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% This script calculates
% 1) The fraction of Ire1 concentrated in foci
% 2) The colocalization index (CI), that scores for the fraction of RNA
% foci colocalizing with Ire1

% This script takes as inputs a GFP ('1g.tif'), and a RFP ('1r.tif') image.
% Derived from the RFP image, a crop containing intracellular, non-ER signal,
% is used as a background image ('1b.tif')
% To use this script, move images to be compared into the same directory as
% this file. Then modify this script to call the image by typing the file
% names (including the extension) between the ' ' in the two lines below.

RNAim = imread('1g.tif');
IRE1im = imread('1r.tif');
BGRNDim = imread('1b.tif');

% Total Ire1 fluorescence is defined as the RFP signal above 1.1 times the
% average intensity of the background image.
% Under non-stress conditions, we never observed pixels with an Ire1 signal
% to exceed a 1.5-fold background threshold. Thus, this threshold value is used
% to score for Ire1 in foci.

IRE1_background = 1.1;
threshold_IRE1_foci = 1.5;

% The smallest Ire1 foci visualized was ~10 square pixels. To prevent
% individual bright background pixels from appearing as Ire1 signal in foci,
% we applied a smooting filter

h = ones(5,5) / 25;
IRE1im=imfilter(IRE1im,h,'replicate');

% For HAC1 fluorescence, the thresholds are with respect to the average
% intensity of the image

RNA_background = 1.5;
threshold_RNA_foci = 2.0;

% TotIRE1 and TotRNA define the Ire and RNA signal in those pixels above the
% background cutoff value.

TotIRE1 = IRE1im - IRE1_background*mean(mean(BGRNDim));
TotRNA = RNAim - RNA_background*mean(mean(RNAim));

% IRE1_in_foci and HAC1_in_foci scores the signal of IRE1 and HAC1 in those pixels
% above the threshold that defines foci
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RNA_in_foci = RNAim - threshold_RNA_foci*mean(mean(RNAim));
IRE1_in_foci = IRE1im - threshold_IRE1_foci*mean(mean(BGRNDim));

% To identify the RNA signal colocalizing with Ire1 foci, we first make
% a mask which includes all pixels containing IRE1 in foci

IRE1_foci_mask = im2bw(single(IRE1_in_foci),1);

% Also, we grow this mask by 3 pixels in each direction (to compensate
% for the foci having moved in the time between acquisition of the red and green
% channels)

h2 = ones(7,7);
IRE1_foci_mask_grown = imfilter(IRE1_foci_mask,h2,'replicate');

% Finally, we define RNA in IRE1 foci as RNA in foci which overlaps the enlarged
% IRE1 foci mask

RNA_in_IRE1_foci = IRE1_foci_mask_grown.*single(RNA_in_foci);

% To calculate the colocalization index (fraction of RNA colocalizing with Ire1 vs
TotRNA), CI

num = mean(mean(single(RNA_in_IRE1_foci)));
denom = mean(mean(single(TotRNA)));

if num == 0
   CI = 0;
else
   CI = num/denom;

end

CI

% To calculate the fraction of Ire in foci

IRE1_in_fociQ = single(TotIRE1).*IRE1_foci_mask;
Fraction_IRE1_in_Foci = mean(mean(IRE1_in_fociQ))/mean(mean(TotIRE1))
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