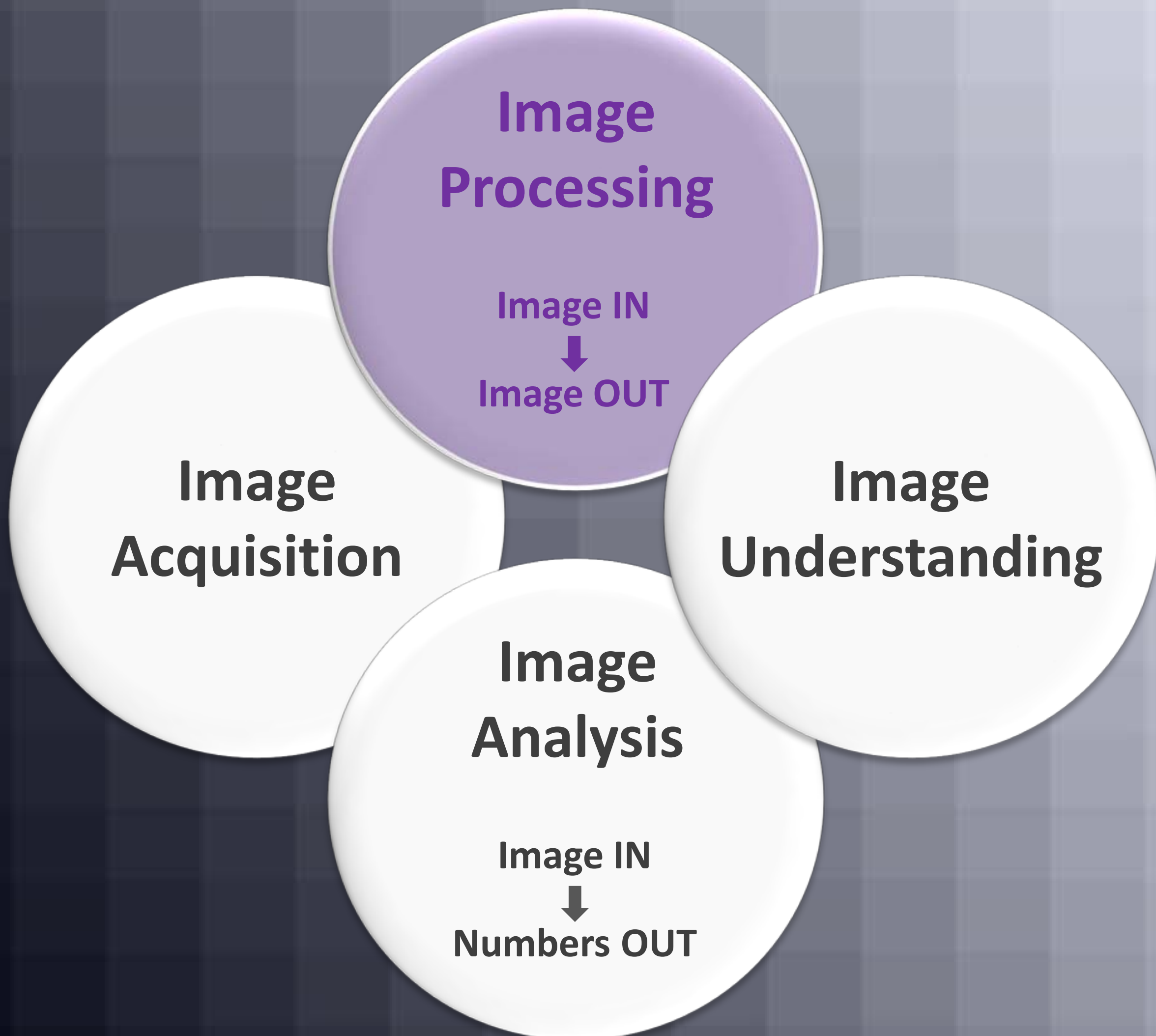
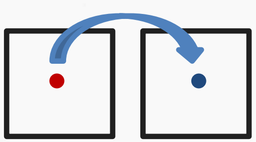


Image Processing
Image IN
Image OUT
Image Acquisition
Image Understanding
Image Analysis
Image IN
Numbers OUT

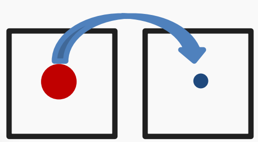
Image Processing

Basic Image Operations

Point Operations



Local Operations



Global Operations

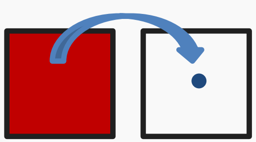


Image Processing

Point Operations

- How do point operations work?
- Point operations on single images
- Point operations combining images

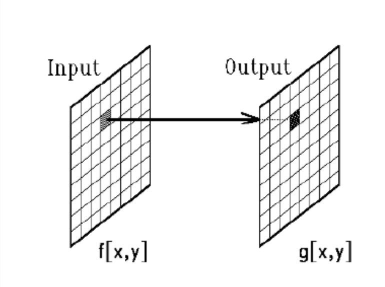
How to…?

Point Operations

= all functions that are performed on each pixel of an image, independent of all other pixels in that image

$$I'(x,y) \longleftarrow f(I(x,y))$$

On single gray level images



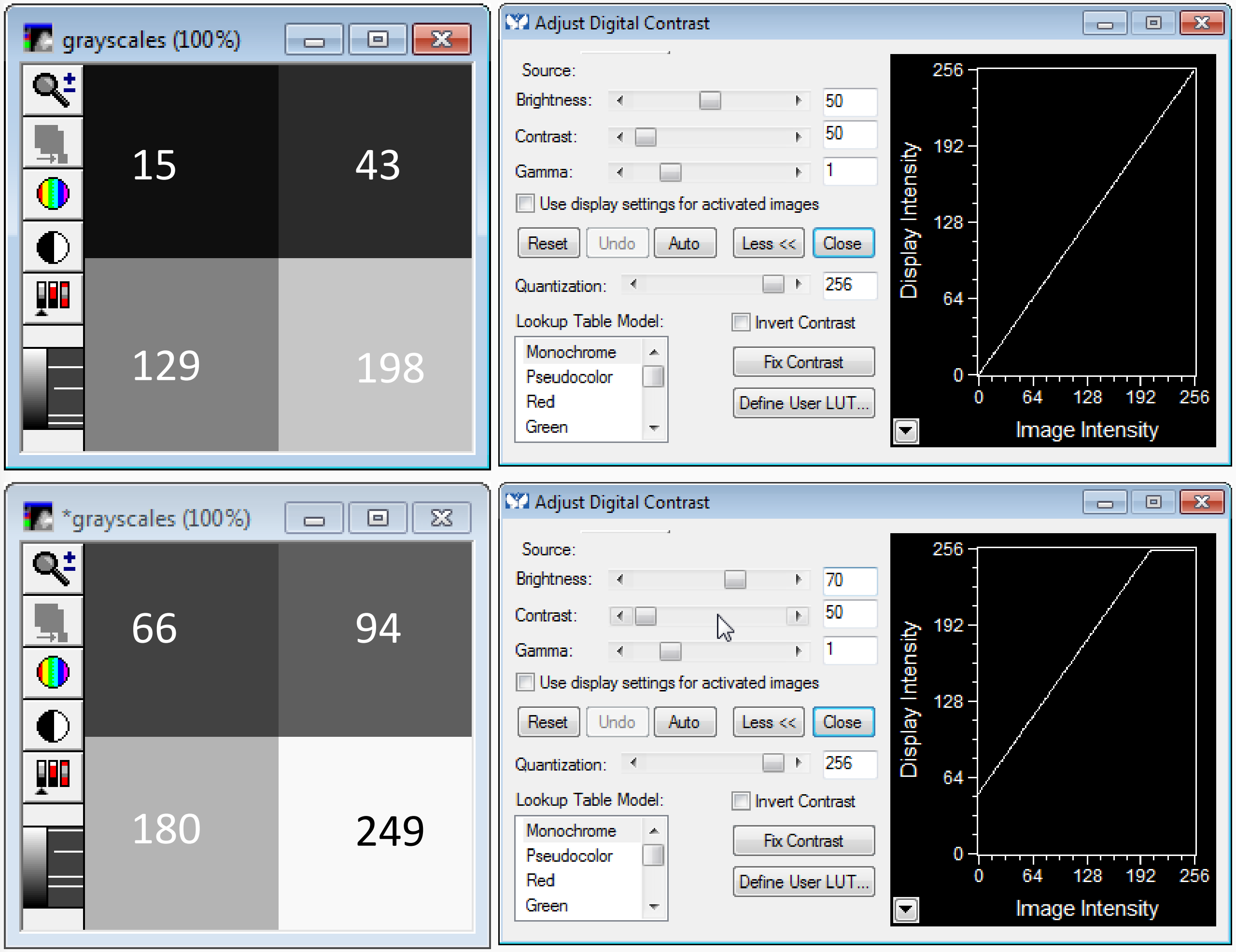
- contrast and brightness
- gammacorrection
- inversion
- thresholding
- arithmetical operations (background subtraction)

combining several images/multiple images (stacks)

- logical operations (on binary images)
- arithmetical operations (addition, subtraction, shading correction)
- minimum / maximum operations
- averaging, median filtering

Brightness

Brightness $I'(x,y) \longleftarrow I(x,y) + \text{Brightness-Increase}$

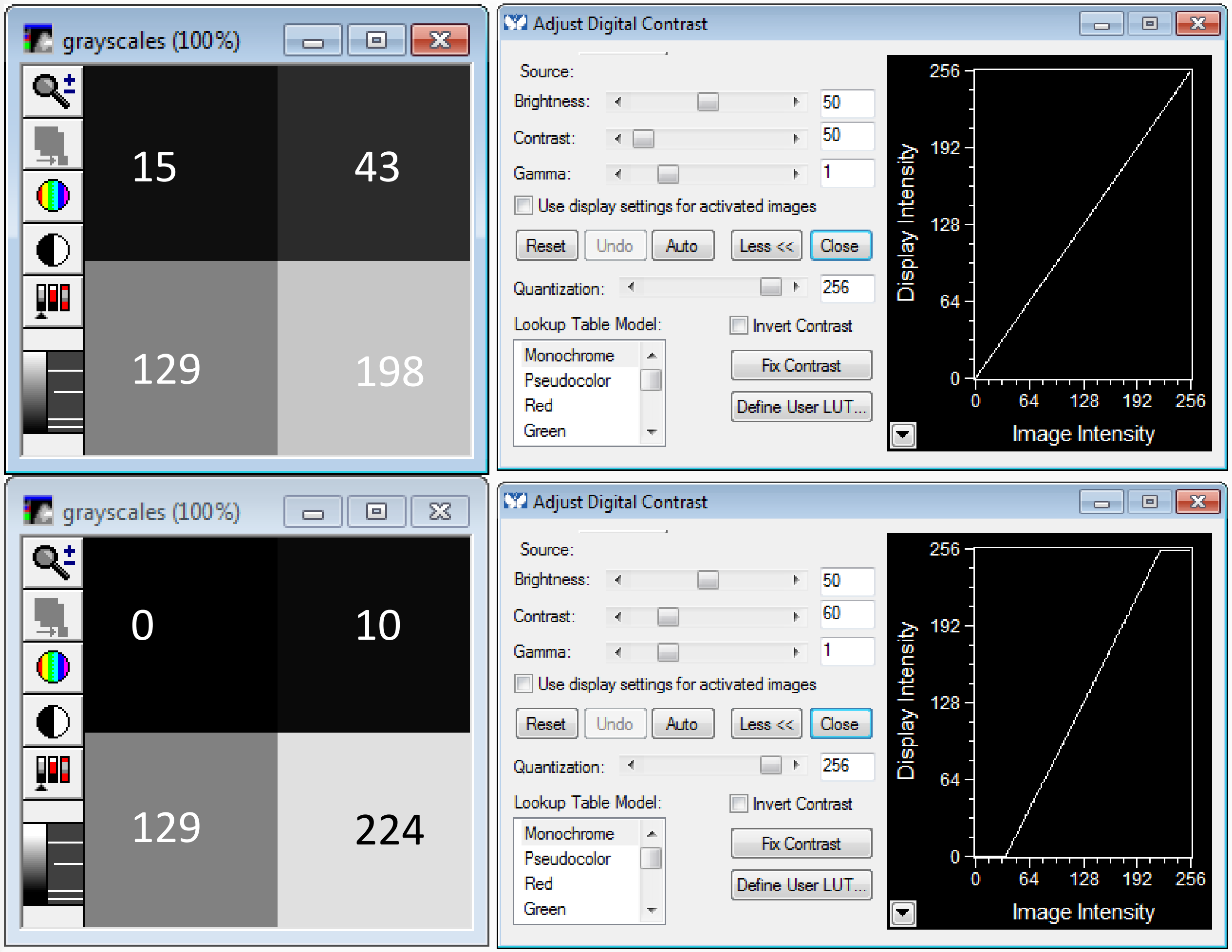


- linear – each pixel is treated the same way
- easy to enter saturation

$I'(x,y) \longleftarrow I(x,y) + 51$

Contrast

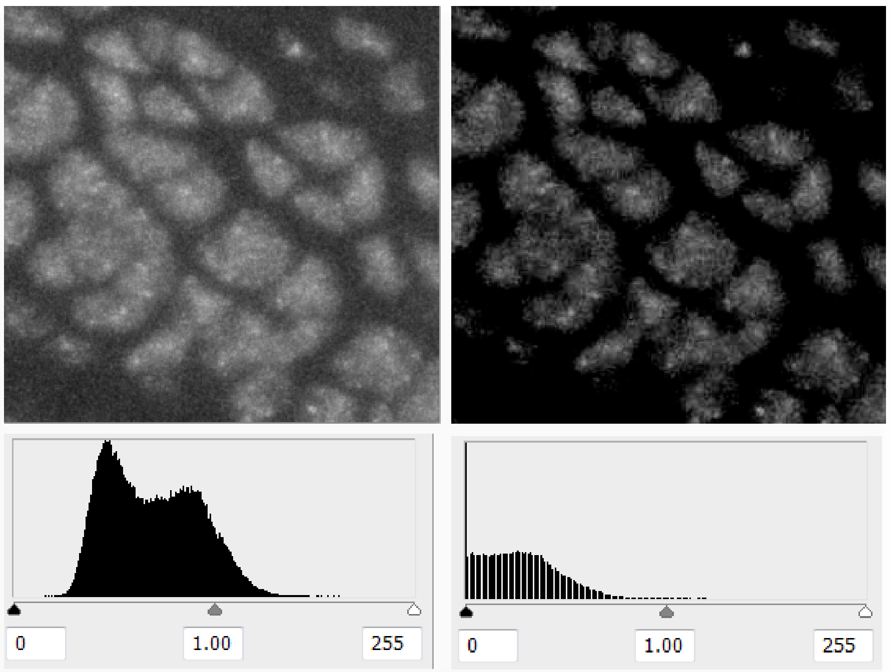
Contrast (outputLevel - 128) = contrastAmount * (inputLevel - 128)
changes slope of the line around the middle (128)

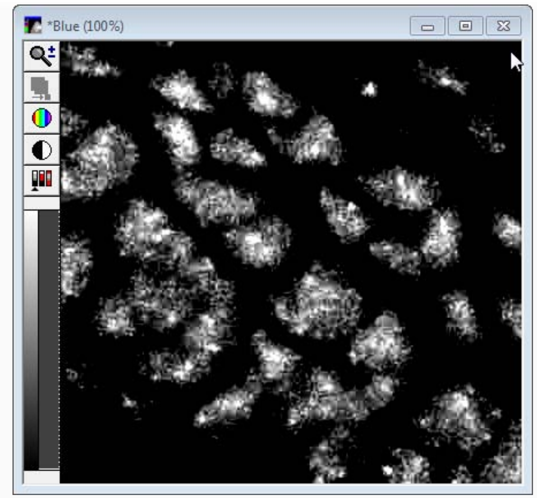


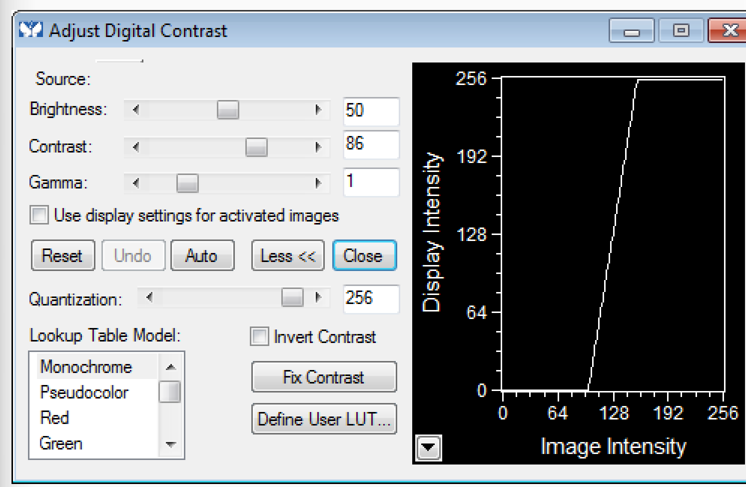
(224 -128) = 1.4*(198 – 128)

Contrast

- linear – each pixel is treated the same way
- information is lost if clipping occurs





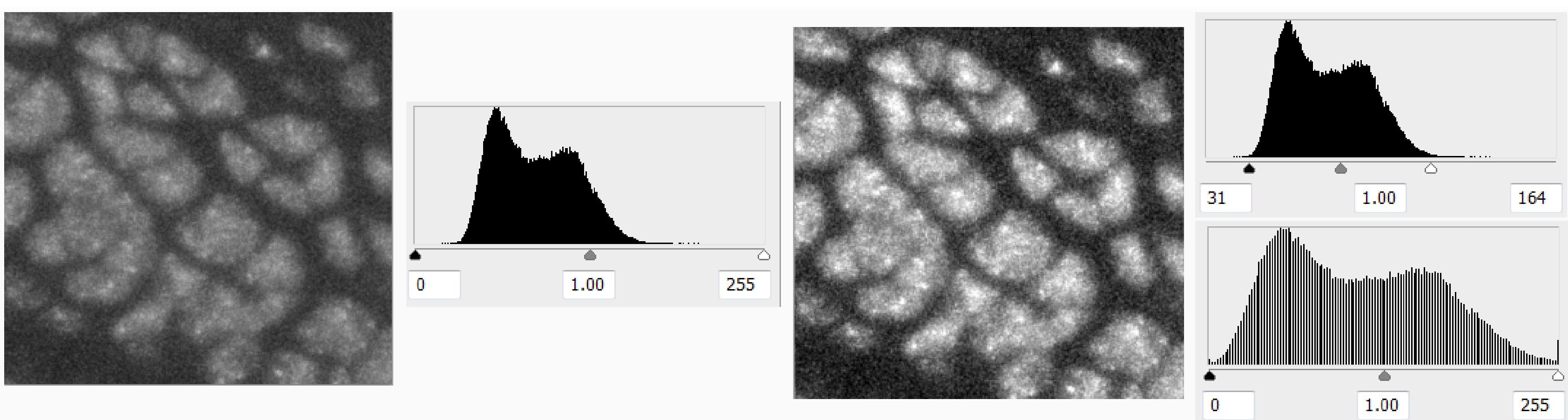


Contrast

Auto -Contrast

maximum and minimum pixel values are found and the histogram is stretched between them linearly

Sometimes a certain percentage is saturated in order to increase mid-tones



What is intensity?

Intensity is somehow measured in numerical pixel values, **BUT** :

- relationship of pixel value to incoming light??
- linearity of detector?
- relationship of pixel value to toner-particles on printed paper?
- linearity of screen?
- …

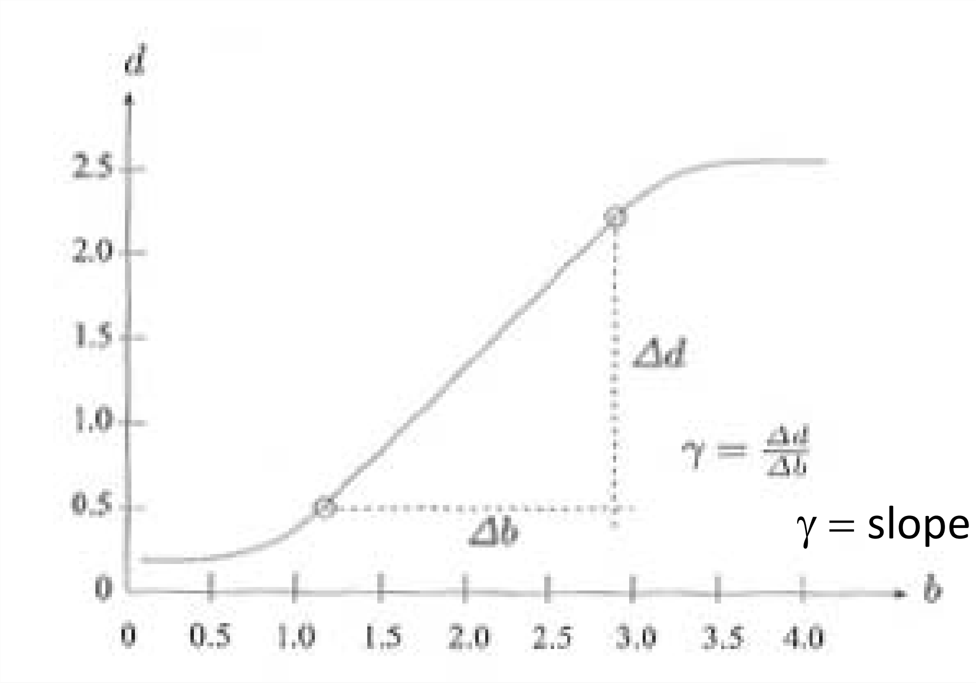
Best would be a calibrated "Intensity Space"
> not possible > correction needed

Easiest point operation to compensate for different characteristics of in- and output device:

Gammacorrection

Why gamma γ ?

Density of the film

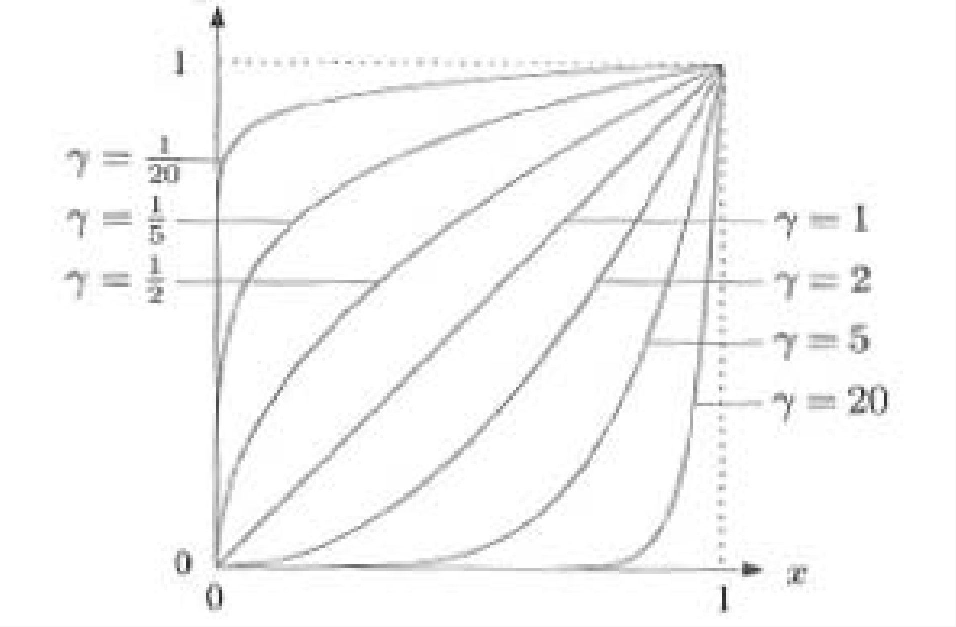


γ = slope of linear increase

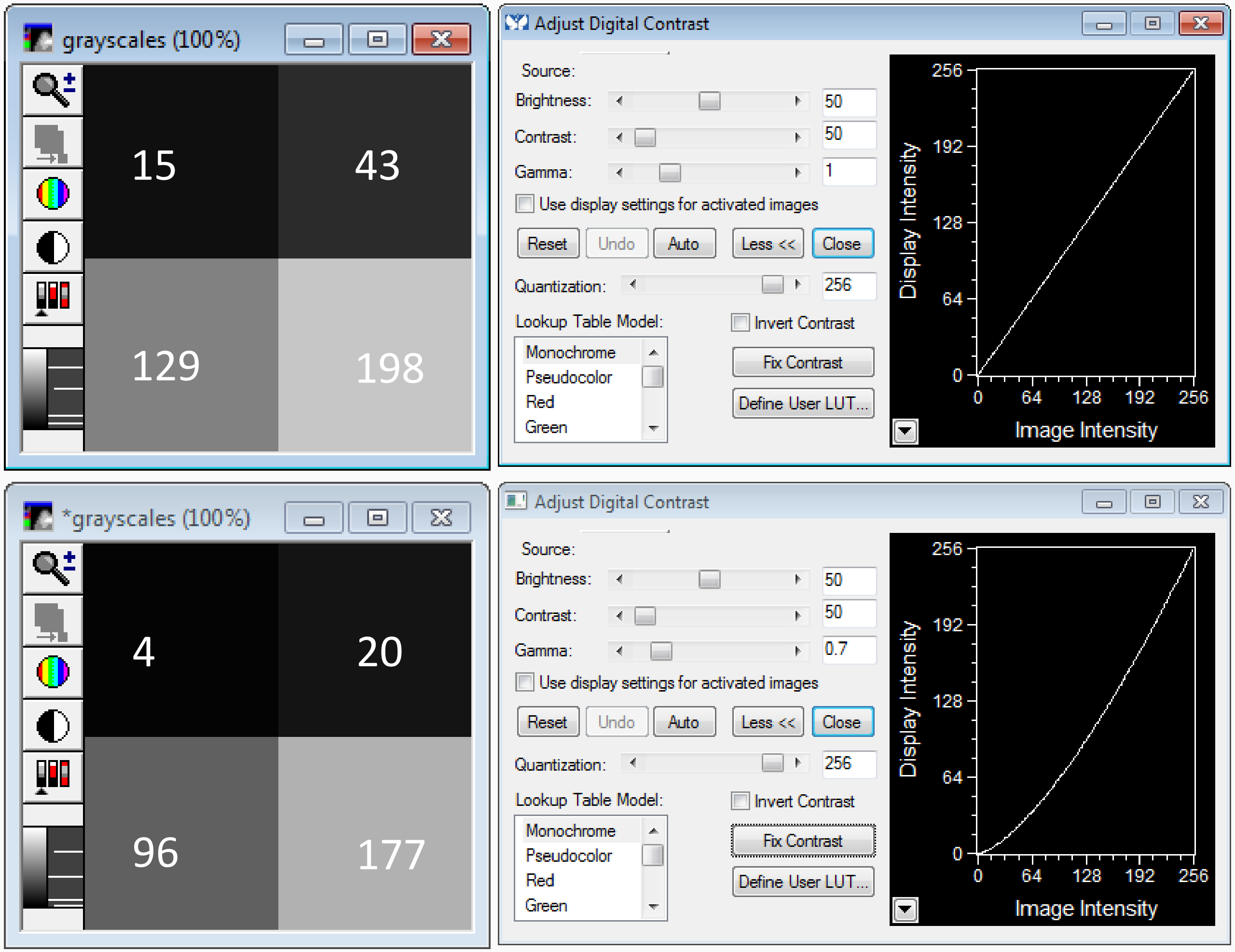
logarithm of the film's exposure to light

Gamma function:

$$y = x^{\gamma}$$



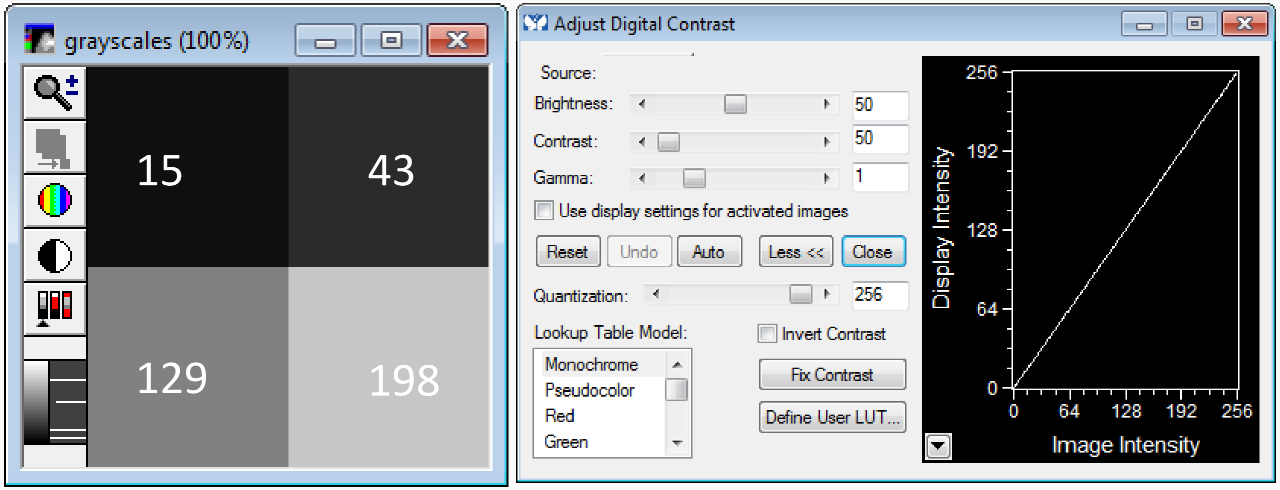
Gammacorrection



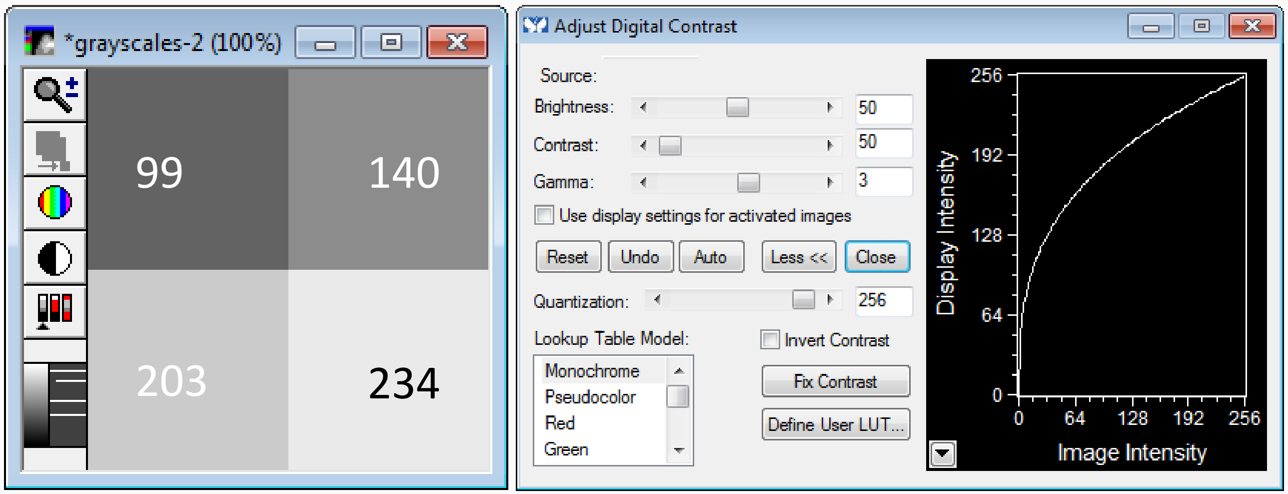
$\gamma = 1$

$\gamma = 0.7$

Gammacorrection

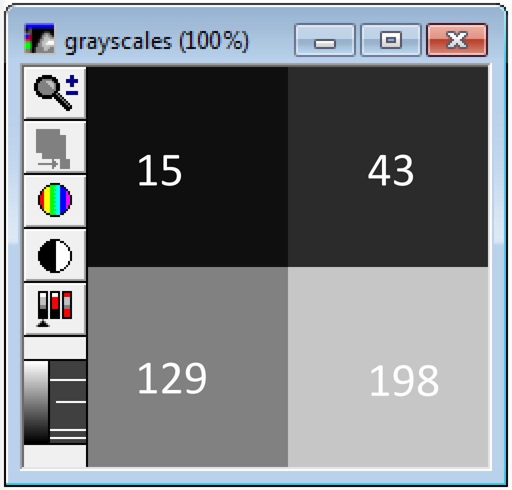


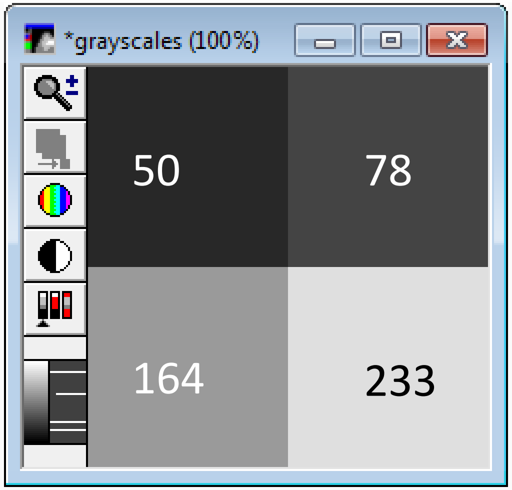
$\gamma = 1$

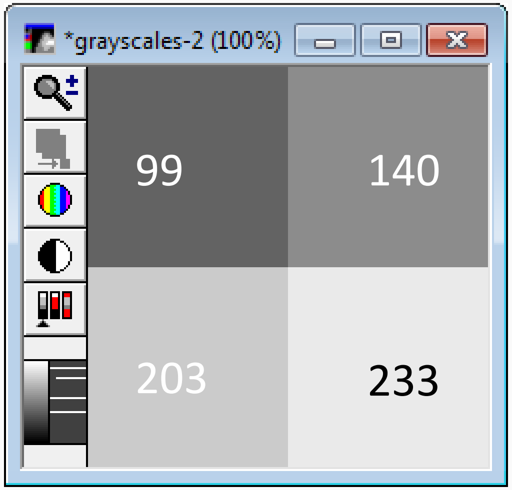


$\gamma = 3$

Gammacorrection





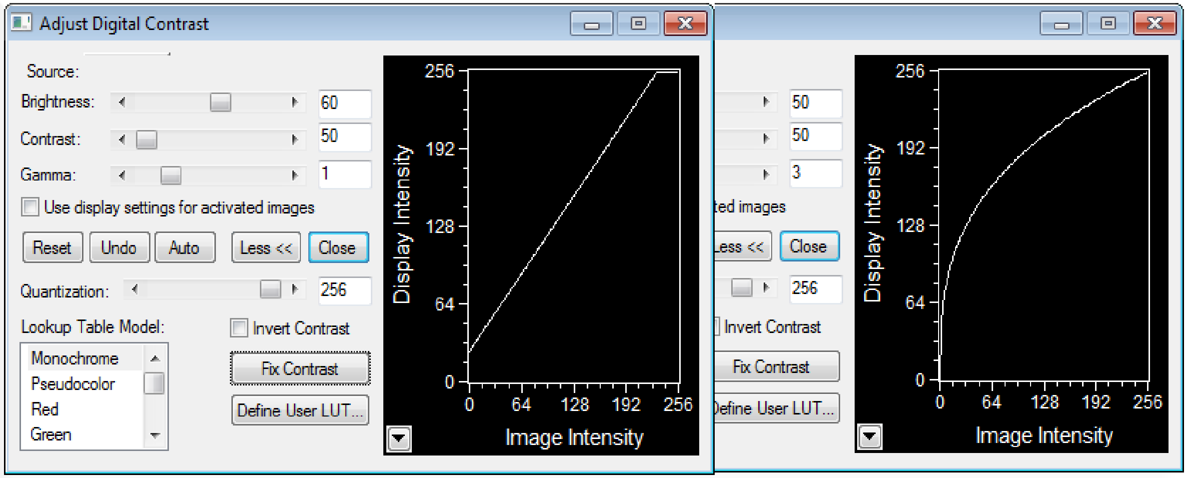


$\gamma = 1$

Brightness = 60

$\gamma = 3$

Not linear –
Results can be changed



Histogram Equalization

Histogram Equalization = a method in image processing of contrast adjustment using the image's histogram. It usually increases the global contrast of many images.

$$p_x(i) = p(x = i) = \frac{n_i}{n}$$

L being the total number of gray lev
normalized to [0,1].

Let us also define the *cumulative d*

$$cdf_x(i) = \sum_{j=0}^{i} p_x(j),$$

which is also the image's accumula

We would like to create a transform

$$cdf_y(i) = iK$$

for some constant K. The propertie

$$y = T(x) = cdf_x(x)$$

Notice that the T maps the levels ir
applied on the result:

$$y' = y \cdot (\max\{x\} - \mathrm{m}$$

All pixels in the input image with densities in the region D_A to $D_A + dD_A$ will ha
$D_B + dD_B$. The surface areas $h_A(D_A)dD_A$ and $h_B(D_B)dD_B$ will therefore

$$h_B(D_B) = h_A(D_A) \div d(D_A)$$

where $d(x) = \frac{df(x)}{dx}$.

This result can be written in the language of probability theory if the histogram h is
intensity levels:

$$p_B(D_B) = p_A(D_A) \div d(D_A)$$

In the case of histogram equalization, the output probability densities should all be
considered is 0). The transfer function (or point operator) necessary to achieve th

$$d(D_A) = D_M * p_A(D_A)$$

Therefore,

$$f(D_A) = D_M \int_0^{D_A} p_A(u)du = D_M * F_A(D_A)$$

where $F_A(D_A)$ is simply the cumulative probability distribution (*i.e.* cumulative h
an output histogram which is flat!

A digital implementation of histogram equalization is usually performed by defining

$$f(D_A) = max(0, round[D_M * n_k/N^2)] - 1)$$

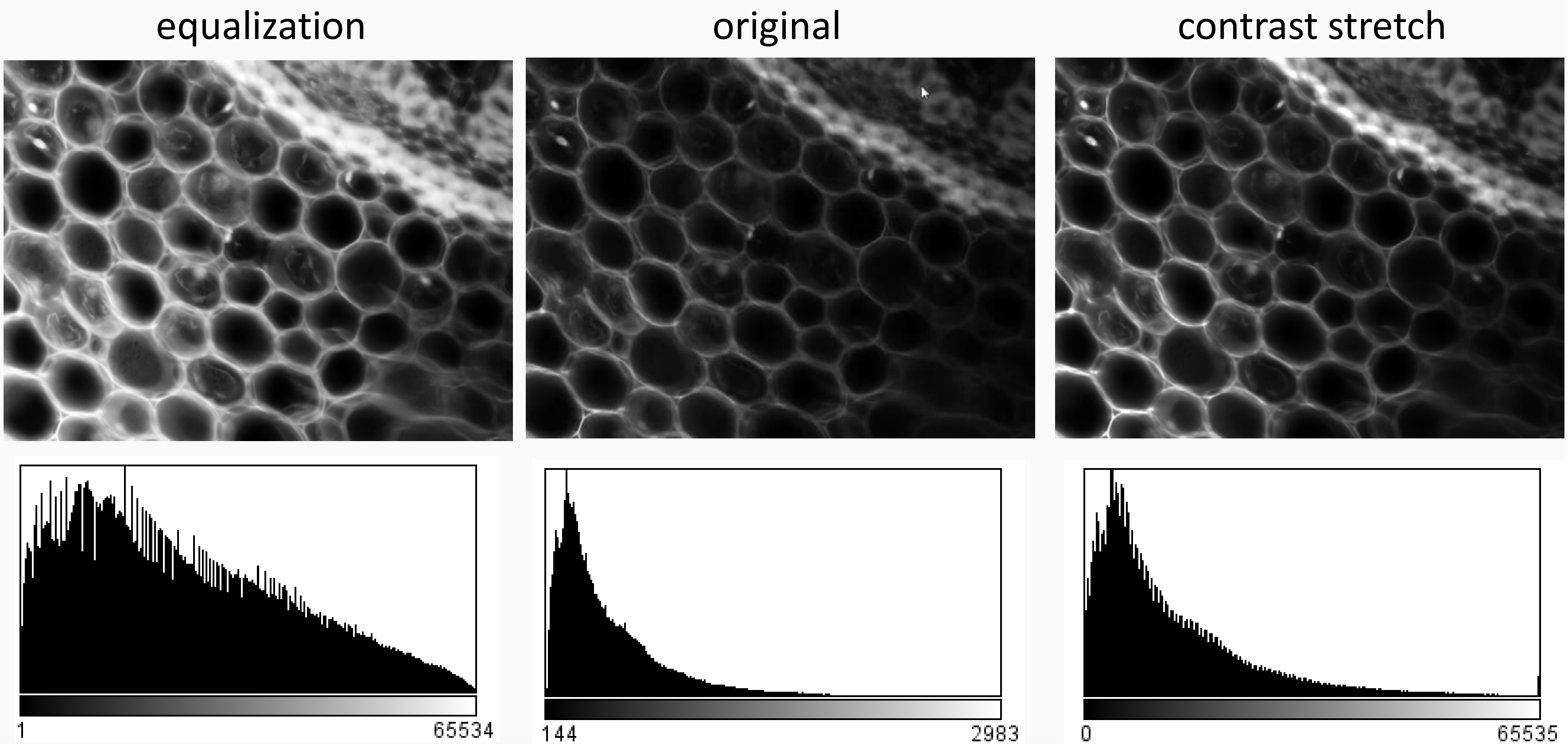
$$L - 1)\Big)$$

ution function (in this case 1), M × N giv
rey levels used (in most cases, like this

the bottom row of the 7th column.) The r

$$\mathrm{nd}\,(0.714286 \times 255) = 182$$

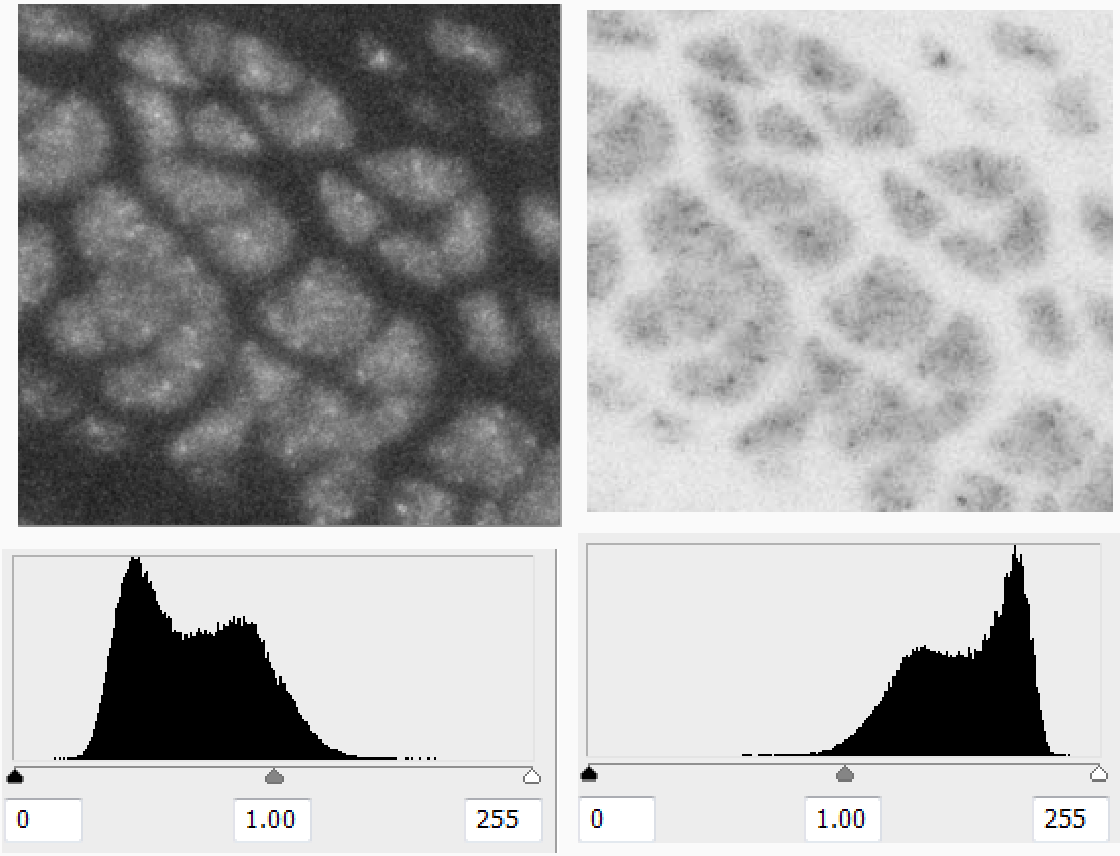
Histogram Equalization



Not linear –
Results can be changed

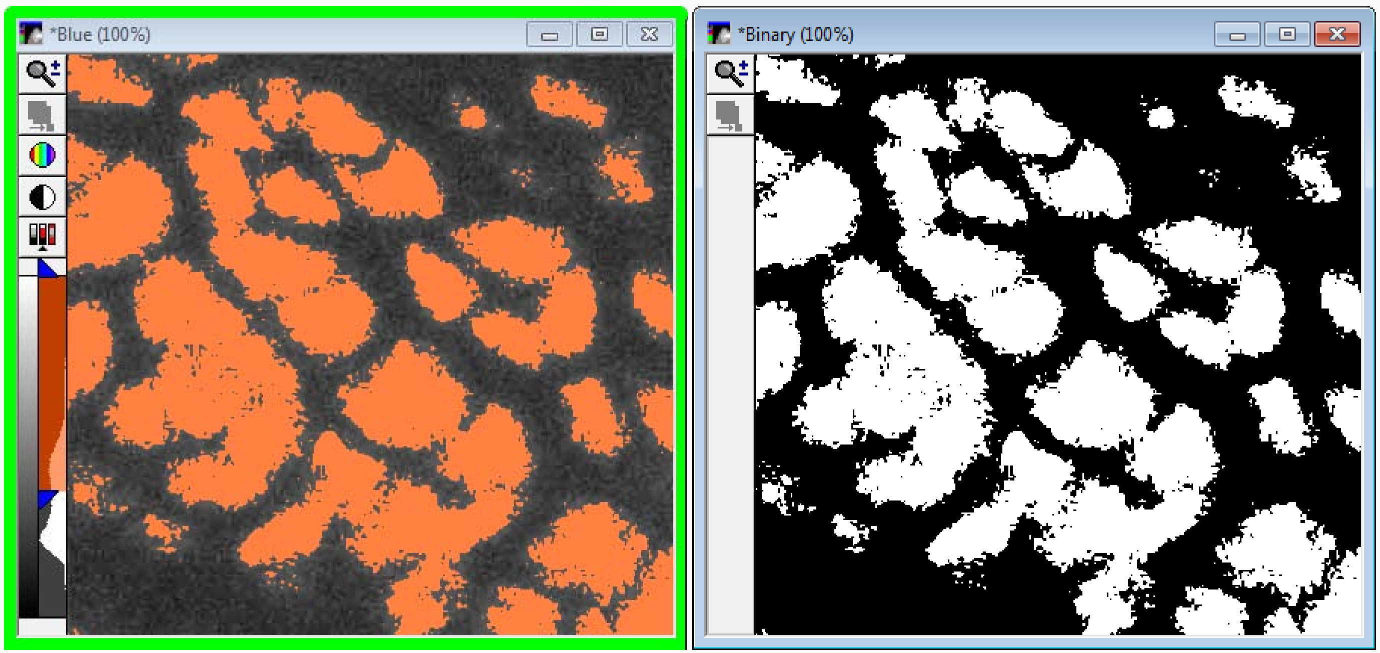
Inversion

$$I'(x,y) \longleftarrow p_{max} - I(x,y)$$



Thesholding = dividing pixels in two classes (object and background)
= simplest form of segmentation
- creates binary image (0 .. background, 1 .. Object)

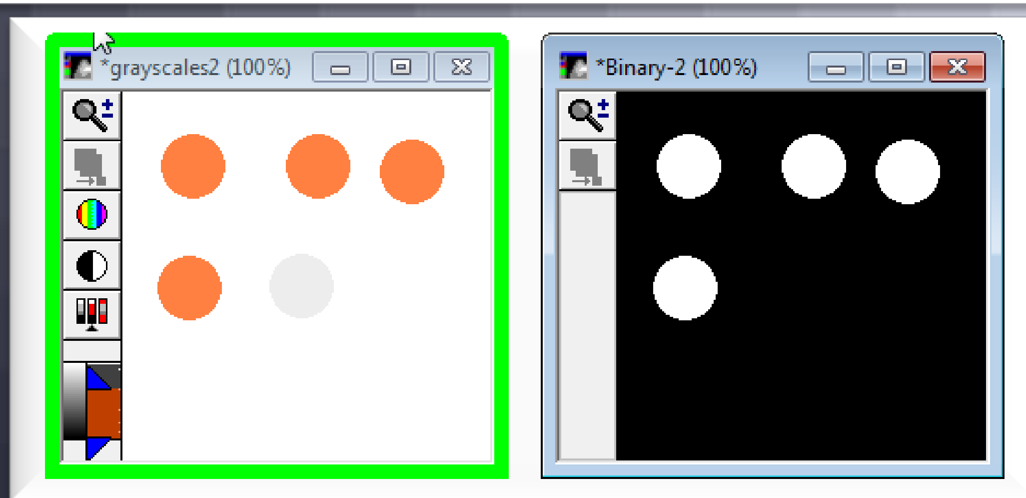
threshold above: intensity above threshold is object (bright object on dark background)
each pixel with intensity value > thresh = 1; with intensity value < thresh = 0



Thresholding

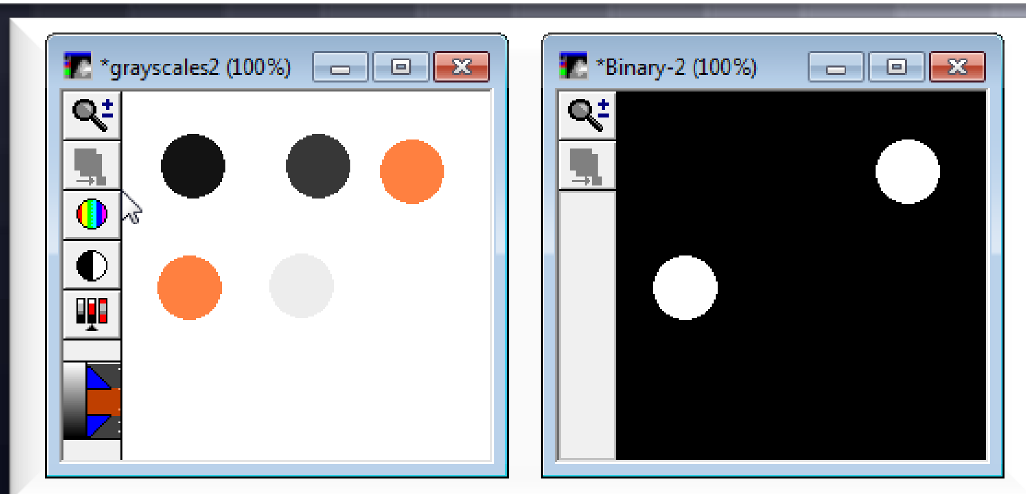
threshold below: intensity below threshold is object (bright object on dark background)

pixel intensity value < thresh = 1; > thresh = 0



threshold inside: a pixel is labeled "object" if its value is between two thresholds

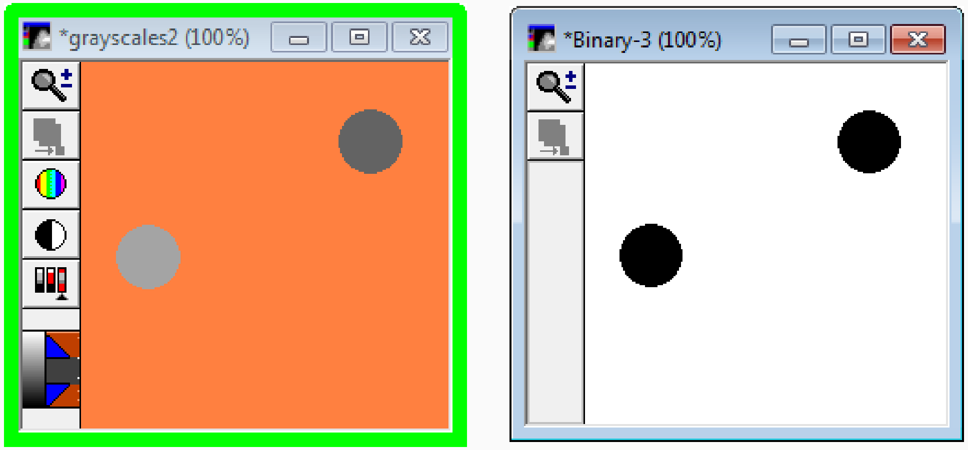
thresh1 < pixel intensity value > thresh2 = 1;



Thresholding

threshold outside: a pixel is labeled "object" if its value is outside two thresholds

pixel intensity value< thresh1 or pixel intensity value > thresh2 = 1



Background Correction

subtraction of a background image or a fixed value

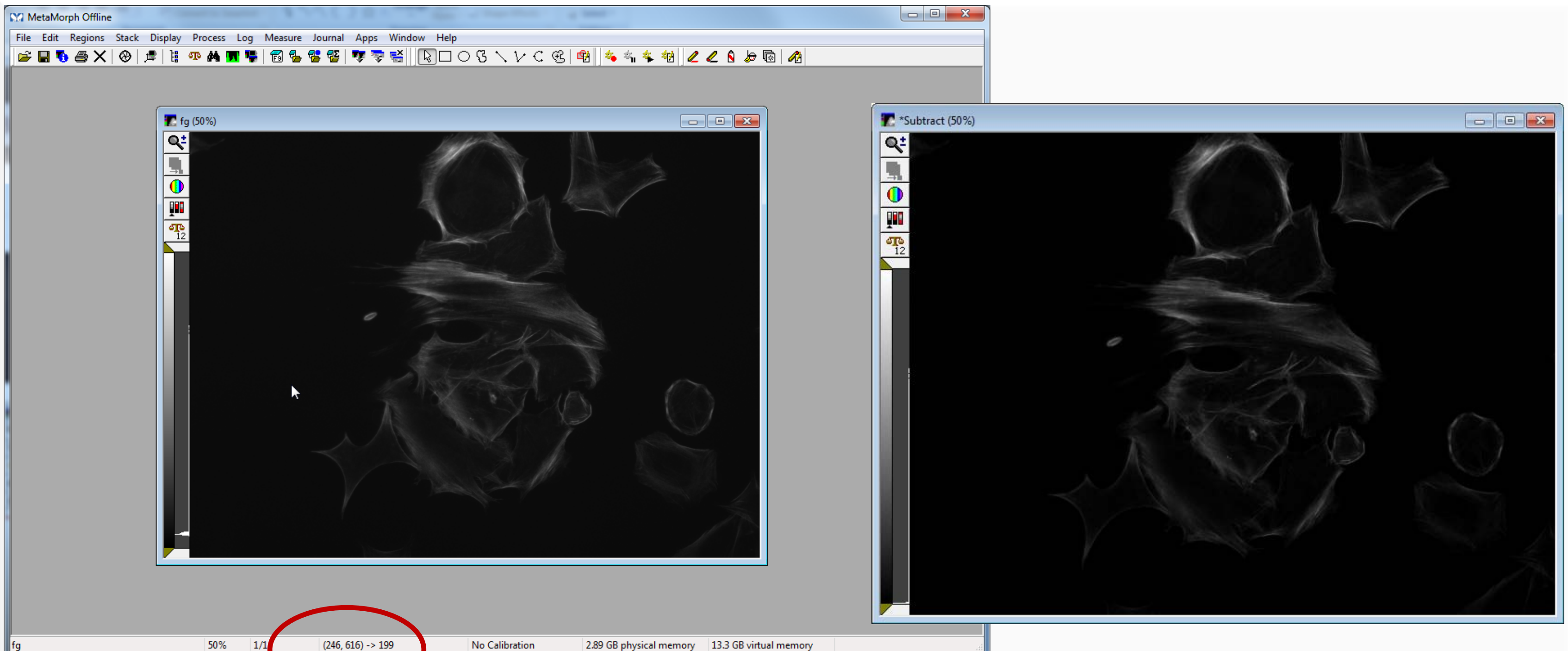
Shading Correction

division by a shading reference image to correct for uneven illumination

Background Correction

subtraction of a background image or a fixed value

Result = Source image – Constant value

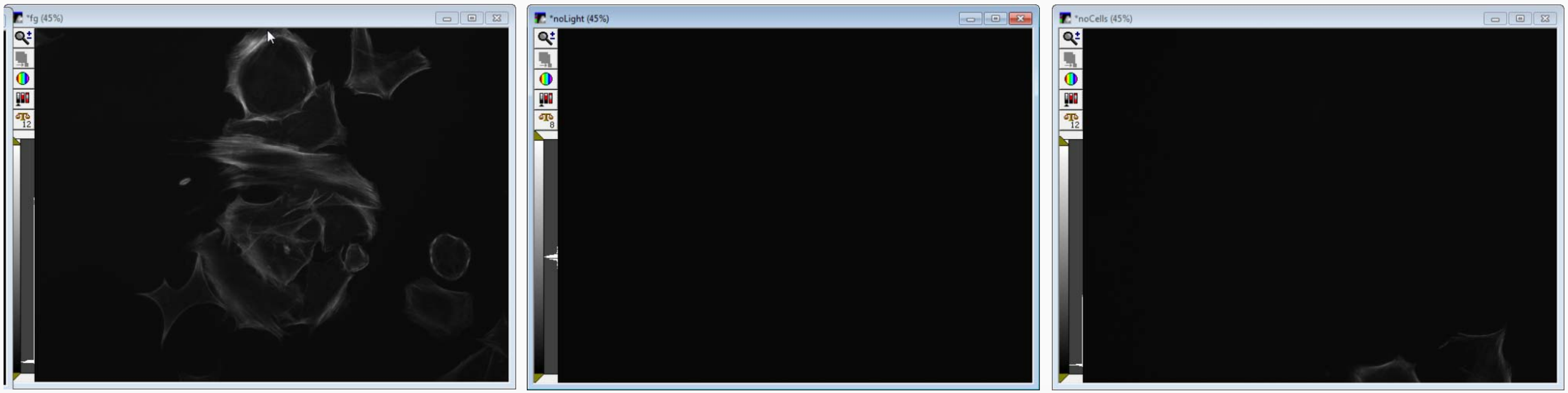


Source image - 199

Background Correction

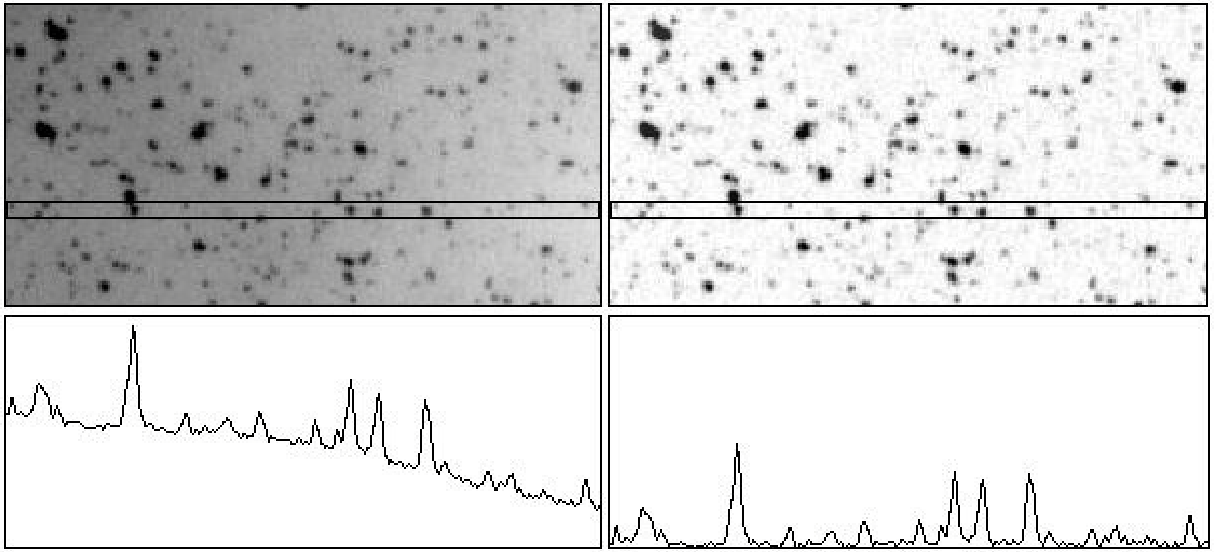
Result = Source image – Background image + Offset

Image	No light on camera	Empty position on slide



Background Correction

Subtract Background using “Rolling Ball” algorithm



Before

After

Shading Correction

division by a shading reference image to correct for uneven illumination

With acquired reference image

(Current Protocols in Cytometry – Metamorph):

White Reference Image: fl: Image of uniform fluorescence slide (dye or plastic)

bf: Image of empty slide/region

Background Reference Image: Image of Background Fluorescence

- illumination turned off (preferred) or light blocked to camera
- should be taken for white reference and specimen image individually

$$\text{Corrected Image} = \frac{\text{Specimen Image} - \text{Background Reference Image} + \text{Offset}}{\text{White Reference Image} - \text{Background Reference Image}} * \text{Scaling Factor}$$

Scaling Factor: manual selected, max. or mean of white reference

Shading Correction

$$\text{Corrected Image} = \frac{\text{Specimen Image} - \text{Background Reference Image} + \text{Offset}}{\text{White Reference Image} - \text{Background Reference Image}} * \text{Scaling Factor}$$

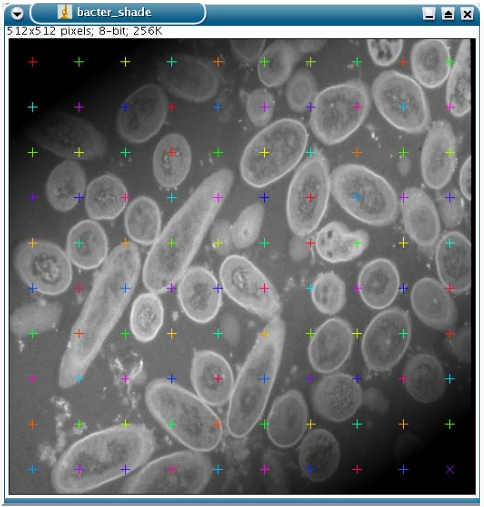
Shading Reference = White Reference Image – Background Reference Image

$$\text{Corrected Image} = \frac{\text{Specimen Image} - \text{Background Reference Image} + \text{Offset}}{\text{Shading Reference}} * \text{Scaling Factor}$$

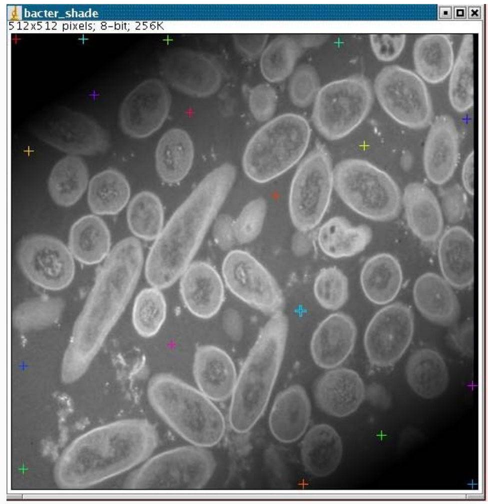
Simplified: $$\text{Corrected Image} = \frac{\text{Specimen Image}}{\text{Shading Reference}} * \text{Scaling Factor}$$

Shading Correction

without acquired reference image

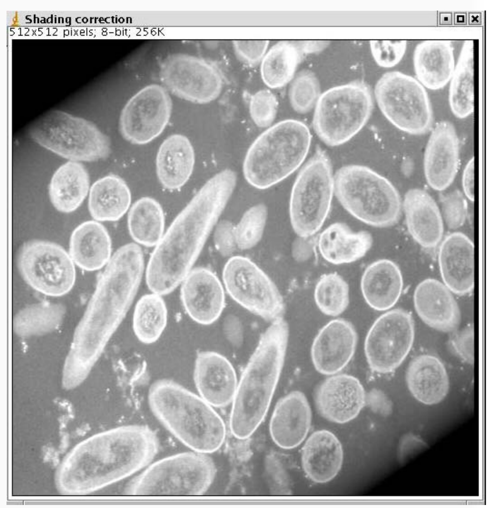


automatic

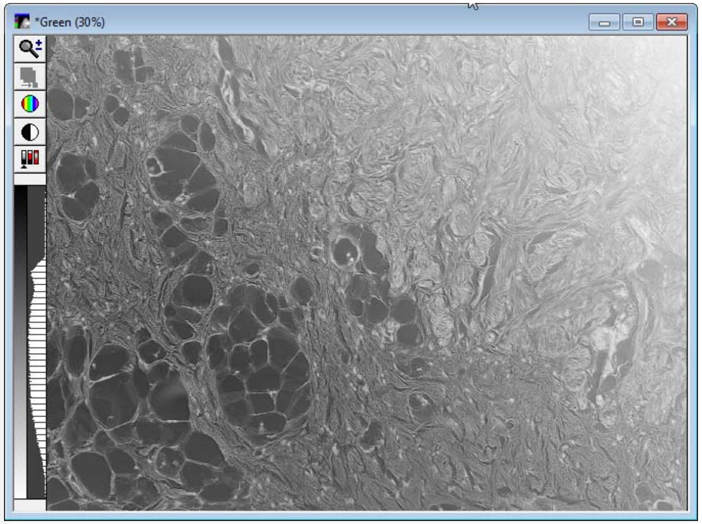


Semi-automatic

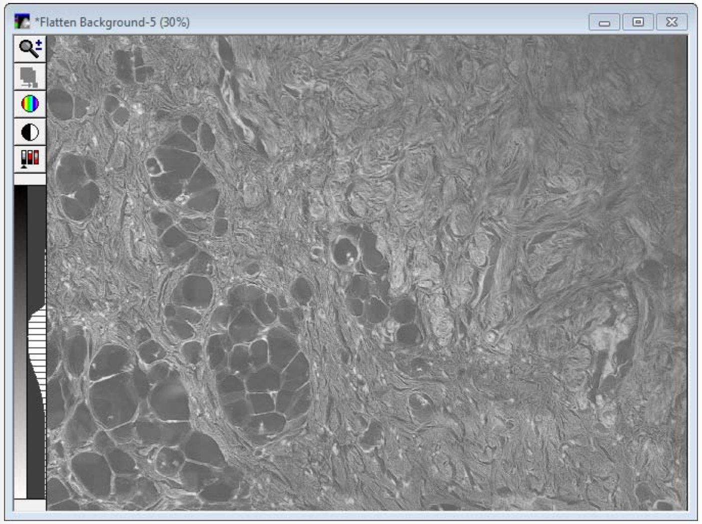
Calculated shading image



result



Input



result

Exercise

- do Shading correcting on fl_sample.tiff using the bg, white, and bg-white reference images
- do Subtract Background
- do shading correction using the A_posteriori_shading_correction plug-in automatic and semi-automatic
- compare results using the threshold tool