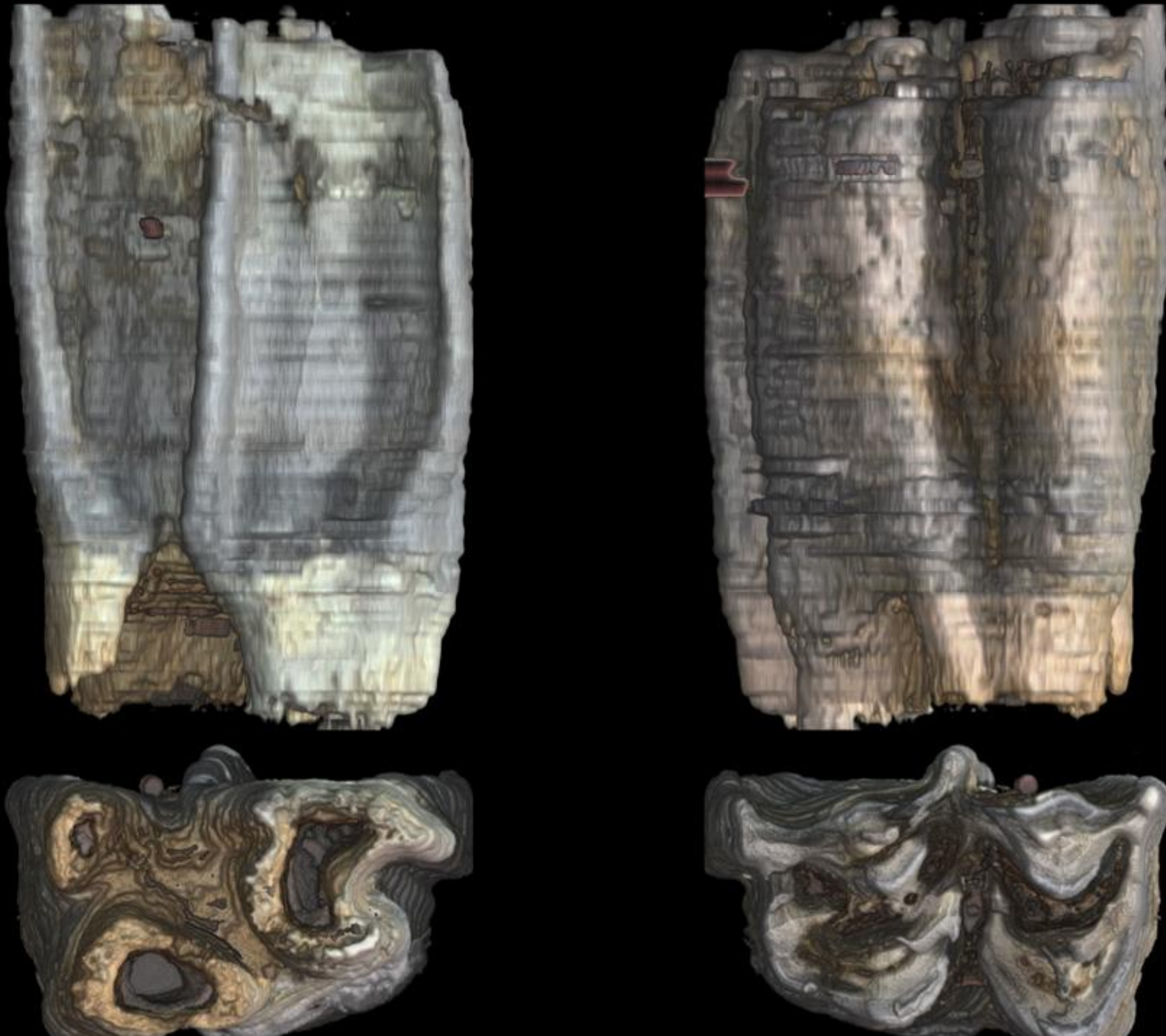
Synchrotron X-ray imaging and palaeontology:

Paul Tafforeau, ESRF

Palaeontologists are using tomography to investigate internal structures of fossils.

It consists into serial slices into the specimen that are then virtually stacked together using a computer.

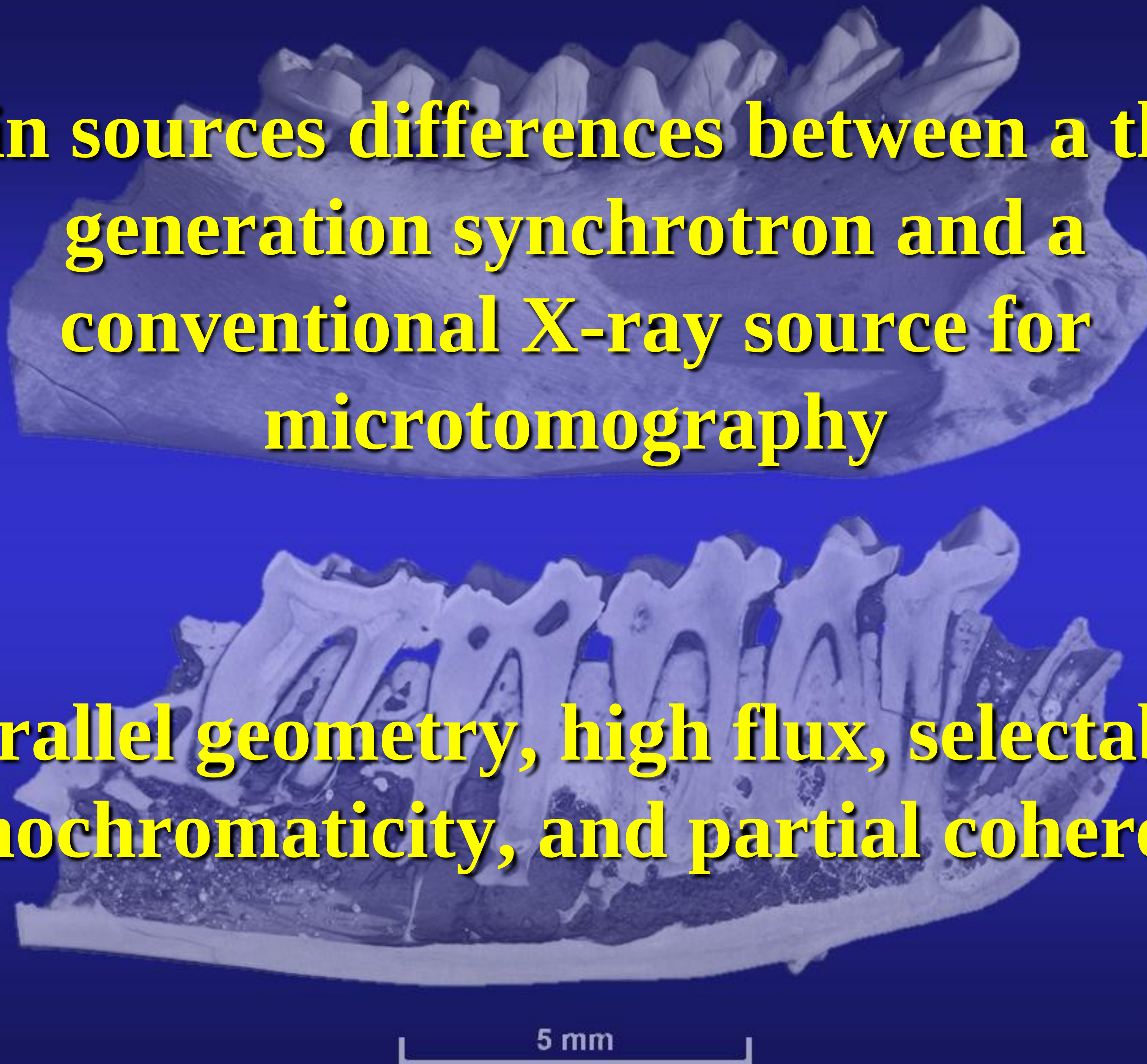


But on important specimens, physical sections are generally unconceivable.

Non-destructiveness is crucial

X-ray microtomography (especially using synchrotrons) is a much better solution

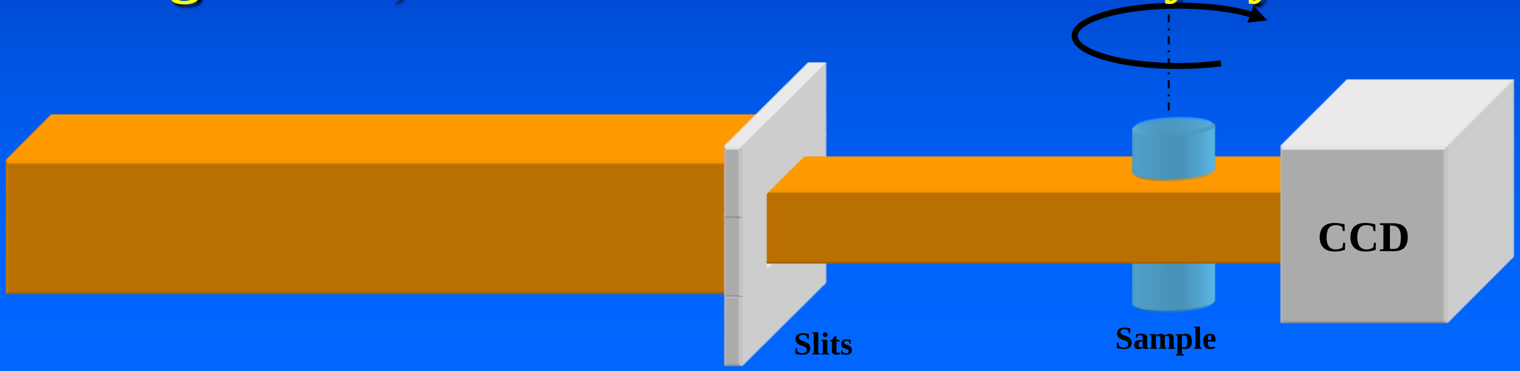
Main sources differences between a third generation synchrotron and a conventional X-ray source for microtomography



parallel geometry, high flux, selectable monochromaticity, and partial coherence

Beam geometry

Parallel geometry (synchrotron source) exact reconstruction algorithm, the resolution is limited only by the detector



Conical geometry (laboratory source) approximate reconstruction algorithm, the resolution depends on the detector and of the source properties (size, divergence)

Modified from P. Bleuet

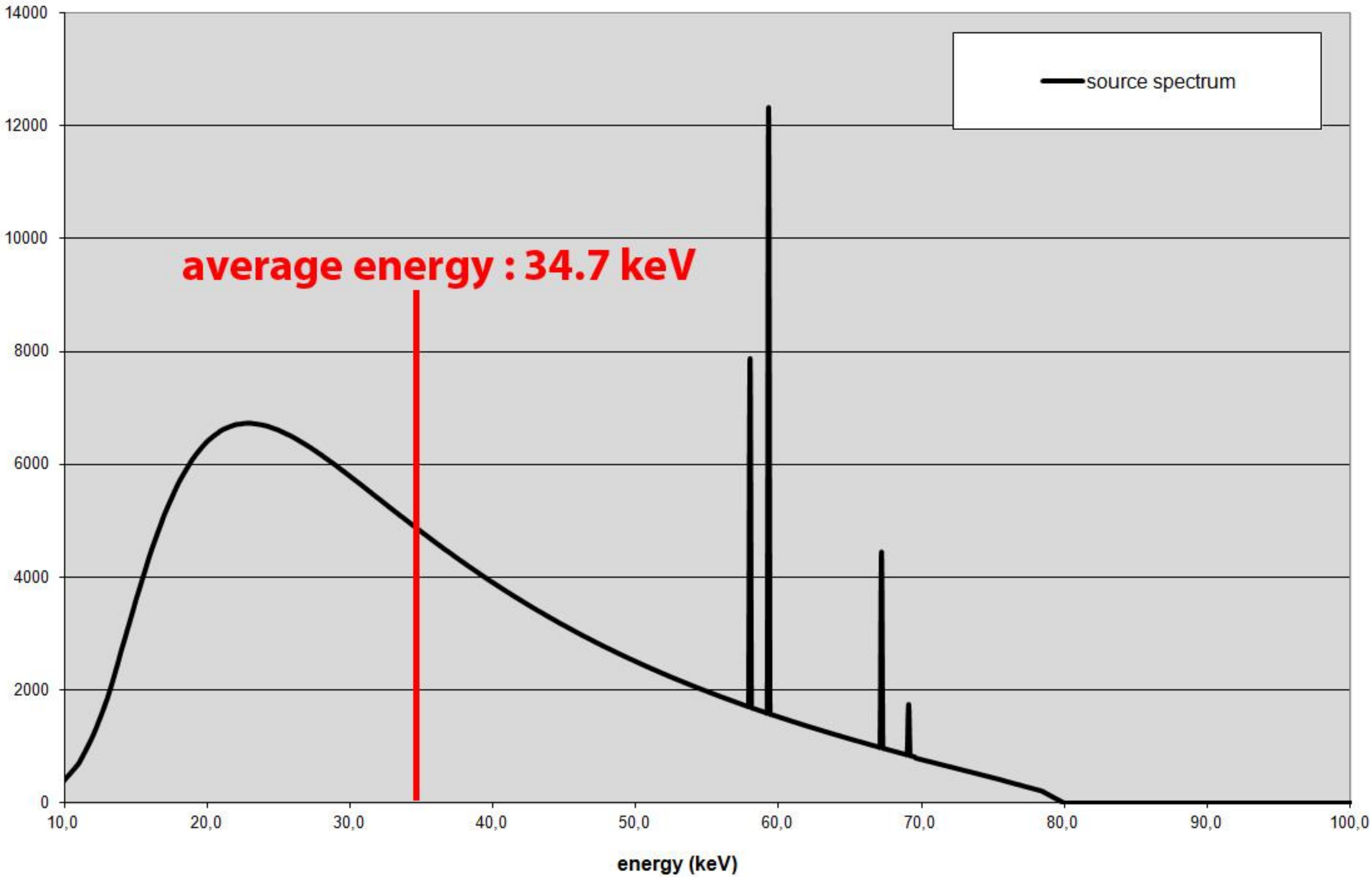
Monochromaticity

- Beam hardening is a differential absorption of the X-ray spectrum by the sample, the low energies being more absorbed than the high ones.

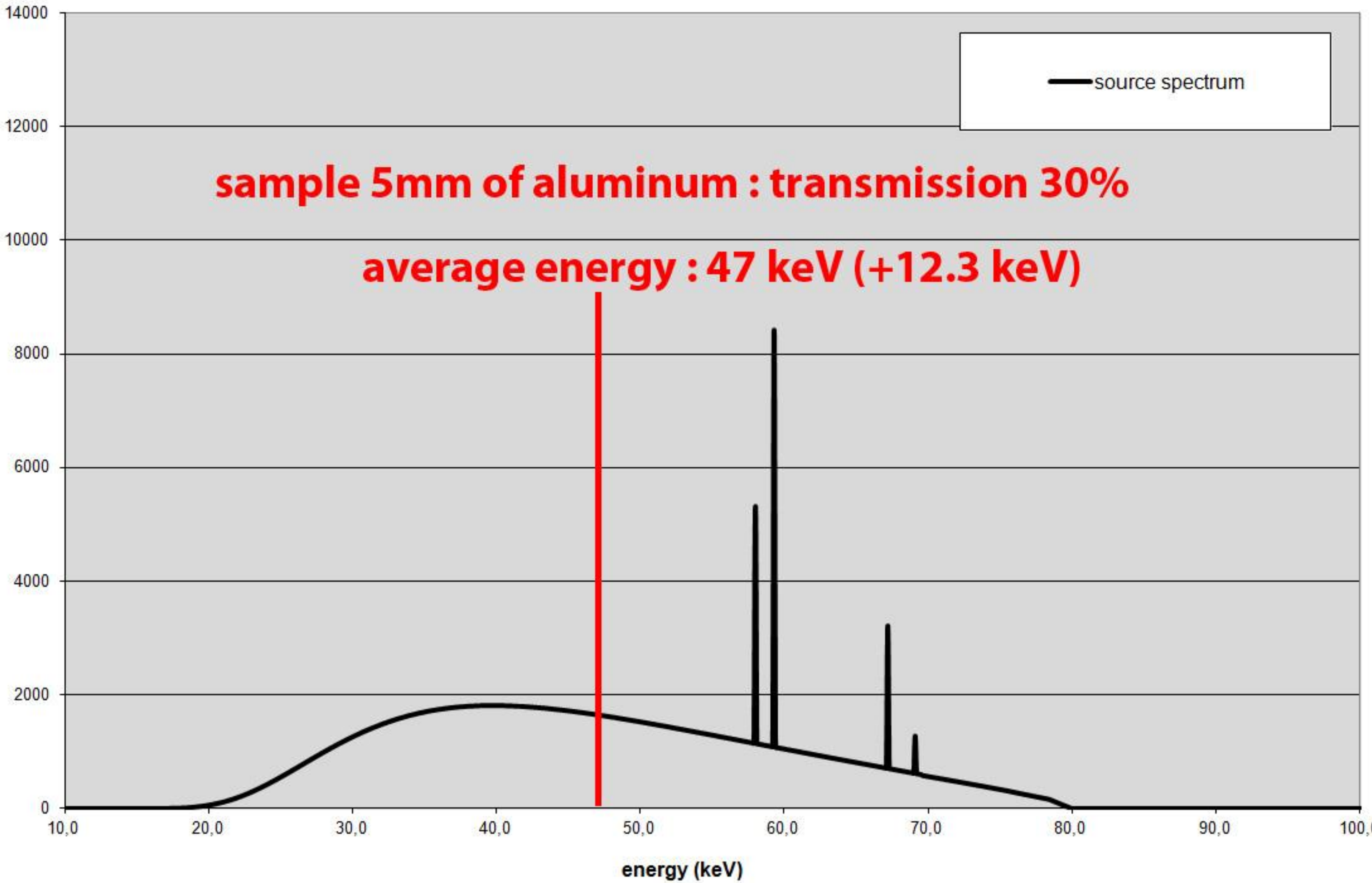
- It leads to misleading reconstructed data with typical brightening of the sample borders and linking of dense structures.

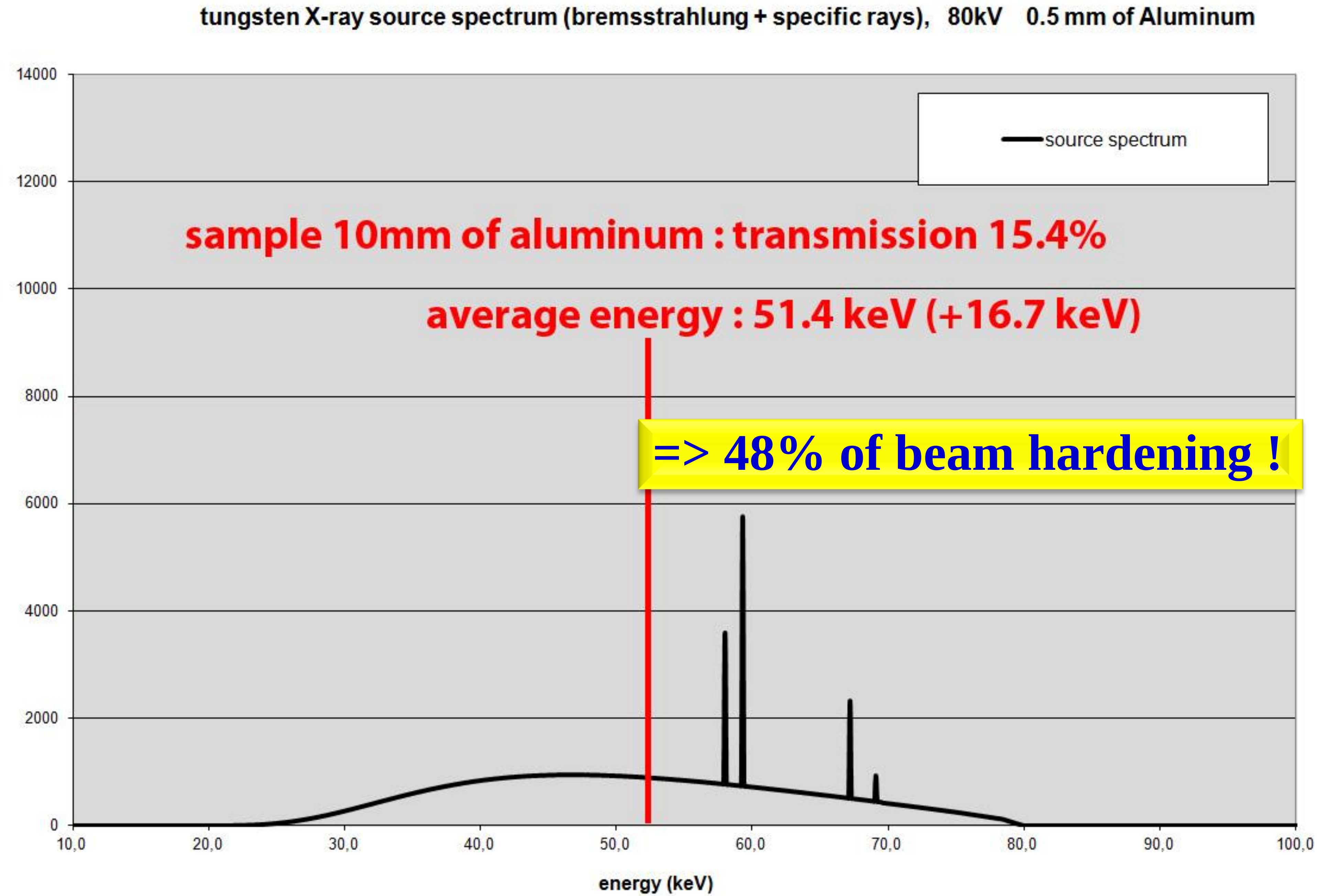
- It is due to wide X-ray spectrum and represents one of the very important drawback of conventional CT.

tungsten X-ray source spectrum (bremsstrahlung + specific rays), 80kV 0.5 mm of Aluminum



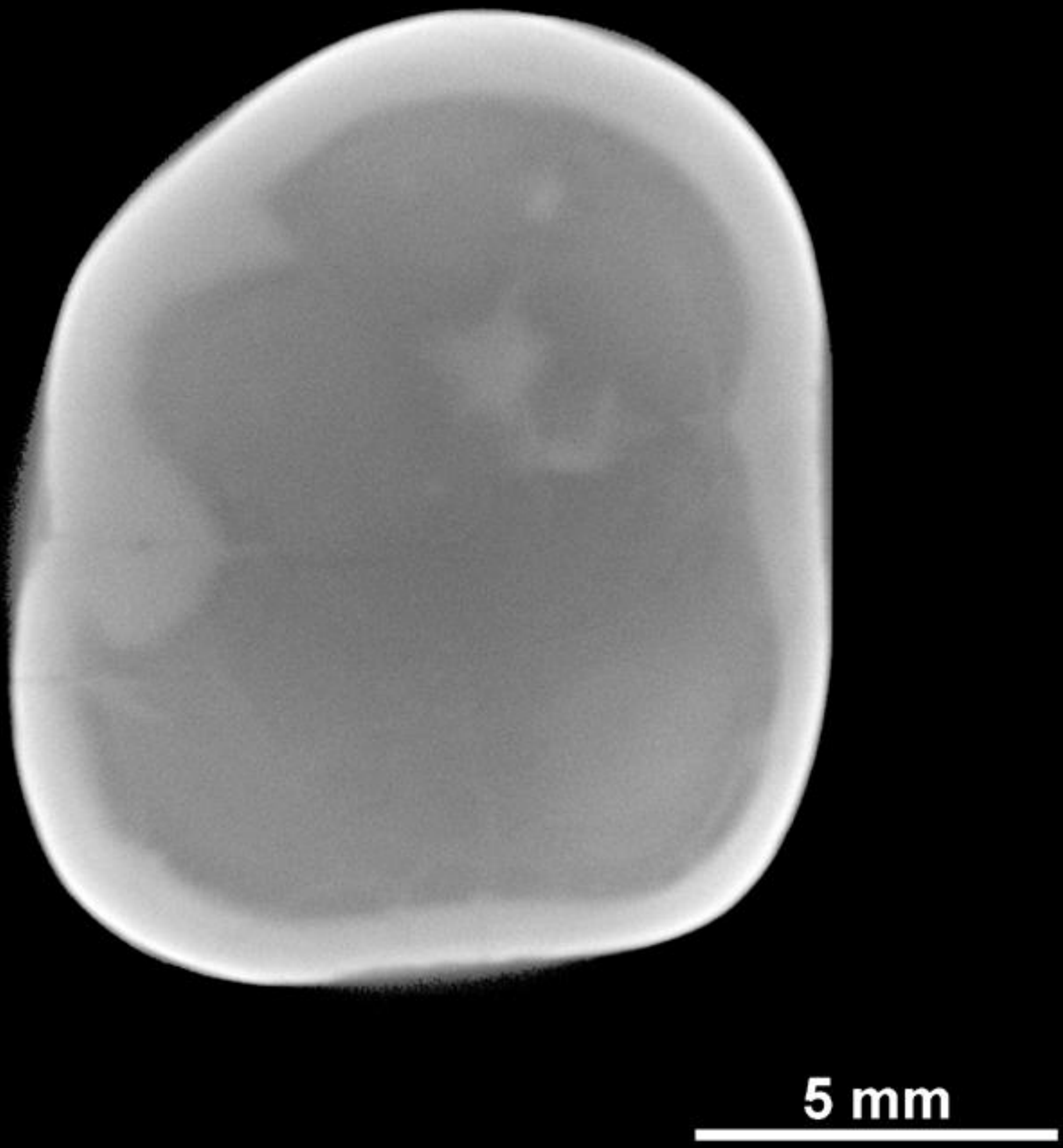
tungsten X-ray source spectrum (bremsstrahlung + specific rays), 80kV 0.5 mm of Aluminum



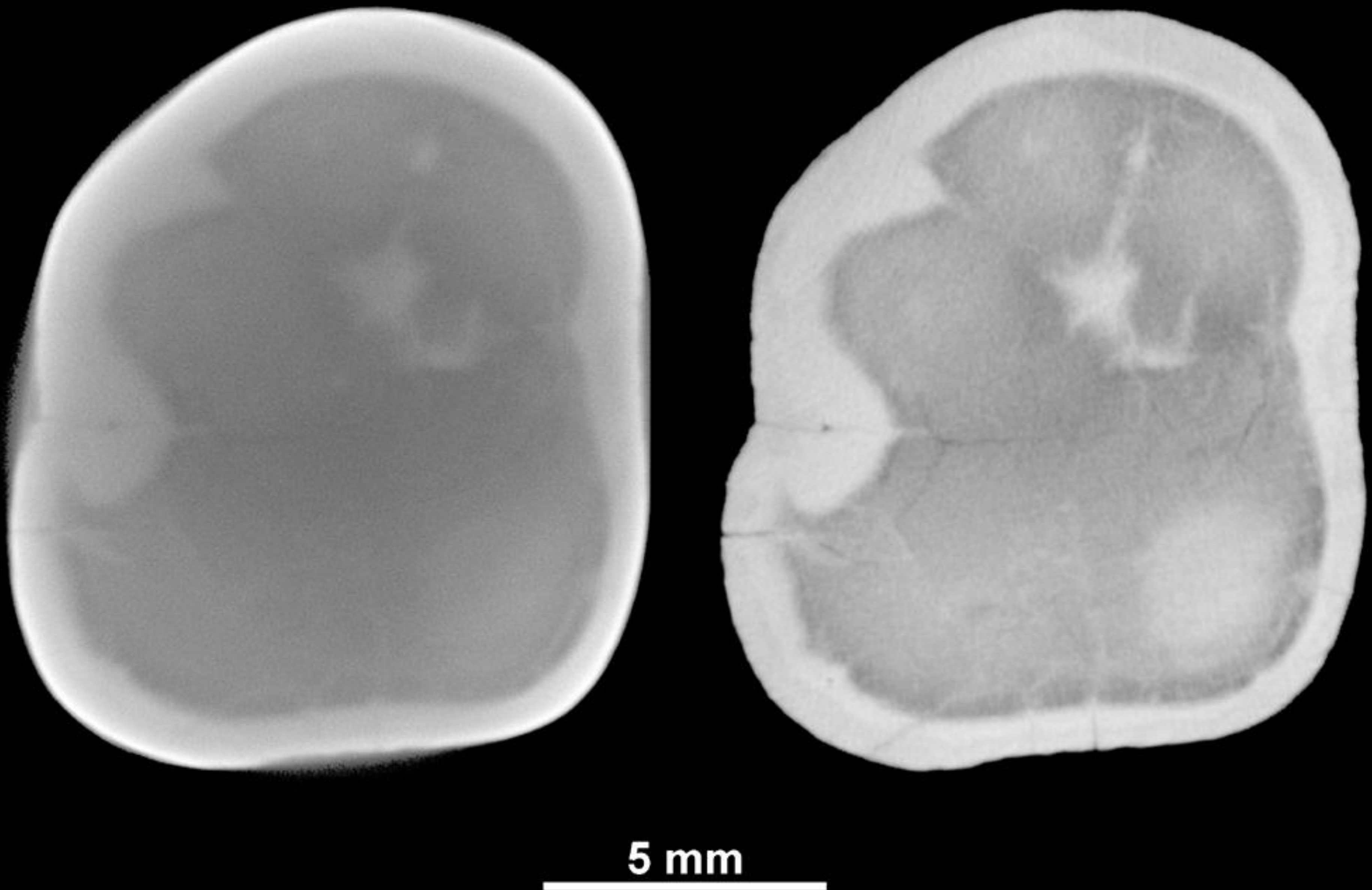


tungsten X-ray source spectrum (bremsstrahlung + specific rays), 80kV 0.5 mm of Aluminum
14000
12000
10000
8000
6000
4000
2000
0
10,0
20,0
30,0
40,0
50,0
60,0
70,0
80,0
90,0
100,0
energy (keV)
source spectrum
sample 10mm of aluminum : transmission 15.4%
average energy : 51.4 keV (+16.7 keV)
=> 48% of beam hardening !

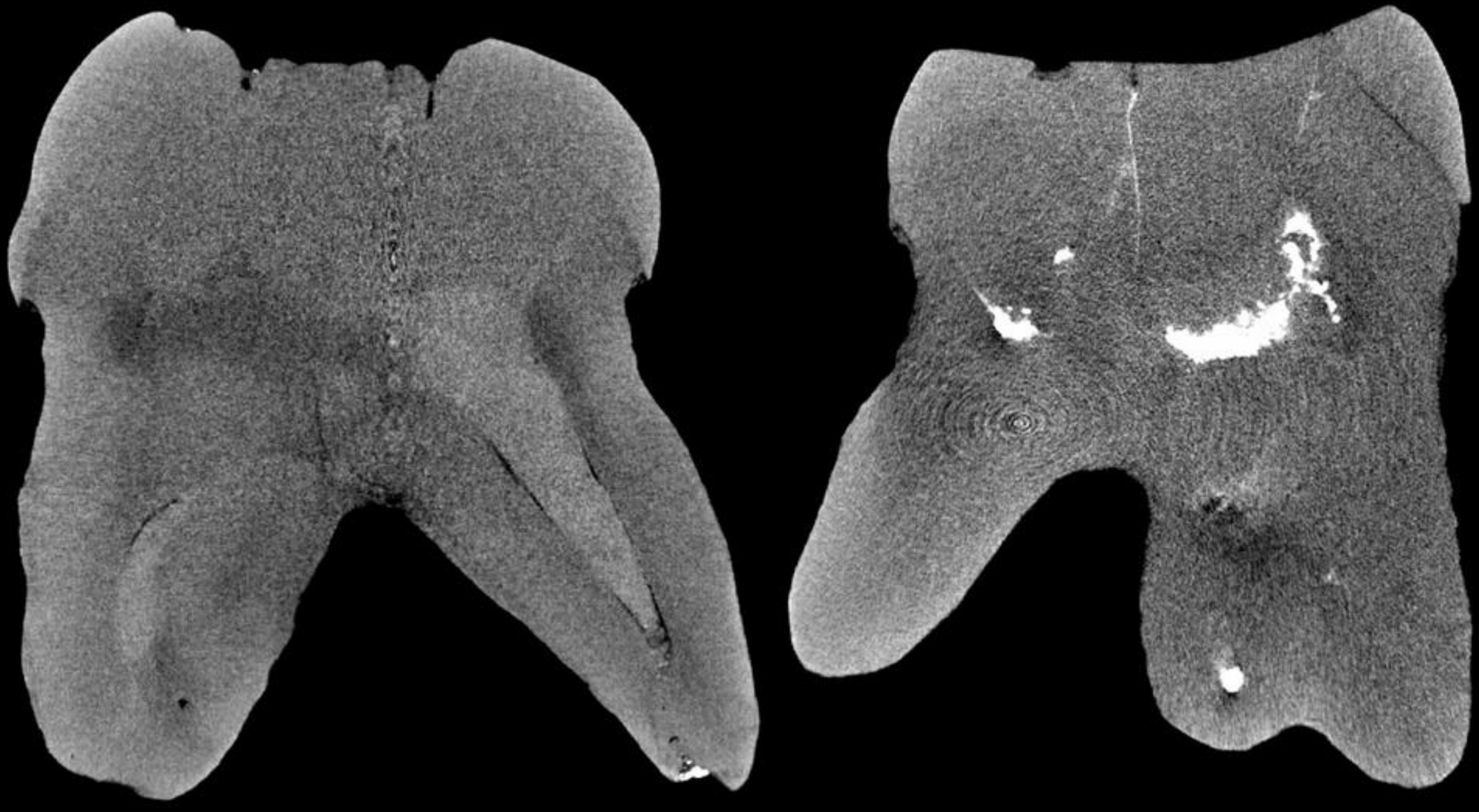
slice obtained on conventional X-ray microtomograph without any filtering or algorithmic correction: Strong beam hardening



Same slice obtained on the ESRF ID19 beamline using monochromatic beam, free of beam hardening.



conventional microtomograph used to image the Trinil molars, putative *Homo erectus* remains



Smith et al. 2009

Synchrotron imaging with monochromatic beam on ID19

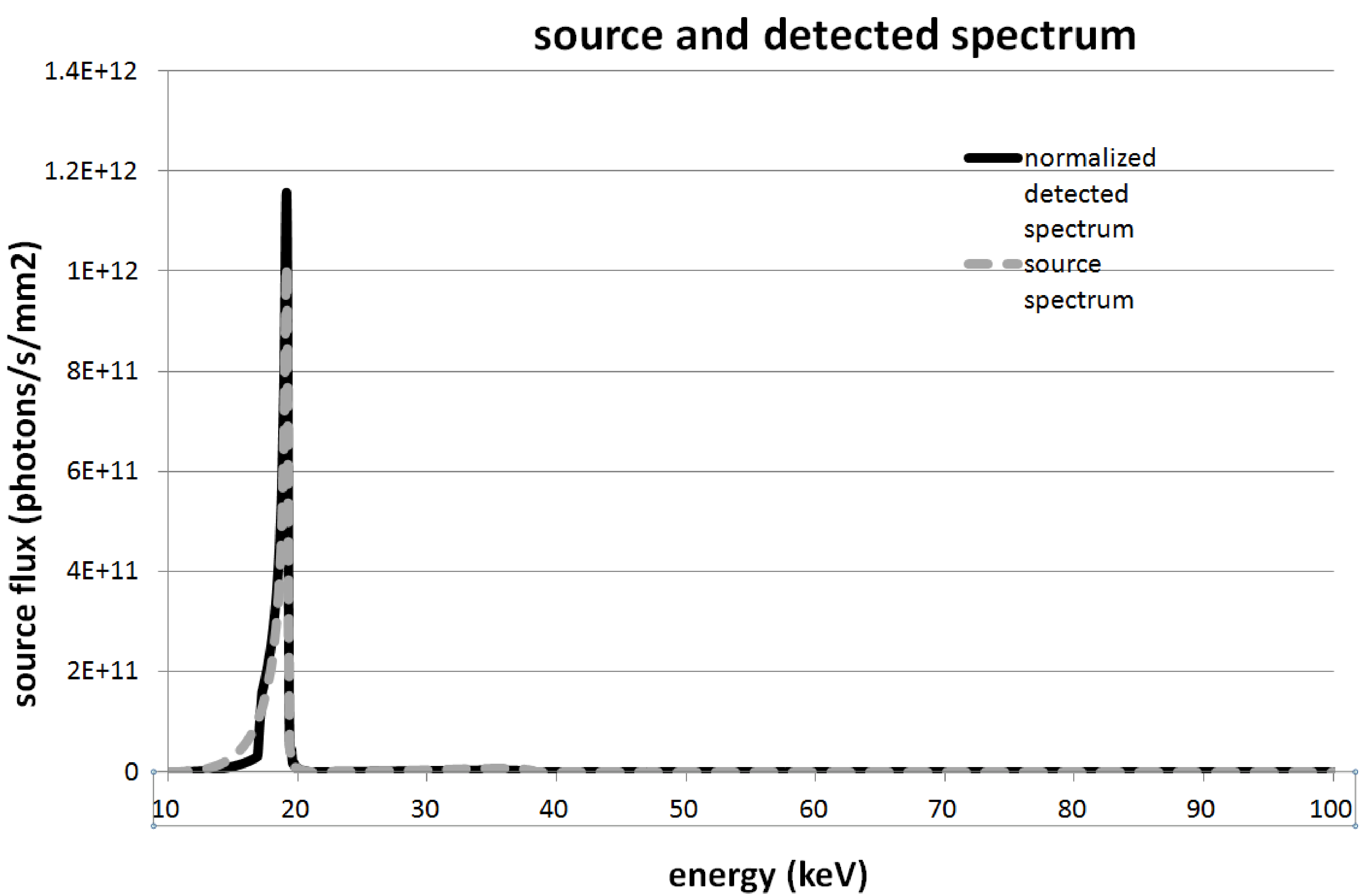
Smith et al. 2009

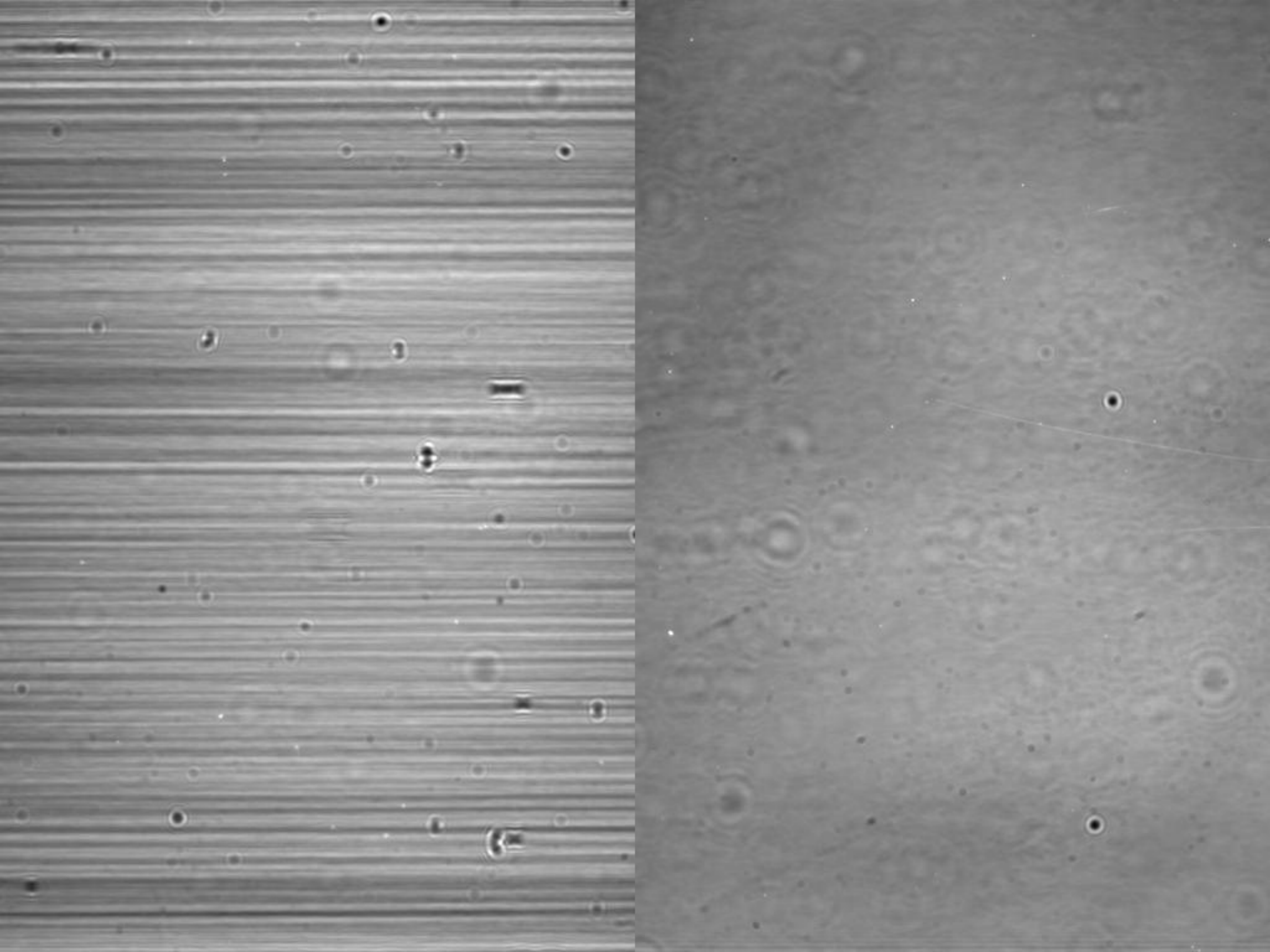
In 2010, ID19 moved from mostly monochromatic to nearly fully polychromatic operations, but with high control level of the spectrum shape.

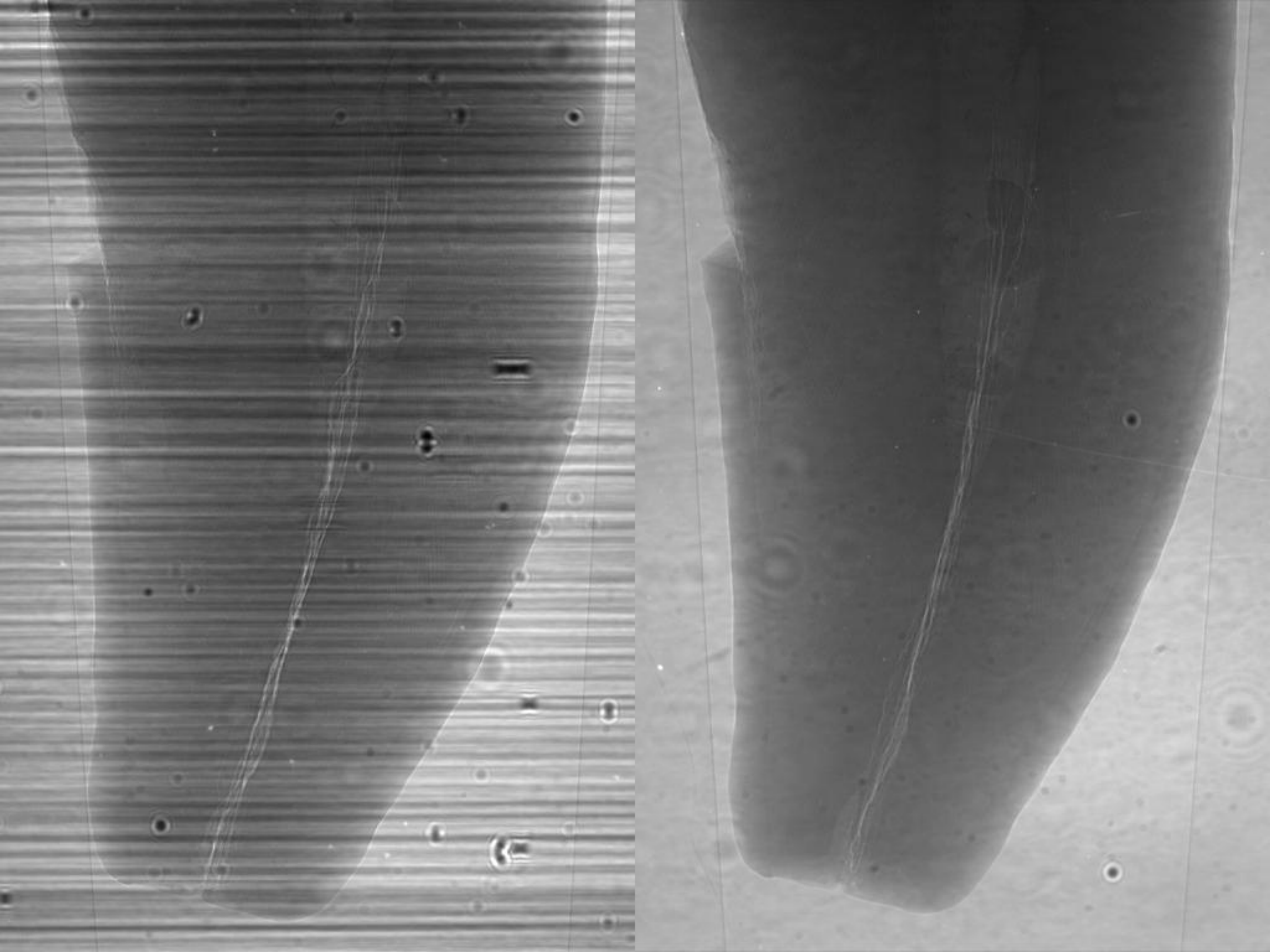
- It is not a “new” technique, but the spectral properties of these beams are now so good that in many cases they can be considered as **nearly monochromatic**

- Several configurations have been developed, covering an energy range from **19 to 250 keV**, with partially tunable flux, bandwidth and beam size

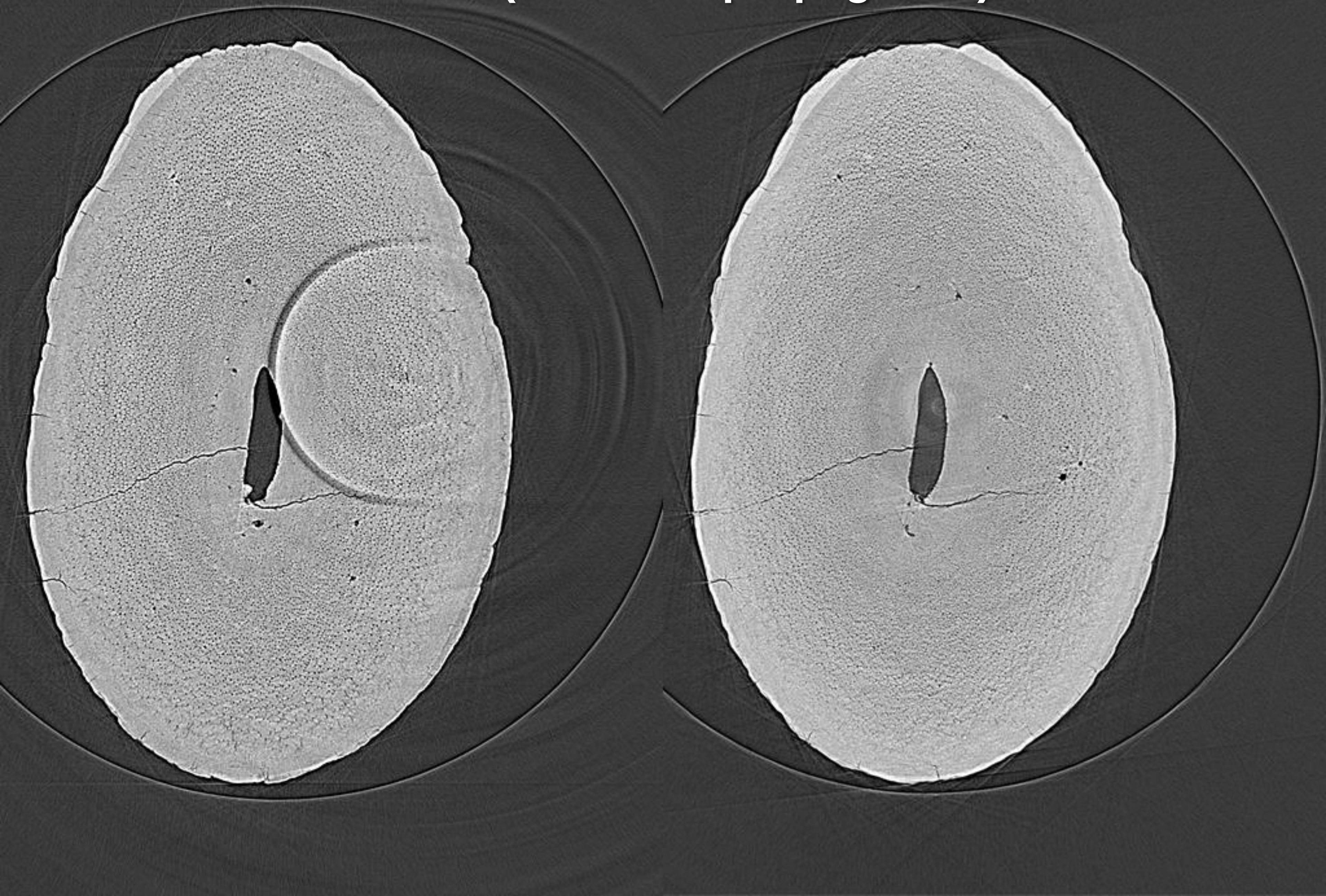
- It **replaced monochromatic** configurations for most of topics on ID19 and BM5.

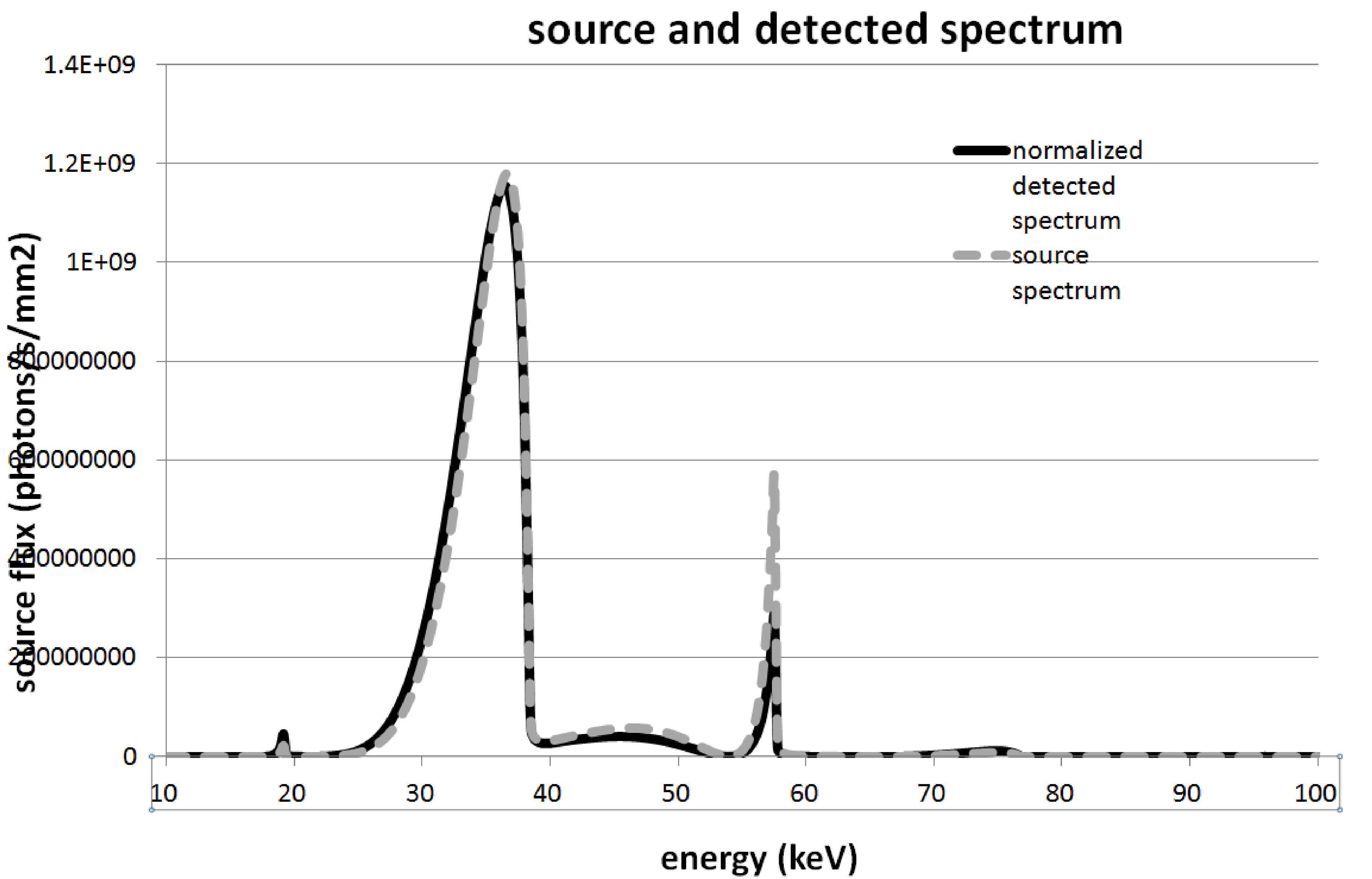

source and detected spectrum
1.4E+12
1.2E+12
1E+12
8E+11
6E+11
4E+11
2E+11
0
source flux (photons/s/mm2)
normalized detected spectrum
source spectrum
10
20
30
40
50
60
70
80
90
100
energy (keV)





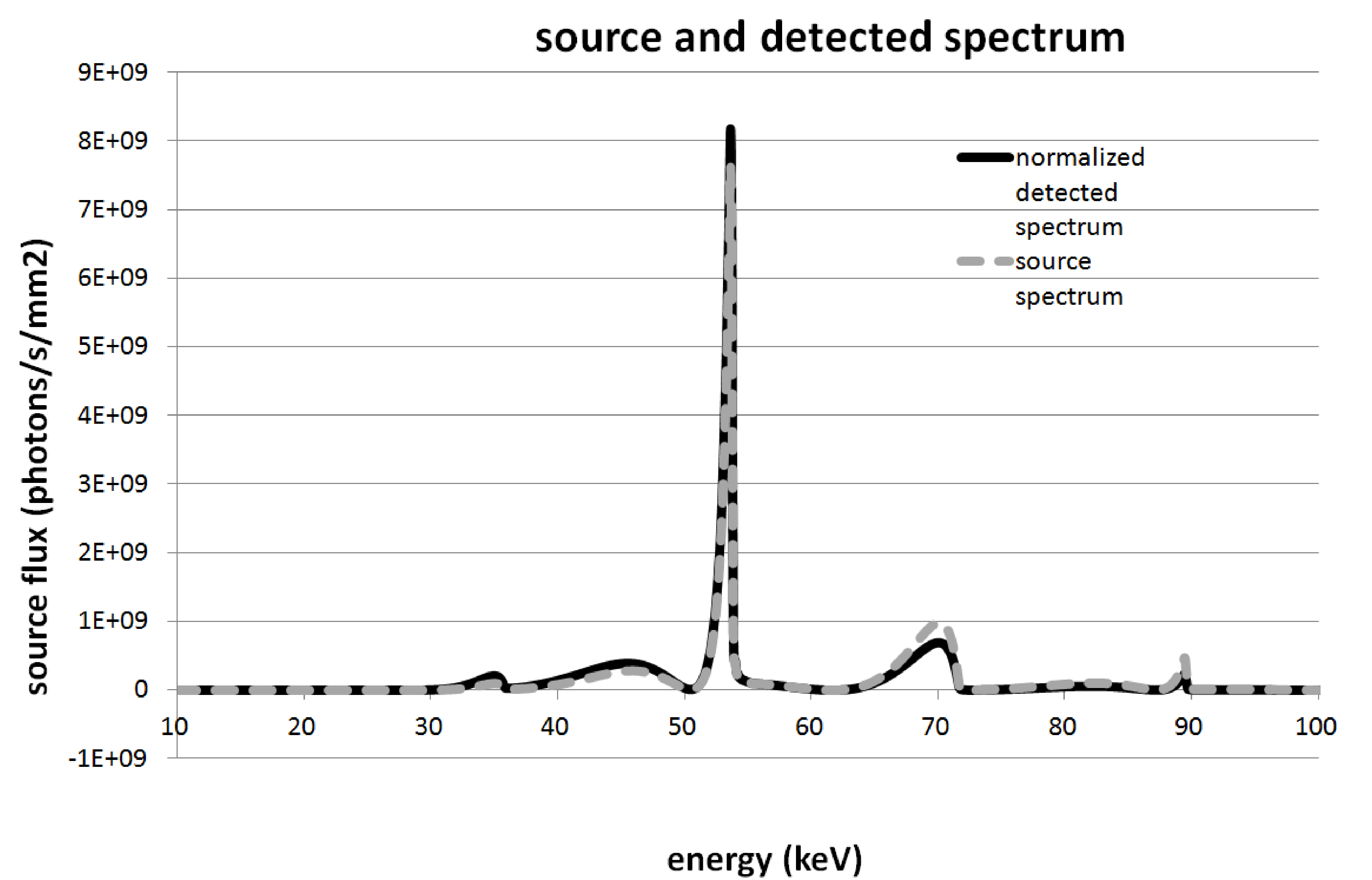
20 keV multilayer vs. 19.1 keV pink beam at 0.7 microns (50 mm of propagation)



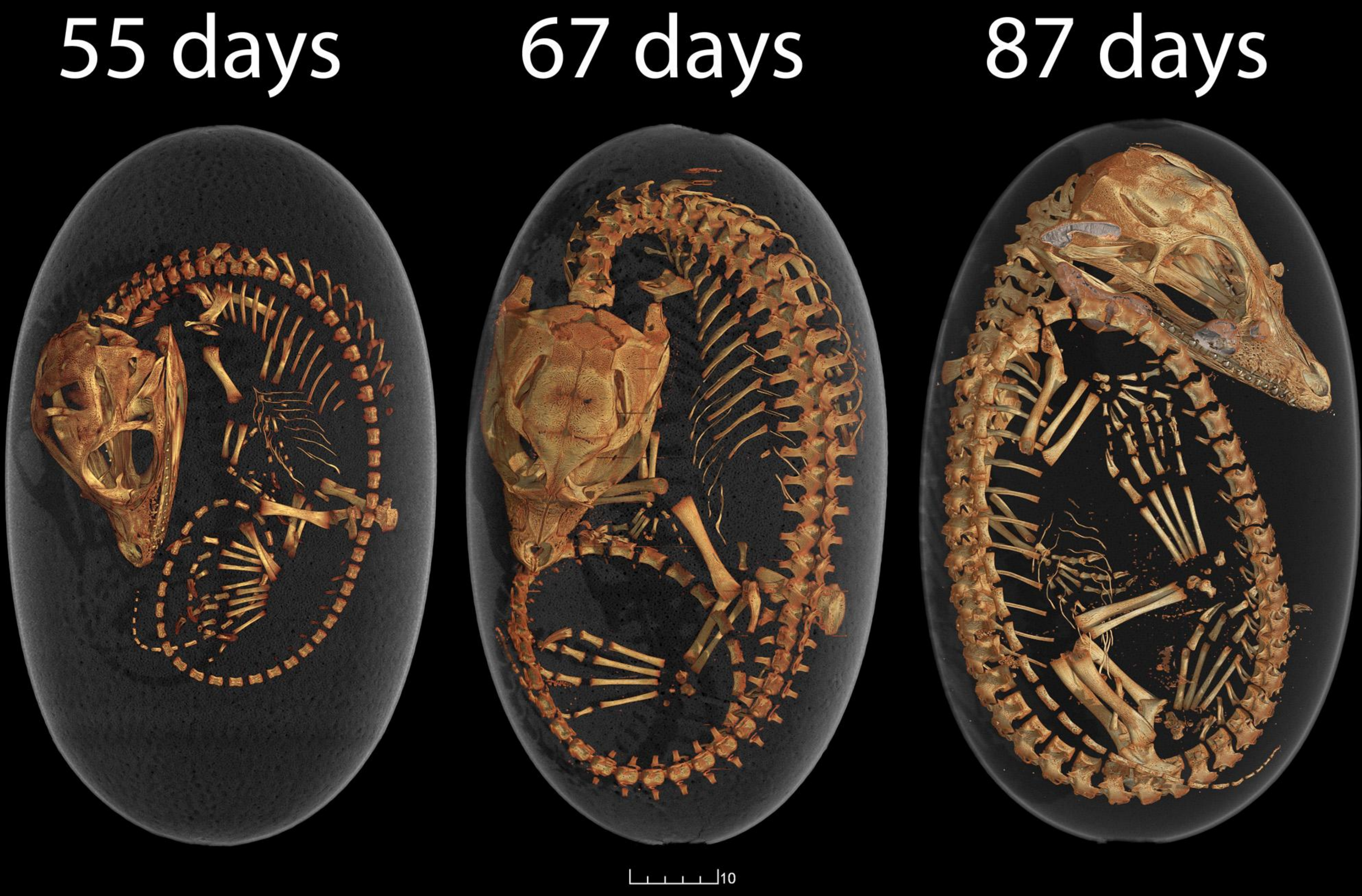

source and detected spectrum
1.4E+09
1.2E+09
1E+09
800000000
600000000
400000000
200000000
0
source flux (photons/s/mm2)
10
20
30
40
50
60
70
80
90
100
energy (keV)
normalized detected spectrum
source spectrum

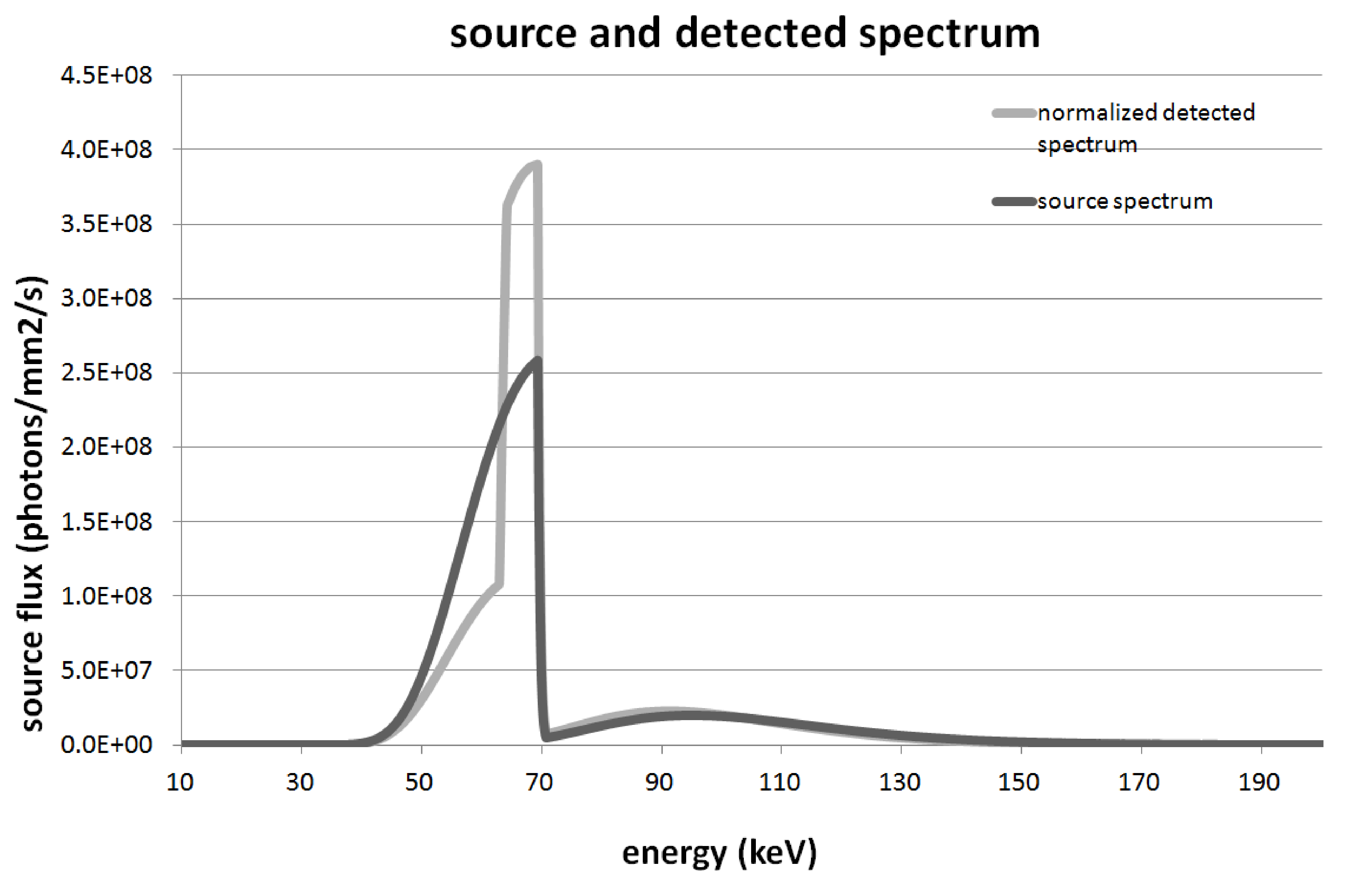
31 keV pink beam at 7.5 microns (1m of propagation)

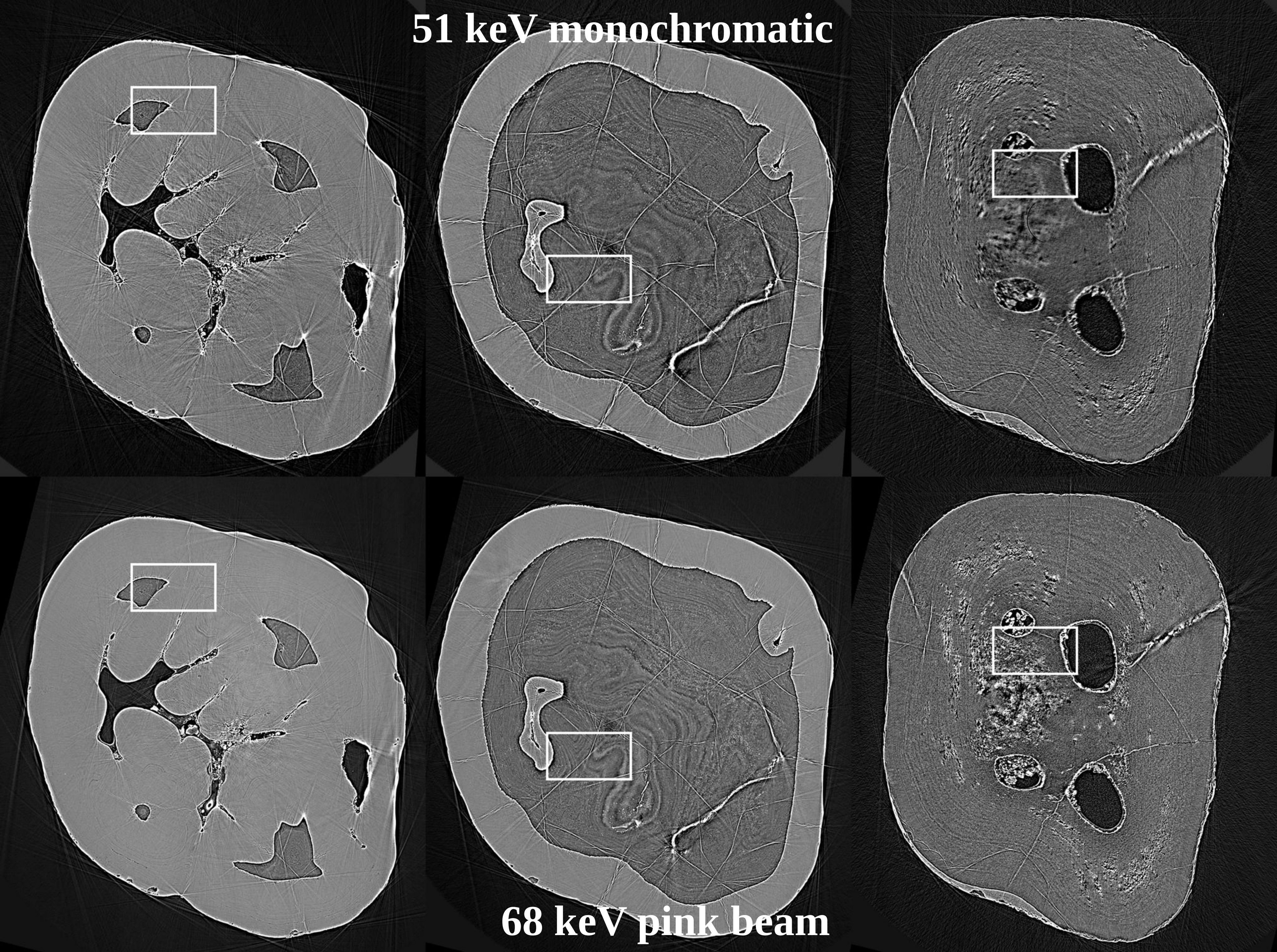
31 days old crocodile embryo, from the Pierrelatte crocodile farm

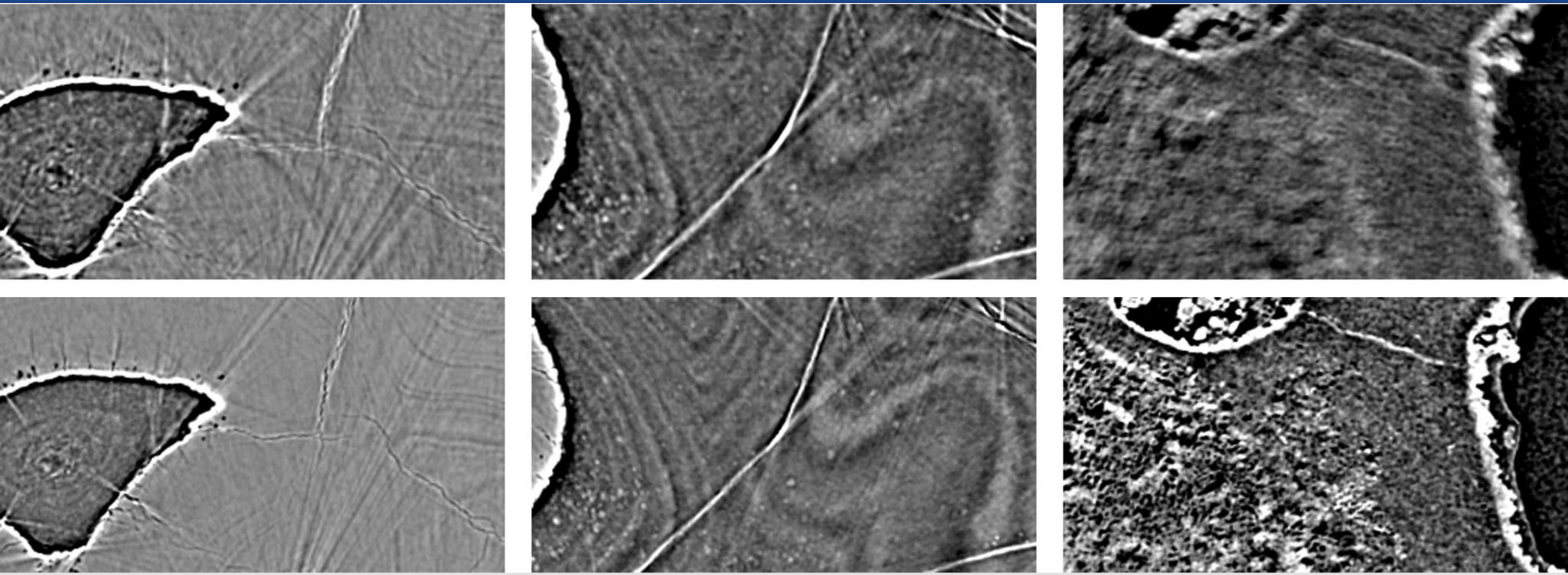

source and detected spectrum
9E+09
8E+09
7E+09
6E+09
5E+09
4E+09
3E+09
2E+09
1E+09
0
-1E+09
source flux (photons/s/mm2)
10
20
30
40
50
60
70
80
90
100
energy (keV)
normalized detected spectrum
source spectrum

51 keV pink beam at 30 microns (4m of propagation)



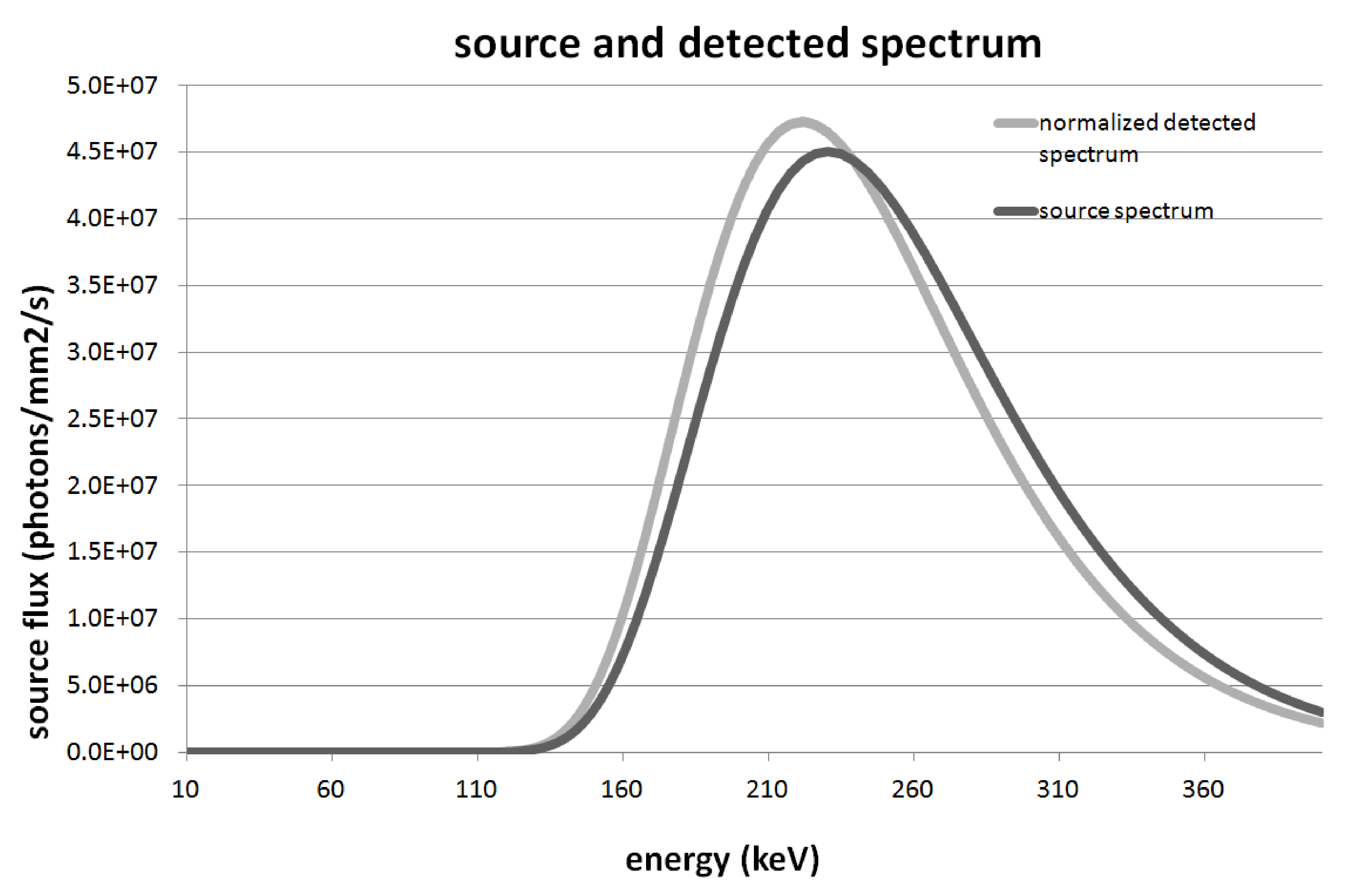

source and detected spectrum
source flux (photons/mm2/s)
4.5E+08
4.0E+08
3.5E+08
3.0E+08
2.5E+08
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
10
30
50
70
90
110
130
150
170
190
energy (keV)
normalized detected spectrum
source spectrum


51 keV monochromatic
68 keV pink beam

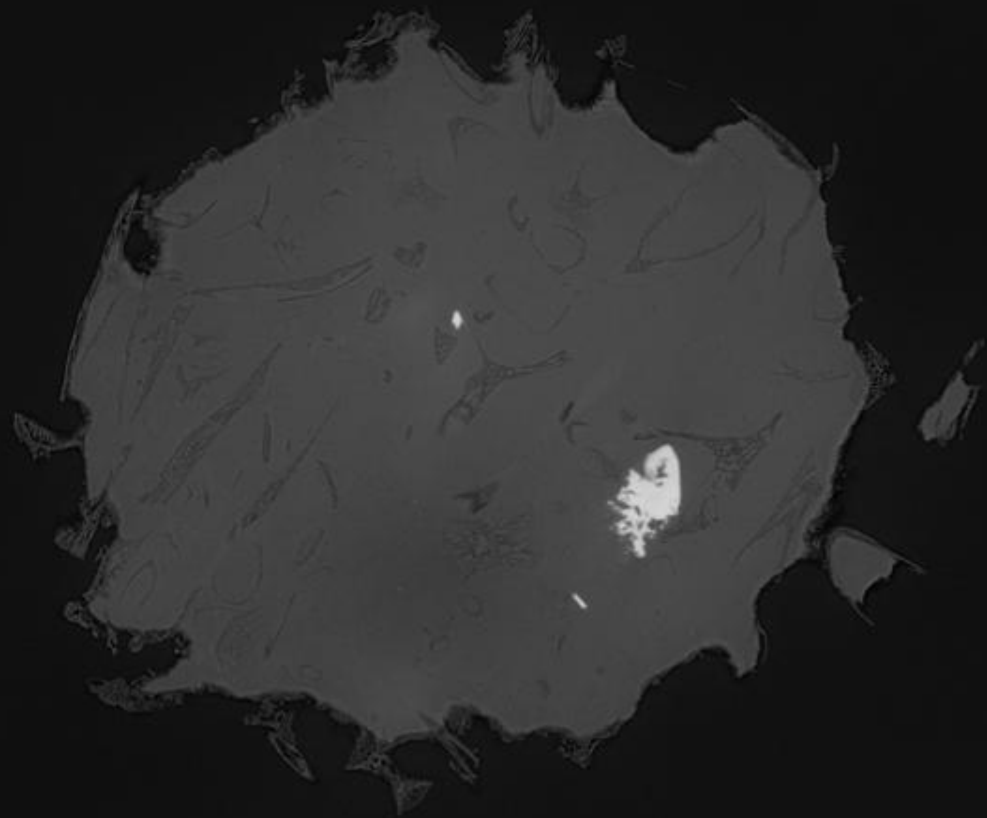


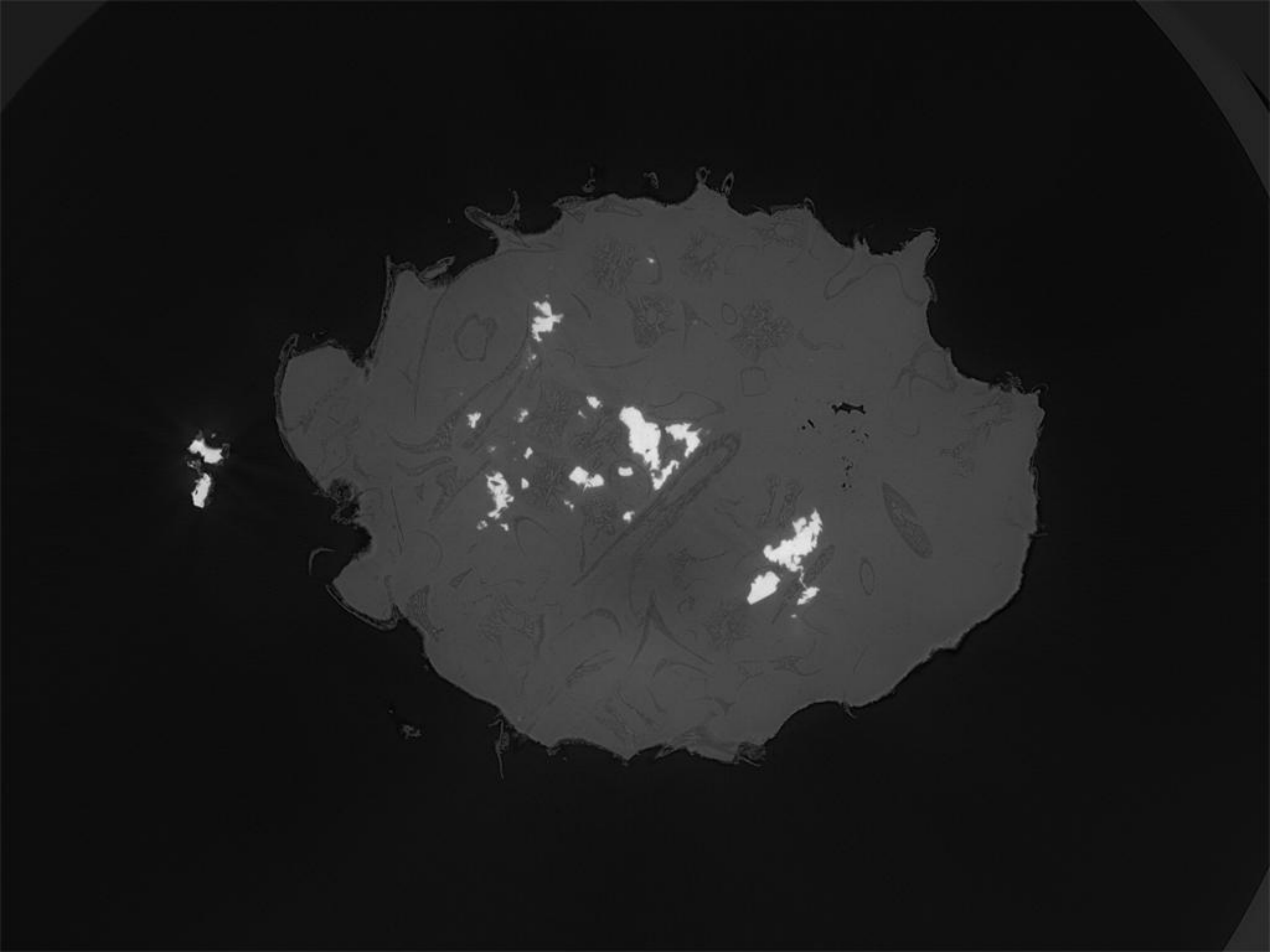
- Faster scans (2 hours vs. 12 hours)m higher signal to noise ratio, higher energy, less risk of decohesion, less ring artefacts, no detectable beam hardening (~3%).

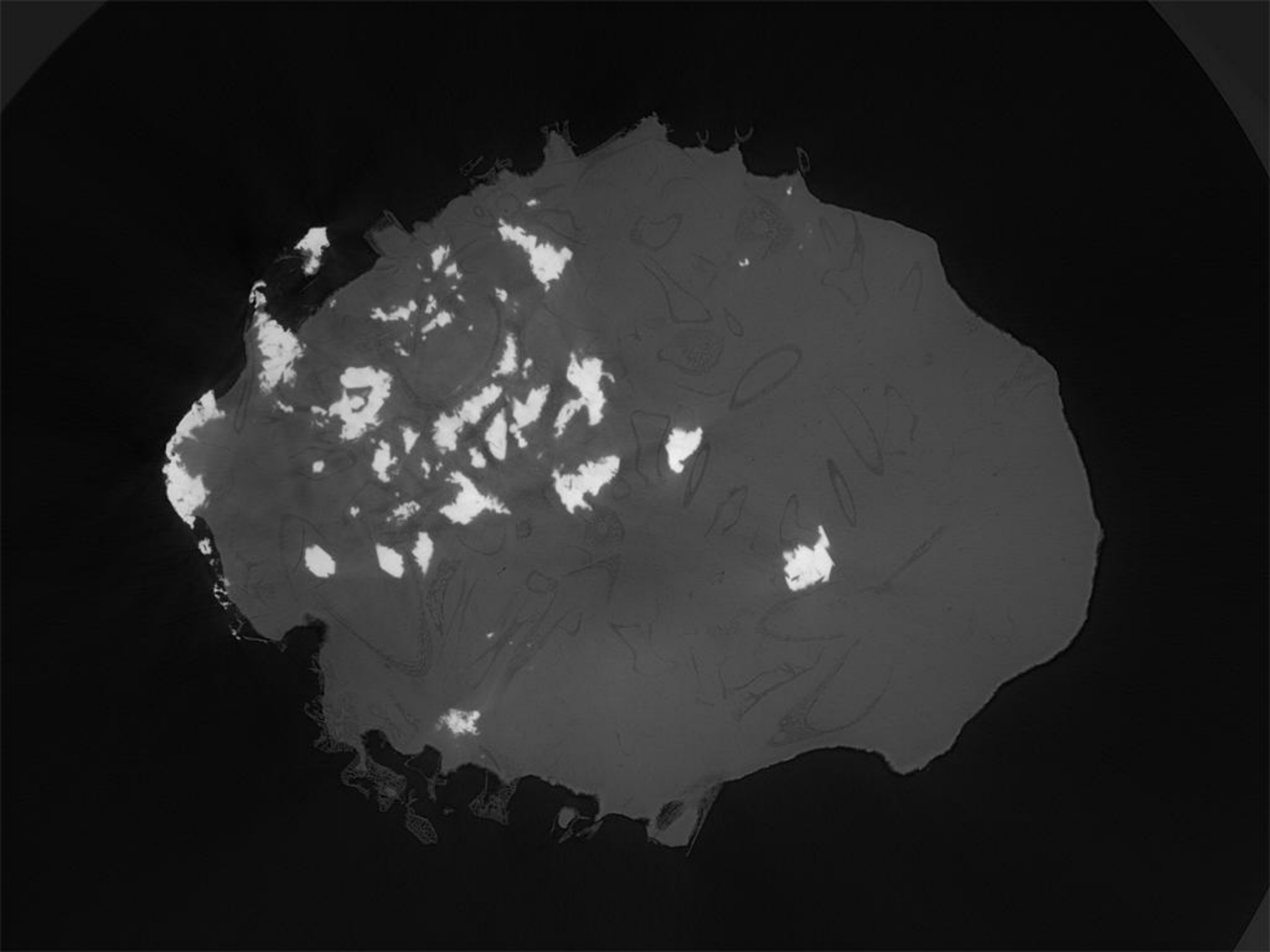
- All hominid teeth are now scanned with pink beams for incremental lines investigations

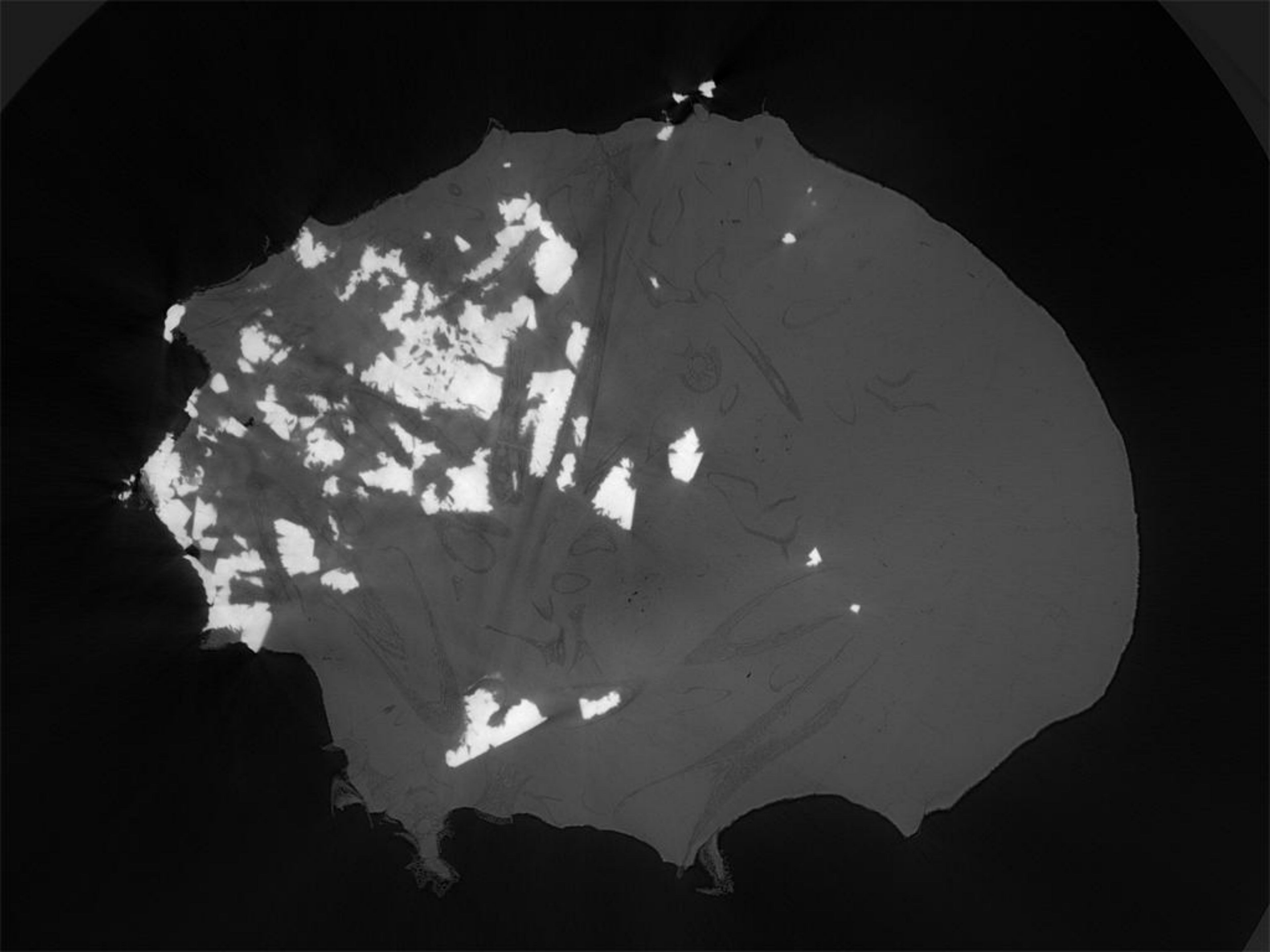

source and detected spectrum
normalized detected spectrum
source spectrum
source flux (photons/mm2/s)
5.0E+07
4.5E+07
4.0E+07
3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
10
60
110
160
210
260
310
360
energy (keV)

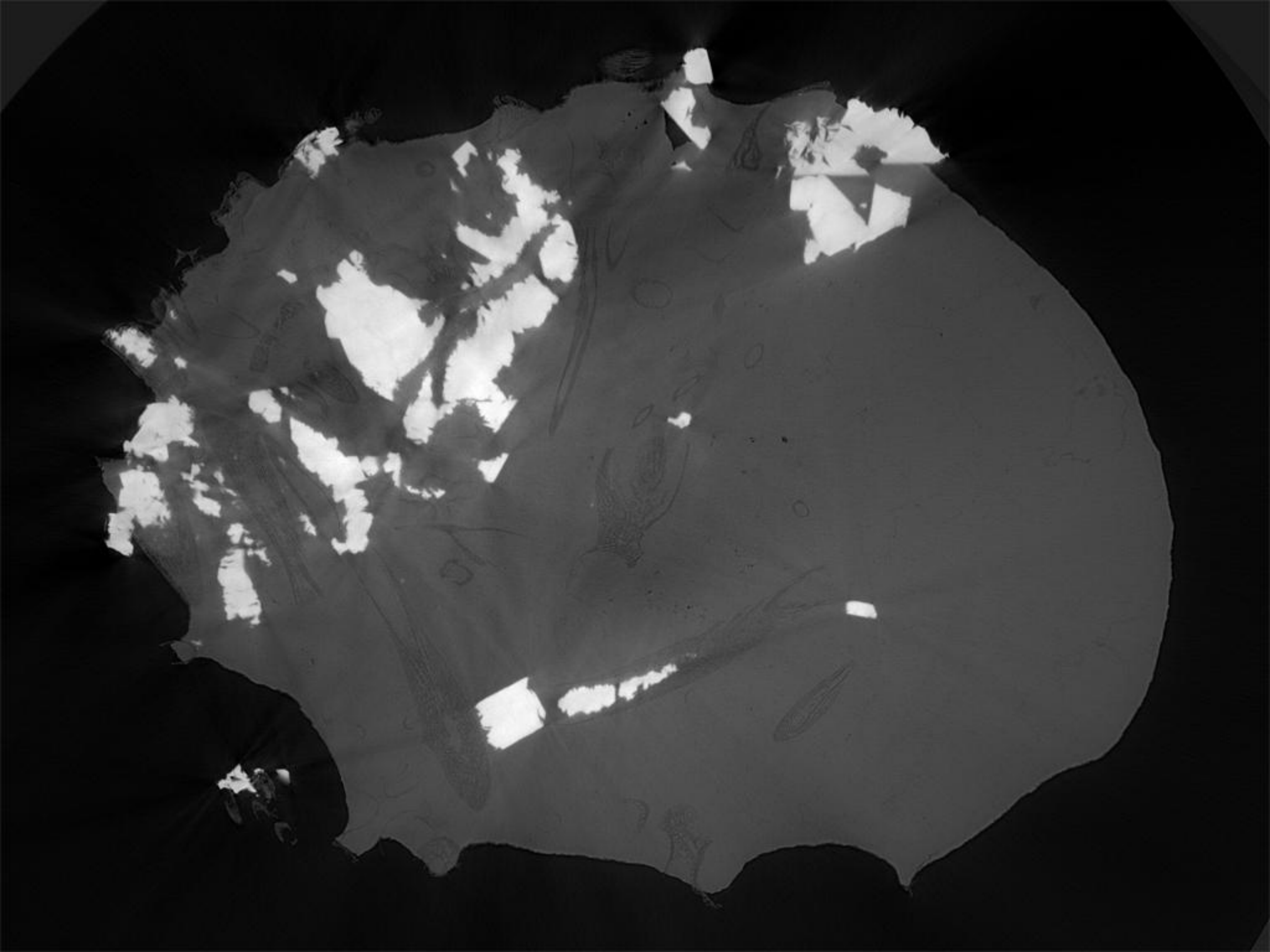
Therizinosauroid embryo, spectrum pic around 240 keV filtered with 7 mm of cooper and 2mm of tungsten

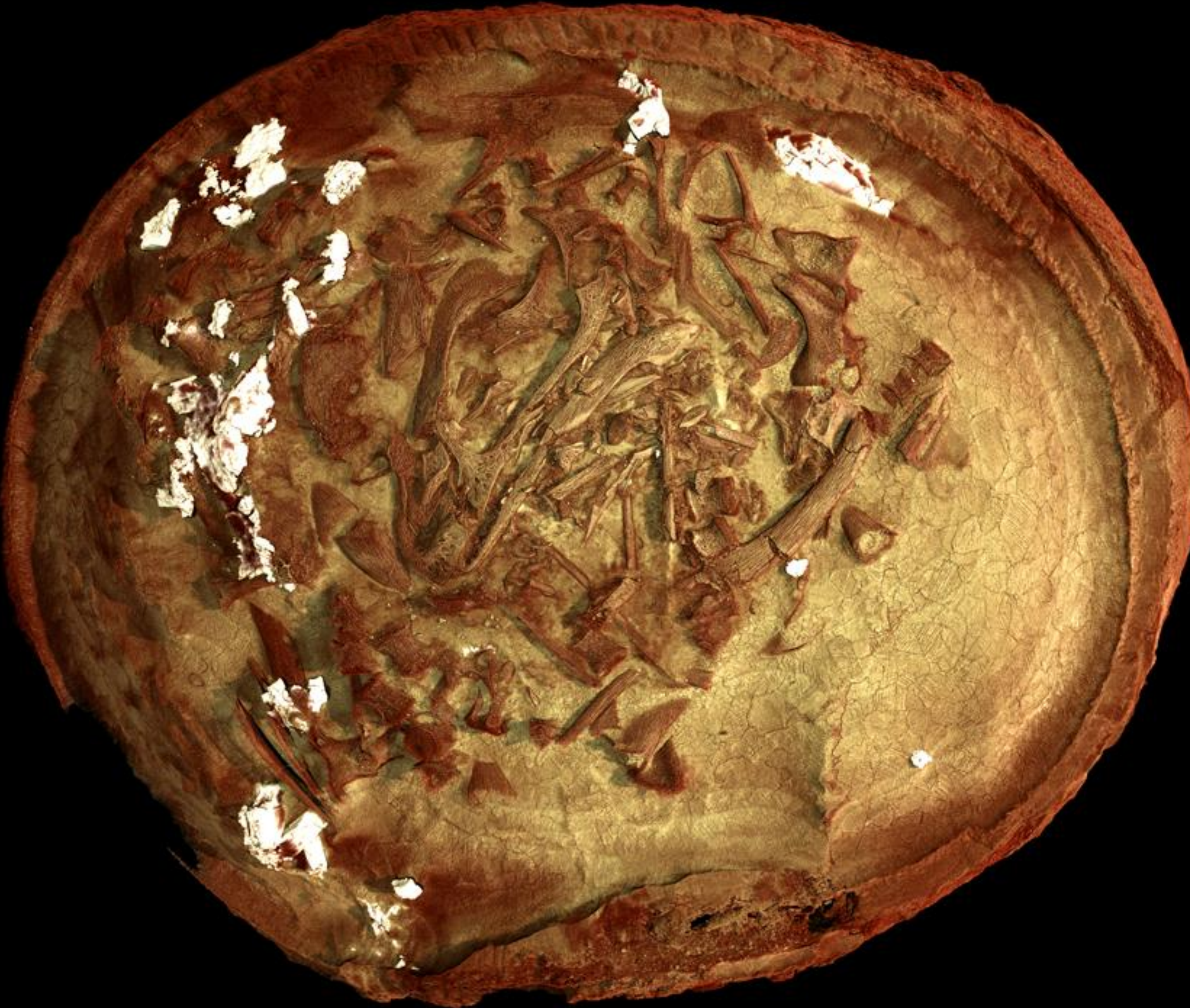


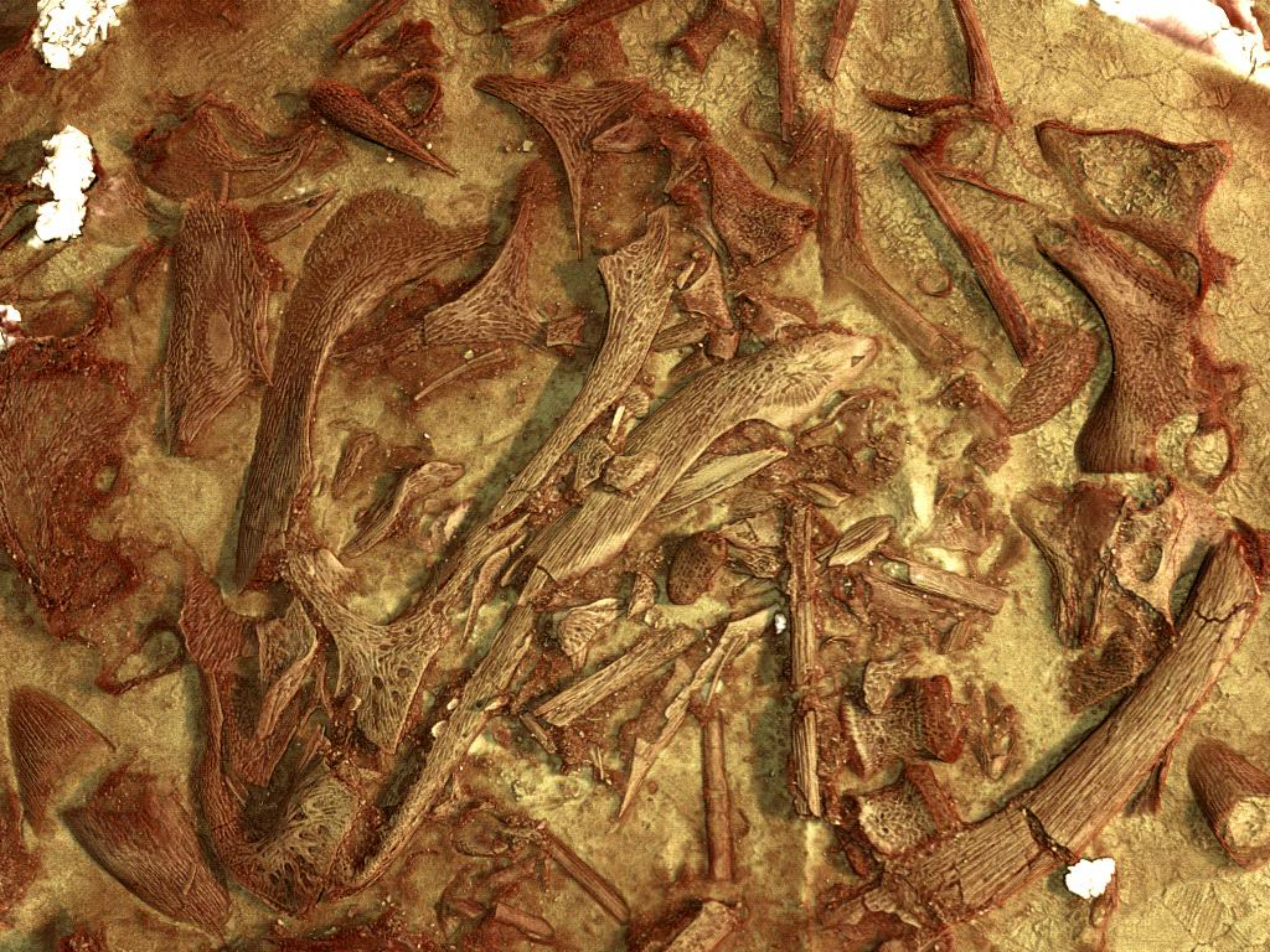




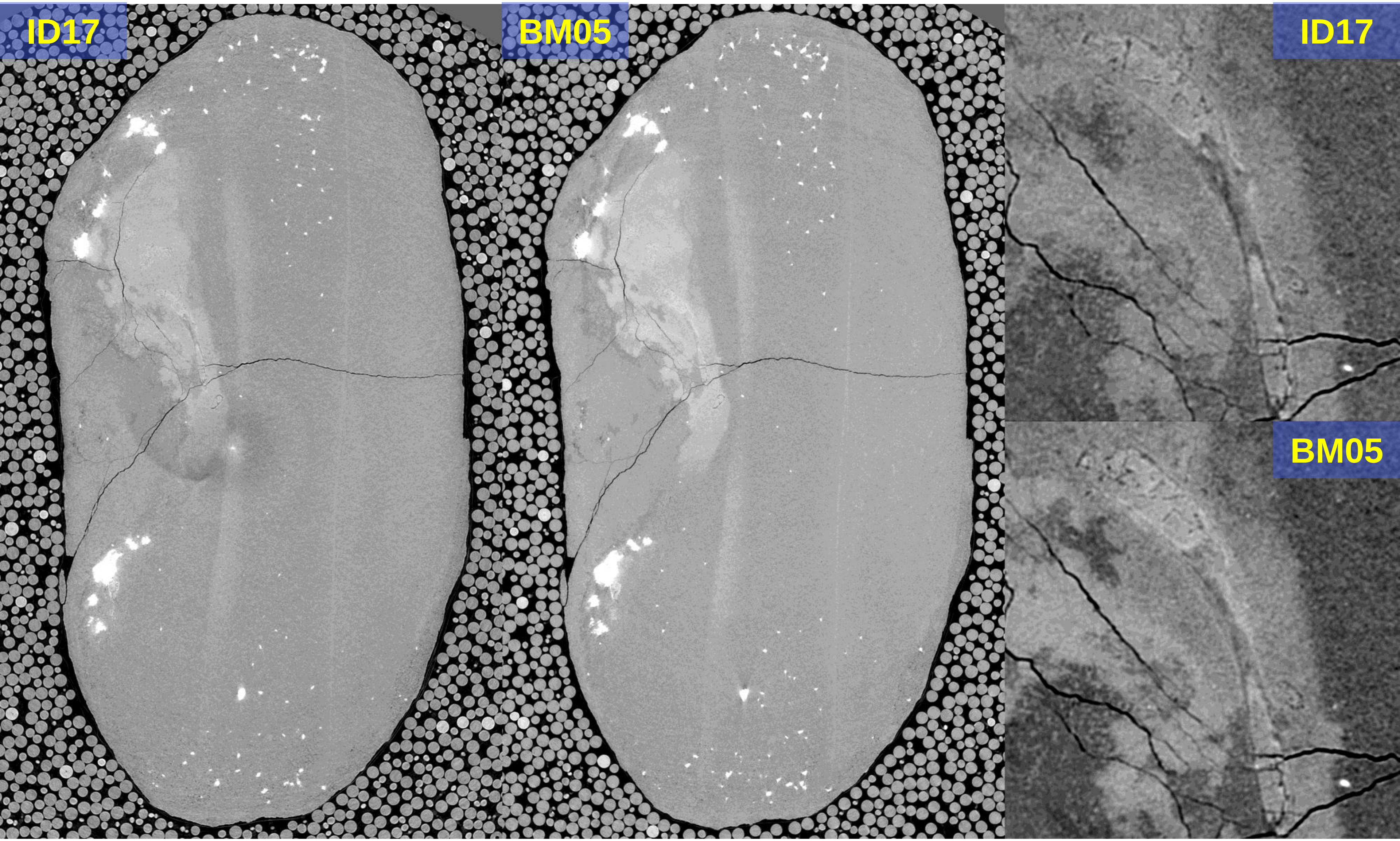








COMPARISON OF RESULTS ON LARGE FOSSIL FROM ID17 AND BM5

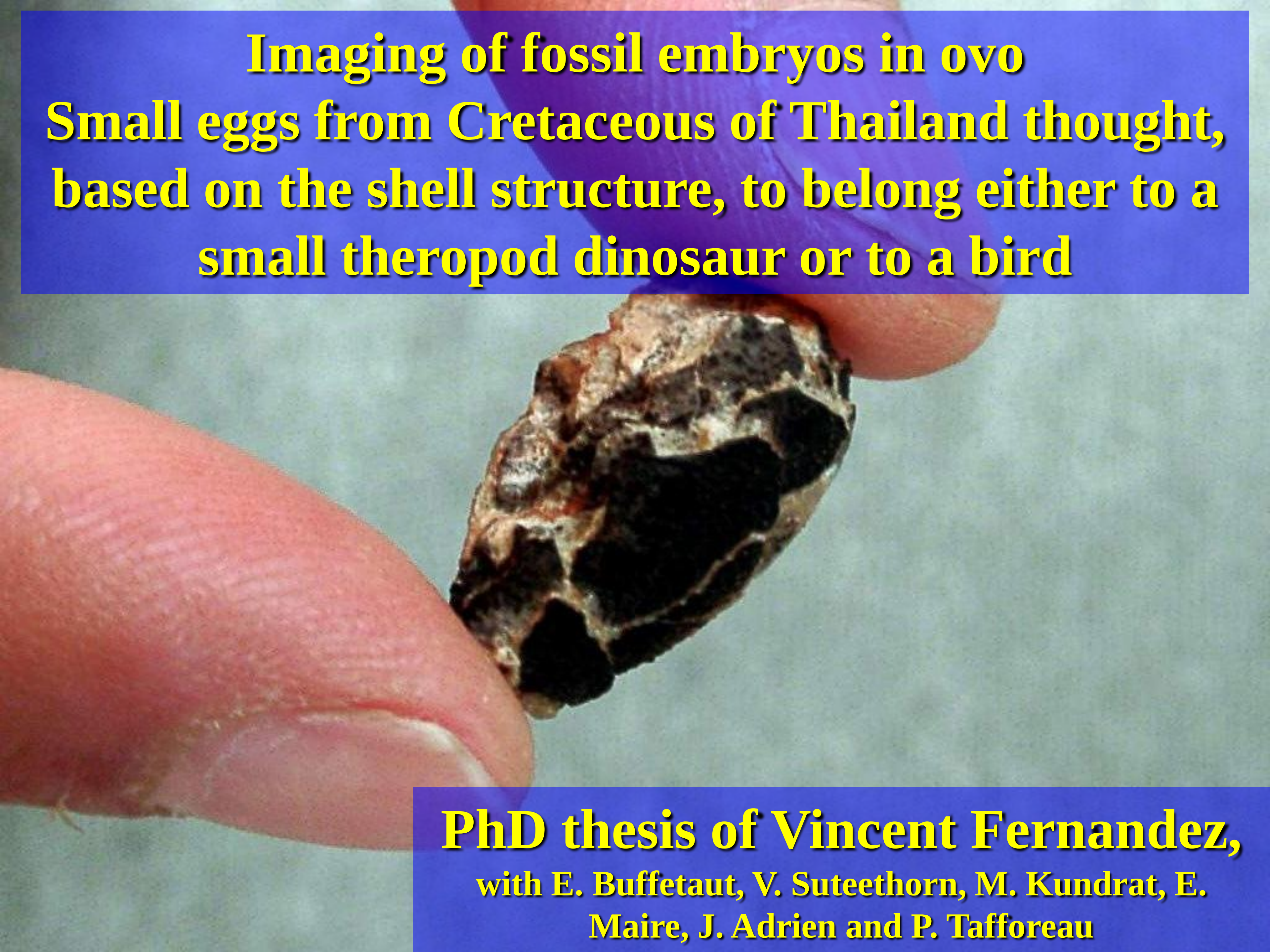


160 keV monochromatic on ID17 vs. 170 keV average polychromatic on BM5

What really made the success of synchrotron imaging in palaeontology :

<u>the propagation phase contrast</u>

First application on a fossil in 2002, since then it replaced absorption.


Imaging of fossil embryos in ovo
Small eggs from Cretaceous of Thailand thought, based on the shell structure, to belong either to a small theropod dinosaur or to a bird
PhD thesis of Vincent Fernandez,
with E. Buffetaut, V. Suteethorn, M. Kundrat, E. Maire, J. Adrien and P. Tafforeau

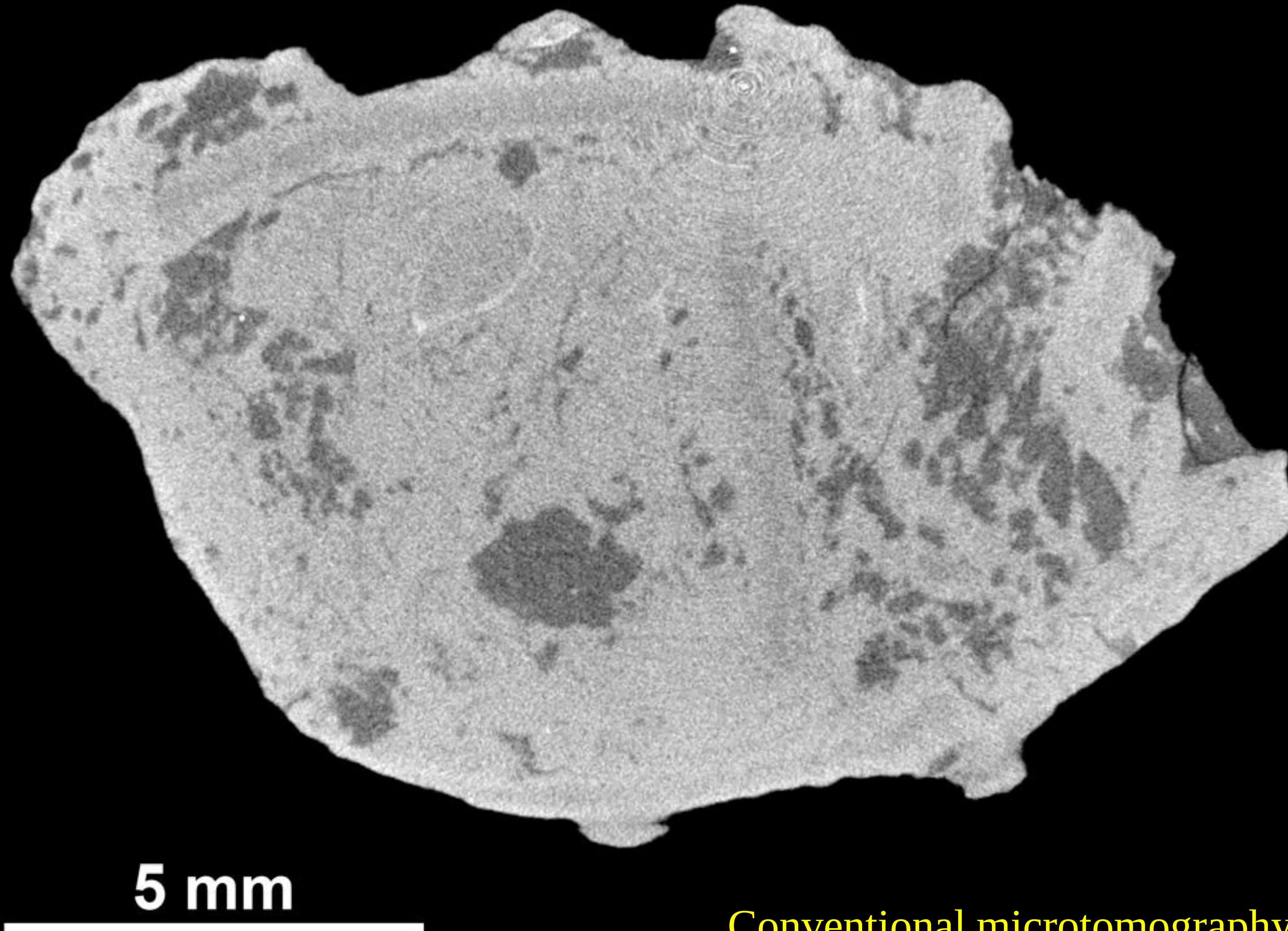
It is impossible to know what animal laid an egg without seing the embryo inside !

L'âge de glace 3

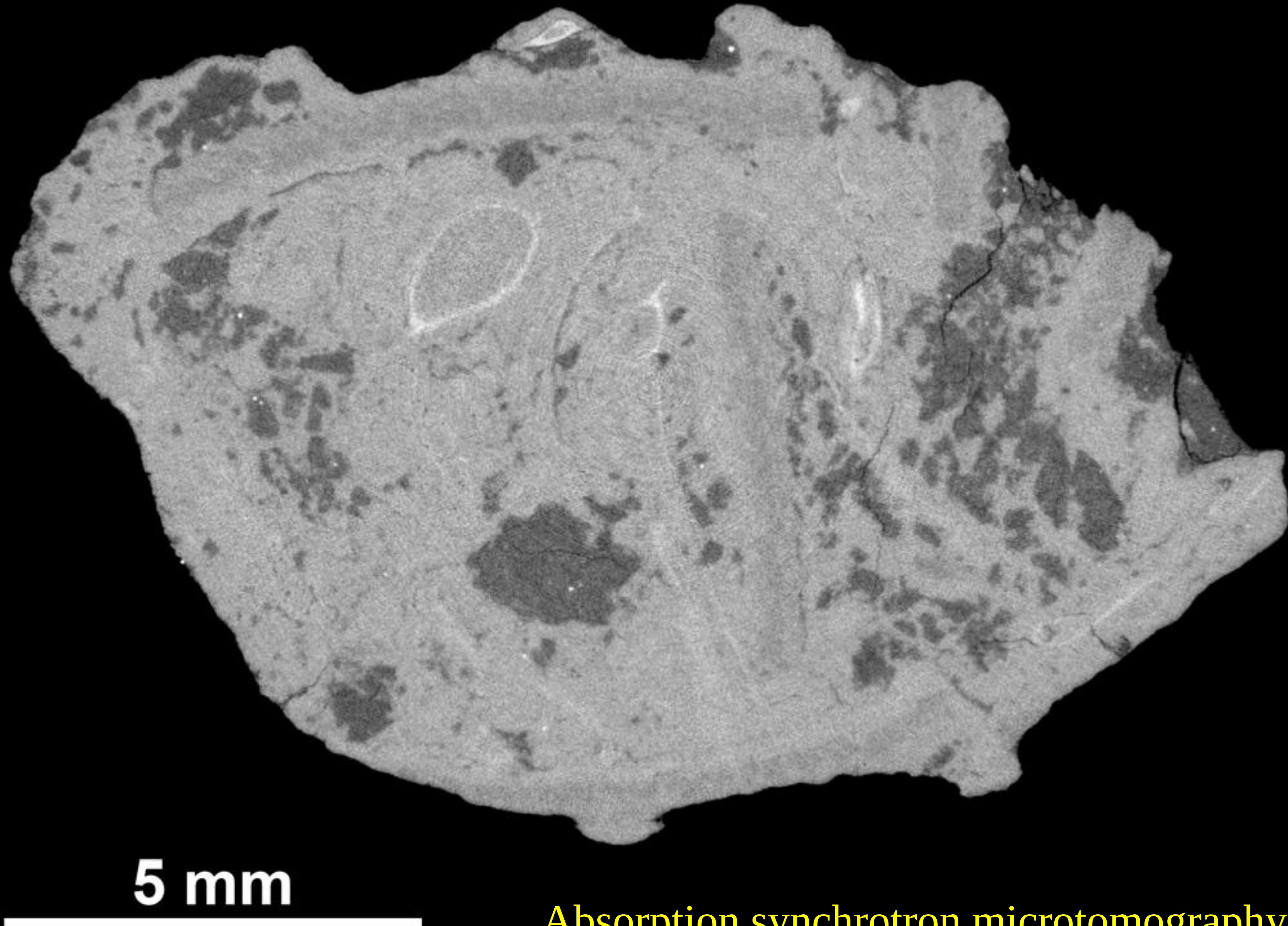
Small dinosaurs ?

•L'âge de glace 3, le temps des dinosaures

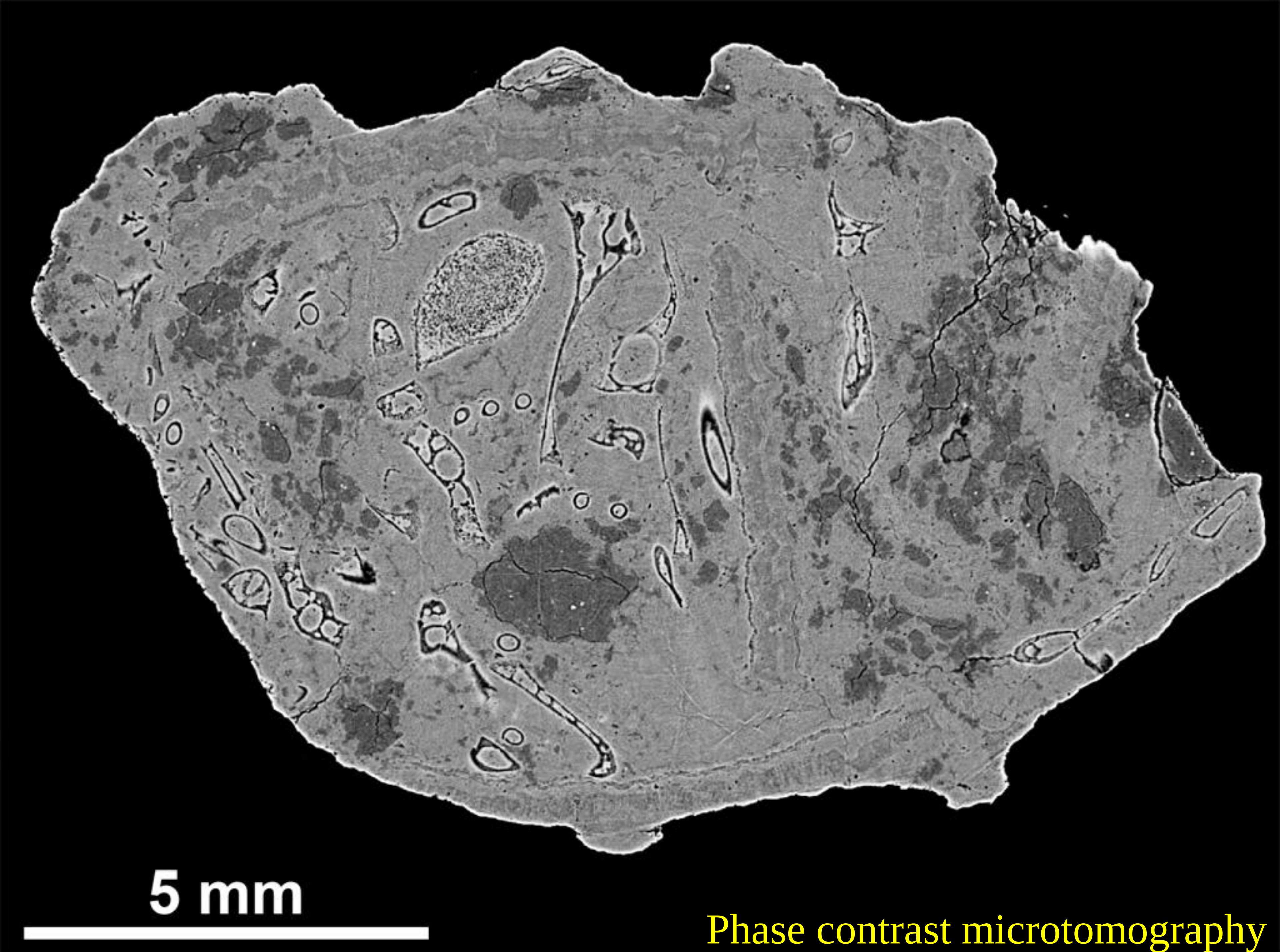
Primitive birds ?



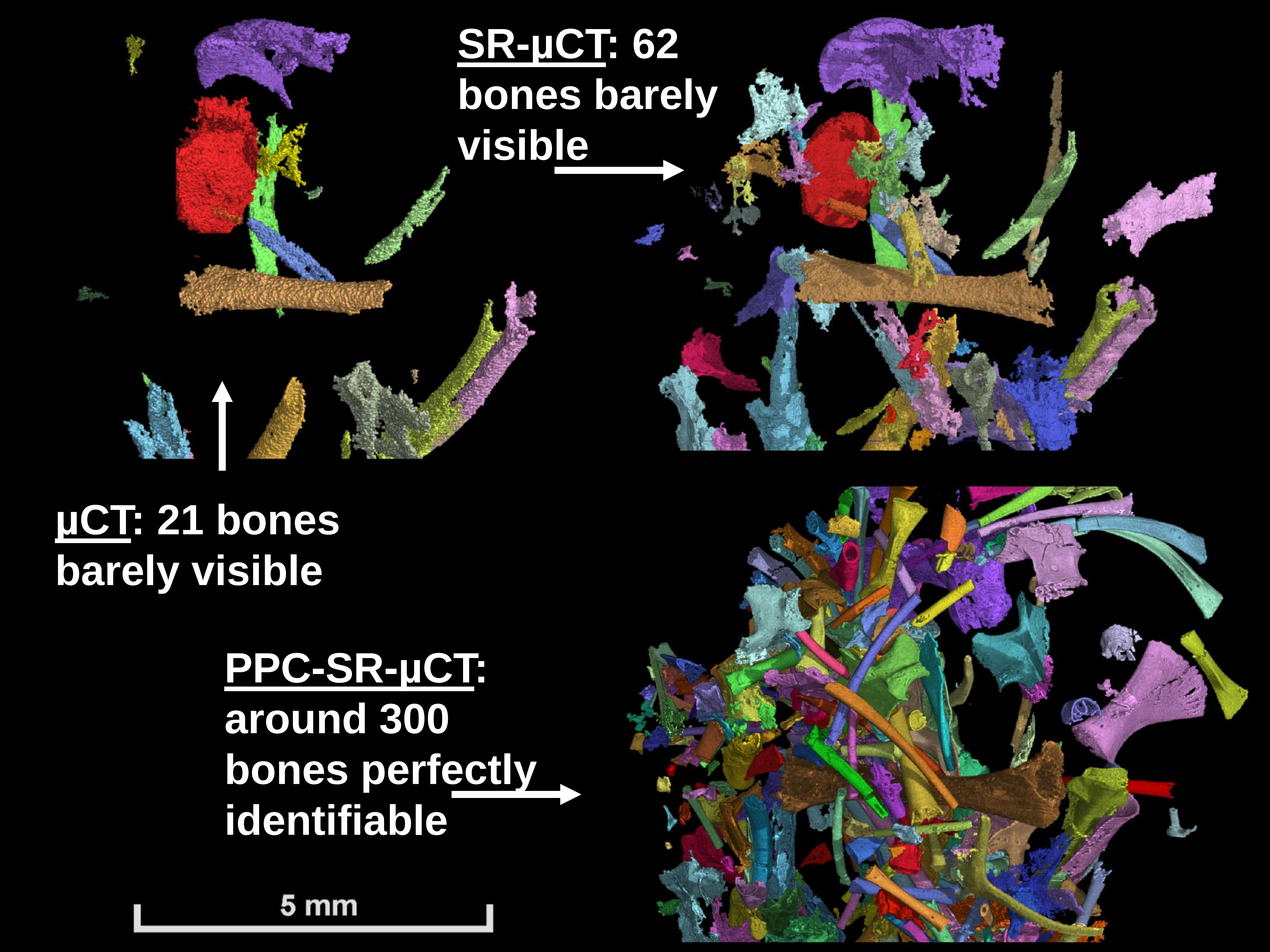
Conventional microtomography

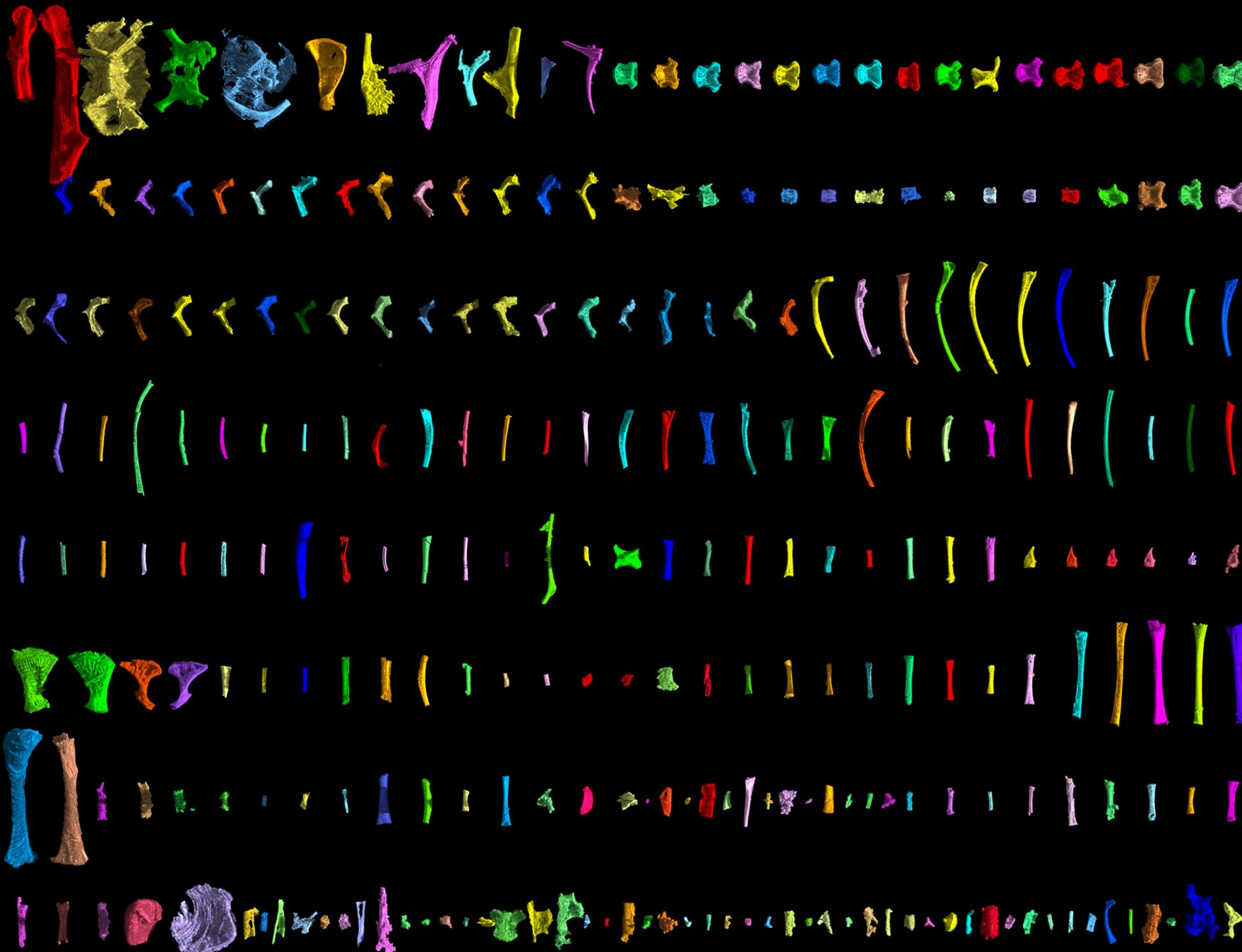


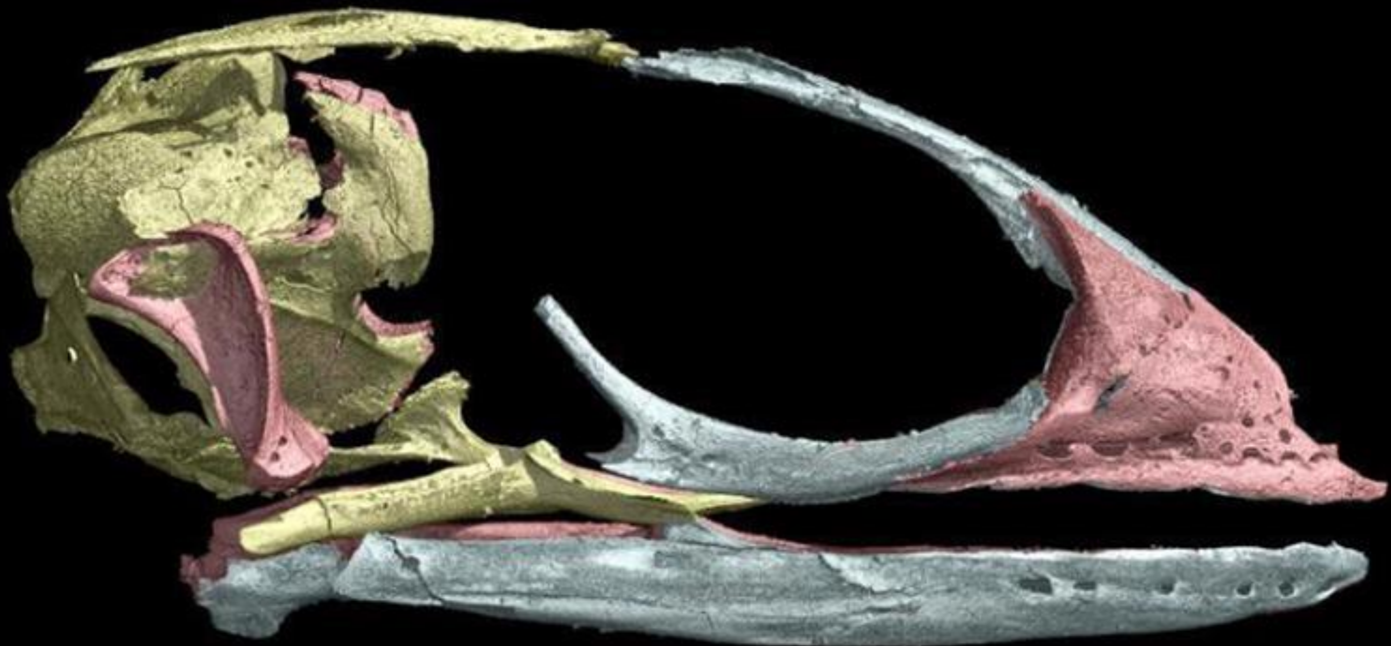
Absorption synchrotron microtomography



Phase contrast microtomography


SR-µCT: 62 bones barely visible
µCT: 21 bones barely visible
PPC-SR-µCT: around 300 bones perfectly identifiable
5 mm





Neither dinosaurs, nor birds. These eggs finally belong to lizards !

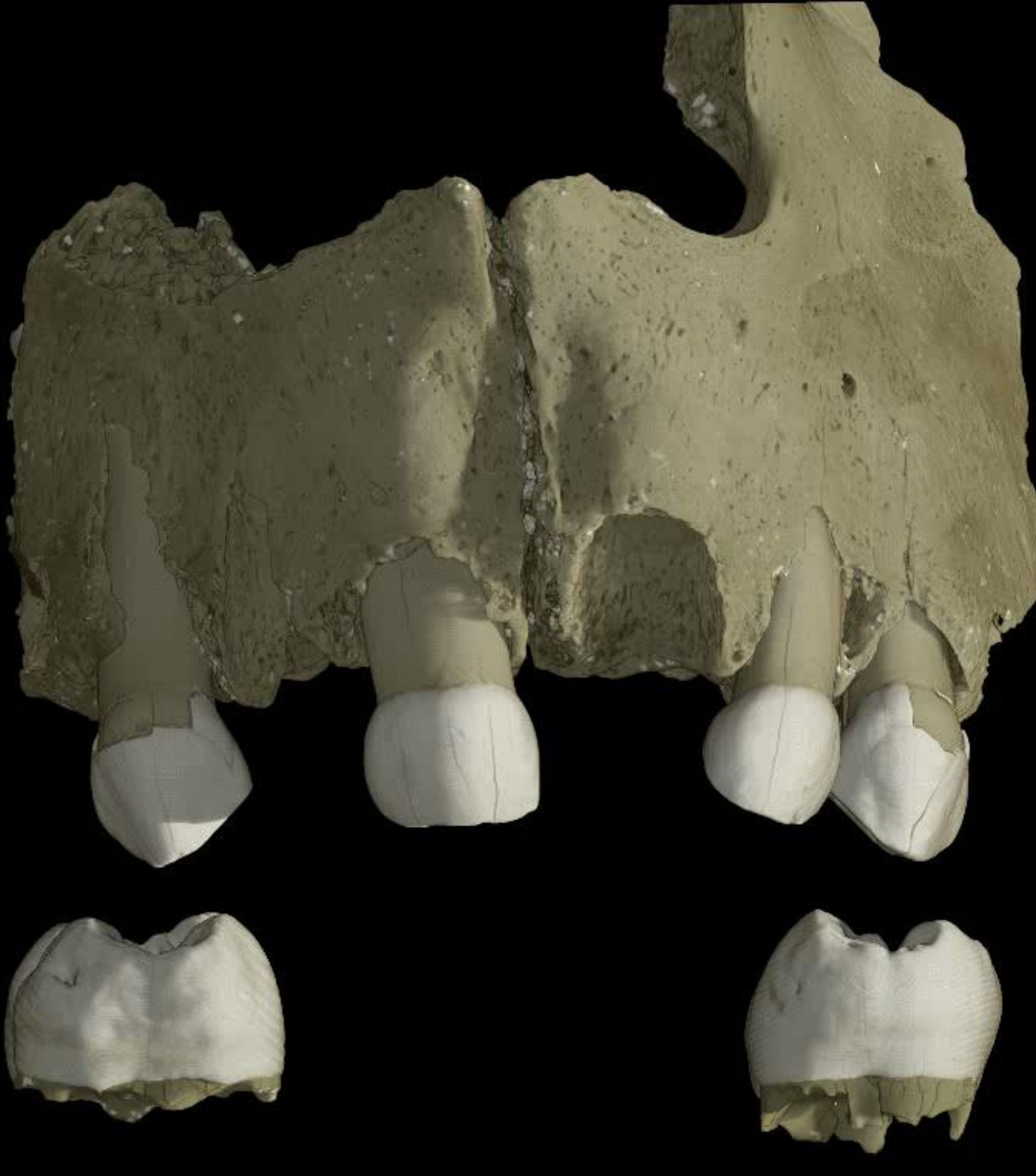
Nothing is better than a direct evidence.

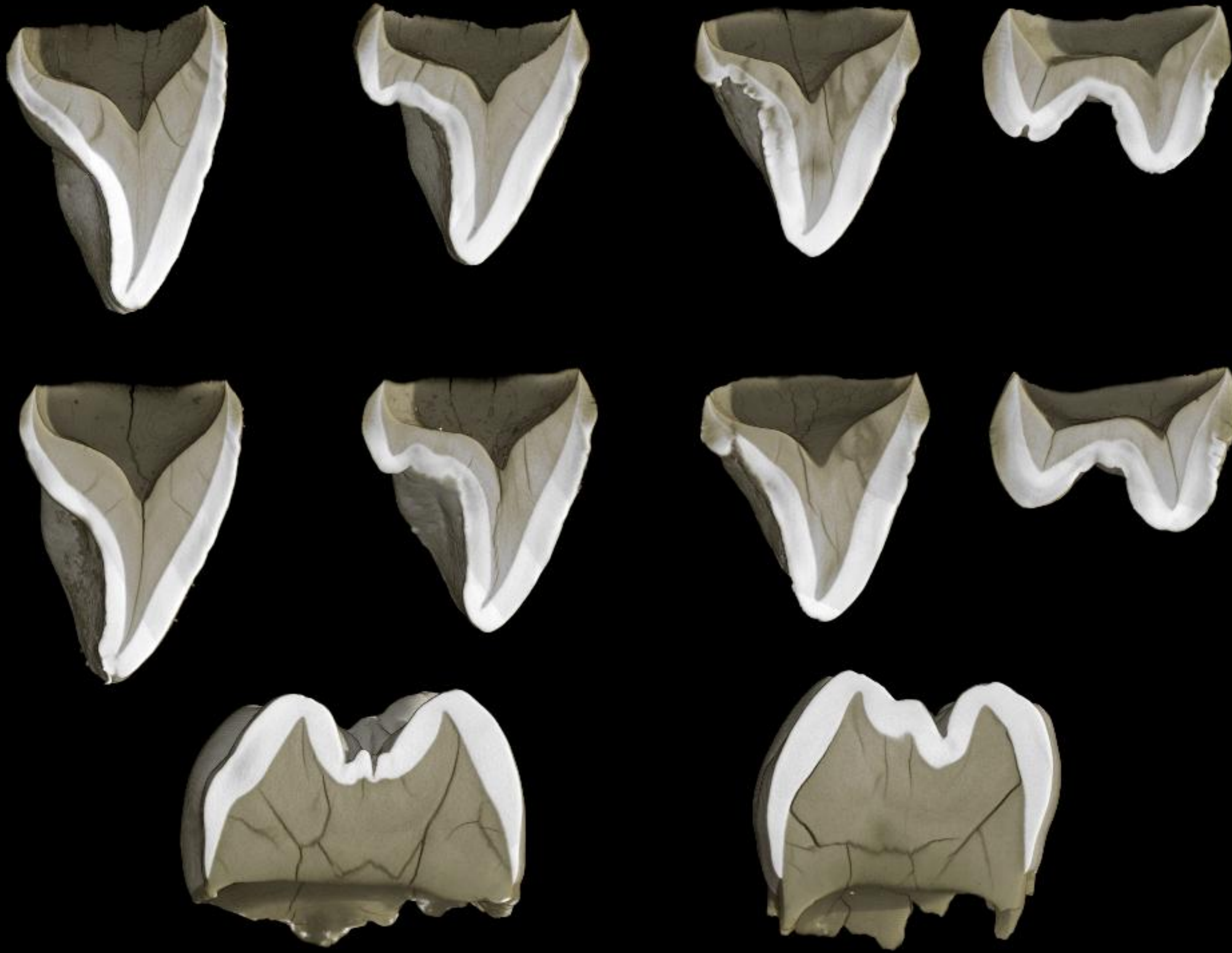
Propagation phase contrast multiscale analysis:

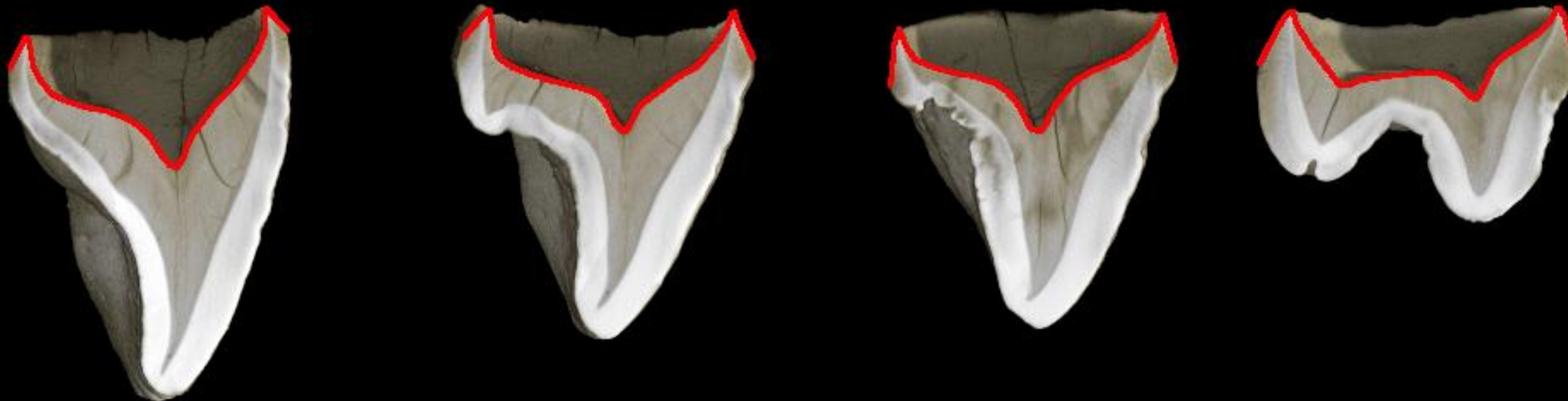
The revolution of the non-destructive virtual palaeohistology

Age at death determination of the Engis 2 Neanderthal child.

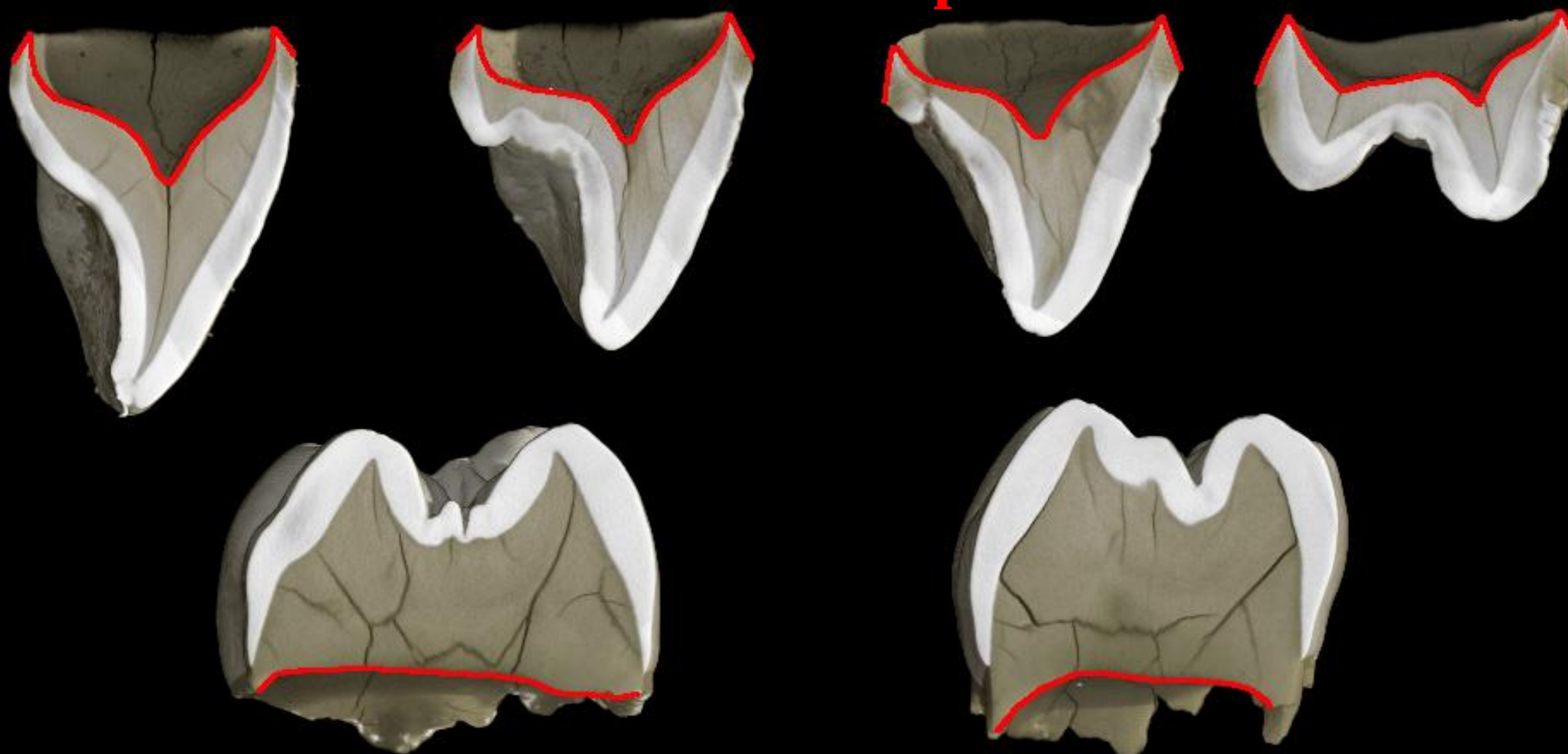
30 000 – 50 000 ans, Belgique

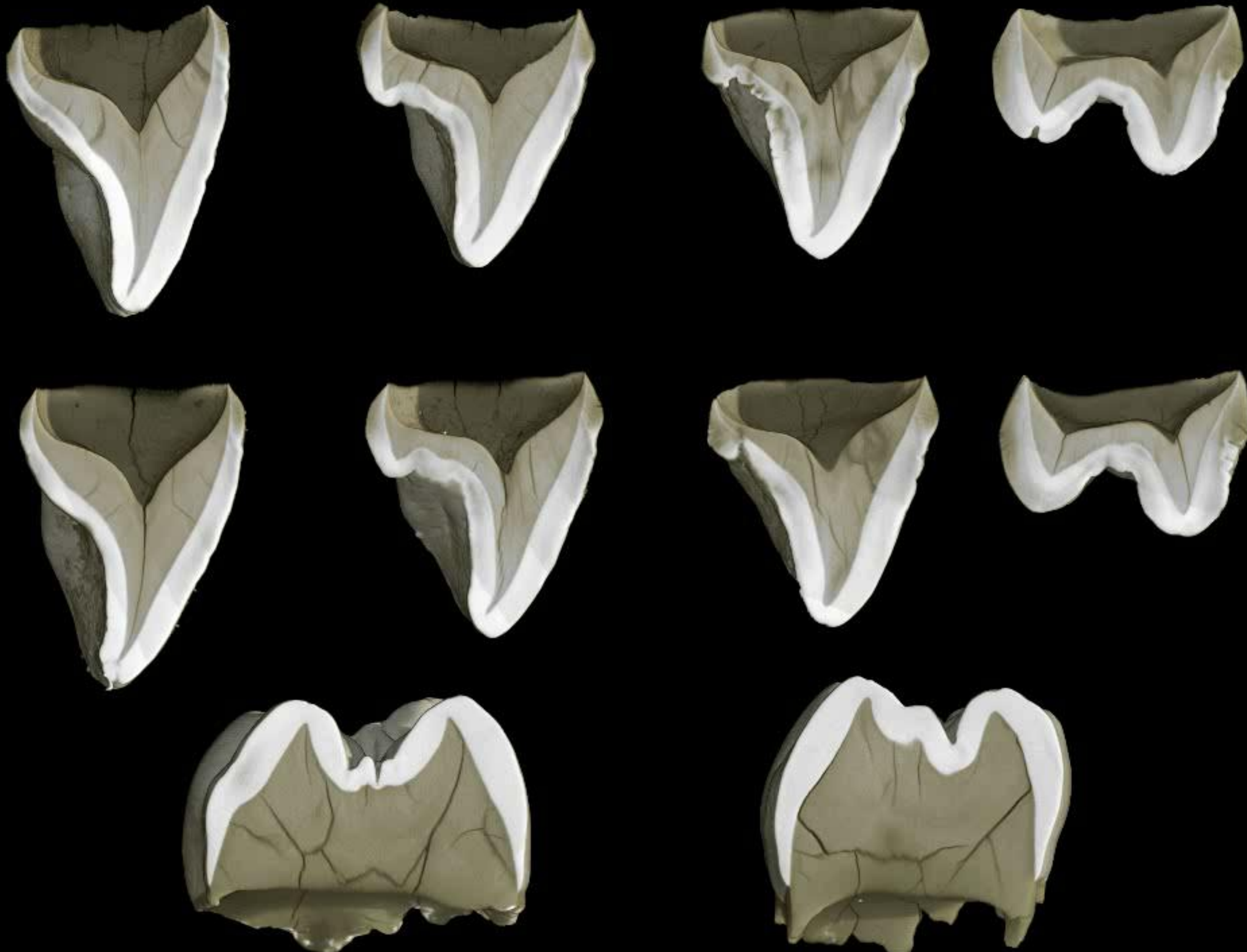


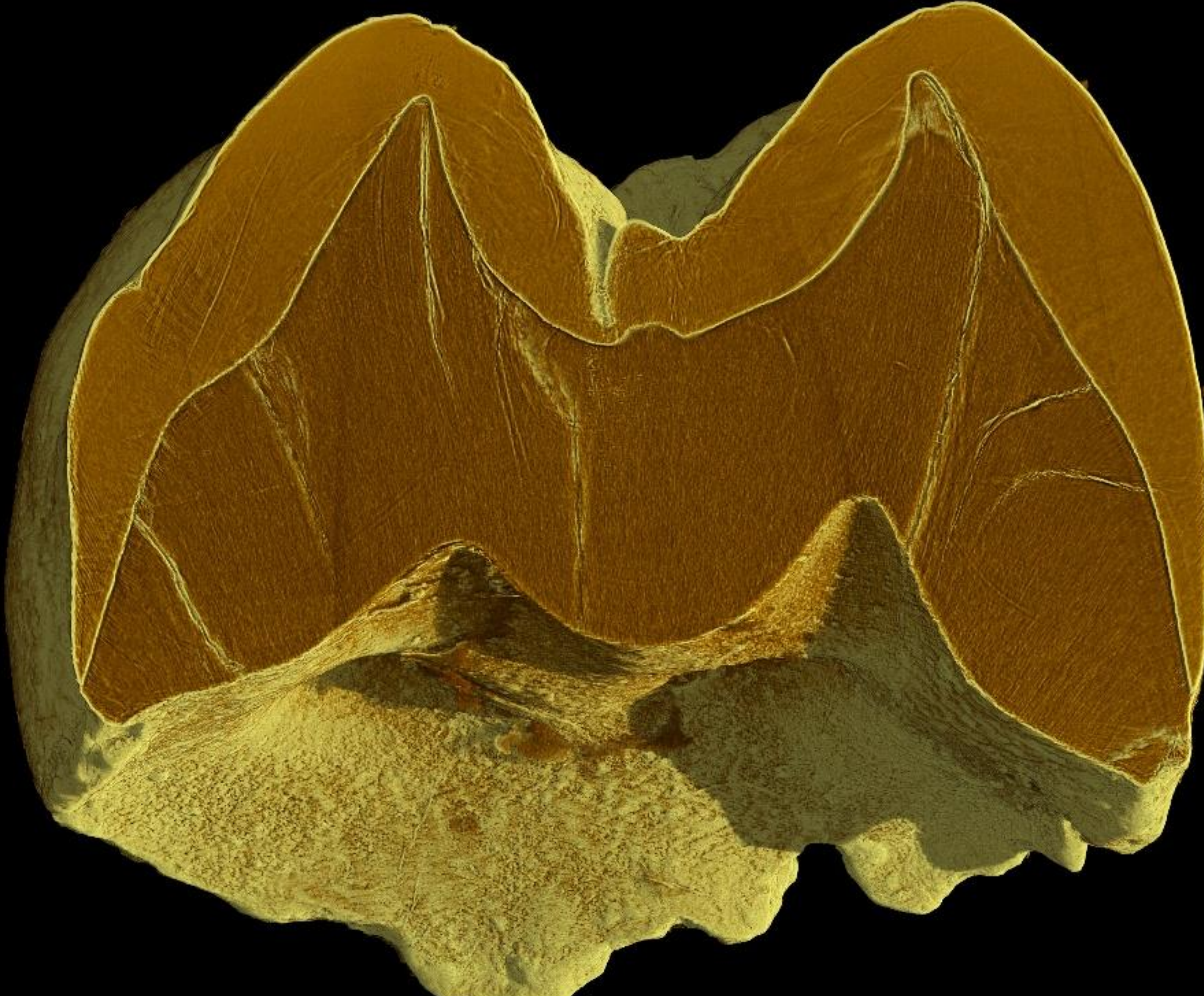


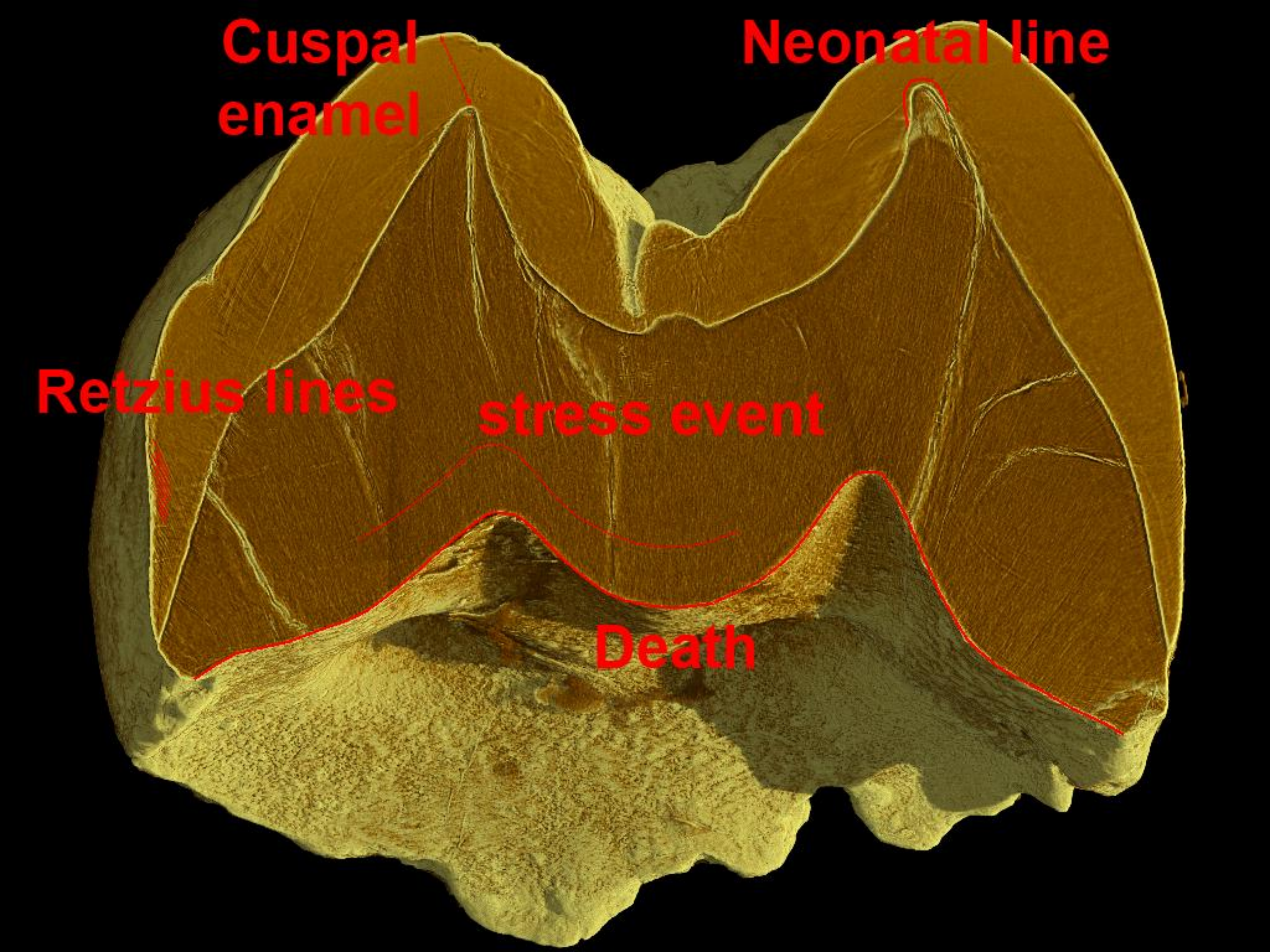


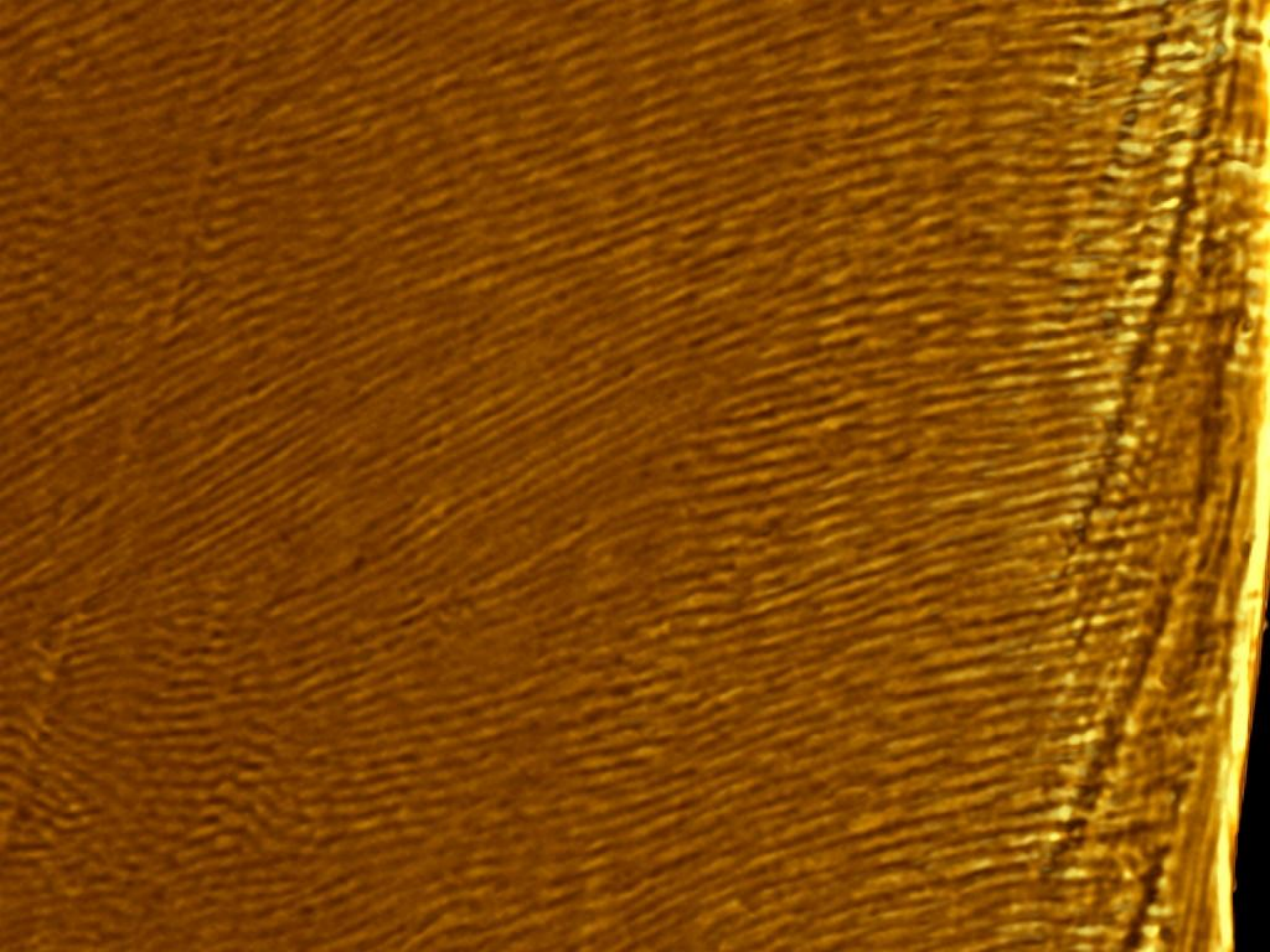
Death is recorded in all the permanent teeth








Cuspal
enamel
Neonatal line
Retzius lines
stress event
Death




8 days periodicity between two consecutive Retzius lines

Age at death calculation

Measured age at death

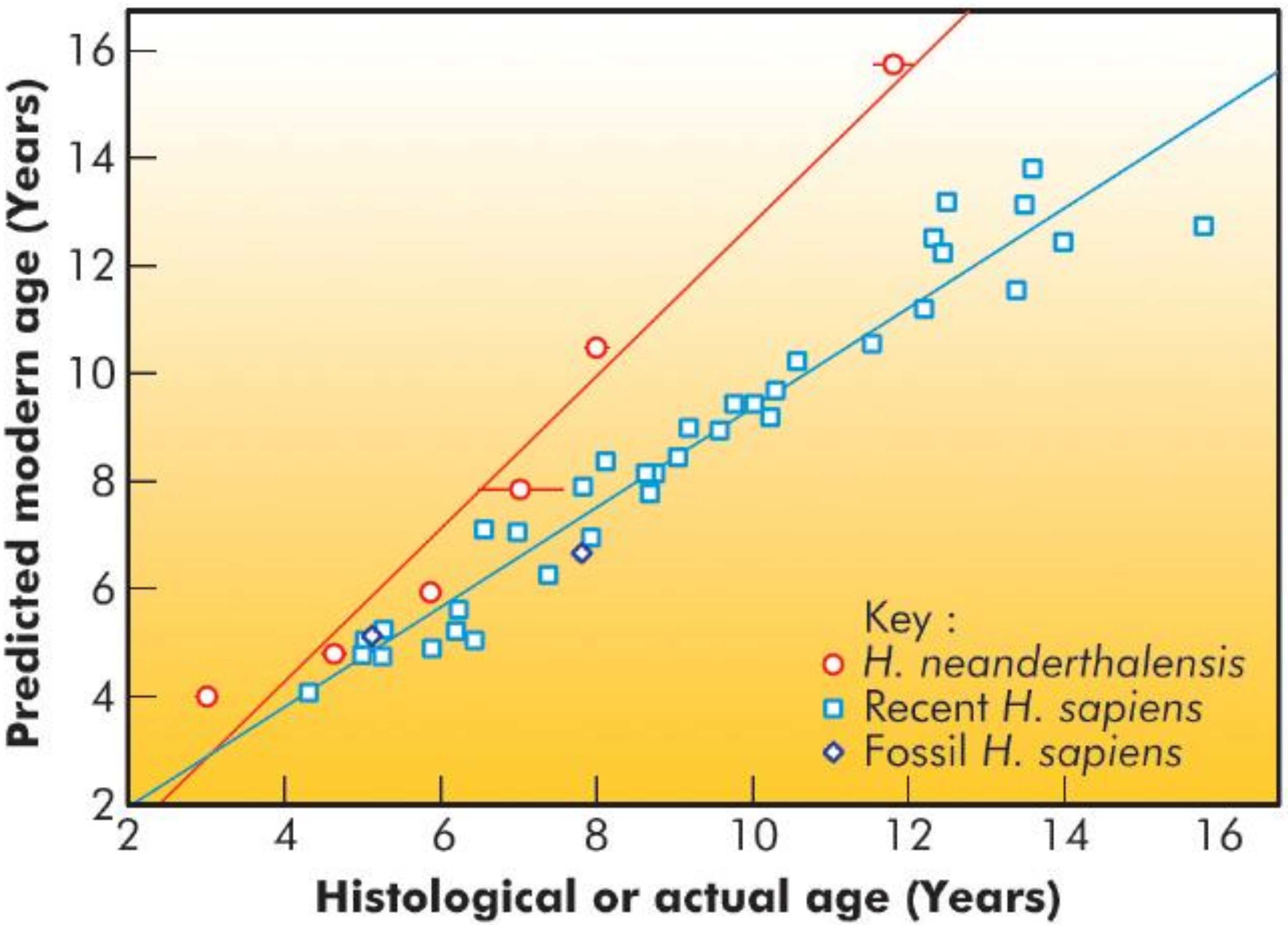
||

(number of long period lines) * periodicity

(137) * 8 = 1096 days

Engis 2 child was 3 years old when it died, not 4. Same approach on several other fossils demonstrated that in average Neanderthals were developing faster than *Homo sapiens*

Comparison estimated / measured age



Smith et al. (2007a,b) PNAS

Phase retrieval process

Going from edge detection to quantitative phase map by holo-tomography

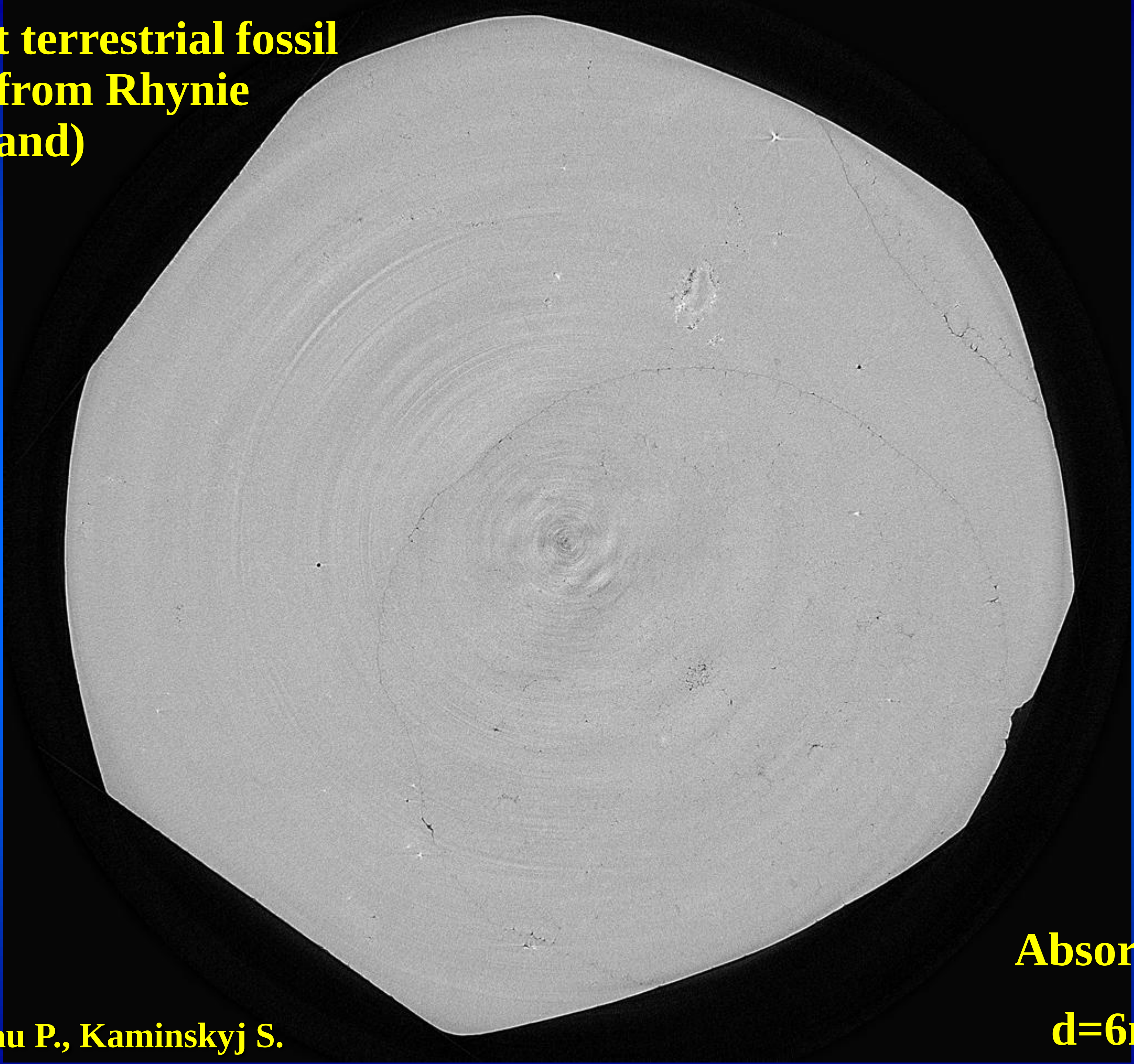
Phase retrieval based on several propagation distances

Repeated during Sample rotation

Very powerful technique for phase retrieval, and historically the first one to have been successfully used on fossils.

The main limitations are the strong requirements on acquisition protocol and quite complex data processing, especially for dense specimens

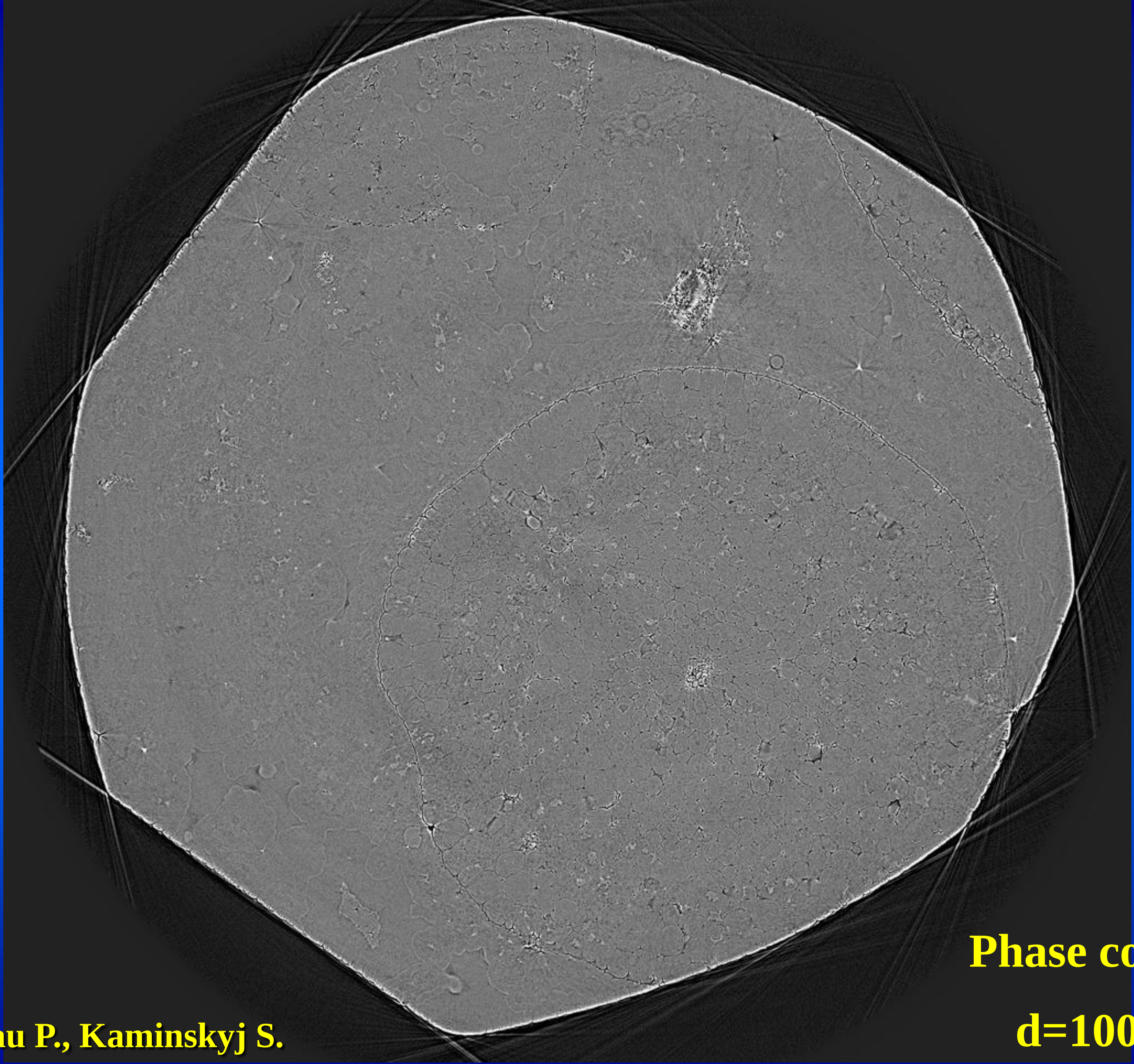
Oldest terrestrial fossil plant from Rhynie (Scotland)



Absorption

d=6mm

Tafforeau P., Kaminskyj S.



Phase contrast

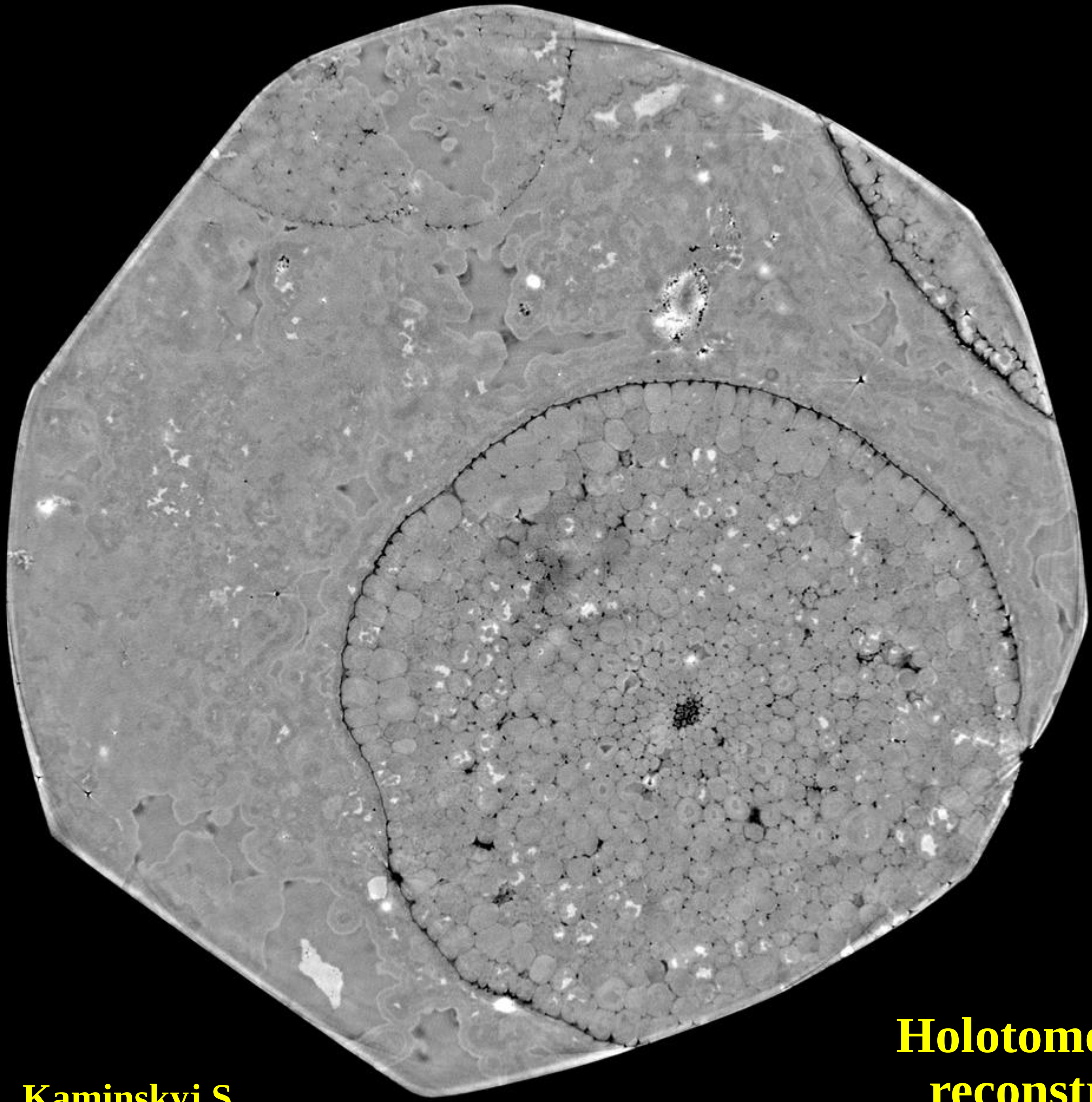
d=100mm

Tafforeau P., Kaminskyj S.

Phase contrast

d=300mm

Tafforeau P., Kaminskyj S.



Holotomographic reconstruction

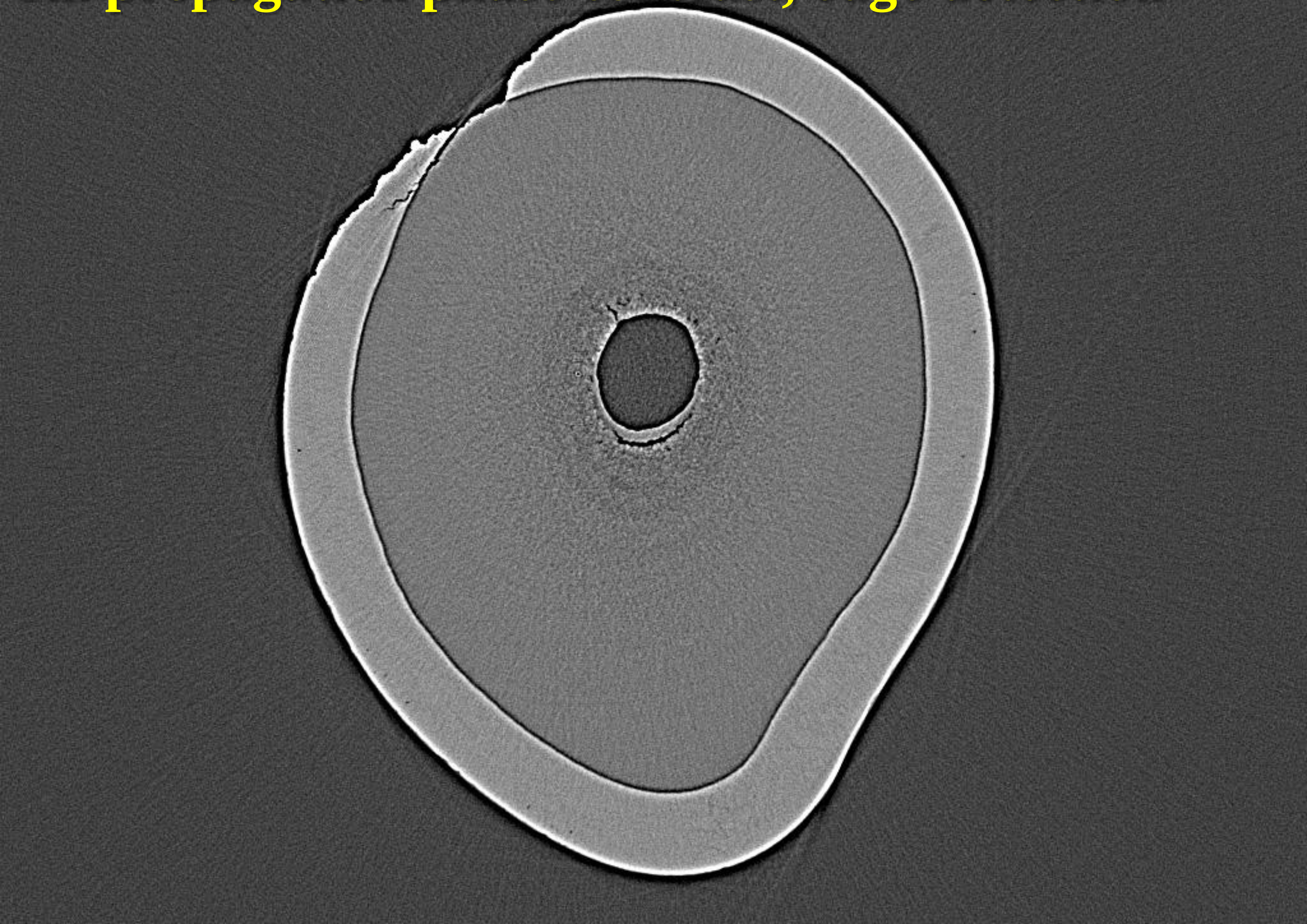
Tafforeau P., Kaminskyj S.

A major breakthrough for phase imaging of fossil (but not only) :

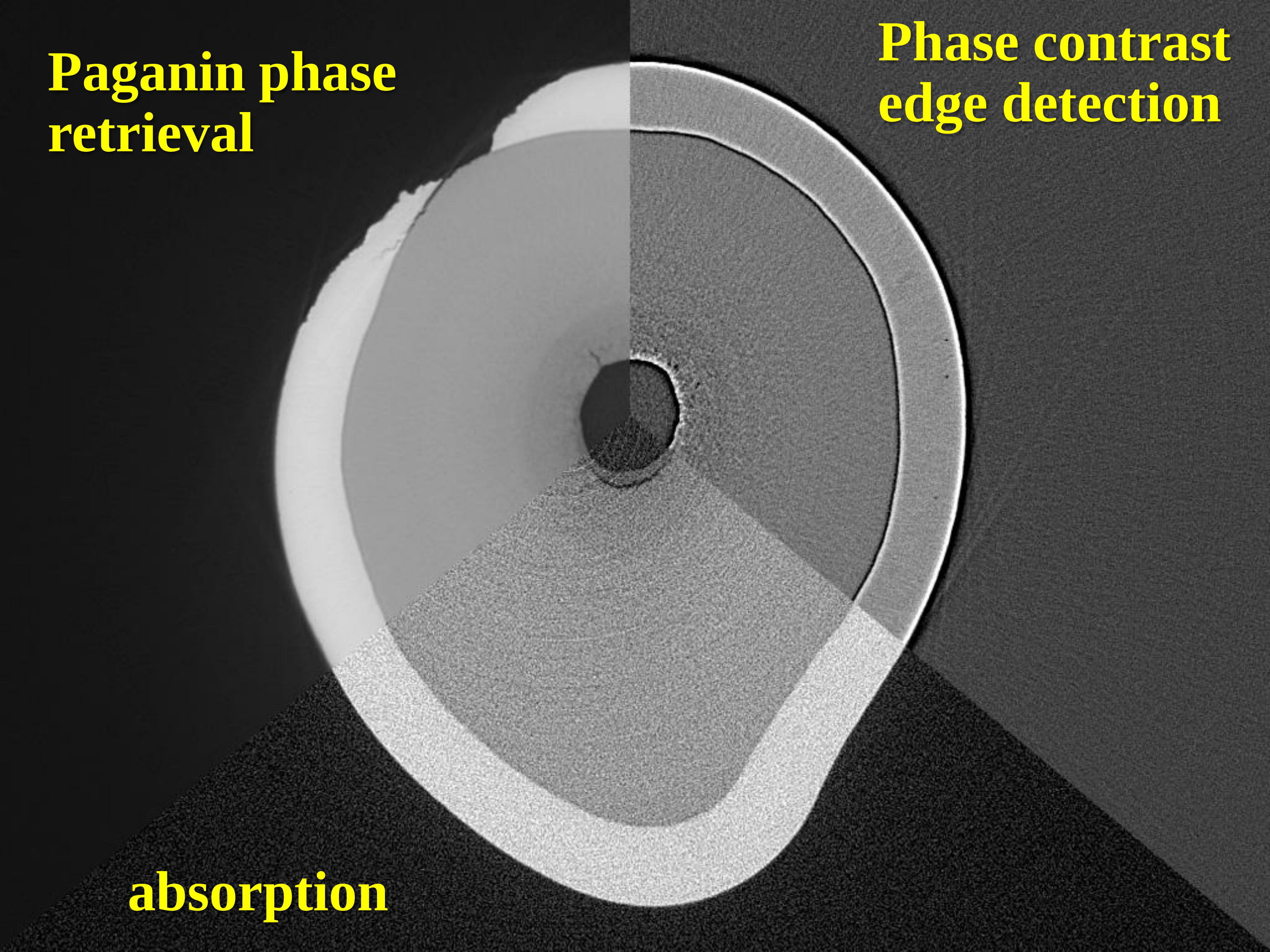
Single distance phase retrieval using modified approach of Paganin *et al.*


absorption

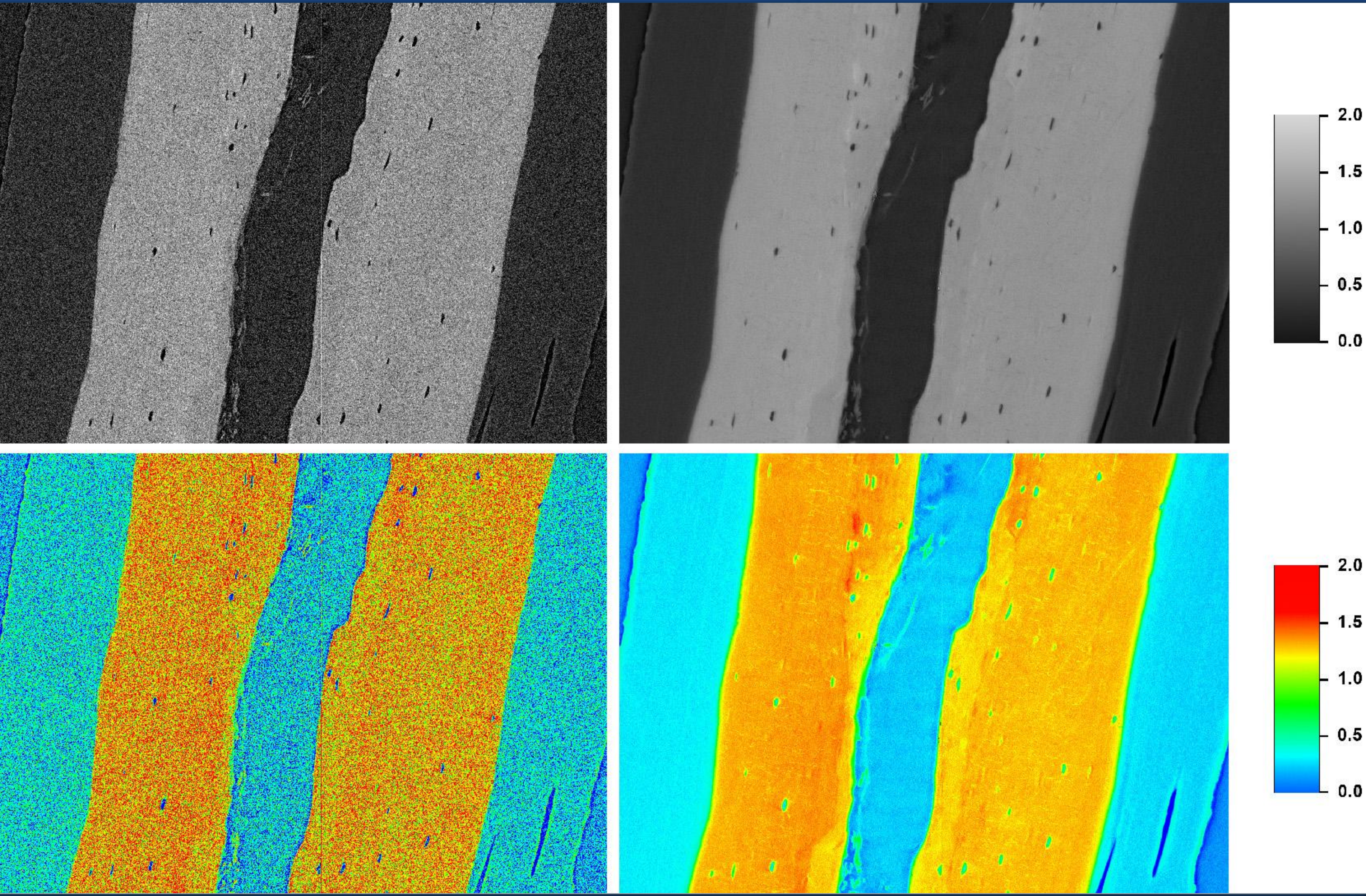
1m propagation phase contrast, edge detection



Reconstruction after single distance phase retrieval


Paganin phase retrieval
Phase contrast edge detection
absorption

19.1 keV pink beam at 0.7 microns



Reconciling X-ray microtomography of recent specimens and paleogenetics: simple technical solutions and good practices

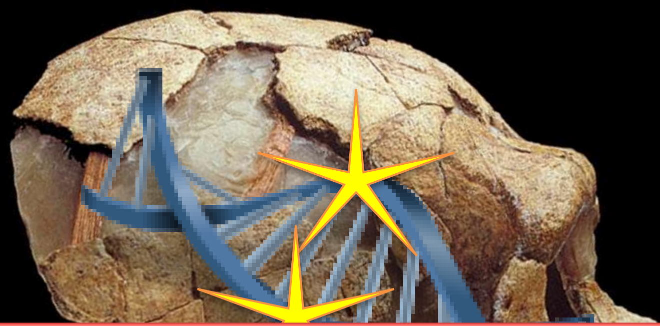
P. Tafforeau, A. Le Cabec, A. Immel, M. Bonazzi, V. Schuenemann, A. Herbig, H. Temming, B. Viola, J.-J. Hublin and J. Krause

Australopithecus sediba: **1.97 My**

Imaged at the ESRF in 2009

Carlson,..., **Tafforeau**, ... ***Science***, 2011

Homo neanderthalensis: **36 Ky**



Extremely hot topic in palaeoanthropology:

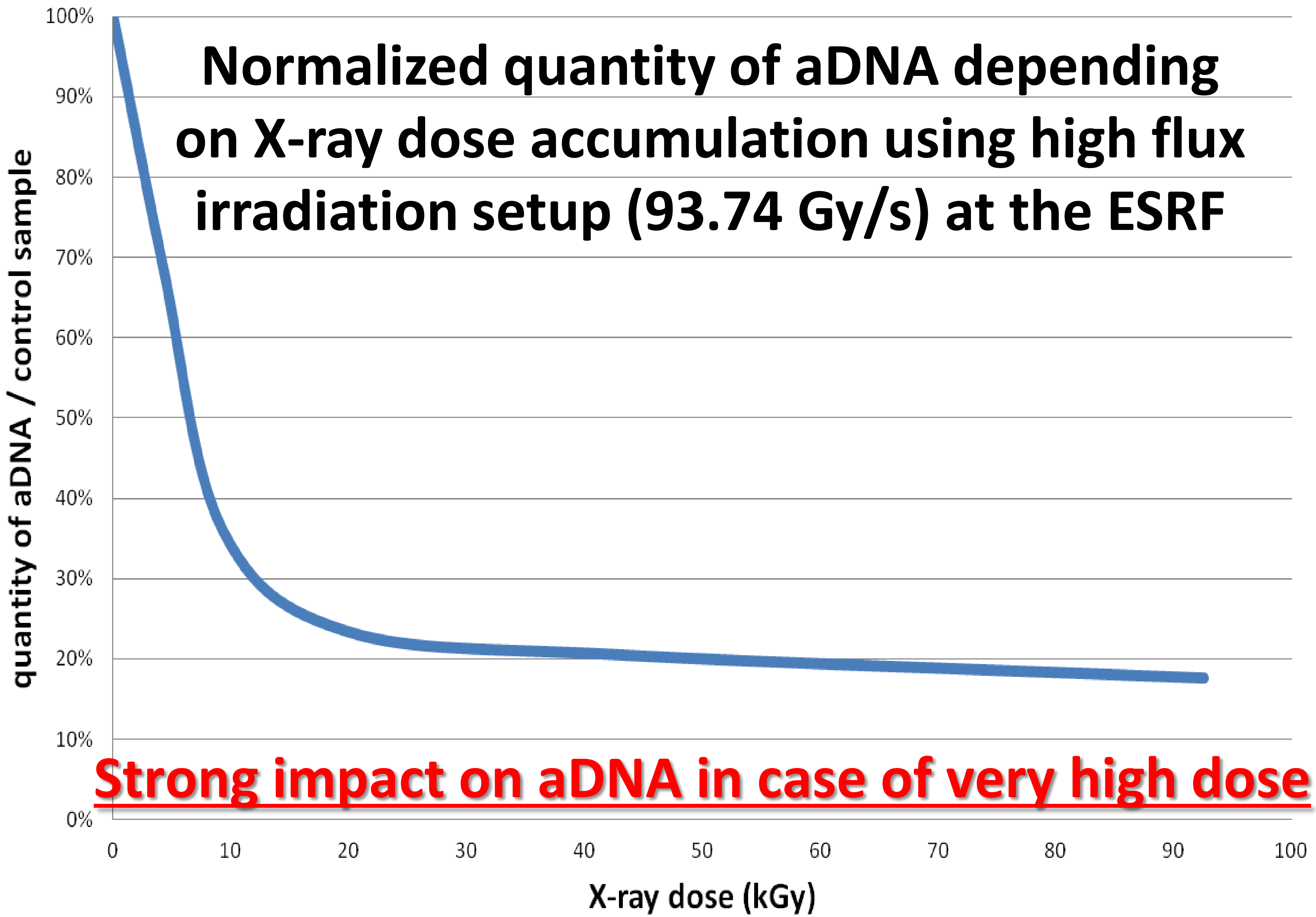
High risk of loosing access to recent hominids for X-ray imaging if not solved

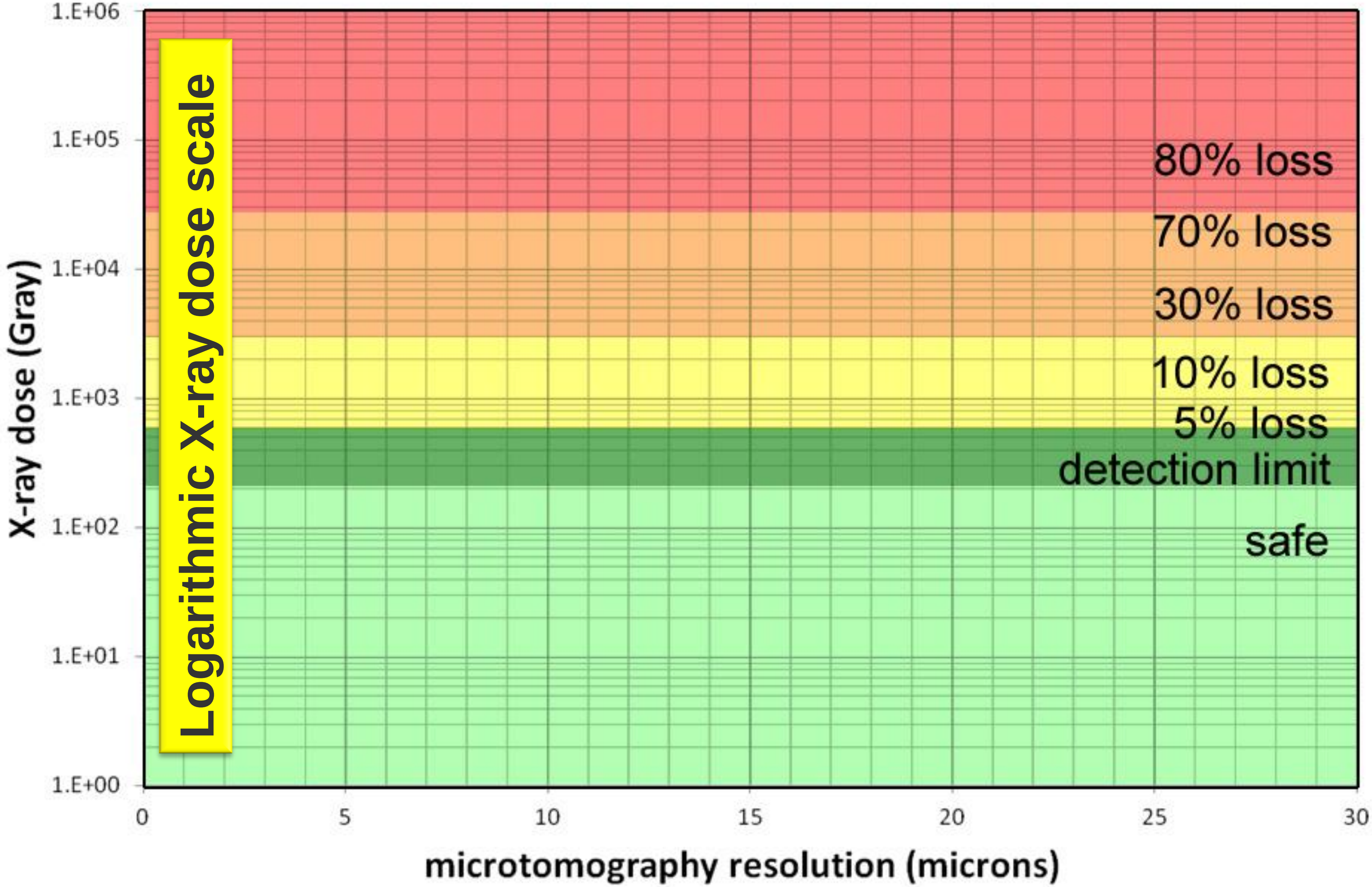
Old fossils:

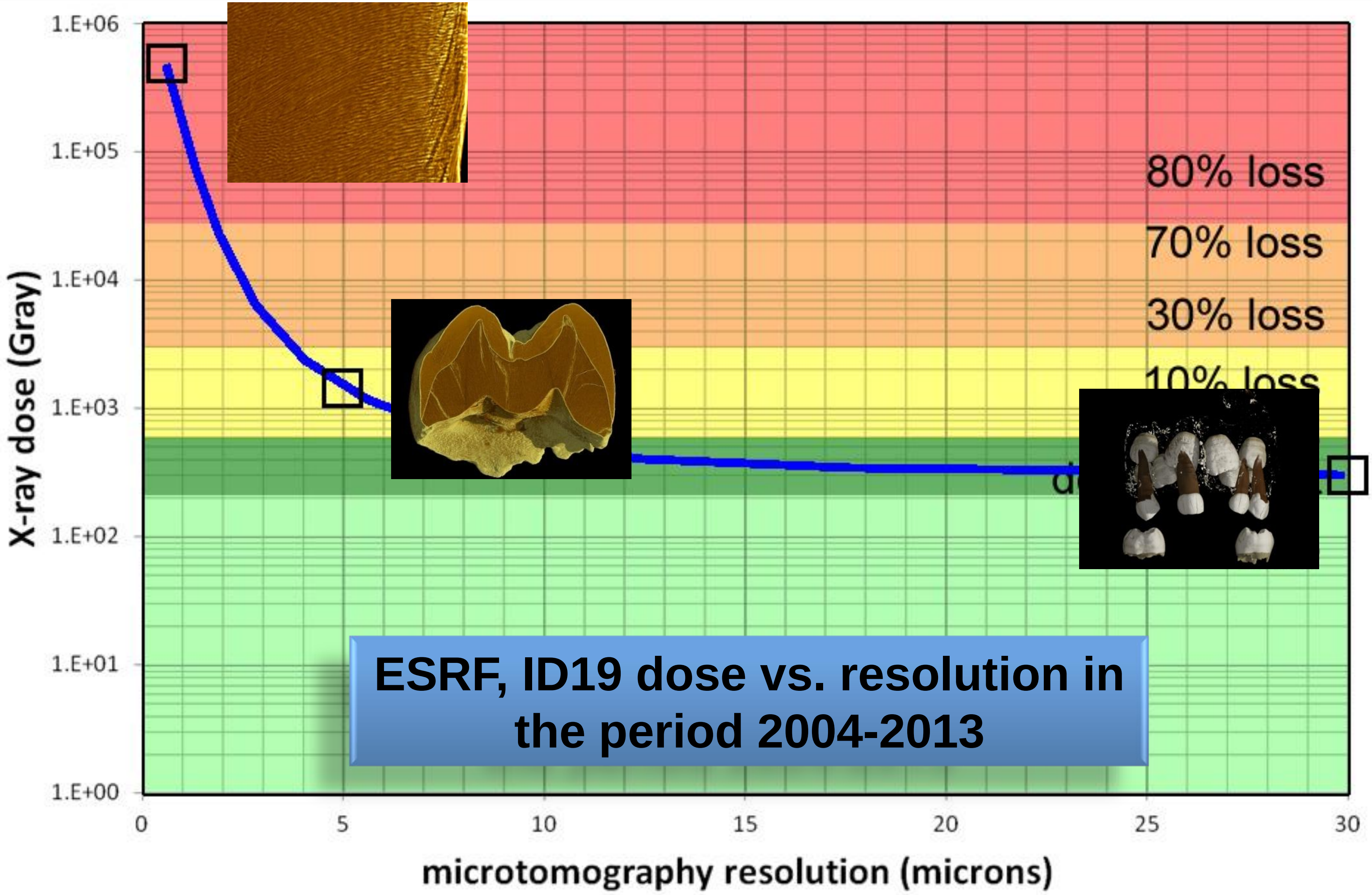
No risk for X-ray imaging

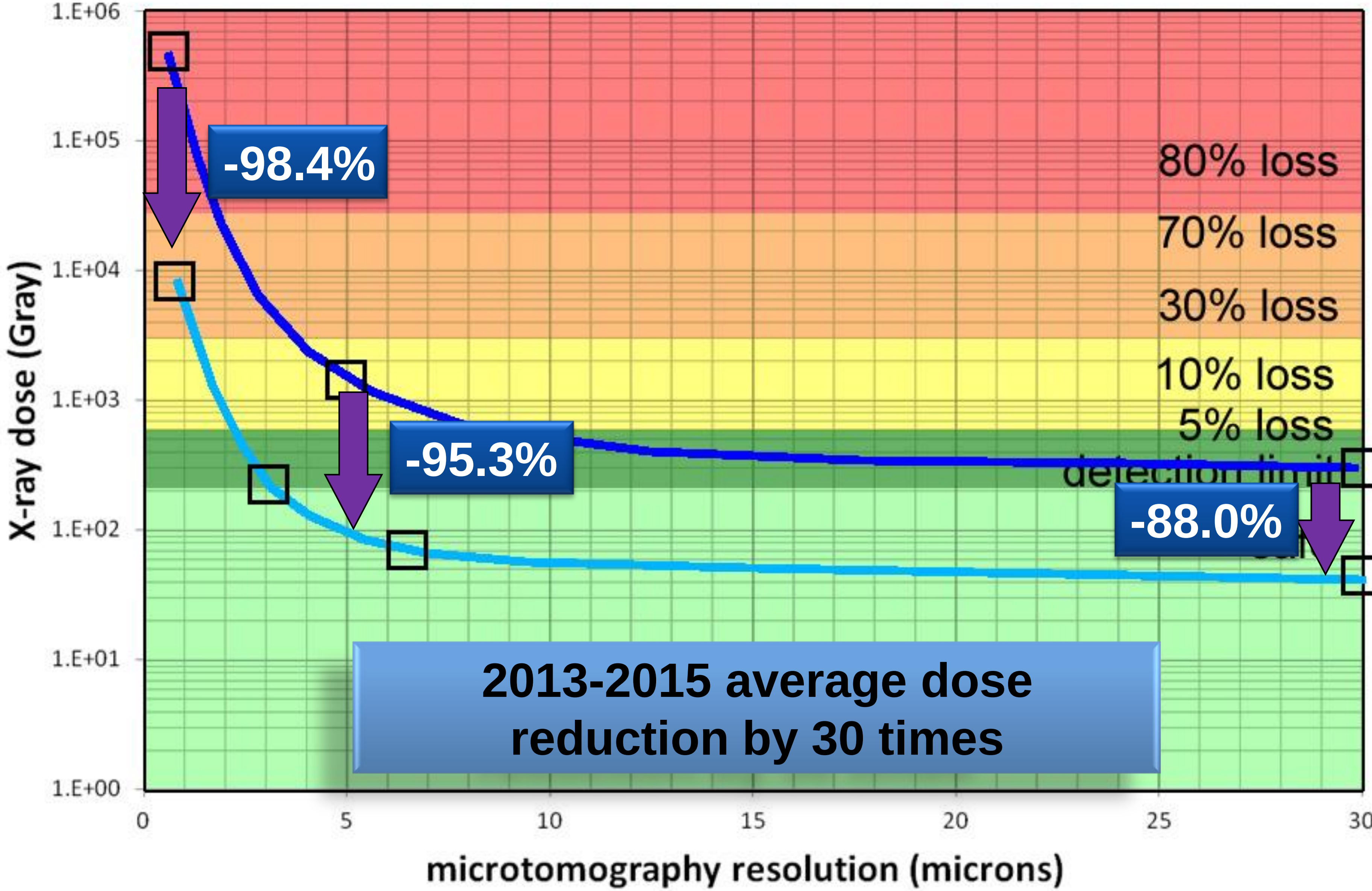
Recent fossils:

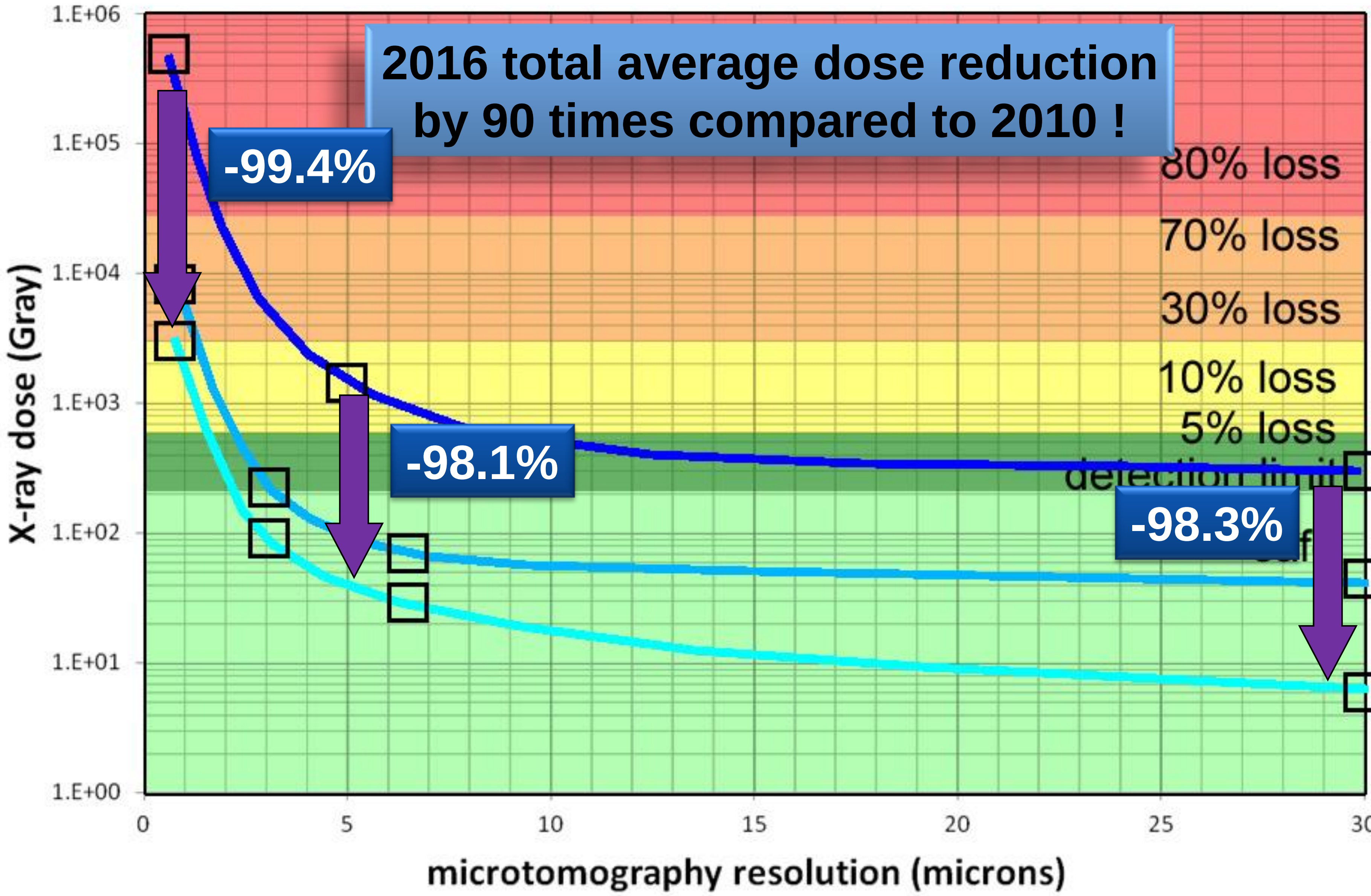
Risk of aDNA degradation

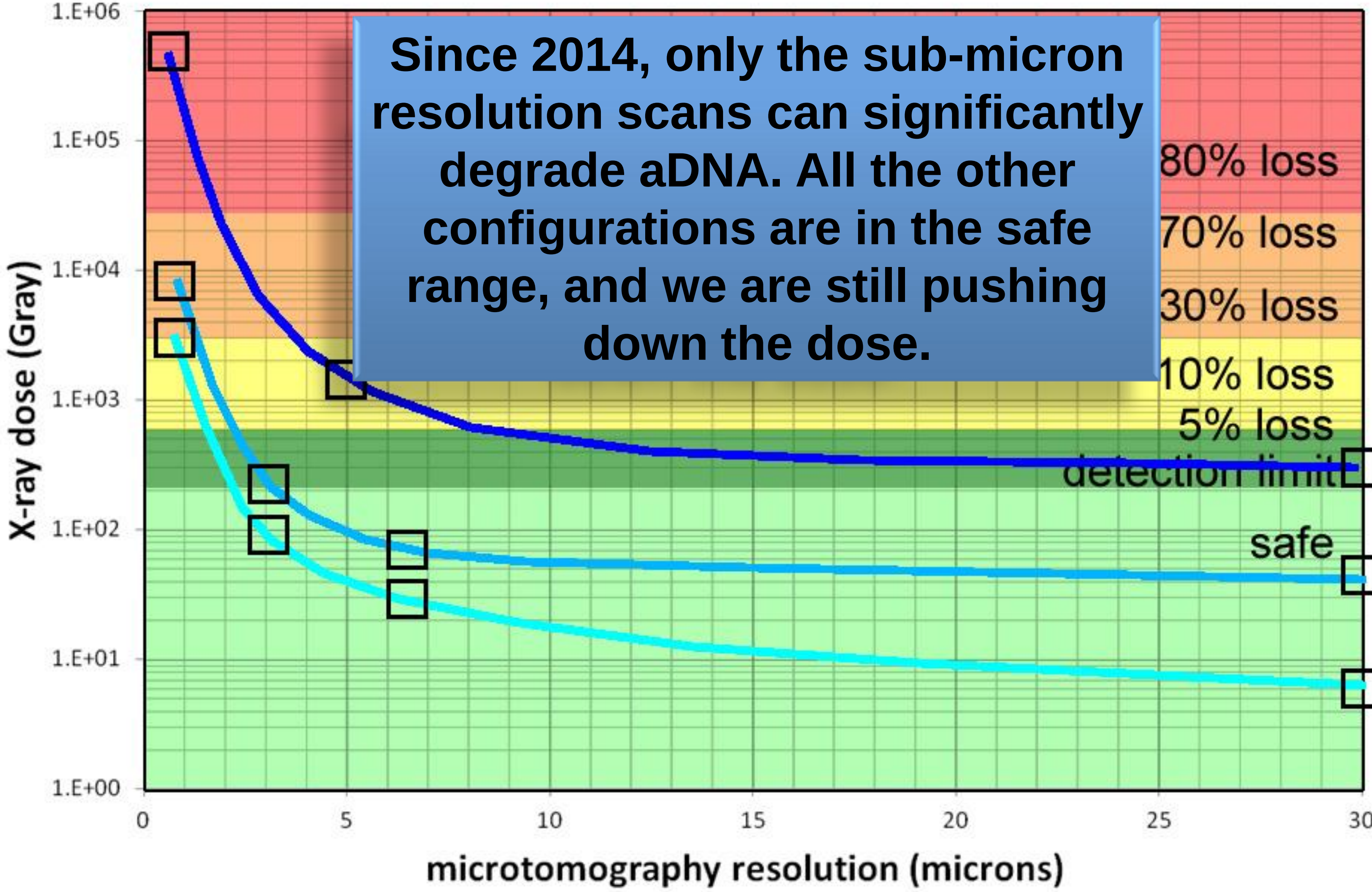

Normalized quantity of aDNA depending on X-ray dose accumulation using high flux irradiation setup (93.74 Gy/s) at the ESRF
quantity of aDNA / control sample
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
10
20
30
40
50
60
70
80
90
100
X-ray dose (kGy)
Strong impact on aDNA in case of very high dose


1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
X-ray dose (Gray)
Logarithmic X-ray dose scale
80% loss
70% loss
30% loss
10% loss
5% loss
detection limit
safe
0
5
10
15
20
25
30
microtomography resolution (microns)

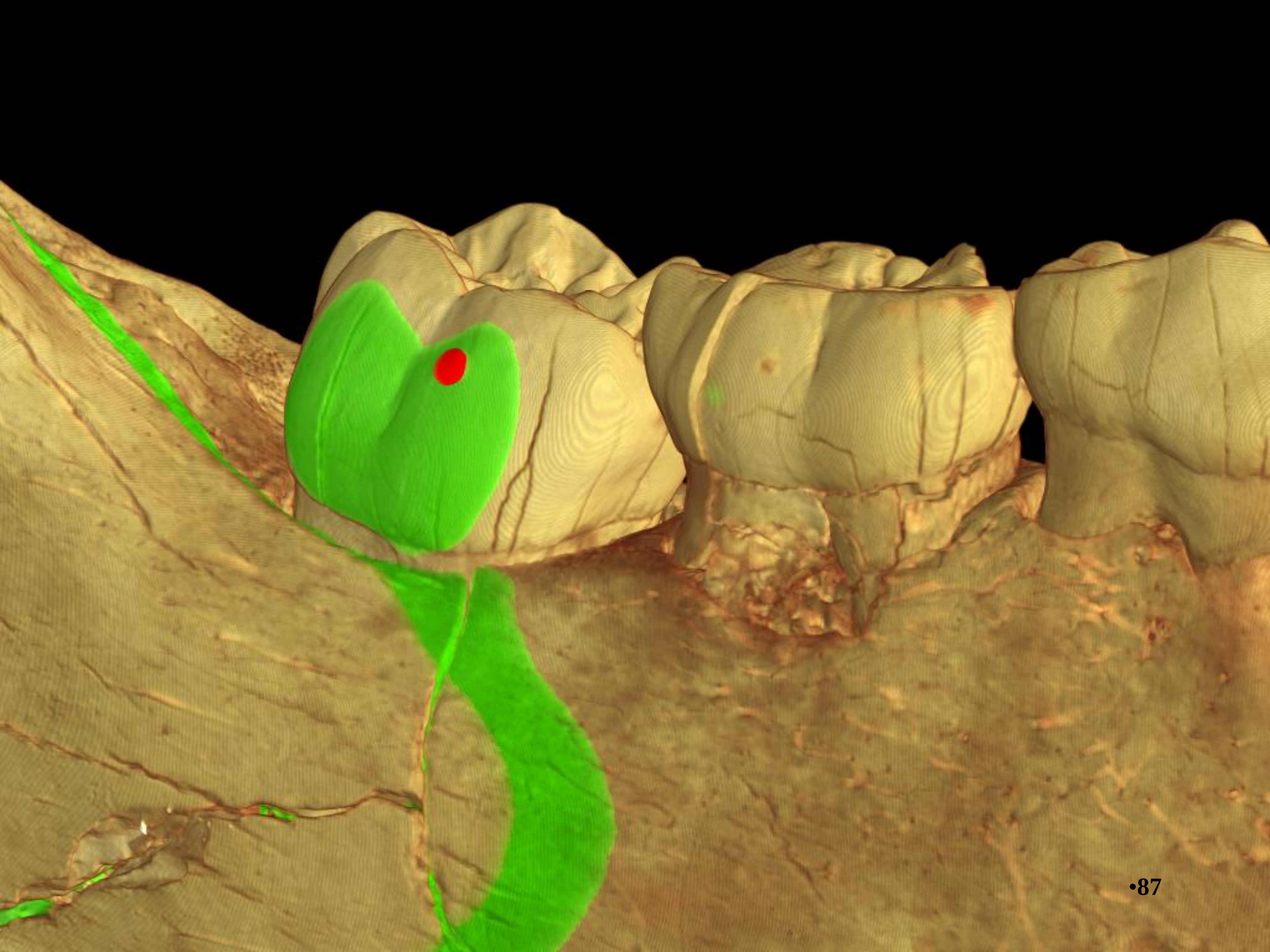

ESRF, ID19 dose vs. resolution in the period 2004-2013
X-ray dose (Gray)
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
0
5
10
15
20
25
30
microtomography resolution (microns)
80% loss
70% loss
30% loss
10% loss

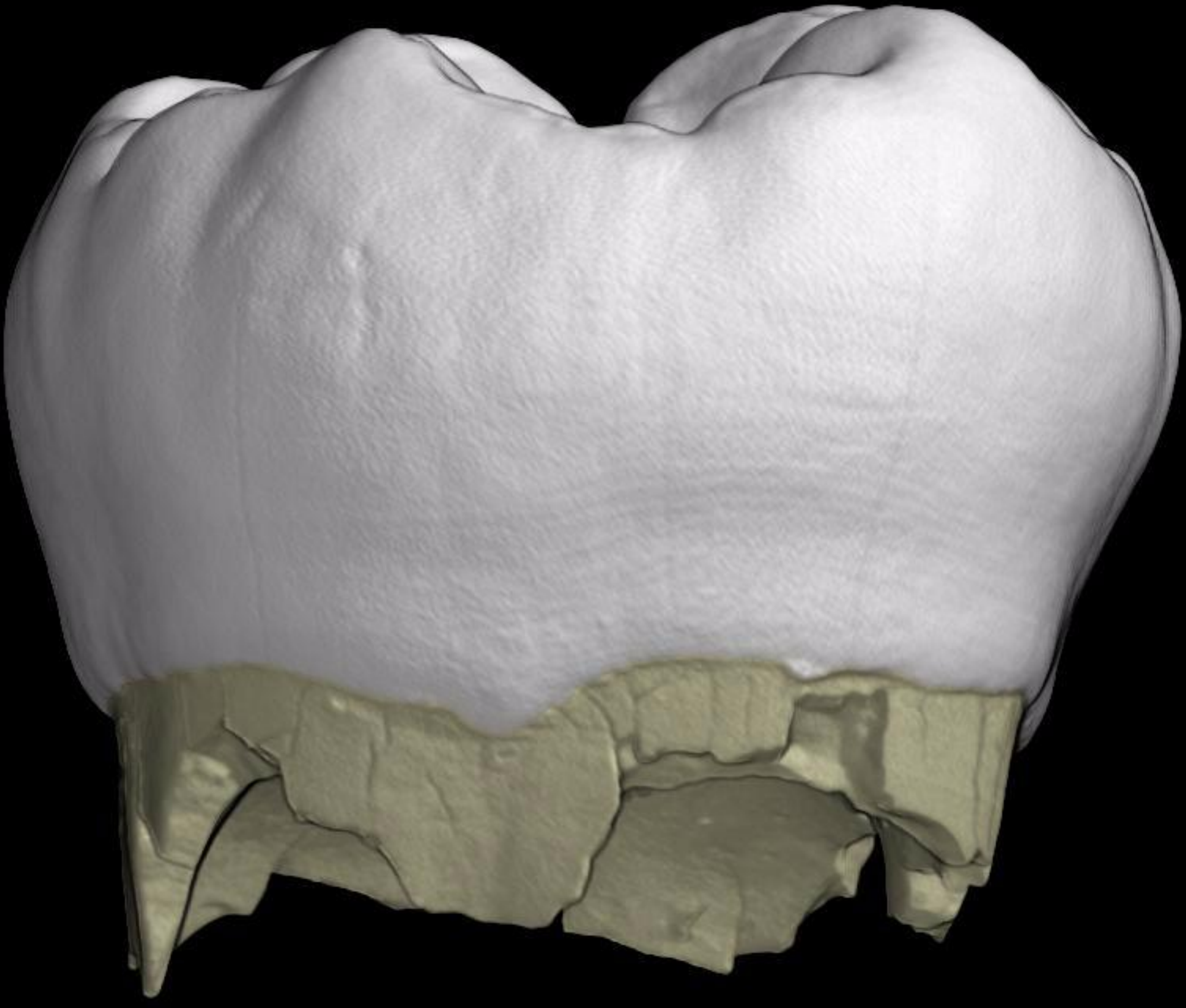

1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
X-ray dose (Gray)
0
5
10
15
20
25
30
microtomography resolution (microns)
80% loss
70% loss
30% loss
10% loss
5% loss
-98.4%
-95.3%
-88.0%
2013-2015 average dose
reduction by 30 times

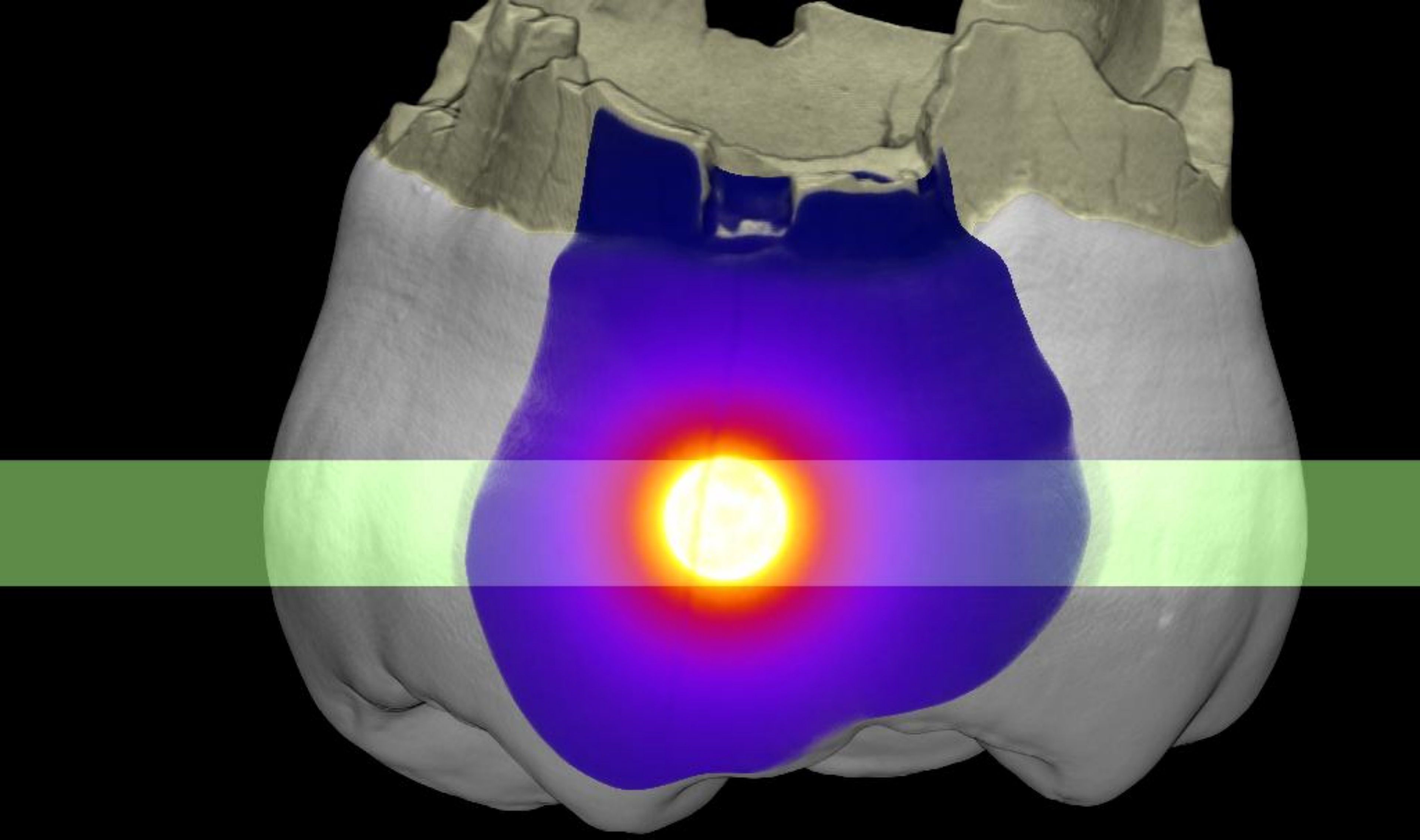

2016 total average dose reduction
by 90 times compared to 2010 !
-99.4%
-98.1%
-98.3%
80% loss
70% loss
30% loss
10% loss
5% loss
detection limit
X-ray dose (Gray)
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
0
5
10
15
20
25
30
microtomography resolution (microns)



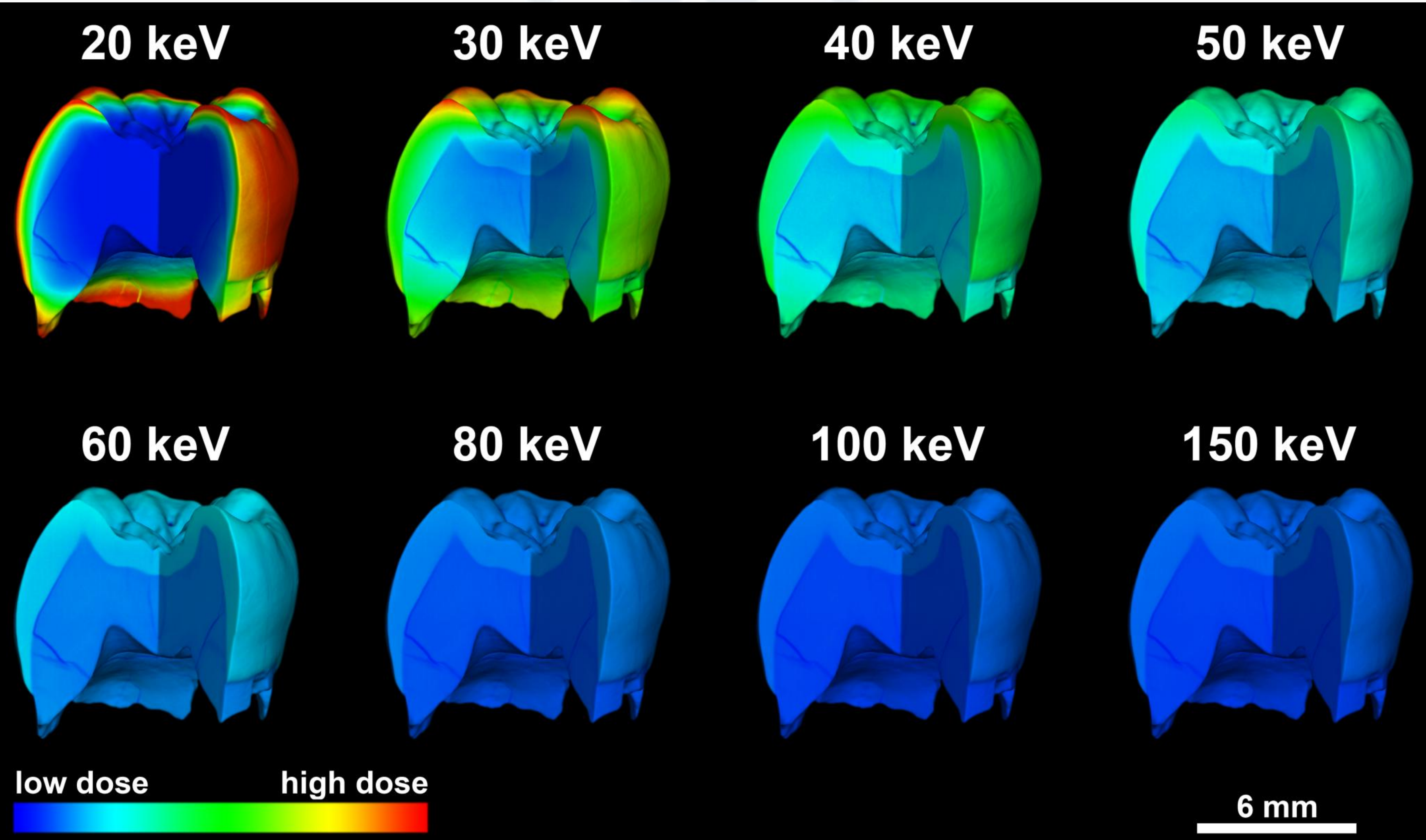
Since 2014, only the sub-micron resolution scans can significantly degrade aDNA. All the other configurations are in the safe range, and we are still pushing down the dose.







EFFECT OF THE X-RAY SPECTRUM : 3D dose deposition on a fossil tooth depending of energy for constant photon flux



TO BE CONTINUED...

Thank you for your attention

Aknowledgments

All the staff of the beamlines ID19, ID17 and BM05, as well as all the people in administration, technical services, computing, control, maintenance... i.e. all the people at the ESRF that are working every day to make such kind of scientific success story happening.

All our collaborators around the world that allows us to work on so exceptional fossils and to develop new research approaches in palaeontology.

Special thanks to José Baruchel who made all this possible 17 years ago by helping a crazy young PhD student in palaeontology to access for the first time to a synchrotron beamline.