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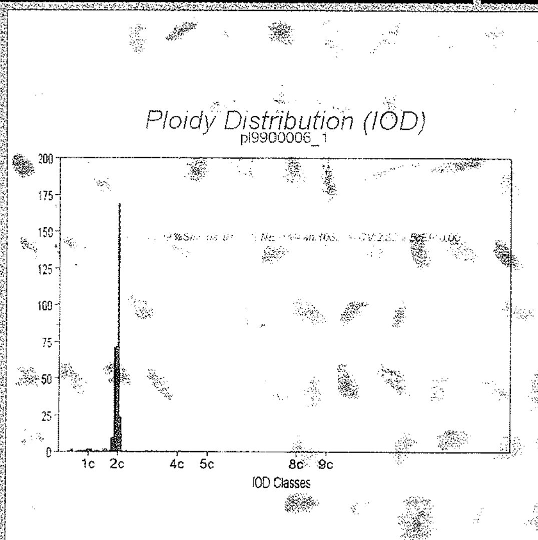
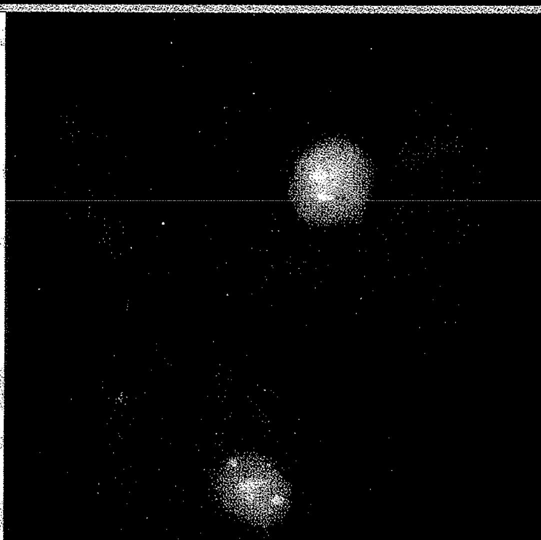
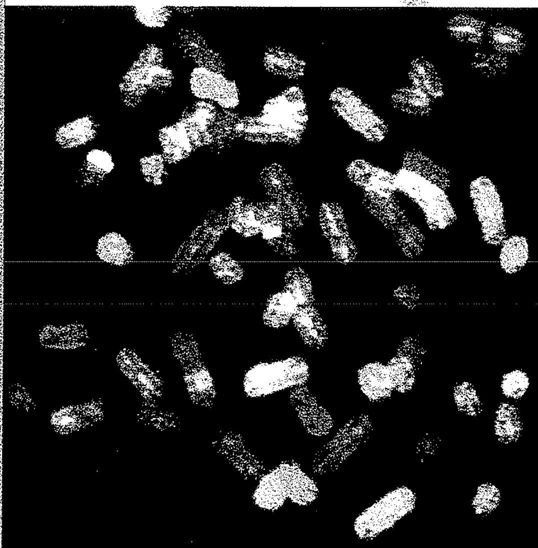
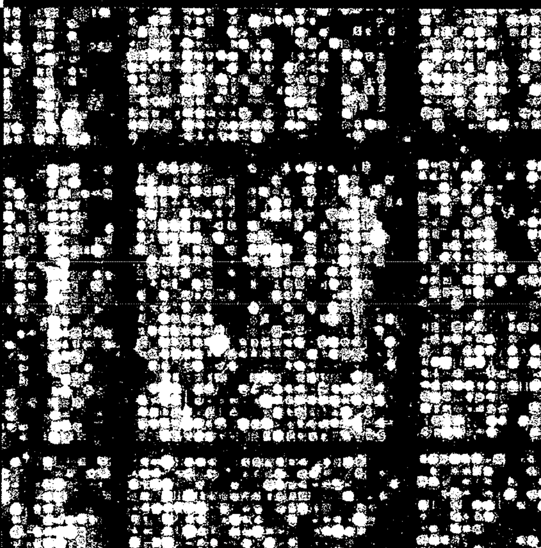
MOLECULAR AND GENETIC PATHOLOGY
DIAGNOSTIC CYTOMETRY AND HISTOMETRY
NEW MEDIA AND TECHNOLOGY









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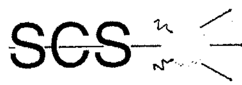
ABSTRACTS



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1

TOOLS FOR MULTIPARAMETRIC ANALYSIS IN THE CLINICAL FLOW CYTOMETRY LABORATORY AT THE EVE OF THE Y2K

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Since the availability of monoclonal antibodies in 1975, the complexity of flow cytometric analysis had increased continuously: From the determination of percentage of positive cells, on a single parameter fluorescent histogram, up to 11 correlated parameters measured recently by the group of Herzenberg, on a prototype machine.
New bench top instruments, with capability of four to six fluorescence measurements, allow us the use of powerful multiparametric methods in clinical applications. With the optimal use of the infinite space of analysis created by the combination of six or eight parameters and the simultaneous use of mixture of multiple probes, we are able to study most of the cell types present in a sample in a single test tube.
The data analysis time, needed to set gates and statistical regions manually, increase exponentially with the number of parameters used. To overcome this drawback automatic softwares has been developed and some of these are reviewed here.
From this review it is obvious that introducing some kind of artificial intelligence could improve data analysis software currently in use. Moreover, development in this way, should be one of the solution for a true automation in the flow cytometry laboratory.

2

AUTOMATIC ALIGNMENT OF HISTOLOGICAL SECTIONS FOR 3D RECONSTRUCTION AND ANALYSIS

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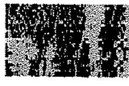
We have developed a new algorithm to automatically align images of histological or anatomical sections. First a displacement field between two successive images is computed using a block matching method. Then we compute the best rigid transformation that approximates this field. By stacking the aligned sections, we obtain a 3D image from which we can reconstruct the 3D surface of the anatomical structure.
The results were obtained from 26 automatically registered images (768x576 pixels of 2.5x2.5 µm with an inter-section gap of 8 µm) of sections of an endometrical adeno-carcinoma. The 3D reconstruction shows its complexity and the alternation of the papillar, tubular pattern, and solid zones. Thus, it allows us to evaluate quantitatively the ratio between the proliferating tissue mass, and the adaptative stroma or the conjonctive tissue.

3D surface reconstruction

slices YZ



slices XZ

slices XY

3

A MULTICENTER EVALUATION OF FLOW CYTOMETRY FOR LOW COUNTING LEUKOCYTES IN LEUKODEPLETED RED BLOOD CONCENTRATES

E. Hardy, N. Marpaux, S. Bégué, N. Boeri, R. Buffalo, E. Ferminet, D. Grossin, G. Lambert, M. Maurer, M.P. Perrault, T. Peyrard, and M. Masse from the French Produits Sanguins Labiles study group., SFTS, ETS de Franche-Comté, 25020 Besançon, France

To evaluate the performance of flow cytometric (FCM) techniques for counting low numbers of leukocytes (WBC) in red blood concentrates (RBC), the French Produits Sanguins Labiles study group, conducted a multicenter study to compare the results of WBC counting by FCM and the current manual technique in a Nageotte chamber (NC).
10 laboratories participated in this study, distributed in 2 areas. Each laboratory sent to the others 5 samples of fresh leucodepleted RBC, and calibration samples in B area. The samples were analysed by manual counting and by the FCM technique validated in the laboratory.
The correlation between the two techniques were:
R^2= 0.75 in A area (WBC/µl= 0.42±1.04) n= 20 samples
R^2= 0.92 in B area (WBC/µl = 1.36± 2.6) n= 25 samples
For concentrations ≥1 WBC/µl, the CV% was acceptable for all the laboratories (≅ 30%). This limit correspond to 0.3×10^6 WBC/unit, wich is well below the french standard of 1×10^6 residual WBC/leukodepleted blood product.
Altough FCM seems to be less precise than NC for counting very low numbers of leucocytes, this technnique is efficient enough for process validation and routine quality control of leukodepletion.

4

QUALITY CONTROL AND QUALITY ASSURANCE PROTOCOLS FOR DNA IMAGE CYTOMETRY

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The ESACP consensus report on the standardization of diagnostic DNA image cytometry is actually in press in the ACP journal and open for forum discussion through the ESACP server. The QC events will be introduced and illustrated by data obtained from partners in the PRESS project and users of EUROQUANT server. A simulation of various situations will be presented. Briefly, the QC procedure includes six tests: 1) QC for ICM-DNA protocol, 2) QC for preparation stability, 3) QC for corrective factor, 4) QC for diagnostic DNA interpretation, 5) QC for IOD measurements and 6) QC for ICM instrumentation. Any new user is invited to run the QC for ICM-DNA protocol. Depending on the positive or negative issue of this first test, he/she will be invited either to run clinical cases or to check his/her protocol. Protocol checking needs two steps: QC for IOD measurements and QC for ICM instrumentation in order to solve the methodological problems in routine : fixation, staining, image analysis procedure. As soon as the QC for ICM-DNA protocol leads to a positive issue, the user is invited to run clinical samples and respective external and internal references appropriate for evaluation of both stability of protocol (QC for preparation stability) and data calibration (QC for corrective factor). Finally QC for diagnostic DNA interpretation is required.