

# Hyperspectral microscopy

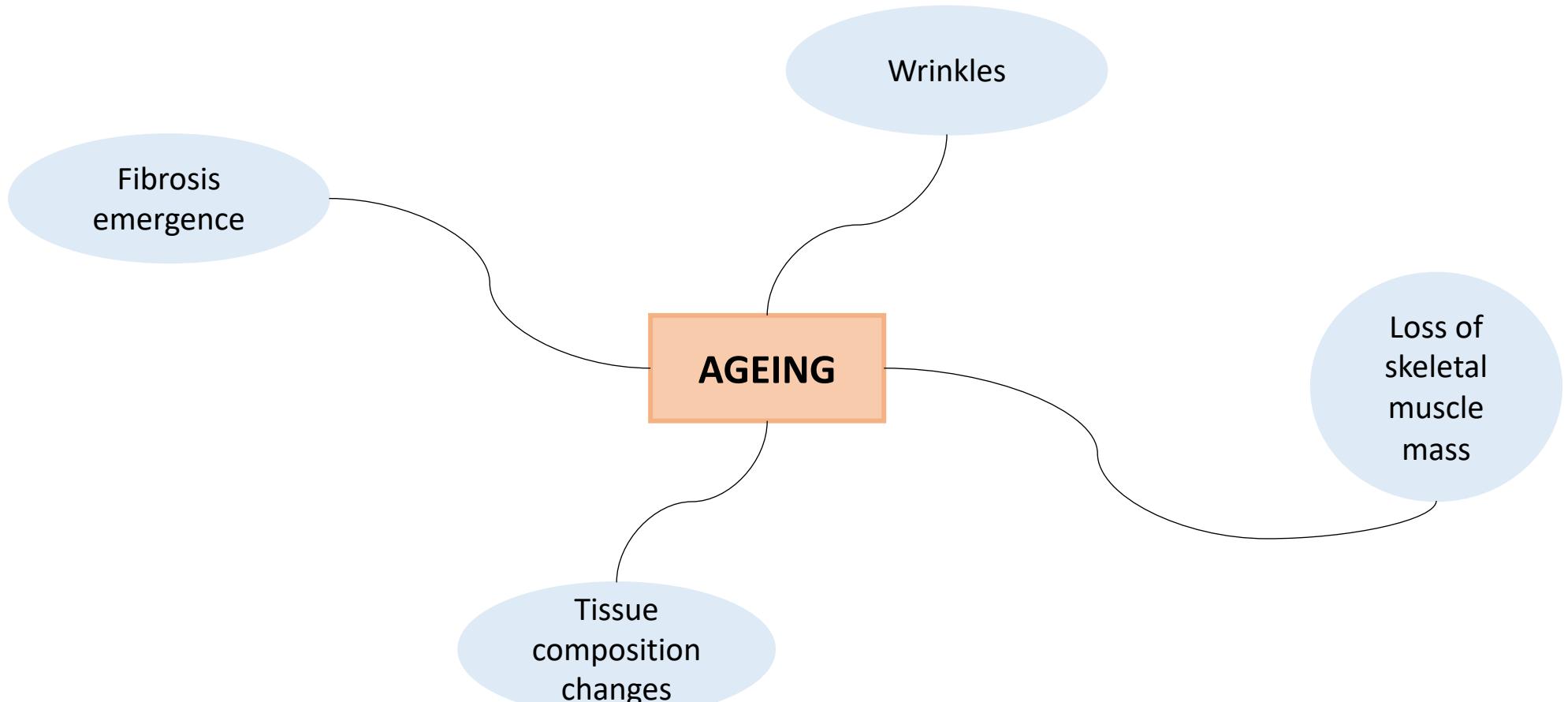
*Under the supervision of: Mr. Pierre Weiss*

*With the help of: Ms. Corinne Barreau, Ms. Coralie Sengenes*

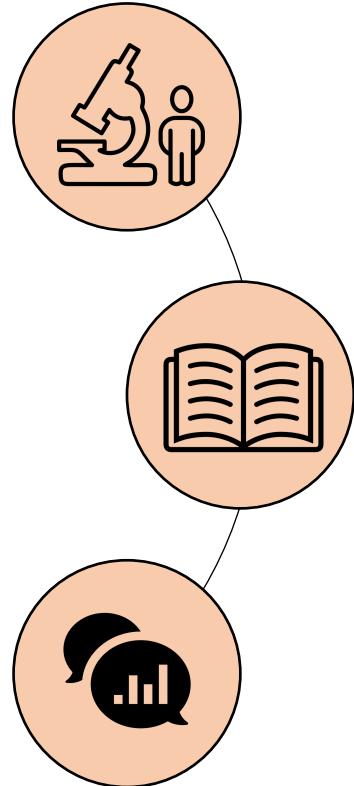
**Quentin Douzery**  
**Alexia Ghozland**

January, 21<sup>st</sup> 2022

# Introduction



# Summary



**Scientific context**

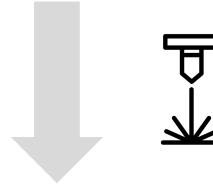
**Materials and methods**

**Results and discussion**

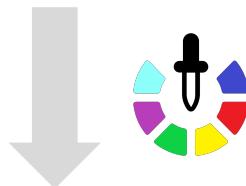
# Scientific context

## What is spectroscopy

How does the light interact with the target?



Spectrum: how much light is emitted from the target?



Spectral signature



Equivalent to a human fingerprint

**Spectroscopy**

Analysis

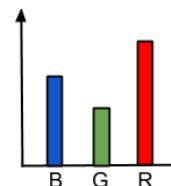
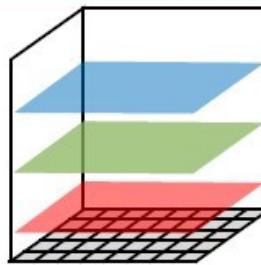
Detection

Identification

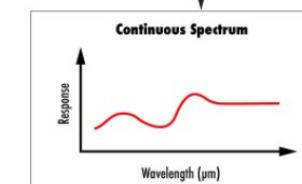
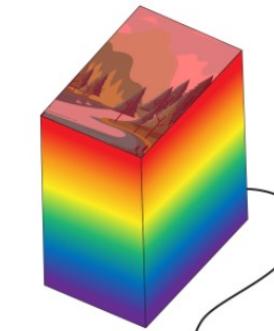
# Scientific context

What is hyperspectral imaging (HSI)

RGB



HSI



For each voxel

Non contact

Non destructive

More informative

Voxel 3D  $\equiv$  Pixel 2D

Does the hyperspectral signature of a muscle of mouse indicate its ageing?

# Scientific context

## Hyperspectral microscopy in our case



6 mice:  
3 young, 3 old



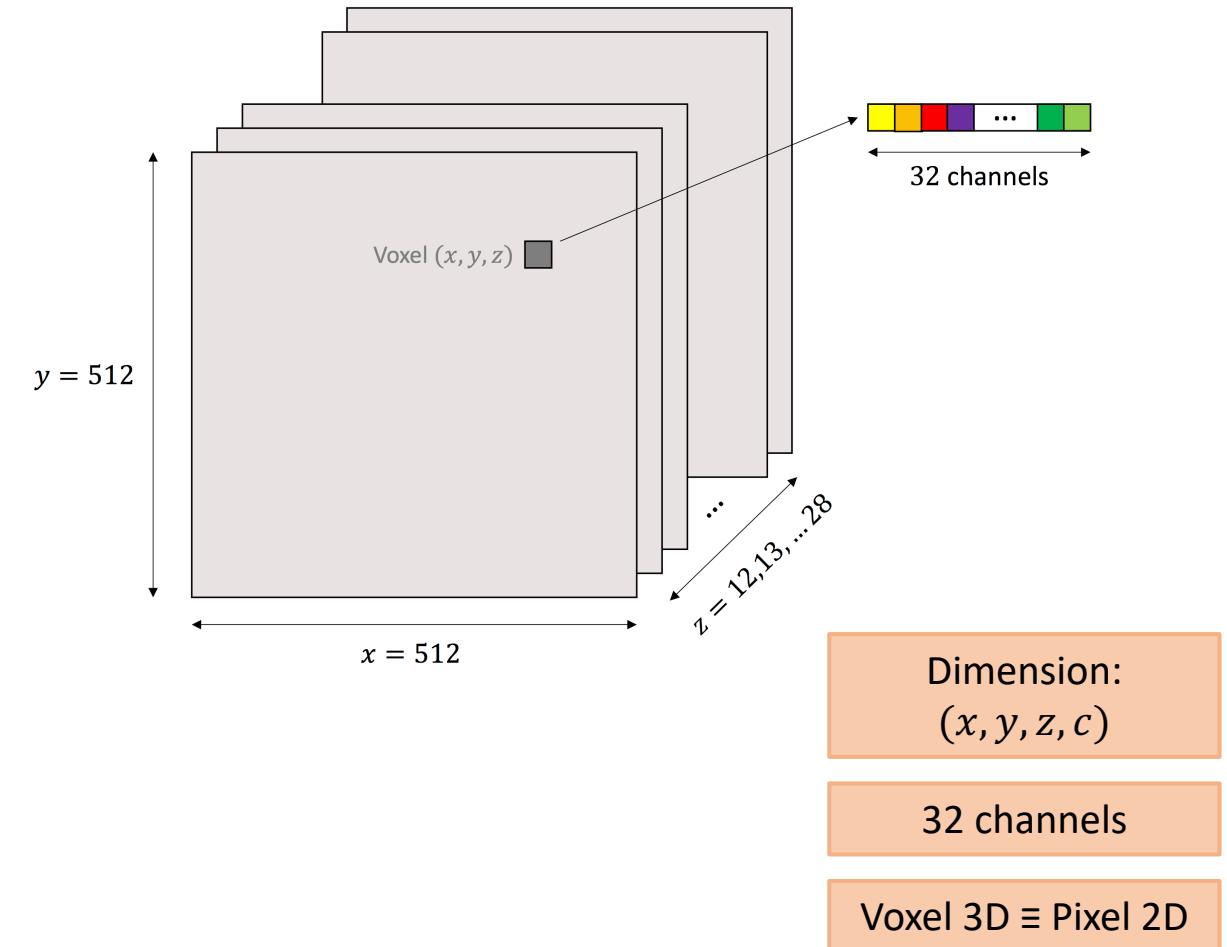
Excitation wavelengths:  
**740nm, 780nm, 820nm**



Light emission:  
fluorescent tissues

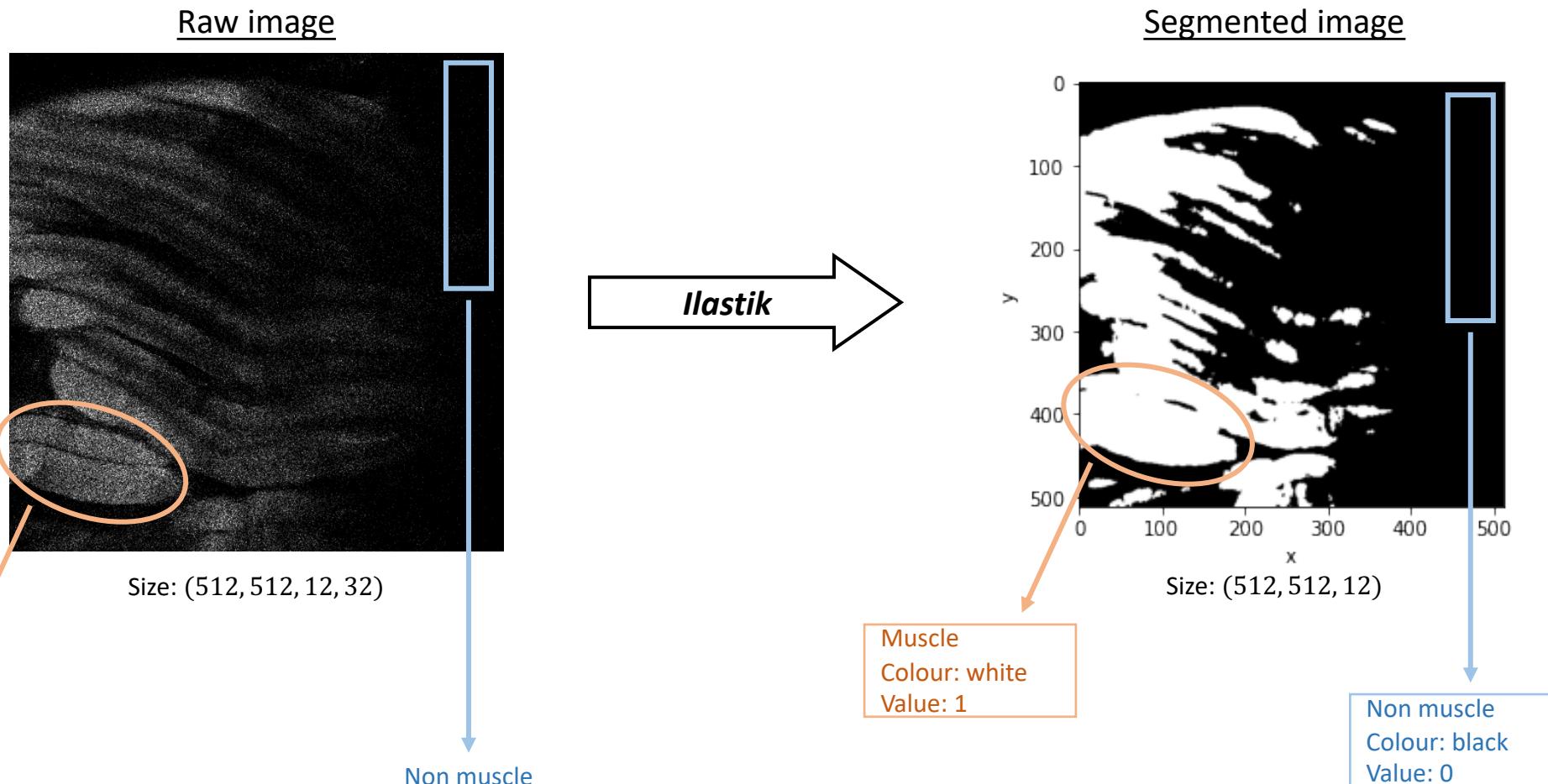


Light collection:  
**410nm – 690nm every 8.9nm**



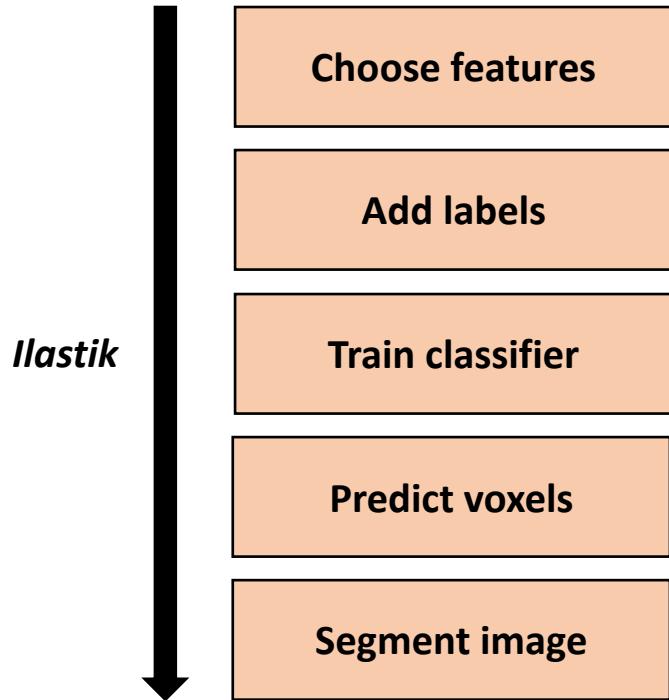
# Materials and methods

## Segmentation of the images



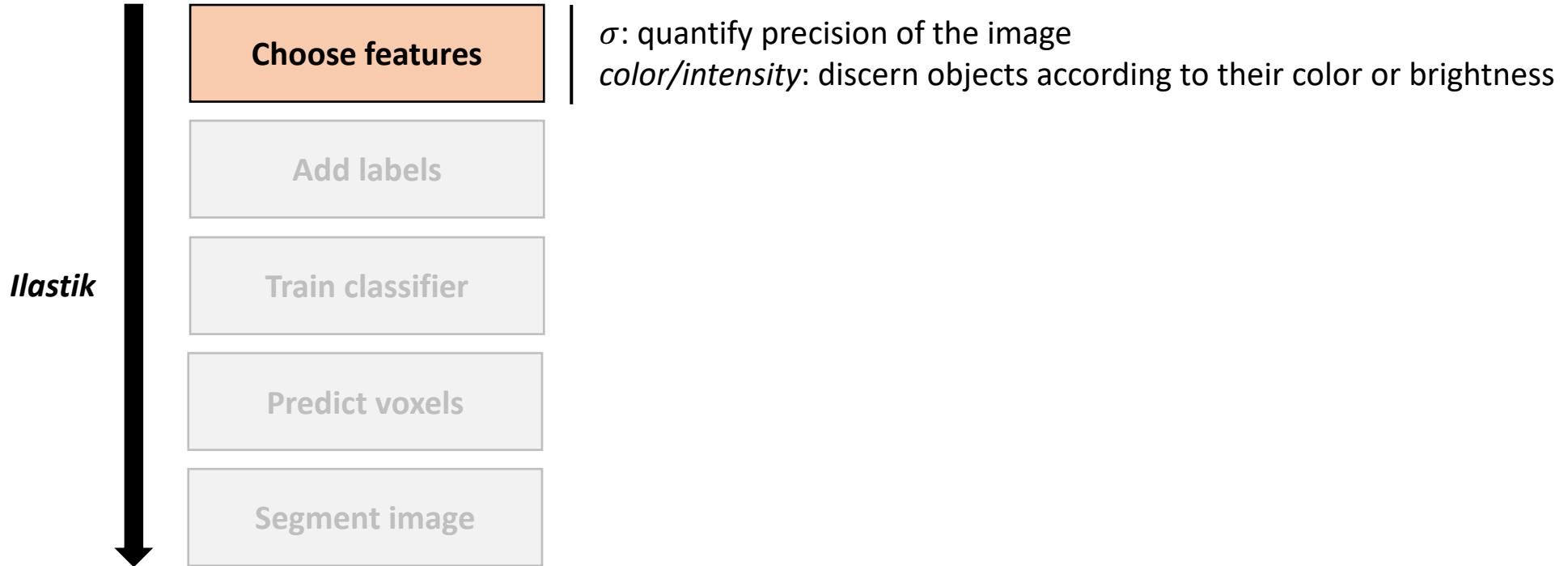
# Materials and methods

## Segmentation of the images



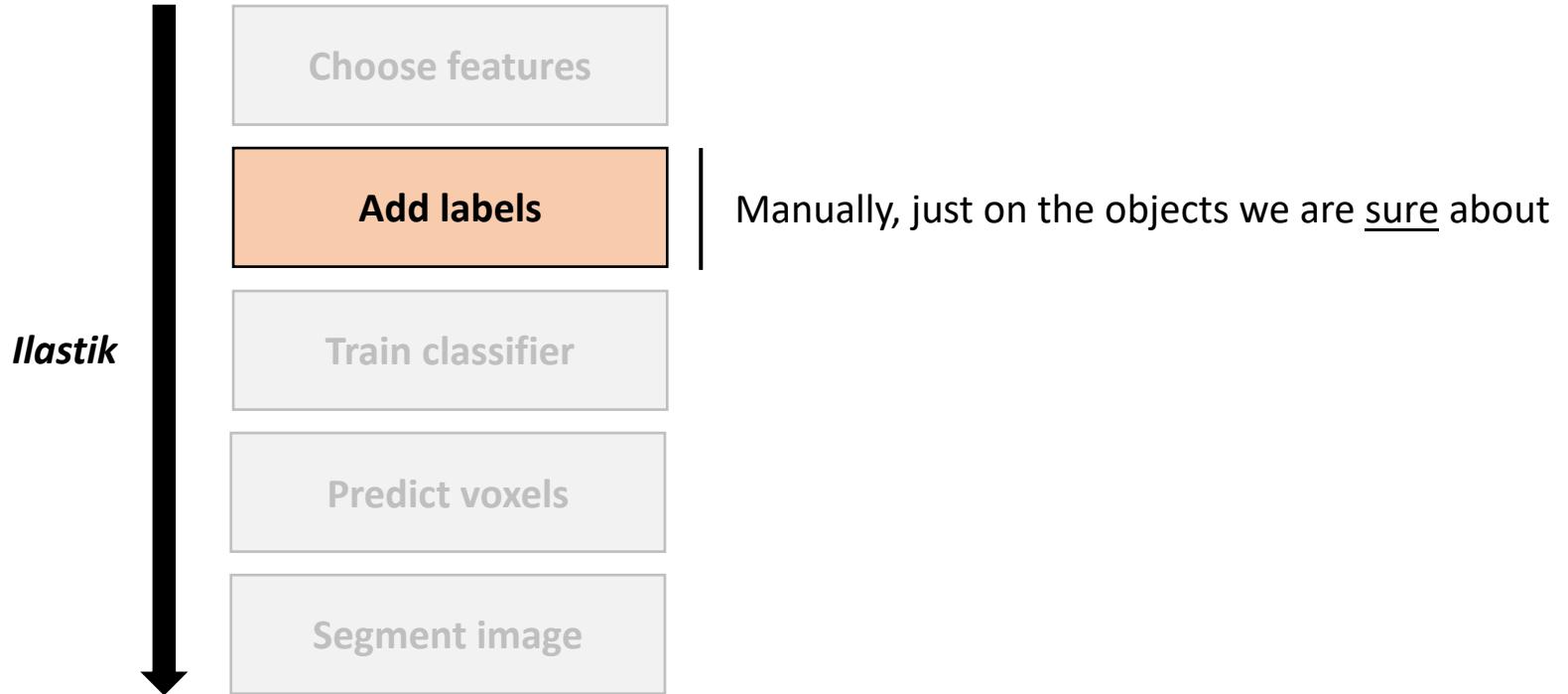
# Materials and methods

## Segmentation of the images



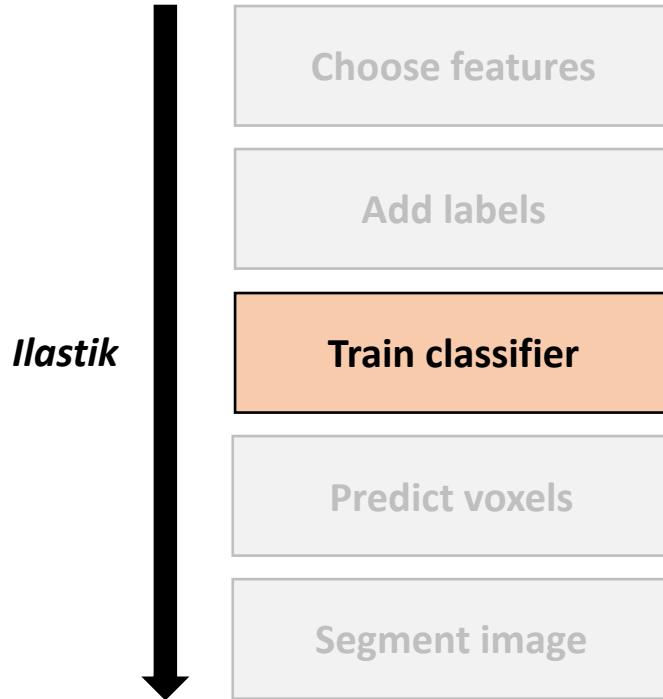
# Materials and methods

## Segmentation of the images

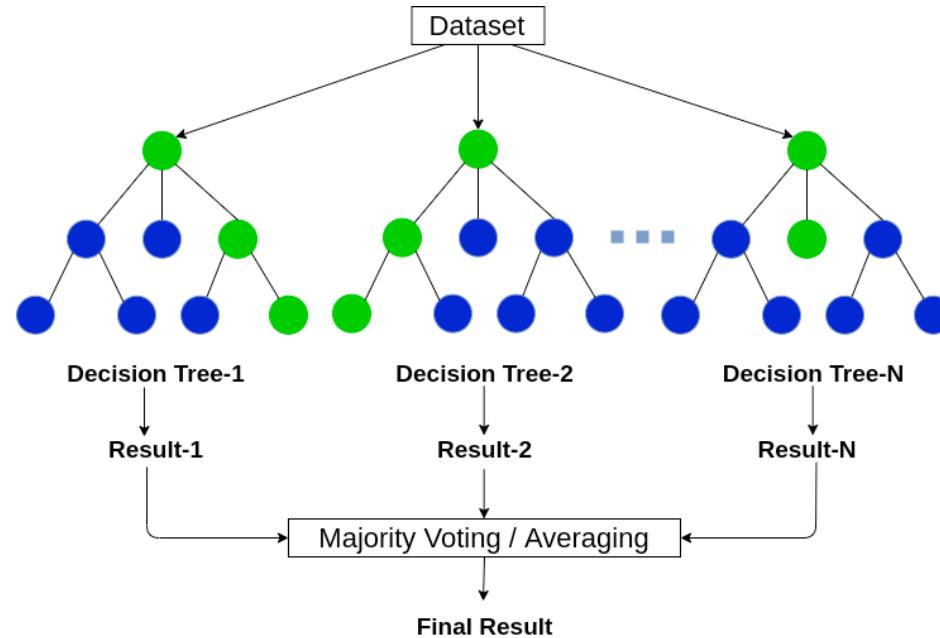


# Materials and methods

## Segmentation of the images

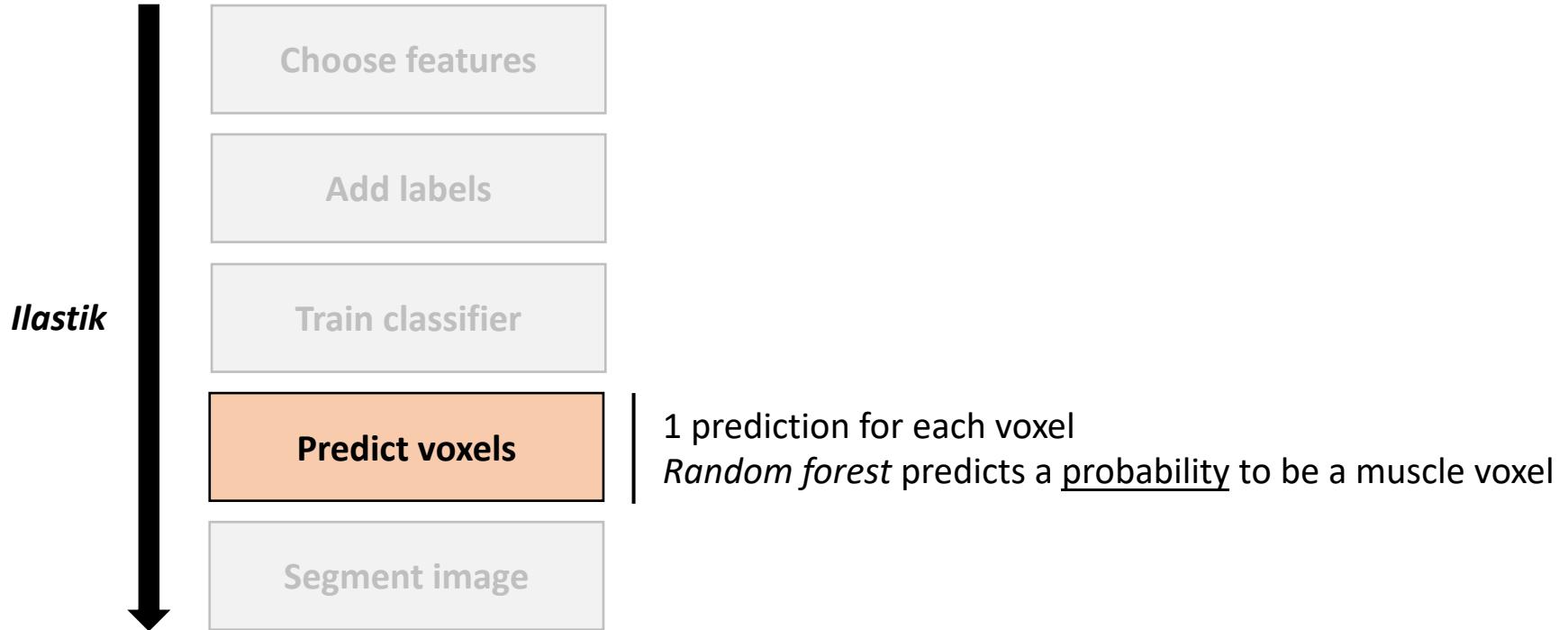


*Random forest:* aggregation of decision trees  
Algorithm trained on each labelled voxel



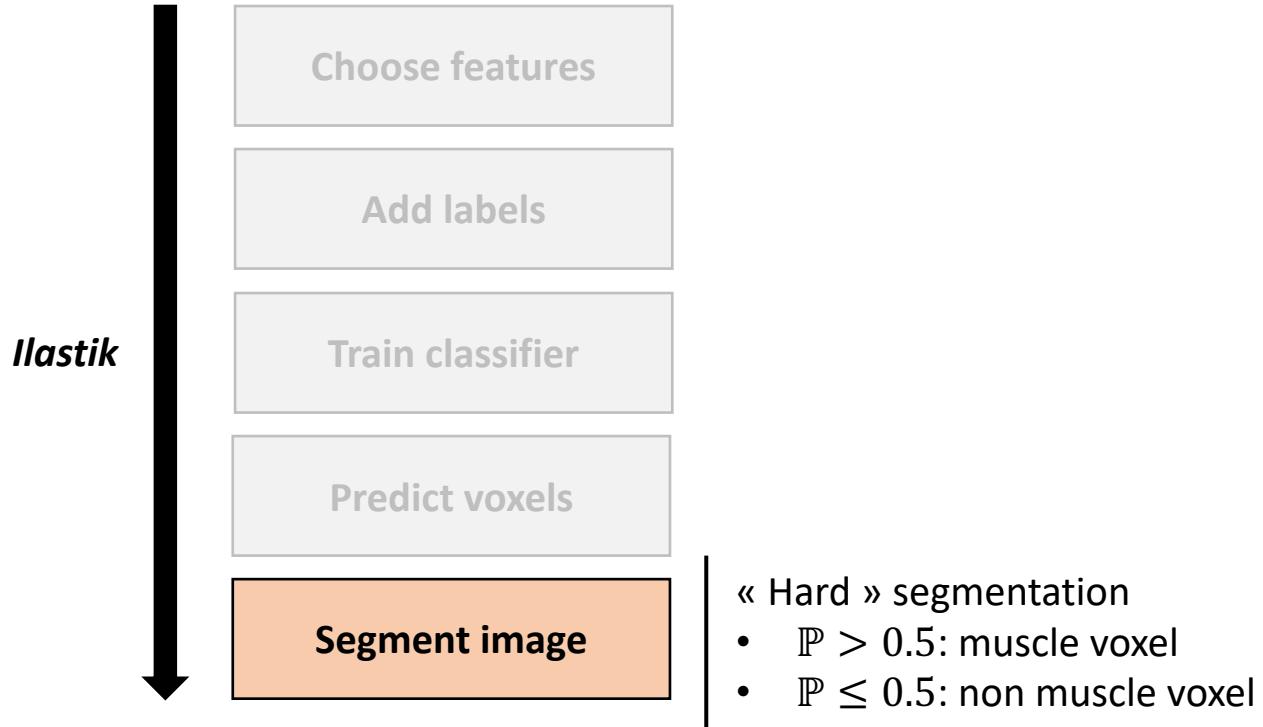
# Materials and methods

## Segmentation of the images



# Materials and methods

## Segmentation of the images



- « Hard » segmentation
- $\mathbb{P} > 0.5$ : muscle voxel
  - $\mathbb{P} \leq 0.5$ : non muscle voxel

# Materials and methods

## Mathematical morphology

### Erosion

Under the kernel:  
If 1 pixel = 0  
► all pixels set to 0

### Dilation

Under the kernel:  
If 1 pixel = 1  
► all pixels set to 1



### Kernel

- Shape (circle, square, etc.)
- Given size (e.g. 5x5 pixels)
- Slides through image

### Opening

Erosion + Dilation

Segmented image

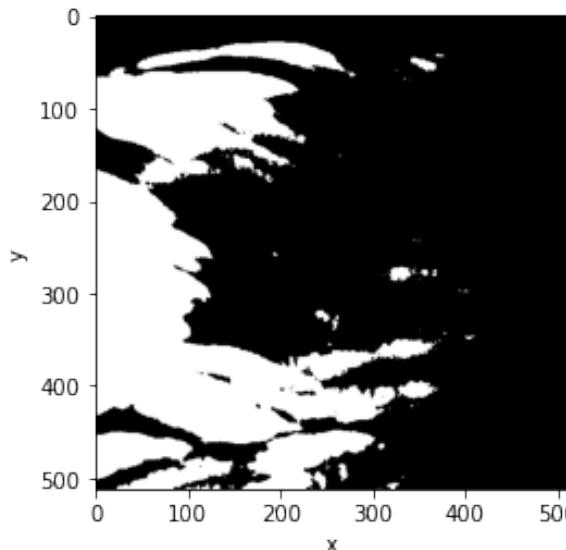
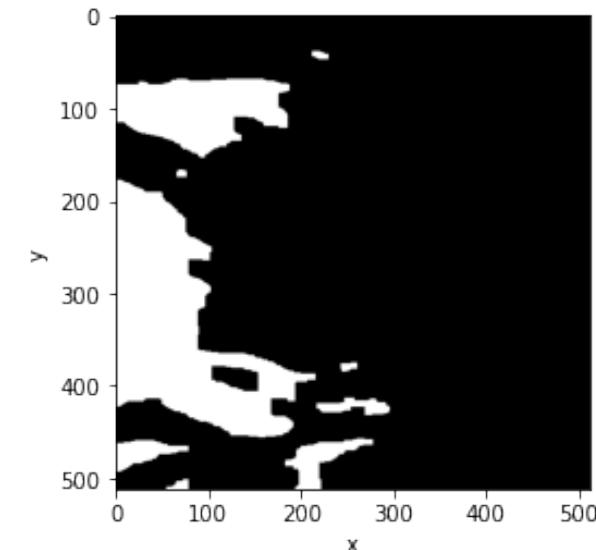
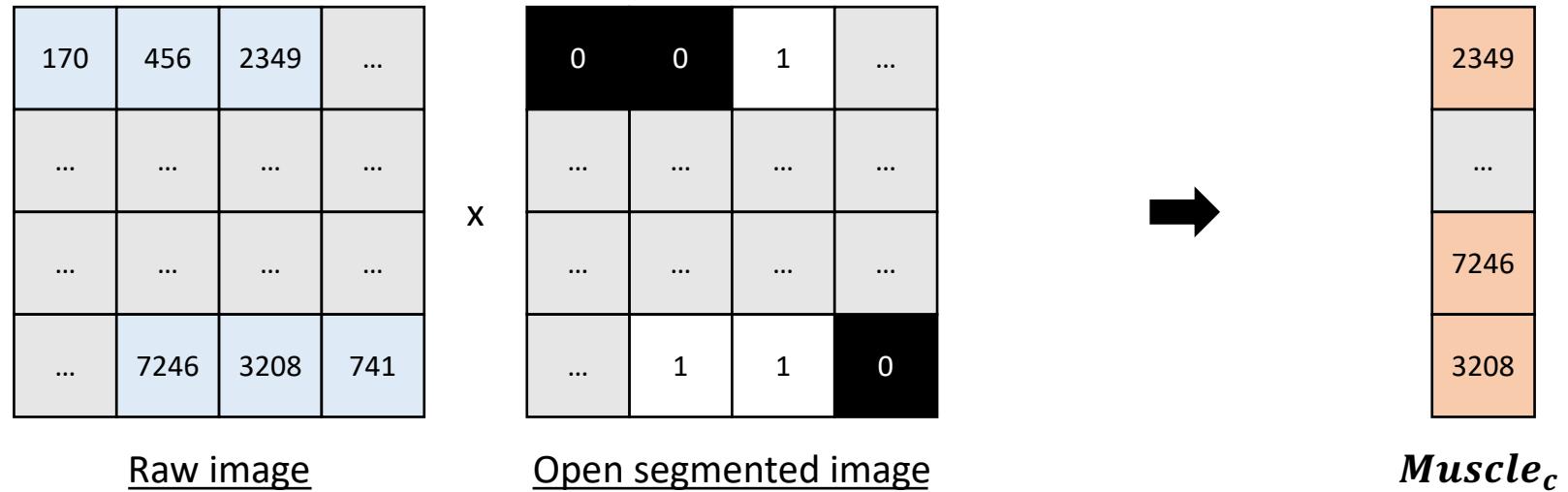


Image after opening + erosion



# Materials and methods

## Spectrum analysis

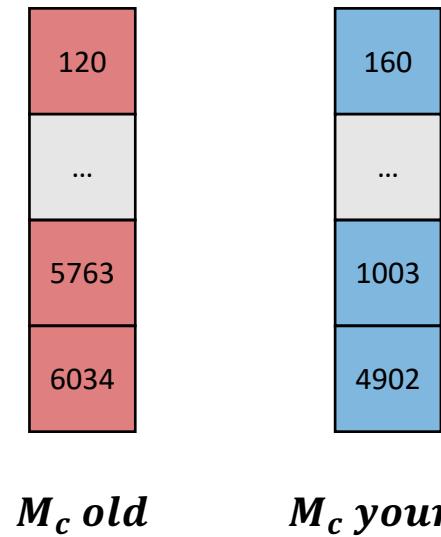


**Muscle<sub>c</sub>** | Array that contains the fluorescence value of all « muscle voxels » for a given channel  $c$

- Compute mean or median of each Muscle<sub>c</sub>
- Step of normalization by the maximum of the means/medians
- Display normalized mean or median spectrum

# Materials and methods

## Mann Whitney U test



Are the distributions of both samples same?

$$\mathcal{H}0: \mathbb{P}(M_c \text{ old} > M_c \text{ young}) = \mathbb{P}(M_c \text{ young} > M_c \text{ old})$$

$$\mathcal{H}1: \mathbb{P}(M_c \text{ old} > M_c \text{ young}) \neq \mathbb{P}(M_c \text{ young} > M_c \text{ old})$$

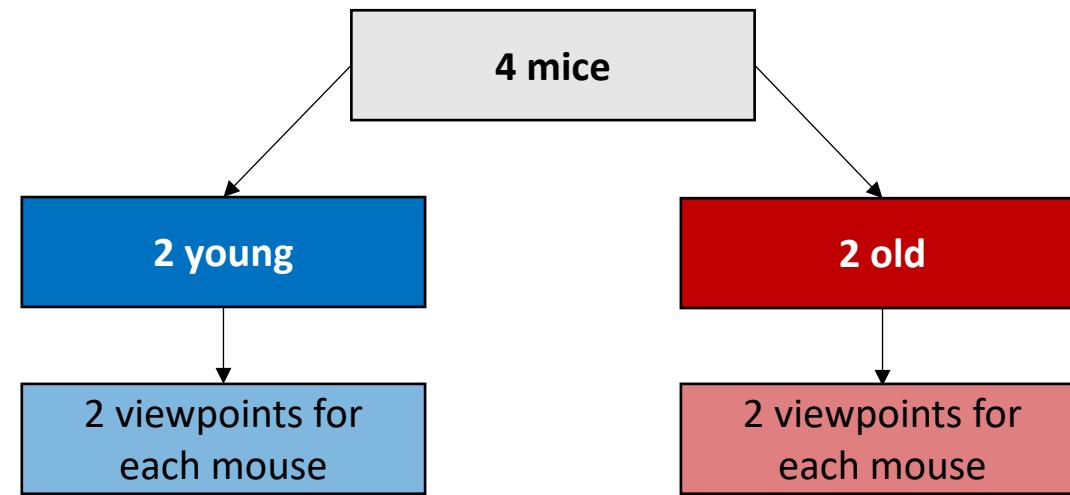
$p$ : probability to reject  $\mathcal{H}0$  wrongly

$p \leq 0.05$   $\mathcal{H}0$  rejected

$p > 0.05$   $\mathcal{H}0$  not rejected

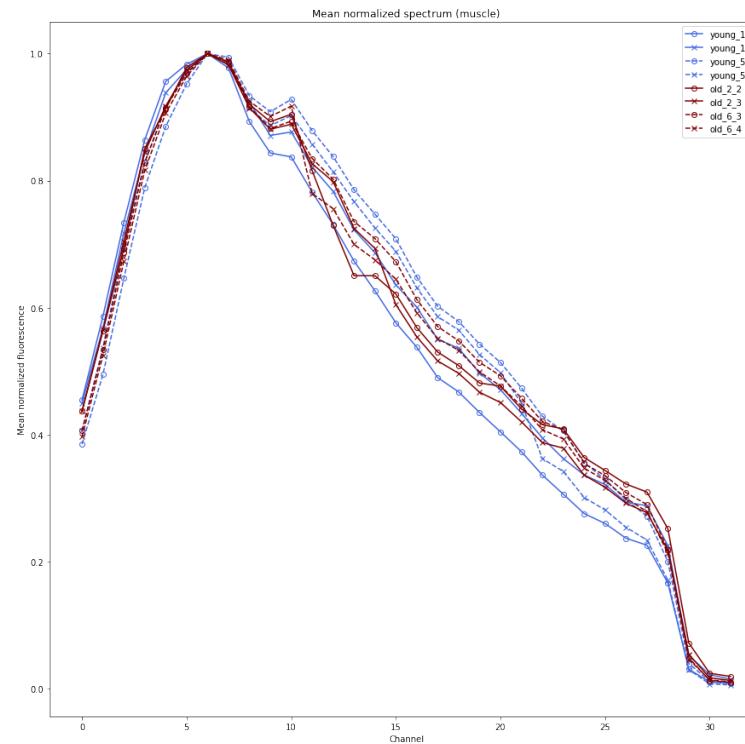
# Results and discussion

## The samples

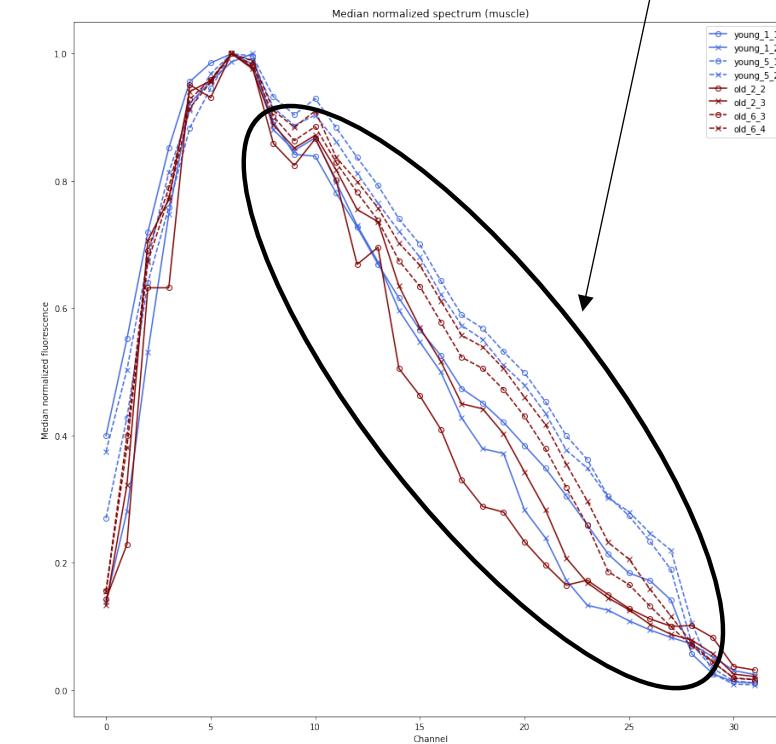


# Results and discussion

## Spectrum analysis



Normalized mean spectrum

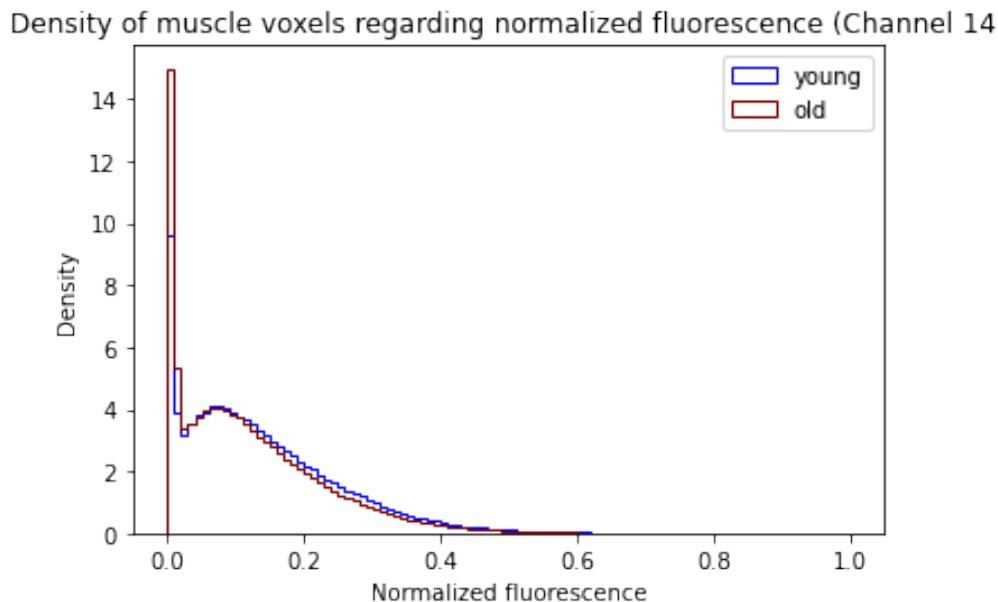


Normalized median spectrum

Some differences  
**BUT** no tendency due to the age

# Results and discussion

## Mann Whitney U test



Comparison young sample vs. old sample

32 p-values

$^{31}/_{32} : p = 0$

$\mathcal{H}_0$  rejected

Different samples distribution according to Mann-Whitney U test

# Results and discussion

## Reservations about conditions of application of Mann Whitney U

1

### Samples are very large

- 200 000 values for a given channel
- Probability laws tabulated for small samples

2

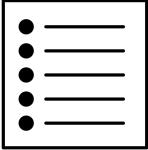
### Observations of each sample are not independent

- Observations come from the same image = same individual

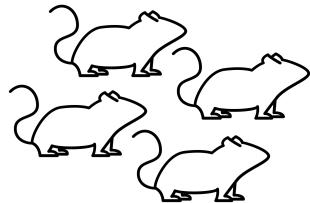
Some assumptions are not fulfilled  
➤ We cannot conclude from the results

# Results and discussion

What next ?



**Conclusion: we did not find out significant differences between young and old hyperspectral signatures**



**Mann Whitney test on normalized mean/median fluorescence for a given channel**

- Requires at least 4 old mice and 4 young mice



**Bright points detection and analysis**

# References

## Websites

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## Images

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<https://www.edmundoptics.fr/knowledge-center/application-notes/imaging/hyperspectral-and-multispectral-imaging/>

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