A semi-automatic approach to measurement of Pancreatic Endocrine Volume Tissue Density

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Abstract— This paper presents a reliable, fast and efficient method for measuring the volume density of pancreatic endocrine volume density. The algorithm segmentates digitized images in three different classes: the endocrine (En), exocrine (Ex) and artifact (At) components. A statistical classifier based on the k -Nearest Neighbour (k -NN) decision rule in the RGB color space was compared with a standard point counting technique. The k -NN rule classifies other pixels in the class that is mostly represented among the k nearest training samples in the RGB space, which is efficiently implemented with a fast k -distance transform algorithm. All extracted areas were quantified in absolute (μm^2) and relative (%) values. The different tissues were point counting determined and their quantifications statistically compared with those obtained semi-automatically. All analyses were performed by an expert pathologist and showed no significant differences between the two approaches.

Keywords— Semi-automatic morphometry, pancreas morphometry, k -NN.

I. INTRODUCTION

Quantification of tissues are not only needed for a better understanding of physiological data, but also to establish proper correlations with the elements seen histologically. For visibility, structures to measure need examination in thin histological sections, which bring in their turn various difficulties such as shrinkage and compression that distort absolute and relative values. Nowadays, most of these difficulties are overcome by using non-biased strategies like point-counting methods [10], [3], whose strength comes from an original mathematical treatment of the relations between three-dimensional structures and the various probabilities of encountering specific objects in a particular dimension. Measurement of pancreatic endocrine tissue density has been usually made by point counting methods [7], [8]. Although its use has come an unavoidable condition for any modern morphometrical study, this procedure is not drawback free: it is time-consuming, requires a skill histologist and is based in geometrical hypotheses that not all organs fit, as it is the case of pancreas. These difficulties have limited the study of the pancreatic endocrine mass, which remains an important point of discussion in a broad spectrum of pancreas pathologies, especially in type II Diabetes Mellitus [6].

This paper presents the development and validation of

a semi-automatic method to quantify the pancreatic endocrine volume tissue. Firstly, a few samples of correctly classified pixels are manually selected to obtain a good representation of the different tissues in one image. Next, these pixels are used to train a statistical classifier that uses the RGB color components as features. The classifier used here is the k -Nearest Neighbors (k -NN) classification rule [1], [2], which is a technique for pattern classification that makes no *a priori* assumption on the distribution probability functions of the different classes. This rule classifies a given pixel within the category most heavily represented amongst its k -Nearest Neighbors in the pattern space. Thus, every pixel in the RGB space is then associated to a determined category, according to the k -NN rule.

II. METHODOLOGY

A. Histological Material and Image Acquisition

Pancreatic paraffine sections ($5\mu\text{m}$ thick) were mounted on glass slides and processed by the immunoperoxidase method. The sections were incubated successively in methanol and normal goat serum with anti-human insulin serum at dilutions of 1/4000, followed by incubations with biotinylated rabbit anti-mouse IgG and streptavidin-peroxidase, respectively. The peroxidase activity was revealed by immersion in a solution of 3,3' diaminobenzidine tetrahydrochloride (DAB). Digital images were taken through a Zeiss microscope equipped with a JVC colour camera coupled to a Matrox Frame Grabber. A Zeiss-Vision KS-400 software was used to handle the images and perform point-counting computation of volume densities with a grid of 250 points as described in [10]. A transversal section of human pancreas was digitized and eight images were randomly selected to perform k -NN and point-counting comparisons. Segmented image surfaces were quantified in both raw pixels and μm^2 .

B. Image Segmentation

Implementing the k -NN rule with a brute-force method to classify F patterns with N prototypes requires $F \times N$ distance computations and $O(F \times N \times \log(N))$ comparisons, which is prohibitive in our case. When the number of possible patterns is smaller than the number of patterns

to classify it is efficient to pre-compute a lookup table for every possible pattern and then classify patterns by accessing this lookup table [9]. The computation of this lookup table is essentially a k -distance transformation problem. Distance transformations are algorithms that compute for every pixel of an image the distance to the nearest pixel of a given object. Here, the k -Euclidean distance transformation is computed by using ordered propagation to scan the pattern space [5]. The k -NN algorithm was implemented in VTK software with a TCL script as interpreter. Parameters were set for k -NN in one image and then applied to the entire set. One expert pathologist performed both point-counting and k -NN measurements. Reliability of k -NN method was assessed by comparing the endocrine tissue volume density with a two-way ANOVA test without replication. Differences were considered to be significant when $p < 0.05$. Data were analyzed with SygmaStat software version 2.0.3 (SPSS).

III. RESULTS

A subsection of a typical pancreas image is illustrated in the upper part of Figure 1. In this case, upper image shows the endocrine surface colored in brown, the exocrine in violet and the artifact component in white.

Fig. 1. Top figure shows an image subset ($428 \times 275 \mu m^2$) in which appears the different features to segmentate. Bottom image corresponds to the segmented subset image.

Three different classes were defined on this image: En, Ex and At. Artifact class corresponds to tissue retraction and is observed as white or transparent areas. Classification of these features were achieved by using 850 (artifact), 725 (En), and 954 (Ex) corrected classified pixels from one image that were then applied to the eight images. Bottom image shows the segmentation results, for a $k = 11$.

A. Point-Counting and k -NN comparisons

Point-counting and k -NN measurements performed in eight randomly selected images. Statistically results are shown in table 1.

Image	PC	k -NN
1	8.04	7.68
2	35.78	40.15
3	6.83	6.63
4	32.37	37.94
5	8.90	7.86
6	10.48	10.56
7	9.80	10.02
8	3.36	3.94

TABLE I
RELATIVE ENDOCRINE MEASUREMENTS ($En/Es + End$) ARE SHOWN FOR POINT-COUNTING AND k -NN METHODS.

Statistical analysis showed no significant difference between methods ($p = 0.22$) and significant inter-image differences ($p < 0.0001$).

IV. CONCLUSION

k -NN opens up a new perspective for morphometrical studies of complex geometry tissues. The method produces fast, robust, fully reproducible, accurate and reliable measurements of the different tissues present in pancreas sections. This contrasts with so far used semi-automatic methods that have shown poor inter-observer reproducibility and low correlation with the point counting method [6], when used for pancreatic morphometry.

REFERENCES

- [1] T.M. Cover and P.E. Hart, "Nearest neighbor pattern classification," *IEEE Trans. on Inf. Theo.*, vol. IT-13, pp. 21-27, 1967.
- [2] E. Fix and J.L. Hodges, "Discriminatory analysis, non-parametric discrimination," Tech. Rep. Project 21-49-004, Rept. 4, Contract AF41(128)-131, USAF School of Aviation Medicine, Randolph Field, Texas, February 1951.
- [3] H.J. Gundersen, T.F. Bendtsen, L. Korbo, N. Marcussen, A. Moller, K. Nielsen, J.R. Nyengaard, B. Pakkenberg, F.B. Sorensen, and A. Vesterby, "Some new, simple and efficient stereological methods and their use in pathological research and diagnosis," *APMIS*, vol. 96, pp. 379-394, 1988.
- [4] P.E. Hart, "The condensed nearest neighbor rule," *IEEE Trans. on Inf. Theory*, vol. 14, pp. 515-516, 1968.
- [5] O. Cuisenaire and B. Macq, "Fast k -nn classification with an optimal k -distance transformation algorithm," *Proc. of the 10th European Signal Processing Conference*, pp. 1365-1368, September 2000.
- [6] Kahn, R. and G. Weir, "Joslin's Diabetes Mellitus 13th edition," chapter: Pathology of the pancreas in Diabetes Mellitus, Foulis, A. and A. Clark, 1994, Lea & Febiger, pp 255-281.
- [7] Rahier, J. and R.M. Goebels and J.C. Henquin, "Cellular composition of the human diabetic pancreas," *Diabetologia*, 24, 1983, pp. 366-371.
- [8] Rahier, J. and S. Loozen and R.M. Goebels and M. Abraham, "The haemochromatotic human pancreas: a quantitative immunohistochemical and ultrastructural study," *Diabetologia*, 30, 1987, pp. 5-12.
- [9] Warfield, S. , "Fast k -NN classification for multichannel image data," *Patt. Recog. Letters*, 17, 1996, pp. 713-721.
- [10] E.R. Weibel, "Stereological methods I: Practical methods for biological morphometry," London: Academic Press, 1989.