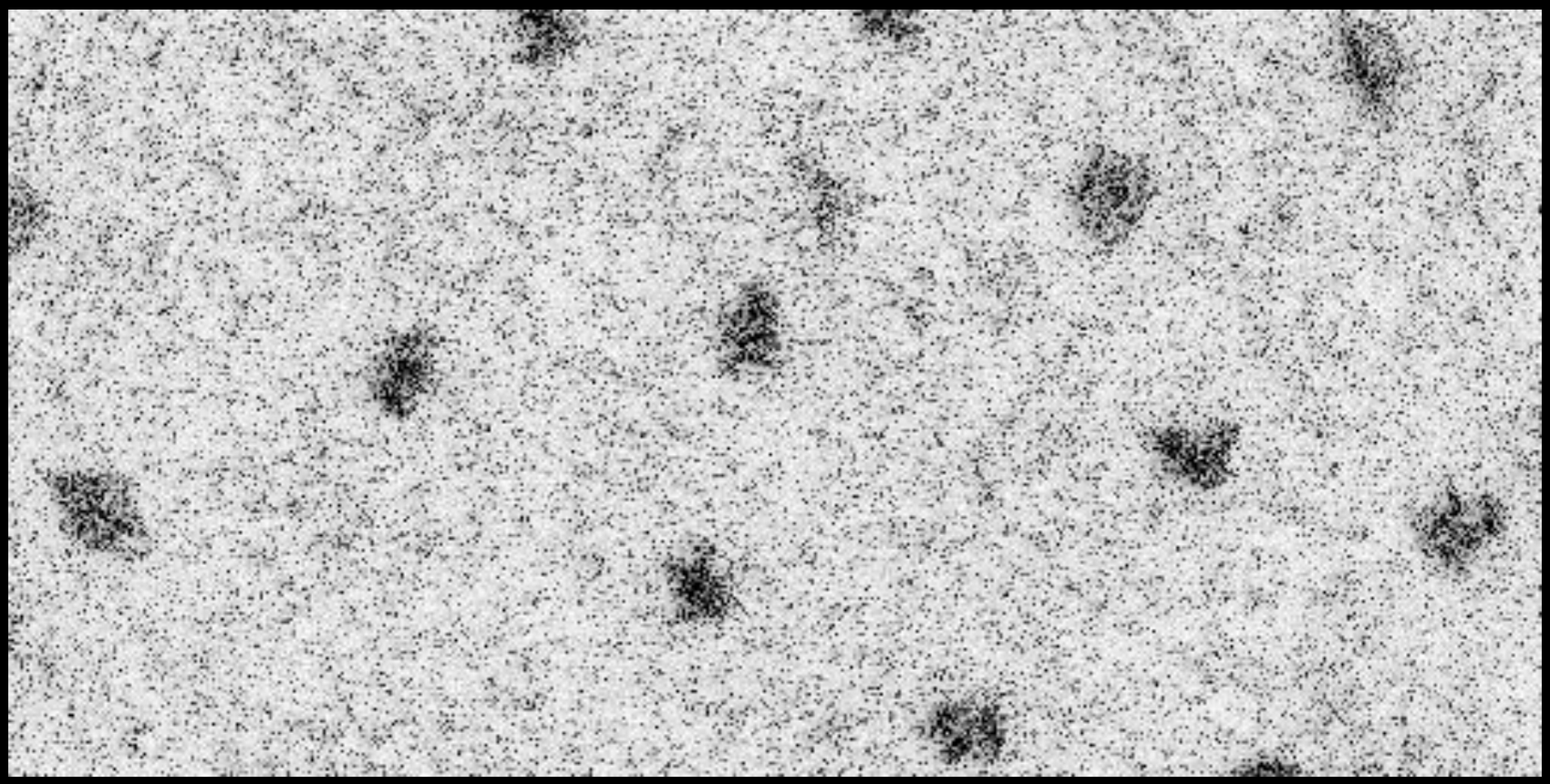
Fundamentals of image processing and image analysis

BIOL 542
Vincent Boudreau
28.11.2017

Why process images from a microscope?



Images are often pretty, but the really useful information is in the pixels

Why process images from a microscope?

In the real world, images need processing all the time…

Terahertz time-gated spectroscopic imaging for content extraction through layered structures

Camera Culture
MIT Media Lab

Authors:
A. R. Sanchez, B. Heshmat*, A. Aghasi, M. Zhang, S. Naqvi, J. Romberg, R. Raskar

Why process images from a microscope?

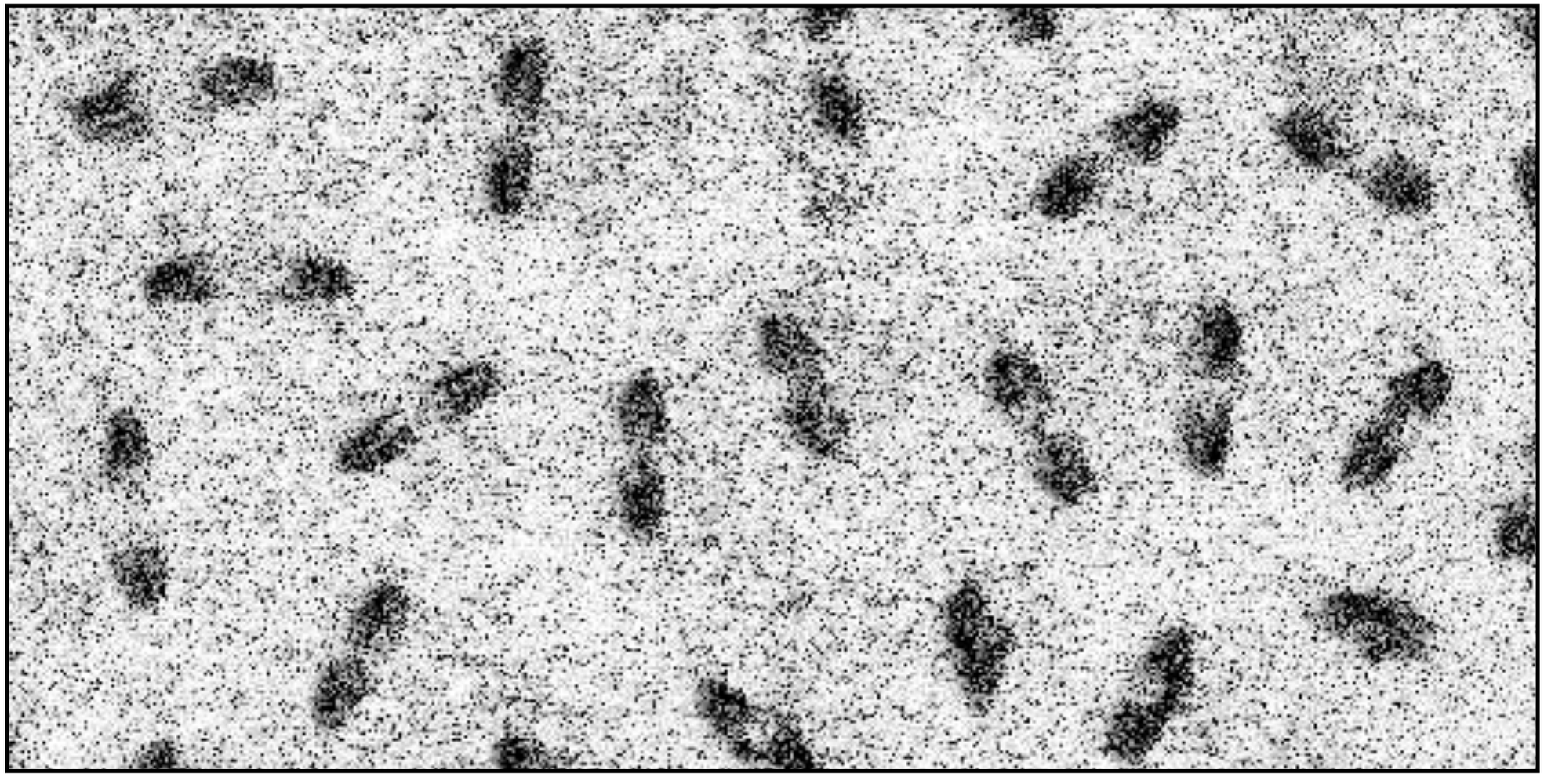
In the real world, images need processing all the time…

Why process images from a microscope?

In the real world, images need processing all the time…

Starting from the pixel up

What makes up an image?



Starting from the pixel up

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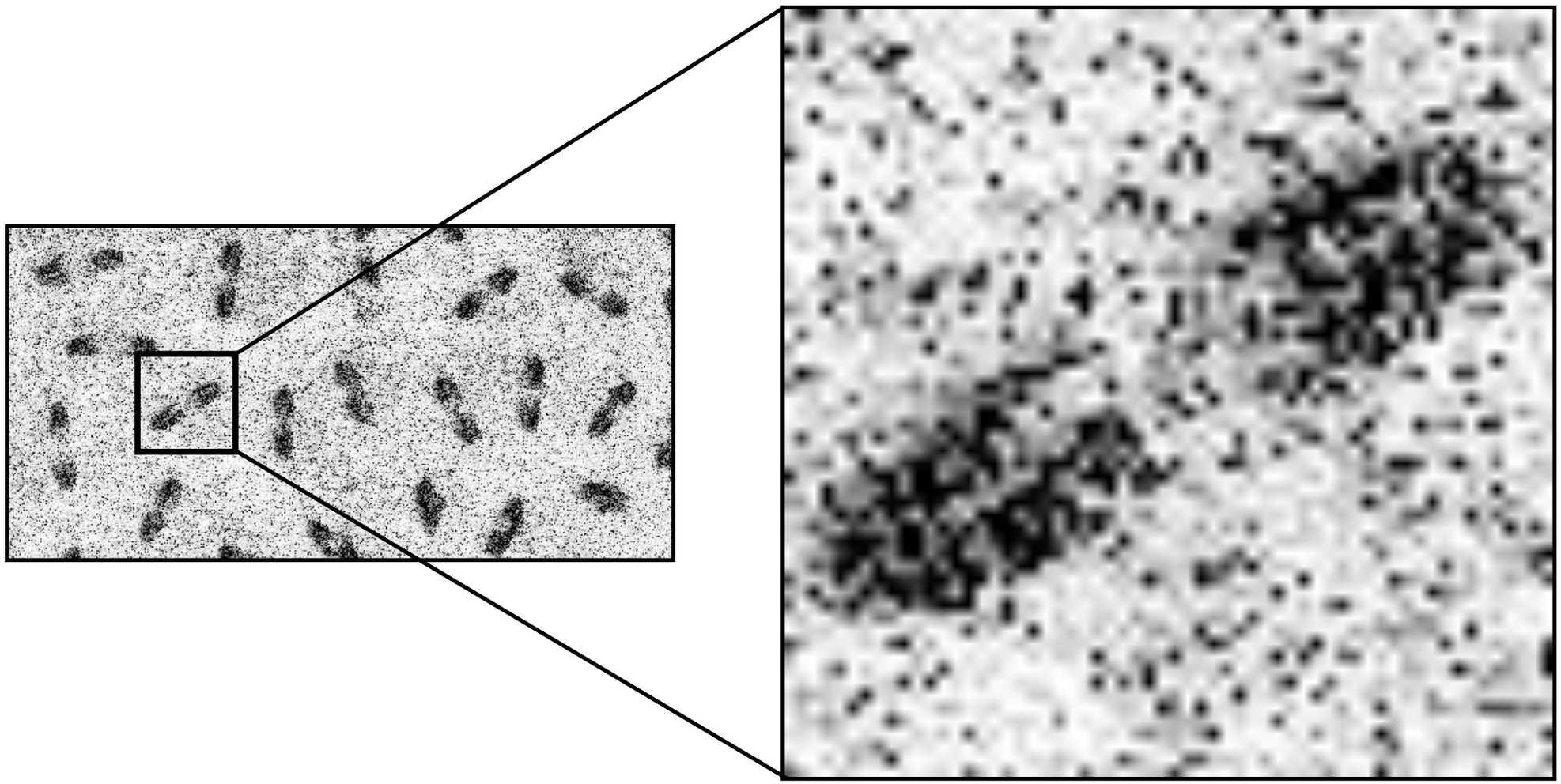

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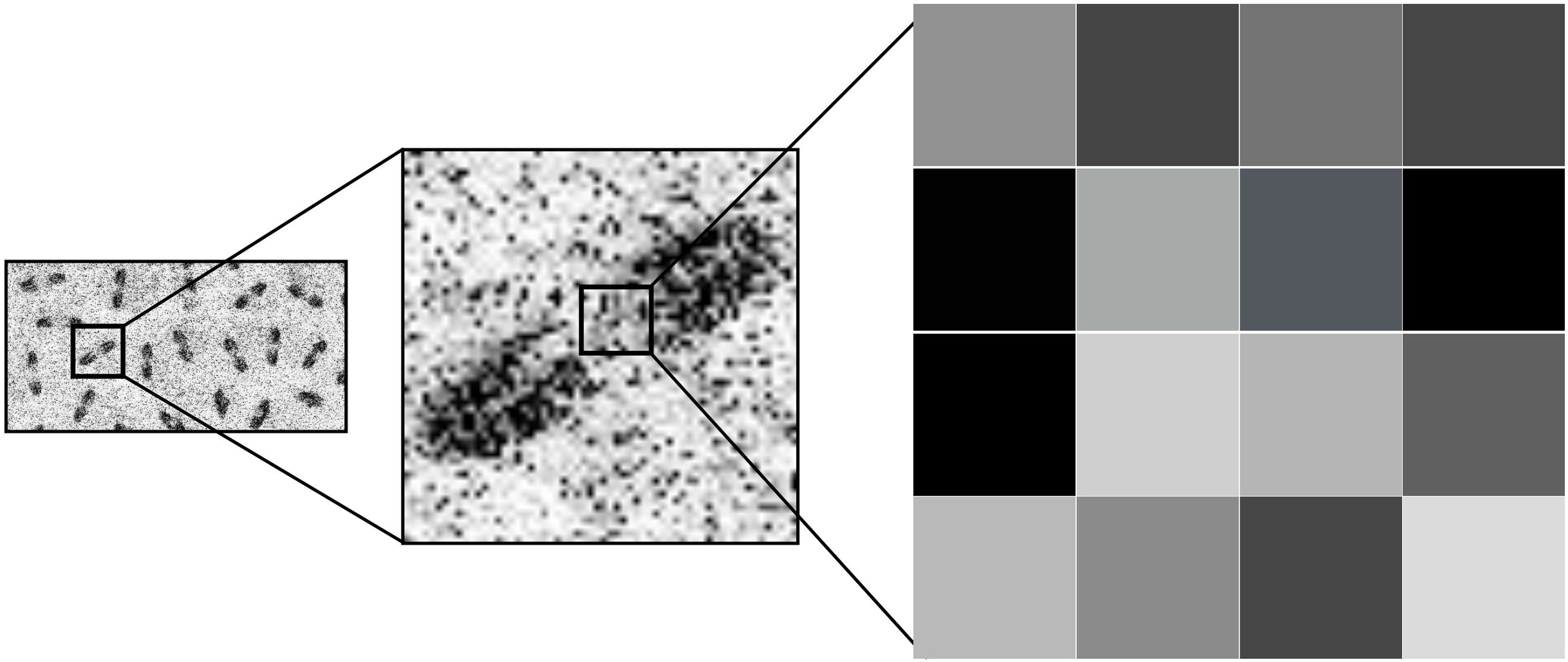

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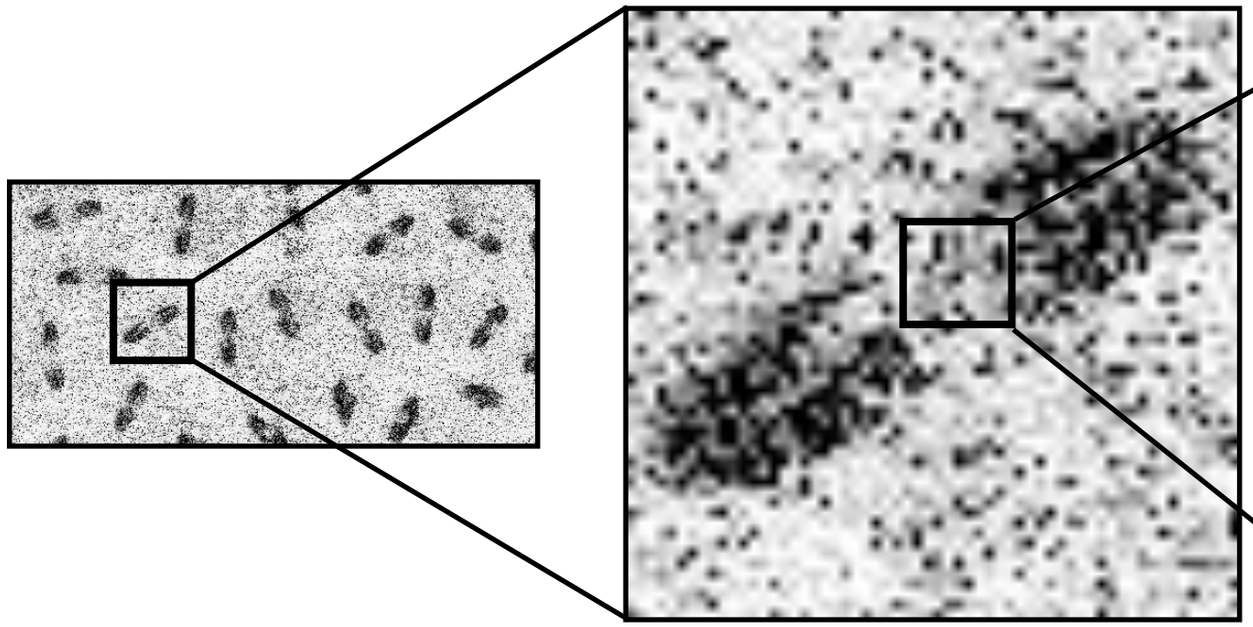
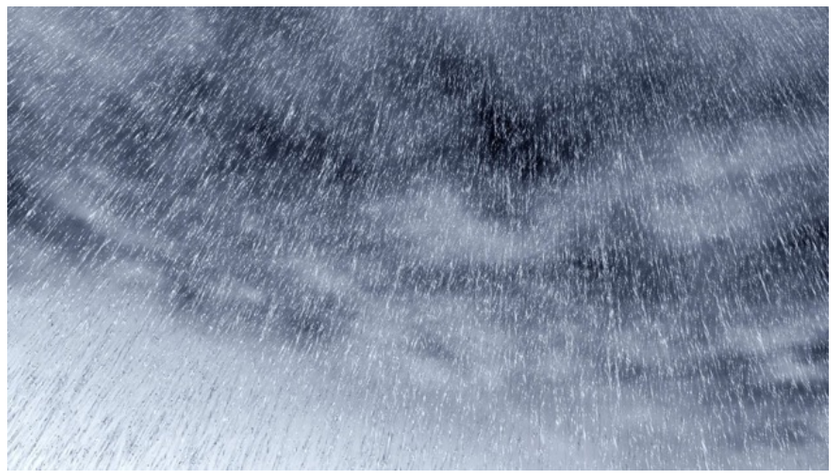


175	236	150	151	166	201	110	144	128	3	102	100	204	184	101
148	168	127	138	0	8	177	202	166	44	12	81	83	153	0
208	201	169	237	146	164	196	157	108	167	0	0	7	7	148
3	153	93	198	132	228	202	250	160	168	96	79	154	159	153
0	239	82	207	169	183	46	210	6	0	79	124	4	132	16
0	165	152	177	198	98	0	162	147	205	160	120	0	5	106
254	199	135	125	149	218	4	132	15	154	4	0	4	116	6
148	112	1	255	188	113	164	163	183	224	205	157	34	124	0
223	113	217	219	208	198	190	208	202	121	159	152	35	23	180
162	226	162	230	207	158	210	65	67	162	1	71	163	228	175
164	197	0	217	207	189	153	22	93	146	6	228	4	20	251
1	244	243	0	221	180	178	196	213	5	205	53	200	153	126
163	2	169	218	218	245	255	165	197	217	225	208	236	191	255
214	224	255	194	204	217	200	247	199	92	121	196	255	198	208
196	231	176	254	253	55	221	203	199	255	105	59	202	216	0

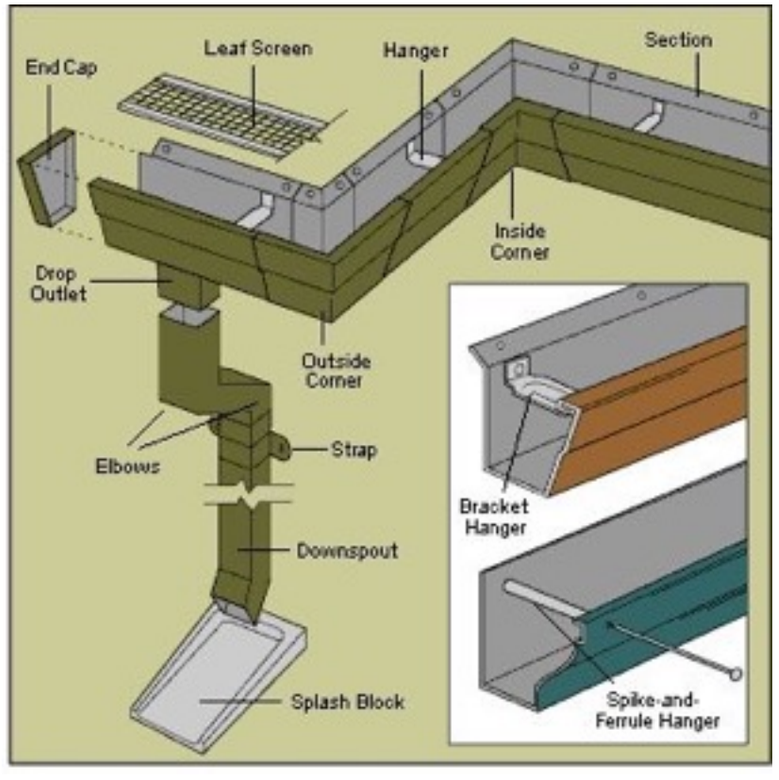
What’s a pixel?

What's a pixel?

Photons…

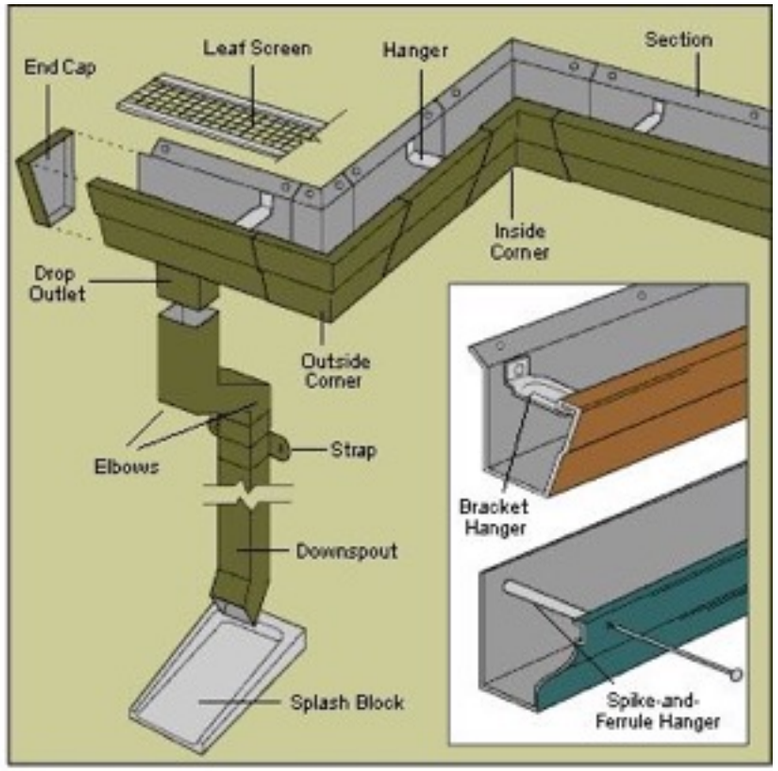


Microscope body…



What's a pixel?

Microscope body…



Pixels!

What’s a pixel?

Pixel value,
gray value,
gray level,
colours,
…

Pixels!

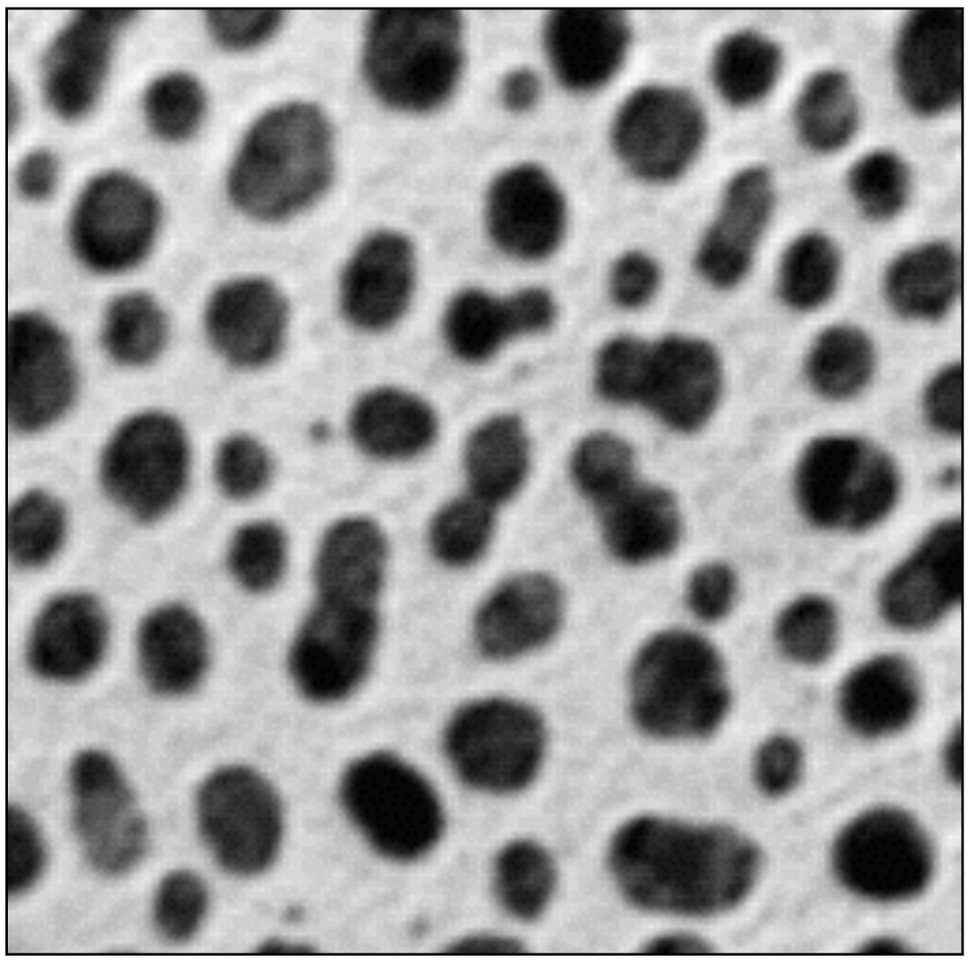
Bit-depth and dynamic range

$$\#\text{ gray values} = 2^{\#\text{bits} * \#\text{colours}}$$

$$256\text{ gray values} = 2^{8 * 1}$$

$$65536\text{ gray values} = 2^{16 * 1}$$

From gray values to masks

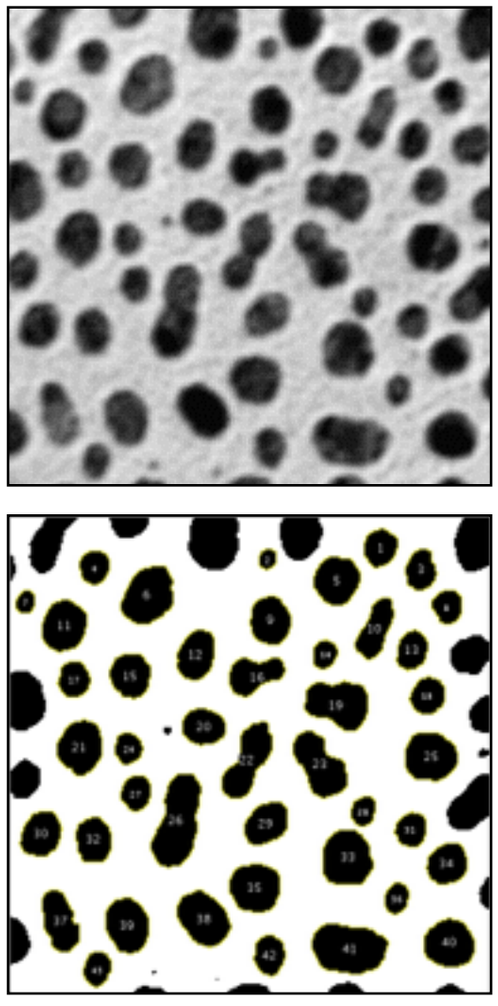


gray values = 2 #bits * #colours

↓

Finding relevant objects

From gray values to masks

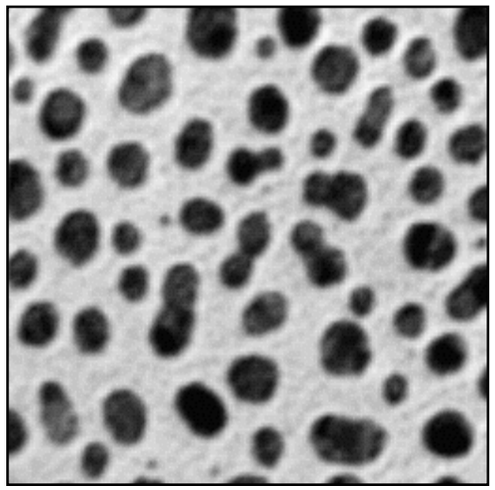


gray values = 2 #bits * #colours

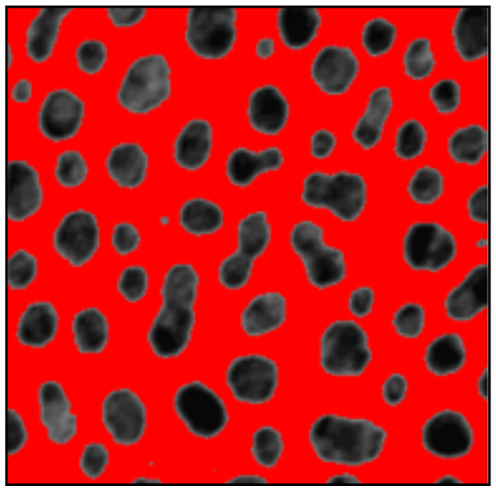
Finding relevant objects

Creating a mask

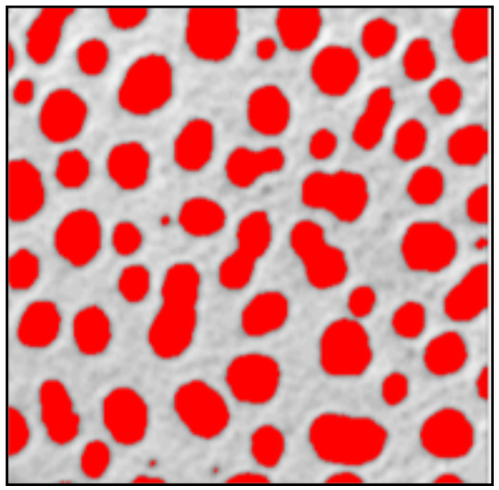
Thresholding - fluorescence intensity



All grays



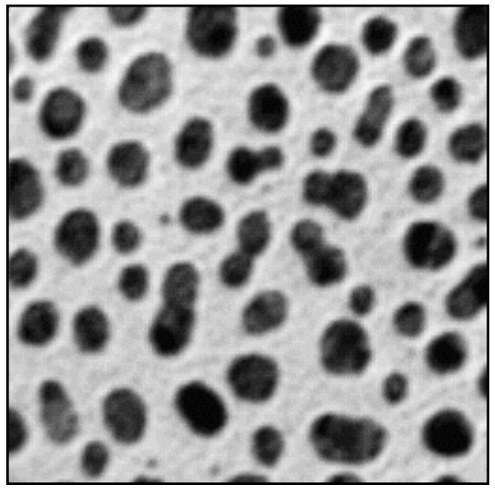
Low grays



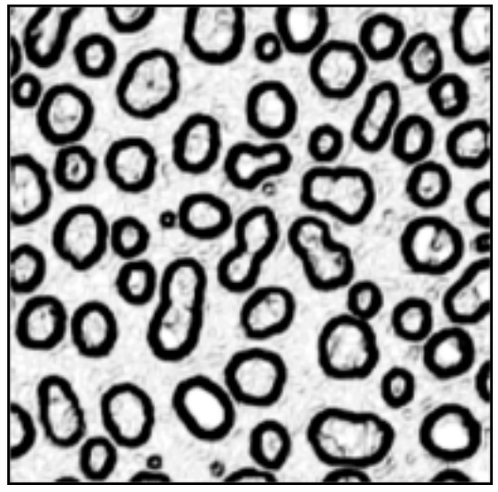
High grays

Creating a mask

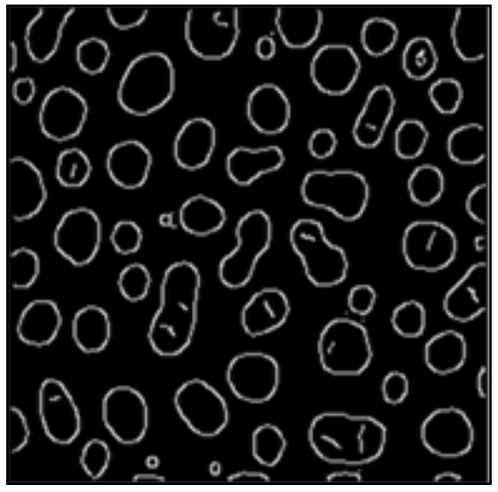
Edge detection



All grays



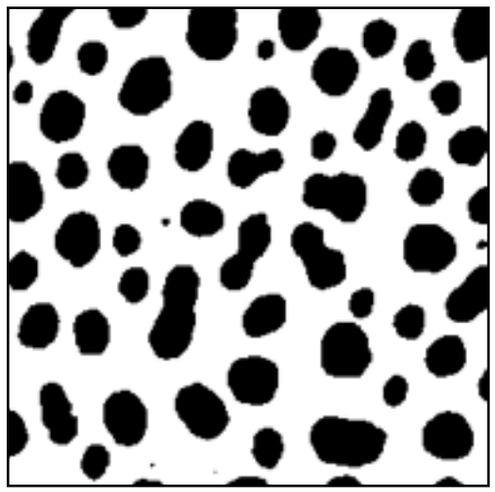
Variance filter
(not yet binary)



Canny edge detection

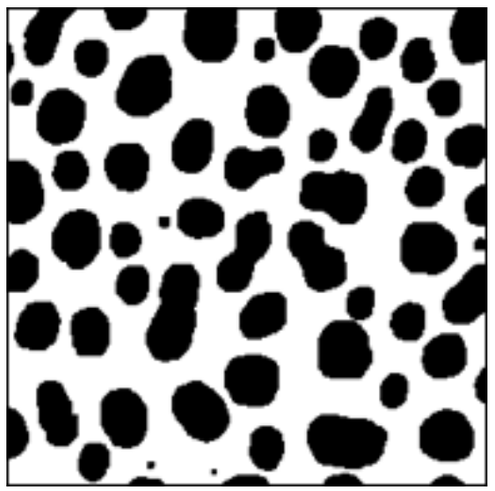
Adjusting a mask

Filters



Fluorescence intensity thresholded mask

Erode
(or minimum filter of 1)



Dilate
(or maximum filter of 1)

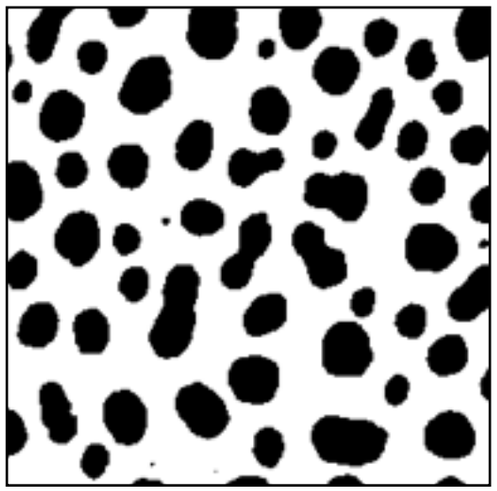
Filtering kernels

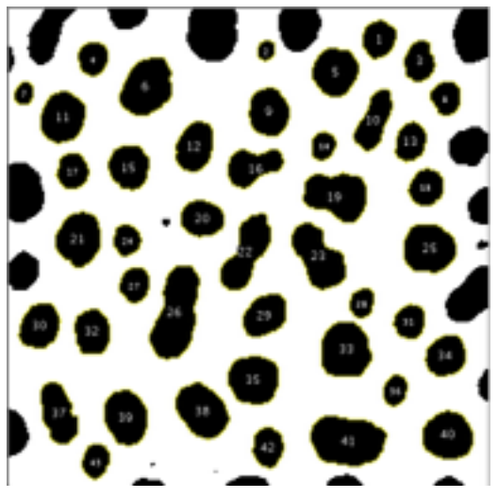
(here: circular radius of 1.5 pixels)

0	0	$\frac{1}{13}$	0	0
0	$\frac{1}{13}$	$\frac{1}{13}$	$\frac{1}{13}$	0
$\frac{1}{13}$	$\frac{1}{13}$	$\frac{1}{13}$	$\frac{1}{13}$	$\frac{1}{13}$
0	$\frac{1}{13}$	$\frac{1}{13}$	$\frac{1}{13}$	0
0	0	$\frac{1}{13}$	0	0

Generate quantitative information

Images are data in themselves, but contain more information than what is immediately available





Area
Perimeter
Circularity
Aspect ratio
Longest distance
…

Tutorial #1

Quantify nuclear size

Tutorial #2

Quantify nuclear fluorescence intensity

Processing exercises

Intensity distribution + dynamic range

Filtering

Thresholding

Analyzing particles

Measurements

Tutorial 1: shape descriptors - nuclei

- Open image: File > Open Samples > HeLa cells
- Separate channels: Image > Color > Split Channels
- Duplicate the nucleus image for #2
- Thresholding: Image > Adjust > Threshold
- Add to ROI manager: Analyze > Analyze Particles
- Adjust measurements: Analyze > Set Measurements > Area + Perimeter + Shape descriptors + Feret's diameter
- Measure: From ROI manager, click measure

Tutorial 2: fluorescence intensity

- Open image: Select original nucleus image
- Overlay: Image > Overlay > From ROI manager
- Adjust measurements: Analyze > Set Measurements > Mean + STD deviation + Min & Max + Mean, etc.
- Measure: From ROI manager, click measure

Tutorial 3: macro recorder

- Do #1 with macro recorder on
- Run it on new image…