Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary information:

A rapid and effective vignetting correction for quantitative microscopy

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Supplementary Figure Legends:

Figure S1: Calculation of $CV_I(x,y)$ allows for efficient detection of background information of images in the training set.

Detection of pixels containing only background information using *in silico* generated images analyzed in Figure 1. (A) Representative histograms of the $CV_I(x,y)$ and mean intensity for each pixel across the images of the training set for 70 objects/field and 700 objects/field, respectively. Gaussian fit of the histogram of the $CV_I(x,y)$ that is used for determining the *cutoff* is shown in red. (B) Mapping of pixels with a $CV_I(x,y) < cutoff$. An image representing the average of pixel intensities across the images of the training set is shown (grey) and all pixels identified to contain only background information using the algorithm are indicated in red.

Figure S2: Application of vignetting correction on bright field images.

(A) Bright field images taken from Hela cells grown on plastic substratum imaged using a 60x objective before and after vignetting correction. (B) Pixel intensities along the line indicated by the dotted line in (A) before and after correction.

Figure S3: Application of vignetting correction restores object intensity and variability on artificially distorted images.

Object intensities from images as in Figure 1A were analyzed using our algorithm and CellProfiler software for comparison. (A and C) Object intensities of original images, after distortion and after subsequent corrections are shown as the mean +/- SD for images containing 70 or 700

objects in images representing two different illuminations (Y and C in Figure 1A). (B and D) Histograms of object intensities from images analyzed in (A and C, respectively). Only histograms for images containing 700 objects are shown.

Figure S4: Vignetting correction significantly affects critical parameters of image analysis in a typical quantitative microscopy experiment.

(A) The distribution of GFP intensities of cells before and after vignetting correction from the experiment shown in Figure 3 is plotted. (B) The coefficient of variation (CV) of GFP intensity from cells as in (A) is shown.

Supplementary Tables:

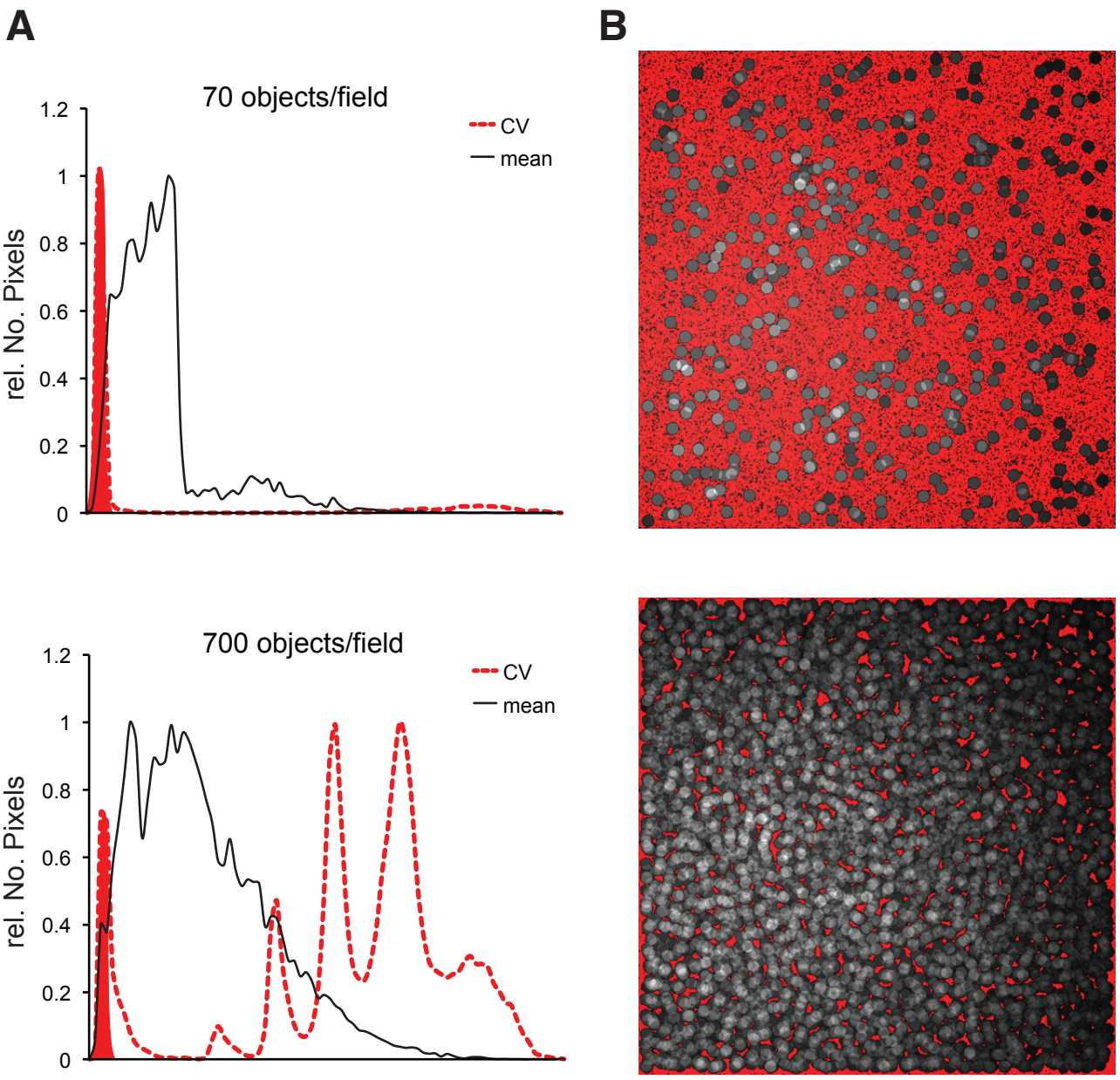
Table S1: Yeast strains used in this study

Strain	Relevant genotype	Reference
ySS4	BY4741, *CDC19-GFP::His3*	OpenBiosystems
ySP257	W303, *MAT*a *leu2::LEU2-pSTL1-qVenus; his3::HIS3-pRPS2 qCFP*	this study

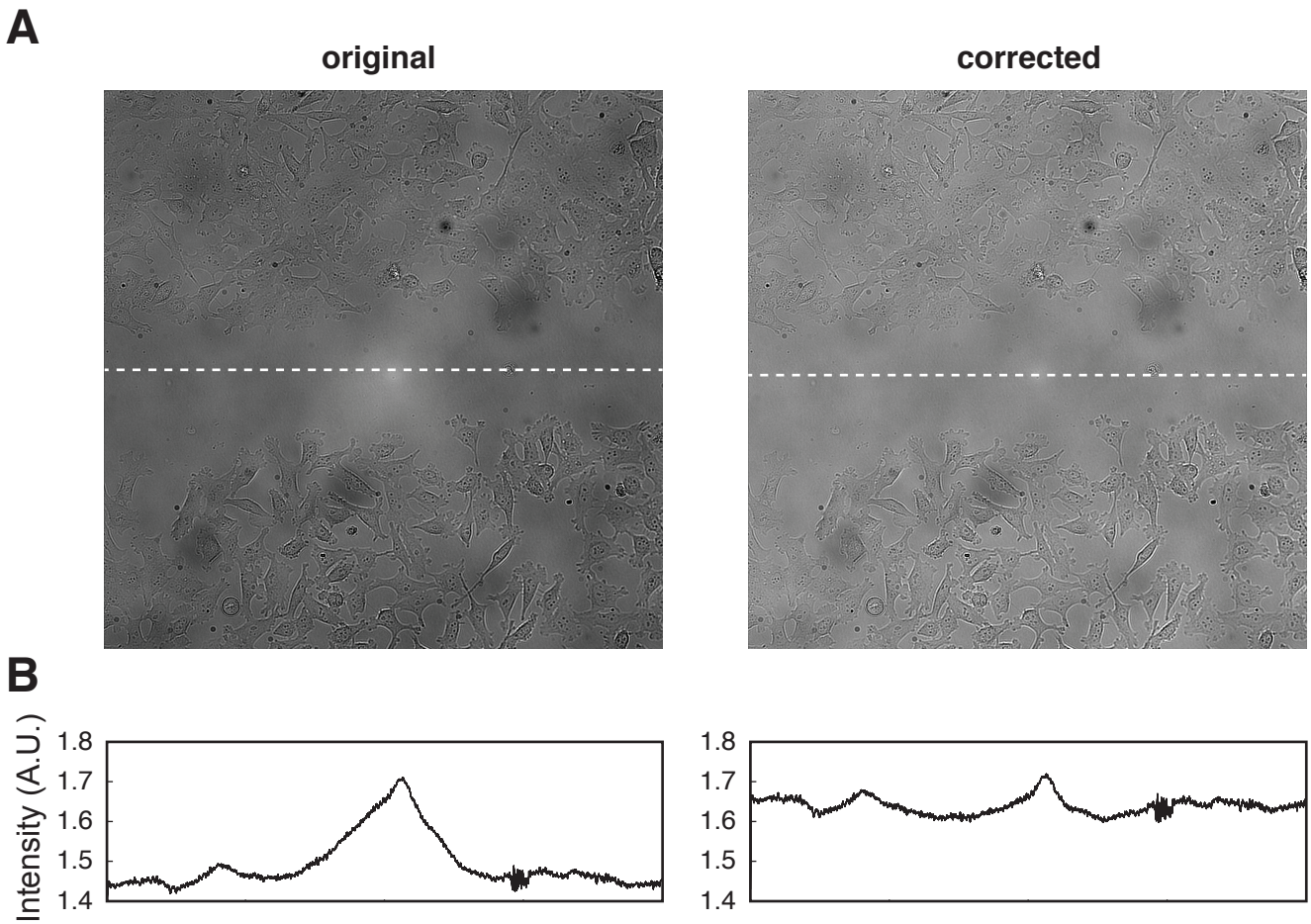
Implementation of the vignetting correction algorithm

```
function corrFactor = flatnessCorrectionIllum_interp2_withFit(imagesForTrainingSet)

% this function uses a cell array containing the images of the training set as an
% input and returns the scaled correction factor. Format for the training set:
% imagesForTrainingSet{numImagesInTrainingSet}(imgSizeX, imgSizeY)

% retrieve parameters of the training set

numImagesInTrainingSet = length(imagesForTrainingSet);

imgSizeX = size(imagesForTrainingSet{1},1);
imgSizeY = size(imagesForTrainingSet{1},2);

% perform median filtering of all images of the training set to minimize noise

for k=1: numImagesInTrainingSet
    trainingSet(:,:,k) = medfilt2(imagesForTrainingSet{k},[10 10],'symmetric');
end

% sort pixels across the training set

trainingSet=sort(trainingSet,3);

% calculate the CV_I(x,y) for each pixel

if (numImagesInTrainingSet > 4)
    CVImage = std(trainingSet(:,:,2:numImagesInTrainingSet-1),0,3)…
              … ./mean(trainingSet(:,:,2:numImagesInTrainingSet-1),3);
else
    CVImage = std(trainingSet,0,3)./mean(trainingSet,3);
end

CVImageToFlatten = CVImage(:,:,1);

% calculate histogram of CV_I(x,y)

hist_max = max(CVImageToFlatten(:));
hist_min = min(CVImageToFlatten(:));

histX = 0:1:100;
histX = double(histX./100);
histX=histX*(hist_max-hist_min)*1.25+(9*hist_min-hist_max)/8;

hist_CVimg = hist(CVImageToFlatten(:),histX);
startValue_a = max(hist_CVimg);
startValue_b = mode(CVImageToFlatten(:));

% fit single Gaussian function to determine the cutoff

gaussianOptions =
    fitoptions('Method','NonlinearLeastSquares','Lower',[0,0,0],'StartPoint', …
    …[startValue_a,startValue_b,0.1],'MaxIter',10000);

gaussianFittype =
    fittype( @(a1,b1,c1,x) a1*exp(-((x-b1)/c1).^2),'options',gaussianOptions);

histogrammFit = fit(histX',hist_CVimg',gaussianFittype);

% calculate representation of the Gaussian distribution for vizalization

fittedGaussian =
    histogrammFit.a1*exp(-((histX-histogrammFit.b1)./histogrammFit.c1).^2);

% Display histogram and Gaussian fit

figure (1)
clf(1)
hold on
plot(histX,hist_CVimg)
plot(histX,fittedGaussian,'r')
hold off

% determine cutoff as mean + SD from the Gaussian fit
```

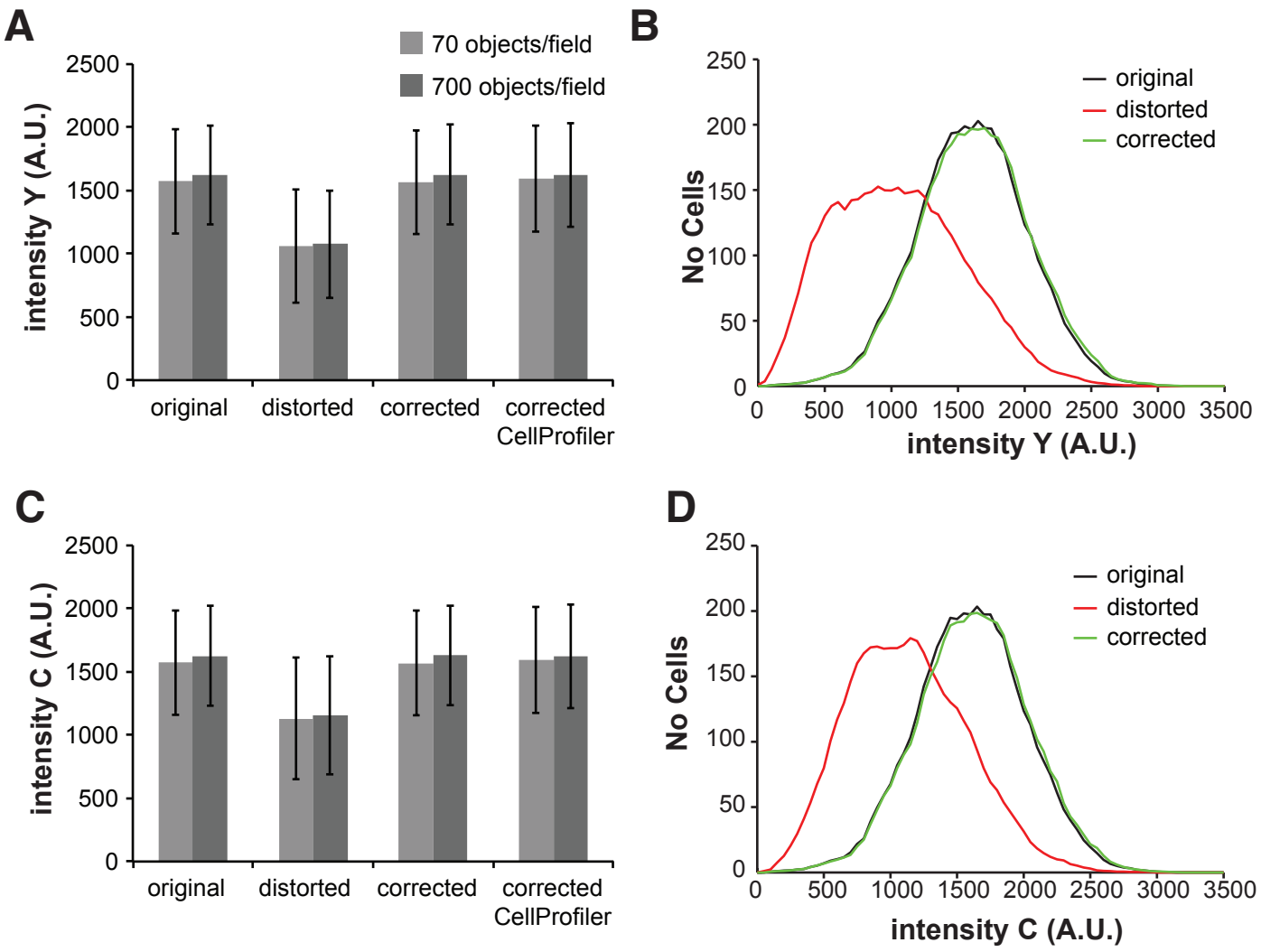
```
cutoff = histogrammFit.b1+histogrammFit.c1;



% sample randomly 500 pixels with CV1(x,y) < cutoff  as a starting point for the correction
% factor

c=0;

while c<500
    i= randi(imgSizeX);
    j= randi(imgSizeY);
    if (CVImageToFlatten(i,j)<cutoff)
        c=c+1;
        X(c) = i;
        Y(c) = j;
        if (numImagesInTrainingSet > 4)
            Z(c) = mean(trainingSet(i,j,2:numImagesInTrainingSet-1));
        else
            Z(c) = mean(trainingSet(i,j,:));
        end
    end
end

% fit correction function using LOESS model

fittedFactor = fit([X',Y'],Z','loess');

% calculate correction factor over a sparse grid representing the entire image using
% the calculated fit

X1 = 1:100:imgSizeX;
X1 = [X1 imgSizeX];

Y1 = 1:100:imgSizeY;
Y1 = [Y1 imgSizeY];

for i=1:length(X1)
for j=1:length(Y1)
    corrFactor_grid(i,j)= fittedFactor(X1(i),Y1(j));
end
end

% interpolate correction factor over the entire image

x1 = 1:1:imgSizeX;
y1 = 1:1:imgSizeY;

corrFactor = interp2(X1,Y1',corrFactor_grid,x1,y1','spline');

%normalize correction factor

corrFactor = corrFactor/max(corrFactor(:));

% display correction factor

figure (2)
clf(2)
surf(corrFactor,'EdgeColor','none')
```



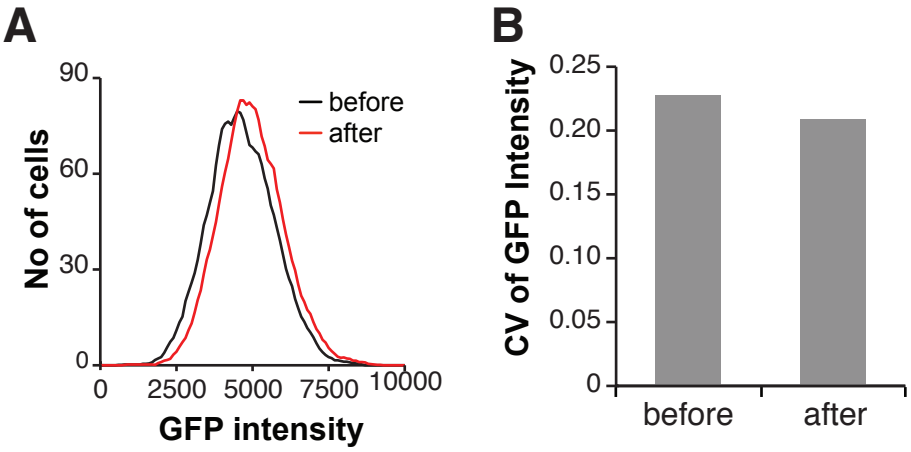
Lee et al., Figure S1



Lee et al., Figure S2



Lee et al., Figure S3



Lee et al., Figure S4