Quantitative microscopic image analysis by Active Contours

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Abstract

This article presents a complete framework to characterise morphological modifications of biological objects by data quantification from microscopic images analysis in the particular application of molecular reorganization during the early immune response. The image processing consists in 1) extraction of cells contour and 2) quantification of molecules involved in the reorganisation of the cell surface delimited by the extracted contour.

Our analysis uses multimodal images, in order to detect the best information on each kind of image, and is based on active contours to extract cells contours and morphological operators to quantify the molecular reorganisation.

1 Introduction

The aim of the work is to propose a computerised scheme to characterise morphological modifications of biological objects, observed during the early steps of the immune response. The image processing consists in extracting cell contours in order to detect the deformation in shape and to quantify the concentration of molecules involved in the immune response.

The defense of an organism against infectious agents depends on finely regulated interactions between different cell types of the immune system. Among these cells, T lymphocytes play a central regulatory role in the immune response. The presence of microorganisms is detected by T lymphocytes *via* surface receptors capable of recognising molecular components of the infectious agent (antigens). If an antigen is detected, the T lymphocyte undergoes a series of rapid changes involving various membrane and cytoplasmic components. Thus the T lymphocyte immediately changes its shape in order to increase the surface of contact with the antigen presenting cell (APC). Moreover, a highly ordered reorganisation of membrane receptors, intracellular signalling molecules, and cytoskeletal components occurs in the contact zone between both cells.

A crucial aspect of the analysis is to measure the magnitude of the local molecular reorganisation that occurs in the contact area between the T cell and the antigen presenting cell and to try to correlate it with morphological deformation of the T cell. This requires quantitative microscopy image analysis.

Two types of microscopic images are available: phase contrast images (see Figure 1), and fluorescent images. In the former, well defined information can be extracted about the cell morphology. In the latter type of images, cells are labelled with fluorescent antibodies, either against DNA (see Figure 3) to label the nucleus of the cell (*DAPI image*), or against the molecules (see Figure 2) which seems to be involved in the immune response (*Actin or Ezrin image*). Morphological information are very poor on this type of images, but they are very useful to localise cells in the image (*DAPI Image*), or to visualise and quantify the molecular reorganisation (*Actin or Ezrin image*).

The system developed system consists in two steps:

contour cell extraction and detection of morphological deformation from phase contrast image;

quantization over the fluorescent image (*Actin or Ezrin image)*, in the cell areas detected during the first step.

Contour extraction has been widely studied in image processing and a number of methods have been proposed, as for example, the Canny-Deriche filter [1]. Classical methods however usually need post-processing steps to obtain contours that are connected and closed.

The technique of active contours (snakes), first presented by Kass *et al.* [2], is used in many applications, including edge detection, shape modelling, segmentation, pattern recognition, and object tracking ([3], [4], [5], [6], [7]). These techniques always produce closed contours, and are well adapted to segment biological images.

Two main approaches of active contours exist: the parametric and the implicit representations. The latter are also called the level sets [8], which include the work of Caselles *et al.* [9] and their concept of geodesic snake.

The parametric approach of active contours is regarded as being more efficient than the implicit one [10]. The main advantage of the implicit representation is its ability to change automatically the contour topology during the deformation, which is not necessary in this application. We therefore use parametric representation of the snake in this study.

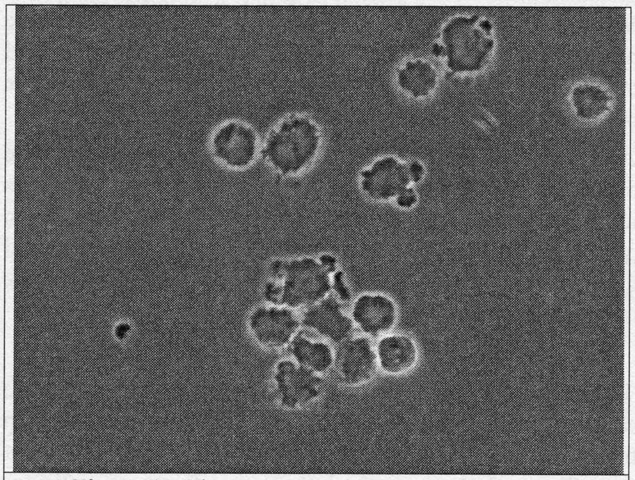

Figure 1 : Phase contrast image, in this image the cell morphology can be extracted

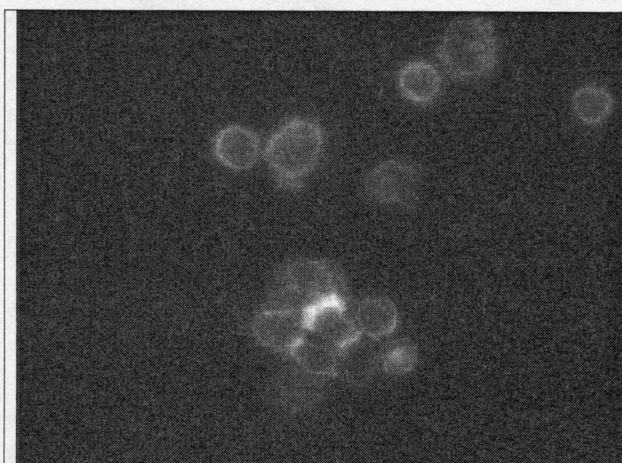

Figure 2: Fluorescence image, this image shows the concentration of marked molecular (actin or ezrin)

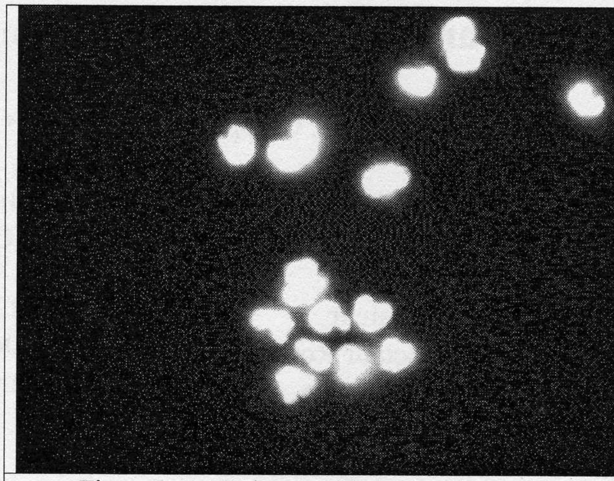

Figure 3: DAPI image, this image shows the cell nuclei

2 Methodology

2.1 Detection of morphological deformation

To quantify the evolution of the cells morphology, cell contours have to be extracted in a first step.

A parametric snake is a deformable model, represented by a parametric curve $x(s) = [x(s), y(s)]$, $s \in [0,1]$, which moves on the image to minimise the functional:

$$E = \int_0^1 \frac{1}{2}[E_{\text{int}}(\mathbf{x}(s)) + E_{ext}(\mathbf{x}(s))]ds$$

It can be represented as energy-minimising curve guided by internal and external forces which tend to move it towards the desired features. The internal forces depend on the geometry of the curve, its elasticity and its ability to shrink or to bend. It is defined as:

$E_{\text{int}}(\mathrm{x}(s)) = \alpha|\mathrm{x}_s|^2 + \beta|\mathrm{x}_{ss}|^2$, where α characterises the elasticity of the curve, and β its rigidity.

The external energy E_{ext} is given by:

$E_{ext} = -\kappa|\nabla f(\mathbf{x})|$, where f is the edge map, typically defined as $f = |\nabla I|$ or $f = |G_\sigma * \nabla I|$ and κ is a scalar parameter set to 0.5. The external forces are derived from the features of the image such as lines and edges which attract the snake. The curve converges to the edge under the action of the potential forces $-\nabla E_{ext}$.

A curve which minimises the functional E must satisfy the Euler-Lagrange equation:

$$\begin{cases} (\alpha x_s)_s - (\beta x_{ss})_{ss} - f_x = 0 \\ (\alpha y_s)_s - (\beta y_{ss})_{ss} - f_y = 0 \end{cases}$$

A solution for the snake can be found by treating the function $\mathbf{x}$ as function of both s and time t. Then, a solution of the energy minimisation is a stationary solution of the evolution equations:

$$\begin{cases} x_t(t) = \gamma\left[(\alpha x_s)_s - (\beta x_{ss})_{ss} - f_x\right] \\ y_t(t) = \gamma\left[(\alpha y_s)_s - (\beta y_{ss})_{ss} - f_y\right] \end{cases}$$

A snake can be seen as an elastic curve which deforms and adjusts its initial shape on the basis of additional image information and such as to provide a continuous contour.

One main problem with active contours is the step of initialisation. Indeed, as the functional is not convex, many solutions can be found and the curve may converge to a wrong result. Several improvements and reformulations have been proposed to overcome this problem, like multiresolution approaches [4], pressure forces [3], and dynamic programming [11]. In [5], Xu and Prince proposed the Gradient Vector Field (GVF) model which allows for flexible initialisation of the snake and helps convergence to boundary concavities. This model has been found to be well adapted to our application, in particular because of this flexible initialisation and its good convergence performances compared to other methods (classical and balloon).

2.1.1 Gradient vector field

The gradient Vector Field model is a classical parametric snake, in which a non potential vector field $\mathbf{v}(x,y) = (u(x,y), v(x,y))$ is introduced in the external force expression and is computed as the diffusion of the gradient vector:

$$\begin{cases} x_t(t) = \gamma\left[(\alpha x_s)_s - (\beta x_{ss})_{ss} + u\right] \\ y_t(t) = \gamma\left[(\alpha y_s)_s - (\beta y_{ss})_{ss} + v\right] \end{cases}$$

By definition, this vector field minimises the energy functional:

$$\varepsilon = \quad \mu\left(u_x^2 + u_y^2 + v_x^2 + v_y^2\right) + |\nabla f|^2 |v - \nabla f|^2 \, dxdy$$

where μ is the GVF parameter, and its value for our images, is set to 0.2.

And therefore satisfies the Euler-Lagrange equations:

$$\begin{cases} \mu\nabla^2 u - \left(f_x^2 + f_y^2\right)\left(u - f_x\right) = 0 \\ \mu\nabla^2 v - \left(f_x^2 + f_y^2\right)\left(v - f_y\right) = 0 \end{cases}$$

The GVF field can also be computed as the stationary solution of the following evolution equations:

$$\begin{cases} u_t(x,y,t) = \mu\nabla^2 u - \left(f_x^2 + f_y^2\right)\left(u - f_x\right) = 0 \\ v_t(x,y,t) = \mu\nabla^2 v - \left(f_x^2 + f_y^2\right)\left(v - f_y\right) = 0 \end{cases}$$

This vector field increases the capture range of the active contour (see figures 4 and 5).

We have used both traditional snakes and the GVF approach to segment these images. The edge map f taken here is simply the negative of the image intensity (the object boundaries appear dark) and the GVF gives the best results.

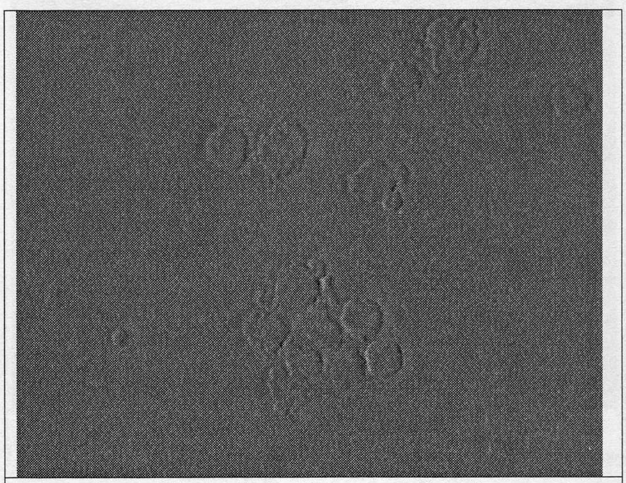

Figure 4: Gradient on x direction

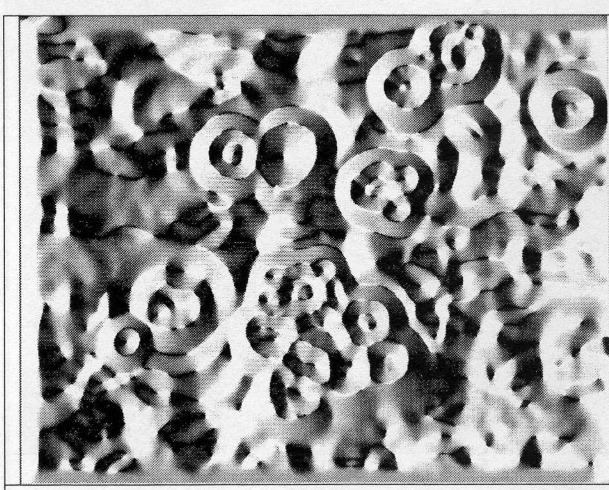

Figure 5: GVF on x direction

2.1.2 Initialisation

The initialisation step begins with the processing of the DAPI fluorescent image, where the cell nuclei are only visible. This image is automatically thresholded using the Otsu's method [12] and then the Deriche's operator is applied, followed by the extraction of contour points (see Figure 6). Morphological operators (opening and erosion) are used to separate nuclei that are joined.

But in the cytoplasm, organelles, which is seen as texture and local edge on the image, can stop the snake evolution (see Figure 7). Each active contour is initialised with the ellipse circumscribed to each nucleus detected in the DAPI image, to overcome this problem, which gives a better result (see Figure 8).

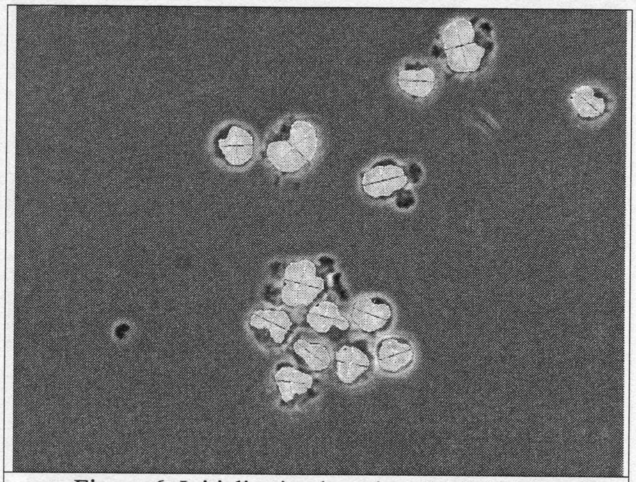

Figure 6: Initialisation based on the DAPI image segmentation superimposed on the phase contrast image

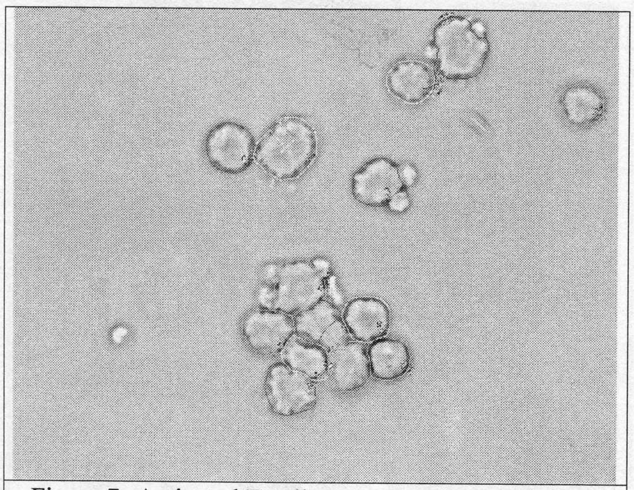

Figure 7: Activated T cells: segmentation result from the snake superimposed on the phase contrast image when initial contours are extracted from the nuclei

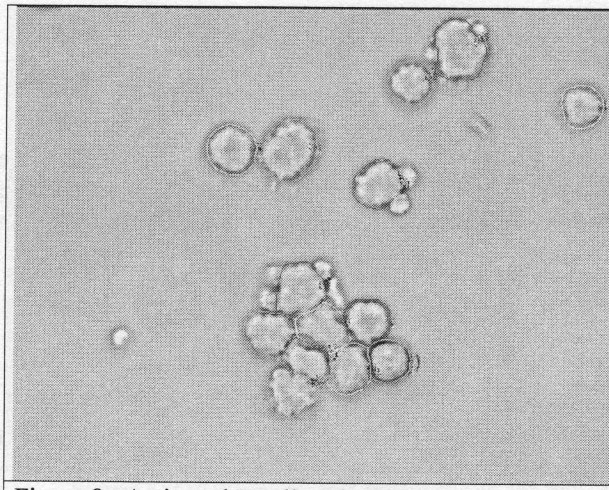

Figure 8 : Activated T cells: a better segmentation result from the snake superimposed on the phase contrast image

2.1.3 Shape measures

A non active cell is characterised by a compact and generally convex shape, close to an ellipse, whereas the activated cell is less compact and non convex (see Fig. 9).

Hence, the pattern compactness parameter computed on the extracted contours will help to characterise the shape deformation.

We suppose that a non activated cell has a nearly circular shape. To measure deviations from this shape, we used a normalised compactness parameter:

$C_n = 1 - \dfrac{4\pi}{C}$, where C, the compactness parameter is defined by: $\dfrac{P^2}{A}$, where P and A are respectively the perimeter and the area object.

For a perfect disk, $C_n = 0$, but because of the discrete framework, it is only approximately to zero. For activated cells, the value parameter is higher than 0.45 and lower for non activated cells (see Fig. 9).

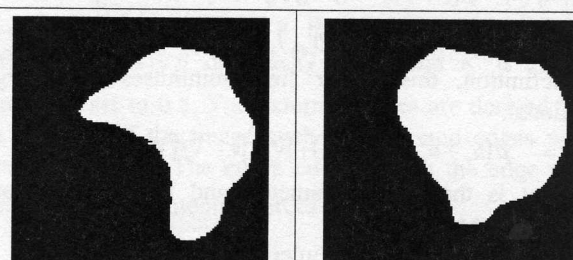

Figure 9 : activated (left) where C_n =0.61, and non activated (right) T cells where C_n =0.36

2.2 Quantification of molecular relocalisation

In this study, a local molecular reorganisation means an accumulation of these molecules in the contact area of the cells (see Figure 10). The system must distinguish the activated cells and the non activated ones. Cells activation occurs only in T cells. Theorically, a deformation is done between APC and T Cells (cf. figure 10).

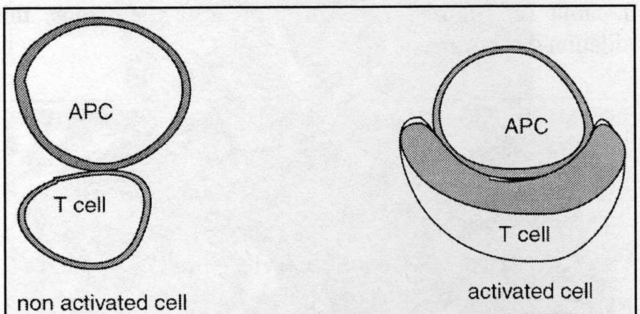

Figure 10 : Configuration of cells interaction

The quantification of the molecule which is involved in the cell deformation, is done with contours (see Figures 14,16) superimposed on the fluorescence image (see Figures 15,17). The quality of the result directly depends on the extracted contour.

It is not possible to obtain directly the number of molecules, as the relation between image intensity and number of molecules is unknown. Neither exists an absolute parameter of quantification because the comparison of the deformation of cells is done between different cells. The sequence images analysis will be another step.

We define the quantification parameter as the ratio between the number of pixels which intensity is above than a threshold and belonging to the accumulation area over the total number of pixels which are brightened in the fluorescent image (see Figure 11).

Morphologic and boolean operators are used to quantify molecular reorganisation (see Figure 12).

Successive erosions are performed on the region image derived from the extracted contours (cf. figure 12 and algorithm). The number of erosion is defined on activated cell: when there is no more brightened pixel.

Algorithm

```
   In: Contours extracted
   For each contour,
      Create   the   region   from   the
contour: R0
   While (condition)
      erode the image -> R1
      make the difference: R0-R1 = M1
      do  the boolean operation: M1 And
          fluorescence image -> I3
      threshold I3 with the value
      determined with the help of
      Otsu's segmentation -> I^i_4
   Do a boolean Or operation on I^i_4
   Out:   Number   of   all   "activated"
pixels for each cell
```

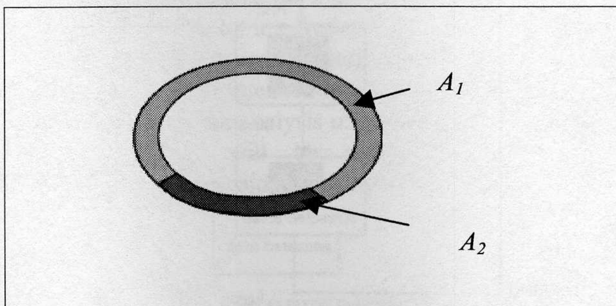

Figure 11: Relocalisation parameter: $\dfrac{A_2}{A_2 + A_1}$,

where A_2 is the touching area between two cells

The first results give values of relocalisation parameter which are more than 0.9 for activated cells and less than 0.4 for non activated cells.

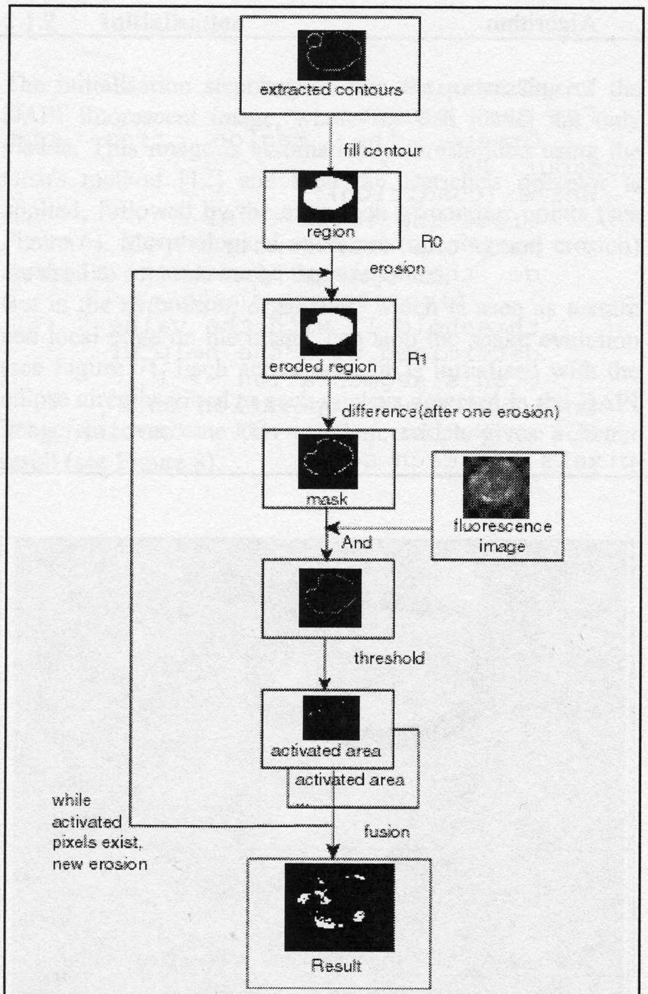

Figure 12 : Methodology to quantify molecular reorganisation

The threshold value is determined with two Otsu segmentations on fluorescence image (cf. figures 13a and 13 b).

Figure 13a: First Ostsu' segmentation

Figure 13b: Second Ostsu' segmentation

To estimate the number of pixels with high intensity in the accumulation region, the system has to find the points belonging to the contact areas. This is done by computing the distance between the points of two contours (C1, C2).

$$P \in C^1 \cap C^2,\ d(C^1_{P_i}, C^2_{P_j}) = 0$$

3 Results

Because of the halo created by the phase contrast image the contours extracted are dilated compared to the real contour (cf. figures 14, 16). With a few erosions, this dilation disappears.

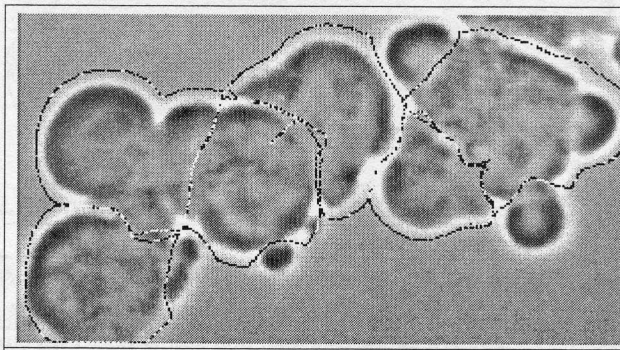

Figure 14 : Activated T cells: segmentation result from the snake superimposed on the phase contrast image

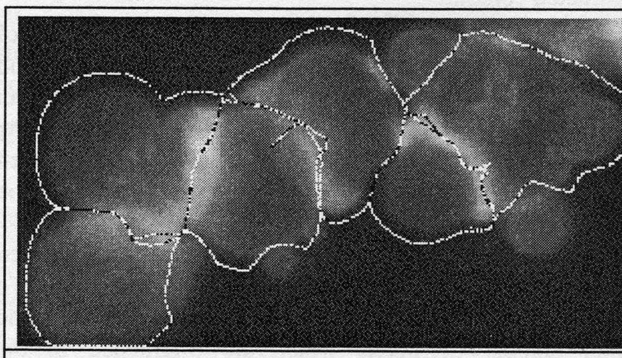

Figure 15 : Activated T cells: segmentation result from the snake superimposed to the fluorescence image

In the fluorescent image of APC cells, with the 3D effect, due to the projection (see Figure 18), the extraction contour has detected molecules in those cells. But as we know that molecules are only reorganized in the T cell, the accumulation value from the APC is added to the neighbour T cell.

For activated cells, the value parameter is higher than 0.45 and lower for non activated cells (see Fig. 10).

For activated T cells, the parameter relocalisation value is a more than 0.9 and for the non activated it is less than 0.4.

These results show that the discrimination between activated and non activated cells is well done.

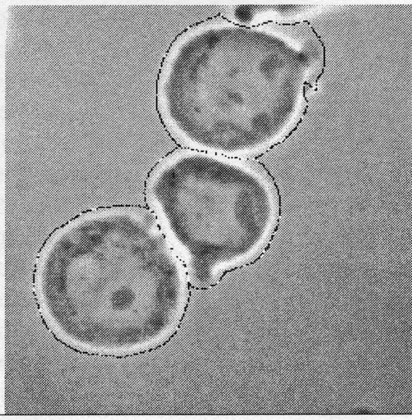

Figure 16 : Non activated T cell: segmentation result from the snake superimposed on the phase contrast image

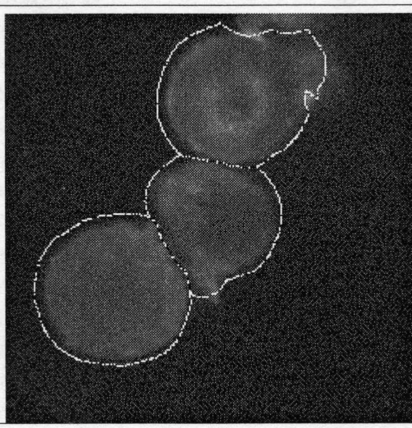

Figure 17 : Segmentation result from the snake superimposed to the fluorescence image

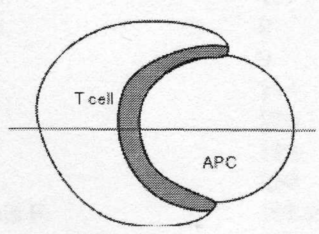

Figure 18 : An ortho view of the focal plane, which explains the fact that the molecular is detected in the APC cell.

4 Conclusion

In this paper, a method to quantify the deformation cells during the immune response is proposed. It has two steps and is based on a snake segmentation to extract the morphology of the cell on the phase contrast image and morphological operators to define the quantification parameter.

The first results are very encouraging as we have shown the correlation between morphologic deformation and the concentration of molecules in this deformation and could discriminate cell activation.

To improve extraction contour step near the accumulation areas, the edge map should integrated also the information of edge from fluorescence image and not only the intensity image. In particular, the extraction contour step near the accumulation areas need the improvement of the edge map. This could be done by integrating the information of edges from other modality images such as fluorescence image. For this, we are also intended to use the wavelets [13].

Furthermore with the analysis of a sequence of images, the cell deformation will be more relevant and the quantification data could be more accurate.

5. REFERENCES

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