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A COMPUTER CONTROLLED DATA MANAGING SYSTEM FOR MULTIPARAMETER FLOW CYTOMETRIC ANALYSES

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A data transfer system has been developed for writing non-classified cell data containing 3- or 4 parameter measurements directly and rapidly on magnetic tape. Thus all data are stored. This makes possible subsequent multiple analyses of a single probe at any time. Furthermore the system provides for the reading of hardware-stored histograms for three different multichannel analysers. Special FORTRAN IV software enables the evaluation of measurement data in various ways and the plotting of histograms.

Key words: Multiparameter flow cytometry; data managing system; cell sizing.

Data analyses of 3 or 4-parameter cell measurements require a special kind of data evaluation because there are no commercial pulse height analysers available for more than two parameters and in addition it is not possible to display the histogram on a monitor. A common technique is to use the principle of «gating» (3), which means that three or more parameters are measured, but only two parameters are stored and displayed, depending on the remaining parameters. The drawback to this technique is that it requires the choice of gating parameters to be made without knowledge of the resulting measurement. A fast data transfer system has therefore been developed which allows the on-line storage of four non-classified data for each cell on magnetic tape. The system is managed by a minicomputer. Thus all data, without any reduction, are available for later multiple analyses concerned with many different aspects of a single probe. Another advantage is the possibility of rapid, time-dependent measurements. Only three parameters are in use in our preliminary system (volume, fluorescence 1, fluorescence 2).

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Special FORTRAN IV software enables the data evaluation of the measurements by computer.

The system is, in addition, capable of reading hardware-stored histograms out of three different pulse height analysers.

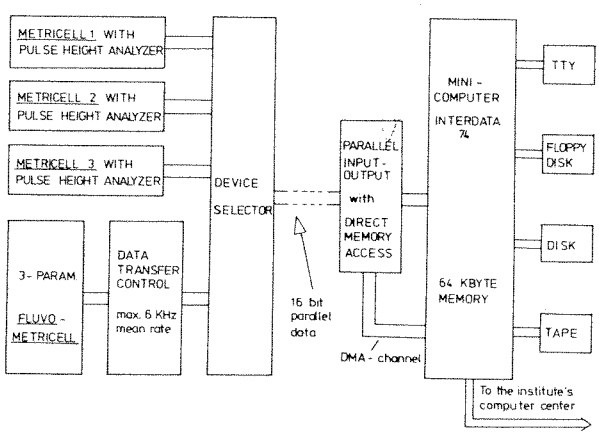


Fig. 1. Schematic view of the complete data transfer system.

INSTRUMENTATION AND BASIC FUNCTION

The complete system is managed by an INTERDATA 74 minicomputer with 64 kbyte memory. (Fig. 1.) The peripheral devices are teletype, disk, floppy-disk, and magnetic tape. An additional DMA controller enables

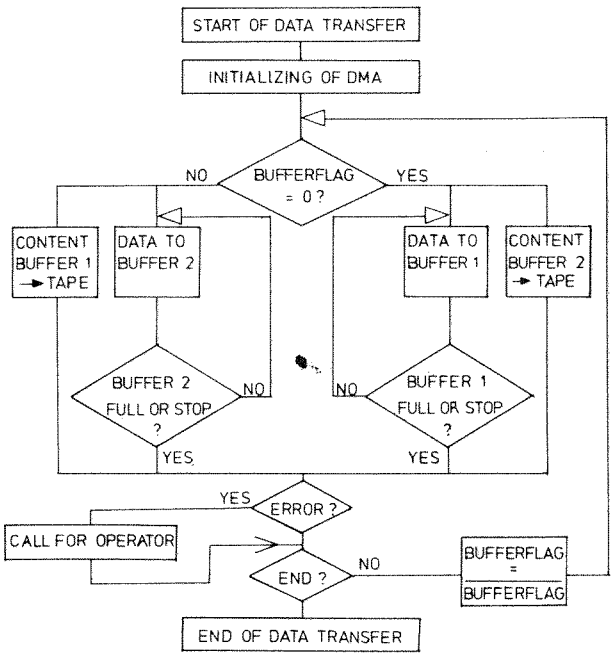


Fig. 2. Flow chart of data processing with the «change of buffers» for fast on-line data transfer. While data are written in buffer 1 (2), the contents of buffer 2 (1) are written on magnetic tape.

data transfer by direct memory access. Data exchange between the minicomputer and the Institute's computer centre is also possible. The device selector connects one of three pulse height analysers or the data transfer controller with the computer. In this way the data flow from three METRICELL (1) volume analysers and the 3-parameter FLUVO-METRICELL (2) may be managed. The data transfer controller processes data handling over the DMA-channel. Each cell measured by the FLUVO-METRICELL triggers the transfer of two 16-bit data items. The first item contains two 7-bit data of volume and fluorescence 1, the second item 7-bit data of fluorescence 2. The remaining seven bit may be used for a fourth parameter. Two bit of each 16-bit data item enable a later data identification. The minimum time interval between the transfer of data from two different cells can be less than 3 μsec. The fastest mean transfer rate is about 6 kHz. It is determined by the time necessary to write the data on tape.

Because of the fast processing, all 16 bits of one data item are transferred in parallel between the device selector and the input/output interface of the computer.

Three 2-parameter histograms (vol/f1, vol/f2, f1/f2) may be displayed alternatively for immediate control during the measurement.

In the computer, data are processed by the «change of buffers» technique. This means that one buffer is filled with data by DMA-access while the contents of the other buffer are written on magnetic tape. (Fig. 2.) Afterwards, the buffers are changing. One buffer stores 2048 data items, containing the data from 1024 cells. The maximum transfer rate of 6 kHz could be raised by enlarging the buffers. The data analysis classifies the 3-parameter data in three 2-parameter histograms with 64 × 64 channels. The histograms are printed and stored on disk or floppy-disk. Afterwards a 3-parameter block, reduced to 22 × 22 × 22 channels, is evaluated and also stored. The channel-limits of this block may be selected in such a way that only the part of the complete histogram of interest is reduced to 22 × 22 × 22 channels. More complicated evaluations exceeding the capability of the minicomputer, are possible in the Institute's computer centre to which data may be transferred (4,5).

RESULTS

For a first test, 3-parameter pulses have been simulated by a generator as shown in Fig. 3. The result are three 2-parameter isoamplitude plots. (Fig. 4.) These histograms make possible a first critical examination of a measurement and show, whether a more complicated data evaluation is worthwhile.

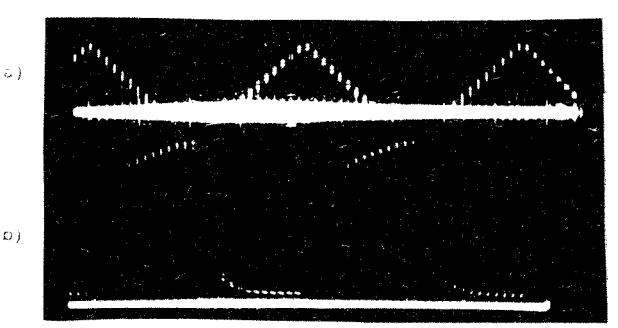
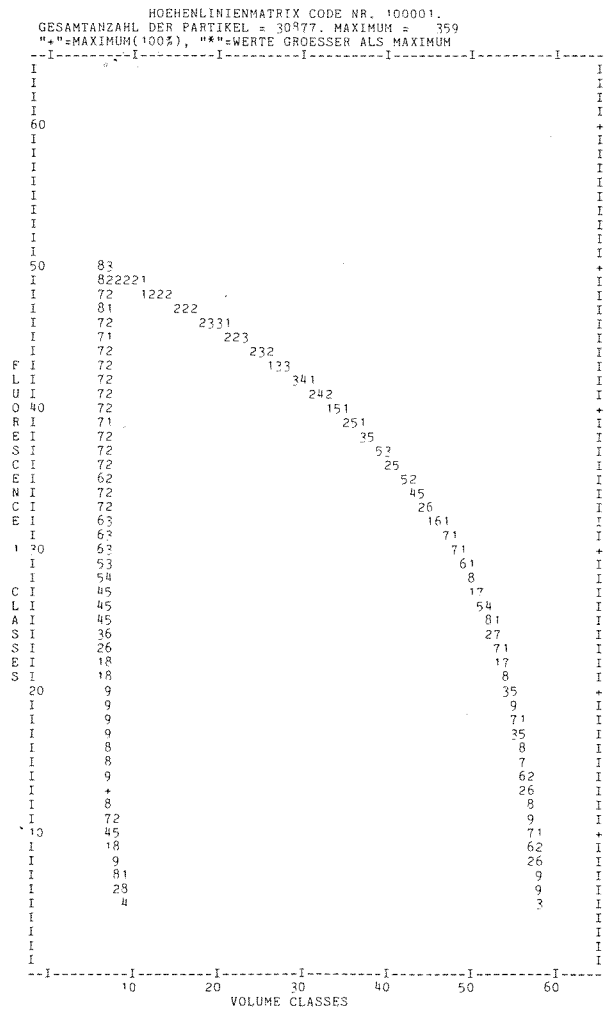
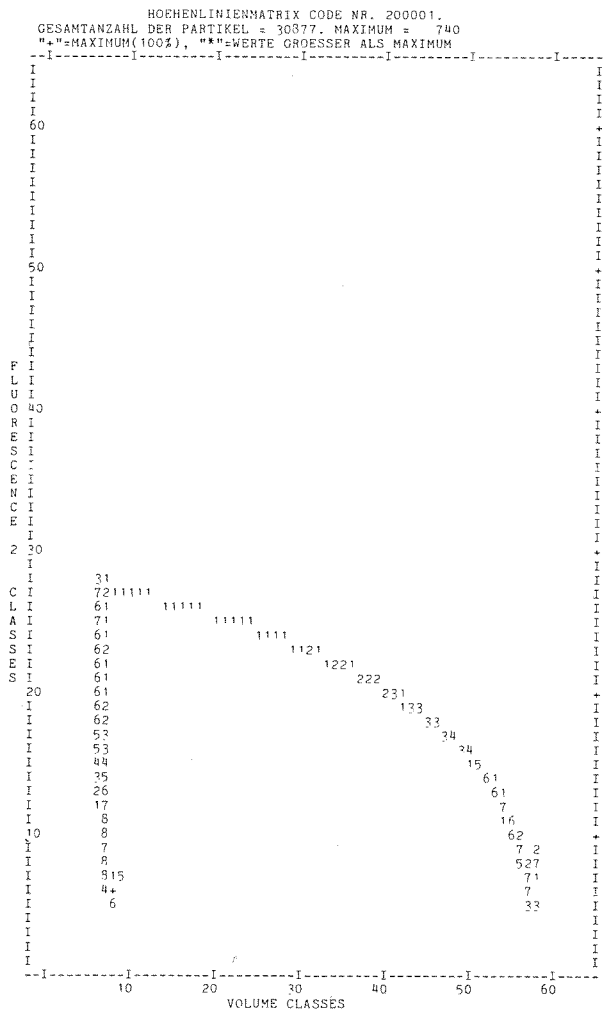


Fig. 3. Pattern of the test pulses simulating a 3-parameter measurement.
a) shows the pulse distribution of the volume channel;
b) shows the pulse distribution of the fluorescence 1 channel;
The pulses of fluorescence 2 are the same as those of fluorescence 1, but differ in their amplitudes; (amplitude f2 = 0.7 amplitude f1)





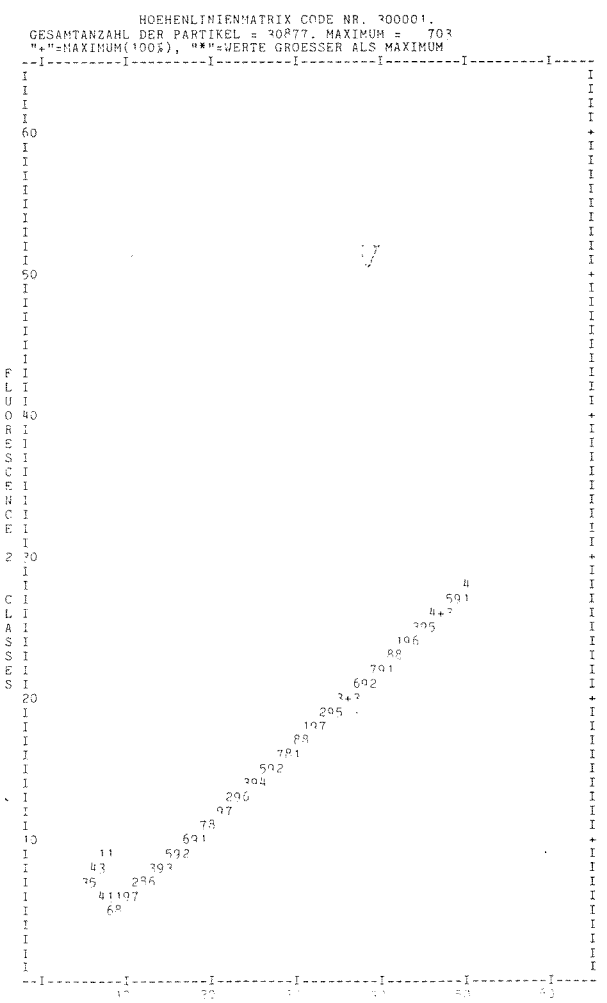


Fig. 4. Isoamplitude plots of the test measurement with pulses as shown in Fig. 3. The plots are normalized to the maximum amplitude (+) as 100%. The numbers from 0 – 9 mark 10% classes. Histogram classes with number 0 (0 – 9.99%) are not printed. The resulting pattern of the fluorescence 1/fluorescence 2 histogram must be a straight line because of the constant amplitude ratio.

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