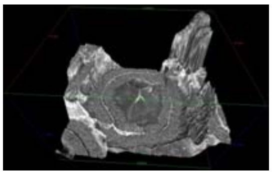
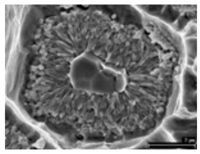
3D Microscopy and FIB Nanotomography

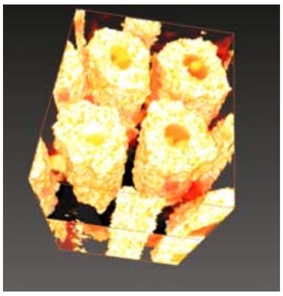


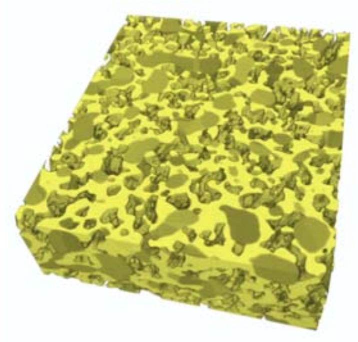


Marco Cantoni

CIME

mxc-133, 34816





Introduction to Tomography

- **Tomography** is imaging by sections or sectioning. A device used in tomography is called a **tomograph**, while the image produced is a **tomogram**.
- The method is used in medicine, archaeology, biology, geophysics, oceanography, materials science, astrophysics and other sciences.
- In most cases it is based on the mathematical procedure called **tomographic reconstruction**.
- The word "tomography" is derived from the Greek *tomos* (slice) and *graphein* (to write).

Wickipedia

Introduction to Tomography

- **Tomography** is a method in which a 3-D structure is reconstructed from a series of 2-D projections (images) acquired at successive tilts (Radon 1917).
- First developed for use in medical imaging (1963, Nobel Prize for Medicine in 1979) using X-rays, ultrasound and magnetic resonance (e.g. 'cat-scans')..
- Found further application in geology, astronomy, materials science, etc…

P. Midgley, tomo workshop in Berlin

Introduction to Tomography

Recording

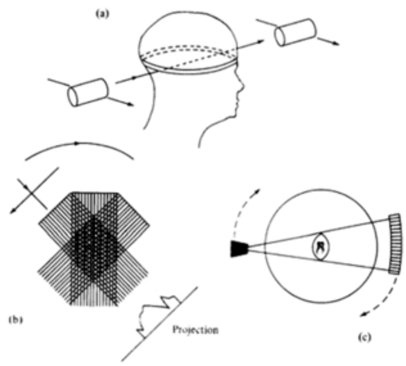
- Series of 2D images
- Destructive: serial sectioning, FIB
- Non-destructive: X-rays, TEM

Reconstruction and « viewing »

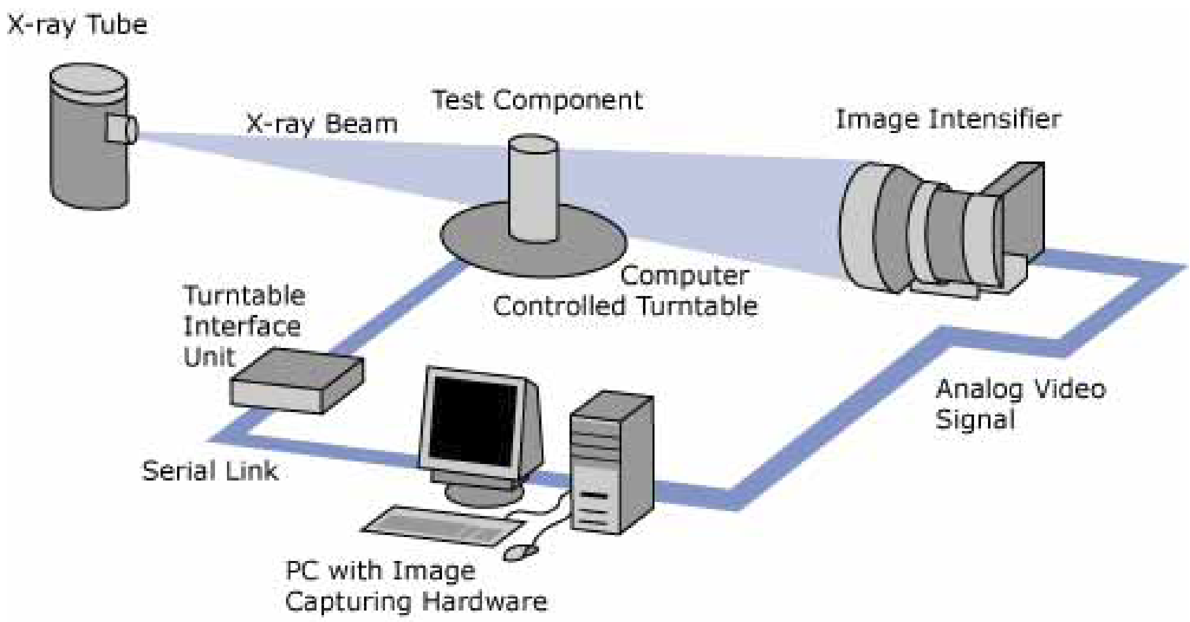
- Registration (alignment of images)
- Back-projection, reconstruct (tilt-series)
- **Tomogram**
- Segmentation (image processing), extraction of the desired information

3D imaging in medicine

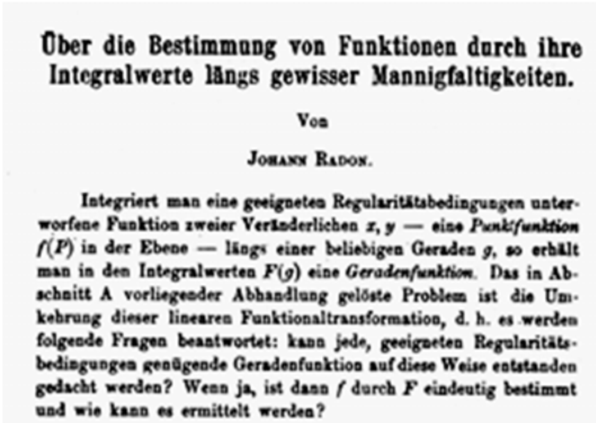
- Non-invasive methods are preferred!
- The disadvantage of conventional X-radiographs is its inability to discriminate between organs of close absorptivity or overlapping organs in the viewing direction.
- X-ray computed tomography overcomes that limitation:
- X-radiographs are made in many different directions and combined mathematically to to reconstruct cross-sectional maps.
- reconstruction tomography or computer assisted tomography.



Tomograph



Radon 1917



Über die Bestimmung von Funktionen durch ihre Integralwerte längs gewisser Mannigfaltigkeiten.

Von

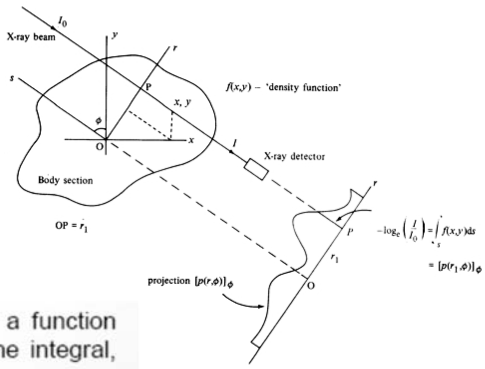
JOHANN RADON.

Integriert man eine geeigneten Regularitätsbedingungen unterworfene Funktion zweier Veränderlichen x, y — eine *Punktfunktion* $f(P)$ in der Ebene — längs einer beliebigen Geraden g, so erhält man in den Integralwerten $F(g)$ eine *Geradenfunktion*. Das in Abschnitt A vorliegender Abhandlung gelöste Problem ist die Umkehrung dieser linearen Funktionaltransformation, d. h. es werden folgende Fragen beantwortet: kann jede, geeigneten Regularitätsbedingungen genügende Geradenfunktion auf diese Weise entstanden gedacht werden? Wenn ja, ist dann f durch F eindeutig bestimmt und wie kann es ermittelt werden?

Ber. Sächs. Akad. Wiss. Leipzig, Math. Phys. Kl. 69, 262 (1917)

English translation in: Deans, S.R. (1983) The Radon transform and its applications. John Wiley & Sons, NY)

Radon Transform

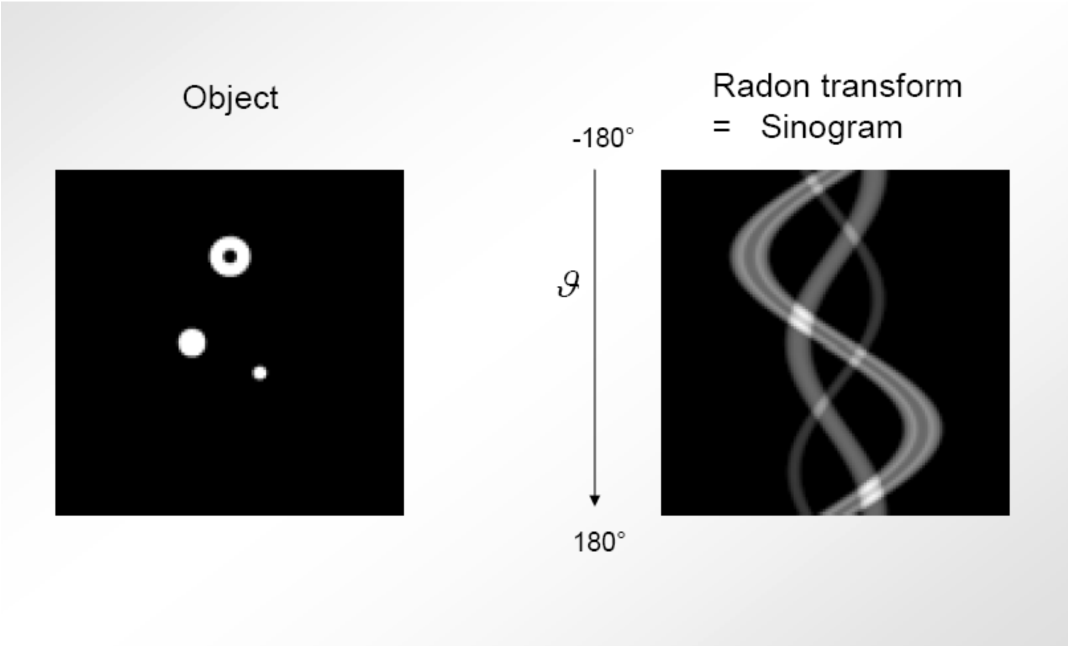


The paper defines the Radon transform R as the mapping of a function $f(x,y)$, describing a real space object D, by the projection, or line integral, through f along all possible lines L:

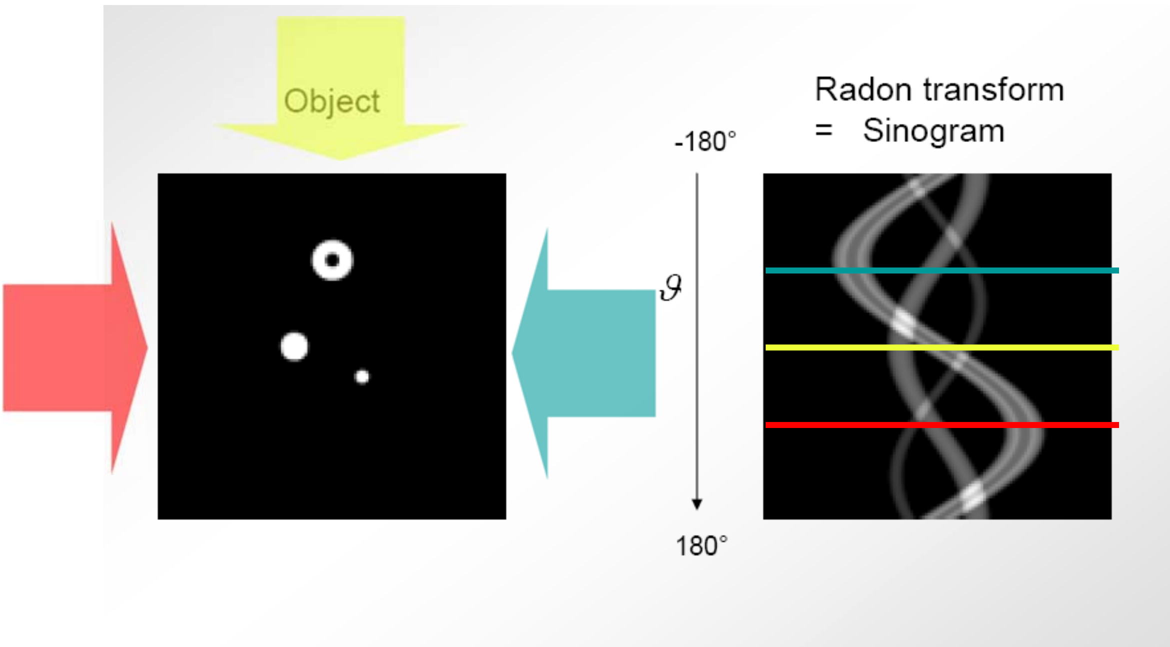
$$Rf = \int_L f(x,y)ds,$$

A discrete sampling of the Radon transform is geometrically equivalent to the sampling of an experimental object by some form of transmitted signal: a projection. ***The consequence of such equivalency is that the reconstruction of an object f(x,y) from projections Rf can be achieved by implementation of the inverse Radon transform***

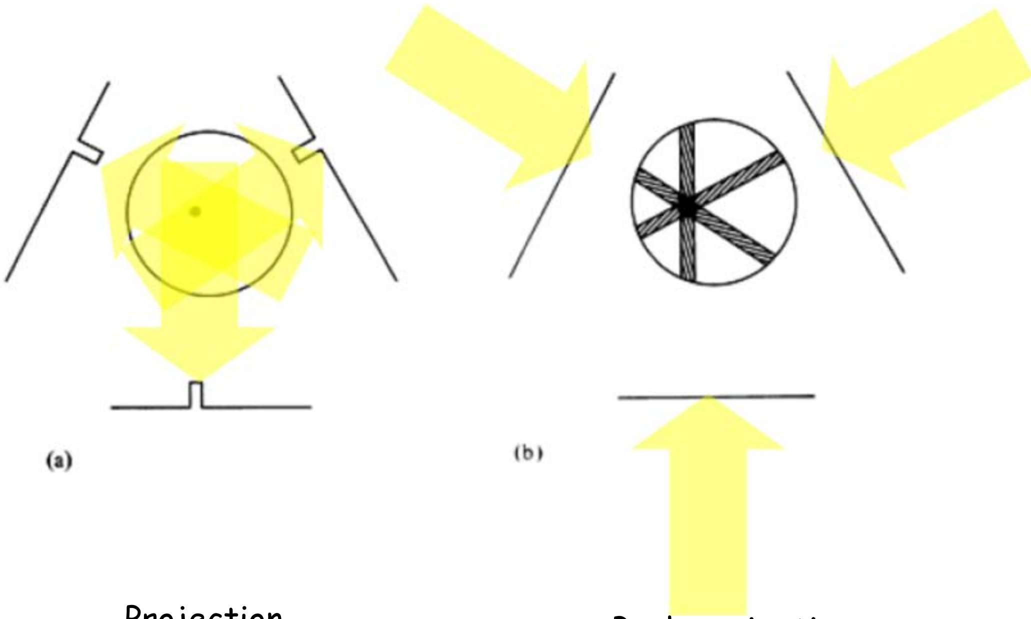
Radon Transform



Radon Transform



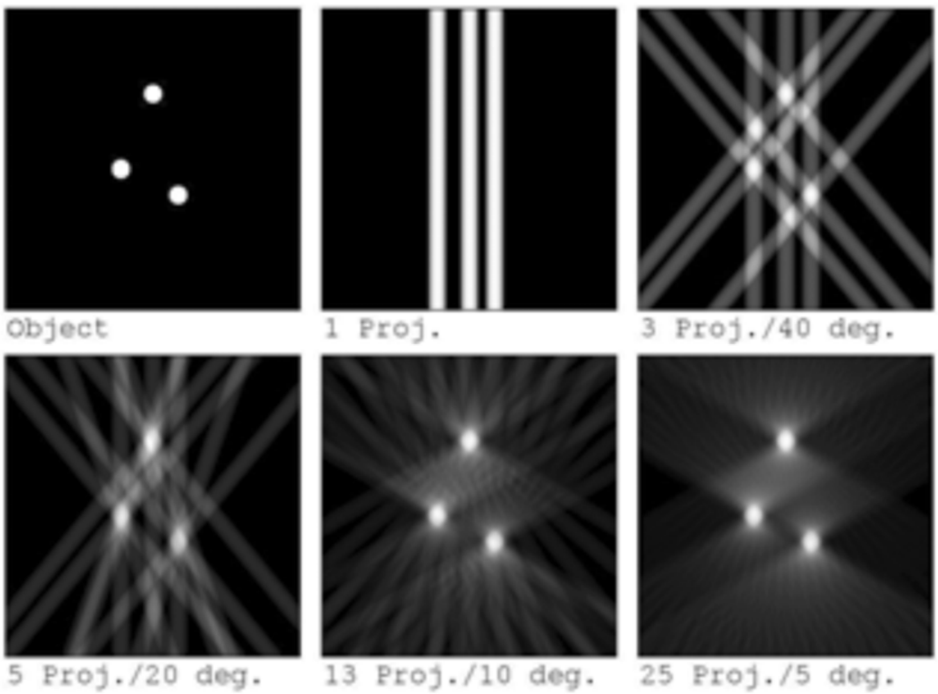
Back projection



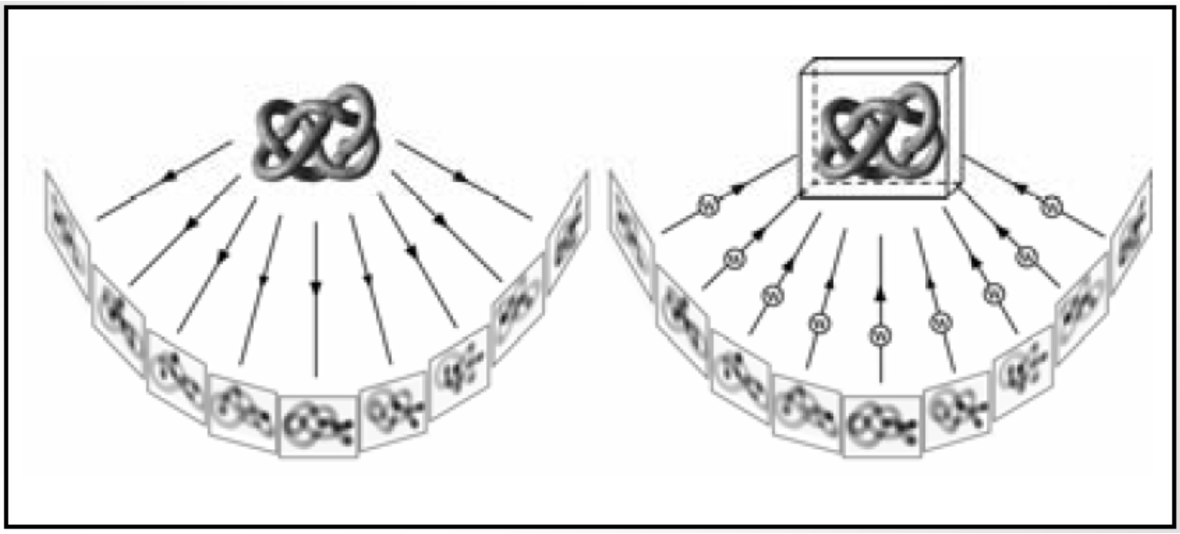
Projection recording

Back projection reconstructing

Back projection



back projection

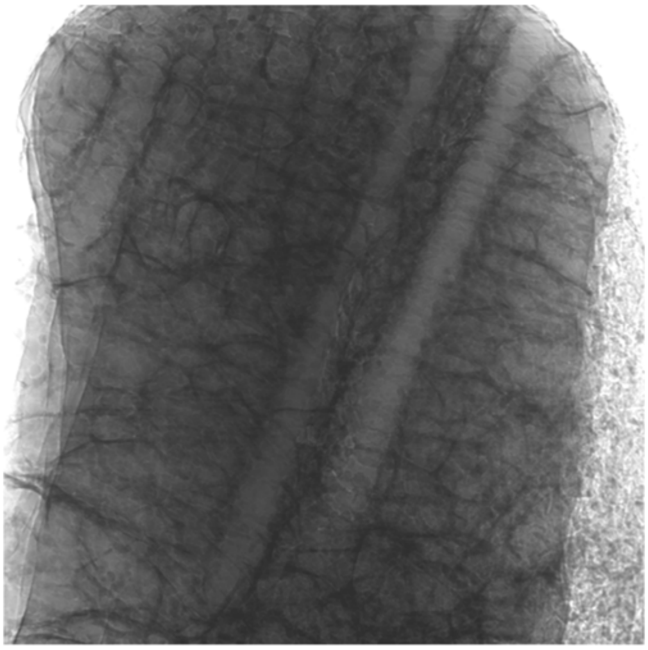


Tomography in medicine

3D imaging in materials science

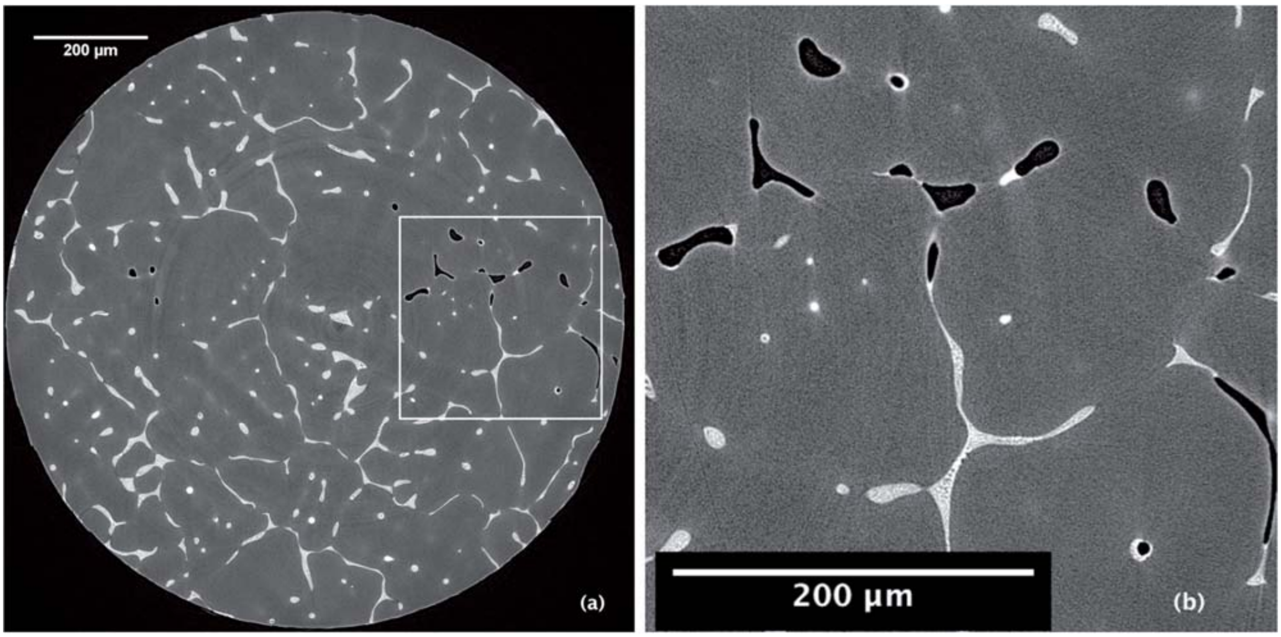
360degree X-ray tomography
Milan Felberbaum
STI-IMX-LSMX

Cylinder of an Al-Cu Alloy



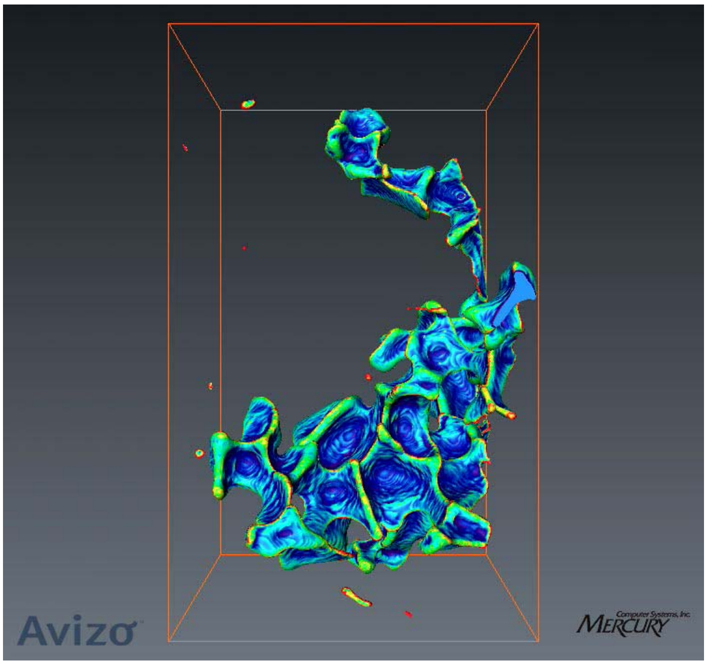
3D imaging in materials science

Tomogram



3D imaging in materials science

Reconstructed pore

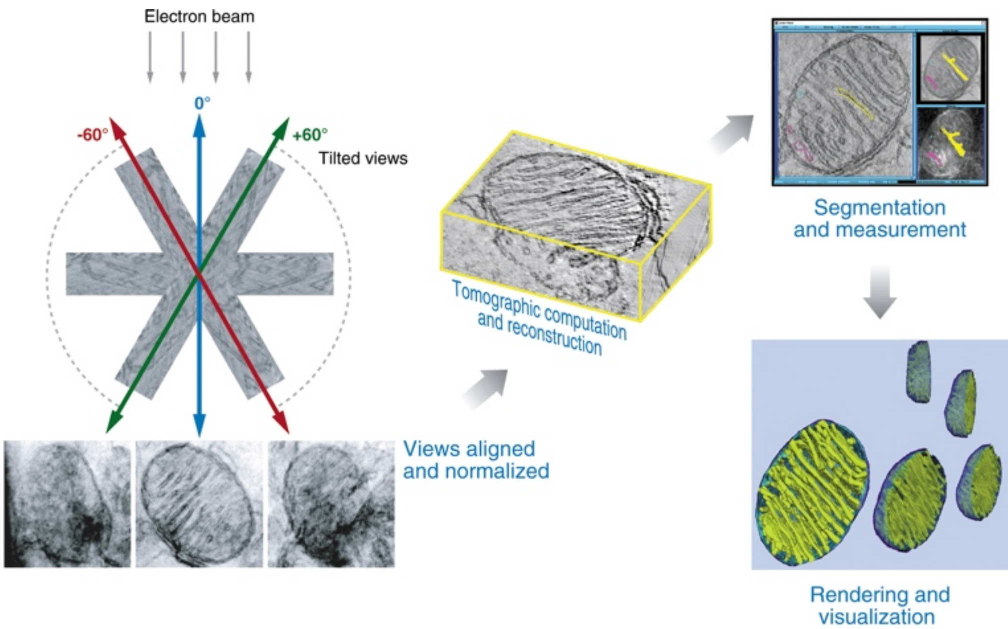


Tomography with electrons

Stopping range for electrons (99% absorbed)

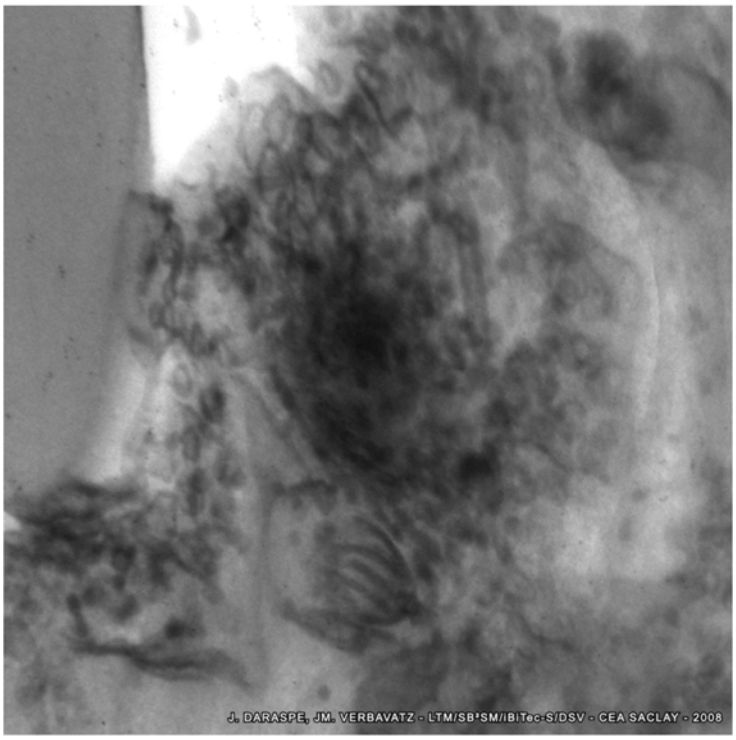
Element (specific weight)	4-Be 1.84 g/cm^3	13-Al 2.7 g/cm^3	29-Cu 8.93 g/cm^3	82-Pb 11.3 g/cm^3
X-rays Cu-Kα λ=1.54 Å Mo-Kα λ=0.71 Å	16 mm 83 mm	0.35 mm 3.3 mm	0.10 mm 0.10 mm	0.017 mm 0.034 mm
Neutrons λ≈1.08 Å	89 m	6 m	0.26 m	14 m
Électrons λ=0.037 Å à 100 kV λ=0.020 Å à 300 kV	39 μm	42 μm ~330 μm	11 μm	0.6 μm

Bio-EM, Tomography

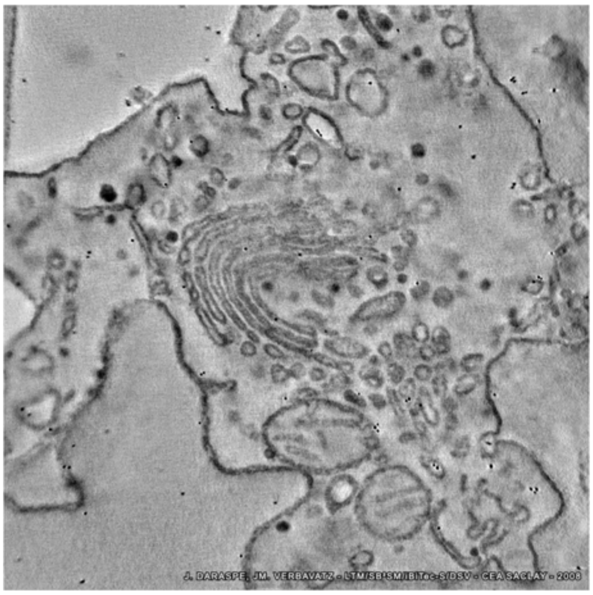


Frey TG, et al. 2006.
Annu. Rev. Biophys. Biomol. Struct. 35:199–224

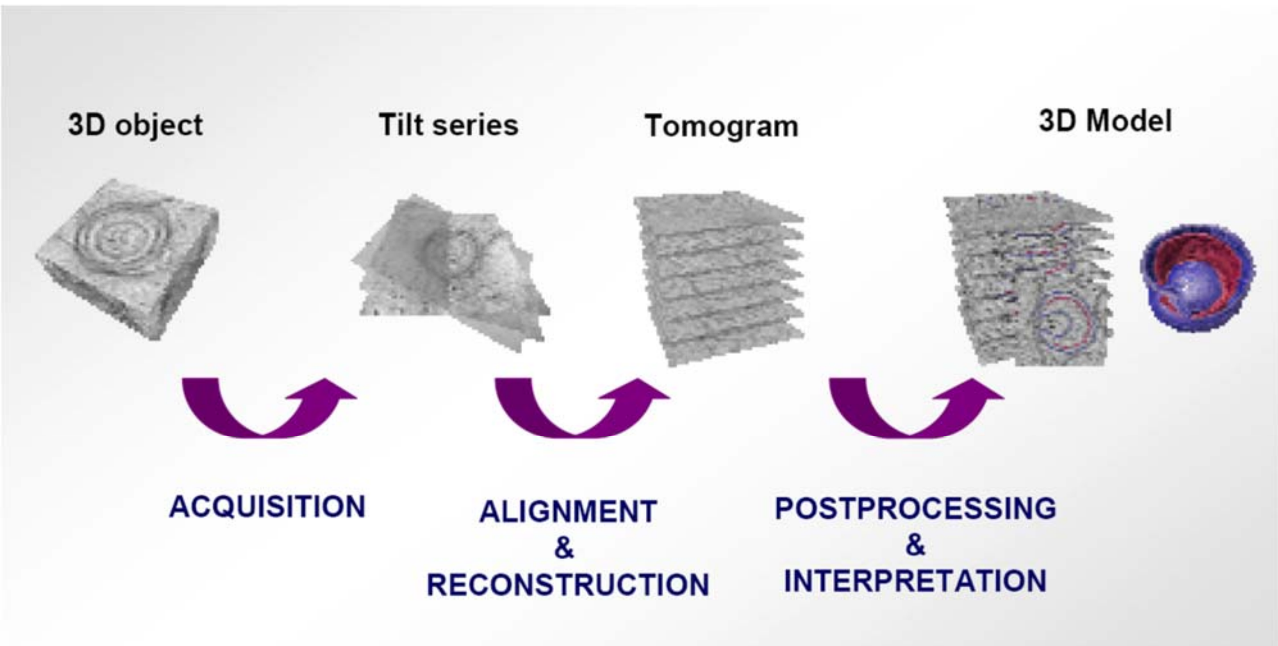
Tilt series, -60 … +60 degree tilt



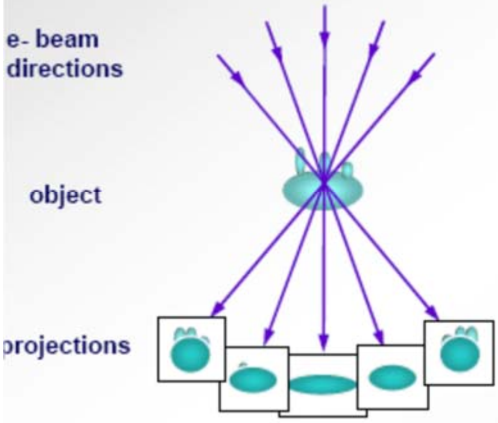
Tomogram



Tomo workflow



resolution



IDEAL SITUATION:

As many images as possible from ALL orientations (-90° to 90°)

RESOLUTION, *d*

Crowther, DeRosier, and Klug, 1970

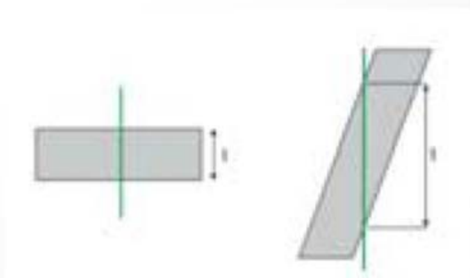
$$d = \pi \frac{D}{N}$$

D, diameter of the object
N, number of projections

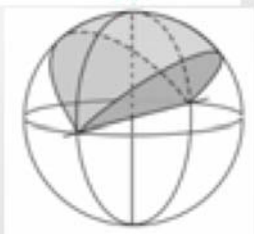
2 nm with 141 images of a 100 nm object

geometrical limit, the missing wedge

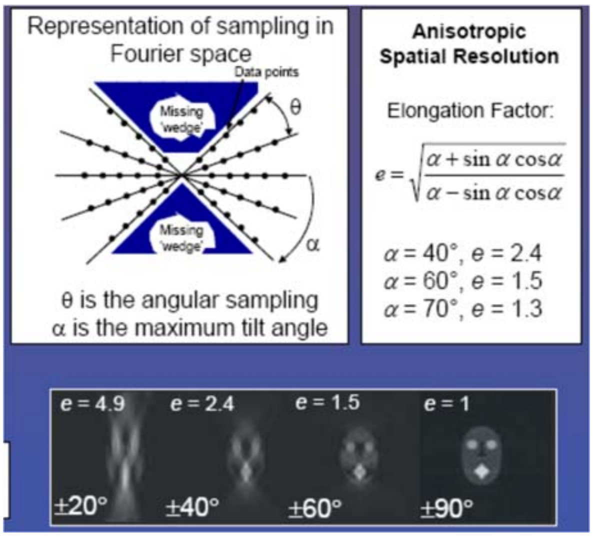
- There is a limit in the tilt angle we can reach (~±70°) due to:
  - Design of the holder
  - Grid bars
  - Increasing thickness of the specimen with high tilt angles

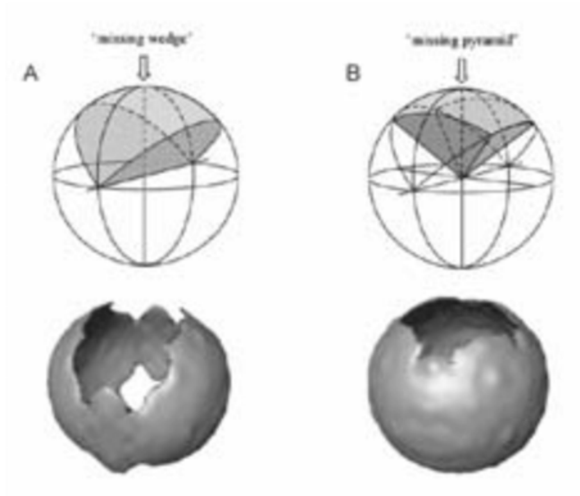


- This is known as the missing wedge problem (missing information, loss of resolution)

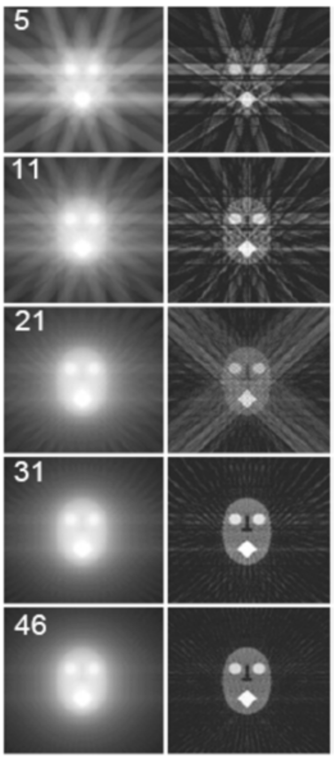


Missing wedge



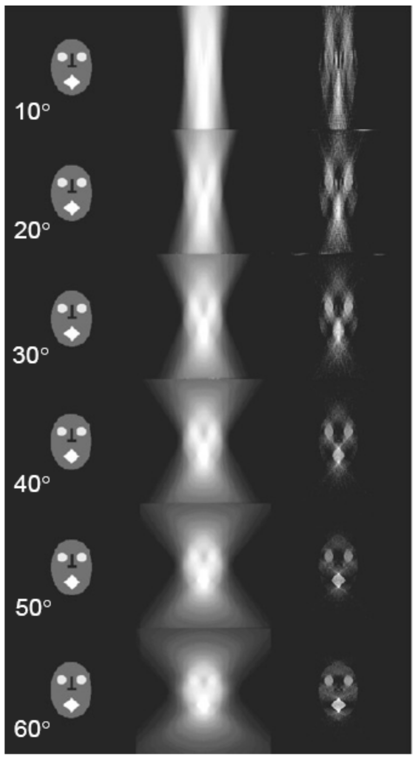


Weighted back projection WBP



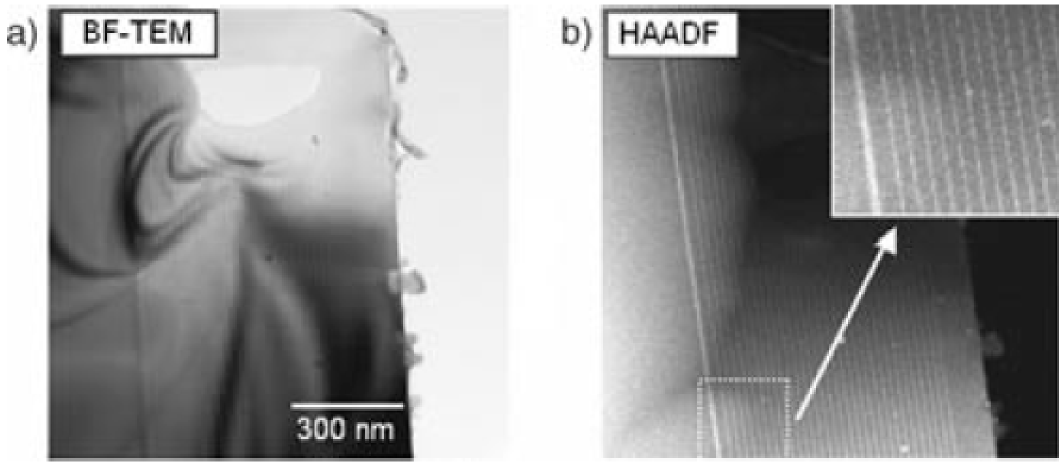
Limited number of projections

Limited tilt



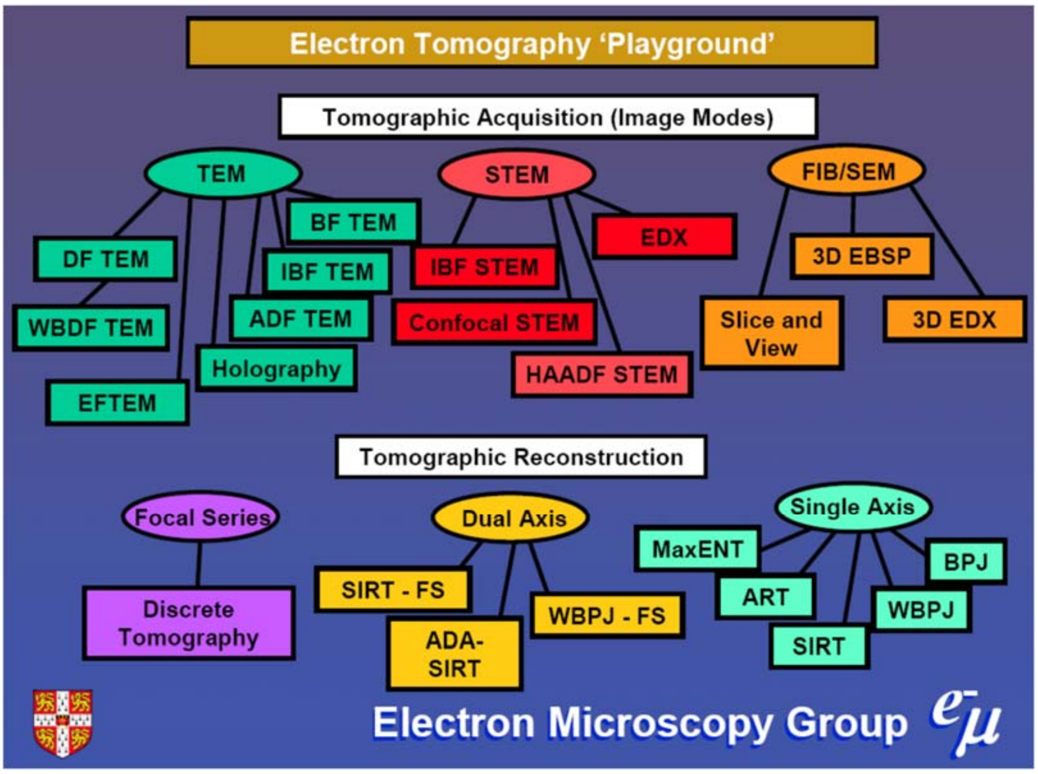
projection requirement

- projection requirement: monotonically varying function of a physical property: mass-thickness dominant in biological samples !



Si-Ge multiple quantum well structure

Tomography in Electron Microscopy

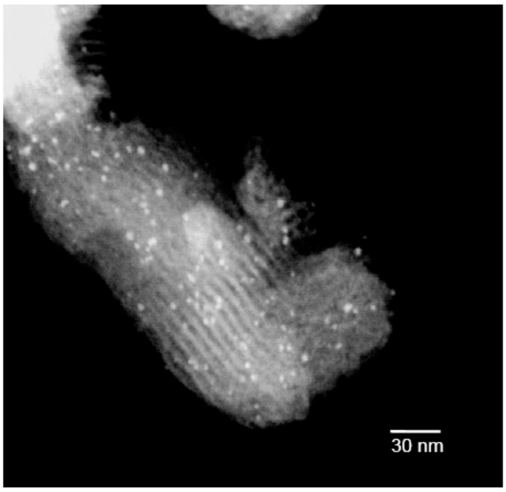


From P. Midgley

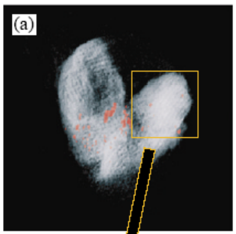
Tomography with HAADF (z-contrast)

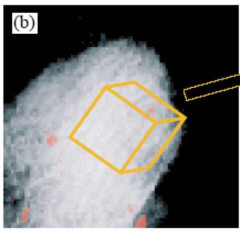
nanoparticle bimetallic catalysts supported on mesoporous silica

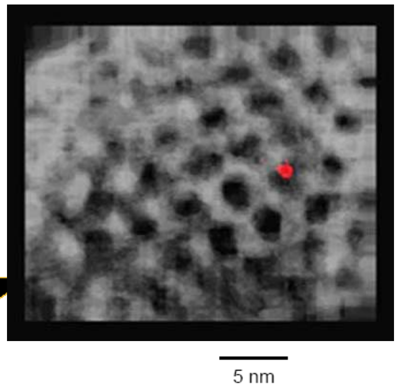
Dogan Ozkaya,Paul Midgley;
Catalysis Letters 60 (1999) 113–120



STEM HAADF: heterogeneous catalyst composed of Pd_6Ru_6 nanoparticles (~ 1 nm) on mesoporous silica support with mesopores of ~ 3 nm diameter.







Pd_6Ru_6 nanoparticles anchored to the wall of mesopore

Electron Tomography in materials science

- Acquisition and alignment of typically 2x71 tilted images
- Back-projection: missing wedge
- Projection requirement (image contrast)
- Thickness limitation (samples rather thin)
- …
- …

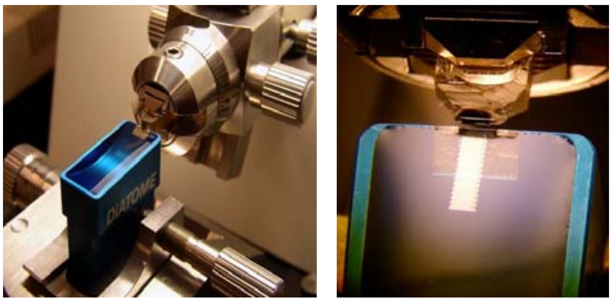
Serial sectioning in Bio-EM

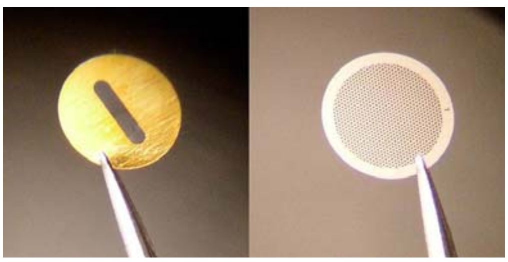
- Direct acquisition of « Tomogram »
- Serial sectioning of biological samples

- No equivalency in Materials Science

3D Imaging, Electron Tomography in Bio-EM

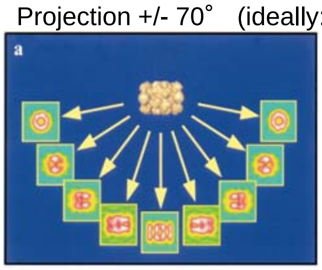
- Serial sectioning

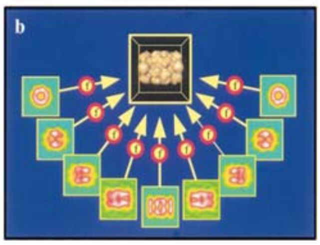




- Tilt series Tomography

Projection +/- 70° (ideally: +/-90°)





Back-projection (reconstruction)

Tomography in Bio-EM
advantages/disadvantages and solutions

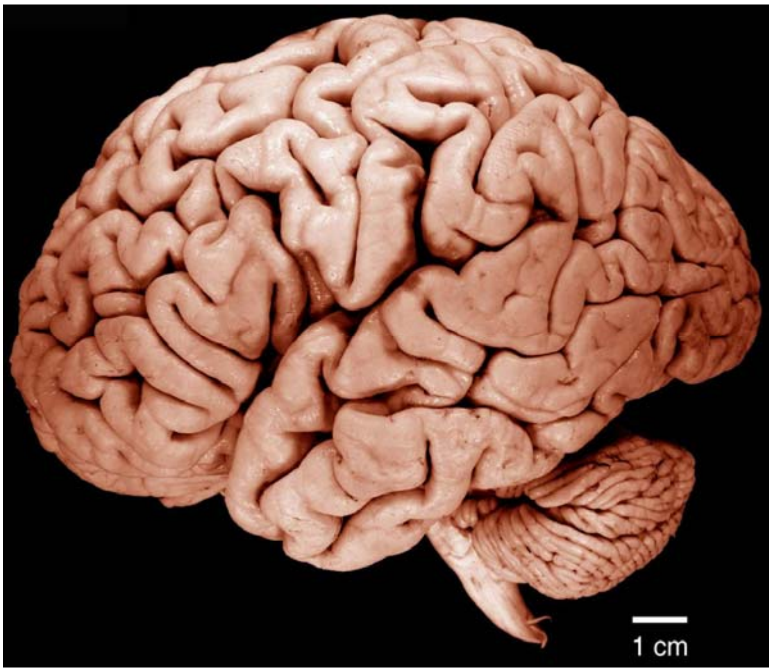
Serial sectioning
- Time consuming
- Artifacts
- Limited resolution in z direction (typ. 50nm)

Tilt series Tomography
- Limited in volume (thickness)
- Missing wedge
- Stability of stage and sample

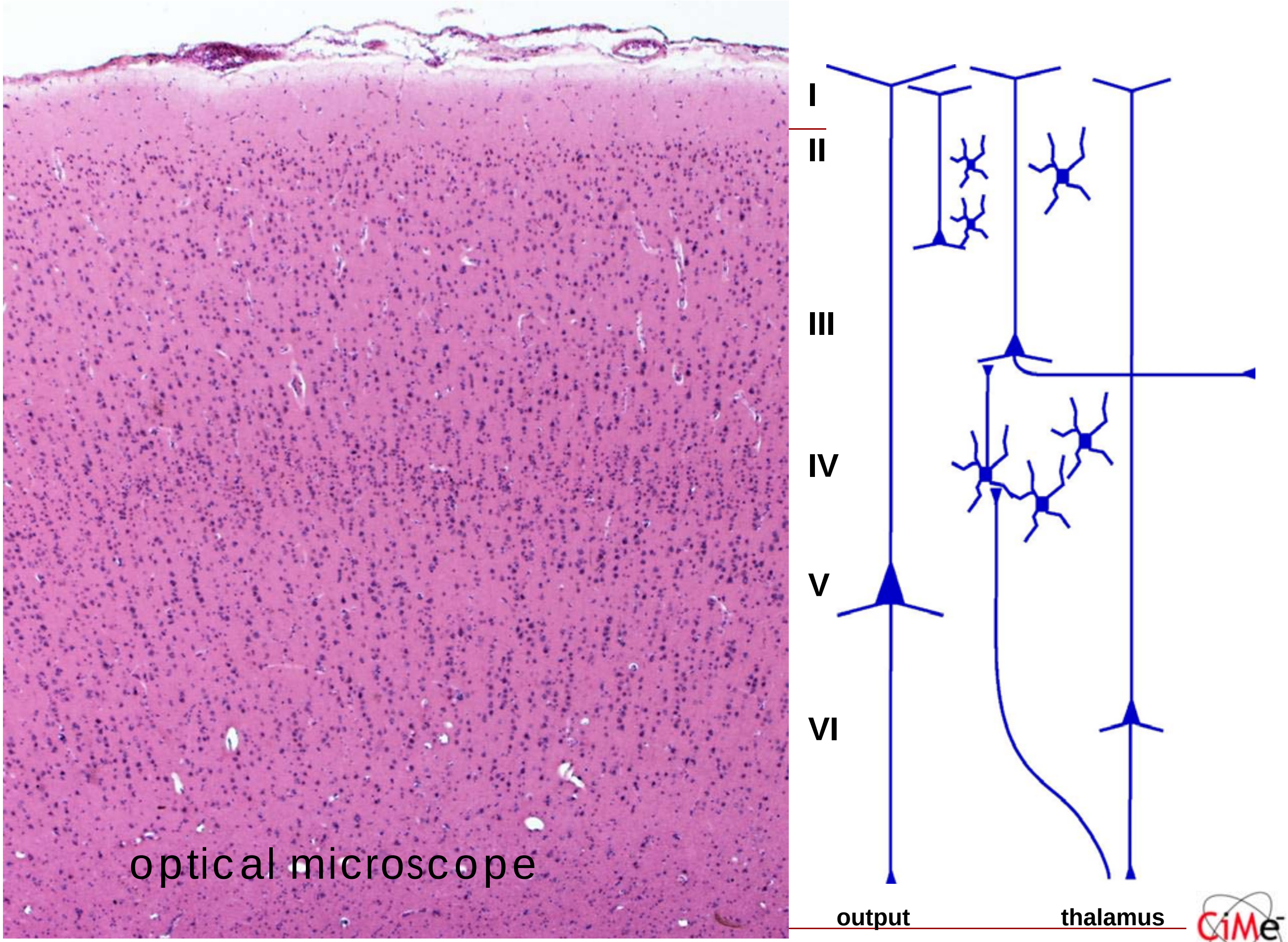
Sample: low contrast +/- (lots of C)

Sample preparation: (Cryo-)fixation, staining, dehydration, resin infiltration

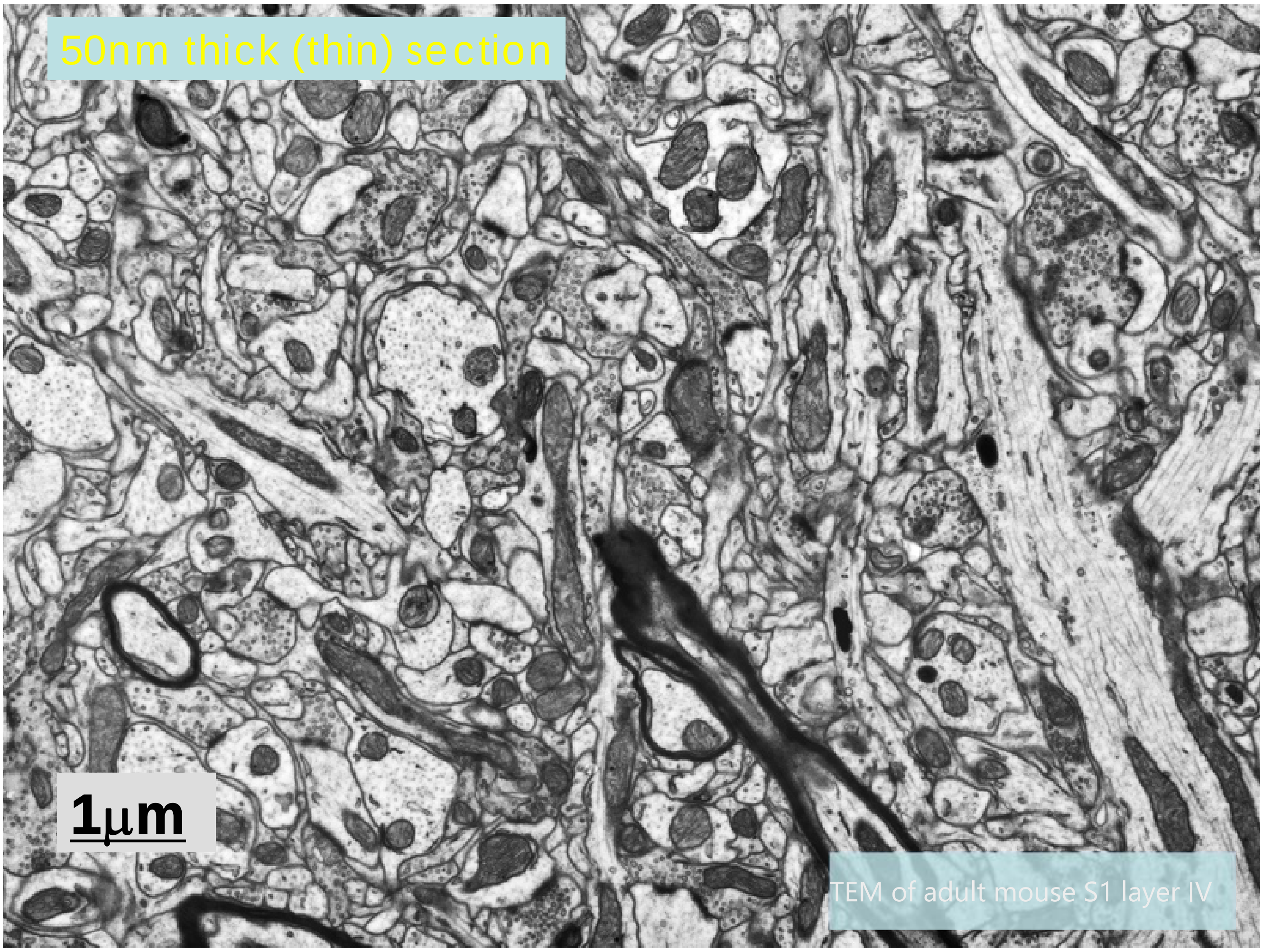
One of the biggest challenge in Life Science



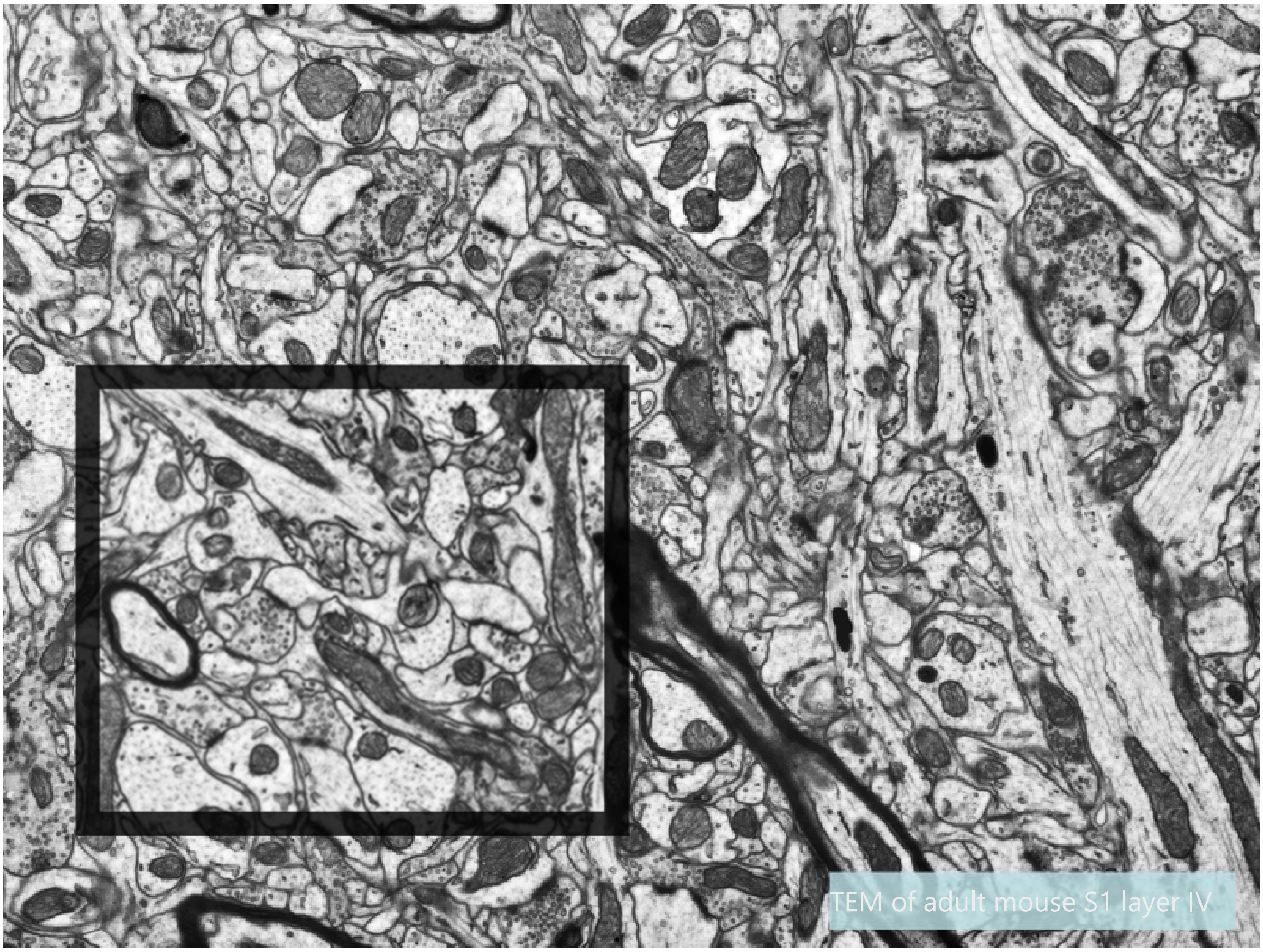
~1’000’000’000 neurons
~10’000’000’000 connections

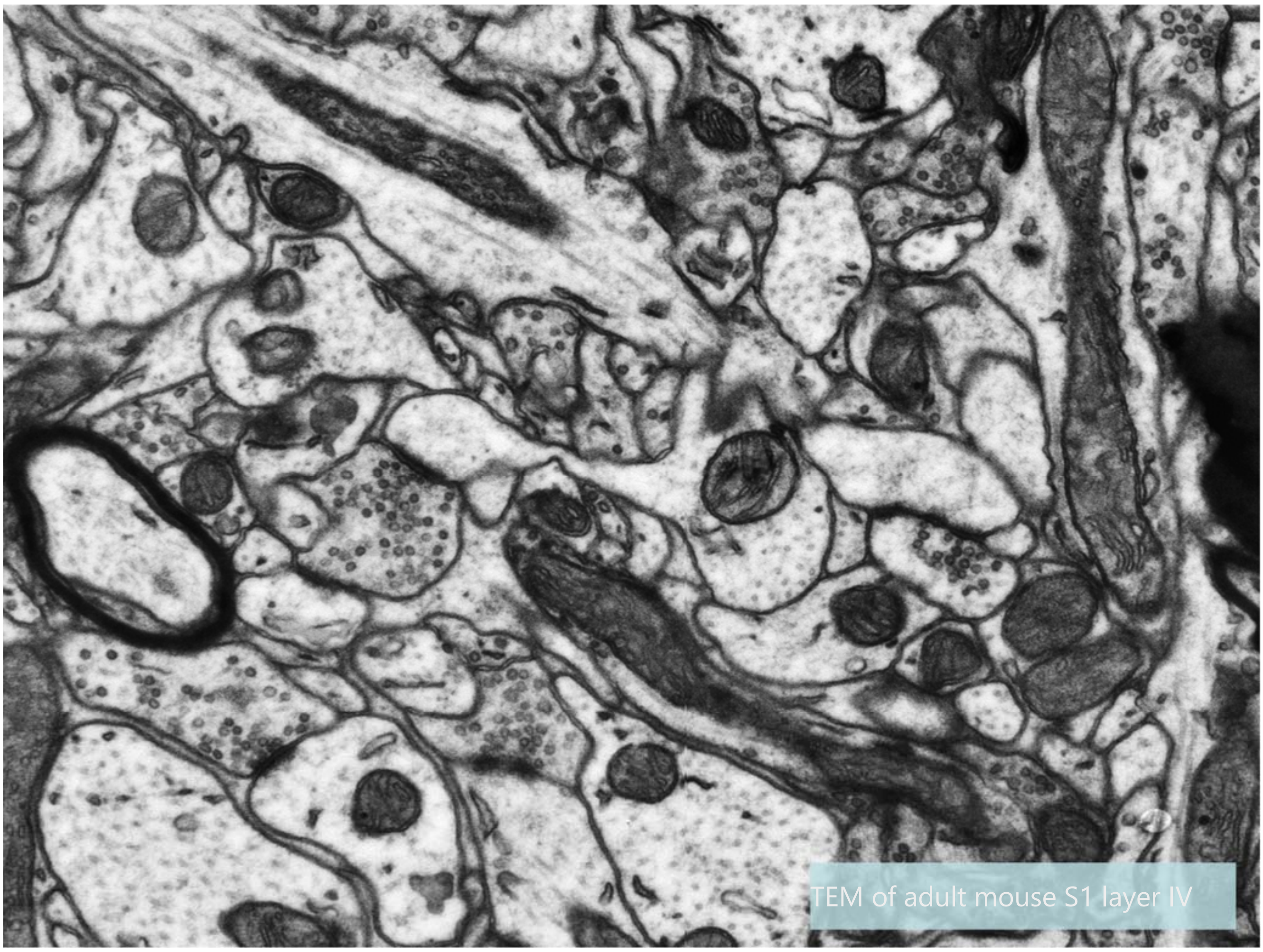


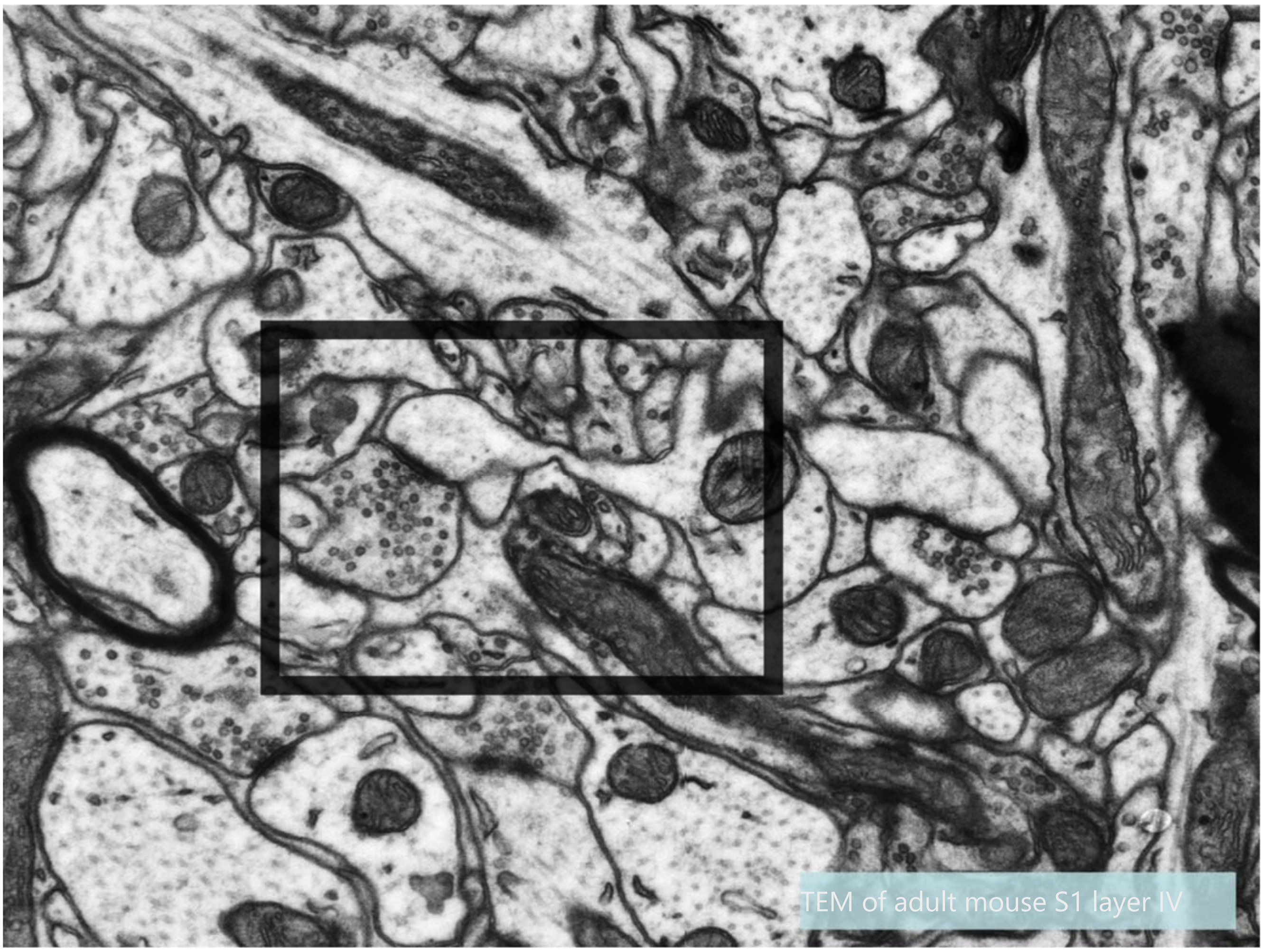
I
II
III
IV
V
VI
optical microscope
output
thalamus

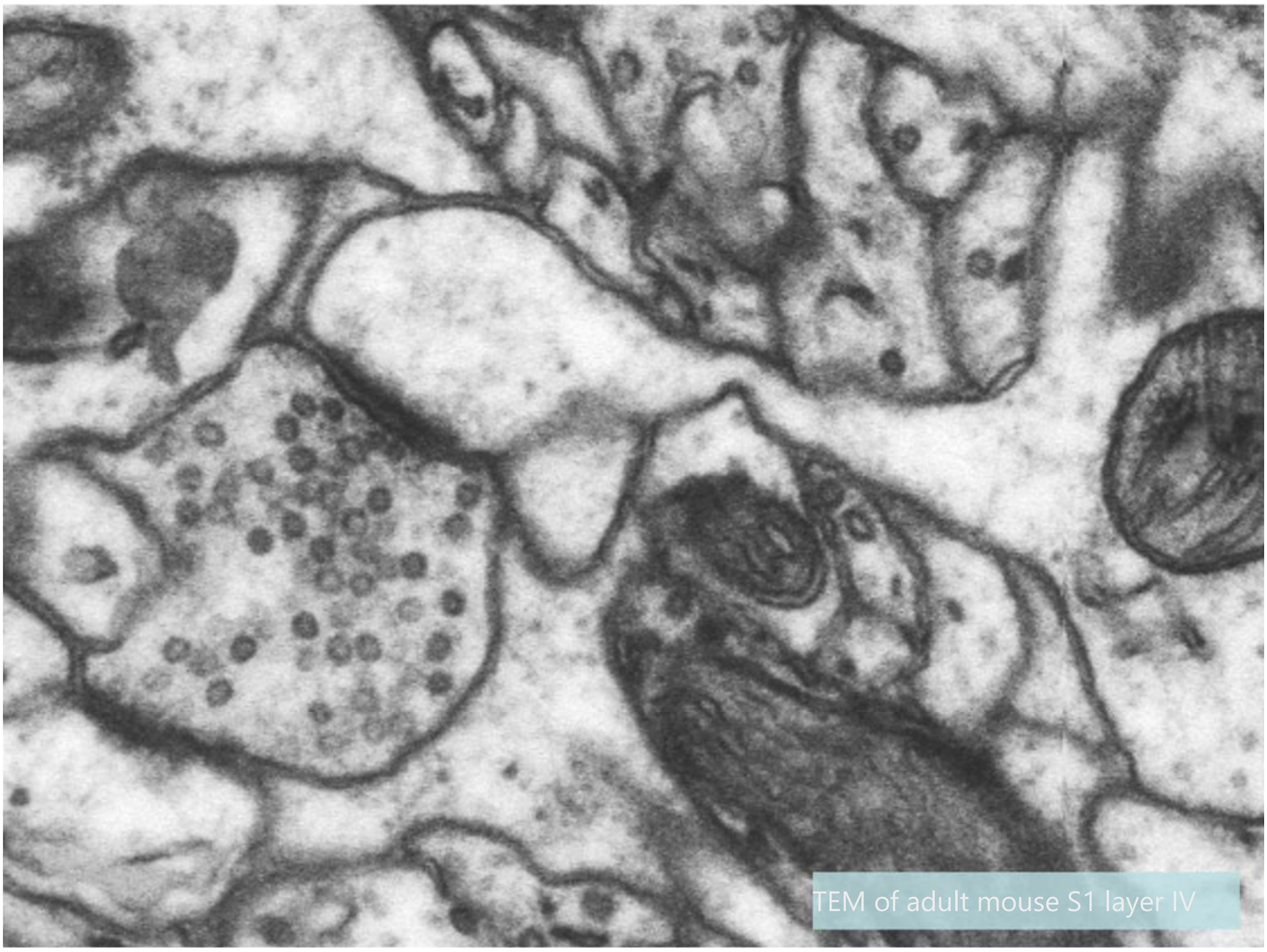


50nm thick (thin) section
1μm
TEM of adult mouse S1 layer IV

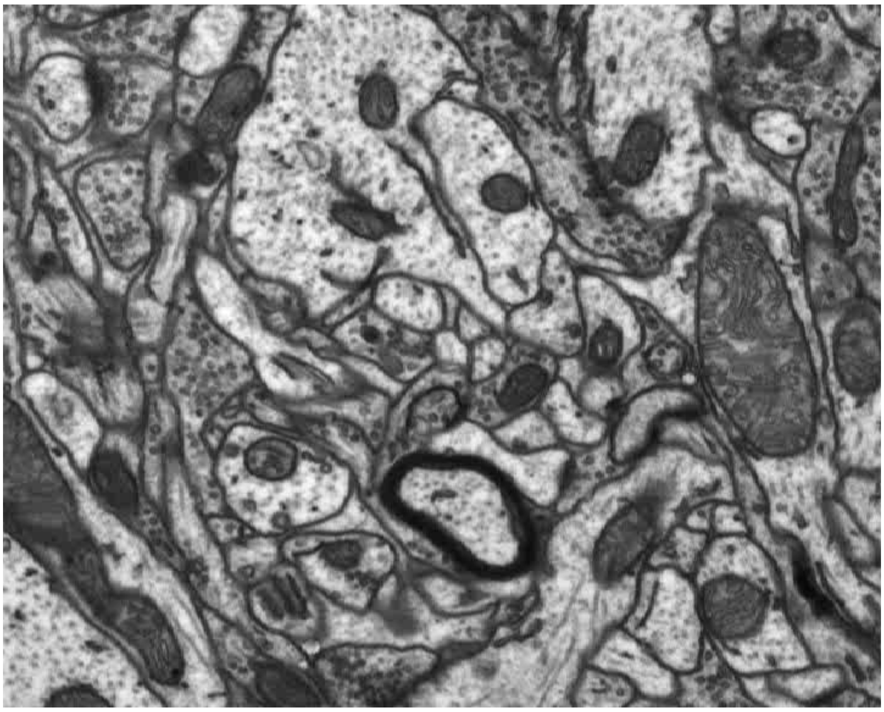

TEM of adult mouse S1 layer IV


TEM of adult mouse S1 layer IV


TEM of adult mouse S1 layer IV


TEM of adult mouse S1 layer IV

Serial sectioning by TEM



Manual, time consuming, Artifacts, lost slices, alignment

The next challenge: segementation and image analysis