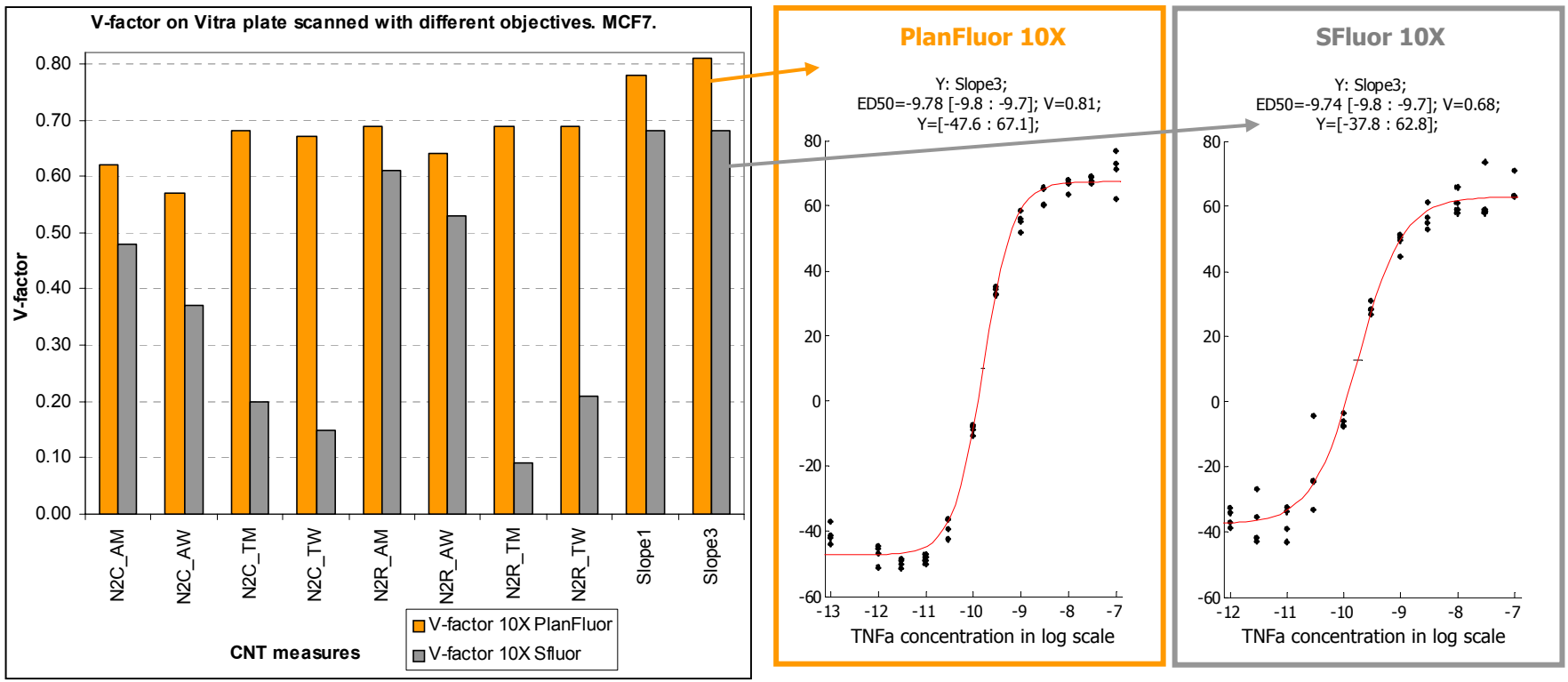
Depth of Field and Thickness of Cells in a Widefield Imaging System (1)

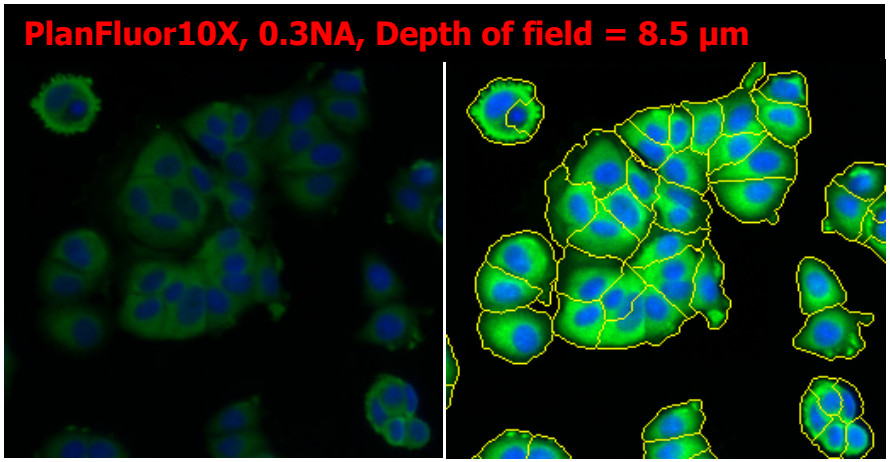
Dependency of assay quality on the depth of field was studied on cytoplasm to nucleus translocation assay by comparing objectives with the same magnification but different numerical aperture. Difference in image brightness was equalized by adjusting integration times. The Vitra plate from the SBS image library was scanned two times: with a PlanFluor 10X 0.3NA objective and with a SFluor 10X 0.5NA objective (Nikon). PlanFluor objective gives better results for all measures. SFluor objective shows reduced dynamic range and increased variation among replica wells. Measures based on total amount of fluorescence (as opposed to average) suffer the most.

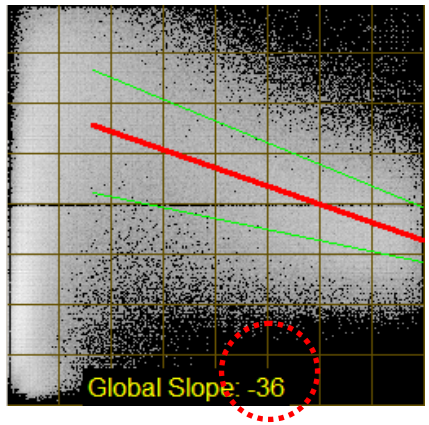


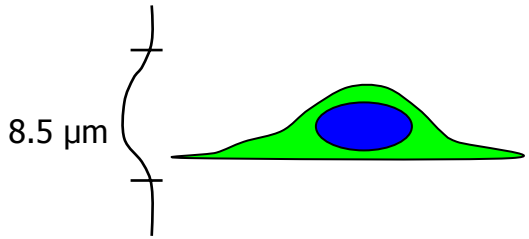
For algorithm description see:

Comparison of several classes of algorithms for cytoplasm to nucleus translocation at www.ravkin.net

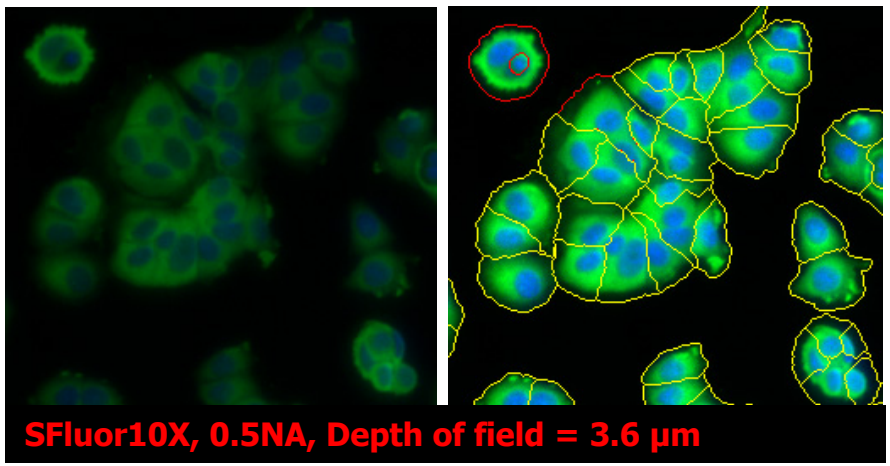
Depth of Field and Thickness of Cells (2)

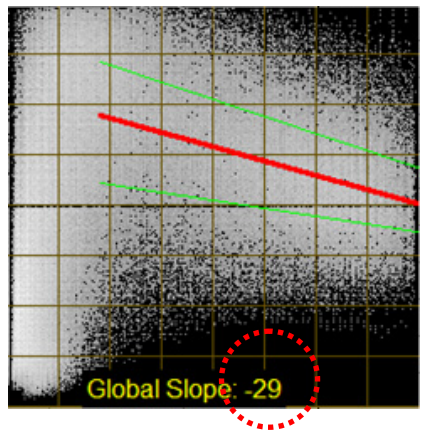


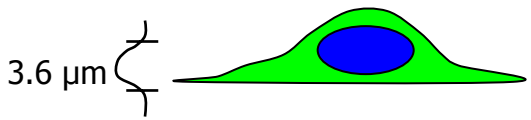




Original | Normalized | 2D distribution | Depth of field and thickness of cells







Our hypothesis is that this result is due to the relative thickness of cells and the depth of field of the two objectives. The depth of field of 0.3NA PlanFluor 10X is about 8.5 µm, and the depth of field of 0.5NA SFluor 10X is about 3.6 µm. The thickness of attached cells is probably between these two values. If the cell is thicker than the depth of field of the objective it appears fuzzy. This makes negative cells look less negative and reduces the dynamic range of the measurements. This also makes focusing more difficult and can increase variability due to inaccurate focusing.