

# Flat Field Correction

## *Correction for Uneven Illumination*

Even under the best of imaging conditions, the illumination across a field of cells isn't quite uniform. This is due to imperfections (i.e. slight misalignments, marks, dust, and additional physical properties) contributed by each optical element (bulb, filters, mirrors, objectives) within a light path.

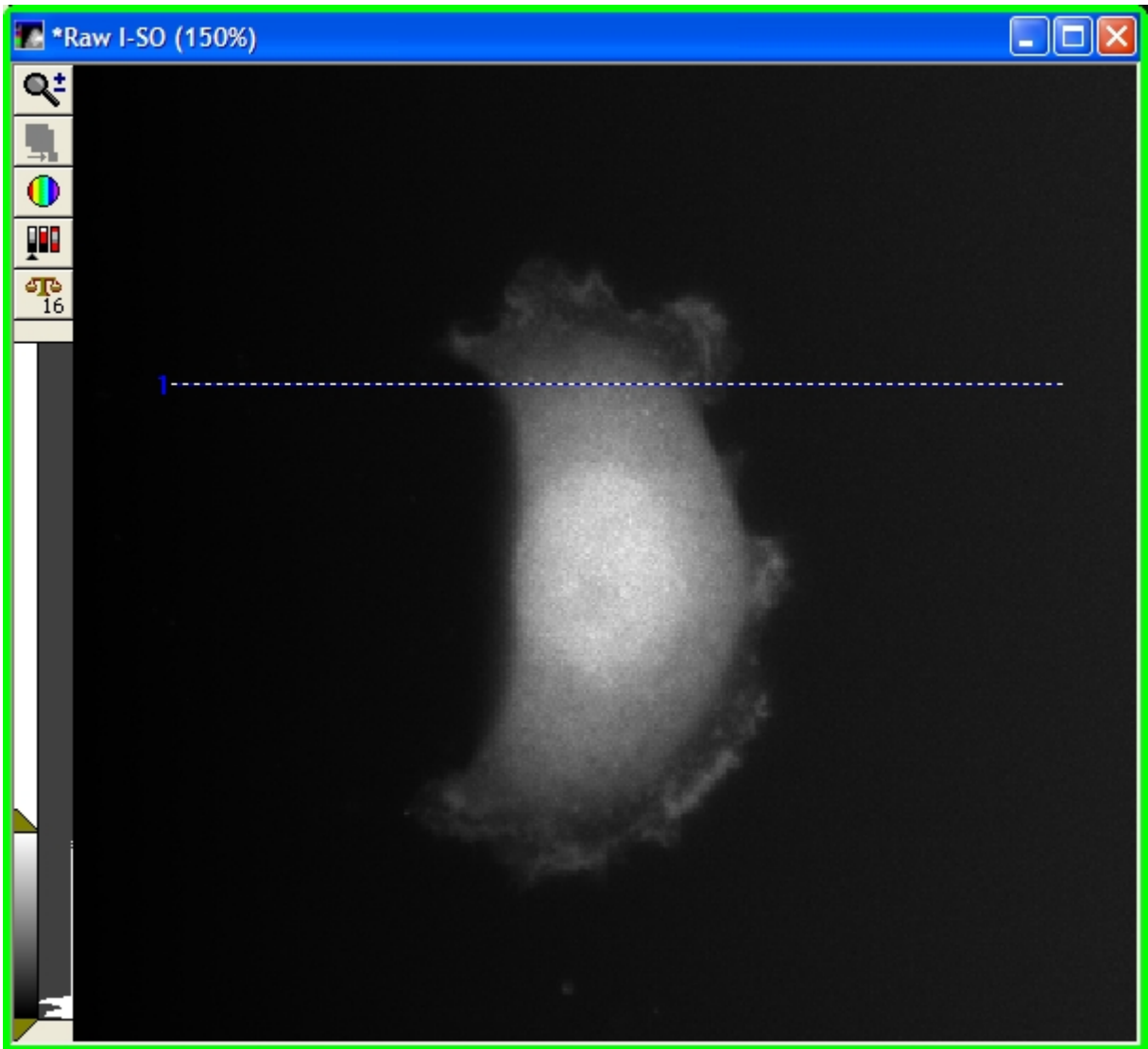
Flat Field Correction is a commonly used approach for this problem. For Flat Field Correction (also called Shading Correction), an image of a Blank field (containing no cellular or fluorescent material) is collected for each channel (I-SO and EGFP) using the identical exposure times and acquisition settings as if one were collecting images of cells. The "blank" field should be an empty area of a coverslip in the same focal plane as the cells.

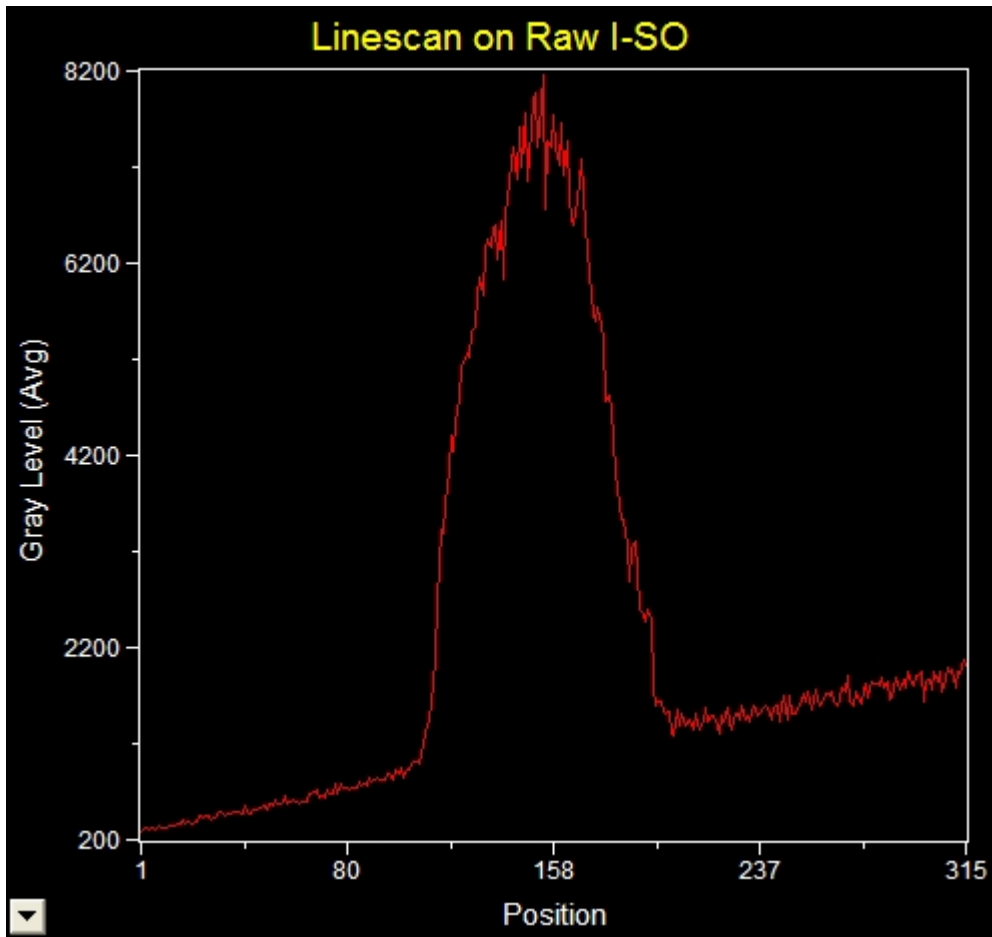
Flat field adjustment is then simple division, on a pixel-per-pixel basis, of each raw image by its conjugate blank field with rescaling.

$$\text{Shading\_Corrected\_Image} = (\text{Raw Image} \times 1000) / (\text{Blank\_Image})$$

## ***Considerations:***

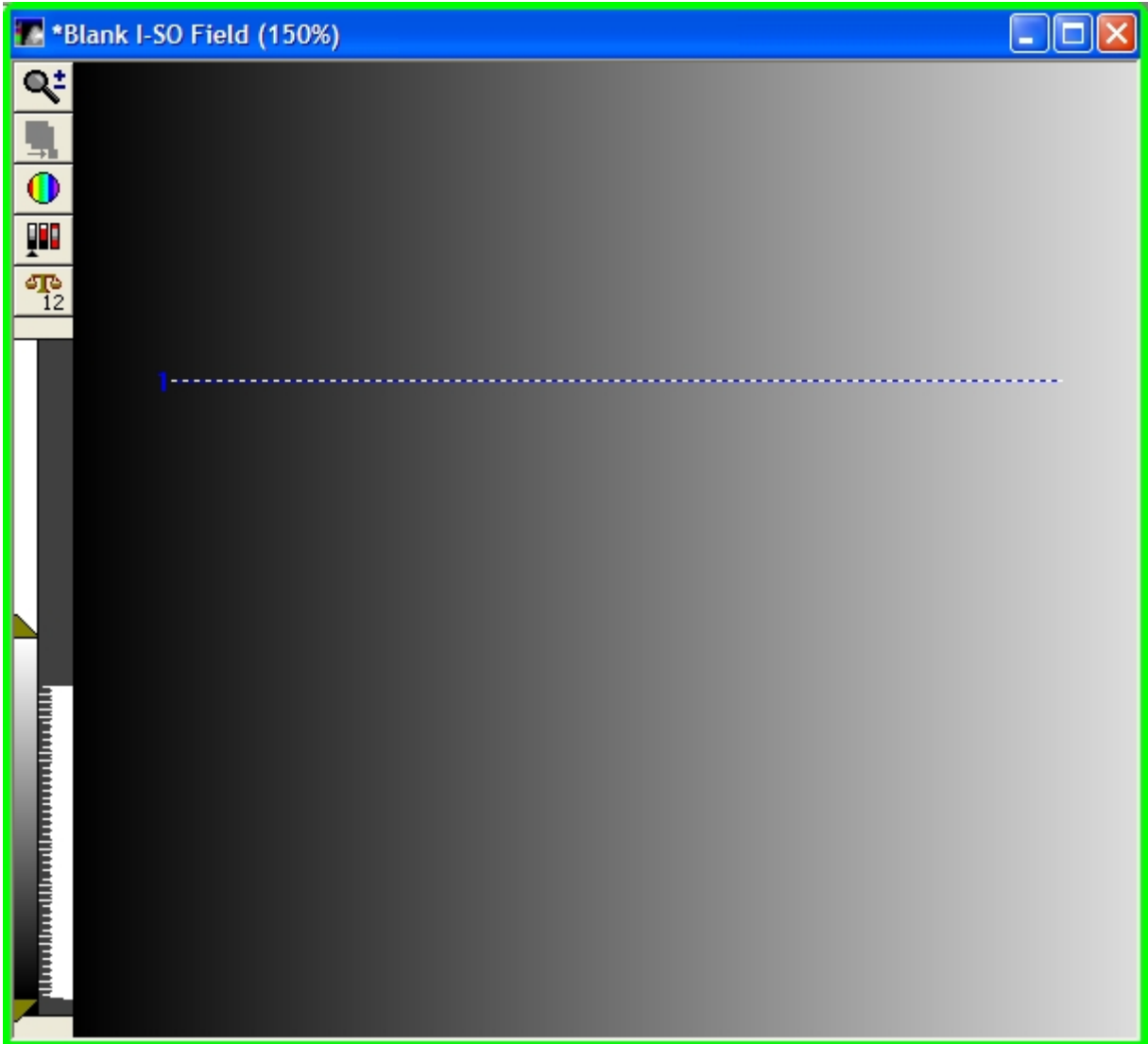
- Minimize Uneven illumination by Aligning the Fluorescent Bulb (Prior to Image Acquisition)
- Shading-Correction (Blank field) Images should not contain images of Fluorescing material (i.e. cellular debris)
- Shading Correction Images should be inspected and compared to Raw Images to ensure they have similar intensity values (in non-cell areas)
- In Flat Field-corrected Images the cell-free regions should be nearly uniform; however, their intensity values will remain above zero
- Collect a new set of Correction images for each coverslip
- Collect a new set of Correction Images each time an optical component is selected or aligned
- All Shading-corrected images should be rescaled by the same factor (i.e "1000"). This is because Floating point errors, due to rounding off algorithms, which can be introduced during Mathematical manipulation of digital images by Image Processing Software.

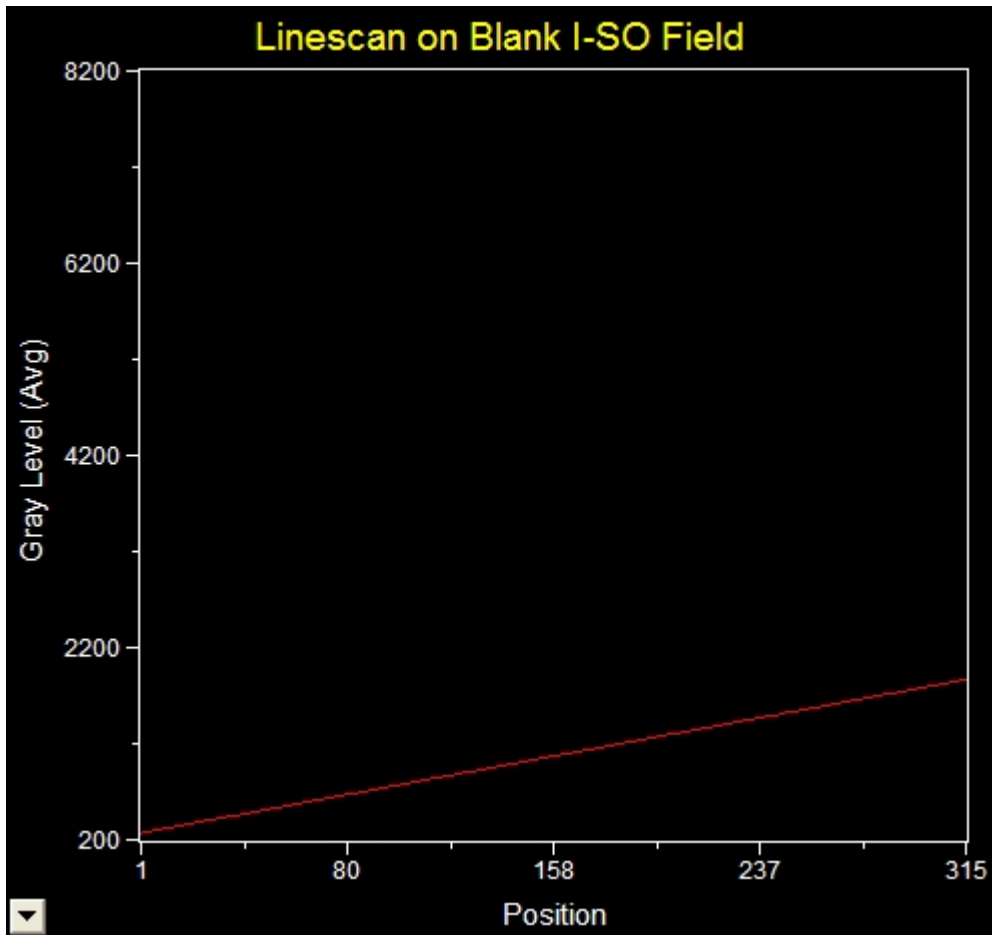




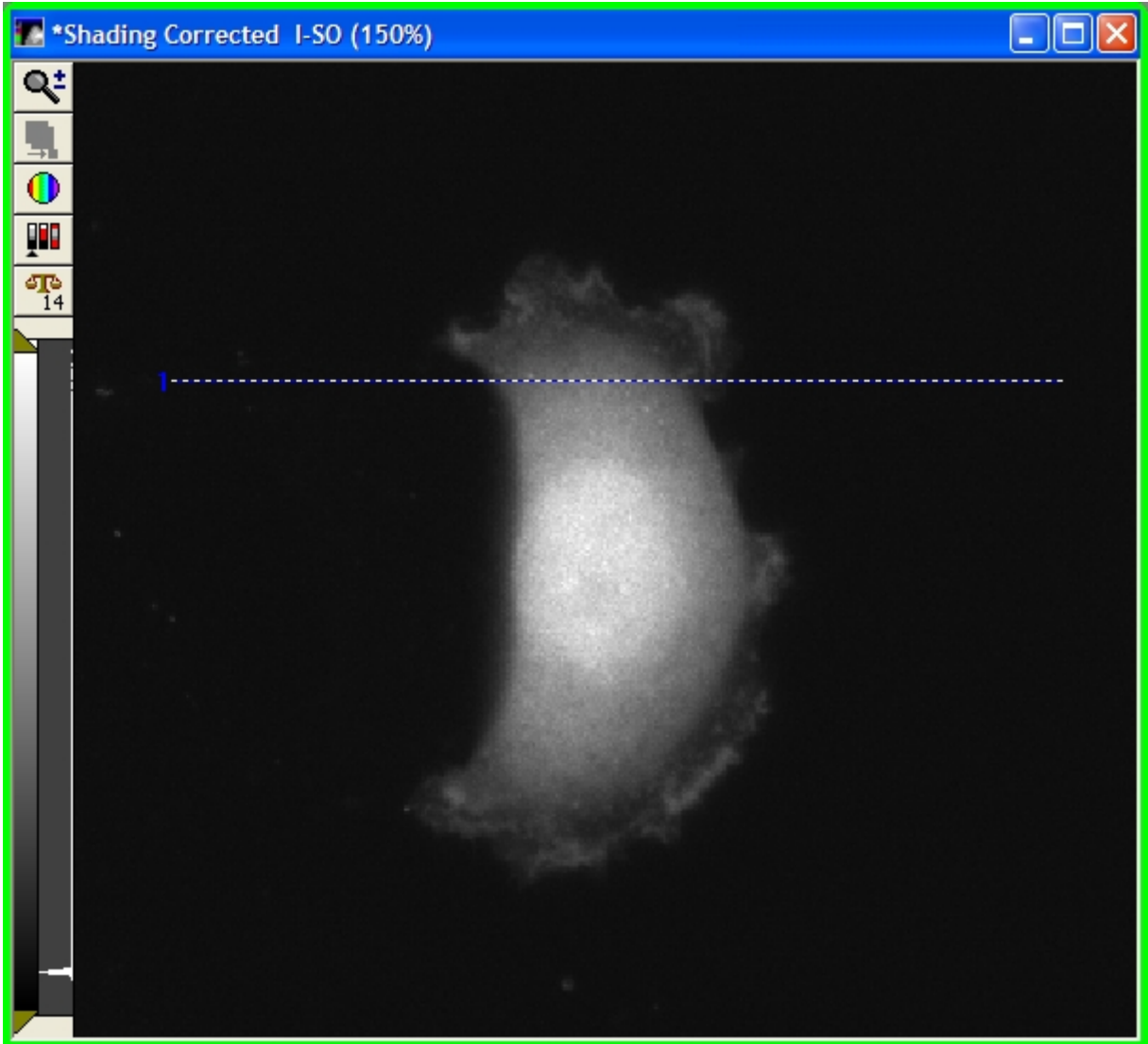
Left: **The untreated I-SO image has uneven shading.**

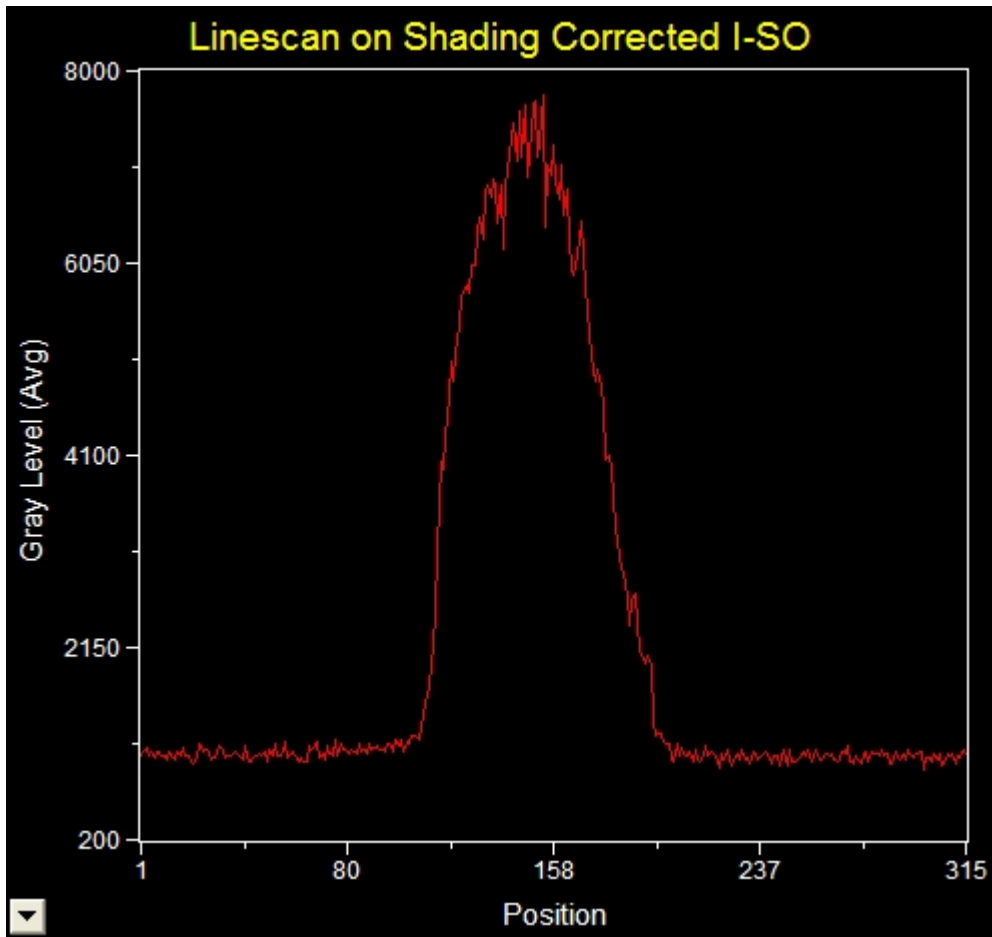
Right: **A graph of a linescan of I-SO's intensity values (dotted line) show a gradient from left to right**





Left: A blank field collected for shade correction  
Right: Intensity values, from linescan (dotted line) are shown in the graph.  
The background values in this example again increase from left to right.





**Left** The Shade-Corrected I-SO image was created using the two images above.  
**Right:** The background values, although above zero, now are now fairly even across the non- cellular regions of the image. Contributions to cell intensity resulting from uneven illumination have been compensated.