

Backscattered electron SEM of early childhood caries

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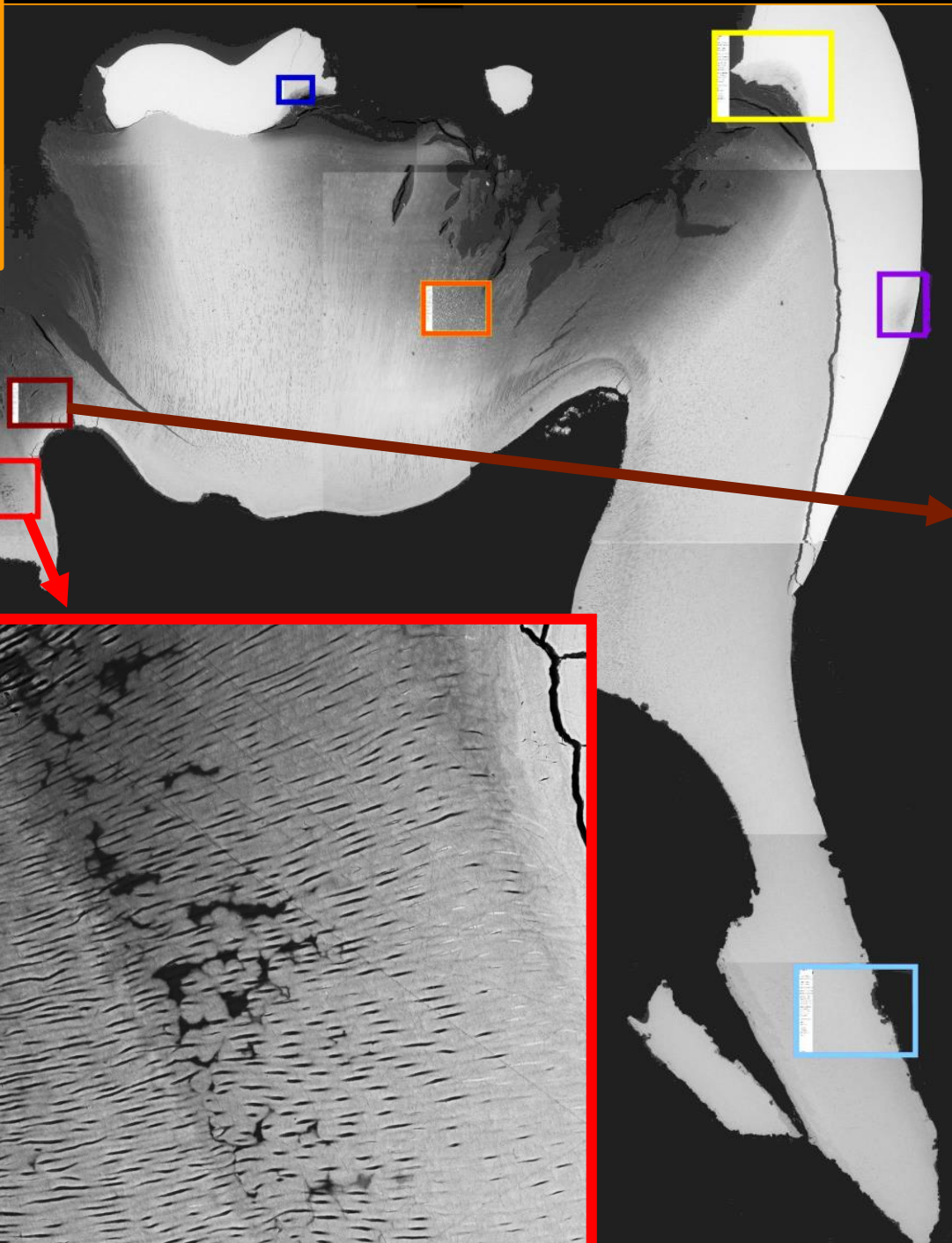
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No conflicts of interest to disclose

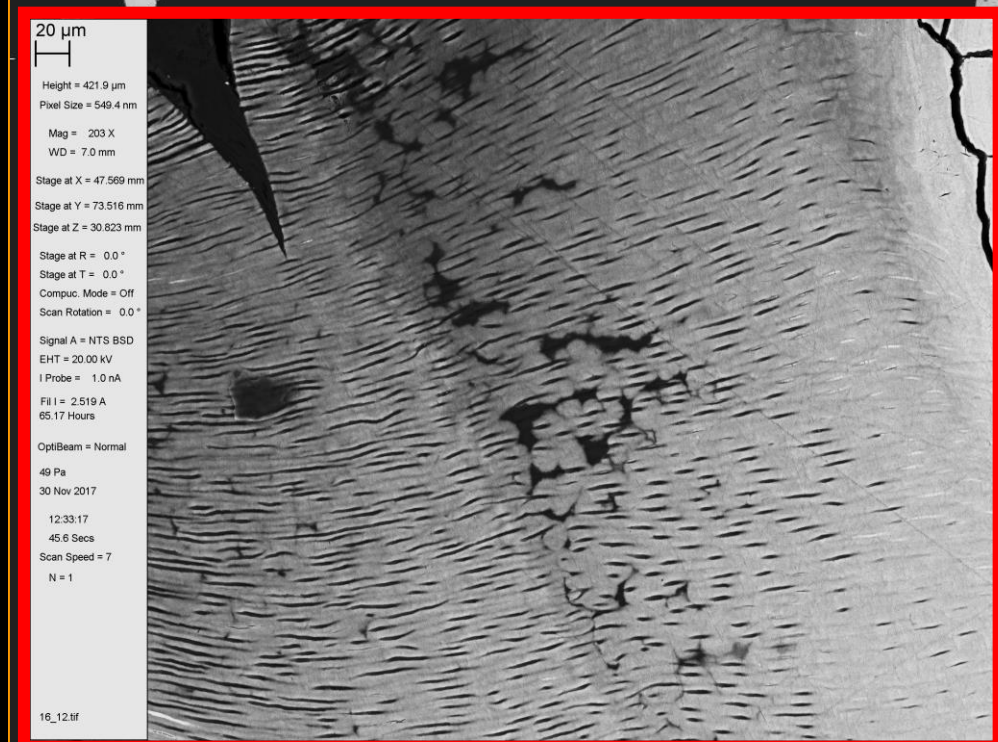
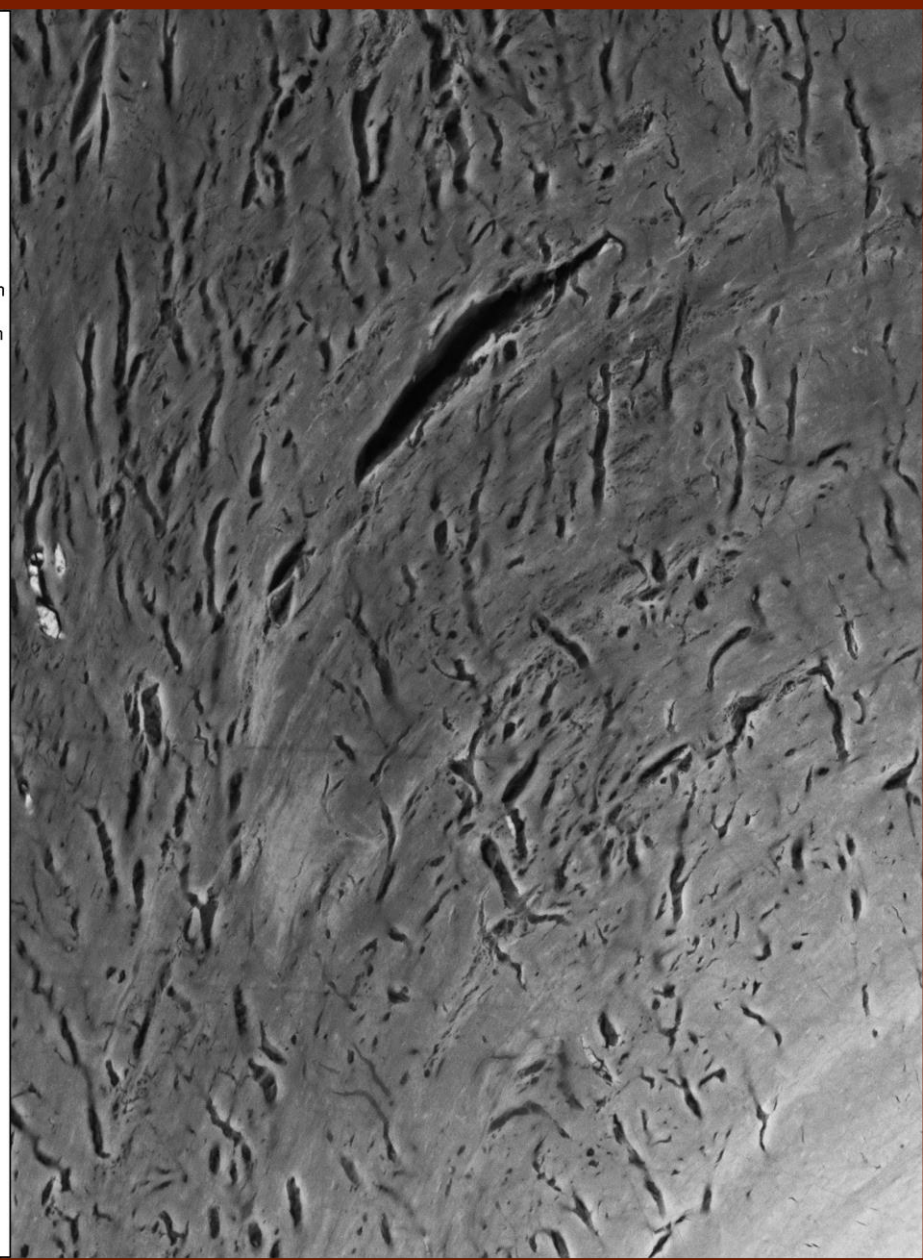
Objectives and Methods

- Early childhood caries is a major health problem
- We looked for structural and compositional changes in primary molar decay
- Deciduous molars preserved in 70% ethanol, obtained from **anonymised** collection. Age at extraction estimated from remaining root lengths in the range 4 to 8 years. **Photographed**
- Reason for extraction most likely pulpitis - in every case there was a **deep penetrating carious lesion**
- Further dehydration to 100% ethanol, then xylene, and embedding in PMMA from monomer
- Teeth bisected and **vertical section surfaces polished flat. Photographed**
- Compositional contrast = **mineral concentration dependent imaging** using 20kV backscattered electron scanning electron microscopy,
 - **uncoated** [means we can examine number of polishing levels with no further preparation],
 - **50Pa** chamber pressure
 - Fields **montaged** to cover entire specimen & higher magnifications for **detail**
- Blocks later stained with **iodine vapour** to study residual organic matrix and invading microbiota.

Montage
Low mag
and details
e.g. #16
Lower E



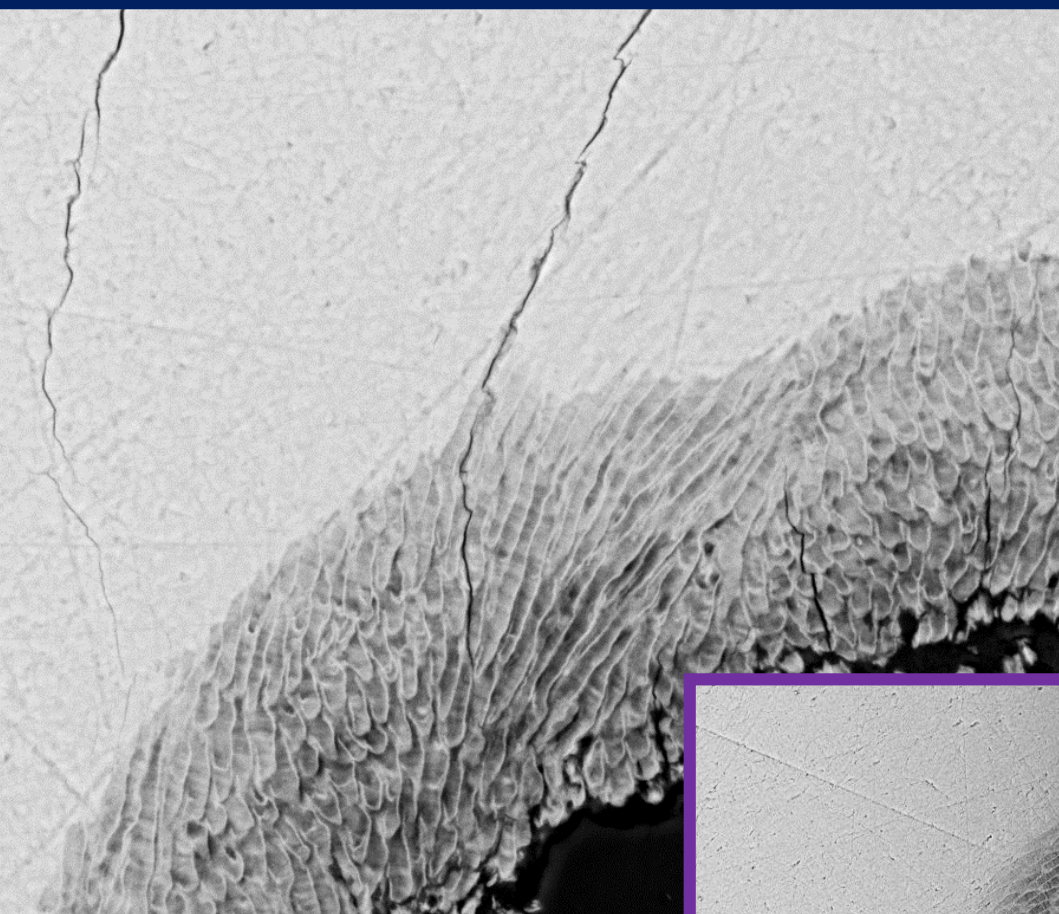
10 μ m
H
Height = 342.7 μ m
Pixel Size = 446.3 nm
Mag = 250 X
WD = 7.0 mm
Stage at X = 47.233 mm
Stage at Y = 72.888 mm
Stage at Z = 30.823 mm
Stage at R = 0.0 °
Stage at T = 0.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.519 A
65.20 Hours
OptiBeam = Normal
49 Pa
30 Nov 2017
12:34:56
45.6 Secs
Scan Speed = 7
N = 1
16_13.tif



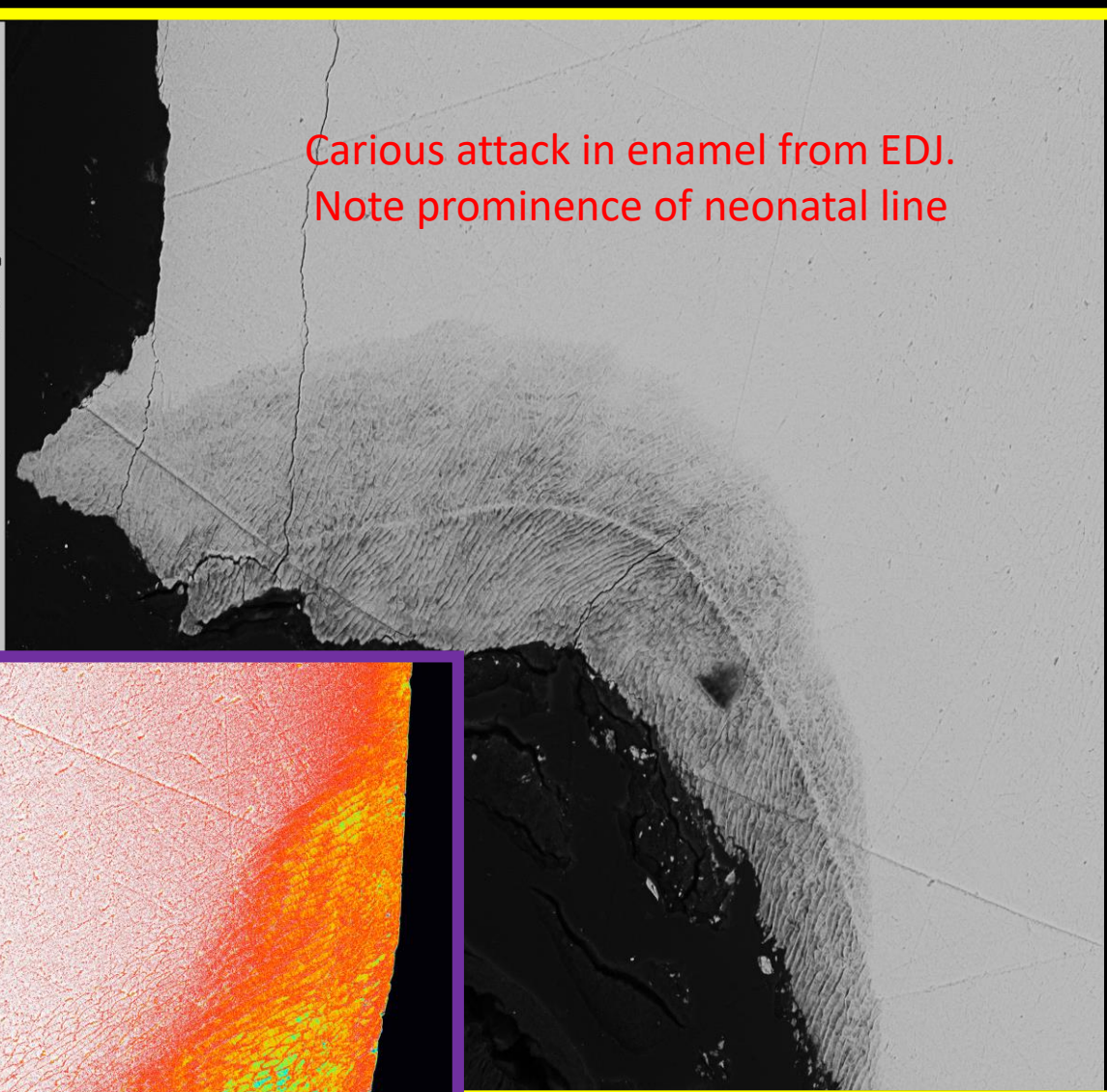
20 μ m
H
Height = 421.9 μ m
Pixel Size = 549.4 nm
Mag = 203 X
WD = 7.0 mm
Stage at X = 47.569 mm
Stage at Y = 73.516 mm
Stage at Z = 30.823 mm
Stage at R = 0.0 °
Stage at T = 0.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.519 A
65.17 Hours
OptiBeam = Normal
49 Pa
30 Nov 2017
12:33:17
45.6 Secs
Scan Speed = 7
N = 1
16_12.tif

Invasive spread in dentine parallel with incremental layering
of collagen during its development.
? Motile invasive species

10 µm
Height = 176.2 µm
Pixel Size = 229.4 nm
Mag = 487 X
WD = 7.0 mm
Stage at X = 45.175 mm
Stage at Y = 70.349 mm
Stage at Z = 30.823 mm
Stage at R = 0.0 °
Stage at T = 0.0 °
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Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.519 A
65.45 Hours
OptiBeam = Normal
49 Pa
30 Nov 2017
12:49:51
20.2 Secs
Scan Speed = 9
N = 1
16_22.tif

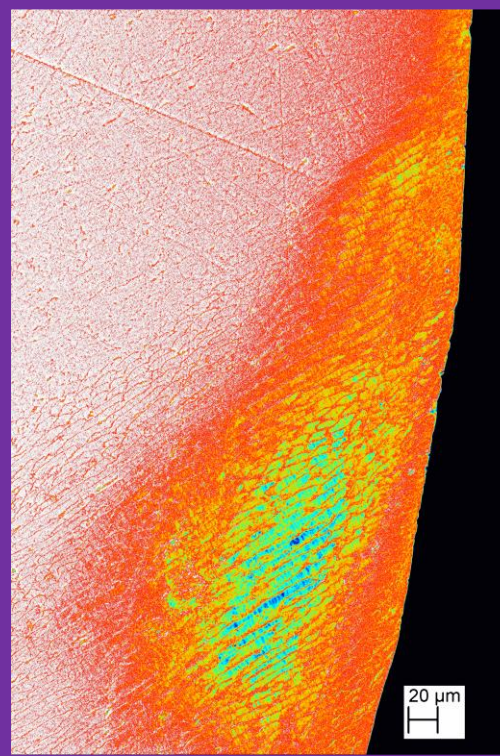
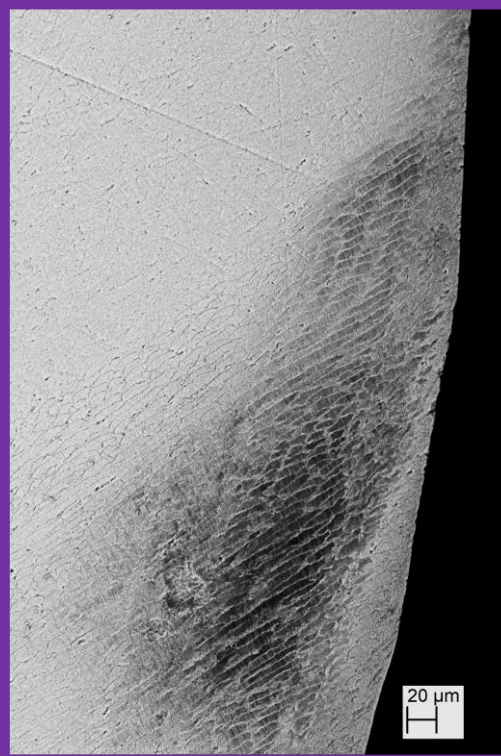


20 µm
Height = 661.6 µm
Pixel Size = 861.5 nm
Mag = 130 X
WD = 7.0 mm
Stage at X = 41.376 mm
Stage at Y = 70.289 mm
Stage at Z = 30.823 mm
Stage at R = 0.0 °
Stage at T = 0.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.519 A
65.35 Hours



Carious attack in enamel from EDJ.
Note prominence of neonatal line

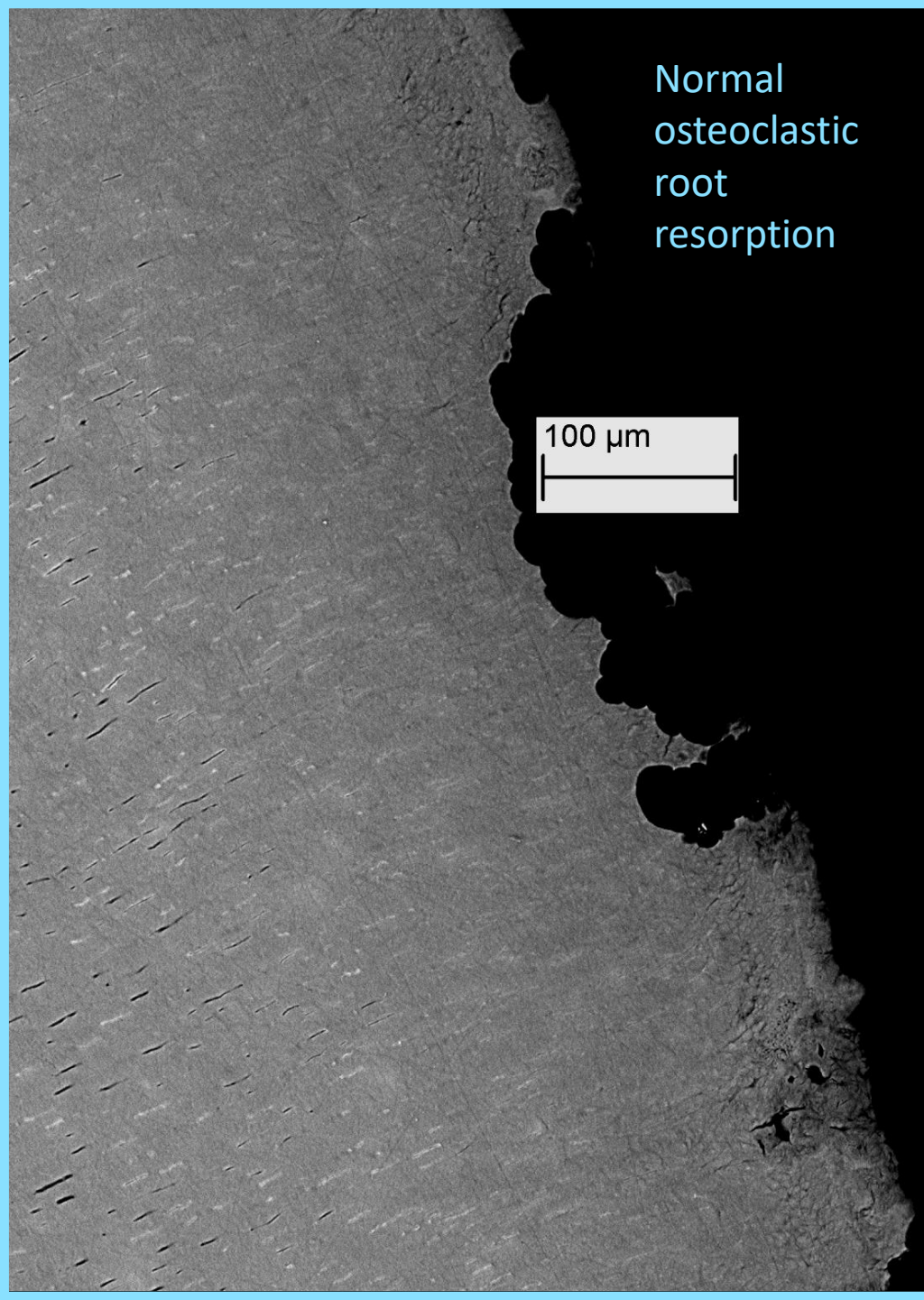
Carious attack in enamel from EDJ;
prominent cross-striations and mineral
deposition within the prism boundary
discontinuity spaces



Interproximal carious lesion
in enamel & pseudocoloured

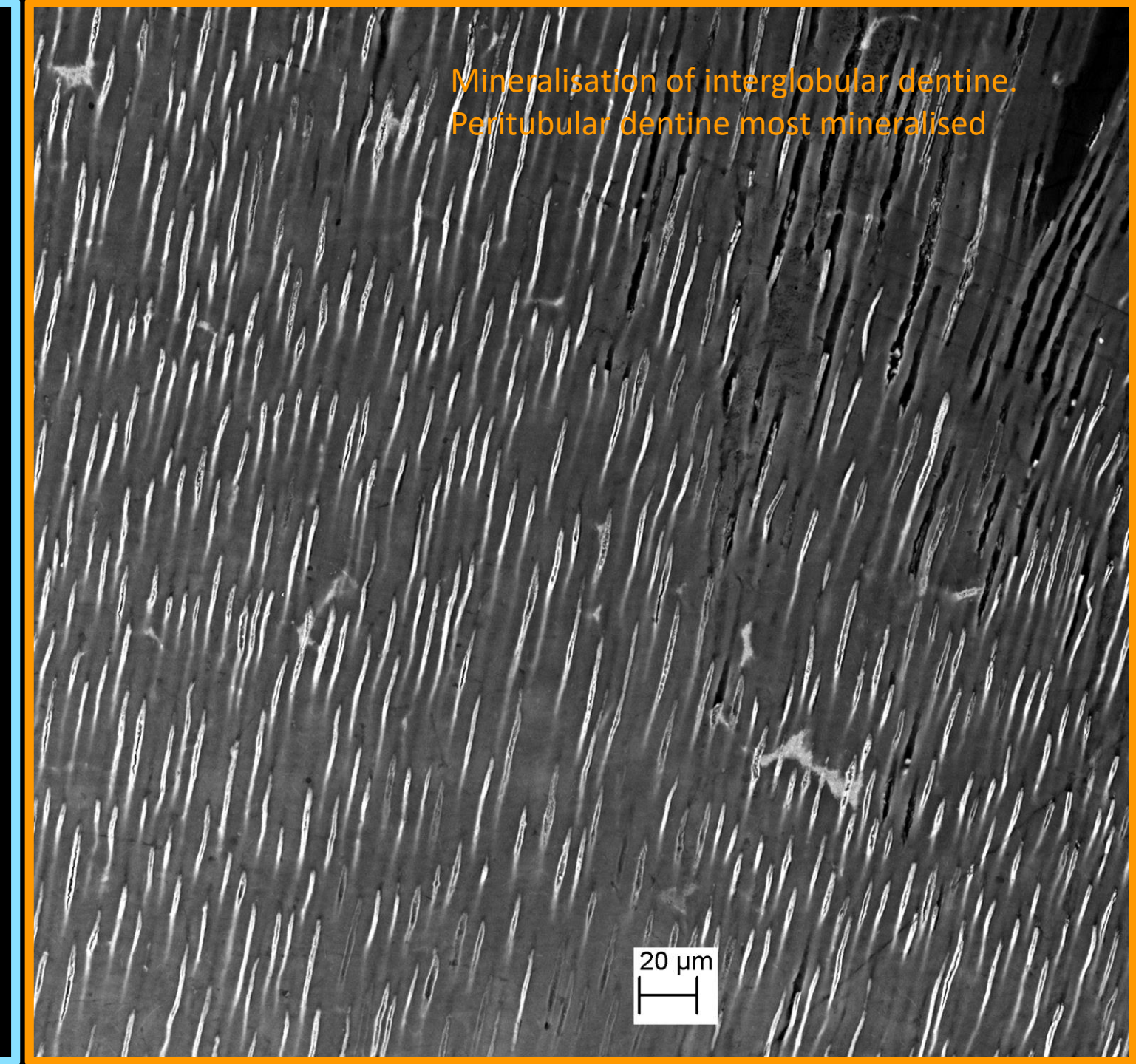
Normal
osteoclastic
root
resorption

100 μm

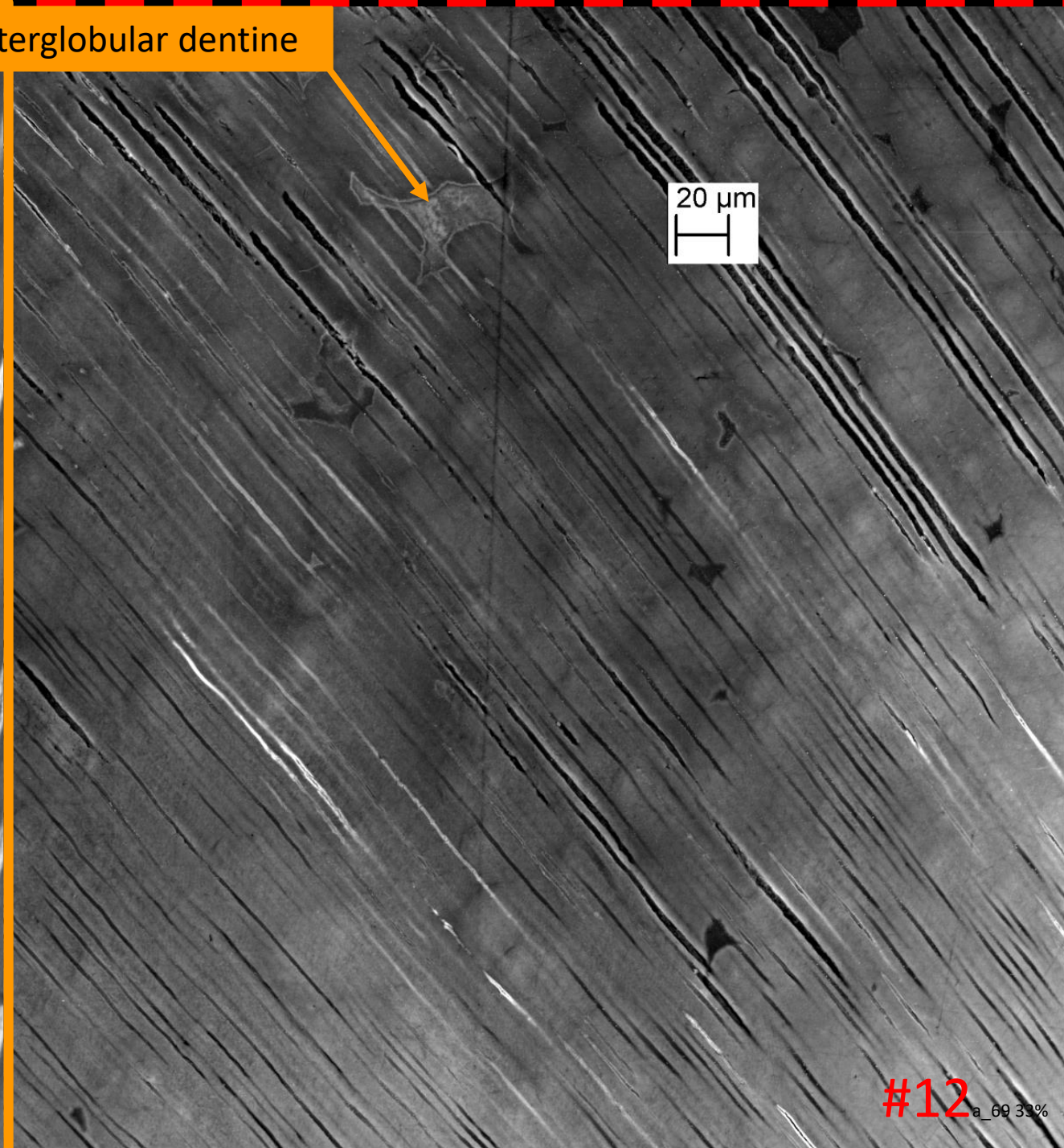
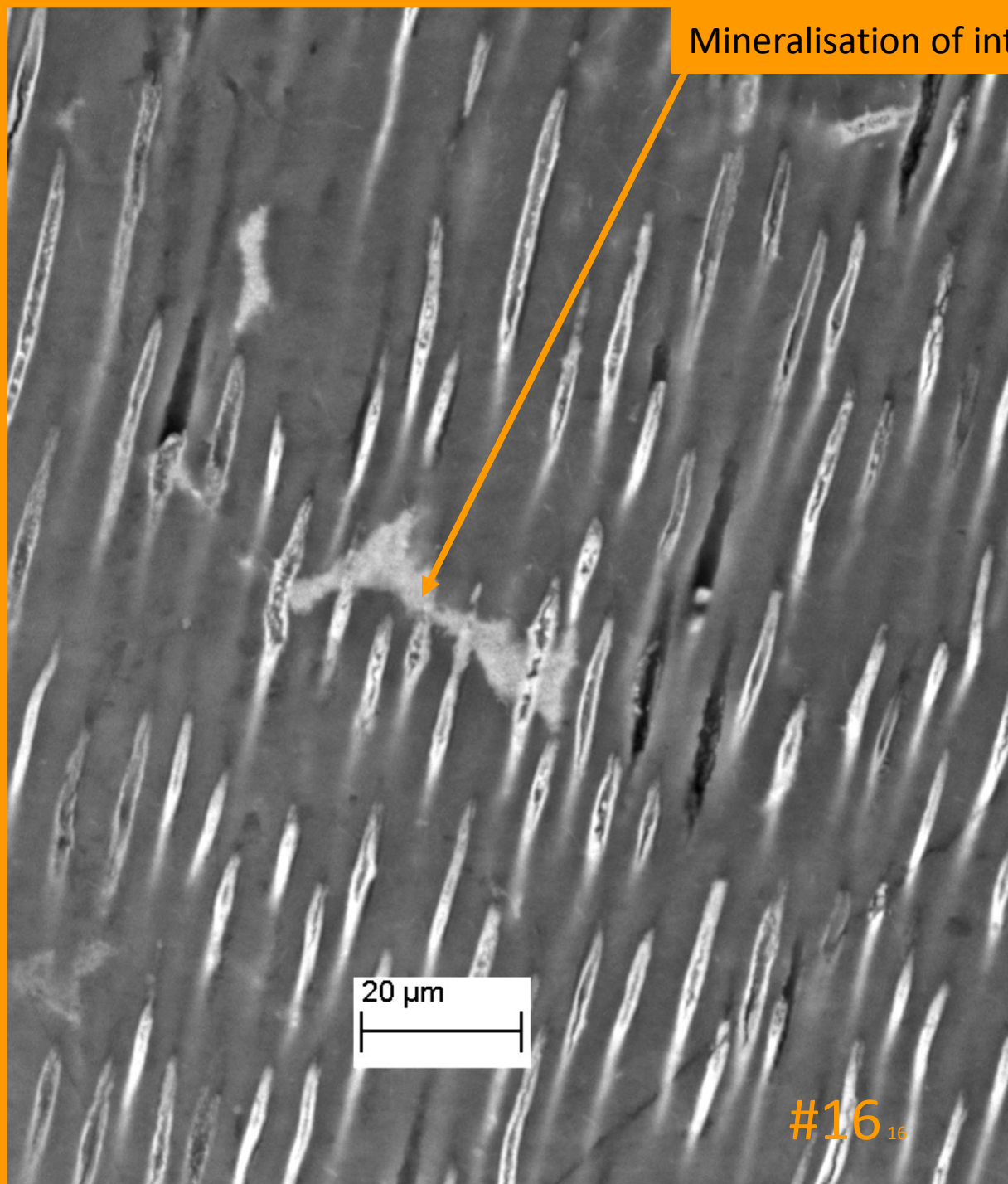


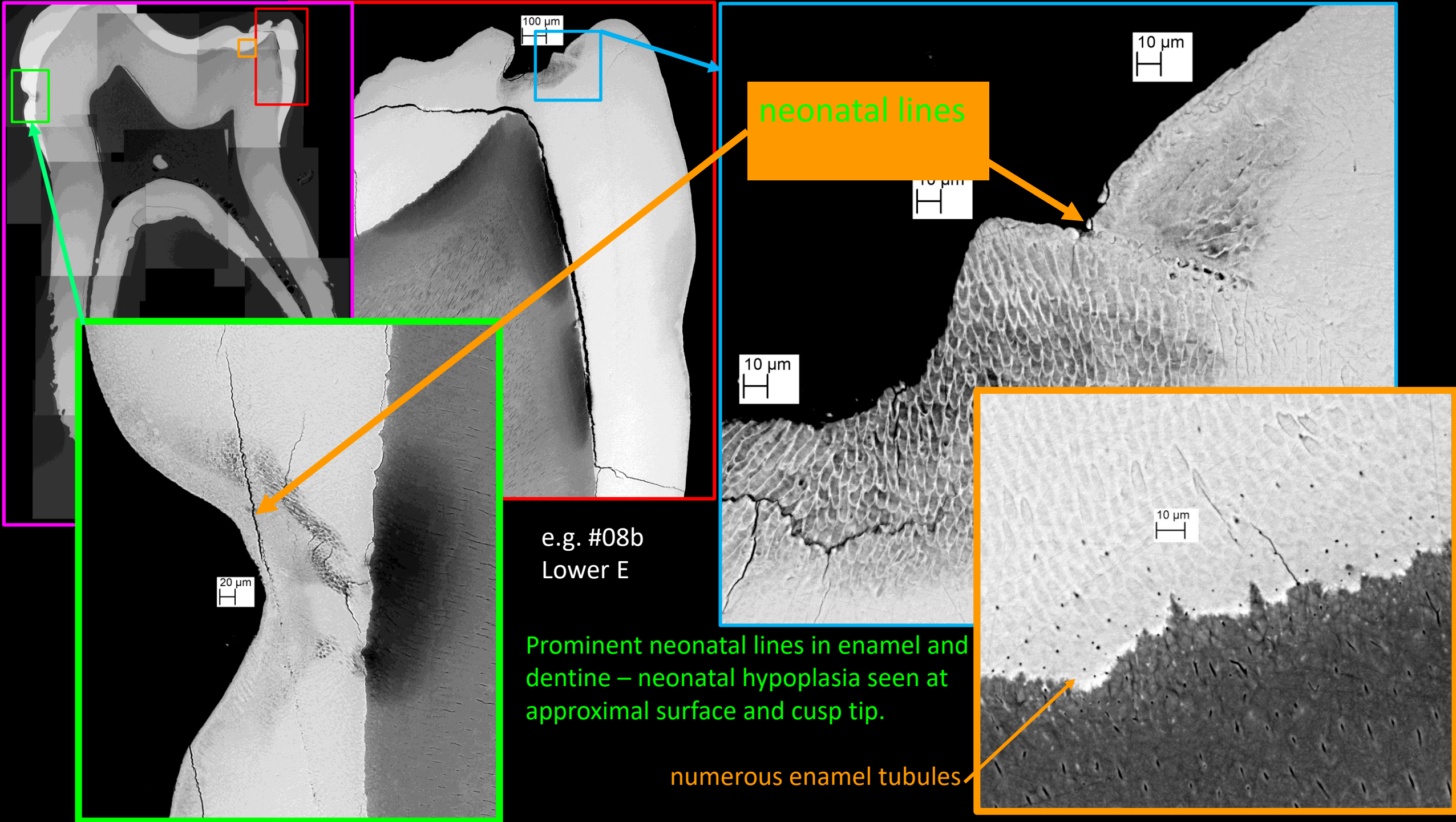
Mineralisation of interglobular dentine.
Peritubular dentine most mineralised

20 μm

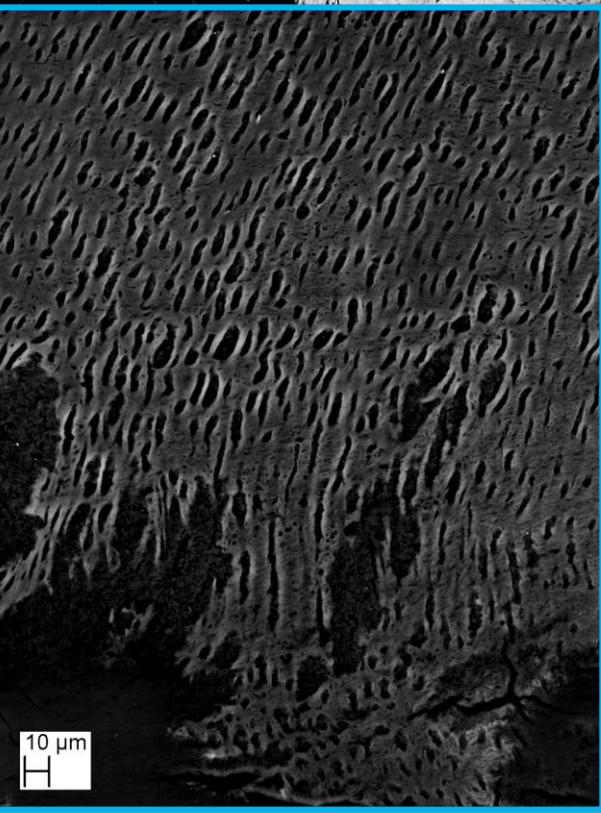
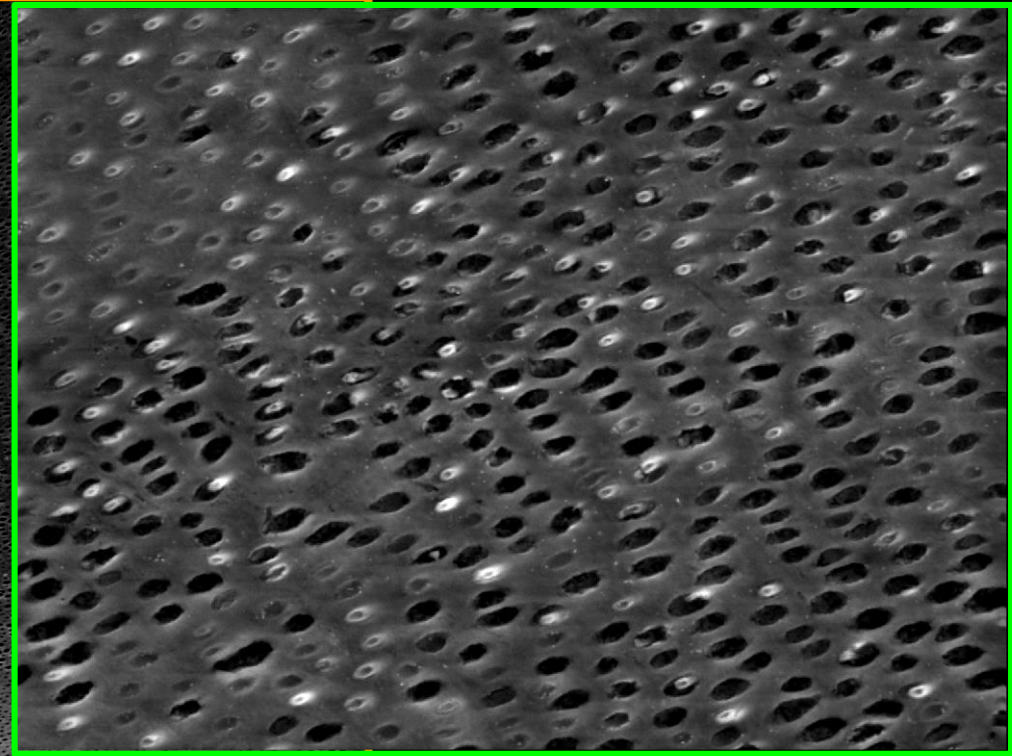


Mineralisation of interglobular dentine



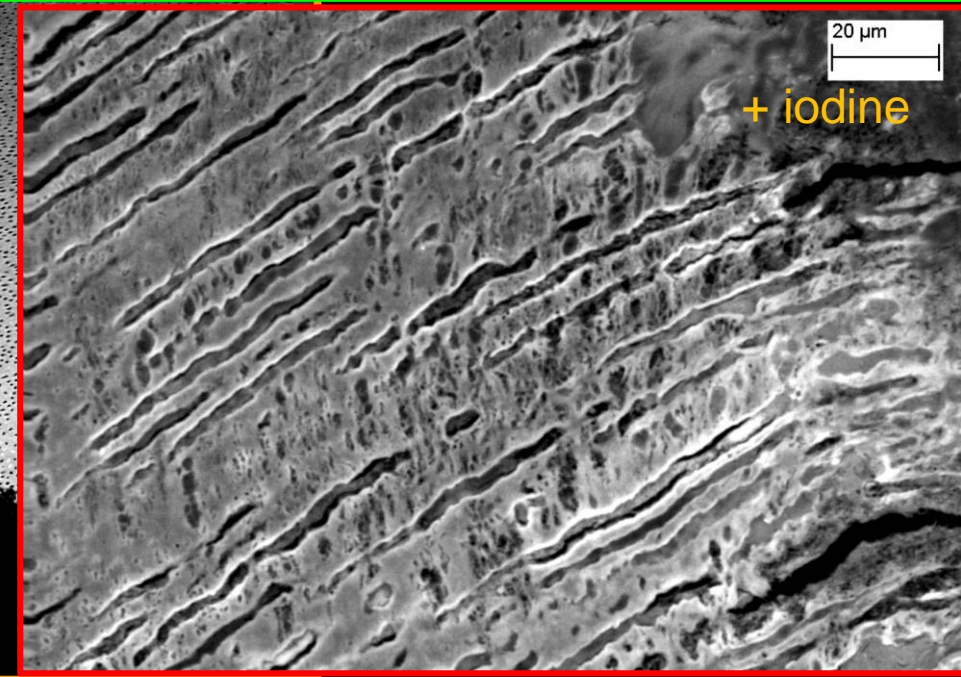
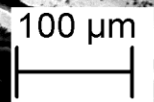


grossly carious dentine



#01_{a 369 371 372}

01b 619



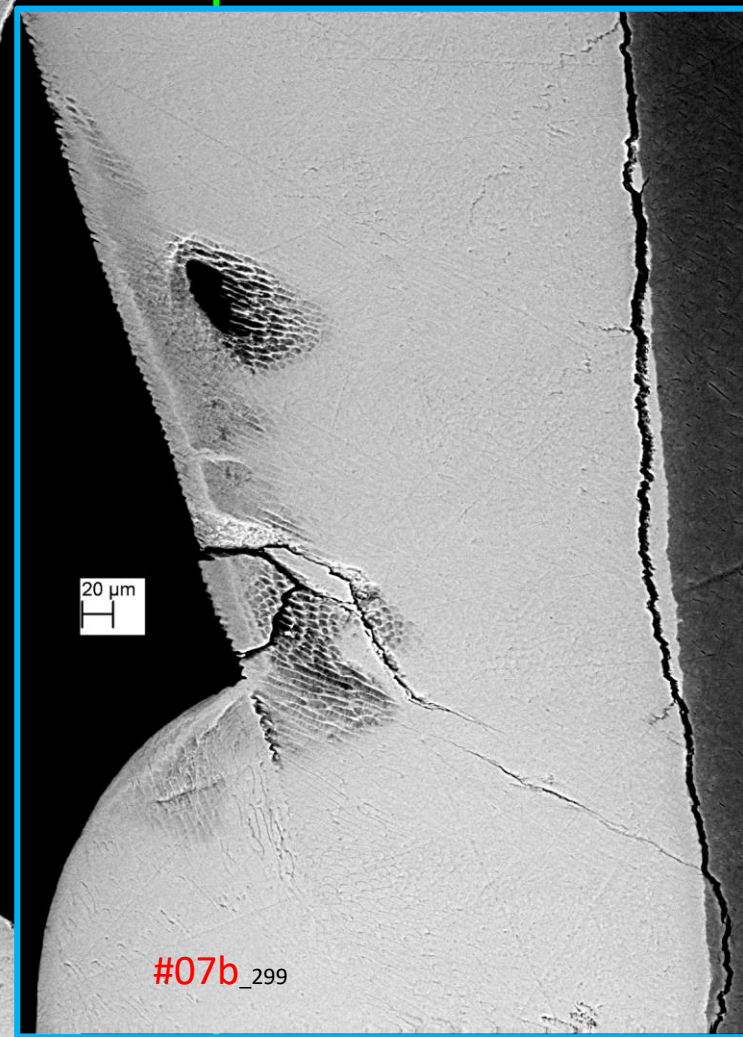
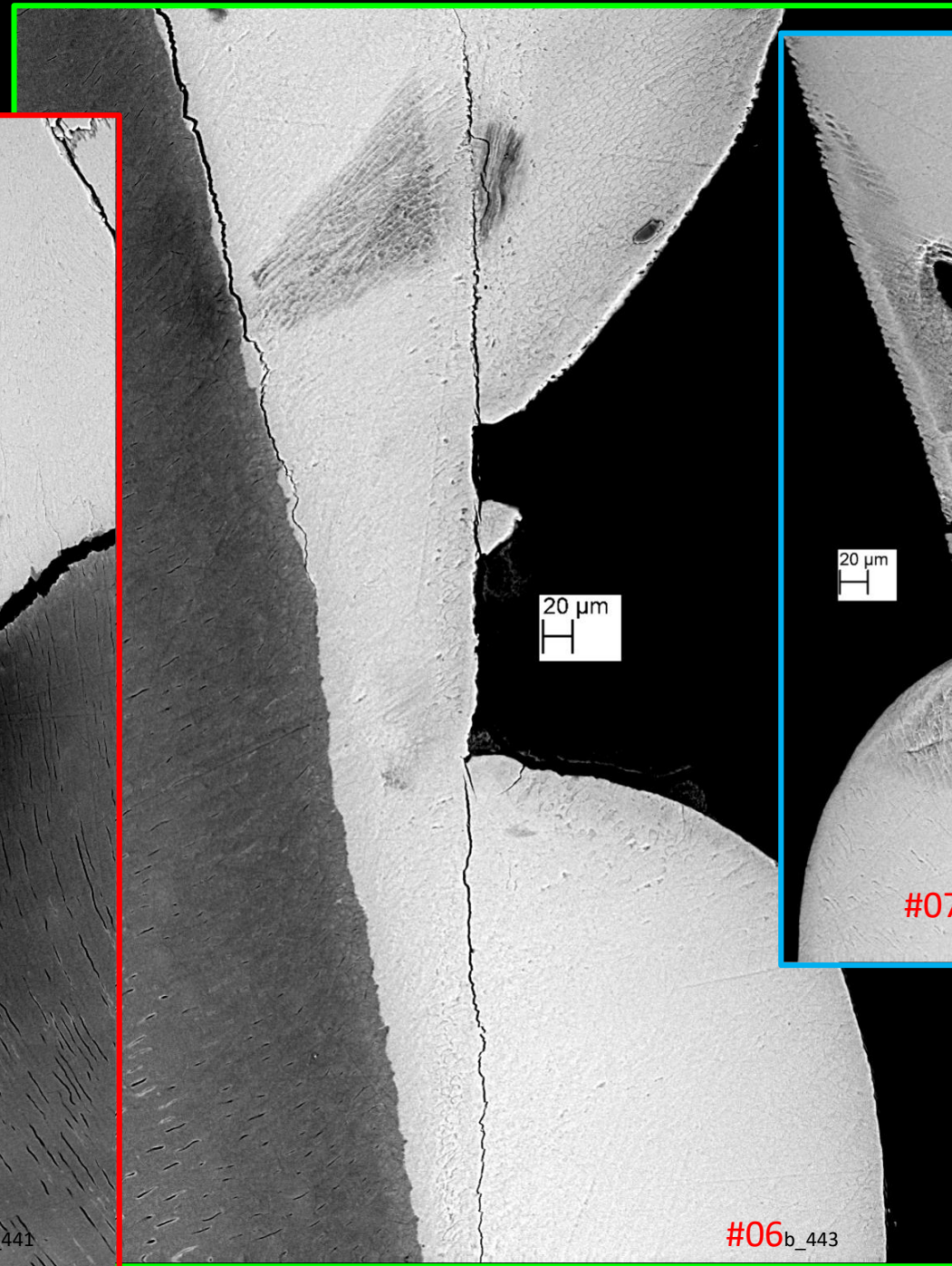
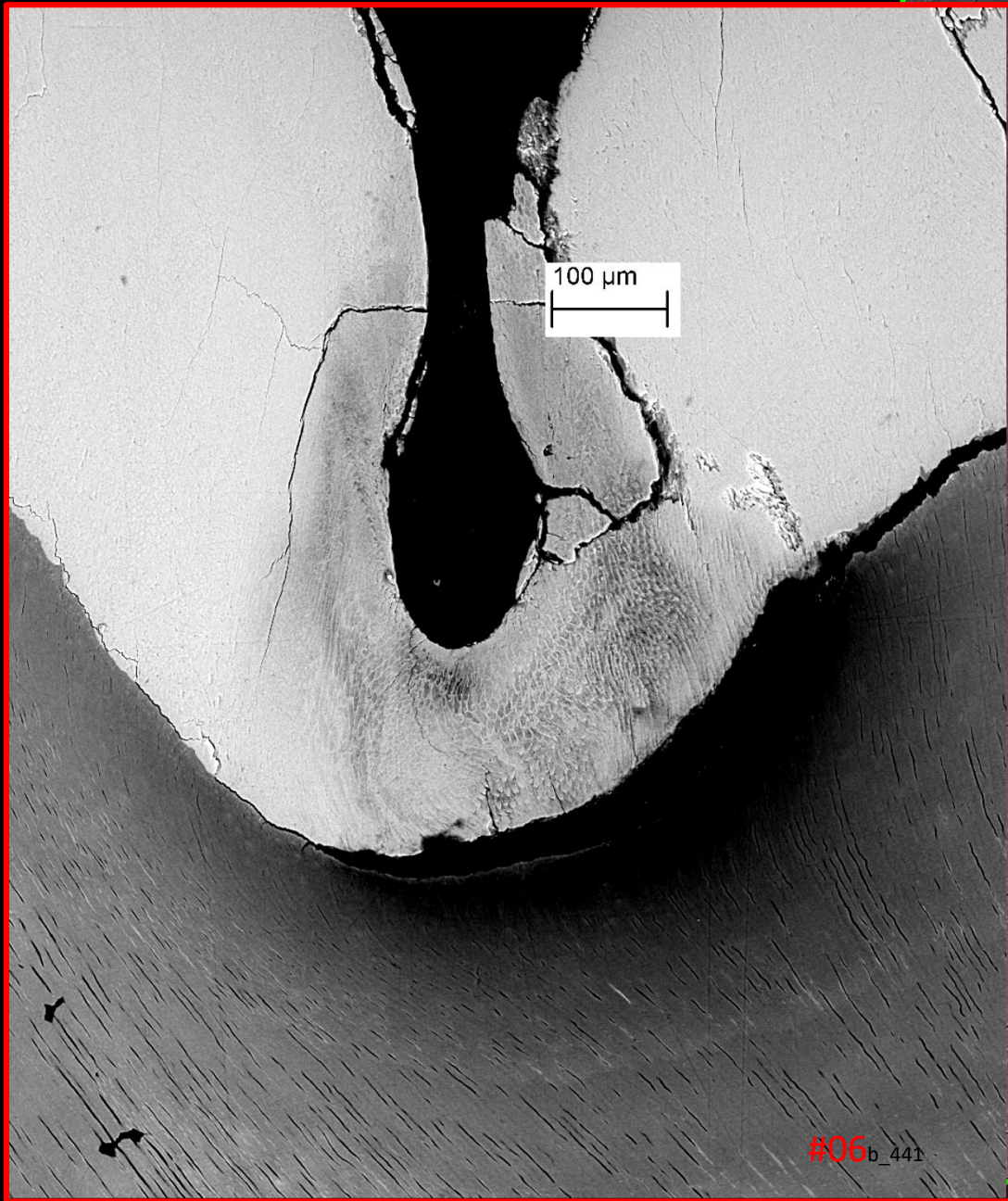
20 µm

+ iodine

Above: secondary dentine: top right PTD remnants in grossly carious dentine.
Left and right: ? fungal invasion.

10 µm

Fissure caries



Two more cases of neonatal hypoplasia

Results and Conclusions and THANKS

- We studied disease which must have spread rapidly through dentine
- Much destruction of enamel occurred from within, attacking enamel from the EDJ
- Neonatal enamel hypoplasia an important factor in several cases
- Even most demineralised carious dentine showed hyperdense 'peritubular dentine' (PTD)
 - or locally expanded tubules, probably fungal invasion (e.g., *Candida* spp.)
 - or multiple fine tubes crossing the tubule axis in the collagen fibre direction of the dentine matrix, a hallmark of motile invasive species (e.g. *Capnocytophaga* spp.)
- Patches of interglobular dentine (IGD), normally unmineralised, sometimes more densely calcified than surrounding carious matrix
 - This is the first report of addition of mineral to IGD in association with remineralisation phenomena in caries
- Calcospheritic de-mineralisation pattern indicates different state of intra- vs. inter-collagen mineral
- Caries involves many microbial species which cannot be identified by morphology alone
- but there are many morphological types of dentine caries.
- In future work, it might be possible to reach some spp. identification, with, e.g., Matrix Assisted Laser Desorption/Ionization Time Of Flight {MALDI-TOF} mass spectrometry