Advanced Image Analysis

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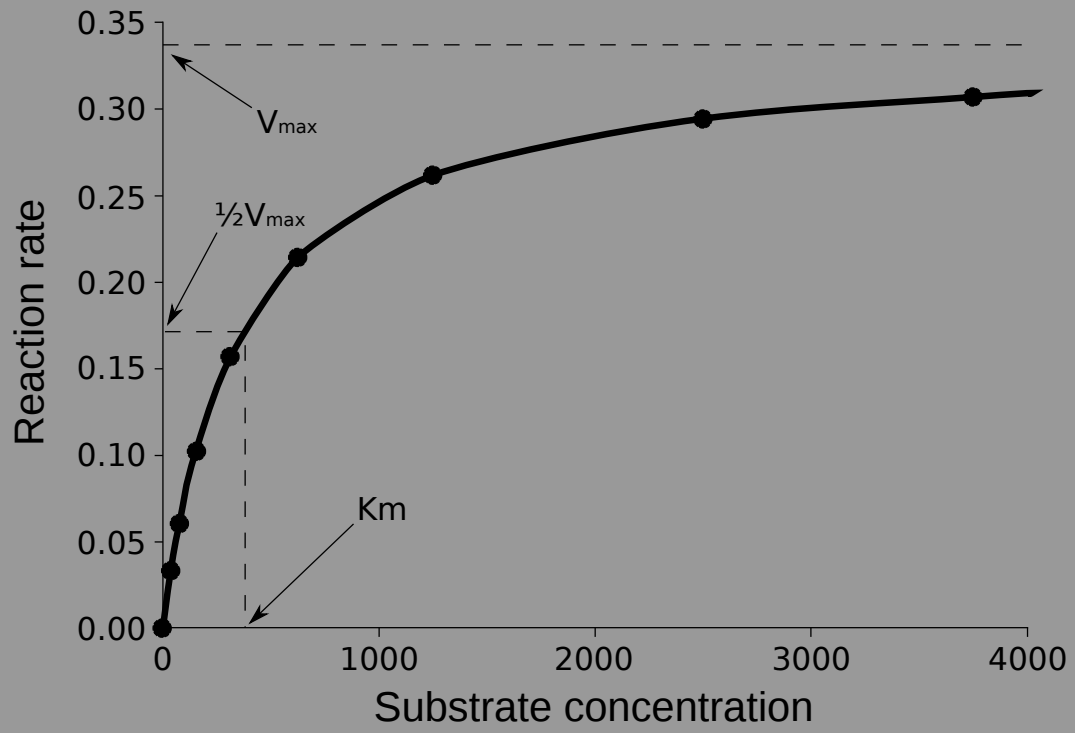
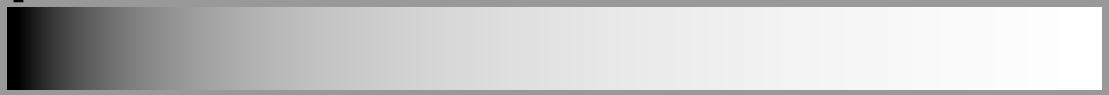
Advanced BioImaging Unit
(the basement)

Micron Advanced Microscopy Course, 2015

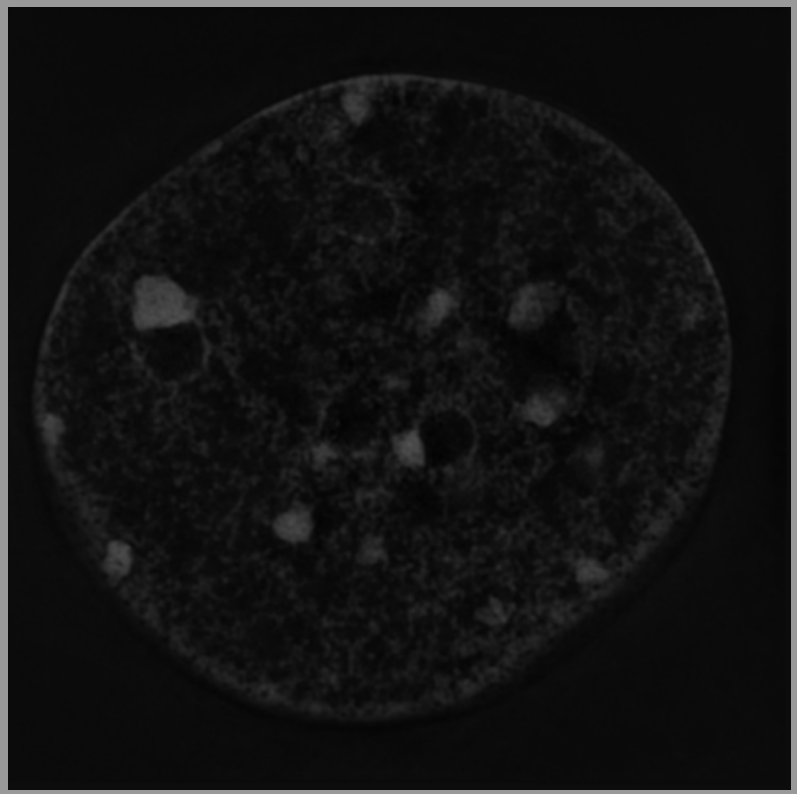
Images as Signals

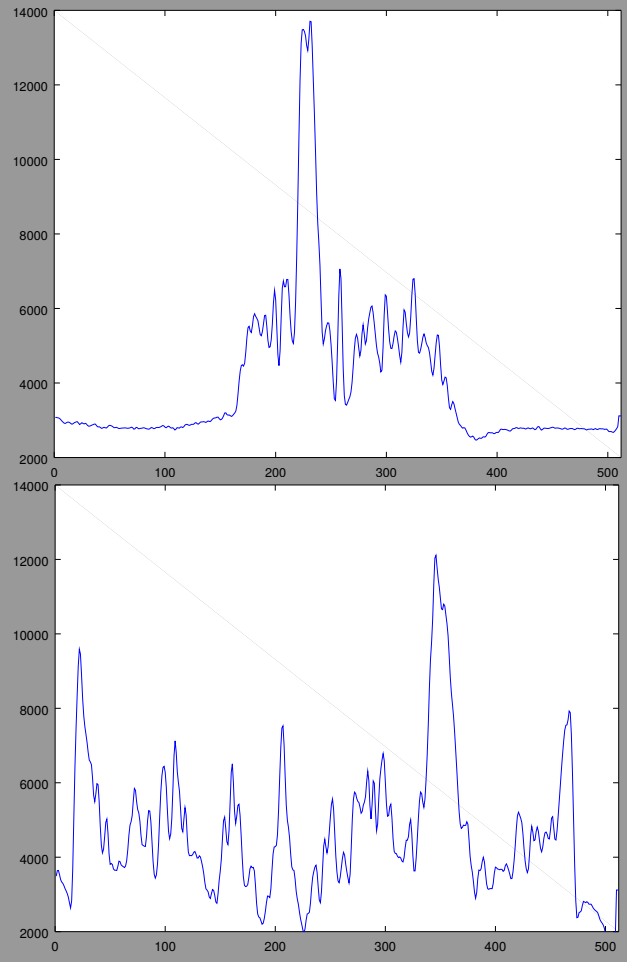


[0.000 0.175 0.233 0.263 0.280 0.292 0.300 0.306 0.311]

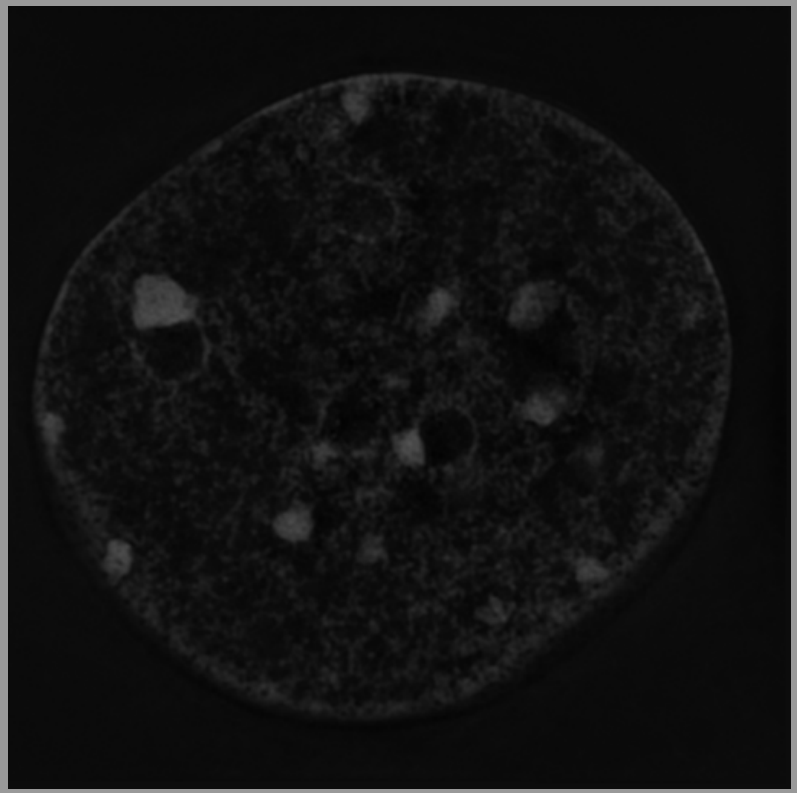


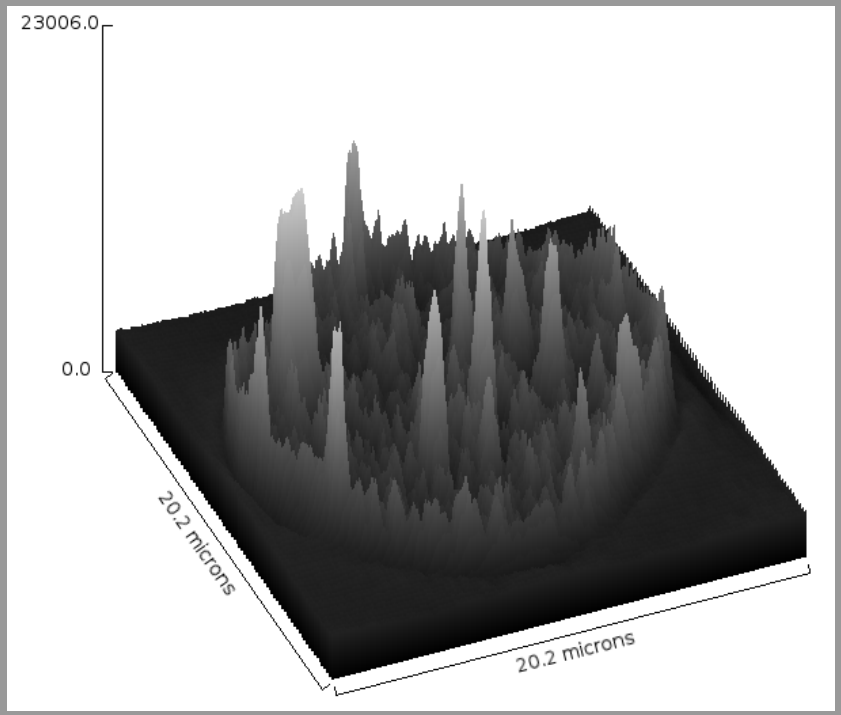
Images as Signals



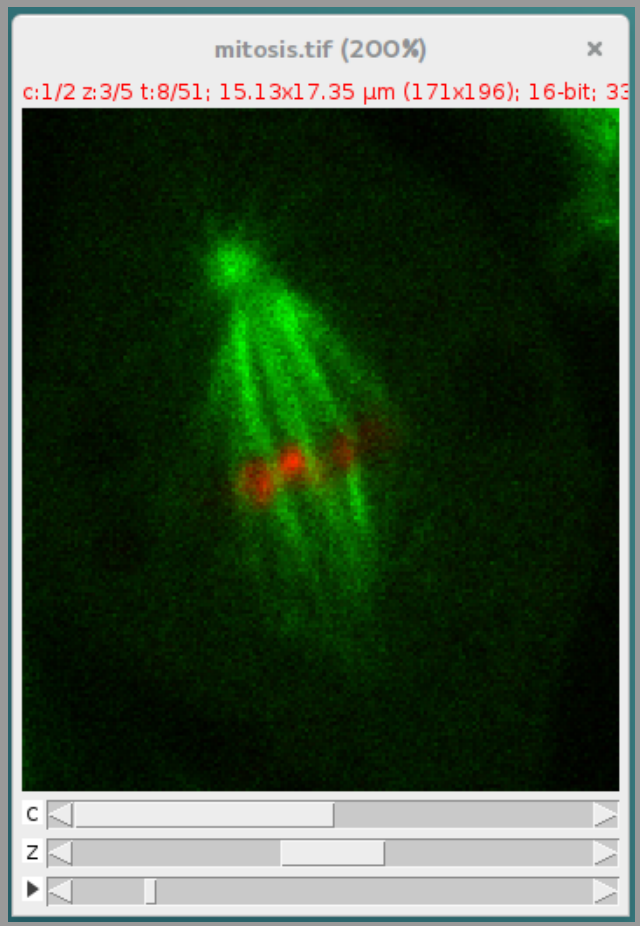


Images as Surfaces





Images as ND Arrays

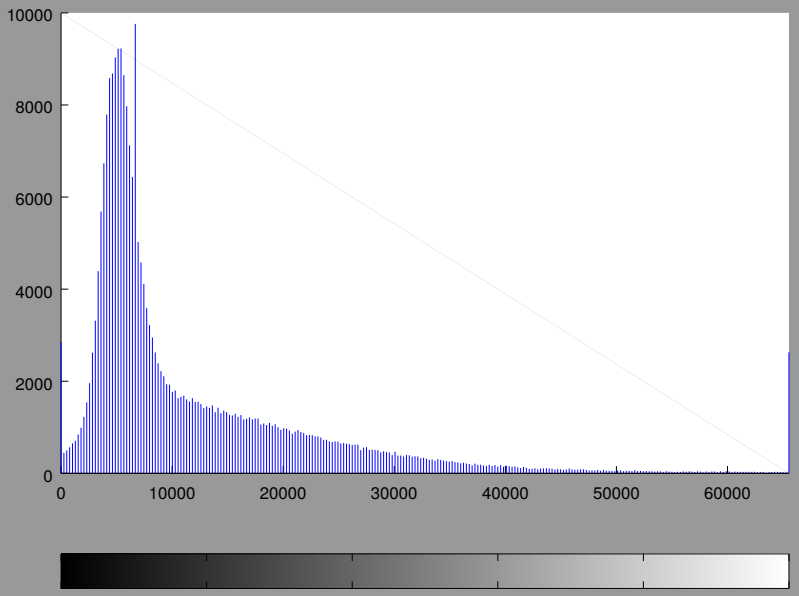


- x and y
- time
- z (volume)
- wavelength
- phase
- stage angle

Think "data", not "picture"

Bit depth / dynamic range

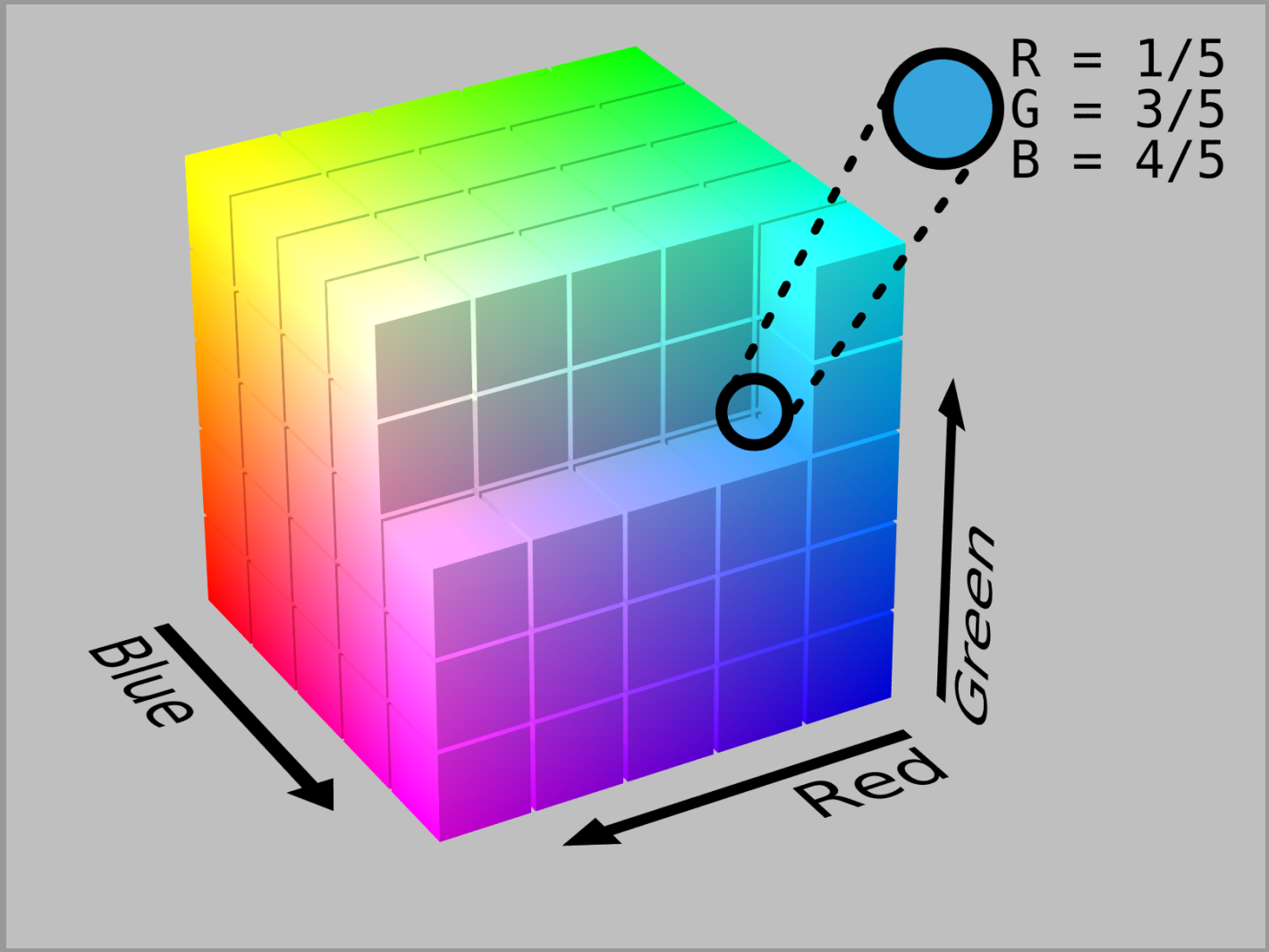
2	2
$2 * 2$	4
$2 * 2 * 2$	8
...	
2^7	128
2^8	256
2^9	512
2^{10}	1024
2^{11}	2048
...	
2^{15}	32768
2^{16}	65536



More pixels in the same bin, less dynamic range.

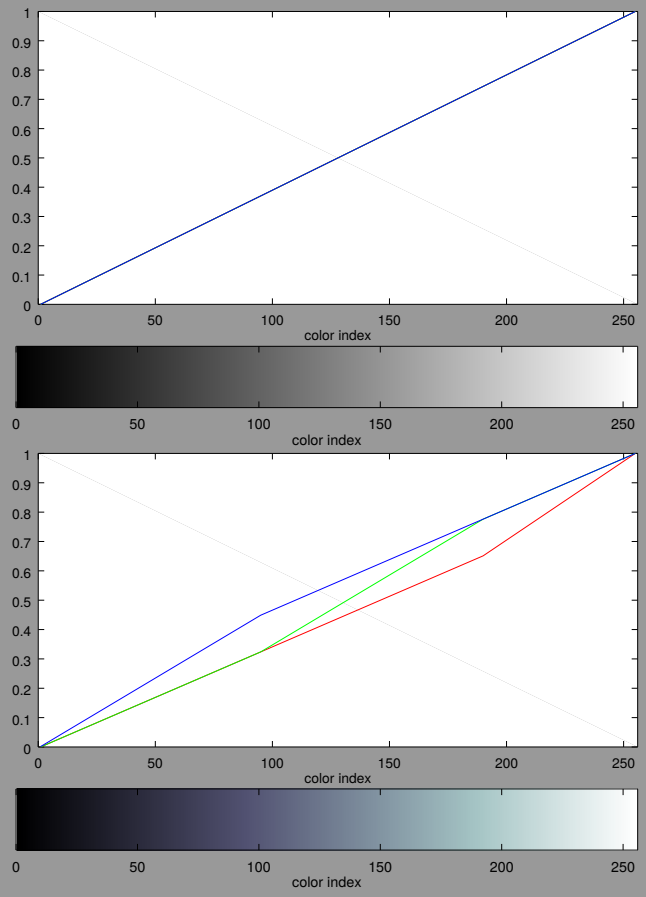
There is actually no limit.

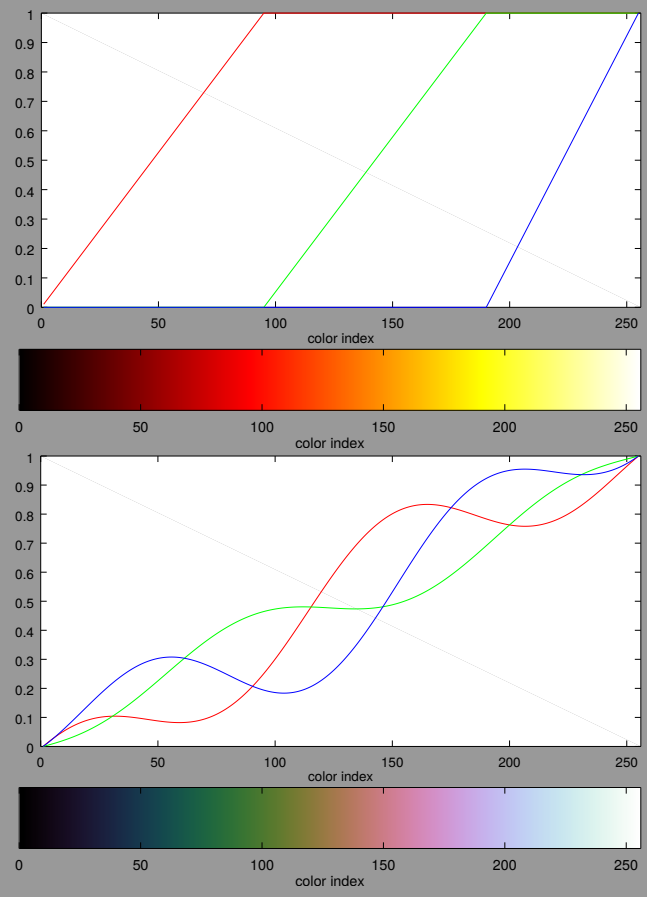
RGB



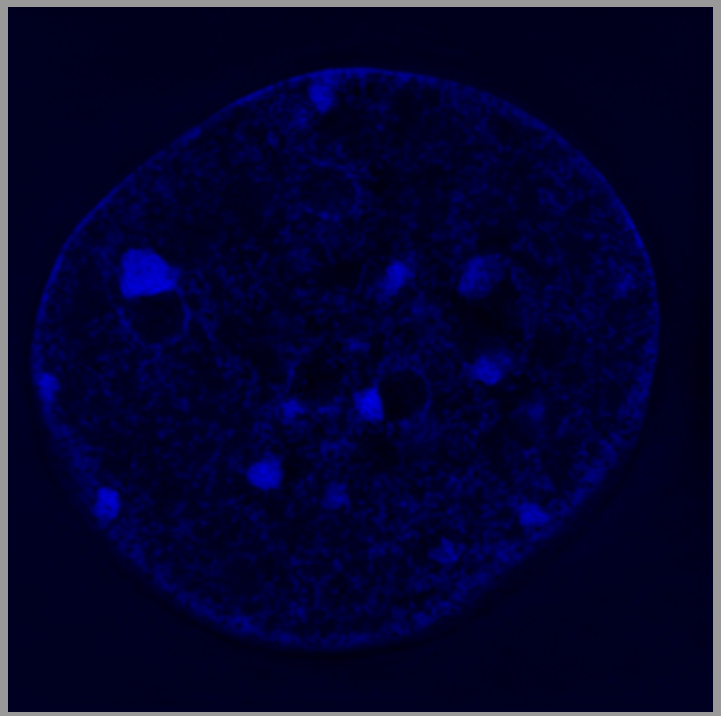
If you ever get an RGB image, you did something wrong.

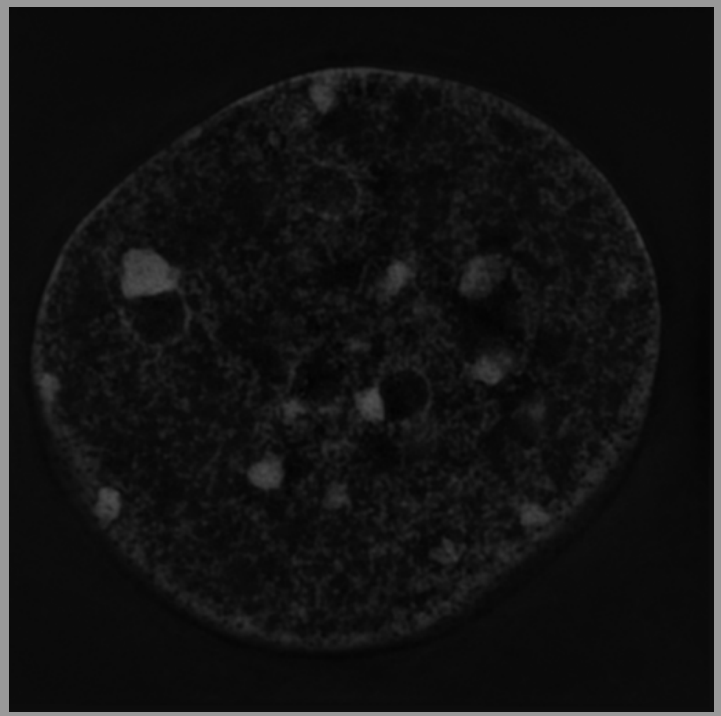
Lookup tables or colormaps





Old faithful





Typical problems

Concentration

Protein expression, number of complexes.

Co-localization

Do two overlap and correlate?

Dynamics

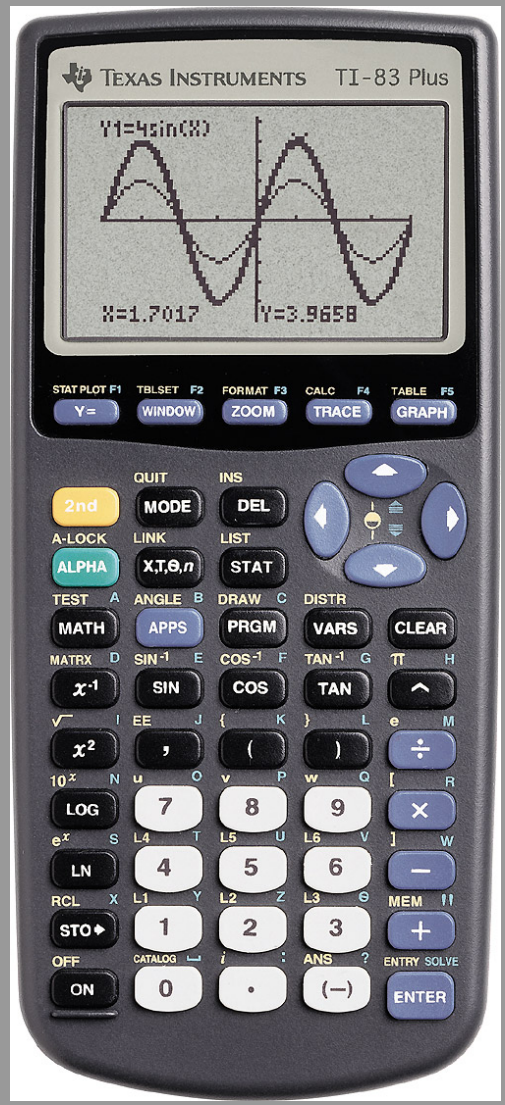
How fast does it move?

All require identifying a region of interest.

Logical (binary) images

Very useful as masks

Tools for image analysis



Tools for image analysis

ImageJ / FIJI

Python with NumPy

Octave

R

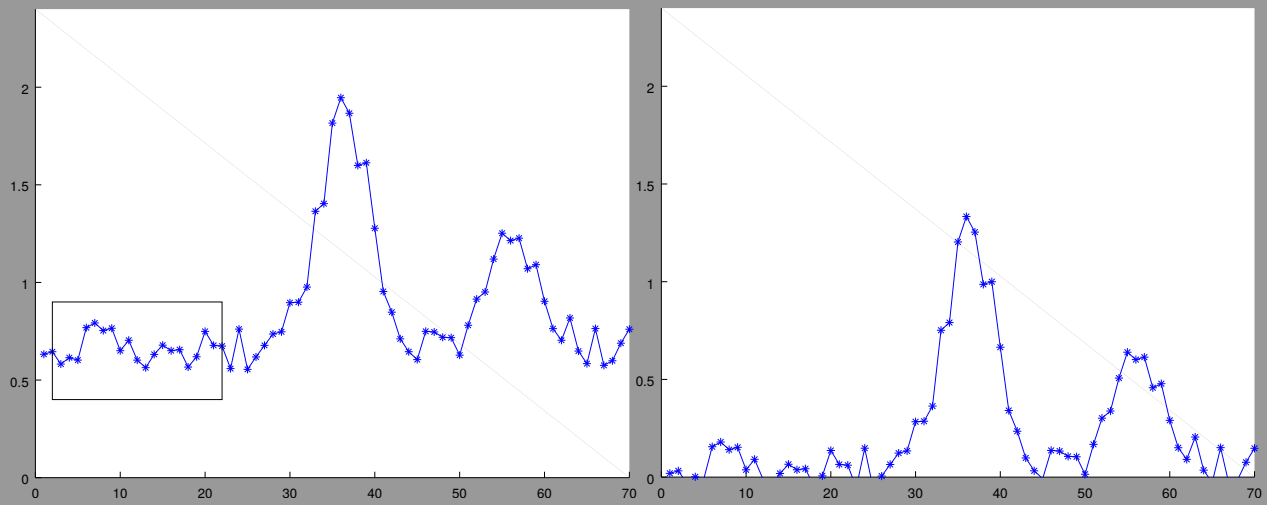
- CellProfiler
- Icy
- Omero
- Imaris
- softWoRx
- Volocity
- Matlab
- Metamorph
- Image-Pro Plus
- Huygens
- Mathematica
- ...

Files and metadata

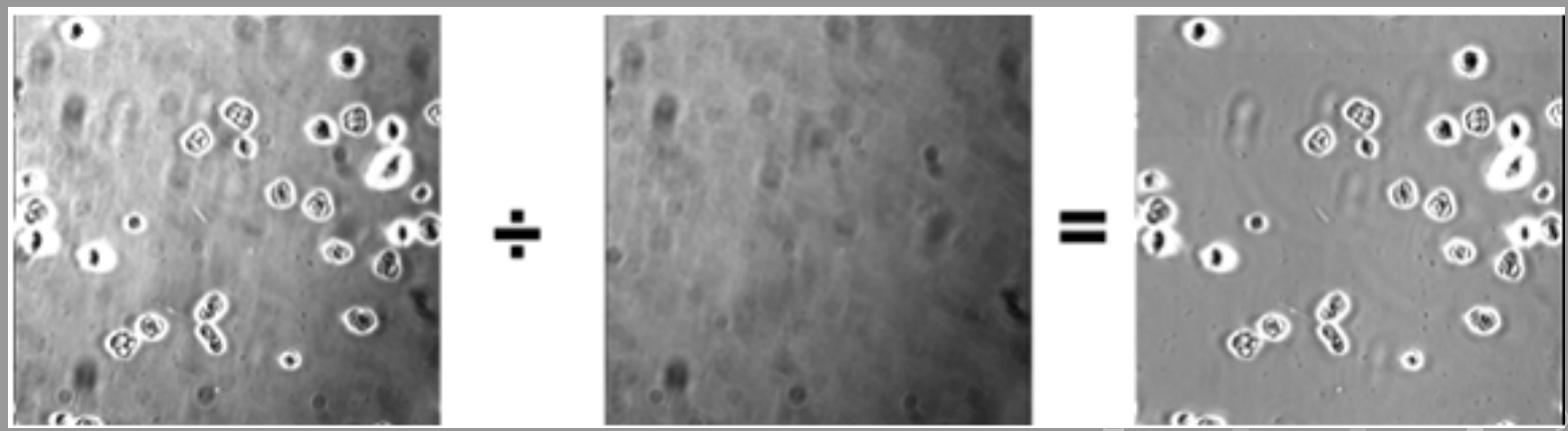
- why some many formats?
- bioformats and ome-tiff
- metadata saved may be format dependent
- use libraries and applications that respect you

Background correction

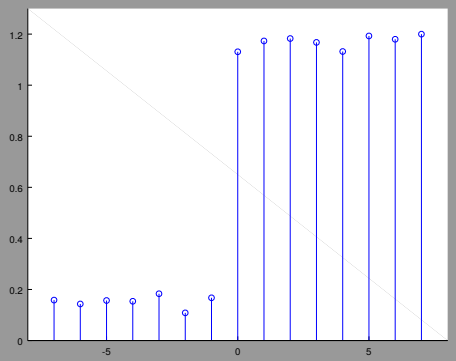
- Subtract mean of a known background region (darks).
- Many cameras (not-microscopes) do this.



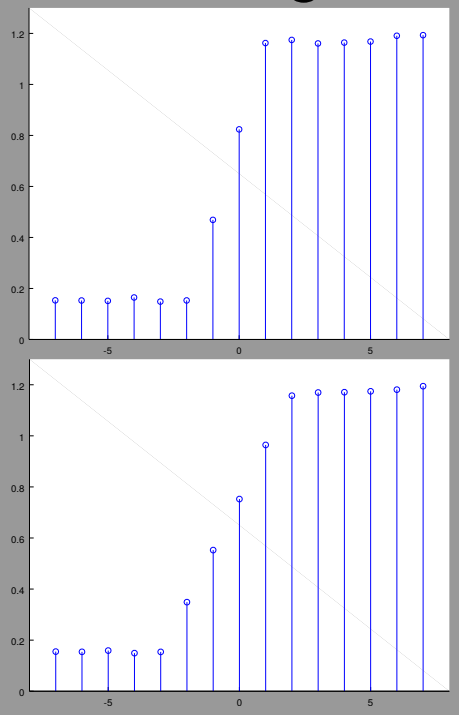
- Correction for uneven illumination (divide by flats)



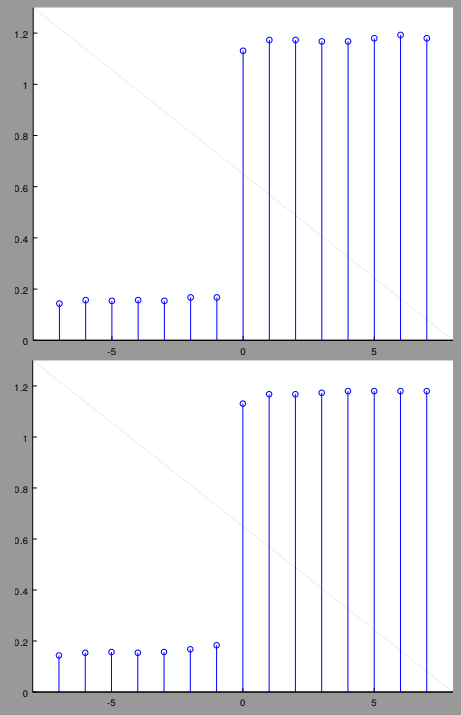
Local means



Average

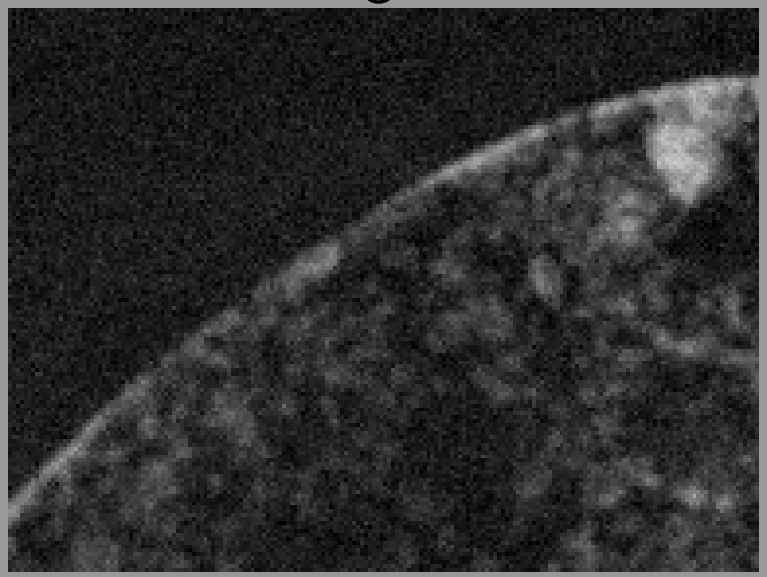


Median

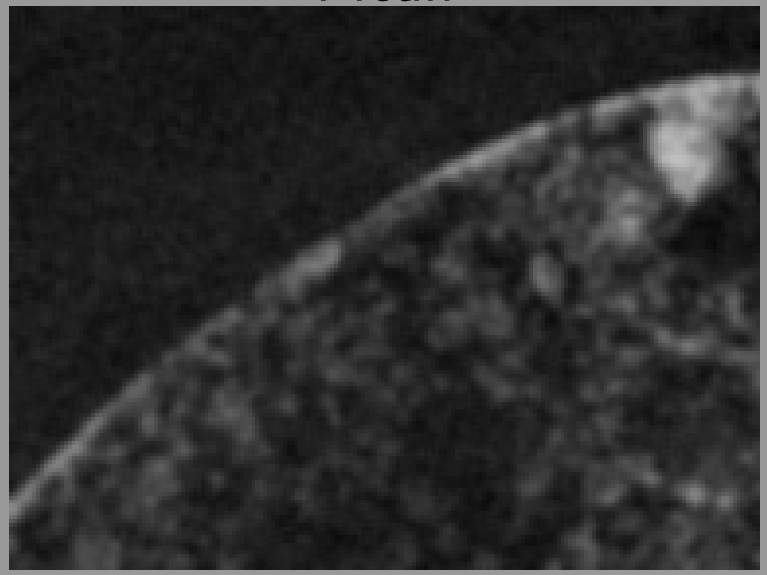


Local means

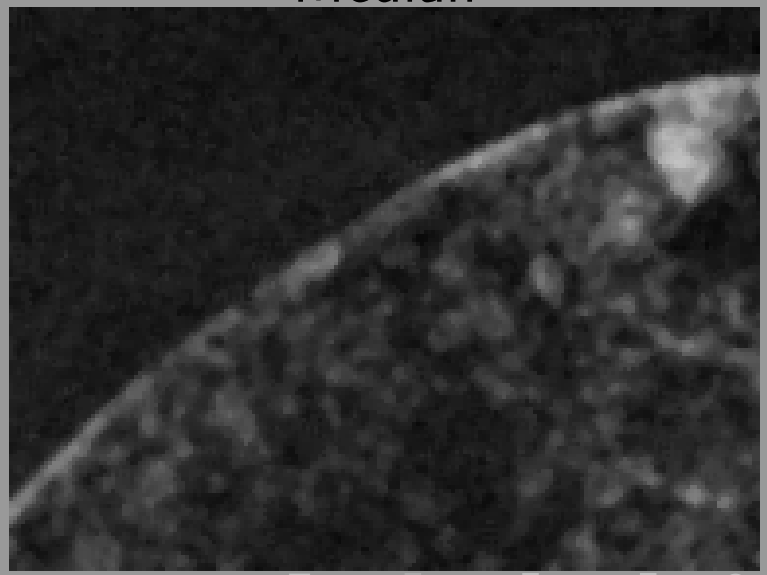
Original



Mean

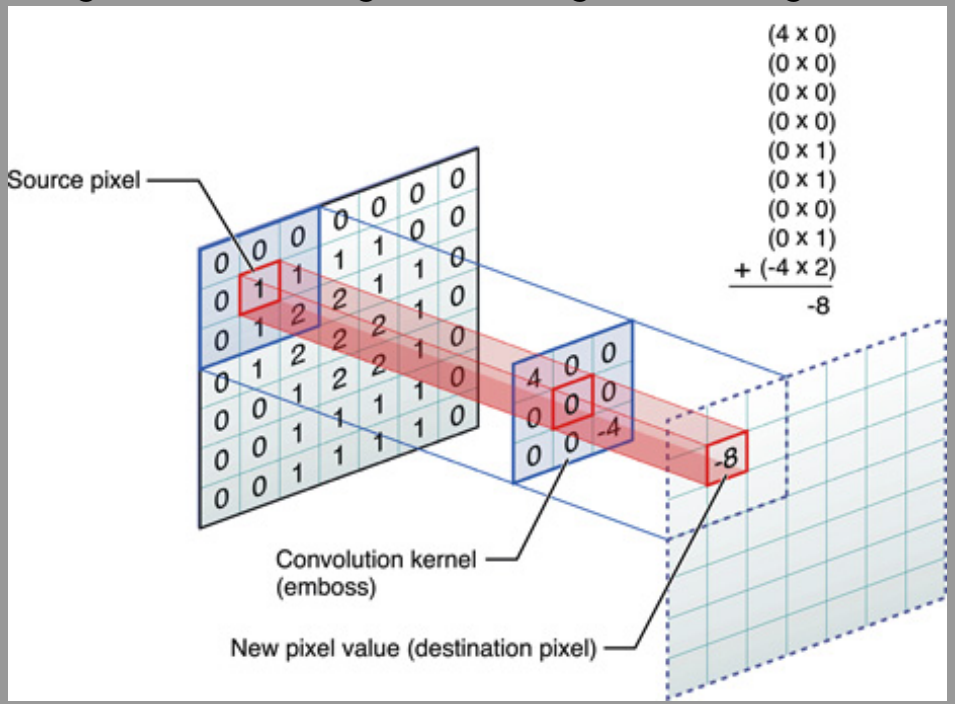


Median



Mean as convolution kernel

$$\frac{4+5+6}{3} = 4 \times \frac{1}{3} + 5 \times \frac{1}{3} + 6 \times \frac{1}{3}$$



$$\begin{bmatrix} 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \end{bmatrix}$$

3x3 mean kernel

$$\begin{bmatrix} 1/25 & 1/25 & 1/25 & 1/25 \\ 1/25 & 1/25 & 1/25 & 1/25 \\ 1/25 & 1/25 & 1/25 & 1/25 \\ 1/25 & 1/25 & 1/25 & 1/25 \\ 1/25 & 1/25 & 1/25 & 1/25 \end{bmatrix}$$

5x5 mean kernel

Gaussian filter

as weigthed mean

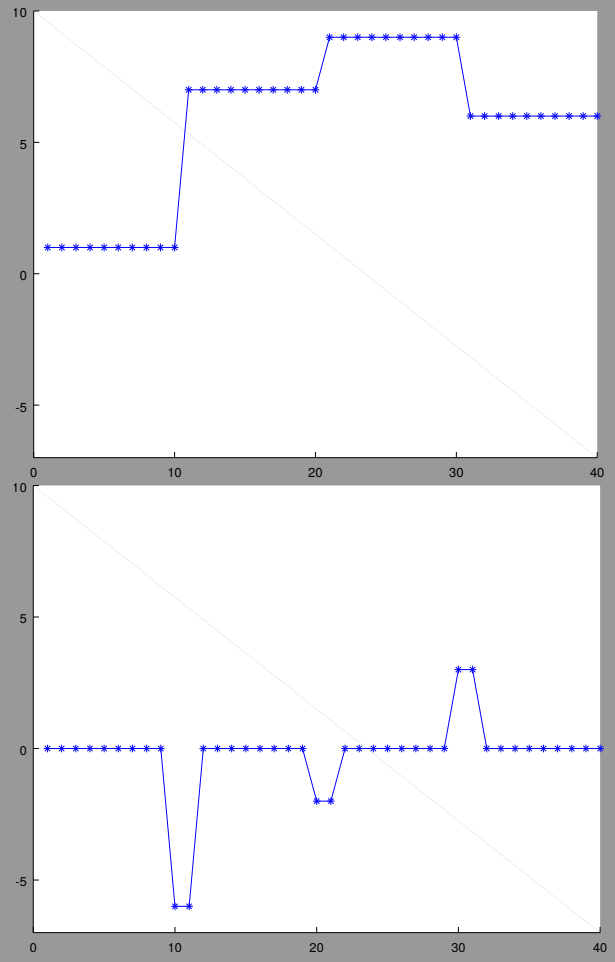
$$\begin{bmatrix} 0.011 & 0.014 & 0.017 & 0.018 & 0.017 & 0.014 & 0.011 \\ 0.014 & 0.019 & 0.023 & 0.024 & 0.023 & 0.019 & 0.014 \\ 0.017 & 0.023 & 0.027 & 0.029 & 0.027 & 0.023 & 0.017 \\ 0.018 & 0.024 & 0.029 & 0.030 & 0.029 & 0.024 & 0.018 \\ 0.017 & 0.023 & 0.027 & 0.029 & 0.027 & 0.023 & 0.017 \\ 0.014 & 0.019 & 0.023 & 0.024 & 0.023 & 0.019 & 0.014 \\ 0.011 & 0.014 & 0.017 & 0.018 & 0.017 & 0.014 & 0.011 \end{bmatrix}$$

Edge detection

Sobel operator

1D filter

$$\begin{bmatrix} -1 & 0 & +1 \end{bmatrix}$$



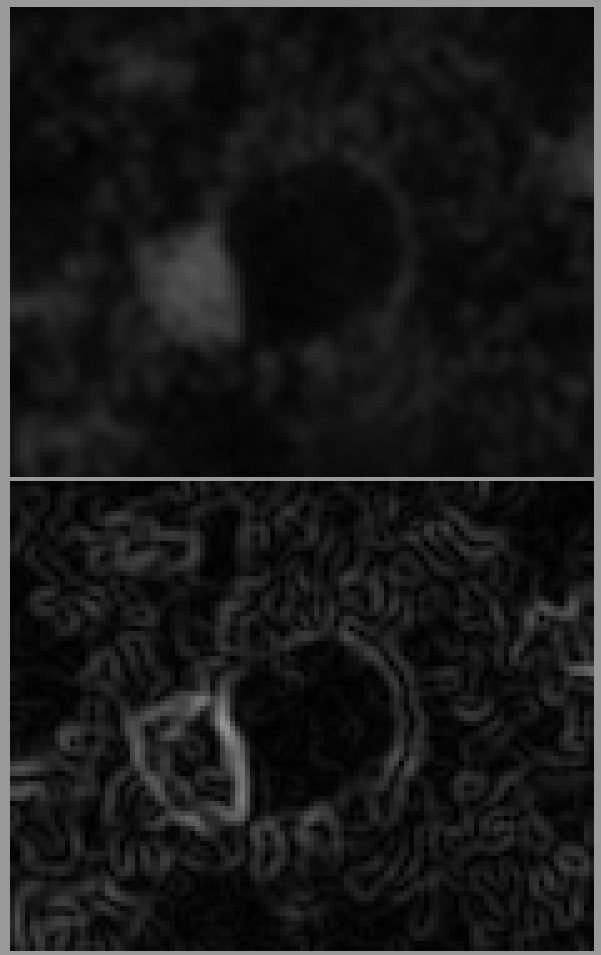
Edge detection

Sobel operator

2D filter(s)

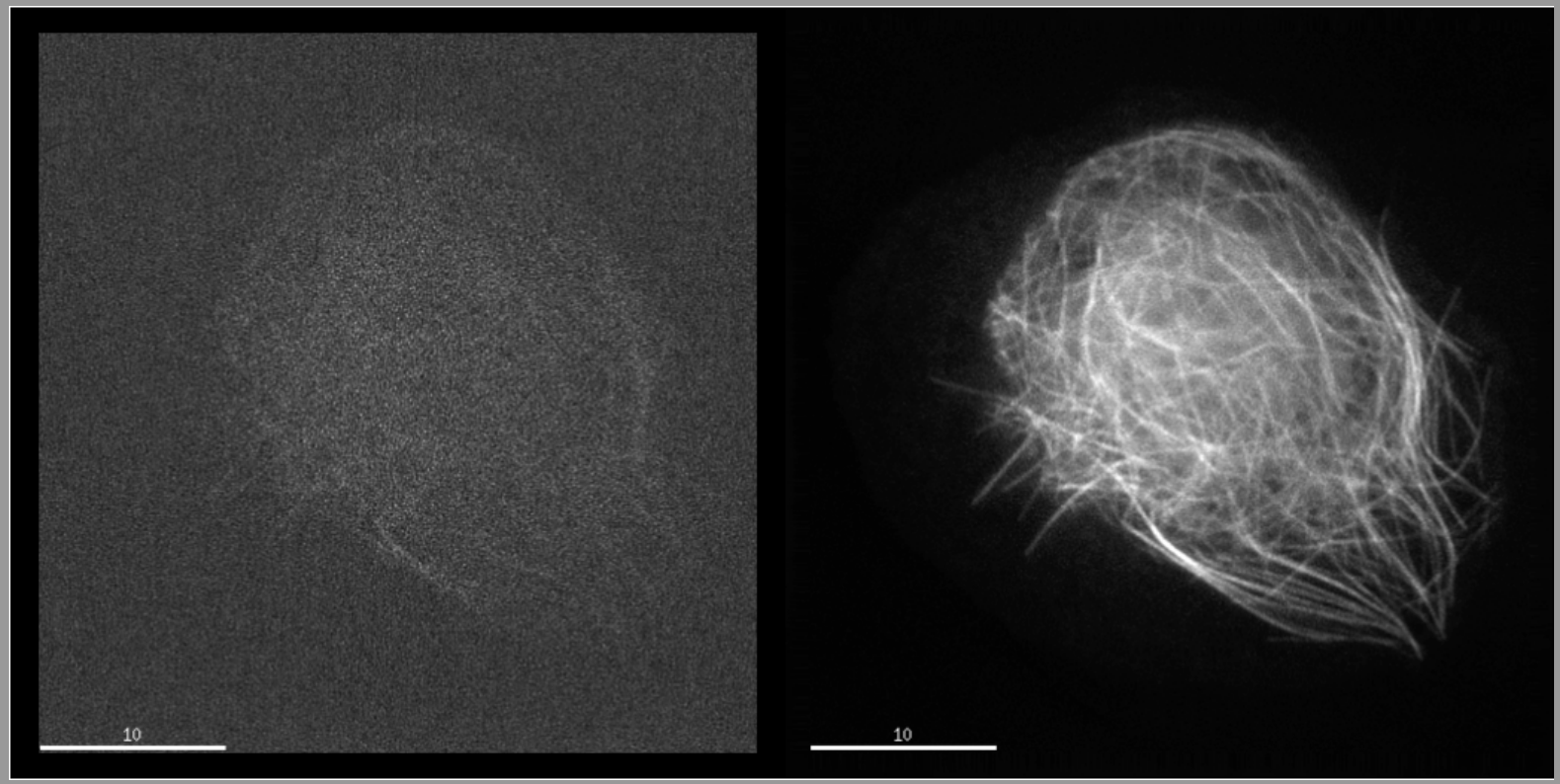
$$\begin{bmatrix} -1 & 0 & +1 \\ -2 & 0 & +2 \\ -1 & 0 & +1 \end{bmatrix}$$

$$\begin{bmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ -1 & +2 & +1 \end{bmatrix}$$

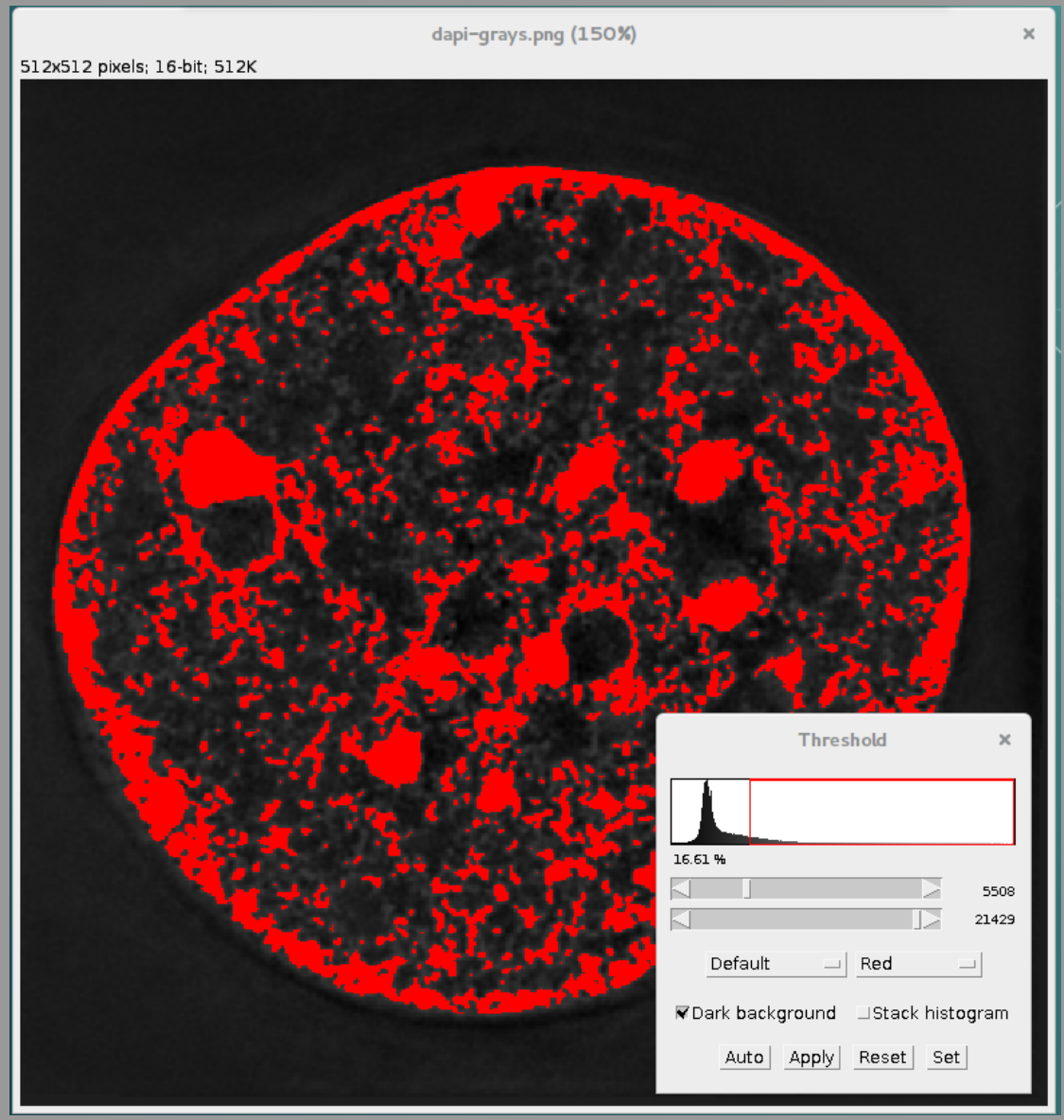


Non-local means

patch based denoise

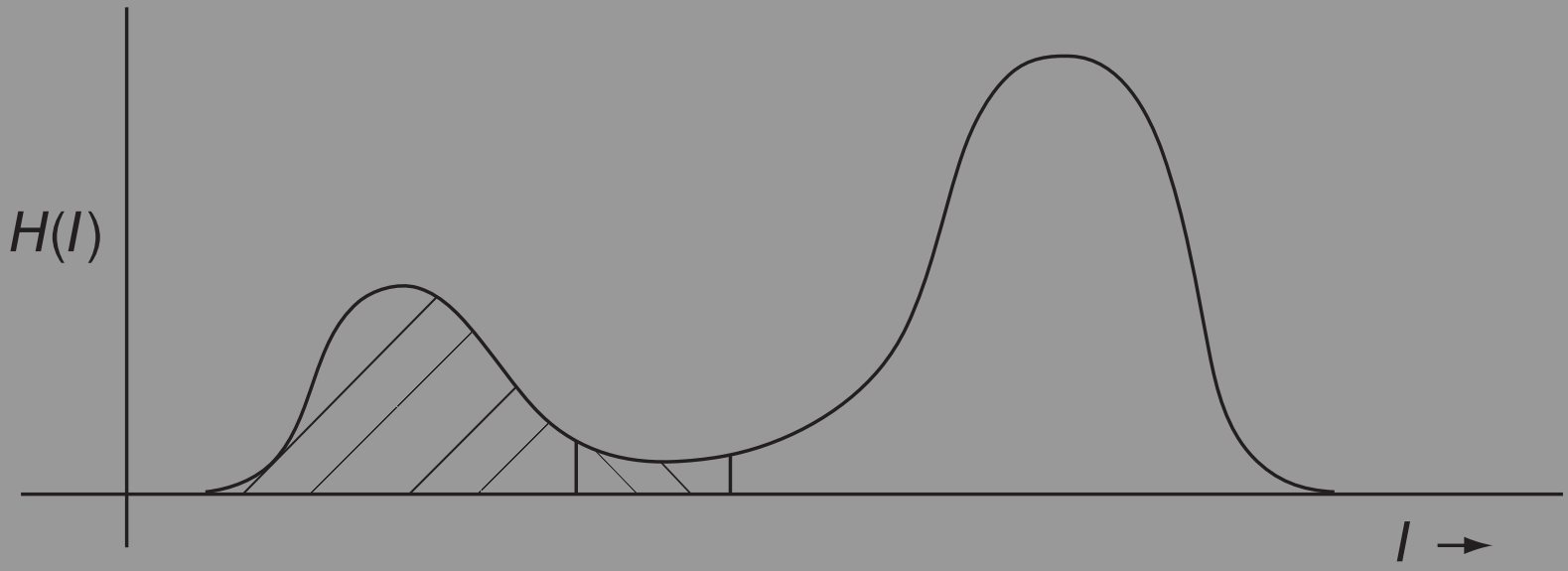


Manual threshold



Automatic threshold

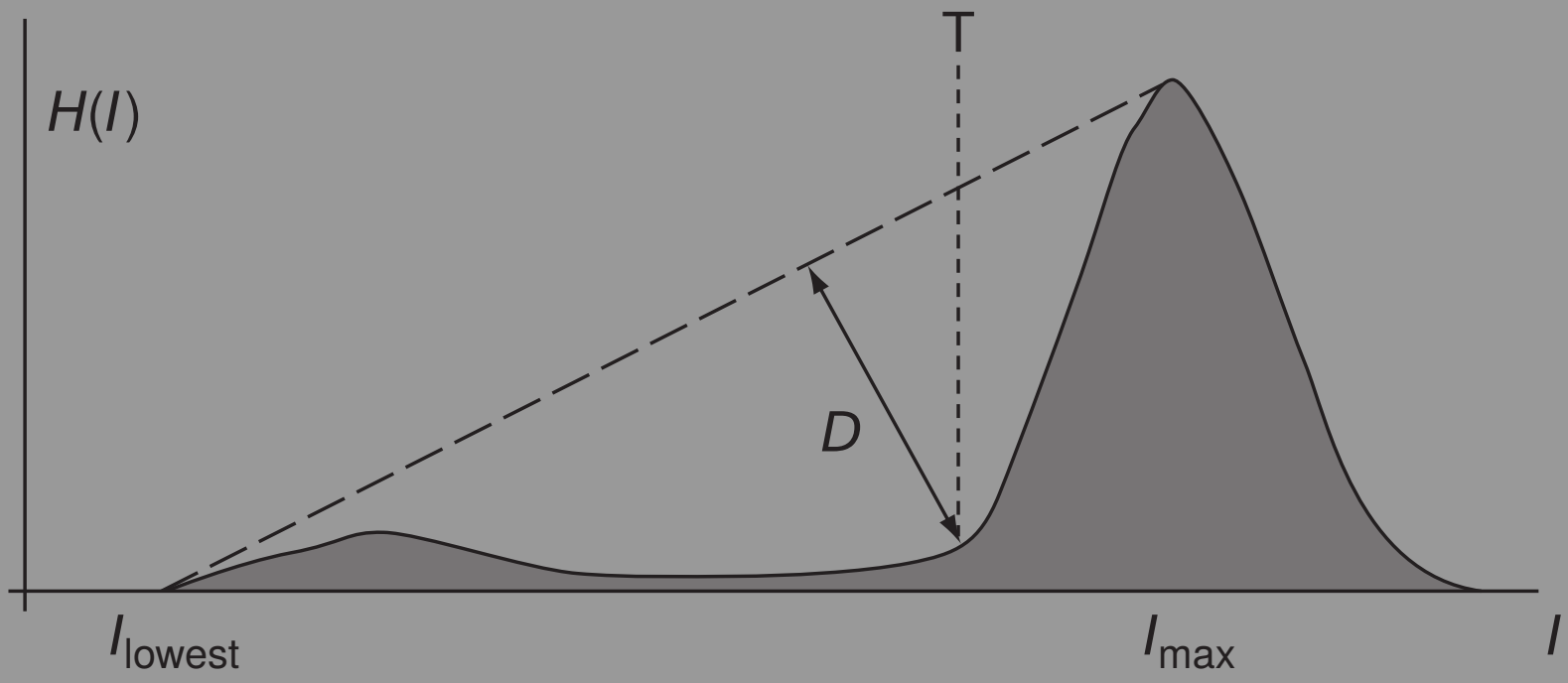
Otsu's algorithm



- bimodal histogram
- reduce intra-class variance (spread)

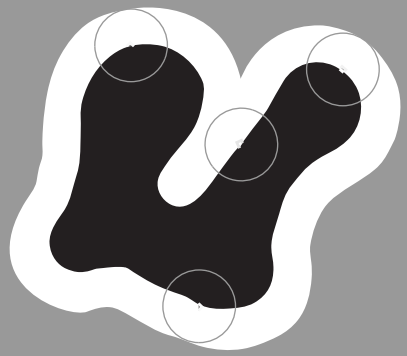
Automatic threshold

Triangle algorithm

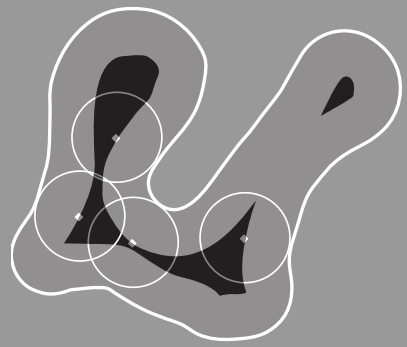


- histogram smoothing (mean filter)

Erosion and dilation



Dilation

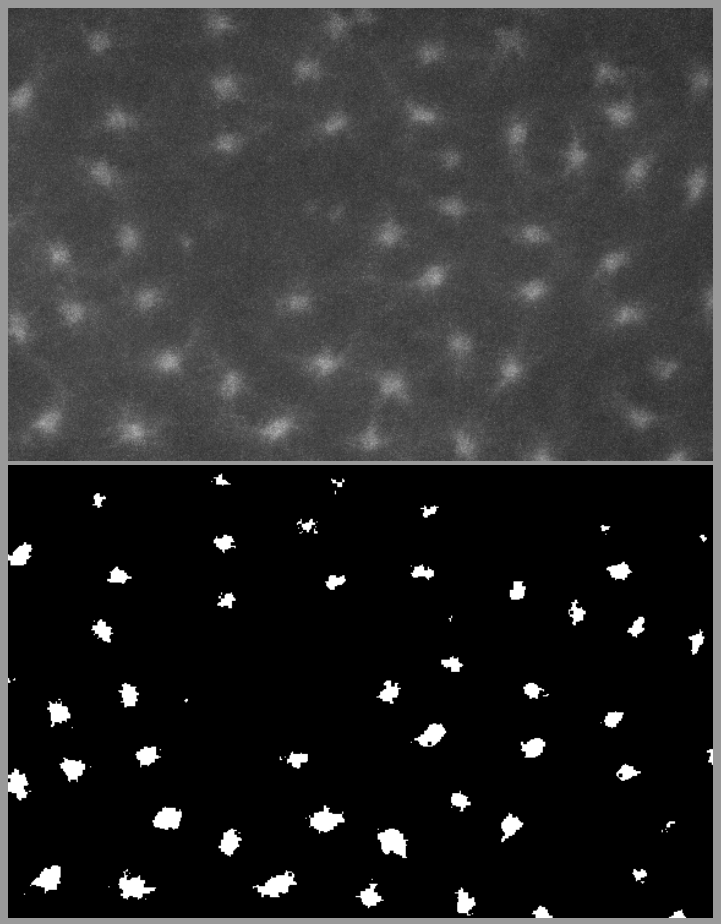


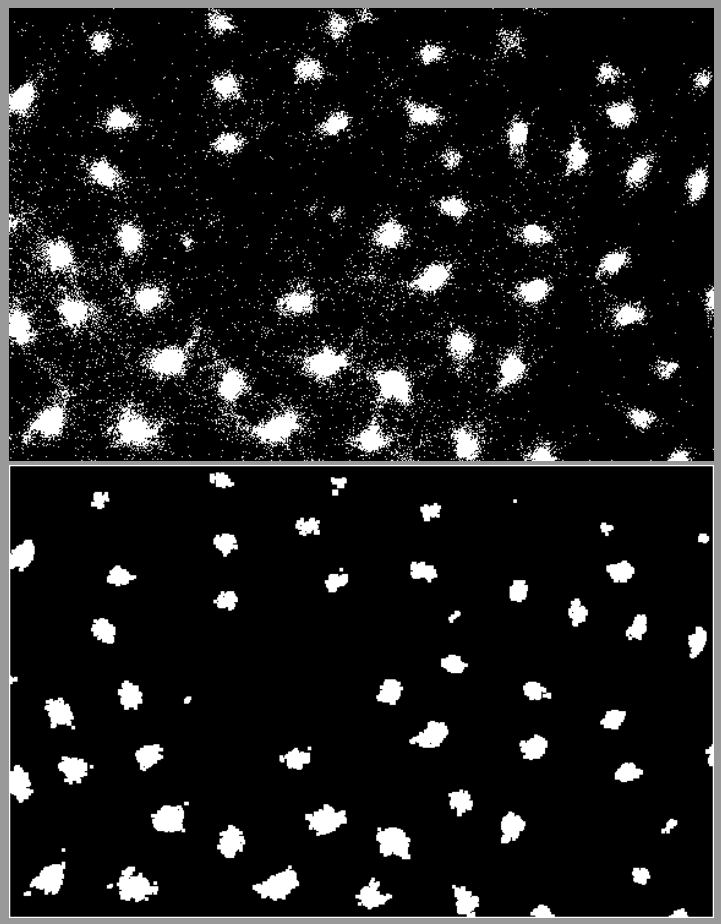
Erosion

Closing

Opening

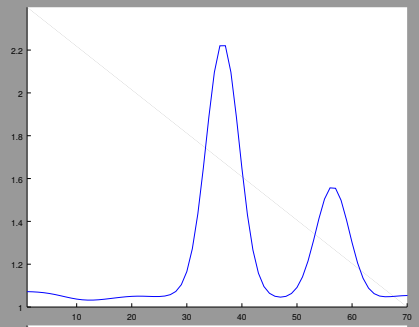
Erosion and dilation



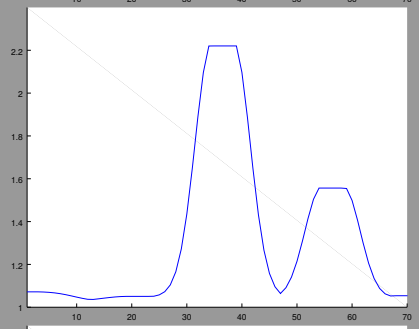


Bottom hat

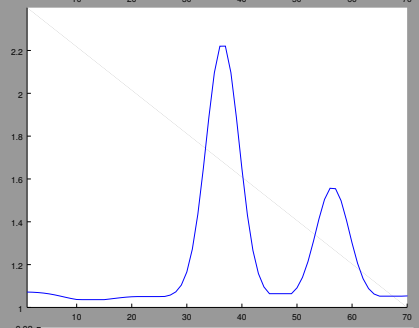
Original



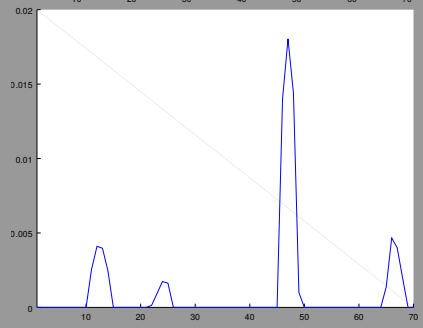
Dilation

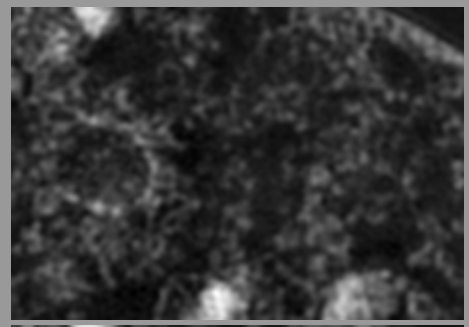


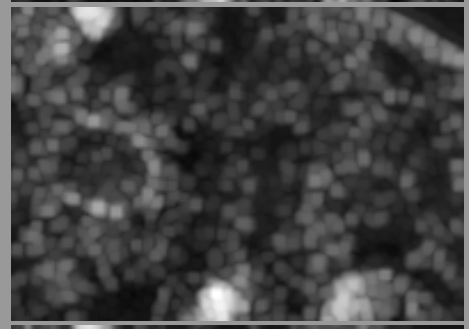
Closing

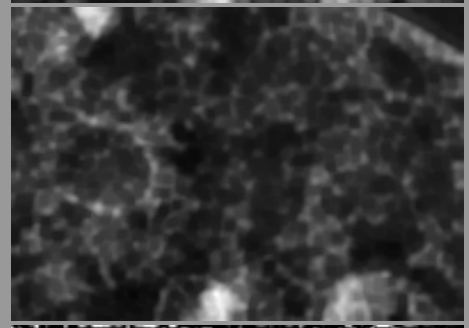


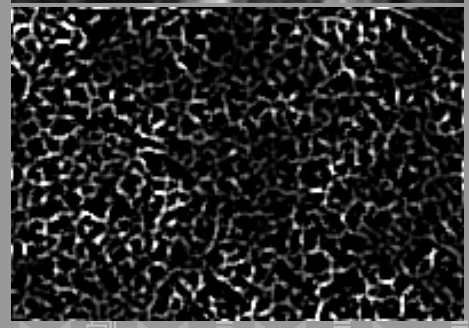
Bottom hat
(minus image)











Watershed

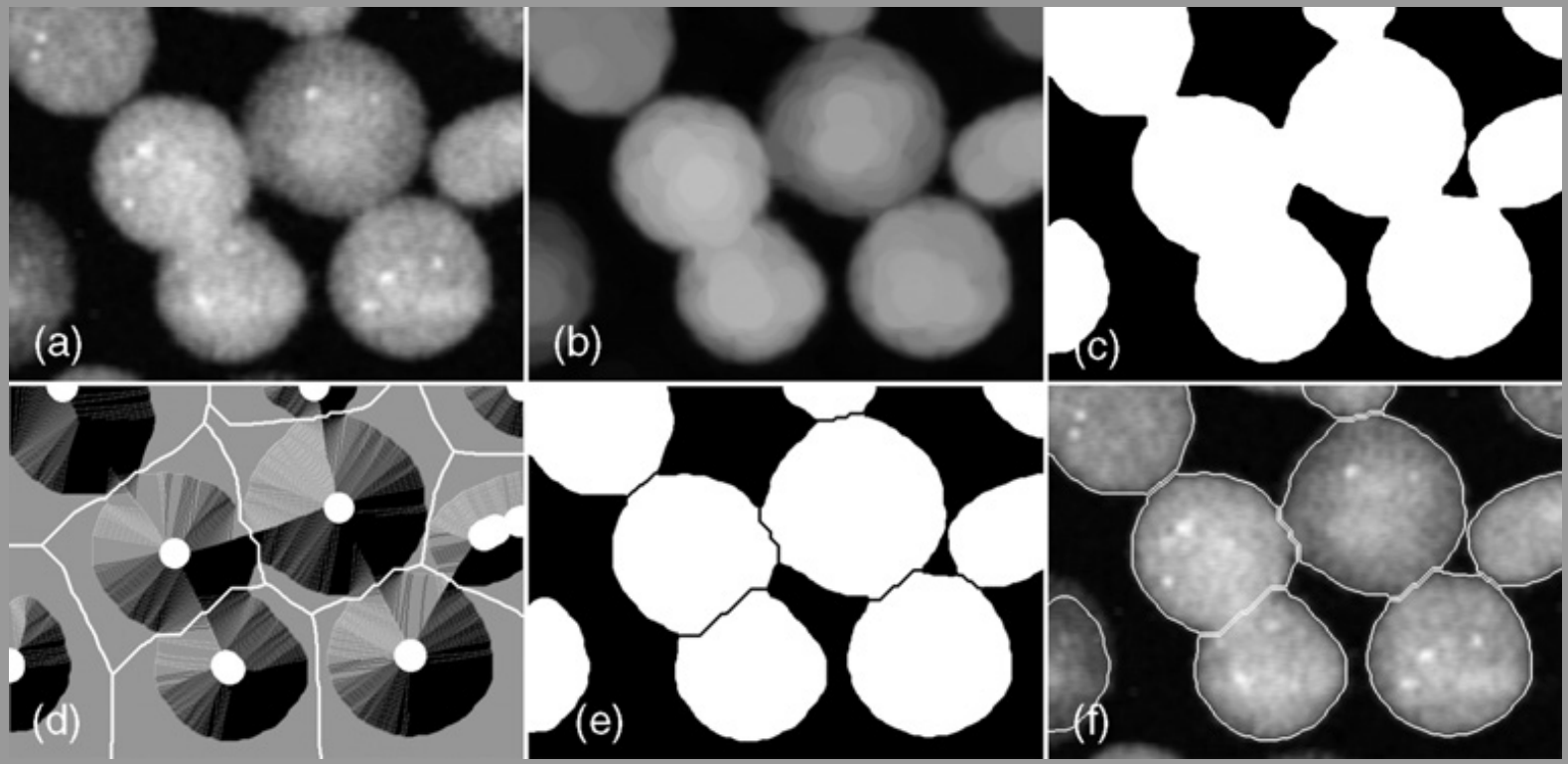
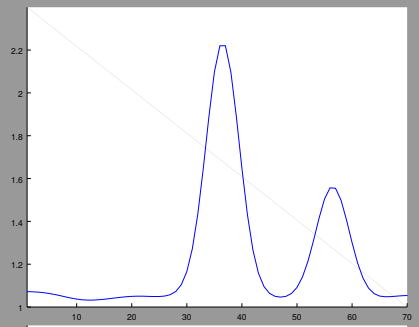
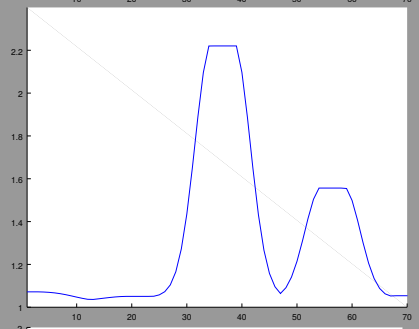


Image gradient

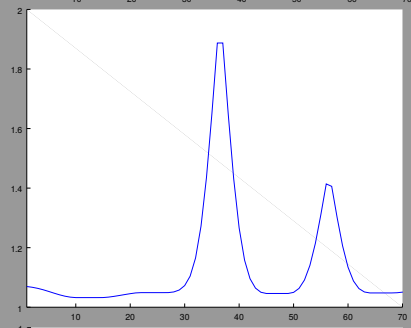
Original



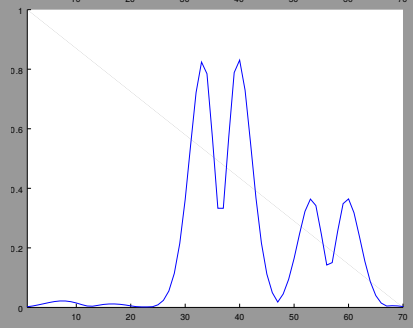
Dilation

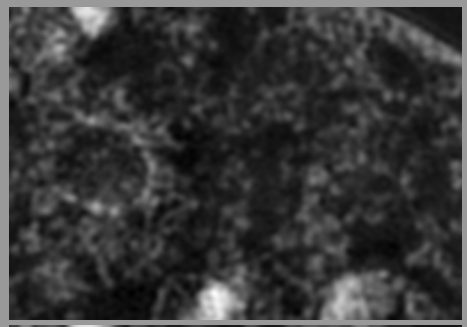


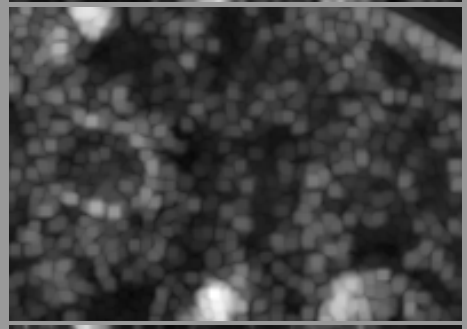
Erosion

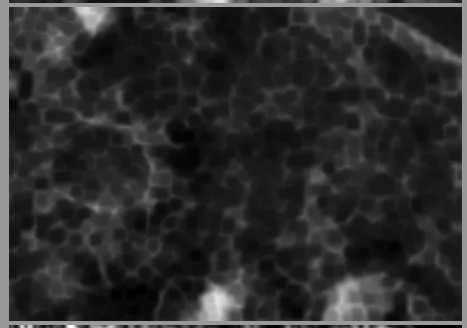


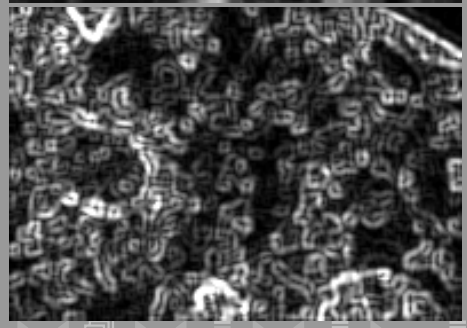
Gradient
(dilate - erode)



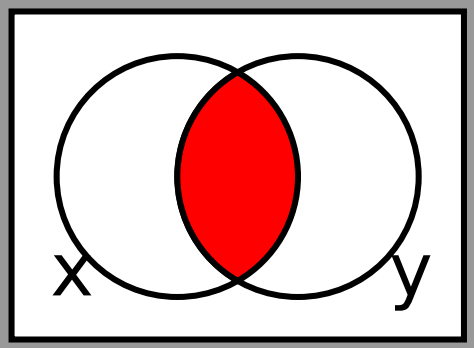




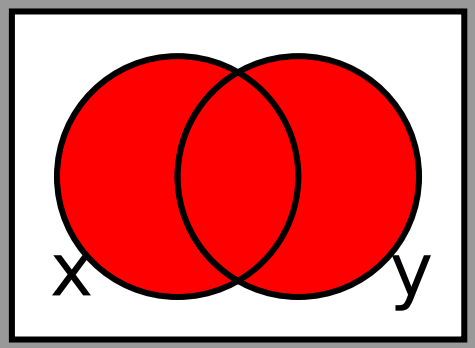




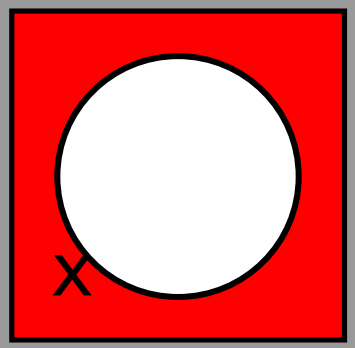
Logical operations



x AND y



x OR y



NOT x

Object properties

Particle/Region/ROI properties/measurements

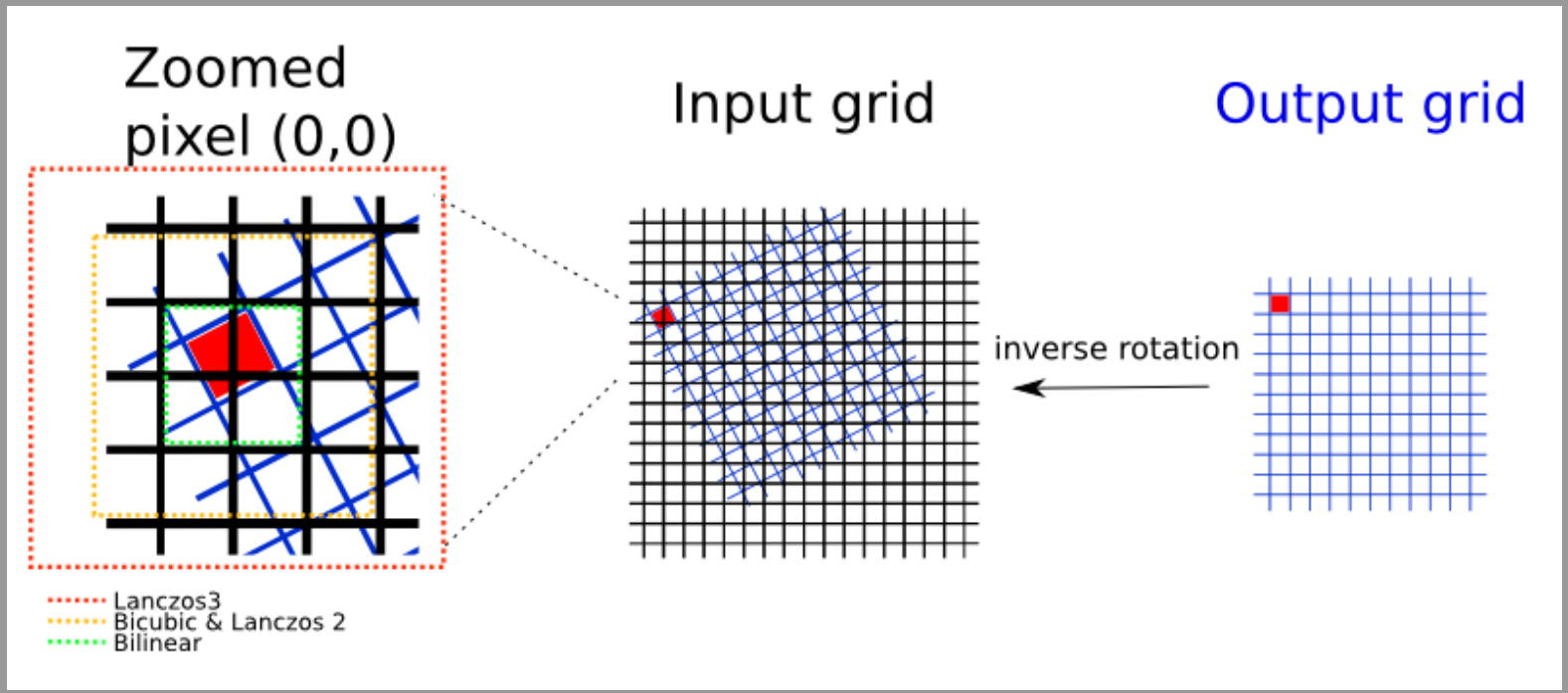
These are always one button or one line of code. The only problem is getting to this point.

- area
- eccenctricity
- centroid
- center of mass
- integrated density
- min and max
- perimeter

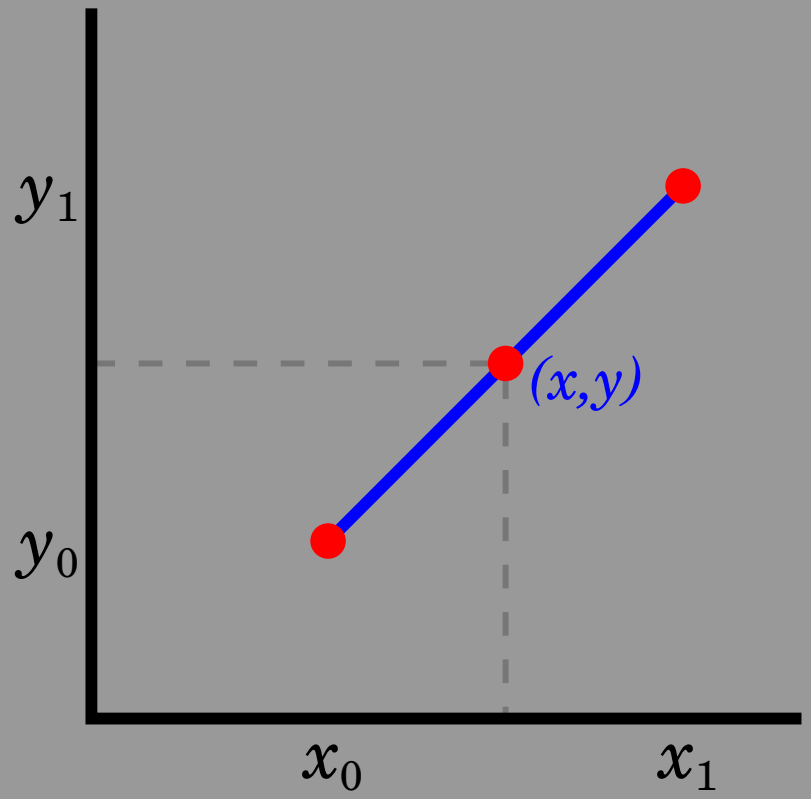
geometric transformation

What happens when you:

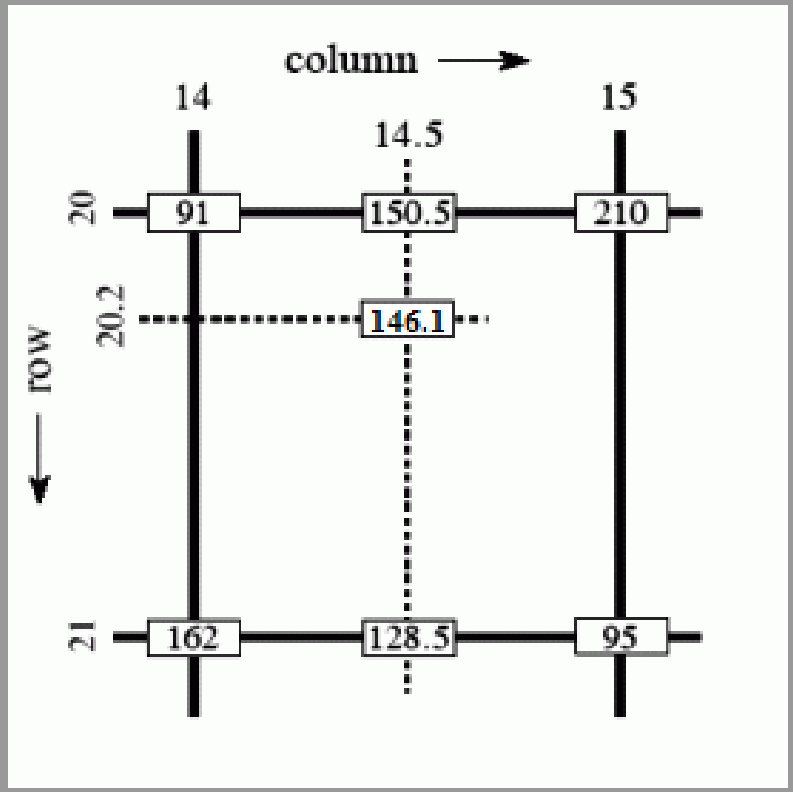
- rotate
- align
- translate
- stretch



Linear interpolation



Bilinear interpolation



A final word

Limitations such as:

- only black and white;
- only 8 bit;
- only 2D images;
- only 3D images;

are limitations of the implementation.

Summary

- Images are just N dimensional array of numbers
- Mathematical operations can be extended to images
- Thresholding to create masks
- Filters for processing image
- Morphology to identify shapes