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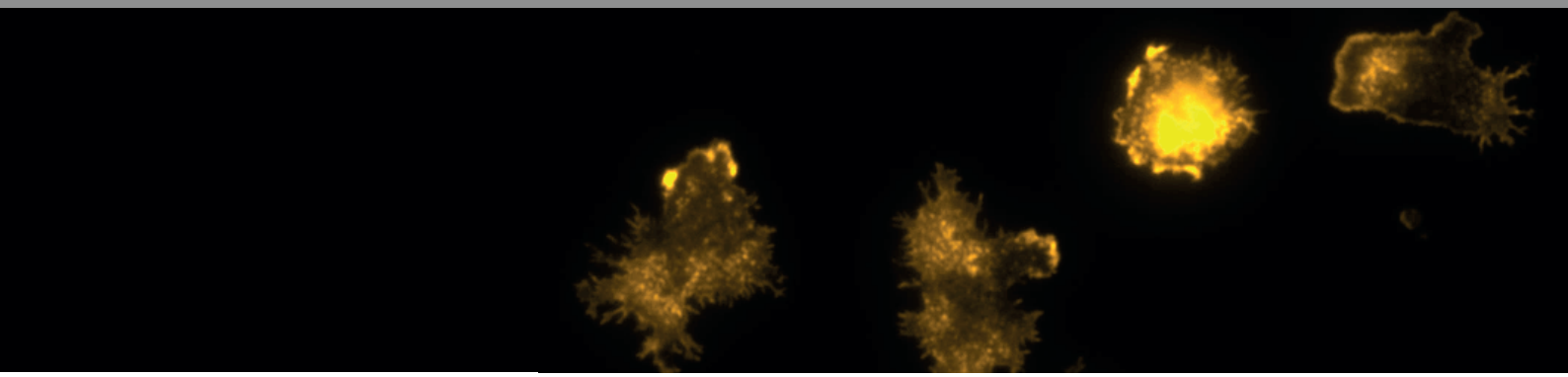

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ANALYSIS OF B CELL SPREADING ON IMMOBILIZED ANTIBODIES WITH WHOLE SLIDE IMAGING

Introduction

Whole slide scanning technologies enable the acquisition of massive amounts of multiple-stained fluorescence images with a high quality. Applied to investigate B cell spreading on immobilized antibodies, the images allow for evaluations based on single cells. But manual assessment of large cell numbers is a tedious, error-prone and time consuming task. The application of automated image processing and analysis algorithms is strongly required to solve this task in adequate amount of time.

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Materials and Methods

For the investigation of B cell spreading, the microscope slides with 8 wells were coated with either αBCR (rat anti BCR monoclonal) or αCD19 (rat anti CD19 monoclonal).

F-actin was specifically stained intracellularly with Phalloidin-Rhodamin, nuclei were stained with DAPI (see fig. 1).

For the segmentation of the images a multi-channel approach is applied. First, the nuclei in the DAPI channel are detected and segmented (see fig. 2). Then the cytoskeleton in the F-actin channel is delineated based on nuclei shapes and positions (see fig. 3). Parameters of these algorithms are automatically adapted with respect to a small, hand labeled set of reference images. To capture the effect of cell spreading, the cell area and the cell circularity is calculated for each cell separately.

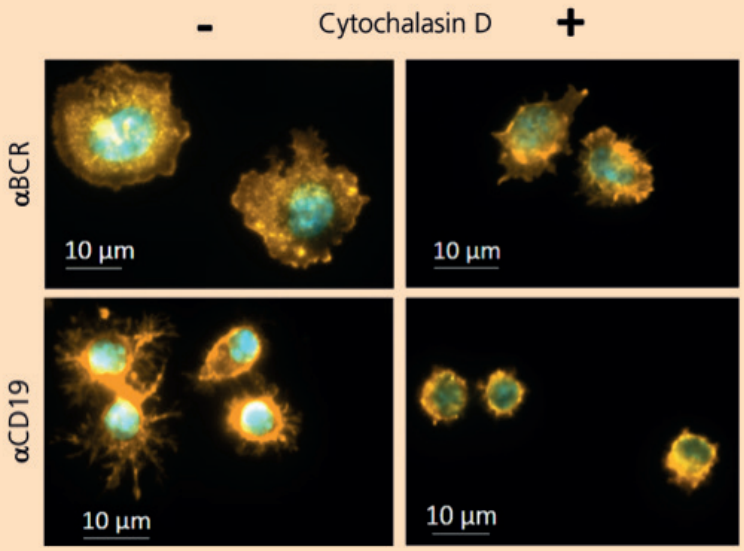

Figure 1: Different cell spreading behavior of LPS activated B cells treated with (right) or without (left) Cytochalasin D on immobilized antibodies BCR or CD19. Immunofluorescent staining was performed for F-Actin (Phalloidin-Rhodamin) and DNA (DAPI).

Results

For nuclei, the performance measured with two-fold cross validation and the combined Jaccard metric is $p_n = 0.73$. For F-actin cytoskeleton, combined Jaccard performance was $p_c = 0.64$. Analysis shows that LPS-activated primary murine B cells attached on glass slides spread significantly less on anti CD19 mAb than on anti BCR mAb as well in absence as in presence of Cytochalasin D (see fig. 4).

Conclusion

Preparation protocol and the proposed algorithm allow the quantification of large numbers of fluorescent labeled and fixed B-cells attached to glass slides. The proposed method allows rapid screening of cytoskeletal effector molecules of mouse mutants suspected to effect cytoskeletal rearrangement.
Besides the current experiment, the applied algorithms can be easily adapted and extended to investigate similar biological experiments.

Reference

Wiesmann, V., Reimer, D., Franz, D., Hüttmayer, H., Mielenz, D., & Wittenberg, T. (2015). Automated high-throughput analysis of B cell spreading on immobilized antibodies with whole slide imaging. Current Directions in Biomedical Engineering, 1(1), 224-227.

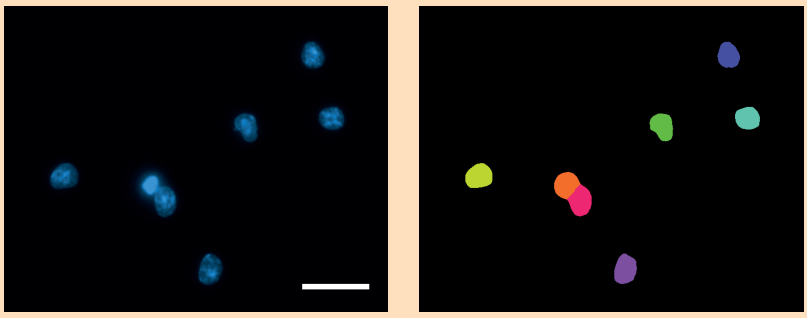

Figure 2: Exemplary segmentation result for nuclei: part of original DAPI image (left); segmentation result (right); scale bar corresponds to 20µm.

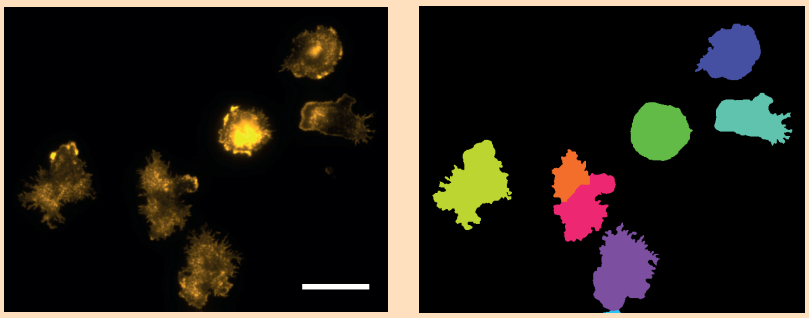

Figure 3: Exemplary segmentation result for F-actin cytoskeleton: brightness adapted part of original Phalloidin-Rhodamin image(left); segmentation result (right); scale bar corresponds to 20µm.

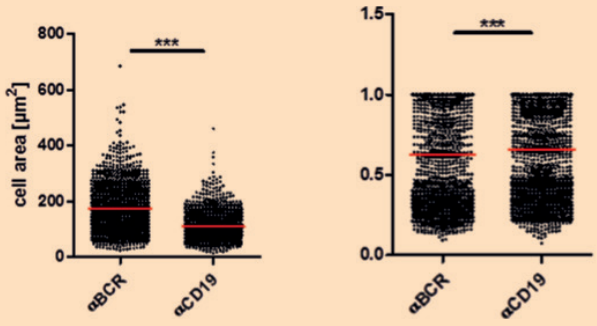

*Figure 4: Measurements depict highly significant results in cell area and circularity between B cells spreading on immobilized BCR or CD19 antibodies. Significance according to Mann-Whitney-U test (***p= 0.0001 - 0.001). Red line represents the mean.*