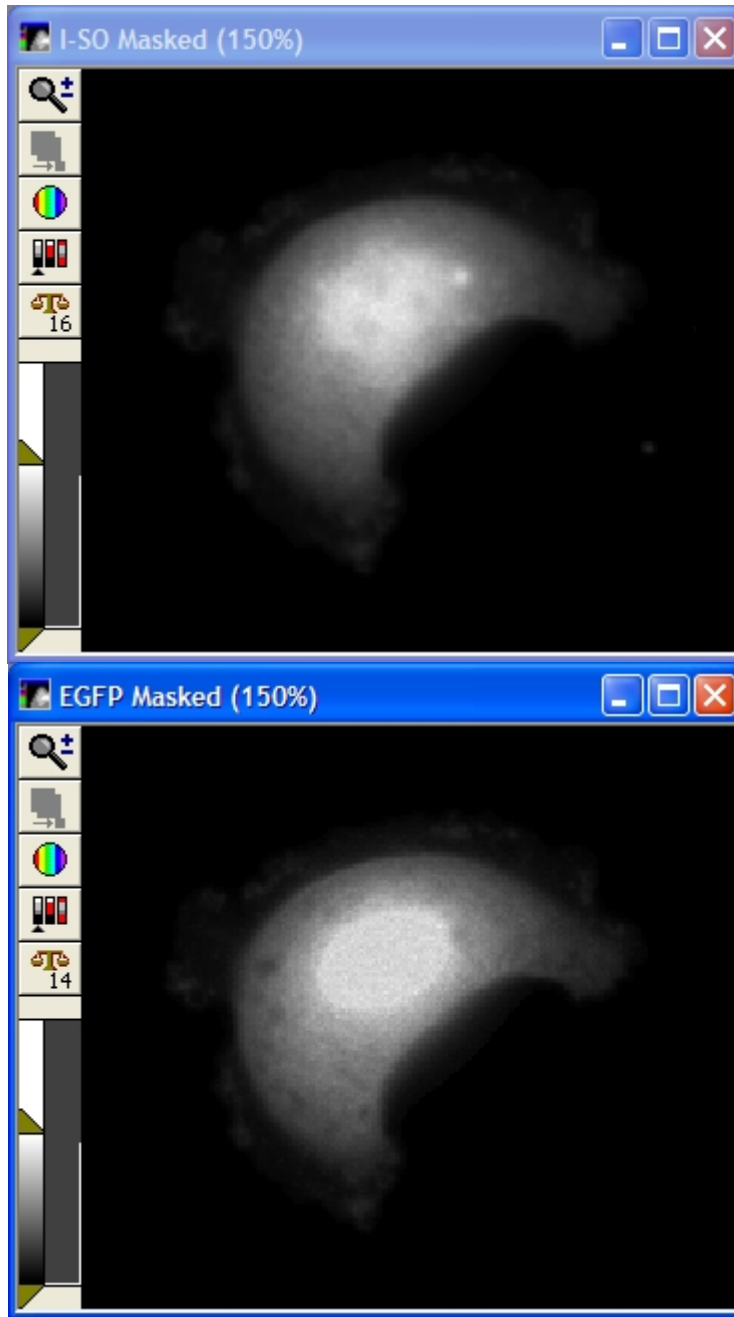


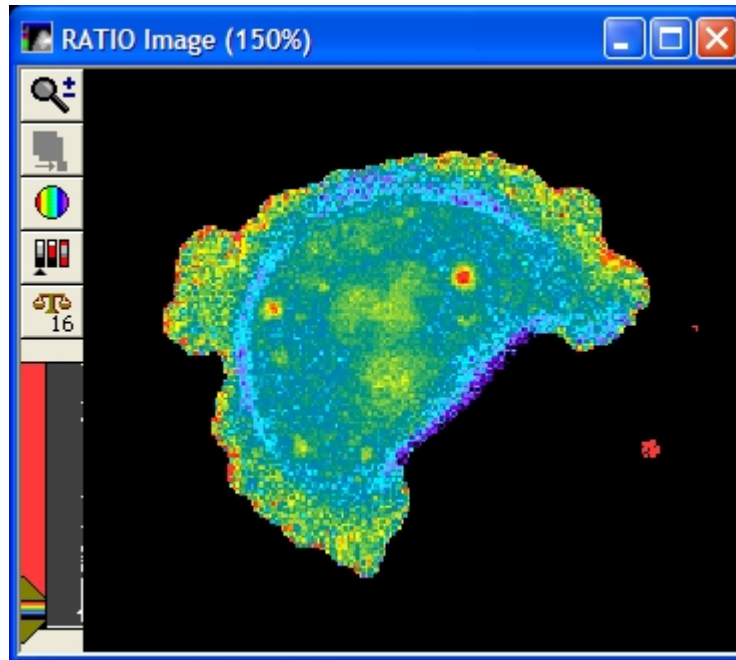
Ratio Images

A Ratio image is created by dividing a corrected (uneven illumination, background-subtracted, photo bleaching, registered, and masked) I-SO image by the corrected EGFP image on a pixel by pixel basis and rescaling. We typically map the resultant ratio image to a pseudocolor Look Up Table which is adjusted so that the high intensity regions are red and yellow and the non-cellular background regions are black. The high intensity values for a Ratio image correlate with the activated Cdc42 enzyme distribution.

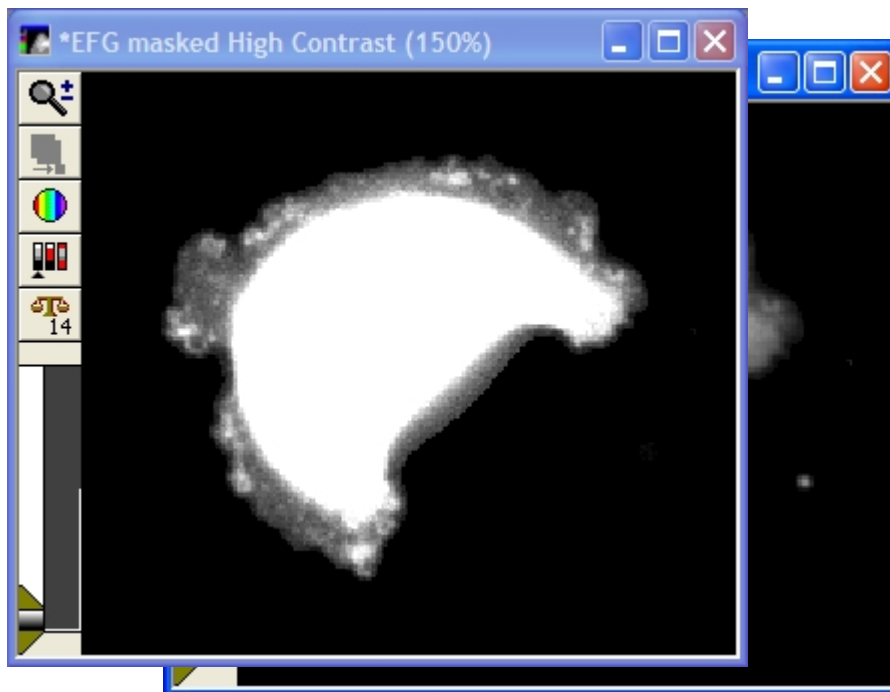
Considerations:

- For Correction of Photobleaching please refer to MatLab Routines found at this site (Hodgson)
- If the images have not been registered correctly there may be uniform high or low values (colors) along the Ratio Cell's edge.
- A Pseudo Color Look Up table helps emphasize intensity differences.
- If comparing two Ratio Images make sure the color LUT refer to the same intensity levels.
- If the I-SO and EGFP images are collected sequentially using a single-camera system, then motion artifacts may be seen in Ratio Images as areas of high or low values (see Hodgson).





Left to Right: Masked I-SO (Numerator Image), Masked EGFP Image (Denominator Image), and Resultant Ratio Image. The Ratio Image shows the areas of Cdc42 activity correlating to a pseudocolor look up table



Left: Increasing the contrast of the I-SO image one can see localized regions correlating to I-SO staining which may be obscured in

the Raw and processed I-SO images (for example Above Left). However, the regions of Cdc42 activity (Red regions in Ratio Image above) do not correspond linearly to regions containing high I-SO! The regions of Cdc42 activity can be visualized as a Ratio of I-SO to EGFP (Right) probe values.