Automated cell-detection technologies for science and diagnostics

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Introduction

Automated microscopy technologies (e.g. TissueFAXS/ TissueGnostics GmbH, Vienna, Austria) already allow high throughput screens of large tissue samples in order to determine localization and interaction of proteins and other biomolecules. In this novel approach termed cytomics, molecular architecture and functionality of these systems is investigated. Rapid, automated cell-detection technologies aiming for computer-based cell recognition in a tissue-context for observer-independent, quantitative and reproducible evaluation of the obtained pictures, as well as quantification of cell-associated markers/parameters are of growing importance in research and diagnostics (e.g. TissueQuest/TissueGnostics). However, automated in-situ identification of special cellular shapes (e.g. multi-nuclei cells) or recognition of large tissue areas as well as sub-cellular structures has not been assessed thoroughly so far.

Therefore, our project aims to develop a versatile system for holistic pattern-recognition based on human-like interpretations. It will be primarily applied on tissues that are not able to be analyzed satisfactorily with the current state-of-the-art technology (TissueQuest/ TissueGnostics). These include i) colon cancer specimens with alterations in tissue structure and organization and ii) tissues with multinuclear cell types such as human placenta or bone.

Aims

Combining classical digital image-processing and pattern recognition approaches with machine-learning techniques the following specific issues will be addressed:

i/ Automated recognition of large (> 1 field of view; FOV), interconnected tissue structures (e.g. placental chorionic villi, tumour areas)

ii/ Automated recognition of multinuclear cells (e.g. syncytiotrophoblast, osteoclast)

Important prerequisite for computer-based recognition of areas larger than 1 FOV (aim i/) is an automated image stitching process that aligns images at sub-pixel resolution [4].

Methods

Paraffin-embedded tissue samples (Normal small intestine, healthy term placenta) were derived from Clin. Dept. Pathol. and Dept. Obstet. Gynecol., MUW, respectively. Sections (4µm) were de-paraffinized and antigen retrieval was performed. Unspecific binding sites were blocked and sections incubated with primary (see Figure legend) and respective fluorescence-conjugated (Alexa Fluor-488, Invitrogen) secondary antibodies. Nuclei were labelled with DAPI (Roche) and samples embedded in Fluoromount GTM (SouthernBiotech). Fluorescent Images were recorded by automated slidescan using the TissueFAXS (TissueGnostics; 20x objective). Pictures were sampled with an Overlap. All adjacent overlaps were used to merge the respective images by using template-matching function of OpenCV. The lossless stored images were read by using LibTIF.

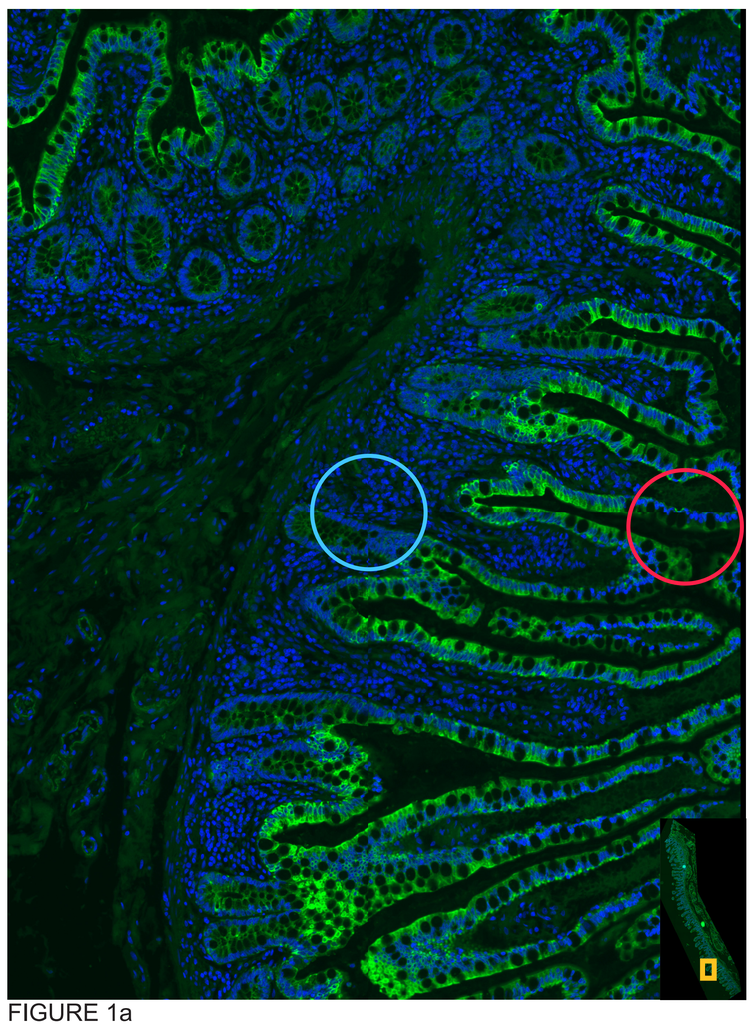

FIGURE 1a

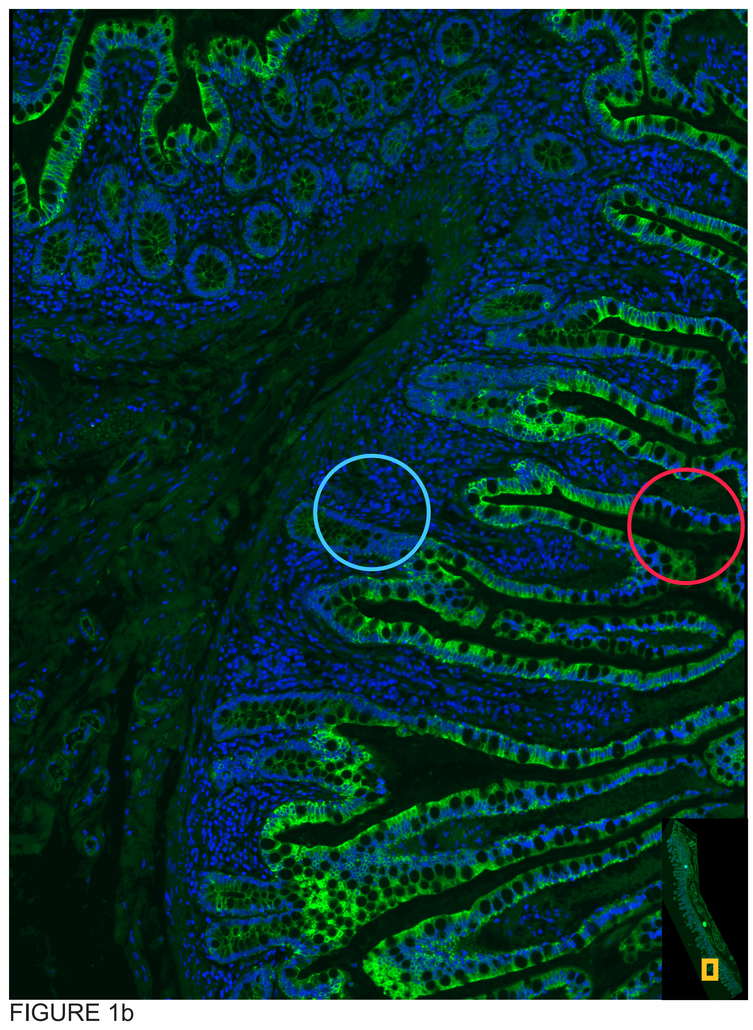

FIGURE 1b

Human normal small intestine was processed as described in Methods using rabbit polyclonal anti-keratin 8 (NeoMarkers Inc, San Antonio TX).The section shown above is a 2x2 selection of a 7x10 region. FOVs were stitched classically (Figure 1a) or using a newly developed algorithm (1b). Stitching improvement is especially visible in encircled areas.

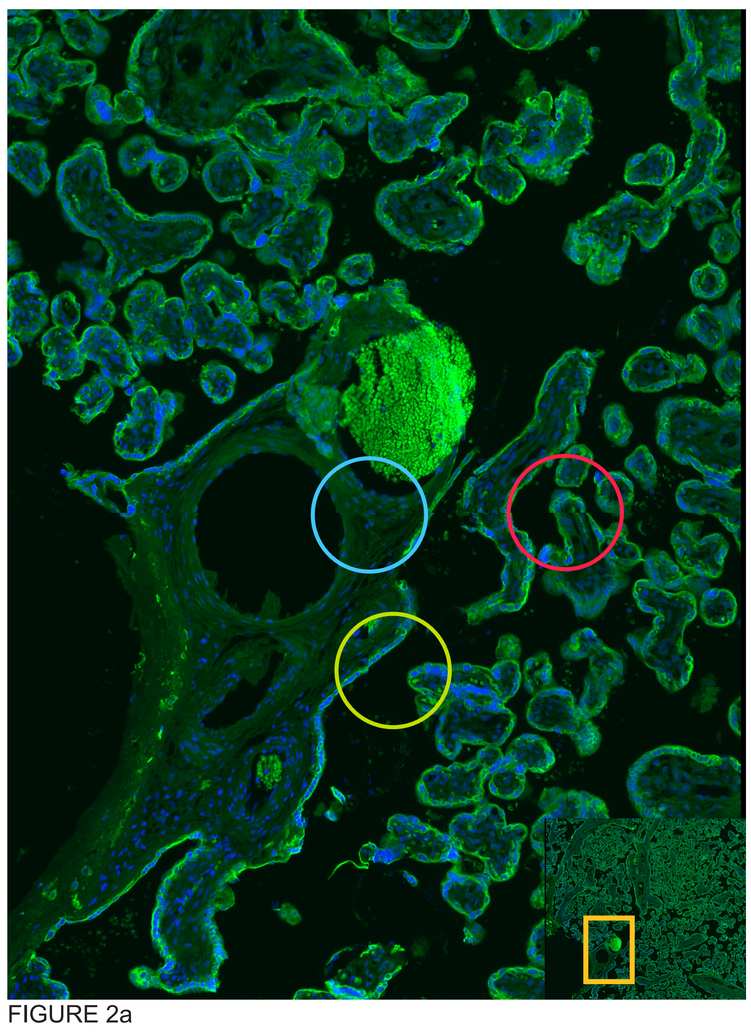

FIGURE 2a

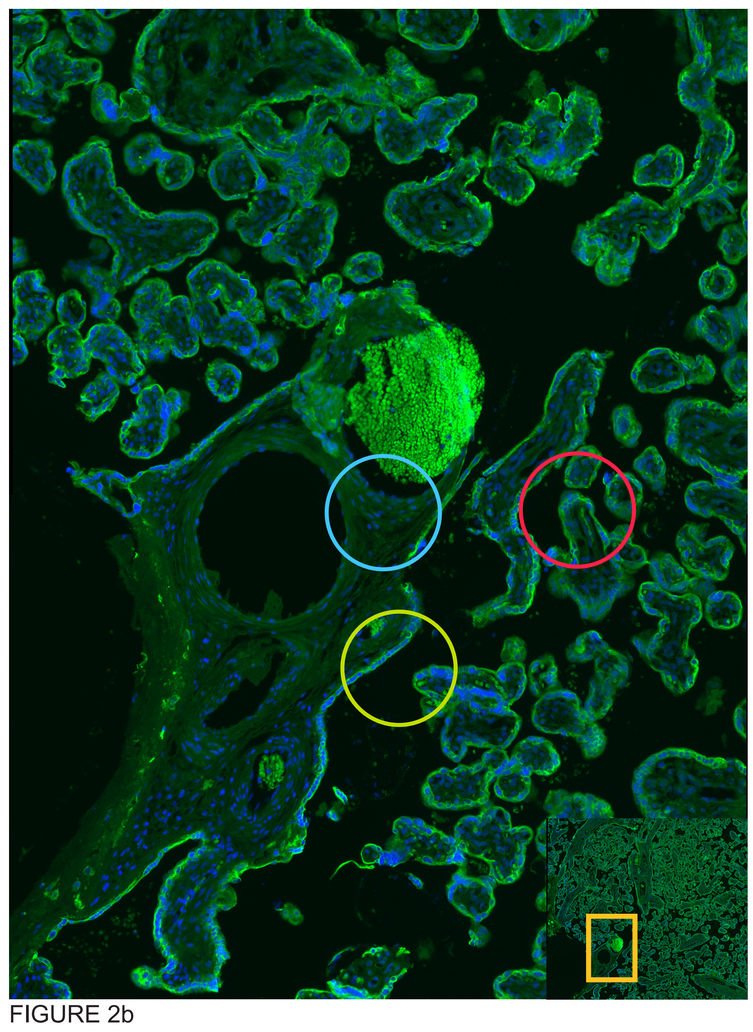

FIGURE 2b

Human term placental chorionic tissue was processed as described in Methods using mouse monoclonal anti-cytokeratin 7 (Dako). The section shown above is a 2x2 selection of a 3x5 region. FOVs were stitched classically (Figure 2a) or using a newly developed algorithm (2b). Stitching improvement is especially visible in encircled areas.

Results

Following automatic acquisition, a multiplicity of captured pictures is routinely stitched to create an overview as demonstrated in Figure 1a (Colon tumour) and 2a (Placental chorionic tissue). New stitching algorithms developed in this project significantly improve the alignment of the single pictures as demonstrated in Figure 1b and 2b and will allow for automated recognition of large tissue areas (aim i).

Conclusion

It is essential for smart cell analysis to operate on perfectly stitched images, because, otherwise, automated cell detection is very limited without knowledge of a tissue as a whole.

References

[4] A Computational Framework for Ultrastructural Mapping of Neural Circuitry, James R. Anderson et al, March 2009, Issue Of PLoS Biology

Acknowledgements

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