Thurs Oct 16th 2014 Rare bone diseases meeting Stockholm

Rarer insights from rarer microscopies in the study of the rare bone disease AKU

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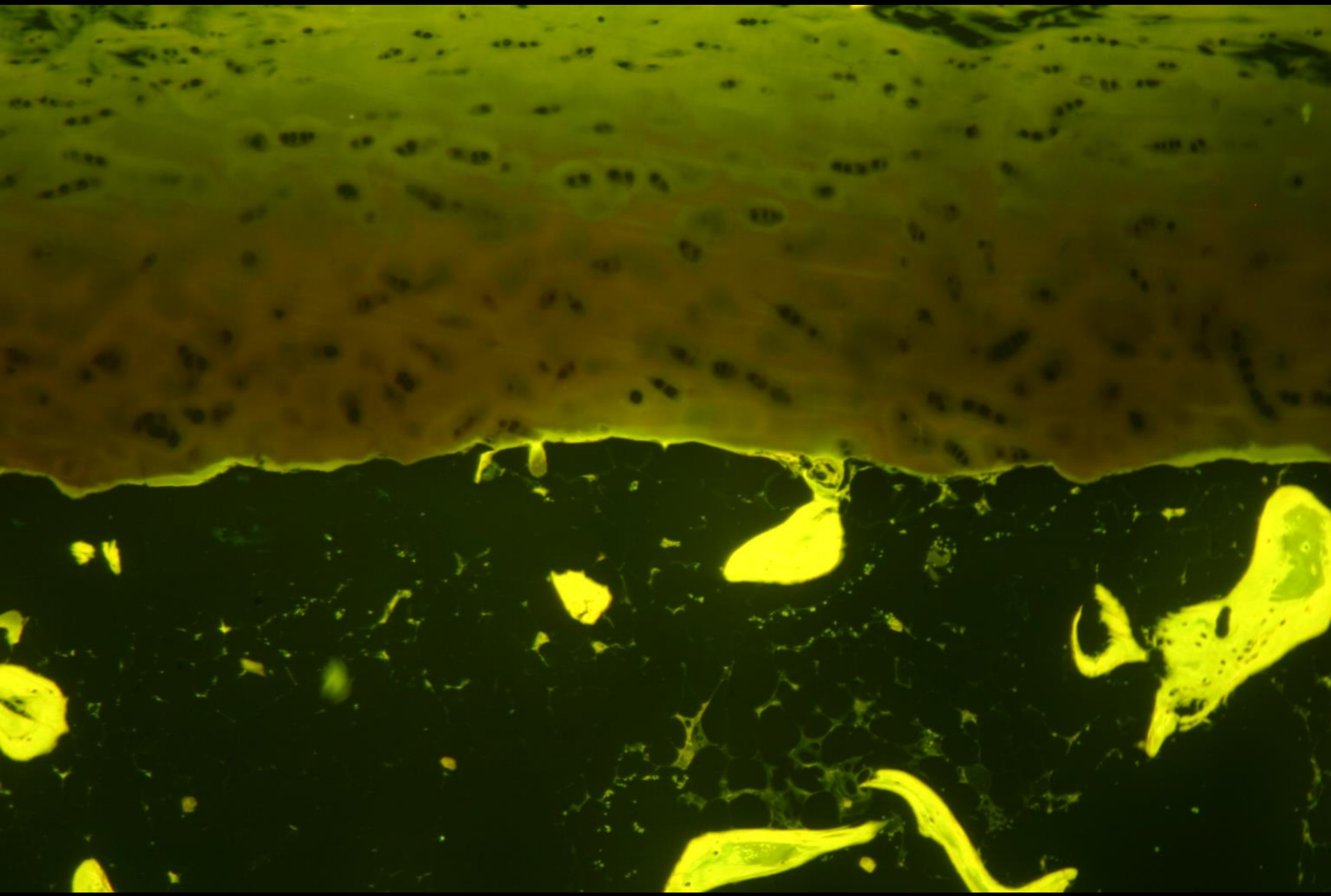
NORMAL EQUINE DISTAL MC3

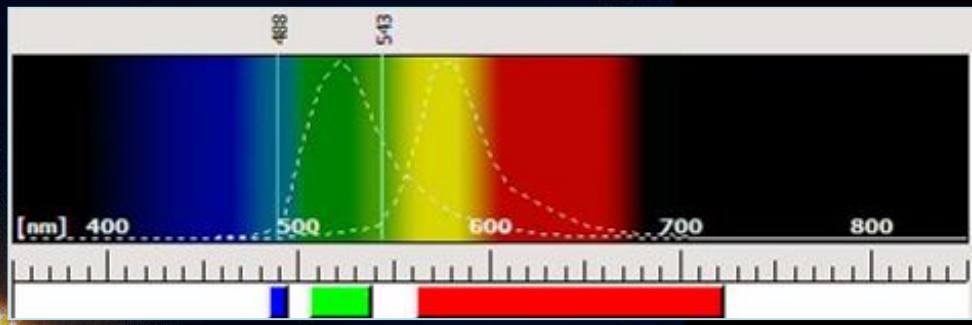


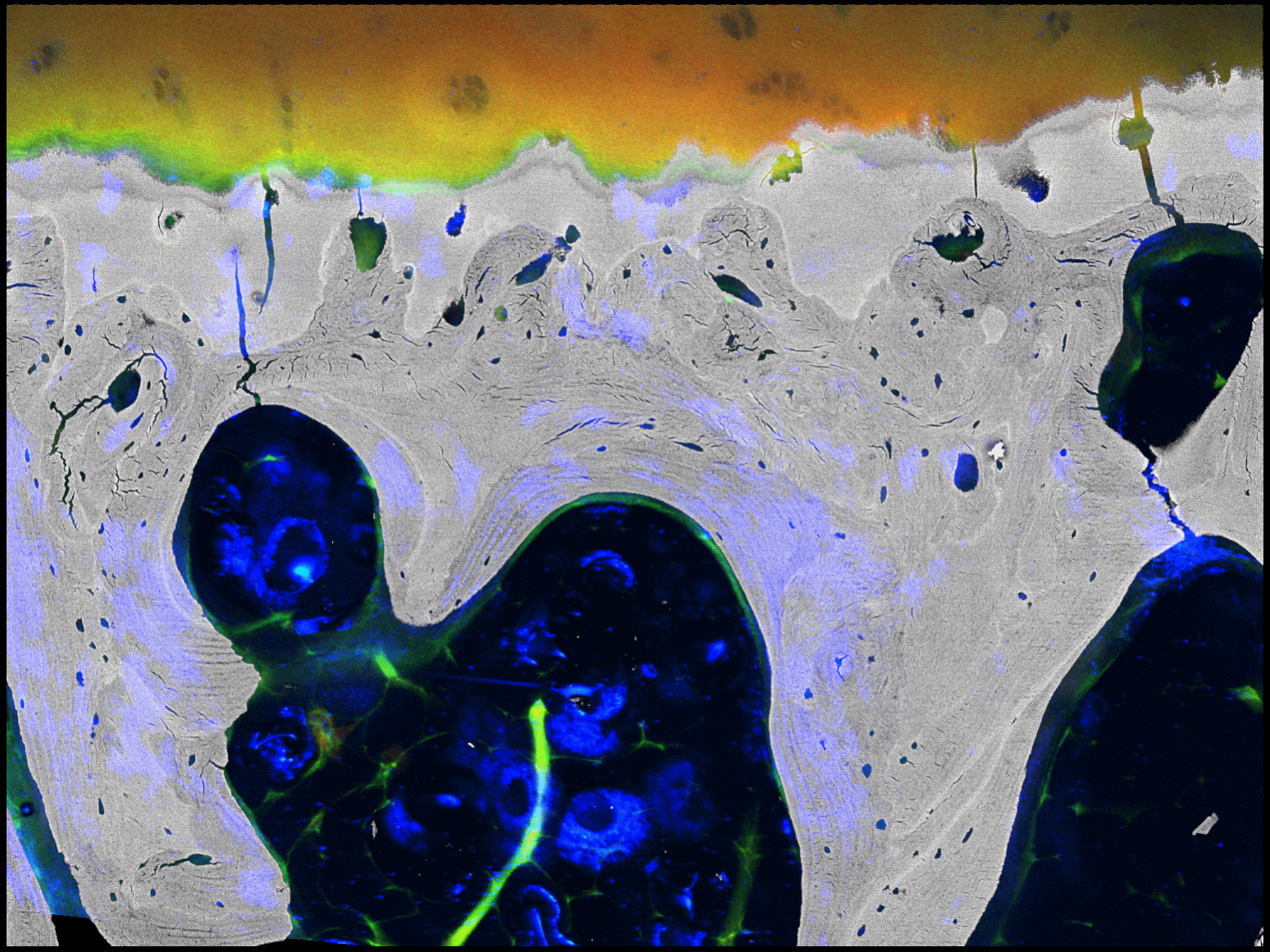
Edge 3D head LM autofluorescence,
One of stereo pair

AKU04 IMG_9343.JPG 18/02/2010

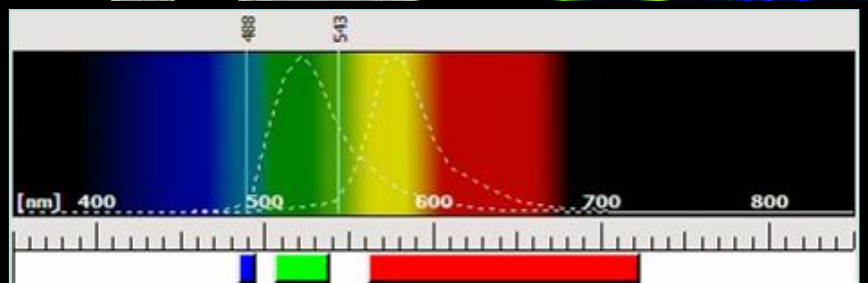
aku_15a_20_50Xsem_10xCSLM.gif

Embedded in PMMA, polished block, Confocal autofluorescence LM married to 20kV BSE SEM, movie

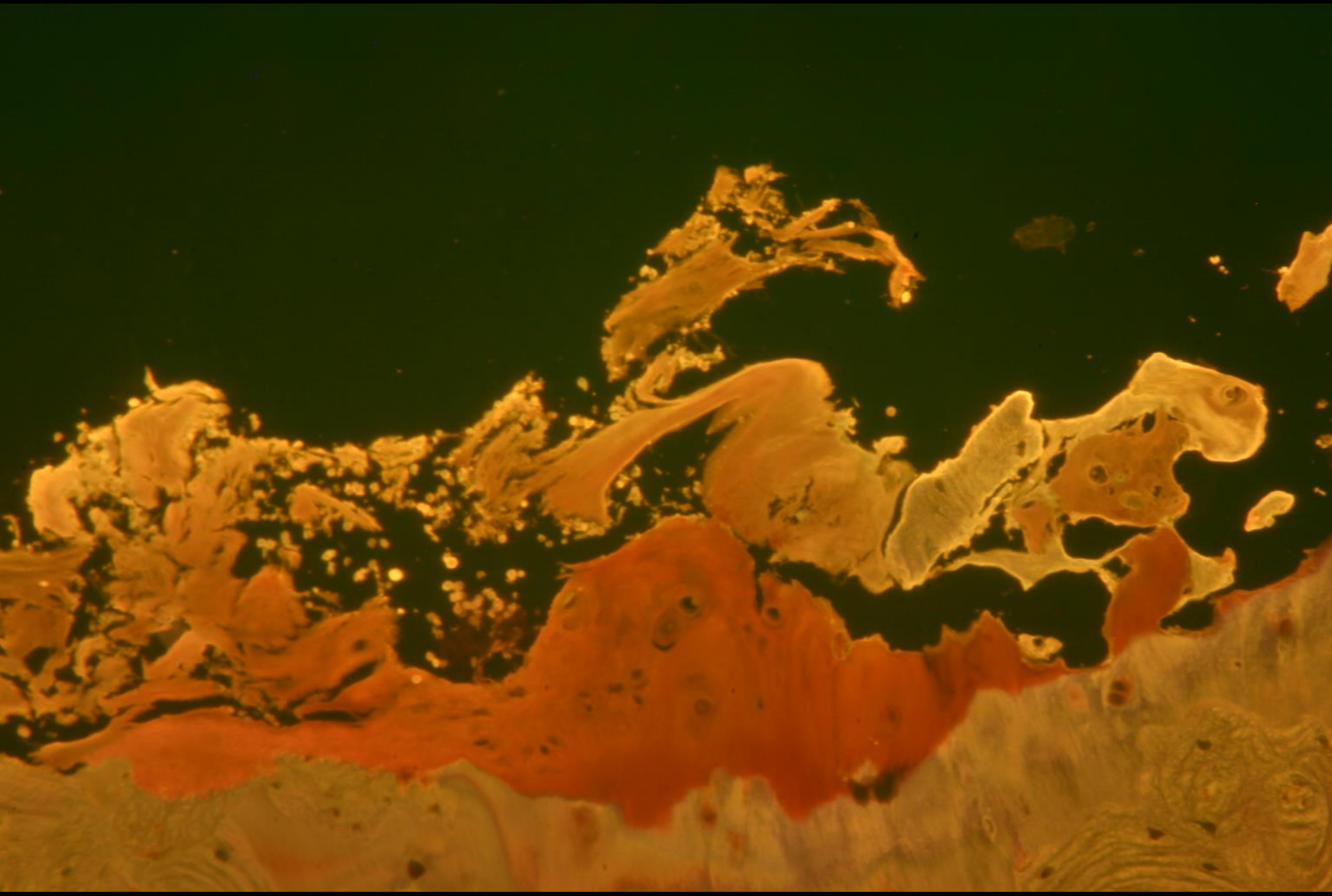




aku_15b_23_66Xsem_10xCSLM7fr.gif



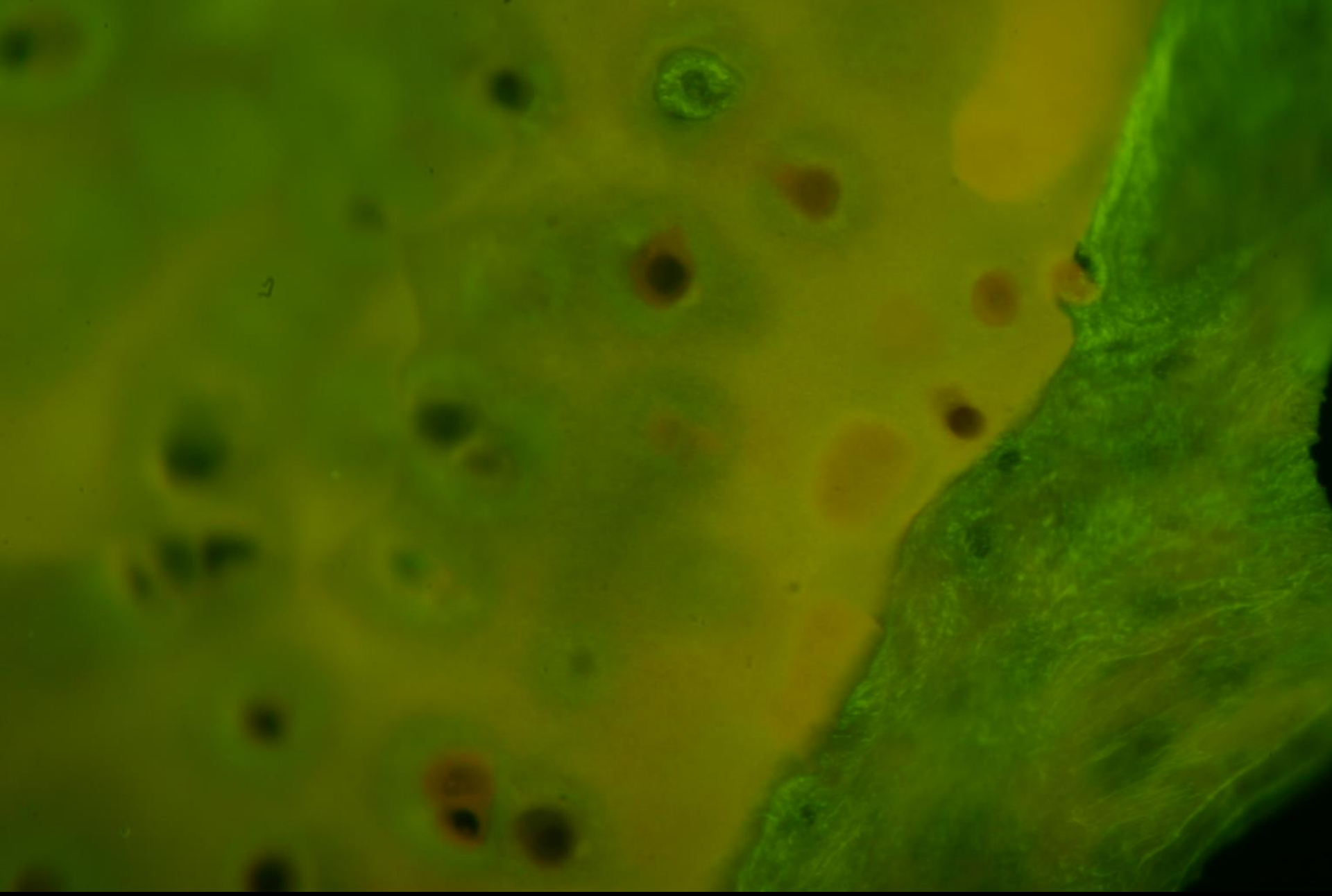
Embedded in PMMA, polished block, Confocal autofluorescence LM married to 20kV BSE SEM, movie



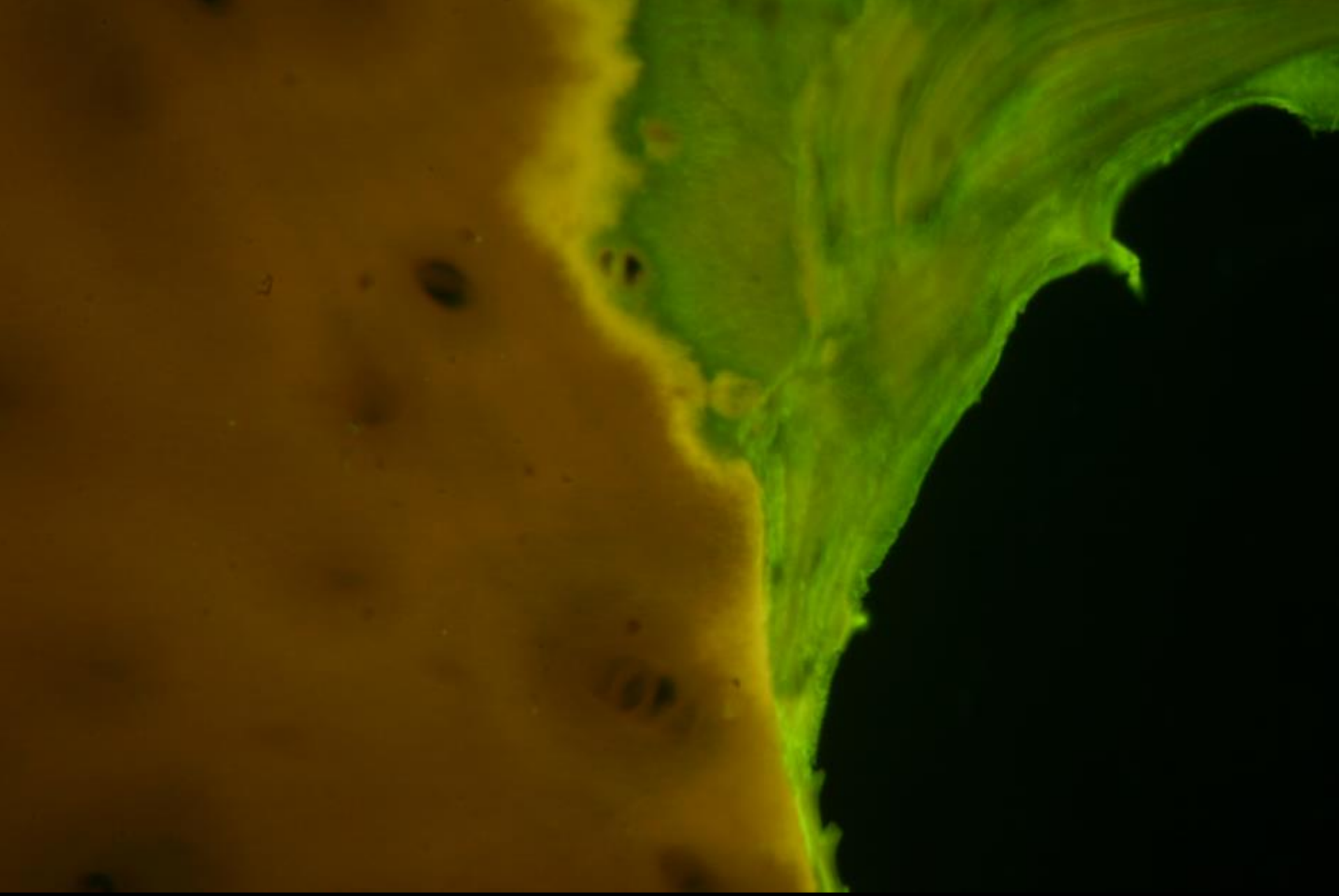
aku3081_l_9263.JPG

aku\20100222edge\aku10bdeminCC\IMG_9660.JPG

Edge 3D head LM autofluorescence,
One of stereo pair



f:\aku\20100217edge\l_9209.JPG



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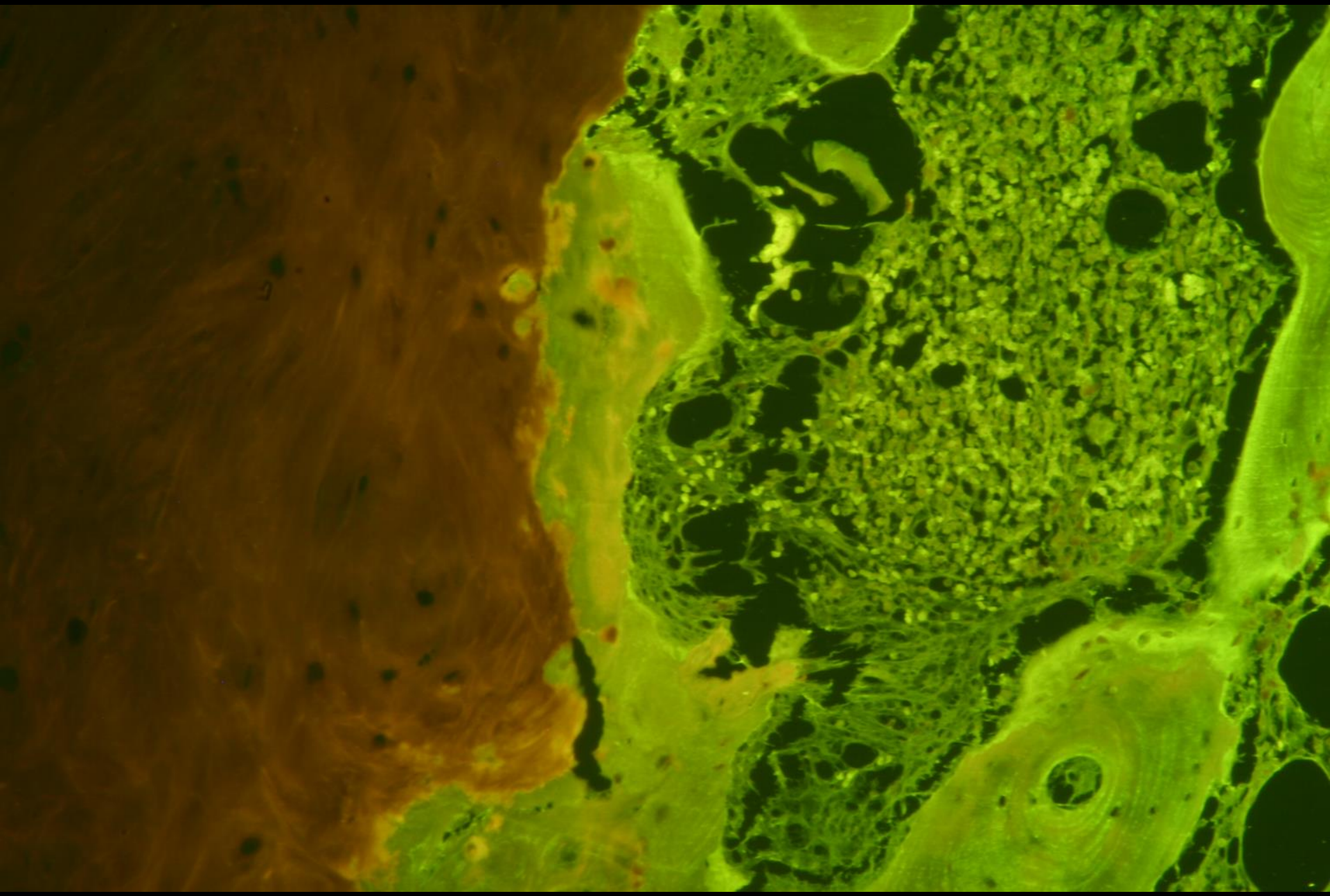
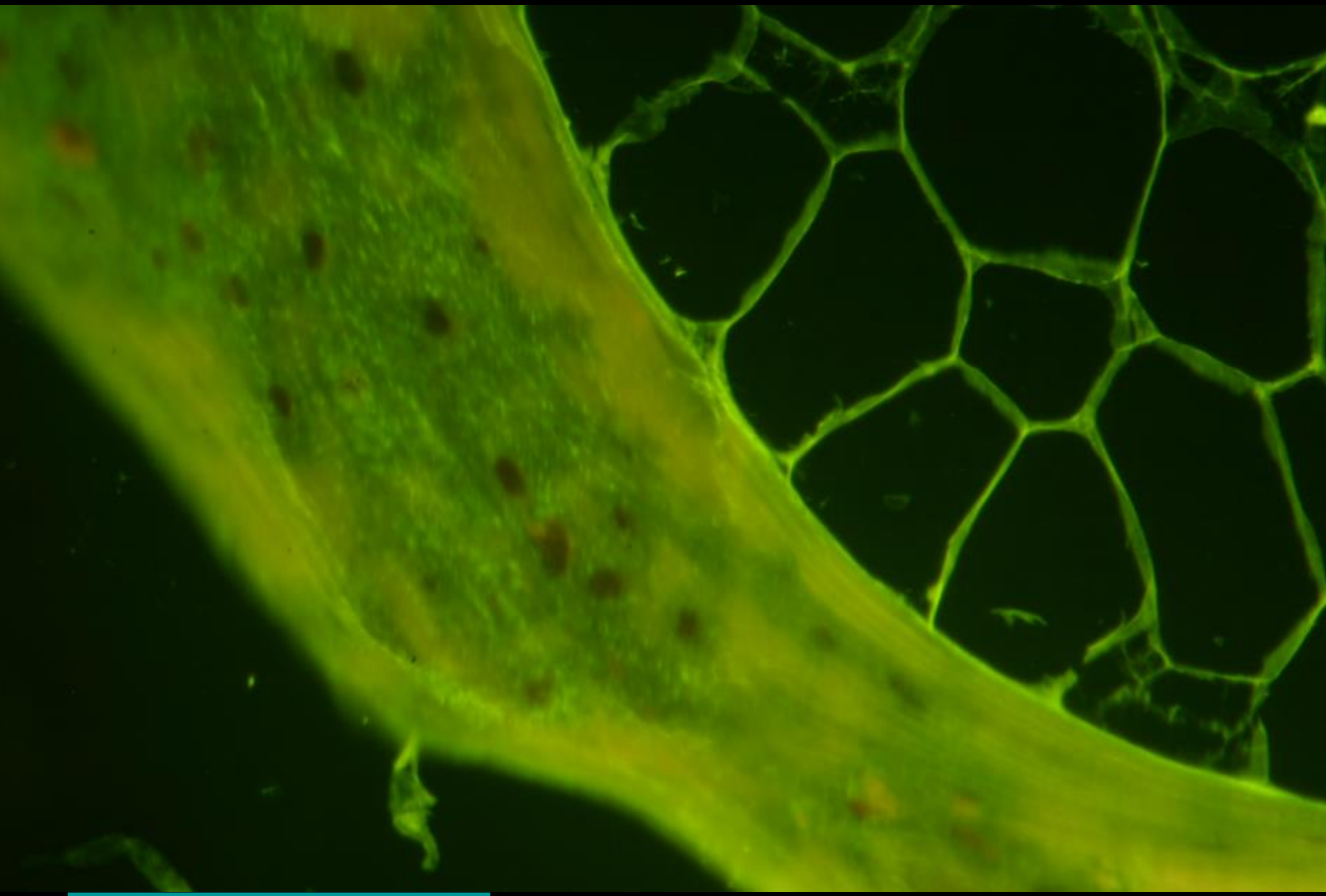
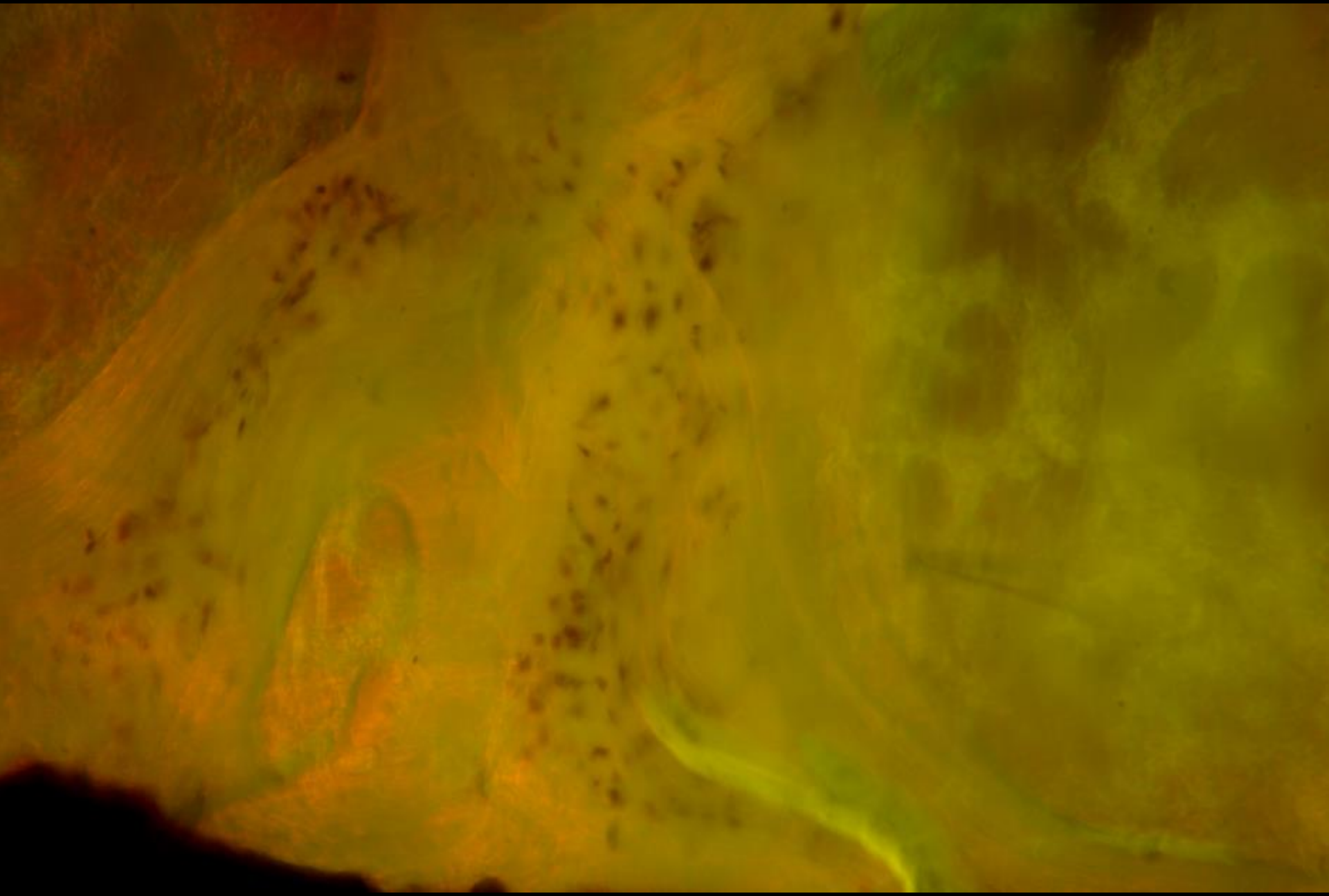


aku3r83_r_9621.JPG

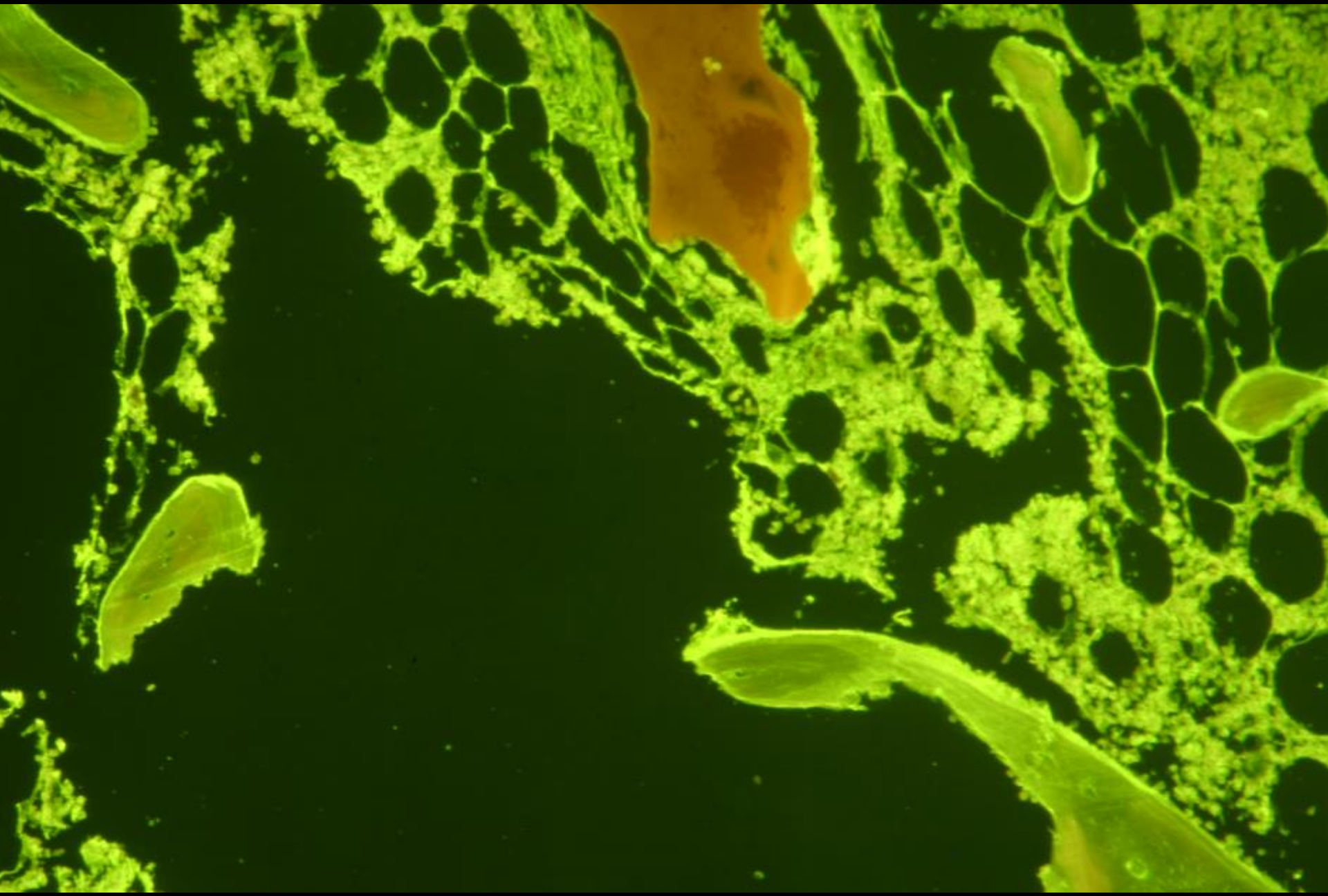


Aku3q085\IMG_9328

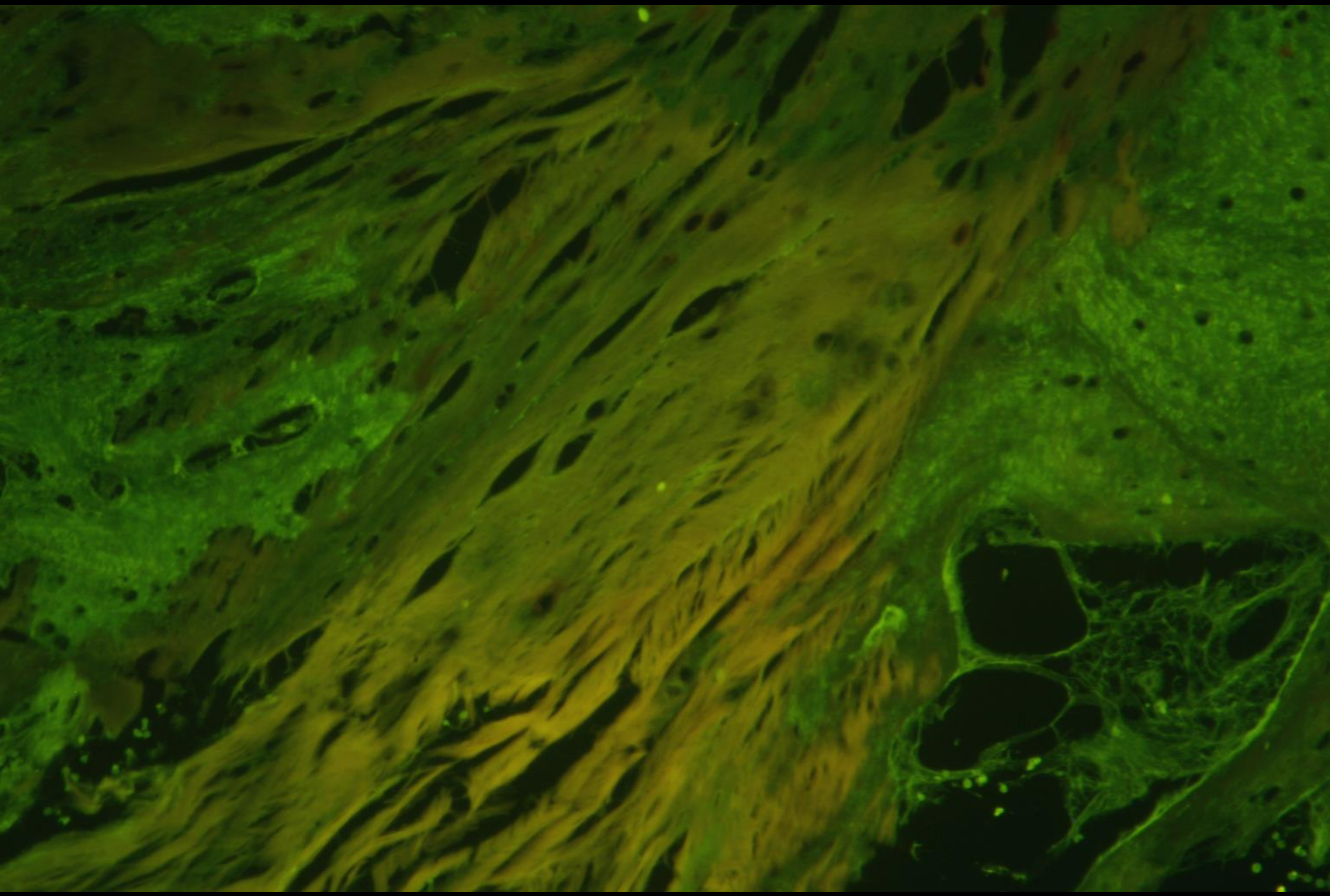
Edge 3D head LM autofluorescence,
one of stereo pair



IMG_9378.JPG



aku3082_l_9297.JPG



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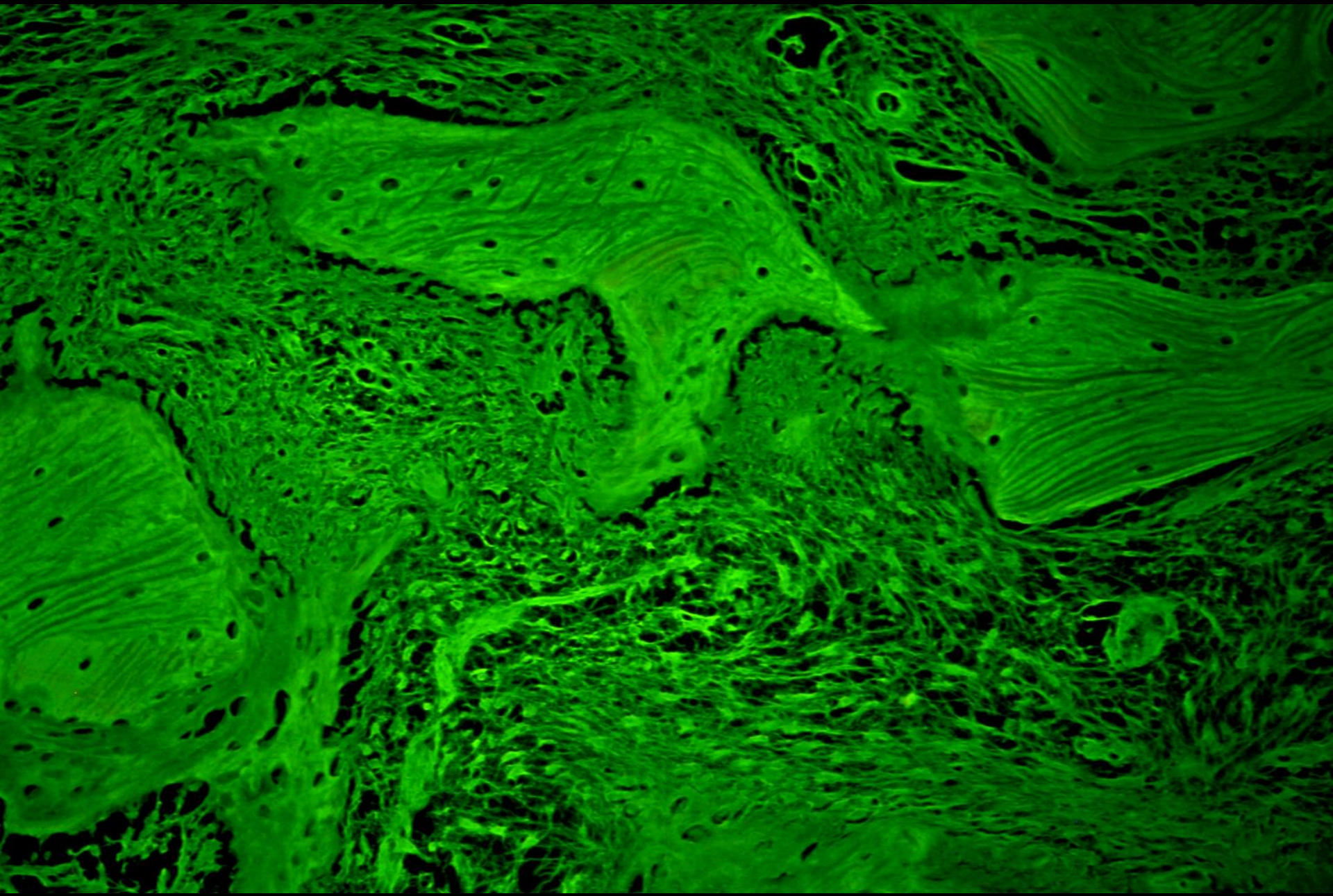
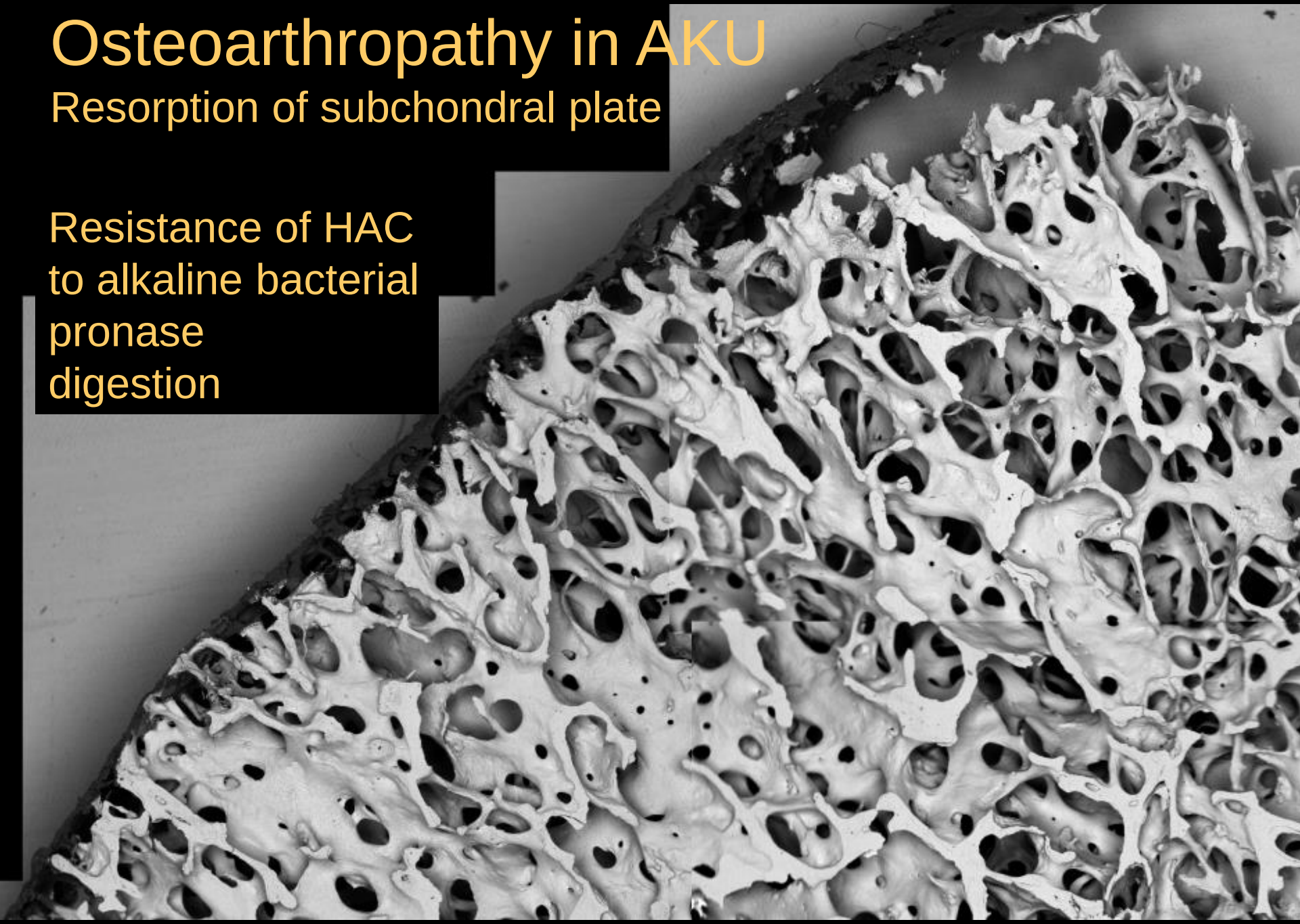


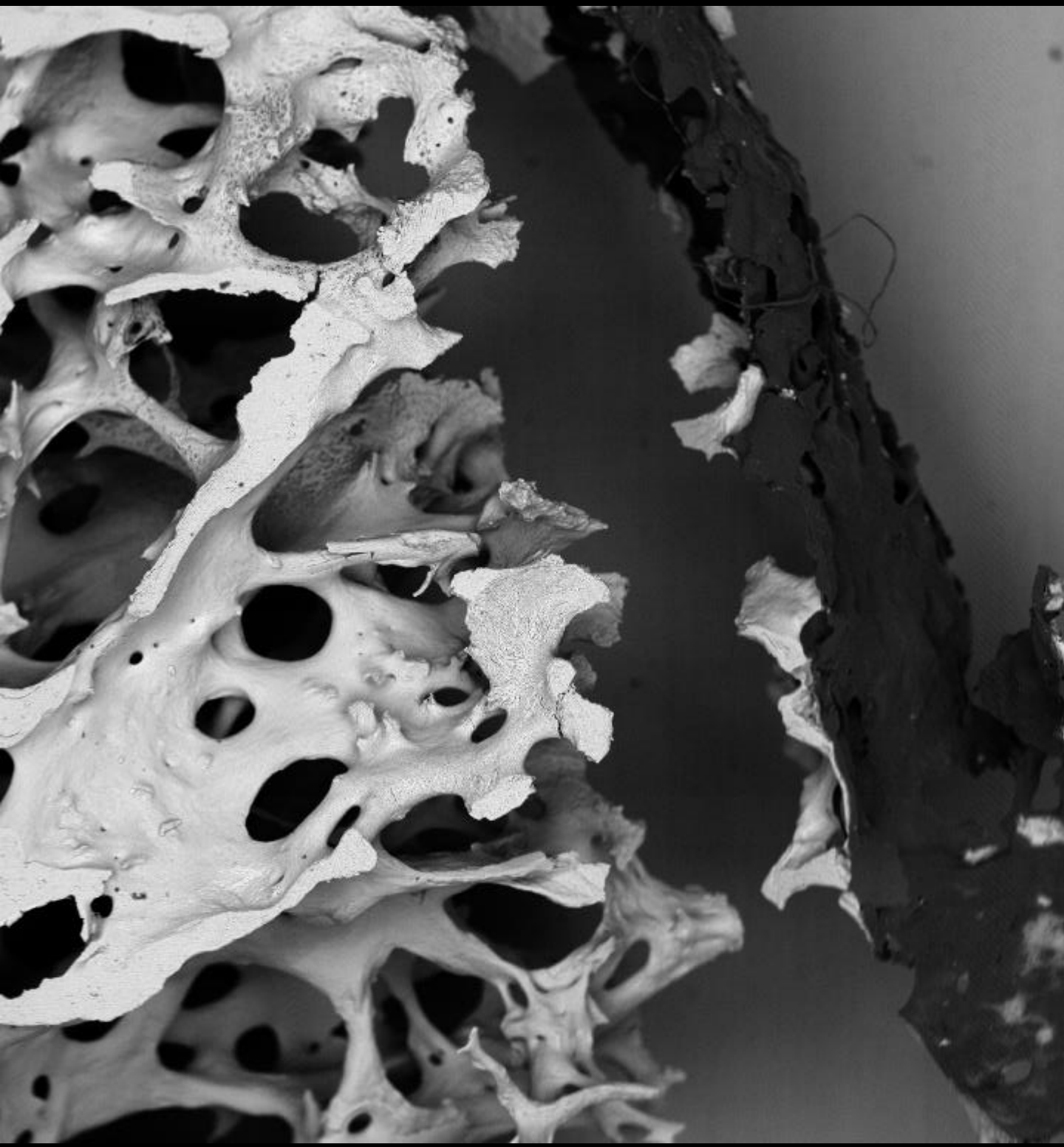
aku3081_I_9247&bc.JPG

Osteoarthropathy in AKU

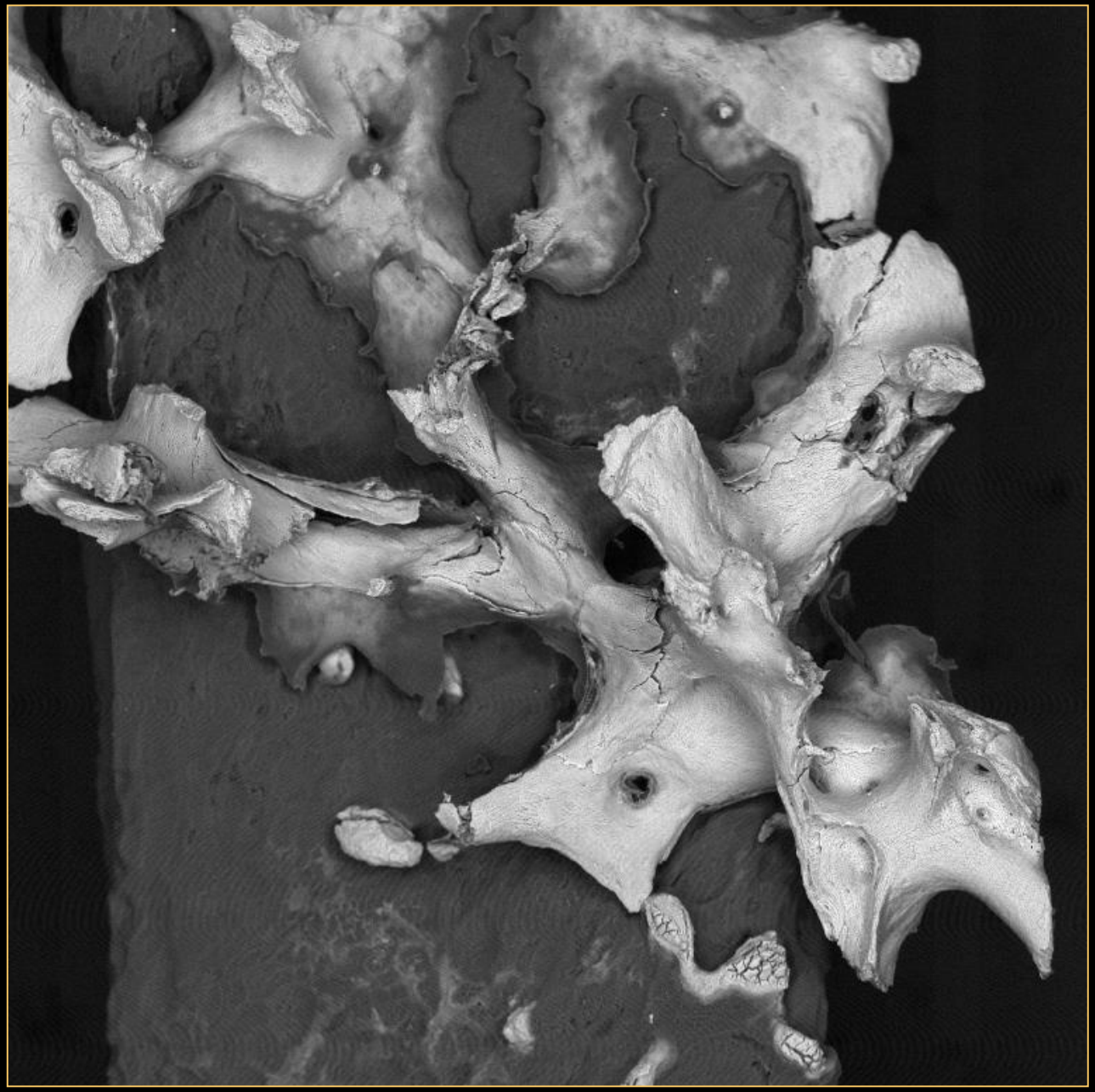
Resorption of subchondral plate

Resistance of HAC to alkaline bacterial pronase digestion



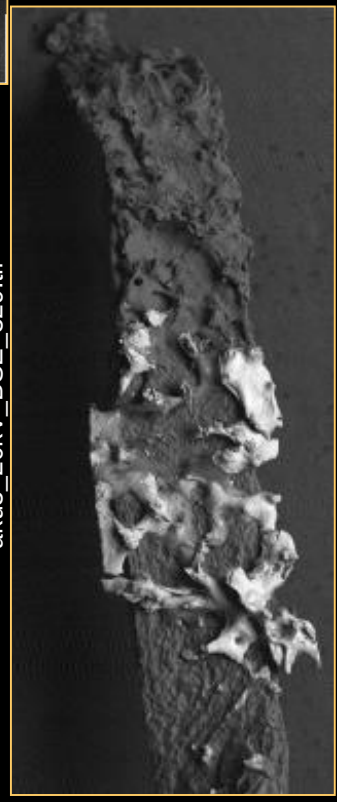


aku3-0-6-17-15

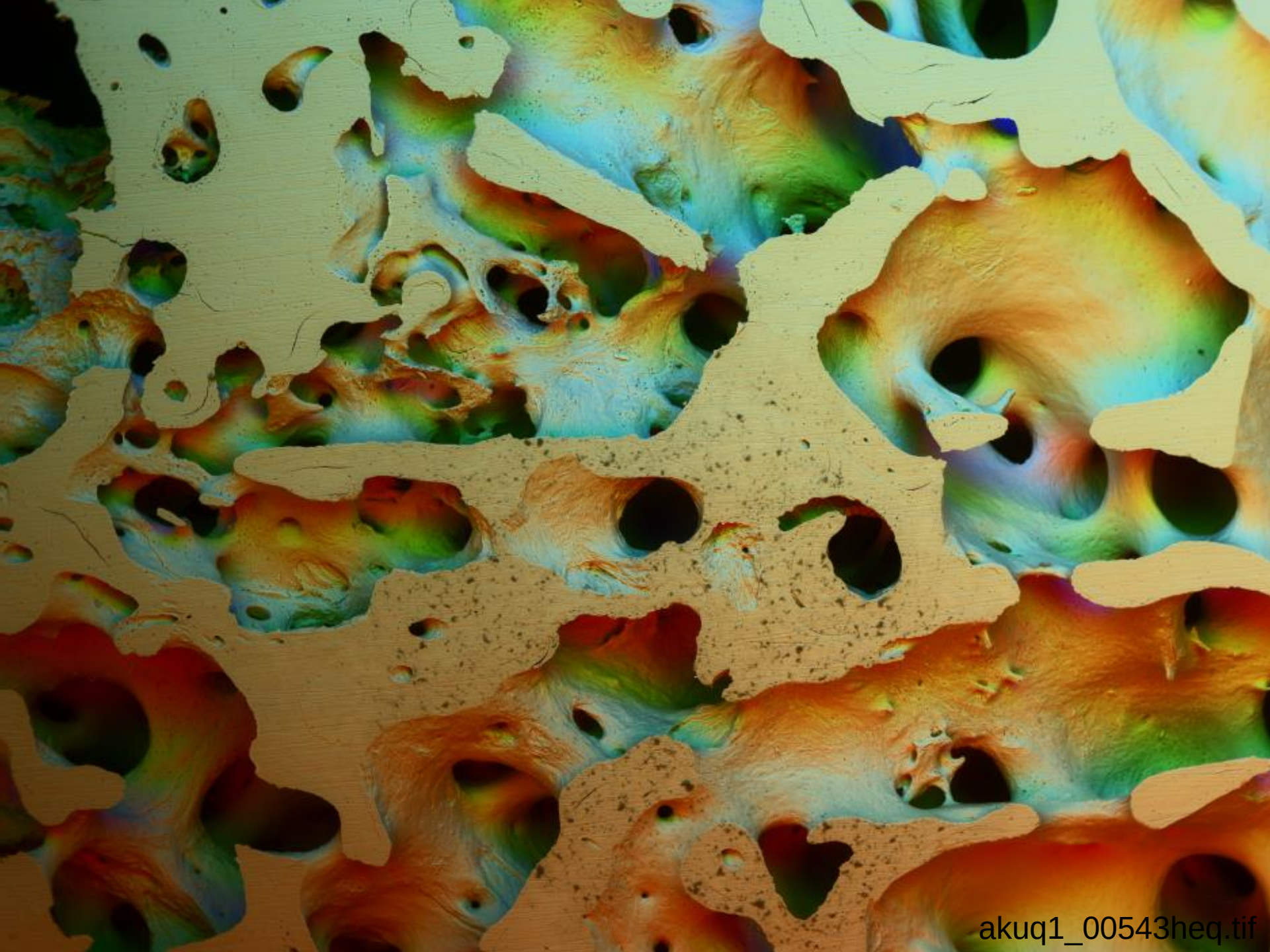


aku3_20kV_BSE_4033.tif 2010 02 23 AK

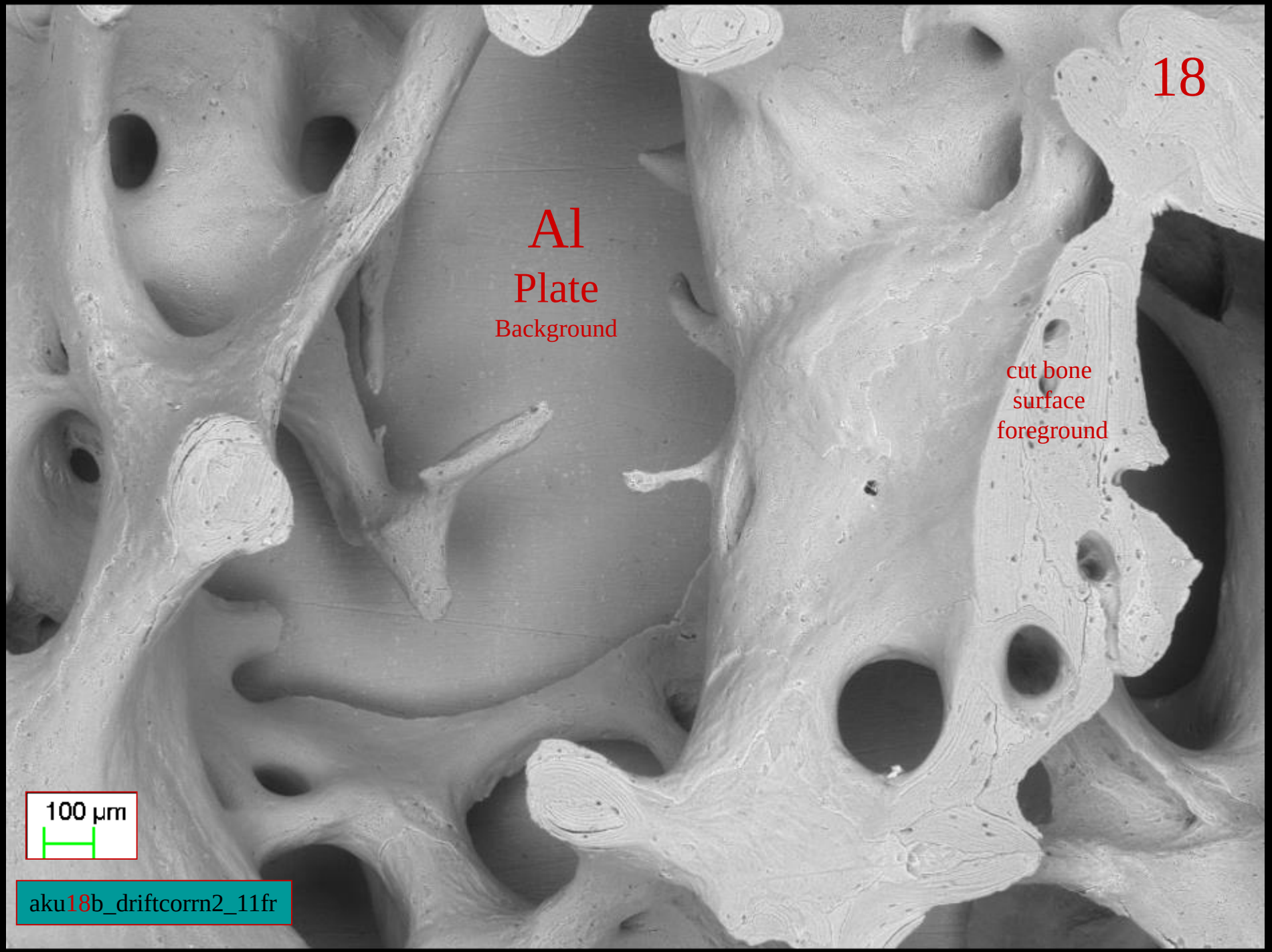
aku3_2kV_SE_strip_mont.tif



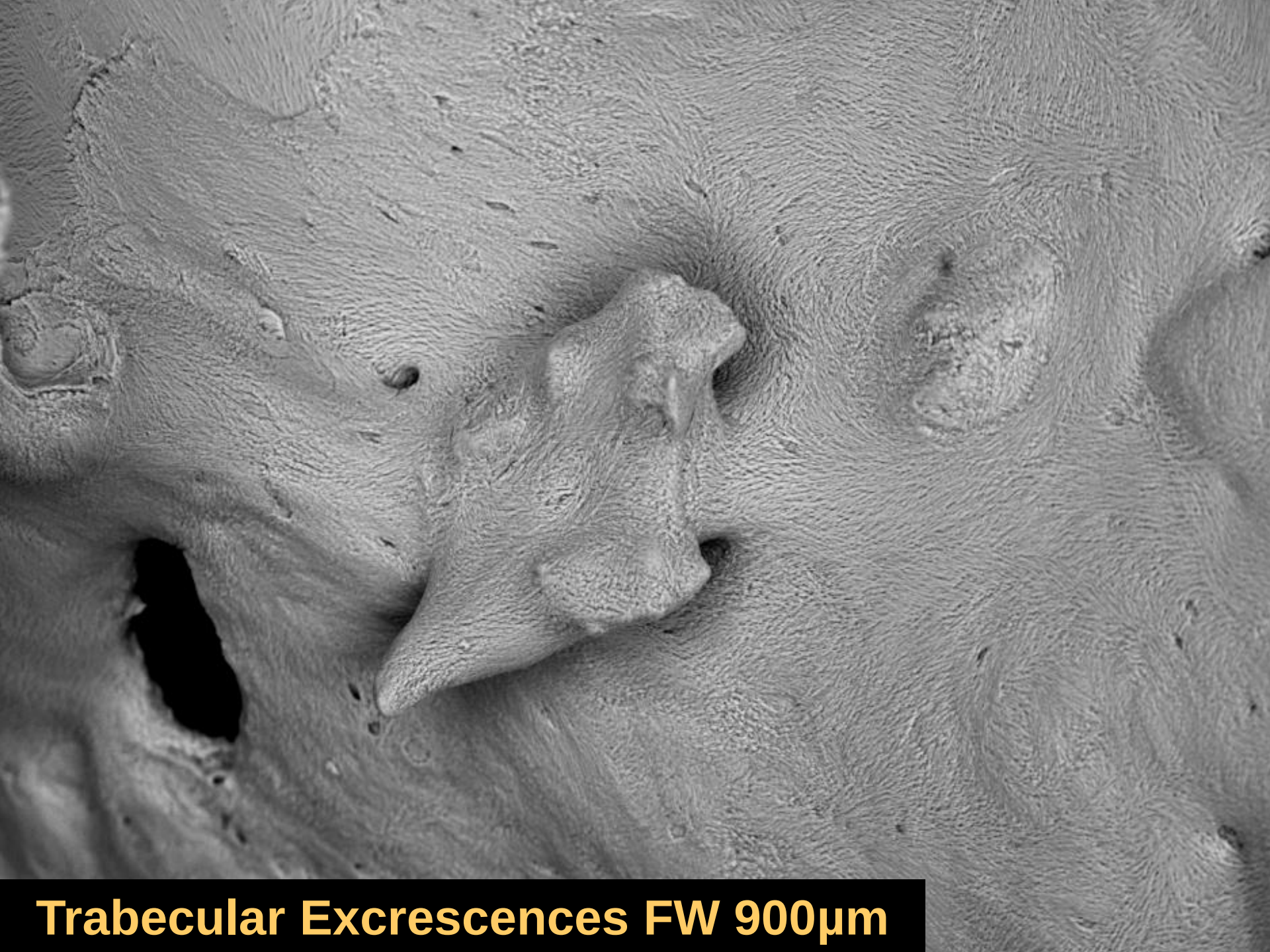
aku3_20kV_BSE_329.tif



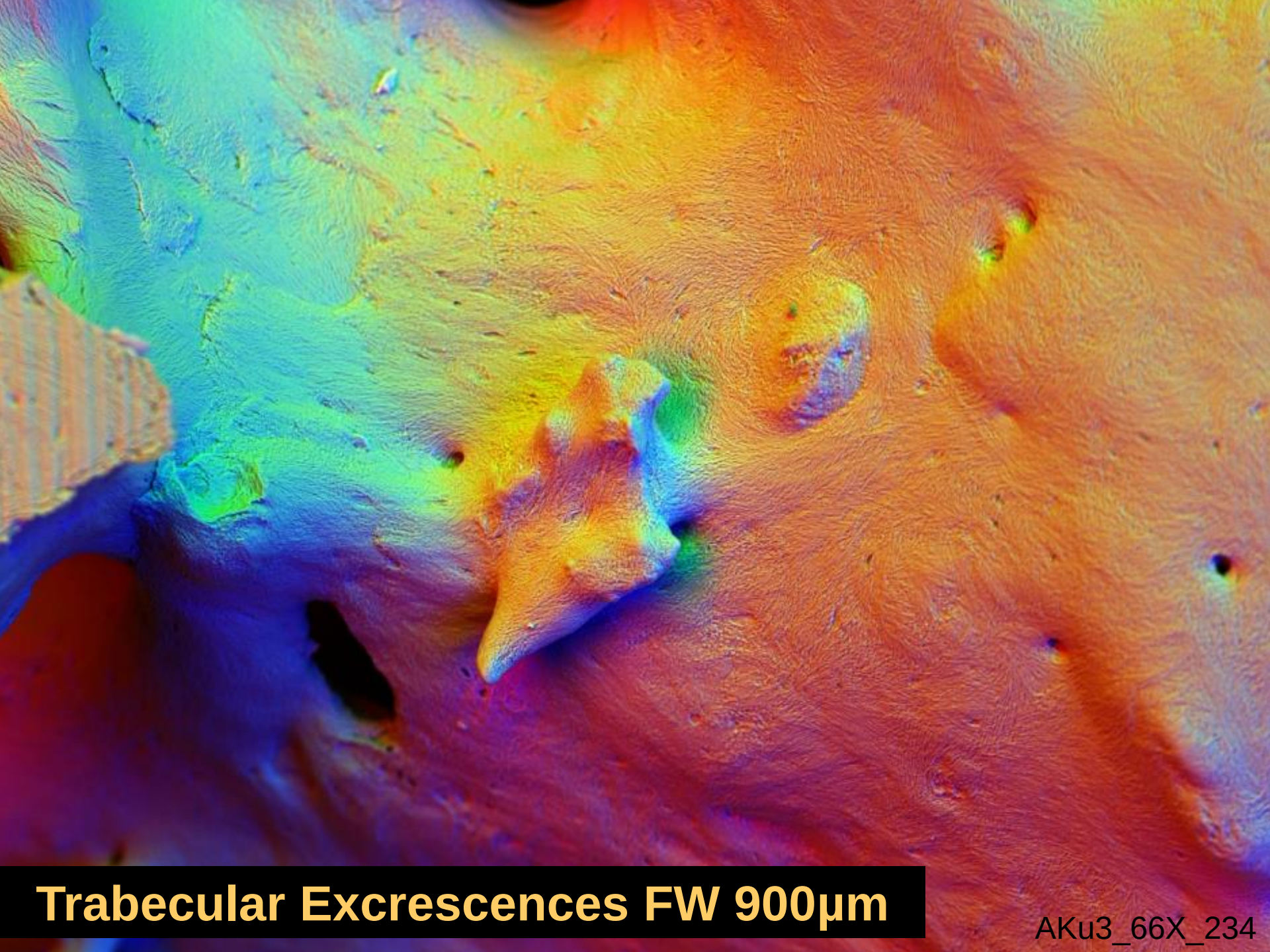
akuq1_00543heq.tif

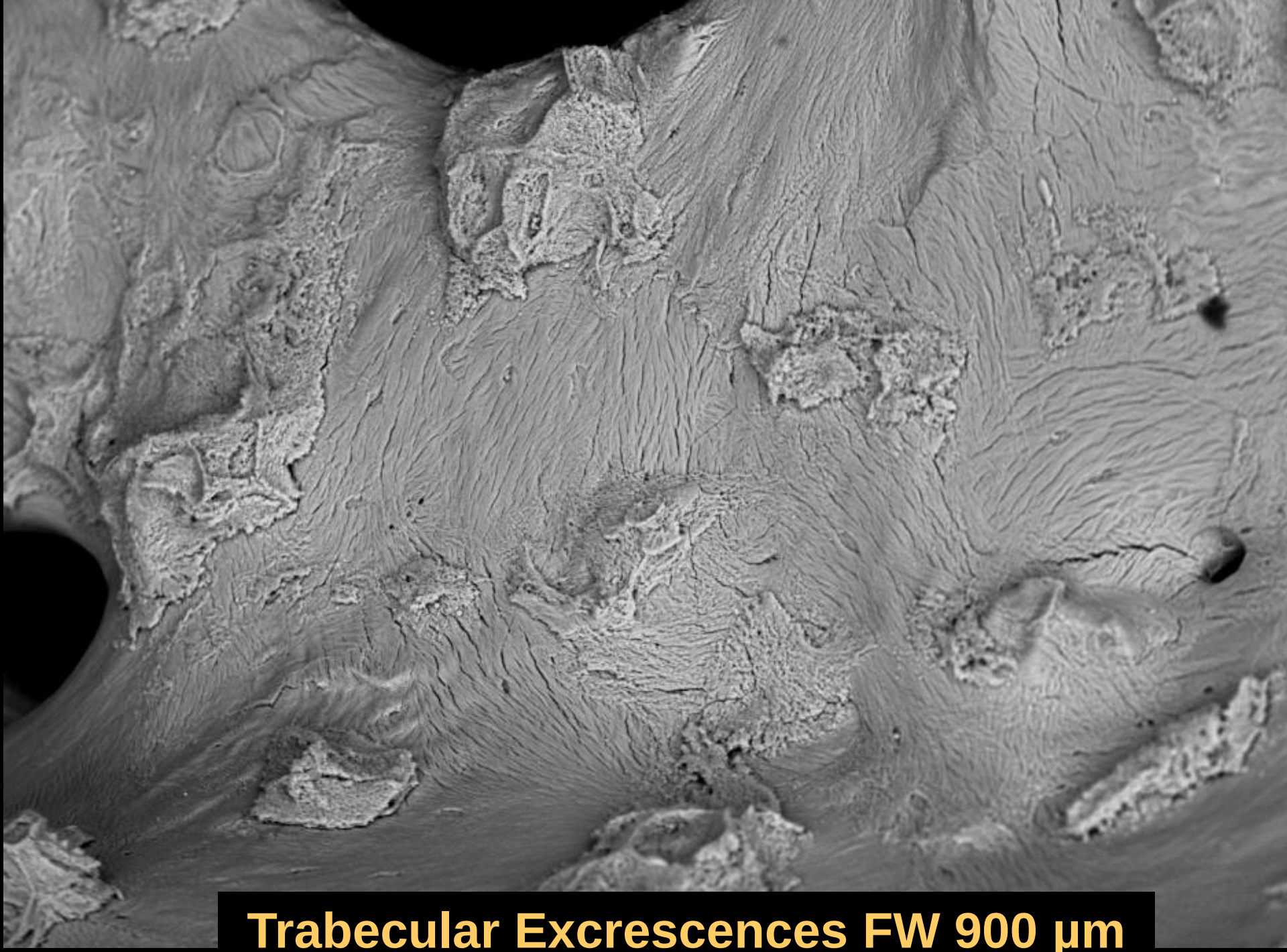

18
Al
Plate
Background
cut bone
surface
foreground
100 µm
aku18b_driftcorrn2_11fr


Trabecular Excrescences

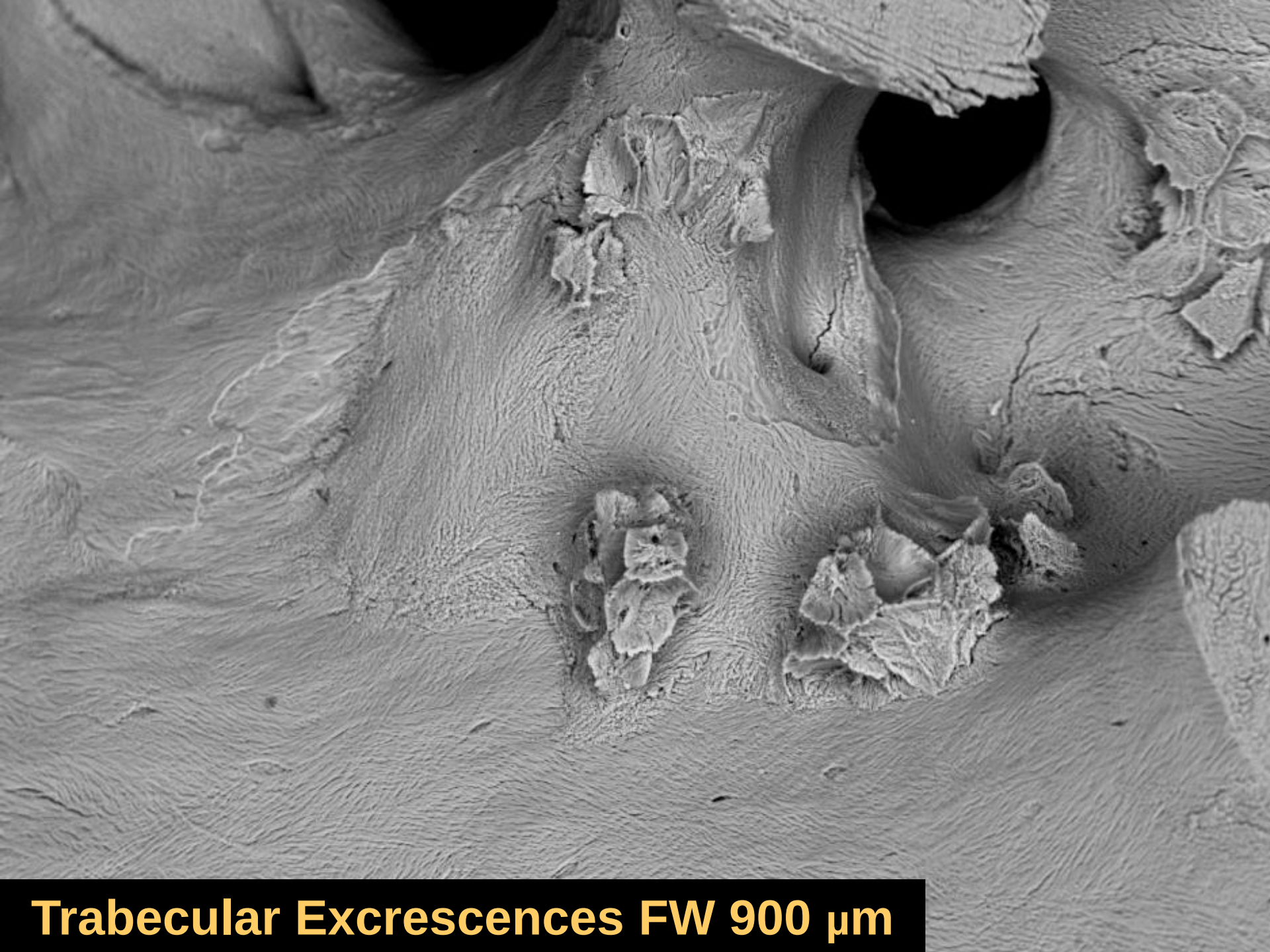


Trabecular Excrescences FW 900µm

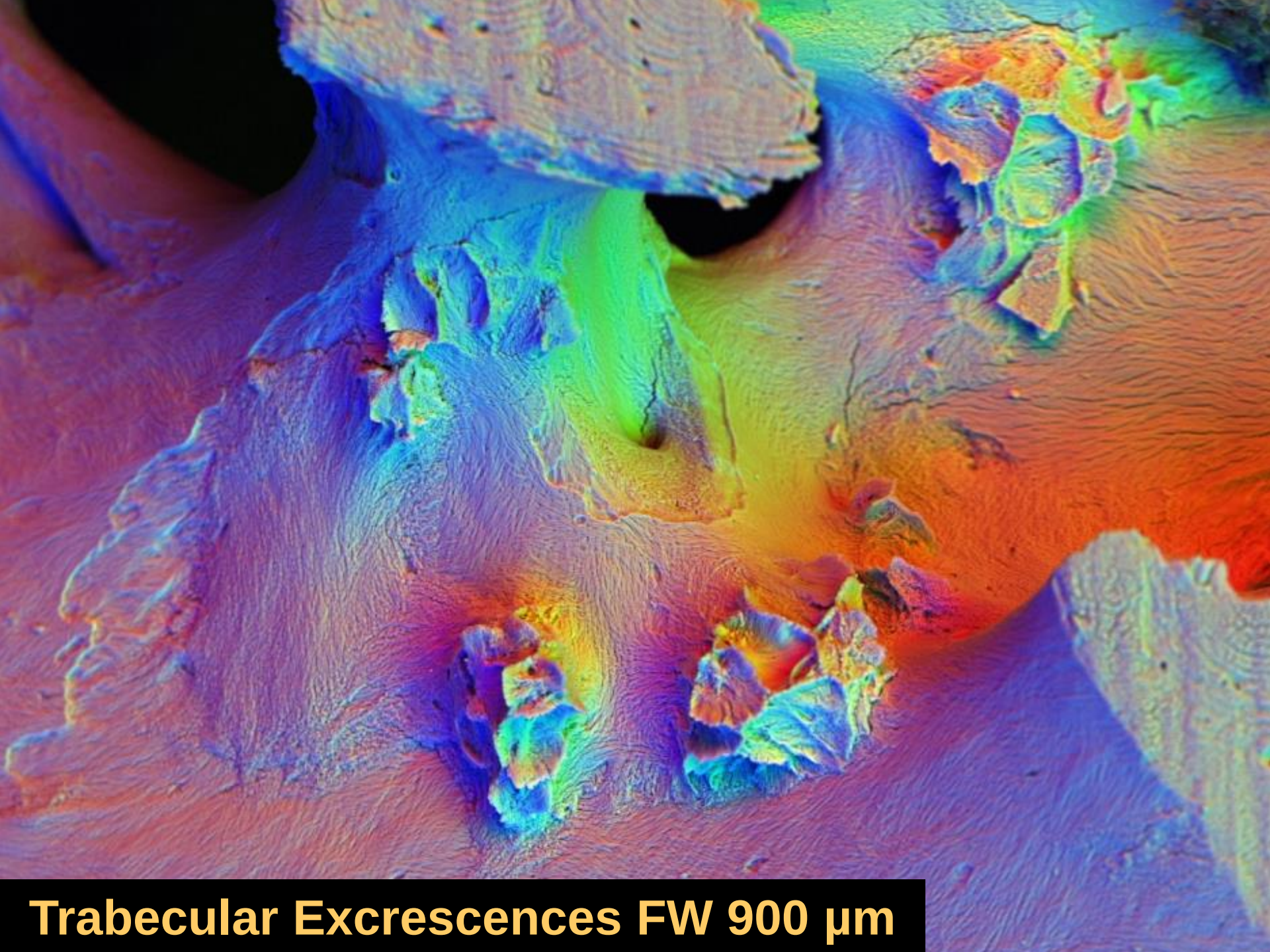

Trabecular Excrescences FW 900µm
AKu3_66X_234



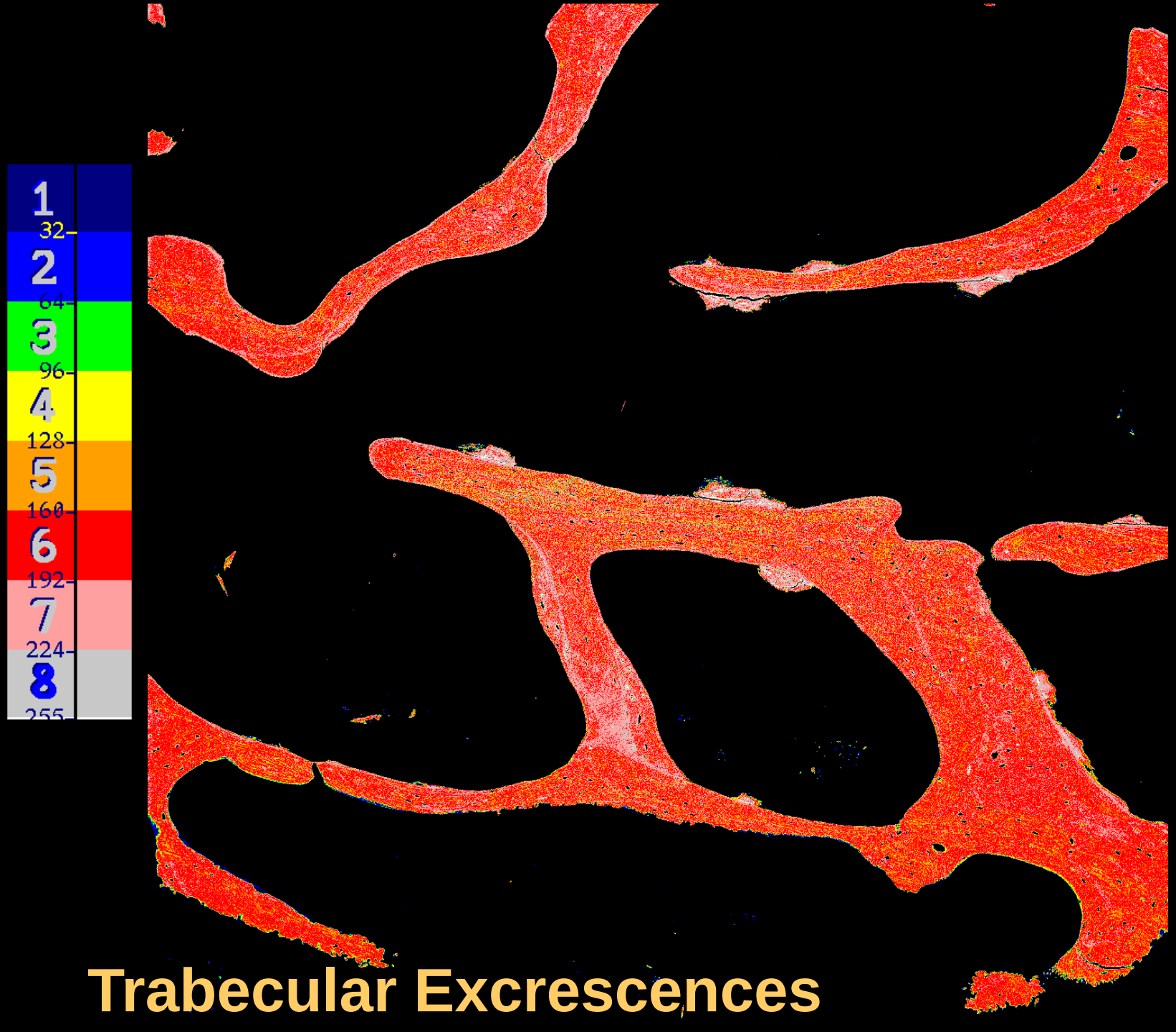
Trabecular Excrescences FW 900 µm



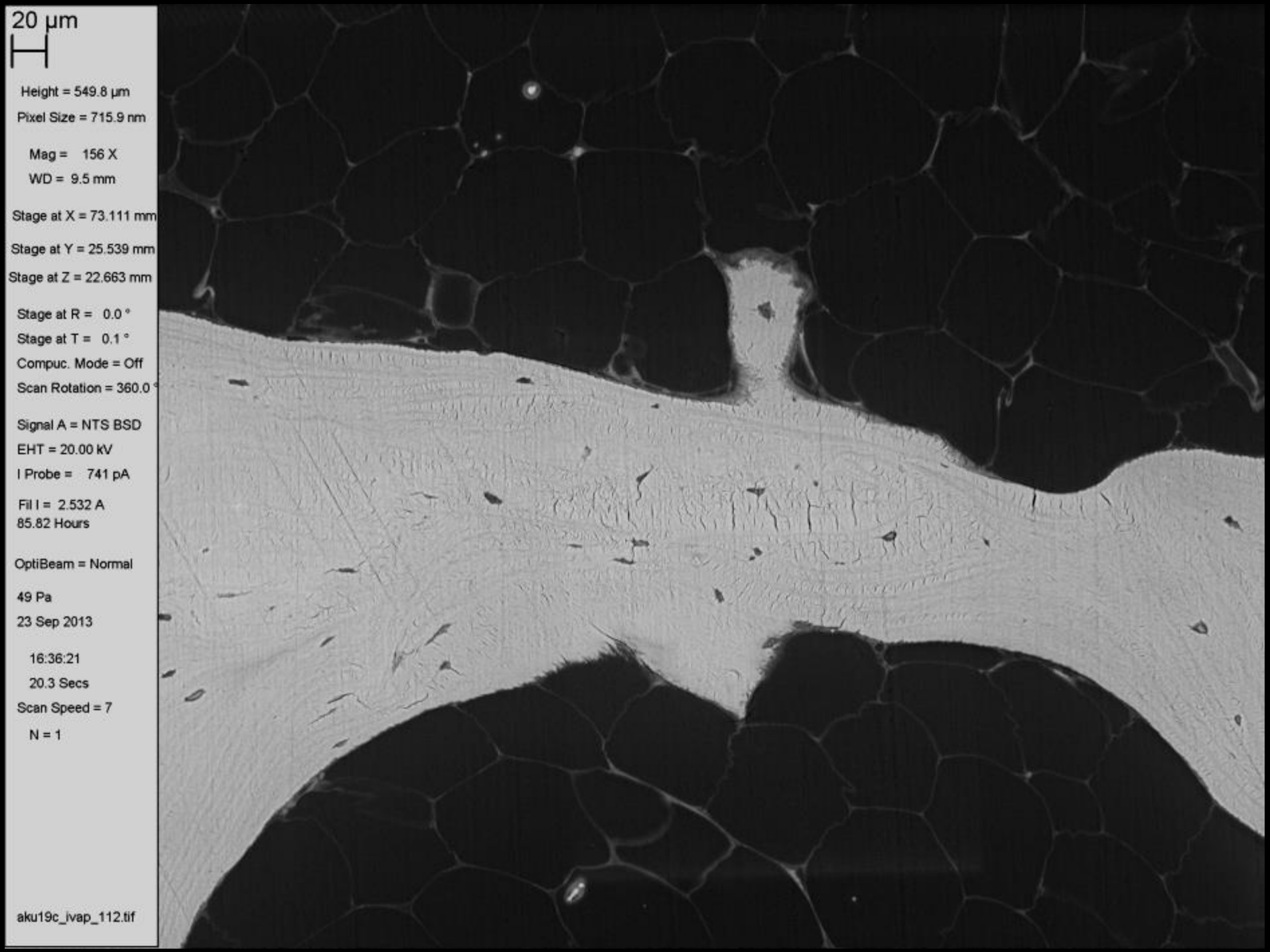
Trabecular Excrescences FW 900 µm



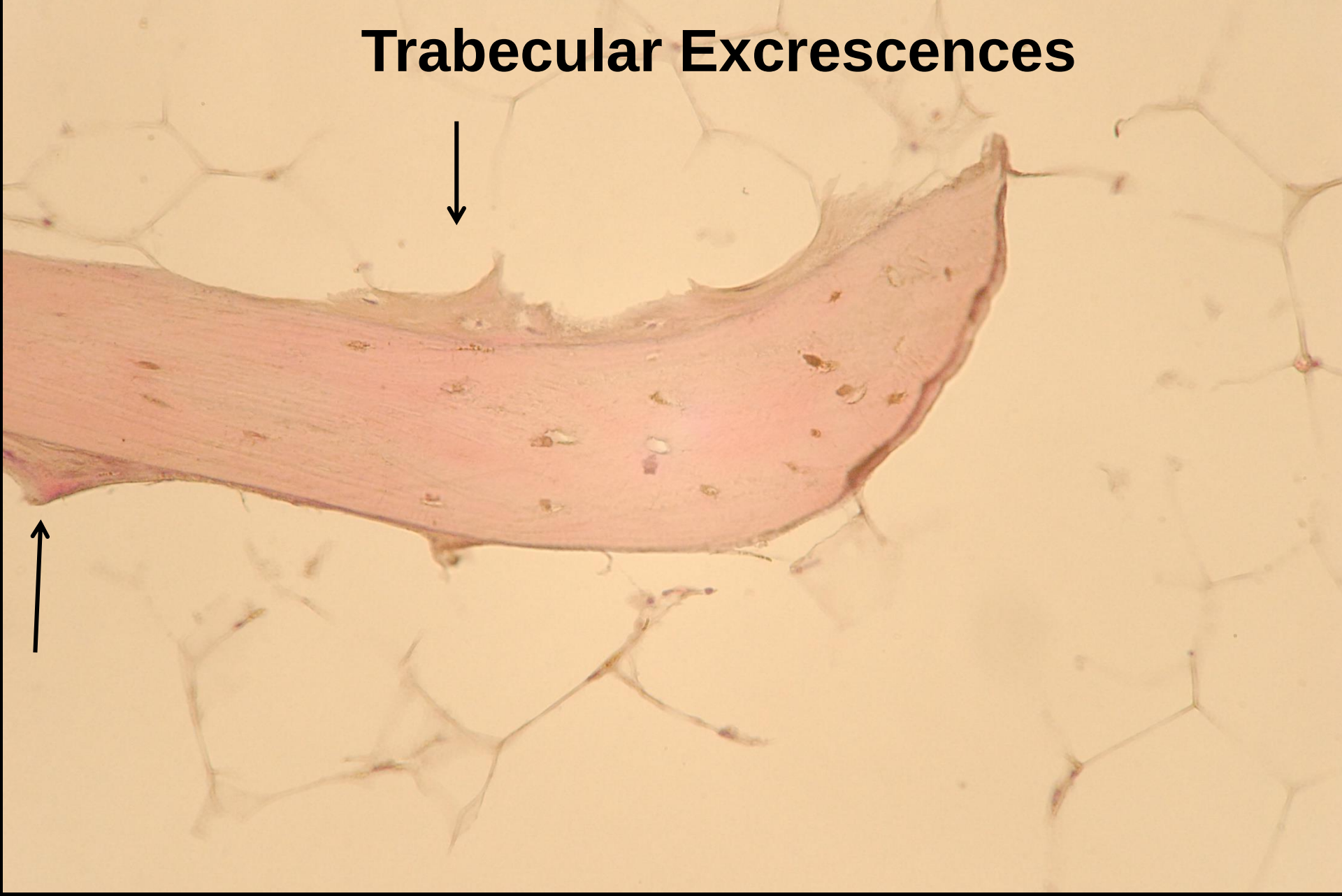
Trabecular Excrescences FW 900 µm



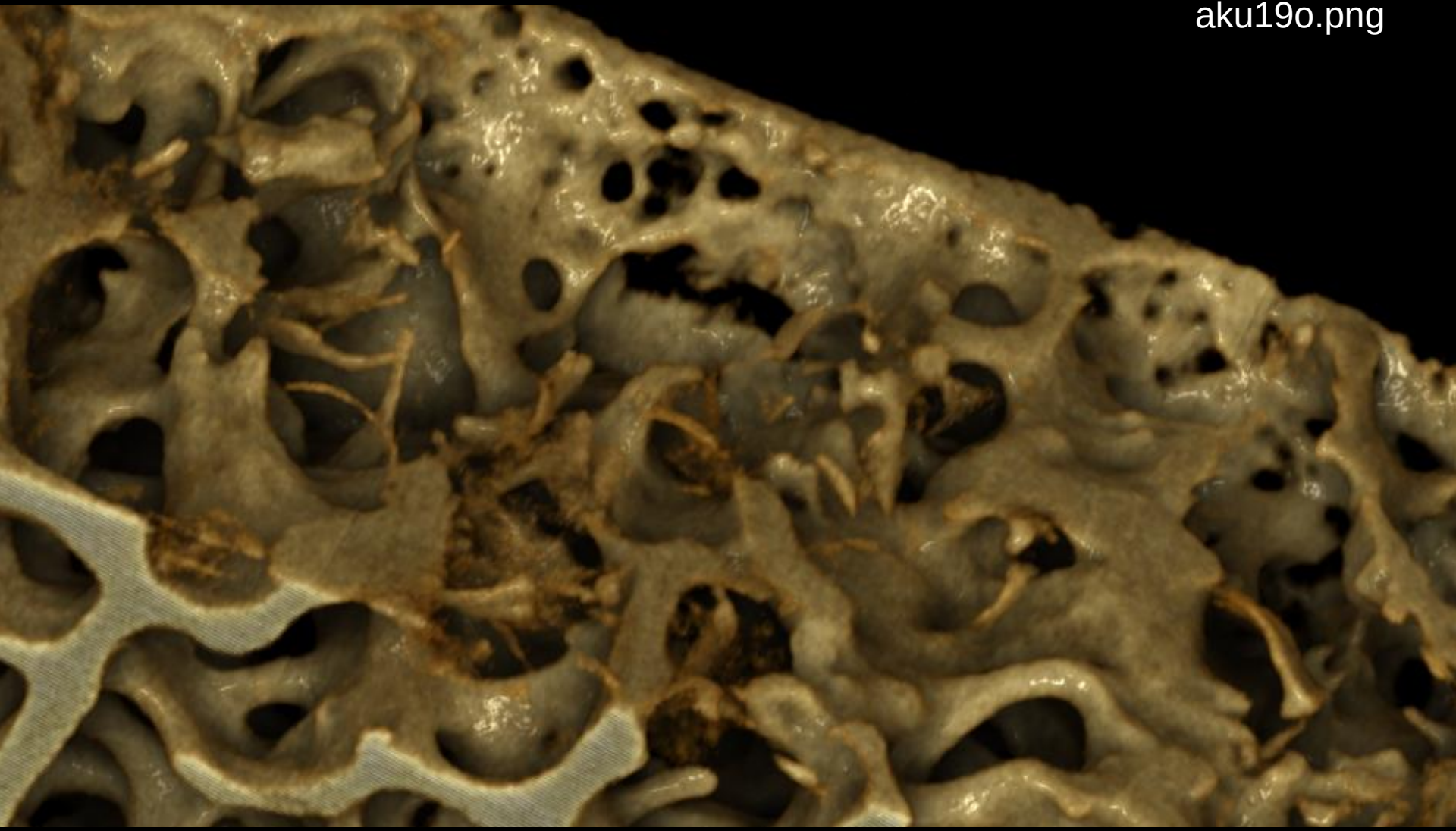
Trabecular Excrescences


20 µm
Height = 549.8 µm
Pixel Size = 715.9 nm
Mag = 156 X
WD = 9.5 mm
Stage at X = 73.111 mm
Stage at Y = 25.539 mm
Stage at Z = 22.663 mm
Stage at R = 0.0 °
Stage at T = 0.1 °
Compuc. Mode = Off
Scan Rotation = 360.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 741 pA
Fil I = 2.532 A
85.82 Hours
OptiBeam = Normal
49 Pa
23 Sep 2013
16:36:21
20.3 Secs
Scan Speed = 7
N = 1
aku19c_ivap_112.tif

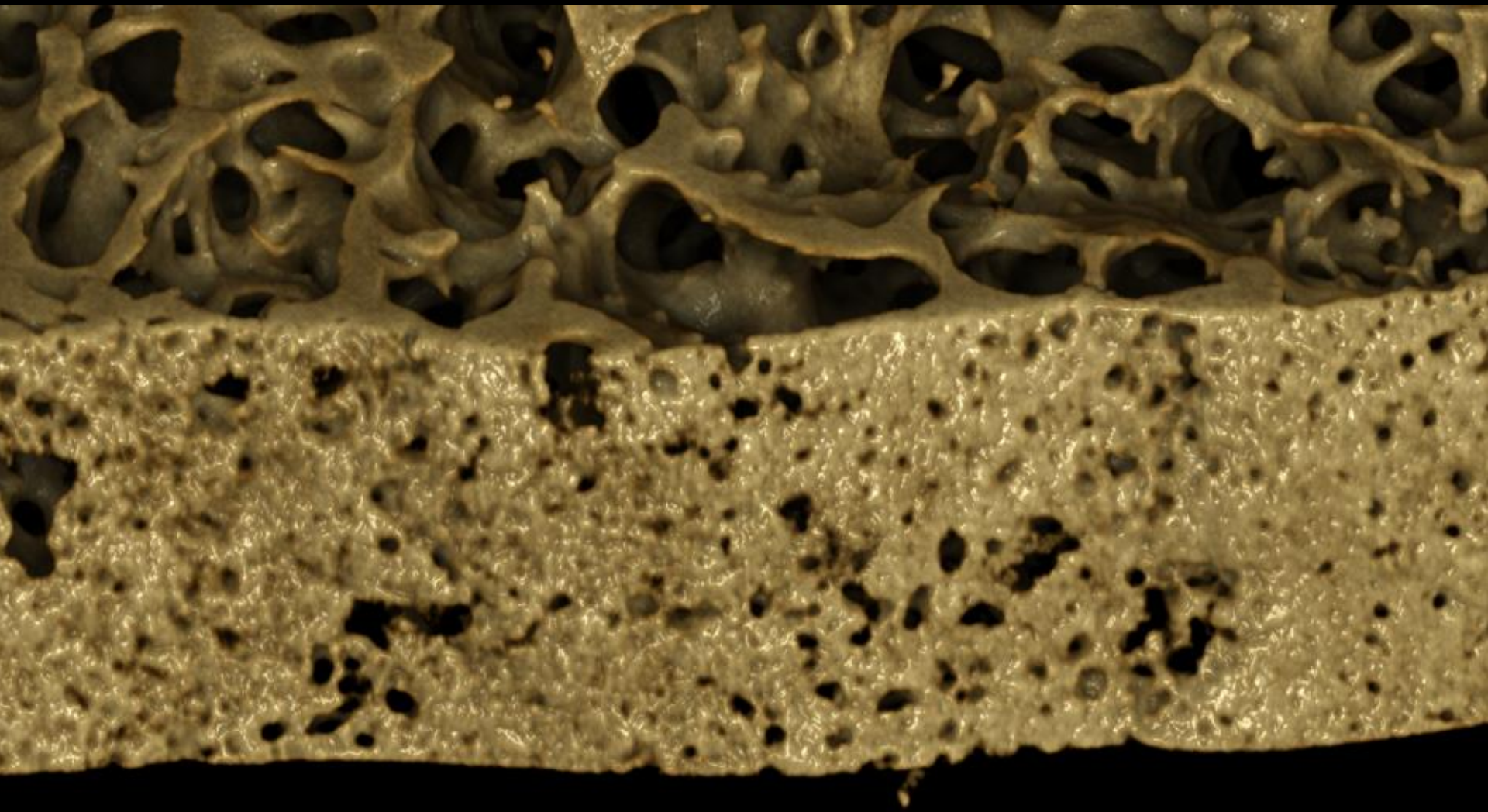
Trabecular Excrescences



Eur Cell Mater 2012 23 300

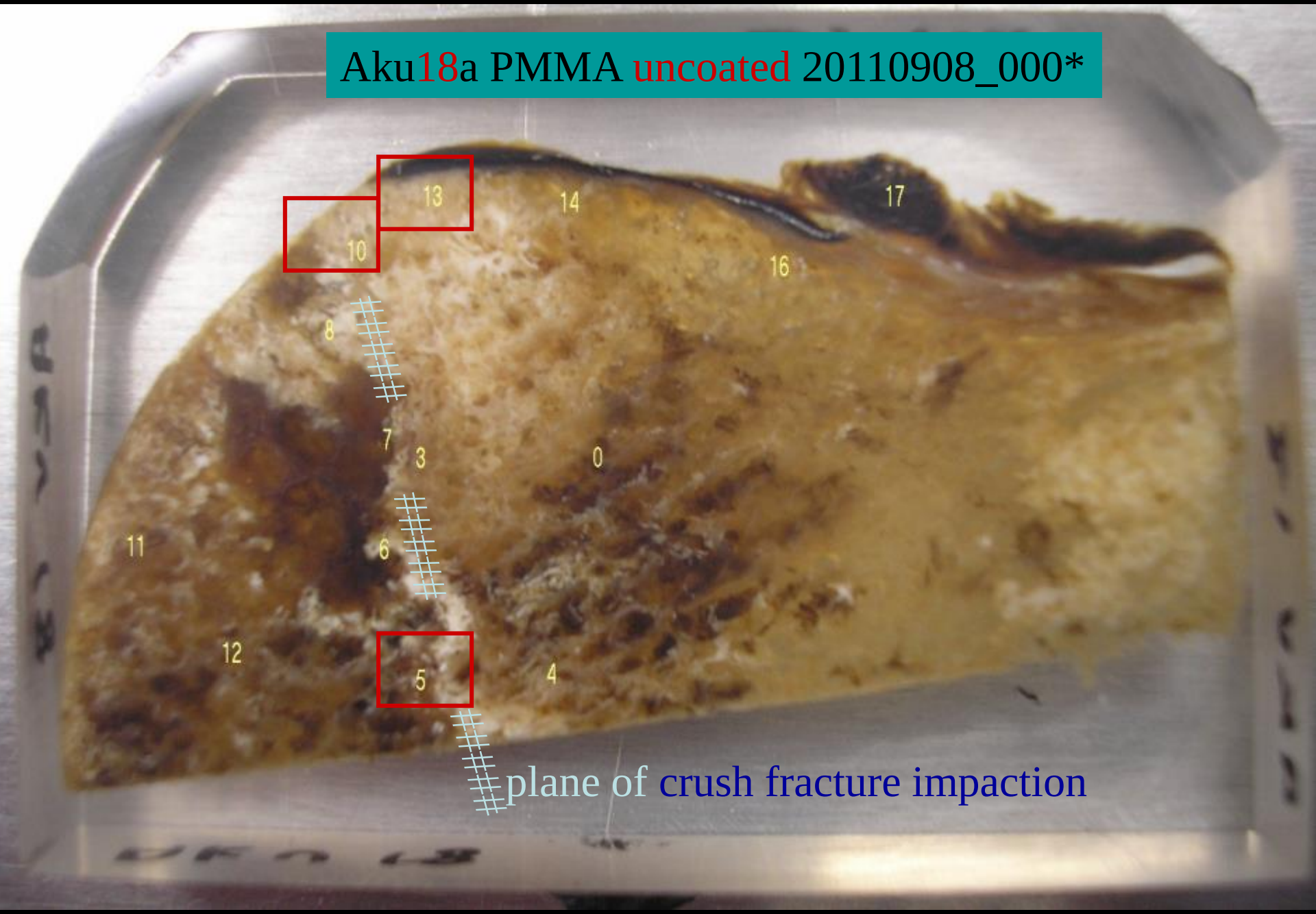


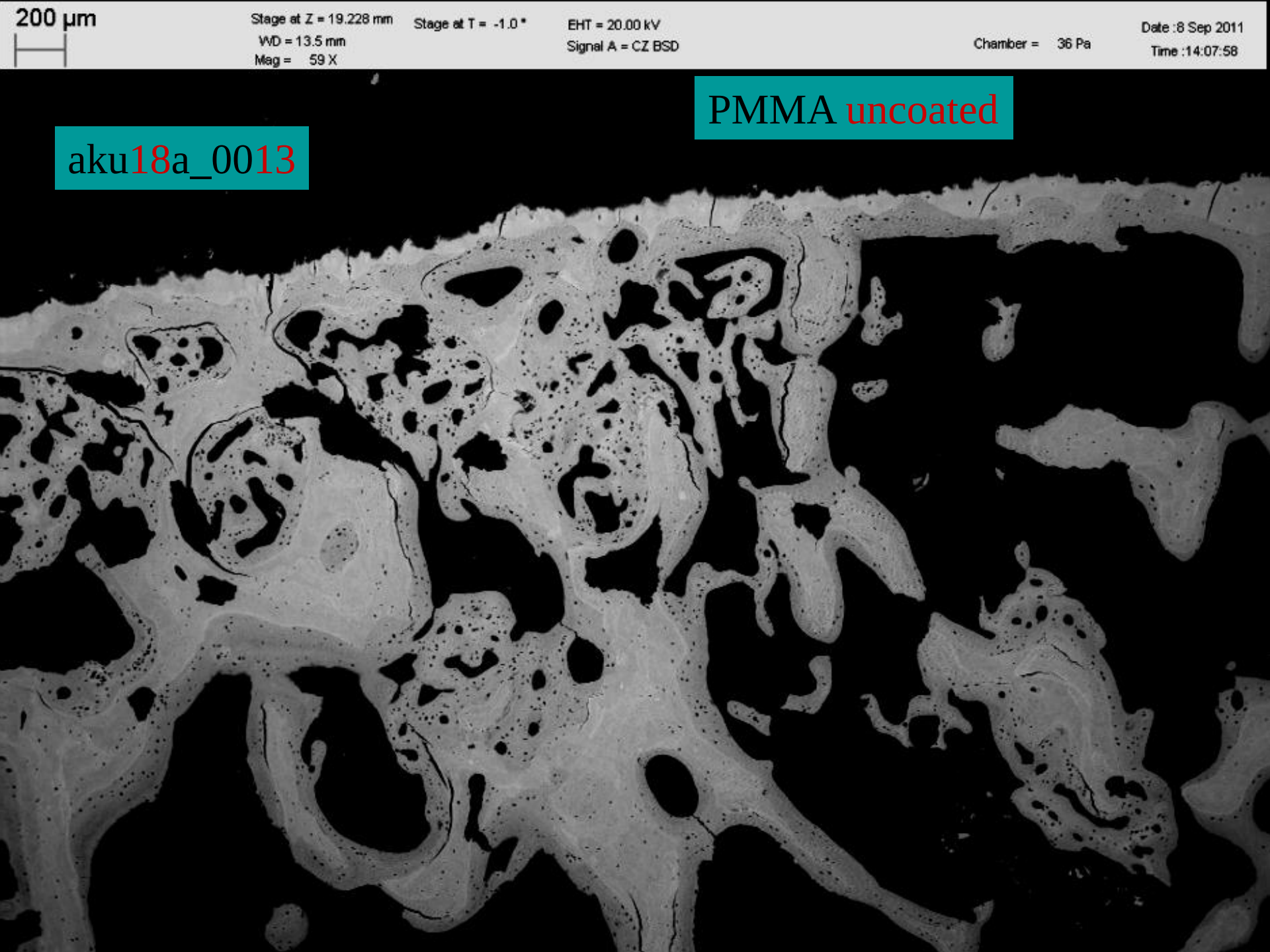
Microtomography

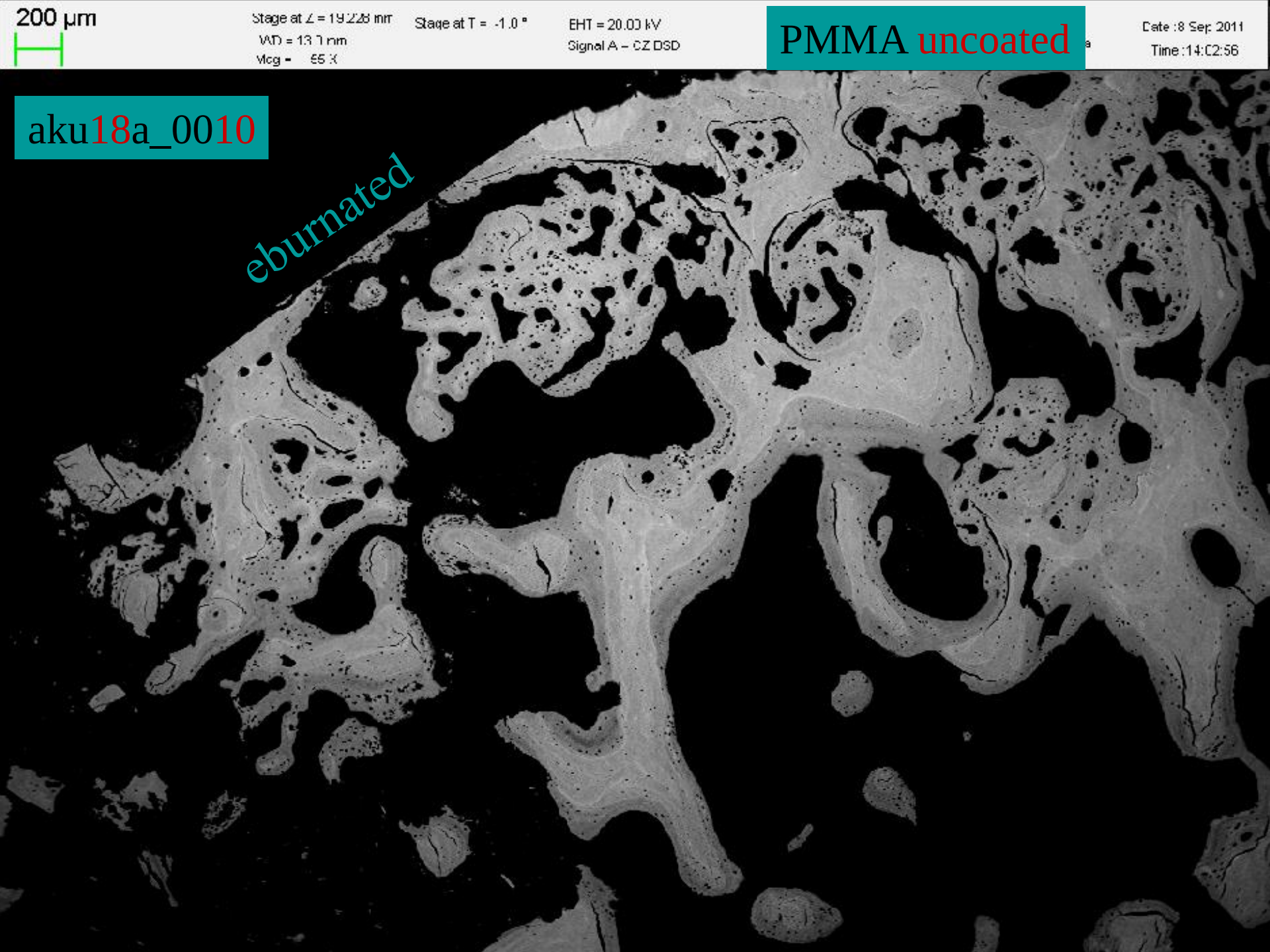


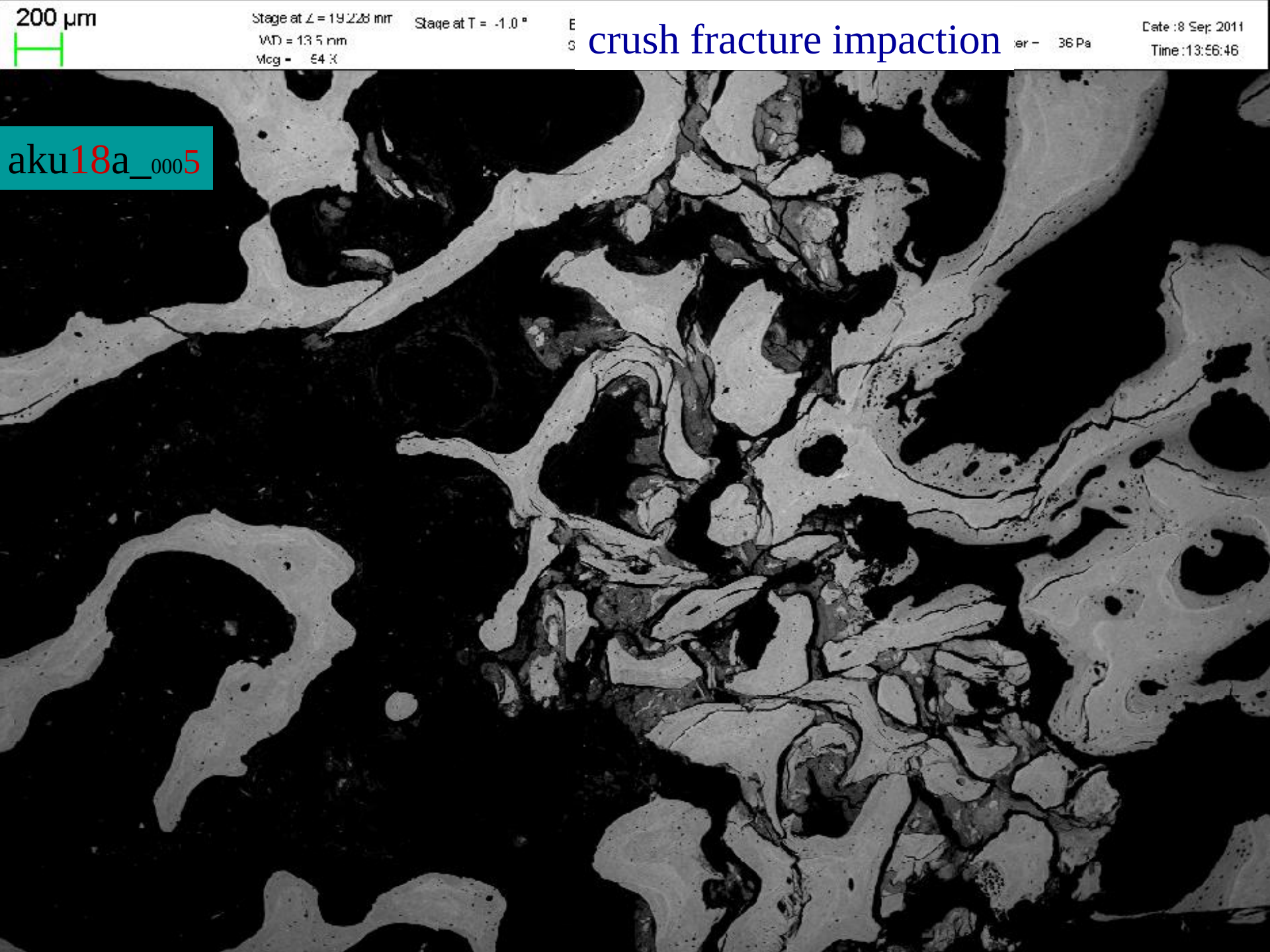
Microtomography

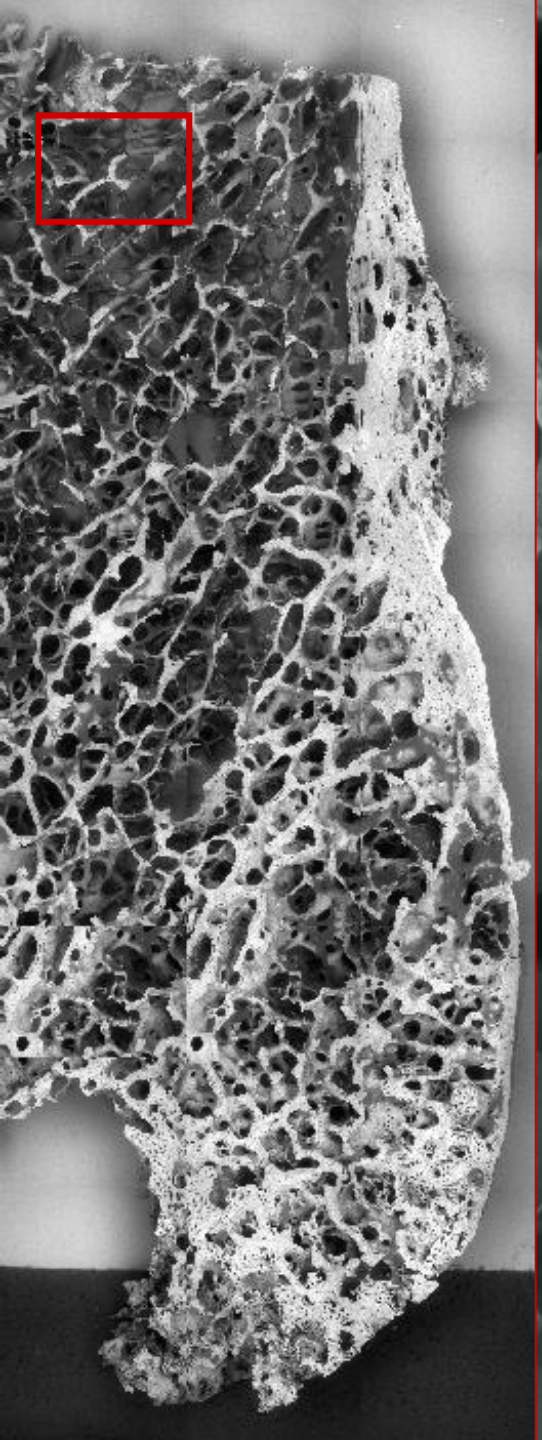
Some features are common to / with common OA


Aku18a PMMA uncoated 20110908_000*
13
14
17
10
16
8
7
3
0
11
6
12
5
4
plane of crush fracture impaction

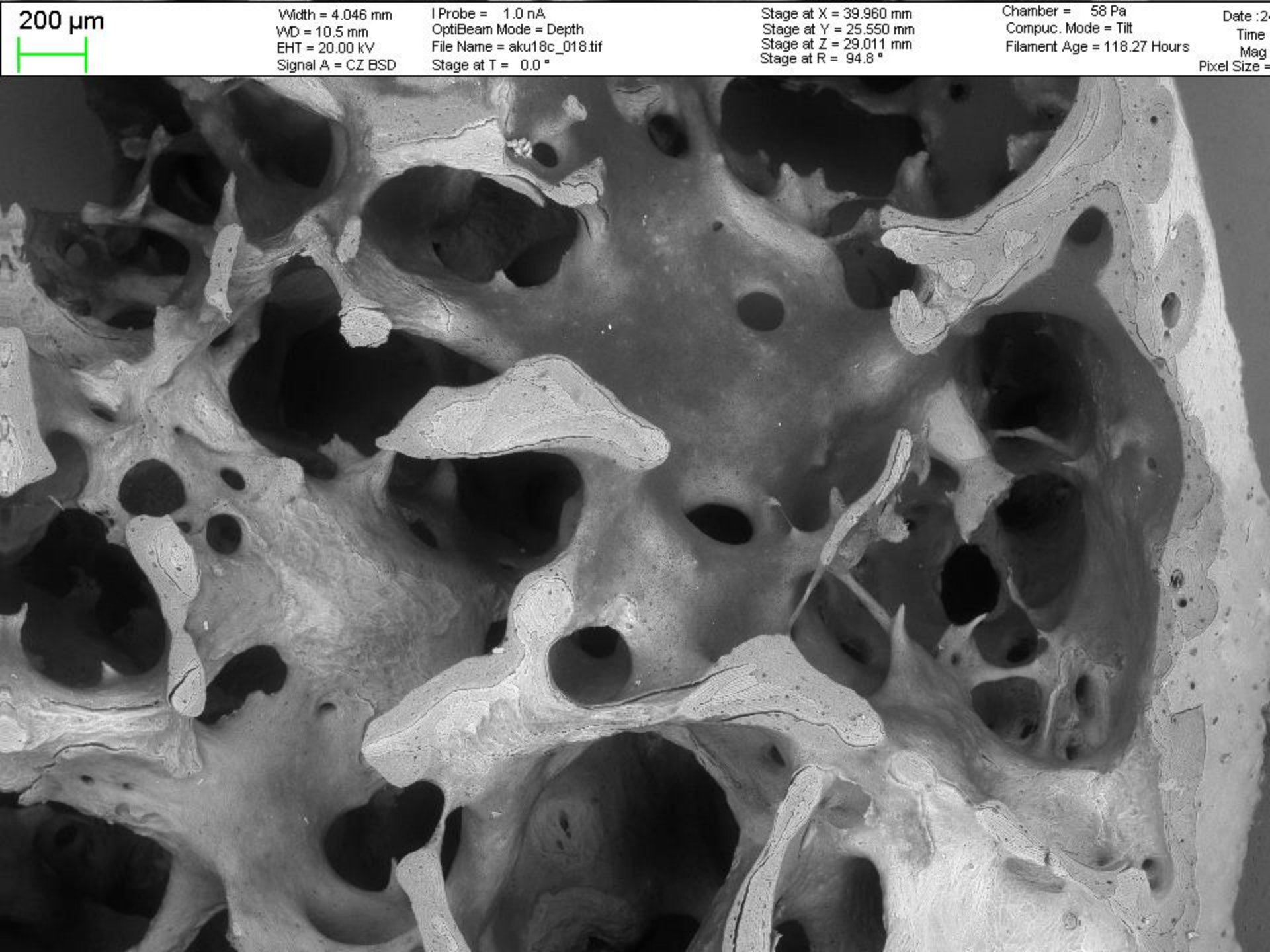

200 µm
Stage at Z = 19.228 mm
WD = 13.5 mm
Mag = 59 X
Stage at T = -1.0 °
EHT = 20.00 kV
Signal A = CZ BSD
Chamber = 36 Pa
Date :8 Sep 2011
Time :14:07:58
PMMA uncoated
aku18a_0013

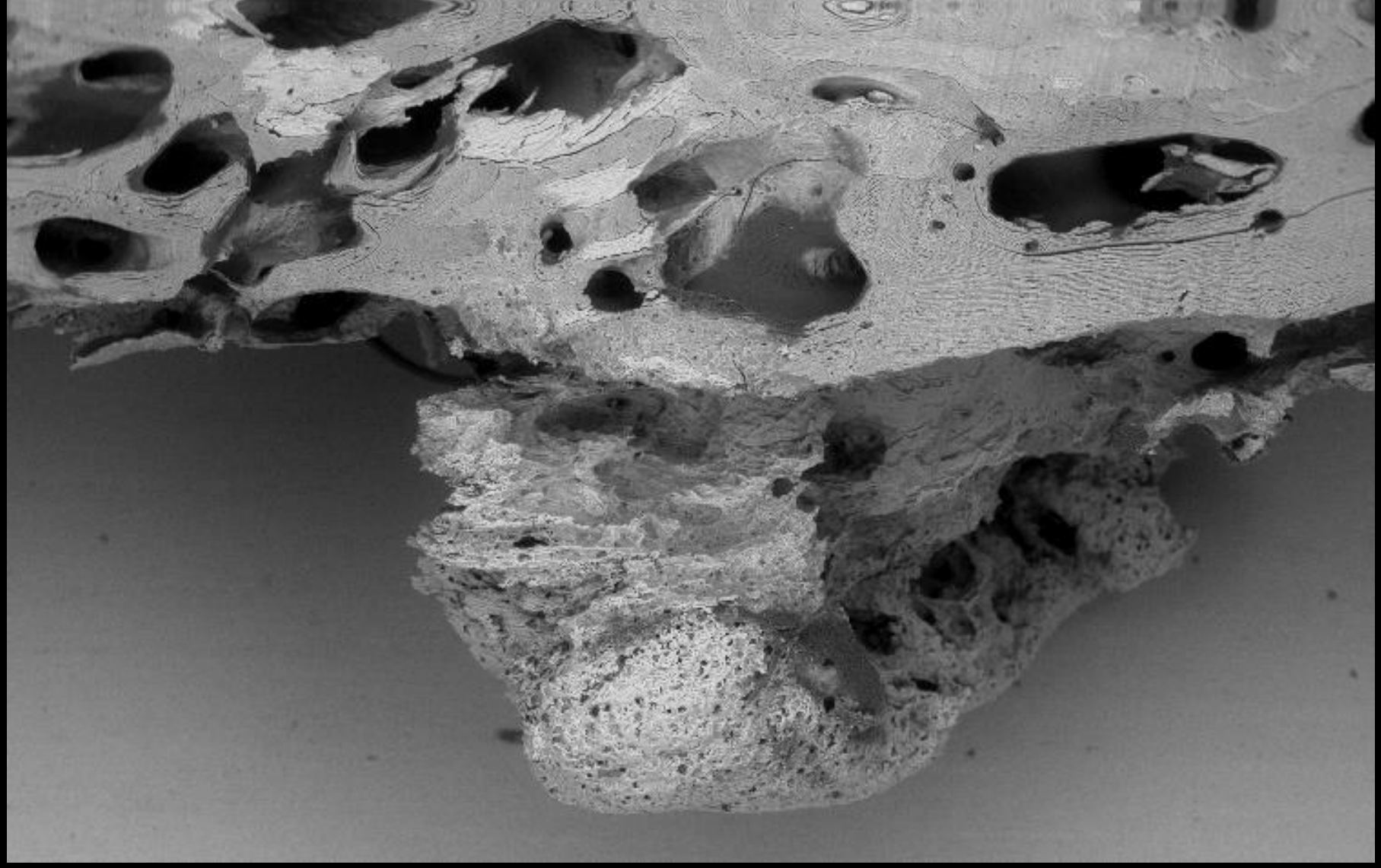

200 µm
Stage at Z = 19.228 mm
WD = 13.1 mm
Mag = 55 X
Stage at T = -1.0 °
EHT = 20.00 kV
Signal A = CZ DSD
PMMA uncoated
Date :8 Sep 2011
Time :14:02:56
aku18a_0010
eburnated


200 µm
Stage at Z = 19.228 mm
Stage at T = -1.0 °
VAD = 13.5 mm
Mag = 64 X
crush fracture impaction
Date :8 Sep 2011
Time :13:56:46
aku18a_0005



aku18b_24Xr_10_1 macerated NB excess low density bone or osteoid


200 µm
Width = 4.046 mm
WD = 10.5 mm
EHT = 20.00 kV
Signal A = CZ BSD
I Probe = 1.0 nA
OptiBeam Mode = Depth
File Name = aku18c_018.tif
Stage at T = 0.0 °
Stage at X = 39.960 mm
Stage at Y = 25.550 mm
Stage at Z = 29.011 mm
Stage at R = 94.8 °
Chamber = 58 Pa
Compuc. Mode = Tilt
Filament Age = 118.27 Hours
Date :2
Time
Mag
Pixel Size =



Lumps and bumps in the neck region in AKU samples

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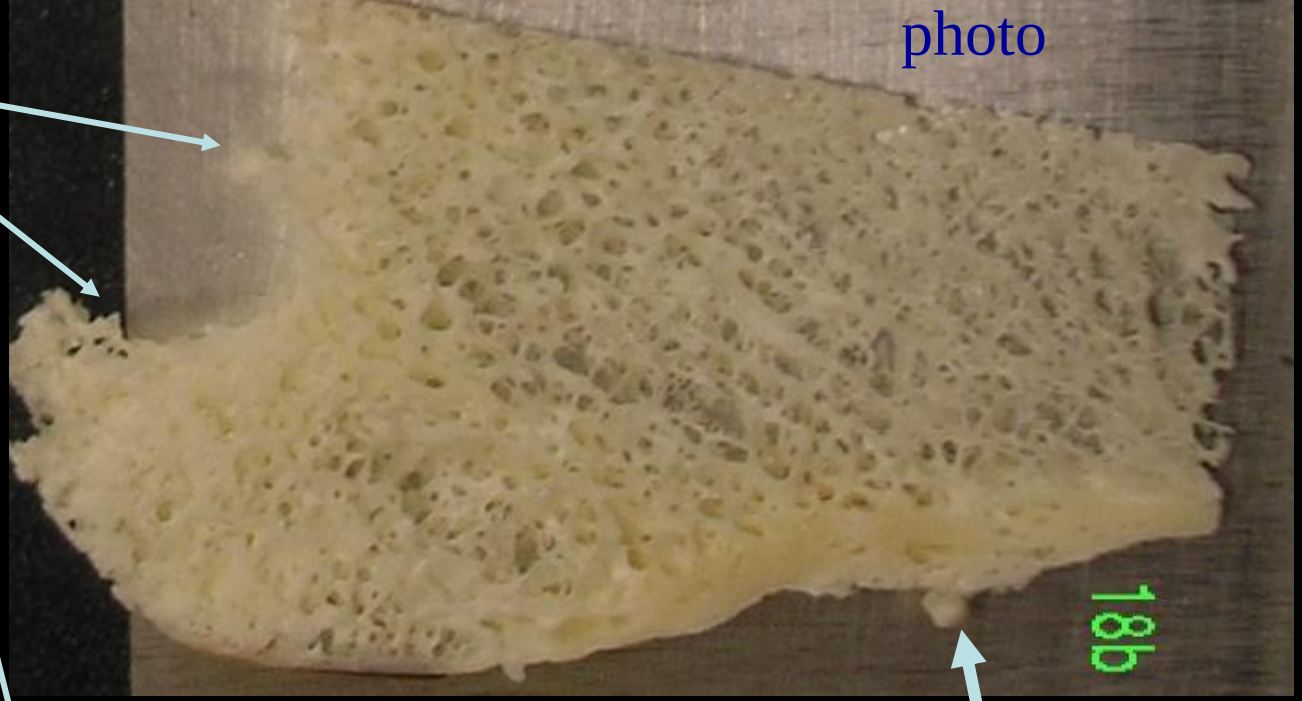
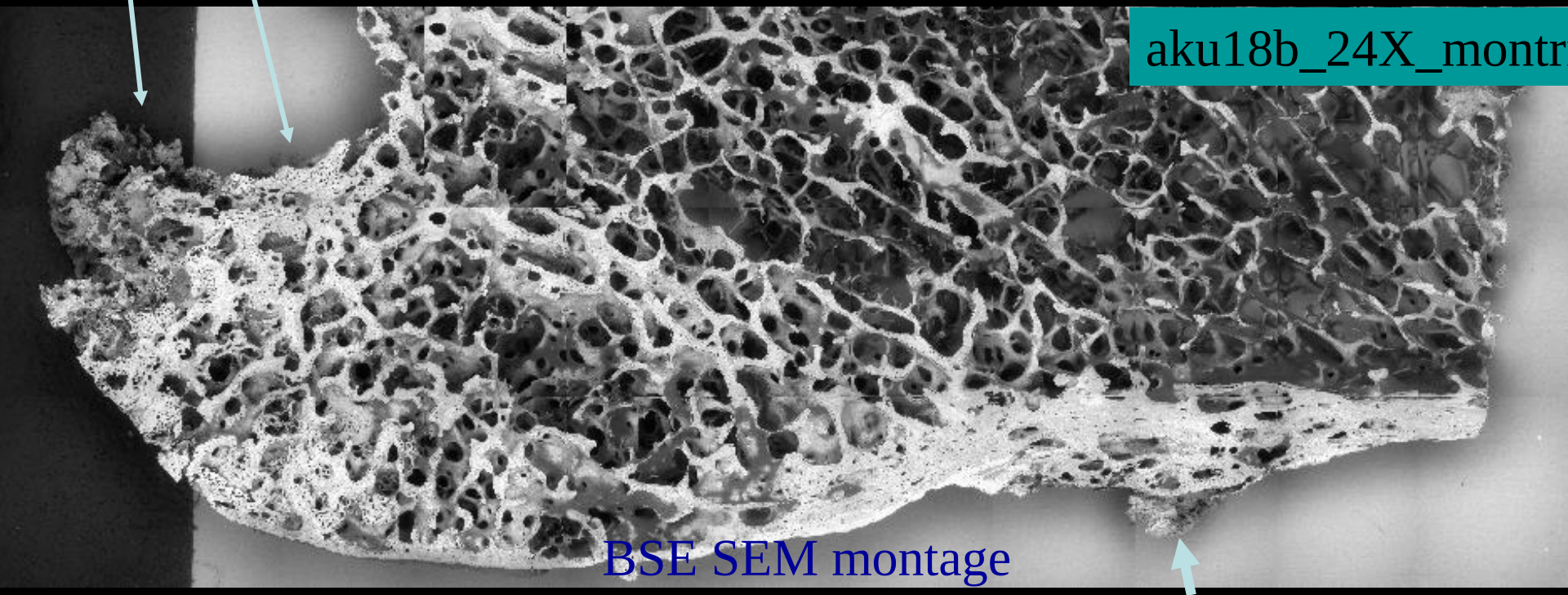
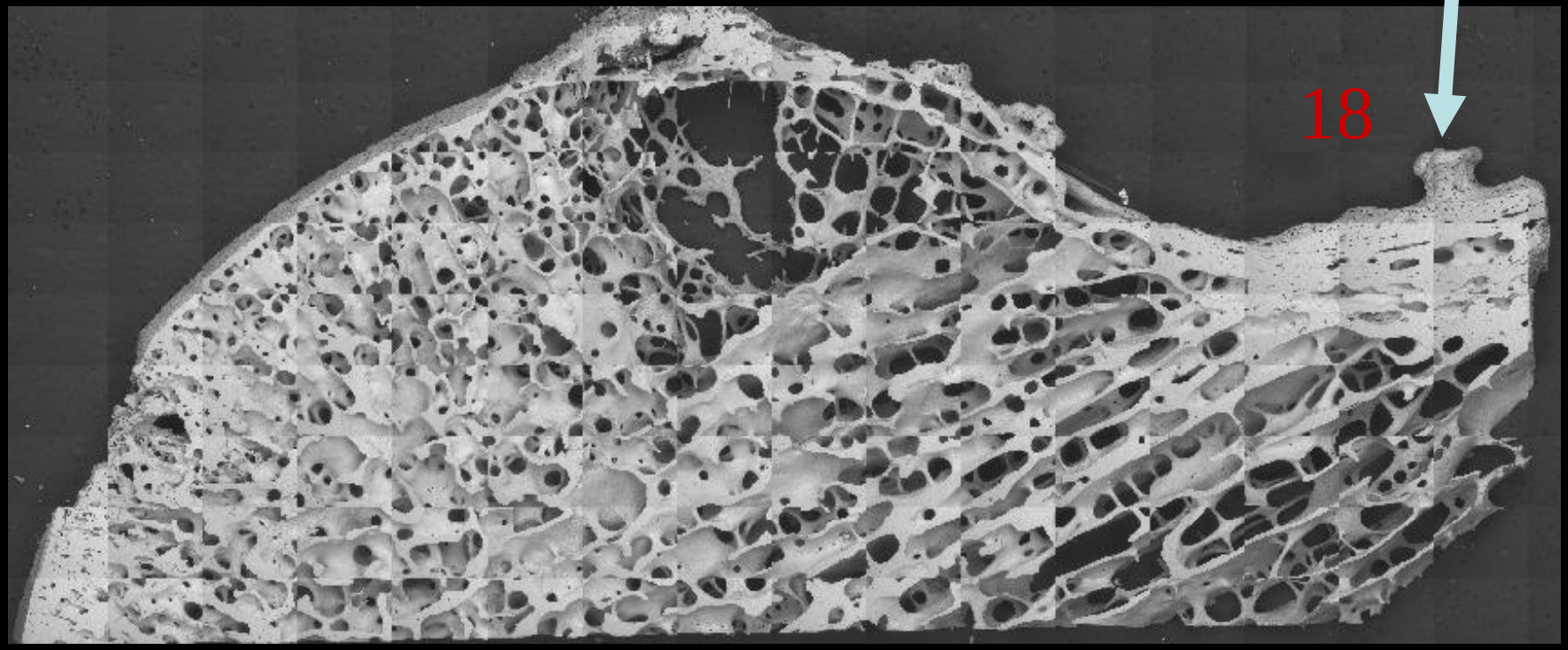
photo



aku18b_24X_montr



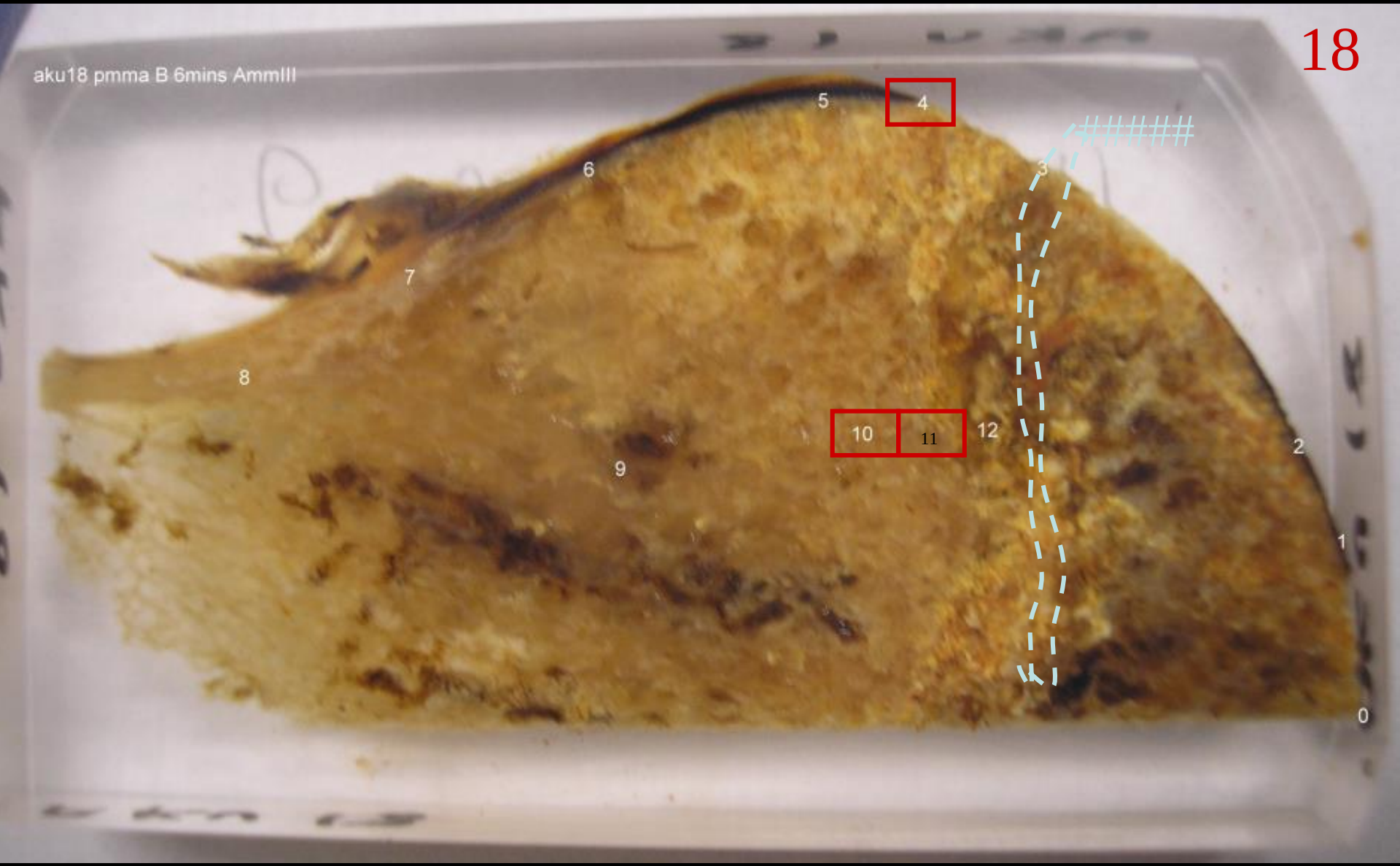
BSE SEM montage

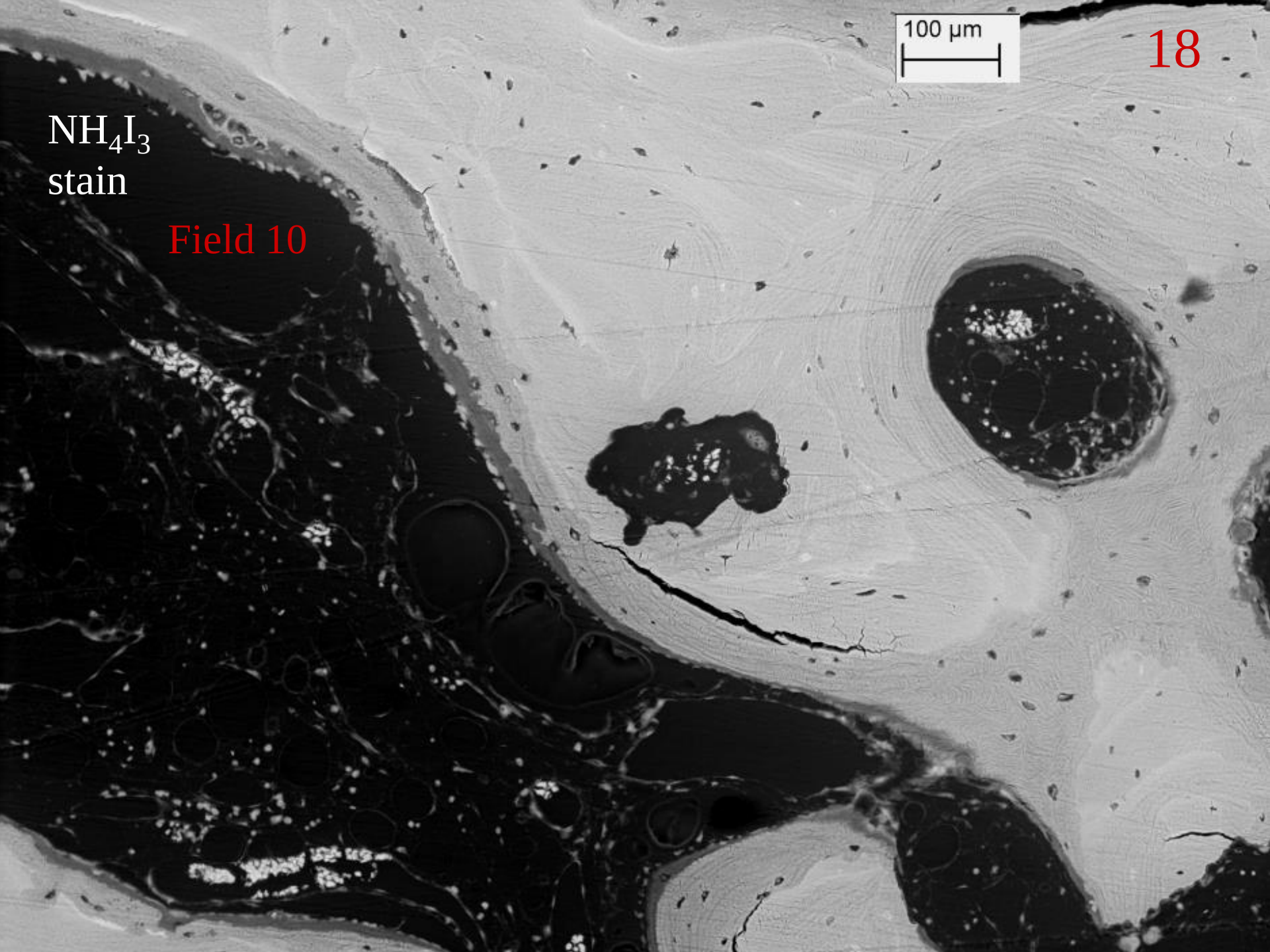


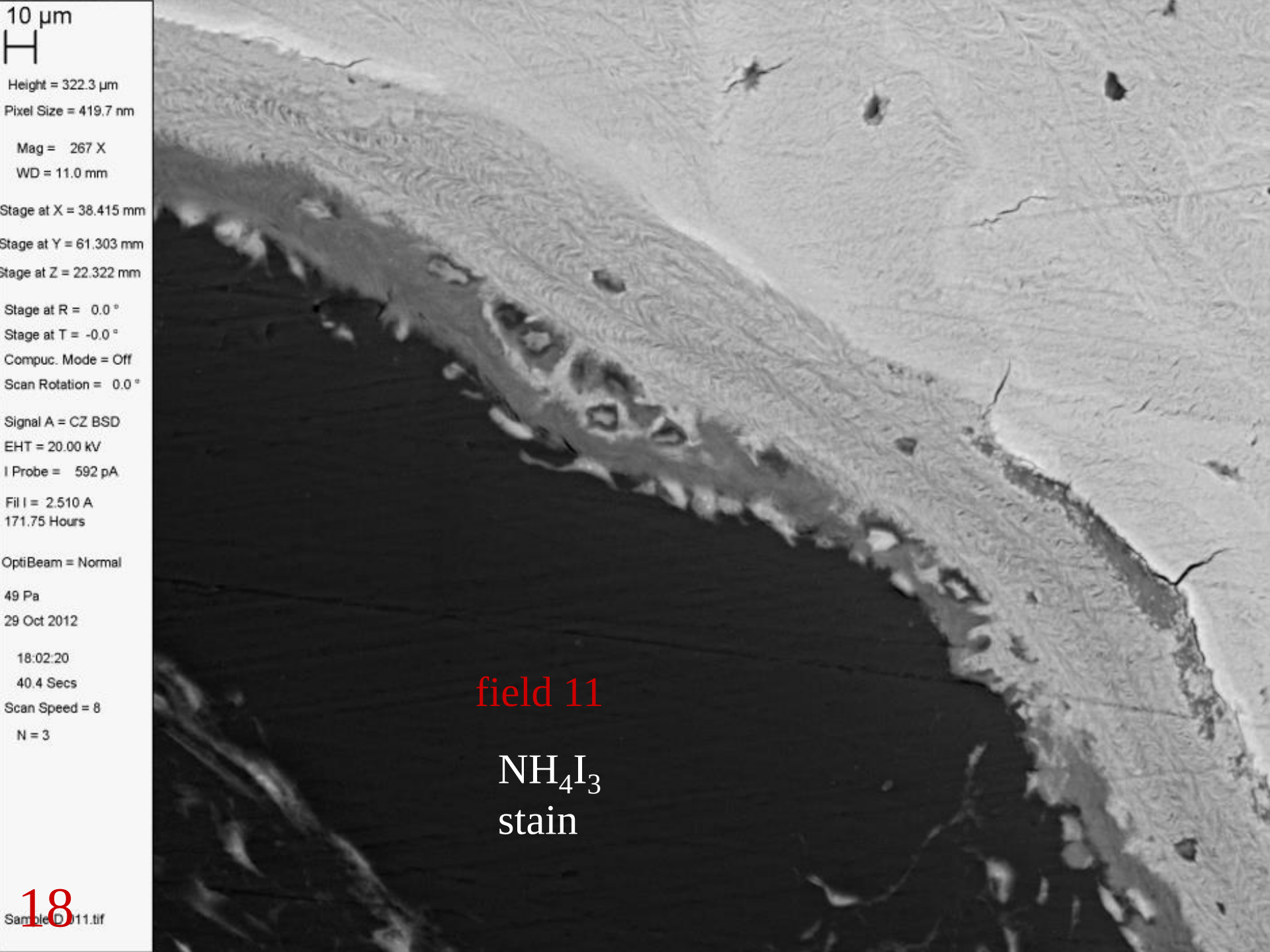
BSE SEM montage

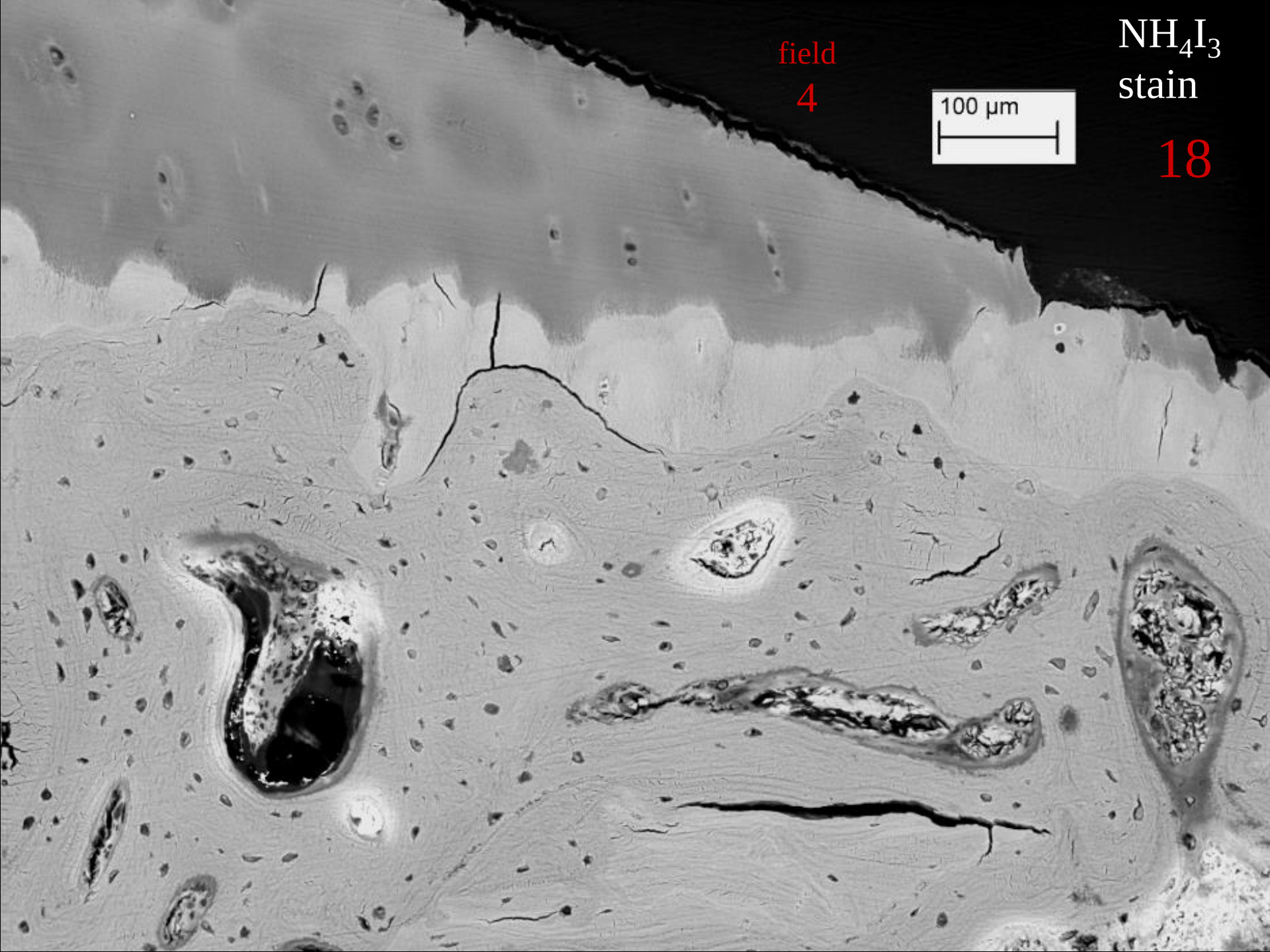

Aku18 B macerated
67x30deg123heq
18

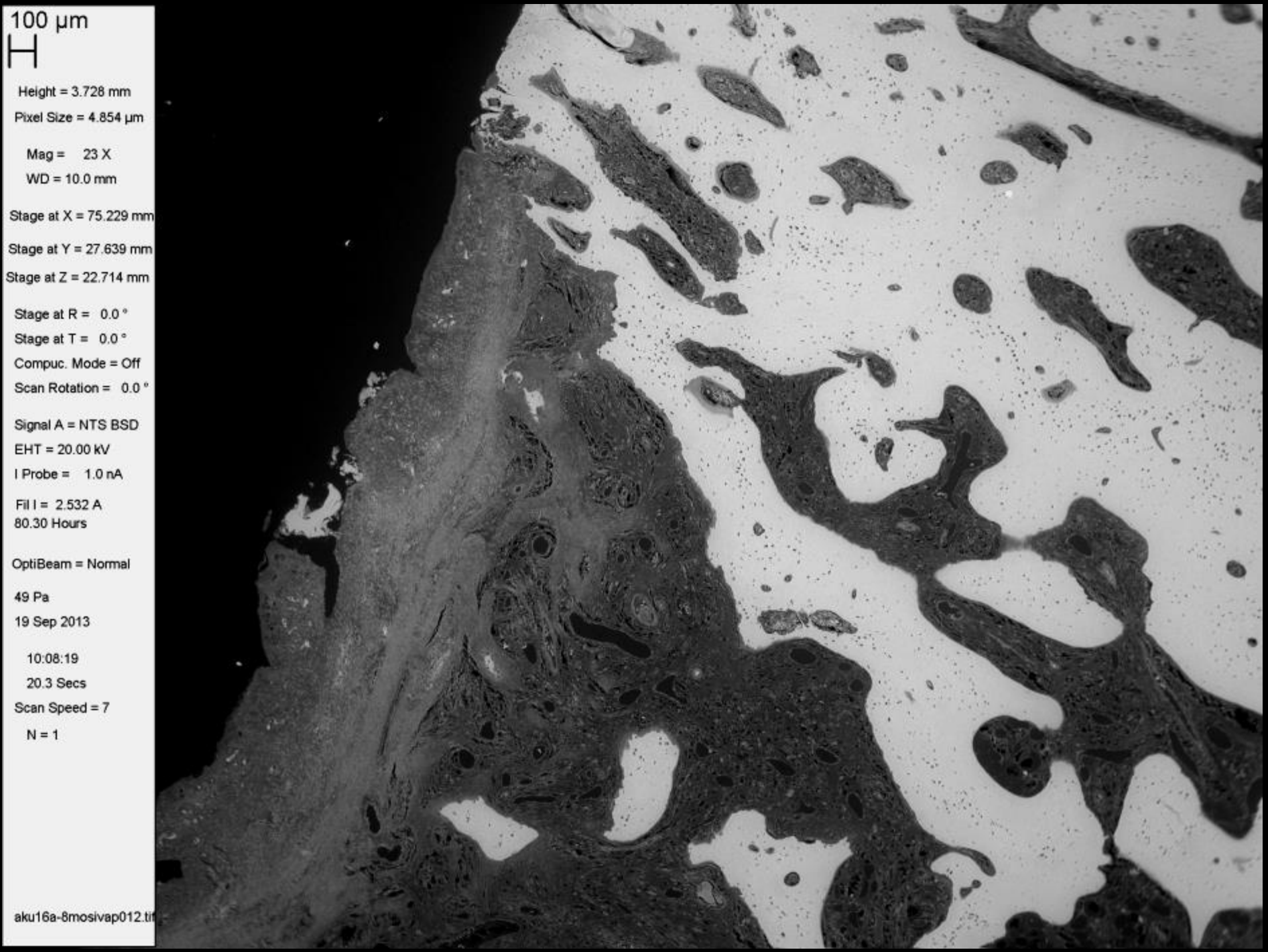
Triiodide or iodine vapour staining

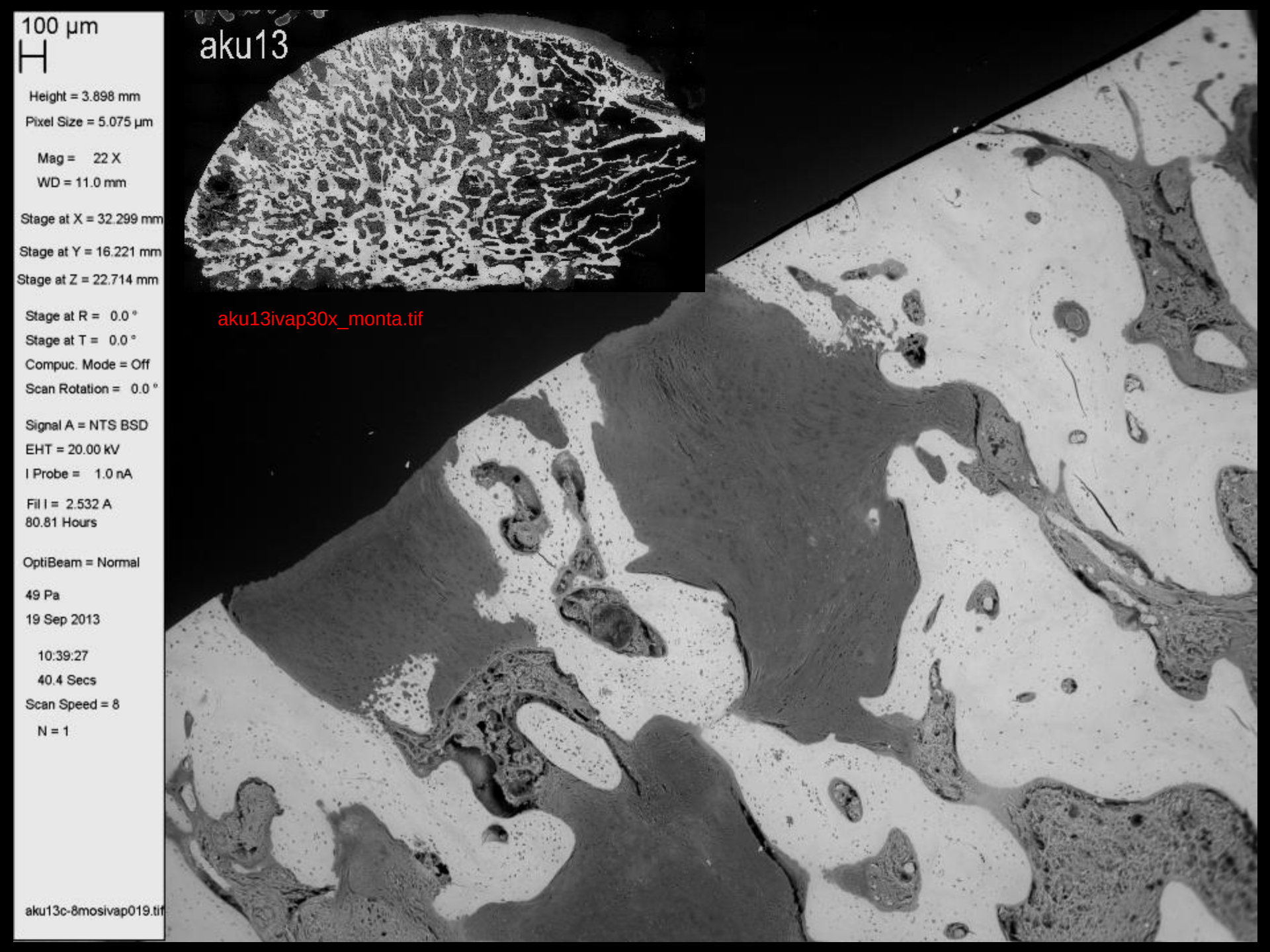


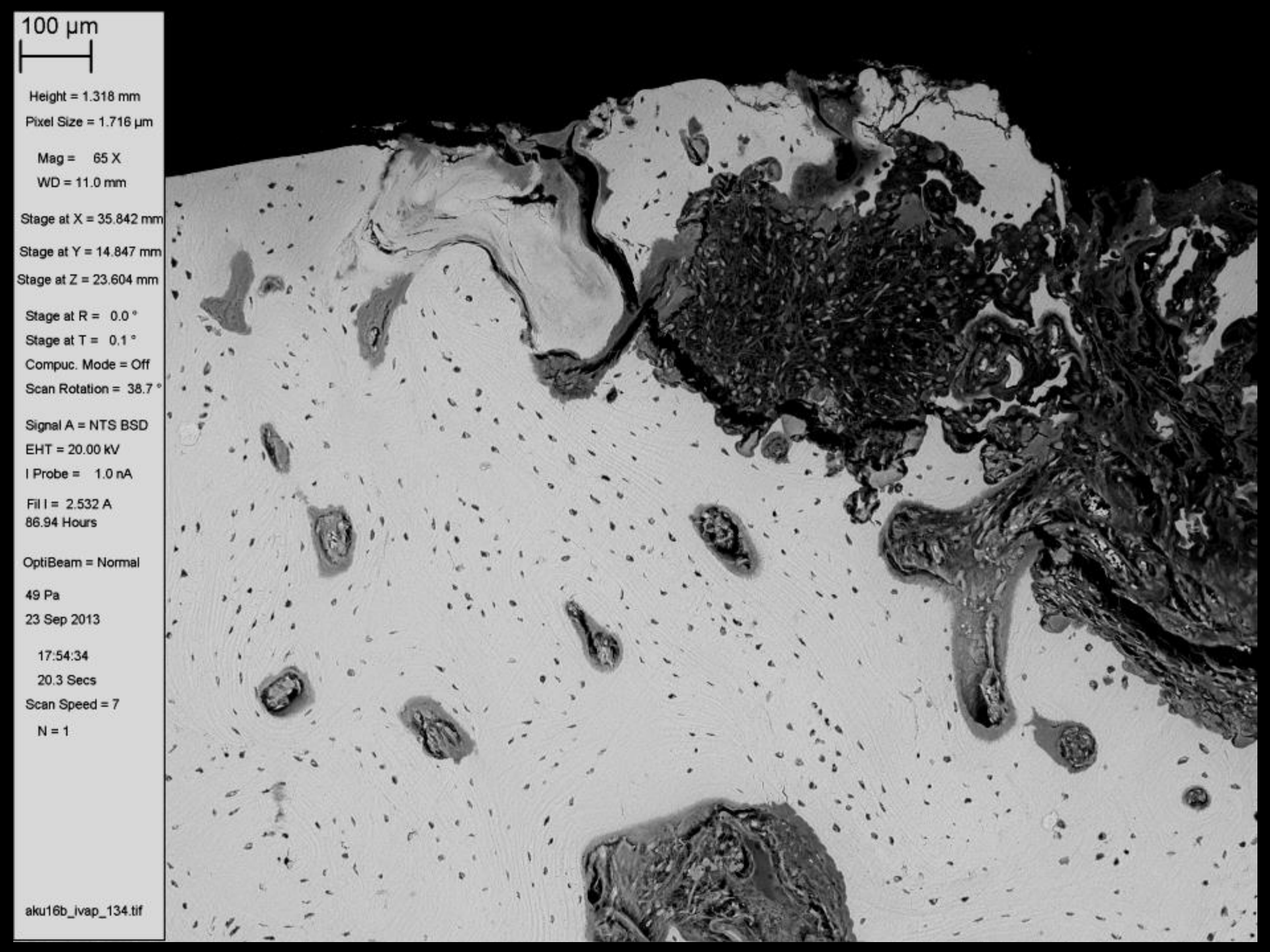

100 µm
18
NH_4I_3
stain
Field 10

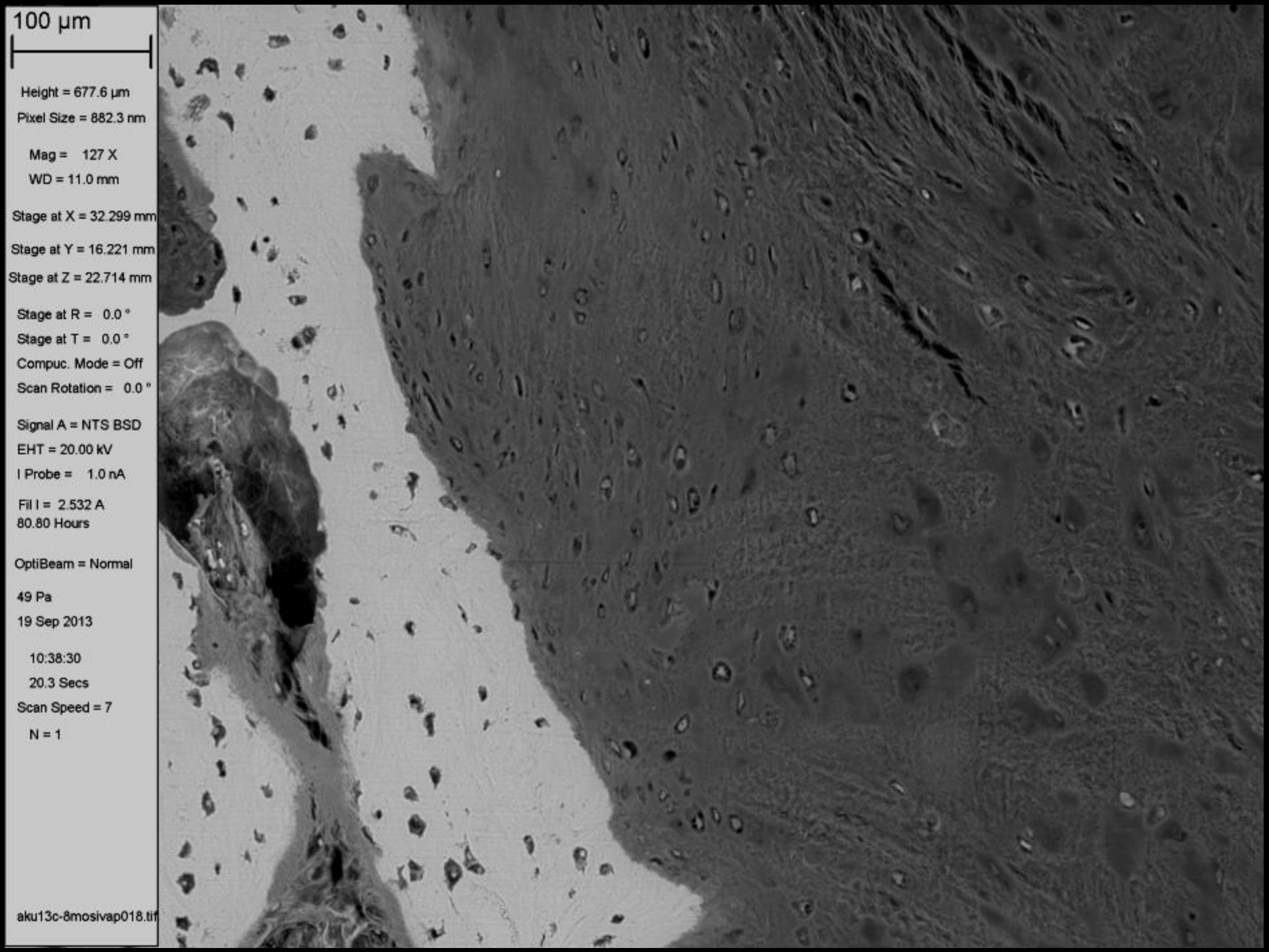

10 µm
Height = 322.3 µm
Pixel Size = 419.7 nm
Mag = 267 X
WD = 11.0 mm
Stage at X = 38.415 mm
Stage at Y = 61.303 mm
Stage at Z = 22.322 mm
Stage at R = 0.0 °
Stage at T = -0.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = CZ BSD
EHT = 20.00 kV
I Probe = 592 pA
Fil I = 2.510 A
171.75 Hours
OptiBeam = Normal
49 Pa
29 Oct 2012
18:02:20
40.4 Secs
Scan Speed = 8
N = 3
field 11
NH_4I_3
stain
18

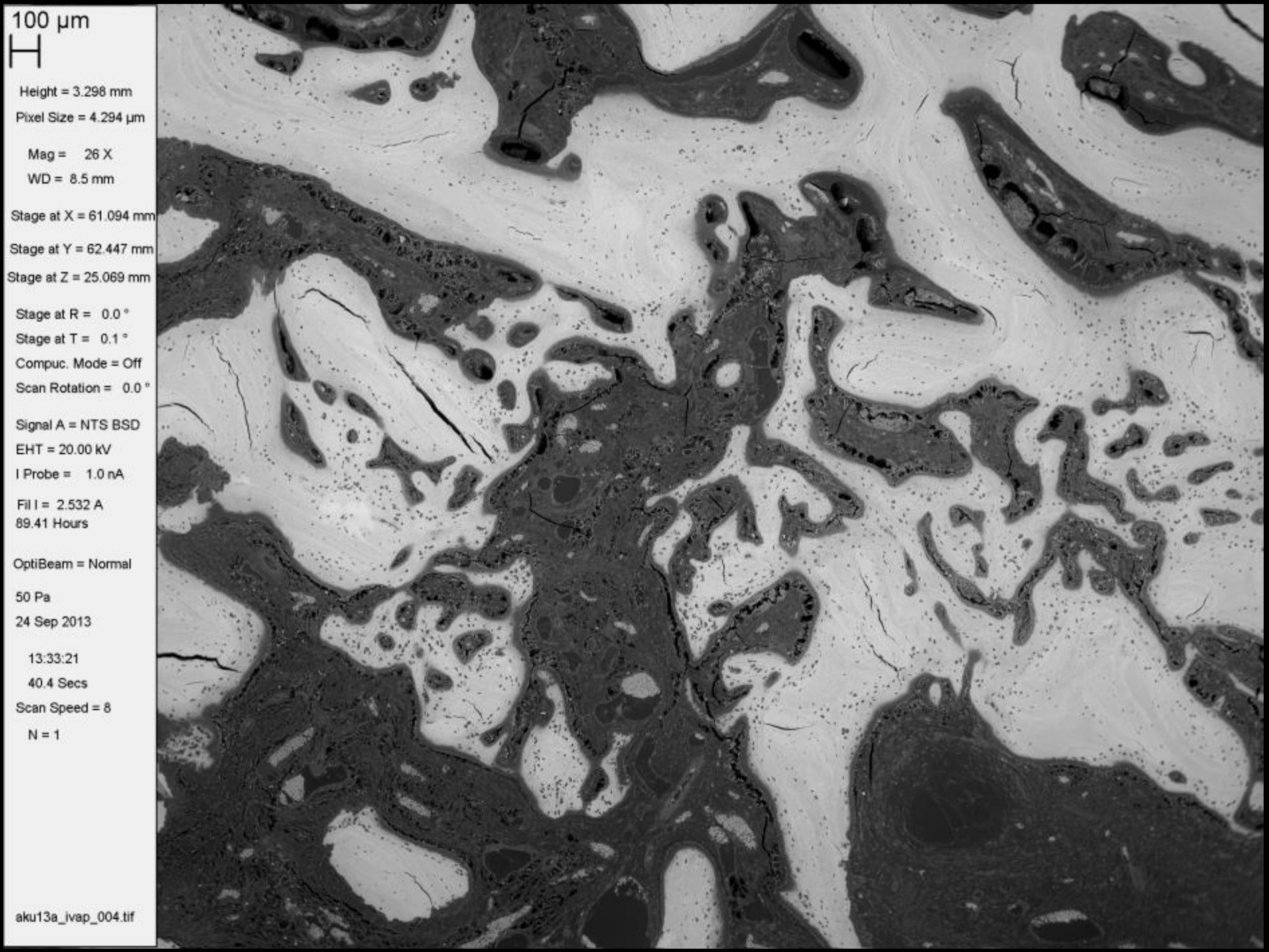

field
4
100 µm
NH_4I_3
stain
18

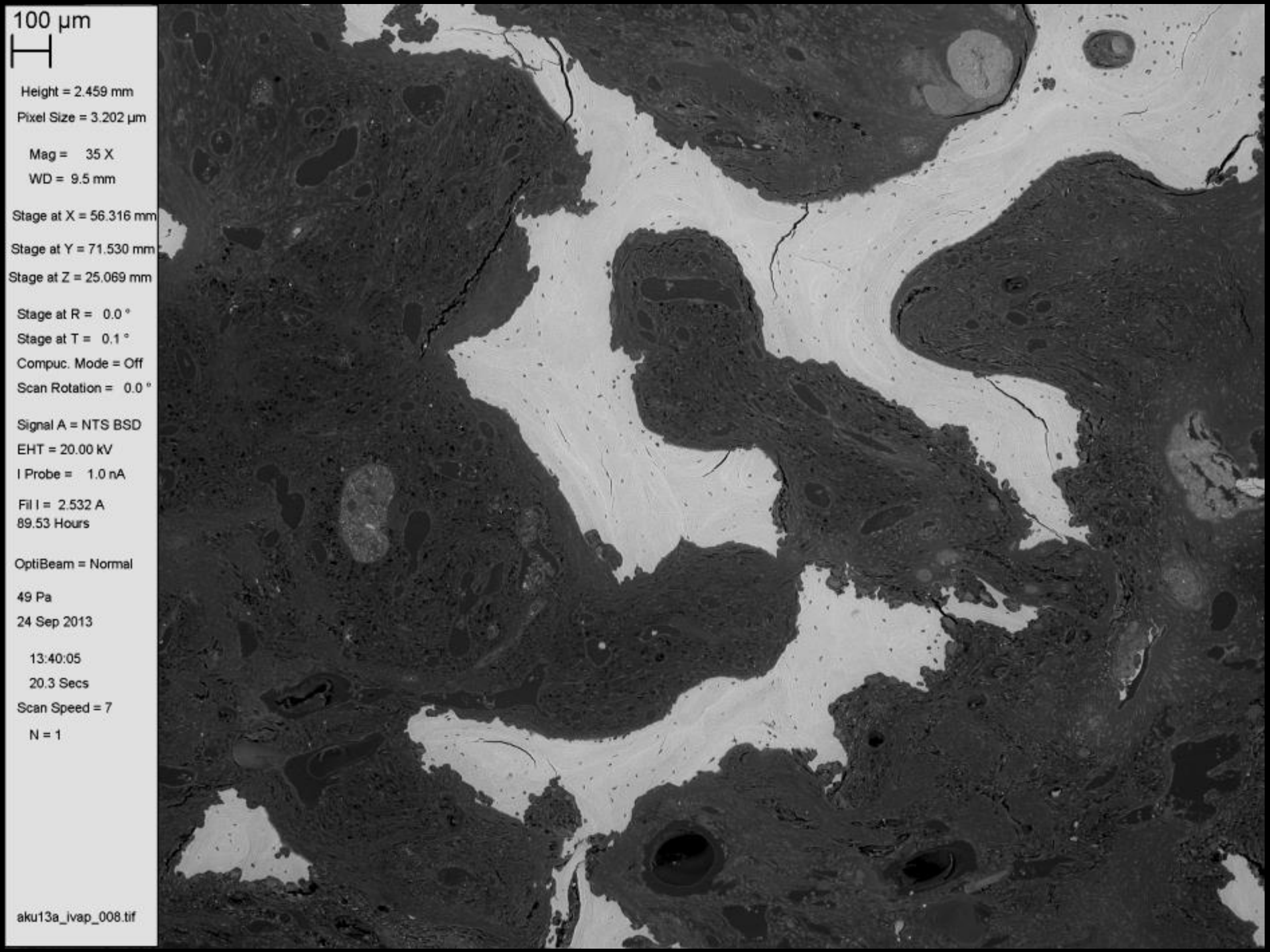

100 µm
Height = 3.728 mm
Pixel Size = 4.854 µm
Mag = 23 X
WD = 10.0 mm
Stage at X = 75.229 mm
Stage at Y = 27.639 mm
Stage at Z = 22.714 mm
Stage at R = 0.0 °
Stage at T = 0.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.532 A
80.30 Hours
OptiBeam = Normal
49 Pa
19 Sep 2013
10:08:19
20.3 Secs
Scan Speed = 7
N = 1
aku16a-8mosivap012.tif


100 µm
H
Height = 3.898 mm
Pixel Size = 5.075 µm
Mag = 22 X
WD = 11.0 mm
Stage at X = 32.299 mm
Stage at Y = 16.221 mm
Stage at Z = 22.714 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 = 2.532 A
80.81 Hours
OptiBeam = Normal
49 Pa
19 Sep 2013
10:39:27
40.4 Secs
Scan Speed = 8
N = 1
aku13c-8mosivap019.tif
aku13
aku13ivap30x_monta.tif


100 µm
Height = 1.318 mm
Pixel Size = 1.716 µm
Mag = 65 X
WD = 11.0 mm
Stage at X = 35.842 mm
Stage at Y = 14.847 mm
Stage at Z = 23.604 mm
Stage at R = 0.0 °
Stage at T = 0.1 °
Compuc. Mode = Off
Scan Rotation = 38.7 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.532 A
86.94 Hours
OptiBeam = Normal
49 Pa
23 Sep 2013
17:54:34
20.3 Secs
Scan Speed = 7
N = 1
aku16b_ivap_134.tif


100 µm
Height = 677.6 µm
Pixel Size = 882.3 nm
Mag = 127 X
WD = 11.0 mm
Stage at X = 32.299 mm
Stage at Y = 16.221 mm
Stage at Z = 22.714 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 = 2.532 A
80.80 Hours
OptiBeam = Normal
49 Pa
19 Sep 2013
10:38:30
20.3 Secs
Scan Speed = 7
N = 1
aku13c-8mosivap018.tif

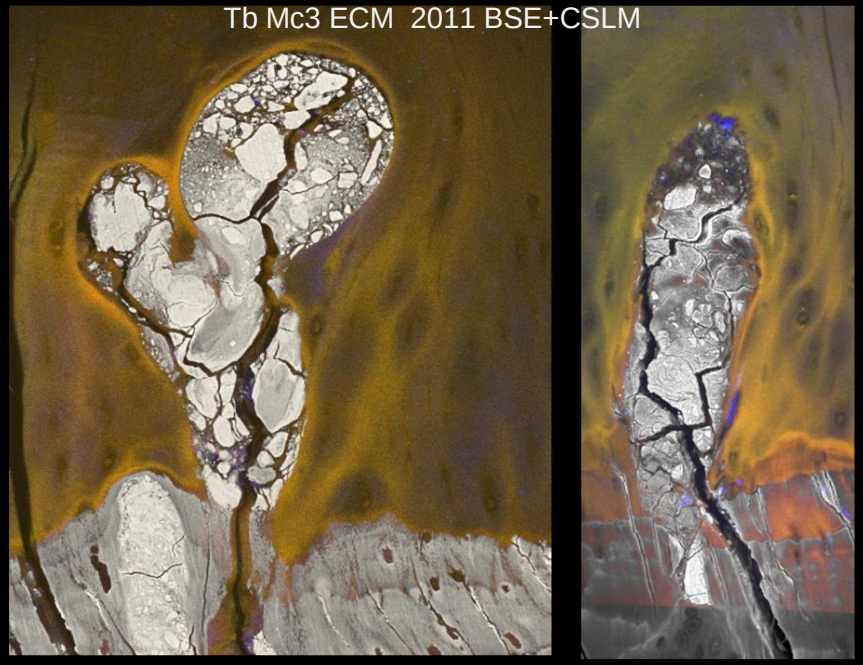

100 µm
Height = 3.298 mm
Pixel Size = 4.294 µm
Mag = 26 X
WD = 8.5 mm
Stage at X = 61.094 mm
Stage at Y = 62.447 mm
Stage at Z = 25.069 mm
Stage at R = 0.0 °
Stage at T = 0.1 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.532 A
89.41 Hours
OptiBeam = Normal
50 Pa
24 Sep 2013
13:33:21
40.4 Secs
Scan Speed = 8
N = 1
aku13a_ivap_004.tif


100 µm
Height = 2.459 mm
Pixel Size = 3.202 µm
Mag = 35 X
WD = 9.5 mm
Stage at X = 56.316 mm
Stage at Y = 71.530 mm
Stage at Z = 25.069 mm
Stage at R = 0.0 °
Stage at T = 0.1 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.532 A
89.53 Hours
OptiBeam = Normal
49 Pa
24 Sep 2013
13:40:05
20.3 Secs
Scan Speed = 7
N = 1
aku13a_ivap_008.tif

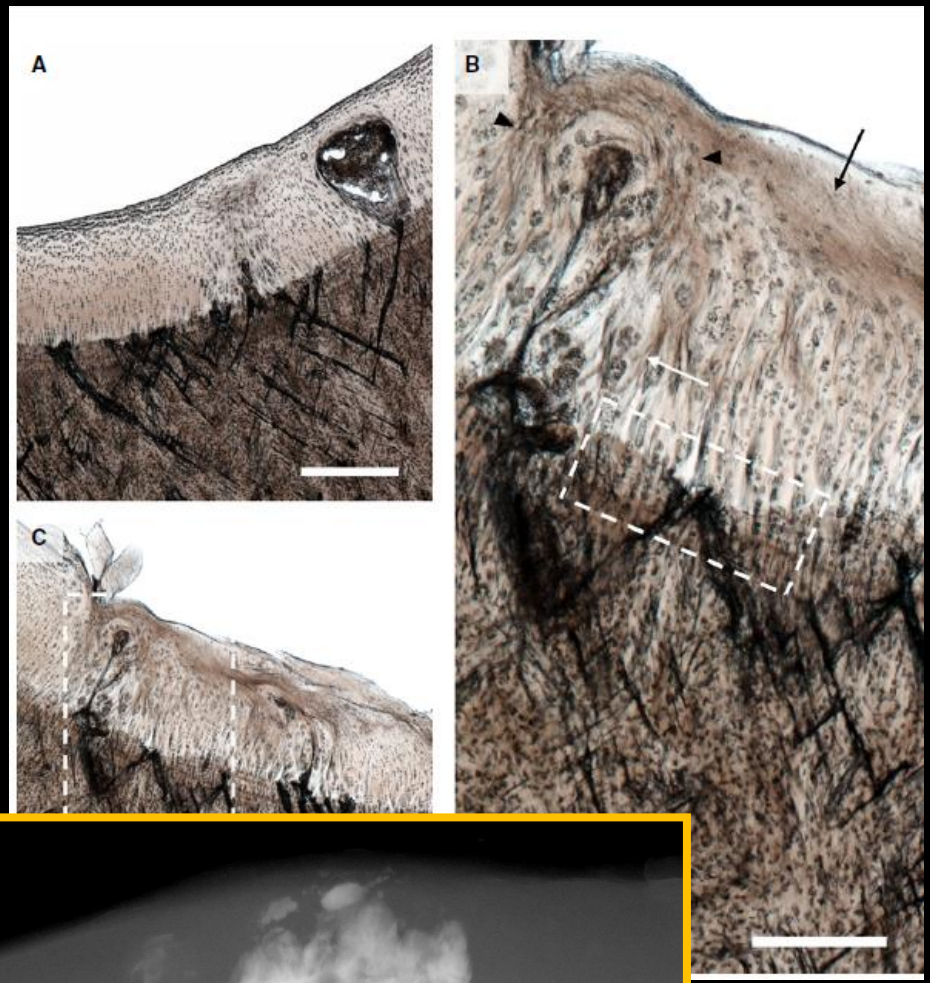
PRODs

HDMPs

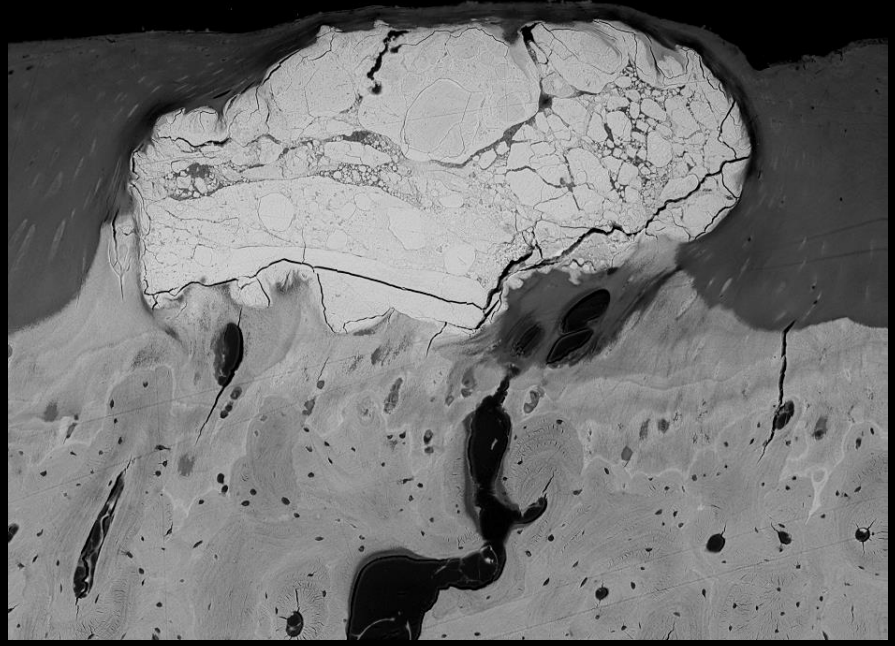
Tb Mc3 ECM 2011 BSE+CSLM

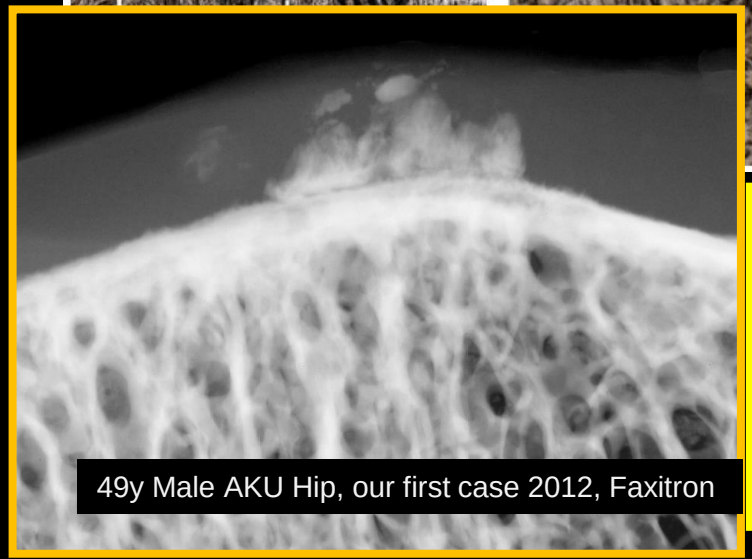


Tb Mc3 J Anat 2014 partly demin LM Turley…Broom

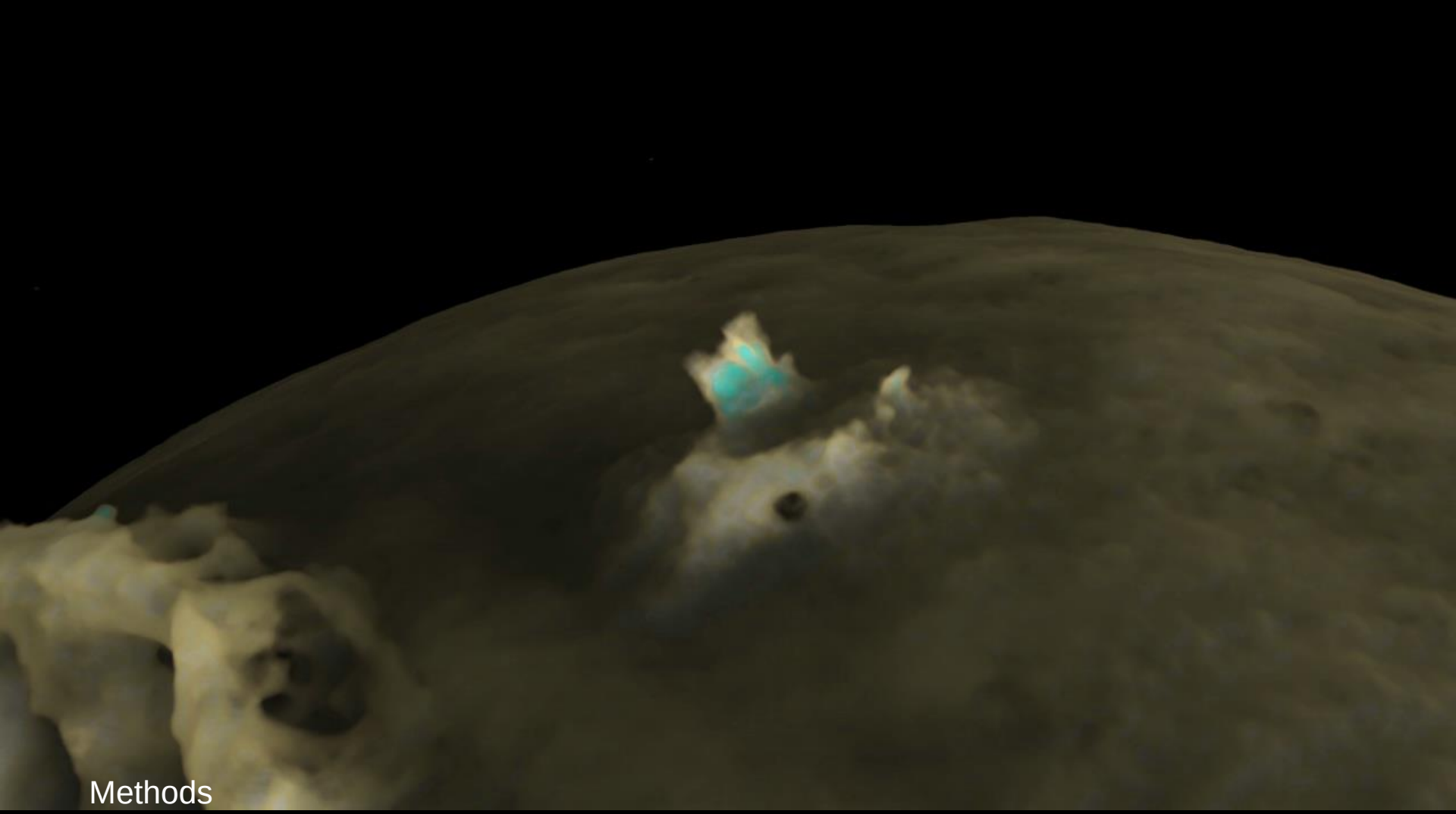


Icelandic Tarsals ECM 2014 MRI+CT+BSE+Iodine+CSLM





MRI DESS
XMT 26um
Faxitron
PMMA
XMT 6um
BSE
Triiodide



Methods

Remove the head of femur > MRI DESS, best resolution 230um

XMT high contrast x-ray microtomography, MuCat, 26um, 96 hours scanning >

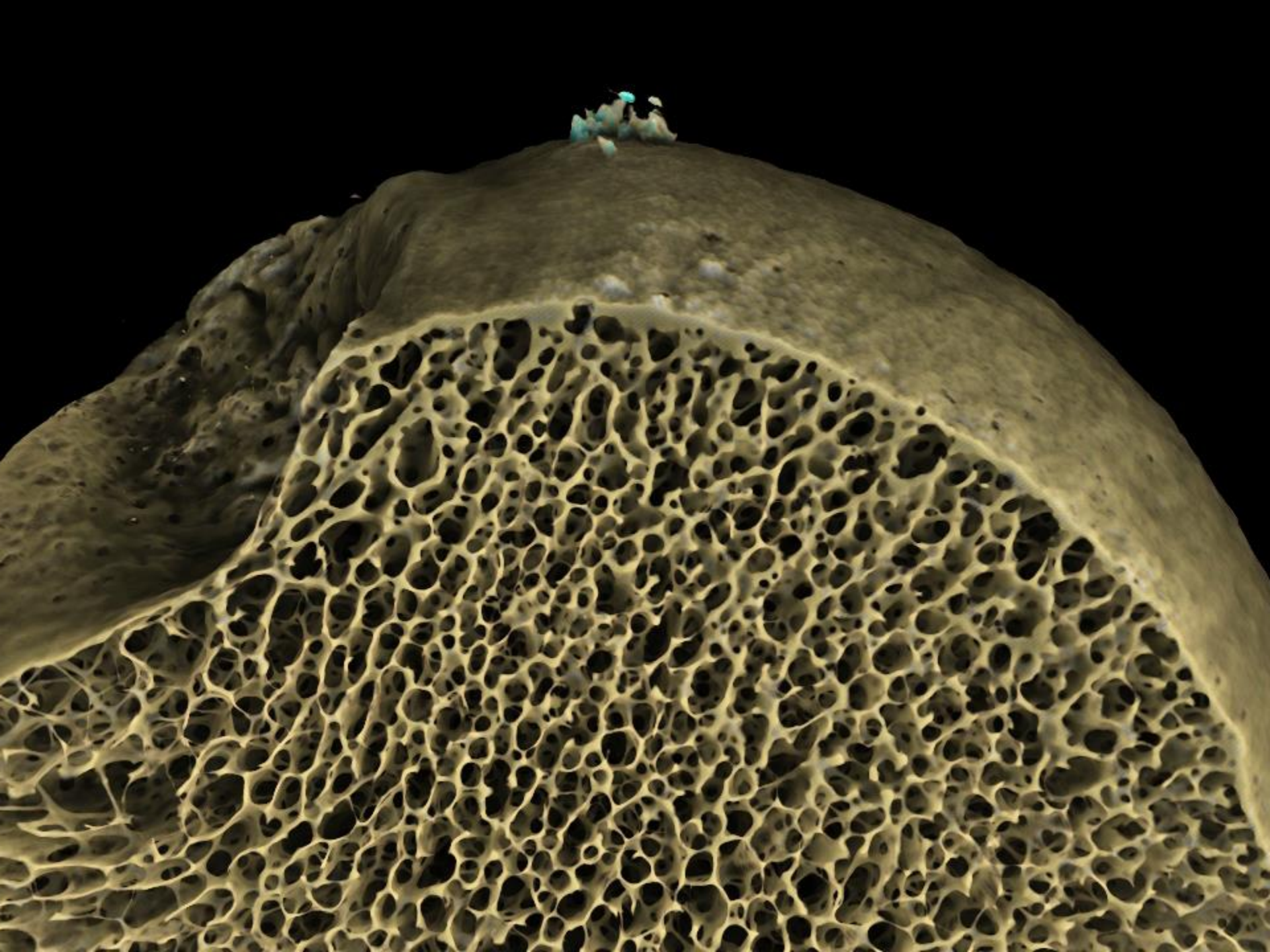
Find the regions of interest, Slice the femoral head, Embed in PMMA

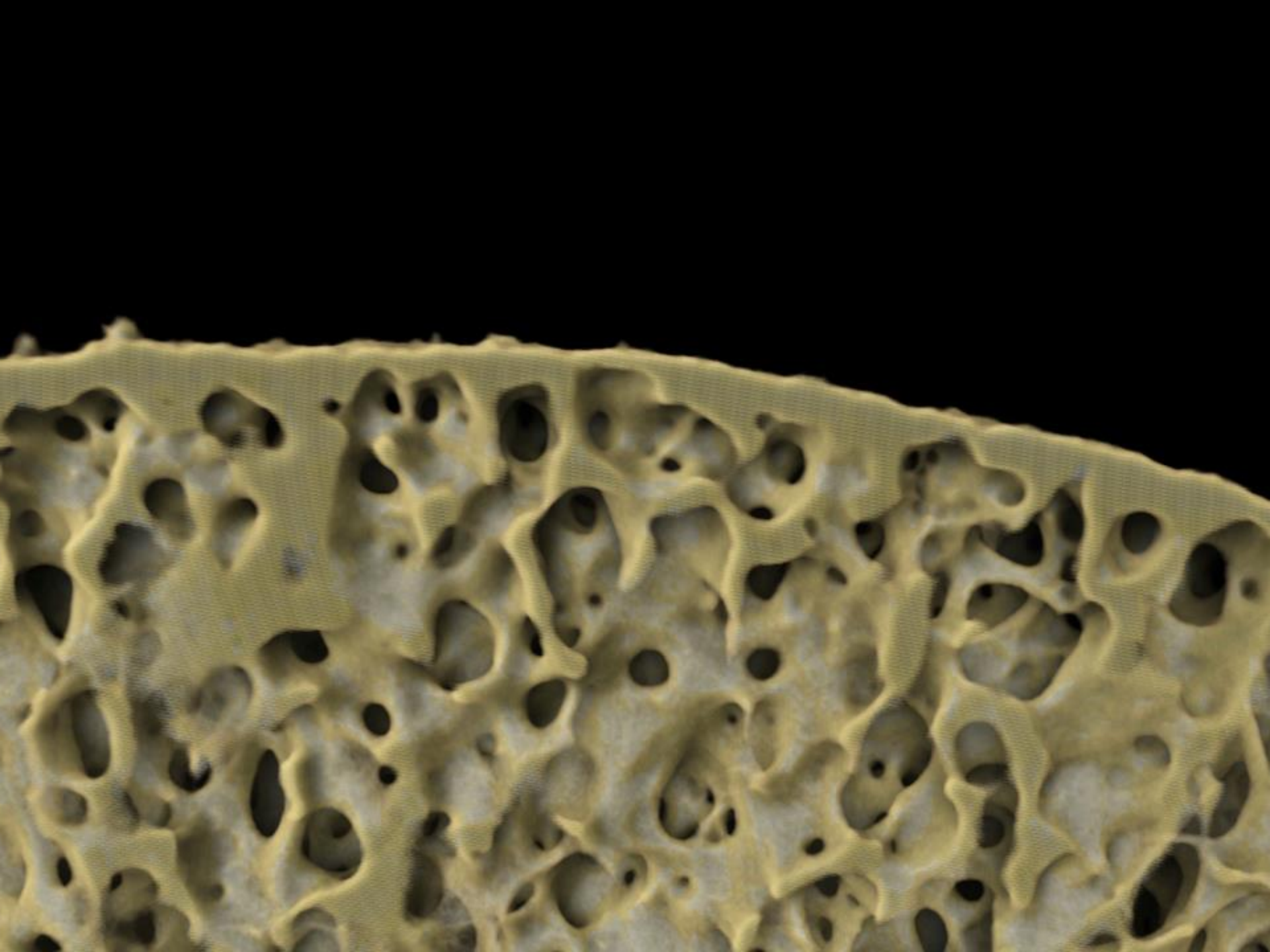
Digital microradiography of slices, Faxitron, 35kV > cut out & polish blocks

More XMT on blocks, Scanco uCT40, 6um voxels

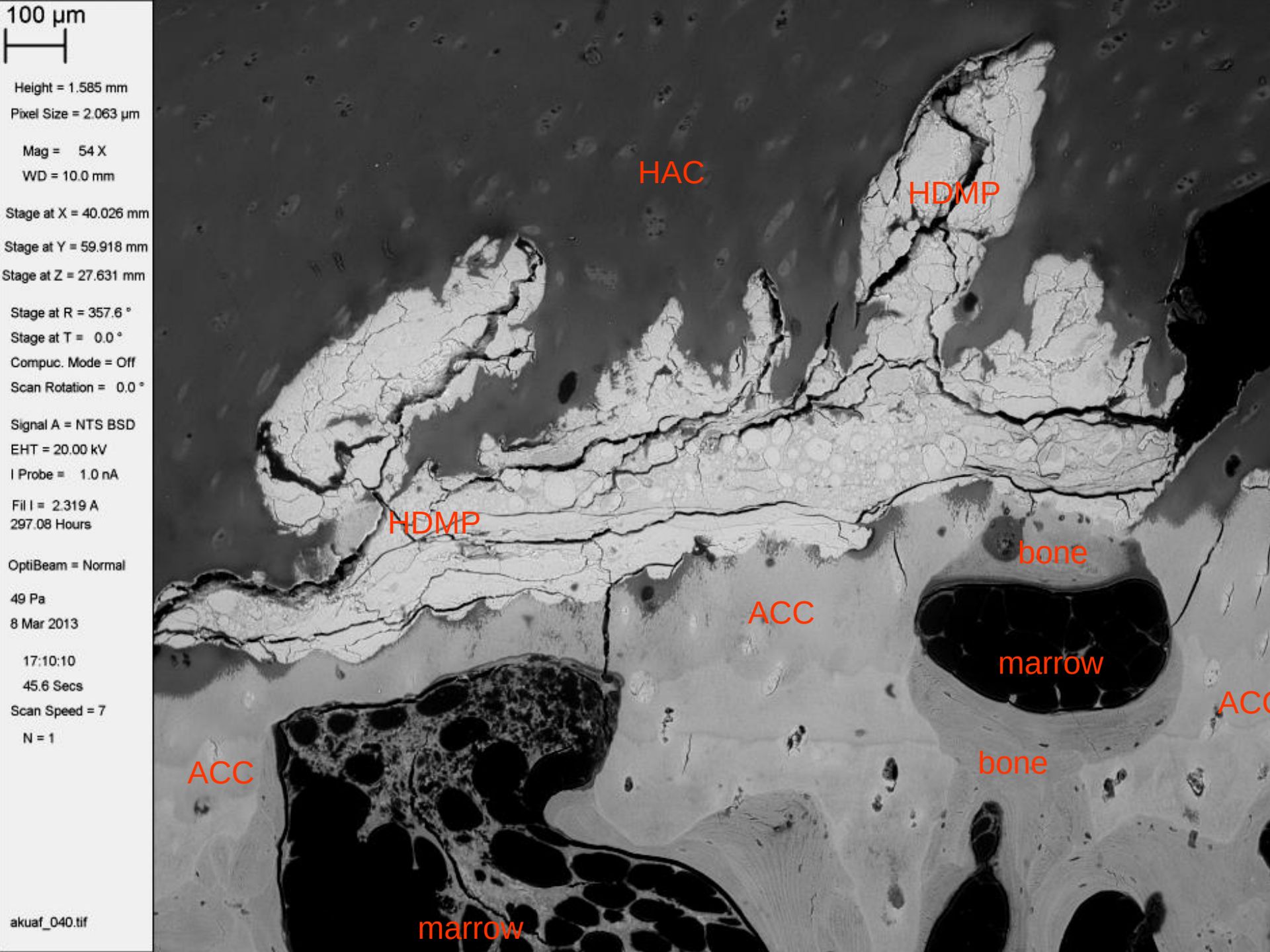
BSE SEM, wide field and < 0.2um resolution imaging of mineral concentration

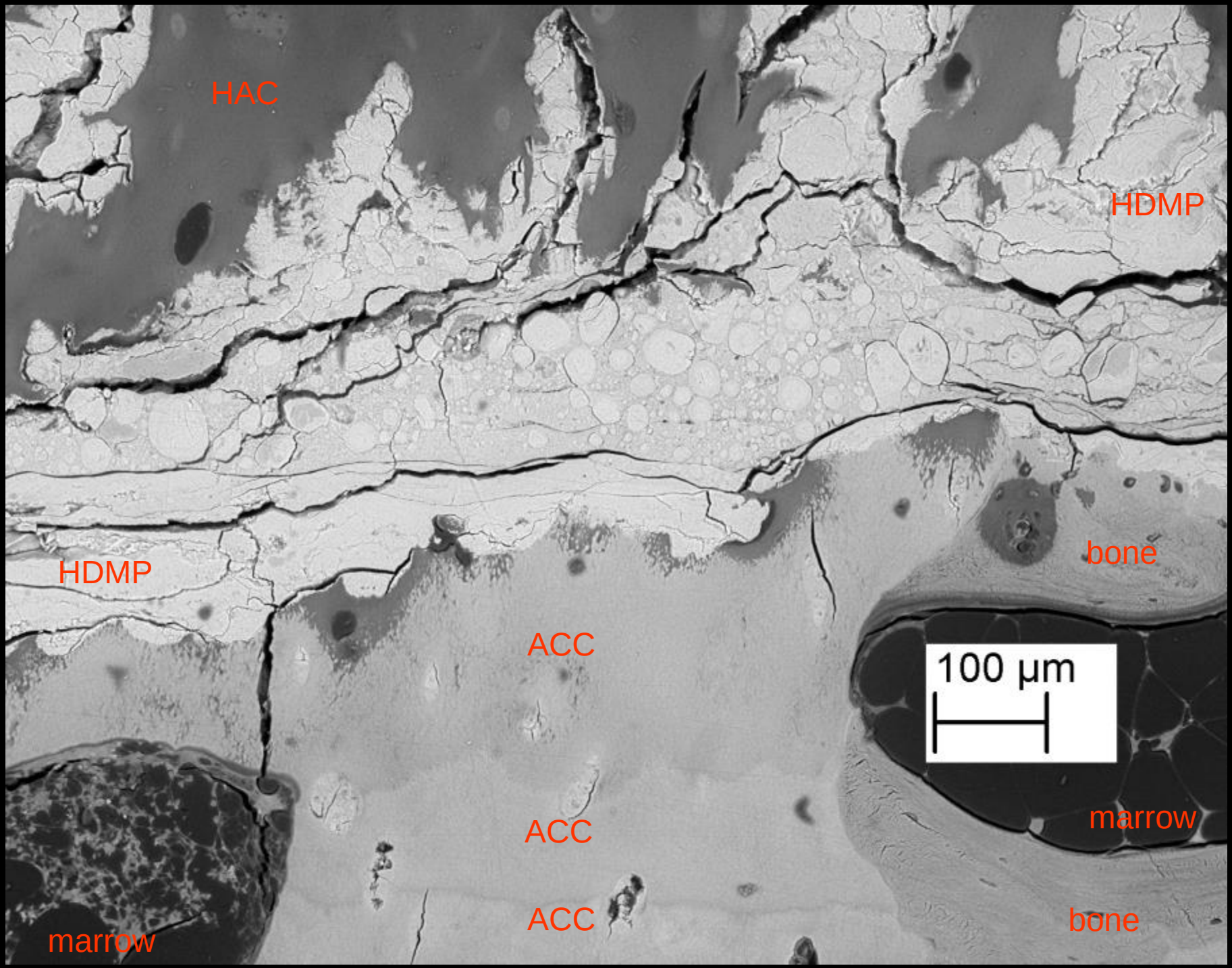
Repeat BSE after staining triiodide, Serial re-polishing and re-imaging

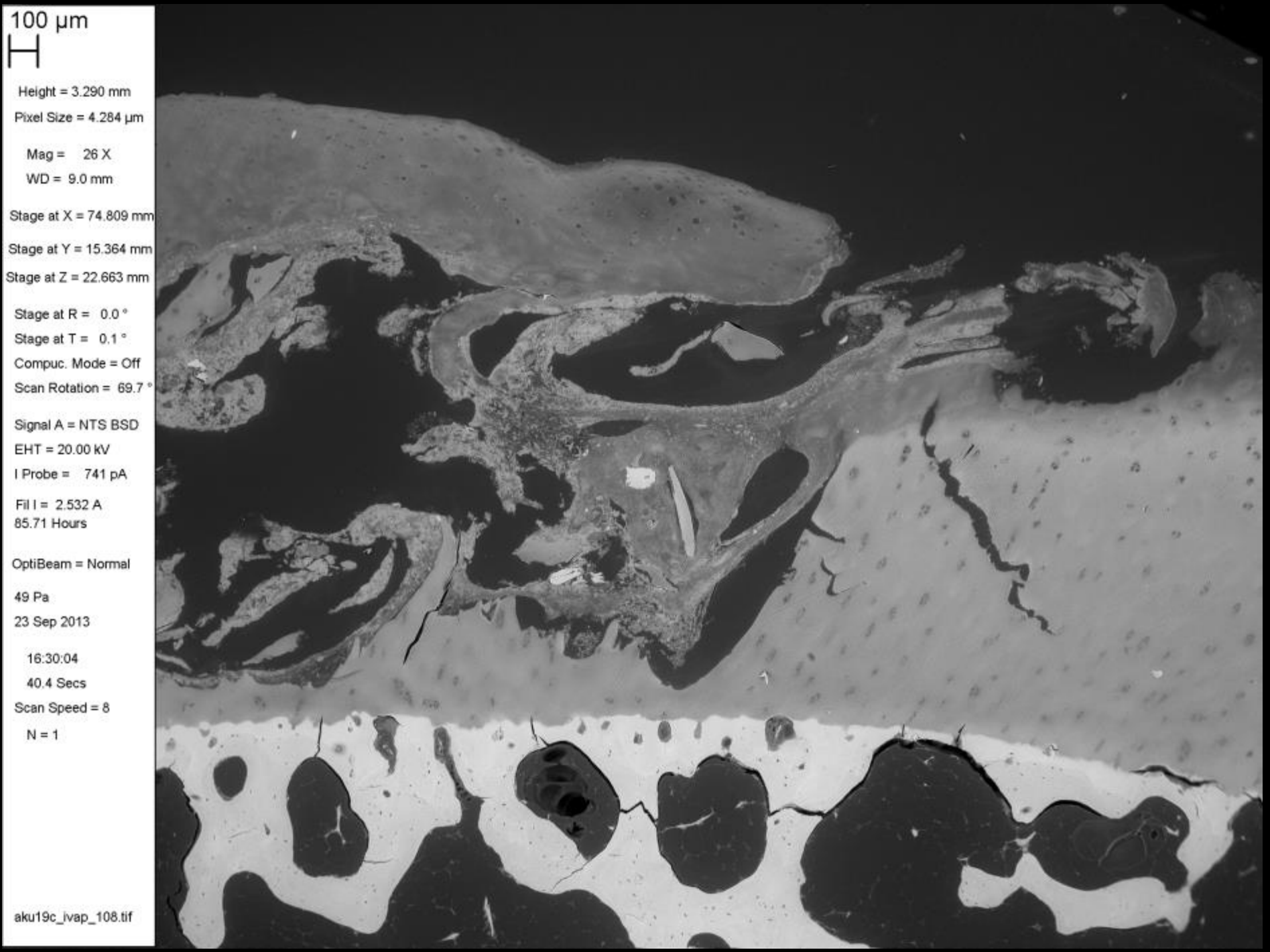




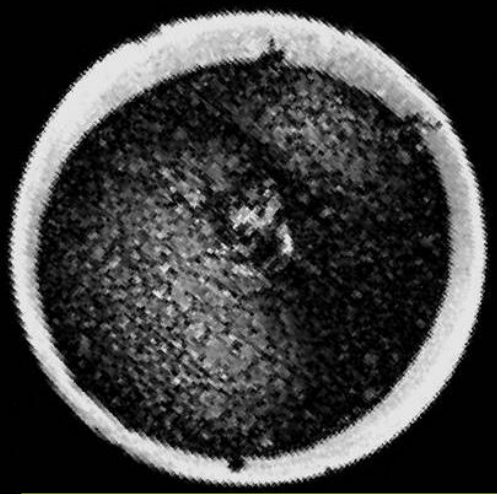

a
c
e
g

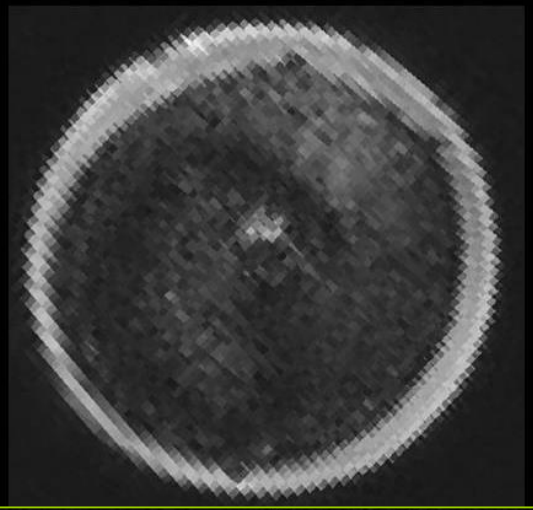

100 µm
Height = 1.585 mm
Pixel Size = 2.063 µm
Mag = 54 X
WD = 10.0 mm
Stage at X = 40.026 mm
Stage at Y = 59.918 mm
Stage at Z = 27.631 mm
Stage at R = 357.6 °
Stage at T = 0.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.319 A
297.08 Hours
OptiBeam = Normal
49 Pa
8 Mar 2013
17:10:10
45.6 Secs
Scan Speed = 7
N = 1
akuaf_040.tif
HAC
HDMP
HDMP
bone
ACC
marrow
ACC
ACC
bone
marrow

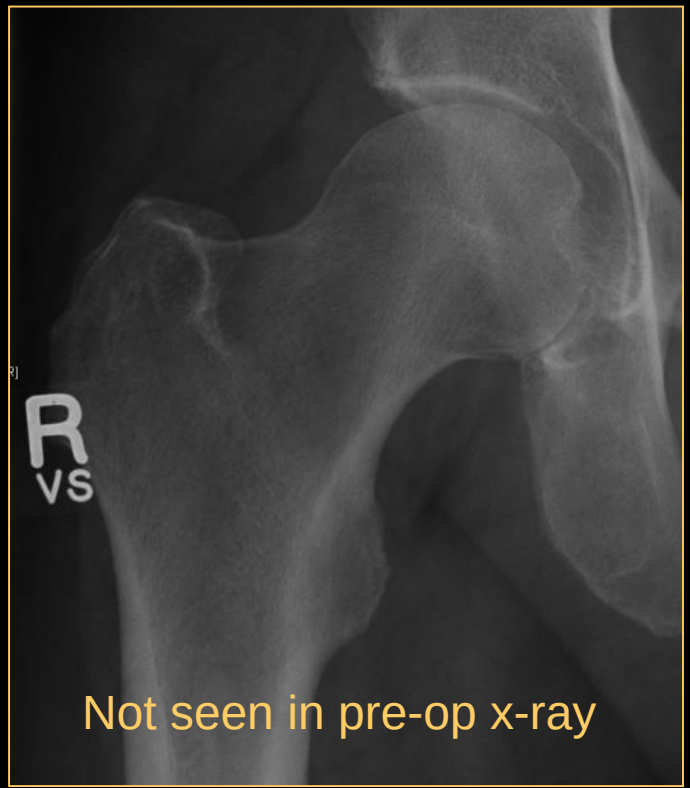

HAC
HDMP
HDMP
bone
ACC
100 µm
ACC
marrow
ACC
bone
marrow


100 µm
Height = 3.290 mm
Pixel Size = 4.284 µm
Mag = 26 X
WD = 9.0 mm
Stage at X = 74.809 mm
Stage at Y = 15.364 mm
Stage at Z = 22.663 mm
Stage at R = 0.0 °
Stage at T = 0.1 °
Compuc. Mode = Off
Scan Rotation = 69.7 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 741 pA
Fil I = 2.532 A
85.71 Hours
OptiBeam = Normal
49 Pa
23 Sep 2013
16:30:04
40.4 Secs
Scan Speed = 8
N = 1
aku19c_ivap_108.tif

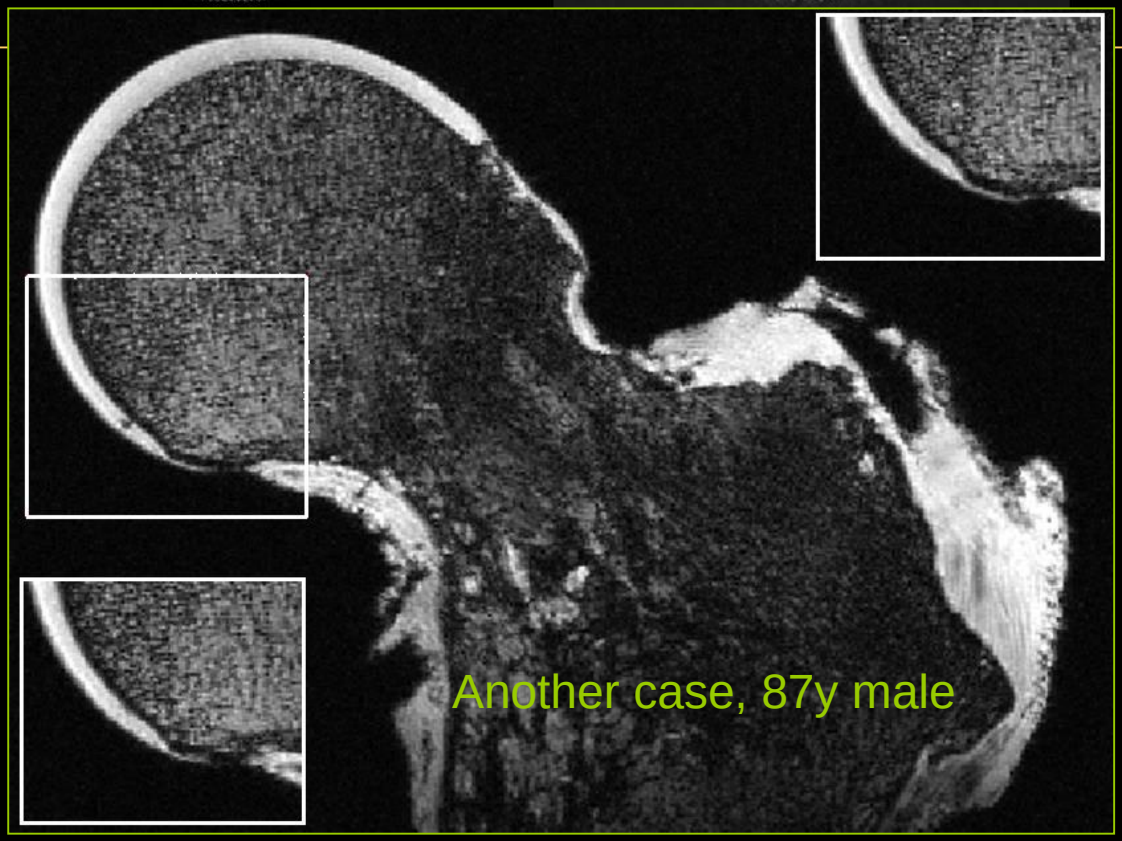
Hypointense HDMP be found with MRI DESS in isolated bones







Not seen in pre-op x-ray



Another case, 87y male

Indian J Radiol Imaging. 2013 Jan;23(1):101-5. doi: 10.4103/0971-3026.113628.

A simplified staging system based on the radiological findings in different stages of ochronotic spondyloarthropathy.

Jebaraj I1, Chacko BR, Chiramel GK, Matthai T, Parameswaran A.

QMUL: Mo Arora, Graham Davis, David Mills, Tomas Zikmunt
Liverpool: Jim Gallagher, Ranga Lakshminarayan, Nathan Jeffery, Jonathan Jarvis, Jane Dillon, Peter Wilson
Cambridge: Duncan Batty And Tim Cox

Barts and The London School of Medicine and Dentistry

UNIVERSITY OF LIVERPOOL