Bioimage Informatics

EPFL

Mock exam

Series of Exercises 2025

Name

Sciper

Signature

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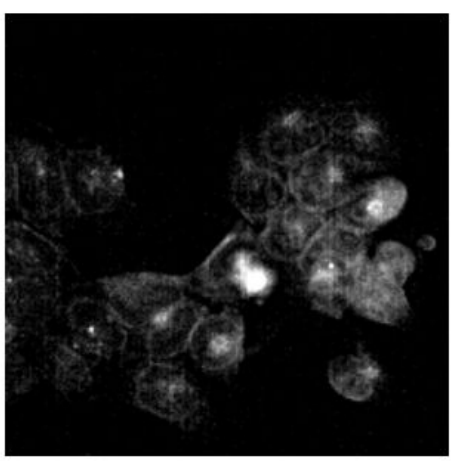
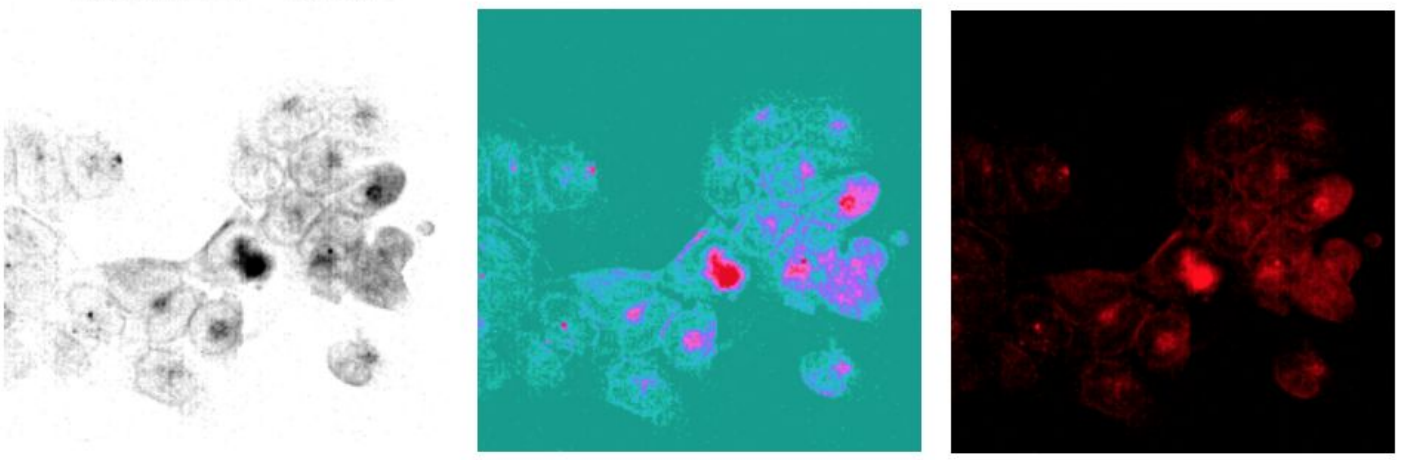
Color LUT

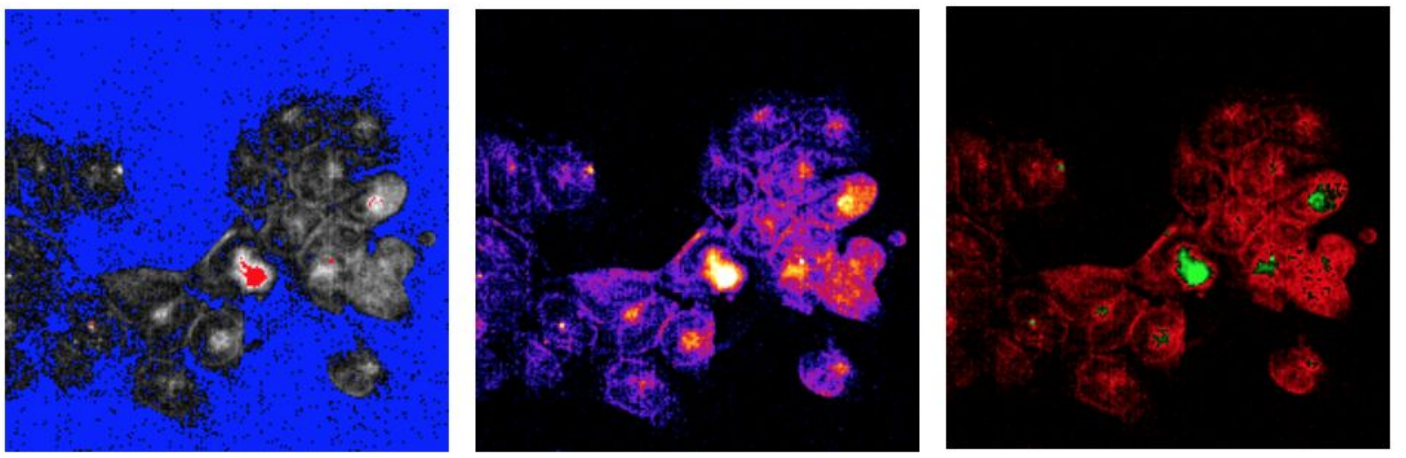
The series of images (A-F) has been generated by changing the LUT in FIJI starting form the input image with the gray LUT.
For each image, select the correct LUT among the following LUT (Fiji terminology):
{blue, cyan, fire, green, gray, hilo, inverted gray, ice, magenta, red, red green, spectrum, thermal, unionjack, yellow}



input (LUT= gray)



A B C



D E F

ANSWER

A

B

C

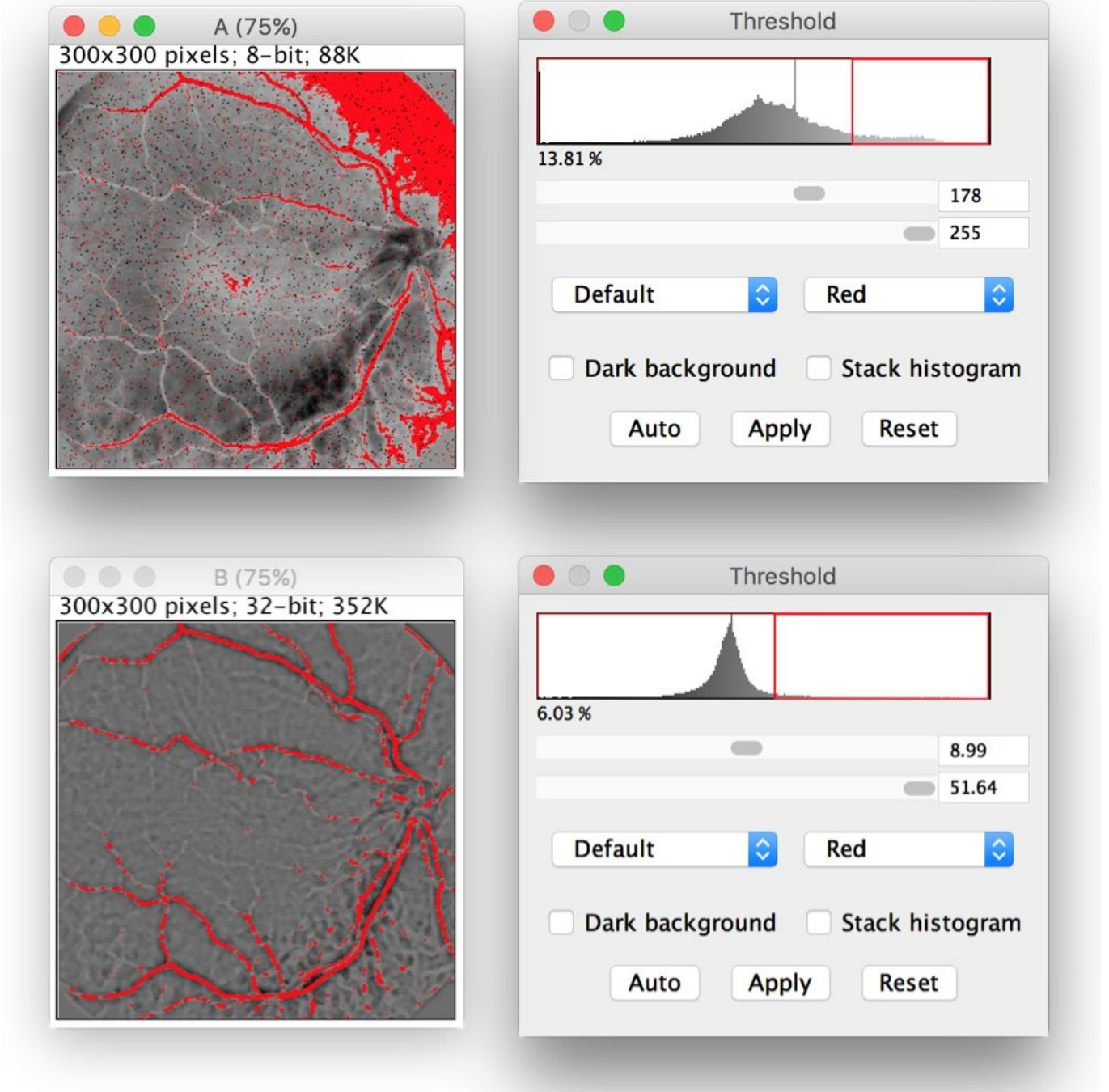
D

E

F

Preprocessing

Propose a preprocessing with simple image processing filters to go from the image A to the image B. The corresponding histograms are provided at right.

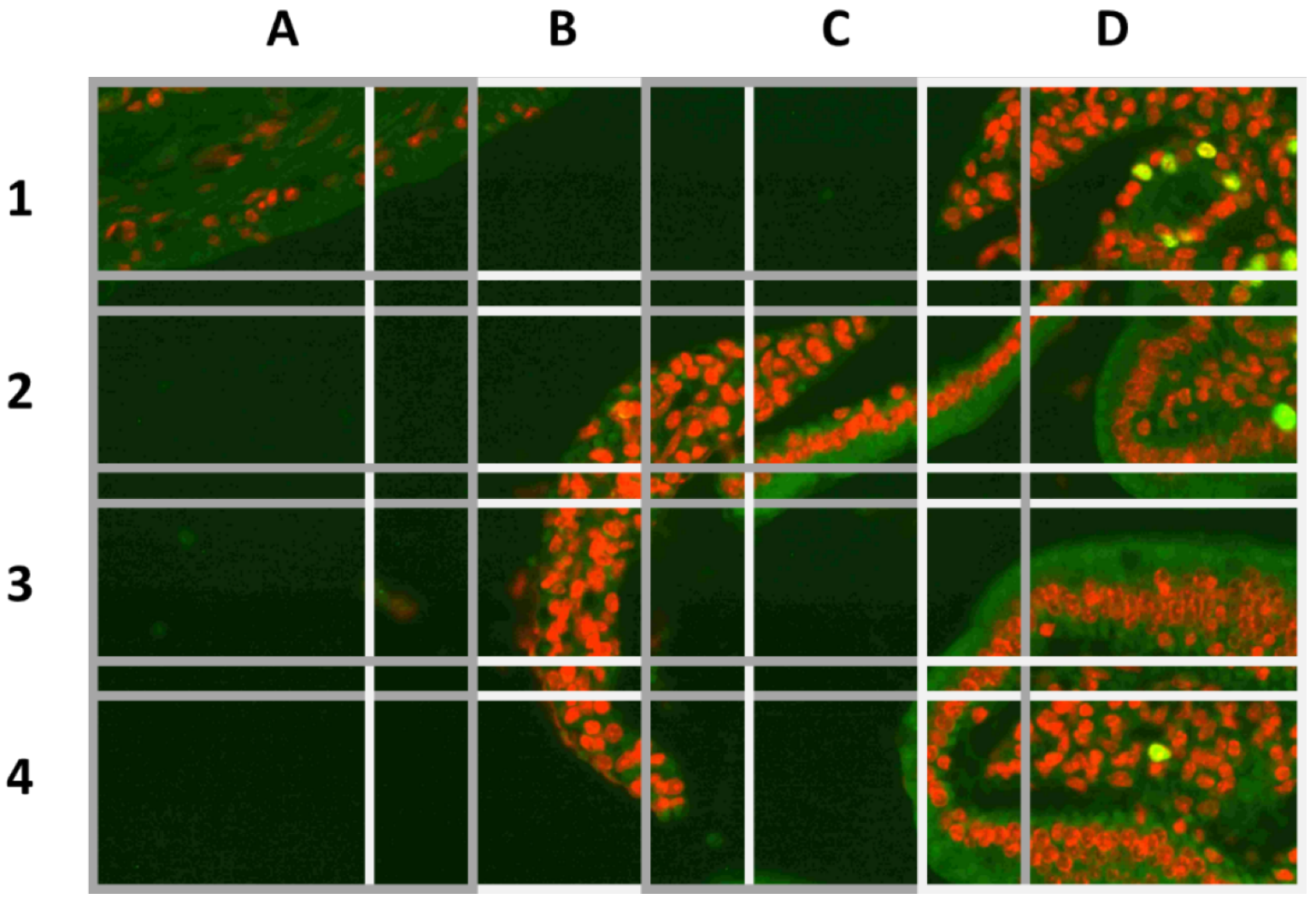


ANSWER

Write your proposed preprocessing

Stitching with Overlap

In an experiment 16 images were acquired with some overlap in order to image a large piece of tissue (see image below). The individual images shall be aligned and assembled with the so called Scale Invariant Feature Transform (SIFT). A priori knowledge from the acquisition process shall not be taken into account..



Question 2.a
Indicate three pairs of images where the above-mentioned stitching approach will most probably fail.
Give a short explanation.

Question 2.b
Which of the two channels in the image displayed in red and green is better suited for stitching?
Give a short explanation.

MSD Classification - Computation

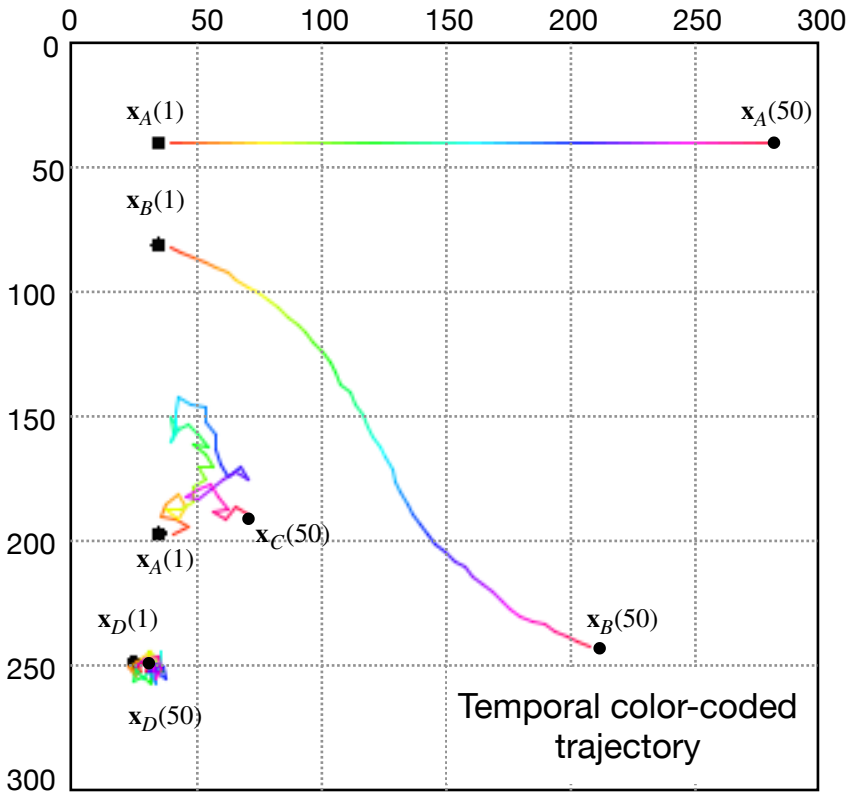
We want to characterize the diffusivity of the 4 black particles using the mean square displacement (MSD) in the following experiment: 4 particles are moving in a sequence of 50 (2D) images ; their displacement from one frame to the next frame is always of 5 pixels. A anomalous diffusion model is fit to MSD function $\mathbf{m_I}$ of the trajectory $\mathbf{T_I}$ to identify the parameters $\mathbf{a_I}$ and $\boldsymbol{\alpha_I}$.

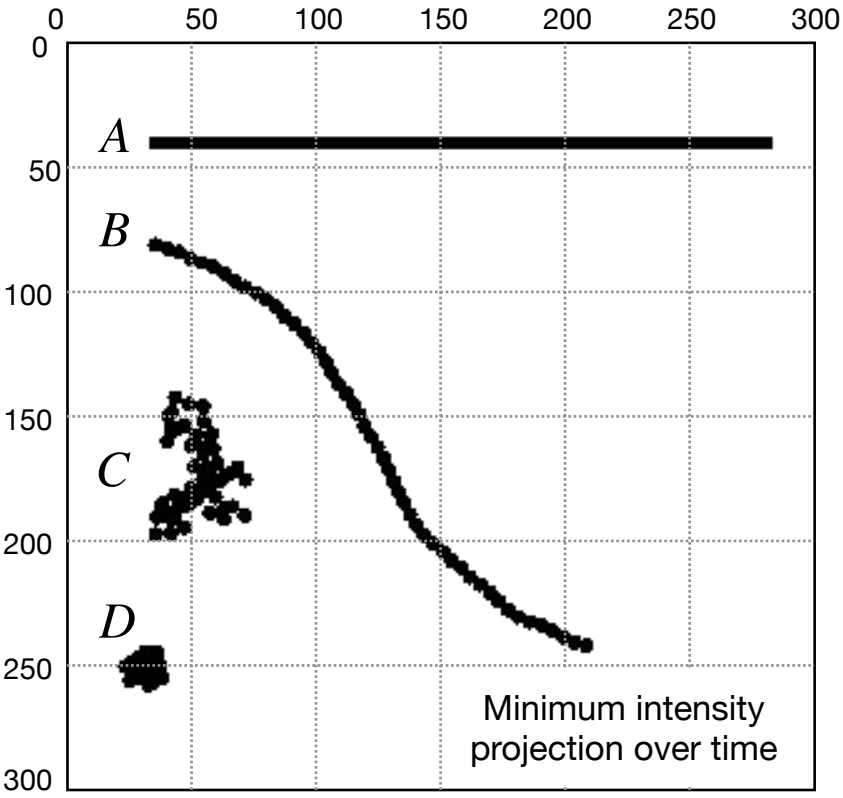
Coordinate (x,y) of the particle I at the image t: $\mathbf{x}_I(t)$

Trajectory of the particle I: $T_I = \left[\mathbf{x}_I(1), \mathbf{x}_I(2), \ldots \mathbf{x}_I(50)\right]$

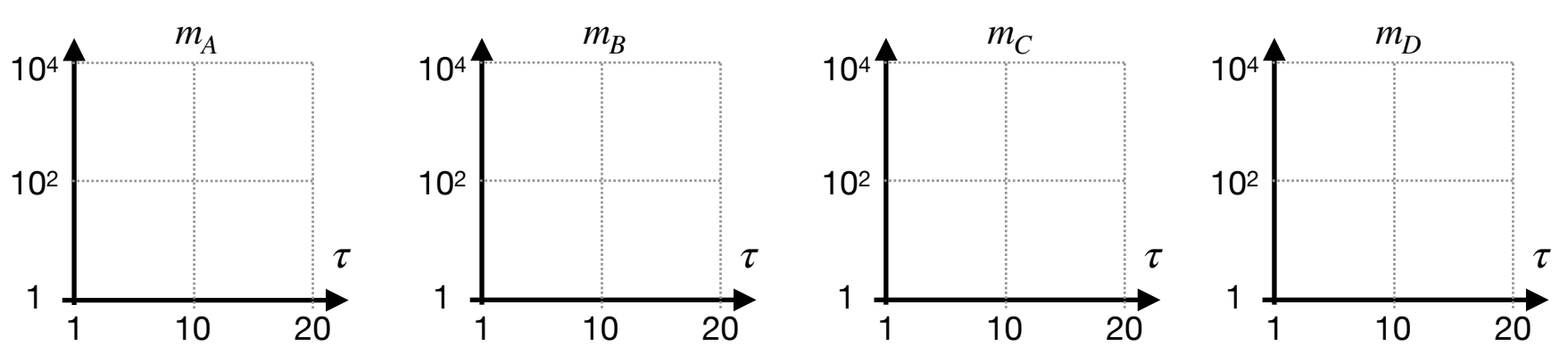
Mean square displ.: $m_I(\tau) = |\,\mathbf{x}_I(t) - \mathbf{x}_I(t+\tau)\,|^2$

Anomalous diffusion model: $m_I(\tau) = a_I\ \tau^{\alpha_I}$





Question A.
Draw a rough graph representing the MSD function m_I for each particle A, B, C, and D.



Question B.
Circle with the closest value $\boldsymbol{\alpha}$ of the MSD and circle the type of diffusion for each of the 4 particles.

	Approximated value of the parameter $\boldsymbol{\alpha}$			Type of diffusion		
Particle A	0.1	1	2	normal diffusion	super-diffusive	sub-diffusive
Particle B	0.1	1	2	normal diffusion	super-diffusive	sub-diffusive
Particle C	0.1	1	2	normal diffusion	super-diffusive	sub-diffusive
Particle D	0.1	1	2	normal diffusion	super-diffusive	sub-diffusive

Projection over Time

Time projection

The input images are two sequences of 81 frames C1 and C2. The sequence C3 is the difference between C2 and C1. For illustration, we show the frame t=22 of the two sequences.

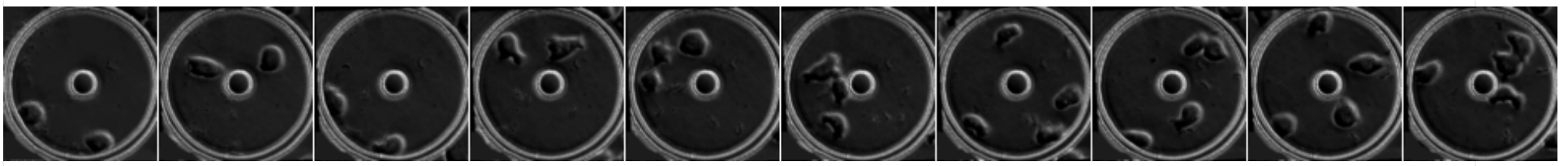
QUESTION

1) The images A-D are computed time projections. For each projections, find the kind of projection and the source sequence, C1, C2, or C3.

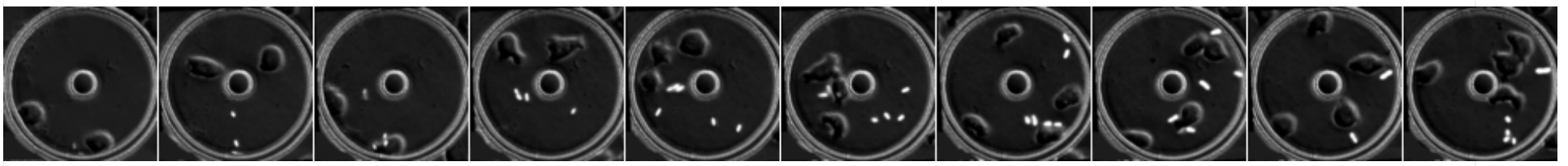
2) The sequence F1 was obtained by a convolution with a 1D filter in the time axis with a 3-tap filter. Guess the 3 values of the filter mask.

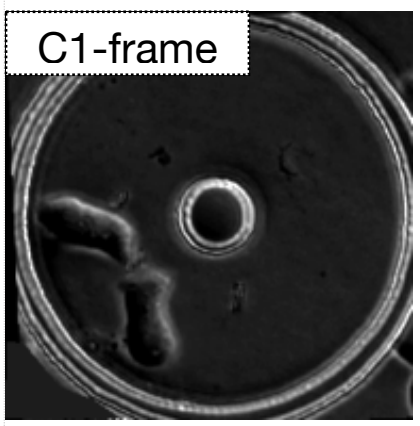
3) Same question for F2

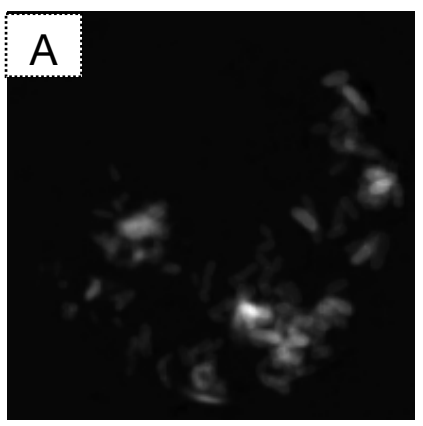
C1 frames: 0 to 80

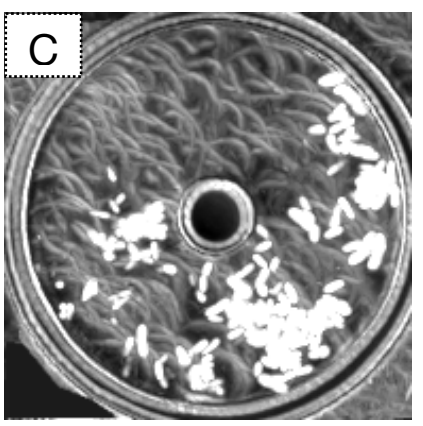


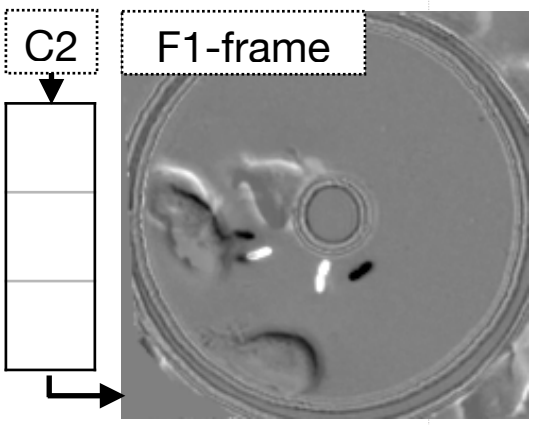
C2 frames 0 to 80

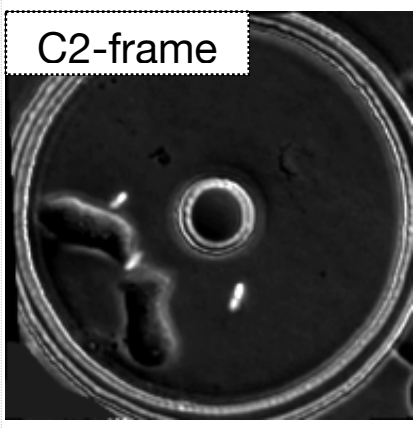


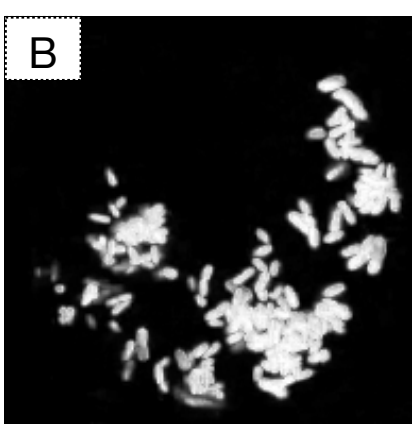


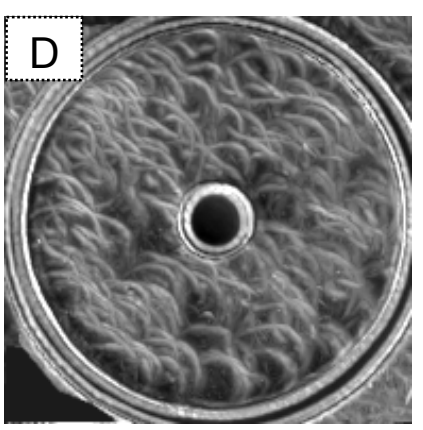


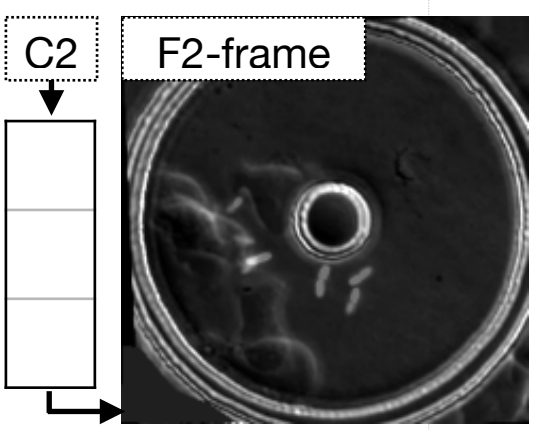








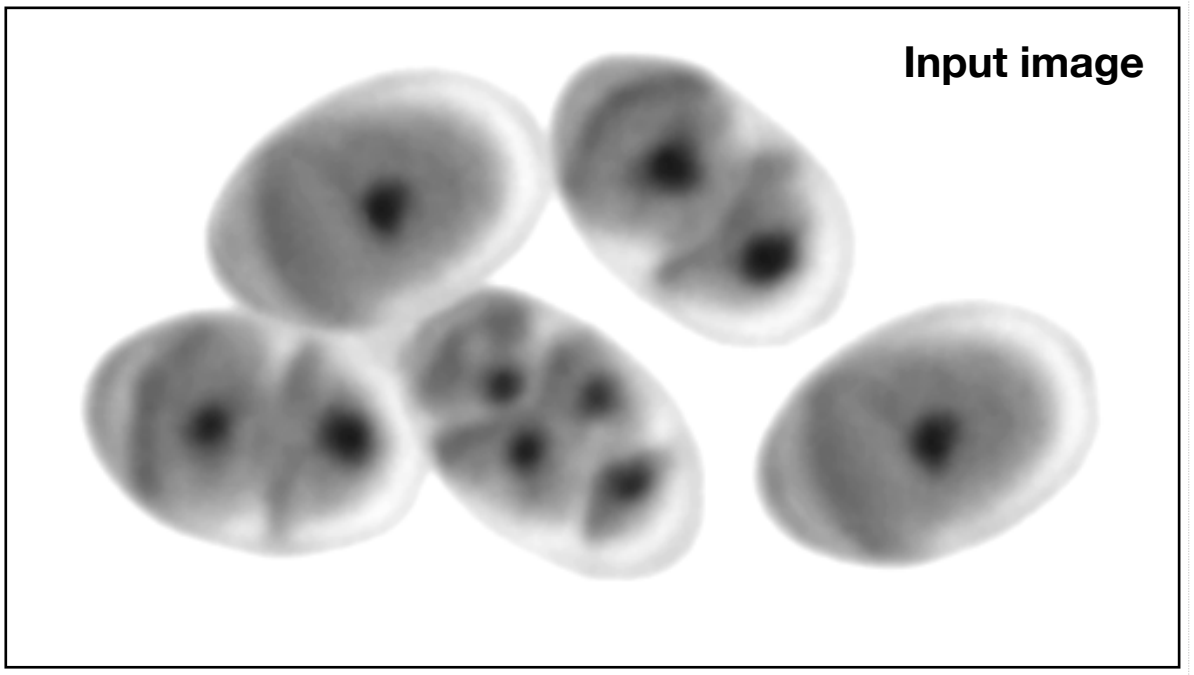




Watershed

The Watershed is a one the most used algorithm to segment packed objects in bioimages. There are two variations of this algorithm:

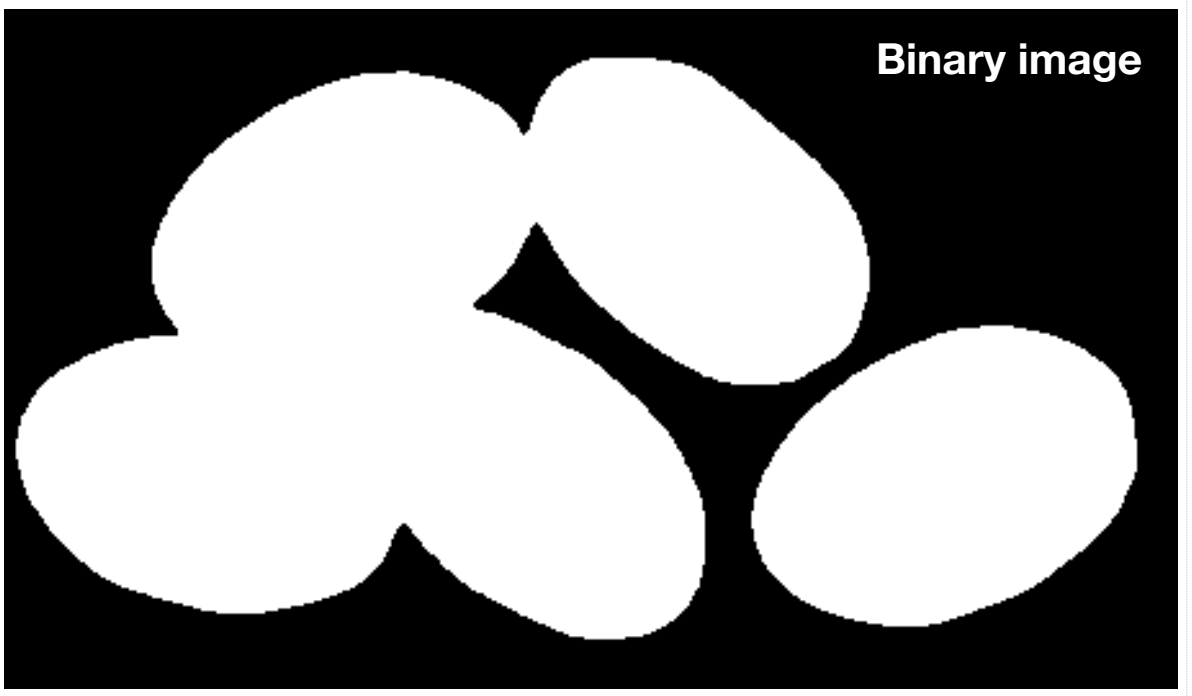
- the classic watershed
- the graylevel watershed



Question A

The **Classic Watershed** is working on a binary image. The watershed flooding is applied on the image obtained by distance map transform. This is the watershed implements by ImageJ.

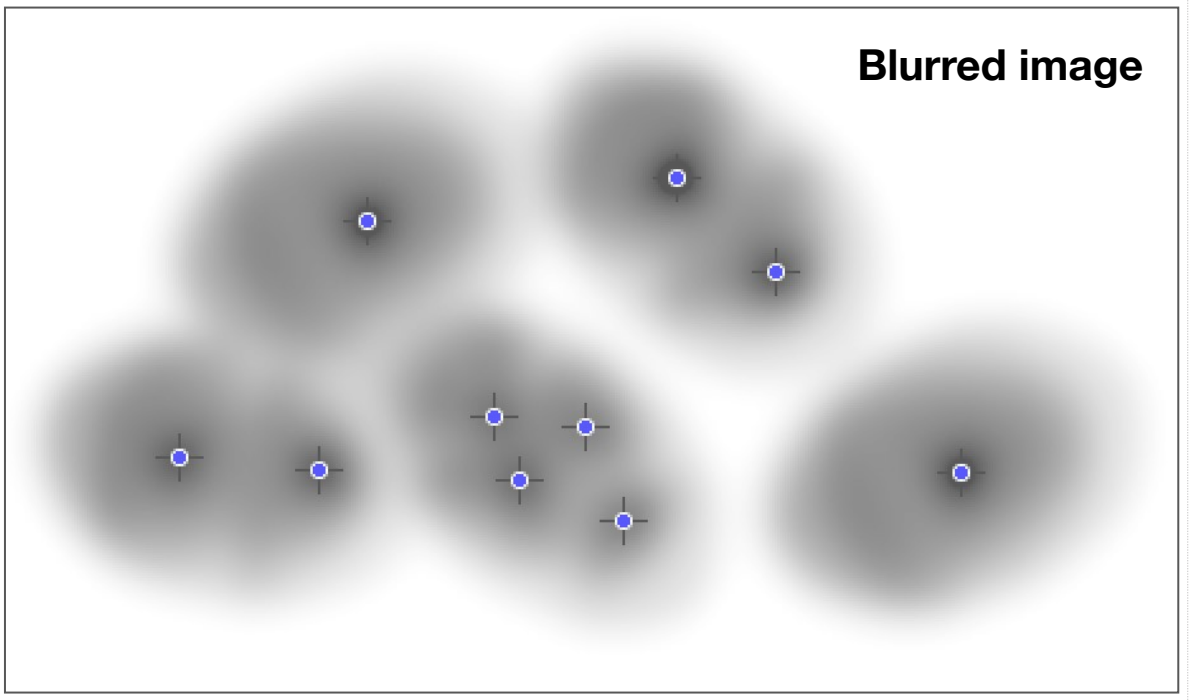
Draft the dams lines built by the classic watershed algorithm on this binary image (write only on the white area).



Question B

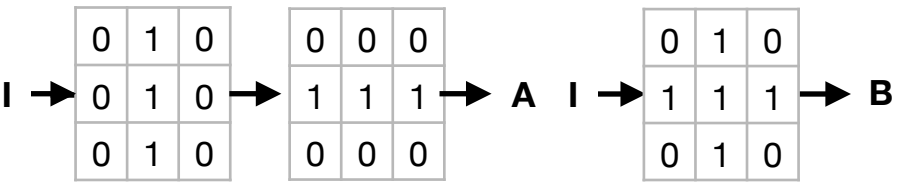
The **Graylevel Watershed** is working on a 8-bit image. The watershed flooding is performed on the intensity level starting from the local minima (seeds). To avoid the over segmentation, the images is strongly blurred.

Draft the dams lines built by the graylevel watershed algorithm on this blurred image. The blue dots denote the local minima.



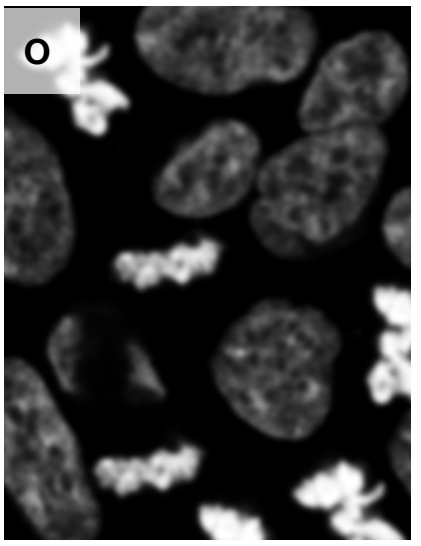
MCQ Digital Filter

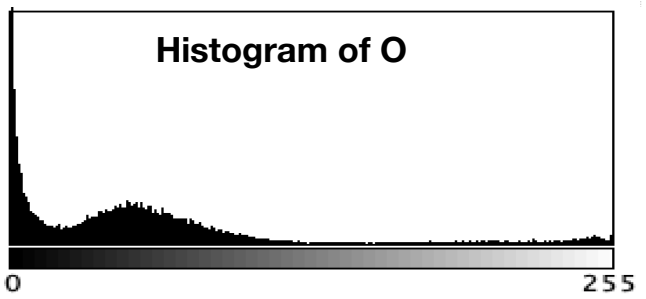
Put the appropriate check mark for each question. If you have already put a check mark but you want to change your mind, then explicitly write True or False or Void. Correct answer: **+1** - No answer: **0** - Wrong answer: **-0.5**

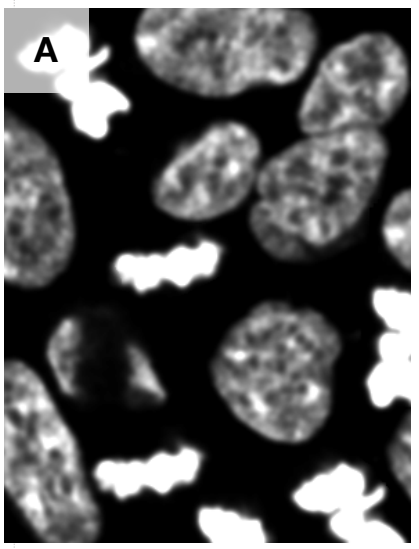
TRUE	FALSE	Questions
☐	☐	Mean filter with a 7x7 filter kernel is a edge-preserving smoothing filter in 2D images
☐	☐	Median filter with a 7x7 filter kernel is a edge-preserving smoothing filter in 2D images
☐	☐	Applying a threshold T following by a median filter (5x5) gives always the same results than applying a median filter (5x5) following a threshold T in 2D images
☐	☐	The output of the 2D Gaussian (σ_x=3, σ_y=3) filter in XY following by 1D Gaussian filter (σ_z=3) in Z is exactly the same than the output of a 3D Gaussian filter (σ_x=3, σ_y=3, σ_z=3).
☐	☐	A circular convolution in spatial domain corresponds to a pointwise multiplication in Fourier domain
☐	☐	We apply a 1D Difference of Gaussian filter only in the time direction to a sequence of images. This is a low-pass filter in the time direction.
☐	☐	The equalization of histogram is a smoothing digital filter
☐	☐	A 2D Gaussian filter is characterized by its standard deviation σ. s applying a Gaussian filter with σ=3 twice equivalent to applying a single Gaussian filter with with σ=6
☐	☐	A convolutional neural network (CNN) with 3 layers of 3x3 filters and ReLU, using stride = 1 and padding = 1, has a receptive field of 7 x 7
☐	☐	The convolutional neural network (CNN) with 2 layers of 3x3 filters and ReLU activations, with 2 channels per layer has 58 parameters
☐	☐	The image A and B are the results of 2 following filters. A and B will have the exact sam content 

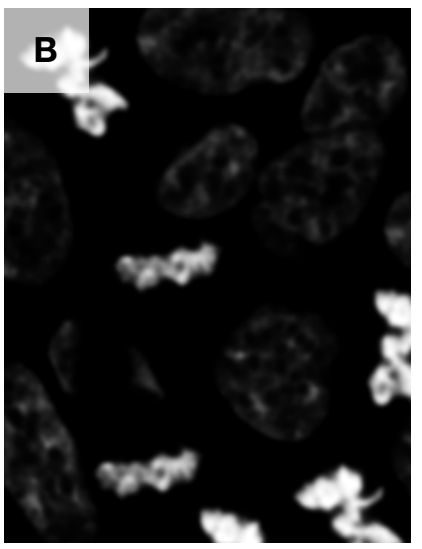
Histogram Matching

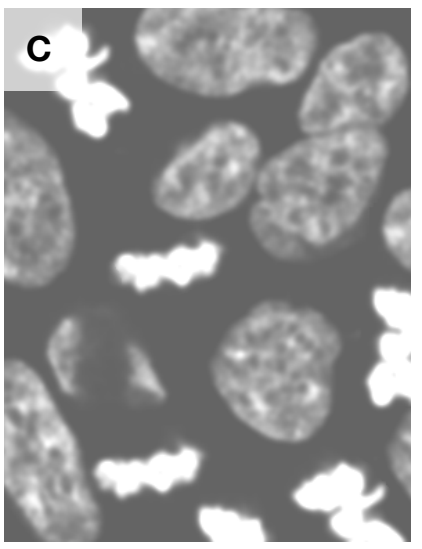
We provide an input 8-bit image O and its histogram. Then, we apply 4 classic image-processing operations that gives 4 output images A, B, C, and D.
Fill the table below, to match the 4 operations with the 4 output images [A-D] and with the 4 corresponding histograms [H1-H4].

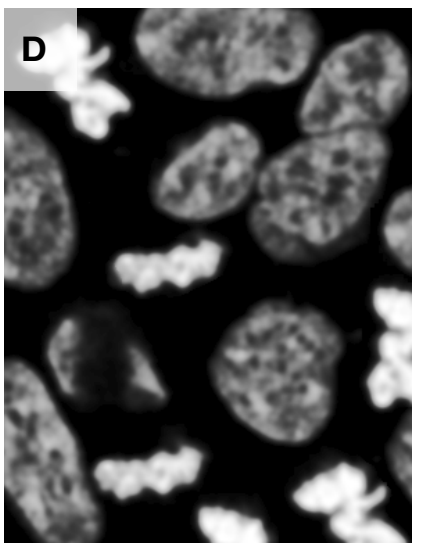


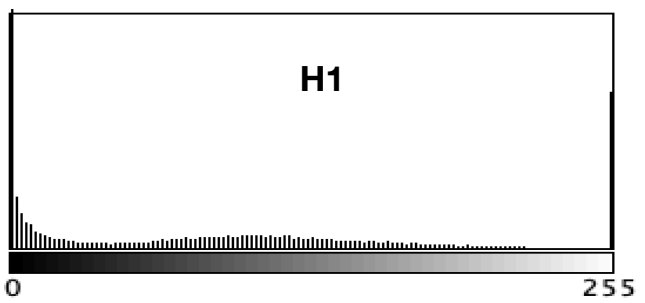


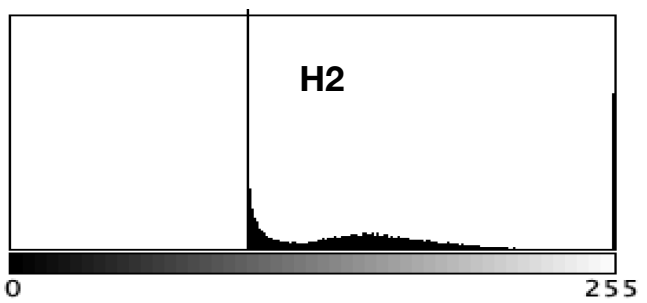


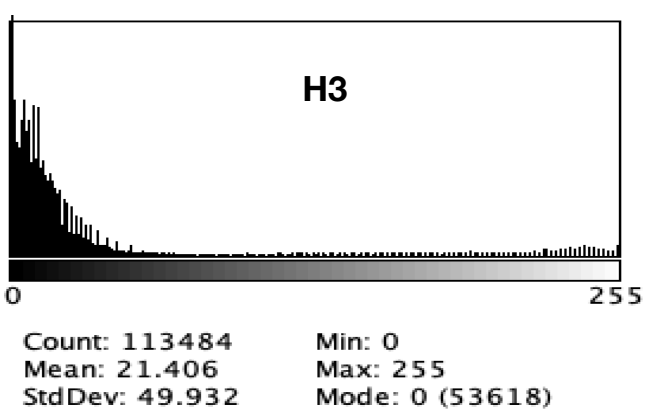


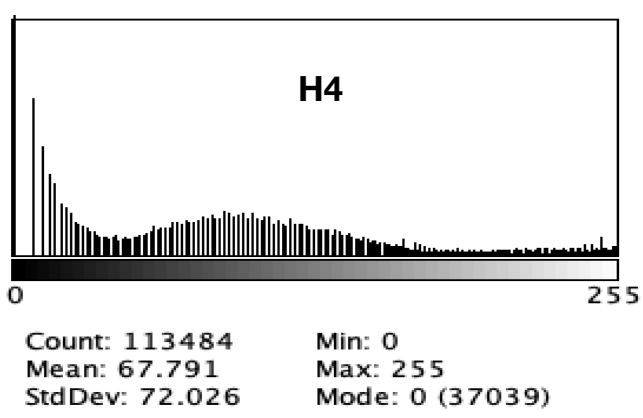












Operation	Image	Histogram
Add a value of 100		
Pixelwise multiplication by a factor of 2		
Apply a Gamma correction by 2		
Apply an histogram equalization		

MCQ Super Resolution Microscopy

Put the appropriate check mark for each question. If you have already put a check mark but you want to change your mind, then explicitly write True or False or Void. Correct answer: **+1** - No answer: **0** - Wrong answer: **-0.5**

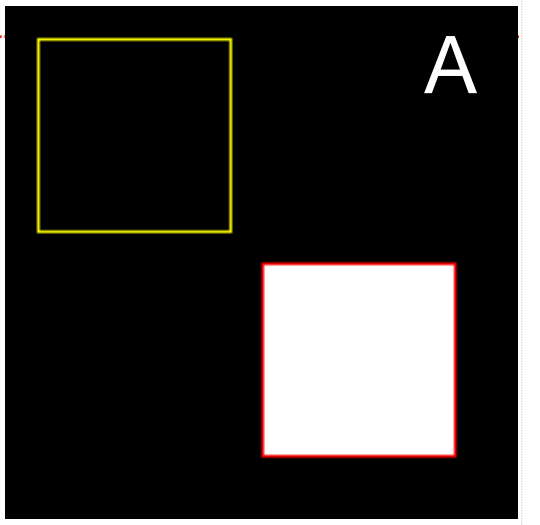
TRUE	FALSE	Super-resolution microscopy
☐	☐	Among these three well known techniques of 2D super-resolution microscopy {SIM, SMLM, STED} this is SIM which provides the best spatial resolution.
☐	☐	Stitching two consecutive images allows the super-resolution effect.
☐	☐	Among these three well known techniques of super-resolution microscopy {SIM, SMLM, STED} this is the SMLM which requires the longest time of acquisition to obtain a good super-resolved image.
☐	☐	The diffraction limit defined by Ernst Abbe is equal to: NA / (2 λ) where NA is the numerical aperture and λ is the wavelength
☐	☐	In light microscopy, the super-resolution state starts when the resolution is less than ~250 µm.

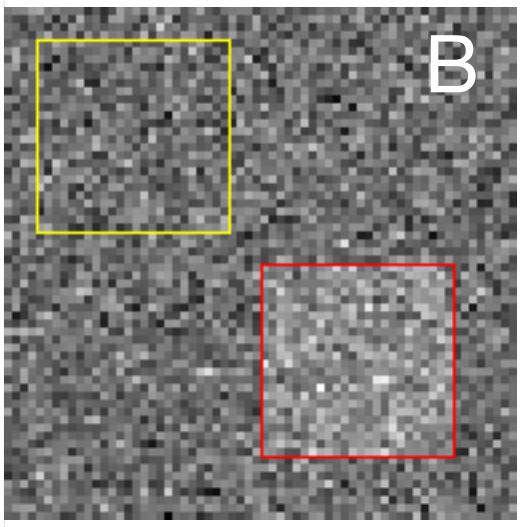
TRUE	FALSE	Single-molecule localization microscopy
☐	☐	The double-helix phase mask is one of the technique to get the Z position of emitters and finally to have a 3D localization microscopy.
☐	☐	PALM is one of the acquisition setting that allows single-molecule localization microscopy.
☐	☐	In SMLM, the super-resolution mode is due to the separation of the emission of the fluorescence in time.
☐	☐	In SMLM, the deconvolution step is required to remove the out-of-focus
☐	☐	In SMLM, the drift correction is required to compensate the derive of the microscope.
☐	☐	In SMLM, the computation of the center of mass in a small region of interest centered around a bright spot is enough to achieve super-resolution.
☐	☐	In SMLM, the simplified theoretical localization precision is given by the formula p = s / sqrt(N) where s is the size of the PSF and N the number of photon.

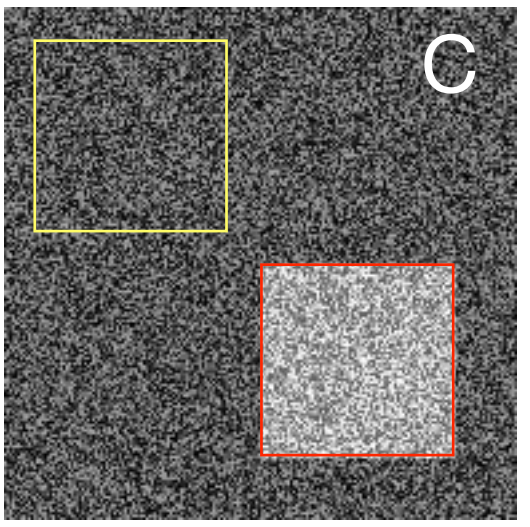
Estimation of Noise

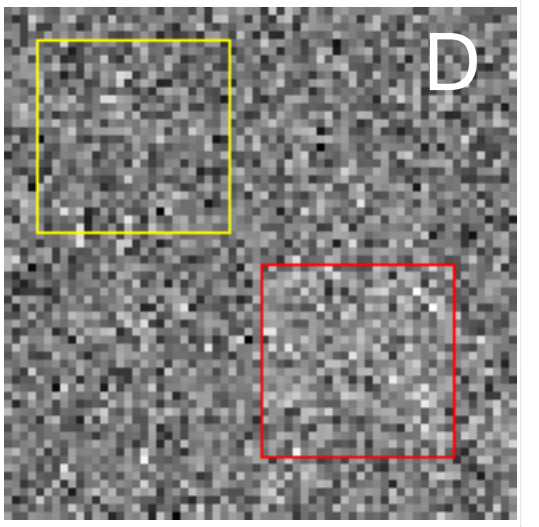
We provide the statistics of in two different areas, signal in red and background in yellow of the 6 following images. All images have a baseline of 100.

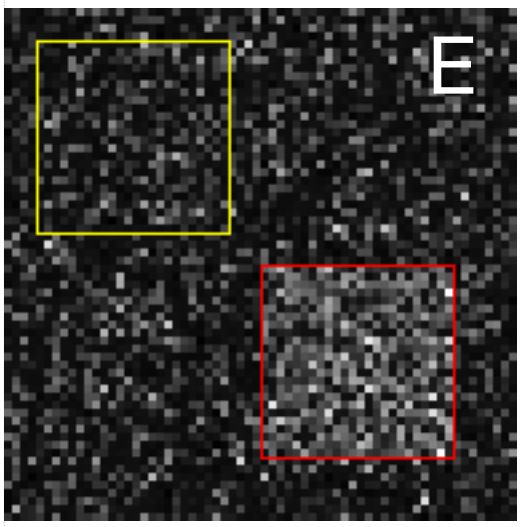
Question: fill the table below
- **SNR** in dB, give a value to the closest integer.
- **Model of Noise** = {Noiseless, Gaussian, Poisson, Speckle}
- **Type of Noise** = {Pixelwise, Structural}
- **Appropriate for image analysis** = {Yes, No}

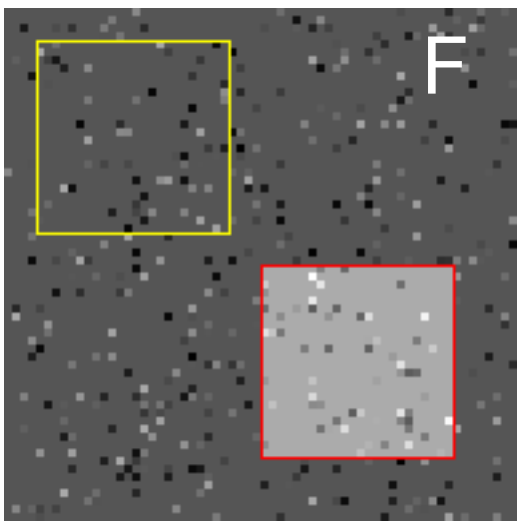


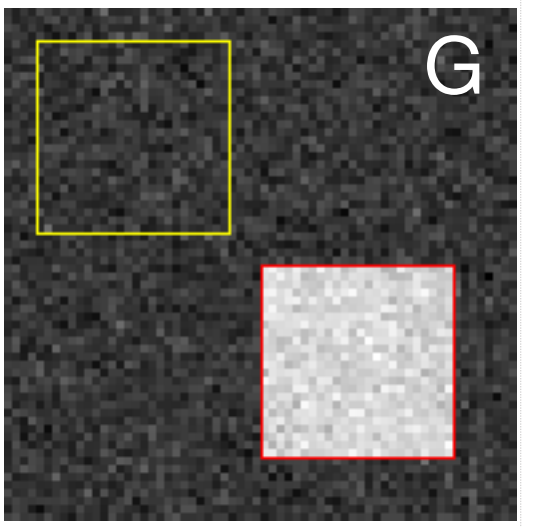












		Max	Min	Mean	Standard Deviation	SNR in dB	Model of the noise	Type of noise	Appropriate for image analysis
A	Signal	1100	1100	1100	0				
	Background	100	100	100	0				
B	Signal	505	-68	201	103				
	Background	389	-203	93	103				
C	Signal	2099	100	1094	482				
	Background	1100	-900	95	483				
D	Signal	441	-126	147	102				
	Background	431	-227	98	102				
E	Signal	147	98	112	10				
	Background	139	97	104	7				
F	Signal	120	101	110	2				
	Background	109	90	100	2				
G	Signal	1441	858	1102	100				
	Background	372	-195	99	101				

Reminder: $\log_{10}(0.5) = -0.3$; $\log_{10}(5) = 7$; $\log_{10}(2) = 0.3$

Instance Segmentation of Nuclei

Compare three methods of instance segmentation to segment fluorescently labelled nuclei/cell. Here, we use the most common/standard implementation of these methods

- the classic **Watershed** algorithm which is working on a binary ImageJ (Watershed of ImageJ)
- the active contour (**Snake**) on a parametric curve
- the **Stardist** model

Question For each of these blocks, draw 3 arrows to connect the method with the right statement

Watershed	It is required to have an initialization of the shape close to object to detect
Snake	It is required to have a "clean" binary image without holes and spurious points
Stardist	It is required to have a training phase with many annotated examples

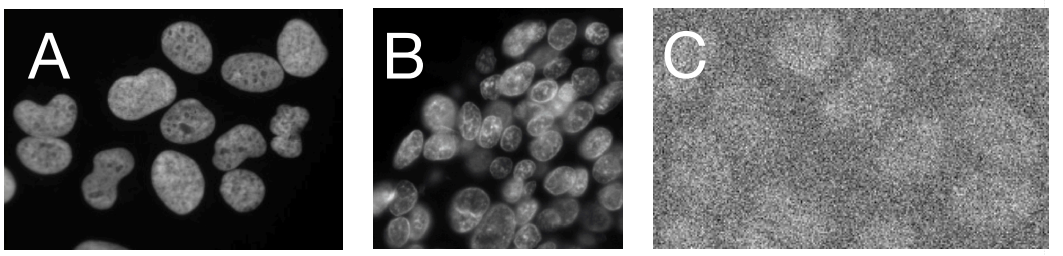
Watershed	It allows detection of objects in presence of high quantity of noise
Snake	It allows detection of objects even with some overlap
Stardist	It allows detection of objects in binary image

Watershed	It is able to perform an instance segmentation of the shape that corresponds to the predefined model
Snake	It is able to perform an instance segmentation of only the roundish shape
Stardist	It is able to perform an instance segmentation of any kind of shape

Watershed	In image processing, it is known as a deep-learning method
Snake	In image processing, it is known as a variational method
Stardist	In image processing, it is known as a morphological method

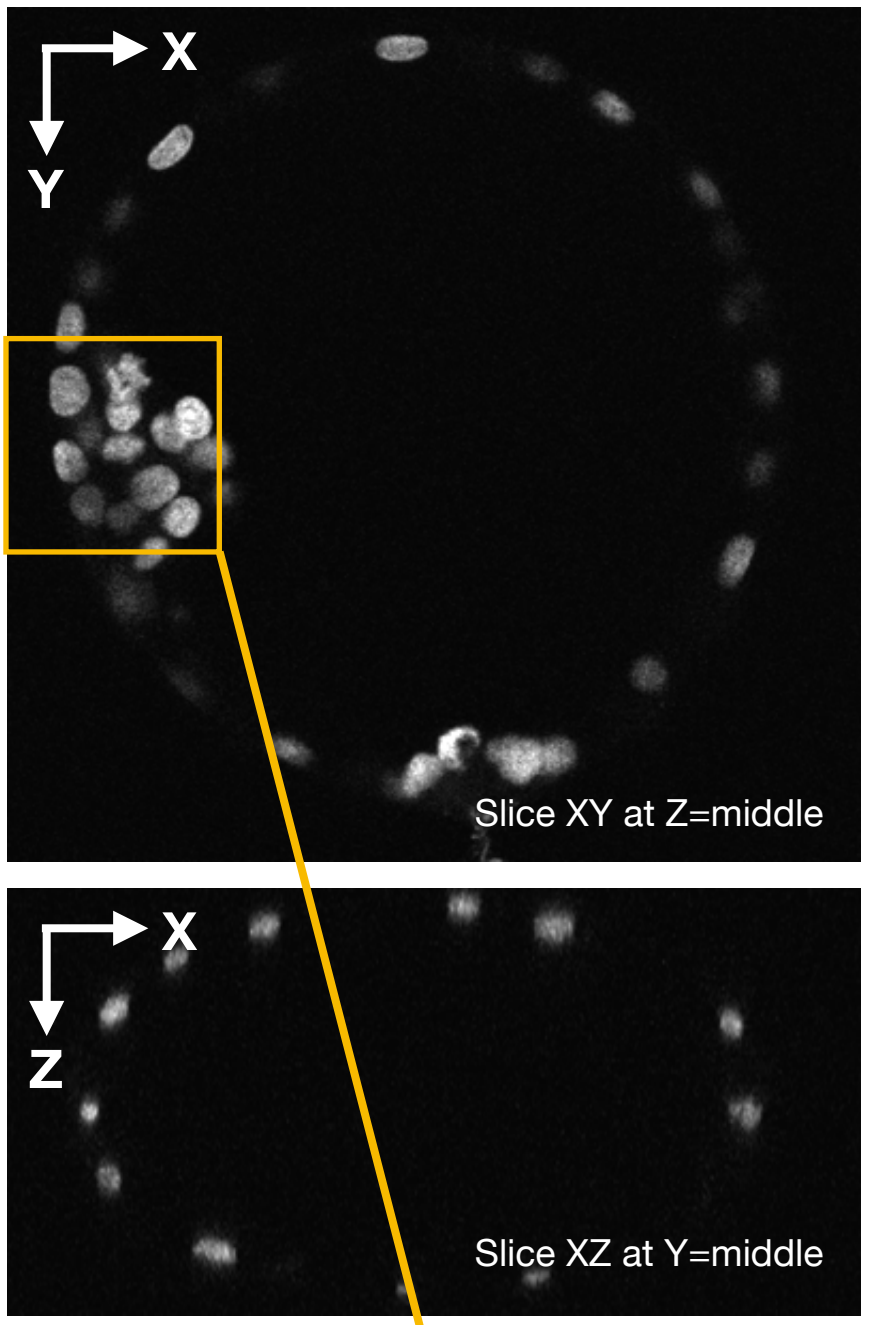
Which methods will you recommend to a biologist to segment the nuclei in these images?

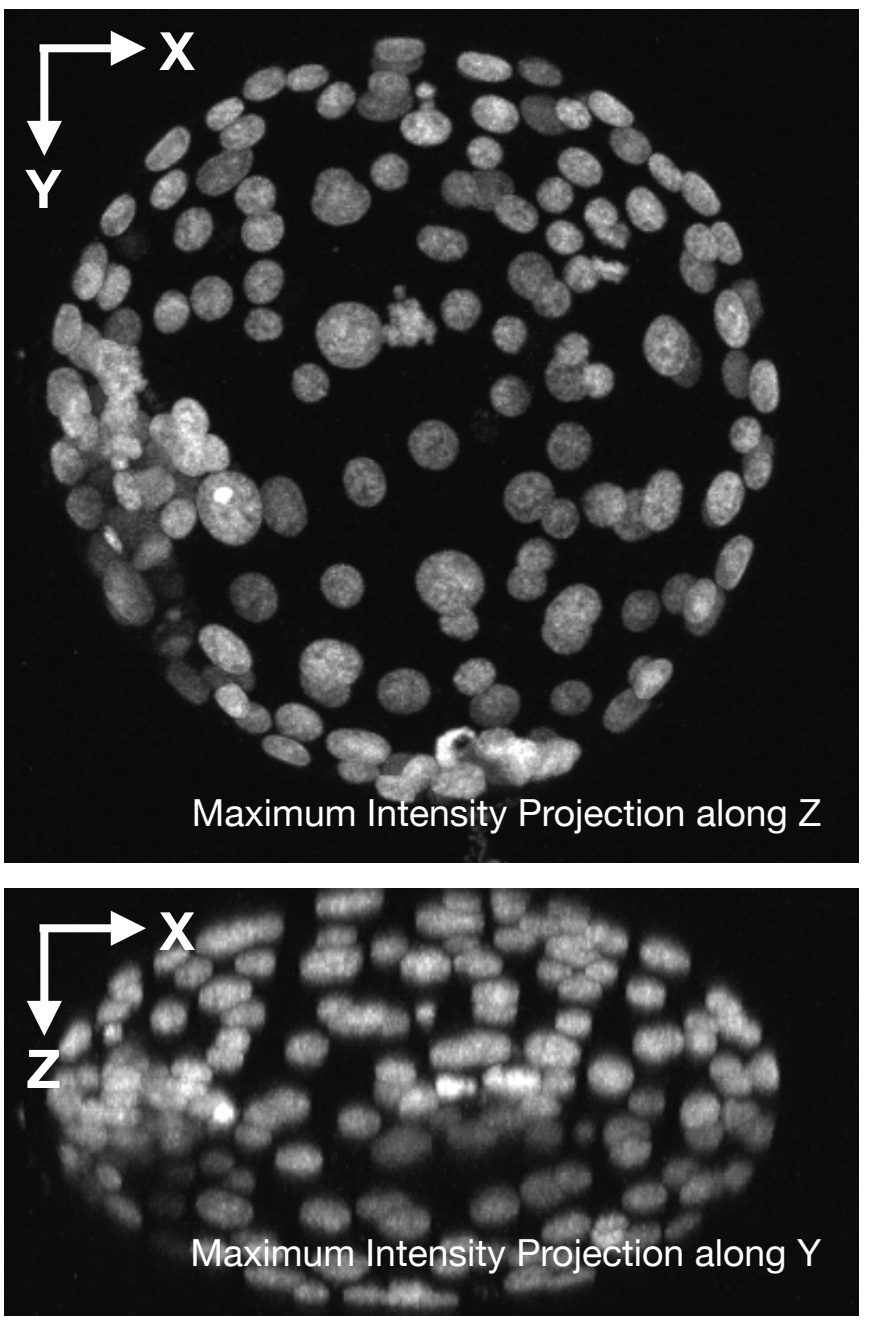
Watershed	A
Snake	B
Stardist	C

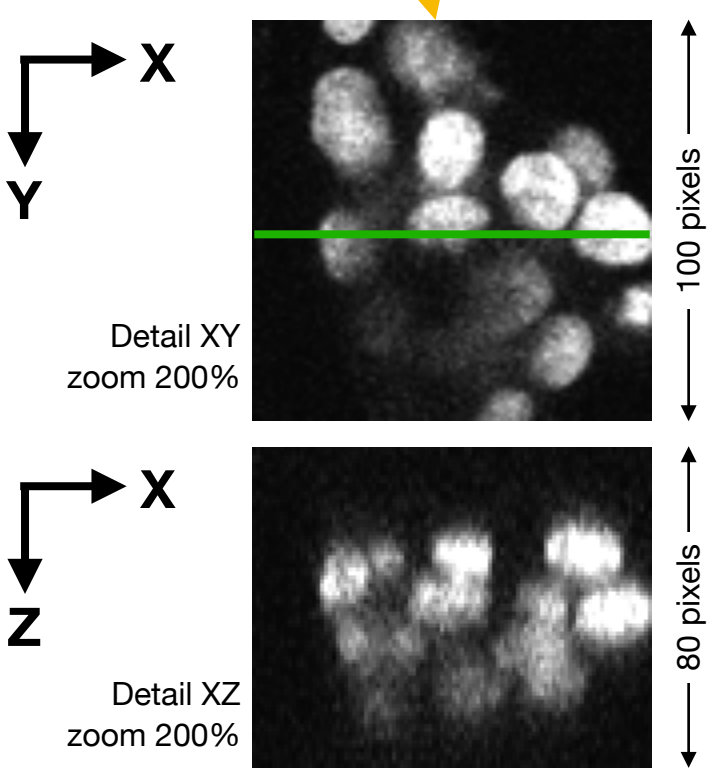


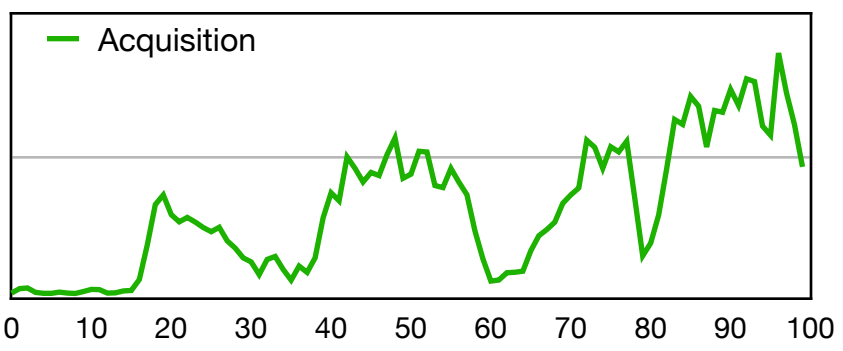
Reminder: $\log_{10}(0.5) = -0.3$; $\log_{10}(5) = 7$; $\log_{10}(2) = 0.3$

Image Quality for Analysis Introduction







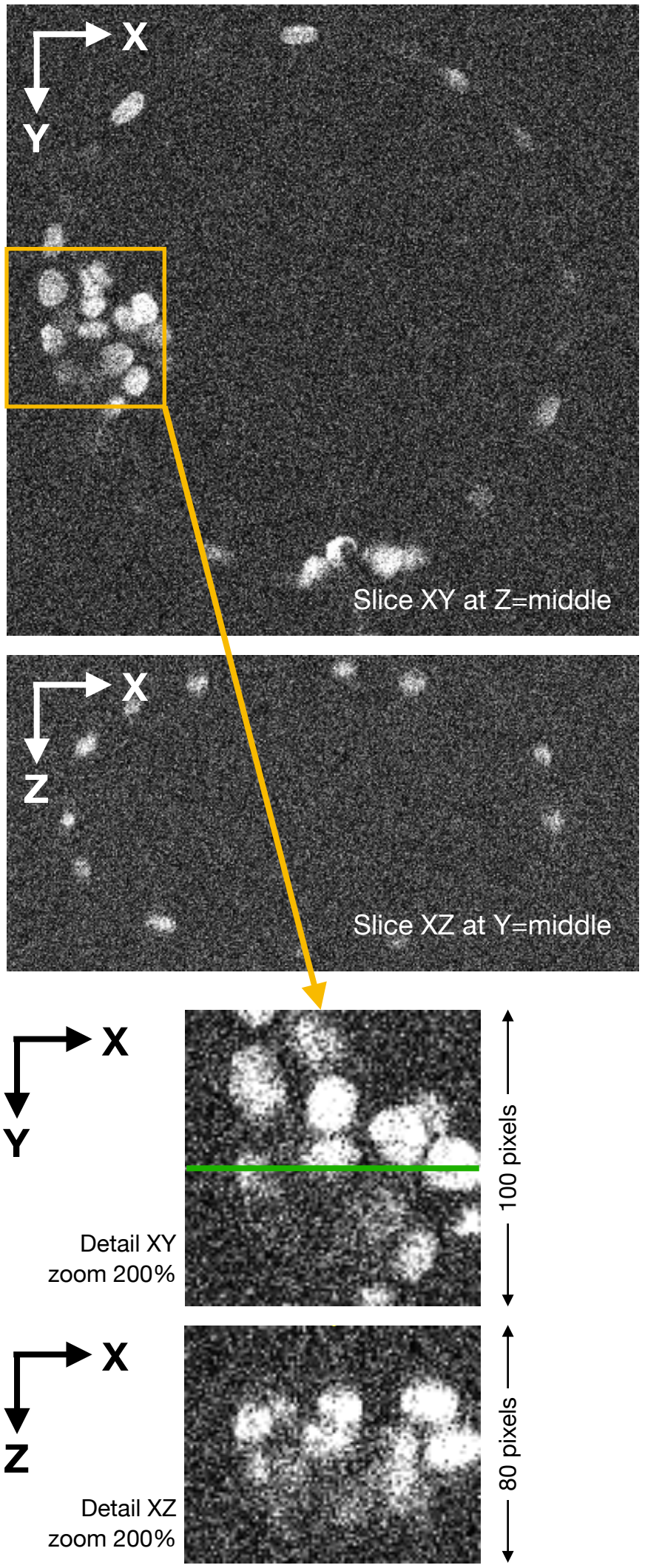


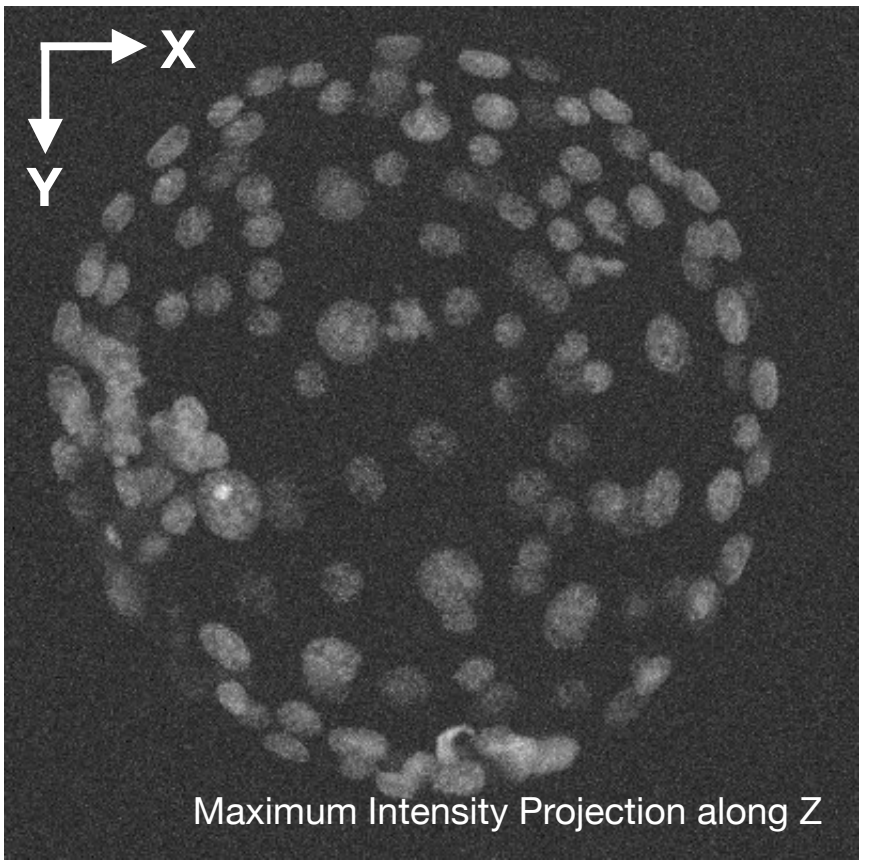
We found an image-analysis workflow to segment almost every nucleus in this 3D image. It is based on the difference of intensity (Ostu and Watershed 3D), no pre-processing steps were necessary.
Using the segmented masks, it is it also possible to quantify the fluorescence expression by computing the mean intensity for each single nuclei.

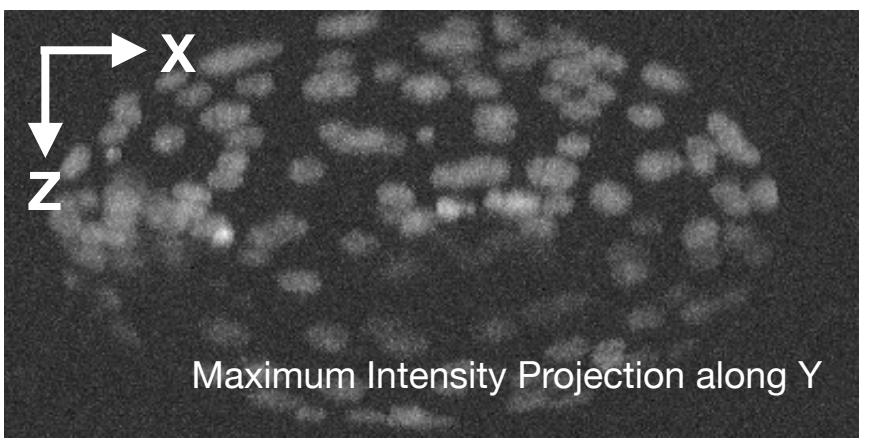
In the 4 next pages, we provide the same 3D image acquired in various degraded acquisitions. In the 4 next Questions you are to indicate if the segmentation and if the quantification are possible.

There is no answer to give at this page, it is only the presentation of the dataset.

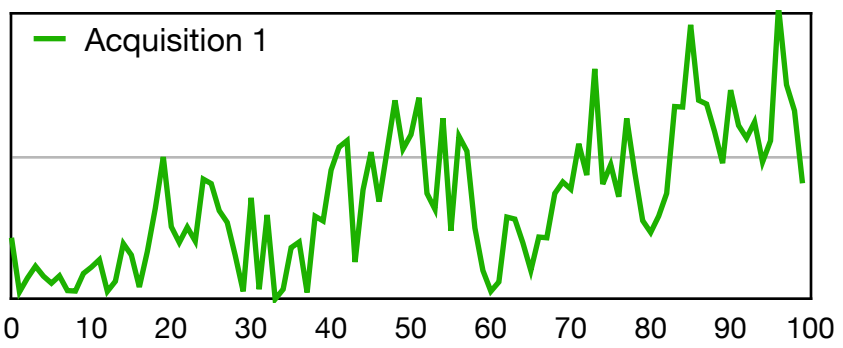
Image Quality for Analysis 1







Intensity profile along the green line in the detail XY



Question

Is it possible to use this 3D image to perform a reasonable intensity-based segmentation?

- ☐ **NO**
- ☐ **YES WITHOUT PRE-PROCESSING**
- ☐ **YES WITH A PRE-PROCESSING**

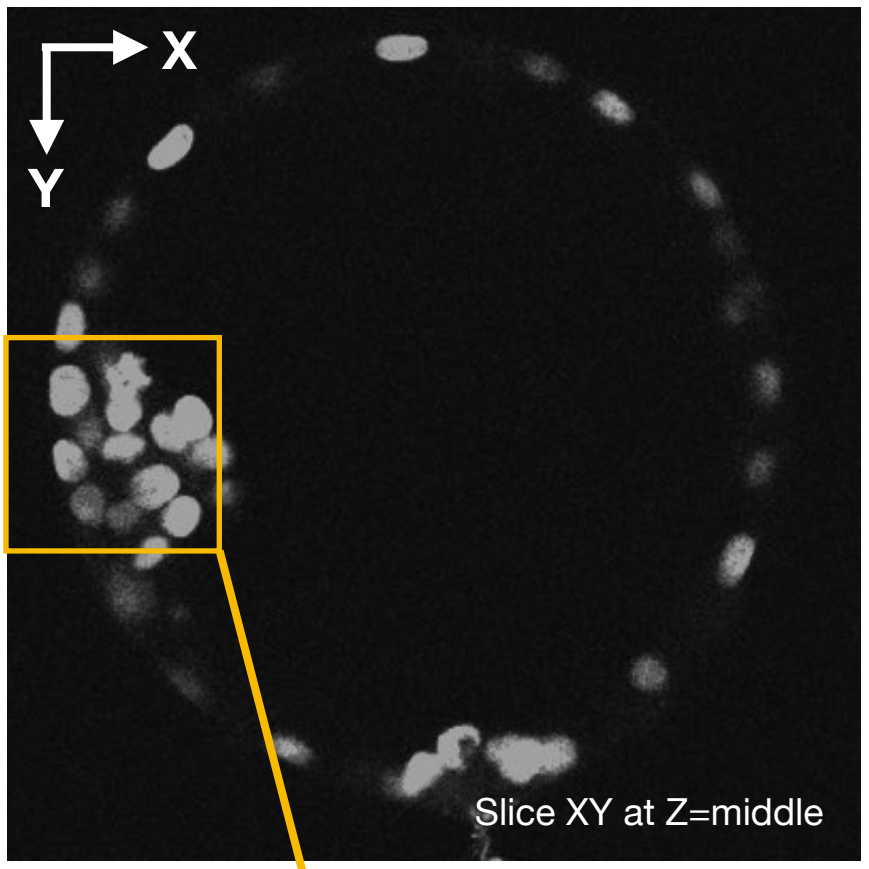
If a pre-processing is required, name it and justify your choice.

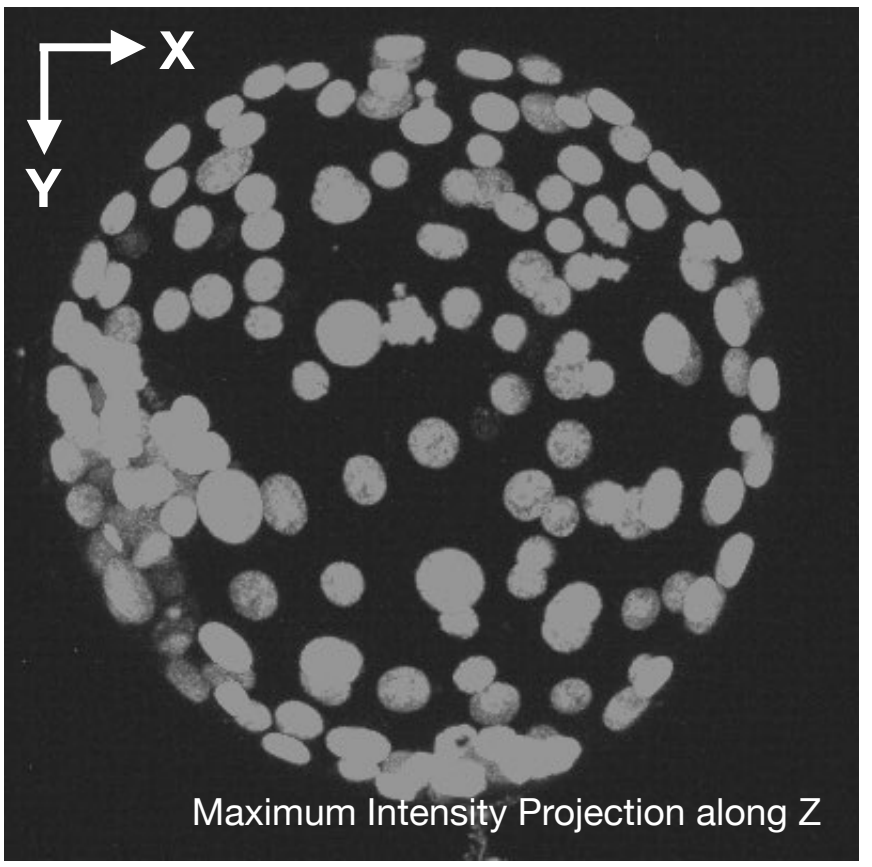
please, only few words are necessary

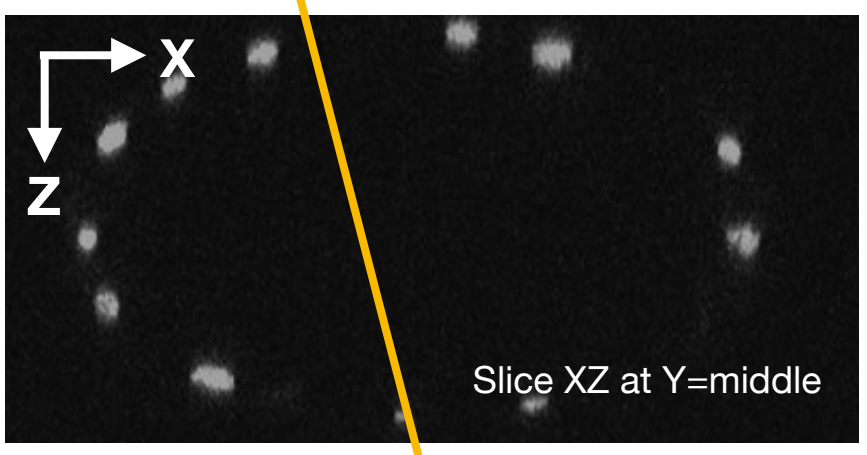
f the intensity-based segmentation gives reasonable masks around the nuclei, is it possible to use these masks to quantify the fluorescence expression?

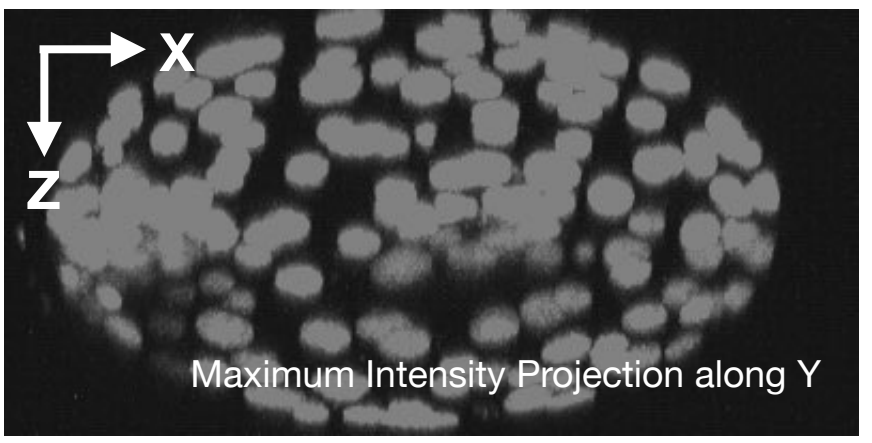
☐ **NO** ☐ **YES**

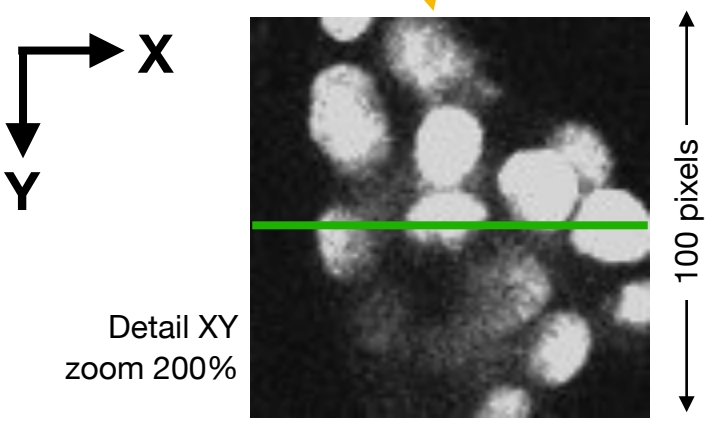
Image Quality for Analysis 2

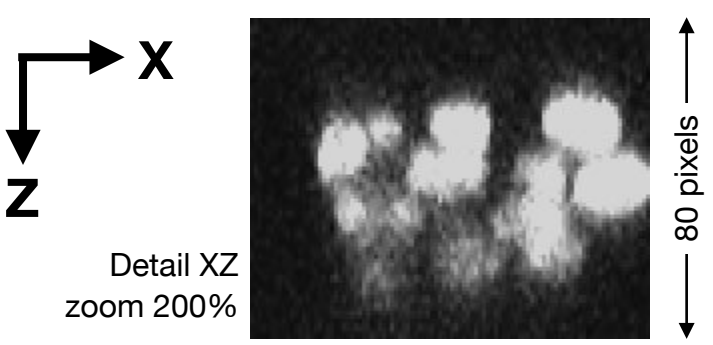




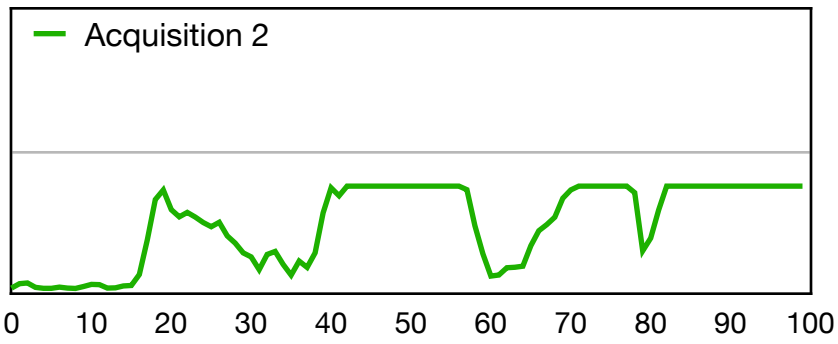








Intensity profile along the green line in the detail XY



Question

Is it possible to use this 3D image to perform a reasonable intensity-based segmentation?

- ☐ **NO**
- ☐ **YES WITHOUT PRE-PROCESSING**
- ☐ **YES WITH A PRE-PROCESSING**

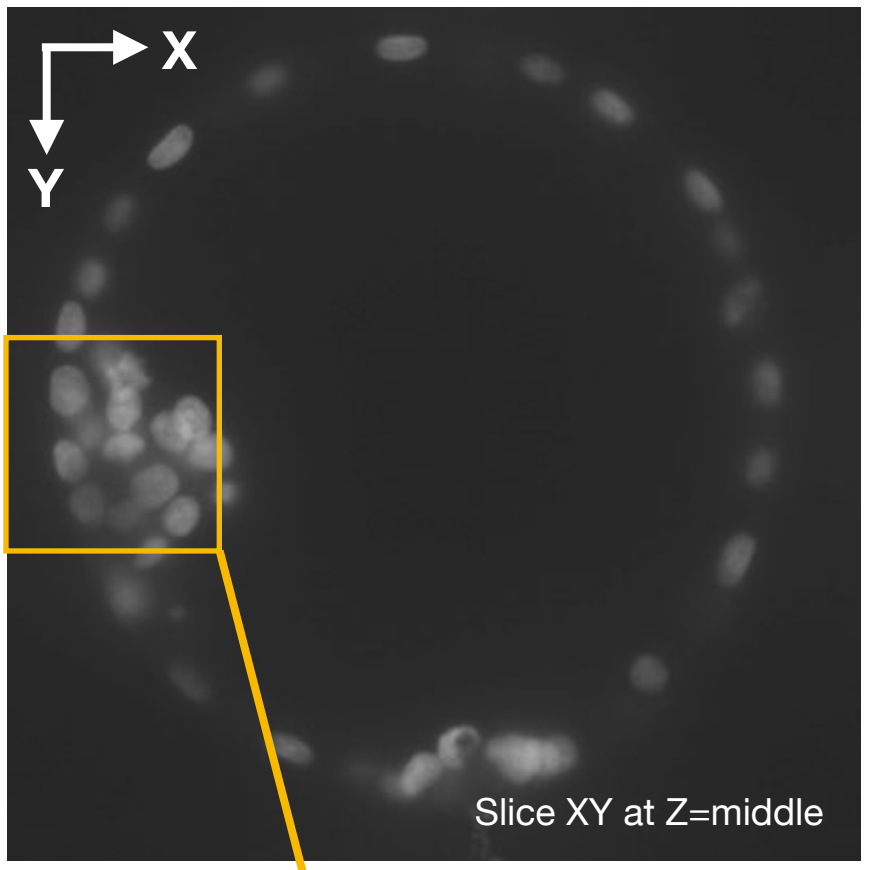
If a pre-processing is required, name it and justify your choice.

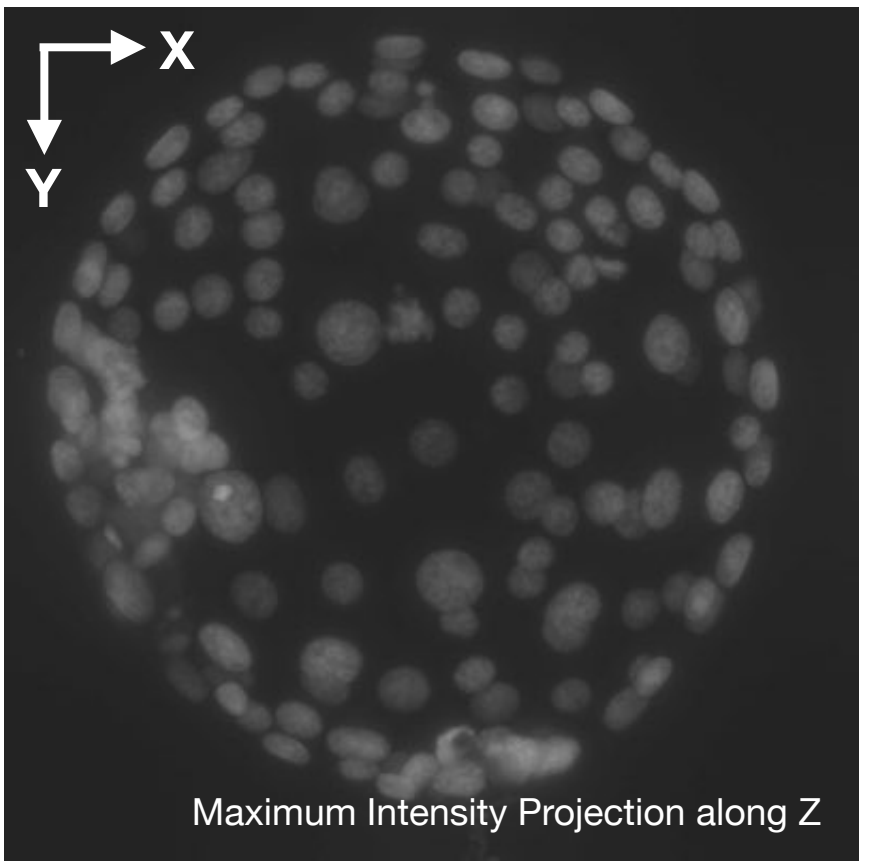
please, only few words are necessary

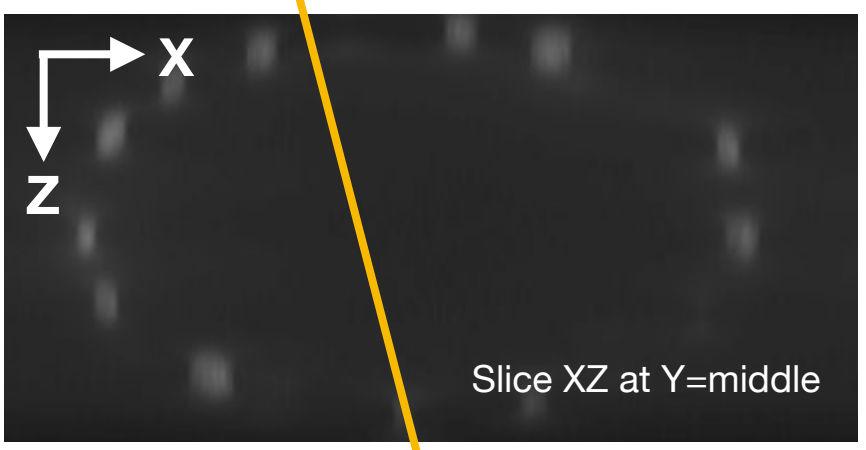
If the intensity-based segmentation gives reasonable masks around the nuclei, is it possible to use these masks to quantify the fluorescence expression?

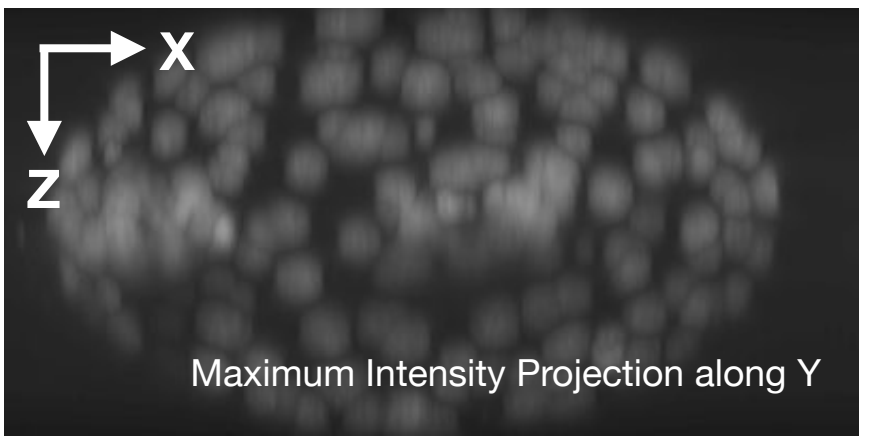
☐ **NO** ☐ **YES**

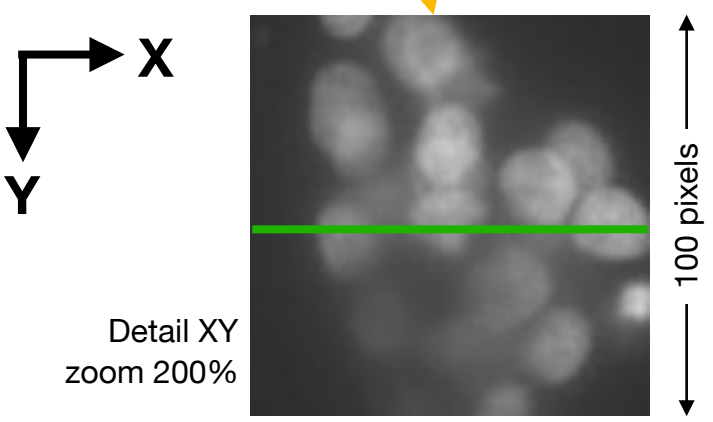
Image Quality for Analysis 3

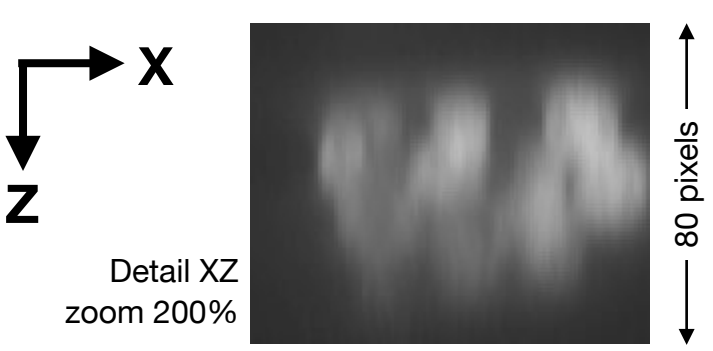




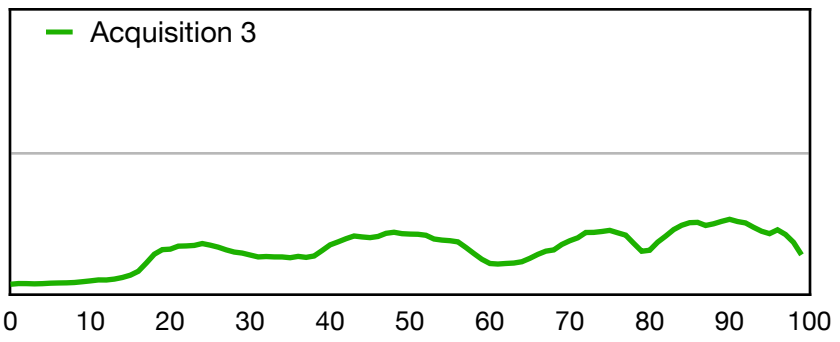








Intensity profile along the green line in the detail XY



Question

Is it possible to use this 3D image to perform a reasonable intensity-based segmentation?

- ☐ **NO**
- ☐ **YES WITHOUT PRE-PROCESSING**
- ☐ **YES WITH A PRE-PROCESSING**

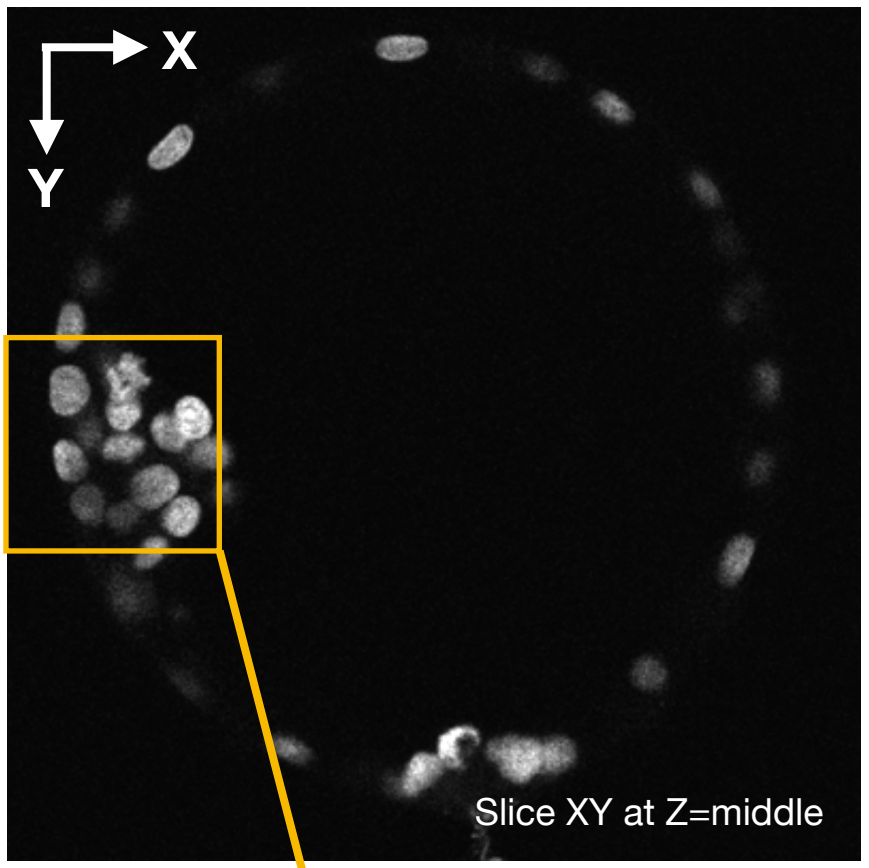
If a pre-processing is required, name it and justify your choice.

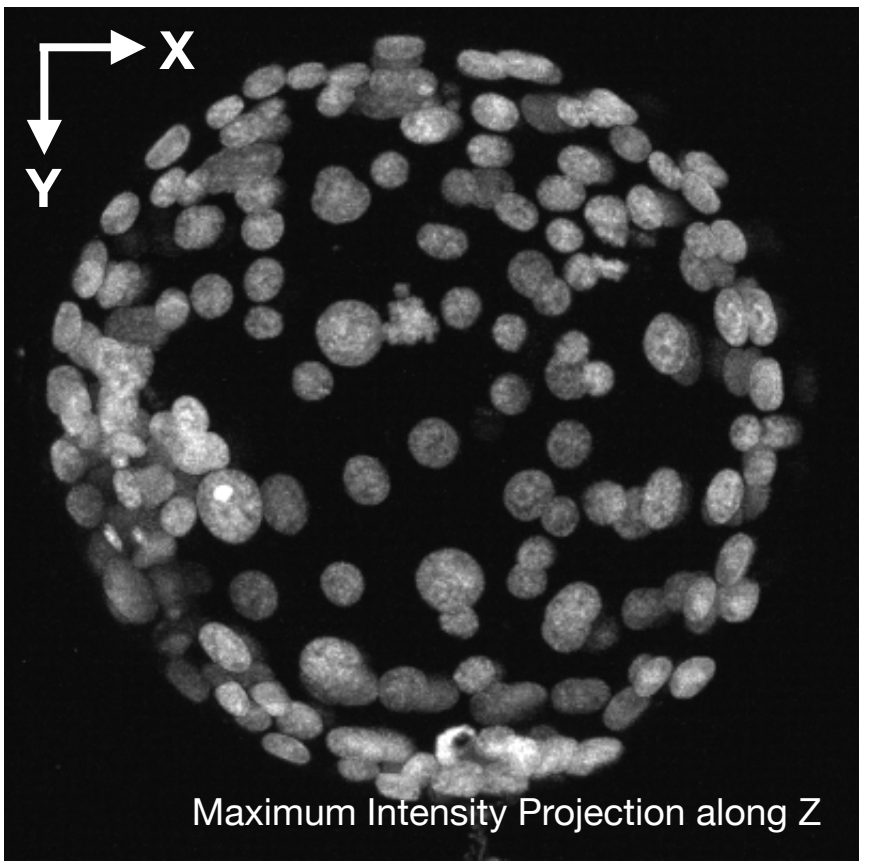
please, only few words are necessary

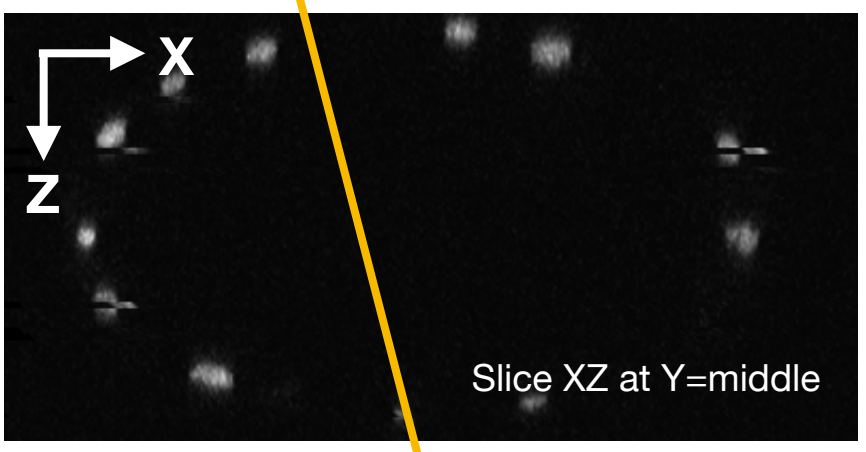
If the intensity-based segmentation gives reasonable masks around the nuclei, is it possible to use these masks to quantify the fluorescence expression?

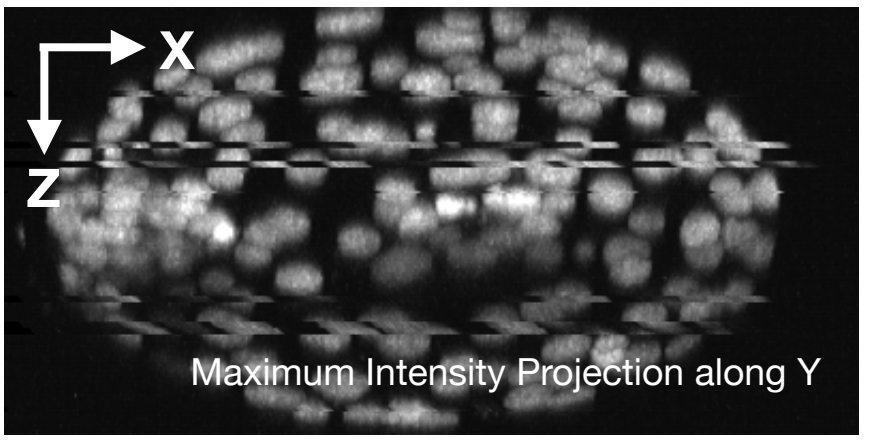
☐ **NO** ☐ **YES**

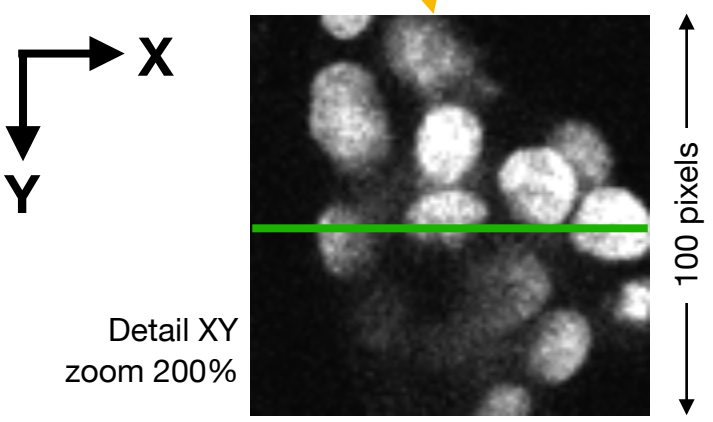
Image Quality for Analysis 4

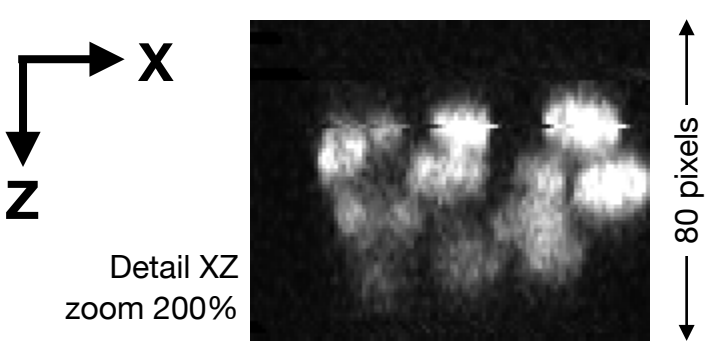




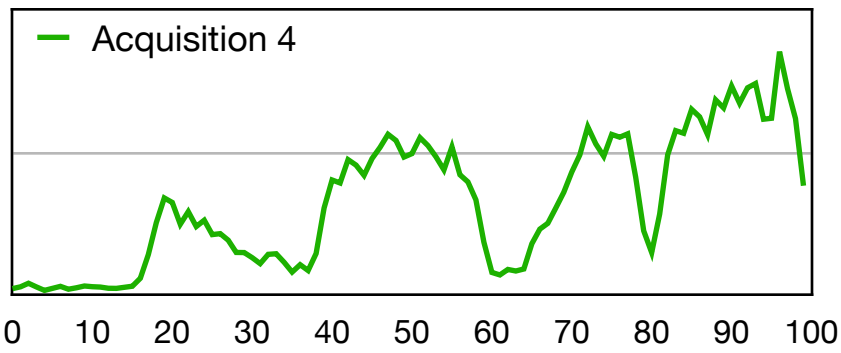








Intensity profile along the green line in the detail XY



Question

Is it possible to use this 3D image to perform a reasonable intensity-based segmentation?

- ☐ **NO**
- ☐ **YES WITHOUT PRE-PROCESSING**
- ☐ **YES WITH A PRE-PROCESSING**

If a pre-processing is required, name it and justify your choice.

please, only few words are necessary

If the intensity-based segmentation gives reasonable masks around the nuclei, is it possible to use these masks to quantify the fluorescence expression?

☐ **NO** ☐ **YES**