

Focal Points

Application Note FP-122



Biolmaging Systems

Using Flat Field Correction to Compensate for Shadowing Effect When Imaging Microtiter Plates

To address concerns by many researchers of the parallax effect observed when imaging all 96 wells of a microtiter plate with a CCD camera, we have introduced the Flat Field Correction (FFC) plate. When imaging (fluorescence, chemiluminescence, colorimetric, and bioluminescence) a 96 well plate, a shadowing or reflective effect occurs in the periphery columns and rows of the microtiter plate decreasing signal strength. The wall height of the well optically interferes with the signal along the edges of the microplate whereas the center of the plate does not show this interference. Under some experimental conditions the well height may have an effect on the signal captured from these outlying wells. Unlike mathematical corrections that are applied after image capture, the flat field lens allows full utilization of signal strength prior to image capture resulting in unadulterated data generation.

The wall height, made evident in Figure A, appears as either shadows or bright areas within each microtiter well.

By the addition of the FFC plate (Figure B), the wall height is effectively eliminated by optical parallax correction.

Normal use of the FFC plate includes any application where a luminescent signal is directed up through the microtiter plate.

Place a transparent bottom microplate on top of a UV transilluminator, white light source or any other UVP light transilluminator, and place the Flat Field Correction plate on top the microtiter plate. Alternatively, a black-bottomed microtiter plate can be used for bioluminescent applications. Focus on the center of the plate and acquire the image.

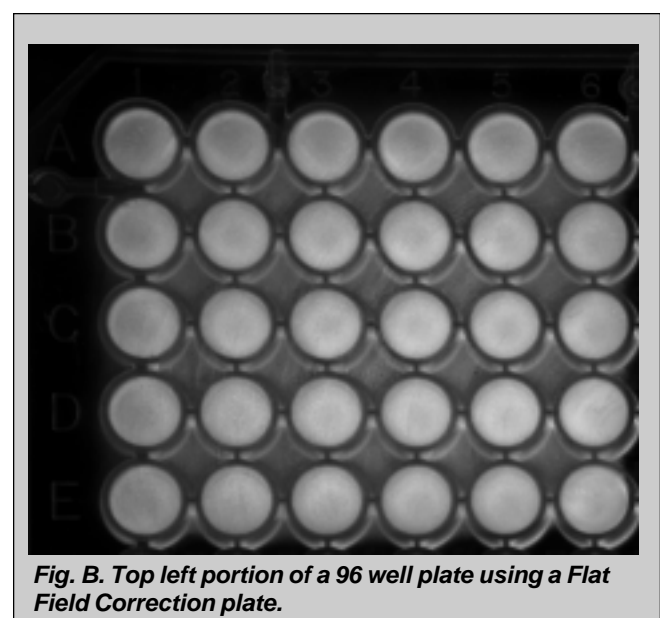
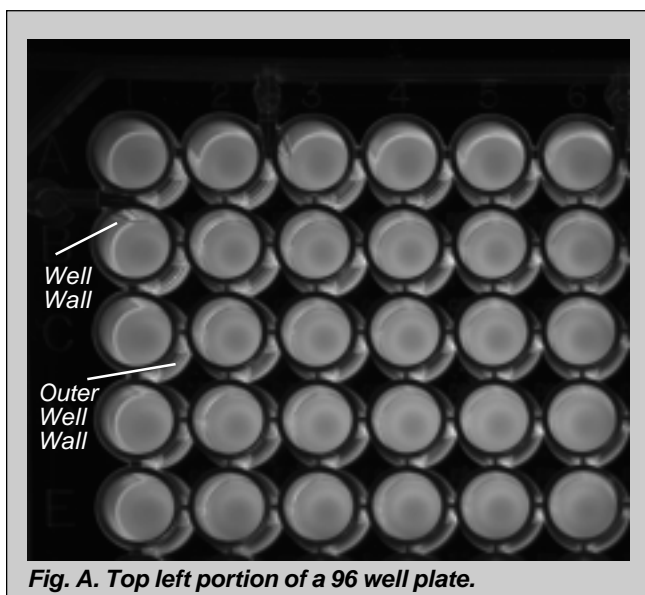
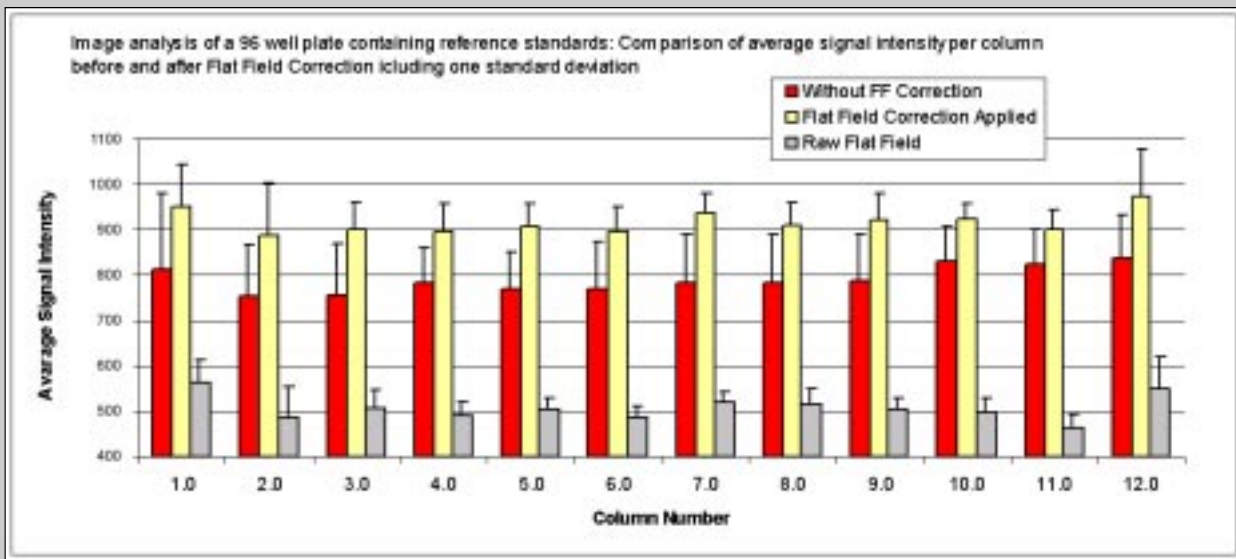
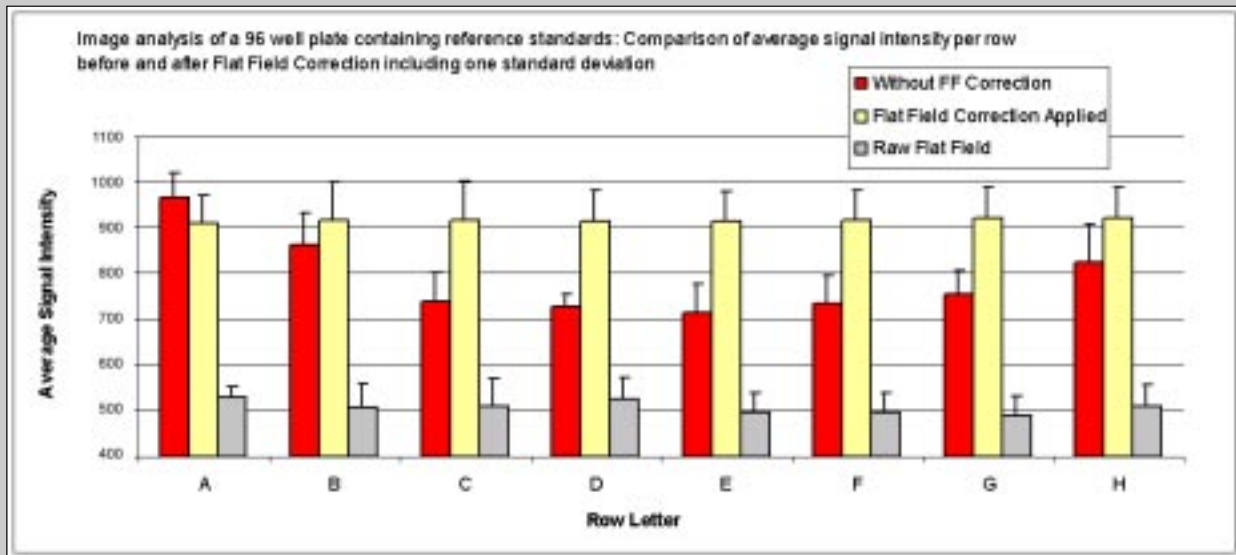


Image analysis of a reference plate before and after the Field correction:

A 96 well microplate containing the luminescent standard LumiQuant was imaged using a Bioimaging System; both with and without the Flat Field Correction plate. The image was acquired using a UV 302nm transilluminator light source and a SYBR gold filter. The images were captured with and without the field correction plate. In the graphs below, the *Raw Flat Field* signal was increased using

LabWorks software to levels (*Flat Field Correction Applied*) near or equal to those prior to Flat Field Correction (*Without Flat Field Correction*). A comparison was made between row and column signal intensity averages showing the effect of parallax without the leveling effect of the Flat Field Correction plate.

Overall the FFC Plate is used to obtain accurate data by eliminating parallax effect and decreasing standard deviations and inter-well variance.



ORDERING INFORMATION

Flat Field Correction Plate Part Number 98-0070-02
For more information or to order a FFC plate, contact UVP's

Biolmaging Systems Specialists at (800) 452-6788 / (909) 946-3197 in the US or in Europe call +44(0)1223-420022.



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