UNIVERSITÀ DEGLI STUDI DI TORINO

Neuroscienze "Rita Levi Montalcini" Dipartimento di eccellenza

جامعة الملك عبدالله للعلوم والتقنية
King Abdullah University of Science and Technology

NICO
Neuroscience Institute Cavalieri Ottolenghi

The Neuroanatomical Basis of Brain Energy Metabolism:

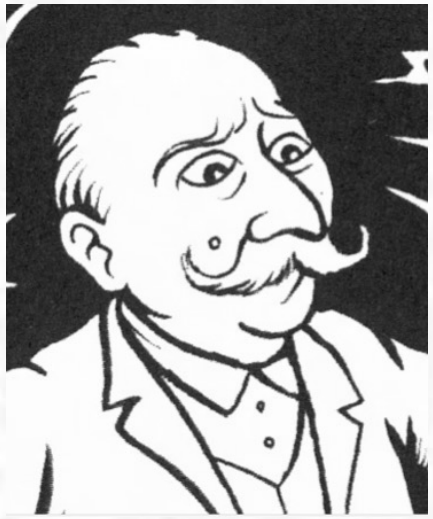
Investigating Neuroanatomical basis of Brain-Energy Metabolism using 3D models and VR tools.

Corrado Calì, PhD

Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano (TO), Italy
Department of Neuroscience, Unversity of Torino (TO), Italy
King Abdullah University of Science and Technology, BESE Division, Thuwal, Saudi Arabia

Astrocytes: the metabolic partner

Santiago Ramón y Cajal (1852 - 1934)



Camillo Golgi (1843 - 1926)

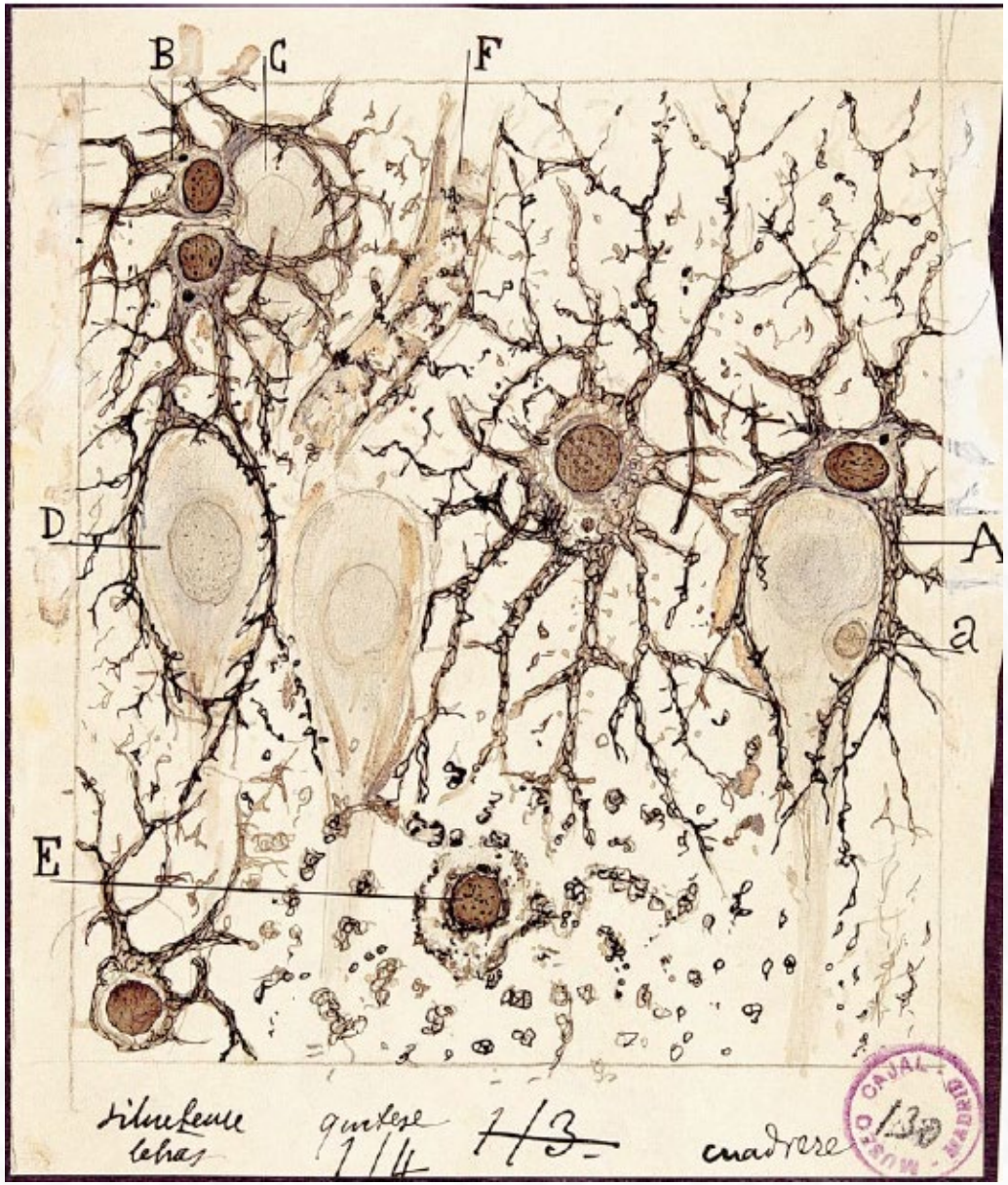
"Neuroglia of the pyramidal layer and stratum radiatum of the Ammon horn. Adult man autopsied three hours after death. Chloride of gold.

(A) big astrocyte embracing a pyramidal neuron.

(B) twin astrocytes forming a nest around a cell

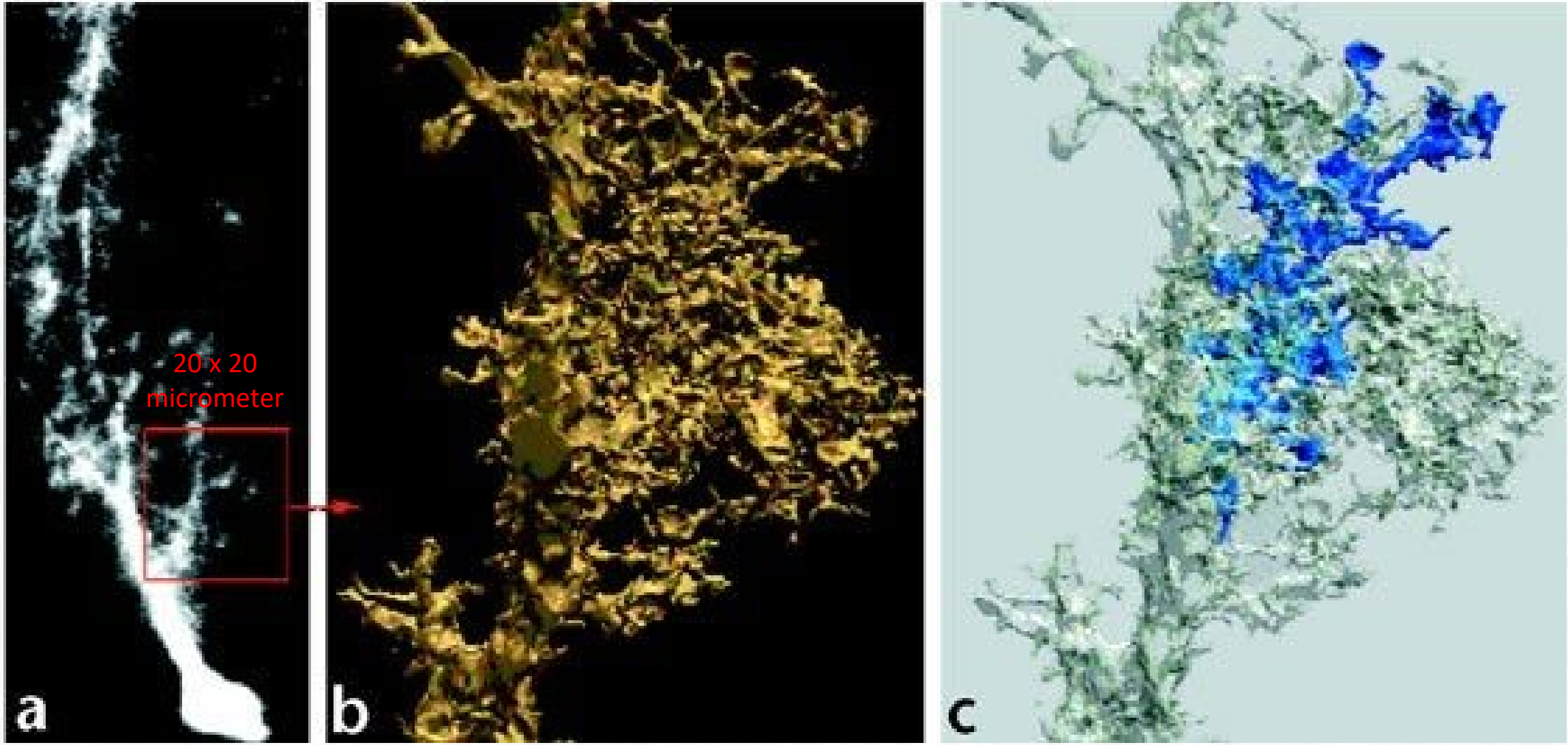
(C) while one of them sends two branches forming another nest (*D*).

(D) cell with signs of autolysis".



Ramòn y Cajal, S Histologie de systeme nerveux de l'homme et des vertebras, 1911

Recent history of 3D Glia Electron Microscopy



450 serial sections
22.5 micrometer

Grosche et al., Nature Neuroscience, 1999

Recent history of 3D Glia Electron Microscopy

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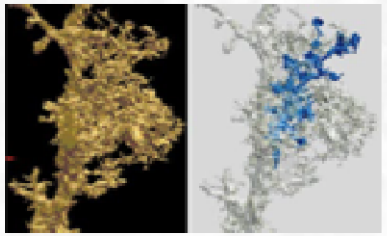
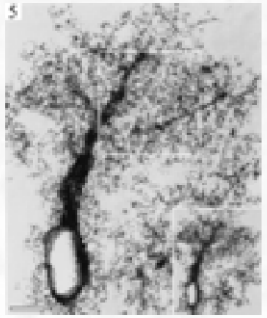
1999 — Grosche et al., Nature Neuroscience



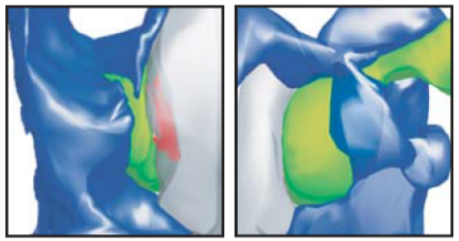
Bergmann Glia

2004 — Hama et al., Journal of Neurocytology



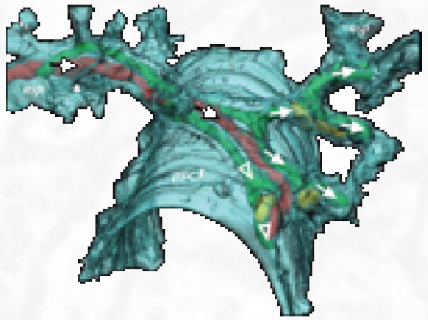
Astrocyte, dentate gyrus

2006 — Genoud et al., Plos Biology



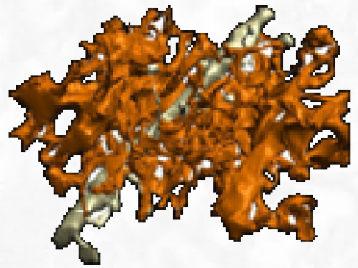
Layer IV Somatosensory Cortex, perisynaptic astrocytic processes

2010 — Mathiesen et al., Glia



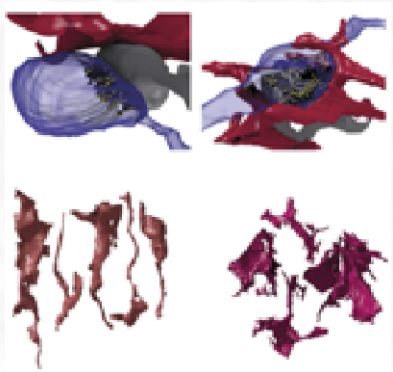
CA1, Astrocytic endfoot

2014 — Ostroff et al., Journal of Comp. Neurology



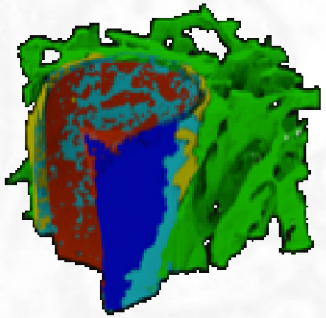
Amigdala, astrocytic process, neuropil

2015 — Korogod et al., eLife



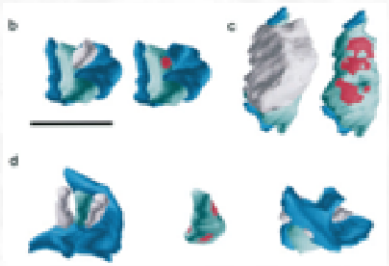
Barrel cortex, astrocytic processes, neuropil

2016 — Cali et al., Journal of Comp. Neurology



CA1 perivascular and endfoot process

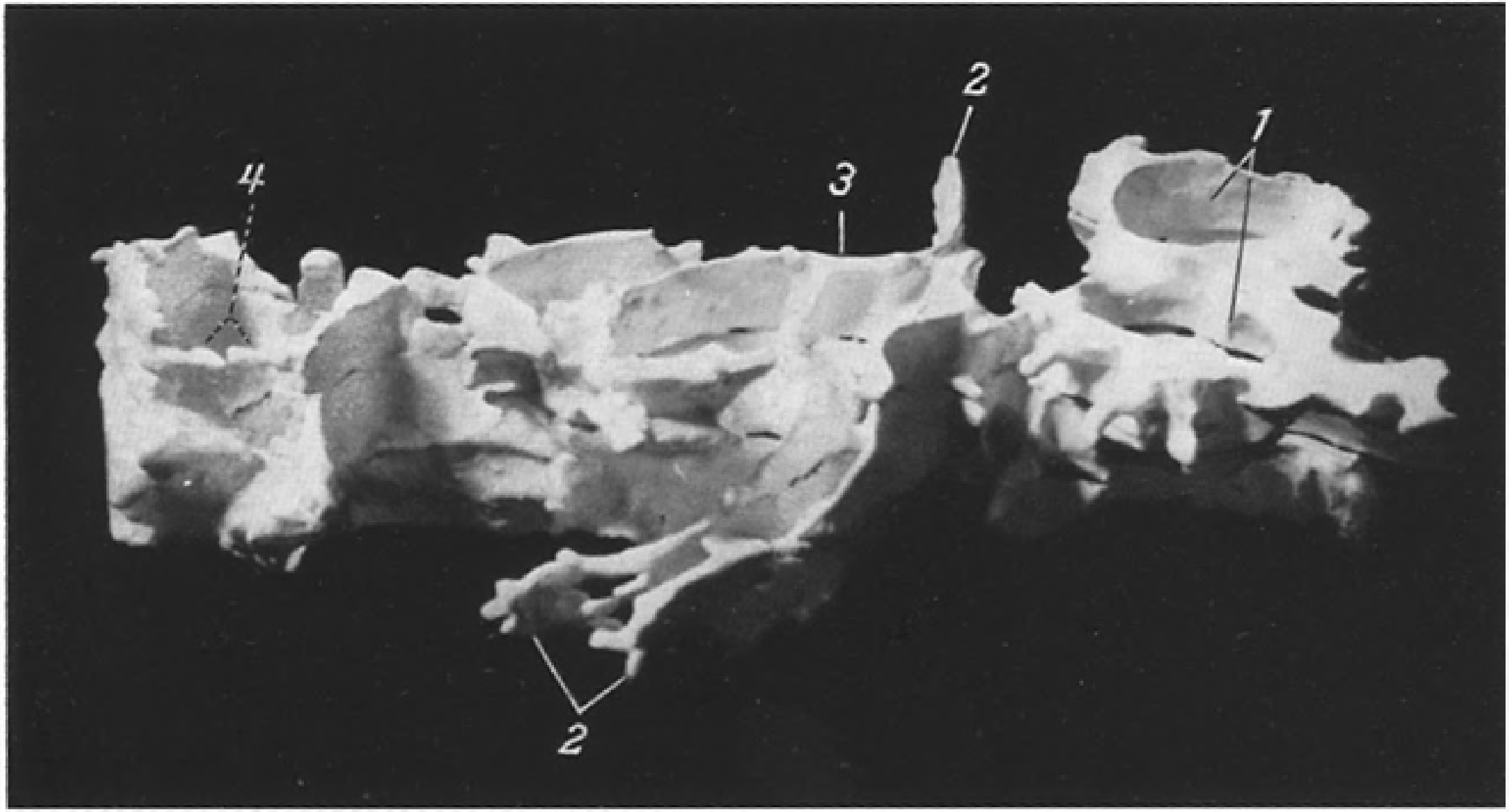
Ventura and Harris, Journal of Neuroscience



Hyppocampal perisynaptic astrocytic processes

Calì et al., Journal of Translational Neuroscience, 2018

Prehistory of 3D Glia Electron Microscopy



Wolff, Zeitschrift fiir Zetlforschung, 1965

Prehistory of 3D Glia Electron Microscopy

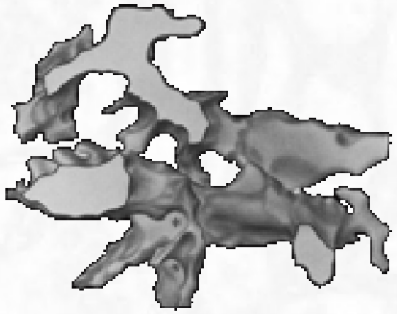
Stentaas and Stentaas, **Zeitschrift fiir Zetlforschung**

Astrocytic process, peripheral plexus

Spacek, Z. Zellforsch

Astrocytic process, cerebral cortex

Spacek, Anatomy and Embryology



Astrocytic process, cerebral cortex

1965 1968 1969 1971 1985

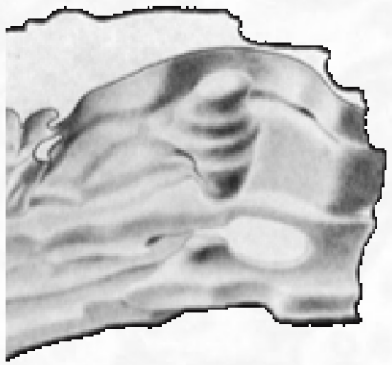
Wolff, **Zeitschrift fiir Zetlforschung**

Astrocytic process, cerebral cortex

Poritsky, Journal of Comparative Neurology

Astrocytic process, peripheral plexus

Spacek, Z. Zellforsch

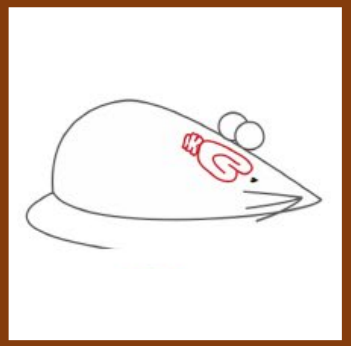


Astrocytic process, ventrobasal nucleus

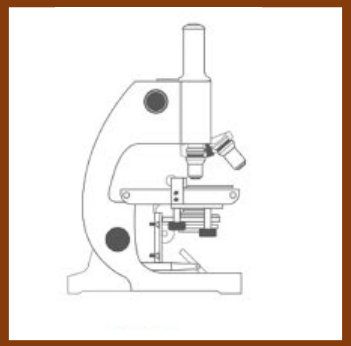
3DEM on astrocytes is 55 years old.

Why is this a good moment to study the 3D Structure of astrocytes?

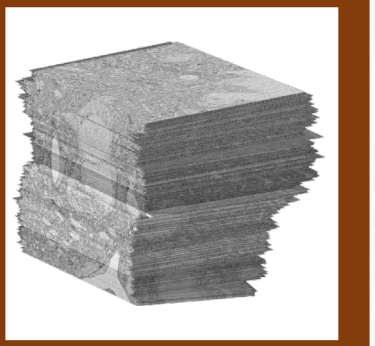
Adapting Connectomics to Glia



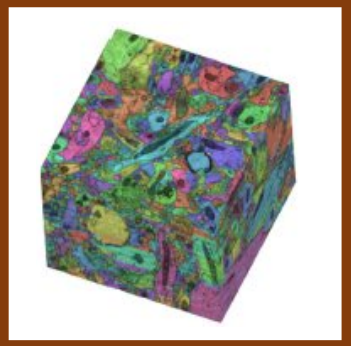
Mouse Brain



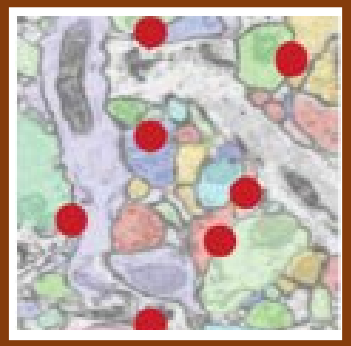
Electron Microscope



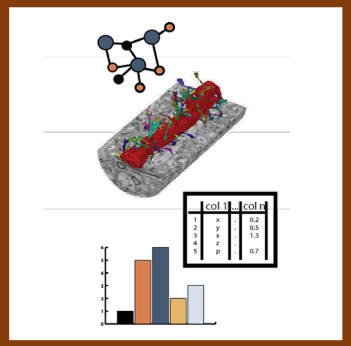
Volume



Segmentation



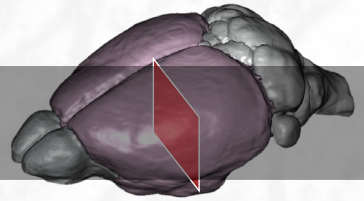
Labeling



Visualization & Analysis

We adapted an approach developed for connectomics to develop analytical techniques for the study of the ultrastructure of glial cells

Adapting Connectomics to Glia



P14 Rat brain

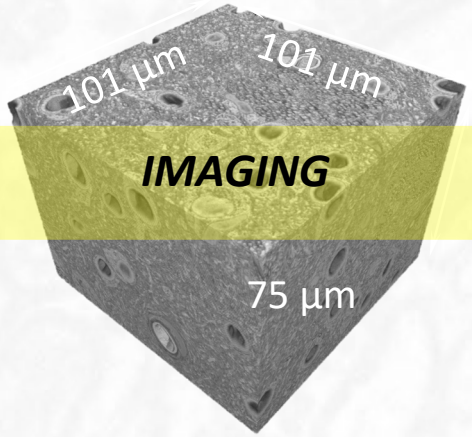
Sample

Somatosensory cortex

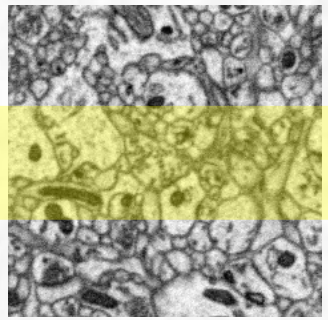
Preparation

Layer VI Block (To 3View)

3View SEM



High resolution EM stack



Segmentation

Coggan, Calì et al., Frontiers in Neuroscience, 2018

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Ultrastructural characterization of astrocytes from sparse reconstruction from 3DEM

DEPARTMENT OF NEUROSCIENCE "RITA LEVI MONTALCINI"

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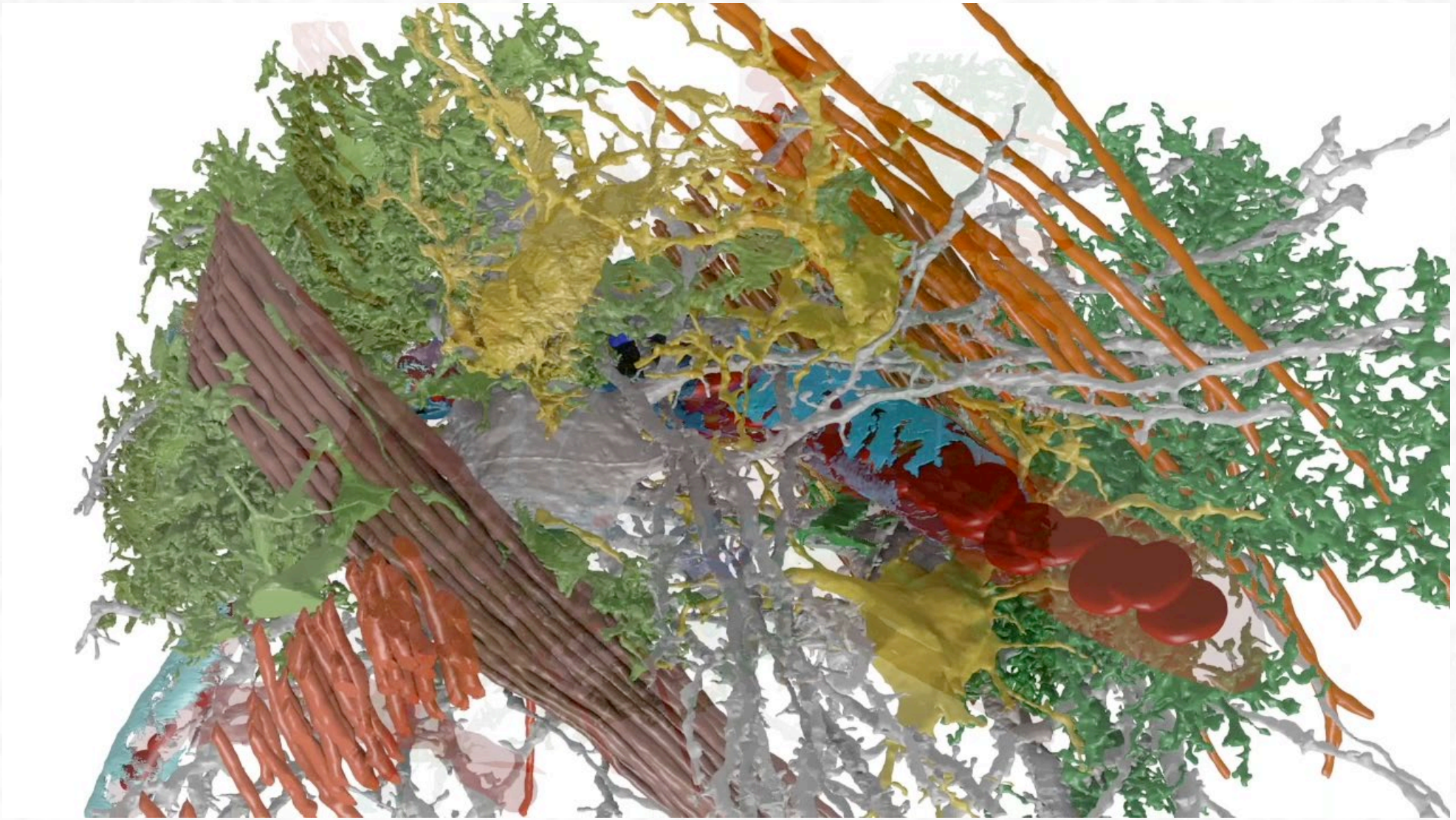


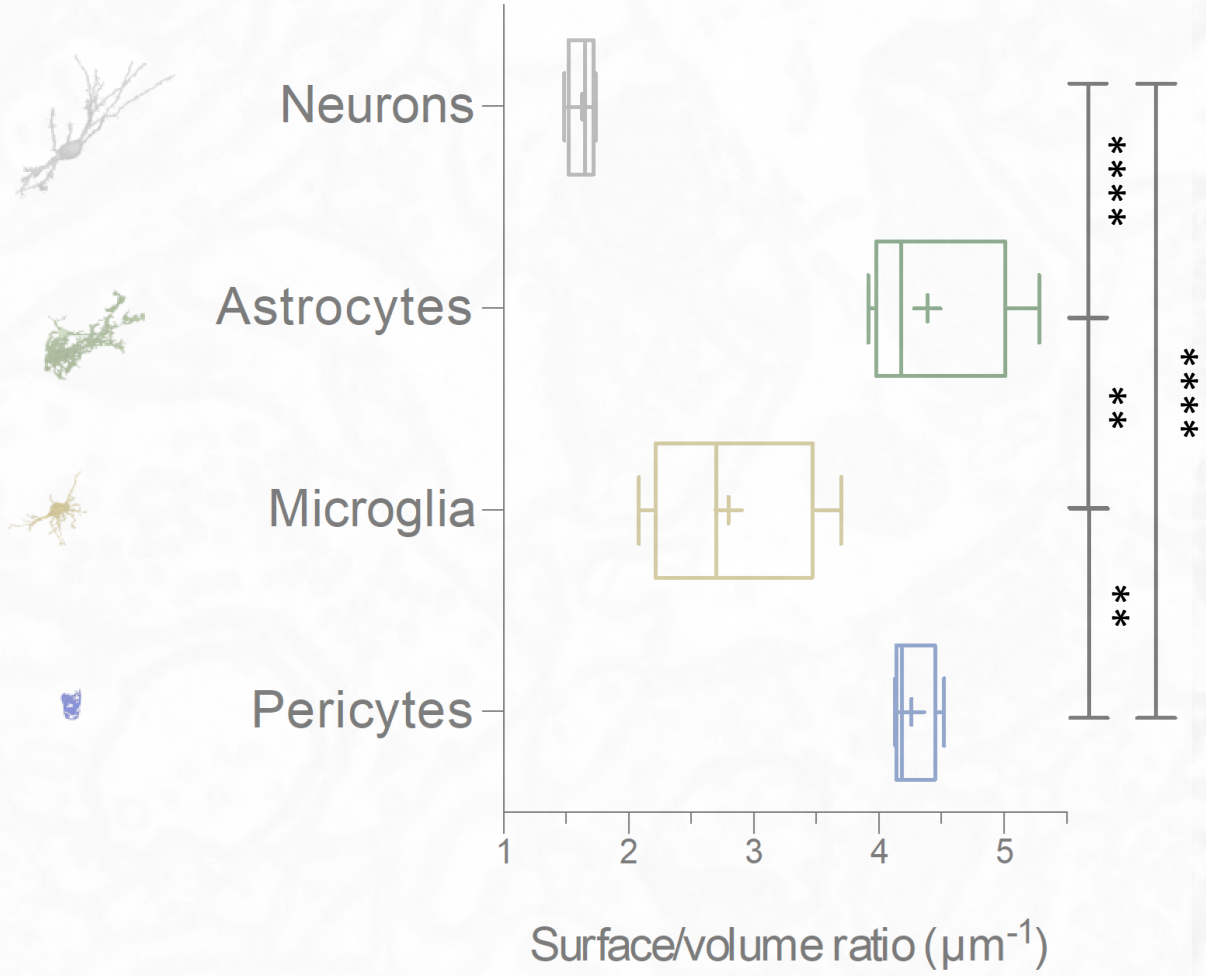
Manual pipeline:
TrakEM2
Slower, more reliable

Sami-automated pipeline:
iLastik, oversegmentation
TrakEM2, proofreading
Faster; proofreading very labor-intensive

Structure	Reconstructed
Nuclei	186
Blood Vessels	2
Myelinated axons	213 (4 bundles)
Neurons	4
Astrocytes	4
Microglia	4
Pericytes	4
Full morphologies	**16**

Calì et al., Progress in Neurobiology, 2019

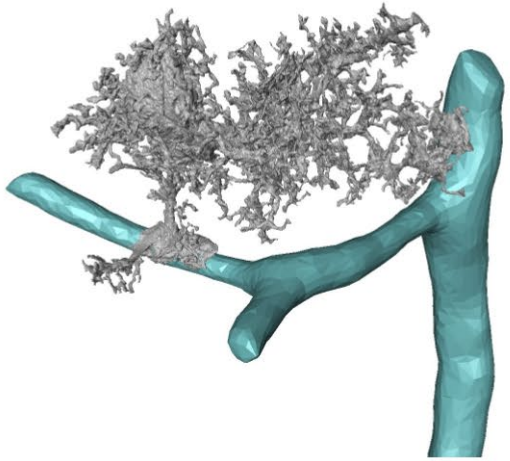
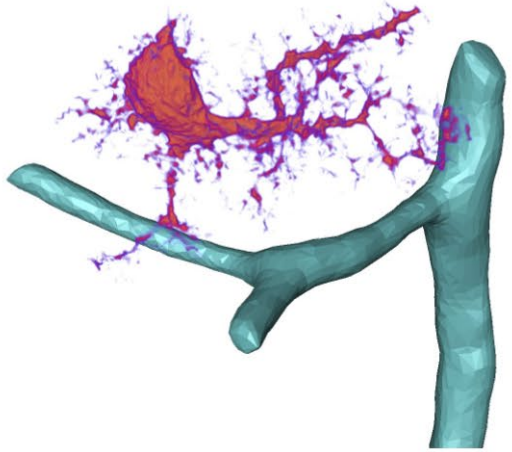
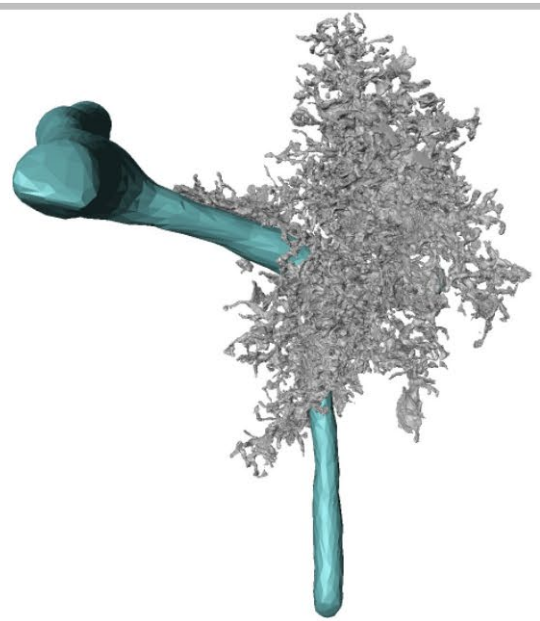
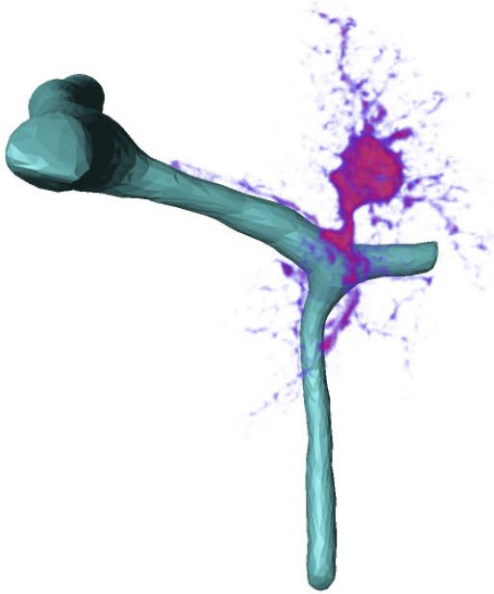
Ultrastructural characterization of astrocytes from sparse reconstruction from 3DEM



- Neurons have a less complex morphology and therefore a smaller surface to volume ratio
- Astrocytes have a much fractal morphology therefore a higher surface to volume ratio
- Is an index of how much the cell is interfacing with its environment versus its tendency of processing signals

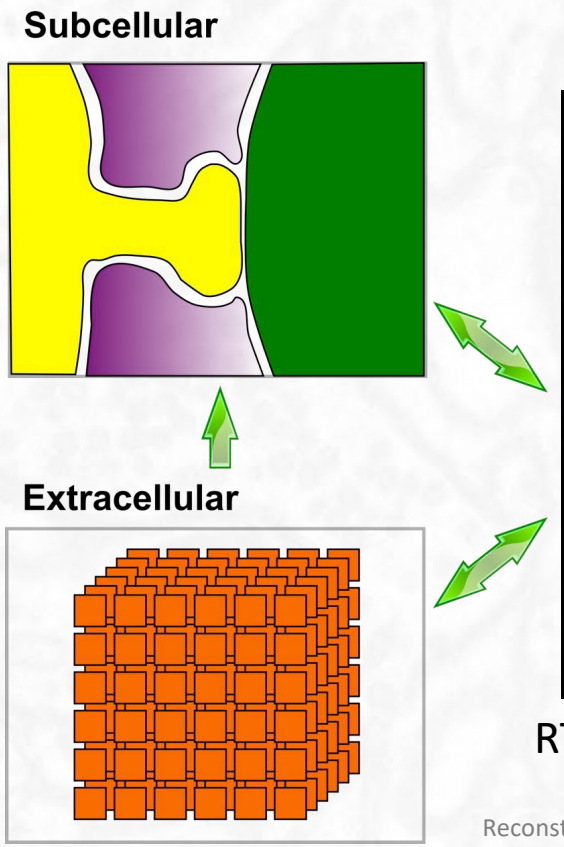
Calì et al., Progress in Neurobiology, 2019

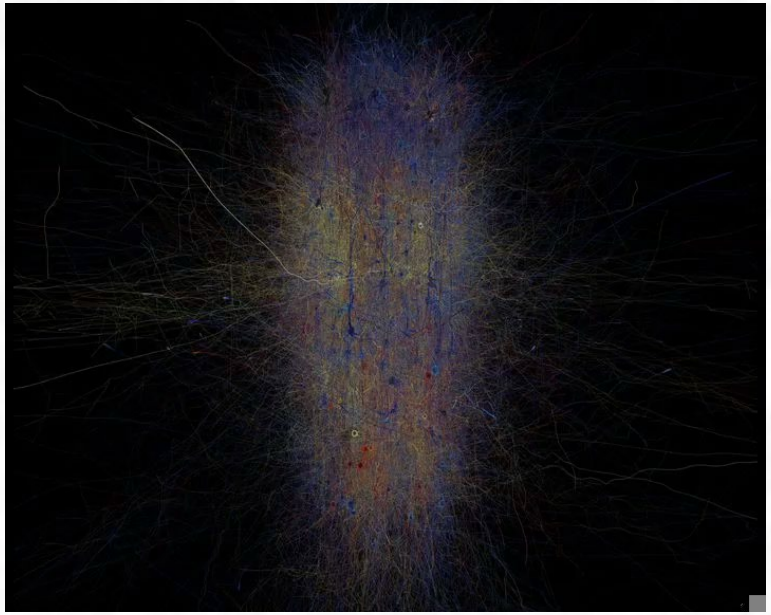
Ultrastructural characterization of astrocytes from sparse reconstruction from 3DEM

Full mesh	Thinned volume rendering	Qualitative Assessment
		Astrocyte 1 Primary processes: 4 Total Branches: 1853 Perivascular processes: 2
		Astrocyte 2 Primary processes: 4 Total Branches: 6001 Perivascular processes: 1

Calì et al., Progress in Neurobiology, 2019

What is the value in providing a detailed morphological description of astrocytes?





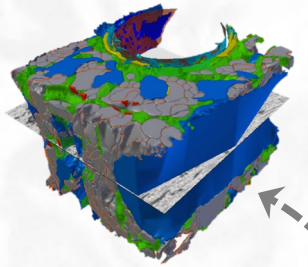
RTNeuron: simulation of a 1mm^3 rat brain

Reconstruction and simulation of Neocortical Microcircuitry. Markram et al., 2015, *Cell*

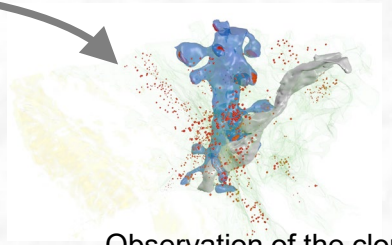
Compartmentalization: does it matter?

- Branching order (complexity)
- Microdomains/subdomains
  - Ontology
  - Function

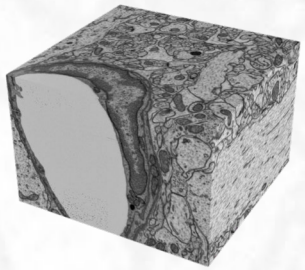
VR Analysis of astrocytic morphologies



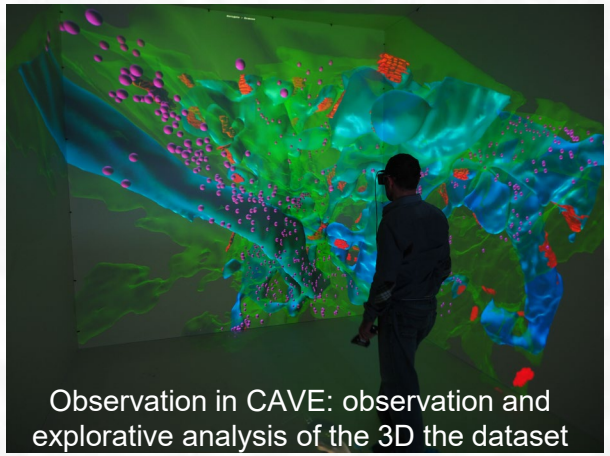
3D Reconstruction



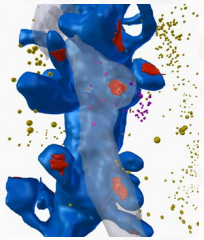
Observation of the cloud of points



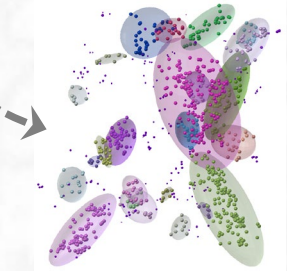
Serial Section Electron Microscopy



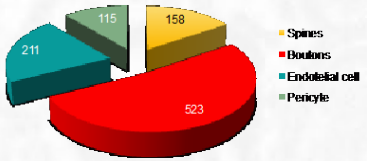
Observation in CAVE: observation and explorative analysis of the 3D the dataset



Hypotheses: non random distribution



Clustering (DBSCAN)



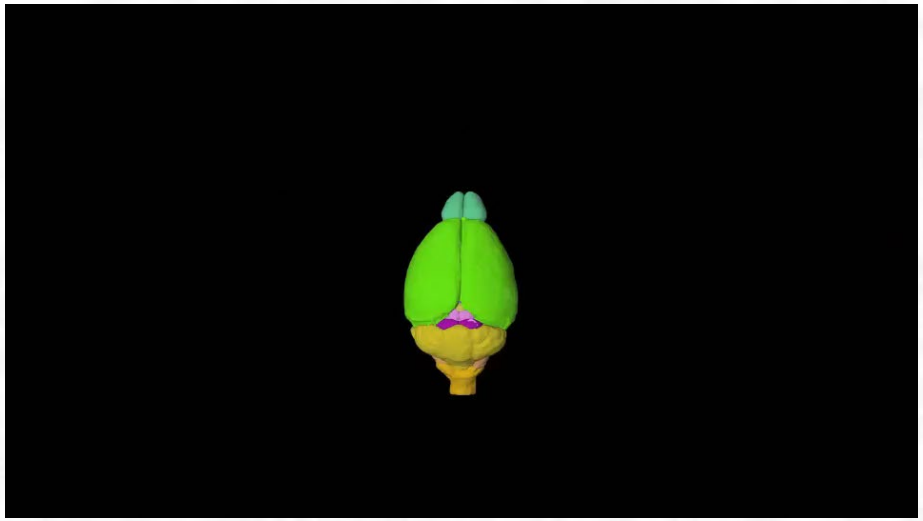
Quantitative analysis of the distribution in 3D

Calì et al., Journal of Comparative Neurology, 2015

VR Analysis of astrocytic morphologies

Collaborative analysis sessions on large-scale display

Agus, Calì et al., Computer Graphics Forum, 2019

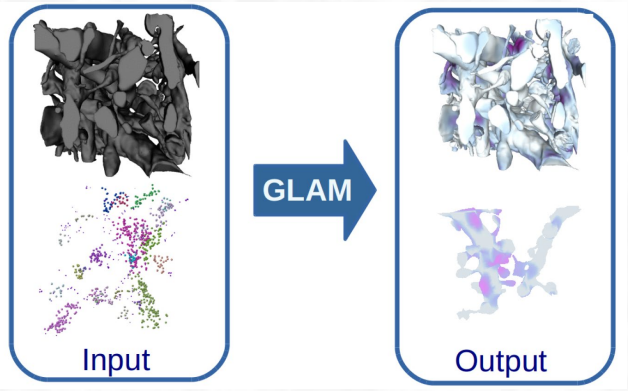


Calì et al., 2015 Journal of comparative neurology

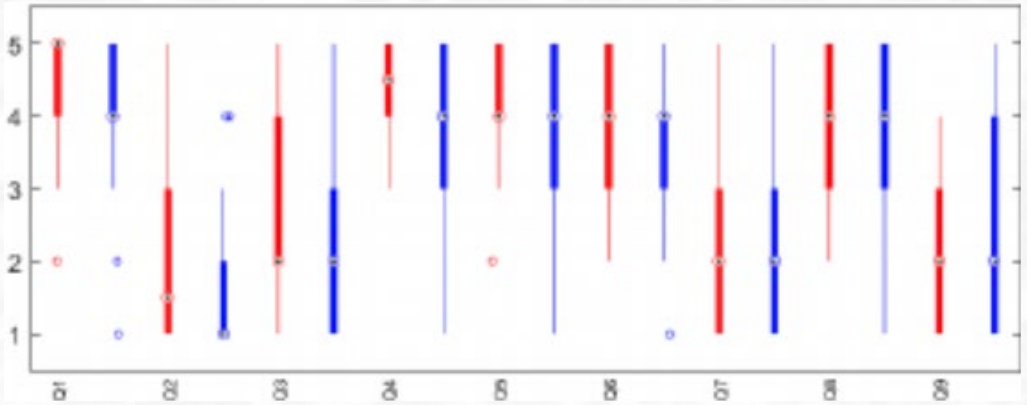
Calì et al., Progress in Neurobiology, 2019

Boges et al., Computers & Graphics, 2020

VR Analysis of astrocytic morphologies



Question	Results (Likert scale: 1=disagree 5=agree) VR - HTC Avg	VR - HTC σ	Desktop- Mono Avg	Desktop- Mono σ	ANOVA $F(2, 27)$	ANOVA p
Q1: I think that i would like to use this application frequently.	4.384	0.726	4.115	1.066	1.48	0.235
Q2: I found the application unnecessarily complex.	1.923	1.274	1.654	0.955	1.204	0.283
Q3: I think that i would need technical support for using this application.	2.539	1.539	2.308	1.341	1.696	0.204
Q4:I like using the interface of this application.	4.345	0.555	3.846	1.655	4.684	0.04
Q5:I think that most people would learn to use this application quickly.	4.115	0.746	3.731	1.325	2.617	0.118
Q6:I felt very confident using the application.	4.154	0.855	3.692	1.101	5.294	0.03
Q7: I needed to learn a lot of things before I could use this application.	2.115	1.306	2.231	2.025	0.355	0.556
Q8: the information provided by the application was clear and helpful.	4.039	0.912	3.769	1.545	1.093	0.306
Q9: I felt it difficult to interact and control the system.	1.923	0.954	2.500	2.180	4.851	0.037

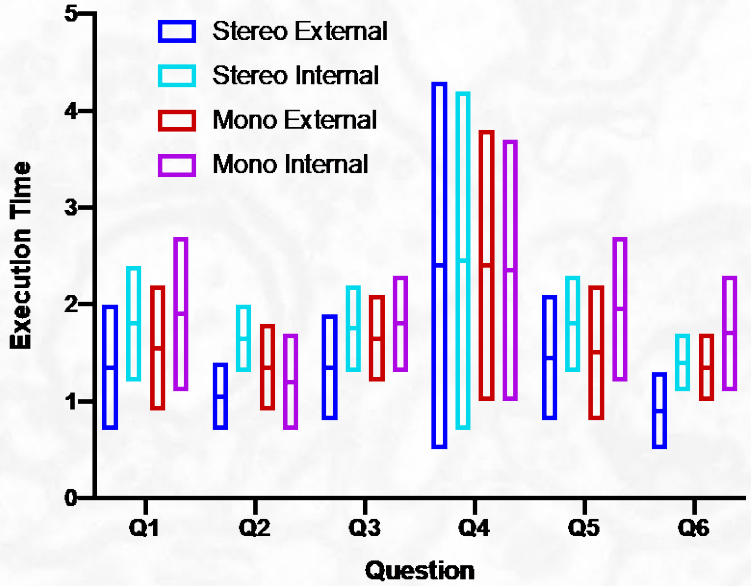


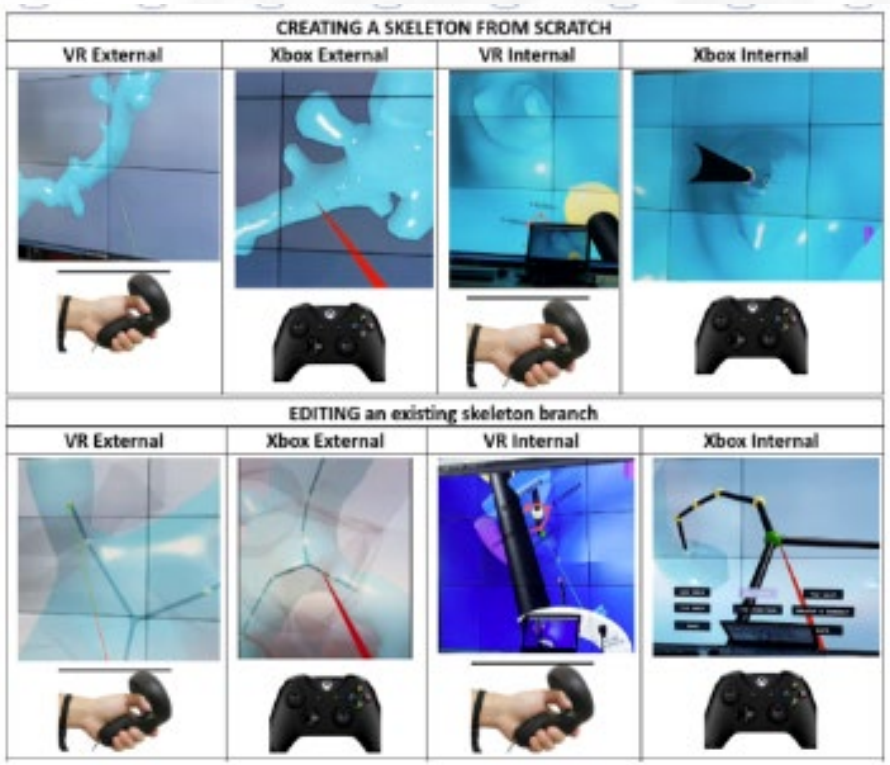
VR **Desktop**

- Users overall preferred VR over desktop ("wow effect?")
- Astrocytes were easier to analyze in VR because of visual cluttering
- Few users suffered from "VR sickness"

Agus et al., Computers and Graphics, 2018

VR Analysis of astrocytic morphologies





Likert scale: 1=low, 5=high

Q1 : How mentally demanding was it?
Q2 : How physically demanding was it?
Q3 : How hurried was the pace?
Q4 : How successful were you?
Q5 : How hard did you have to work ?
Q6 : How stressed were you?

Boges et al., Progress in Neurobiology, 2020

Augmented Reality for Neurosurgeons

Industrial transition

Marzo 2021

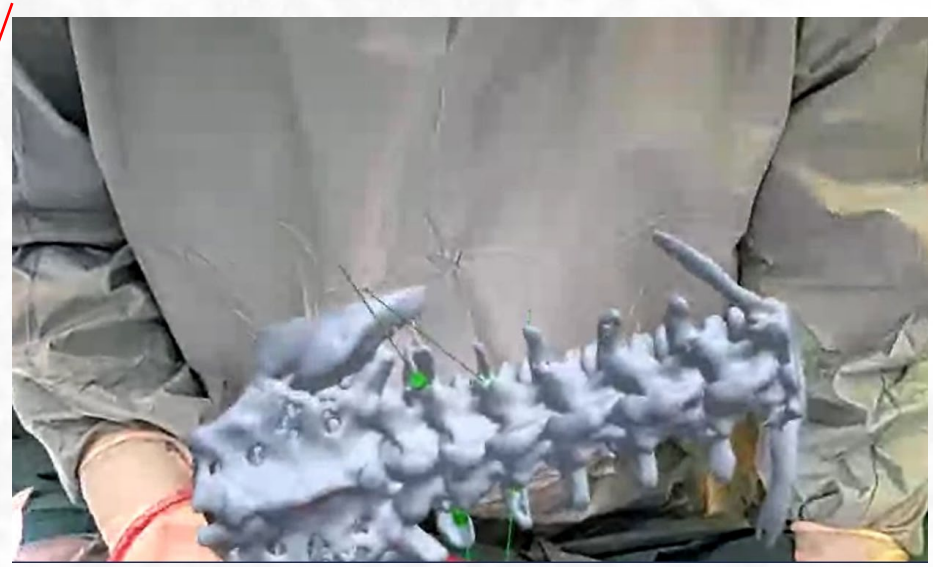
Dott. Paolo Regolo

Grandenigo (Humanitas), Torino

Lumbar Arthrodesis

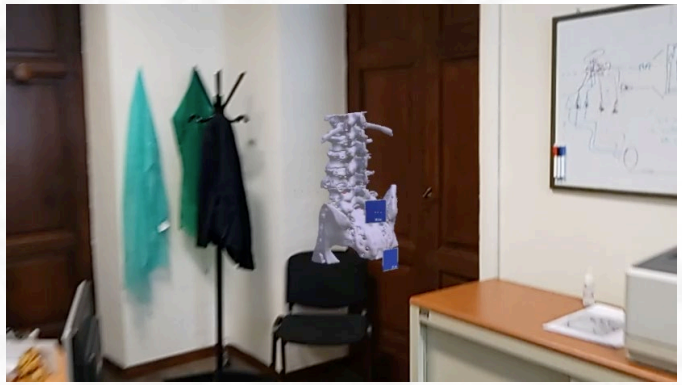
Prototype: Hololens 2

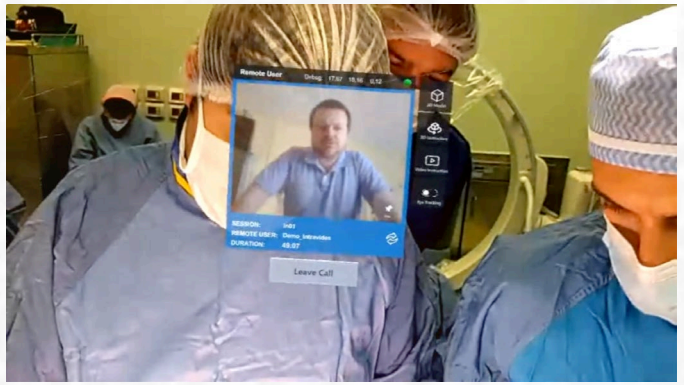
Software: ARSistant (Xonne, Parma)



Industrial transition

- **Navigation**
  - QR-Code
  - Fiducial points during imaging
- **5G Transmission**
  - Remote connection
  - Mulituser room (education)
- **Alternative Use Cases**
  - Education (Human Anatomy, Workshop)
  - Surgery of other anatomical districts
  - Soft Tissue
  - Planning





Conclusions

Astrocytes 3D structure is highly fractal (high SVR)

Hetereogeneity of subdomains/microdomains (SVR spans from 3 to 14)

VR / AR is a promising tool for ultrastructural analysis of their morphology

Quantitative analysis can be performed with custom tools depending on analytical needs

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