Optical **P**rojection **T**omography Microscopy (**OPT**)

Martin Čapek, PhD

Laboratory of advanced microscopy and data analyses

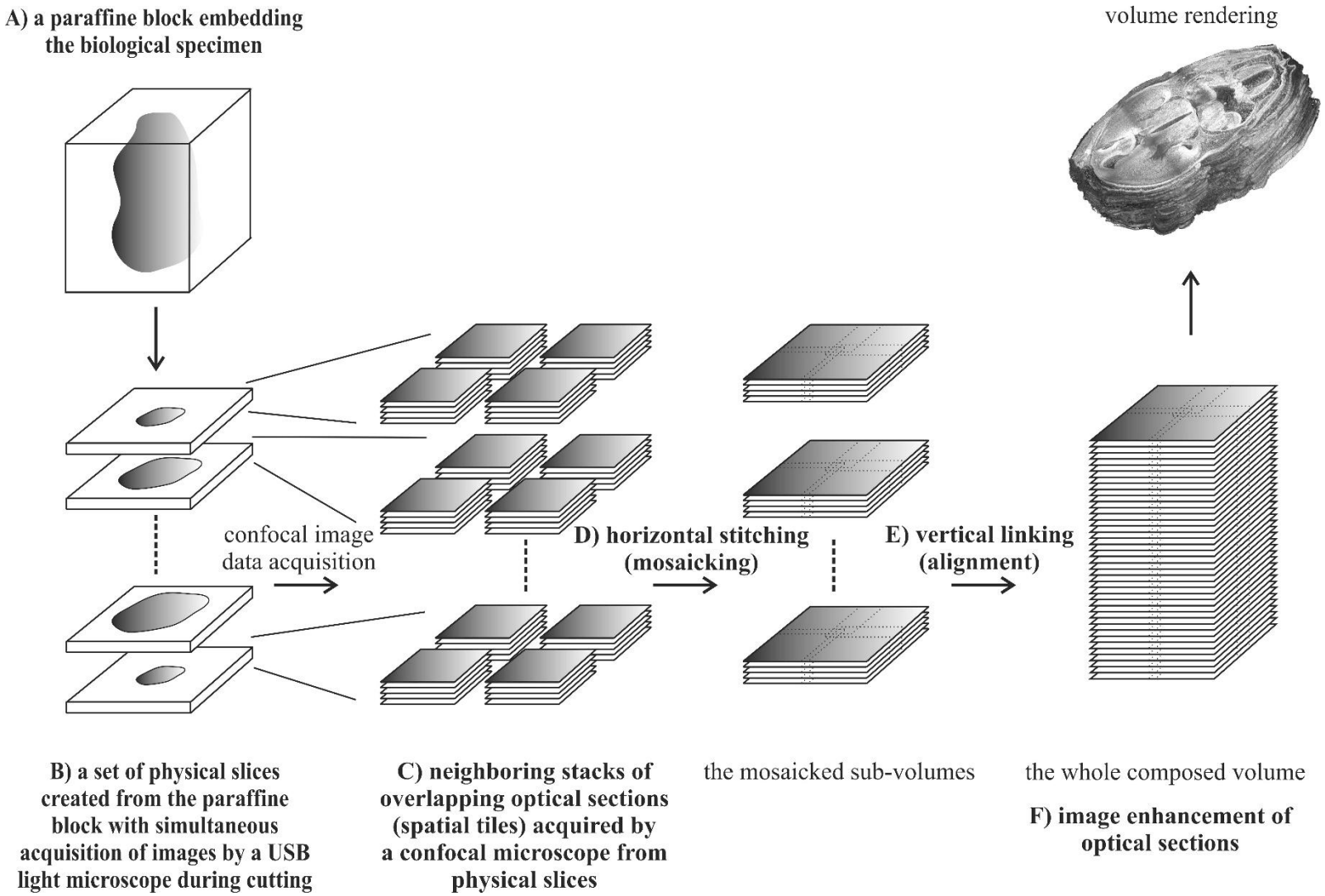
Institute of Physiology CAS

OPT – main motivation

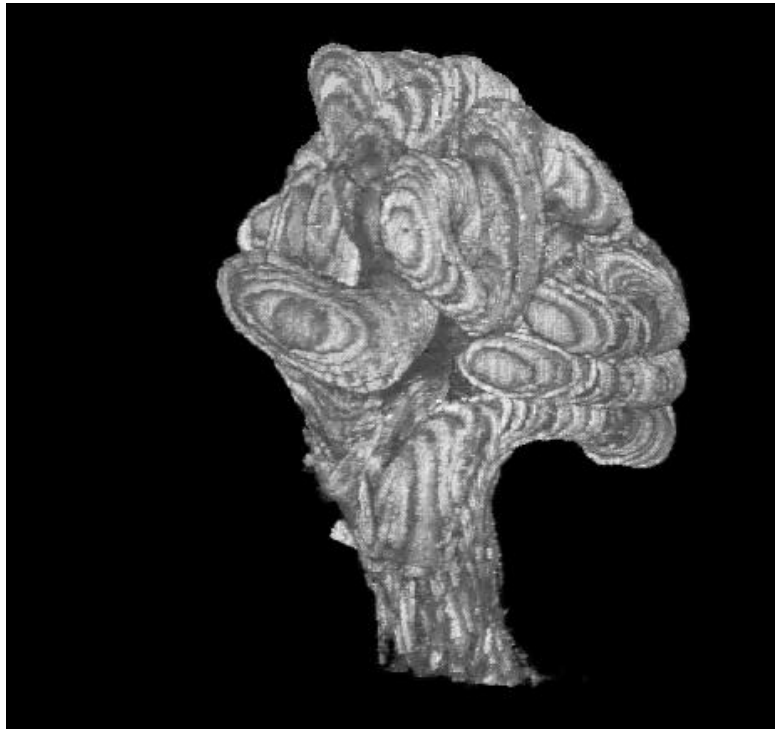
Visualization of relatively big 3D specimens…

…without the necessity of serial (mechanical) histological sectioning

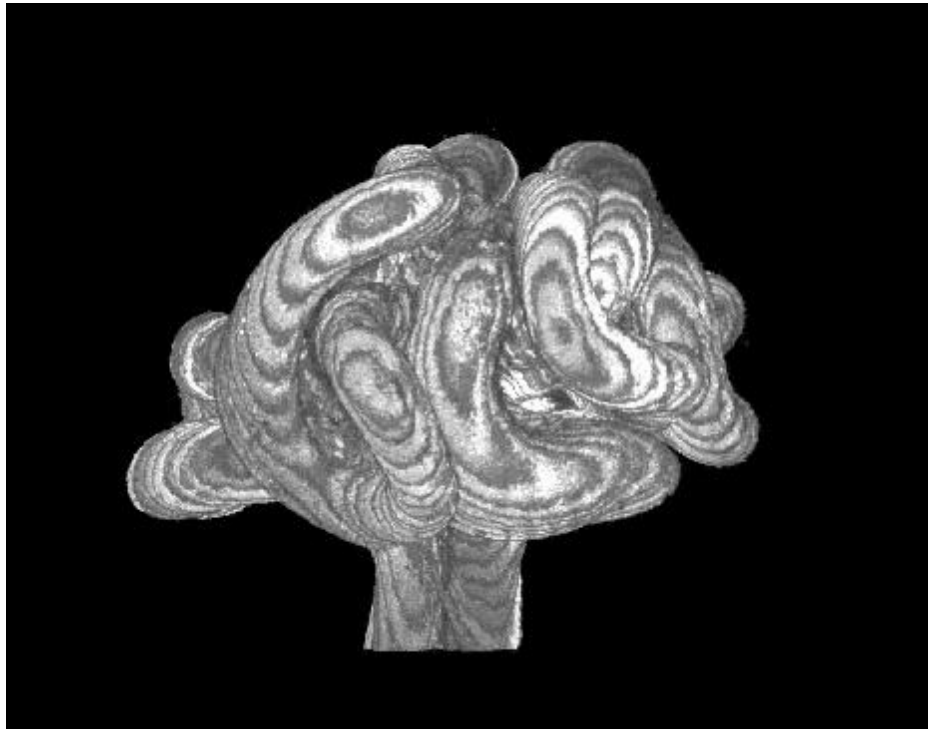
Serial histological sectioning + digital volume reconstruction



Volume reconstructions of intestines of 17-day-old rat embryos from confocal data

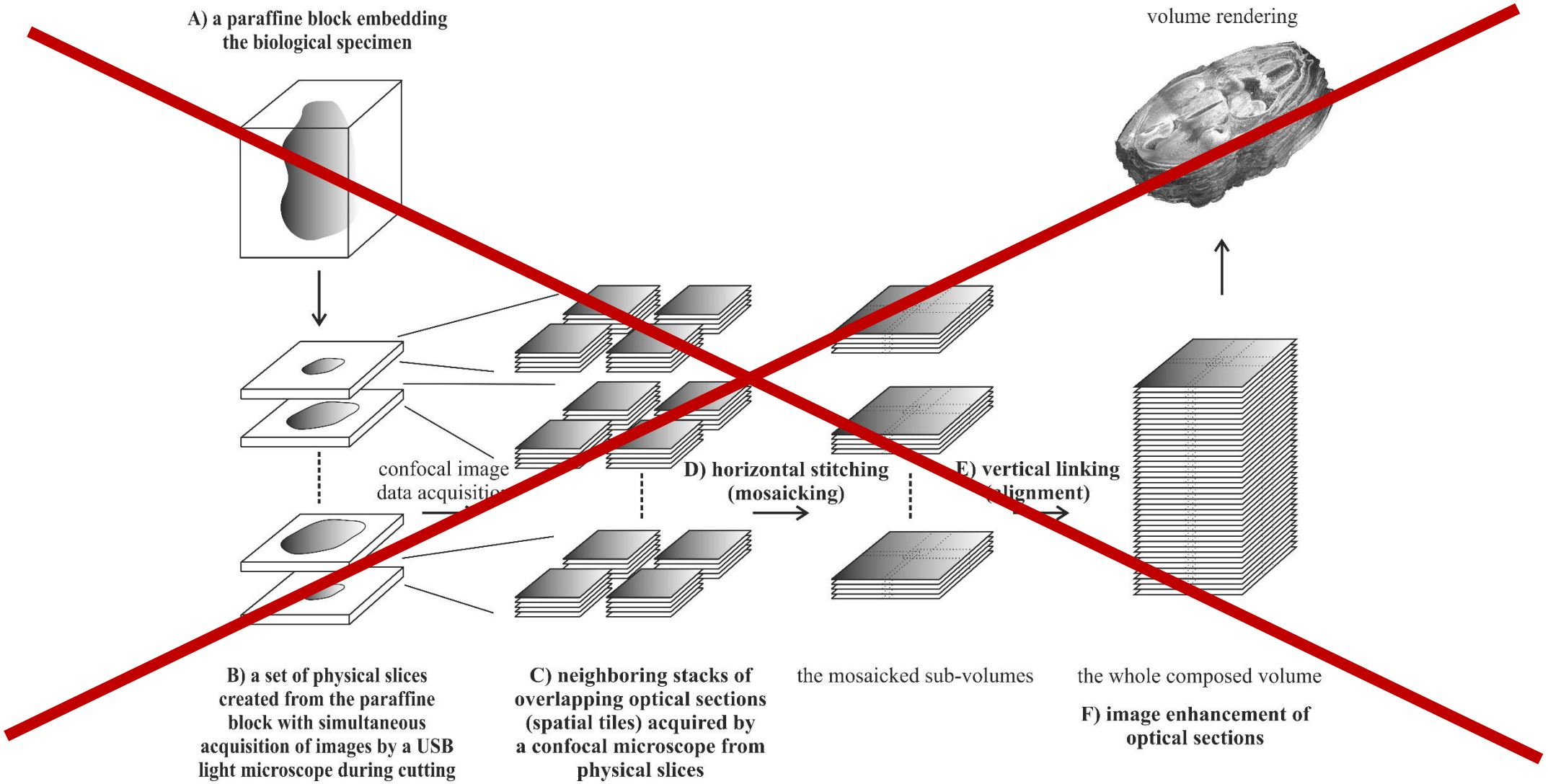


Resolution: 1496 x 1519 x 316 voxels
79 physical slices,
from 3 to 9 fields of view per each phys. slice



1433 x 1433 x 252 voxels
63 physical slices,
from 3 to 9 fields of view per each phys. slice

Serial histological sectioning + digital volume reconstruction



Main principle of computed x-ray tomography

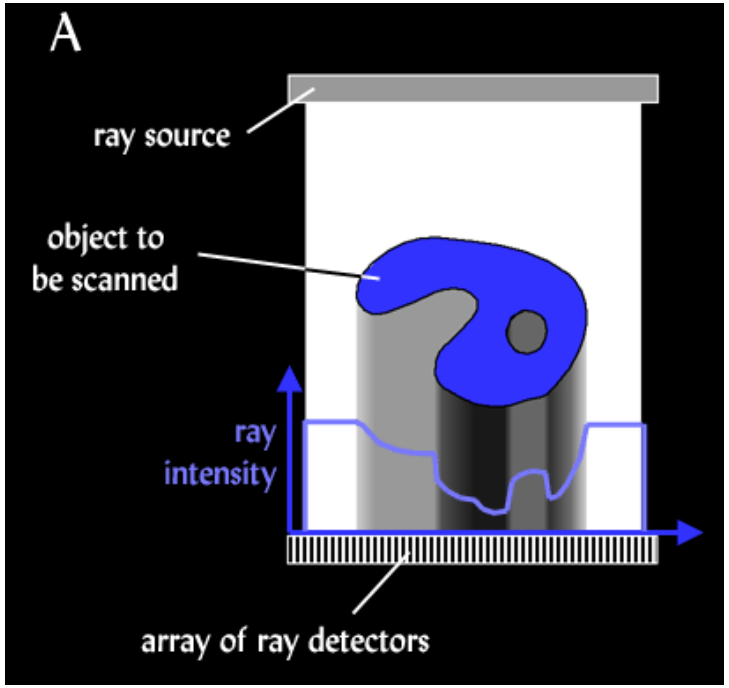
- Developed by **Sir Godfrey Newbold Hounsfield** (the end of 60s).

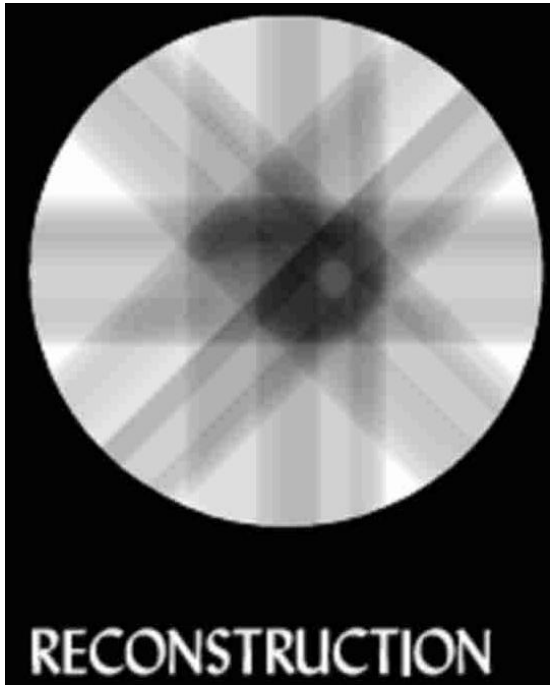
- The **idea** was that the internal structure of the subject could be determined by **being x-rayed at various angles** and subsequently **analyzing the individual absorption values of tissues…**

- = the basic idea of **computed tomography (CT).**

- The first X-ray medical computer tomograph (September 1971, London, Atkinson Morley's Hospital).

Principle of computed tomography

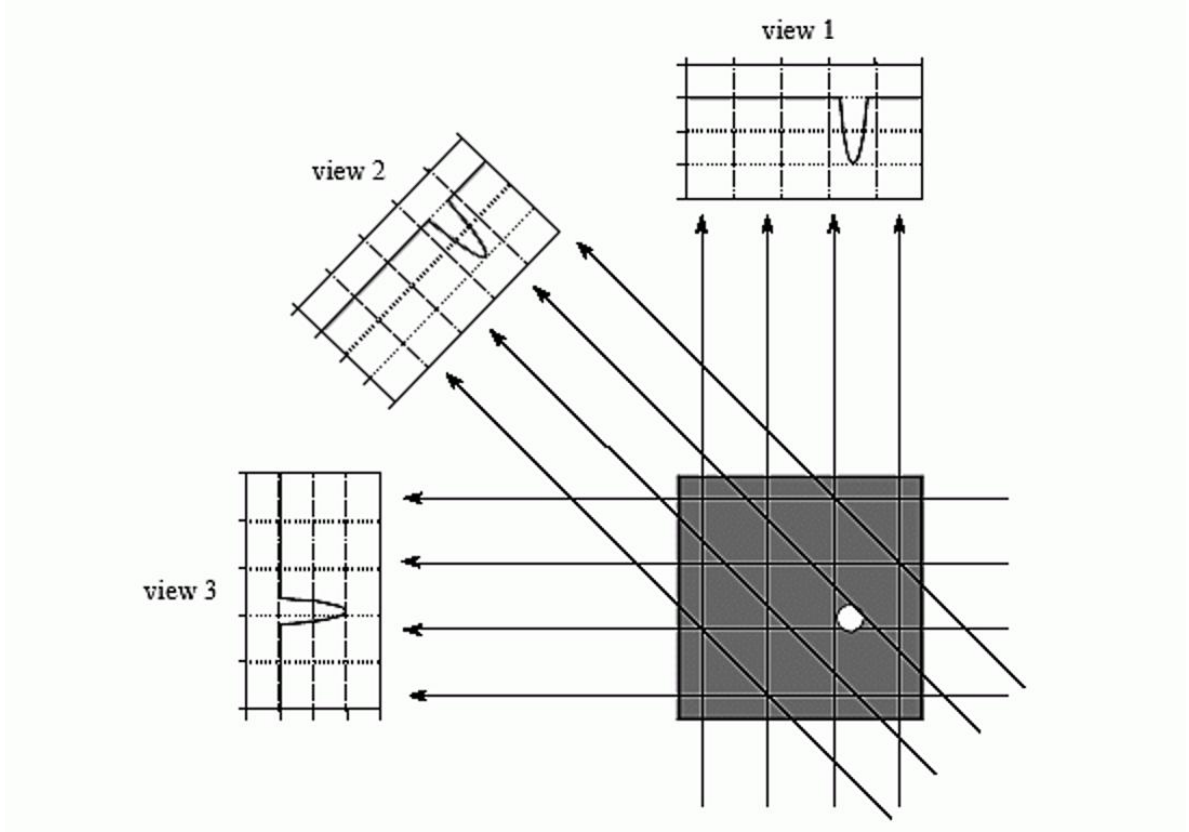




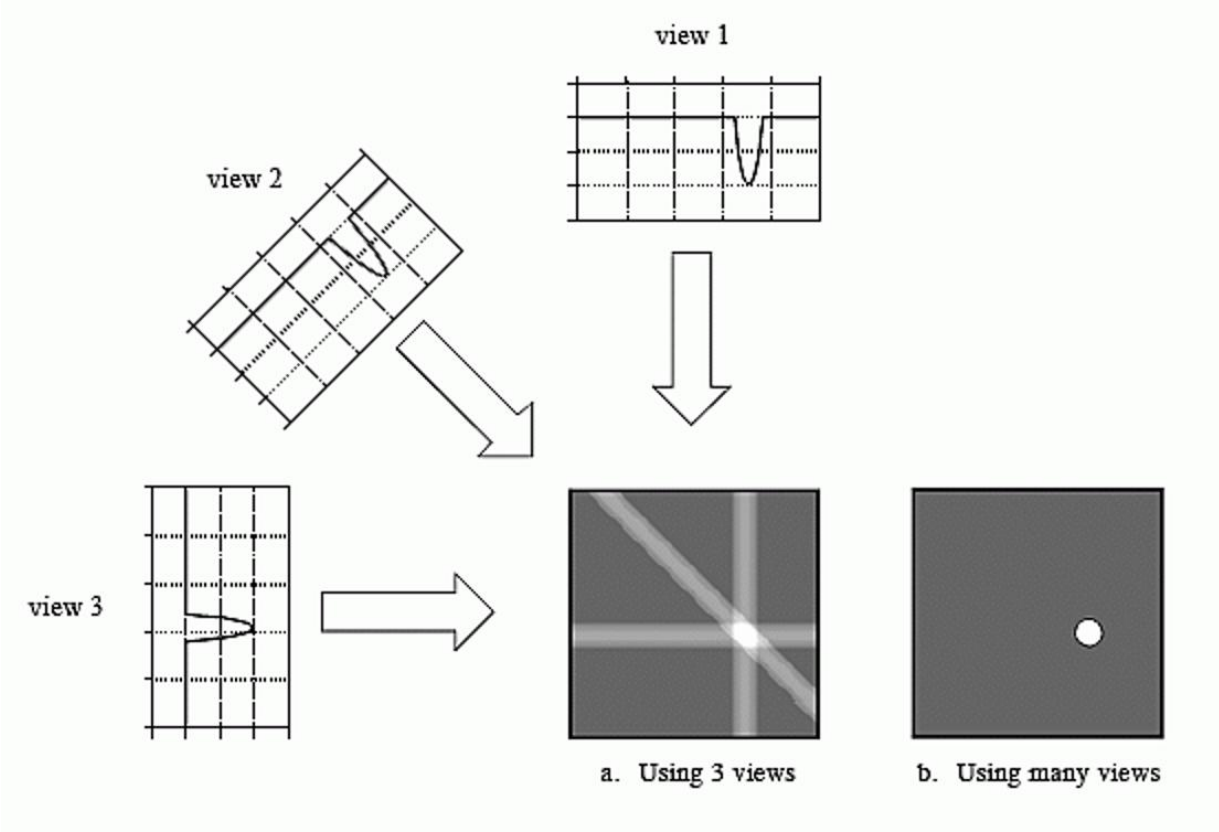
- For reconstruction – **an algorithm of back projections**.
- The result – **3D image showing internal arrangements and structures.**

http://www.emouseatlas.org/emap/about/collaborations/OPT/tomography.html

Principle of computed tomography (2D case)



Acquisition

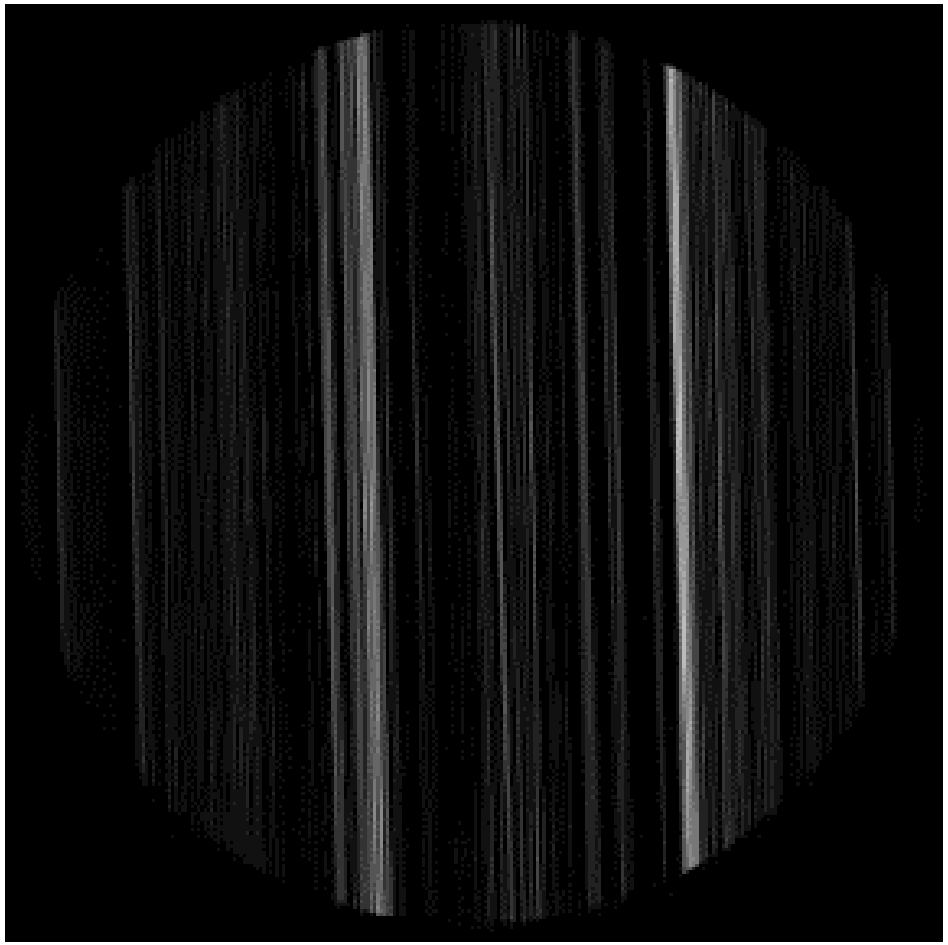


Reconstruction – back projections

https://www.dspguide.com/ch25/5.htm

Algorithm of back projections

Section through an E10.5 mouse embryo



http://www.emouseatlas.org/emap/about/collaborations/OPT/tomography.html

Principle of **optical** projection tomography

- Instead of using **X-rays** for screening the object…

  … we use **infrared or visible light**

  (laser, LED, halogen lamp, UV lamp etc.).

OPT – the first scientific article

- James Sharpe, et al.: *Science 19 April* ***2002***

Optical Projection Tomography as a Tool for 3D Microscopy and Gene Expression Studies

James Sharpe,* Ulf Ahlgren, Paul Perry, Bill Hill, Allyson Ross, Jacob Hecksher-Sørensen, Richard Baldock, Duncan Davidson

Current techniques for three-dimensional (3D) optical microscopy (deconvolution, confocal microscopy, and optical coherence tomography) generate 3D data by "optically sectioning" the specimen. This places severe constraints on the maximum thickness of a specimen that can be imaged. We have developed a microscopy technique that uses optical projection tomography (OPT) to produce high-resolution 3D images of both fluorescent and nonfluorescent biological specimens with a thickness of up to 15 millimeters. OPT microscopy allows the rapid mapping of the tissue distribution of RNA and protein expression in intact embryos or organ systems and can therefore be instrumental in studies of developmental biology or gene function.

The ability to analyze the organization of biological tissue in three dimensions has proven to be invaluable in understanding embryo development, a complex process in which tissues undergo an intricate sequence of movements relative to each other. A related goal is the mapping of gene expression patterns onto these 3D tissue descriptions (*1*). This information provides clues about the biological functions of genes and also indicates which genes may interact with each other. Gathering this data has become one of the clear challenges of the genomics era.

A number of techniques for obtaining 3D information about biological tissue have recently been developed or improved (*2*–*7*). Methods for the digital reconstruction of thin serial sections have become increasingly automated (*2*,

Medical Research Council, Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK.

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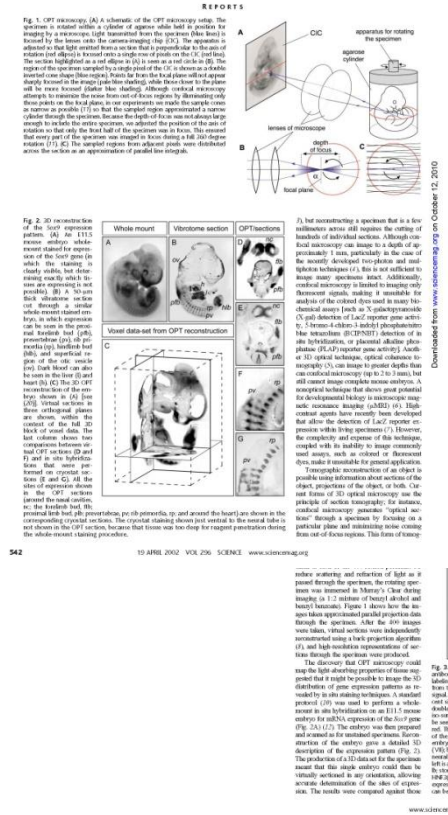
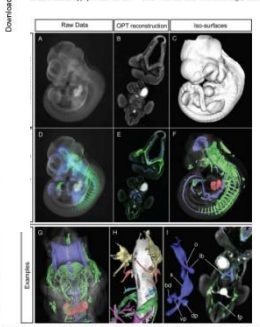
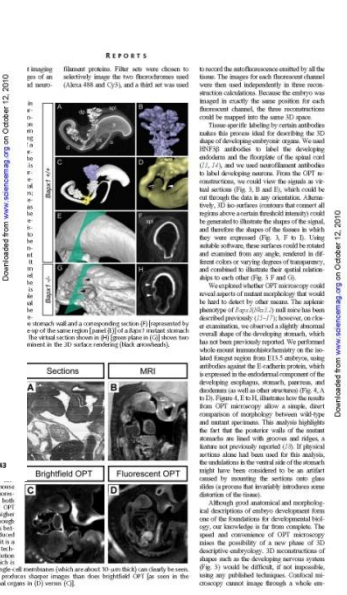




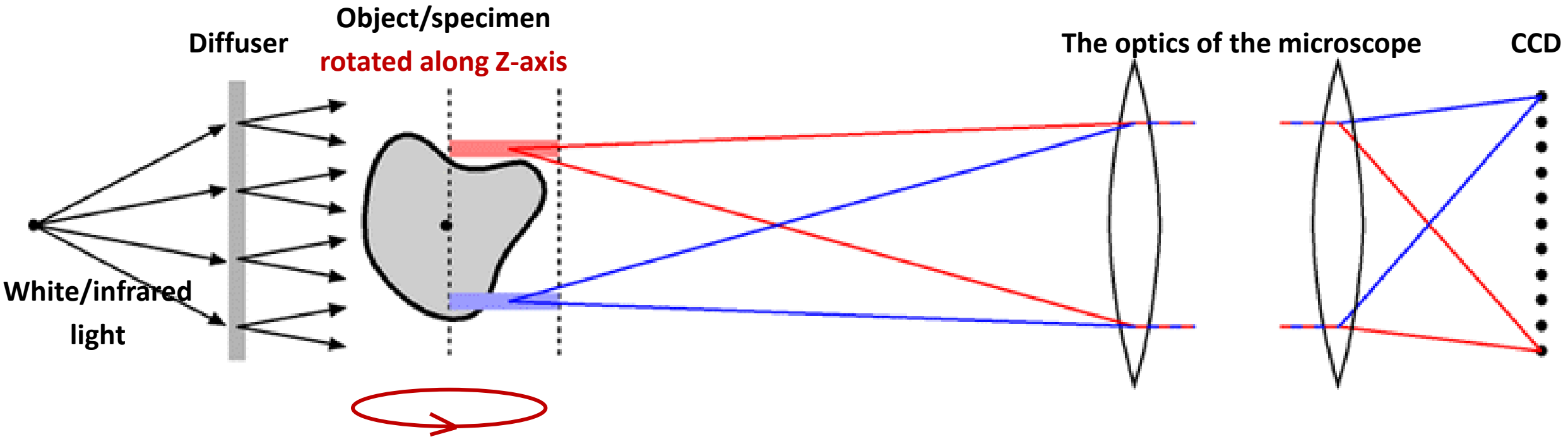


OPT – two modes

1. **Transmission** optical projection tomography

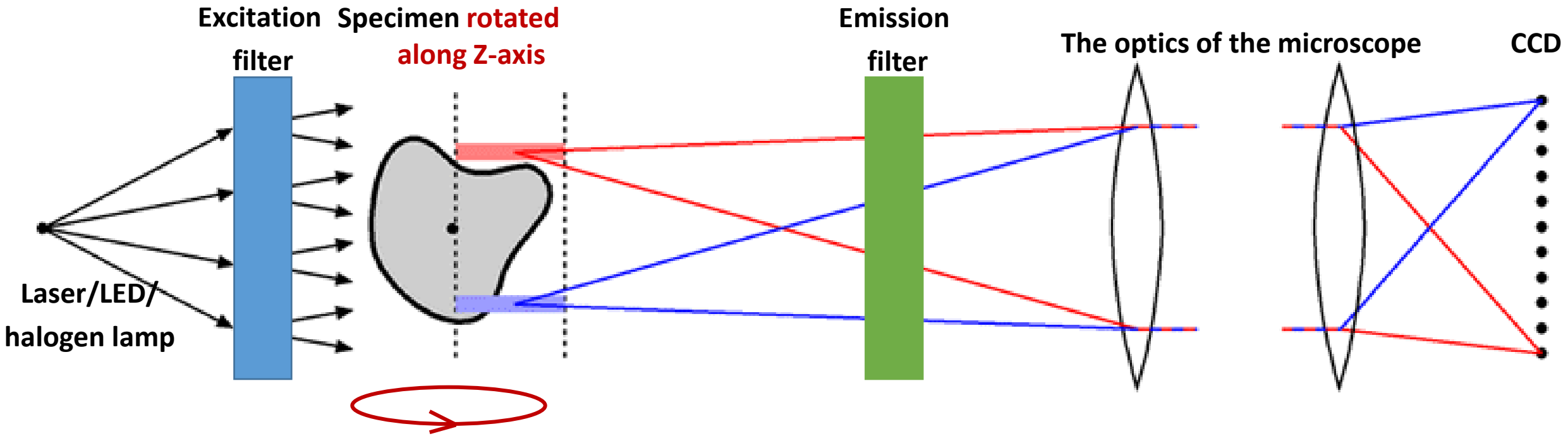
2. **Fluorescence** optical projection tomography

Transmission optical projection tomography



Specimen is immersed in a chamber with water or clearing solution

Fluorescence optical projection tomography



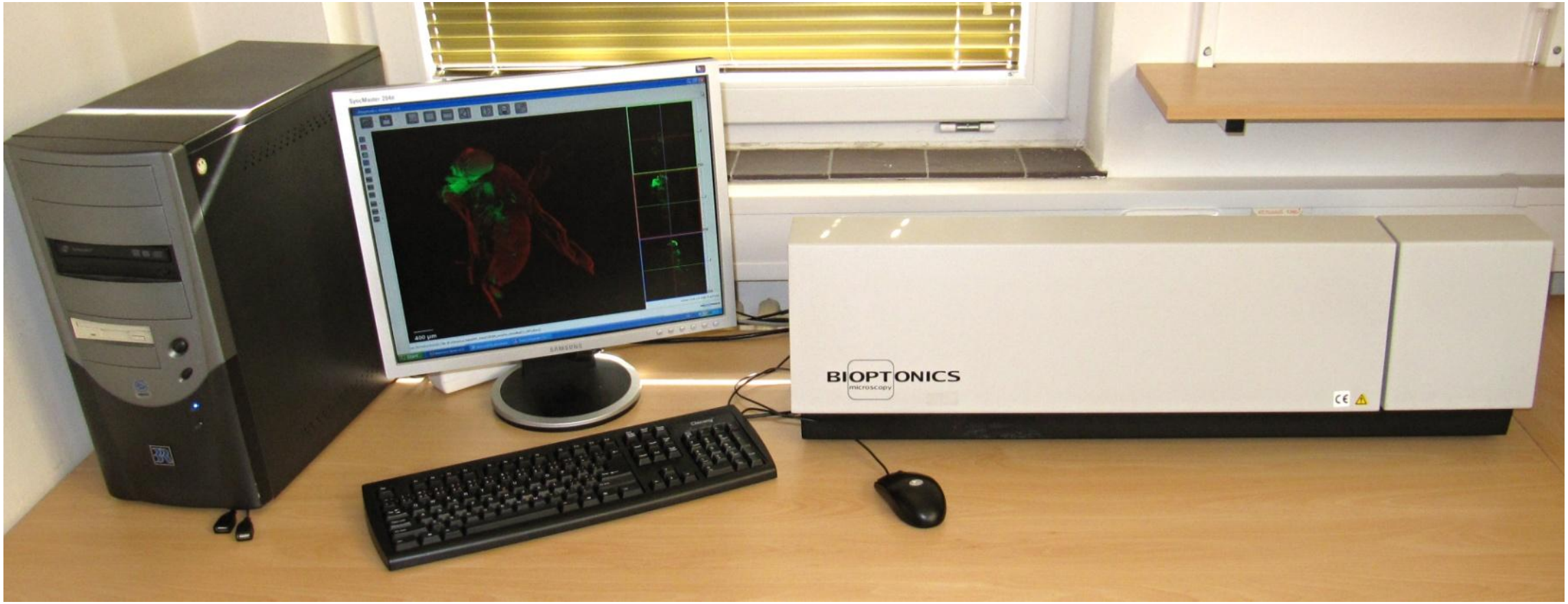
Specimen is immersed in a chamber with water or clearing solution

Two OPT scanners installed in IPHYS CAS

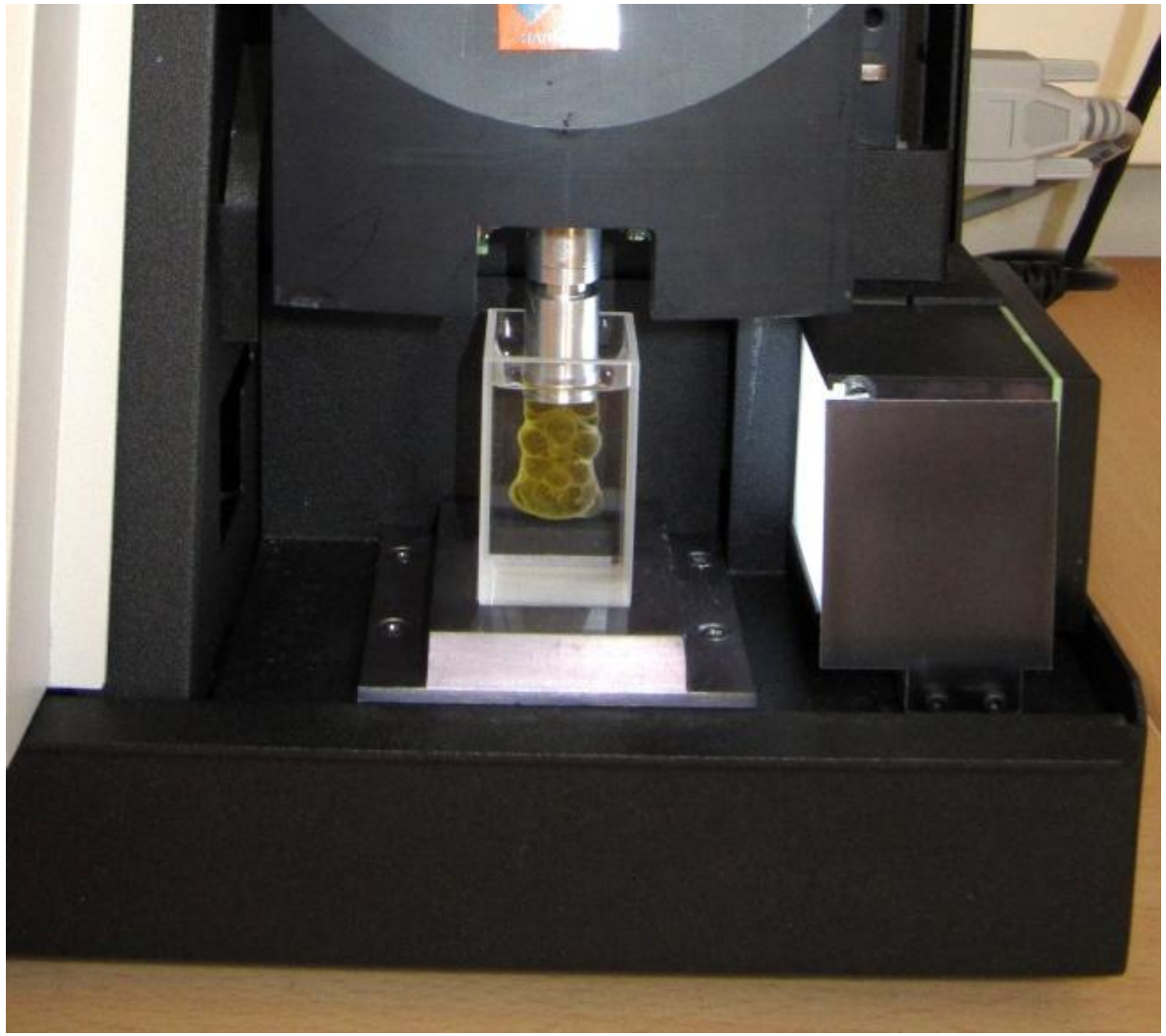
1. **Bioptonics OPT 3001 Scanner** (Medical Research Council Tech, Edinburgh, UK / Skyscan, Belgium)
   - Adapting the field of view to the size of the specimen by **zooming**;
   - Using **high intensity of the excitation light (UV Lamp)**, thus big and thick specimens can be scanned and visualized easily.
   - GFP1, GFP+, Cy3, Cy5

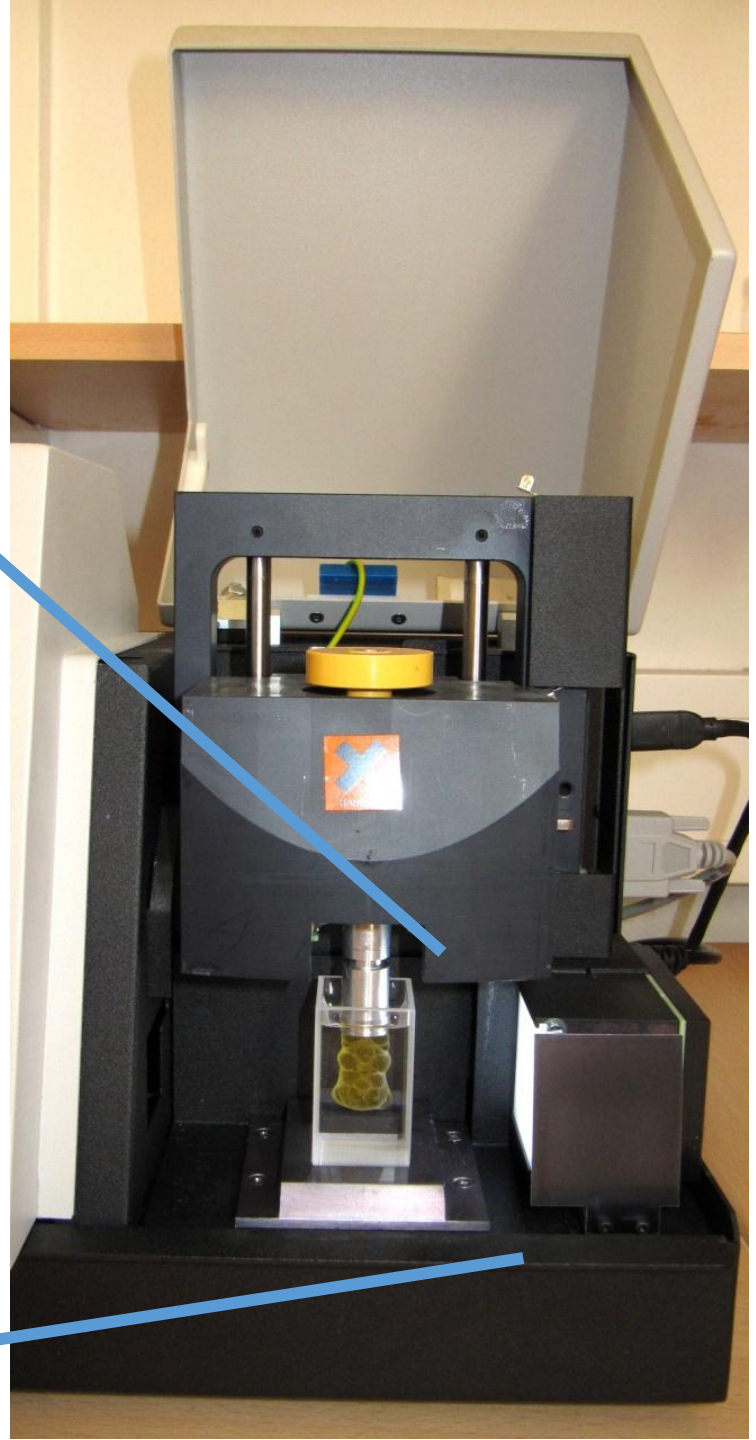
2. **OPT Scanner Milano** (developed in cooperation with Technical University in Milano, Dr. Andrea Bassi)
   - Constructed **from components available on the market**.
   - **High-quality optics**, but without zoom.
   - **Sensitive EM-CCD camera** giving sharp images of projections.
   - GFP1, GFP+, Cy5

Bioptonics OPT 3001 Scanner

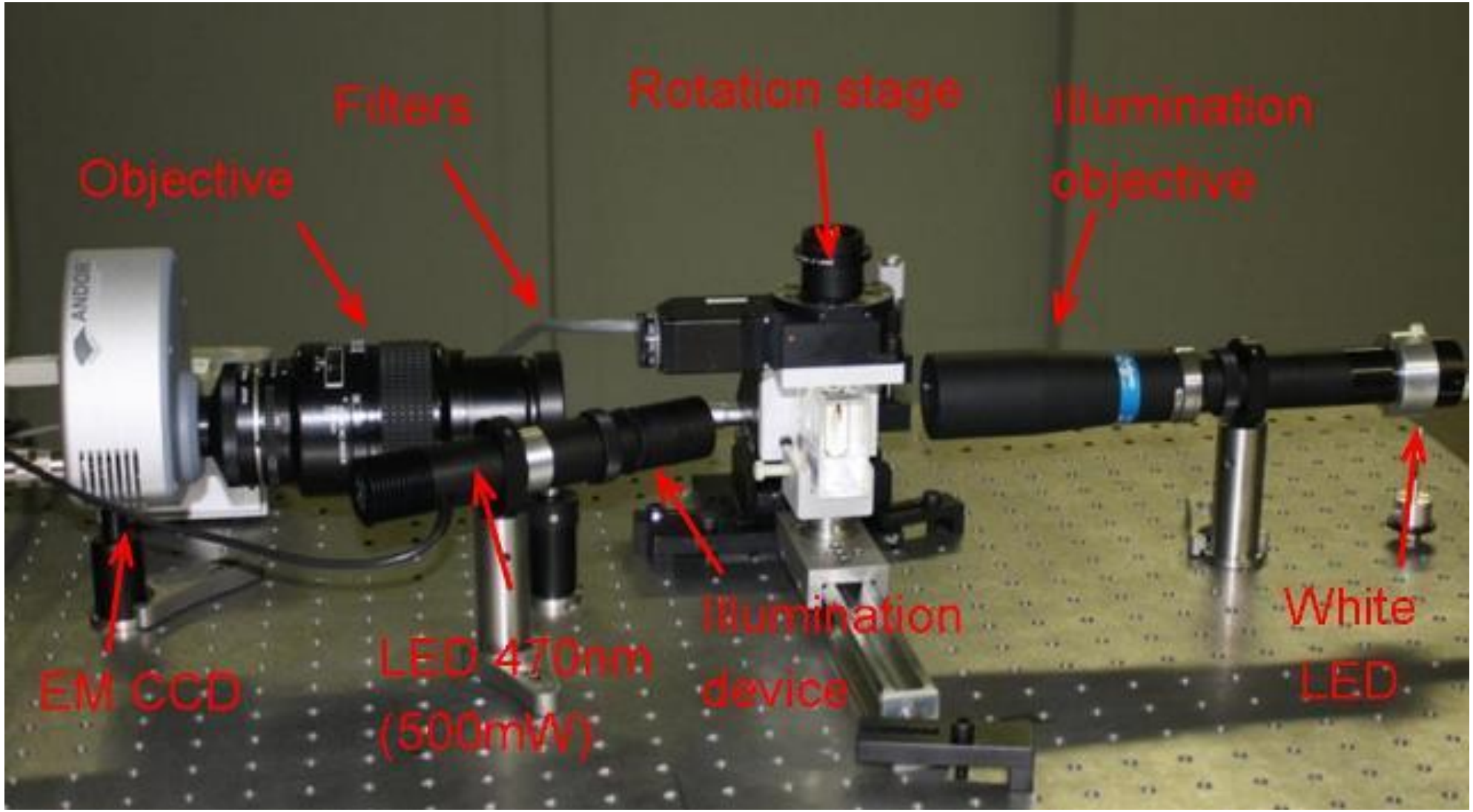


Bioptonics OPT 3001 Scanner





OPT Scanner Milano



Samples

- **Sizes:** the diameter of specimens can be in the range 0.1-10 mm;

- **Conditions:**
  - **Fixed** specimens;
  - **Translucent!** specimens…
  - … or **the clearing!** of specimens is required (BABB, Cubic, …);
  - **Large specimens** can be glued directly to the holder,
  - …but **small specimens** must be embedded in agarose;

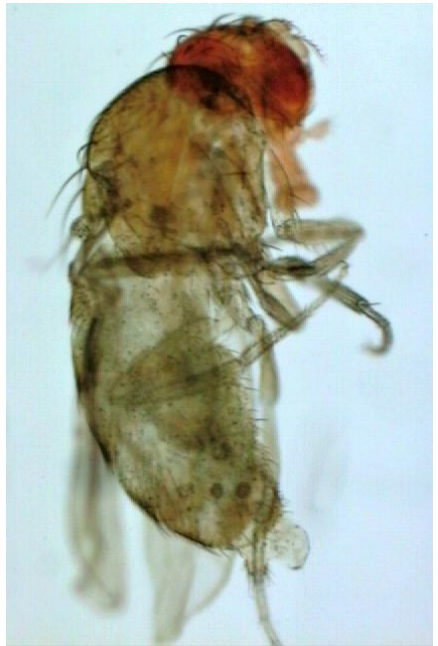
Optical clearing

= making specimens **more translucent** chemically or electrophoretically.

Basic chemical approach:

1. Dehydration in ethanol/methanol.
2. Immersing in the solution of **BABB** (**B**enzyl **A**lcohol + **B**enzyl **B**enzoate, 1:2; RI=1.55)

Water RI=1.33
Tissues RI~1.56



Drosophila Melanogaster,
thanks to Dr. Martin Zápotocký,
IPHYS CAS

Clearing – other possible methods

- DBE – tetrahydrofuran (dehydration) & dibenzylether (clearing)
- Scale – water based, urea + glycerol + Triton
- Clarity – embedding in acrylamide + electrophoresis
- **CUBIC1** – water based, urea + N,N,N',N'-tetrakis(2-hydroxypropyl) ethylenediamine + Triton

… SeeDB, ClearT, ScaleS, µDISCO etc.

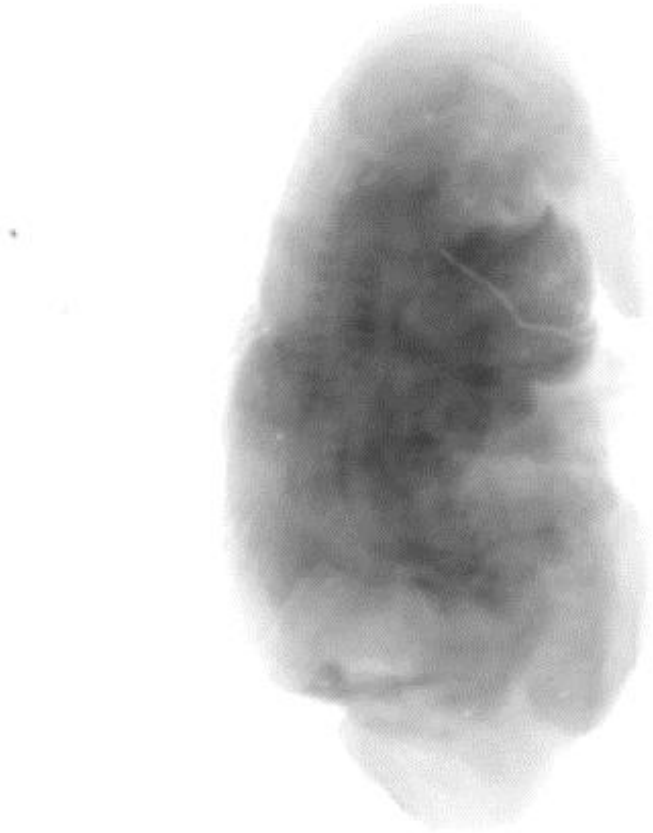
Kolesová, Čapek, Radochová, Janáček, Sedmera: Comparison of different tissue clearing methods and 3D imaging techniques for visualization of **GFP-expressing mouse embryos and embryonic hearts**, **Histochem Cell Biol (2016) 146:141–152.**

OPT – Pros & Cons

- Visualization of **large 3D specimens** without serial histological sectioning
- Low photo-damage

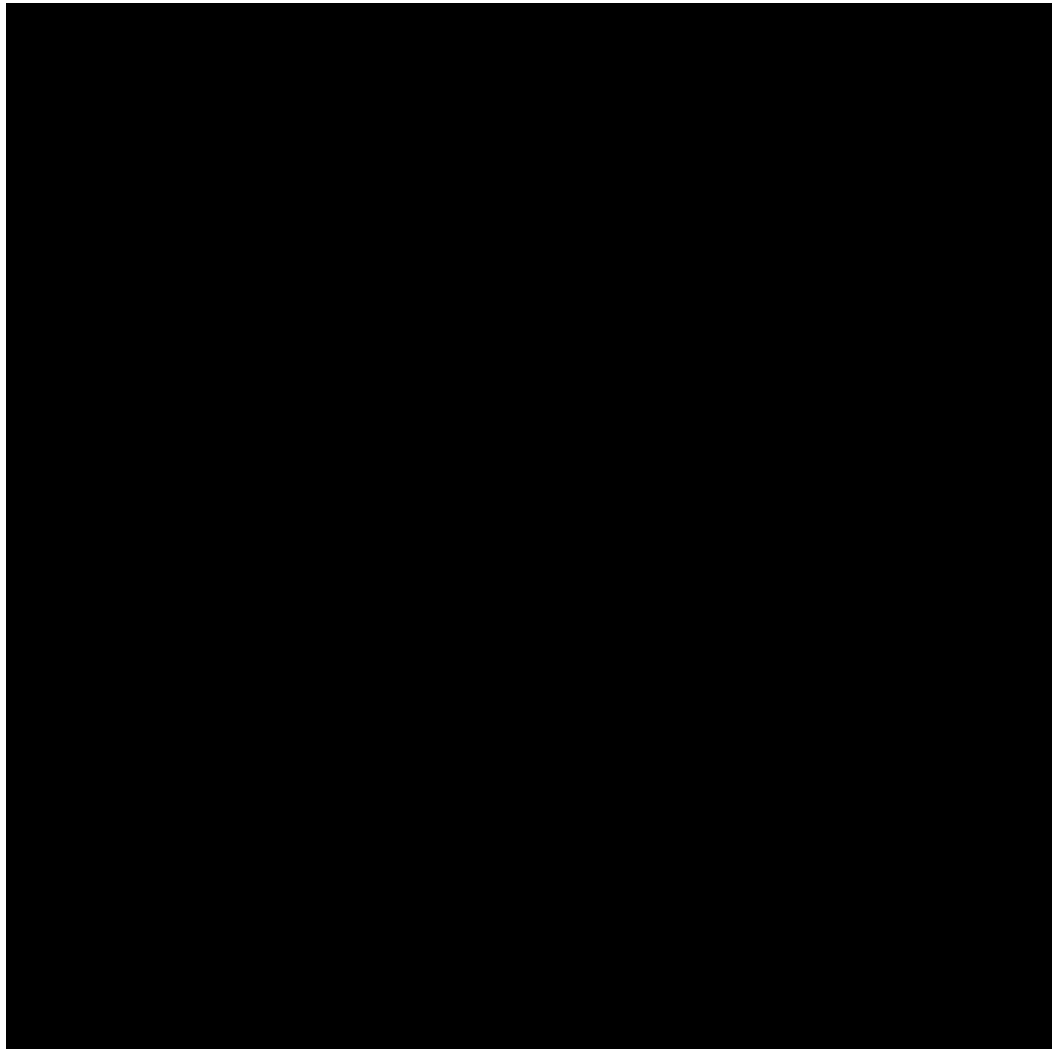
- Visualization of inherently **translucent** specimens …
- … or the **clearing** of specimens is required (**fixed specimens only**).
- Relatively demanding image post-processing and reconstruction.

Acquired data – 401 projections: 360° by 0.9°



Mouse embryo, ED12.5, expression of connexin40:eGFP, **CUBIC1**, embedded in agarose; Prof. David Sedmera, DSc., IPHYS CAS

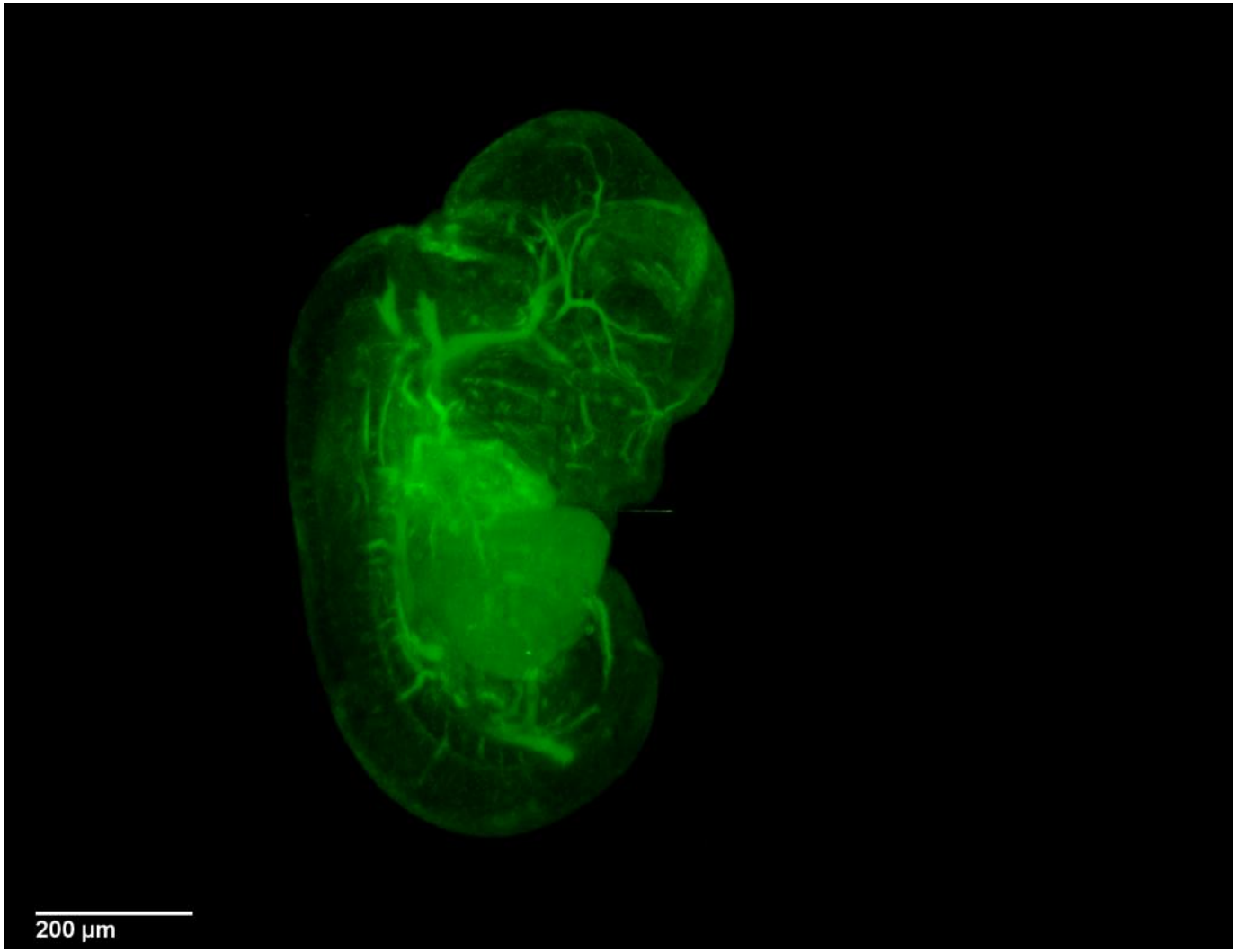
Reconstructed tomography sections



Mouse embryo, ED12.5, expression of connexin40:eGFP, **CUBIC1**, embedded in agarose; Prof. David Sedmera, DSc., IPHYS CAS

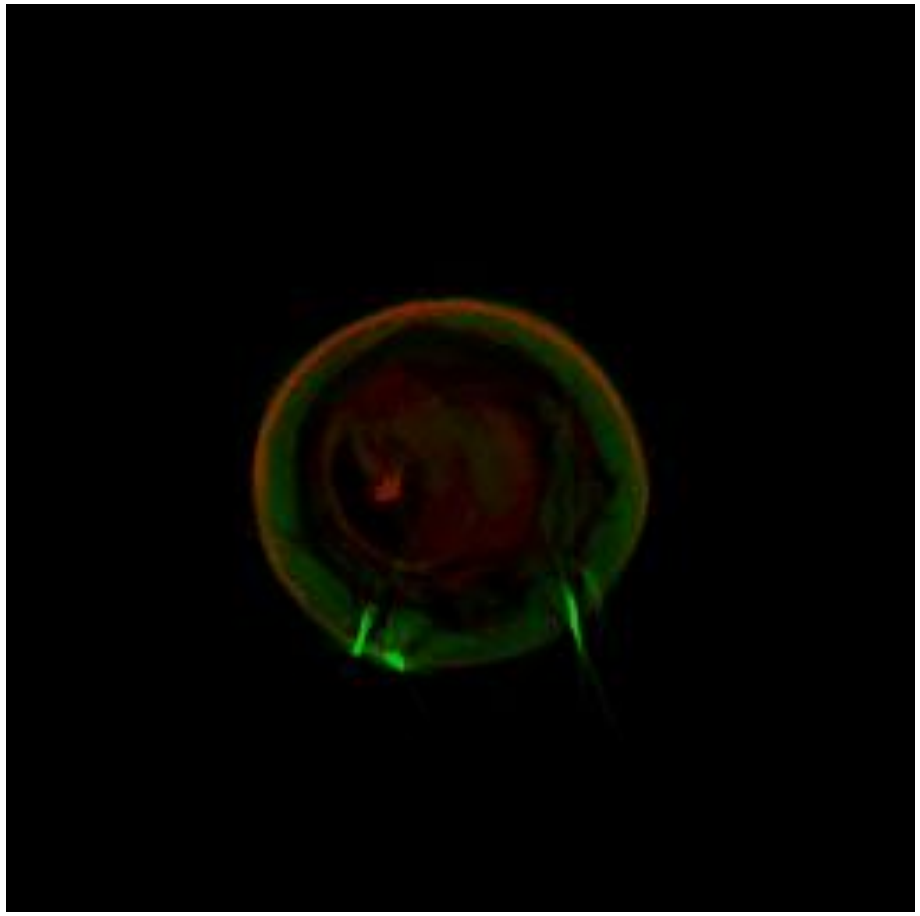
3D visualization

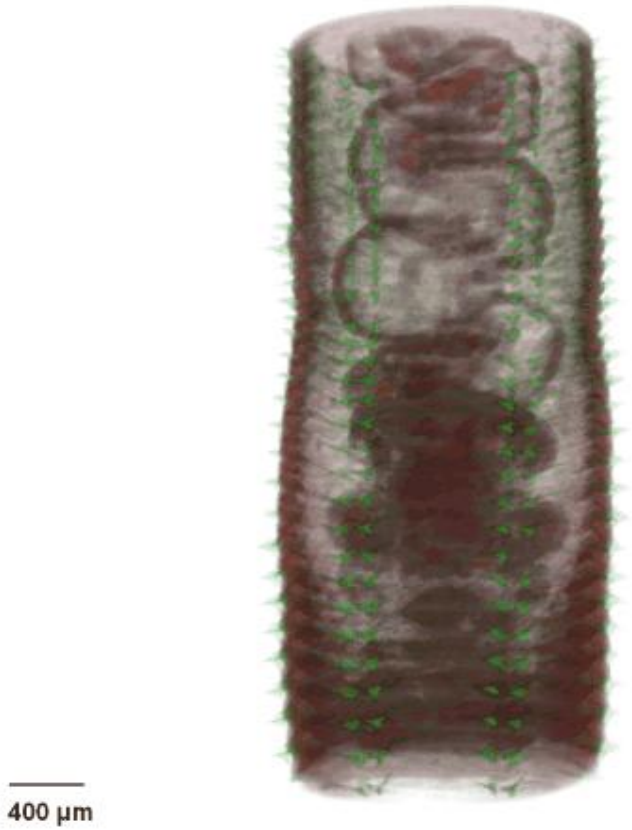
Main blood vessels in the body and in the brain, a heart, a liver…



Mouse embryo, ED12.5, expression of connexin40:eGFP, **CUBIC1**, embedded in agarose; Prof. David Sedmera, DSc., IPHYS CAS

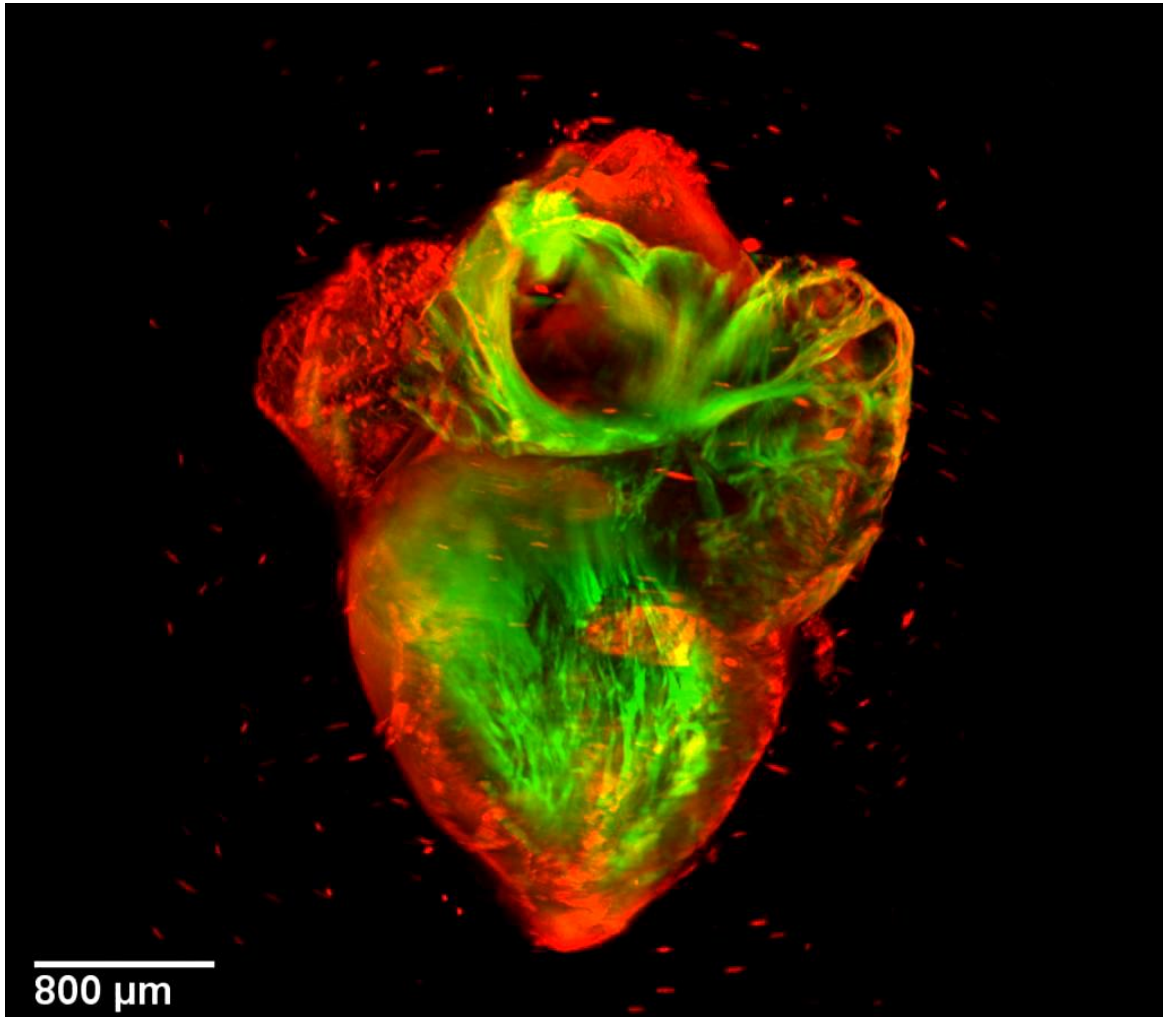
Earthworm





Transmission (red/grey), autofluorescence (green), **BABB**, embedded in agarose; Dr. Škanta, IMIC CAS

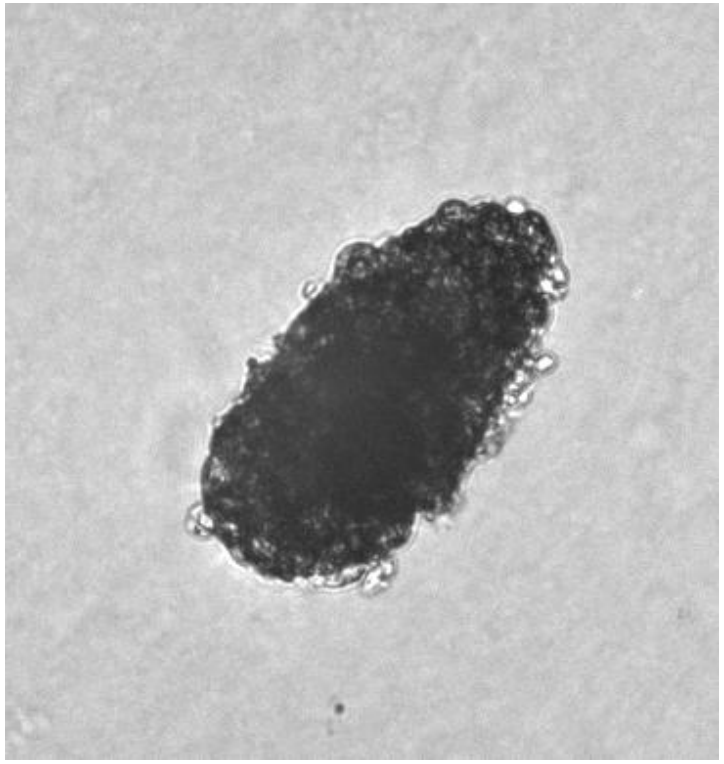
ED16.5 mouse heart

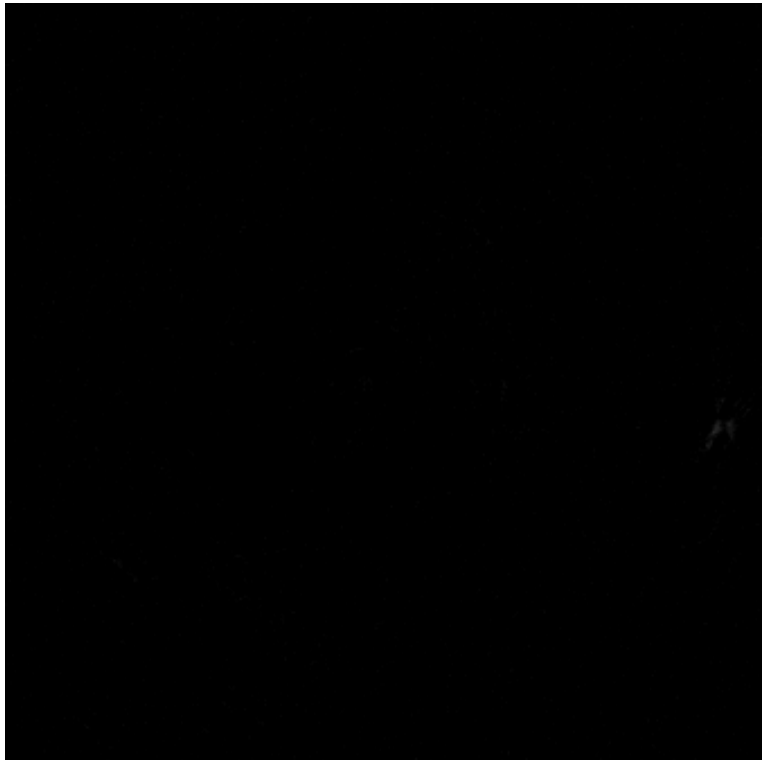


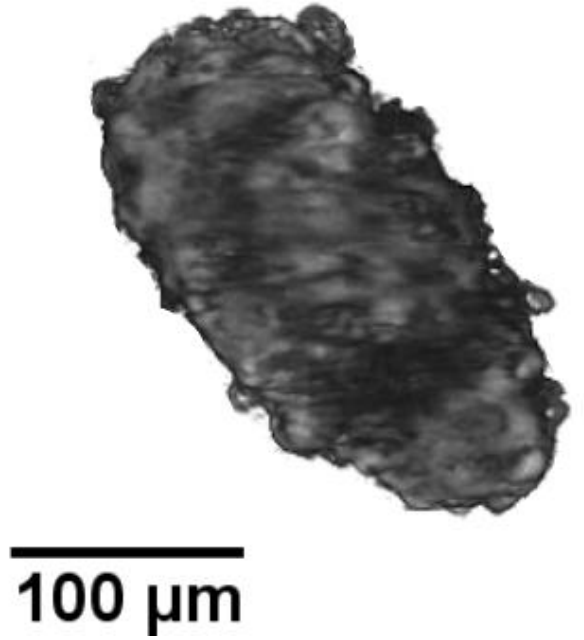
Expression of connexin40:eGFP in the atria and cardiac conduction system (green), **SCALE**; Prof. David Sedmera, DSc., IPHYS CAS

Volume analysis of Langerhans islets

- Cooperation with Dr. David Habart, IKEM, Prague
- Pancreatic islets are isolated from a surgically removed pancreas and used to treat diabetes by islet transplantation, which is a less invasive treatment than a full pancreas transplantation.
- Freshly isolated islets (human, rat, mouse, pig).
- Autofluorescence or staining with dithizone, embedded in agarose.
- OPT applied to individual islets and **the acquired images used to obtain ground truth islet volumes**.

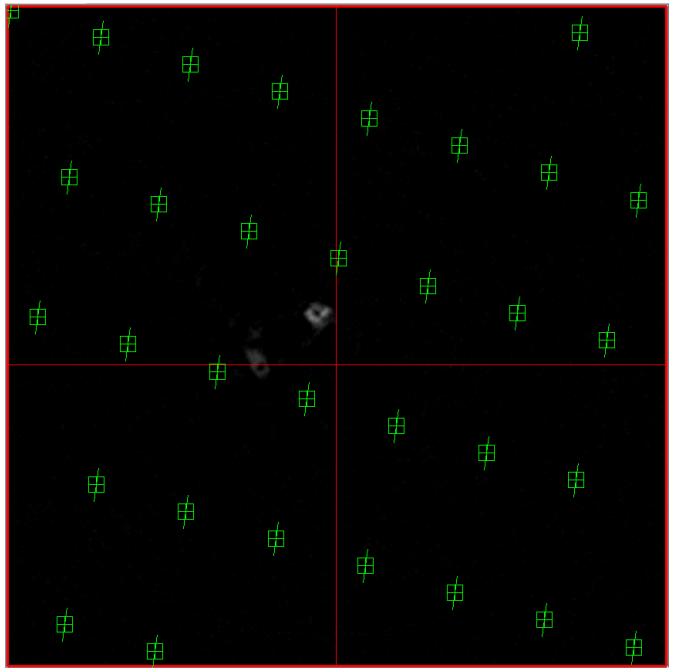


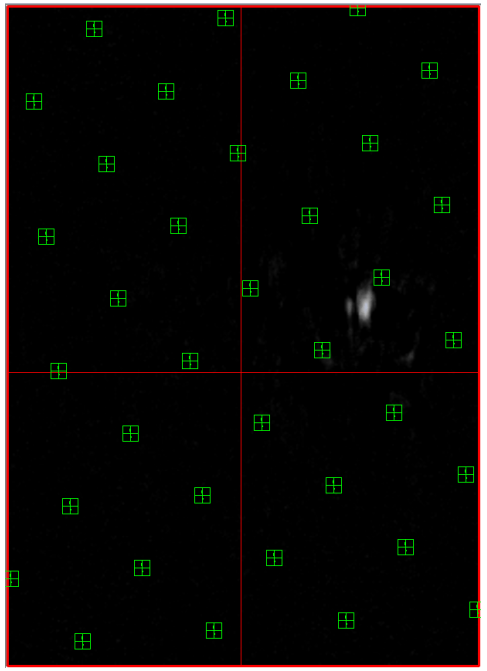


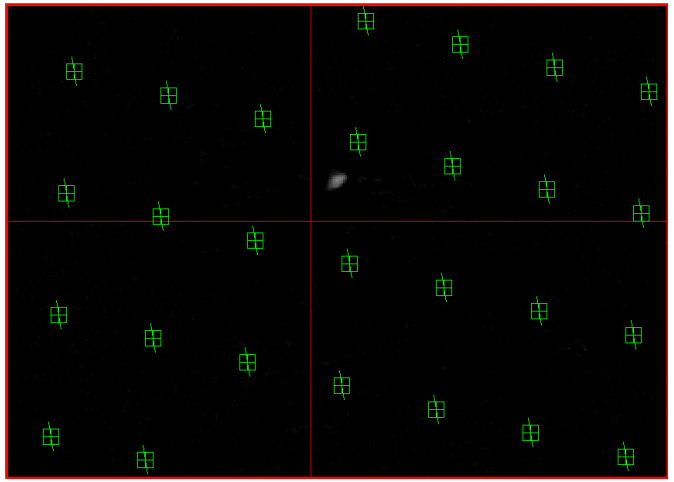


Autofluorescence, Dr. Barbora Radochová, IPHYS CAS.

Volume analysis of Langerhans islets



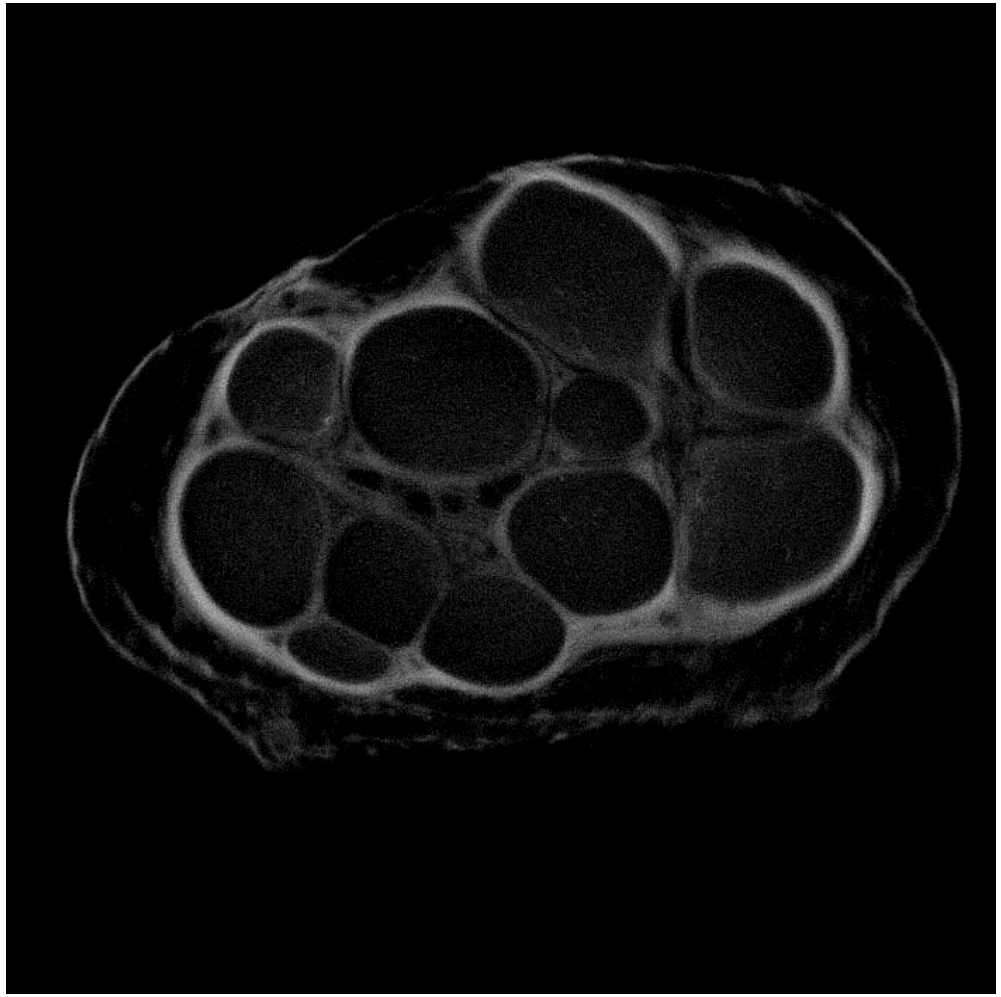


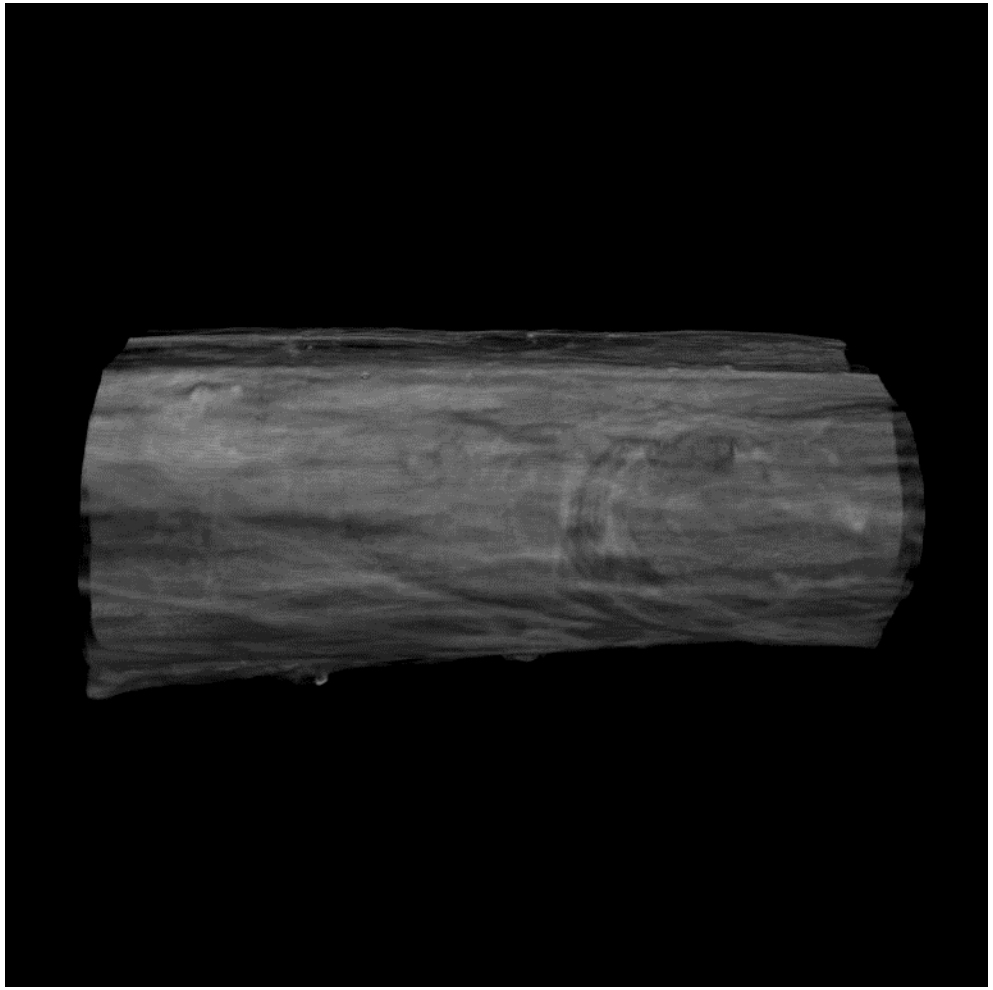


- 3D stereology – Fakir measurement
- Volumes of individual islets ranged between 0.4-64 nl

Dr. Barbora Radochová, IPHYS CAS.

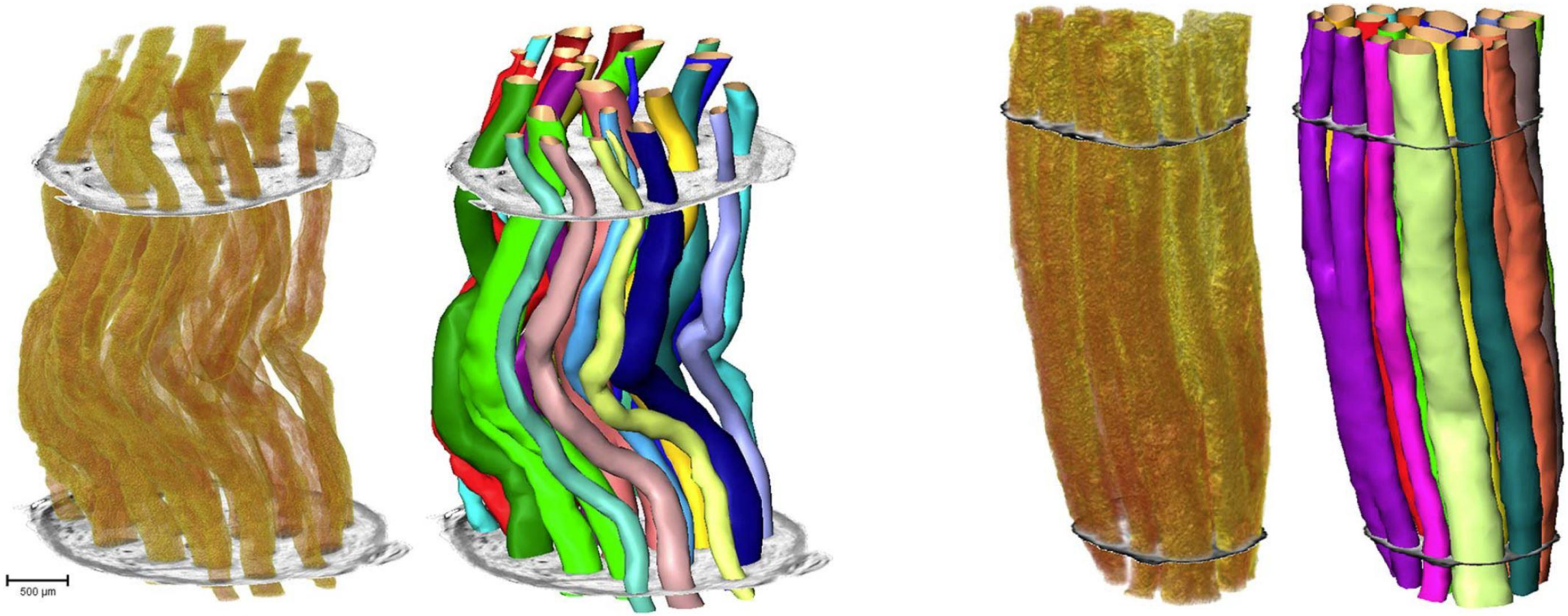
Study of morphology of pig/human nerve fascicles (cooperation with Uni Med Ljubljana, Slovenia)





Autofluorescence, BABB; Dr. Barbora Radochová, IPHYS CAS.

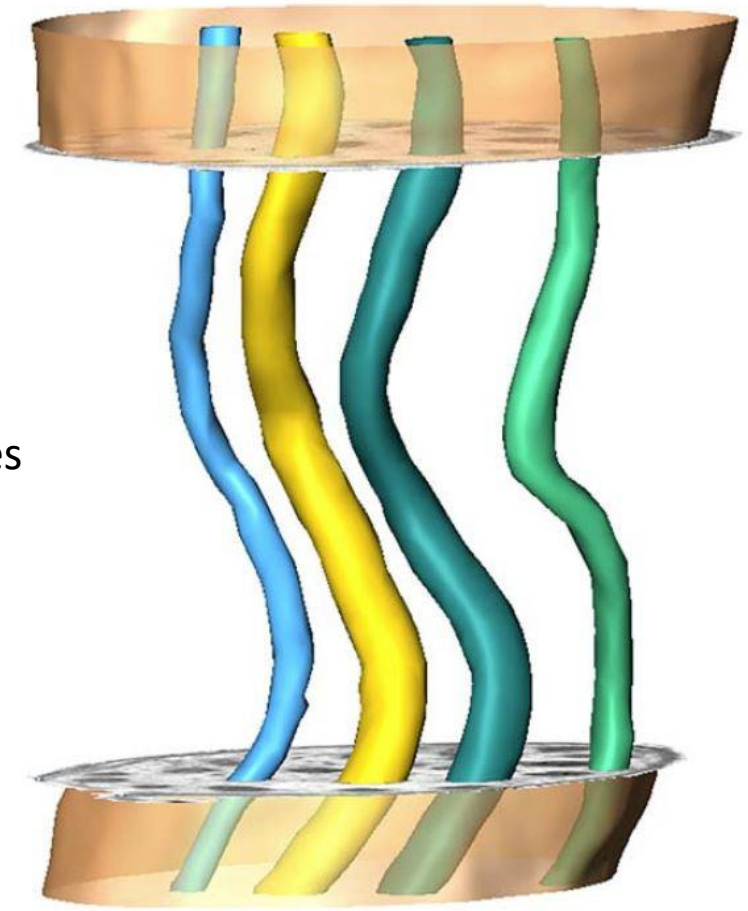
3D recontruction of fascicles of median and lingual nerves (Uni Med Ljubljana, Slovenia & Uni Barcelona, Spain; Amira sw)

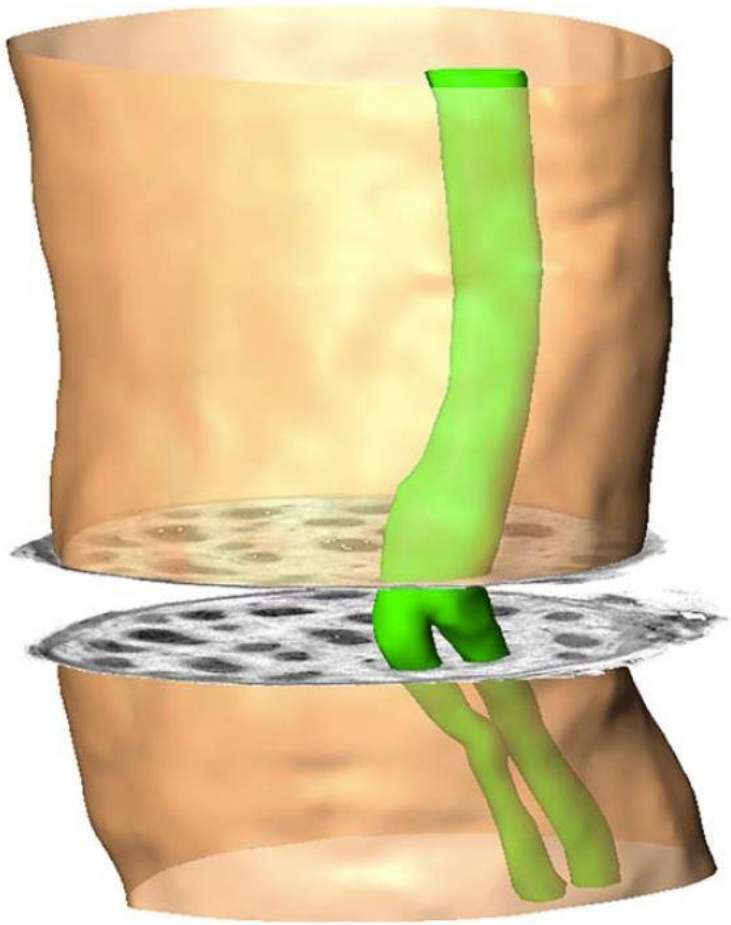


Prats-Galino, Čapek, Radochová et al.: 3D Reconstruction of Peripheral Nerves from Optical Projection Tomography Images, **Clinic Anatomy** 31:424-431, 2018.

3D recontruction of fascicles of median and lingual nerves (Uni Med Ljubljana, Slovenia & Uni Barcelona, Spain)

Single nerve fascicles



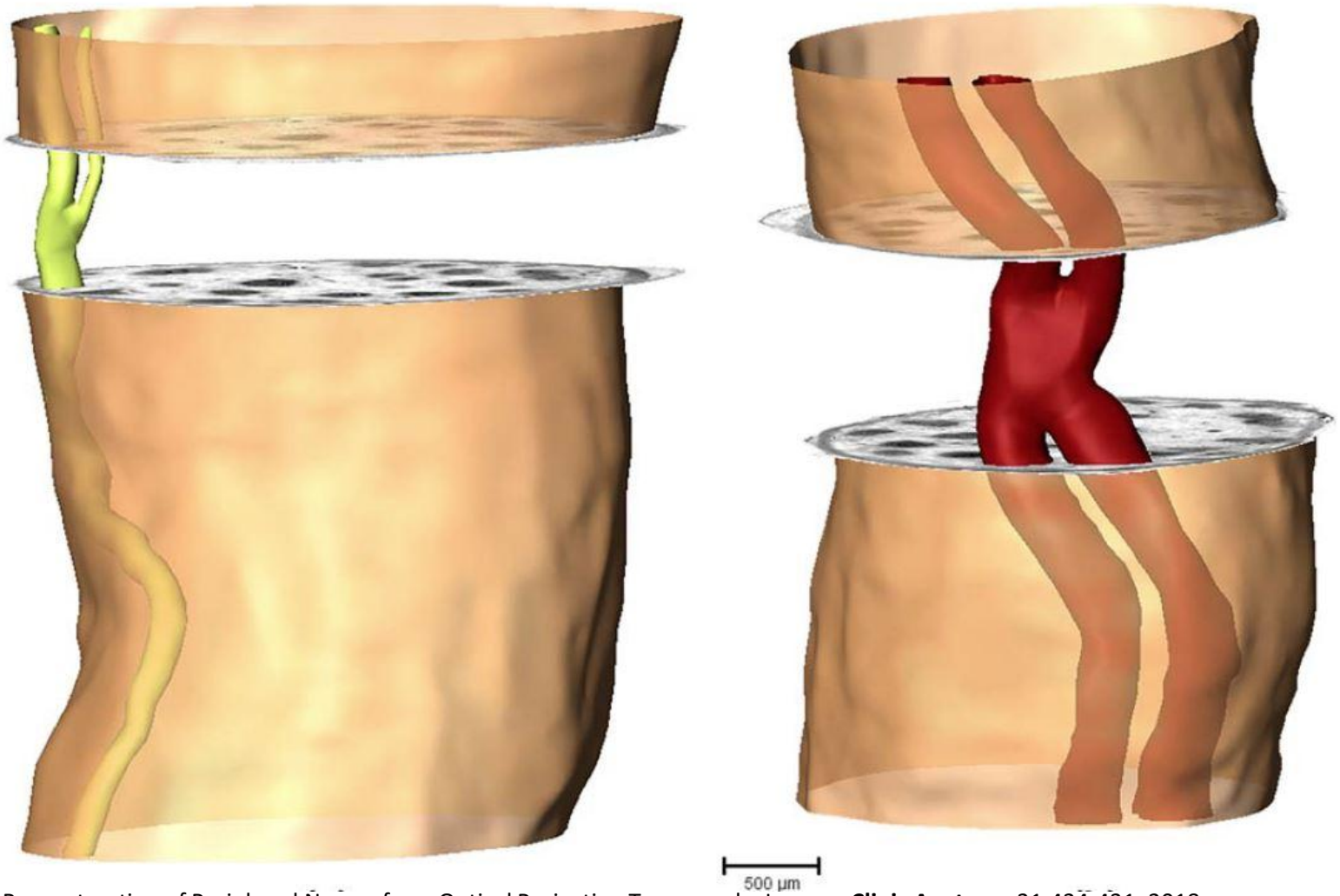


Inverted Y-shape

Prats-Galino, Čapek, Radochová et al.: 3D Reconstruction of Peripheral Nerves from Optical Projection Tomography Images, **Clinic Anatomy** 31:424-431, 2018.

3D recontruction of fascicles of median and lingual nerves (Uni Med Ljubljana, Slovenia & Uni Barcelona, Spain)

Y-shape

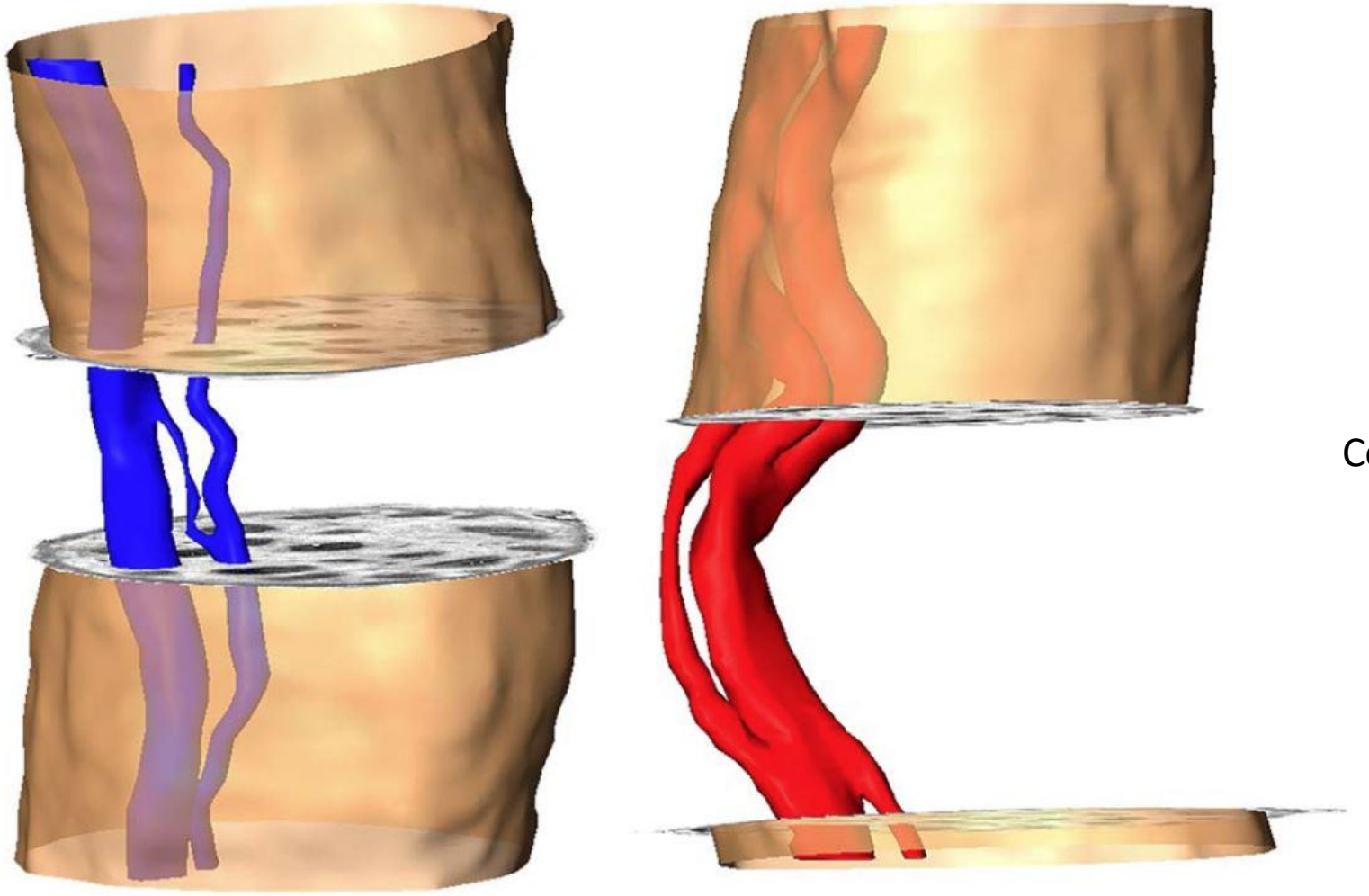


X-shape

Prats-Galino, Čapek, Radochová et al.: 3D Reconstruction of Peripheral Nerves from Optical Projection Tomography Images, **Clinic Anatomy** 31:424-431, 2018.

3D recontruction of fascicles of median and lingual nerves (Uni Med Ljubljana, Slovenia & Uni Barcelona, Spain)

Fascicular interconnection



Complex interconnection

Prats-Galino, Čapek, Radochová et al.: 3D Reconstruction of Peripheral Nerves from Optical Projection Tomography Images, **Clinic Anatomy** 31:424-431, 2018.

Thanks for your attention!