

Automatic Cell Segmentation

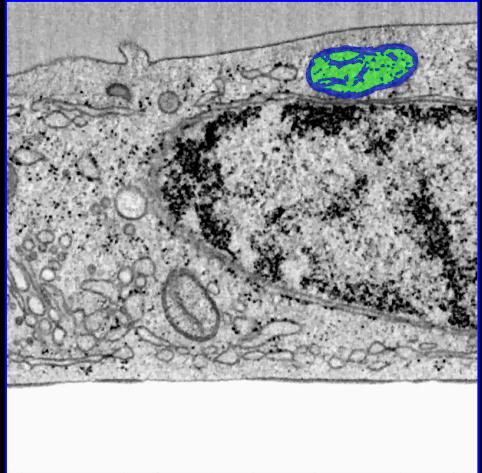
Rohit Batra

Lippincott-Schwartz Lab

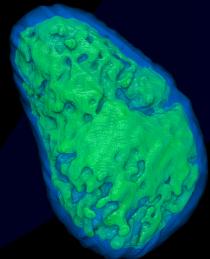
COSEM Project Team



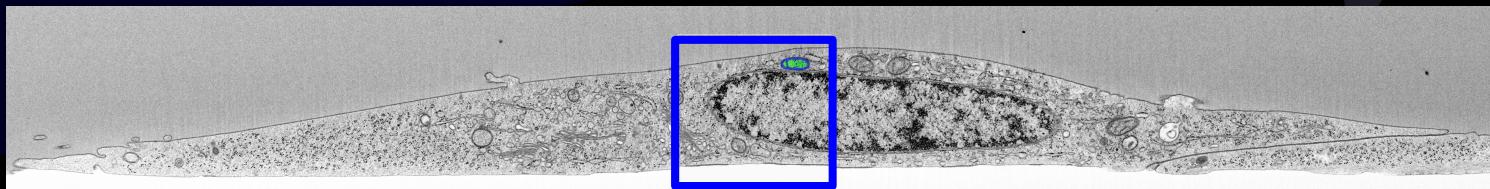
Size & Scaling



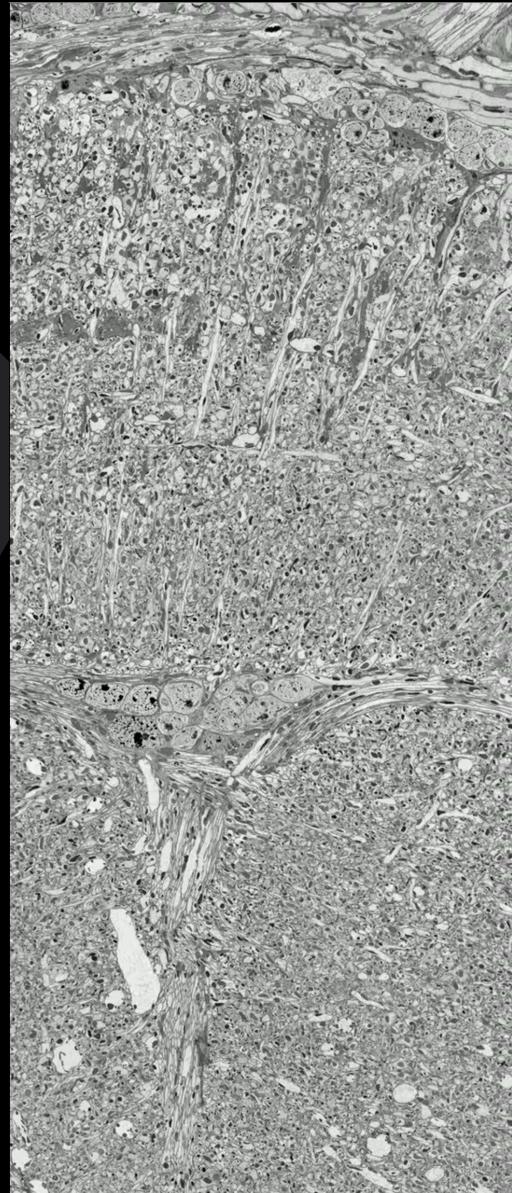
Perinuclear Region
 $\sim 5 \times 5 \times 5 \mu\text{m}^3$



Single Mitochondria
 $\sim 0.5 \times 0.5 \times 1 \mu\text{m}^3$



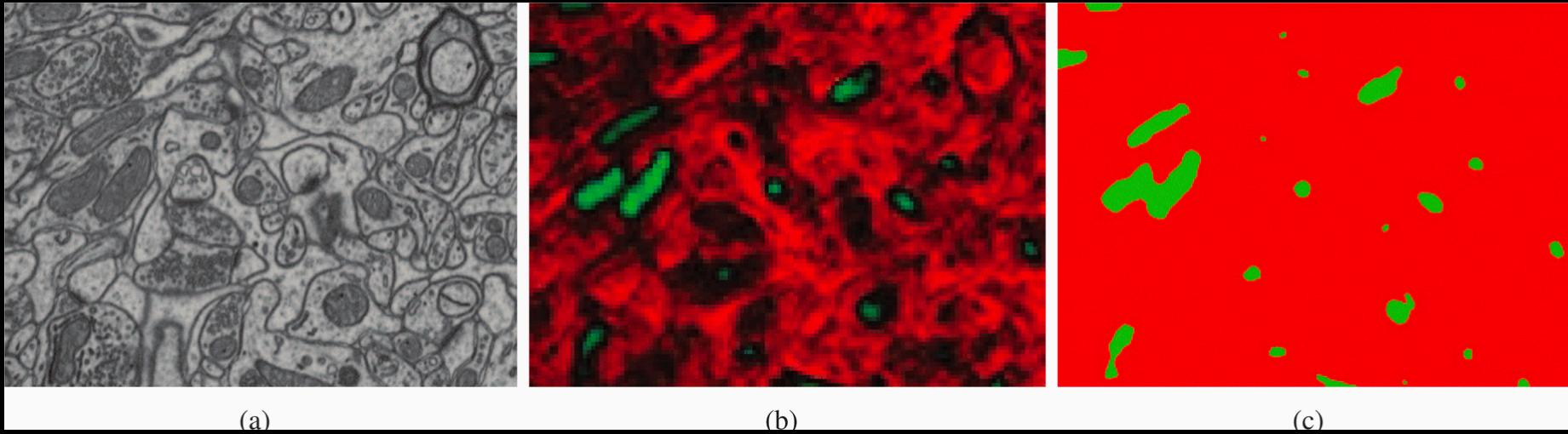
Whole Cell
 $\sim 10 \times 20 \times 20 \mu\text{m}^3$



Drosophilla Optic lobe
 $150 \times 64 \times 40 \mu\text{m}^3$

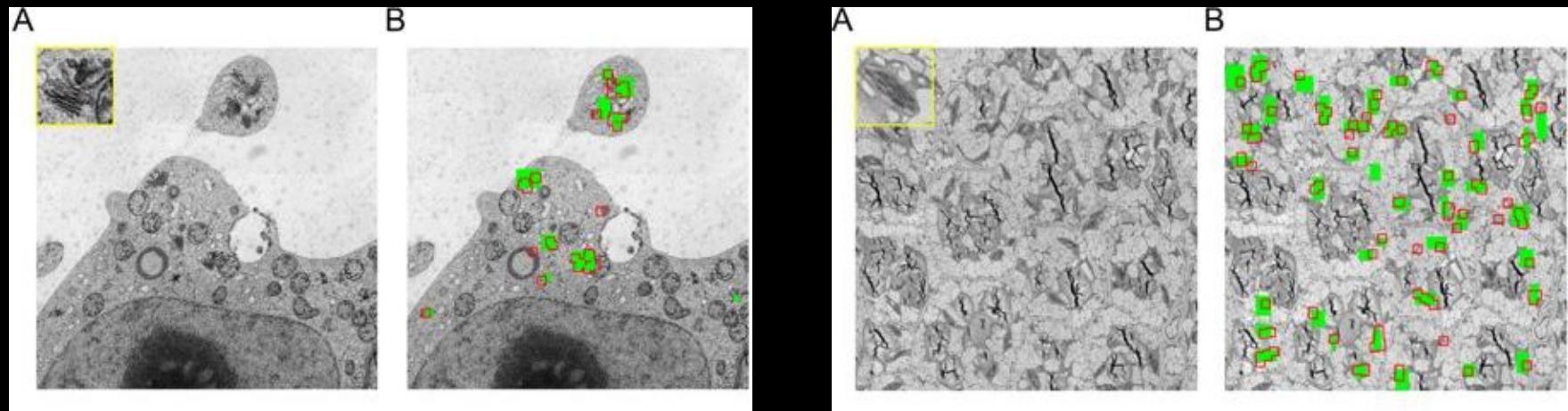
Current Attempts

Automatic Segmentation of Mitochondria



Oztel, IEEE

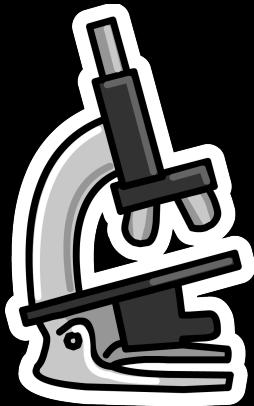
Semi-Automatic Segmentation of Golgi Stacks & Chloroplasts



Higaki, Nature

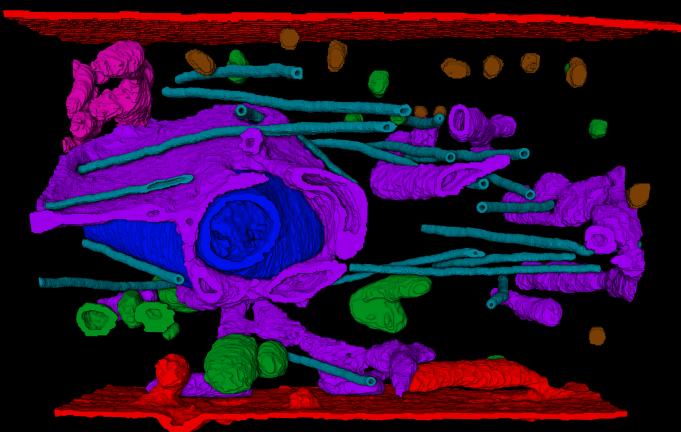
Procedure

1. Gather FIB-SEM data

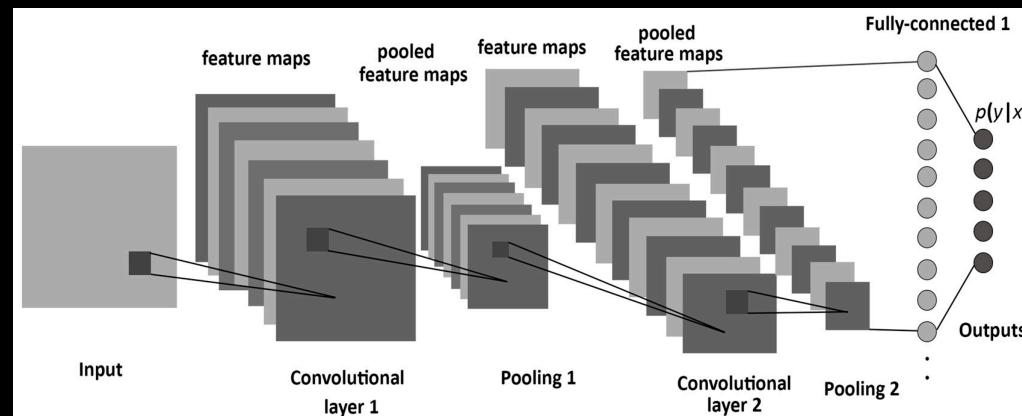


<https://es.kisspng.com/png-optical-microscope-clip-art-microscope-552955/>

2. Develop the ground truth



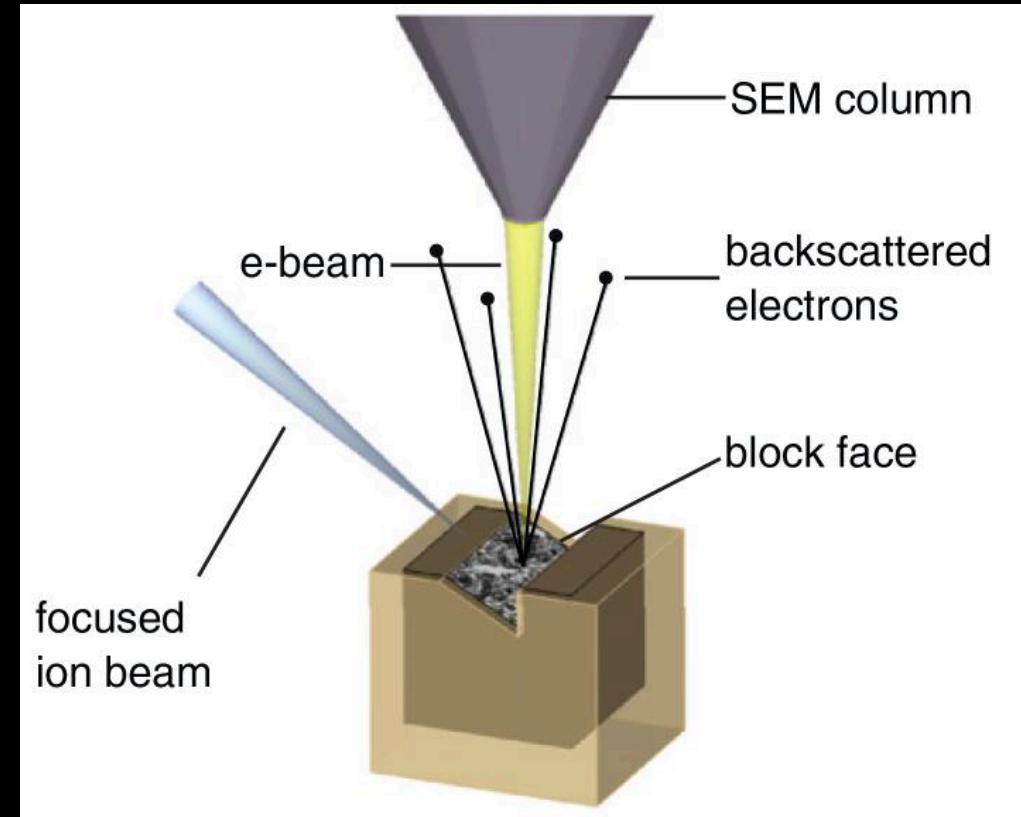
3. Train & refine the machine model



<http://www.mdpi.com/1099-4300/19/6/242>

FIB-SEM Microscopy

- Focused Ion Beam Scanning Electron Microscopy
- Gallium ions
- Ion Beam can slice thinner than traditional hot knife
- 2-8 nm voxel size



https://www.embl.de/services/core_facilities/em/services/fibsem/

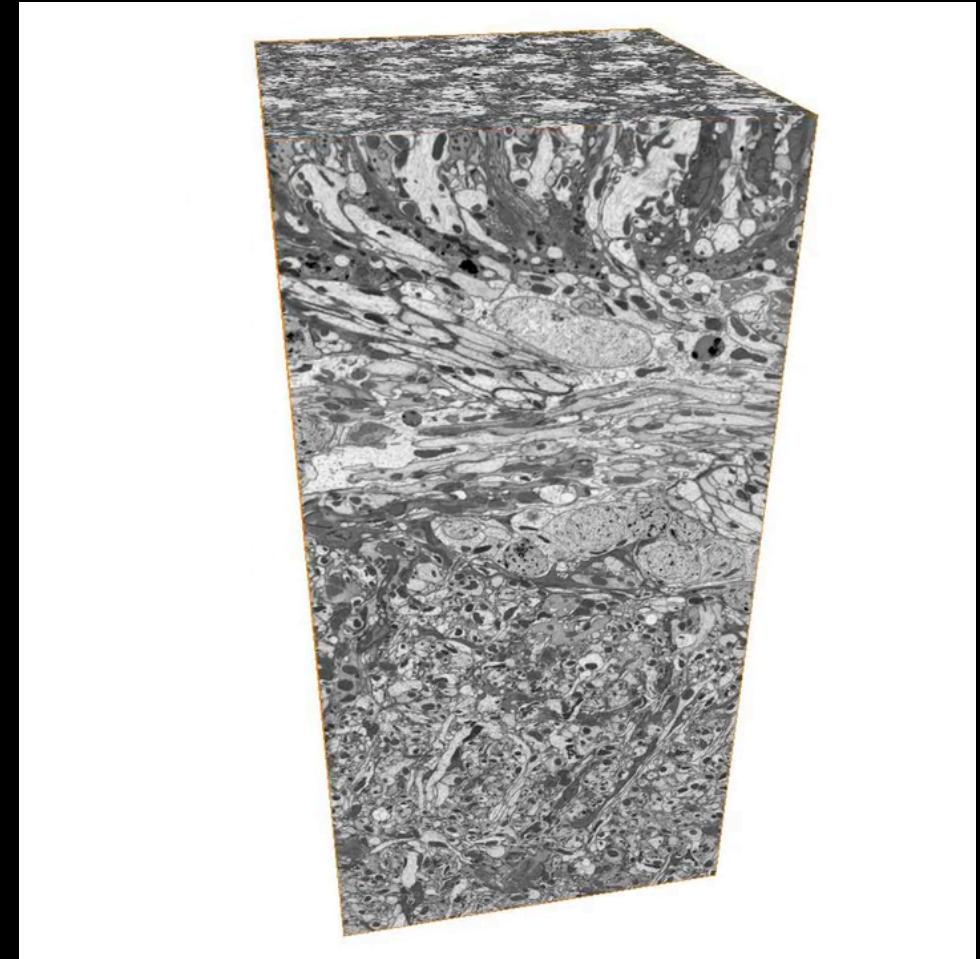
FIB-SEM Microscopy

Benefits:

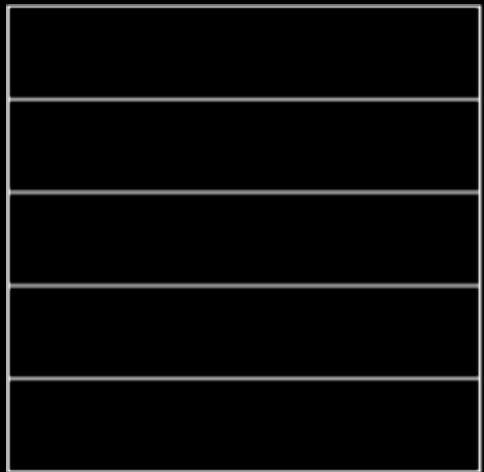
- Isotropic resolution.
- Ability to gather more precise samples.
- Ability to gather large 3D volumes.

Tradeoffs:

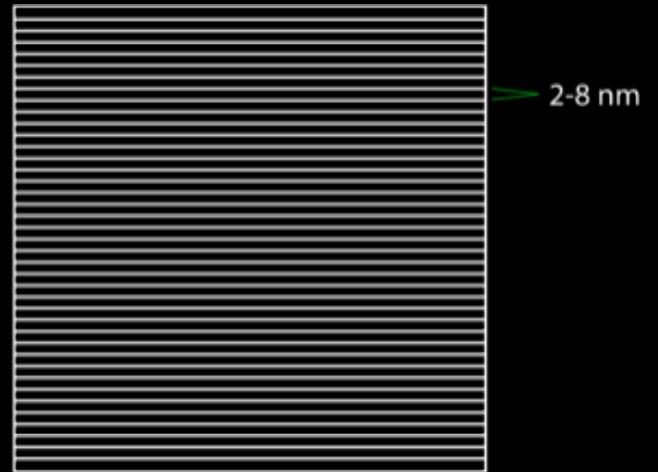
- Samples are destroyed by ion beam.
- Lower resolution as volume increases.



Janelia



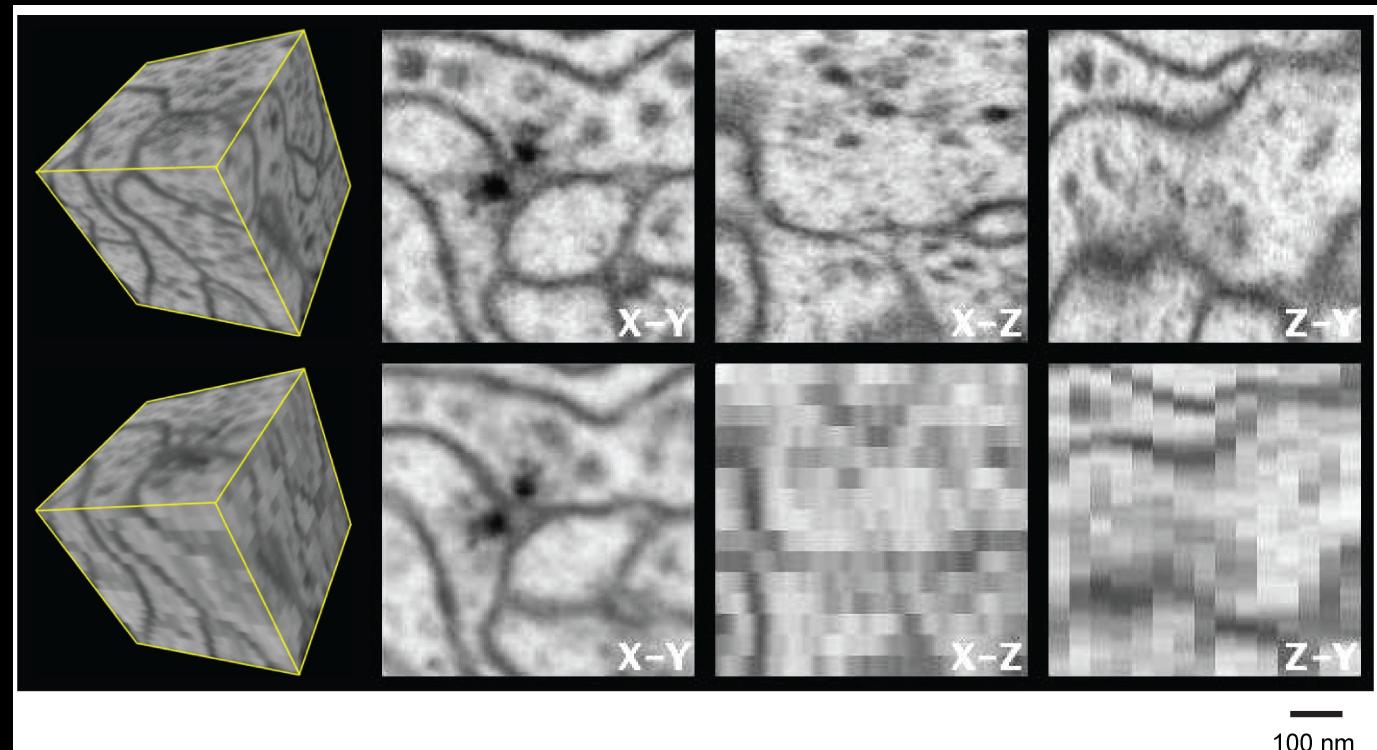
ssTEM



FIB-SEM

FIB-SEM

ssTEM



100 nm

Structures being segmented:

Cytoskeletal Elements

- Centrosome
- Microtubules
- Actin stress fibers

Membrane Bound Organelles

- ER
- ER Exit Sites
- Golgi
- Lysosome
- Lipid Droplets
- Mitochondria
- MVB
- Endosomes
- Autophagosome
- Vesicles
- Peroxisomes

Nucleus

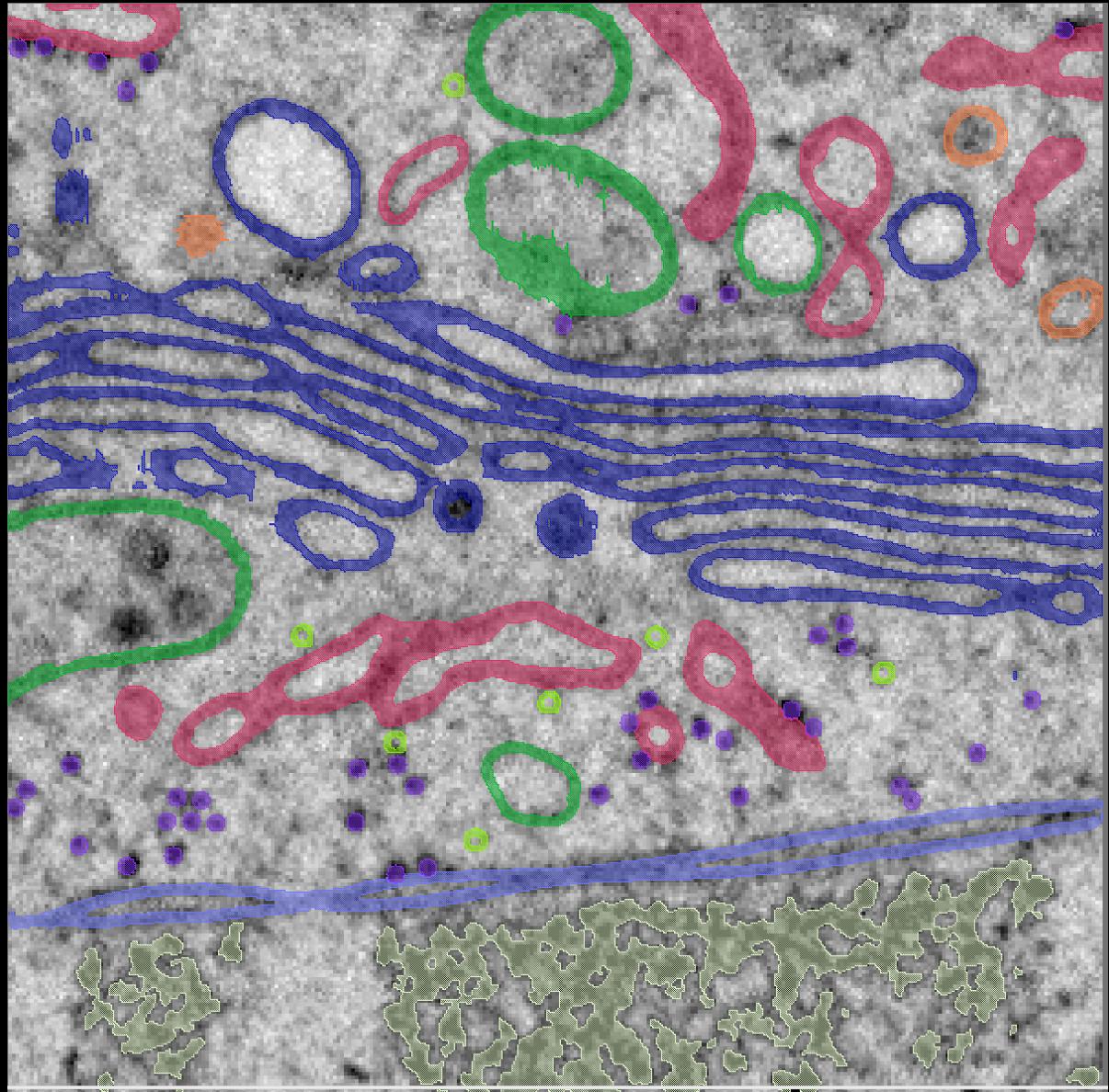
- Nucleur envelope
- Chromatin

Proteins Complexes:

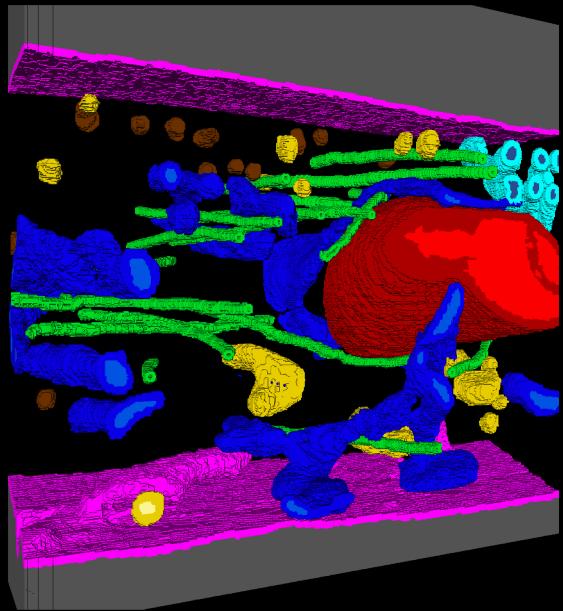
- Ribosomes

Other structures:

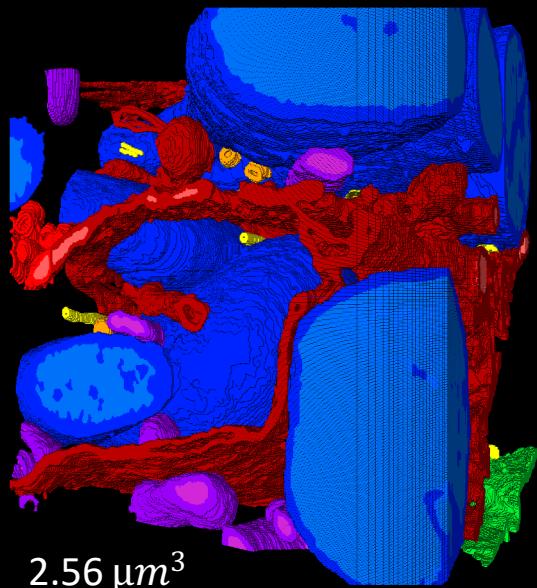
- Plasma Membrane
- Endocytic events
- Cytosol
- Extracellular Space



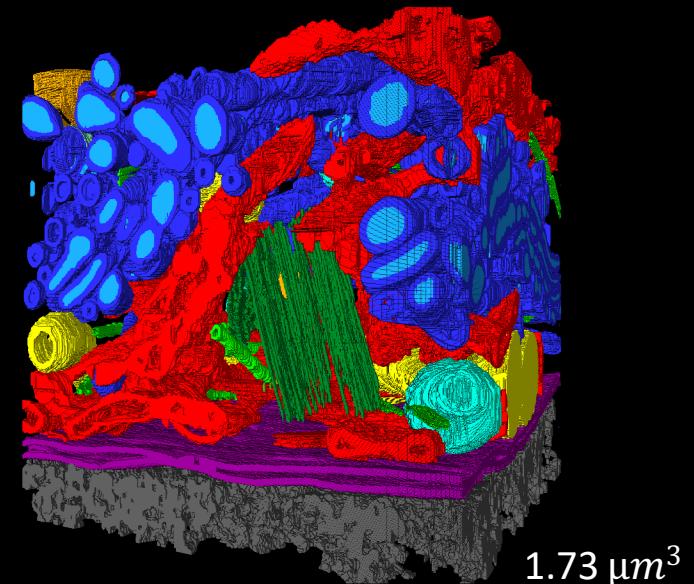
Annotated Volumes:



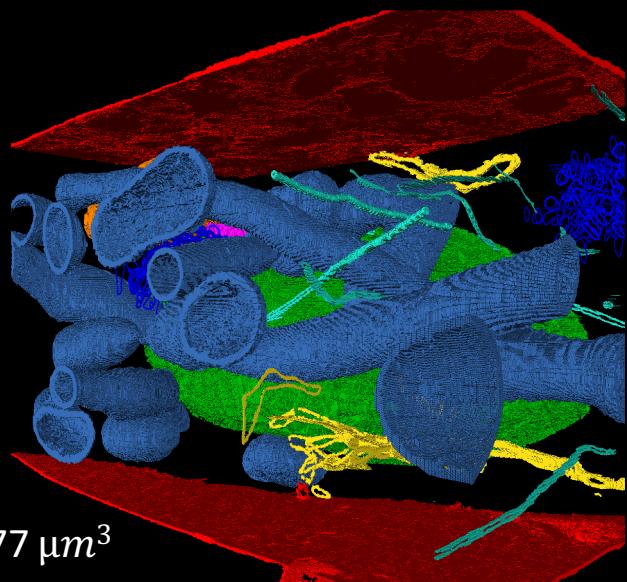
$1.6 \mu m^3$



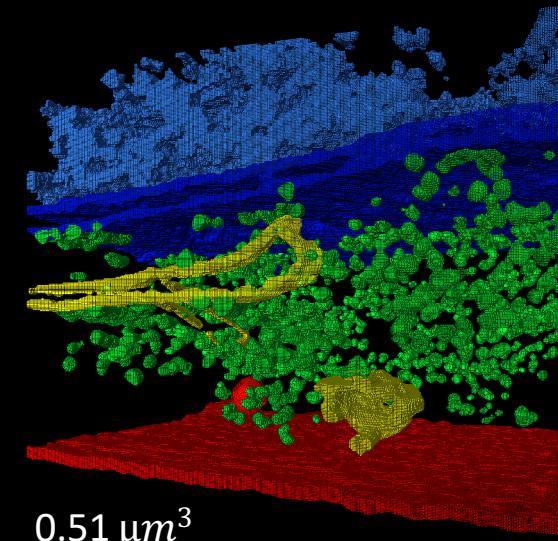
$2.56 \mu m^3$



$1.73 \mu m^3$



$32.77 \mu m^3$

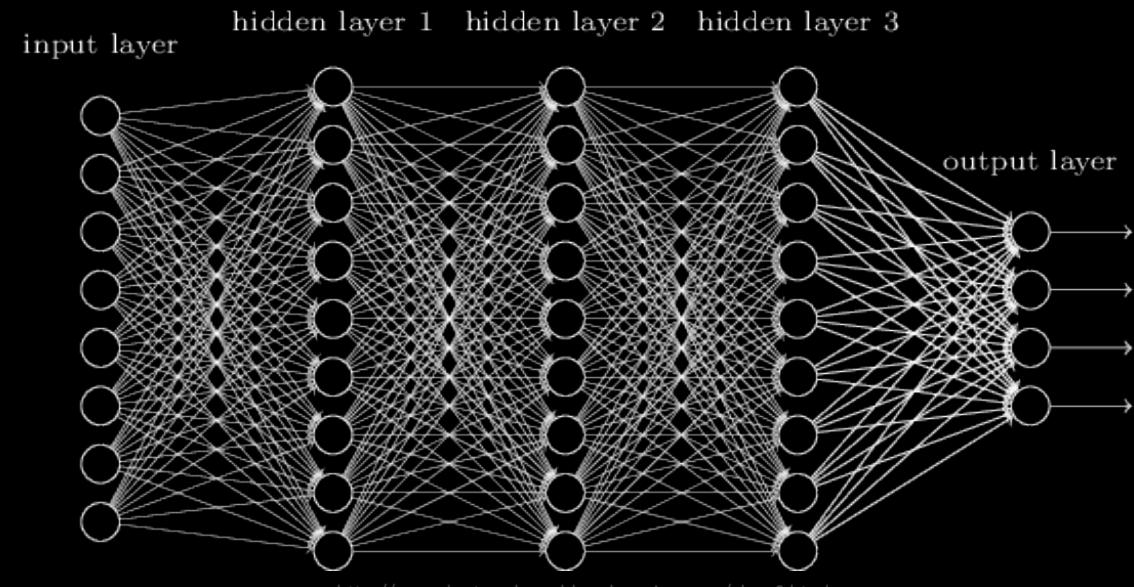


$0.51 \mu m^3$

Machine Learning

Convolutional Neural Network

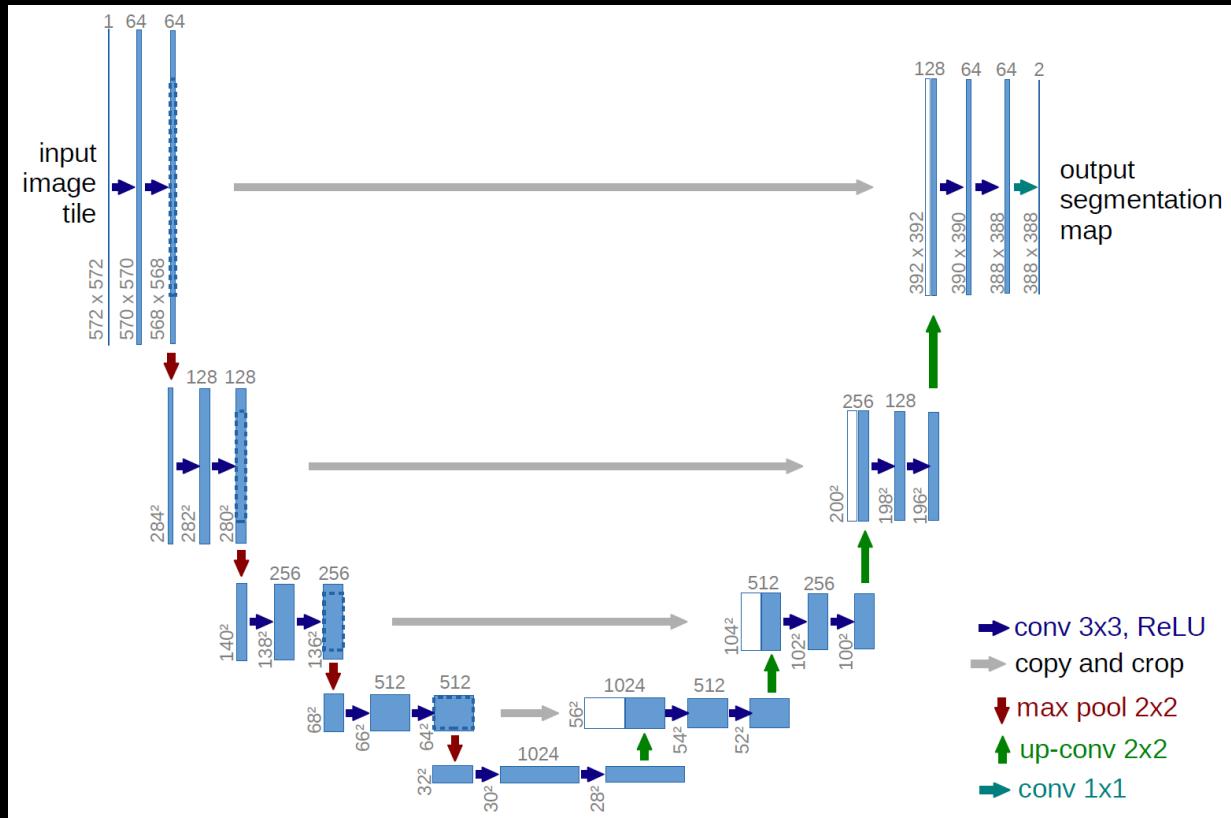
- 12 Hyperparameters
- Network learns and generates its own features based on ground truth data
- Cross-entropy loss function is used to optimize neural network
- Prediction, candidate extraction, detection



<http://neuralnetworksanddeeplearning.com/chap6.html>

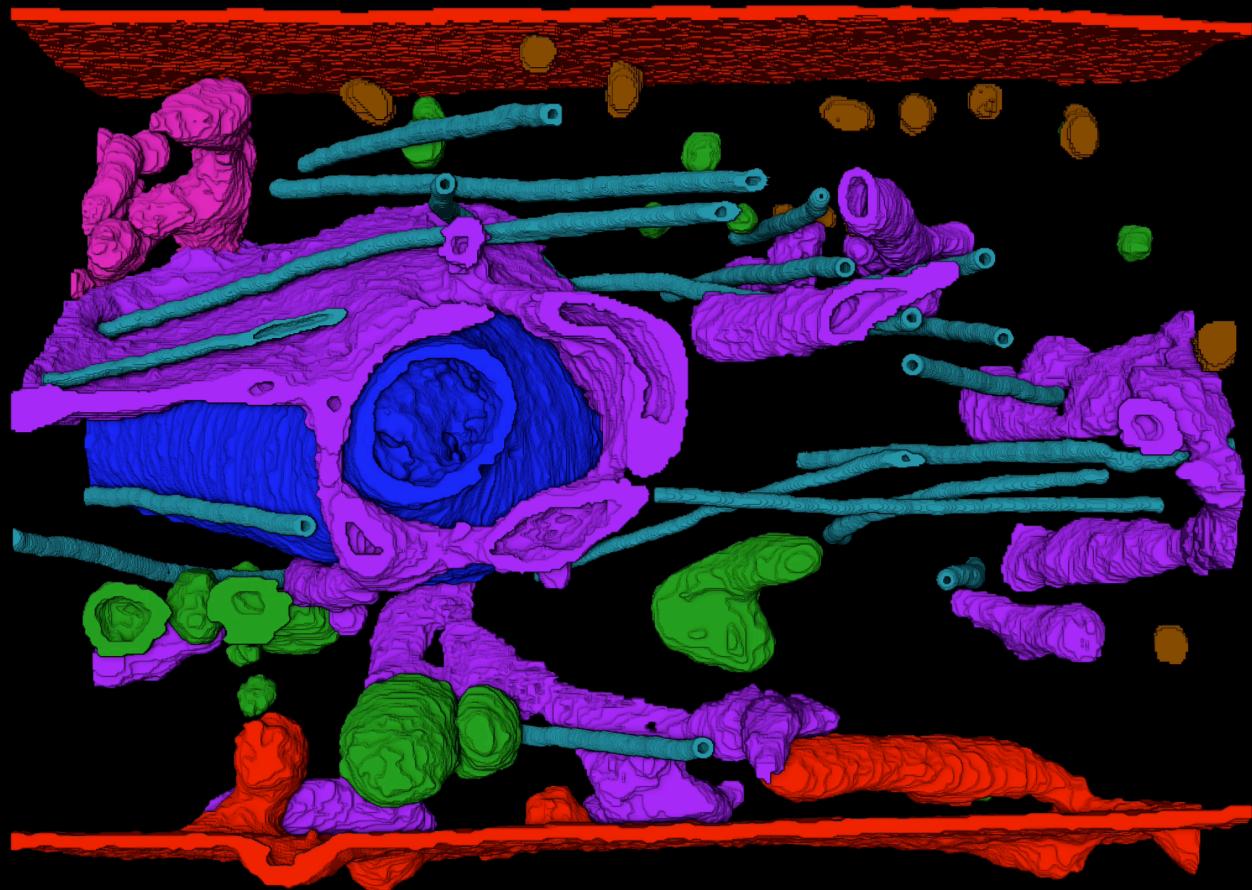
U-net Architecture

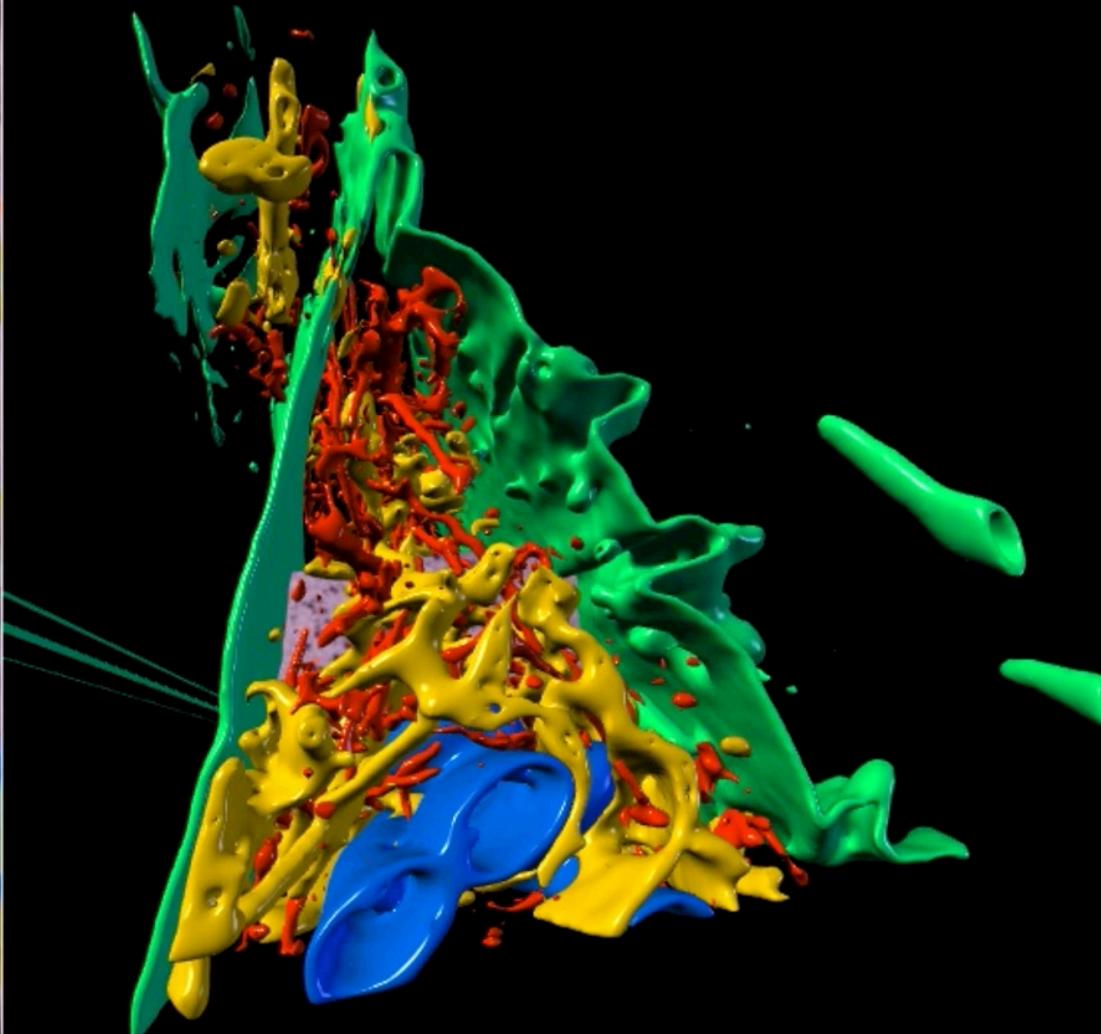
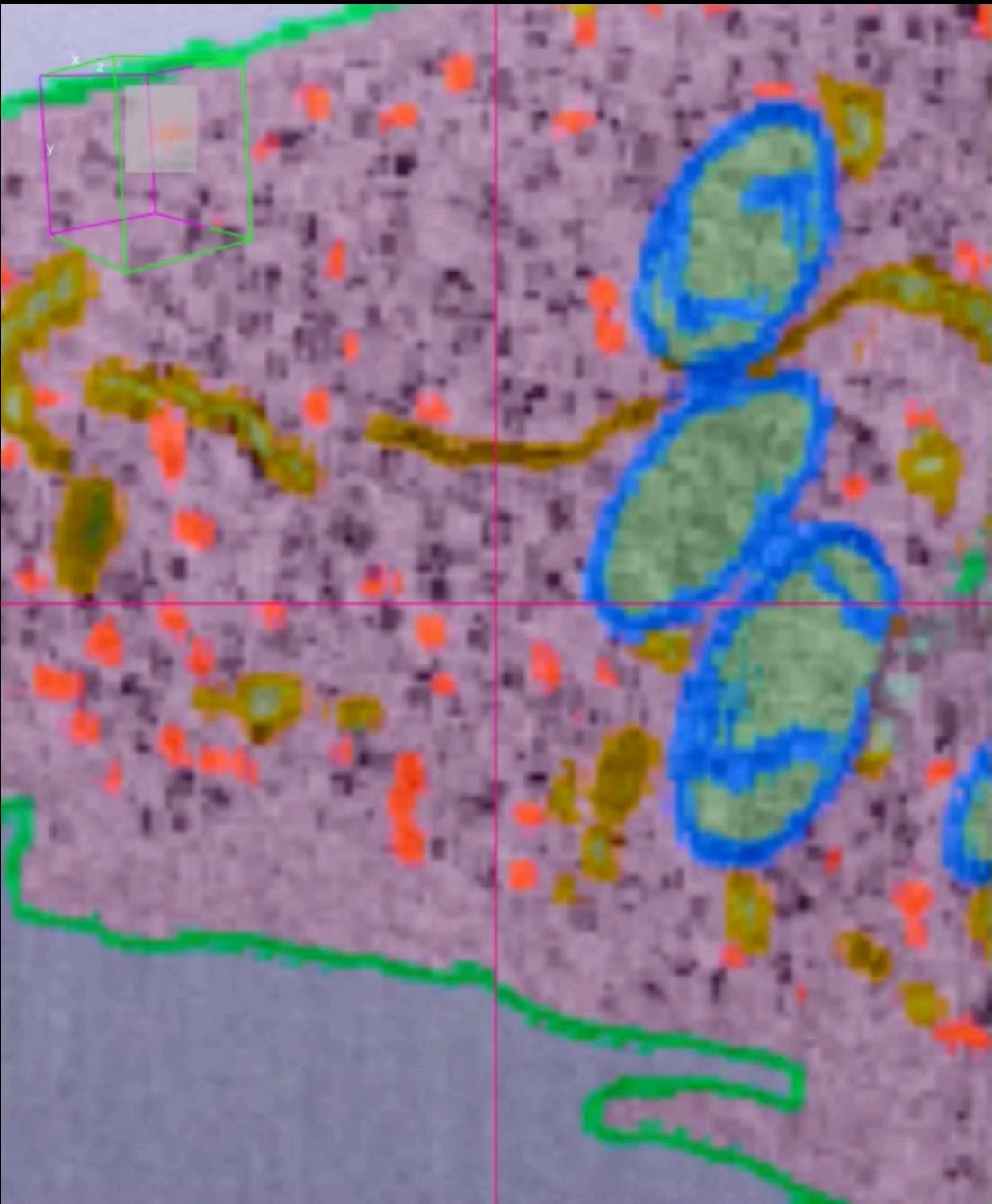
- Type of neural network constructed specifically for image segmentation
- Requires less ground truth data than typical neural network
- Machine segmentation is faster than typical neural network



<https://arxiv.org/pdf/1505.04597.pdf>

- Plasma Membrane
- Mito Membrane
- ER
- ER Exit Site
- Microtubules
- Endosomes
- Vesicles





Summary

- Tool will aid in the classification of cell and synapse types in FIB-SEM data
- Informed predictions about synaptic weight and direction
- Build a bridge between connectomics and cell biology targeted efforts
- Tool will be open sourced

Acknowledgements

- Dr. Weigel
- Dr. Lippincott-Schwartz
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- Dr. Hess
- Dr. Saalfeld
- Dr. Funke

