Sharks, skeletons and spectral imaging: Exploring the hidden life of chondrocytes and tessellated cartilage

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CORBEL shared services for life-science

PID: 2358 - Morphology and structure of chondrocytes and their association with mineralizing tissues in shark and ray cartilage

User: **Mason Dean**, Max Planck Institute of Colloids & Interfaces, Berlin, Germany

RI 1: EMBRC Oceanographic Observatory Banyuls-sur-Mer, France

RI 2: EuBI ICFO, Super Resolution Node, Barcelona, Spain - **Maria Marsal et al**


Mason
Mélanie
Maria
Júlia
Pascal
Emilio
Michael
Sylvie
Marina


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Institute
X
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Founded 1992
Markus Antonietti (Colloid Chemistry)
Reinhard Lipowsky (Theory & Bio-Systems)
Helmuth Möhwald (Interfaces; until 2013)

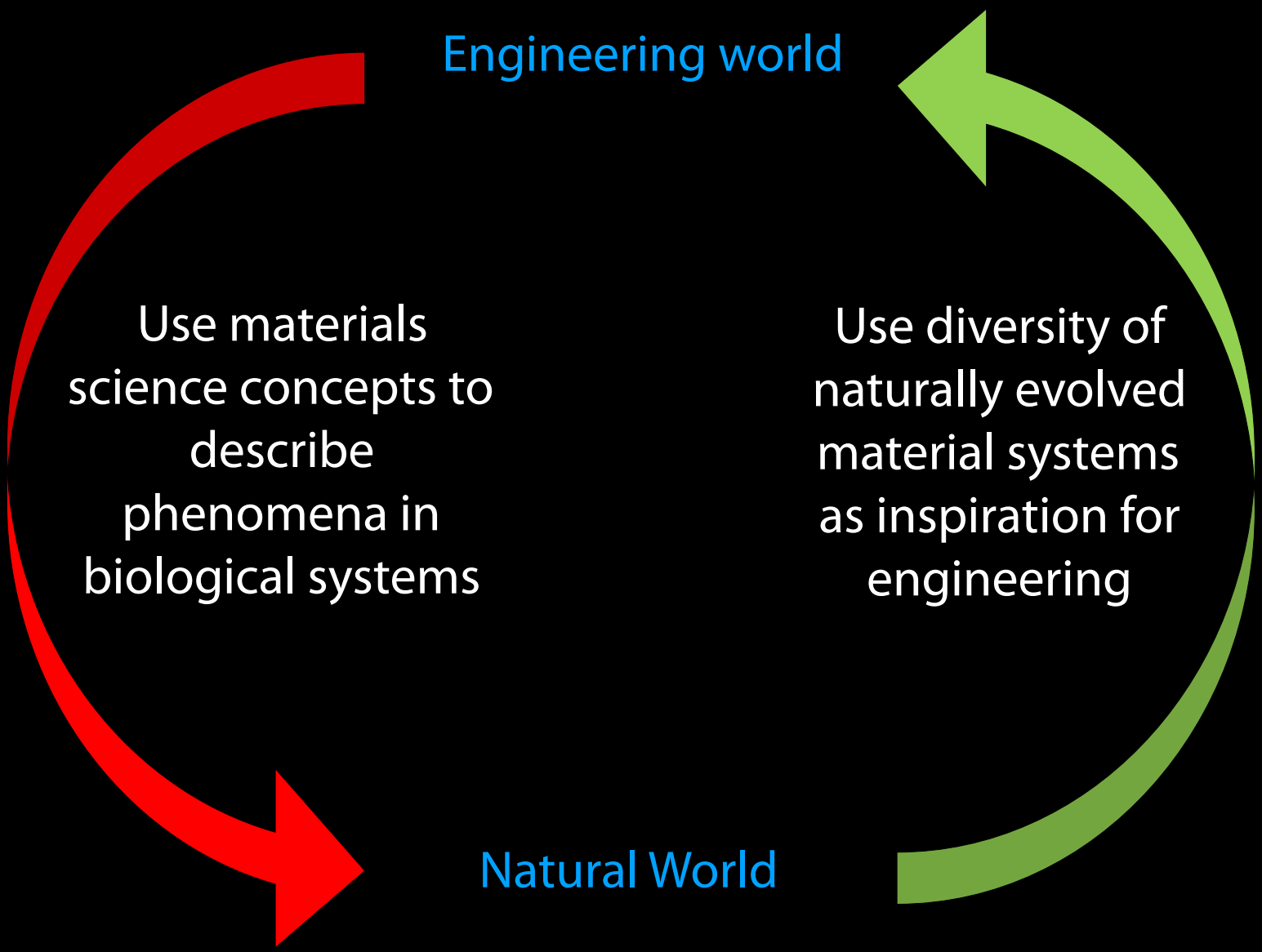
Since 2003
Peter Fratzl (Biomaterials)

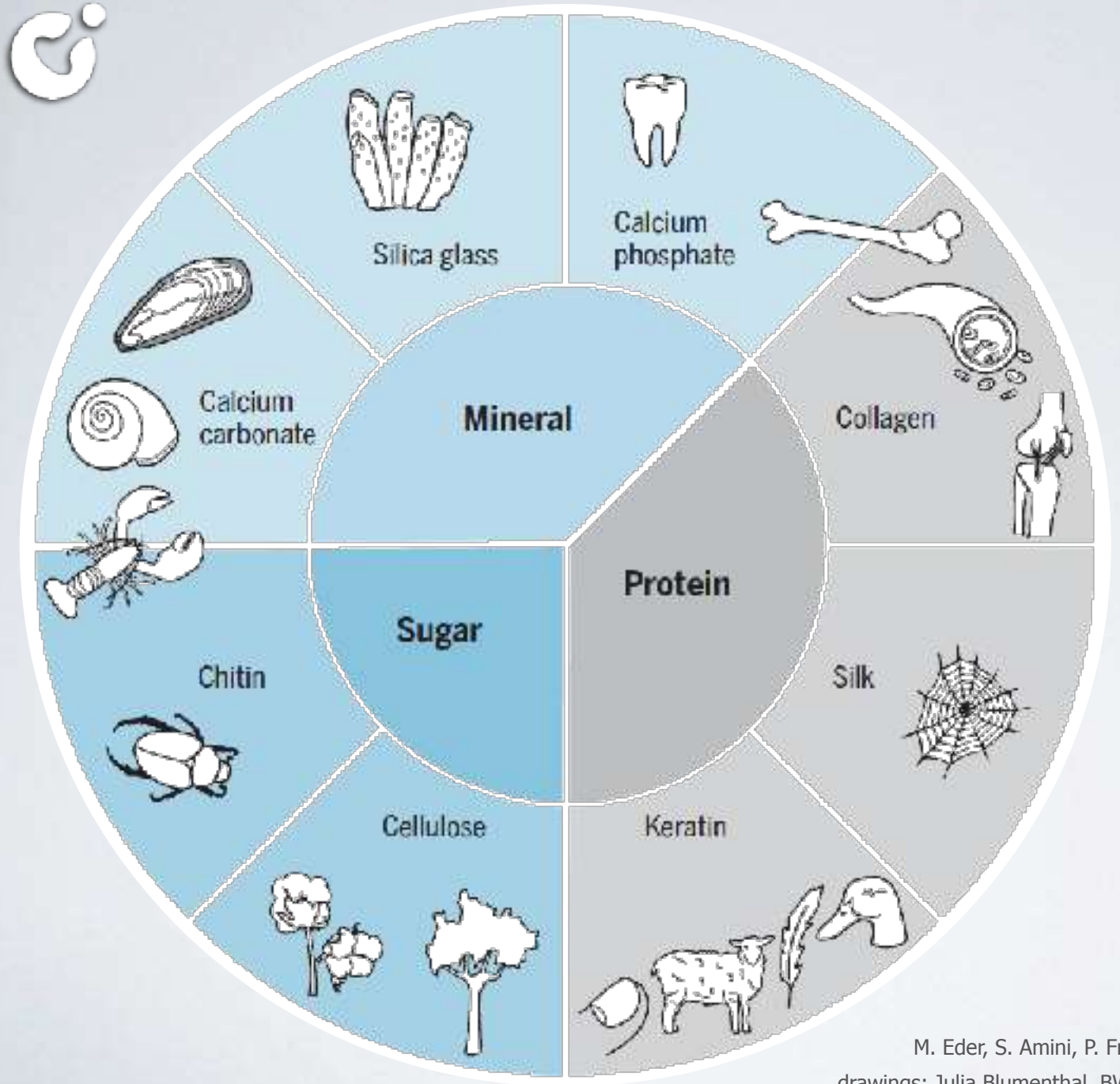
Since 2008
Peter Seeberger (Biomolecular Systems)

Since 2014 – W2 Max Planck Group
Kerstin Blank (Mechano-bio-chemistry)

External members:
Jürgen Rabe (HU Berlin - Physics)
Ulrich S. Schubert (FSU Jena - Chemistry)
Joanna Aizenberg (Harvard U – SEAS)

Biological Materials Sciences





Few base components

A diversity of structures

M. Eder, S. Amini, P. Fratzl, Science **2018** (November 2)

drawings: Julia Blumenthal, BWG & Kunsthochschule Weißensee

Department of Biomaterials

Sensory biomaterials

Yael Politi

Cécile Bidan

Bacterial biofilms

Biomolecular interfaces

Emanuel Schneck

Water biomolecule interaction

Luca Bertinetti

Hierarchical materials

Wolfgang Wagermaier

Plant Material Adaptation

Michaela Eder

Skeletal material evolution

Mason Dean

Mechanobiology

Richard Weinkamer

Amaia Cipitria

Extracellular matrix in disease & regeneration

Material quality in osteoporosis, Genetic bone diseases, regeneration

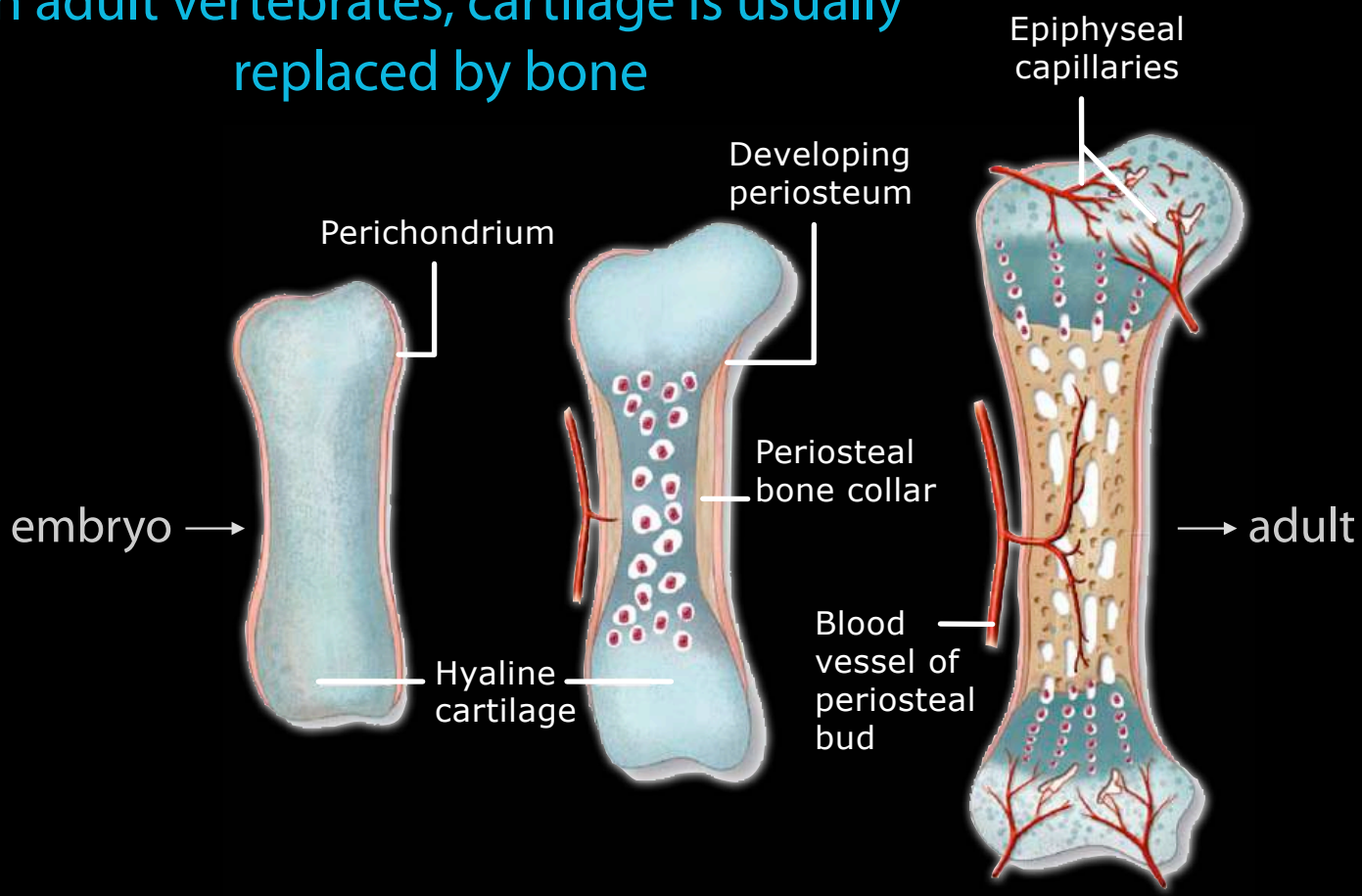
Berlin-Brandenburg School for Regenerative Therapies

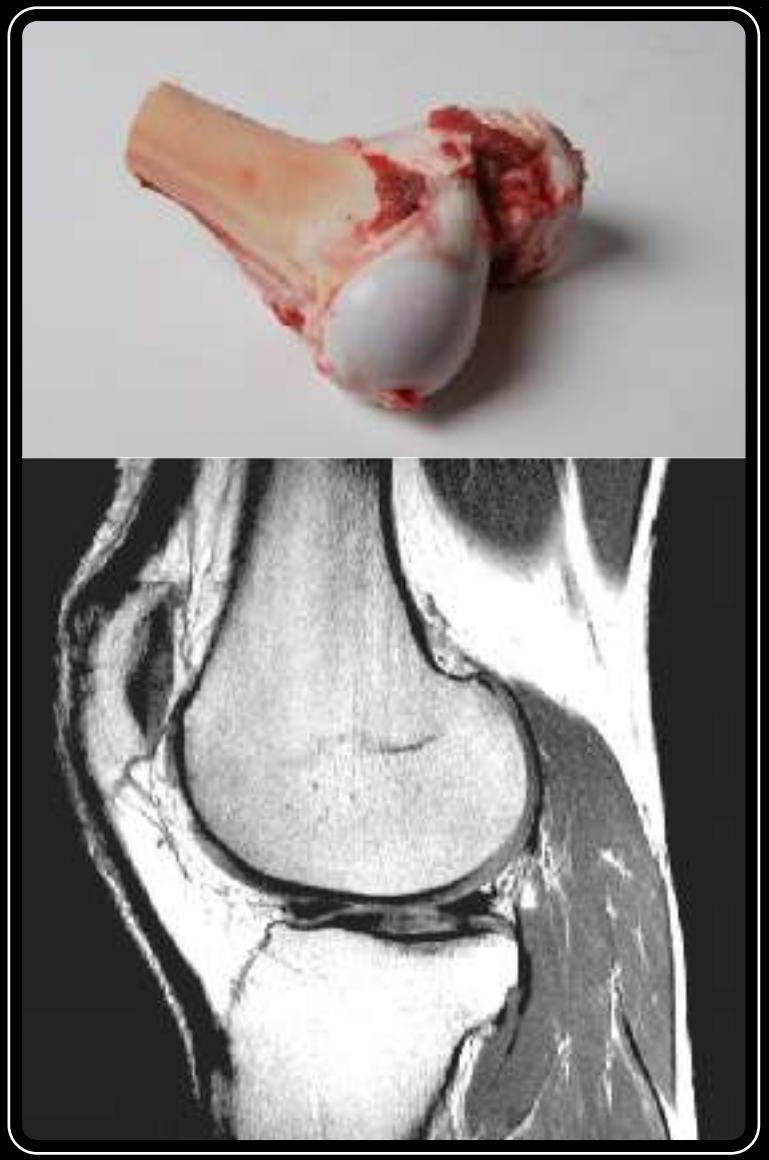
Biological materials → materials sciences

Biological materials → medical application

The scientific questions

In adult vertebrates, cartilage is usually replaced by bone

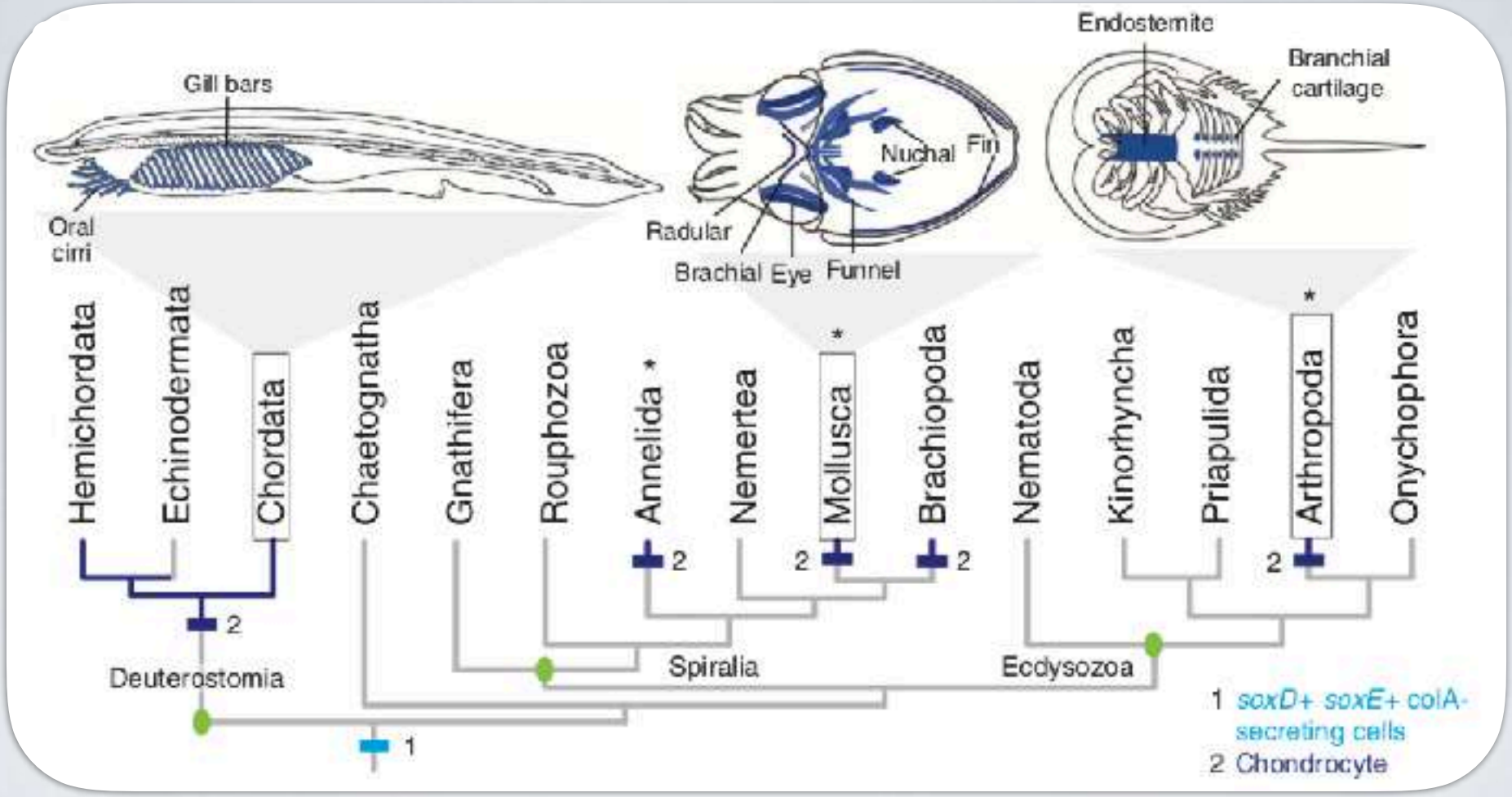




Cartilage is...

- √ Incapable of repair
- √ Aneural
- √ Avascular

Cartilage is stubborn.

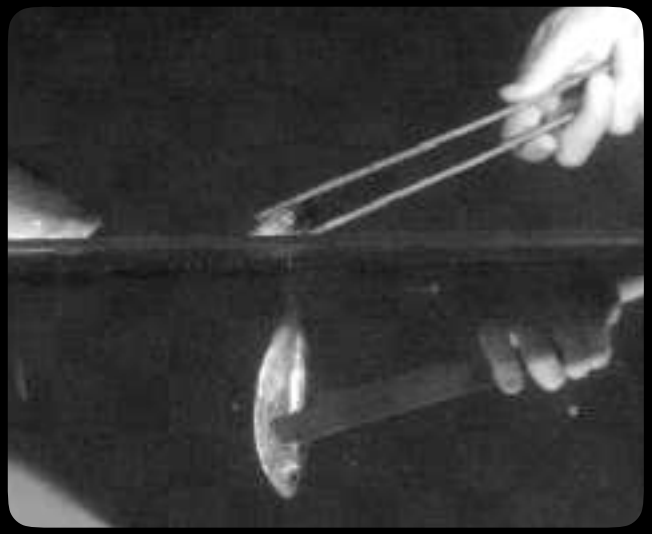


Nature 533, 86-89, 2016

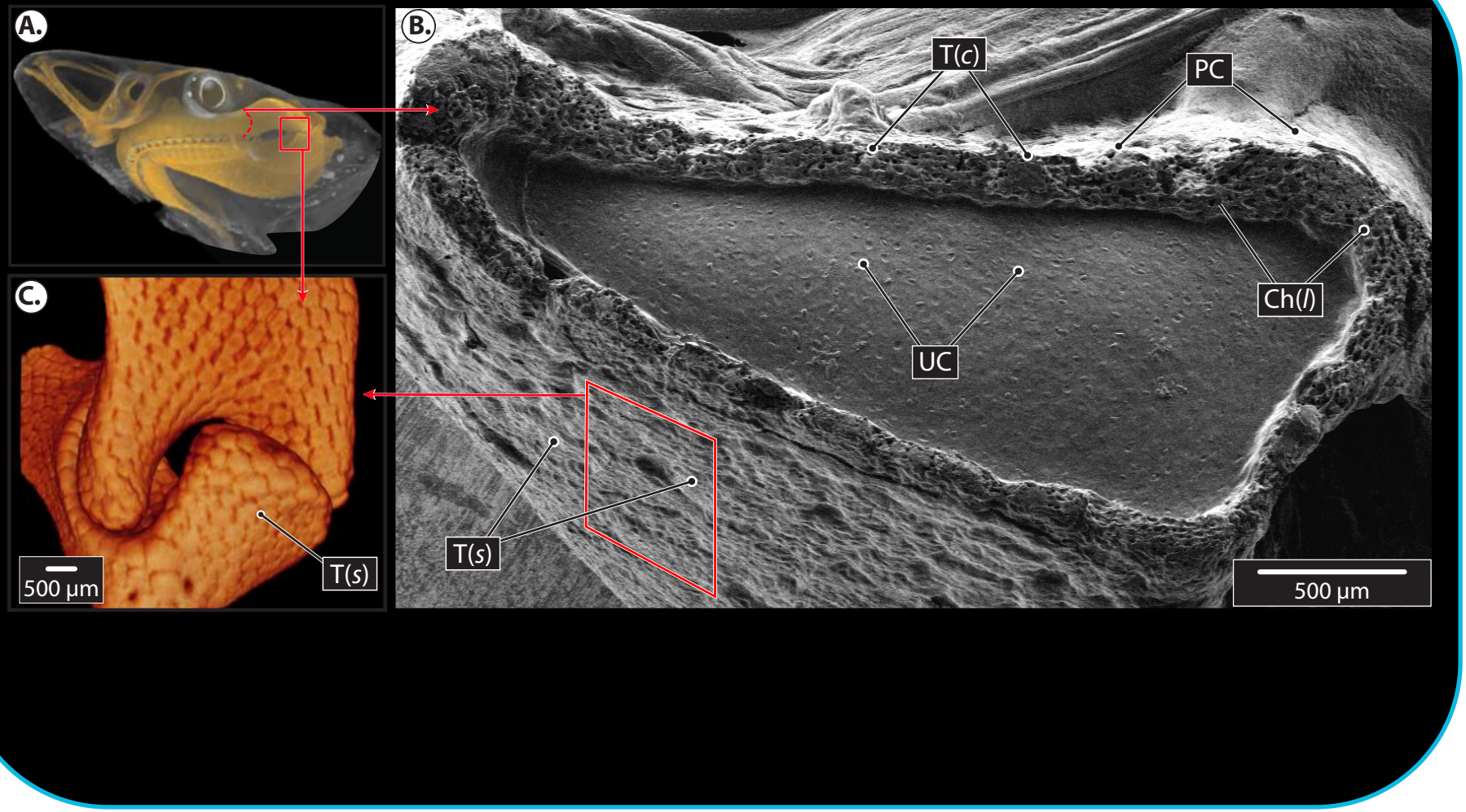
Current Biology 26, R667-R668, 2016

Cartilage is fickle.

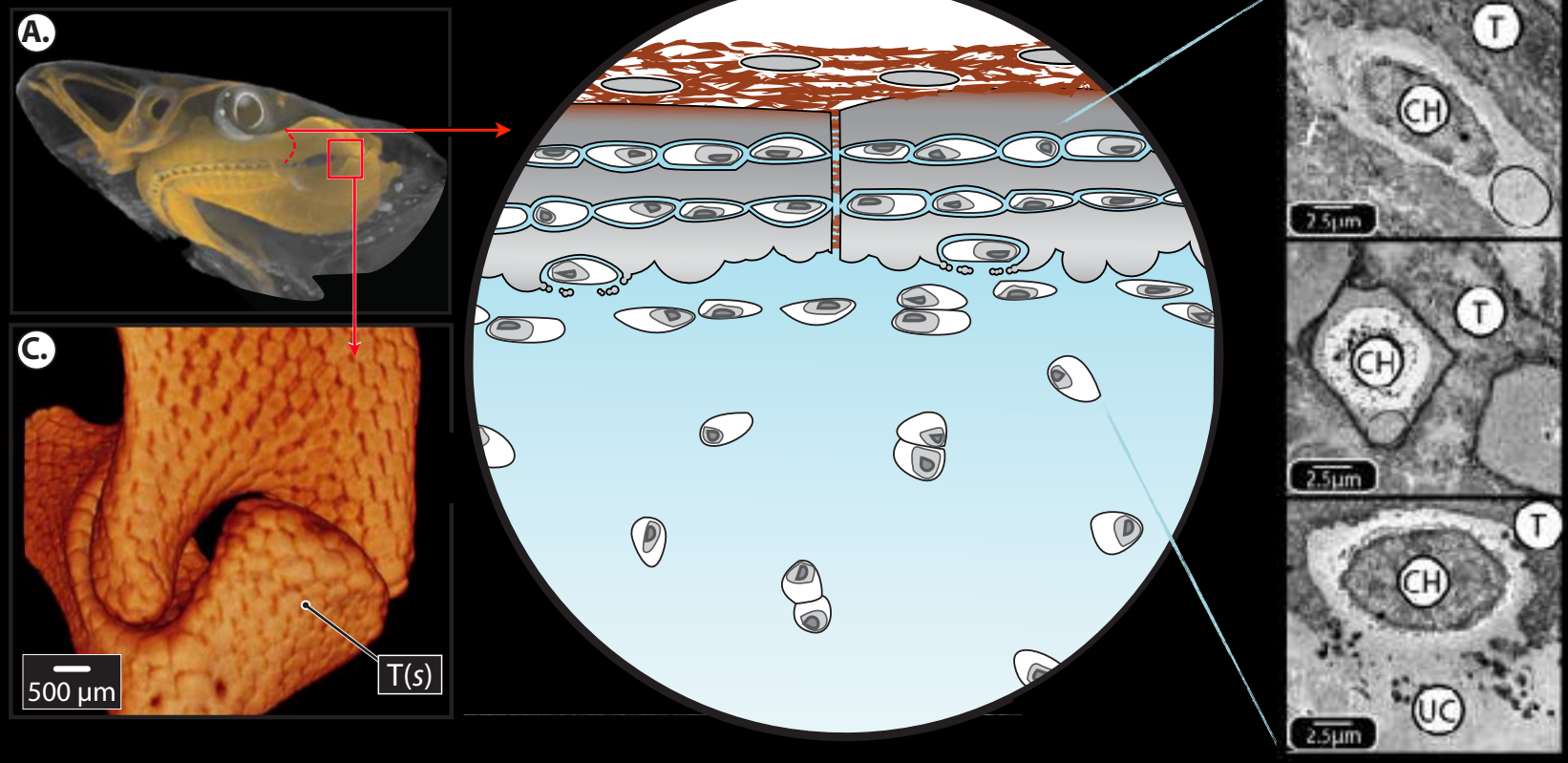
The scientific questions



The scientific questions

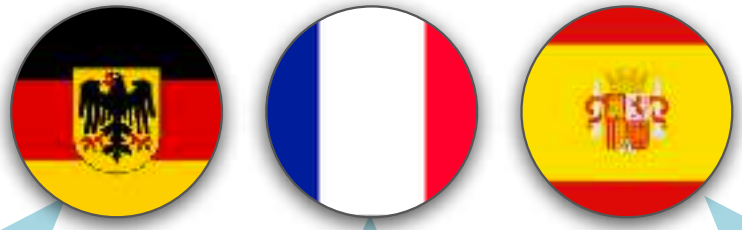


The scientific questions



Can shark cartilage be developed as a model for cartilage biology?
…skeletal mineralization? …chondrocyte longevity?

Experience with CORBEL joint service provision



Biological context

Material & structural characterization

Animal collection & care

Tissue prep & histology

Light microscopy

Label-free microscopy

Development of a live cell staining and imaging protocol for tissues collected in ~~Portugal~~ and imaged in Spain France!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Pyrenees
Mediterranean
Marine labs

Observatoire Océanologique de Banyuls – sur – Mer

Founded in 1881 by Henri de Lacaze - Duthiers

Observatoire Océanologique de Banyuls – sur – Mer

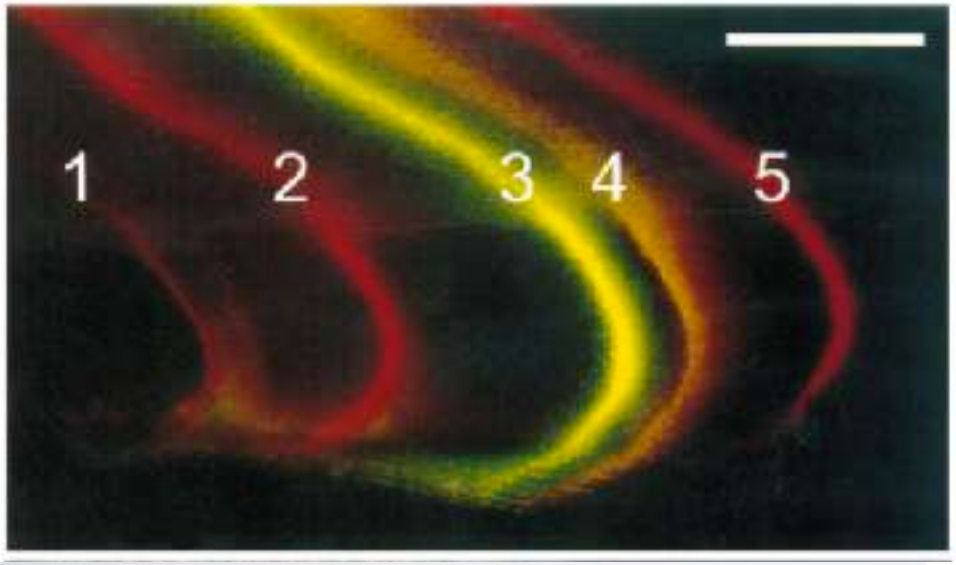
Pierre and Marie Curie University

French National Center for Scientific Research

Aquarium

Laboratories

Raja clavata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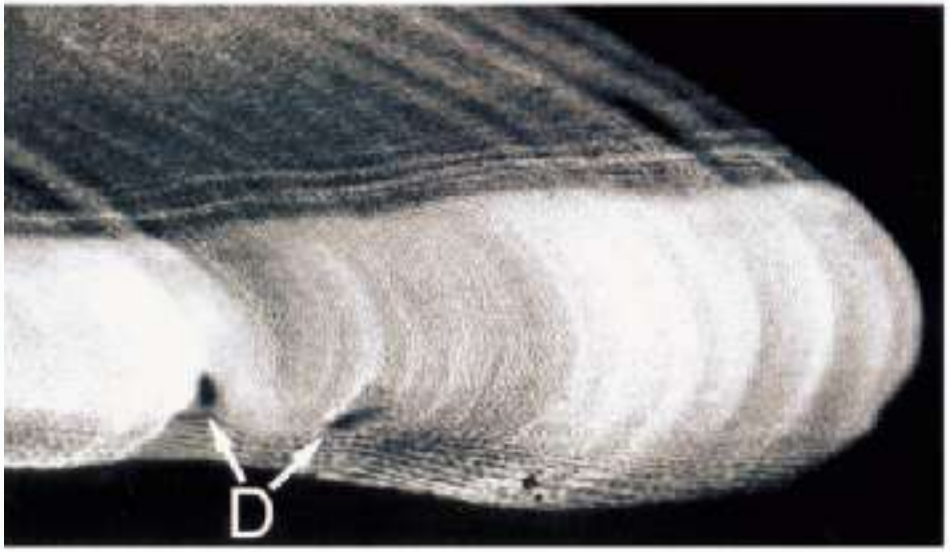
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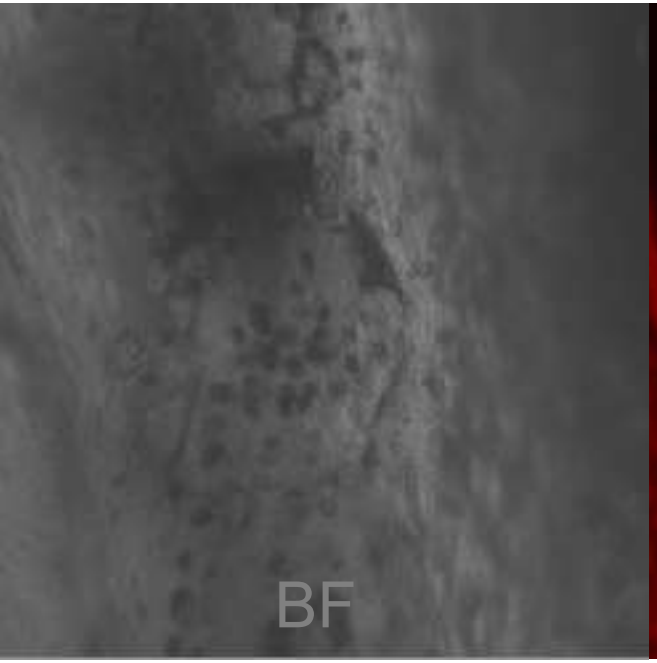
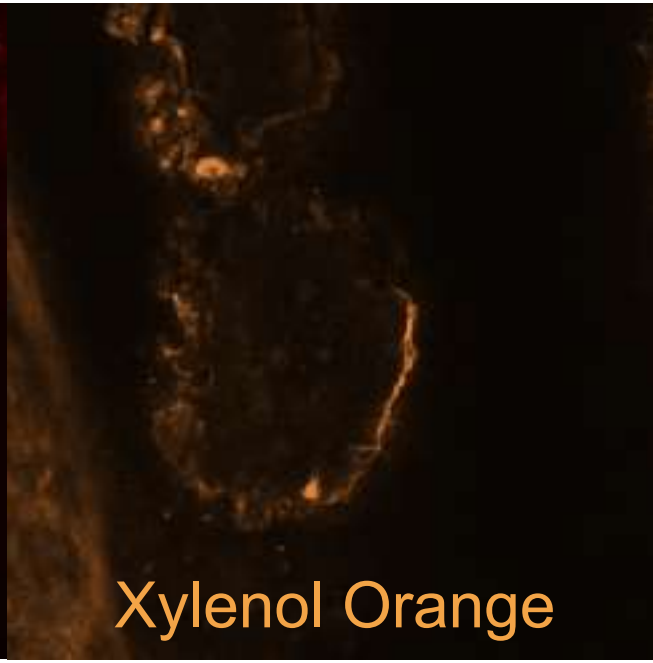
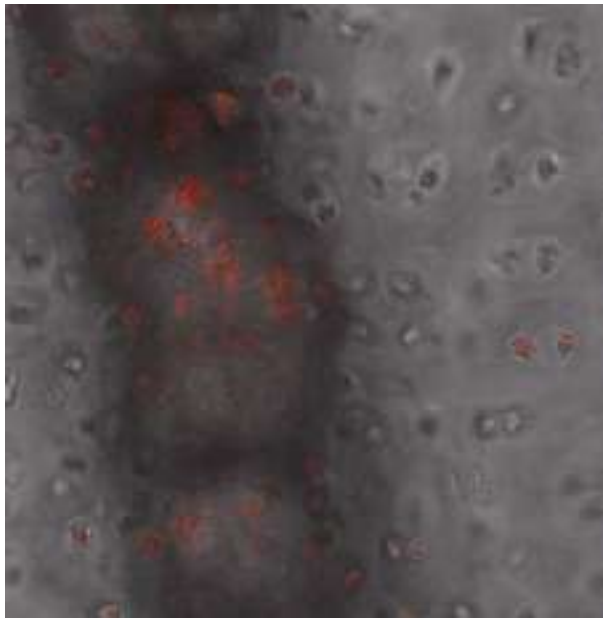
Raja clavata

Raja clavata

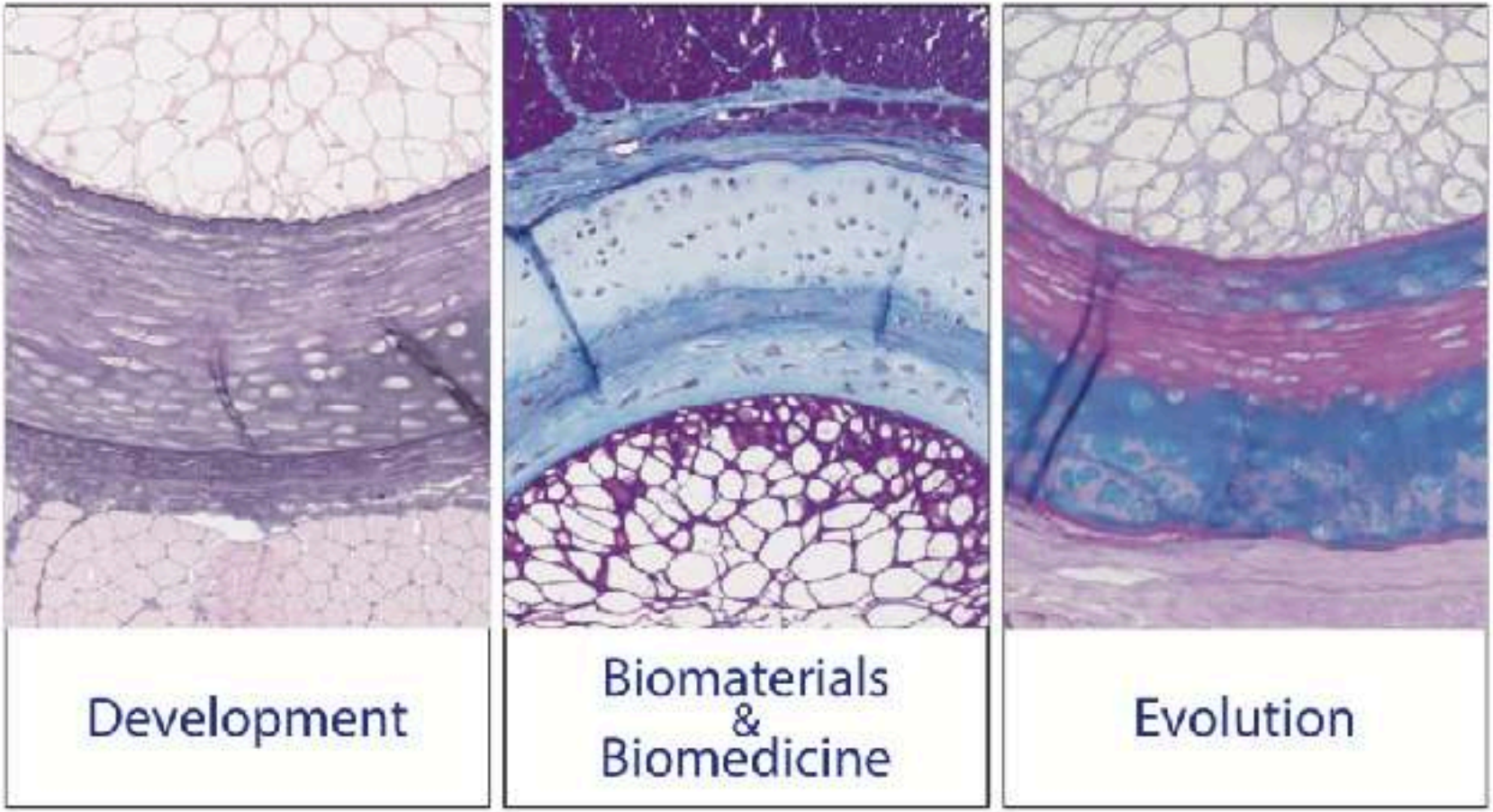






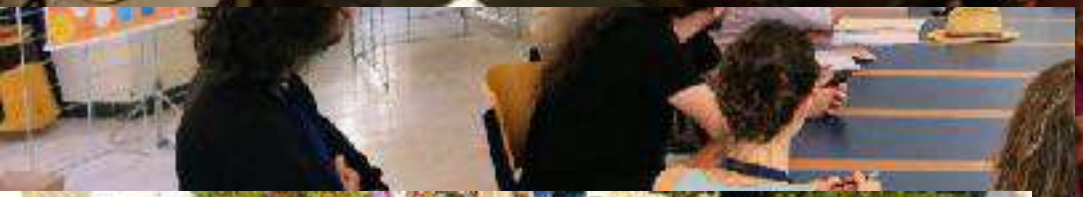
Comparative Cartilage Biology

Conference, June 24-26 2019



https://ccbconference.wixsite.com/ccb2019


BANYUL

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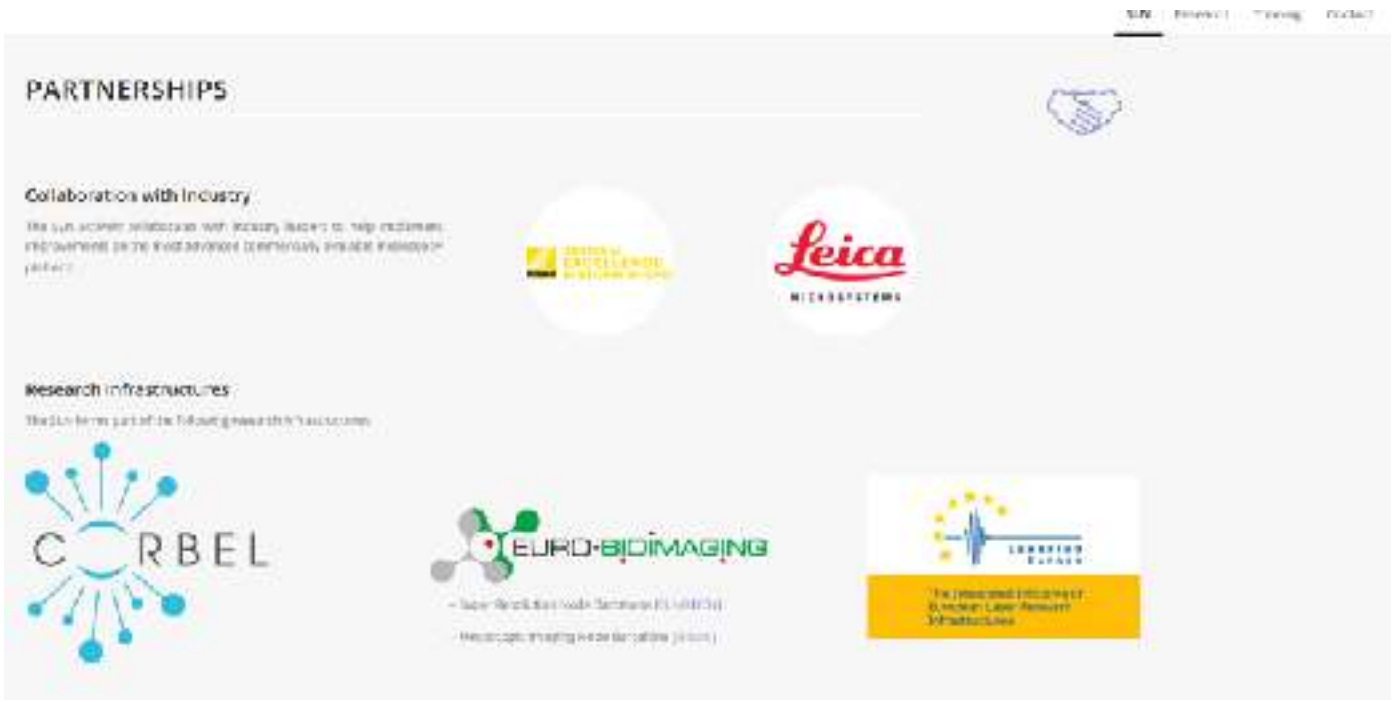
ICFO Research Team Super-resolution Light Microscopy & Nanoscopy



http://sln.icfo.eu/

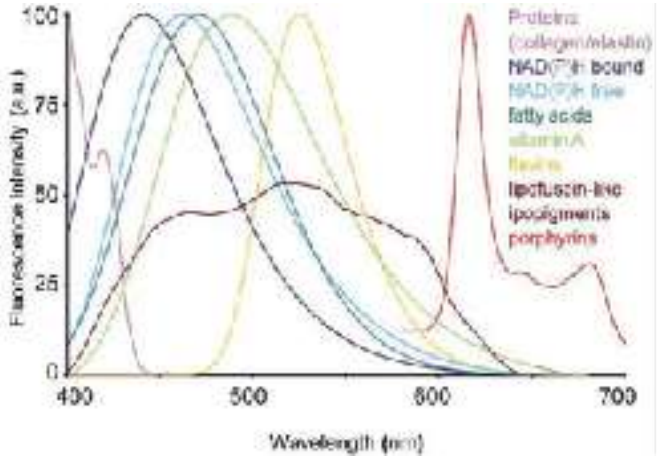
label-free imaging modalities

- Hyperspectral Laser Scanning Confocal Microscopy
- multiphoton approaches (Two-Photon Excited Fluorescence (TPEF) and Second Harmonic Generation (SHG)


CFO

Autofluorescence (AF)

- AF is emission of light when biological samples are excited with a proper wavelength, due to the presence of intrinsic molecules acting as endogenous fluorophores



Croce and Bottiroli 2014

Natural fluorophores

- Metabolic states
- pathologies

- there is a strict relationship between the AF and the morphofunctional properties of biological tissues

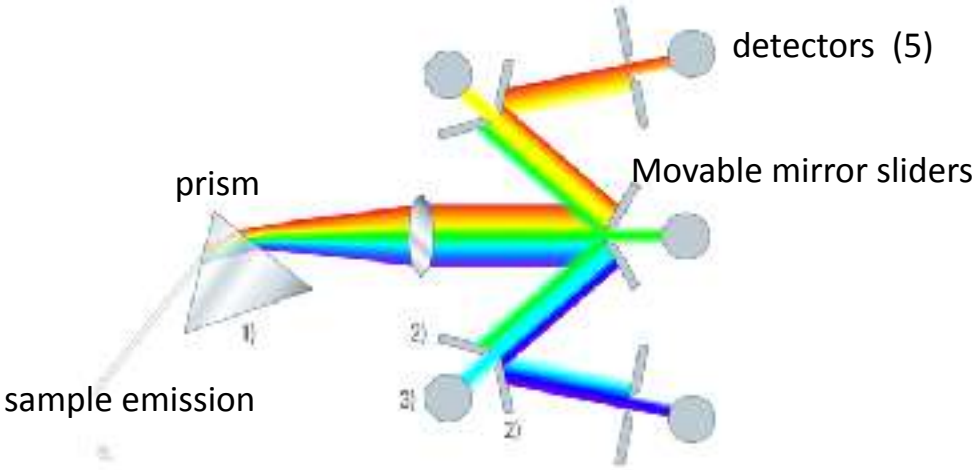
"Optical Biopsy"

✓ Diagnostic information *in situ*
✓ Non-invasive or minimally invasive
✓ No need of tissue removal
✓ No exogenous markers

AF detection and data interpretation

1. Current instrumentation for detection

Multispectral imaging with Leica SP8 confocal microscope: White light laser + SP (spectral) detector



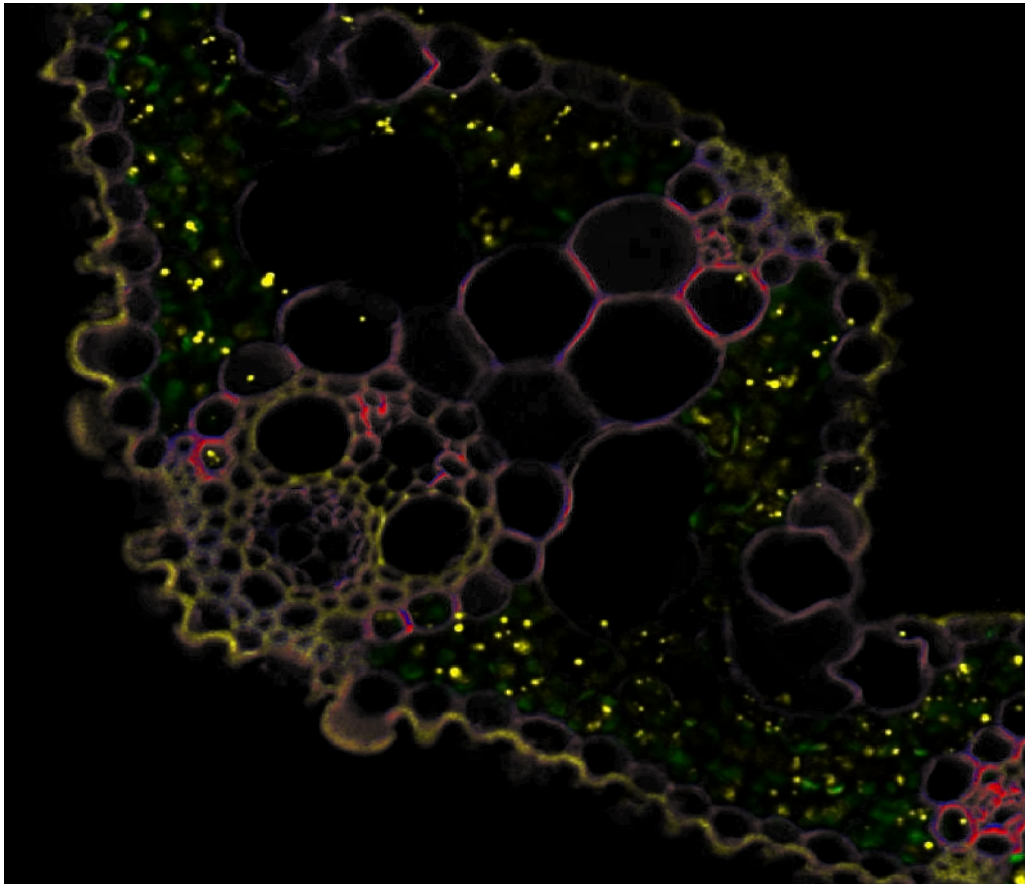
Adapted from Leica microsystems

White light laser 470-670nm, 1nm spectral resolution

The SP detector is a multi-band detection system. Every slit transmits the desired wavelength band for the different channels

AF detection and data interpretation

2. Analytical procedures (mathematical algorithms)



(Gómez-Sánchez et al., 2020 submitted)

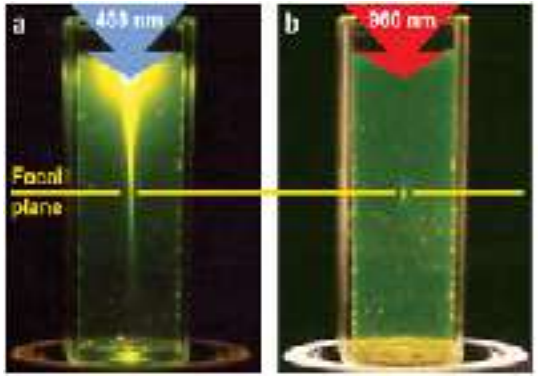
Autofluorescence of a rice leaf cross-section analysed by Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS)

Distribution of components are shown in false color:

- vesicles (yellow),
- lignin A (red),
- lignin B (blue)
- and chlorophyll (green)

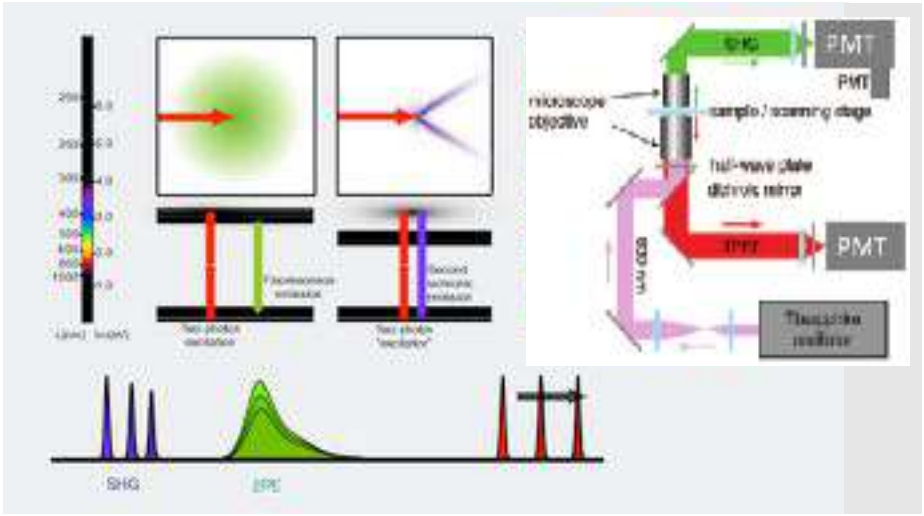
Image acquired in ICFO and analyzed by the Chemometric Group of the University of Barcelona. (Gómez-Sánchez, A.; de Juan, A).

Second Harmonic Generation (SHG) imaging & two-photon excitation fluorescence (TPEF)



Linear Excitation

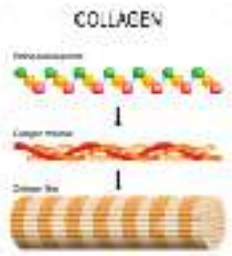
Non-linear Excitation

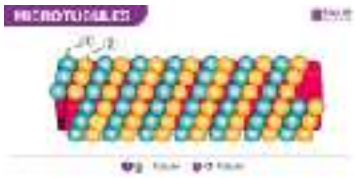


Emilio J. Gualda

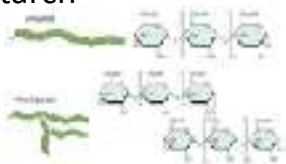
Sources of Second Harmonic Generation

Non-centrosymmetric materials: chiral molecules, highly ordered structures.



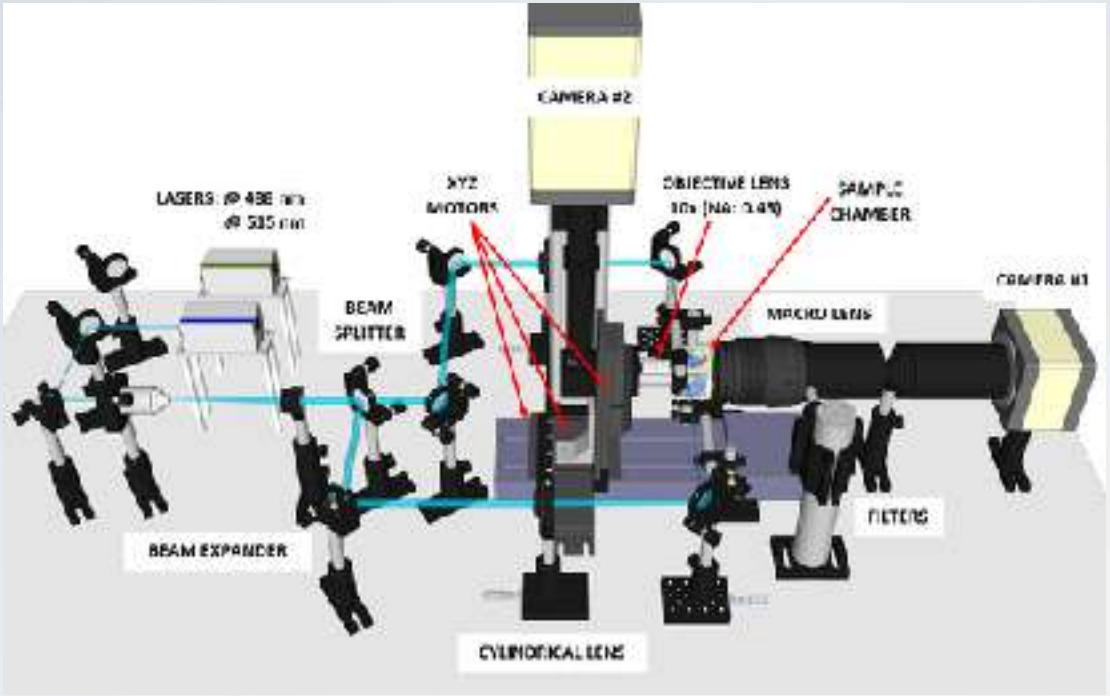


Starch

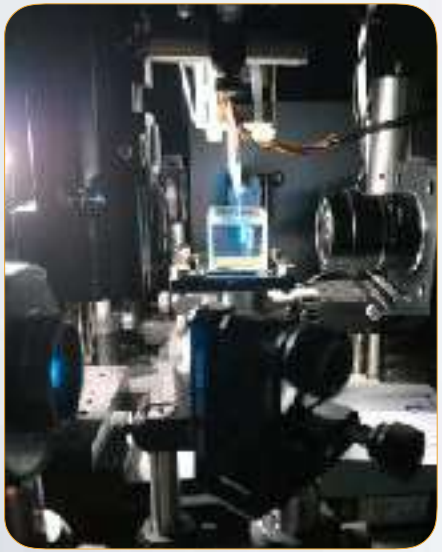


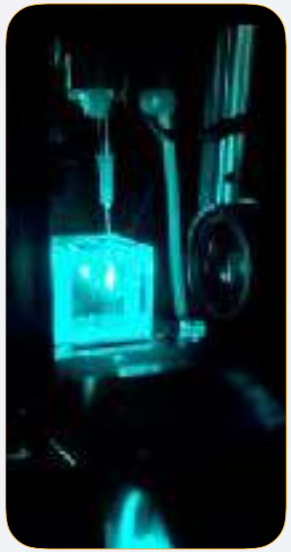
Light-sheet fluorescence microsocopy

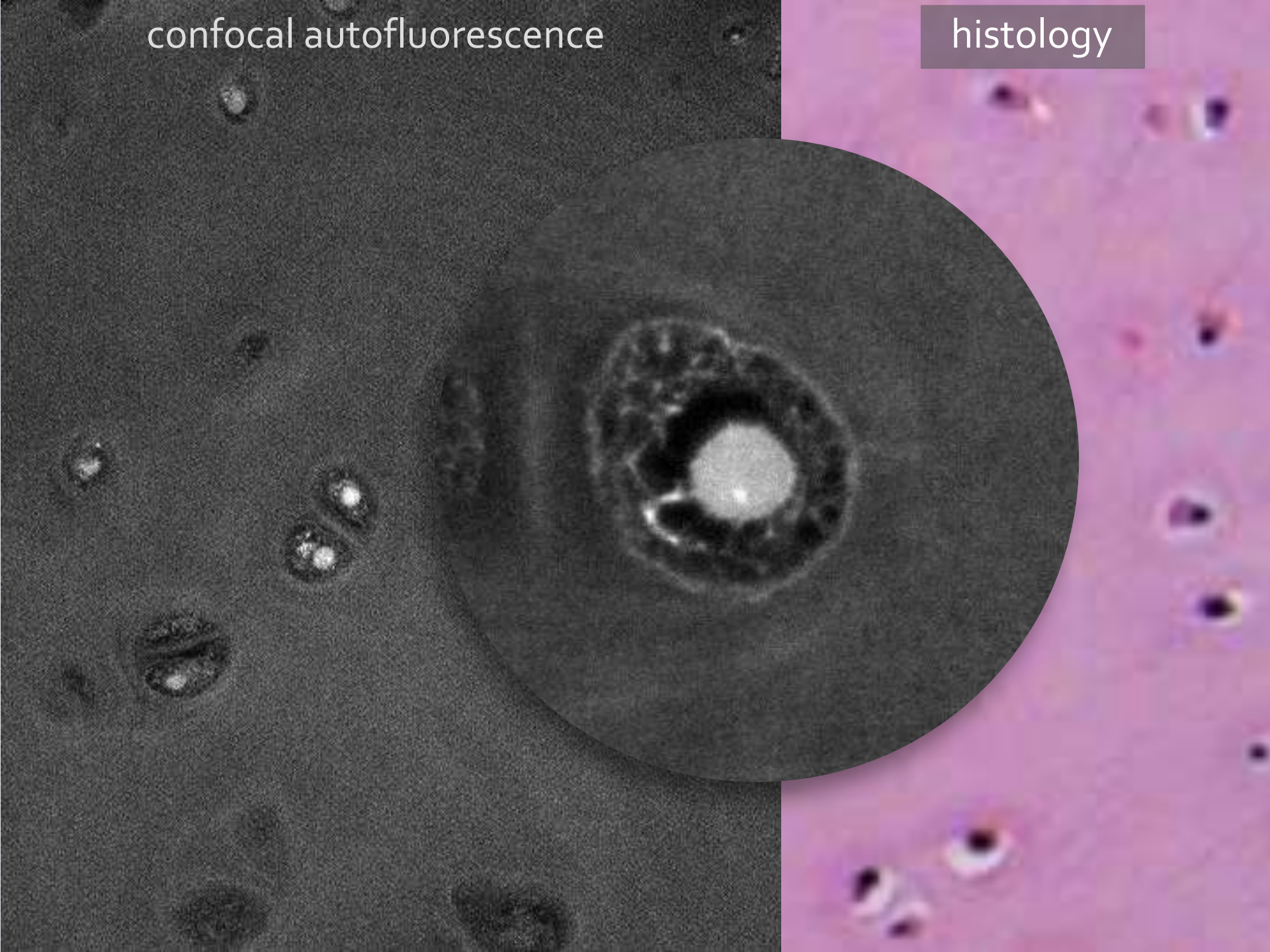
The sample is illuminated with a plane of light. Illumination and detection are perpendicular

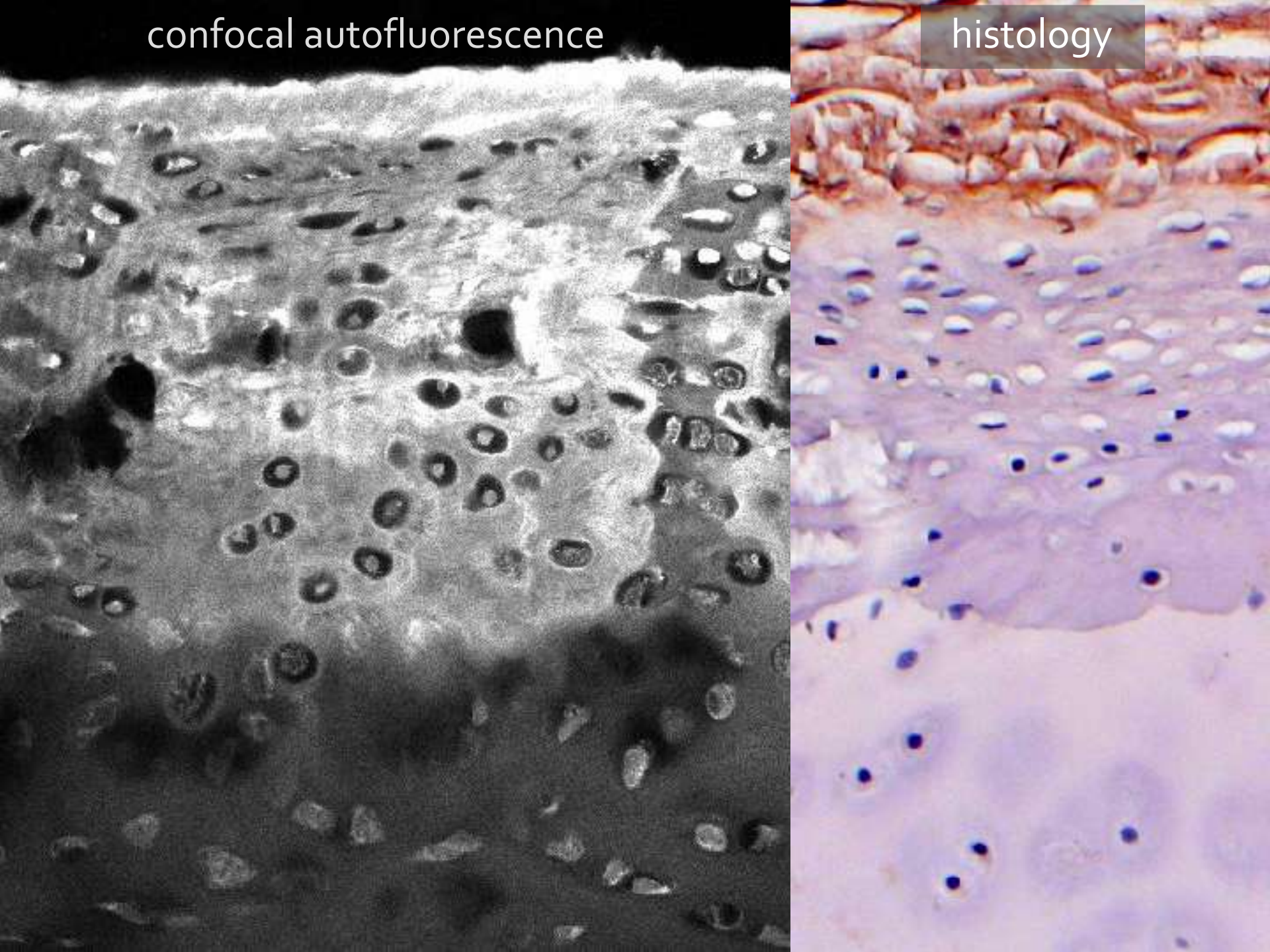


- Low photobleaching
- Fast imaging
- Big samples
- Multi-view acquisition






confocal autofluorescence
histology


confocal autofluorescence
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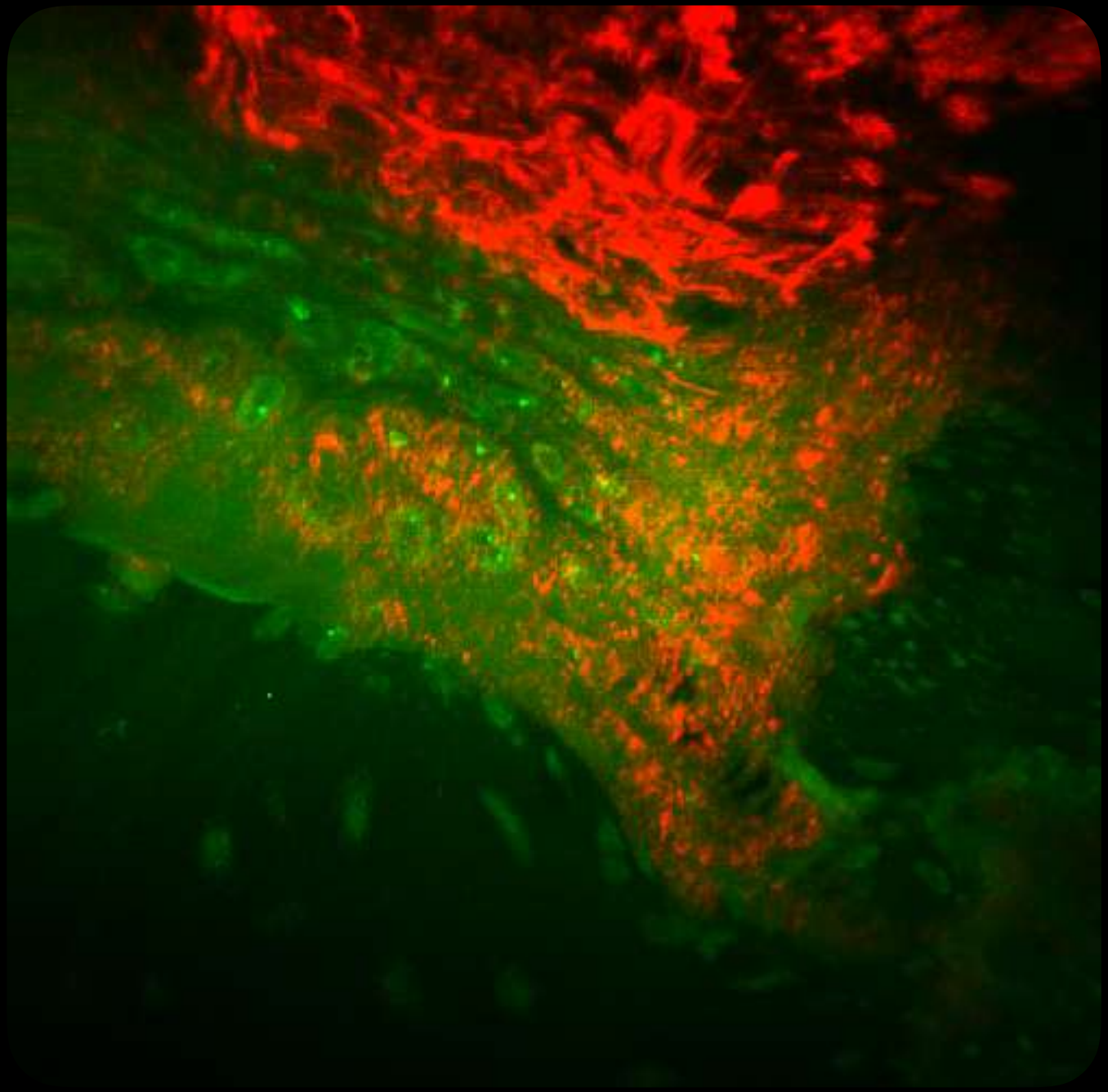
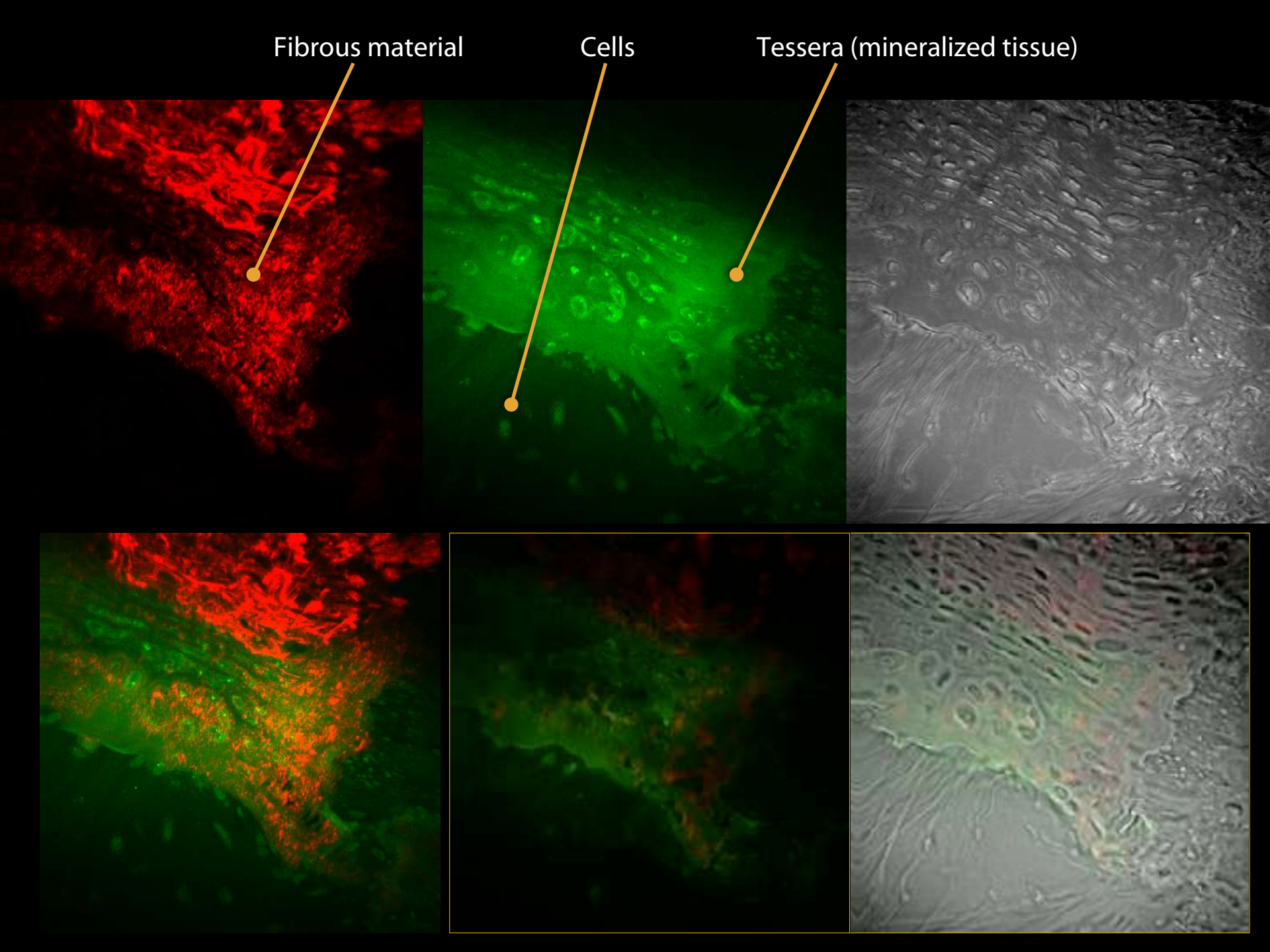
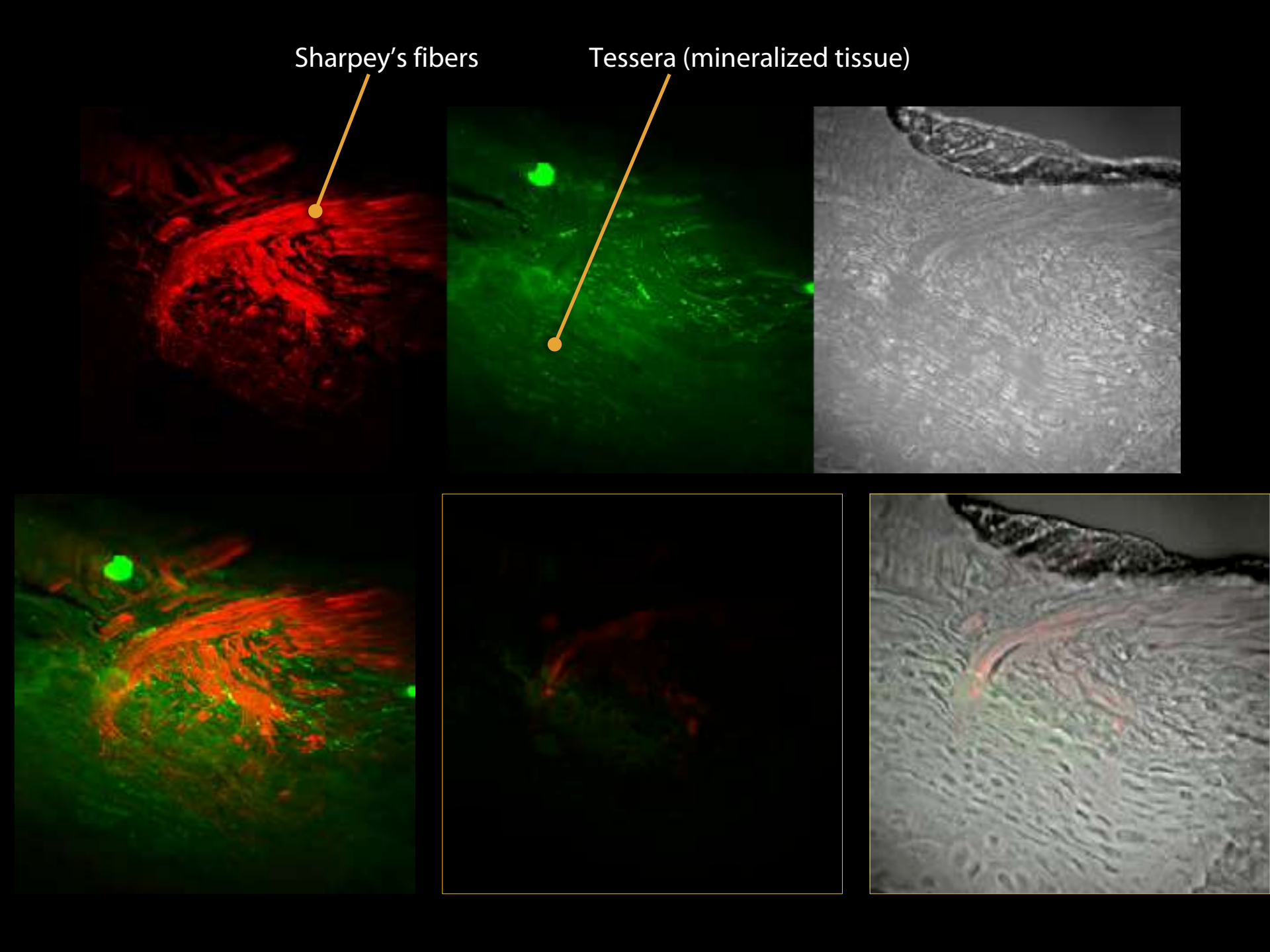
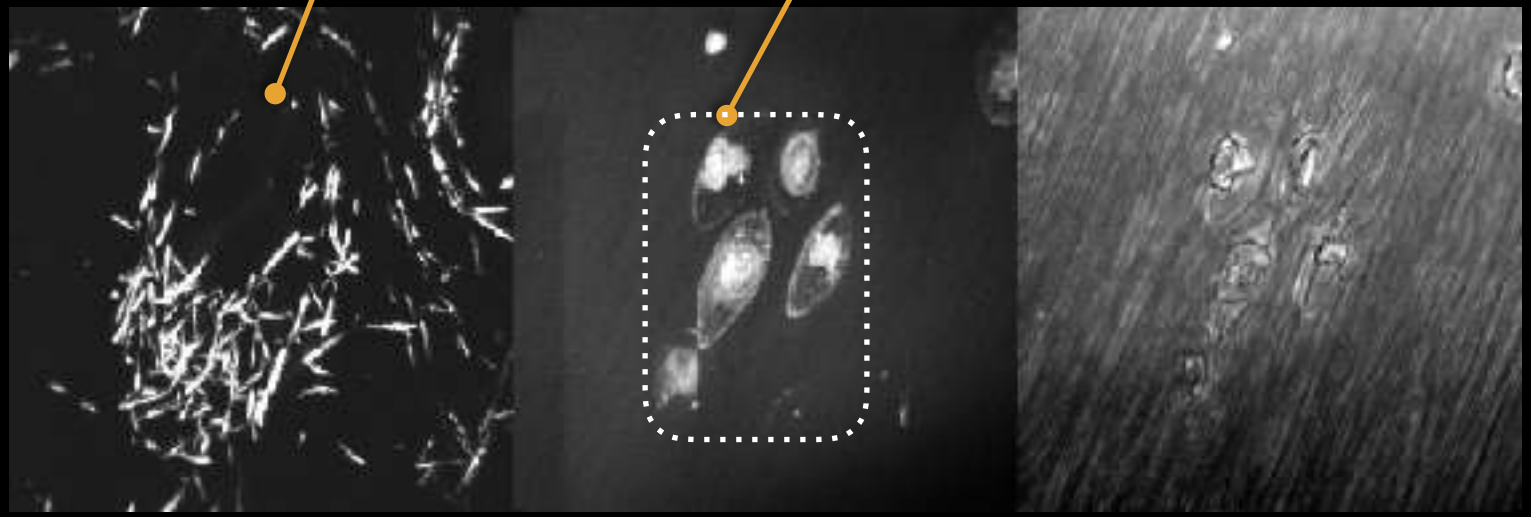


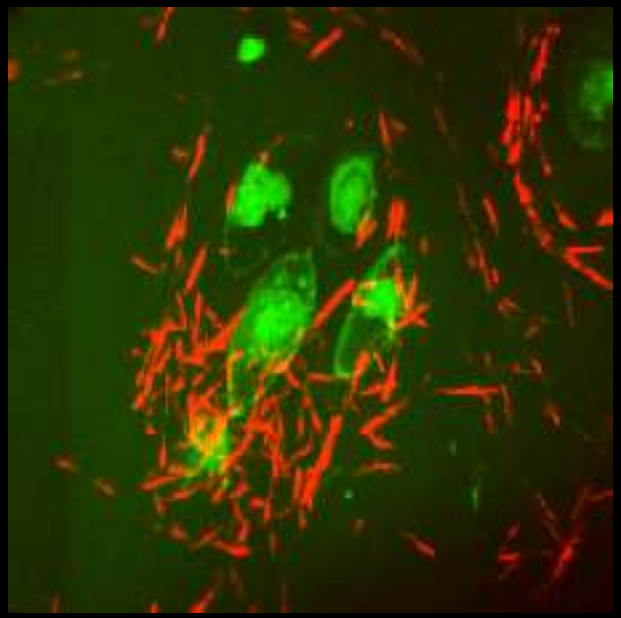

Fibrous material
Cells
Tessera (mineralized tissue)

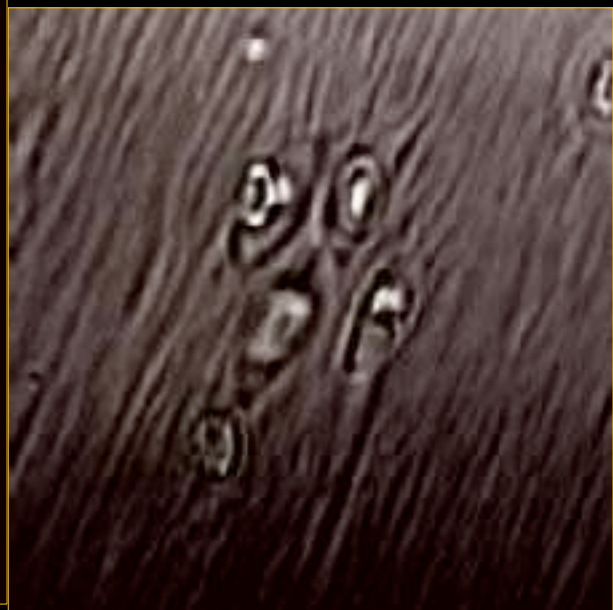

Sharpey's fibers
Tessera (mineralized tissue)

Pericellular envelope?

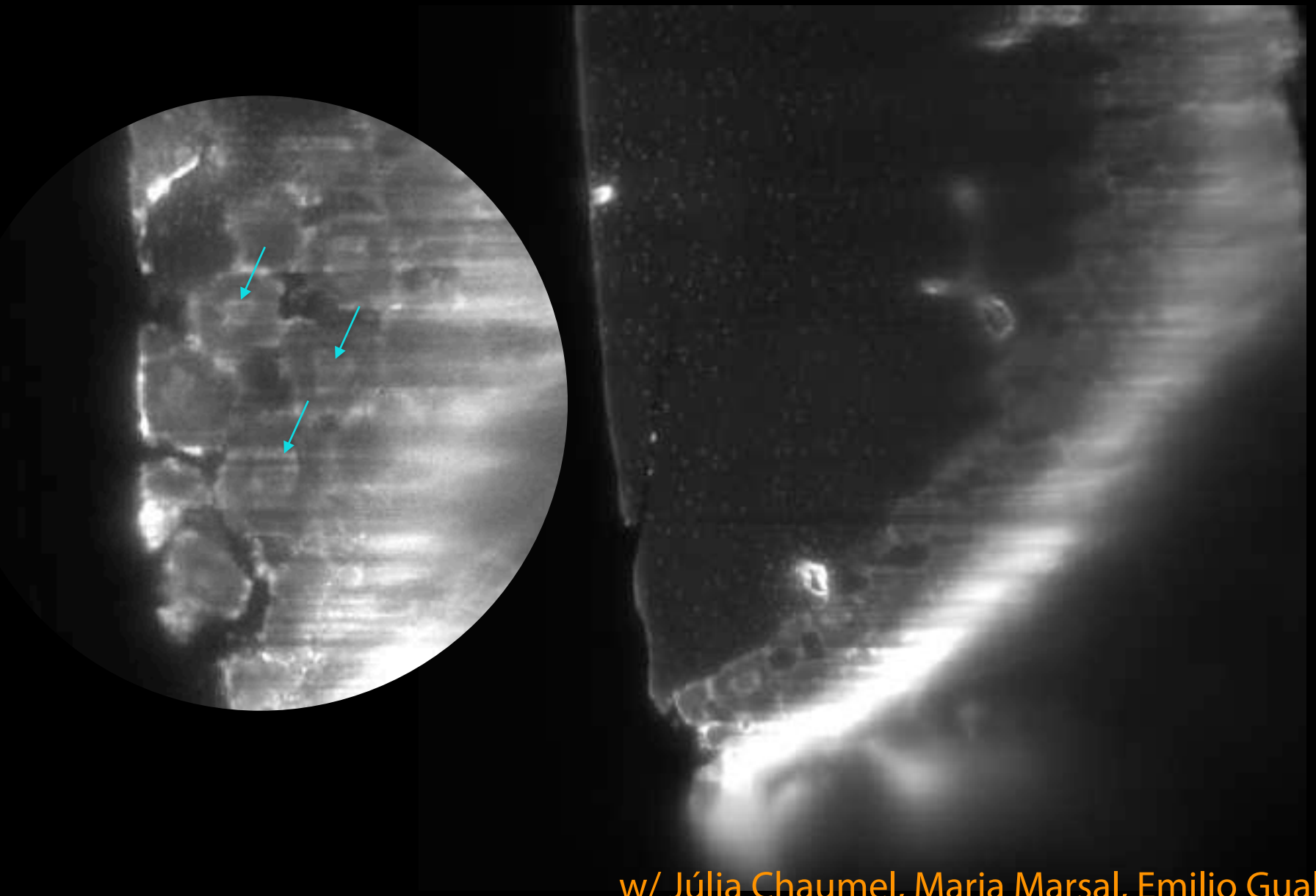
Isogenous cell groups



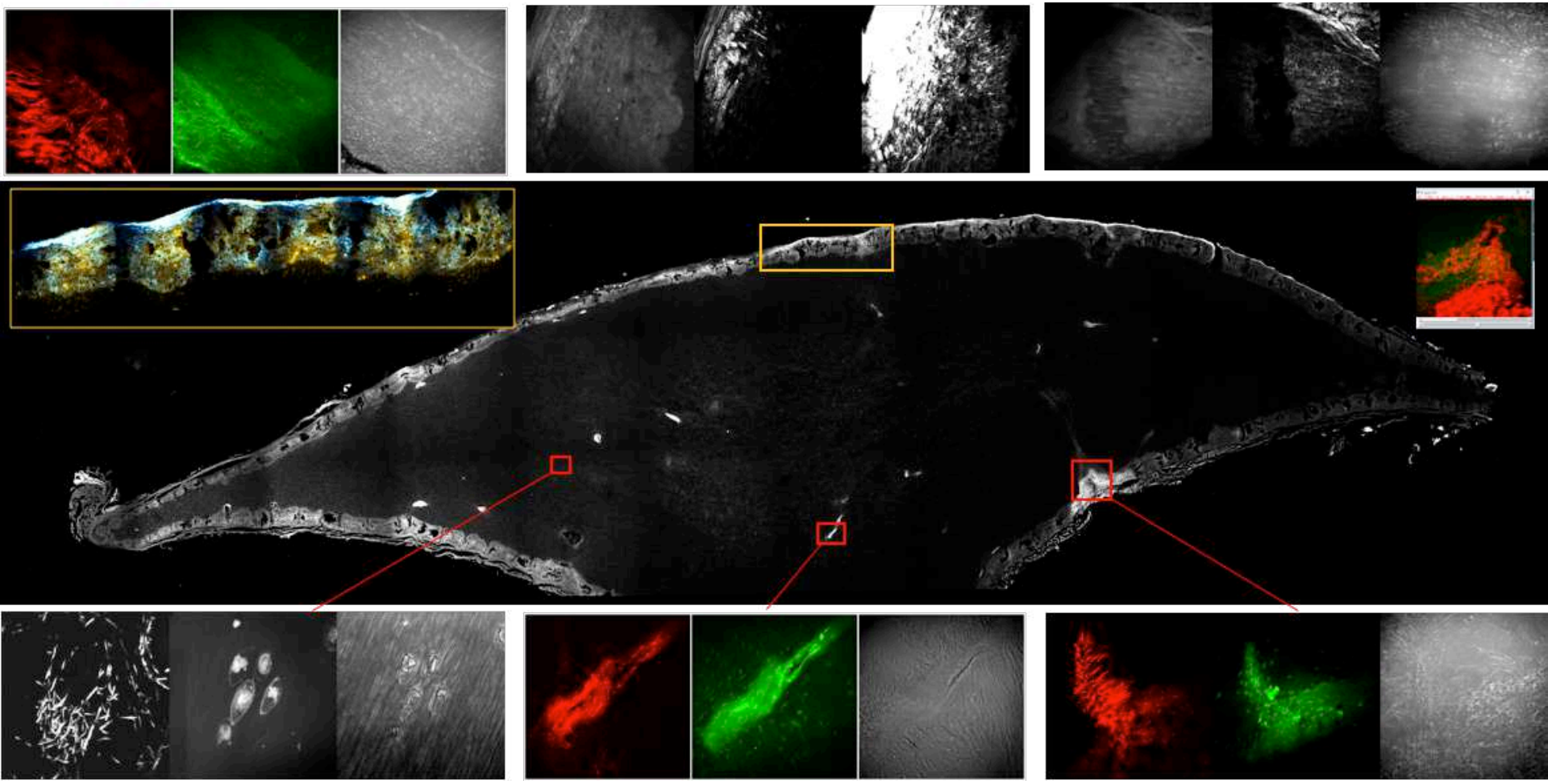




Light sheet imaging of tessellated cartilage



w/ Júlia Chaumel, Maria Marsal, Emilio Gualda



What we've learned:

- Fluorochromes can be used to study skeletal growth
  (except when they can't)

What we've learned:

- Fluorochromes can be used to study skeletal growth
  (except when they can't)
  - Autofluorescence is annoying
    (except when it isn't)

What we've learned:

- Fluorochromes can be used to study skeletal growth
  (except when they can't)
  - Autofluorescence is annoying
    (except when it isn't)
  - Label-free imaging is a powerful tool for less-accessible tissues
    (period.)

THANK YOU!

- Frauke Leitner
- Pablo Loza-Alvarez, Jordi Andilla & Marina Cunquero
- Mélanie Debiais-Thibaud & the OOB Staff
- Paolo Gavaia & CCMAR

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