Tumor Detection in Fluorescent Tissue Microarrays Enables High-Throughput Analysis of Multiple Cancer Biomarkers

Automation in immunohistological image processing is currently an essential technological development taking place in the clinical hunt for objective biomarkers in research and diagnostics. In cancer research one of the most important but also extreme challenges is the development of methods for the automatic separation of tumor and stroma tissue.

An important method routinely used in this context is the Tissue Microarray (TMA) technology, introduced in 1998. TMAs allow the simultaneous immunohistochemical analysis of several hundred tissues on a single slide. The manual detection of tumor regions in Tissue Microarrays is routinely based on the quantitative analysis of protein levels by pathologists or other experts. Yet manual detection is subjective, time consuming and most importantly suffers from intra and inter-observer variability.

A route to objectivity in histological tumor evaluation is automation and quantification by software routines. This will require computational methods capable of automatically identifying tumor areas and differentiating them from the stroma in a high quality microscopic image of the tissue, obtained by a virtual slide scanner.

To overcome the key problems of bright field staining caused by the objective and automatic capturing of distinct biomarker signals, we use fluorescence staining, PD Dr.-Ing. Niels Grabe, Scientific Head of the TIGA center explains. Although fluorescence helps in the quantification of individual cells, it does not per se help in differentiating tumor and stroma. Consequently fluorescence stained tissue slides are frequently counterstained with DAPI (4'6-diamidino-2-phenylindole) taking the role of a conventional background stain.

For scanning our fluorescence stained TMAs we used the Nanozoomer 2.0 HT scan system capable of scanning whole slides in high resolution Bernd Lahrmann (PhD student) tells us. Glass slides were scanned at 20x magnification (resolution of 0.46µm/pixel). The slide scanner automatically detects the region of interest that contains the array of cores and also determines automatically a valid focal plane for scanning. Single core images were located and extracted from the TMAs using template matching.

As no histological biomarker is available which would exclusively stain tumor tissue, pathology routinely uses morphological criteria as a spatial reference system. To combine the advantages of fluorescence with automatic image acquisition and processing we developed an algorithm for tumor-stroma separation and classification in immunofluorescence histological slides solely from a DAPI background stain explains Bernd Lahrmann.

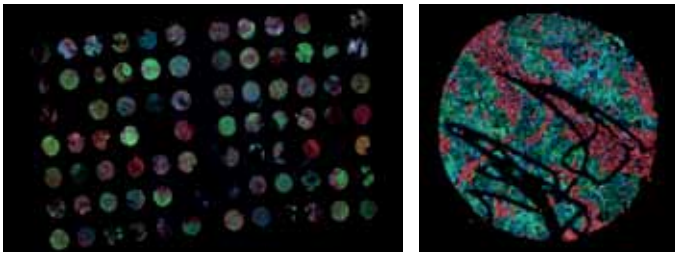

Figure 1: Microscopic Image examples of TMA slides (left) and Cores (right). Representation of all 3 channels of a fluorescence stained core in RGB color space. Red representing stromal Marker (CK19), green the tumor marker (Vimentin) and blue the DAPI channel highlighting the cell nuclei.

Due to the restriction to a single color channel this is inherently challenging. We formed cell graphs based on the topological distribution of the tissue cell nuclei and extracted the corresponding graph features. By using topological, morphological and intensity based features we could systematically quantify and compare the discrimination capability individual features contribute to the overall algorithm. We found that when classifying fluorescence tissue slides in the DAPI channel, morphological and intensity based features clearly outpace topological ones which have been used exclusively in related previous approaches. We assembled the 15 best features to train a support vector machine based on Keratin stained tumor areas. Figure 2 shows an artificial sketch of 3 different cell types (tumor cells, lymphocytes and fibroblasts) and a cell graph representation of this sketch. Cells are depicted as nodes and the links between them represent biological relations. Cell graphs are used to train a SVM for the classification step.

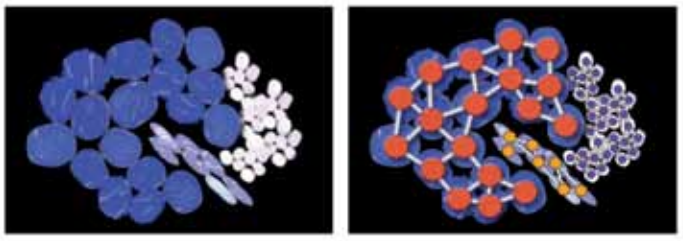

Figure 2: Conceptional representation of cell graphs.
(a) Artificial sketch of 3 different cell types: tumor cells (blue), lymphocytes (white) and fibroblast (purple). **(b)** Cell graph representation of (a). Cells are depicted as nodes and the links between them represent biological relations.

On a test set of TMAs with 210 cores of triple negative breast cancers our classifier was able to distinguish between tumor and stroma tissue with a total overall accuracy of 88%. Figure 3 shows the results of the image processing steps. Cells classified as tumor in green, stroma in blue.

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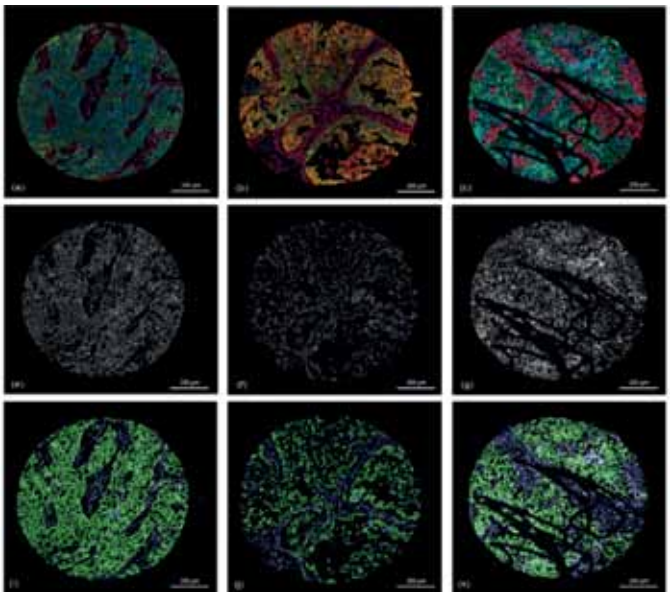

Figure 3 Results of classification. (a-c) show the original RGB core images; (e-g) showing the corresponding DAPI channel as an intensity image of the cores (a-c); (i-k) show the results of the classification step, green = cells classified as tumor cells, blue = cells classified as stroma cells.

Our method yields first results on the discrimination capability of features groups which is essential for an automated tumor diagnostics. Also it provides an objective spatial reference system for the multiplex analysis of biomarkers in fluorescence immunohistochemistry.

The TIGA Center is a cooperative project which started in 2007 at the University Heidelberg with the goal of establishing a bioinformatics platform dedicated to the quantitative analysis and modeling of tissues. A strong emphasis is placed on clinically relevant research projects.

PD Dr.-Ing. Niels Grabe

Bernd Lahrmann

At the heart of the TIGA's technology platform are automated microscopic scanners for whole slide imaging of glass slides. By integrating such imaging systems in a technical pipeline ranging from organotypic in vitro cell cultures to computational tissue modeling the TIGA generates a wealth of yet unexploited clinically highly relevant tissue data.

PD Dr.-Ing. Niels Grabe is the scientific head of the TIGA center, Dipl.-Bioinf. Bernd Lahrmann is PhD student in medical informatics at the TIGA center.

For further information see "Lahrmann B, Halama S, Sinn HP, Schirmacher P, Jaeger D, Grabe N. Automatic Tumor-Stroma Separation in Fluorescence TMAs Enables the Quantitative High-throughput Analysis of Multiple Cancer Biomarkers, PLoS ONE. December 2011;Vol 6(12):e28048" and http://tigacenter.bioquant.uni-heidelberg.de/

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