FYZIOLOGICKÝ ÚSTAV AV ČR

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Image analysis and 3D visualization

Microscopy methods in biomedicine, October 17th, 2025

http://www.biomed.cas.cz/~janacek/

01 Imaging

Microscopes, confocal, multiphoton
Electron microscopes
Tomographs

02 Data

Digital images
Triangulated surfaces
Spatial graphs

03 Measurement or visualization

Characteristics – numbers, statistics

Images, videos – using visual cues

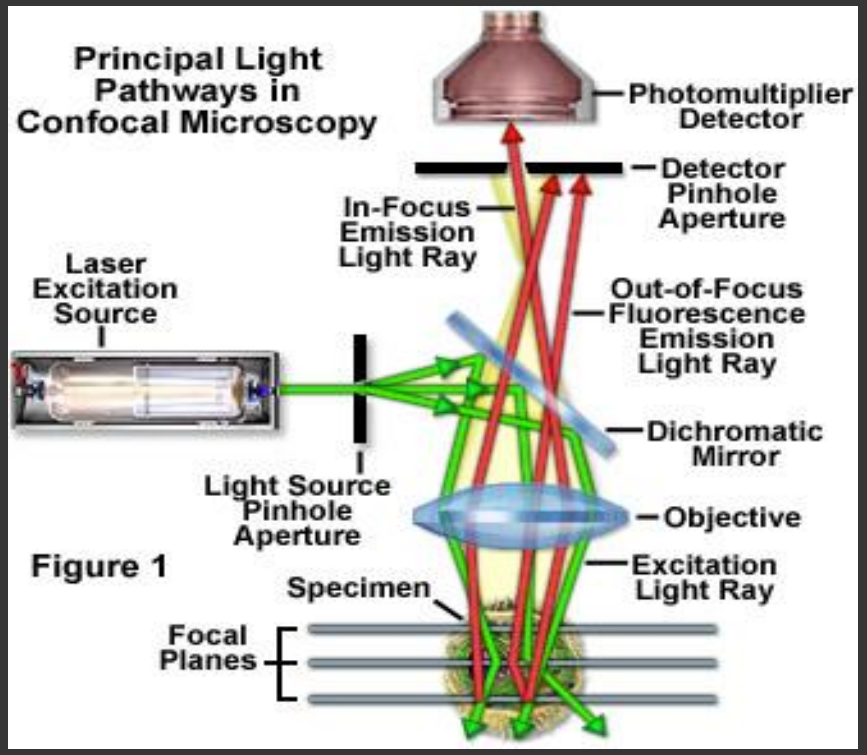
3D: imaging, analysis and virtual models

- We extract information (construct models) for visualization or measurements
- The models are constructed from spatial data obtained by tomography, confocal microscopy etc., keeping the most important properties of the structures
- Visualization uses computer devices and human spatial imagination (visual cues: colour, stereopsy, motion, shading, texture).

Spatial data: I) 3D optical images

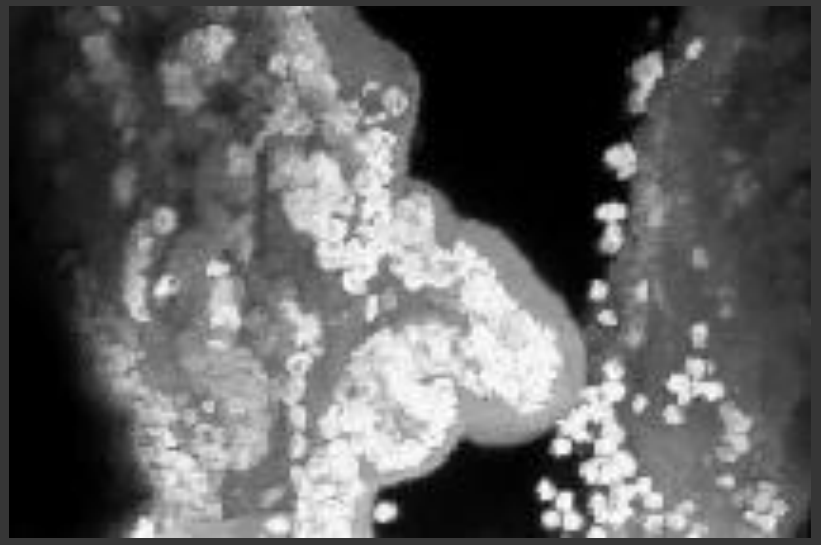
- Are created using visible light: microscopy, optical tomography.
- Light scattering and light attenuation in 3D.
- Thick slices are prone to shrinkage.
- Specific staining either by penetrating dye or by incorporating fluorescent protein into structure of interest.

Confocal microscopy



http://www.microscopyu.com/articles/confocal/confocalintrobasics.html

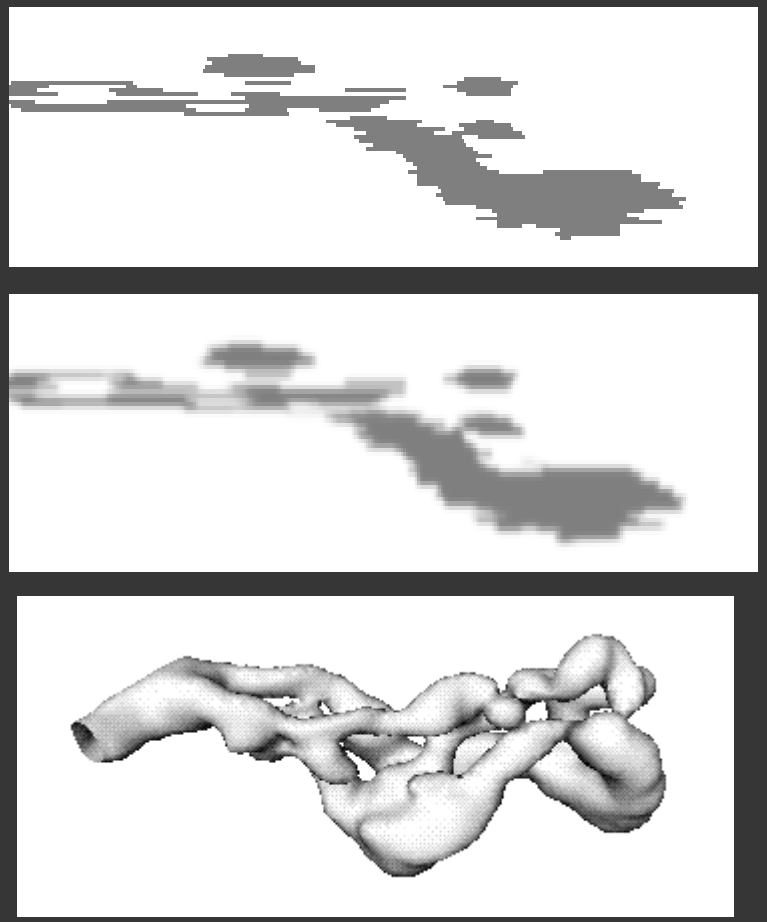
Placenta, MIP, manual segmentation



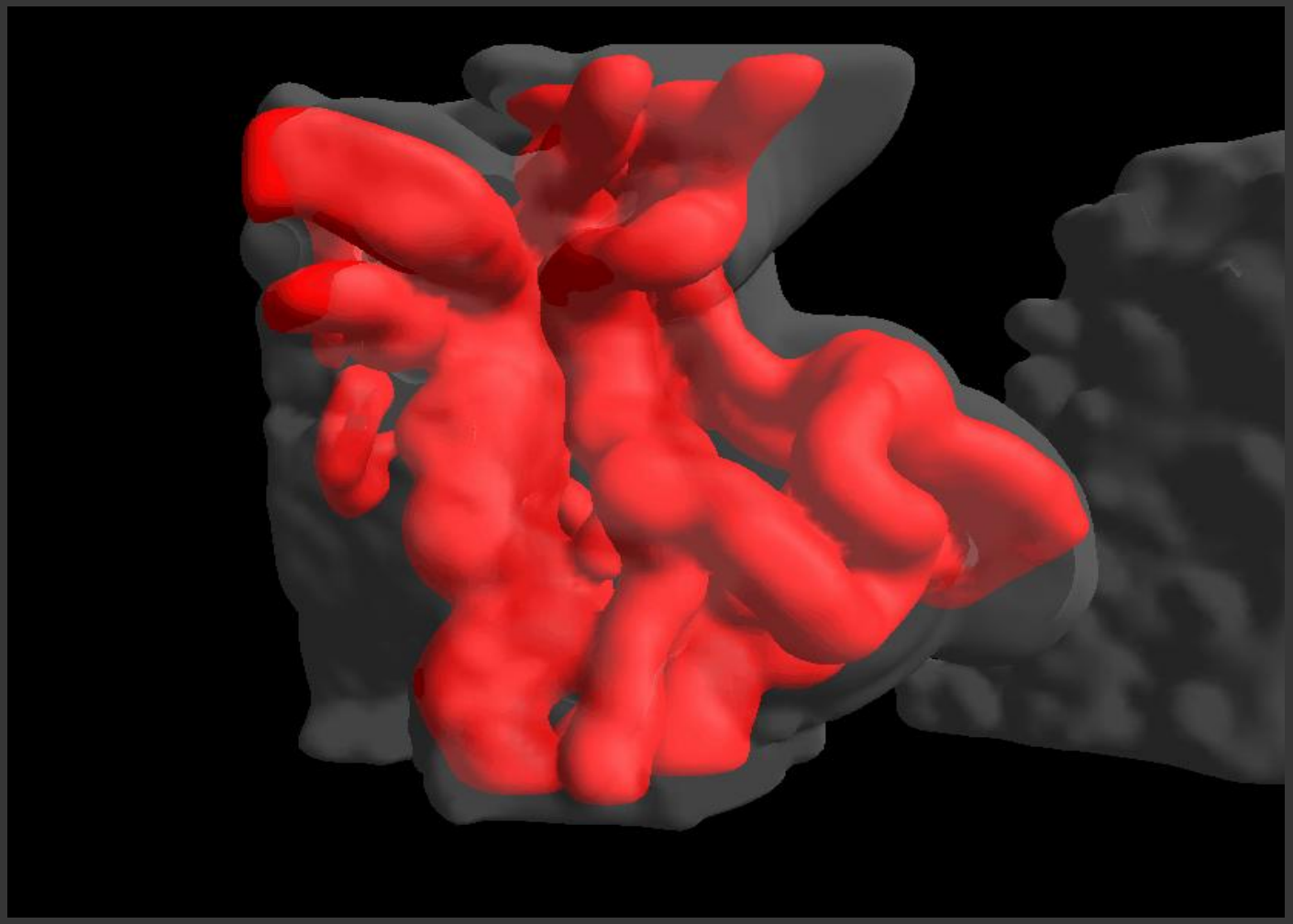
MIP – maximal projection

Surface model

- Drawn polygons
- Gaussian
- Marching cubes



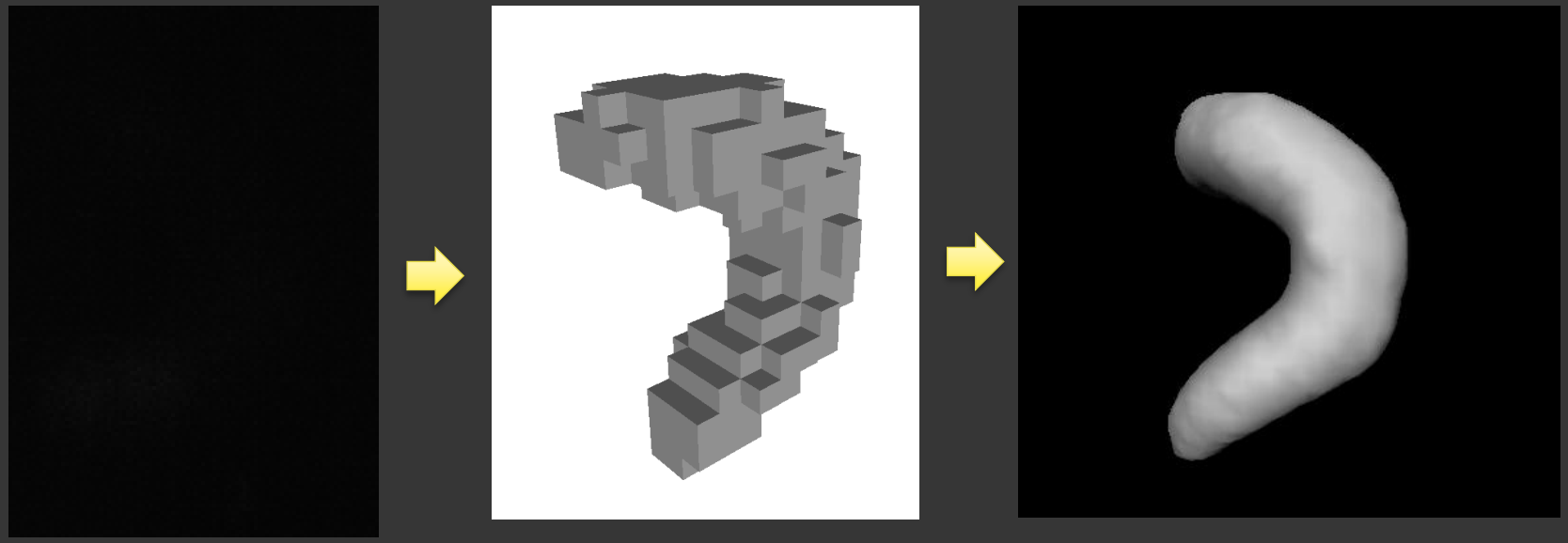
Surface rendering



Measurable 3D data

- 3D segmented (binary) images
- Surface models
- Line models

Binary and surface representation



Basic parameters

- Number, Euler characteristics [m^0]
- Length, perimeter [m^1]
- Area, surface area [m^2]
- Volume [m^3]

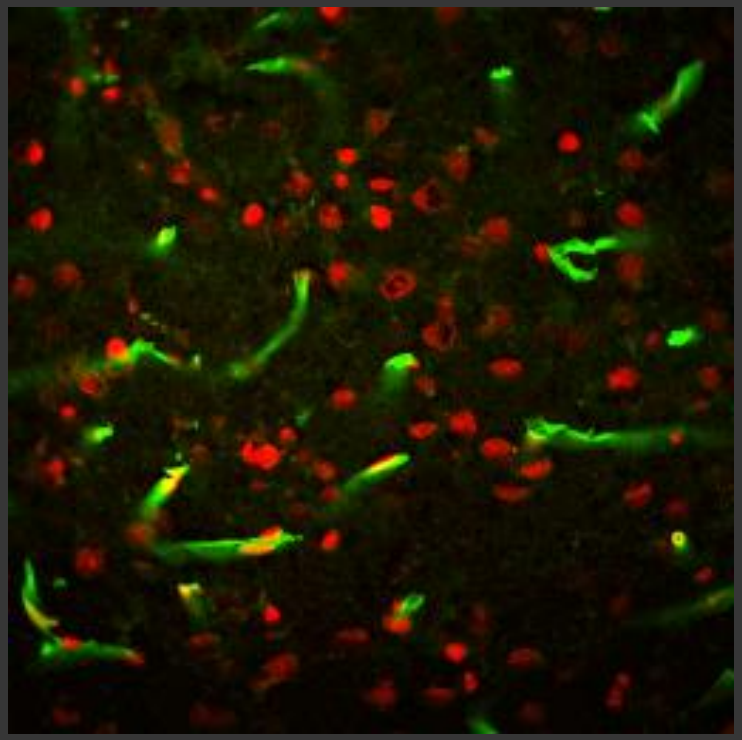
Intensities, weighted averages

- Intensity: value divided by volume of ROI, e.g. L/V, V/V
- Averages of particle values weighted by other value, e.g. volume weighted average volume

Derived characteristics

- Shape factors (sphericity)
- Anisotropy
- Fractal dimension

Capillaries in rat brain



Cortex (gray matter)

Capillaries stained by intravenous application of biotynilated lectin

Cell nuclei stained by propidiumiodide

BioRad 1024, Loma Linda University Hospital,

John Archambeau, Vivien Mao, excitation 488 and 567 nm,

40x (N.A. = 1.3), 512 x 512 x 35 voxels; voxel 0.52 x 0.52 x 2 μm^3

Filtration

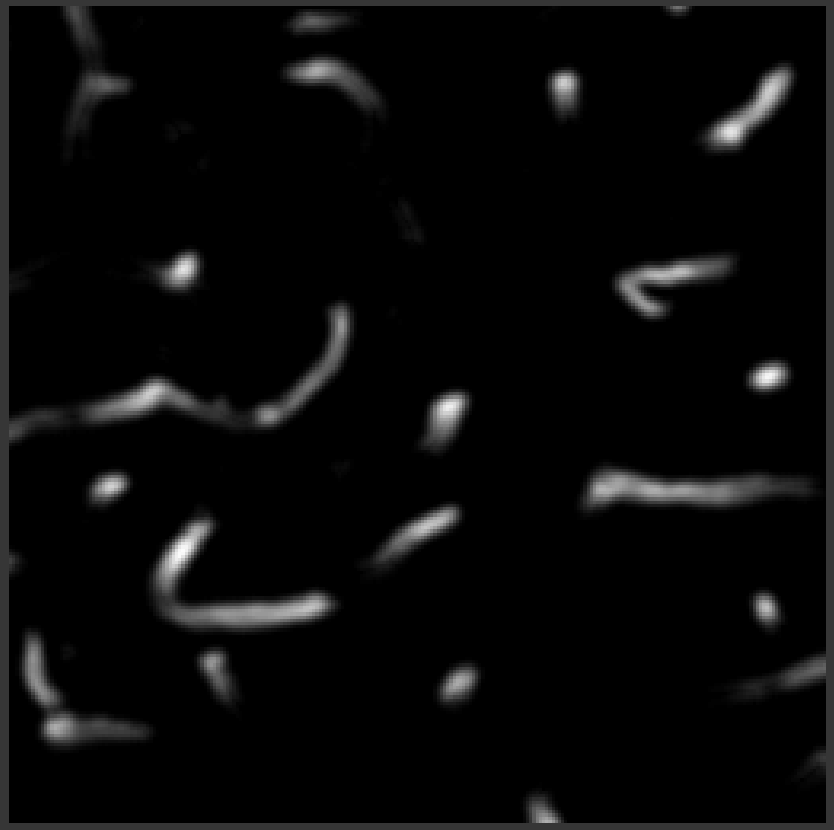
Green channel

Contrast enhanced with depth (1x to 3x).

Resampling (1/2)

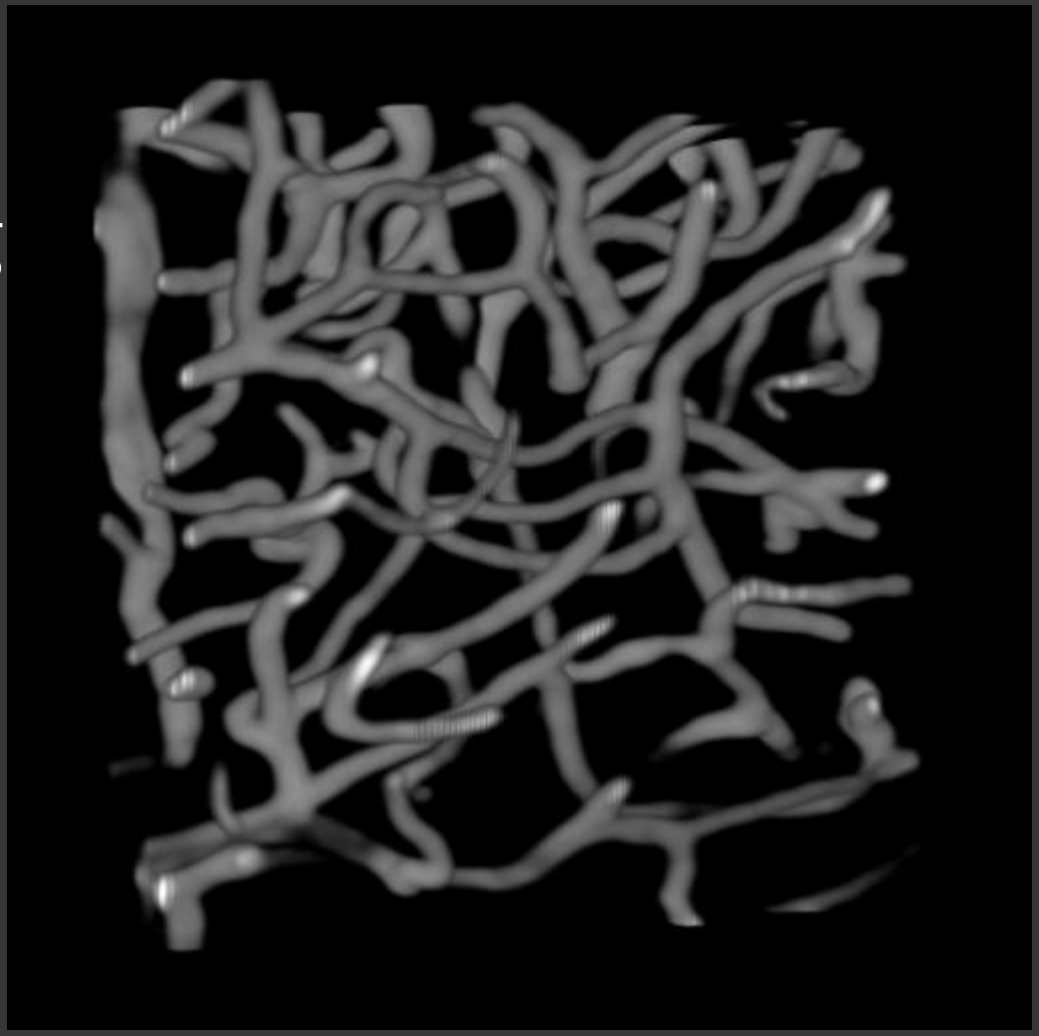
3D Gaussian filter (σ=2pix).

3D Lipschitz top-hat filter (slope=2/pix).

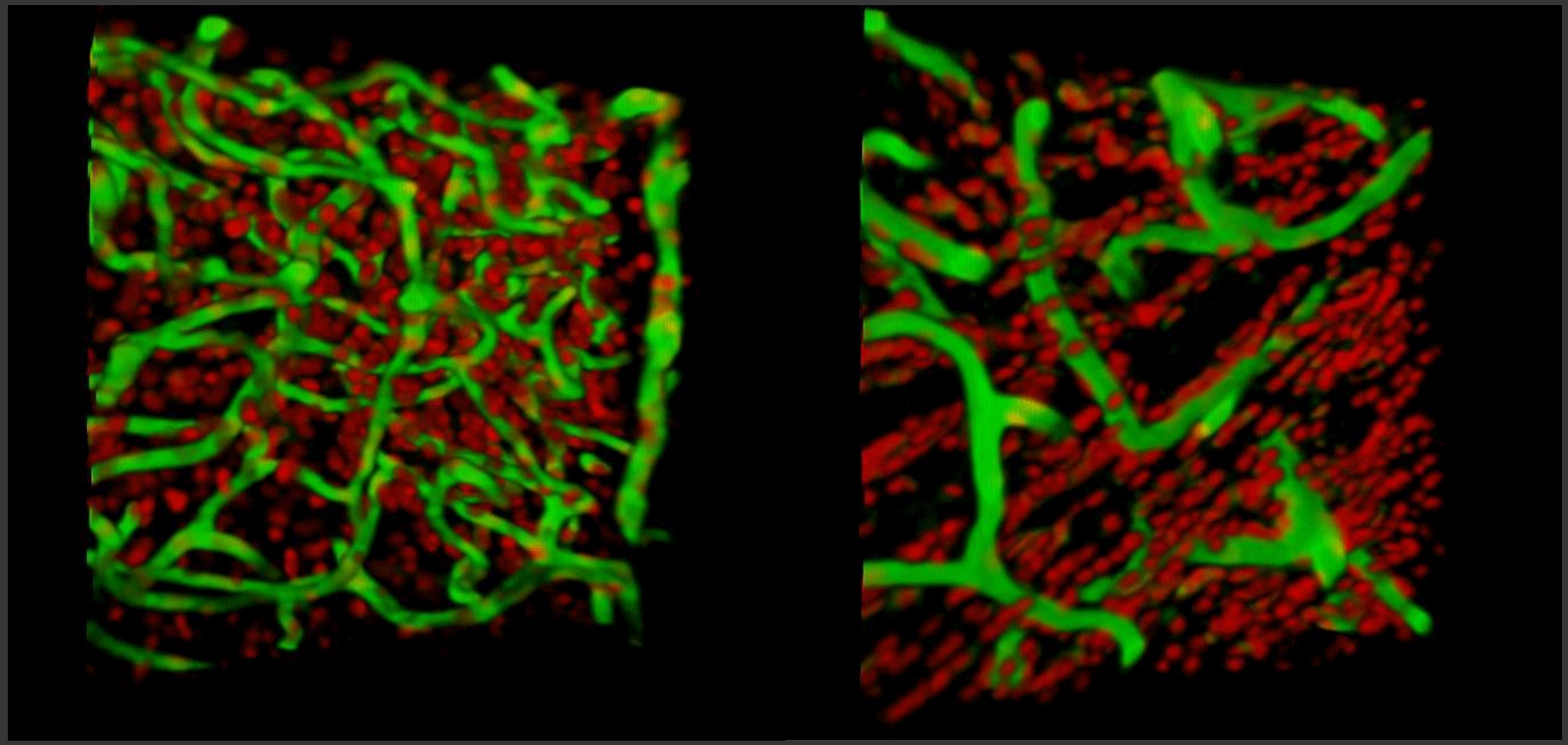


Visualization

- Volume rendering VolumePro 1000 dedicated
- PCI card.



Gray and white matter



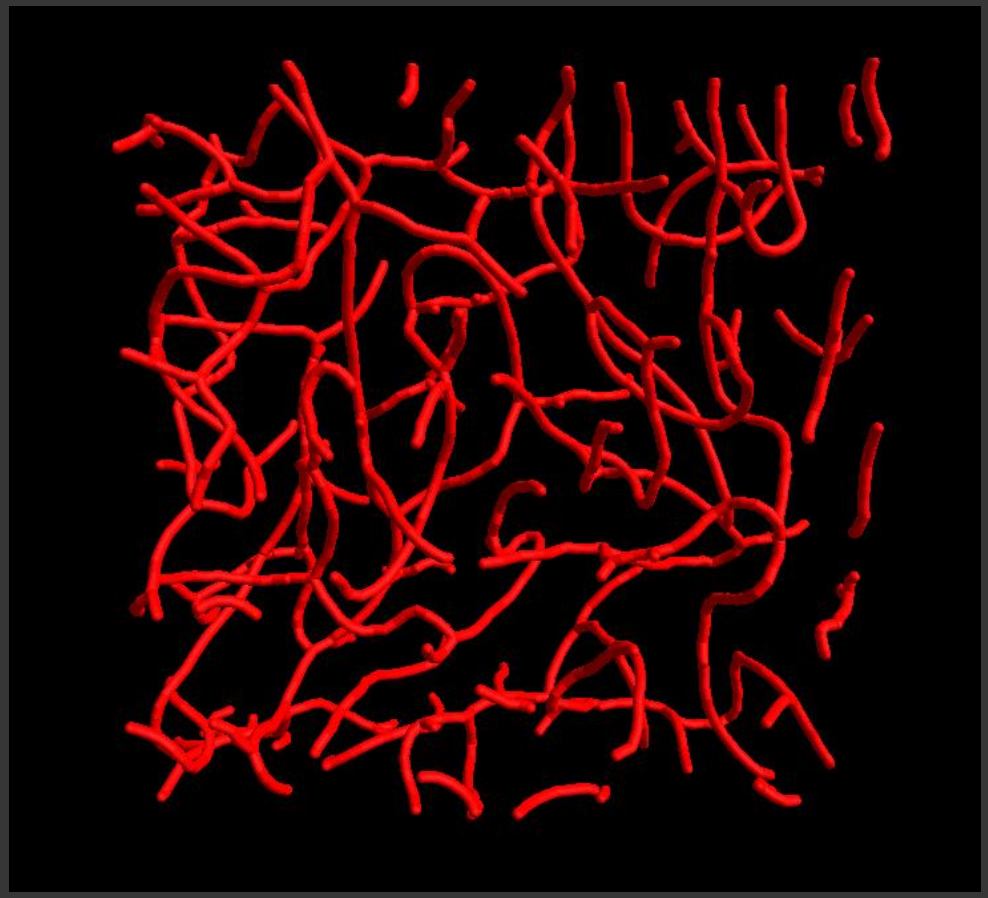
Cortex

Corpus callosum

Volume rendering, nVidia, OpenGL 3D texture

Skeletonization of capillaries

- Threshold of grayscale image
- 3D skeletonization of binary image
- Tracing the skeleton between branchings and endpoinds : segments



Length density

- Total length / ROI volume

Cortex (N=6):	$1.1 \pm 0.2\ 10^{-3}\ \mu m^{-2}$
Corpus callosum (N=4):	$0.5 \pm 0.1\ 10^{-3}\ \mu m^{-2}$

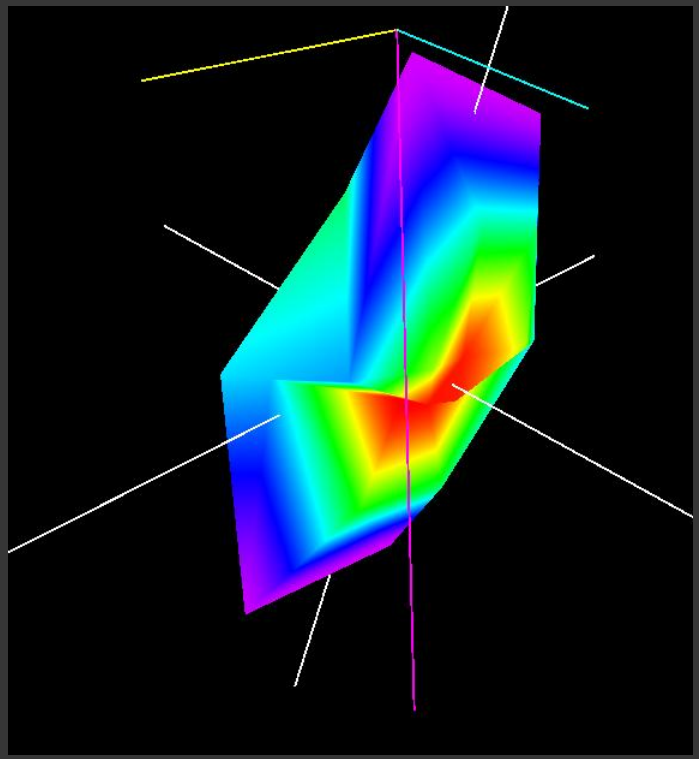
Branching density

- Number of branchings / ROI volume

Cortex (N=6):	$17 \pm 2\ 10^{-6}\ \mu m^{-3}$
Corpus callosum (N=4):	$6 \pm 3\ 10^{-6}\ \mu m^{-3}$

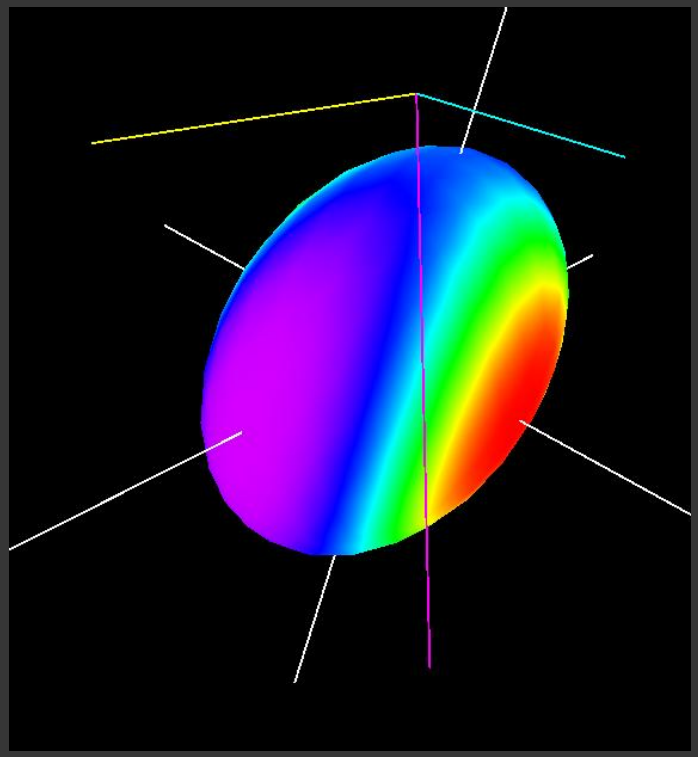
Directional orientation - Rose of directions

- Regularized histograms on sphere S_2 (symmetric)



Positive definite tensor

- Structure tensor: Length weighted sum of the segments tensors
- $T=\sum_{k=1..3} \lambda_k \cdot v_k \otimes v_k^T$.
- Ellipsoid:



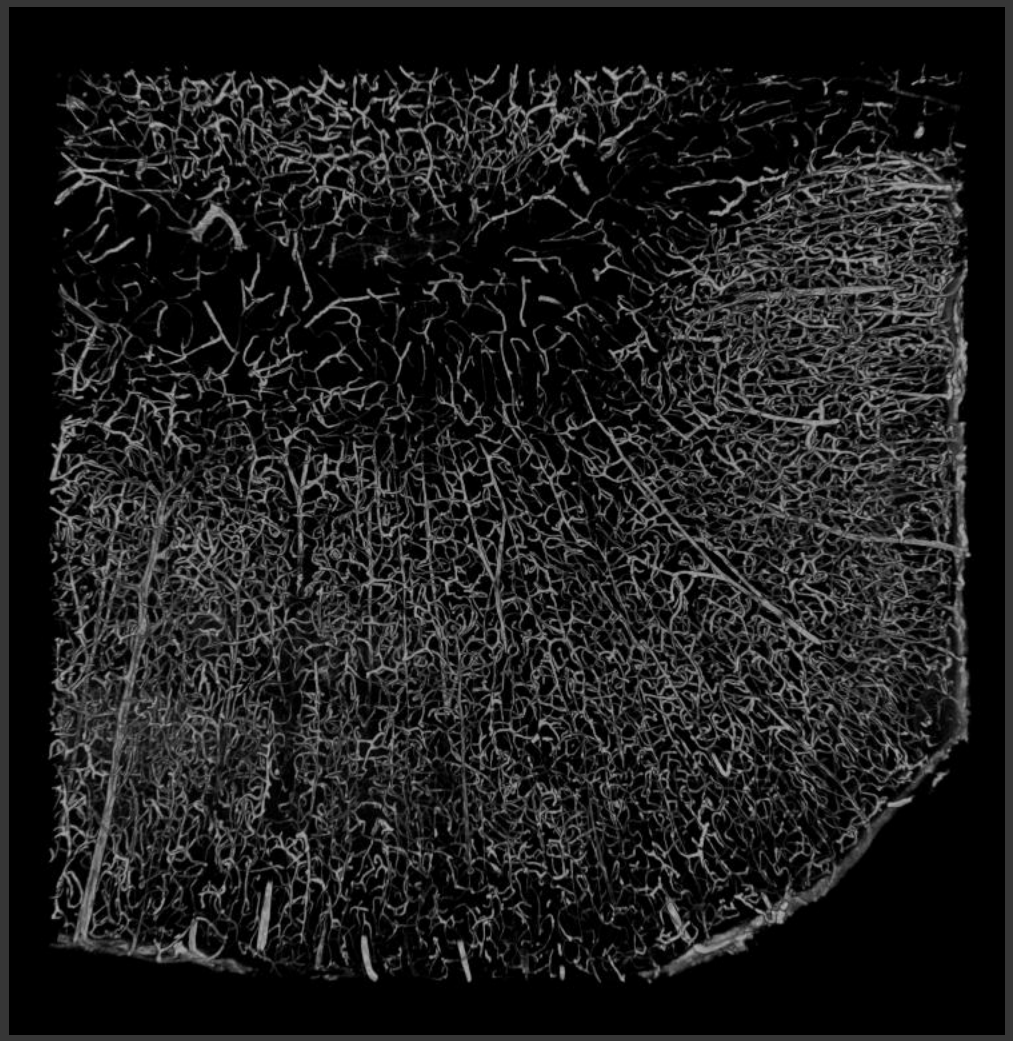
Tensor eigenvalues

- Main direction of capillaries correspond to the eigenvector with biggest eigenvalue.
- Anisotropy e.g.:

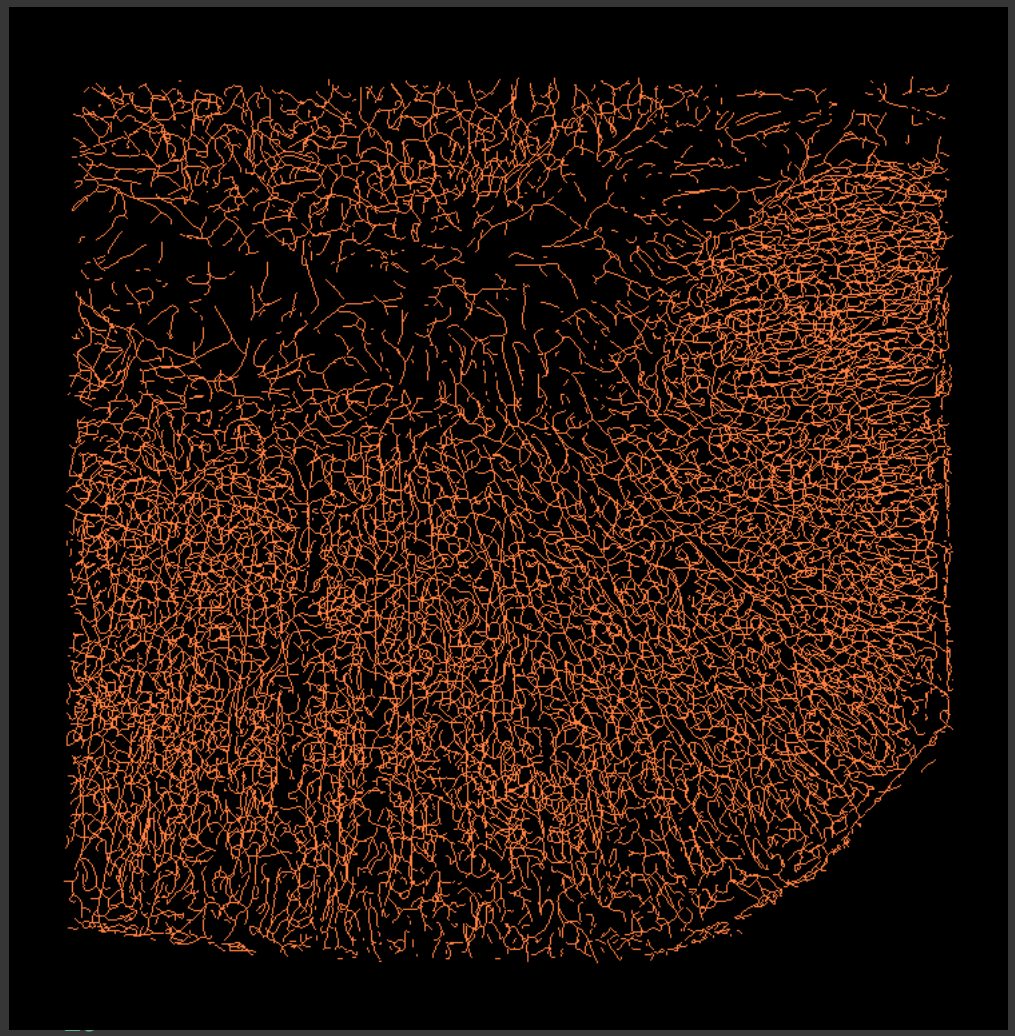
$$(\lambda_{max} - \lambda_{min}) / \lambda_{avg}.$$

Cortex (N=6):	0.4 ± 0.1
Corpus callosum (N=4):	0.6 ± 0.2

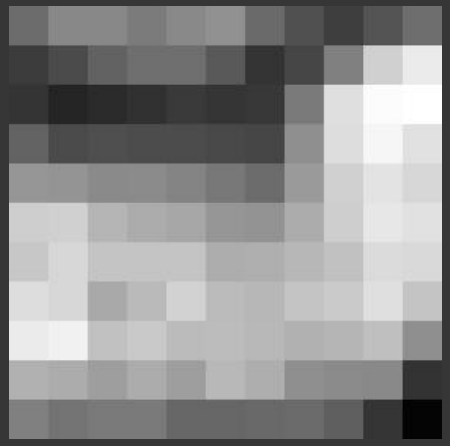
Mozaic of images

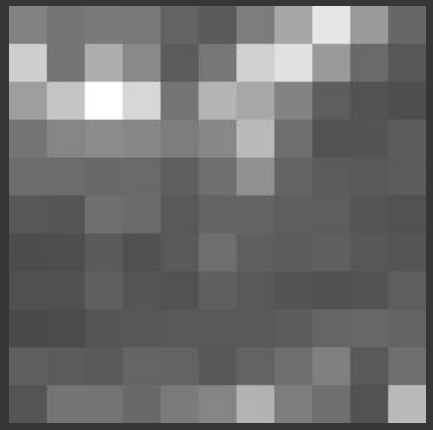


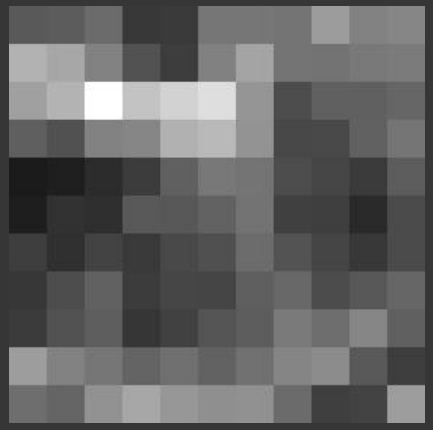
Skeletonization of mozaic



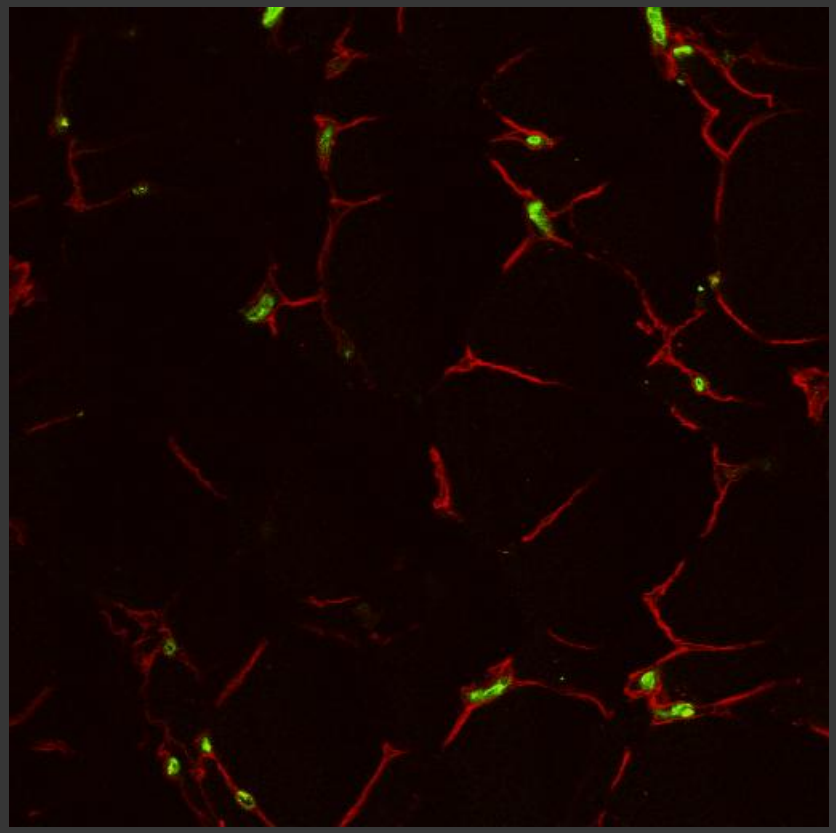
Capillaries parameters maps: L_V, length, anisotropy





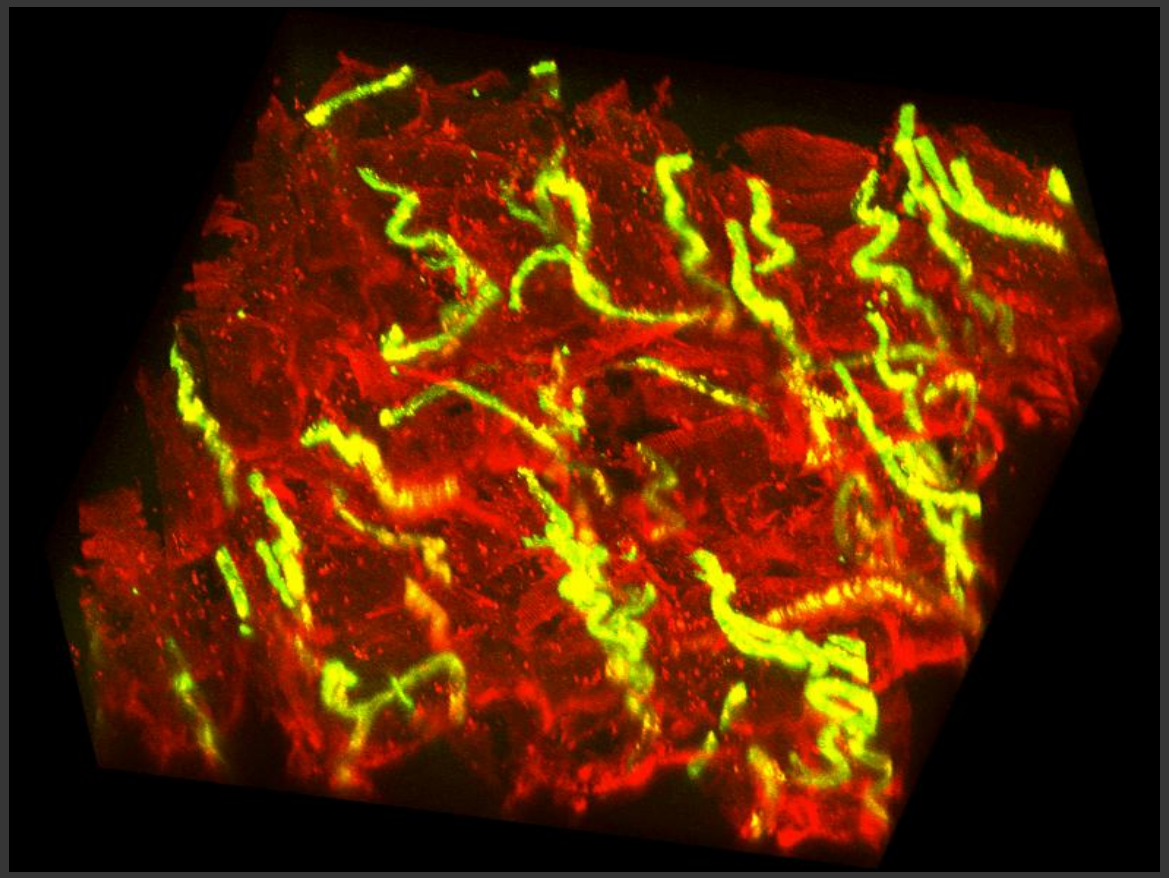


Capillaries in skeletal muscle



- Prof. Ida Eržen, Medical faculty, Ljubljana

Maximal projection



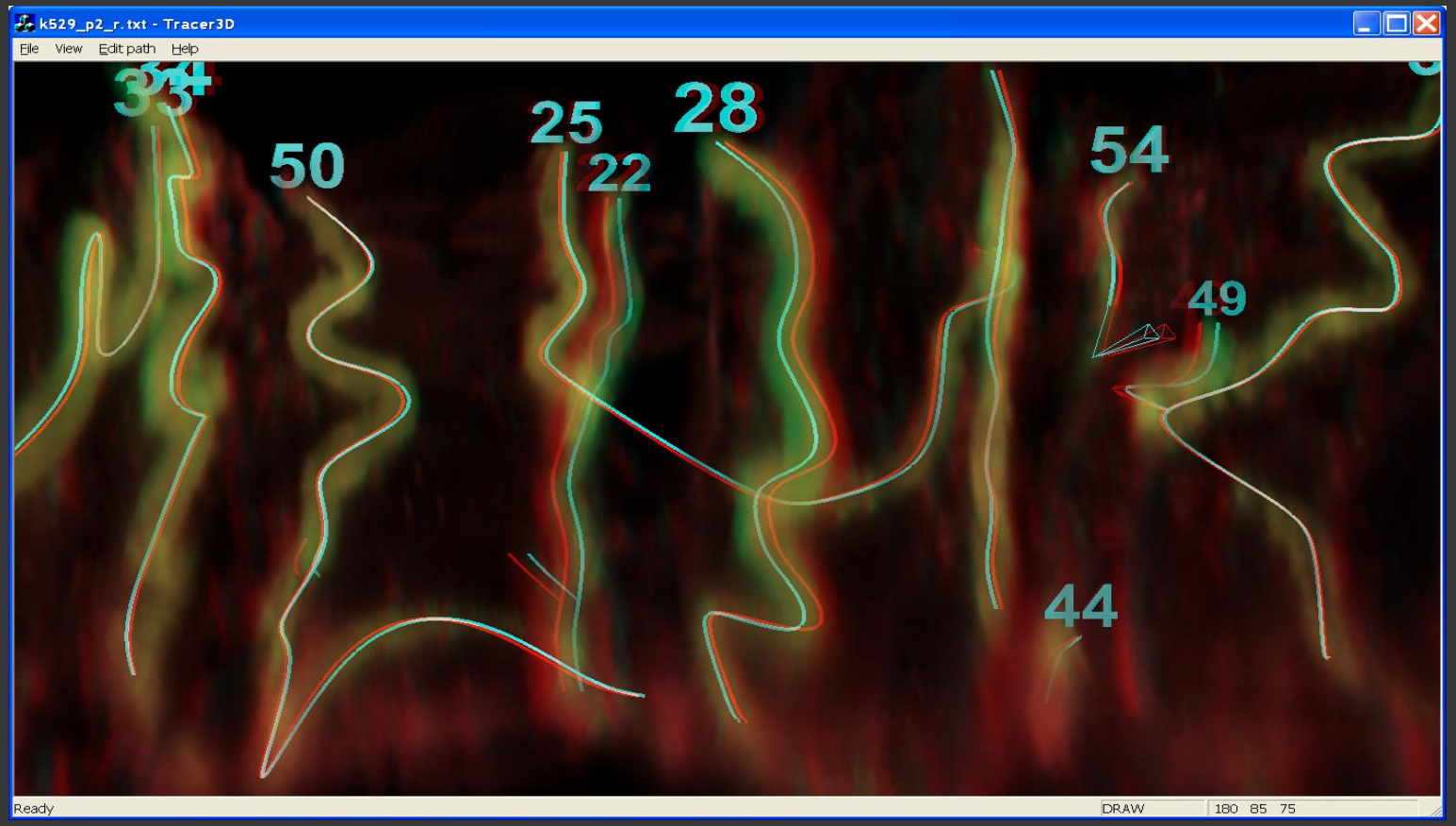
Editing 3D line segment model

- Stereoscopic rendering of image, model and cursor controlled by haptic device and 3D position controller.
- Feedback – force proportional to the image gradient – allows precisely locate the cursor.
- Model is moved by 6DOF controler

6DOF controler and haptic device

http://www.biomed.cas.cz/~janacek/

Anaglyph stereo



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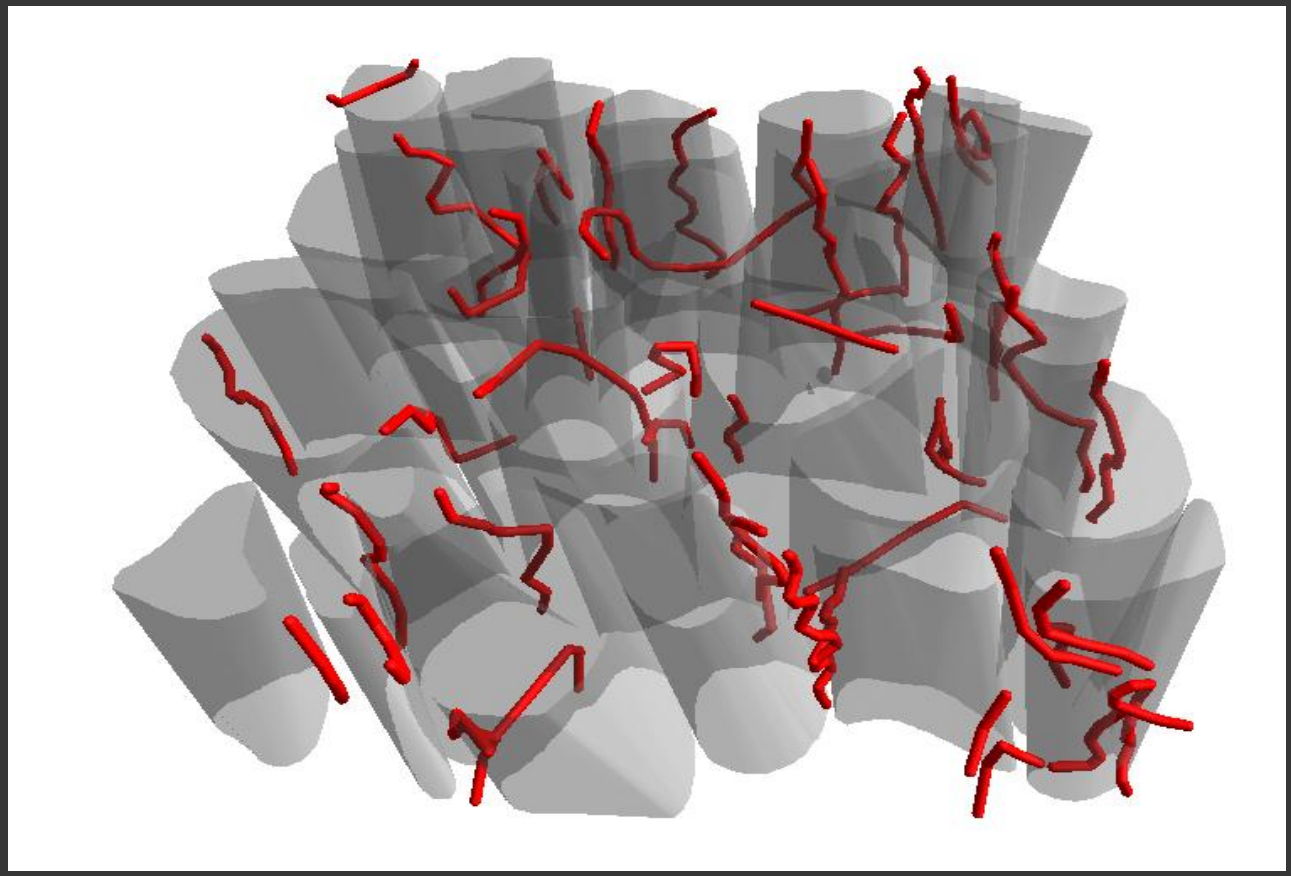
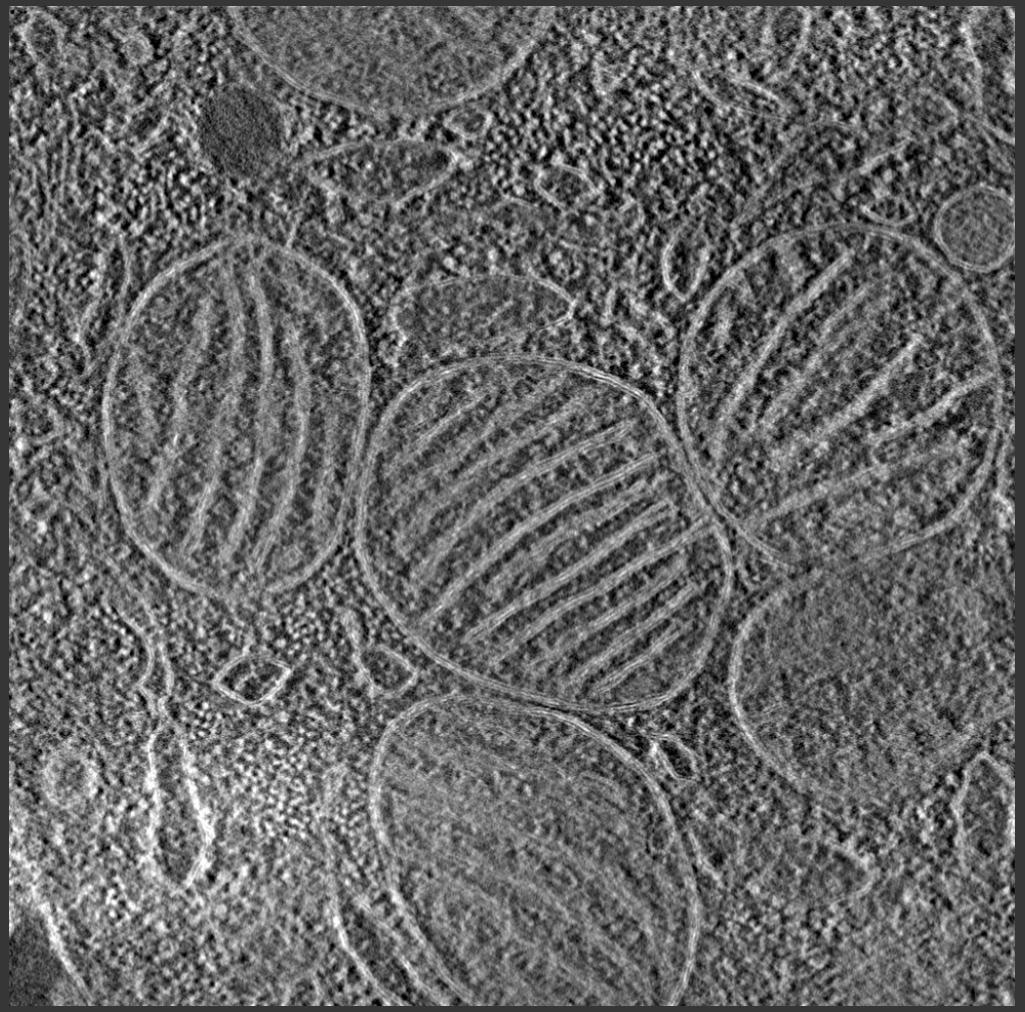
Segmented muscle

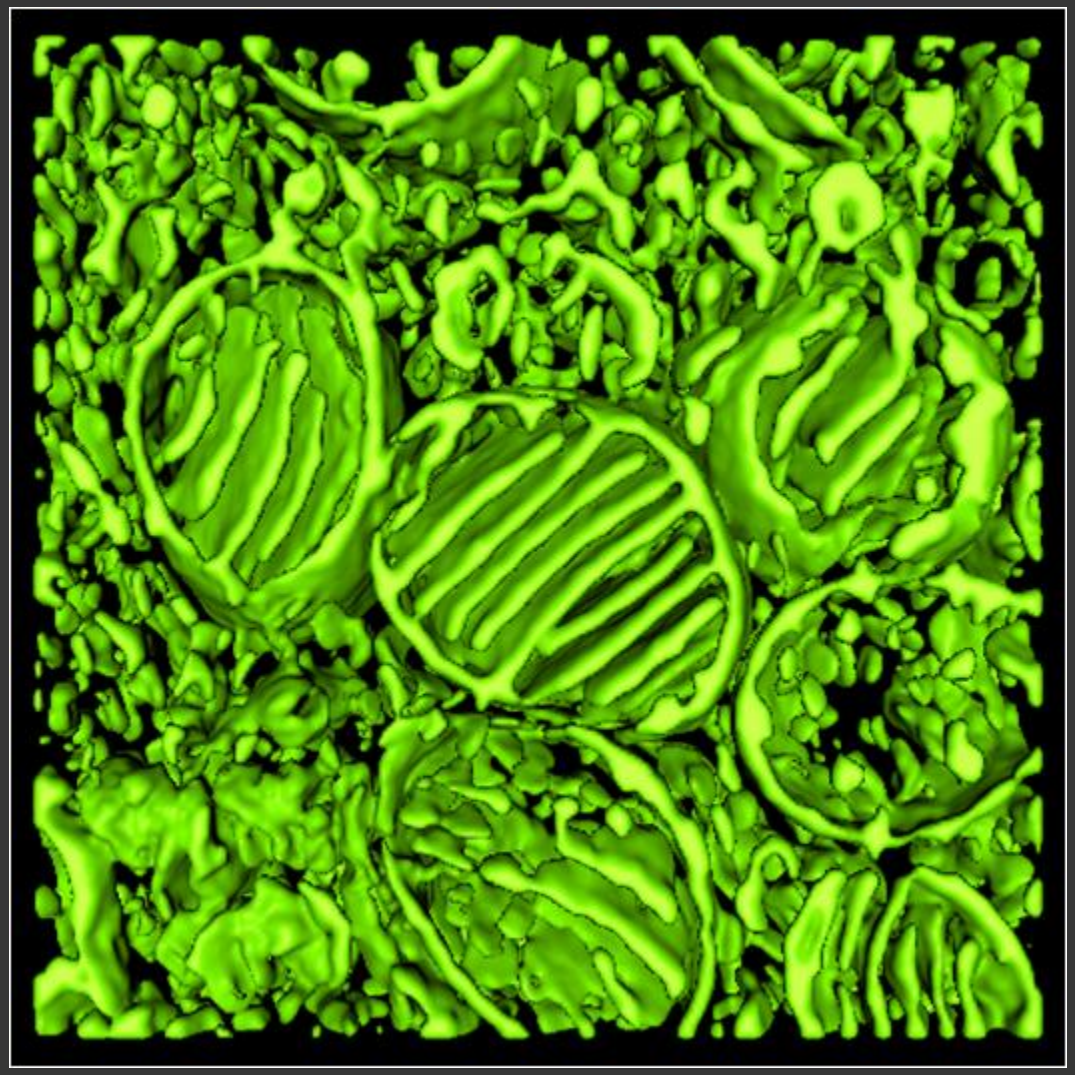


Spatial data: II) 3D TEM of mitochondria (Dr. Walther, Ulm)

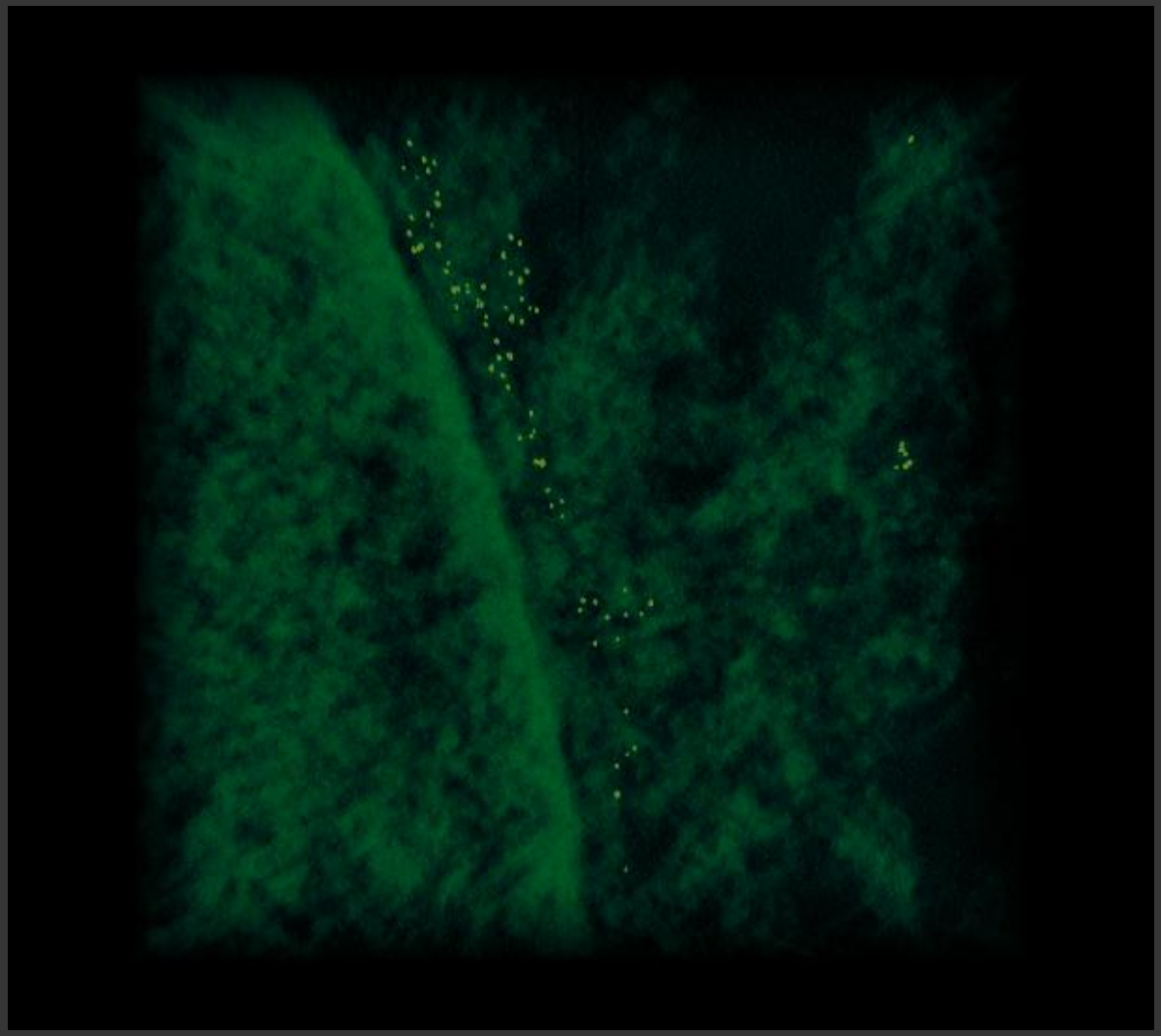


http://www.biomed.cas.cz/~janacek/

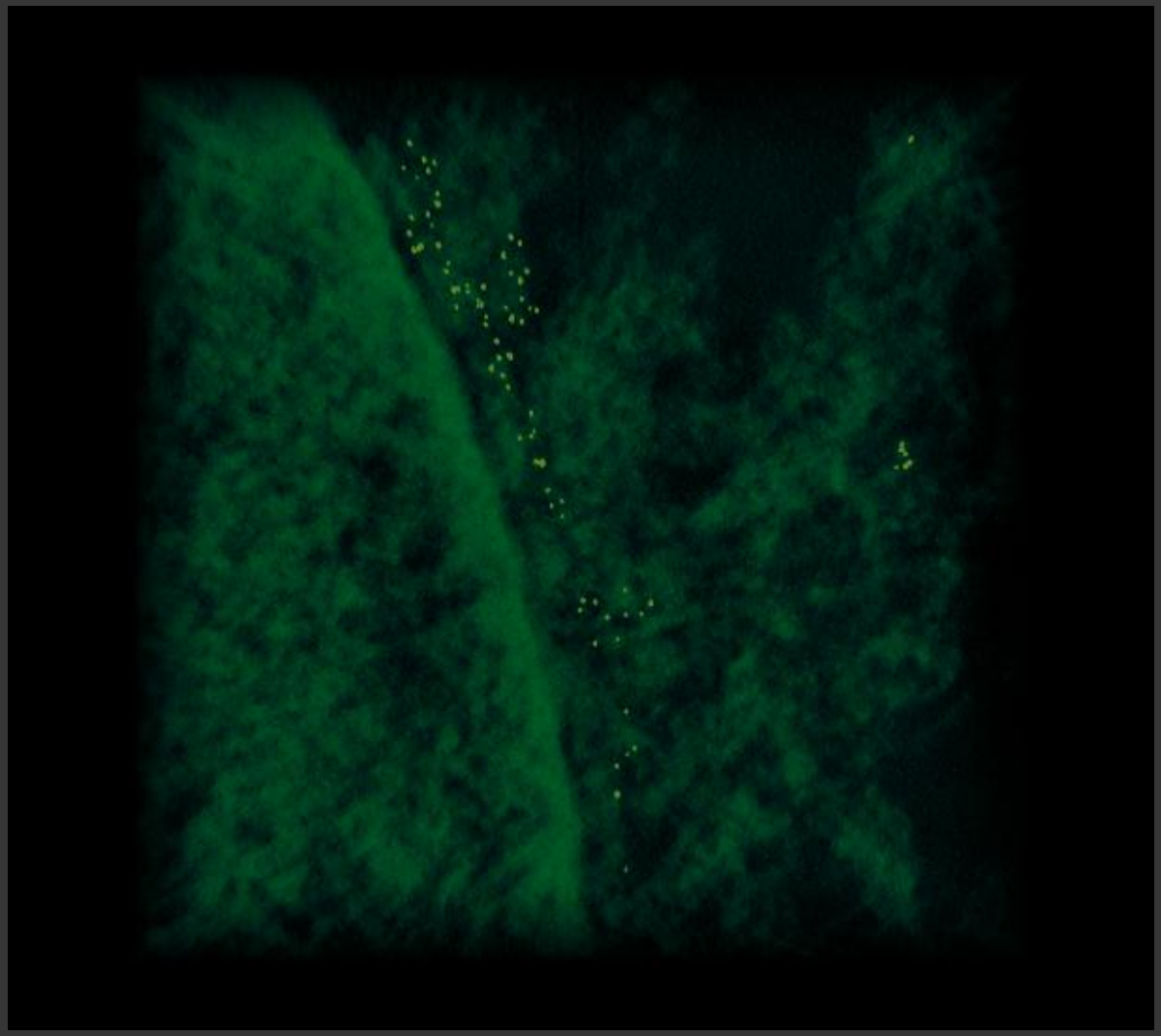
Reconstruction



Reconstruction in IMOD

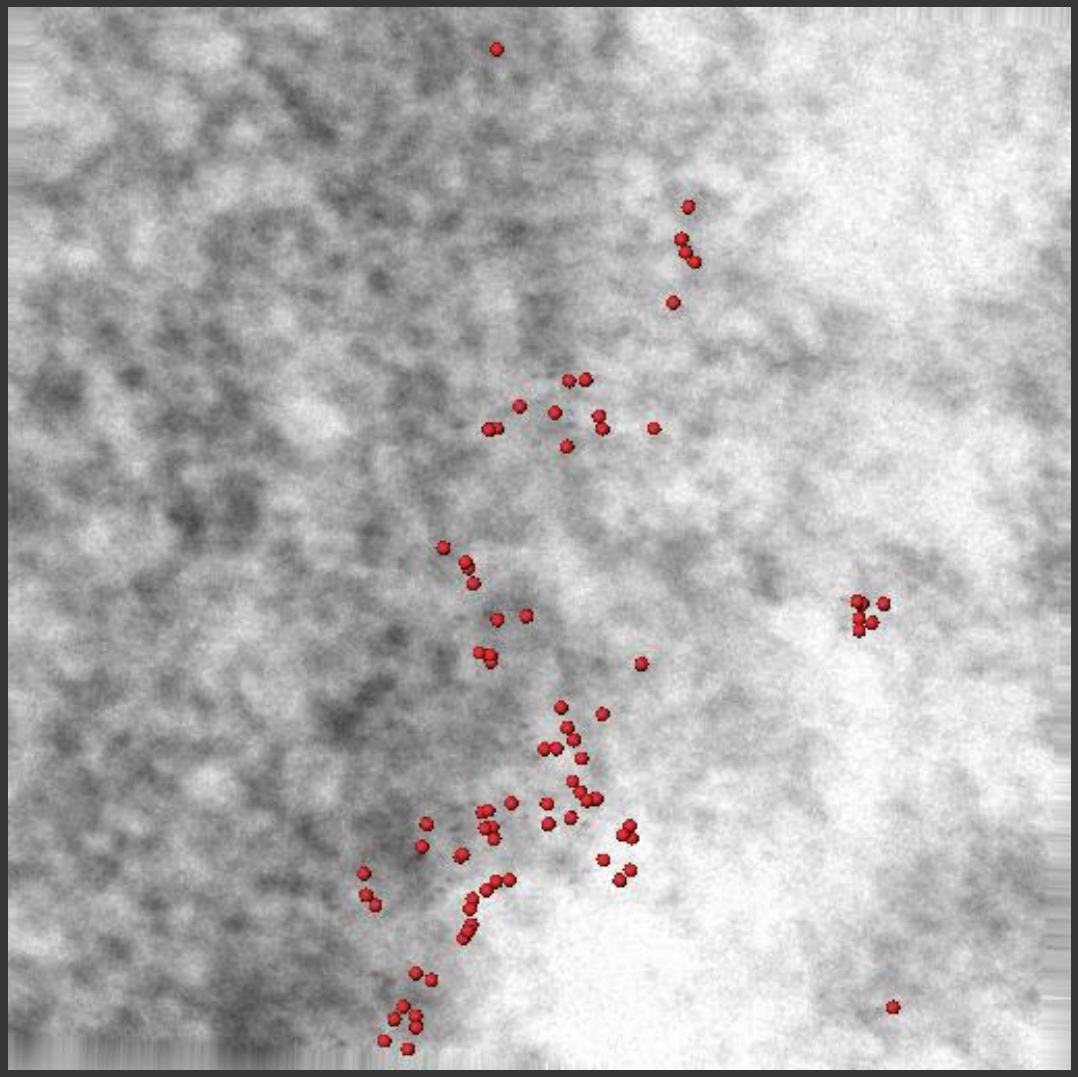


Reconstruction in IMOD



http://www2.biomed.cas.cz/~janacek/

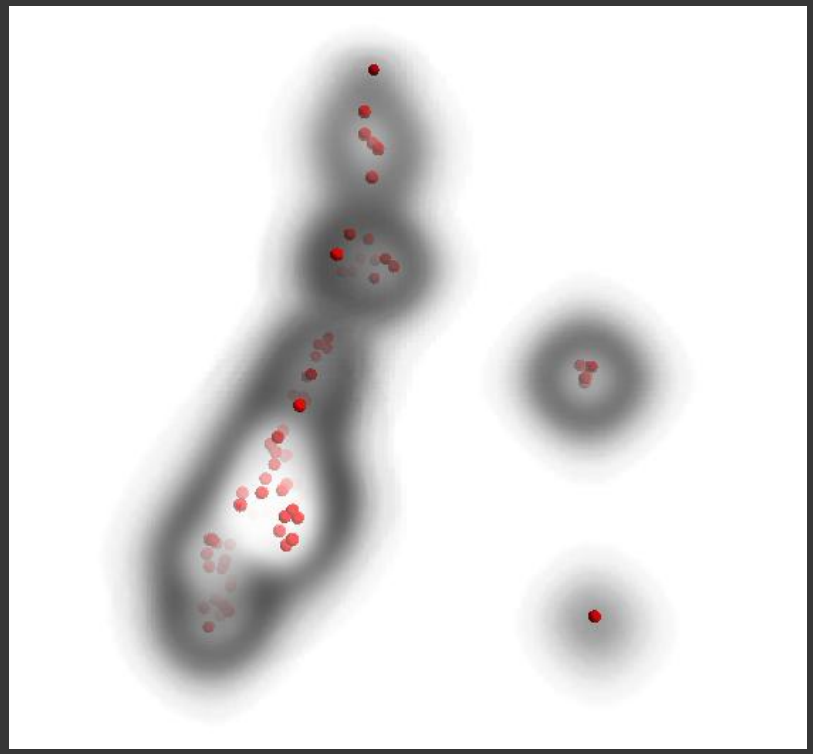
3D TEM, point data



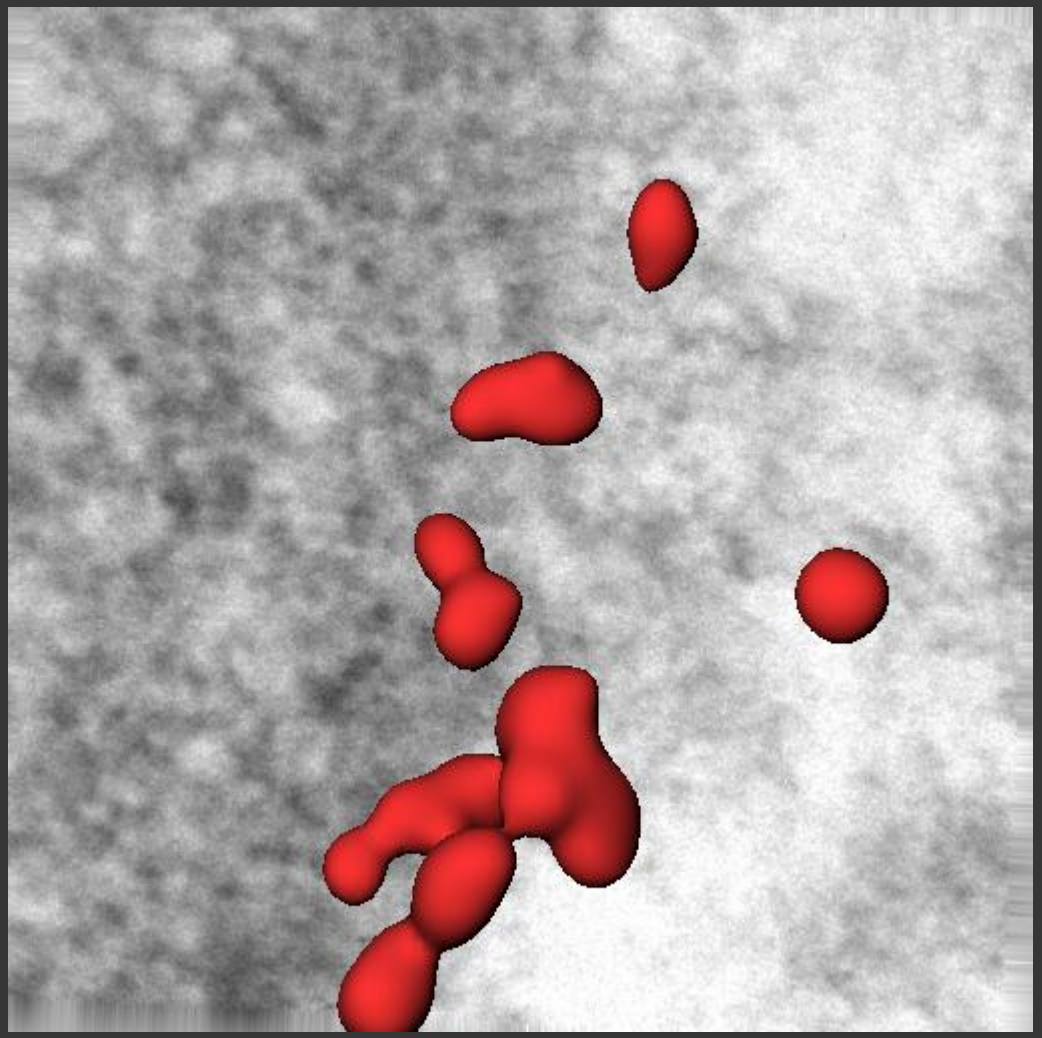
http://www2.biomed.cas.cz/~janacek/

Kernel density estimate

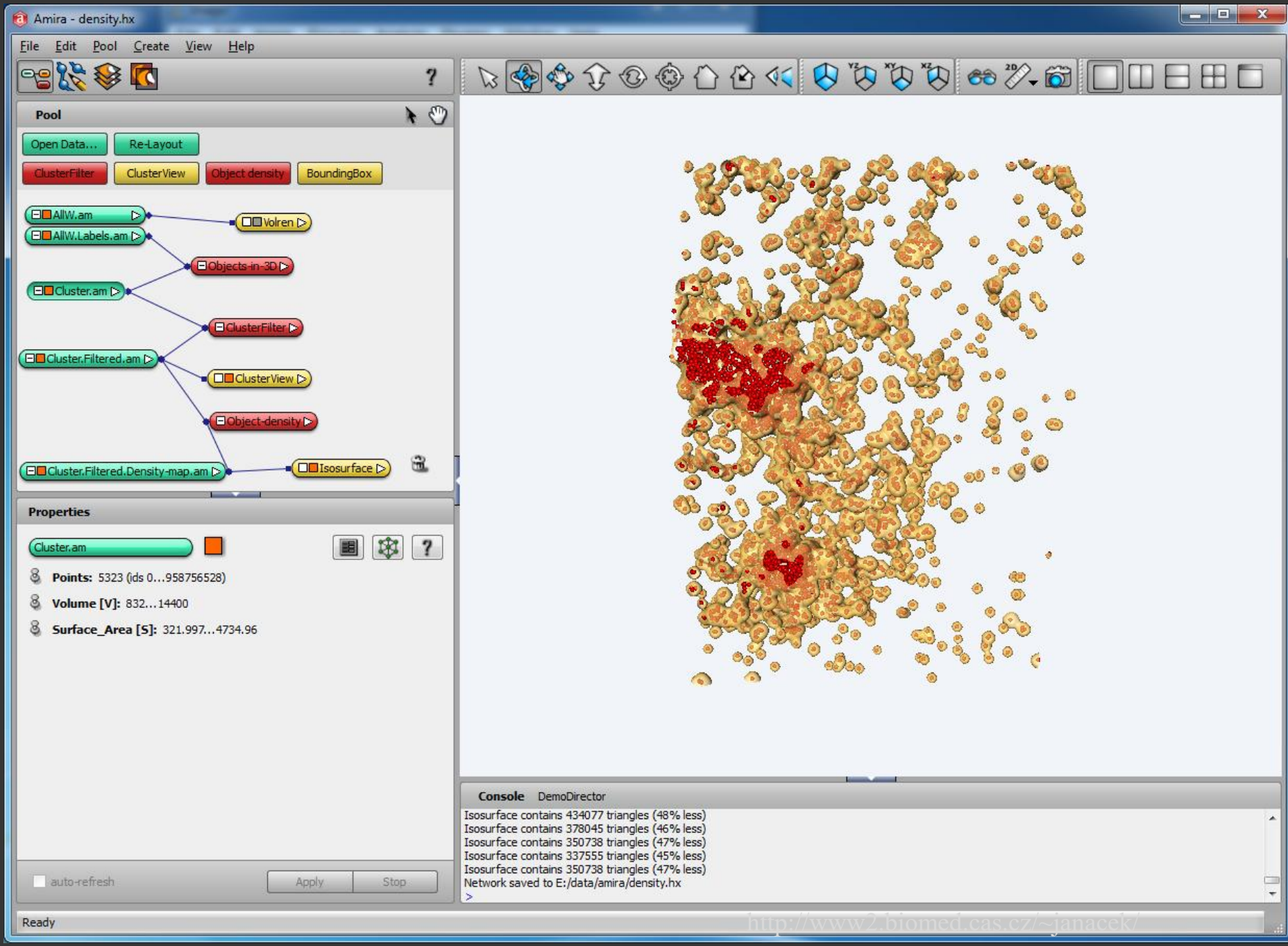
$$\lambda(x) = \sum_{y} e^{-\frac{\|x-y\|^2}{h}}$$



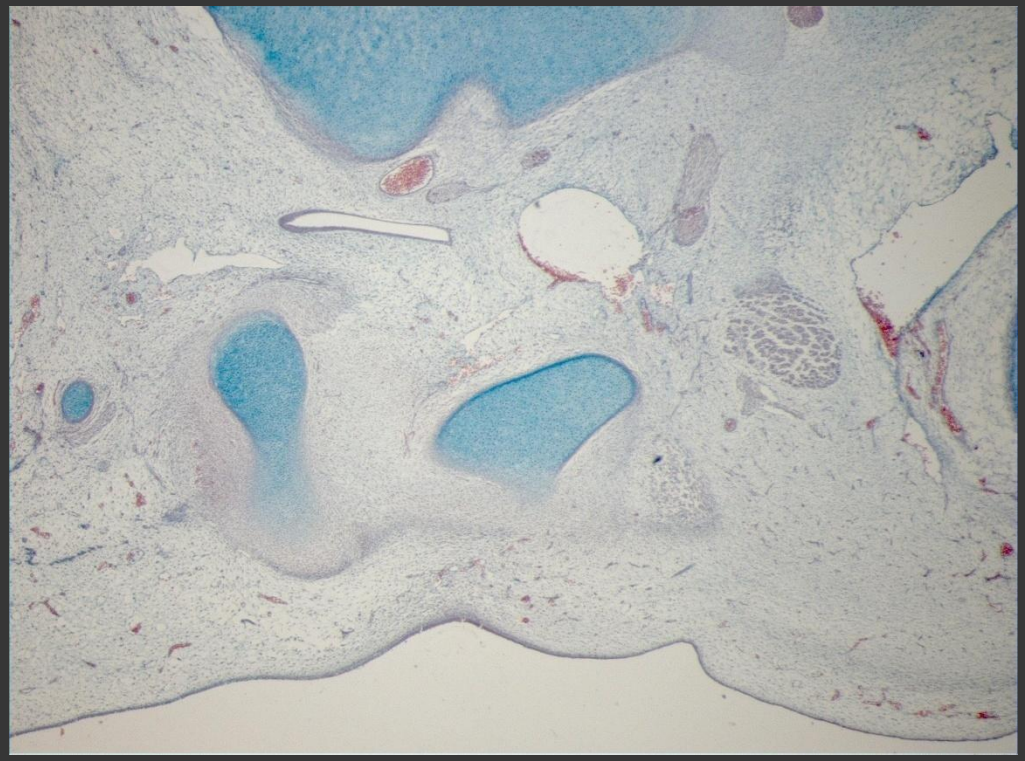
Thresholded intensity



http://www2.biomed.cas.cz/~janacek/


Amira - density.hx
File Edit Pool Create View Help
Pool
Open Data... Re-Layout
ClusterFilter ClusterView Object density BoundingBox
AllW.am
AllW.Labels.am
Cluster.am
Cluster.Filtered.am
Cluster.Filtered.Density-map.am
Volren
Objects-in-3D
ClusterFilter
ClusterView
Object-density
Isosurface
Properties
Cluster.am
Points: 5323 (ids 0...958756528)
Volume [V]: 832...14400
Surface_Area [S]: 321.997...4734.96
auto-refresh
Apply
Stop
Console DemoDirector
Isosurface contains 434077 triangles (48% less)
Isosurface contains 378045 triangles (46% less)
Isosurface contains 350738 triangles (47% less)
Isosurface contains 337555 triangles (45% less)
Isosurface contains 350738 triangles (47% less)
Network saved to E:/data/amira/density.hx
>
Ready
http://www2.biomed.cas.cz/~janacek/

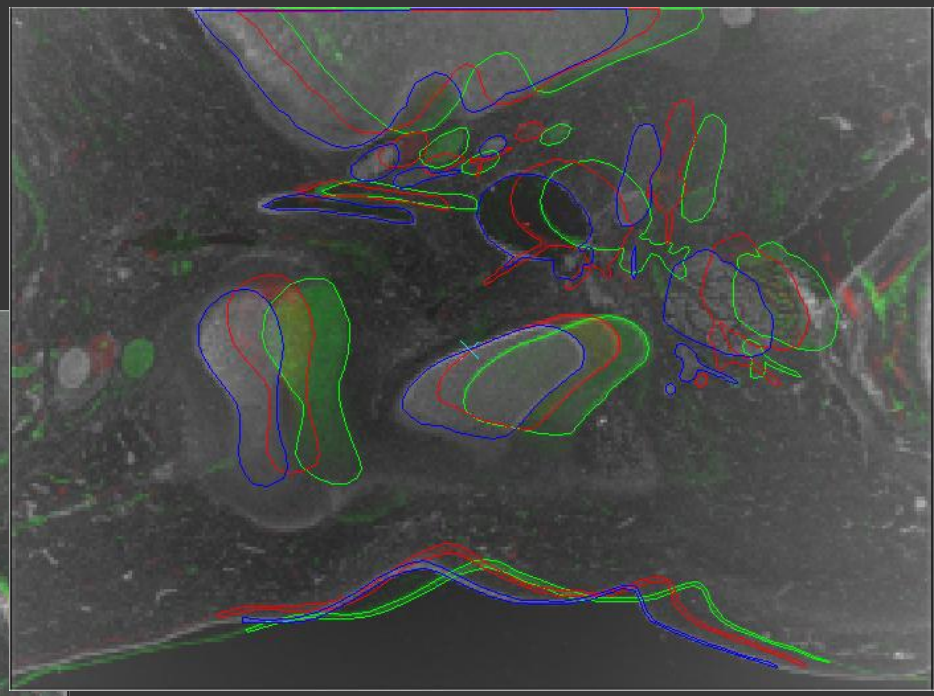
Spatial data: III) 3D reconstruction from sections

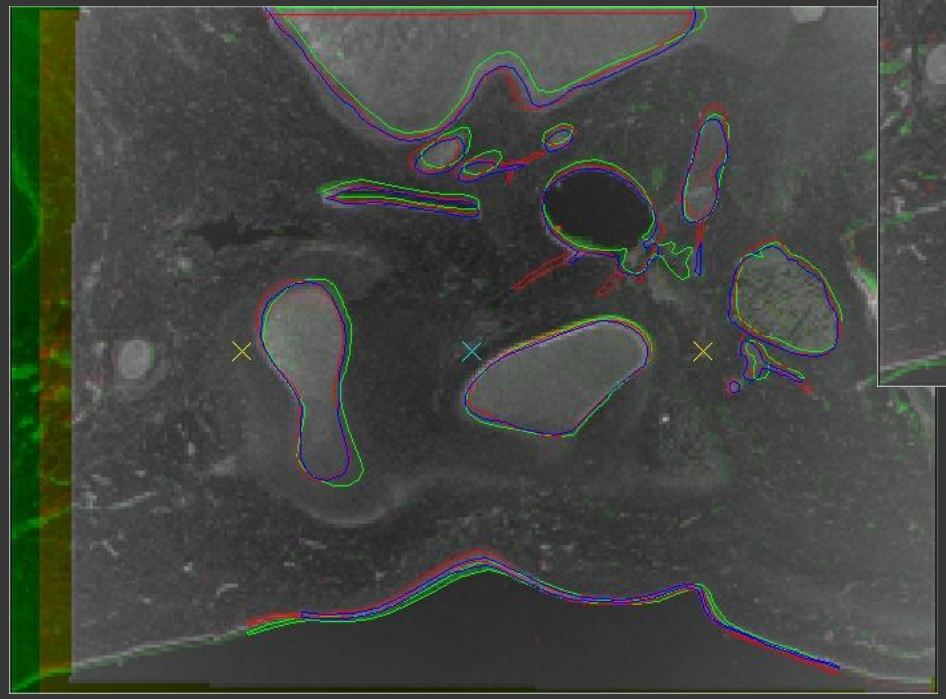


- Ostrich embryo, columela, Dr. Kundrát

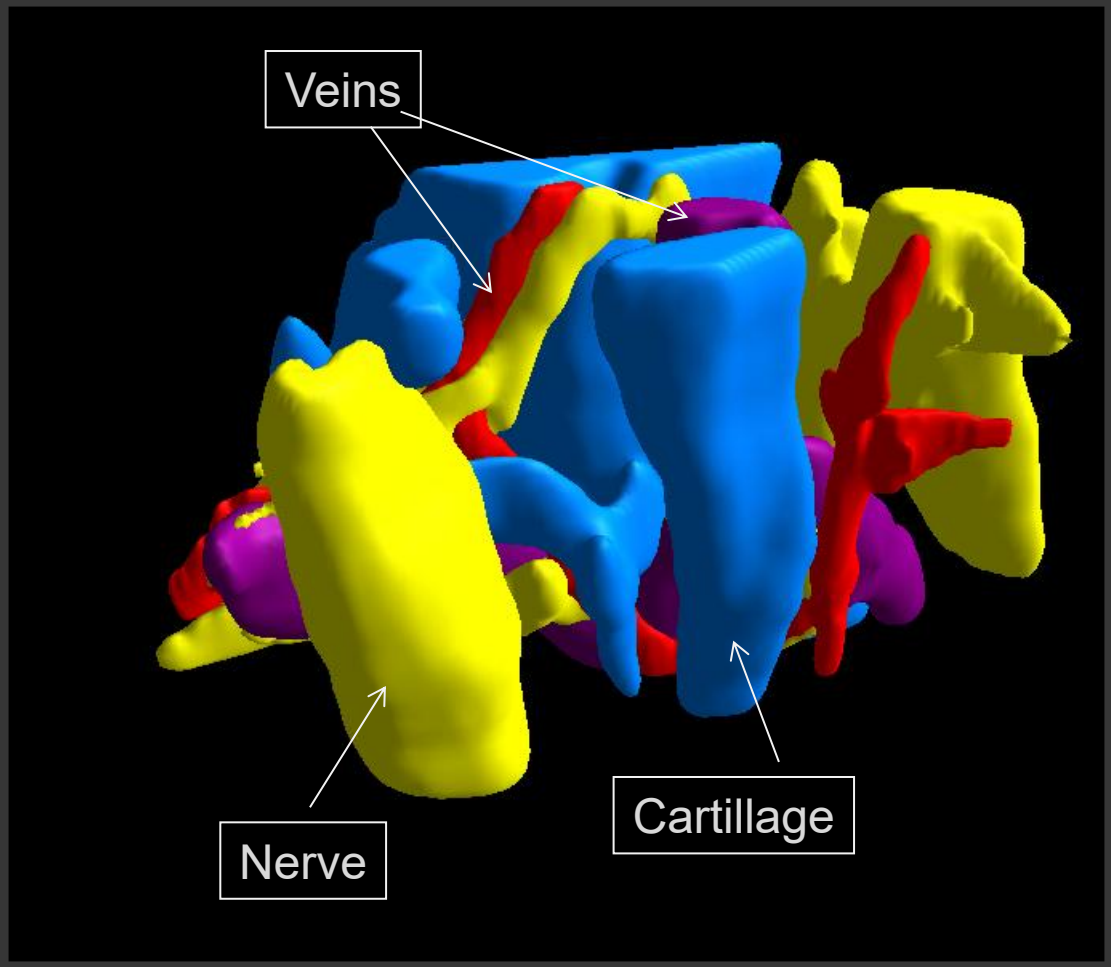
Registration

- Program ImagReg1



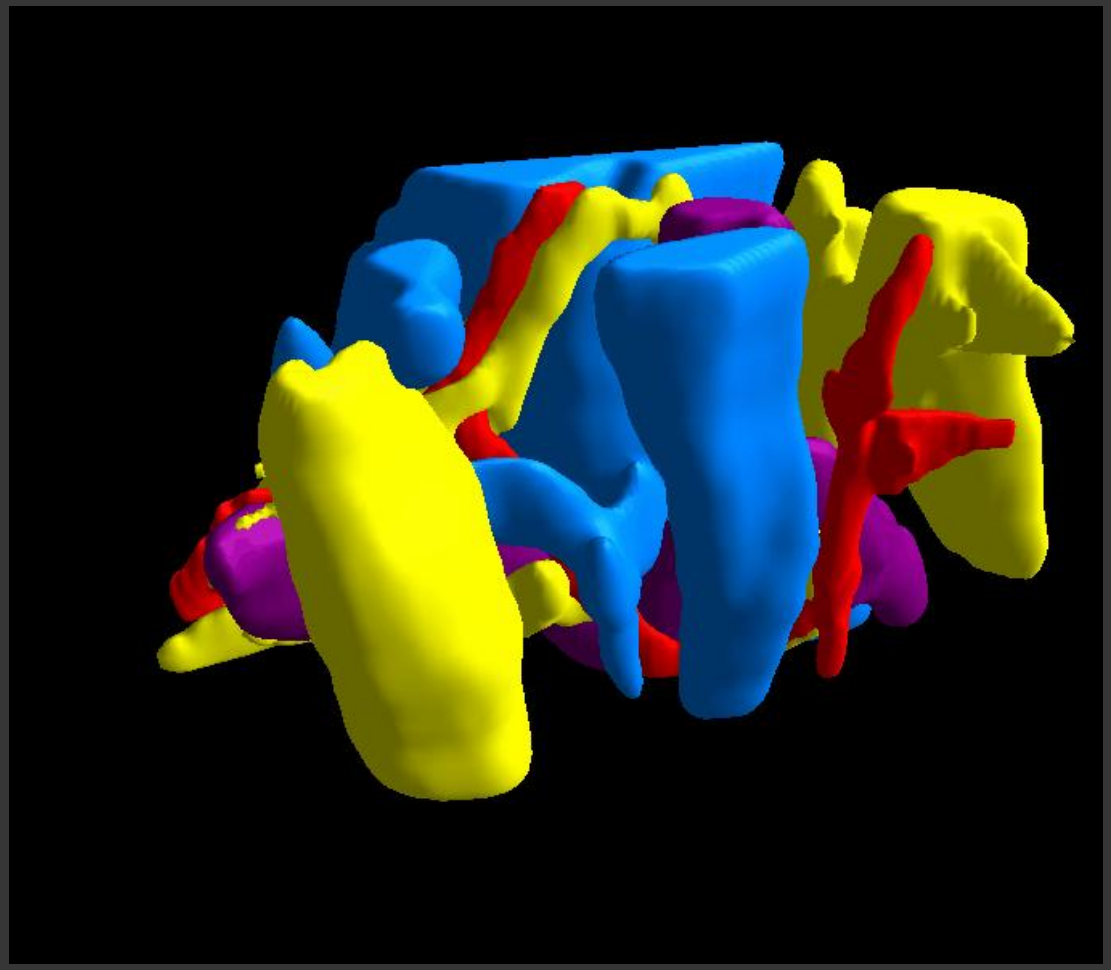


3D model



http://www.biomed.cas.cz/~janacek/

3D model



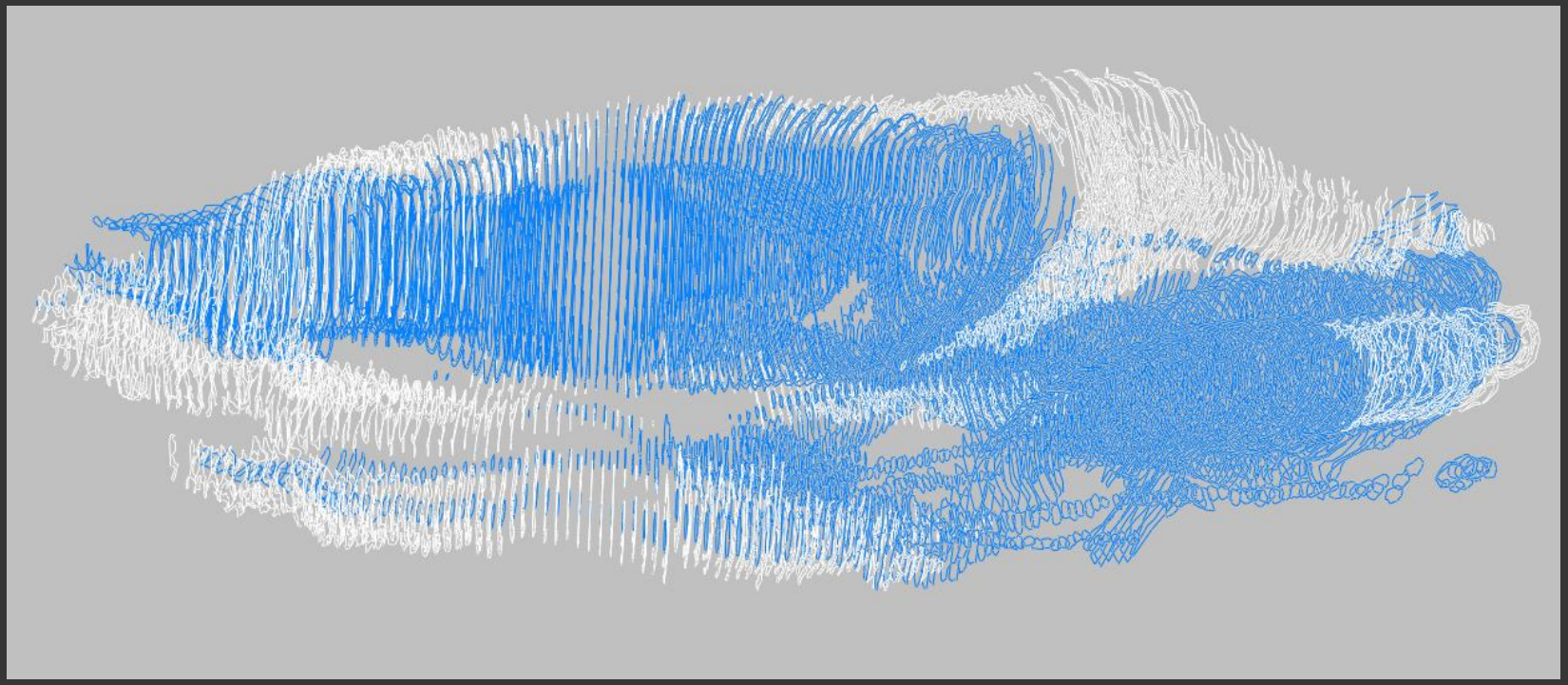
Discontinuous registration

- Deformations and fissures
- Correspondence of deformed sections by minimisation of functional (smoothness – similarity).

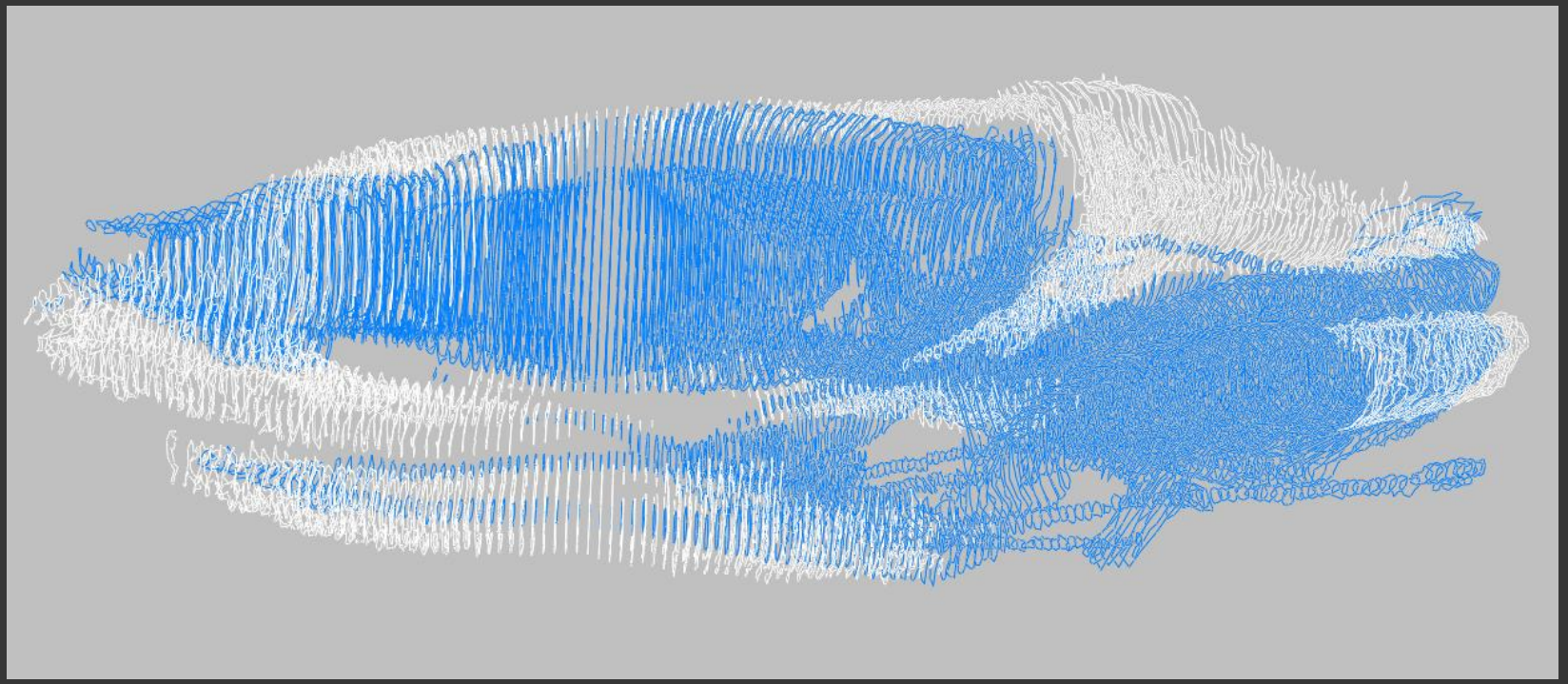
Turtle embryo head

- Dr. Kundrát, Mgr. Tvarožková

Standard registration



Discontinuous registration



Spatial data: IV) 3D roentgen tomography

- 3D samples partially opaque to roentgen irradiance
- Bones (fosilized)
- CT, micro CT, synchrotron

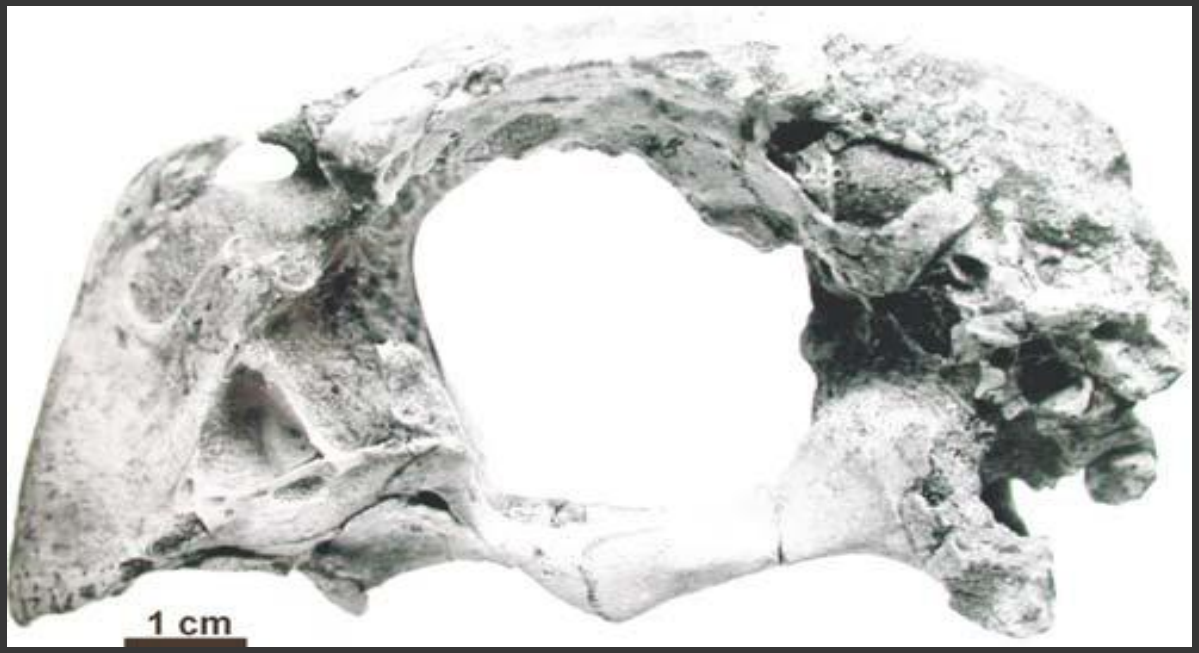
Conchoraptor gracilis Barsbold 1986 (Theropoda, Maniraptora), Dr. Martin Kundrát, Uppsala

- Late Cretaceous epoch

Conchoraptor gracilis Barsbold 1986

- Found by Polish-Mongolian Palaeontological expedition 1971 at the Hermiin Tzav locality (Upper Cretaceous), Gobi Desert, Mongolia
- Institute of Paleobiology of the Polish Academy of Sciences, ZPAL MgD-I/95

ZPAL MgD-I/95 specimen



Conchoraptor gracilis Barsbold 1986

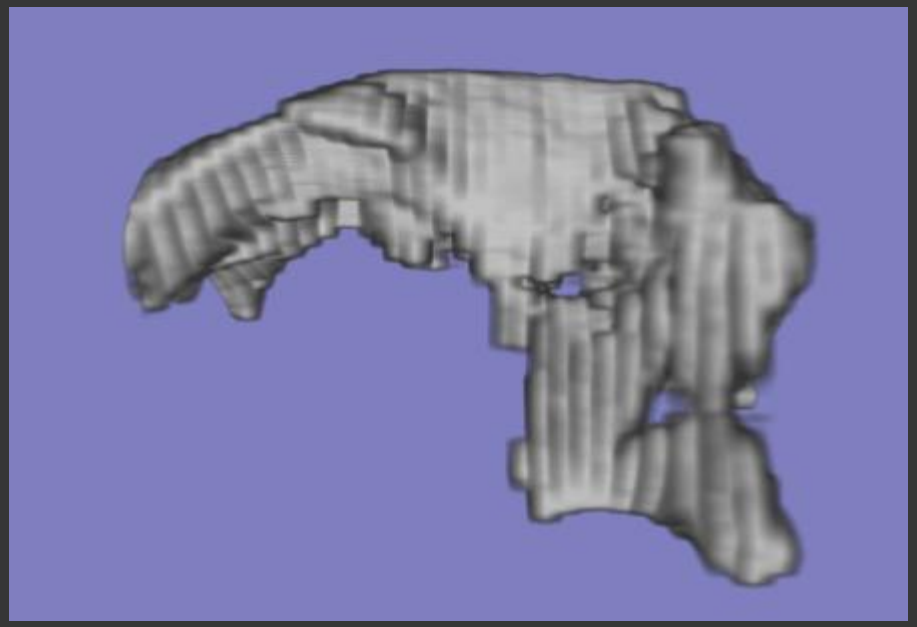
Restored profile by Vladimír Rimbala

CT scan

CT scan

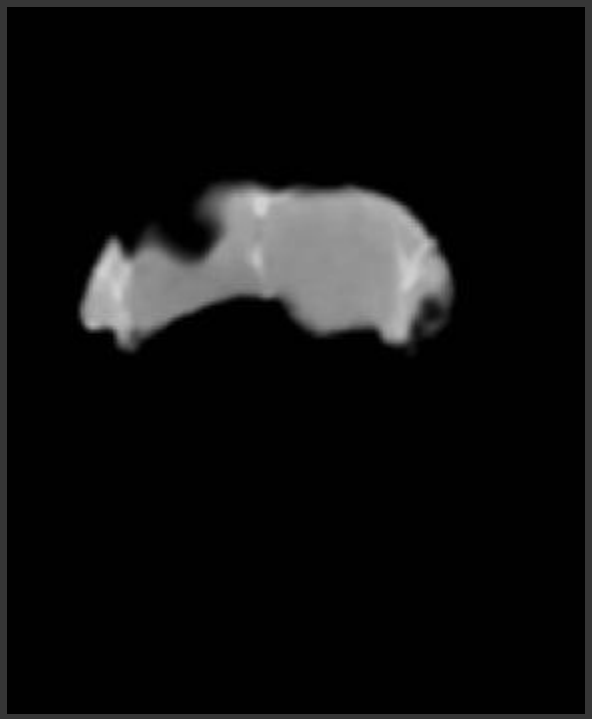
- scanned at the CT Facility of the MRI Unit (Pace Plus; General Electric) of the First Faculty of Medicine, Charles University, Prague

Volume rendering of CT scan

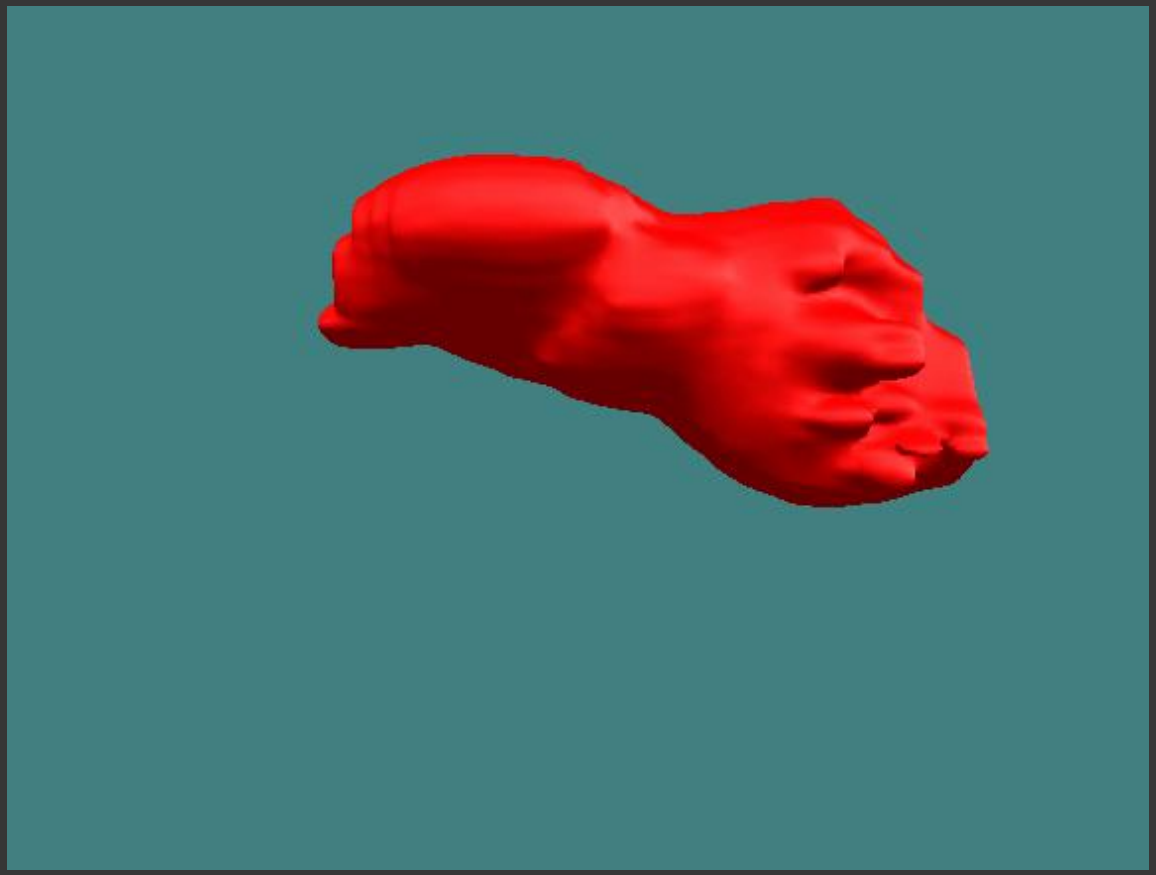


Endoneurocranium reconstruction

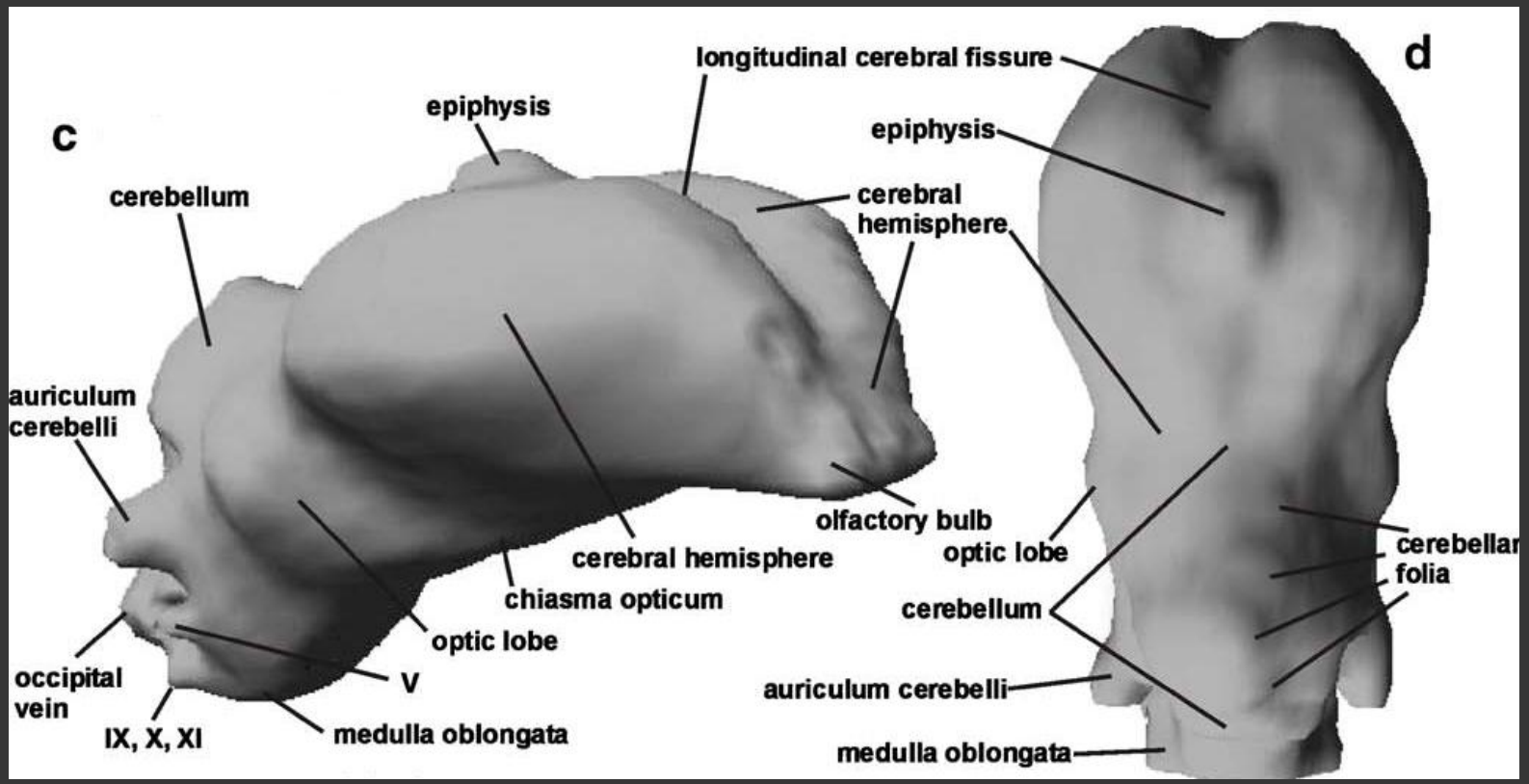
- Interactive drawing of virtual endocast contours.
- Volumetric surface reconstruction (Marching cubes by W. Lorensen).
- Ellipse (ViDiTo, Slovakia) with custom made modules



Surface rendering of endoneurocranium



Anatomy of *Conchoraptor* brain



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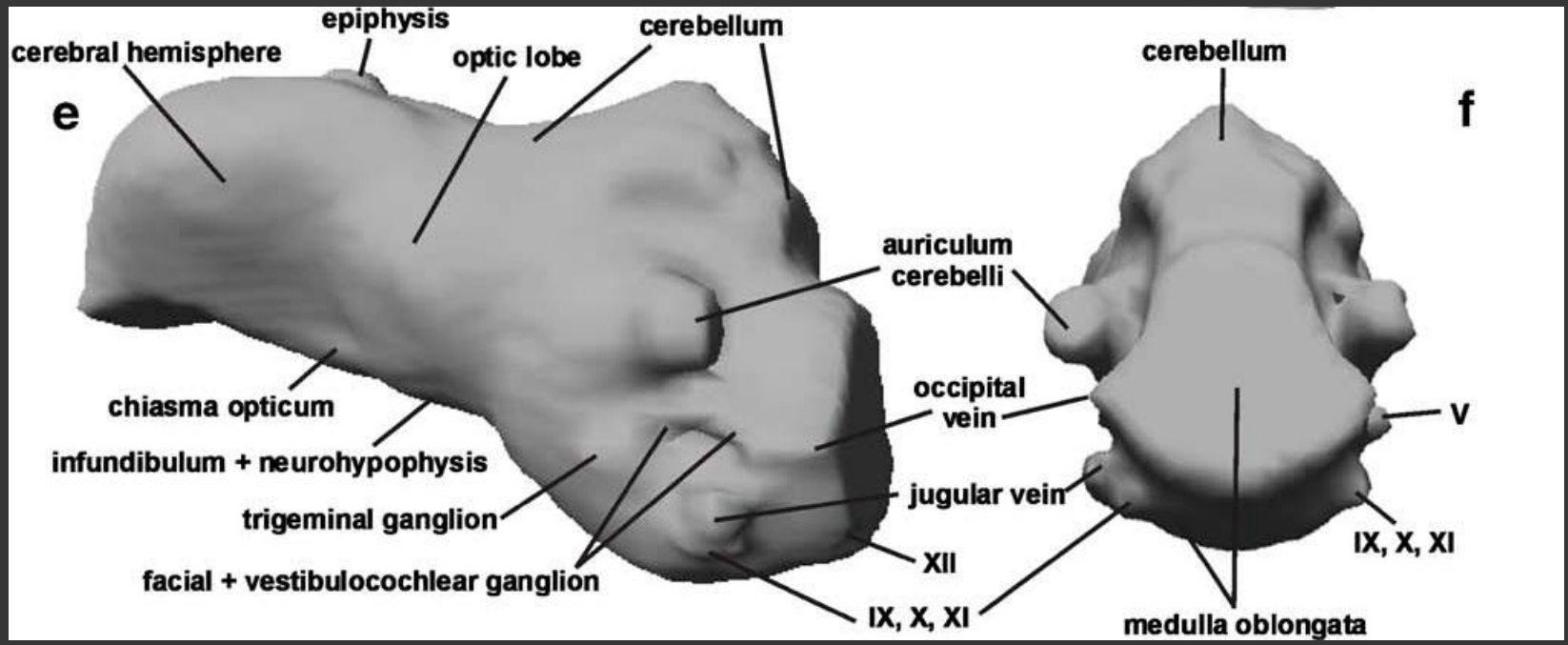
Anatomy of *Conchoraptor* brain

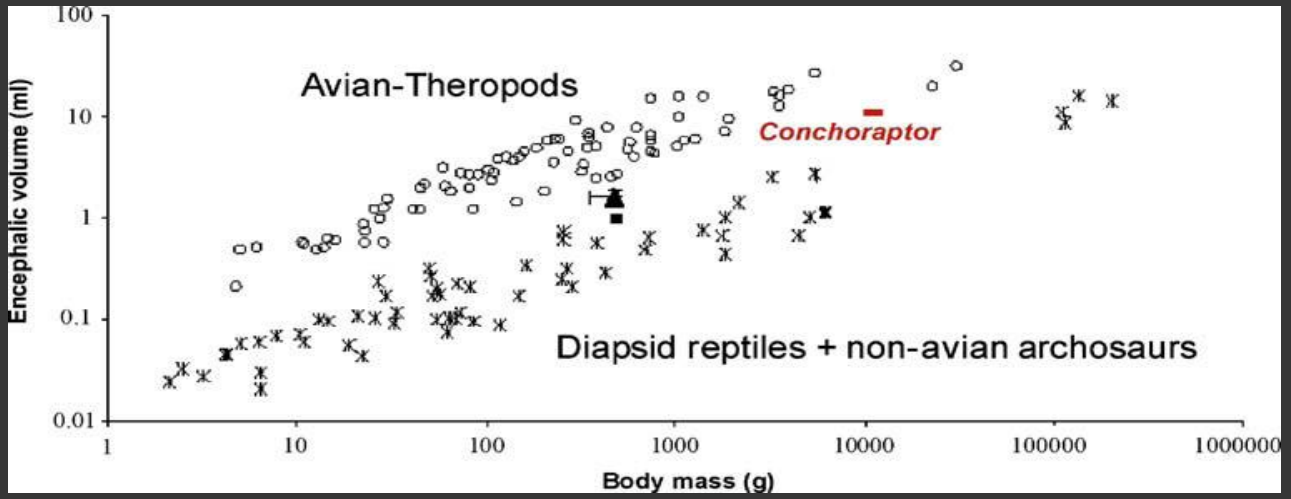


The brain volume

- V – brain volume
- d_i – ith section thickness
- A_i – ith section area

$$V = \sum_{i=1}^{n} d_i A_i$$

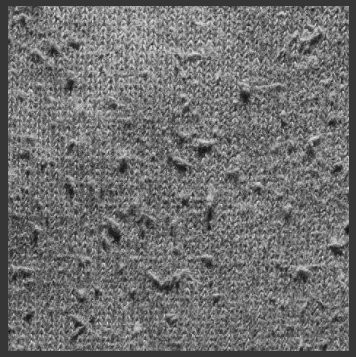
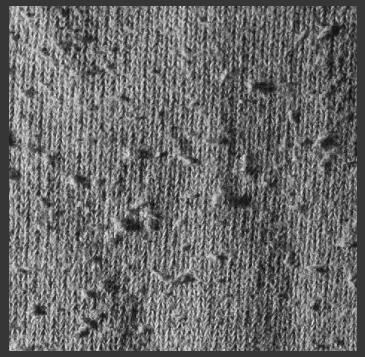
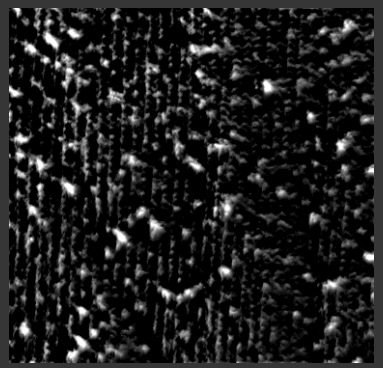
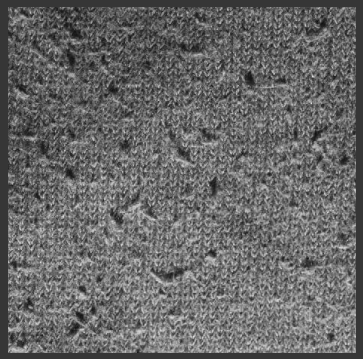
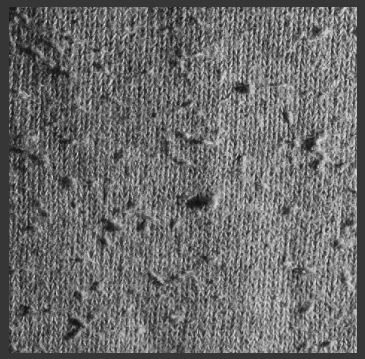
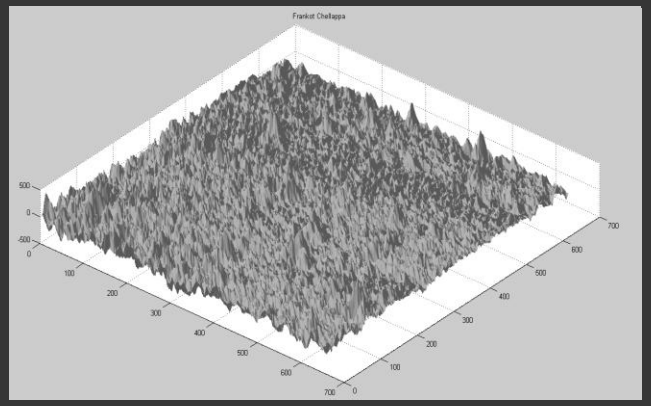
Conchoraptor brain volume: 14.6 ml



Spatial data: V) 3D imaging

- Photogrammetry: multiview or stereo, structure from motion; structure from shading or texture.
- Kinect (IR laser speckles)
- Laser range finder
- Optical coherence spectroscopy

Shape from shading

Ing. Techniková, TUL

Fabric pilling analysis

3D software

- ImageJ, FiJi, MorpholibJ
- 3DSlicer
- Amira, Avizo
- 3DStudioMax
- Imaris