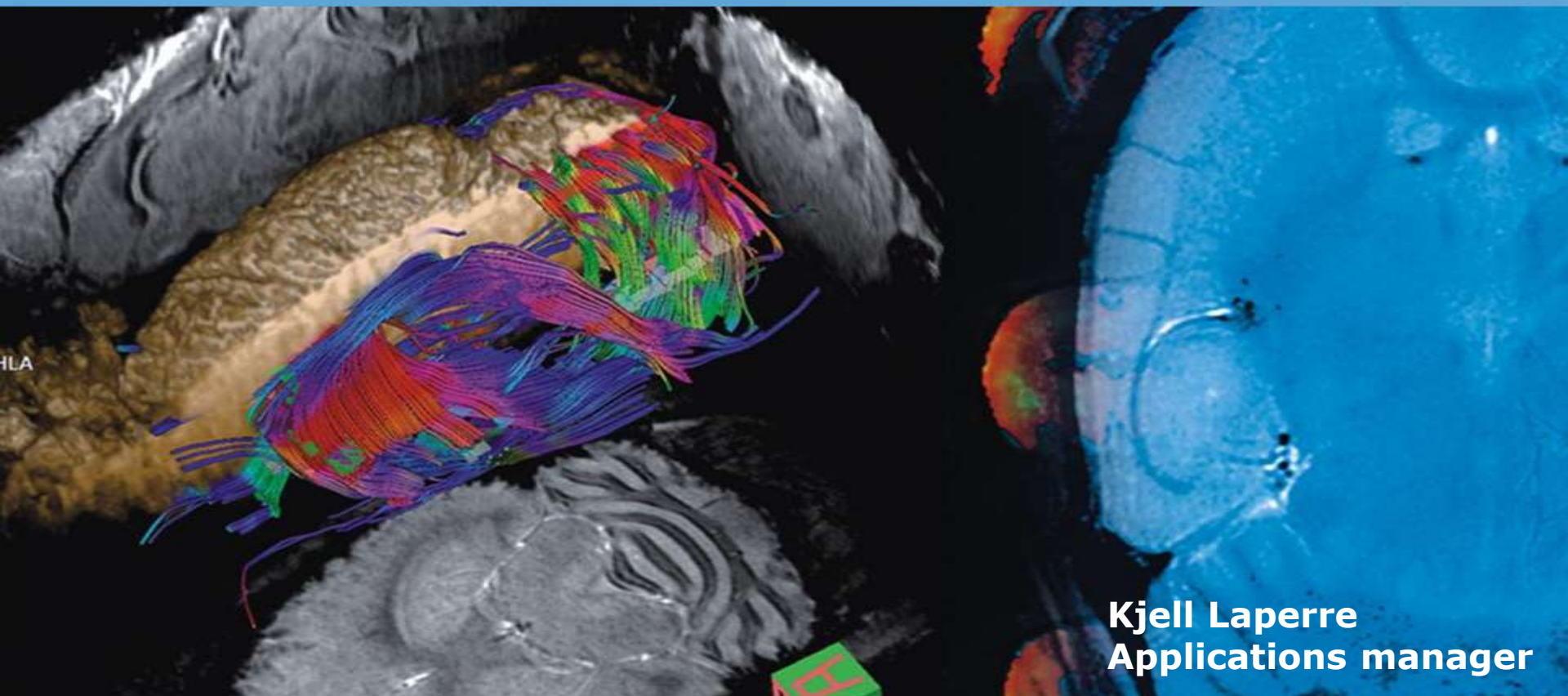


# Bone imaging and analysis with microCT

## Exploring the possibilities



**Kjell Laperre**  
**Applications manager**

# Overview



Background

Technology

Applications

Systems & Software

- Background
- Technology
- Applications
- Systems & software

# Background



Background

Technology

Applications

Systems & Software

- Why analyze bones?
  - Developmental studies
  - Genetic screens (phenotyping)
  - Disease progression
    - Osteoporosis
    - (Osteo)arthritis
    - Fractures
    - ...
  - Drug evaluation/treatments
  - ...



<http://www.livescience.com/>

# From histology to microCT

Background

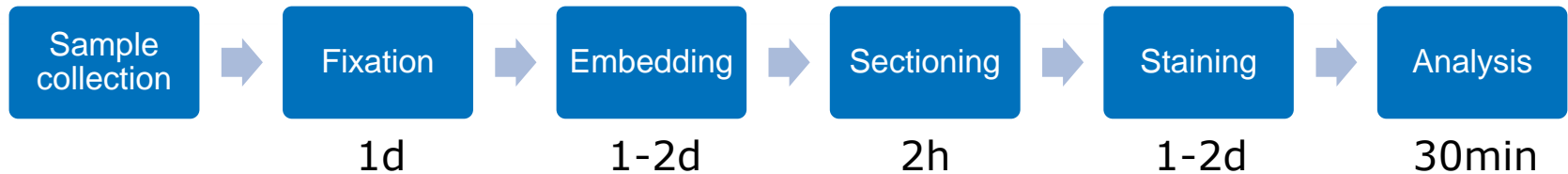
Technology

Applications

Systems & Software

- **Histology**

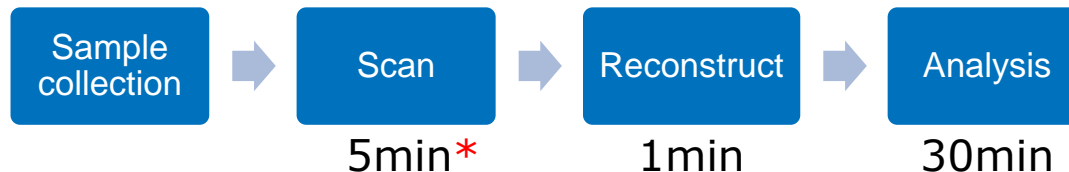
*Counted per sample*



Advantages: resolution, multiple staining options

Disadvantages: not time-efficient, invasive (artefacts), 2D info only (3D interpolation), large number of animals

- **MicroCT**



\* SkyScan1275

Advantages: 10-15x more time-efficient (workload), true 2D & 3D info, stronger statistics & animal reduction

Disadvantages: less staining options (but histology can be done afterwards), resolution *in vivo* (radiation)

# Technology

## What is microCT?

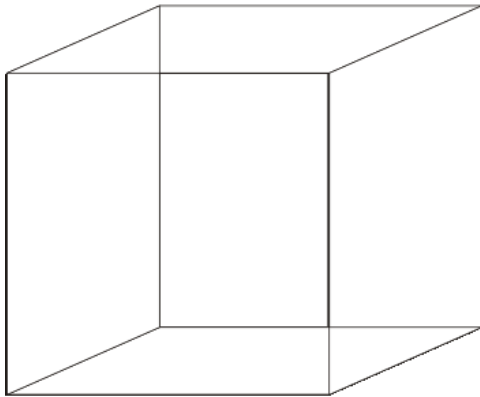


Background

Technology

Applications

Systems & Software




635  $\mu\text{m}$



*In vivo*

 9  $\mu\text{m}$

*Ex vivo*

 0.1  $\mu\text{m}$

# What is micro-CT?

Background

Technology

Applications

Systems & Software

- Micro-CT = Micro-computed tomography
- Imaging technique making use of X-rays
- When X-rays pass through different types of tissue, they are deflected and absorbed (attenuated) to different degrees depending on the atomic number  $Z$
- CT measures the attenuation of X-rays in the object at different angles
- CT results in 2D- and 3D-images and information
- No histological slicing

# MicroCT in 3 steps

Background

Technology

Applications

Systems & Software

## 1. "Scan"

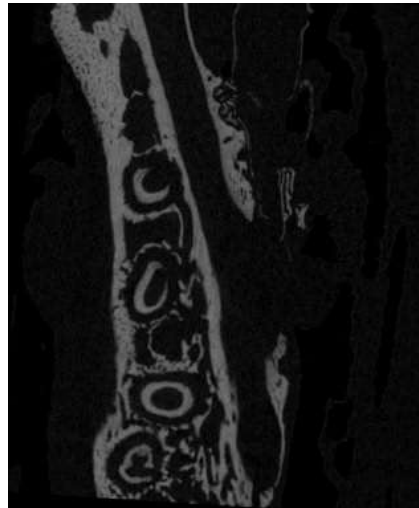
A set of 2D x-ray "projection" images are taken over a rotation of the imaging axis *in vivo* or *ex vivo* non-destructively.



Example: jaw bone

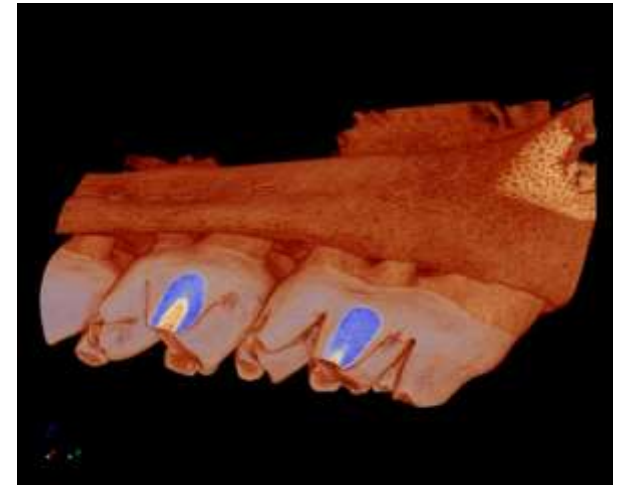
## 2. "Reconstruction"

The "projection" images are processed by a backprojection method to create a stack of cross-sectional slices.



## 3. "Analysis and visualisation"

The reconstructed crosssection slices are processed into 3d models and used for morphometric and density measurements.



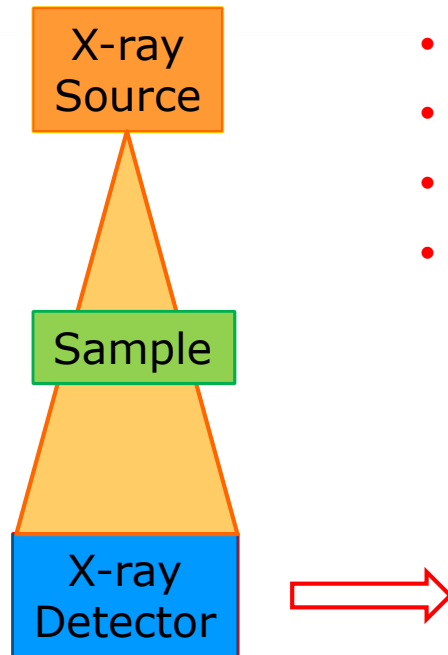
# Step 1: Scan

Background

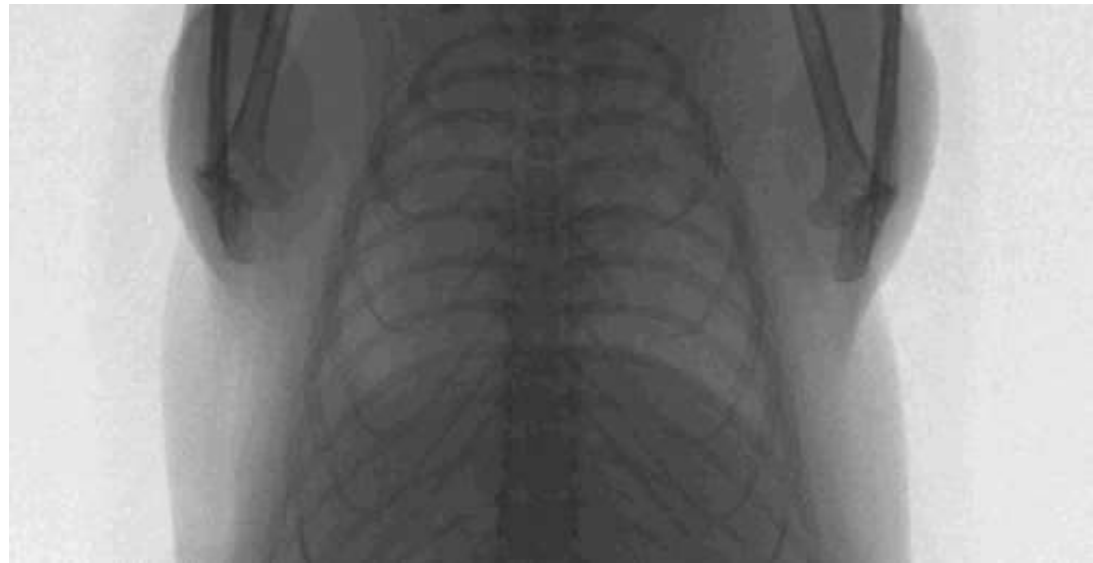
Technology

Applications

Systems & Software



- Shadow image / projection / Röntgen photo
- 2D image of a 3D object
- No thickness information!
- Acquired at multiple angles
- Non destructive: no slicing





# Step 1: Scan



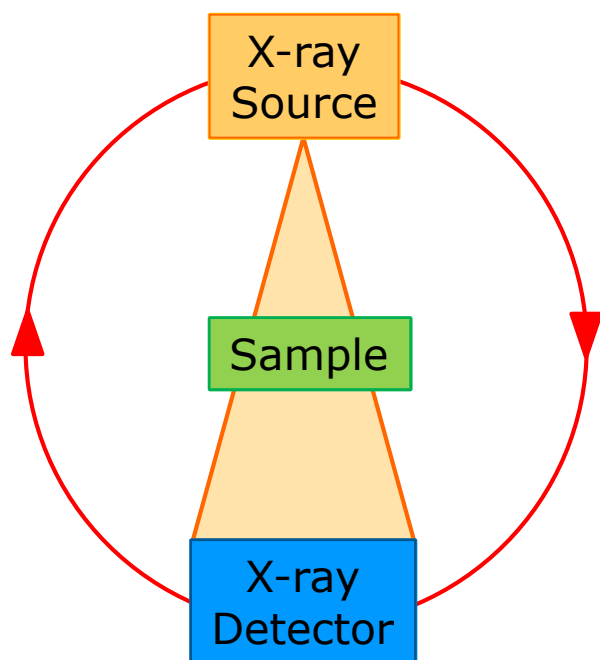
Background

Technology

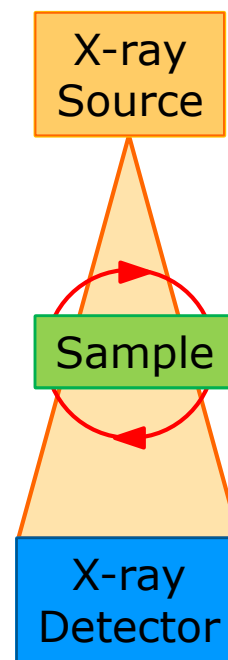
Applications

Systems & Software

## ***In vivo systems***



## ***Ex vivo systems***



## Step 2: Reconstruction

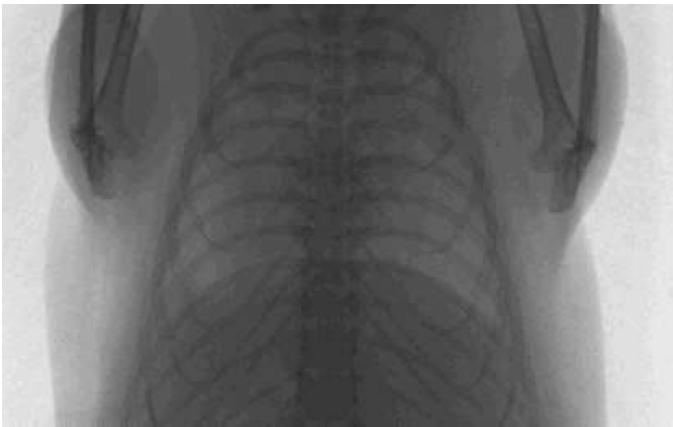
Background

Technology

Applications

Systems & Software

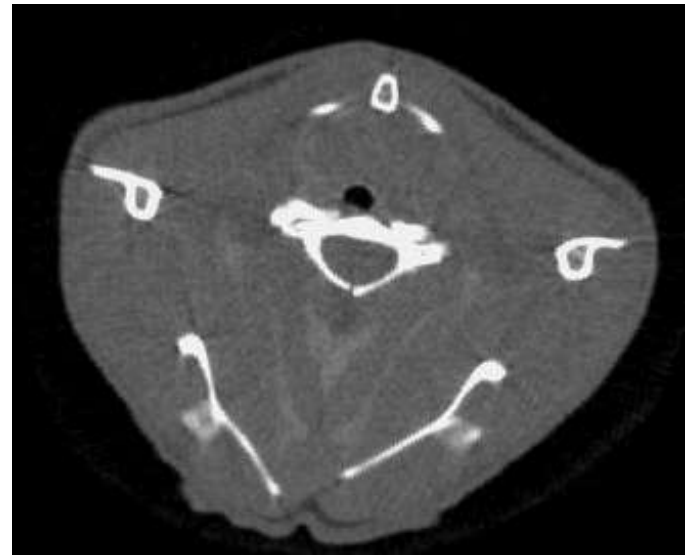
### Projection images



2D at multiple angles  
without thickness information

Reconstruction  


### Reconstructed images



Stack of images containing  
3D information

## Step 2: Reconstruction

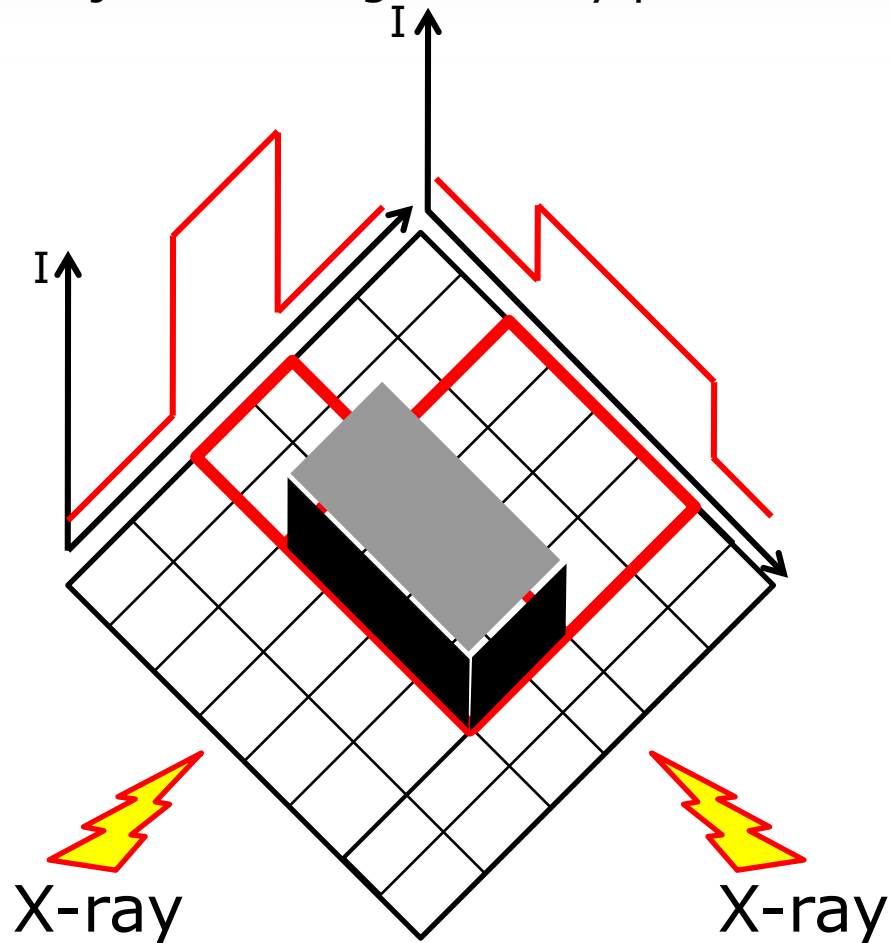
Background

Technology

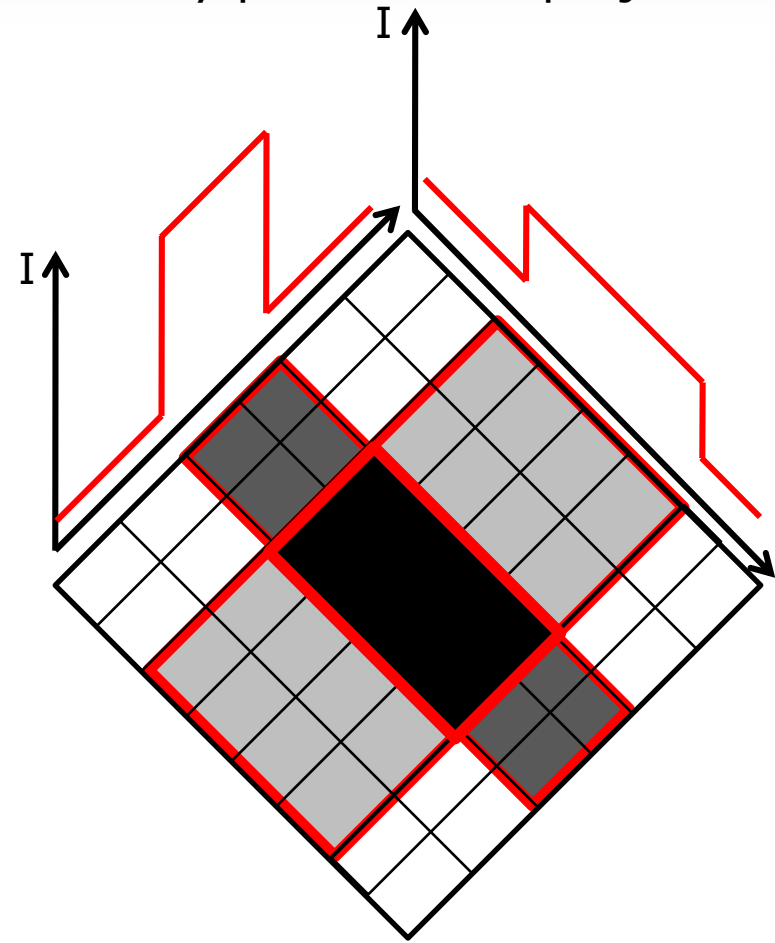
Applications

Systems & Software

Projection image: density profile



density profile: Back projection



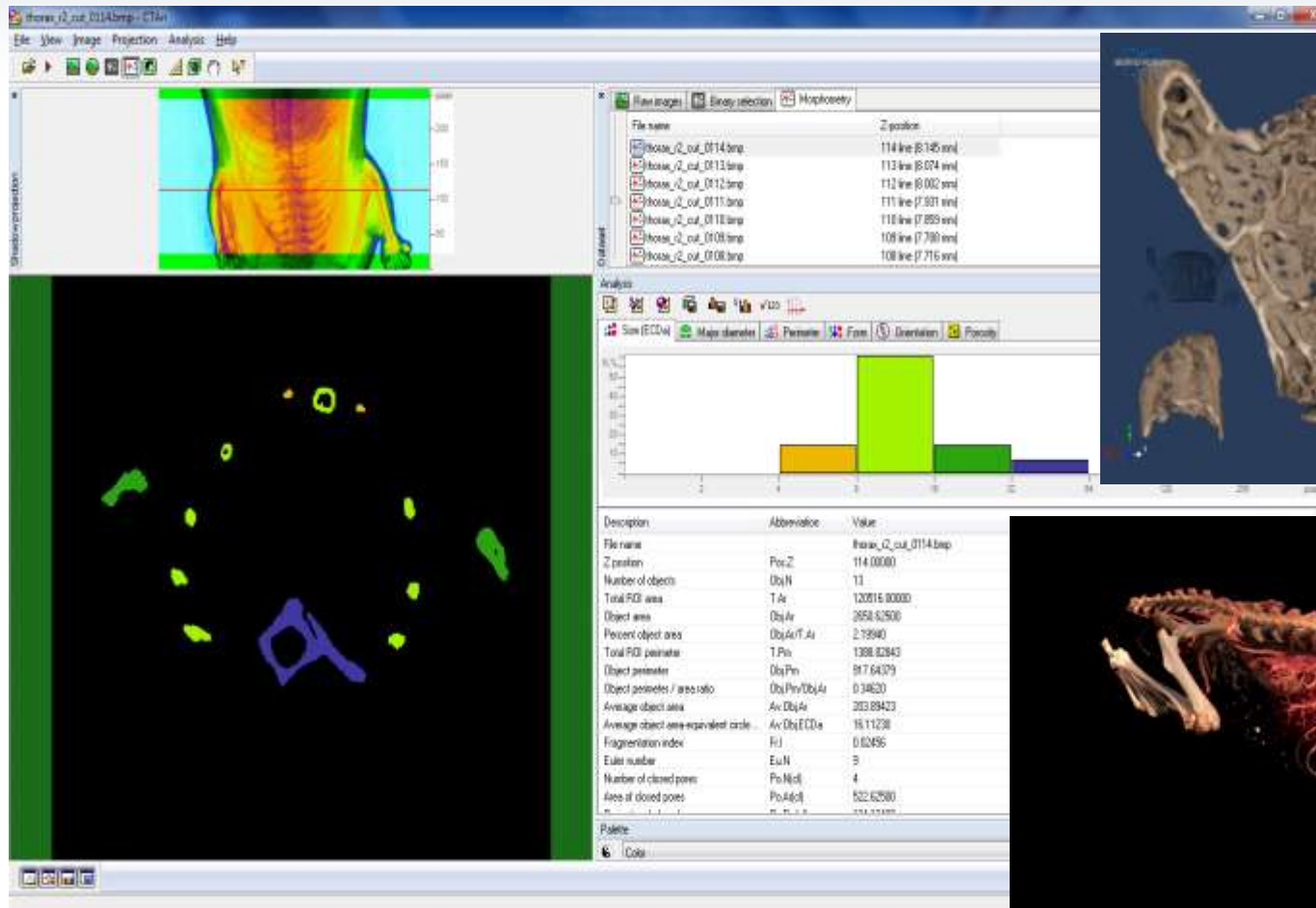
# Step 3: Analysis & 3D viewing

Background

Technology

Applications

Systems & Software



# Applications: What can be seen & analyzed?

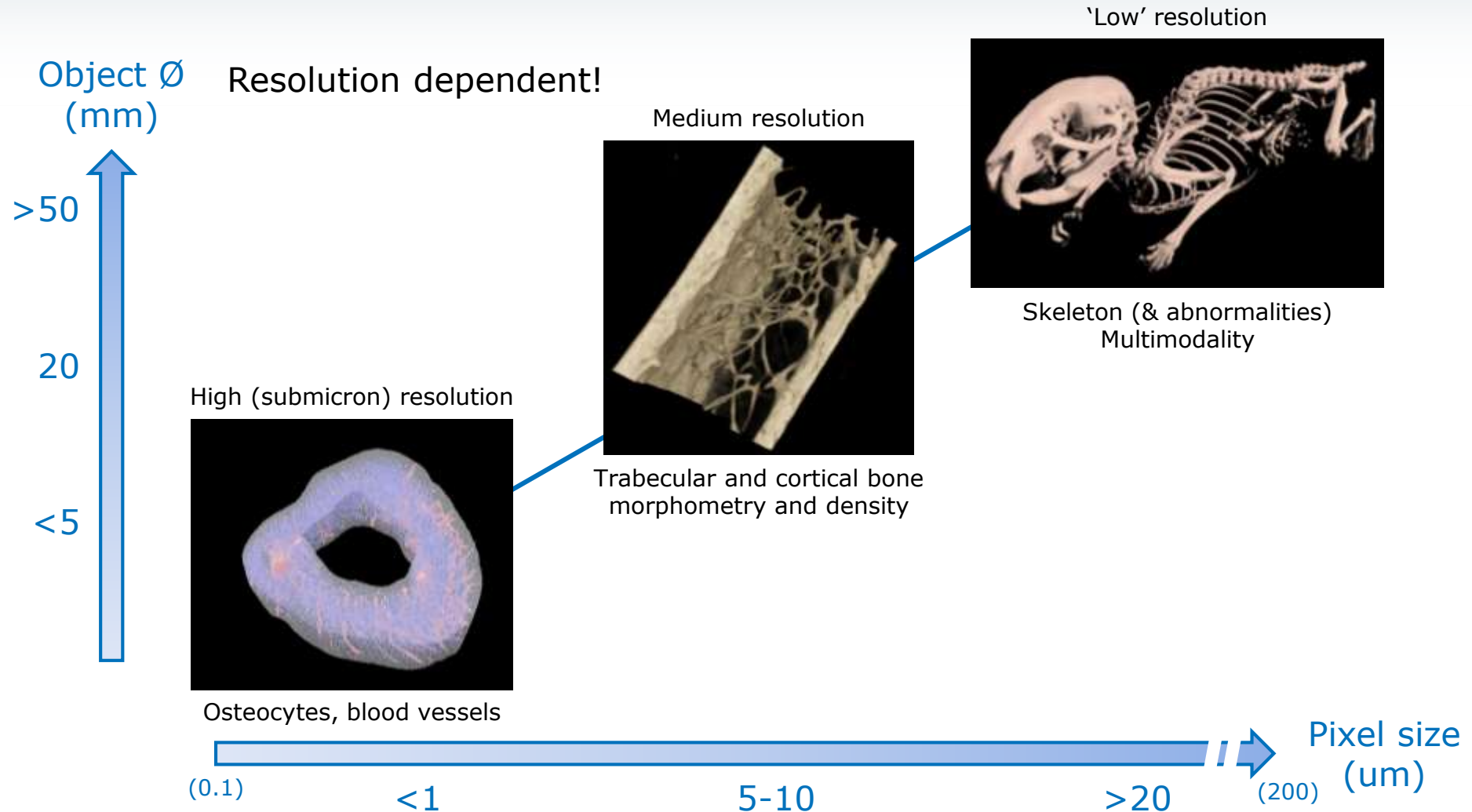


Background

Technology

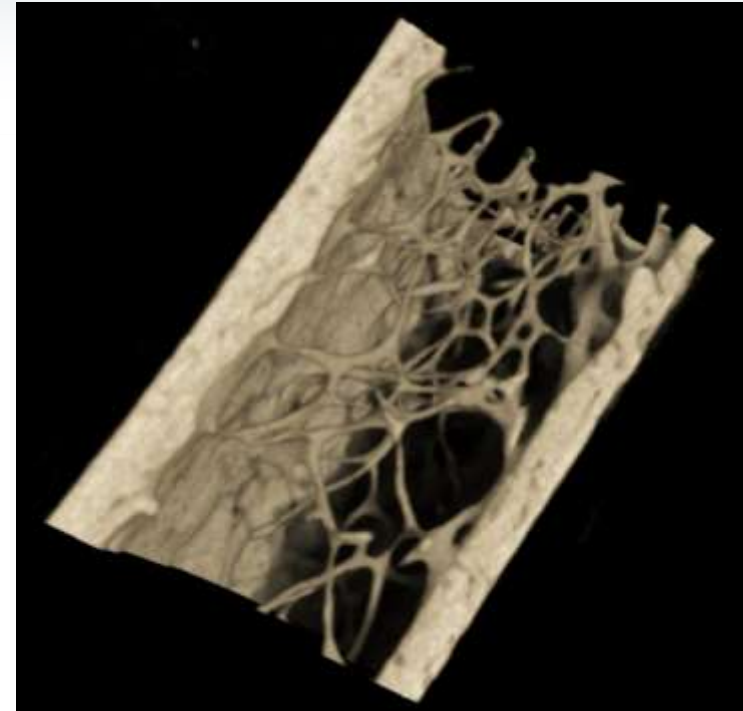
Applications

Systems & Software



- **Resolution?**  
5-20um pixel size
- **What can you see?**  
Trabecular and cortical structures  
mice, rat, rabbit, sheep, human, etc  
Blood vessels, cartilage
- **What can you analyze?**  
Density (BMD, BMC)  
Trabecular and cortical bone parameters

<i>Trabecular bone</i>	<i>Cortical bone</i>
TBV	BV
TbTh	Porosity
TbSp	CtTh
TbN	CSA
Anisotropy	Inertia
...	...



# Medium resolution

## Trabecular and cortical bone morphology *ex vivo*

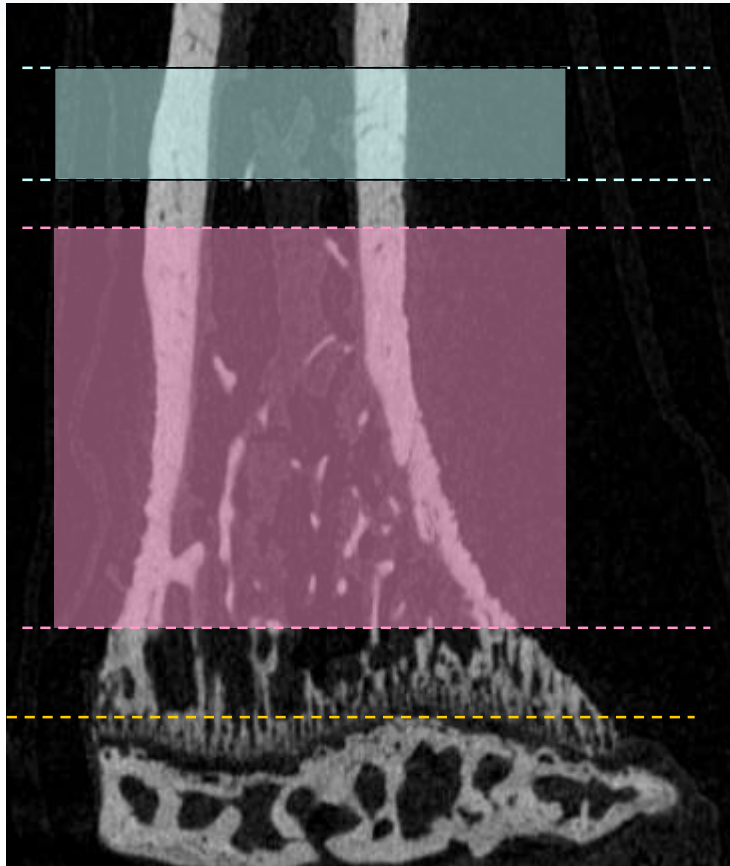


Background

Technology

Applications

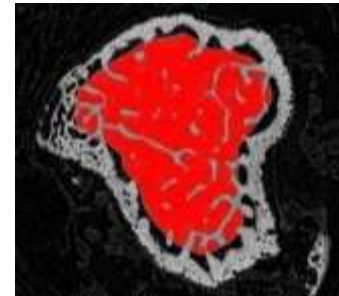
Systems & Software



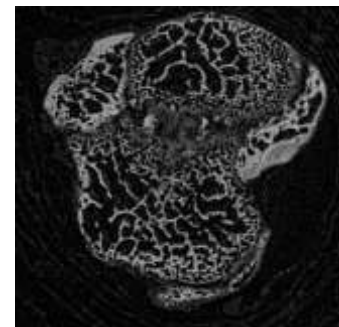
Reconstructed cross-section through a mouse tibia  
SkyScan 1272, 5µm pixel size



Cortical ROI  
Manual or fully  
automated selection  
in the diaphysis



Trabecular ROI  
Manual or fully  
automated selection  
in the metaphysis



Reference point:  
Manual selection of  
the growth plate

Analysis in 3D!!



# Medium resolution

## Trabecular and cortical bone morphology *in vivo*

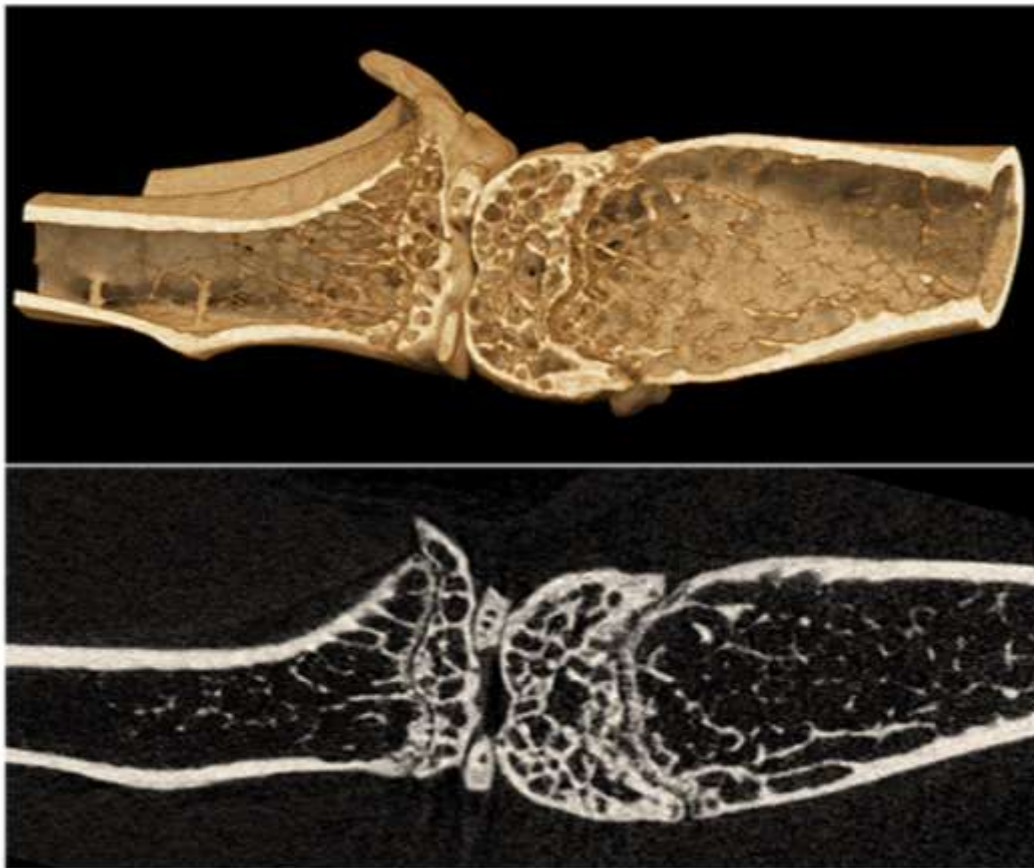


Background

Technology

Applications

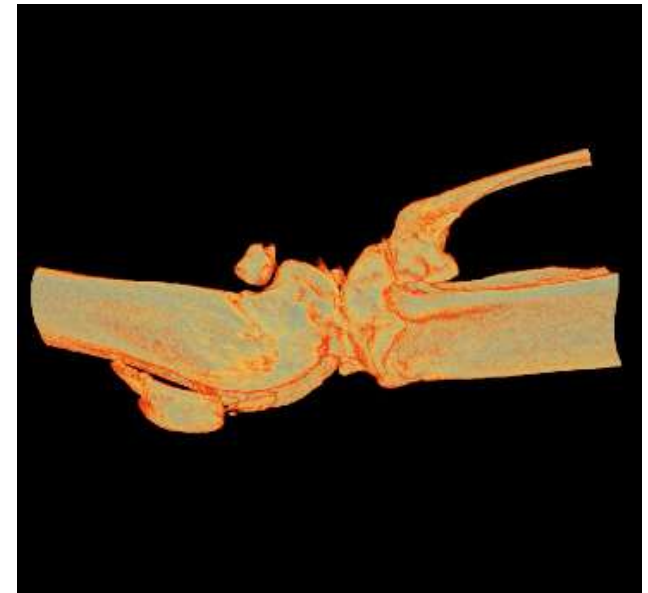
Systems & Software



Volume rendered 3D model of (top + right) and reconstructed cross-section through (bottom) a mouse hindlimb *in vivo*

SkyScan 1176, 9 $\mu$ m pixel size

- *In vivo* 9 $\mu$ m pixel size scans of a mouse hindlimb with a radiation dose of 450mGy (stay below 500 mGy!).





# Medium resolution Vertebra analysis



Background

Technology

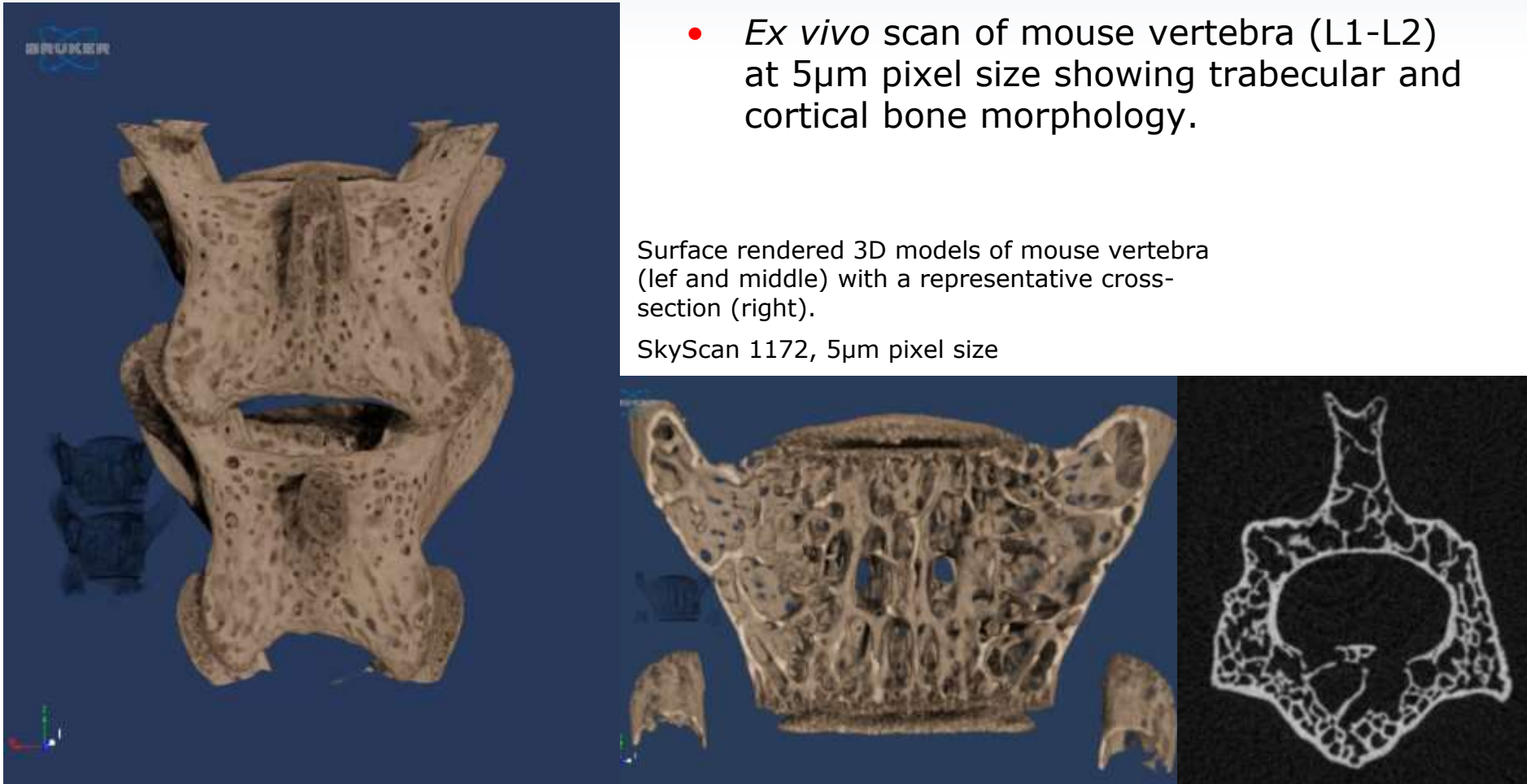
Applications

Systems & Software

- *Ex vivo* scan of mouse vertebra (L1-L2) at 5 $\mu$ m pixel size showing trabecular and cortical bone morphology.

Surface rendered 3D models of mouse vertebra (left and middle) with a representative cross-section (right).

SkyScan 1172, 5 $\mu$ m pixel size



# Medium resolution

## Calvaria developmental analysis



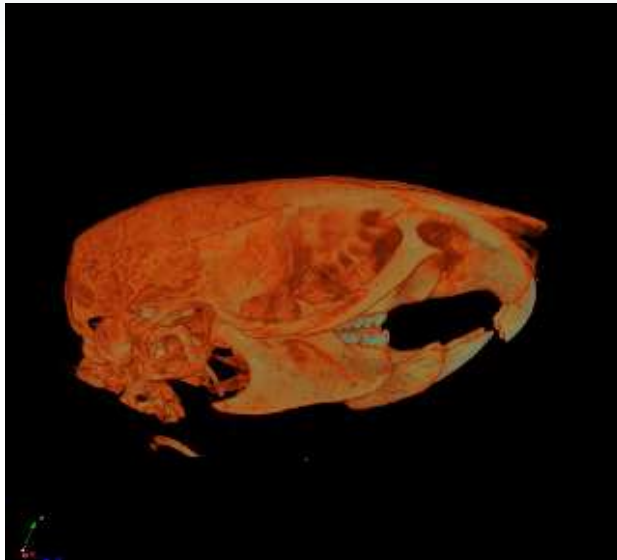
Background

Technology

Applications

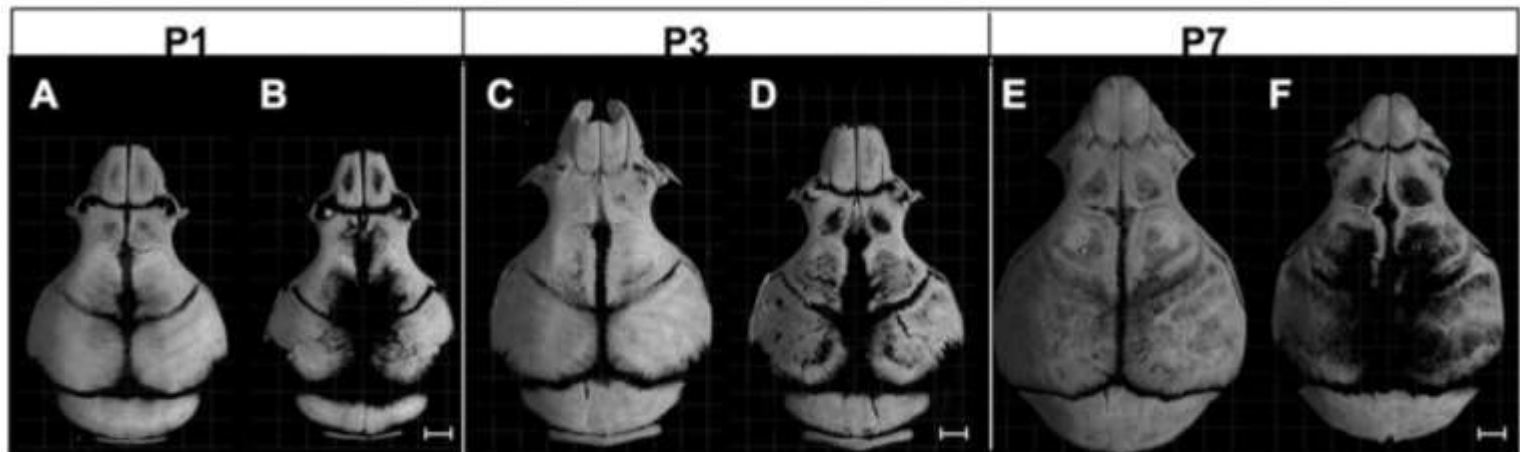
Systems & Software

- Skull shape variation analysis is achieved through selection of multiple specific reference points.



Left: Volume rendered 3D model of an *in vivo* scan of a mouse head (SkyScan 1176, 9 $\mu$ m pixel size)

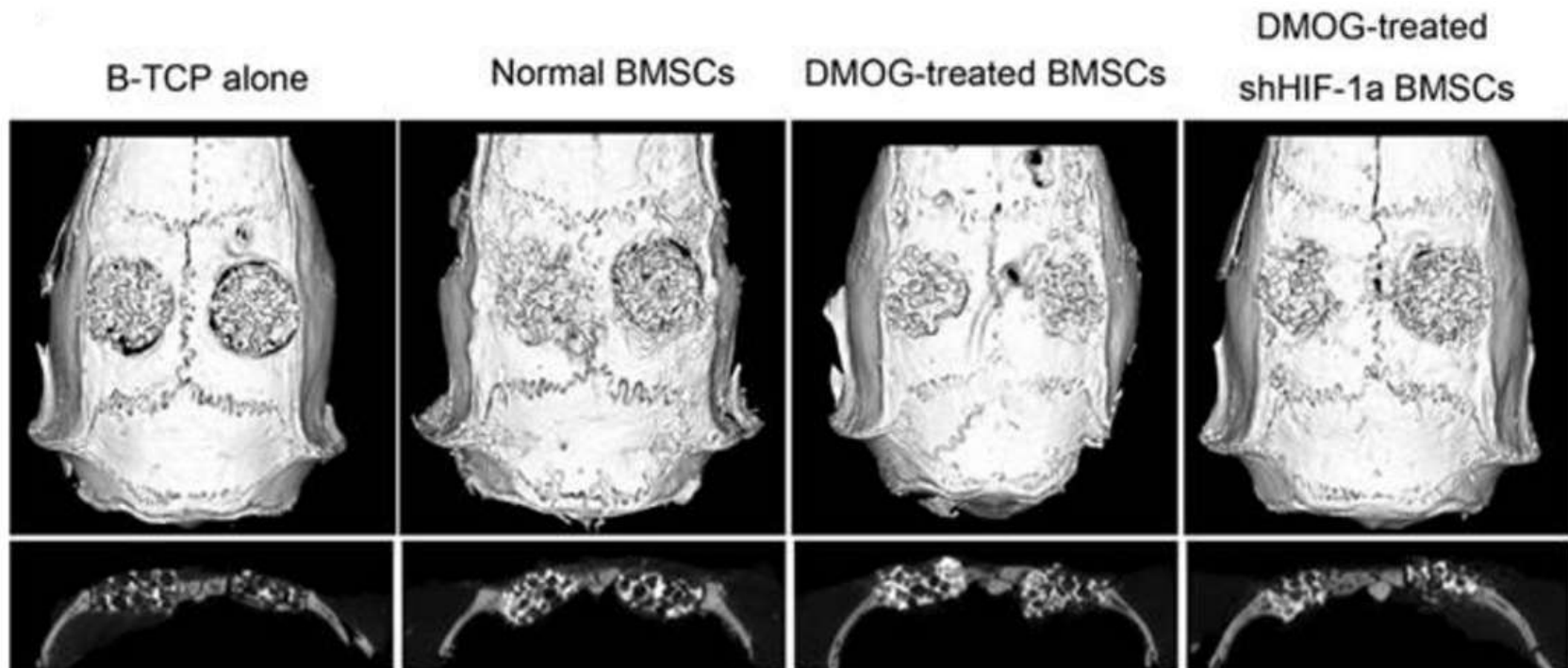
Right: Volume rendered 3D models of neonatal mouse skulls showing developmental retardation (SkyScan 1172, 12 $\mu$ m pixel size) Zhou *et al*, Development 2009 136: 427-436



# Medium resolution

## Calvaria defect analysis

- Treatment strategies for the healing of large defects are often evaluated in mouse calvaria.



Volume rendered 3D model of mouse calvaria (top) with corresponding cross-sections (bottom)  
(SkyScan 1076, 18 $\mu$ m pixel size)

# Medium resolution

## Fracture healing



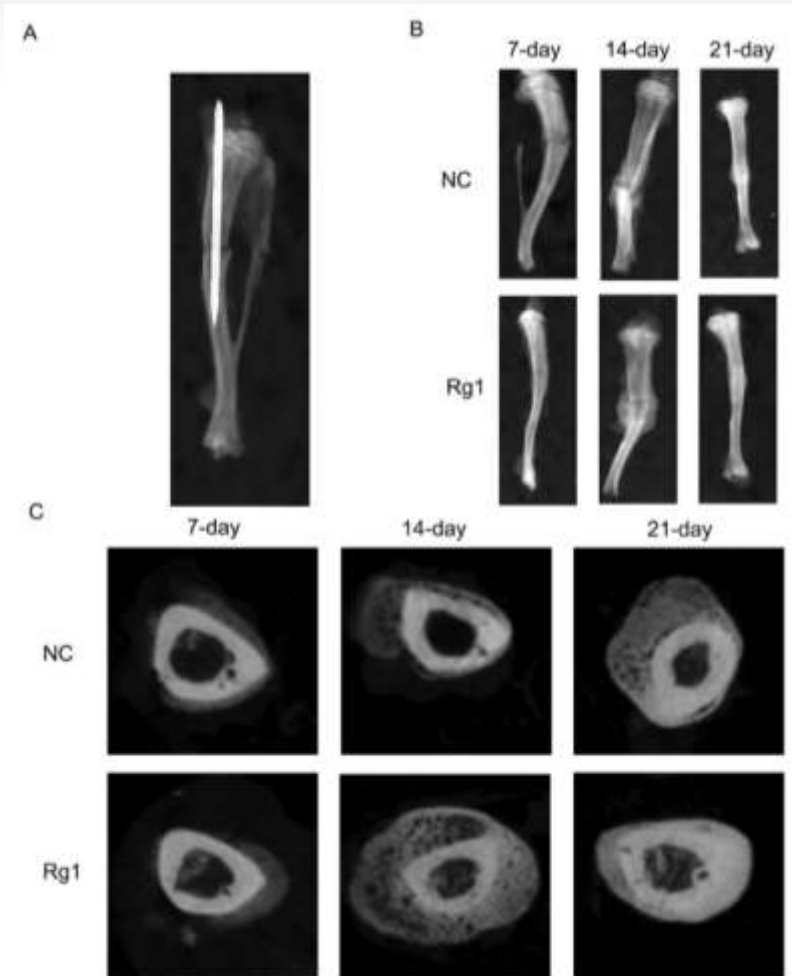
Background

Technology

Applications

Systems & Software

- 3D microCT analysis allows *in vivo* follow-up of long bone fracture repair. Callus formation can be visualized and quantified without slicing or removal of the stabilizing pin.



Radiographs of a tibia fracture model (top) and corresponding cross-sections through the fracture callus at several timepoints.

SkyScan 1176

# Medium resolution

## Tissue engineering

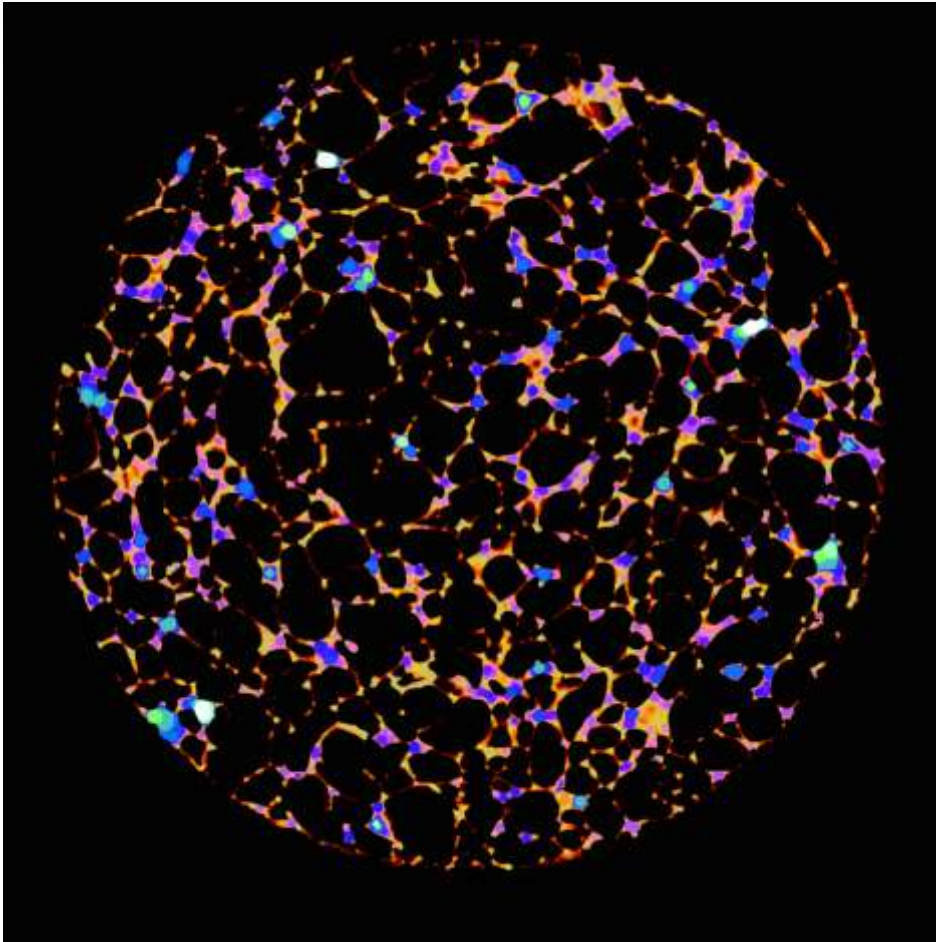


Background

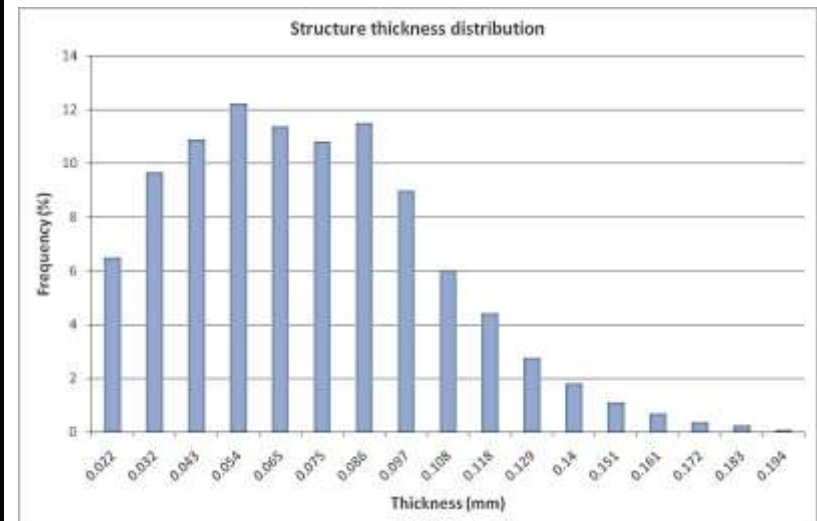
Technology

Applications

Systems & Software



- MicroCT analysis allows true 3D model-independent measurement of
  - Structure thickness
  - Structure separation
  - Open vs closed porosity
  - % object volume
  - ...





# Medium resolution Orthopedics



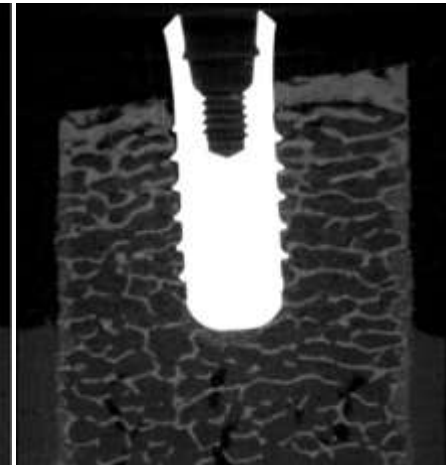
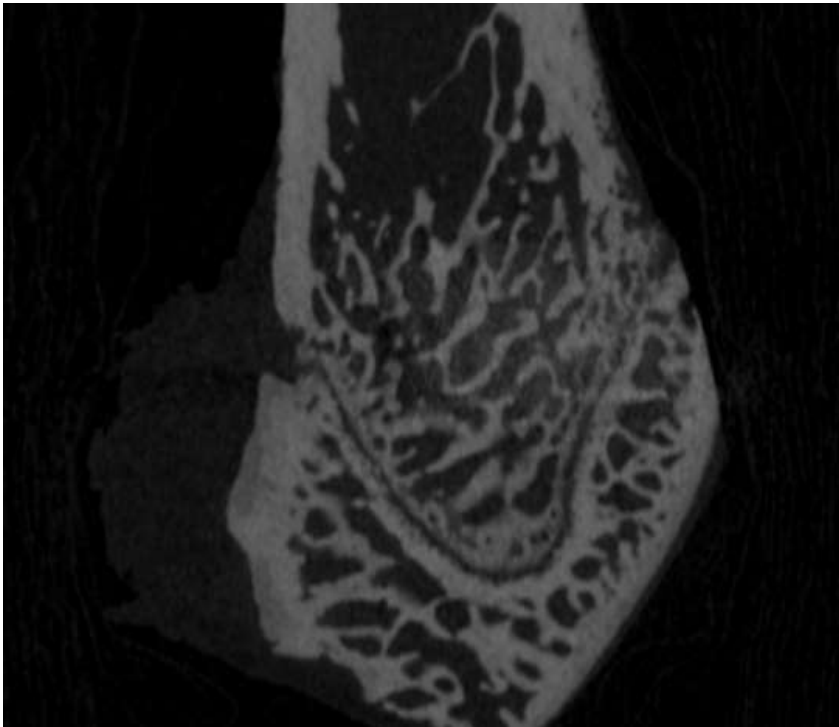
Background

Technology

Applications

Systems & Software

- Titanium implants can be imaged with minimal artefacts, allowing osteo-integration analysis in 3D



Reconstructed cross-sections through a rat distal femur (left) and pig rib (above) containing titanium implant (screw).

SkyScan 1272

# Medium resolution

## Osteo-integration analysis



Background

Technology

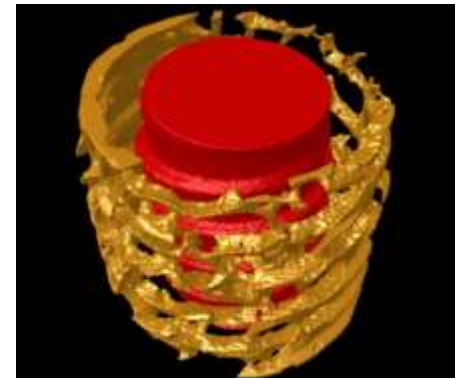
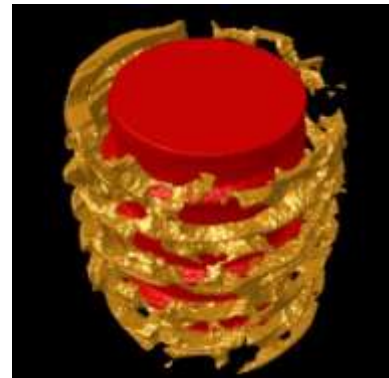
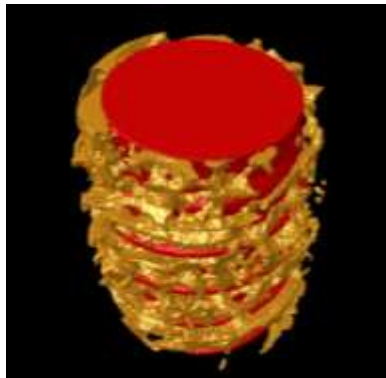
Applications

Systems & Software

- The bone-implant contact surface (2D) as well as the bone volume (3D) can be calculated at varying distances from the implant surface.

Reconstructed cross-sections through an implant zone (left) with surface rendered 3D models showing trabecular bone in virtual rings at increasing distances from the implant surface (below).

SkyScan 1272



# Medium resolution

## Osteoporosis progression



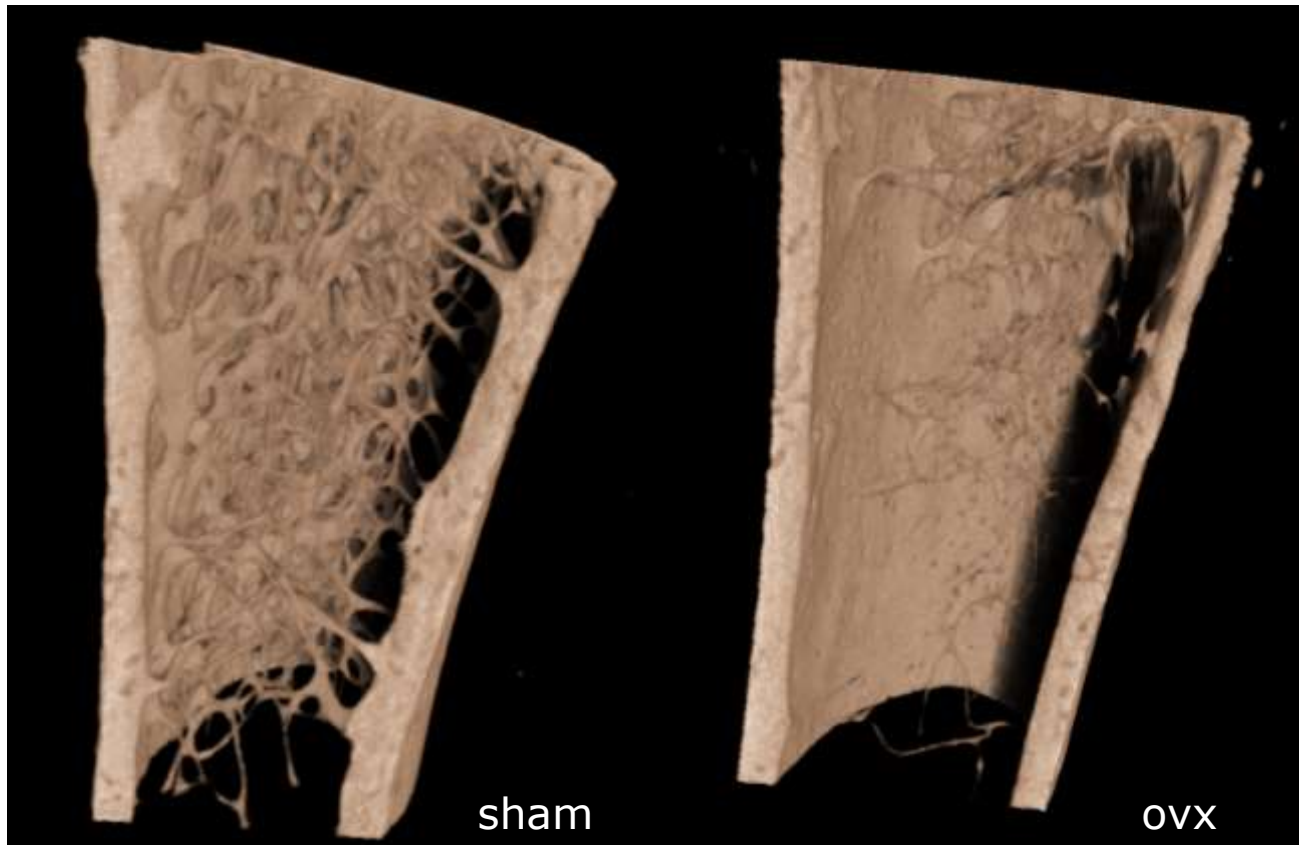
Background

Technology

Applications

Systems & Software

- Mouse tibia scan (metaphysis) with 5 $\mu$ m pixel size, illustrating trabecular bone loss upon ovariectomy



Volume rendered 3D model of a mouse tibia: sham-operation (left) or ovariectomy (right)

SkyScan1272



# Medium resolution

## Bone tumor progression

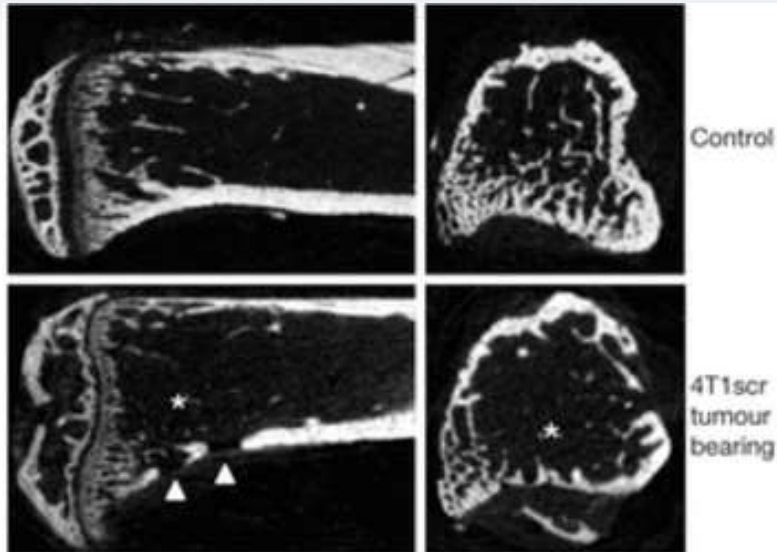


Background

Technology

Applications

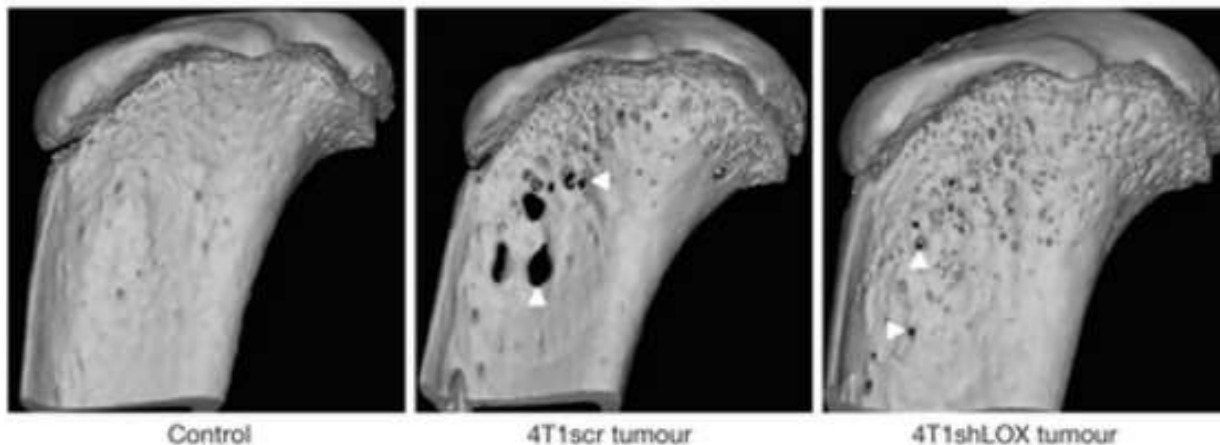
Systems & Software



- Tumor progression in long bones can be followed over time by means of bone resorption (osteolytic phenotype).

Reconstructed cross-sections through a mouse tibia (top) and corresponding surface rendered 3D models of the proximal tibia (bottom) showing bone resorption due to bone tumor growth.

SkyScan 1172



Cox *et al*, Nature. 2015 Jun 4;522(7554):106-10

# Medium resolution

## Evolution of osteoarthritis



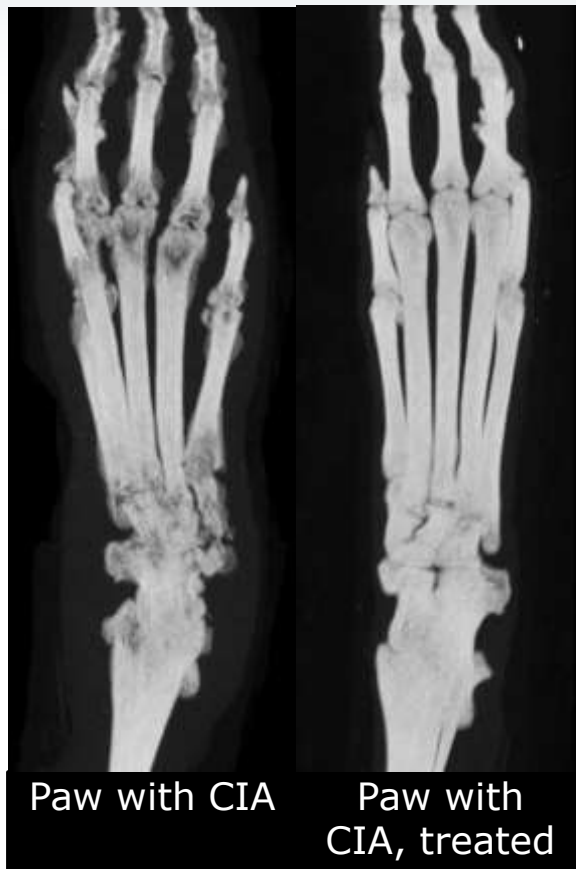
Background

Technology

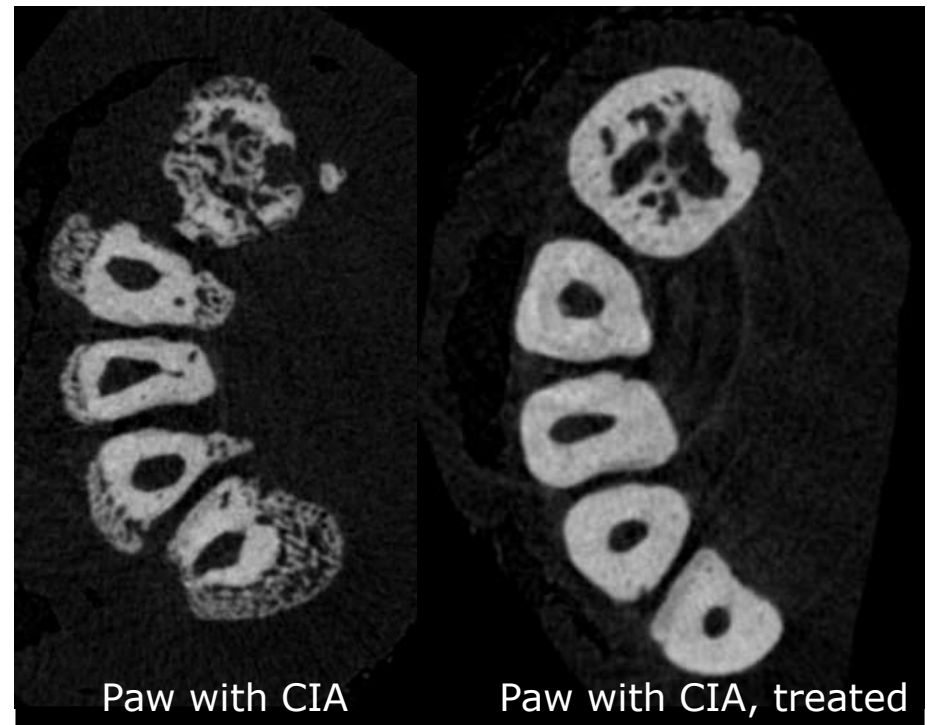
Applications

Systems & Software

- Periosteal reaction around the tarsals is characteristic of CIA and quantifiable due to its lower density (and hence grey value).



MIP projection of a mouse paw (left) and corresponding cross-sections through the tarsals (right)



# Medium resolution Cartilage analysis



Background

Technology

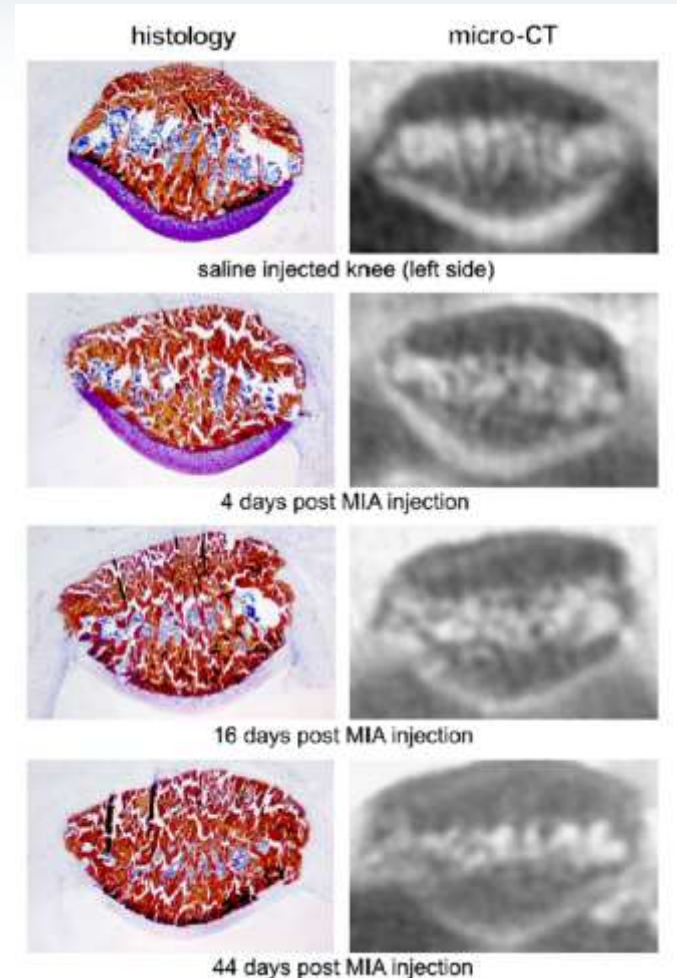
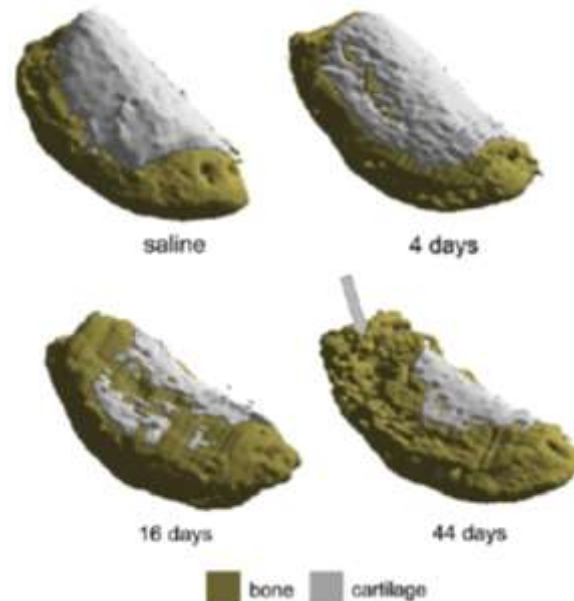
Applications

Systems & Software

- The contrast agent HEXABRIX 320 allows *in vivo* visualization and measurement of joint cartilage thickness and degradation over time.

Surface rendered 3D models of mouse femoral condyles (left) and representative cross-sections (right) showing cartilage degradation over time.

SkyScan 1076



Piscaer et al, Osteoarthritis Cartilage. 2008 Sep;16(9):1011-7

# Medium resolution

## Vascular network in bone marrow



Background

Technology

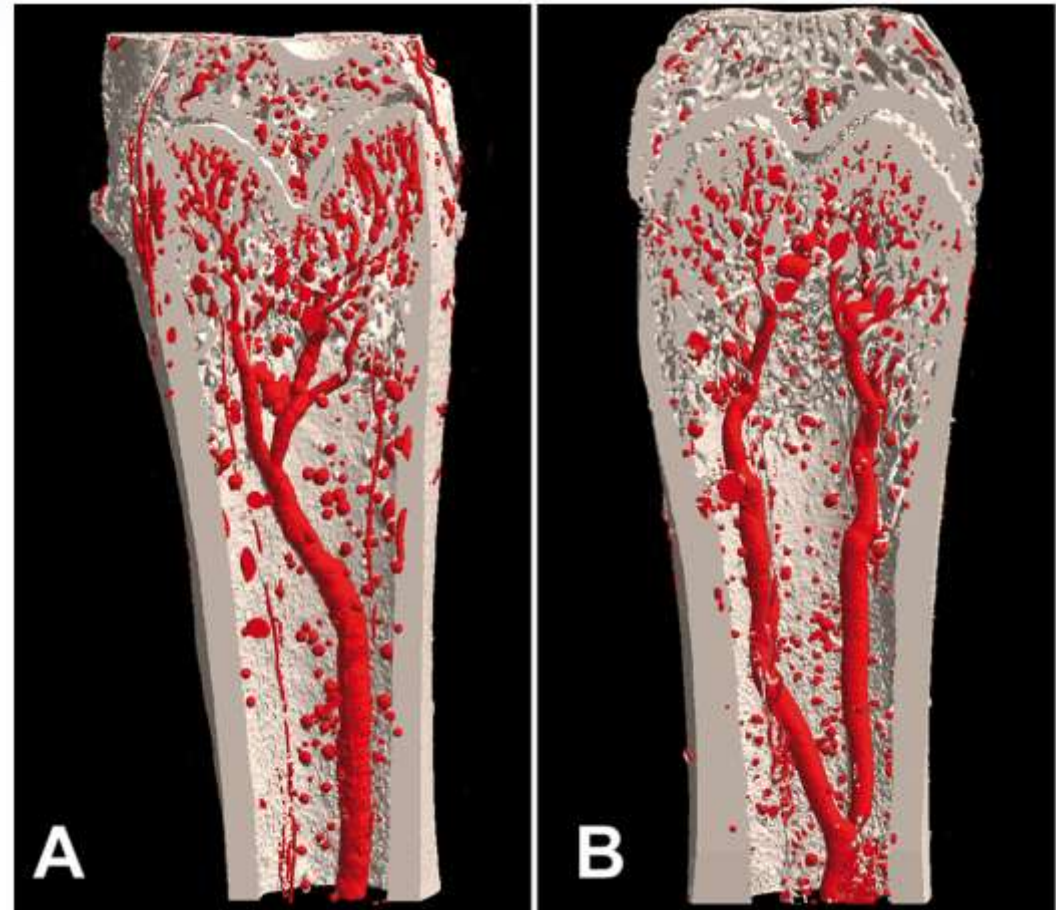
Applications

Systems & Software

- Perfusion of the vascular network with contrast agent allows to visualize the vascular network inside long bones.

Surface rendered 3D models of mouse femora with the vascular network (large vessels) in red.

SkyScan 1172, 9µm pixel size



Nyangoga et al, *PLoS One*. 2011 Mar 28;6(3):e17336



# High (submicron) resolution



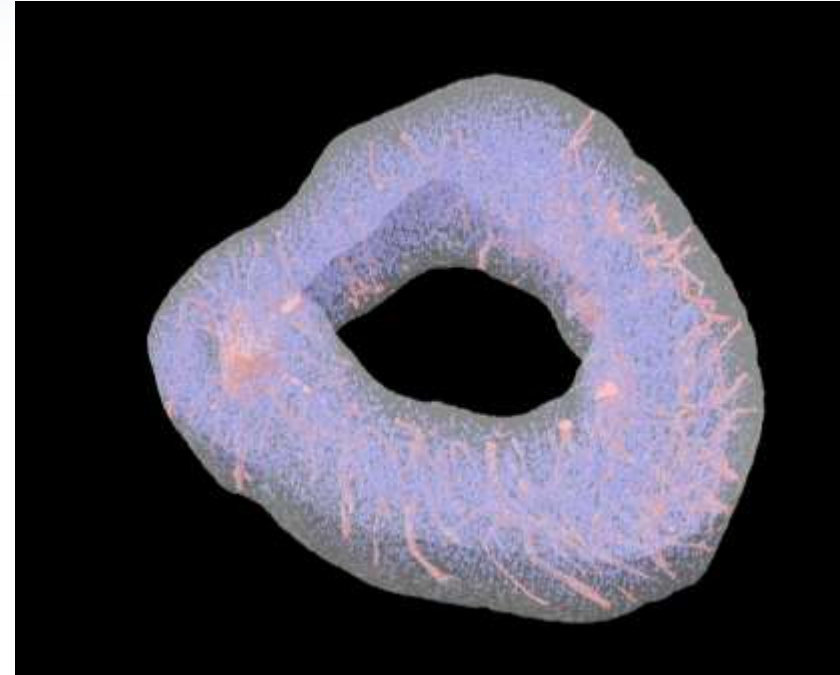
Background

Technology

Applications

Systems & Software

- **Resolution?**
  - <1µm pixel size (down to 100nm)
- **What can you see?**
  - Osteocyte lacunae
  - Blood vessels inside bone
  - Trabecular and cortical structures
- **What can you analyze?**
  - Density (BMD, BMC)
  - Osteocyte size, shape and orientation
  - Vascular network inside bone
  - Trabecular and cortical bone parameters



# High (submicron) resolution

## Osteocytes and blood vessels in mouse tibia

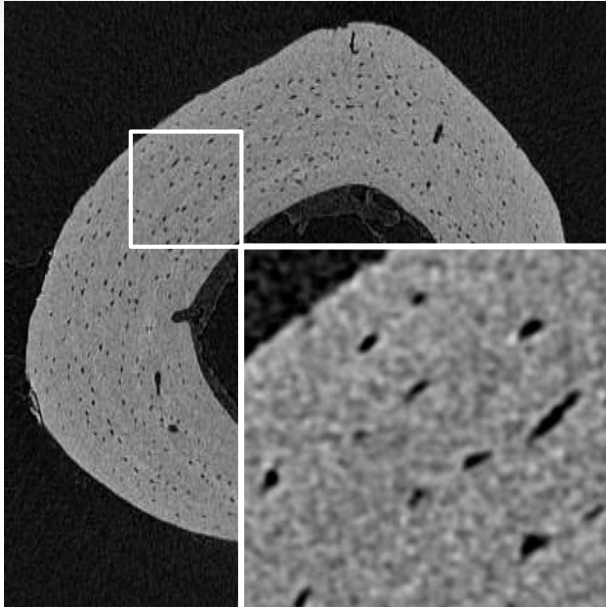


Background

Technology

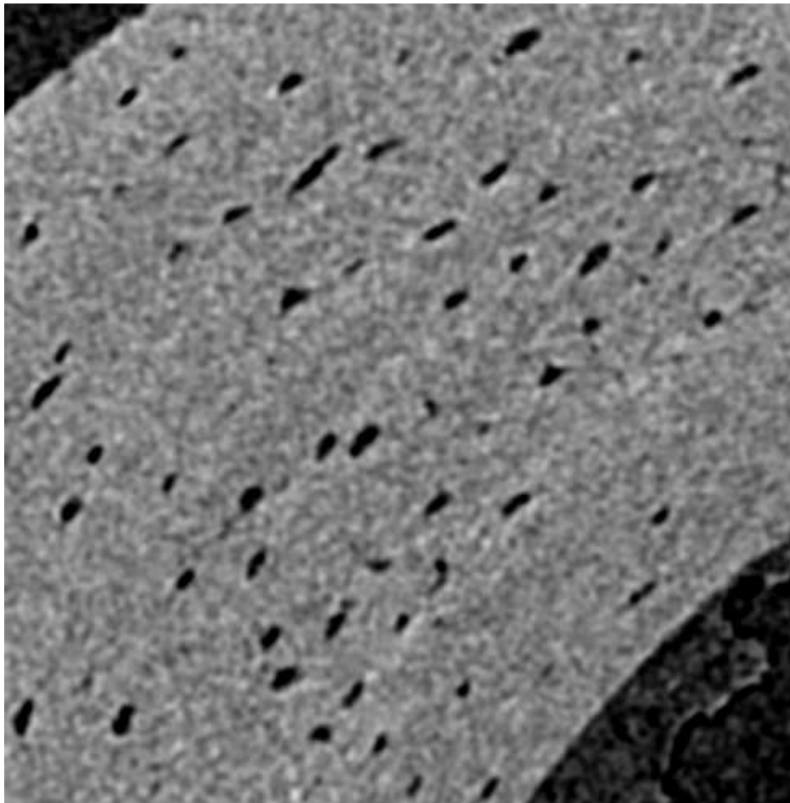
Applications

Systems & Software



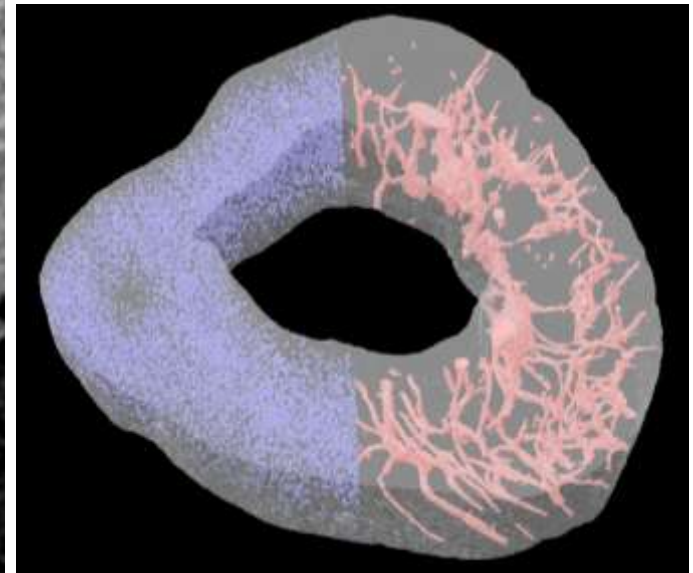
SkyScan2211

- Mouse tibia (diaphysis), scanned at 500nm pixel size, showing all small pores inside the cortical bone, containing osteocytes (closed pores) or blood vessels (open pores)



Left & Middle: reconstructed cross-sectional slice

Right: 3D surface rendered model showing osteocyte lacunae (blue) and blood vessels (red)



# High (submicron) resolution Osteocytes in mouse fibula

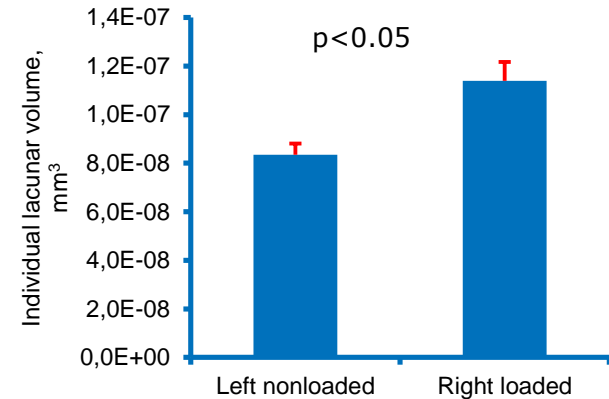
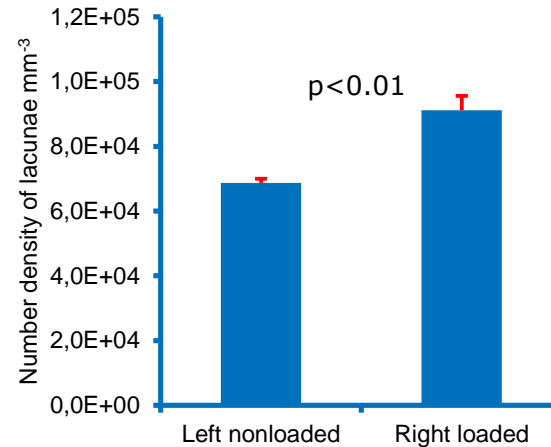
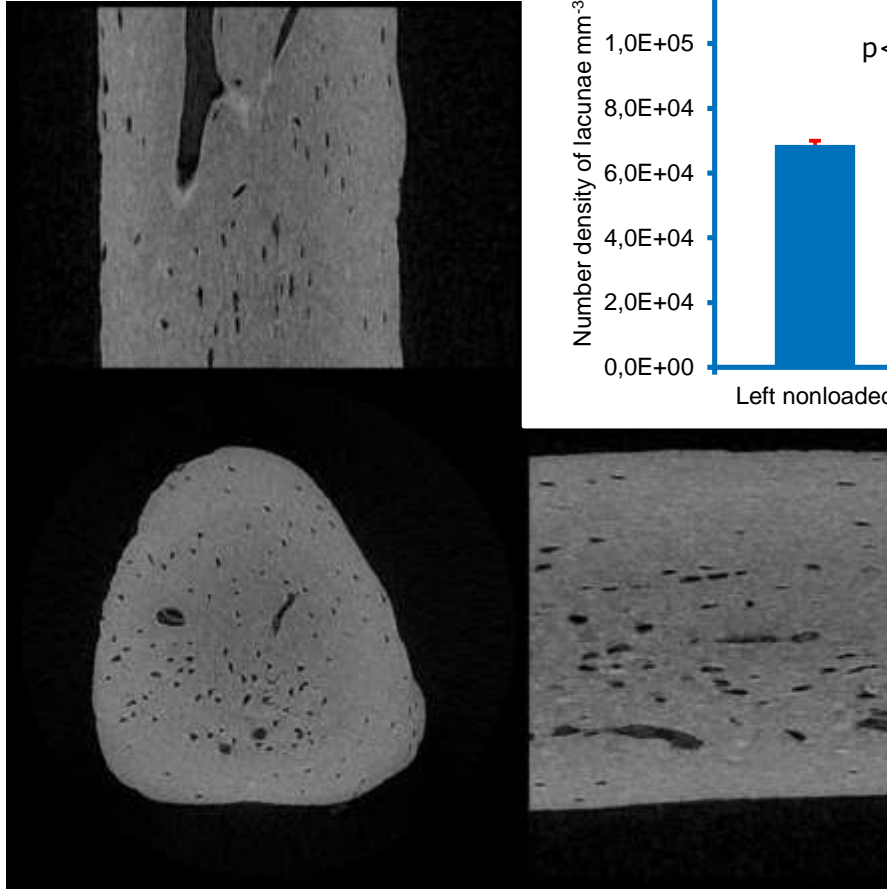


Background

Technology

Applications

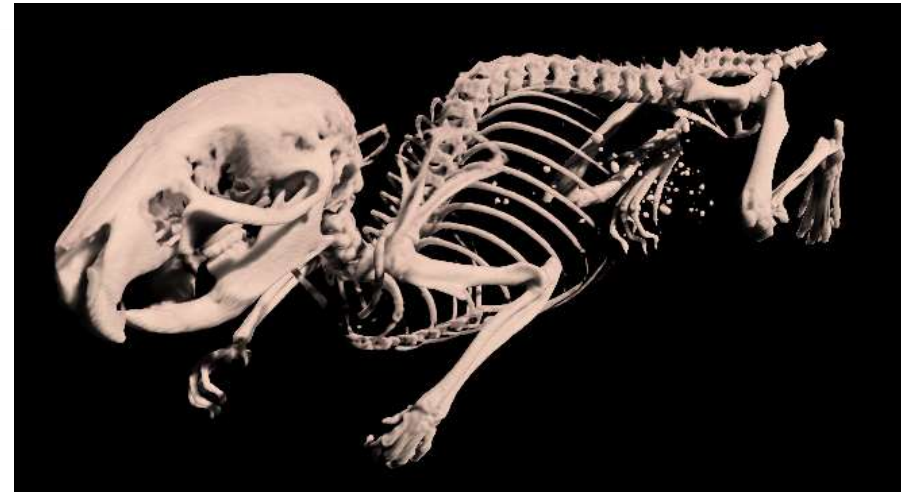
Systems & Software



- Mouse fibula, scanned at 400nm pixel size, showing an increase of the density and size of osteocyte lacunae upon mechanical loading

3 Orthogonal cross-sectional slices through a mouse fibula  
SkyScan 2011

- **Resolution?**
  - >20um pixel size (up to 200um)
- **What can you see?**
  - Skeleton (+ soft tissue/fat)
  - Small bones without internal details
    - mice, rats, ...
  - Large bones with internal details
    - human, sheep, ...
- **What can you analyze?**
  - Trabecular and cortical morphology large bones
  - Major skeletal abnormalities/zoological classification
  - Multimodality: use microCT data for anatomical reference (image registration)





# Low resolution

## Large bone (sheep) with internal structures



Background

Technology

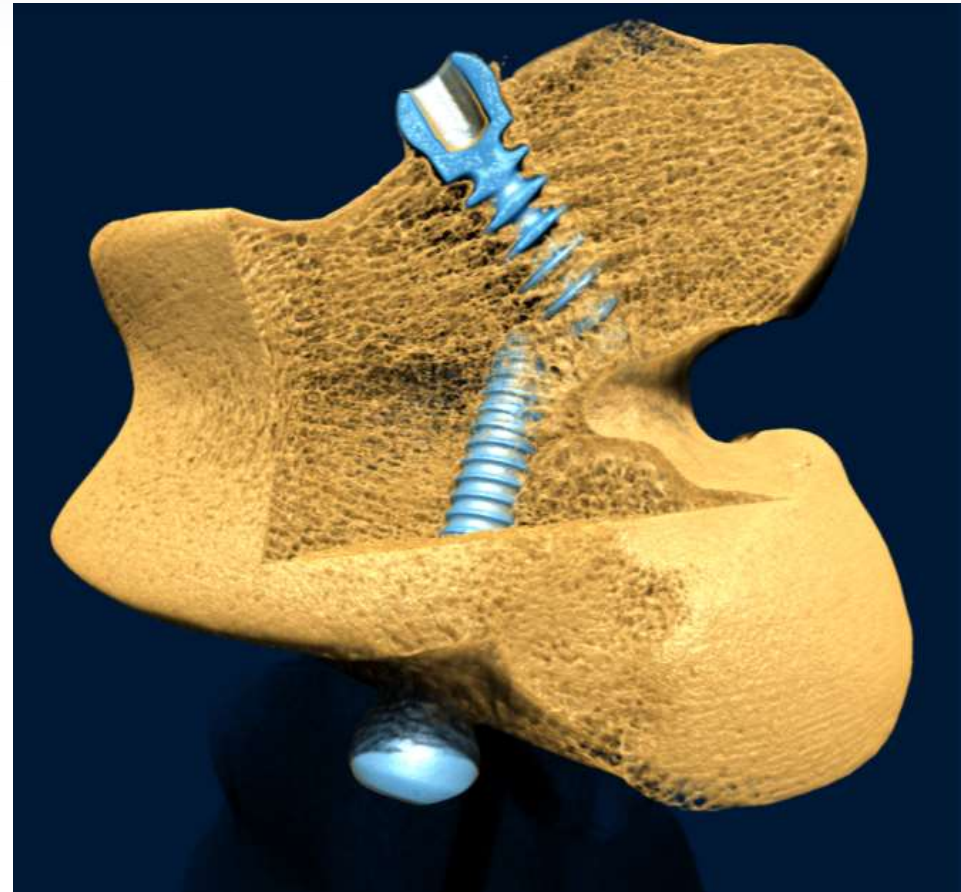
Applications

Systems & Software

- Sheep bone (femur) with 2 titanium screws, scanned at 37  $\mu\text{m}$  pixel size.
- Morphometric analysis includes calculation of the cortical and trabecular bone parameters, as well as the bone-implant contact zone.

Volume rendered 3D model of a sheep femur  
with 2 titanium screw implants

SkyScan 2211



# Low resolution

## Mouse skeletal abnormalities



Background

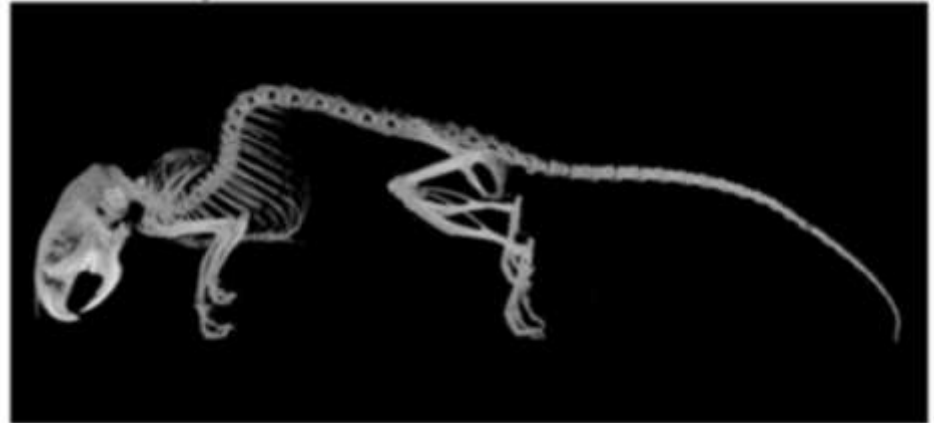
Technology

Applications

Systems & Software

- Full body microCT analysis of mice at 35  $\mu\text{m}$  pixel size, showing kyphosis upon ageing in  $P4ha1^{+/-};P4ha2^{-/-}$  mice (48 weeks of age).

$P4ha1^{+/-};P4ha2^{+/-}$



$P4ha1^{+/-};P4ha2^{-/-}$



SkyScan 1176

Aeo et al, [J Biol Chem](#). 2015 Jul 3;290(27)

# Low resolution Zoology



Background

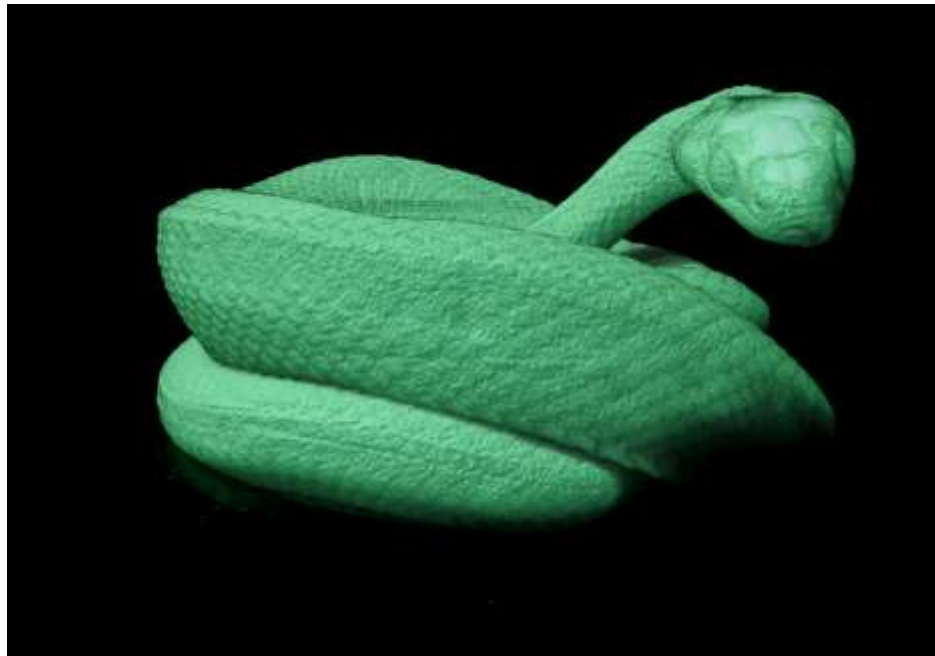
Technology

Applications

Systems & Software



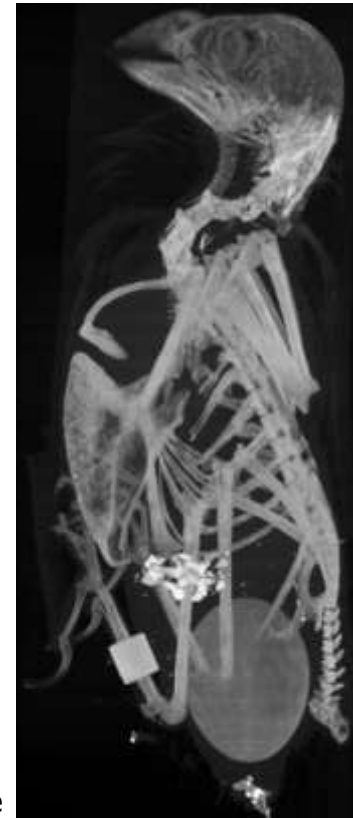
- Zebrafish
- SkyScan1172
- 4.4µm pixel size



- Ammonite
- SkyScan1173
- 51µm pixel size

- Snake
- SkyScan1173
- 40µm pixel size

- Bird
- SkyScan1173
- 35µm pixel size



# Low resolution Multimodality

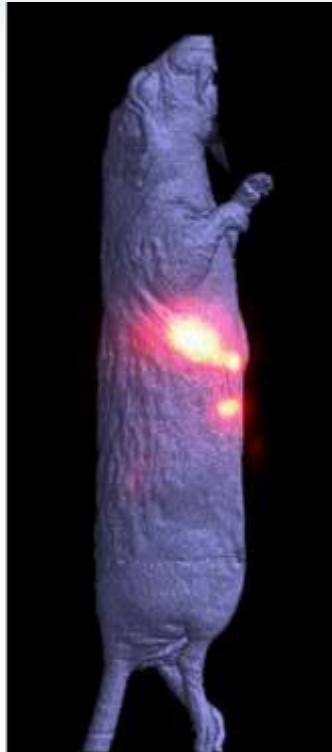


Background

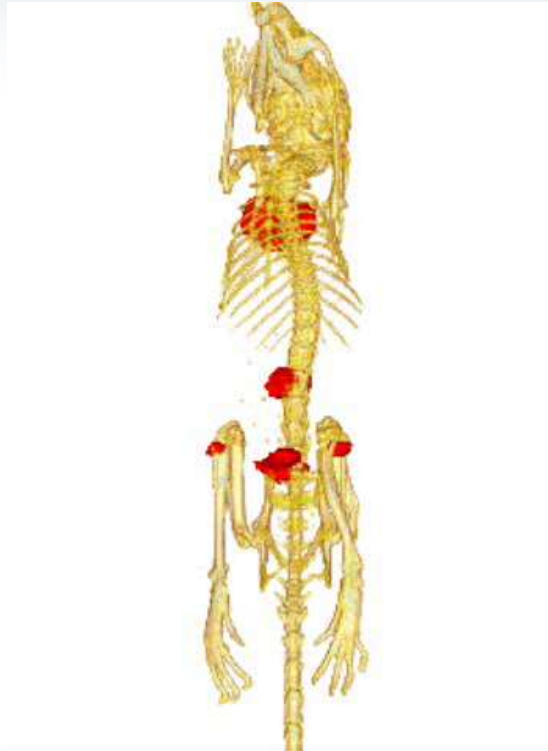
Technology

Applications

Systems & Software

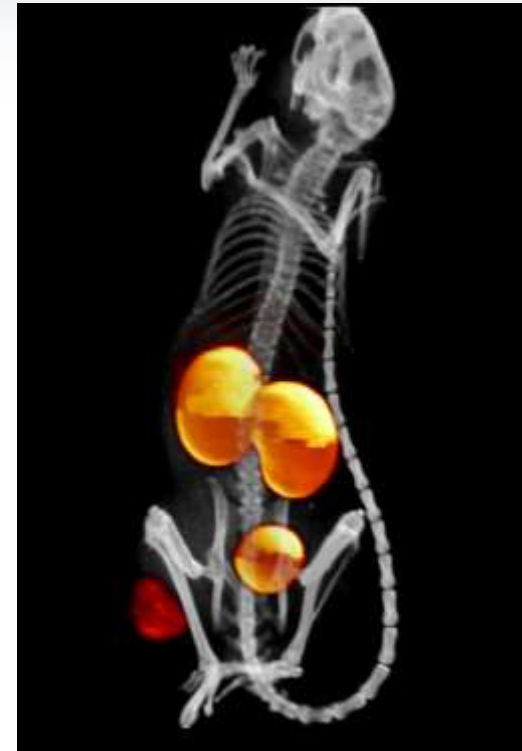


BLI (XtremeII)  
+  
micro-CT



fluorescence  
+  
micro-CT

*University of Leiden*



SPECT  
+  
micro-CT

*Brussels University*

# Systems & Software

## What system to use?



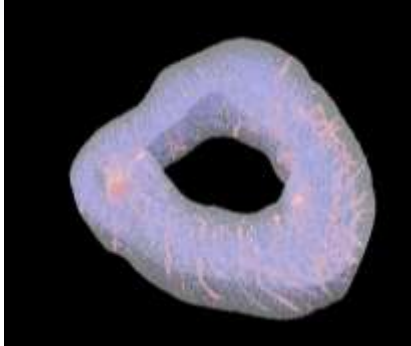
Background

Technology

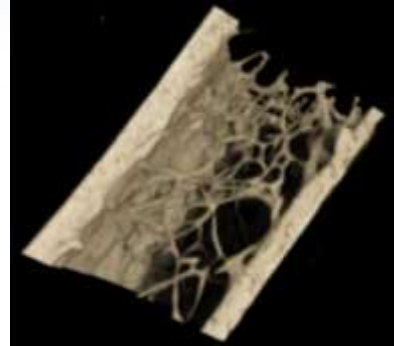
Applications

Systems & Software

High (submicron) resolution



Medium resolution



Low resolution



<1 $\mu$ m

5-10 $\mu$ m

>20 $\mu$ m

SkyScan1176

SkyScan1278

In vivo  
scanning

SkyScan1272

SkyScan1275/1173

SkyScan1174

Ex vivo  
scanning

SkyScan2211

# Bruker microCT: *In vivo* product line



Background

Technology

Applications

Systems & Software

## SkyScan 1176



High resolution

9-18-35  $\mu\text{m}$  px size

11 Mpx CCD camera

20-90kV source

Samples:

Ø max 6.8cm

⇅ max 20cm

## SkyScan 1278



High Throughput (<8sec)

50-200  $\mu\text{m}$  px size

3 Mpx FP camera

20-65kV source

Samples:

Ø max 8cm

⇅ max 20cm

Physiological monitoring and synchronized scanning

Low X-ray dose scanning



# Bruker microCT: *Ex vivo* product line



Background

Technology

Applications

Systems & Software

SkyScan 1174



Compact

<6 $\mu$ m px size

1.3 Mpx CCD camera

20-50kV source

Samples:  
 $\varnothing$  max 3cm  
 $\updownarrow$  max 5cm

SkyScan 1275



High throughput

<5 $\mu$ m px size

3 Mpx FP camera

20-100kV source

Samples:  
 $\varnothing$  max 10cm  
 $\updownarrow$  max 12cm

Automated scanning  
 Sample changer

SkyScan 1173



High energy

<5 $\mu$ m px size

5 Mpx FP camera

20-130kV source

Samples:  
 $\varnothing$  max 14cm  
 $\updownarrow$  max 20cm

SkyScan 1272



High resolution

<0.35 $\mu$ m px size

11-16 Mpx CCD camera

20-100kV source

Samples:  
 $\varnothing$  max 7.5cm  
 $\updownarrow$  max 7cm

Sample changer  
 Automated scanning

SkyScan 2211



Multiscale nanoCT

<100nm px size

3 Mpx FP camera  
 11 Mpx CCD camera

20-190kV source

Samples:  
 $\varnothing$  max 20cm  
 $\updownarrow$  max 20cm

Sample changer

# SkyScan software suite



Background

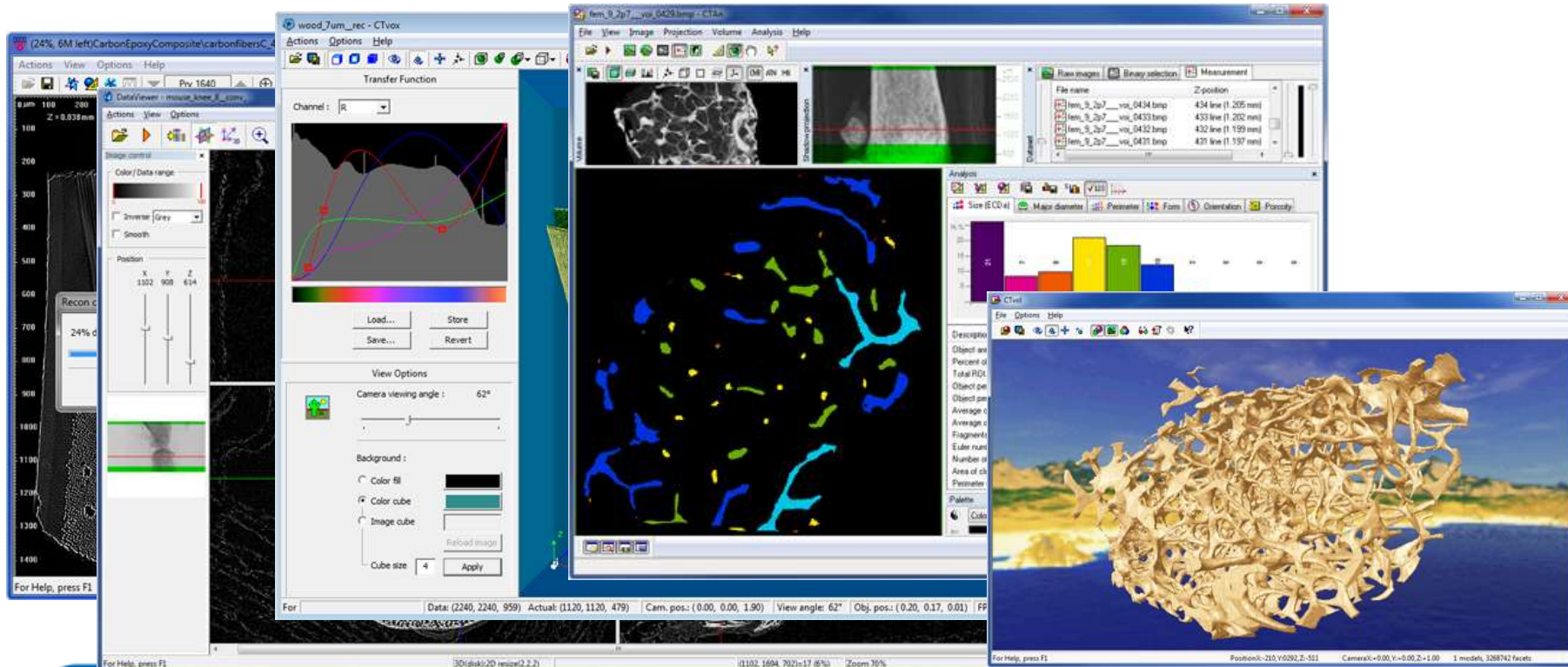
Technology

Applications

Systems & Software

Supplied software package includes:

- Control program (integrated scanner and sample changer control)
- Reconstruction: Nrecon with GPU-acceleration or Insta-recon
- Visualization and registration = DataViewer
- Volume rendering with export to mobile devices = CTvox
- 2D/3D image analysis = CTan+CTvol





# After sale support: all inclusive!

Background

Technology

Applications

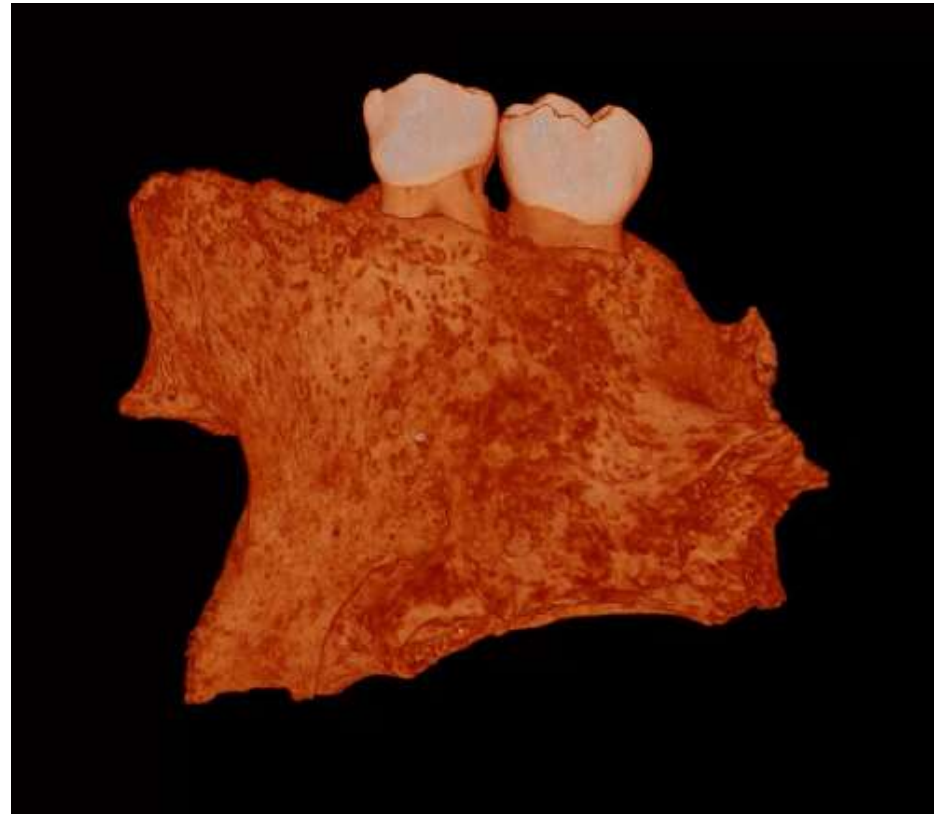
Systems & Software

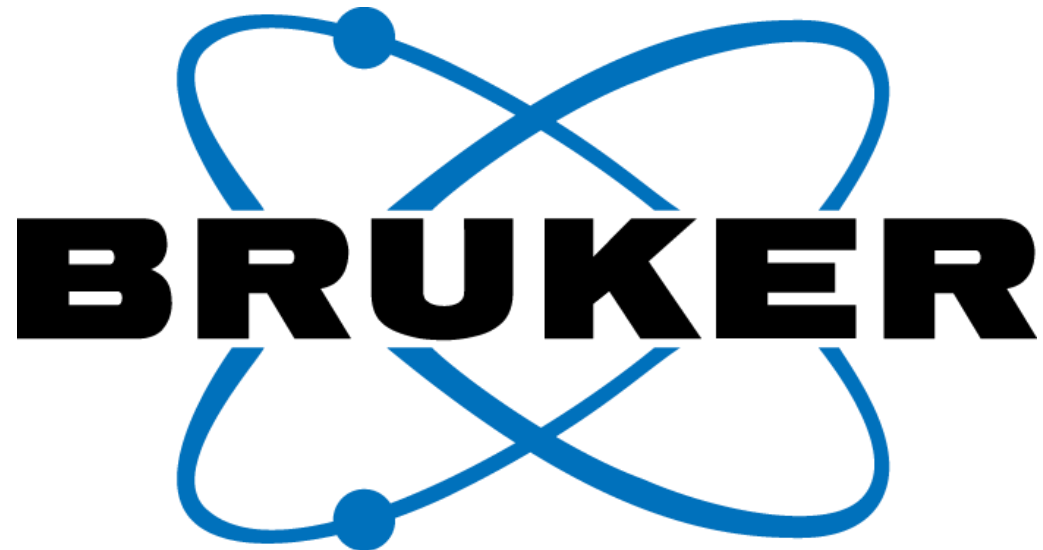
- Applications training
  - Initial: on site
  - Advanced: on site or at HQ
- Applications mail hotline
- Monthly microCT newsletter academy
  - News, updates and conference info
  - Method and application notes
  - Webinar training sessions
- User meetings

# Got inspired?

- [Applications.bmct@bruker.com](mailto:Applications.bmct@bruker.com)
- [www.bruker-microCT.com](http://www.bruker-microCT.com)
- Check website for our local representative (distributors section)
- Get your test sample scanned free of charge!

Mandible and teeth  
from a Saxon child,  
1500 years ago.





Innovation with Integrity

Would you like to learn more? Contact a customer service representative.