Digital processing of leading front of neutrophil chemotaxis by in real time image analysis. An advanced system for the detection of inhibiting factors in immunoproliferative malignancies.

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Patients with neoplasias of the immune system frequently show a deficiency in humoral and cell-mediated immunity. In particular, an impairment of accessory cell-mediated immunity may be present, because of intracellular defects and humoral inhibiting factors. In recent years we have been involved in the detection and partial characterization of one (or more) factor(s) blocking neutrophil chemotactic functions, named Serum Leukocyte Inhibiting Factor (S-LIF). Using the Boyden chamber method and the leading front technique, S-LIF was found in Hodgkin's Disease (57%), Non-Hodgkin Lymphoma (62%), B-Chronic Lymphatic Leukemia (66%), Malignant Paraproteinemia (92%), with interesting clinical implications and correlation with stage and other prognostic parameters. Unfortunately a chemical characterization of S-LIF is quite difficult, because the first needful step is a serum fractionation and a chemotactic study using each fraction is required. In our experience 36 fractions for each sample (obtained by Sephadex G-200 columns) had to be tested. As every study is carried out in triplicate, evaluating neutrophil random and stimulated motility, by microscopic observation of almost 10 randomly selected fields for each microporous filter, the study of a single serum needed more than 2,300 lectures! So we developed a specific image processing software capable of performing a computerized lecture of neutrophil chemotaxis by an automatic system consisting of: TV-Camera connected with a Leitz Ortholux microscope, Personal Computer HP 286 provided by a video board PIP-1024 for image acquisition, microscope table moved by an electromechanical system driven by the PC. The program acquires the video signal from the TV-Camera at the first focal plane; then - by means of derivation and thresholding computation on acquired data - calculates a number proportional to the number of cells focused in the field and displays it; subsequently the program checks the value of the number and drives the microengine to the next plane until no more cell is found, exploring all the leading front (with depht steps of 10 µm ; finally the whole number sequence is printed. In the present study 30 filters (20 with normal migration, 10 with inhibited migration) were evaluated, exploring 310 microscopic fields. The comparison between the usual microscopic lecture and the digital computation showed a very close linear correlation ($r = 0.99$, $p < 0.001$). Only 6 s are needed for each lecture; in about a minute the program is able not only to display the value of the leading front but also the number of cells in each explored field, supplying a lot of information which allow us to reduce the number of the randomly selected fields. The system may be used also to perform different kinds of digital processing for morphological and densitometric studies, which may be employed in the evaluation of tests exploring other neutrophil functions, such as phagocytosis, NBT reduction, chemotaxis under agarose.