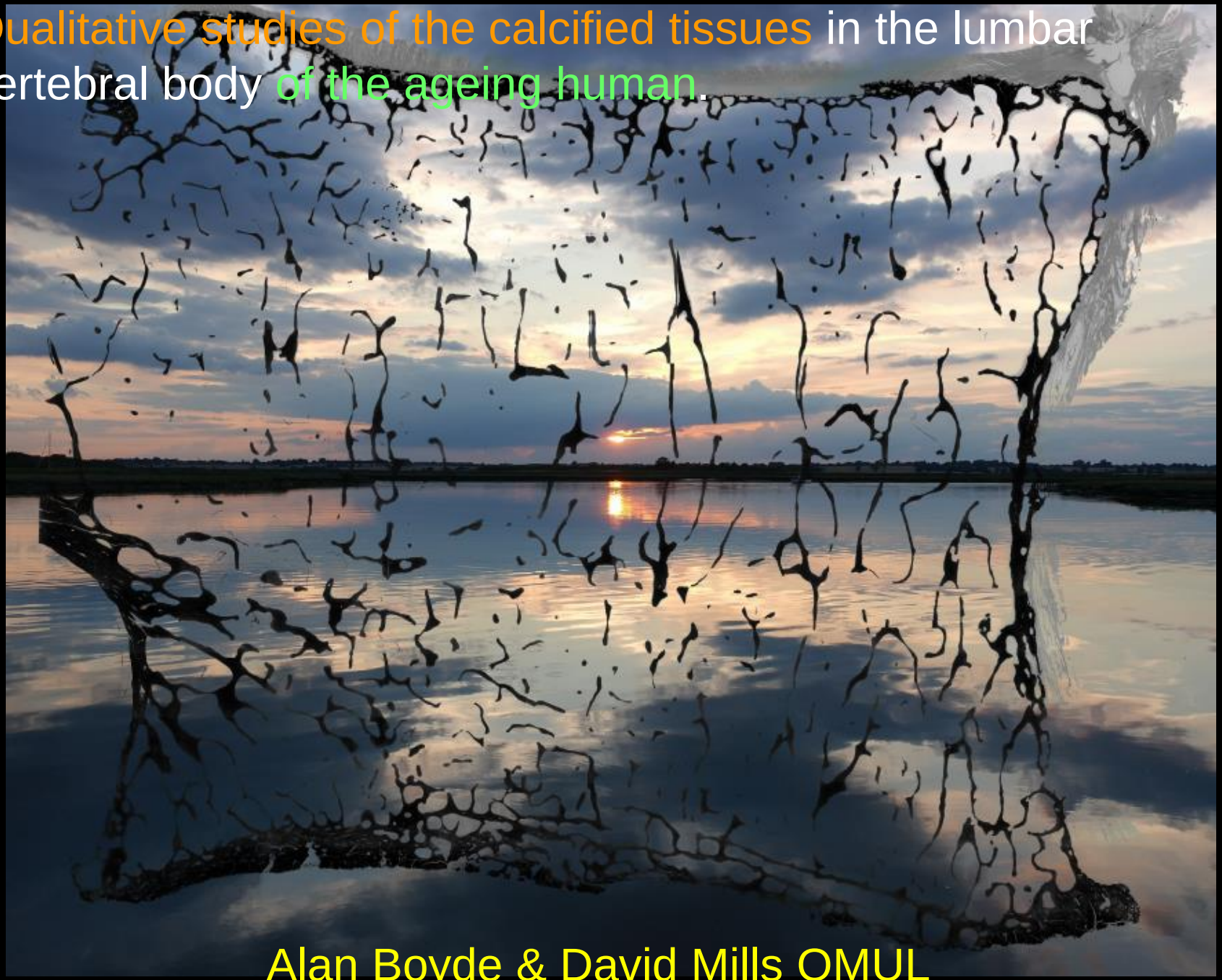
Qualitative studies of the calcified tissues in the lumbar vertebral body of the ageing human.



Alan Boyde & David Mills QMUL

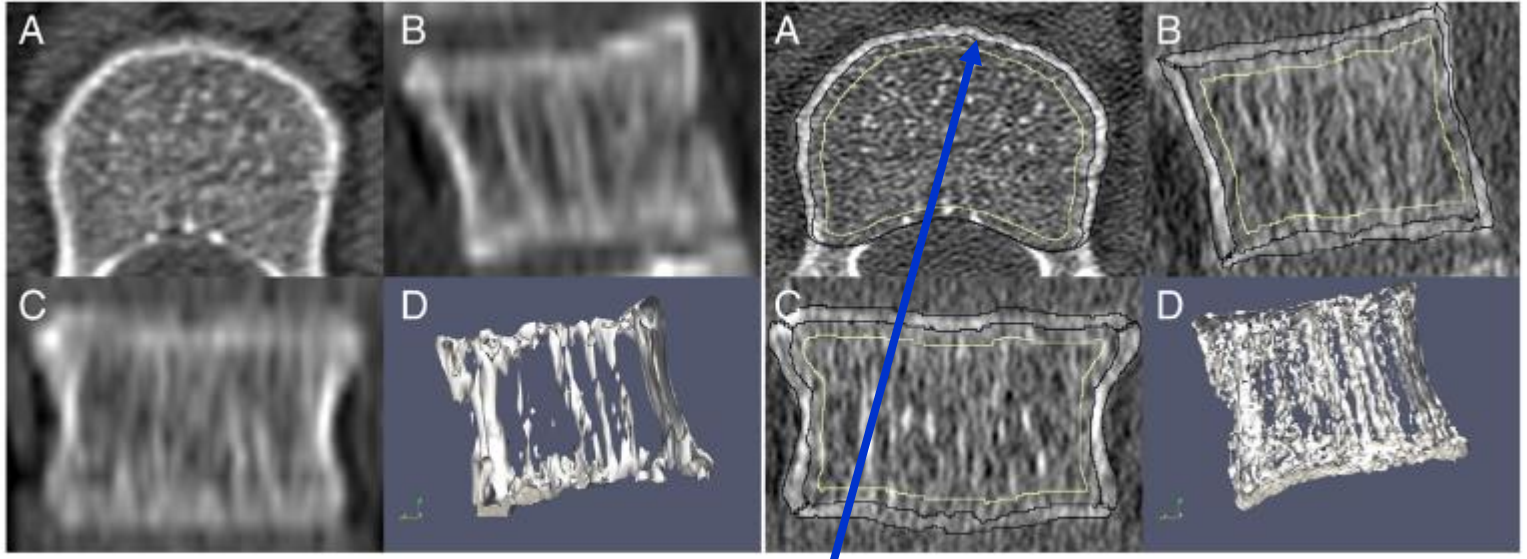


Fig. 1. Depiction of L1 vertebra scanned in QCT (left panels) and HRQCT (right panels) in transversal (A), coronal (B), and sagittal (C) views. A rendered, sagittal slice of 10 mm thickness (D) shows the trabecular structure in 3-D. The difference in image quality is apparent, especially in the lower slice thickness of HRQCT (0.3 vs. 2.5 mm). In the HRQCT images, the outer, double line (black) denotes the cortical region of interest, the inner line (white) the trabecular one.

•Aim - understand cortical thickness in clinical imaging

ELSEVIER

Bone 52 (2013) 568–577

Original Full Length Article

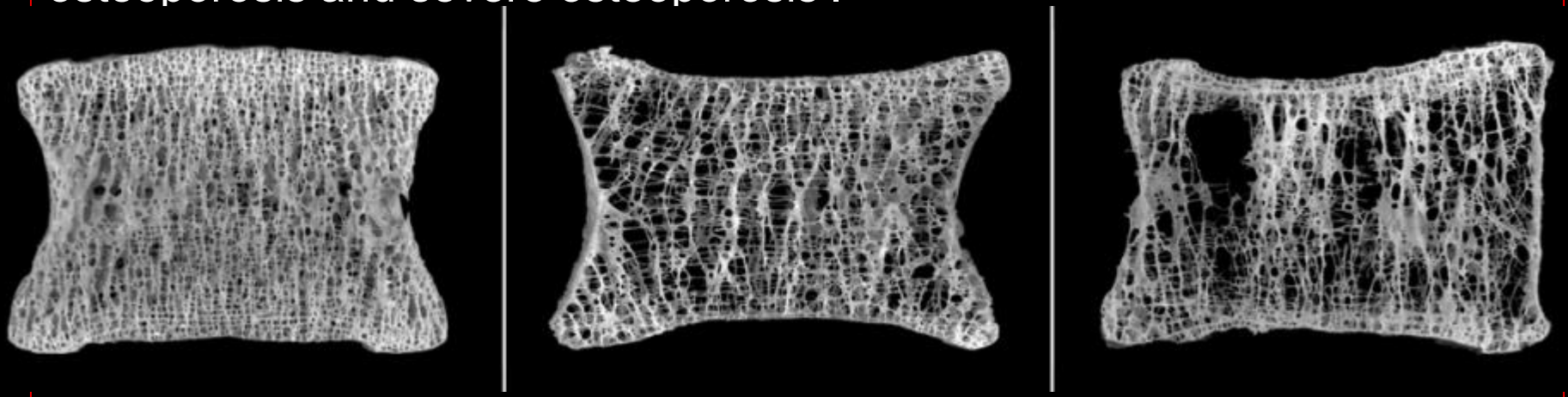
High resolution quantitative computed tomography-based assessment of trabecular microstructure and strength estimates by finite-element analysis of the spine, but not DXA, reflects vertebral fracture status in men with glucocorticoid-induced osteoporosis

Christian Graeff [a,*,1], Fernando Marin [b], Helmut Petto [b], Ole Kayser [c], Andreas Reisinger [d], Jaime Peña [a], Philippe Zysset [d,2], Claus-Christian Glüer [a]

[a] *Sektion Biomedizinische Bildgebung, Klinik für Diagnostische Radiologie, Universitätsklinikum Schleswig-Holstein, Kiel, Germany*

New York Times, June 2nd 2016.

Caption read ‘CT scans show the progression of one patient’s vertebra over a six- to eight-year period, from normal bone density to moderate osteoporosis and severe osteoporosis’.



30 year female | 88 year female | 89 year male

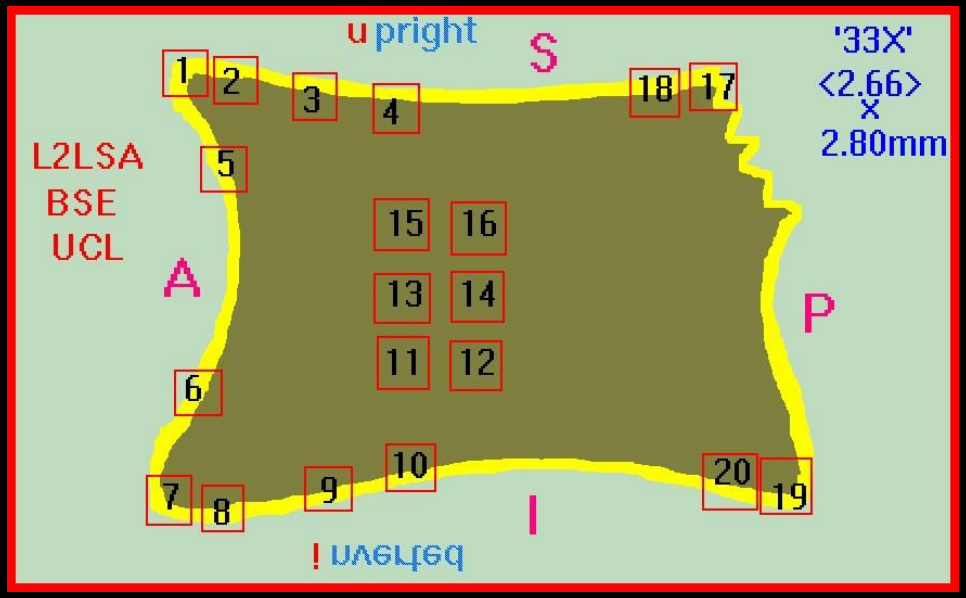
Photographs of 3 mm slices of L4 obtained at post mortem, published in 3D photographic study of cancellous bone in human fourth lumbar vertebral bodies. Anatomy and Embryology 189:259-274, 1994

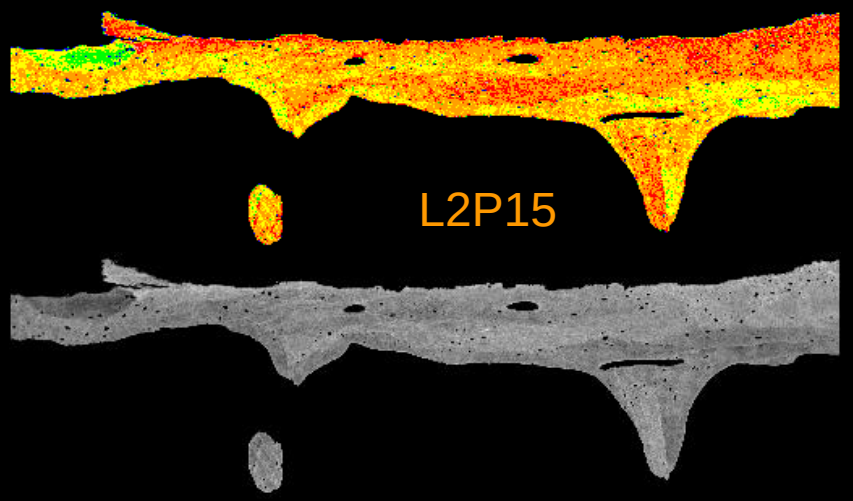
Freedom of the press

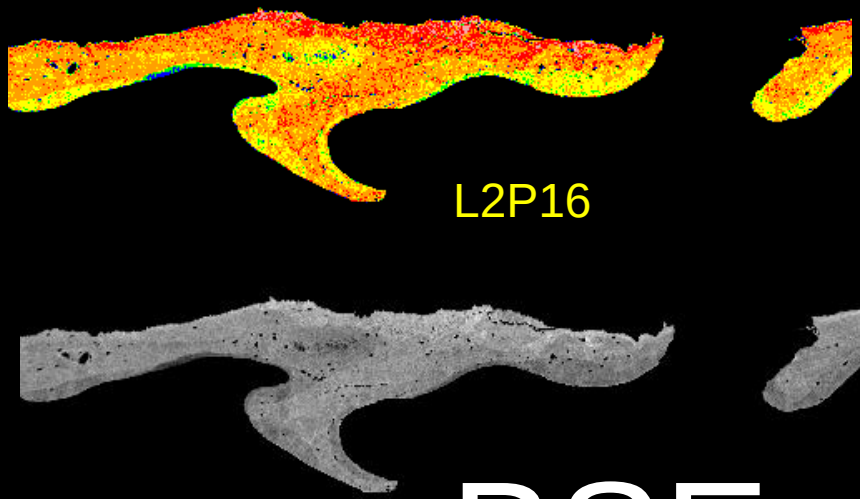
M & M & Aims

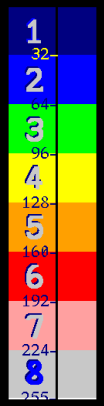
- 1992:- qBSE study of degree of mineralisation of 'bone' in L2 – L4 bodies (38 male, 31 female, 70±15 years, European Union Concerted Action Biomed 1 "Assessment of ***bone quality in osteoporosis***")
- 2012:- SEM & XMT re-imaging of L2 and ~ 50 L4 collected at UCL 1989-1993, some embedded, majority macerated
- qBSE SEM topography free PMMA block surfaces
- BSE SEM iodine stained blocks to reveal uncalcified matrix
- High-contrast x-ray microtomography
- Thin ground sections cut from block faces
  - by laser ablation, Rowiak LaserSolutions GmbH Hannover
  - studied by SEM, polarised light microscopy and after staining
- understand cortical thickness in clinical imaging

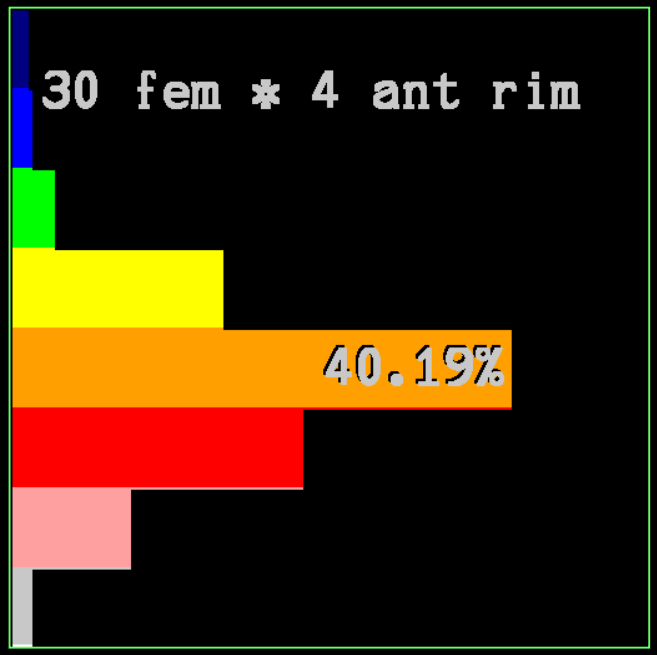
‘Mineral quantitation of human bone by analysis of backscattered electron images’. Zeiss DSM962 with Kontron external control computer. Grant application started 1982. MRC Funded 1992. Moved to Whitechapel 2003. Scrapped 2014. SEM No 5


upright
S
'33X'
<2.66>
x
2.80mm
L2LSA
BSE
UCL
A
P
I
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

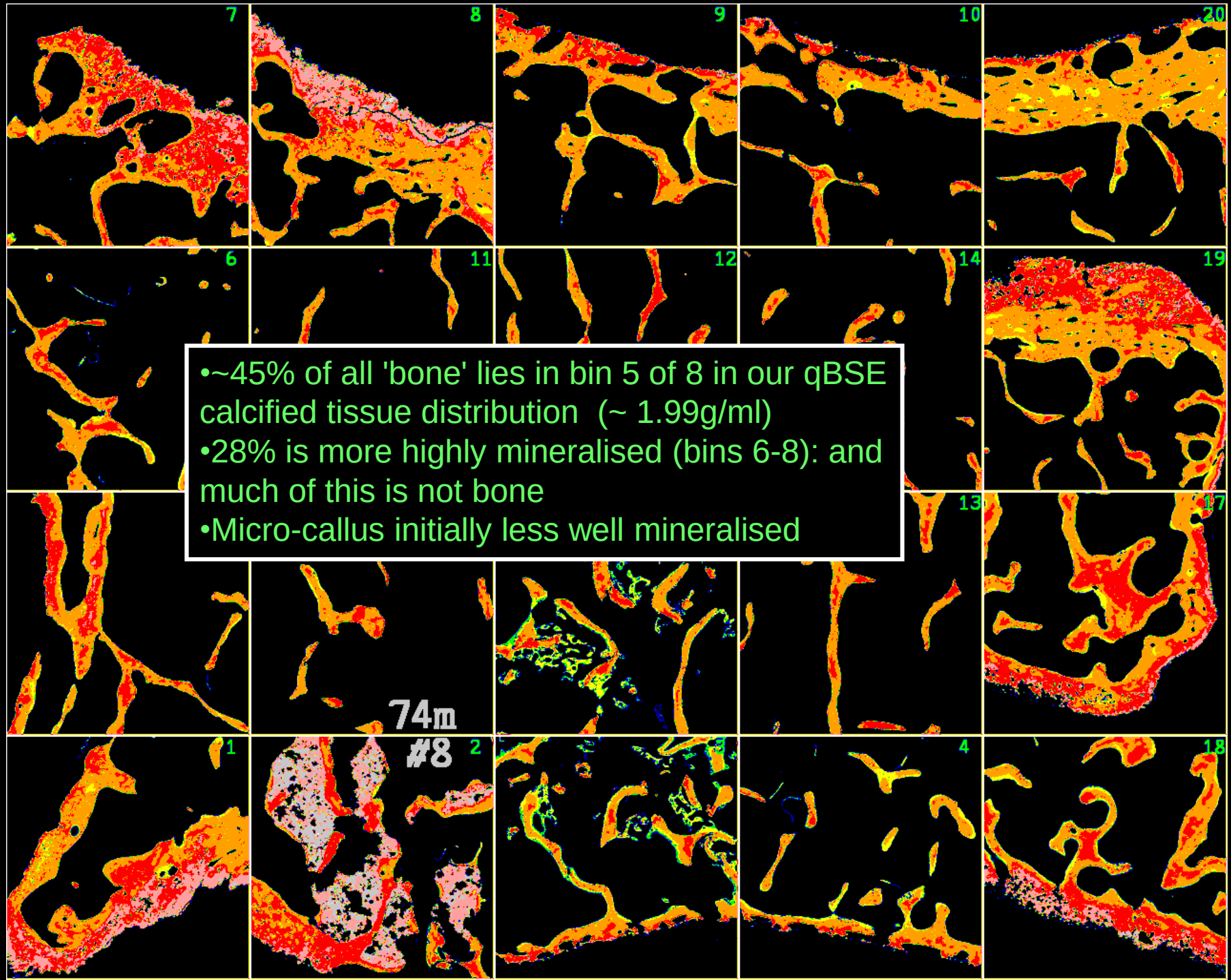

L2P15


L2P16


1
32
2
64
3
96
4
128
5
160
6
192
7
224
8
255


30 fem * 4 ant rim
40.19%

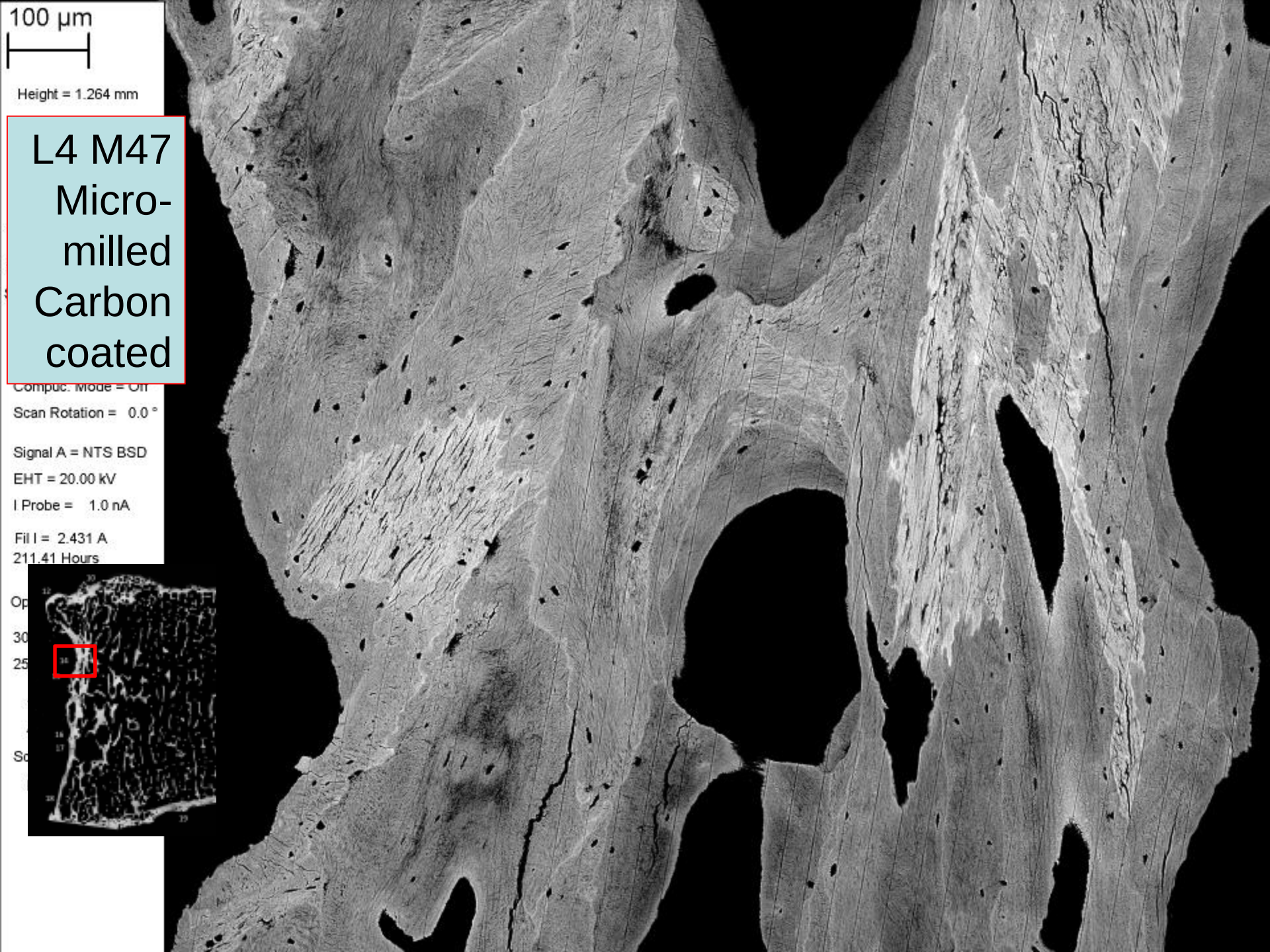
qBSE

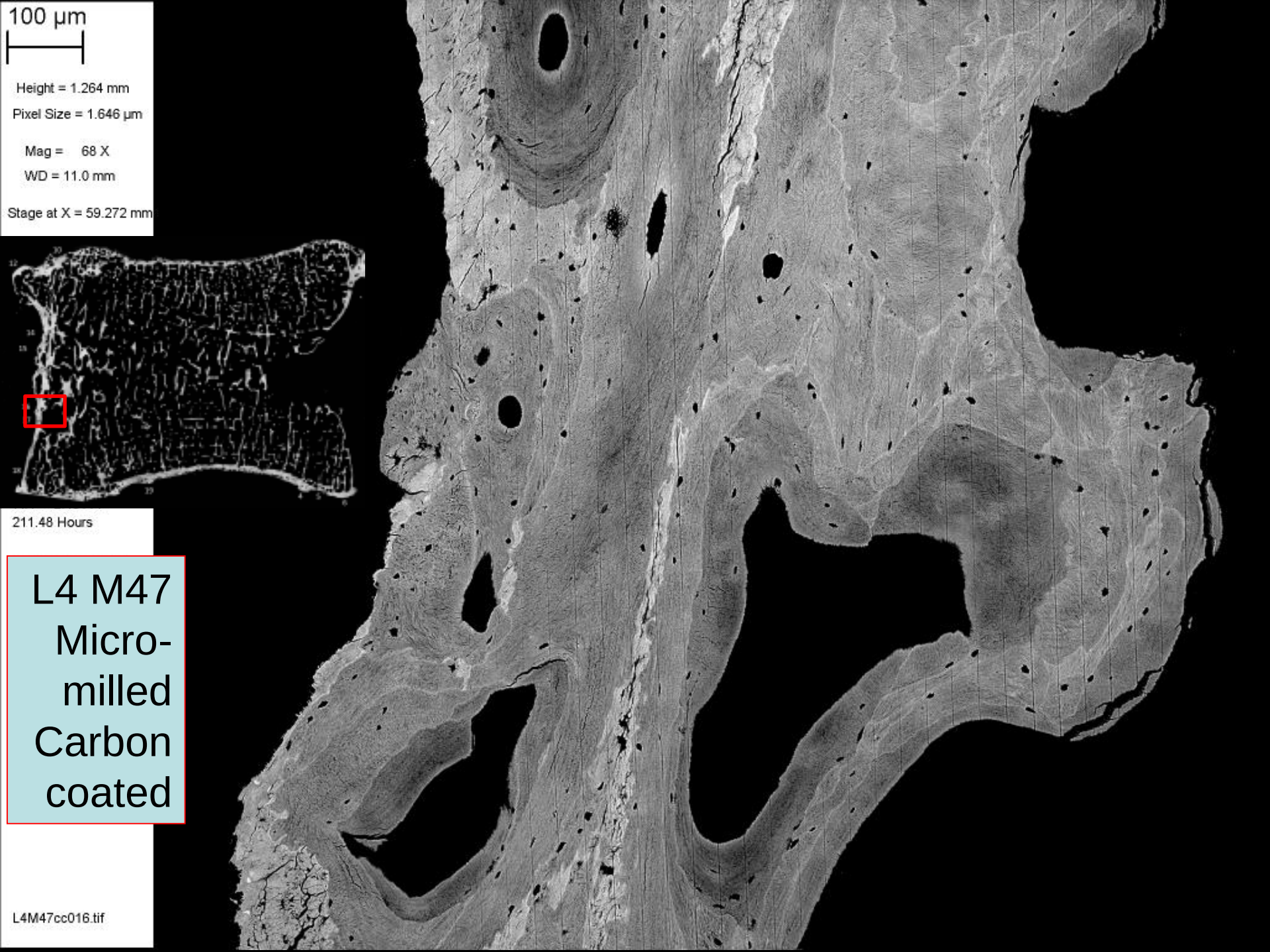


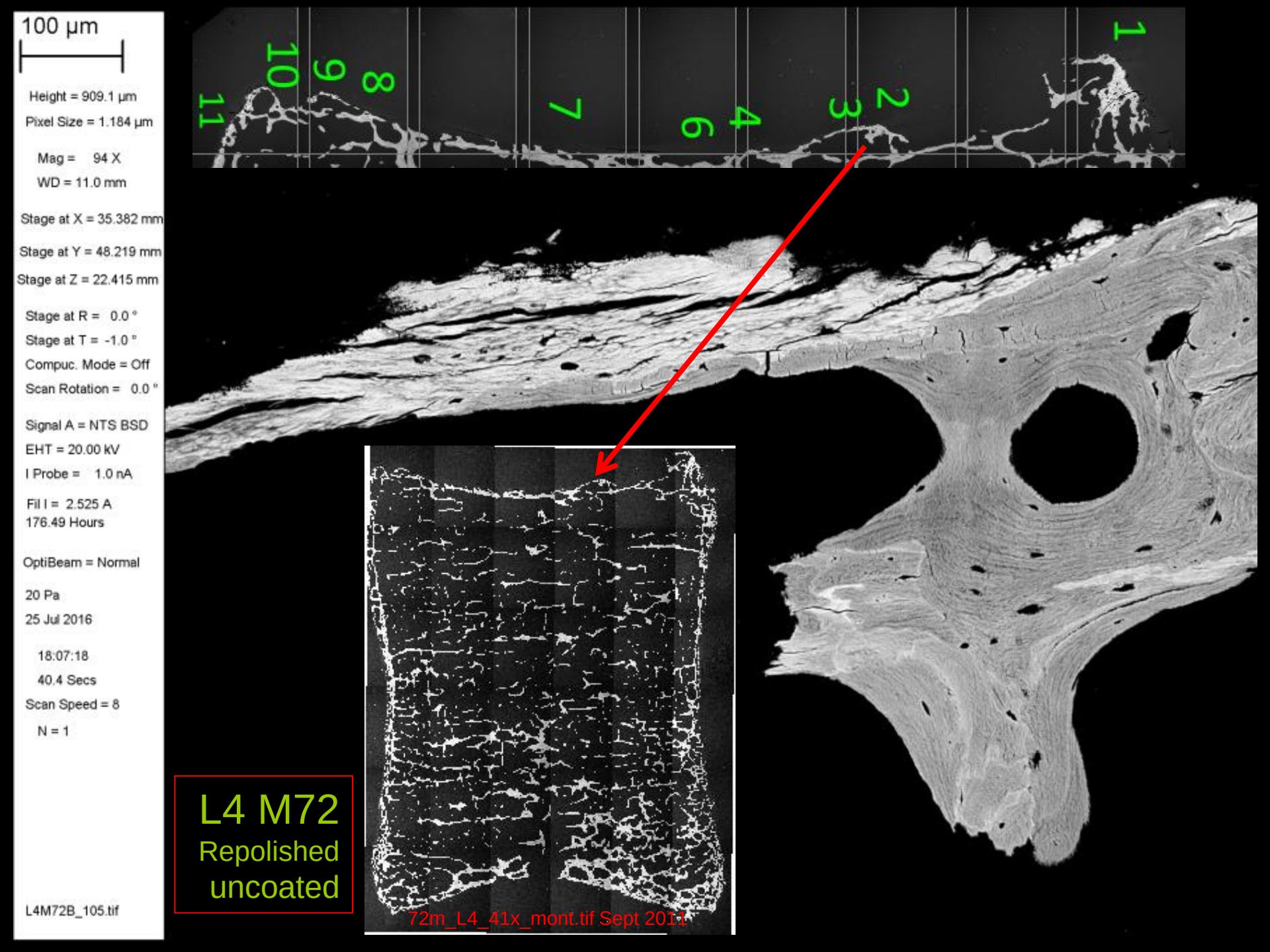
- ~45% of all 'bone' lies in bin 5 of 8 in our qBSE calcified tissue distribution (~ 1.99g/ml)
- 28% is more highly mineralised (bins 6-8): and much of this is not bone
- Micro-callus initially less well mineralised

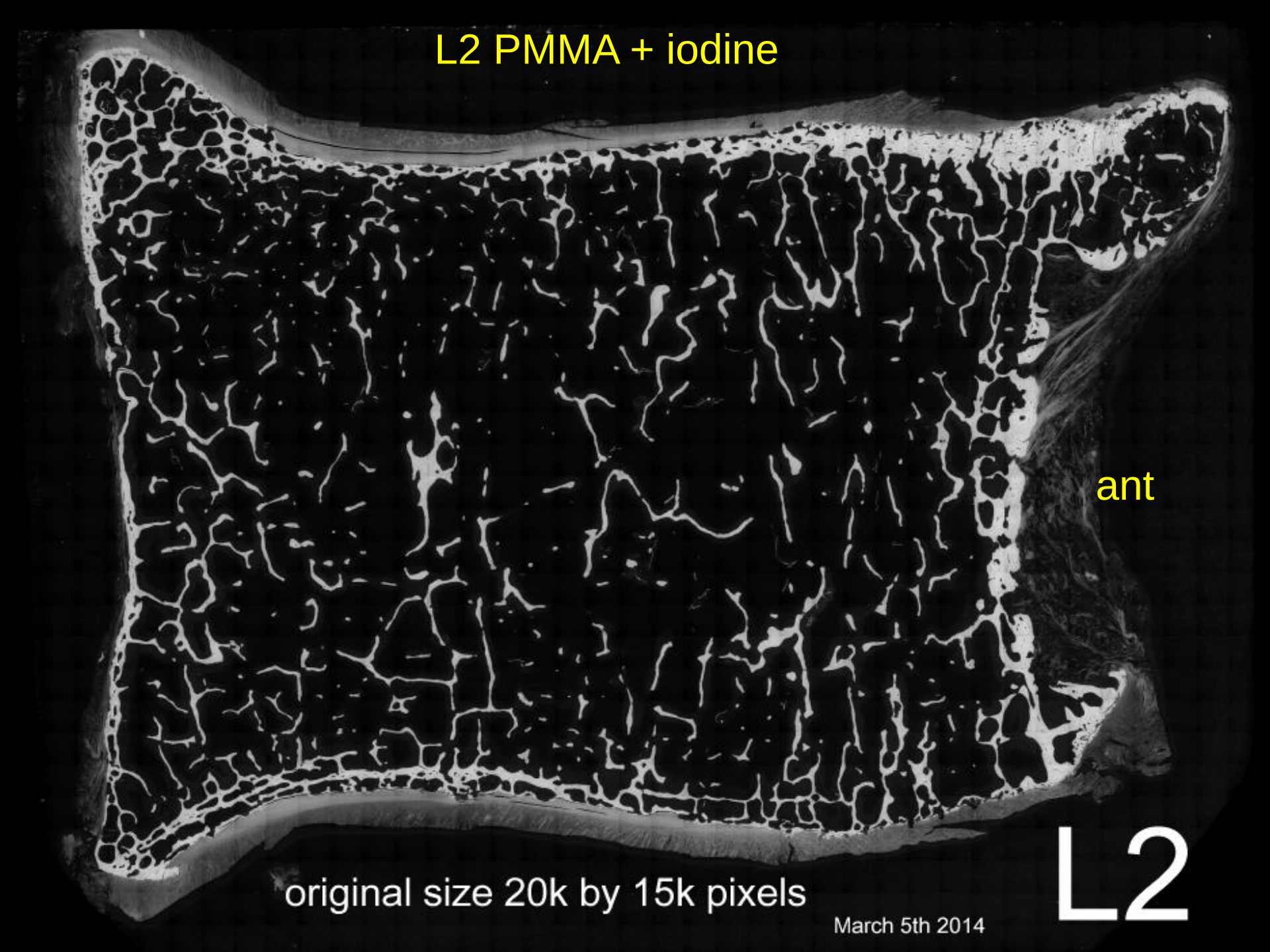
SEM No 6. 2011 self funded. Zeiss EVO MA10. ‘bad vacuum’. Quick turnaround. Uncoated samples. Big pictures

IODINE Z 53


100 µm
Height = 1.264 mm
L4 M47
Micro-
milled
Carbon
coated
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.431 A
211.41 Hours


100 µm
Height = 1.264 mm
Pixel Size = 1.646 µm
Mag = 68 X
WD = 11.0 mm
Stage at X = 59.272 mm
211.48 Hours
L4 M47
Micro-
milled
Carbon
coated
L4M47cc016.tif


100 µm
Height = 909.1 µm
Pixel Size = 1.184 µm
Mag = 94 X
WD = 11.0 mm
Stage at X = 35.382 mm
Stage at Y = 48.219 mm
Stage at Z = 22.415 mm
Stage at R = 0.0 °
Stage at T = -1.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.525 A
176.49 Hours
OptiBeam = Normal
20 Pa
25 Jul 2016
18:07:18
40.4 Secs
Scan Speed = 8
N = 1
L4M72B_105.tif
1
2
3
4
6
7
8
9
10
11
L4 M72
Repolished
uncoated
72m_L4_41x_mont.tif Sept 2011


L2 PMMA + iodine
ant
original size 20k by 15k pixels
March 5th 2014
L2

Iodine vapour staining, dry

100 µm

Height = 3.176 mm

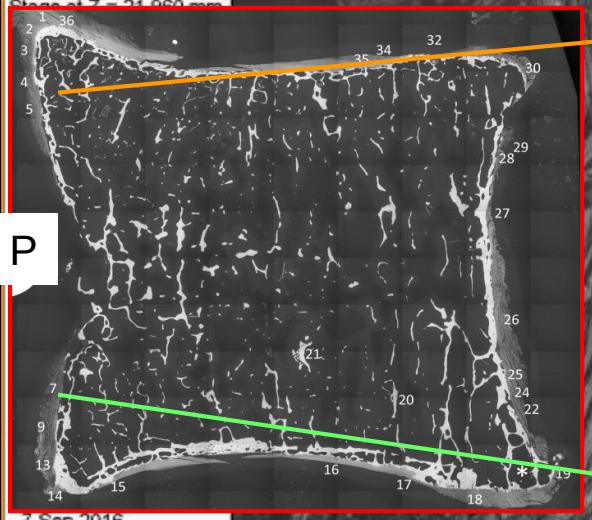
Pixel Size = 4.135 µm

Mag = 27 X

WD = 8.5 mm

Stage at X = 54.563 mm

Stage at Y = 55.222 mm



P

7 Sep 2016

11:01:04

40.4 Secs

Scan Speed = 8

N = 1

02ivap513_08.tif

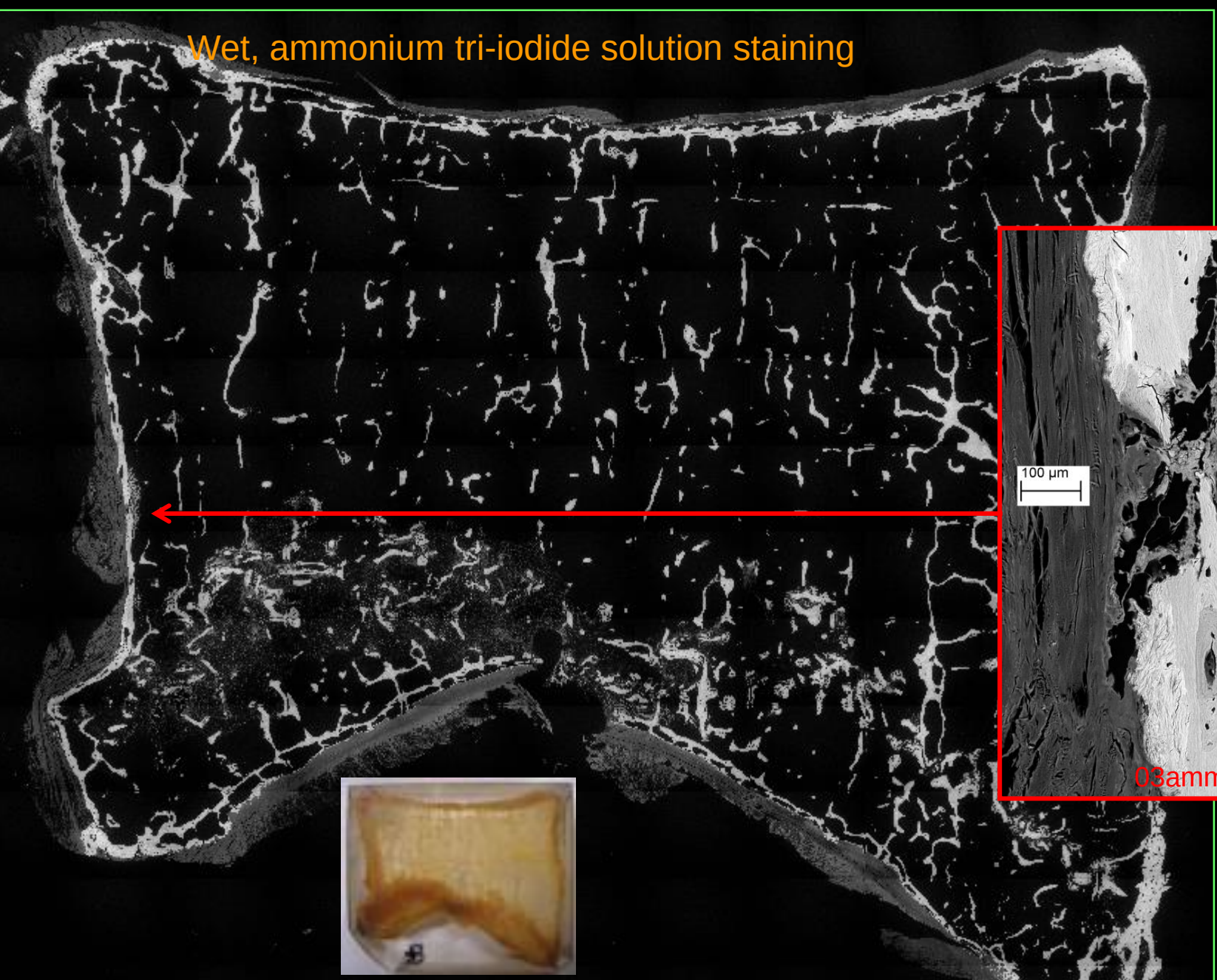
bone marrow outside bone organ

100 µm

bone marrow outside bone organ

02ivap513_06.tif

Wet, ammonium tri-iodide solution staining

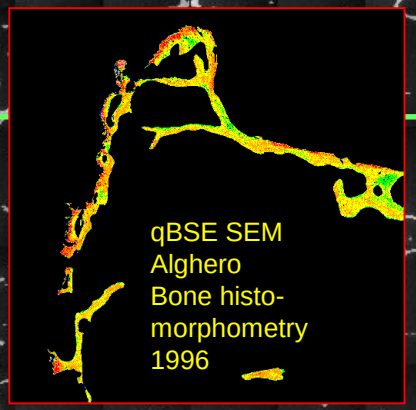


thickness and sickness of the cortex

L2 PMMA + iodine

>XMT 2016

XMT 2016



L2 #62
92F

L2 #62
92yF
Iodine
Vapour
stained

Iodine stained montage 2014

bone
marrow
outside
bone

bone
marrow
inside
bone

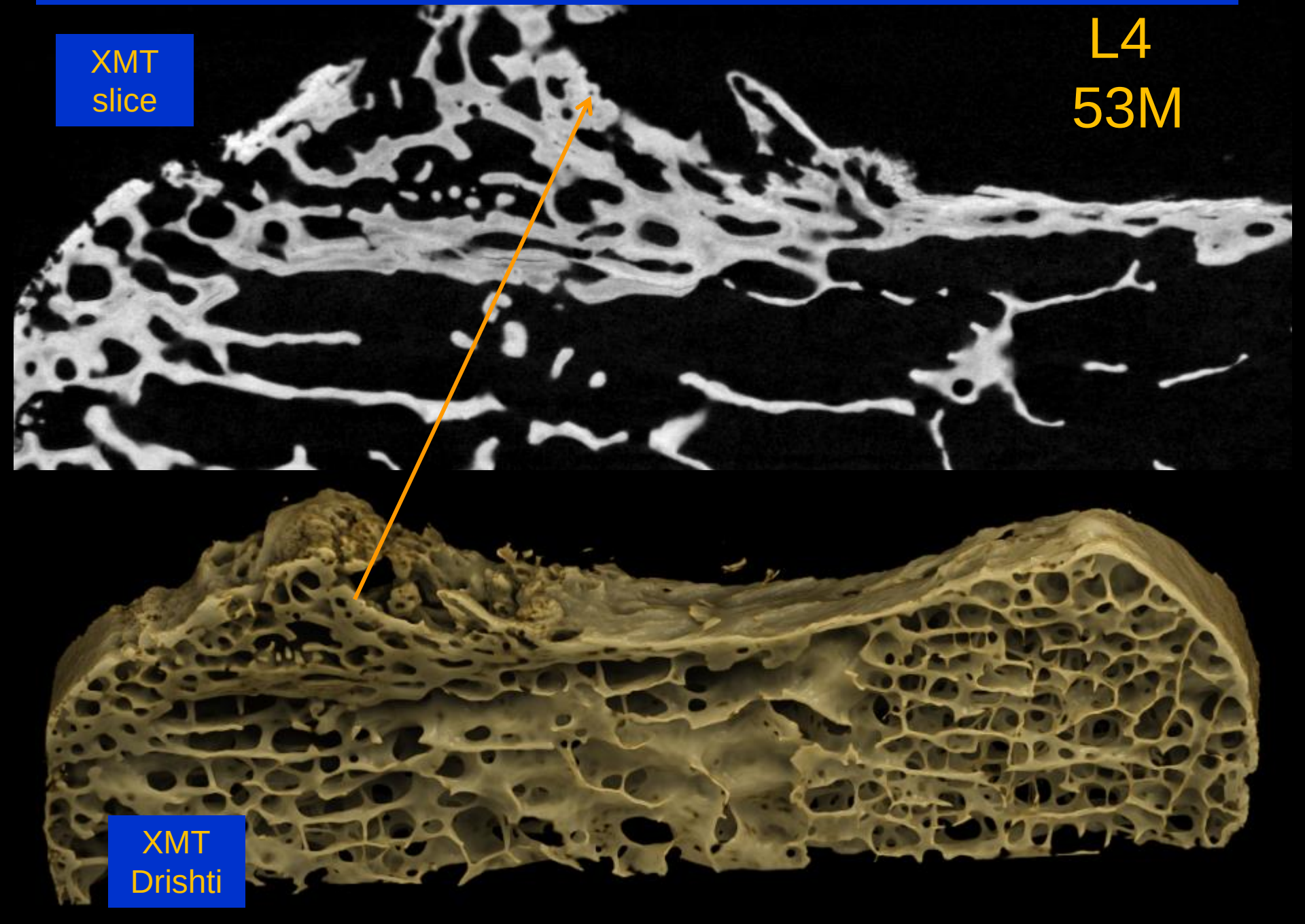
bone
marrow
inside
bone

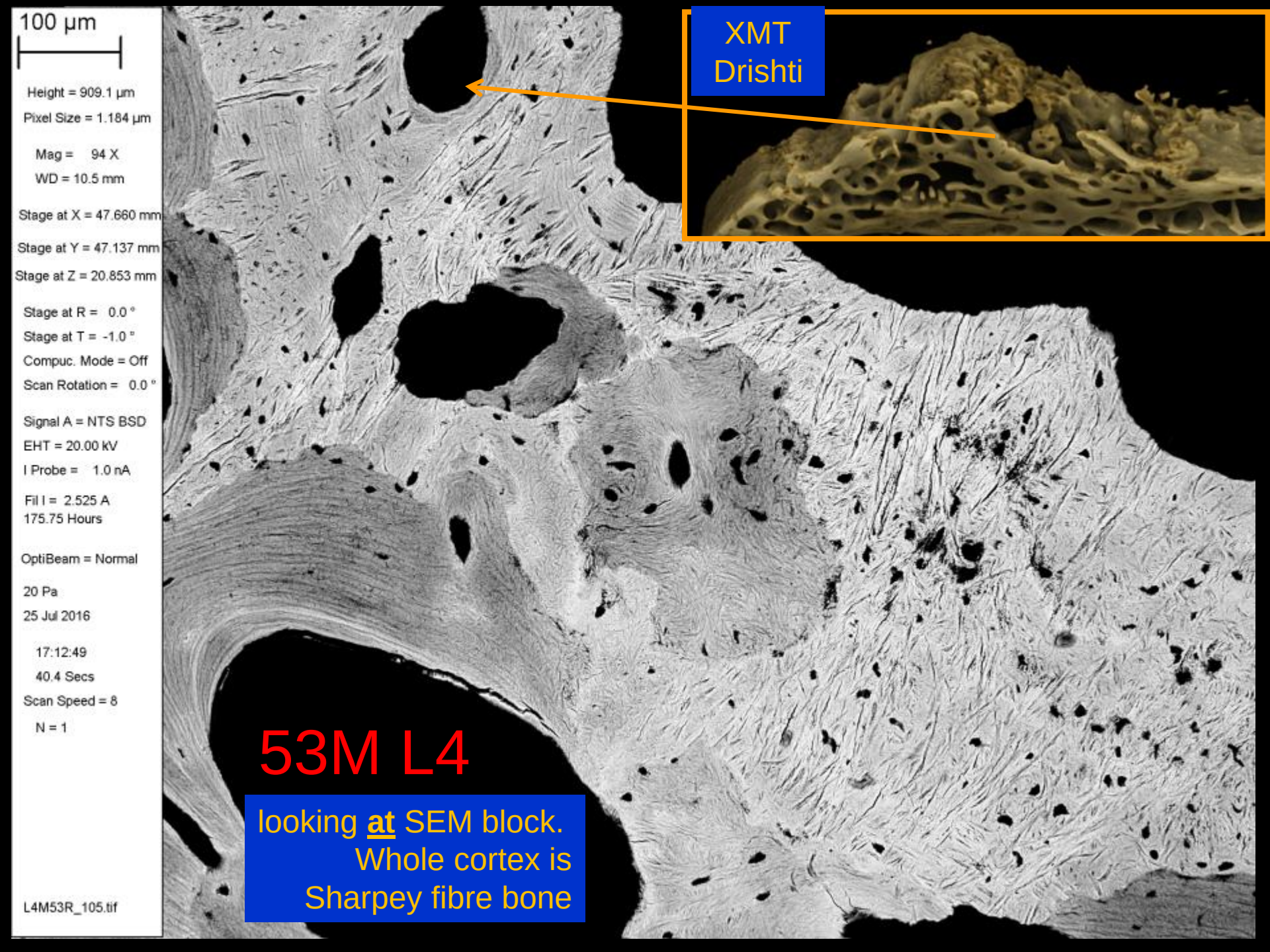
100 µm

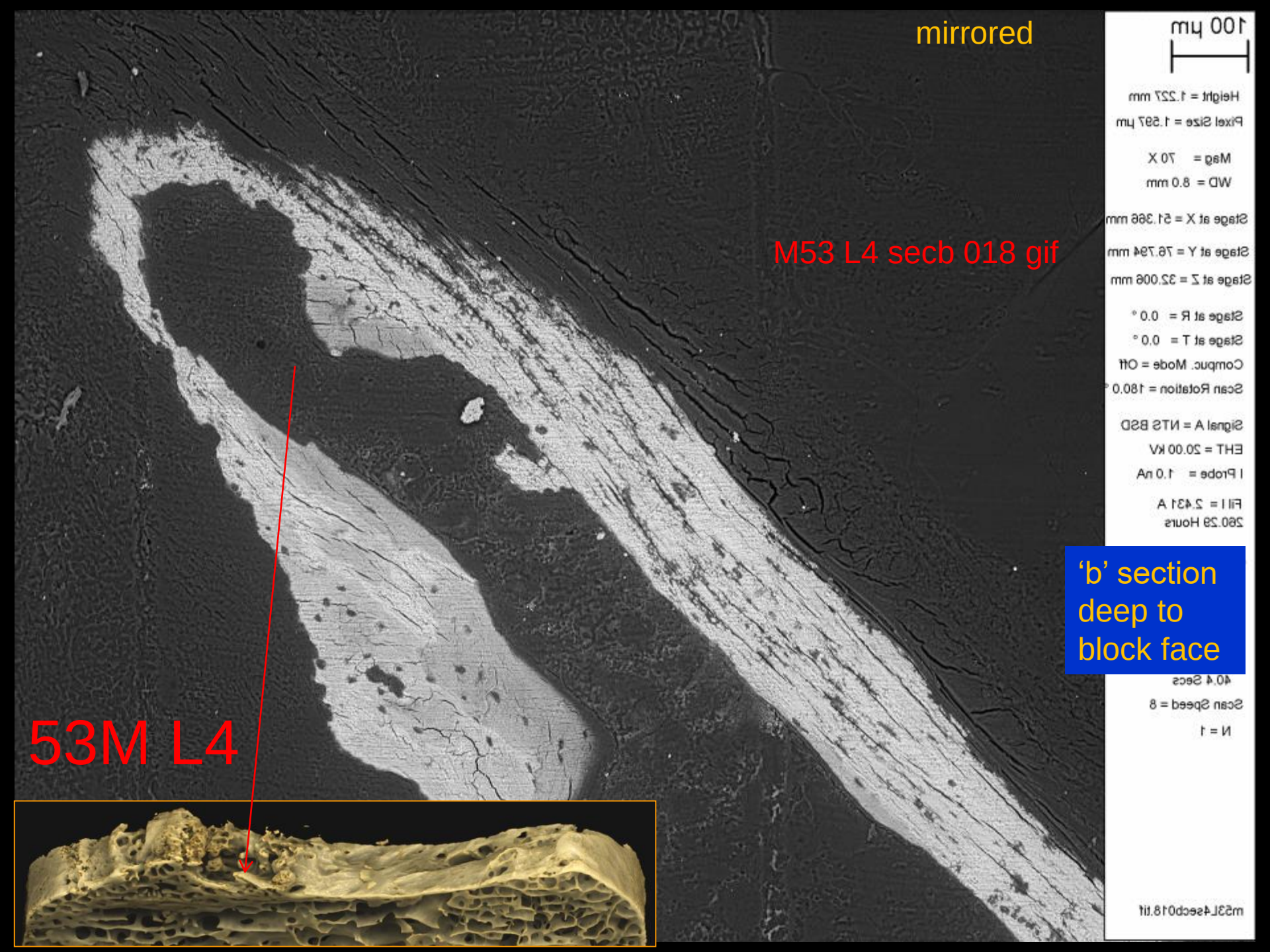
Height = 3.430 mm

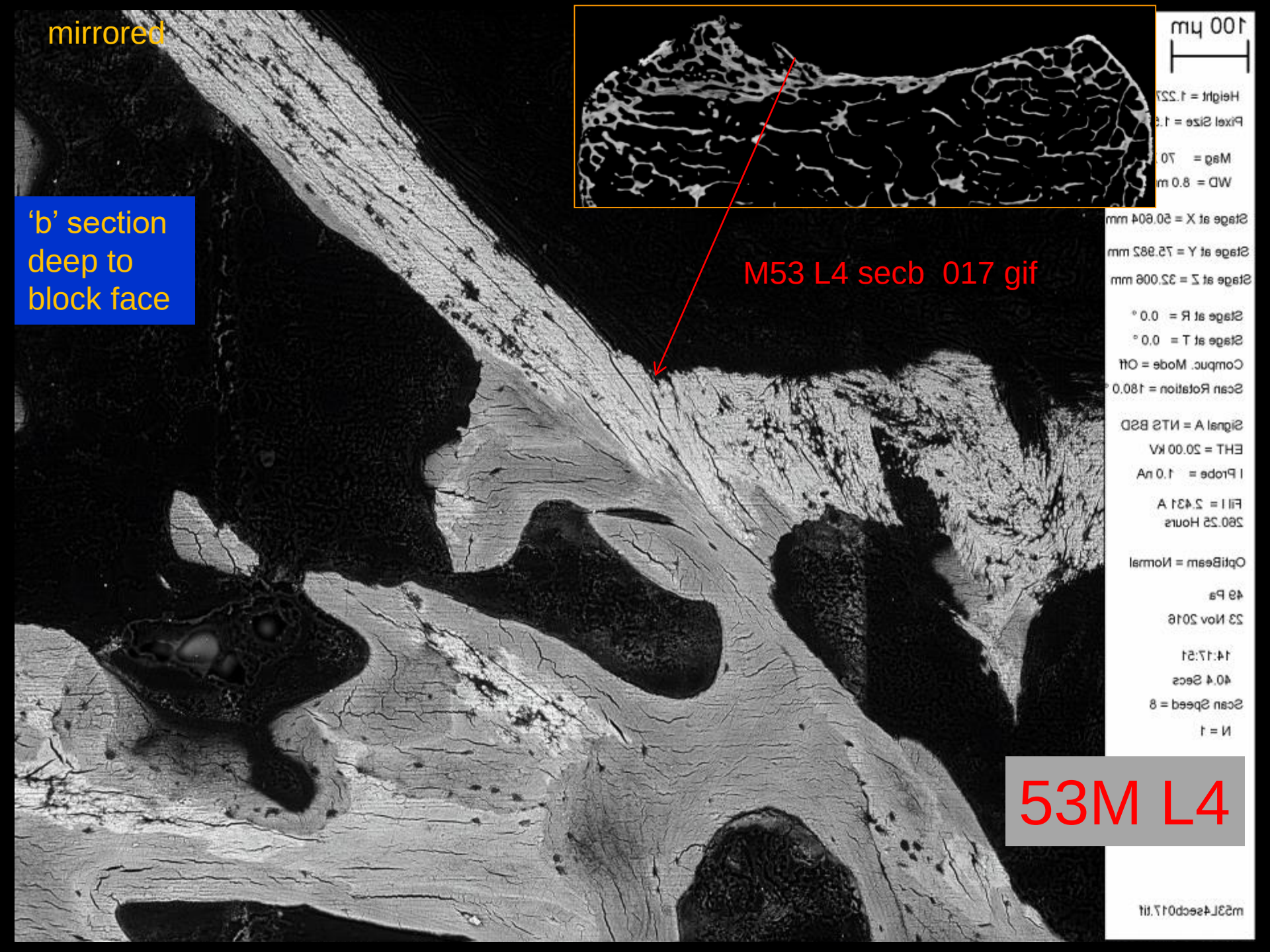
Iodine vapour staining, dry:
staining still there after 2 years

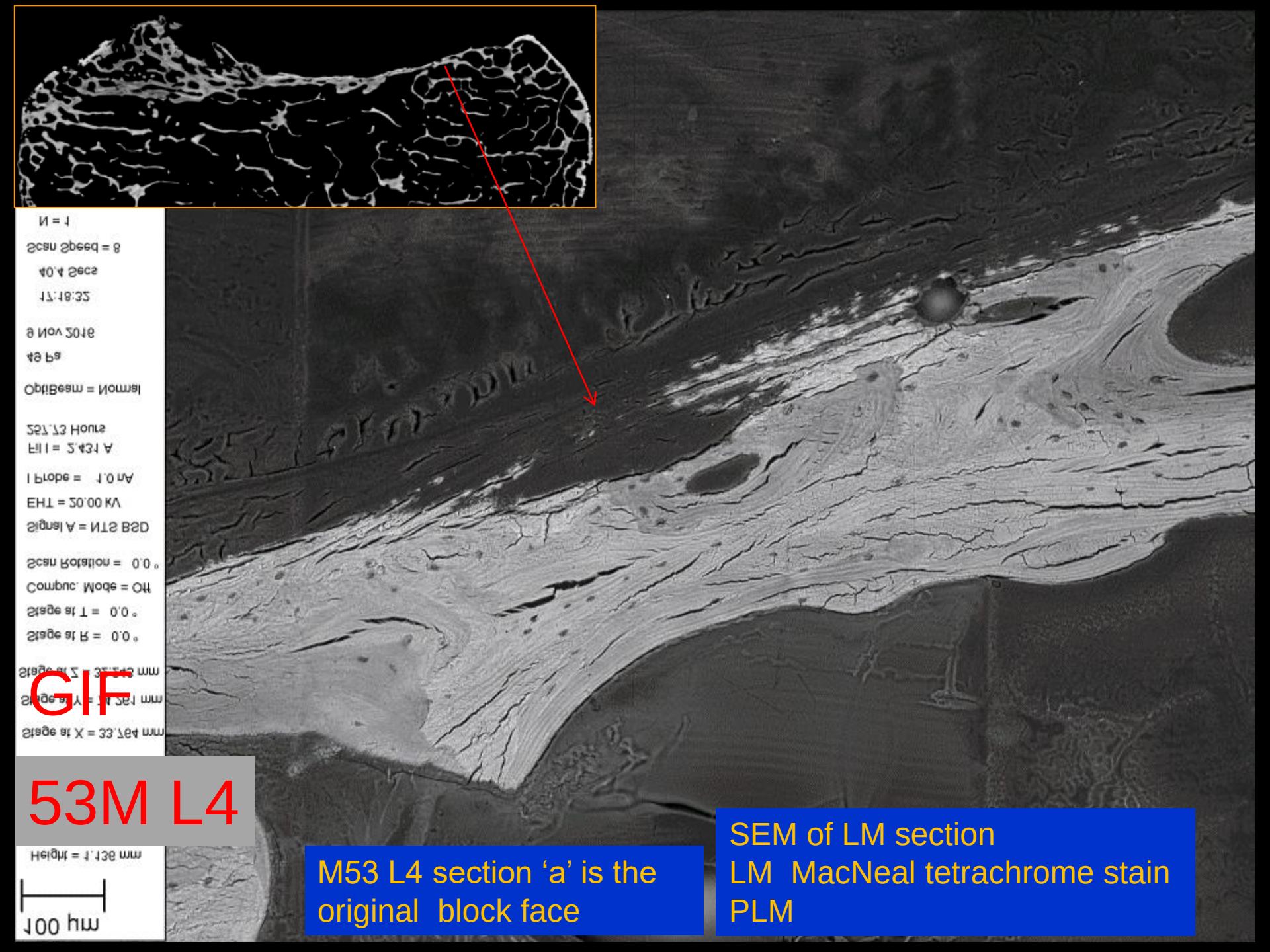
Orientation same as looking **at** SEM block, arrow shows Sharpey fibre cortex

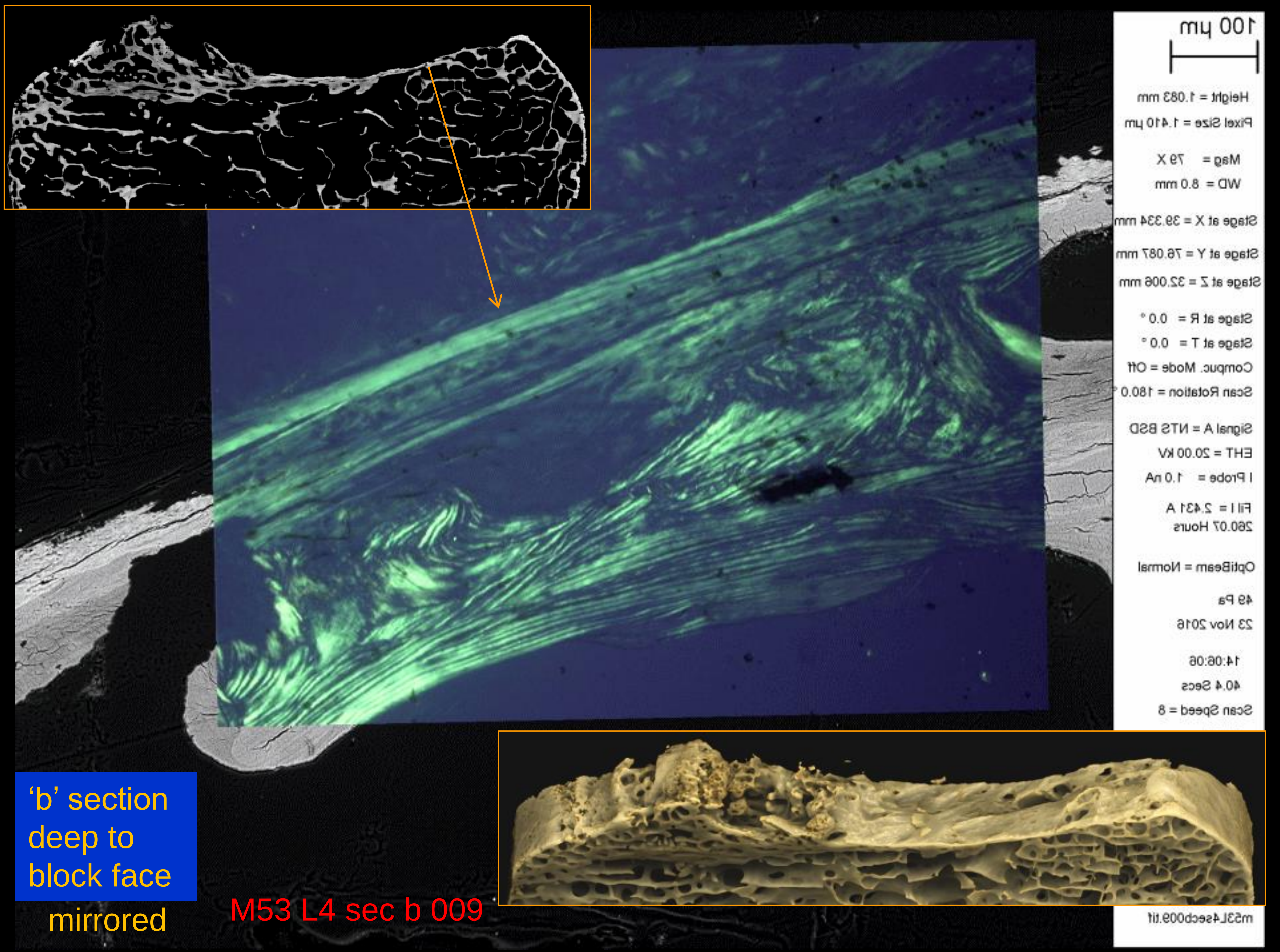


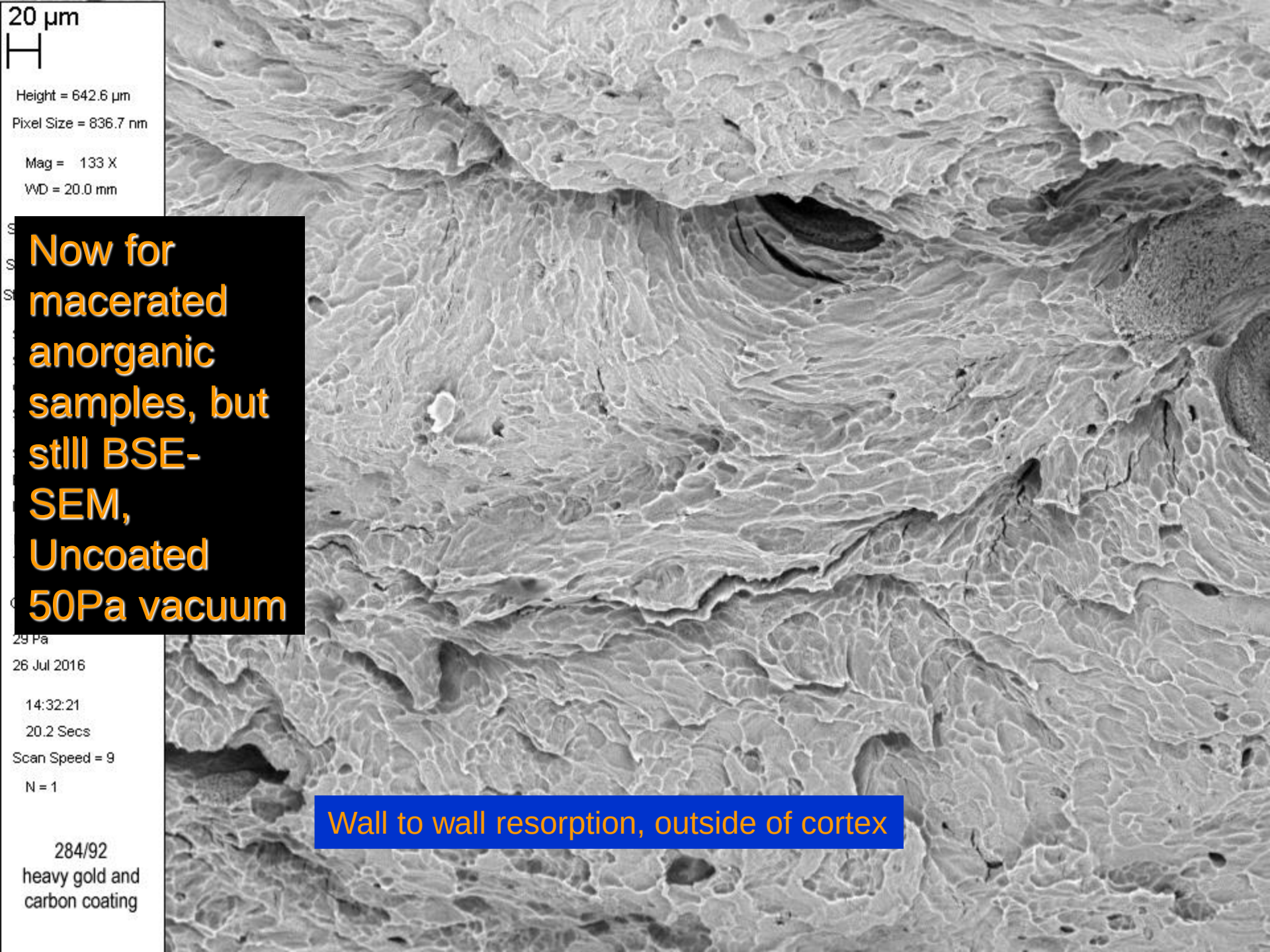

100 µm
Height = 909.1 µm
Pixel Size = 1.184 µm
Mag = 94 X
WD = 10.5 mm
Stage at X = 47.660 mm
Stage at Y = 47.137 mm
Stage at Z = 20.853 mm
Stage at R = 0.0 °
Stage at T = -1.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.525 A
175.75 Hours
OptiBeam = Normal
20 Pa
25 Jul 2016
17:12:49
40.4 Secs
Scan Speed = 8
N = 1
L4M53R_105.tif
XMT
Drishti
53M L4
looking at SEM block.
Whole cortex is
Sharpey fibre bone

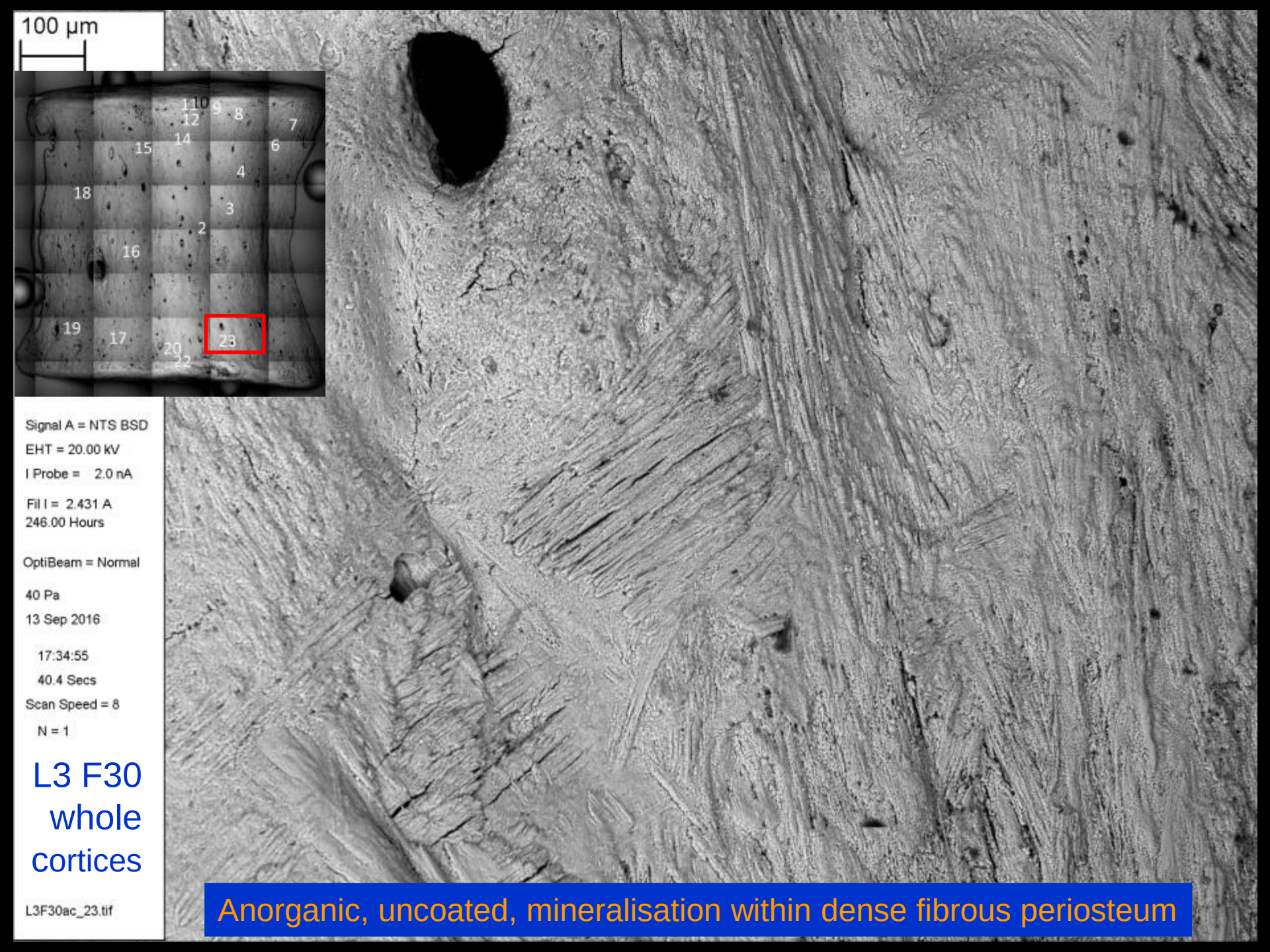

mirrored
M53 L4 secb 018 gif
53M L4
‘b’ section deep to block face

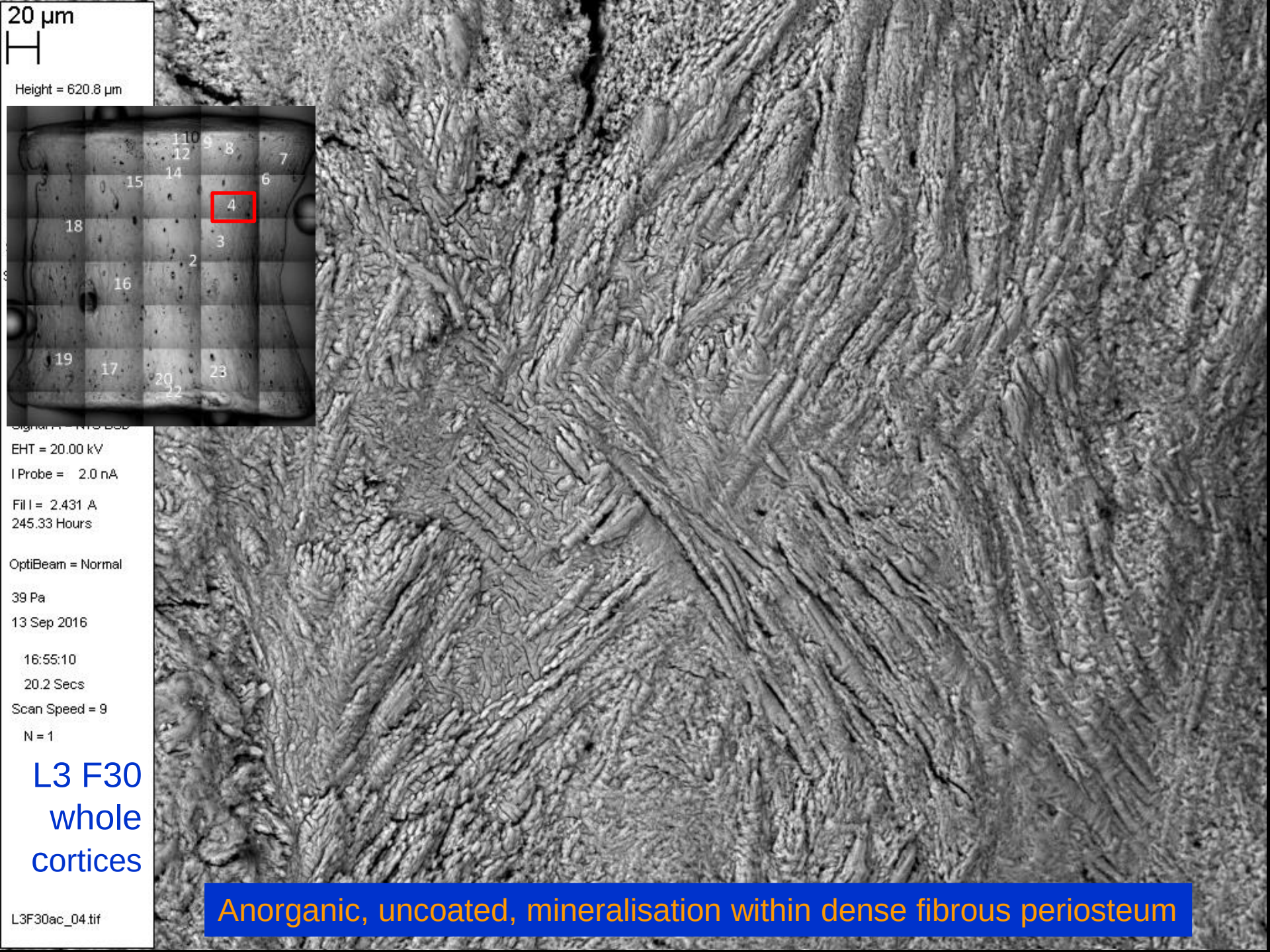

mirrored
‘b’ section deep to block face
M53 L4 secb 017 gif
53M L4

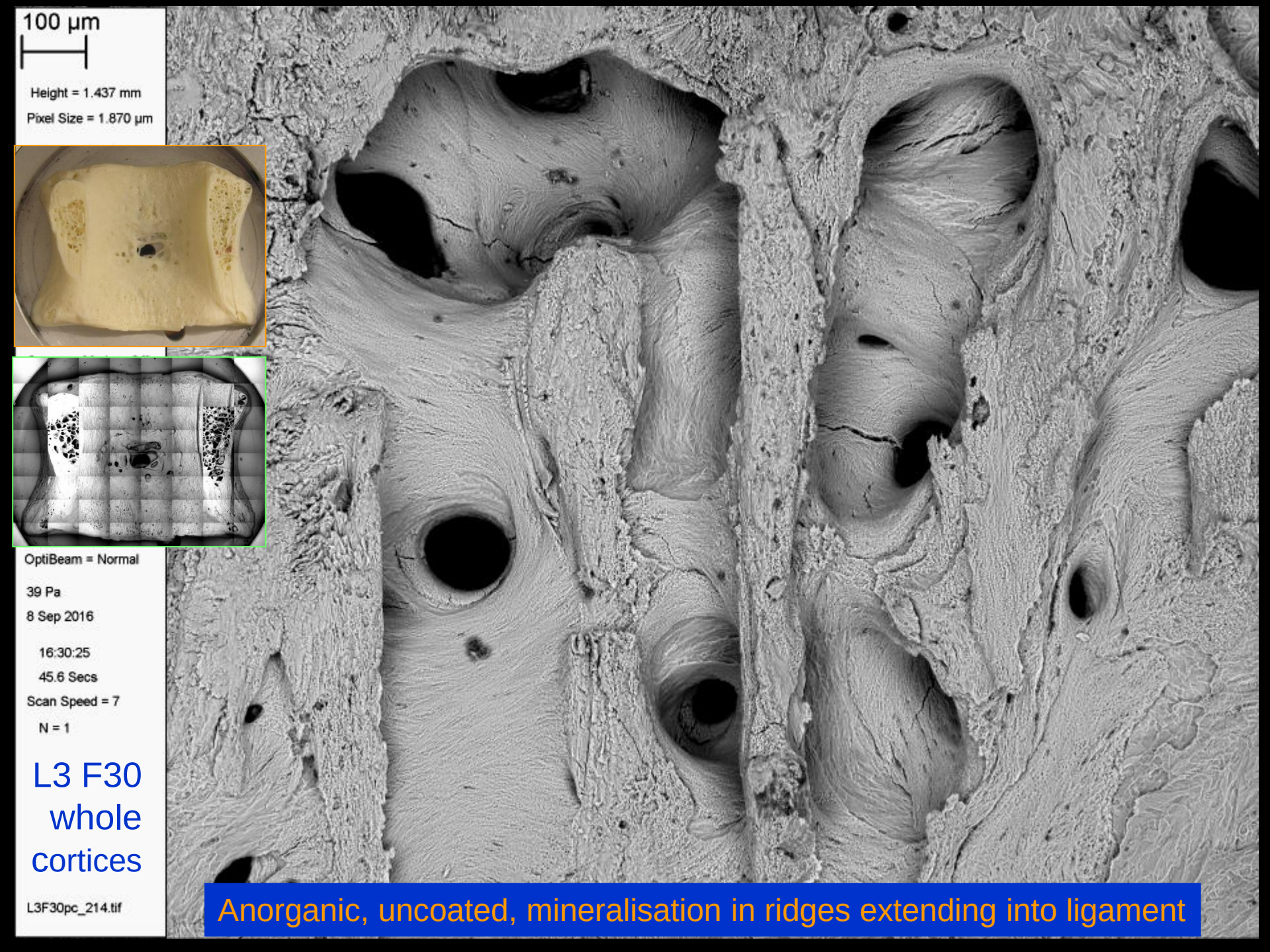

GIF
53M L4
M53 L4 section ‘a’ is the original block face
SEM of LM section
LM MacNeal tetrachrome stain
PLM

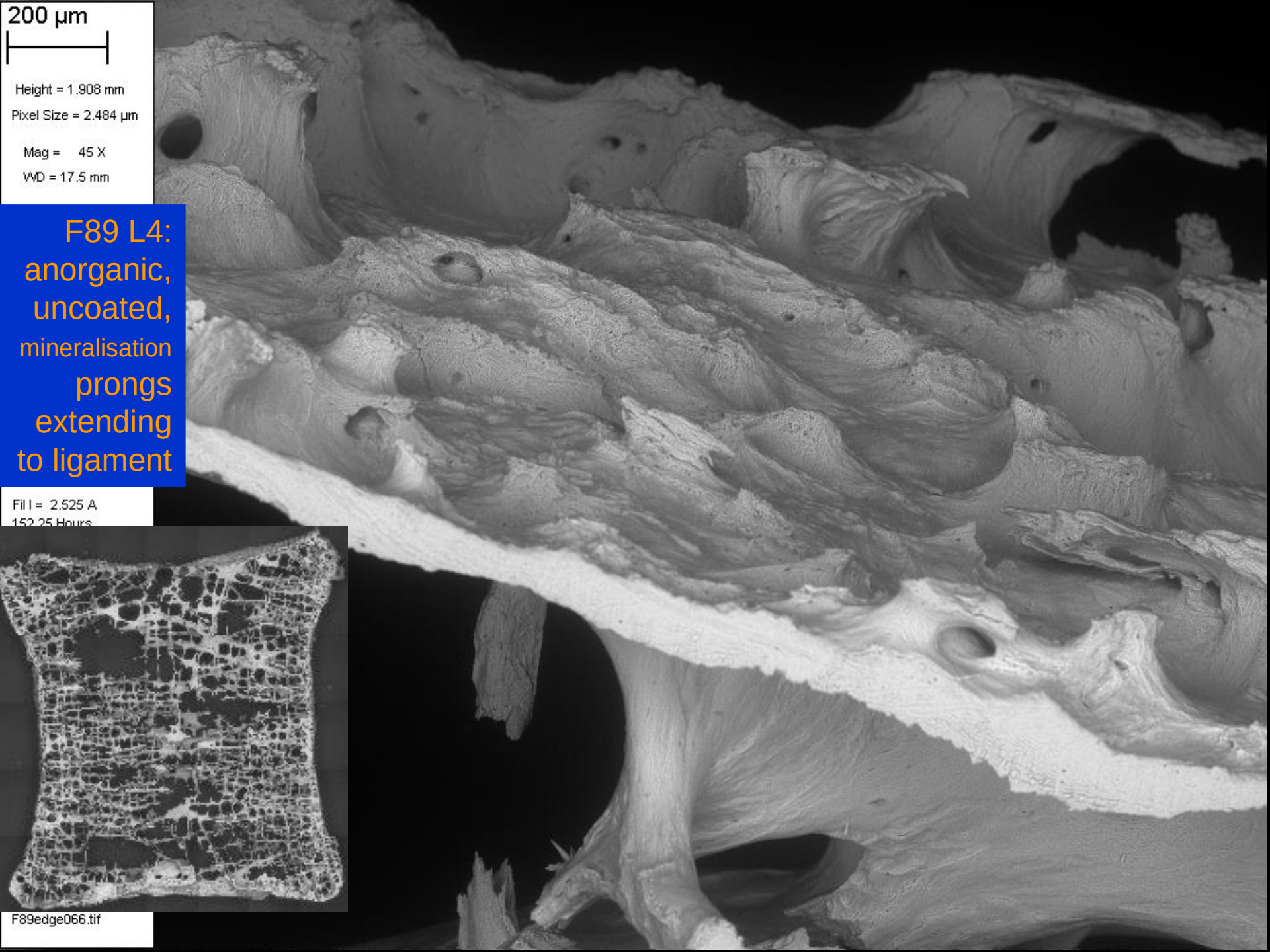

100 μm
Height = 1.083 mm
Pixel Size = 1.410 μm
Mag = 79 X
WD = 8.0 mm
Stage at X = 39.334 mm
Stage at Y = 76.087 mm
Stage at Z = 32.006 mm
Stage at R = 0.0 °
Stage at T = 0.0 °
Compuc. Mode = Off
Scan Rotation = 180.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 1.0 nA
Fil I = 2.431 A
260.07 Hours
OptiBeam = Normal
49 Pa
23 Nov 2016
14:06:06
40.4 Secs
Scan Speed = 8
m53L4secb009.tif
'b' section deep to block face
mirrored
M53 L4 sec b 009

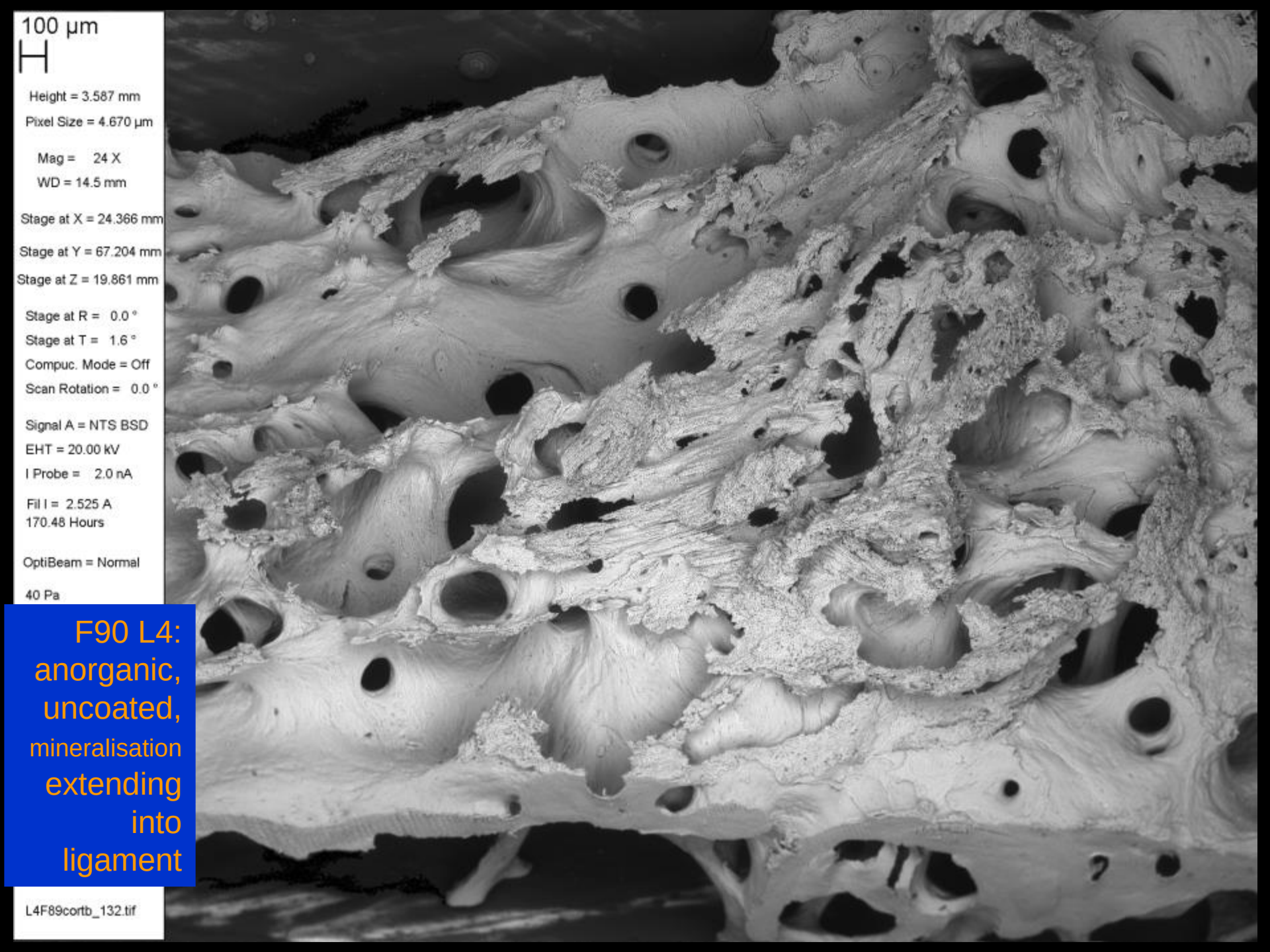

20 µm
Height = 642.6 µm
Pixel Size = 836.7 nm
Mag = 133 X
WD = 20.0 mm
Now for macerated anorganic samples, but stIll BSE-SEM, Uncoated 50Pa vacuum
29 Pa
26 Jul 2016
14:32:21
20.2 Secs
Scan Speed = 9
N = 1
284/92
heavy gold and carbon coating
Wall to wall resorption, outside of cortex

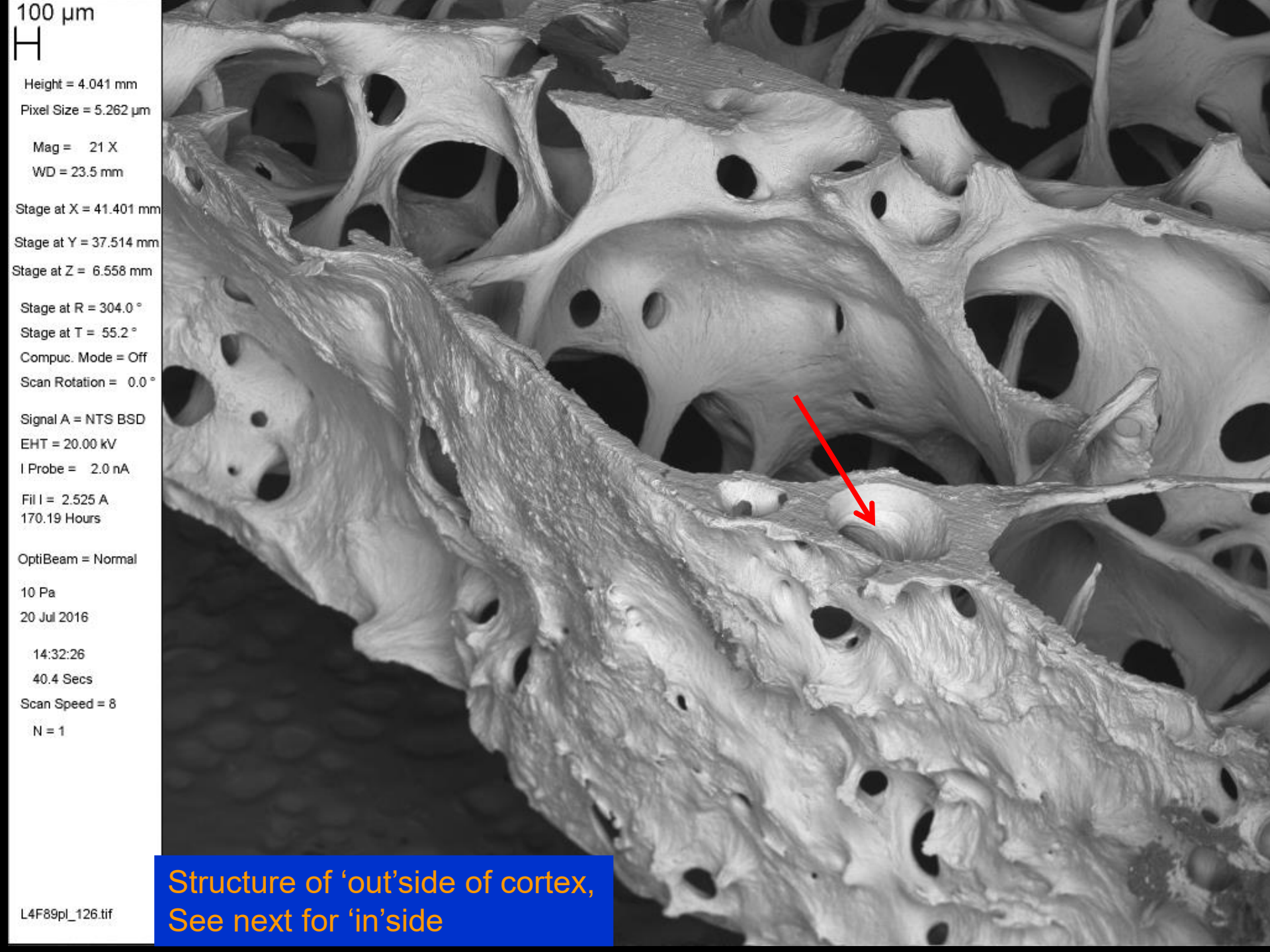

100 µm
11
10
9
8
12
7
14
15
6
4
18
3
2
16
19
17
20
23
22
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 2.0 nA
Fil I = 2.431 A
246.00 Hours
OptiBeam = Normal
40 Pa
13 Sep 2016
17:34:55
40.4 Secs
Scan Speed = 8
N = 1
L3 F30 whole cortices
L3F30ac_23.tif
Anorganic, uncoated, mineralisation within dense fibrous periosteum

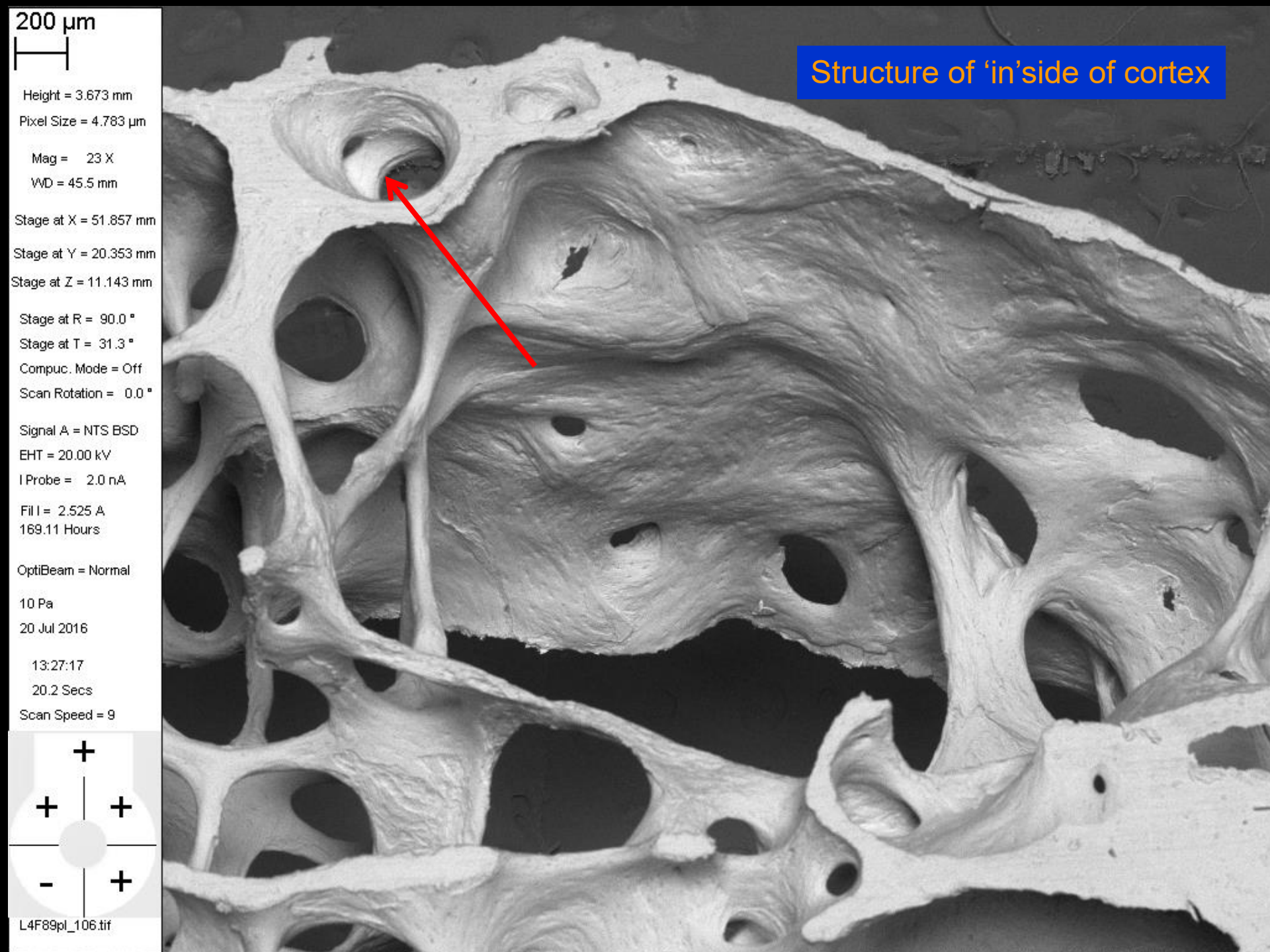

20 µm
Height = 620.8 µm
EHT = 20.00 kV
I Probe = 2.0 nA
Fil I = 2.431 A
245.33 Hours
OptiBeam = Normal
39 Pa
13 Sep 2016
16:55:10
20.2 Secs
Scan Speed = 9
N = 1
L3 F30 whole cortices
L3F30ac_04.tif
Anorganic, uncoated, mineralisation within dense fibrous periosteum

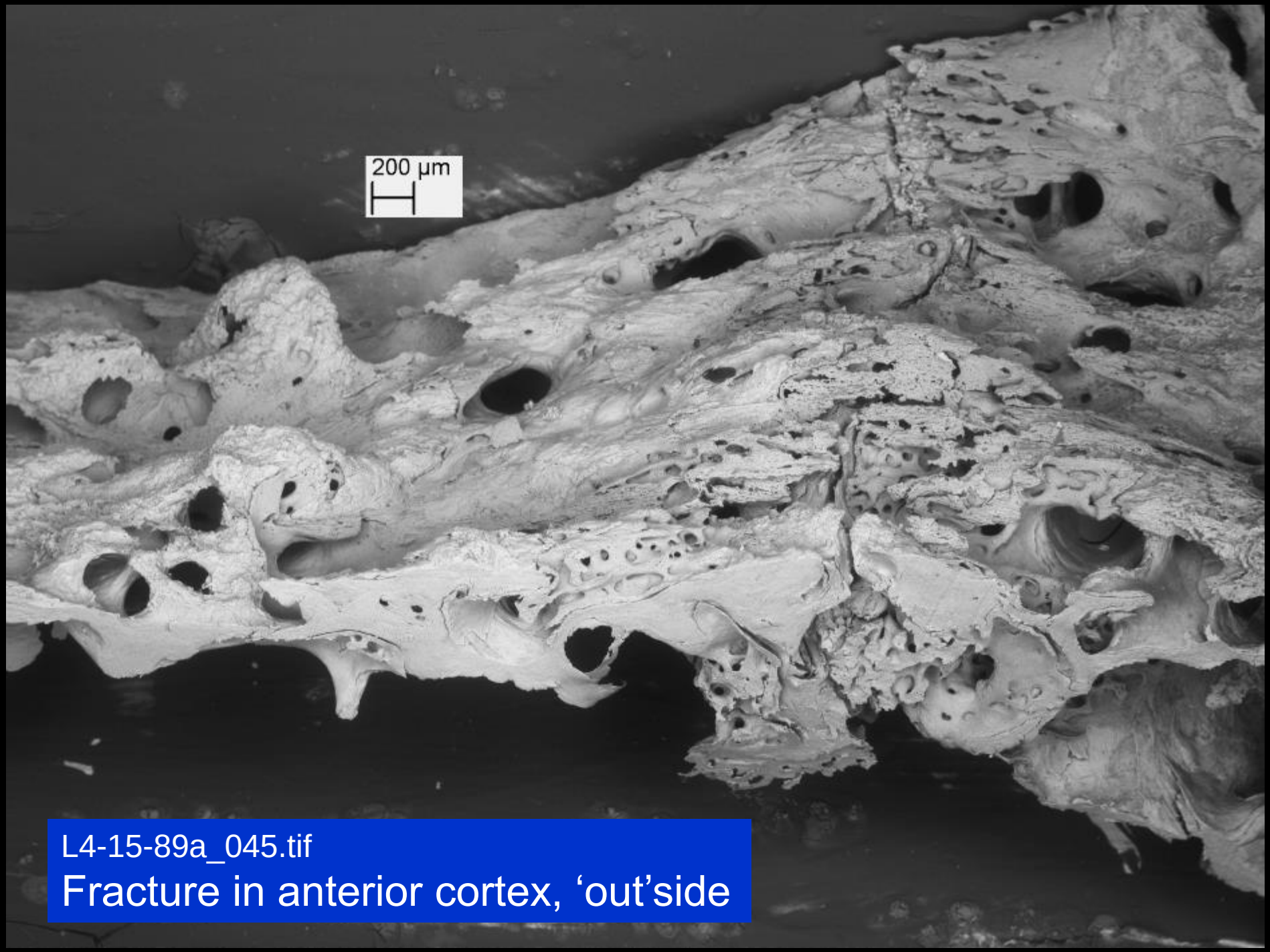

100 µm
Height = 1.437 mm
Pixel Size = 1.870 µm
OptiBeam = Normal
39 Pa
8 Sep 2016
16:30:25
45.6 Secs
Scan Speed = 7
N = 1
L3 F30 whole cortices
L3F30pc_214.tif
Anorganic, uncoated, mineralisation in ridges extending into ligament


200 µm
Height = 1.908 mm
Pixel Size = 2.484 µm
Mag = 45 X
WD = 17.5 mm
F89 L4: anorganic, uncoated, mineralisation prongs extending to ligament
Fil l = 2.525 A
F89edge066.tif


100 µm
Height = 3.587 mm
Pixel Size = 4.670 µm
Mag = 24 X
WD = 14.5 mm
Stage at X = 24.366 mm
Stage at Y = 67.204 mm
Stage at Z = 19.861 mm
Stage at R = 0.0 °
Stage at T = 1.6 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 2.0 nA
Fil I = 2.525 A
170.48 Hours
OptiBeam = Normal
40 Pa
F90 L4: anorganic, uncoated, mineralisation extending into ligament
L4F89cortb_132.tif

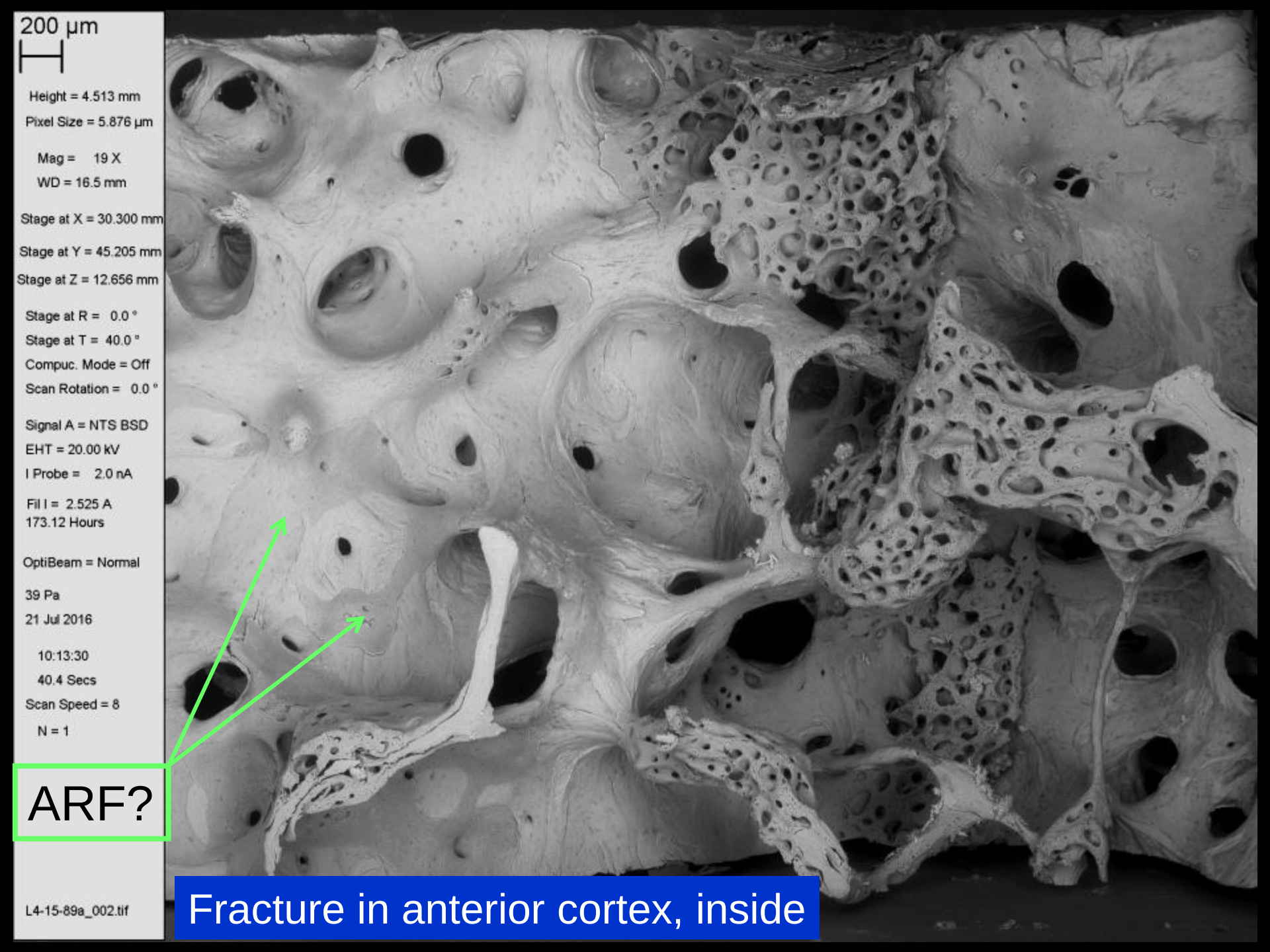

100 µm
H
Height = 4.041 mm
Pixel Size = 5.262 µm
Mag = 21 X
WD = 23.5 mm
Stage at X = 41.401 mm
Stage at Y = 37.514 mm
Stage at Z = 6.558 mm
Stage at R = 304.0 °
Stage at T = 55.2 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 2.0 nA
Fil I = 2.525 A
170.19 Hours
OptiBeam = Normal
10 Pa
20 Jul 2016
14:32:26
40.4 Secs
Scan Speed = 8
N = 1
L4F89pl_126.tif
Structure of ‘out’side of cortex,
See next for ‘in’side

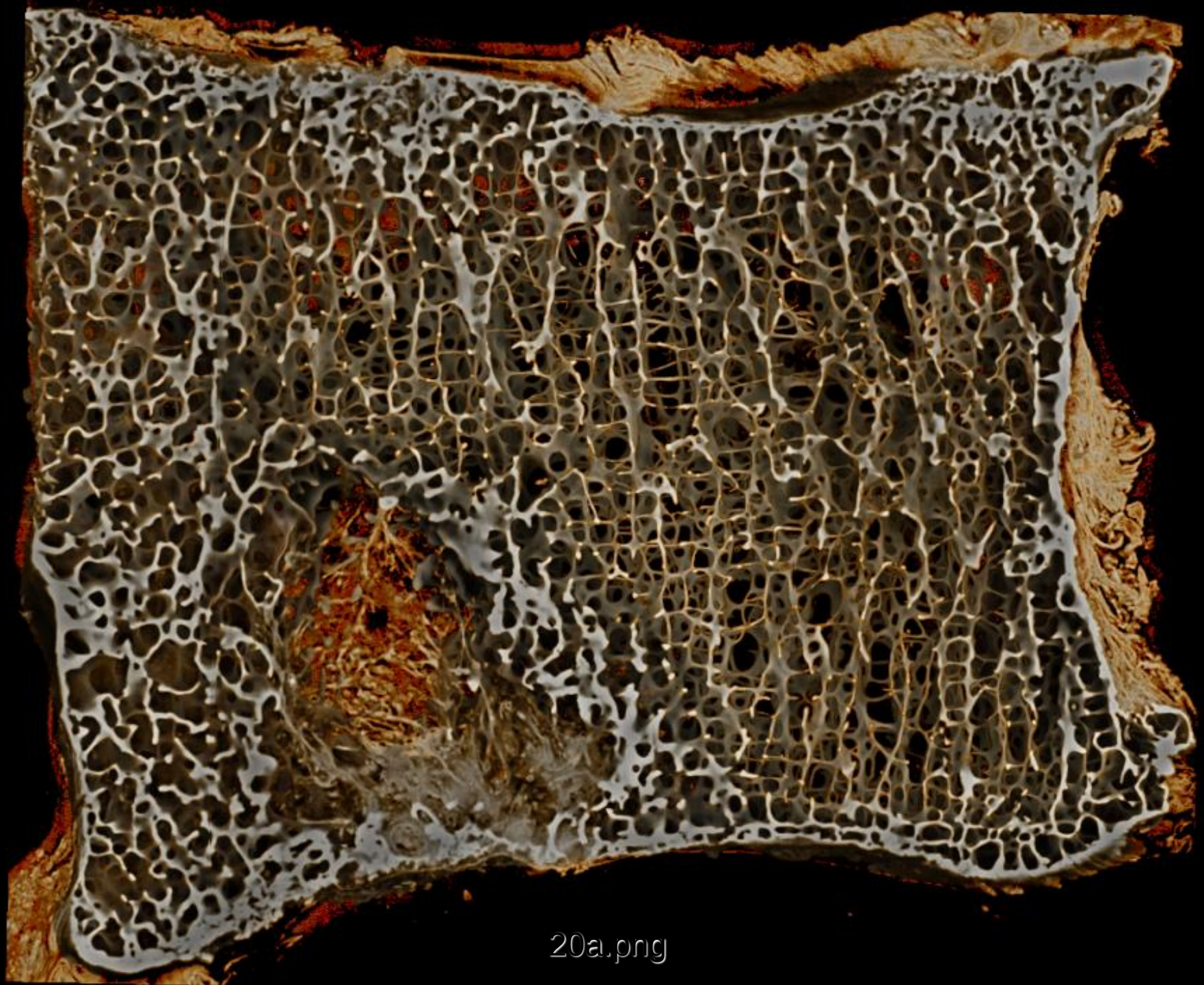

200 µm
Height = 3.673 mm
Pixel Size = 4.783 µm
Mag = 23 X
WD = 45.5 mm
Stage at X = 51.857 mm
Stage at Y = 20.353 mm
Stage at Z = 11.143 mm
Stage at R = 90.0 °
Stage at T = 31.3 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 2.0 nA
Fil I = 2.525 A
169.11 Hours
OptiBeam = Normal
10 Pa
20 Jul 2016
13:27:17
20.2 Secs
Scan Speed = 9
L4F89pl_106.tif
Structure of ‘in’side of cortex



L4-15-89a_045.tif

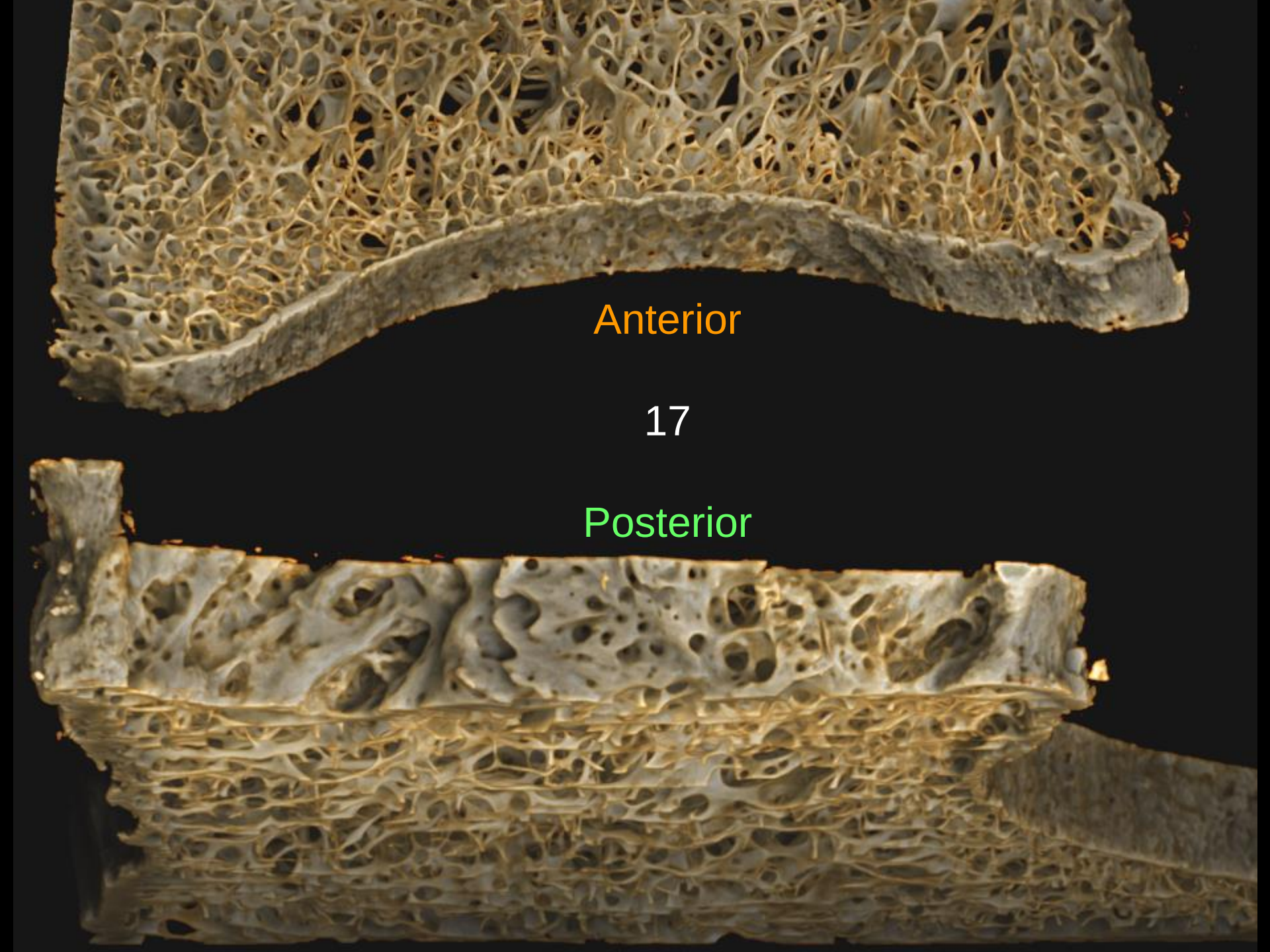
Fracture in anterior cortex, ‘out’side

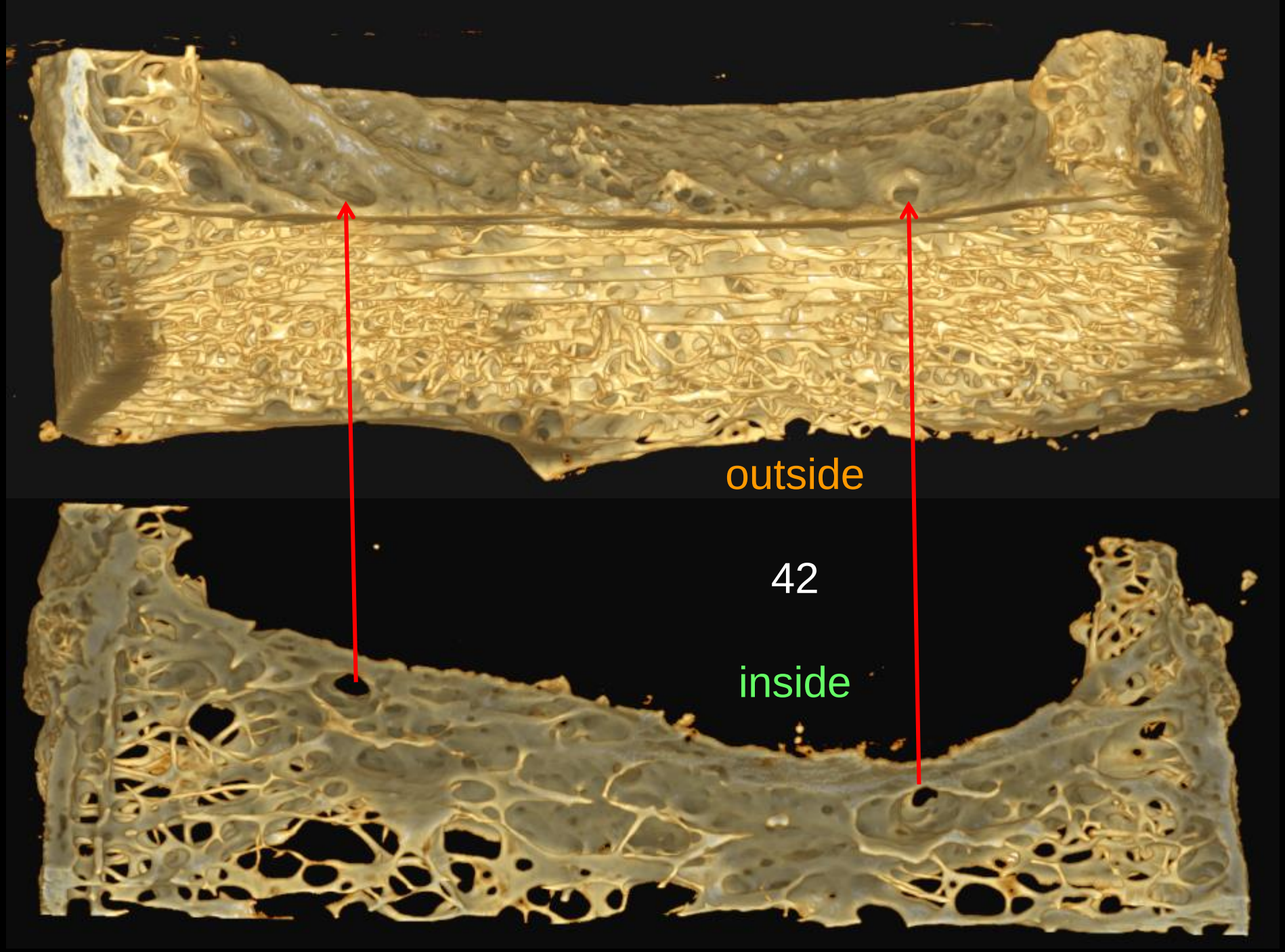

200 µm
Height = 4.513 mm
Pixel Size = 5.876 µm
Mag = 19 X
WD = 16.5 mm
Stage at X = 30.300 mm
Stage at Y = 45.205 mm
Stage at Z = 12.656 mm
Stage at R = 0.0 °
Stage at T = 40.0 °
Compuc. Mode = Off
Scan Rotation = 0.0 °
Signal A = NTS BSD
EHT = 20.00 kV
I Probe = 2.0 nA
Fil I = 2.525 A
173.12 Hours
OptiBeam = Normal
39 Pa
21 Jul 2016
10:13:30
40.4 Secs
Scan Speed = 8
N = 1
ARF?
L4-15-89a_002.tif
Fracture in anterior cortex, inside

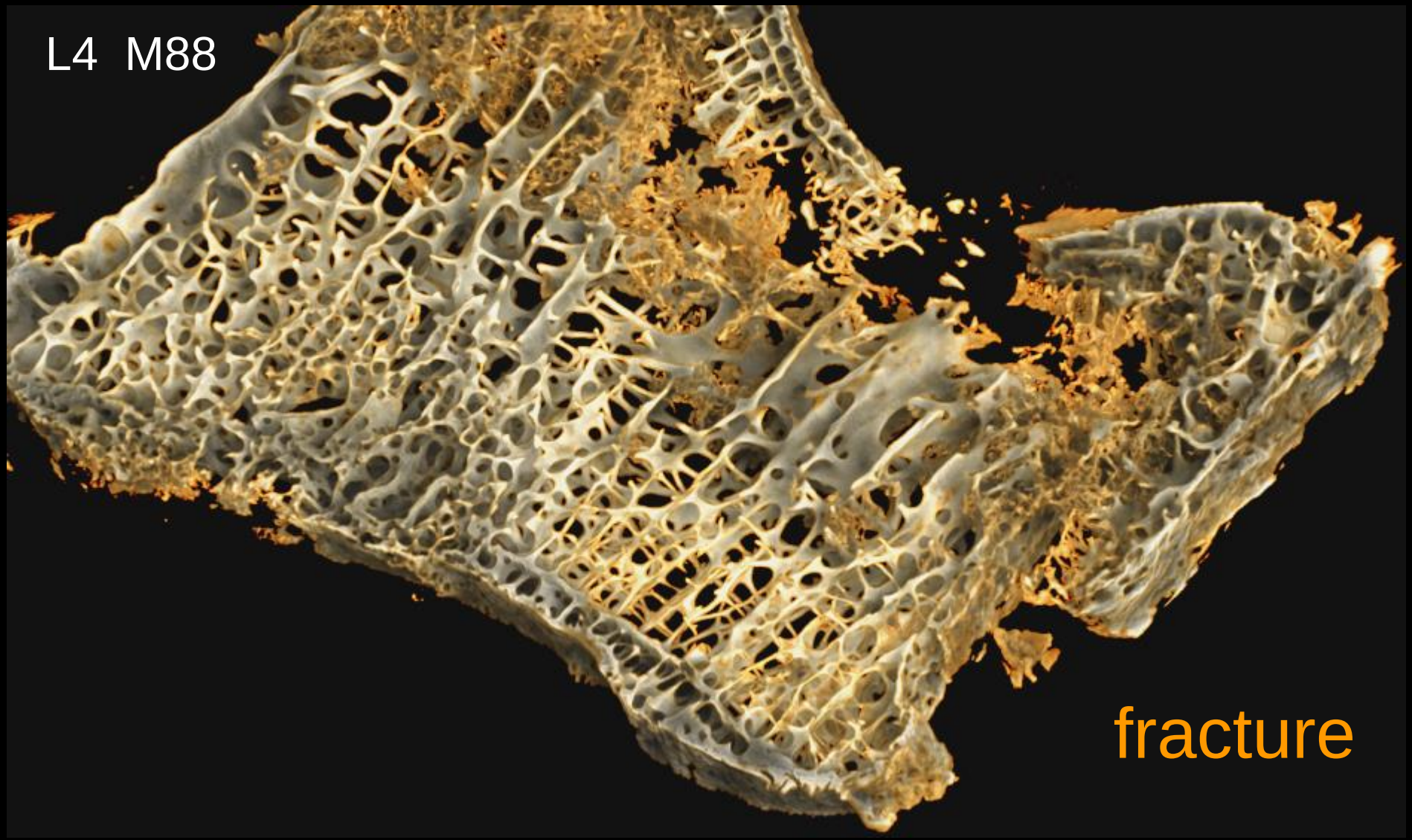


20a.png

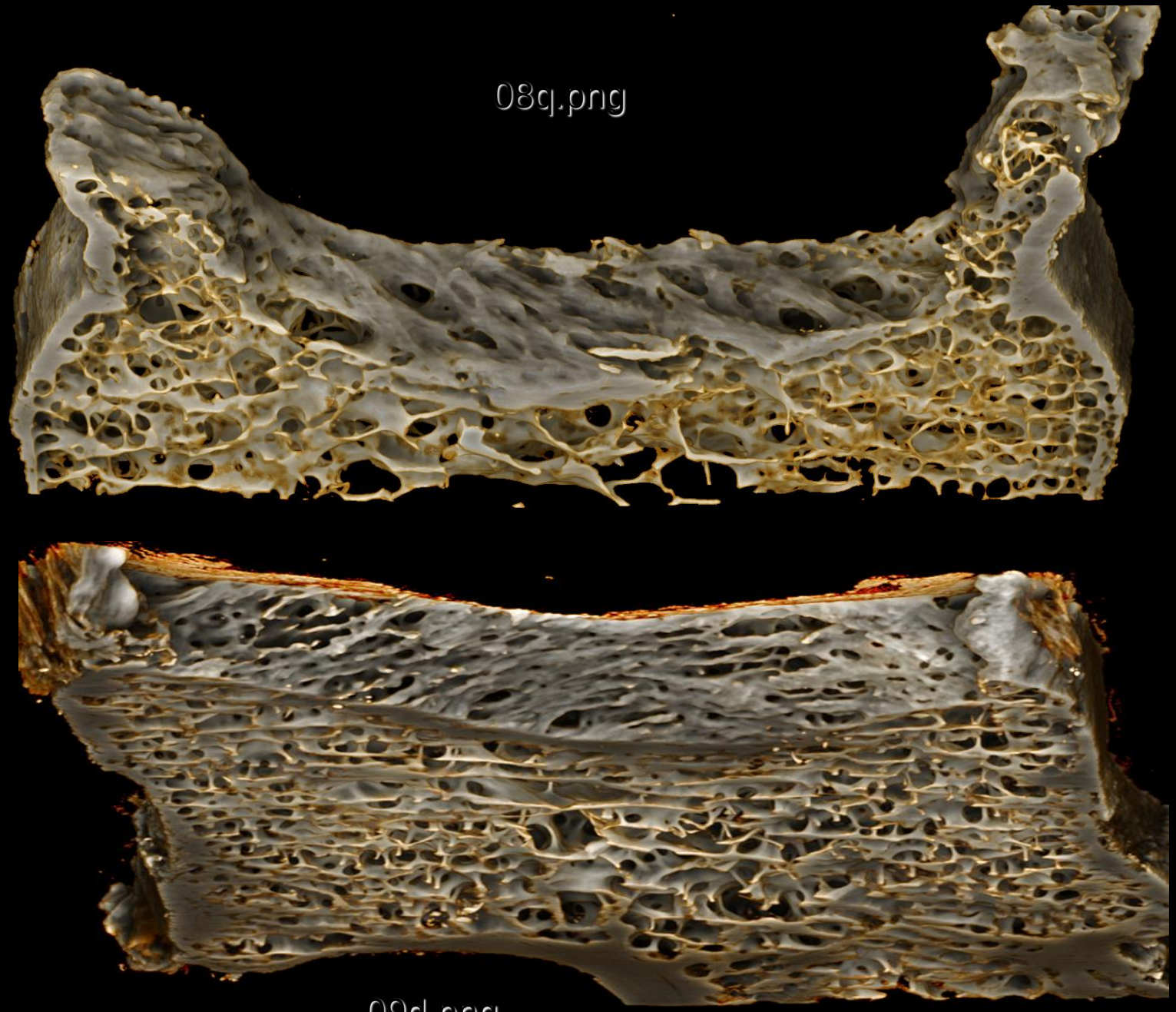

26

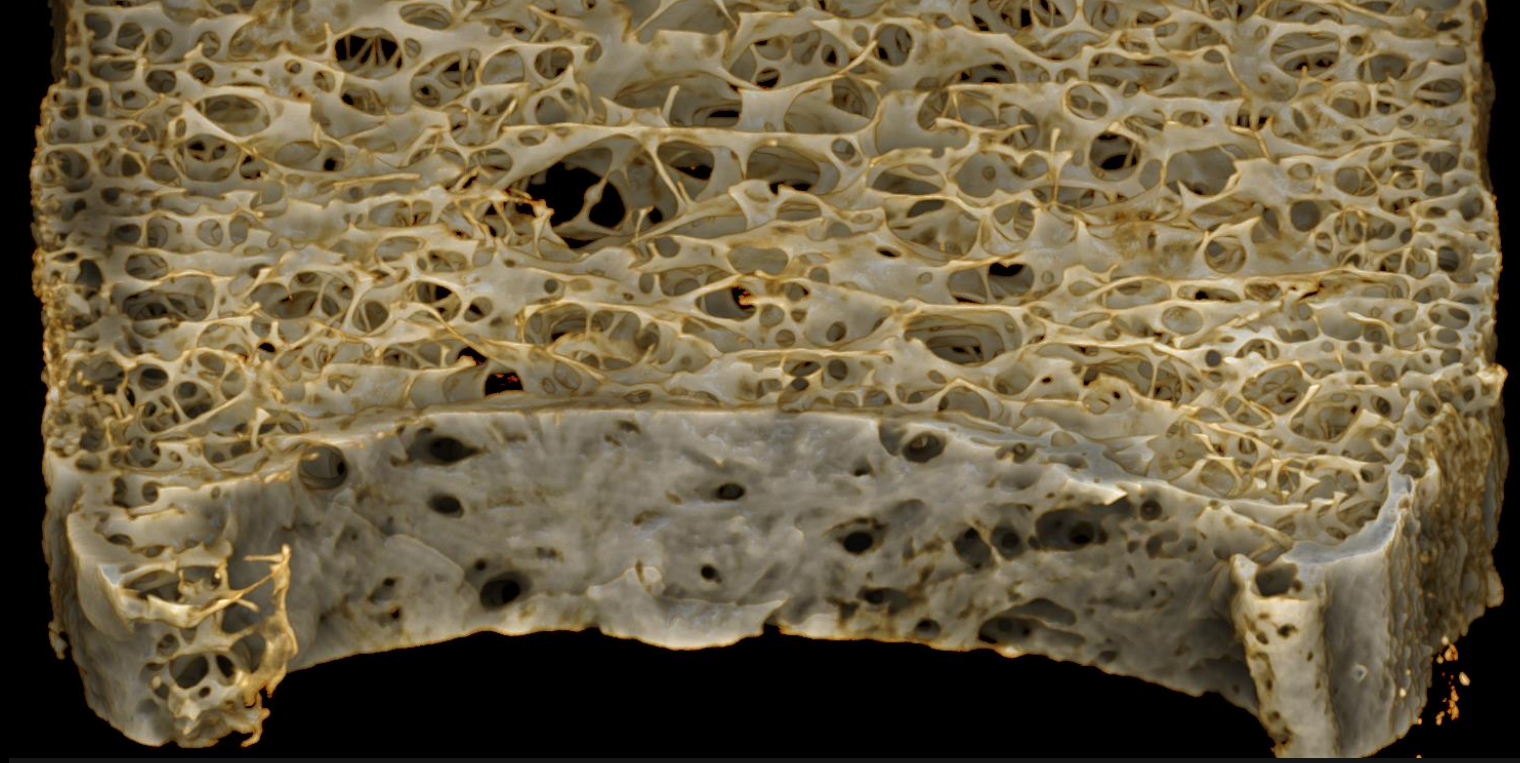

Anterior
17
Posterior


outside
42
inside

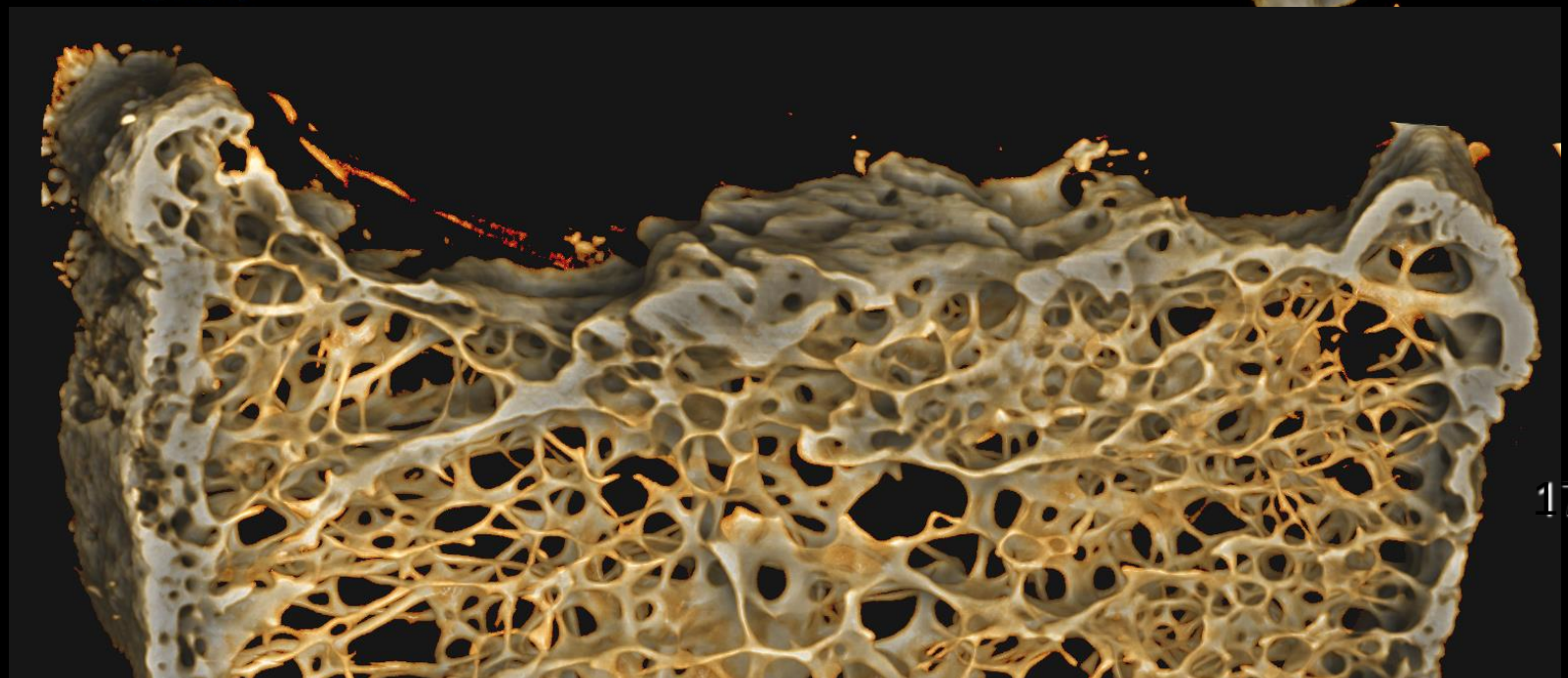


We now have high contrast resolution XMT (uCT) for most of our samples


08q.png
09d.png



13m.png



17j.png

- central compression fractures involve highly calcified cartilage or fibrocartilage of the end-plate
- anterior wedge compression fractures and collapse fractures include cortex, which may be very thin and partially absent, but can be very thick < great range
- Cortex contains highly calcified (1) Sharpey fibre bone, (2) fibrous periosteum and (3) ligament
- The highly calcified non-bone matrices should be factored into thinking about mechanical properties of the whole bone organ and in guesstimating cortical thickness from clinical imaging

Thanks, XMT from David Mills >

Heiko Richter, Rowiak, Hannover

- Bones contain several calcified matrices which are not bone
- These are placed at the ‘out’sides of bone organs
- They are less well organised to prevent fracture
- They are more brittle, and prone to cleave
- Hence failure of vertebral bodies may partly depend on minority, foreign phases
- don’t neglect them