Whole Brain Mapping with X-rays

Yeukuang Hwu 胡宇光

Institute of Physics, Academia Sinica

台大物理 27/10/2020

Collaborators: A true international effort!

- Taiwan
  - Keng S. Liang, Ting-Kuo Lee, Maw-Kun Wu, Shih-Chang Lee, En-Te Hwu, Ming-Li Chu, Chih-Hsiun Lin (Academia Sinica): phase retrieval, reconstruction, magnetic nanoparticles, microfluidity
  - Ann-Shyn Chiang, Chia-Wei Li (Life Science, Tsing Hua U.): cell biology, shell fish, fossil, fire fly
  - Hong-Ming Lin (Tatung U): electron chemistry, battery
  - Yu-Tai Ching (NCTU), David Chien (UCS-St. Marcos): Image Processing, reconstruction
  - Chung-Shi Yang, Kelvin K. C. Tsai (NHRI): nanomedicine, microangiogenesis
  - Ann Chen, Maria Ka, Dueng-Yuan Hong (TSGH): CKD vessel imaging
  - Y. F. Hu, S. K. Tsai (TTY Pharmaceutics): (drug delivery, pharmacokinetics)
  - Y. C. Yang, Hong-I Yeh, Yu-Jen Chen (Mackay Memorial Hospital): (artery disease, tumor development)
- Japan: T. Ishikawa, Y. Kohmura, K. Sawada, Y. Joti (RIKEN/SPring-8 Center)
- Korea: Jung-Ho Je (POSTECH), Doyoung Noh (GIST), Jun Lim, Jae-Hong Lim (PAL)
- China: Jun Hu, Chunhai Fan, Lihua Wang, Xiaoqing Cai, Chichao Zhang, Ying Zhu
- Singapore: Eng Soon Tok, Alvin Teo, Chian-Ming Low, Gan-Moog Chow, H. O. Moser (NUS)—drug delivery, polymer blend, electrochemistry, neurobiology.
- France: Cyril Petibois, Sophie Javerzat, Michele Moenner (U. Bordeaux, brain tumor angiogenesis), Patrick Soukiassian
- USA: Yong S. Chu, Wah-Keat Lee, Qun Shen, B. Lai, (APS), Wen-An Chiou (U. Maryland), John Boeckl (US Air Force Lab)–TXM
- Switzerland:
  - G. Margaritondo (EPFL)
  - Rolf Gruetter (EPFL)

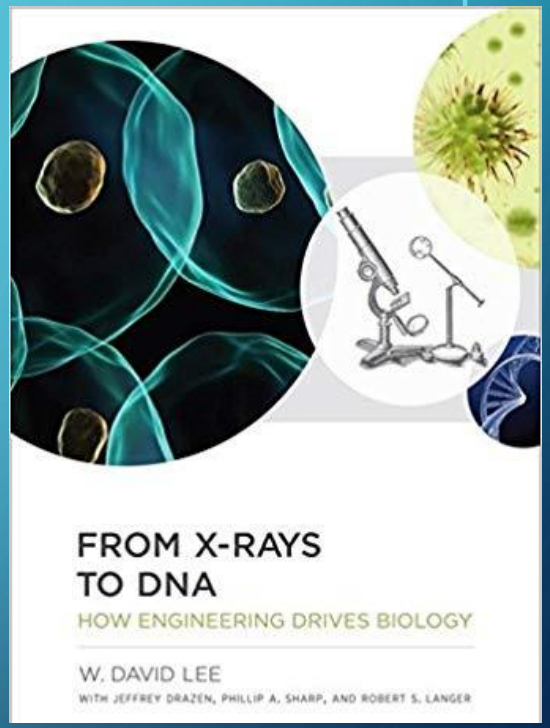
NanoX Team (Current Members)

- X-ray microscopy:
  - Hsiang-Hsin Chen (陳翔欣), Shun-Min Yang (楊舜閔), Tsung-Tse Lee (李宗澤), Cheng-Huan Hsu (徐晟桓), Ching-Yu Chiou(邱鏡宇)
- X-ray Nanosynthesis:
  - Ming-Tsang Lee (李旻倉)
- Nanofabrication of X-ray optics:
  - Mei-Chun Chen (陳玫君), Yu-Ting Jian (簡郁庭)
- Bioimaging:
  - Shun-Min Yang (楊舜閔), Hsiang-Hsin Chen (陳翔欣), Yi-Yun Chen (陳怡云), Chia-Ru Chang (張家如), Ya-Sian Wang (王雅嫻), Shiou-Jin Chiou (邱繡謹), Cheng Jyun Yang (楊程鈞)

- Visitors: Cyril Petibois, Pei-Feng Chen, Keng S. Liang, Benoit Recur

Innovation in physics and instrumentation has opened new eras of biology and medicine

- Microscopy
- Spectroscopy
- X-rays scattering
- Mass spectrometry
- Nuclear Medicine
- NMR, SPECT, PET, CT, ultrasound, OCT
- …



And, More Recently..

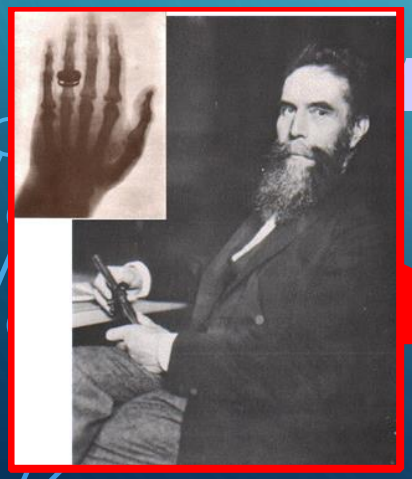
- Laser confocal scanning microscopy + fluorescent protein
- Protein crystallography
- Cryo-electron microscopy

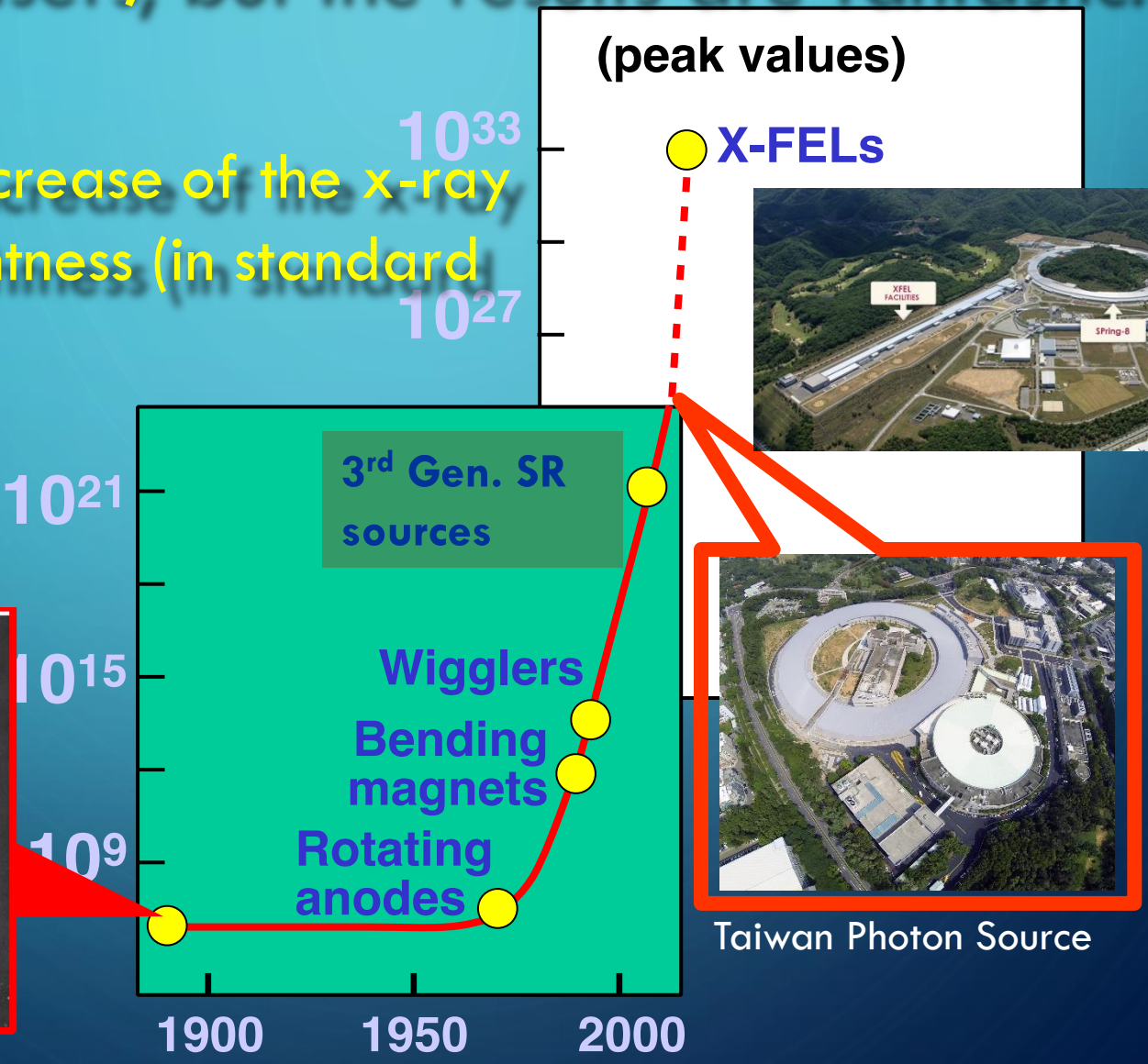
The breakthroughs in x-ray imaging

- Phase contrast
- Nano-resolution
- Elemental contrast
- High speed for 3D imaging

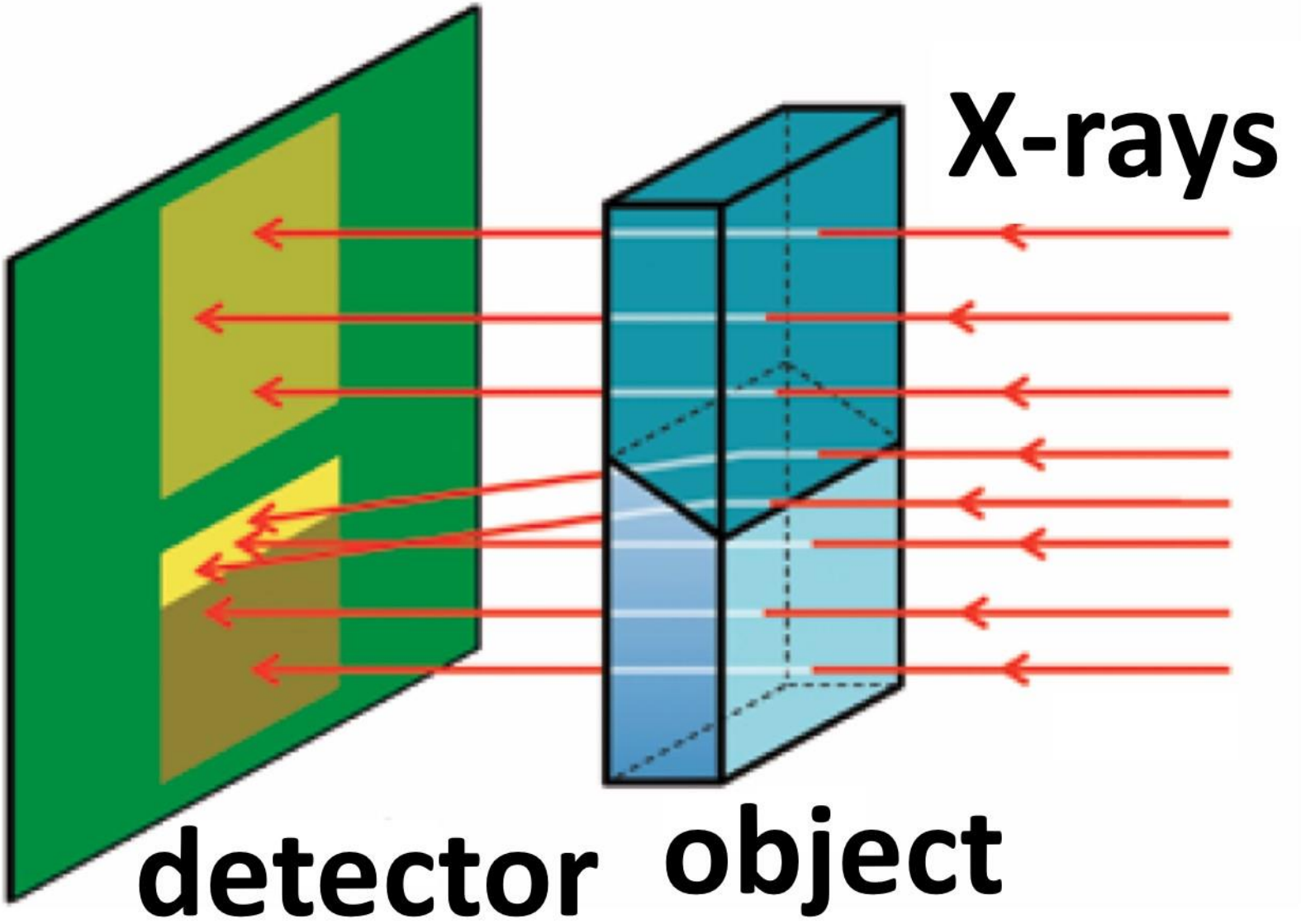
It took decades to obtain the first x-ray free electron lasers, but the results are fantastic:

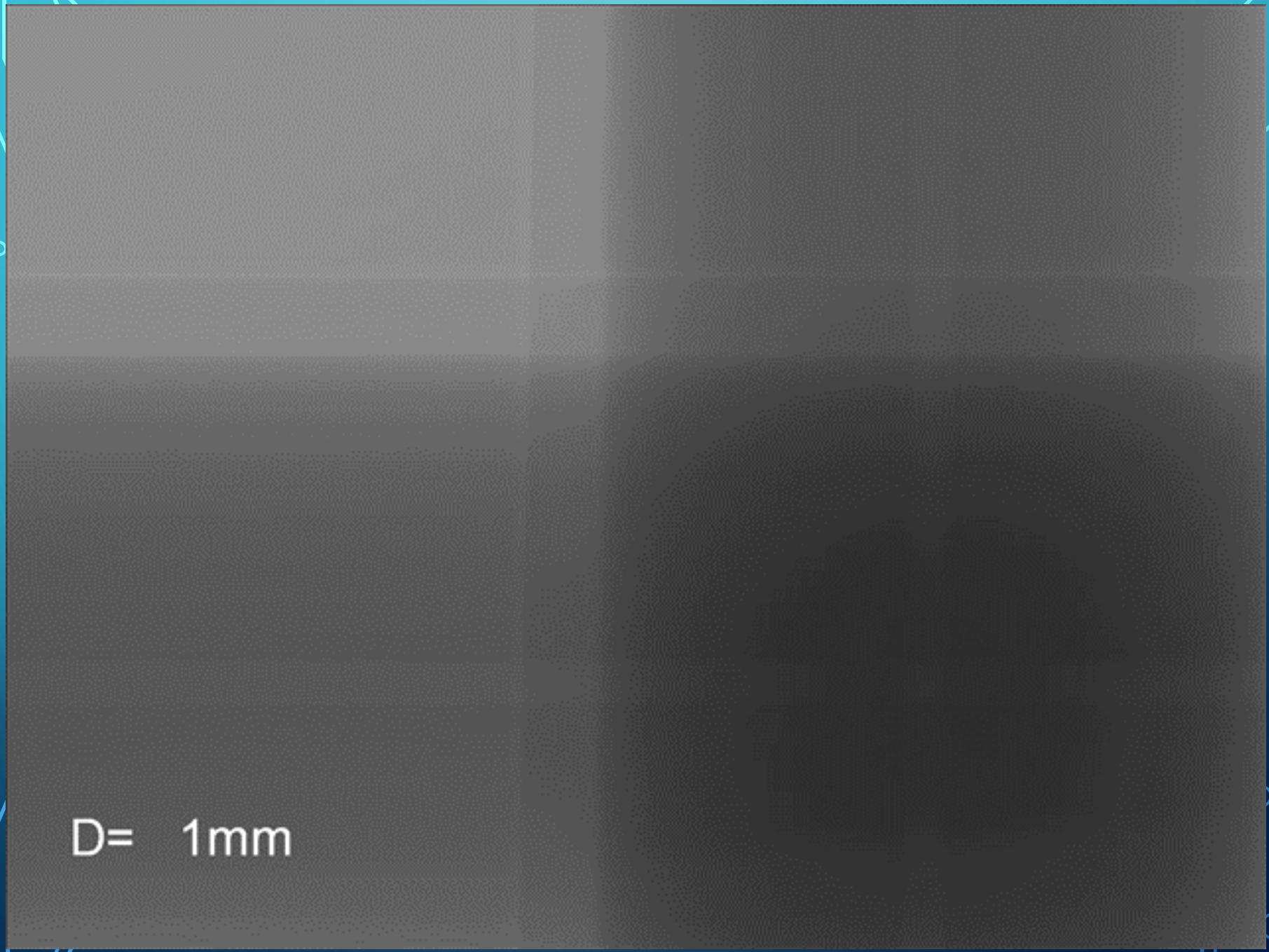
Historical increase of the x-ray source brightness (in standard units)





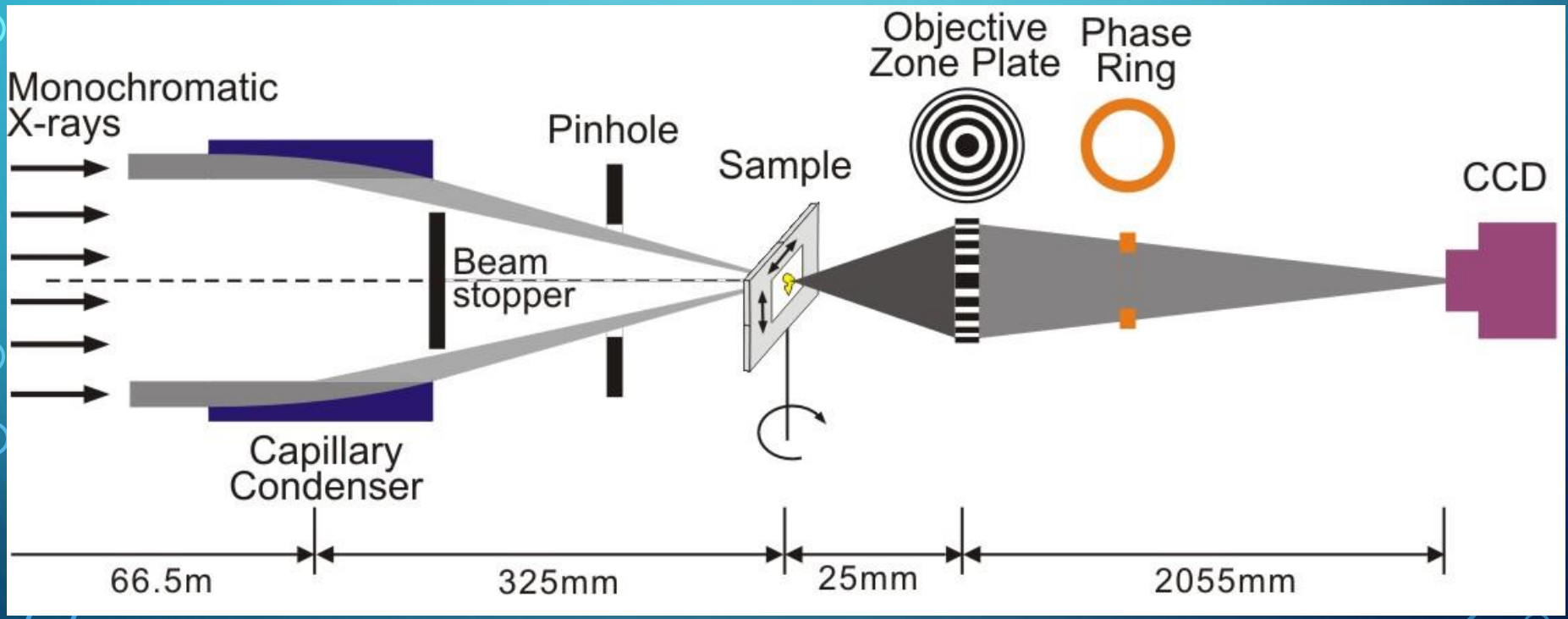
Taiwan Photon Source


X-rays
detector
object

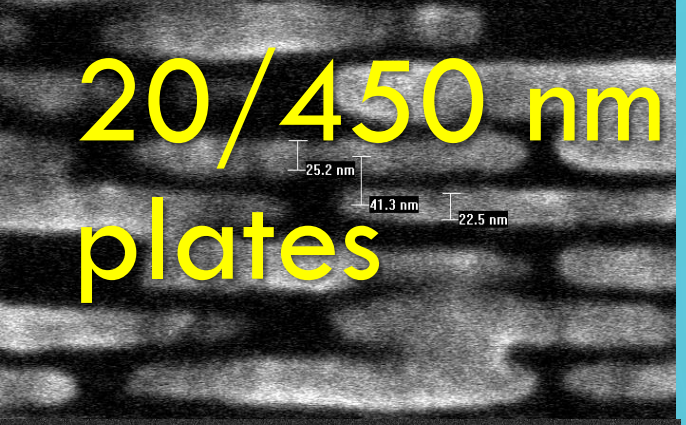

D= 1mm

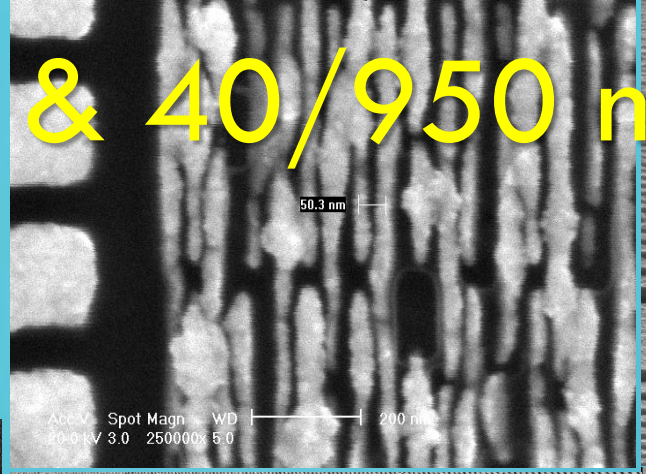
The second breakthrough: nanoscale resolution

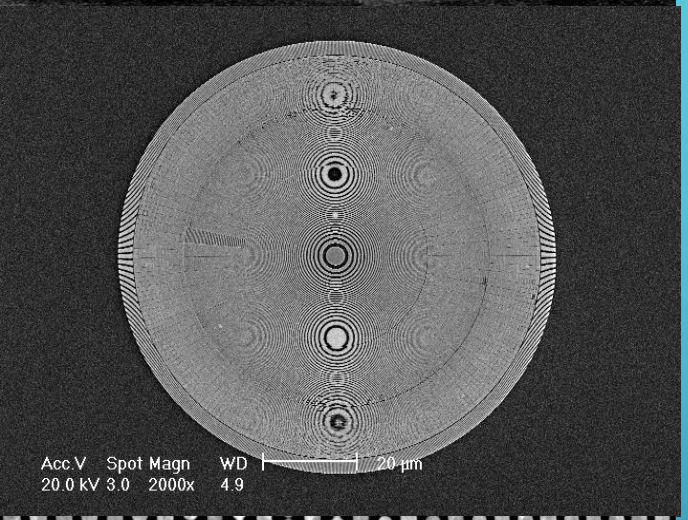
Transmission x-ray microscopy with Fresnel zone plate optics

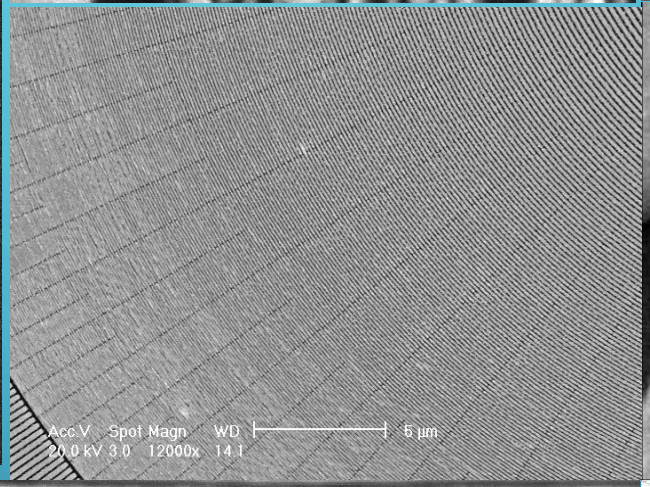


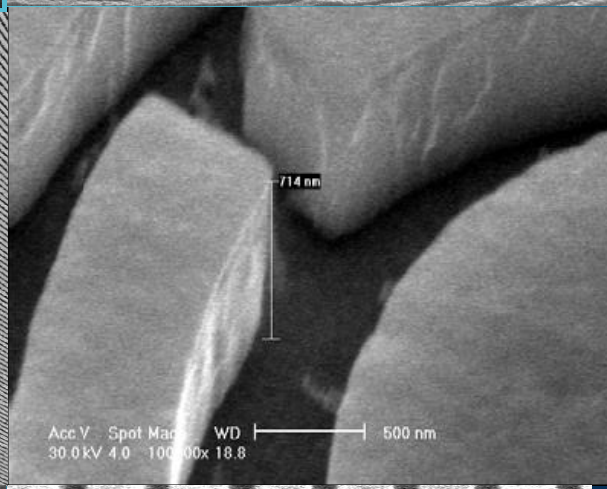
20/450 nm & 40/950 nm Au zone plates

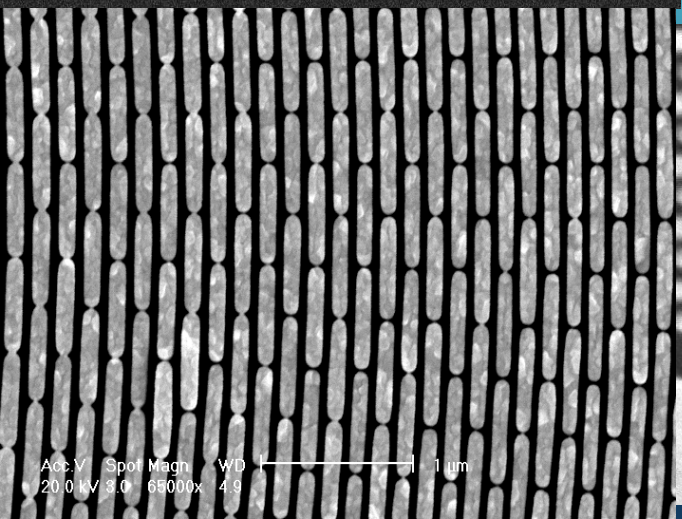


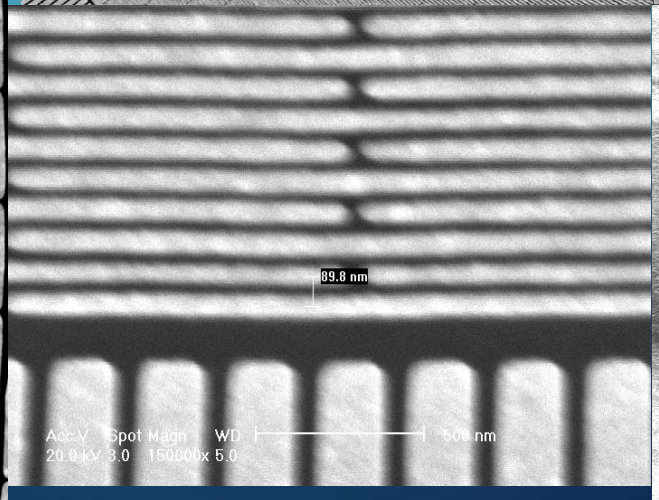


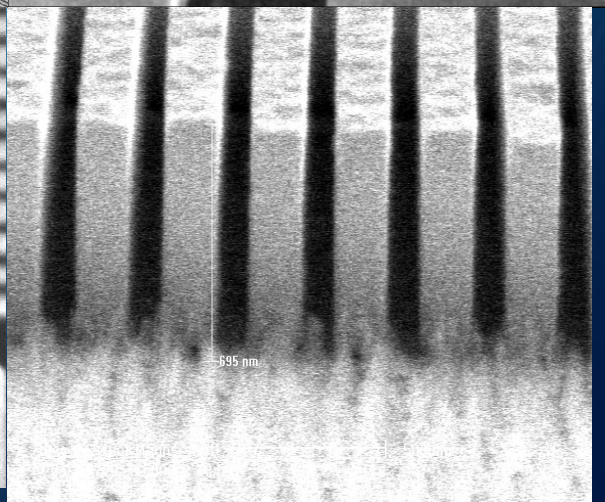












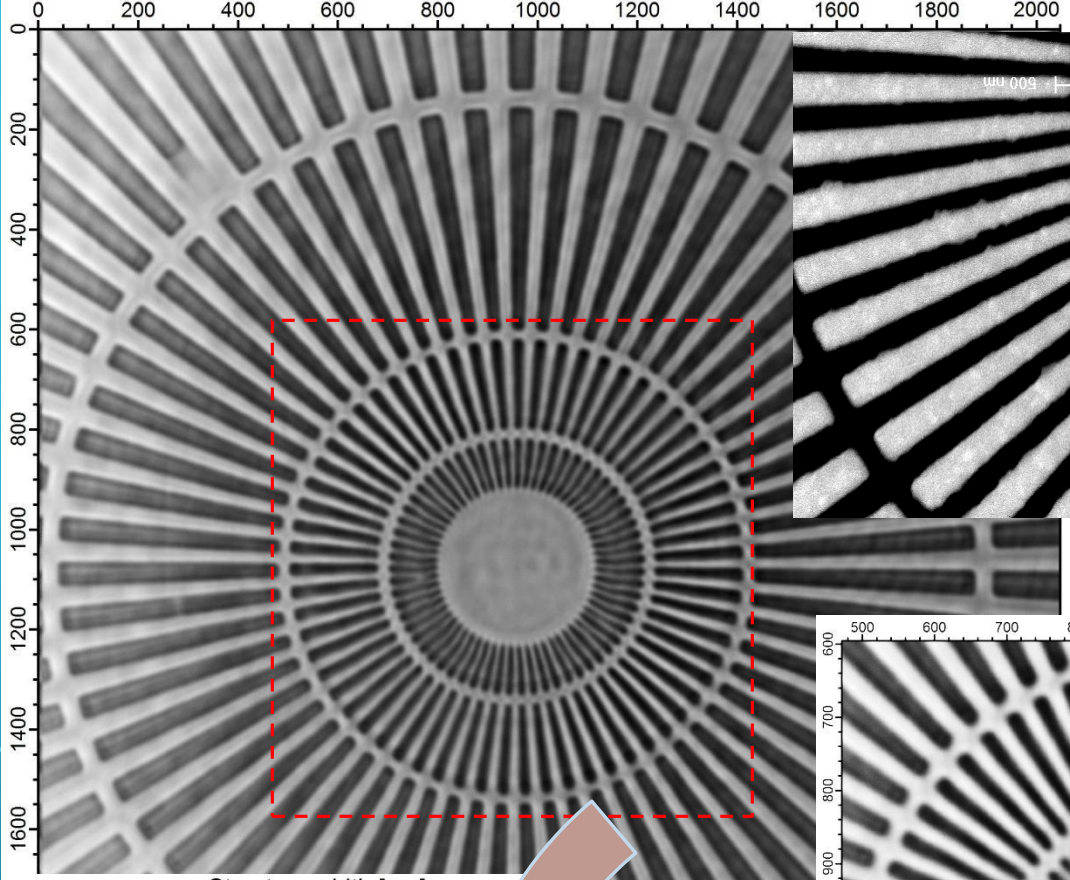
Star Pattern Image

Finest line width: 20nm
Au thickness: 300nm

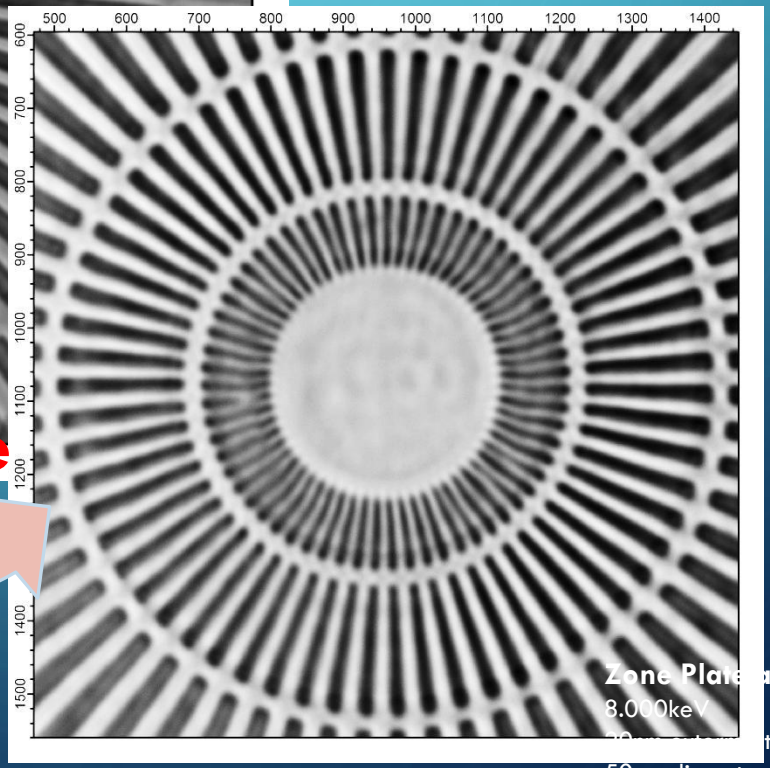
Pixel size: 2.51nm
FOV: 5.14um

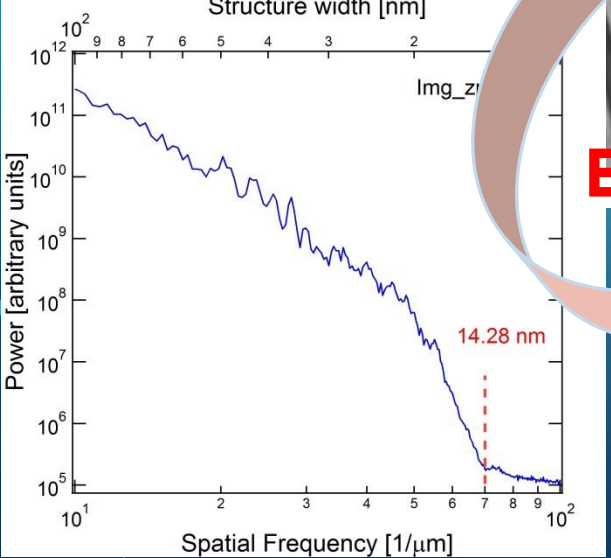
No binning
8 sec exposure
60 frames averaged

4 pieces of diffuser



Enlarge

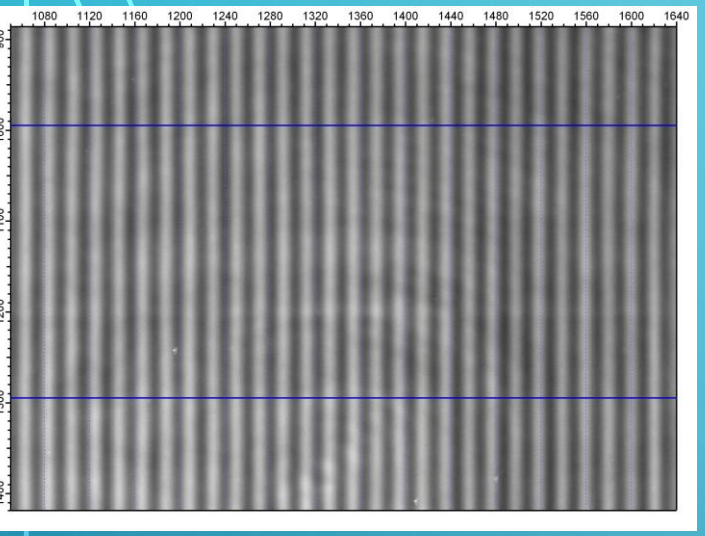


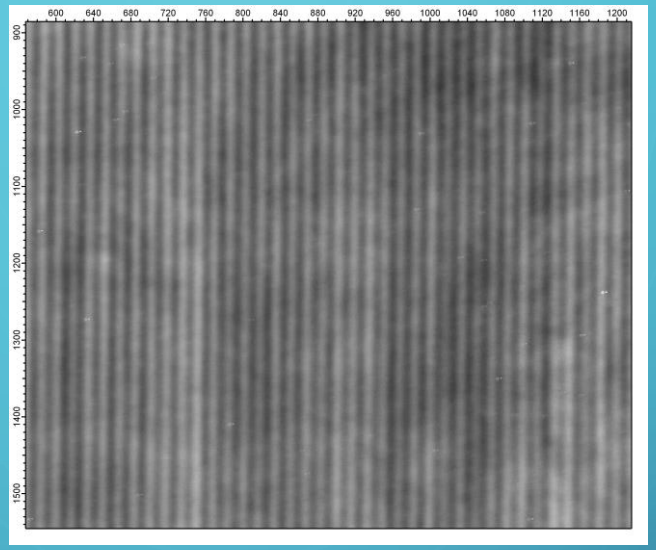


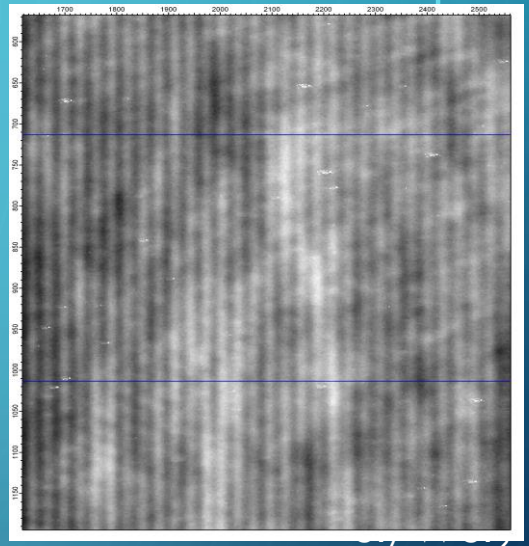
Zone Plate applied (ZP m2-6):
8.000keV
t zone width
50um diameter
400nm thickness

Si/W multilayer test patterns

H. R. Wu, et al. Nanoresolution Radiology of Neurons, J. Phys. D 45, 242001 (2012).



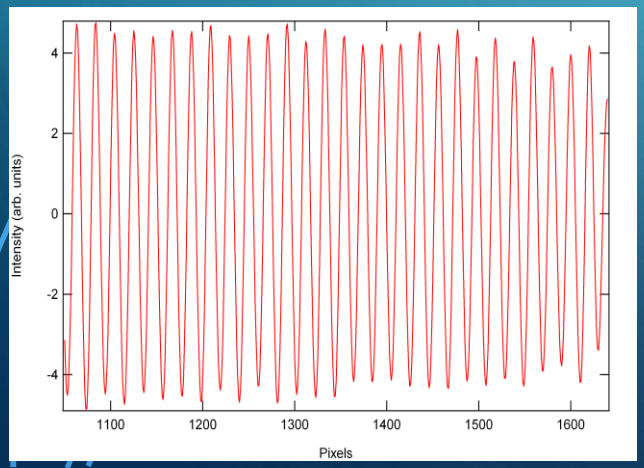


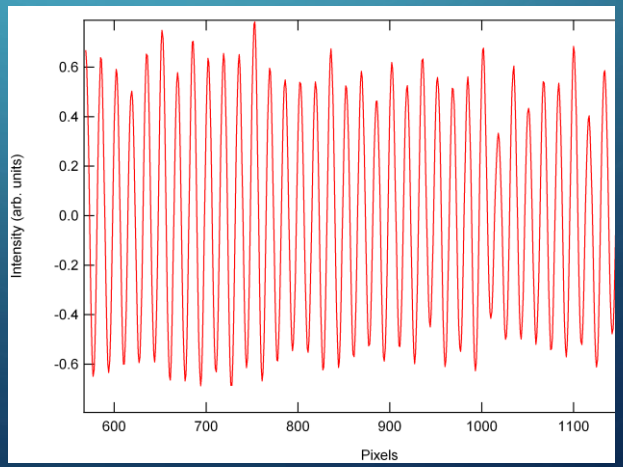


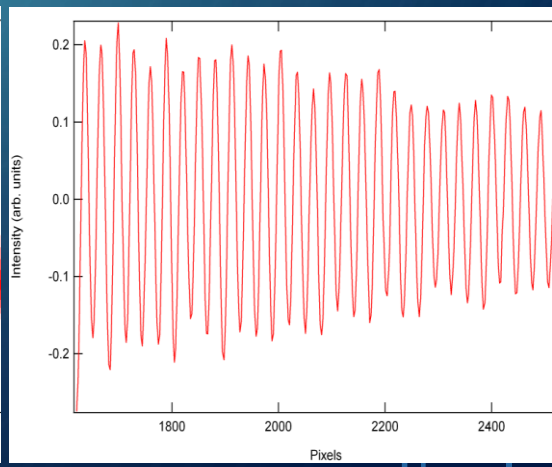
Finest line width = 25nm

20 nm

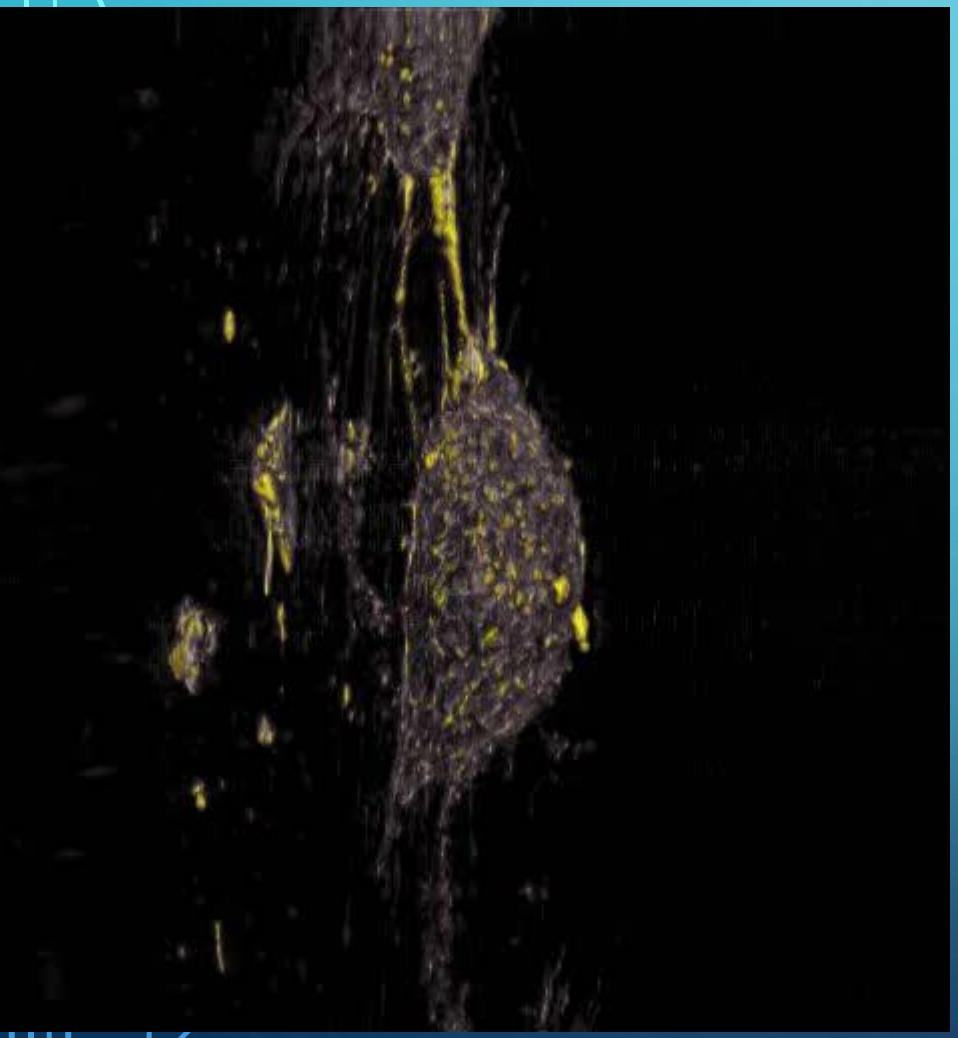
15 nm

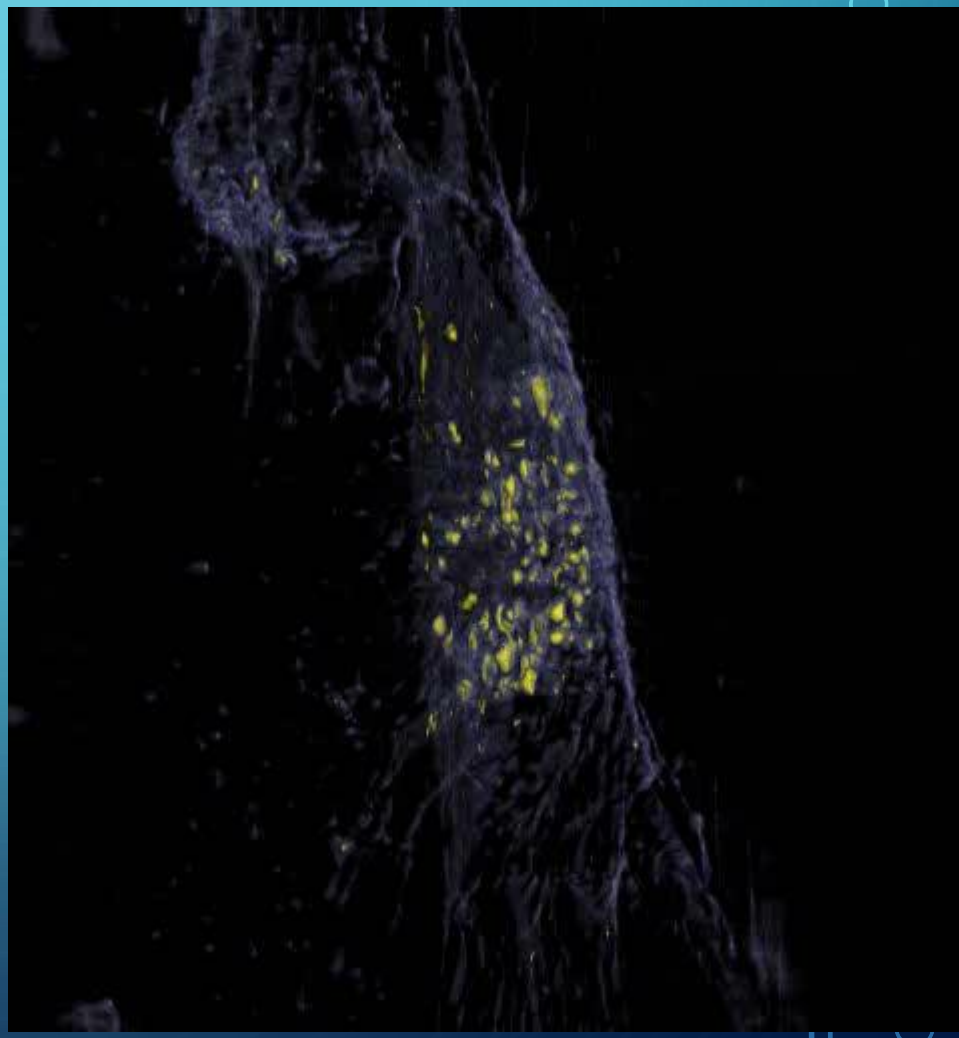




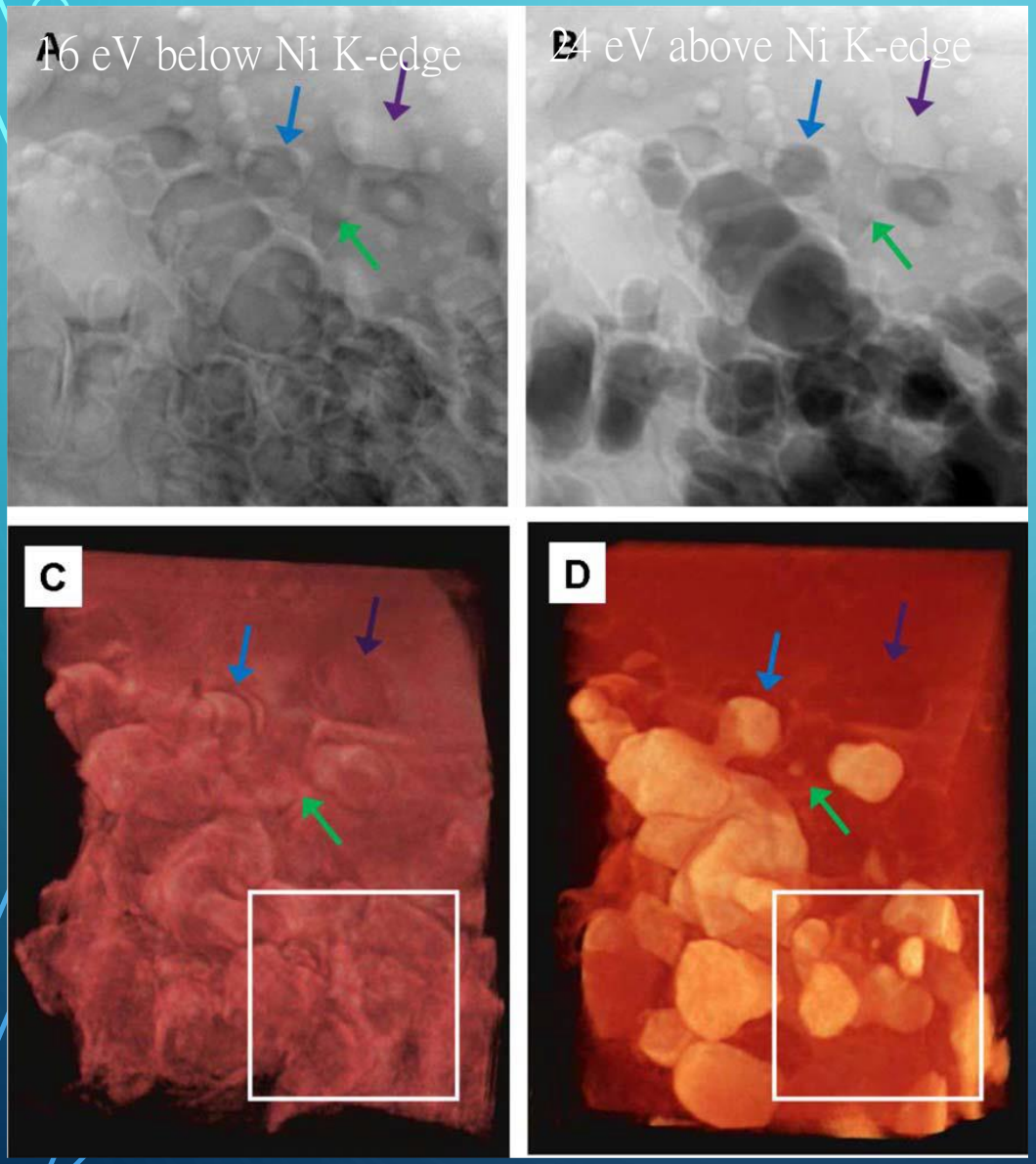


HeLa cells with AuNPs on culture dish





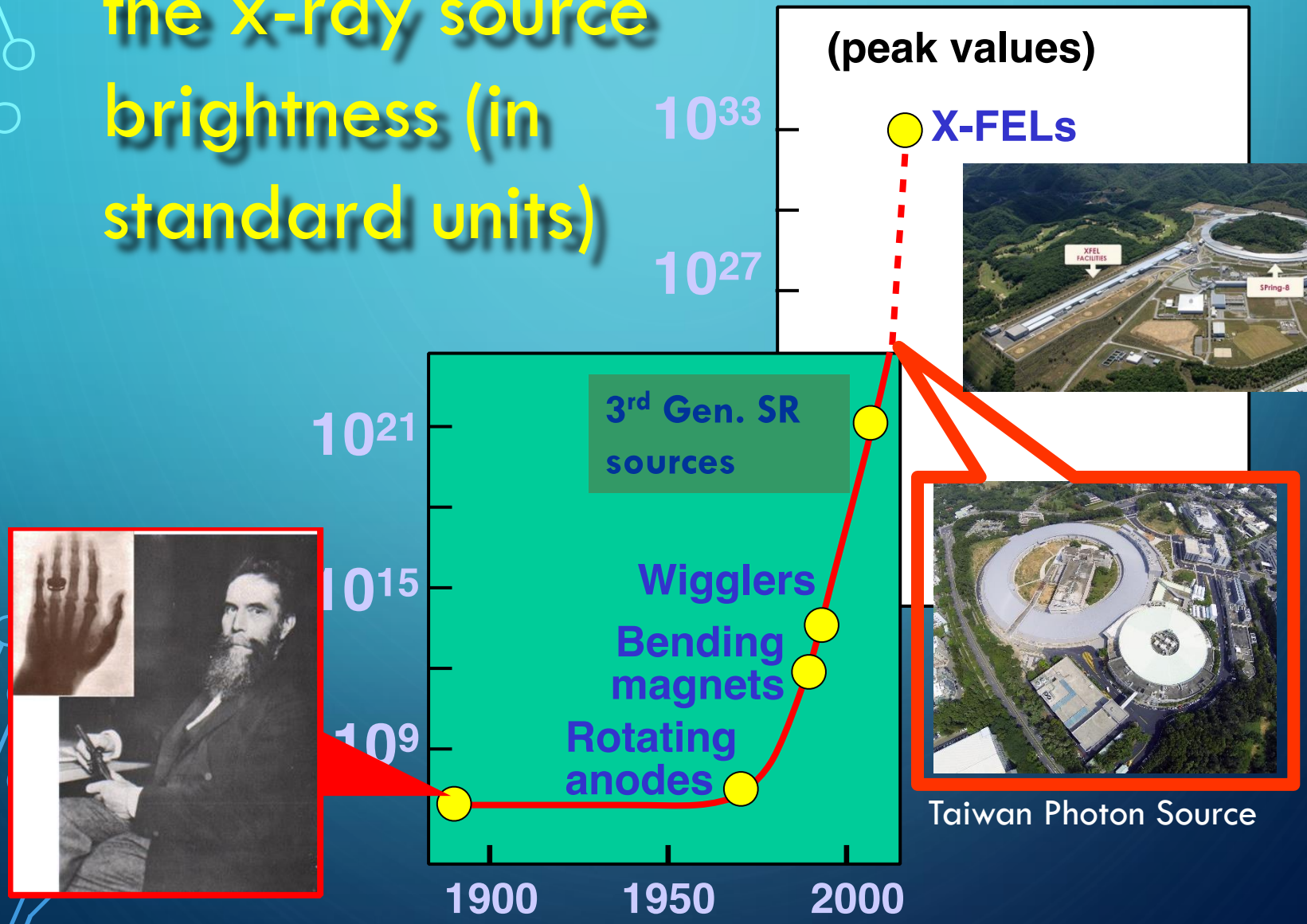
Element mapping with nanoresolution



J Electrochem Soc **157**, B783 (2010) **Figure 2**

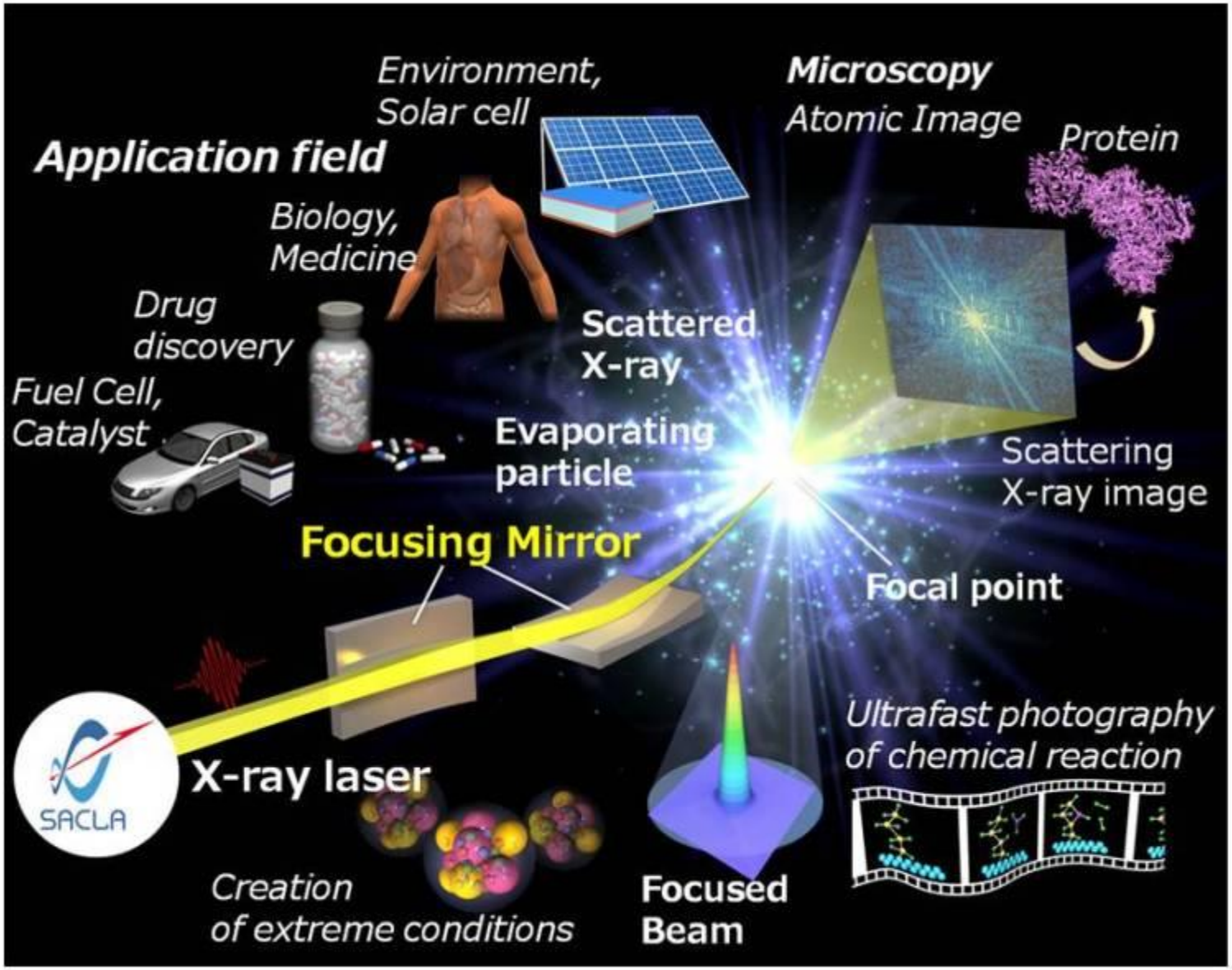
- Ni – YSZ (yittria-stabilized zirconia) anode
- Ni K-edge: 8.333 keV
- Spatial resolution: 38.5 nm
- Box size: 5 μm

Historical increase of the x-ray source brightness (in standard units)

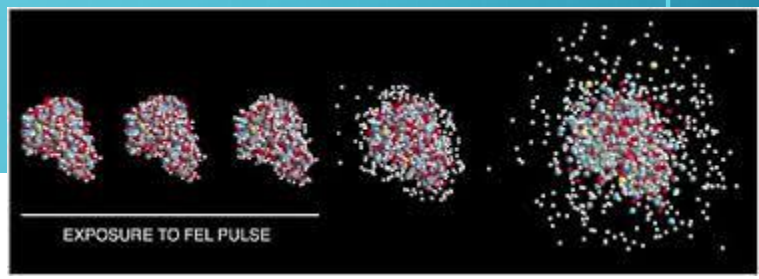


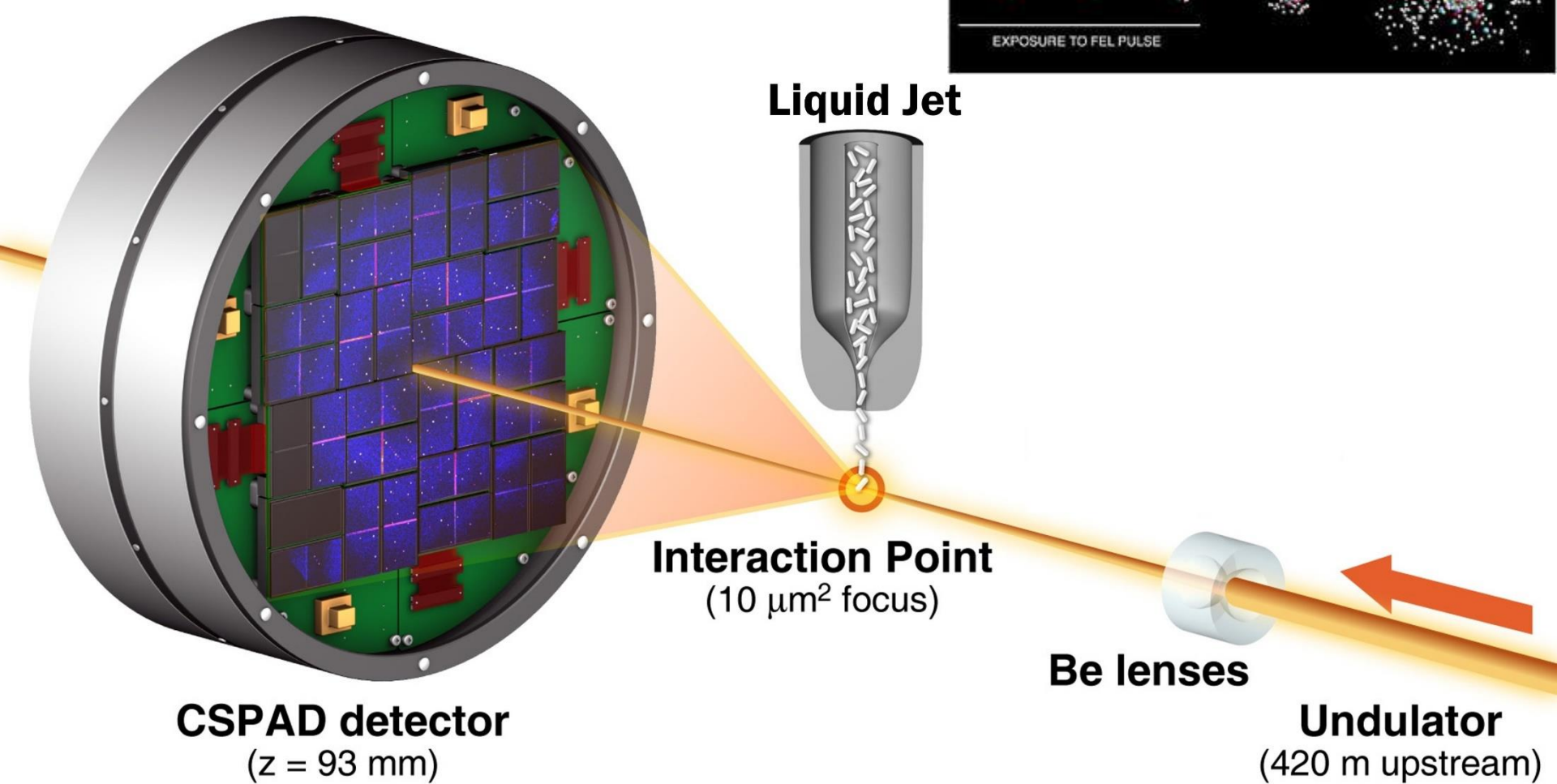
Phase contrast: the first breakthrough

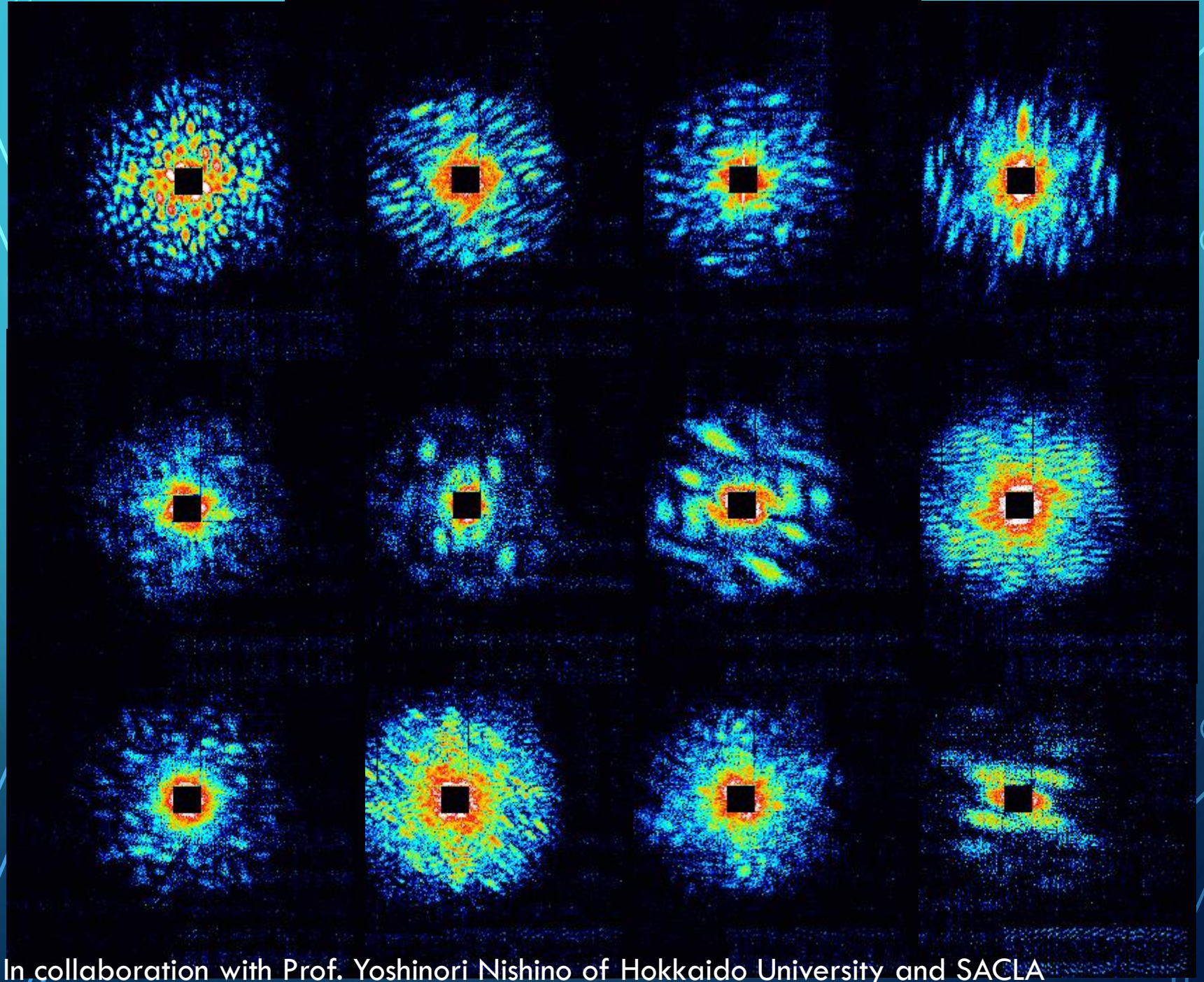
The XFEL: Toward the ultimate x-ray source


Environment,
Solar cell
Microscopy
Atomic Image
Protein
Application field
Biology,
Medicine
Drug
discovery
Fuel Cell,
Catalyst
Scattered
X-ray
Evaporating
particle
Scattering
X-ray image
Focusing Mirror
Focal point
SACLA
X-ray laser
Ultrafast photography
of chemical reaction
Creation
of extreme conditions
Focused
Beam

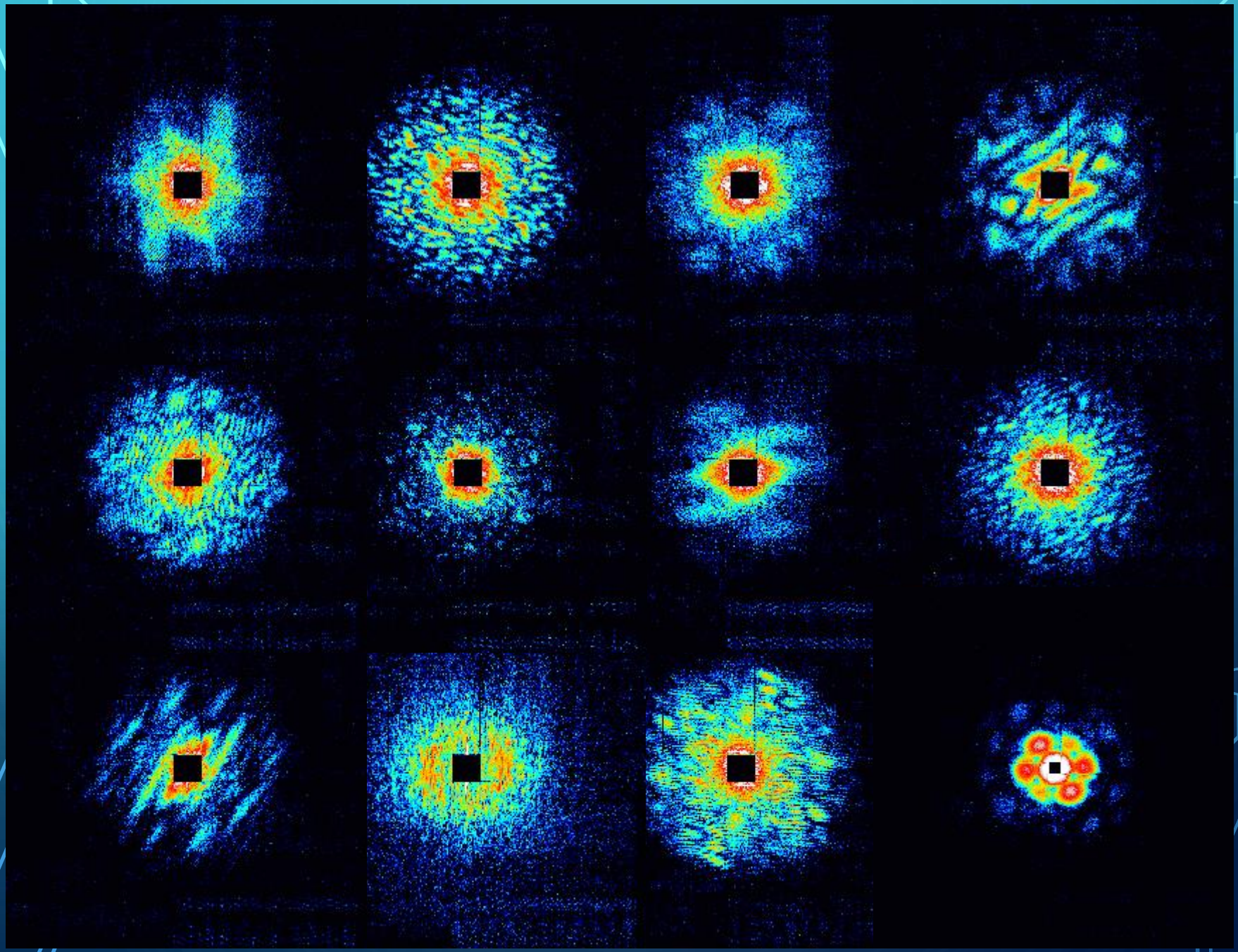
“Detect and Destroy”



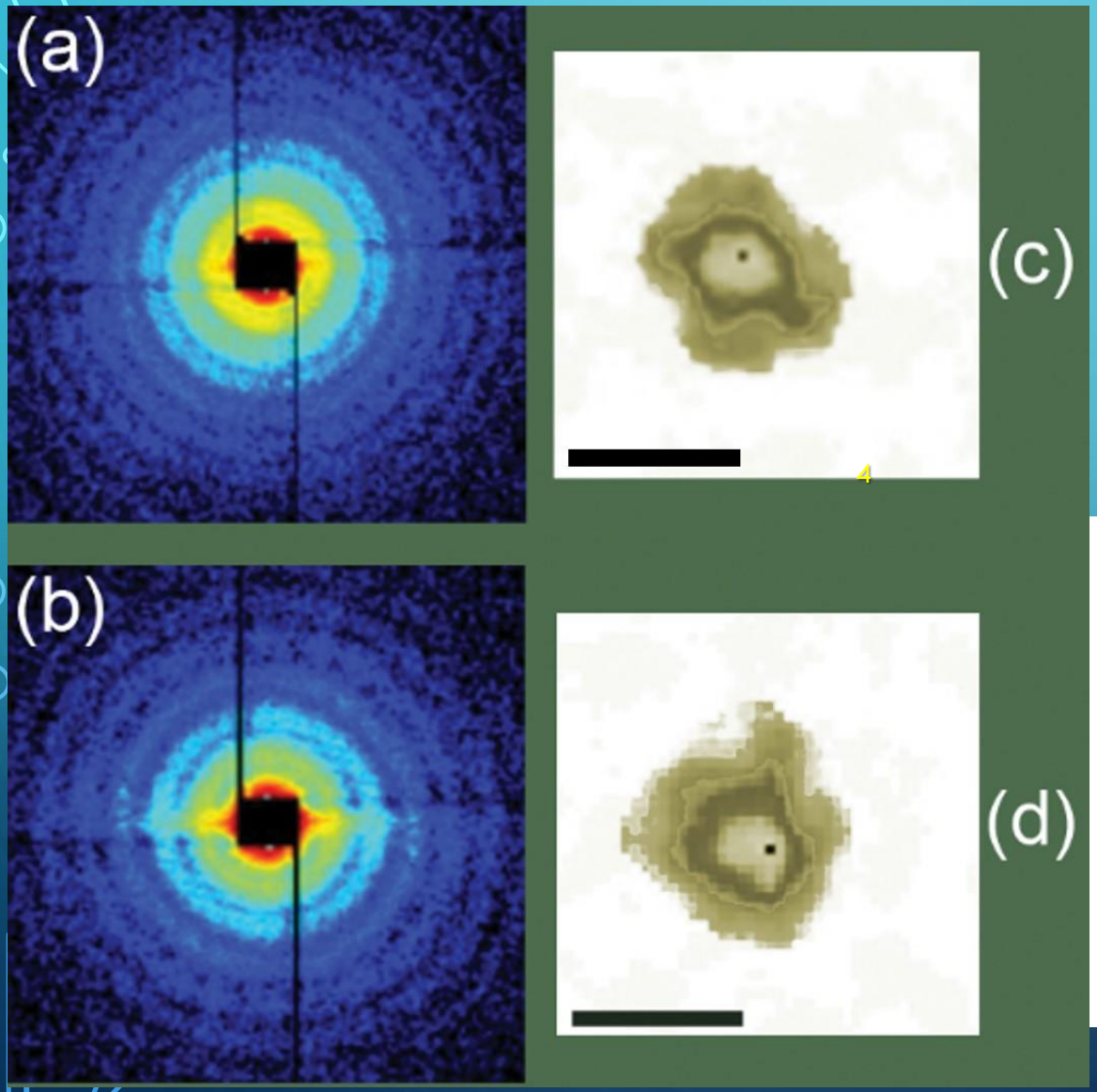


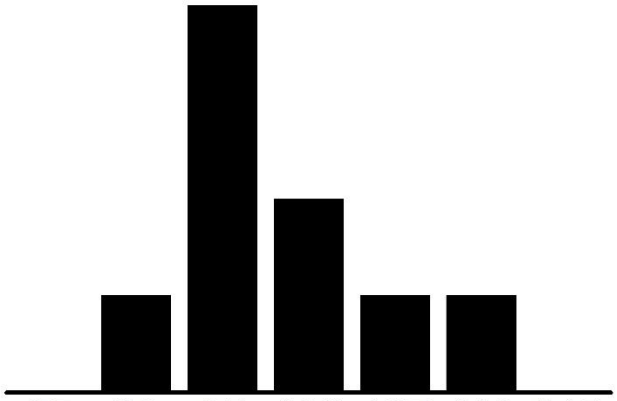


In collaboration with Prof. Yoshinori Nishino of Hokkaido University and SACLA

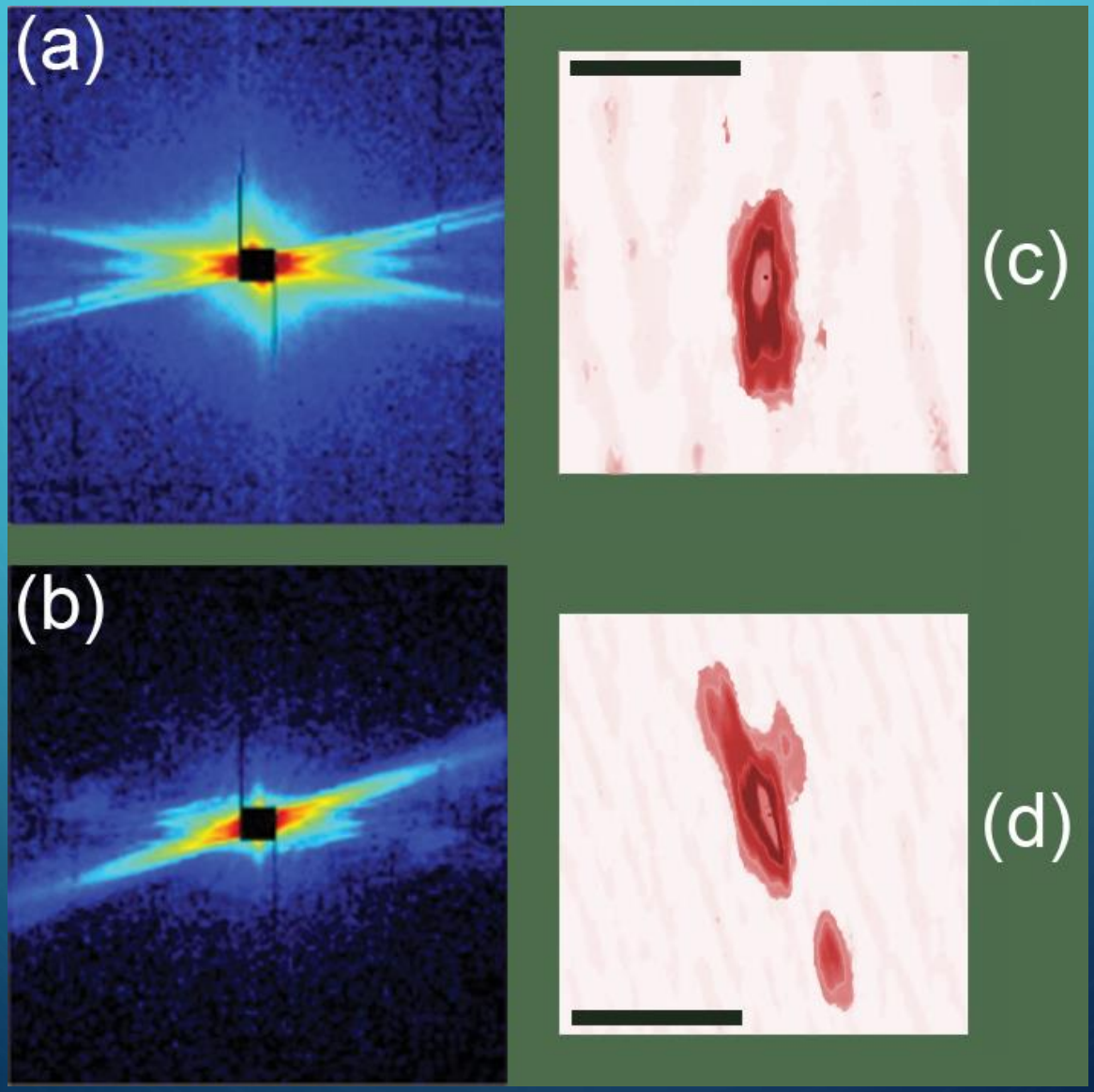


Blank liposome ($(NH_4)_2SO_4$ inside)

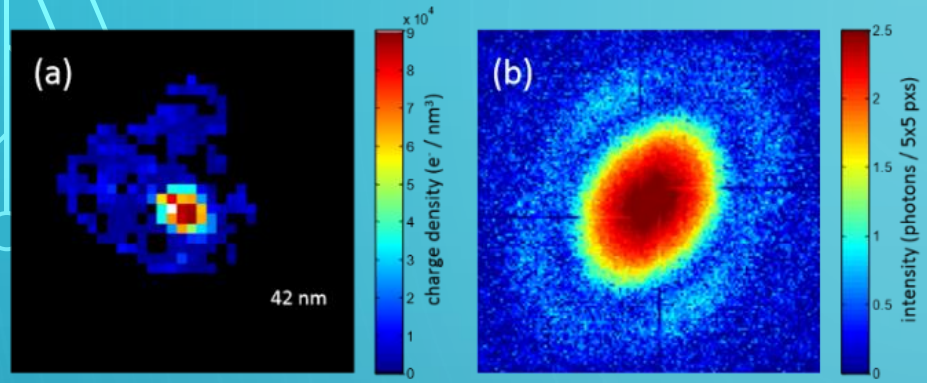




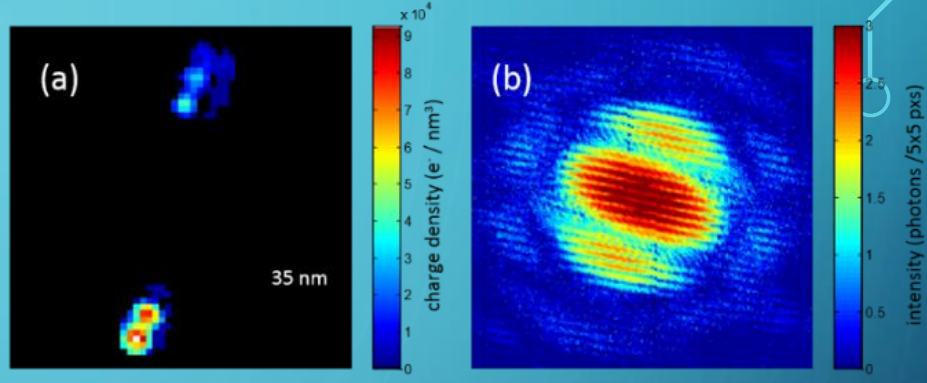
Doxorubicin loaded liposome



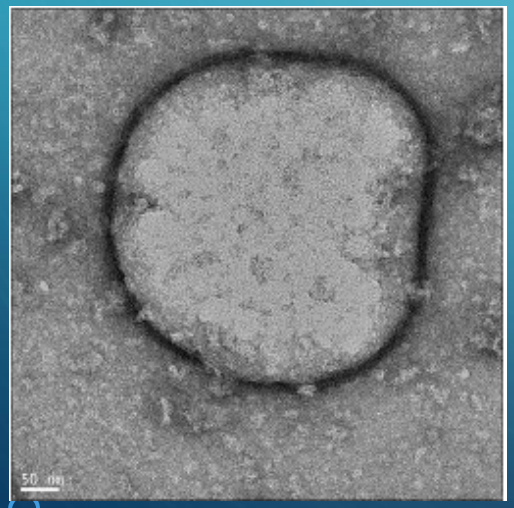
Coherent diffraction imaging (XCDI)

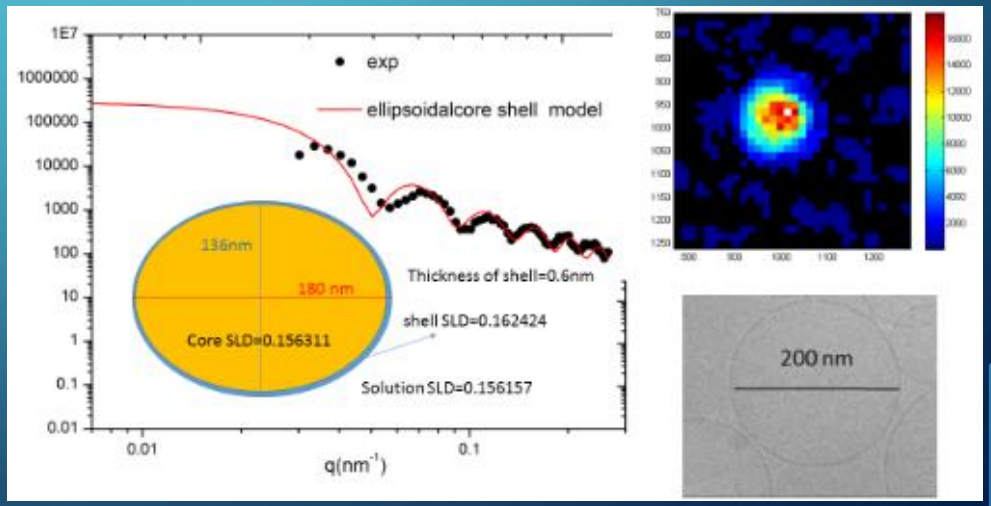


A single 40 nm AuNP



Two 10 nm AuNP



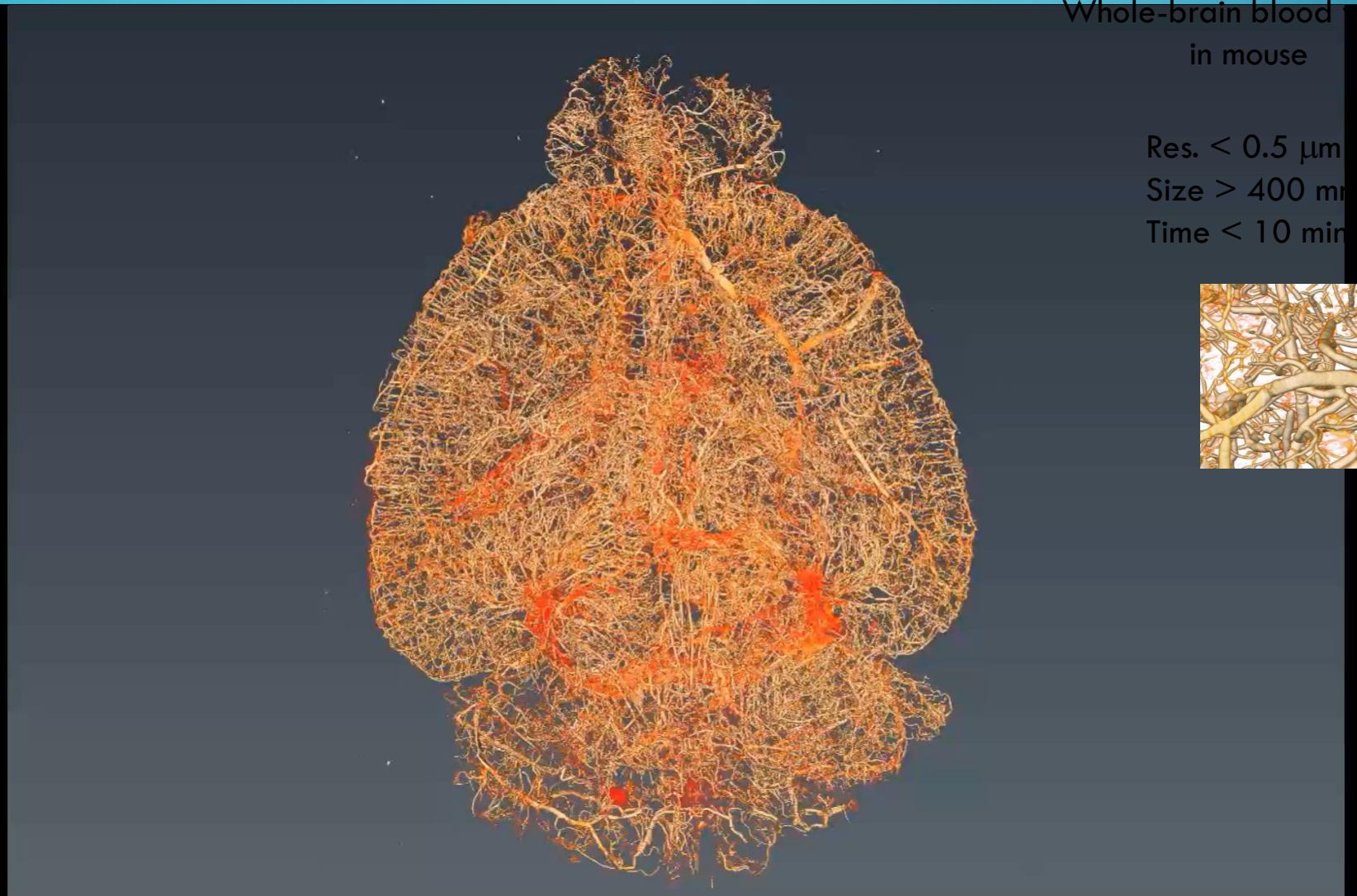


A single 200 nm liposome

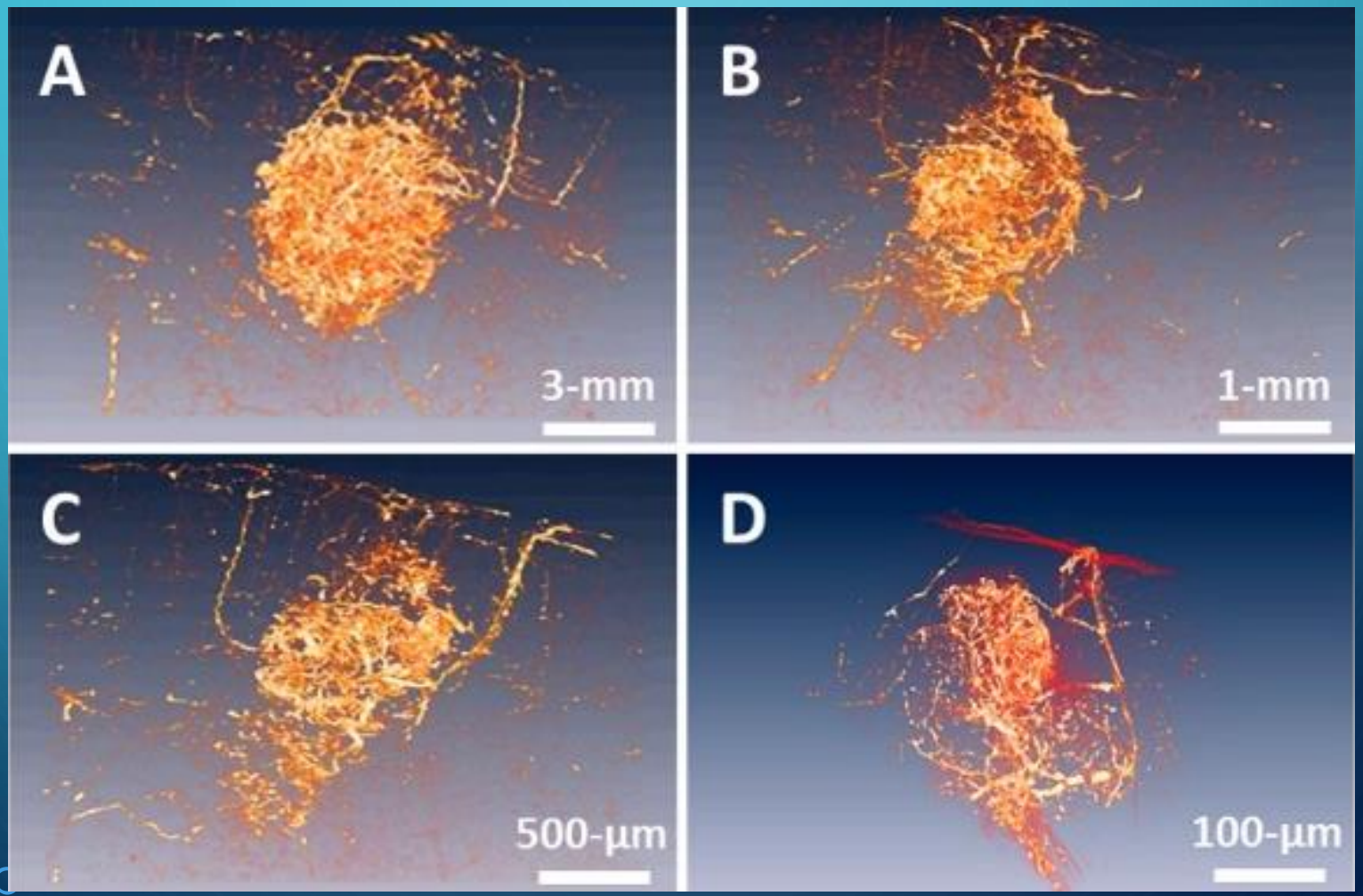
Tumor Induced Angiogenesis

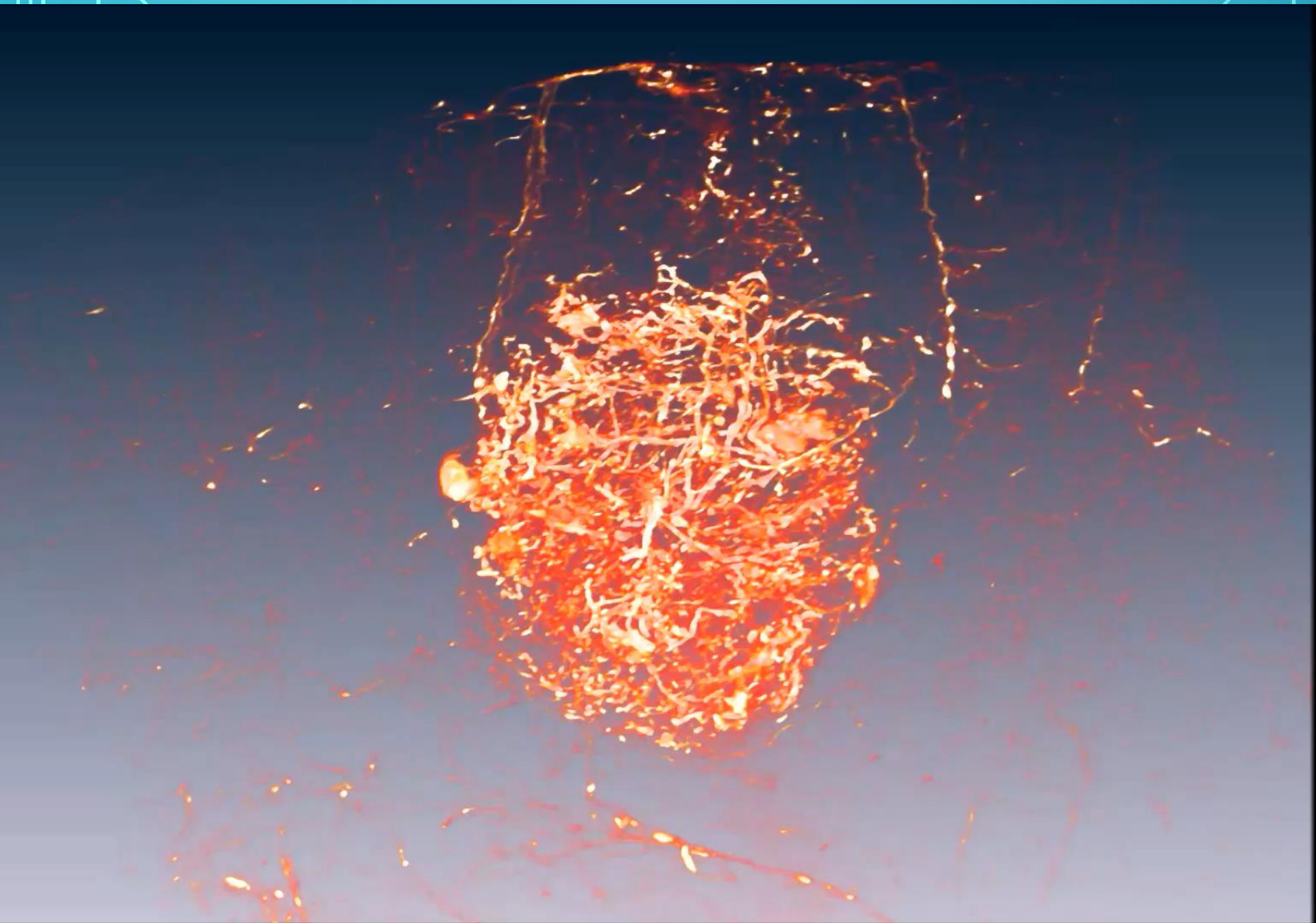
Cyril Petibois et al. (U. Bordeaux), DY Hong et al. (TSGH),

Large tissue, high resolution and speed

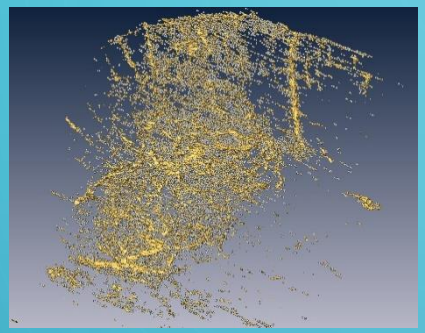
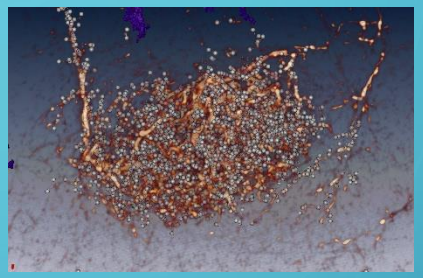
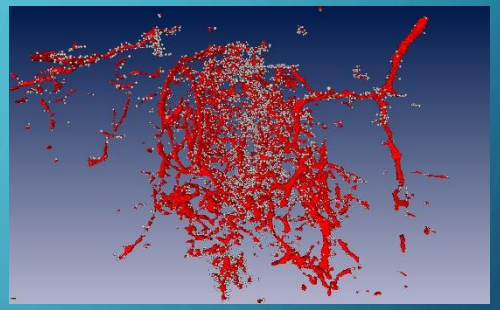


X-ray imaging of angiogenic microvasculature of glioblastoma



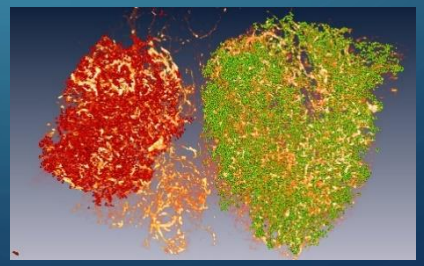


Solid glioma tumors

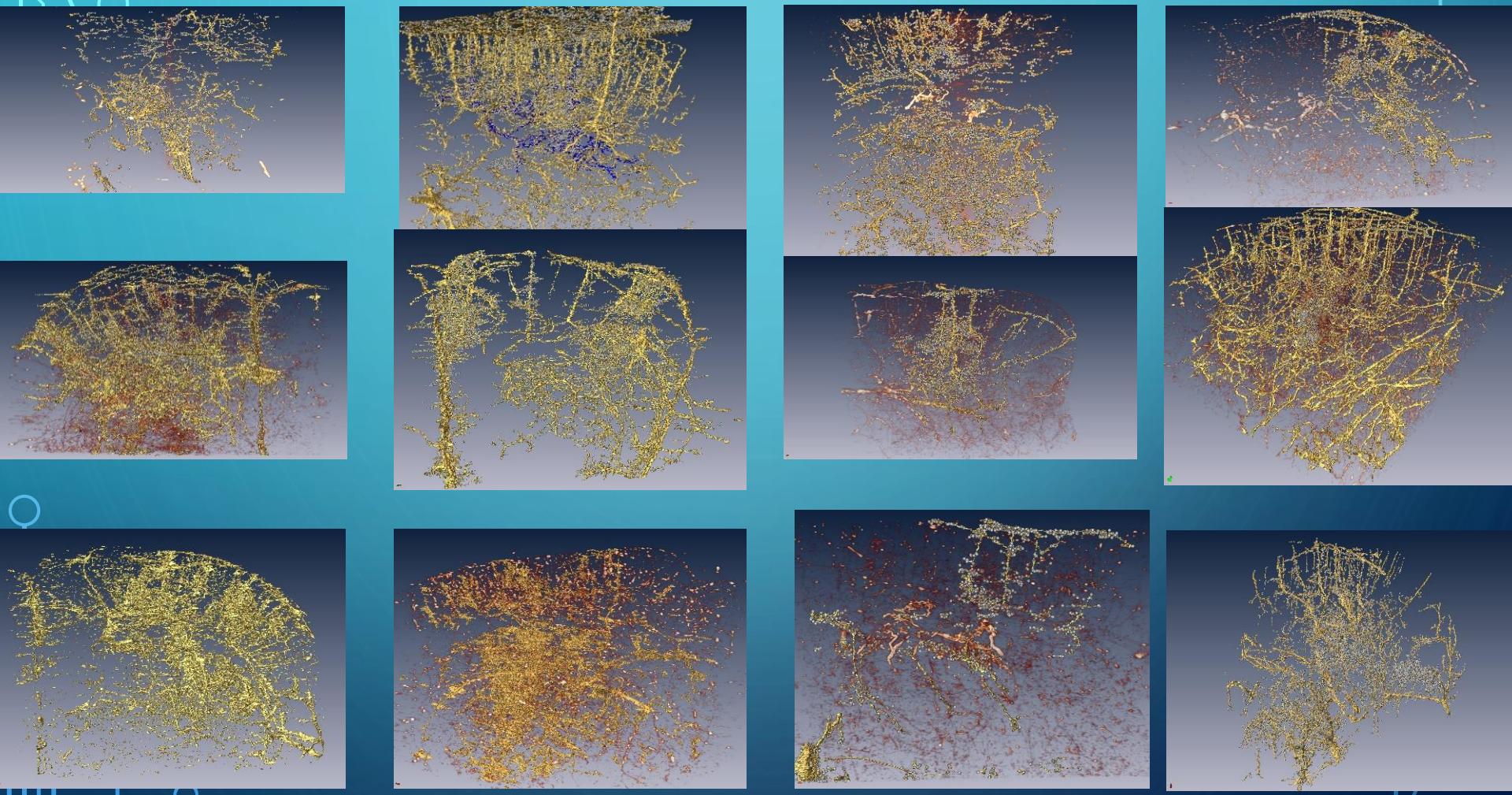







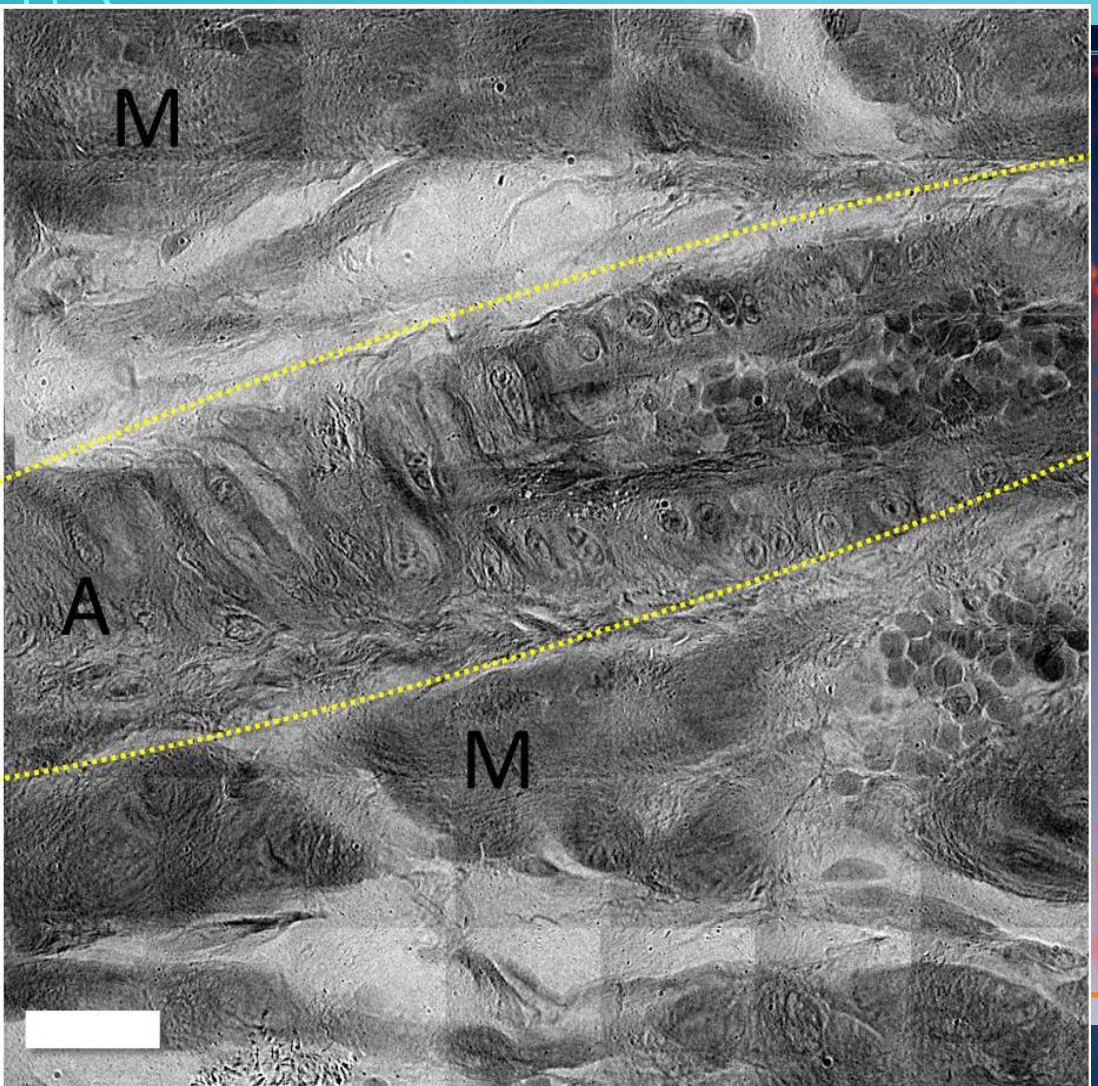


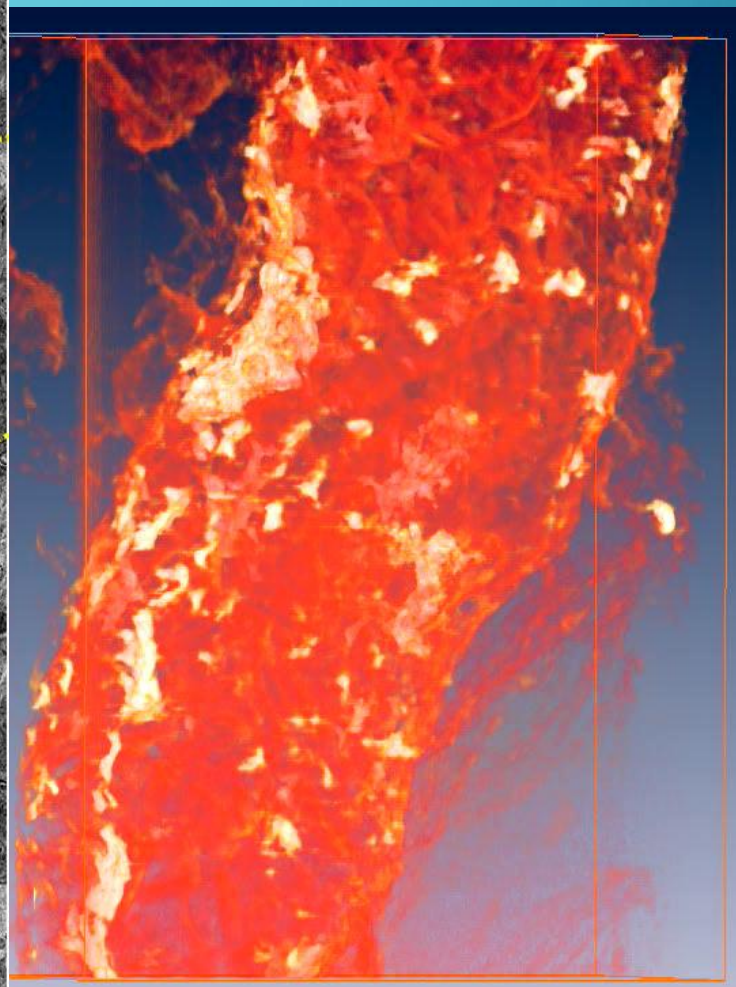




Diffused glioma tumors



TXM Images of a microvessel





What do we expect to learn?

- Small differences in microvasculature between different models
- Effect of treatment
- Inhomogeneity in microvasculature with respect to tumor
- Microvasculature with respect to the growth and metastasis of tumor
- Drug development and usage

There are more problems in brains with neurons (dementia) than vessels (cancer)

- Can X-rays image neurons?
- Can X-rays image blood vessels at the same time?
- Can X-rays study neuro-vascular interaction?
- On dead specimens, of course.

WHY BRAIN?

The Economist

JANUARY 6TH–12TH 2018

The message of protests in Iran

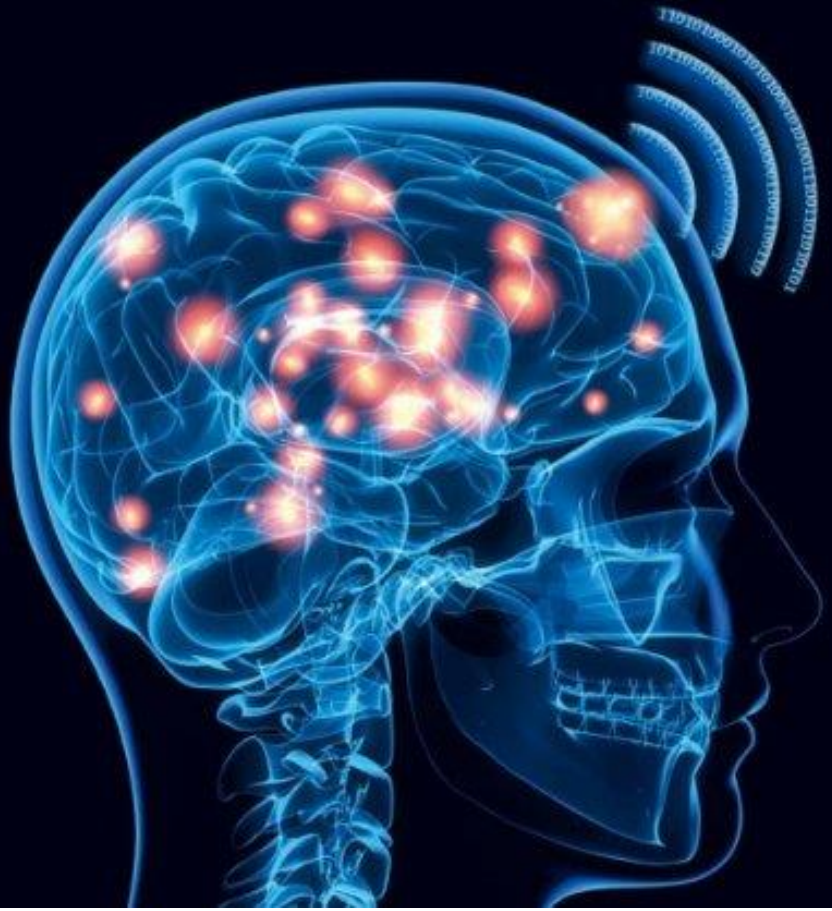
Blue-state Republicans: no right left

Education lessons from Pakistan

The world's worst airports

The next frontier

When thoughts control machines



Mapping the Brain: an Historical Mission for Science and Technology

- A fundamental research objective
- An effective way to understand and cure brain diseases
- Potential for a broad social impact
- Important technological byproducts:
  - Advanced imaging technologies
  - New computational strategies
  - Artificial intelligence

THE CHALLENGES:

No present technique can image an entire animal brain down to individual synapses with all the required performances:

- High speed and high throughput
- High spatial resolution in 3 D

…we must do better!

Synchrotrons for Neuroscience -- an Asia-Pacific Strategic Enterprise

An effort to image the neural network of a whole human brain with proven performance of synchrotron x-ray tomography and a collective effort

SYNAPSE: an international partnership with a milestone research mission

First objective: mapping the neuron connections of an entire human brain by 2023

Mapping the Brain: an Historical Mission for Science and Technology

- A fundamental research objective
- An effective way to understand and cure brain diseases
- Potential for a broad social impact
- Important technological byproducts:
  - Advanced imaging technologies
  - New computational strategies

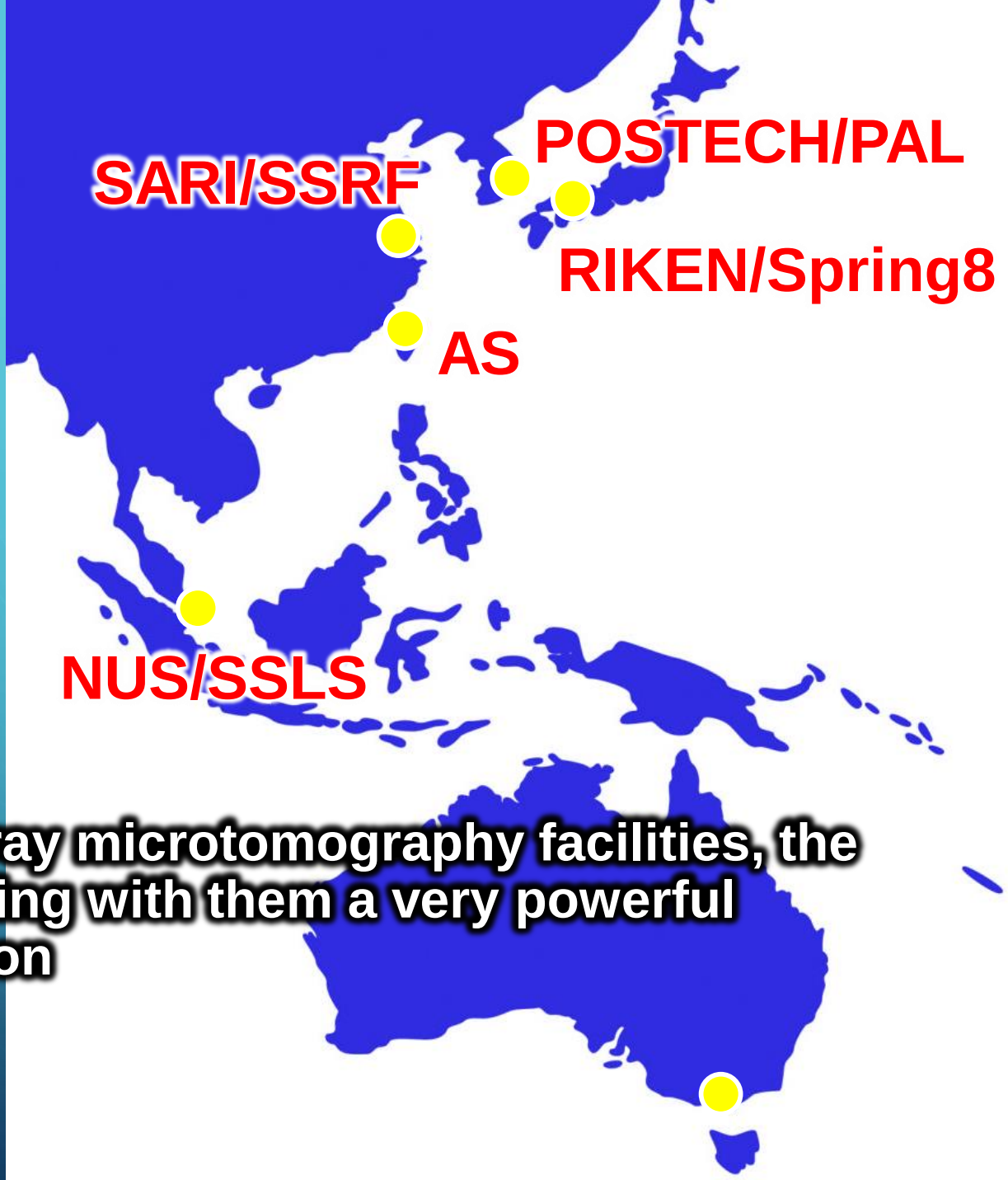
IT TAKES DECADES FOR MAPPING ONE HUMAN BRAIN WITH THE PRESENT X-RAY TOMOGRAPHY PERFORMANCES!

We must accelerate!

- Parallel image taking at multiple facilities
- Automated specimen sectioning
- Automated specimen mounting and alignment
- High-speed data transfer and sharing for all partners
- New, efficient strategies for reconstruction

SYNAPSE is the solution

SYNAPSE:
core partners with top-level synchrotron facilities



In addition to x-ray microtomography facilities, the core partners bring with them a very powerful extended coalition


Memorandum of Understanding
Synchrotrons Coordinated Imaging & High Performance Computing
SYNAPSE
Signing Ceremony
Synchrotron for Neuroscience:
an Asia-Pacific Strategic Enterprise
SSRF
ANSTO
Memorandum of Understanding
Synchrotrons Coordinated Imaging & High Performance Computing
Signing Ceremony
SYNAPSE
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ANSTO

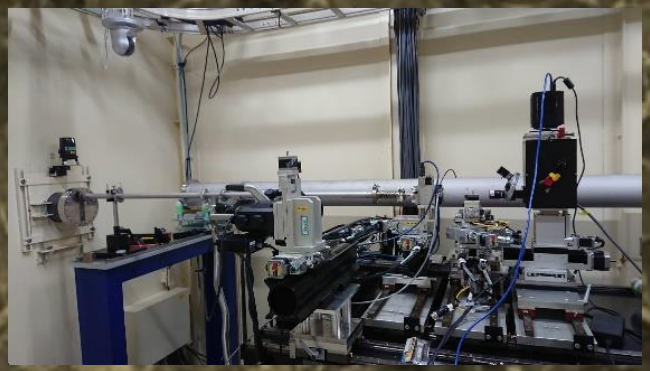

Memorandum of Understanding
Synchrotrons Coordinated Imaging & High Performance Computing
Signing Ceremony
SYNAPSE
Synchrotron for Neuroscience:
an Asia-Pacific Strategic Enterprise

CONCENTRATE ON 3D IMAGING:

New radiology techniques combined with other advanced microscopies

- For overall connection mapping: phase-contrast microtomography (0.3 µm resolution in all 3D directions)
- To explore in detail synapses and neuron connections: nanotomography with <10 nm resolution
- To obtain detailed 3D maps of special regions: electron microscopy/continuous sectioning, optical super-resolution microscopy, with nm resolution
- To analyze the whole drosophila brain and large parts of the mouse brain obtaining functional information: Confocal (or Light Sheet) + *FocusClear* imaging
- For single-molecule mapping of drosophila and mouse brains: Super-resolution microscopy with *FocusClear*
- high resolution functional imaging: Raman, IR spectromicroscopy
- Fusing-in low resolution functional imaging, i.e. fMRI
- …plus other tasks using other frontline techniques

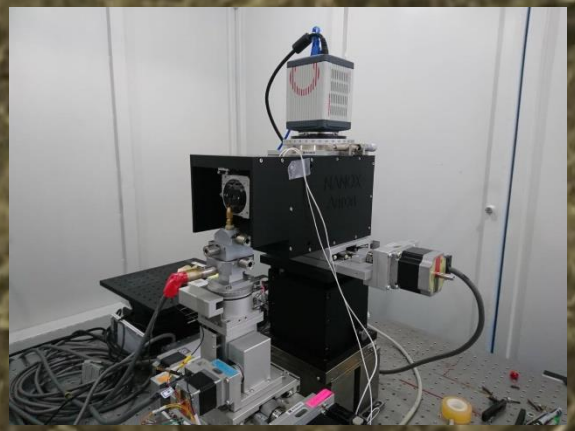
Pohang Light Source II 7C



SPring-8 BL32B2

Taiwan Photon Source BL2A

Shanghai Synchrotron
Radiation Facility BL 09



Singapore Synchrotron
Light Source PCXT

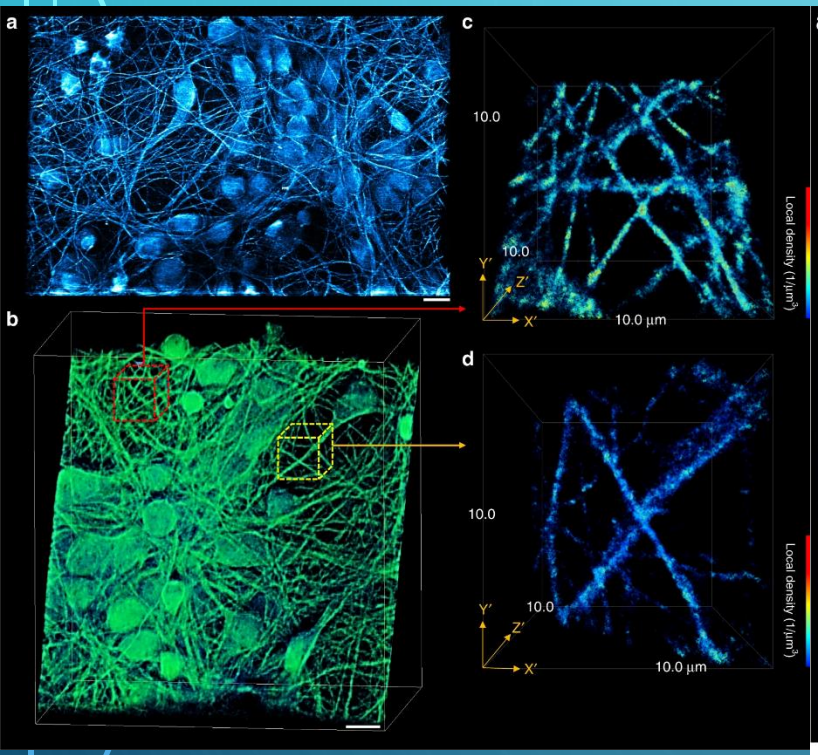
SYNAPSE:
core partners with top-level synchrotron facilities

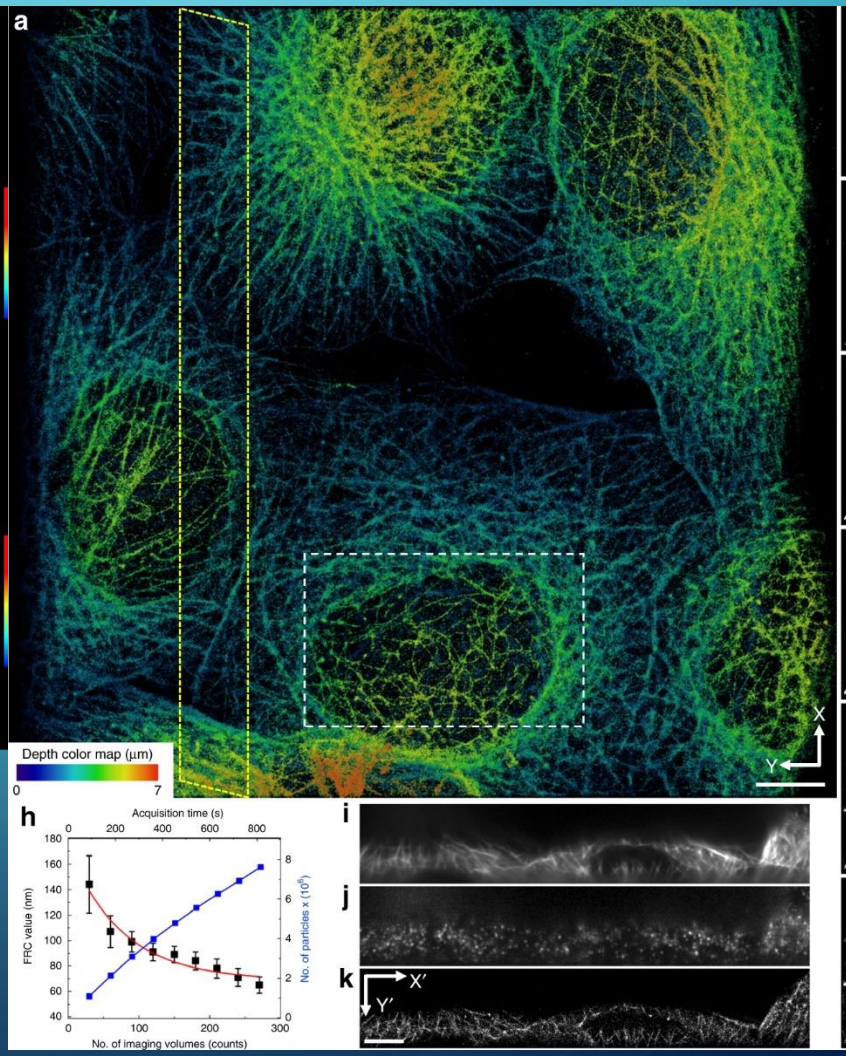
In addition to x-ray microtomography facilities, the core partners bring with them a very powerful extended coalition

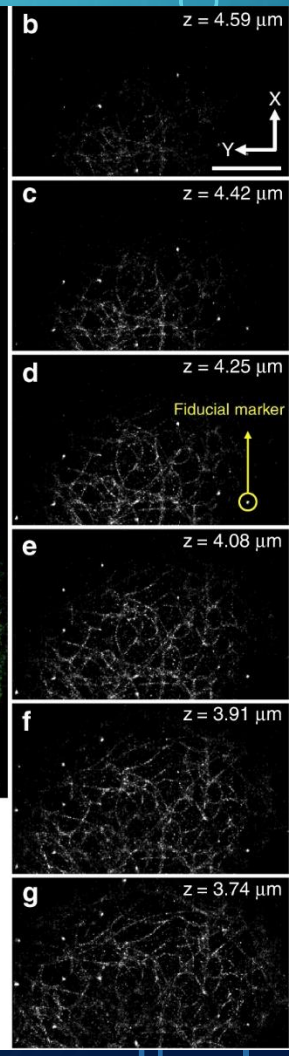
Concentrate on 3D imaging: new radiology techniques combined with other advanced microscopies

- For overall connection mapping: phase-contrast microtomography (0.3 µm resolution in all 3D directions)
- To explore in detail synapses and neuron connections: nanotomography with <10 nm resolution
- To obtain detailed 3D maps of special regions: cryo-electron microscopy with nm resolution
- To analyze the whole drosophila brain and large parts of the mouse brain obtaining functional information: Confocal FocusClear imaging
- For single-molecule mapping of drosophila and mouse brains: Super-resolution microscopy with FocusClear
- …plus other tasks using other frontline techniques

Lightsheet Localization super-resolution Microscopy







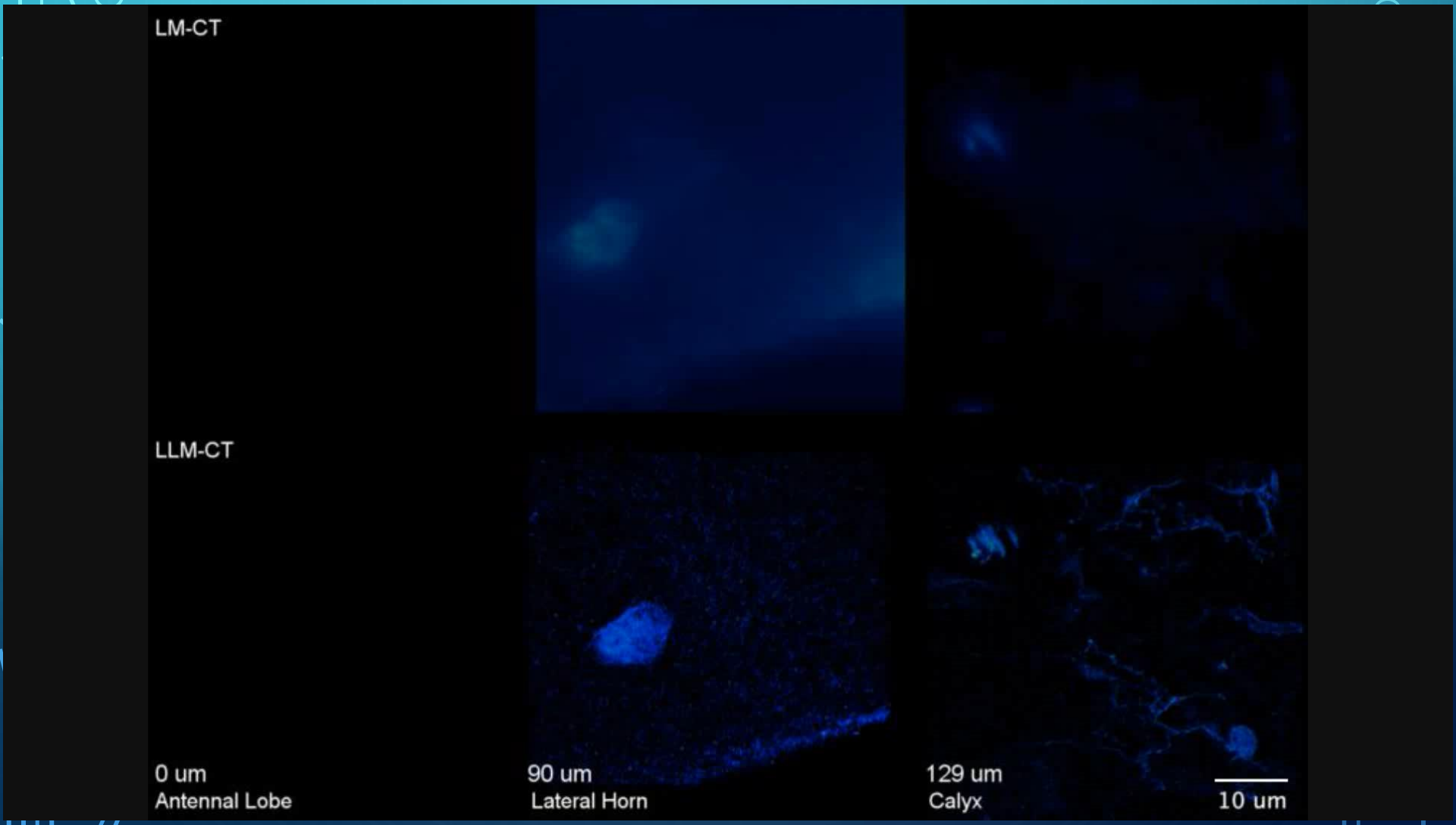
Communications Biology, **2**, 177 (2019)

2.7×10^4 µm^3/s

250 image averaging, 15 min

75 nm resolution

Lattice lightsheet microscopy w/ tissue clearing



nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-12715-3 OPEN

Rapid single-wavelength lightsheet localization microscopy for clarified tissue

Li-An Chu[1,2,10], Chieh-Han Lu[2,3,9,10], Shun-Min Yang[2], Yen-Ting Liu[3], Kuan-Lin Feng[4], Yun-Chi Tsai[3], Wei-Kun Chang[1], Wen-Cheng Wang[3], Shu-Wei Chang[3], Peilin Chen[3], Ting-Kuo Lee[2], Yeu-Kuang Hwu[2], Ann-Shyn Chiang[1,2,4,5,6,7,8*] & Bi-Chang Chen[1,3*]

Optical super-resolution microscopy allows nanoscale imaging of protein molecules in intact biological tissues. However, it is still challenging to perform large volume super-resolution imaging for entire animal organs. Here we develop a single-wavelength Bessel lightsheet method, optimized for refractive-index matching with clarified specimens to overcome the aberrations encountered in imaging thick tissues. Using spontaneous blinking fluorophores to label proteins of interest, we resolve the morphology of most, if not all, dopaminergic neurons in the whole adult brain ($3.64 \times 10^7\ \mu m^3$) of *Drosophila melanogaster* at the nanometer scale with high imaging speed ($436\ \mu m^3$ per second) for localization. Quantitative single-molecule localization reveals the subcellular distribution of a monoamine transporter protein in the axons of a single, identified serotonergic Dorsal Paired Medial (DPM) neuron. Large datasets are obtained from imaging one brain per day to provide a robust statistical analysis of these

Hwu et al. BMC Biology (2017) 15:122
DOI 10.1186/s12915-017-0461-8

BMC Biology

QUESTION AND ANSWER Open Access

Q&A: Why use synchrotron x-ray tomography for multi-scale connectome mapping?

Yeukuang Hwu[1*], Giorgio Margaritondo[2*] and Ann-Shyn Chiang[1,3*]

Abstract

To understand how information flows and is used in the human brain, we must map neural structures at all levels, providing visualizations similar to those of Google Earth for continents, countries, cities, and streets. Unfortunately, the imaging and processing techniques currently used in connectomics projects cannot achieve complete mapping for the brains of large animals within the timespan of a typical research career. However, feasible improvements in x-ray imaging would change this situation. This Q&A discusses synchrotron x-ray tomography, an exciting new approach for in situ mapping of whole-brain wiring diagrams at multiple levels of spatial resolution.

In a Presidential Lecture at the 2016 Annual Meeting of Society for Neuroscience, you presented the use of synchrotron x-ray tomography for mapping whole body connectomes in *Drosophila*. Why do we need x-ray imaging for connectomics?

Recent advances in imaging technologies open the door to mapping a complete wiring diagram of the human brain, a approach to mapping a small part of the human connectome at least for the foreseeable future.

We sought an alternative to these techniques on deciding that we wanted to map the whole body connectome of *Drosophila* in order to understand the neural basis of its behavior. X-ray microscopy offered advantages in speed and resolution made more apparent and exploitable since its development using modern synchrotron facilities [1, 2]. To meet the challenge of connectome mapping, however, certain performance improvements were required, notably to handle large specimens—the entire animal brain and body—with sufficient image contrast and tunable resolution to detect the fine connections. Overcoming these obstacles took more than a decade, but as a result x-ray imaging is now, in our view, the technique of choice for whole-body connectome mapping.

Many of the advanced x-ray imaging techniques of today are based on synchrotron sources. Synchrotron x-rays offer high brightness with deep-penetration for in situ visualization at a high speed of internal structures within a large tissue—similar to medical computer tomography, but with much better spatial resolution for obtaining multi-level views, ranging from a large brain

Brain size

	Volume (mm^3)	Number of Neurons	brain/body Ratio	Number of Synapse	Density of neurons ($/mm^3$)
drosophila	$2x10^{-2}$	$2.5x10^5$ ($1.3x10^5$)			$1.25x10^7$
mouse	450	$7.1x10^7$	1:40	10^{11}	$1.57x10^5$
marmoset		$6.4x10^8$			
human	$1.2x10^6$	$8.6x10^{10}$	1:50	10^{14-15}	$7.2x10^4$

Source of information: Wikipedia

Big Brain, Big Data

TECHNOLOGY FEATURE

BIG BRAIN, BIG DATA

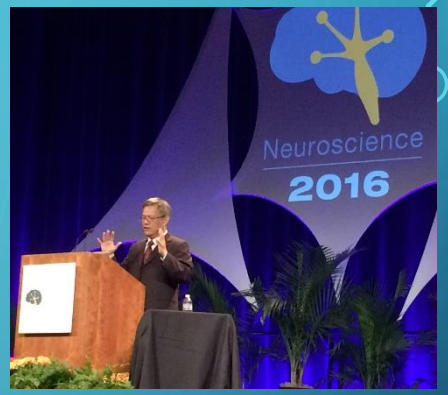
Neuroscientists are starting to share and integrate data — but shifting to a team approach isn't easy.

Diffusion magnetic resonance imaging is just one of many data types that researchers are working out how to handle to bring the brain into focus.

BY ESTHER LANDHUIS

As big brain-mapping initiatives go, Taiwan's might seem small. Scientists there are studying the humble fruit fly, reverse-engineering its brain from images of single neurons. Their efforts have produced 3D maps of brain circuitry in stunning detail.

Researchers need only a computer mouse and web browser to home in on individual cells and zoom back out to intertwined networks of nerve bundles. The wiring diagrams look like colourful threads on a tapestry, and they're clear enough to show which cell clusters control specific behaviours. By stimulating a specific neural circuit, researchers can cue a fly to flap its left wing or swing its head from side to side — feats that roused a late-afternoon crowd in November at the annual meeting of the Society for Neuroscience in San Diego, California.



When it comes to the brain, 'complete' can be a moving target. But so, too, is the neuroscience toolset. During his Society for Neuroscience talk, Chiang lamented that it's taken ten years to map half the fly brain. Working with physicists at Taiwan's Academia Sinica, Chiang's team has started to use a technique called synchrotron X-ray tomography to boost data-acquisition speed dramatically.

"It took less than 10 minutes to image a fly brain containing thousands of Golgi-stained single neurons," says Chiang, whose crew is now trying the method in mice and pigs. They plan to integrate confocal and X-ray images on a single platform from which scientists can download data. "With synchrotron X-ray imaging, mapping the human connectome at single-neuron resolution is now more realistic," Chiang says. How easy it will be to meld the maps with other data remains to be seen. ■

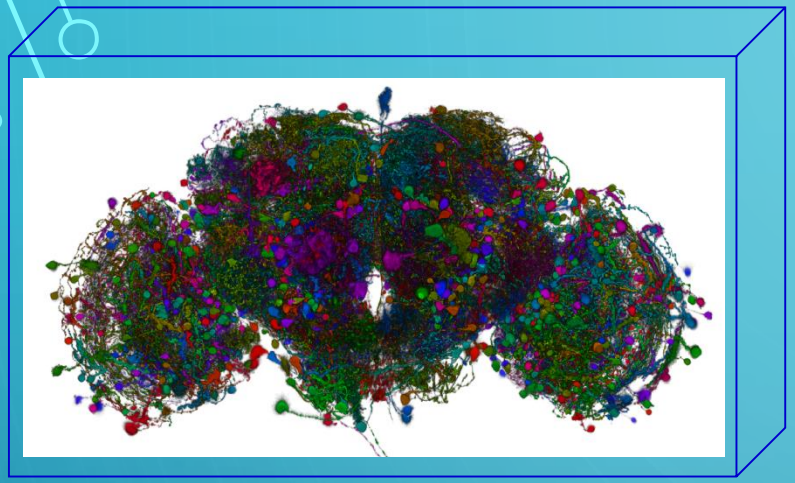
Esther Landhuis *is a freelance science writer in the San Francisco Bay Area of California.*

Nature http://www.nature.com/nature/journal/v541/n7638/full/541559a.html#references

Nature blogs http://blogs.nature.com/naturejobs/2017/01/26/new-neuroscience-tools-for-team-science-in-big-data-era/

Scientific American https://www.scientificamerican.com/article/neuroscience-big-brain-big-data/

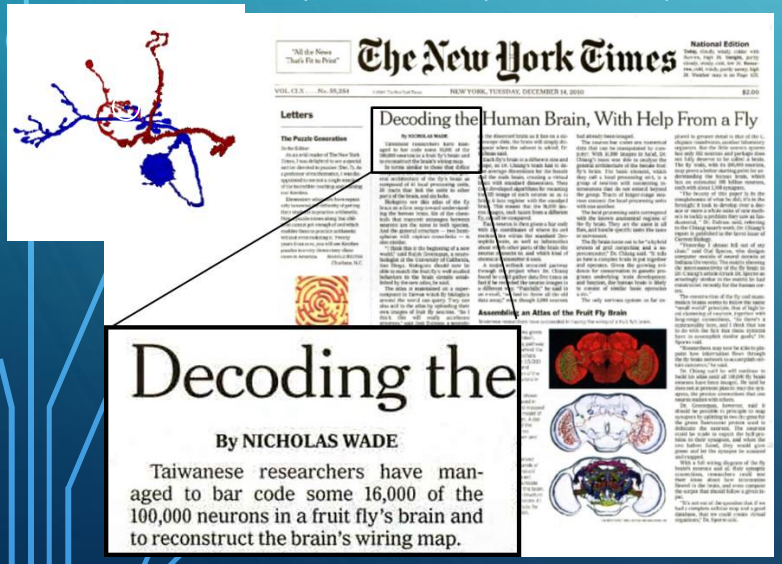
Small Brain, Big Data



500 μm × 300 μm × 200 μm

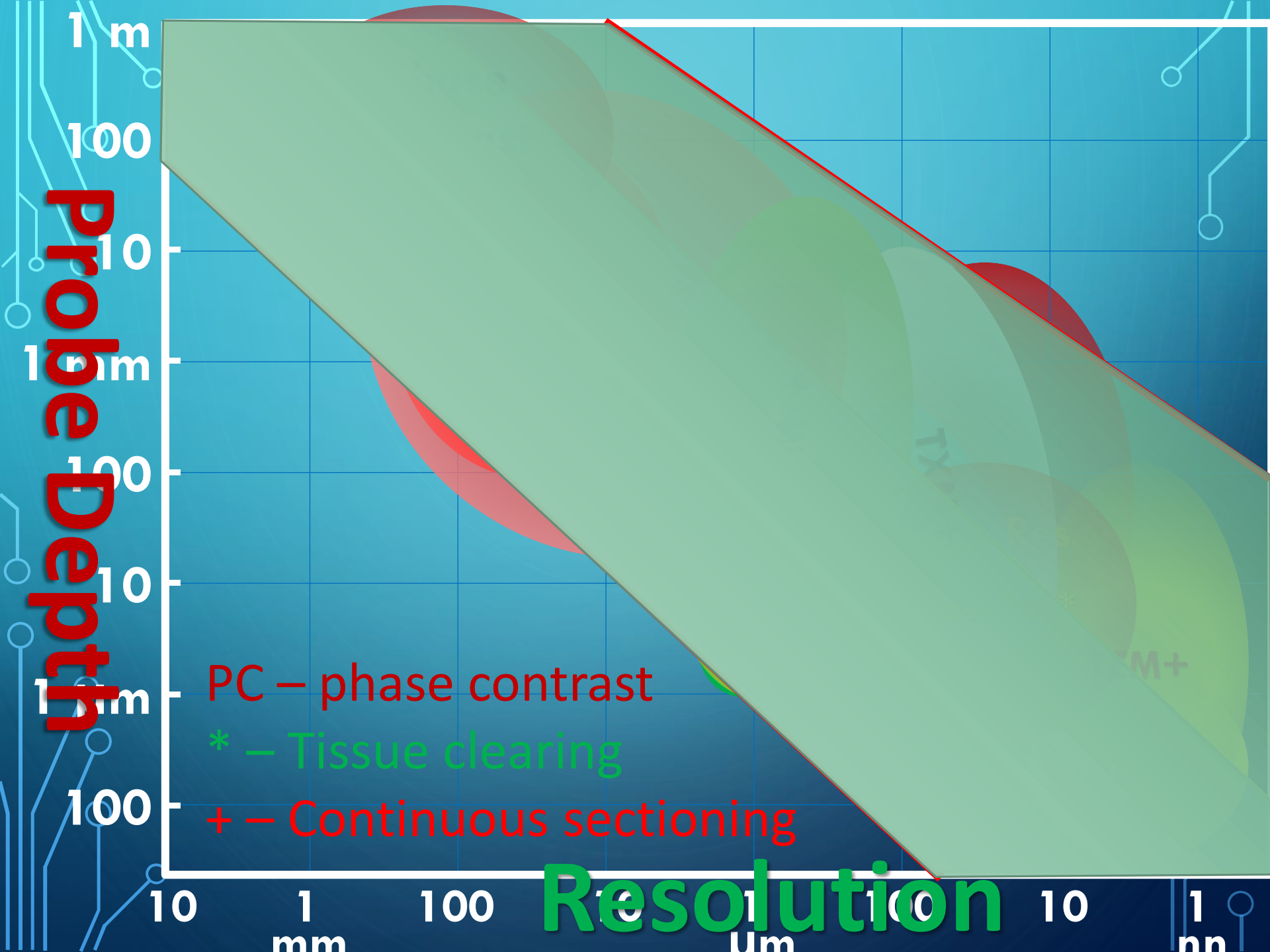
Fluorescence confocal microscopy on connectome mapping

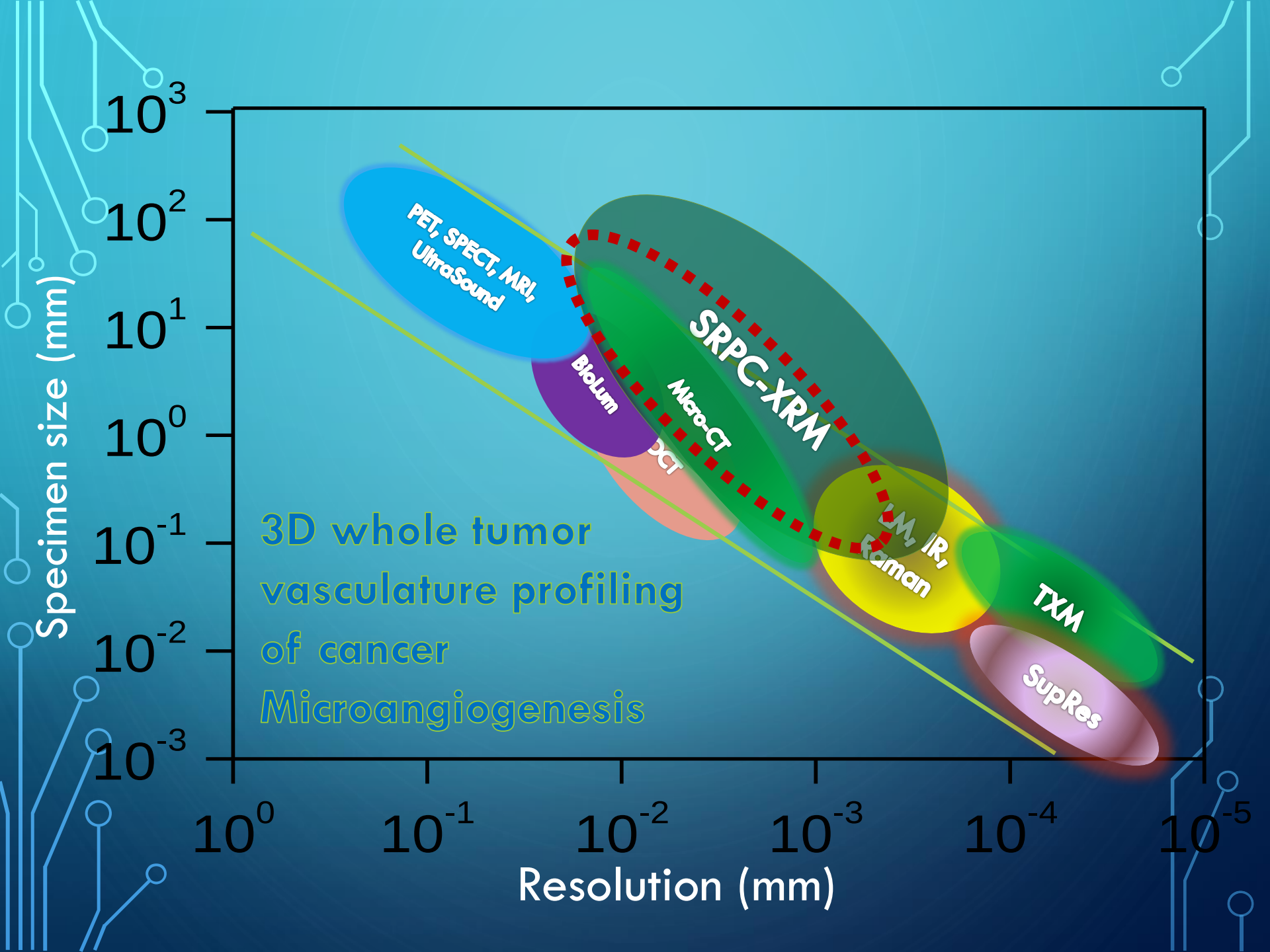
- Drosophila Brain: ~135,000 neurons
- Resolution：500 nm
- Data amount： ~10 TB
- Time to complete： ~20 year/10 microscopes
- Human brain: 85 billion neurons
- Time to complete： ~17,000,000 年



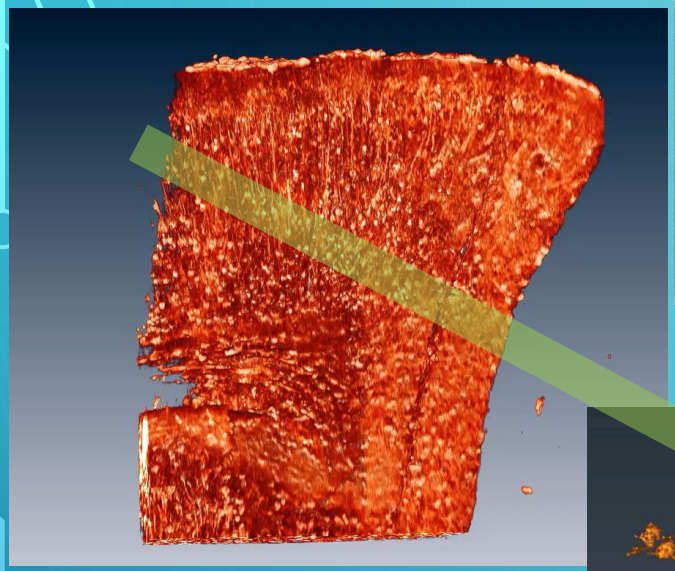
3D X-ray data

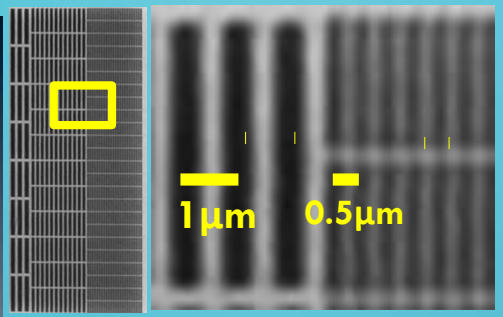
- Single fly brain: ~100GB
- Single mouse brain: ~360TB
- Complete the map with 100 brains: ~36 PetaByte (PB)!
- Human/mouse brain volume: 2500 !


Probe Depth
1 m
100
10
1 mm
100
10
1 μm
100
10
1 nm
Resolution
PC – phase contrast
* – Tissue clearing
+ – Continuous sectioning

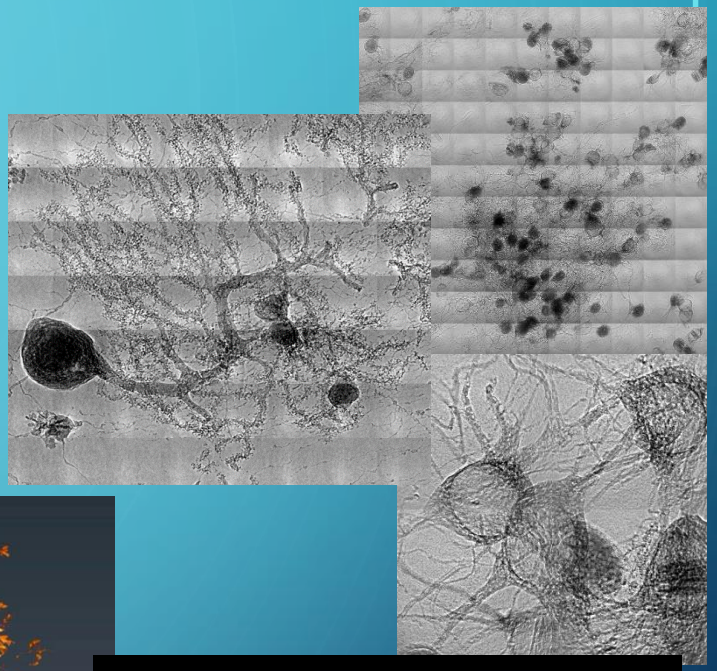

Specimen size (mm)
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
Resolution (mm)
PET, SPECT, MRI, UltraSound
BioLum
Micro-CT
SRPC-XRM
LM, IR, Raman
TXM
SupRes
3D whole tumor vasculature profiling of cancer Microangiogenesis

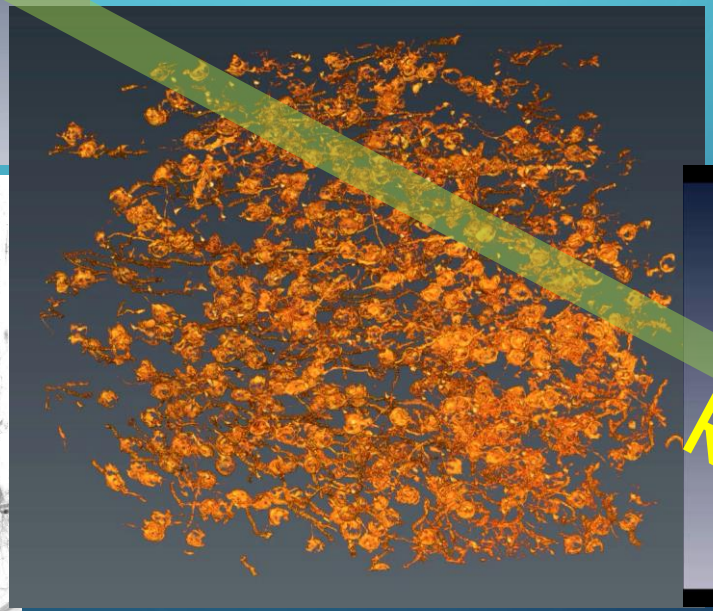
X-ray imaging — From Animals, Organs, Neurons to Synapses

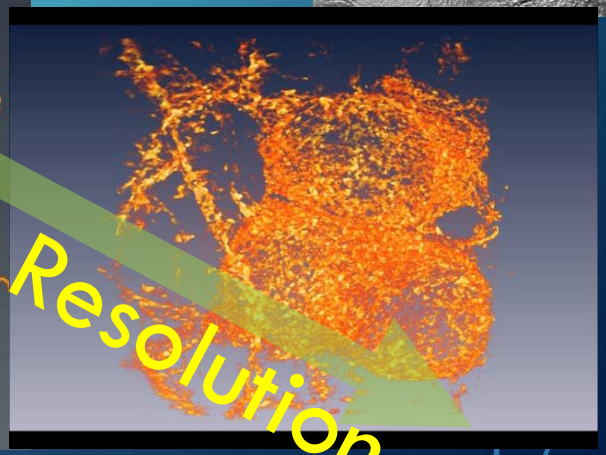


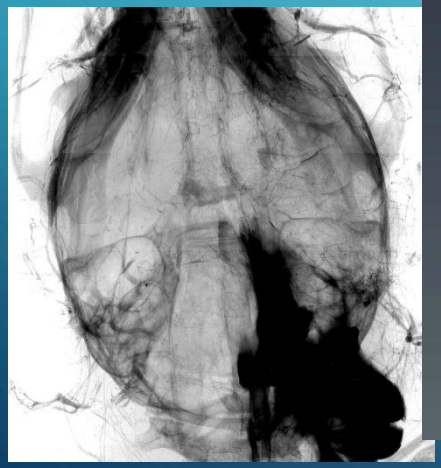


< 0.5 µm resolution (µCT)









Resolution

400 nm

Mouse brain

20 nm

3D imaging with AXON

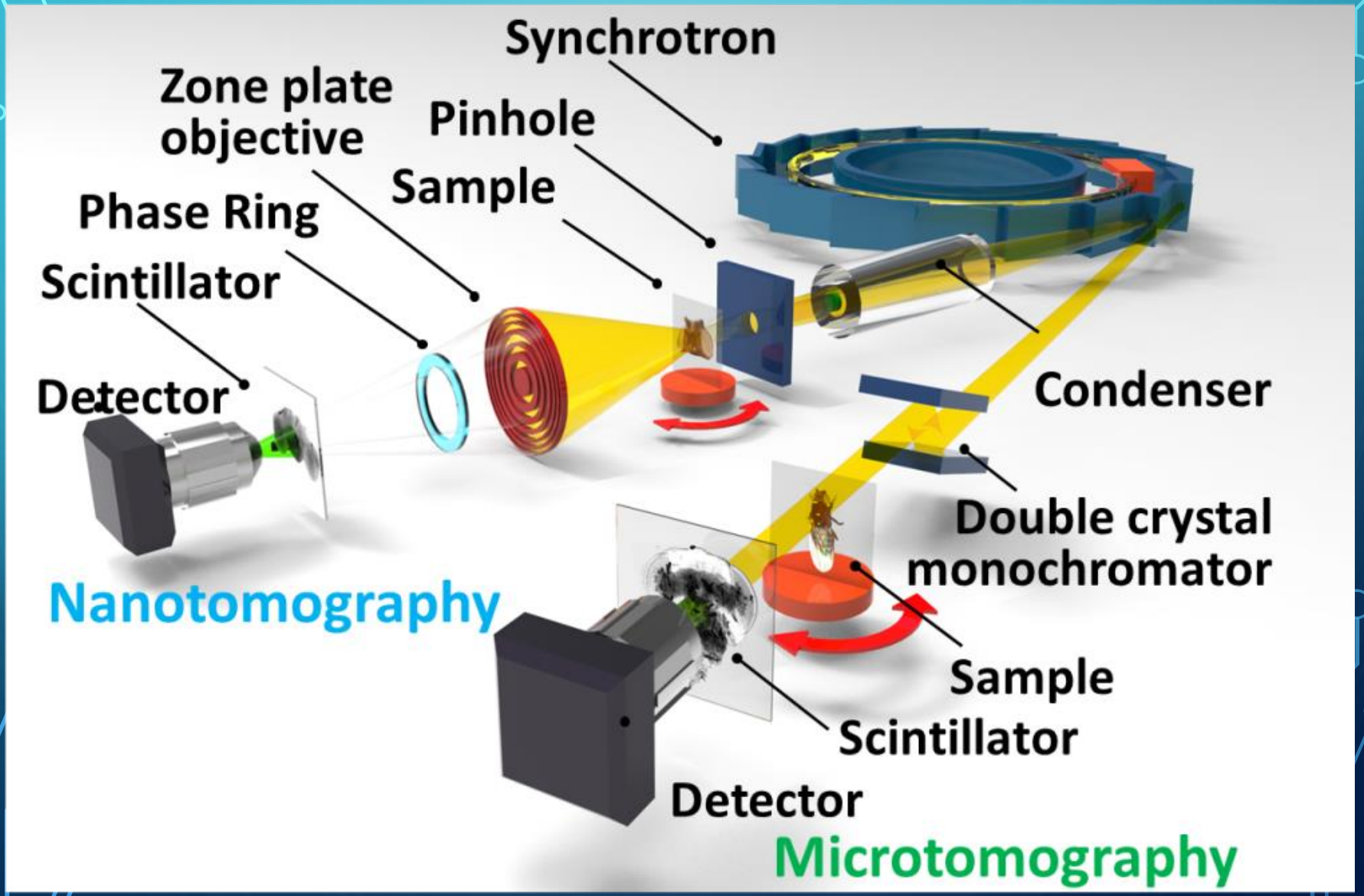
Accelerated

X-ray

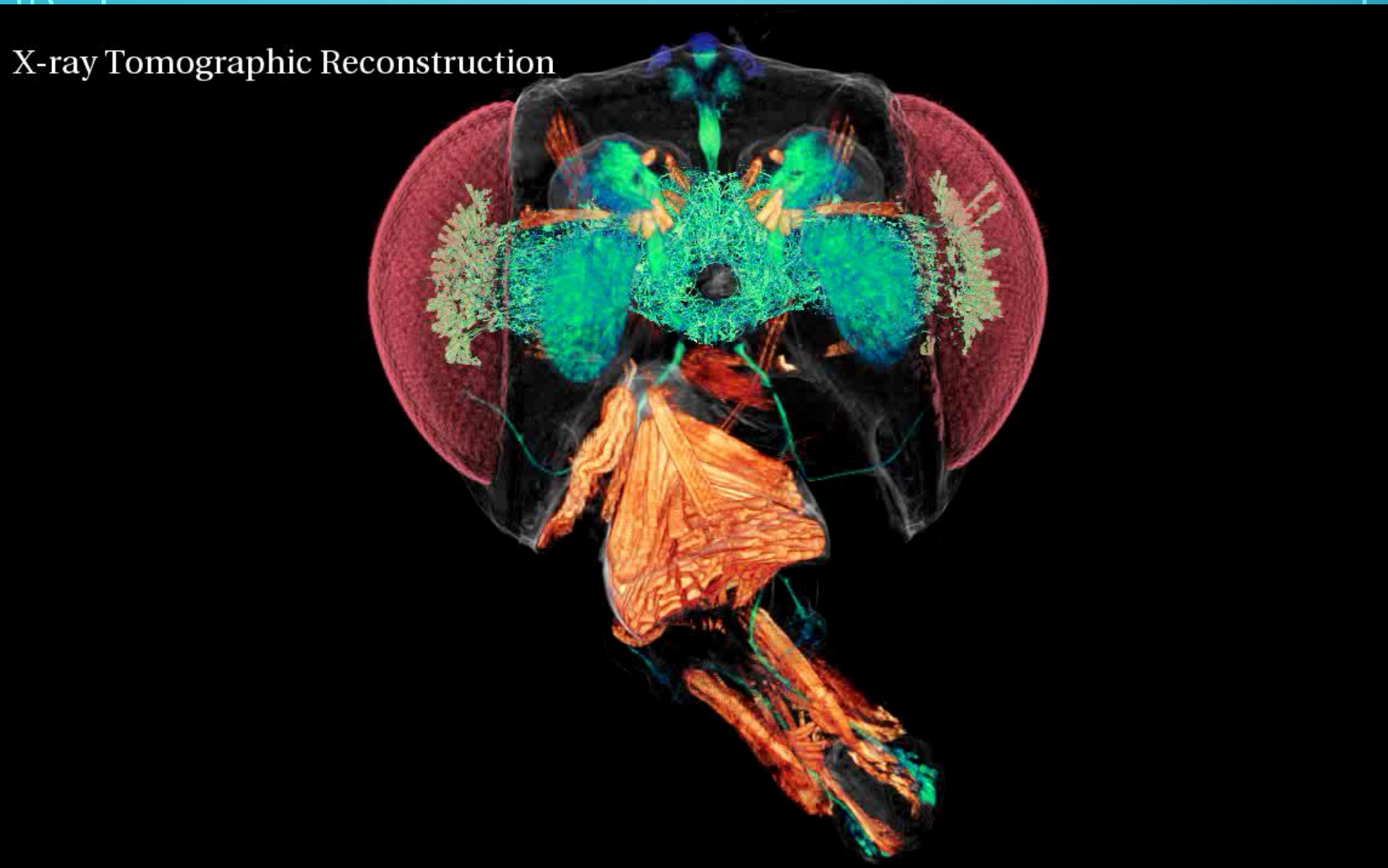
Observation for

Neurons

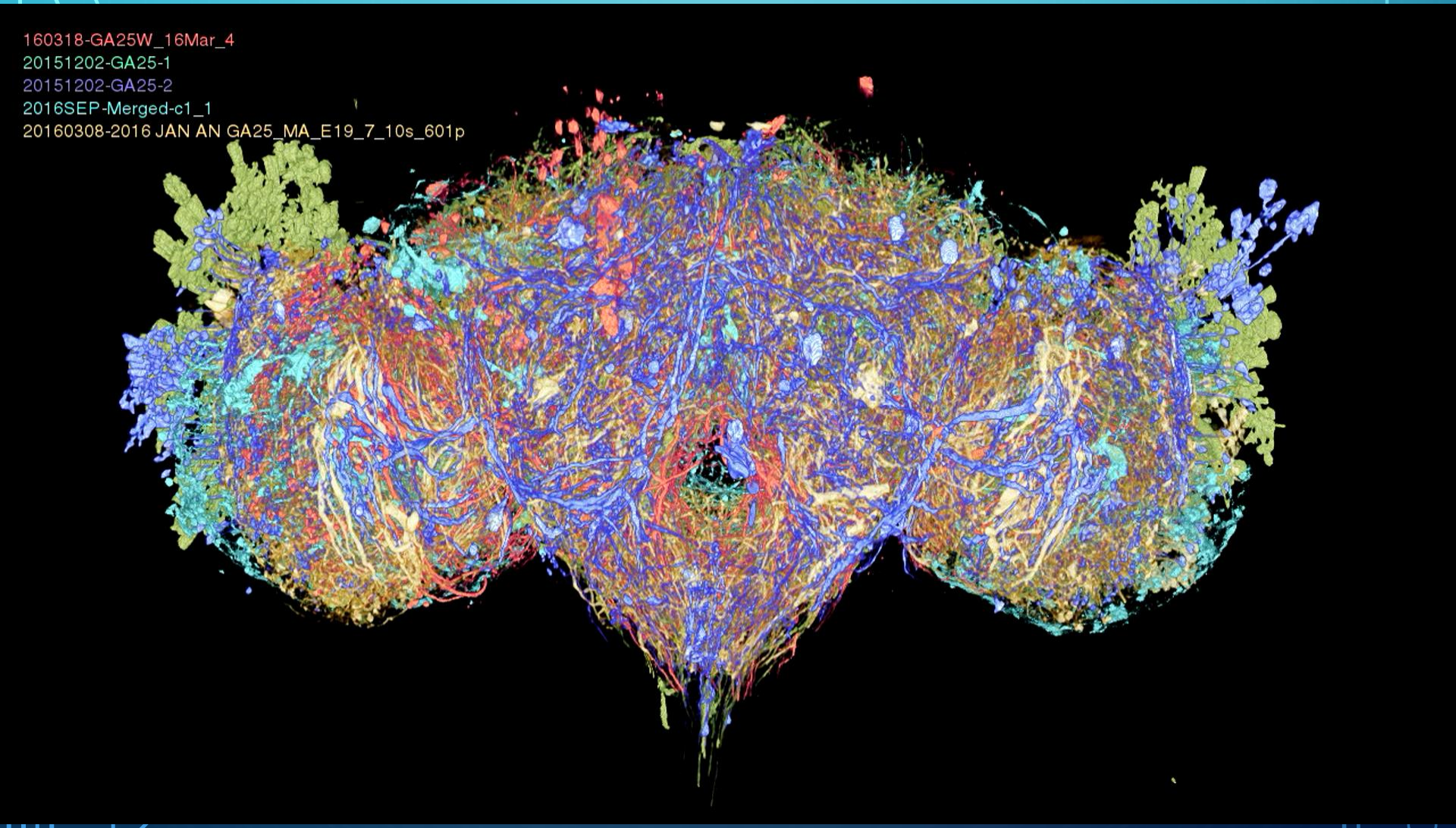
AXON Implementation: TLS, APS, PLS-II, TPS..



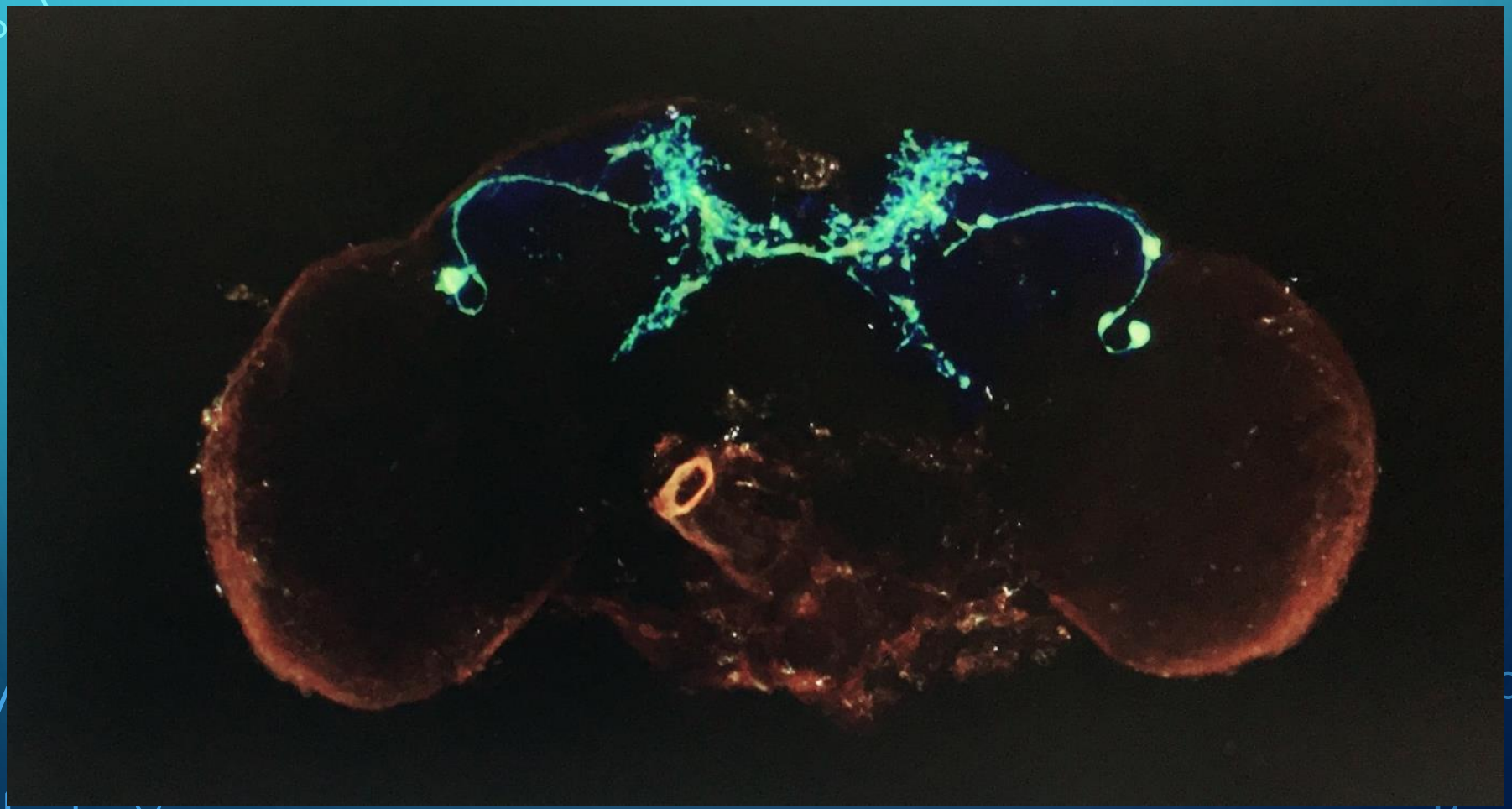
Whole brain imaging without sectioning



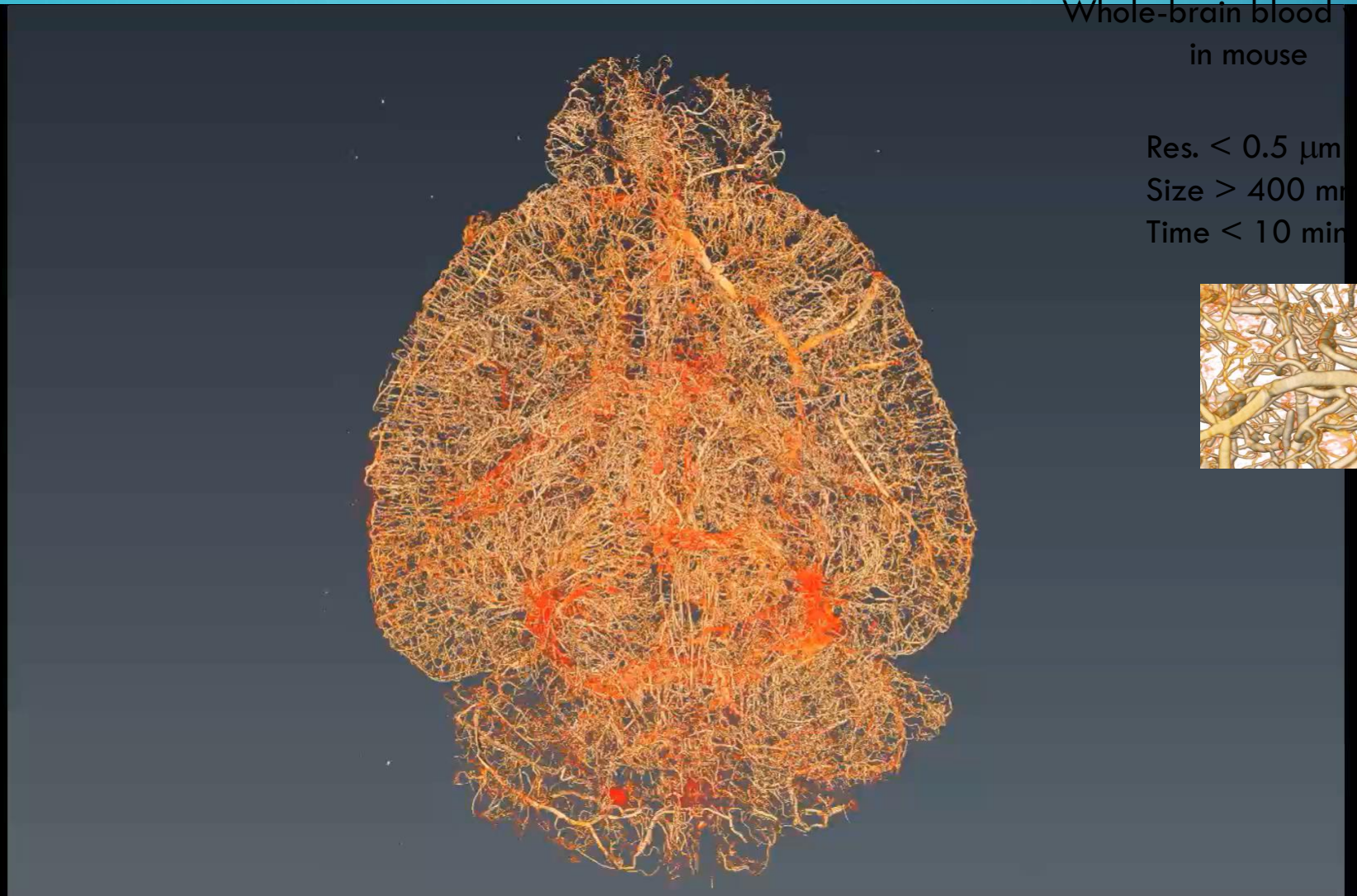
Merging Different Brains



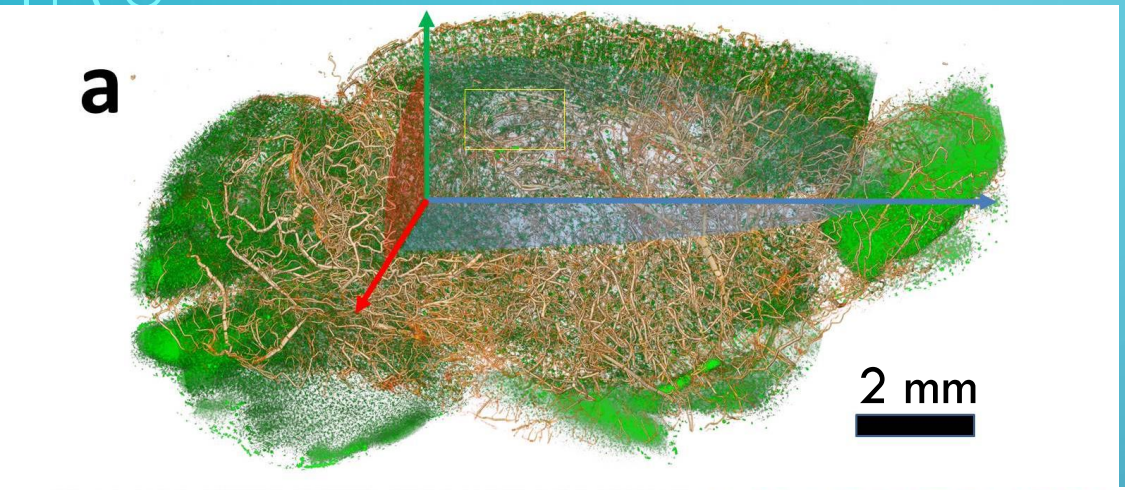
DAL neurons
enzmet/gold toning/gold enhance

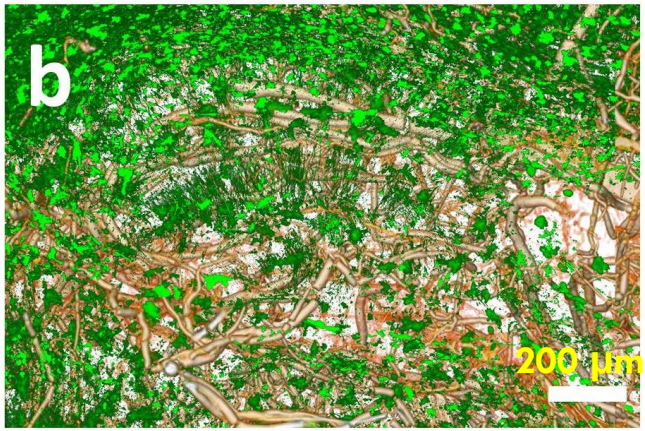


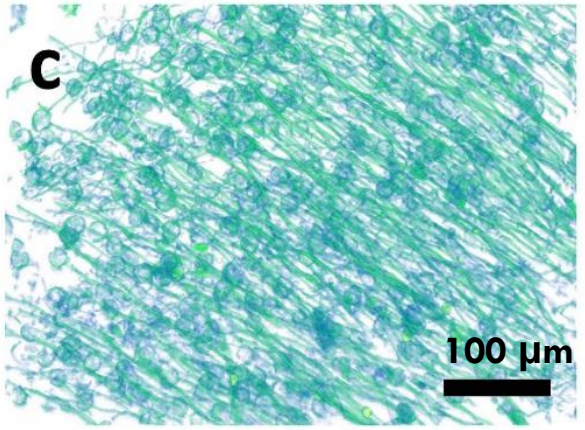
Large tissue, high resolution and speed



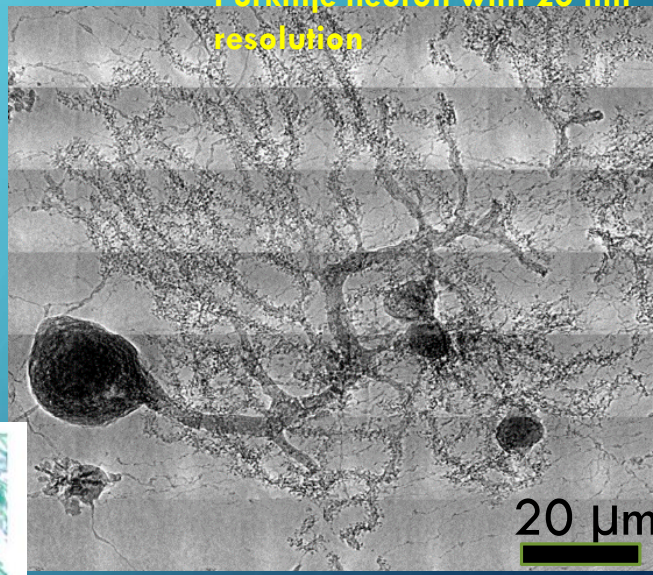
AXON on Mouse Brains



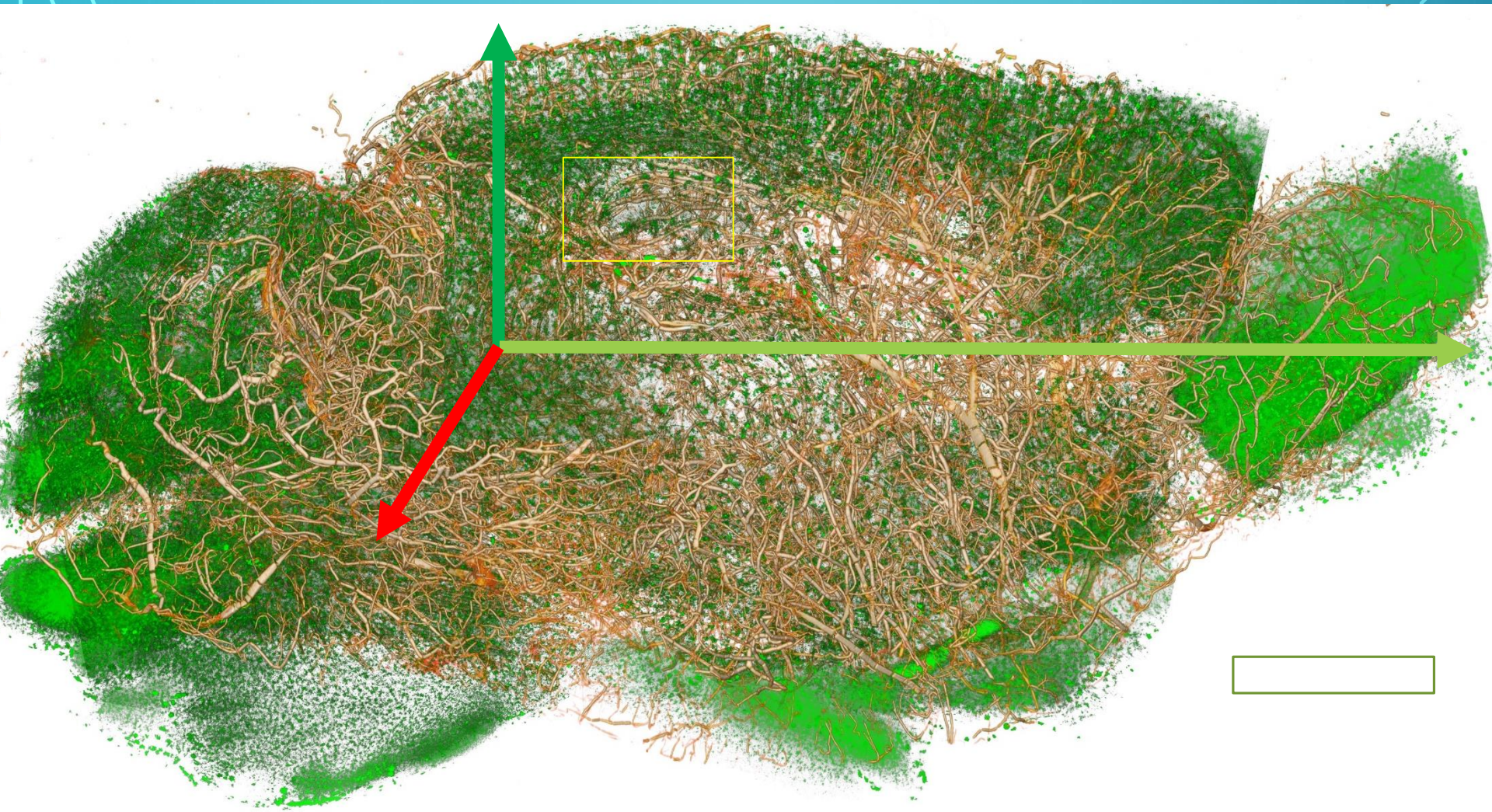


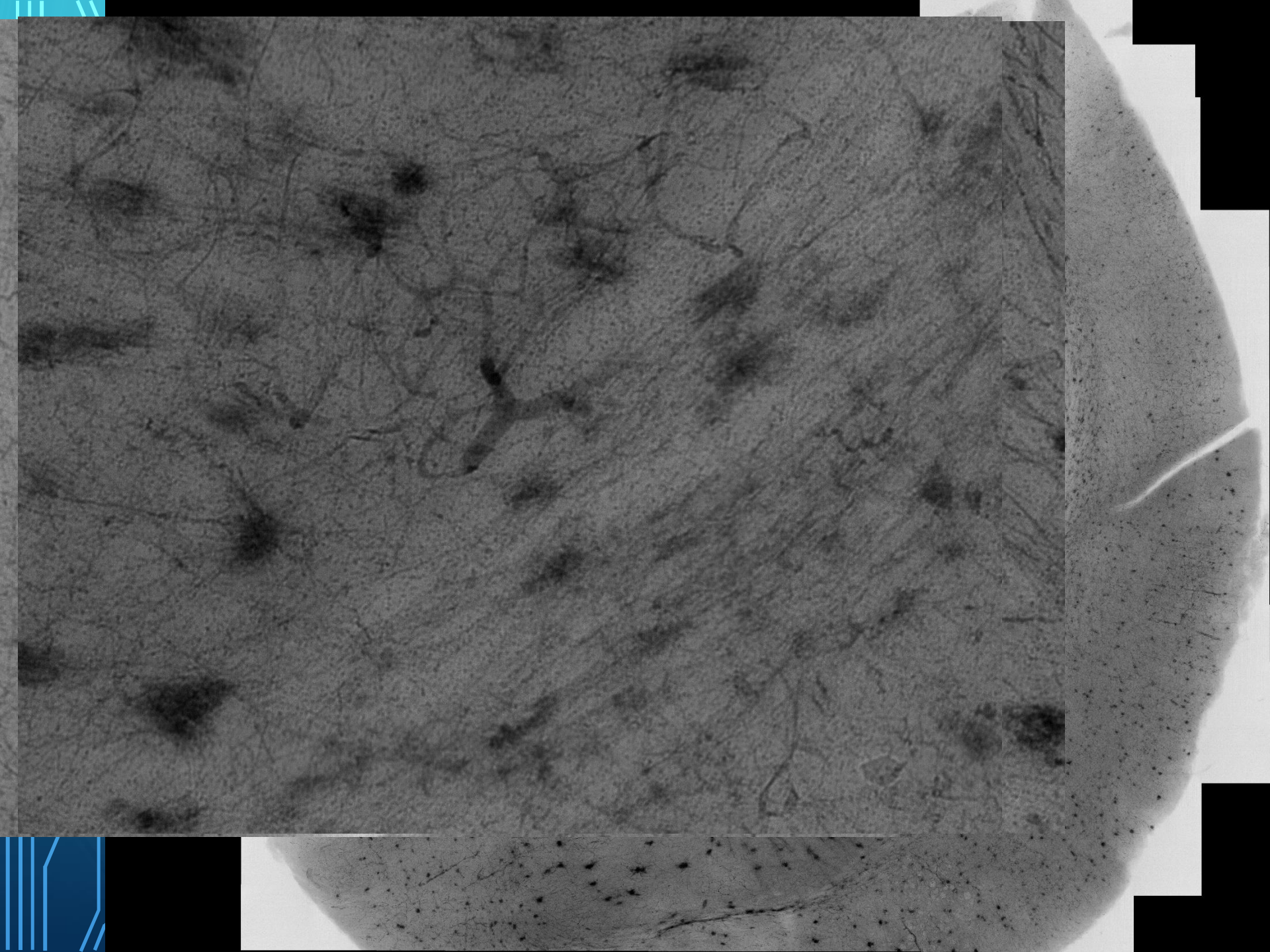


X-ray imaging of a mouse Purkinje neuron with 20 nm resolution

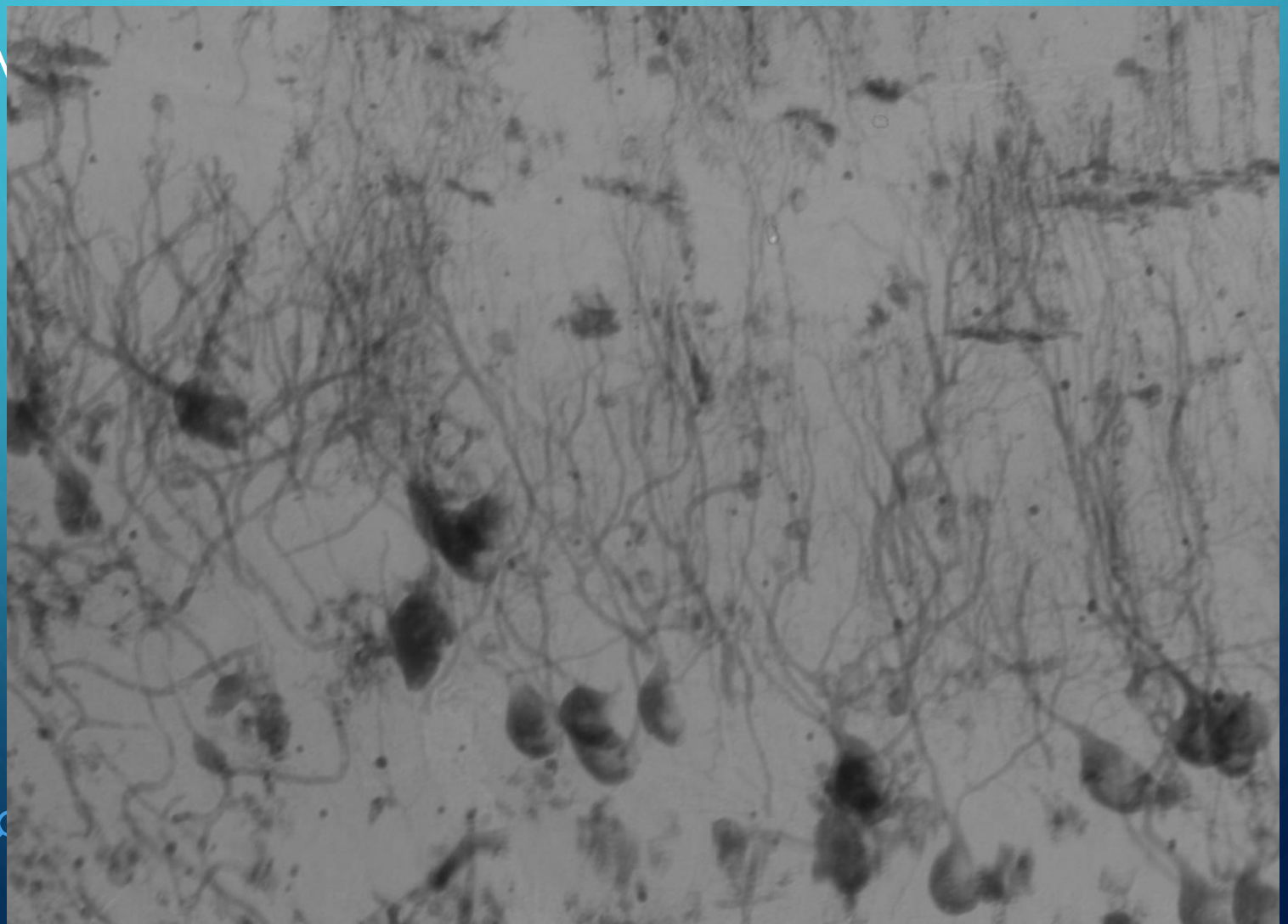


X-ray imaging of a whole mouse with 0.5 µm resolution. Tomography image shows blood vessels (golden) and neurons (green)

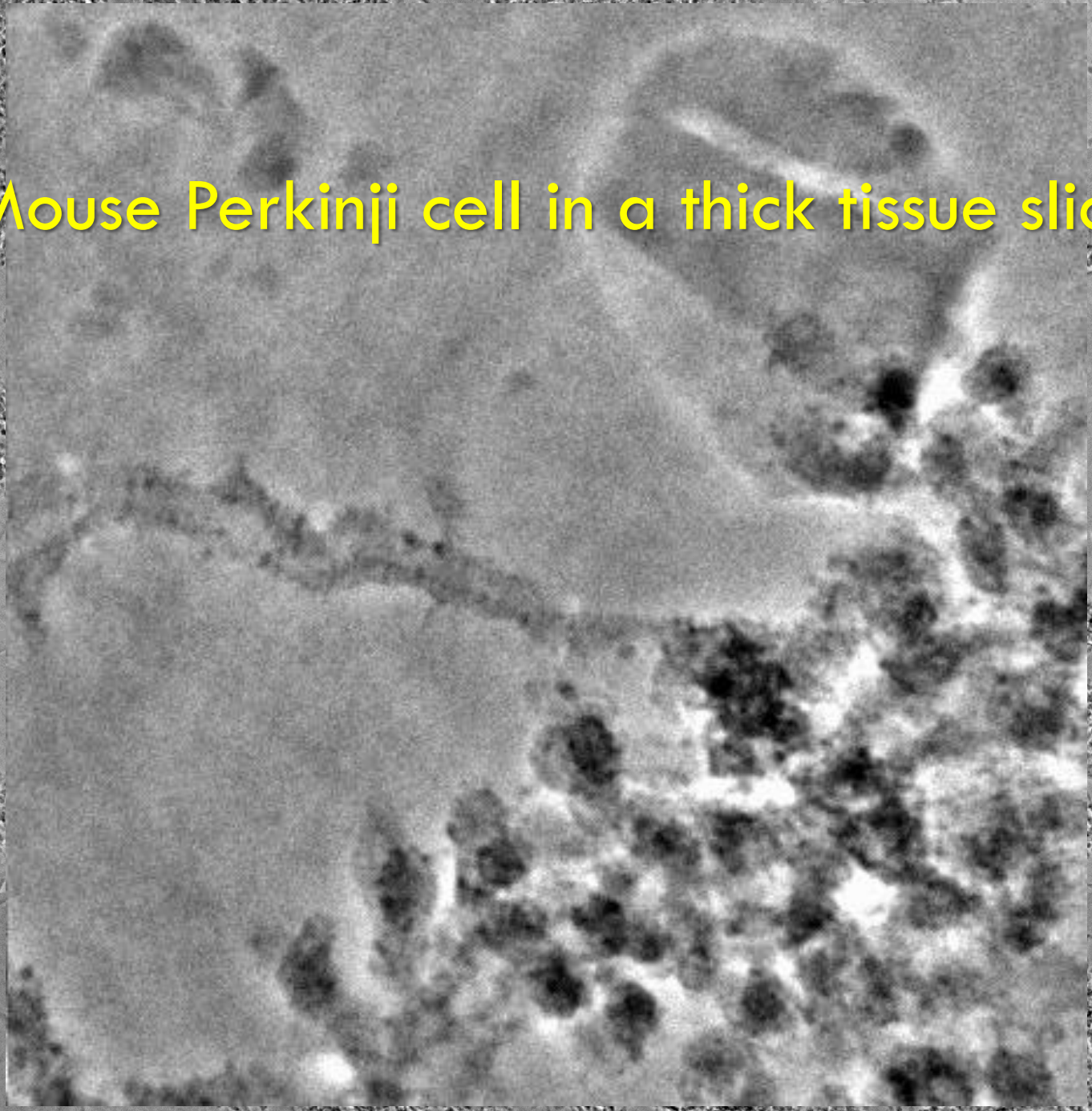




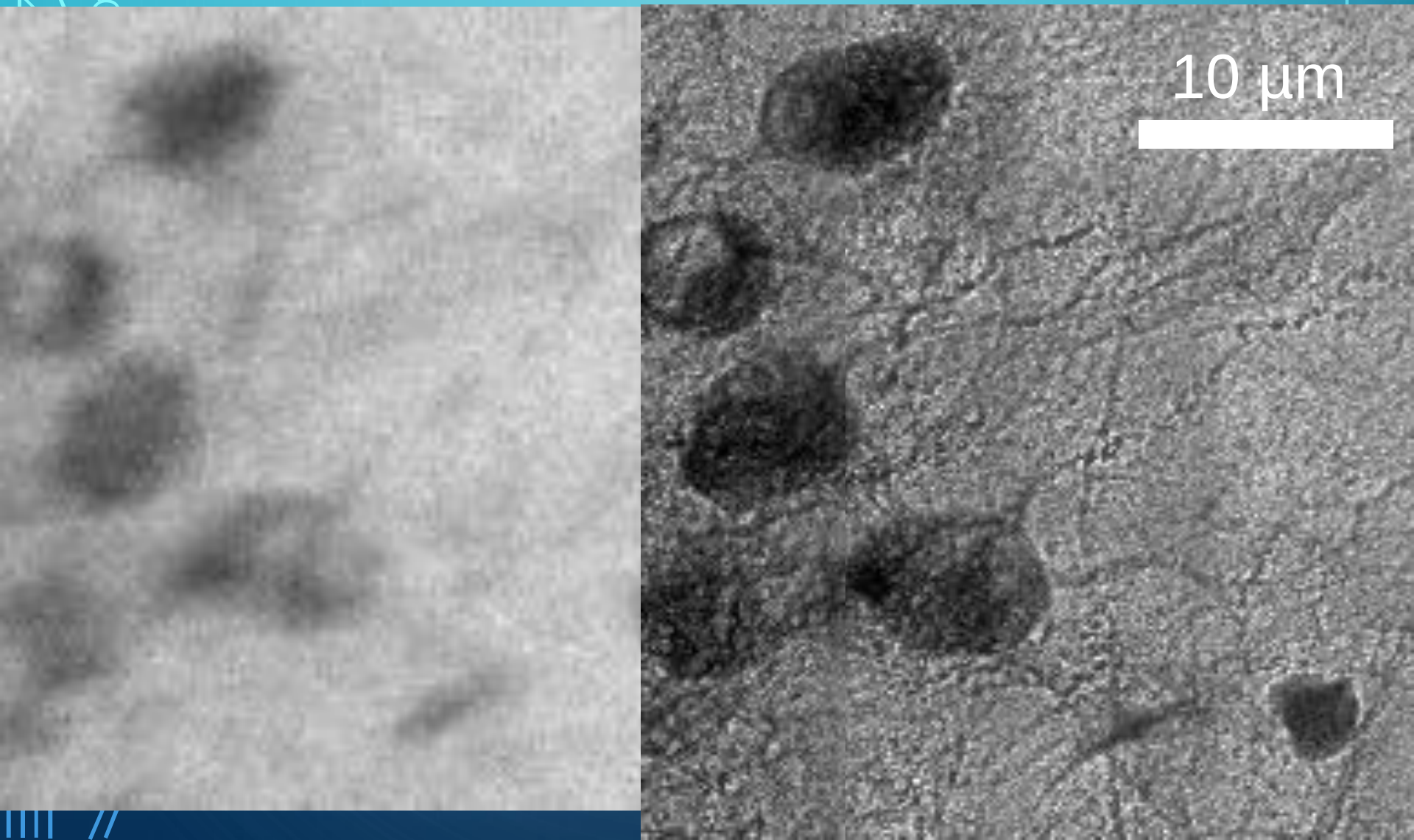
Recent progress – whole mouse brain imaging



Mouse Perkinji cell in a thick tissue slice



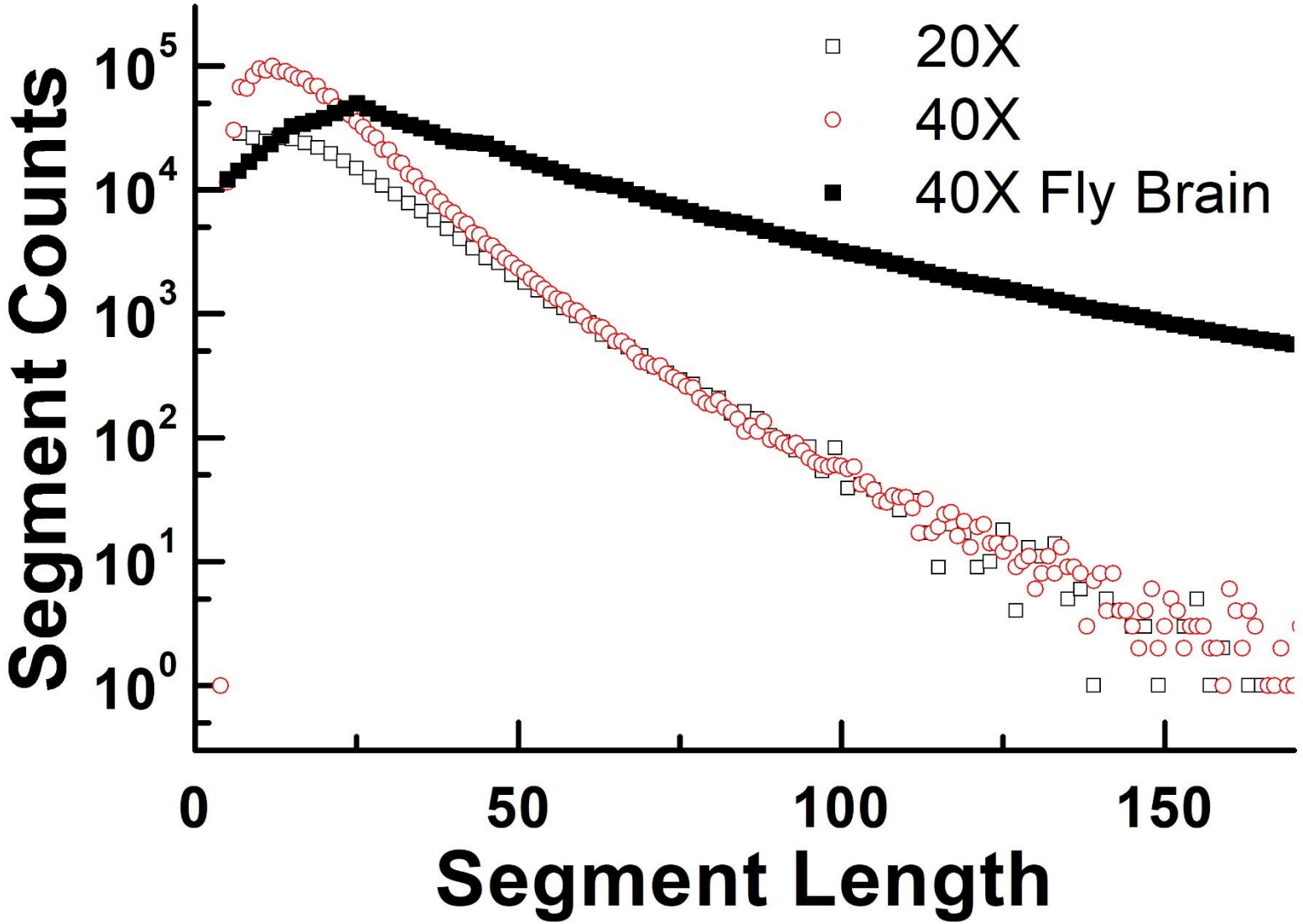
Same specimen, different resolution



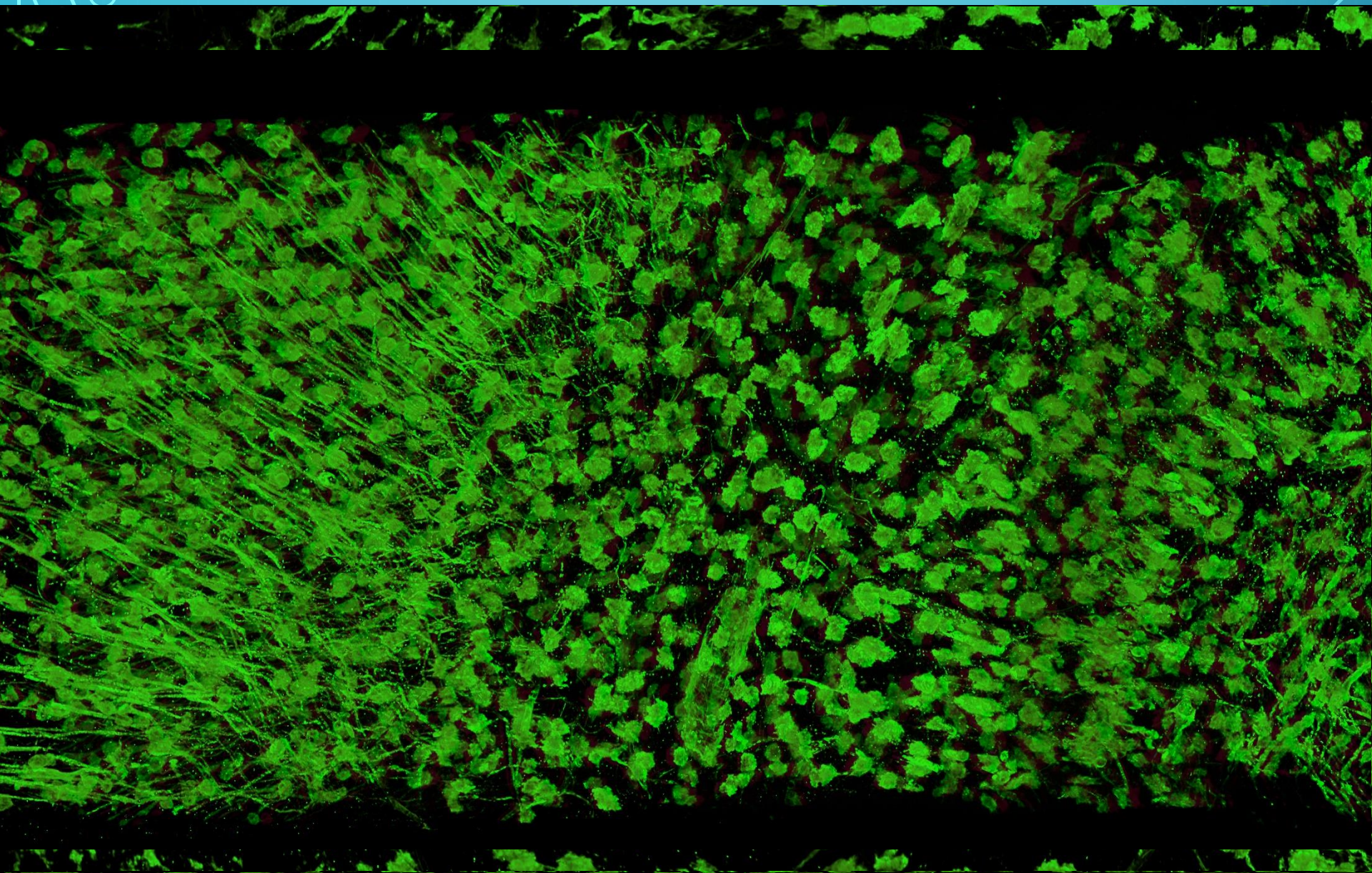
Same specimen, different resolution

20x, NA = 0.75, resolution ~0.5 µm

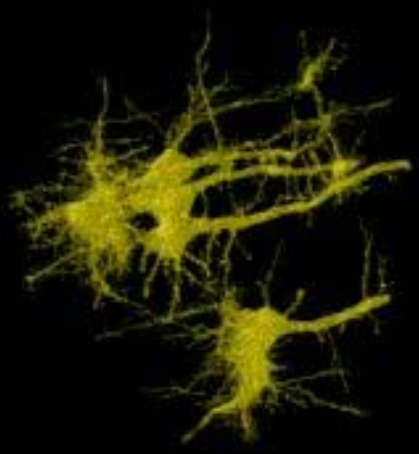
40x, NA = 0.95, resolution ~0.3 µm


Segment Counts
10^5
10^4
10^3
10^2
10^1
10^0
0
50
100
150
Segment Length
20X
40X
40X Fly Brain

And speed..



Mouse brain, $(0.8\ \mu m)^3$

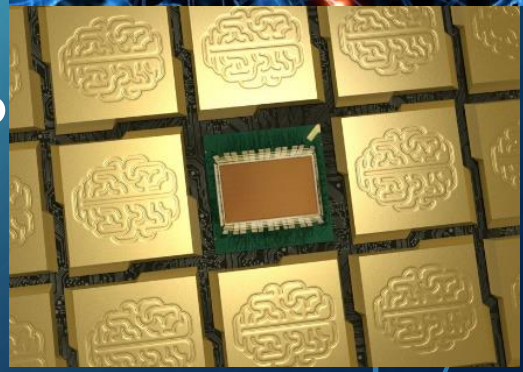


Technology impacts — Beyond connectome

- X-ray imaging: phase contrast radiology and tomography, nanotomography
- Super-resolution imaging
- 3D continuous sectioning
- 3D spectromicroscopy
- 3D pathology
- Big Data analysis
- Neuromorphic computing & AI

Brain inspired computation, neurosynaptic computing, neuromorphism and artificial brains

- Power consumption
  - 86 billion neurons, ~1000 synapses per neuron
  - >trillion transistor, >10 GW
  - Human brain 20 W!
- Simulate Brain from neuron (HBP)
- Neurosynaptic computer using neuromorphic chip
- Artificial Intelligence



Flybrain Simulators

- 130,000 neurons
- Build with real connectome map
- With input and output terminals for simulations
- Functional information included in the simulations
- Understand how fruit fly brain function (with a super computer)
- Better algorithm and computer architecture for AI
- Simulate and understand brain diseases, and the cure

Next steps:

- Sub-10 nm imaging
- Multi-modality imaging
- X-ray molecular/functional imaging
- X-FEL applications?
- Laser wavefront accelerators?

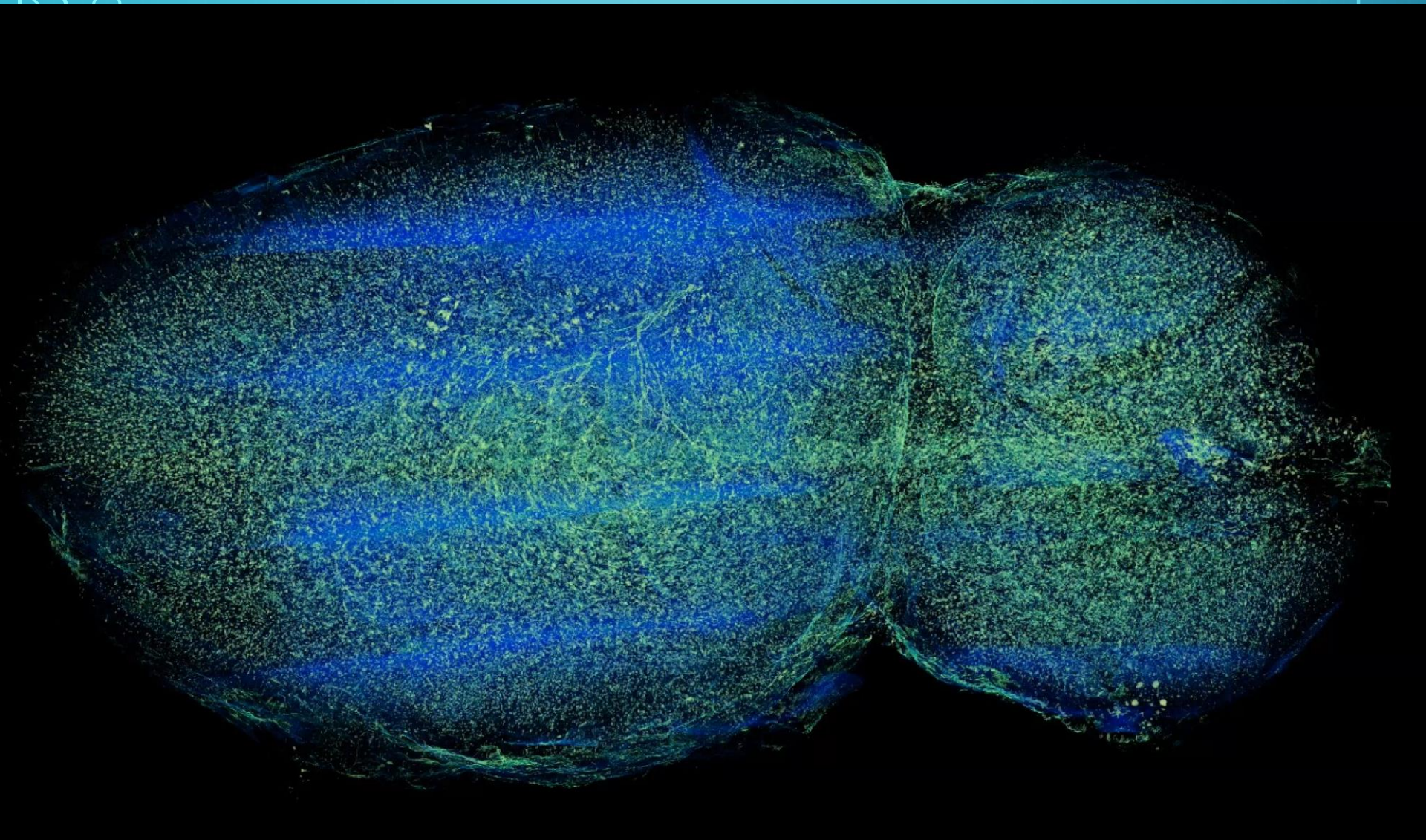
Toward Sub-10 nm

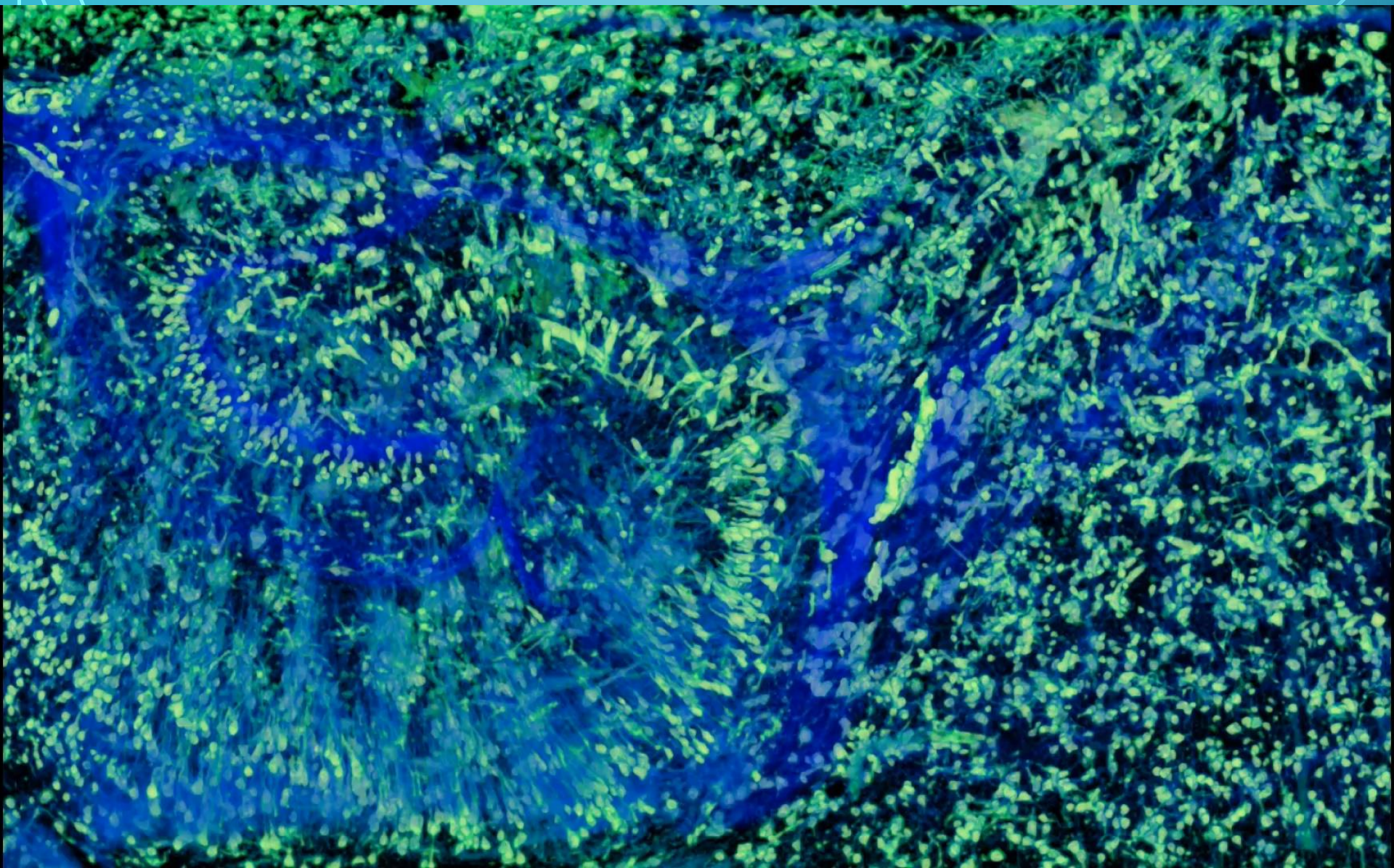
- Must be an international effort
- Mobilization of resources and user base
- Focus on novel configurations and applications
- A consortium of beamlines at NSRRC, SPring-8, PLS-II, BSRF (Beijing), SSRF (Shanghai), SSLS, NSLS-II (Singapore) …

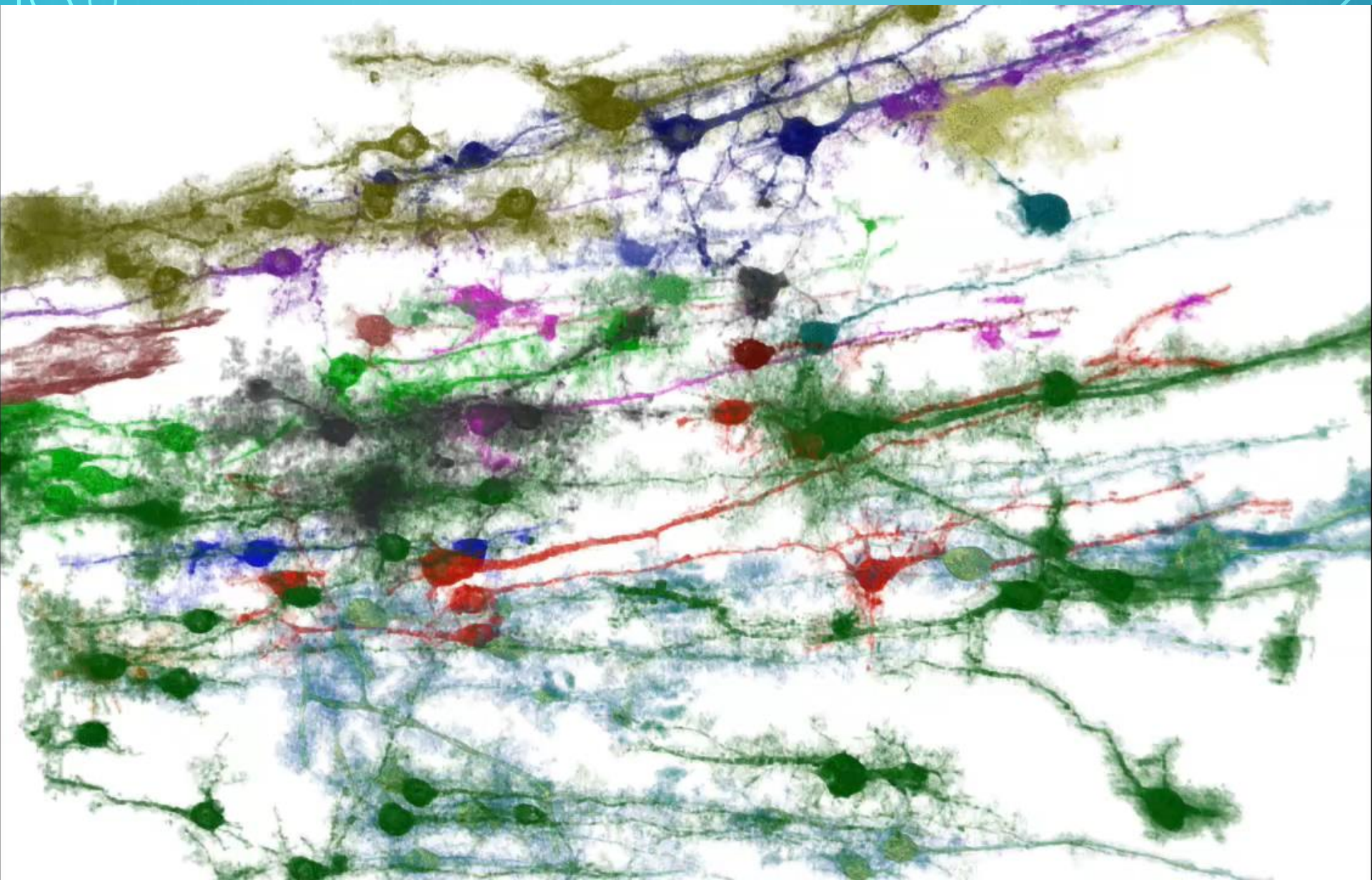
Objectives – to map in 3 years:

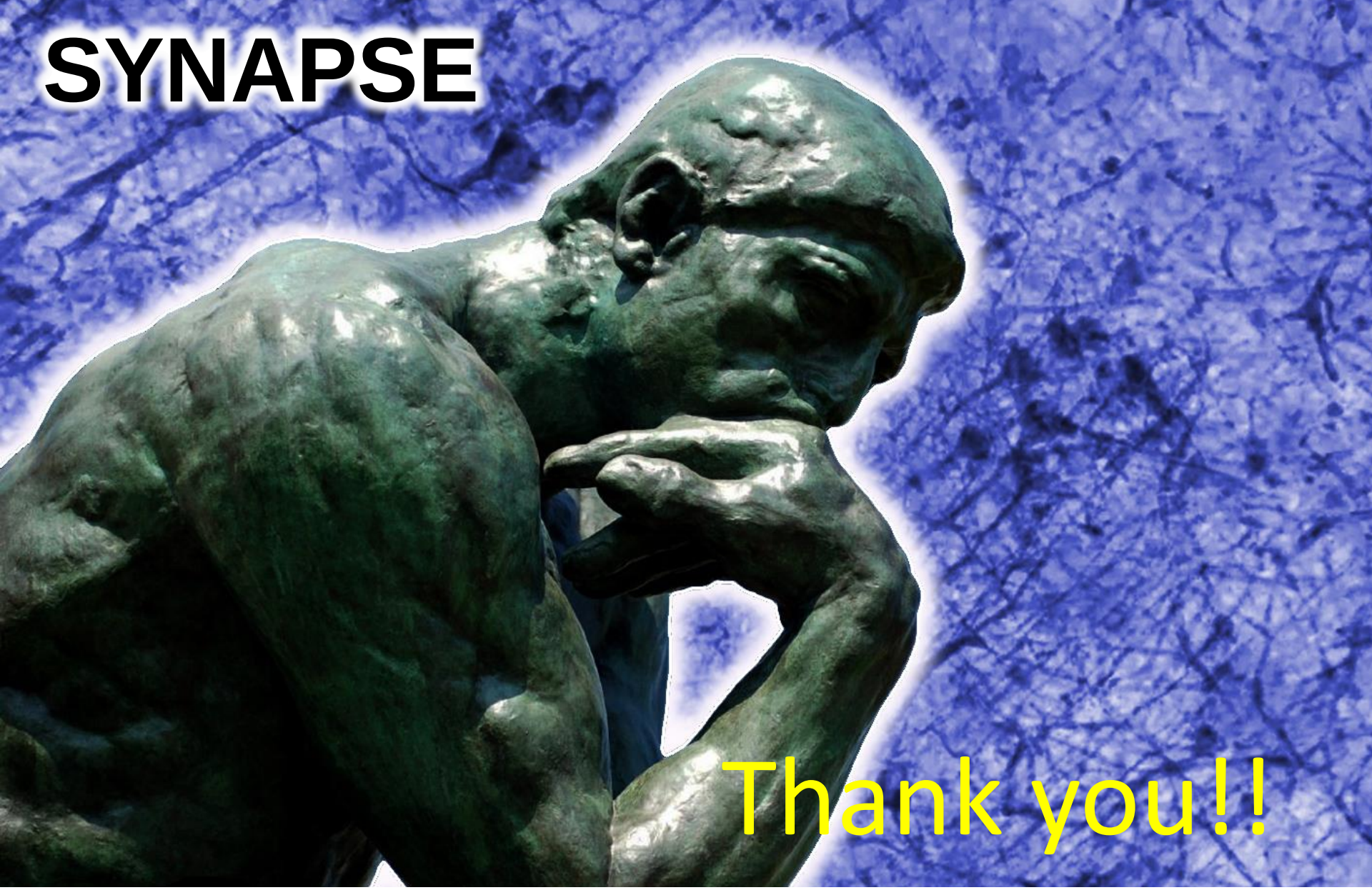
- One whole human brain
- 200 whole mouse brains
- Develop the microtomography technique for >100x improvement in speed to enable complete human connectome mapping

Whole brain w/ 1 μm resolution



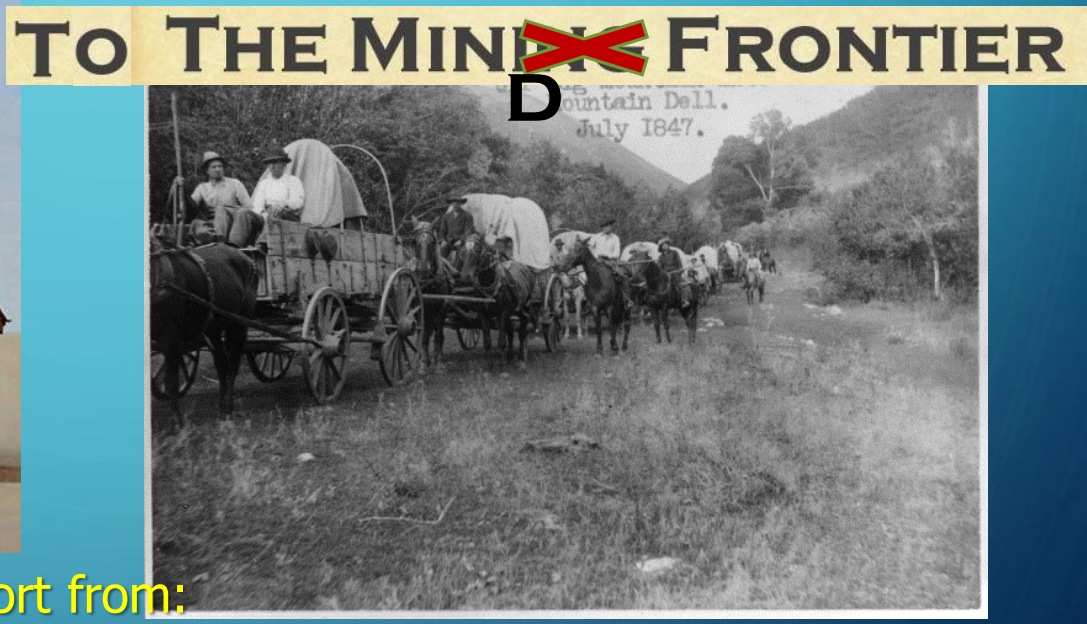






2023: groundbreaking progress in human brain knowledge brought by the Asia-Pacific countries

Go fast and go far with friends! (from China, France, Germany, Japan, Korea, Singapore, Switzerland, US, ..)



Thanks for the funding support from:

- Ministry of Science & Technology
- National Program for Nanoscience and Nanotechnology
- Academia Sinica Thematic Projects
- ANR-MOST, INSERM-MOST
- RIKEN-MOST
- USAF-MOST

The SYNAPSE strategy: data acquisition and management

Data size:

- Our experience from the tomography of a 0.5^3 mm^3 volume containing one drosophila brain:
  - Size of each raw projection image: 32 MB
  - Tomography set (1000 projection images): 32 GB
  - Reconstructed images for volume rendering: ≈128 GB
- Scaling to one mouse brain: $[450/(0.5)^3]$ x 128 GigaByte ≈ 460 TB
- Limited staining rate (~5%) ➔100 mouse brains required for complete map: ~46 PB
- Scaling to one human brain (2,700 times the mouse volume): 460 TB x 2,700 ≈ 1,240 PB

Total image-taking times at current speed:

- ~5 min for a $(0.5)^3$ mm^3 volume:

  For one mouse brain: $(450/0.5^3)$ x 5 min ≈ 1.8×10^4 minutes ≈ 12.5 days

  For 100 mouse brains: 1250 days ≈ 3.4 years

- ~15 min for a 1 mm^3 volume (with a 4K x 4K detector):

  For one human brain: 1.2×10^6 x 15 min ≈ 1.8×10^7 minutes ≈ 34 years

  For 100 human brains: 3,400 years

We must increase the throughput. Without new technologies, by:

- Increasing the number of synchrotron beamlines used in parallel, coordinated within SYNAPSE
- Reducing the number of projection images for each tomography, from 1000 to 100.
  - With new algorithms
  - With artificial intelligence

It becomes thus realistic to map one human brain in 4 years – the first SYNAPSE objective

Further throughput increases possible with new technologies:

- Staining rate increase from 5% to 30% (demonstrated but not optimized). The number of brains for a complete map could decrease to <20.
- Faster imaging: Higher x-ray flux (10x), higher detector sensitivity (5x) and better algorithms (to further reduce the number of projections) – overall, the image acquisition speed could be increased by two orders of magnitude.

The image taking time for full human brain mapping could be reduced to a few years

Image taking is not all: data managing is a big challenge!

- Tomography reconstruction for drosophila: currently, 10 times slower than image acquisition
- To perform one automatic segmentation and tracing within a comparable time, it takes now the largest computing facility in Taiwan, NCHC
- Commercial graphic workstations cannot handle the rendering and visualization of the reconstructed data set
- Another big challenge: creating and managing the database
- Further complication: adding functional information to the map

Crucial data handling tasks:

- Image morphing, warping and fusion
- Correlation identification
- Tracing, segmentation
- Database architecture and optimization
- Database access
- Visualization

Adding functional information:

- Using the connectome as the skeleton/grid.
- Adding local high-resolution 3D information on synapse connections with:
  - X-ray tomography with 10 nm resolution.
  - Super-resolution 3D fluorescence microscopy (with tissue clearing)
  - Super-resolution vibrational spectromicroscopy (Nano-IR and Nano-Raman)
  - Continuous sectioning with electron and optical microscopy
- Chemical information in large region:
  - Functional MRI, PET & SPECT imaging (with 100 µm resolution)
  - 3D IR (with <3 µm resolution)
- Simulations based on the structural and functional information

Other technological developments targeted by SYNAPSE:

- Algorithms and related software
- Computer hardware: machine-learning graphic station
- Automation in specimen handling
- Automation in image acquisition
- Artificial Intelligence for automation of image processing
- Image taking hardware: specimen manipulation, detectors and high-brightness X-ray sources

Why us?
We are uniquely qualified!

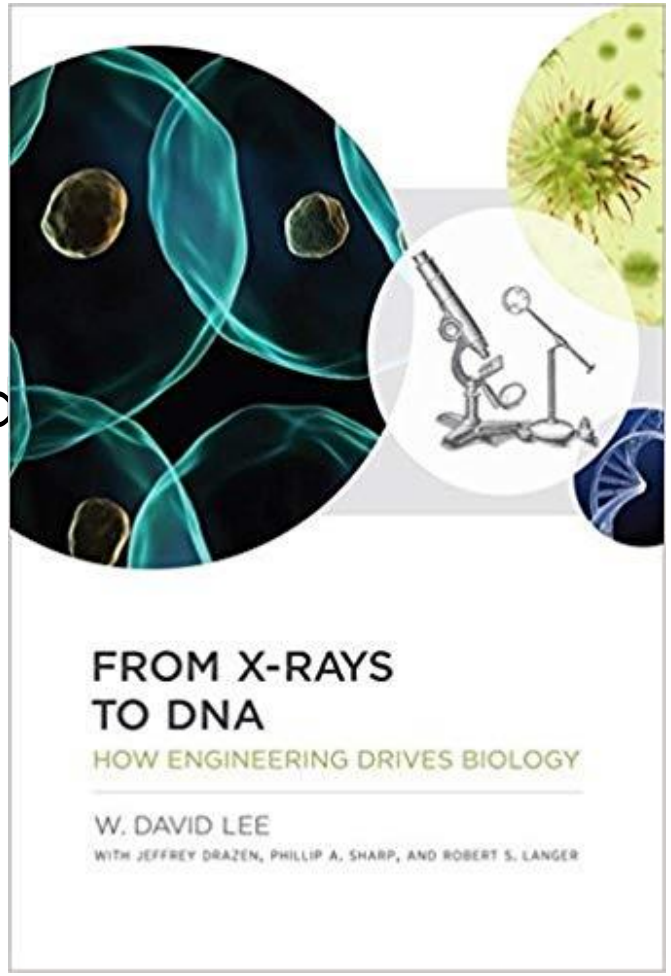
- We pioneered the special techniques required for the SYNAPSE initiative, including:
  - X-ray phase-contrast tomography
  - World-record x-ray microscopy
  - Deep tissue super-resolution microscopy
- We have a solid record of relevant previous accomplishments and all the required know-how for the core and support techniques
- The new partnership is built on long-standing and very successful collaborations

Sizes of Brains:

	Volume (mm^3)	Number of neurons	Brain/ body ratio	Number of synapses	Density of neurons (mm^{-3})
drosophila	$2x10^{-2}$	$2.5x10^5$ ($1.3x10^5$)			$1.25x10^7$
mouse	450	$7.1x10^7$	1:40	10^{11}	$1.57x10^5$
marmoset		$6.4x10^8$			
human	$1.2x10^6$	$8.6x10^{10}$	1:50	10^{14}-10^{15}	$7.2x10^4$

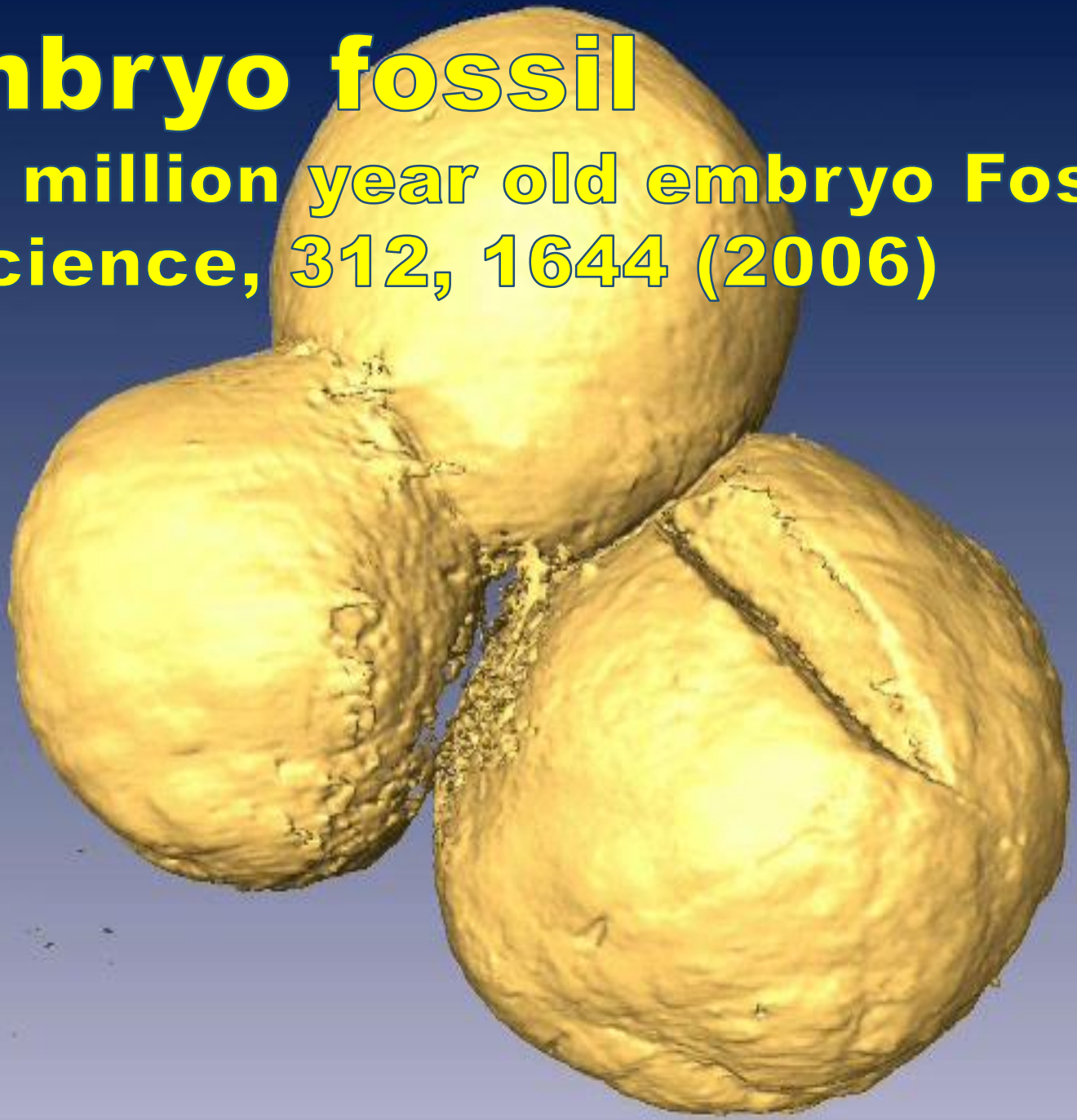
Physics opens the new era of biology and medicine, again and again.

- Microscopy
- Spectroscopy
- X-rays
- Protein crystallography
- NMR, SPECT, PET, CT, ultrasound
- Mass spectrometry
- Nuclear Medicine
- Digital Camera
- …

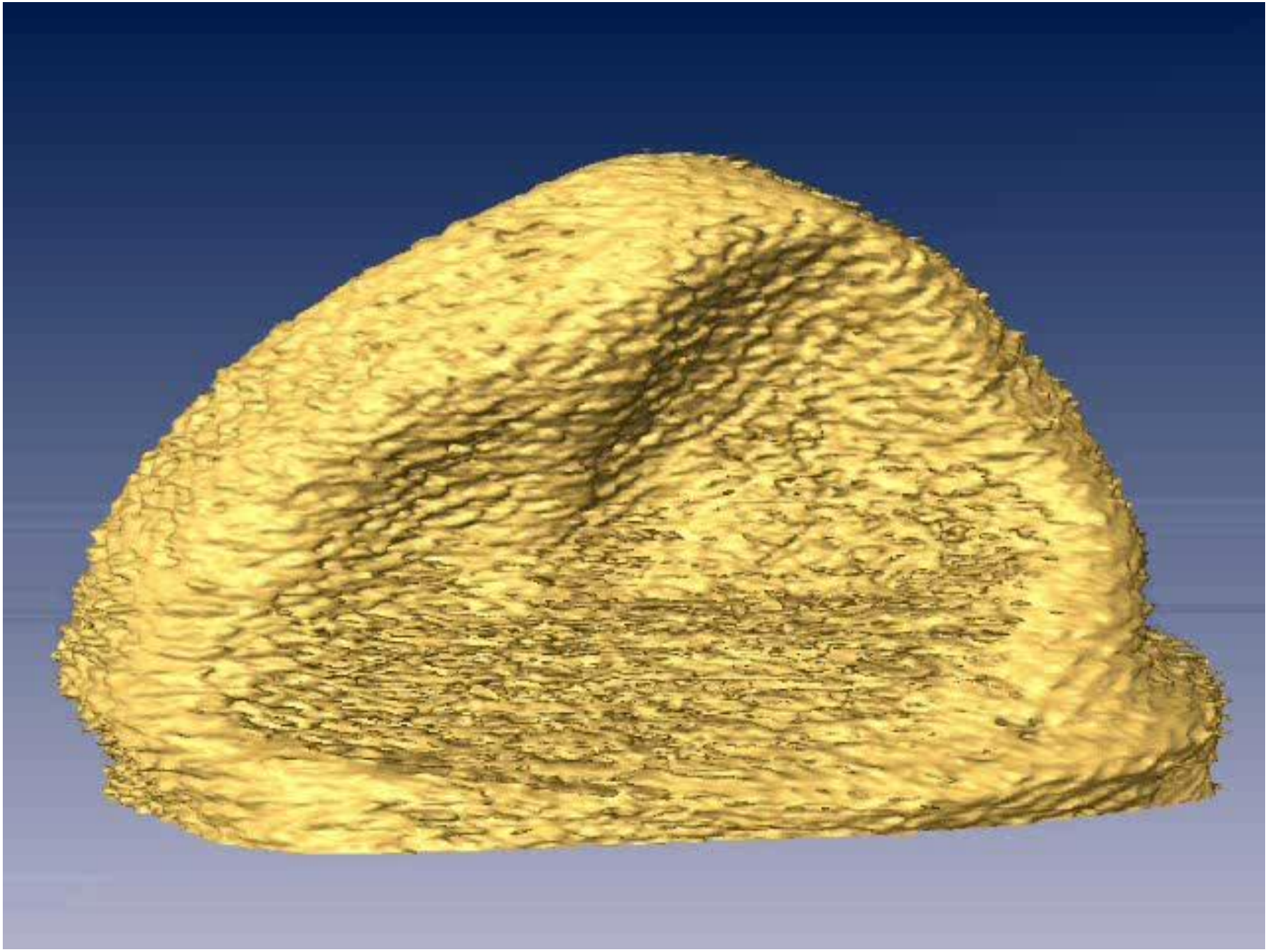


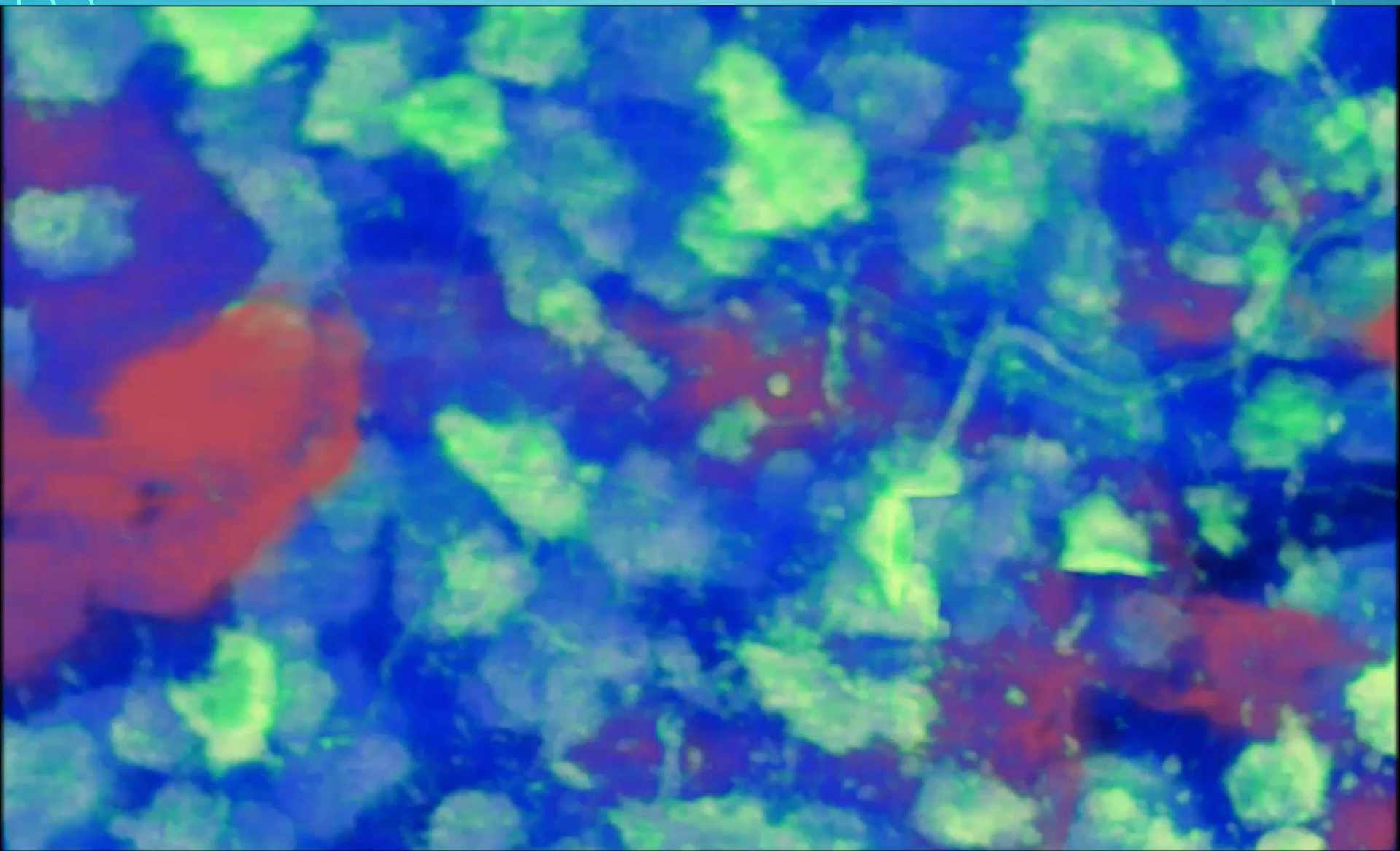
Embryo fossil

540 million year old embryo Fossil -- Science, 312, 1644 (2006)



540 million year old embryo fossil



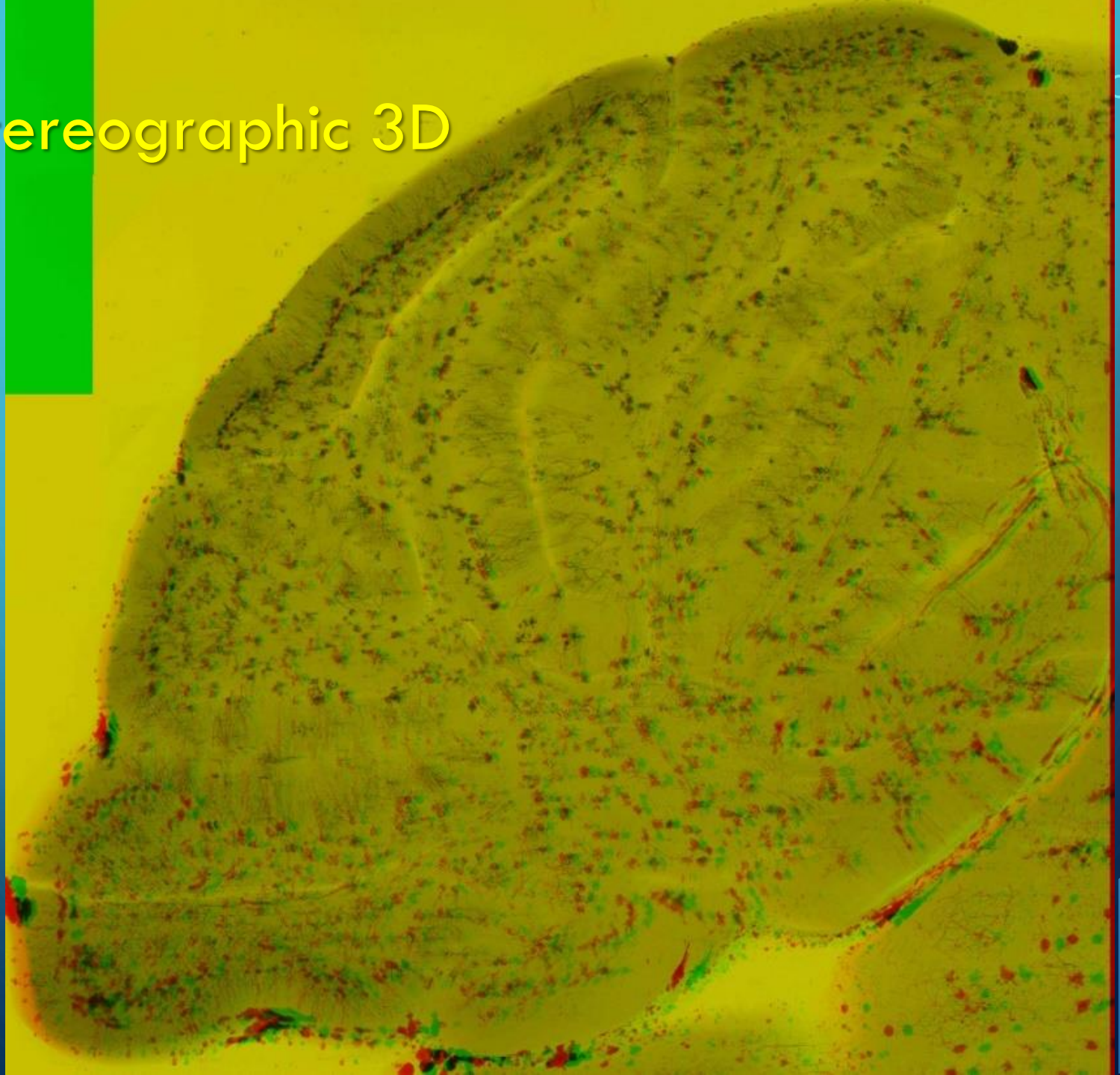


Concentrate on imaging

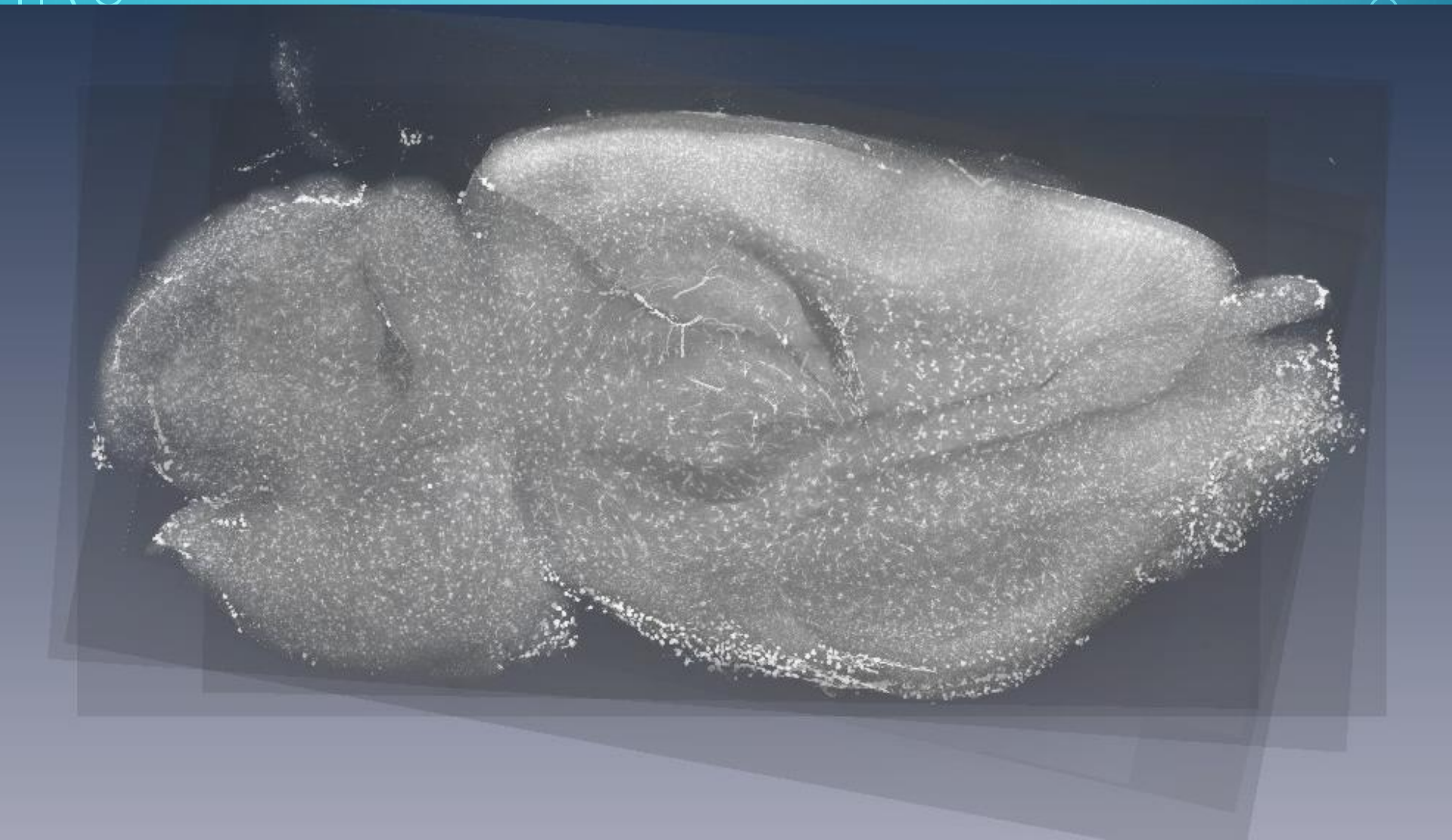
Structure and Network:

- X-ray phase contrast microtomography, 0.3 μm resolution in all 3D directions: to provide the wiring map
- X-ray nanotomography: 10 nm to provide the structure and location information of targeted synapse and neuron connections
- Confocal + *FocusClear*® : Full drosophila brain and regions of mouse brain with functional information
- Cryo-EM: small region 3D map down to nm resolution.
- Super-Res + *FocusClear*® : Full drosophila brain and small region of mouse brain but concentrate on single molecule detection

Stereographic 3D



Volumetric view



ONE STEP FURTHER…

Chian Ming Low, Eng Soon Tok, Alvin Teo, Tin-wee Tan (National University of Singapore)

Zhong Zhong & Hua Hau (中中與华华)

Monkey Hippocampus (*Macaca Fascicularis*)

<u>Hippocampal Formation</u>

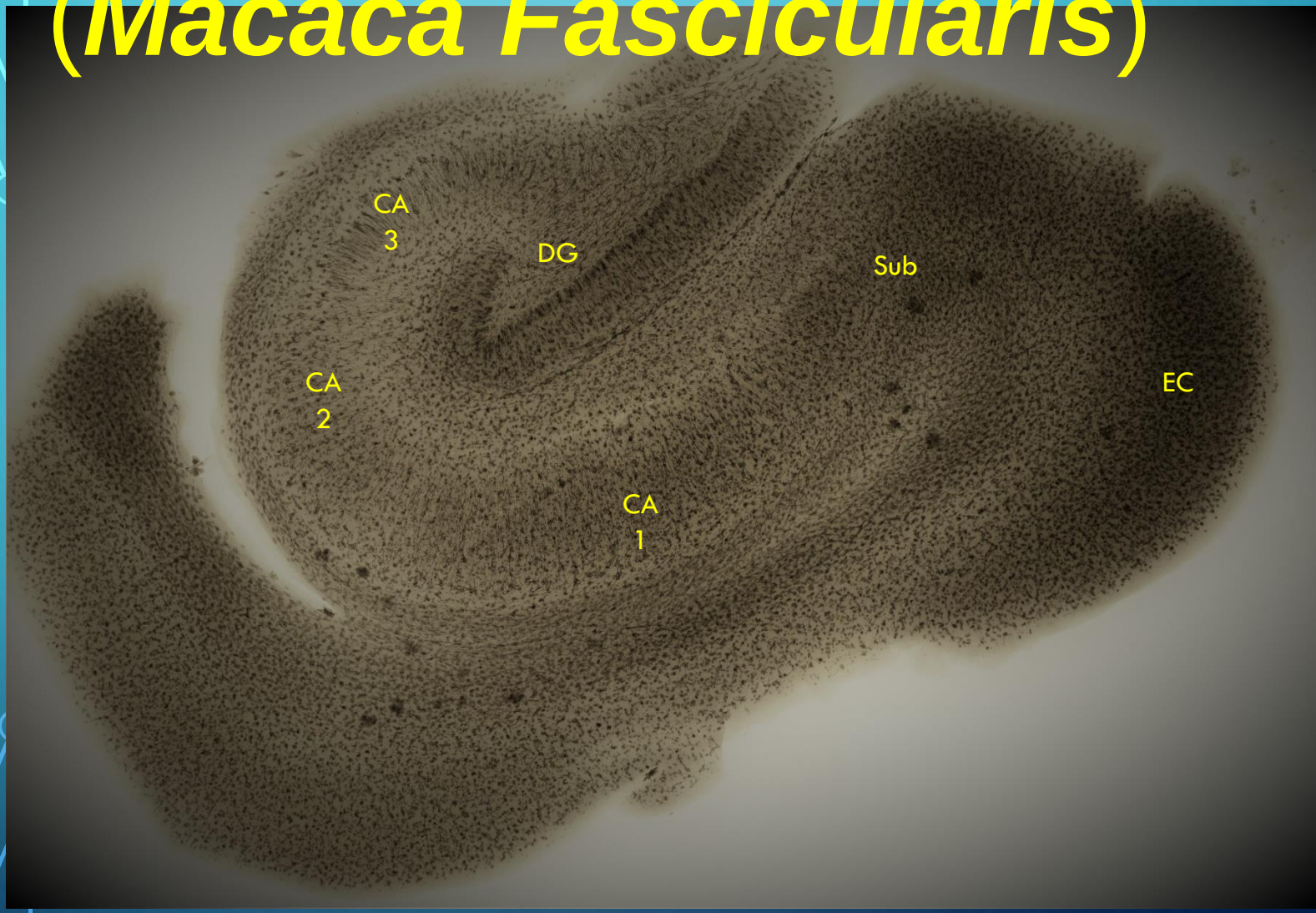
DG – Dentate Gyrus

CA – Cornu Ammonis

Sub – Subiculum

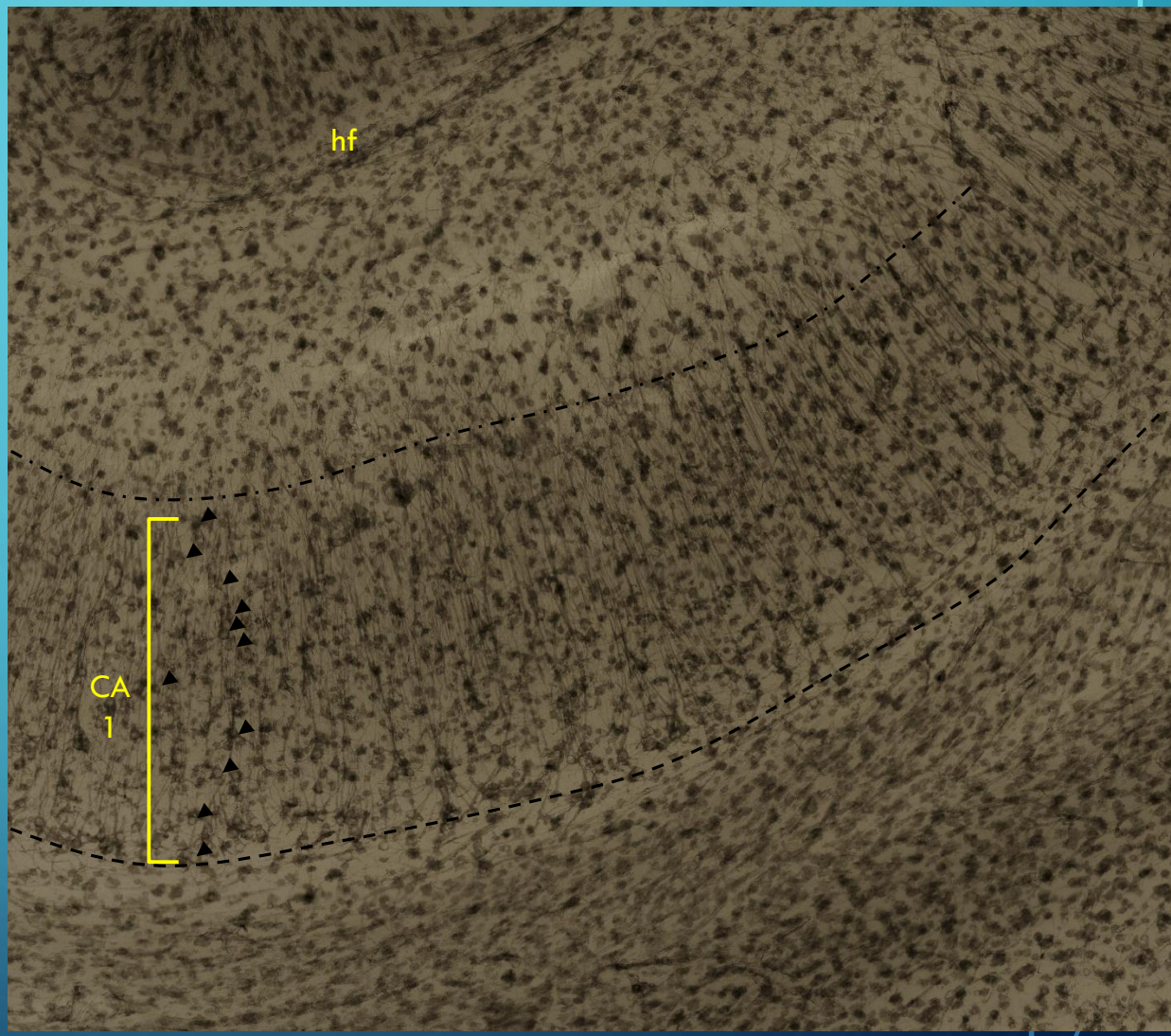
EC – Entorhinal Cortex

Note: hippocampal formation consists of these regions – DG, CA3, CA2, CA1, Subiculum & Entorhinal cortex…you can also call it hippocampus (a more 'general' term)

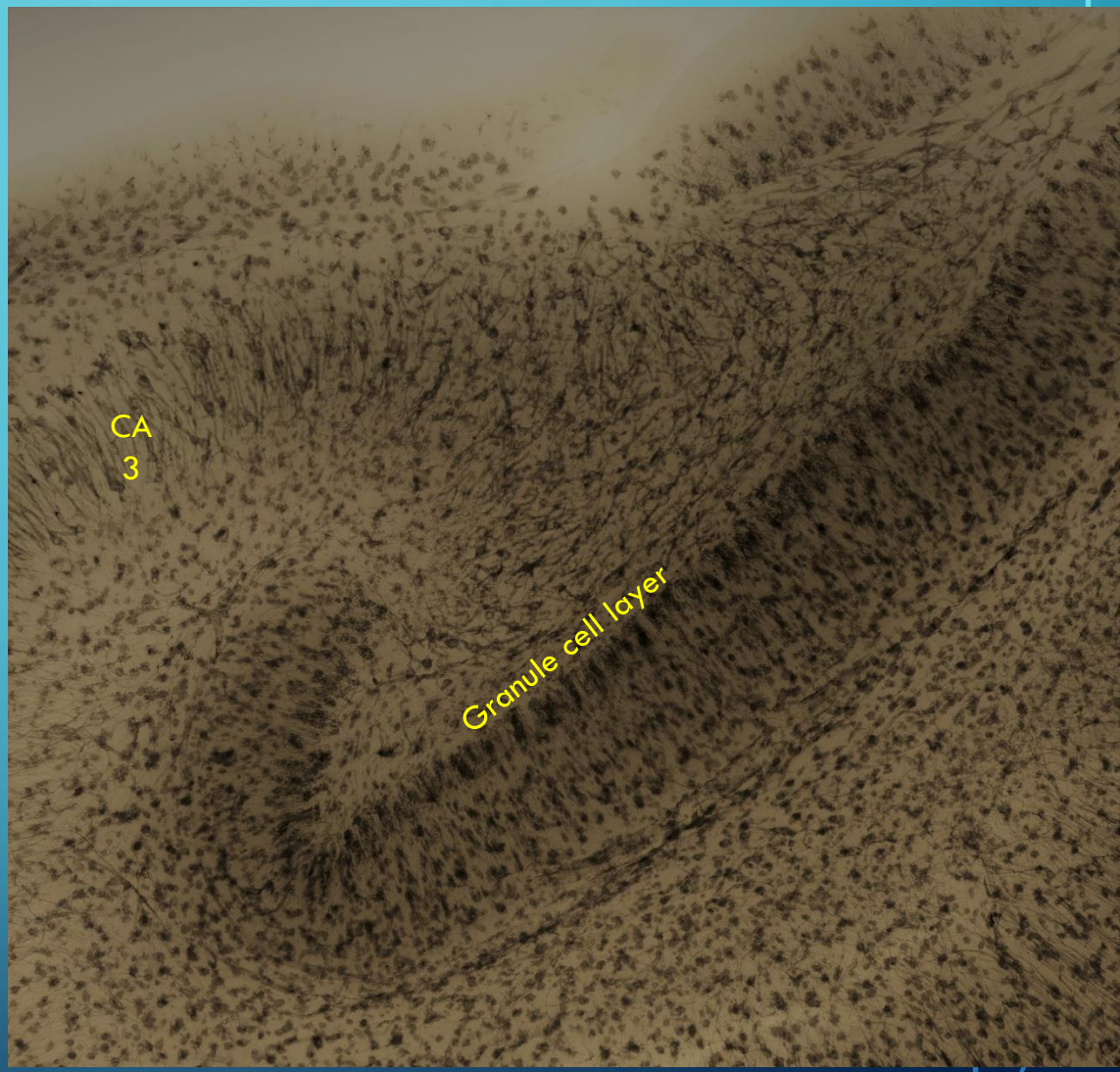


CA1	Rat	Monkey
# Divisions	1-2	7
# Cell Thick	5	10-15
Boundary between PCL -SR	Clear	Less clear
Boundary between CA1-Subiculum	Clear	Less clear
Entorhinal Cortex – laminar organization	Less clear (e.g. Layers V-VI) 2 divisions	Clear (e.g. Layers V-VI) 7 divisions

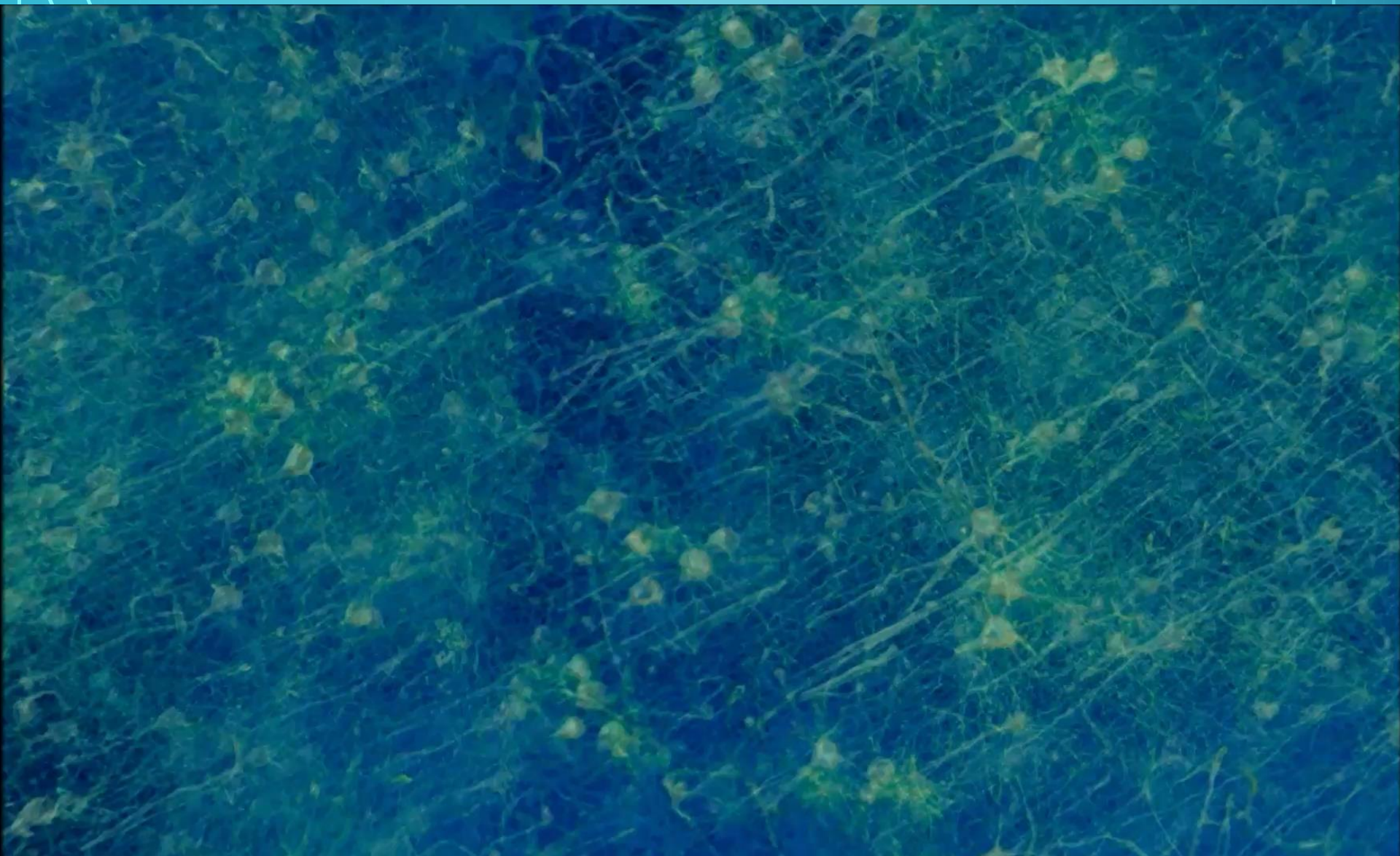
hf – hippocampal fissure (a 'line' that DG is separated from CA1)
Arrowhead – neuron soma



Dentate Gyrus



Mouse brain 2x2x20 mm^3



Besides Image Taking:

- Full biological characterization
- Specimen preparation: novel labeling procedures
- Coordinated use of different imaging facilities at partner countries, to reach the required throughput
- Standardization and mutual validation for all participating facilities
- Advanced image processing with new computer techniques
- Full data access for all partners and eventually to public

The core partners bring with them a very powerful extended coalition:

- Through NUS/SSLS: research teams from university (NUS, NTU, NYP), public institutions (NCSS)
- Through SARI/SSRF: research teams from universities (Shanghai Jiao Tong U., Shanghaitech U.), public institutions (SINAP, IoN)
- Through POSTECH/PAL: research teams from universities (POSTECH, KAIST), medical institutions (ASAN, Samsung, SNU, Yonsei)
- Through RIKEN/Spring8: public institutions (RIKEN QBiC-BDR, BSI)
- Through ANSTO: research teams from universities (U. Sydney, U. Wollongong), public institutions (Australian Synchrotron, Brain & Mind Center)
- Through AS: research teams from universities (NTU, NTHU, NCTU, NCKU), public institutions (ITRI, NHRI, NHPC), medical institutions (NTUH, Chang Gun H, Mackay H, TSGH, VGH, Chinese Medical UH, Taipei, Medical UH, Kaohsung MU, Cheng Kung UH) and from the private sector (TTY Pharma, Delta Electronics)