TMA-Oriented Tool for Automatic Indentification of Pathological Areas in Human Colon Tissues

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Motivation

One of the most promising biomolecular approaches of recent years in cancer research field is the Tissue MicroArray (TMA) technique [1]. It relies on the use of formalin-fixed paraffin-embedded (FFPE) tissue samples, designated donor blocks. Cores from the donor blocks are punched and organized as a matrix in a new paraffin recipient block, named TMA. The intrinsic high parallelism of a matrix leads to a decrease of time and reagents costs and the reuse of scarce resources such as human tissues. The array design and content can be highly customized, according to the aim of the experiments, and may be based on phenotype or genotype features, allowing either to examine differences among diseases, or to study the effect of drugs on certain tissues. The major bottleneck of this promising technique is the selection, on each donor block, of the areas where cores have to be punched, through the judgment of hematoxylin-eosin (HE) slides of tissue donor blocks. The technique is innovative and up to now few specific tools [2,3] have been developed to support pathologists in exploiting TMA experiments, especially in the core punching evaluation step. Even converting the HE slides into digital format, none of the existing tools provides automatic approaches for this phase. In this context, it is clear how the impact of TMA technique may be greatly improved by designing suitable and efficient algorithms to analyse HE stained tissue morphology. The presented work deals with the automation of the region identification phase, in the context of one of the most frequent human genetic disease: the colon cancer. The final aim of the work is obtaining a software able to offer useful representations of colon tissue images, automatically differentiating between normal and pathological areas and providing a final discrimination, which may support the pathologist during the diagnosis phase.

Methods

Few morphological features are commonly used to differentiate normal and pathological colon tissues. Normal samples are characterized by a particular distribution of nuclei, which occupy only a small area of cells and are mainly localized on the edge of glands. Furthermore, cells are round in shape and strongly produce mucus, while glands are separated each other by a space at least suitable for one layer of cells. On the other hand, pathological colon tissue presents nuclei which extend over the whole area of the cell, proceeding toward the centre of the glands. Moreover, cells take a cigar shape, highly decreasing their mucus production and glands move close to each other. Since features mostly able to discern normal and pathological tissues concern the quantity of mucus and nuclei distribution, the developed image processing algorithm focus on an analytical evaluation of them. In the algorithm implementation we exploited ImageJ software, one of the most reliable free and open source Java based image analysis existing tool.

Results

The HE stained tissue images are processed to identify all glands within the sample. This step is performed by highlighting glands: the whole image is scanned to find objects border, defined by colour similarity. Objects contours are outlined exploiting a ROI (Region Of Interest) approach, which is employed to segment the image. The parameters values (mucus and nuclei presence) are computed separately for each object. An example of the described procedure is shown in Fig.1. Such information is compared with empirically defined thresholds and, according to achieved results, analysed tissue is automatically judged as a normal or pathological sample. Thresholds definition is currently in progress, because it should be validated on external images, coming from specialized tissue biobanks. A user friendly visualization is achieved by labelling image areas using different colours, according to the output (green border for normal tissue and red border for pathological ones), and by assembling them again in the original whole tissue structure image.

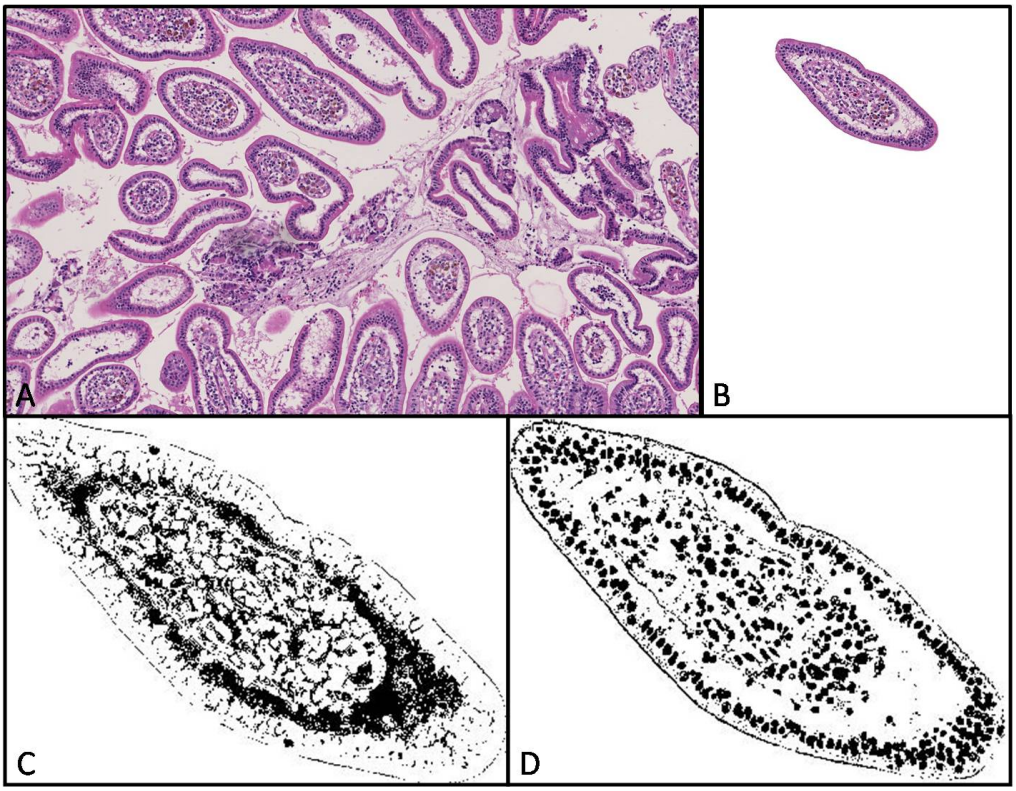

Fig.1: The original HE colon tissue image (A); the automatic gland extraction (B); the automatic evaluation of mucus (C) and nuclei (D) presence and quantification. The comparison with the empirically found thresholds leads to the final diagnosis.

References

[1] Kononen J. et al., Nat Med., 1998; 4: 844–847.
[2] http://genome-www.stanford.edu/TMA/
[3] http://tmaj.pathology.jhmi.edu/

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