Breakout Session 3: Track B

Development of Software for the Optimization and Normalization of 3D Electron Microscopic Data Acquisition to Facilitate Use and Reuse of AI/ML-Based Image Analysis Tools

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Development of software for the optimization and normalization of 3D electron microscopic data acquisition to facilitate use and reuse of AI/ML-based image analysis tools

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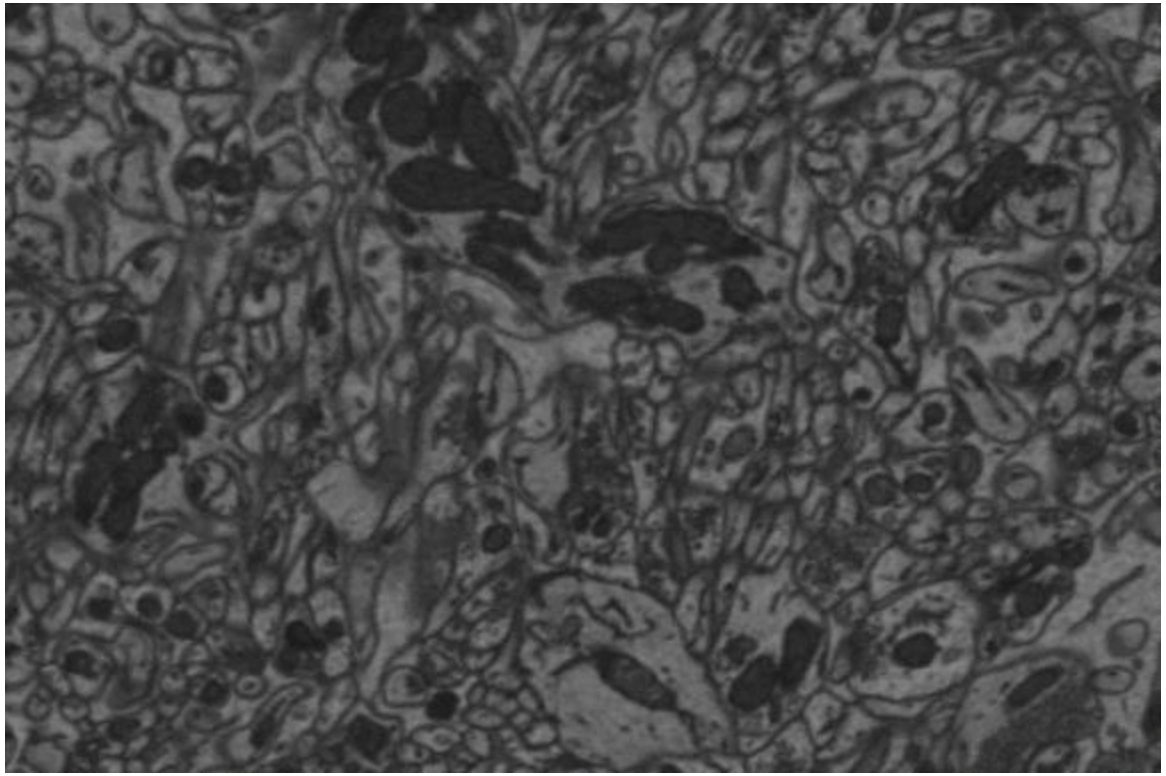
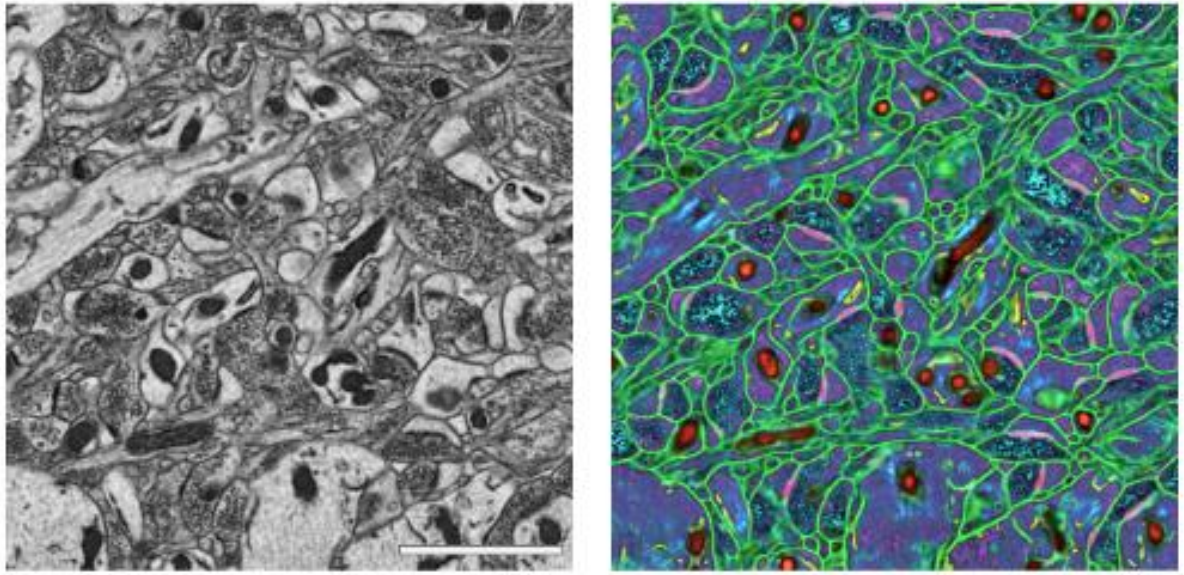
UC San Diego

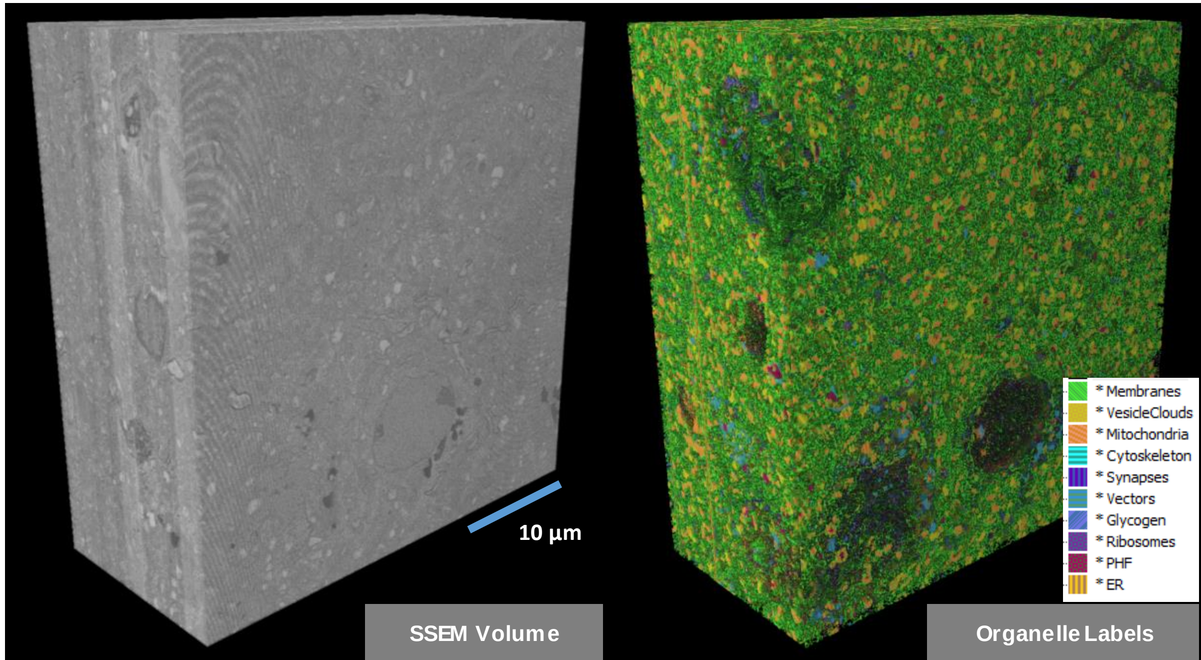
Converting Raw Image Data into Segmentation Data with Deep Neural Networks

Region Segmentation



Organelle Segmentation

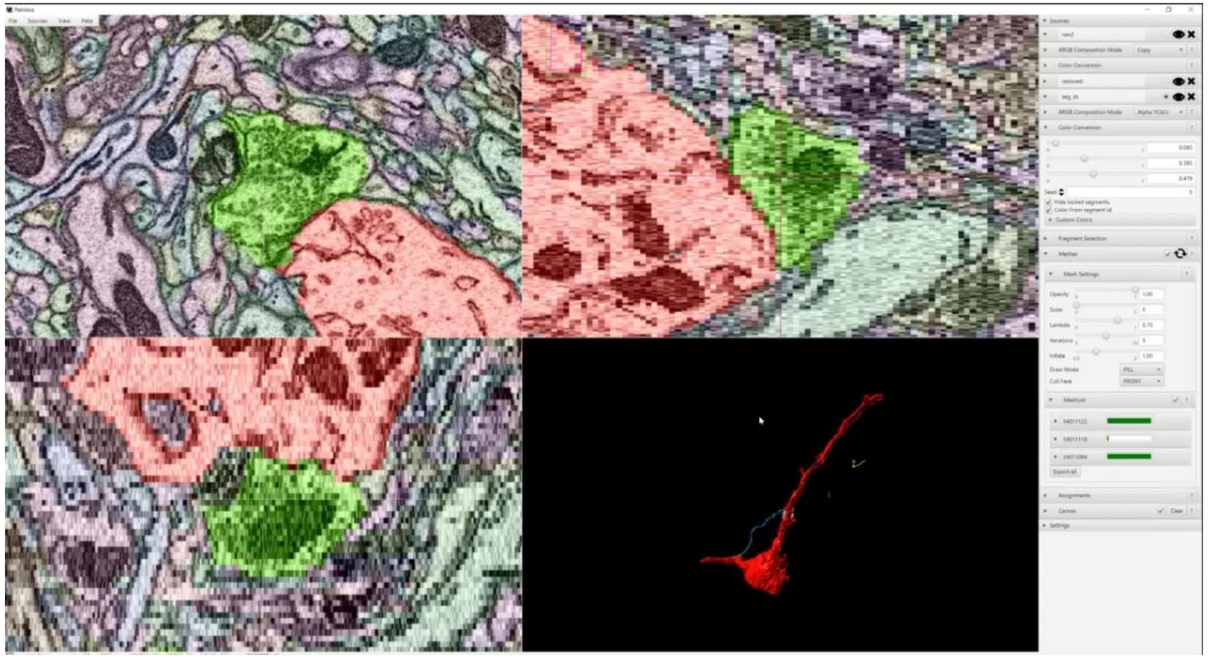




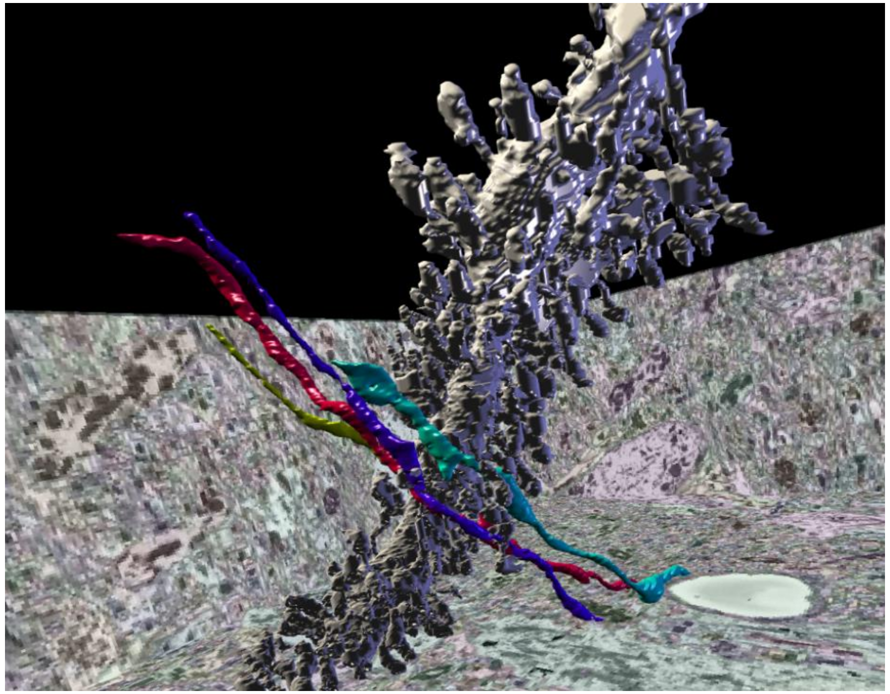
Converting Segmentation Information into Data-enriched Reconstructions

Software-assisted tracing

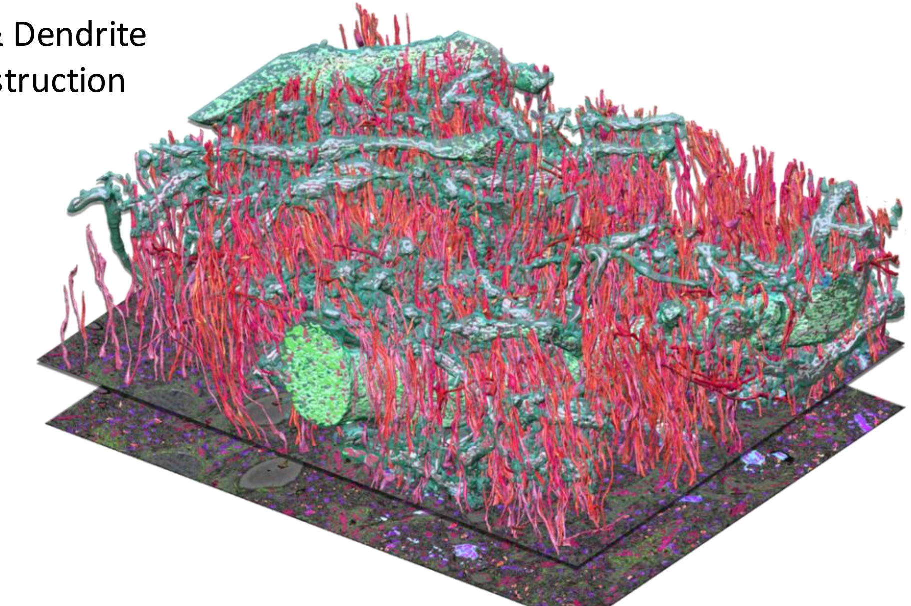
Using Region Labels and *Paintera* software



Spiny dendrite and presynaptic inputs



Axon & Dendrite Reconstruction



More information:
M. Madany, K. Marcus, S. Peltier, M. H. Ellisman and I. Altintas, "NeuroKube: An Automated and Autoscaling Neuroimaging Reconstruction Framework using Cloud Native Computing and A.I.," 2020 IEEE International Conference on Big Data (Big Data), Atlanta, GA, USA, 2020, pp. 320-330, doi: 10.1109/BigData50022.2020.9378053.

Focus of this work

Is not the optimization of the ML/AI tools but rather the optimization of the acquisition of the raw image files that feeds to the ML/AI tools.

Challenge

Generally the end user of a SEM, visually inspects an image and determines by eye if the image is "***good enough***" before proceeding with the acquisition of the protracted 3D Blockface SEM data.

Goal

Provide the end-user with tools to **"measure"** the quality of the image before he/she proceeds with the 3D Blockface SEM data acquisition.

Photo Credit : AutoTopNL on youtube/www.autoevolution.com

Image metrics the software tools will measure

Resolution

Fourier transform of an image, which represents the intensity of frequency spectrum of the contents of the image plotted radially. Fourier analysis is the most reliable method for measuring Resolution of a SEM image. The tool measures the resolution completely automatically, but determining the boundary threshold between signal and noise.

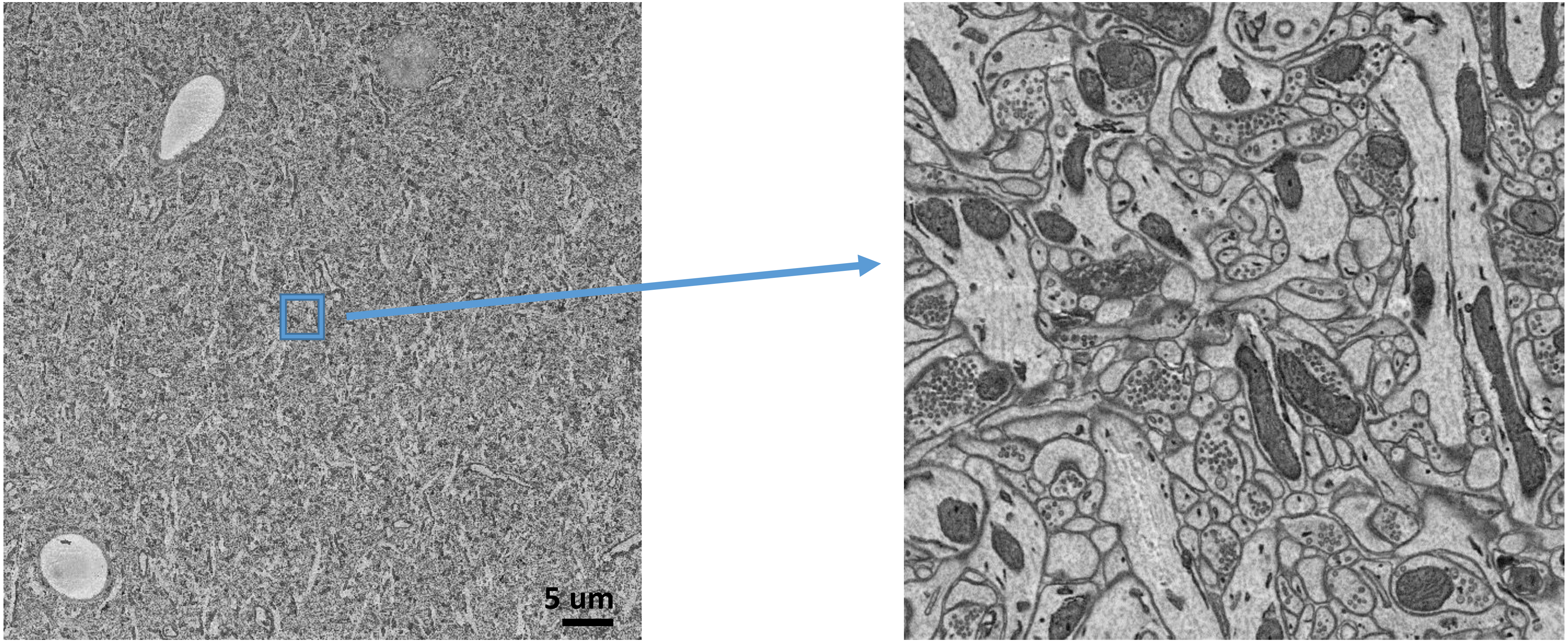
Signal-to-Noise (SNR)

The SNR is calculated by computing the cross correlation of two images acquired on the same object, based on the premise that signal is correlated between the two images but noise is uncorrelated. When the image detail is correlated over many pixels, than cross correlation can be substituted by auto correlation, and SNR can be computed by a single image.

Field of View (FOV) distortions

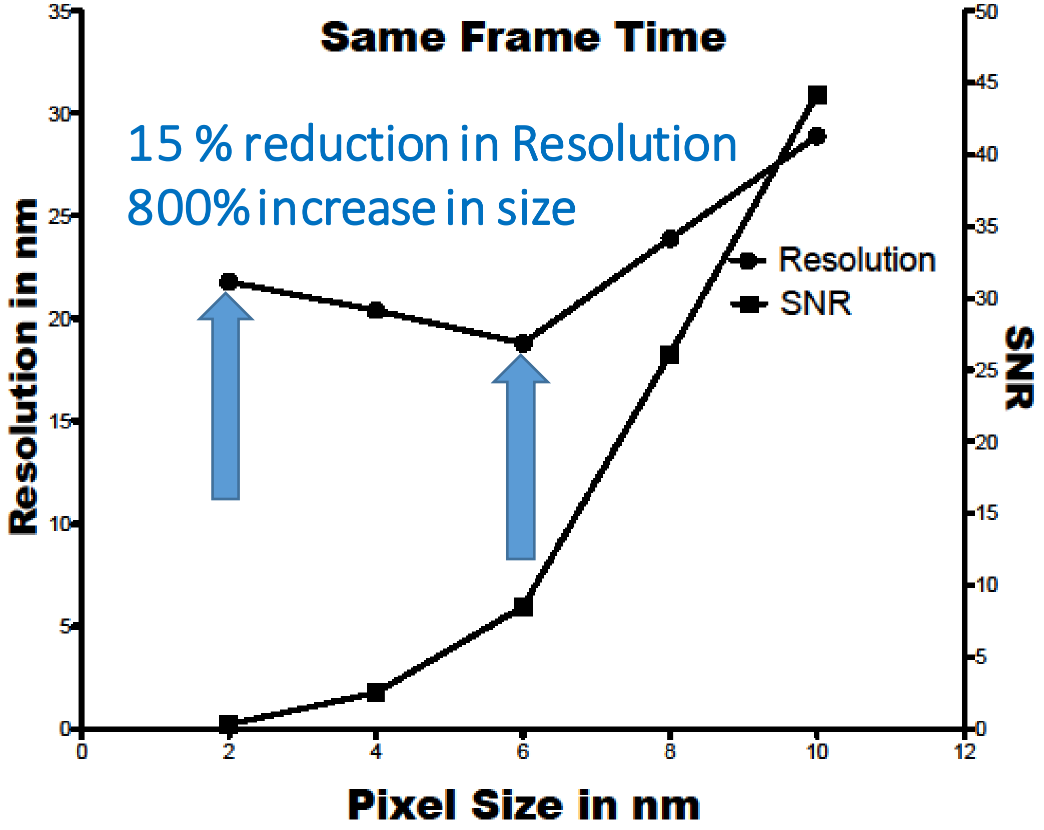
The FOV of a 3D Blockface SEM data is typically very large ~ X microns, and can result in deterioration at the edges of the image. This can be quantified by computing the stigmation at different grids in the image.

Sample used to make the measurements



6 month old Mouse Hippocampus CA1.
Stained for standard 3 View SBEM protocol (includes reduced Osmium stain, post-stained with UA and Pb).
Imaged with Zeiss Sigma 300 Blockface SEM.

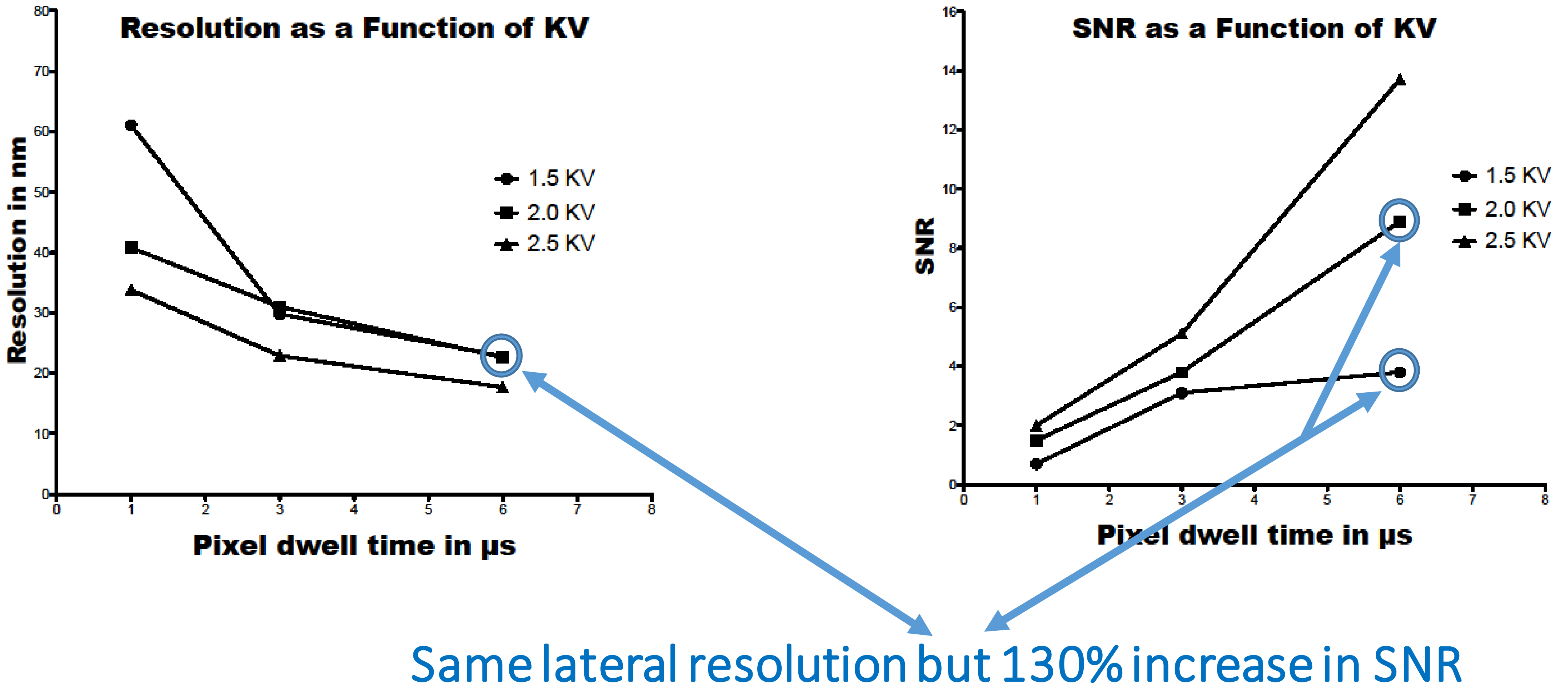
Total Image Acquisition time kept constant



Pixel Size	Dwell time	Image Size
2 nm	0.5 µs	760 MB
4 nm	2 µs	190 MB
6 nm	4.5 µs	85 MB
8 nm	8 µs	48 MB
10 nm	12.5 µs	31 MB

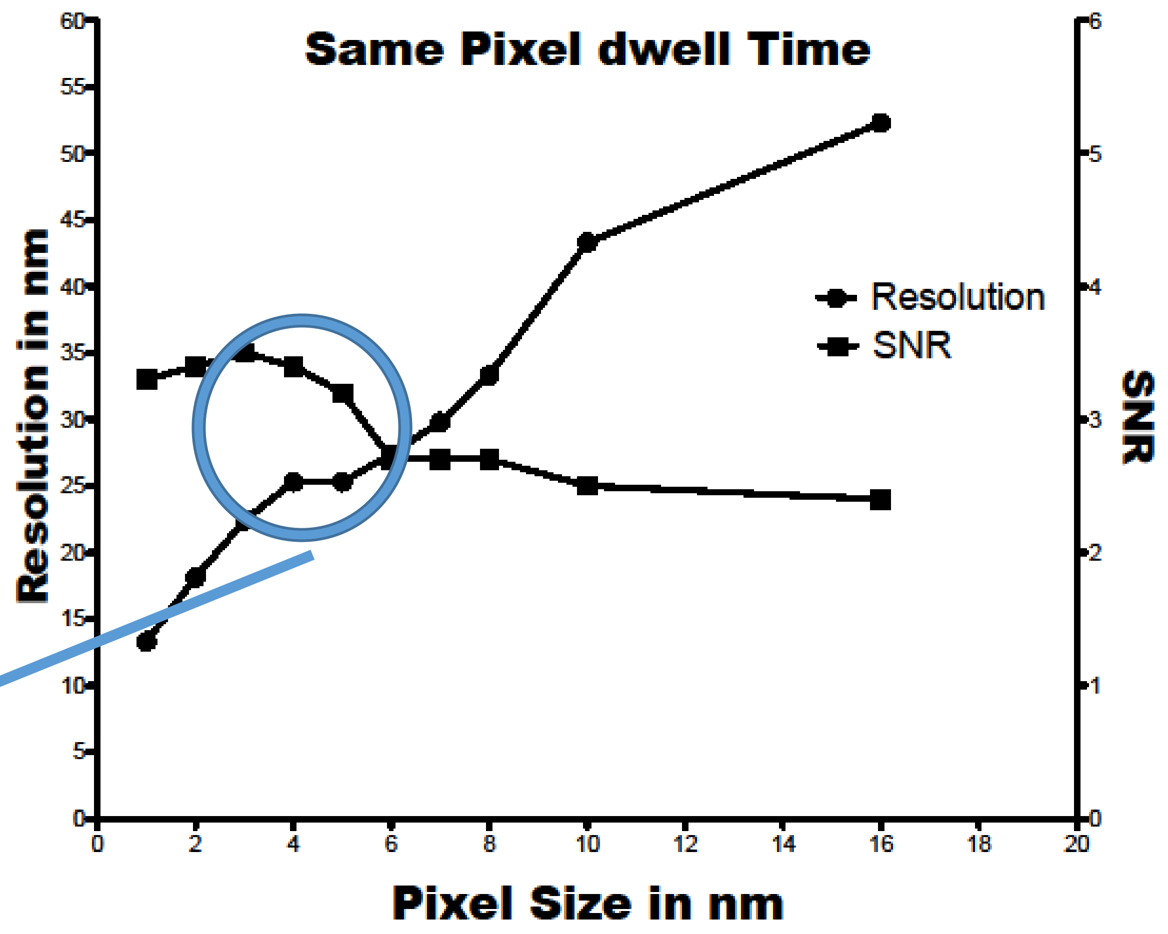
Practically, the total acquisition time is dictated by the beam dose the sample can tolerate without beam damage.

Pixel size kept constant, variations in beam energy and acquisition time



At Low KV, the depth resolution increases, generally at the expense of lateral resolution.

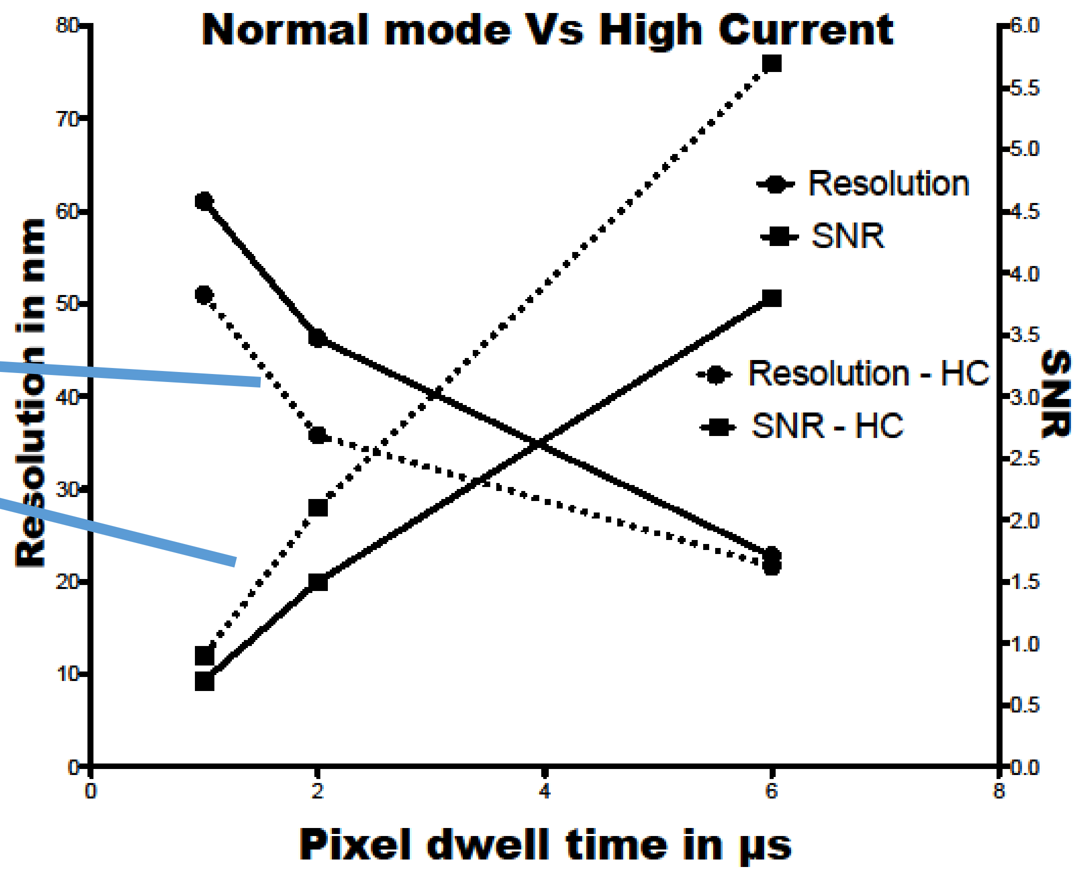
Pixel dwell time kept constant, pixel size varied



Optimum in terms of Resolution, SNR, beam damage, total acquisition time.

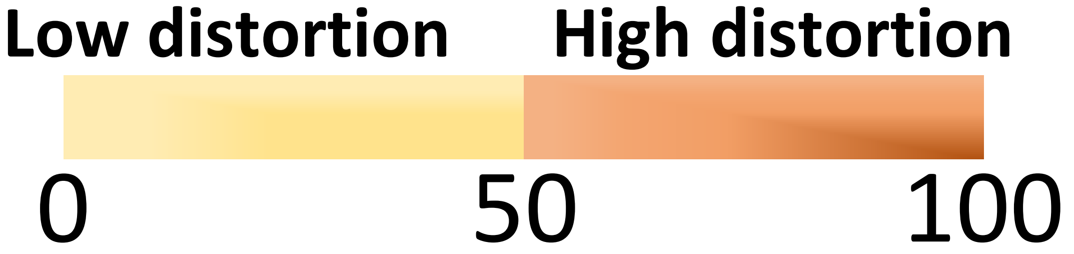
Effect of beam current

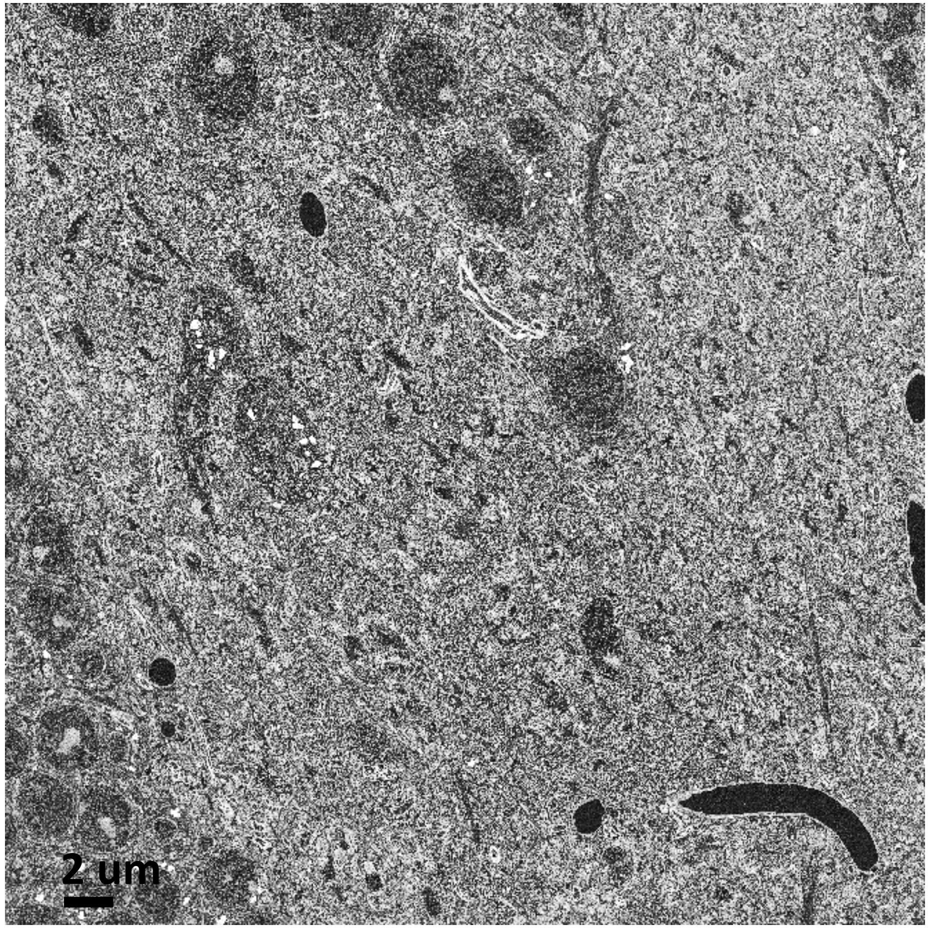
High current mode provides both better resolution and SNR



Theoretically, Normal mode has smaller probe/beam diameter and should provide better resolution; High current mode should have better SNR due to its higher beam current.

Field of View (FOV) distortions





9	32	13	30	21
8	35	13	30	27
27	25	23	41	38
59	25	5	27	22
65	59	48	34	29

Summary

- We have developed a tool by which the end user of the microscope can measure the image quality by varying imaging different parameters, and determine the most optimum conditions.
- This will allow the Biologist/Microscopist who is collecting the data and the AI/ML data scientist who is processing the data to communicate to each other, and target specific image acquisition strategy for the task at hand.
- Improves efficiency of transfer learning for AI/ML segementation for similar datasets, thereby reducing computational time and cost.

Future Work

- We are currently in the process of collecting 3D volumes for some of the different conditions that were mentioned. We would like to perform the 3D reconstruction, segmentation and rendering of these datasets to determine how the initial parameters of image acquisition affect the quality of final reconstruction and optimal strategies to normalize the data.

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