A comparative analysis of standard microtiter plate reading versus imaging in cellular assays.

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Authors: Paul J Bushway, Mark Mercola, Jeffrey H Price

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Public Summary:

Scientific Abstract:
We evaluated the performance of two plate readers (the Beckman Coulter [Fullerton, CA] DTX and the PerkinElmer [Wellesley, MA] EnVision) and a plate imager (the General Electric [Fairfield, CT] IN Cell 1000 Analyzer) in a primary fluorescent cellular screen of 10,000 Molecular Libraries Screening Center Network library compounds for up- and down-regulation of vascular cell adhesion molecule (VCAM)-1, which has been shown to be up-regulated in atherothrombotic vascular disease and is a general indicator of chronic inflammatory disease. Prior to screening, imaging of a twofold, six-step titration of fluorescent cells in a 384-well test plate showed greater consistency, sensitivity, and dynamic range of signal detection curves throughout the detection range, as compared to the plate readers. With the same 384-well test plate, the detection limits for fluorescent protein-labeled cells on the DTX and EnVision instruments were 2,250 and 560 fluorescent cells per well, respectively, as compared to 280 on the IN Cell 1000. During VCAM screening, sensitivity was critical for detection of antagonists, which reduced brightness of the primary immunofluorescence readout; inhibitor controls yielded Z' values of 0.41 and 0.16 for the IN Cell 1000 and EnVision instruments, respectively. The best 1% of small molecule inhibitors from all platforms were visually confirmed using images from the IN Cell 1000. The EnVision and DTX plate readers mutually identified approximately 57% and 21%, respectively, of the VCAM-1 inhibitors visually confirmed in the IN Cell best 1% of inhibitors. Furthermore, the plate reader hits were largely exclusive, with only 6% agreement across all platforms (three hits out of 47). Taken together, the imager outperformed the plate readers at hit detection in this bimodal assay because of superior sensitivity and had the advantage of speeding hit confirmation during post-acquisition analysis.