Automatic analysis of tumor budding in colorectal cancer specimens

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Introduction

During progression of colorectal cancer, the epithelial structures lose their normal morphology due to their intense und unorganized proliferation.

Epithelial-mesenchymal transition occurring in proliferation sites induces an increase in the cell mobility as the cellular adhesion is lost (1). Epithelial structures lose single cells or small groups of cells which spread in the neighboring stroma, a phenomenon called tumor budding (2). Such small epithelial structures move further into the blood and lymphatic vessels and generate new proliferation sites.

The detaching process is noticed in both center (intratumoral budding, IB) and peripheral part of the tumor (peritumoral budding, PB).

Recent studies focused on the importance of IB for diagnosis and prognosis of colorectal cancer, by measuring and proposing various scores for grading the tumor budding. However, manual evaluation of tumour budding is a very tedious and time consuming task.

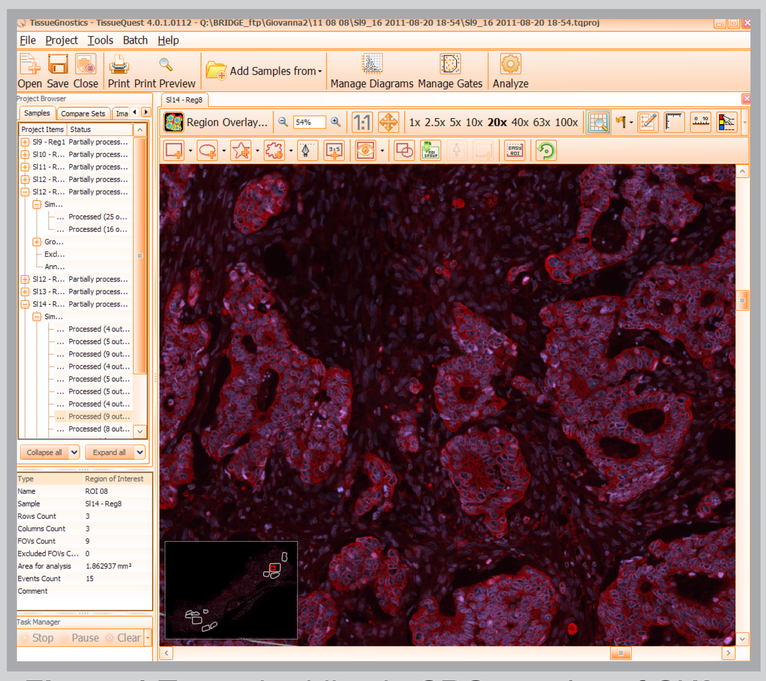

Figure 1 Tumor budding in CRC: overlay of CK8-TexasRed in red and DAPI in light blue

Aims

Our work focused on developing automated tools for recognition and scoring of tumor budding in colon cancer tissue sections.

This approach required realization of fast and robust algorithms which should deal with large amount of data and a high degree of variability specific to such biological samples.

As input, the tools will be able process, analyze and compare different regions of interest manually marked by the user on a virtual representation of the tissue section.

The methods will provide an easy user-interface as well as capabilities for integration into existing biomedical image analysis platforms.

Further analysis using other algorithms will be possible, combinining additional morphological and intensity-related measurements.

Results

1. Algorithm workflow

As a first step, overall Tissue Mask is detected using the tissue auto-fluorescence present in the GFP channel. This mask is used on TexasRed channel for assessing the expression of the CK-8, thus detecting the epithelial area. DAPI information is used in addition to the CK-8, in order to include the whole cell area into the measurement, and not only the cytoplasma. Each epithelial structure is then labeled and its area is measured. Labels smaller than the equivalent area of 3 epithelial cells are counted and their density is assessed as ratio of total count divided by area of the region of interest (Fig. 2).

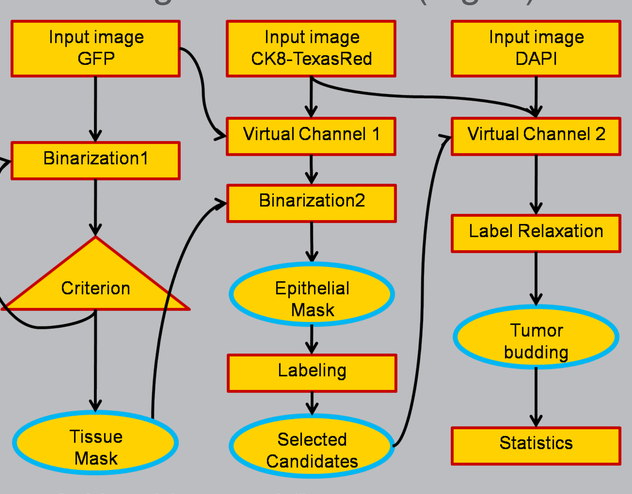

Figure 2 Algorithm workflow

2. Distributed image processing

Due to algorithm complexity and amount of data (n=20402 Fields of View, each with 3 images), a reasonably powerful up-to-date PC would need only for the image analysis about 6-18 hours when run on a single thread (processor core). During algorithm development, tests, as well as for final measurements, many runs are necessary, making the single-threaded approach an unacceptable solution. Thus, a distributed approach was implemented using a small cluster of 4 personal computers (PC), with a total number of 20 processor cores running at 3 GHz. Special code was designed to split the totality of images among the PCs, balancing each PC's load according the their number of available cores. Once the image processing results are available, they are automatically gathered for final area measurement of each epithelial structure (Fig. 3). The overall noticed analysis speed was between 13x-15x faster when compared against the classical single-threaded approach.

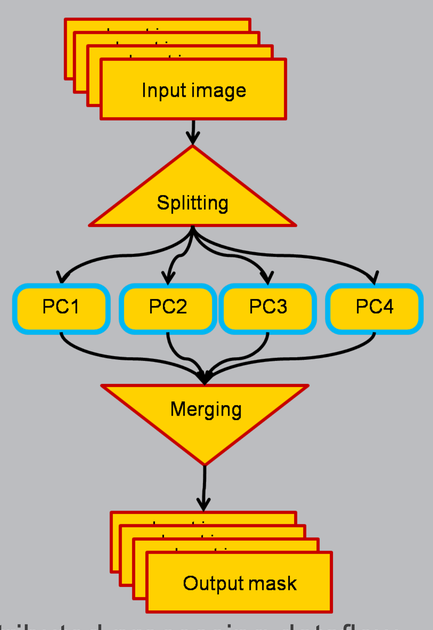

Figure 3 Distributed processing dataflow

3. Analysis

Regions of interests were manually marked to define IB and PB respectively, using integrated annotation features of TissueQuest. Area of each epithelial structure was measured and final statistics were exported in Microsoft Excel format. No statistical relevant differences were noticed in IB and PB budding density between the two groups of patients.

Conclusion

The newly realized computer-aided approach for tumor budding measurement can be used for assessment of CRC speciment.

Similar budding densities were noticed in tumor center when compared to peripheral tumor area, in both groups of patients, leading to the conclusion that the IB has a low correlation with the liver metastasis in G2 colon cancer.

The new method was realized in a modular way opening possibilities for further combination with other measurement capabilities targeting different morphological and phenotypic features as well.

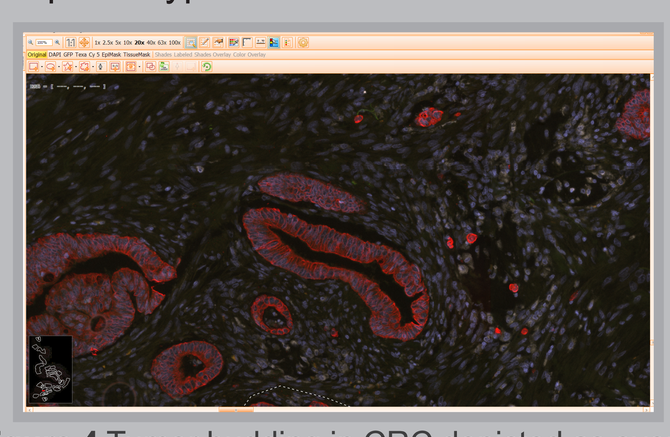

Figure 4 Tumor budding in CRC depicted as overlay of CK8-TexasRed in red and DAPI in light blue.

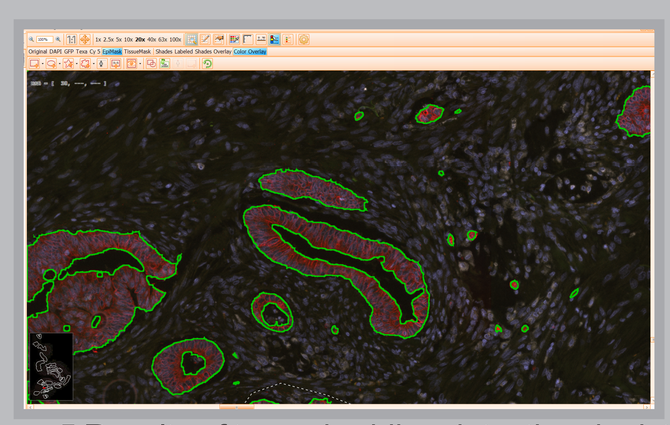

Figure 5 Results of tumor budding detection depicted as overlayed contours in light greeen

Methods

The algorithms assessed 8 samples from patients with colorectal cancer, from which 4 already developed liver metastasis. Regions of interest with normal mucosa from two healthy patients as well as with non-cancerous adjacent mucosa from CRC samples were added to the analysis group.

The paraffin embedded sections were stained in immunofluorescence for keratin-8 revealing the location of the epithelial cells.

The entire tissue sections were scanned with an automated microscopy system (TissueFAXS™, TissueGnostics GmbH) using DAPI, TexasRed and GFP filter sets.

The scans were augmented with the image processing results of our method and imported in TissueQuest. IB and PB were defined as region of interest. Bud area threshold was set according to examples detected in the images and budding density was extracted as count divided by area.

References:

1) Gosens et al. Loss of membranous Ep-CAM in budding colorectal carcinoma cells. Mod Pathol. 2007;20:221–232.

2) Hase et al. Prognostic value of tumor 'budding' in patients with colorectal cancer. Diseases of the Colon & Rectum, Vol 36, Nr 7 (1993), 627-635

Acknowledgements

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