Figure 2-figure supplement 3

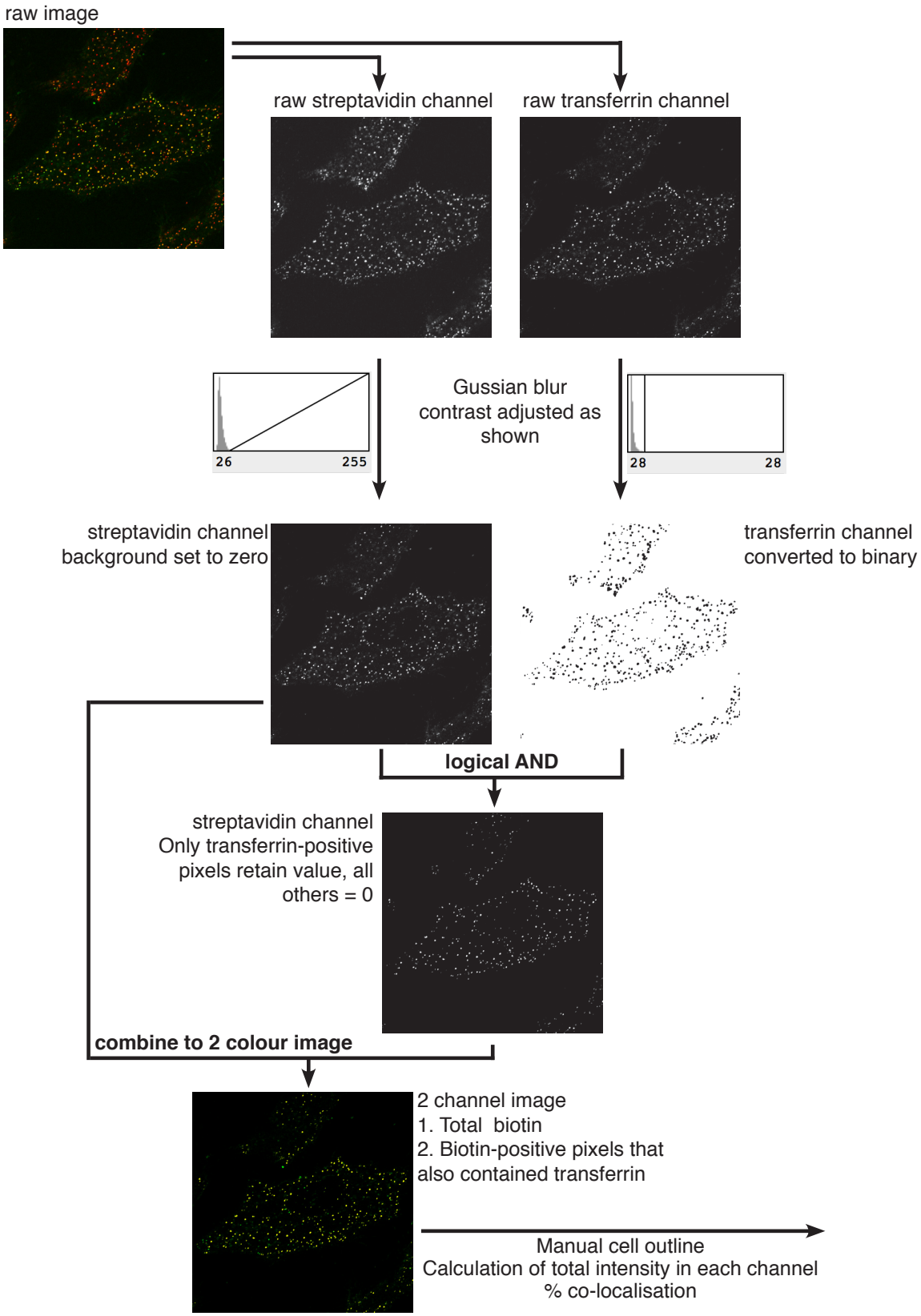

Figure 2-figure supplement 3. Quantification of percent co-localisation. All processing was carried out in Image J. 2 channel raw images were acquired by confocal microscopy. The channels were separated, subjected to Gaussian blur with σ=0.7, and then contrast adjusted using the histogram of pixel intensities as shown. In the streptavidin / biotin channel, the base of the histogram was used to set pixel intensity=0, maximal pixel intensity was not altered. In the transferrin channel, which is used to generate a binary mask, pixel intensity=0 and maximal pixel intensity were both set to the base of the histogram of pixel intensities as shown. Following dilation of positive pixels in the binary mask a logical “AND” operation was carried out to isolate those pixels in the streptavidin channel that also are positive in the transferrin binary mask. This image was combined with the original biotin image in a 2 colour overlay, and manually drawn regions of interest were used to calculate total pixel intensity in the biotin channel, and total pixel intensity in the same channel from transferrin-positive pixels.