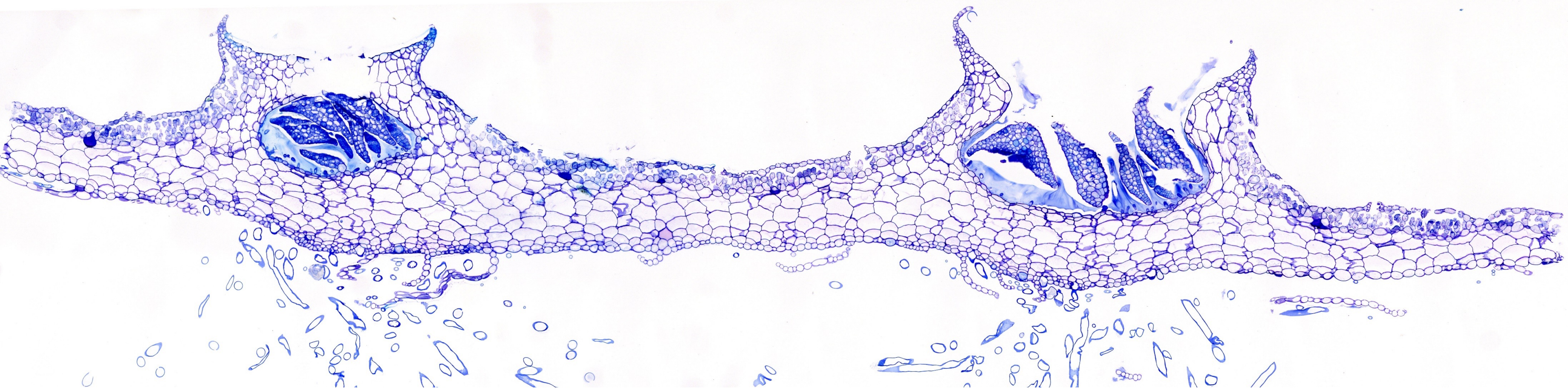
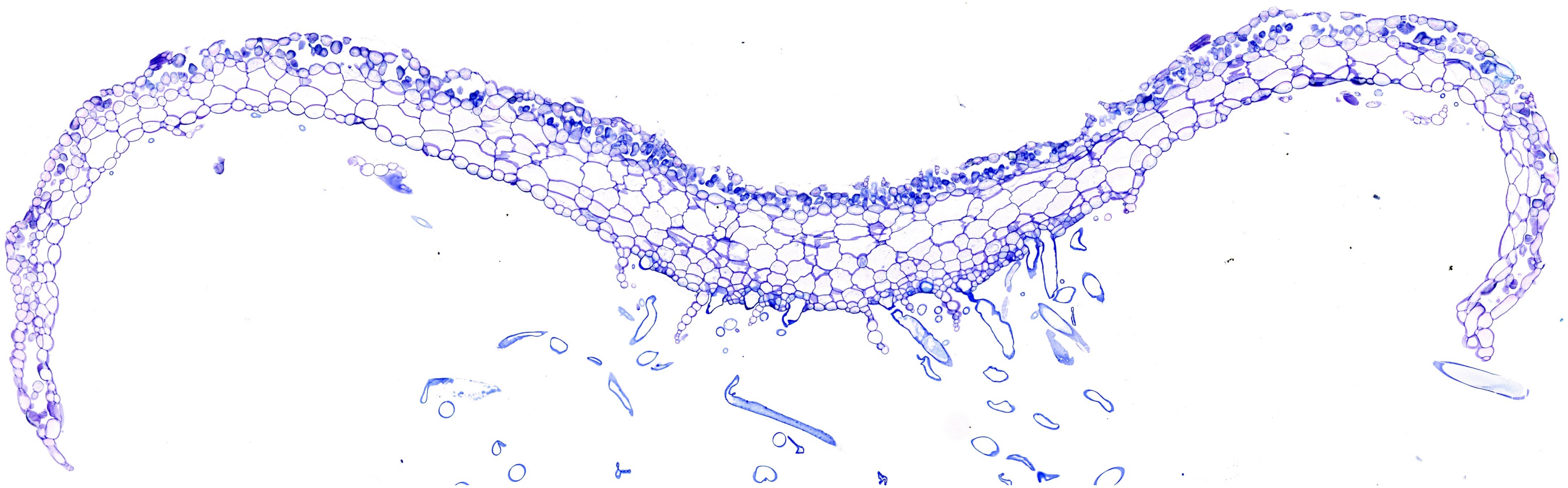
# Mise en évidence et quantification du collagène de type 1 sur des coupes transversales d’aortes thoraciques et de cœur de souris

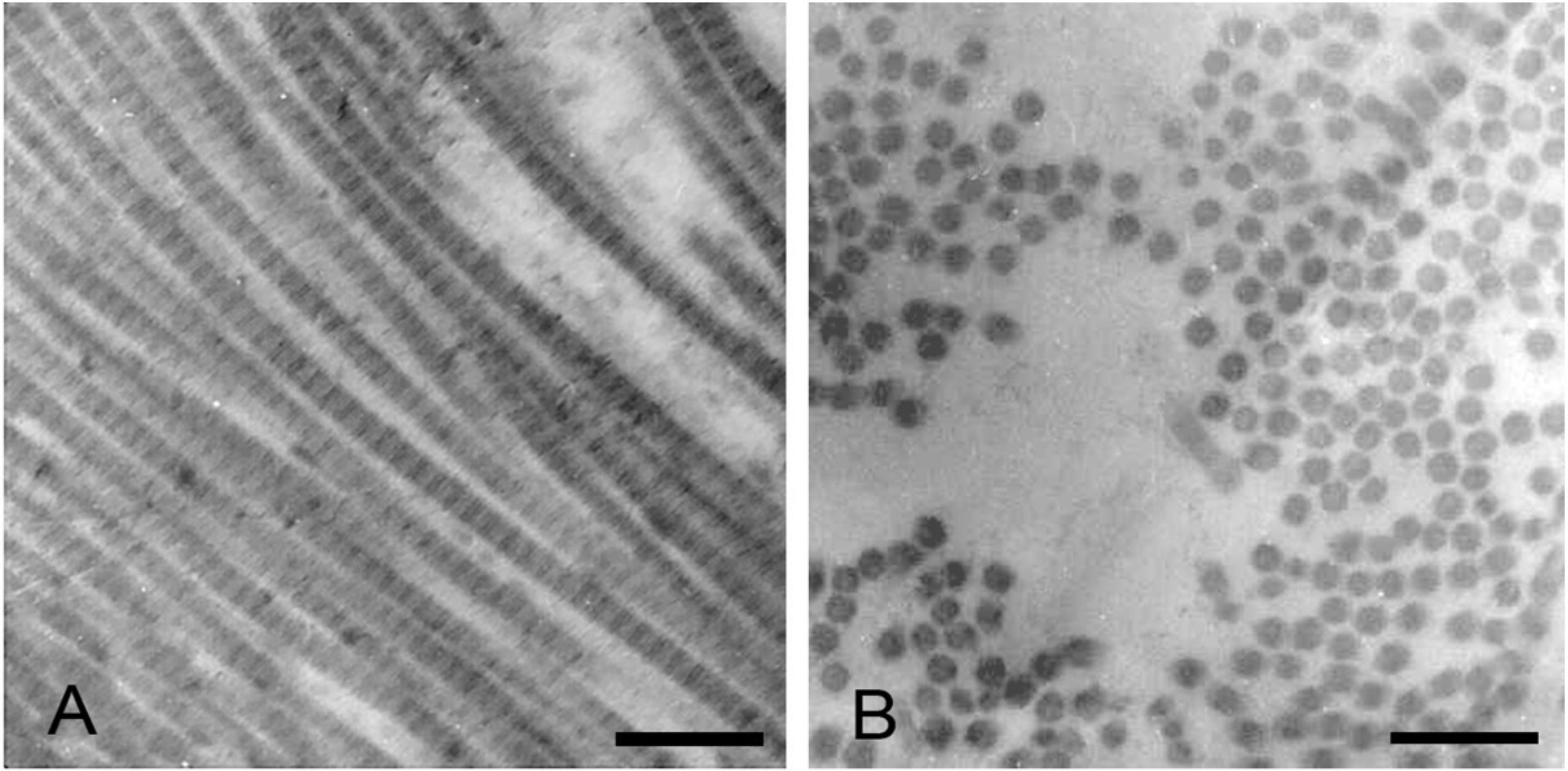
Florian LAURENT, Christophe DURLET, Gabriele SORCI, Jean-Louis CONNAT

# Des plantes aussi intéressantes!!



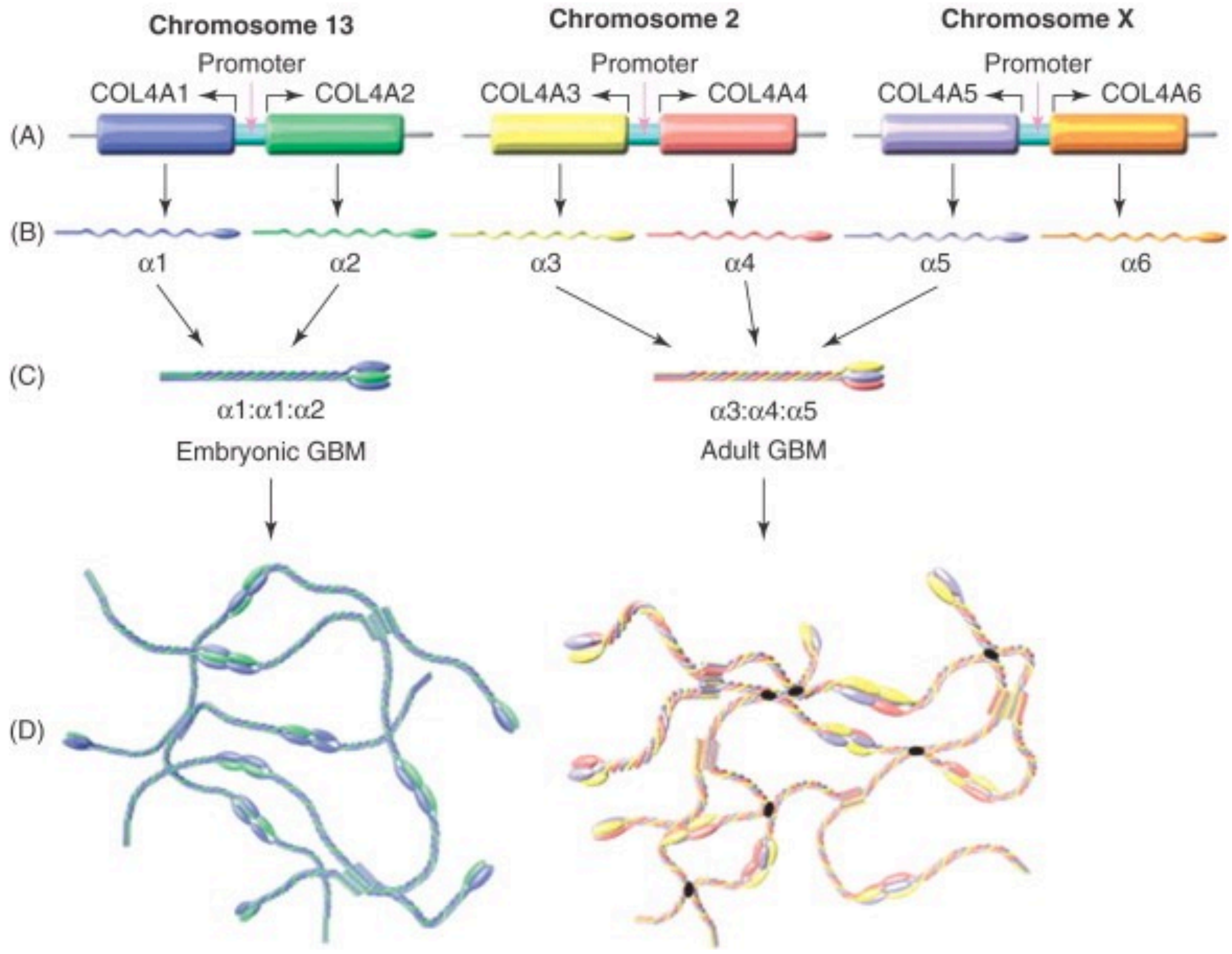


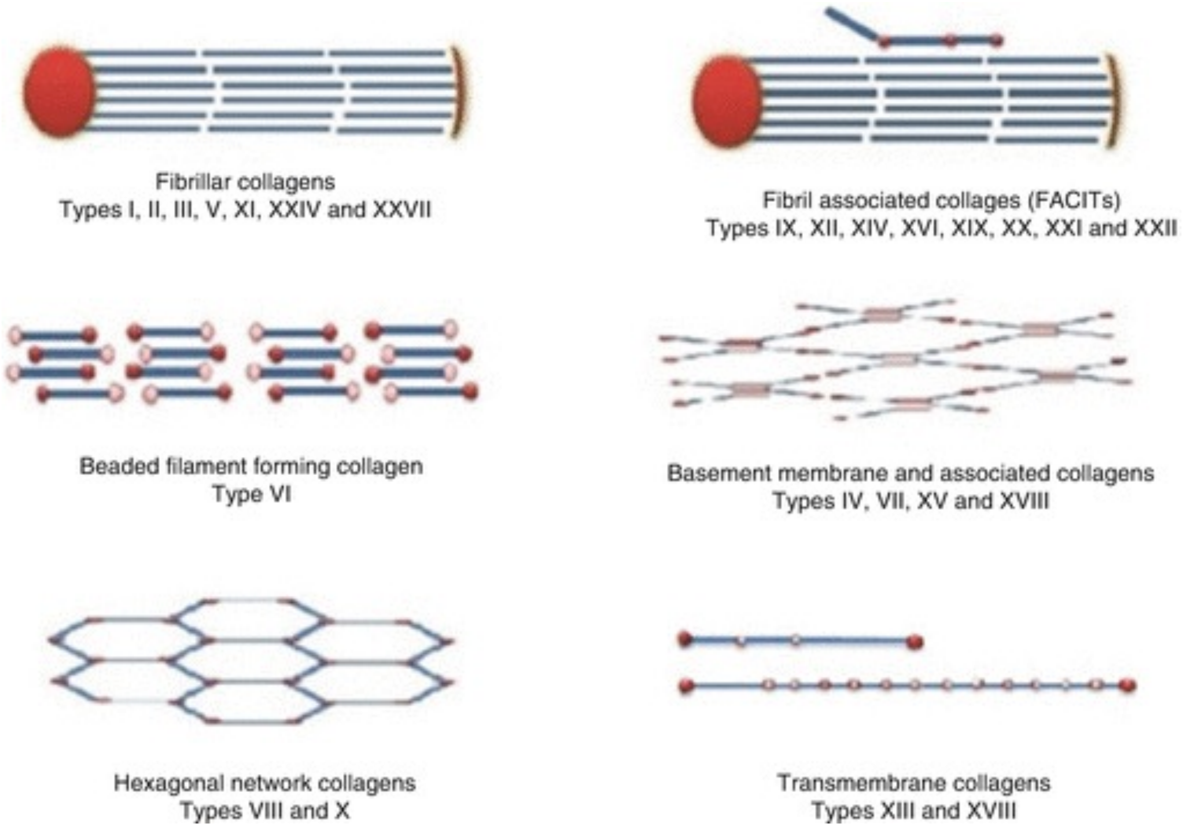
# Le collagène, une structure fascinante



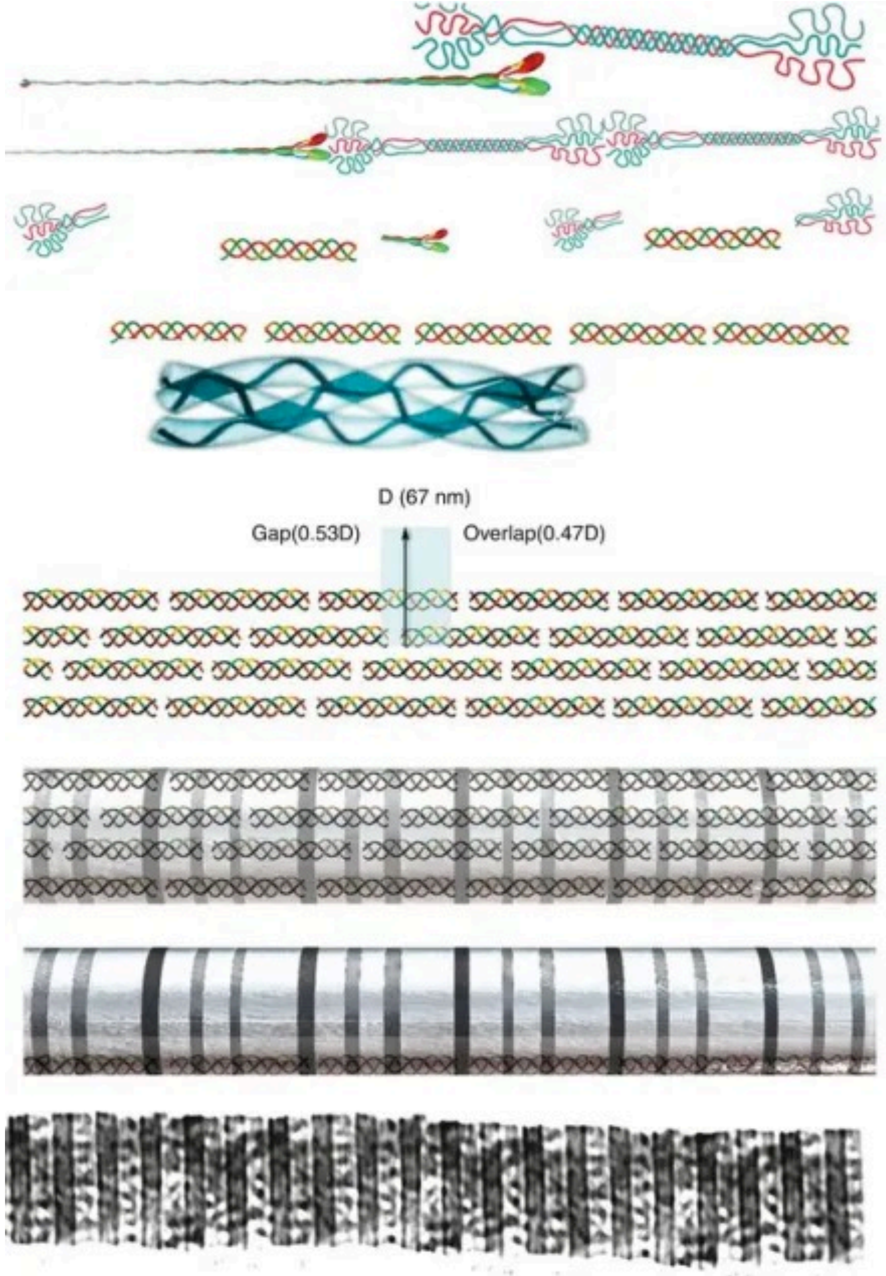

Berillis et al., 2013

# Les types de collagènes



# La formation de super-structure de collagène




Procollagen triple helix (300nm)

N-terminal peptide and C-terminal covalent crosslinking

Cleavage of propeptide C and N and tropocolagen molecule by N- and C proteinases

Self-assembly of tropocollagen into microfibril catalyzed by lysyl oxidase

The molecules are staggered from each other by about 67 nm (D)

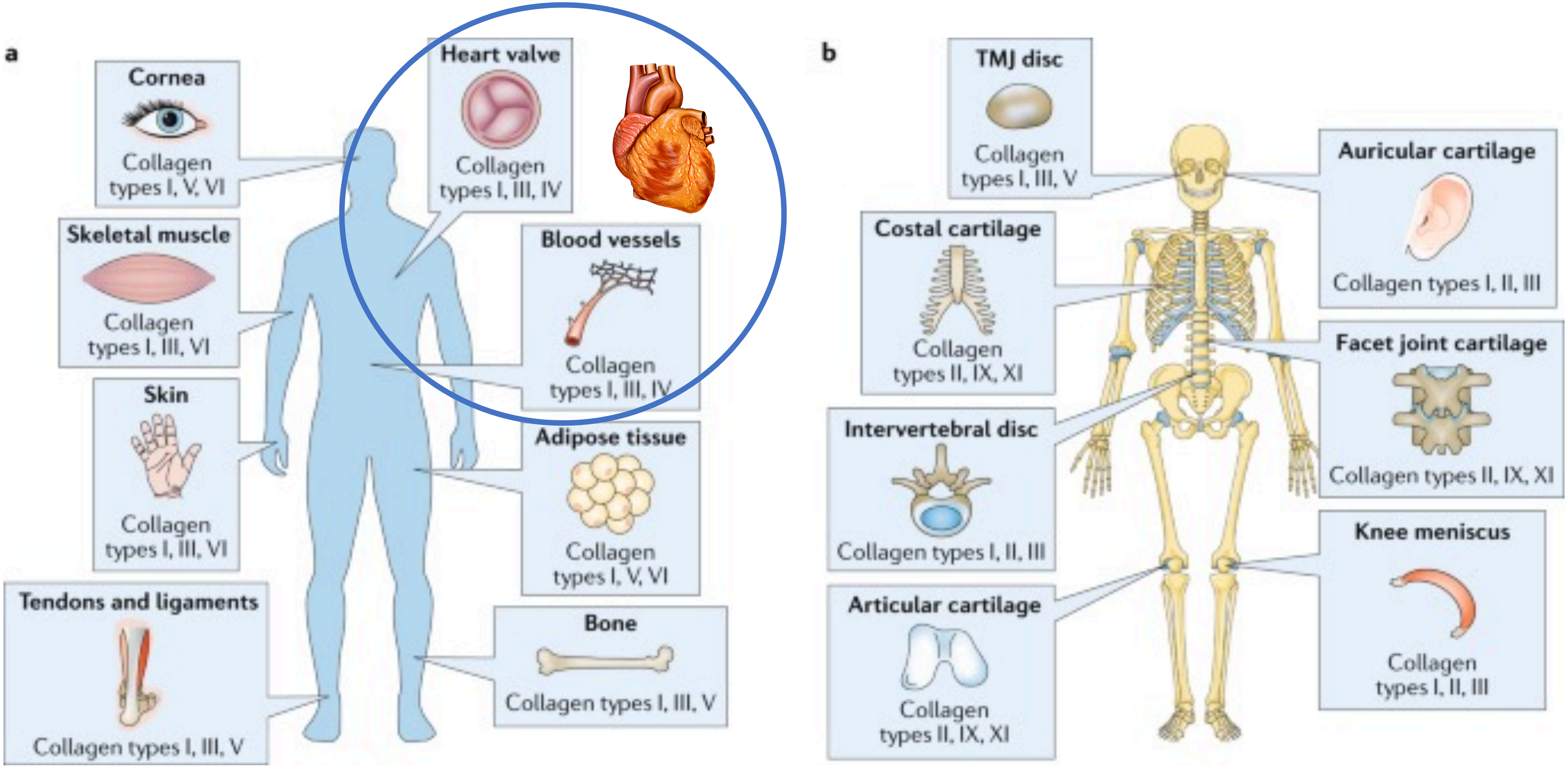
Overlap and gap regions

Assembled mictofibrils form the collagen fibril

Gap (lacunar) regions and overlap regions of microfibrils give the striate aspect of collagen fibers

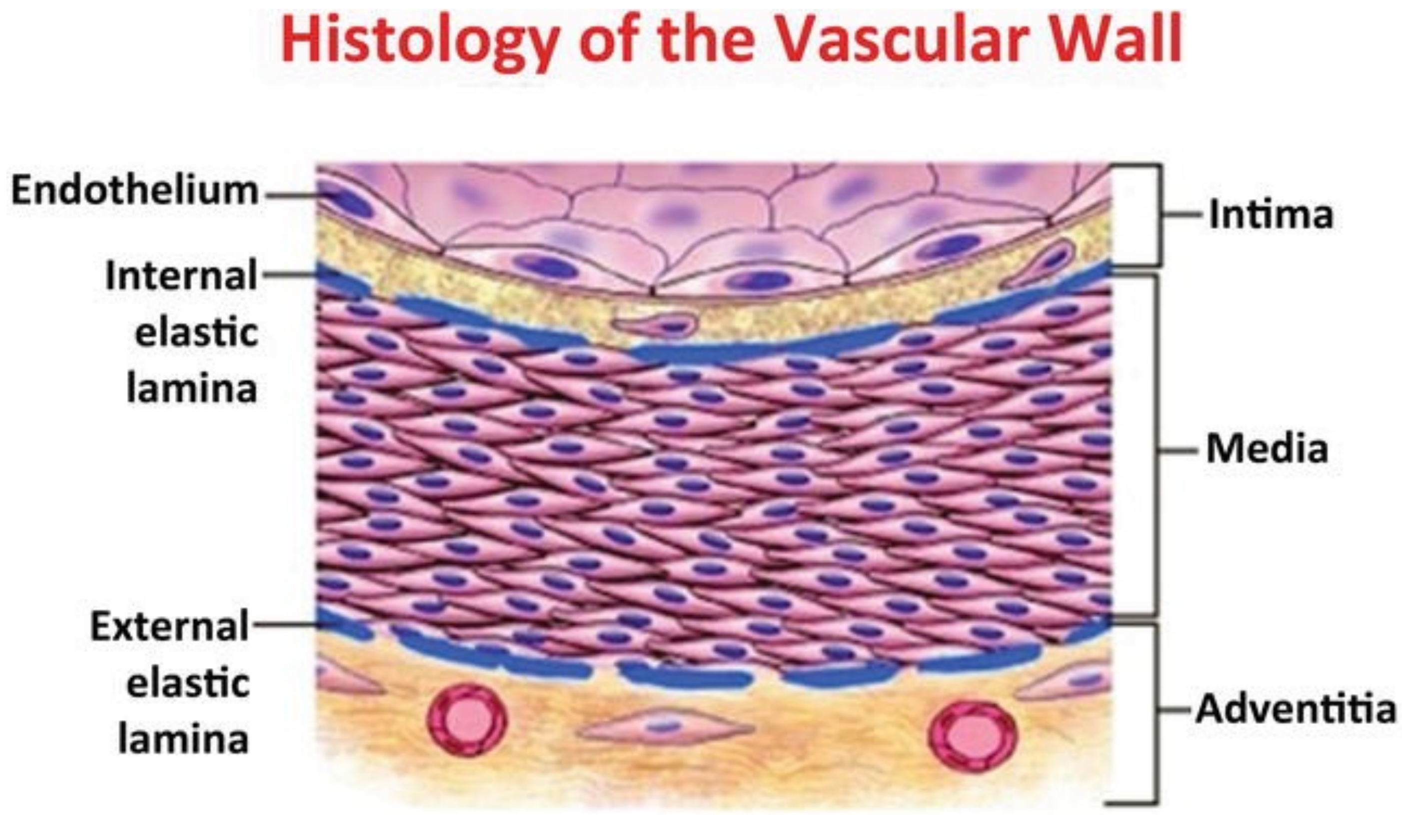
Microscopic aspect of a collagen fibril

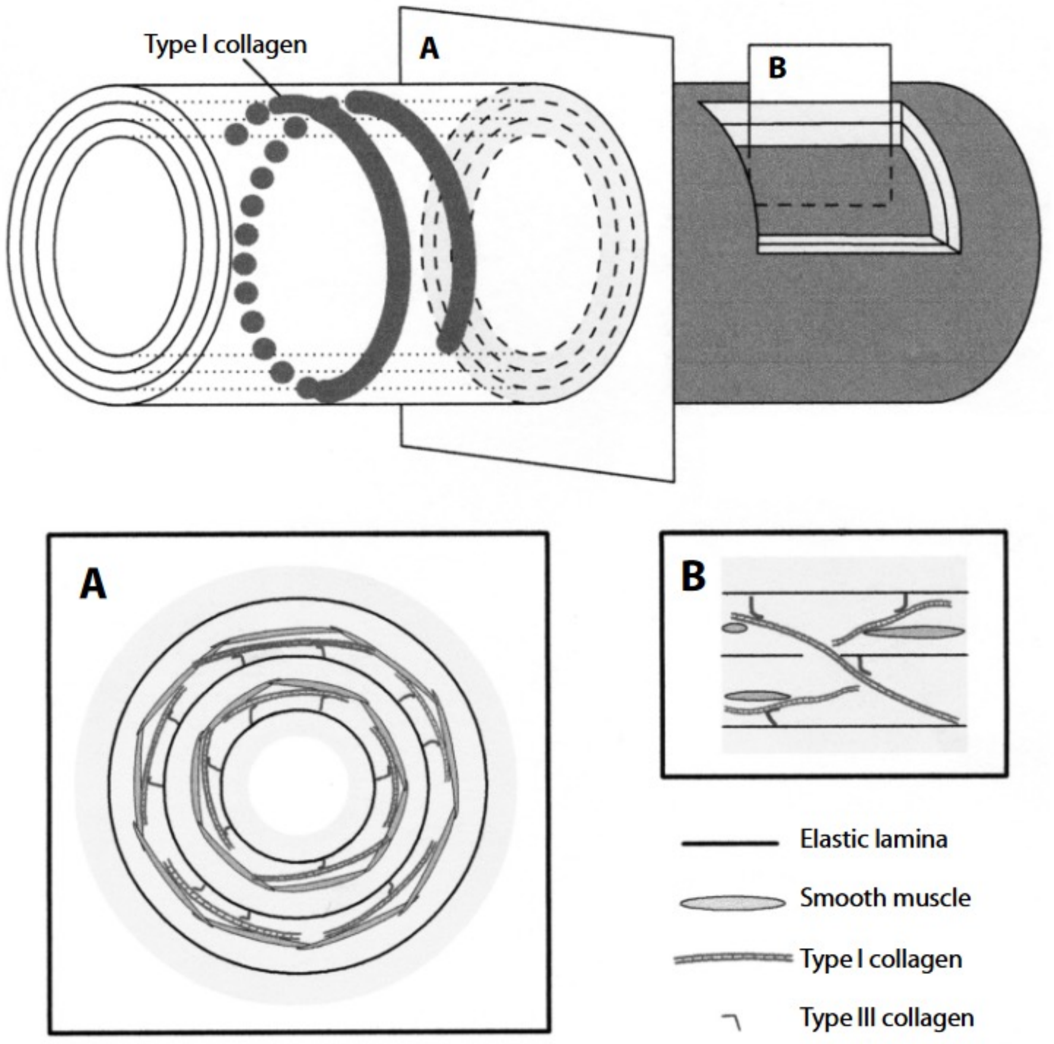
# Différents types de collagènes selon les tissus




Bielajew et al., . Nat Rev Mater 5, 2020

# Repartition du collagène dans les vaisseaux

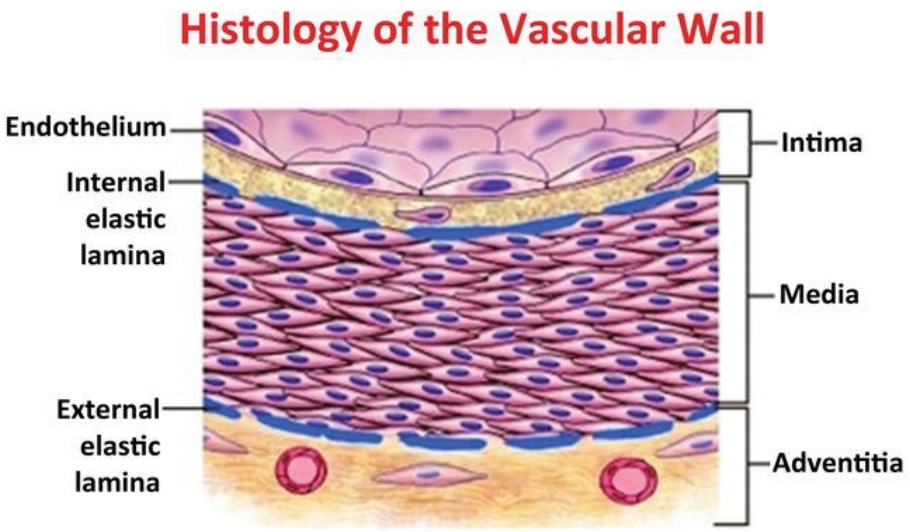


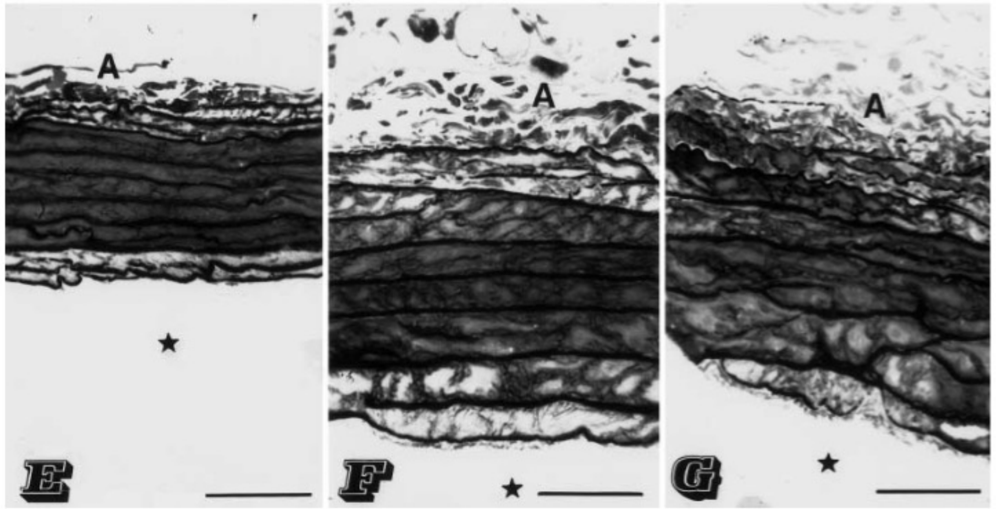




Schema des parois aortiques

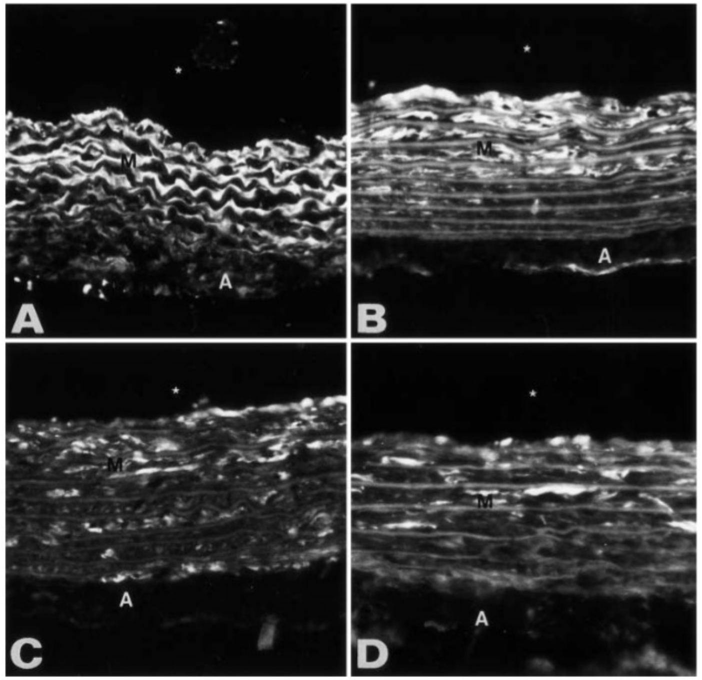
Silver et al., 2001

# Vieillissement des parois aortiques



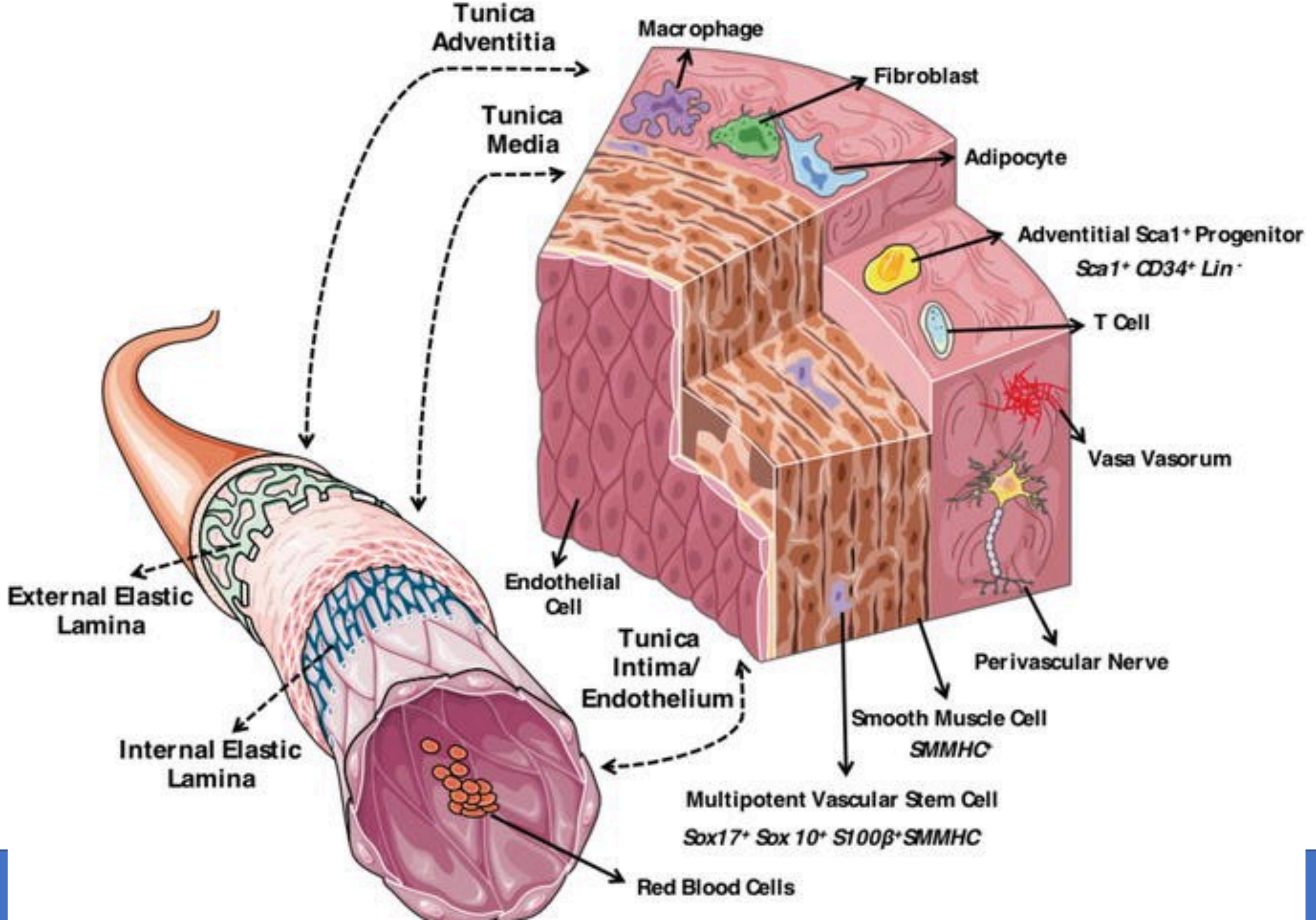

2 months 24 months 29 months



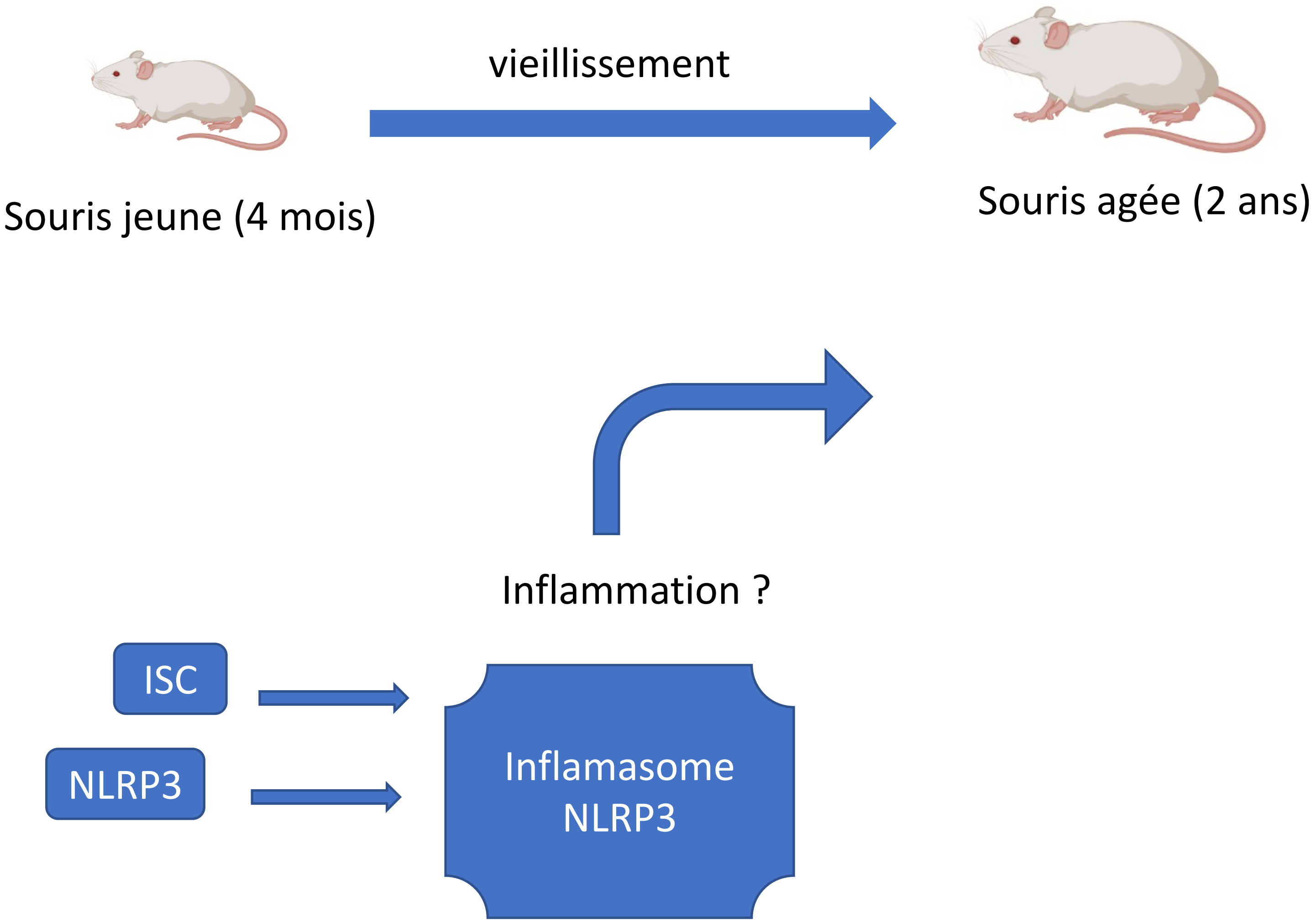

IHC
For Desmin

Connat et al., Anat Embryol, 2001

# Plasticité CML



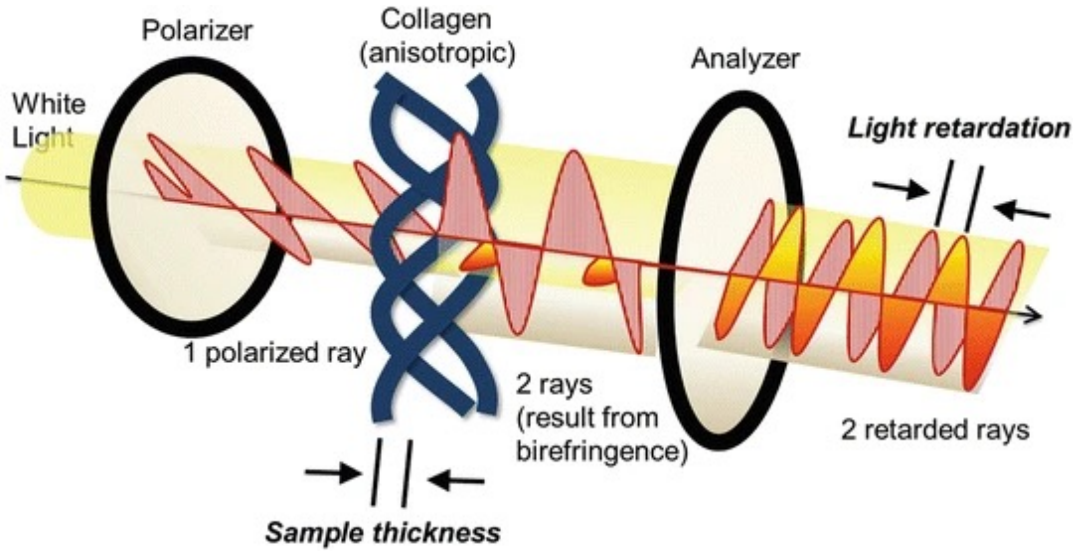
# Contexte de l'étude

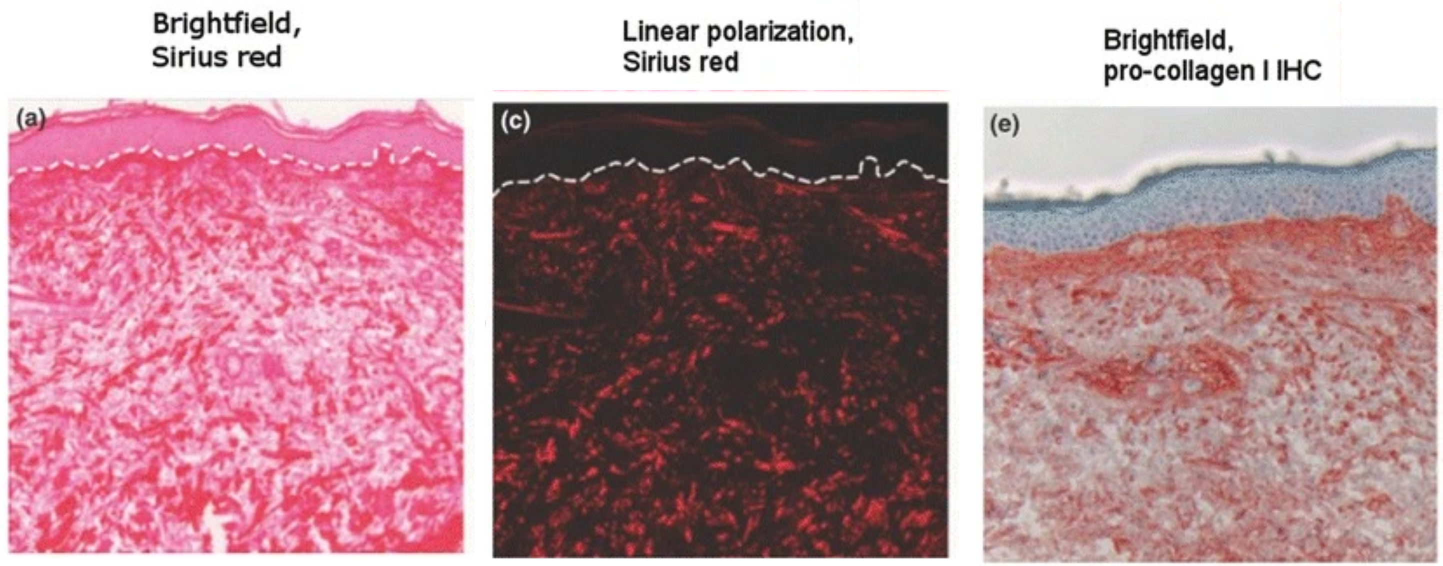


# Les différentes méthodes pour détecter et quantifier le collagène

| Modality | Resolution | FOV | Speed | Advantages | Disadvantages |
|---|---|---|---|---|---|
| SAXS/WAXS | 0.1–100 nm | 1 mm | >1 h | Provides nanoscale information about collagen ultrastructure and orientation. | Averaged measurements through the thickness of the tissue; long acquisition times. |
| Confocal/ multiphoton | 1 µm | 1 mm | <30 Hz | Can be combined with polarimetry, exogenous (e.g., CHP) and endogenous contrast (e.g., autofluorescence, SHG) for fibril-level evaluation of collagen. | Low penetration depths compared to other techniques, risk of thermal damage or photobleaching. |
| Polarimetry (QPLI/PLM) | Tunable based on focusing optics | Tunable based on focusing optics | 20–70 Hz | Sensitive, quantitative, and fast detection of collagen fiber alignment strength and orientation, can be combined with other modalities. | Biological tissue is highly scattering that can sometimes result in loss of polarization information. |
| SALS | 50 µm | >10 mm | >20 min | Wide tissue characterization of structural properties and fiber alignment | Long data acquisition times and destructive preparation. |
| OCT/OCE | 1–10 µm | 1–5 mm | <30 Hz | Can image relatively deeper, quicker, and at a higher resolution than some other techniques. Correlation with histology lends towards use in optical biopsies. | Relatively high cost and complex instrumentation of imaging system. |
| PAI | 20 µm | 5–10 mm | <30 Hz | Powerful imaging of highly absorbing elements like hemoglobin, useful in visualizing vascular changes. | Collagen absorbance is only at a relative maximum over other tissue components in >1,300 nm wavelength range. |
| Brioullin microscopy | 10 µm | 1–5 mm | <1 Hz | Local measurements of mechanics without tissue excision or external perturbation. | Longitudinal modulus does not correlate with elastic modulus for incompressible materials. |

# Détecter le collagène de type I




Coupes de peau
Laure Rittié, 2017

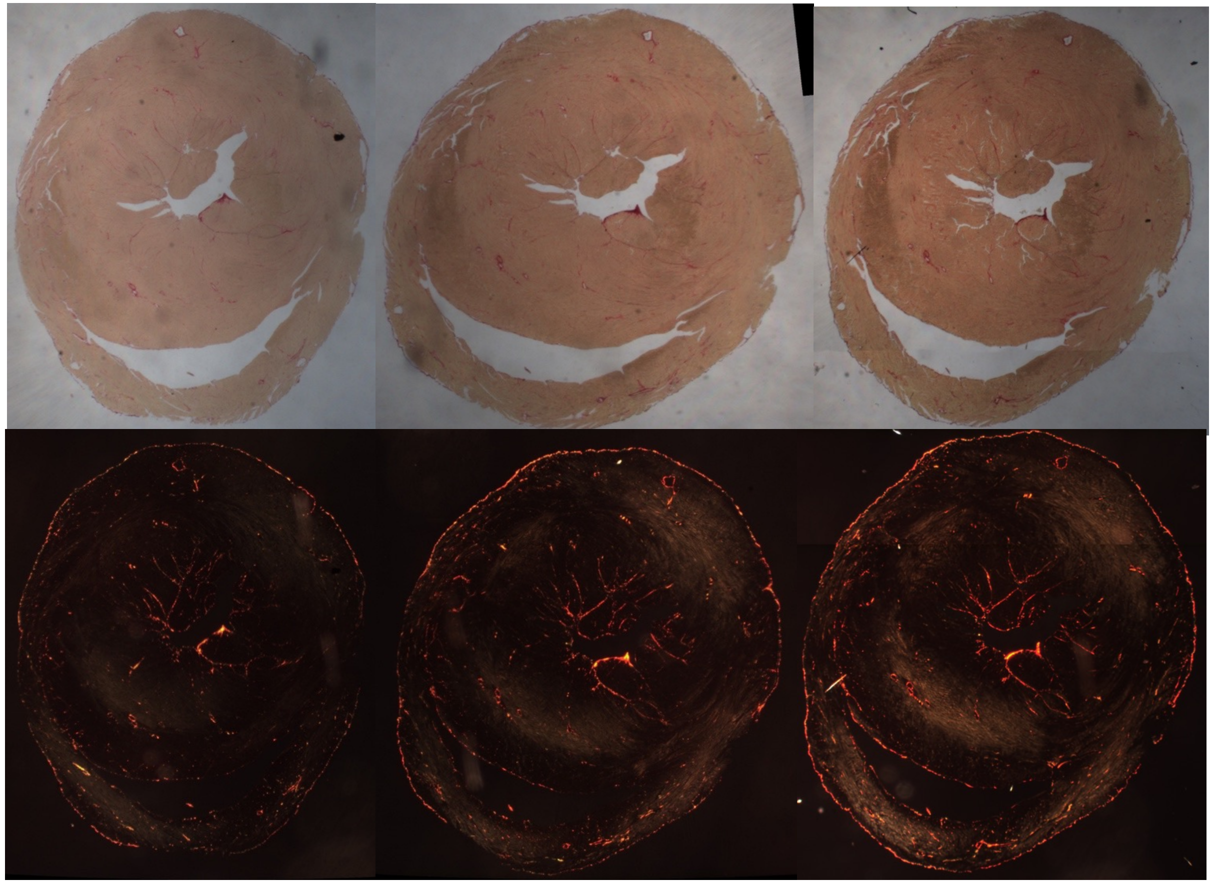
# Précautions à avoir lors de la prise d’image

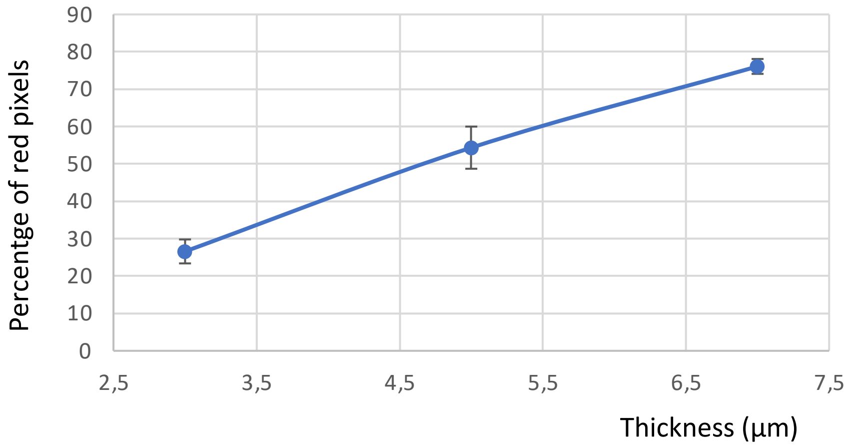
Epaisseur coupe

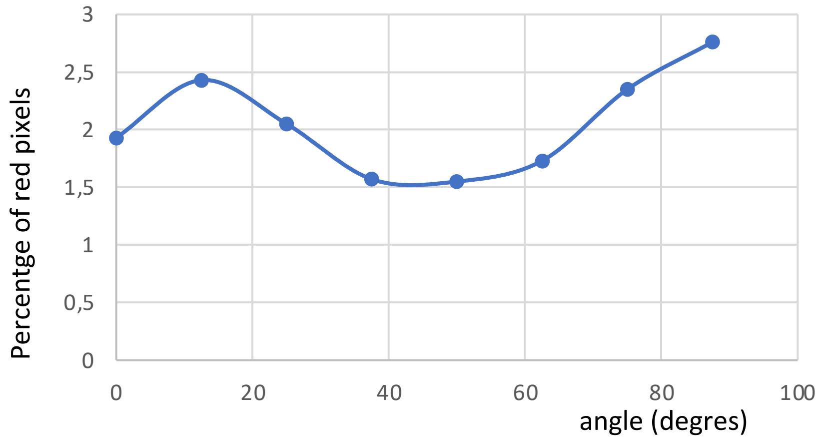
| | 3 µm | 5 µm | 7 µm |
|---|---|---|---|

Fond clair

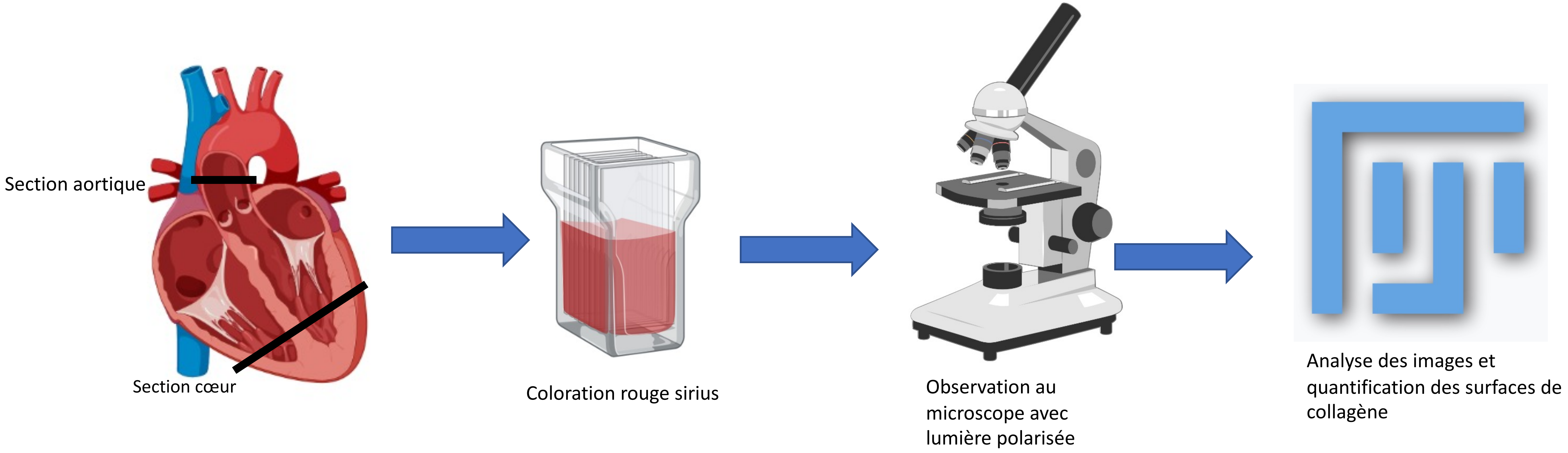
Rouge sirius





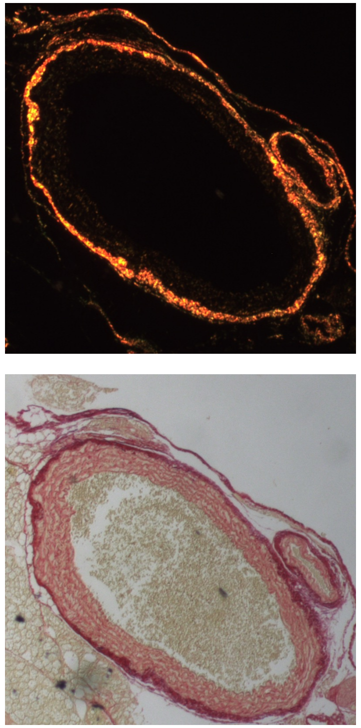



# Schéma des étapes d'acquisition des images

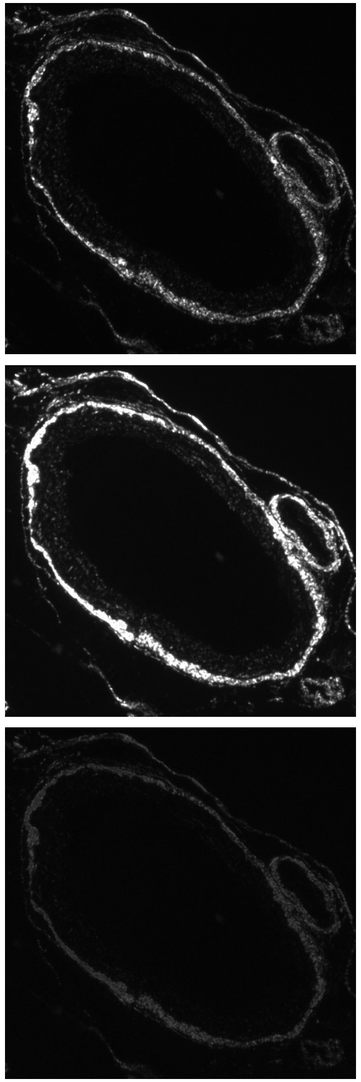


# Protocol d’analyse des images

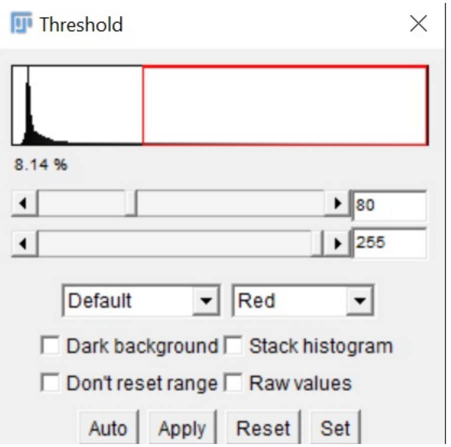
## Détourage

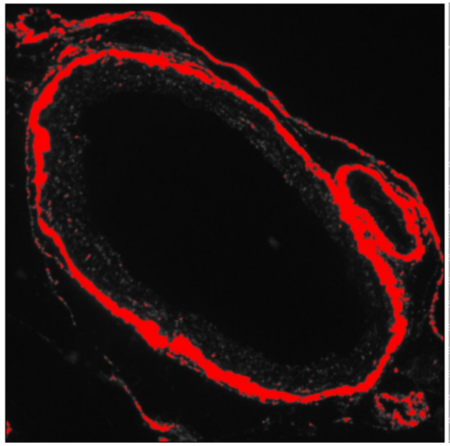


## Sélection du bon canal



## Seuillage



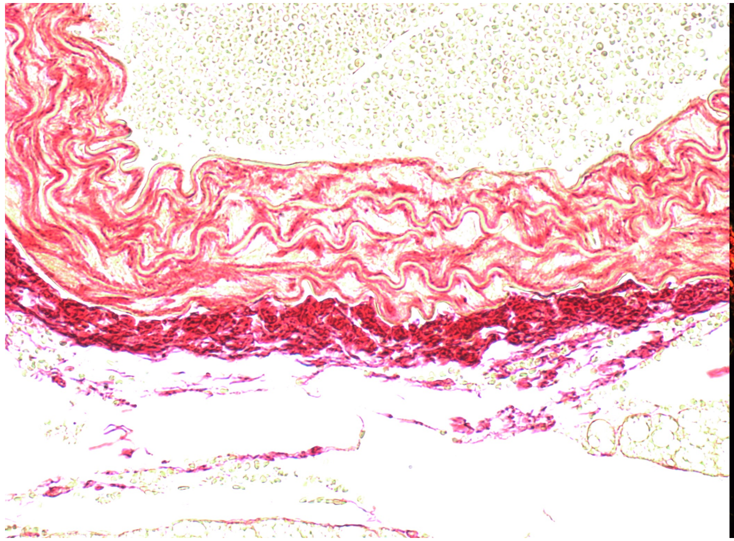



## Récupération des résultats

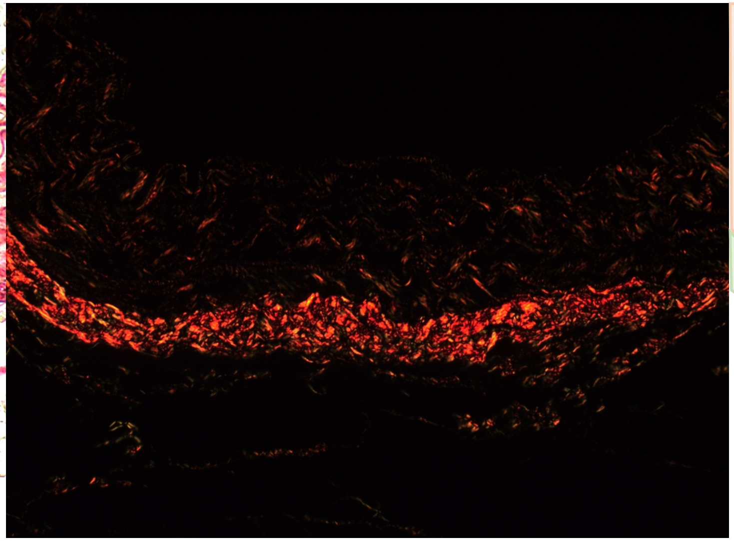
| fond génétique | age | info N° animal | replicat | Threshold | %pixels rouges adventice | %pixels rouges media |
|---|---|---|---|---|---|---|
| ASC | 4M | 1 | 1 | 40 | 31,34 | 4 |
| ASC | 4M | 2 | 1 | 40 | 23,55 | 2,3 |
| ASC | 4M | 3 | 1 | 40 | 23,05 | 1,15 |
| ASC | 4M | 4 | 1 | 40 | 27,43 | 4,91 |
| ASC | 4M | 5 | 1 | 40 | 19,55 | 1,36 |
| ASC | 4M | 6 | 1 | 40 | 18,67 | 1,95 |
| ASC | 4M | 7 | 1 | 40 | 19,69 | 0,66 |
| ASC | 4M | 8 | 1 | 40 | 19,65 | 0,42 |

# Des tissus spécifiques peuvent être isolés

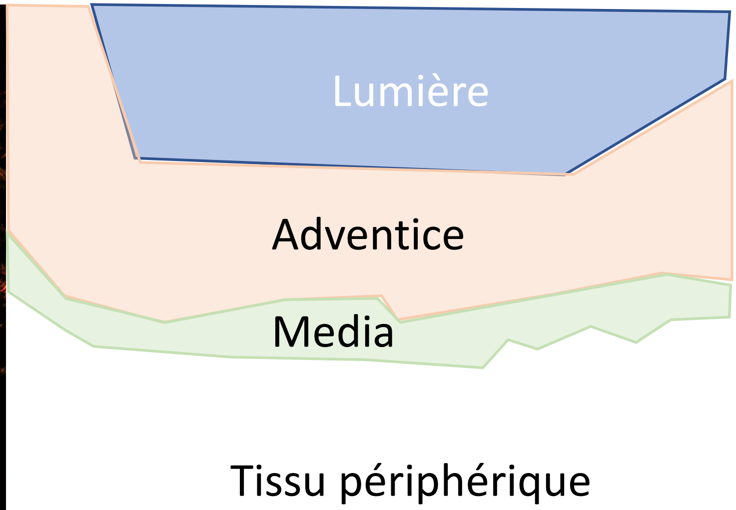
Fond clair



Rouge sirius



Segmentation




- Séparation des canaux
- Seuillage
- Mesure des pixels

# Utilisation de macro pour une semi-automatisation

Recorder

Record: Macro   Name: Macro.ijm   Create   ?

```
open("C:/Users/Lab common/Documents/Florian_stuff/zzOthers/aorte WT 2A C3 R2 RS.tif");
selectWindow("aorte WT 2A C3 R2 RS.tif");
run("Split Channels");
selectWindow("aorte WT 2A C3 R2 RS.tif (blue)");
selectWindow("aorte WT 2A C3 R2 RS.tif (green)");
selectWindow("aorte WT 2A C3 R2 RS.tif (red)");
setAutoThreshold("Default");
//run("Threshold...");
//setThreshold(93, 255);
setOption("BlackBackground", false);
run("Convert to Mask");
```

De l’enregistrement des actions ….

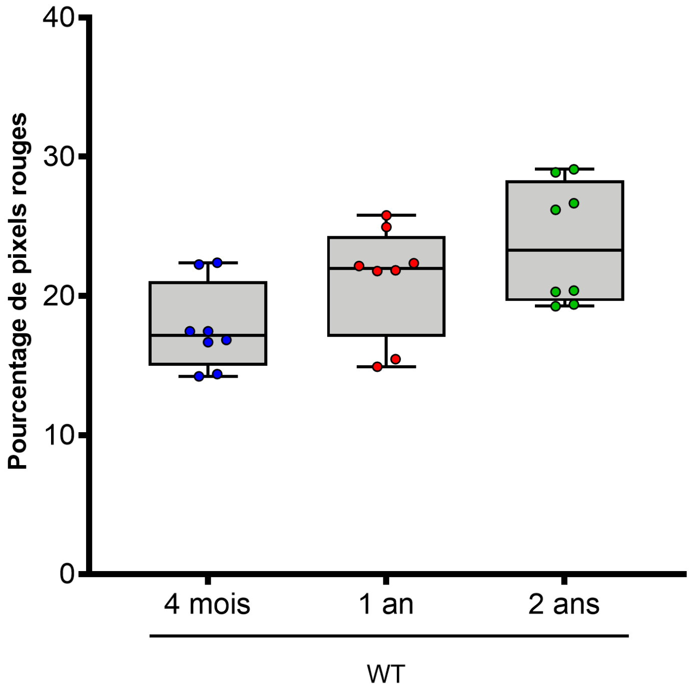
…. À une macro

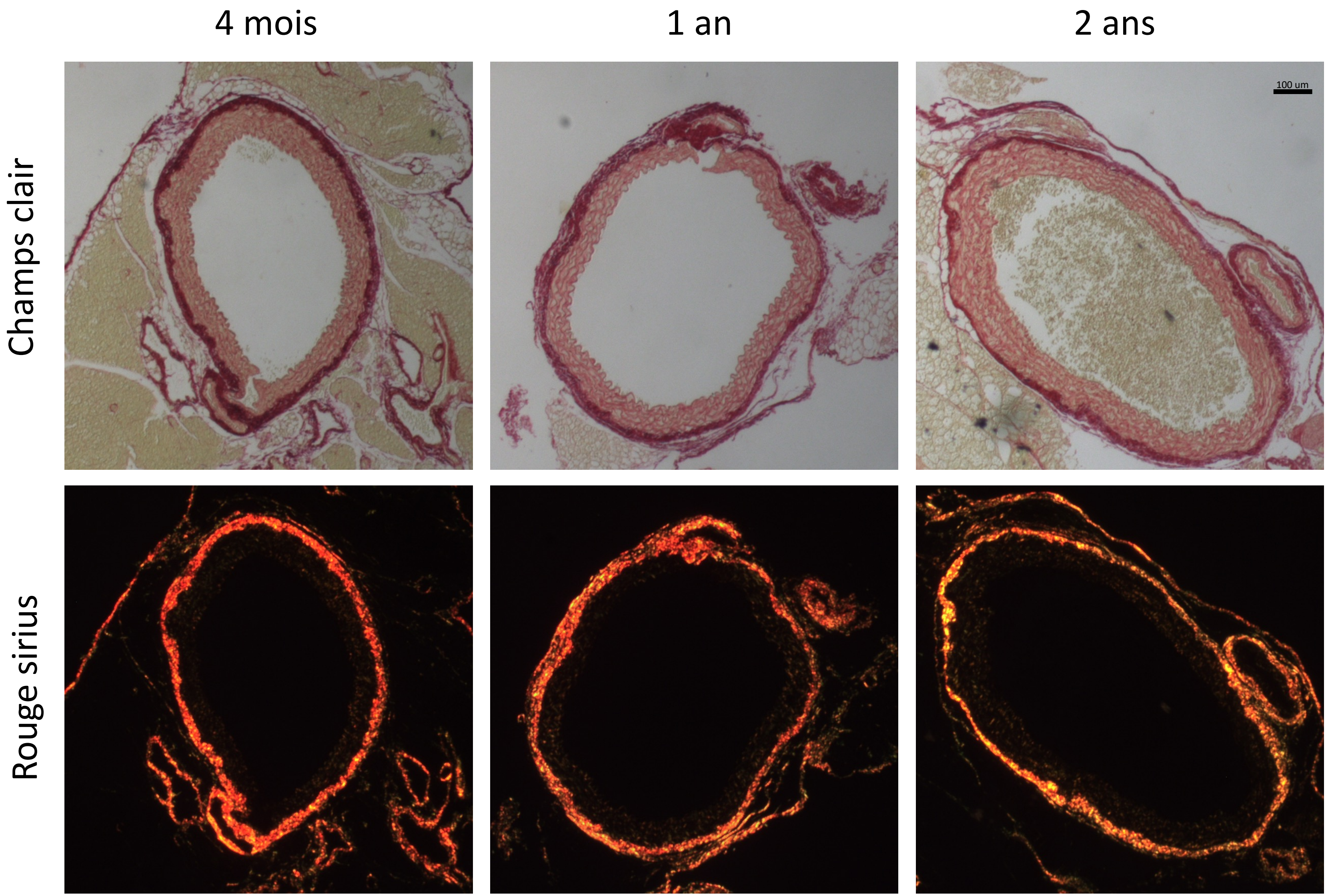
```
macro "Red-only"
{
setAutoThreshold("Default")
setThreshold(80,90);
run("Analyze Particles...","size=0-Infinity circularity=0,00-1,00 show=Nothing summarize")
setAutoThreshold("Default")
setThreshold(90,100);
run("Analyze Particles...","size=0-Infinity circularity=0,00-1,00 show=Nothing summarize")
setAutoThreshold("Default")
setThreshold(100,110);
run("Analyze Particles...","size=0-Infinity circularity=0,00-1,00 show=Nothing summarize")
setAutoThreshold("Default")
setThreshold(110,120);
run("Analyze Particles...","size=0-Infinity circularity=0,00-1,00 show=Nothing summarize")
```

Extrait de la macro utilisée

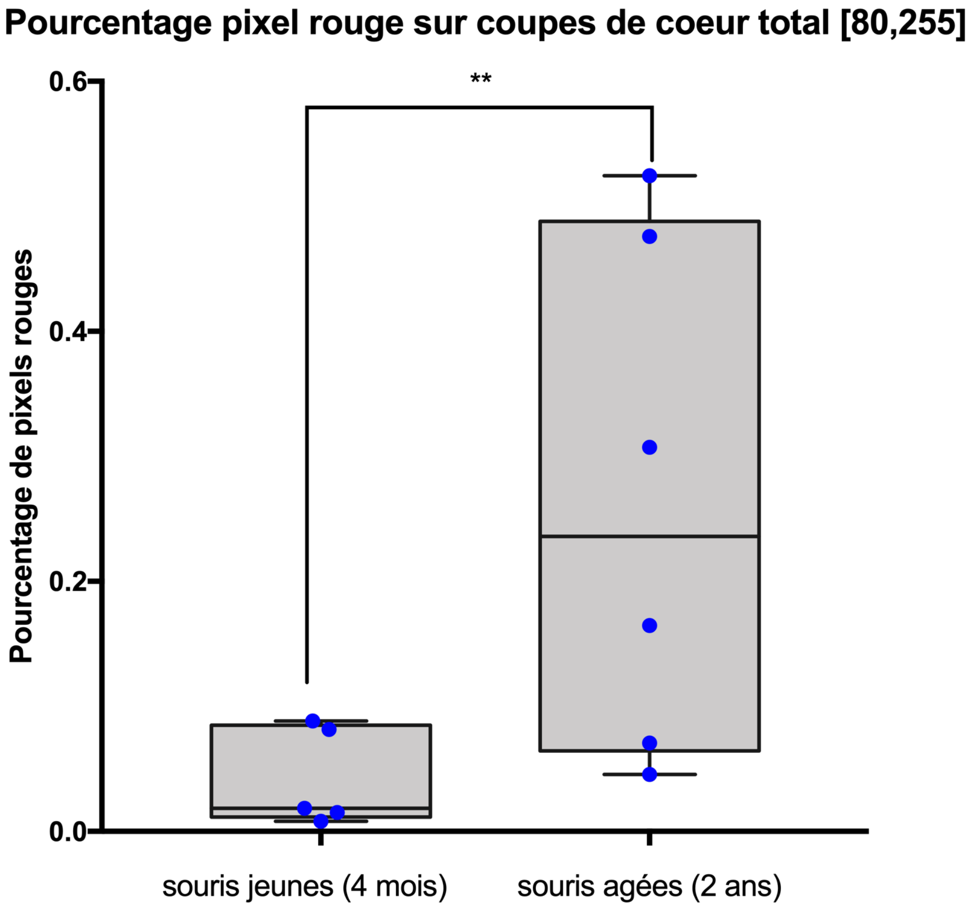
ChatGPT est notre “ami”

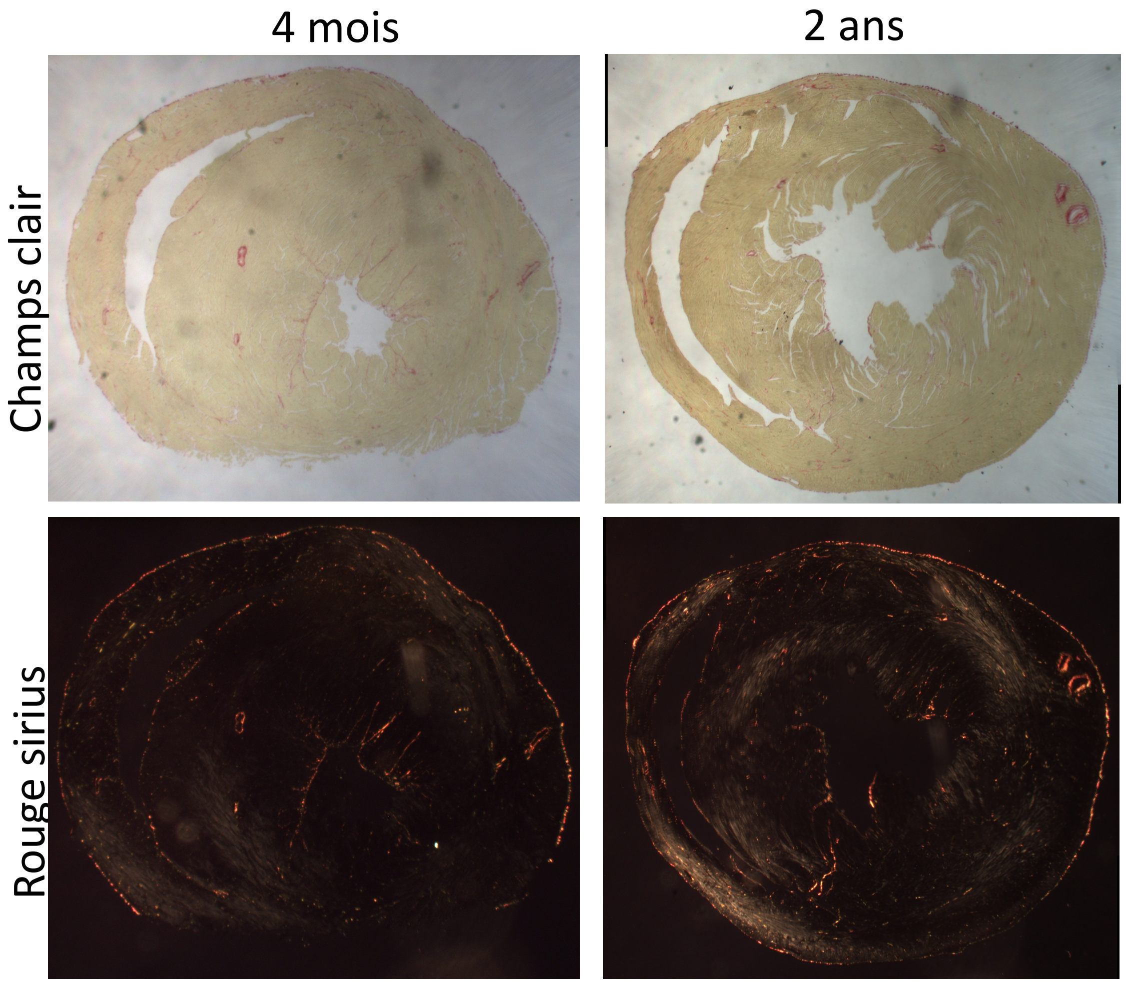
# On peut mesurer l'augmentation de collagène dans la media



# Le collagène peut aussi être mesuré dans les coupes de coeur



# Conclusions

- Une technique applicable pour quantifier le collagène de Type I dans les tissus aortiques et cardiaques
- La technique est peu chère et ne nécessite pas une formation avancée pour être utilisée
- Les autres types de collagènes sont aussi détectables avec la même technique

# Merci beaucoup pour votre attention

Remerciements:

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Florian Bouthiaux et Joanna Loglisci

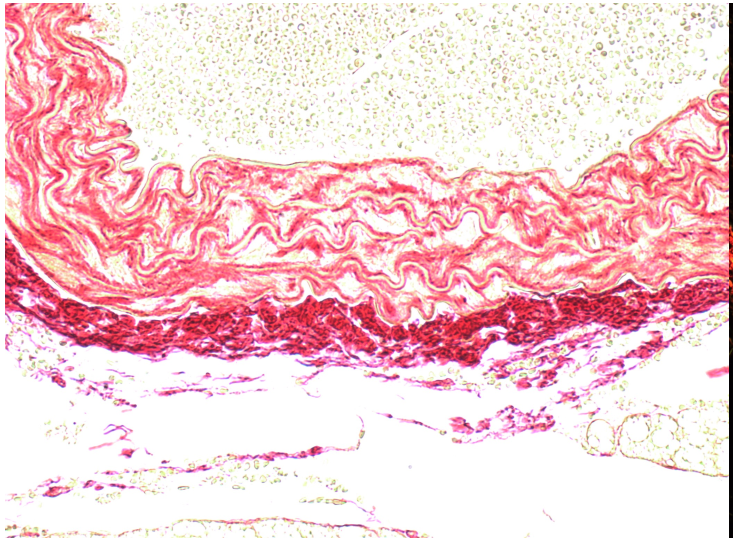
Christophe Durlet

Plateforme DimaCell de Dijon

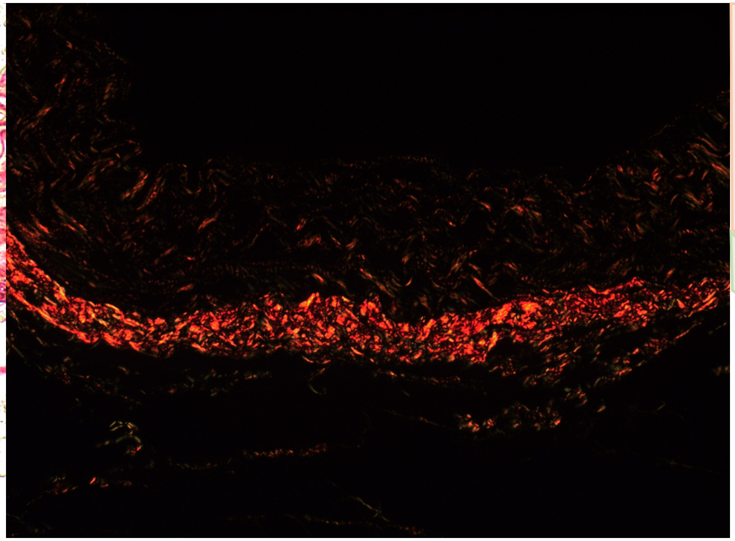
Laboratoire Chop, Université de Lausanne

# Semi-automatization de la quantification

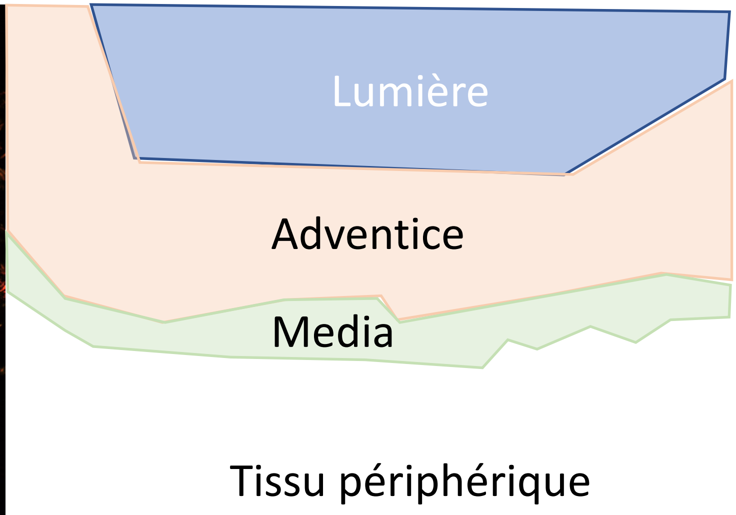
Fond clair



Rouge sirius

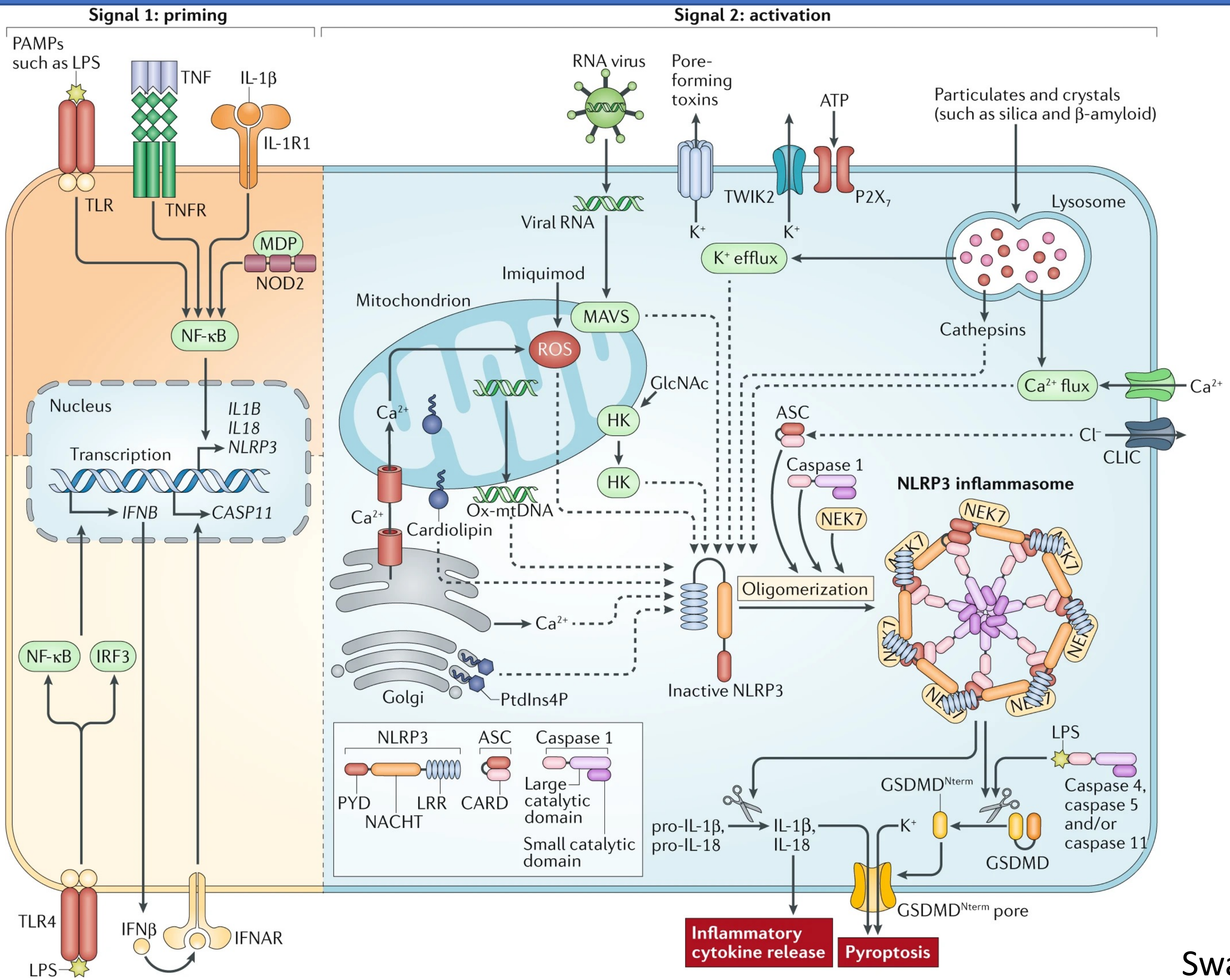


Segmentation



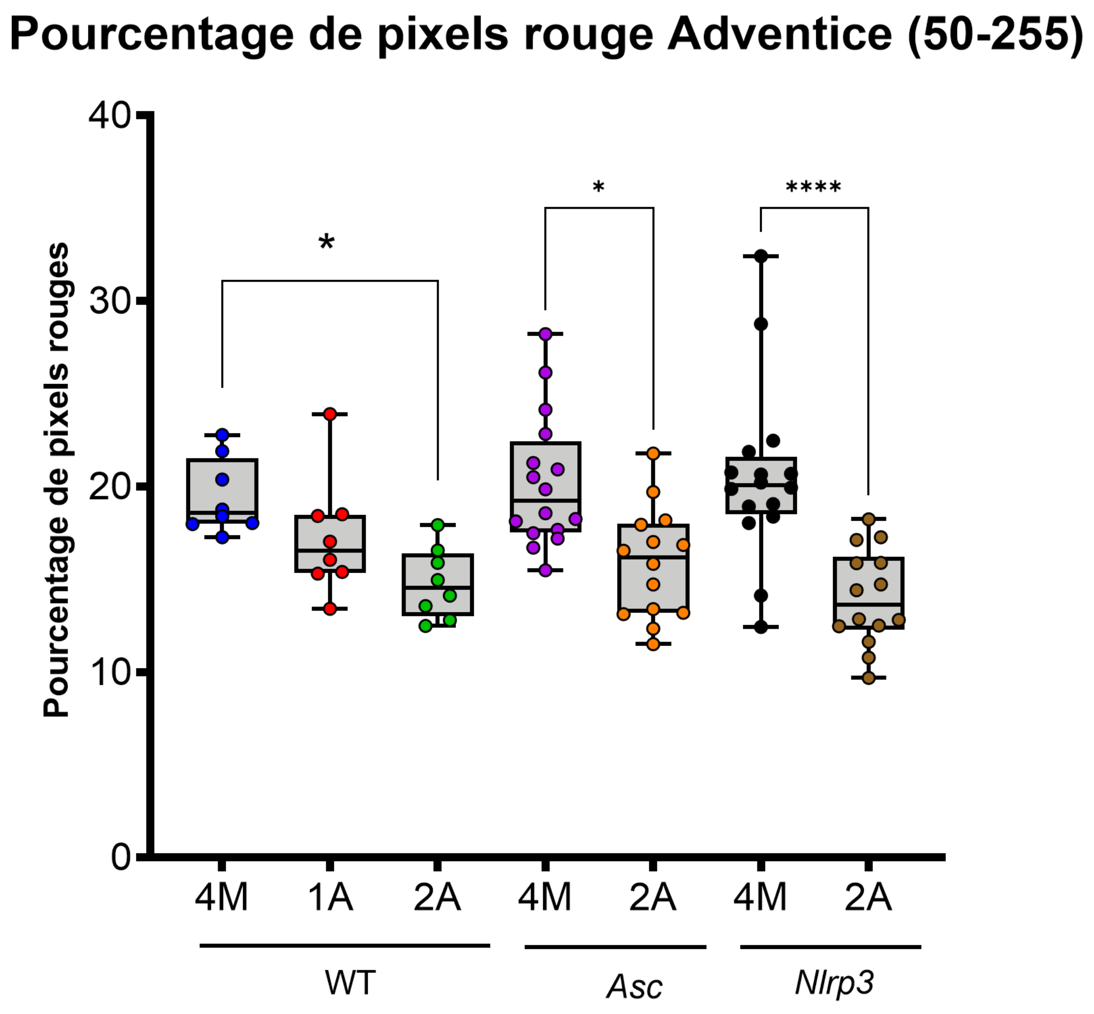

Séparation des canaux RGB → Seuillage par tranche d'intensité → Mesure de la surface positive

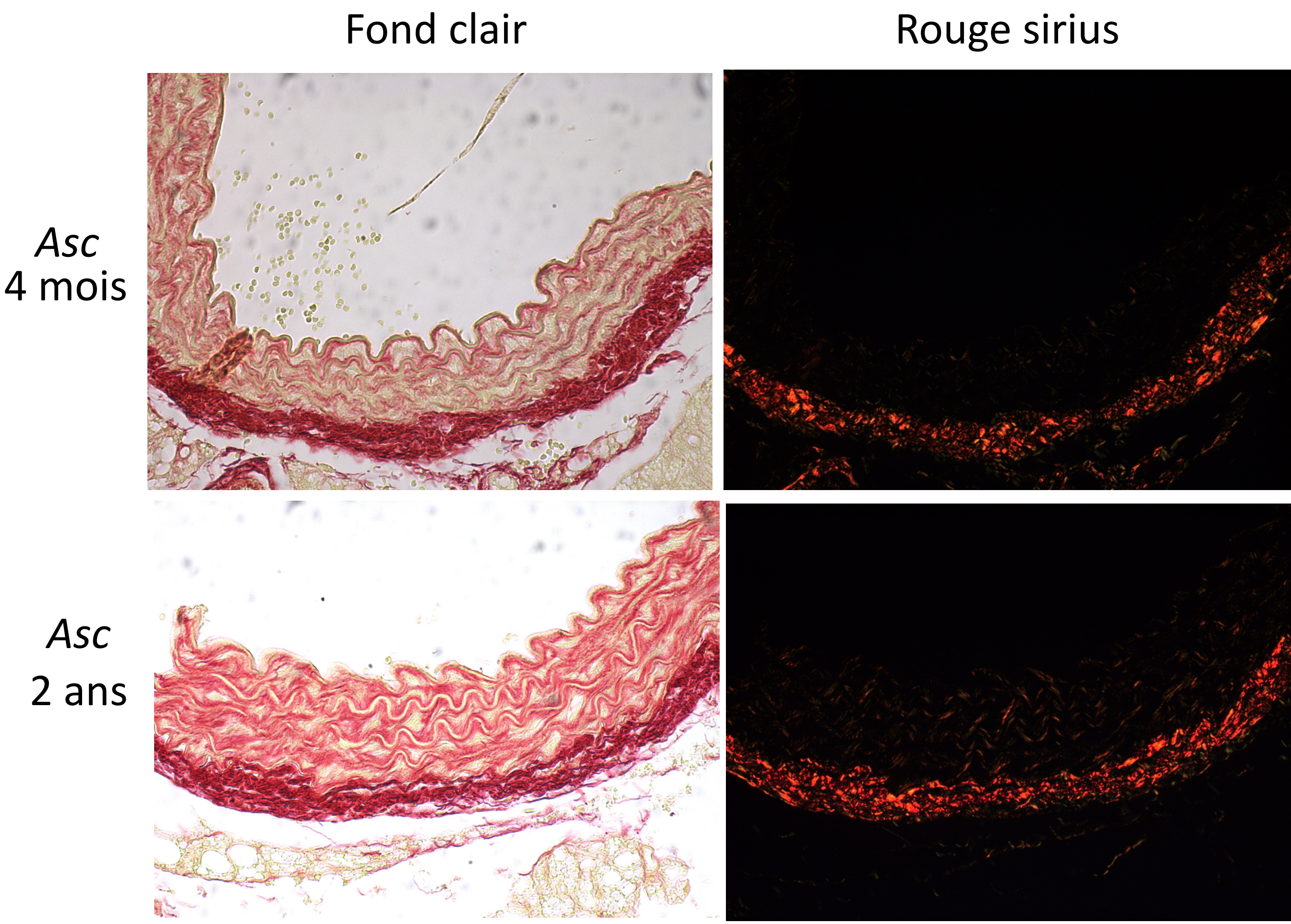
# Inflammasome NLRP3




Swanson et al, 2019

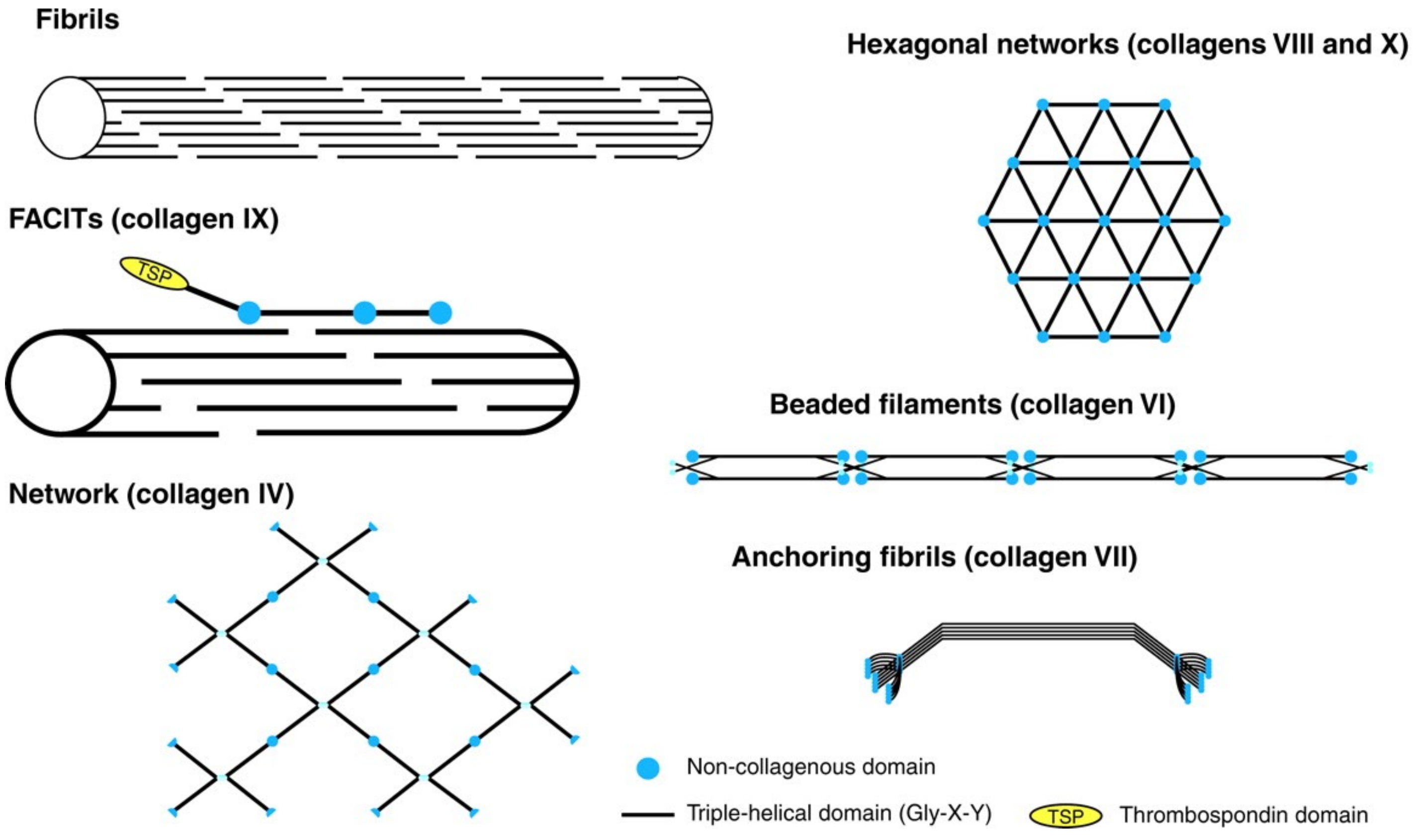
# Cette technique permet d’observer des differences selon les fonds génétiques des souris



# La grande diversité des collagènes

| Type | Gene | Disease | References, databases |
|---|---|---|---|
| I | COL1A1<br>COL1A2 | Osteogenesis imperfecta<br>Ehlers–Danlos syndrome | Marini et al. (2007)<br>Dalgleish (1997, 1998)<br>www.le.ac.uk/genetics/collagen<br>Bodian and Klein (2009)<br>http://collagen.stanford.edu/ |
| II | COL2A1 | Spondyloepiphyseal dysplasia<br>Spondyloepimetaphyseal dysplasia,<br>Achondrogenesis, hypochondrogenesis<br>Kniest dysplasia, Stickler syndrome | Bodian and Klein (2009)<br>http://collagen.stanford.edu/ |
| III | COL3A1 | Ehlers–Danlos syndrome | Dalgleish (1997, 1998)<br>www.le.ac.uk/genetics/collagen<br>Bodian and Klein (2009)<br>http://collagen.stanford.edu/ |
| IV | COL4A1 | Familial porencephaly<br>Hereditary angiopathy with nephropathy, aneurysms and muscle cramps syndrome | Van Agtmael and Bruckner-Tuderman (2010) |
| IV | COL4A3<br>COL4A4 | Alport syndrome<br>Benign familial haematuria | Van Agtmael and Bruckner-Tuderman (2010) |
| IV | COL4A5<br>COL4A6 | Alport syndrome<br>Leiomyomatosis | Bateman et al. (2009), Van Agtmael and Bruckner-Tuderman (2010) |
| V | COL5A1<br>COL5A2 | Ehlers–Danlos syndrome | Callewaert et al. (2008) |
| VI | COL6A1<br>COL6A2<br>COL6A3 | Bethlem myopathy<br>Ullrich congenital muscular dystrophy | Lampe and Bushby (2005) |
| VII | COL7A1 | Dystrophic epidermolysis bullosa | Fine (2010) |
| VIII | COL8A2 | Corneal endothelial dystrophies | Bateman et al. (2009) |
| IX | COL9A1<br>COL9A2 | Multiple epiphyseal dysplasia | Carter and Raggio (2009) |
| IX | COL9A3 | Multiple epiphyseal dysplasia<br>Autosomal recessive Stickler syndrome | Carter and Raggio (2009) |
| X | COL10A1 | Schmid metaphyseal chondrodysplasia | Grant (2007) |
| XI | COL11A1 | Stickler syndrome<br>Marshall syndrome | Carter and Raggio (2009) |
| XI | COL11A2 | Stickler syndrome<br>Marshall syndrome<br>Otospondylomegaepiphyseal dysplasia<br>Deafness | Carter and Raggio (2009) |
| XVII | COL17A1 | Junctional epidermolysis bullosa-other | Has and Kern (2010) |
| XVIII | COL18A1 | Knobloch syndrome | Nicolae and Olsen (2010) |




Sylvie Ricard-Blum, Cold Spring Harb Perspect Biol, 2011