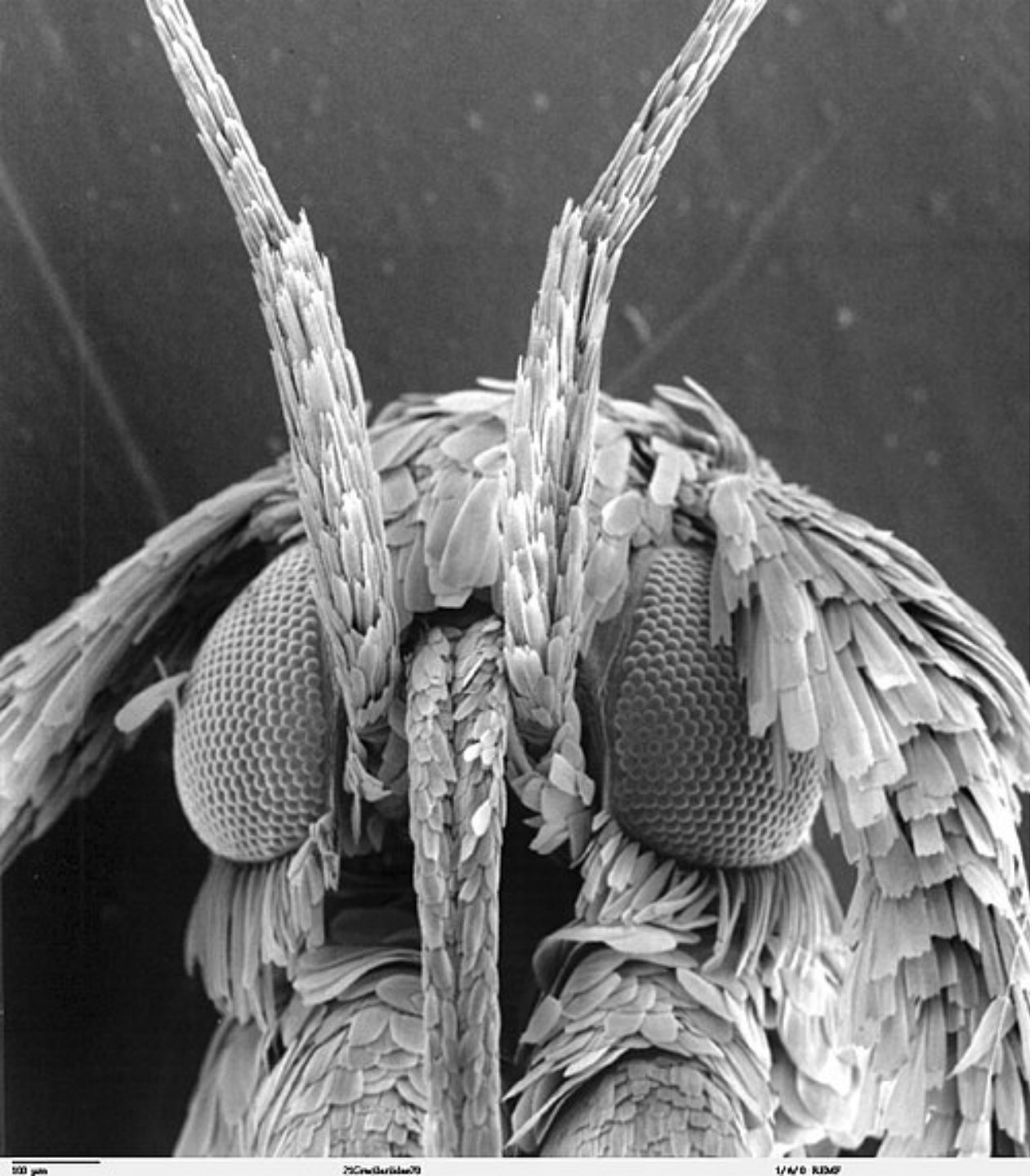
Southampton
University Hospitals NHS Trust

NHS

University of Southampton

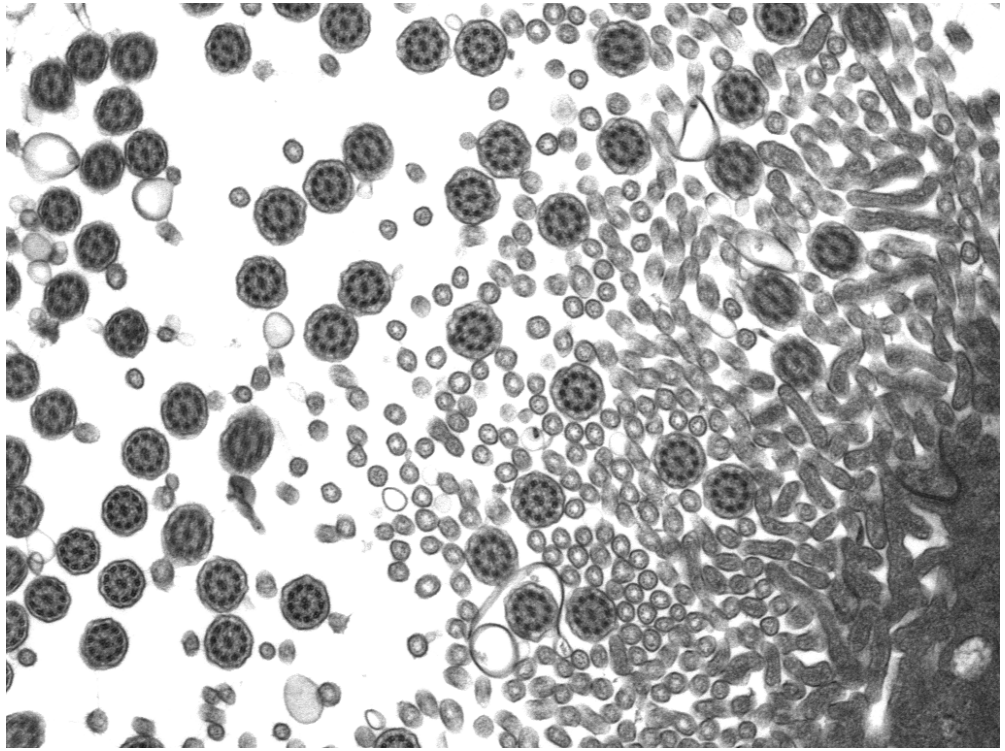
Could SEM be the new TEM?

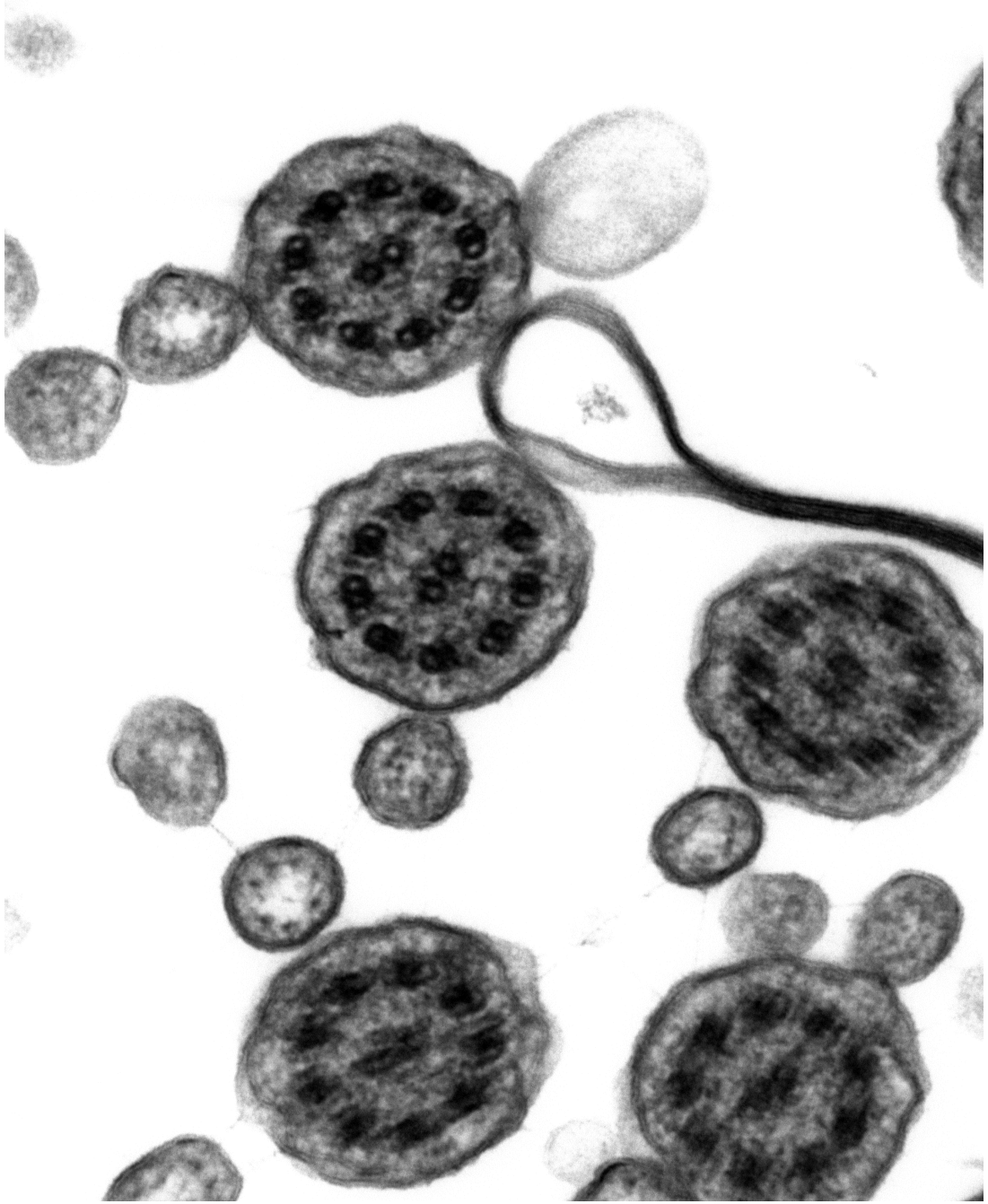
Patricia Goggin

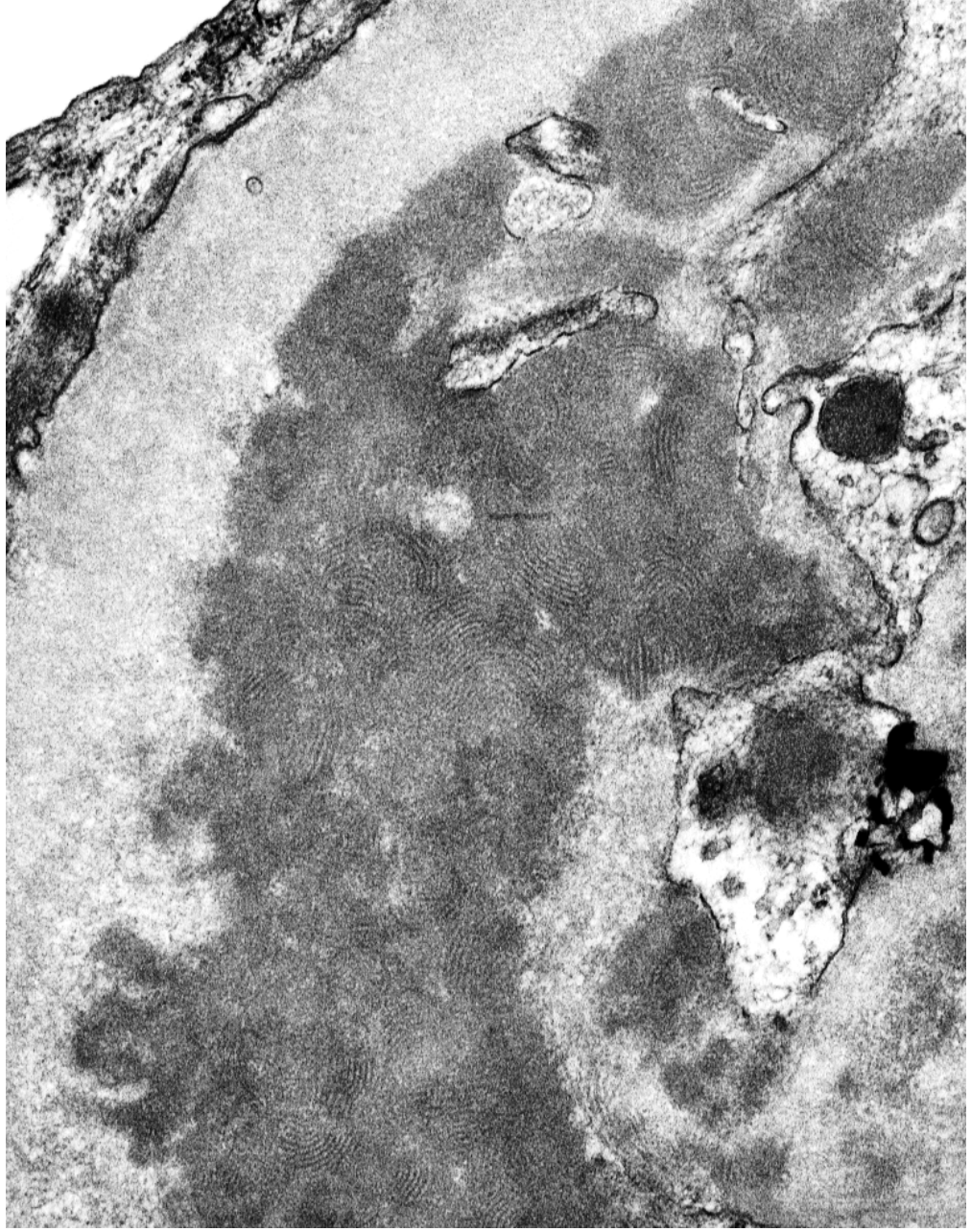


SEM or TEM?

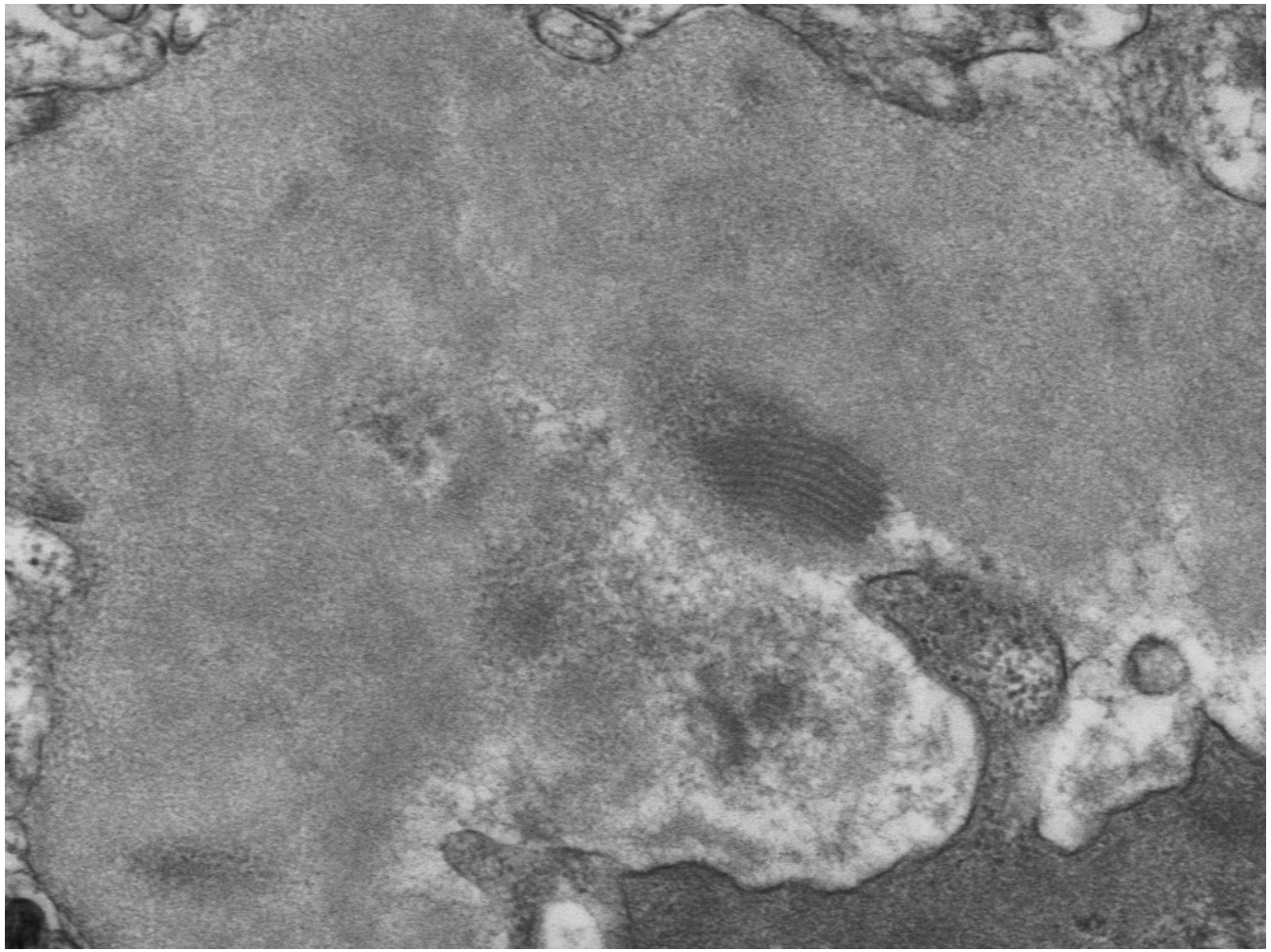
SEM or TEM?







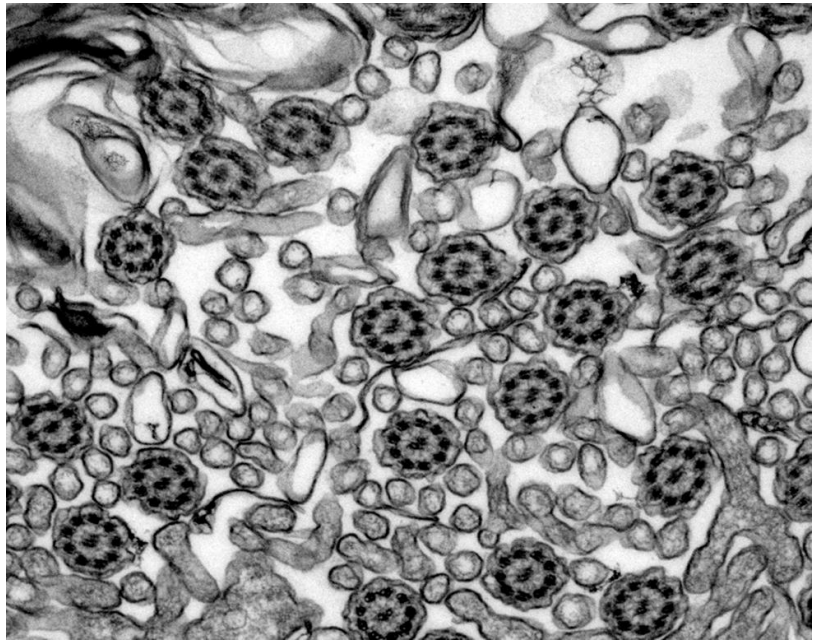
SEM or TEM?

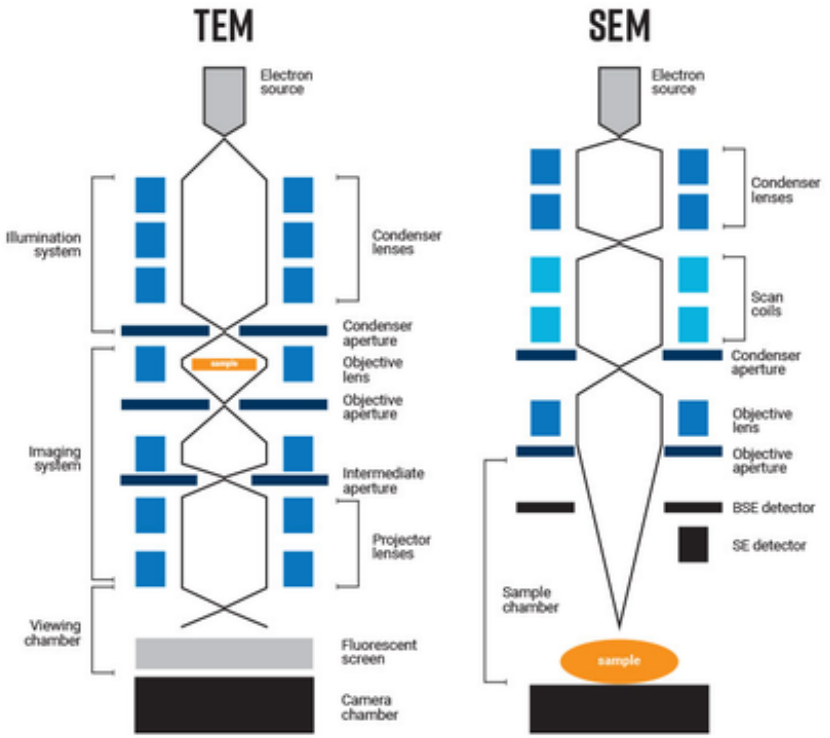


SEM v TEM

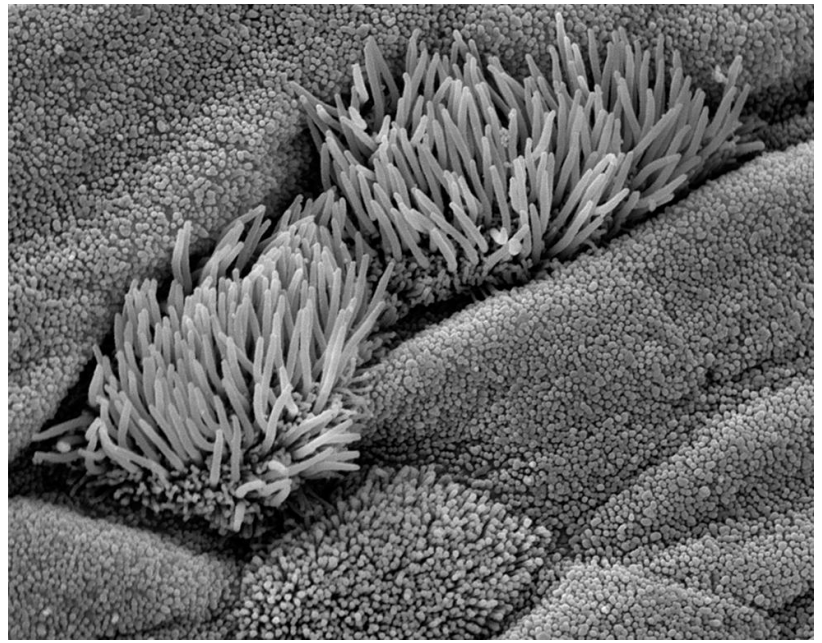








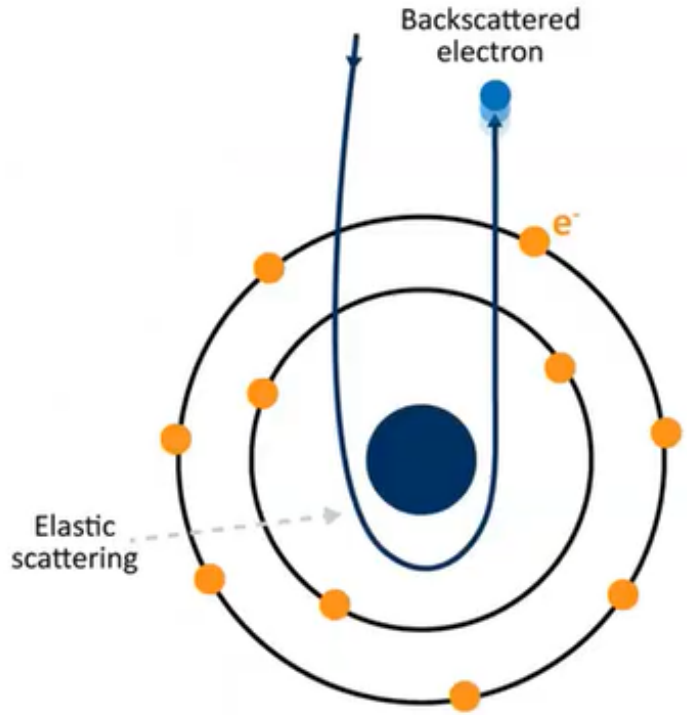
Schematic diagram of TEM vs SEM column design, highlighting the major components.



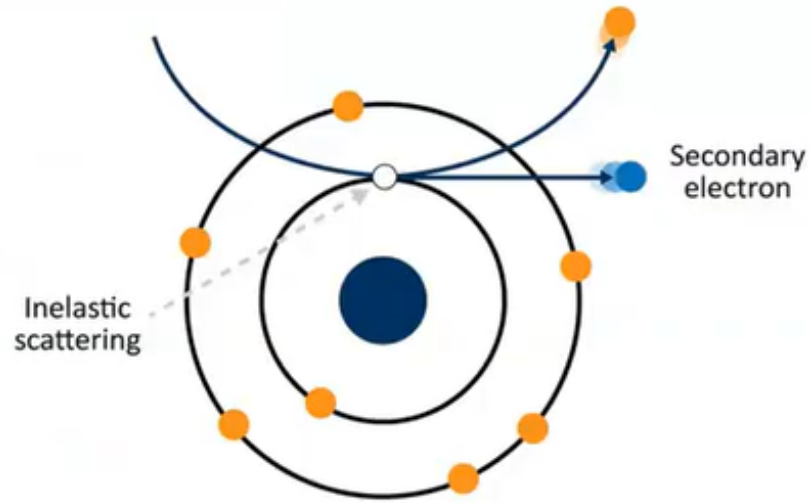
Improvements in SEM

- FEG - > HRSEM
- Improved BSE and STEM detectors
- Table top ‘portable’ instrumentation
- Automated scan generation systems
- Serial Block Face
- Focussed Ion Beam

Electrons in SEM



BSE



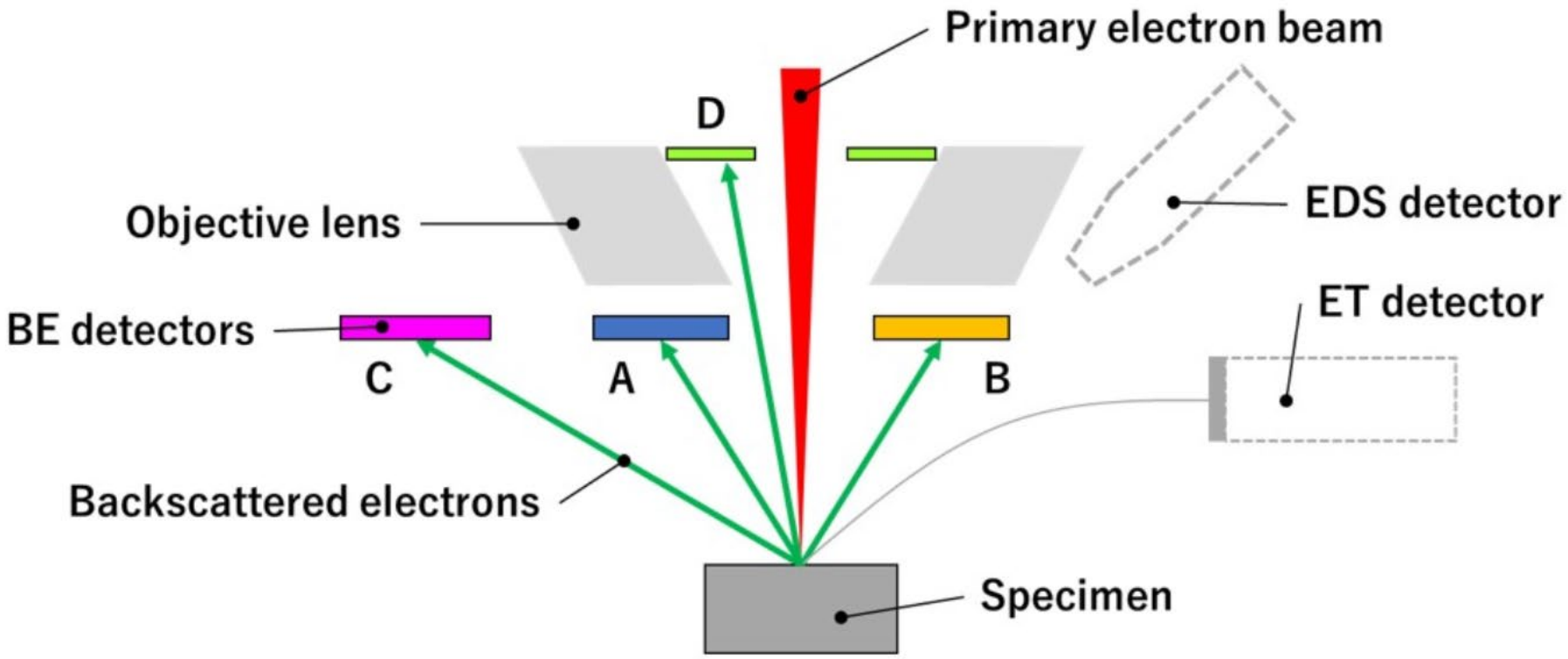
SE

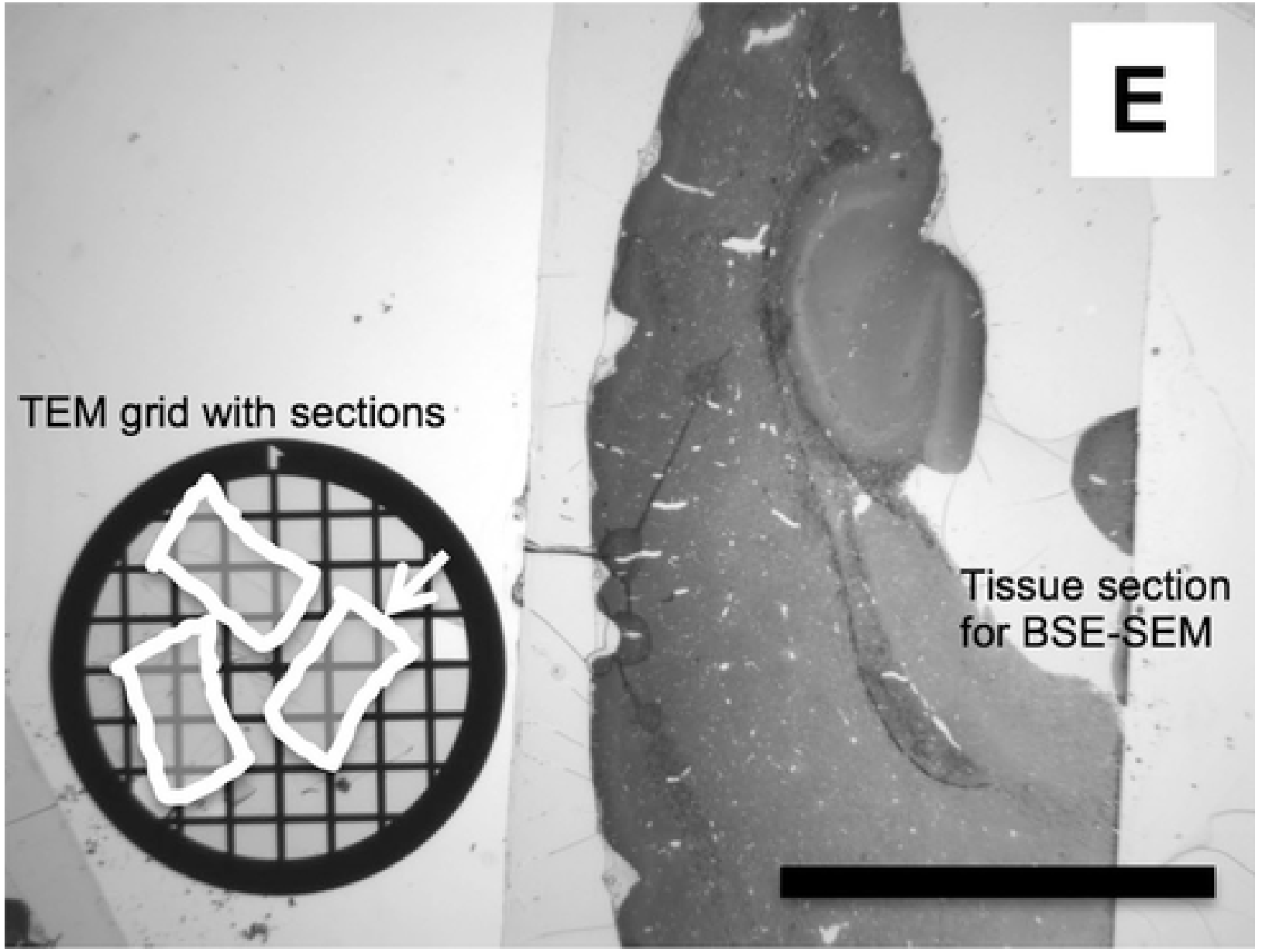
- BSE are reflected back after elastic interactions between the beam and the sample.
- BSE come from deeper regions of the sample.
- BSE reflect high sensitivity to differences in atomic number; the higher the atomic number, the brighter the material appears in the image.

- SE originate from the atoms of the sample.
- SE originate from surface regions. SE imaging can provide more detailed surface information.

https://www.nanoscience.com/

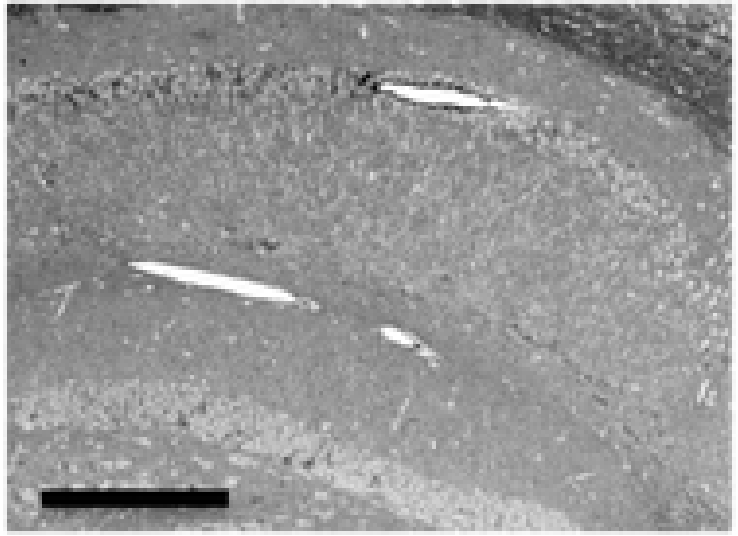
BSE


Primary electron beam
D
Objective lens
EDS detector
BE detectors
ET detector
C
A
B
Backscattered electrons
Specimen



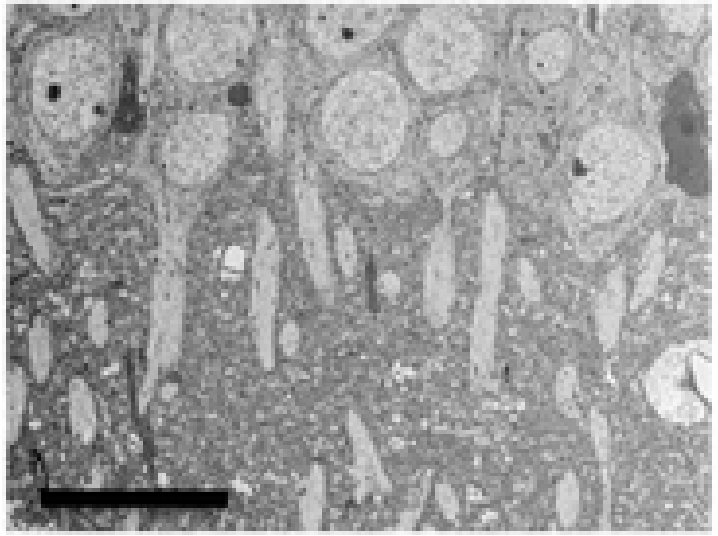
Reichelt et al, J Histochem Cytochem, 2019

Histology
(tissue scale)
Find ROI, lesions or tissue landmarks.
50-250x



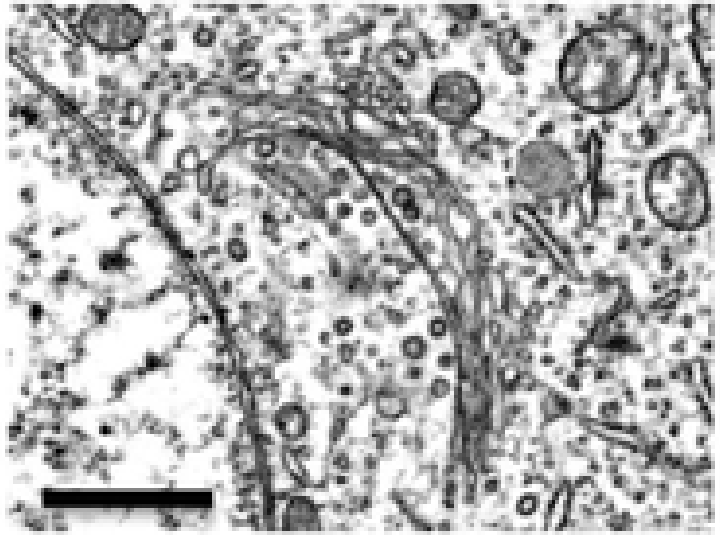
300 µm

Ultra-Histology
(cell scale)
Wide-field and high resolution mapping.
500-2500x



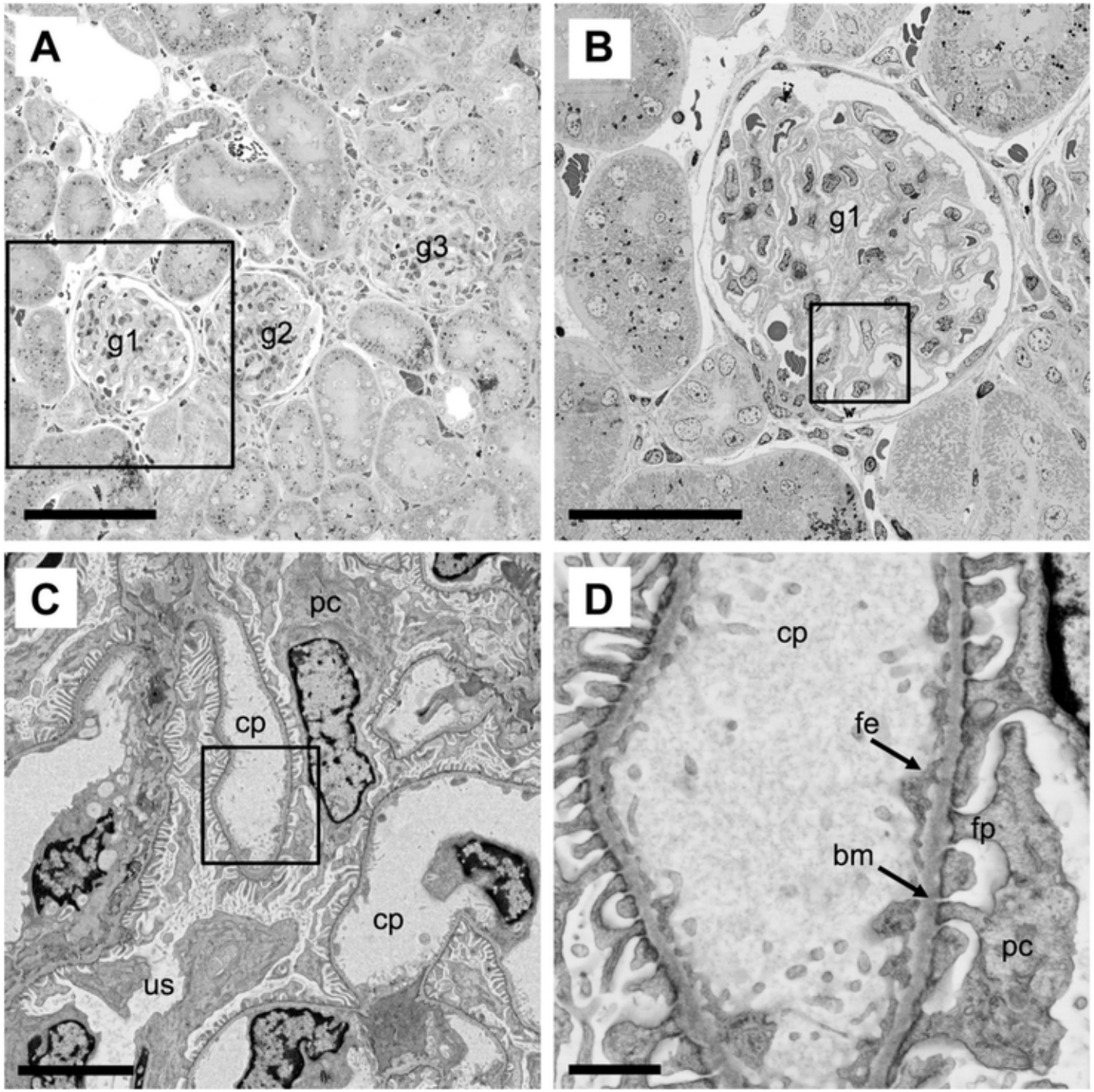
20 µm

Ultra-Structure
(organelle scale)
High resolution with pixel size 1-3 nm.
> 5000x



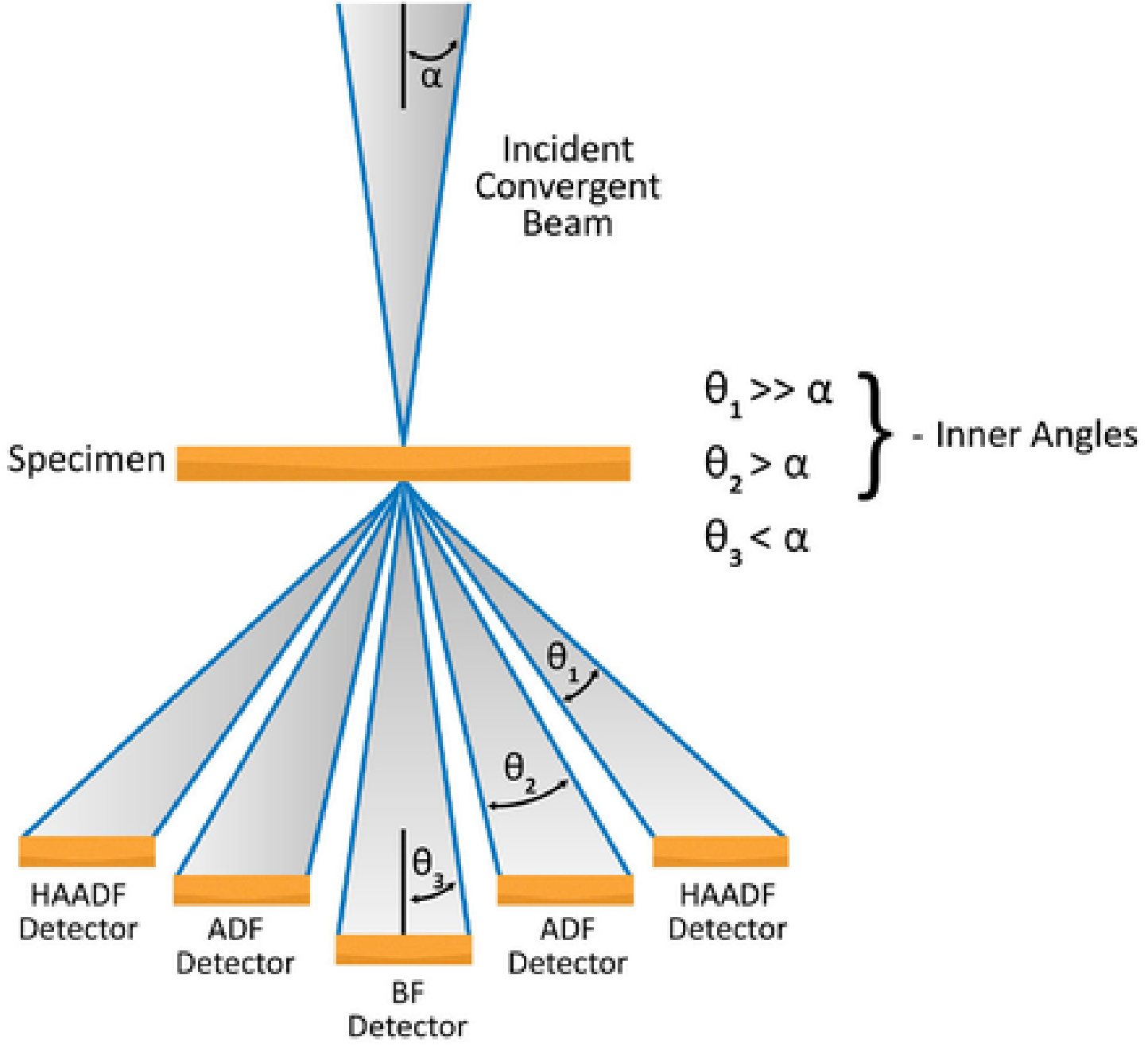
1 µm

Reichelt et al, J Histochem Cytochem, 2019



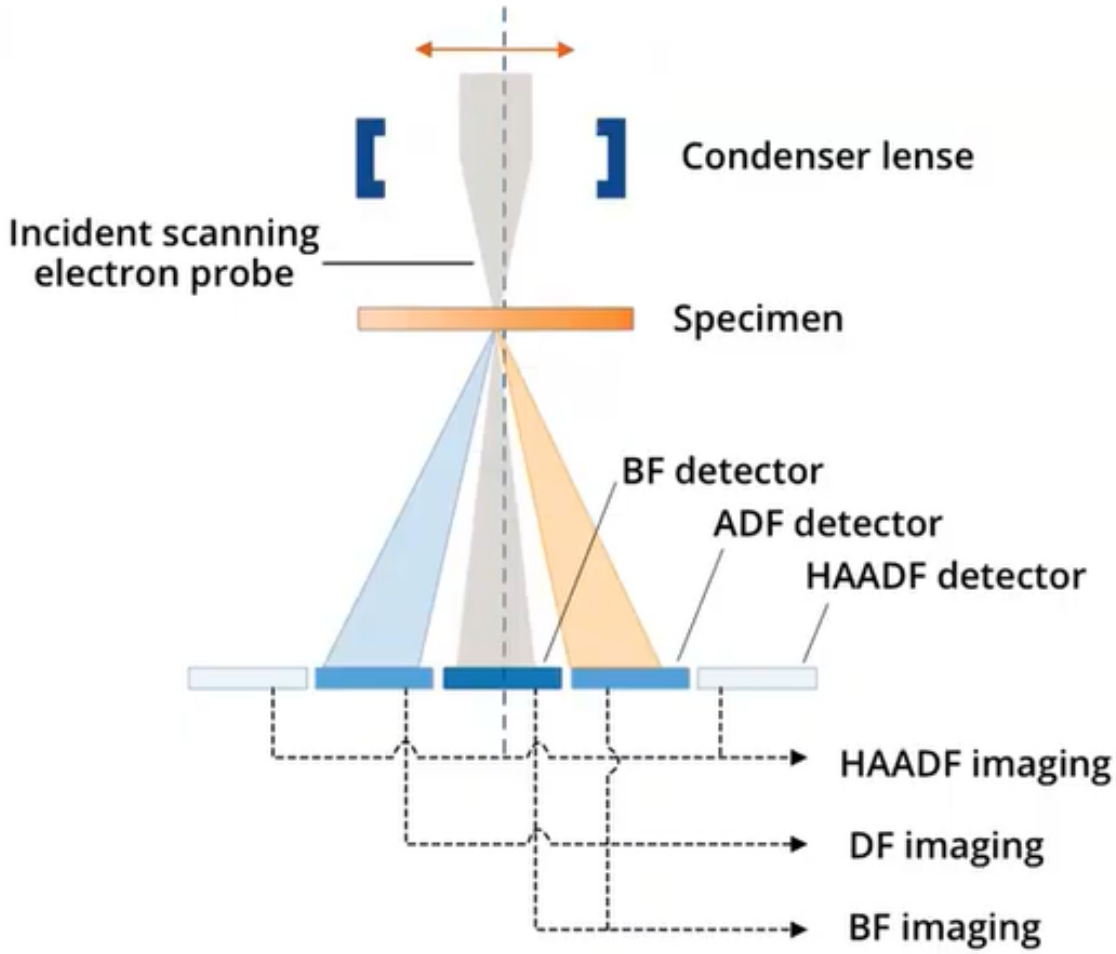
Reichelt et al, J Histochem Cytochem, 2019

STEM


α
Incident
Convergent
Beam
Specimen
θ1 >> α
θ2 > α
θ3 < α
- Inner Angles
θ1
θ2
θ3
HAADF
Detector
ADF
Detector
BF
Detector
ADF
Detector
HAADF
Detector

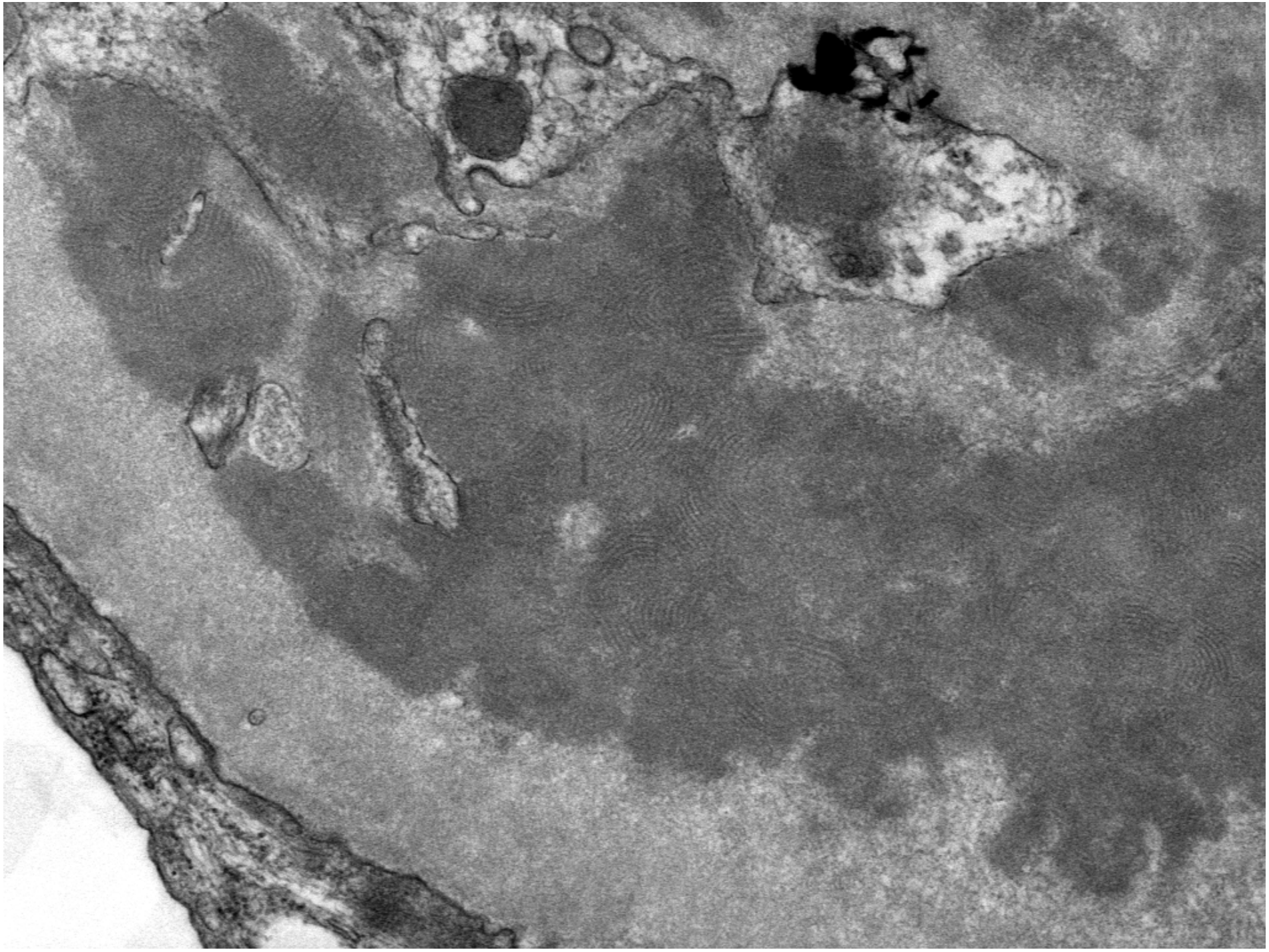
New generation STEM

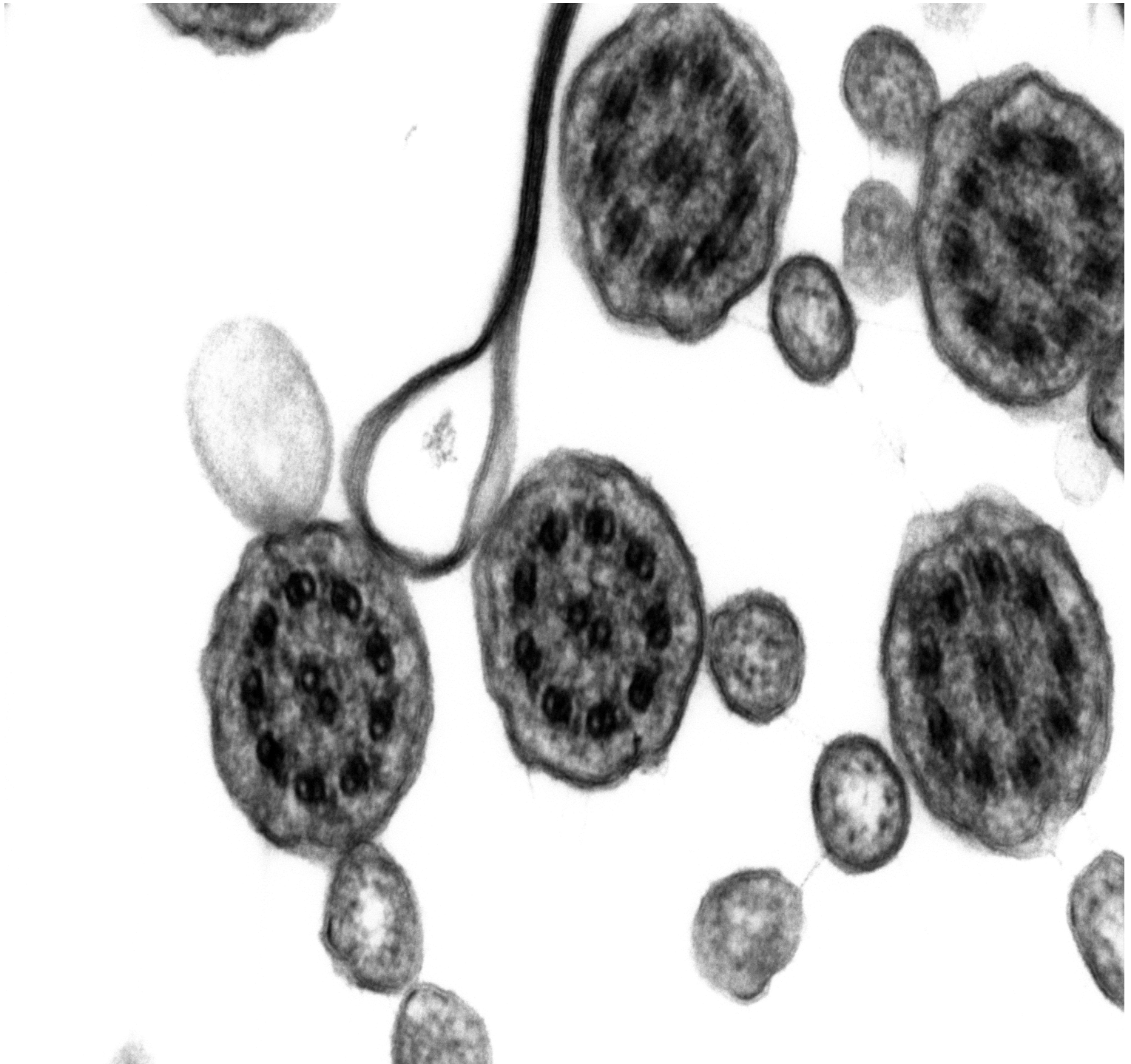
- ❖ STEM- a focused probe is rastered over an area of interest- a signal is recorded at each probe position to form an image.
- ❑ Bright field: The BF detector is a disc that collects all of the electrons directly transmitted on the optical axis of the microscope.
- ❑ Annular Dark-field: The ADF is an annular detector, concentric with the BF detector, allowing the BF signal to pass through while collecting the diffracted and/or scattered electrons.
- ❑ High-angle annular Dark-field: The HAADF detector is a large annular detector that collects electrons scattered at very high angles (Z Contrast imaging).



https://myscope.training/TEM_STEM_detectors

Zeiss Gemini SEM 360
Operating at 20 kV
7.5 um aperture
STEM detector





Zeiss Gemini SEM 360
Operating at 20 kV
7.5 um aperture
STEM detector

Specimen holders

TEM



Phenom Pharos G2 Desktop





HRSEM/STEM









Diagnostic renal pathology in rural, regional and remote Australia: The compelling need for cost-effective solutions The Desktop Electron Microscope Initiative (DEMI)

Murray Killingsworth PhD, FRMS, FFSc (RCPA)[1-4], **Tzipi Cohen-Hyams** PhD[2-4]

[1]NSW Health Pathology

[2]South West Sydney Clinical Campus, School of Clinical Medicine, UNSW Sydney

[3]School of Medicine, Western Sydney University

[4]Correlative Microscopy Group, Ingham Institute for Applied Medical Research

EM diagnostic pathology - NSW

Transmission Electron Microscopy (TEM)

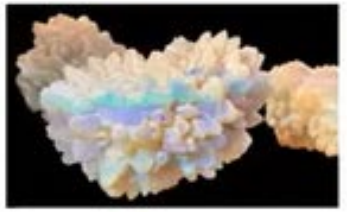
- Used in anatomical pathology, microbiology, virology, haematology
- **NSW – 8.55 million people (high CALD)**
- Increasing incidence of renal pathology from CKD, diabetes, obesity, renal transplantation
- Public Health EM labs decreasing (5 > 2)
- Existing instruments ageing
- Staff shortages
- No current training institutions



This is where the diagnostic journey could start







White Cliffs (opal mining)

Population =

White Cliffs Health Service

The Royal Flying Doctor Service runs a General Practice clinic each week and regular dental clinics, with supporting allied health, mental health and specialist nursing staff.

Patients requiring hospitalisation are transported to Broken Hill.

Services provided by registered nurses, also on call for emergencies. Visiting specialists as required.

Services available

- General medicine
- Immunisation
- Mental health services
- Pathology
- Wound management



Renal disease diagnosis

- 2 tier system metro v rural and remote
- Longer TAT
- Less opportunity for Clinical Trial involvement

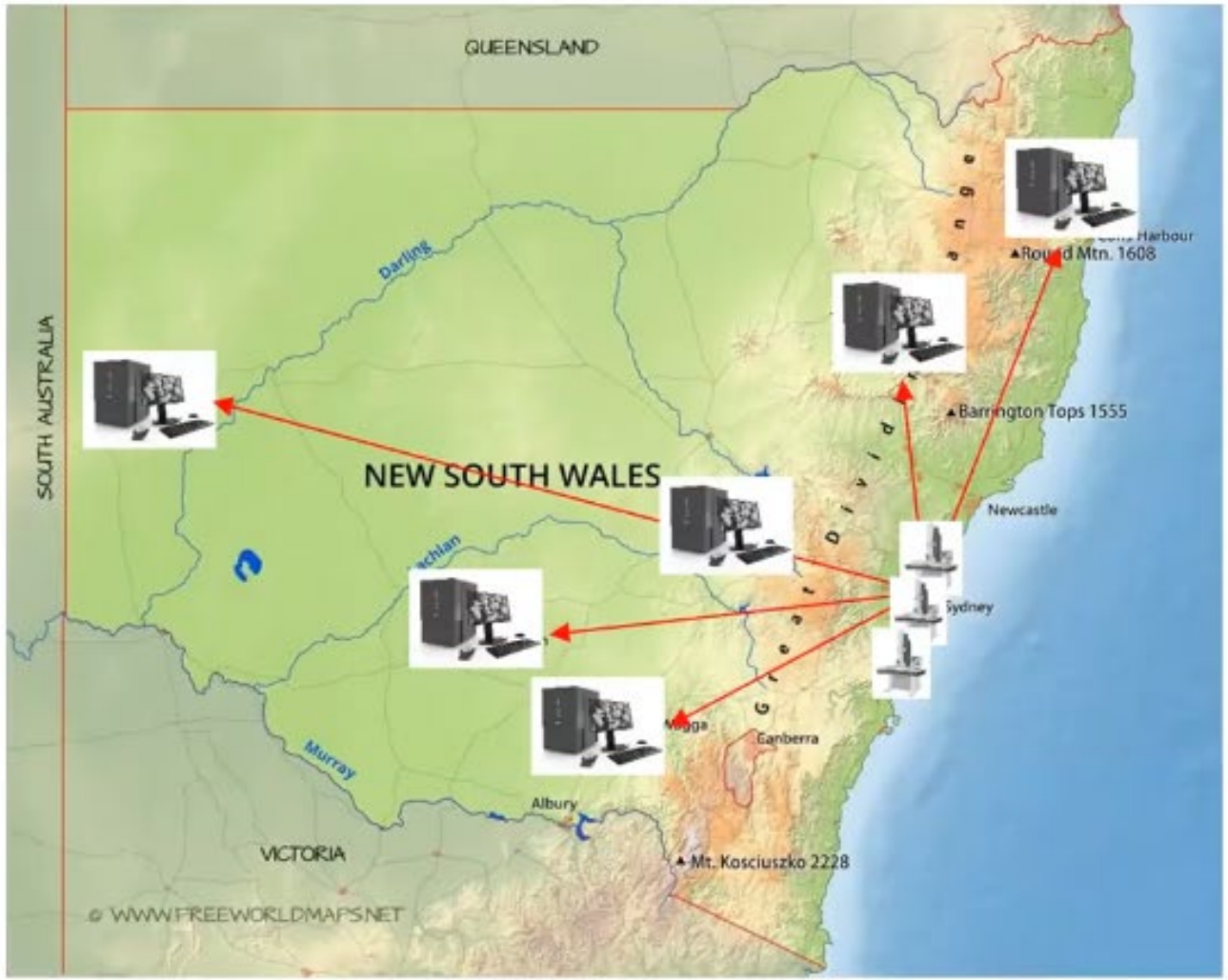
New model of EM service delivery

Implementation:

Use existing NSWHP laboratories with existing Histology/IHC benches

Connect to Sydney hub by digital network for "expert review"

High-resolution EM imaging of grids provided from hub sites if required



Awards



The Desktop Electron Microscope Initiative (DEMI). Winner - 2023 NSW Health Pathology "Pioneering Spirit/Innovation" Award



The Desktop Electron Microscope Initiative (DEMI). One of 4 Finalists for the 2023 NSW Health Awards

Pharos G2 20 kV desktop EM





The Desktop Electron Microscope Initiative (DEMI). **Winner - 2023 NSW Health Pathology "Pioneering Spirit/Innovation" Award**



The Desktop Electron Microscope Initiative (DEMI). One of 4 **Finalists for the 2023 NSW Health Awards**

Broken Hill Base Hospital



Broken Hill Base Hospital

About the facility:
Broken Hill Hospital is a rural teaching hospital that supports 10 remote Health Services.
Providing a 10-bed Emergency Department as well as general medical, surgical, obstetric, paediatric, dialysis, oncology unit and acute mental health beds, operating theatres, specialist palliative care, intensive and coronary care units.
Support services include radiology and pathology departments, as well as a wide range of allied and primary health services. Inpatient beds: 27 medical acute beds including palliative care priority beds, 21 surgical beds, 5 ICU/CCU beds, 6 maternit
health inpatient beds, 10 s
dialysis chairs, 3 operating
Special Care Nursery Level



An EM provides more than just a diagnosis

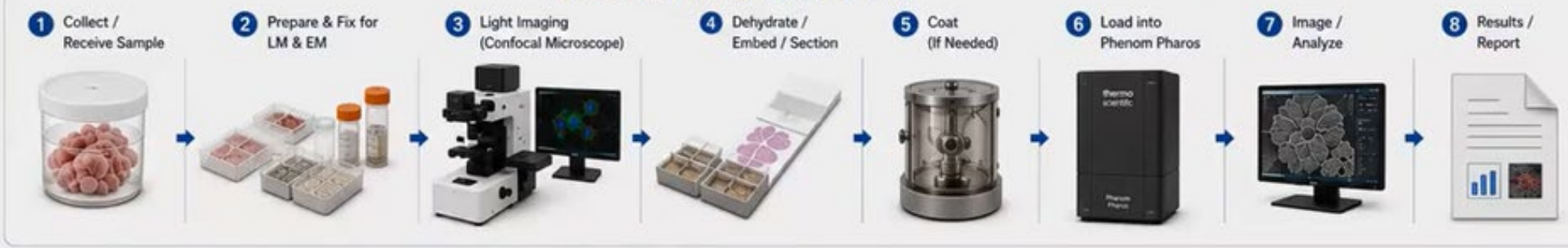
- Provides **Diagnostic** and **Research** infrastructure
- Attracts clinicians with an academic interest
- Superior diagnosis when included
- Faster turnaround times for biopsy diagnosis
- Fosters an academic environment – clinical trials
- Chemical analysis platform

MOBILE ELECTRON MICROSCOPY LAB

Sample Preparation to Imaging



WORKFLOW: FROM SAMPLE TO IMAGE

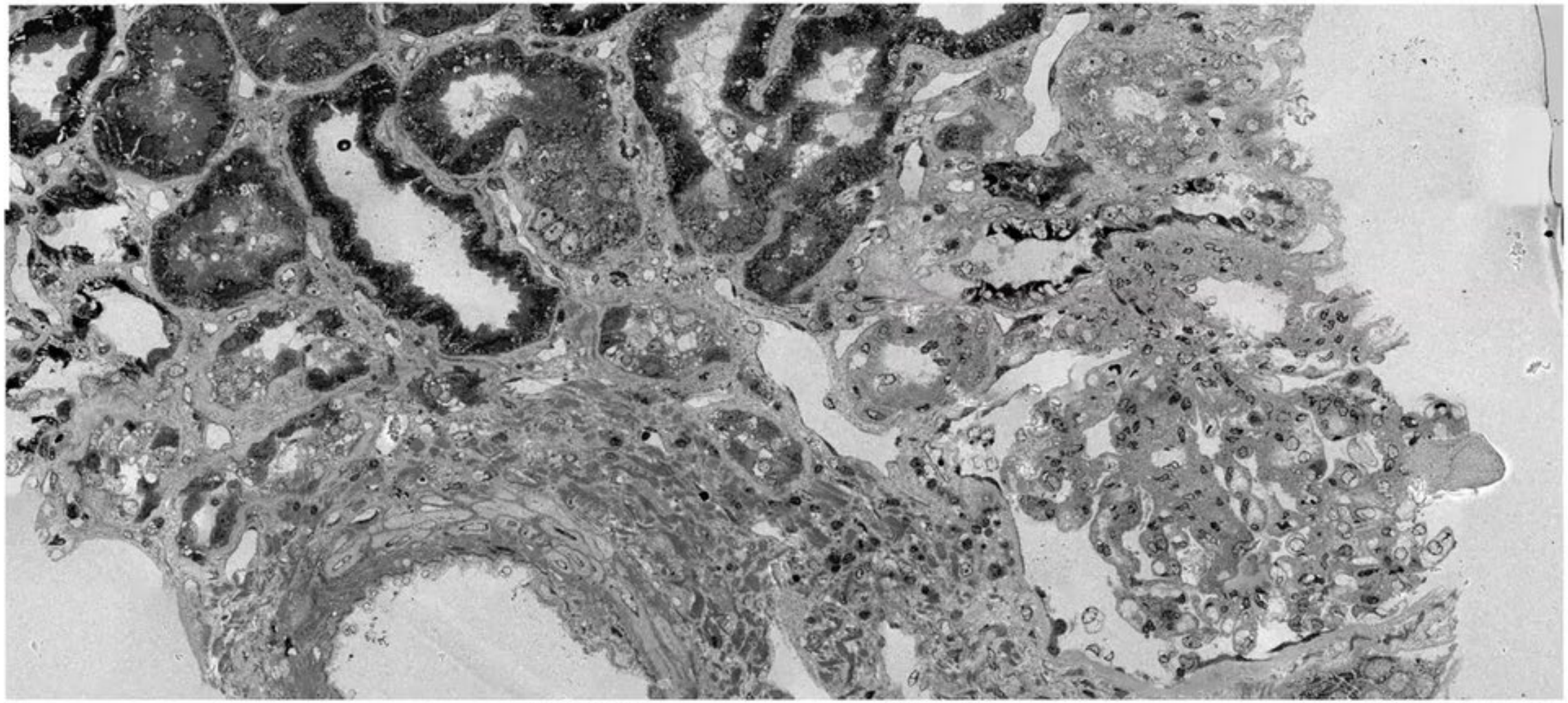


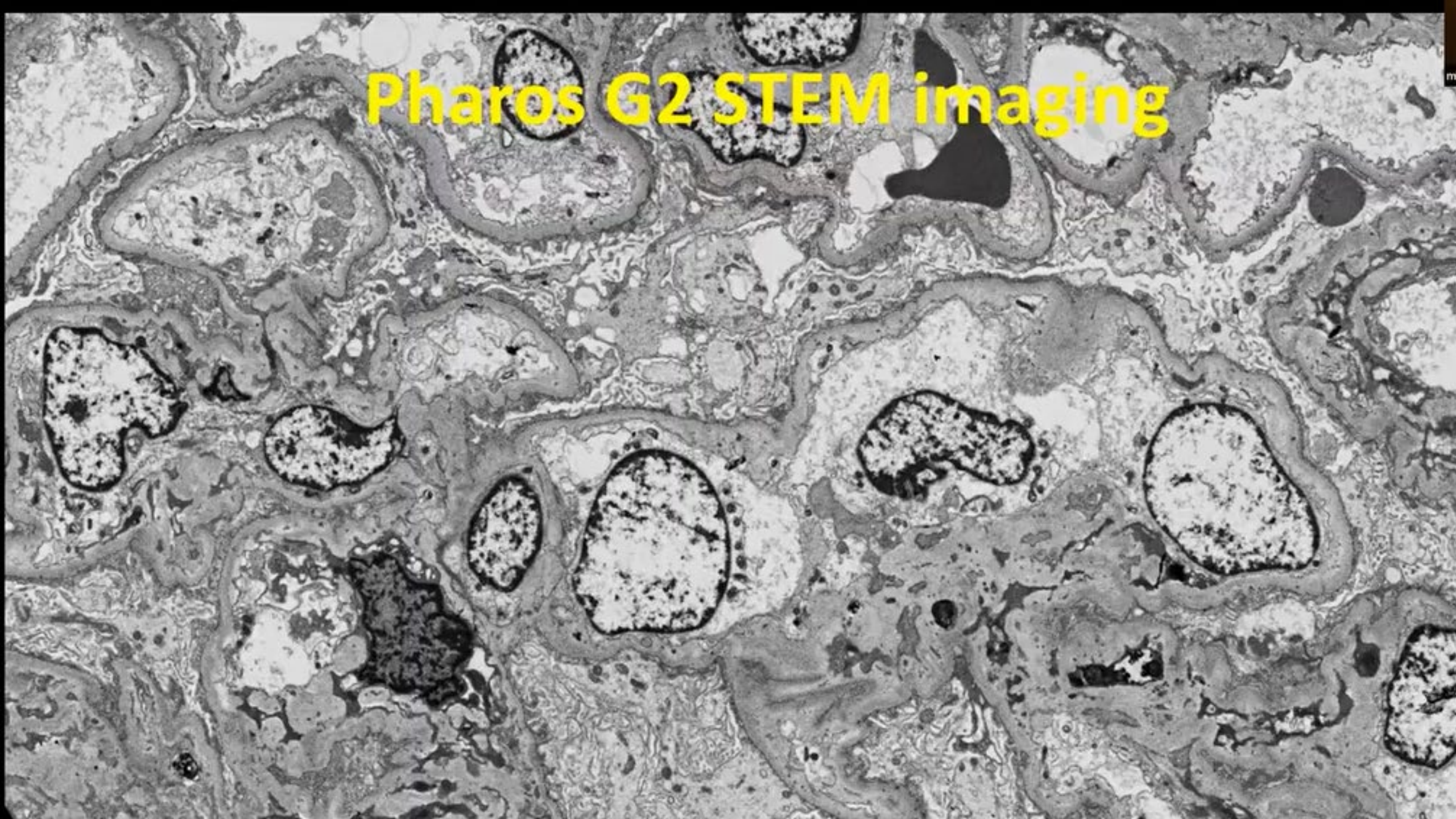
EM examination is fundamentally important – but what are the barriers to implementation?

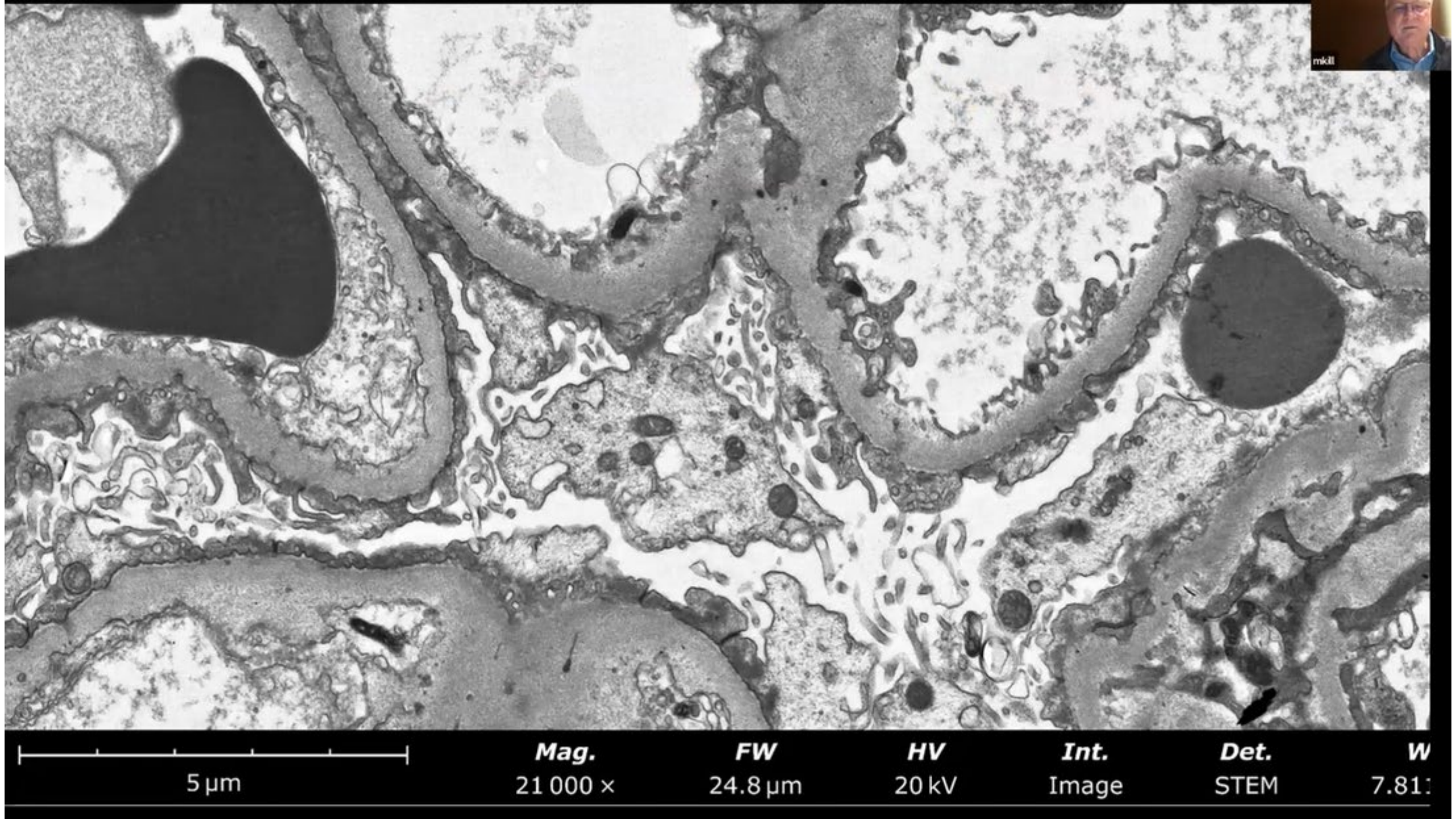
- **Expense of equipment** for regional institutions
- Lab space requirements
- Requirement for “mature” Anatomical Pathology
- Existing workforce – skill levels
- Access to knowledge and technical back-up
- Lack of regional training institutions
- Centralisation of complex tests…..
- The will to improve things….



Pharos G2 backscatter imaging




Pharos G2 STEM imaging


mkill
5 µm
Mag.
21 000 ×
FW
24.8 µm
HV
20 kV
Int.
Image
Det.
STEM
W
7.81

Does the G2 have enough resolution?

Renal casemix NSW Health Pathology 2023:	*Resolution adequate?*
IgA	**YES**
FSGS	**YES**
Minimal change disease (MCD)	**YES**
Pauci-immune GN	**YES**
Diabetes	**YES**
Hypertension / ischaemia	**YES**
Tubular interstitial nephritis	**YES**
Mesangioproliferative GN	**YES**
Amyloid	**YES**
Fibrillary GN	Underassessment
Tx protocol	**YES**

TEM Vs SEM

	SEM	TEM
Type of electrons	Scattered, scanning electrons	Transmitted electrons
High tension	~5–30 kV	~60–300 kV
Specimen thickness	~70-300nm	Typically <100 nm
Type of info	Surface compositional contrast, surface morphology, surface topography (2D/3D)	Phase or diffraction contrast image through sample's surface and internal structure (2D)
Max. magnification	Up to ~1–2 million times	More than 50 million times
Max. Field of View (FOV)	Large	Limited
Optimal spatial resolution	~0.5 nm	<50 pm
Signals analysed	Backscattered electrons, secondary electrons, X-rays	Transmitted electrons, X-rays
Image formation	Electrons are captured and counted by detectors, image on PC screen	Direct imaging on fluorescent screen or PC screen with CCD
Detection mode	Back scattered, STEM (BF, HAADF)	BF, HAADF
Operation	Same sample preparation as for TEM	Laborious sample preparation, trained users required

- Generally, TEMs are more complex to operate. TEM users require intensive training before being able to operate them. Special procedures need to be performed before every use, with several steps included that ensure that the electron beam is perfectly aligned.

https://www.thermofisher.com/

Advantages of SEM

- Large field of view: Can image millimetre-scale tissue sections
- Digital zoom capability: Navigate from low magnification to ultrastructure seamlessly
- Faster workflows: Reduces time for ultrastructural analysis
- Easier automation
- Simpler preparation than TEM – thicker sections or block, robust substrate allows reimaging
- High contrast from lower voltages – less damage
- Enables correlation with light microscopy (easier ROI targeting)
- Ease of use
- Reduced costs in purchase, operation and maintenance
- Small footprint

Limitations of SEM

- Lower resolution than TEM (no true atomic detail)
- Mostly surface or near-surface imaging
- Charging - May require coatings or specialised prep for biological samples
- Less suitable for:
  - Internal organelle detail
  - Viral / macromolecular ultrastructure

Advantages of TEM

- Highest resolution
- Direct imaging of internal structures
- Essential for:
  - Crystal structure
  - Viral particles
  - Subcellular ultrastructure

  - Validated over many years
  - Familiar and interpretable by pathologists

⚠️ Slide: Limitations of TEM (from ZEISS comparison)

Limitations of TEM

- Cost
- Small sample size (limited by TEM grids)
- Grid bars can block parts of the image
- Requires ultrathin sectioning (high skill)
- Time-consuming workflows Difficult to:
  - Correlate with light microscopy
  - Locate regions of interest

Conclusion

- SEM is evolving into a high-throughput, multiscale imaging tool
- Particularly powerful for:
  - Pathology
  - Large area and volume tissue analysis
- TEM remains irreplaceable for atomic and high res ultrastructure
- Future direction: SEM for screening + TEM for in depth analysis?

Next steps

- Loan microscope
- Validation study
- Prospective trials
- Health Economics study
- Grant funding application

Thanks to

- Steve Furzeland, Zeiss
- Murray Killingsworth
- Tzipi Cohen Hyams
- Lucy Collinson
- Candice Roufosse



Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Invited review

Scanning electron microscopy as a new tool for diagnostic pathology and cell biology

Tzipi Cohen Hyams[a,c,d,*], Keriya Mam[e], Murray C. Killingsworth[a,b,c,d]

Advanced search

Journal of Histochemistry & Cytochemistry

Official Journal of THE HISTOCHEMICAL SOCIETY

Impact Factor: 1.5
5-Year Impact Factor: 2.8

Free access | Research article | First published online August 6, 2019 | Re-use permissions

Unobstructed Multiscale Imaging of Tissue Sections for Ultrastructural Pathology Analysis by Backscattered Electron Scanning Microscopy

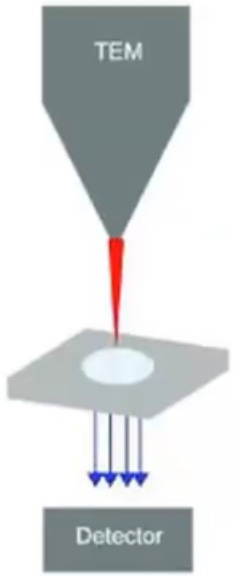
Mike Reichelt, Meredith Sagolla, [...], and Joshua D. Webster +1 View all authors and affiliations

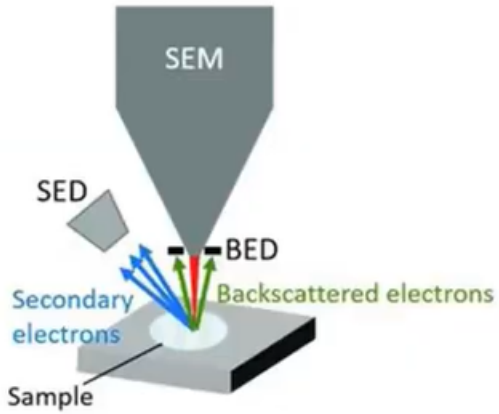
Volume 68, Issue 1 | https://doi.org/10.1369/0022155419868992

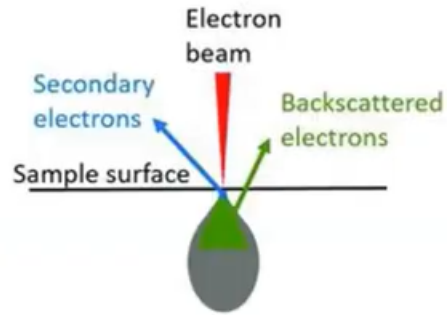
- SEM is not replacing TEM—but in pathology, it may provide a viable alternative to TEM and become a useful tool due to speed, scale, and workflow efficiency.

TEM Vs SEM









TEM Detection

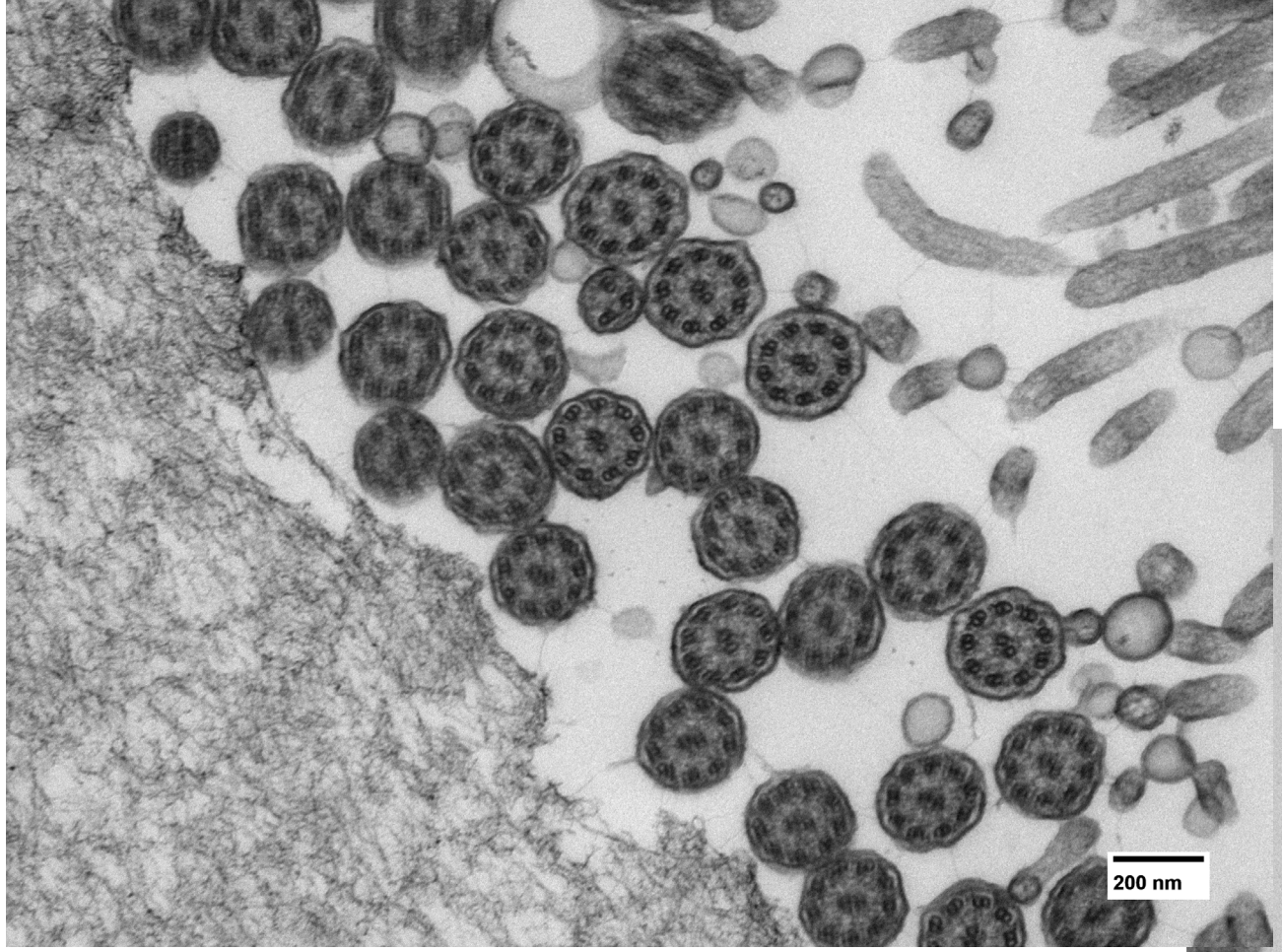
- **Electrons pass through the thin sample causing some scattering by metal atoms that stains in the sample (ultrastructure).**
- **Chemical composition (X-rays)**

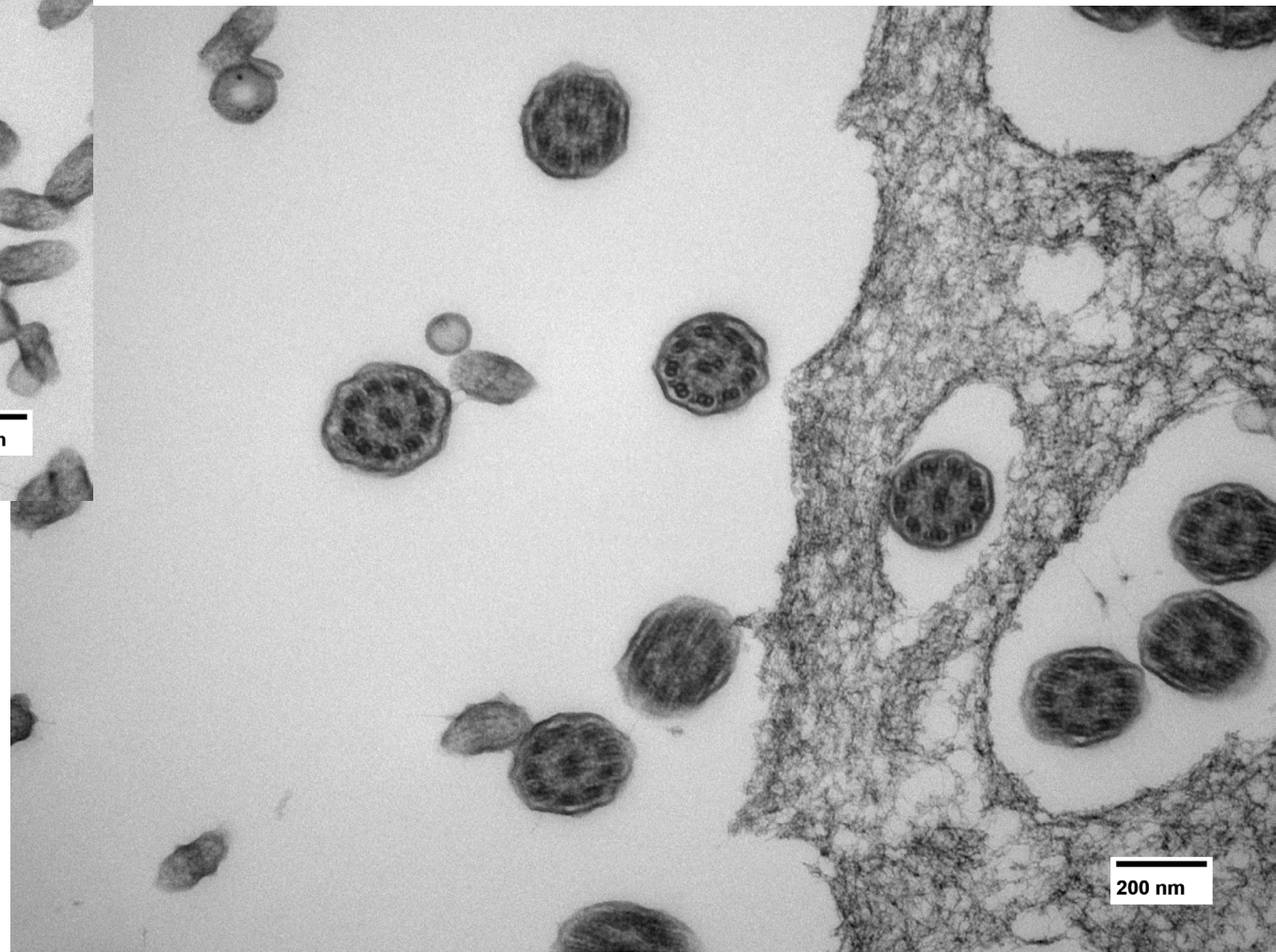
SEM signals:

- **Secondary electrons (topology and morphology)**
- **Back-scattered electrons (ultrastructure and chemical Information)**
- **Chemical composition (X-rays)**

DOI:10.3389/fonc.2020.00608

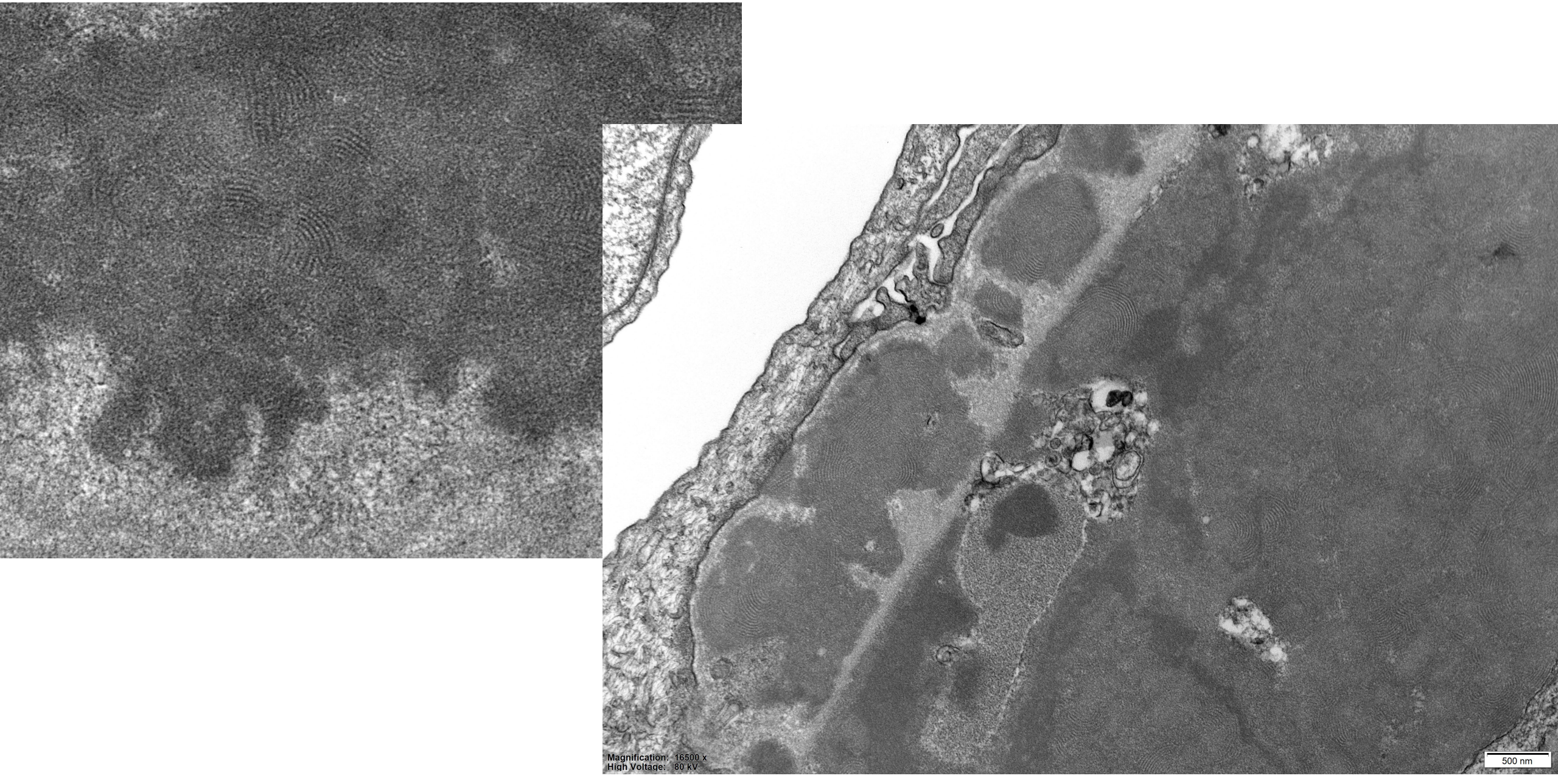
SEM or TEM?





Why do we need an alternative?

- TEM is expensive
- TEM is complex
- We don’t always need the very highest mag/resolution


Magnification: 16500 x
High Voltage: 80 kV
500 nm

New generation high-resolution SEMs



- ❑ Low cost and maintenance
- ❑ Easier Acquisition
- ❑ More accurate - More and better information is possible
- ❑ “Google Earth” View
- ❑ Automation
- ❑ 3D imaging
- ❑ Multi modal Correlation (LM and EM with chemical analysis)