Introduction to Digital Image Analysis

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NIC

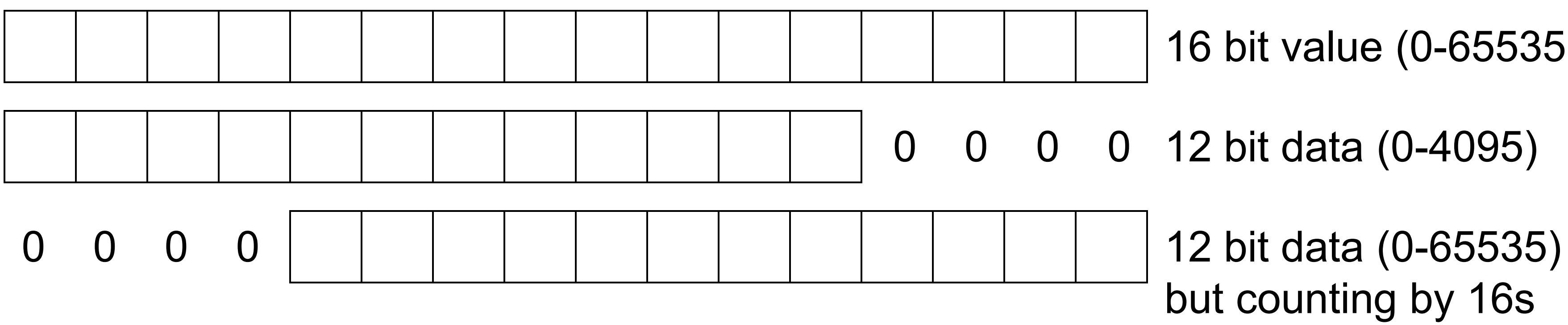
Bitdepth

- Digital cameras have a specified bitdepth = number of gray levels they can record

- 8-bit $\rightarrow 2^8 = 256$ gray levels
- 10-bit $\rightarrow 2^{10} = 1024$ gray levels
- 12-bit $\rightarrow 2^{12} = 4096$ gray levels
- 14-bit $\rightarrow 2^{14} = 16384$ gray levels
- 16-bit $\rightarrow 2^{16} = 65536$ gray levels

Bitdepth and file formats

- Standard imaging formats, like tiff, are always 8 or 16 bit (because 8 bits = 1 byte)



Color Images

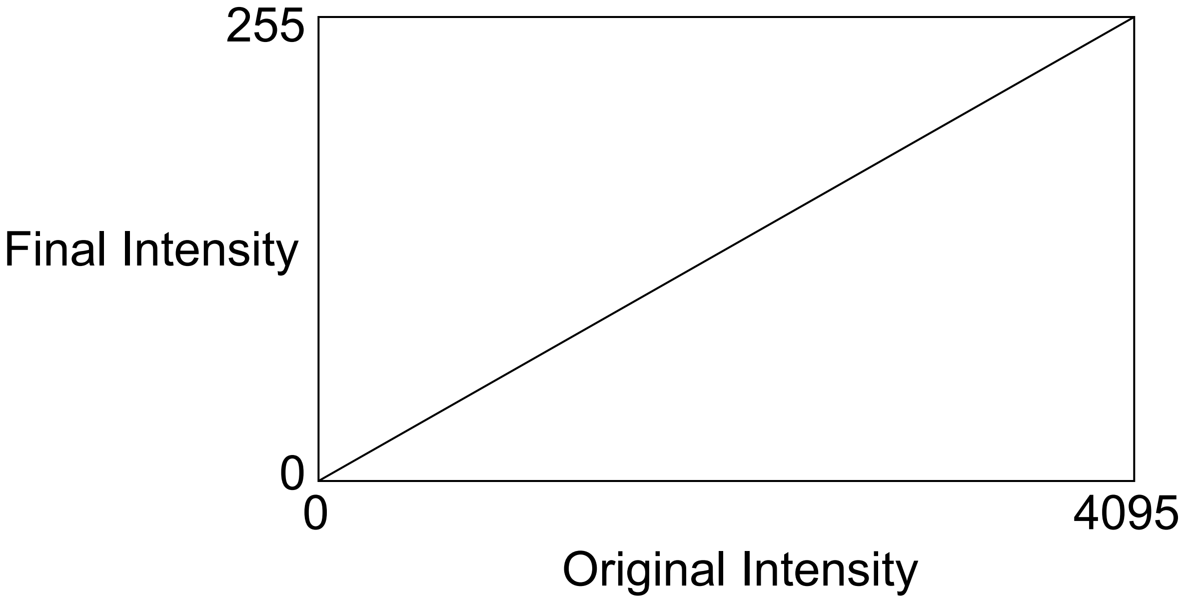
- Color images are made up of three gray scale images, one for each of red, green, and blue
- Can be 8 or 16 bits per channel

File Formats

- Most portable: TIFF
  - 8 or 16-bit, lossless, supports grayscale or RGB
- OK: JPEG2000, custom formats (nd2, ids, zvi, lsm, etc.)
  - Lossless, supports full bitdepth
  - Custom formats often support multidimensional images
  - Not so portable
- Bad: Jpeg, GIF, BMP, etc.
  - Lossy and / or 8-bit

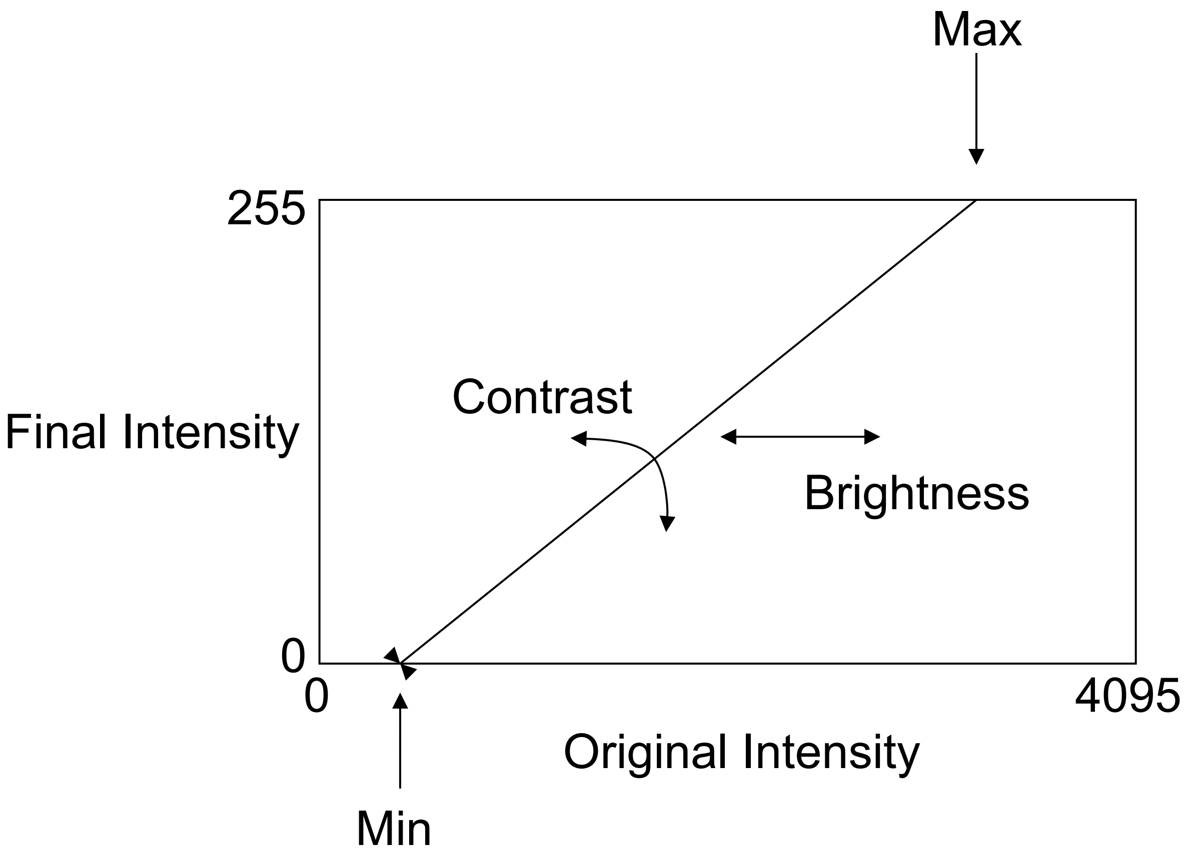
Intensity scaling

- Computer screens are 8-bit
- Publishers also want 8-bit files

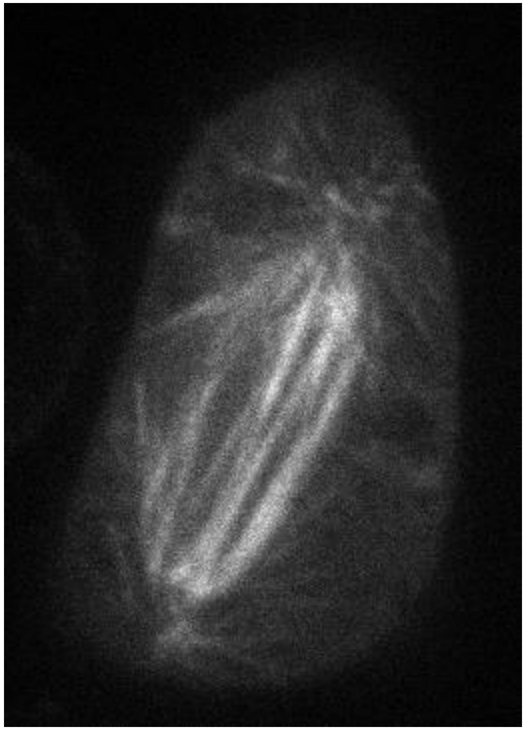


You lose information in this process – values 4080-4095 all end up as 255

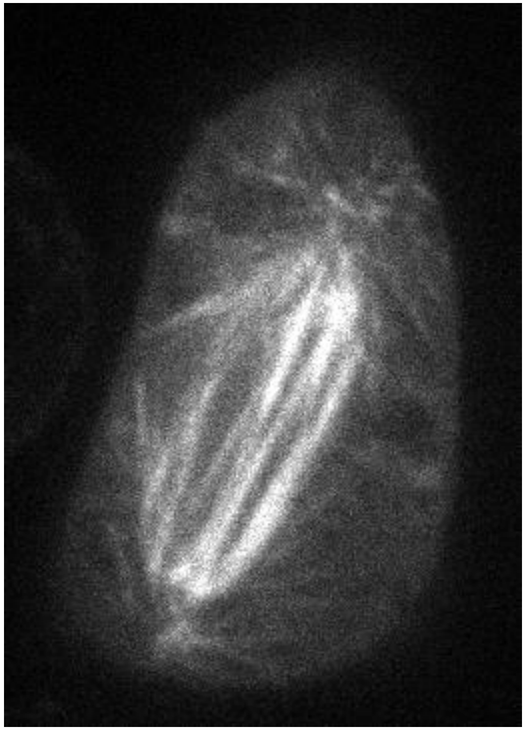
Intensity scaling



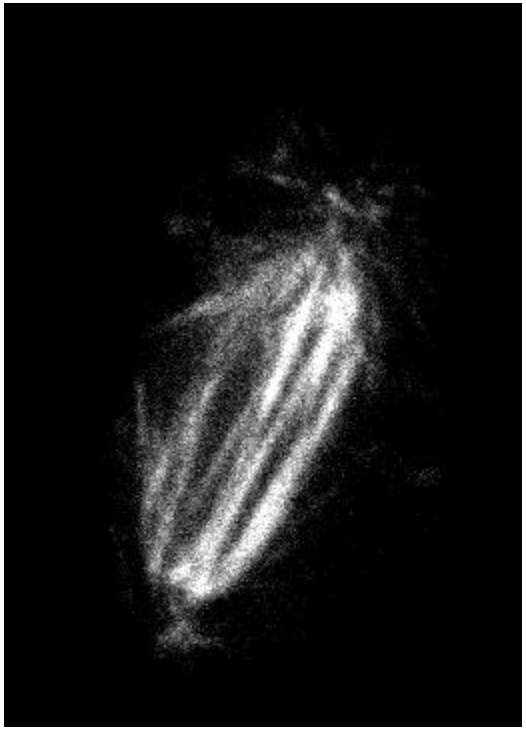
Effect of scaling



Scaled to min / max
(874 / 25438)



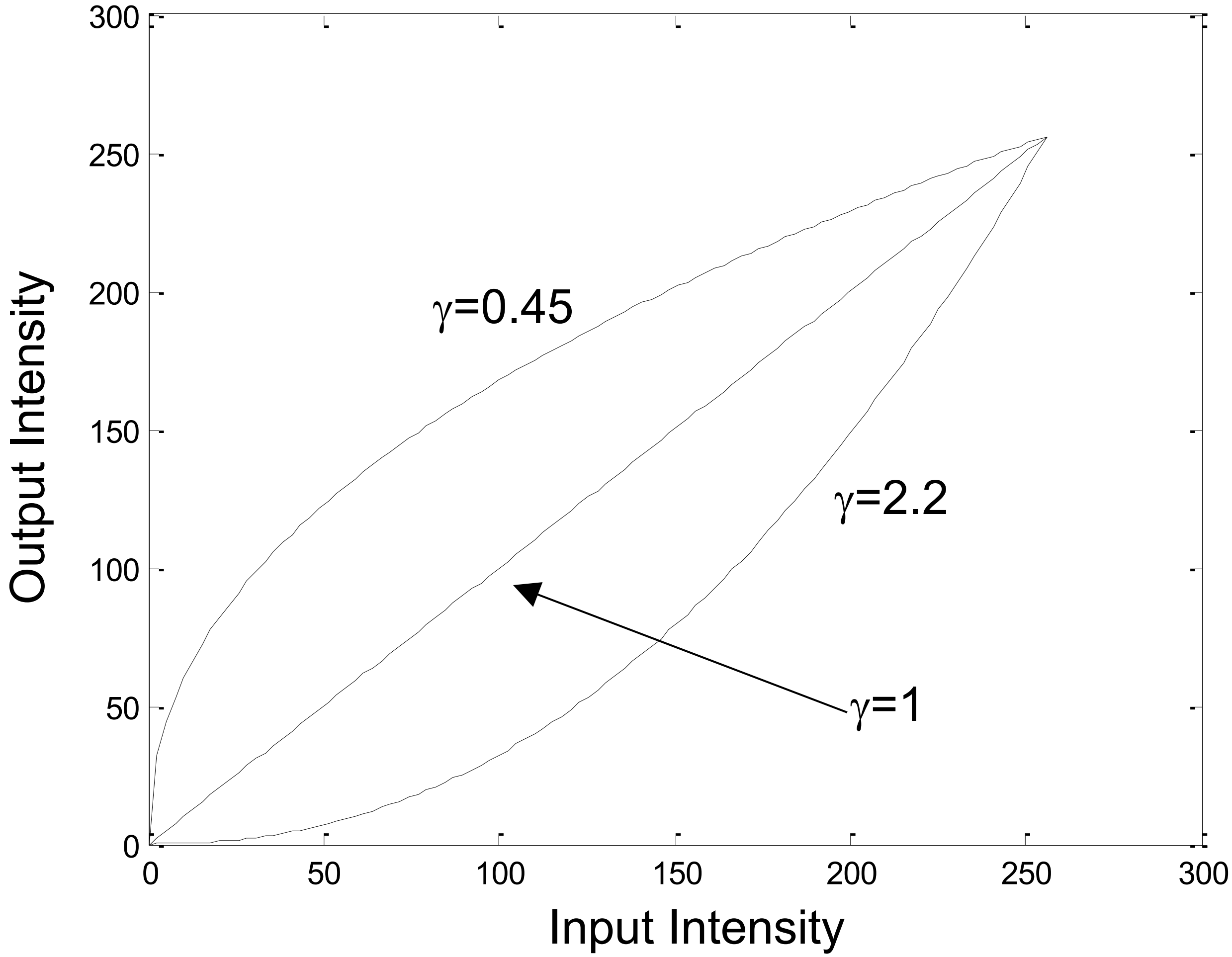
(874 / 19200)



(6400 / 18432)

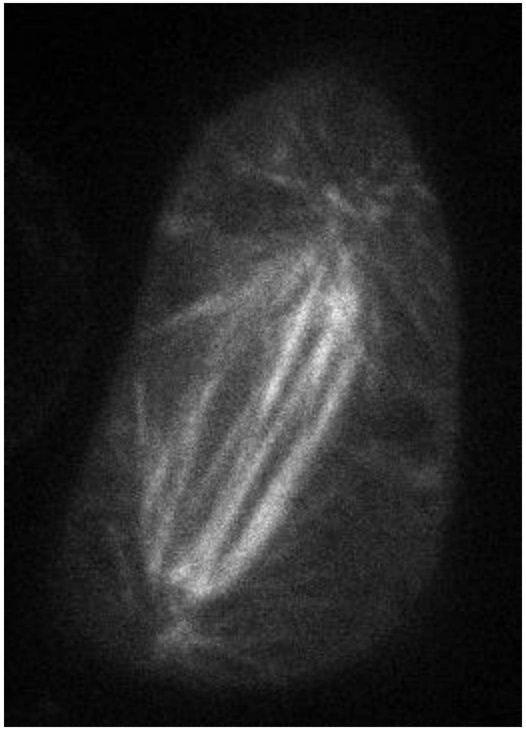
Drosophila S2 cell with mCherry-tubulin (Nico Stuurman)

Gamma correction

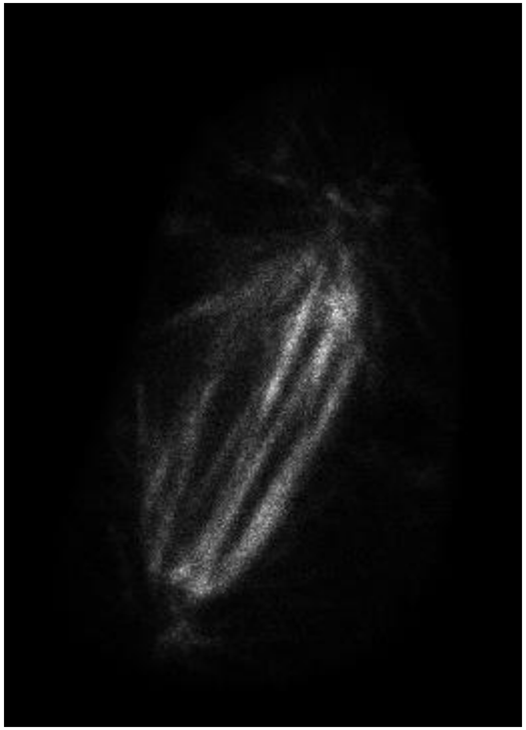


Other contrast stretching transforms….

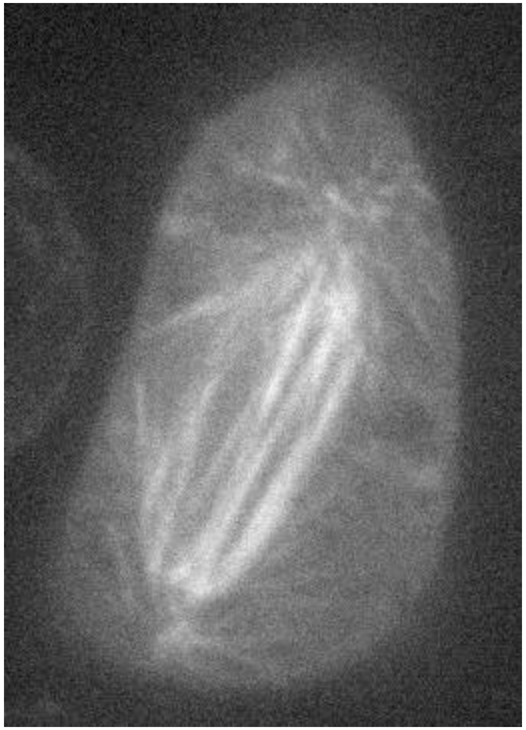
Effect of gamma



Scaled to min / max
(874 / 25438), $\gamma = 1$



Scaled to min / max
(874 / 25438), $\gamma = 2.16$



Scaled to min / max
(874 / 25438), $\gamma = 0.45$

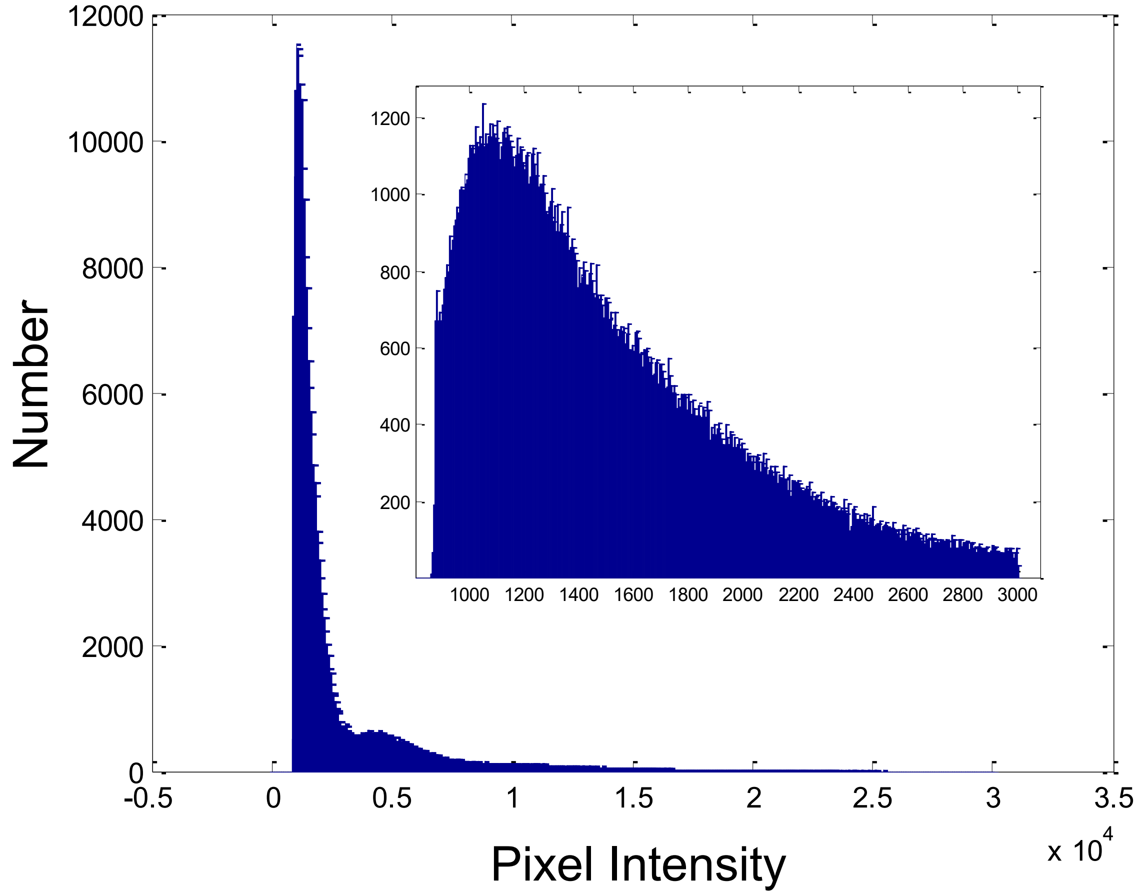
What are acceptable image manipulations?

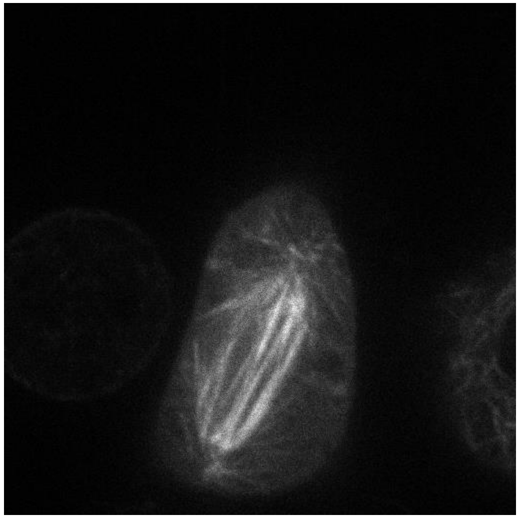
- JCB has the best guidelines
  - http://jcb.rupress.org/misc/ifora.shtml#image_aquisition
  - http://jcb.rupress.org/cgi/content/full/166/1/1
- Brightness and contrast adjustments ok, so long as done over whole image and don't obscure or eliminate background
- Nonlinear adjustments (like gamma) must be disclosed
- No cutting and pasting of regions within an image (e.g. individual cells)

Background correction

- Cameras have a non-zero offset
- There can also be background fluorescence due to media autofluorescence, etc.
- Want to correct for this by background subtraction
  - Camera dark image
  - Estimate background from image

Estimating background from image

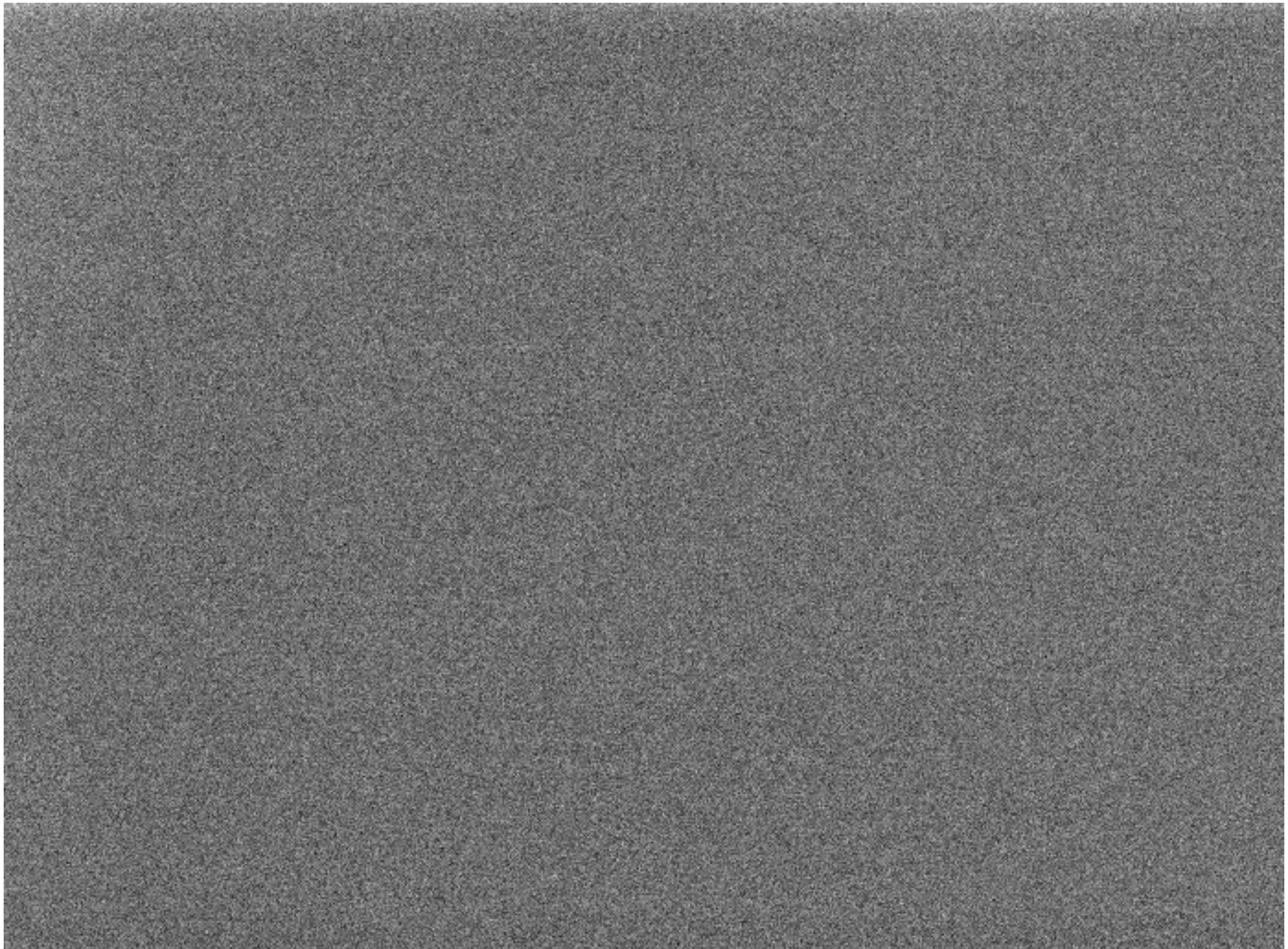




Dark image

- Acquired with no light going to the camera
  - Allows you to measure instrument background
  - Can detect what’s real background autofluorescence

Dark image



Shading correction

- Measure and correct for nonuniformity in illumination and detection
- Image a uniform fluorescent sample

Shading correction

Correction procedure

$I_{meas} = I_{true} * \text{Shading} + \text{Dark}$

$I_{true} = (I_{meas} – \text{Dark}) / \text{Shading}$

Good to do on all images

More formally…

$$I(x,y) = F(x,y) * Ex(x,y) * Det(x,y) + Dark(x,y)$$

Fluorophore distribution

Excitation Intensity distribution

Detection efficiency

If no photobleaching, then Shading = Ex(x,y)*Det(x,y) is a good approximation

If photobleaching…

I(x,y) = (F(x,y) / k*Ex(x,y)) * [1-exp(-k*Ex(x,y)*t)] * Det(x,y) + Dark(x,y)

Yuck

If you have significant photobleaching and significant excitation non-uniformity, shading correction breaks down

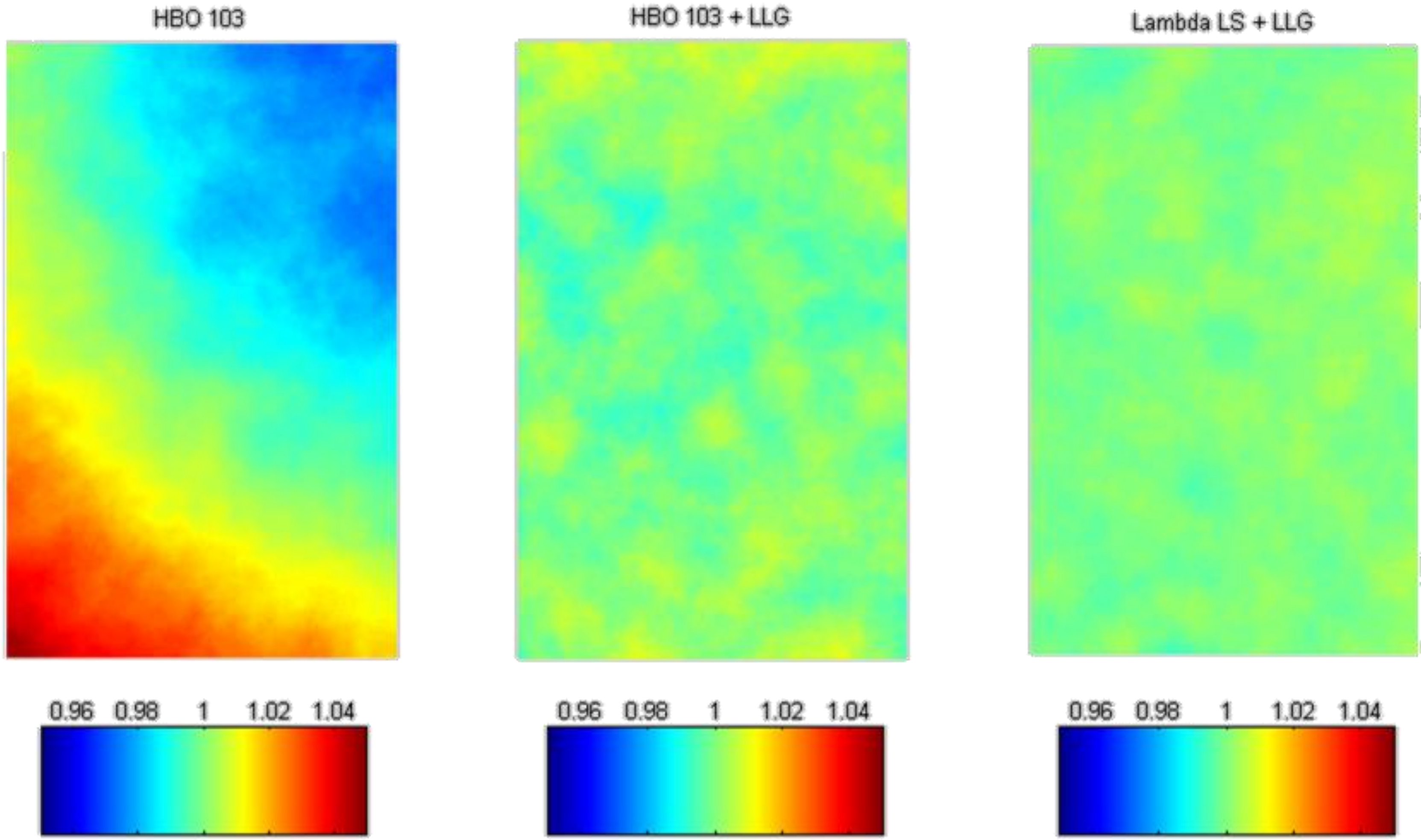
Can actually lead to contrast inversion (!)

Measuring Excitation and Detection Distributions

- Take photobleaching time-series on uniform thin-film of fluorophore
- Photobleaching rate at each pixel gives intensity
- Variance not explained by intensity distribution gives detector sensitivity distribution

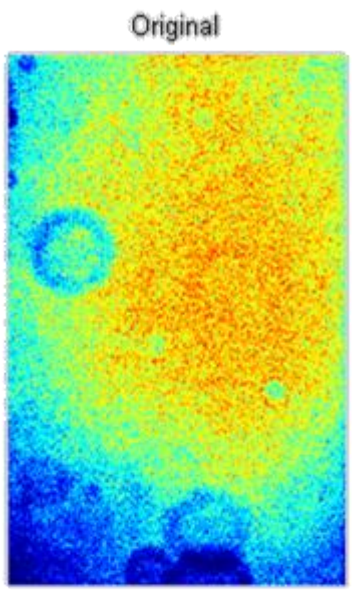
Zweir et al. 2004, Ghauharali et al. 1998,1999

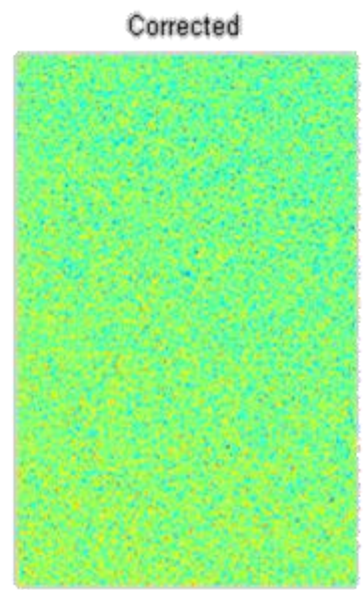
Illumination Uniformity



Correcting

- Once excitation distribution is relatively flat, correction for detector distribution is once again linear.





Back to digital image analysis

- Filering

1	1	1	
1	1	1	Averaging / Smoothing
1	1	1	

0	1	2	1	0	
1	6	10	6	1	
2	10	16	10	2	Gaussian smoothing
1	6	10	6	1	
0	1	2	1	0	

How this works

1	1	1
1	1	1
1	1	1

$$(10+11+22+13+8+10+20+20+15)/9 = 14$$

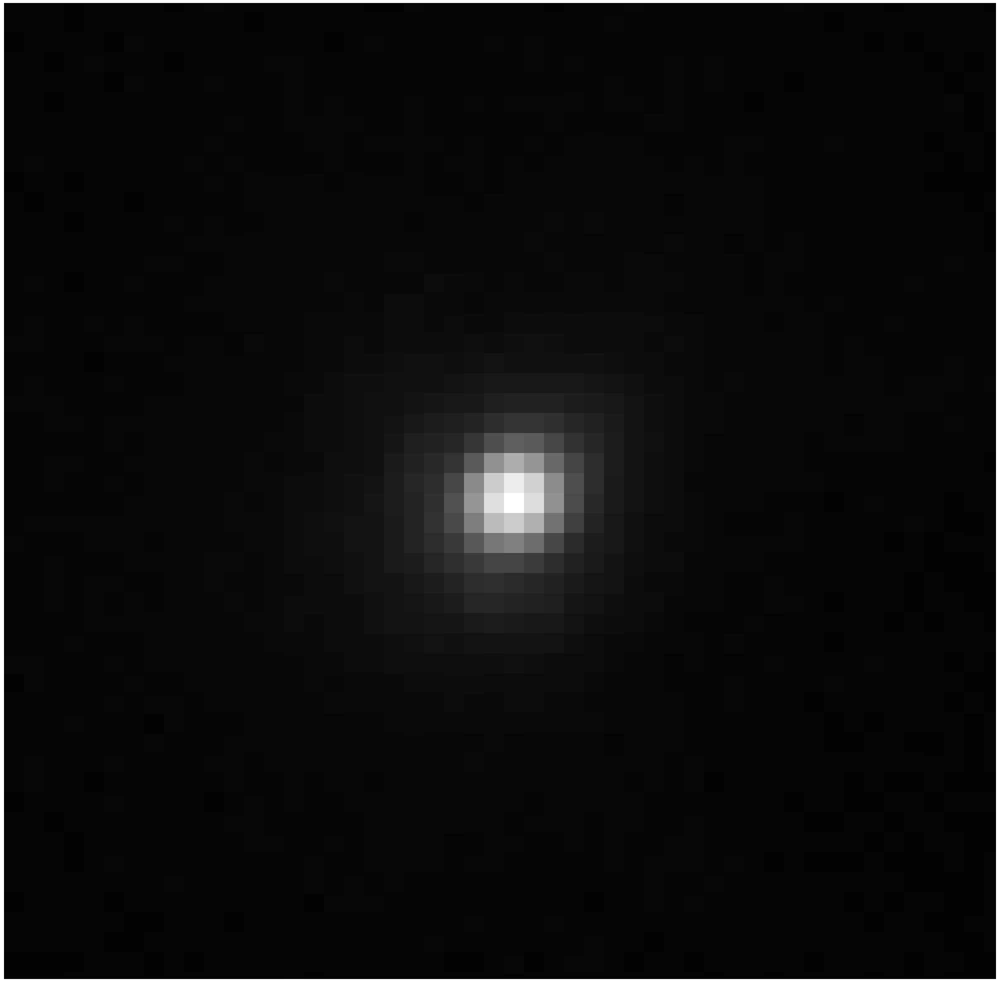
Multiply corresponding
pixels and sum

10	11	22	5	7
13	14	10	5	24
20	20	15	23	14
0	3	17	15	8
7	11	6	15	12

Why smooth?

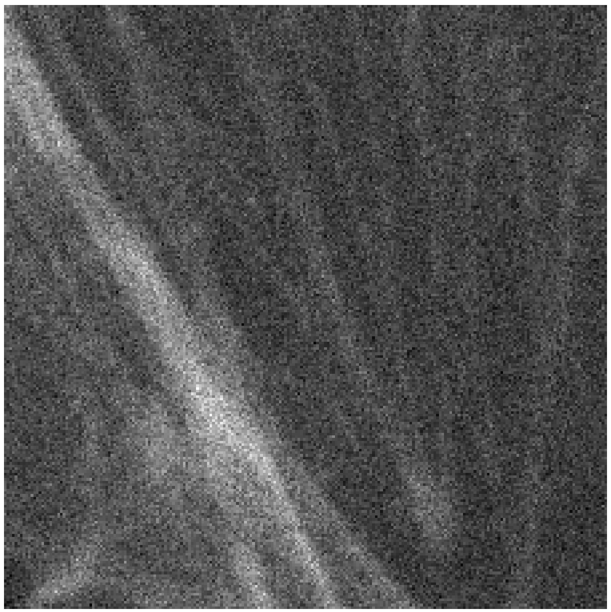
- If your image is sampled appropriately (at Nyquist) the point spread function will be spread out over multiple pixels
- Properly exploiting this redundancy requires deconvolution
- But smoothing helps
- Also reduces single pixel noise artifacts that can't be real

Actual PSF



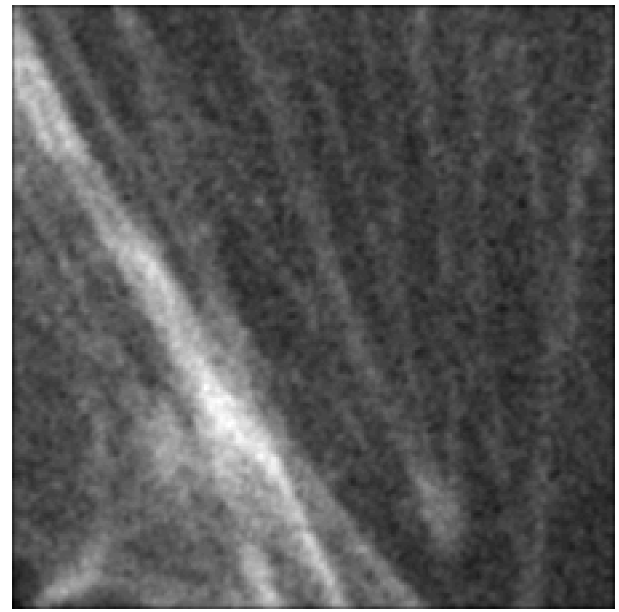
Why smooth?

- Averages redundancy and suppresses noise



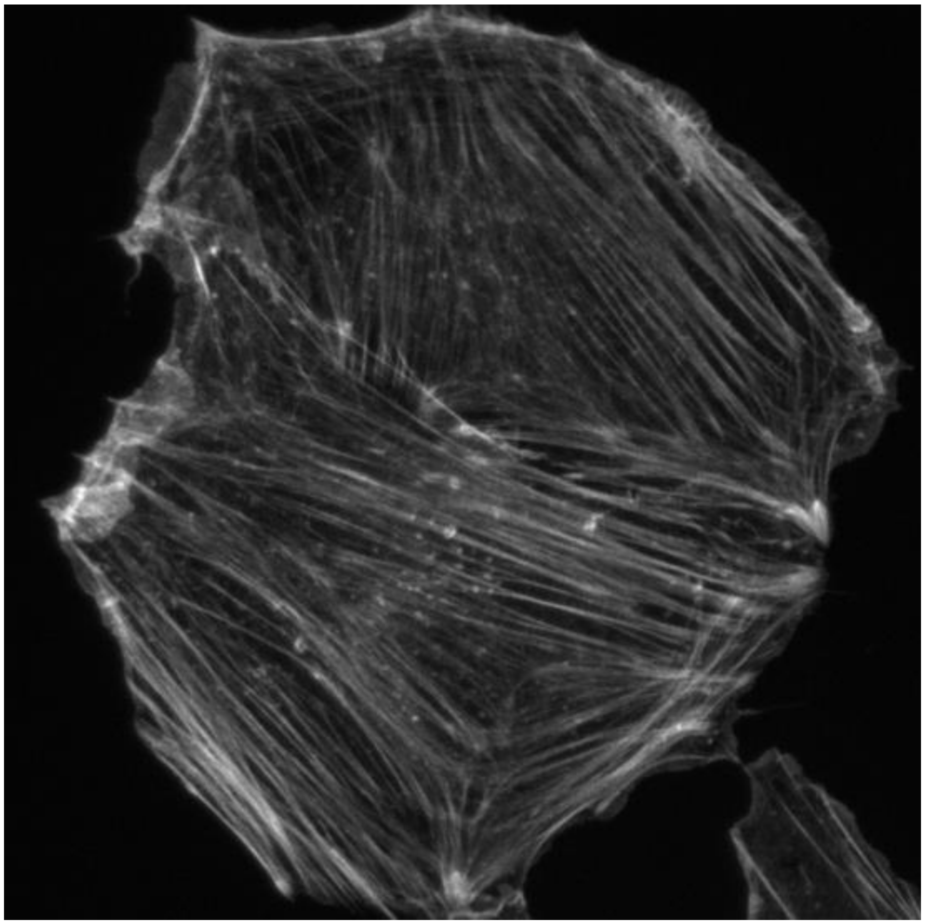
10 photons/pixel average

5 e- read noise

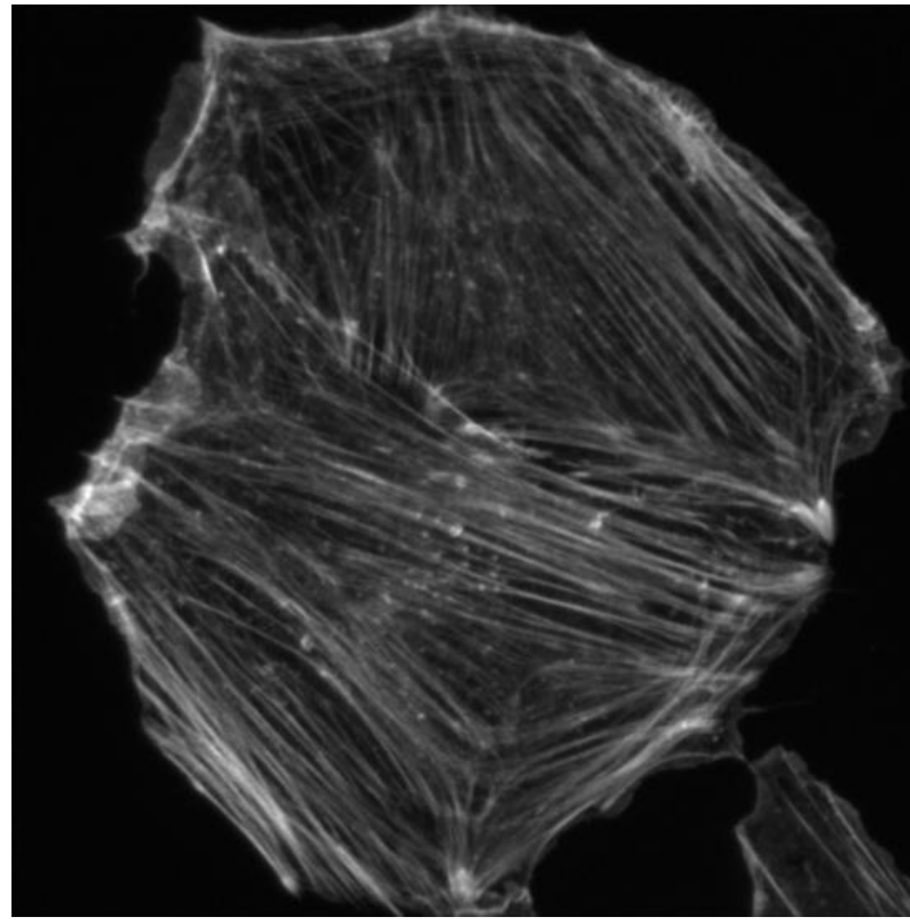


Gaussian smoothing filter, σ = 1 pixel

Smoothing



Original



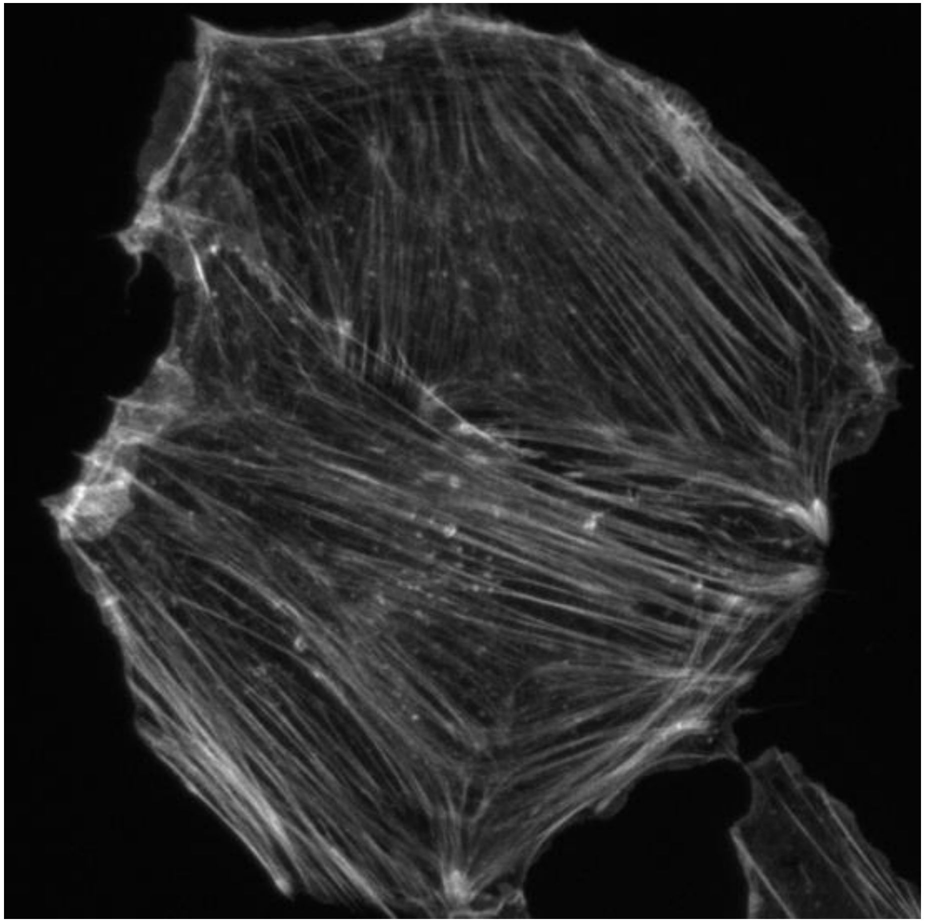
Gaussian filtered, $\sigma = 0.6$

Other filters

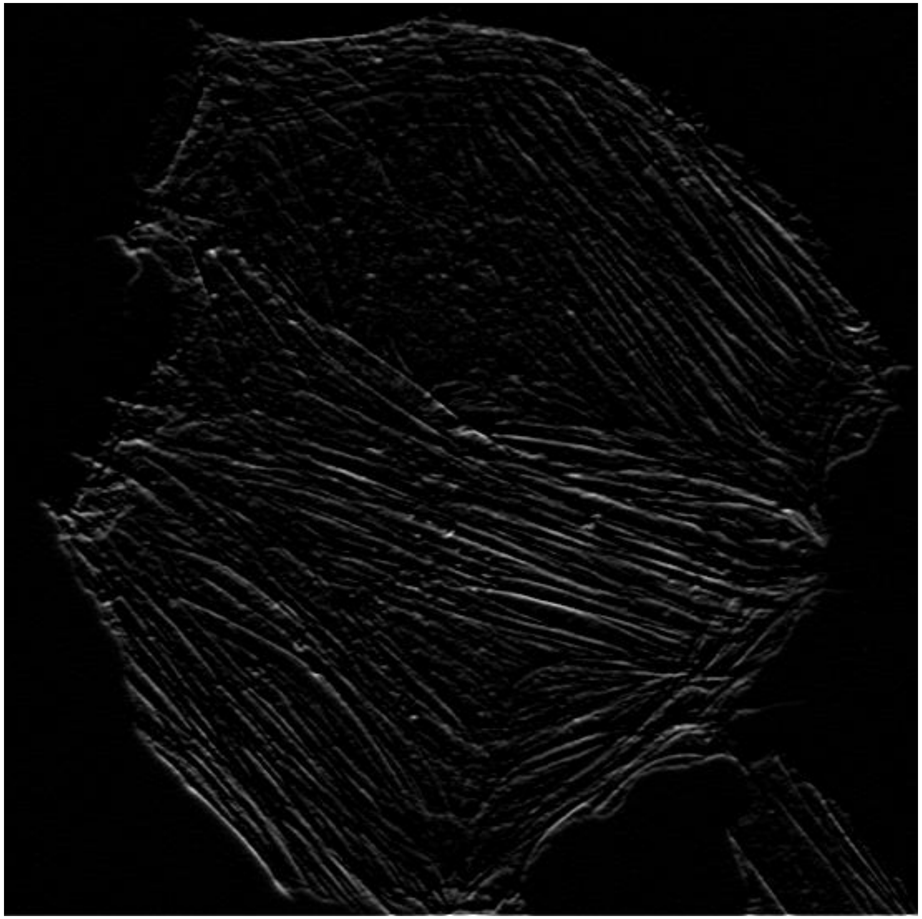
- Edge Detection

1	1	1		1	2	1
0	0	0		0	0	0
-1	-1	-1		-1	-2	-1

Edge Detection



Original



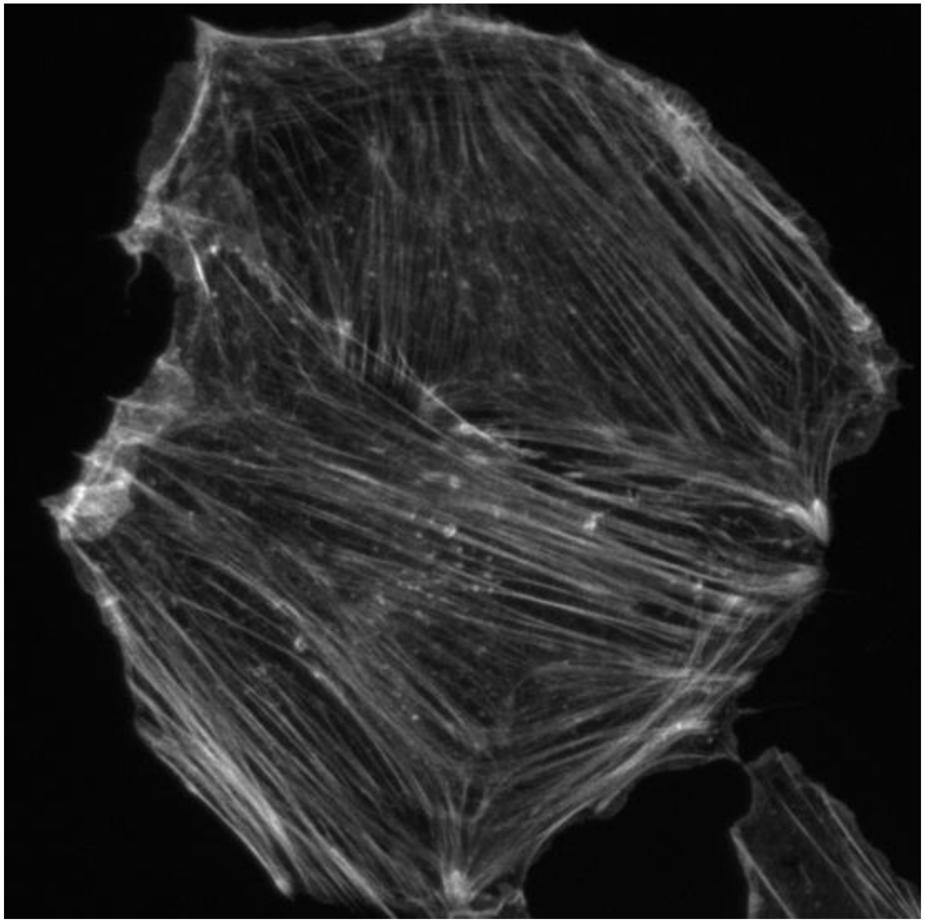
Horizontal edge detection

Other filters

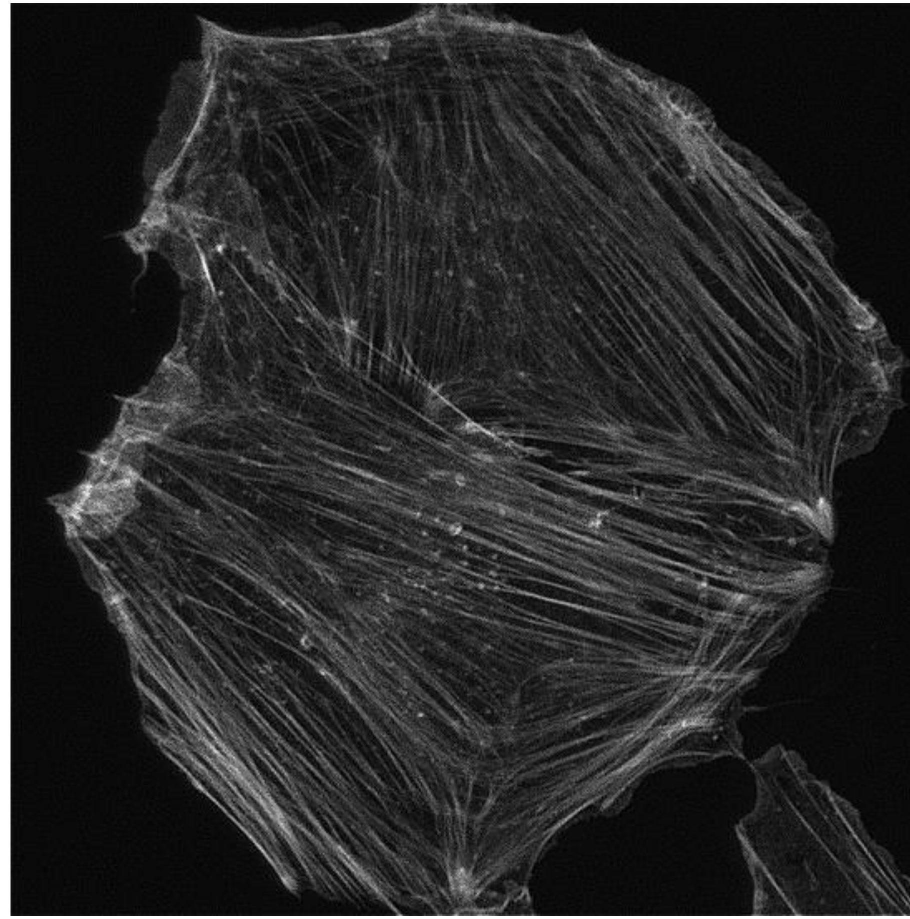
- Unsharp masking

-1	-4	-1
-4	26	-4
-1	-4	-1

Unsharp Masking



Original

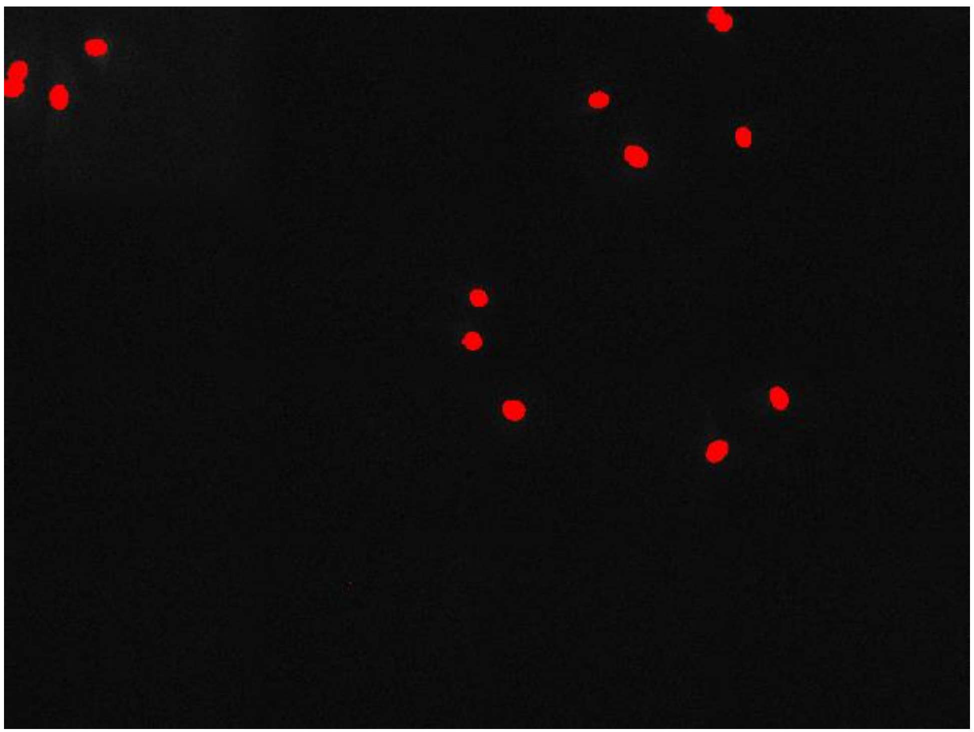


Unsharp masked

Nonlinear filters

- Can do things like median filtering – replace center pixel by median value within box
- Good for smoothing while maintaining edges

Thresholding



Pixel Intensity

One problem with this approach…

- It’s biased towards brighter objects
- Ideally would use second channel to independently define objects to measure

Binary images

- Thresholding gives you a binary image; 1 inside an object, 0 elsewhere
- This can be used to identify objects
- It can also be manipulated

Binary operations: dilation and erosion

0	0	0	0	0	0	0
0	0	0	1	0	0	0
0	0	1	1	1	0	0
0	0	1	1	1	1	0
0	1	1	1	1	0	0
0	0	1	1	0	0	0
0	0	0	0	0	0	0

$\xrightarrow{\text{erode}}$

0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	1	0	0	0
0	0	0	1	1	0	0
0	0	1	1	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

Binary operations: dilation and erosion

0	0	0	0	0	0	0
0	0	0	1	0	0	0
0	0	1	1	1	0	0
0	0	1	1	1	1	0
0	1	1	1	1	0	0
0	0	1	1	0	0	0
0	0	0	0	0	0	0

dilate →

0	0	0	1	0	0	0
0	0	1	1	1	0	0
0	1	1	1	1	1	0
0	1	1	1	1	1	1
1	1	1	1	1	1	0
0	1	1	1	1	0	0
0	0	1	1	0	0	0

Other binary operations

- Sequential erosion and dilation – tends to smooth objects
- Hole filling
- Removing objects at borders

Further reading

- Lectures available online: nic.ucsf.edu/edu.html

- Gonzalez, Woods, and Eddins: Digital Image Processing (Using Matlab)
- Pawley: Handbook of Biological Confocal Microscopy