Streamlined Antibody-Binding Assay Using High-Throughput Microscopy

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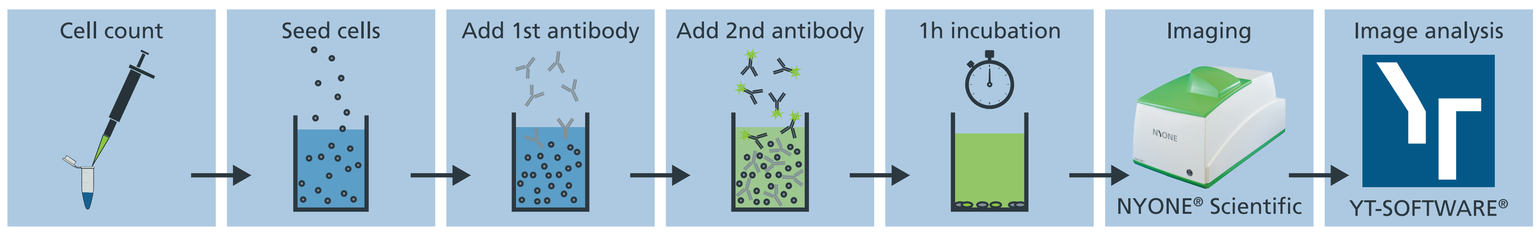
Introduction

The production of high-affinity, antigen-specific antibodies is crucial for various purposes in research, diagnosis, and therapy. Antibody-producing cells are commonly generated using hybridoma technology and single cell cloning (SCC). However, the selection of high-producing clones is challenging and requires a reliable method. The assay should need minimal amounts of antibody sample and no wash steps. Therefore, we aimed to develop a simple homogeneous antibody-binding assay using our automated microscope NYONE® Scientific. As a reference model, we used an anti-HER2 antibody with the HER2-expressing breast cancer cell line SK-BR-3 as target cells and HER2-negative MDA-MB-468 cells as control. Cells were seeded into 384-well plates, followed by primary anti-HER2 antibody and fluorescence-labeled secondary antibody. The entire 384-well plate was imaged in less than 6 minutes, and the images were analyzed using the Suspension Cell AB Binding (1F) application of YT-SOFTWARE®.

Benefits

- Screening without plate washing
- Increased reproducibility and accuracy through simple assay design
- Fast imaging for high-throughput screening
- Detection of weak signal with high sensitivity up to 1 ng/mL
- Same device and software as for single cell cloning

Method

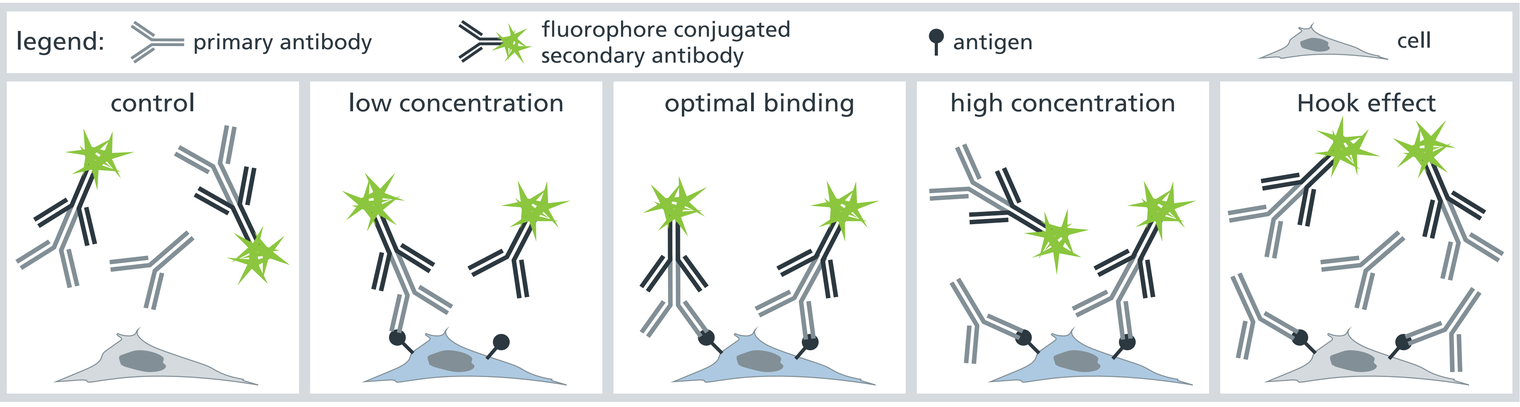


Application

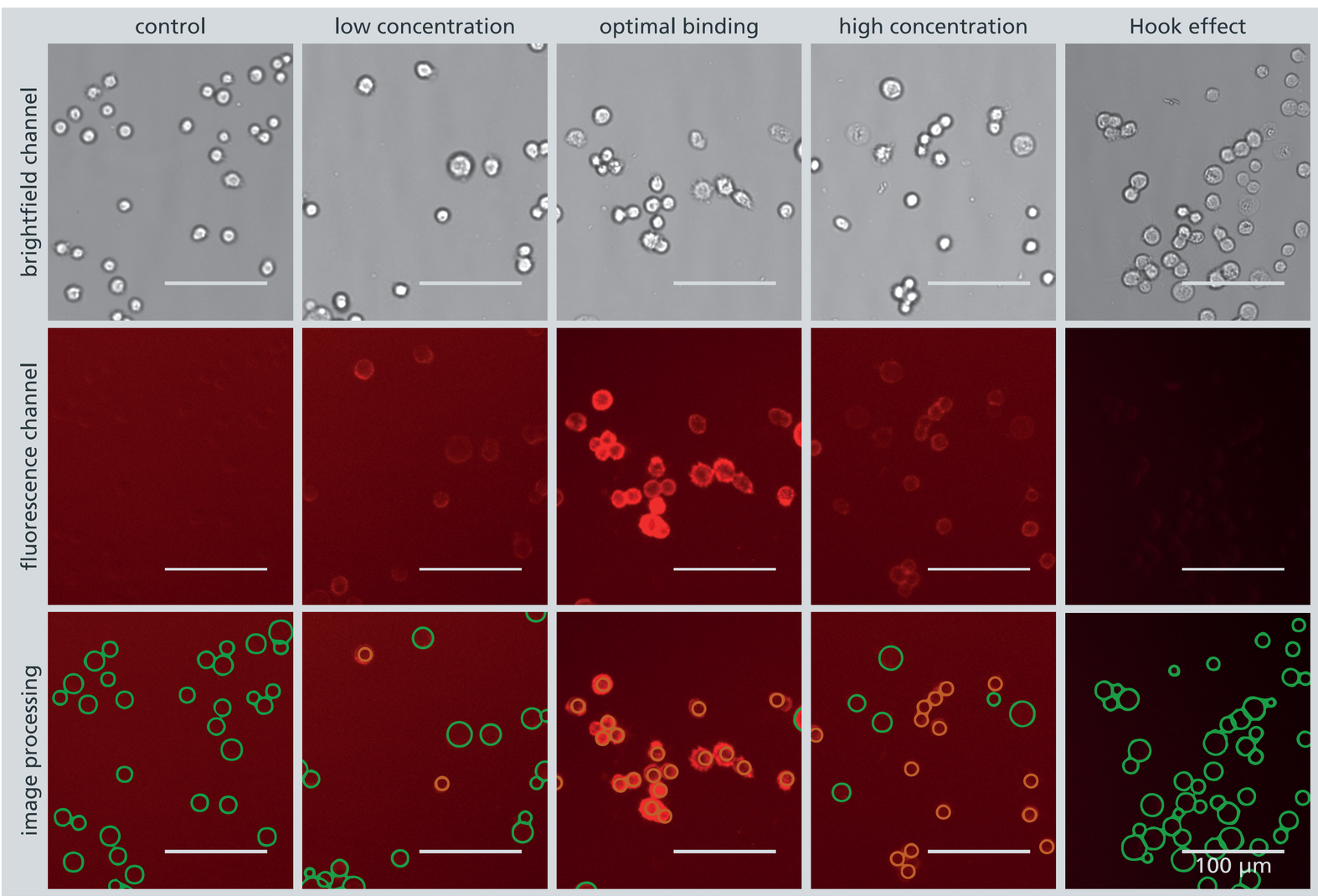
Suspension Cell AB Binding (1F)

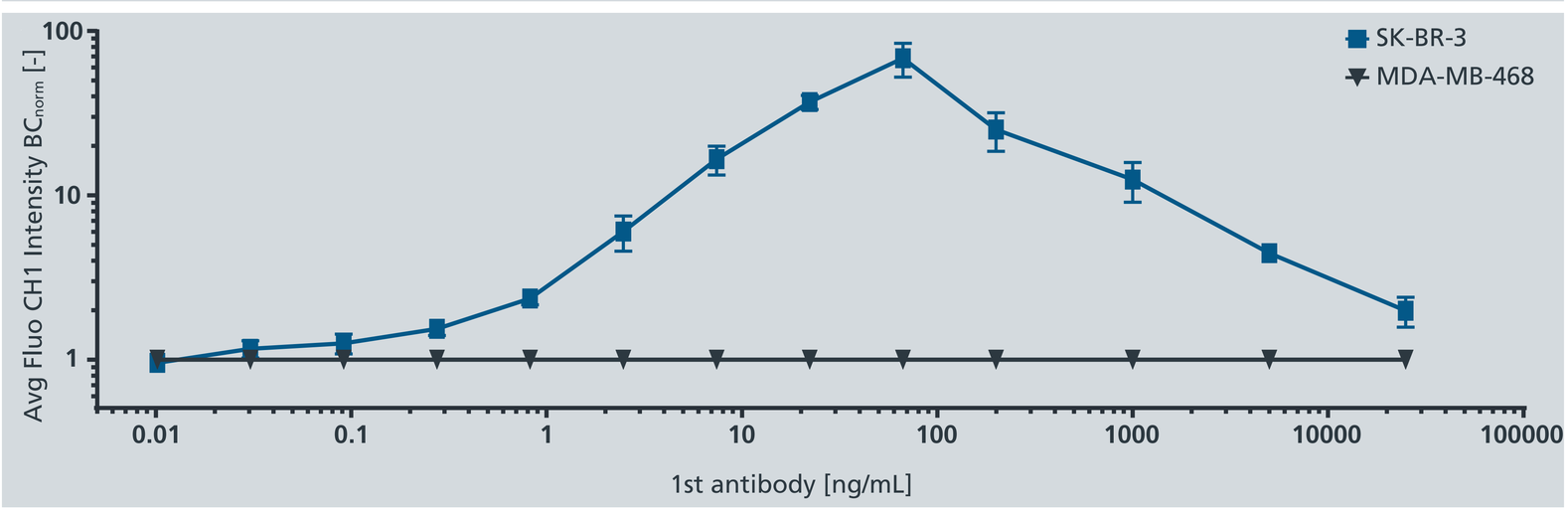
detection of the cells in the brightfield channel → determination of fluorescence intensity within the cells (background corrected) → labeling of fluorescence-positive cells

Principle



Results





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Institut für Experimentelle Tumorforschung