# Microscope Image Analysis

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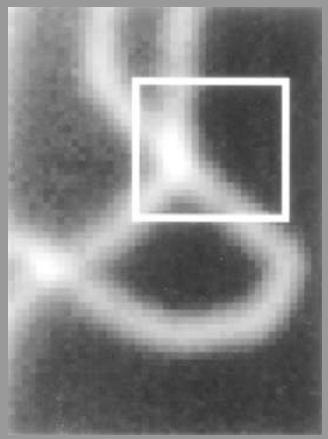
Micron Advanced Microscopy Course, 2019

# Microscope Image Analysis in 3 parts

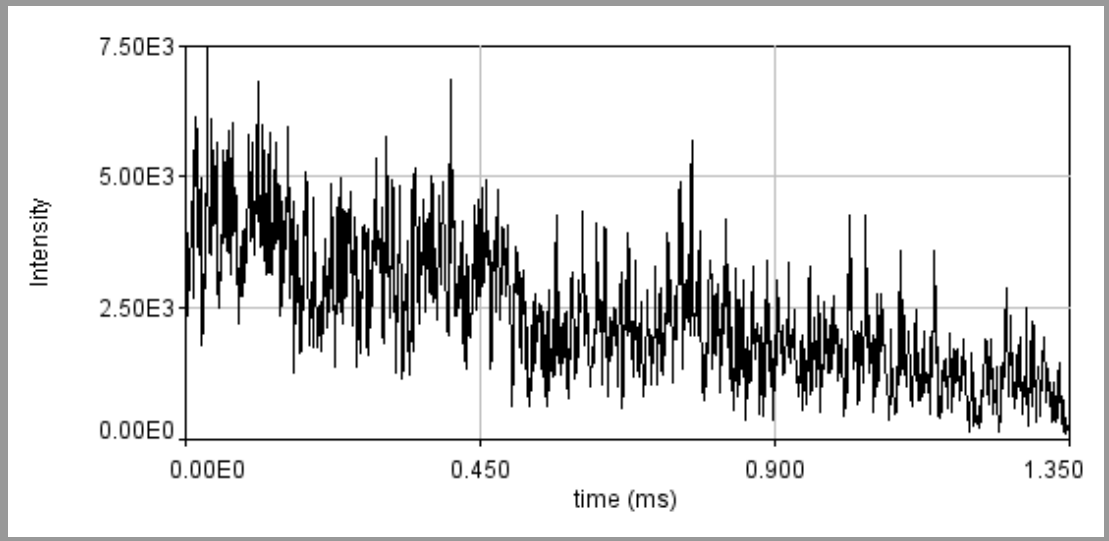
1. What is in a microscope image
   - What is in a image?
   - Image display
   - Image acquisition
2. Careful with your data
   - File formats
   - OMERO
   - Figure preparation
3. Images as N dimensional numeric arrays
   - N dimensional images
   - Spatial filters
   - Morphology
   - Connected components
   - Tools

# Pixel data

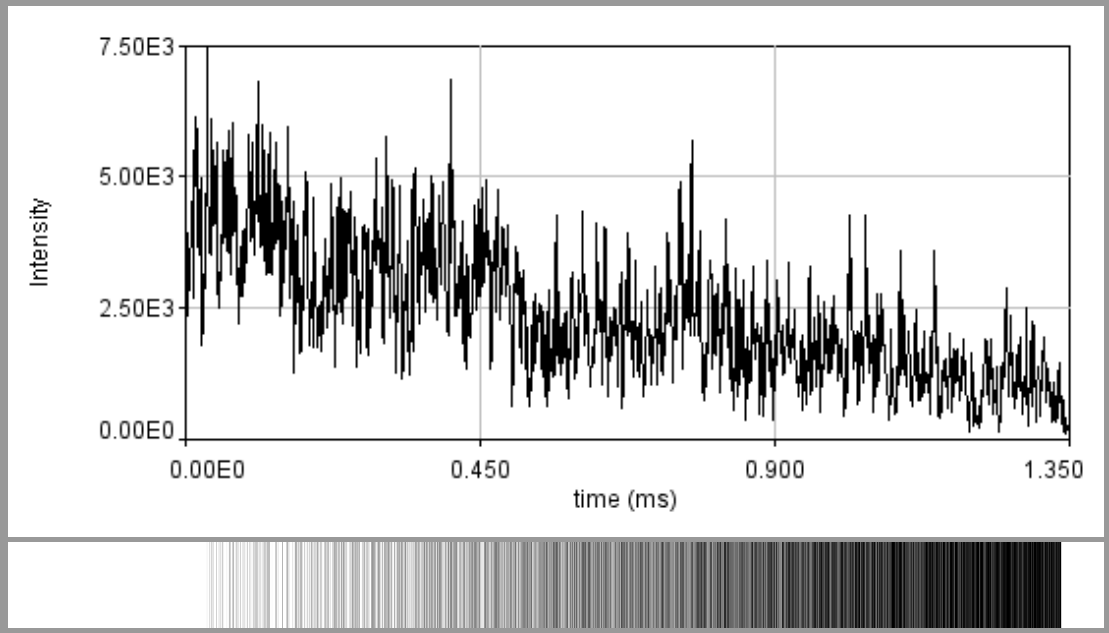


| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 63 | 84 | 119 | 172 | 219 | 225 | 182 | 135 | 79 | 51 | 36 | 24 | 23 | 19 | 15 | -1 | 14 | 14 | 8 | 0 | -4 | 7 | 18 |
| 78 | 84 | 104 | 170 | 223 | 224 | 196 | 118 | 84 | 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 |

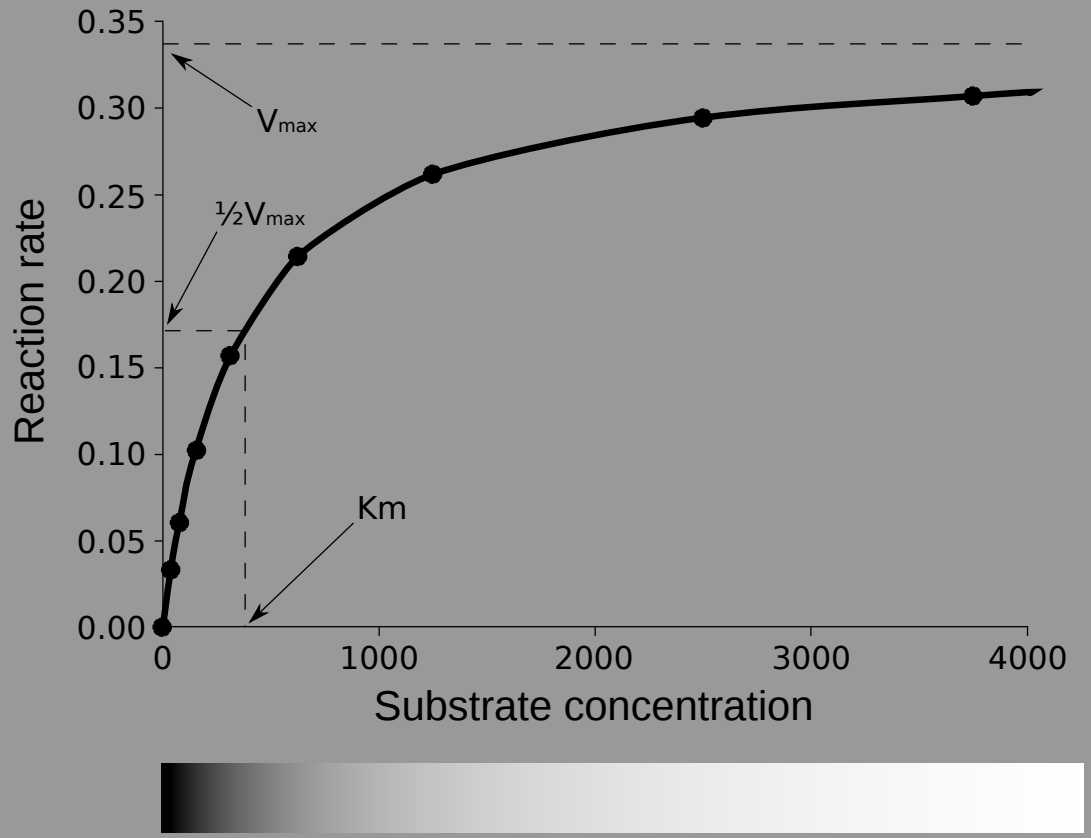
# Images as Signals



# Images as Signals

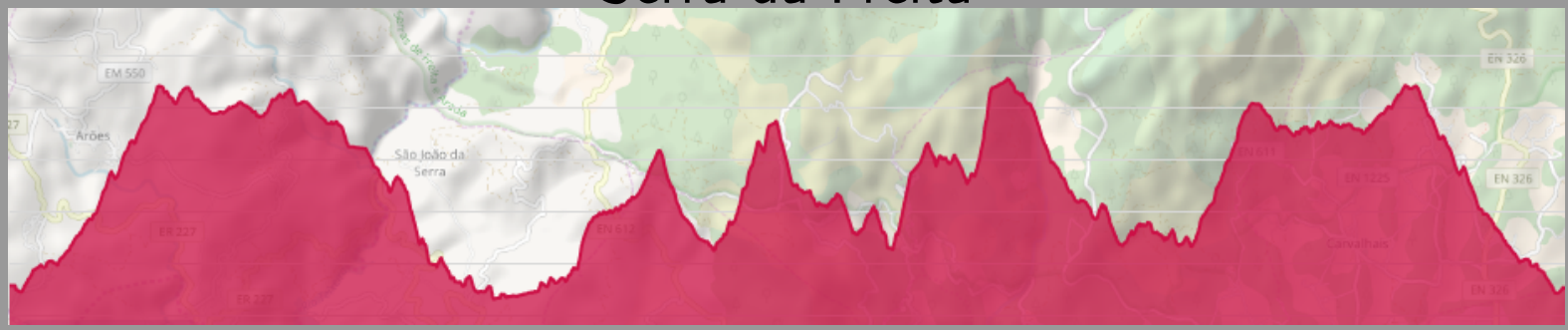


# Images as Signals

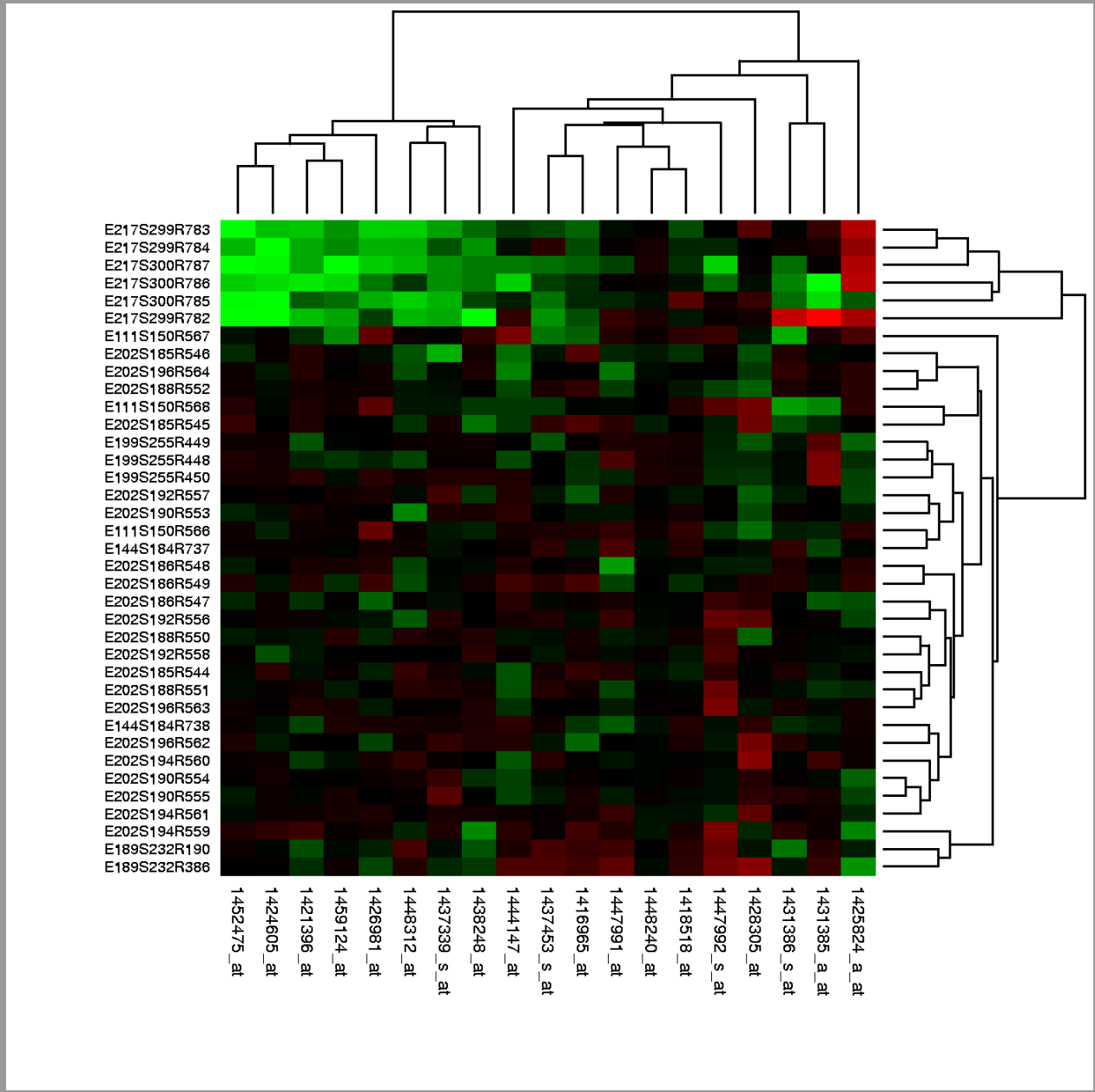


# Images as Signals

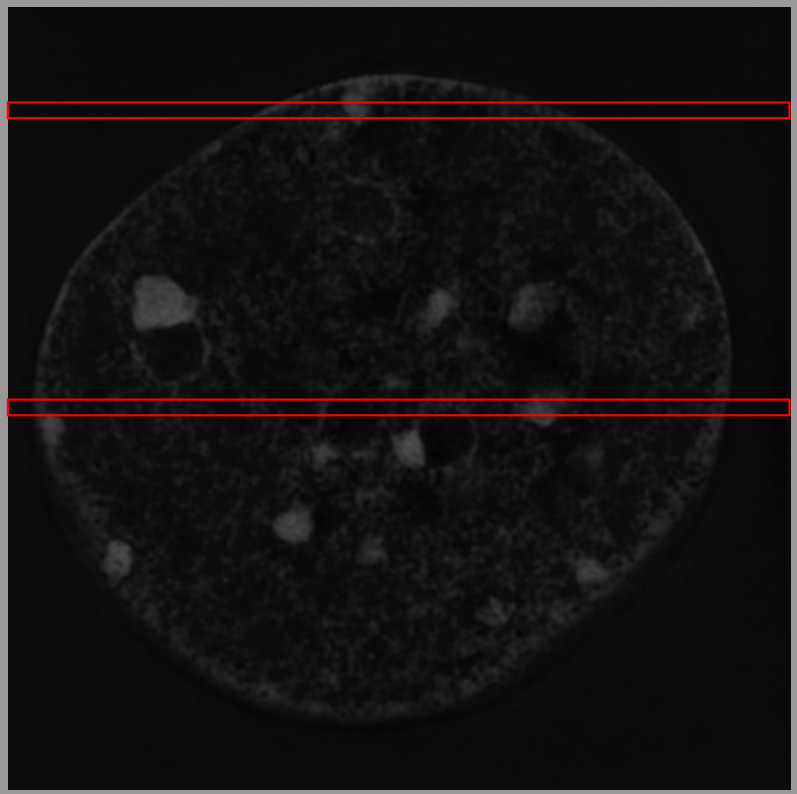
## Serra da Freita



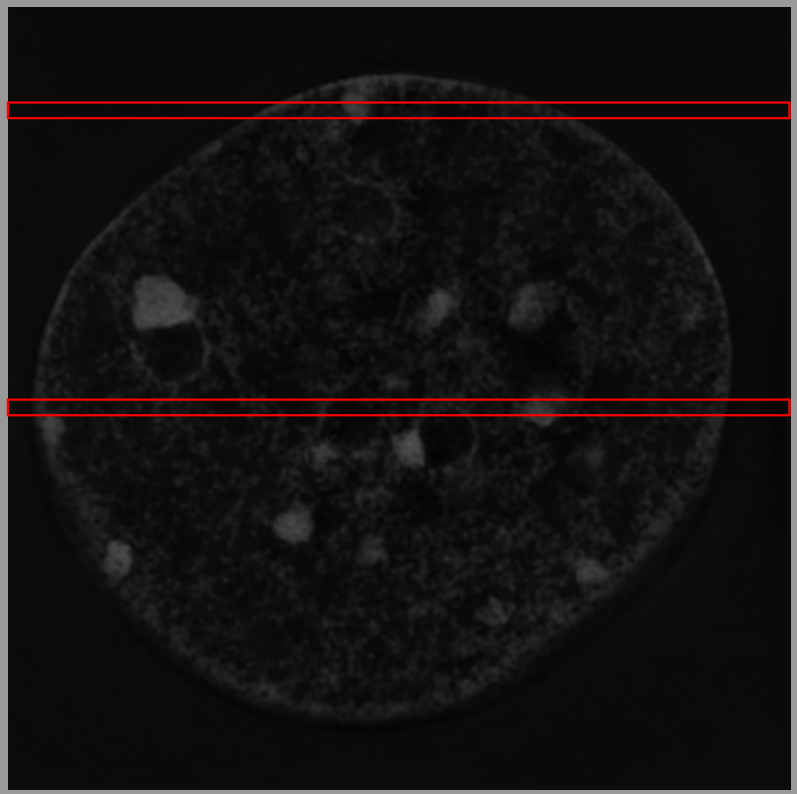
# Images as Signals

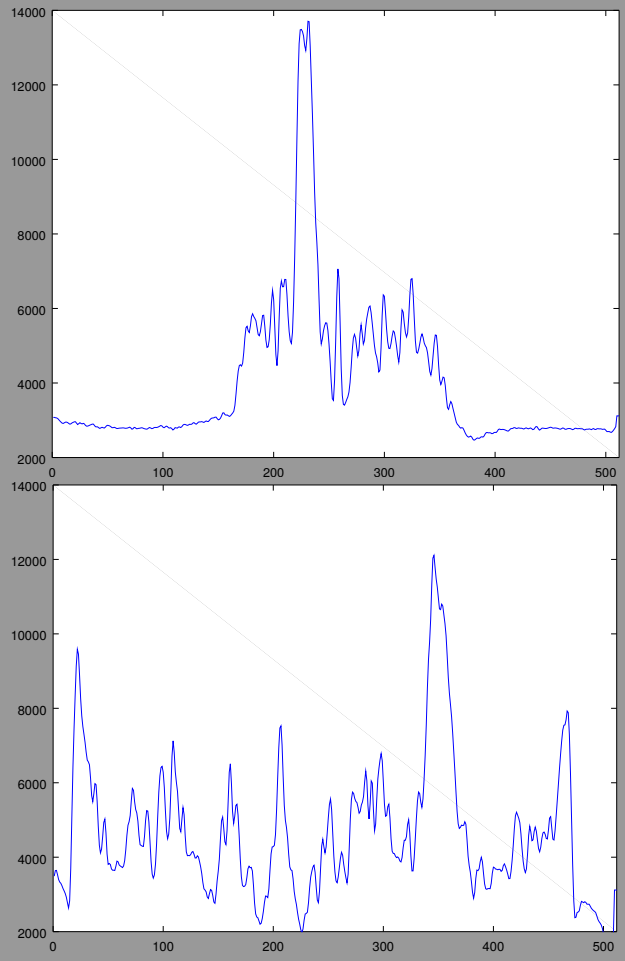


# Images as Signals

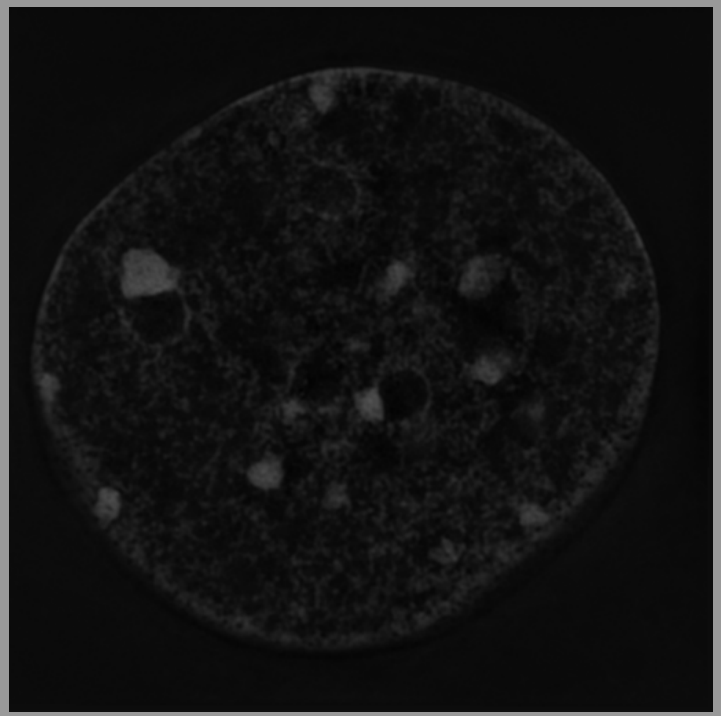


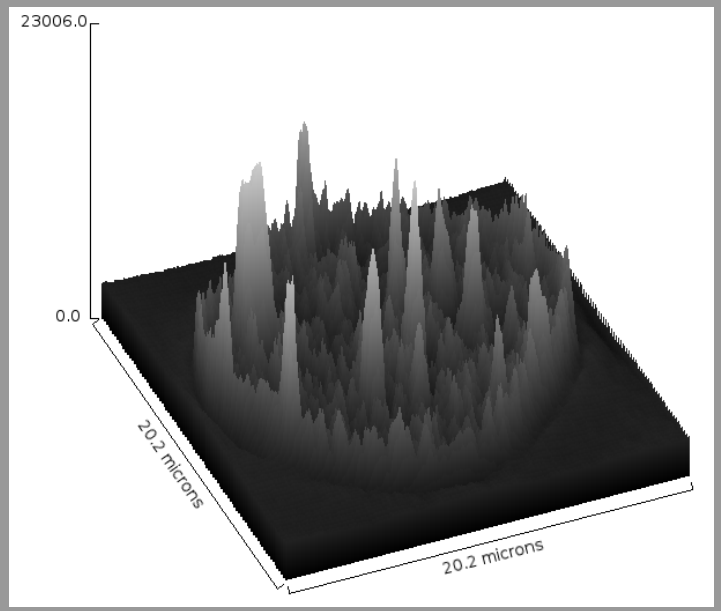
# Images as Signals



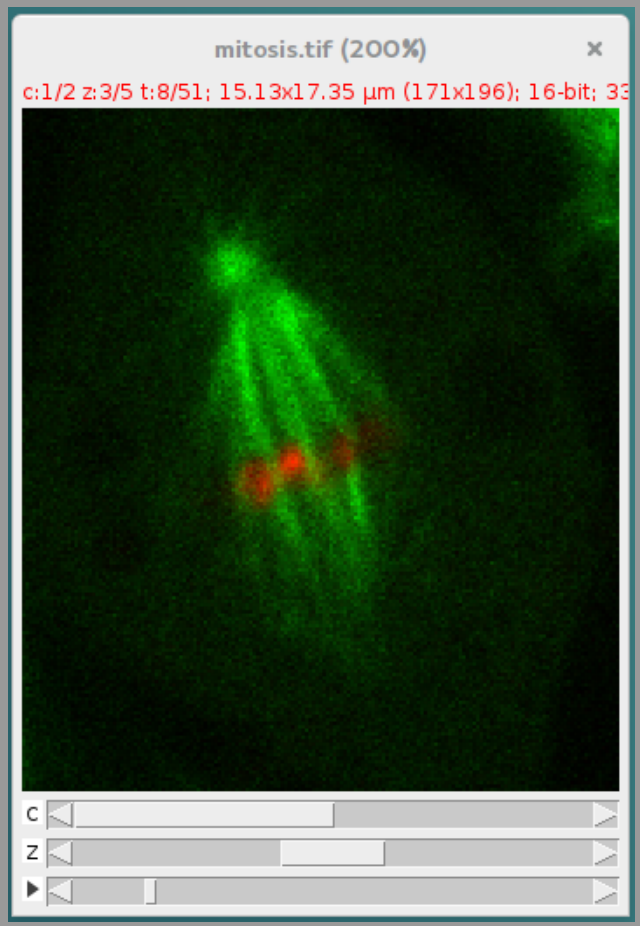


# Images as Surfaces





# Images as ND Arrays

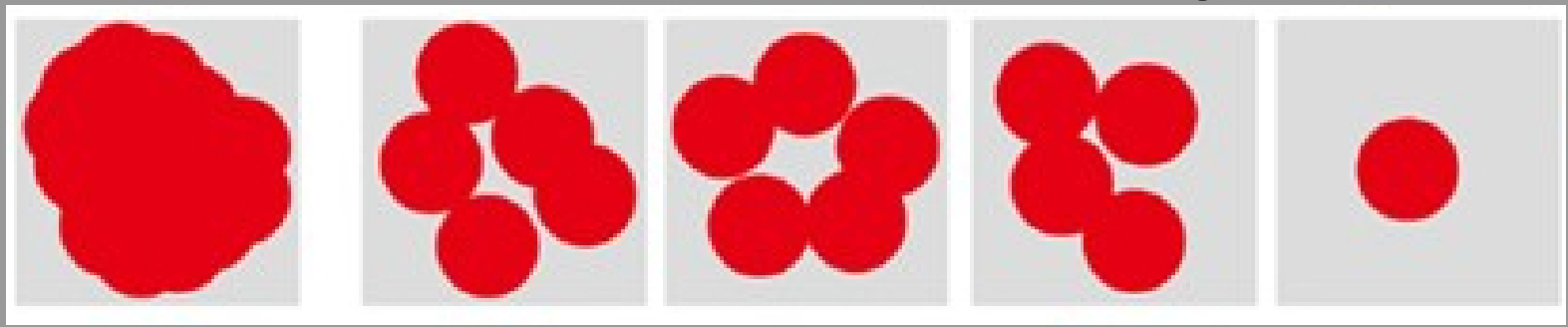



- x and y
- time
- z (volume)
- wavelength
- phase
- stage angle

Think "data", not "picture"

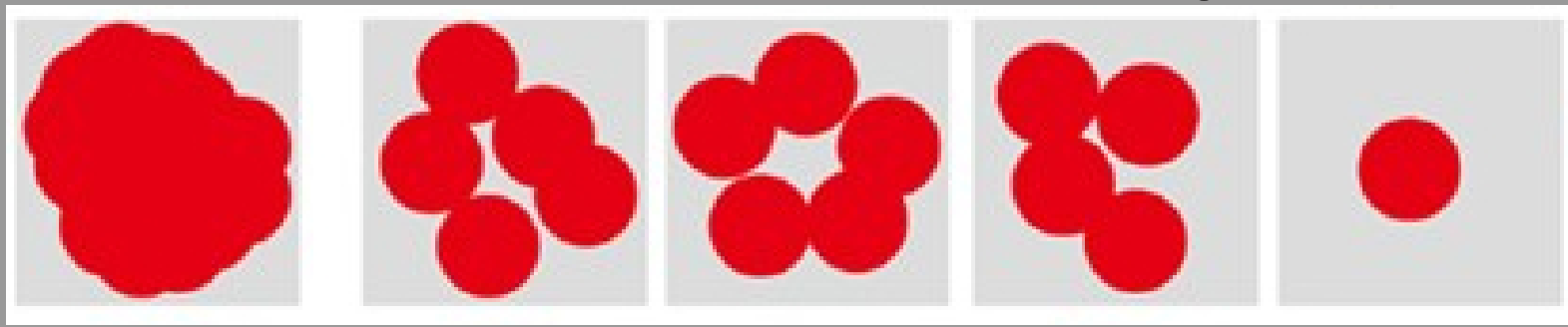
# Localisation Microscopy

## Diffraction limited fluorescence images

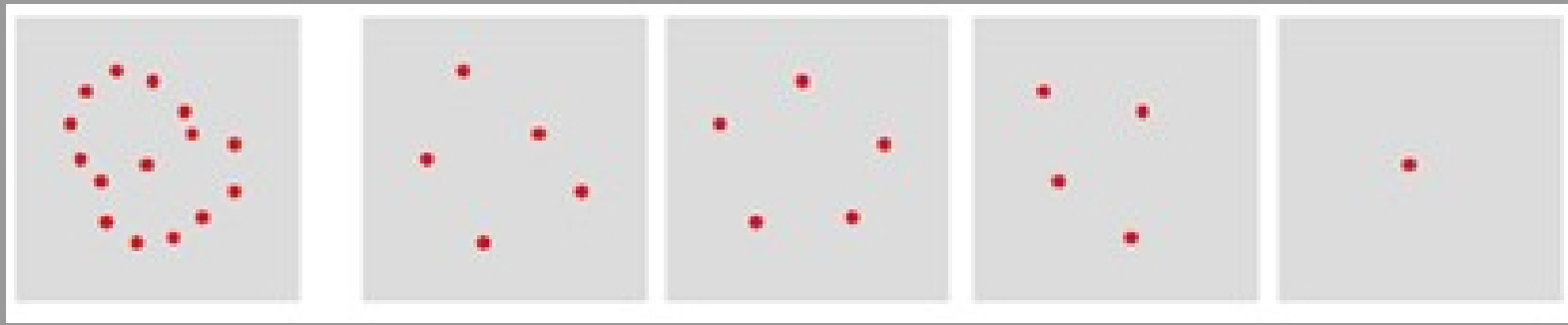


# Localisation Microscopy

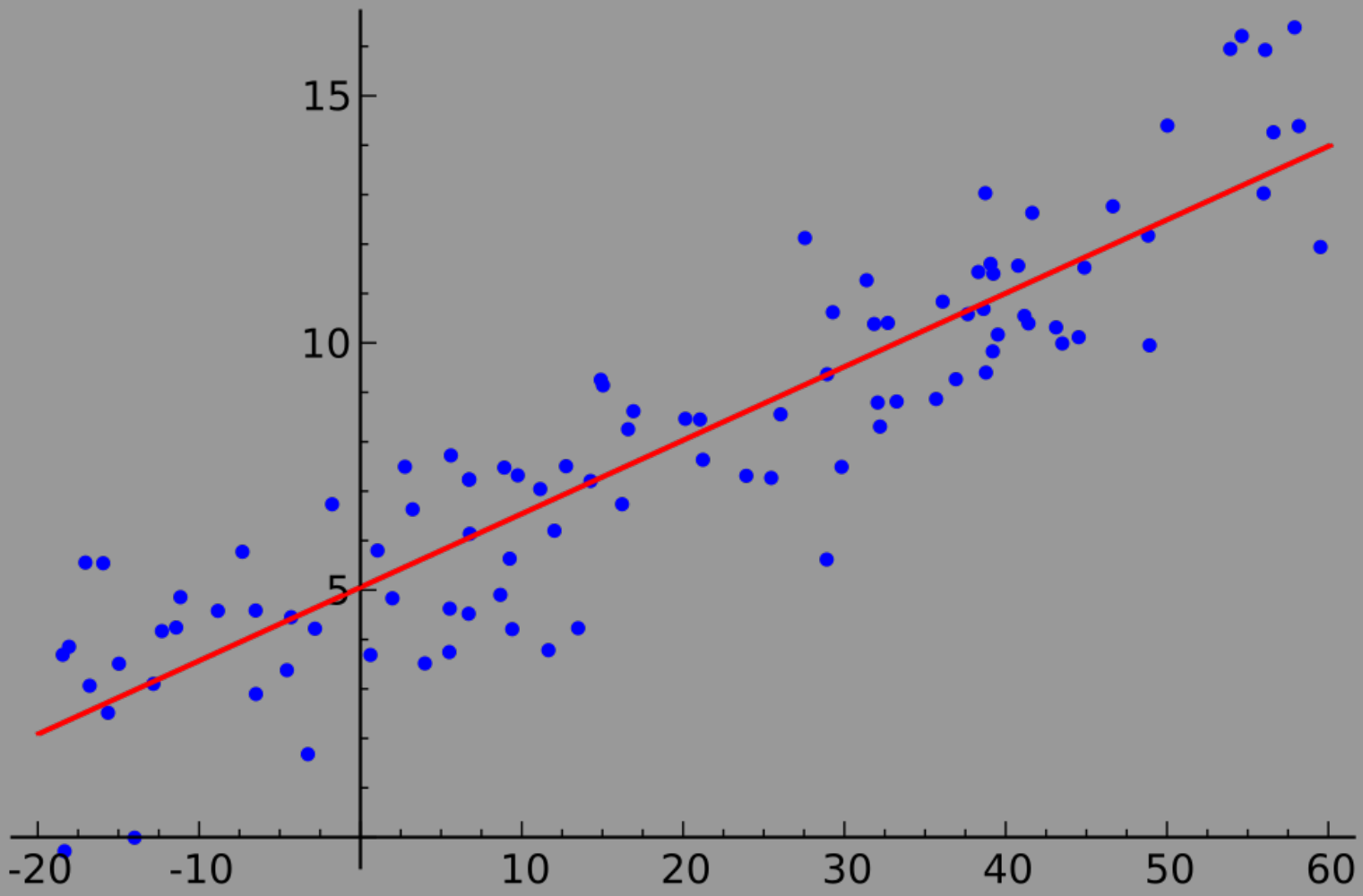
## Diffraction limited fluorescence images



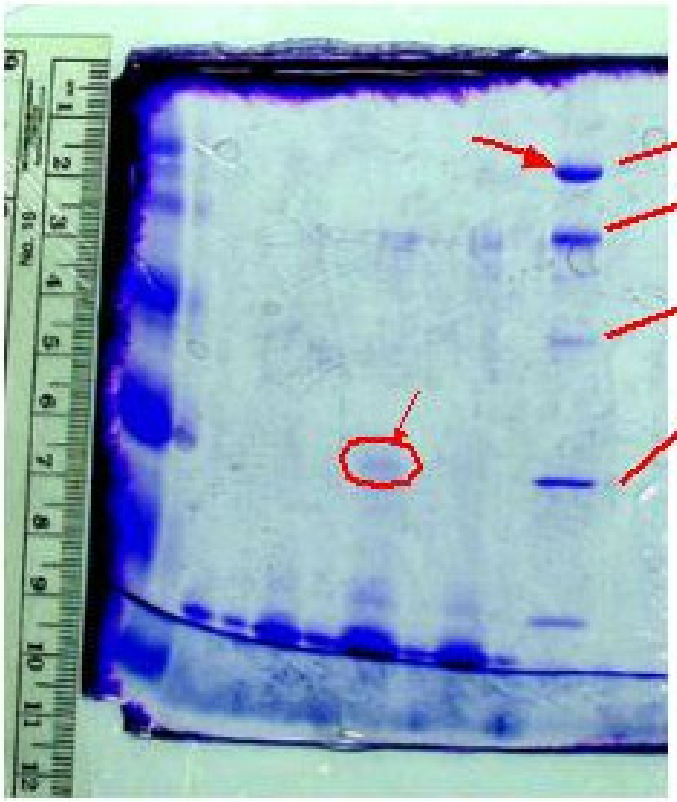
## Localised fluorophores



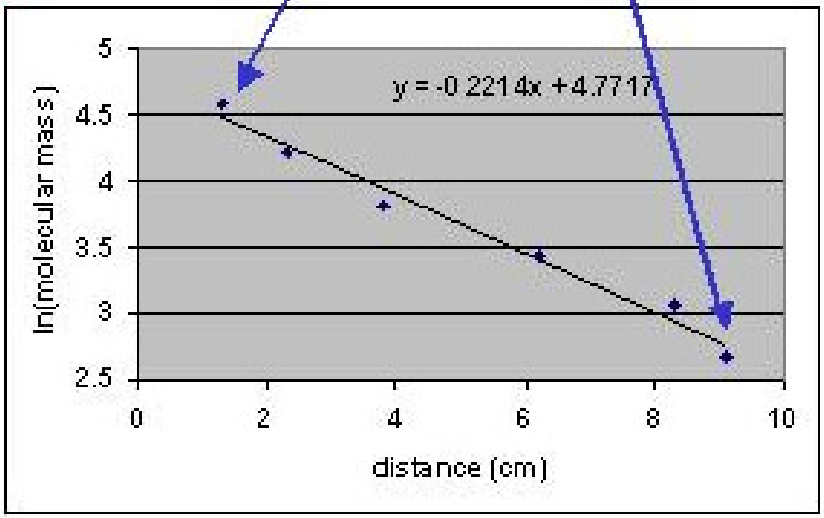
# Fitting



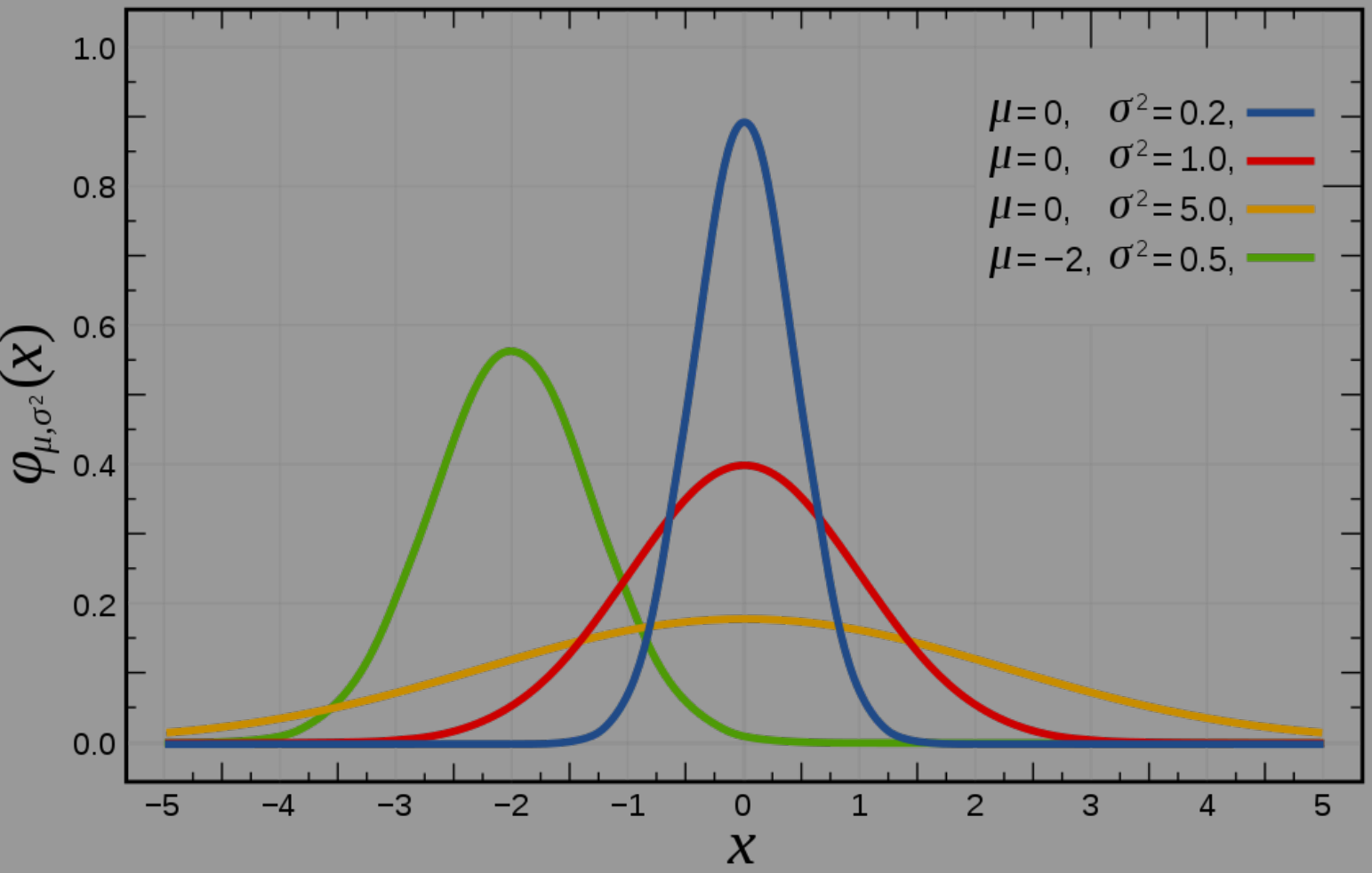
# Fitting



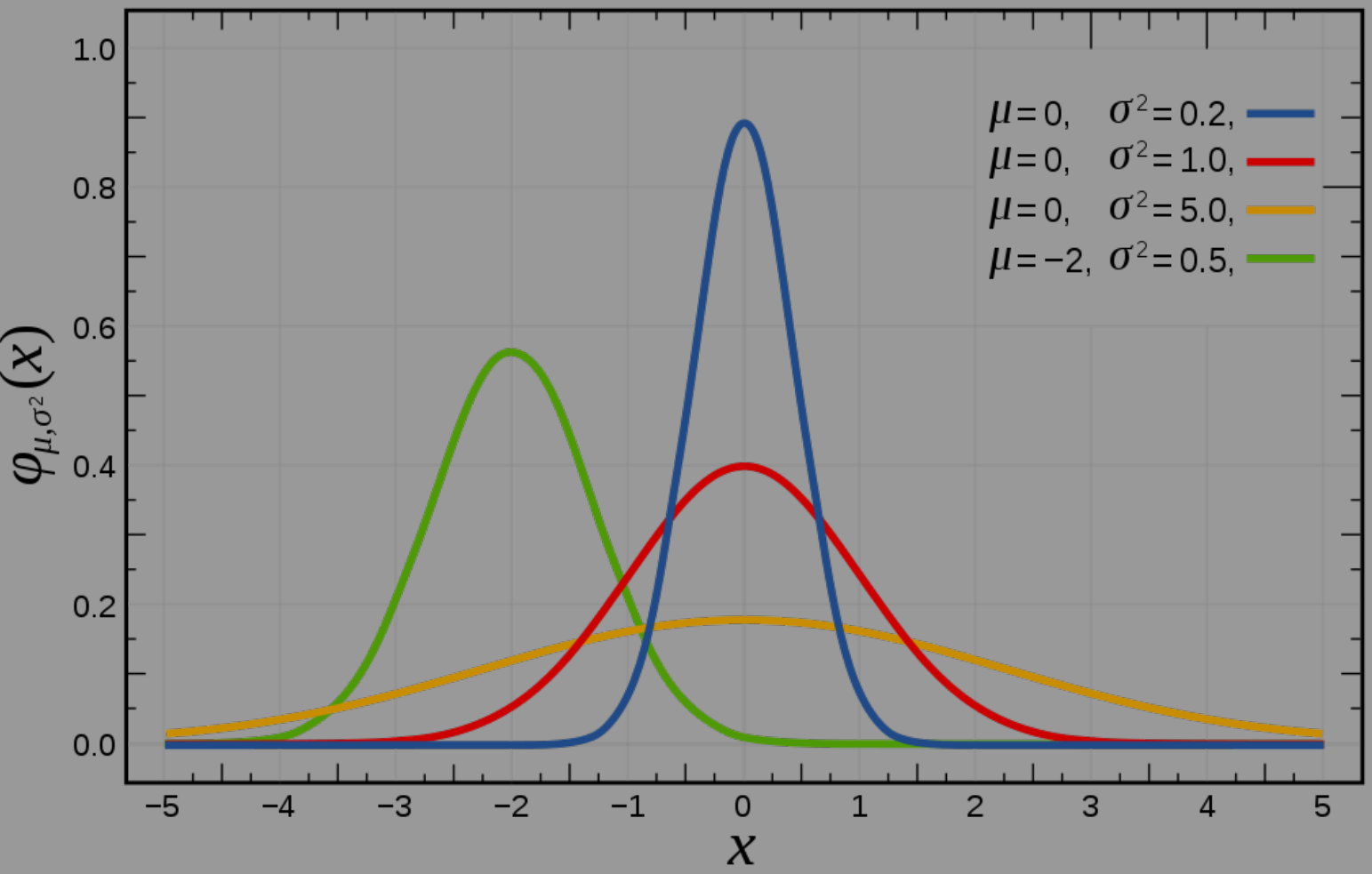
| molec. mass | distance | ln(mass) |
|---|---|---|
| 97.4 | 1.3 | 4.57883 |
| 68 | 2.3 | 4.21951 |
| 45 | 3.8 | 3.80666 |
| 31 | 6.2 | 3.43399 |
| 21.5 | 8.3 | 3.06805 |
| 14.3 | 9.1 | 2.66026 |



# Fitting

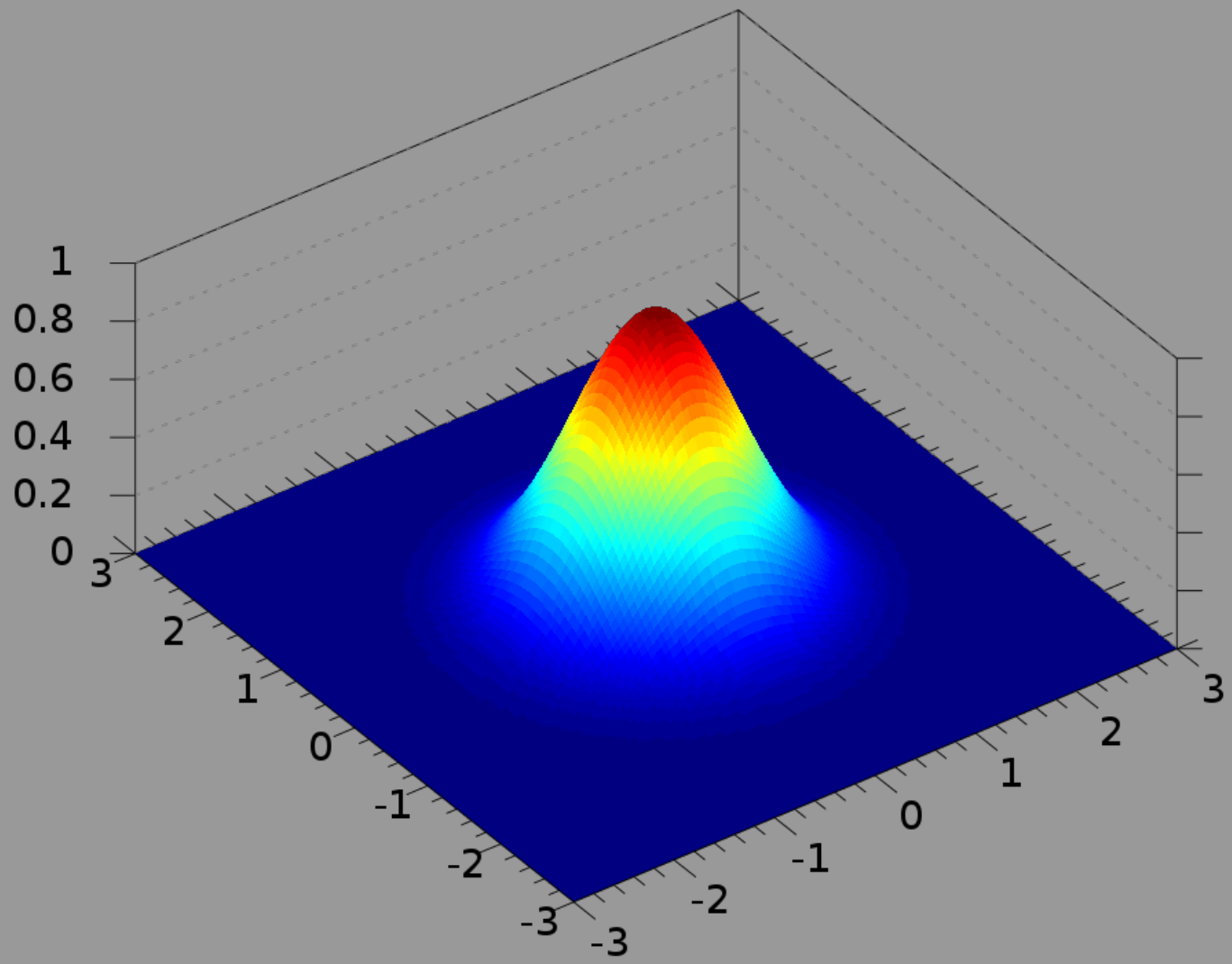


# Fitting




Check line profile of a bead.

# Fitting



# Localisation imaging

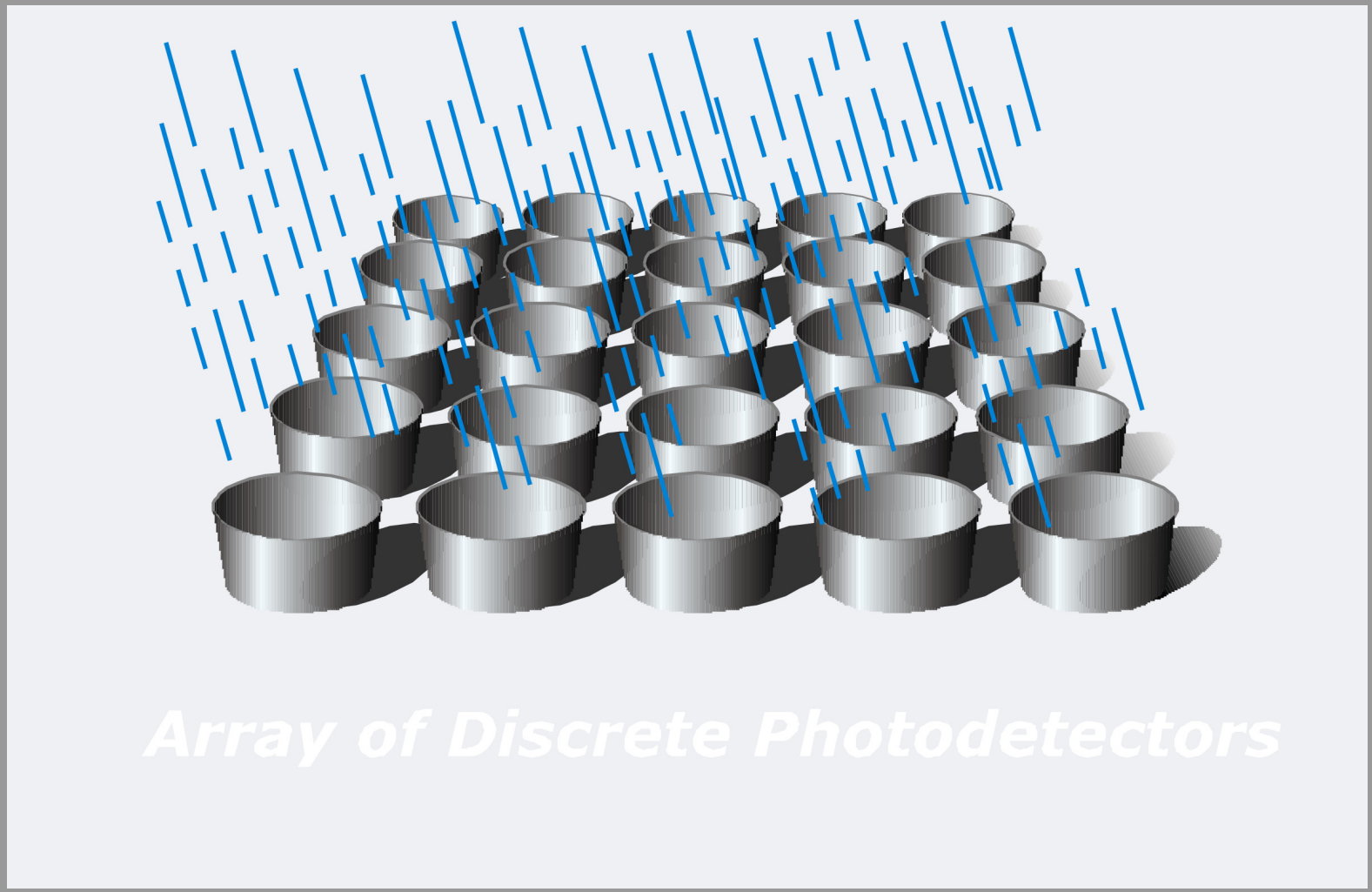
Table of coordinates.

# Localisation imaging

Table of coordinates.
What is the pixel size?

# Direct record



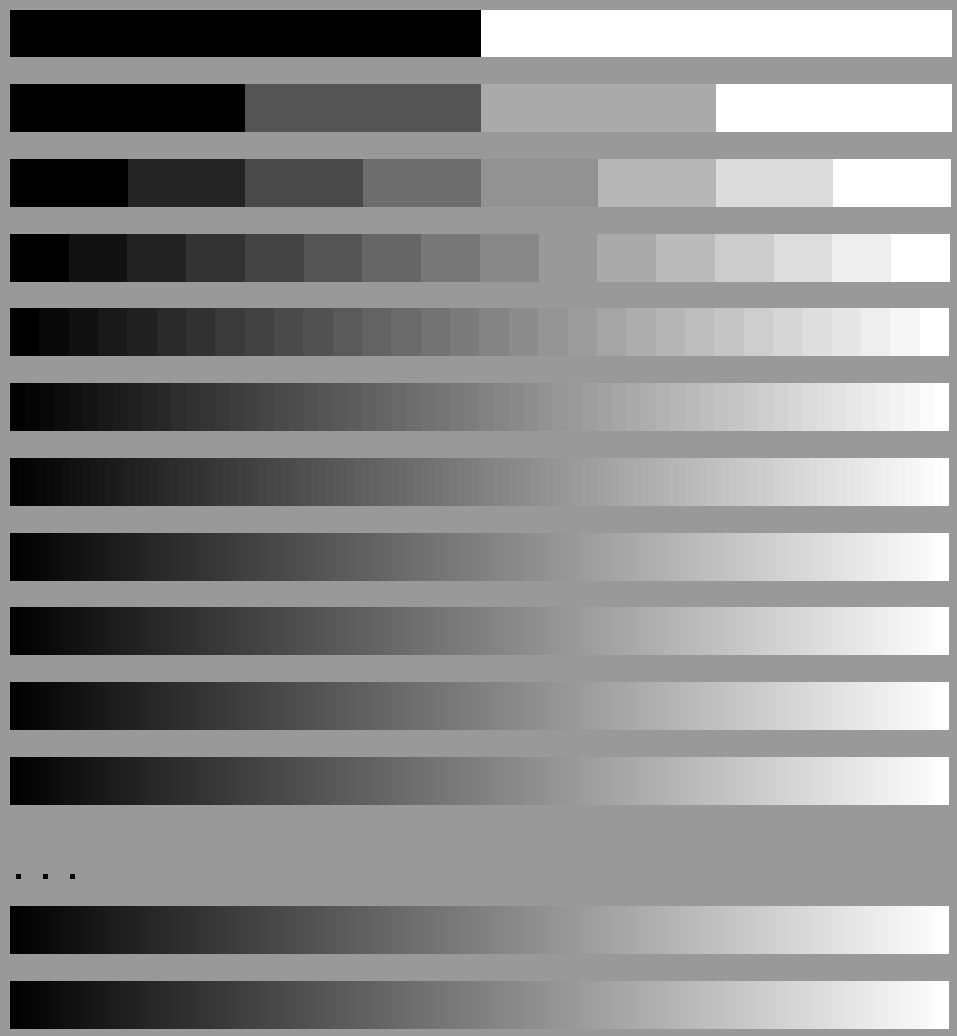


# Image Reconstruction

- Localisation Microscopy (STORM/PALM)
- Structured Illumination

Two step process

# Integer types

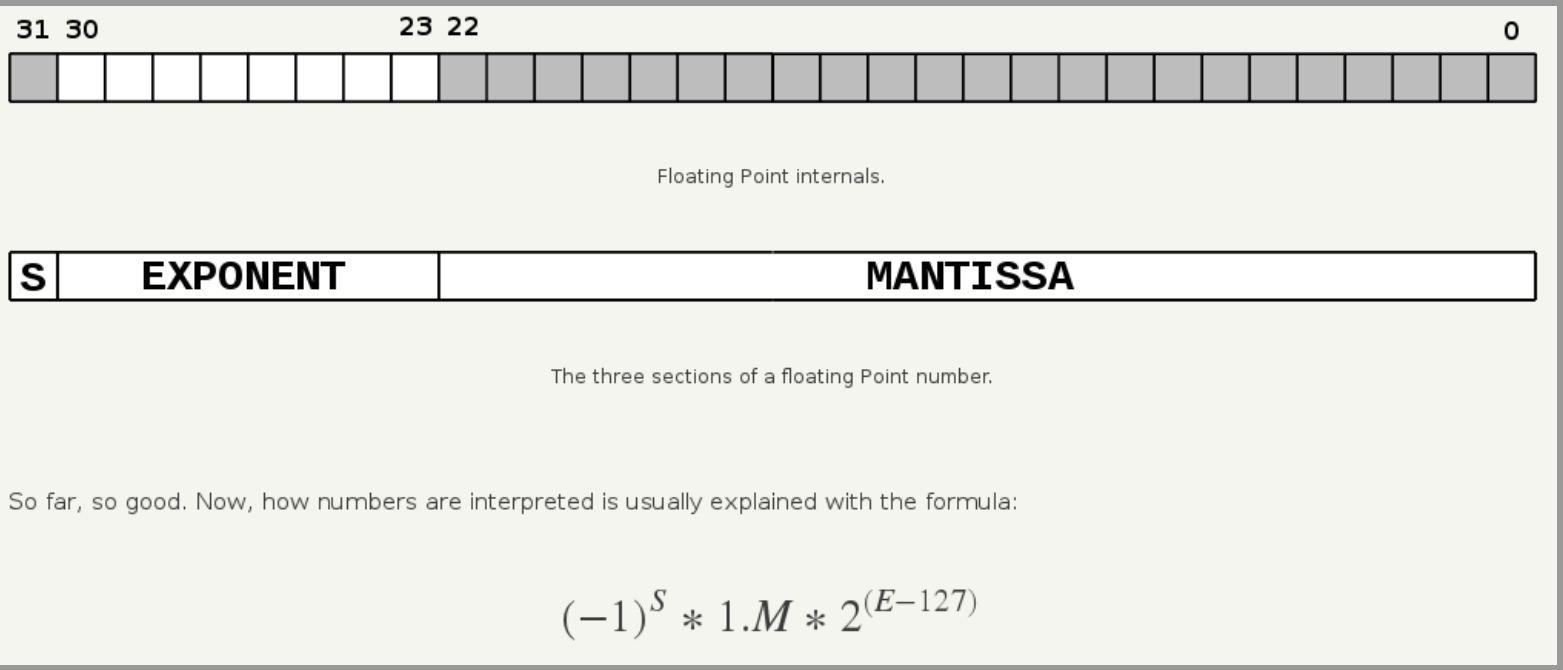


| | |
|---|---|
| $2^1$ | 2 |
| $2^2$ | 4 |
| $2^3$ | 8 |
| $2^4$ | 16 |
| $2^5$ | 32 |
| $2^6$ | 64 |
| $2^7$ | 128 |
| $2^8$ | 256 |
| $2^9$ | 512 |
| $2^{10}$ | 1024 |
| $2^{11}$ | 2048 |
| ... | |
| $2^{15}$ | 32768 |
| $2^{16}$ | 65536 |

# Integer types

| | unique values | min | max |
|---|---|---|---|
| $2^1$ | 2 | -1 | 0 |
| $2^2$ | 4 | -2 | 1 |
| $2^3$ | 8 | -4 | 3 |
| ... | | | |
| $2^8$ | 256 | -128 | 127 |
| $2^{32}$ | 4294967296 | -2147483648 | 2147483647 |

# Floating point

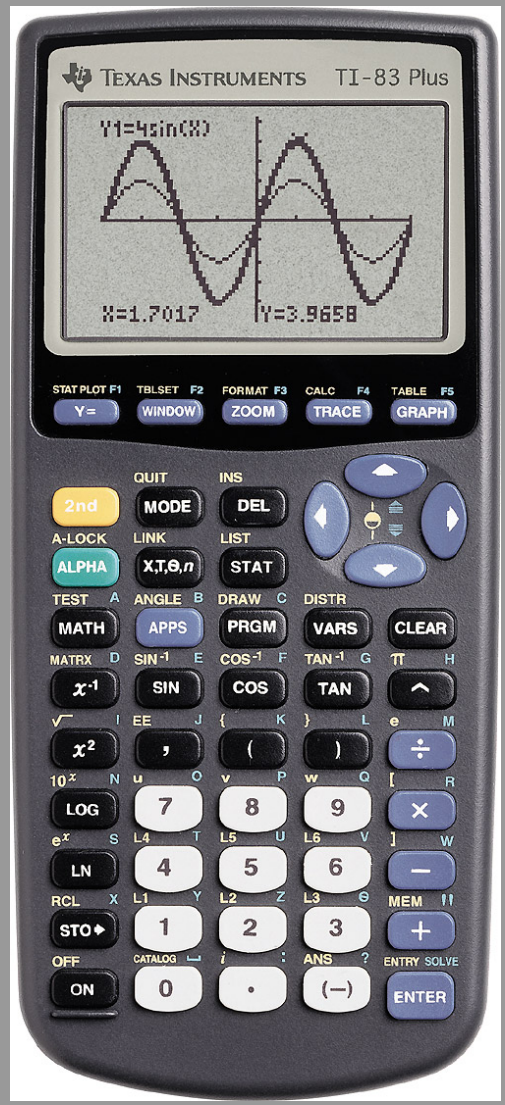



So far, so good. Now, how numbers are interpreted is usually explained with the formula:

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

Floating points — sometimes incorrectly called 32 bit.

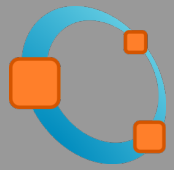
# Tools for image analysis

# Tools for image analysis

ImageJ / FIJI

Python with NumPy



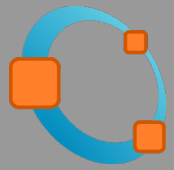
Octave

R

# Tools for image analysis

ImageJ / FIJI

Python with NumPy



Octave

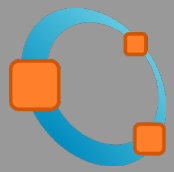
R

- CellProfiler
- Icy
- OMERO
- KNIME

# Tools for image analysis

ImageJ / FIJI

Python with NumPy



Octave

R

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

# ImageJ

- *de facto* standard in medical sciences
- libre software (free and open source)
- massive helpfully massive community

ImageJ1

ImageJ2

FIJI

# Typical problems

**Concentration**

Protein expression, number of complexes.

**Co-localization**

Do two overlap and correlate?

**Dynamics**

How fast does it move?

# Typical problems

**Concentration**

Protein expression, number of complexes.

**Co-localization**

Do two overlap and correlate?

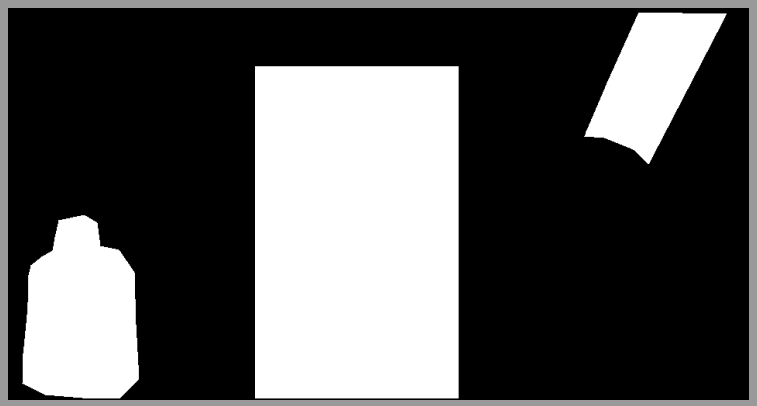
**Dynamics**

How fast does it move?

All require identifying a region of interest.

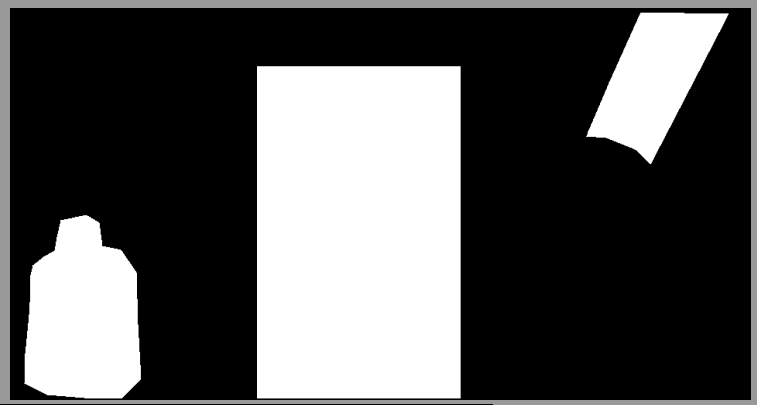
# Logical (binary) images

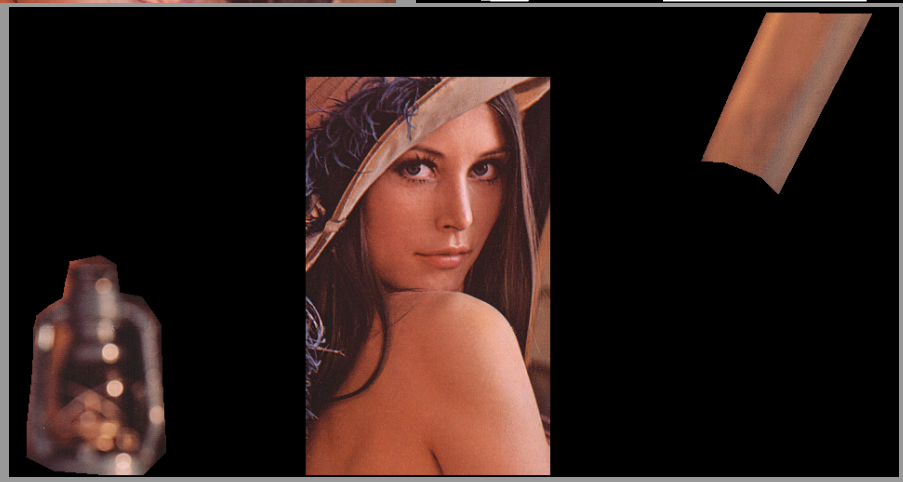
## Very useful as masks



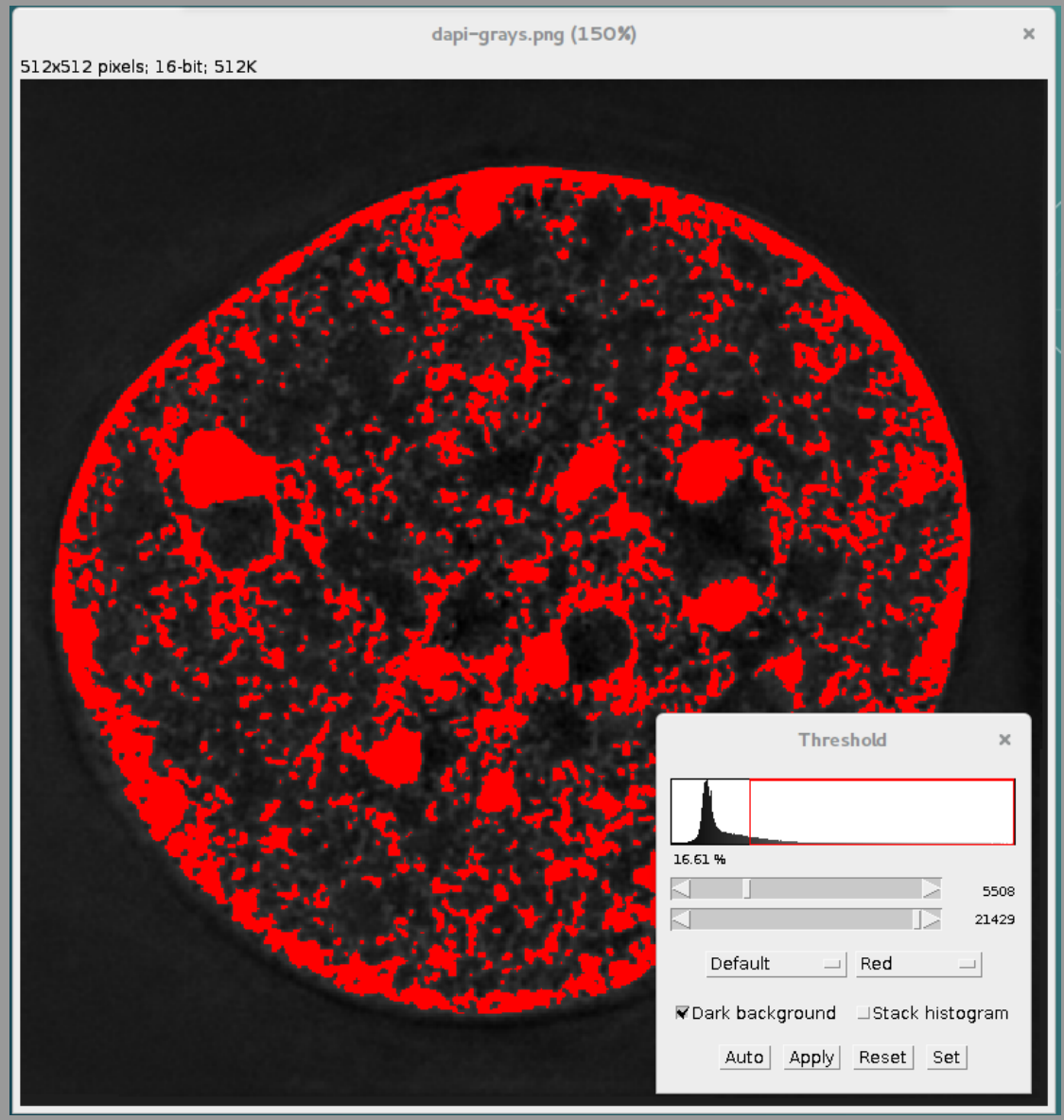
# Logical (binary) images

## Very useful as masks



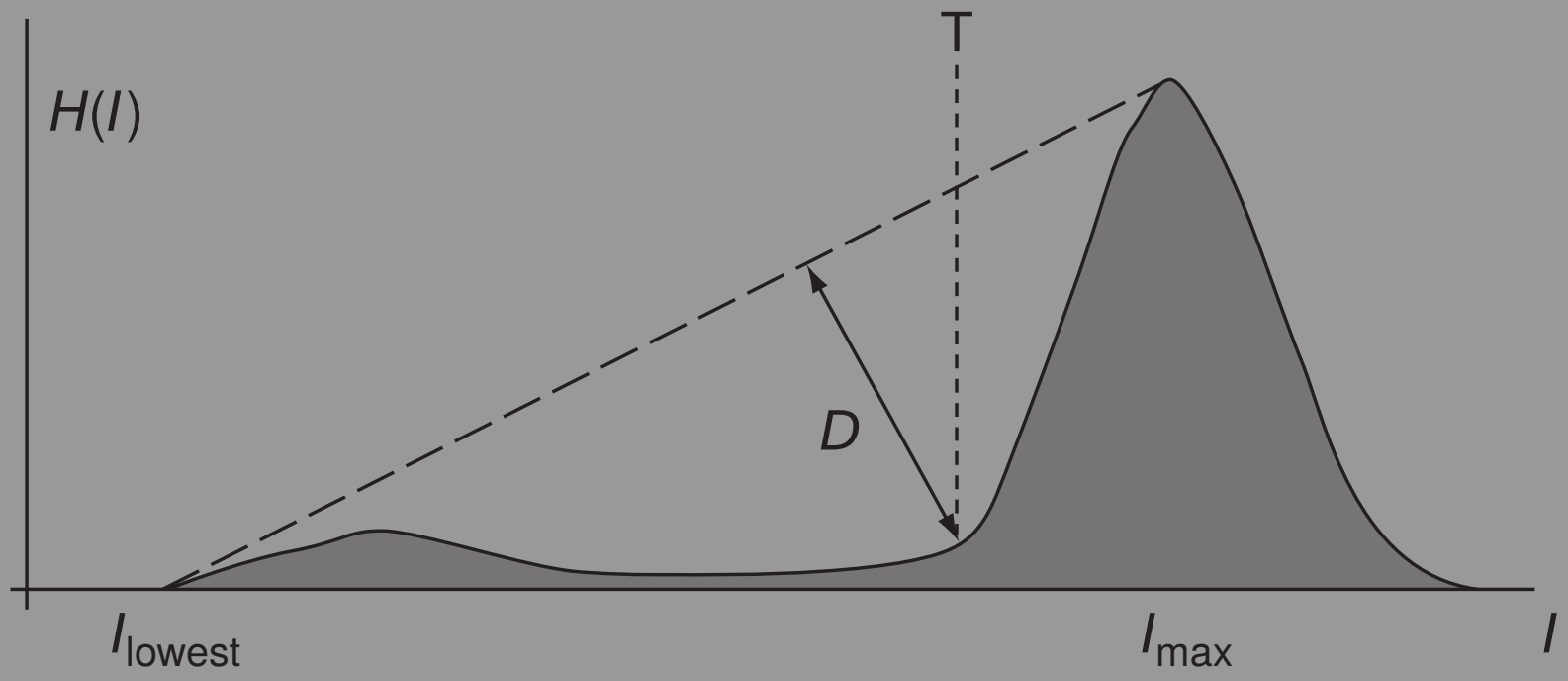


# Manual threshold



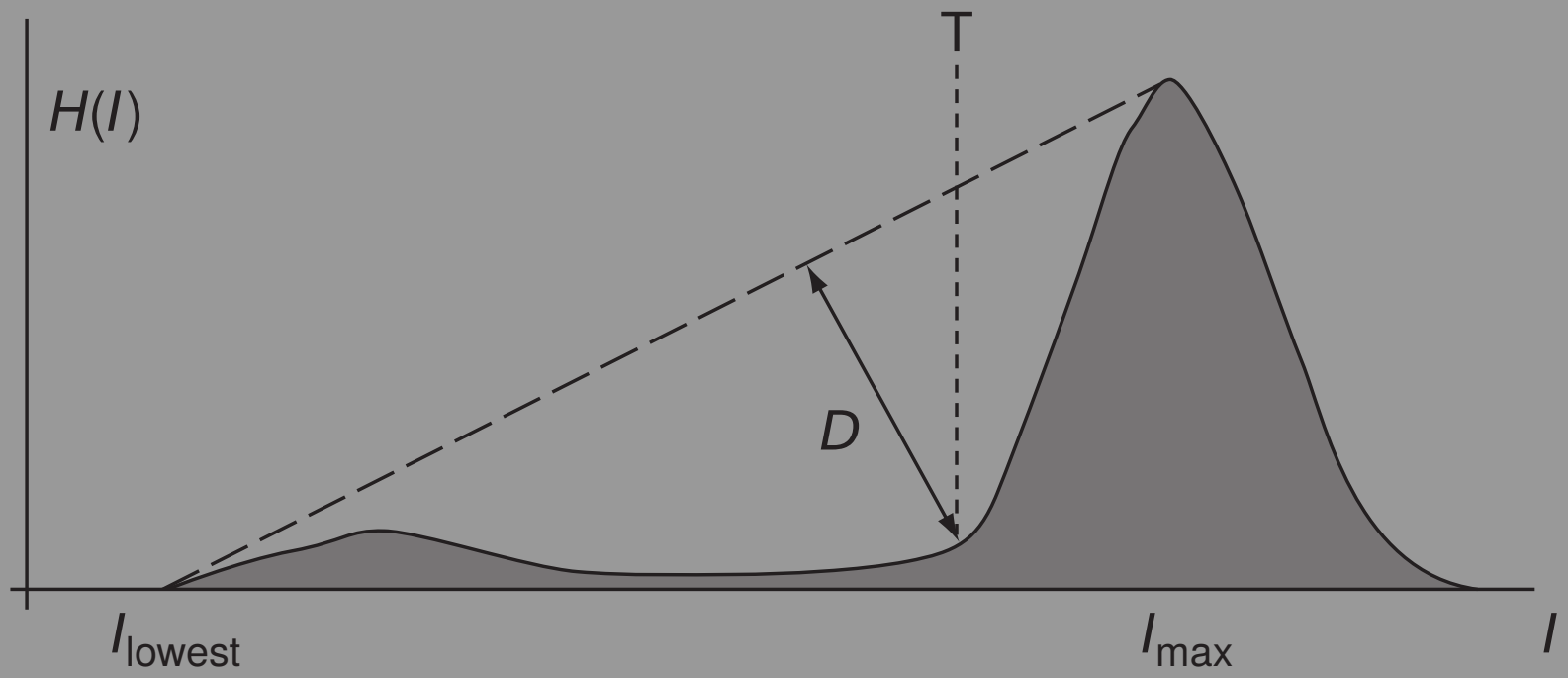
# Automatic threshold

## Triangle algorithm



# Automatic threshold

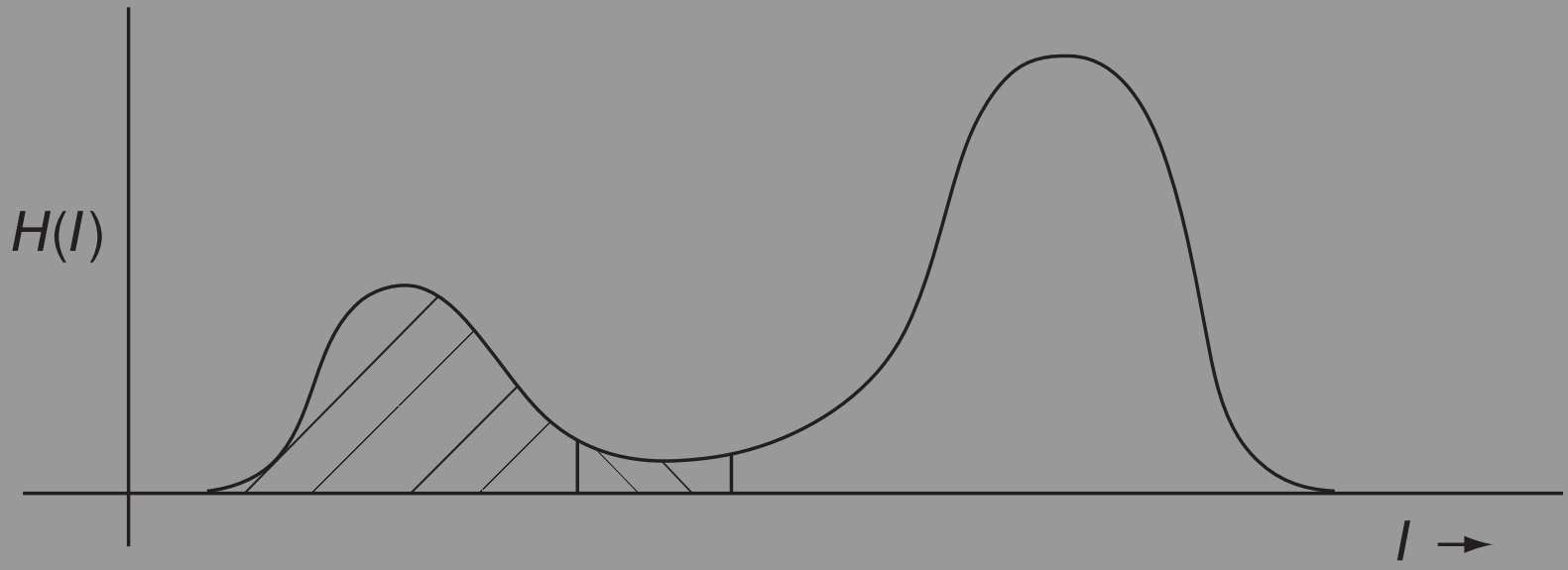
## Triangle algorithm




- histogram smoothing (mean filter)

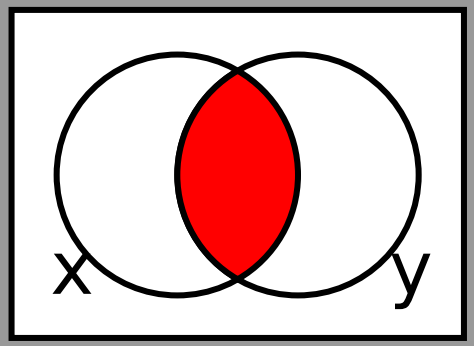
# Automatic threshold

## Otsu's algorithm

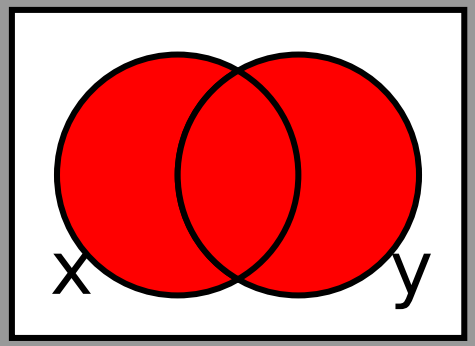



- bimodal histogram
- reduce intra-class variance (spread)

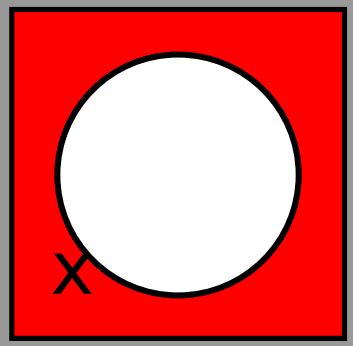
# Logical operations




x AND y

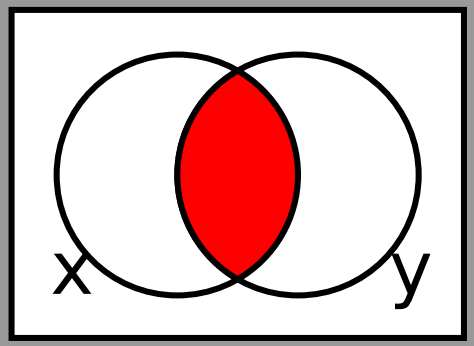



x OR y

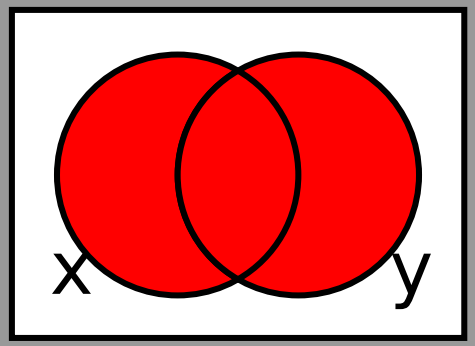



NOT x

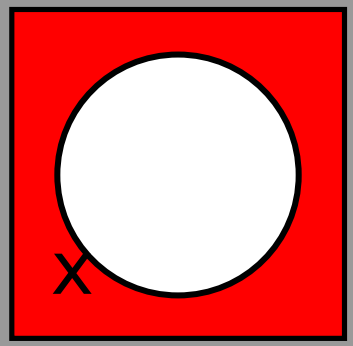
# Logical operations




x AND y



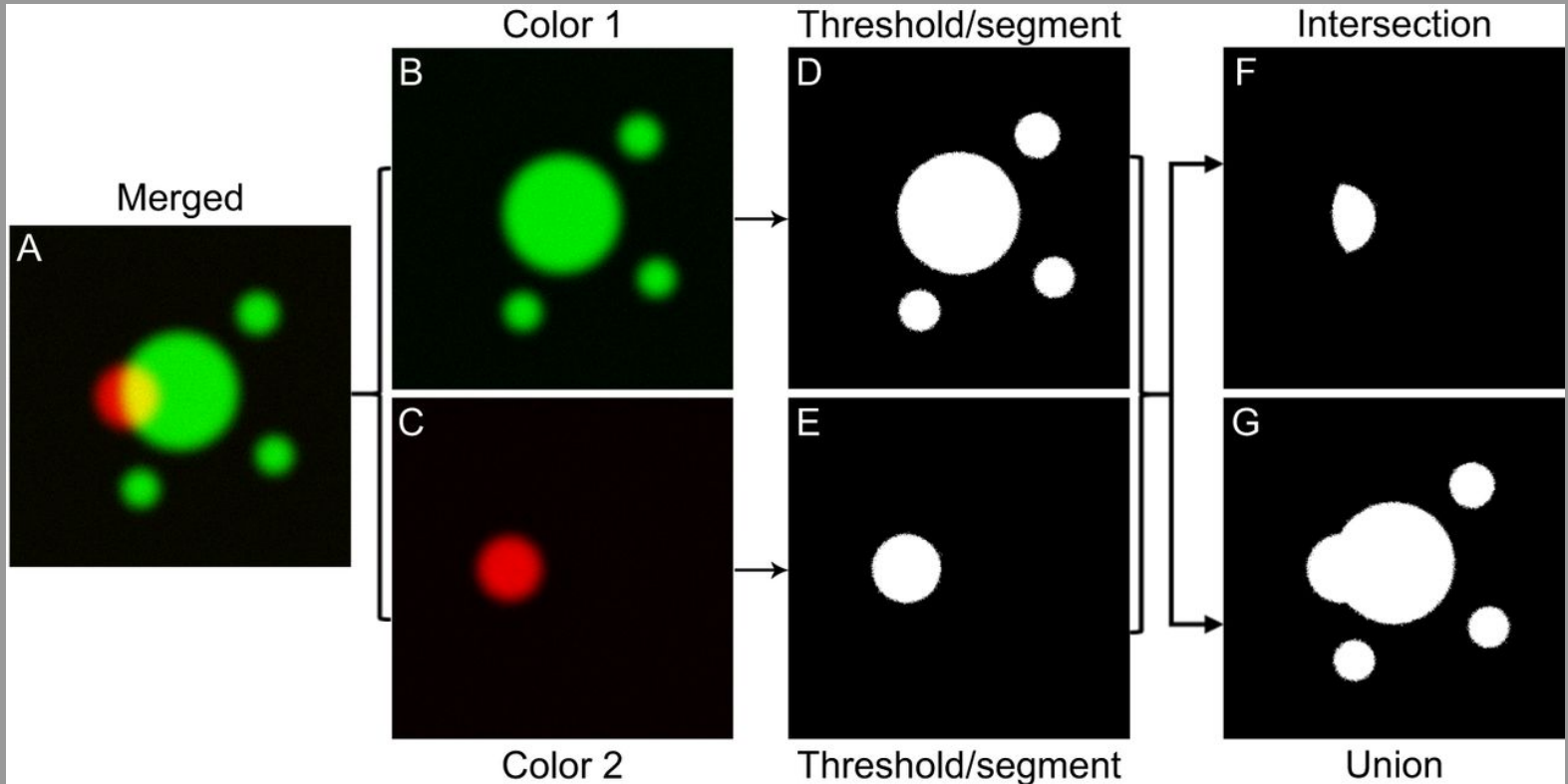

x OR y



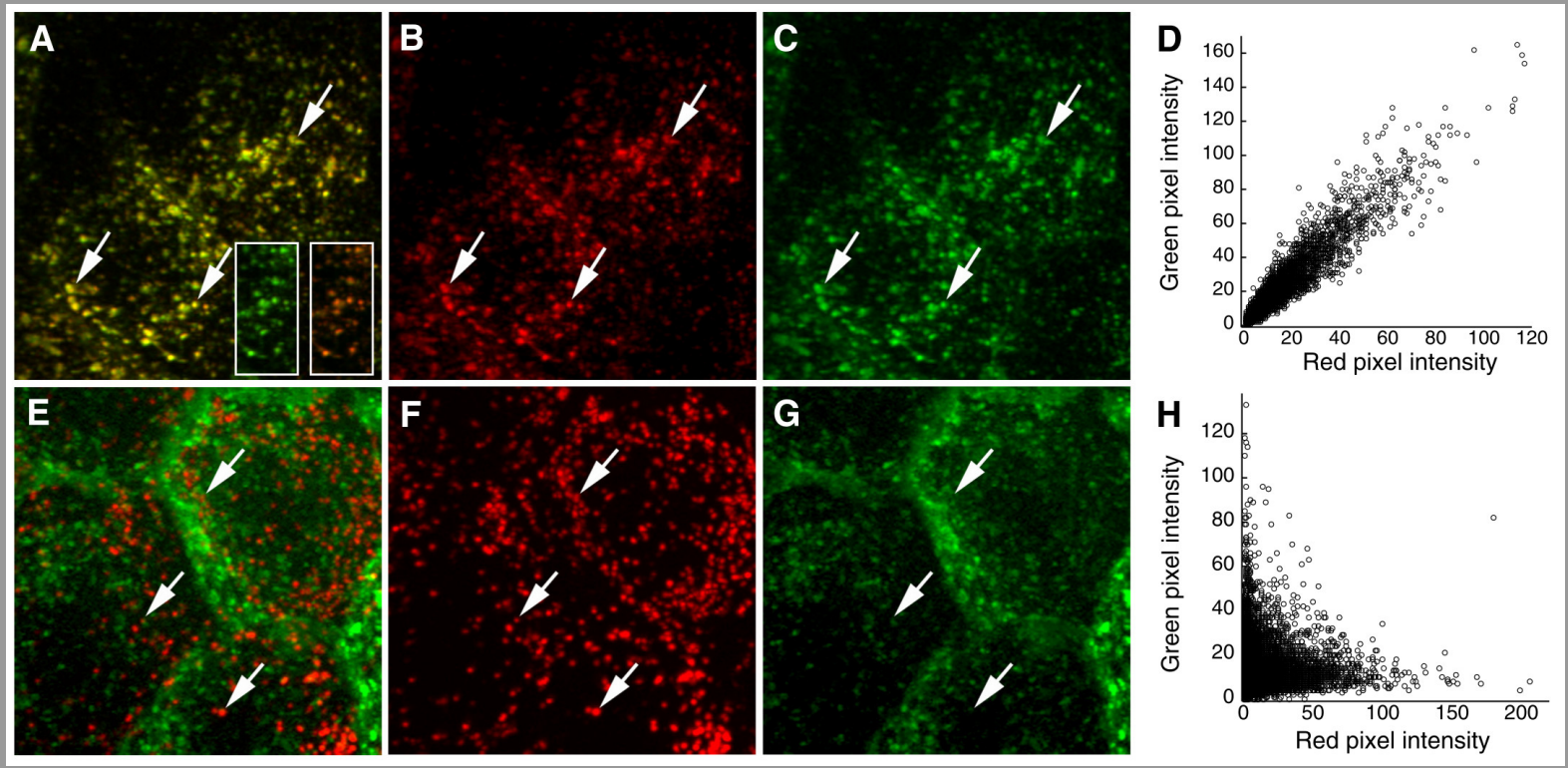

NOT x

Example: split plant cells with cell membrane.

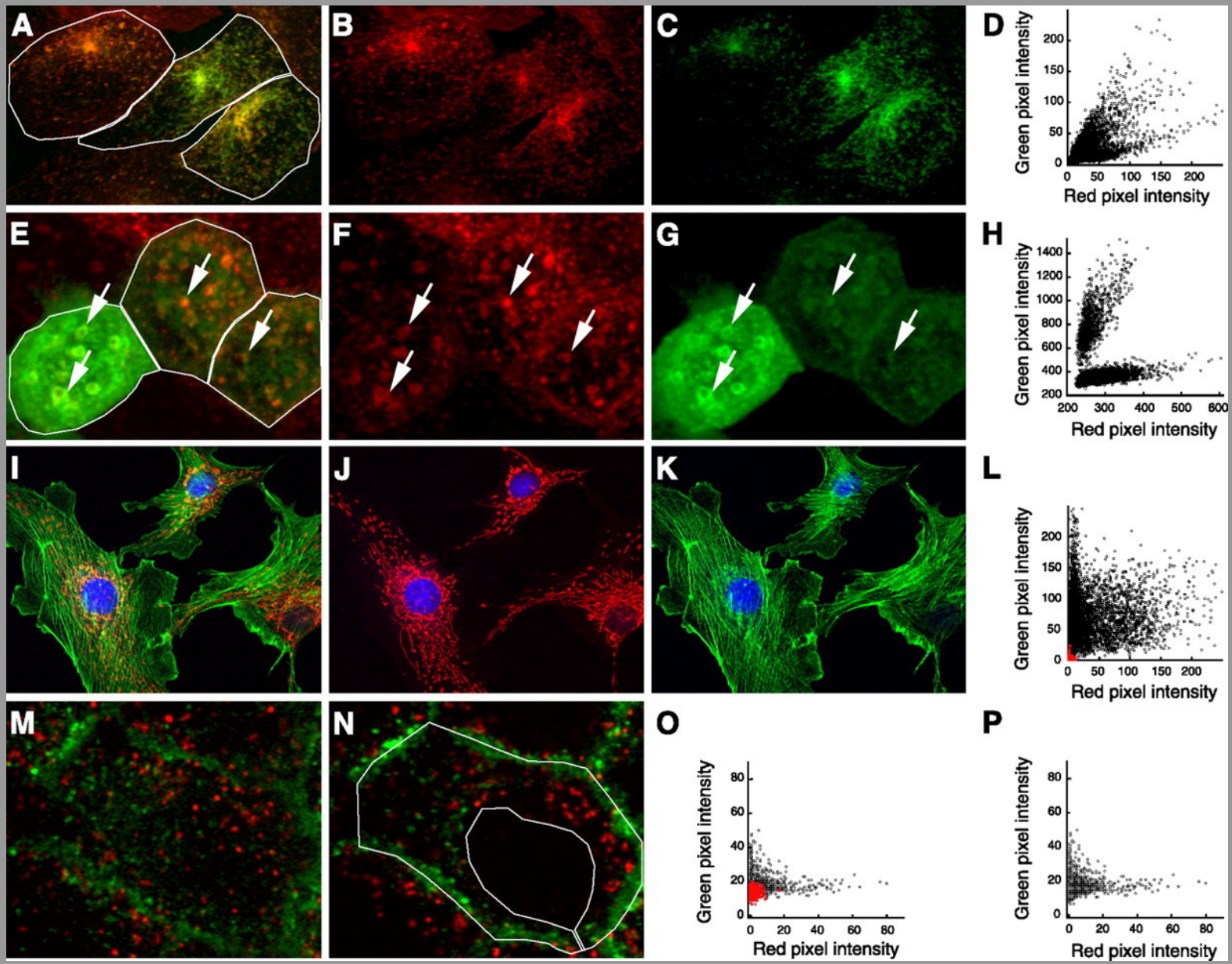
# colocalisation



# colocalisation



# colocalisation



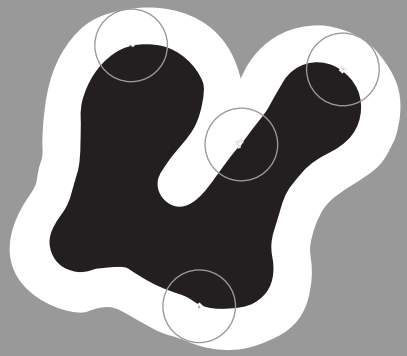
# Object properties

## Particle/Region/ROI properties/measurements

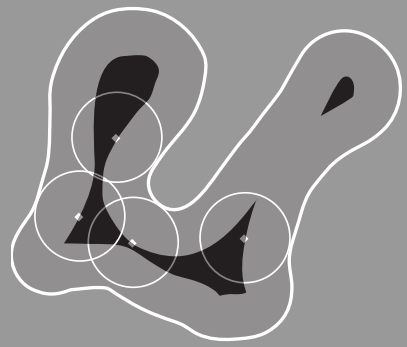
These are always one button or one line of code. The only problem is getting to this point.

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

# Erosion and dilation



Dilation

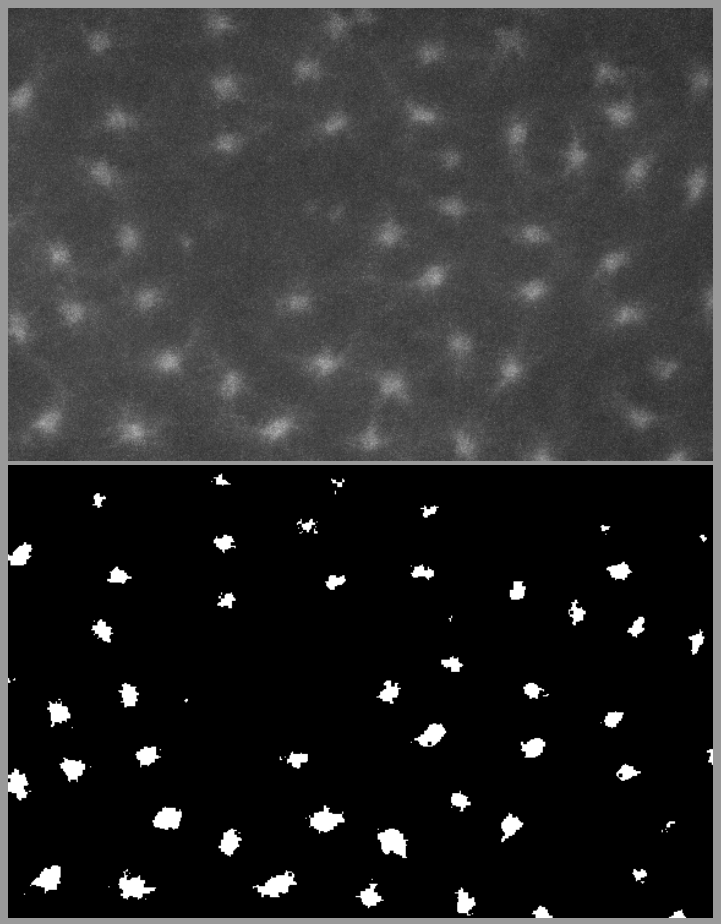


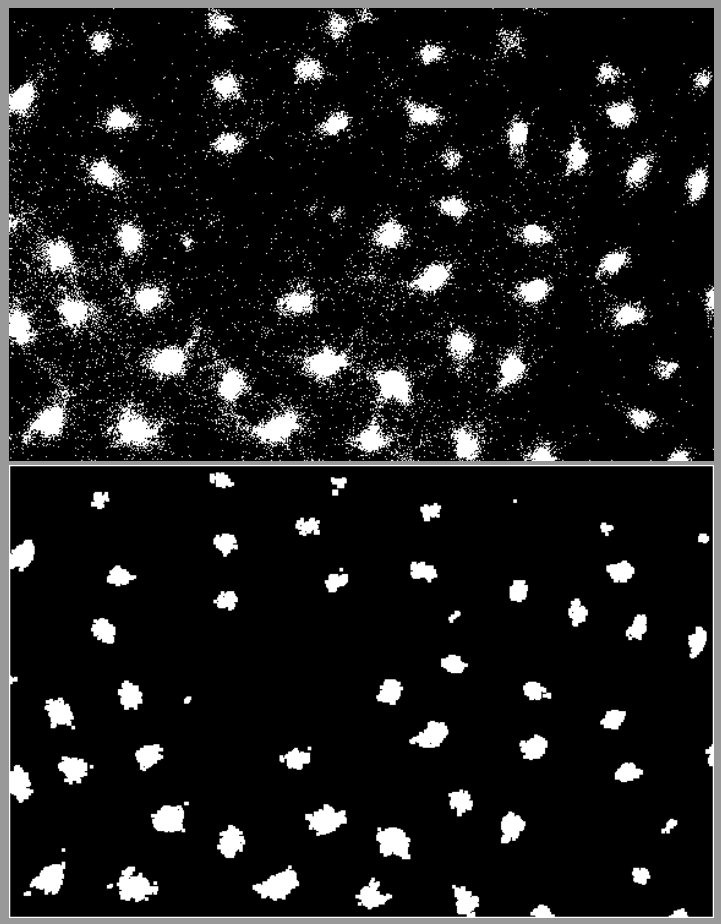
Erosion

Closing

Opening

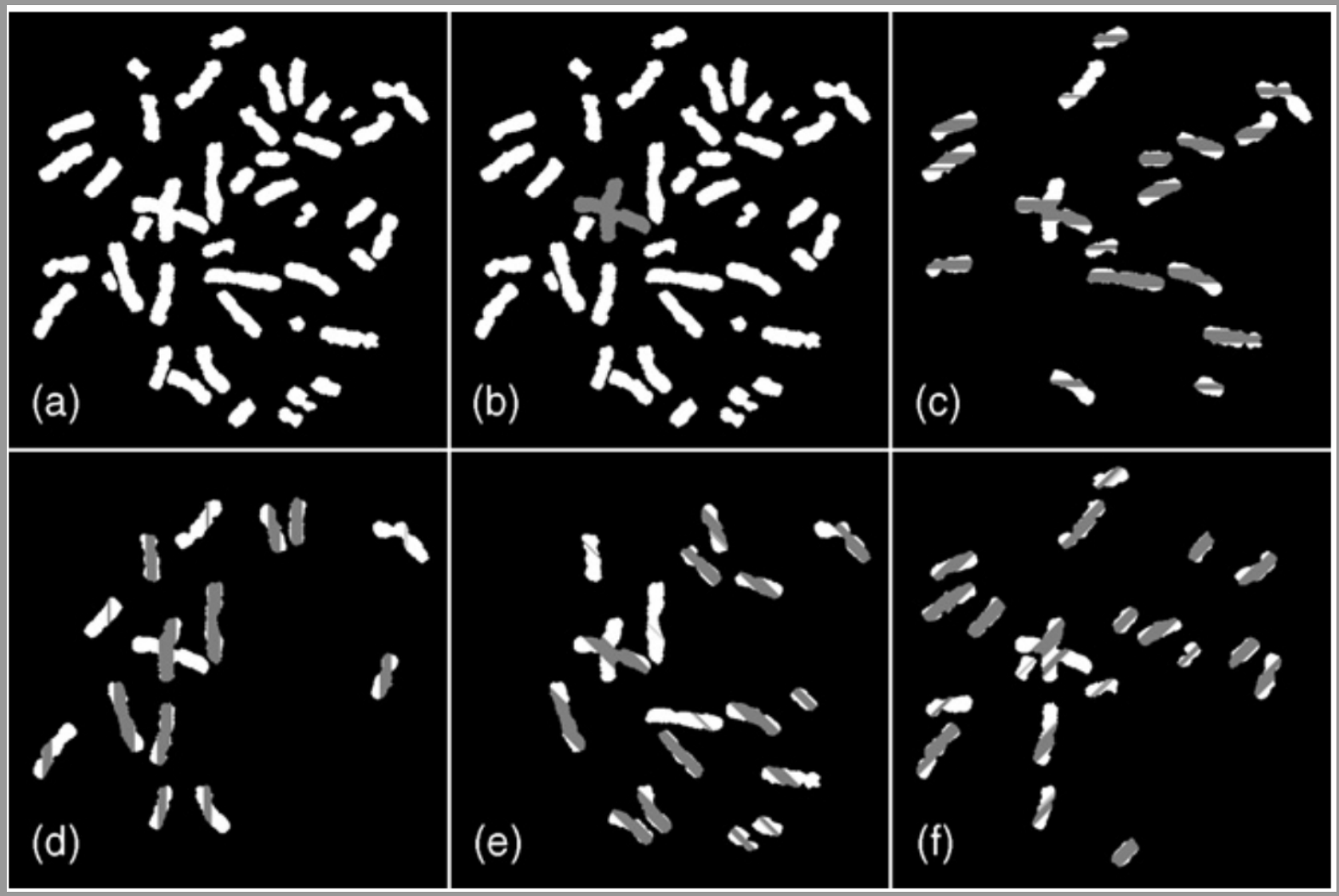
# Erosion and dilation



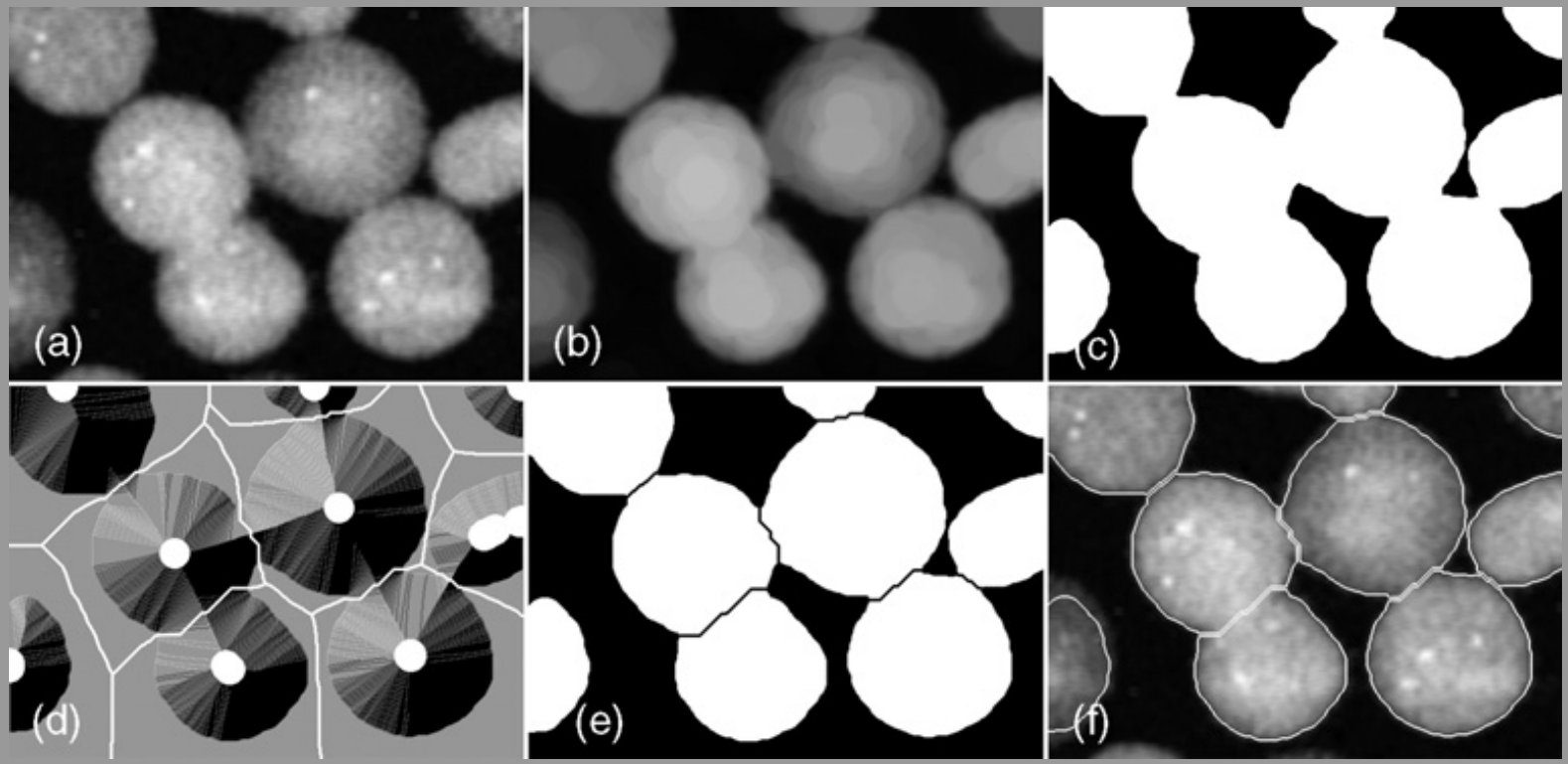


# Reconstruction from markers



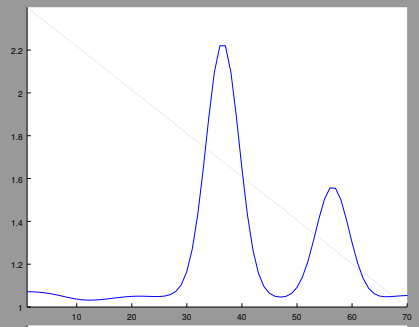


# Watershed

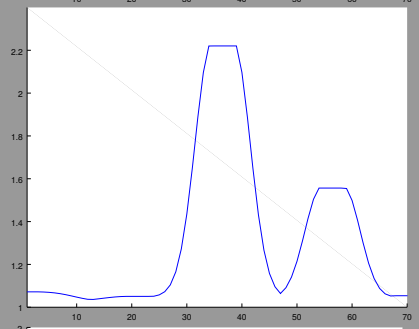


# Image gradient

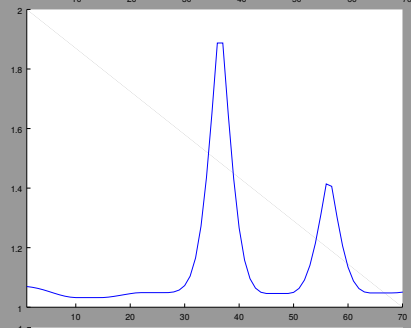
Original



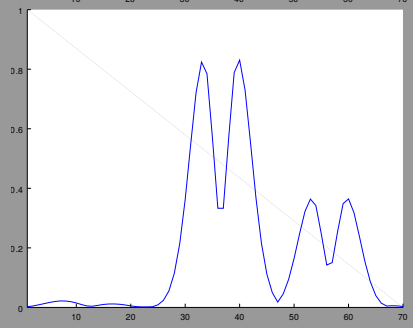
Dilation

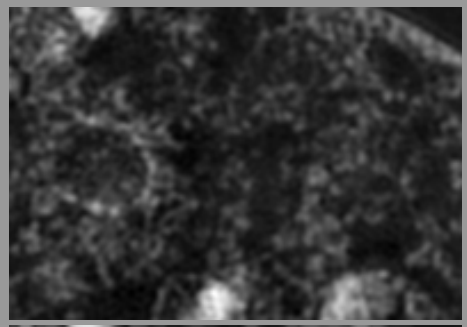


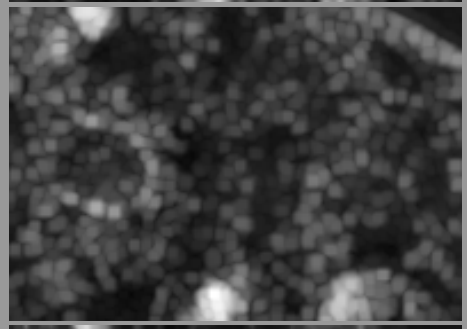
Erosion

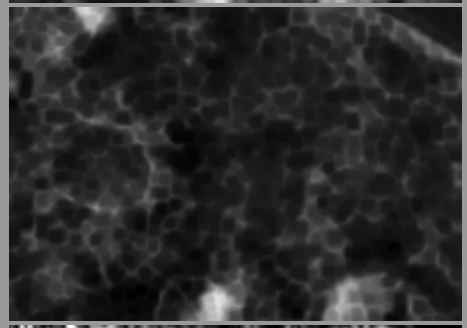


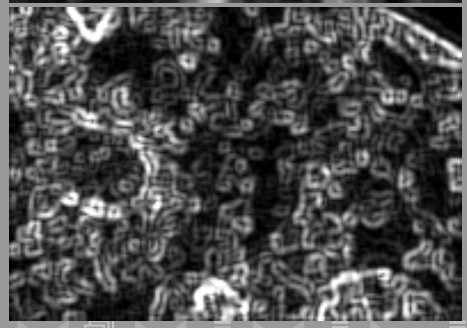
Gradient
(dilate - erode)





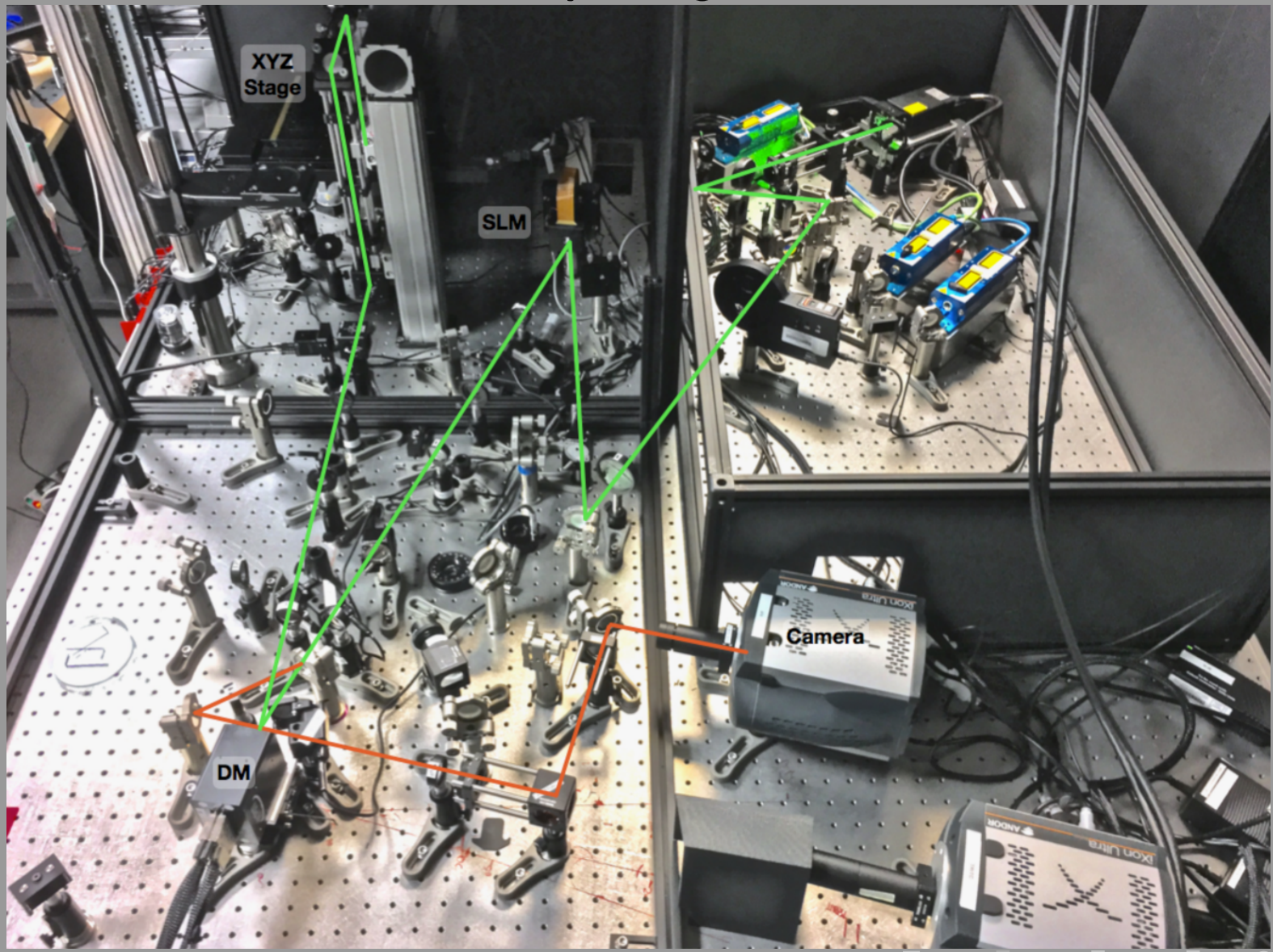






# Moving image

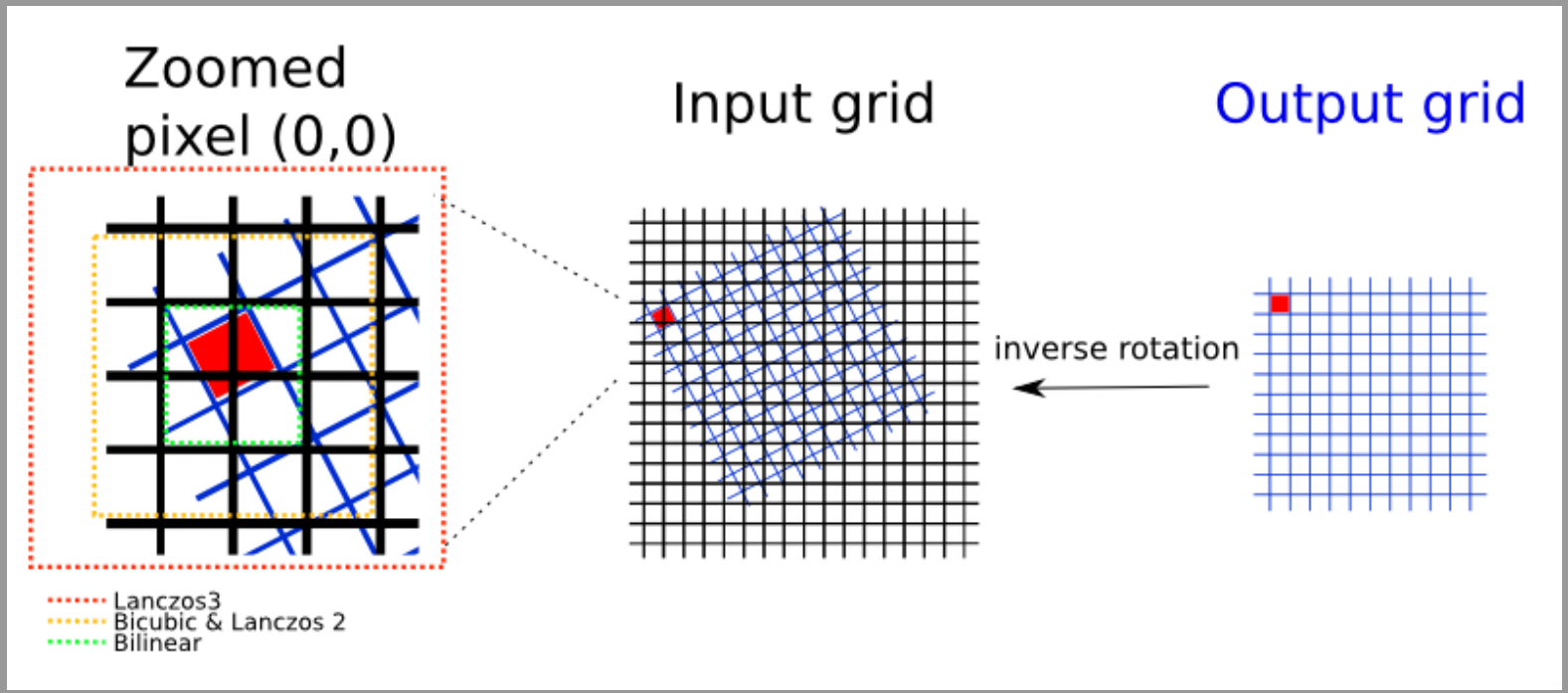
## Why doing it?



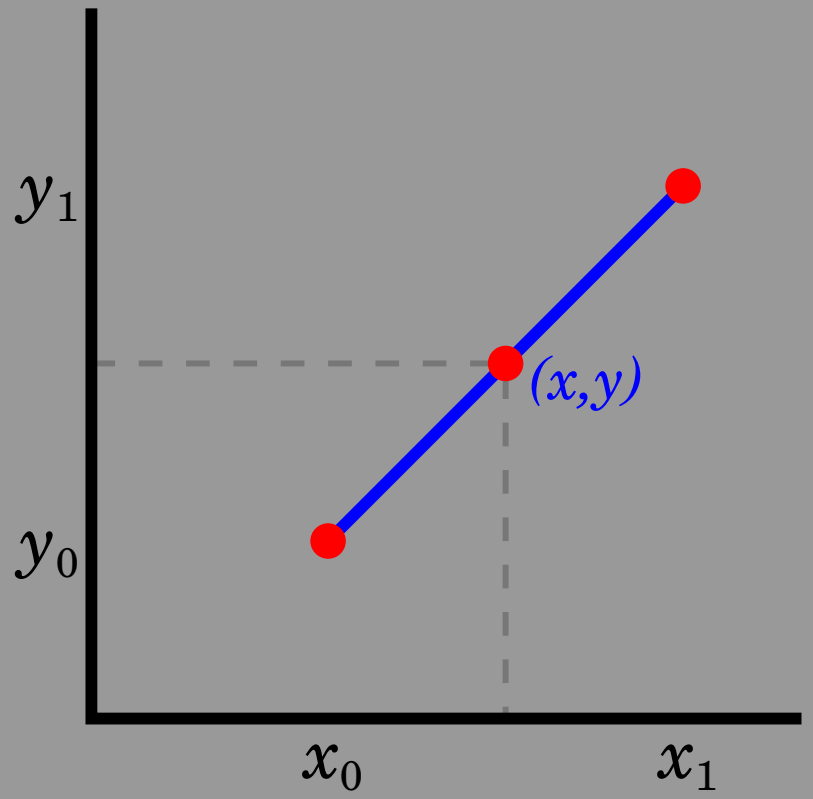
# geometric transformation

What happens when you:

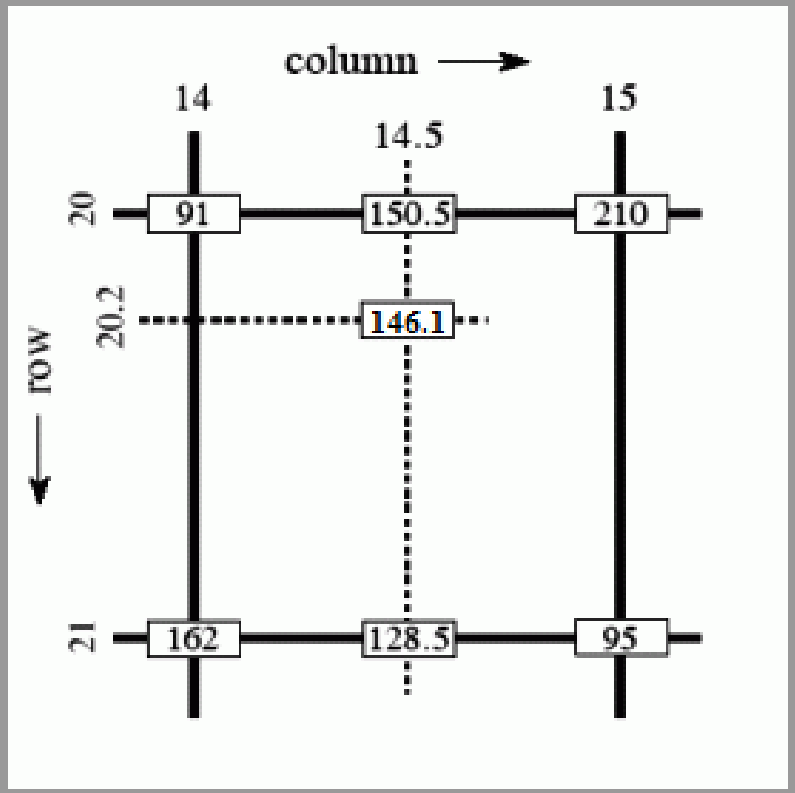
- rotate
- align
- translate
- stretch



# Linear interpolation

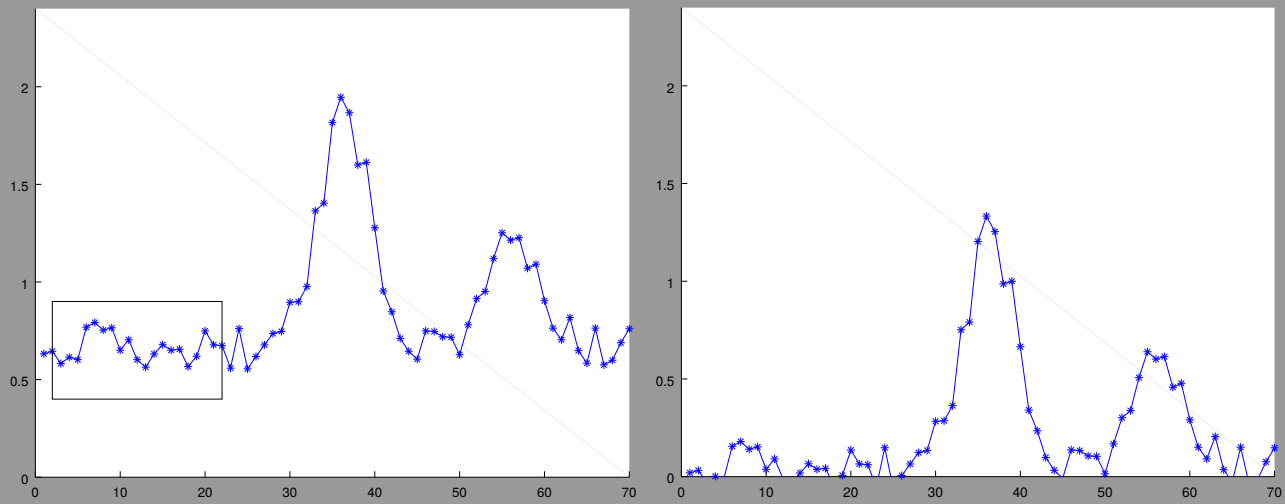


# Bilinear interpolation

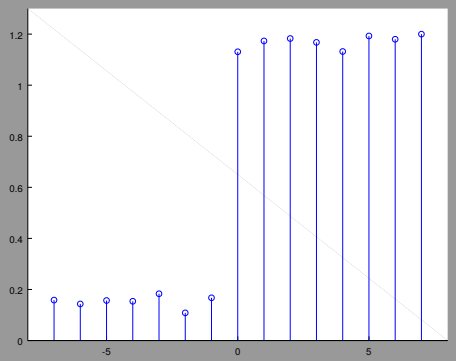


# Background correction

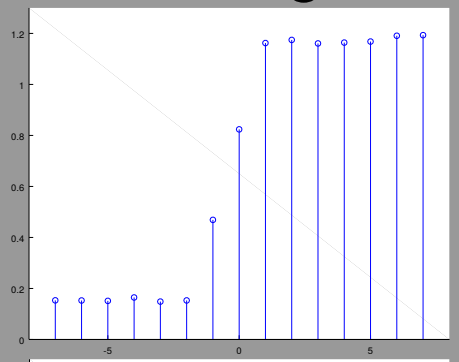
- Subtract mean of a known background region (darks).
- Many cameras (not-microscopes) do this.

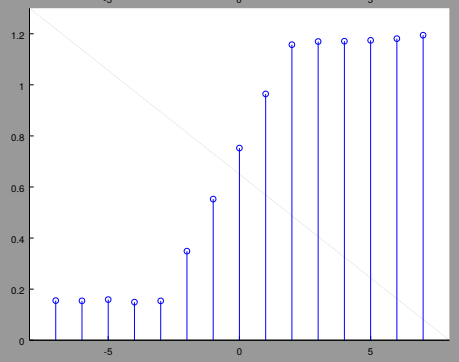


# Local means

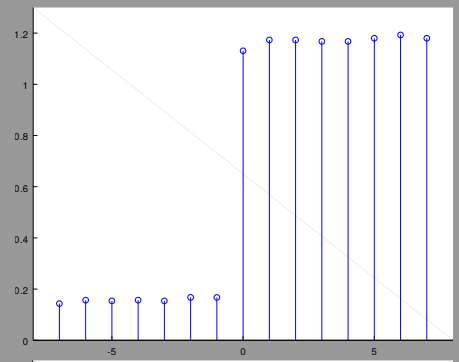


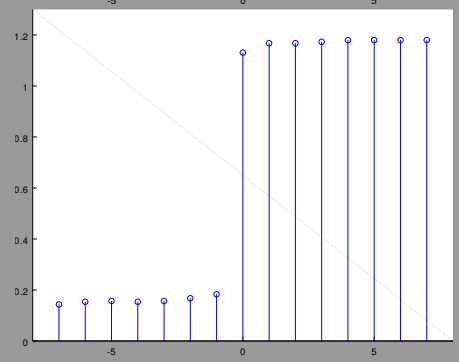
Average





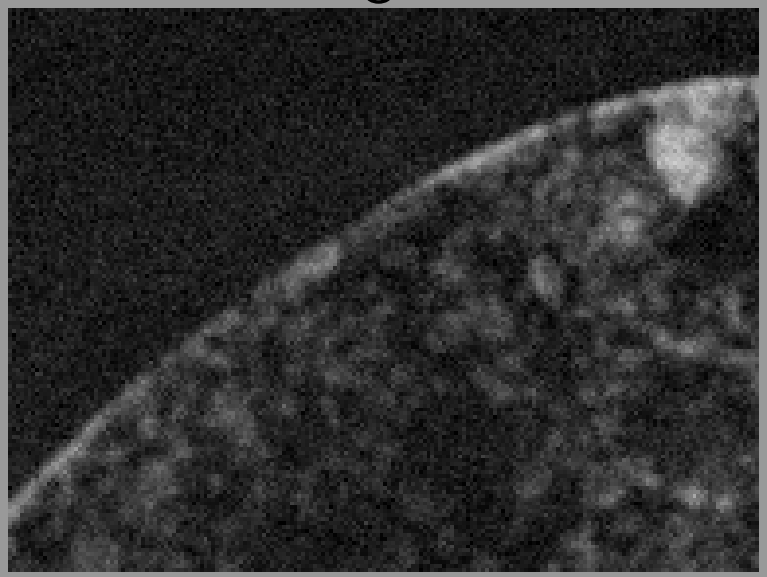
Median



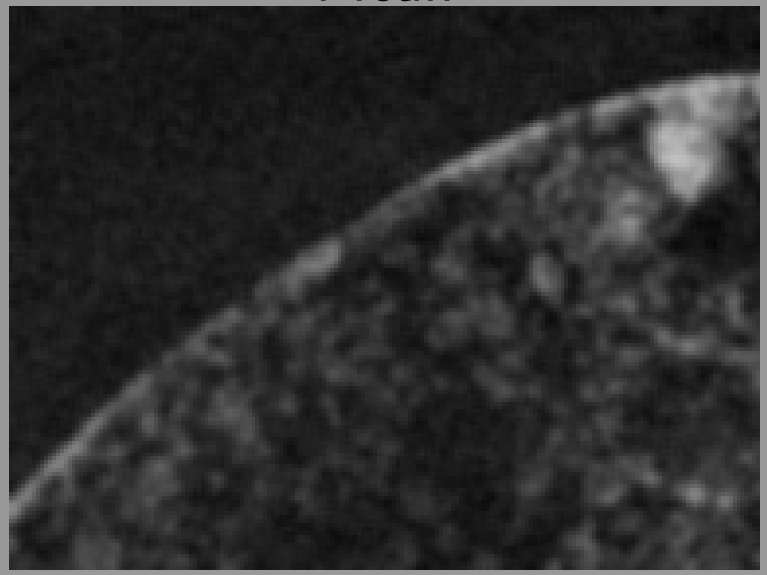


# Local means

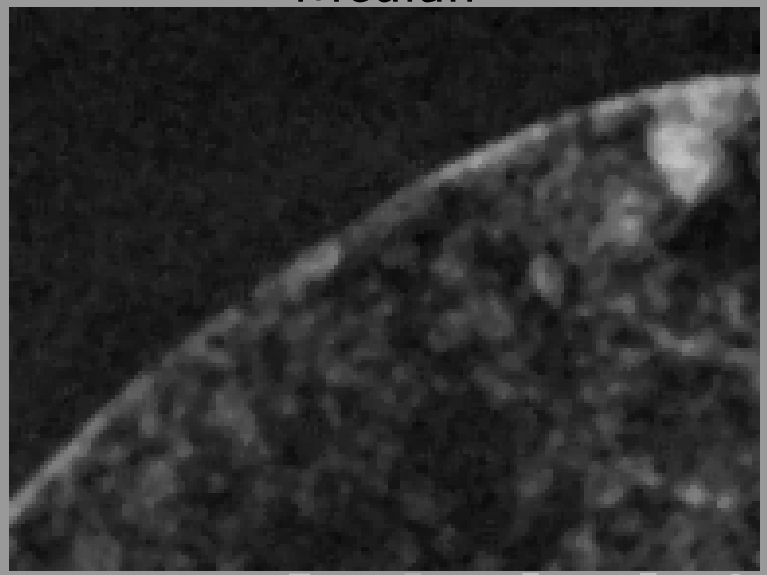
Original



Mean

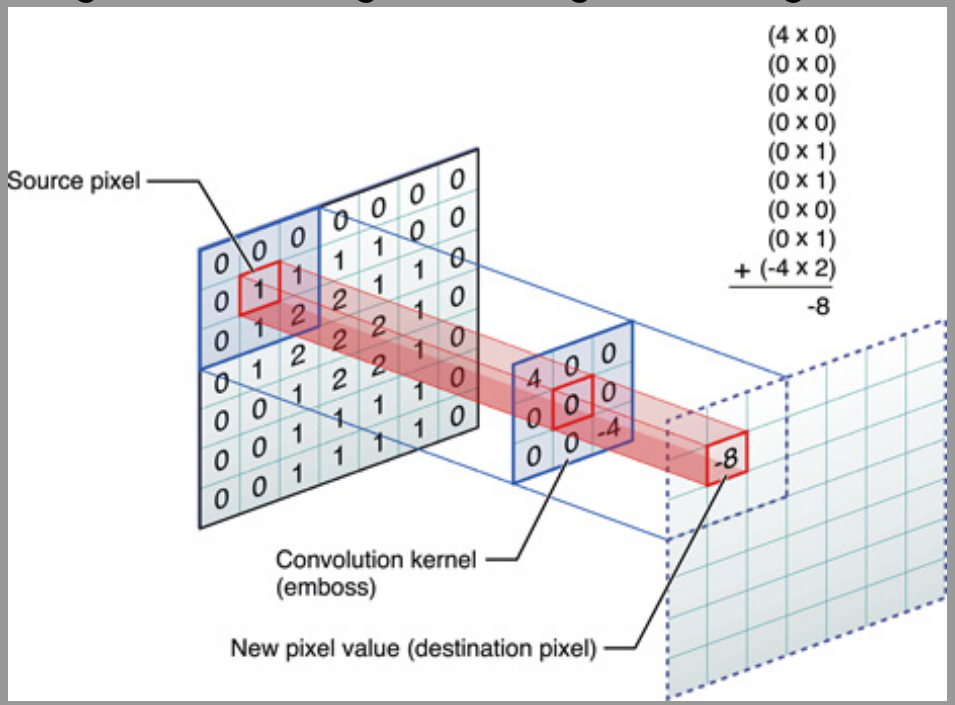


Median



# Mean as convolution kernel

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




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

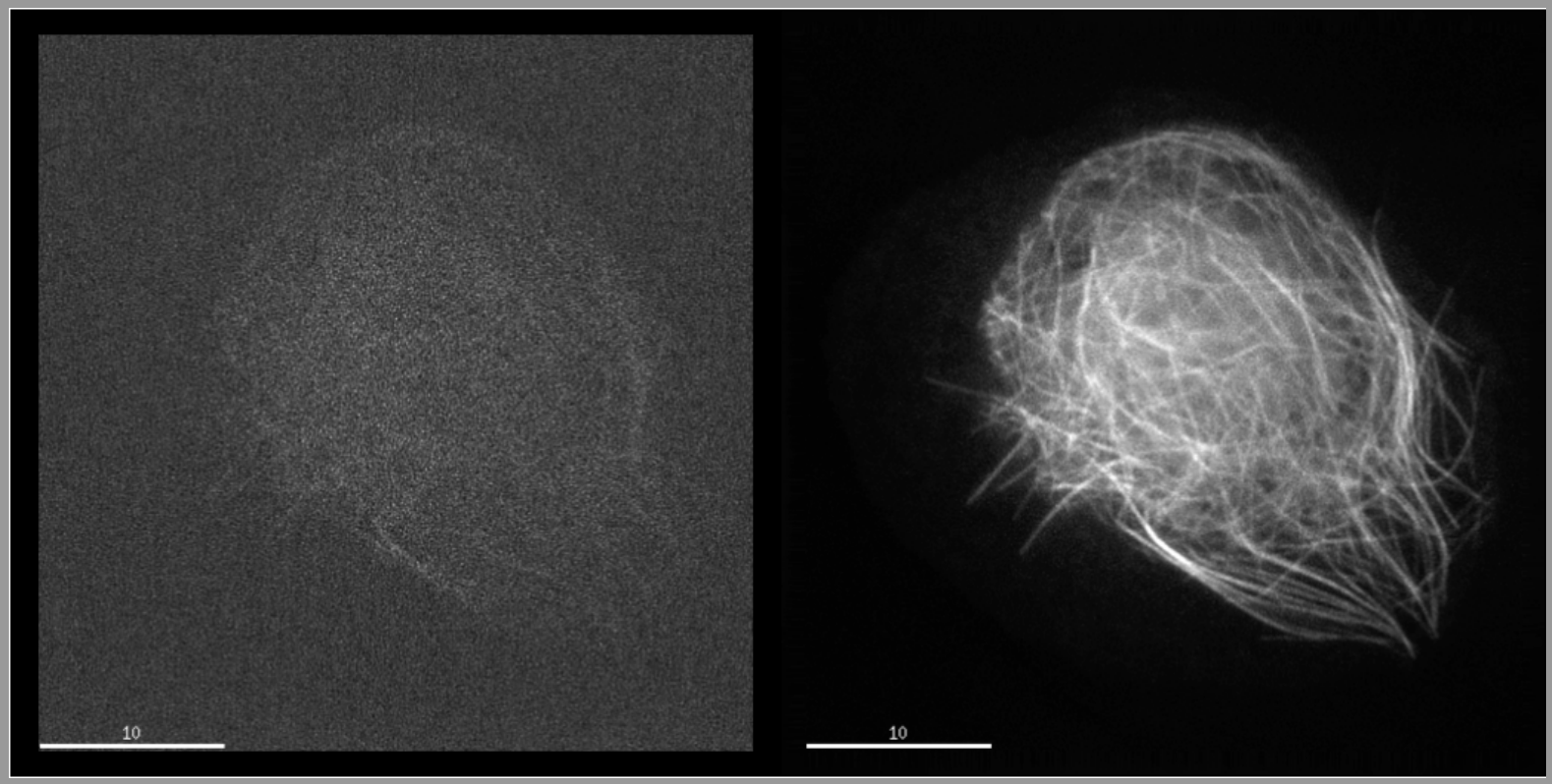
3x3 mean kernel

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

5x5 mean kernel

# Non-local means

## patch based denoise



# Gaussian filter

## as weigthed mean

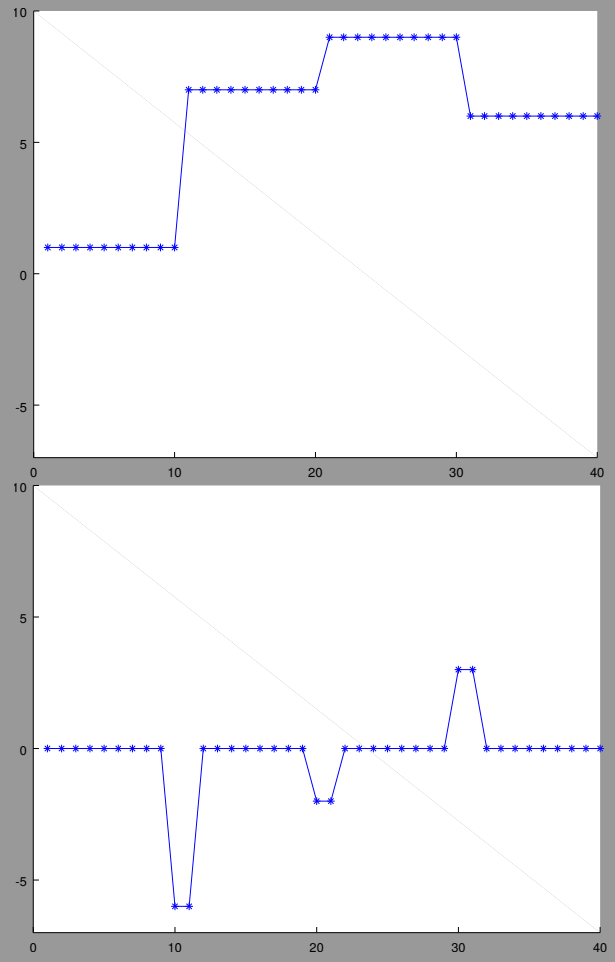
$$\begin{bmatrix} 0.011 & 0.014 & 0.017 & 0.018 & 0.017 & 0.014 & 0.011 \\ 0.014 & 0.019 & 0.023 & 0.024 & 0.023 & 0.019 & 0.014 \\ 0.017 & 0.023 & 0.027 & 0.029 & 0.027 & 0.023 & 0.017 \\ 0.018 & 0.024 & 0.029 & 0.030 & 0.029 & 0.024 & 0.018 \\ 0.017 & 0.023 & 0.027 & 0.029 & 0.027 & 0.023 & 0.017 \\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

# Edge detection

## Sobel operator

1D filter

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



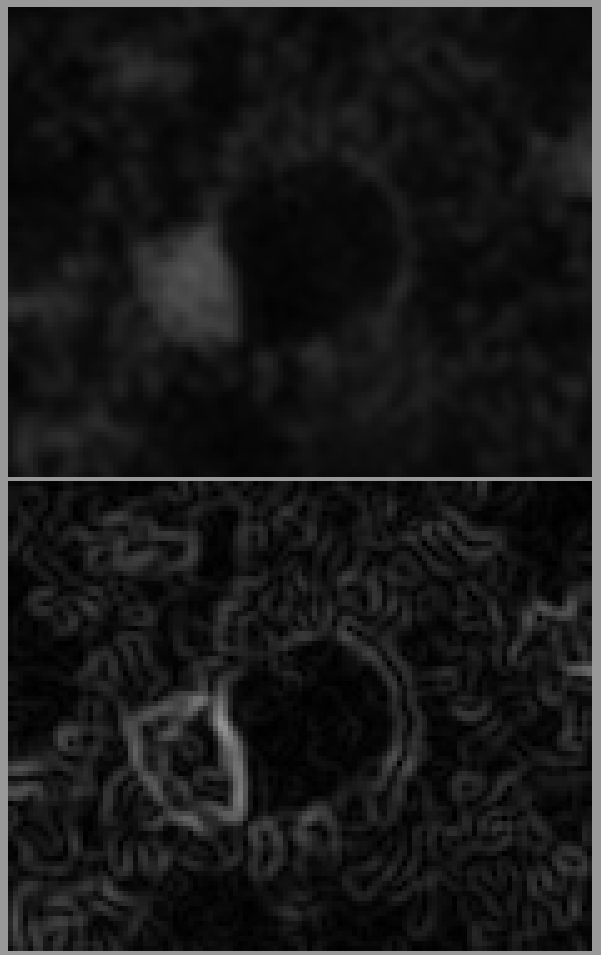
# Edge detection

## Sobel operator

2D filter(s)

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

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



# 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