利用相位與繞射對比強化的動態奈米生醫影像

Extracting Nanoscale Information for Biomedical Imaging in the Phase, Reciprocal Space and Time Domains

Yeukuang Hwu 胡宇光

Institute of Physics, Academia Sinica

政大June 5, 2009

Collaborators:

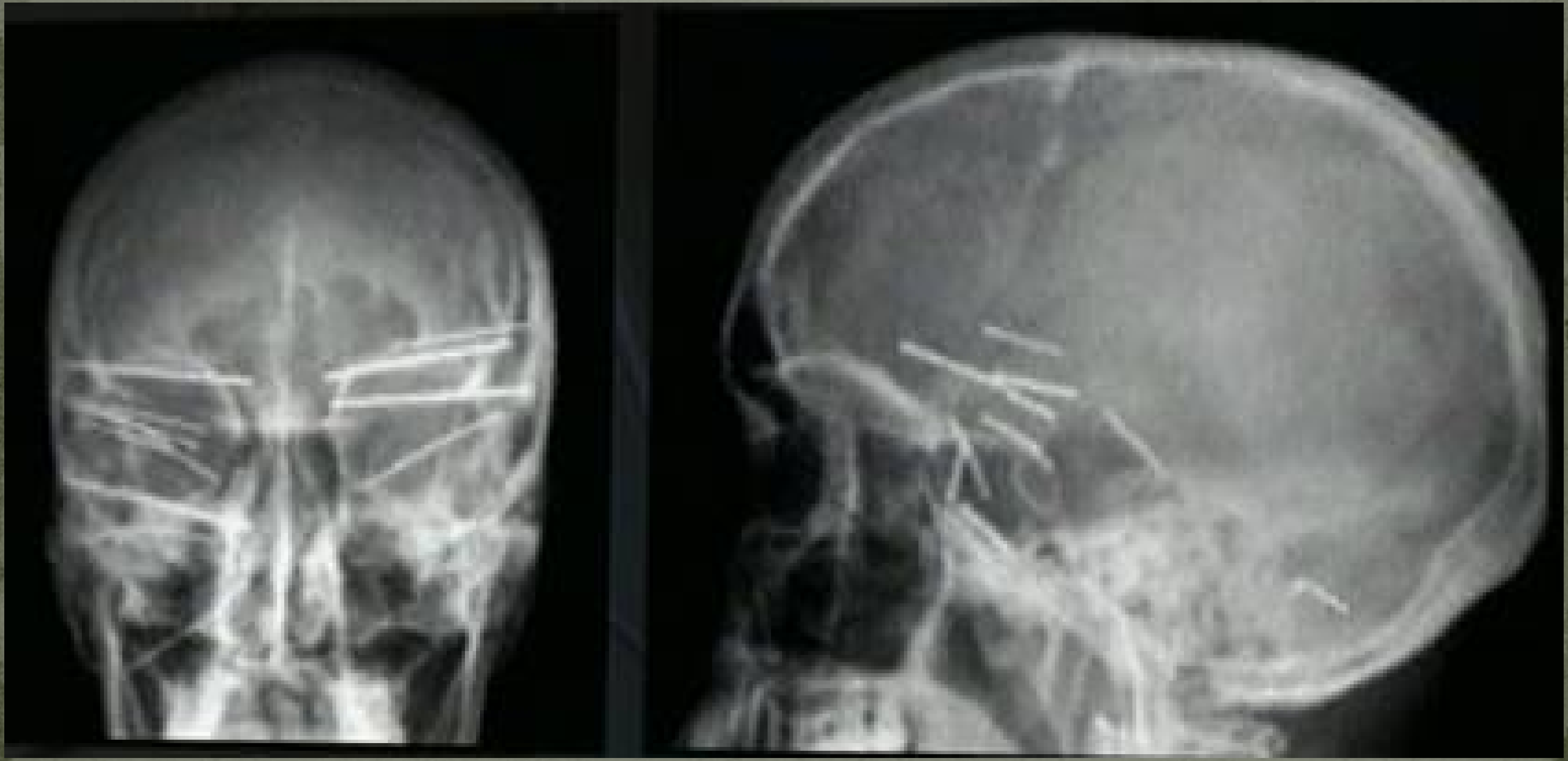
相 XIANG 像

- Taiwan:
  - 殷廣乾、梁耕三 (NSRRC), X-ray microscopy
  - 楊重熙(National Health Research Institutes)—nanomedicine, contrast agents.
  - 陳志雄、張六文(China Steel Co.)—steel, Al and related works, electro-deposition.
  - 林鴻明、吳溪煌(Tatung U) — electron chemistry, battery
  - 李家維、張修明、江安世(Life Science, Tsing Hua U.) —cell, shell fish, fossil, fire fly
  - 陳志成(Yang Ming U.) 、謝昌煥(High Performance Computing Center) — tomography reconstruction
  - 李定國、吳茂昆(Physics, Academia Sinica) — phase retrieval, holography reconstruction, magnetic nanoparticles, microfluidity
  - 陳福榮、開執中 (Materials, Tsing Hua U.) — transport intensity, phase retrieval.
  - TTY Pharmaceutics — Y. F. Hu, S. K. Tsai (drug delivery, pharmacokinetics)
  - 馬偕紀念醫院(Mackay Memorial Hospital) – Eugene Yang, Hung-I Yeh, Shiyu Lin (artery disease, tumor development)
  - 林玉娟(ITRI)—nanoparticle uptake
- USA: Yong Chu, Qun Shen, Jae-Mok Yi, B. Lai, (APS) Wen-An Chiou (U. Maryland)—TXM
- Switzerland:
  - G. Margaritondo (EPFL)
  - T. Liebling and M. Liebling (EPFL) grain dynamics
  - Rolf Gruetter (EPFL)
- Korea:
  - Jung-Ho Je (POSTECH)
- China: 張桂林,章英劍,蕭體喬 (Shanghai Institute of Nuclear Research), 吳自玉,朱佩平(BSRF)
- Singapore: Gan-Moog Chow, Eng Soon Tok, Herbert O. Moser (NUS)—drug delivery, polymer blend, mushroom, oil filters, Orchid, ..
- Germany: G. Fecher, G. Schönhense, C. Felser (JGU, Mainz)—PEEM, magnetic materials
- Russia: T. S. Argunova (IOFFE), M. Yu. Gutkin (Russia Academy of Science)—SiC

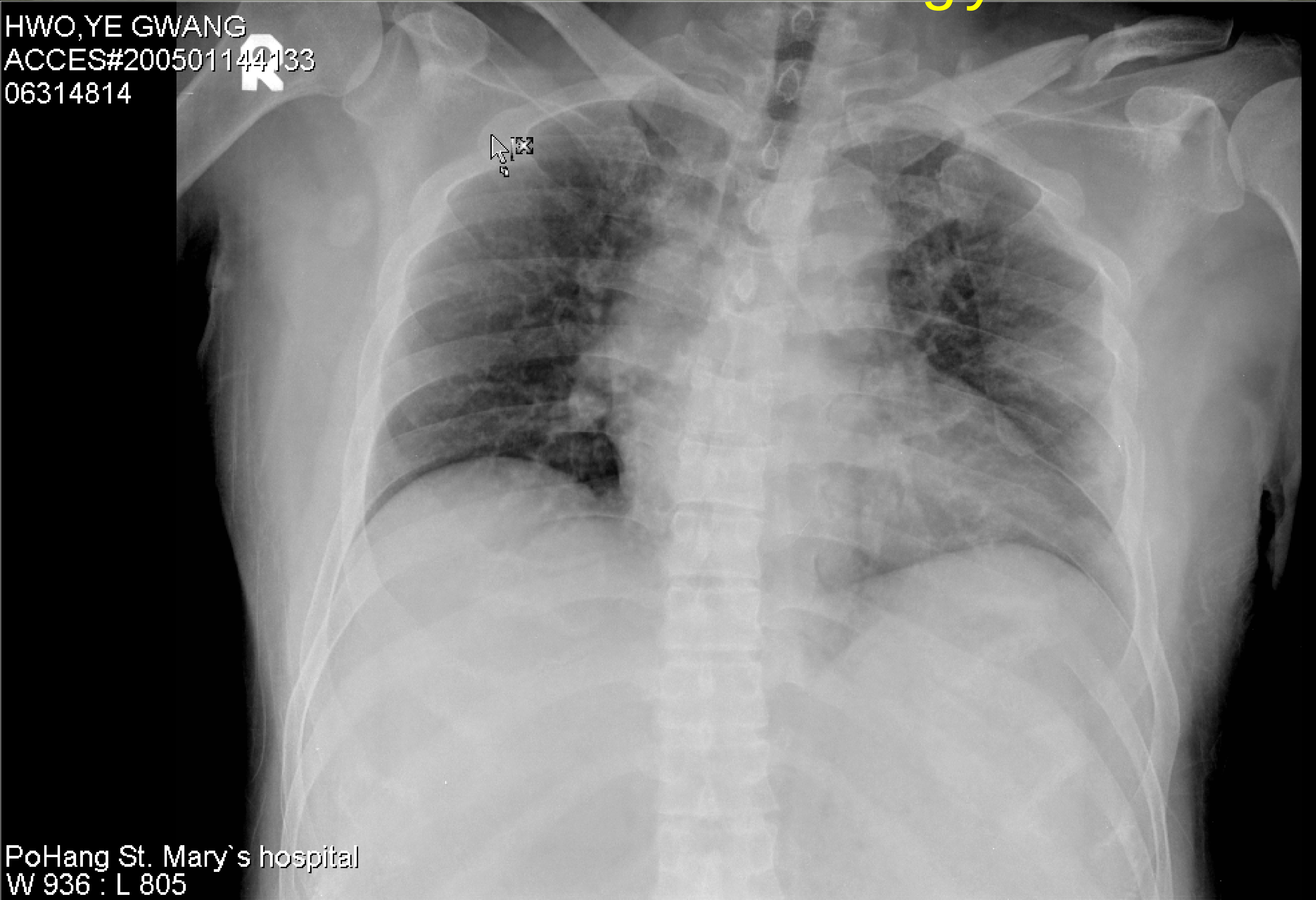
中央研究院

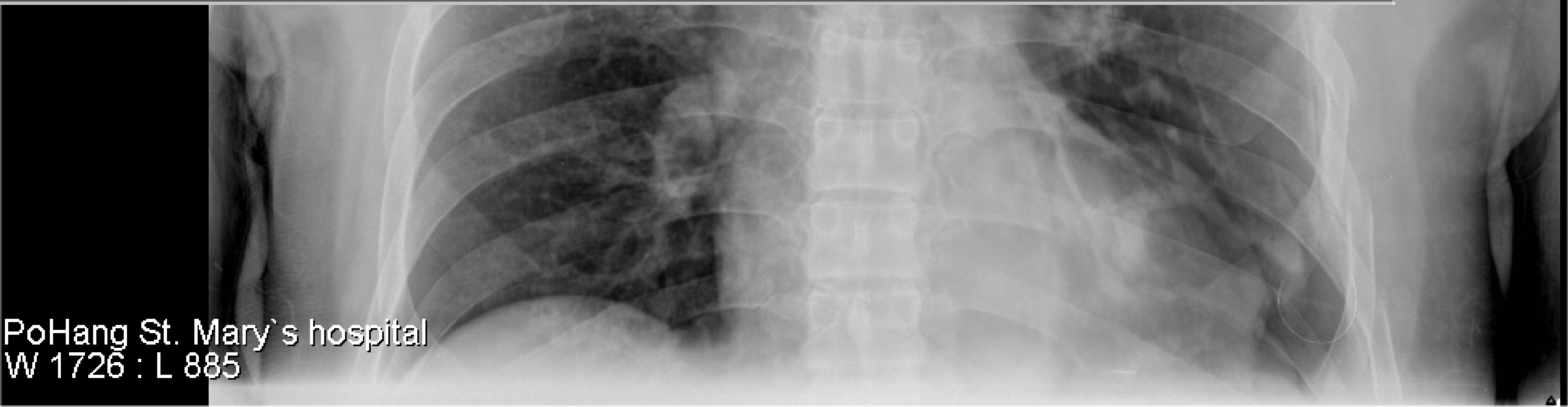
Current NanoX Team

- X-ray microscopy:
    - 王錚亮、華子恩、王雋越、吳學仁、楊博文
- Nanosynthesis:
    - 王長海、劉啟仁、王錚亮、簡浩霖
- Nanofabrication:
    - 羅宗男、劉啟仁、陳語同、許沛良、顏琮閔
- Biomedical:
    - 錢家琪、陳翔欣、華子恩、陳心泰、冷偉華、劉啟仁

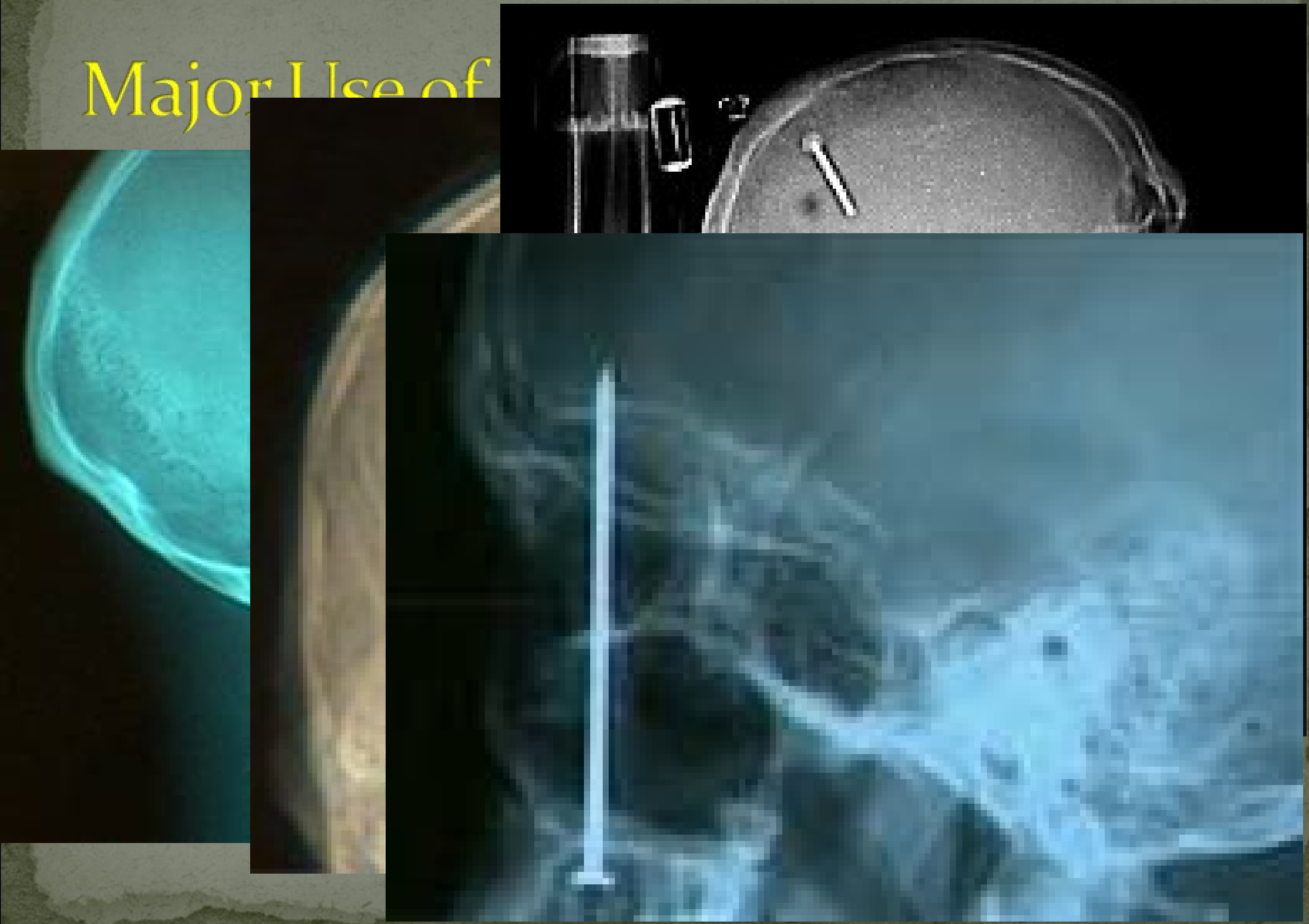


Conventional Radiology




PoHang St. Mary`s hospital
W 1726 : L 885

Major Use of



Microtomography study of Embryo fossils — Some very old (540M years) examples!

REPORTS

Phosphatized Polar Lobe–Forming Embryos from the Precambrian of Southwest China

Jun-Yuan Chen,[1*] David J. Bottjer,[2] Eric H. Davidson,[3] Stephen Q. Dornbos,[4] Xiang Gao,[1] Yong-Hua Yang,[1] Chia-Wei Li,[5] Gang Li,[6] Xiu-Qiang Wang,[1] Ding-Chang Xian,[6] Hung-Jen Wu,[5] Yeu-Kuang Hwu,[7] Paul Tafforeau[8,9]

In developing embryos of some extant spiralian animals, polar lobe formation is one of the symmetry-breaking mechanisms for segregation of maternal cytoplasmic substances to certain blastomeres and not others. Polar lobe formation leads to unique early cleavage morphologies that include trilobed, J-shaped, and five-lobed structures. Fossil embryos similar to modern lobe-forming embryos are recognized from the Precambrian Doushantuo Formation phosphates, Weng'an, Guizhou Province, China. These embryos are abundant and form a developmental sequence comparable to different developing stages observed in lobe-forming embryos of extant spiralians. These data imply that lobe formation is an evolutionarily ancient process of embryonic specification.

The Weng'an fossil fauna from Precambrian Doushantuo phosphates in Weng'an, Guizhou Province, China contains likely fossil representatives of the oldest known metazoans (*1*, *2*). The fossil-bearing

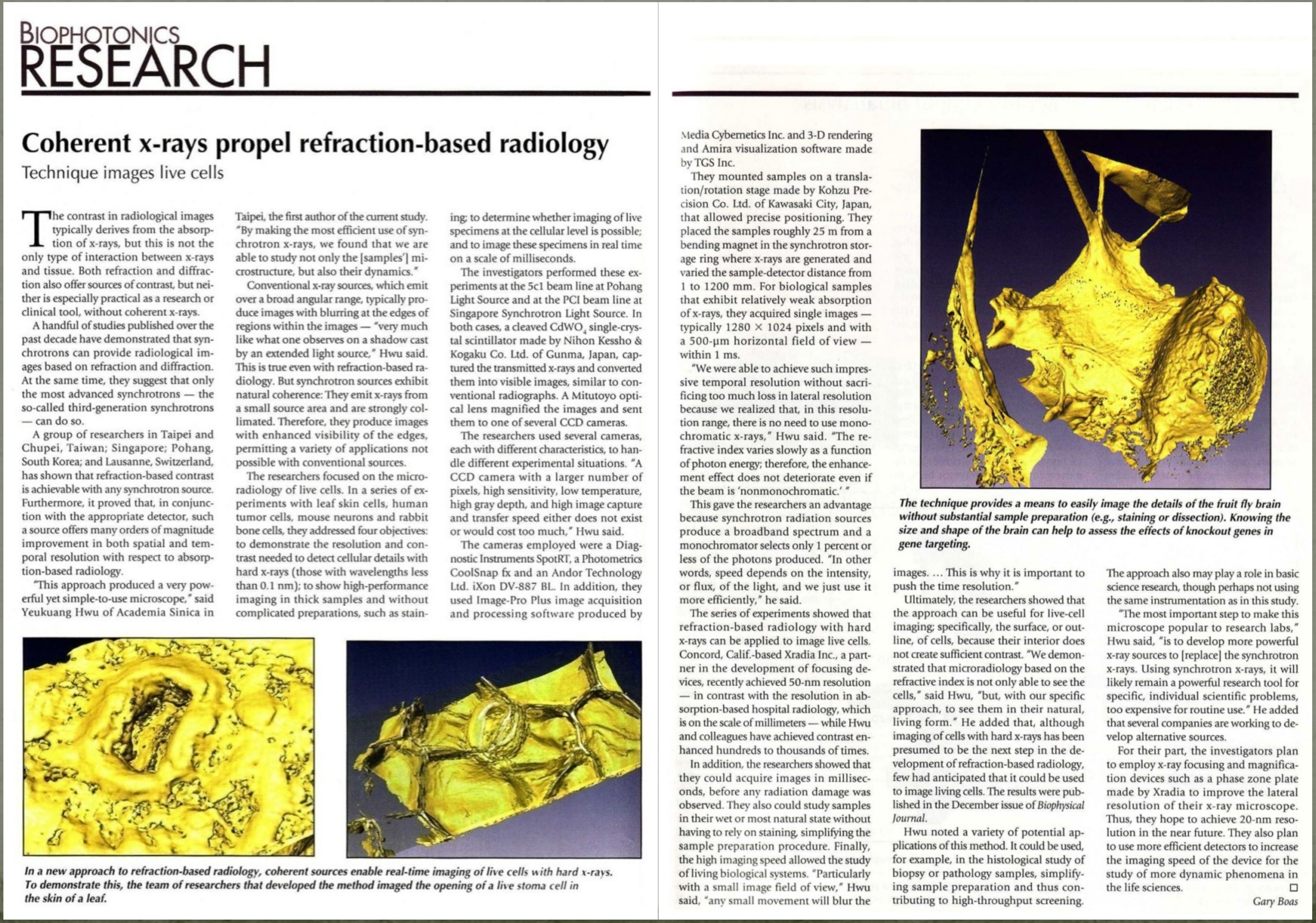
the matrix. Fossil embryos were recovered from the acid residues, coated with gold, and observed and photographed with a Hitachi S2600N scanning electron microscope (SEM); we identified 248 embryos with morphological

Lobe formation is a sequential process of dynamic change, which occurs by the protrusion and then absorption of a cytoplasmic lobe called a polar lobe. The lobe protrudes from the vegetal pole of the embryo at each round of cytokinesis, leading to the formation of dumbbell, three-fold (trefoil), J-shaped, and five-lobed morphologies (Fig. 1). The polar lobe (PL) superficially resembles a blastomere, but the lobe is anucleate and is connected to the CD or D blastomere by a deep constricted neck. Fossil PLs can be recognized by their size and connecting necks and by the complementary relation between their size and that of the CD or D blastomere from which they arose.

In the modern mud snail (*Nassarius obsoletus*) the first PL is protruded soon after fertilization, leading to the formation of a calabash-shaped structure: a large ball attached to a small one (*16*). Similar structures occur in many of our samples from the Weng'an. In *N. obsoletus*, the fertilized eggs at the first-lobe stage bear a superficial resemblance to unequally cleaving two-cell embryos, but they differ in that the embryo consists of a rather large uncleaved fertilized egg with a smaller anucleate lobe. After first cleavage, the larger

Science, **312** p.1644, June 16, 2006

Biophotonic International Magzine



BIOPHOTONICS
RESEARCH

Coherent x-rays propel refraction-based radiology

Technique images live cells

The contrast in radiological images typically derives from the absorption of x-rays, but this is not the only type of interaction between x-rays and tissue. Both refraction and diffraction also offer sources of contrast, but neither is especially practical as a research or clinical tool, without coherent x-rays.

A handful of studies published over the past decade have demonstrated that synchrotrons can provide radiological images based on refraction and diffraction. At the same time, they suggest that only the most advanced synchrotrons — the so-called third-generation synchrotrons — can do so.

A group of researchers in Taipei and Chupei, Taiwan; Singapore; Pohang, South Korea; and Lausanne, Switzerland, has shown that refraction-based contrast is achievable with any synchrotron source. Furthermore, it proved that, in conjunction with the appropriate detector, such a source offers many orders of magnitude improvement in both spatial and temporal resolution with respect to absorption-based radiology.

"This approach produced a very powerful yet simple-to-use microscope," said Yeukuang Hwu of Academia Sinica in Taipei, the first author of the current study. "By making the most efficient use of synchrotron x-rays, we found that we are able to study not only the [samples'] microstructure, but also their dynamics."

Conventional x-ray sources, which emit over a broad angular range, typically produce images with blurring at the edges of regions within the images — "very much like what one observes on a shadow cast by an extended light source," Hwu said. This is true even with refraction-based radiology. But synchrotron sources exhibit natural coherence: They emit x-rays from a small source area and are strongly collimated. Therefore, they produce images with enhanced visibility of the edges, permitting a variety of applications not possible with conventional sources.

The researchers focused on the microradiology of live cells. In a series of experiments with leaf skin cells, human tumor cells, mouse neurons and rabbit bone cells, they addressed four objectives: to demonstrate the resolution and contrast needed to detect cellular details with hard x-rays (those with wavelengths less than 0.1 nm); to show high-performance imaging in thick samples and without complicated preparations, such as staining; to determine whether imaging of live specimens at the cellular level is possible; and to image these specimens in real time on a scale of milliseconds.

The investigators performed these experiments at the 5c1 beam line at Pohang Light Source and at the PCI beam line at Singapore Synchrotron Light Source. In both cases, a cleaved $CdWO_4$ single-crystal scintillator made by Nihon Kessho & Kogaku Co. Ltd. of Gunma, Japan, captured the transmitted x-rays and converted them into visible images, similar to conventional radiographs. A Mitutoyo optical lens magnified the images and sent them to one of several CCD cameras.

The researchers used several cameras, each with different characteristics, to handle different experimental situations. "A CCD camera with a larger number of pixels, high sensitivity, low temperature, high gray depth, and high image capture and transfer speed either does not exist or would cost too much," Hwu said.

The cameras employed were a Diagnostic Instruments SpotRT, a Photometrics CoolSnap fx and an Andor Technology Ltd. iXon DV-887 BL. In addition, they used Image-Pro Plus image acquisition and processing software produced by Media Cybernetics Inc. and 3-D rendering and Amira visualization software made by TGS Inc.

In a new approach to refraction-based radiology, coherent sources enable real-time imaging of live cells with hard x-rays. To demonstrate this, the team of researchers that developed the method imaged the opening of a live stoma cell in the skin of a leaf.

They mounted samples on a translation/rotation stage made by Kohzu Precision Co. Ltd. of Kawasaki City, Japan, that allowed precise positioning. They placed the samples roughly 25 m from a bending magnet in the synchrotron storage ring where x-rays are generated and varied the sample-detector distance from 1 to 1200 mm. For biological samples that exhibit relatively weak absorption of x-rays, they acquired single images — typically 1280 × 1024 pixels and with a 500-μm horizontal field of view — within 1 ms.

"We were able to achieve such impressive temporal resolution without sacrificing too much loss in lateral resolution because we realized that, in this resolution range, there is no need to use monochromatic x-rays," Hwu said. "The refractive index varies slowly as a function of photon energy; therefore, the enhancement effect does not deteriorate even if the beam is 'nonmonochromatic.' "

This gave the researchers an advantage because synchrotron radiation sources produce a broadband spectrum and a monochromator selects only 1 percent or less of the photons produced. "In other words, speed depends on the intensity, or flux, of the light, and we just use it more efficiently," he said.

The series of experiments showed that refraction-based radiology with hard x-rays can be applied to image live cells. Concord, Calif.-based Xradia Inc., a partner in the development of focusing devices, recently achieved 50-nm resolution — in contrast with the resolution in absorption-based hospital radiology, which is on the scale of millimeters — while Hwu and colleagues have achieved contrast enhanced hundreds to thousands of times.

In addition, the researchers showed that they could acquire images in milliseconds, before any radiation damage was observed. They also could study samples in their wet or most natural state without having to rely on staining, simplifying the sample preparation procedure. Finally, the high imaging speed allowed the study of living biological systems. "Particularly with a small image field of view," Hwu said, "any small movement will blur the images. ... This is why it is important to push the time resolution."

The technique provides a means to easily image the details of the fruit fly brain without substantial sample preparation (e.g., staining or dissection). Knowing the size and shape of the brain can help to assess the effects of knockout genes in gene targeting.

Ultimately, the researchers showed that the approach can be useful for live-cell imaging; specifically, the surface, or outline, of cells, because their interior does not create sufficient contrast. "We demonstrated that microradiology based on the refractive index is not only able to see the cells," said Hwu, "but, with our specific approach, to see them in their natural, living form." He added that, although imaging of cells with hard x-rays has been presumed to be the next step in the development of refraction-based radiology, few had anticipated that it could be used to image living cells. The results were published in the December issue of *Biophysical Journal*.

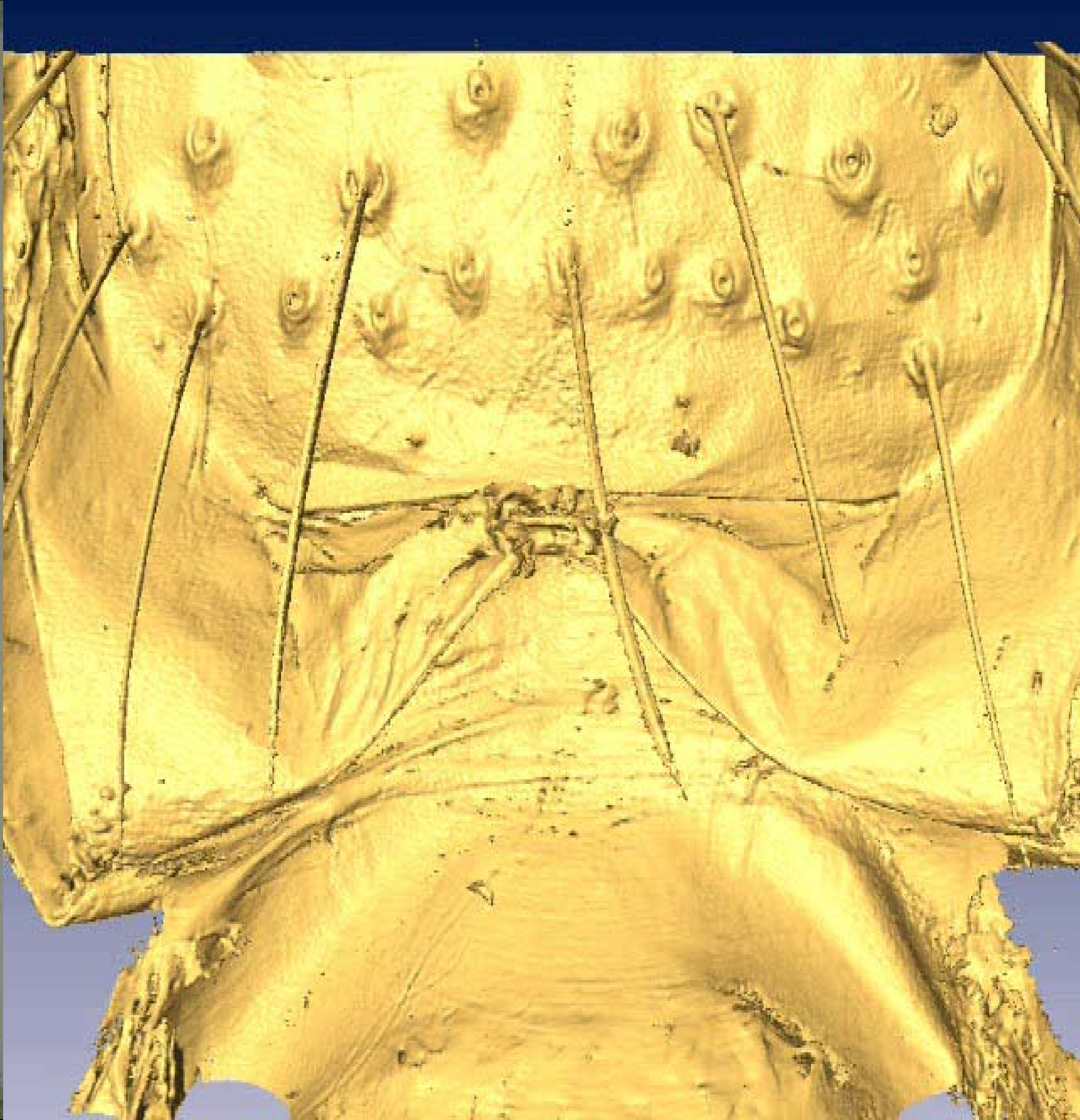
Hwu noted a variety of potential applications of this method. It could be used, for example, in the histological study of biopsy or pathology samples, simplifying sample preparation and thus contributing to high-throughput screening. The approach also may play a role in basic science research, though perhaps not using the same instrumentation as in this study.

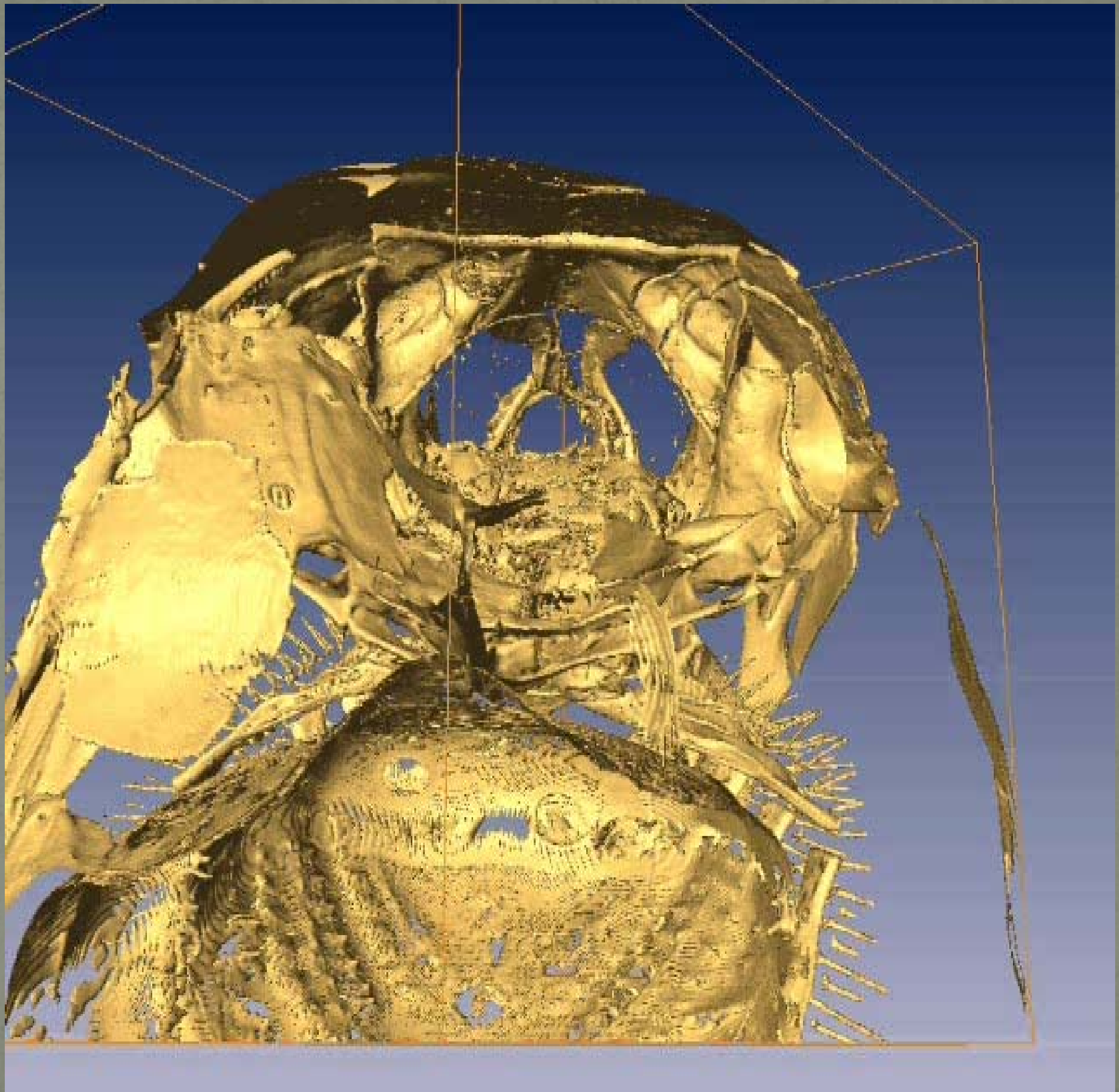
"The most important step to make this microscope popular to research labs," Hwu said, "is to develop more powerful x-ray sources to [replace] the synchrotron x-rays. Using synchrotron x-rays, it will likely remain a powerful research tool for specific, individual scientific problems, too expensive for routine use." He added that several companies are working to develop alternative sources.

For their part, the investigators plan to employ x-ray focusing and magnification devices such as a phase zone plate made by Xradia to improve the lateral resolution of their x-ray microscope. Thus, they hope to achieve 20-nm resolution in the near future. They also plan to use more efficient detectors to increase the imaging speed of the device for the study of more dynamic phenomena in the life sciences. □

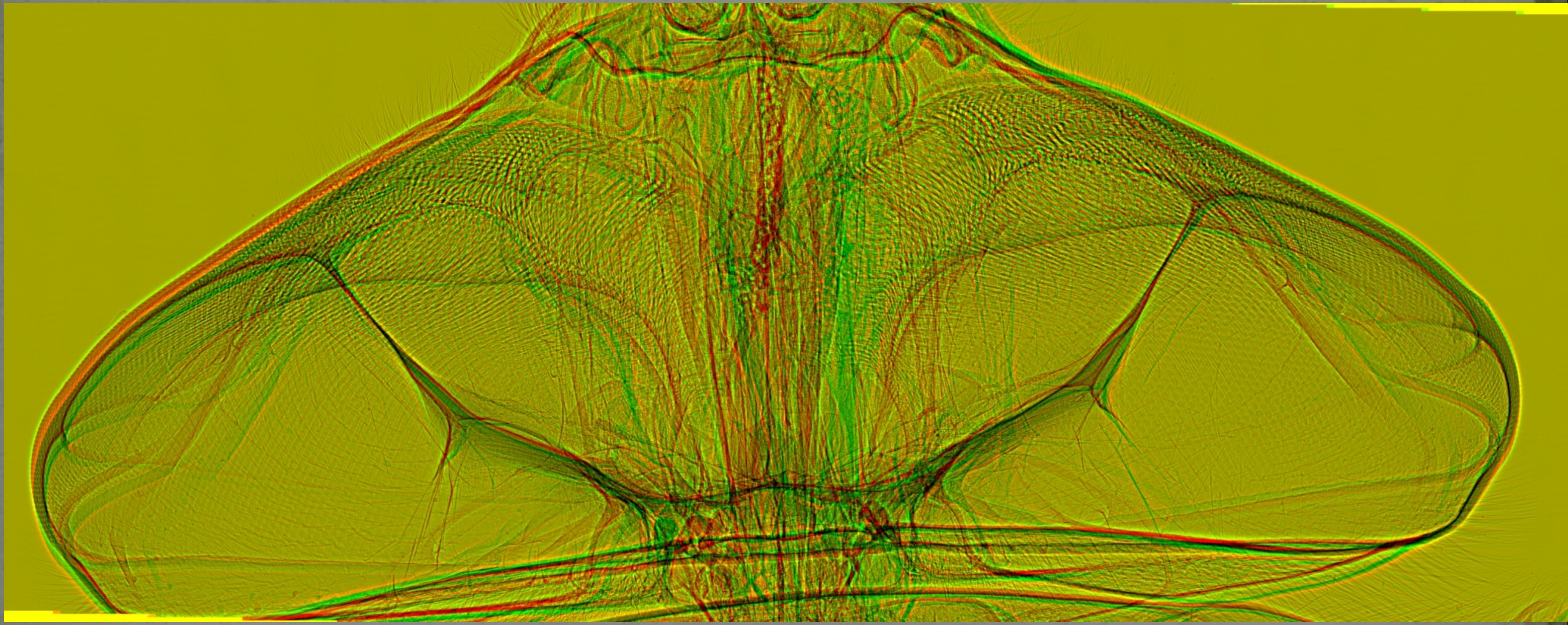
Gary Boas

Mosquito



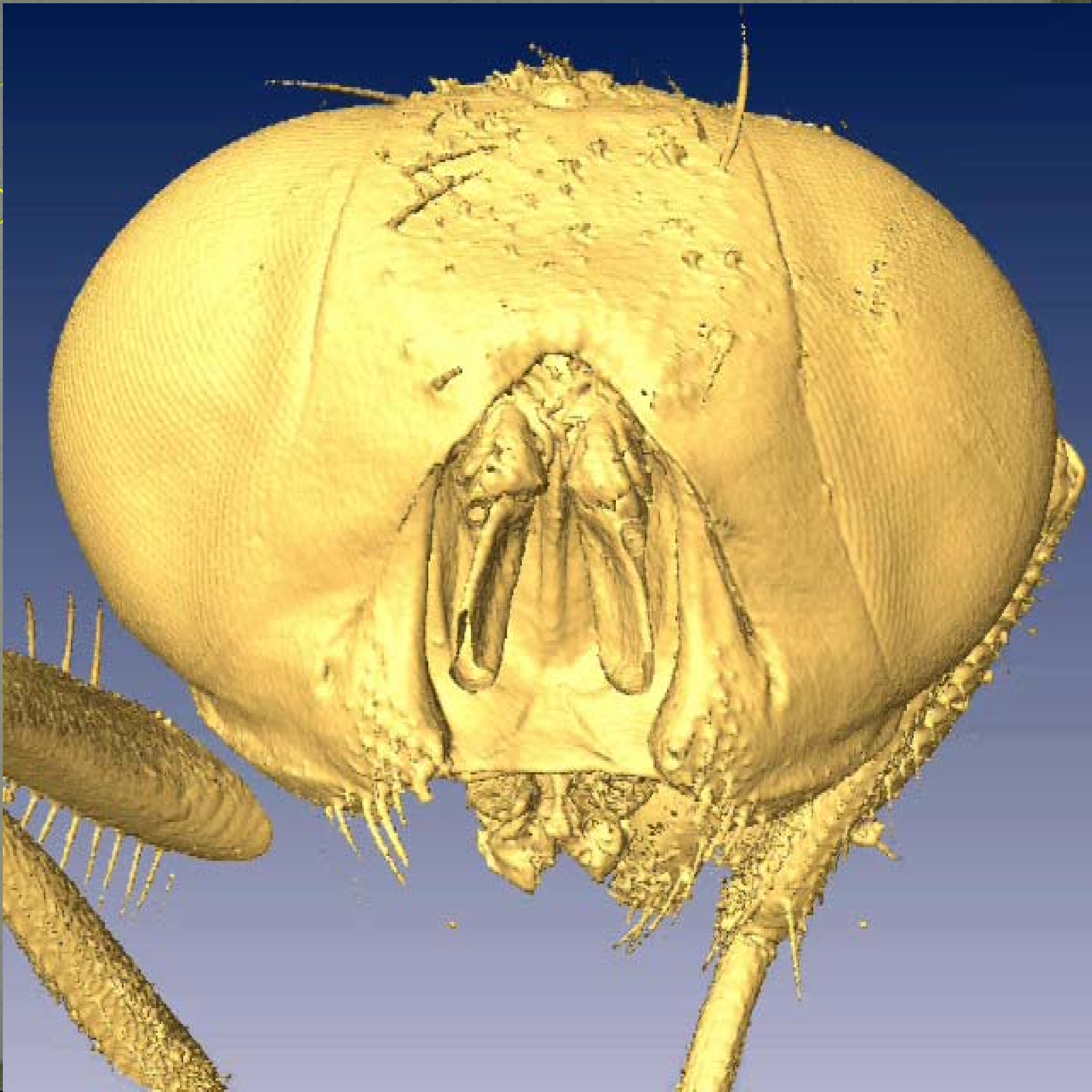


House Fly—A stereograph

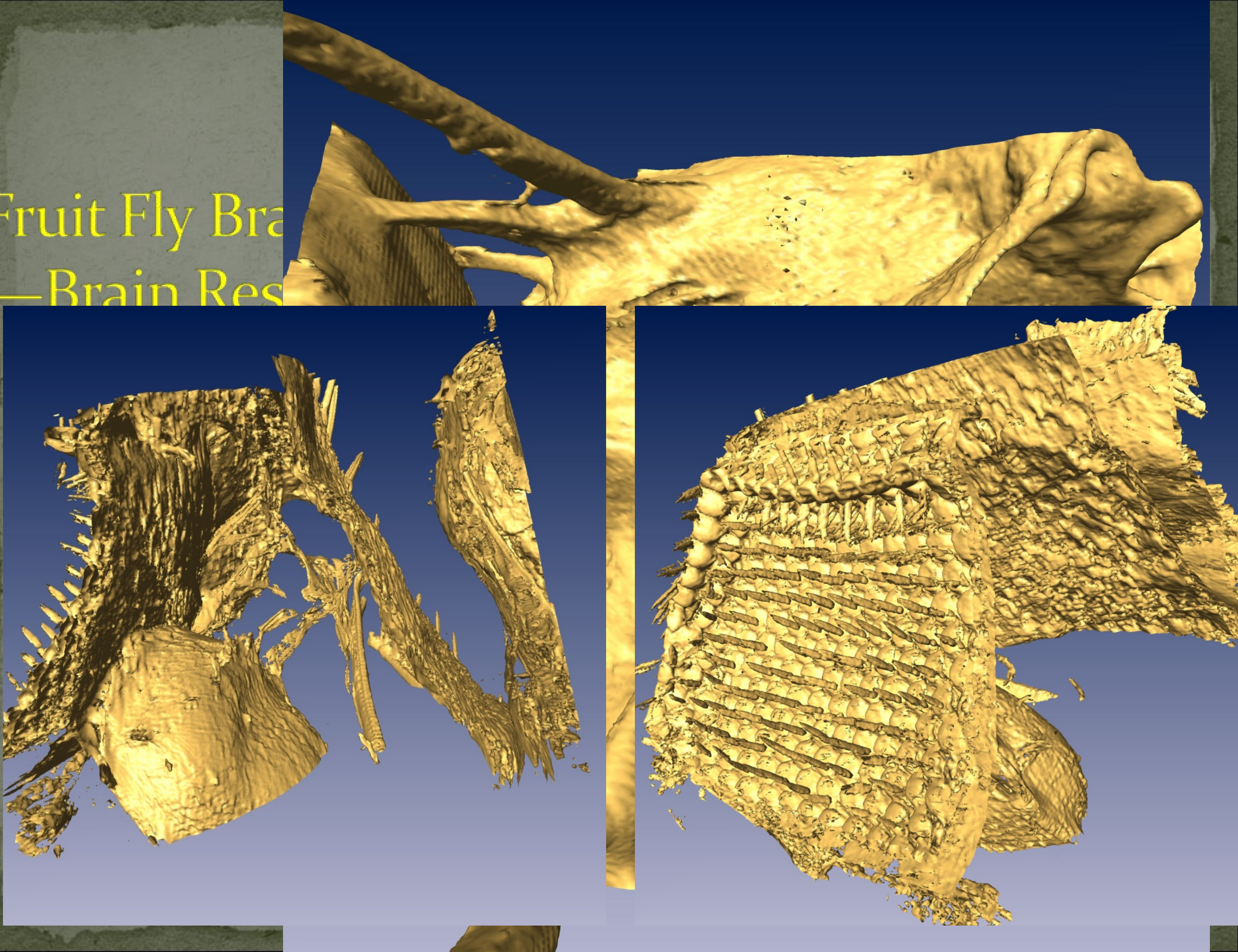


Head of a fl

3D microtomo

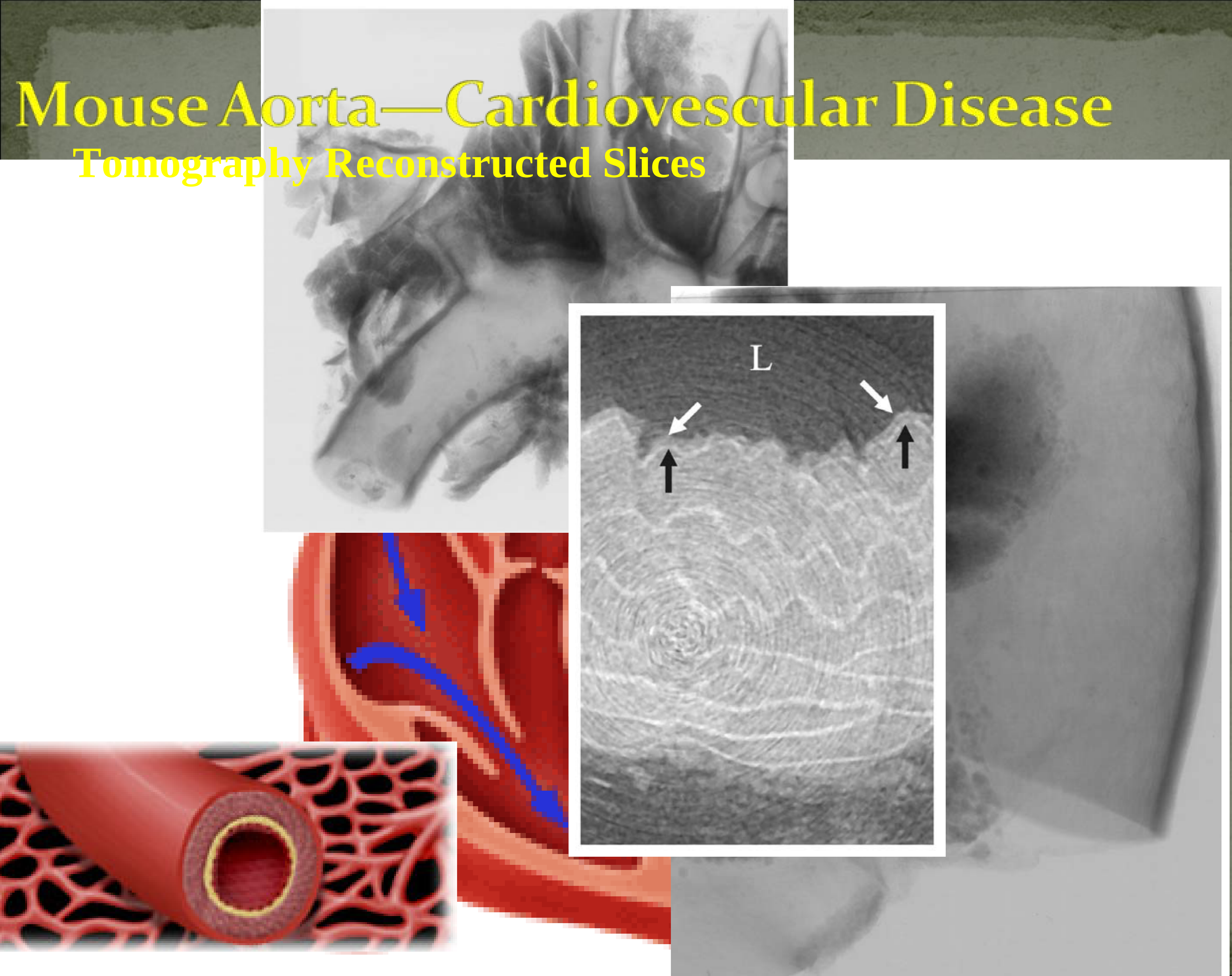


Fruit Fly Bra
—Brain Res



Mouse Aorta—Cardiovescular Disease

Tomography Reconstructed Slices

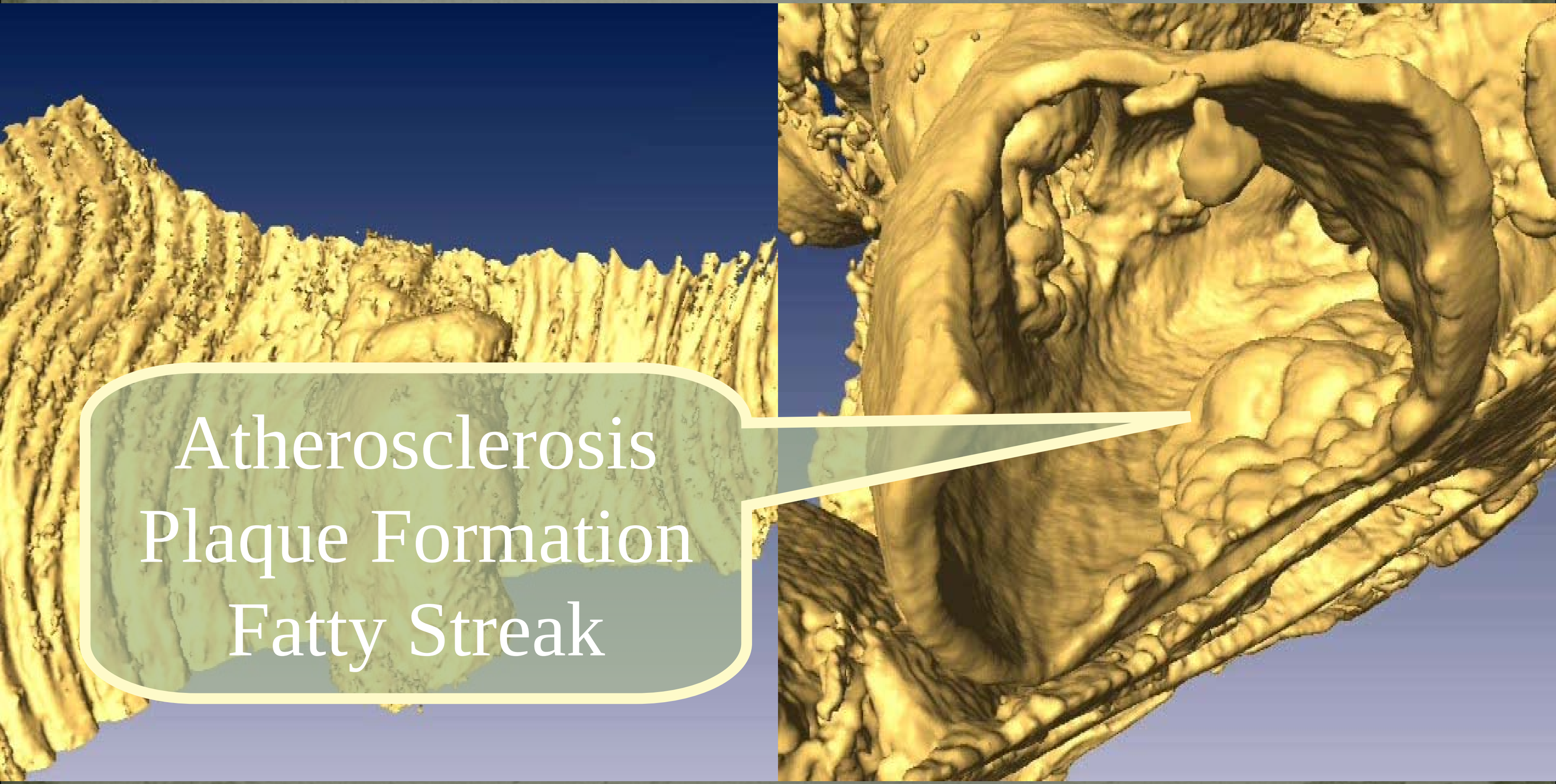


Microtomography of mouse aorta

cleus

Tomo

" Endo-microscope" Visualization of Cardiovascular Disease



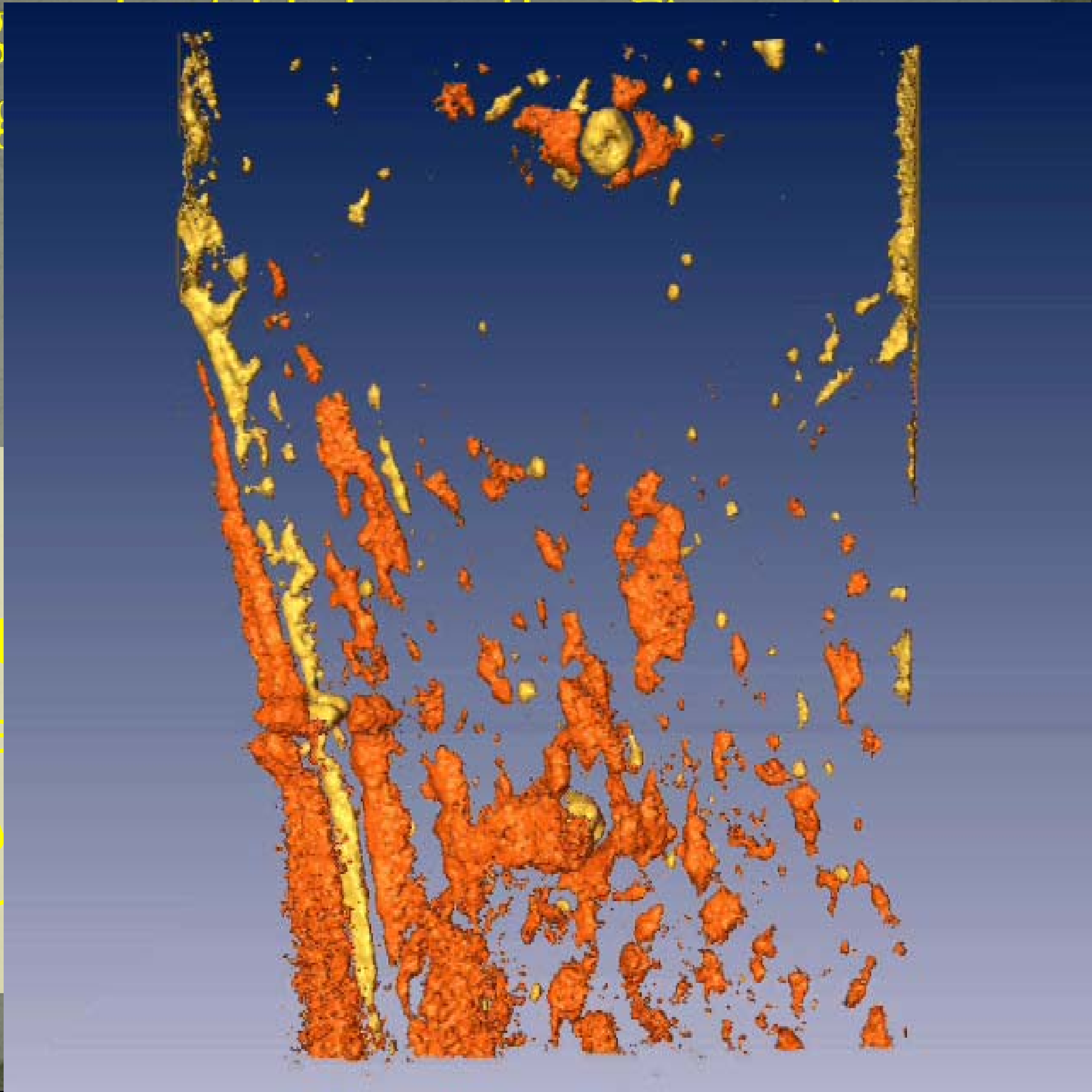
Atherosclerosis
Plaque Formation
Fatty Streak

Nanoradiog
cellular radiog

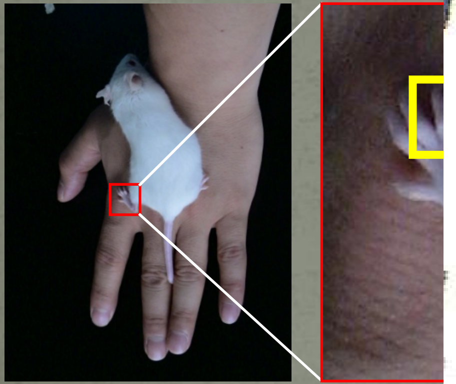
Nanote

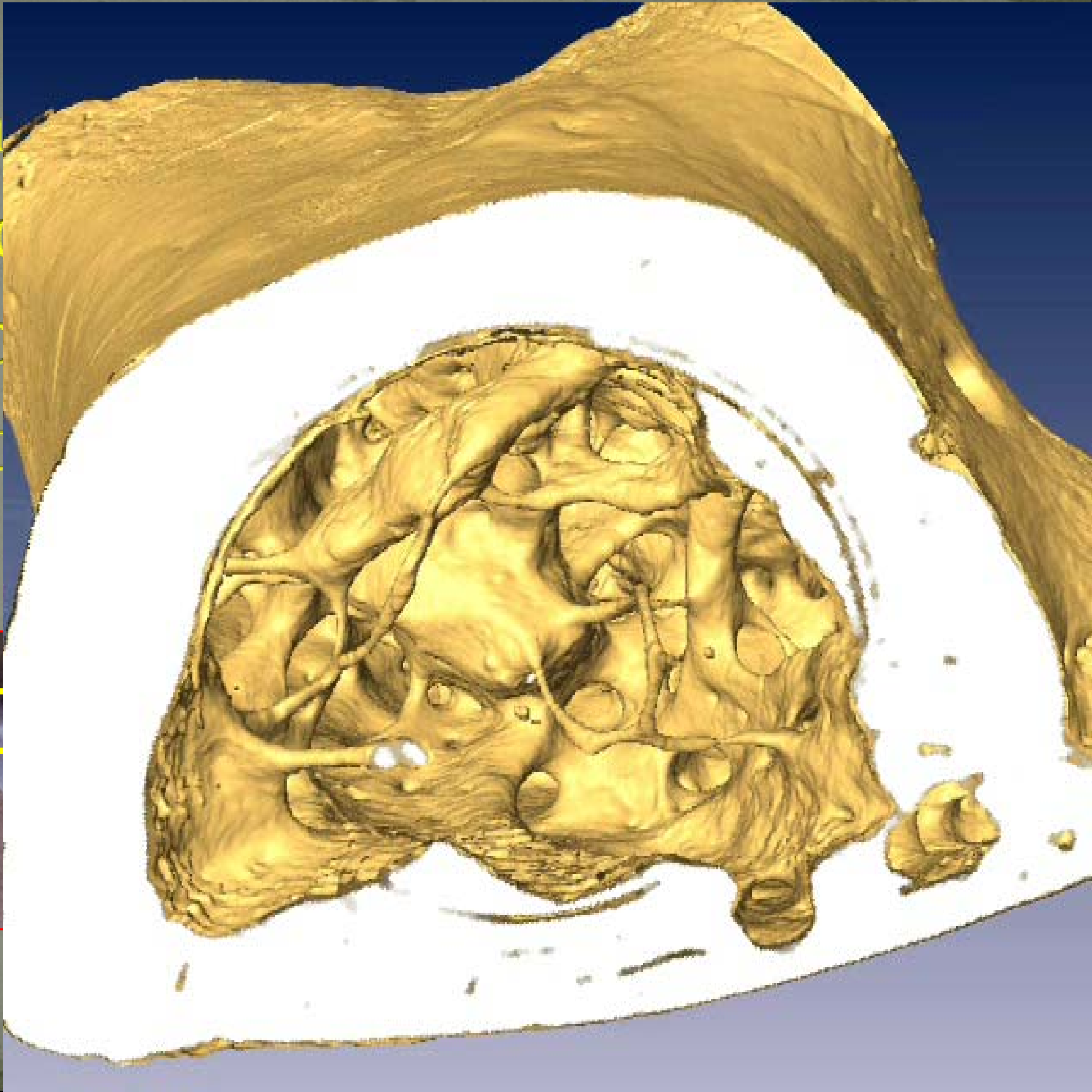
Locate position of Nano-Au in Cell

- Nanopart
  - Early de
  - Therapy
- Health ar

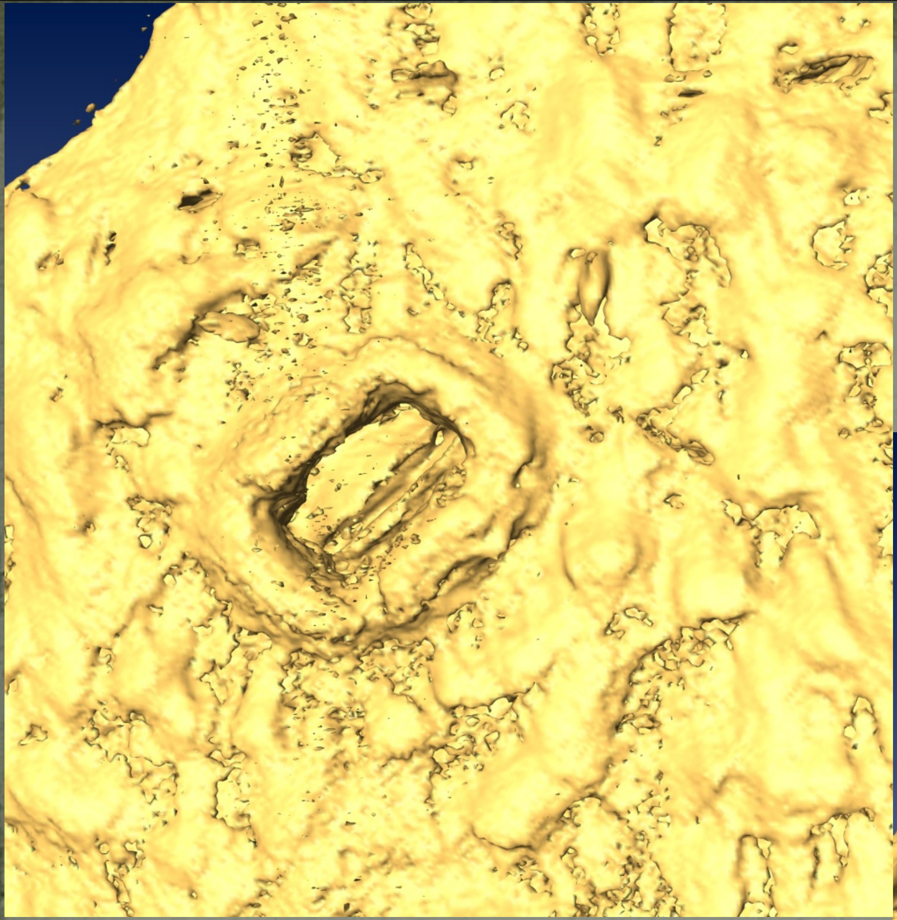


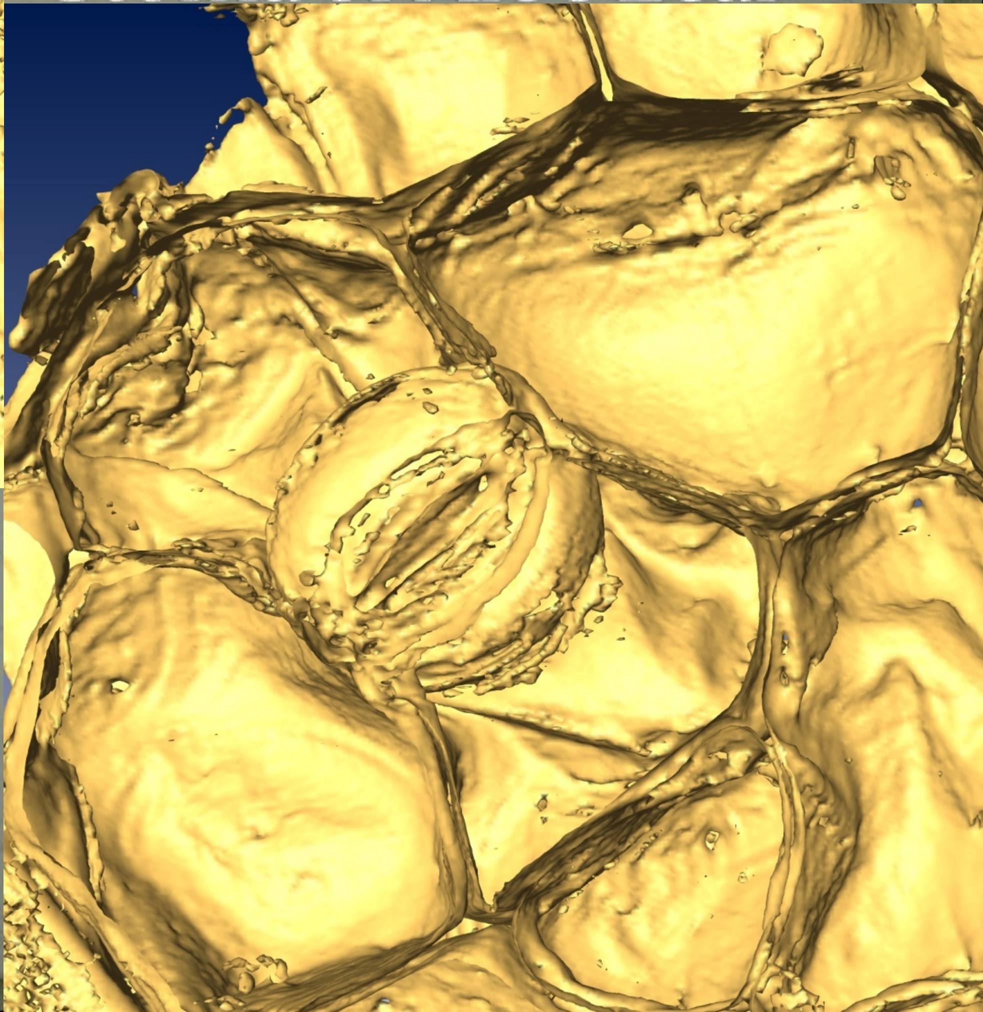
Implan
Bone Fo
Orthop
Biomat

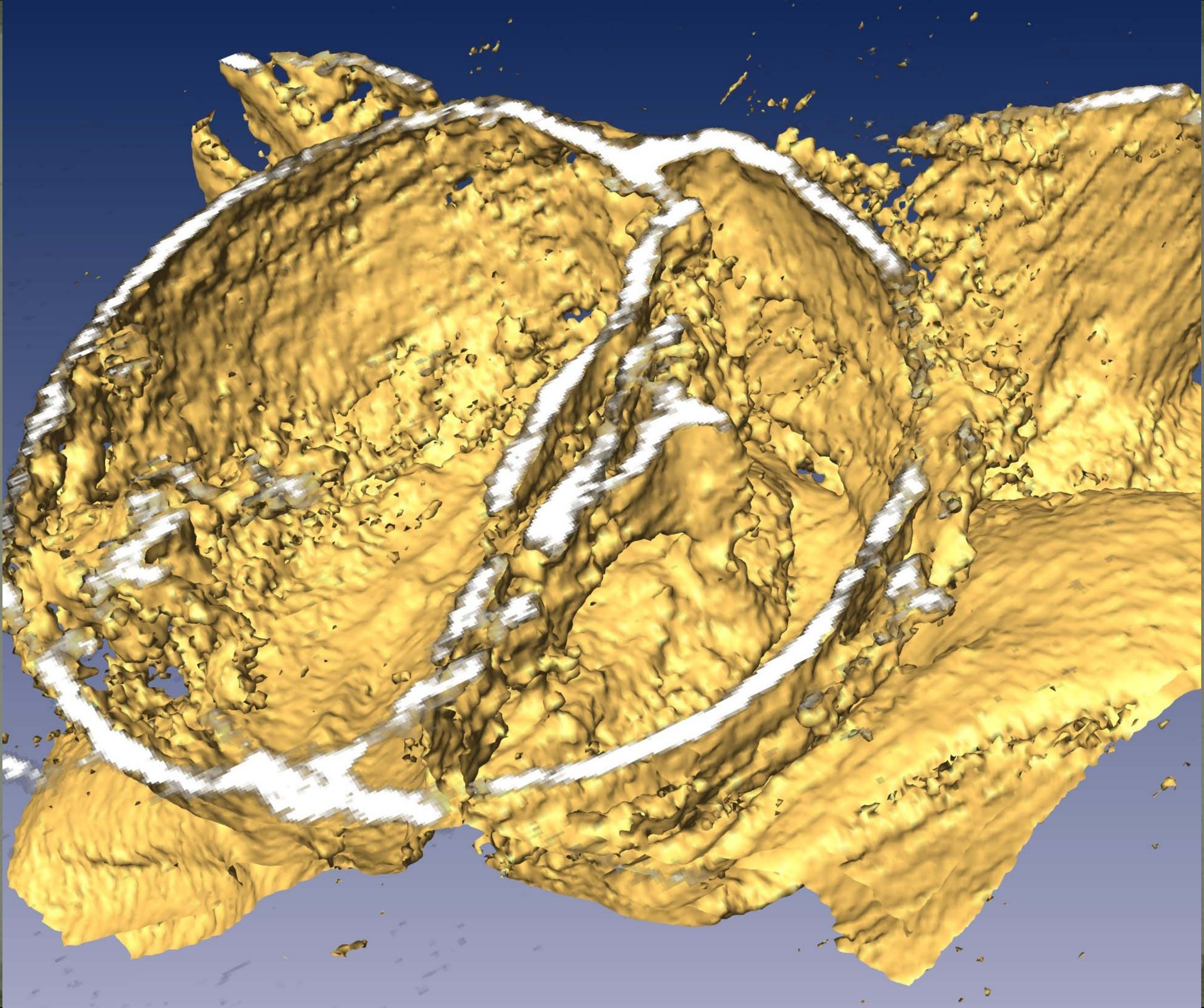




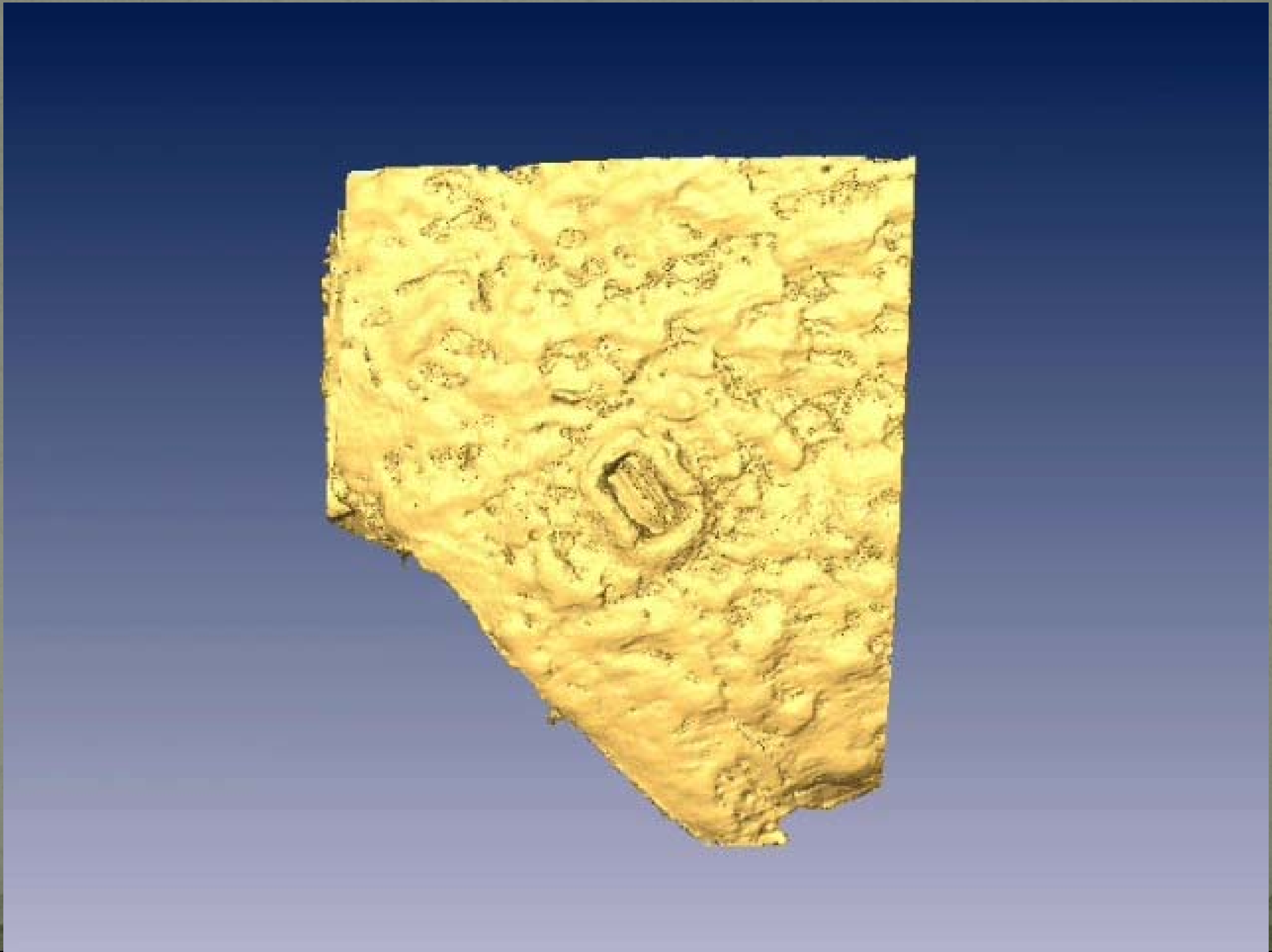
Stoma of Aloe Leaf





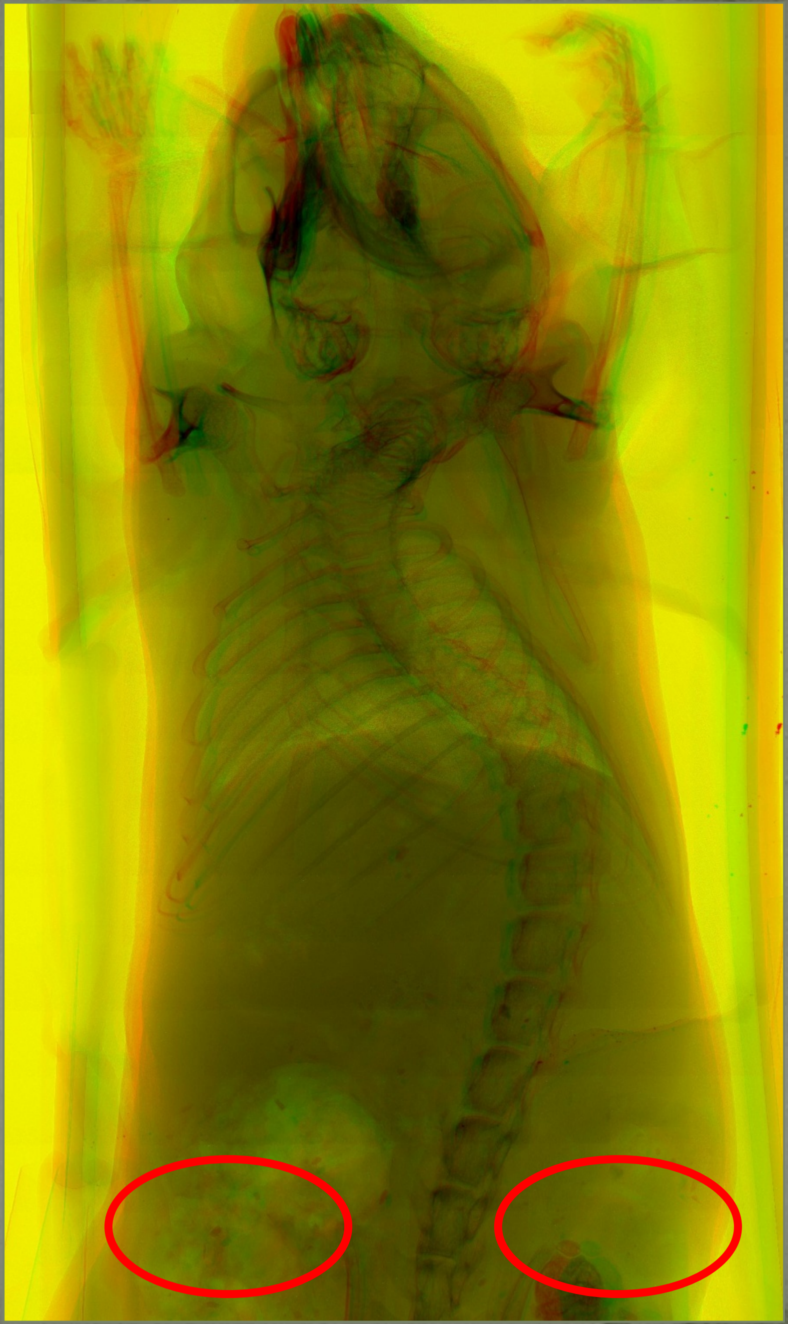


3D Tomography of a Stoma of Aloe Leave



Animal Imaging and Early Diagnosis of Diseases

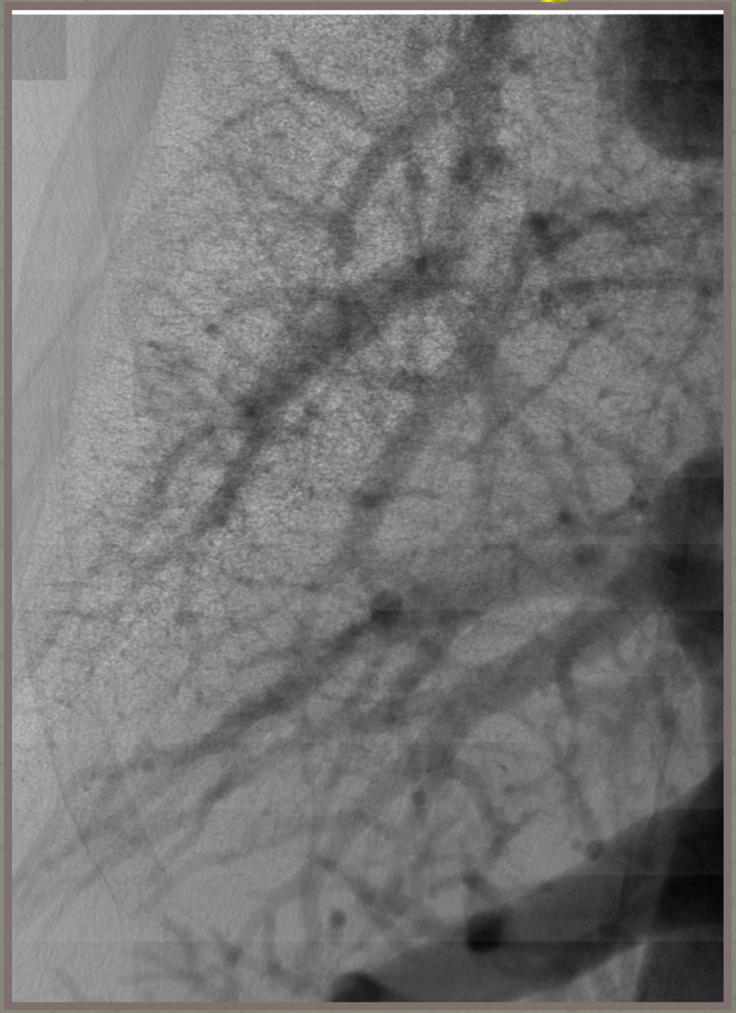
Live imaging of small animals Mouse X-ray Stereographys— Imaging of tumors



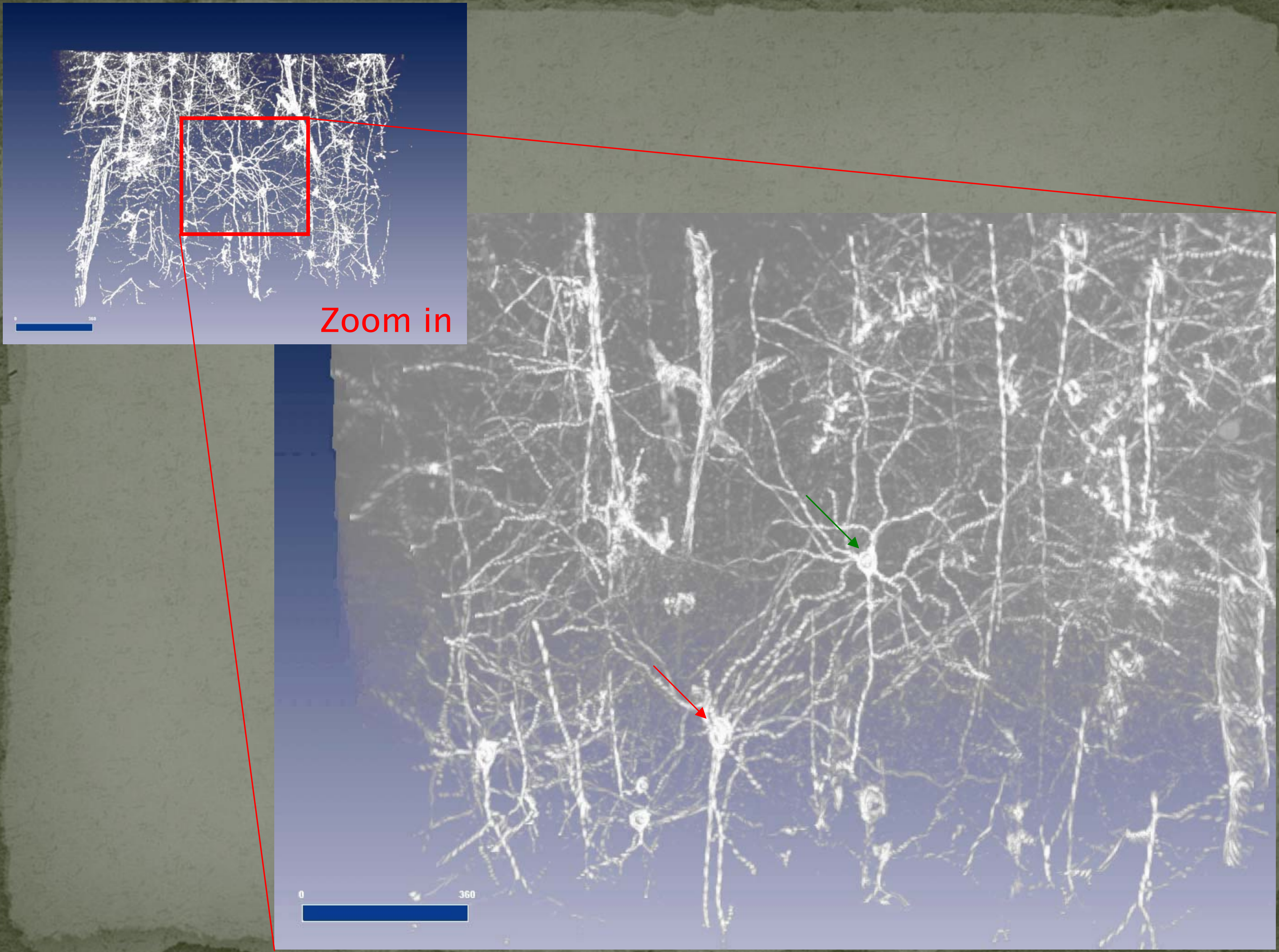
Tumors

Mouse Kidney and Lung with hydrophobic Iodine contrast agent

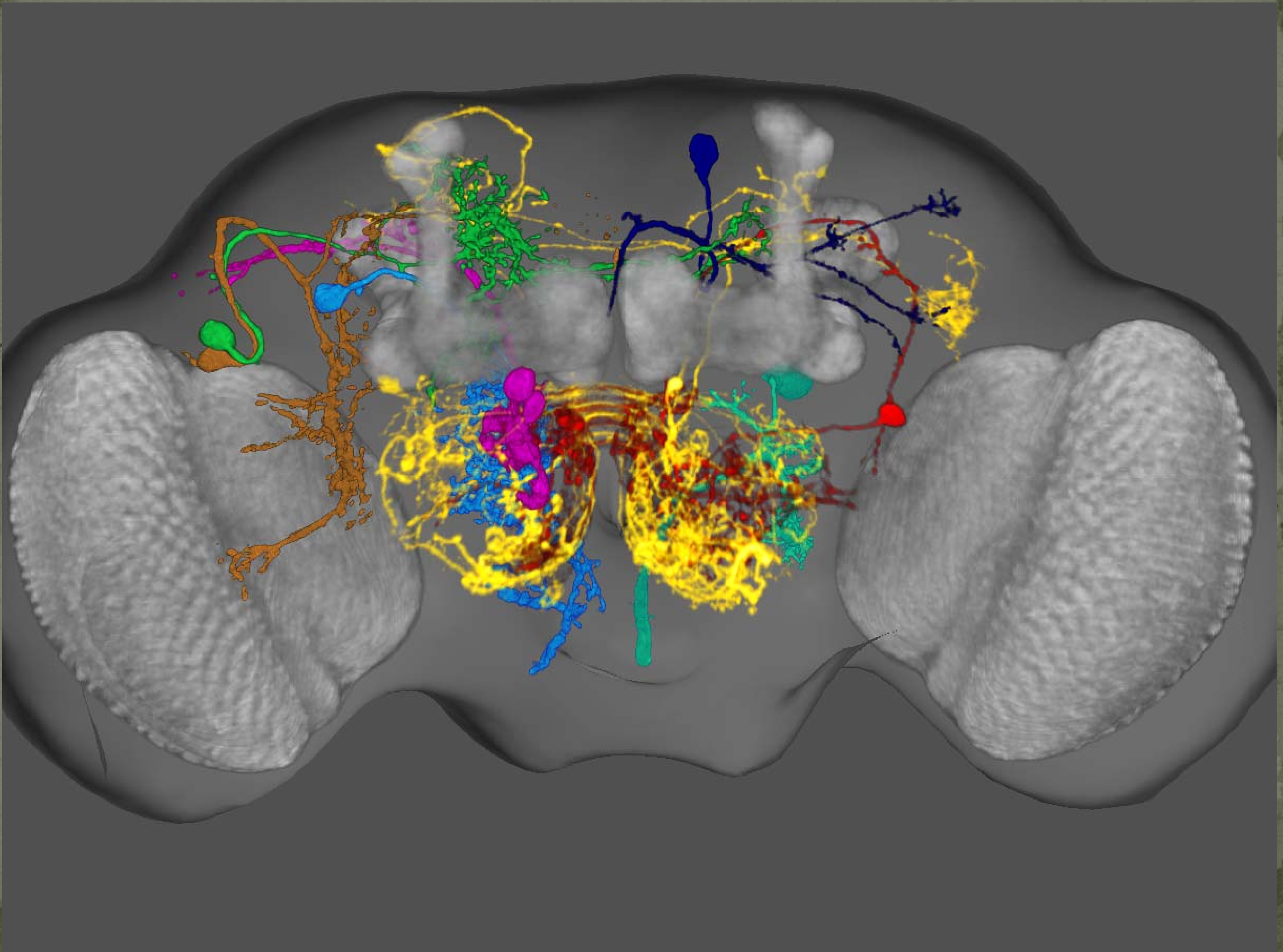
Kidney

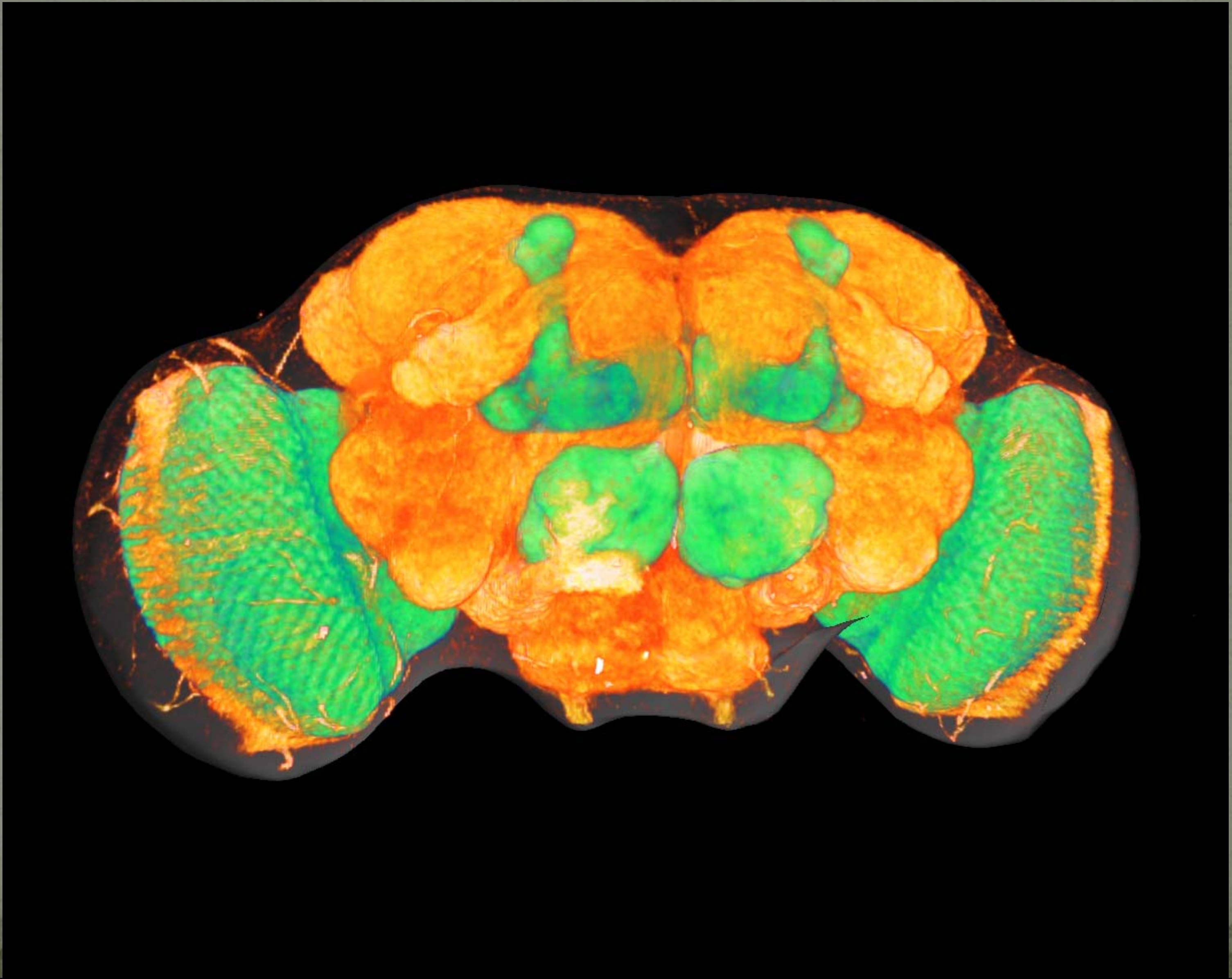


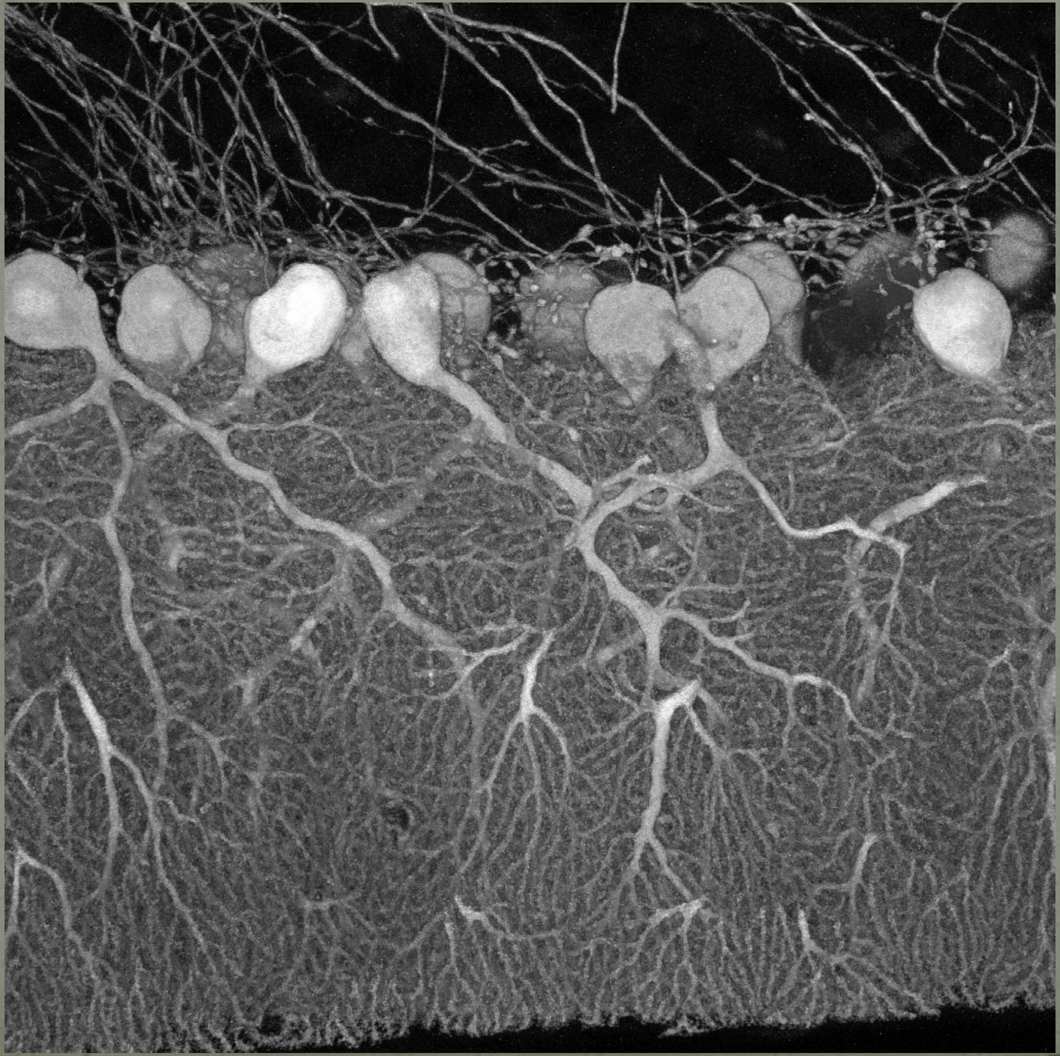
Lung


0
360
Zoom in
0
360

NanoImaging & Nanofabrication — Imaging Sub-Cellular Structures w/ 30nm Resolution



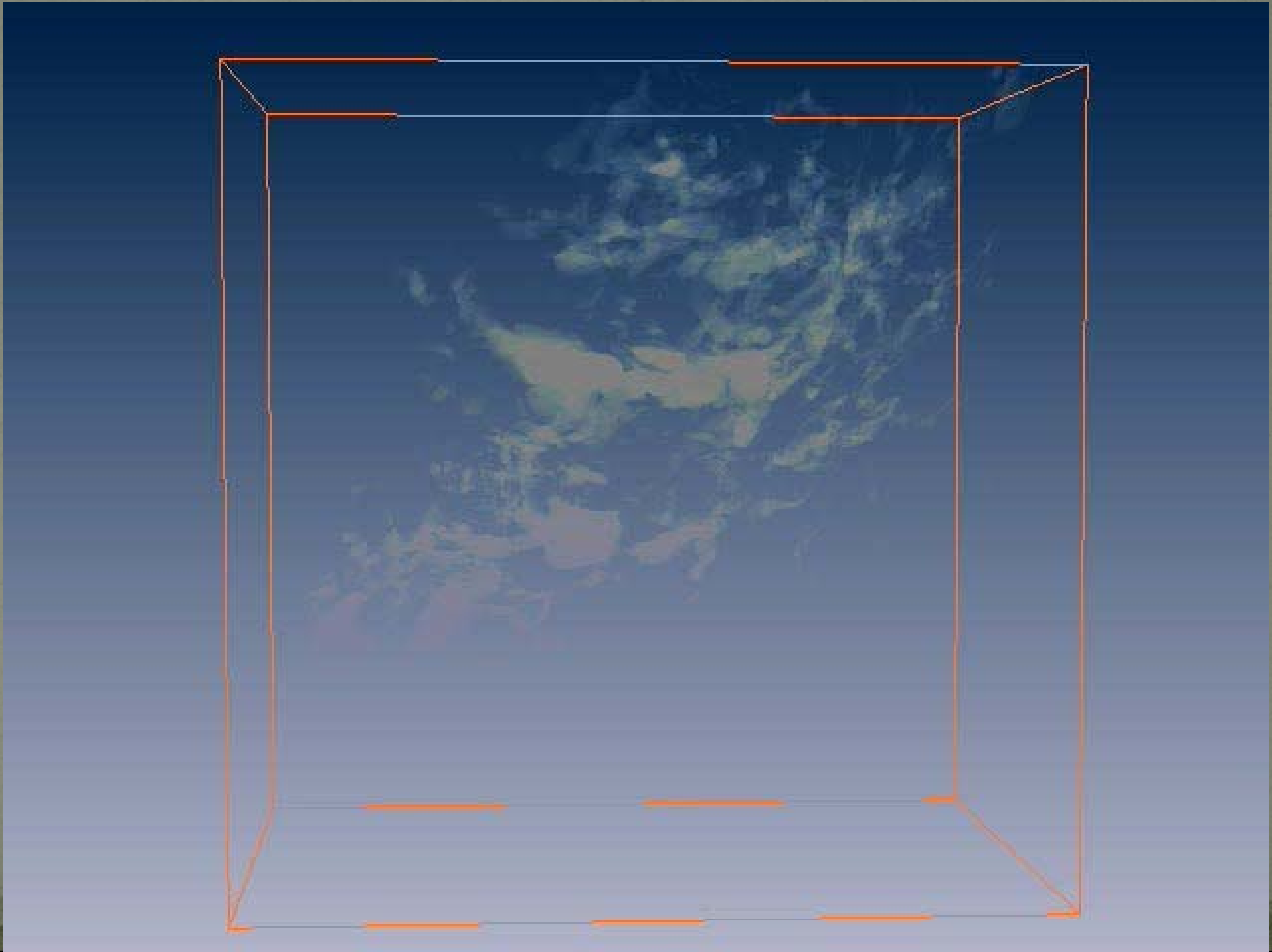




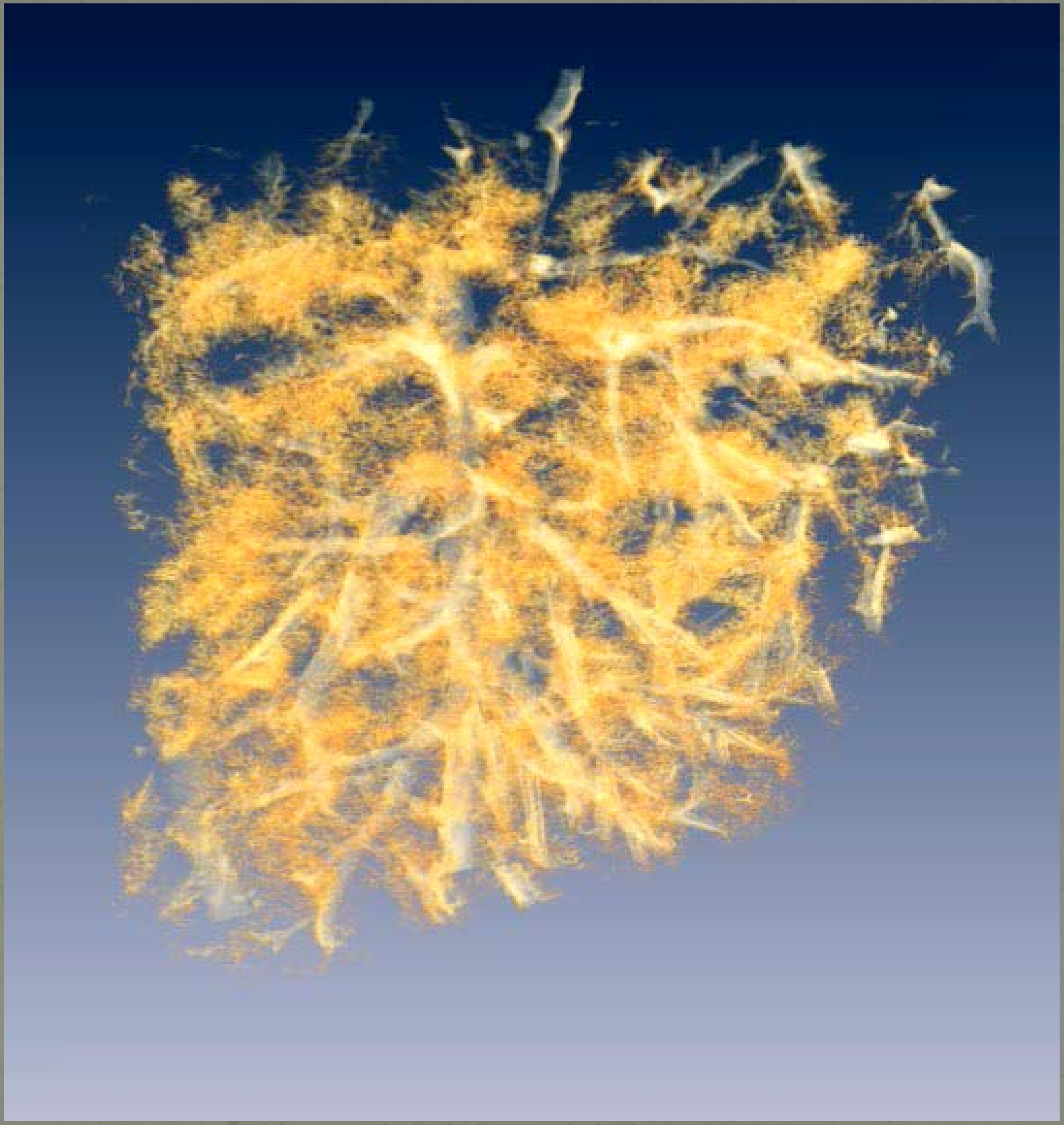
Biomedical Imaging

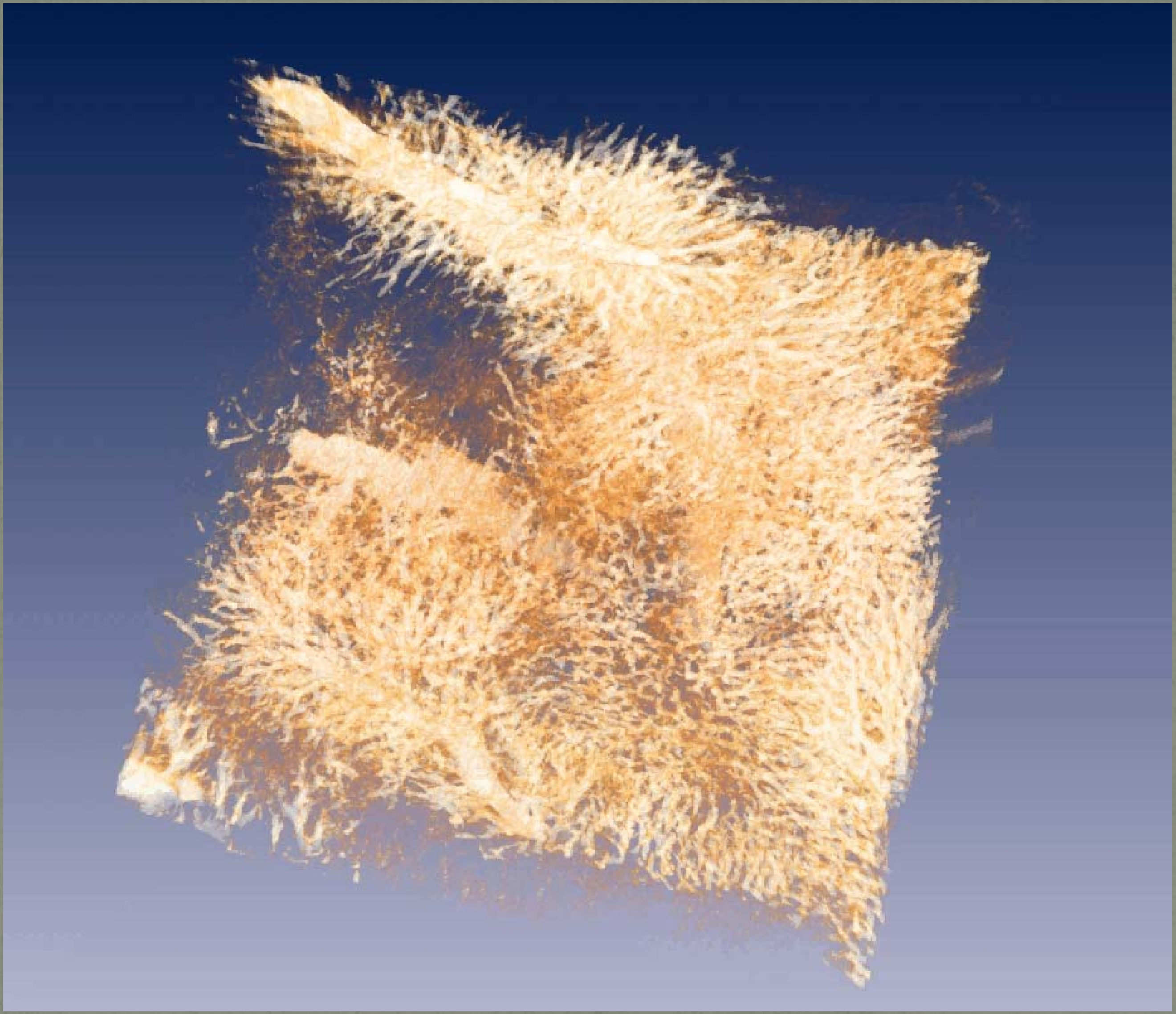
Microangiograph and microangiogenesis

Microvessels at tumor

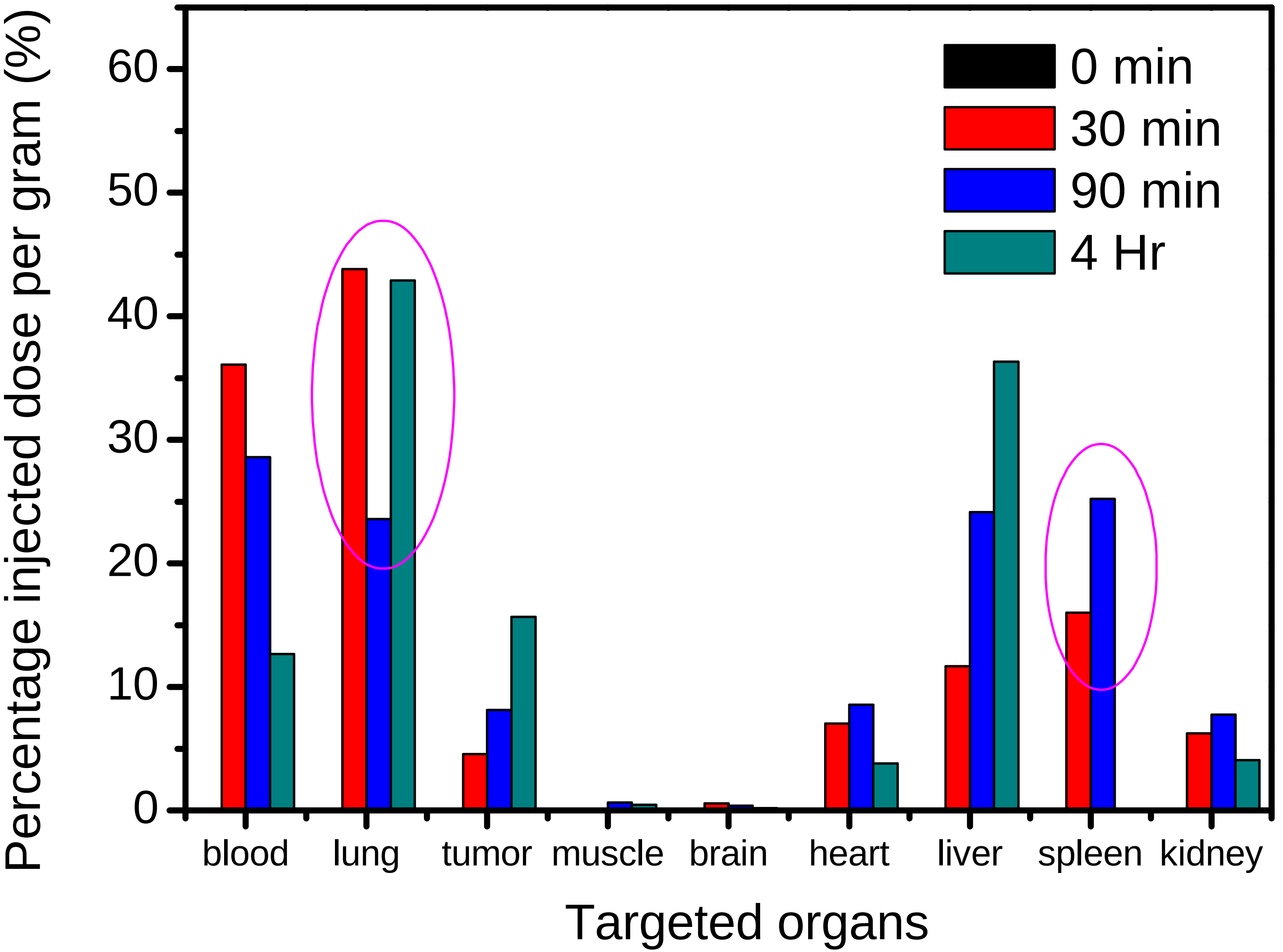


Microangiogenesis

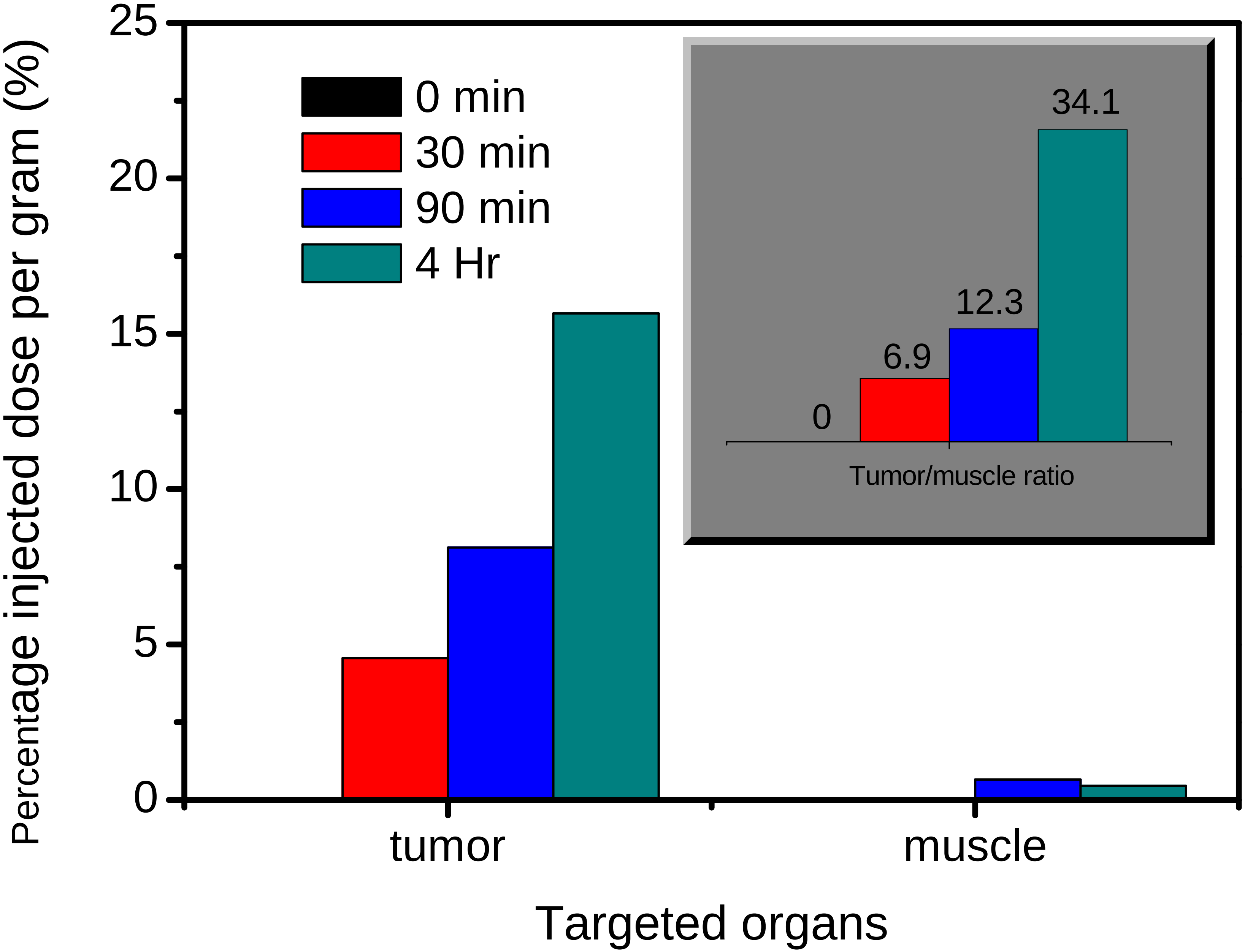




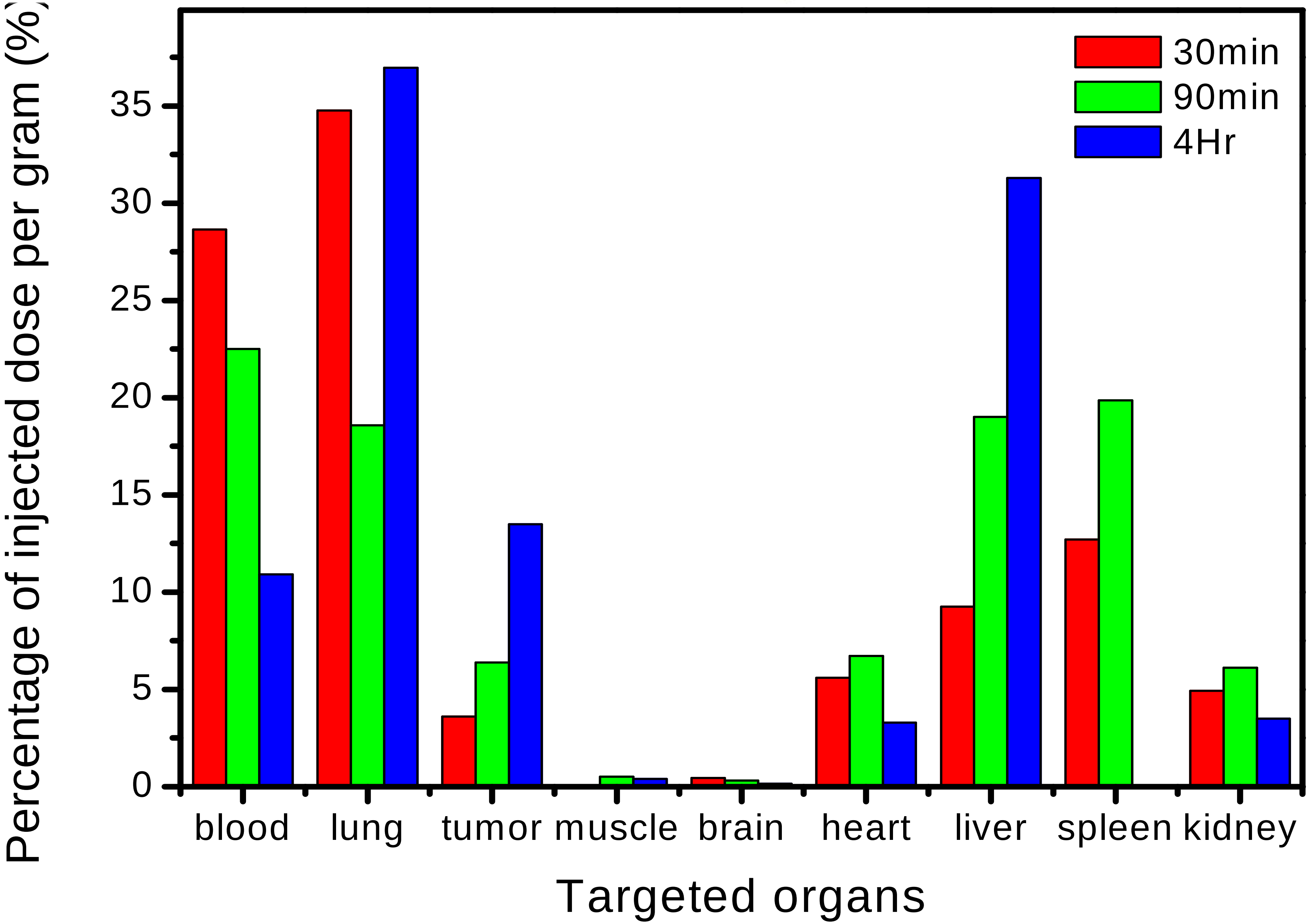
PEG-AuNP Pharmacokinetic



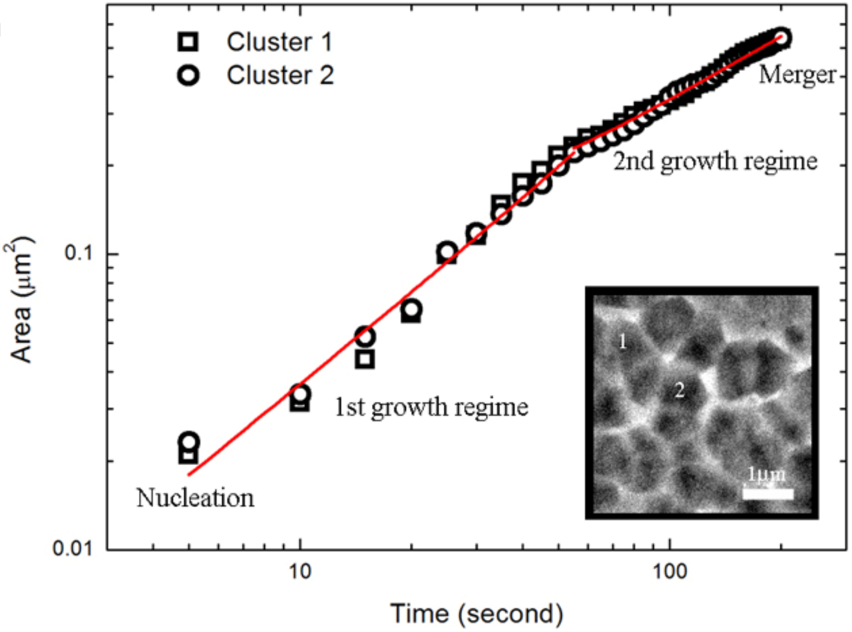
AuNP in tumor and muscle



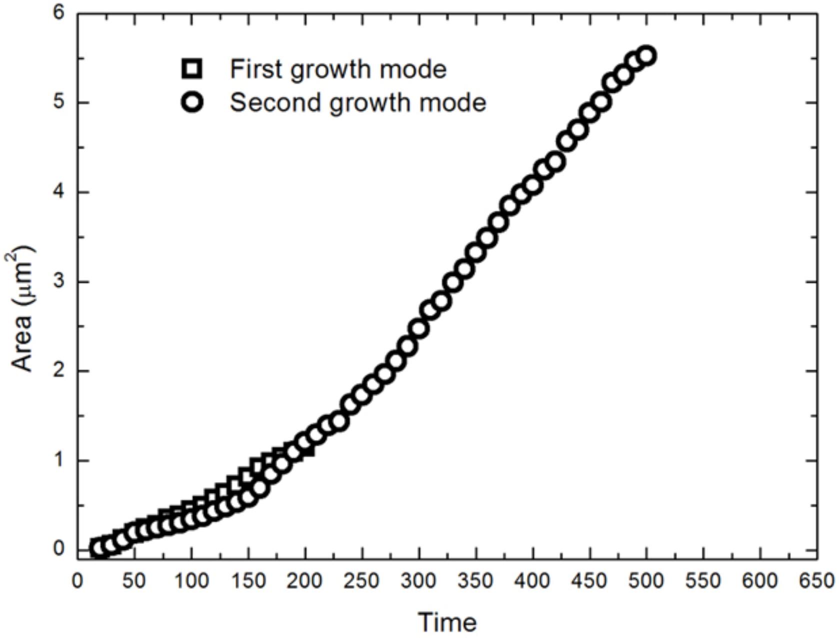
Normalized % of injected AuNP



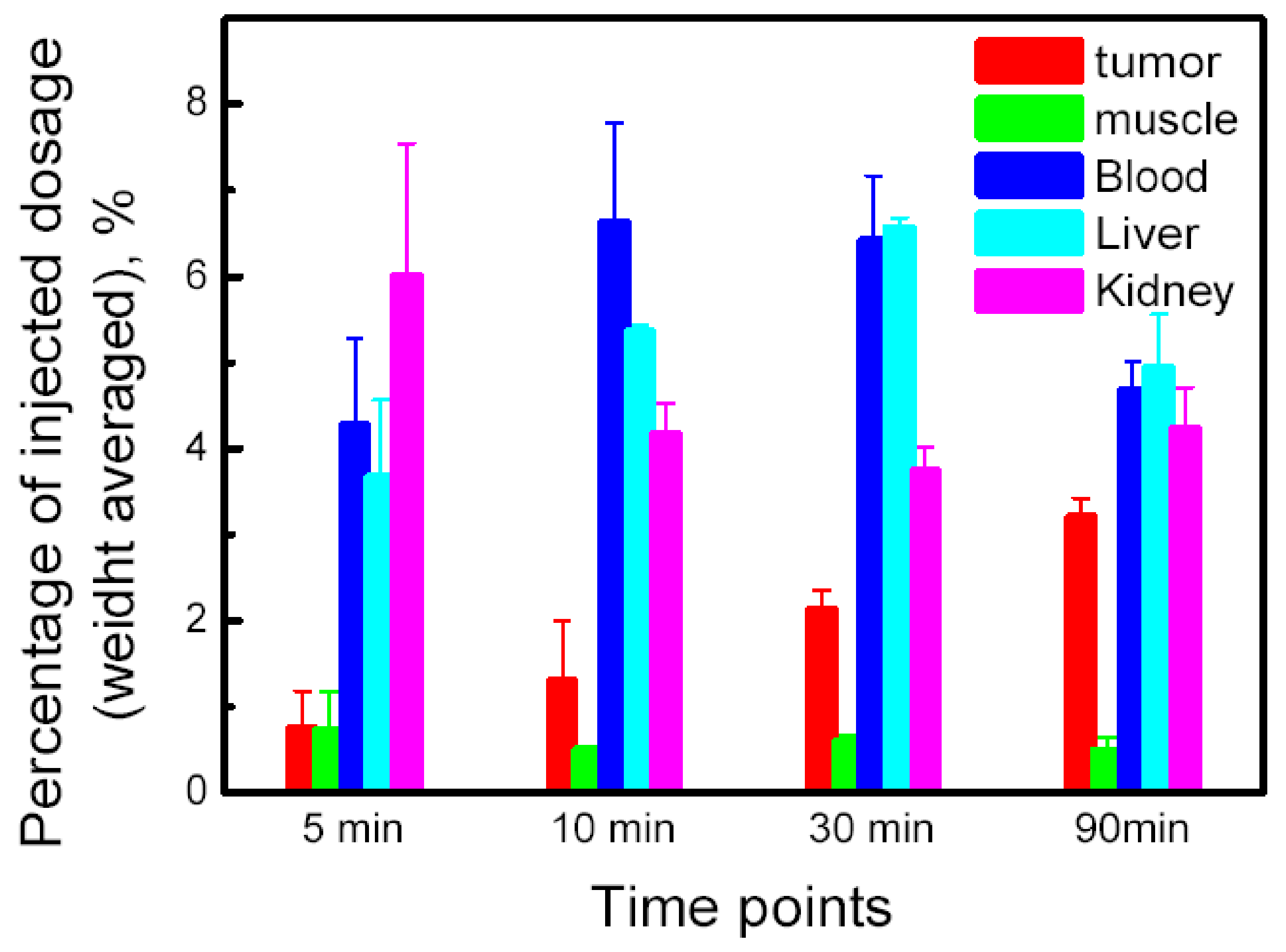
(a)


Cluster 1
Cluster 2
Merger
2nd growth regime
1st growth regime
Nucleation
1
2
1μm
Area (μm^2)
0.1
0.01
10
100
Time (second)

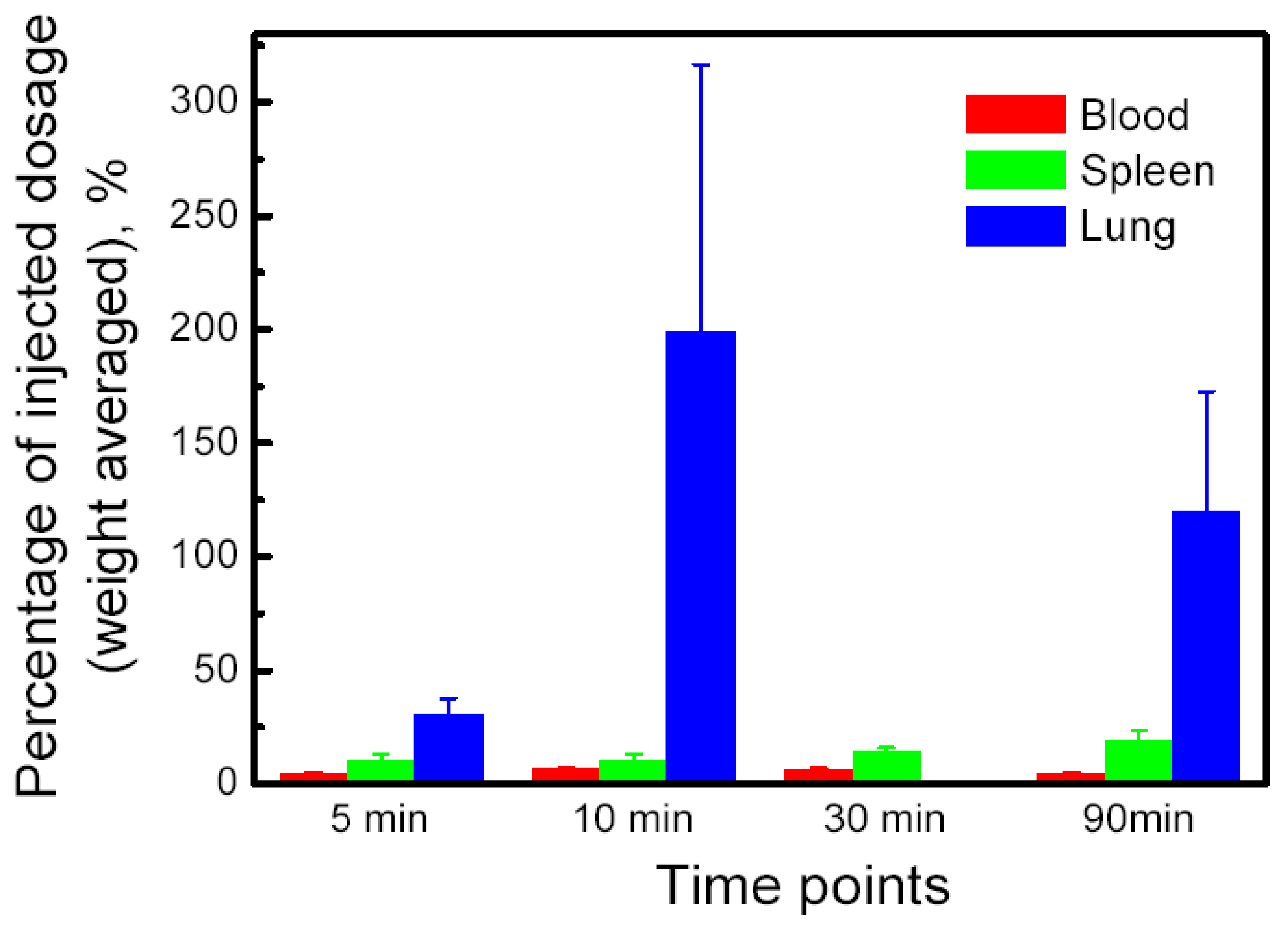
(b)


First growth mode
Second growth mode
Area (μm^2)
0
1
2
3
4
5
6
0
50
100
150
200
250
300
350
400
450
500
550
600
650
Time

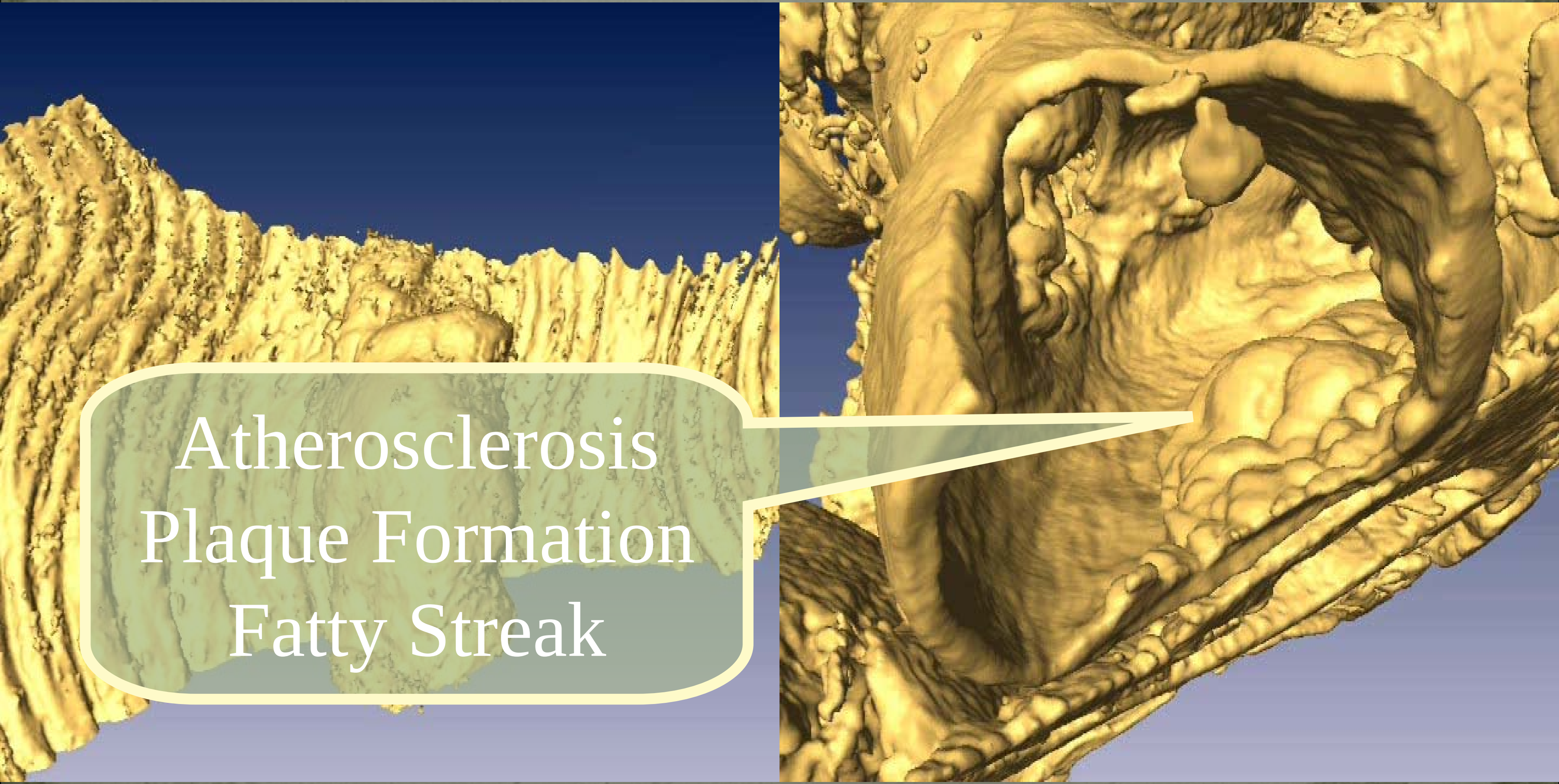
Gold nanoparticles



Gold nanoparticles



” Virtual Endo-microscope”
Visualization of Cardiovascular Disease



Atherosclerosis
Plaque Formation
Fatty Streak

Summary

- We have achieved THE BEST X-ray microscopy technology
- We used phase contrast and high temporal resolution for critical biomedical research.
- Crucial for TPS