THE USE OF A ROBOT-ARM FOR AUTOMATED SAMPLE PREPARATION IN FLOW-CYTOMETRY

V.Manta, J.Treumer, G.Valet

Mildred-Scheel-Labor für Krebszellforschung, Max-Planck-Institut für Biochemie, D-8033 Martinsried, FRG

1. INTRODUCTION

The measurement of flow-cytometric samples and the evaluation of the results can be performed automatically within a short time. Fast and automated sample preparation becomes, therefore, of increasing interest.

The preparation of flow-cytometric samples often consists of the addition of a small quantity of dye or of calibration particles followed by an incubation time for intracellular dye accumulation (1). The addition of cytostatic drugs to cell samples for pretherapeutic cytostatic drug assays in cancer patients is a similar task (2). Indirect immunofluorescence assays as used for automated cancer cell detection (3) are more complicated to prepare since several incubations and cell washings by centrifugation are necessary until the sample is ready for measurement in a flow-cytometer. The goal of this study was to build a multi-purpose biochemical work station with a low cost, programmable robot arm and several tools which could accomplish such tasks.

2. DETAILED PRESENTATION

The sample recipients are microtiter plates with a variable number of wells. Typically 96 well plates containing 150µl cell suspension per well are used. This well size is most appropriate for flow-cytometric work since flow-cytometers measure sample volumes between 20 and 50µl per minute. Pipetting is accomplished by 5 pipettes equipped with removable plastic tips. 3 pipettes take sample volumes between 0 and .1ml, 0 and 1ml and 0 and 5ml. 1 pipette simultaneously takes 12 samples to cover one horizontal row and 1 pipette pipets 8 samples to fill one vertical row of a microtiter

CLINICAL CYTOMETRY AND HISTOMETRY
ISBN 0-12-143240-8

plate per pipetting step. The microtiter plates are transported with a plate grip which is a removable robot arm tool similarly as the pipettes. The BIOROB work station also contains several instruments (fig.1) such as a 5000rpm centrifuge whith two bukets to simultaneously centrifuge two microtiter plates and a microtiter plate photometer and fluorimeter. Further instruments at present are a suction device to empty discardable supernatants, a plate shaker, a temperature controlled plate incubator, a pipet tip remover and a reference point to control whether a pipet has correctly taken a pipet tip from the pipet tip rack. The robot arm (Cobra RS3, Sekuria, 6100 Darmstadt, FRG) has 6 motion axes and is controlled by a robot controller containing a Z80 microprocessor and a RS232C interface for communication. The tools and instruments are controlled by a tool controller (Microcomputer K.Schedler, D-8980 Oberstdorf, FRG) which also contains a Z80 microprocessor and an RS232C interface. The tool controller operates the step motors of the pipettes and of the plate grip but controls also the power switches and timers for the centrifuge and the plate shaker. It furthermore calculates the position of the centrifuge bukets from an angular decoder mounted onto the centrifuge axis, it energizes the magnet of the pipet tip remover and it reads the closure of contact from the reference point when this point is touched by a pipet tip. It is planned to include an analog-digital converter into the tool controller to be able to read analog signals from instruments such as pH-meters or conductivity meters. The robot arm controller and the tool controller are both operated via two RS232C interfaces by the BIOROB mainprogram which resides in an 8 or 16bit personal computer. A Z80 based Formula-1 computer with C/PM operating system or an IBM-XT or IBM-AT computer under MS-DOS were so far used for this purpose. The computer RS232C interface for the tool controller can be multiplexed to permit direct data input and data calculations by the computer from devices ssuch as balances or photometers which are serially equipped with a RS232C interface.

The BIOROB work station can be programmed with macro instructions such as: "MOVE FROM TO, PIPET, CENTRIFUGE, SHAKE, INCUBATE, PHOTOMETER". The macro instructions are embedded in the programming language PASCAL (TURBO-PASCAL). Non computer trained persons can write programs with the macro instructions alone, programmers can call the macro instructions within normal PASCAL-programs. All positions of the robot arm, of the tools and of the instruments are given in metric x, y, z-coordinates which facilitates programming. Spatial positions which are difficult to predetermine e.g. due to static bending of the robot arm under load can be precisely approached by teaching along the x, y, and z-axis until the desired position is reached. Coordinates outside the reach of the robot-arm or inside the robot arm, inside tools, inside instruments or beneath the table are not accepted as input. The BIOROB program system knows the maximum excursion of all joints of the robot-arm and furthermore the physical size of all tools, instruments and microtiter plates. The program automatically calculates a path around known obstacles when the robot-arm comes close to such "forbidden zones".

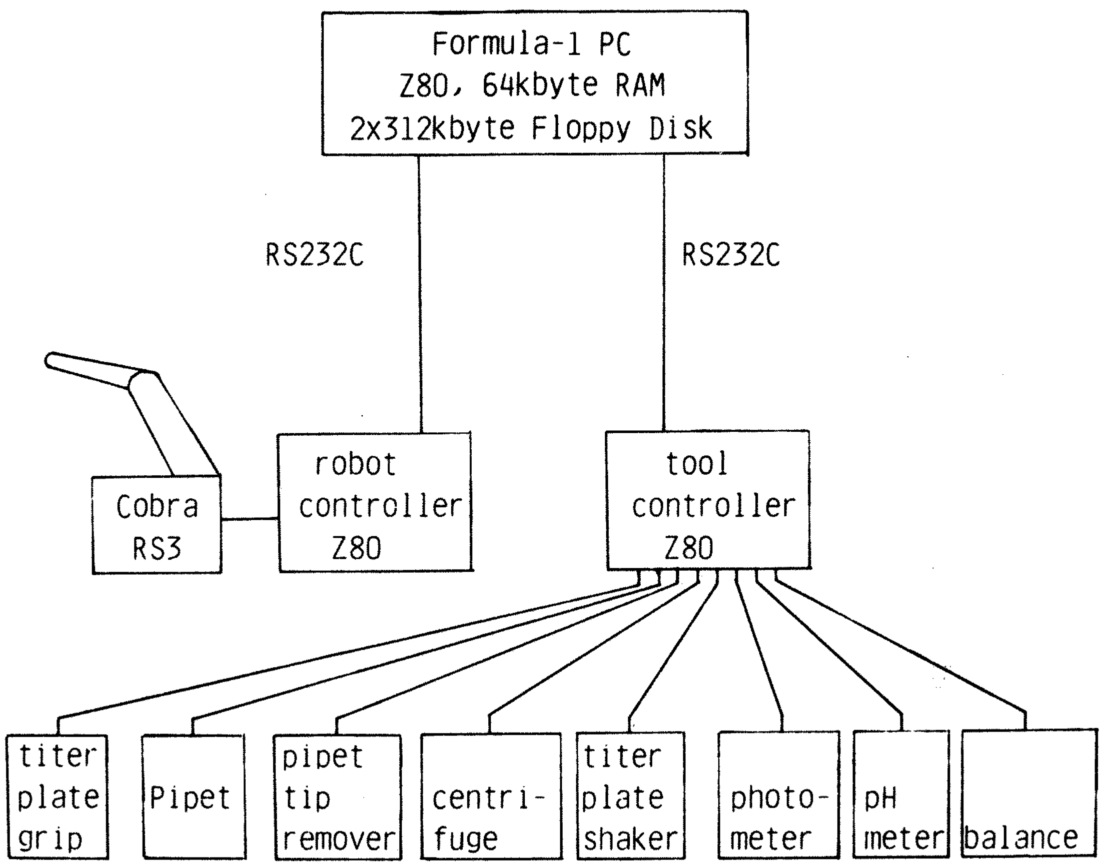

Fig.1 Schematic representation of the BIOROB work station

1. Valet G. (1984) Blut 9, 83-90
2. Valet G., Warnecke H.H., Kahle H. (1984) Blut 9, 37-43
3. Valet G., Rüssmann L., Wirsching R. (1984) J.Clin.Chem. Clin. Biochem. 22,935-942

Clinical Cytometry and Histometry

Edited by

G. Burger

Institut für Strahlenschutz, Neuherberg, FRG

J. S. Ploem

University of Leiden, The Netherlands

and

K. Goerttler

Deutches Krebsforschungszentrum, Heidelberg, FRG

1987

ACADEMIC PRESS

Harcourt Brace Jovanovich, Publishers

London San Diego New York Berkeley
Boston Sydney Tokyo Toronto

INTERNATIONAL SYMPOSIUM
ON
CLINICAL CYTOMETRY AND HISTOMETRY

Abstracts

Schloss Elmau
Federal Republic of Germany
April 20 - 26, 1986

Sponsored by

The Society for Analytical Cytology
The Gesellschaft für Strahlen- und Umweltforschung mbH München
The Concerted Action on Automated and Analytical Cytology of the European Communities