DIGITAL IMAGING TO ASSESS ISLET VIABILITY

Giada La Scalia, PhD, Giuseppe Aiello, PhD, Boyd Carr, MS, Luca Cicalese, MD, and Cristiana Rastellini, MD

University of Texas Medical Branch, Texas Transplant Center, Department of Surgery, Galveston, Texas.

University of Palermo, Dipartimento di Tecnologia Meccanica, Produzione e Ingegneria Gestionale, Palermo, Italy

Leonardo Engineering And Research S.R.L.
www.leonardoengineering.net

INTRODUCTION

Pancreatic islet transplantation is emerging as a therapeutic approach for patients affected by diabetes. The technique has been proven successful, but limitations have been identified. One of the major challenges of the procedure is the counting of the isolated pancreatic islets, which is currently jeopardized by subjectivity and inaccuracy. Determination of the accurate islet number is a crucial factor in determining the correlation between the isolation product and clinical outcome. In the proposed study, we have developed software capable of objectively evaluating islet numbers and other viability variables by image analysis. This software is based on image processing and feature extraction algorithms for recognition of the area of interest. This is the first step toward standardization of the isolation outcome and potential clinical success predictability.

BACKGROUND

Pancreatic islet transplantation has been proven successful, but is still experimental due to lack of standardization and data reproducibility. Major limitations have been identified in the multiple steps of the procedure, which has re-opened investigative avenues that can benefit from innovative technologies. Clinical islet allotransplantation is dependent upon the ability to achieve a high yield and purity of islets isolated from a human pancreas, obtained from cadaveric donor. Pancreatic islets are isolated using a modification of the semi automated method as previously described (1). Isolated islets are stained with DTZ and counted by an operator on a representative sample using an inverted microscope. The count, that takes into consideration the different sizes with the use of a graduated microscopy ocular, is then normalized using mathematical conversion to Islet Equivalents (IEq: islets of an average diameter of 150 micron). The final preparation is then transplanted into the liver of diabetic patients by percutaneous cannulation of the portal vein. At the present time, counting is subjective and inaccurate and cannot be correlated to *in vivo* data. Islet quality assessment (number, purity and preservation) is crucial to determine the relationship between isolation and clinical outcome and move forward in standardizing the procedure.

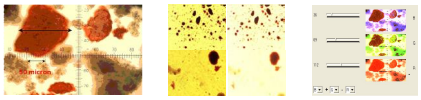
AIM

The proposed study aims to develop and test the effectiveness of digital imaging analysis to evaluate isolated islet samples representative of isolation and establish a correlation with clinical outcome. In order to achieve this goal we have developed software capable of objectively evaluate the variables by image analysis.

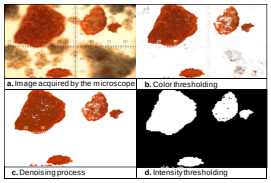
MATERIALS & METHODS

Image processing consists of performing mathematical calculations on a digitized input image to generate an output which can be an image with different characteristics suitable for subsequent feature extraction.
The image analysis procedure was performed as follows:

Image acquisition plays a fundamental role in effective image analysis and feature recognition. Since the image processing phase is based upon color thresholding, it is crucial that acquired images have the same chromatic characteristics. For such reason the staining process variables (preparation, type and concentration) as well as the microscopy setups employed (lights, lenses or focus leads) must be established and standardized. Different staining techniques and microscopy setups may lead to significantly different results.



Our analysis begins with a preliminary extraction of islets attained by DTZ staining (fig.a). The extraction of the regions with a specific color is then essential for the preliminary capture of the features. The color thresholding filter is our first tool to proceed with the initial extraction. This filter involves the demarcation of a histogram range, delimited by a low and high threshold, for each channel of the image. Each channel is independent in the thresholding analysis; features are then extracted on the basis of their color rather than their intensity (fig.b). According to this procedure, six thresholds must be properly established. However, the result obtained is typically affected by sparse residual pixels which do not pertain to features but have similar chromatic characteristics and must be removed.
Statistical averaging schemes were adopted to remove noise while keeping the integrity of the image information obtained (fig.c). Light intensity thresholding was applied in order to obtain a 2-level (BW) image for efficient feature extraction. Intensity thresholding consists of segmenting the image according to its light intensity levels. By adopting image intensity as the thresholding parameter, color information is discarded and the image is translated into standard grey-scale format. In order to perform such segmentation, a proper intensity threshold was selected manually; however, automated methods may also be employed (2). As a result of the thresholding process, a Black and White (BW) image (fig.d) is finally obtained.



On this image, the number of pixel thresholds for exclusion or inclusion of islets can be set and size and purity can be identified. The same procedure with different color thresholds can identify embedded islets and provide morphological information.

Feature extraction is the process to identify the regions of interest (ROI) within an image. The proposed procedure requires the preliminary establishment of the background color and feature color (e.g. black and white) in the thresholded image and the definition of a function that assigns value 0 to all the background and 1 to all the feature pixels. The algorithm hunting the regions of interest scans each line of the image from the top-left corner to the bottom-right corner. Each time a black pixel representing a feature is found, a rectangular region is grown until the whole feature is framed. In such condition all the borders of the rectangular frame do not cross the feature anymore. The region of interest is defined as the set of pixels belonging to the rectangular area within the 4 coordinates of the top-left corner (a,b) and the bottom right corner (c,d) where the following conditions are satisfied.
R=rectangular region of interest
R(a,b,c,d) /

Where *(x0,y0)* are the coordinates of the initial feature pixel found during the scanning process, and *t* the separation threshold, i.e. the maximum number of feature pixels that can be found in a separating border.
Once the whole feature has been framed into the rectangular region *R*, the rectangle area is erased and feature surface is calculated by:

The scan process requires sequential repeating of this process until the whole image is processed.

RESULTS

The above discussed procedure aims to automatically determine the amount and the size of islets of Langerhans in a microscopic image. Effectiveness of the proposed method is confirmed by the preliminary results carried out on test images, although a deeper investigation is necessary in order to measure the robustness and reliability of the approach on an adequate number of samples. The following figure (fig. c) gives the result on a complex image, containing approx. 170 islets, obtained via an optimized microscopy setup in the acquisition process. In more detail, fig. a is the acquired digitized image; fig. b is the output of the image processing phase, and fig. c the final result of the feature extraction process. The image represents a highly fragmented feature distribution which is typically difficult to process for traditional feature extraction algorithms, and considerably time consuming for professional experts. Time required for computation was approximately three seconds on a 2 ghz intel core due duo with 2 gigs of RAM processing RGB bitmaps of 1280 X 960 with 72 dpi resolution. Computational times depend upon the number and size of features. With the experimental setup, the result obtained was judged satisfactory by the experts, thus confirming the helpfulness of the proposed system.

a. Image in "C" lens **b.** Color thresholding **c.** Color thresholding

CONCLUSIONS

Digital image analysis has been proposed here as a new technology for assessing the amount of islets in a microscopy image of the samples of isolated pancreatic islets. Standardization of this information is fundamental to predict clinical transplant outcome. The main goal achieved in our study is the establishment of a methodology for fully automated feature extraction of microscopic images. The methodology here proposed, however, involves several thresholds and operational parameters which ultimately determine the quality of the results. Proper optimization of such parameters should be carried out. In addition, the reliability and robustness of the proposed system should be quantified measuring errors on a proper number of samples and comparing the results with the traditional manual process. Further investigation is required. The approach proposed brings significant innovation to the evaluation and likelihood of clinical outcome prediction, since it allows fast, precise and standardized evaluation of the islet number and size. Additionally, this technology allows removing the subjectiveness in the evaluation process, preventing errors and miscounting deriving from multiple components like: fatigue, bias, interpretation and inexperience. Finally, the automatization and fast delivery of our method allows testing of multiple samples with better estimation of the success probability. The possibility of implementing additional indicators related, for example to the shape and the contours of the islets, may also initiate new evaluation criteria to be employed within structured multi-criteria evaluation techniques for measuring the likelihood of transplant success. We believe this initiates a new area of research that can benefit the islet evaluation procedure and the entire islet transplant field.

REFERENCES

1. Ricordi C., Rastellini C., 1995. Automated method for pancreatic islet separation. In: Ricordi, C. (Eds.), Methods in Islet Transplantation, RG Landes, and Austin, TX, pp. 433–438.
2. 2. Sahoo PK, Soltani S, Wong AKC. 1998. A survey of thresholding techniques. *Compt. Vis. Graph. Image Proc.* 41:233-60.

Cristiana Rastellini, MD
University of Texas Medical Branch
6.312C John Sealy Annex
301 University Boulevard
Galveston, TX 77555-0533
Office 409-772-2412, Fax 409-747 7364
e-mail crrastel@utmb.edu