

# Electron Microscopy Of Brain Tissue Fixed By Focused Beam Microwave To Preserve Glycogen





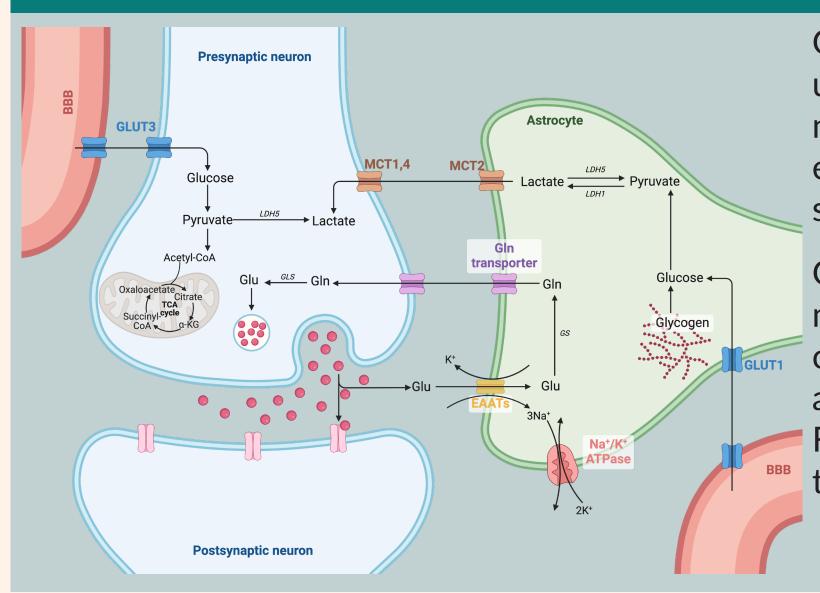
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Cavalieri Ottolengh

# Introduction



Glycogen, the main energy reserve in the brain. It is predominantly stored in astrocytes under normal physiological conditions where it serves as a metabolic buffer during neurotransmission.<sup>1</sup> Growing evidence links glycogen metabolism crucial in highly energetic processes such as long-term memory formation, learning-dependent synaptic stabilization, memory consolidation, and the maintenance of long-term potentiation.<sup>2,3</sup>

Glycogen is prone to rapid degradation post-mortem, making conventional fixation methods inadequate for preserving its native levels and distribution. This represents a challenge when aiming to accurate quantifiy and visualize brian glycogen distribution at different stages of memory consolidation process.3 Hence in this study, we employ Focused Beam Microwave (FBMW) fixation as a rapid euthanasia and fixation method to preserve glycogen in conditions that better reflect in vivo conditions.

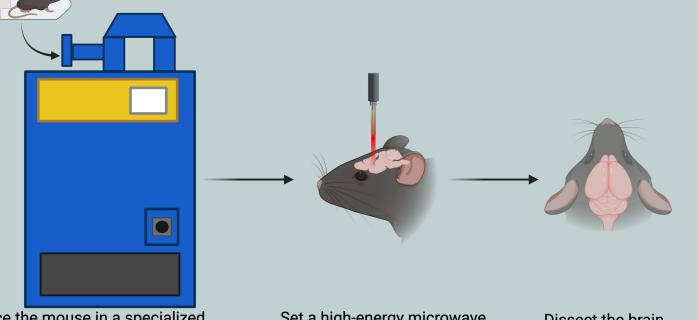
# Objectives

- Assess the ultrastructural integrity of brain tissue fixed using FBMW using transmision electron microscopy (TEM) and Volume Scaning Electron Microscopy (volSEM).
- Evaluate the effectiveness of FBMW fixation in preserving glycogen granules in brain tissue compared to conventional PFA & GA fixation.
- Develop a segmentation and 3D reconstruction pipeline for glycogen granules and organelles using electron microscopy data.

# Methodology

### **Microwave Fixation**

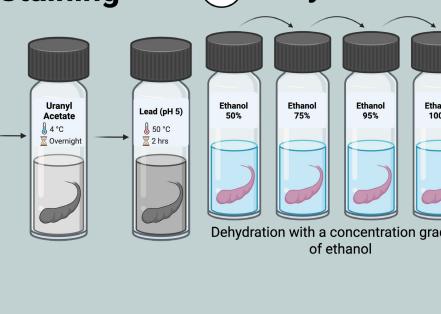
### Sample Preparation

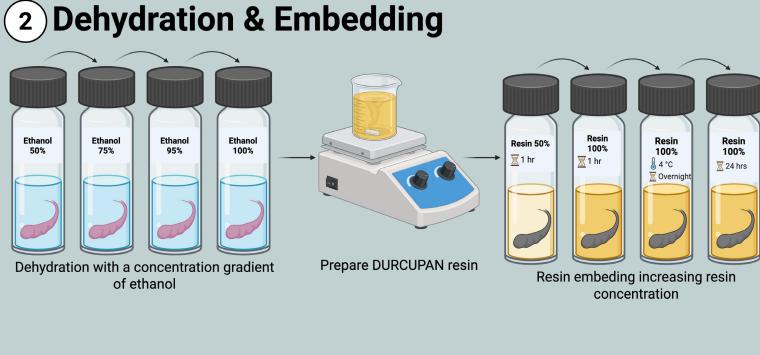


mouse for 600 or 850 ms.

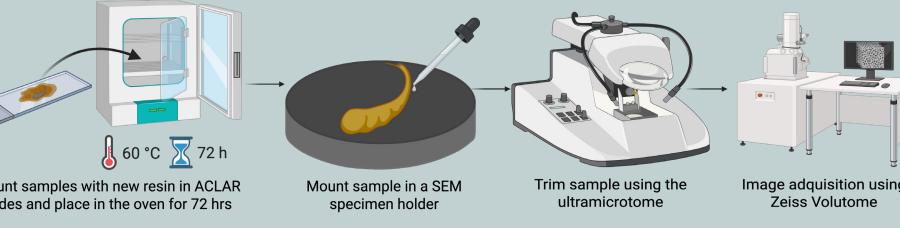
for further use.

1 Post-fixation & Staining



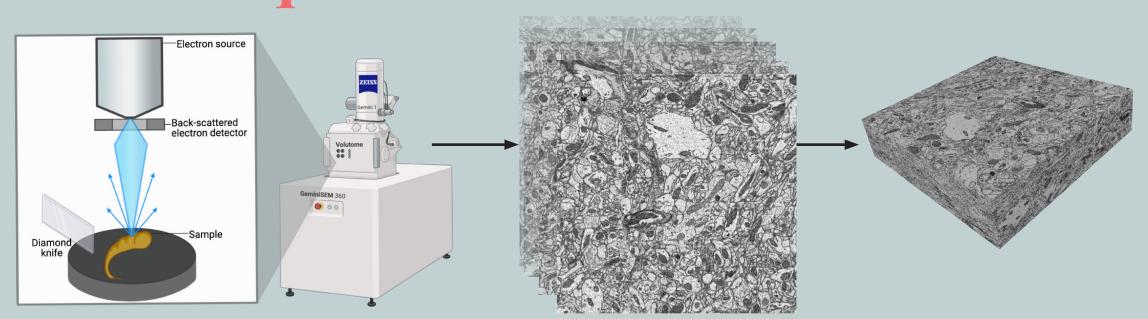


(3) Sectioning & Image Adquisition



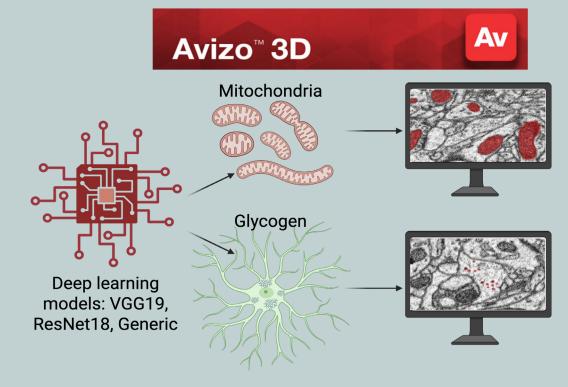
### Data Adquisition

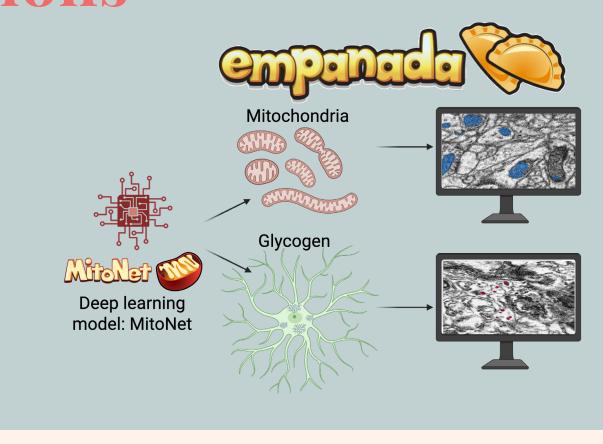
tubulous animal holder with a



### Segmentation and 3D Reconstructions





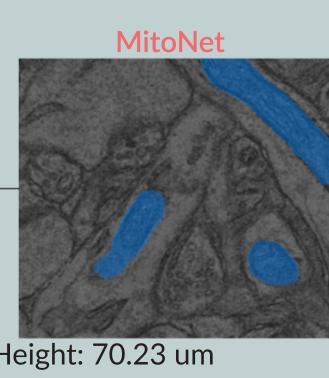


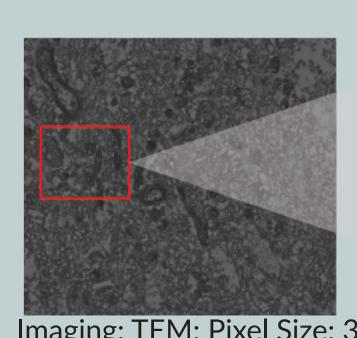
## Results

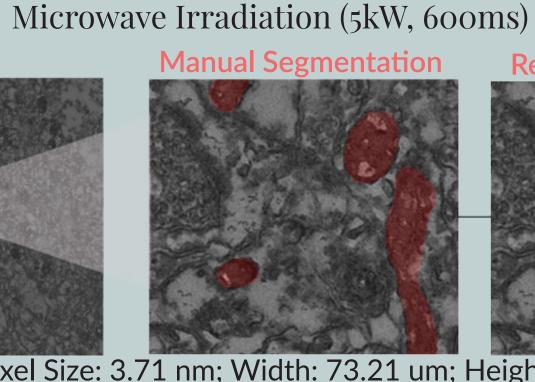
#### Comparison of Mitochondria & Glycogen Segmentation in Perfused and Microwaved Samples

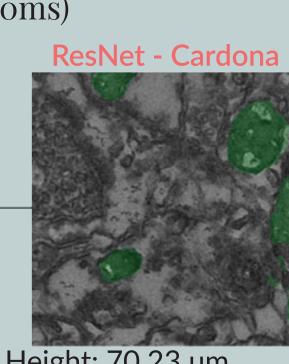
PFA+GA Perfusion

**Manual Segmentation** 







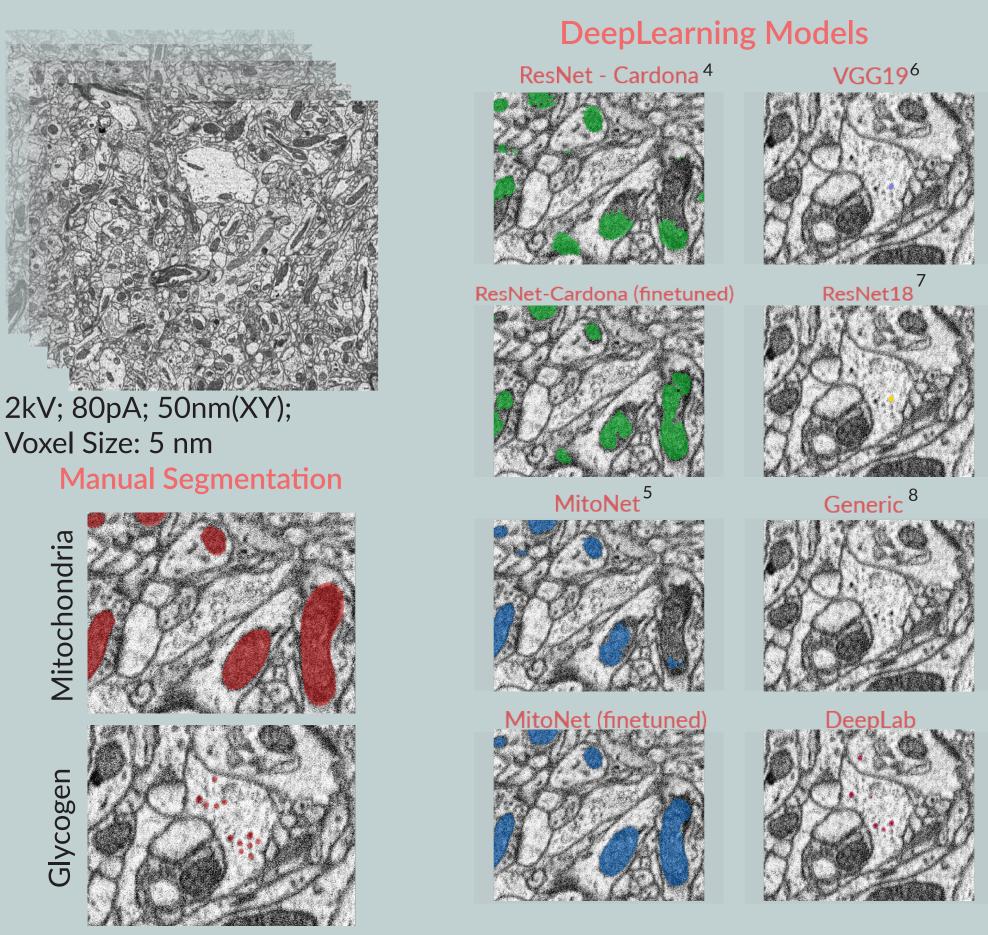


Imaging: TEM; Pixel Size: 3.71 nm; Width: 73.21 um; Height: 70.23 um

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Segmentation and Reconstruction in 3D stacks

PFA+GA Perfusion

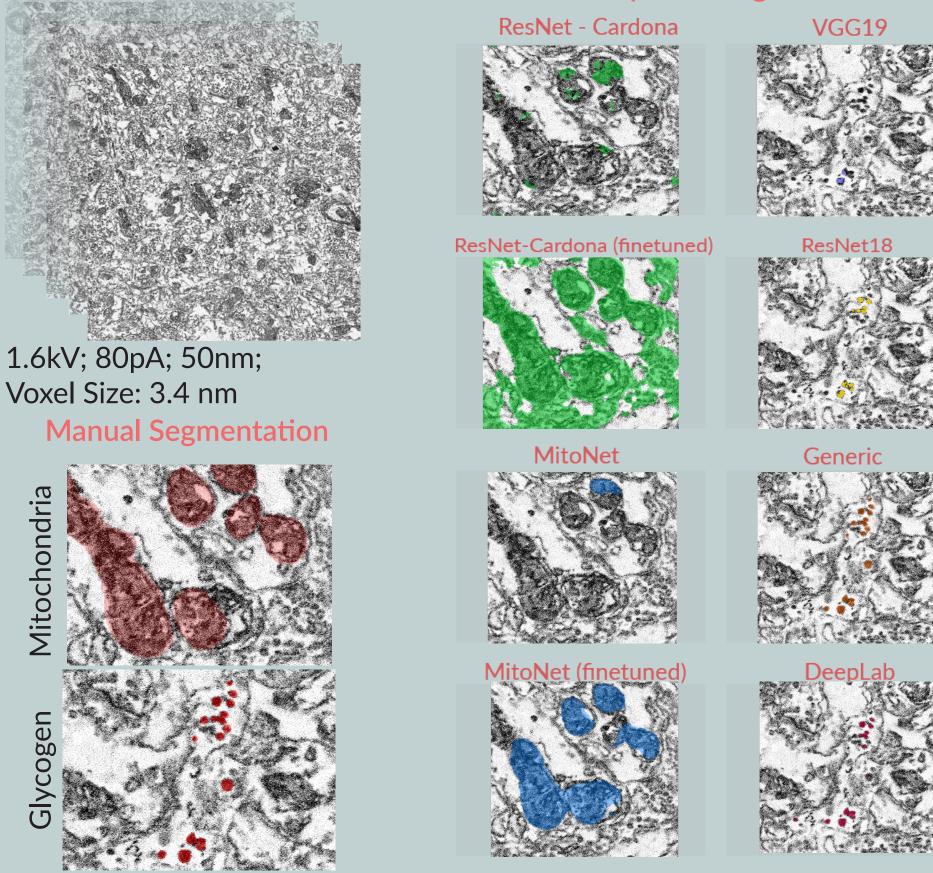


#### Deep learning models for mitochondria segmentation

Model	Type of data	Training time	IoU Score
ResNet - Cardona	Perfused	Pretrained	0.407
ResNet - Cardona (finetuned)	Perfused	00:09:38	0.479
MitoNet	Perfused	Pretrained	0.399
MitoNet (finetuned)	Perfused	00:04:18	0.736
ResNet - Cardona	Microwaved	Pretrained	0.058
ResNet - Cardona (finetuned)	Microwaved	00:06:43	0.262
MitoNet	Microwaved	Pretrained	0.046
MitoNet (finetuned)	Microwaved	00:06:20	0.603

#### Microwave Irradiation (5kW, 600ms)

**DeepLearning Models** 



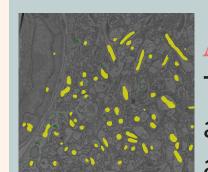
#### Deep learning models for glycogen segmentation

reep learning models for grycogen segmentation							
Model	Type of data	Training time	IoU Score				
VGG19	Perfused	02:17:25	0.024				
ResNet18	Perfused	01:53:16	0.0196				
Generic	Perfused	03:19:18	0.0153				
DeepLab	Perfused	02:06:05	0.135				
VGG19	Microwaved	02:16:11	0.139				
ResNet18	Microwaved	01:45:30	0.288				
Generic	Microwaved	03:14:19	0.815				
DeepLab	Microwaved	02:01:46	0.489				

# Future Directions

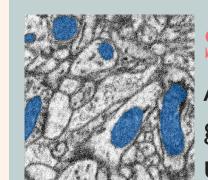
#### **Multi-structure Segmentation**

Continue segmentation efforts to include brain samples fixed under different microwave fixation settings.



### Advanced Segmentation Techniques

Test and validate novel semi-automated and automated segmentation tools to enhance the accuracy, speed, and reproducibility of structure identification in microwaved-fixed tissue.



#### **Spatial Co-localization Studies**

Analyze the 3D spatial distribution of glycogen granules relative to mitochondria. We aim to understand how glycogen positioning may support localized energy demands.



#### Synaptic Association Analysis

Explore the association between the localization of glycogen granules and post-synaptic densities. Measurements of glycogen-synapse distances will provide ultrastructural evidence linking energy metabolism with synaptic function.

### Acknowledgements

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### References

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