## Optimization of Large-Scale Imaging with Scanning Electron Microscopy

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## Y

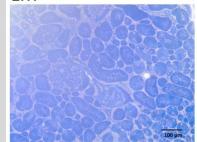
## **Imaging with Electrons**

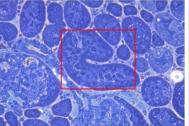
- Scanning Electron Microscope (SEM)
- Transmission Electron Microscope (TEM)

Comparison of light microscopy (LM) and electron microscopy images of sections of mouse kidney.

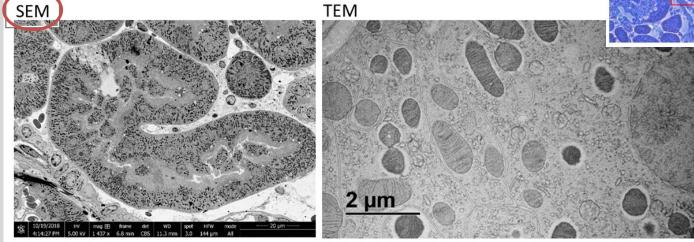
Images provided by Dr. Reiner Bleher, Northwestern University.

#### LΜ





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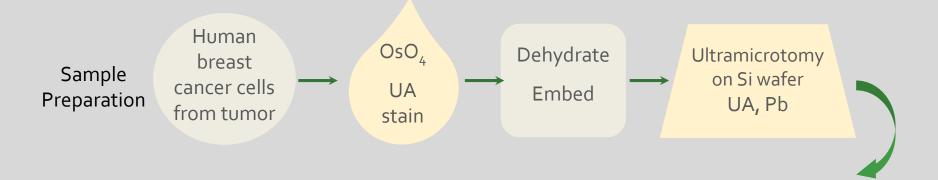








## Large-Scale Imaging with SEM



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## **Image Quality**

### Signal to Noise Ratio (SNR)

SNR = 
$$10 \cdot \log_{10} \left[ \frac{\sum_{0}^{n_x - 1} \sum_{0}^{n_y - 1} [r(x, y)]^2}{\sum_{0}^{n_x - 1} \sum_{0}^{n_y - 1} [r(x, y) - t(x, y)]^2} \right]$$

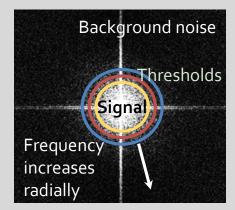
Average signal power

Average background noise power



## **Spatial Resolution**

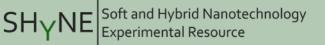
#### Fourier-based method



# Cropped FFT power spectrum with thresholding



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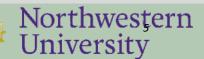


# How do we get the highest quality image?

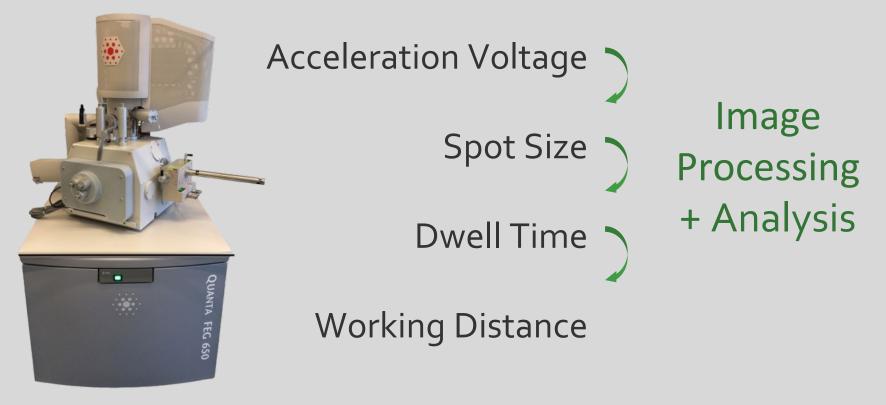
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## Approach



#### Quanta FEG 650 SEM



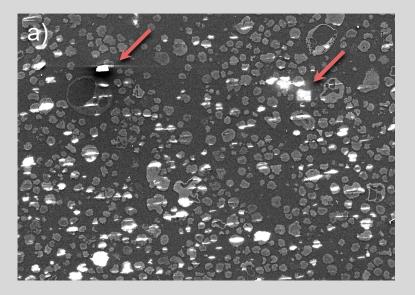




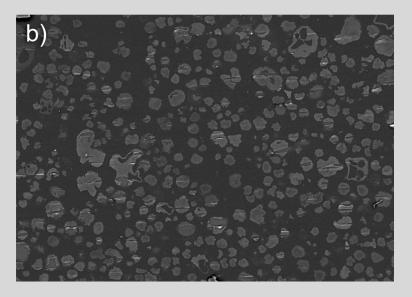


## SE vs. BSE

#### Secondary electrons (SE): surface topography



#### ➤ Backscatter electrons (BSE): material composition



#### Human breast cancer cells from tumor SE (left) vs BSE (right) 20 kV, 200x (cropped)

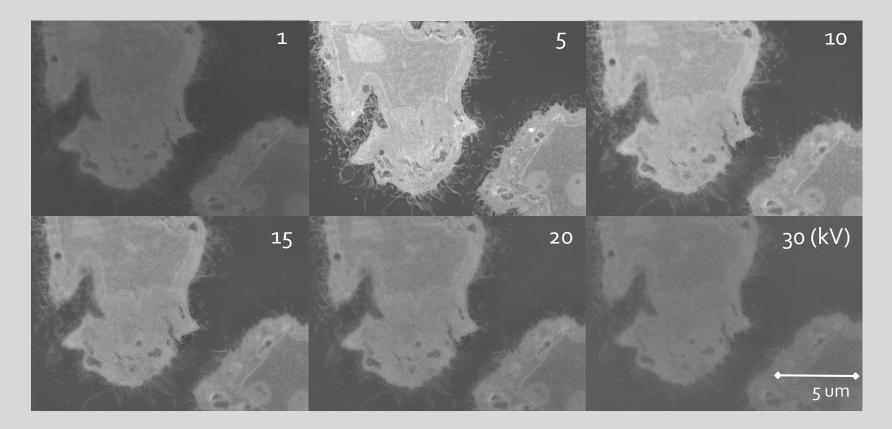






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## Results: Acceleration Voltage



BSE 10kx: 30 µm aperture, 3.5 spot size, 5 µs dwell time, 10 mm working distance, contrast adjusted

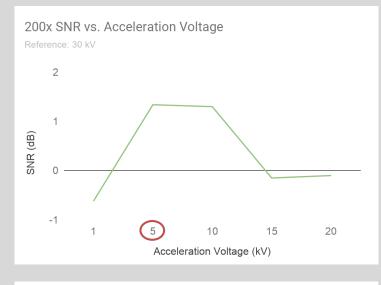
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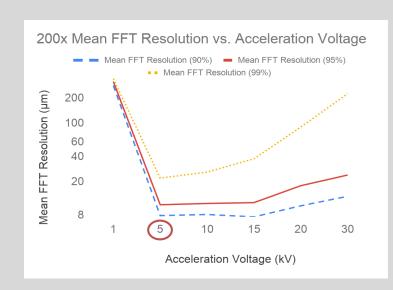
## **Results: Acceleration Voltage**

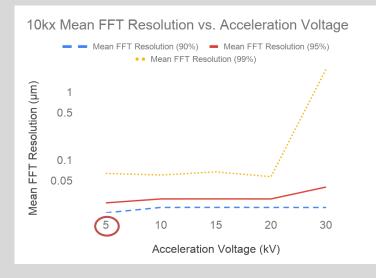


#### 10kx SNR and Acceleration Voltage

Reference: 1 kV



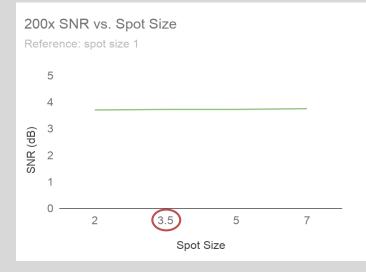


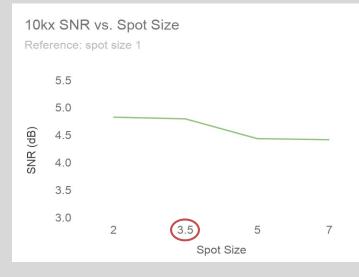






## **Results: Spot Size**





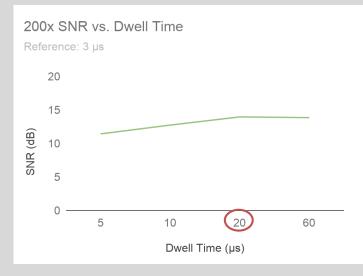


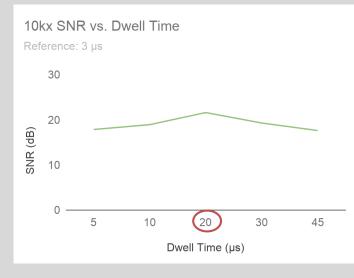




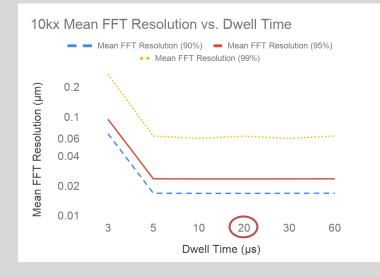
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## **Results: Dwell Time**









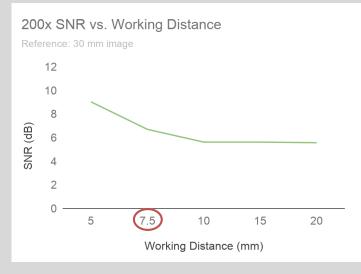






## Y

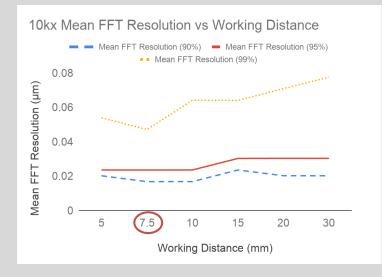
## **Results: Working Distance**











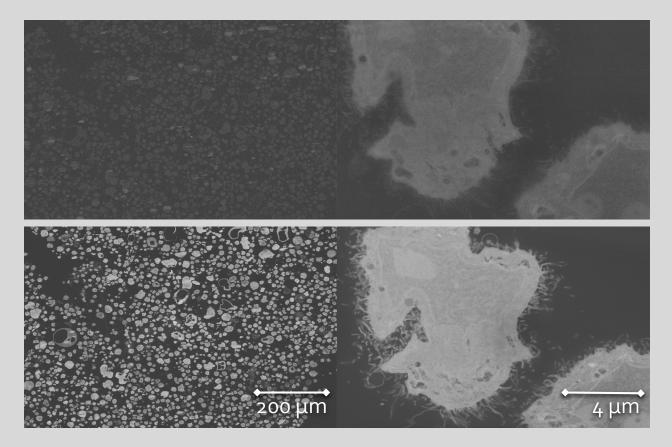








## **Final Images**



Human breast cancer cells from tumor 200x (left), 10kx (right)

(top) 1 kV, 3.5 spot size, 5 µs dwell time, 10 mm working distance (bottom) 5 kV, 3.5 spot size, 20 μs dwell time, 7.5 mm working distance

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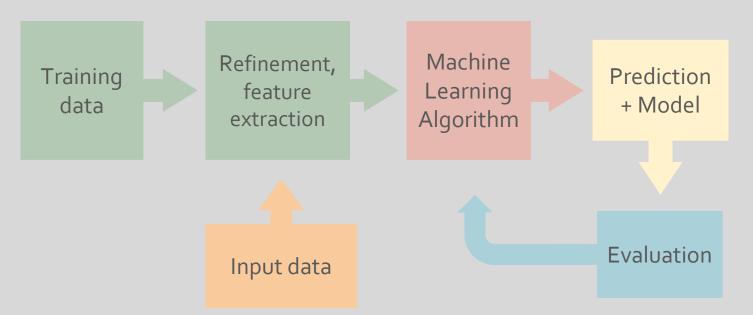


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## **Future Work**

#### > Process of optimization -> Machine learning



#### > Other quality metrics: **contrast transfer function (CTF)**







## Acknowledgements

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