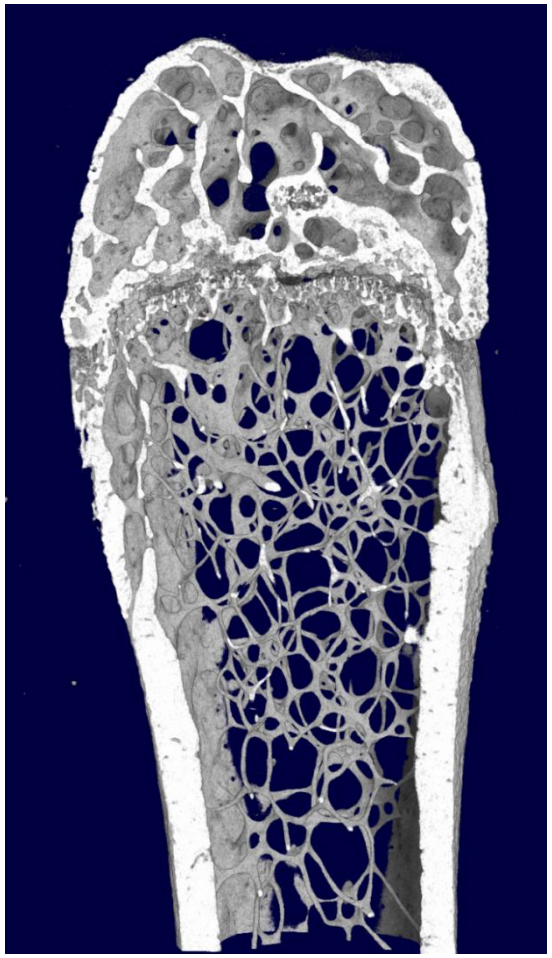
Using Micro-CT Imaging for the Phenotyping and Analysis of Bone Architecture



Rob van ’t Hof

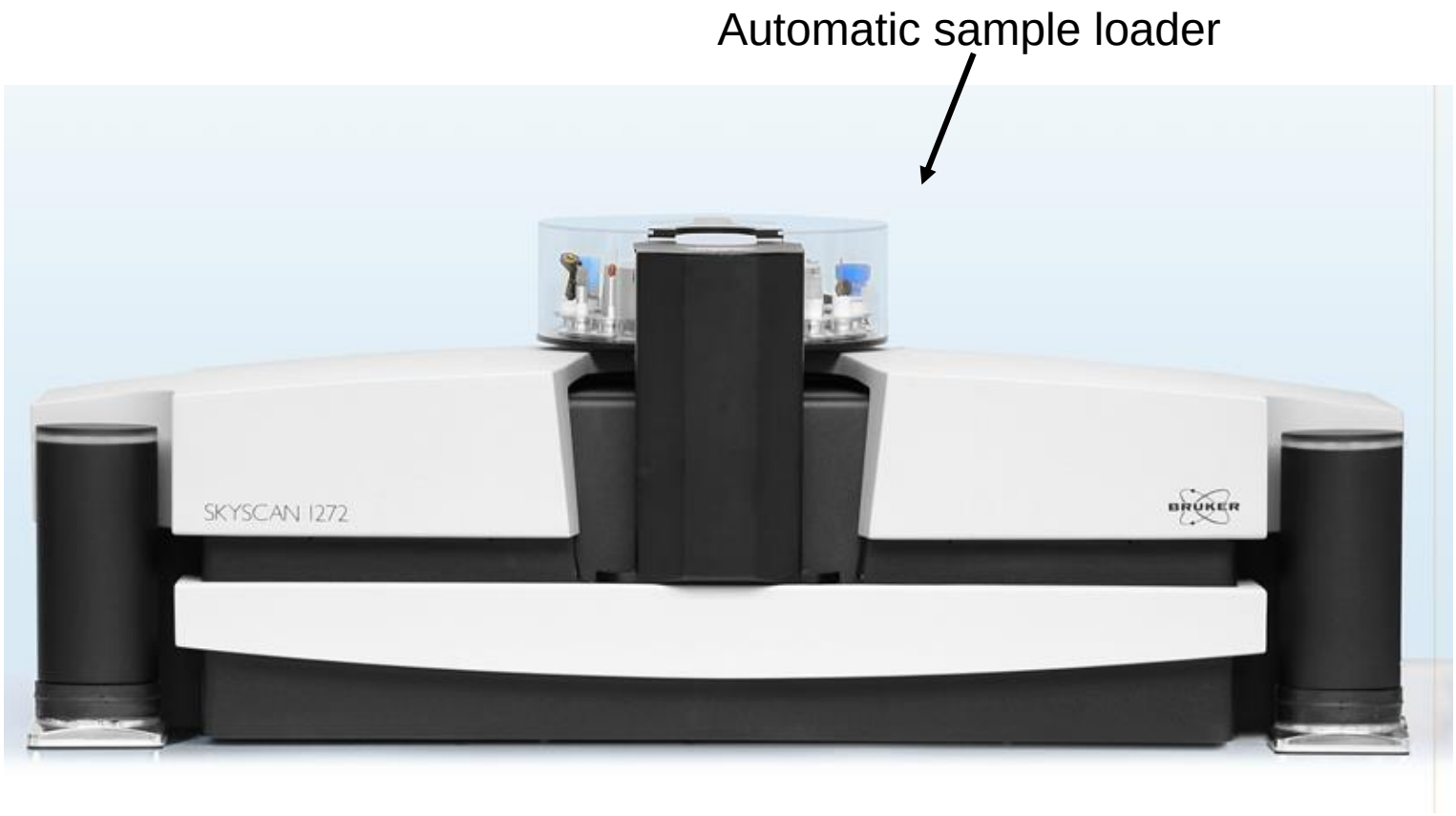
Institute of Ageing and Chronic Disease

UNIVERSITY OF LIVERPOOL

Simple X-ray Image

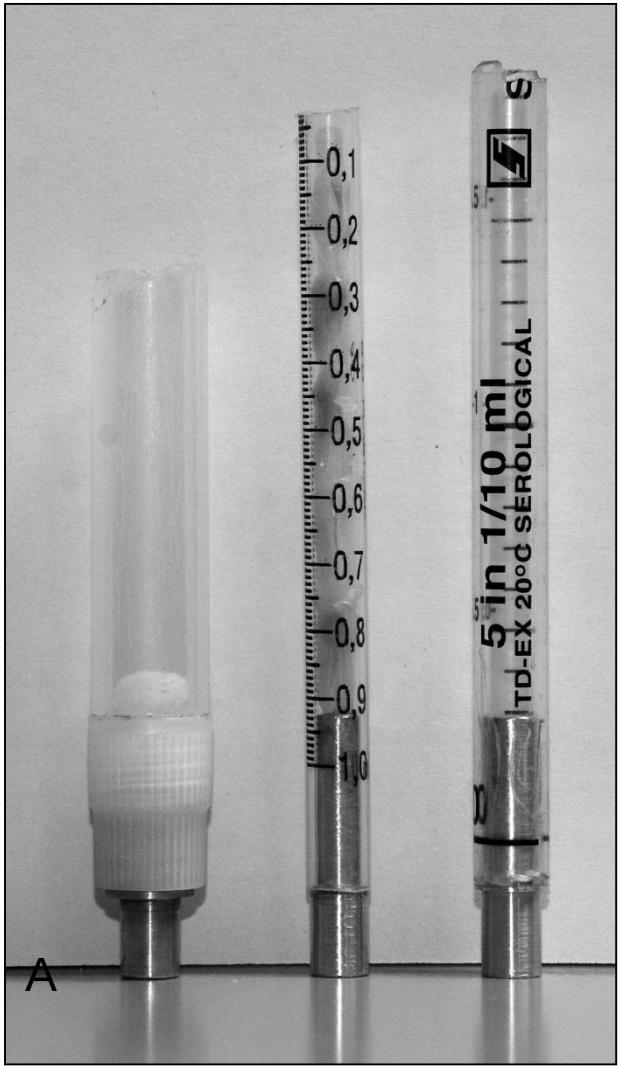
[PatientID]: OMAR, [Access#]: WHOLE, [Name]: PDB, 41, [Gender]: , [Time]: 2006/12/15 12:11:33
[File]: I20061215121133, [StudyID]: , [Study]: , [Proc]: , [Position]:
[Physician]: , [TechID]: , [Tech]: , [Station]: FAXITRON, [Institution]:

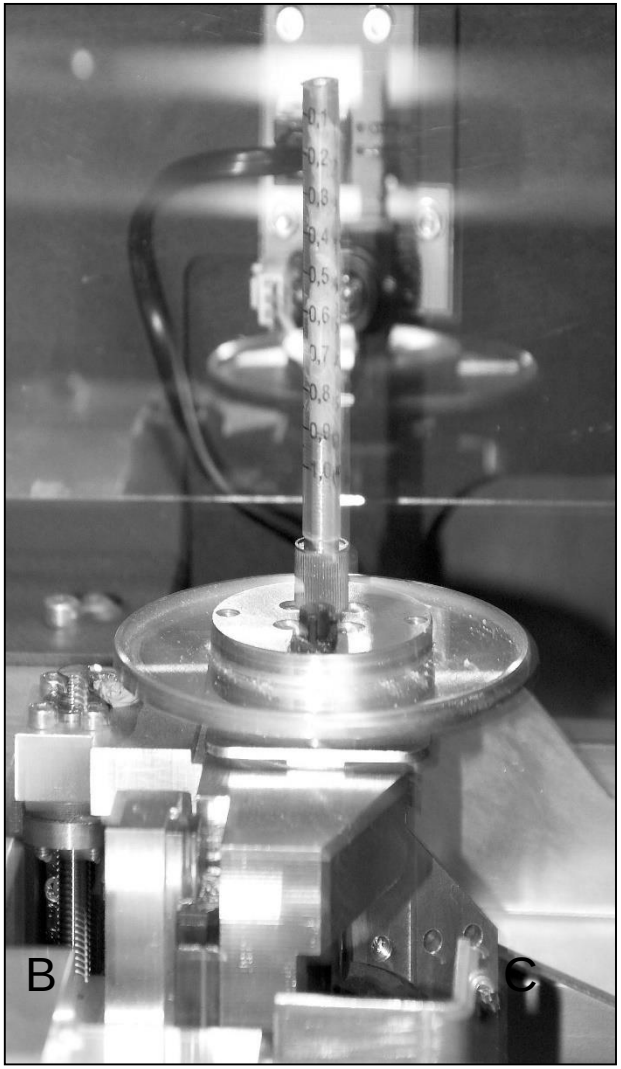
The Hardware

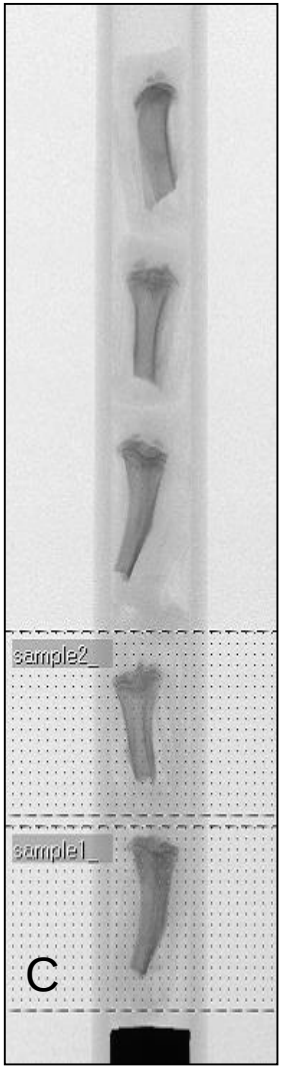


Skyscan 1272

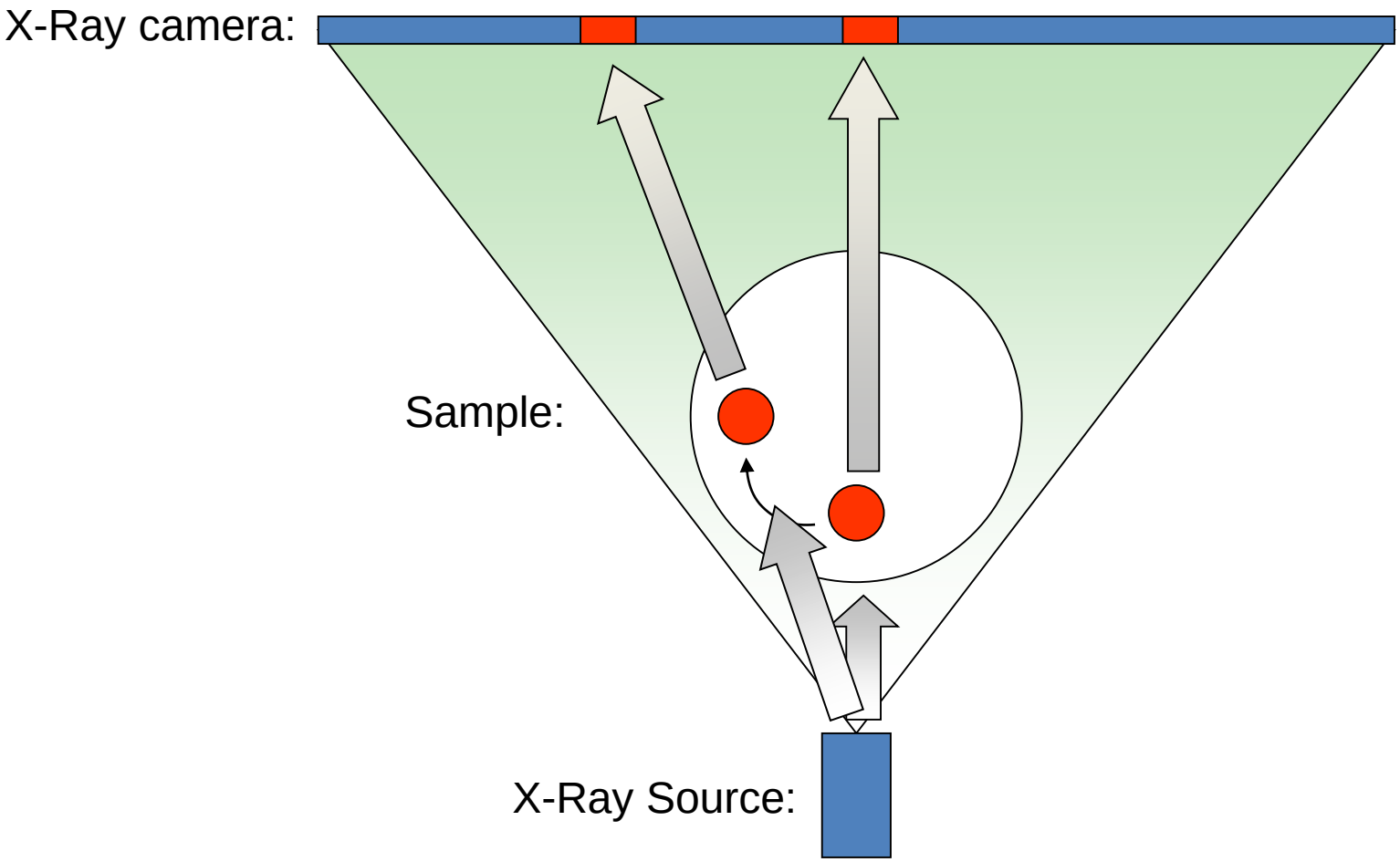
Sample Holders for the desktop Scanner







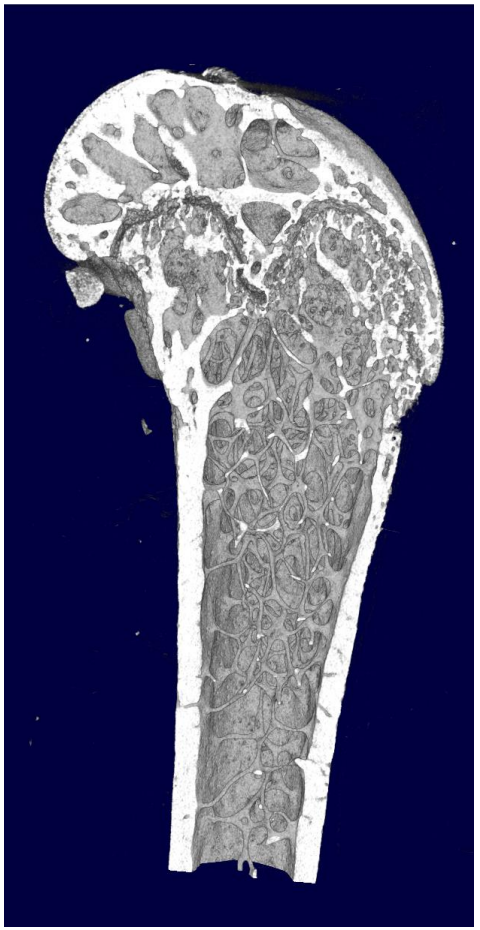
Principle of µCT Imaging



Principle of µCT Imaging

Result: 3D Image

- Visualise
- Virtual sectioning
- Measure



Steps for getting our µCT Data

-Collect X-ray shadow projections

- Perform cone-beam reconstruction

- Identify area to be measured

- measure architectural parameters in 3D

-Optionally make pretty pictures in 3D visualisation software

Main Scanner Settings

1. Resolution/magnification

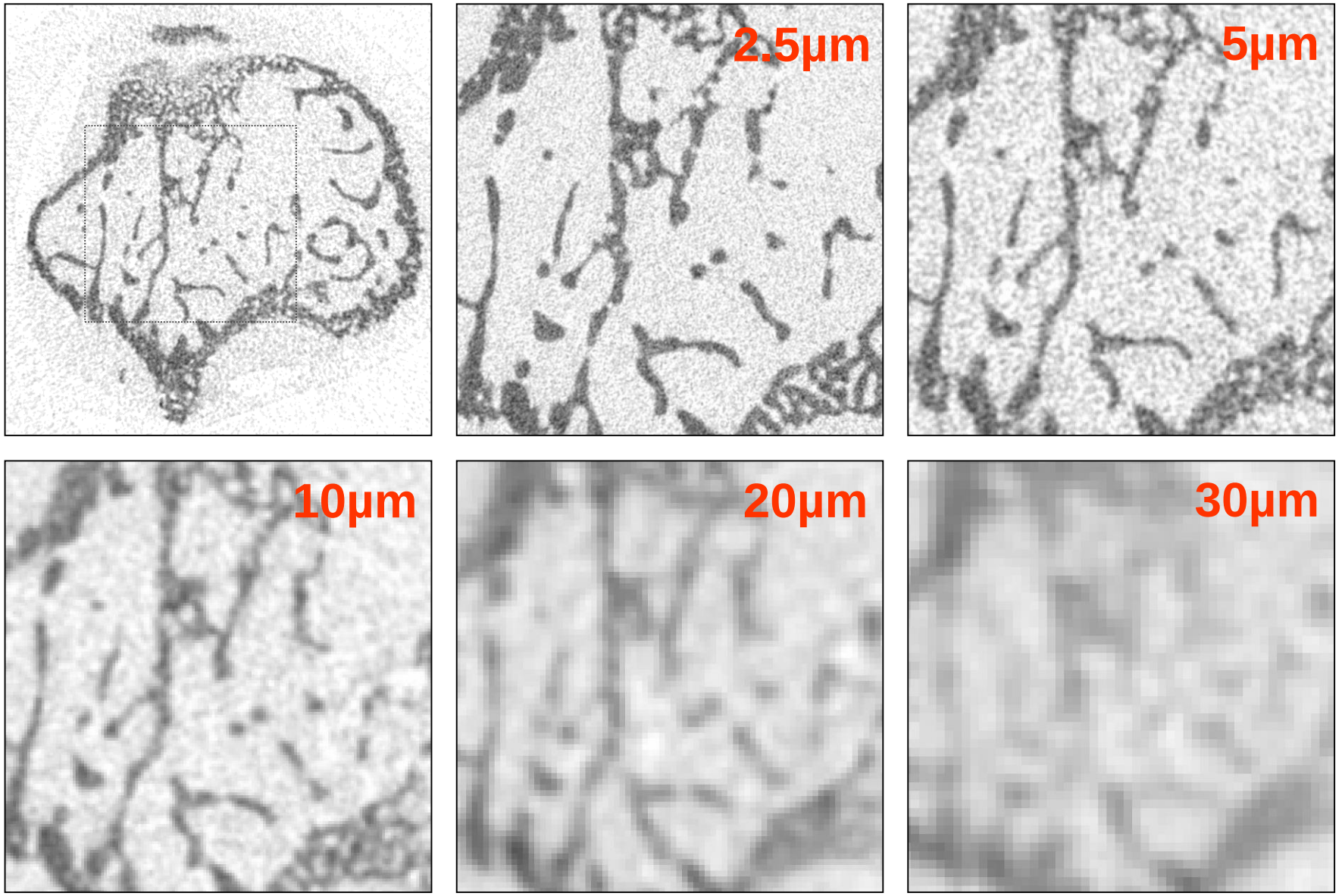
- Between 0.5-34 µm
- 5µm fine for mouse trabecular bone
- 10-20 µm fine for rat and human samples

2. X-Ray voltage and current

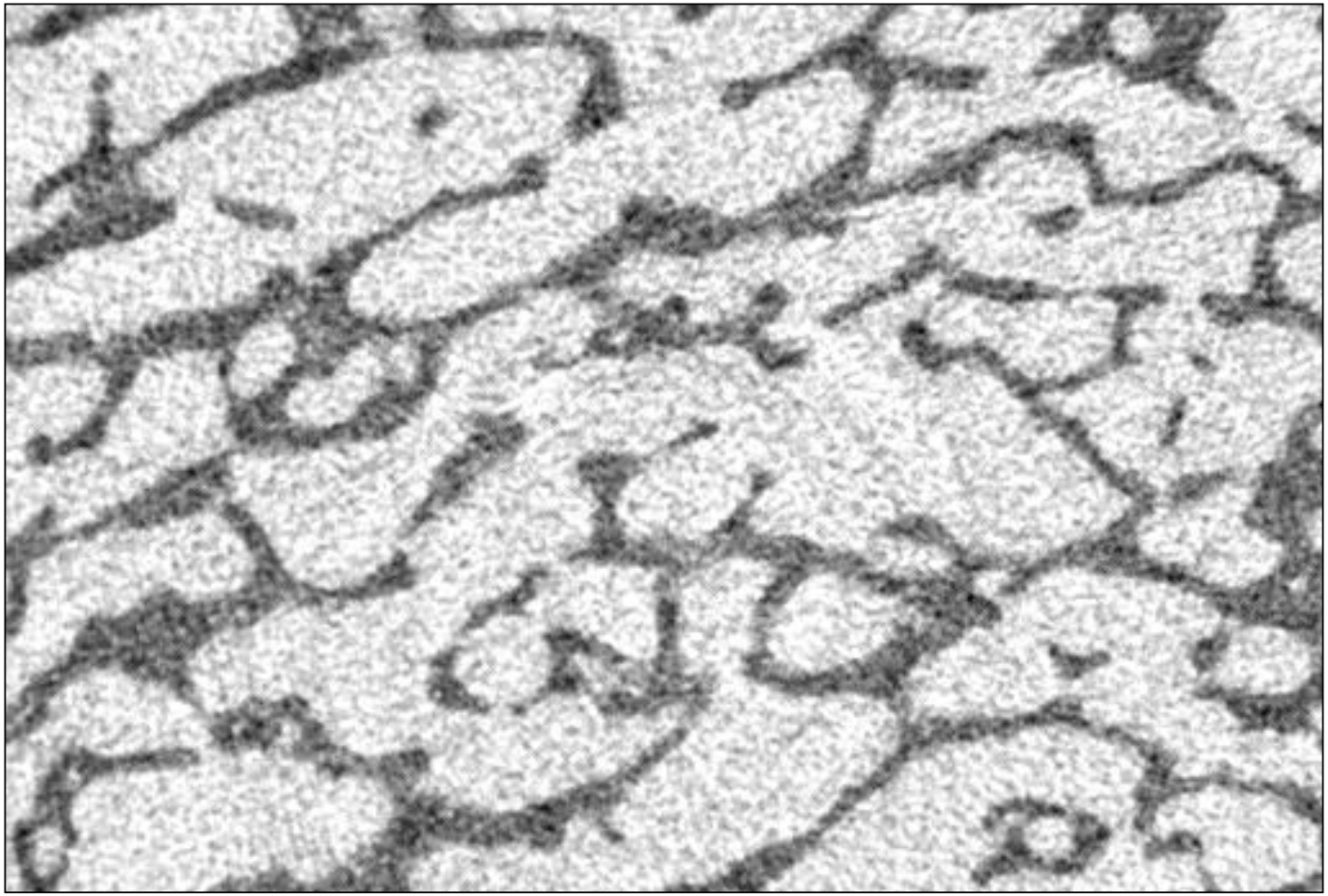
- Instrument dependent
- For mouse bones 50kV and 200mA

3. Rotation angle stepsize

Effect of Resolution



Human Bone biopsy at 20 µm



Why not always scan at best quality ?

1. Scantime

- **7 min or >1h per scan**

2. Filesize

- **2GB or >30 GB per scan**

3. RAM is limited

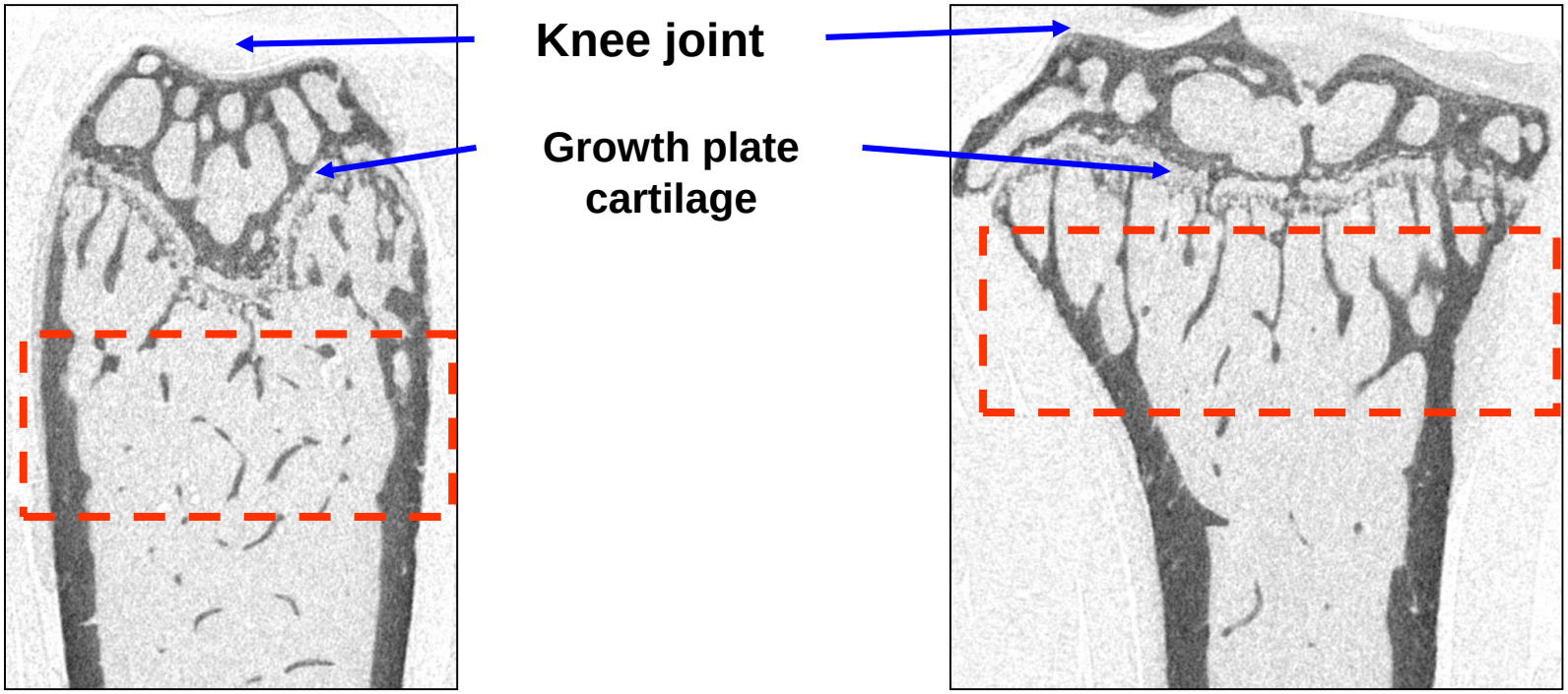
- **Whole dataset needs to be in RAM for 3D analysis**
- **5µm dataset takes up 2GB during analysis**
- **64-bit windows with 16GB or more RAM helps**

4. Analysis speed

- **5 min or overnight per sample**

Standard Bone Volume Analysis

1. **Select Top and bottom slice**
   - **200 slices for 1mm bone length**

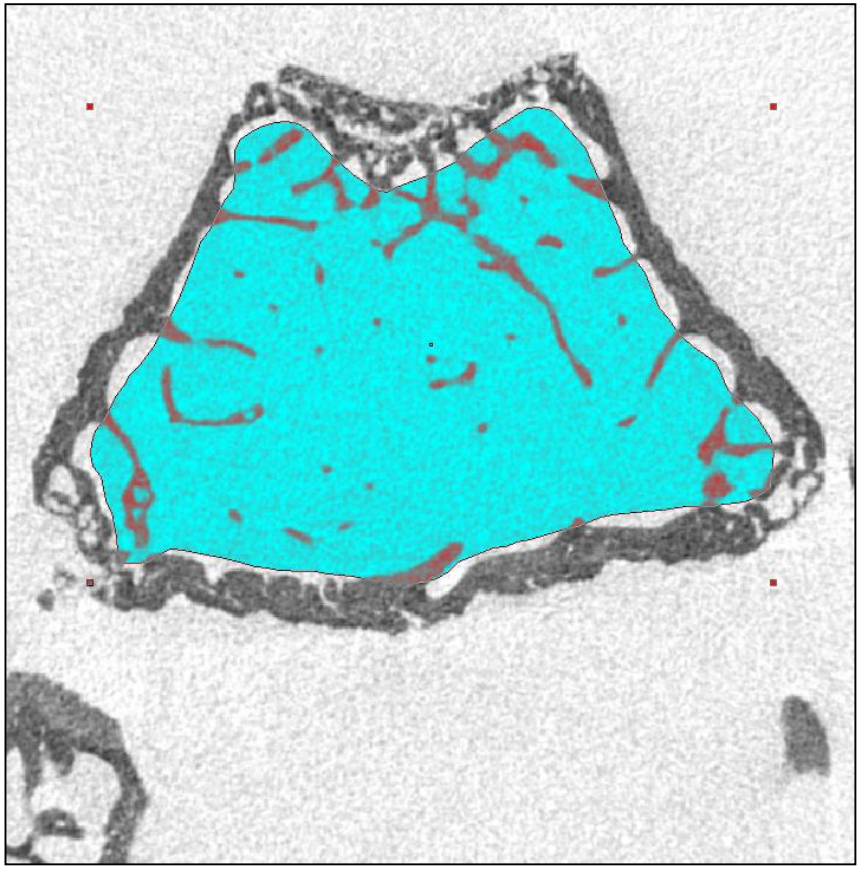


Standard Bone Volume Analysis

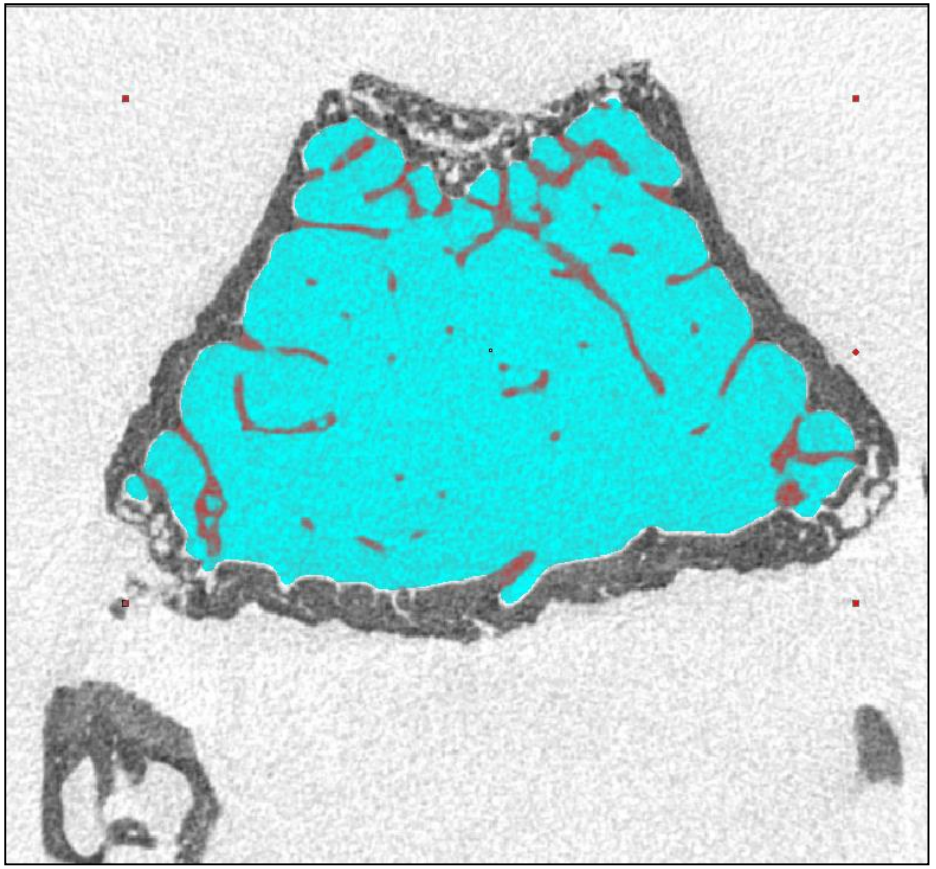
1. Select Top and bottom slice
   - 200 slices for 1mm bone length
2. Separate Trabecular and cortical bone
   - By drawing a number of key slices
3. Determine threshold for bone
   - Usually around 100
4. Run the Analysis
   - Usually done through a saved macro

Automating Separation of trabecular and Cortical Bone

Manual



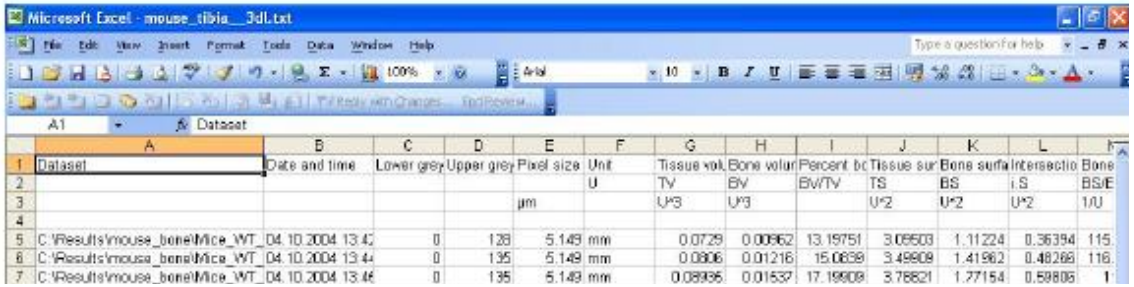
Automatic



Our analysis workflow

- **Straighten reconstructed scan in Dataviewer**
- **Save trabecular and cortical VOI as sub datasets**
- **Analyse in CTAn using macro**
  - Use batman to analyse multiple datasets
  - Steps in trabecular analysis macro:
    - Reduce noise using median filter
    - Perform threshold
    - Series of operations to identify trabecular bone
    - 3D analysis of bone parameters
- **Scan+Analysis capacity: 30-40 mouse knees/day**

Important Output Data



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Dataset	Date and time	Lower grey	Upper grey	Pixel size	Unit	Tissue vol	Bone volur	Percent bc	Tissue sur	Bone surfa	Intersectio	Bone
2						U	TV	BV	BV/TV	TS	BS	i.S	BS/E
3					µm		U^3	U^3		U^2	U^2	U^2	1/U
4													
5	C:\Results\mouse_bone\Mice_WT_	04.10.2004 13:42	0	128	5.149	mm	0.0729	0.00962	13.19751	3.09503	1.11224	0.36394	115.
6	C:\Results\mouse_bone\Mice_WT_	04.10.2004 13:44	0	135	5.149	mm	0.0806	0.01216	15.0839	3.49809	1.41962	0.48268	116.
7	C:\Results\mouse_bone\Mice_WT_	04.10.2004 13:46	0	135	5.149	mm	0.08936	0.01537	17.19909	3.78821	1.77154	0.59806	1

BV/TV: % Bone Volume

Tb.Th: Trabecular Thickness

Tb.Sp: Trabecular Separation

Tb.N: Trabecular Number

Tb.Pf: Trabecular Pattern Factor

- Indicator of trabecular connectivity
- Connectivity Density may be better alternative

SMI: Structure Model Index

- Indicator of plate<-> Rod-like trabecula

Effect of scan resolution on commonly used µCT measurements

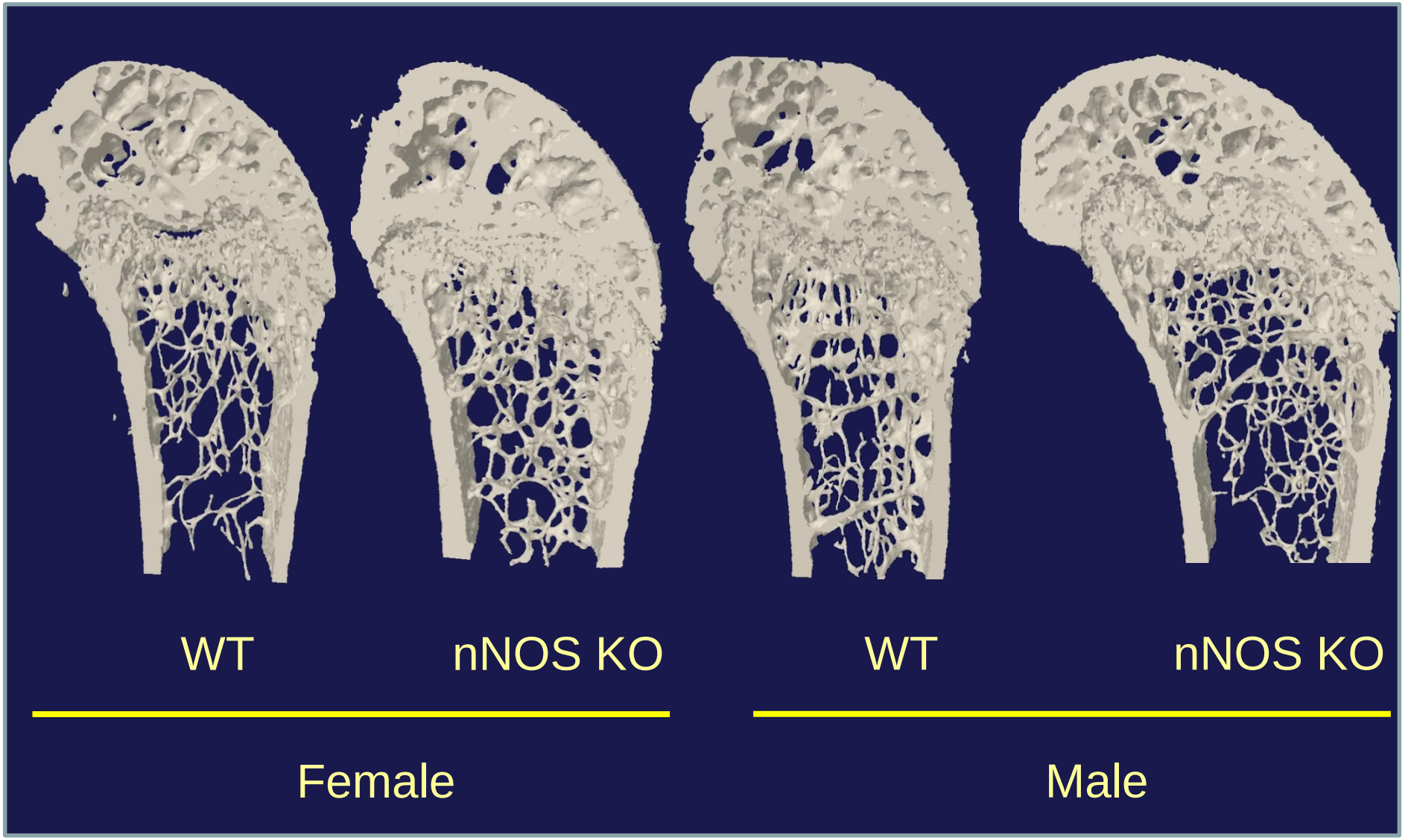
Specimen	Resolution	BV/TV	Tb.Th	Tb.Sp	Tb.N	SMI	Conn.Dn
	µm	%	µm	µm	mm^{-1}		mm^{-3}
Mouse Tibia	2.5	9.05	45.40	279.89	2.15	1.91	459.69
Mouse Tibia	5	9.17	47.94	288.79	1.91	2.10	269.88
Mouse Tibia	10	9.75	62.27	362.93	1.57	2.27	93.26
Mouse Tibia	20	10.64	90.66	501.52	1.17	2.51	61.31
Mouse Tibia	30	7.55	111.41	708.19	0.68	2.67	37.41
Human biopsy	10	15.89	159.80	736.41	0.99	1.03	5.88
Human biopsy	20	15.43	174.44	774.31	0.88	1.14	4.66
Human biopsy	30	18.36	210.45	767.90	0.87	1.24	3.92

An Example:

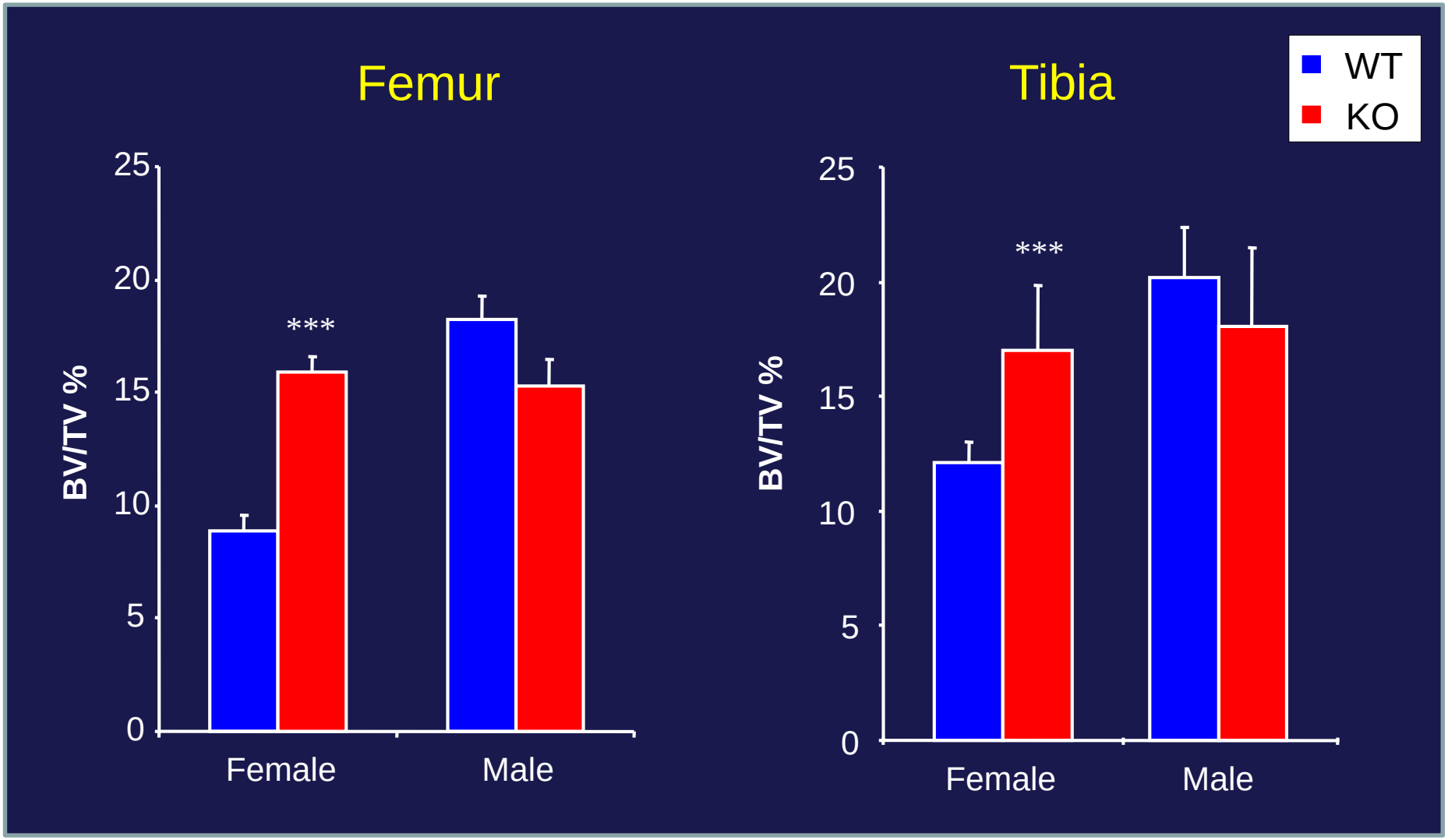
Bone metabolism in nNOS-KO mice

Rob van ‘t Hof, Anna Daroszewska, Lorraine Rose and Stuart Ralston
Paper in preparation

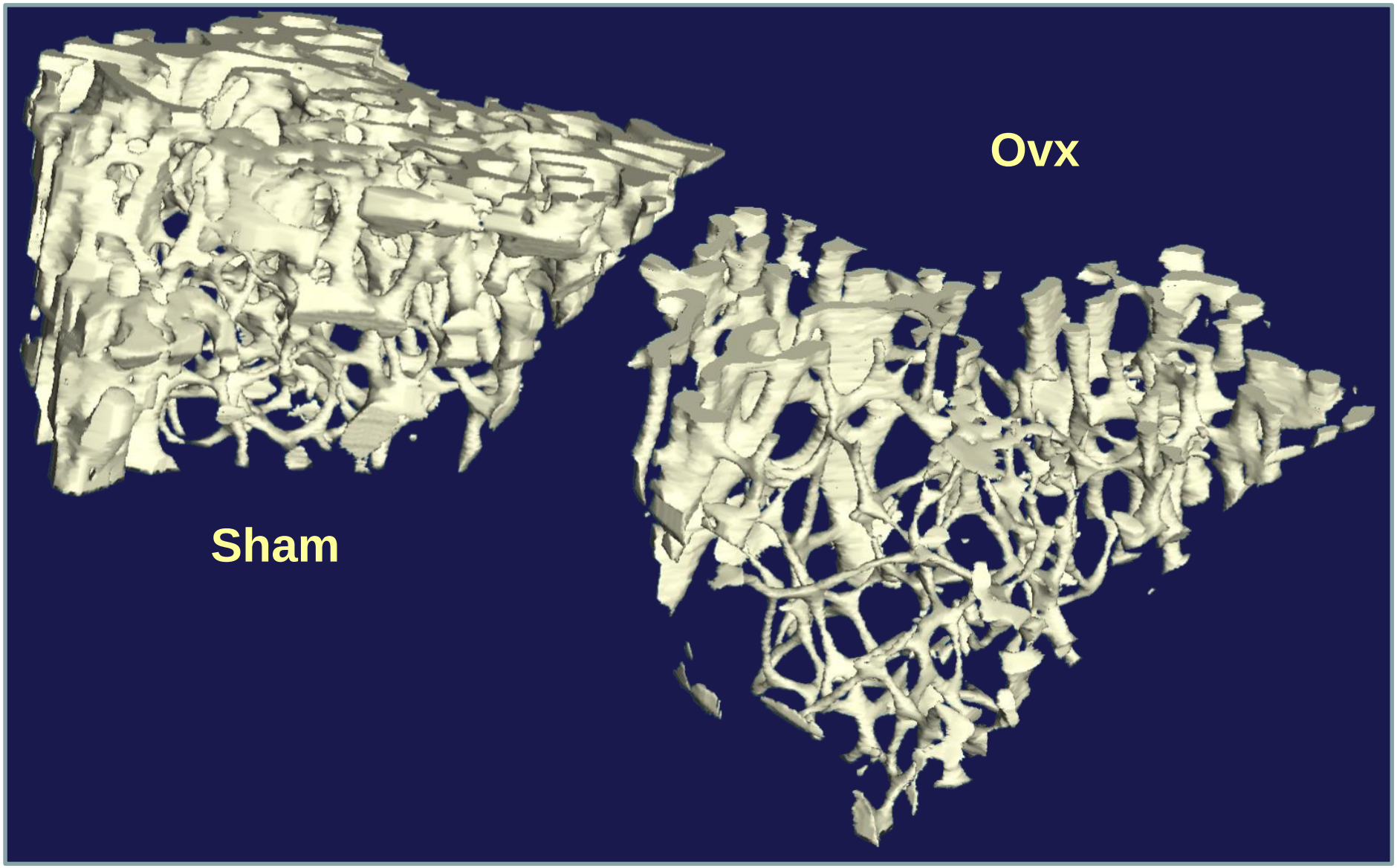
Bone volume and Gender



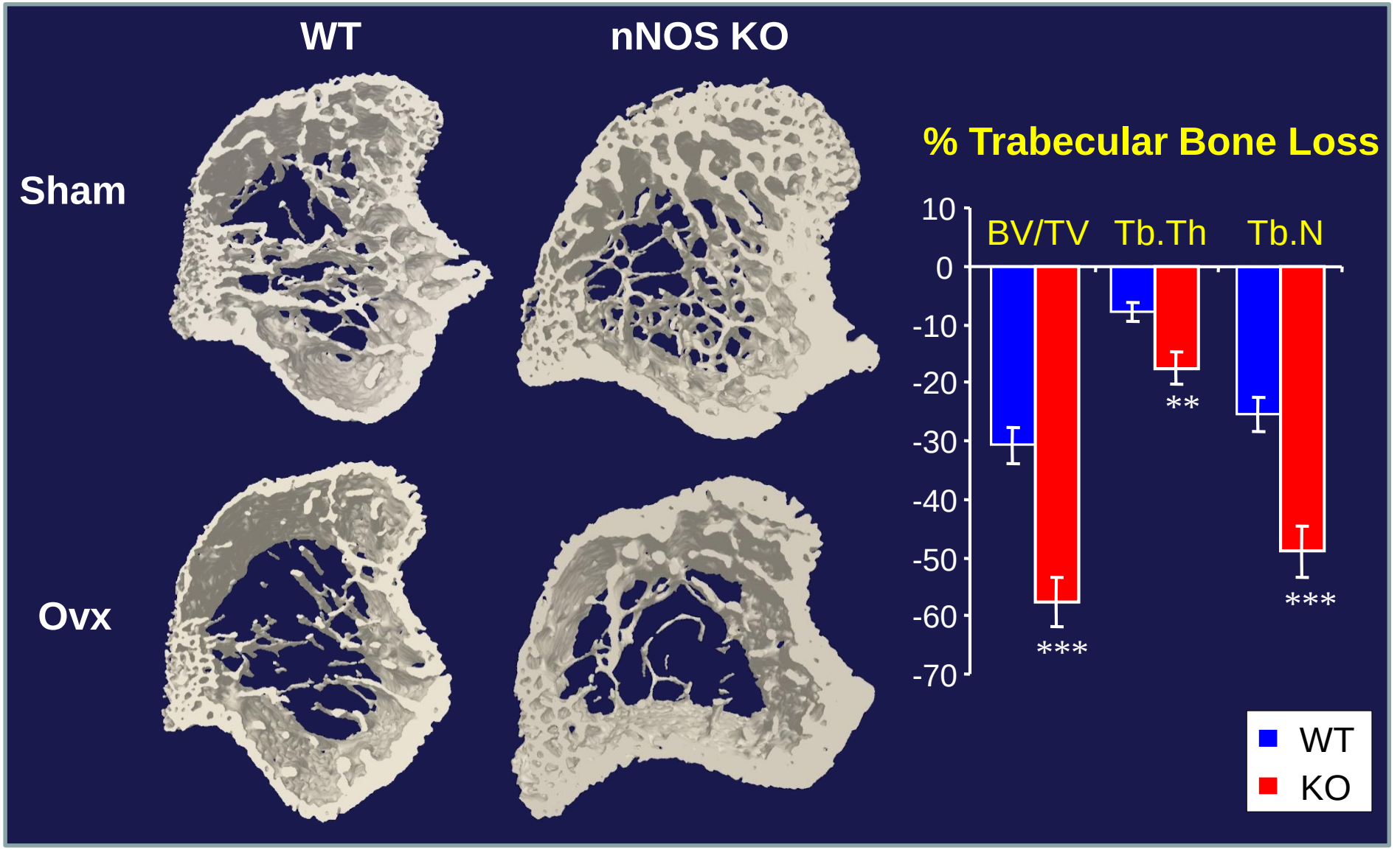
BV/TV



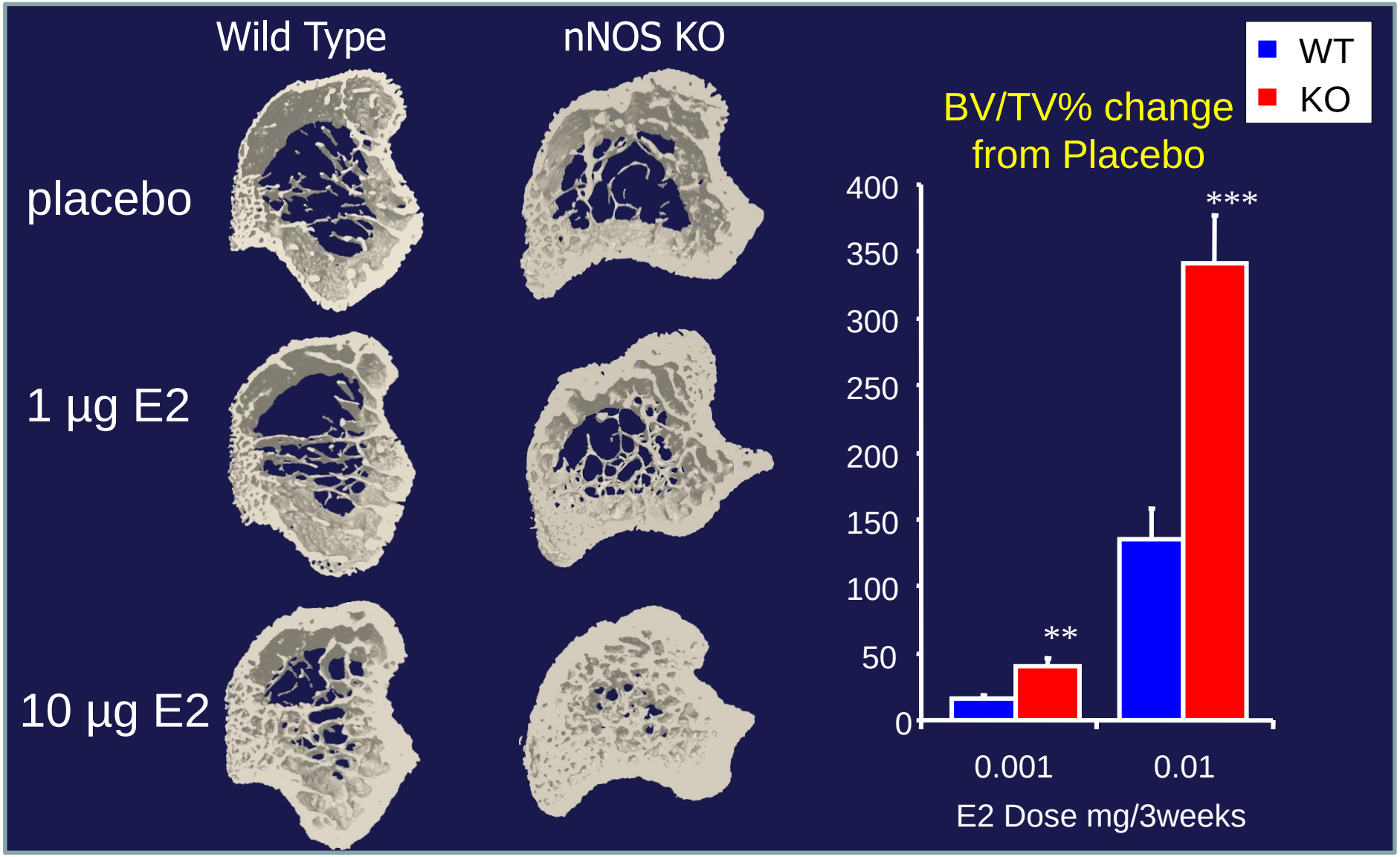
Do nNOS-KO mice loose bone after Ovx?



Ovx-induced Bone Loss is accelerated in nNOS KO mice



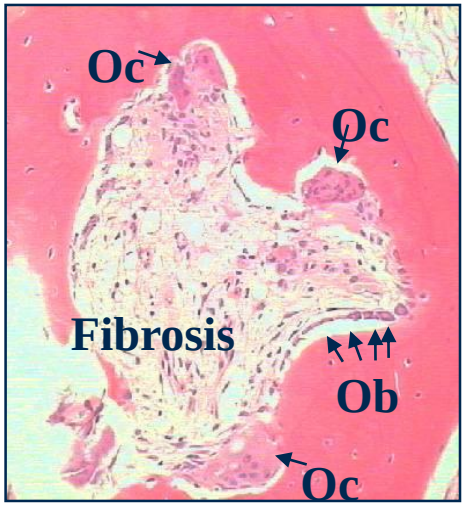
nNOS KO mice are hyper-responsive to estrogen



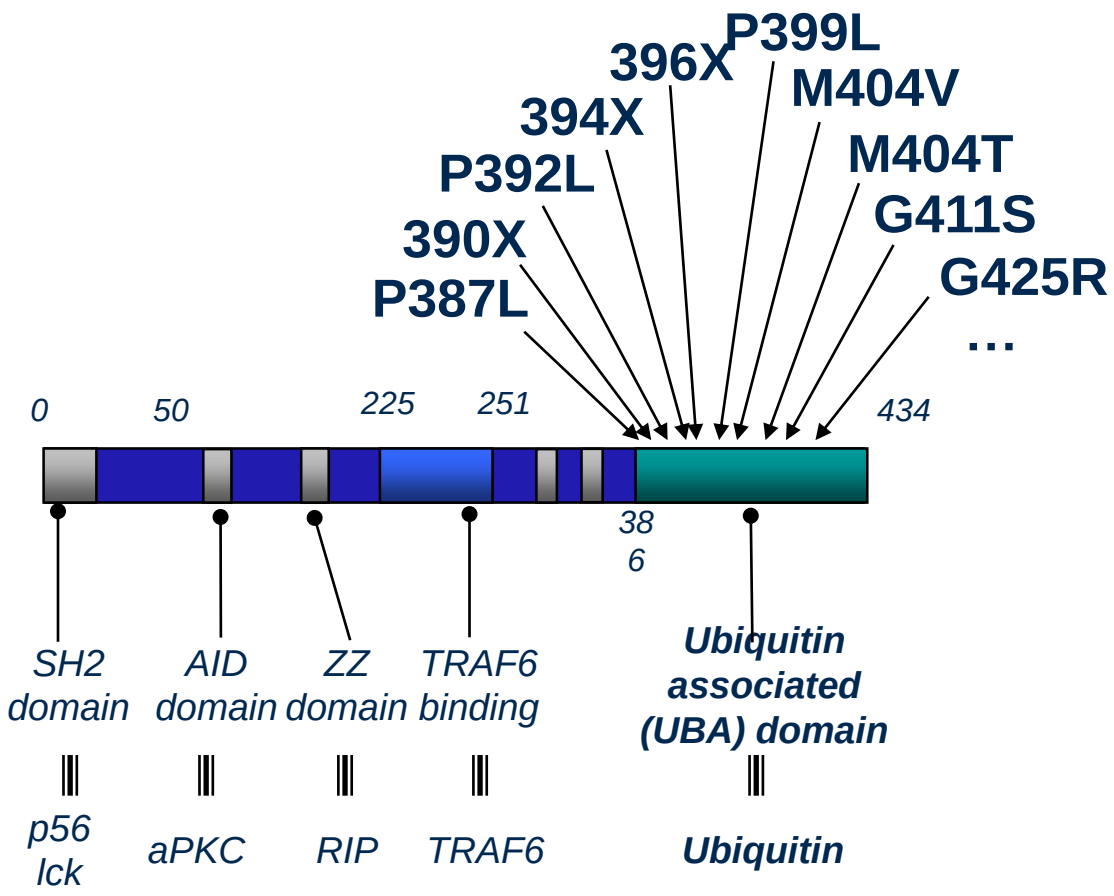
SQSTM1 and Paget's Disease of Bone

Paget's disease of bone

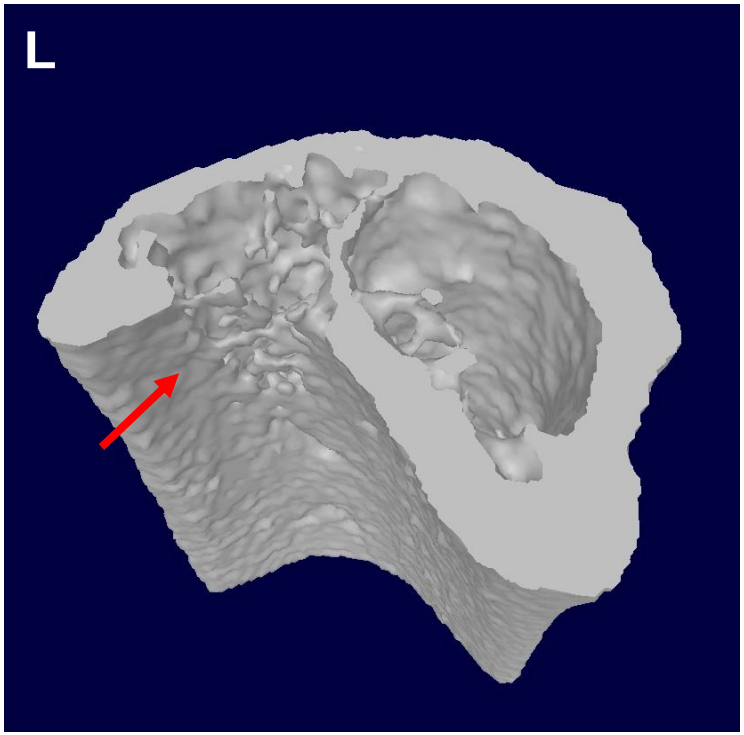
- Common bone disease
  - 3.1% of UK population over 55
- Bone pain, deformity, fractures, osteoarthritis
- Focal areas of increased bone turnover

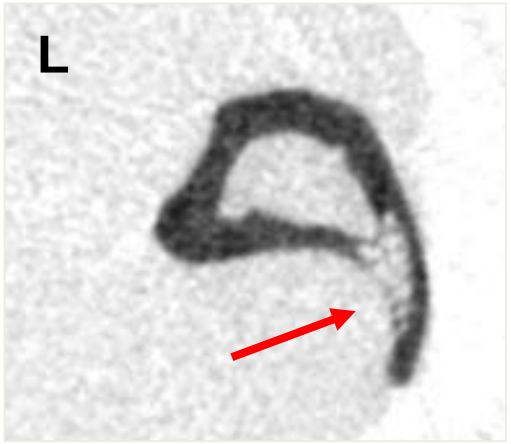


Mutations in Sequestosome1 / p62 UBA domain are associated with PDB



p62^{P394L} mice develop Pagetic-like lesions





12 months old homozygote

- **severe lesion in the left tibia (µCT)**

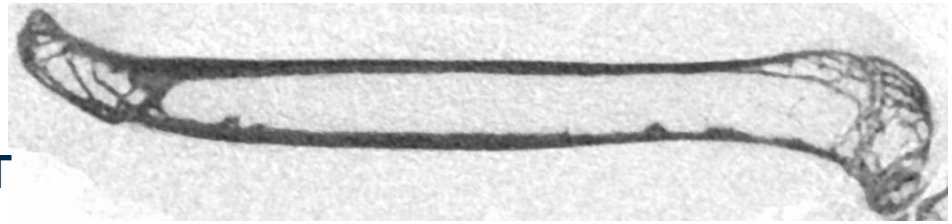
Daroszewska et al 2011 Hum Mol Gen

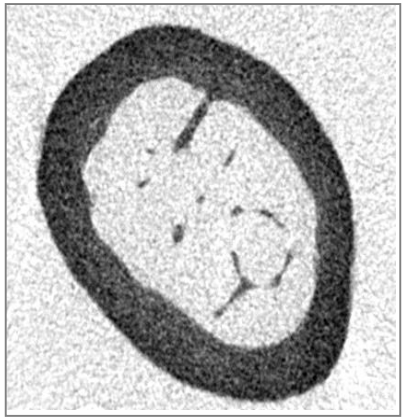
P394L p62 knock-in mice develop Pagetic-like lesions

Femur

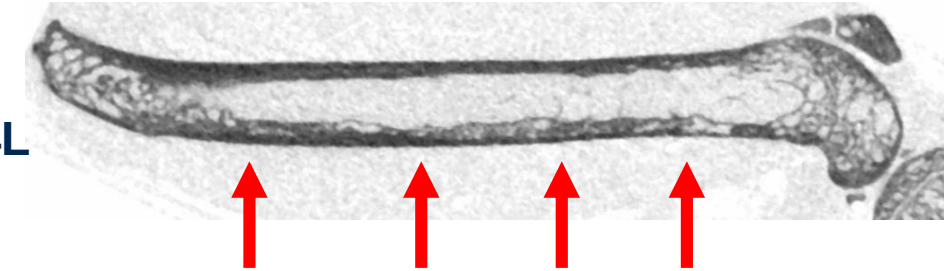
Midshaft Femur

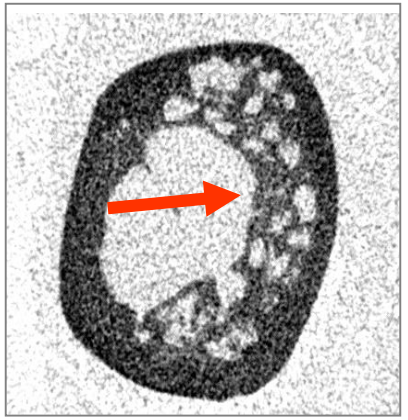
WT





P394L

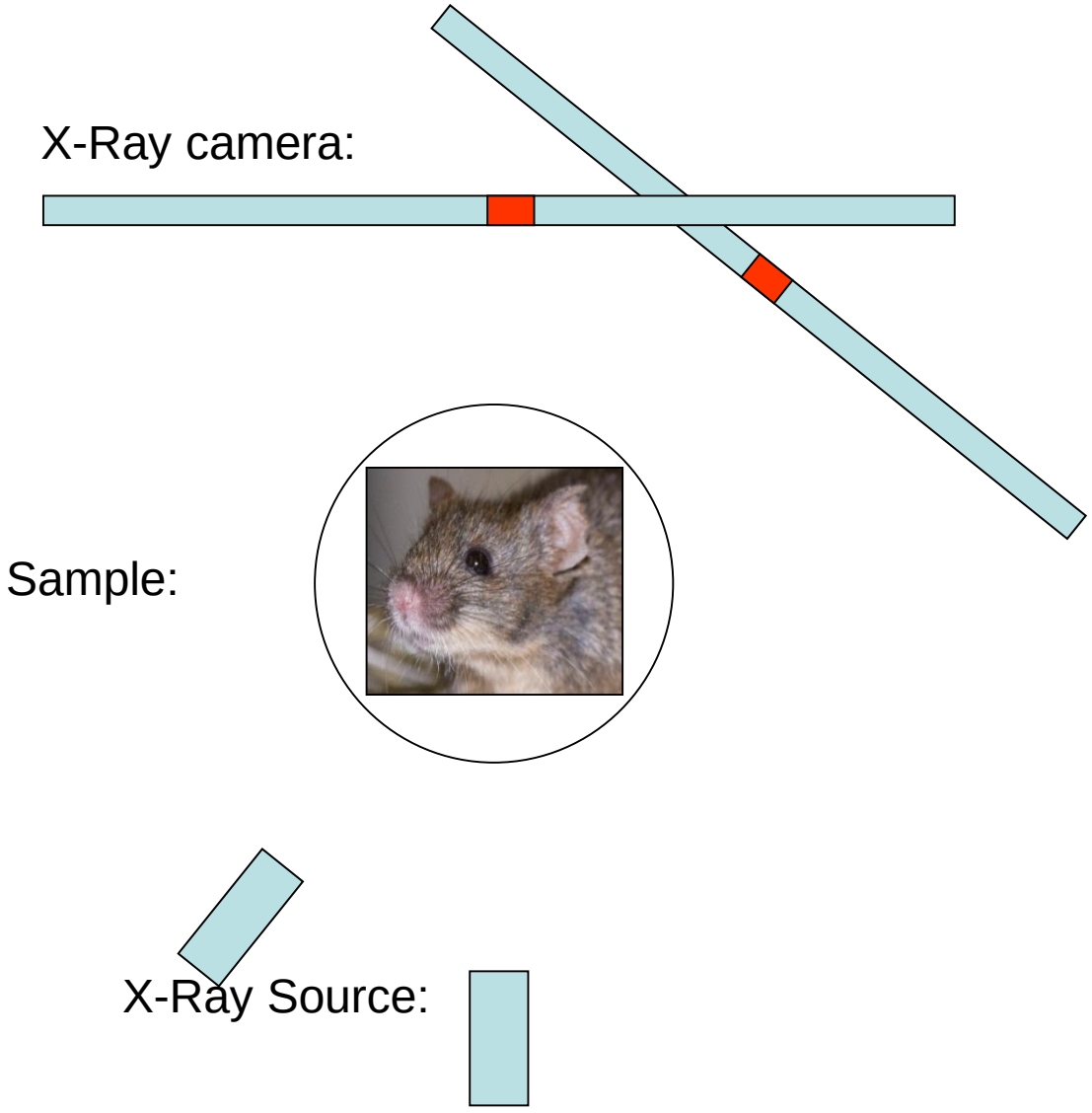




12 month old homozygote

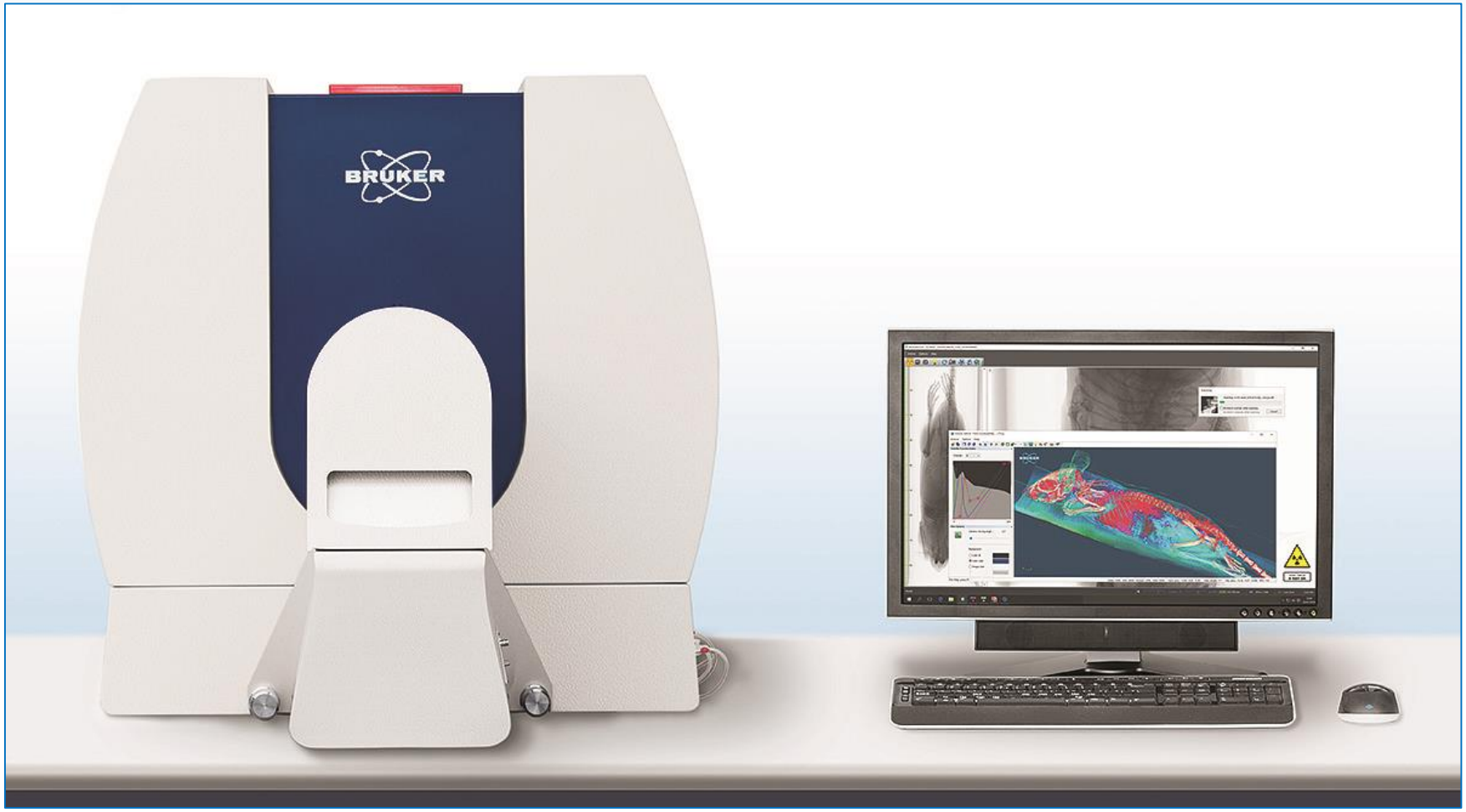
Daroszewska et al. Hum Mol Gen 2011

In Vivo Systems use a gantry to rotate Source and camera



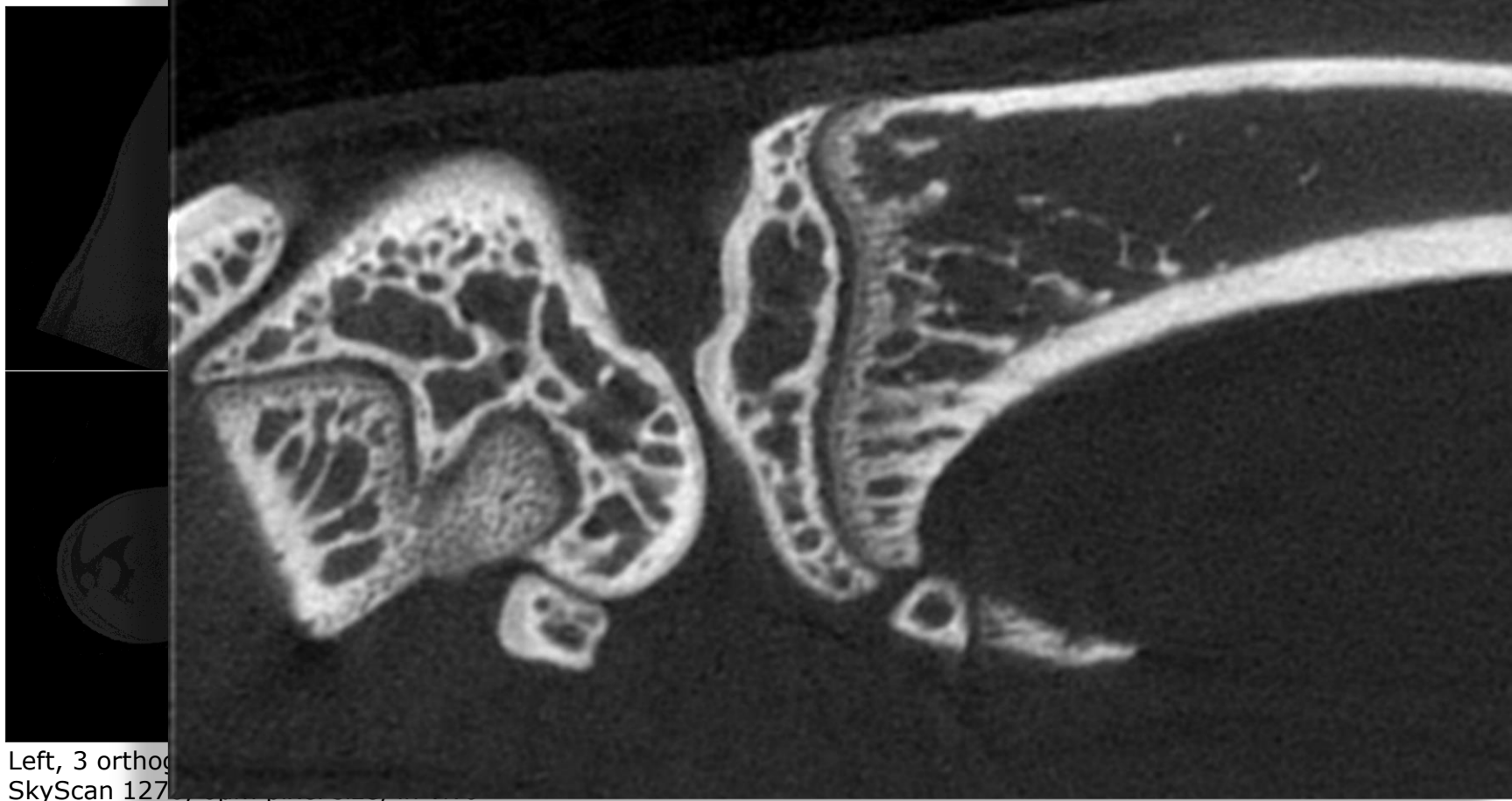
In Vivo µCT System: Skyscan 1076

NEW SkyScan 1276:
The ultimate *in-vivo* X-ray microtomograph



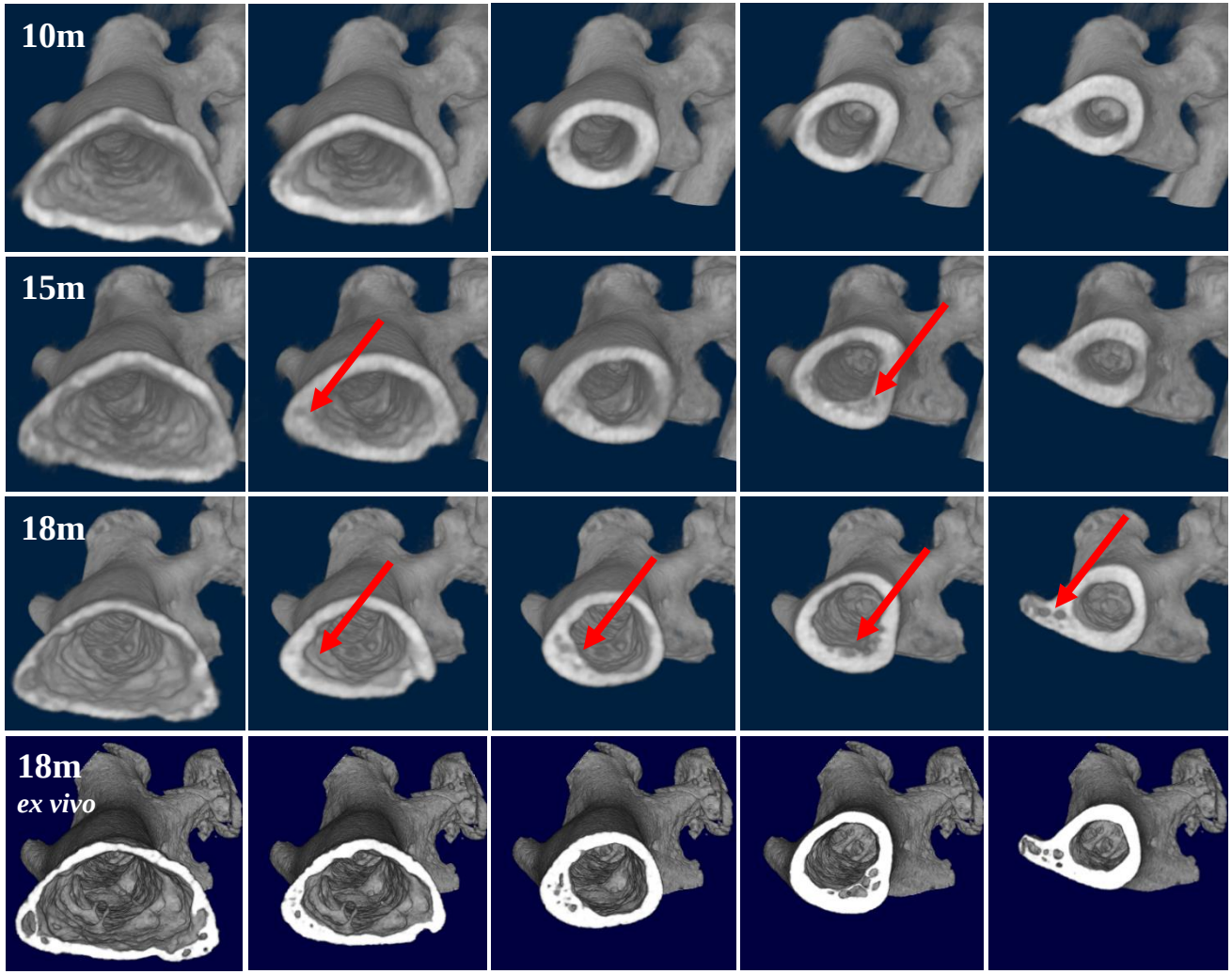
Bone phenotyping *in vivo*

- A 2.5cm long field of view around the knee joint is scanned *in vivo* at 6µm true pixel size in 8 m



Left, 3 orthog
SkyScan 127

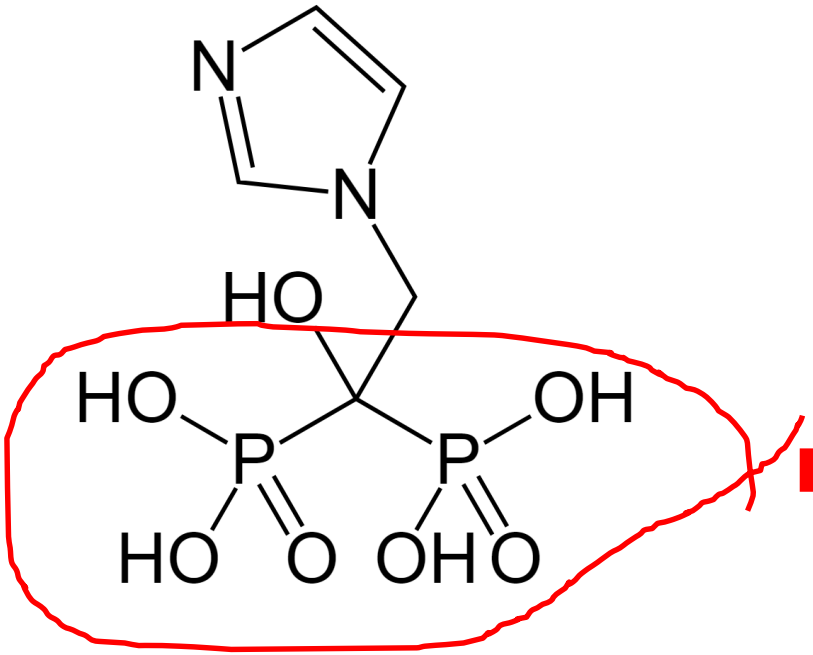
Following P394L p62 lesions over time



Daroszewska et al, DMM 2018

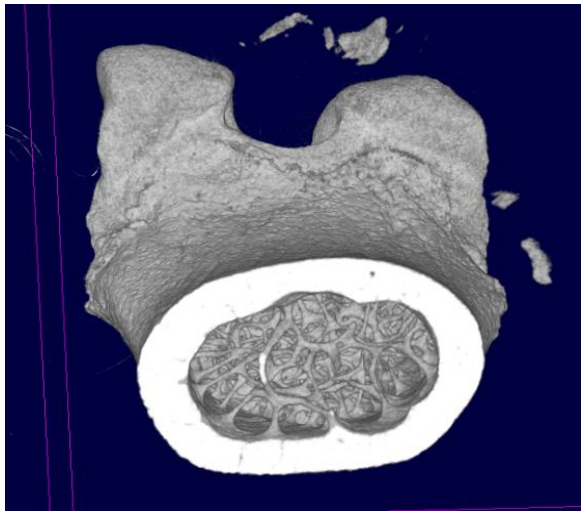
Treatment for Paget's disease

- Main cause believed to be hyperactive osteoclasts
- So: use osteoclast inhibitors!
- Currently most potent osteoclast inhibitor: **Zoledronate**

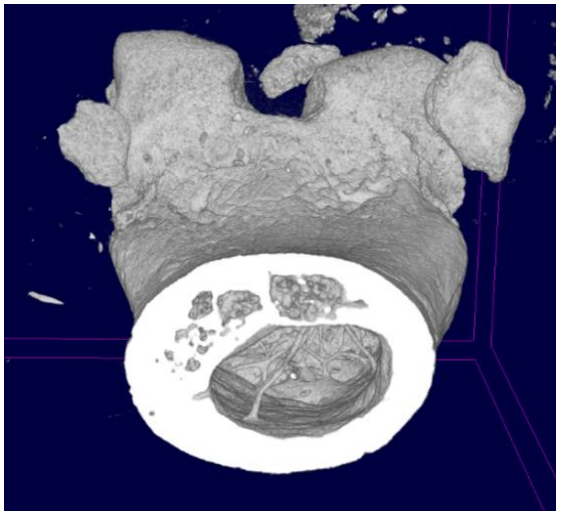


- Bisphosphonate base structure
- High affinity for bone

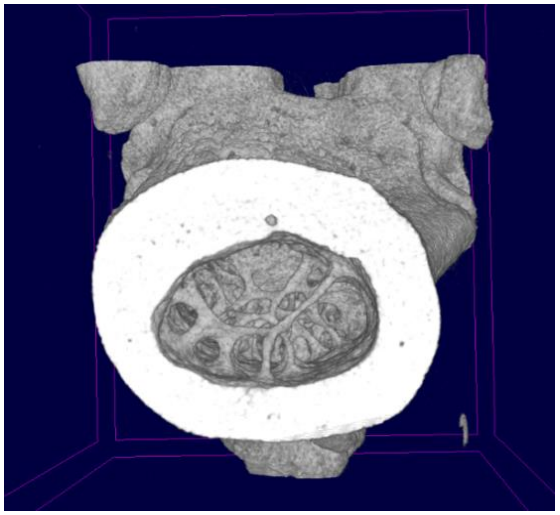
Zoledronate prevents Pagetic lesions in the p62$^{P394L+/+}$ mouse



Wild Type



P394L Vehicle



P394L Zoledronate

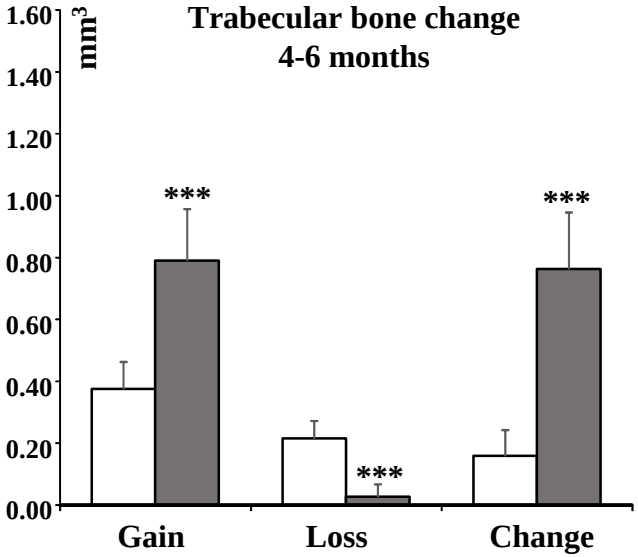
Daroszewska et al, DMM 2018

Measuring bone resorption/formation using in vivo µCT

- Mouse knee scanned in vivo @18µm
- Scanned at 4 months and 6 months
- Scans registered in Dataviewer
- Changes analysed in CTAn

Red: No change
White: Bone lost
Blue: Bone gained

Effect of Zoledronate on bone resorption/formation using in vivo µCT



Daroszewska et al, DMM 2018

Our standard scan:
4.5 µm, 0.5mm Al, 0.3°, no binning

Scantime: 28 min

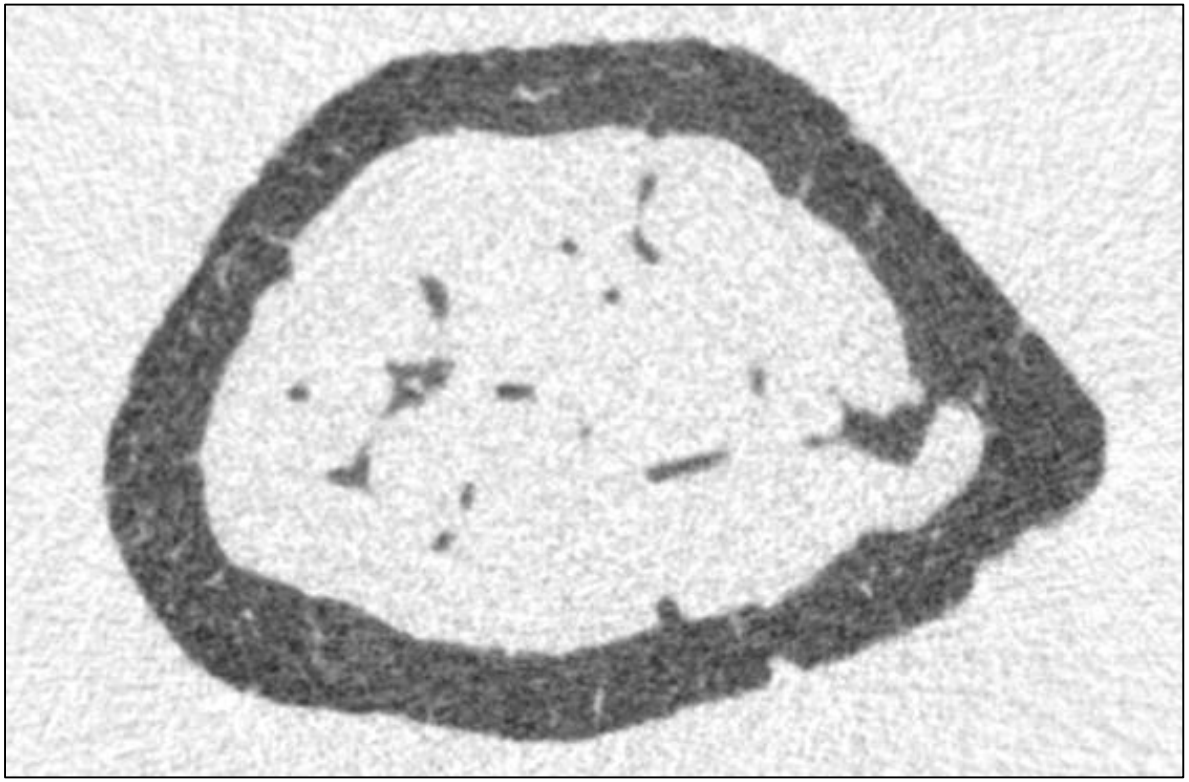
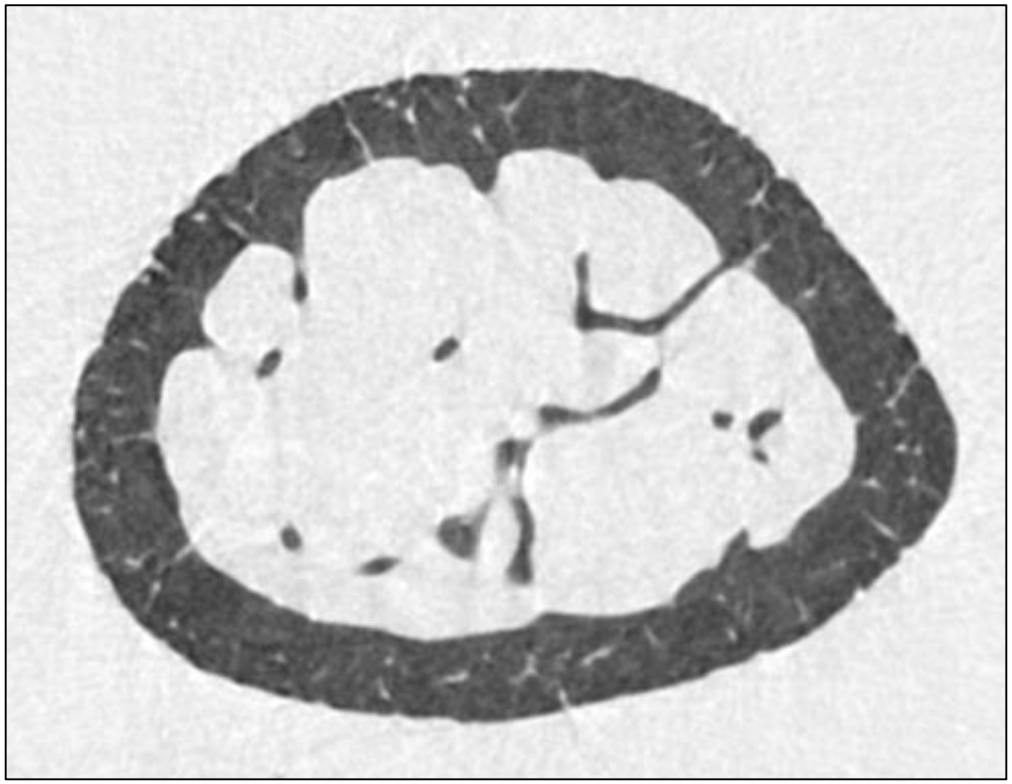


Image Noise!

Improving Signal to Noise: 4.5 µm, 0.5mm Al, 0.3°, 2x2 binning

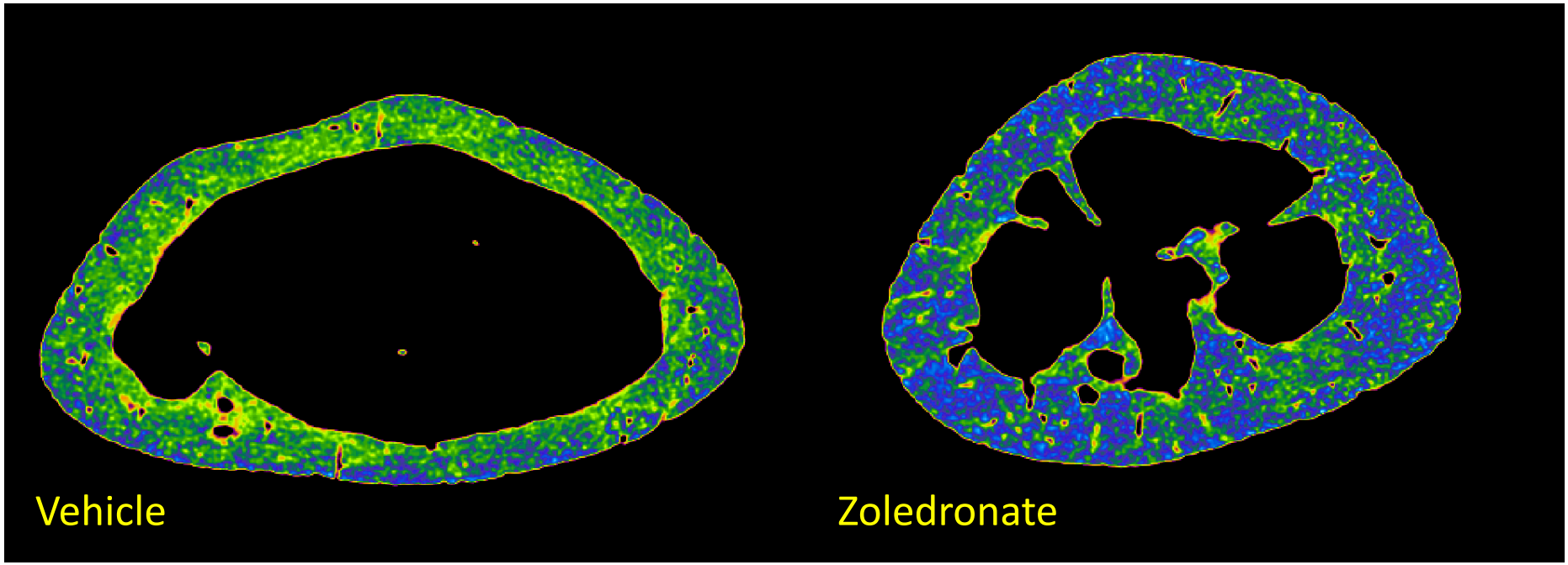
Scan time: 1h 20 min

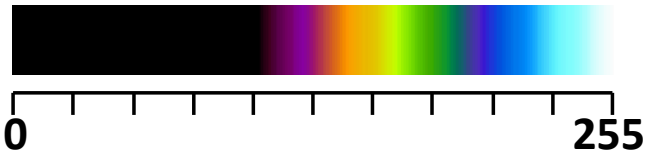


4x Averaging

Downside: Scan time and Reduced field of view.

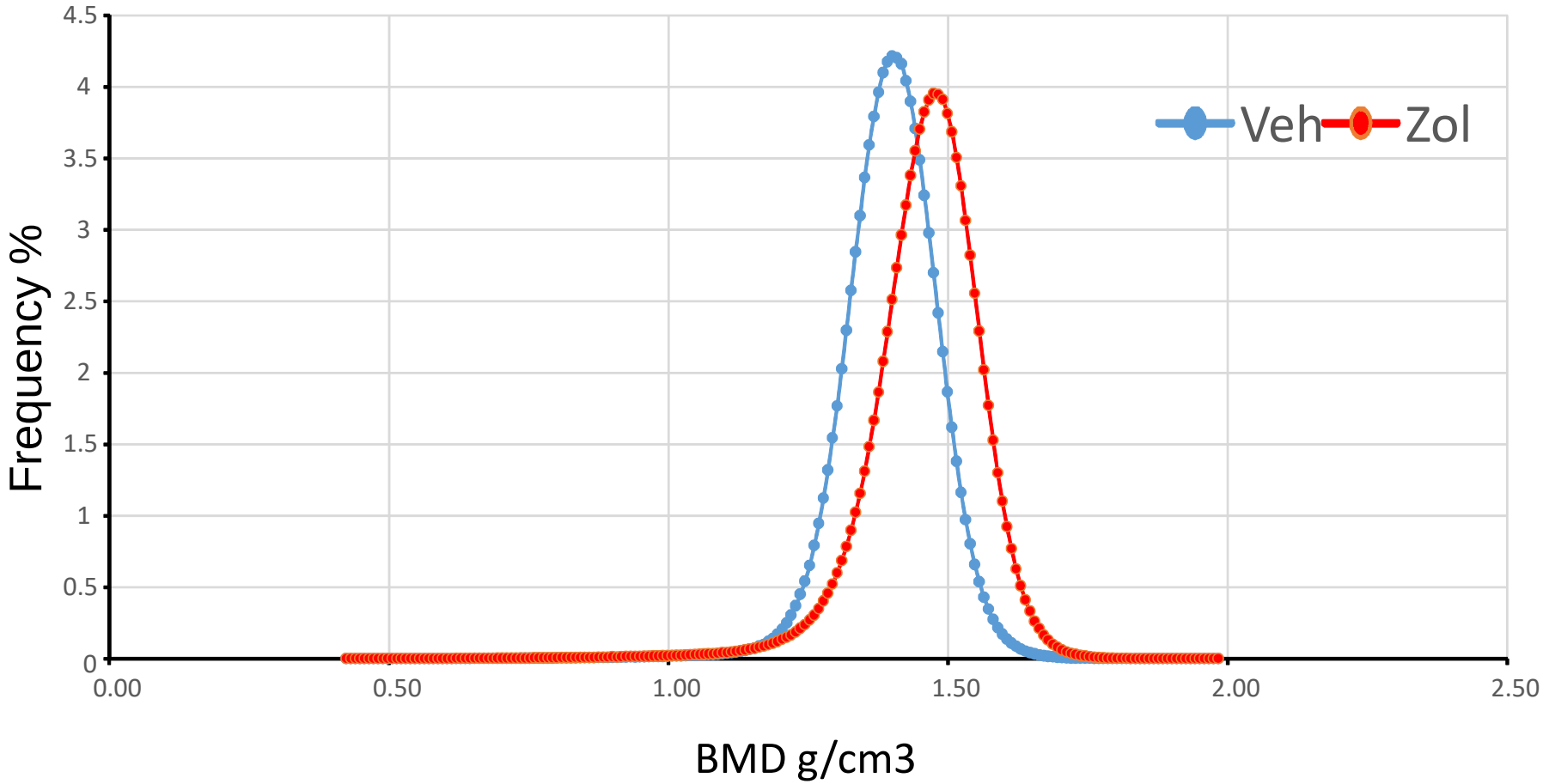
Long term treatment with Zoledronate leads to hypermineralised bone





Daroszewska et al, DMM 2018

Long term treatment with Zoledronate leads to hypermineralised bone



Daroszewska et al, DMM 2018

Acknowledgements

- Anna Daroszewska
- Gemma Charlesworth
- Mandie Prior
- Lorraine Rose