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Digital image analysis of haematopoietic clusters

A. Benzinou[a], Y. Hojeij[a], A.-C. Roudot[b,*]

[a] *ENIB, Laboratoire de Recherche en Electronique, Signal, Optronique et Télécommunications, CP 15, 29608 Brest Cedex, France*
[b] *ESMISAB, Laboratoire de Toxicologie Alimentaire, Technopole Brest-Iroise, 29280 Plouzané, France*

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Summary Counting and differentiating cell clusters is a tedious task when performed with a light microscope. Moreover, biased counts and interpretation are difficult to avoid because of the difficulties to evaluate the limits between different types of clusters. Presented here, is a computer-based application able to solve these problems. The image analysis system is entirely automatic, from the stage screening, to the statistical analysis of the results of each experimental plate. Good correlations are found with measurements made by a specialised technician.

1. Introduction and background

Haematopoiesis is a physiological phenomenon which allows the renewal of blood cells. In an adult, haematopoiesis takes place in the bone marrow and for the foetus in the liver.

The progenitors proliferate rapidly to increase tissue mass and then they differentiate into mature blood cells in response to humoral growth factors and local cytokines in their particular environment [1]. They are then called haematopoietic progenitors. It is possible to distinguish erythroid progenitors which give red blood cells, granulocyte and megakaryocyte progenitors. Unlike stem cells, haematopoietic progenitors can be quantified but not directly. In fact, although it is impossible to identify them morphologically or immunologically speaking, it is possible to make a culture specific to each lineage. Thus, each of the progenitors gives an aggregate coming from its own proliferation. Quantifying these aggregates allows the number of progenitors in the culture at the beginning of the experiment to be known.

These techniques are used in toxicology when they allow the evaluation of the undesirable effects of drugs or contaminants on haematopoiesis and haematopoietic progenitors.

However, an important problem occurs when using haematopoietic progenitors: aggregate quantification is very long and tedious. In order to quantify aggregates coming from one progenitor, it is necessary to use a culture medium which does not allow cell dissemination inside the medium: cells from the same progenitor have to stay together.

The culture is incubated in this medium and aggregates are then counted with a microscope. The shape and size of these aggregates can be different

* Corresponding author. Tel.: +33 2 98056126.
E-mail address: alain-claude.roudot@univ-brest.fr (A.-C. Roudot).

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because the progenitor population is inhomogeneous. One can find colonies (aggregates with more than 50 cells), macro-clusters (20—50 cells) and micro-clusters (5—20 cells). The cells can be close together or not, but all the possibilities between these two cases exist. Each of these cases is representative of a category of cell: granulocyte or monocyte or a mix of these two kinds.

In this method of analysis [2], it is necessary to be able to score the colonies, macro and micro-clusters, and also to evaluate distribution of colonies taking their shape into account.

Because of all these constraints, quantification of aggregates is difficult. That is why an automatic system should be of real interest in laboratories using these techniques. Different methods were tried in order to evaluate colonies, among them microcapillary methods [3] and imaging methods [4—7]. At this time none was entirely automatic and clearly efficient. From another point of view, they only try to quantify the colonies and leave away the macro and micro-clusters. So, the aim of the present work is to propose an entirely automatic method of analysis of haematopoietic cells clusters.

2. Design considerations

In order to be acceptable, the method must take into account different elements:

- The possibility of moving the plate under the video camera and of digitising numerous pictures representing a large enough area of the plate from a statistical point of view.
- The possibility of automatically reconstructing the image from the different fields of view in order to obtain a numerical picture similar to the optical one.
- The possibility of automatically analysing this last image in order to count the number of micro-clusters, macro-clusters and colonies.
- And lastly, comparing the result obtained from this automatic analysis to the visual analysis made by a specialist of this measurement.

3. System description

3.1. Cell culture

Standard methods already described [8] were used for culturing haematopoietic clusters from mouse bone marrow in agar. After 12 days of incubation in this culture medium, the cells (CFU-Mk in this case) are dehydrated and fixed. Then they are identified using specific colouring techniques. They appear as aggregates of pink cells with blue nucleus.

The toxic effect of a chemical on haematopoietic cells may be seen because of a diminishing number of total aggregates, but also because of a modified ratio between the types of clusters, compared with a test culture. That is the reason why micro-clusters, macro-clusters and colonies are all counted separately.

3.2. Measuring materials

The fixed culture plates are analysed using an inverted microscope (CK2 from Olympus). A video camera (Sony DXC 51) is mounted on this microscope and allows the digitization of the different images and their further analysis on a microcomputer. Because of the ratio between the plate area and the optical field of view, an automatic scanning stage (Märzhäuser Scan IM) is used in order to move the plate under the video camera.

All the analysis of the different images and the stage displacement are made using scripts written with the video analysis package Visilog (from Noesis).

3.3. Automatic covering of the plate

As the test area of the plate is around 300 mm^2, and the field of view one mm square, we choose to use 12×12 field of view in order to cover a sufficiently large area and to be far enough from the limits of the test area. The ratio between the measured area and the total area is then around 1/2, which seems large enough to be representative. It is not an actual problem to increase this ratio, but it seems simpler for the user to be sure that the entire measuring area is always inside the total area, even after changing the plate, than having to verify that fact for each plate, because the coverage is too high and then the possibility of being outside the test area is high. This problem is not a theoretical but a practical one because the plates are manually prepared, and so the test area is not positioned exactly at the same place.

In fact, the final answer to the question will be given by the comparison between the automatic analysis and the visual one.