Quantification of NF-κB translocation by image cytometry. Comparative analysis of adhered and suspended MDA-MB-231 cells.

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The spatial distribution of a protein within a cell can provide researchers with valuable information about the active state as well as the function of a protein. In unstimulated cells, the NF-κB protein is located cytosolic in a complex with the inhibitor IκBα. In response to cytokine stimulation IκBα dissociates from NF-κB, thereby unmasking the nuclear localization signal of NF-κB, which allows nuclear translocation of the transcription factor. This transport of NF-κB into the nucleus is commonly used to quantify the activation of the transcription factor.

Here we use the image cytometer Xcyto® to quantify NF-κB translocation. Xcyto® combines the statistical and numerical power of flow cytometry with the spatial resolution of microscopy. In this study, we found that accurate quantification of NF-κB translocation is highly dependent on whether the cells are in suspension or adhered to a surface. Thus, direct analysis of cells adhered to their growth support provides a significantly increased sensitivity to nuclear translocation compared to cells brought into suspension.

1 Experimental setup for NF-κB translocation quantification in MDA-MB-231 cells.

Figure 1. A: Following treatment with TNF-α or solvent control, cells were either left adhered on a glass surface or brought into suspension by trypsinization and then NBF fixed and immunostained for the NF-κB subunit p65. B: Image representation of adherent cells treated with solvent control or 10 ng/ml TNF-α for 30 minutes. C: Image representation of cells in suspension treated with solvent control or 10 ng/ml TNF-α for 30 minutes. All displayed images were acquired using 20X magnification. 

2 Quantification of NF-κB translocation using similarity score.

Figure 2. The Xcyto® system can calculate a similarity score based on the degree of pixel similarity between two different channels. Thus, we measured the degree of co-localization between the NF-κB channel and the DAPI channel on the whole cell level. A high similarity score indicates high correlation between the DAPI channel and the NF-κB channel and therefore high degree of NF-κB translocation. We adjusted the gate (D1) in the histogram of the control sample (left) so it would result in a positive population of 5%, thereafter we copied the gate into the histogram of the treated sample (right). All displayed images were acquired using 20X magnification.

3 The effects of sample preparation and magnification on quantification of NF-κB translocation.

Figure 3. The average percent positive population for NF-κB translocation for adherent and suspension cells analyzed at 4X and 20X magnification. § denotes significant difference from control sample. * denotes significant difference from 4X suspension.

4 Alternative methods to quantify translocation of NF-κB.

Figure 4. A: Quantification of NF-κB translocation by Nuc/Cyto (Nuc/Cyto) ratio, using the information about NF-κB intensity in the nucleus and cytoplasm levels, provided by the Xcyto® system, we quantified translocation by calculation of the ratio between the nucleus and the cytoplasm intensity of NF-κB-CF488A - the Nuc/Cyto ratio. Error bars show standard deviation. * denotes significant difference from control sample. § denotes significant difference from adherent sample with same magnification. B: Quantification of NF-κB translocation by subtracting nucleus and cytoplasm mean intensity (NRn/Cyto). The absolute difference between the control and treated NF-κB values is displayed. Error bars show standard deviation. * denotes significant difference from adherent sample with same magnification. § denotes significant difference from same sample preparation imaged with 4X magnification.