

AUTOMATIC SINGLE-CELL-BASED ANALYSIS OF siRNA SCREENS BASED ON IMMUNOFLUORESCENCE MICROSCOPY

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INTRODUCTION: We have developed an siRNA-based screening system for the identification of cellular genes potentially involved in virus entry and replication [1]. The screening system is based on cell arrays and high-throughput (HT) fluorescence microscopy. Important issue is the efficient analysis of large number of images.

MATERIALS & METHODS: Our image analysis approach comprises: a novel gradient-based thresholding scheme for cell nuclei segmentation, quantification of virus replication level in the neighbourhood of segmented cell nuclei, localization of regions with transfected cells, cell classification by infection status, and image quality assessment. Image quality assessment includes the detection of out-of-focus images, oversaturated pixels, and uneven cell density. The approach has been applied to more than 55.000 images of cells infected by hepatitis C or dengue virus.

RESULTS: Prior to applying the overall approach, we compared the results of our nucleus segmentation approach with that of adaptive thresholding (AT) as well as AT combined with a technique for the separation of cell clusters (AT-SCC). Our approach correctly segments more than 97.1% of all nuclei, whereas AT only about 78.5% and AT-SCC about 95.8%. Our scheme does not require post processing steps, and therefore it is about twice as fast as AT-SCC on the same hardware. We also evaluated the algorithm for the detection of out-of-focus images. 99% of in-focus images were correctly recognized and all out-of-focus images were rejected. The results of overall approach showed a good agreement with reduced infection rates in positive controls as compared to the infections rates in negative controls. Reduced infection rates were also observed for other siRNAs targeting, e.g., cellular genes involved in viral infection.

CONCLUSIONS: The overall approach allows to fully automatically quantify a large number of images on single cell basis. The experimental results encourage the application to screens from further HT experiments.

[1] Matula P, Kumar A, Wörz I, Erfle H, Bartenschlager R, Eils R, Rohr K. "Single-cell-based image analysis of high-throughput cell array screens for quantification of viral infection," *Cytometry Part A*, (2009), in press.