IMAGE SEGMENTATION TO EVALUATE ISLETS OF LANGHERANS

C. Grimaudo, D. Tegolo, C. Valenti
Dipartimento di Matematica e Applicazioni, Università di Palermo, via Archirafi 34, 90123, Italy

F. Bertuzzi
Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, via Tricomi 1, 90127, Italy

Keywords: Islets of Langherans, implantation advisability, image segmentation.

Abstract: This contribution deals with an unsupervised system to process digital photomicrographs in order to locate and analyze islets of Langherans in human pancreases. The experiment has been conducted on real data and, though we are still going to complete the evaluation of the whole method, we expect to define a set of proper features (e.g. area, perimeter, fractal dimension, shape complexity, texture and entropy) useful for a fast and reliable counting of healthy cells. In particular, this research aims to measure the advisability of a possible implantation in patients affected by type 1 diabetes mellitus.

1 INTRODUCTION

This paper introduces a new system for the automatic analysis of high power magnification photomicrographs of the human islets of Langherans. The cells that make these clusters can be divided into a few classes which include the α cells, that secrete glucagon, and the β cells, responsible for the production of insulin. This research field is of particular interest because of the demand to evaluate the state of these endocrine tissue for preoperative planning in patients that suffer from severe type 1 diabetes mellitus, otherwise scarcely treatable by conventional therapies (Ryan et al., 2005; Shapiro et al., 2006). It has been verified that the probability of obtaining a favorable implantation increases when a large number of viable and purified islets is transplanted in to the patients (Bertuzzi and Ricordi, 2007). In a multivariate analysis aimed to identify some *in vitro* parameters for islet quality or function predictive of *in vivo* graft function of the same islets after their transplantation in diabetic patients, islet morphology (in terms of the maintenance of their round shape profile, similar to what they showed in the native pancreas) was demonstrated to be correlated with 1 month recipient c-peptide production (Ricordi et al., 2001); islet morphology therefore should be considered an indirect parameter of islet viability. These results call for the identification of some standardized strategies to characterize islet morphology and to quantify their degree of maintenance of their native round morphology (Nano et al., 2005).

At present, the analysis is also performed by improving the appearance through image processing softwares or ad hoc systems (Metamorph). A grid is laid on the slide so to fix the islets and to let easily count their different typologies (see Figure 1). This process is done by hand to separate those cells useful to the implantation and obviously it is slow, subjective and liable to errors; an environment to help the expert analyst is therefore desirable both to enhance the quality of the digital photos and to elaborate the images in order to locate automatically the zones of interest.

A variety of methods is already present in literature for both supervised and unsupervised segmentation of photomicrographs depicting cells (Coelho et al., 2002; Tripodo et al., 2006; Montseny et al., 2004; Bak et al., 2004). Usually these techniques are taken back to the elaboration of histograms, application of mathematical morphology, texture analysis, Fourier and wavelet transforms to extract the shapes of the components that have been found. Often the images have noise due to the presence of small artifacts, distortions and blurring introduced by the optical system, inherent inaccuracies due to the lattice

Grimaudo C., Tegolo D., Valenti C. and Bertuzzi F. (2008).
IMAGE SEGMENTATION TO EVALUATE ISLETS OF LANGHERANS.
In *Proceedings of the First International Conference on Bio-inspired Systems and Signal Processing*, pages 72-76
DOI: 10.5220/0001056800720076

(e.g. the thickness of the sample that must be analyzed), imperfections of the coloring of the contrast agent (e.g. due to variations of exposure time and to the quantity of the marker itself).

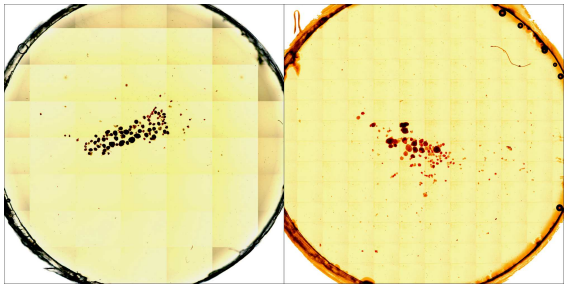


Figure 1: Two slides that put in evidence the presence of a grid to easily count the number of the islets.

We have realized a system that acts through a library of tools to preprocess the data; the segmentation of the various components in the images often requires the intervention of an expert user who locates the promising clusters of cells. This approach can be applied not only to the islets of Langherans, but also to analyze other vital cells (e.g. hepatocytes, bone marrow cells). Finally it should be verified the possibility to apply this strategy also in fixed tissue after different immunohystochemistry staining.

The following Section 2 describes the new environment to elaborate and classify the islets of Langherans. Experimental results are presented in Section 3, while remarks and possible future works are introduced in Section 4.

2 SEGMENTATION OF THE ISLETS

In this paper, we aimed to describe the system which has been developed to provide an unsupervised analysis of the human islets of Langherans (see Figure 2). Different techniques have been implemented to enhance the quality of the images, to segment all components, to distinguish among the cells and to evaluate their conditions in order to quantify the advisability of the implantation.

The photos in our database have been acquired through a digital tool; they suffer from artifacts due to the equipment (e.g. only the center of the image is correctly in focus and a few impurities can be present on the lenses). Predetermined threshold values result in a poor separation between the components of the images, but we have experimentally verified that the Otsu method (Otsu, 1979) is able to compute these optimal values in order to locate the imperfections on the red and green channels of the RGB color space. We have carried out a statistical examination on both the background and foreground to determine their starting threshold values; should the input image be very different from the database we have considered, then, to better calibrate the values, the user can select some regions of interest, representative of the different parts of the islets. Figure 3 shows the previous input image soon after the preprocessing step.

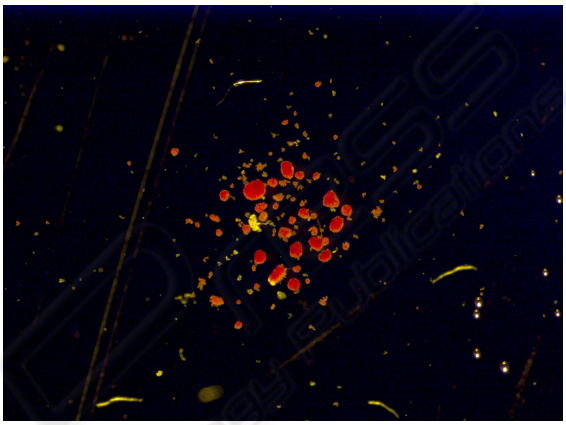


Figure 2: A sample photomicrograph of a cluster of the human islets of Langherans.

We have successfully applied the same adaptive self-tuning technique that has been introduced in (Tripodo et al., 2006) to discriminate between the pure β cells, or the mixed β and exocrine/ductal cells that are highlighted by the marker as red and orange zones respectively, while the yellow parts correspond to dead cells or impurities or simply exocrine/ductal cells. This usually leads to a rough representation of the cells, but a simple median filter is sufficient to remove all small objects (5×5 kernel) and pointlike noise (3×3 kernel). The shape of the cells so far obtained can be further enhanced by the use of a mathematical morphology opening with a structuring element represented by discrete disk of radius 2 (Soille, 2003). In such a way the cells of the islets are better separated and, moreover, we can safely delete all components that are too small (the allowed number of pixels has been pre-defined according to the present magnification power of the microscope). Figure 4 shows the final result obtained on the reference image; another example is reported in Figure 5. We have highlighted the final contour just to easily check the segmentation of the relevant islets.

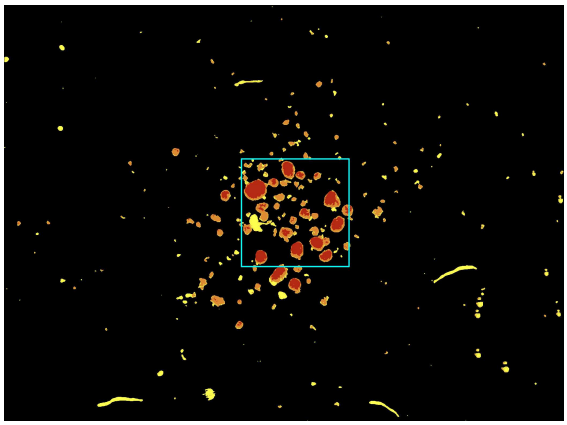


Figure 3: Some artifacts present in Figure 2 have been removed. Due to the huge field of view, in the following we will propose the results relative to the superimposed box.

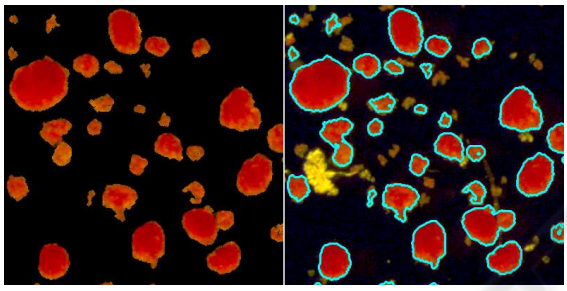


Figure 4: Left: the remaining artifacts and dead cells have been automatically removed from Figure 3. Right: the final contour has been plotted on the input image of Figure 2.

3 EXPERIMENTAL RESULTS

Images have a size of 2088×1550 pixels and were acquired at a sample dilution equal to 2500×, by a stereomicroscope Leica MZ12-5 with a 2× zoom magnification and equipped with a digital camera, able of a 4.34765μm/pixel picture calibration. The set of images we have studied has been obtained by isolating the islets through the automated method from multi-organ donors (Ricordi et al., 1989). After pancreas digestion the islets from 3 preparations have been purified by COBE processor (Vargas et al., 1996) and placed in a culture media for additional 48 hours at 24°C. The islets have been finally stained with dithizone (a vital stain that cross-reacts with zinc) and therefore it has been used to recognize the α, exocrine and ductal cells (in which zinc is absent) from the β cells (rich in zinc).

A set of parameters that describe each kind of cluster of cells has been extracted from the segmented images. The area, the perimeter, the compactness (i.e. the normalized ratio between the area and the squared perimeter) and the eccentricity of the ellipse which approximates the shape of the islet and the measures of convexity/concavity of its edges return a quantitative esteem of its aspect. In particular, compactness and eccentricity measure the roundness: healthy islets should not have protrusions.

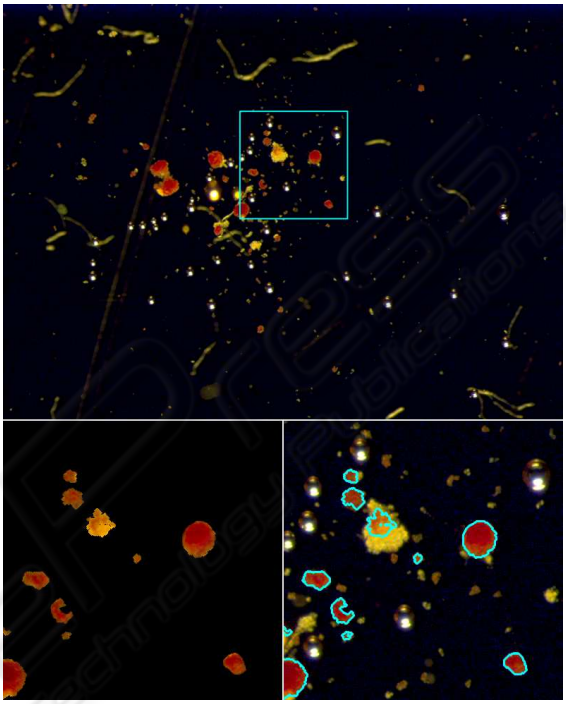
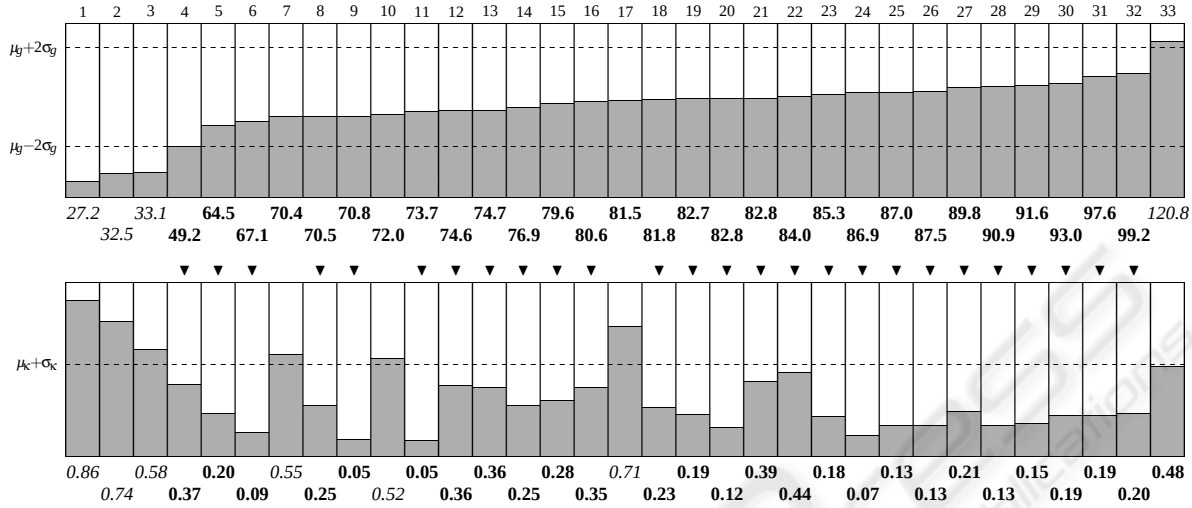


Figure 5: The edges of the islets within the box have been marked in blue.

The amount of information directly deducible from the luminosity of the pixels is another useful characteristic: the more homogenous an islet is, the smaller its local entropy is. We are still investigating on the ability of operators that return marks about the value of local sharpness and textures (which are closely connected to the presence of luminosity gradients).

For each islet $\imath_i$ we compute the product g_i between its average luminosity ℓ_i and its entropy e_i. If we indicate with μ_g and σ_g respectively the mean and the standard deviation of all $g = \ell \times e$, then the islets with a score $|g_i - \mu_g| < 2\sigma_g$ can be considered as promising candidate. A further important characteristic is given by the compactness κ (Rangayyan, 2005): with an analogous approach, the islets till now accepted with a compactness value $\kappa_i < \mu_\kappa + \sigma_\kappa$ are definitely classified as reasonably good. For the sake of clarity, an islet is classified as good if it passes the test on g and then on κ. The final evaluation of the whole input photo of Figure 5 is summarized in Ta-

Table 1: The features of each islet (33 in this example) have been represented by two columns (top: g and bottom: κ). The threshold values are represented by dashed lines and both tests have to be passed: κ reduces the number of candidates already obtained by g (good islets have been marked with a ▼).



ble 1. Figure 6 shows how the system highlights a single islet and proposes its features.

The percentage of the area of the yellow zones (more precisely, the ratio between red and yellow) indicates the purification of islet preparation and the eventual presence of embedded islets, that means islets surrounded by exocrine tissue (Ricordi et al., 1995). The final ratio between the area of good islets and the area of all islets summarizes the goodness of the inspected photomicrograph. Several parameters have been therefore available now by an automated method of analysis for the characterization of an islet preparation in terms of:

- islet number (the number of red clusters);
- islet dimension (the red area);
- islet purification (the ratio between yellow and red areas in the whole preparation);
- percentage of embedded islets (the ratio between red and yellow areas within an islet);
- islet morphology.

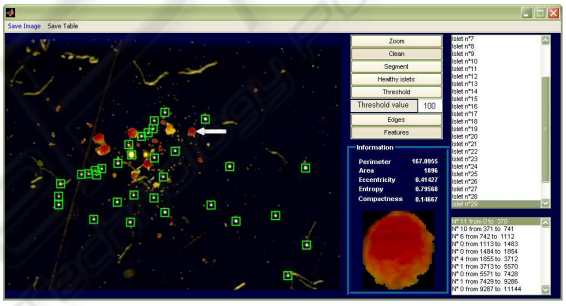


Figure 6: A screenshot of the graphical interface of the system. Small green boxes automatically delimit bubbles (present as artifacts in the photo). A selected islet is pointed out by a white arrow and the values of the relevant features are presented to the user.

4 REMARKS AND FURTHER WORKS

We have introduced an unsupervised system to locate the human islets of Langherans in photomicrographs. These clusters of cells have been characterized in order to define some parameters representative of their number and morphology. The predictive role of these features towards their *in vivo* graft function should be matter of further studies.

From a computer science point of view, the efficiency of the proposed method is still at the testing stage (Altman, 1999) and our system should be considered as a tool to help the experts in obtaining a quantitative esteem of the reliability of the islets in favorable implantation. The final results have been validated by biologists involved in implantations to treat patients affected by severe forms of type 1 diabetes mellitus. It is interesting to note that the methodologies we have applied to segment the components of the photos are quite standard and general enough and that the extracted features can be extended to differentiate between the α and β cells which compose the islets; this is to correlate their peculiarities with information of the state of the patients. Moreover, though preliminary results are encouraging, we are improving the segmentation procedure by including further algorithms based on mathematical morphology and

watershed/level sets.

To the best of our knowledge, our environment is the first attempt to automatically analyze islets of Langherans for implantations. Previous works rely on manual segmentation of their photomicrographs or are too general, thus to require to be adapted in order to process images containing these kind of cells. Therefore, a comparison of the results obtained by our system is still desirable.

Additional projects should be the *in vitro* characterization of the human islet preparations after the staining with vital probes (i.e. propidium iodide, fluorescein diacetate (Barnett et al., 2004; Miyamoto et al., 2000) and probes for apoptosis (Ichii et al., 2005). This should allow the direct quantification of vital, apoptotic and necrotic islets. Finally the automated system for imaging analysis should be applied in fixed tissues after immunostaining for insulin and glucagon thus allowing a complete characterization of islet cell composition (Ichii et al., 2005; Street et al., 2004).

ACKNOWLEDGEMENTS

The authors wish to thank Doctor Domenico Bosco of the Hôpitaux Universitaires de Genève for useful discussions and his kind contribution in providing the input images.

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