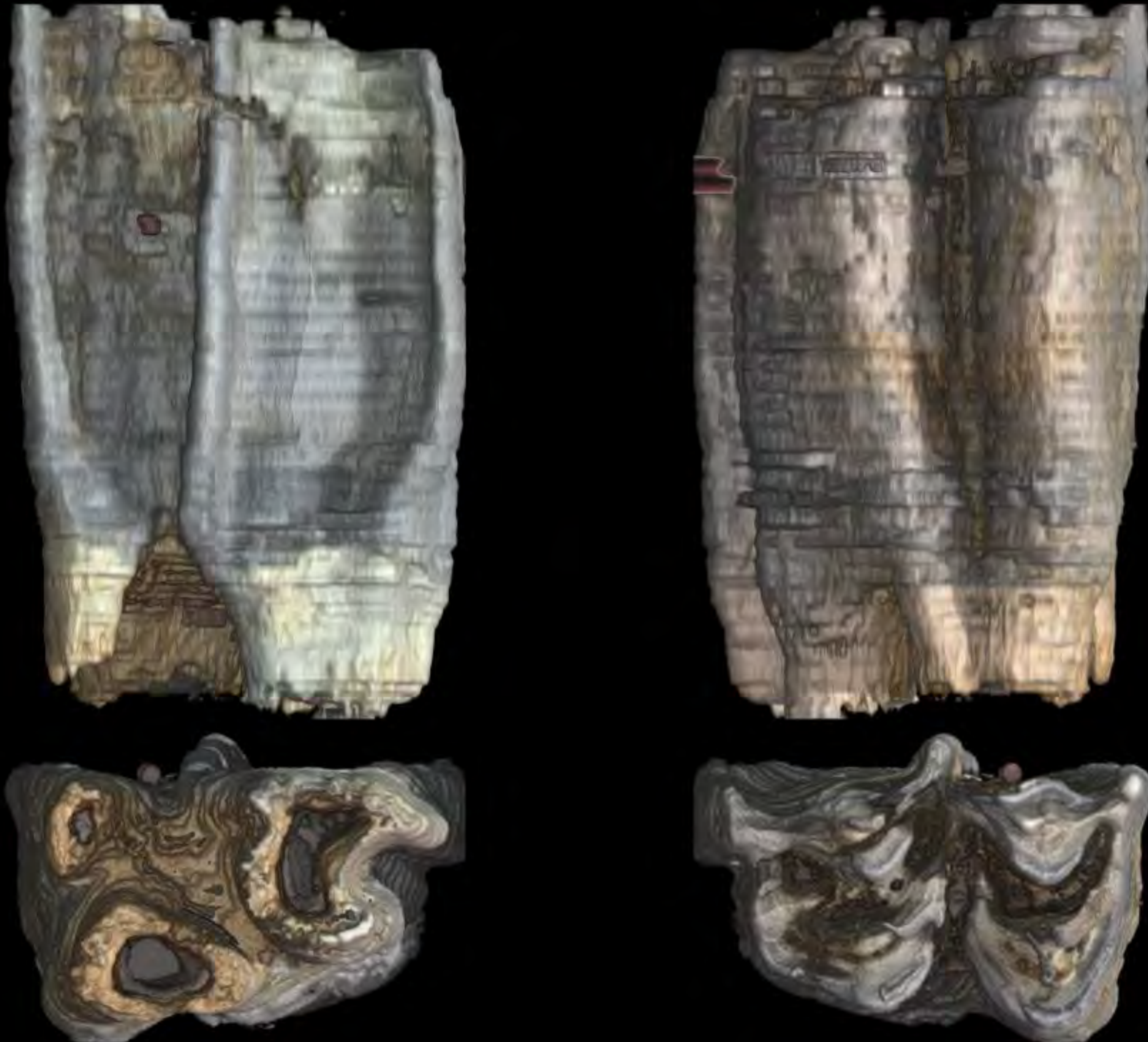
Applications of synchrotron X-ray imaging in palaeontology, a synthesis after 14 years of experiments

Paul Tafforeau

Once upon a time, nearly 17 years ago, in a not so far away palaeontology laboratory, a poor young master student was trying to find a way to study teeth in 3D …

One of these approaches was the so called "poor guy microtomo(graphy)"

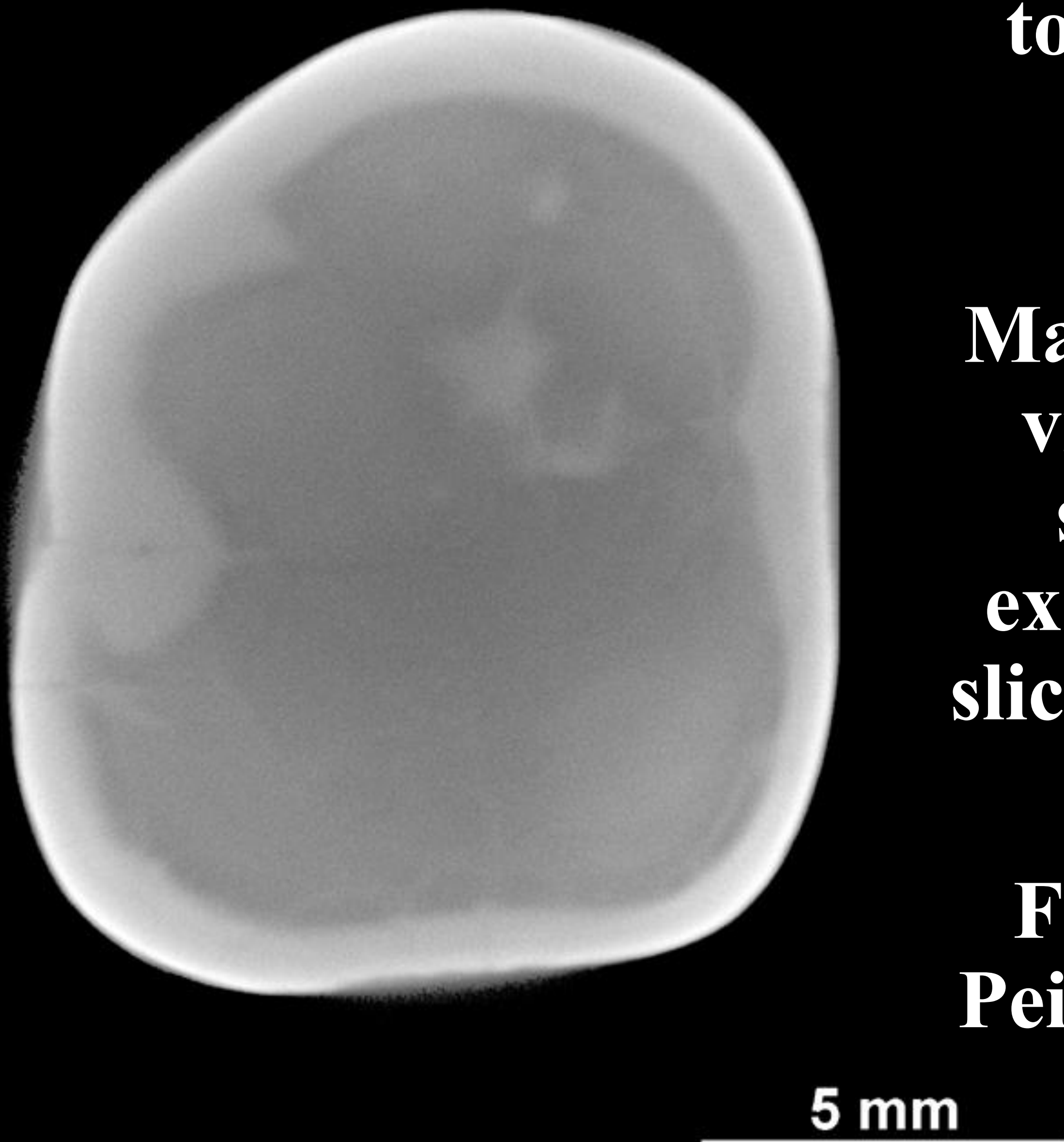
It consists into serial slices into the specimen that are then virtually stacked together on a computer.



But on important or even unique specimens, such as fossil primate teeth, this solution is generally unconceivable

Fortunately, after some time, the young PhD student eared about the possibilities of X-ray microtomography.

Gilles Peix gave him the opportunity of a free test on his laboratory machine at the INSA of Lyon, …



… that turned out not to be a tremendous success.

Main structures were visible, but hardly separable in 3D, except using slice by slice manual selection.

Fortunately, Gilles Peix had another idea

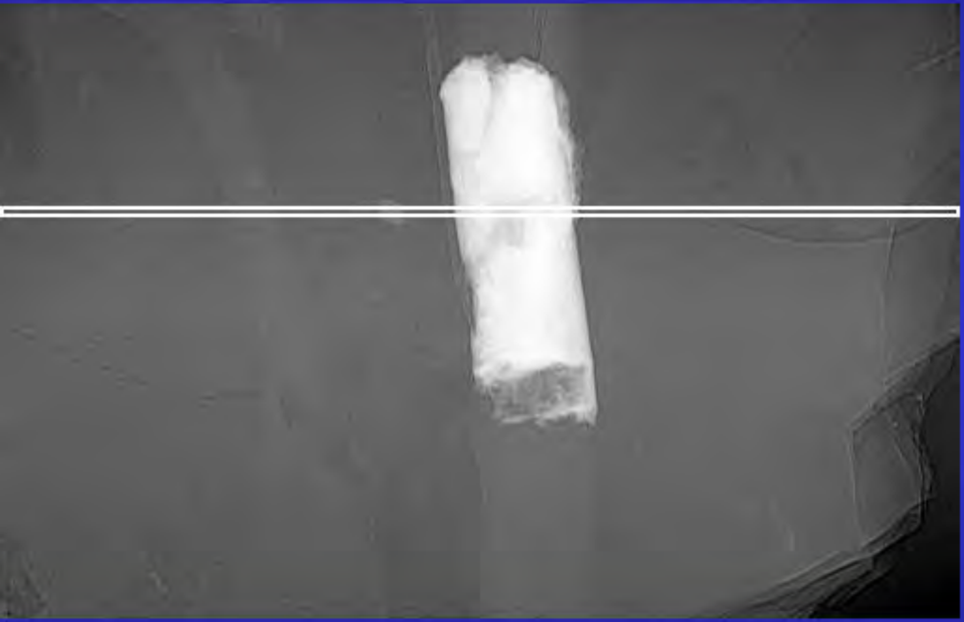
…

… and told to this student to contact José Baruchel who was in charge of a “super-microtomograph” in a big machine called a synchrotron.

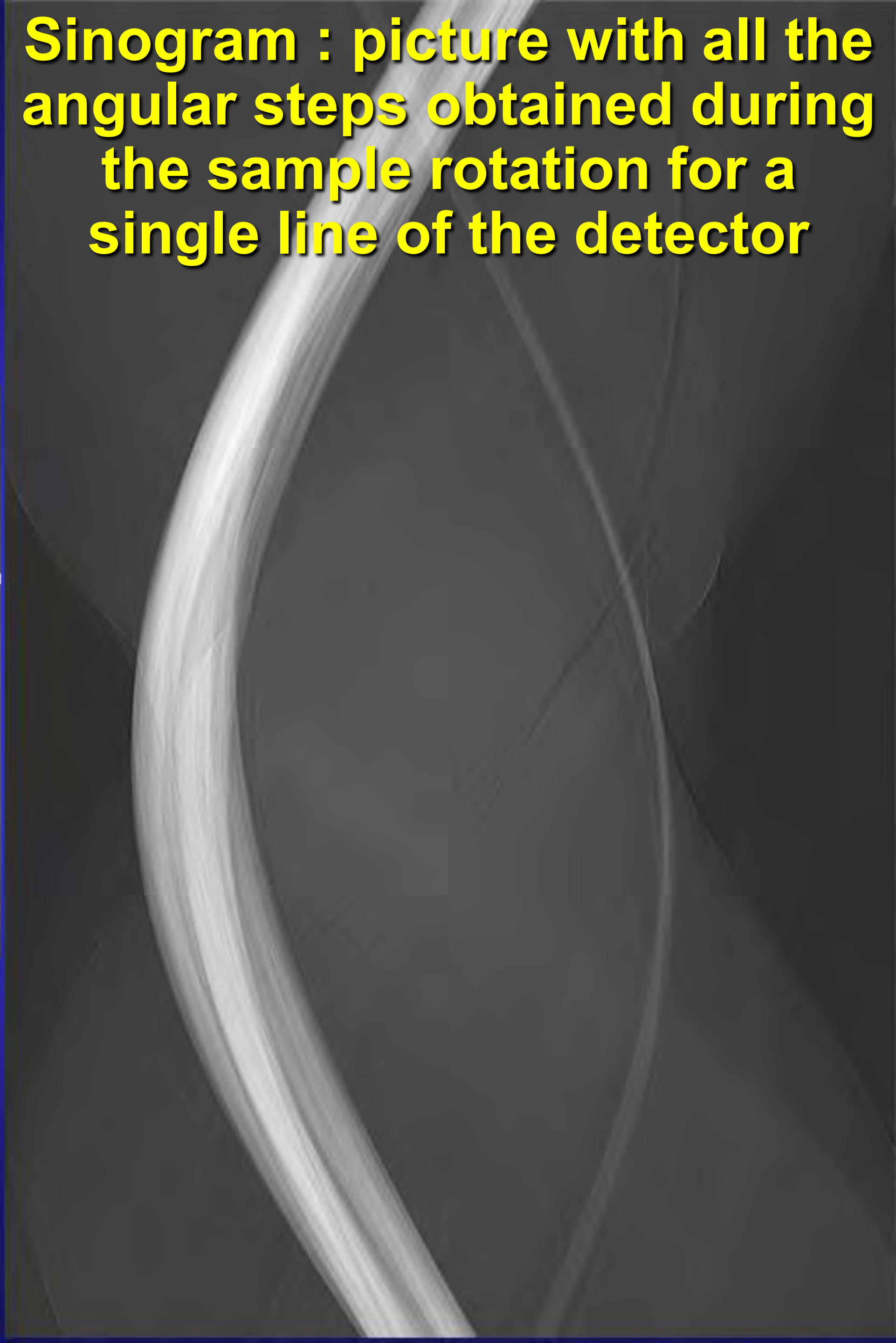
We were in 2000. It was the first ever palaeontology proposal for synchrotron imaging of fossils, and the beginning of our story …

Tomographic reconstruction: the sinogram concept

Example on 100 million years old fossil amber

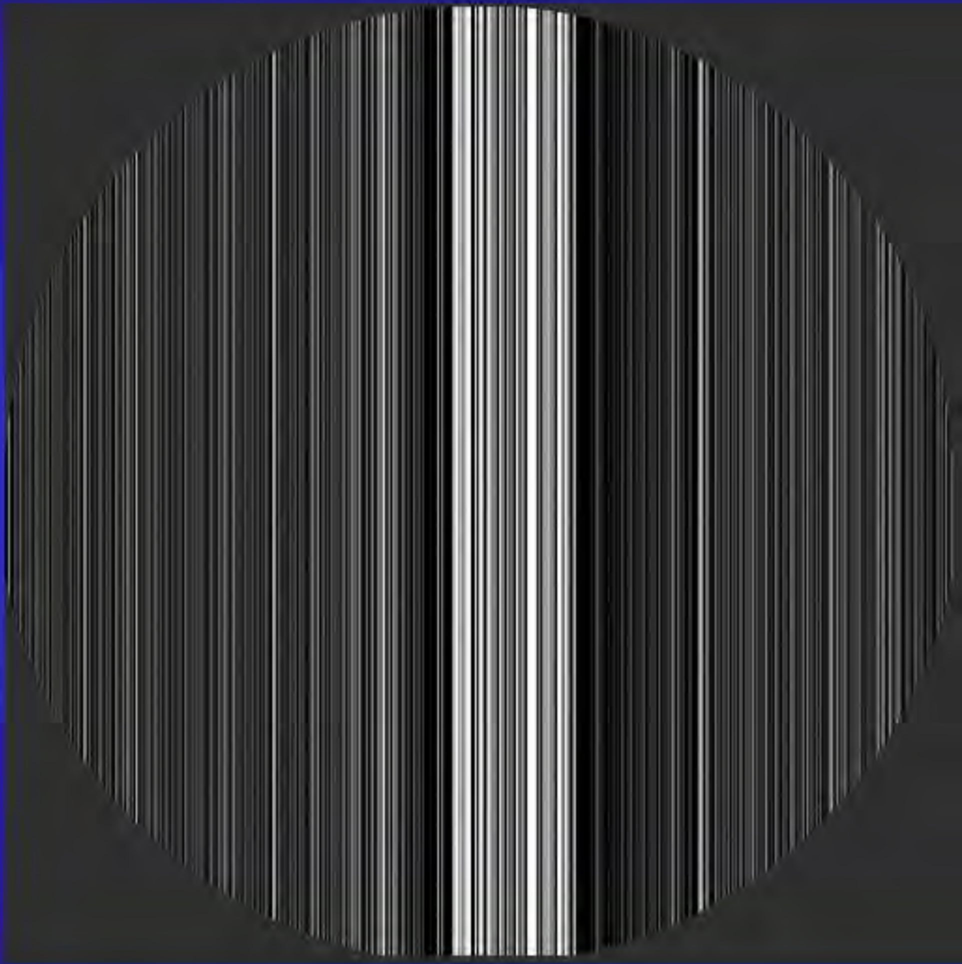


Projection : normalized radiograph of the sample

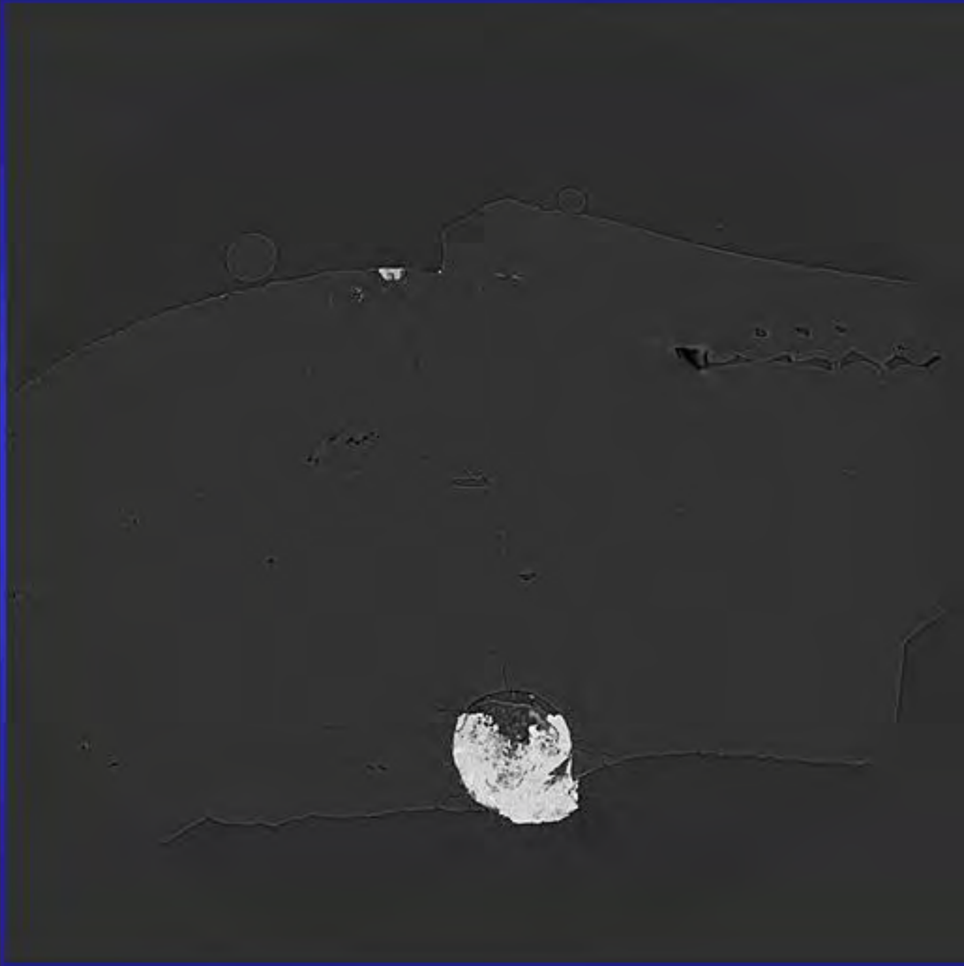


Sinogram : picture with all the angular steps obtained during the sample rotation for a single line of the detector

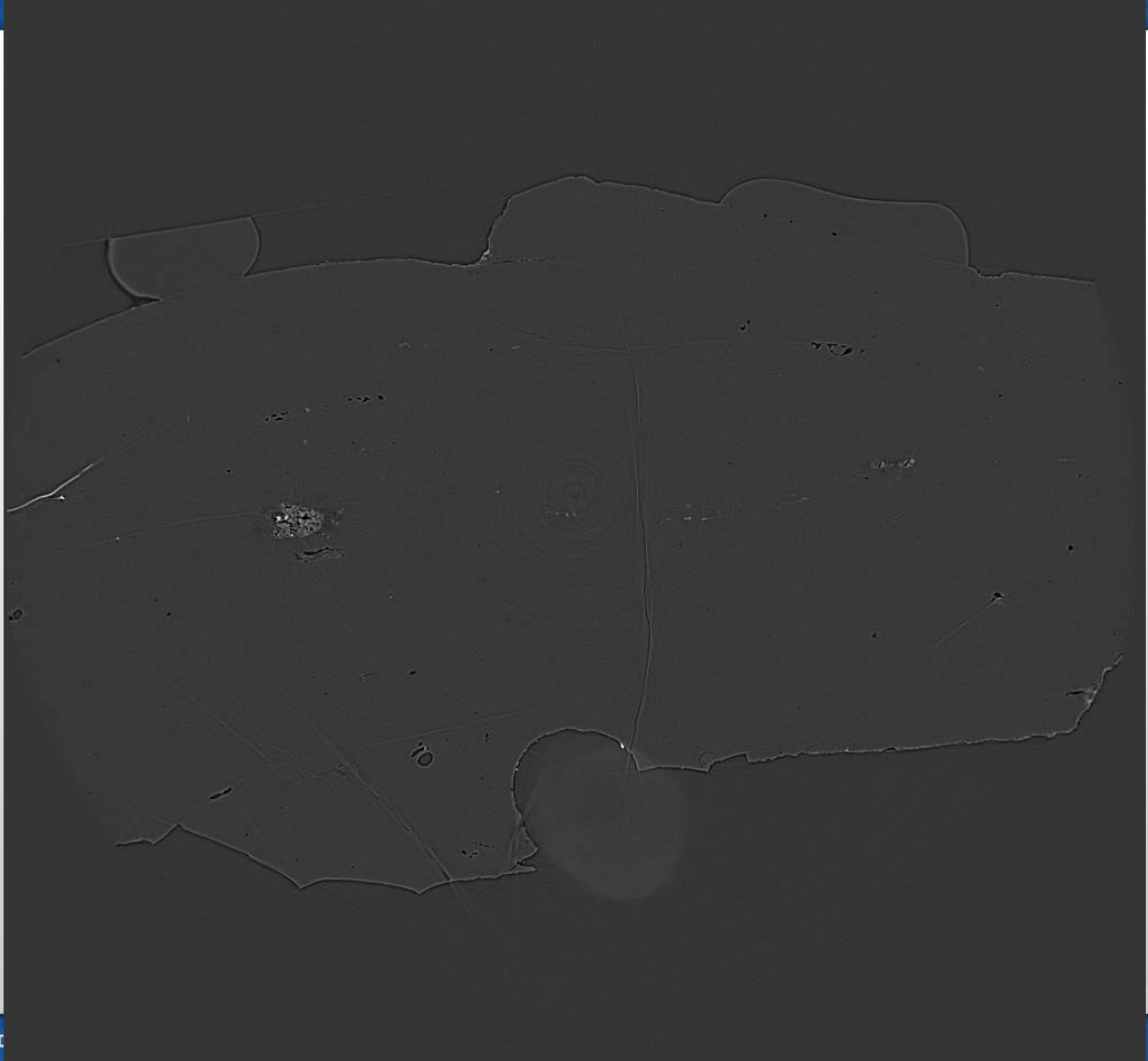
Tomographic reconstruction of the slice corresponding to the sinogram: filtered back-projection



Tomographic reconstruction of the slice corresponding to the sinogram: filtered back-projection



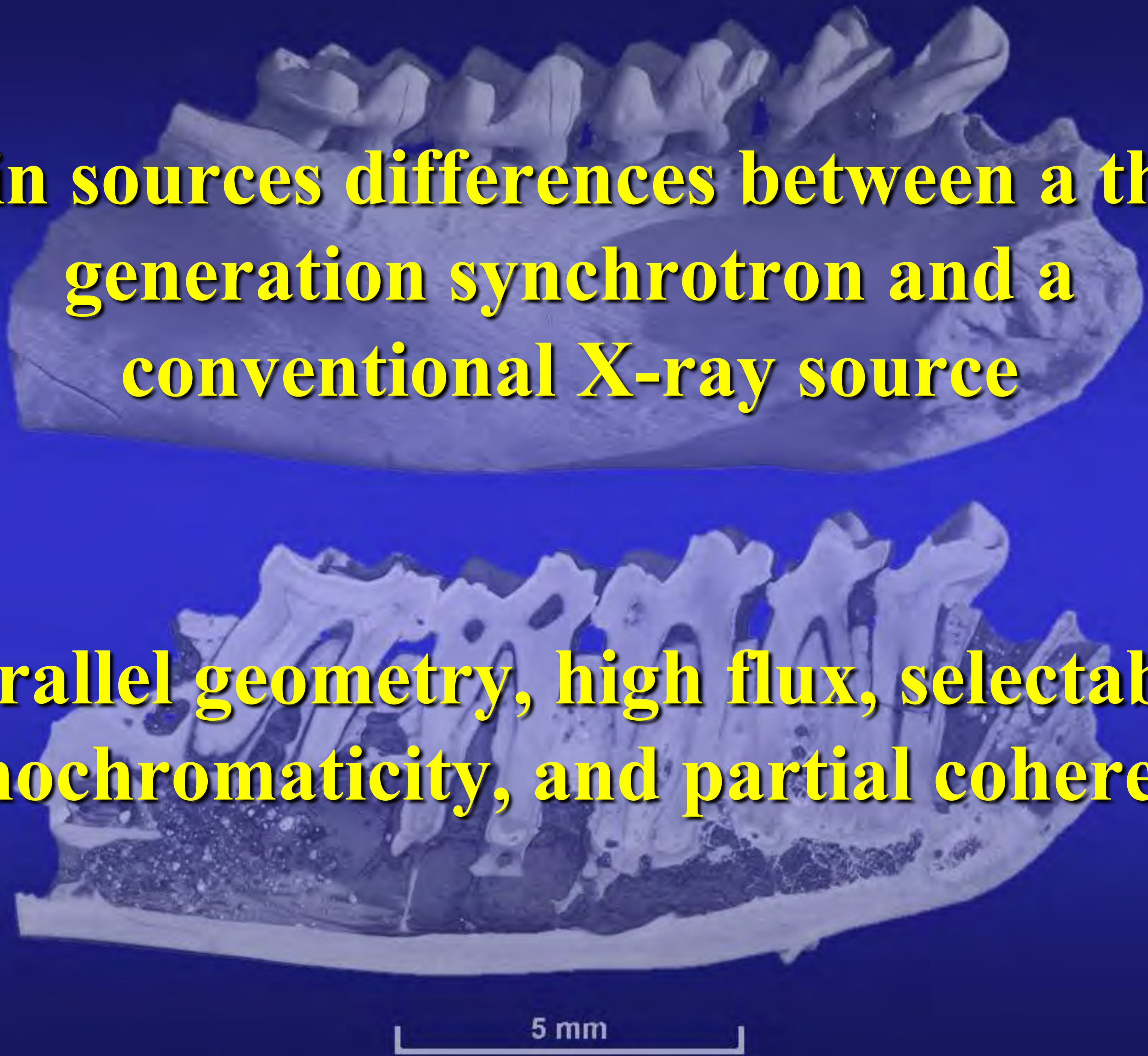
From slices to 3D volume



Processed 3D volume

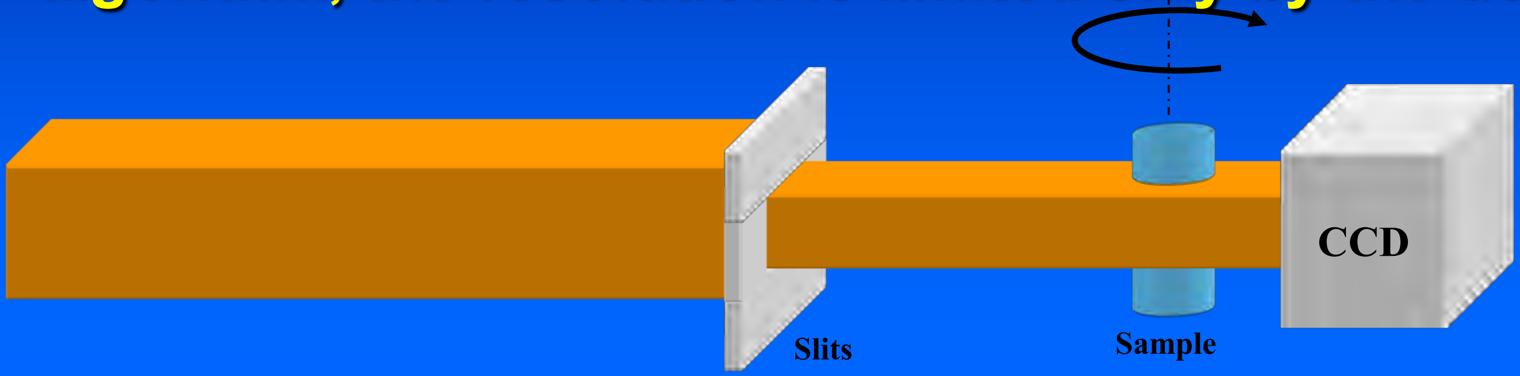
Main sources differences between a third generation synchrotron and a conventional X-ray source

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

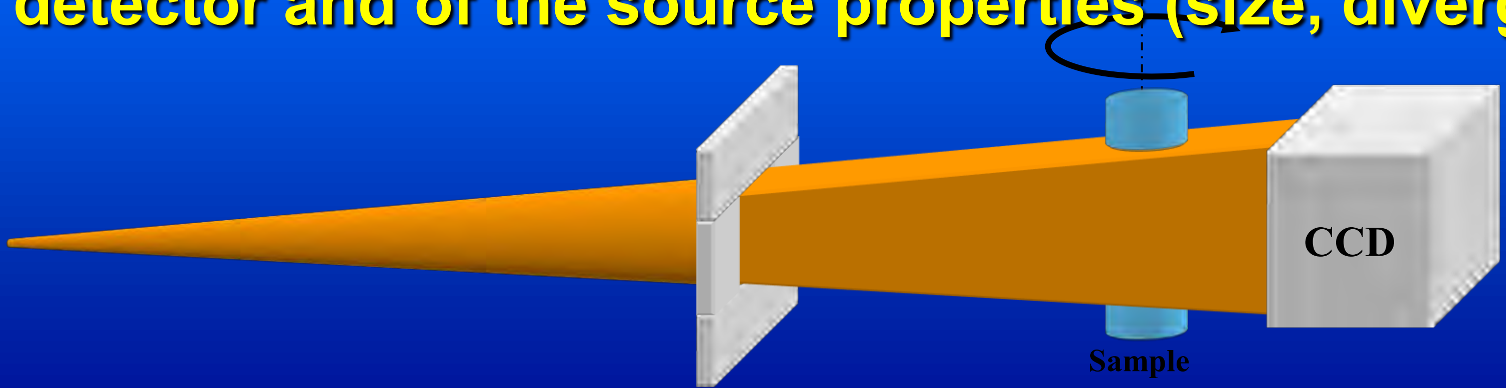


Beam geometry

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

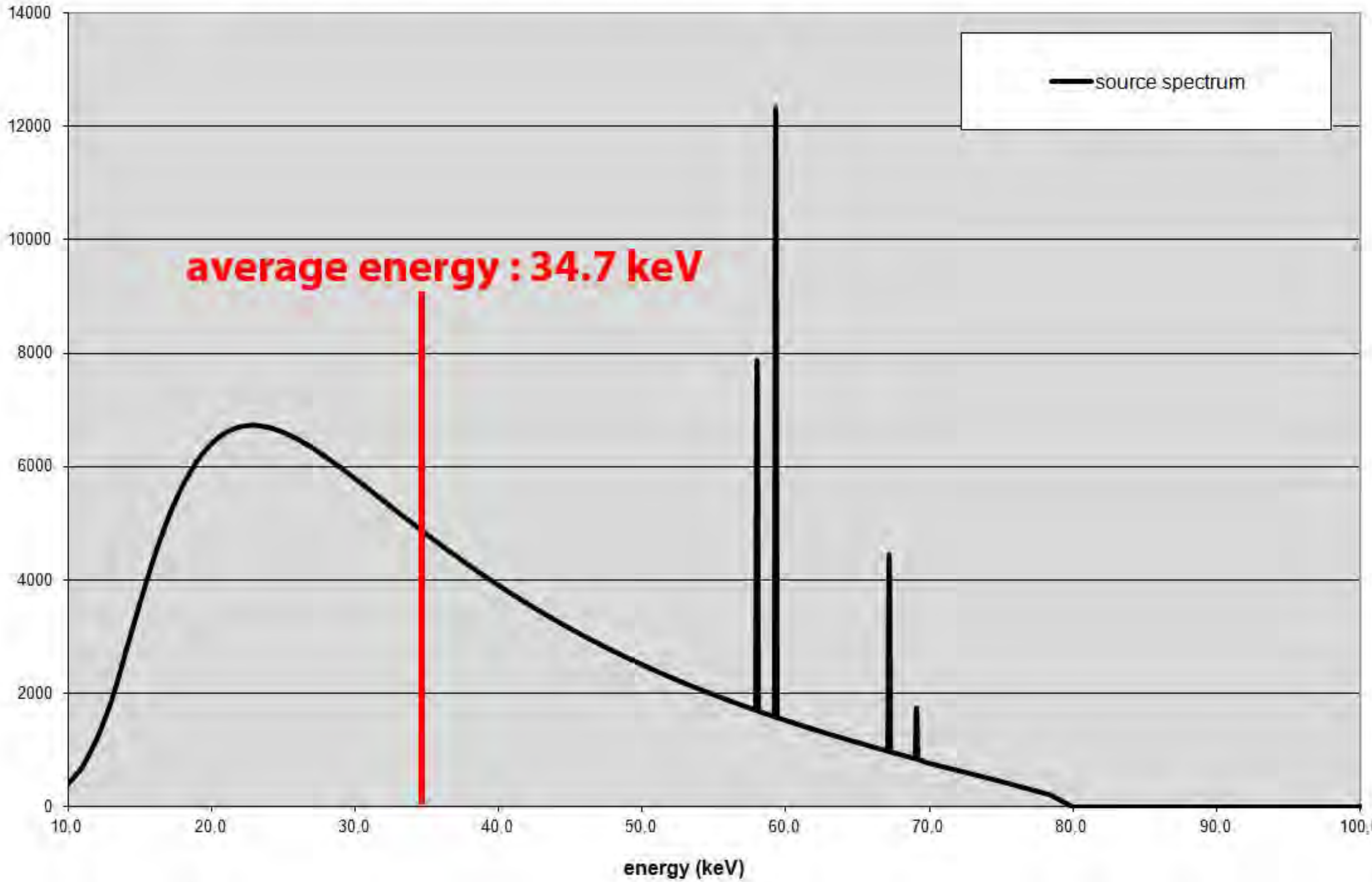
Monochromaticity

- Tomography and microtomography using conventional X-ray sources are subjected to beam hardening effect due to their wide spectrum polychromatic sources.

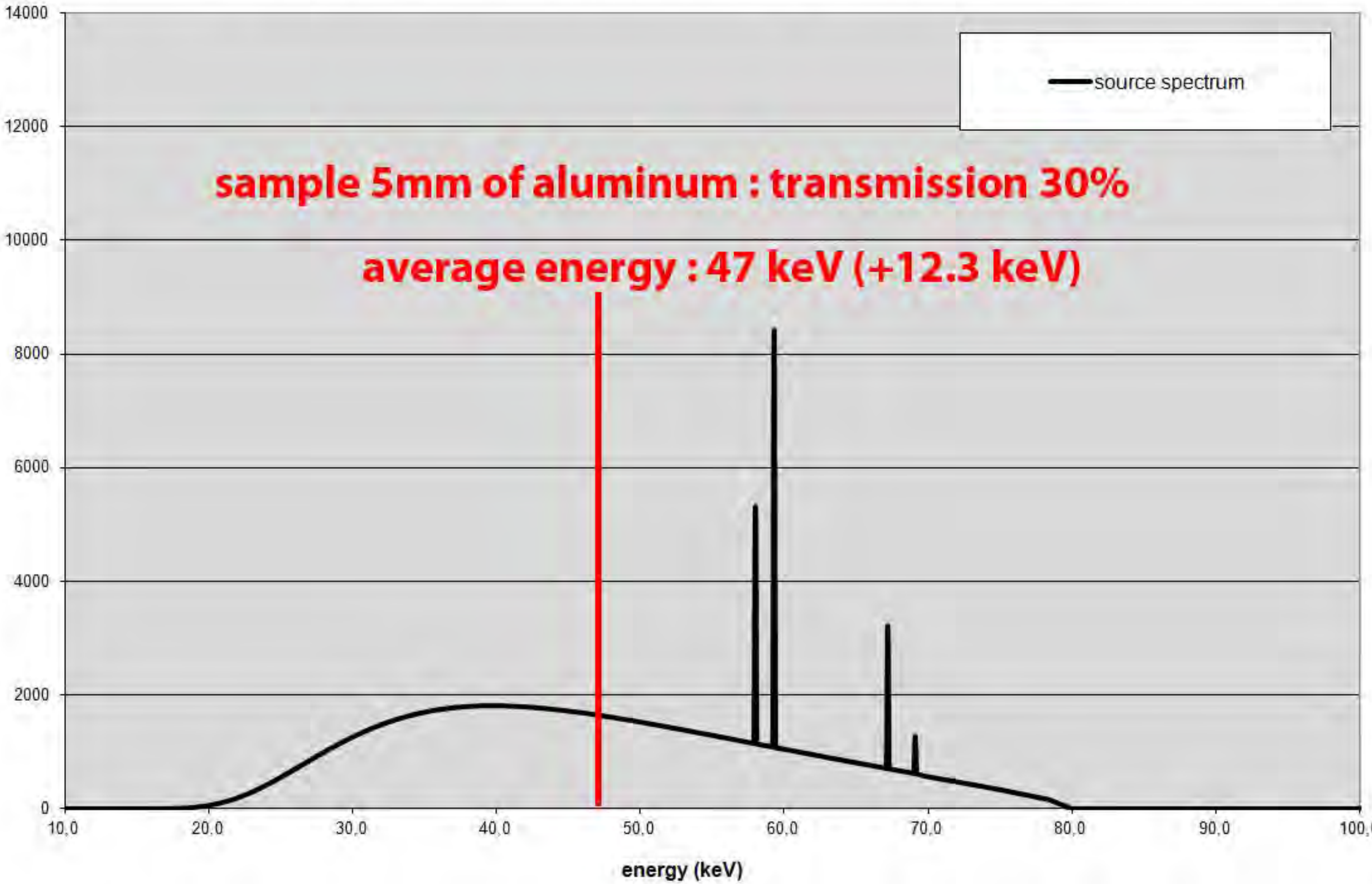
- Beam hardening is a differential absorption of the X-ray spectrum by the sample, the low energies being more absorbed than the high ones. The transmitted beam is then harder (containing in average higher energies) than the incoming beam.

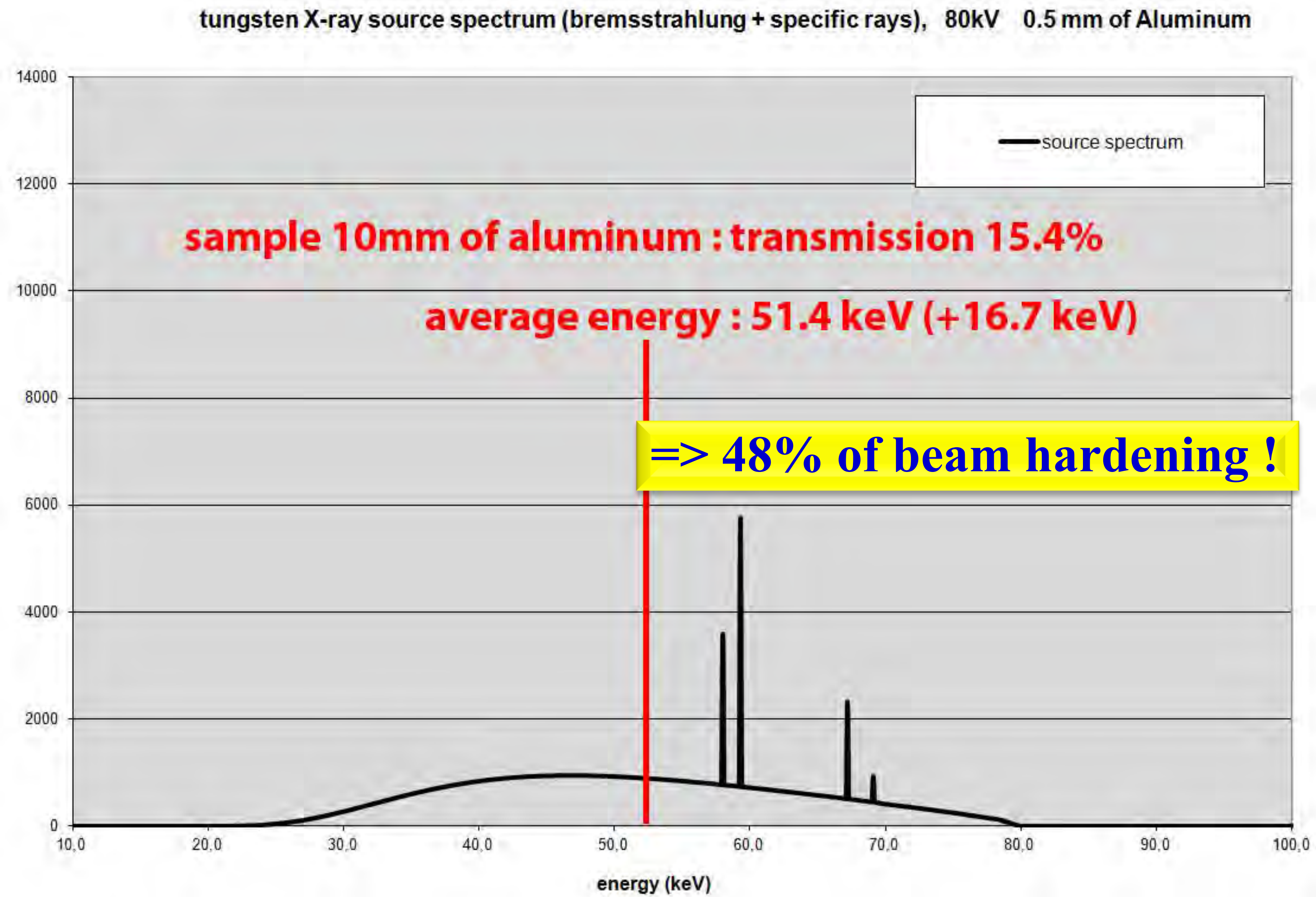
- It leads to misleading reconstructed densities data on the slices with typical brightening of the sample borders and linking of dense structures.

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



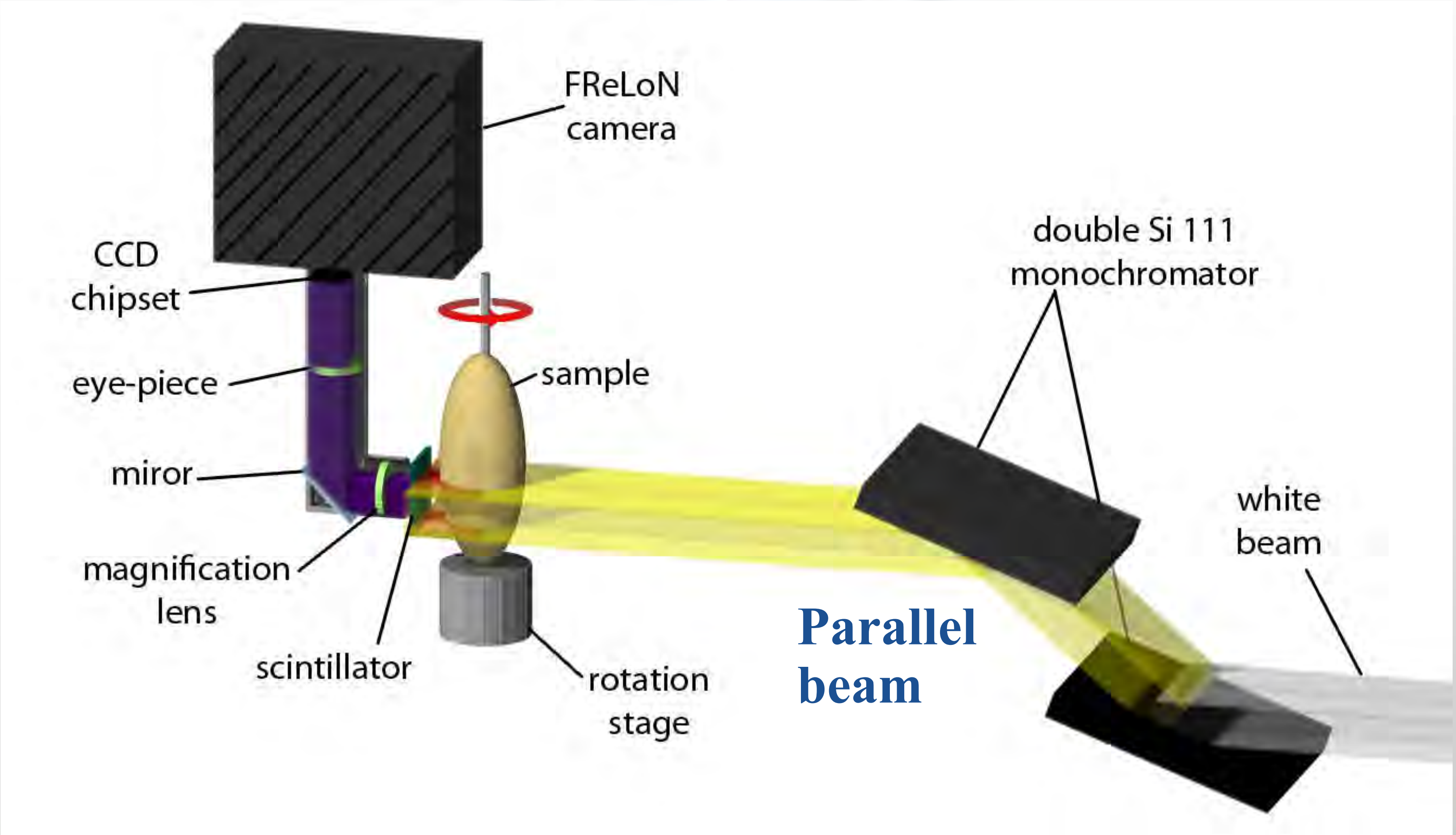
tungsten X-ray source spectrum (bremsstrahlung + specific rays), 80kV 0.5 mm of Aluminum





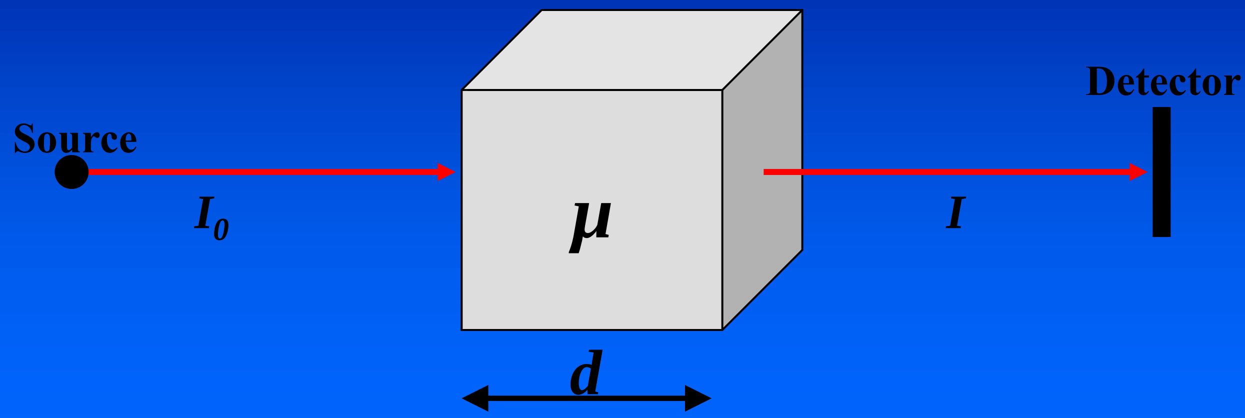
tungsten X-ray source spectrum (bremsstrahlung + specific rays), 80kV 0.5 mm of Aluminum
source spectrum
sample 10mm of aluminum : transmission 15.4%
average energy : 51.4 keV (+16.7 keV)
=> 48% of beam hardening !
14000
12000
10000
8000
6000
4000
2000
0
10,0
20,0
30,0
40,0
50,0
60,0
70,0
80,0
90,0
100,0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

Tomography @ ESRF absorption based CT



Modified from Fernandez

X-ray attenuation by sample with monochromatic beam



$$I = I_0\, e^{-\mu d} \Longrightarrow \mu d = -\ln\left(I/I_0\right)$$

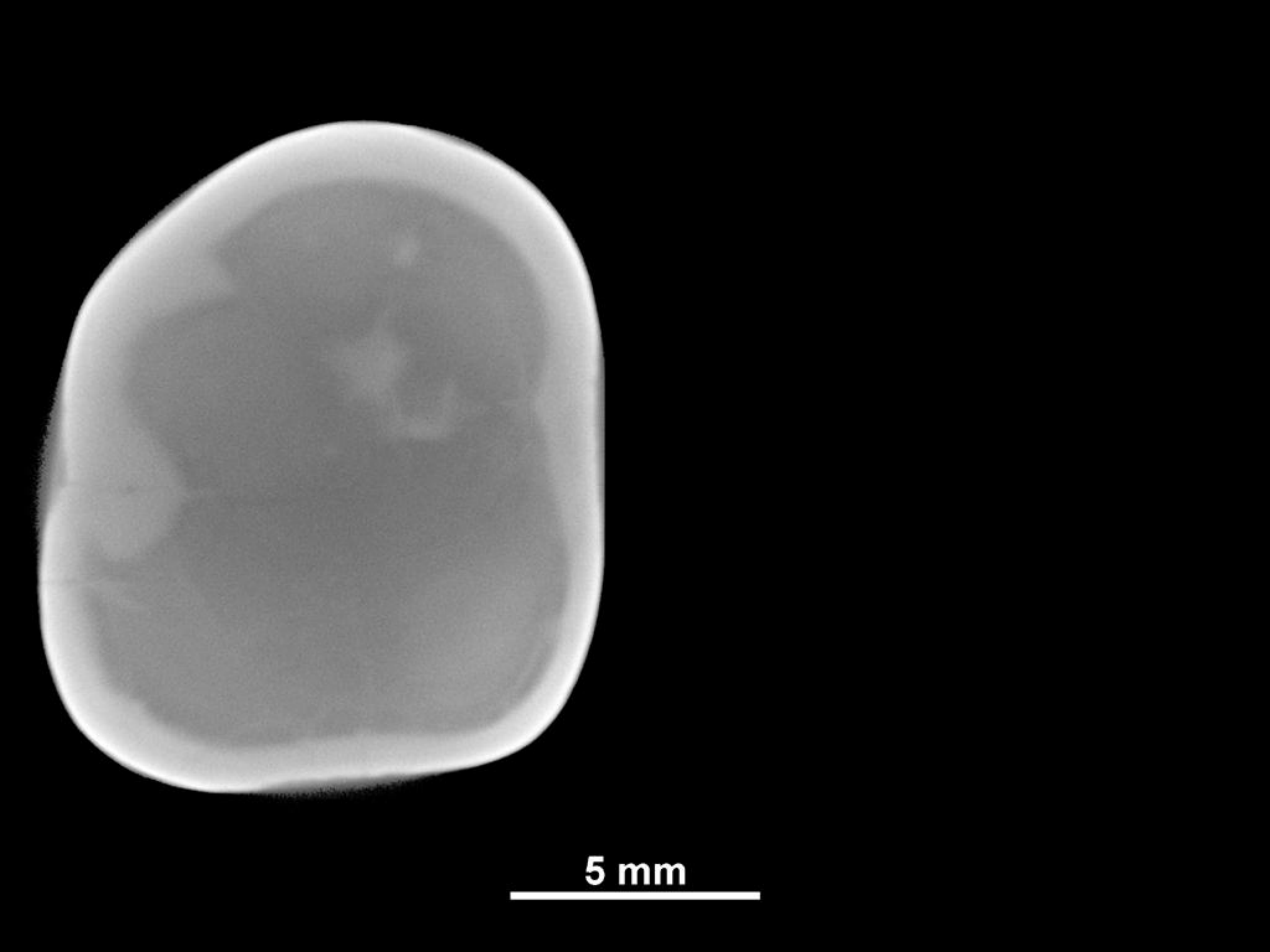
I_0 incident beam

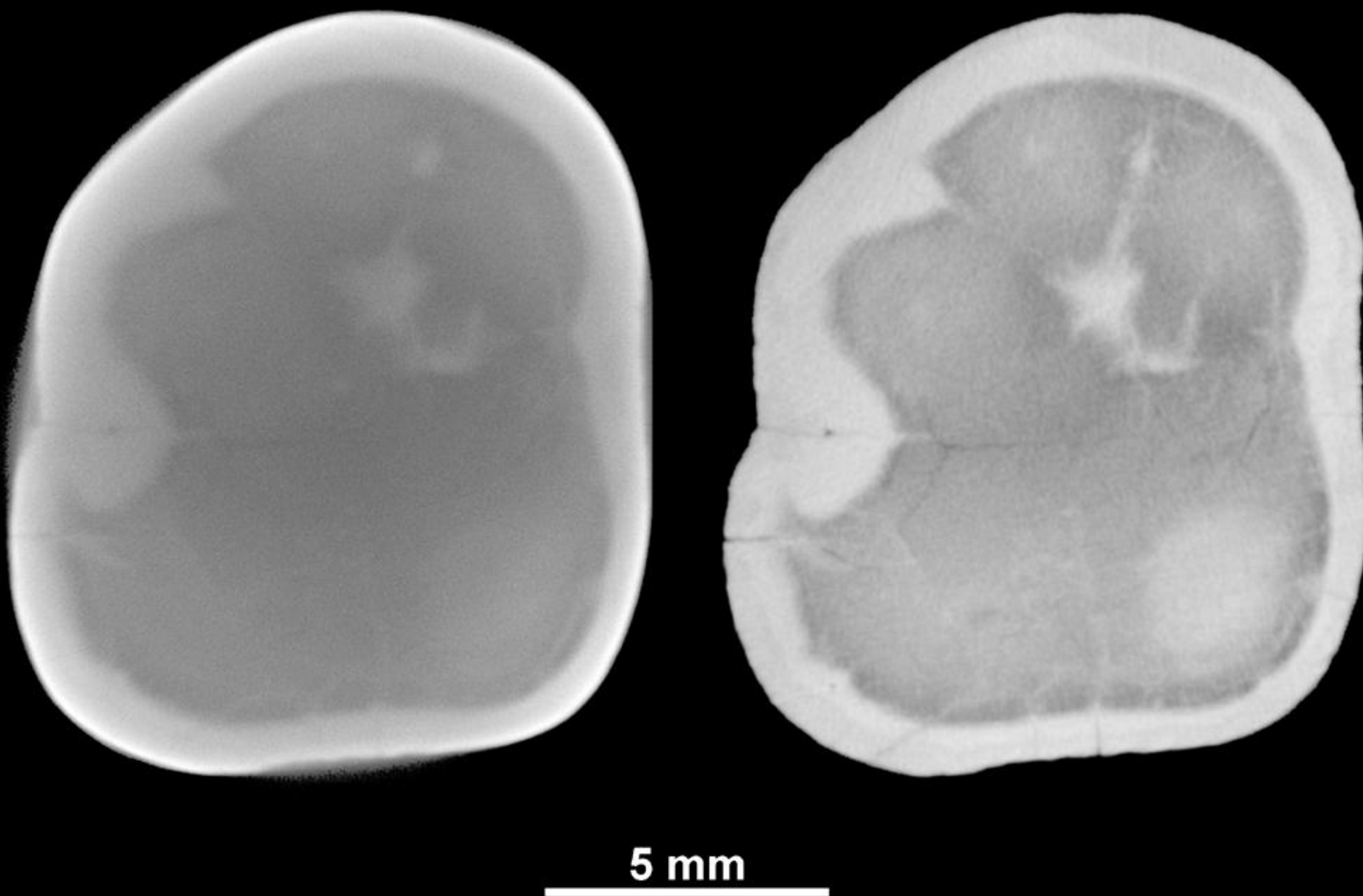
I transmitted beam

μ linear coefficient of absorption (depends on energy and sample composition)

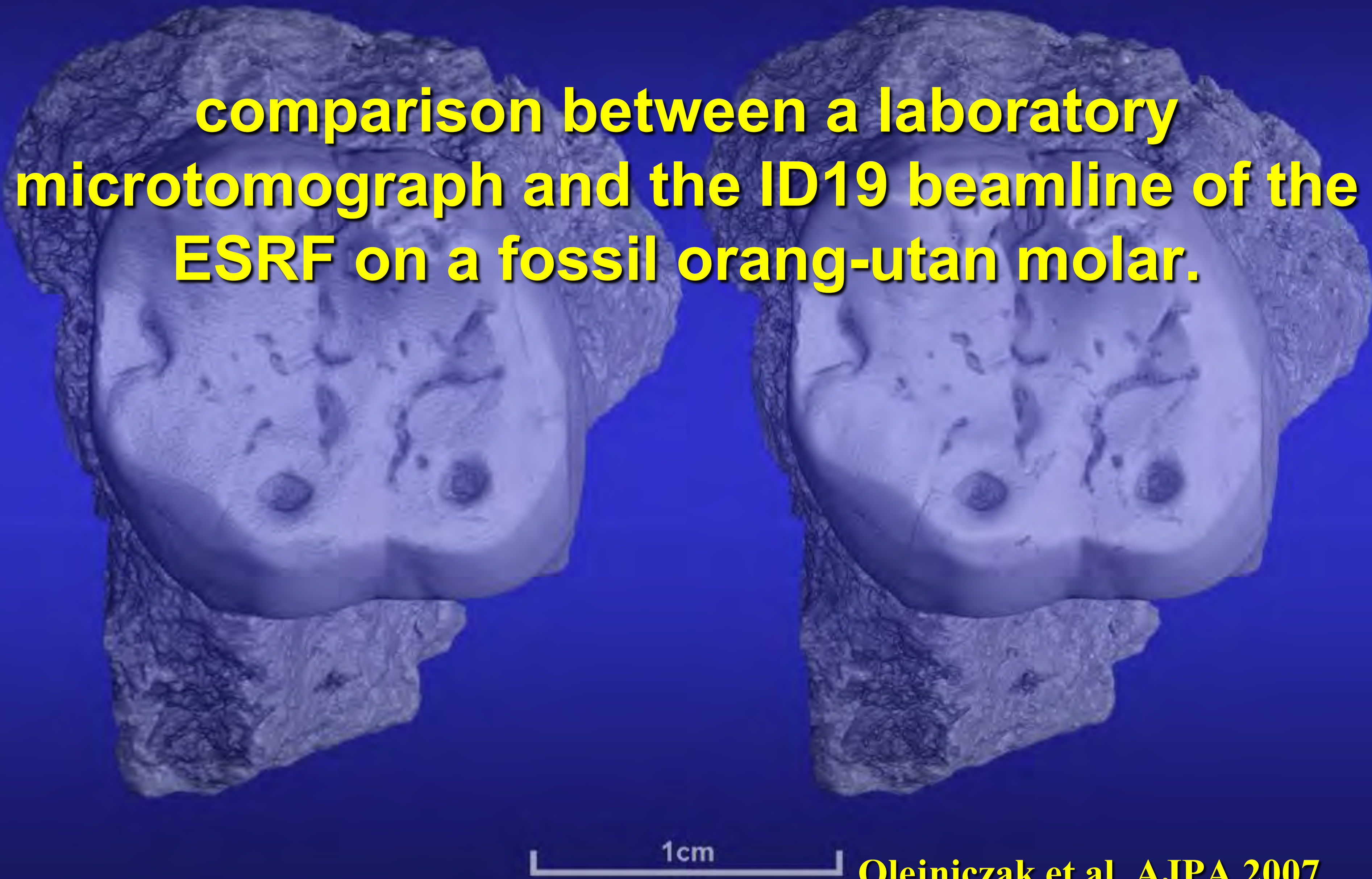
d thickness of sample passed through by the beam

Modified from P. Bleuet


5 mm


5 mm

comparison between a laboratory microtomograph and the ID19 beamline of the ESRF on a fossil orang-utan molar.

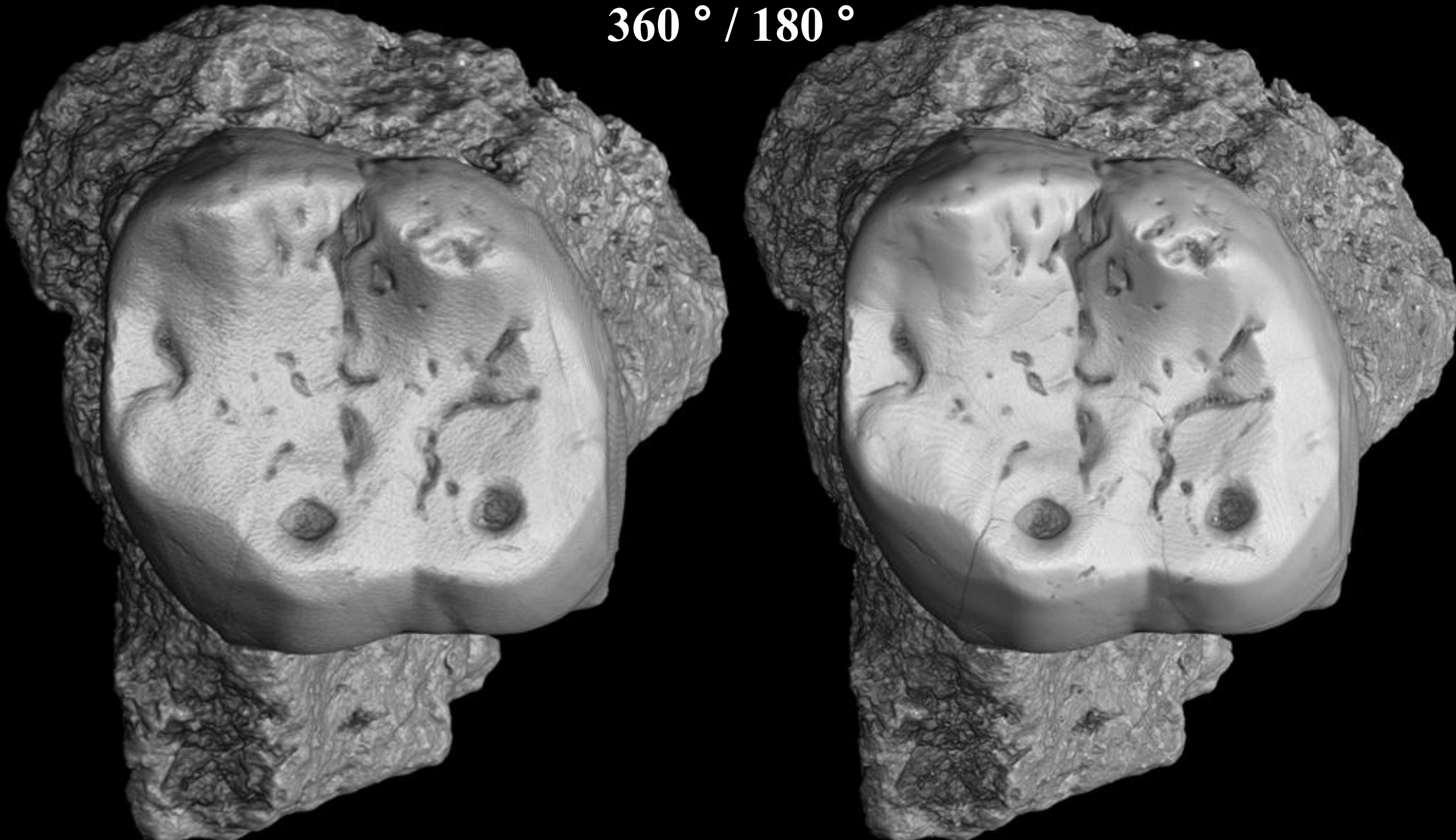


Olejniczak et al. AJPA 2007

Same acquisition parameters : 30 µm voxel size, 0.15° angular step

Differences : 12 hours / 45 minutes, cone beam / parallel beam

360 ° / 180 °

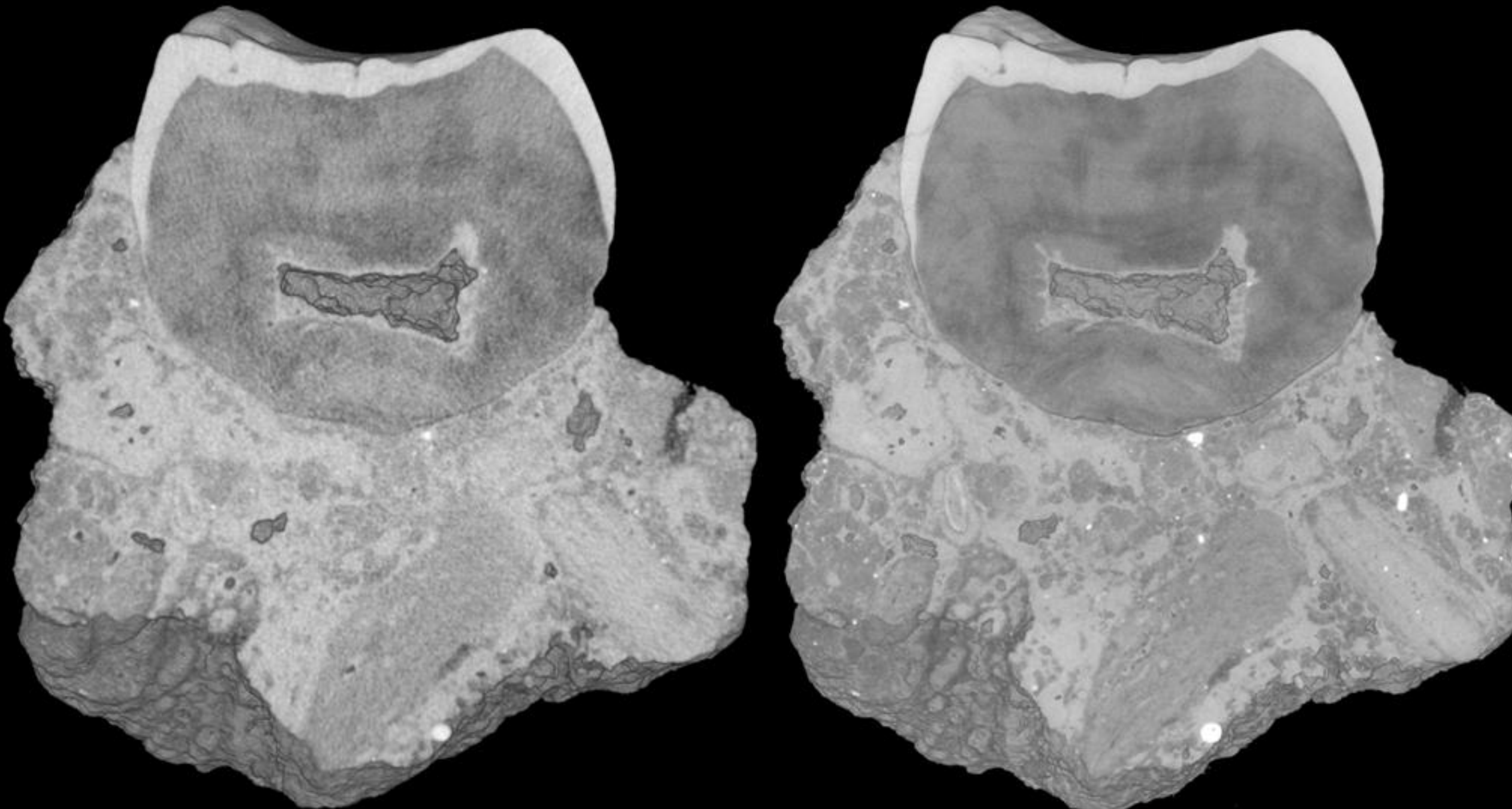


Laboratory microtomography

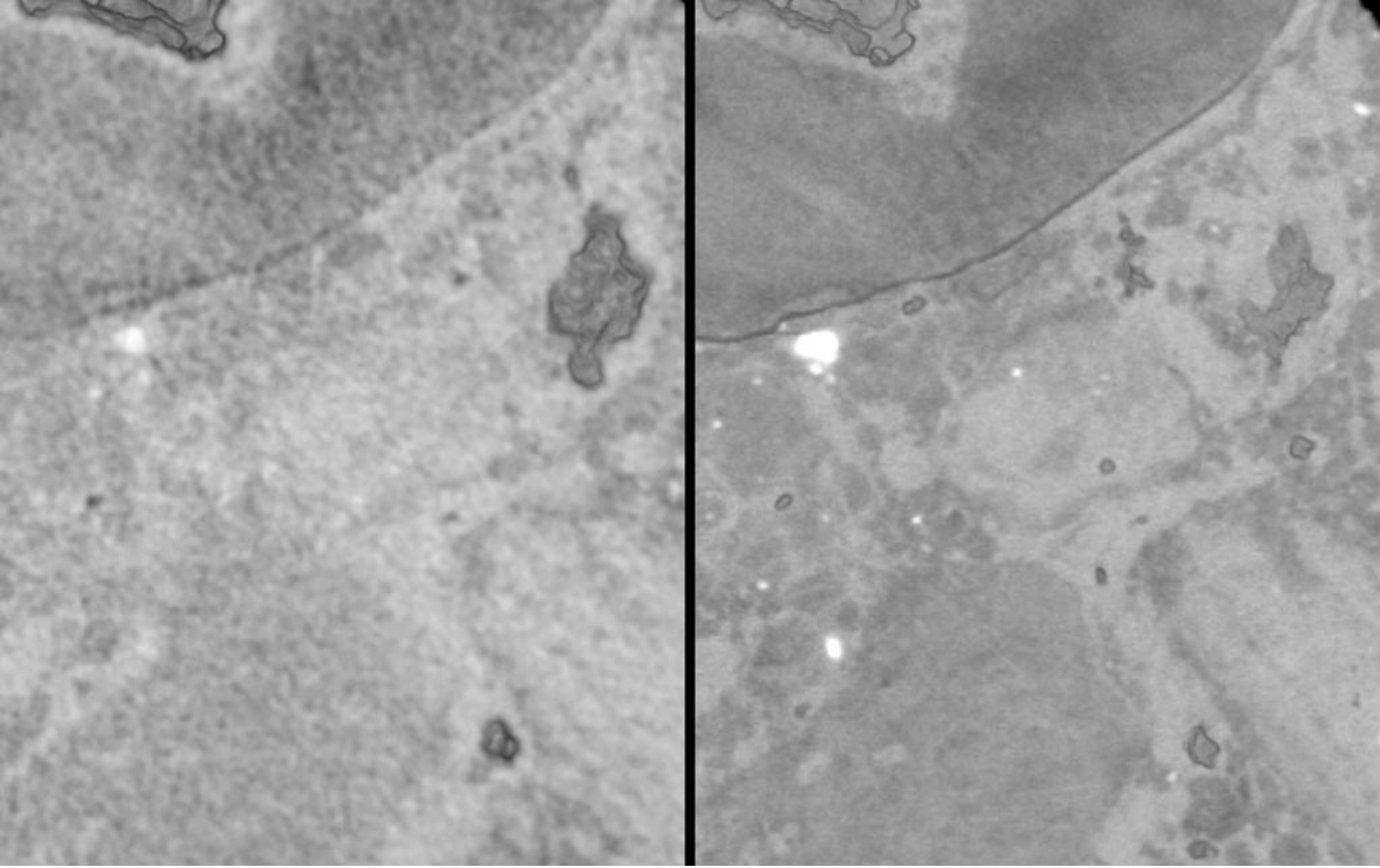
1cm

Synchrotron microtomography

More noisy slice with laboratory scanner
but effective software correction of beam hardening

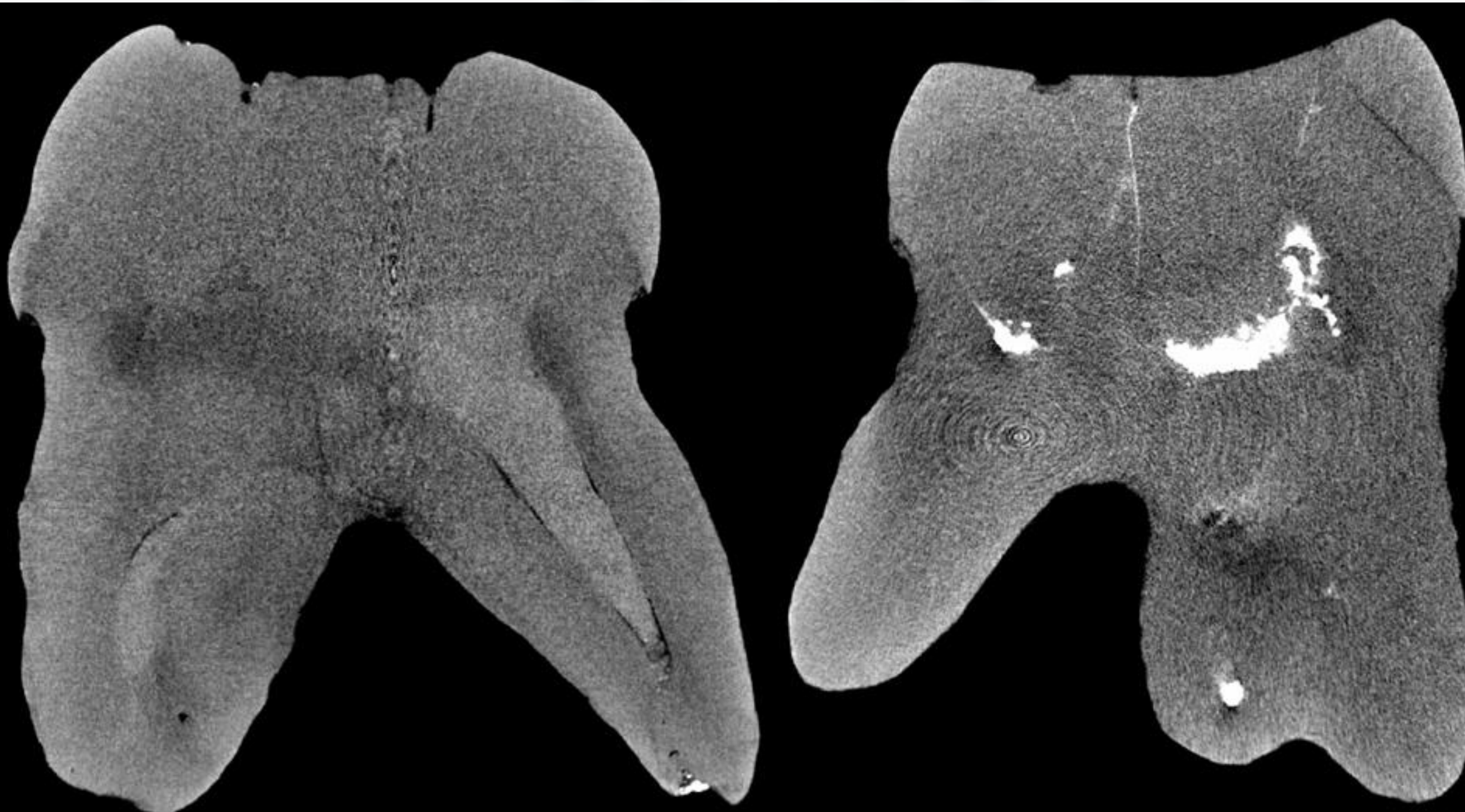


Better signal-to-noise ratio of the right slice
due to the high flux of synchrotron sources



For the same pixel size, smaller details are visible (higher resolution) with the synchrotron, the data quality is clearly higher

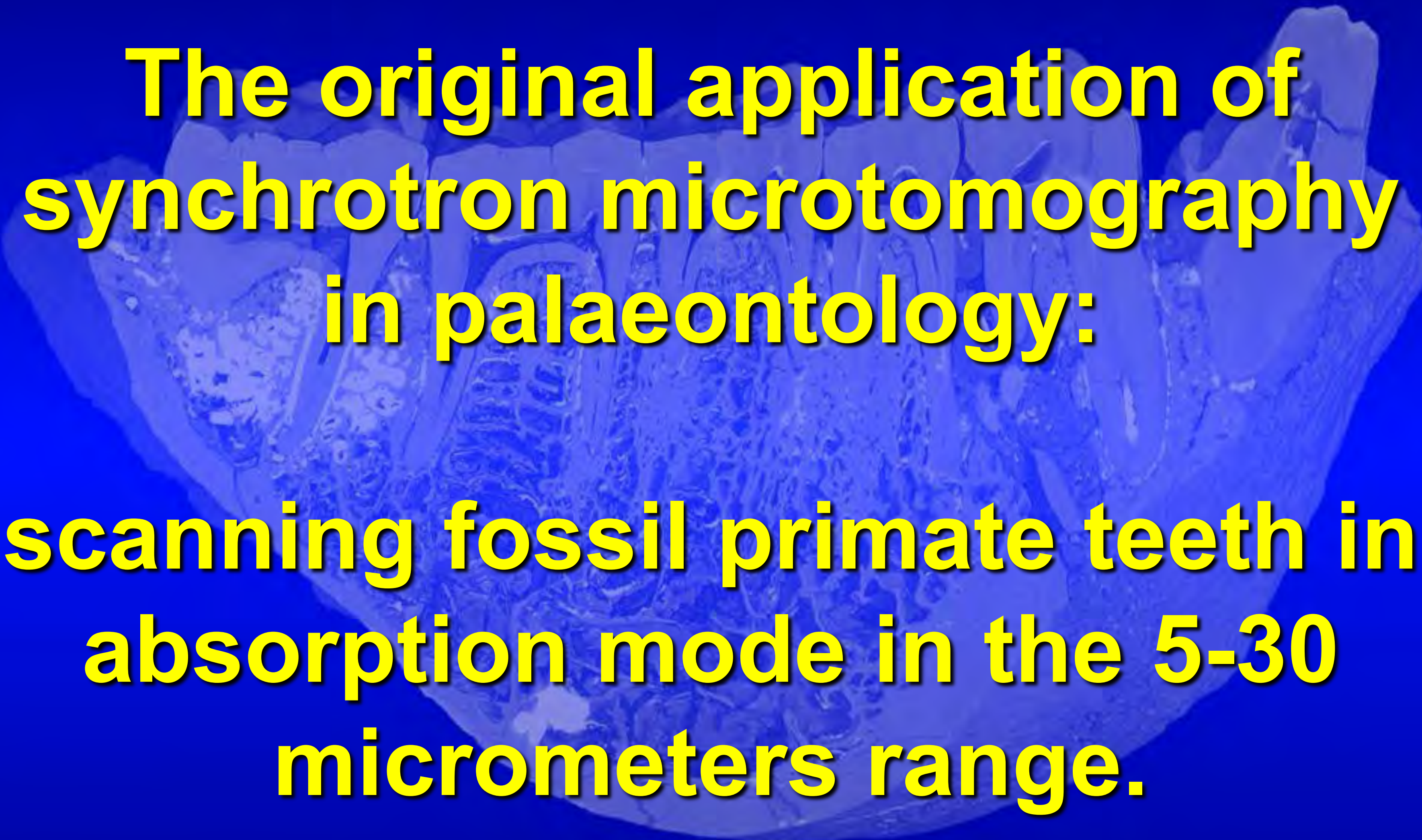
Same conventional microtomograph used to image the Trinil molars, putative *Homo erectus* remains



Smith et al. 2009

Synchrotron imaging with monochromatic beam on ID19

Smith et al. 2009



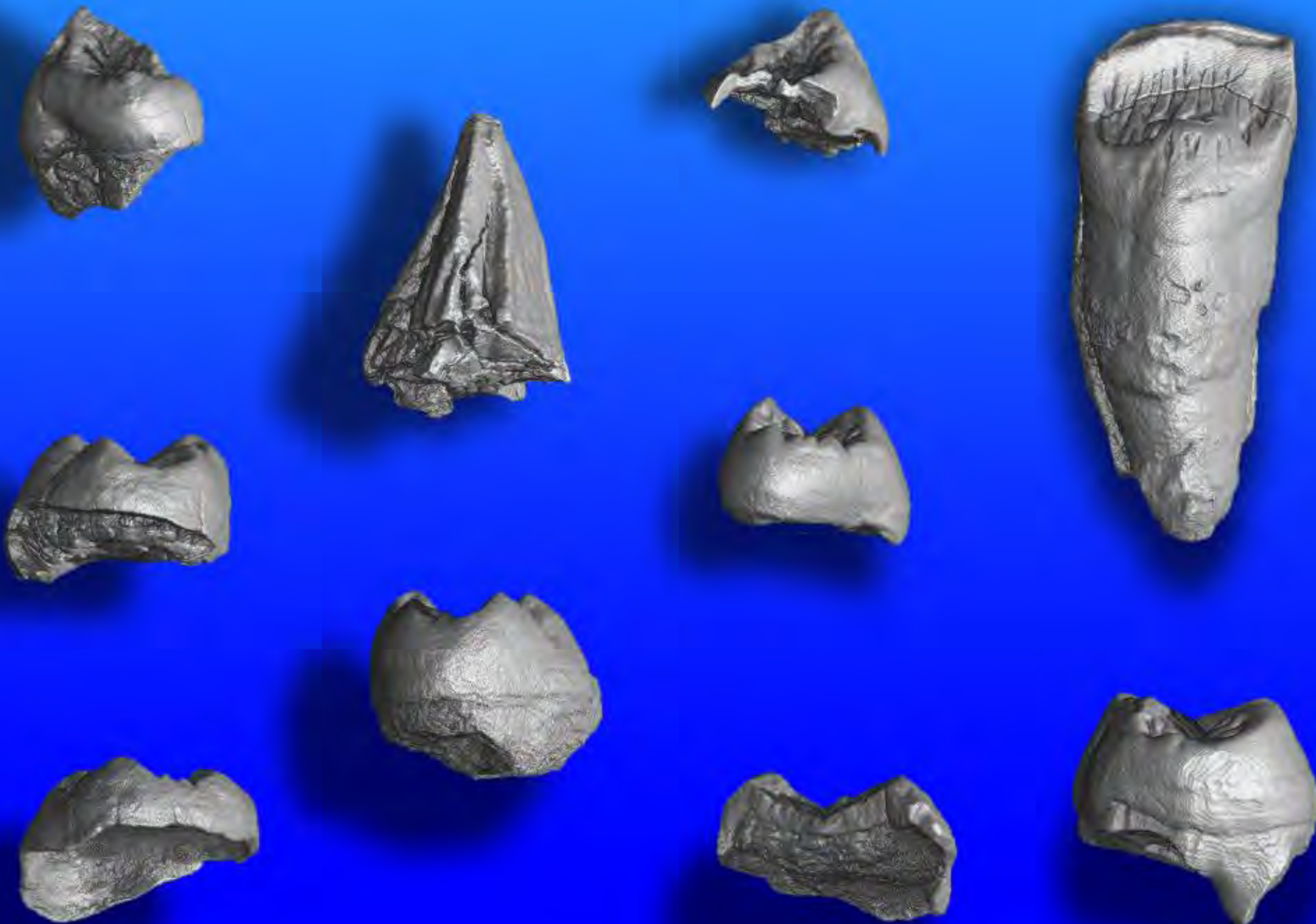
The original application of synchrotron microtomography in palaeontology:

scanning fossil primate teeth in absorption mode in the 5-30 micrometers range.

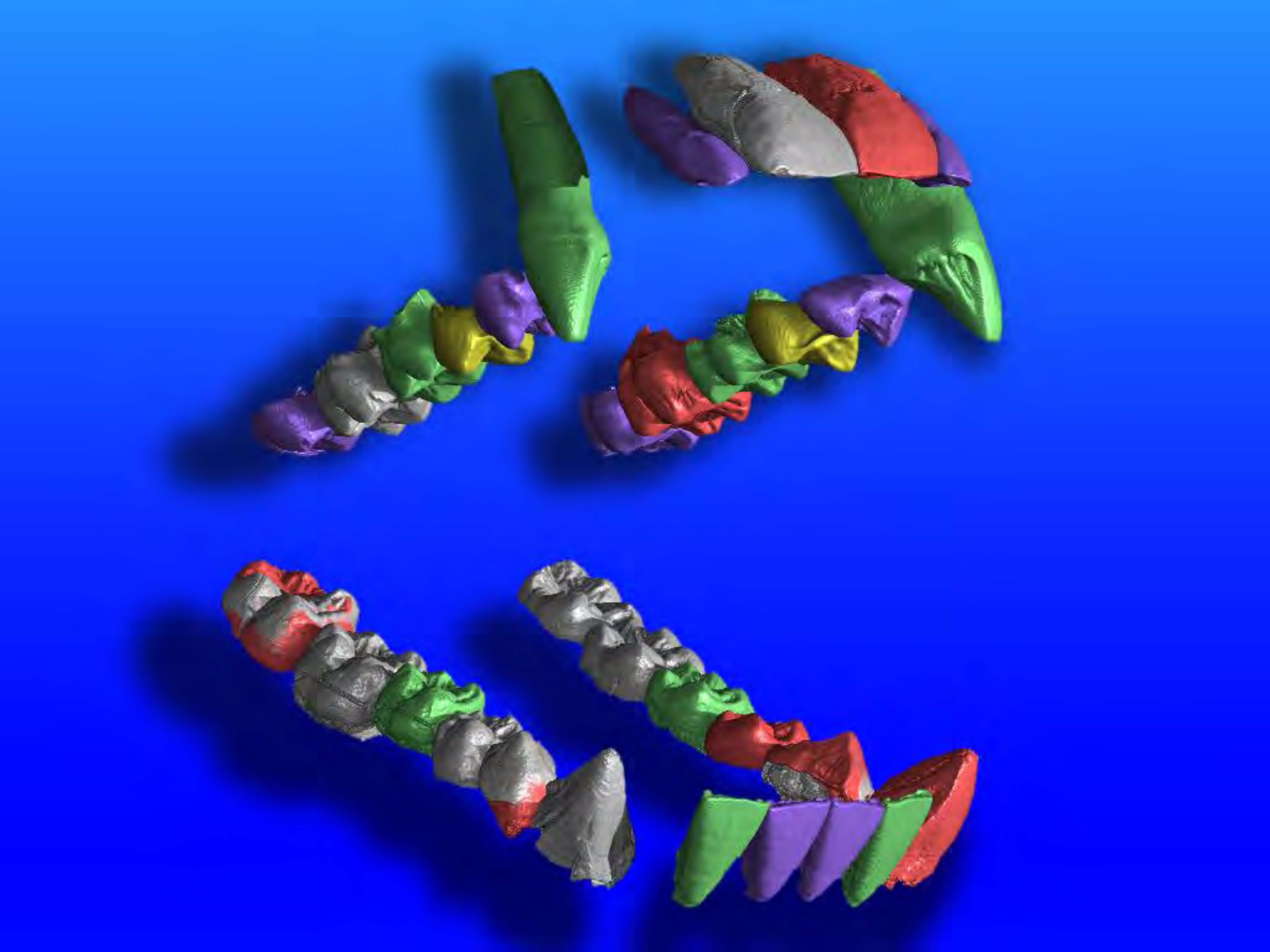
The first palaeontological publication using synchrotron microtomography

Teeth of the Miocene hominoid from Thailand *Khoratpithecus chiangmuanensis*

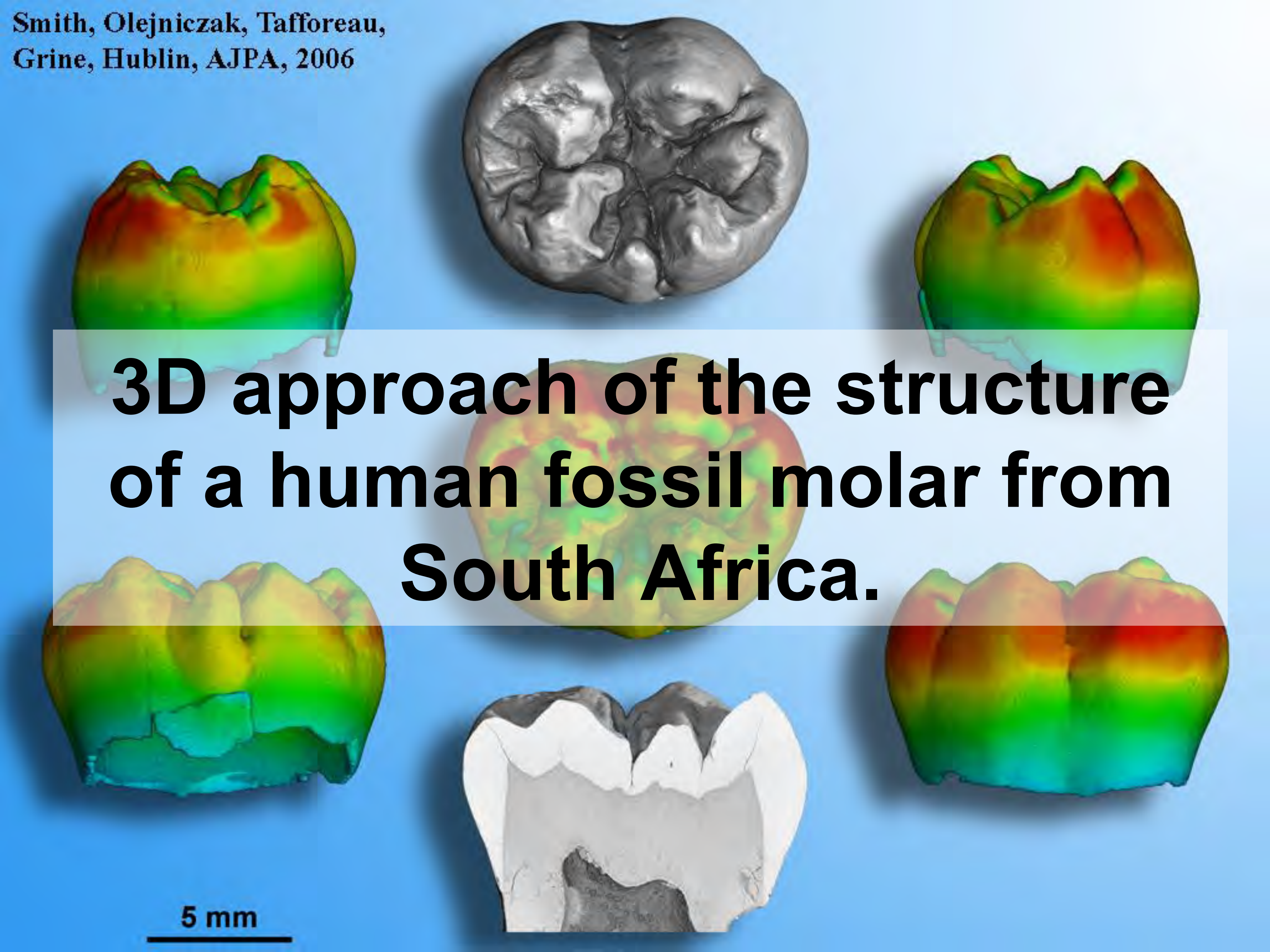
Chaimanee et al. 2003, Nature

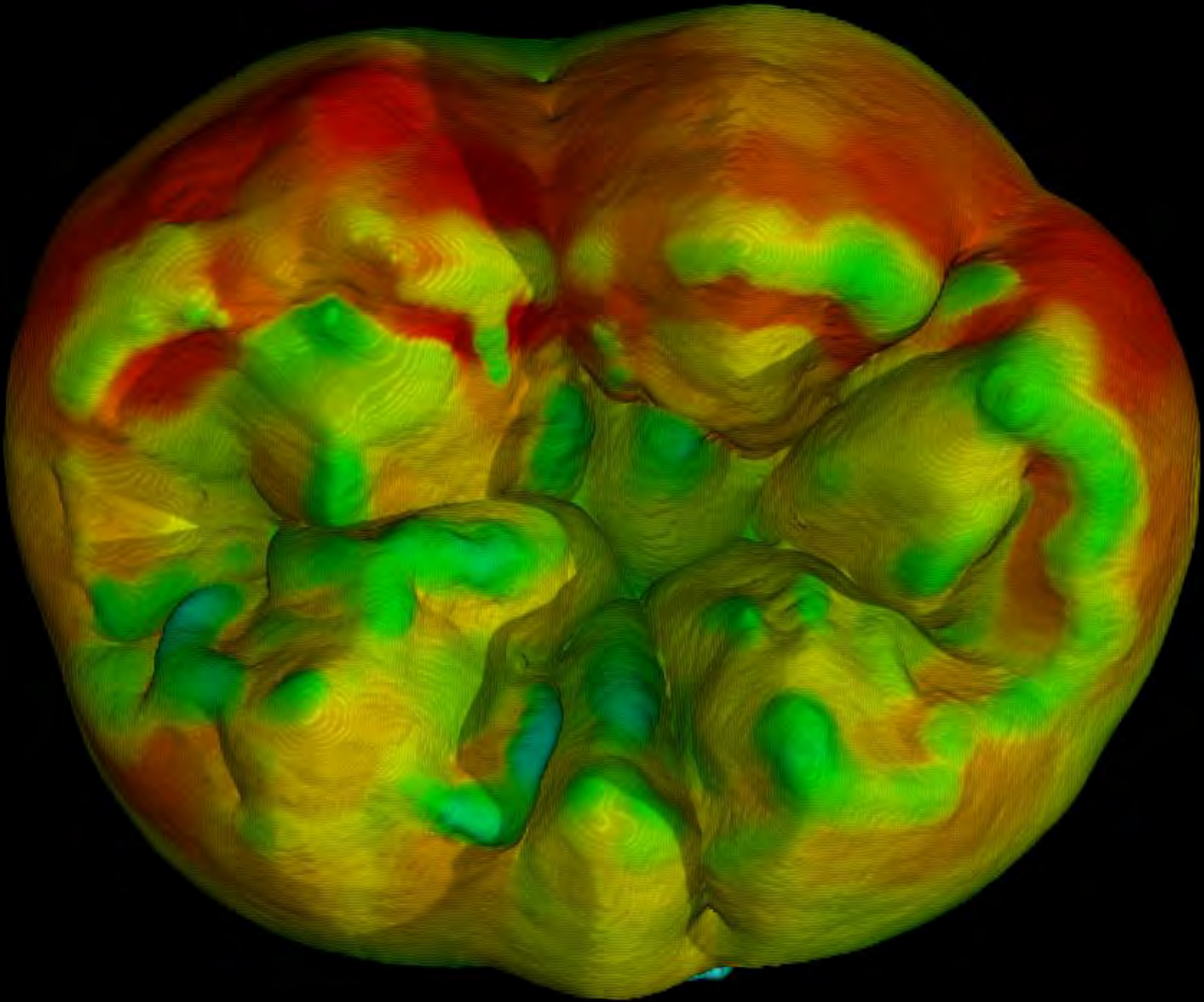


Chaimanee, Jolly, Benammi,Tafforeau, Duzer Moussa and Jaeger. 2003, Nature

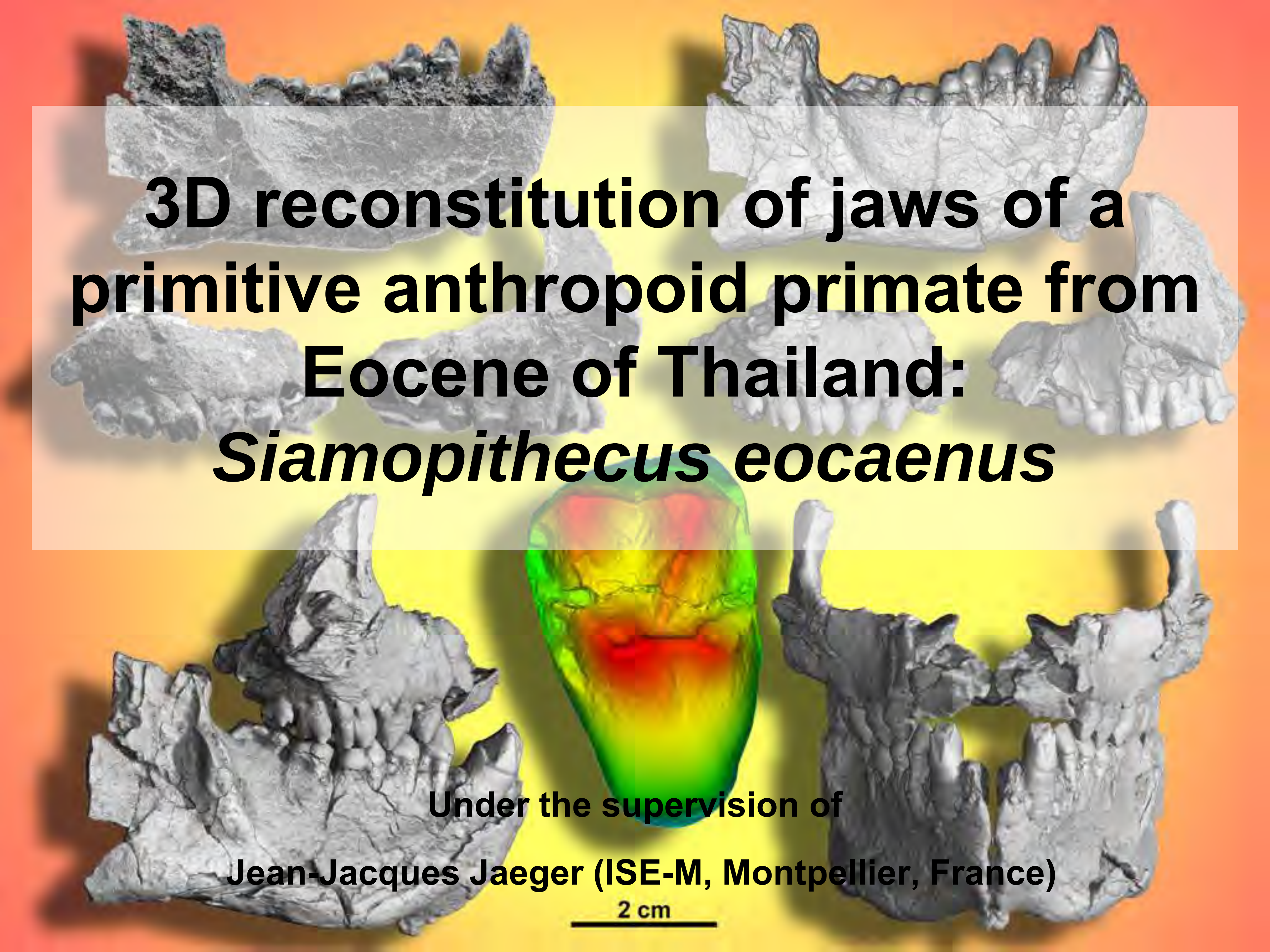


3D approach of the structure of a human fossil molar from South Africa.



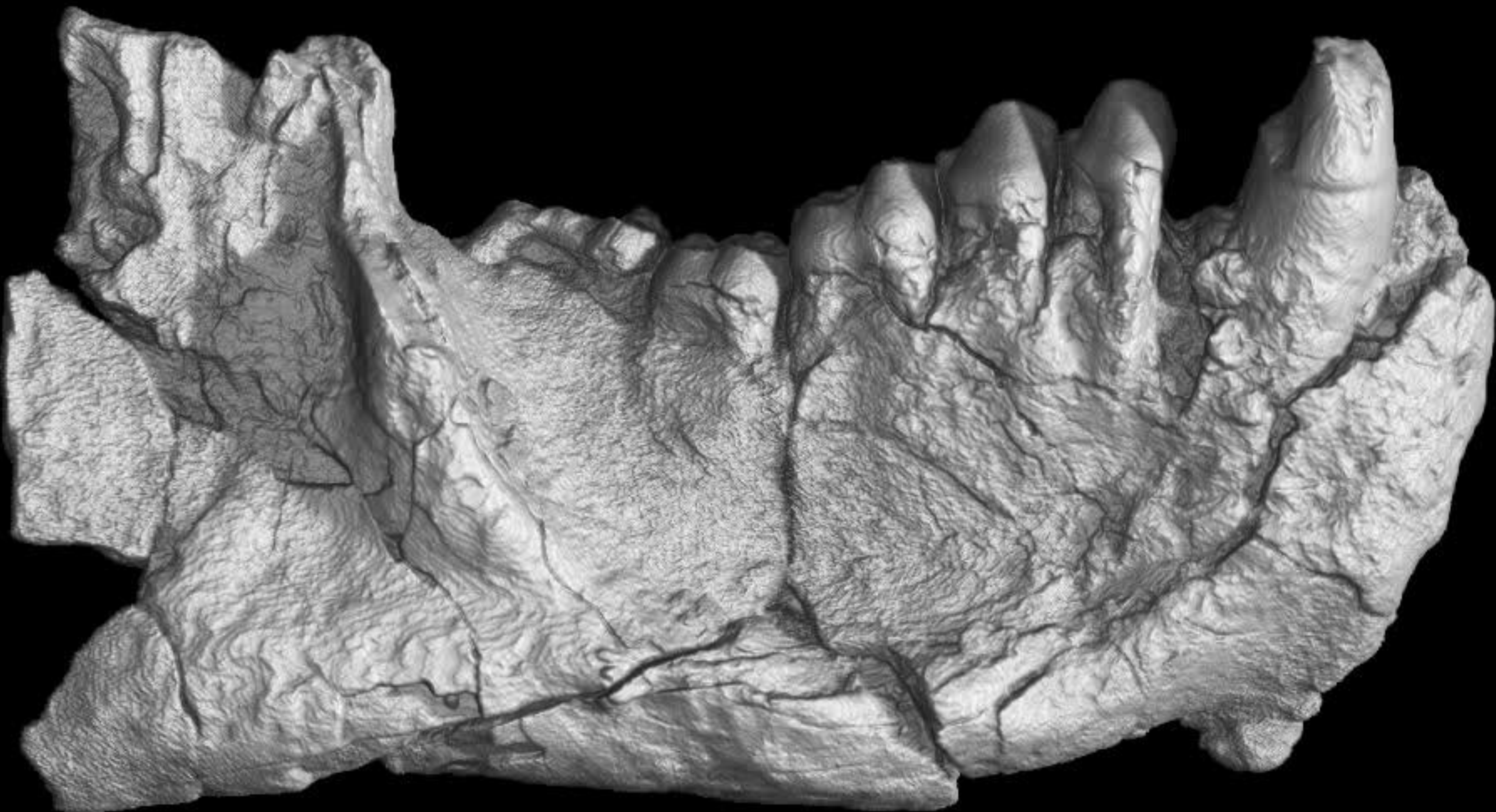


3D reconstitution of jaws of a primitive anthropoid primate from Eocene of Thailand: *Siamopithecus eocaenus*

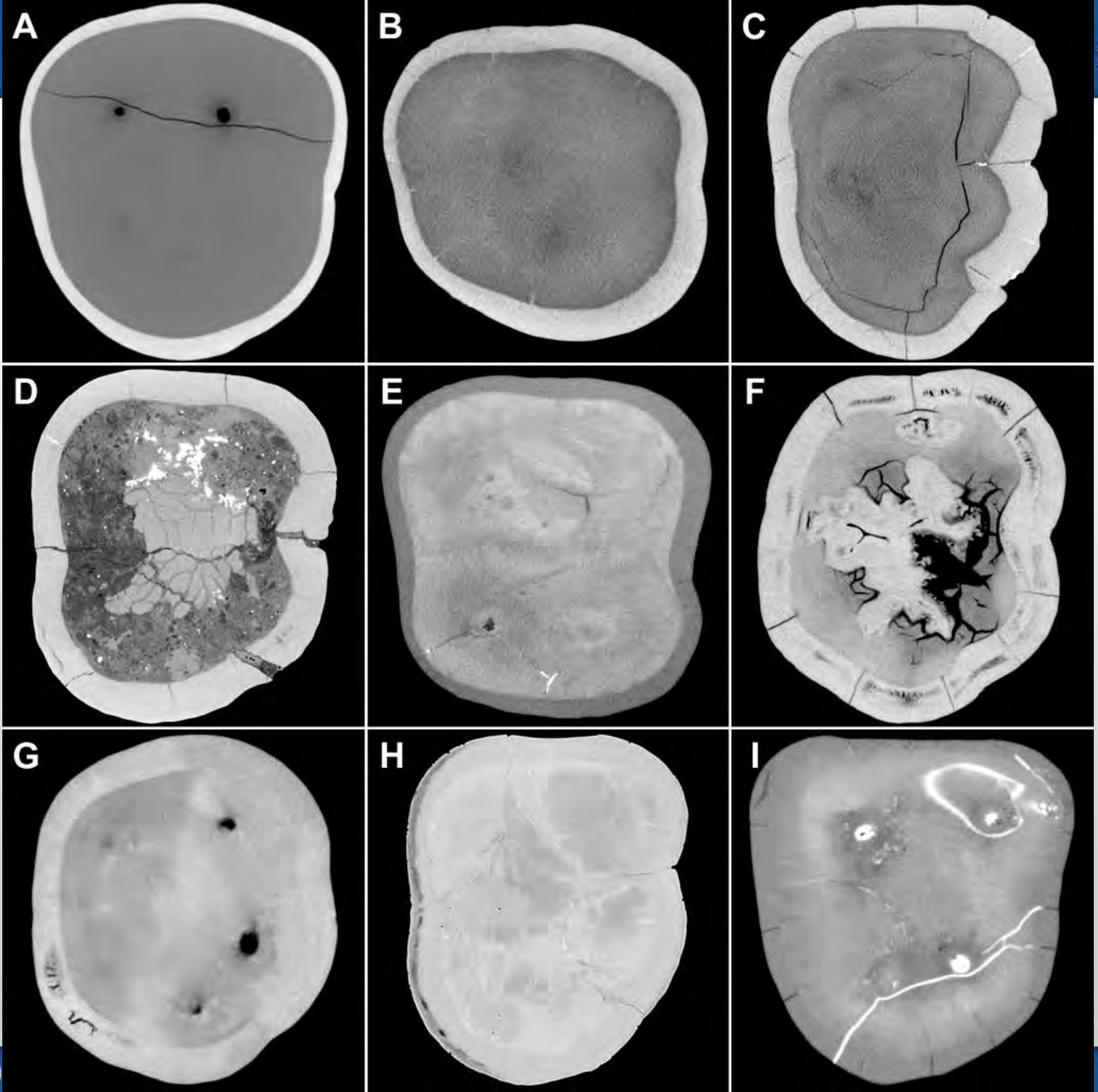


Under the supervision of

Jean-Jacques Jaeger (ISE-M, Montpellier, France)



In such cases, laboratory microtomographs can nowadays reach data quality similar to what was feasible only with synchrotron about a decade ago.


A
B
C
D
E
F
G
H
I

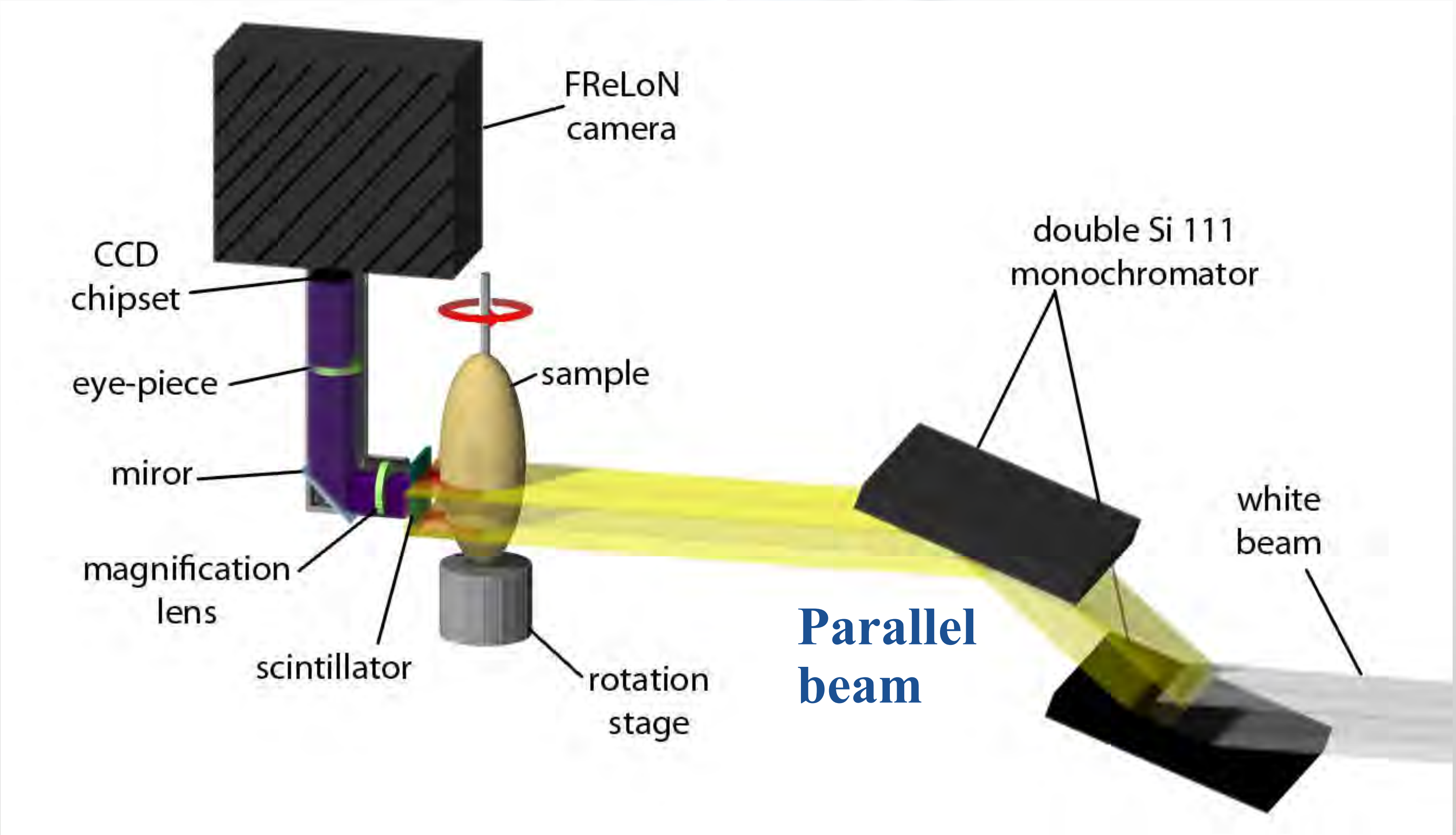
In case of substantial diagenetic modifications, or when very high data quality is required, conventional microtomographs attain their limit.

Synchrotron imaging can push further the limits of absorption X-ray imaging contrast and quality.

When it is not sufficient, here comes what really made the success of synchrotron imaging in palaeontology : <u>the propagation phase contrast</u>

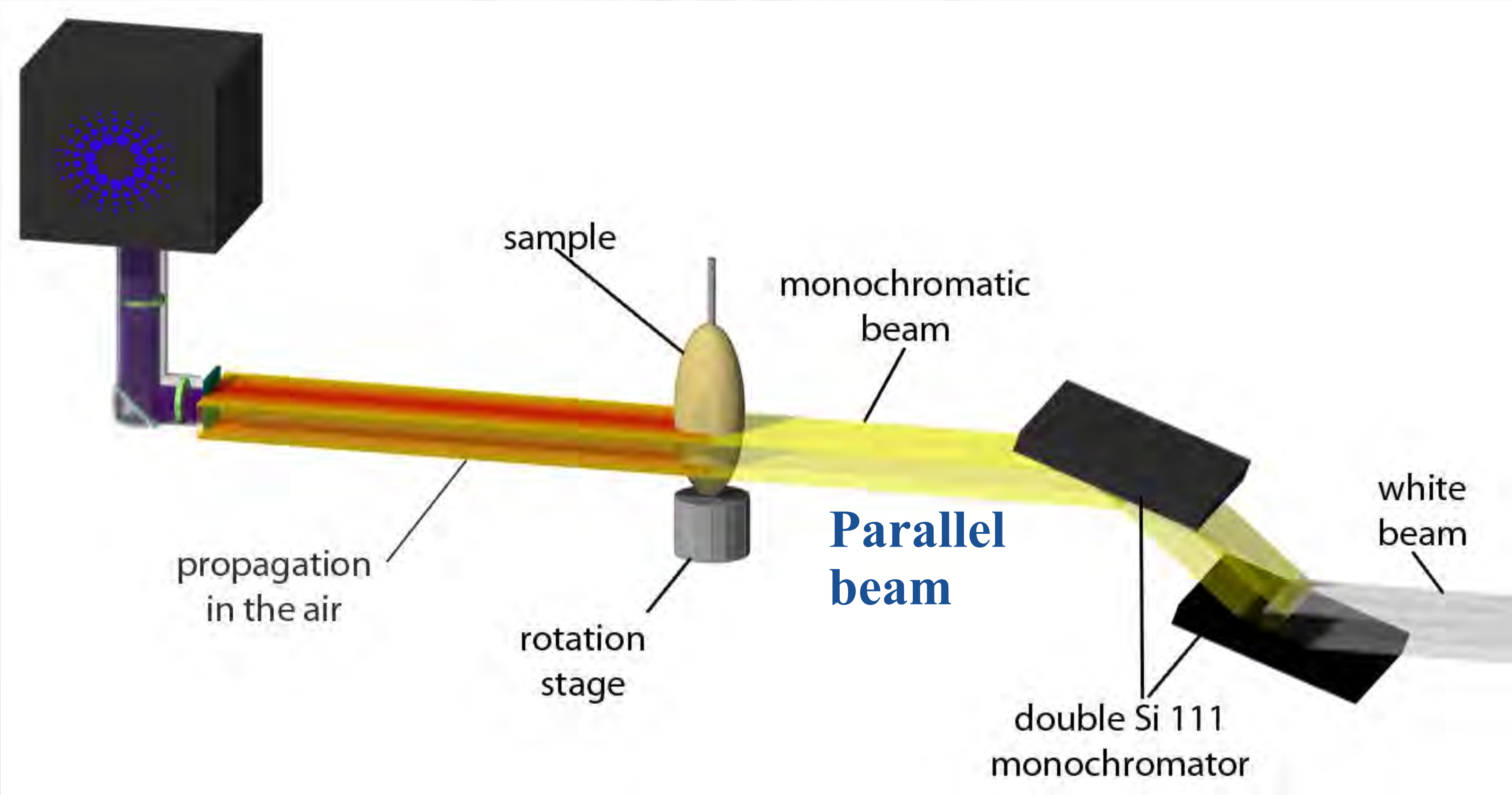
First application on a fossil in 2002, since then it started to replace absorption.

Tomography @ ESRF absorption based CT



Modified from Fernandez

Tomography @ ESRF
propagation phase contrast CT



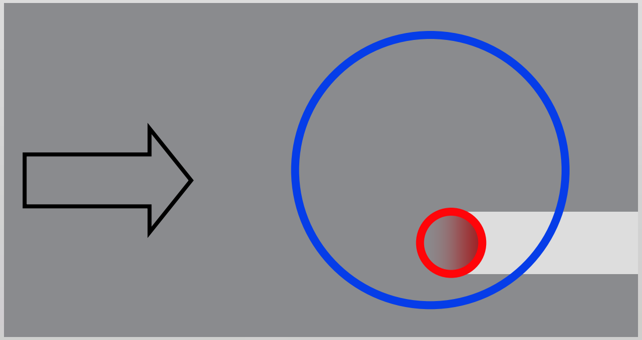
Modified from Fernandez

Propagation Phase Contrast Imaging

Due to the very small source size and to the long distance between the source and the sample, X-ray beams at the ESRF are partially coherent. This property leads to specific imaging techniques based on phase instead of absorption.

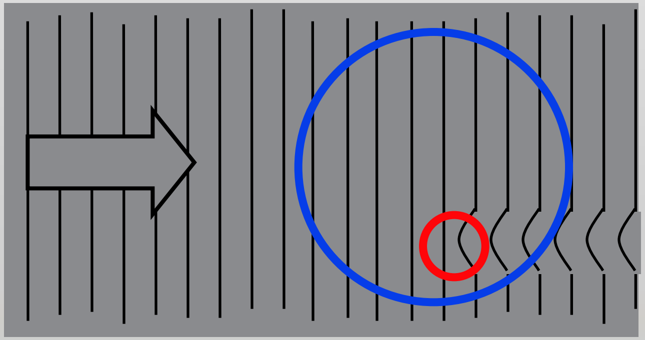
With a third generation synchrotron the easiest way to obtain phase contrast on projections consists in increasing the distance between sample and detector
=> Propagation phase contrast

Absorption



Sample

Phase



Sample

Phase contrast imaging on Cretaceous fossil insects preserved in opaque amber from Charente

Malvina Lak **(Géoscience / ESRF)**

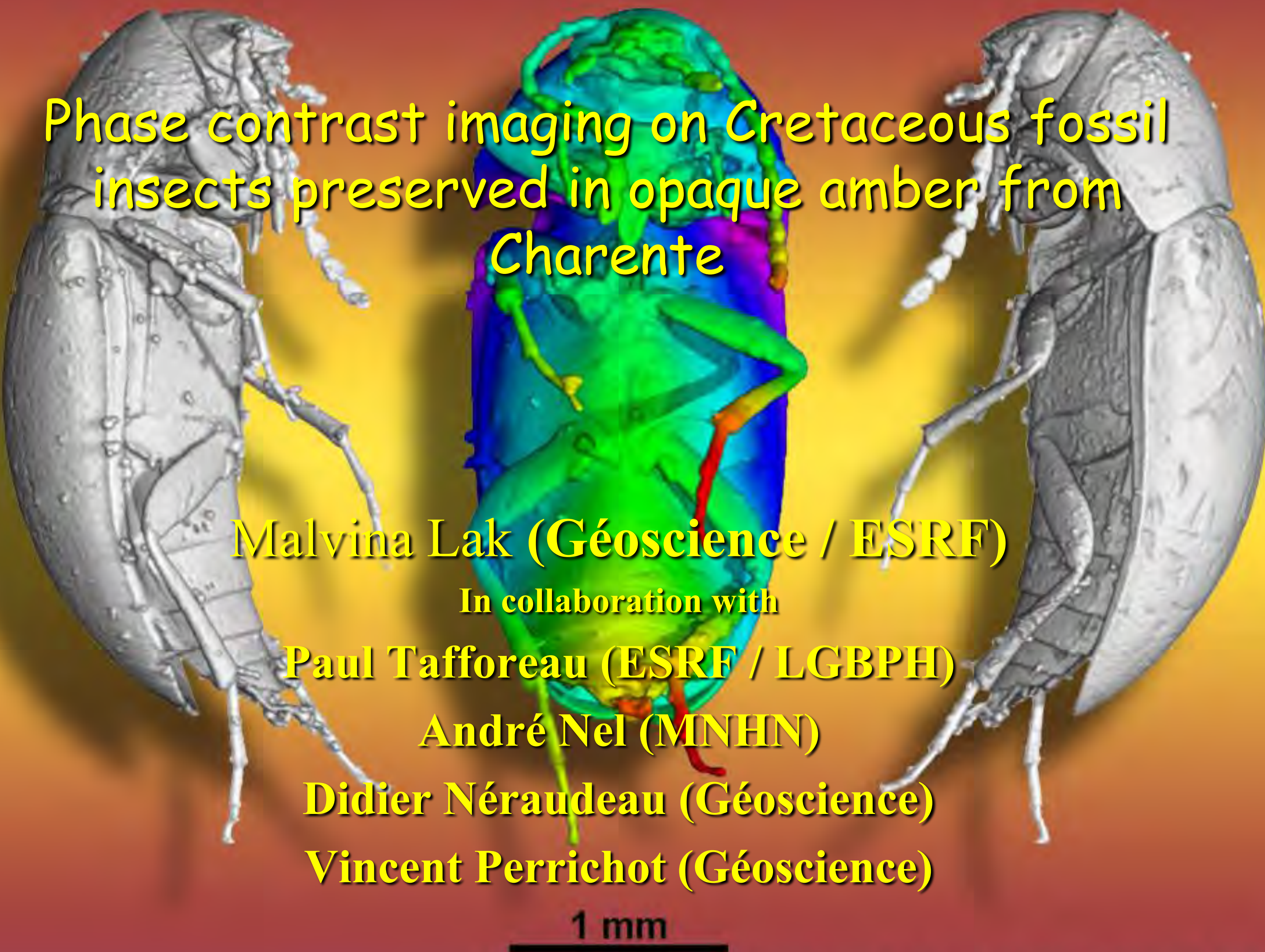
In collaboration with

Paul Tafforeau (ESRF / LGBPH)

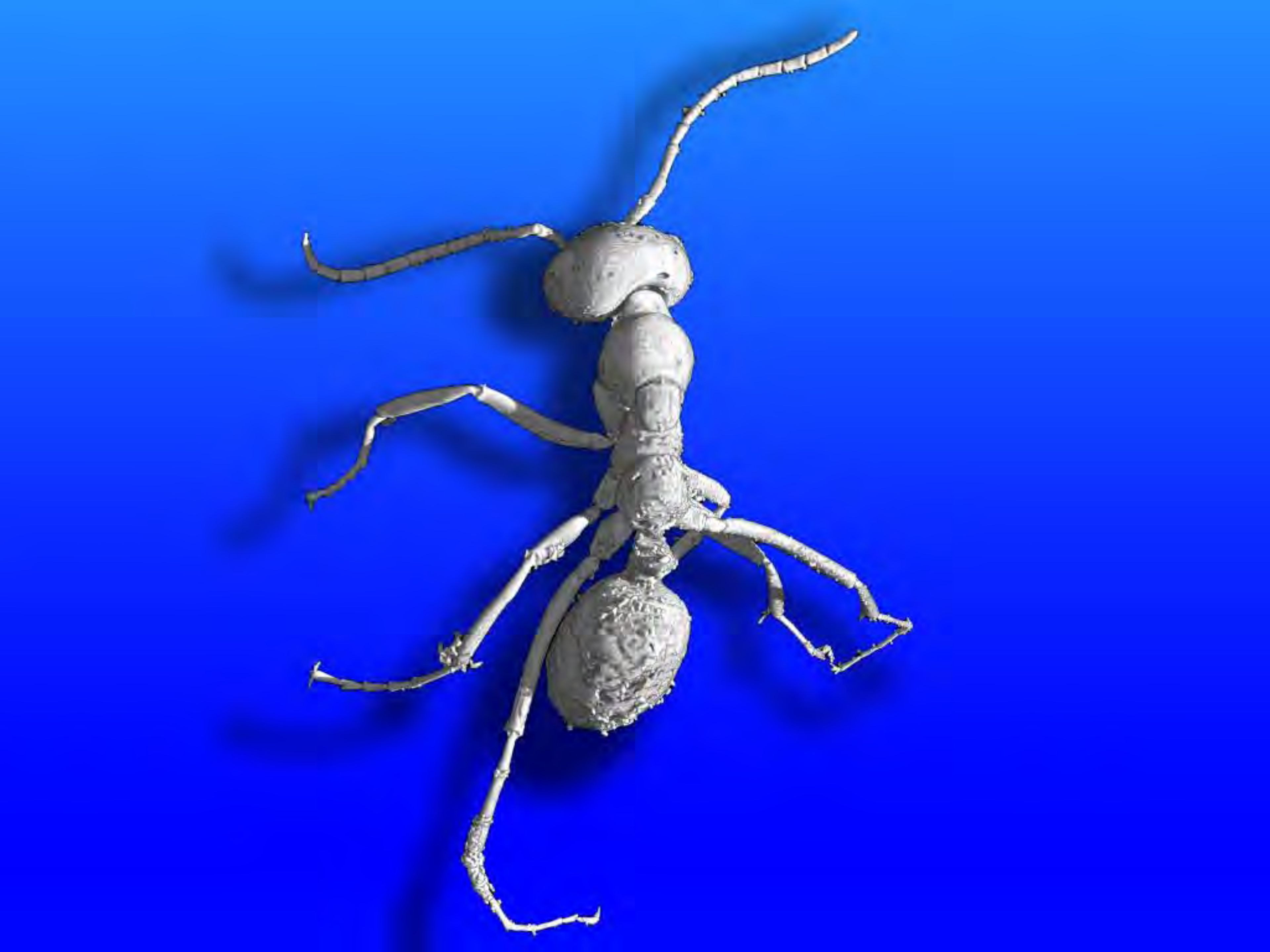
André Nel (MNHN)

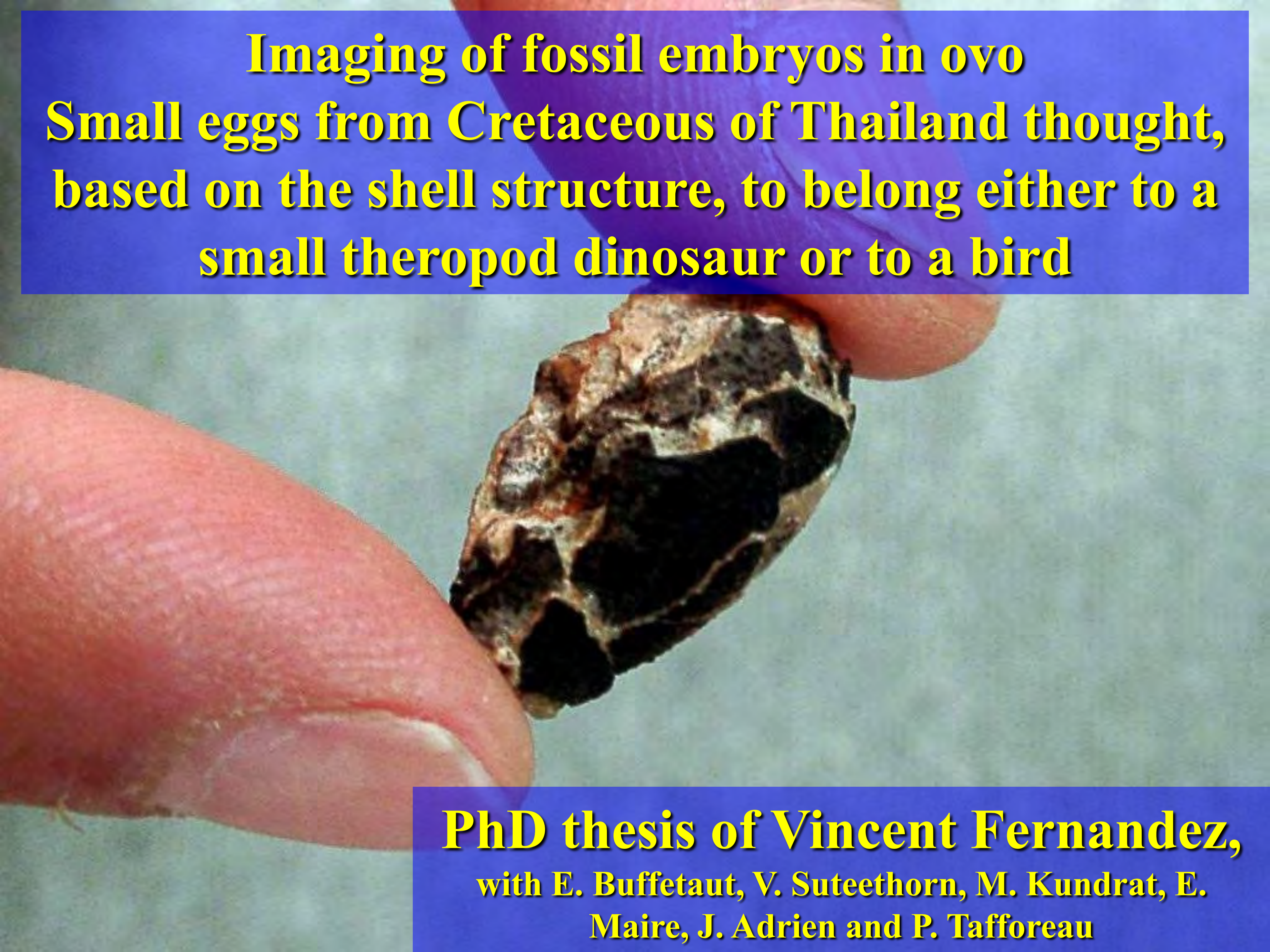
Didier Néraudeau (Géoscience)

Vincent Perrichot (Géoscience)



Tafforeau *et al.*, Applied Physics A, 2006




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

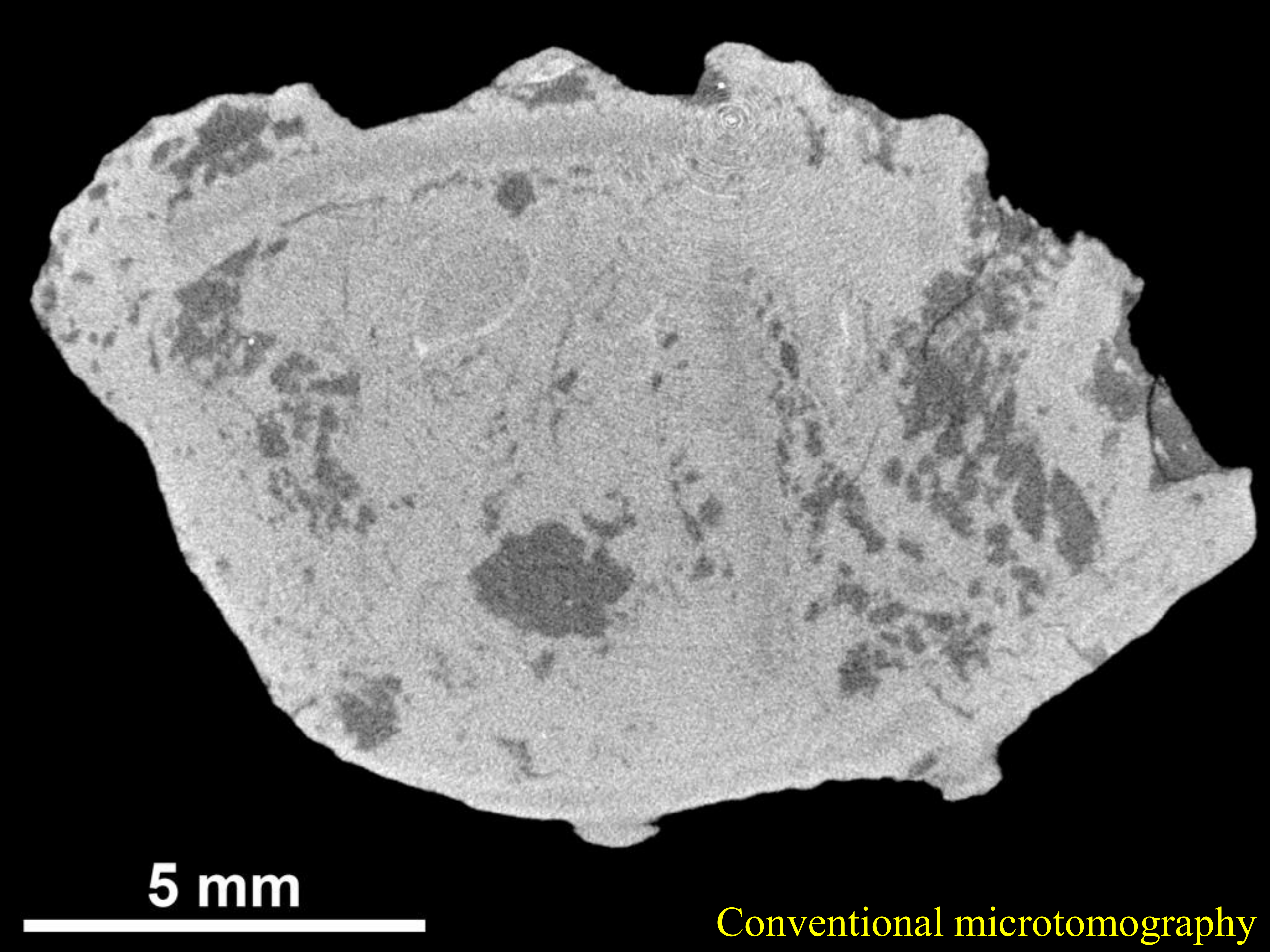
It is impossible to know what animal laid an egg without seing the embryo inside !

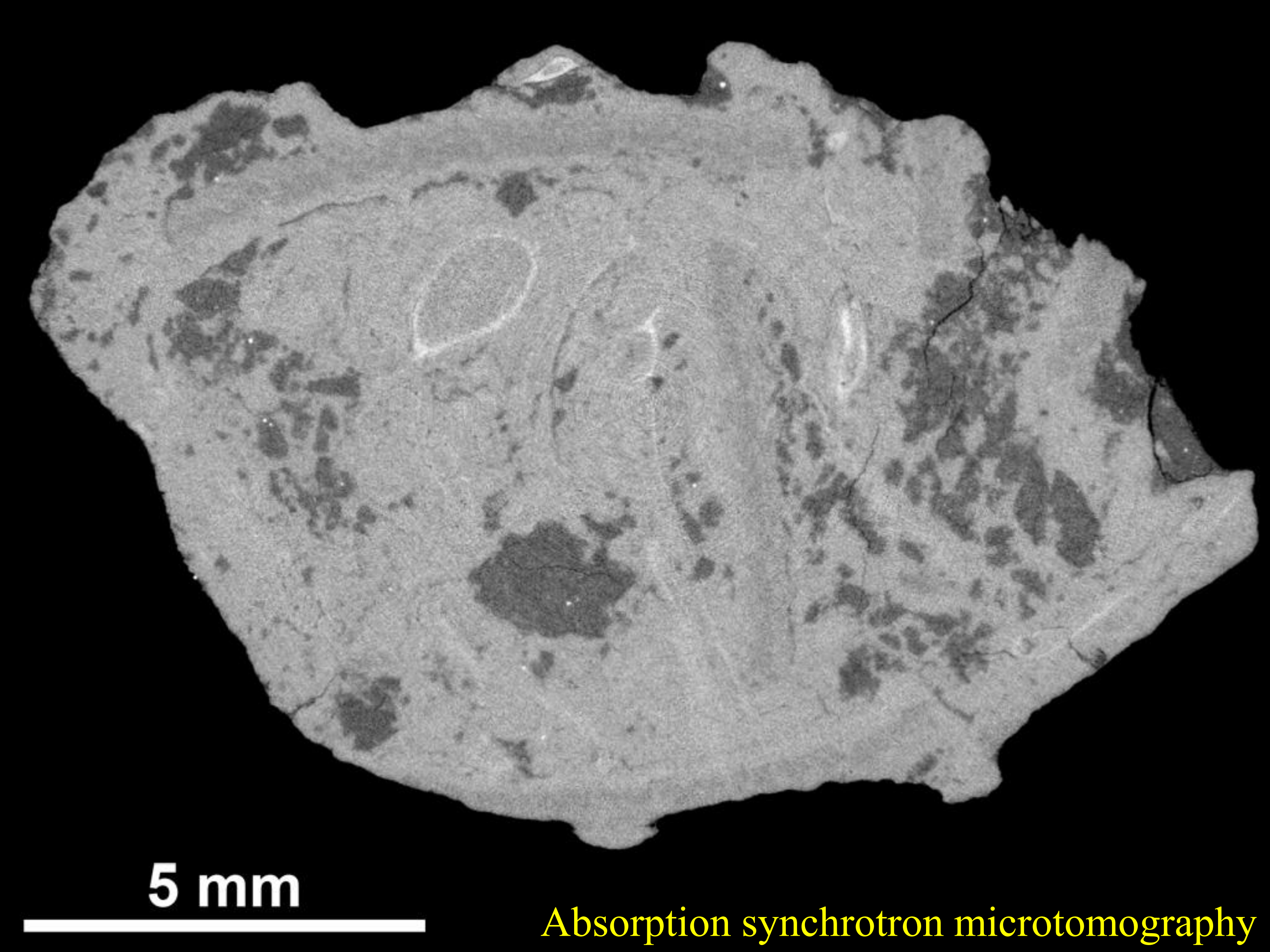
L'âge de glace 3

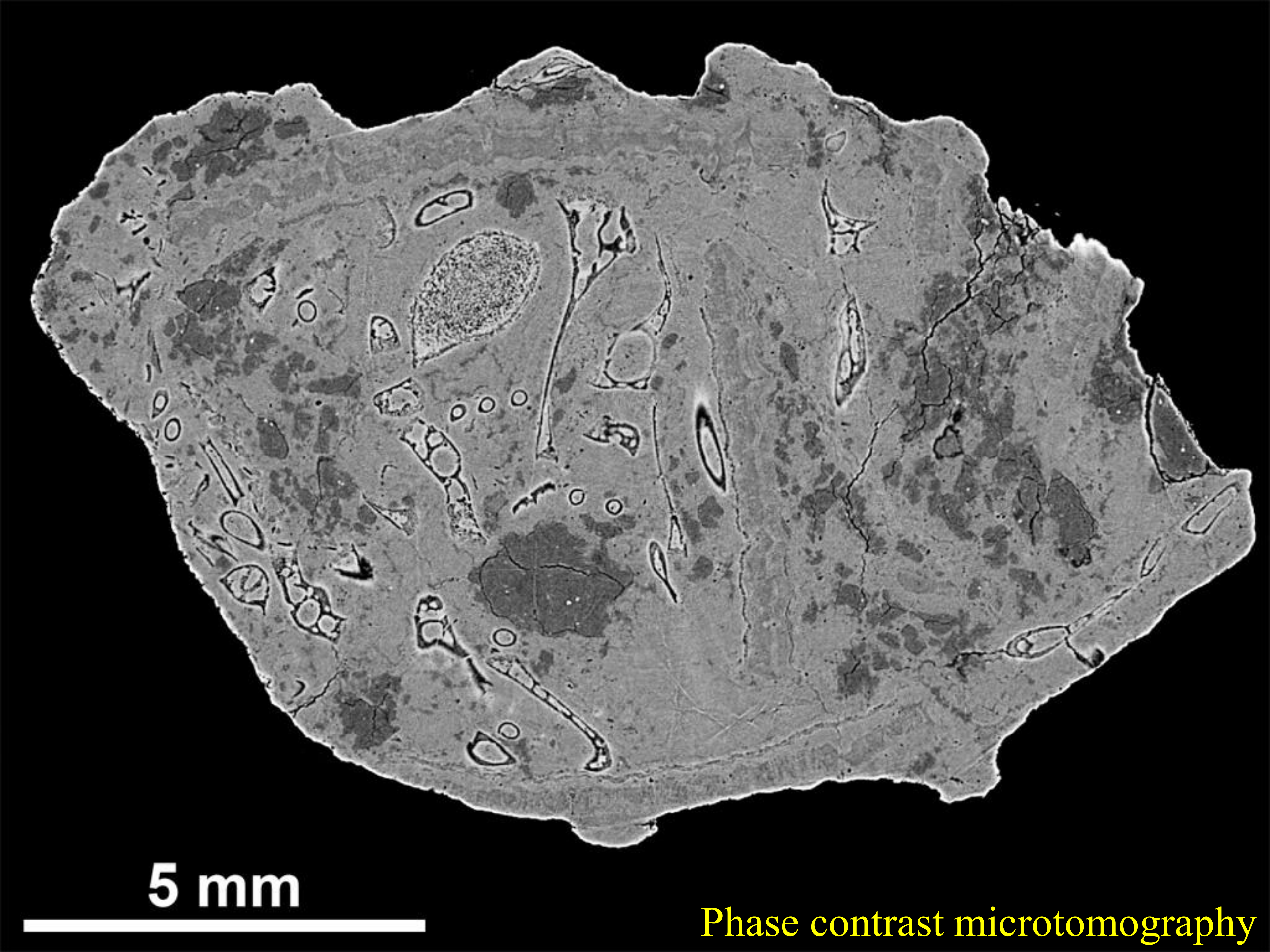
Small dinosaurs ?

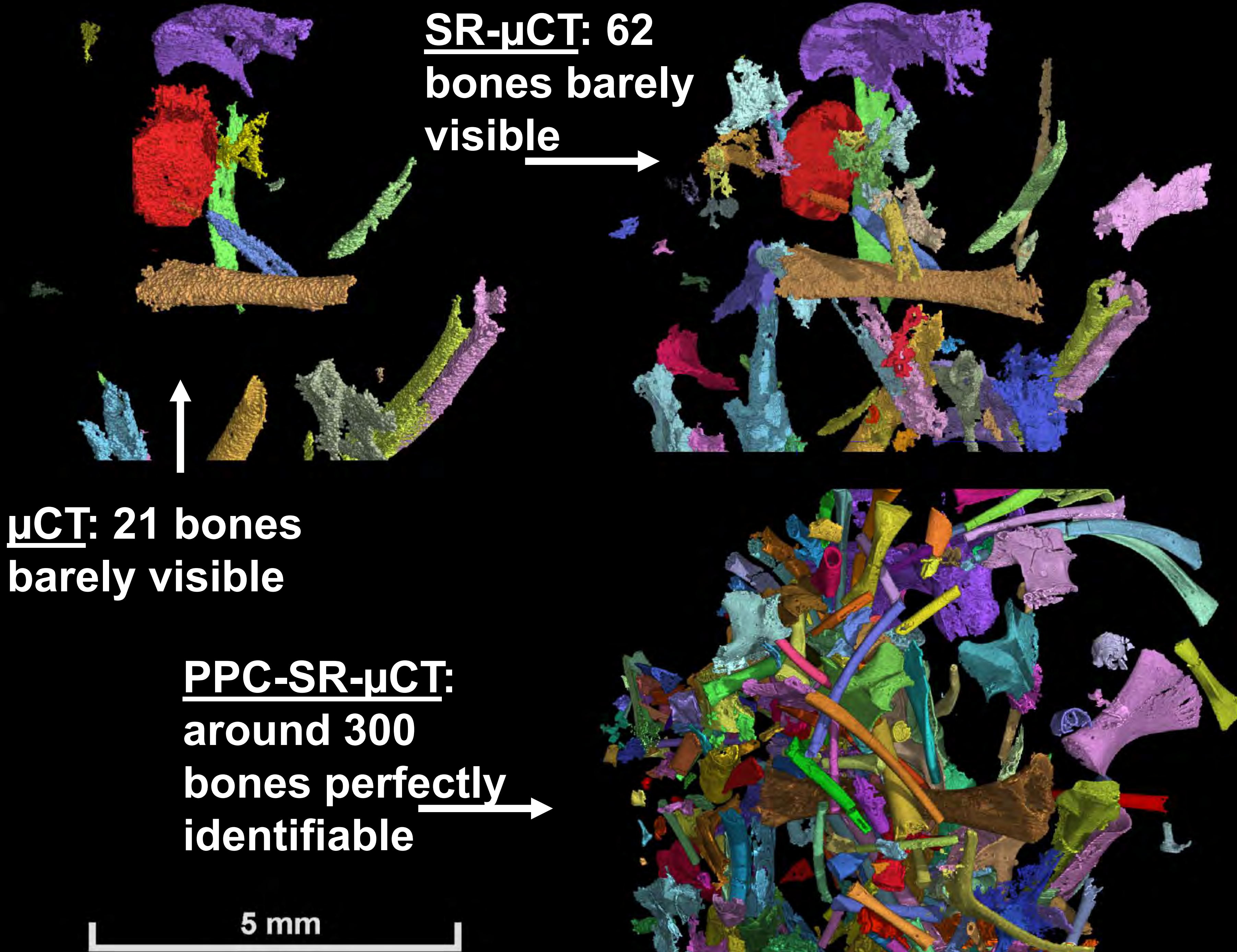
•L’âge de glace 3, le temps des dinosaures

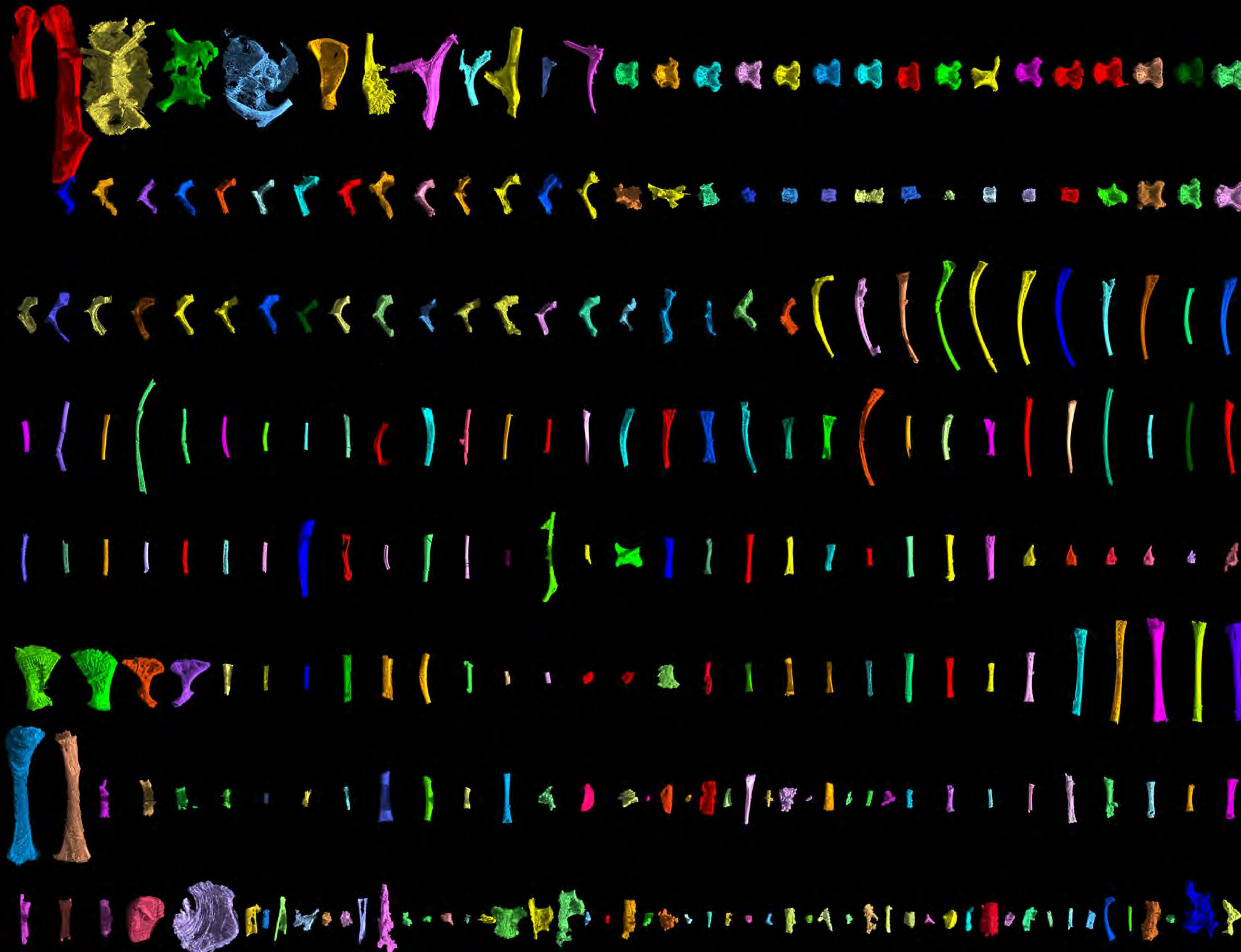
Primitive birds ?


5 mm
Conventional microtomography


5 mm
Absorption synchrotron microtomography

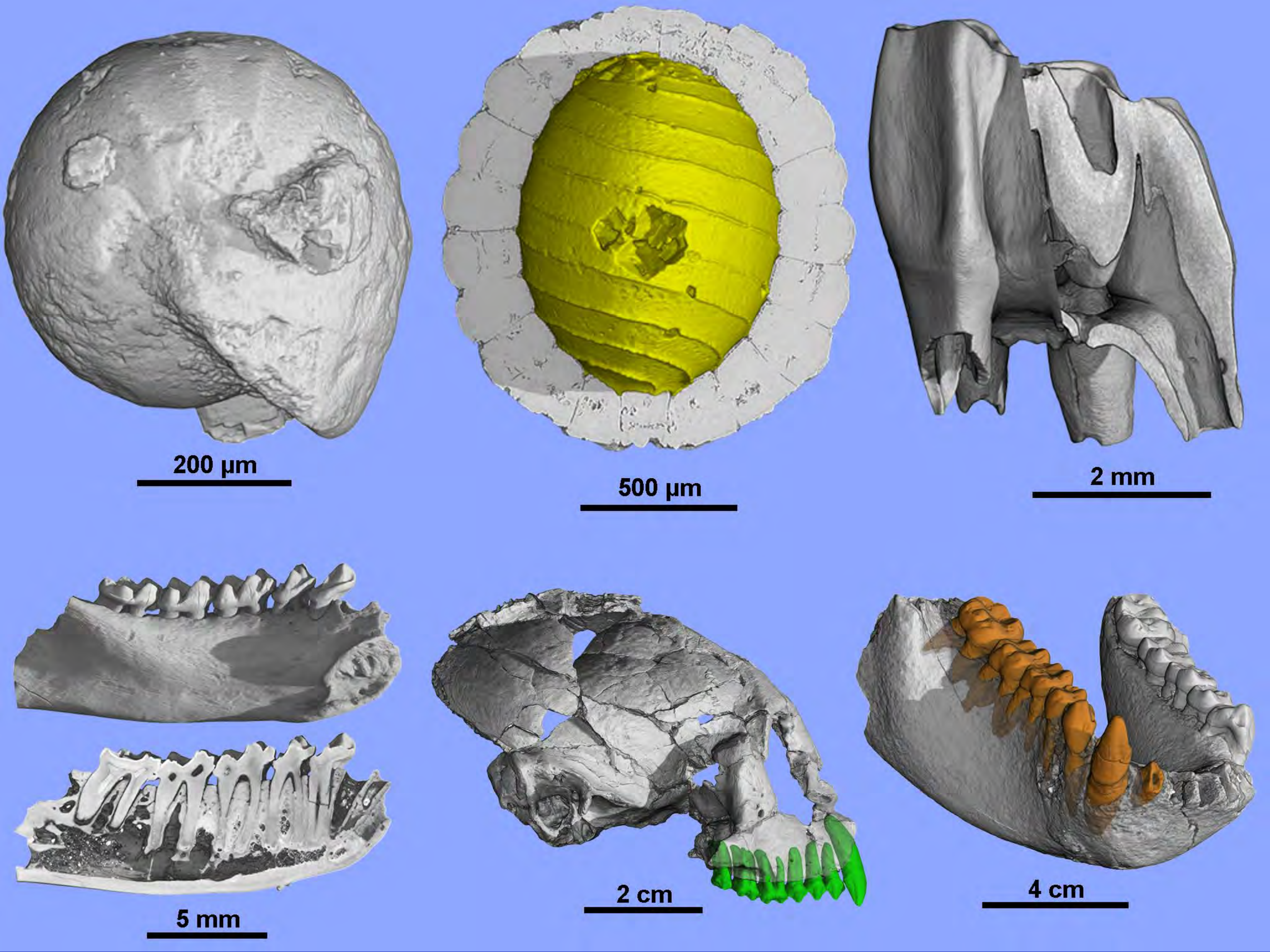

5 mm
Phase contrast microtomography


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

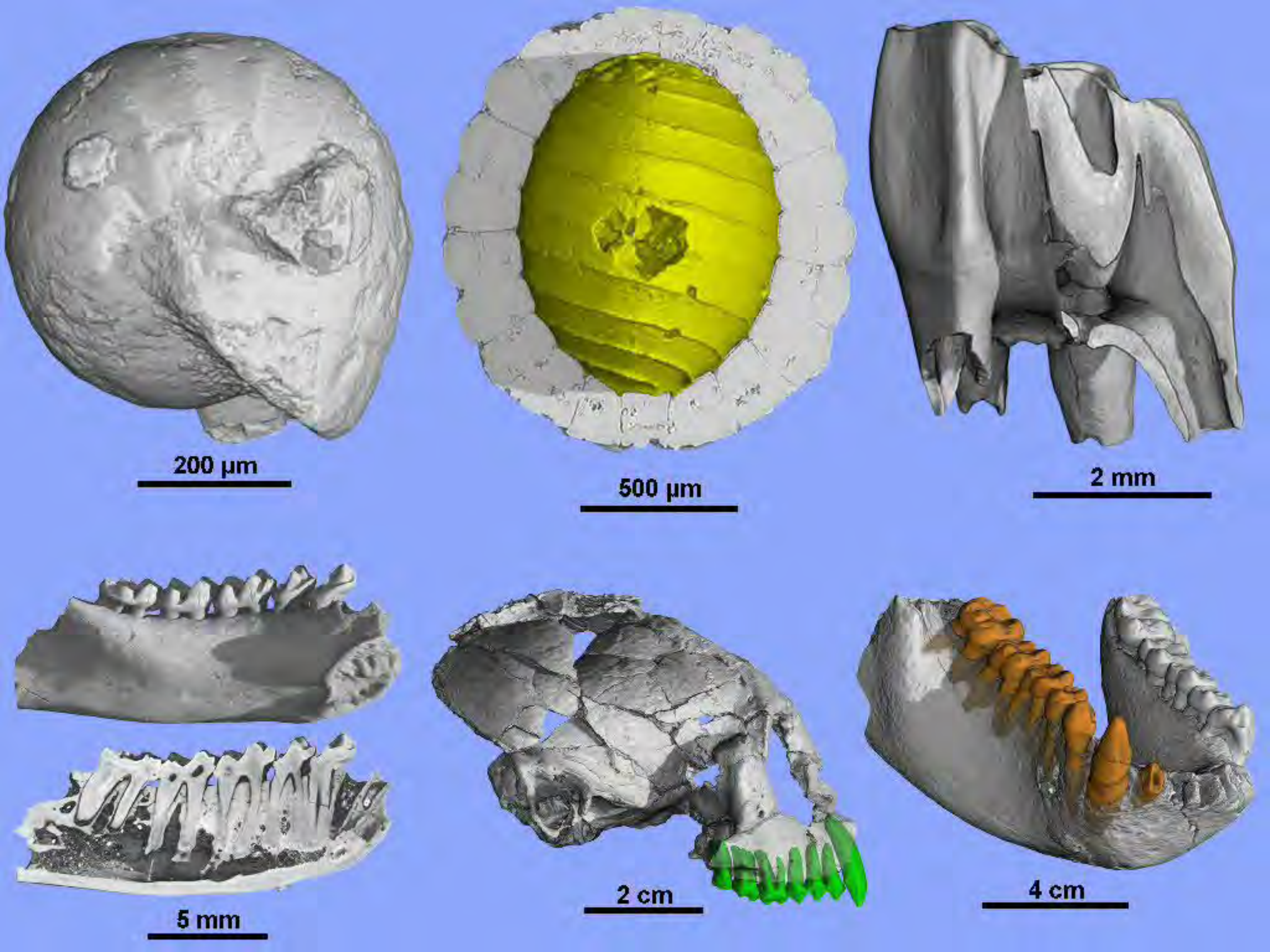


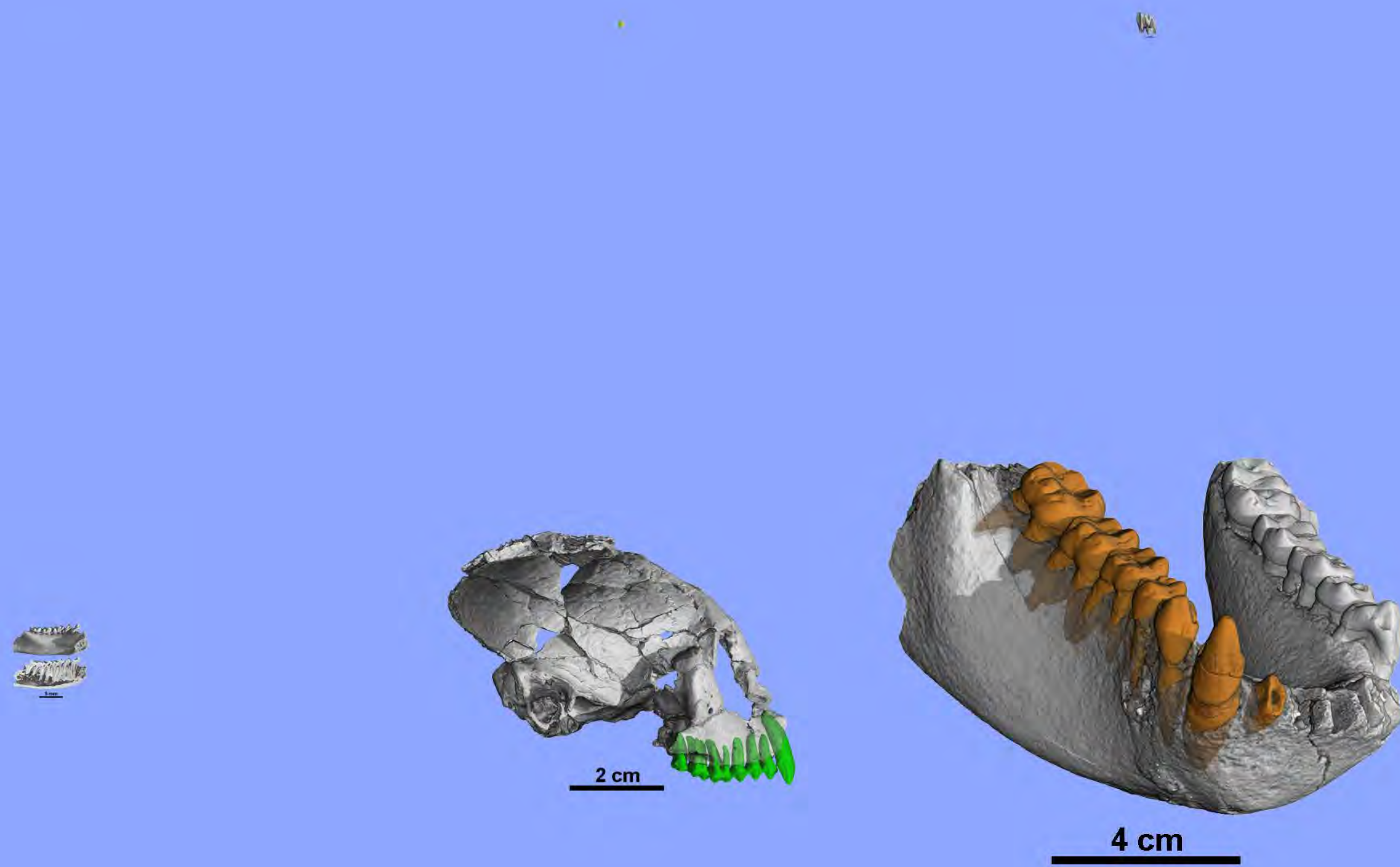
Another aspect of synchrotron imaging in palaeontology:

the high resolution



200 µm
500 µm
2 mm
5 mm
2 cm
4 cm


200 µm
500 µm
2 mm
5 mm
2 cm
4 cm


2 cm
4 cm

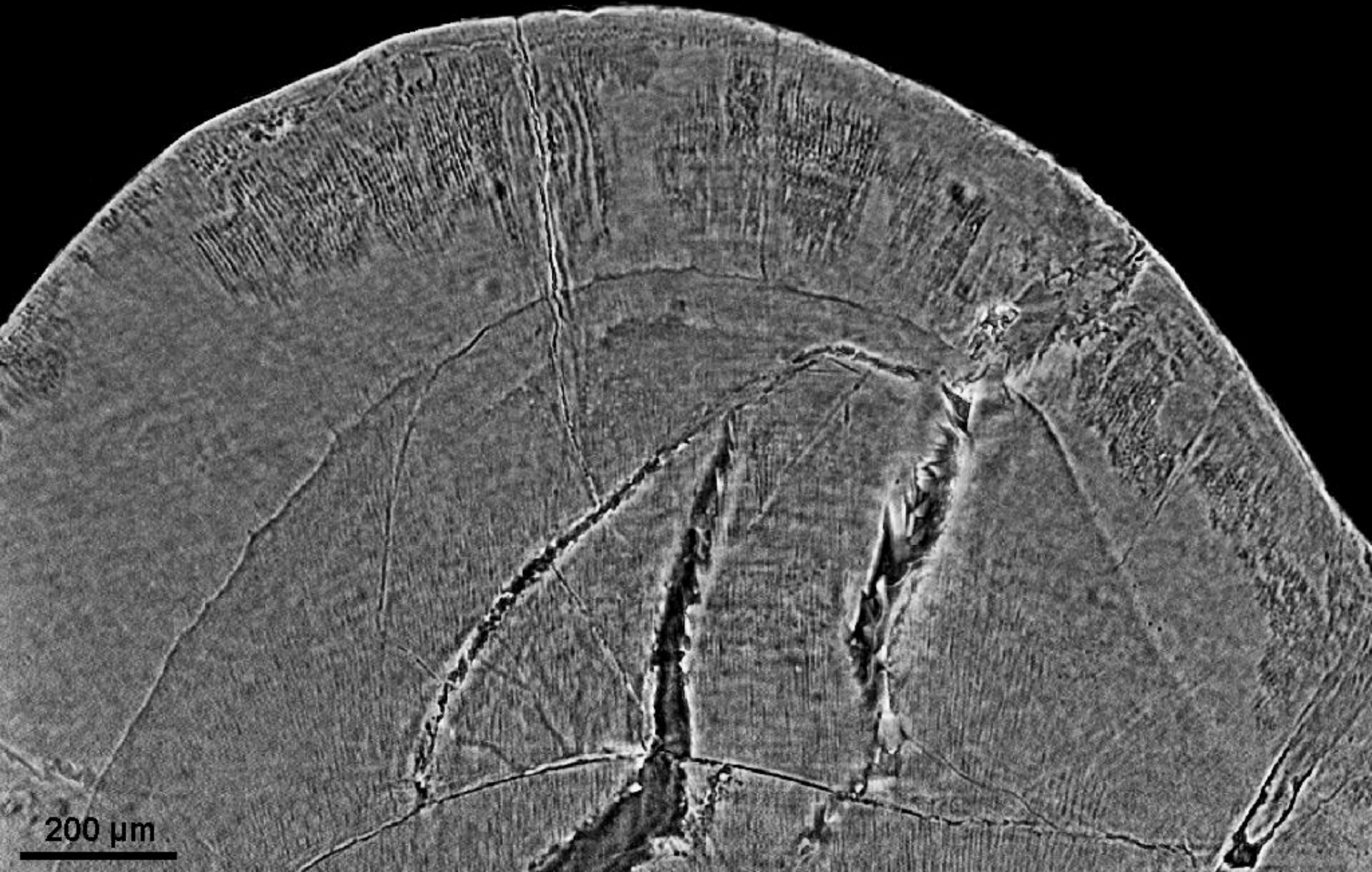
The early origin of synchrotron virtual dental paleohistology (2003)

Eocene Anthropoid from Thailand

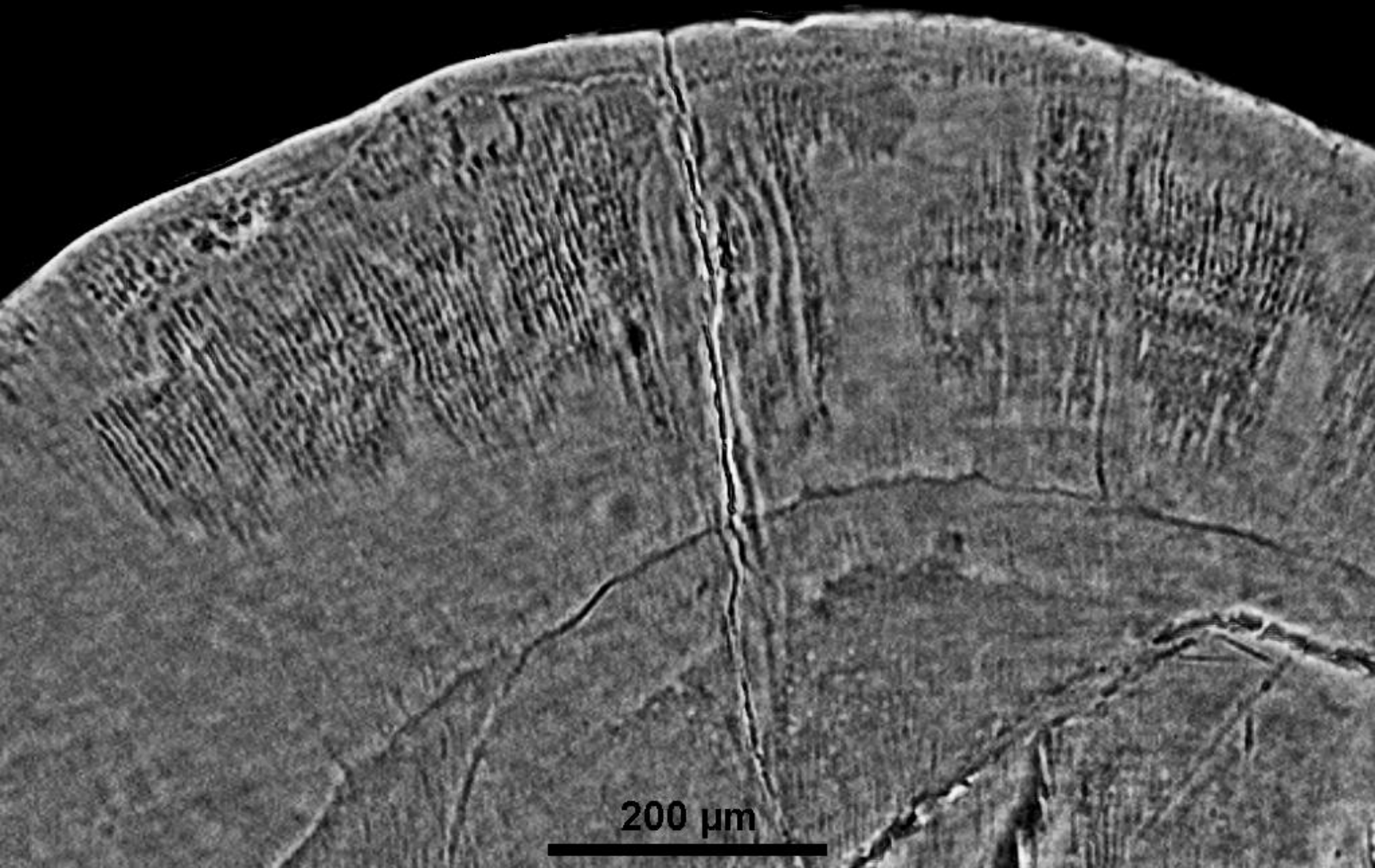
Siamopithecus eoceanus

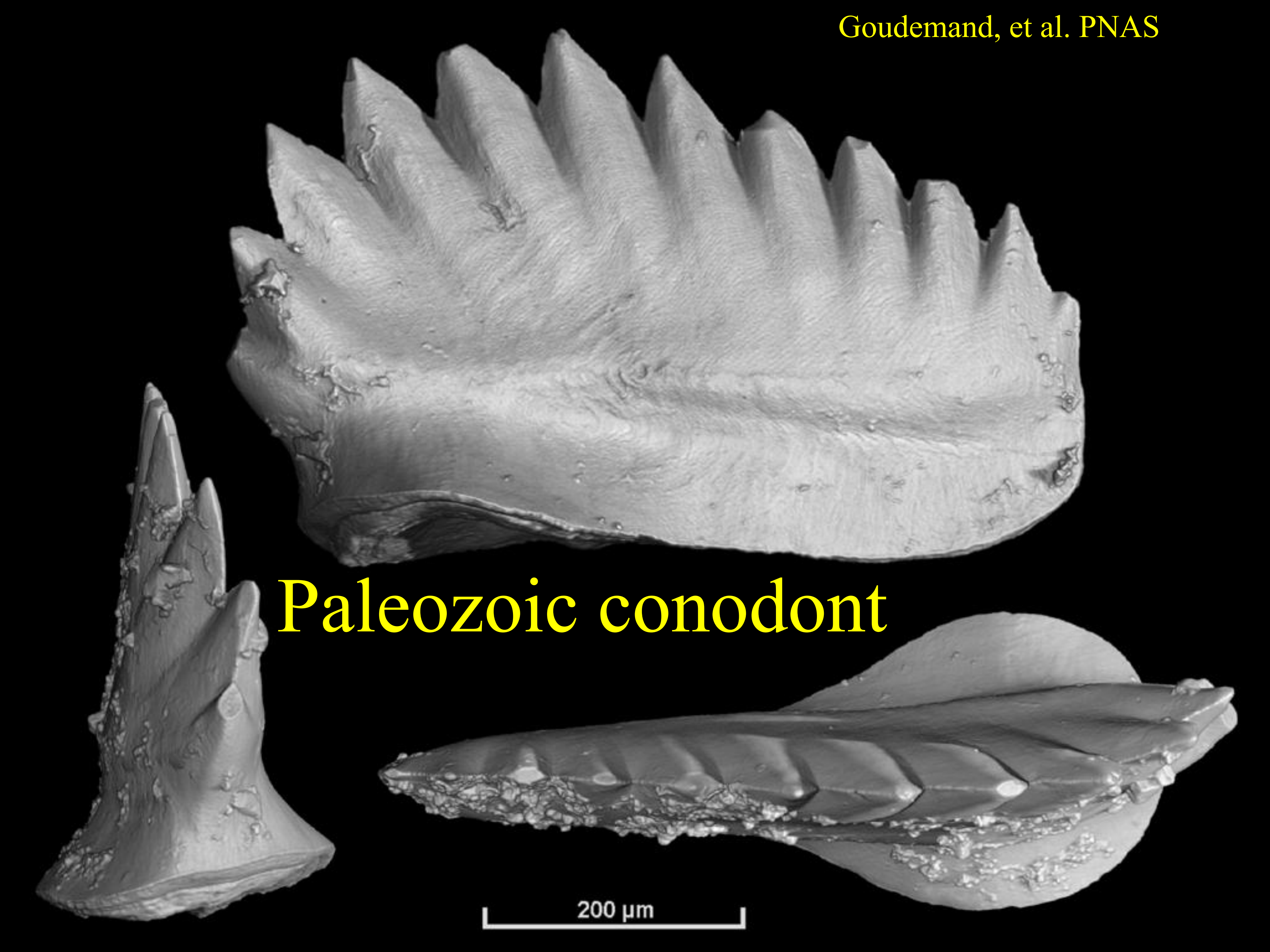
Tafforeau 2004
Tafforeau et al. 2006. J. Appl. Ph. A

Acquisition time 3 hours, low transmission and bad local reconstruction. Maximum amount of enamel around 3 mm (only tips of cusps)

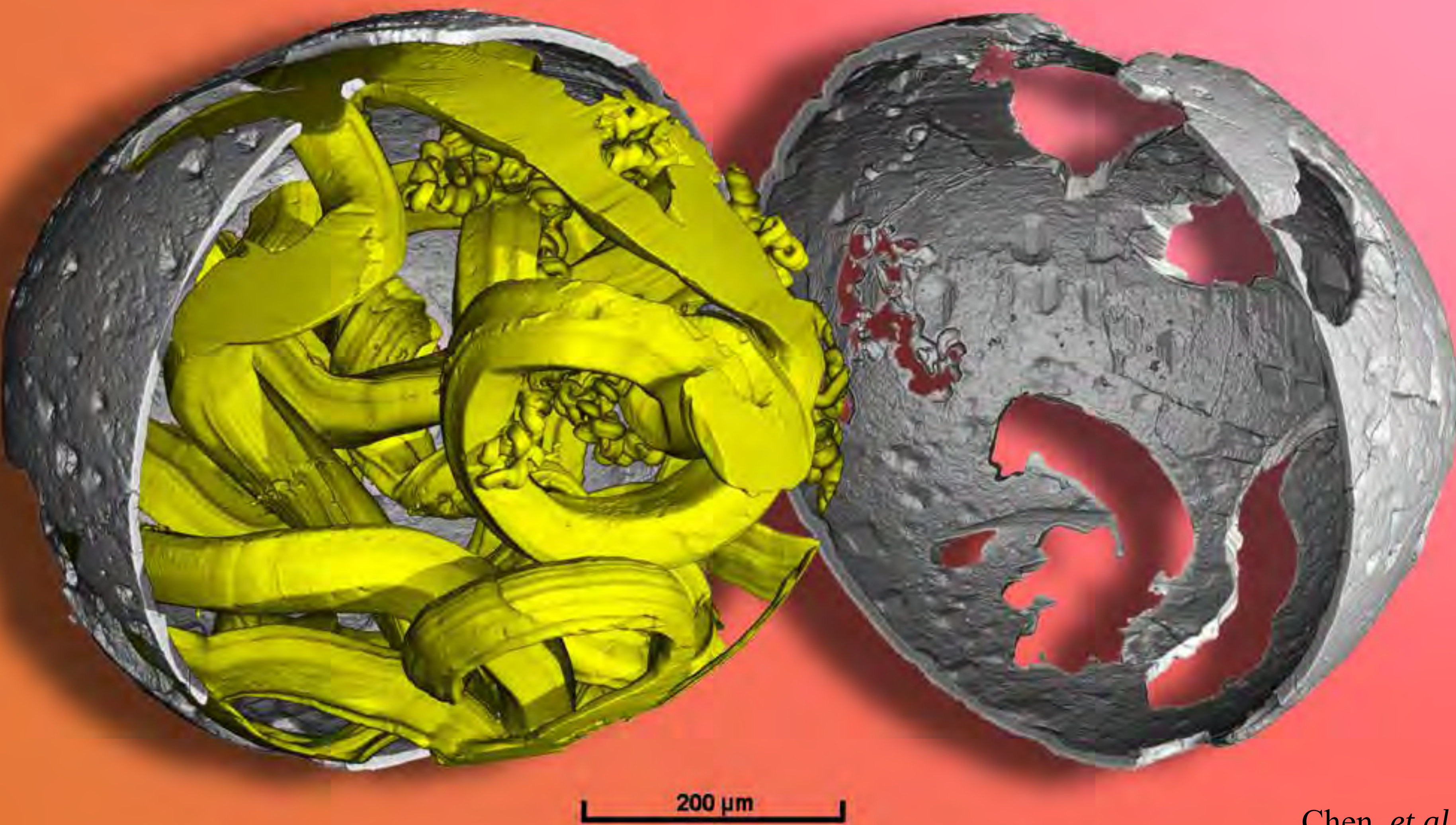


BUT, first observation of enamel prisms and of incremental lines with phase contrast X-ray synchrotron microtomography

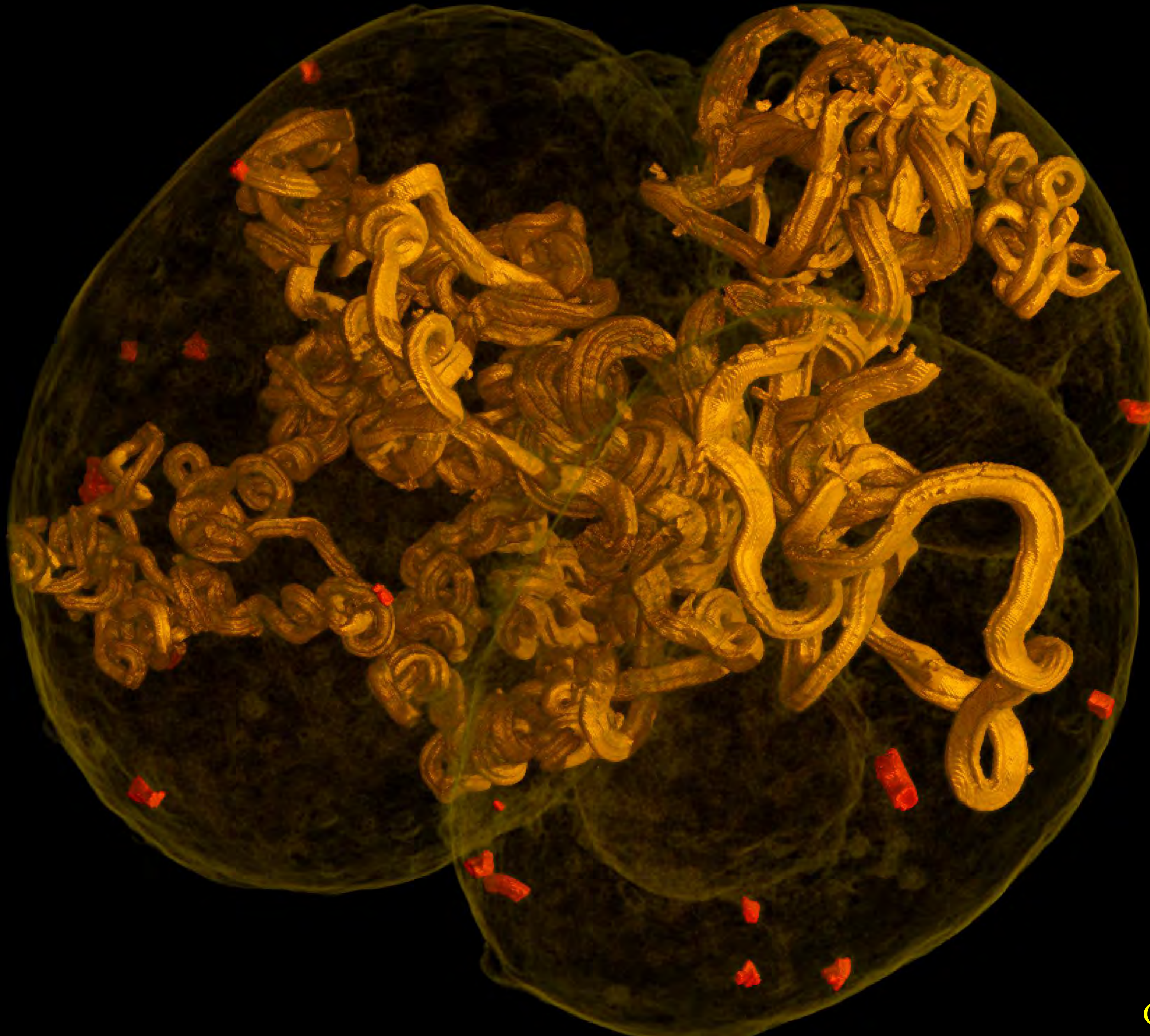



Goudemand, et al. PNAS
Paleozoic conodont
200 µm

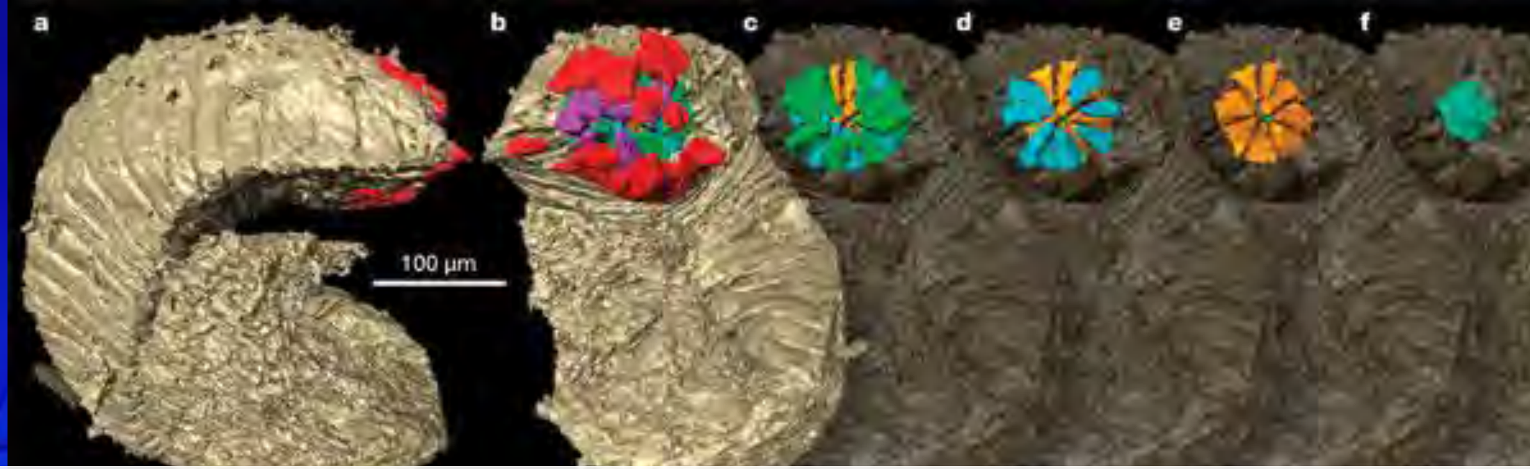
580 million year old embryos from Weng'an with biogenic micro-tunnels



Chen, *et al*



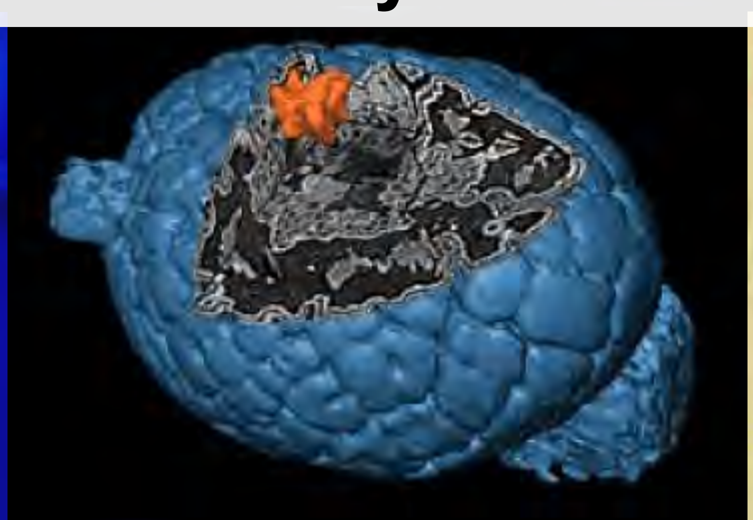
Chen, *et al*



In 2005 another synchrotron enter in the palaeontology field at a high level:

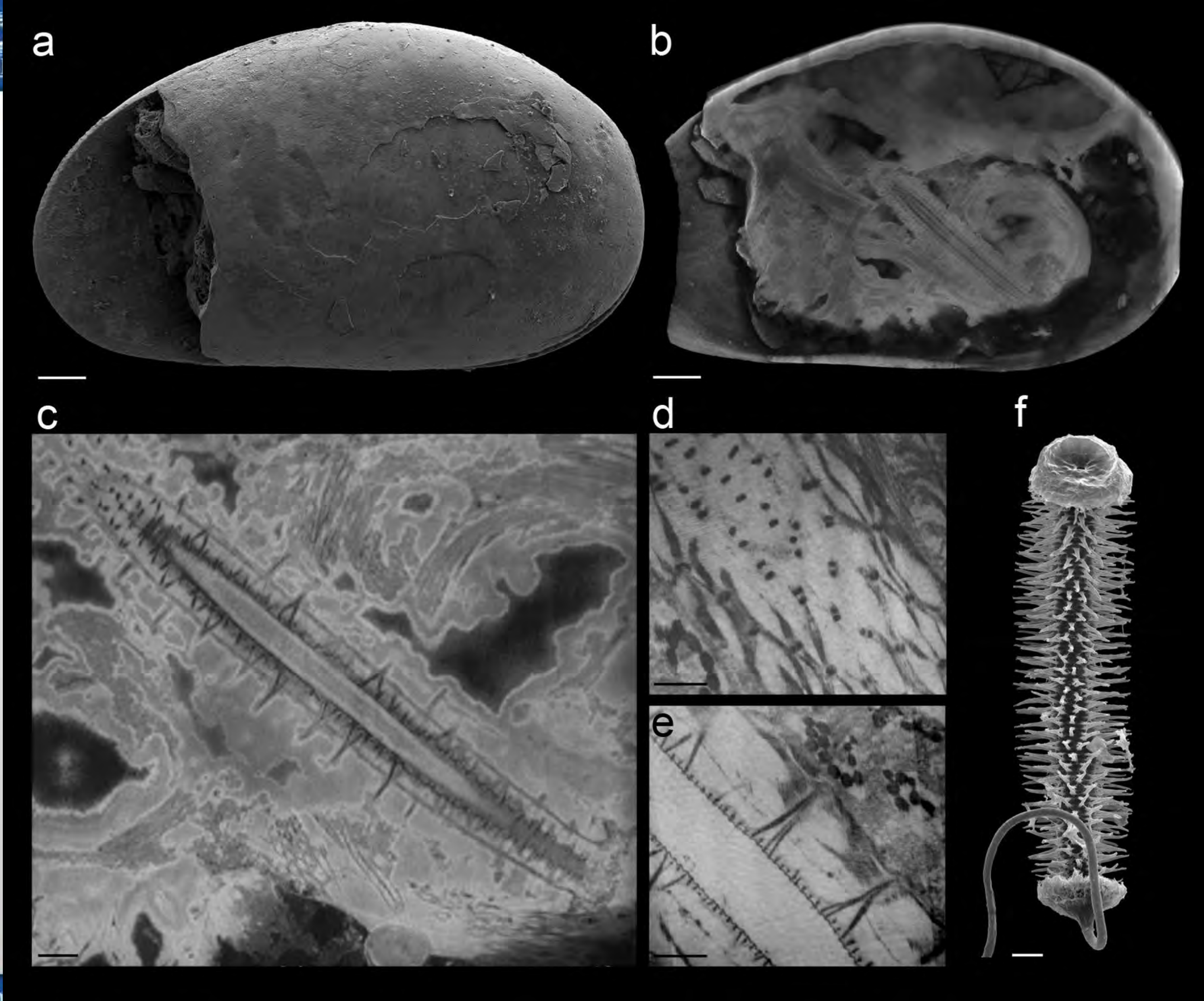
the Swiss Light Source

They work mostly on small samples (<1cm)



Pushing further the resolution: Nanotomography on ID22 (and soon on NINA)

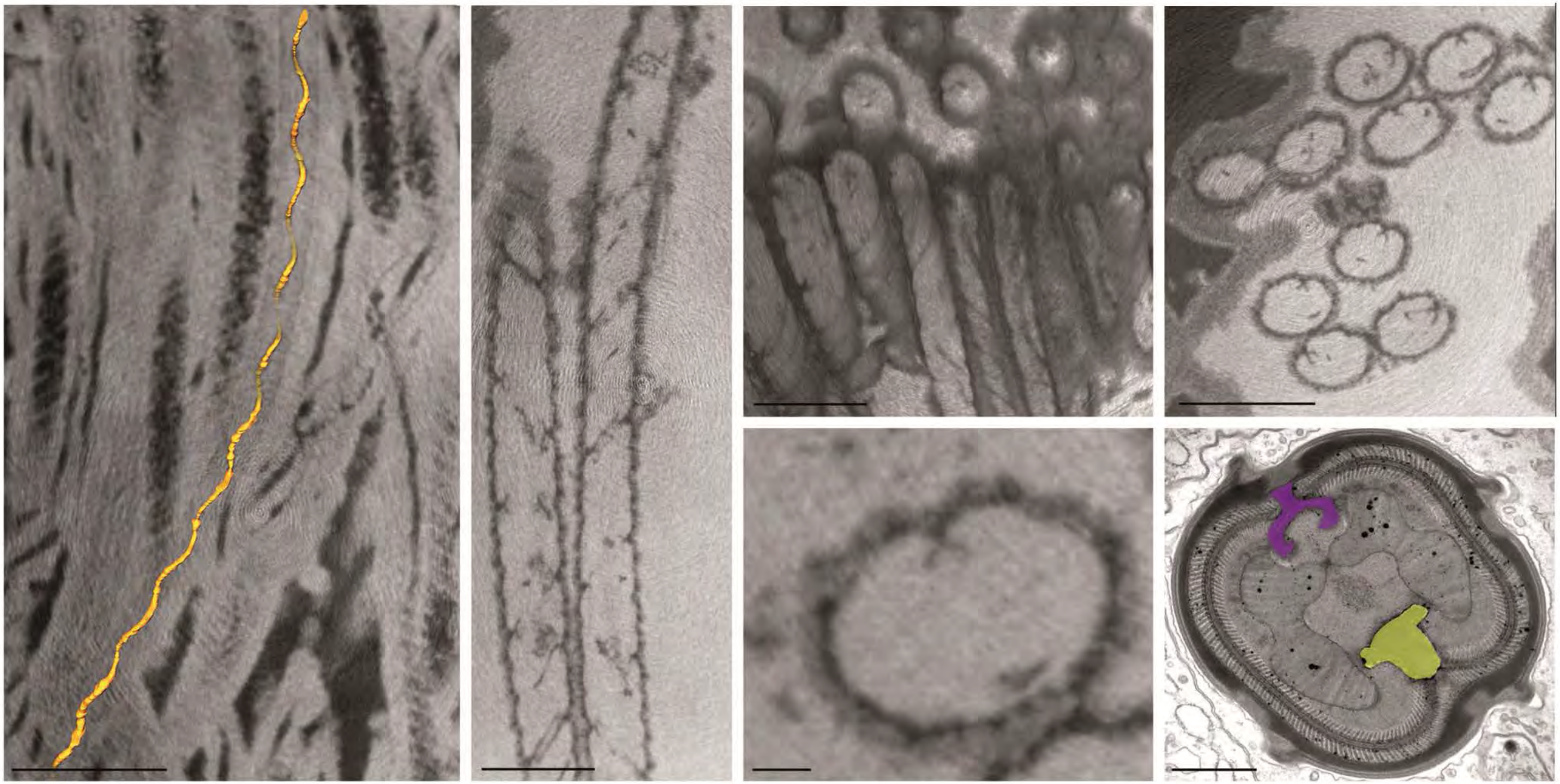
In 2012, some experiments have been performed by associating ID19 down to the micron scale, and ID22NI in order to reach resolution around 50 nm.

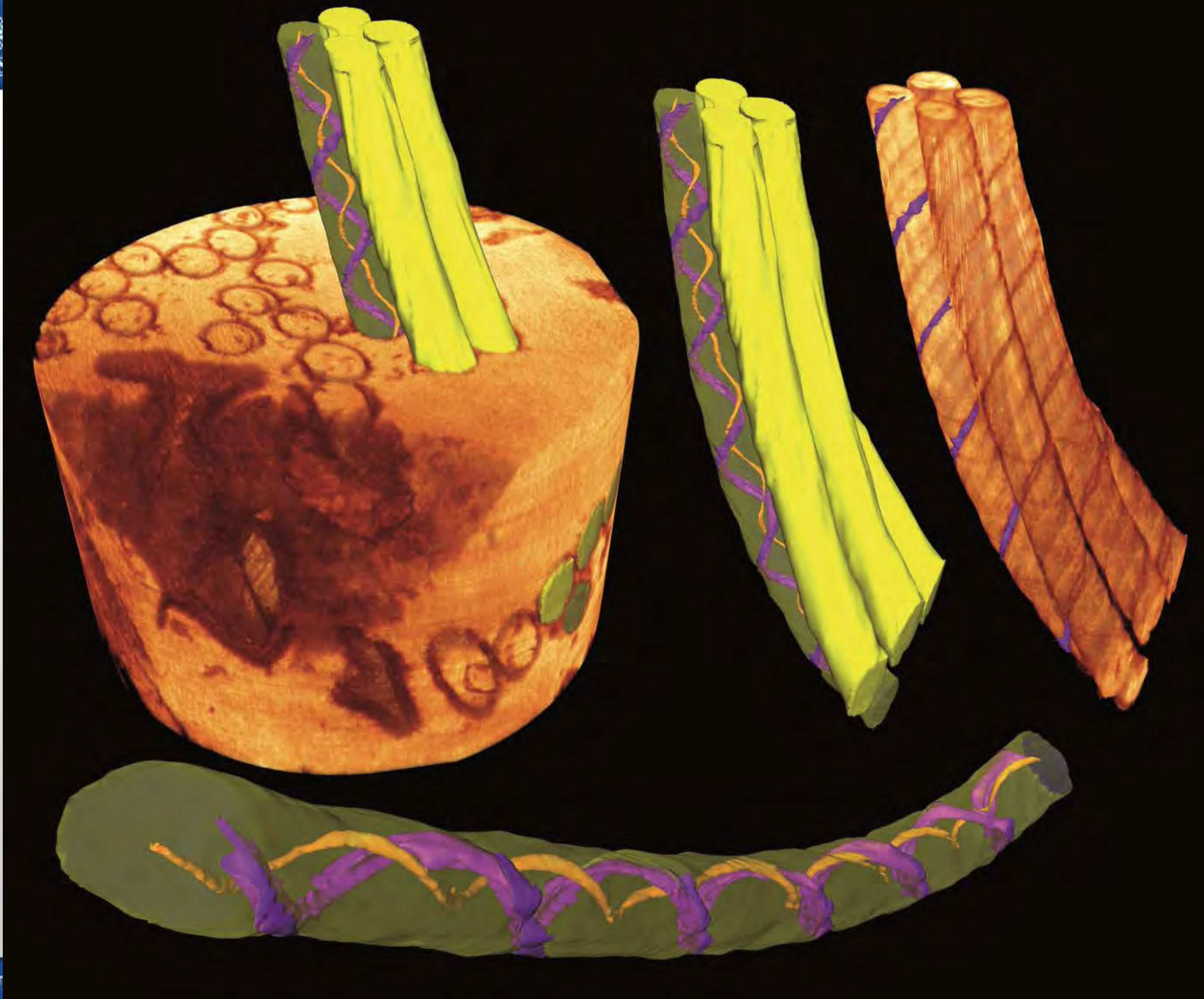


a
b
c
d
e
f


i

25 microns voxel size on ID22NI using holo-nanotomography

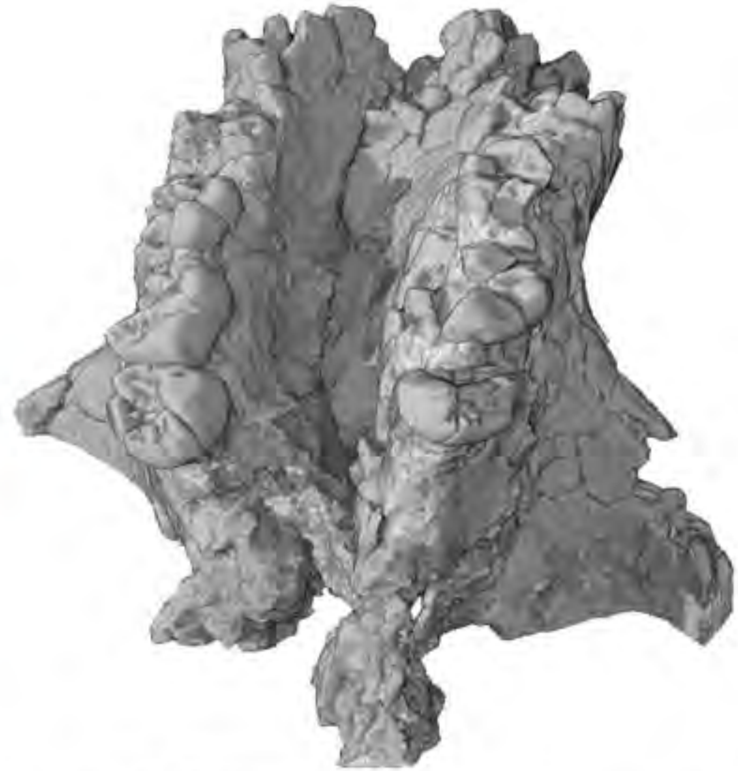


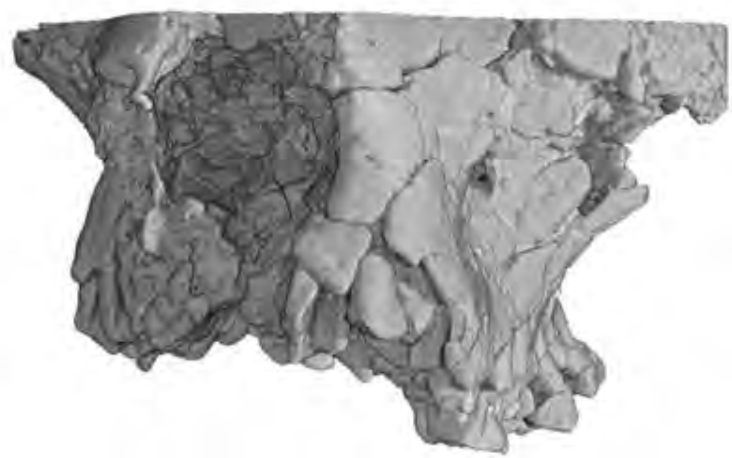


A unique aspect at the ESRF: High quality microtomography on large fossils

project started in 2004, in order to be able to scan *in fine* the skull of Toumaï

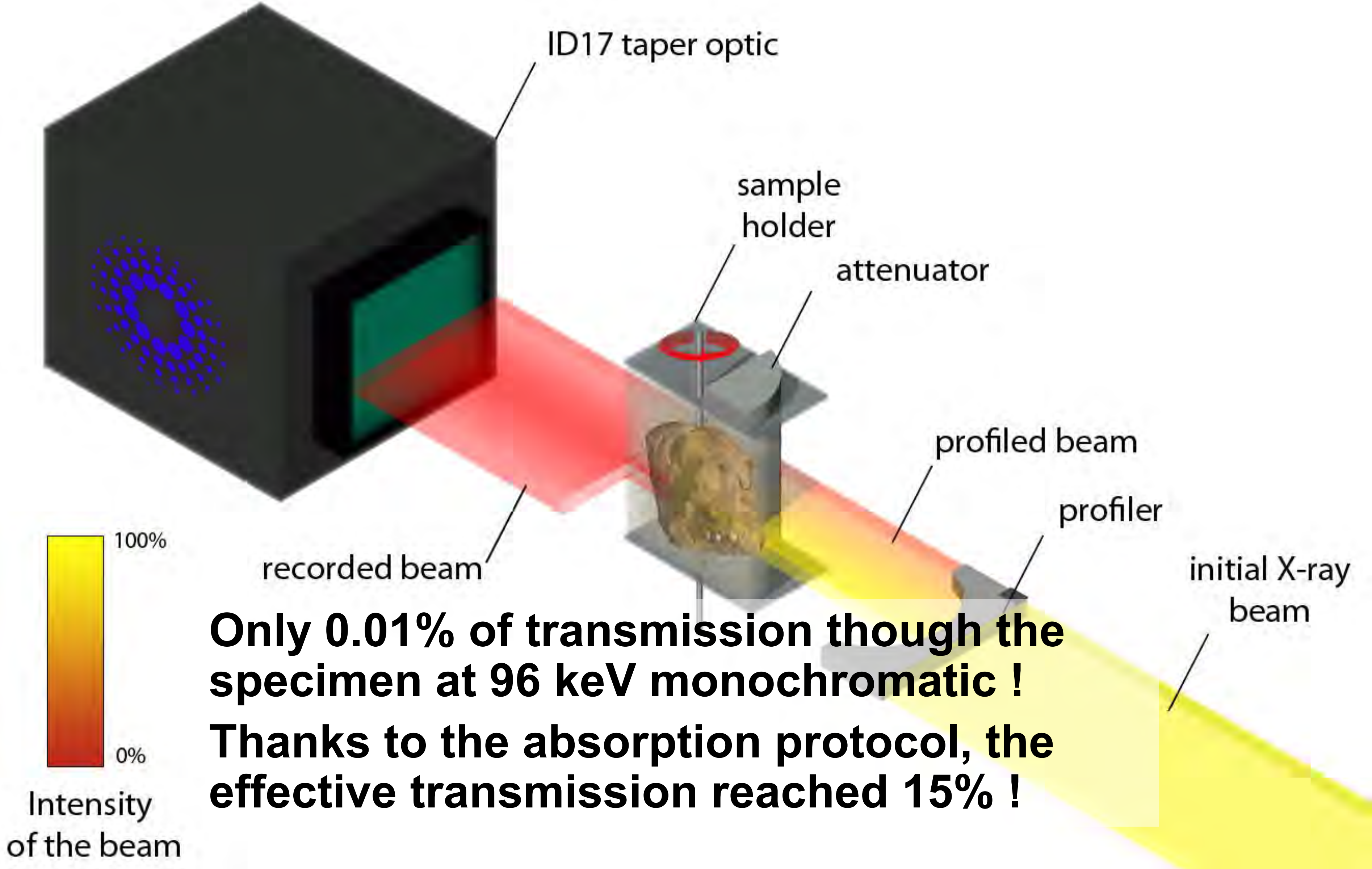
Sahelanthropus tchadensis : Result of the 2004 scan





Tafforeau, Brunet, *et al.*

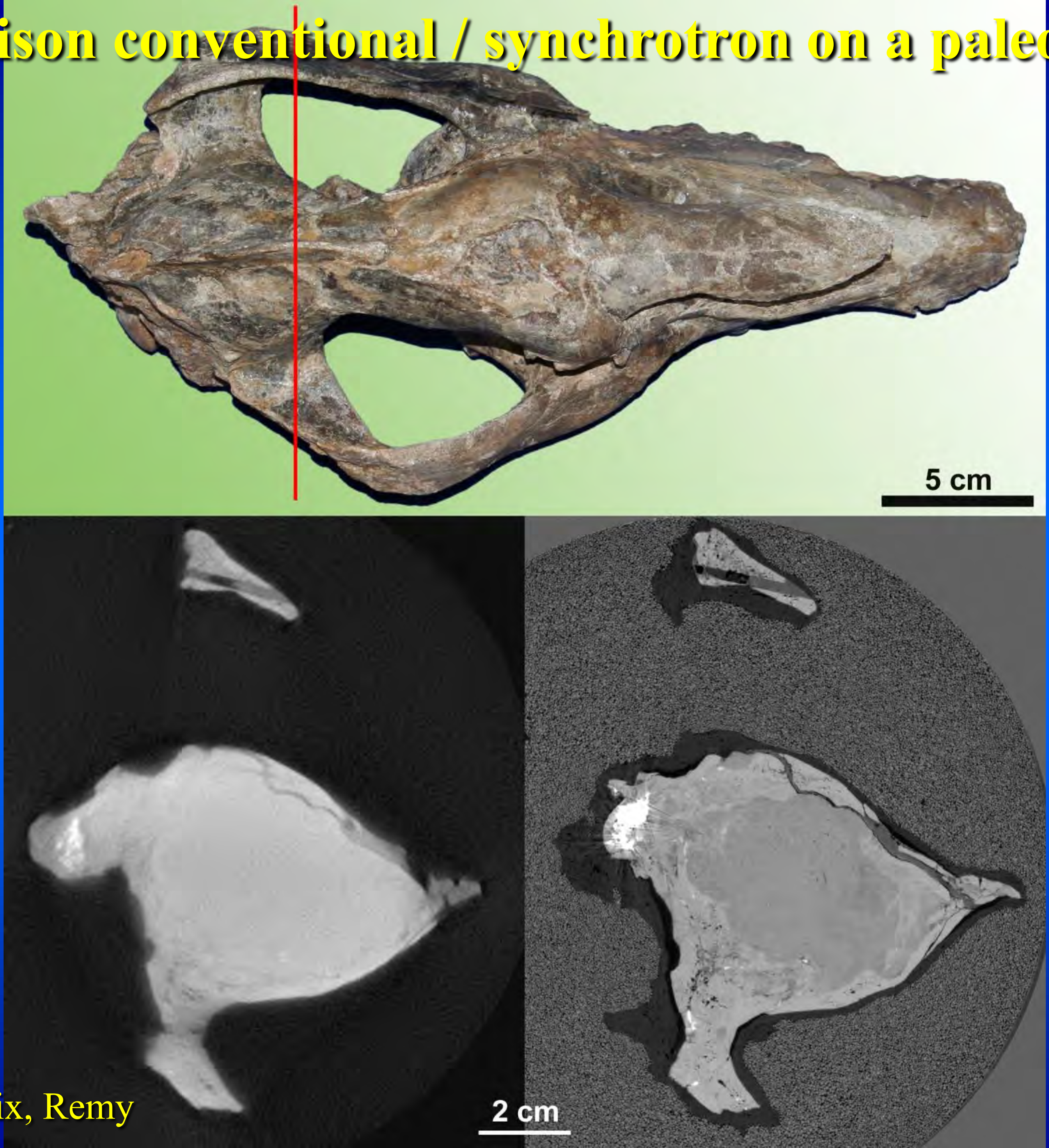
The absorption normalization protocol: how to make an impossible scan possible.



Only 0.01% of transmission though the specimen at 96 keV monochromatic !

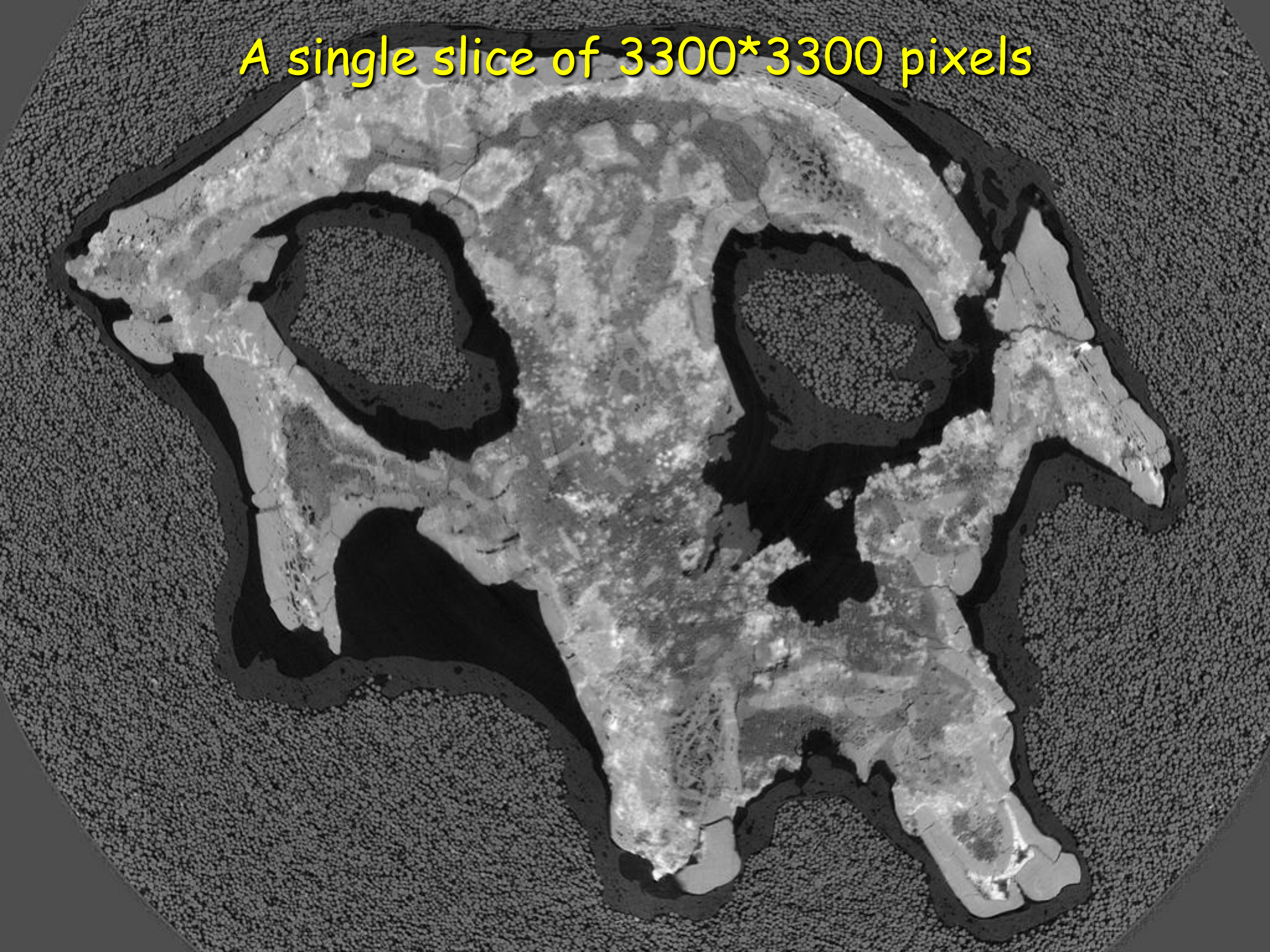
Thanks to the absorption protocol, the effective transmission reached 15% !

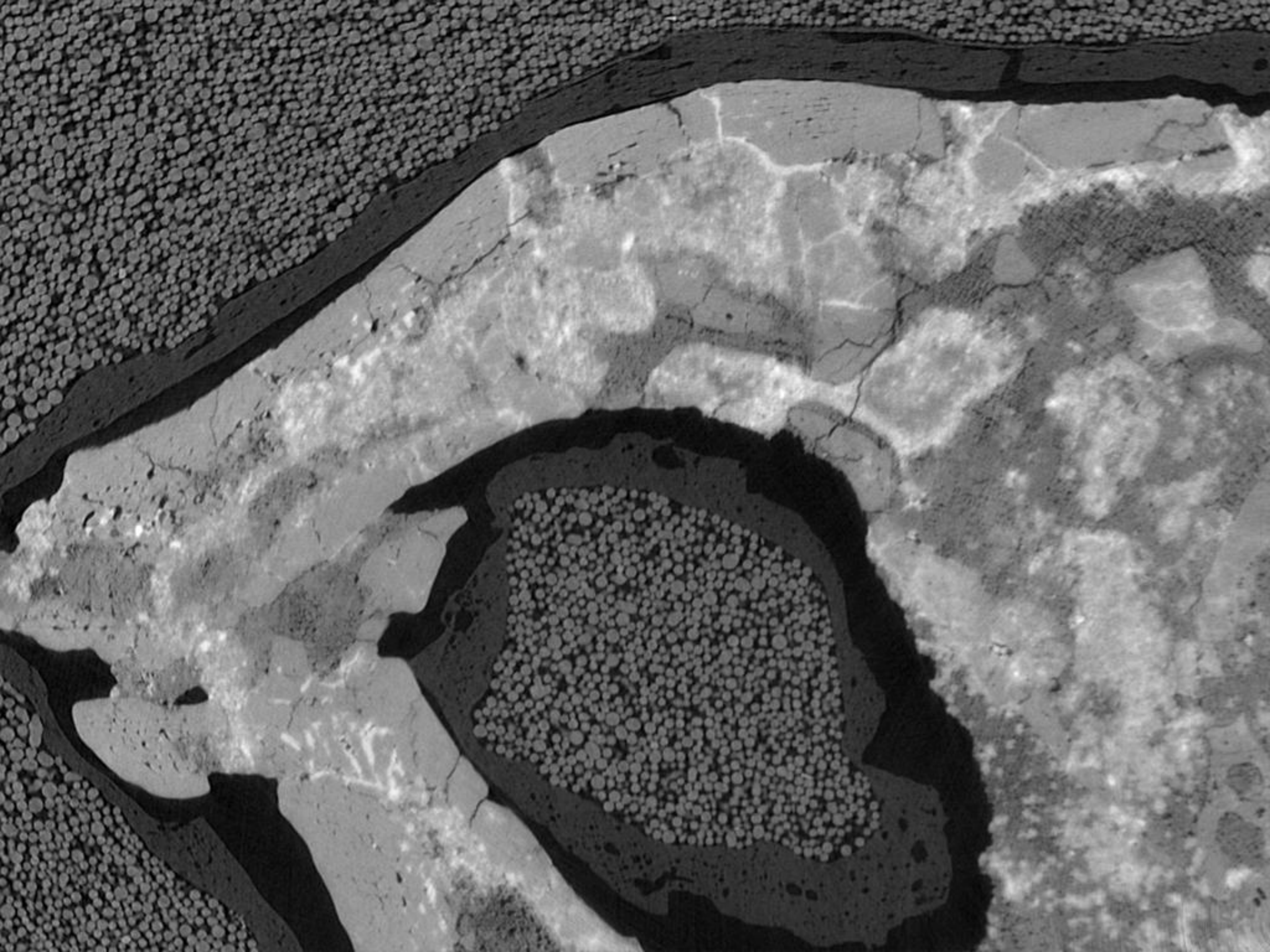
Comparison conventional / synchrotron on a paleotherium

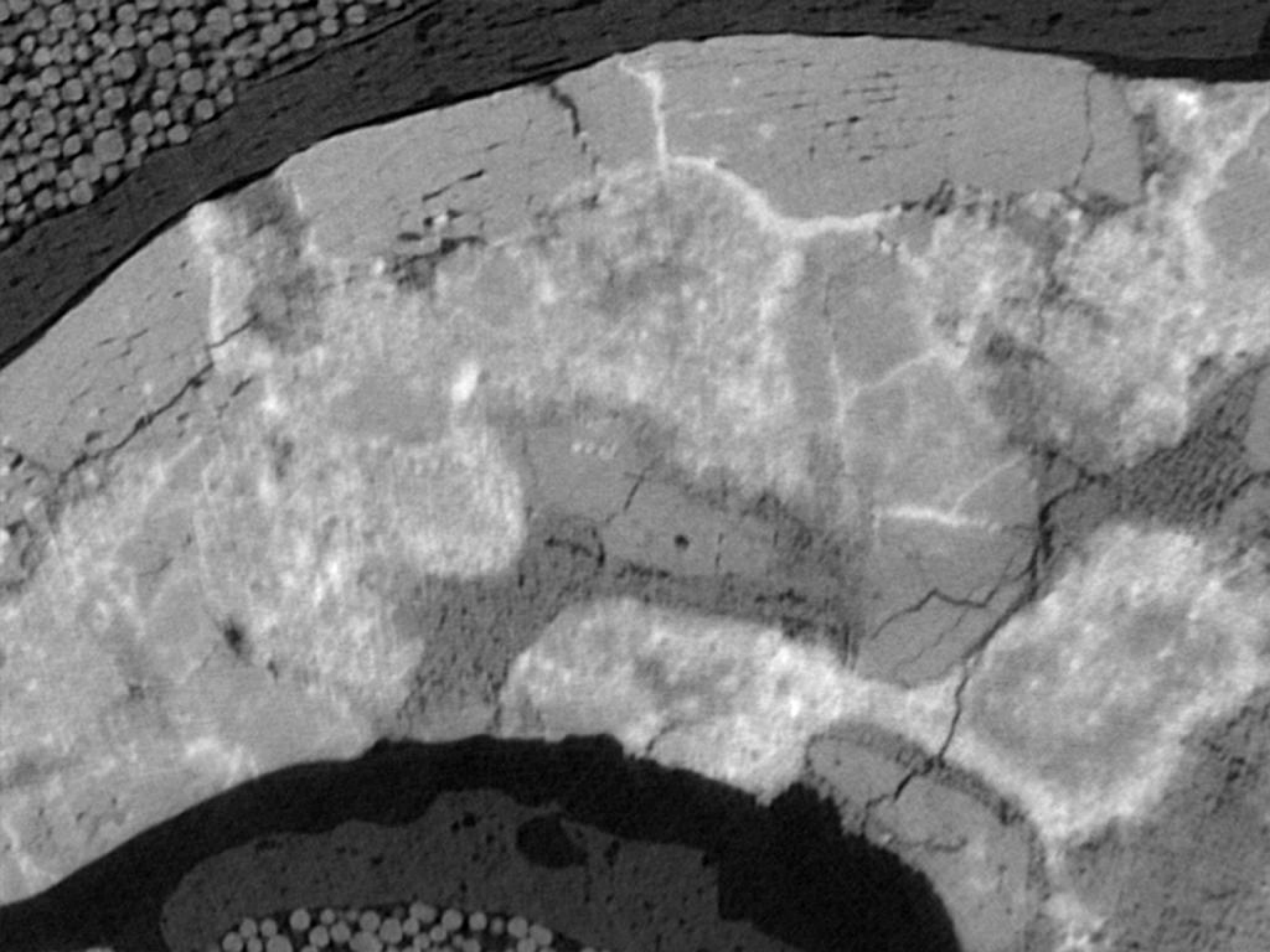


Tafforeau, Peix, Remy

A single slice of 3300*3300 pixels







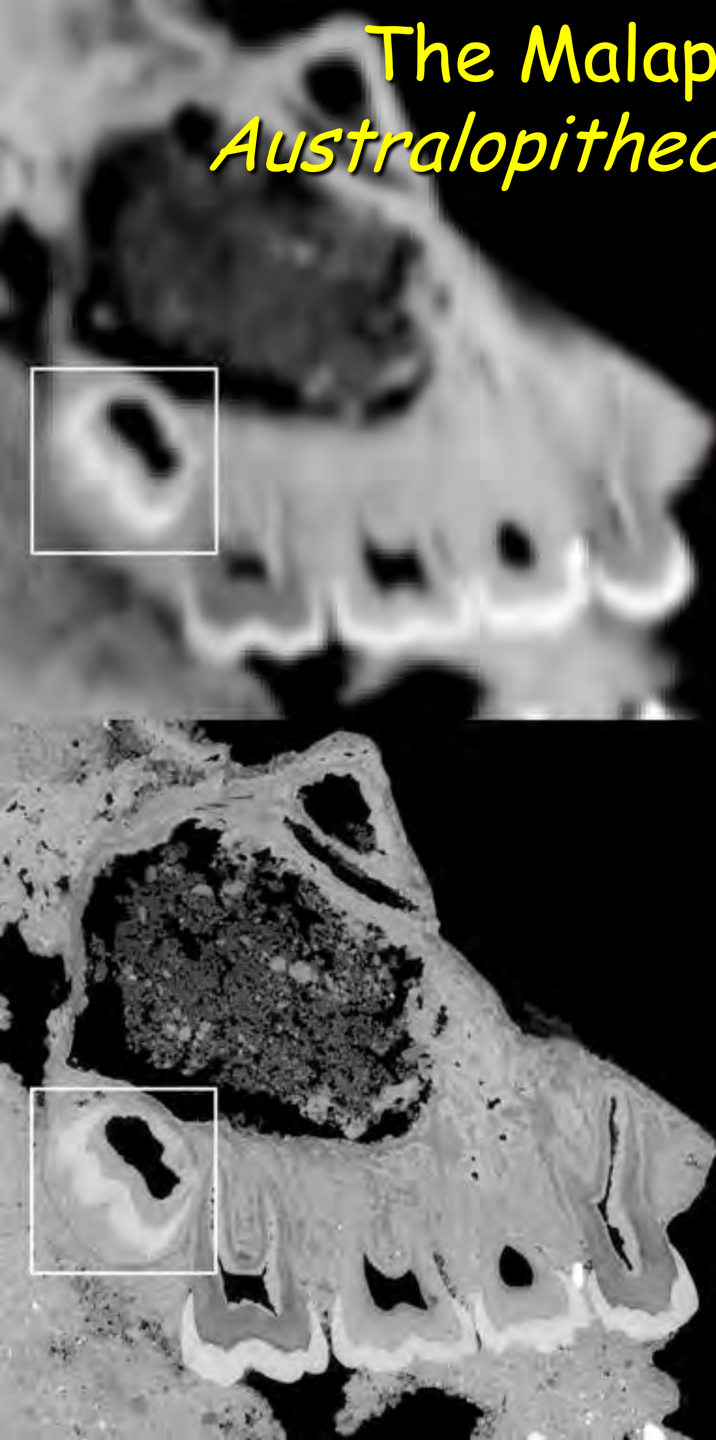
Discovery of two complete skeletons of animals that should have never been together in a burrow of 250 million years in South Africa

Vincent Fernandez, Kris Carlson, Nestor Abdala, Adam Yates, Bruce Rubidge and Paul Tafforeau

Thrinaxodon : Mammalian reptile, close to the origin of mammals

Broomistega : Temnospondyl amphibian, aquatic predator, the best skeleton ever discovered

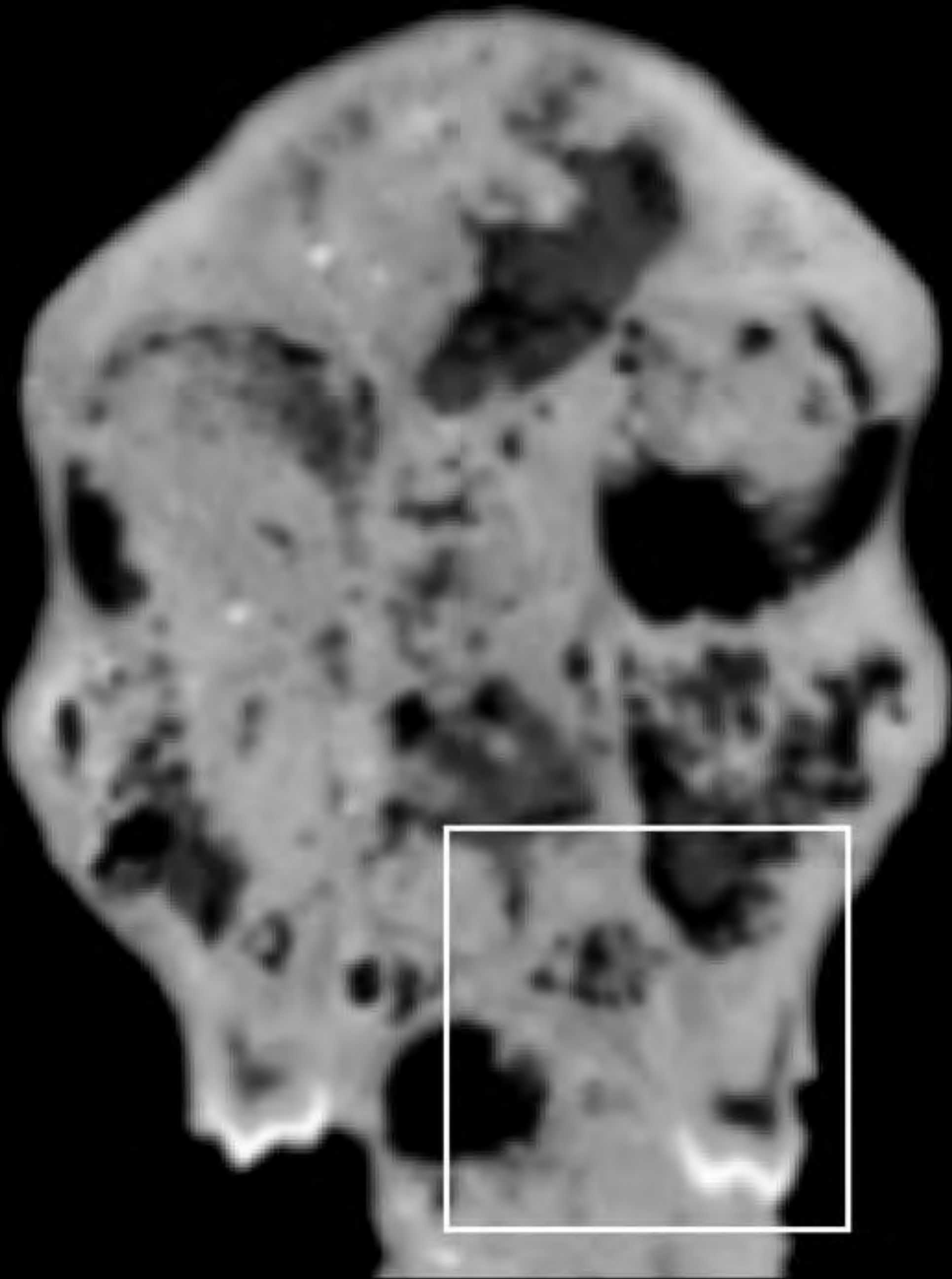
The Malapa hominid study in 2010:
Australopithecus sediba from South Africa

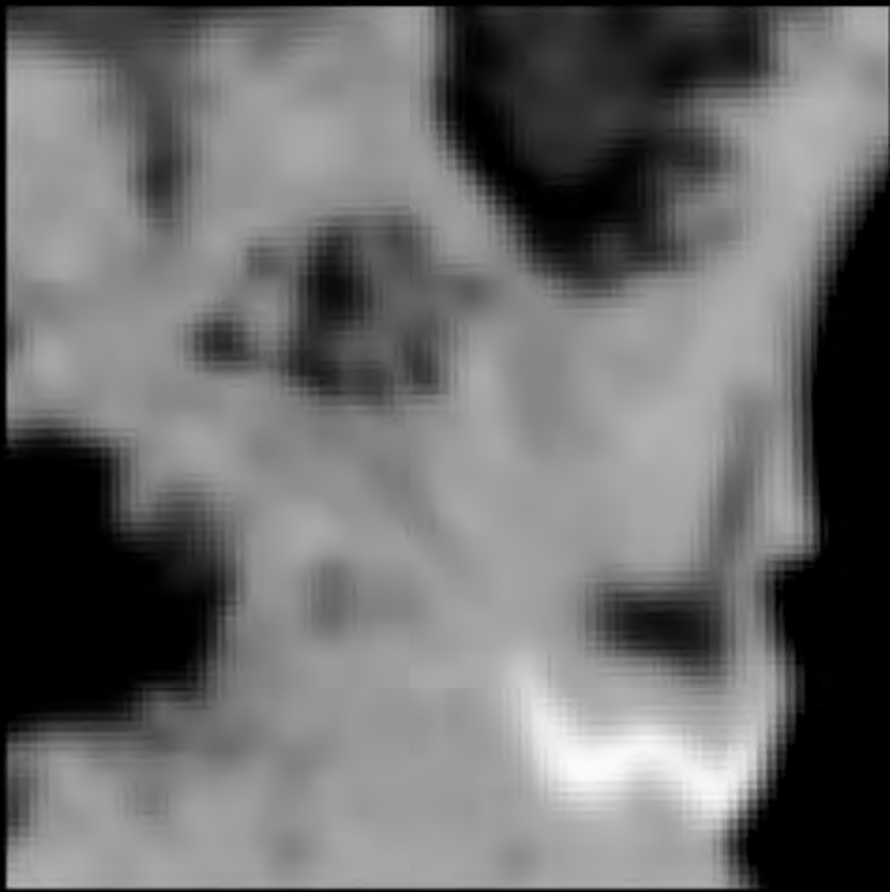


Lee Berger et al.

Wits University

Medical CT





Synchrotron scan on the beamline ID17

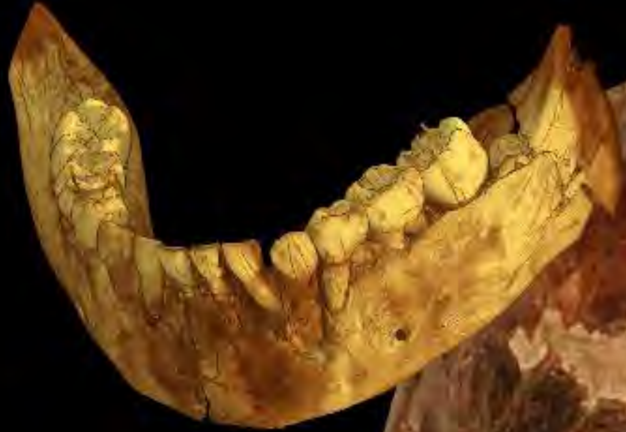
probably the most important advance for palaeoanthropology at the ESRF:

Multiscale analysis of dental development and virtual palaeohistology

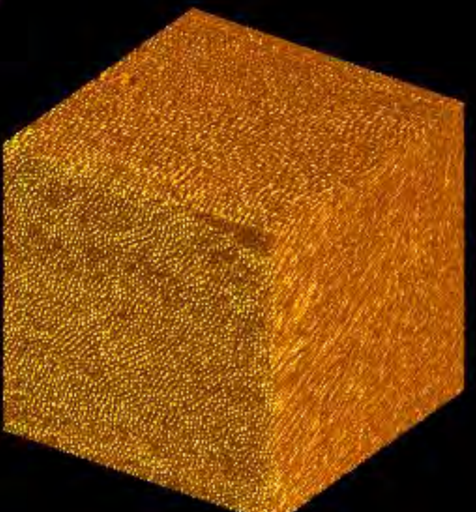
Initiated in 2007 with Tanya M. Smith

Synchrotron non-destructive multiscale analysis of dental development on a 100 000 year old *Homo sapiens* skull from Israel

Paul Tafforeau (ESRF), Tanya Smith (MPI)
Yoel Rak (Tel Aviv University)
and Jean-Jacques Hublin (MPI)







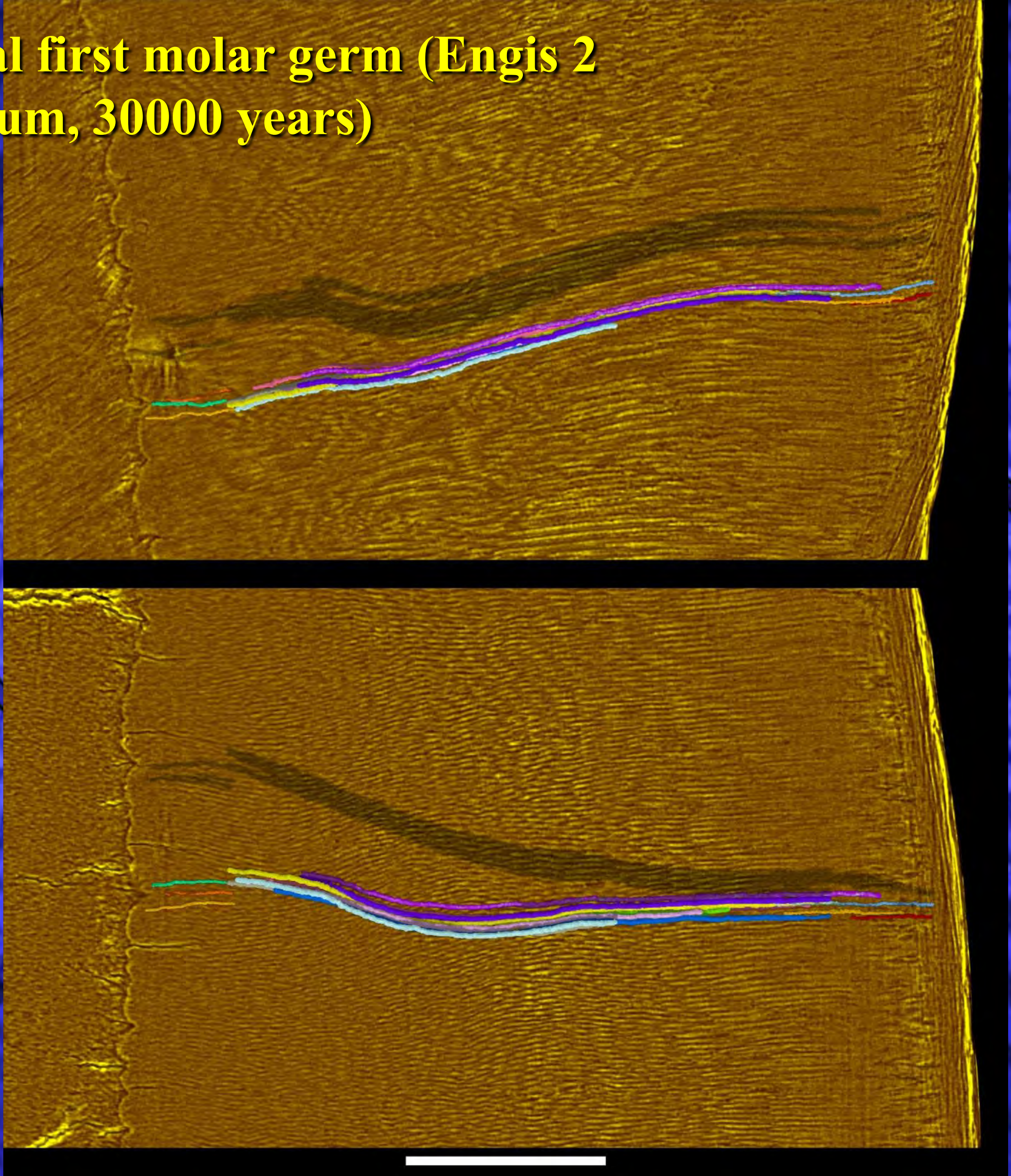
A new level in understanding of enamel microstructure: 3D characterization against time

Going to 4D

Tafforeau *et al.* 2012

Pan troglodytes molar germ

Neanderthal first molar germ (Engis 2 child, Belgium, 30000 years)



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

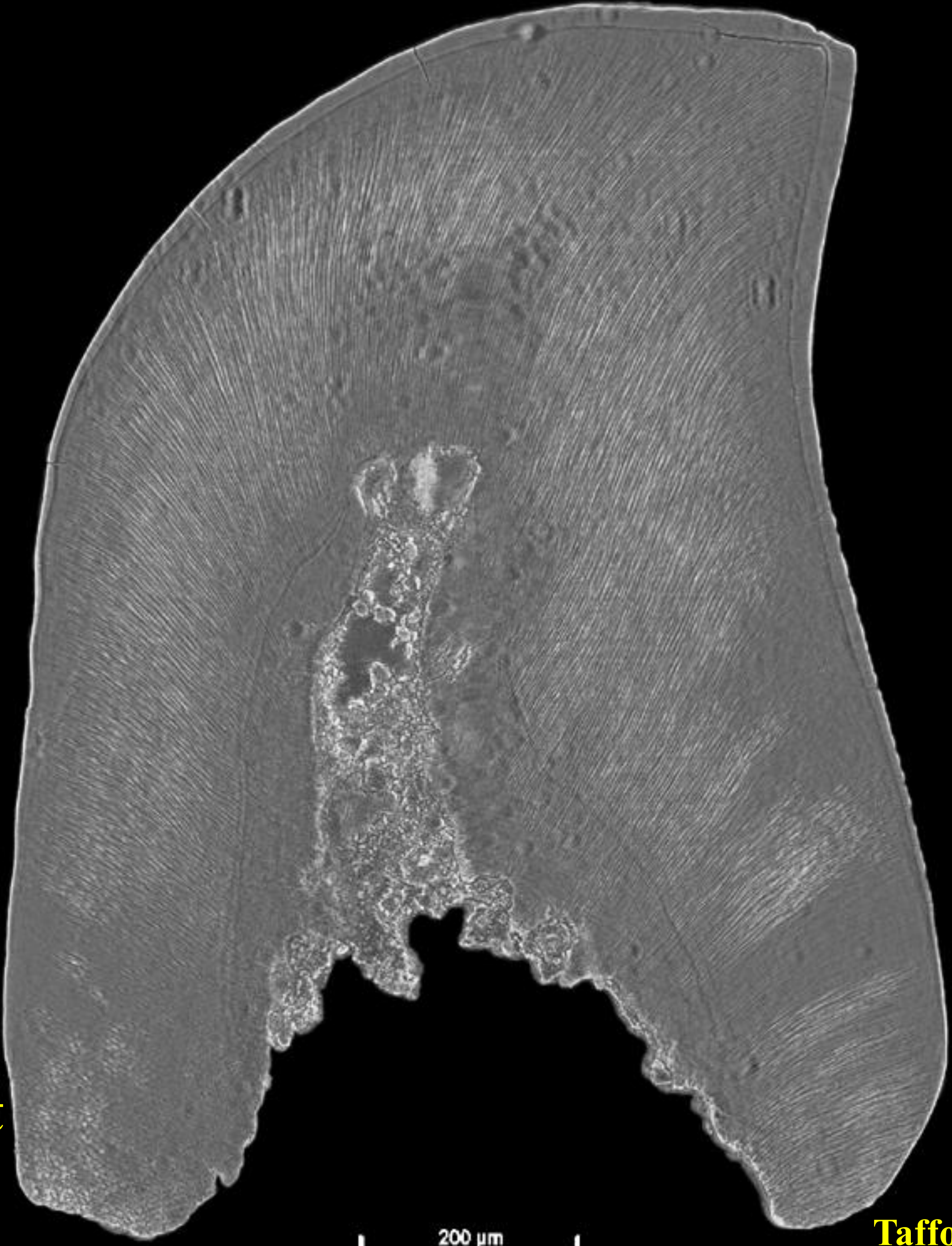
Bird tooth from

Charentes (France)

Absorption

d=6mm

Tafforeau, Pouech, Mazin



Phase contrast

d=20mm

Tafforeau, Pouech, Mazin

Phase contrast

d=70mm

Tafforeau, Pouech, Mazin

Phase contrast

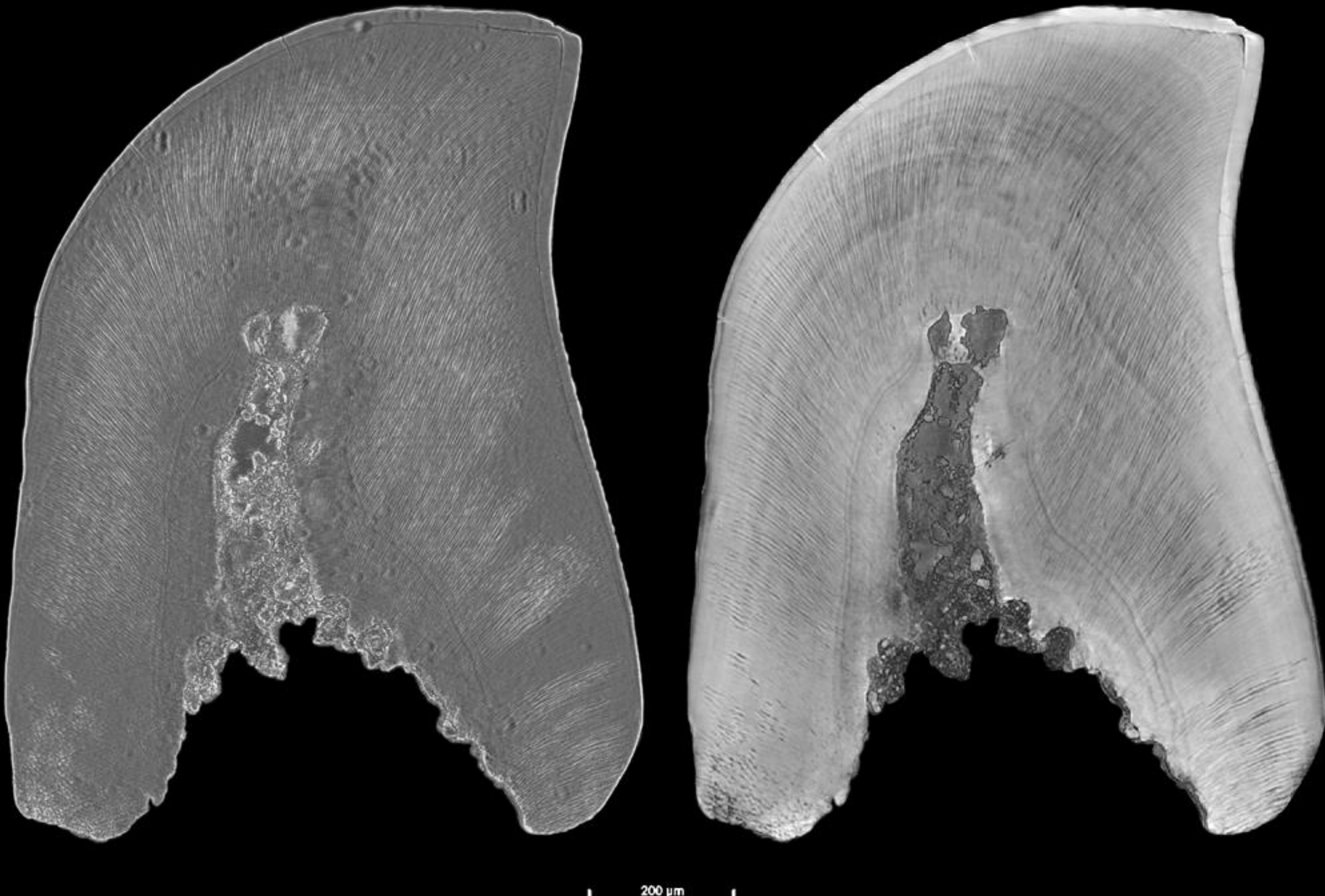
d=150mm

Tafforeau, Pouech, Mazin

Holotomographic reconstruction

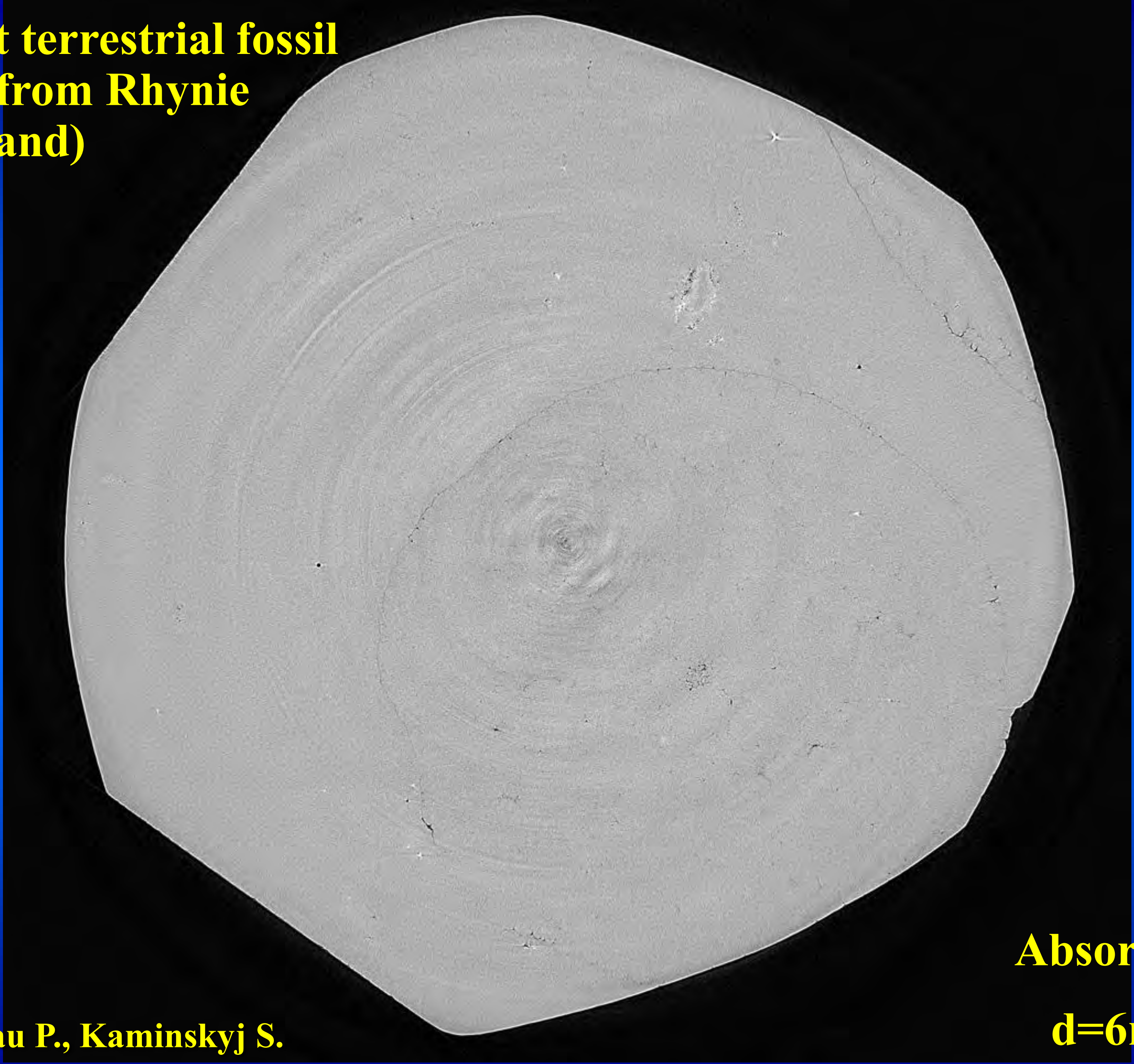
Holotomographic reconstruction using the algorithm developed by P. Cloetens, M. Langer and J.-P. Guigay

Tafforeau, Pouech, Mazin



Tafforeau, Pouech, Mazin

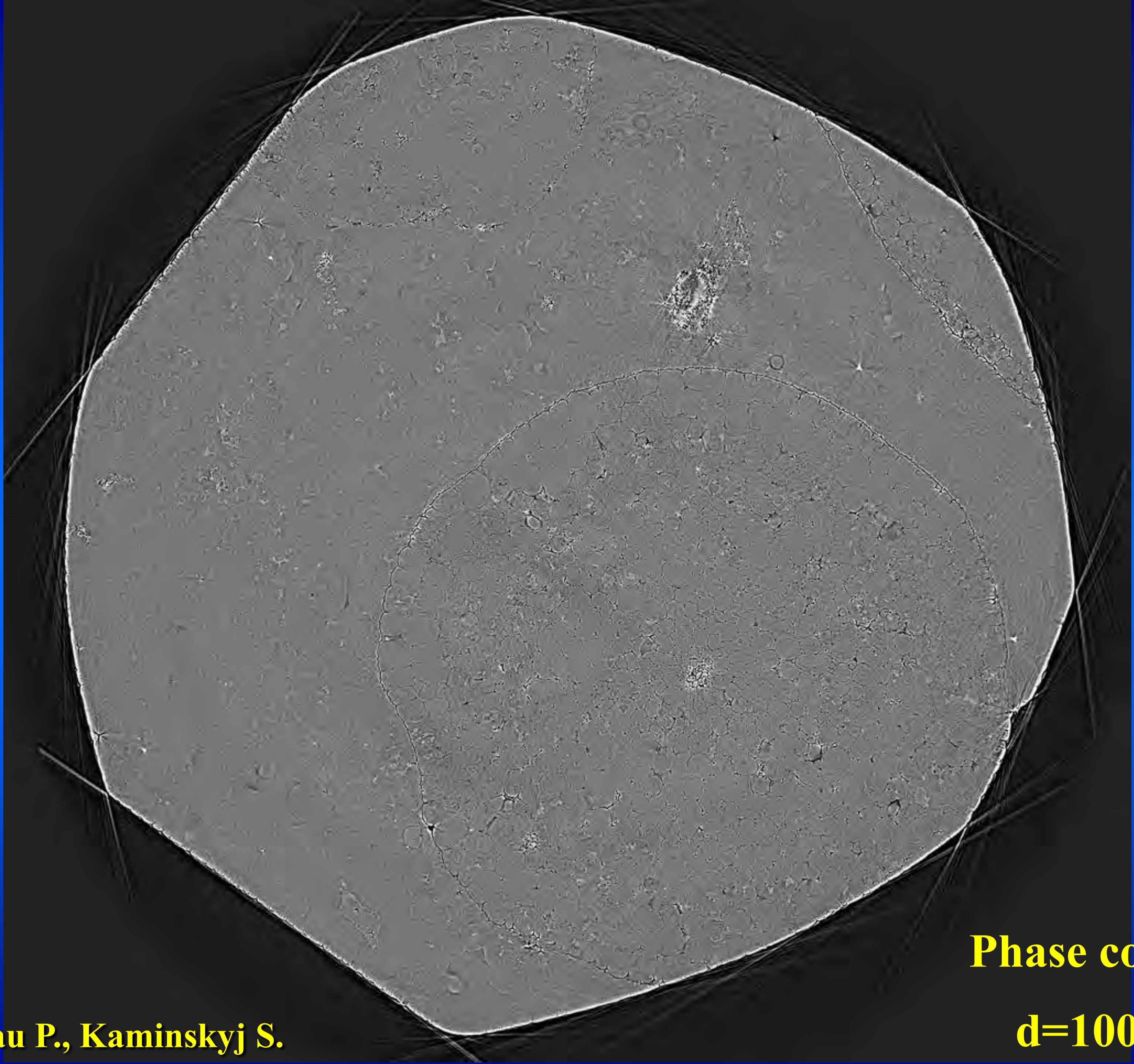
Oldest terrestrial fossil plant from Rhynie (Scotland)



Absorption

d=6mm

Tafforeau P., Kaminskyj S.



Phase contrast

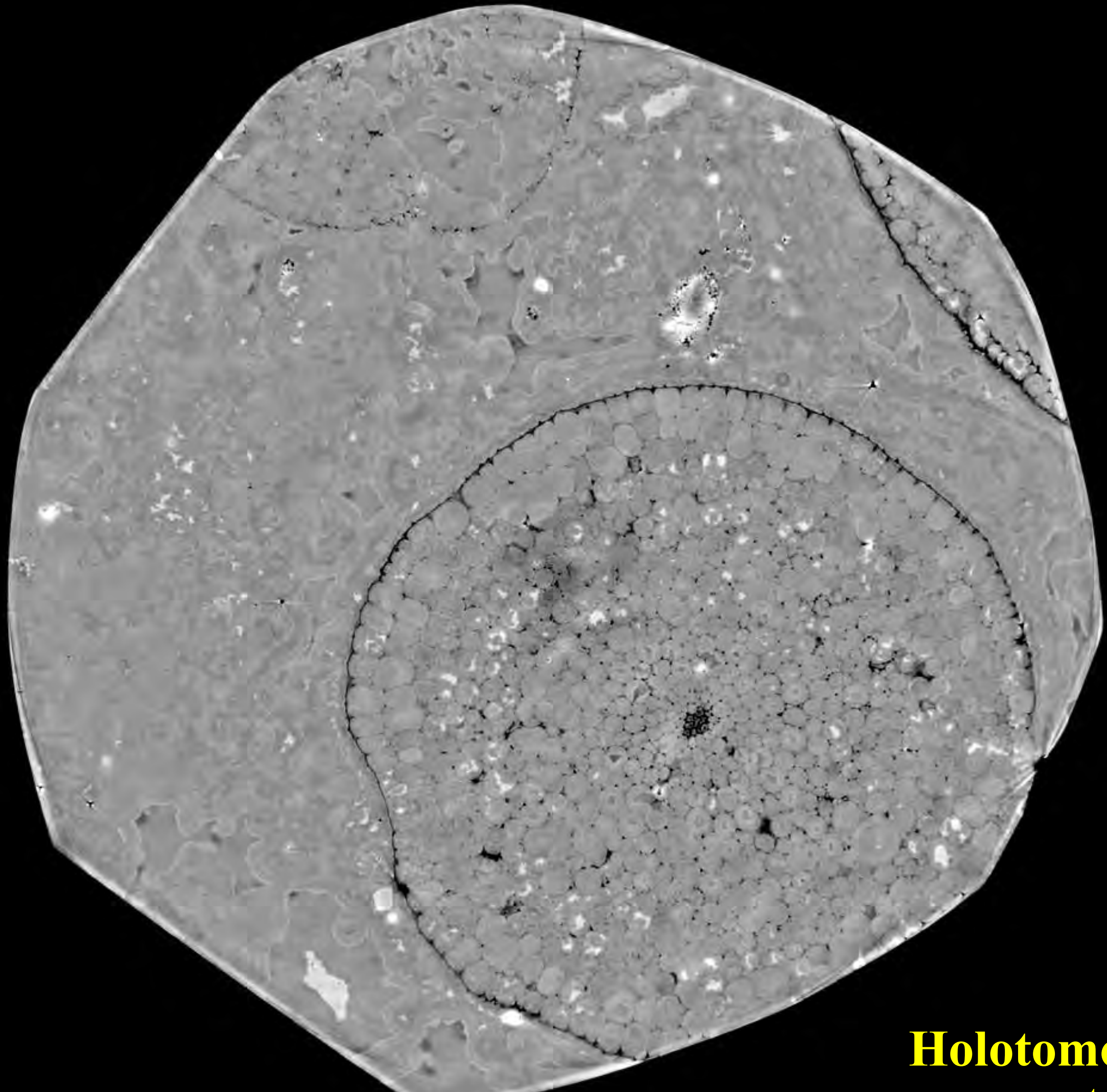
d=100mm

Tafforeau P., Kaminskyj S.

Phase contrast

d=300mm

Tafforeau P., Kaminskyj S.



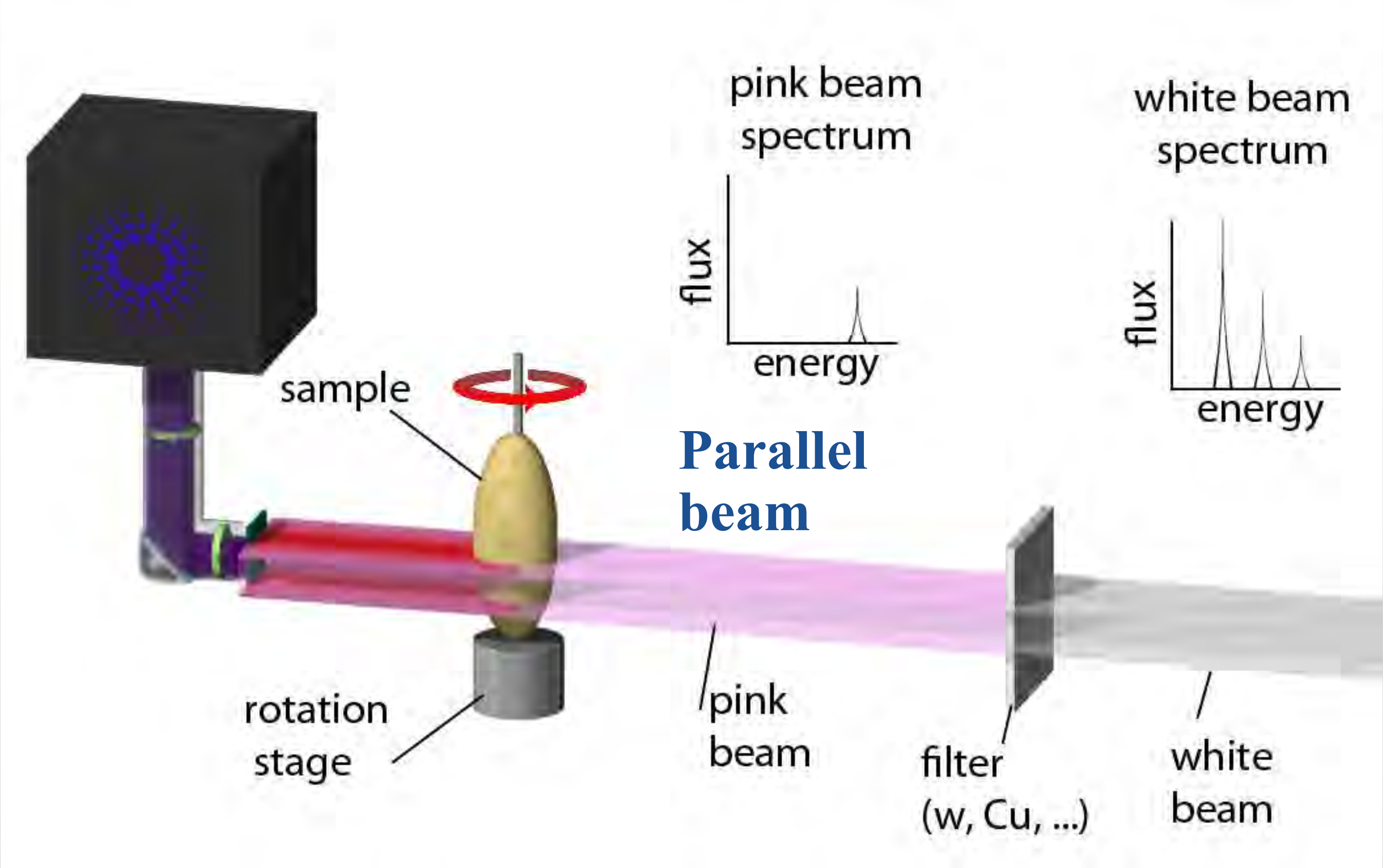
Holotomographic reconstruction

Tafforeau P., Kaminskyj S.

Since 2010, things dramatically changed for imaging of fossils (and of many other samples)

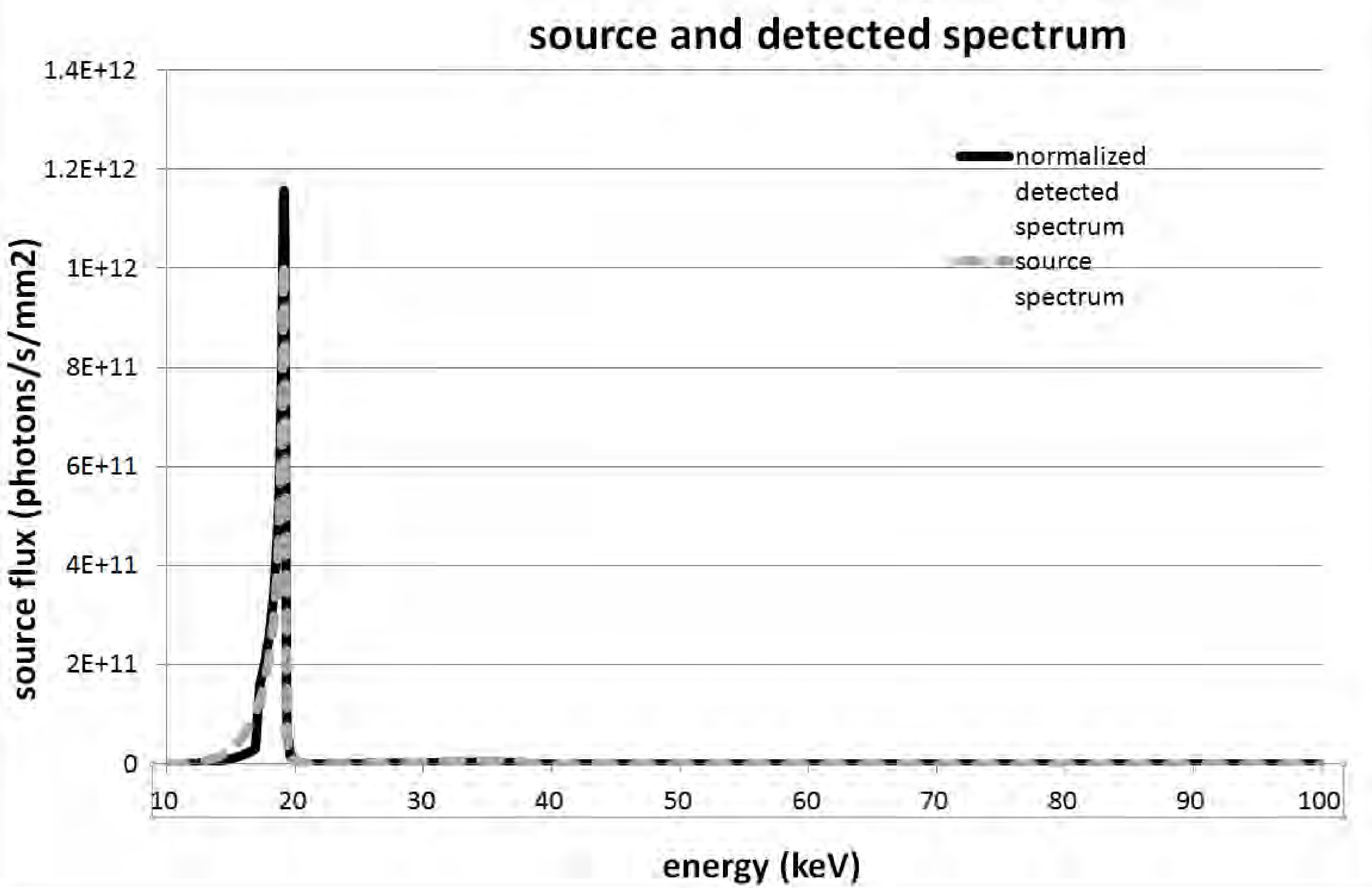
Use of pink beams and single distance phase retrieval approaches on ID19

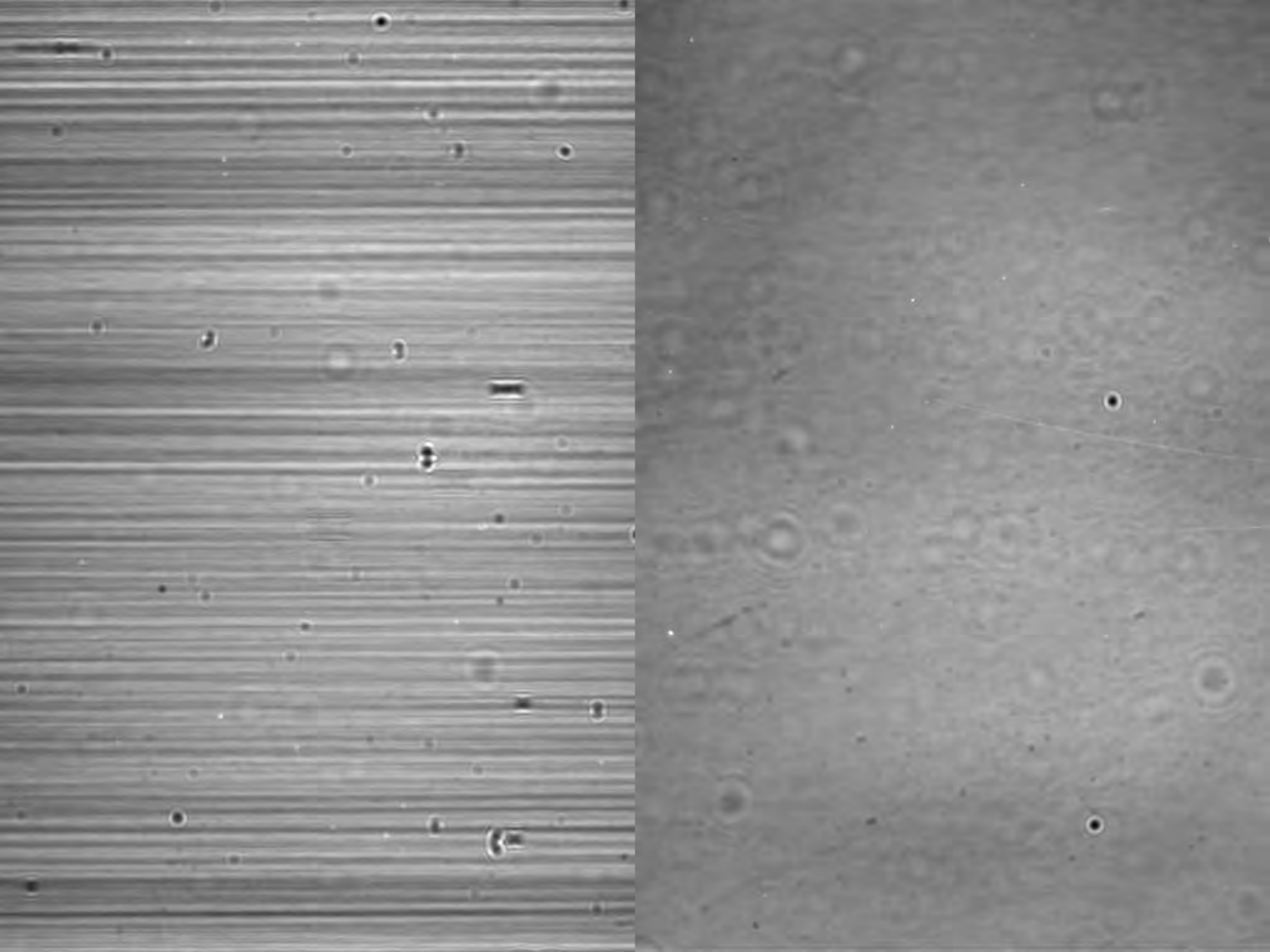
Tomography @ ESRF
filtered white beam CT

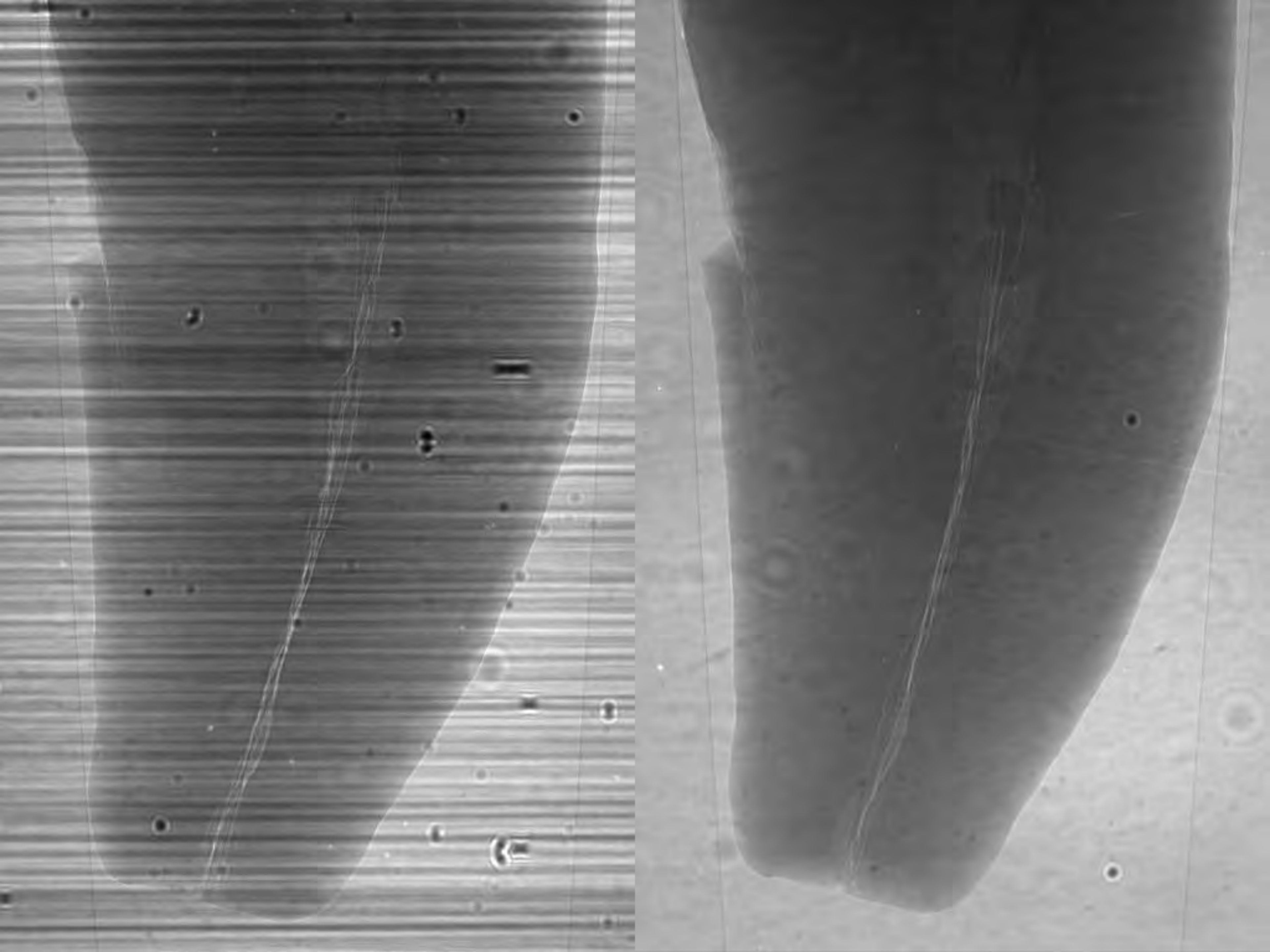


- Pink beams applications for **high quality tomography** is one of the major outreach of last years
- It is not a “new” technique, but the spectral properties of these beams are now so good that in many cases they can be considered as **nearly monochromatic**
- Several configurations have been developed, covering an energy range from **19 to 250 keV**, with partially tunable flux, bandwidth and beam size
- It nearly **replaced monochromatic** configurations for most of topics on ID19 and BM5.

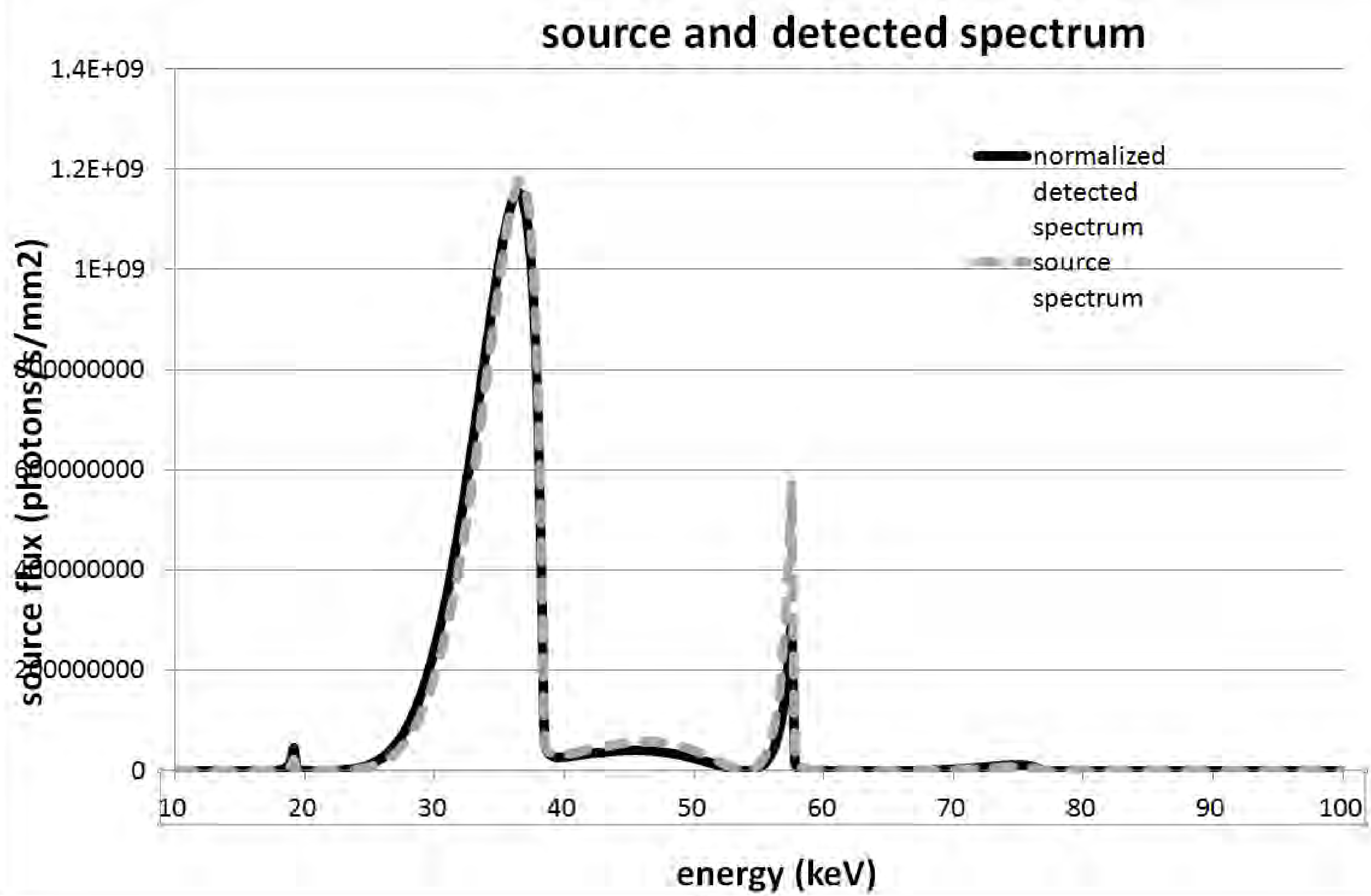
- The principle is based on fitting the source properties with filters and scintillator properties to maximize efficiency in a restricted energy range.
- All the insertion devices can be used (and also BM5), but the most commonly used on ID19 are the wiggler and the U17.6 undulator.
- Coupled with single distance phase retrieval process, pink beams completely changed the way of working on ID19
- The transfocator, and many filters of the ID19 refurbishment improved dramatically the possibilities, especially for high resolution and high energies.


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)
10
20
30
40
50
60
70
80
90
100
energy (keV)
normalized detected spectrum
source spectrum



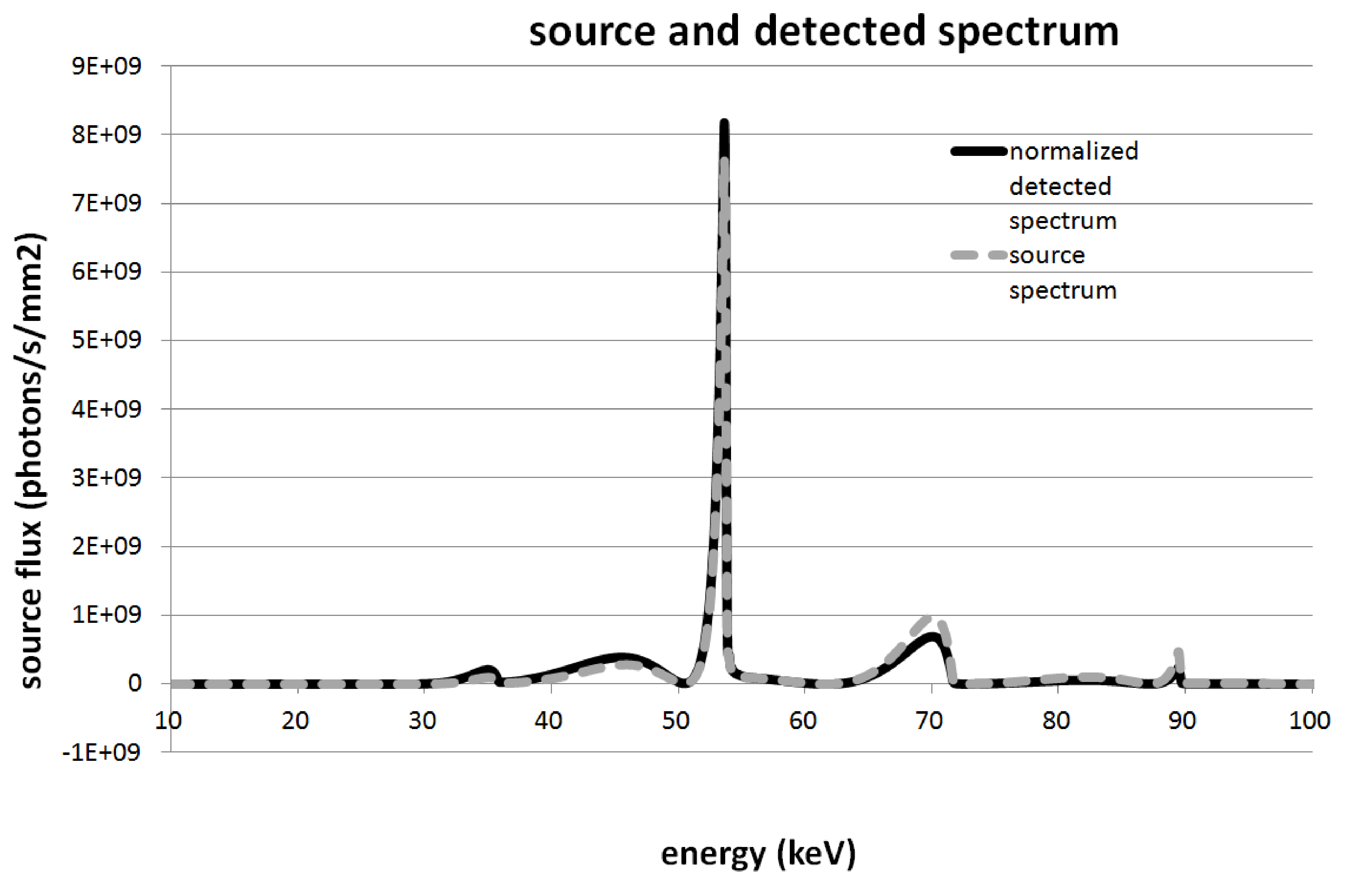


20 keV multilayer vs. 19.1 keV pink beam at 0.7 microns (50 mm of propagation)

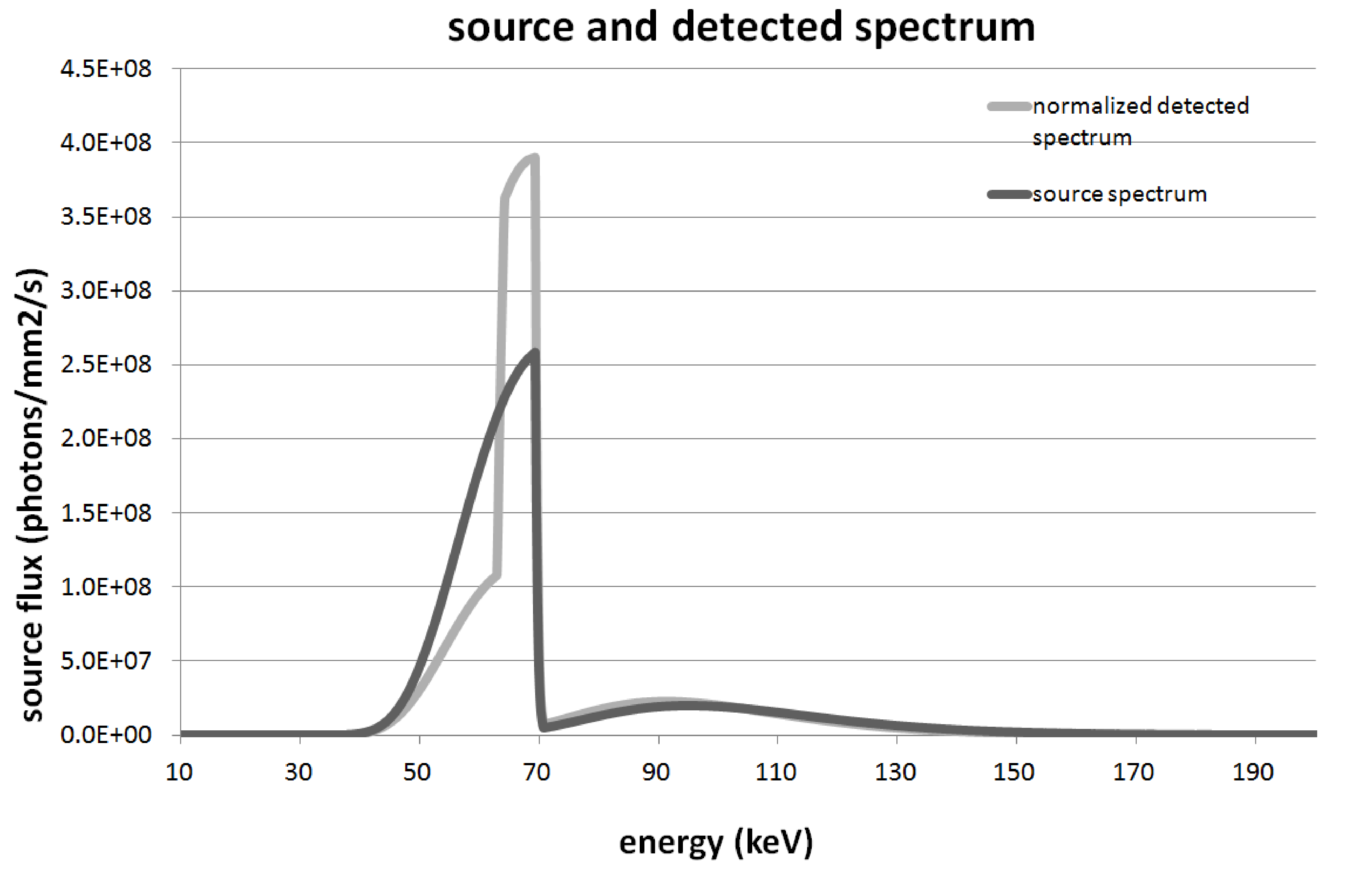

source and detected spectrum
1.4E+09
1.2E+09
1E+09
800000000
600000000
400000000
200000000
0
source flux (photons/s/mm2)
10
20
30
40
50
60
70
80
90
100
energy (keV)
normalized detected spectrum
source spectrum

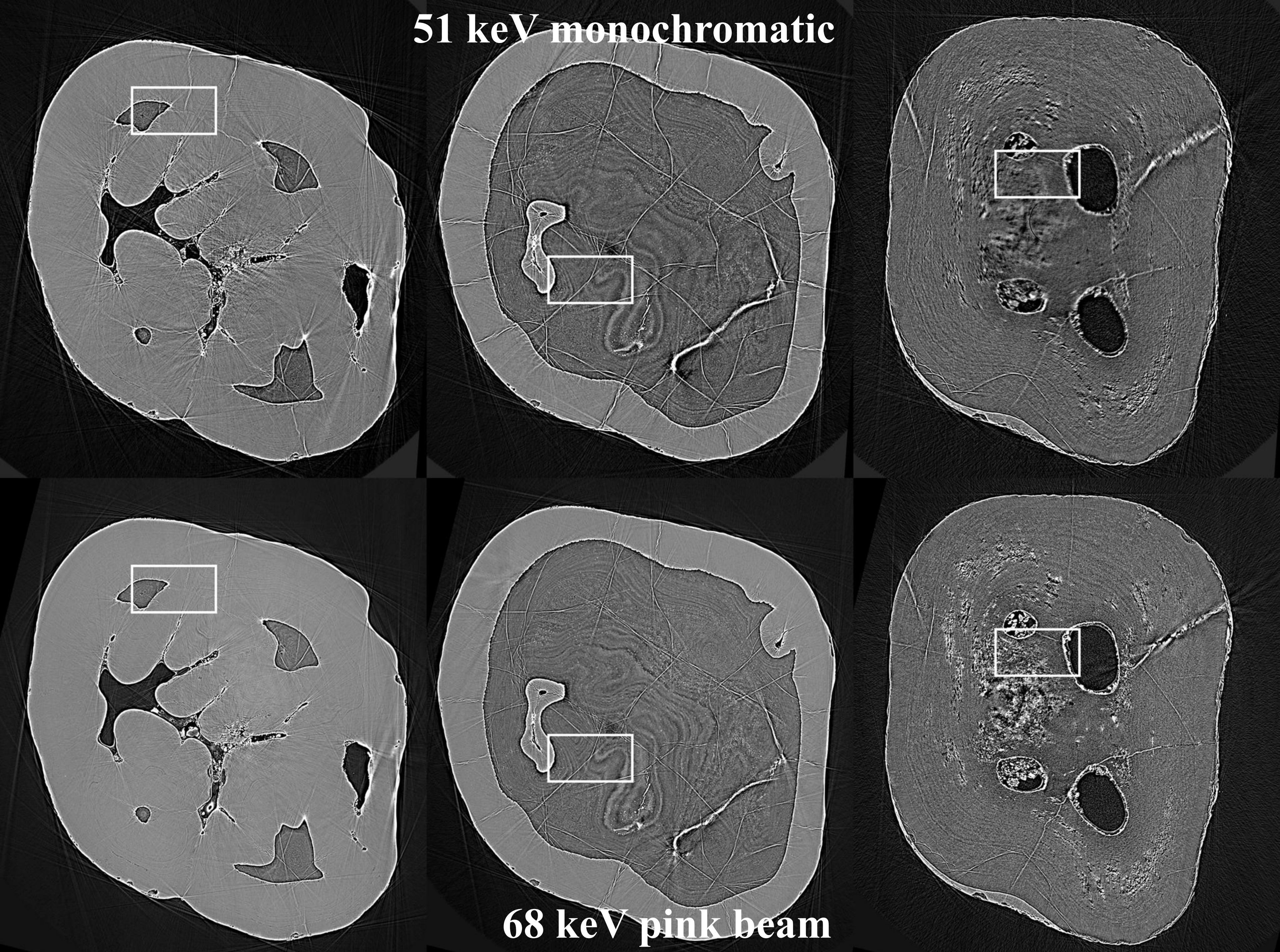
31 keV pink beam at 7.5 microns (1m of propagation)

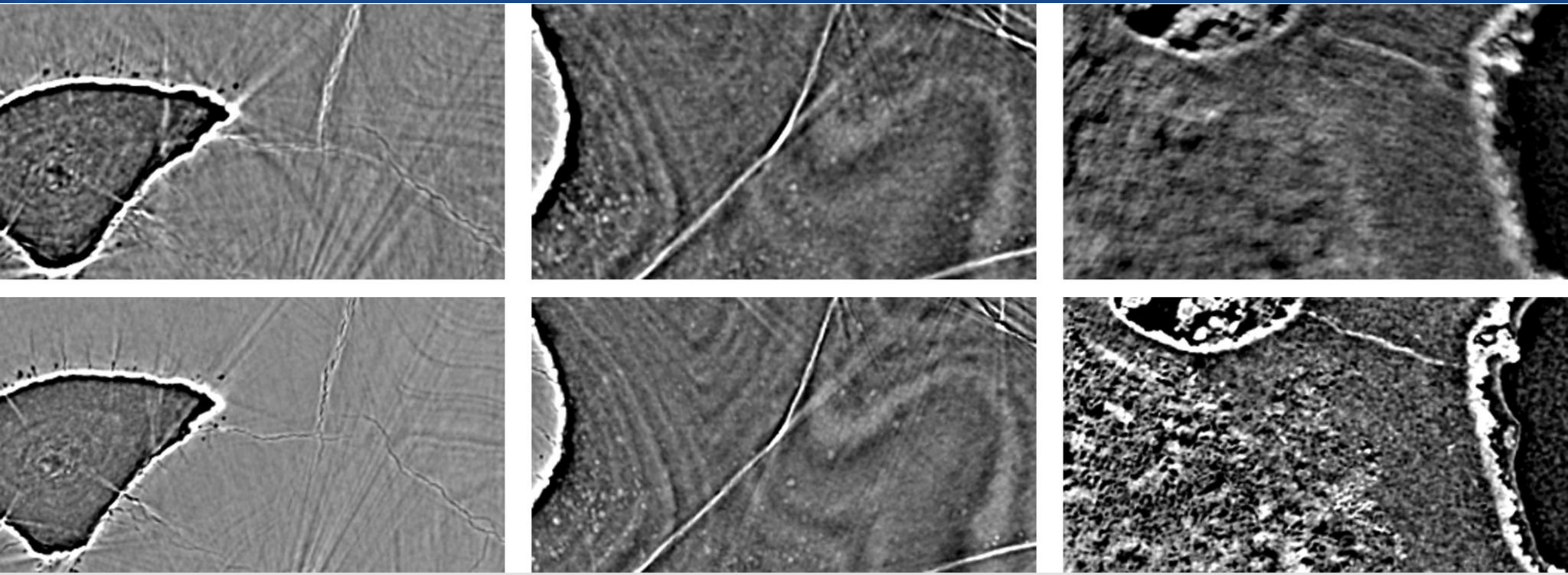
31 days old crocodile embryo, from the Pierrelatte crocodile farm


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

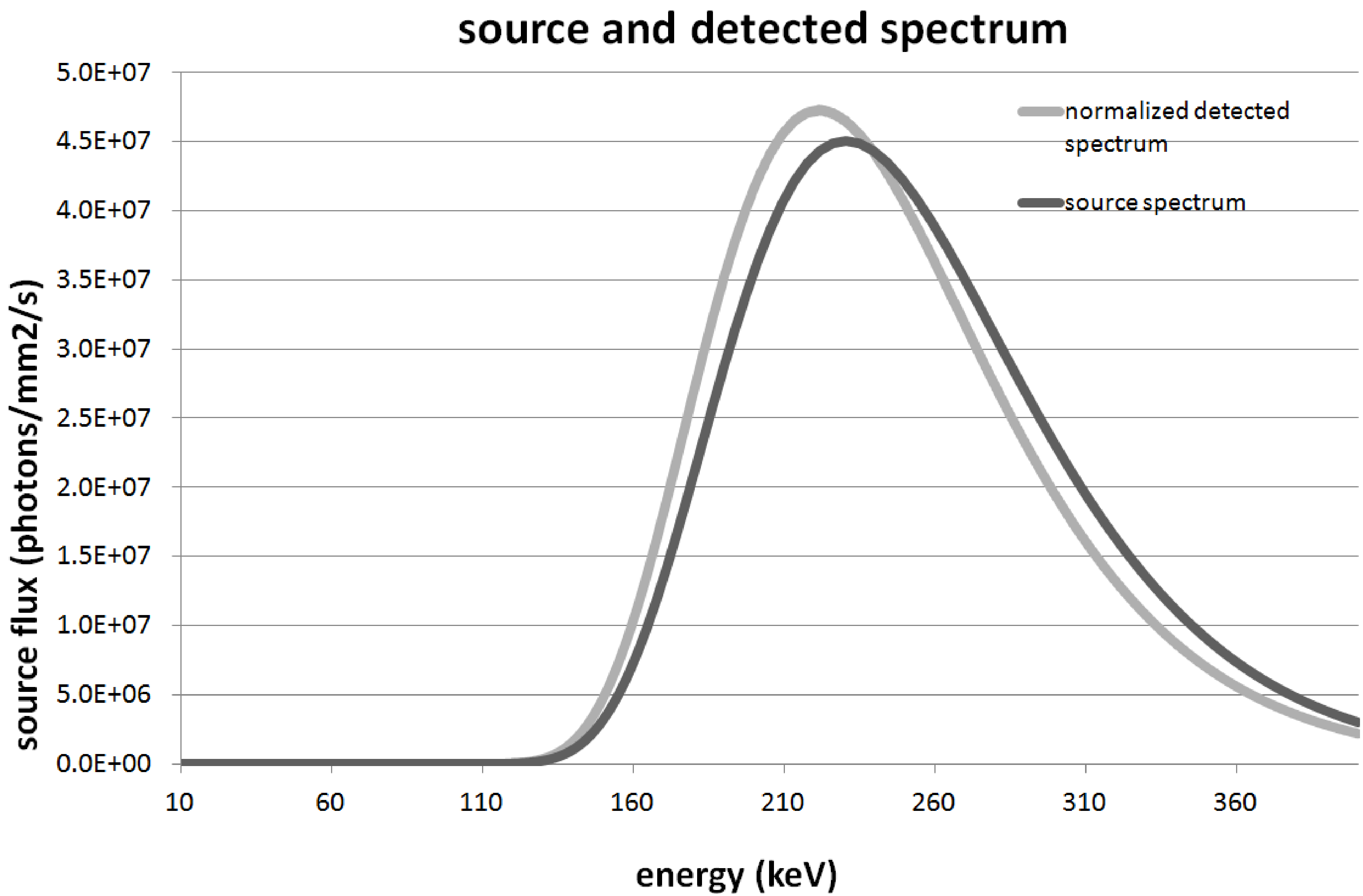
51 keV pink beam at 30 microns (4m of propagation)


source and detected spectrum
normalized detected spectrum
source 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)

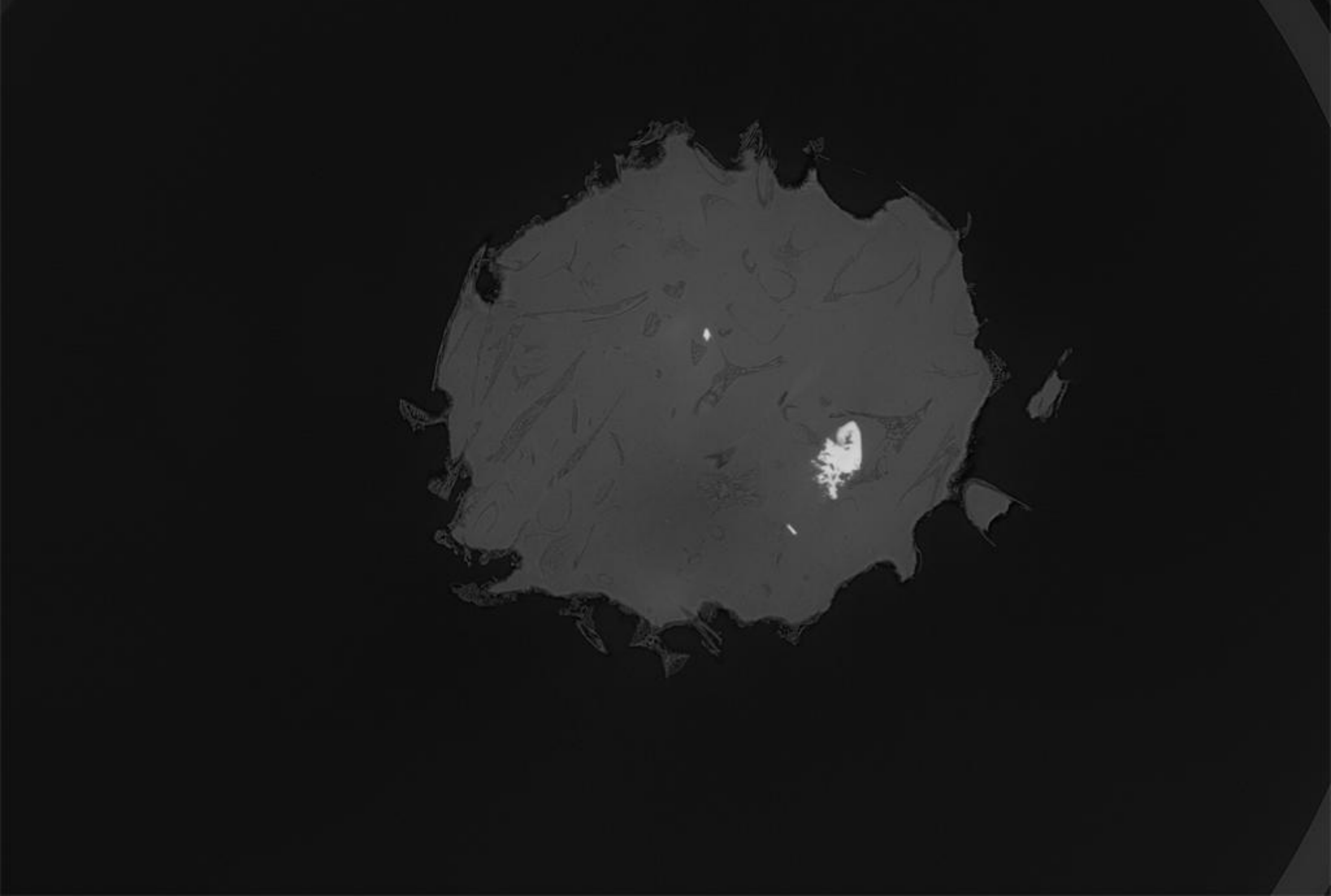

51 keV monochromatic
68 keV pink beam

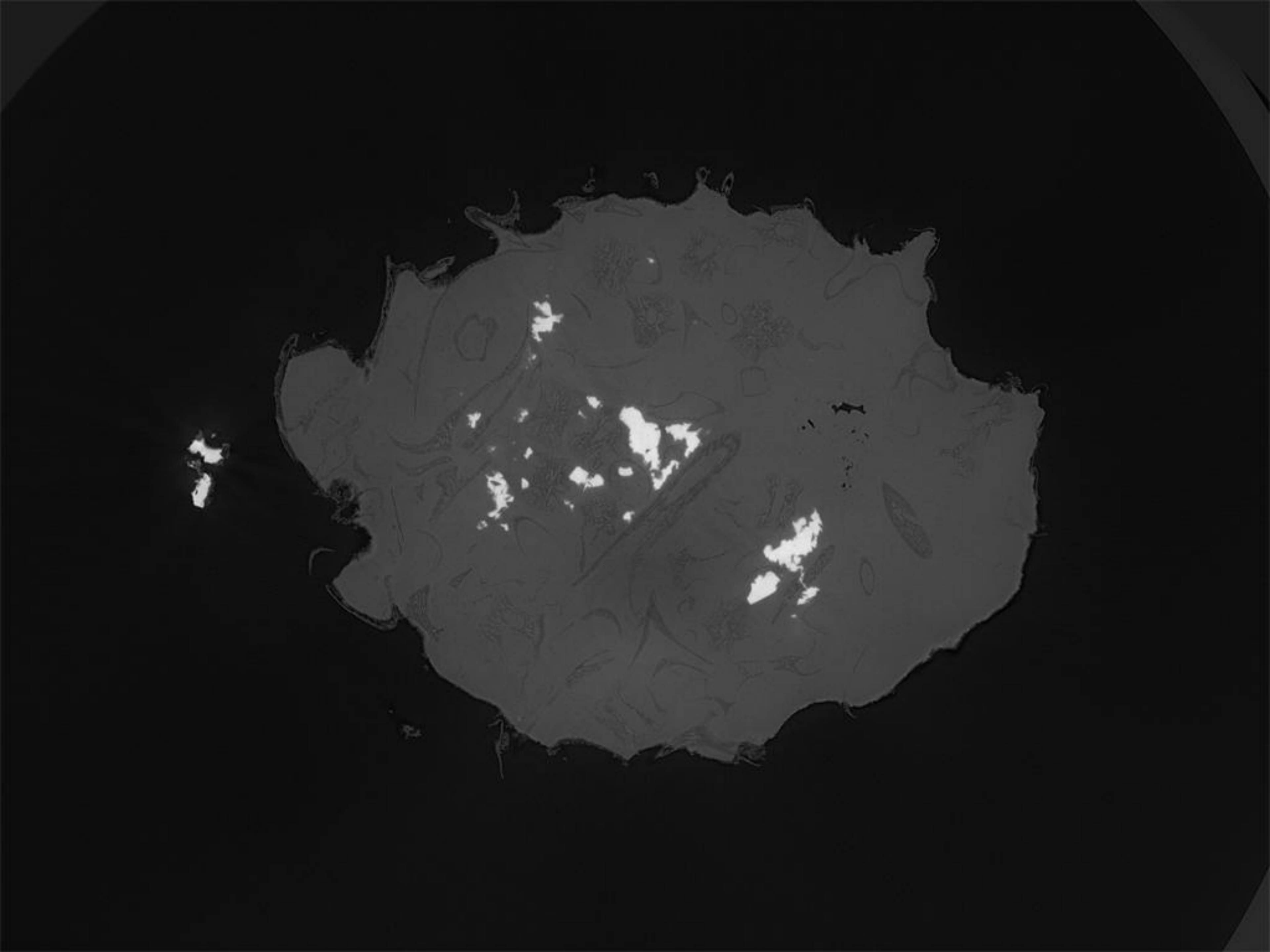


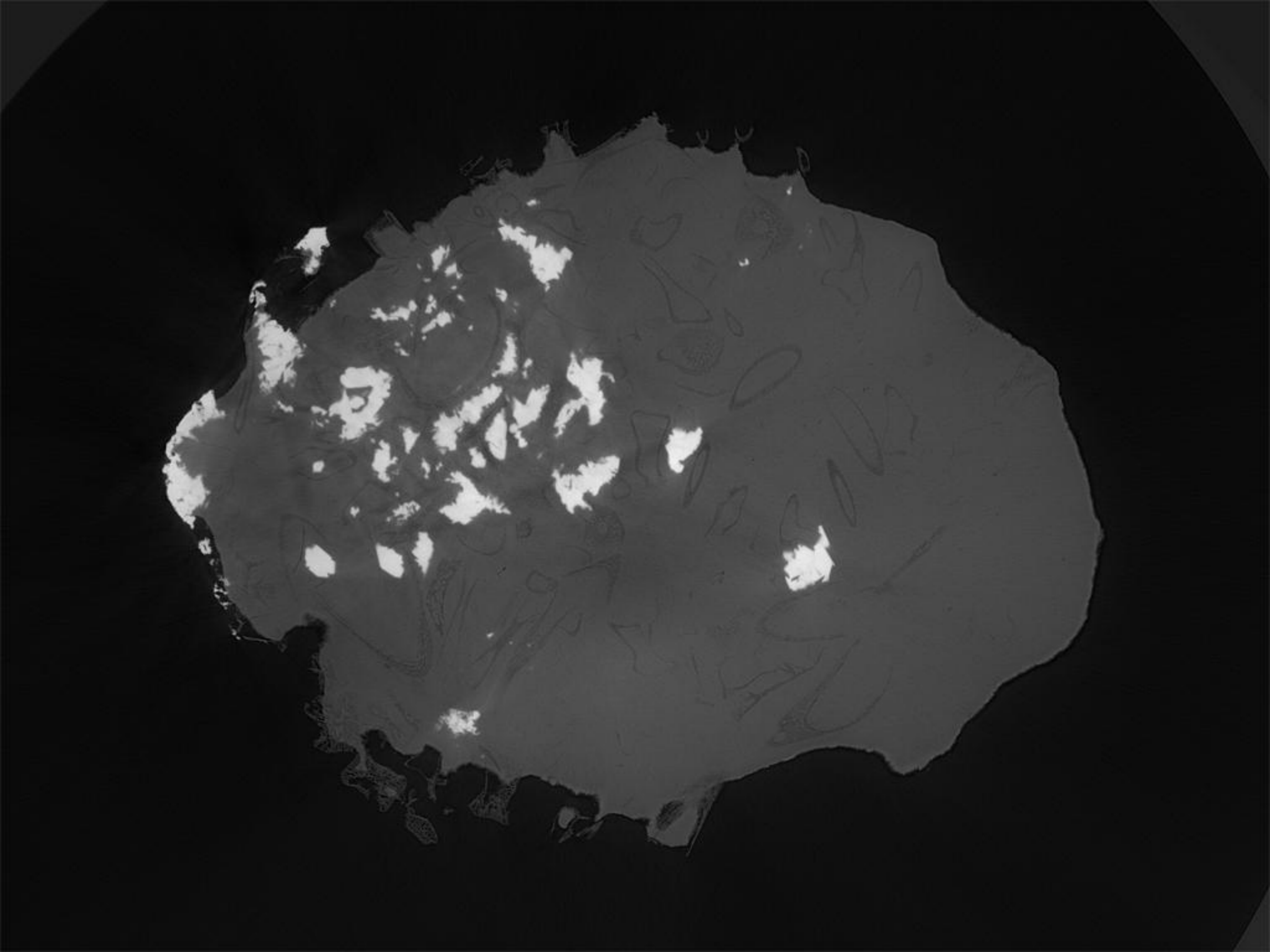
- Faster scans (2 hours vs. 12 hours)m higher signal to noise ratio, higher energy, less risk of decohesion, less ring artefacts, no detectable beam hardening (~3%).
- All hominid teeth are now scanned with pink beams for incremental lines investigations

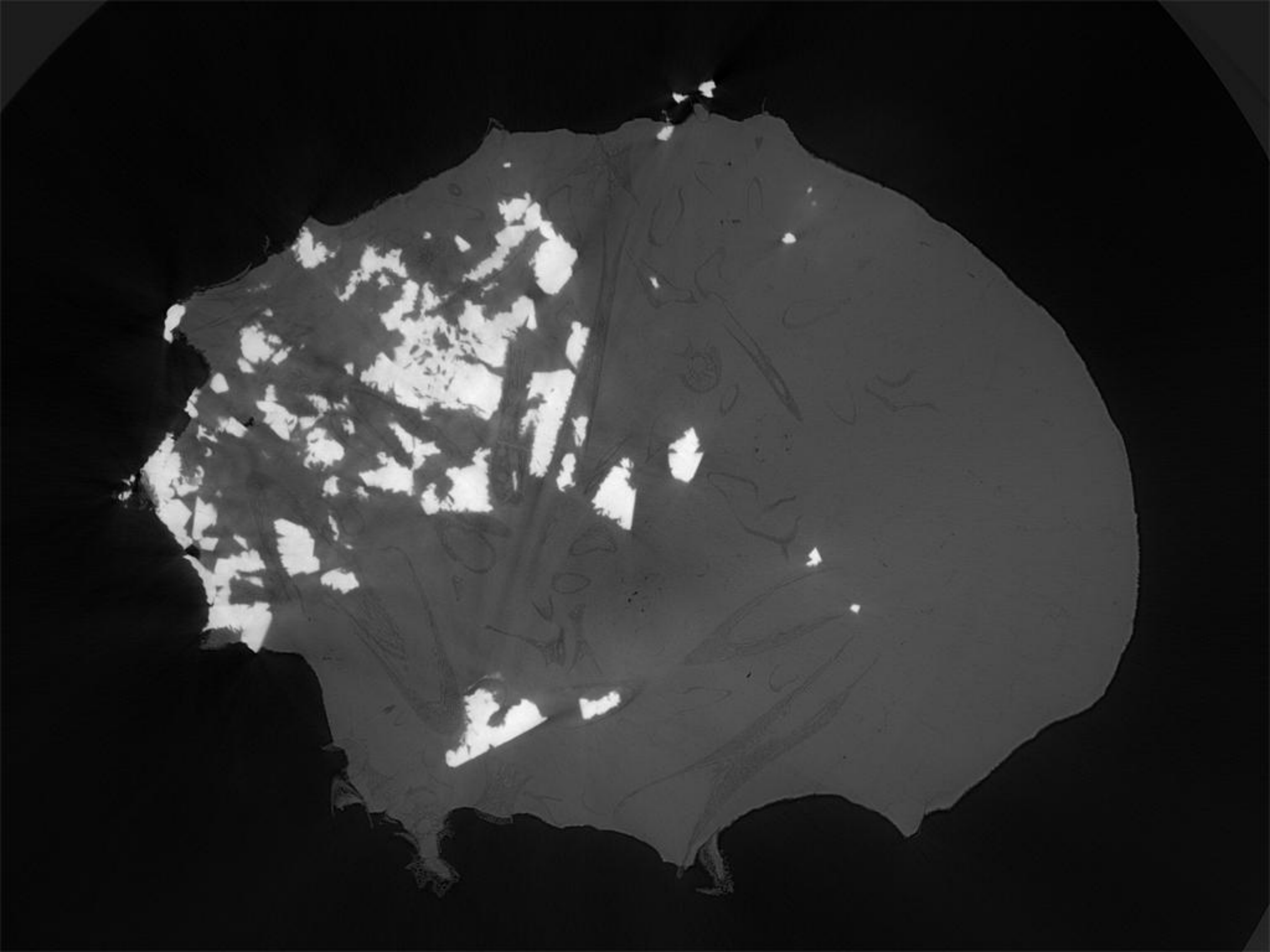

source and detected spectrum
5.0E+07
4.5E+07
4.0E+07
3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
source flux (photons/mm2/s)
10
60
110
160
210
260
310
360
energy (keV)
normalized detected spectrum
source spectrum

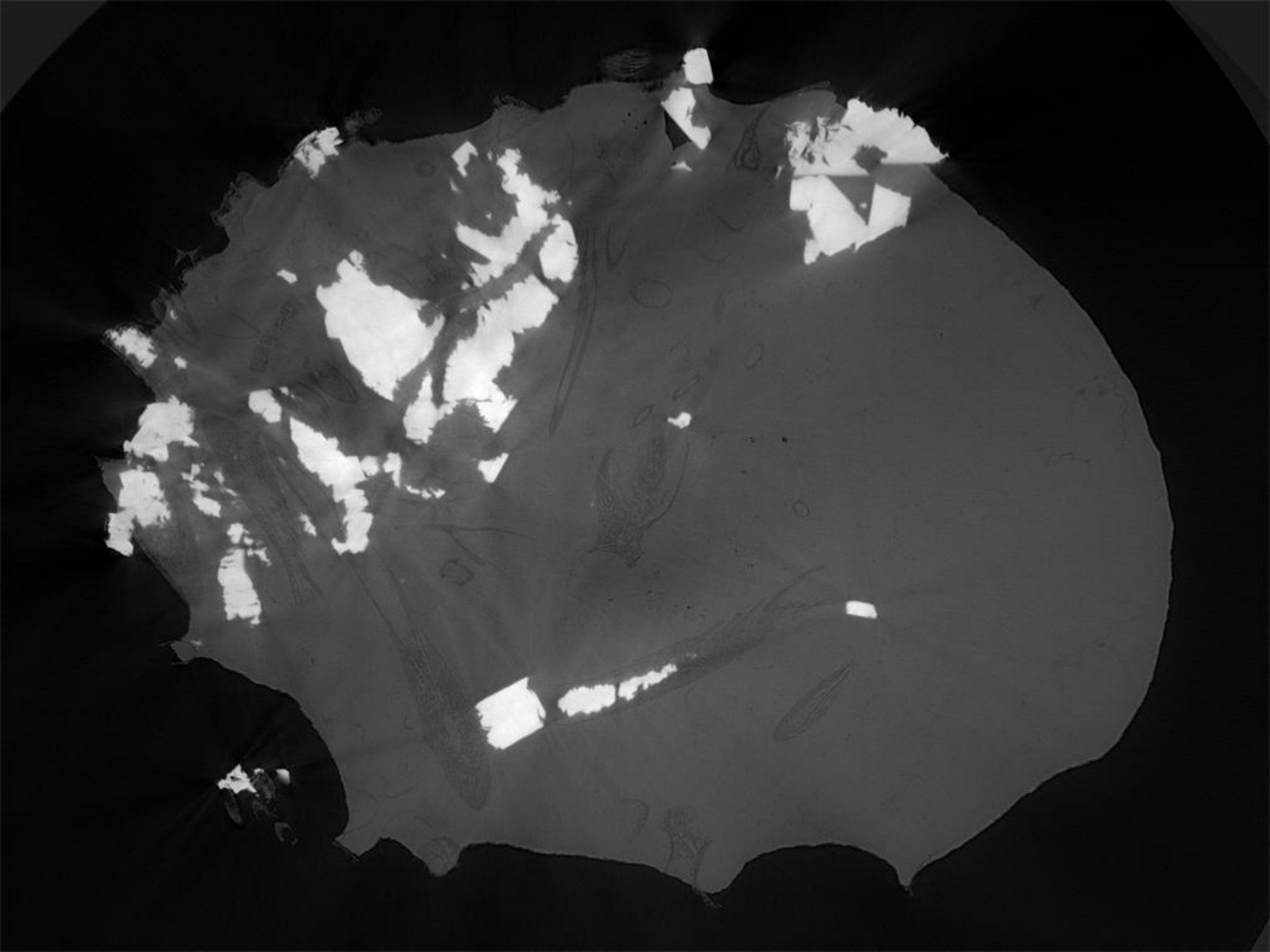
dinosaur embryo, spectrum pic around 240 keV filtered with 7 mm of cooper and 2mm of tungsten











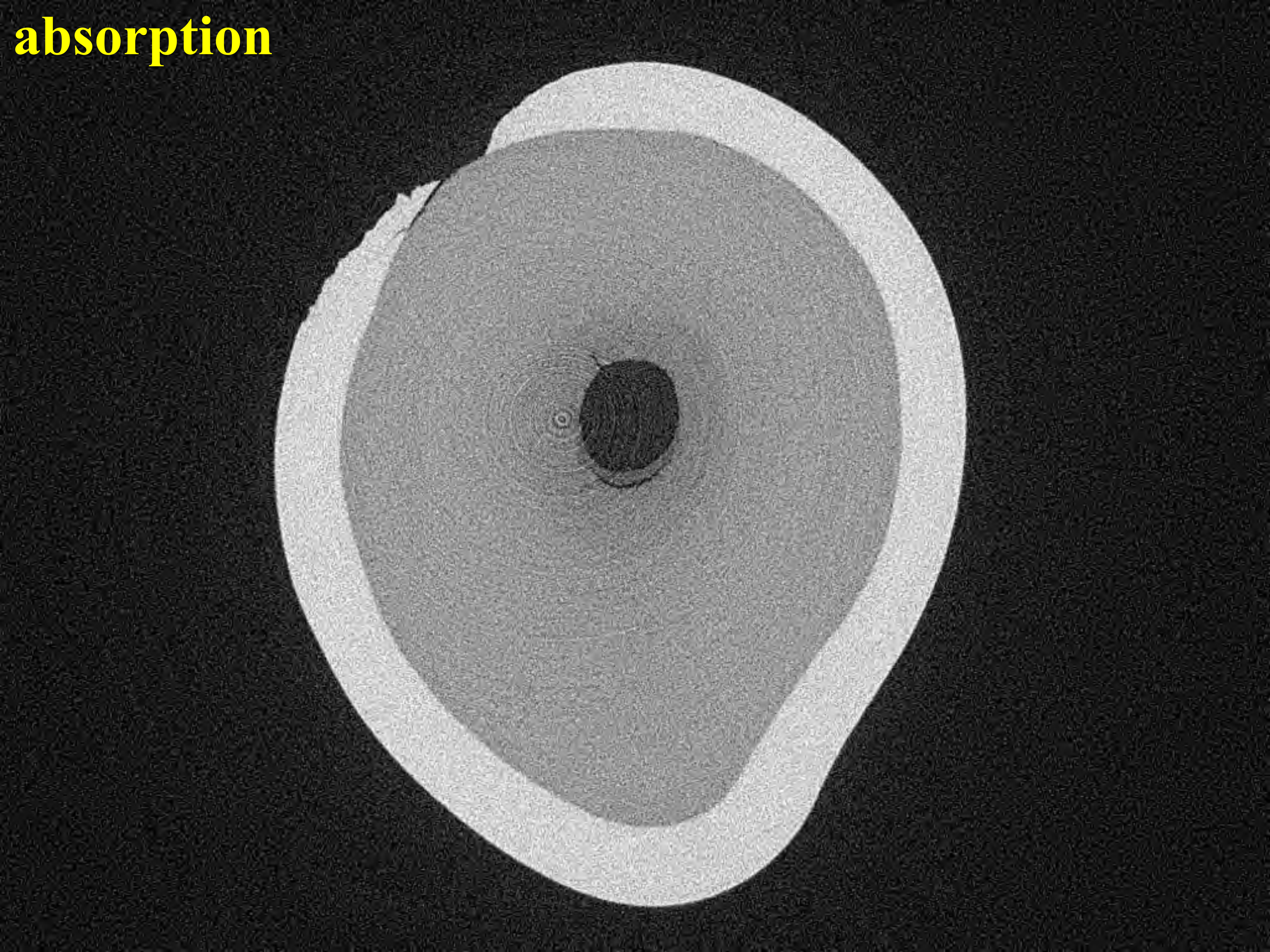
Single distance phase retrieval using modified Paganin's approach

- Phase contrast imaging is one of the most important aspects of synchrotron imaging
- Many phase retrieval systems have been developed throughout time, most of them being very complex (holotomography, GIFM, DEI)
- In 2002, Paganin et al. proposed a very simple algorithm to make phase retrieval in case of non-complex samples

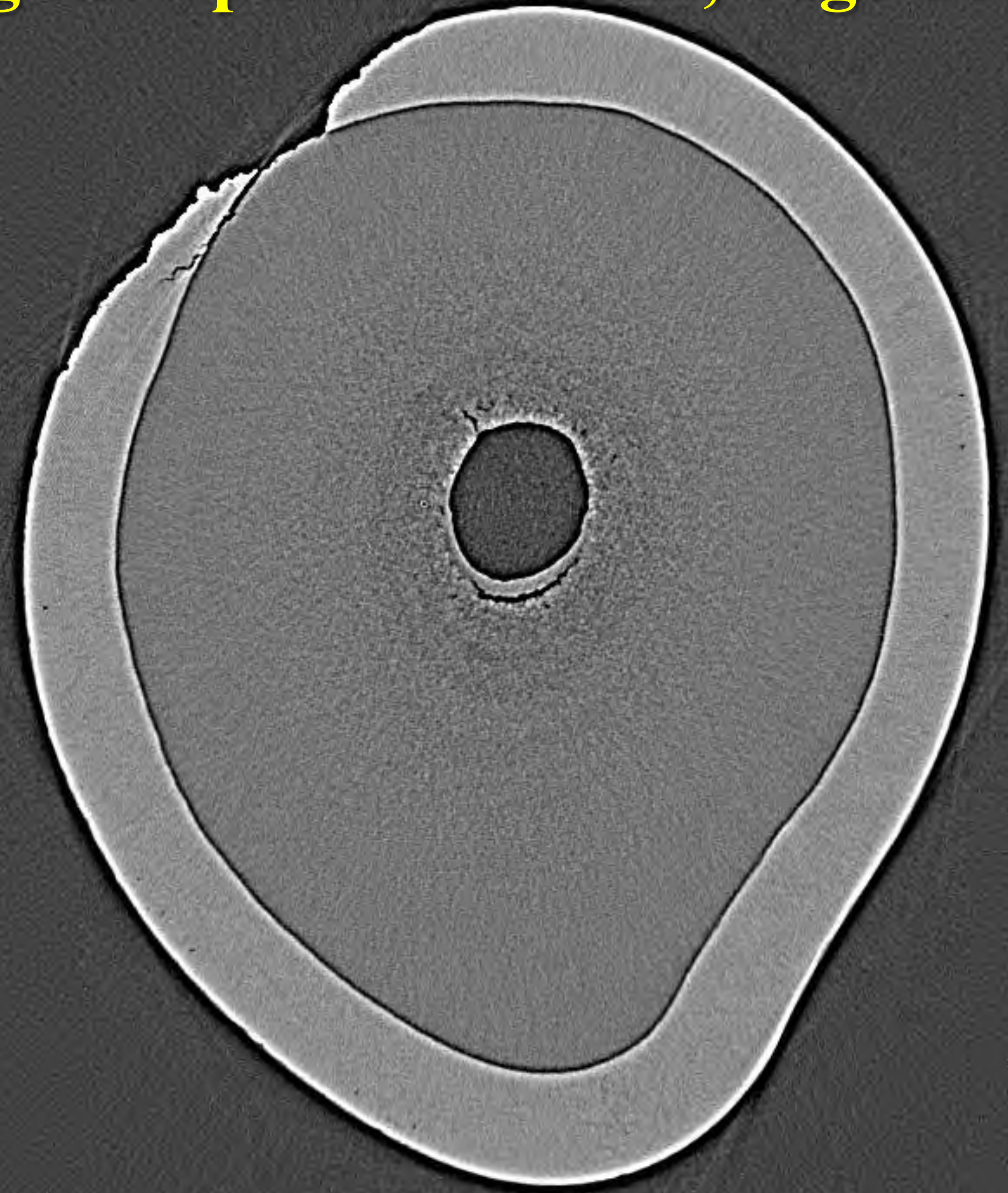
- We implemented this algorithm in PyHST, with some modifications, and it is now widely used (and nearly completely replaced holotomography on ID19)

- It changed in many ways the experiments on fossils and biological samples, but it is now also widely used in industrial applications and material sciences.

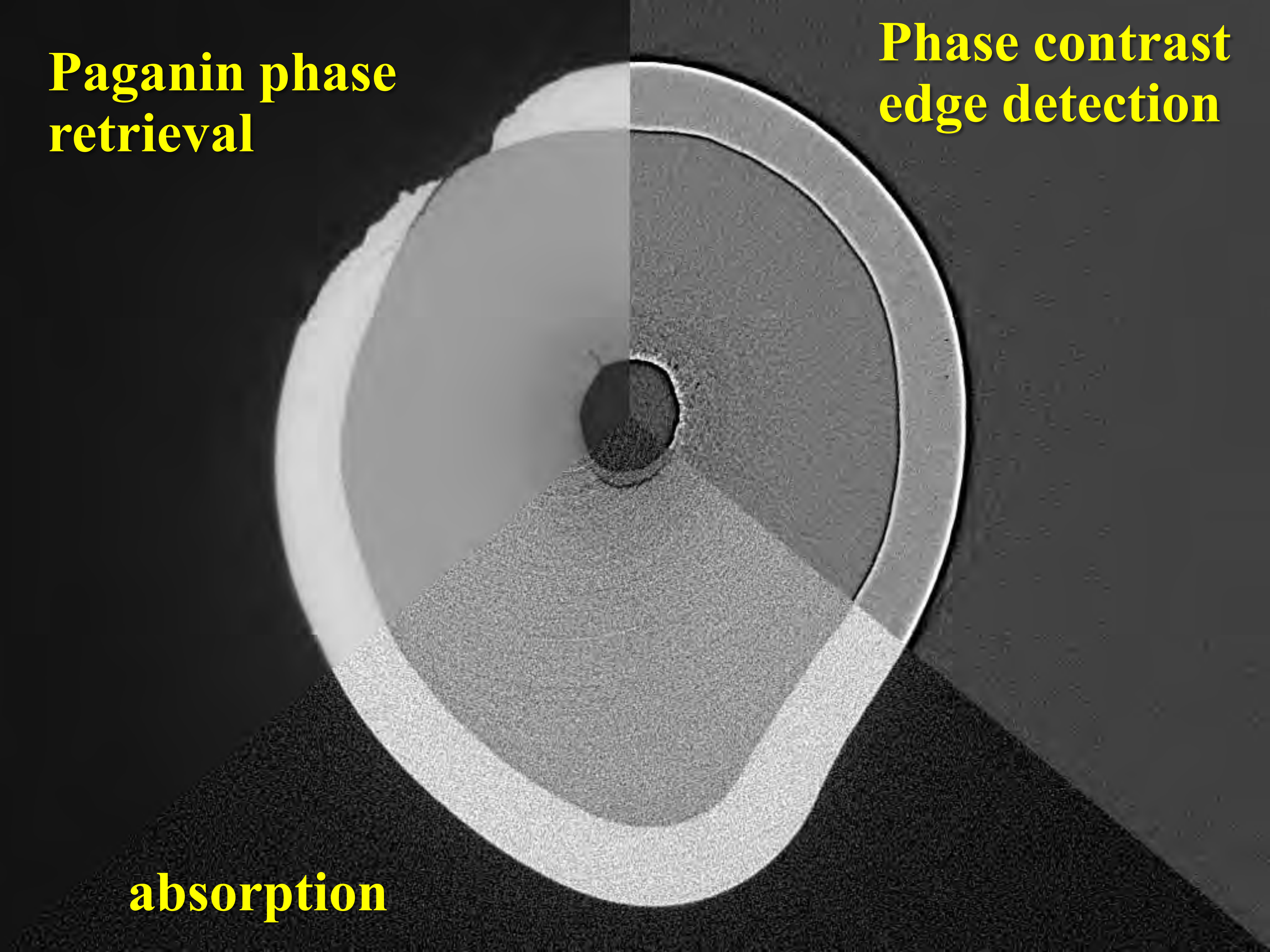
- Coupled with the high quality pink beams, it really made a breakthrough in the last few years, originally on ID19, but now also on BM05 and ID17 (in monochromatic).


absorption

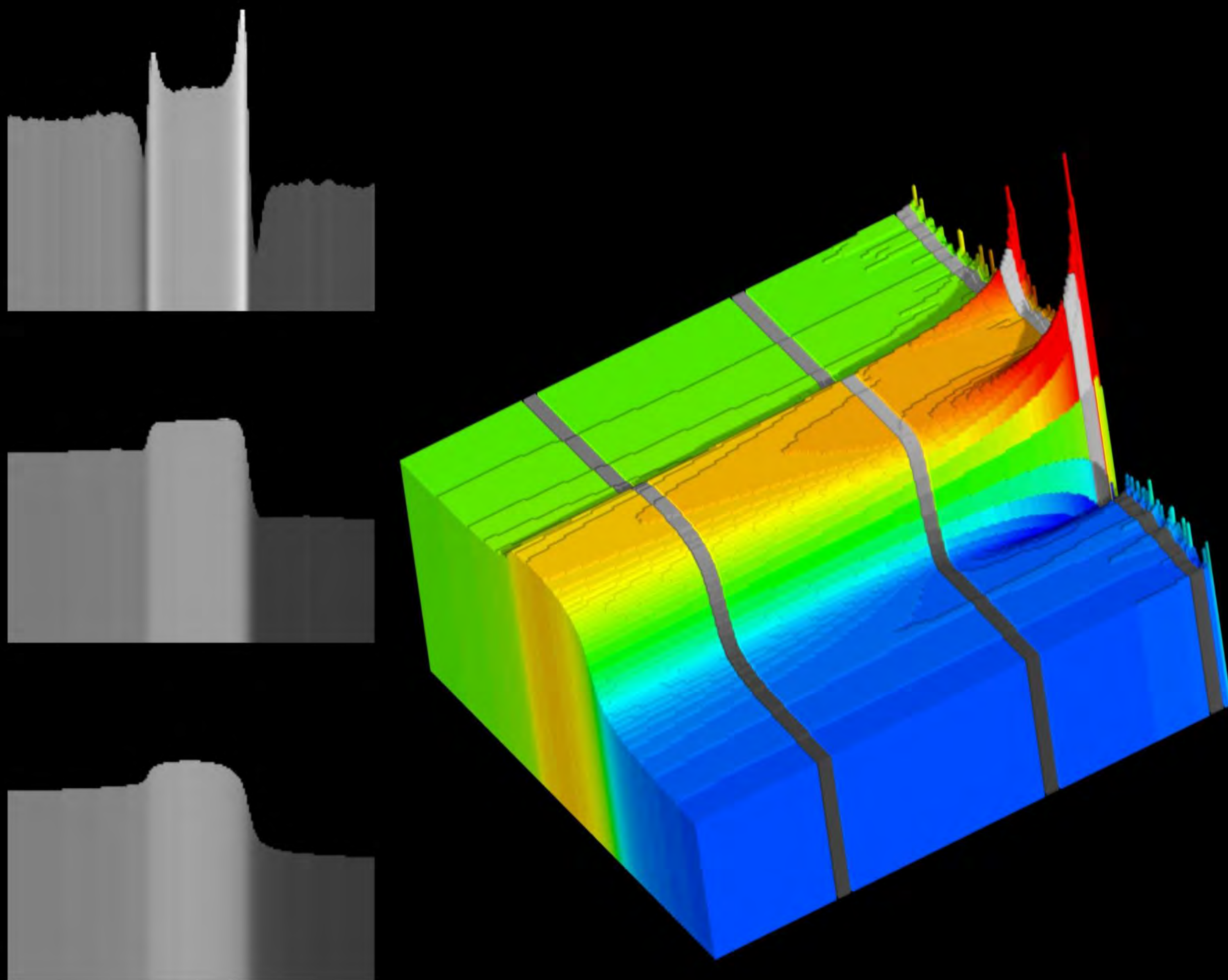
1m propagation phase contrast, edge detection



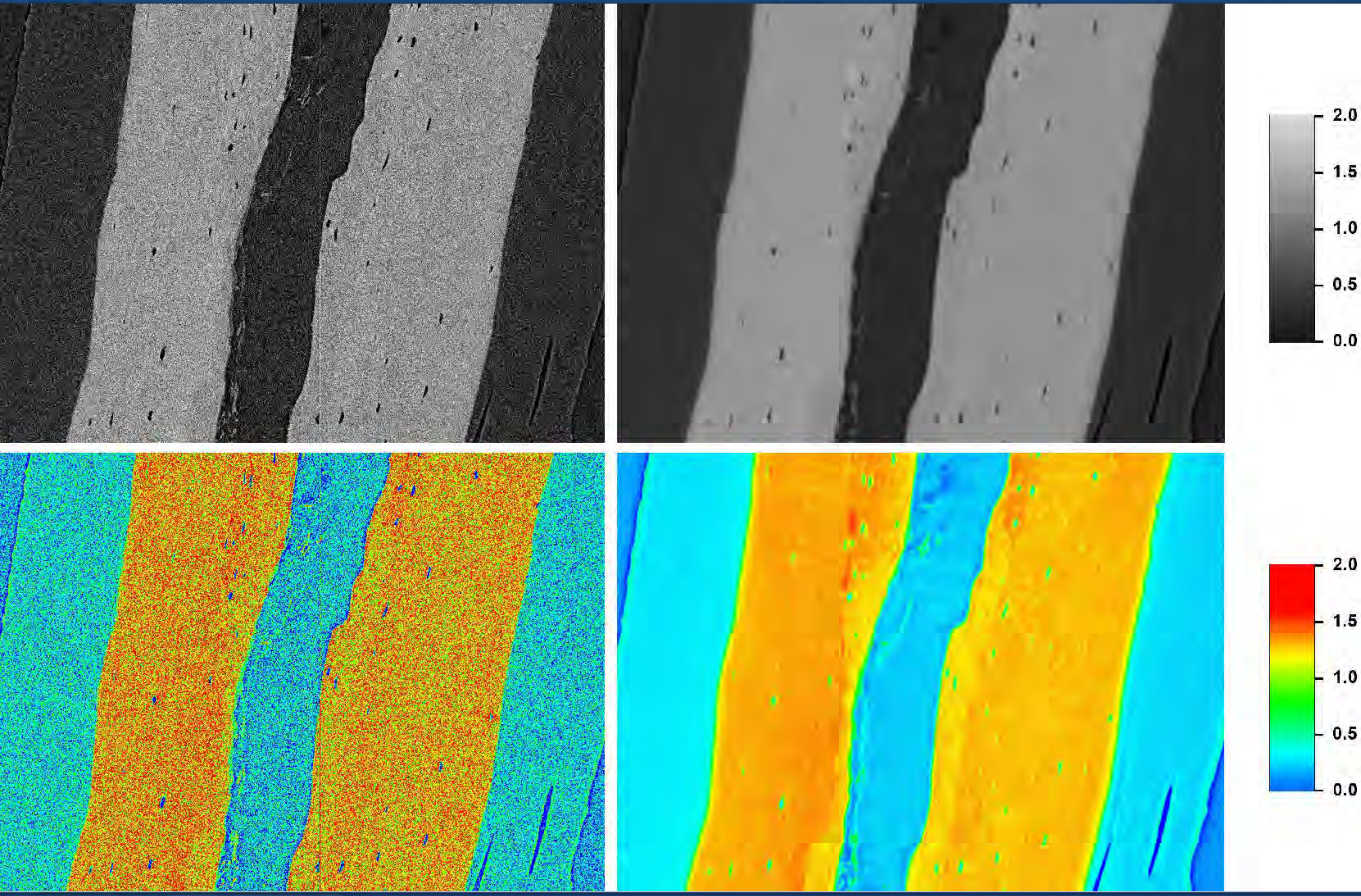
Reconstruction after single distance phase retrieval


Paganin phase retrieval
Phase contrast edge detection
absorption

Effect of the delta/beta (phase/absorption) ratio on the grey levels reconstructions



19.1 keV pink beam at 0.7 microns



General remarks on X-ray effects for optimisation of scanning parameters

- Low energies are more absorbed than high energies, then they are more likely to induce defects in aDNA.
- Monochromatic beams and narrow bandwidth pink beams are less aggressive than wide spectrum, and produce better pictures.
- For equivalent delivered dose, long scans are less aggressive than very rapid scans (dose rate effect).
- When available, phase contrast is far more sensitive than absorption, and then provide better results with less dose (up to 1000 times !).
- Dose is cumulative, i.e. multiple scans lead to higher dose.

Good practices (often common sense) to avoid potential detrimental effects of X-ray imaging on recent fossils

- With conventional sources, **use metallic filters** to remove the low energies in the spectrum (bonus: it reduces the beam hardening).
- **Avoid multiple scans**. Data sharing with open access for scans after publication is part of the solution.
- Avoid large irradiation for scans at high resolution.
- Do not use higher resolution than necessary (producing larger data)
- Avoid unnecessary high resolution scans in areas suitable for aDNA
- Keep track of the **scan history** (with parameters) and locations, especially at high resolution.
- Avoid unnecessary scans not directly linked to real research projects. Technical progress makes unused data rapidly obsolete.
- If only external surfaces are necessary, use other techniques (e.g. laser scanner) to avoid unecessary irradiation and multiple scans.

And the synchrotron revolution in palaeontology is just starting !

2015 will be marked by the multimodal / multicontrast approaches in 3D imaging on fossils.

The ESRF phase 2 upgrade will dramatically increase the possibilities for analysis of fossils thanks to increased coherence and brilliance.

The story continues …

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 and Gilles Peix who made all this possible 15 years ago by helping a crazy young PhD student in palaeontology to access for the first time to a synchrotron beamline.