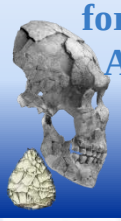


Assessing the impact of X-ray tomography on ancient DNA in recent fossils

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

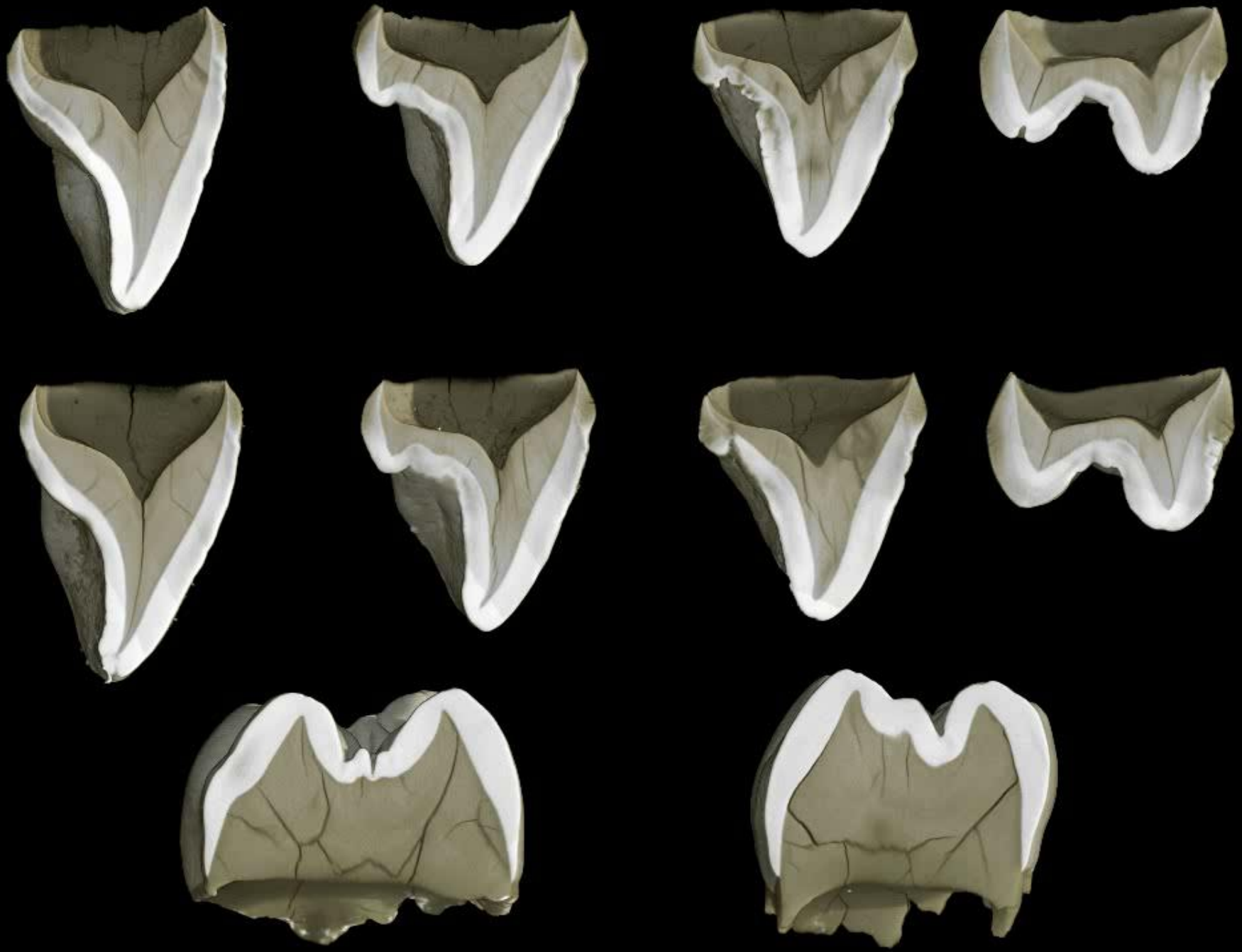
Propagation phase contrast multiscale analysis: The revolution of the non-destructive virtual palaeohistology

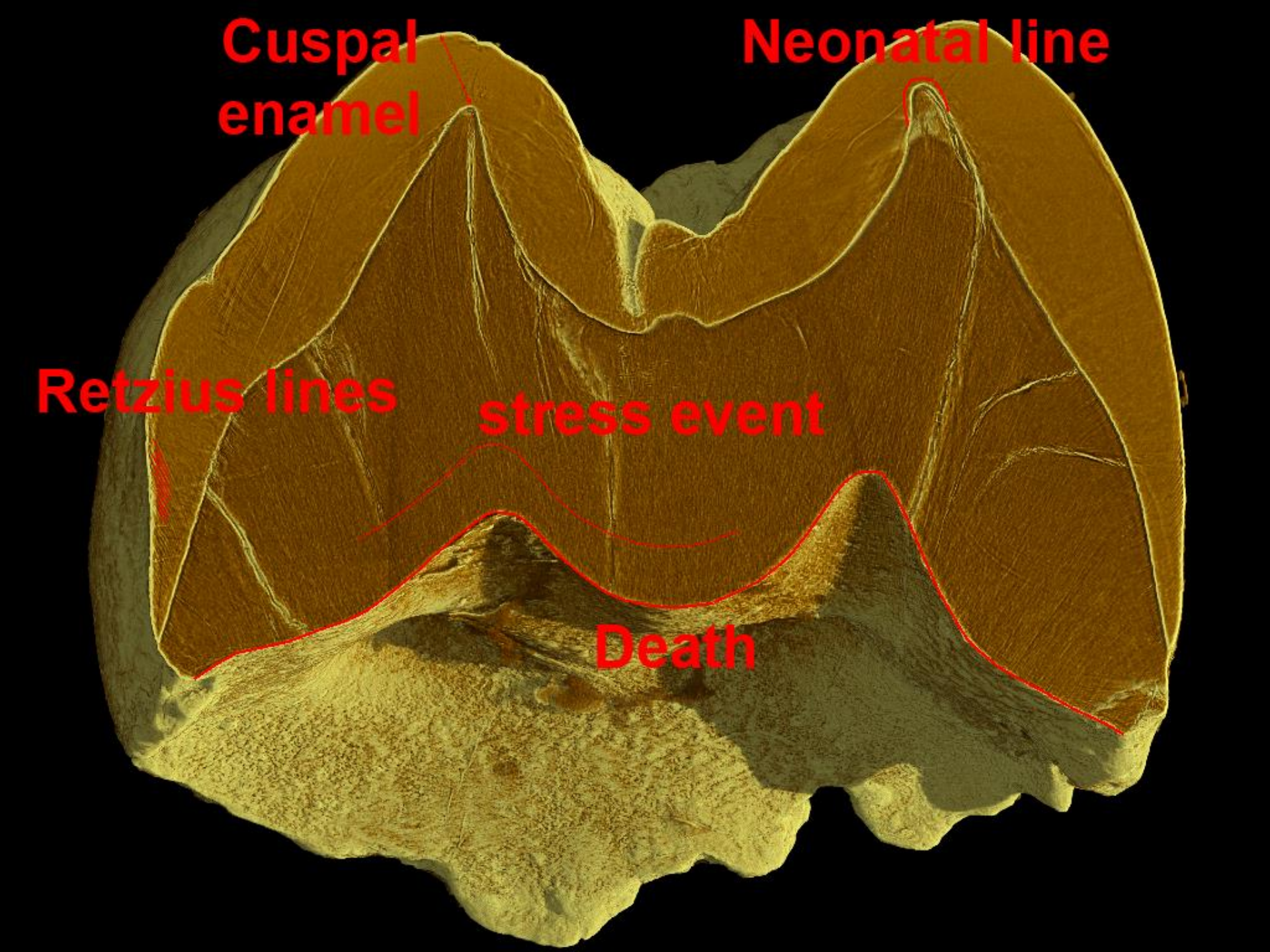
Age at death determination of the Engis 2 Neanderthal child.

30 000 – 50 000 ans, Belgique

Smith *et al.* 2010, PNAS


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

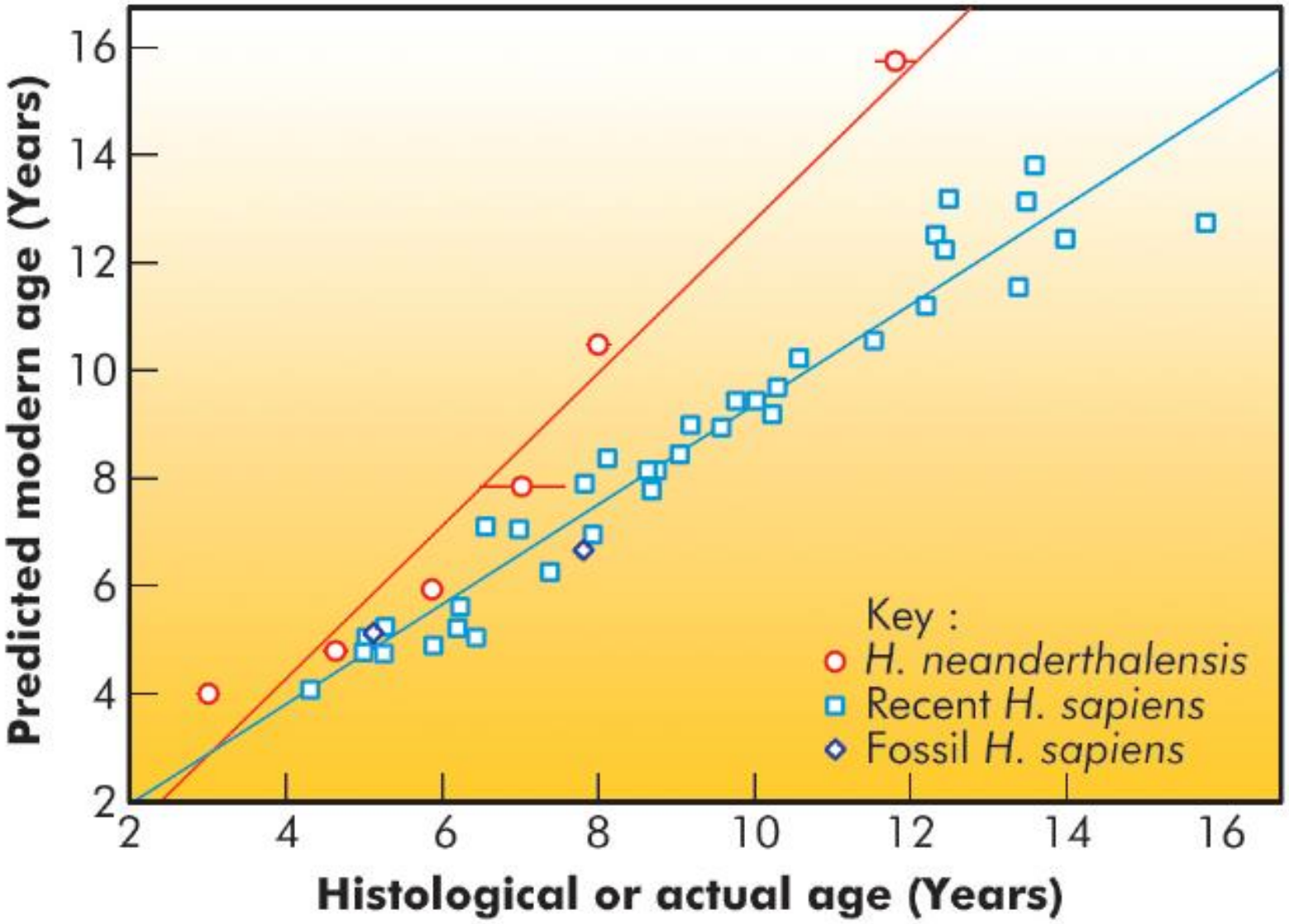
| |

(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



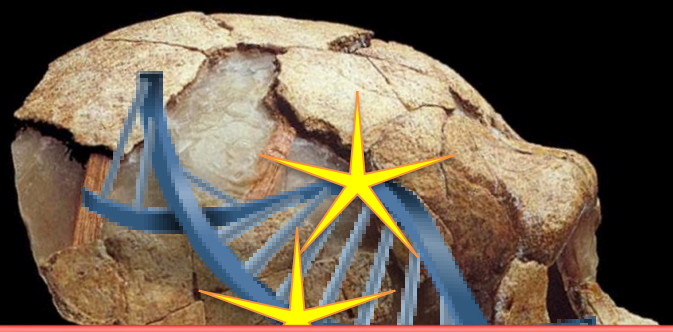
Smith et al. (2010) PNAS

Australopithecus sediba: **1.97 My**

Imaged at the ESRF in 2009

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

Homo neanderthalensis: **36 Ky**



Extremely hot and recurrent topic in palaeoanthropology: How to reconcile X-ray imaging with paleo-DNA investigations ?

Old fossils:

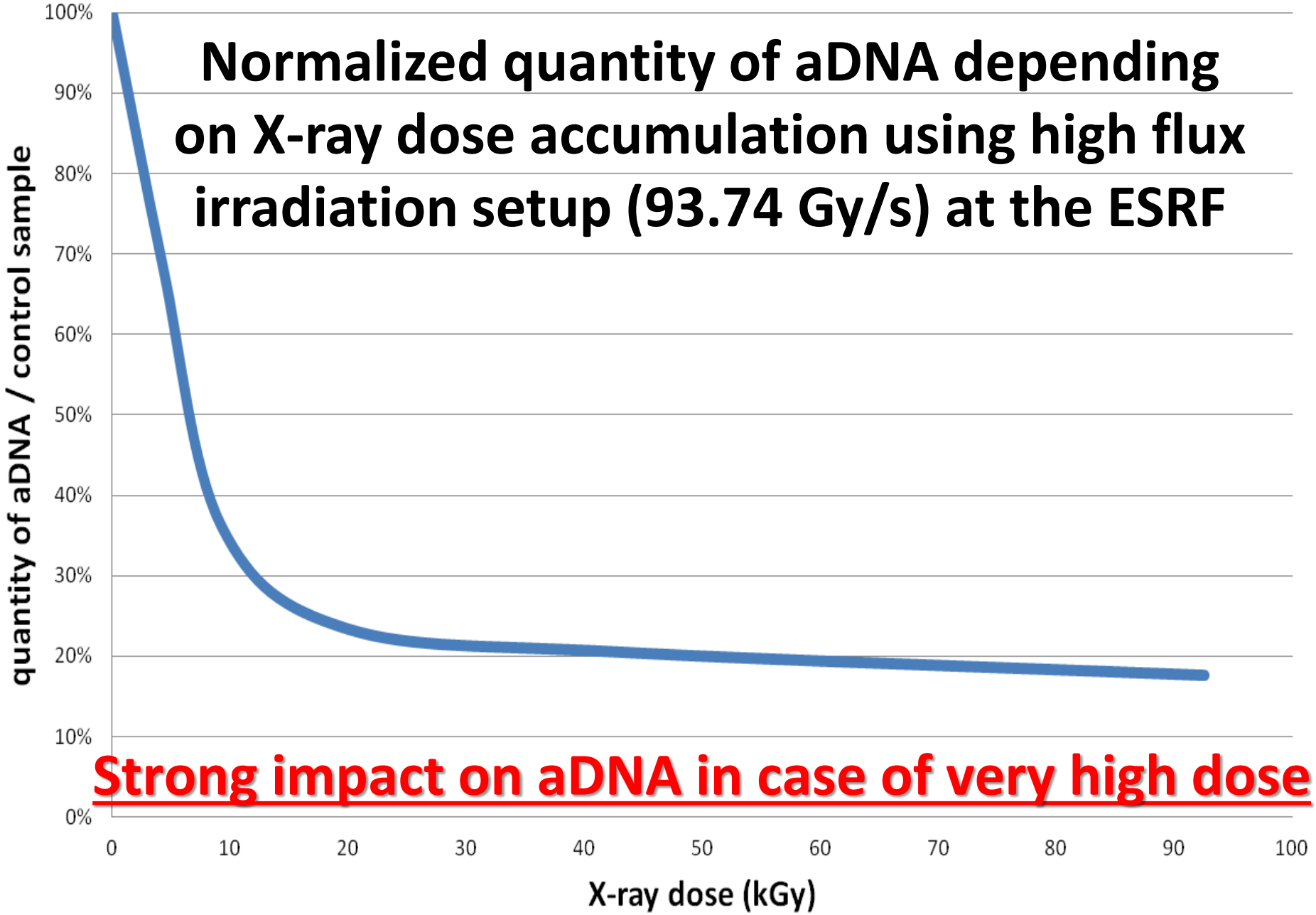
No risk for X-ray imaging

Recent fossils:

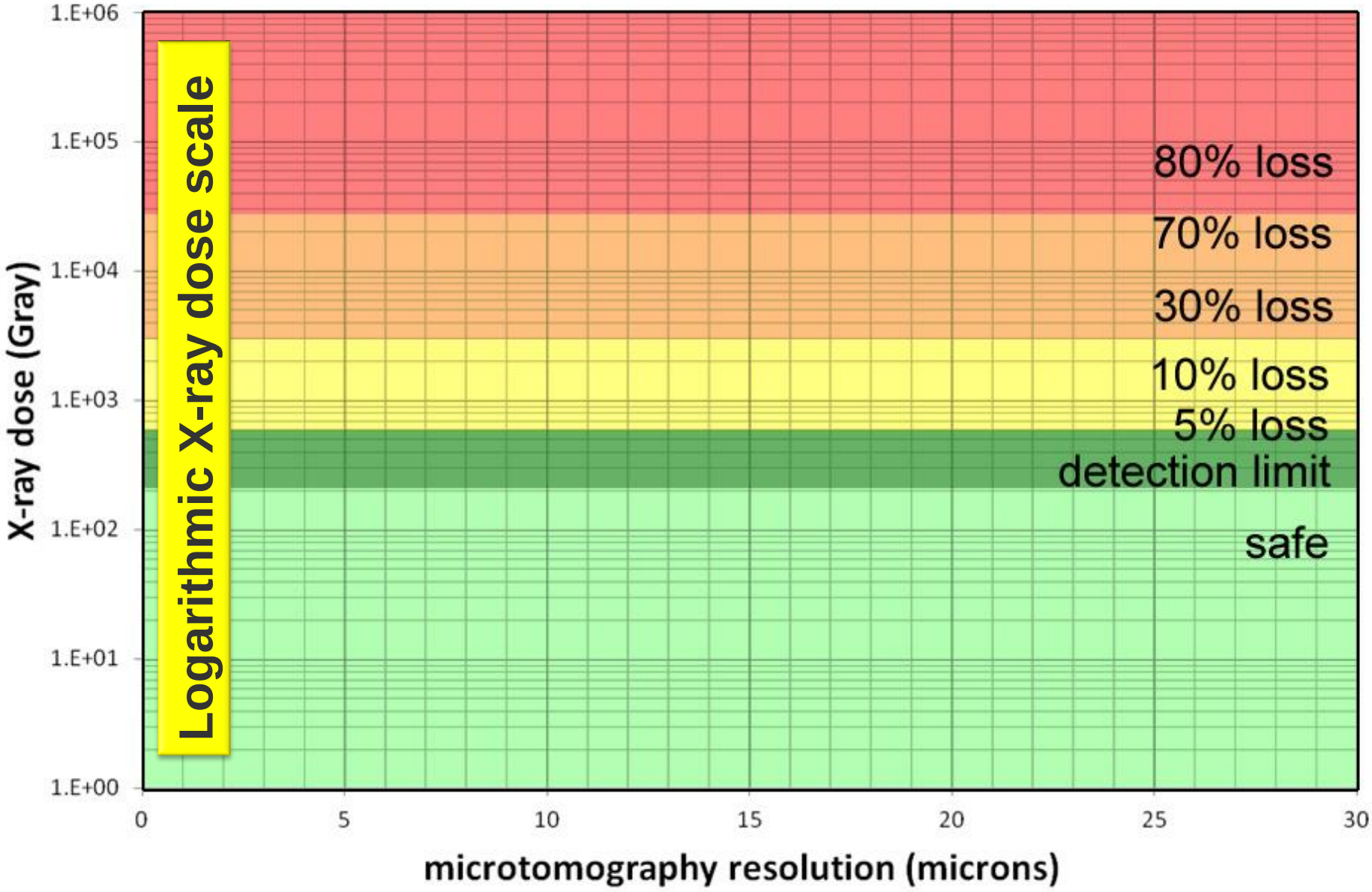
Risk of aDNA degradation

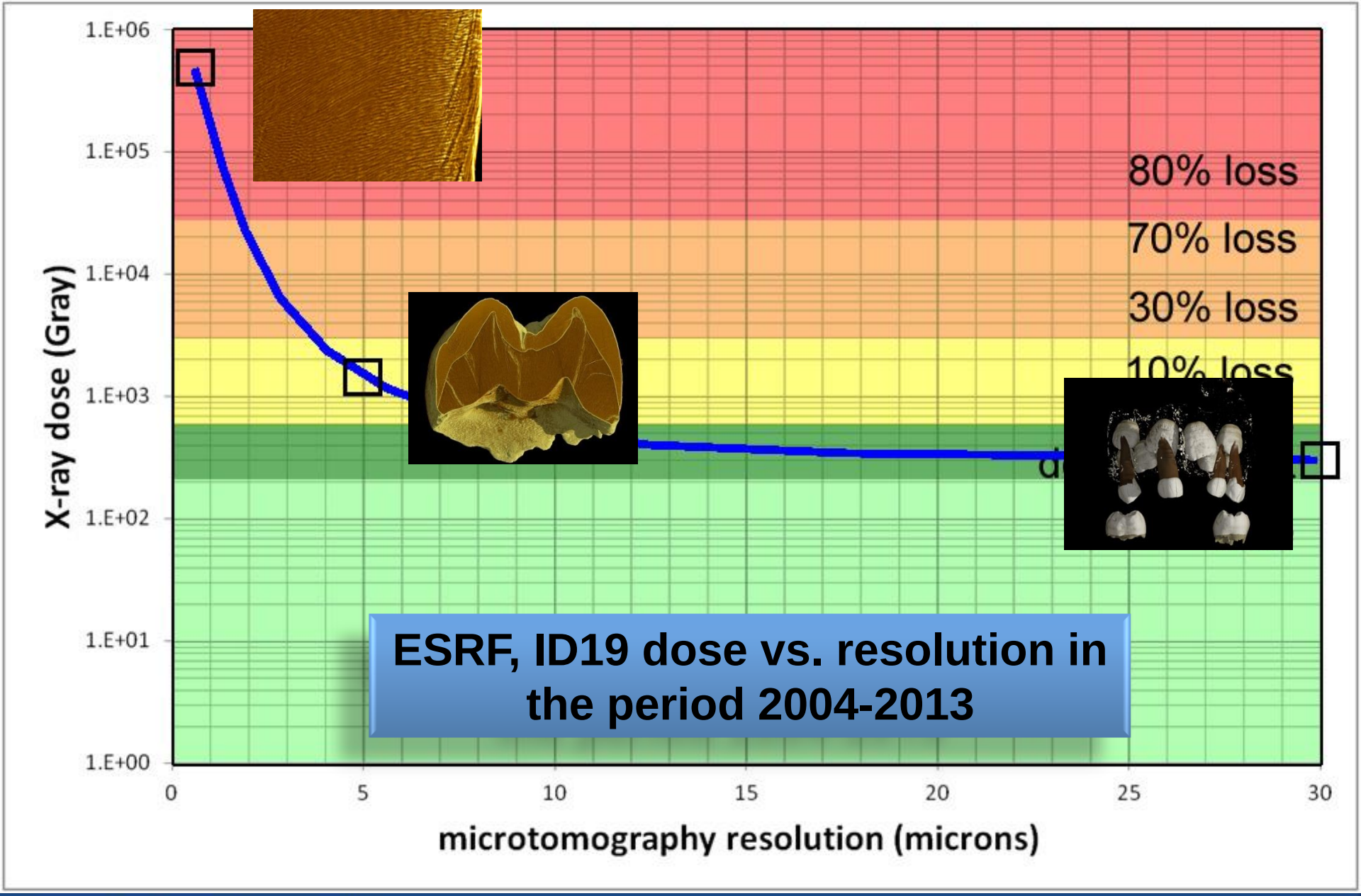
Synchrotron white beam can be very powerful, hence it is necessary to tune carefully the machine to ensure the non-destructive analysis.

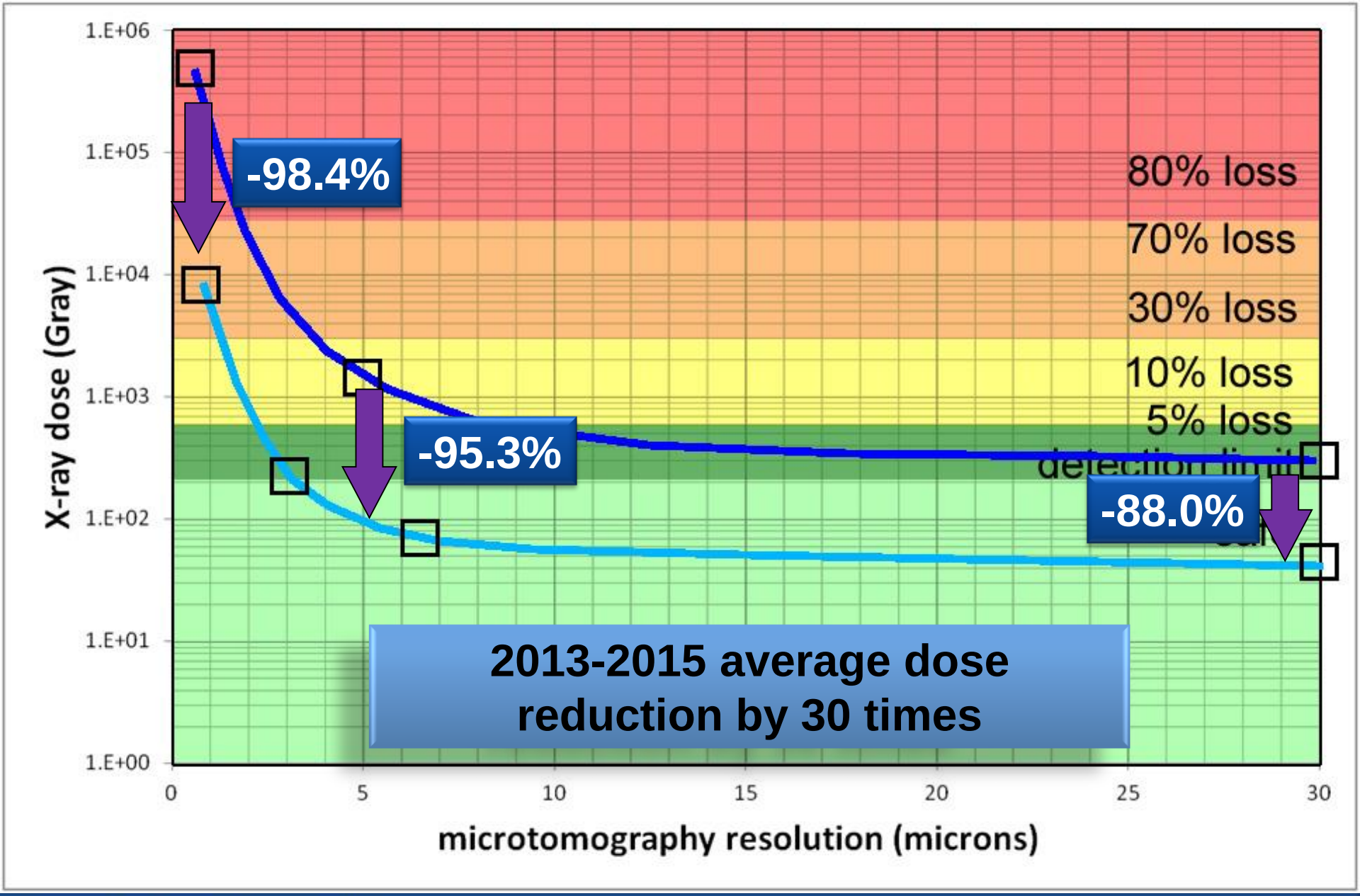
Normalized quantity of aDNA depending on X-ray dose accumulation using high flux irradiation setup (93.74 Gy/s) at the ESRF

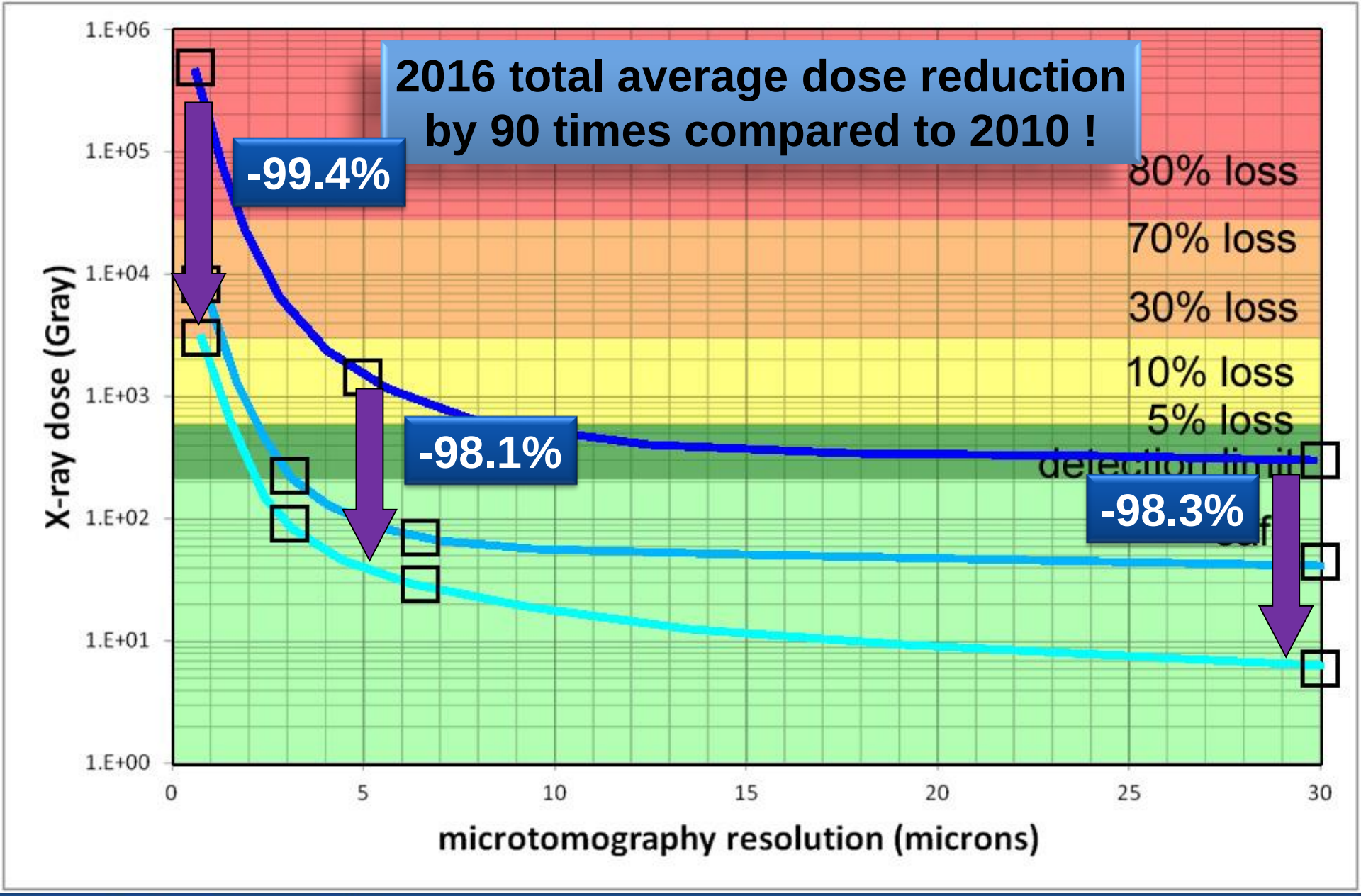


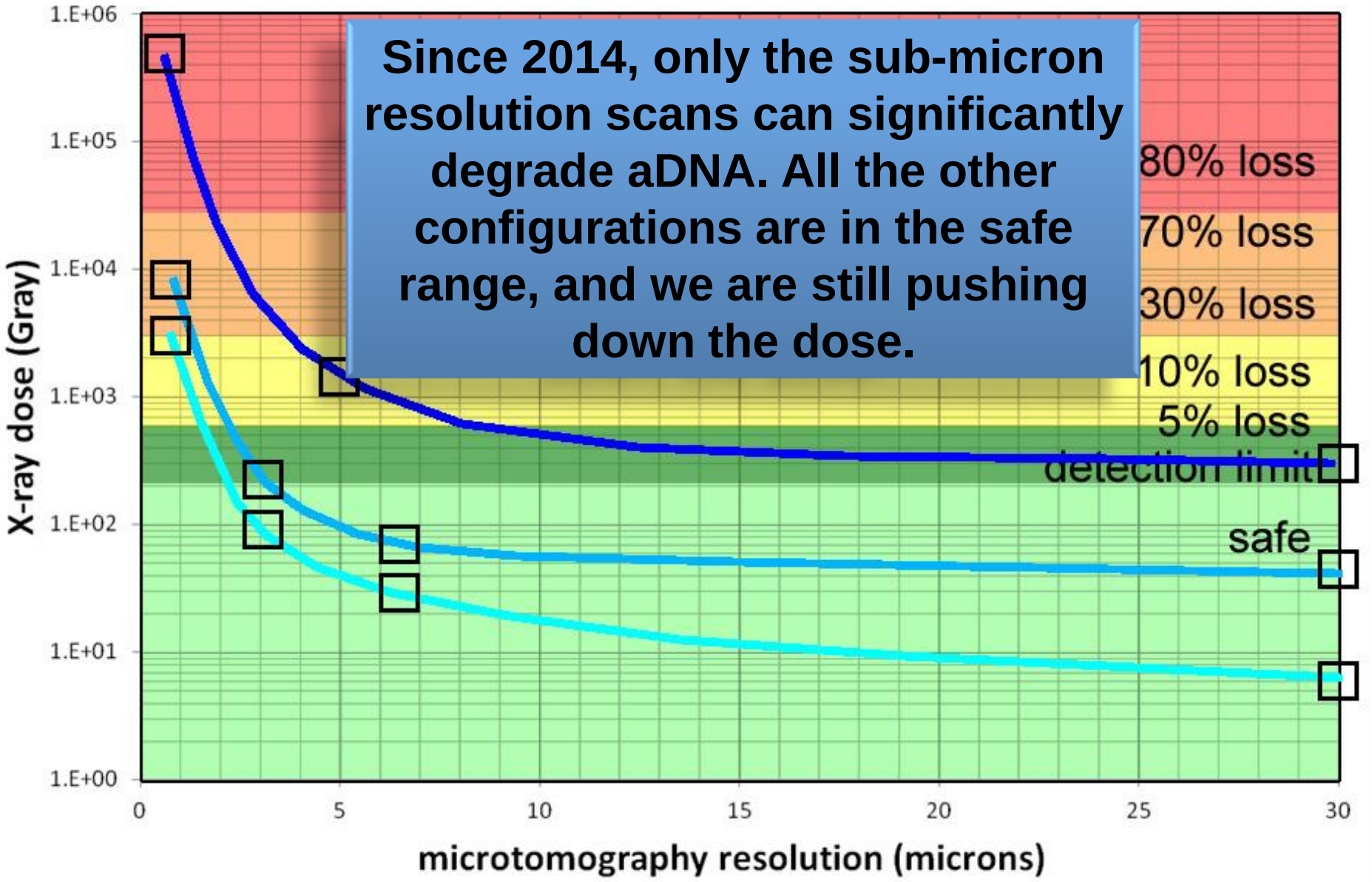
Strong impact on aDNA in case of very high dose

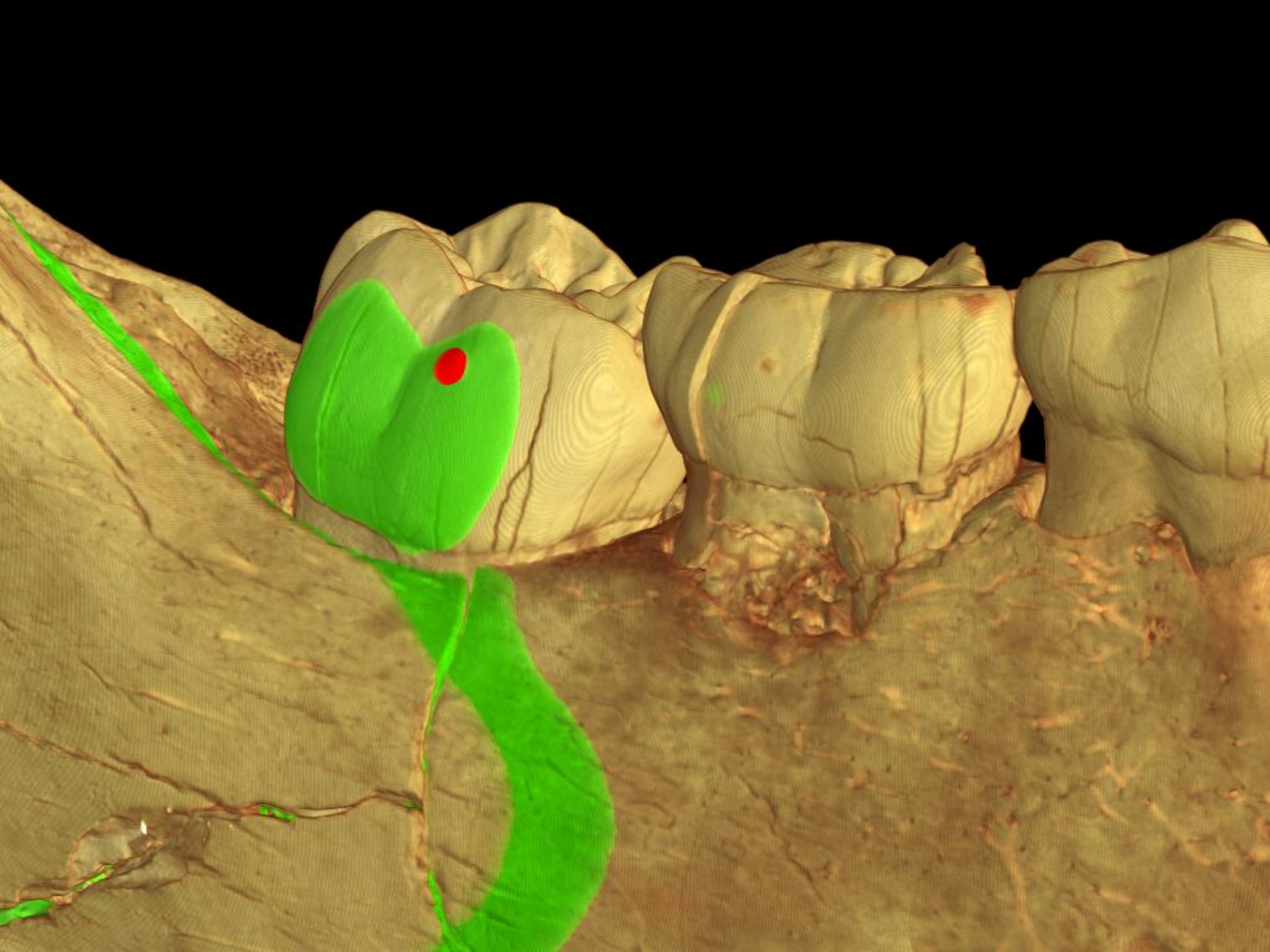

Logarithmic X-ray dose scale
X-ray dose (Gray)
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
0
5
10
15
20
25
30
microtomography resolution (microns)
80% loss
70% loss
30% loss
10% loss
5% loss
detection limit
safe

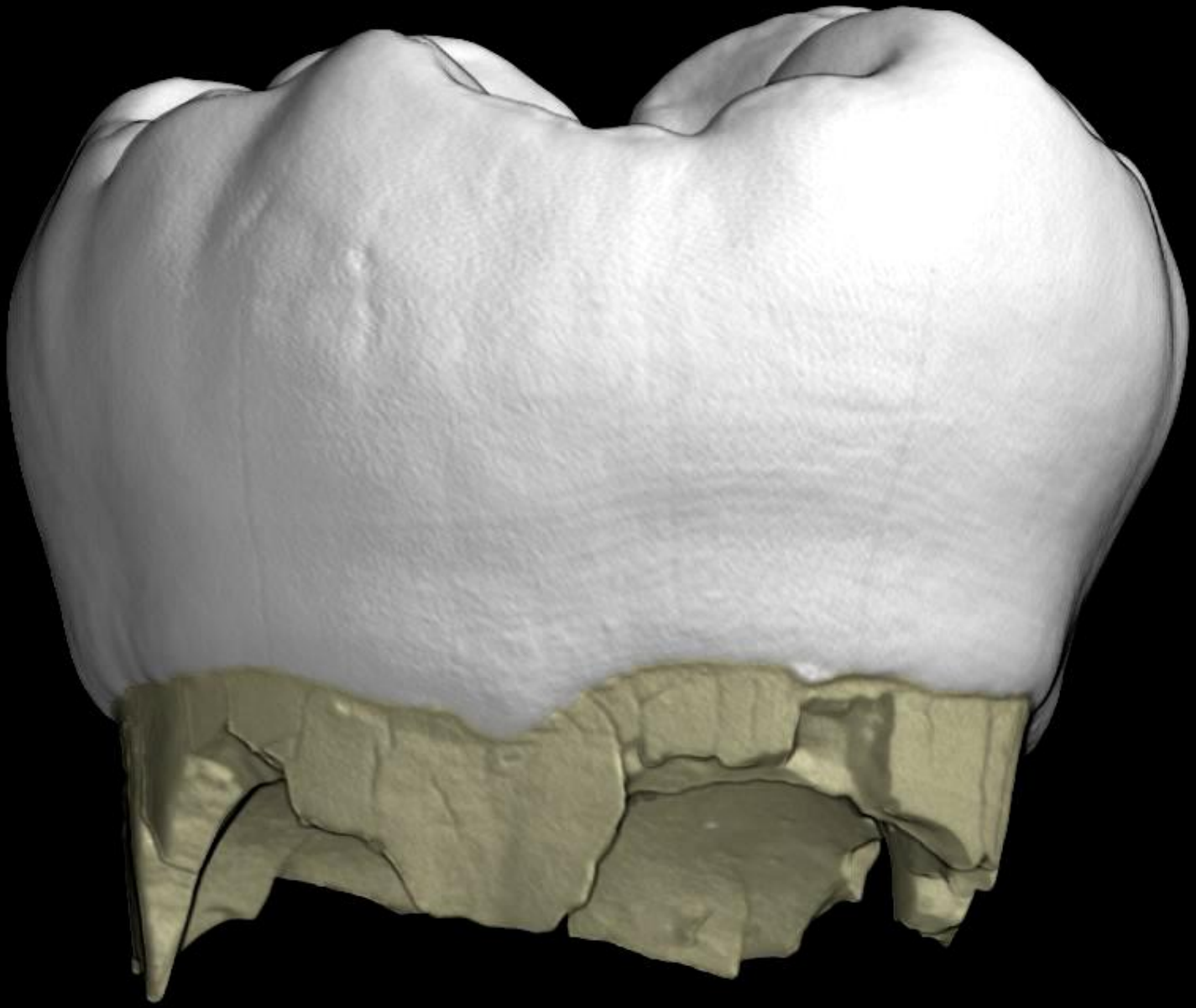

1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
X-ray dose (Gray)
80% loss
70% loss
30% loss
10% loss
0
5
10
15
20
25
30
microtomography resolution (microns)
ESRF, ID19 dose vs. resolution in the period 2004-2013

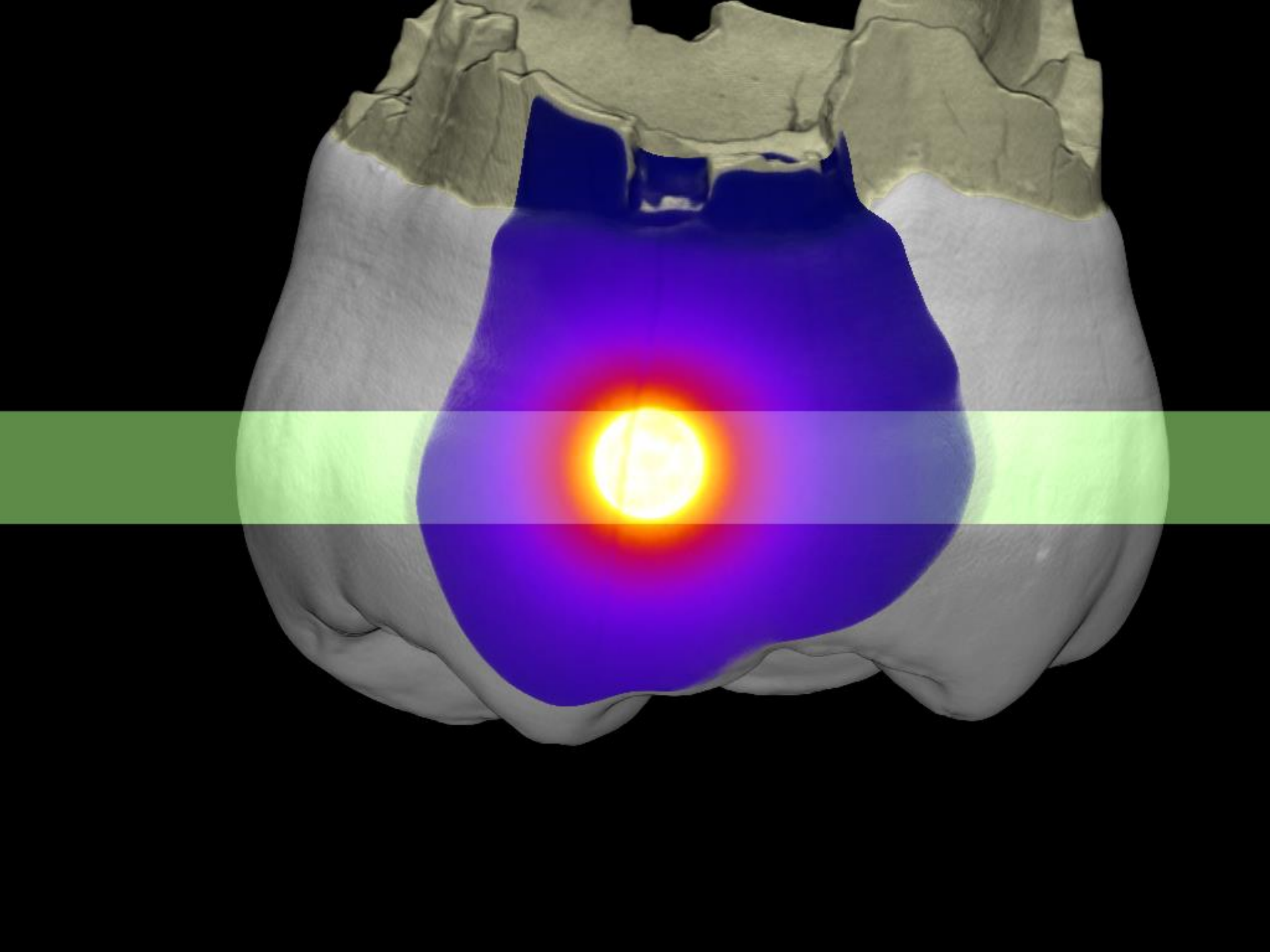

1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
X-ray dose (Gray)
0
5
10
15
20
25
30
microtomography resolution (microns)
-98.4%
-95.3%
-88.0%
80% loss
70% loss
30% loss
10% loss
5% loss
2013-2015 average dose reduction by 30 times


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
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)

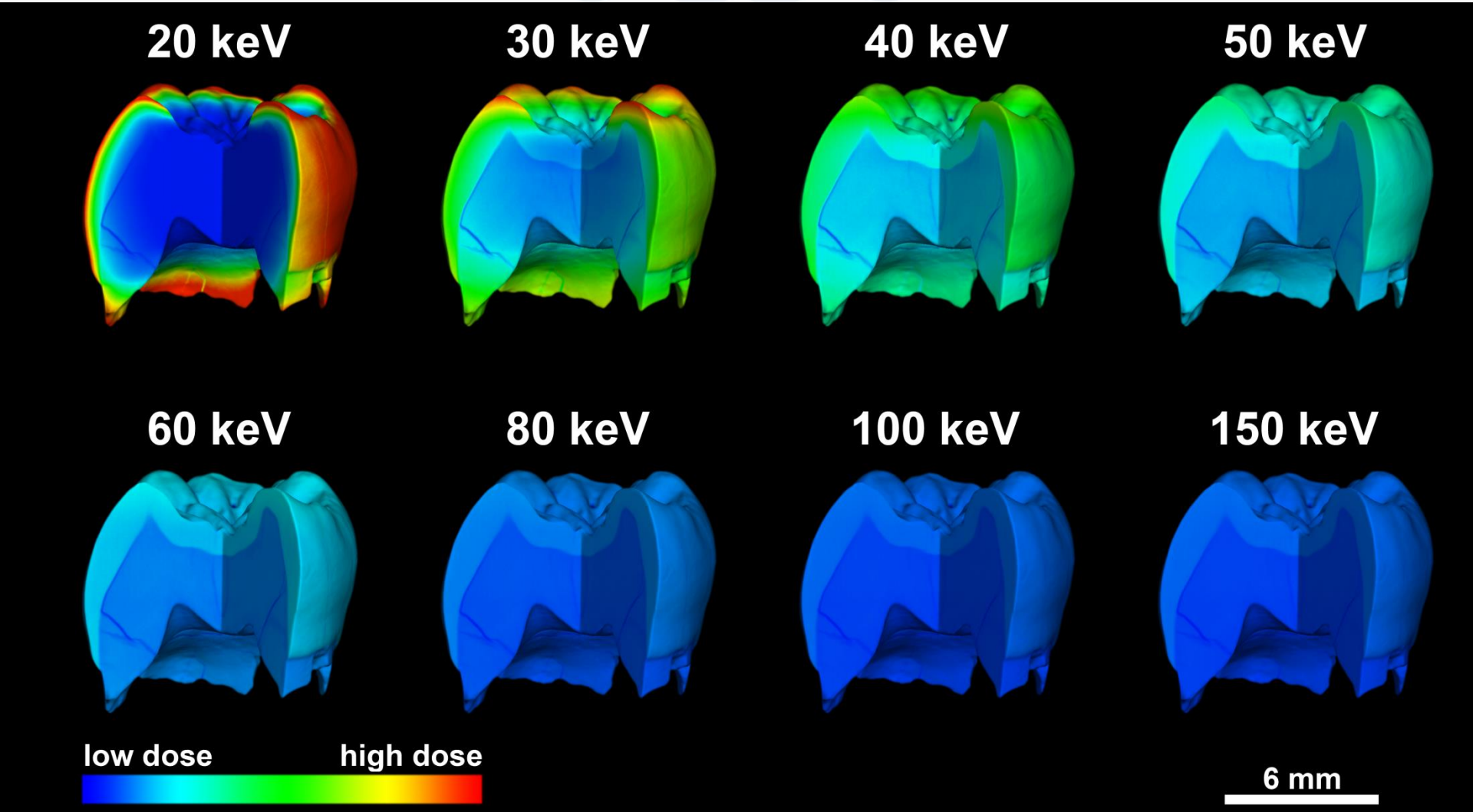

Since 2014, only the sub-micron resolution scans can significantly degrade aDNA. All the other configurations are in the safe range, and we are still pushing down the dose.
80% loss
70% loss
30% loss
10% loss
5% loss
detection limit
safe
X-ray dose (Gray)
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
0
5
10
15
20
25
30
microtomography resolution (microns)







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



GENERAL REMARKS ON X-RAY EFFECTS FOR OPTIMISATION OF SCANNING PARAMETERS

- Absence of water in fossils may be the explanation of the good aDNA resistance to X-ray dose (lower probability of free radicals).
- Low energies are more absorbed than high energies, they are then more likely to induce defects in aDNA.
- Monochromatic beams and relatively narrow bandwidth pink beams are less aggressive than wide spectrum, and produce better pictures.
- For equivalent delivered dose, minutes long scans are less aggressive than rapid scans (effect of dose rate and thermal aspects).
- 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.


Ready for the future, with the ESRF-EBS
Thank you for your attention !