

# Masking Images

## Segmentation, Thresholding and Creating Binary Masks

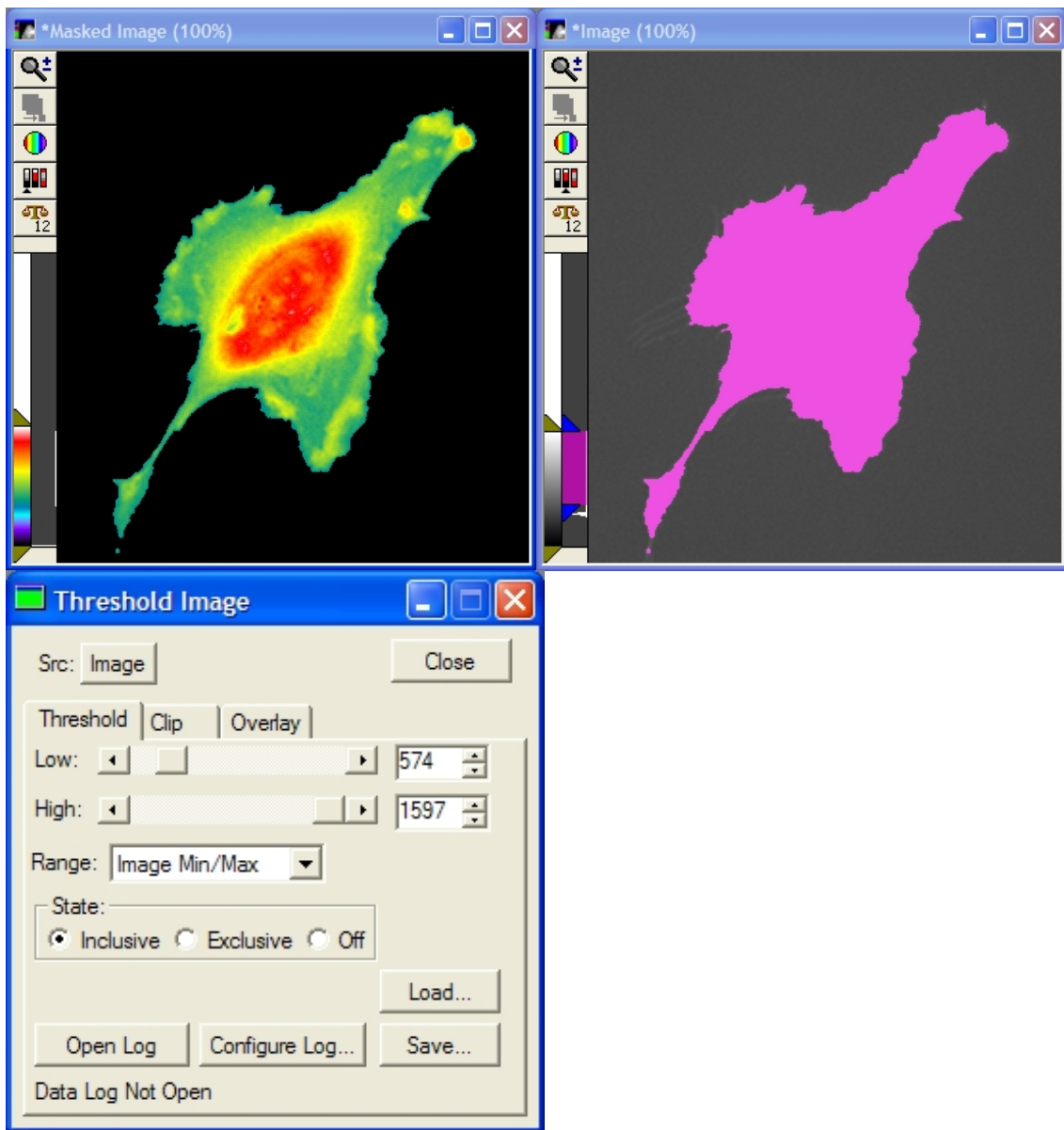
Although Image Masks can be created by manually tracing around a cell's edge, this approach is labor intensive for a single image let alone many multi-plane image stacks. Therefore for image processing, masking is usually a semi-automated endeavor.

Images are first Segmented on the basis of pixel Intensity; this must be done on a plane-by-plane basis for an image stack. In Metamorph the segmented overlay planes are then extracted to a separate image stack for the purpose of creating a Binary Mask. A Binary mask exclusively has pixel intensity values of "0" (background) or "1" (object). Multiplying the I-SO and EGFP Images with their conjugate Binary Mask results in masked-images which only contain values (above zero) for cellular regions.

Masking images allows the Image Processor to discriminate Regions of Interest; these may also be referred to as Areas of Interest or Objects. A second reason to mask images is to prevent artifacts arising from dividing background by background values.

### ***Considerations:***

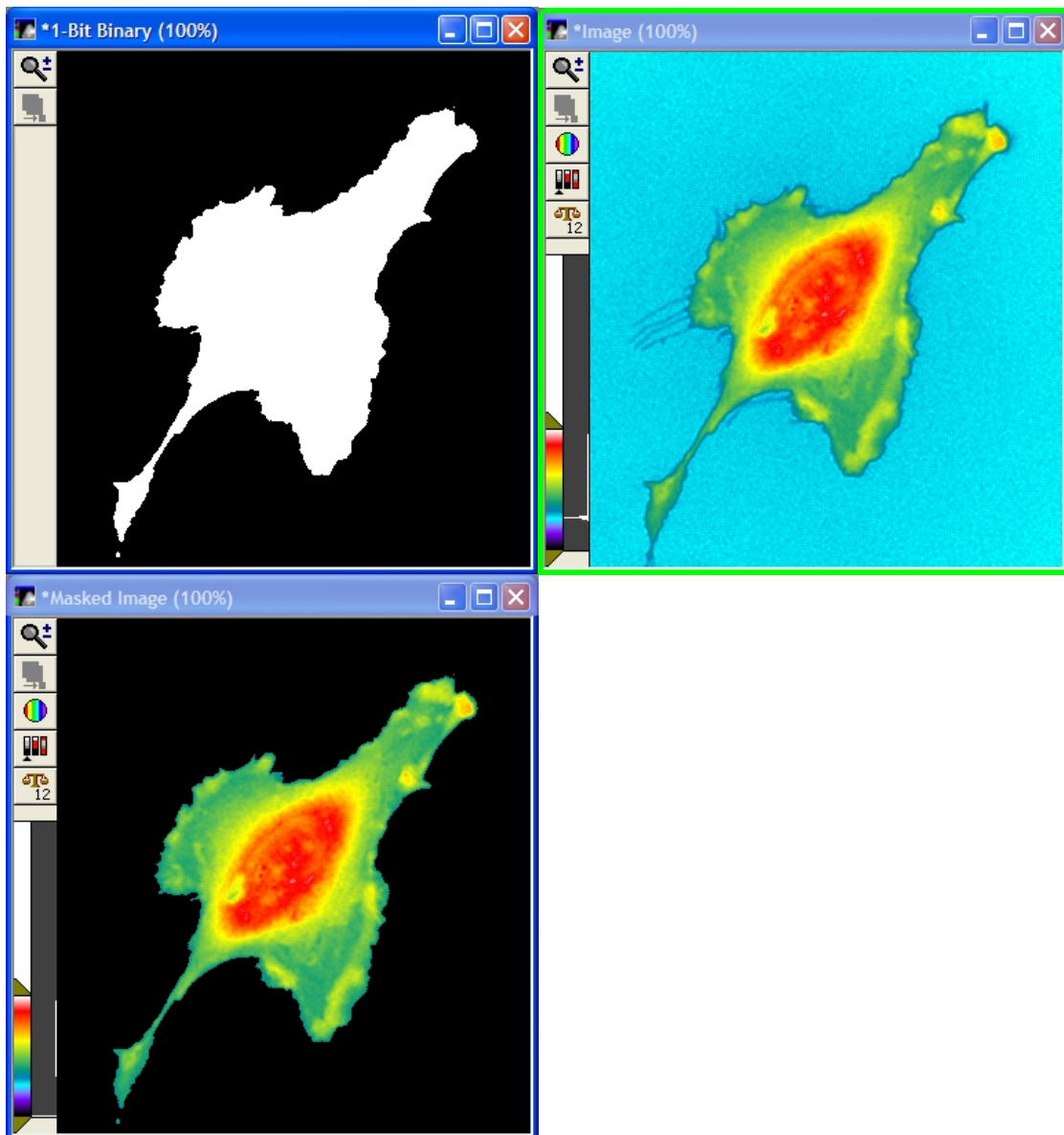
- When Thresholding an Image Stack remember that cells were brighter at earlier time points; inspect the threshold max values for the entire data set.
- Set minimum intensity threshold values (for Segmentation) that do not include much (or any) background regions; this is sometimes difficult.
- Make sure that the resulting mask is a stack of images with the same number of planes (time points) as the image sets.



**Left:** An image to be thresholded as a function of pixel intensity

**Right:** The image has been Segmented and the pink overlay corresponds to the location of the Cell.

**Bottom:** The Thresholding dialog box (Metamorph); a range of intensity values between 574 and 1597 were selected to Segment the Cell.



**Left:** A 1-Bit Mask (having pixel intensity values of 1 (White) and 0 (Black)) was created from Overlay Image (Pink) above.

**Right:** The Image to be masked is pseudo-colored; the background values are very low but are above zero.

**Bottom:** The Masked Image is the product of the 1-Bit Binary Mask (Left) and the Image (Right).