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SEMI-AUTOMATIC QUANTIFICATION OF MRNA EXPRESSION IN WHOLE-SLIDE TISSUE IMAGES

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Introduction/ Background

For complex diseases, commercially available anti- bodies directed again specific protein sometimes lack the specificity required for clinical purpose. Therefore, novel techniques have been proposed recently to better assess the expression status of molecules at the mRNA level, e.g. by in situ hybridization. With such a technique, single-molecule signals can then be quantified on a cell-by-cell basis in whole tissue slides. However, highly heterogeneous expression of mRNA is often observed at the tissue level, requiring the development of accurate and reproducible quantification methods.

Aims

In this work, our aim is to propose and evaluate a methodology for the quantification of mRNA expression in digital slides to help biomedical researchers to analyze their large-scale imaging data. In particular, we will work on a dataset of breast cancer tissue samples and evaluate the accuracy of such a methodology to quantify Breast cancer susceptibility gene 1 (BRCA1) mRNA expression levels. BRCA1 is a tumor suppressor gene associated with the triple negative breast cancer (TNBC) subtype. An accurate technique to determine BRCA1 protein tumoral expression status in TNBC would allow for informed decision and choosing adapted treatments.

Methods

Data: To design and evaluate our methodology, we use the data from [1]. In that study, the BRCA1 mRNA expression was assessed by in situ hybridization using RNAscope technology [2] (ACD-Bioke) for formalin-fixed, paraffin-embedded tissue samples. 88 glass slides were scanned (Hamamatsu, 40X, 0.23µm/pixel) and transferred on a Cytomine server (http://www.cytomine.be/) [3]. These slides correspond to 62 tumours (TNBC subtype). Using Cytomine web manual annotation tools, a total of more than 200 regions of interest were manually drawn by an expert in these whole-slide images. Then, independent assessment was performed by two observers in these ROI: they manually draw point annotations corresponding to tens of thousands of BRCA1 mRNA expression signals.

Algorithms: We have developed the Icytomine plugin to communicate between Icy and Cytomine. It allows the importation of the subtumor images at a desired resolution and the expert annotations from Cytomine and the exportation of detection results from Icy software (http://icy.bioimageanalysis.org) [5]. In order to quantify mRNA signals, we combined several image processing routines in the Icy. Our workflow is composed of three steps: 1) color deconvolution [4] to extract the dark brown staining, 2) detection of spots based on undecimated wavelet transform [6], 3) post-processing to remove artifacts based on size, shape and color features. This transform is fast and ideal for representing isotropic objects. We will use a colocalisation coefficient to quantify the concordance between the experts. For a purpose of automation, we use scripts and protocols of Icy.

Results

An illustration of our results is given in <Figure1>. Our ongoing large-scale empirical study stresses the need for more confident ground-truths hence more robust algorithms. Interestingly, our current strategy detects accurately spot locations. We believe it will help us to create a larger ground-truth dataset using expert proofreading. We will report at the conference our best quantitative results. Overall, we believe that our

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approach will speedup in a confident way the quantification of mRNA signals in whole-slide tissue samples.

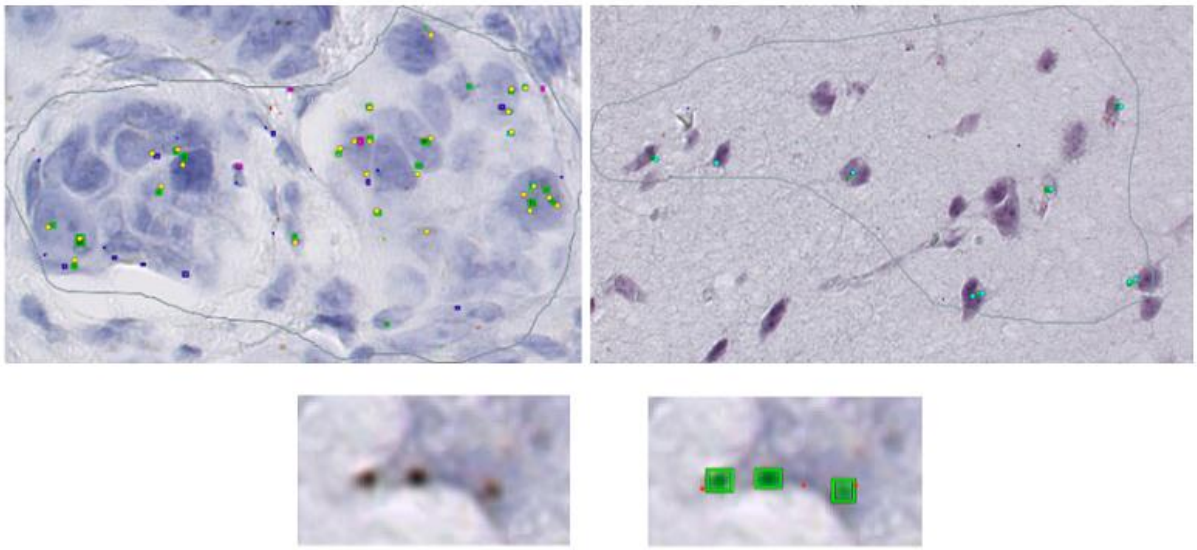

Figure 1.

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