# Problems and Progress in Automating Electron Microscopy Segmentation


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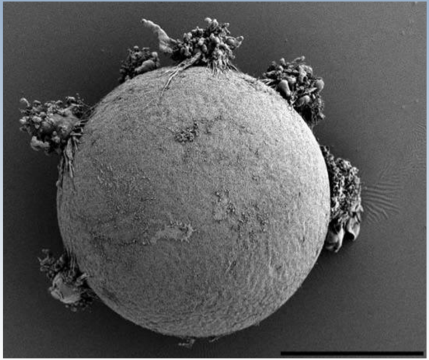
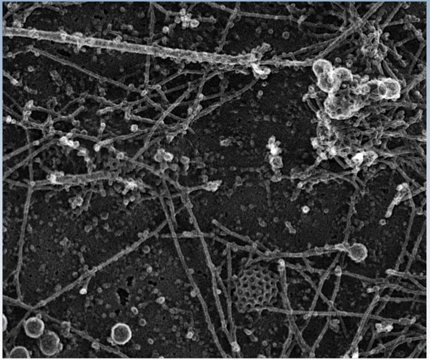
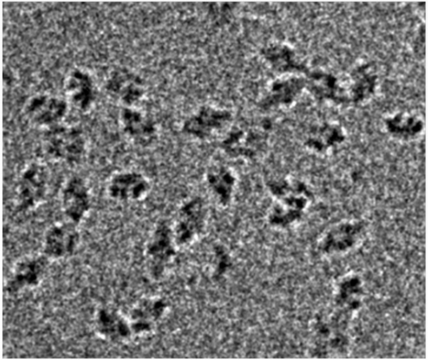
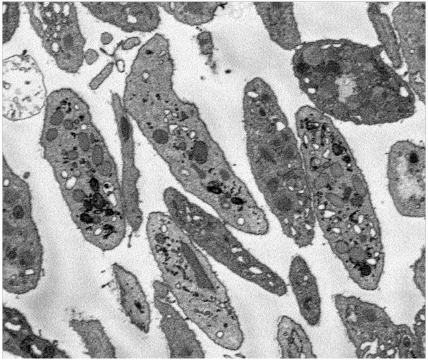
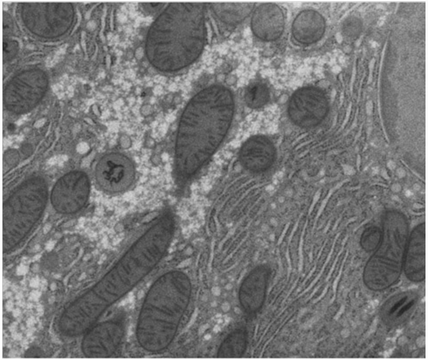
# ELECTRON MICROSCOPY AT THE NIH

The National Institutes of Health (NIH) uses **electron microscopy** (EM) to image biological matter from atomic to macroscopic scales for biomedicine.

The NIH uses many EM hardware platforms across its institutes - SEM, TEM, STEM, SBF-SEM, FIB-SEM, Cryo-EM, etc.

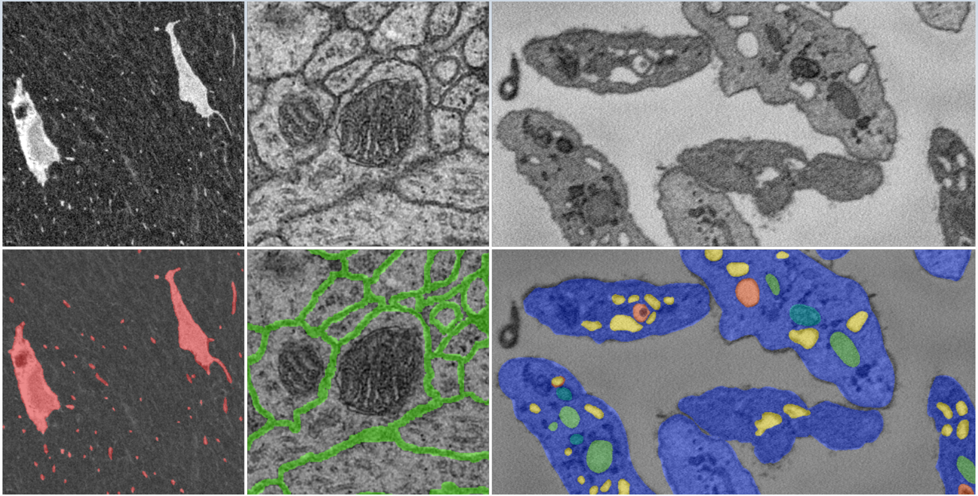
Each EM platform + sample preparation protocol presents different image statistics (noise, resolution, contrast)

New platforms can produce gigapixel 2D images and **teravoxel** 3D images.

# IMAGE SEGMENTATION

**Semantic segmentation:** Partition image pixels into labeled regions corresponding to image content.



# AUTOMATING EM SEGMENTATION

Manual segmentation is infeasible for large EM images.

**Automated segmentation**: algorithmically classify each pixel (and manually correct).

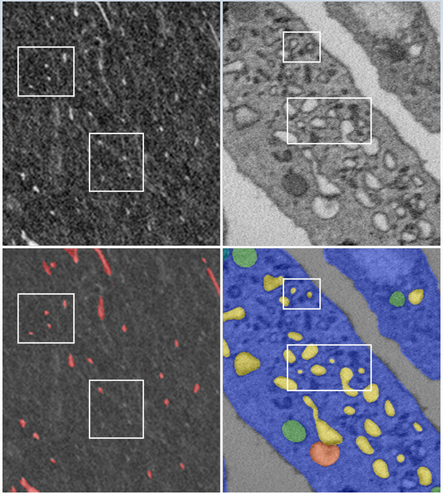
**Practical** automated segmentation: Manual correction of algorithm output is much faster than manual segmentation.

**Challenge**: Automation difficulty is data-dependent. Trivial for some applications, open research problem for others.

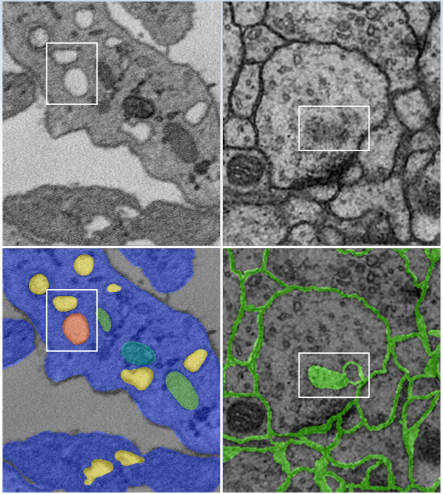
# BIOLOGICAL SEGMENTATION CHALLENGES

A practical segmentation algorithm requires high ($>$ 99.9%) accuracy despite:

**Noise + small objects**



**Difficult label assignment**



# DEEP LEARNING FOR SEGMENTATION

**Deep learning** (DL): Train multilayer neural networks to solve classification and prediction problems.

Neural network approaches achieve state-of-the-art results for challenging segmentation problems, including for biomedicine.

Segmentation network architectures have rapidly evolved since $\sim$2012.

# SEGMENTATION INNOVATION TIMELINE

2012 **Sliding window networks:** Convolutional neural network segments one pixel at a time.

2015 **U-net/Segnet:** Convolutional encoder-decoder neural network segments large image patches simultaneously.

2015 **CRF-RNN:** Fully-connected conditional random fields (CRF) as recurrent neural network (RNN) modules.

2015 **Dilated convolutions:** Replace upsampling/downsampling in u-nets with convolutions with large, sparse receptive fields.

2017 **Deeplab v3:** Natural image segmentation using multiscale dilated convolution paths and CRF-RNNs.

# SEGMENTATION IN 3D

**3D context** is essential for accurate segmentation of 3D image data.

Naive strategy: **full-3D nets**. Replace 2D operations with 3D equivalents in segmentation networks (convolution, pooling). Works poorly with our data.

Drawbacks:

- **Memory**: Larger 3D data means fewer operations fit in GPU memory. Model-parallel multi-GPU networks remain challenging.
- **Anisotropy**: Image *z* resolution is generally worse than *xy* resolution in EM. Full-3D networks do not exploit this.
- **Alignment artifacts**: In SBF-SEM and FIB-SEM, 2D *xy* slices in a 3D image may suffer from alignment jitter.

# HYBRID 3D SEGMENTATION

**Hybrid-3D nets**: Separate 2D and 3D processing network modules.

Existing approach: Segment 2D *xy* slices, combine successive slice segmentations using a sequence-processing RNN.

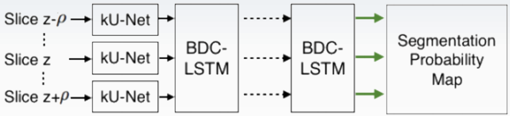



Figure 1: An overview of our DL framework for 3D segmentation. There are two key components in the architecture: $k$U-Net and BDC-LSTM. $k$U-Net is a type of FCN and is applied to 2D slices to exploit intra-slice contexts. BDC-LSTM, a generalized LSTM network, is applied to a sequence of 2D feature maps, from 2D slice $z - \rho$ to 2D slice $z + \rho$, extracted by $k$U-Nets, to extract hierarchical features from the 3D contexts. Finally, a softmax function (the green arrows) is applied to the result of each slice in order to build the segmentation probability map.

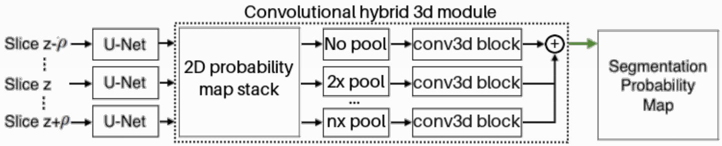
Source: *Combining Fully Convolutional and Recurrent Neural Networks for 3D Biomedical Image Segmentation*, Chen et al., 2016

# FULLY-CONVOLUTIONAL HYBRID 3D SEGMENTATION

Combining 2D segmentations with a sequence-processing RNN works, but the RNN is complicated and may be slow to train.

RNNs good for variable-length sequences, but we can fix our sequence length.

**Fully-convolutional hybrid 3D net**: Use a convolutional module for 2D segmentation, then another one for 3D context aggregation.

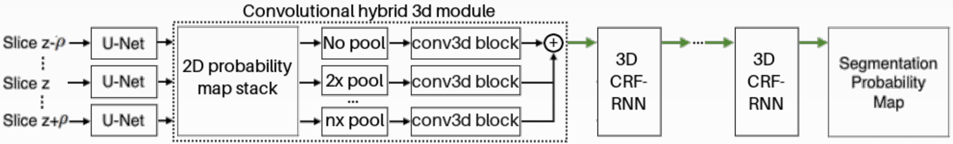


# HYBRID 3D + CRF-RNN

We created **3D CRF-RNN** modules for our data.

Separate *xy* and *z* spatial filtering parameters to account for anisotropy.

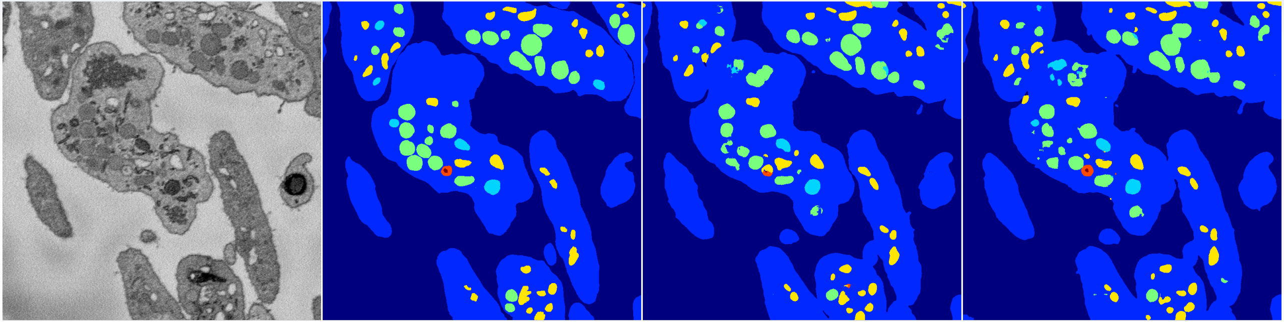
**Full processing stack**: 2D U-nets → hybrid 3D module → 3D CRF-RNN.



# RESULTS

Trained 2D u-nets individually ($\sim$ 100k iterations), then trained a hybrid 3D module + 3D CRF-RNN end-to-end ($\sim$ 1k iterations).

**2D U-net results:** Best 2 out of 150 randomly-generated U-nets.



Platelet data

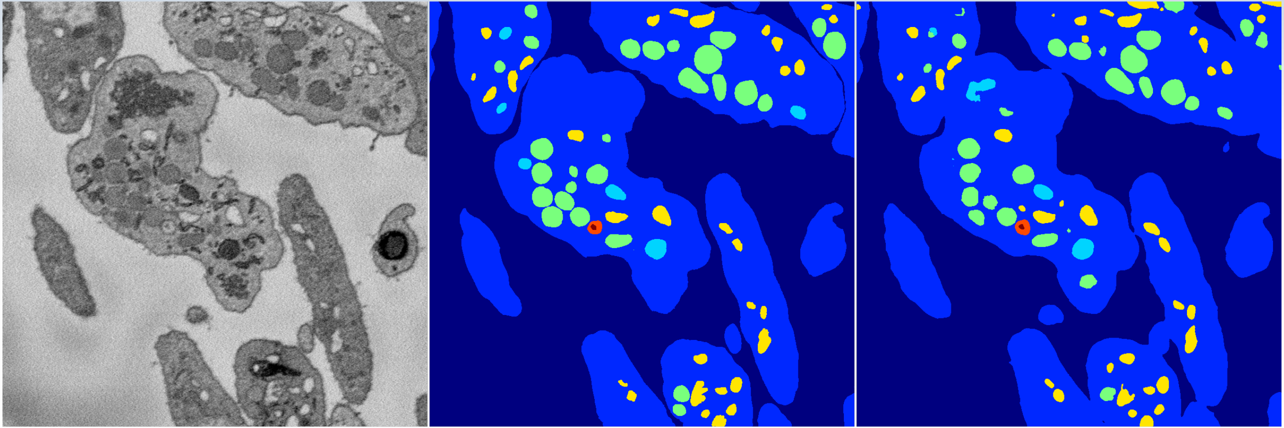
Ground truth

2D U-net 1

2D U-net 2

# RESULTS

## Hybrid 3D + CRF results:



Platelet data | Ground truth | Hybrid 3D + CRF

# FUTURE WORK

Hybrid 3D + CRF still needs some tuning, mainly to prevent **cell merge errors**.

How to combine with network **ensembles**: 2D stage? Right at the end? Both?

**Validate** hybrid 3D module architecture and 3D CRF-RNN hyperparameter choices.

Move on to the hard problem: **generalizing segmentation** across different EM problem instances.

# CONCLUSION

**Ongoing challenge:** Provide segmentation algorithm development solutions that don't require ML expertise in each NIH lab.

3D context can be incorporated cheaply into 3D image segmentation using 2D segmentation networks and a new **hybrid convolutional 3D module** and **3D CRF-RNN** module

Combination of hybrid convolutional 3D module + 3D CRF-RNN boosts performance over 2D segmentation networks alone.