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Nanoscale arrangement of the healthy dentin and comparison with the dentino-genesis imperfecta-affected dentin

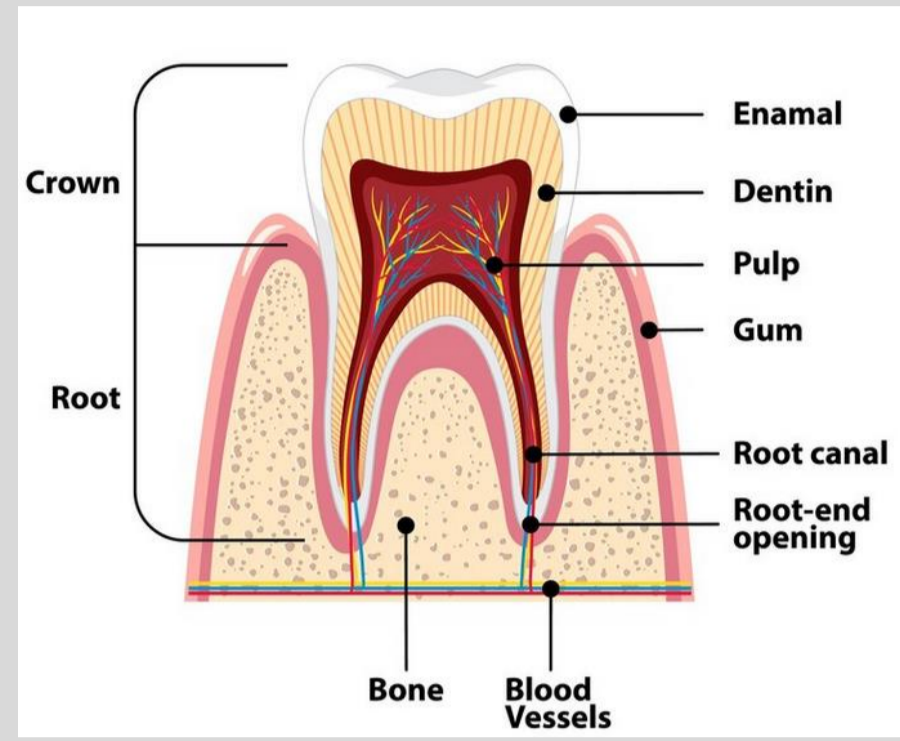
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Introduction



Dentin is a complex biological hard tissue presenting a hierarchical organization at different scales as bones. At the nanoscale, dentin shows an organization involving a mineral phase made of HAP crystals and an organic phase mainly made of CFs.

Dentinogenesis imperfecta (DI)

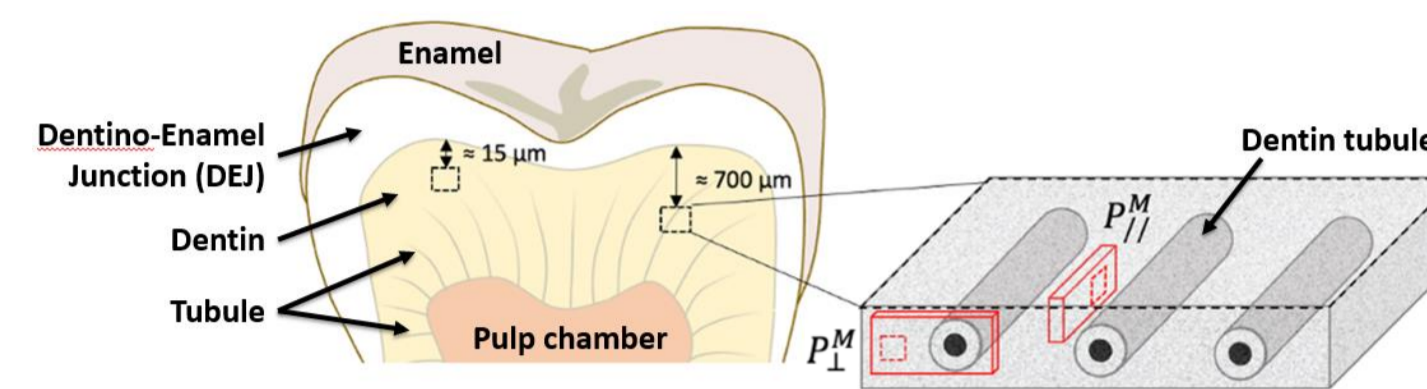
- Rare genetic disorder (1-5/10 000 births)
- Severe anomalous dentin



Method

Both permanent and deciduous teeth were prepared for the control samples. In contrast, a deciduous molar was chosen for DI ones exhibiting damage to type I collagen

FEI ThermoFisher Helios Nanolab 660 focused ion beam for localized sections

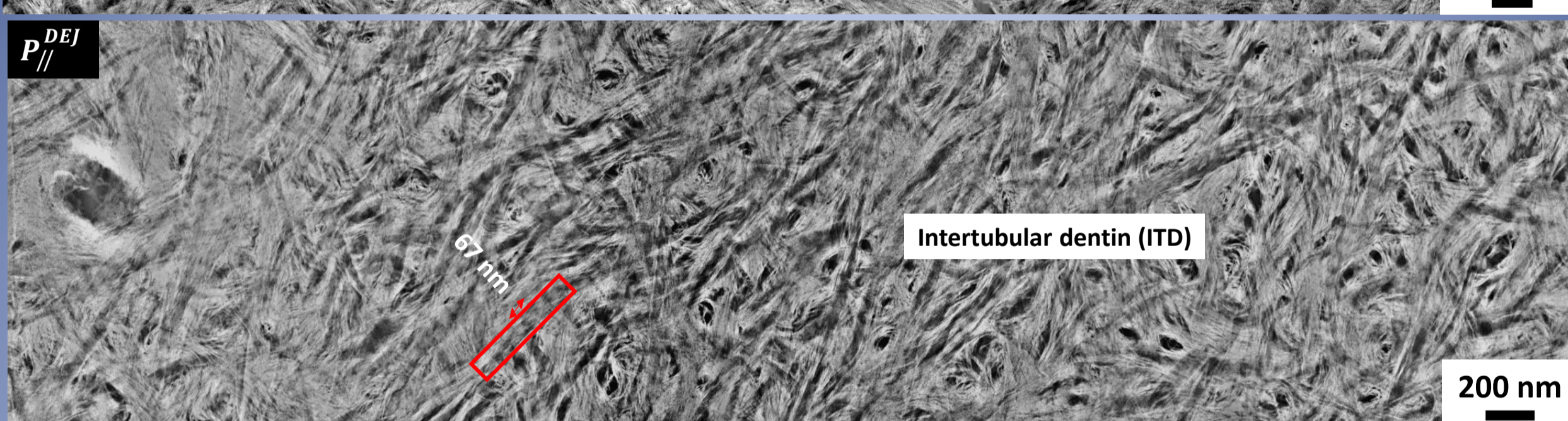
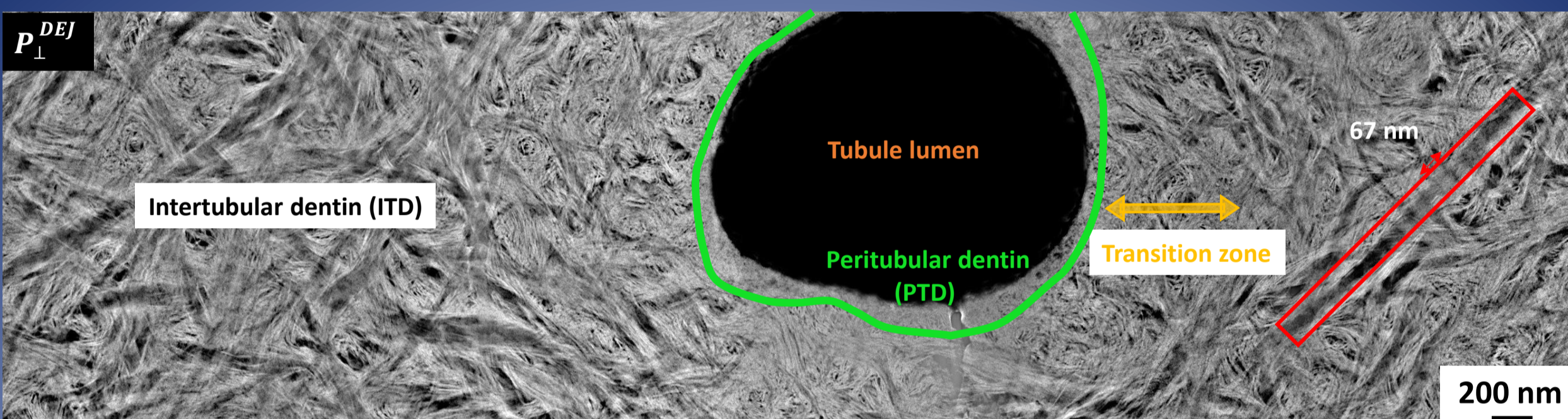


FEI ThermoFisher TITAN³ G2 80-300 transmission electron microscope used in TEM and STEM mode

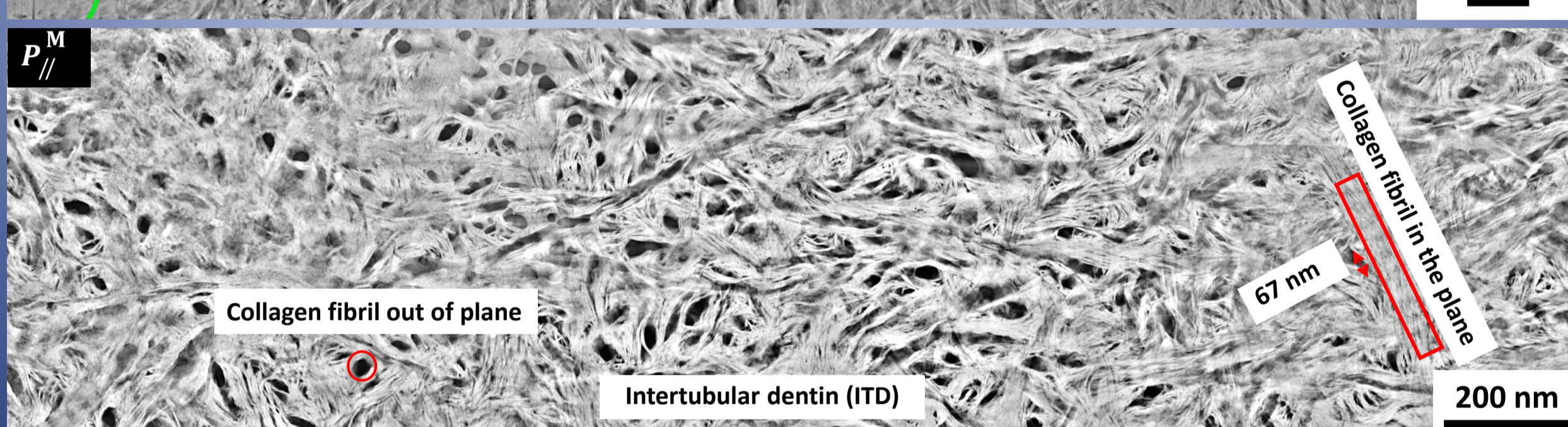
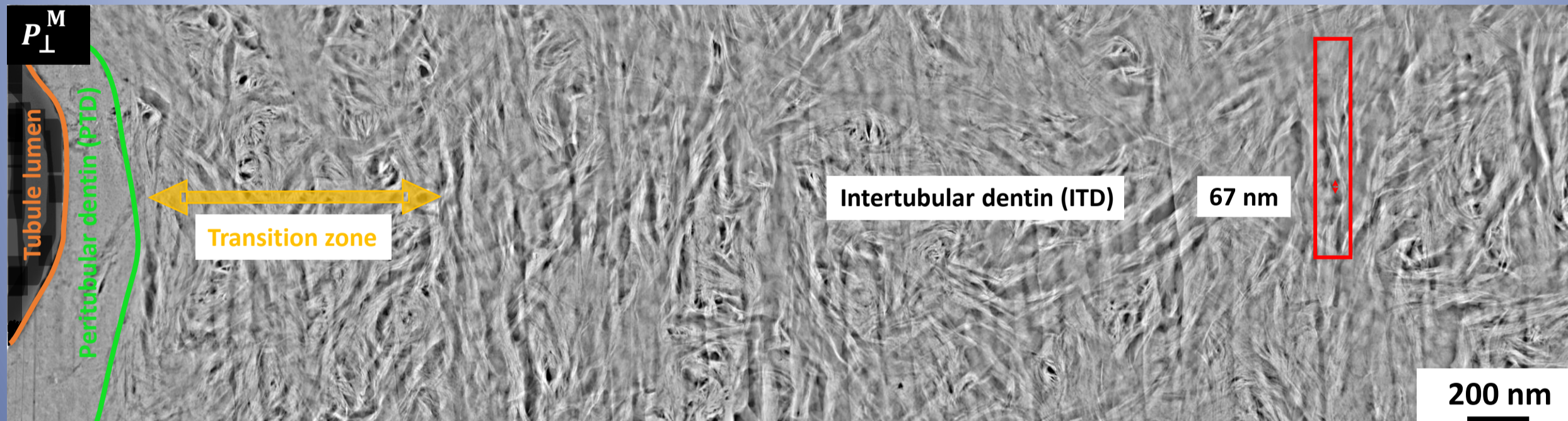
Nanostructure of the healthy dentin [1]

Collagen fibrils (CFs) distribution

HAADF-STEM images of dentin near enamel



HAADF-STEM images of middle dentin



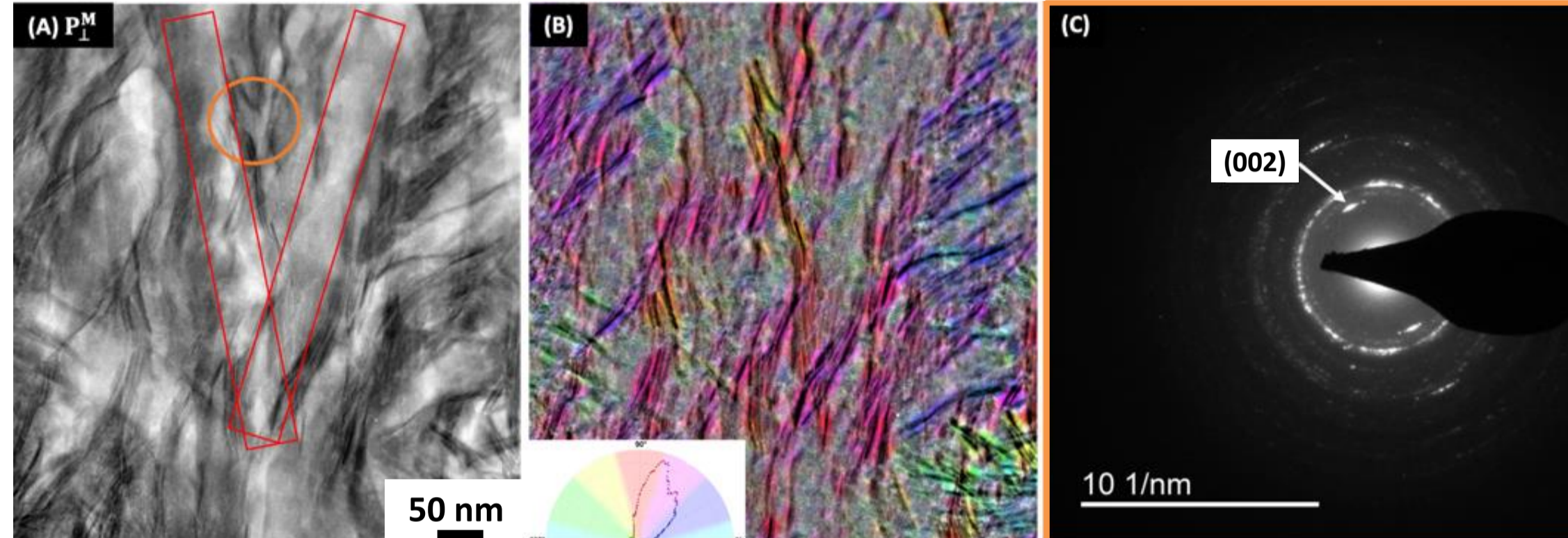
Collagen fibrils (CFs)

- 1- Near enamel: CFs are roughly isotropic
- 2- Middle dentine: CFs mainly lie in planes perpendicular to the tubule axis and create a woven structure

Transition zone between PTD and ITD
Mineralized collagen fibrils are running parallel to the tubule axis, unlike in the rest of ITD. It might explain that cracks initiated in tubules do not propagate in ITD

Hydroxyapatite (HAP) cristal organization

BFTEM image zoomed on some collagen fibrils



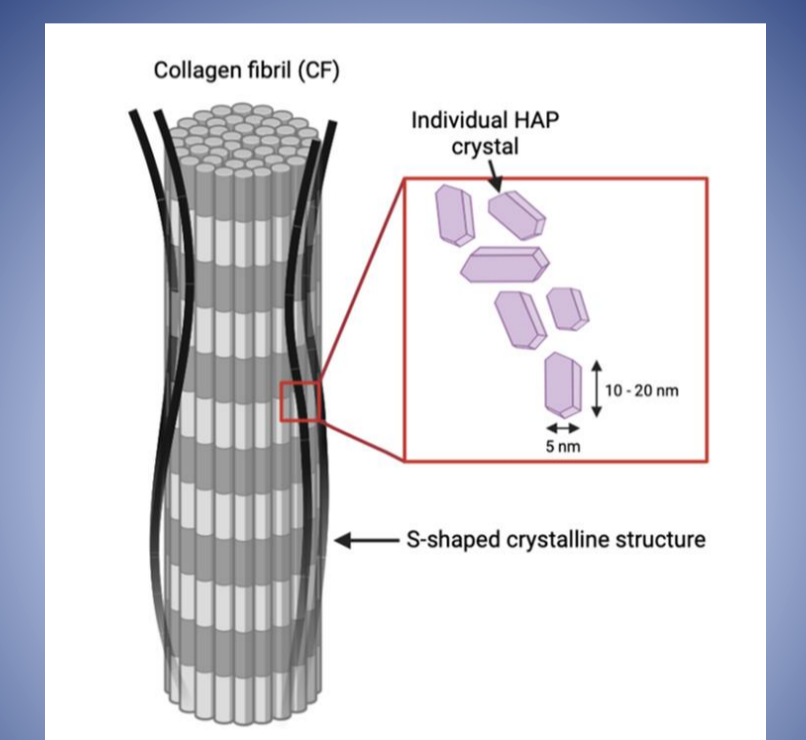
The HAP minerals (in black) surrounding collagen fibrils (red rectangles)

Color-coded map of the BF showing the angle distribution of minerals

Diffraction pattern of the orange circle in figure (a)

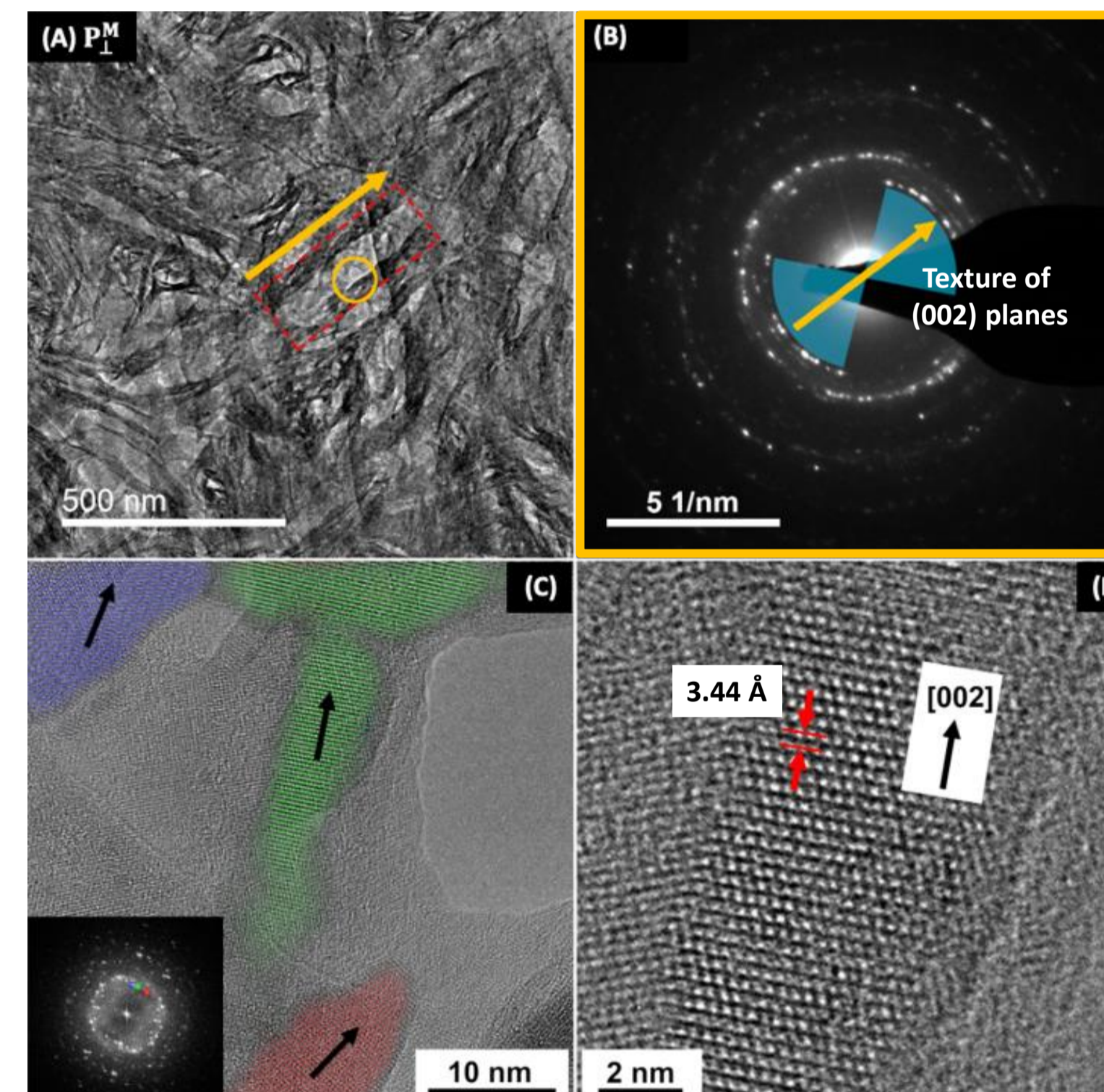
HAP minerals are strongly oriented around the collagen fibrils. The [001] direction is mainly lying parallel to the collagen fibrils as see in the diffraction pattern.

Model proposed by Reznikov et al. [2] for cortical bones



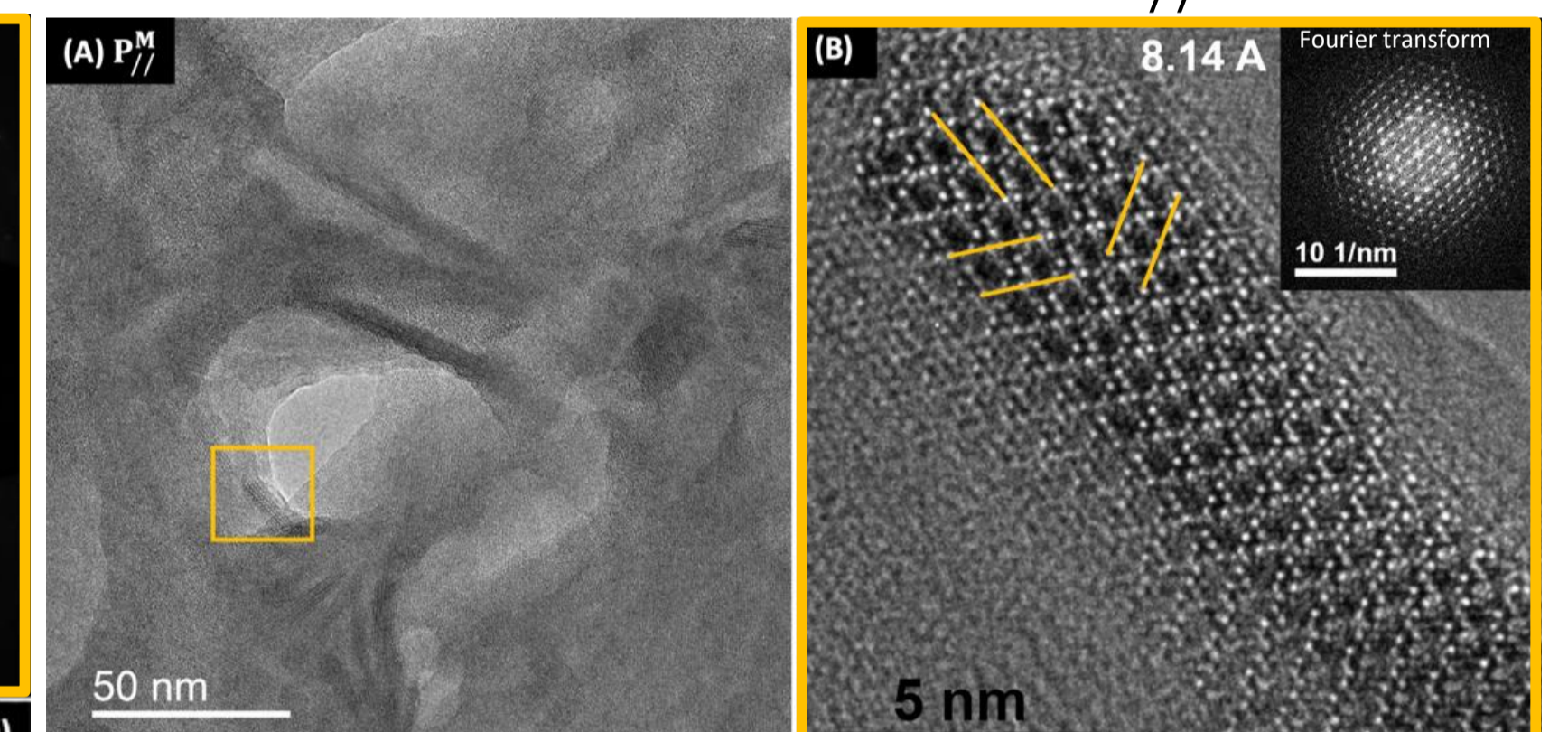
Organization of merged individual HAP crystals with various orientation around adjacent CFs and forming a S-shaped crystalline structure

HRTEM image of HAP crystals in P_{\perp}^M



The diffraction pattern (b) taken on the side of a single CF (a) again shows a preferred orientation of HAP crystals with their [001] direction roughly aligned with the CF one. (c) shows three crystals highlighted by choosing their (002) reflections. (d) Zoom on a single crystal with the (002) interplane distance of 3.44 Å

HRTEM image of HAP crystals in P_{\parallel}^M



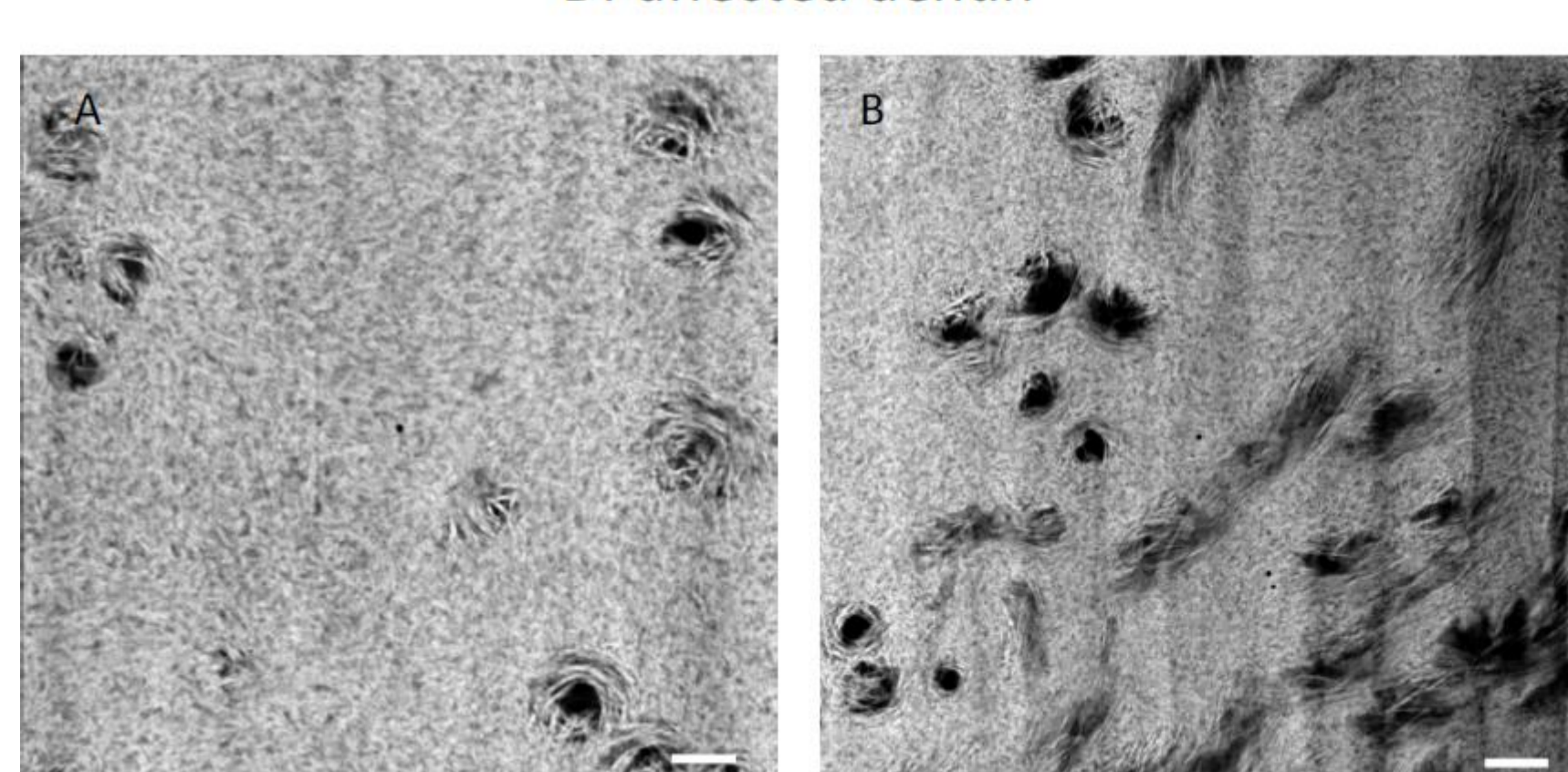
Atomic arrangement of a single HAP crystal seen normal to the c-axis. Periodicities of 8.14 Å characteristic of basal interplane distances are observed in P_{\parallel}^M

1. Single HAP crystals in all samples
 - Width 4-5 nm
 - Length few tens of nanometers
2. Elongated mainly along their [001] c-axis but were also seen elongated in the other crystalline directions (a, b axis).
3. Formation of a S-shape crystalline structure composed of merged single HAP crystals

Dentinogenesis Imperfecta (DI)-affected dentin

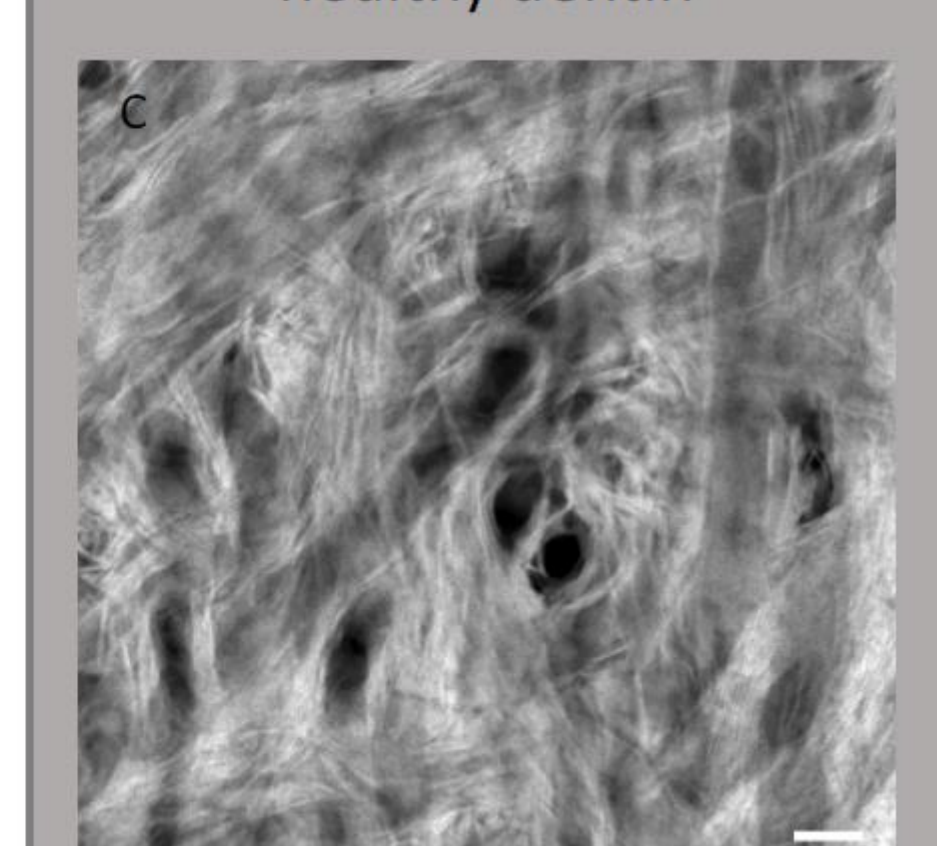
HAADF-STEM images of healthy and DI-affected dentin at the same scale (50 nm)

DI-affected dentin



- 1- Few of no collagen fibrils with varying orientation planes in DI-affected dentine
- 2- Hypermineralization of the dentin: more than 90%

healthy dentin



Mineralization of the healthy dentin around 55% in volume has been evaluated by Vennat et al. 2009 [3]

In DI-affected dentin samples, a drastic decrease in organic content is locally observed associated with a different organization of the remaining collagen fibrils



Modification of the mechanical properties due to a high mineral content:

- Harder
- Stiffer
- More brittle

Conclusions

- Isotropic distribution of CFs close to the enamel than in the middle dentin
- Organization of HAP in the [001] direction around CFs forming S-shaped crystalline structure
- Low mineralization structures reduce the propagation of cracks
- Hypermineralization of DI-affected dentin at the origin of a significant increase of the brittleness

[1] Leclercq et al. Submitted

[2] Reznikov et al., Science, 360, 6388 (2018)

[3] Vennat et al., Dent Mater., 25, 729 (2009)