Advanced Image Analysis

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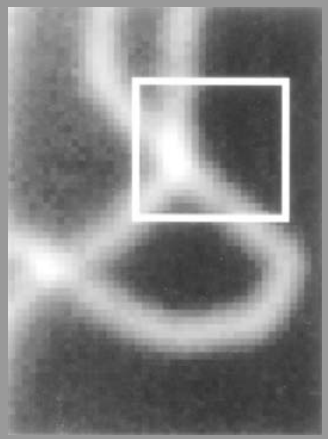
Advanced BioImaging Unit
(the basement)

Micron Advanced Microscopy Course, 2017

Microscope Image Analysis in 3 parts

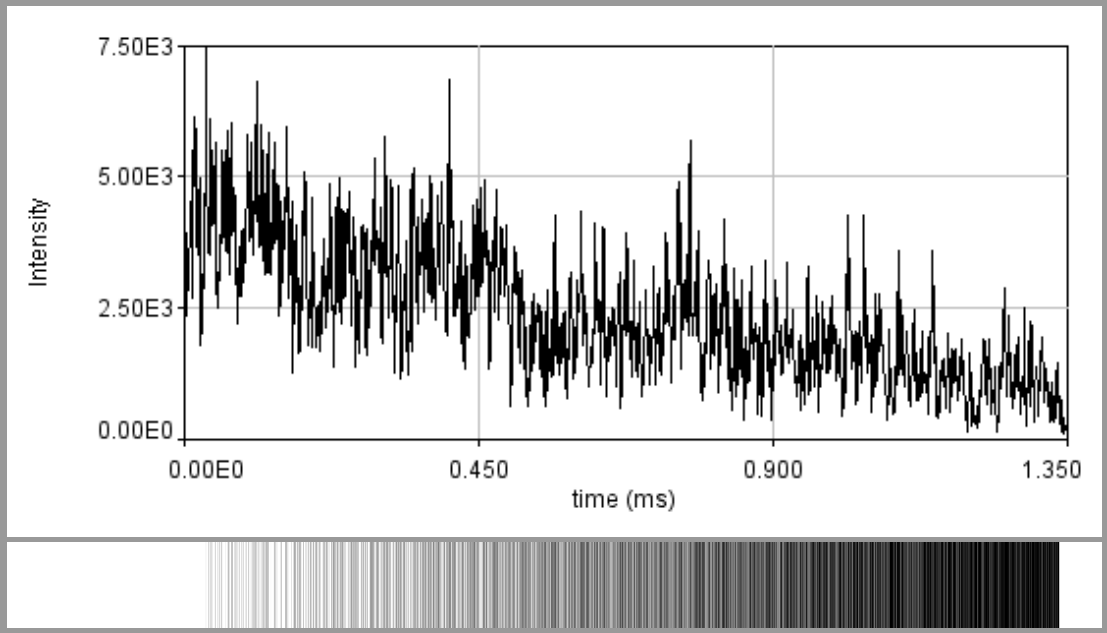
1. What is in a microscope image
   - What is in a image?
   - Image display
   - ImageJ
   - Image acquisition
2. Images as N dimensional numeric arrays
   - N dimensional images
   - Spatial filters
   - Morphology
   - Connected components
3. Don't botch your data
   - File formats
   - Data storage
   - OMERO

Pixel data

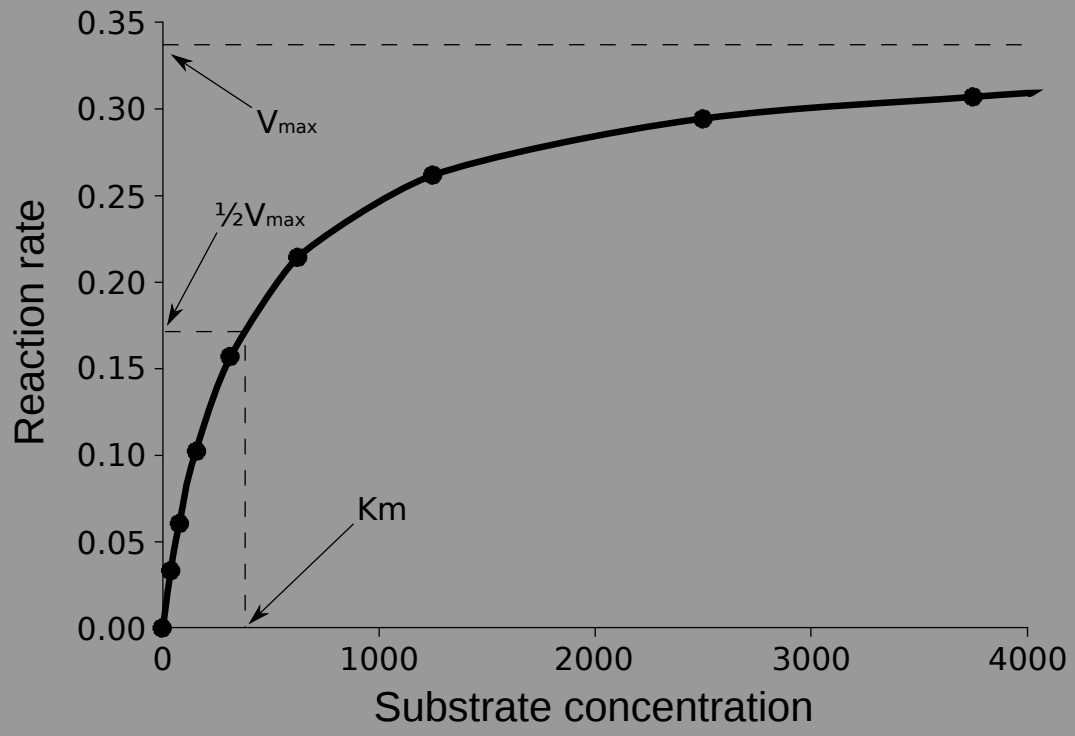


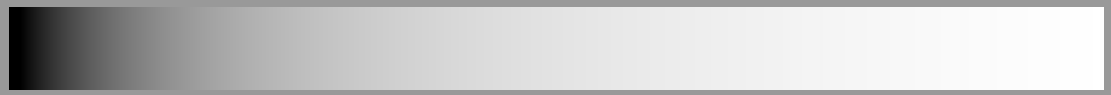
63	84	119	172	219	225	182	135	79	51	36	24	23	19	15	-1	14	14	8	0	-4	7	18
78	84	104	170	223	224	196	118	84	49	36	28	16	11	25	4	15	13	8	-4	9	11	7
61	80	115	153	209	204	170	113	73	46	41	29	9	17	11	11	0	12	-2	2	2	3	23
72	98	121	160	190	207	178	116	68	50	29	22	17	19	7	27	15	9	-3	-4	14	5	8
64	90	132	167	210	214	180	115	71	37	36	31	13	15	9	8	15	6	0	5	-14	4	12
75	93	124	169	216	229	196	107	71	56	19	18	22	24	7	5	15	11	8	-1	12	6	7
97	87	128	193	210	225	193	111	85	47	27	27	21	12	5	2	-1	4	1	-3	7	2	-10
103	108	134	180	201	233	185	115	55	38	26	25	15	20	18	6	2	2	1	4	-3	-13	0
142	132	161	216	238	223	160	90	59	45	17	10	9	13	10	11	4	-9	5	2	7	0	5
172	162	175	231	239	238	155	88	48	28	24	17	15	13	0	14	0	11	-3	4	9	0	-10
226	219	230	260	265	236	161	92	43	31	31	11	5	11	7	13	19	9	18	-11	-9	-2	8
234	247	256	302	311	253	174	97	48	27	12	15	7	7	0	16	8	5	3	-4	0	-6	4
260	263	297	346	349	303	196	126	65	27	30	24	3	6	7	1	12	3	9	0	-2	-13	2
244	293	340	388	399	321	223	130	74	29	24	30	17	4	3	11	0	8	7	-3	-2	-2	-2
209	273	359	423	436	365	264	141	80	57	32	45	13	3	18	8	-7	0	-6	4	-1	-2	-3
176	253	342	430	443	394	291	161	86	59	37	23	18	5	0	7	8	11	1	-3	13	-5	-2
152	218	311	425	470	420	325	208	111	66	52	29	28	9	4	7	8	4	-7	11	-18	-13	-2
129	199	294	413	469	441	384	257	148	111	69	34	20	20	6	3	15	4	-2	-6	-3	-10	9
140	206	294	385	439	442	365	310	223	157	114	76	45	28	9	21	5	15	-4	-13	0	-5	-1
173	233	309	354	392	375	333	303	261	214	135	92	51	47	18	12	13	12	20	-9	4	1	15
221	278	300	321	306	293	286	279	250	231	184	142	108	67	41	18	13	5	8	-8	0	7	5
267	302	291	244	228	211	201	215	241	227	205	184	136	110	68	51	26	11	8	3	0	8	-3
284	279	257	202	133	129	137	151	183	213	209	188	187	155	109	69	49	26	25	8	8	18	-4
275	248	191	143	95	85	87	98	122	166	184	192	206	194	176	135	98	50	44	19	21	0	1

Images as Signals



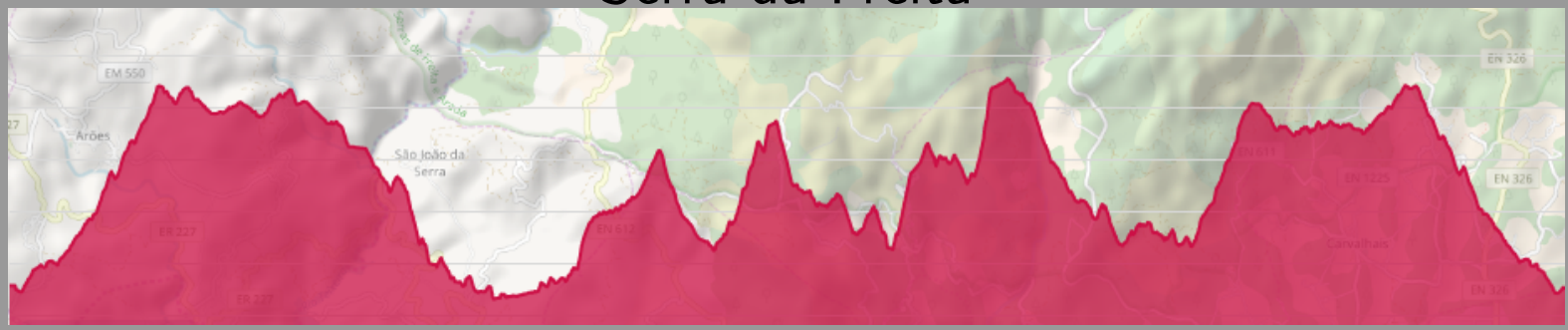
Images as Signals



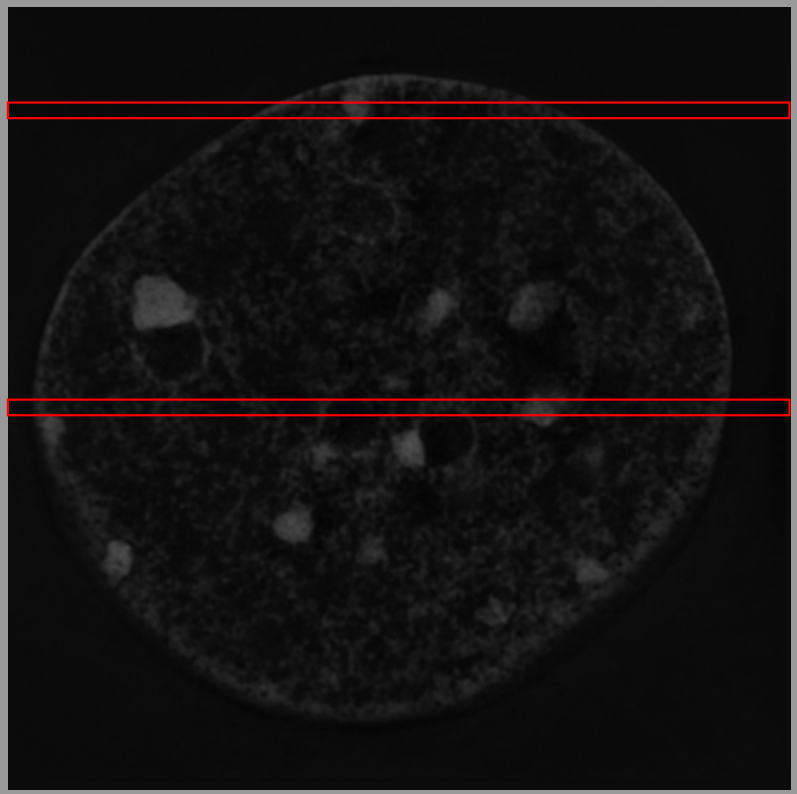


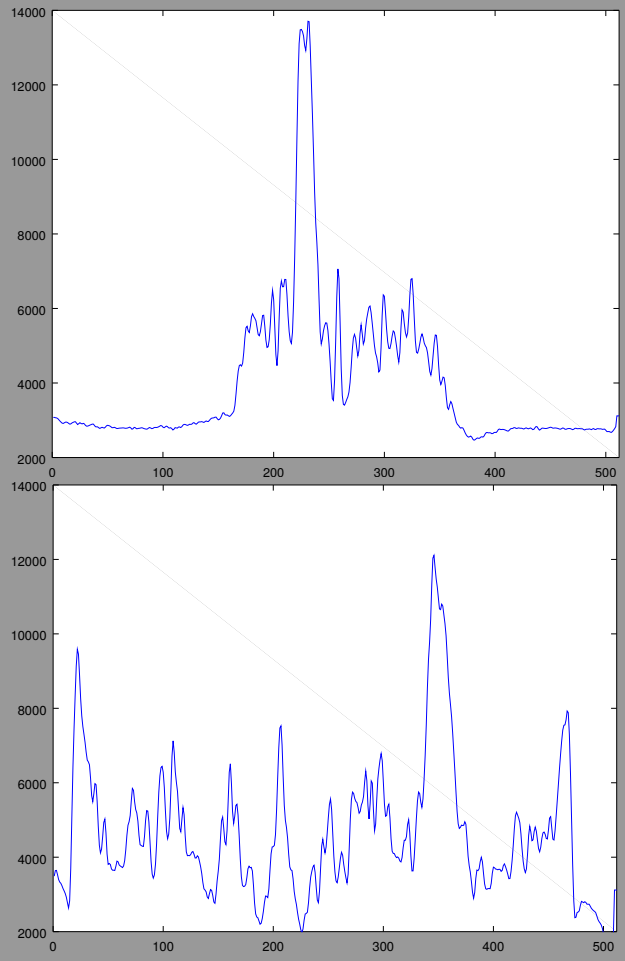
Images as Signals

Serra da Freita

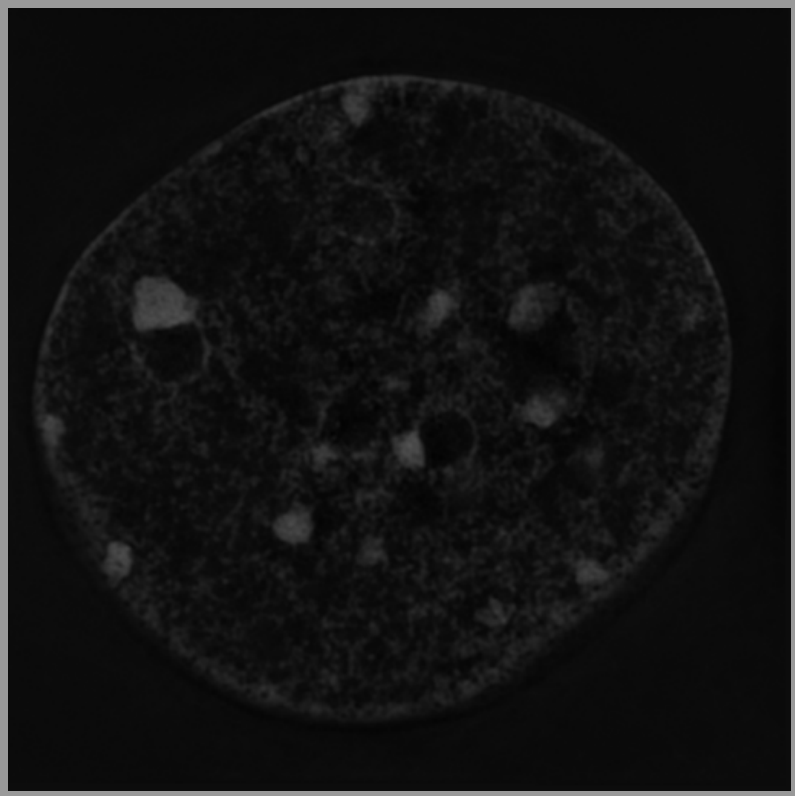


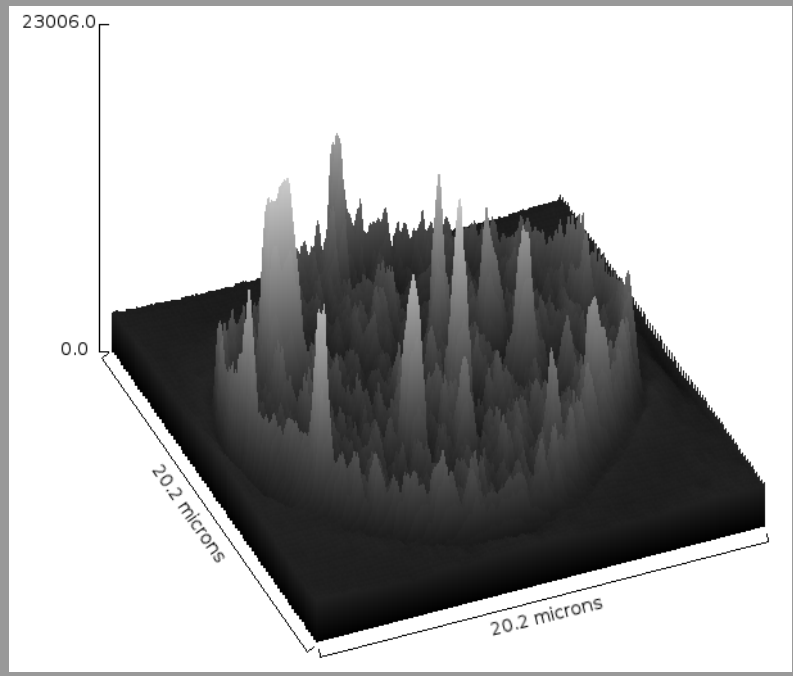
Images as Signals



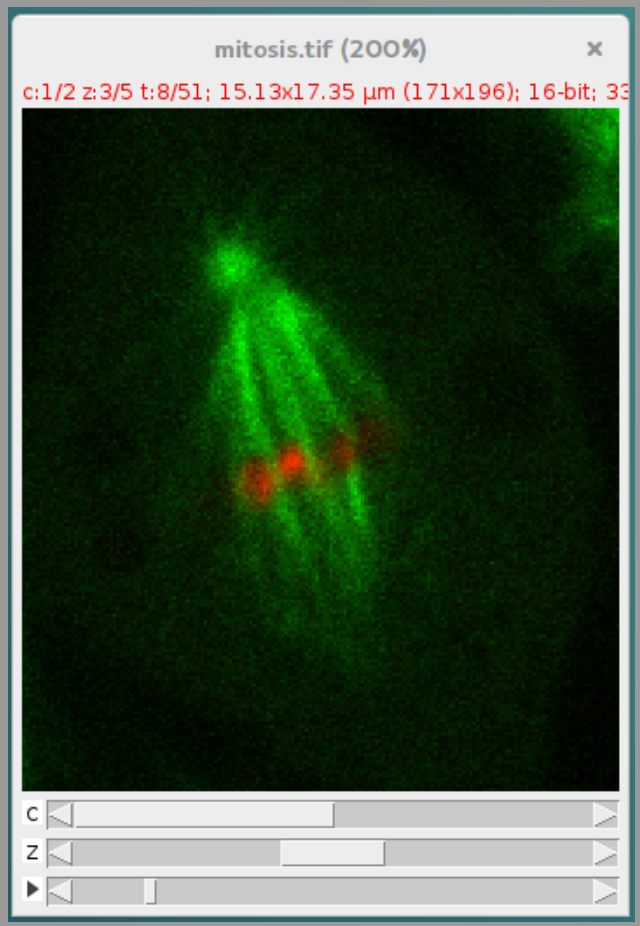


Images as Surfaces





Images as ND Arrays

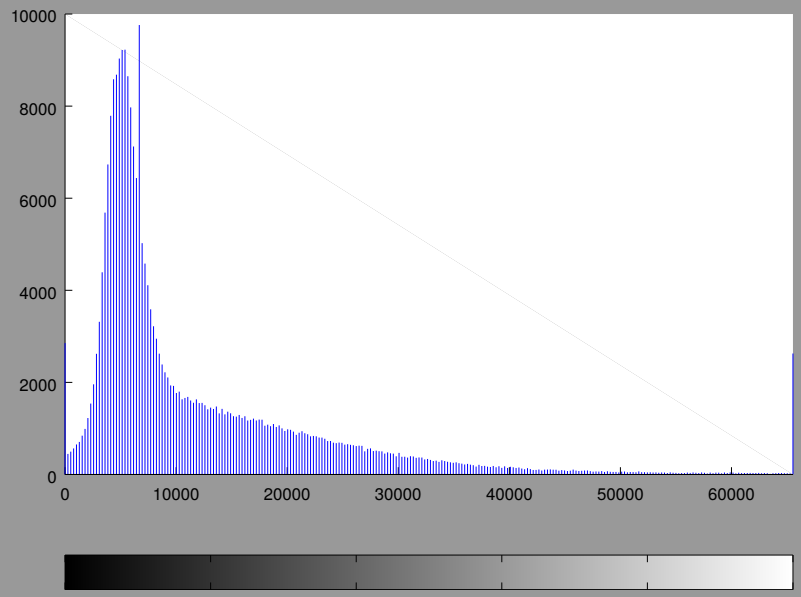


- x and y
- time
- z (volume)
- wavelength
- phase
- stage angle

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.

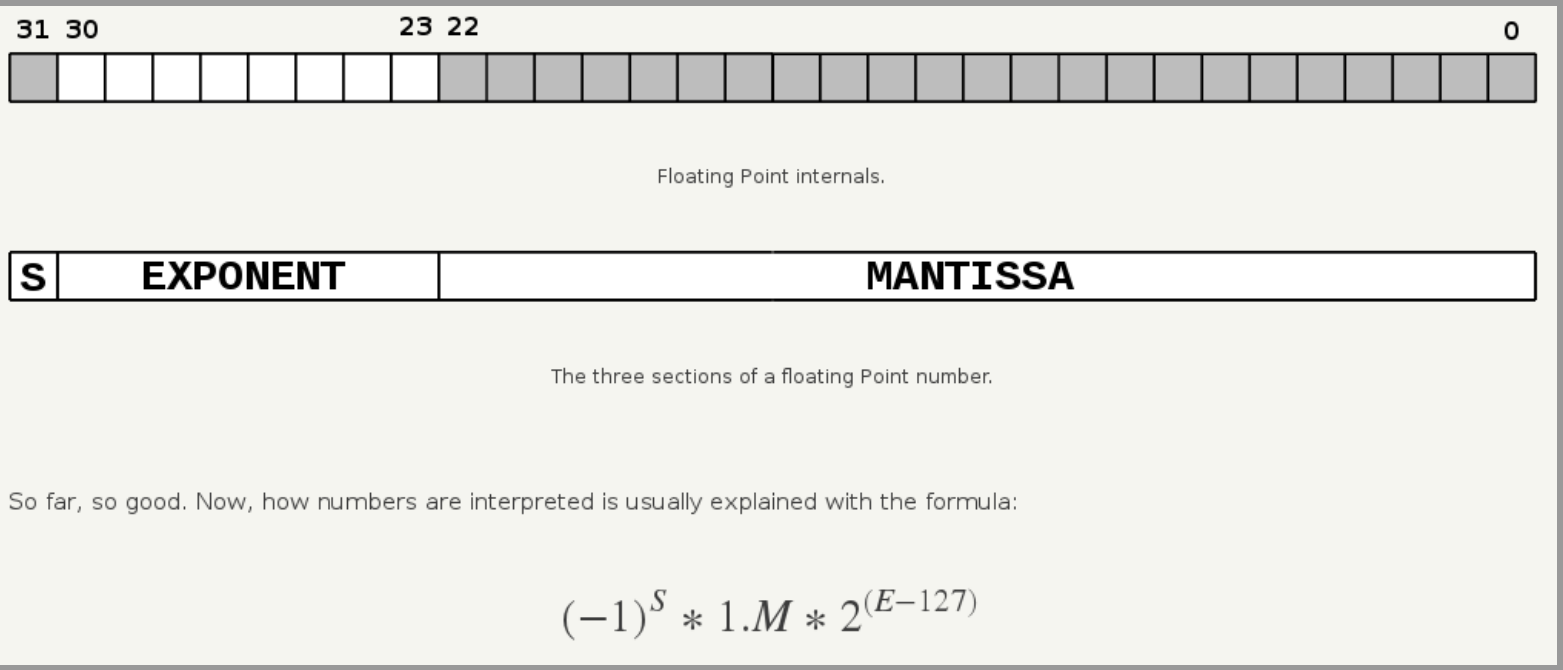
Floating point

There is actually no limit. Example:

$1.2345 = 12345 \times 10^{-4}$

Floating points — sometimes incorrectly called 32 bit.

Floating point



Floating Point internals.

The three sections of a floating Point number.

So far, so good. Now, how numbers are interpreted is usually explained with the formula:

$$(-1)^S * 1.M * 2^{(E-127)}$$

Floating points — sometimes incorrectly called 32 bit.

Typical problems

Concentration

Protein expression, number of complexes.

Co-localization

Do two overlap and correlate?

Dynamics

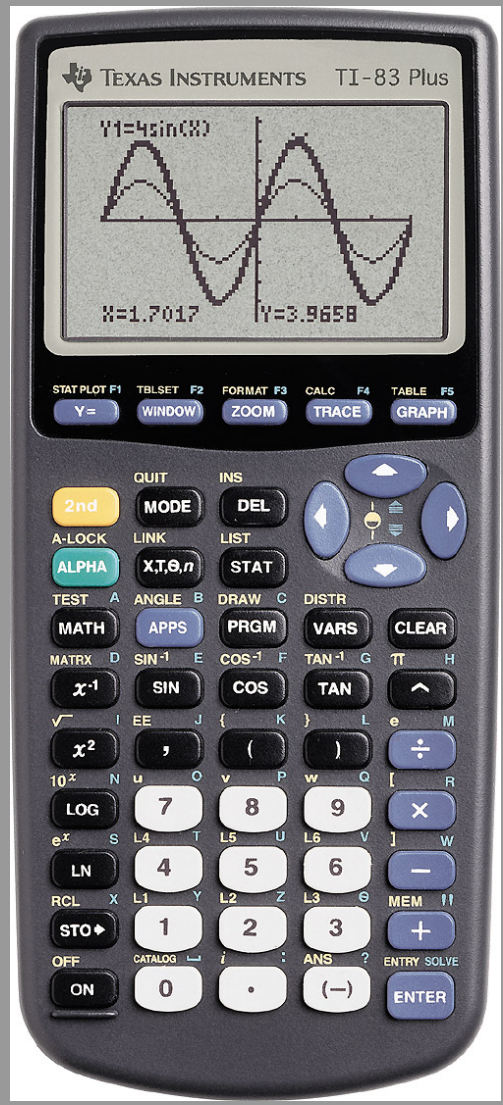
How fast does it move?

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

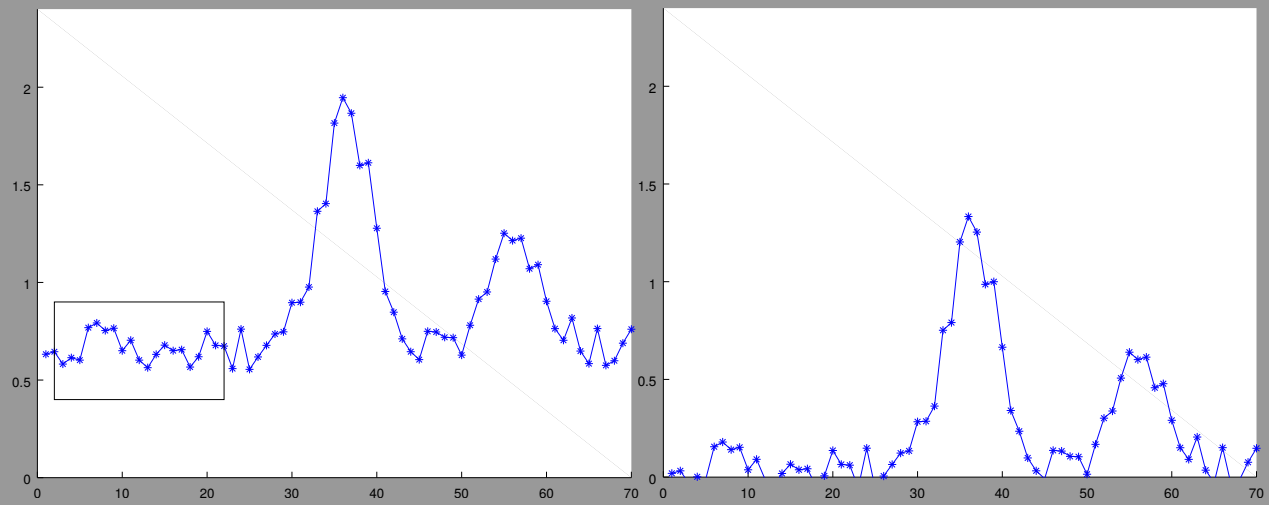
Octave

R

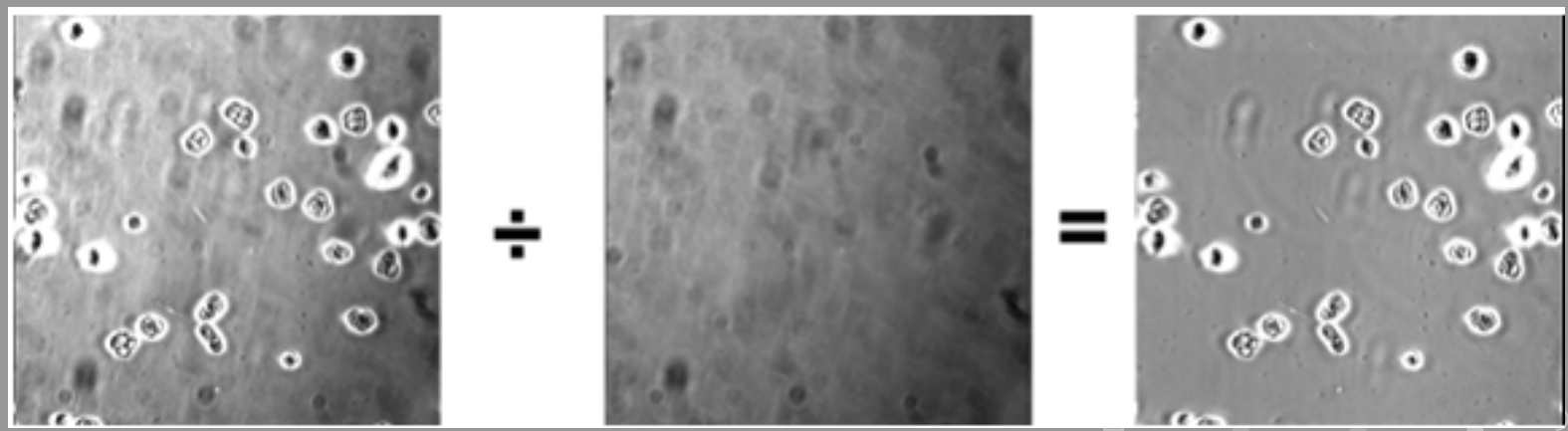
- CellProfiler
- Icy
- Omero
- Imaris
- softWoRx
- Volocity
- Matlab
- Metamorph
- Image-Pro Plus
- Huygens
- Mathematica
- ...

Background correction

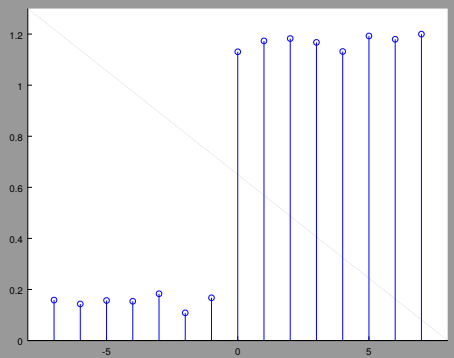
- Subtract mean of a known background region (darks).
- Many cameras (not-microscopes) do this.



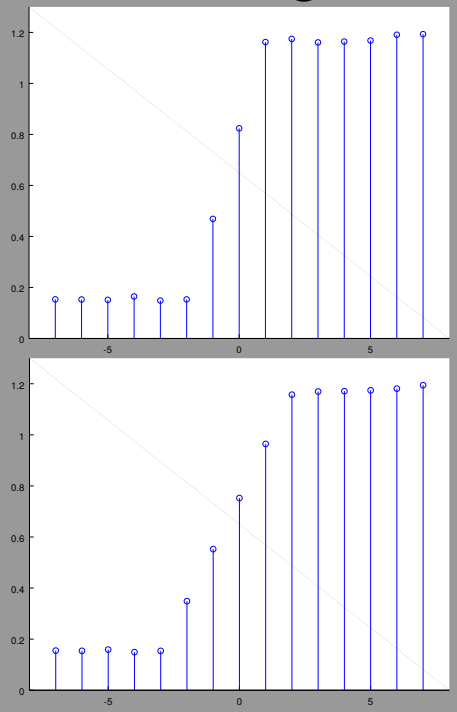
- Correction for uneven illumination (divide by flats)



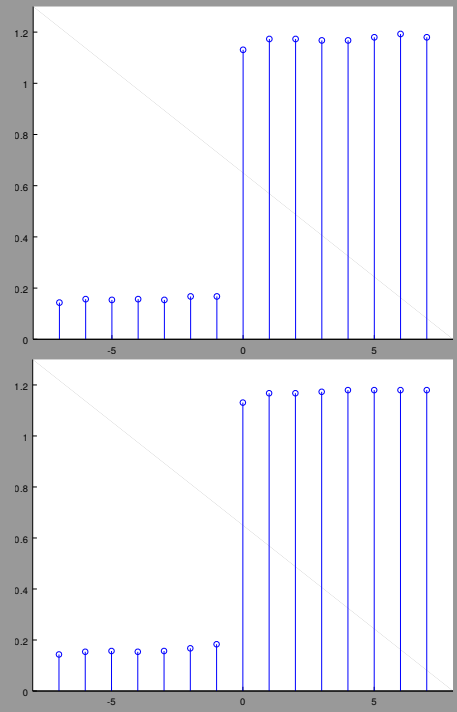
Local means



Average

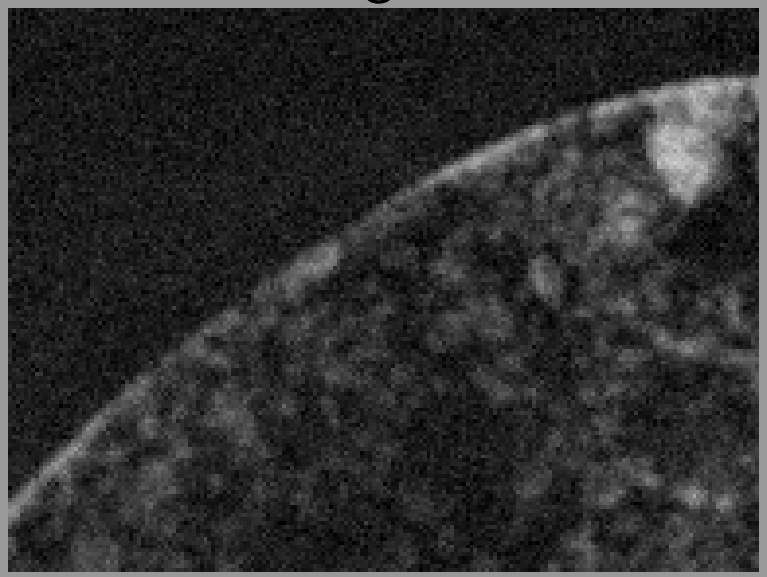


Median

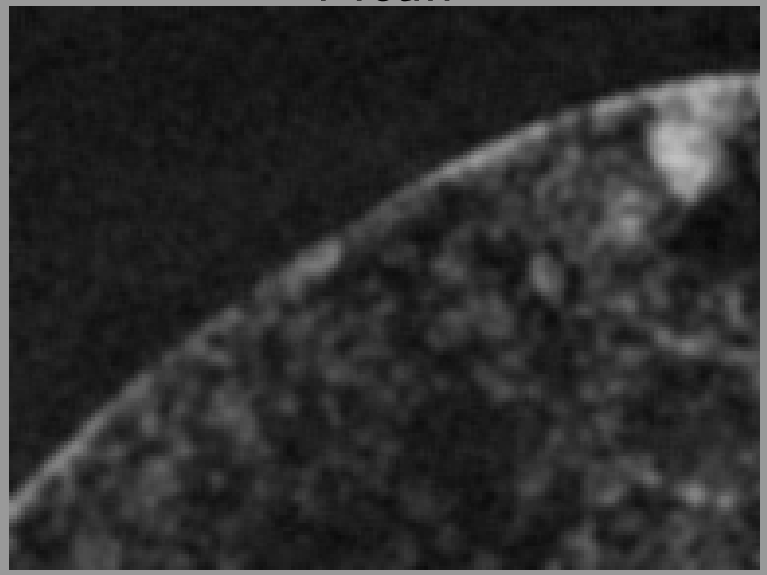


Local means

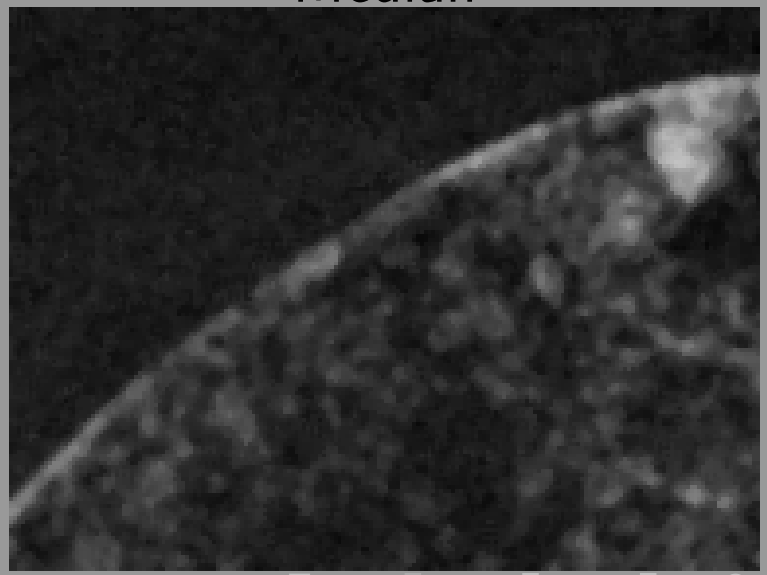
Original



Mean

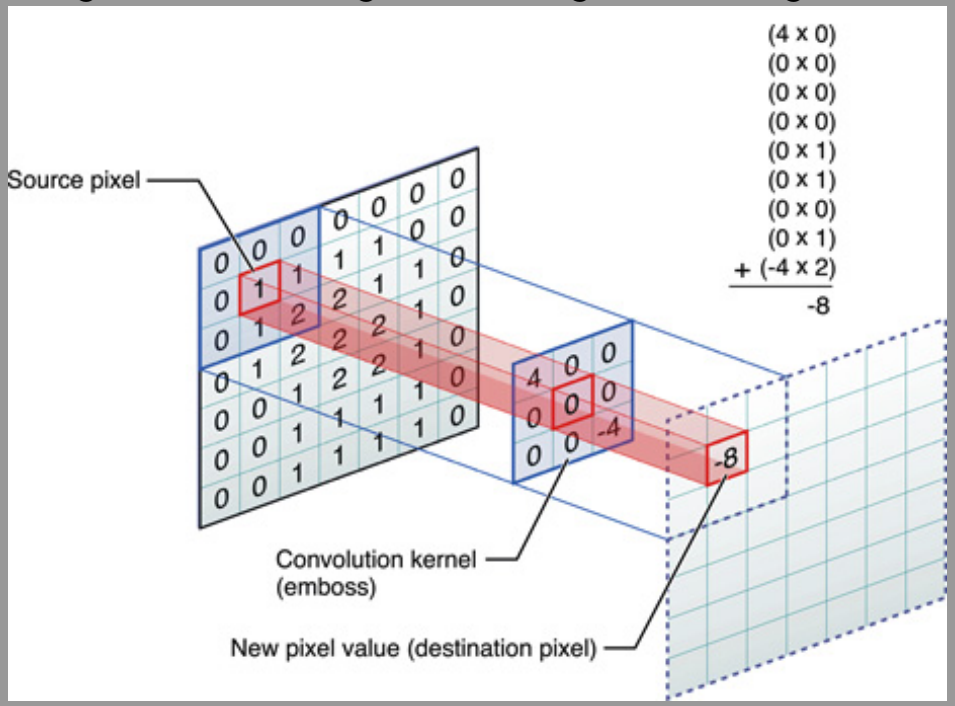


Median



Mean as convolution kernel

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



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

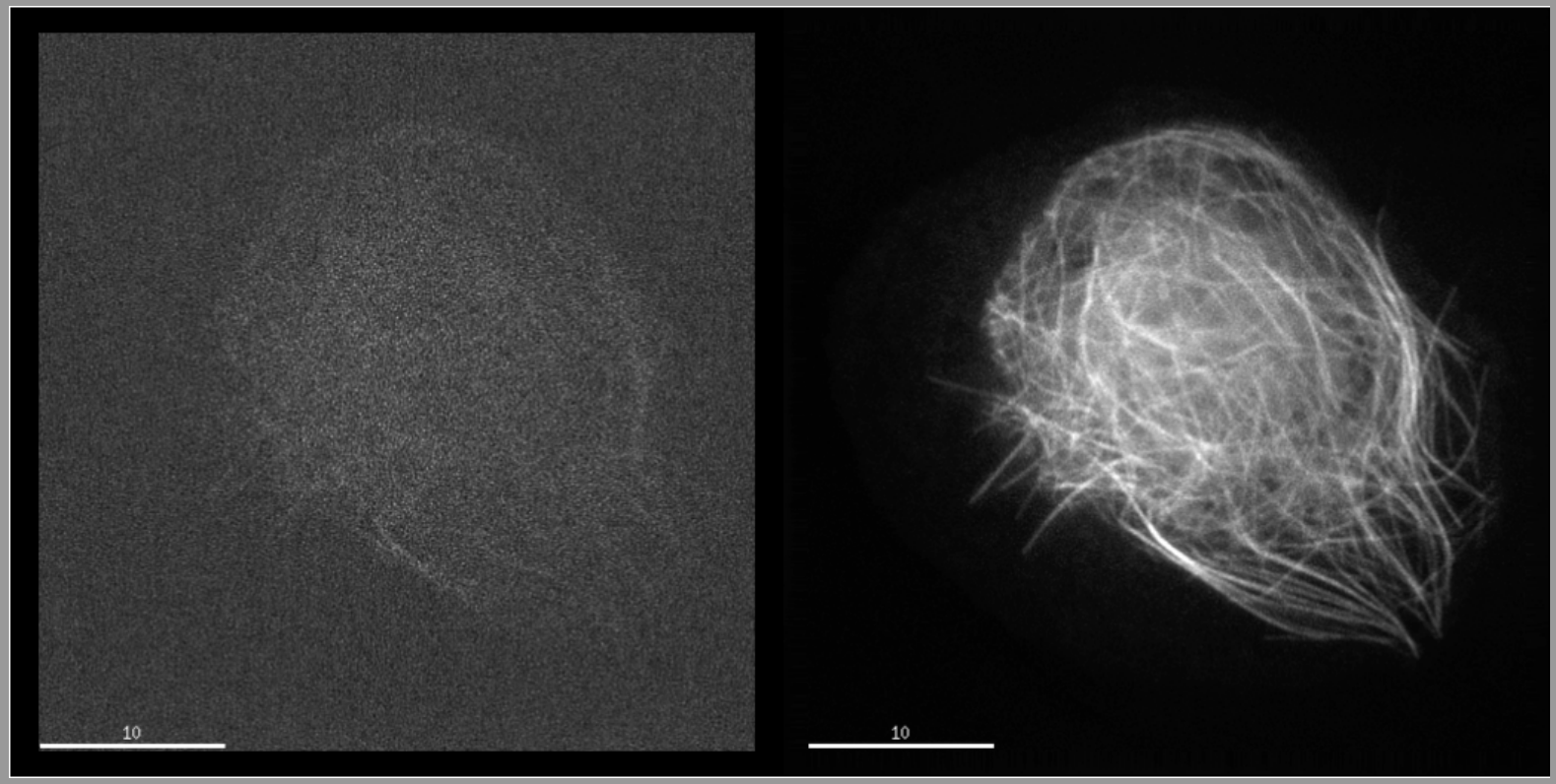
3x3 mean kernel

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

5x5 mean kernel

Non-local means

patch based denoise



Gaussian filter

as weigthed mean

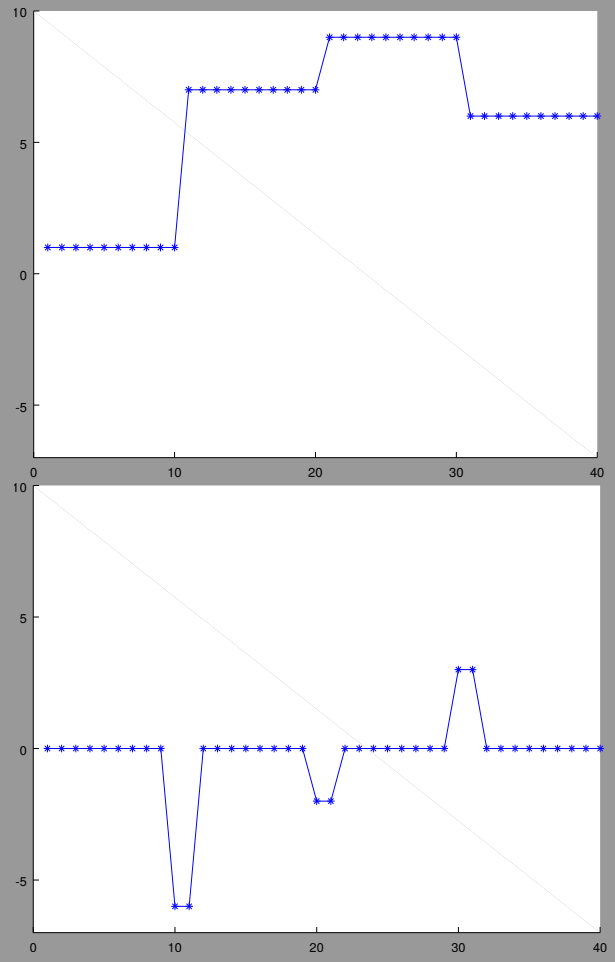
$$\begin{bmatrix} 0.011 & 0.014 & 0.017 & 0.018 & 0.017 & 0.014 & 0.011 \\ 0.014 & 0.019 & 0.023 & 0.024 & 0.023 & 0.019 & 0.014 \\ 0.017 & 0.023 & 0.027 & 0.029 & 0.027 & 0.023 & 0.017 \\ 0.018 & 0.024 & 0.029 & 0.030 & 0.029 & 0.024 & 0.018 \\ 0.017 & 0.023 & 0.027 & 0.029 & 0.027 & 0.023 & 0.017 \\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

Edge detection

Sobel operator

1D filter

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



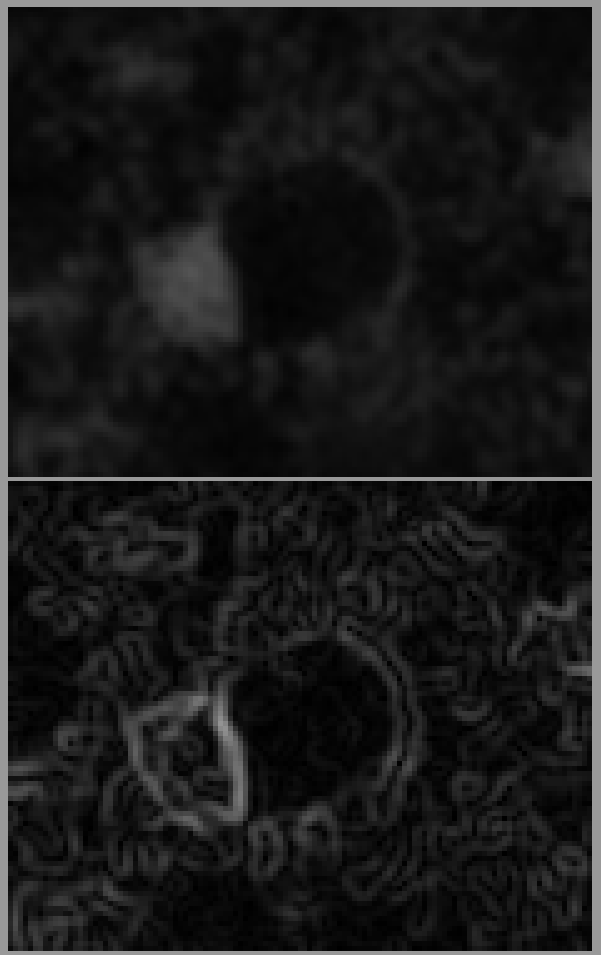
Edge detection

Sobel operator

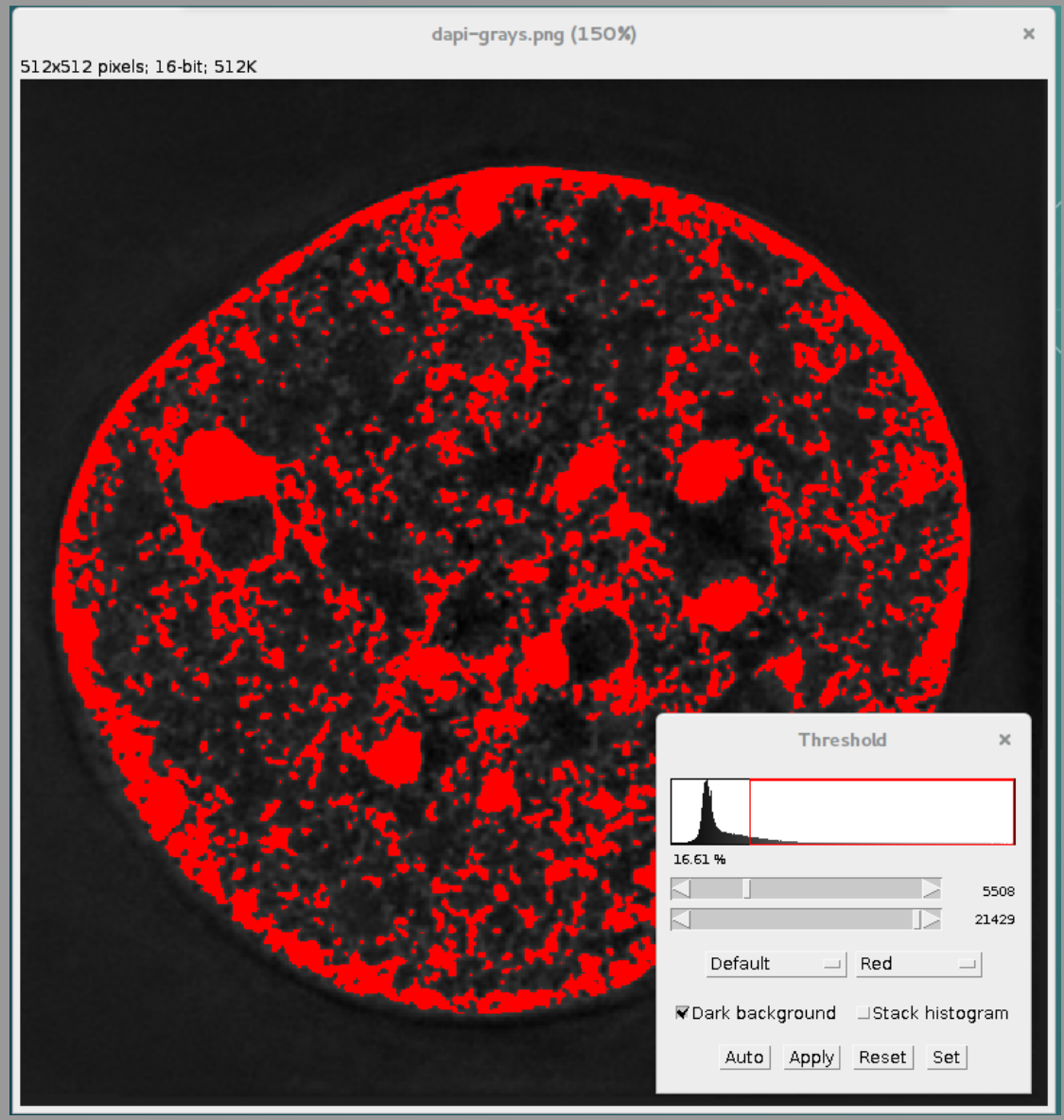
2D filter(s)

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

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

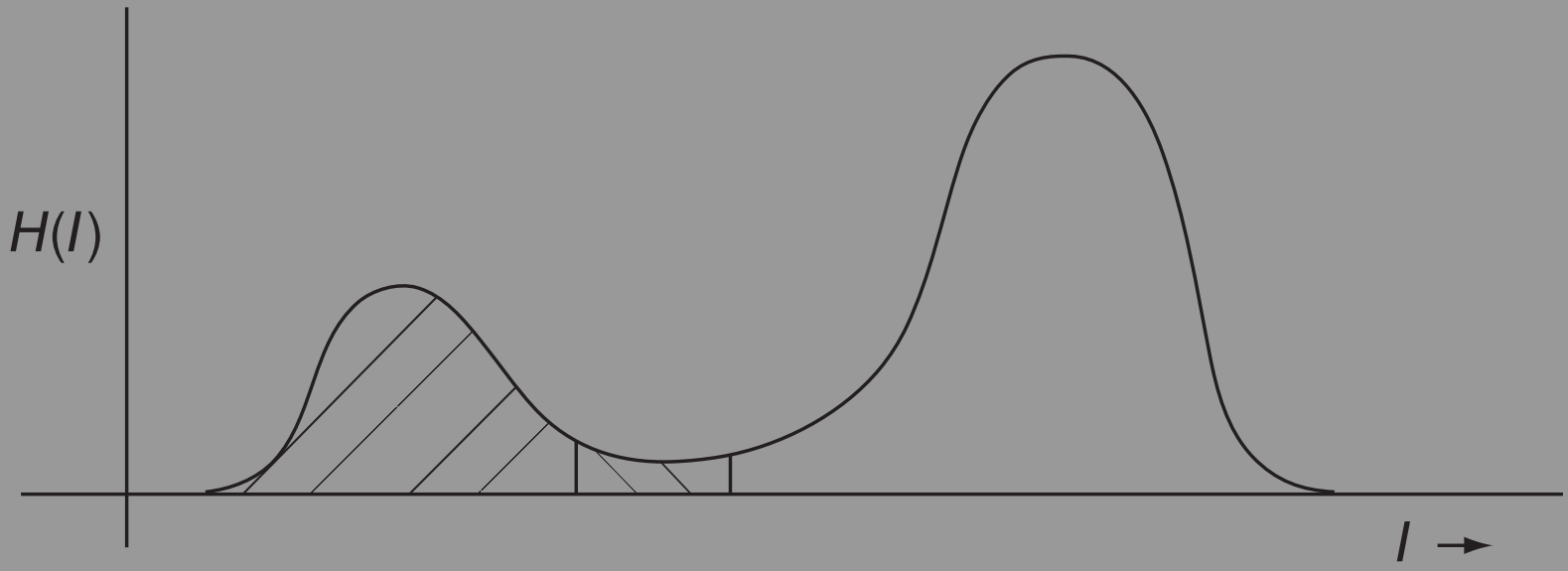


Manual threshold



Automatic threshold

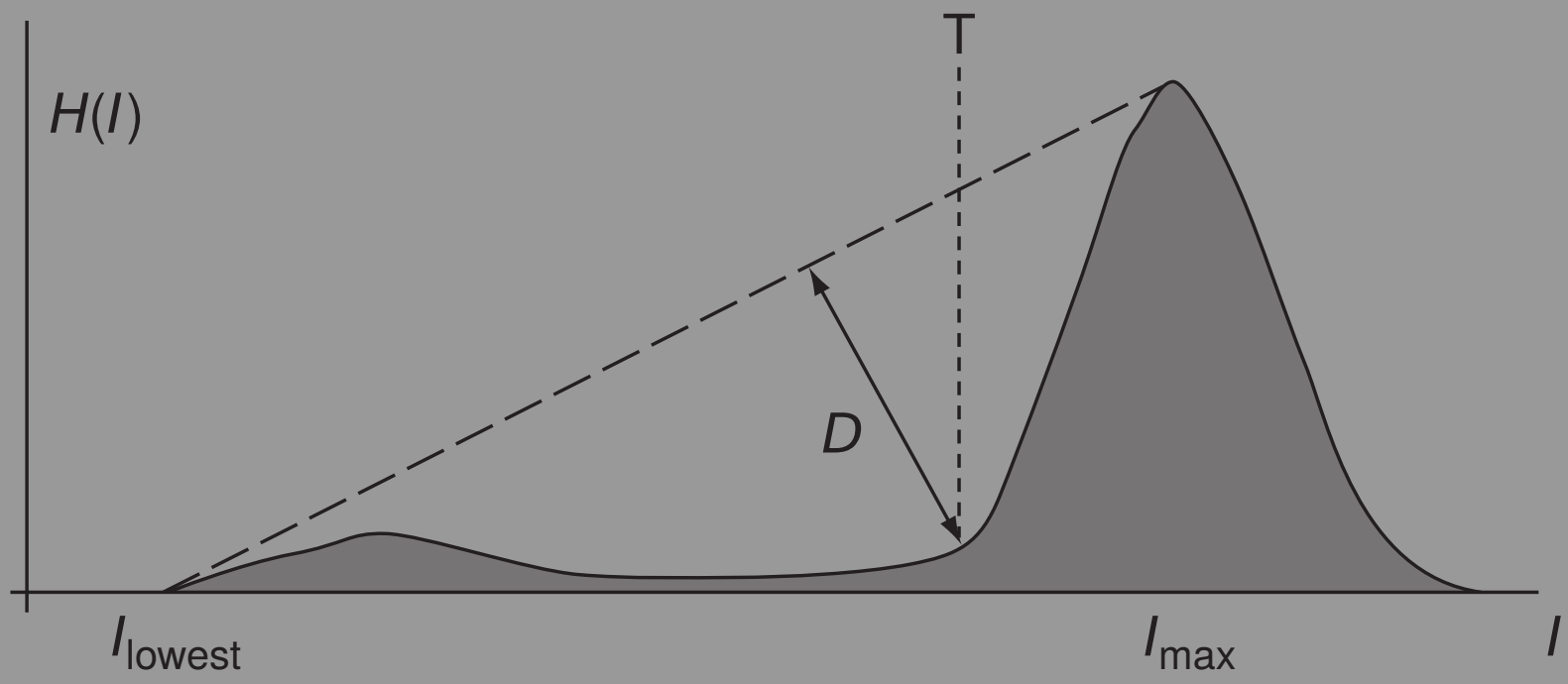
Otsu's algorithm



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

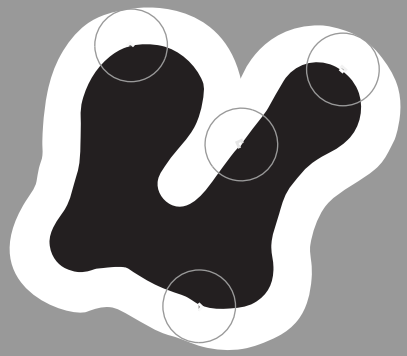
Automatic threshold

Triangle algorithm



- histogram smoothing (mean filter)

Erosion and dilation



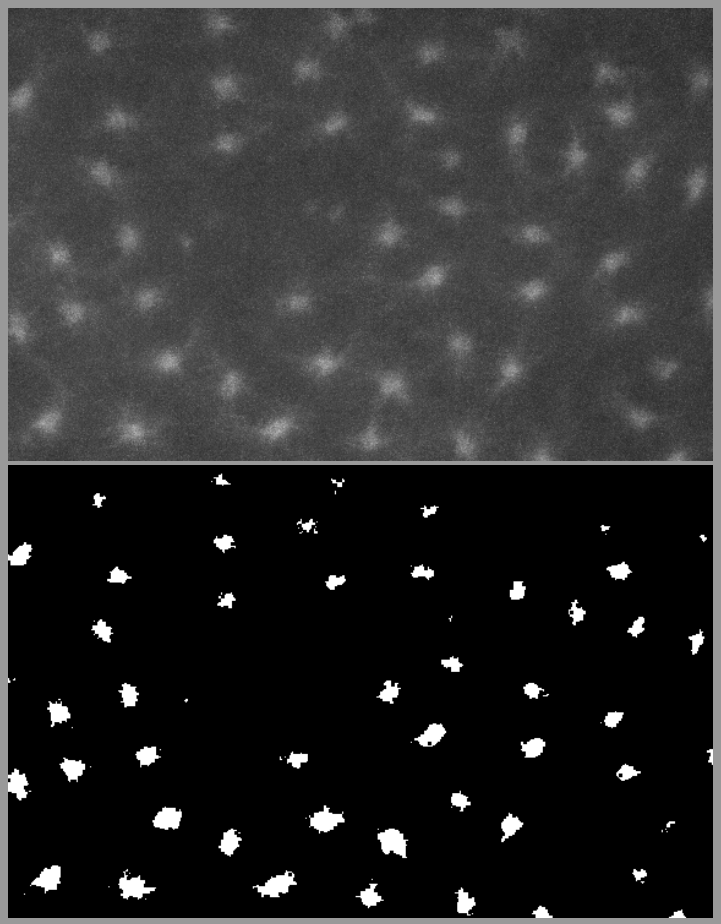
Dilation

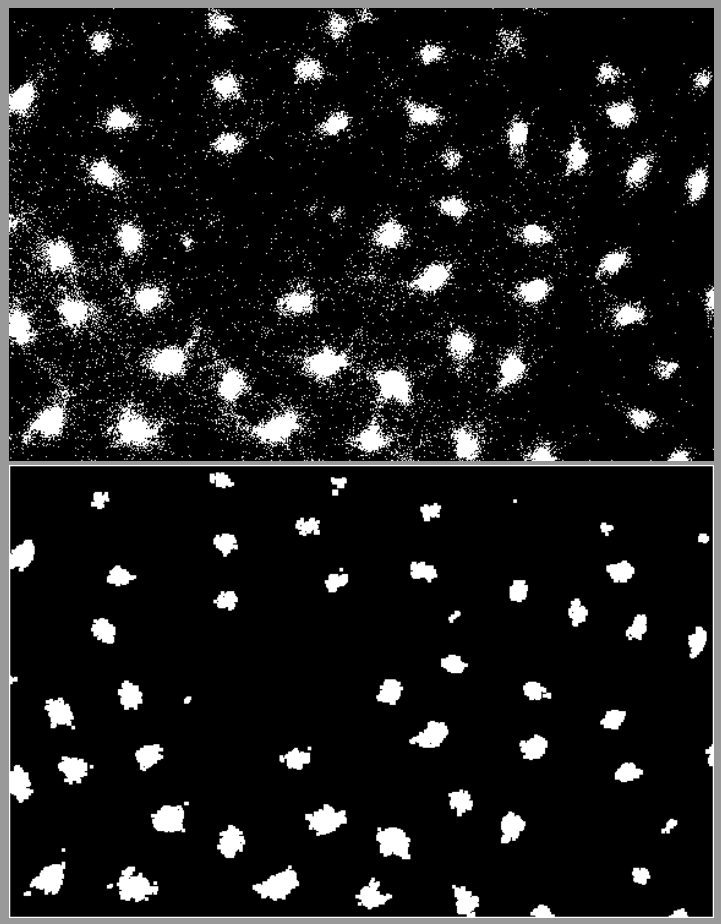
Erosion

Closing

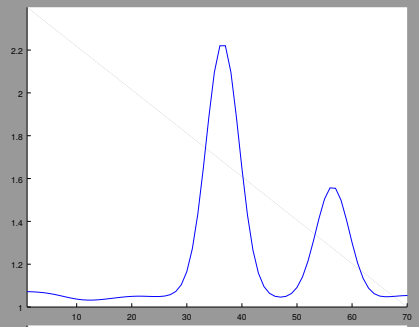
Opening

Erosion and dilation

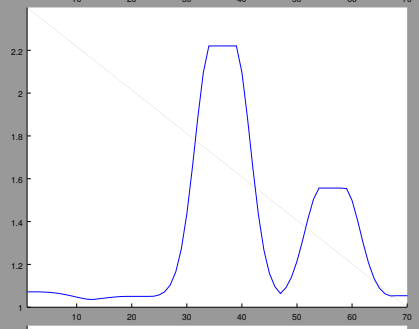




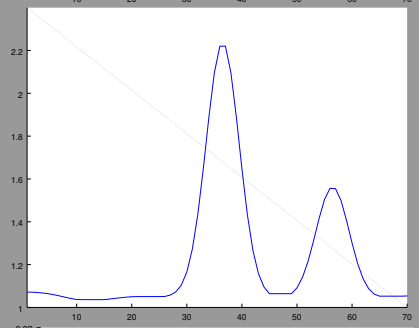
Original



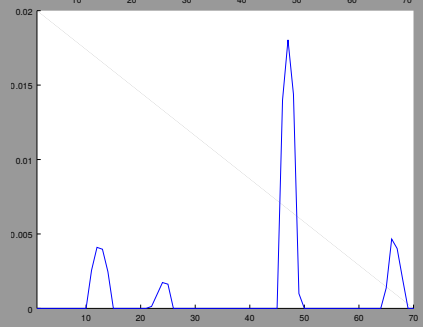
Dilation



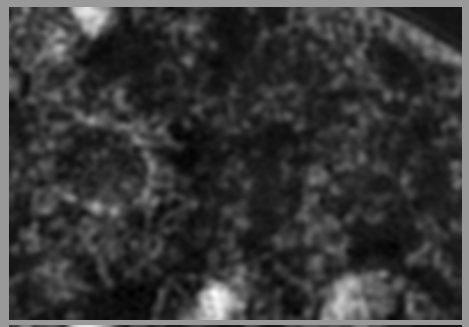
Closing

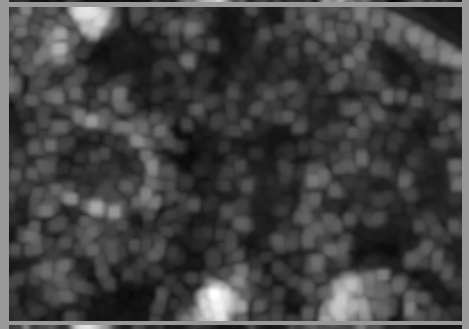


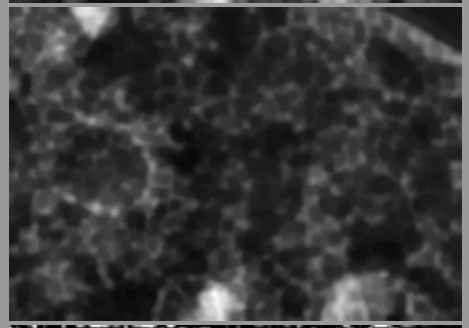
Bottom hat
(minus image)

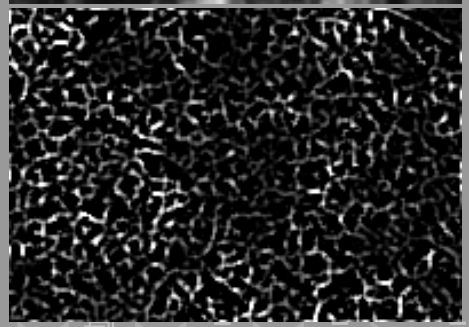


Bottom hat









Watershed

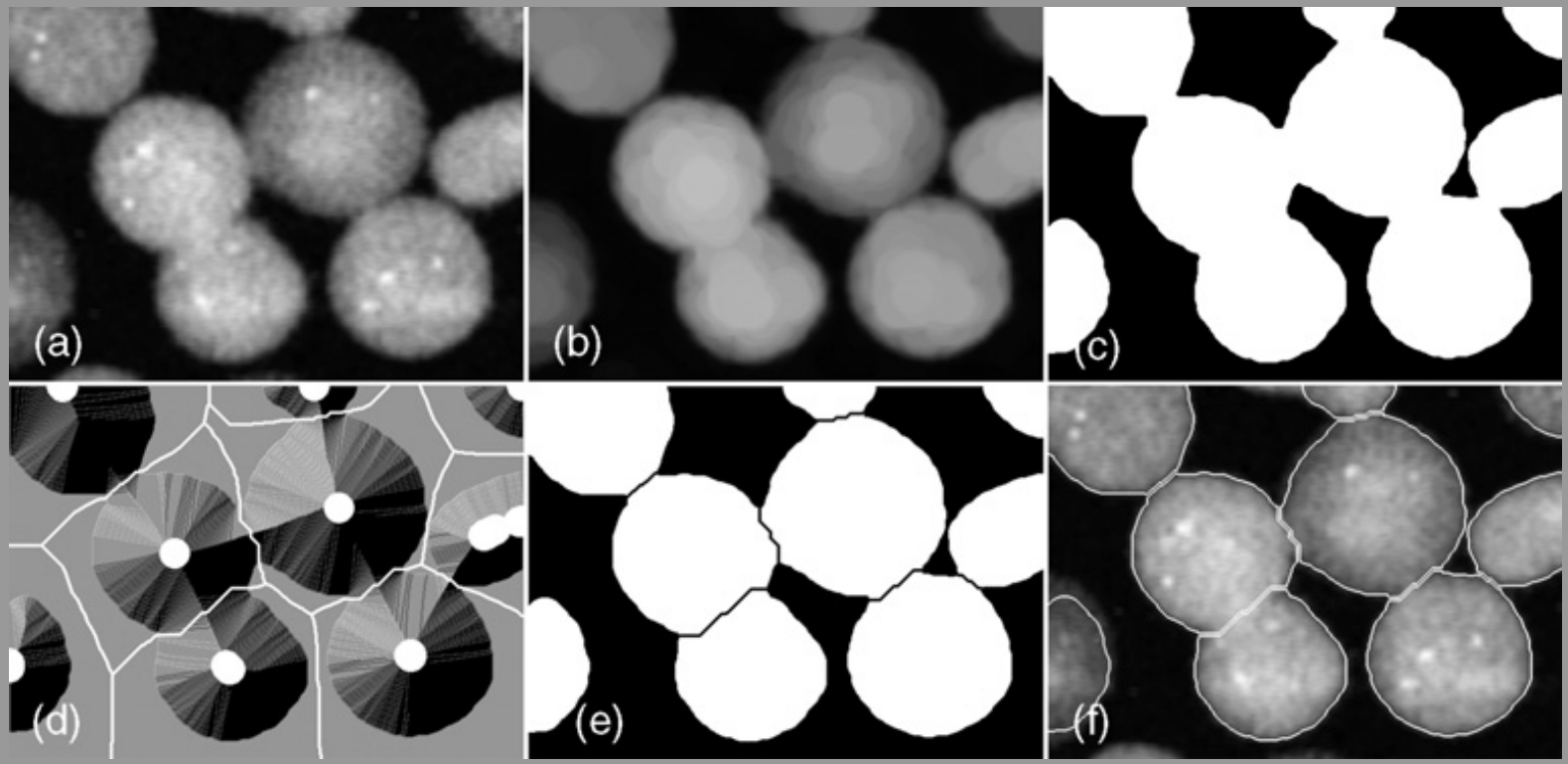
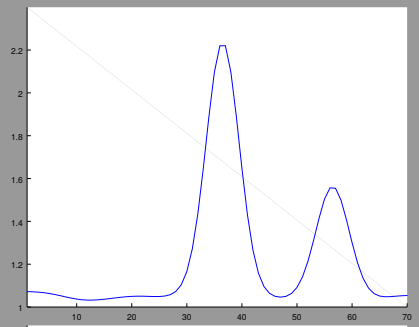
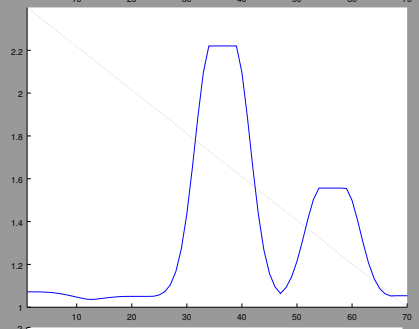


Image gradient

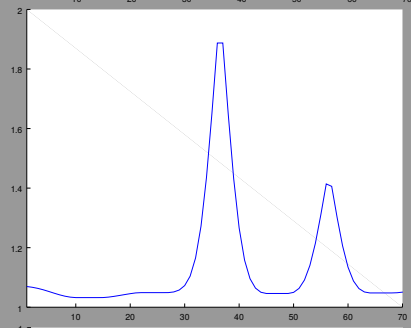
Original



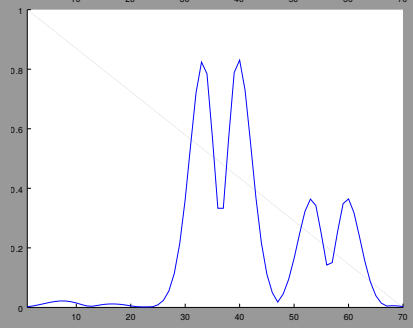
Dilation

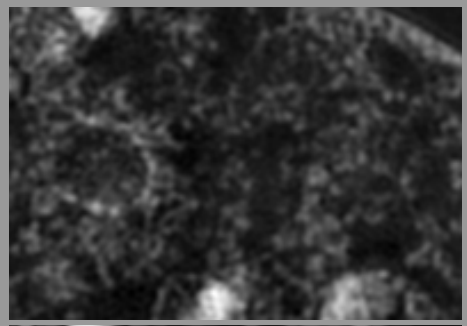


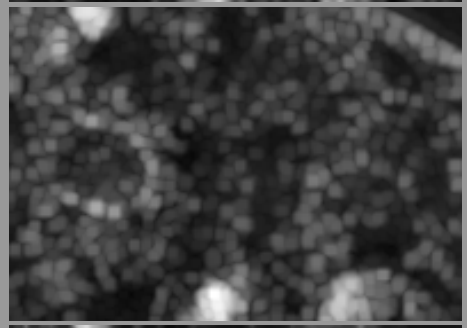
Erosion

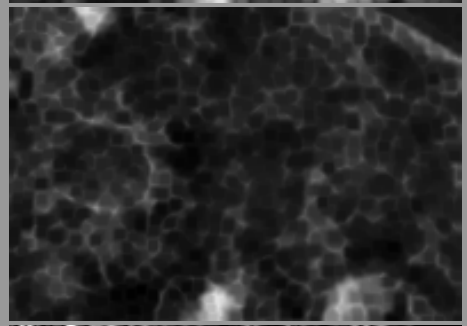


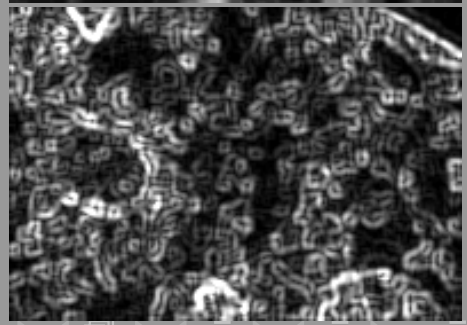
Gradient
(dilate - erode)



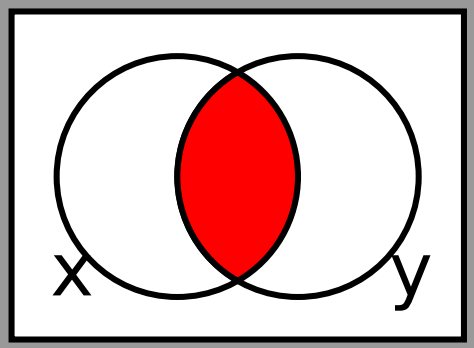




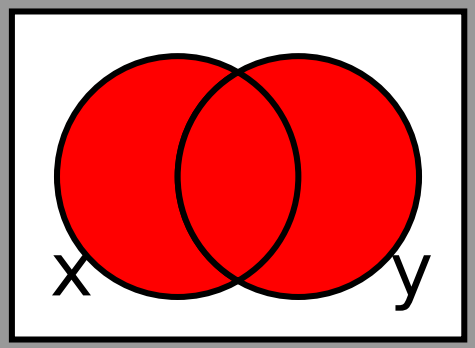




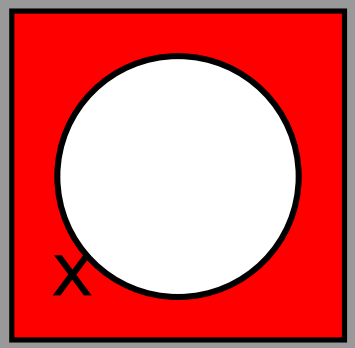
Logical operations



x AND y



x OR y



NOT x

Object properties

Particle/Region/ROI properties/measurements

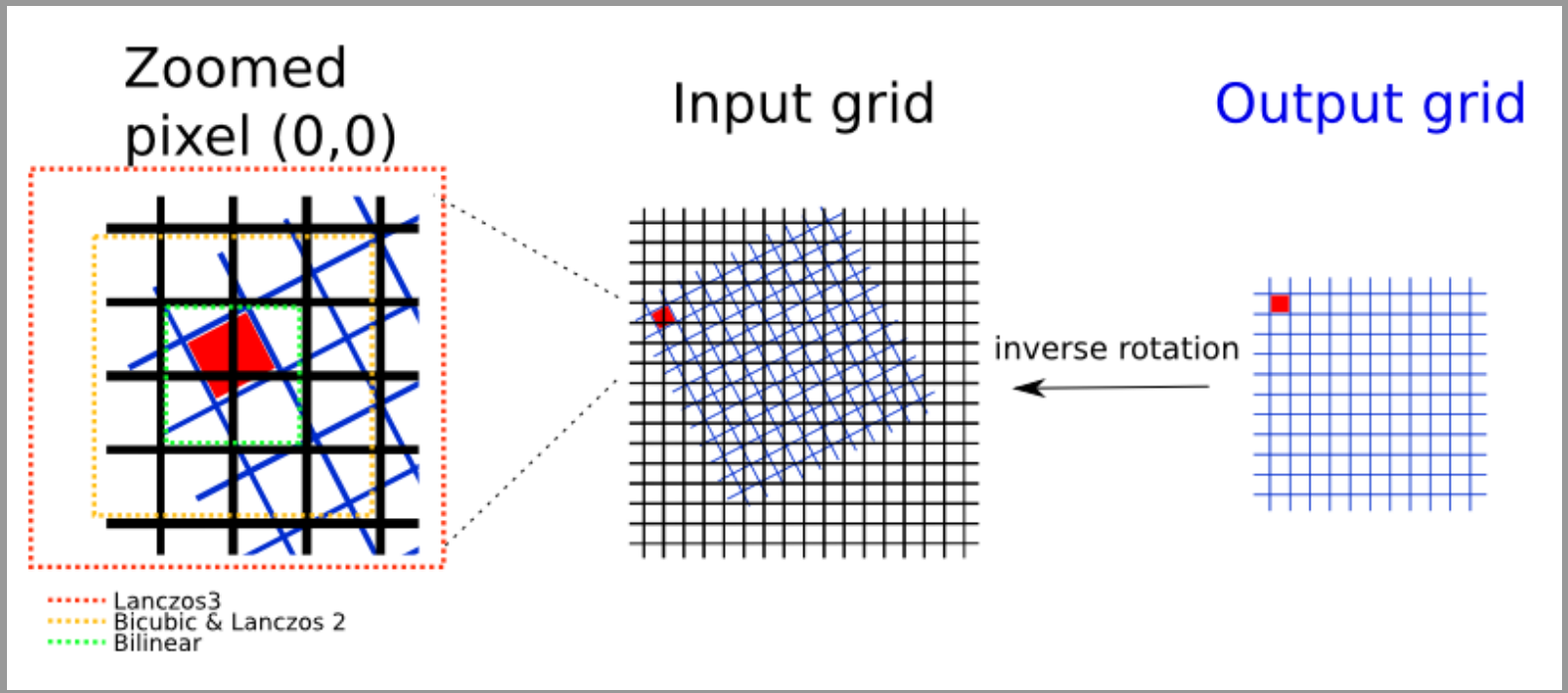
These are always one button or one line of code. The only problem is getting to this point.

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

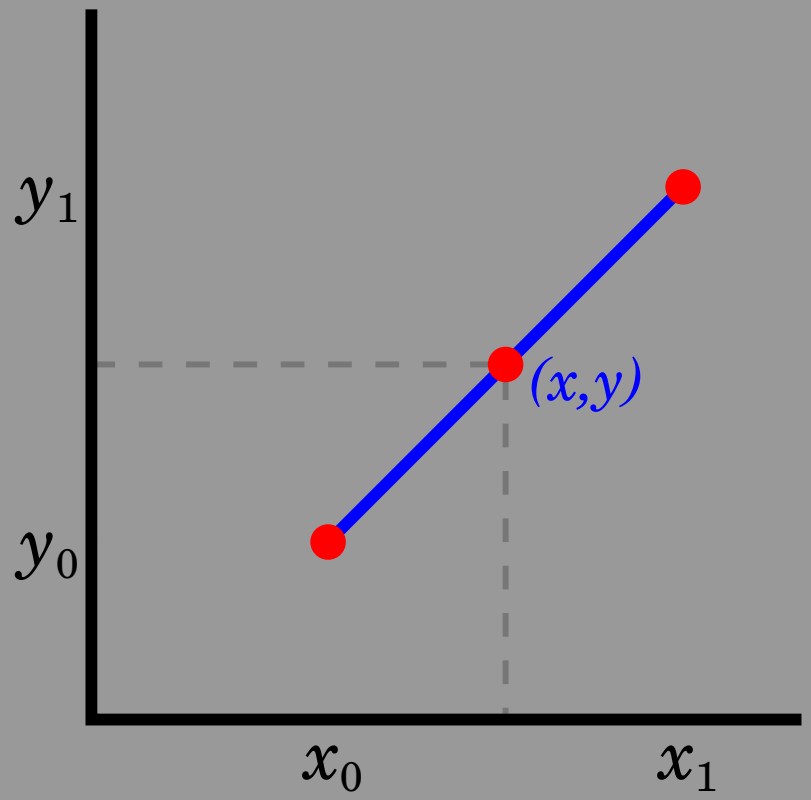
geometric transformation

What happens when you:

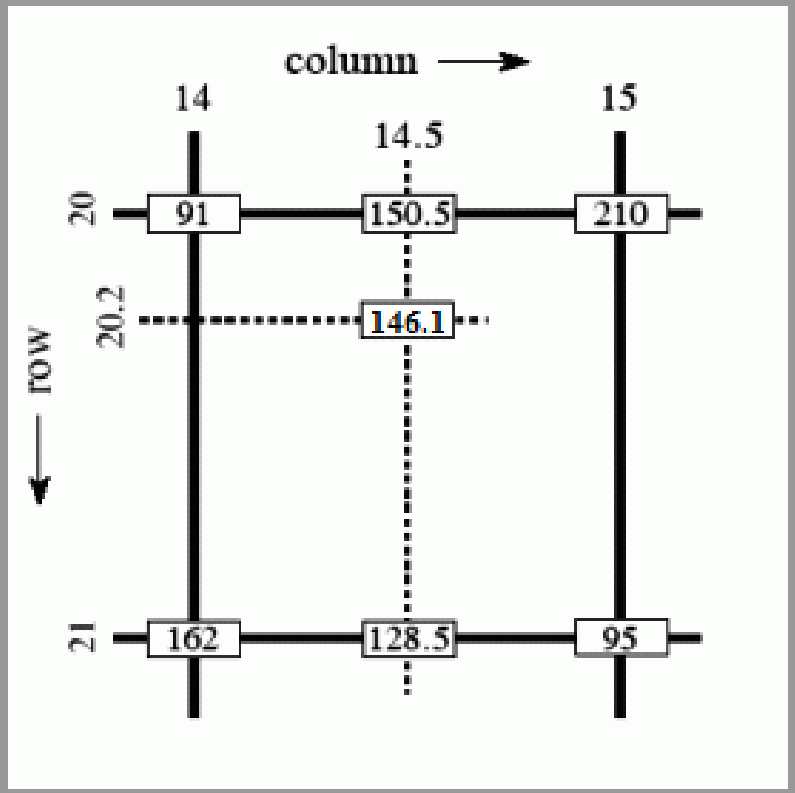
- rotate
- align
- translate
- stretch



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