark-field illumination microscope. This technique has some advantages over the laser-Doppler anemometry. First, quasipermanent visual control of the suspension and a permanent record of the measurements are available. Second, it is possible to select a given fraction of the suspension particles on the slides and to measure their mobility. Third, using conventional optics, the interrogated volume in the suspension has a more suitable geometry than with the laser-Doppler techniques. This factor is particularly important when the electro-osmotic flow is not negligible, because it will alter significantly the resolution and the shape of the spectrum.

The temperature effects which are so important in electrophoresis are also smaller in the proposed method as the use of sinewave bursts reduce the heating by a factor 1.3 for a given amplitude of the particles displacement as compared to a constant field method.[10]

In our first apparatus a serious drawback was its complexity, as many manipulations and side measurements (such as frequency, intensity, and conductance) were necessary. Therefore, we have developed an automated apparatus which we wish to present. It is presently used with photographic detection, however, it could easily be adapted for other detecting devices.

I. GENERAL DESCRIPTION

The apparatus consists of (Fig. 1) a dark-field microscopic examination system with a motorized camera, an electrophoretic cuvette connected to a programmable current source or to a conductimeter, and a control unit.

After having controlled the position of the cuvette walls

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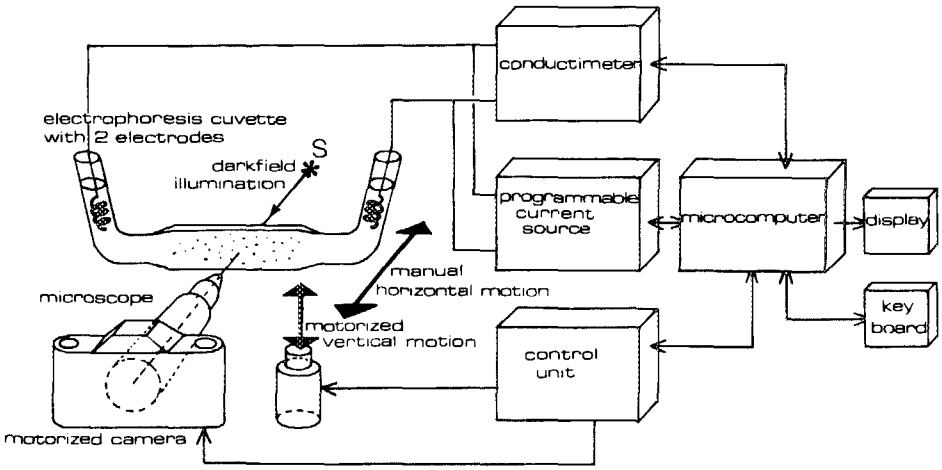

FIG. 1. Outlay of the automated microelectrophoresis apparatus.

through the microscope, the operator feeds the machine with different informations and initiates several measuring cycles, as will be described below. The resulting slides are analyzed through a projection laboratory-made digitizer and a microcomputer (AIM 65) which calculates and prints the electric mobility (corrected to 25 °C) histogram of the suspension.

II. PROGRAM CURRENT SOURCE

The waveforms to be used are stored as numerical values in an EPROM. Actually, only a sinusoidal function has been memorized. These values are read through a MC 6809 microprocessor and transferred through a PIA to a DAC which delivers 10-V amplitude sinewaves (Fig. 2). The output is applied to a second DAC which is used as a programmable attenuator. This configuration allows the generation of an output voltage which is software controlled, regarding shape, frequency, duty cycle, and amplitude. This signal feeds a high-voltage amplifier, similar to the one previously described,[11] except for the class A output stage, which uses 5×2 BU 108 transistors. This amplifier is converted into a current source by a feedback loop; an isolation amplifier is used to eliminate the 400-V common mode voltage (Fig. 3).

The following specifications have been obtained: Maximum output voltage (floating): 800 V p.p.; maximum current: 50 mA; maximum power: 6.5 W; frequency range: 0–15 kHz (voltage amplifier) and 0–50 Hz (current source).

III. CONDUCTIMETER

A simple circuit is used (Fig. 4); different resistors in the feedback loop are automatically switched by the CPU so as to have the maximum usable sensitivity. After adjustment of the general amplification gain, in order to have a simple voltage/conductance ratio, the output is A/D converted and memorized.

The conductance ranges are 2–20 μs, 20–200 μs, and 0.2–2 ms; the measurement precision is better than 0.5%.

IV. CONTROL UNIT

The following operations are performed by this unit (Fig. 5): (1) Generation of a burst of rectangular pulses to drive the stepper motor which is used to move the cuvette vertically (in order to have the same vertical displacement on the photographs, the repetition rate of the driving pulses is automatically changed, linearly with the electric field frequency). The duration of the vertical motion is also controlled by the CPU, according to the required exposure time. To avoid measuring the same particles on two successive photos, another vertical displacement is automatically done after each photograph, with a programmable duration. (2) Generation of a pulse to actuate the camera shutter. (3) Generation of a pulse to actuate the camera electric drive.

V. MEASURING PROCEDURE

Figure 6 illustrates the measuring procedure in the following way:

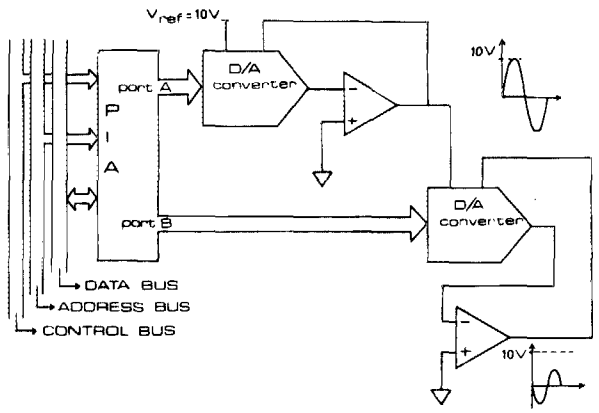

FIG. 2. Simplified diagram of the programmable function generator.

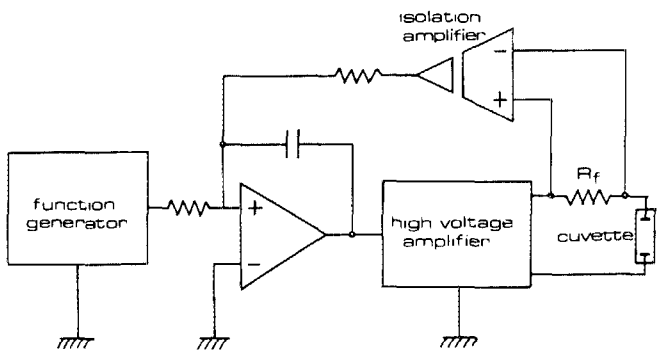

FIG. 3. Simplified diagram of the current source.

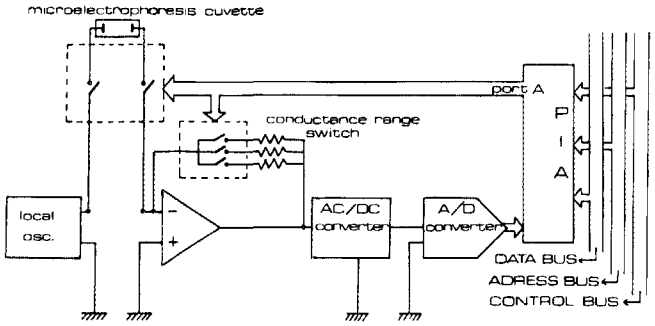

FIG. 4. Simplified diagram of the conductimeter.

(1, 2) The cuvette is filled with the suspension, positioned on the movable bracket and fitted with the electrodes (for details see Ref. 9). The accurate vertical positioning is verified by vertically moving the cuvette and observing a given detail through the microscope. The horizontal motion (along the optical axis) is measured through a transducer feeding a DVM. The position of the two external windows is accurately measured ($\pm 2\ \mu$). The DVM readings for the middle and stationary planes thus can easily be determined knowing the cuvette width. The cuvette is then moved horizontally so that the microscope is focused on one of the stationary planes.

(3) The cuvette conductance G is automatically measured.

(3–7) The operator feeds in the following external parameters: Cuvette cross section S (cm^2); conductivity geometrical factor of the cuvette K (cm^{-1}); value of the estimated mobility M (μ/s/V/cm); chosen value F in the available frequency set (Hz); value of the final displacement amplitude $2A$ (mm p. to p.) on the digitizer screen (a global optical magnification factor γ being previously introduced in the program).

(8) The field strength amplitude (E_0) which is necessary to obtain the wanted displacement amplitude A is automatically calculated and displayed (in V/cm). The resulting current amplitude I_0 is automatically calculated through the relation

$$I_0 = \frac{2\pi FKGSA}{M\gamma}$$

and displayed (in mA). The maximum possible resulting temperature rise ΔT for a duration of two electric field periods is then automatically calculated through the relation

$$\Delta T = \frac{I_0^2}{4.18KGS^2F}\ (°C)$$

and displayed. This value is given for a sine wave, assuming no heat loss (8, 10).

(9) Afterwards, the apparatus ensures that the calculated I_0 value is compatible with the technical specifications (if not, an error message is displayed); then if the operator thinks that the calculated temperature rise is without significant effects, he activates the main measurement program. Otherwise, he can start the measurement procedure again (step 5) either with a lower conductivity suspension or with smaller displacement amplitudes, or with a higher frequency.

(12) When step 11 is completed, the cuvette is vertically moved at the maximum speed to an upper reference position. For security reasons, a manual activation is necessary before each electric field application. After this validation, the motor lowers the cuvette at the controlled speed and the camera shutter is opened. One-half period later, the electric field is applied (for only two periods in our application); the shutter

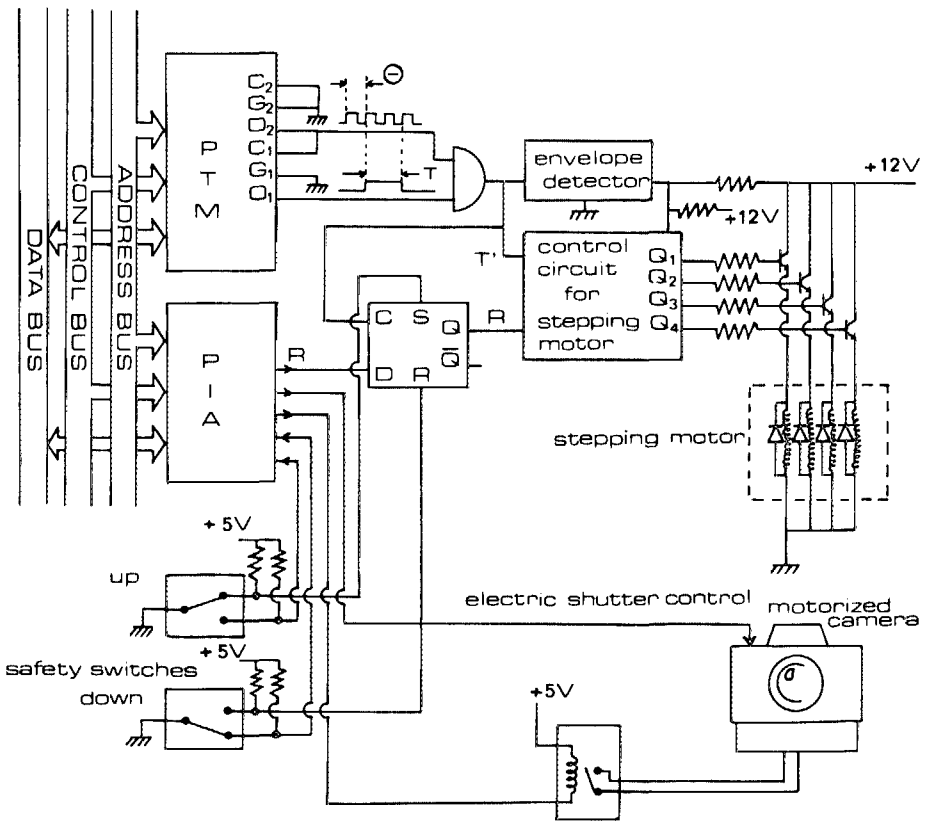

FIG. 5. Simplified diagram of the control unit.

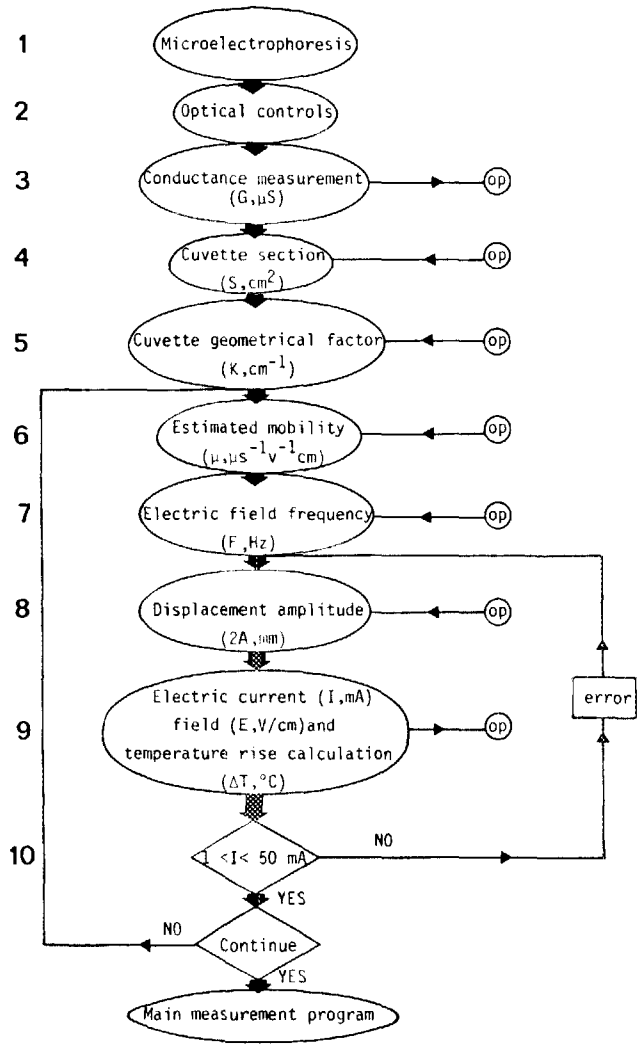

FIG. 6. Flow chart of the interactions between the AMA and the operator (op).

is closed half a period after the end of the wave burst; and the motor is stopped after a total displacement of 335 μ (200 μ during the exposure and 135 μ after the shutter is closed. Three other similar operations can be performed (each time after a normal validation). A drop of liquid is added in one of the cuvette arms every four photographs; this results in a slight horizontal translation of the suspension so that the same cells are not visualized on the successive photographs. After the fourth cycle the cuvette is automatically moved at the upper reference position and the measurement procedure can be started again.

VI. DISCUSSION

In order to obtain only average mobility measurements, eight photographs are taken at the stationary planes. But if a mobility histogram is needed, 16 more photographs are taken in the middle plane.

The displacements are measured on the resulting slides and converted into mobilities as previously described.[9] As an example, Fig. 7 shows a typical mobility histogram of normal human lymphocytes, where the heterogeneity of the population is clearly demonstrated.

The automated electrophoresis apparatus as herein described is routinely used for the analysis of the membrane

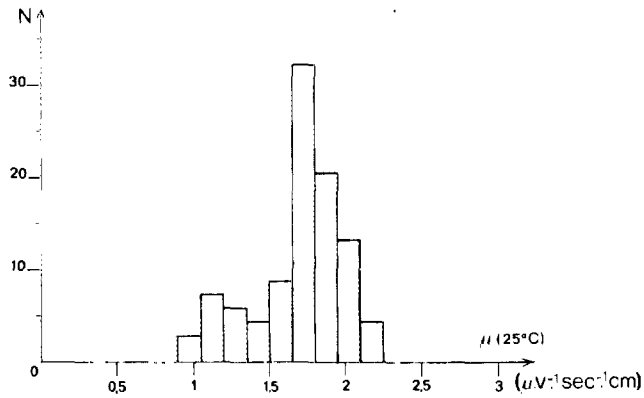

FIG. 7. Mobility histogram of a human lymphocyte suspension (in a 10^{-2}M, *p*H 7.4, phosphate buffer).

electric charge density of human blood cells. Most of the results were obtained with red cells, so as to determine the parameters which affect the value of the mobility, for example, the plasma composition.[12]

The automation results in more reliable and easy experimentation. We wish to point out the advantage of having an automatic conductance measurement. This allows for the control of any temperature drift and increases the reliability. A common practice for temperature control is to use thermostating equipment, however, this is useless. Indeed, the thermal equilibration time can be of the same order of magnitude (or longer) than the electric field duration (because of the low thermal conductivity of the electrophoresis cuvette), so that the temperature control is ineffective when the measurement is performed.

The main part of this apparatus could be used without any modification for other types of detection (such as a video camera or photodiode arrays). This work has been done in partial fulfillment of a thesis.[13]

ACKNOWLEDGMENTS

We wish to acknowledge the expert technical assistance of P. Bec, B. Decrossas, R. Guillet, and J. M. Lepecq, and the efficient secretarial work of N. Decaulne.

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