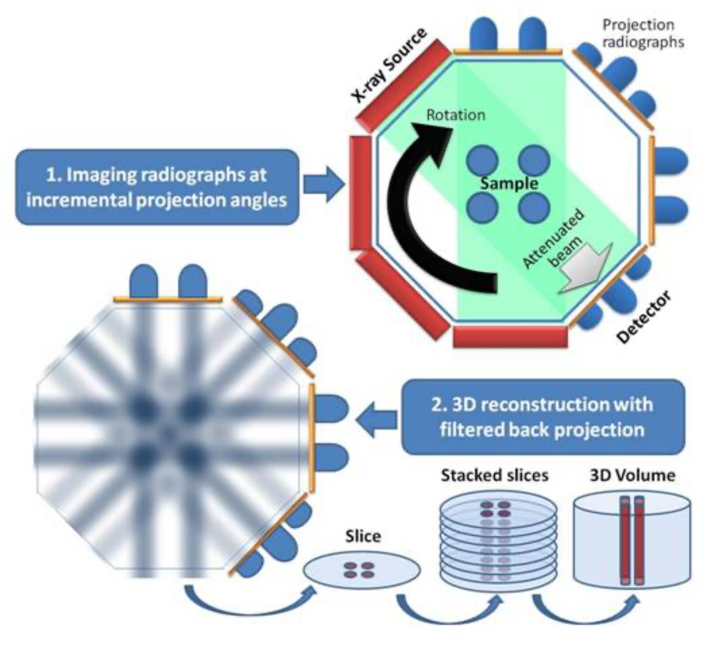
Today: making X-ray tomography better

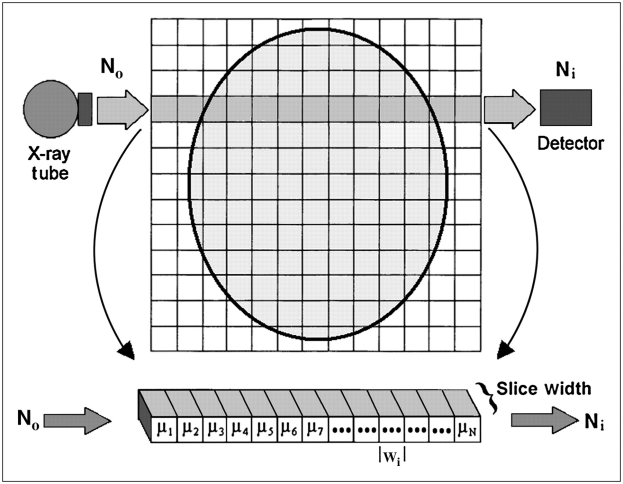
PHY127 FS 2023

Prof. Ben Kilminster
Lecture 10
May 12th, 2023

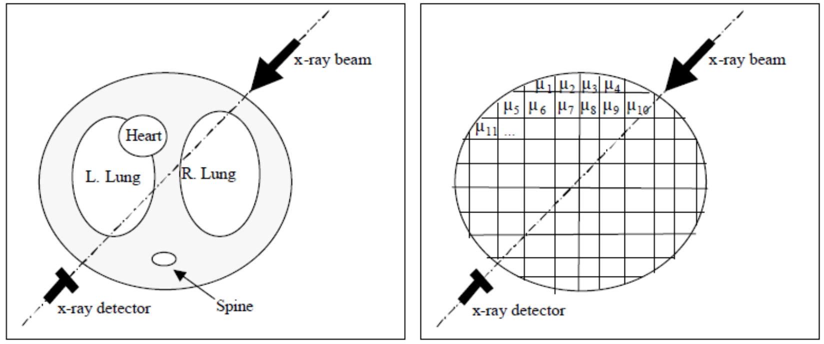
Review :



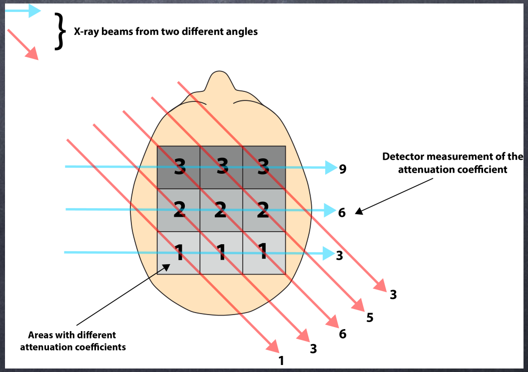
X-ray Source
Projection radiographs
Rotation
Sample
Attenuated beam
Detector
1. Imaging radiographs at incremental projection angles
2. 3D reconstruction with filtered back projection
Stacked slices
3D Volume
Slice



N_o
N_i
X-ray tube
Detector
Slice width
N_o
μ_1 μ_2 μ_3 μ_4 μ_5 μ_6 μ_7 ••• ••• ••• ••• ••• μ_N
N_i
$|w_i|$



x-ray beam
Heart
L. Lung
R. Lung
Spine
x-ray detector
x-ray beam
μ_1 μ_2 μ_3 μ_4
μ_5 μ_6 μ_7 μ_8 μ_9 μ_{10}
μ_{11} ...
x-ray detector



X-ray beams from two different angles
3 3 3
2 2 2
1 1 1
9
6
3
Detector measurement of the attenuation coefficient
3
5
6
3
1
Areas with different attenuation coefficients

CT scan with x-rays of a human body

→ best performance  voxel size of $0.3\ mm^2$

→ gantry revolves around the patient in 0.25 seconds

(less than a heart beat)

(less blurring)

To get better resolution, we can use other techniques

1) smaller set-up with smaller x-ray beam

2) x-ray focussing
   - micro CT
   - nano CT

3) phase-contrast imaging

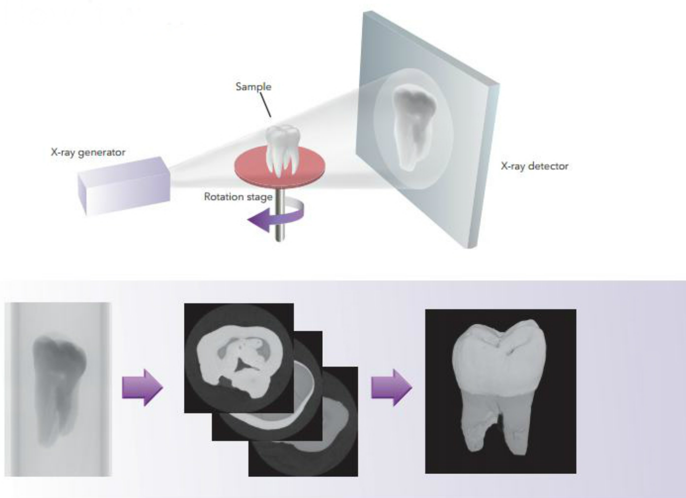
4) more intense x-ray beams (observe time → 4D)

→ synchroton x-rays

→ free-electron laser x-rays

micro CT

- → better resolution than CT scan
- → limited to sample size of 50 cm × 50 cm × 50 cm
- → resolutions of 1 μm (1E-6 m)

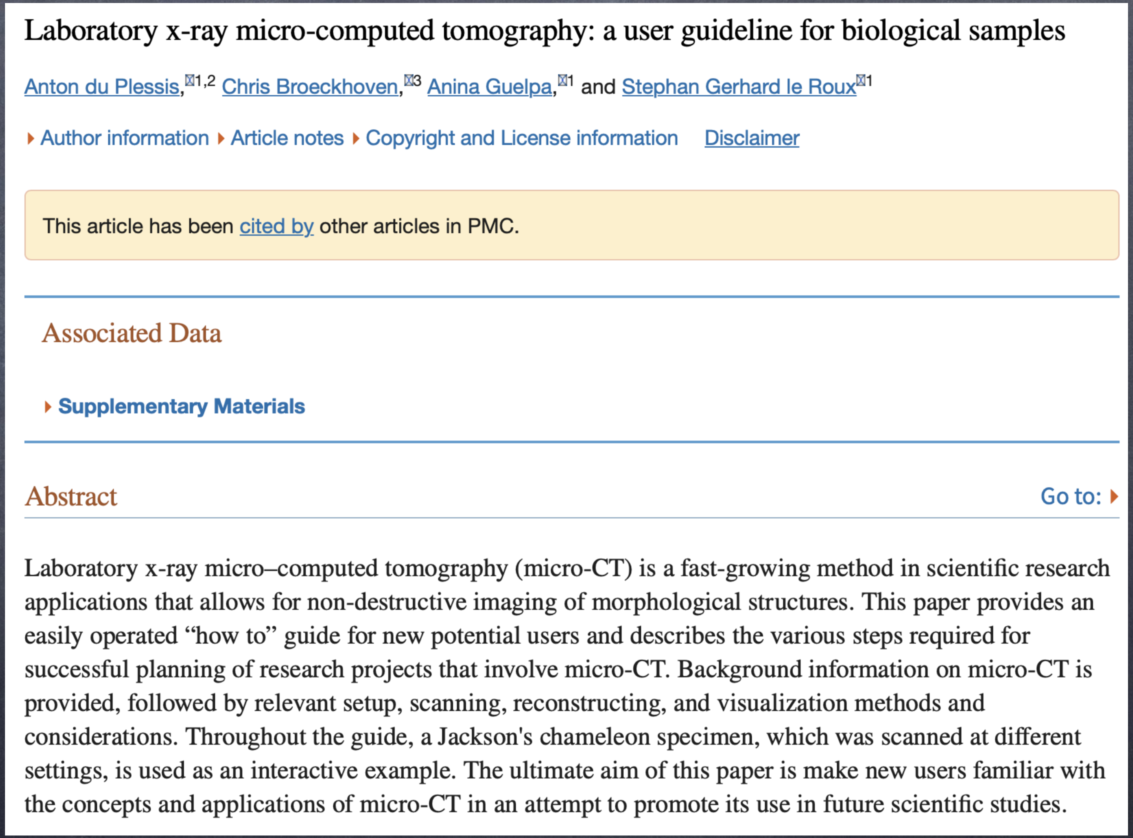


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5449646/

μCT

mCT

(non-destructive)



Laboratory x-ray micro-computed tomography: a user guideline for biological samples

Anton du Plessis,[1,2] Chris Broeckhoven,[3] Anina Guelpa,[1] and Stephan Gerhard le Roux[1]

This article has been cited by other articles in PMC.

Associated Data

Abstract

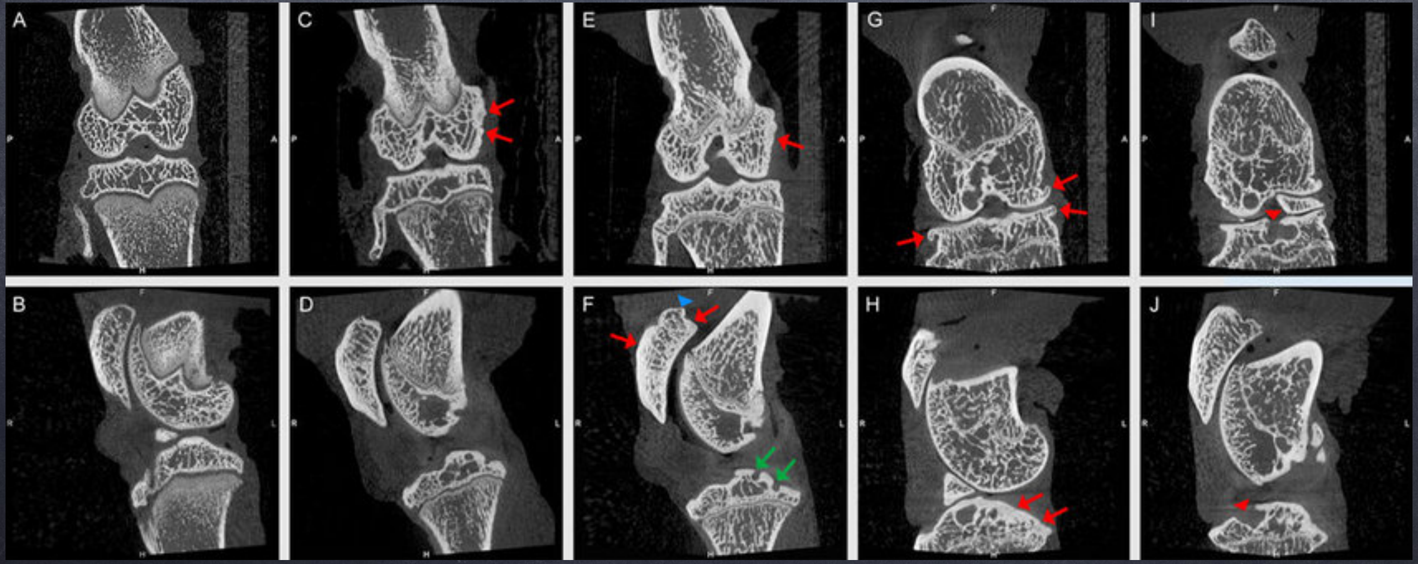
Laboratory x-ray micro–computed tomography (micro-CT) is a fast-growing method in scientific research applications that allows for non-destructive imaging of morphological structures. This paper provides an easily operated “how to” guide for new potential users and describes the various steps required for successful planning of research projects that involve micro-CT. Background information on micro-CT is provided, followed by relevant setup, scanning, reconstructing, and visualization methods and considerations. Throughout the guide, a Jackson's chameleon specimen, which was scanned at different settings, is used as an interactive example. The ultimate aim of this paper is make new users familiar with the concepts and applications of micro-CT in an attempt to promote its use in future scientific studies.

Biological applications of micro-CT

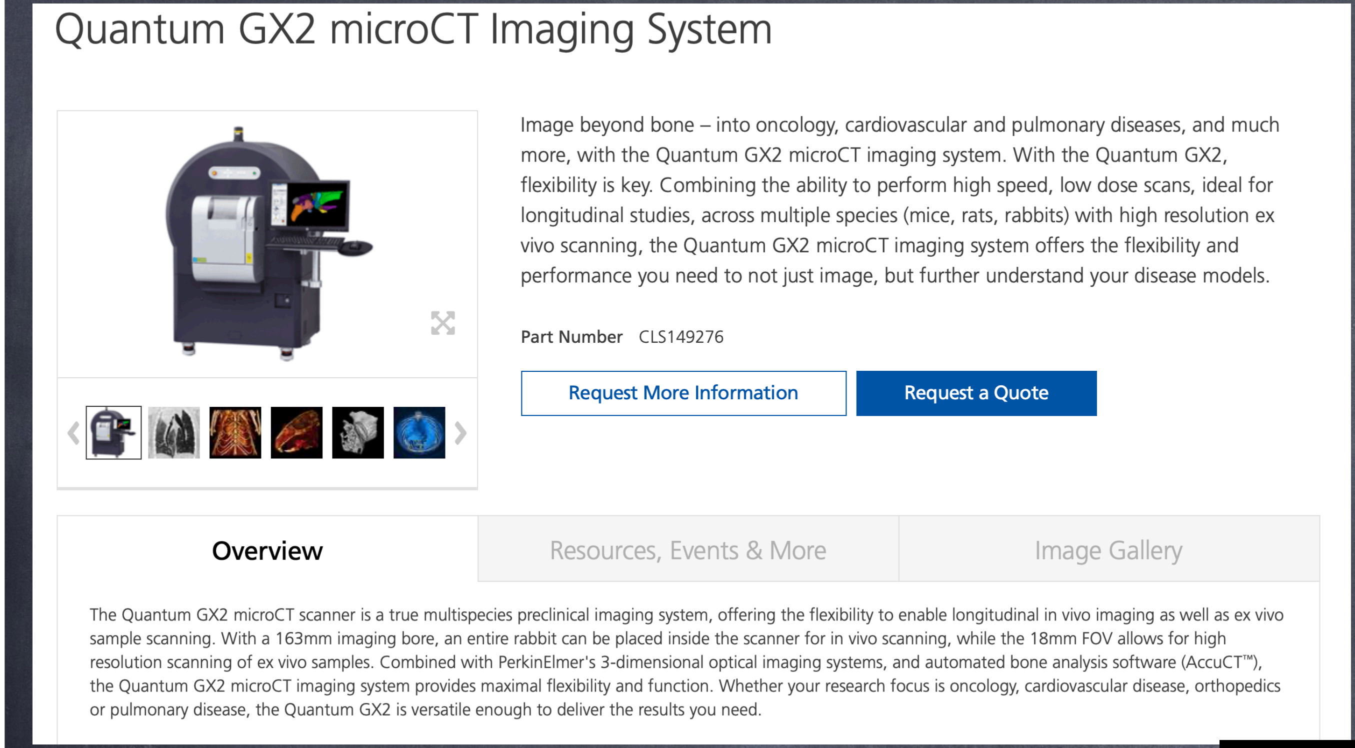
Related Stories

Micro-CT has been successfully applied to biological imaging in the following areas:

- In vivo imaging of head / knee
- Bone analysis
- Lung tumor detection in vivo and ex vivo
- Imaging and quantification of tumors
- Ex vivo imaging of the rabbit brain
- Phenotyping of the mouse kidney
- Imaging of mouse heart calcification and chest of live animals using contrast agents in vivo
- Imaging of tooth and jaw bone in mice



Representative photos from microCT evaluation of knee joints using the novel scoring system. (A) Dorsal and (B) sagittal reconstructions from a 2 month old Hartley guinea pig with no clinically significant OA lesions.



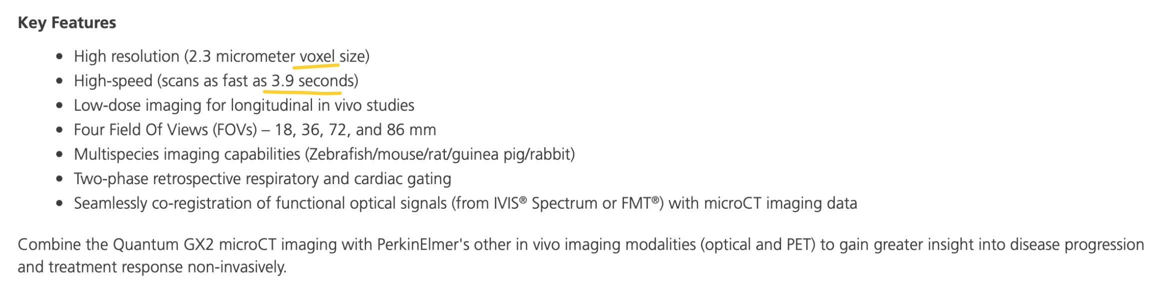
Quantum GX2 microCT Imaging System

Image beyond bone – into oncology, cardiovascular and pulmonary diseases, and much more, with the Quantum GX2 microCT imaging system. With the Quantum GX2, flexibility is key. Combining the ability to perform high speed, low dose scans, ideal for longitudinal studies, across multiple species (mice, rats, rabbits) with high resolution ex vivo scanning, the Quantum GX2 microCT imaging system offers the flexibility and performance you need to not just image, but further understand your disease models.

Part Number CLS149276

The Quantum GX2 microCT scanner is a true multispecies preclinical imaging system, offering the flexibility to enable longitudinal in vivo imaging as well as ex vivo sample scanning. With a 163mm imaging bore, an entire rabbit can be placed inside the scanner for in vivo scanning, while the 18mm FOV allows for high resolution scanning of ex vivo samples. Combined with PerkinElmer's 3-dimensional optical imaging systems, and automated bone analysis software (AccuCT™), the Quantum GX2 microCT imaging system provides maximal flexibility and function. Whether your research focus is oncology, cardiovascular disease, orthopedics or pulmonary disease, the Quantum GX2 is versatile enough to deliver the results you need.

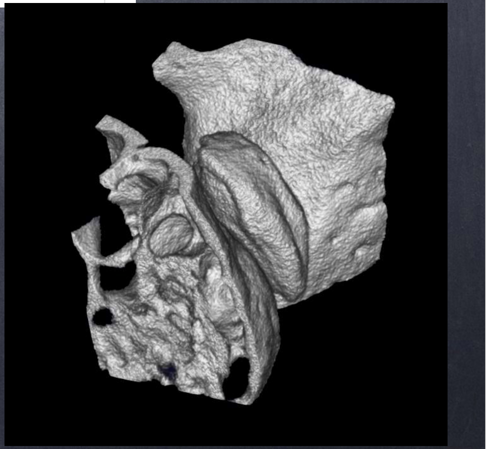
2.3 μm



Key Features

- High resolution (2.3 micrometer voxel size)
- High-speed (scans as fast as 3.9 seconds)
- Low-dose imaging for longitudinal in vivo studies
- Four Field Of Views (FOVs) – 18, 36, 72, and 86 mm
- Multispecies imaging capabilities (Zebrafish/mouse/rat/guinea pig/rabbit)
- Two-phase retrospective respiratory and cardiac gating
- Seamlessly co-registration of functional optical signals (from IVIS® Spectrum or FMT®) with microCT imaging data

Combine the Quantum GX2 microCT imaging with PerkinElmer's other in vivo imaging modalities (optical and PET) to gain greater insight into disease progression and treatment response non-invasively.



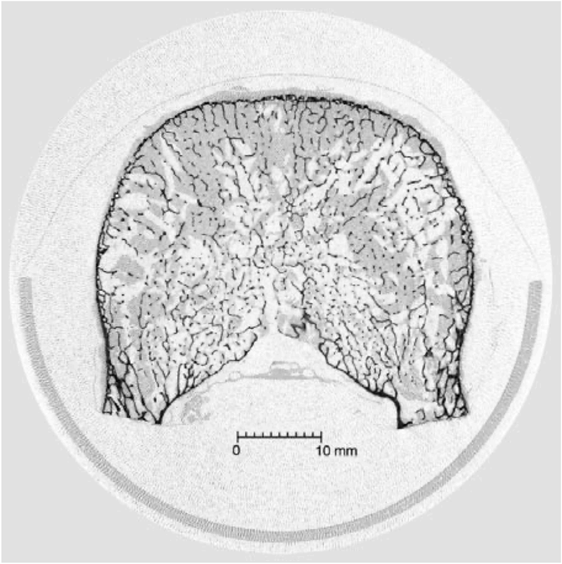


Fig. 1 | *Micro-CT cross-section image of a human vertebral body, at 17.4 μm pixel size (3936 × 3936 pixel, 68.5 × 68.5 mm).*

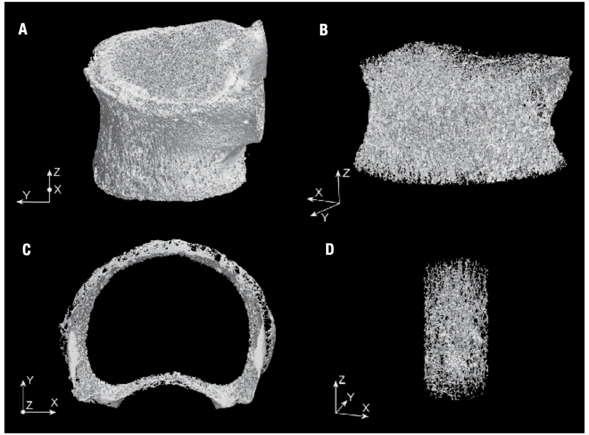
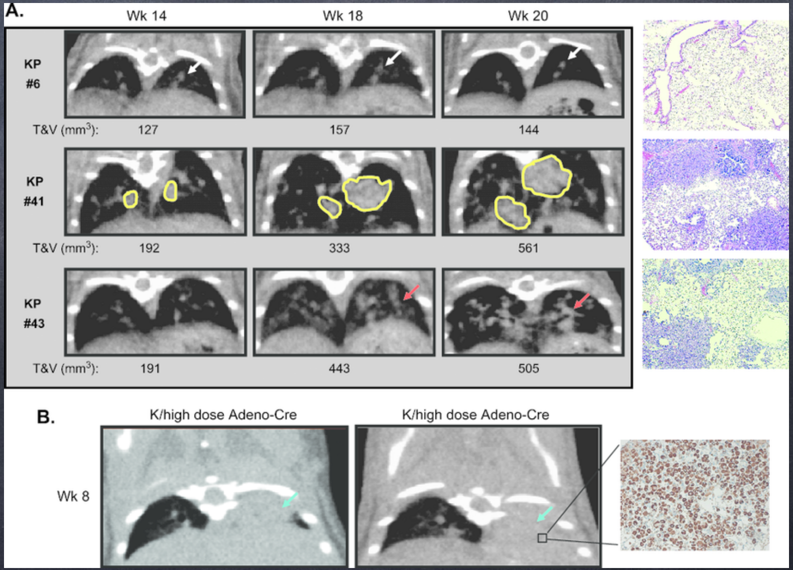


Fig. 2 | *Three-dimensional micro-CT images of a vertebral body scanned at 17.4 μm pixel size. (A) entire vertebral body (B) trabecular bone compartment (VOI) within the endplates, over which the trabecular bone morphometric parameters were calculated (C) cortical bone compartment, superior-inferior view (D) cylindrical subvolume of trabecular bone, 10 mm in diameter, 20 mm in height.*



Lung tumor identification in mouse

University of Zurich^UZH

Home

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- Optical Imaging
- **microCT**
- Body Composition Analysis
- Irradiation
- Preclinical Ultrasound
- MRI

microCT

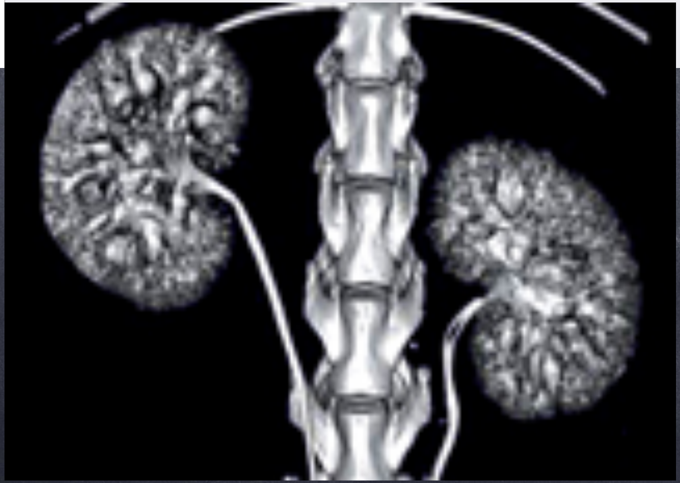
Bildband

Location:

Zurich Integrative Rodent Physiology (ZIRP)
Winterthurer Str. 190
CH-8057 Zürich
Phone: +41446355095
→ directions

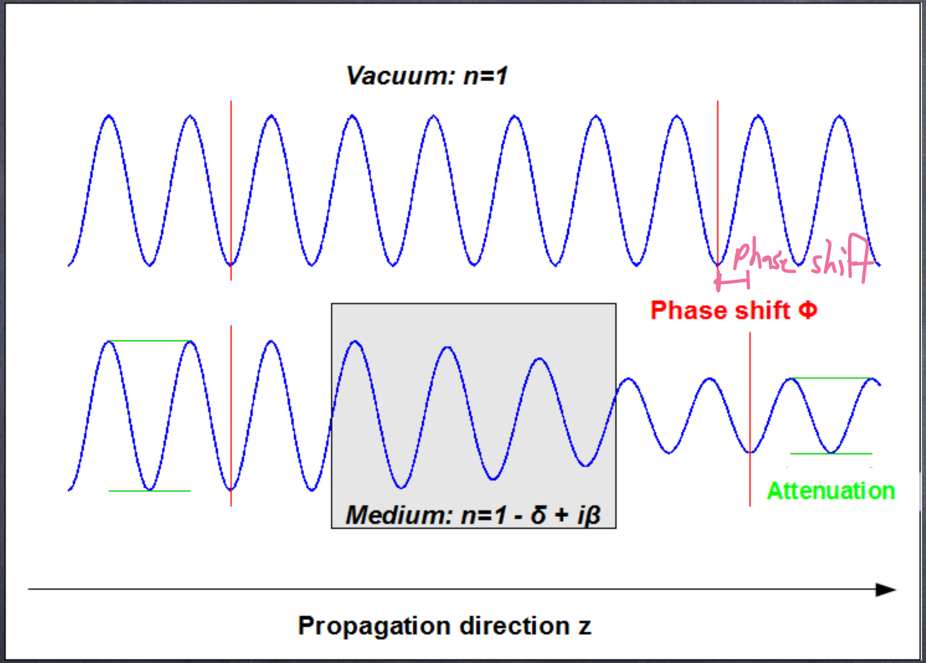
ZIRP news:

news
courses, lectures



Phase-contrast X-ray imaging

x-ray



2 things happen!

1) attenuation
amplitude decreases

2) phase-shift

phase shift is because ~~is a m~~ the wavelength is different in a material with index of refraction, n.

x-ray →

different refraction index

$i^2 = -1$

$$n = 1 - \delta + i\beta$$

complex refraction index

δ ↑ describes change in phase because of change in wavelength

$i\beta$ ↑ describes the absorption (or extinction) coefficient

Electric field wave function ↓

$$E(x) = E_0 e^{inkx}$$

↑ initial

n : index of refraction

k : wave number = $\frac{2\pi}{\lambda}$

x : distance travelled.

$$E(x) = \underbrace{E_0 e^{i(1-\delta)kx}}_{\text{phase shift}} \underbrace{e^{-\beta kx}}_{\text{attenuation}}$$

complex math :

$i^2 = -1$

$$e^{i\theta} = \underbrace{\cos\theta}_{\text{real part which we can measure}} + \underbrace{i\sin\theta}_{\text{imaginary part}}$$

attenuation: $\beta = \frac{\rho_a \sigma_a}{k}$

phase shift: $\delta = \frac{\rho_a p}{k}$

$\sigma_a = 0.02\,[\text{barns}] \left(\frac{k_0}{k}\right)^3 Z^4$

k: wave number $= \frac{2\pi}{\lambda}$

ρ_a: atomic number density

σ_a: absorption cross-section

p: phase-shift cross-section

$p = \frac{2\pi Z r_0}{k}$

Z: atomic number,

r_0: classical electron radius

$1 \text{ barn} = 10^{-28} \text{ m}^2$

k_0: length of wave with $\lambda = 1\text{e-}10$ m

substitute in values: $\delta = \frac{2\pi \rho_a Z r_0}{k^2}$

$\beta = 0.01 \text{ barn } \rho_a k_0^3 \left(\frac{Z}{k}\right)^4$

we see that $\underbrace{\beta \propto k^{-4}}_{\text{attenuation}}, \underbrace{\delta \propto k^{-2}}_{\text{phase shift}}$

For human tissue, δ is 3 orders of magnitude bigger than β

→ The phase shift is much larger than the attenuation.

→ More sensitive to differences in density.

→ k^{-4} vs. k^{-2} means the phase contrast benefit grows with increasing energy

- phase contrast approach has advantages over absorption approach
  - → different dependence on energy
  - → x-rays don't need to be absorbed → less radiation dose
  - → use higher energy x-rays that have less attenuation (less radiation dose)
- in phase-contrast imaging one observes the interference between light passing through a material and light that doesn't pass through.

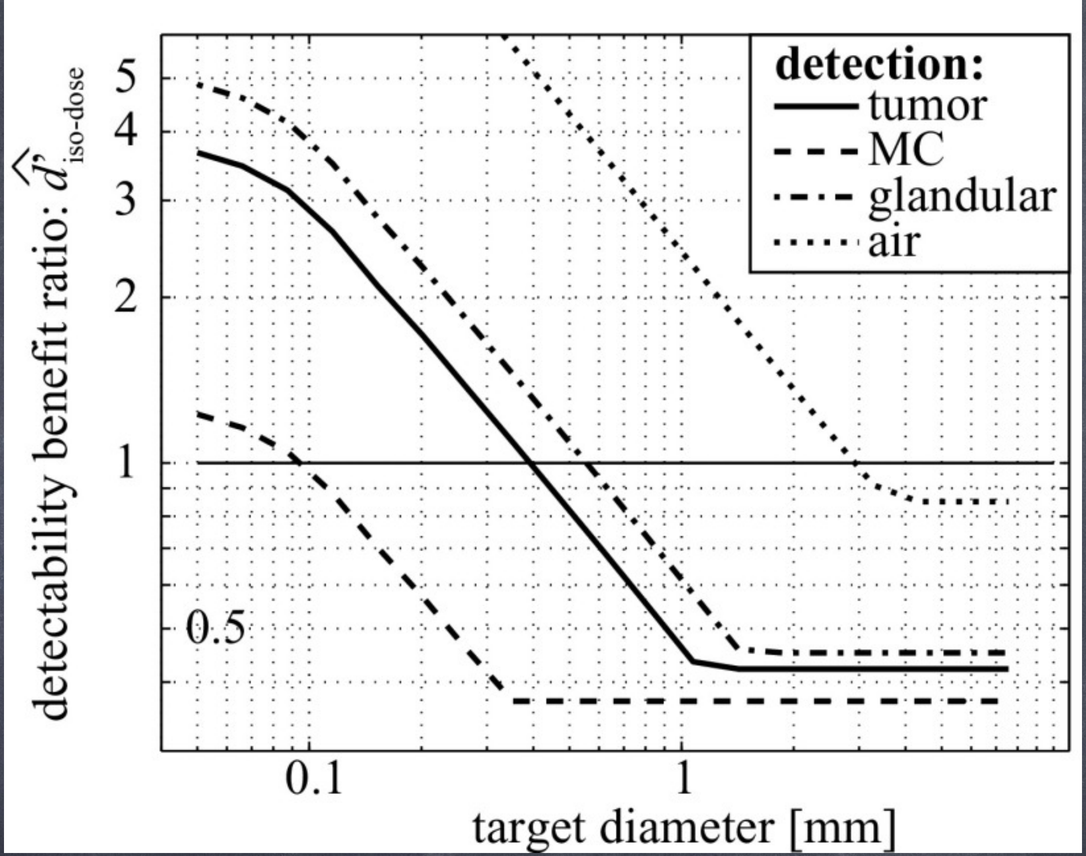
Benefits of phase-contrast imaging:

1) more sensitive to differences in tissue density (tumor detection)
2) higher energy x-rays provide less dose
3) phase shift ~~is~~ in soft tissue is mainly larger than the absorption.

Absorption X-ray vs. phase-contrast X-ray

(In-ear headphone)

better
↑



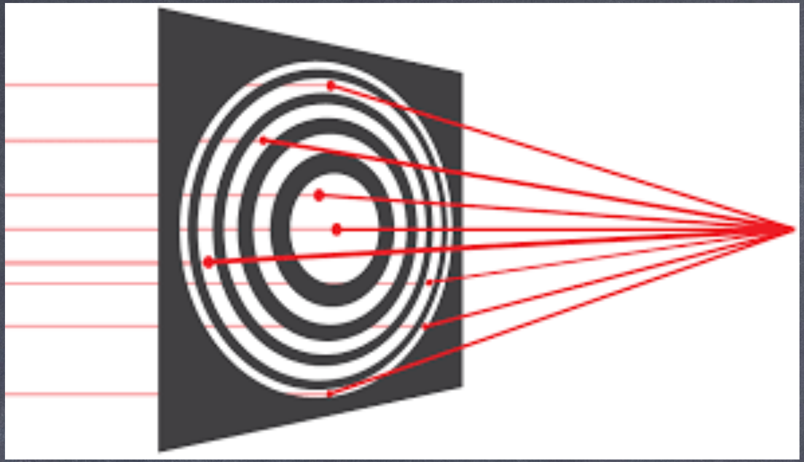
The benefit of phase contrast mammography relative to absorption contrast for (1) a tumor structure (“tumor”), (2) a glandular structure (“glandular”), (3) a microcalcification (“MC”), and (4) an air cavity (“air”) as a function of target size at optimal energy and equal dose.[97]

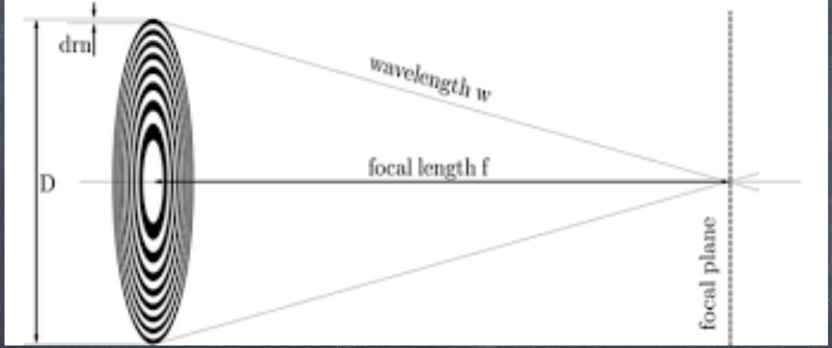
https://en.wikipedia.org/wiki/Phase-contrast_X-ray_imaging#Phase-contrast_x-ray_imaging_in_medicine

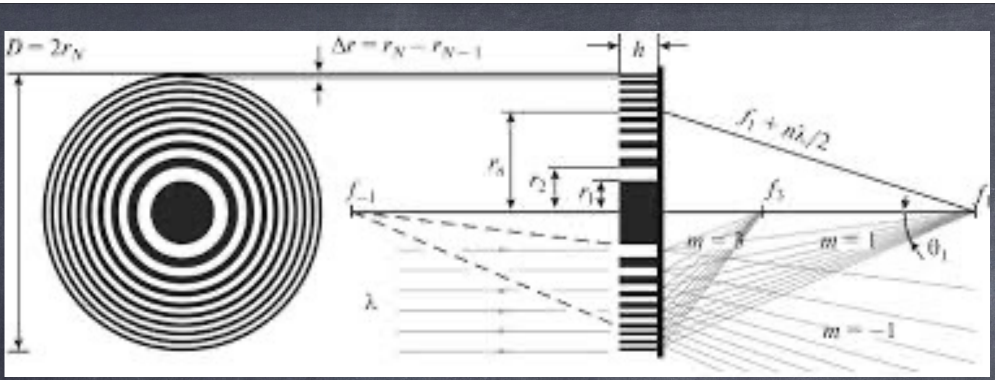
Focussing X-rays

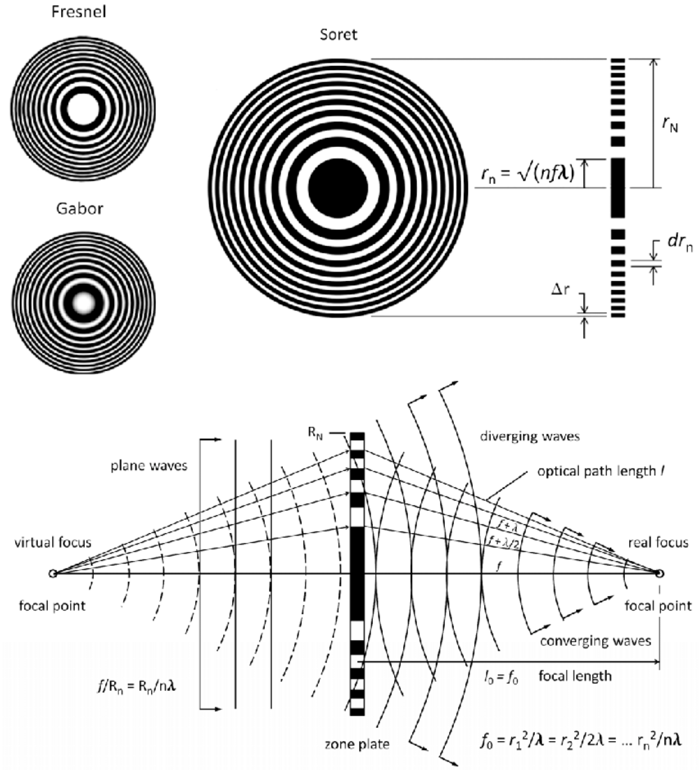
microwaves ($\lambda \sim$ cm)

Fresnel zone plate



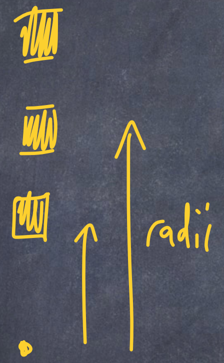

drn
D
wavelength w
focal length f
focal plane


$D = 2r_N$
$\Delta r = r_N - r_{N-1}$
h
$f_1 + n\lambda/2$
r_n
r_2
r_1
f_{-1}
f_3
$m = 3$
$m = 1$
θ_1
λ
$m = -1$


Fresnel
Soret
Gabor
r_N
$r_n = \sqrt{(nf\lambda)}$
dr_n
Δr
R_N
diverging waves
plane waves
optical path length l
$f+\lambda$
$f+\lambda/2$
f
virtual focus
real focus
focal point
focal point
converging waves
$f/R_n = R_n/n\lambda$
$l_0 = f_0$
focal length
zone plate
$f_0 = r_1^2/\lambda = r_2^2/2\lambda = \dots r_n^2/n\lambda$

Fresnel zone plate

to get constructive interference at the focus, the zones (radii) should switch between transparent and opaque at radii where



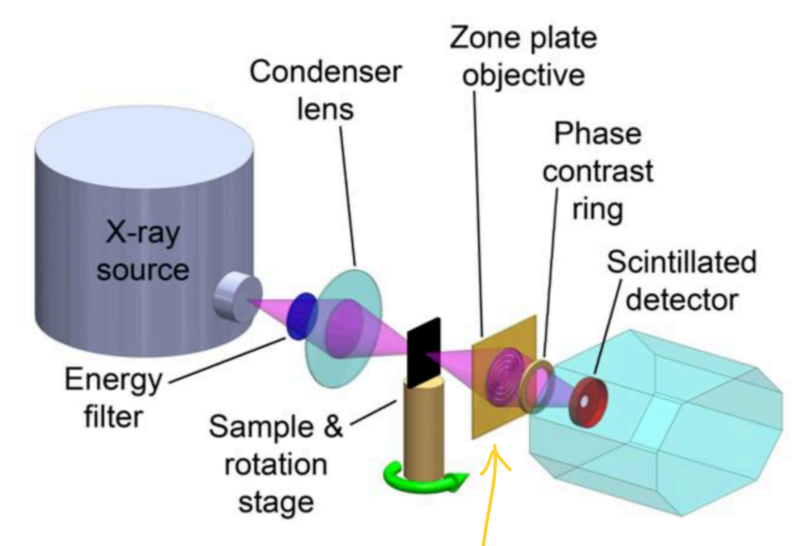
$$r_n = \sqrt{n\lambda f + \frac{1}{4}n^2\lambda^2}$$

n: integer
λ: wavelength of light
f: distance from center of zone plate to the focus plane.

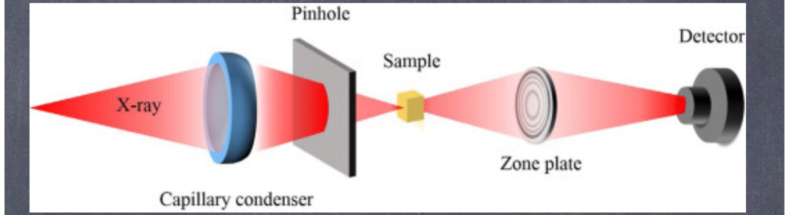
If zone plate is small compared to the focal length f, then

$$r_n \simeq \sqrt{n\lambda f}$$

we can do this with X-rays as well

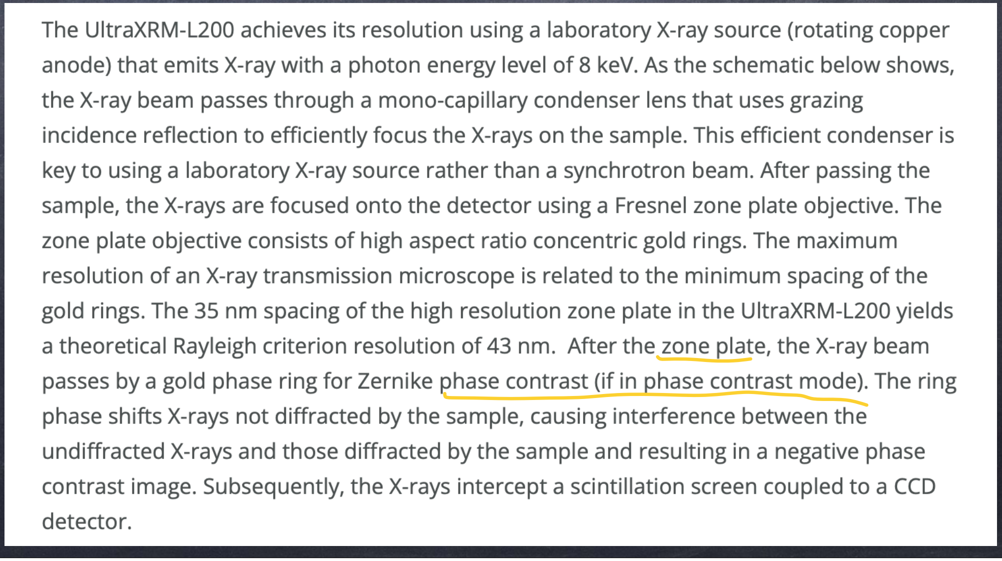


zone plate

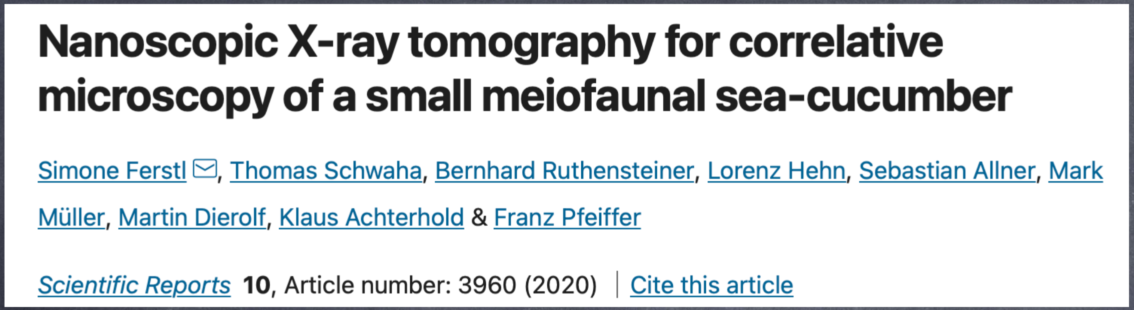


https://www.cmu.edu/me/xctf/facility/index.html

Fresnel zone plate



The UltraXRM-L200 achieves its resolution using a laboratory X-ray source (rotating copper anode) that emits X-ray with a photon energy level of 8 keV. As the schematic below shows, the X-ray beam passes through a mono-capillary condenser lens that uses grazing incidence reflection to efficiently focus the X-rays on the sample. This efficient condenser is key to using a laboratory X-ray source rather than a synchrotron beam. After passing the sample, the X-rays are focused onto the detector using a Fresnel zone plate objective. The zone plate objective consists of high aspect ratio concentric gold rings. The maximum resolution of an X-ray transmission microscope is related to the minimum spacing of the gold rings. The 35 nm spacing of the high resolution zone plate in the UltraXRM-L200 yields a theoretical Rayleigh criterion resolution of 43 nm. After the zone plate, the X-ray beam passes by a gold phase ring for Zernike phase contrast (if in phase contrast mode). The ring phase shifts X-rays not diffracted by the sample, causing interference between the undiffracted X-rays and those diffracted by the sample and resulting in a negative phase contrast image. Subsequently, the X-rays intercept a scintillation screen coupled to a CCD detector.



Nanoscopic X-ray tomography for correlative microscopy of a small meiofaunal sea-cucumber

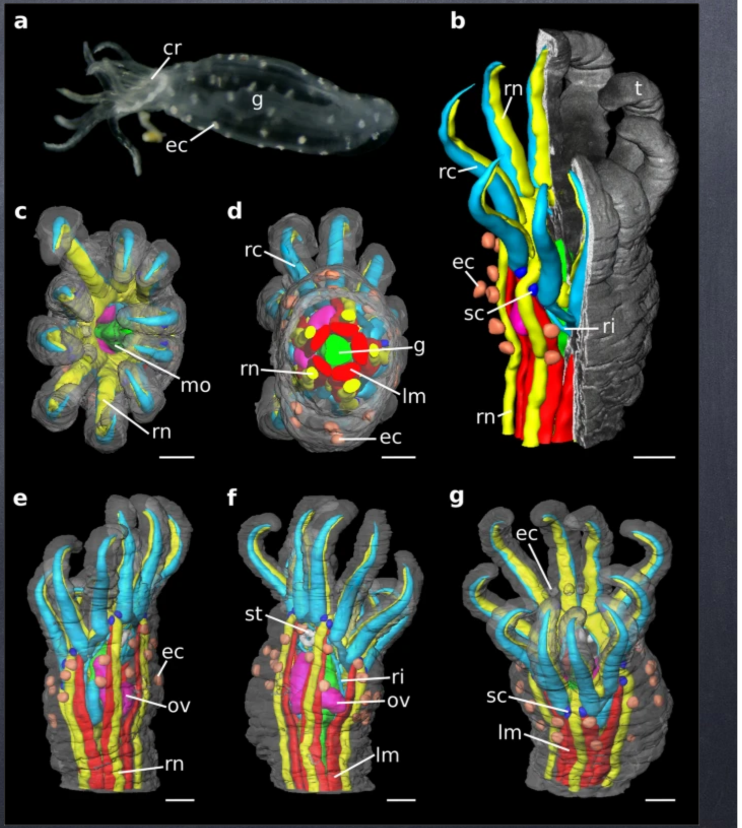
Simone Ferstl, Thomas Schwaha, Bernhard Ruthensteiner, Lorenz Hehn, Sebastian Allner, Mark Müller, Martin Dierolf, Klaus Achterhold & Franz Pfeiffer

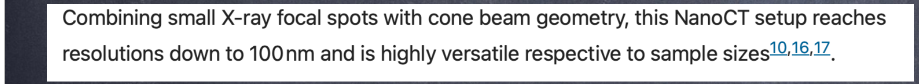
Scientific Reports **10**, Article number: 3960 (2020) | Cite this article

nano CT

https://www.nature.com/articles/s41598-020-60977-5

Worm ~ 1mm length

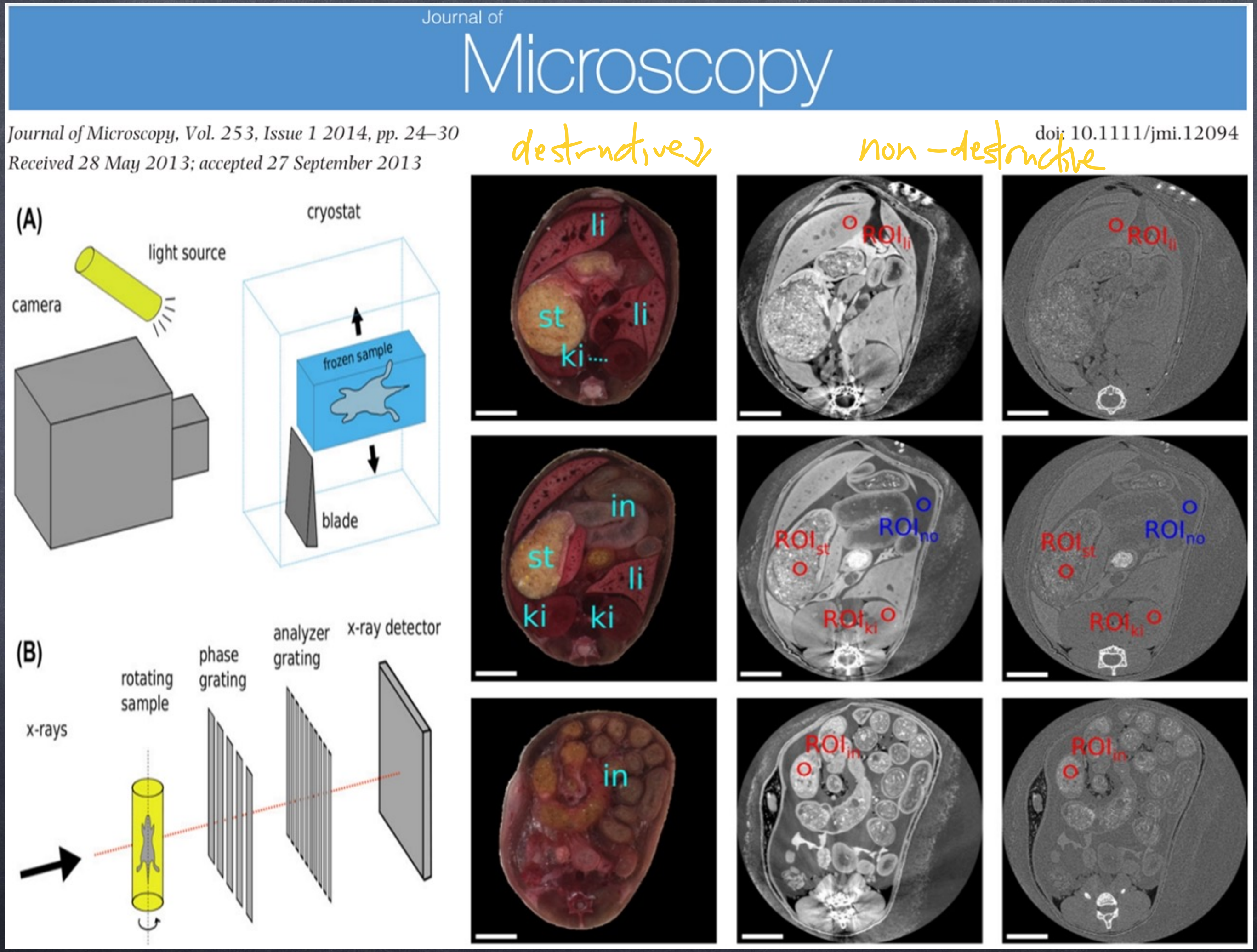




Combining small X-ray focal spots with cone beam geometry, this NanoCT setup reaches resolutions down to 100 nm and is highly versatile respective to sample sizes[10,16,17].

↑

resolutions ~100 nm



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Microscopy

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destructive

non-destructive

visible light

x-ray light

destructive

non-destructive

X-ray sources

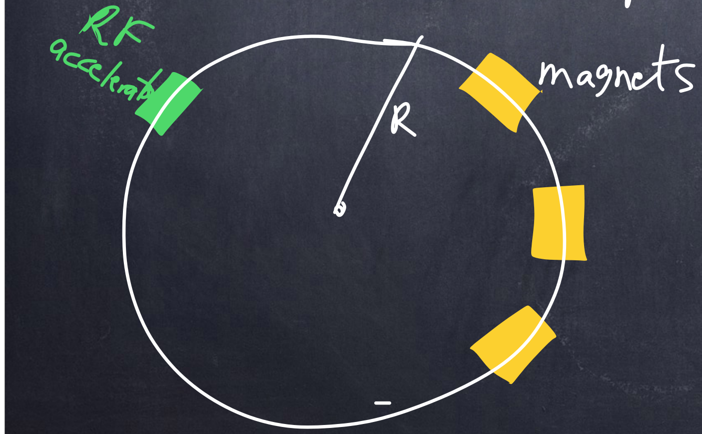
Besides X-ray tubes, X-rays can be produced by synchrotrons.

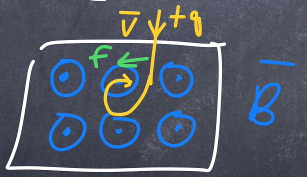
The Large Hadron Collider (LHC) at CERN is the world's largest synchrotron.

The Swiss Light Source (SLS) at PSI is the most relevant for today.

How does a synchrotron work?

$$\bar{F} = q\bar{v}\times\bar{B} \quad \text{(Lorentz force)}$$





$\bar{F}_B = q\bar{v} \times \bar{B} = qvB$ if $\bar{v} \perp \bar{B}$

provides centripetal acceleration, $F_c = \frac{mv^2}{R}$

set $F_B = F_c$

$qvB = \frac{mv^2}{R}$ $\qquad qB = \frac{mv}{R}$

momentum = $p = mv$ (classical momentum)

$B = \frac{p}{qR}$ $\qquad R = \frac{p}{qB}$

relativistic momentum, $p = mv\gamma$

$$\gamma = \frac{1}{\sqrt{1-\frac{v^2}{c^2}}}$$

The orbital frequency $\nu = \frac{\omega}{2\pi}$ $\quad \omega = \frac{v}{R}$ $\quad v$ (velocity)

$$\nu = \frac{v}{2\pi R} = \frac{m v}{m 2\pi R} = \frac{p}{R} \frac{1}{2\pi m} = \frac{p}{\frac{p}{qB}} \cdot \frac{1}{2\pi m} = \frac{qB}{2\pi m}$$

This needs a relativistic correction because time slows down for fast ($v \sim c$) particles,

$$\nu = \frac{qB}{2\pi m} \sqrt{1 - \frac{v^2}{c^2}}$$

In a synchrotron, the magnetic field is increased as the particle momentum increases because the radius R is constant.

So p & B increase together.

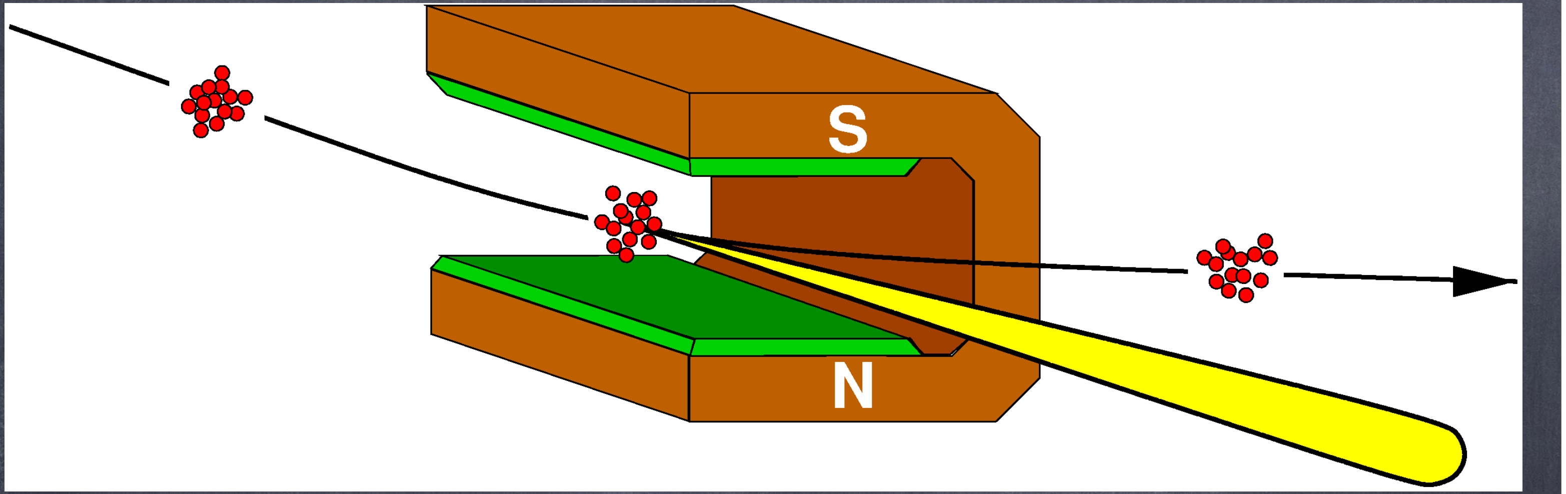
European Synchrotron Radiation Facility (ESRF) in Grenoble, France

13 member countries: France, Germany, Italy, the UK, Spain, Switzerland, Belgium, the Netherlands, Denmark, Finland, Norway, Sweden, Russia

World's largest

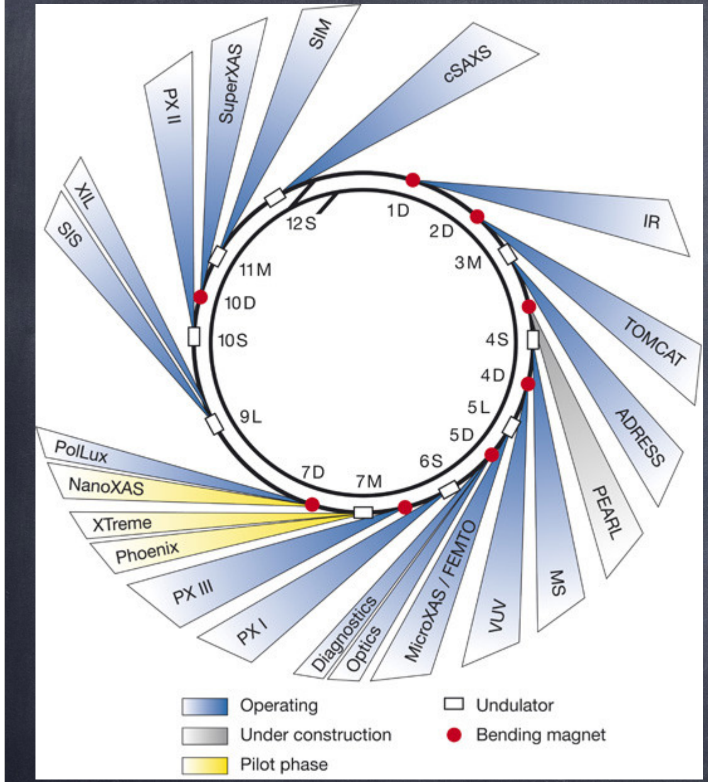
10,000 billion more powerful than X-rays used in the medical field.

Swiss Light Source (SLS) at PSI, Switzerland (45 minutes away)


S
N


e^-
e^-

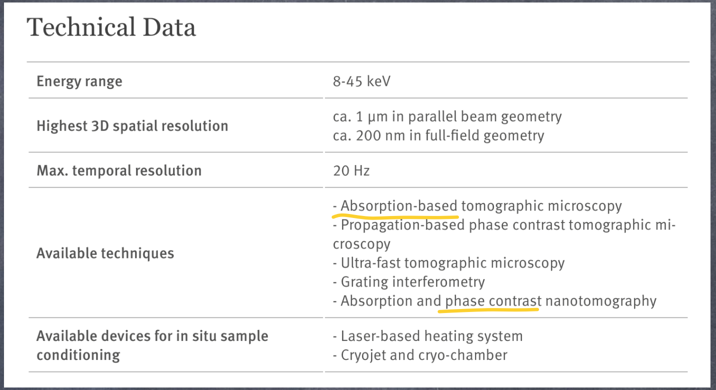
Swiss Light Source (SLS)



TOMCAT (A beamline for TOmographic Microscopy and Coherent rAdiology experimenTs)

https://www.psi.ch/en/sls/tomcat

TOMCAT beam line



Technical Data

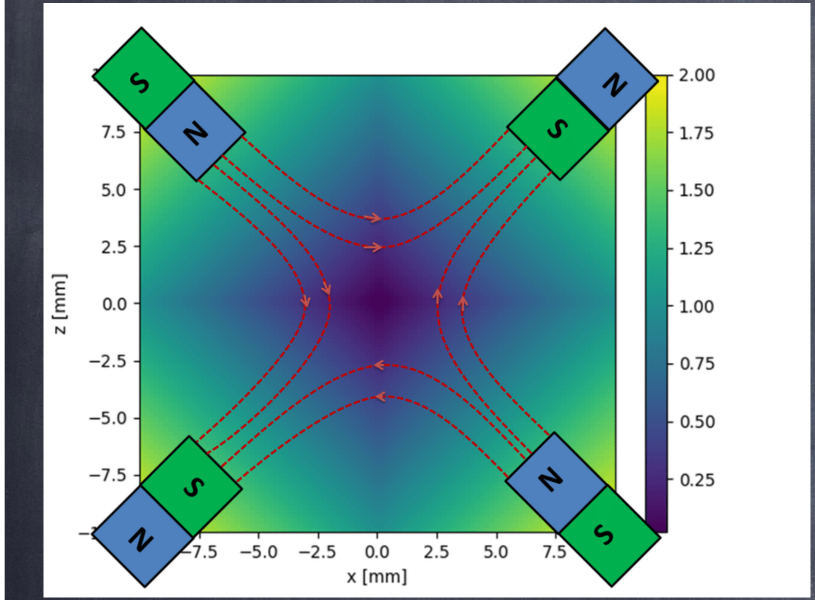
Energy range	8-45 keV
Highest 3D spatial resolution	ca. 1 µm in parallel beam geometry ca. 200 nm in full-field geometry
Max. temporal resolution	20 Hz
Available techniques	- Absorption-based tomographic microscopy - Propagation-based phase contrast tomographic microscopy - Ultra-fast tomographic microscopy - Grating interferometry - Absorption and phase contrast nanotomography
Available devices for in situ sample conditioning	- Laser-based heating system - Cryojet and cryo-chamber

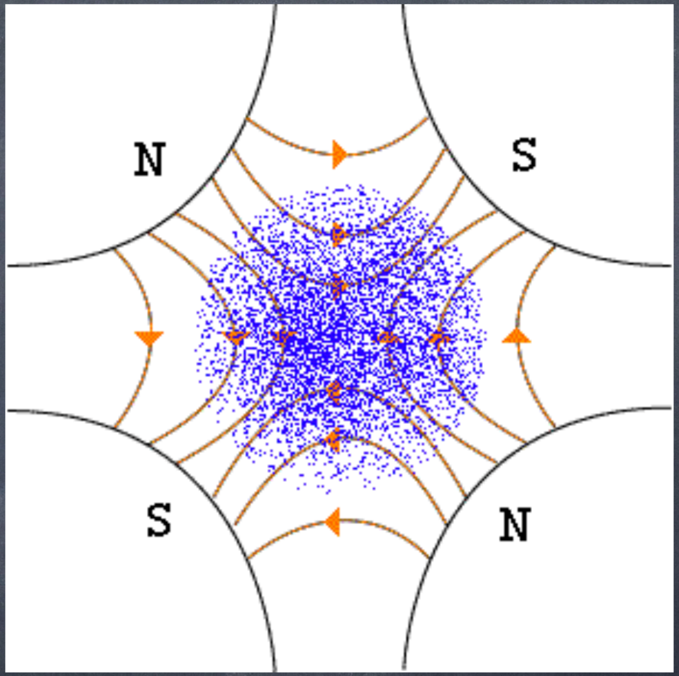
65 nm pixel size (resolution)

Absorption-based and phase contrast imaging are routinely performed with isotropic voxel sizes ranging from 0.16 to 11 µm (fields-of-view (h x v) of 0.4 x 0.3 mm^2 and 22 x 3-7 mm^2, respectively) in an energy range of 8-45 keV. Phase contrast is obtained with simple edge-enhancement, propagation-based techniques [2, 3] or through grating interferometry [4].

A temporal resolution of a few (< 5) minutes can also be achieved with the hard X-ray full-field microscope setup [8] delivering a pixel size of 65 nm for microscopic samples (~75x75 µm^2 field-of-view).

How to focus electrons?

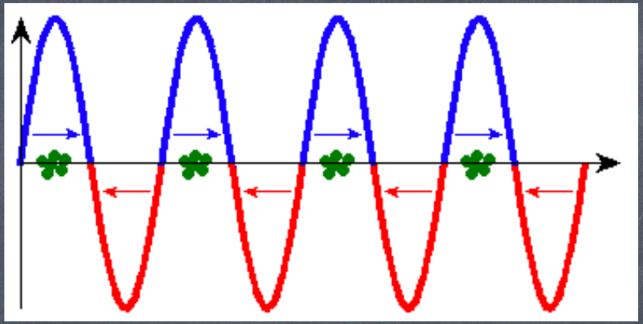


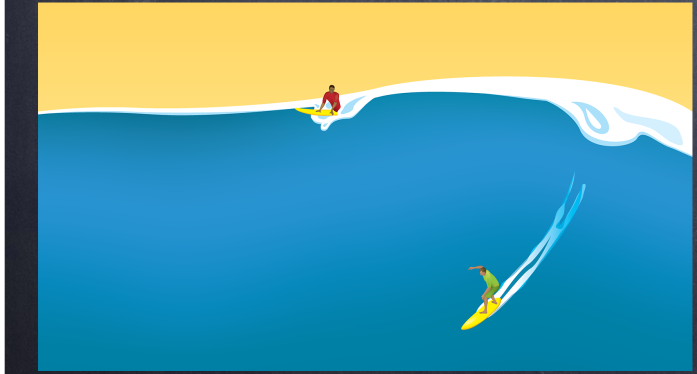


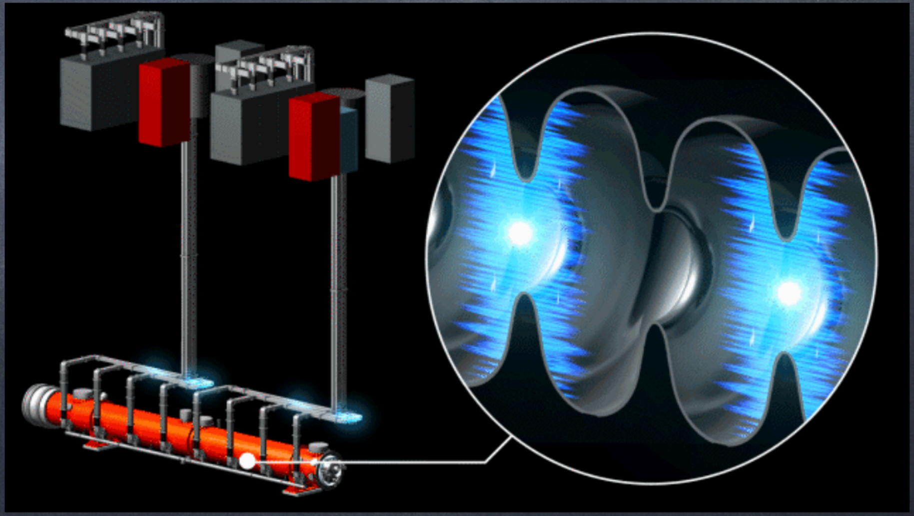
quadrupole magnet

How to accelerate electrons

RF acceleration (YouTube video here https://www.youtube.com/watch?v=mu4m7wSnpD0)







Superconducting RF cavities

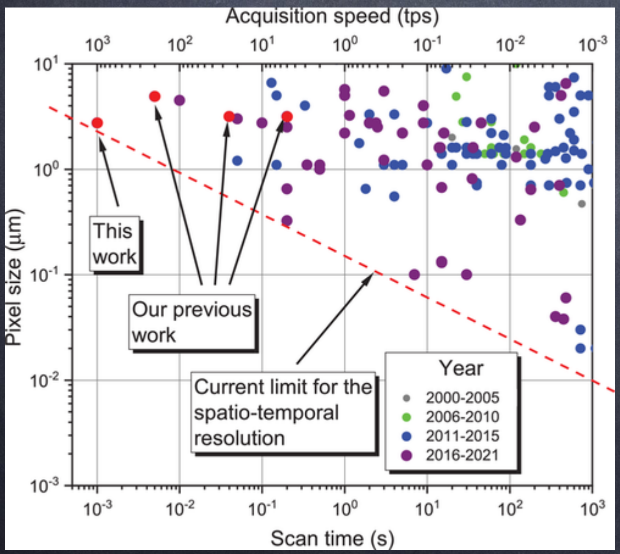
May 2022 News : LCLS-II at Stanford will produce X-ray pulses that are 10,000 times brighter, on average, than those of LCLS and that arrive up to a million times per second – a world record for today's most powerful X-ray light sources.

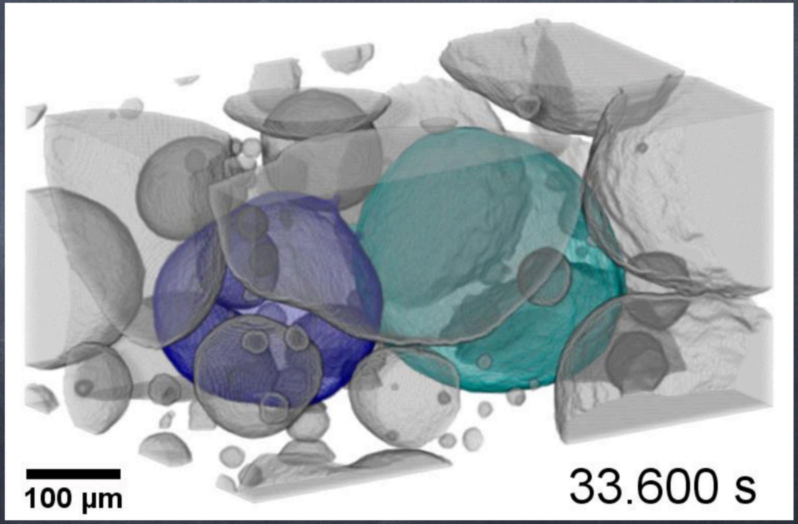
24 September 2021

X-ray microscopy with 1000 tomograms per second

Research Using Synchrotron Light | Materials Research | Matter and Material

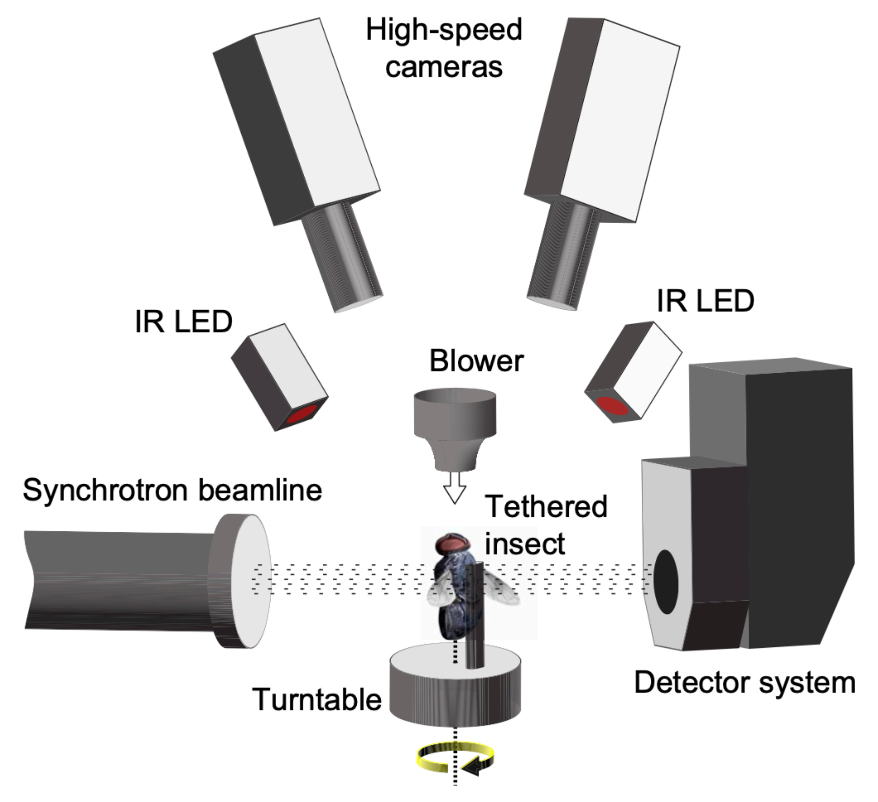
A team at the Swiss Light Source SLS have set a new record using an imaging method called tomoscopy.





100 µm Bubble coalescence

https://onlinelibrary.wiley.com/doi/10.1002/adma.202104659



https://doi.org/10.1371/journal.pbio.1001823

https://pubmed.ncbi.nlm.nih.gov/18682361/

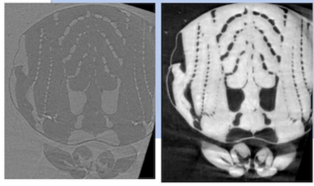
ETH zürich Imperial College London UNIVERSITY OF OXFORD PSI

Muscles and tracheal network *during* flight

Von Prof. Marco Stampanoni / ETHZ und PSI

Surreal X-ray movie reveals how a fly beats its wings

21:00 25 March 2014 by Jacob Aron
For similar stories, visit the Picture of the day Topic Guide



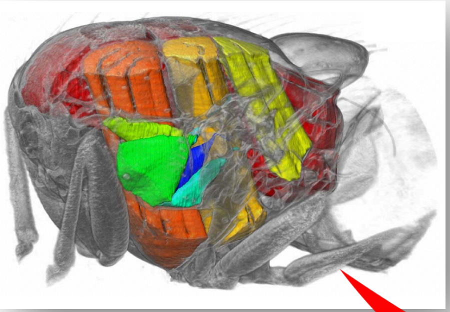
Walker et al., PloS Biology (2014) & Mokso et al., SciRep (2015)

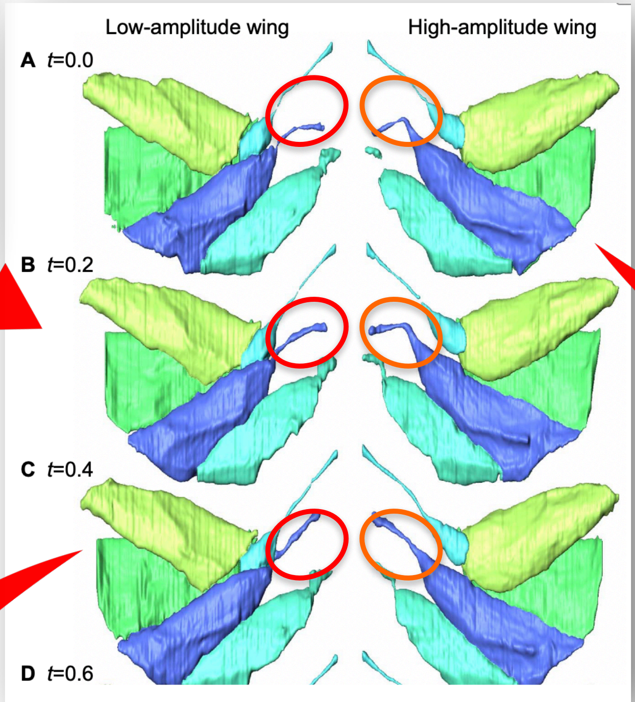
Scientists film inside a flying insect

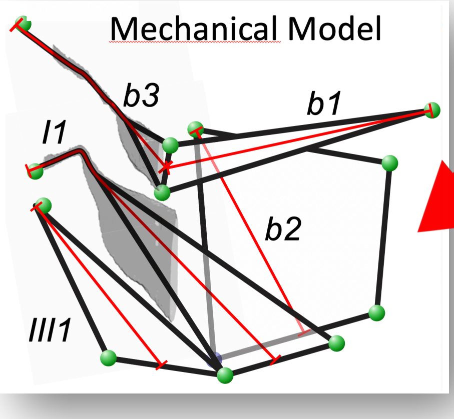
By Victoria Gill
Science reporter, BBC News

26 March 2014 Science & Environment

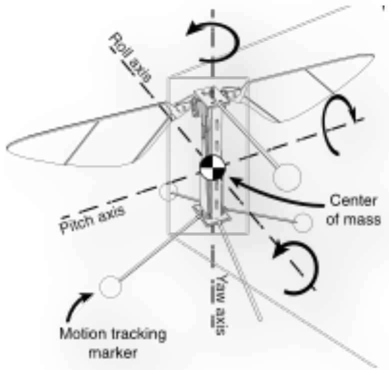
Dynamic X-ray microscopy boosting the development of bio-inspired robotics







Walker S. et al., PloS Biology (2014)



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Jafferis et al., Nature 2019

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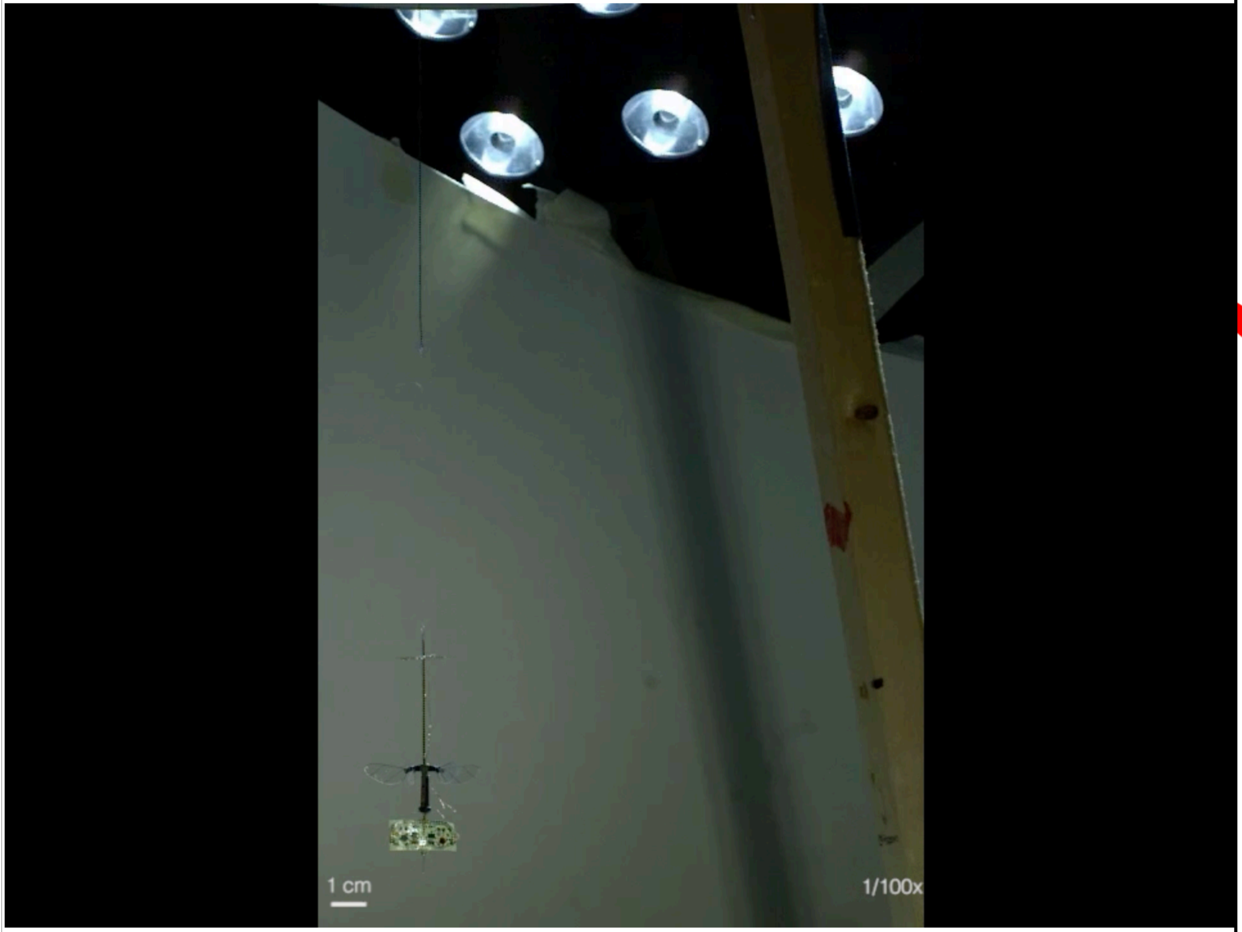
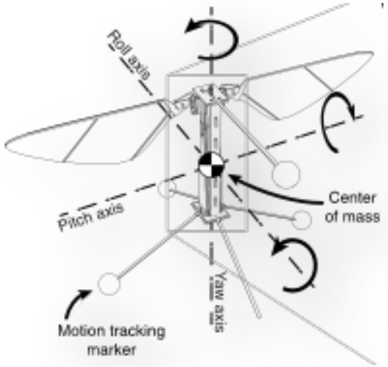


Walker S. et al., PloS Biology (2014)



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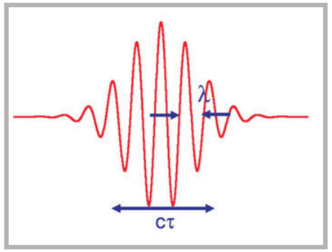
we want 4-D imaging

3-D + time

spatial & temporal resolution

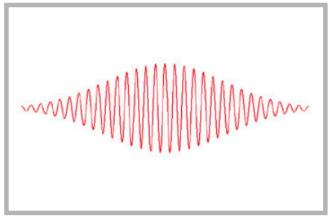


Wanted: short spatial and temporal resolution

laser (good time resolution)

Lasers have poor spacial resolution due to long wavelength

synchrotrons
poor time res.
good pos. res.

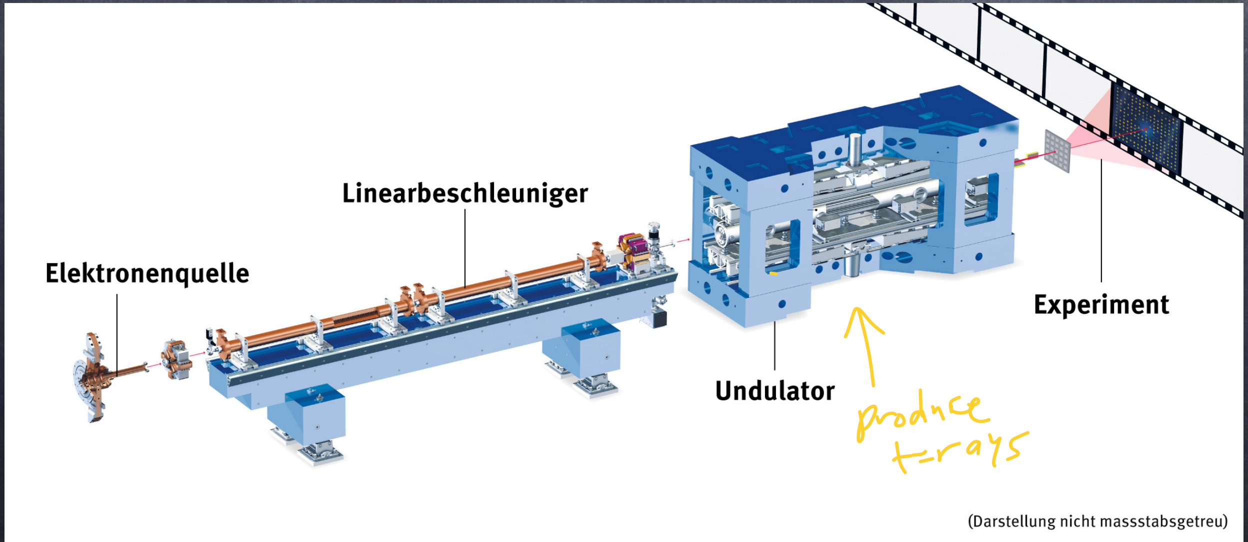


Synchrotrons have a poor temporal resolution due to the long pulse length

(FEL)
Free
Electron
laser

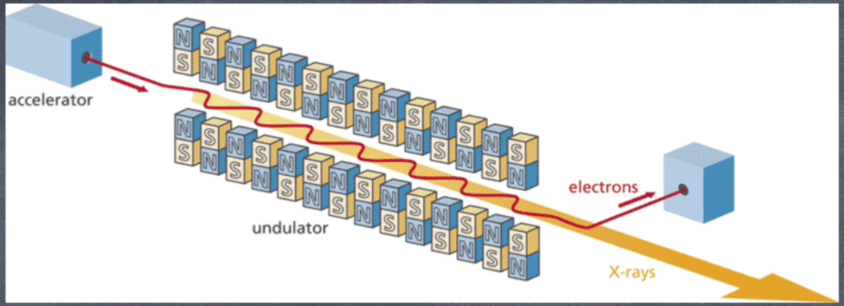
FELs fulfill both demands

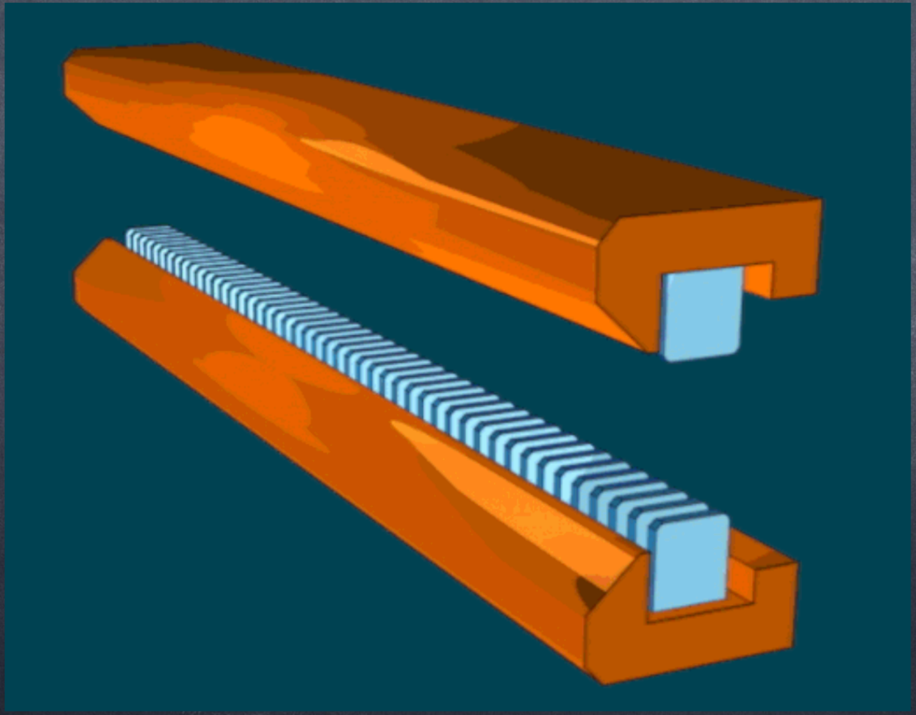
PSI   Swiss FEL



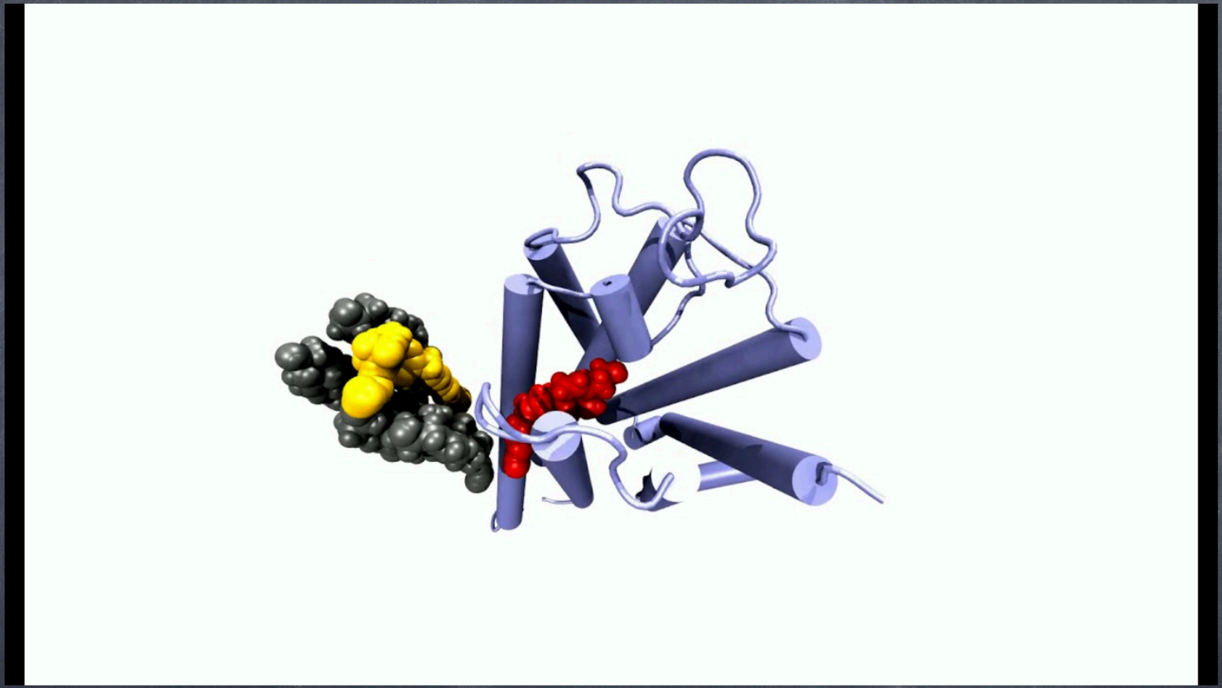
produce x-rays

Undulator





X-ray free-electron laser SwissFEL



Watching receptor proteins change shape

Comparison of time and space resolution	Conventional laser	Synchrotron	Free-electron laser
Wavelength	100 nm	0.1nm	10^{-10} m 0.1 nm
Time pulse	10 fs (10E-15 s)	100 ps (100E-12 s)	10 fs (10E-15 s)
Summary	Can resolve larger scales at ultrafast speeds	Can resolve atomic scale and fast processes	Can resolve atomic scale and ultrafast processes

position resolution

time resolution

Some references :

https://www.microphotonics.com/what-is-micro-ct-an-introduction/
https://www.microphotonics.com/how-does-a-microct-scanner-work/

Laboratory x-ray micro-computed tomography: a user guideline for biological samples
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5449646/

Useful references for a variety of radiation techniques :

https://astronuclphysics.info/Scintigrafie.htm#3

https://astronuclphysics.info/JadRadMetody.htm