Image processing course

M1 immunologie 21 octobre 2021

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Ciml IMMUNOLOGY

cnrs dépasser les frontières

Inserm

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Plan

- Part 1 : Notions on image
- Part 2 : Pre processing
- Part 3 : Analyse
- Part 4 : Segmentation and Colocalisation
- Part 5 : Image for publication

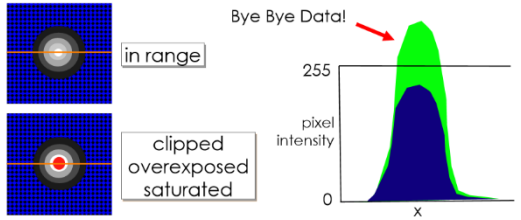
Part 1 : Notions on Image

« un visible qui donne à en voir un autre »

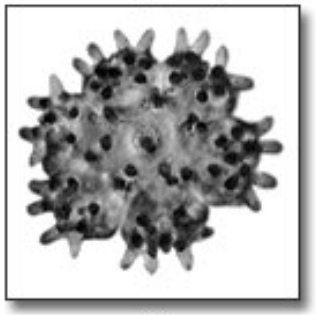
DEFINITION :

- A pixel is a sample not a little square
- Image is define by its number of pixels

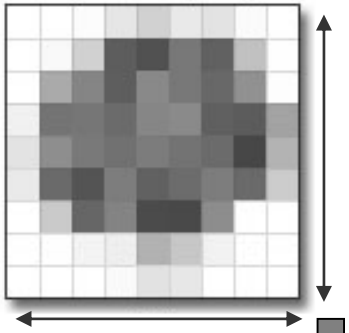
Avoid saturation



Analog Image



Digital Sampling



Pixel Quantization

249	244	240	230	209	233	227	251	255
248	245	210	93	81	120	97	193	254
250	170	133	94	137	120	104	145	253
241	116	118	107	134	138	96	92	163
277	142	121	113	124	115	107	71	179
234	106	84	125	97	108	125	106	204
241	202	102	132	75	73	141	246	252
253	252	244	239	178	199	242	250	245
255	249	244	250	226	231	240	251	253

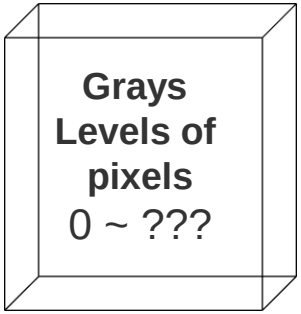
Definition : 9x9

Part 1 : Notions on Image

DYNAMIC :

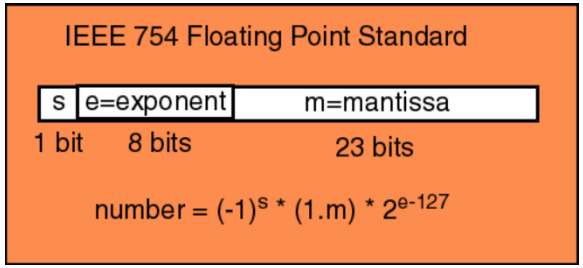
- The dynamic is the range of gray values that pixel can get

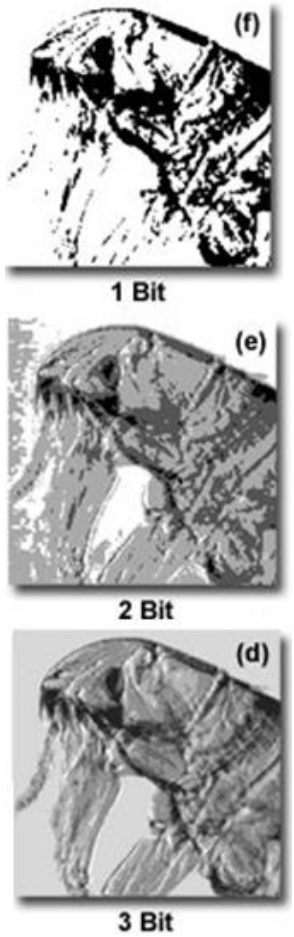
- Eyes can distinguish only around 40 gray levels differences (without image noise)



1 Bit : 2 levels (0 ou 1)
3 Bit : 8 levels
8 Bit : 256 levels = 1 octet (byte)
16 Bit : 65 535 levels = 2 octets (2 bytes)
32 Bit : 4 292 967 296 levels

32 Bits float





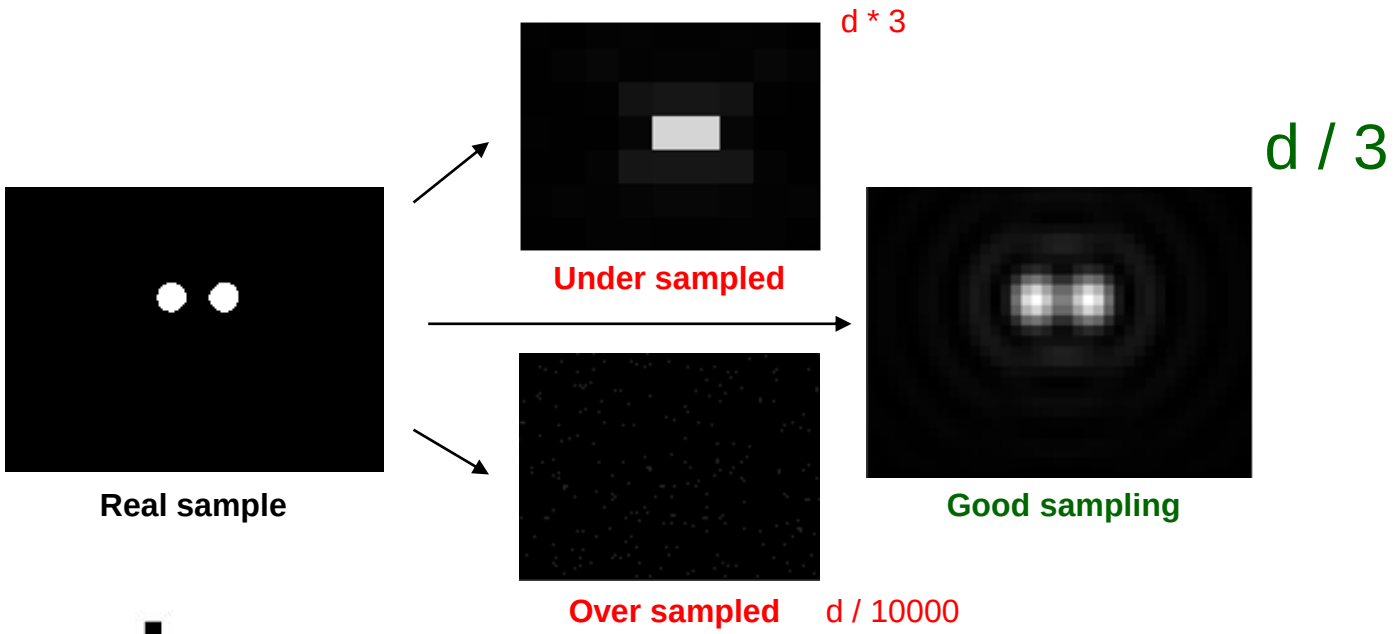
Part 1 : Notions on Image

SAMPLING :

$$d = \frac{\lambda}{2 \times NA}$$

Nyquist – Shannon sampling theory : Proper spatial sampling **2.3 – 3** times smaller than optical resolution (x, y, z)

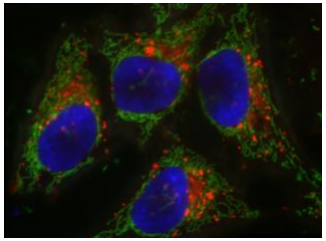
- Sampling cannot improve the optical resolution
- Optimal pixel spacing must be calculate with « Nyquist theory »



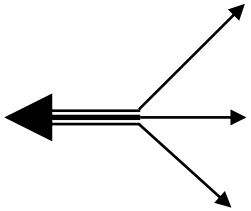
Part 1 : Notions on Image

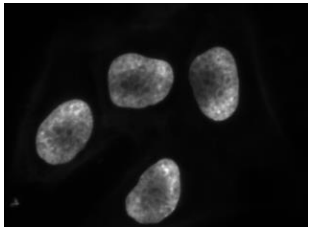
Channels :

- RGB (**R**ed **G**reen **B**lue) are 3 grays images associated
- You can easly split into 3 different images or invert

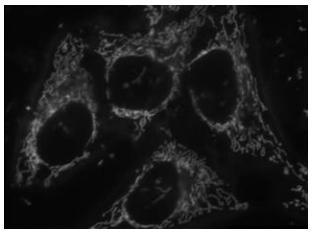


RGB
RGB color

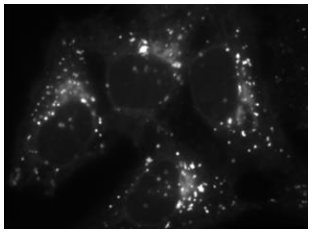




Channel 1 (blue)
8 bits



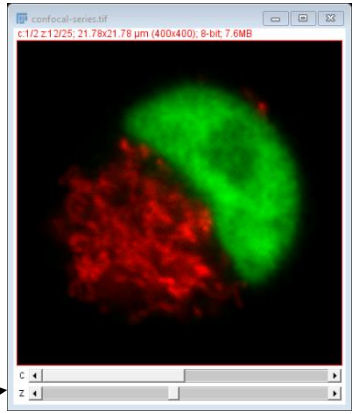
Channel 2 (green)
8 bits



Channel 3 (red)
8 bits

Hyperstack :

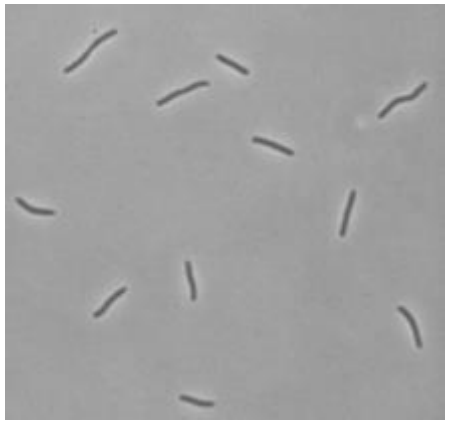
Time, Z slices, channels, multi-positions

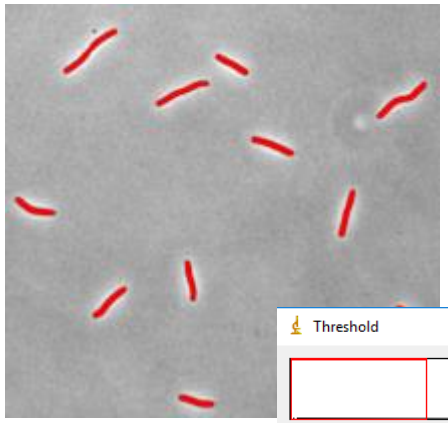


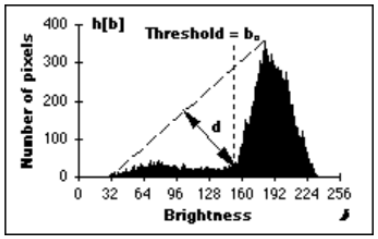
Part 2 : Pre processing

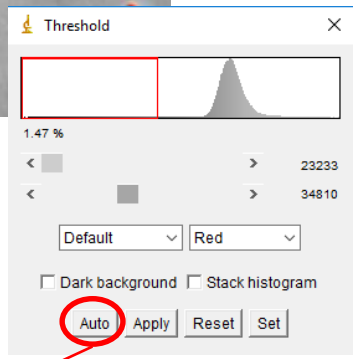
Threshold :

- Use the histogram for determinate manualy or automaticly a threshold value
- Binarise digital image into 2 populations (0 or 255) thanks to theshold value







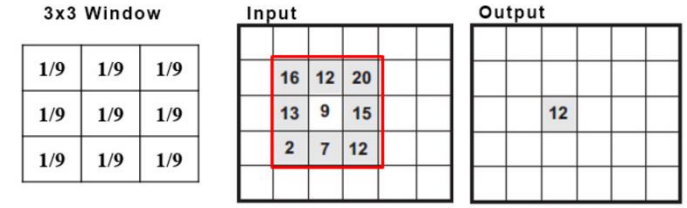


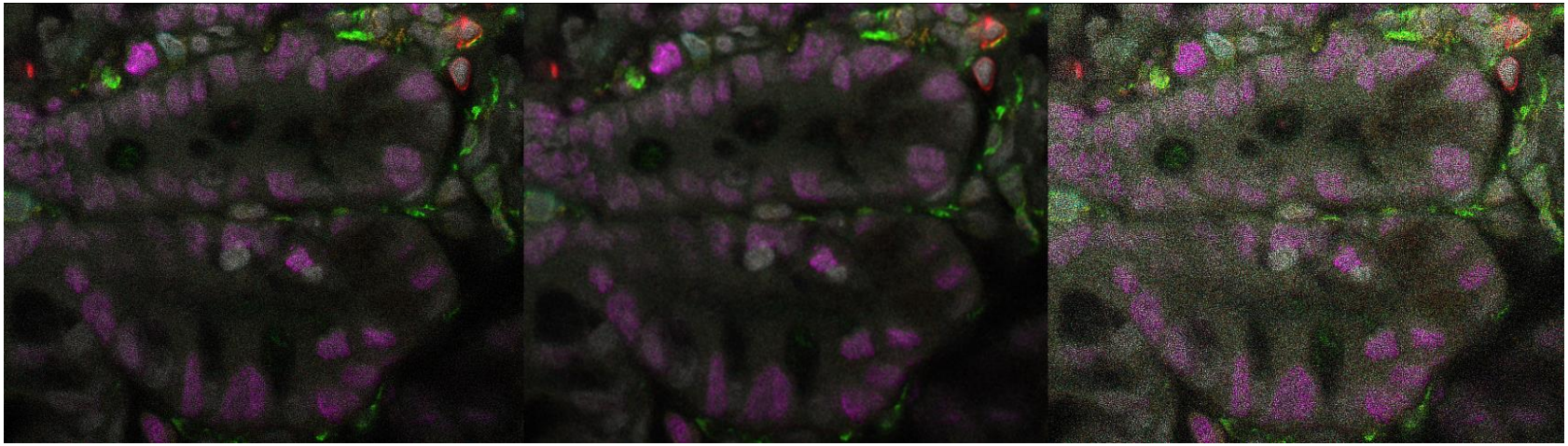
The triangle algorithm is based on finding the value of *b* that gives the maximum distance **d**.

Part 2 : Pre processing

Linear filter

Convolution





1/9	1/9	1/9
1/9	1/9	1/9
1/9	1/9	1/9

Example low-pass filter

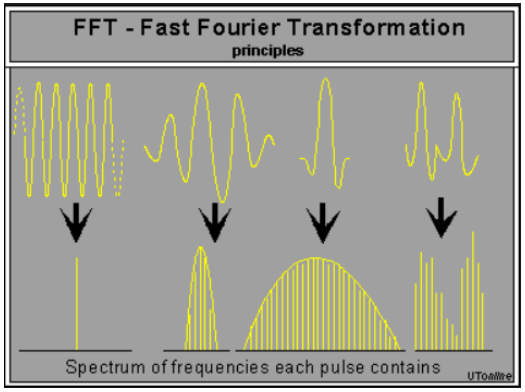
–1	–1	–1
–1	9	–1
–1	–1	–1

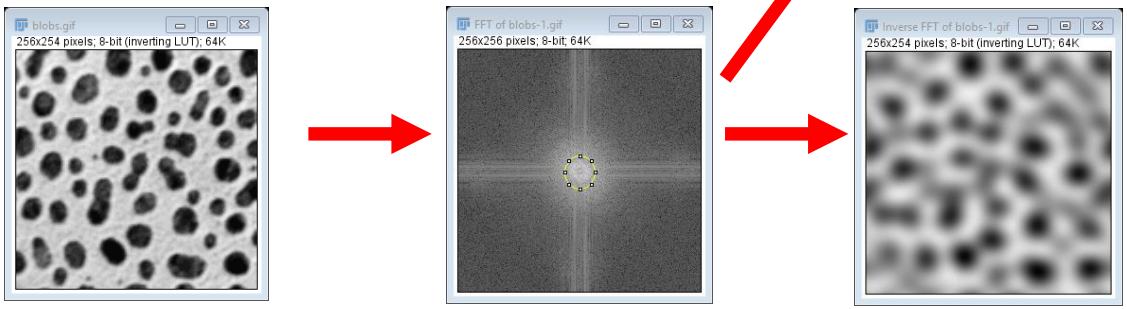
Example high-pass filter

Original → Low Pass filter (smooth) → High Pass filter (sharpen)

Part 2 : Pre processing

Fast Fourrier Transform





High frequency

```
run("FFT");
makeOval(112, 112, 32, 35);
setBackgroundColor(0, 0, 0);
run("Clear");
run("Inverse FFT");
```

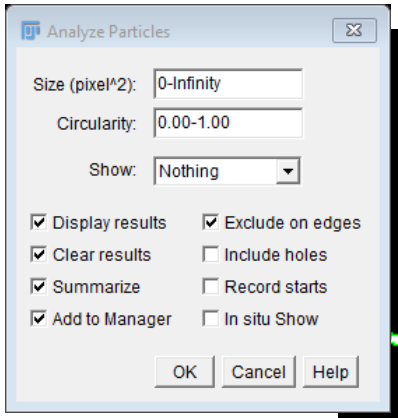
Low frequency

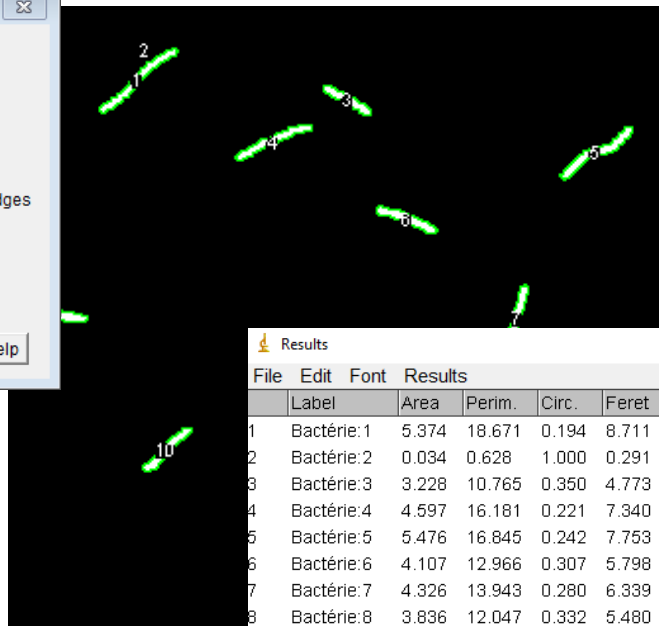
```
run("FFT");
makeOval(112, 112, 32, 35);
setBackgroundColor(0, 0, 0);
run("Clear Outside");
run("Inverse FFT");
```

Part 3 : Analyze

Shape factors

- Use binary image for detect and measure white objects
- You can filter objects with the size and circularity

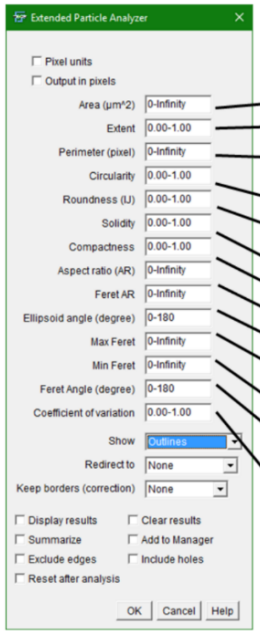


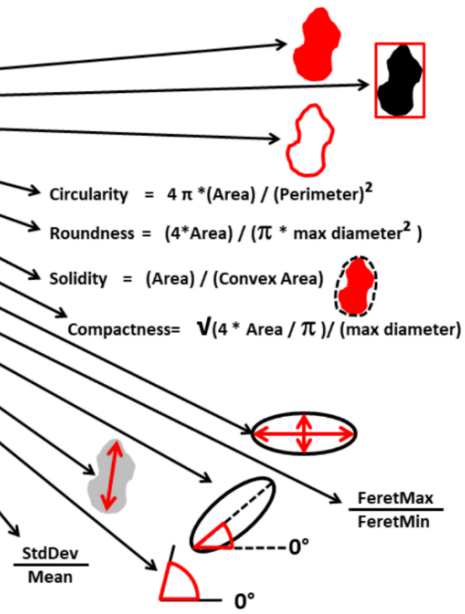


Results

File Edit Font Results

	Label	Area	Perim.	Circ.	Feret
1	Bactérie:1	5.374	18.671	0.194	8.711
2	Bactérie:2	0.034	0.628	1.000	0.291
3	Bactérie:3	3.228	10.765	0.350	4.773
4	Bactérie:4	4.597	16.181	0.221	7.340
5	Bactérie:5	5.476	16.845	0.242	7.753
6	Bactérie:6	4.107	12.966	0.307	5.798
7	Bactérie:7	4.326	13.943	0.280	6.339
8	Bactérie:8	3.836	12.047	0.332	5.480
9	Bactérie:9	3.363	11.661	0.311	5.203
10	Bactérie:10	3.938	12.115	0.337	5.626
11	Bactérie:11	4.310	13.625	0.292	6.176
12	Bactérie:12	3.245	10.361	0.380	4.607

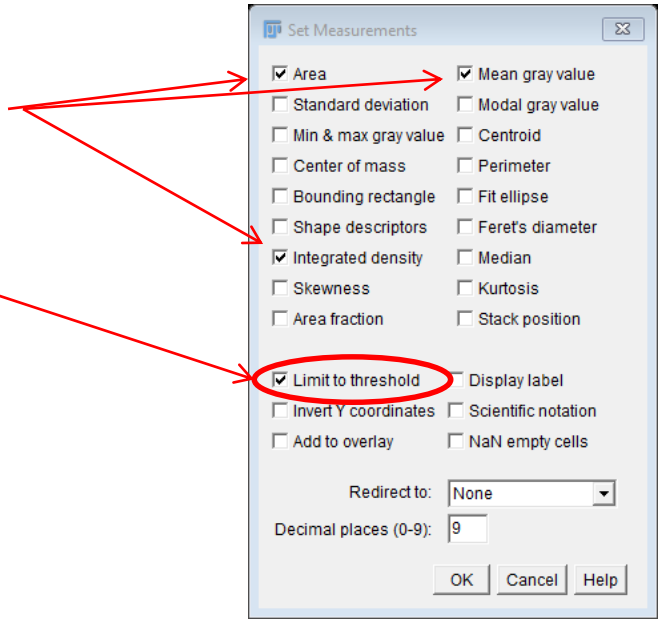


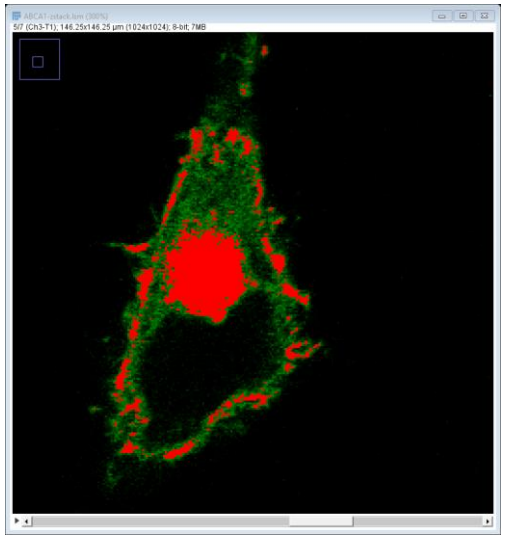


Part 3 : Analyze

Measurements :

- You can check measurements that you want
- You can apply measure only above a threshold





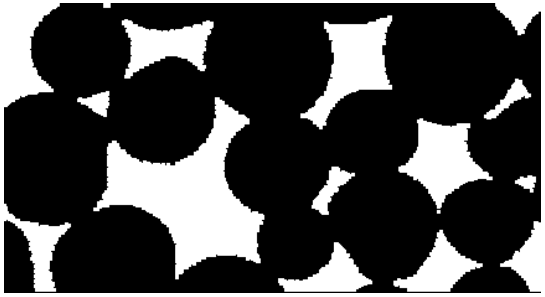
1- Without the option limit to threshold
2 - With the option

Results

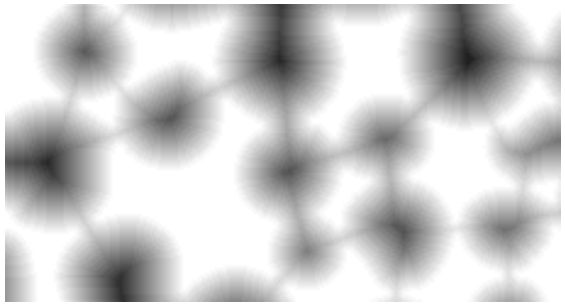
File Edit Font Results

	Area	Mean	IntDen	RawIntDen	MinThr	MaxThr
1	21388.507000304	1.492502213	31922.394020547	1565002	0	0
2	89.851735436	185.716004540	16686.905306150	818079	126	255

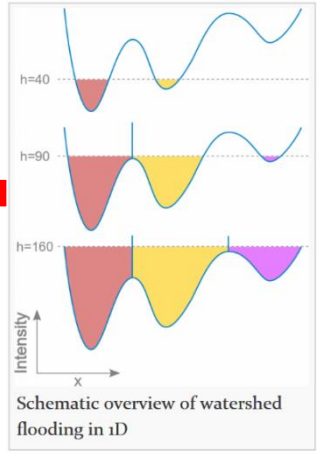
Segmentation by watershed



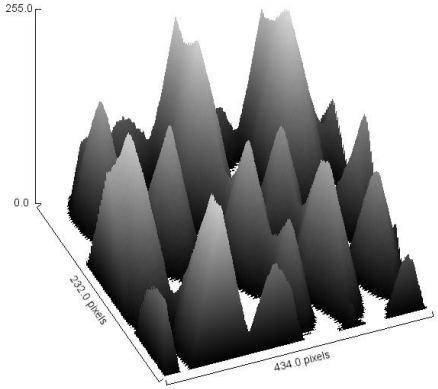
Threshold



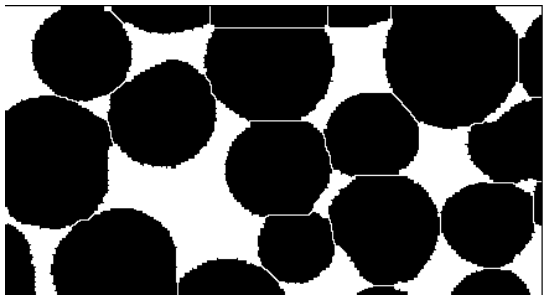
Euclidian distance map flooding



Schematic overview of watershed flooding in 1D



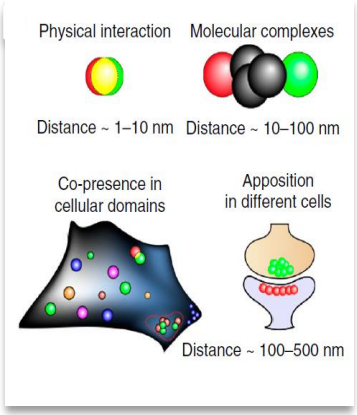
Surface plot



Segmented image

Map distance flooding

Colocalisation



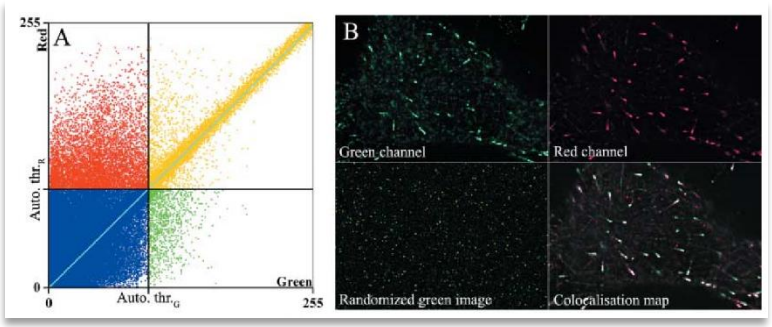
Colocalization Measures

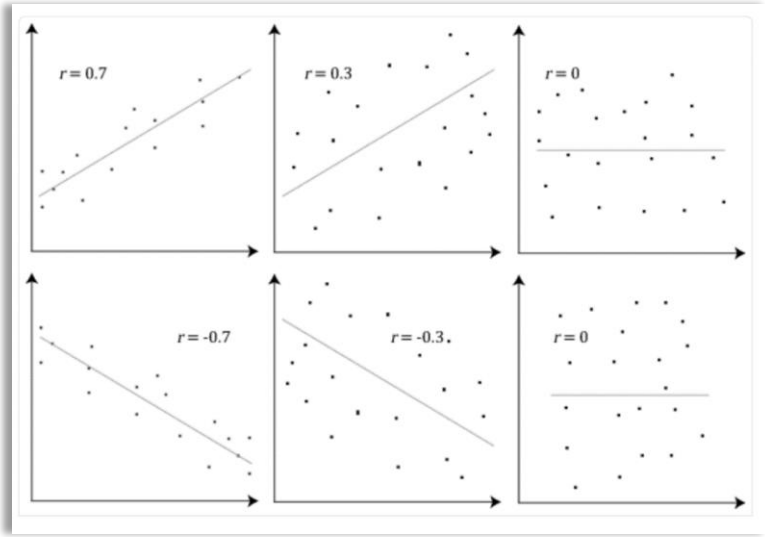
Pearson's correlation coefficient: $r_P = \frac{\sum (I_1 - \bar{I}_1)(I_2 - \bar{I}_2)}{\sqrt{\sum (I_1 - \bar{I}_1)^2 \sum (I_2 - \bar{I}_2)^2}}$

example	r_P	r	k_1	k_2	m_1	m_2
1	0.49	0.58	0.79	0.43	0.99	0.15
2	0.39	0.71	0.81	0.62	0.35	0.24
3	-0.08	0.37	0.24	0.57	0.23	0.38

overlap coefficient: $r = \sqrt{k_1 k_2}$ with $k_1 = \frac{\sum I_1 I_2}{\sum I_1^2}$ and $k_2 = \frac{\sum I_1 I_2}{\sum I_2^2}$

Manders' colocalization coefficients: $m_1 = \frac{\sum I_{1\,\text{coloc}\,2}}{\sum I_1}$ and $m_2 = \frac{\sum I_{2\,\text{coloc}\,1}}{\sum I_2}$





Scatter plot

The Pearson correlation coefficient is a measure of the strength of a linear association between two variables. The algorithm attempt to draw a line of best fit through the data of two variables and r indicates how far away all these data points are in this line of best fit.

Part 4 : Image for publication

Images Save Formats :

- There are destructive formats

JPEG : Joint Photographic Expert Group — for ppt presentation only

- There are non-destructive formats — TIF for publication

TIF : Tagged Image File BMP PNG

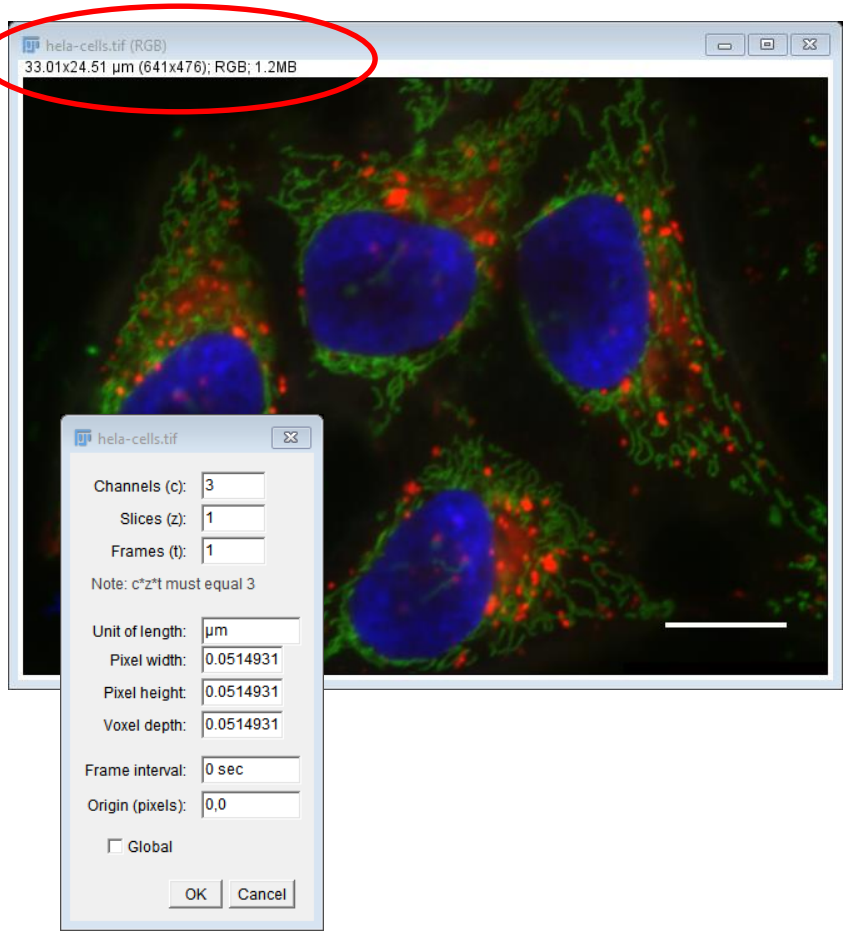
ppi = **p**oint **p**er **i**nch (point par pouce, 1 inch is 2,54 cm)

A definition of 300 ppi correspond to the visual acuity of an image at 25 cm.
Distance between 2 points at 300 ppi: 25,4 mm / 300 = **0,08 mm**.
1 mm^2 contents 144 dots at 300 ppi.

Do not make screen copy

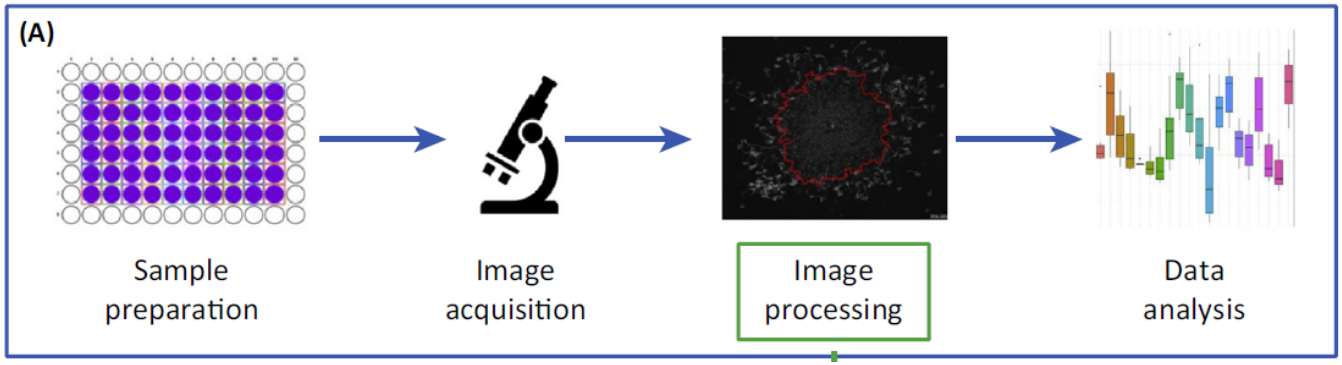
Image for publication

- Raw format have to be stored in dedicated server, user have to work on copy
- All digitized images submitted with the final revision of the manuscript must be of high quality and have resolutions of at least 300 d.p.i. for colour, 600 d.p.i. for greyscale and 1,200 d.p.i. for line art.
- The scale bar width in micron should be indicated in the legend
- Every processing should be also indicated



Conclusion

- Image are data and need to be quantified using image processing tool to give results and produce interpretation on an experiment
- Sample preparation, image acquisition and quantification (+statistics tests) should be done with care (using controls) to avoid artefact



Quizz

Education

1. What is the purpose of image processing and quantification ?

2. Which format is better for publication and to work with, explain why ?

3. How to find the image scaling in image, what about TIF image and jpg image?

1. What is a threshold and a watershed and for what is-it useful for ?

2. What is the size in octet of a image of 1000*1000 pixels containing one channel in 8 bits without compression ?

3. What is the size in cm of an image containing 1000 pixels by 1000 pixels at 600 DPI ?

Question outside this course :

1. What is the vectorized format in comparison to matrix format ?
   Cite an example of vectorized format ? Is the resolution of this format is sensitive to zoom ?

2. What is a Pyramidal format ? In which application is-it useful to use this kind of format ?

3. What advantage to use machine learning in image processing ?

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