

Plan

Basics

Images as Arrays

Bitdepth

Logical images

Tools

Filters

Explained

Convolution

Fancier filters

Segmentation

Threshold

Morphology

Logical operations

Object properties

Interpolation

Summary

Advanced Image Analysis

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Micron Oxford
Advanced Biolmaging Unit
(the basement)

Micron Advanced Microscopy Course, 2016

Microscope Image Analysis in 3 parts

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- 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

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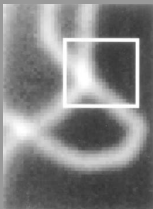
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63	84	119	172	219	225	182	135	79	51	36	24	23	19	15	-1	14	14	8	0	-4	7	18
78	84	104	170	223	224	196	118	84	49	36	28	16	11	25	4	15	13	8	-4	9	11	7
61	80	115	153	209	204	170	113	73	46	41	29	9	17	11	11	0	12	-2	2	2	3	23
72	98	121	160	190	207	178	116	68	50	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	326	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

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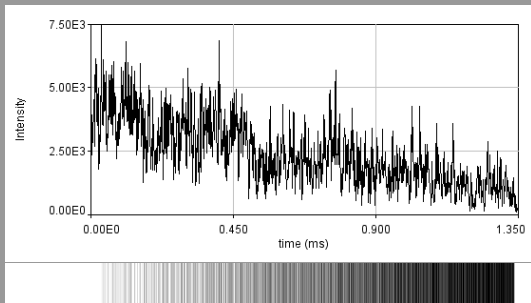
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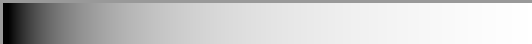
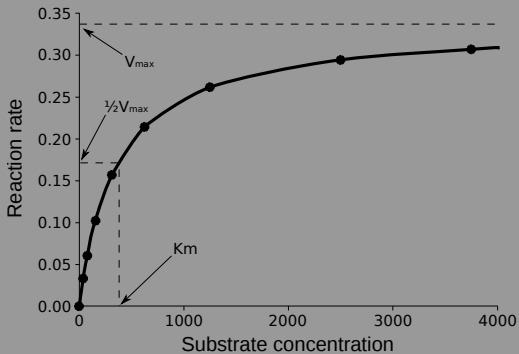
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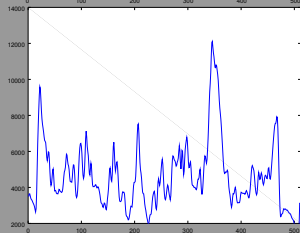
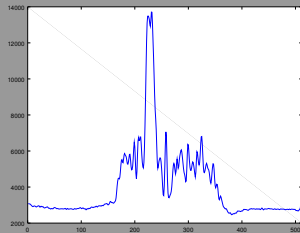
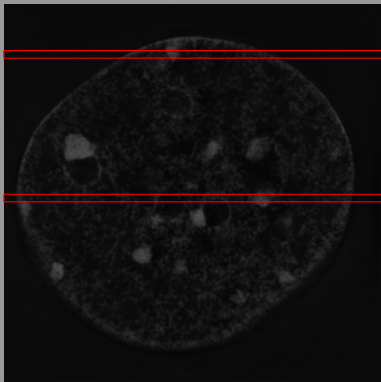
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Images as Surfaces

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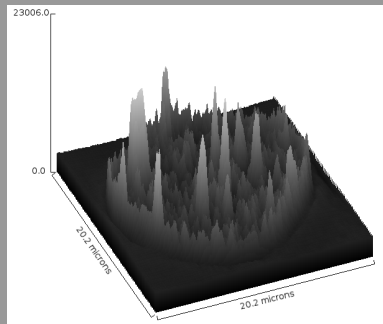
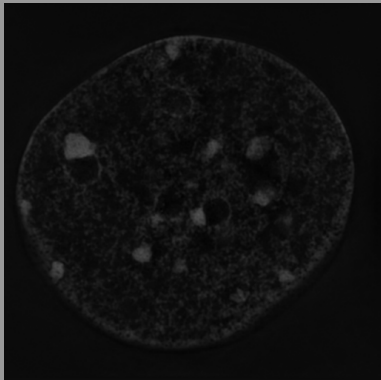
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Images as ND Arrays

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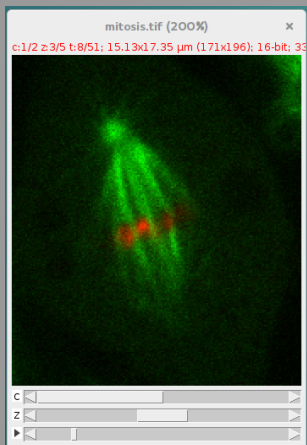
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Summary



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

Think "data", not "picture"

Bit depth / dynamic range

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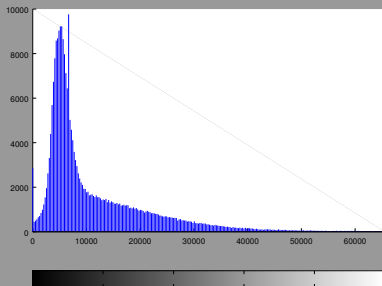
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2	2
$2 * 2$	4
$2 * 2 * 2$	8
...	
2^7	128
2^8	256
2^9	512
2^{10}	1024
2^{11}	2048
...	
2^{15}	32768
2^{16}	65536



More pixels in the same bin,
less dynamic range.

Floating point

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There is actually no limit. Example:

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

Floating points — sometimes incorrectly called 32 bit.

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Summary

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

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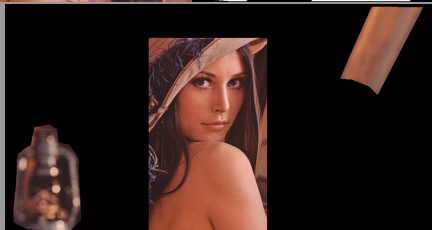
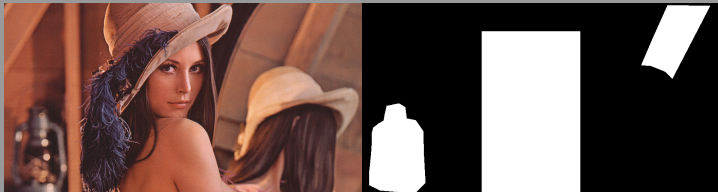
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Tools for image analysis

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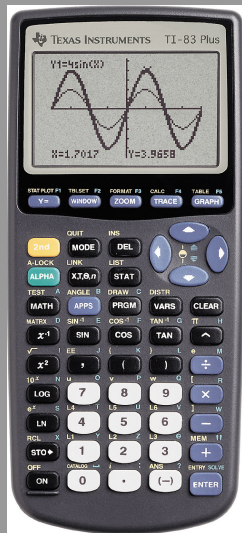
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ImageJ / FIJI



Python with NumPy



Octave

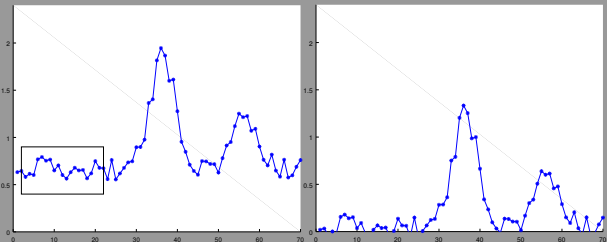


R

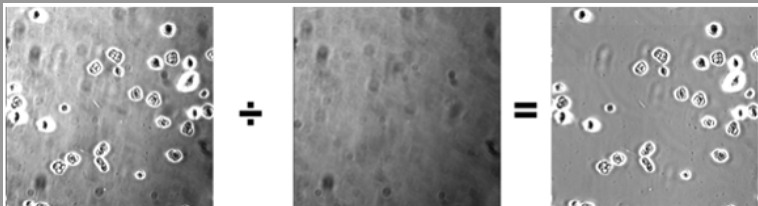
- CellProfiler
- Icy
- Omero
- Imaris
- softWoRx
- Volocity
- Matlab
- Metamorph
- Image-Pro Plus
- Huygens
- Mathematica
- ...

Background correction

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



- Correction for uneven illumination (divide by flats)



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Local means

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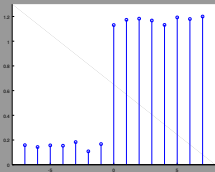
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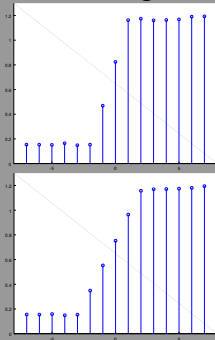
- Threshold
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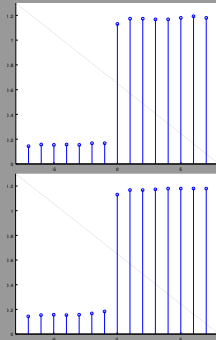
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Average



Median



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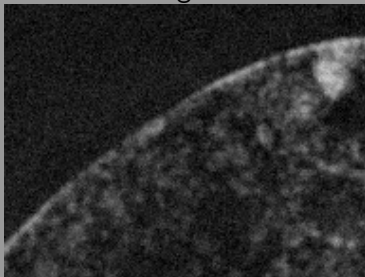
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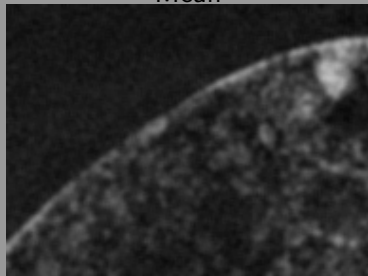
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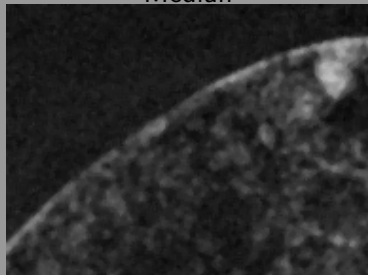


Local means

Mean



Median



Mean as convolution kernel

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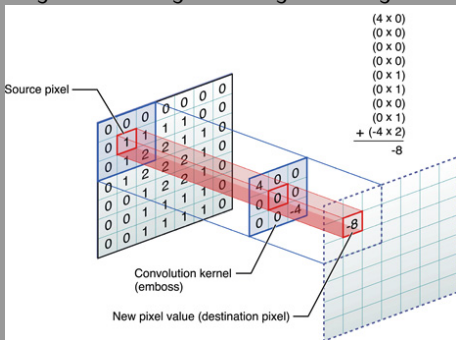
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$$\frac{4+5+6}{3} = 4 \times \frac{1}{3} + 5 \times \frac{1}{3} + 6 \times \frac{1}{3}$$



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

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

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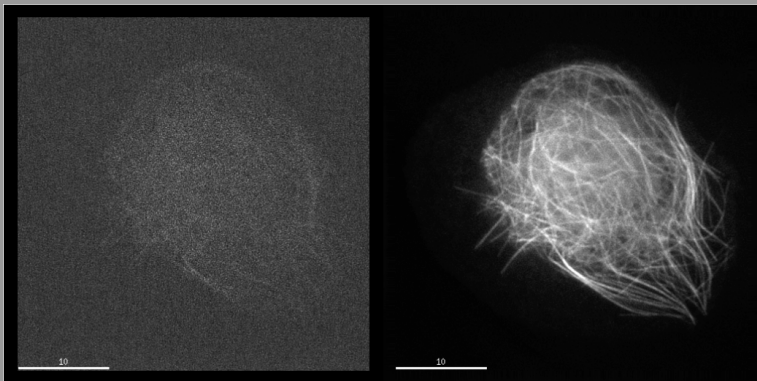
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Gaussian filter

as weighed mean

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

Edge detection

Sobel operator

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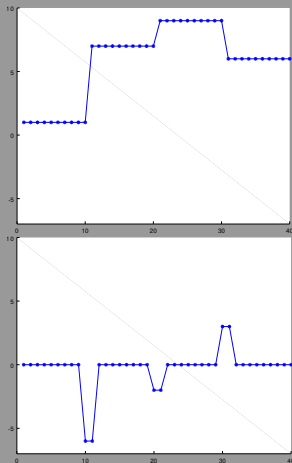
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1D filter

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



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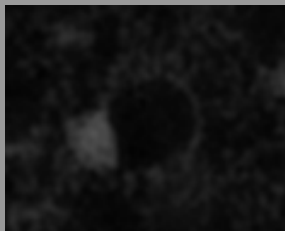
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2D filter(s)

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

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



Manual threshold

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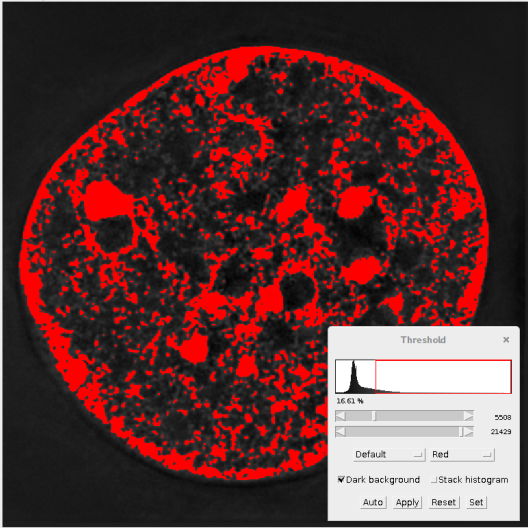
- Threshold**
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dapi-grays.png (150%)

512x512 pixels; 16-bit; 512K



Threshold

16.61 %

5508

21429

Default Red

Dark background Stack histogram

Auto Apply Reset Set

Automatic threshold

Otsu's algorithm

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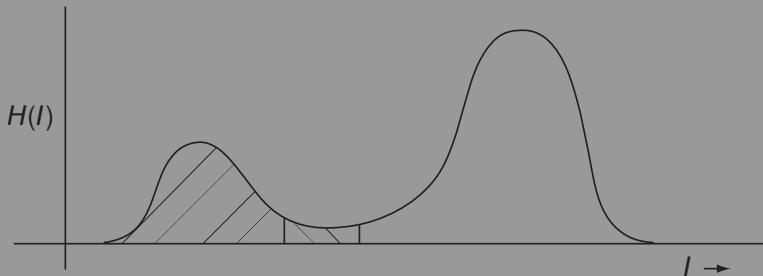
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- bimodal histogram
- reduce intra-class variance (spread)

Automatic threshold

Triangle algorithm

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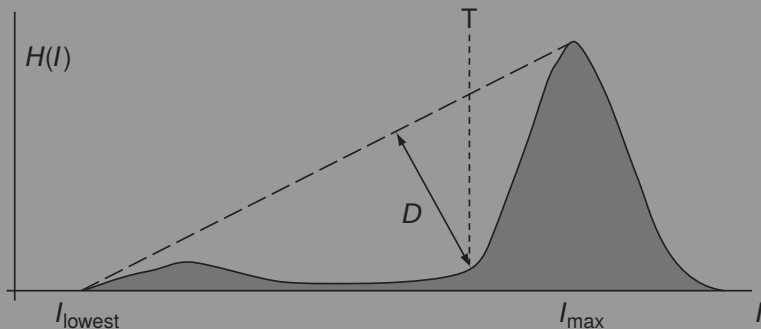
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- histogram smoothing (mean filter)

Erosion and dilation

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Dilation



Closing



Erosion



Opening

Erosion and dilation

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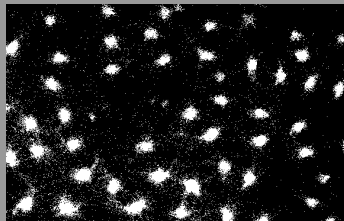
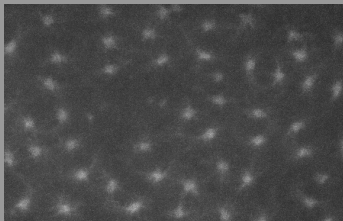
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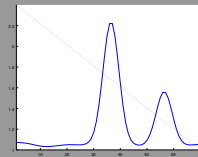
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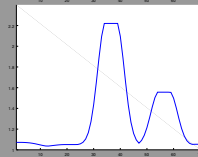
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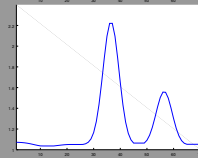
Original



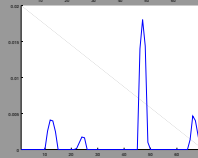
Dilation



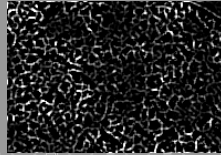
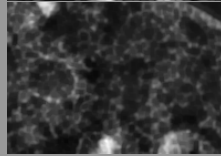
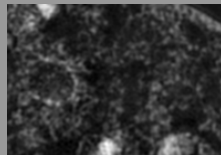
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Bottom hat
(minus image)



Bottom hat



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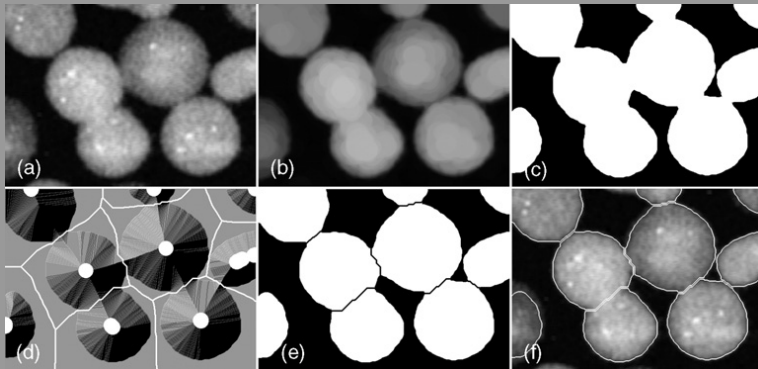
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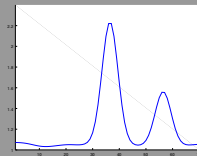
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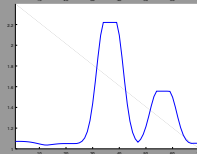
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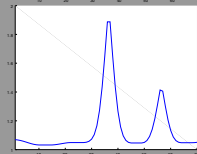
Original



Dilation



Erosion



Gradient
(dilate - erode)

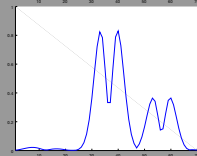
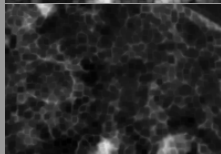
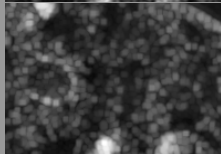
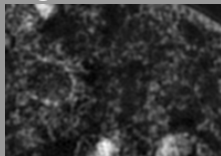


Image gradient



Logical operations

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Basics

Images as Arrays

Bitdepth

Logical images

Tools

Filters

Explained

Convolution

Fancier filters

Segmentation

Threshold

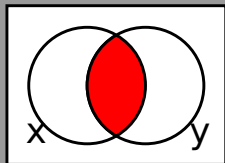
Morphology

Logical operations

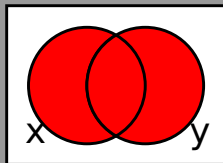
Object properties

Interpolation

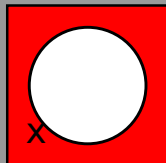
Summary



$x \text{ AND } y$



$x \text{ OR } y$



$\text{NOT } x$

Object properties

Particle/Region/ROI properties/measurements

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Summary

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

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

geometric transformation

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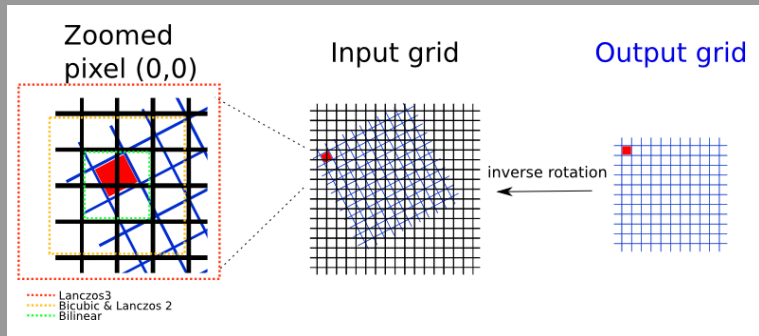
Object properties

Interpolation

Summary

What happens when you:

- rotate
- align
- translate
- stretch



Linear interpolation

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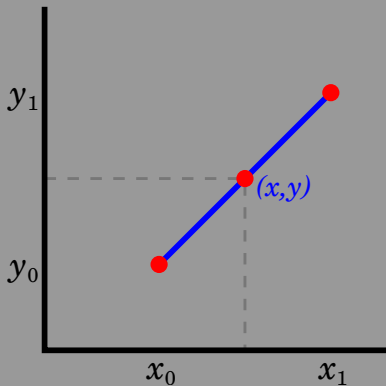
Morphology

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Summary



Bilinear interpolation

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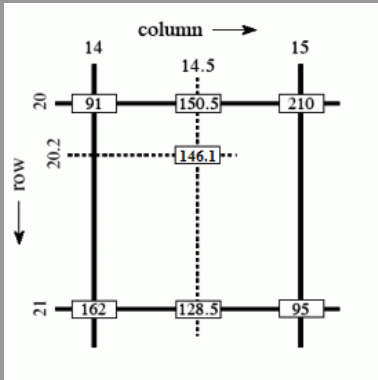
Morphology

Logical operations

Object properties

Interpolation

Summary



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Summary

Limitations such as:

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

are limitations of the implementation.

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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