

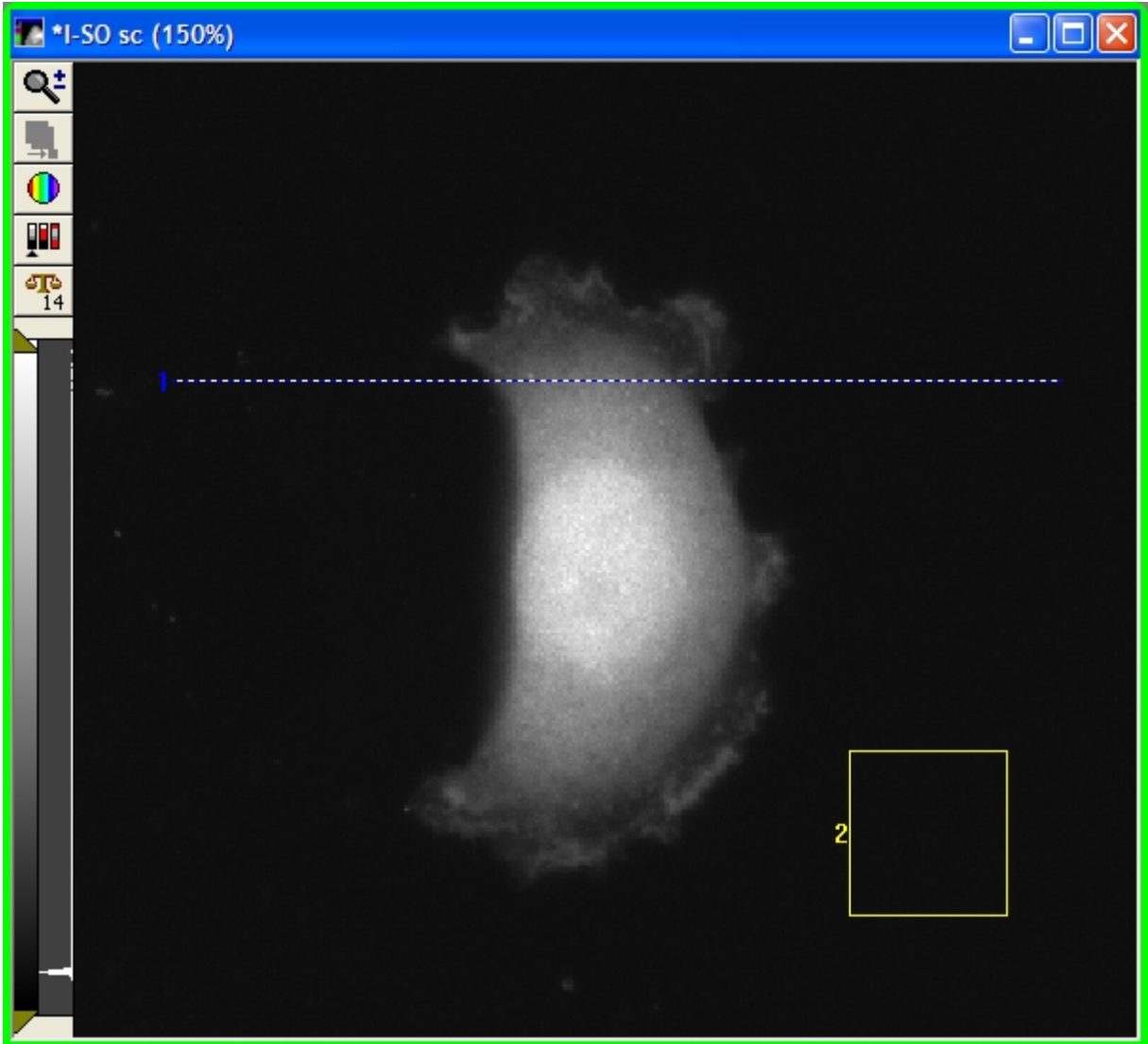
Background Subtraction

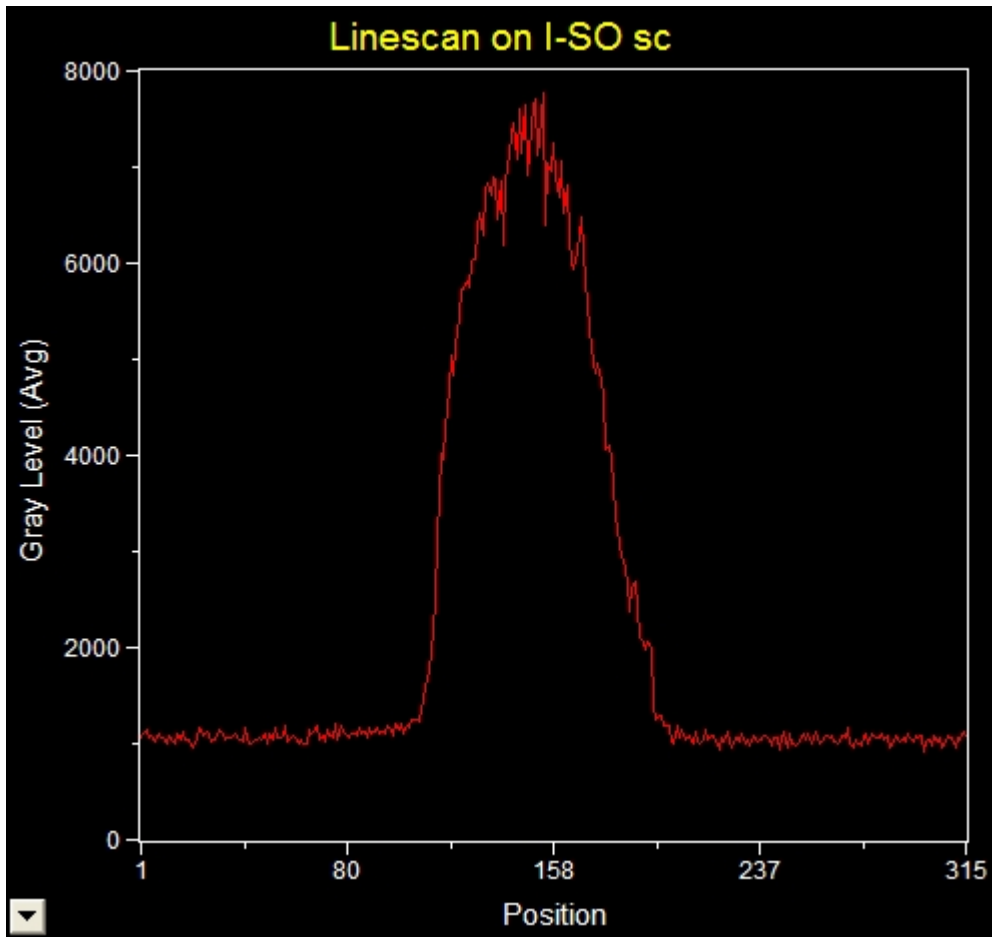
Background Subtraction

Background subtraction is performed by selecting a non-cellular Region of Interest (ROI) for each image or image set and then subtracting the ROI's average pixel intensity value from each pixel of the (shading-corrected) image.

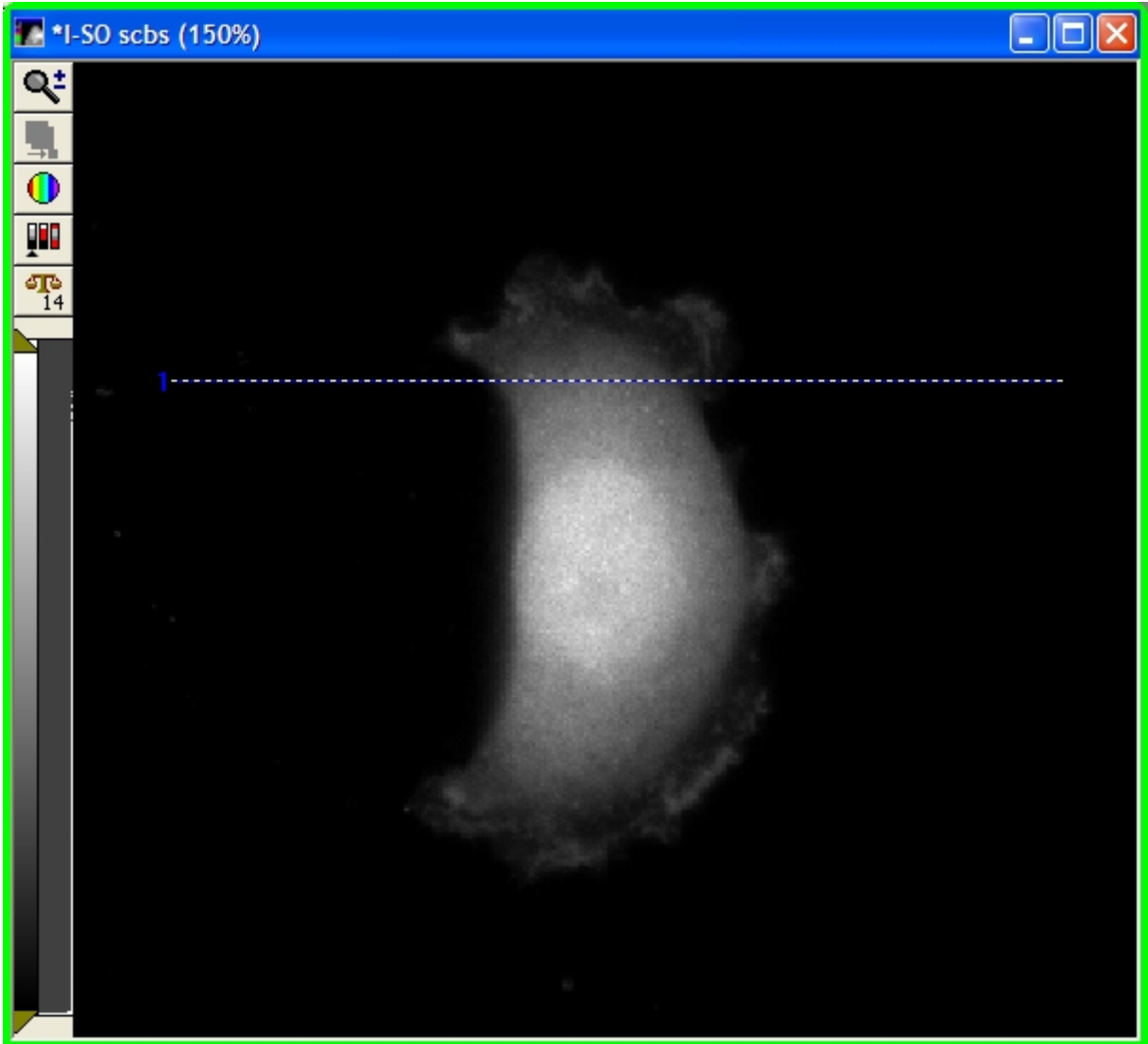
Considerations:

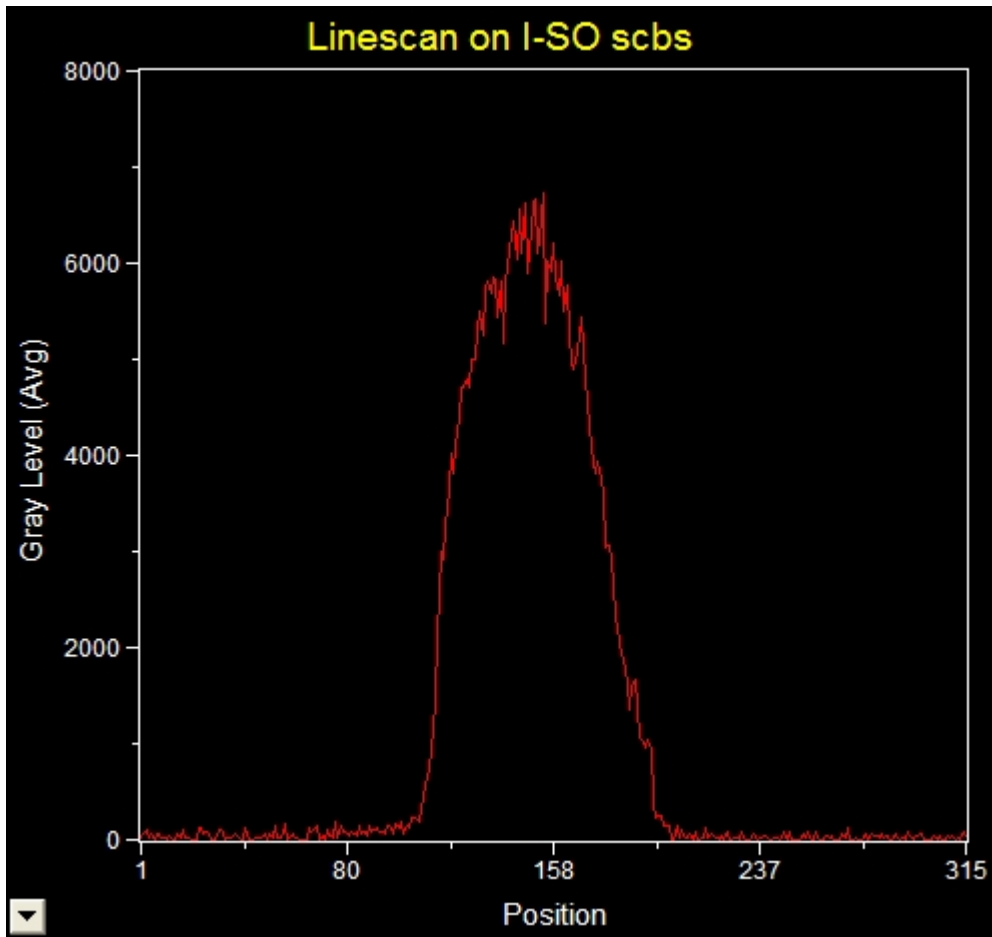
- The Region of Interest (ROI #2 is the yellow square in image below) should be near the object(s) of interest
- The Background value should be calculated on a plane-by-plane basis.
- Care should be taken to ensure that debris did not float through the ROI during the experiment (that is, scan through all planes while selecting ROI)
- If the Background ROI's intensity values exceed those of a cell, then the background-corrected cell values for those areas will be set to "0".





Left: The Shade-Corrected I-SO image (I-SOsc) has an even background.
Right: However its background intensity values (dotted line ROI (Left)) are above zero (Right).





Left: I-SOscbs was created by subtracting a background intensity value (as a constant) for each pixel of I-SOsc.

Right: The intensity values of the cellular region have been normalized to background and are nearly uniform and near-zero.