Cell Ultrastructure and Preservation Considerations for the Hybrid Material Scientist

NU*ANCE* Tech Talk 2022.01.20

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Microscopy Specialist

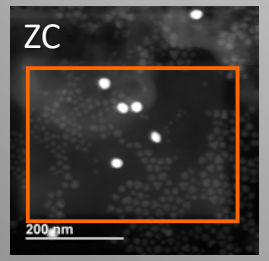
they/them/their's

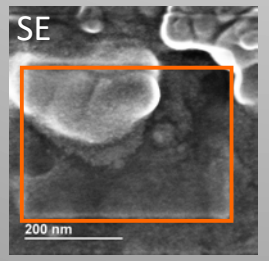
Office: Silverman B545

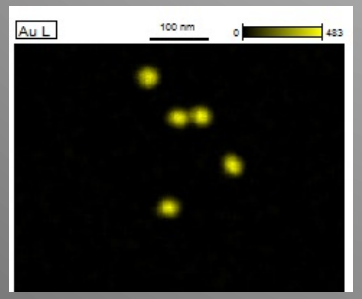
"Happy Microscopy!"

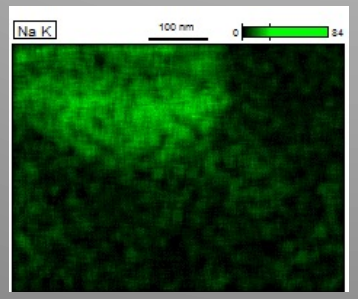
Hitachi HD2300 STEM EDS Upgrade scheduled for next week.

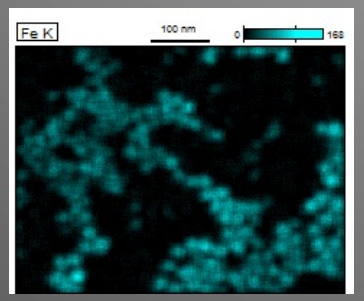
Solid state detectors. Higher count rate, better with lower Z elements, similar resolution as previous system, no liquid nitrogen necessary

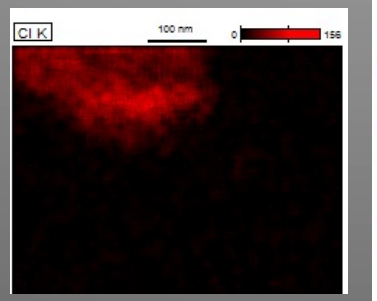


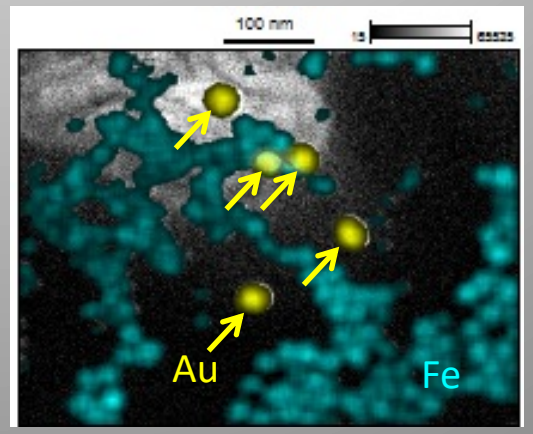


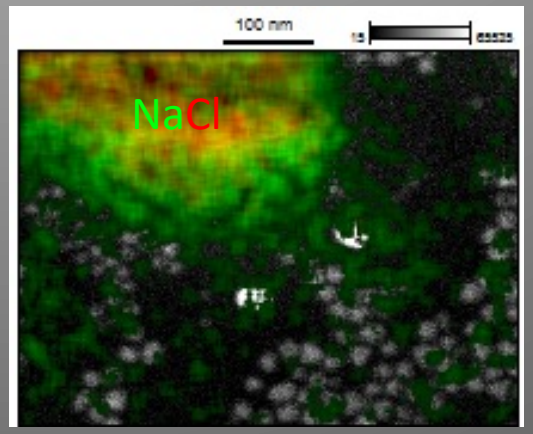


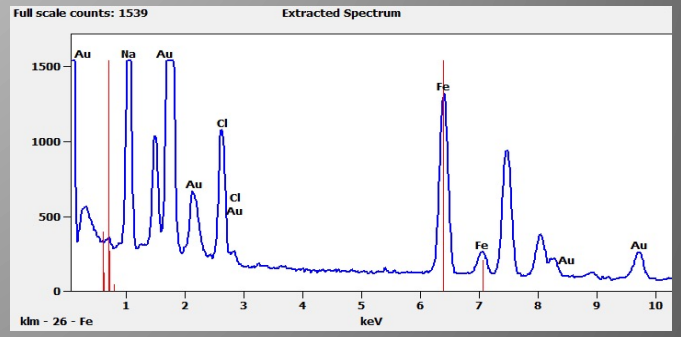


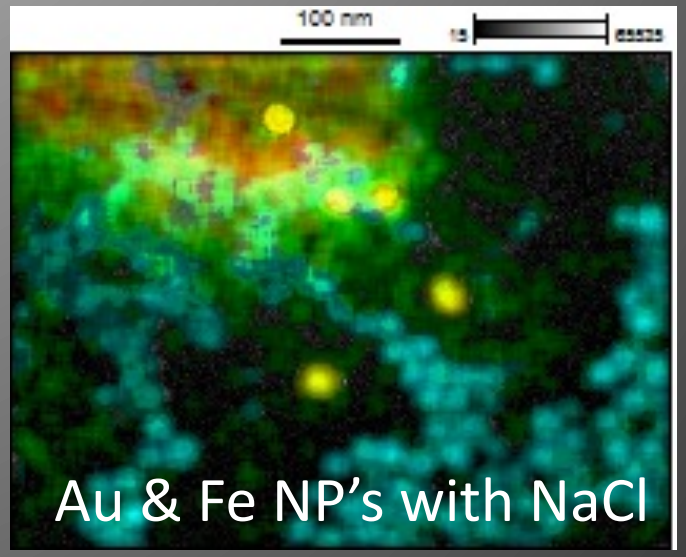






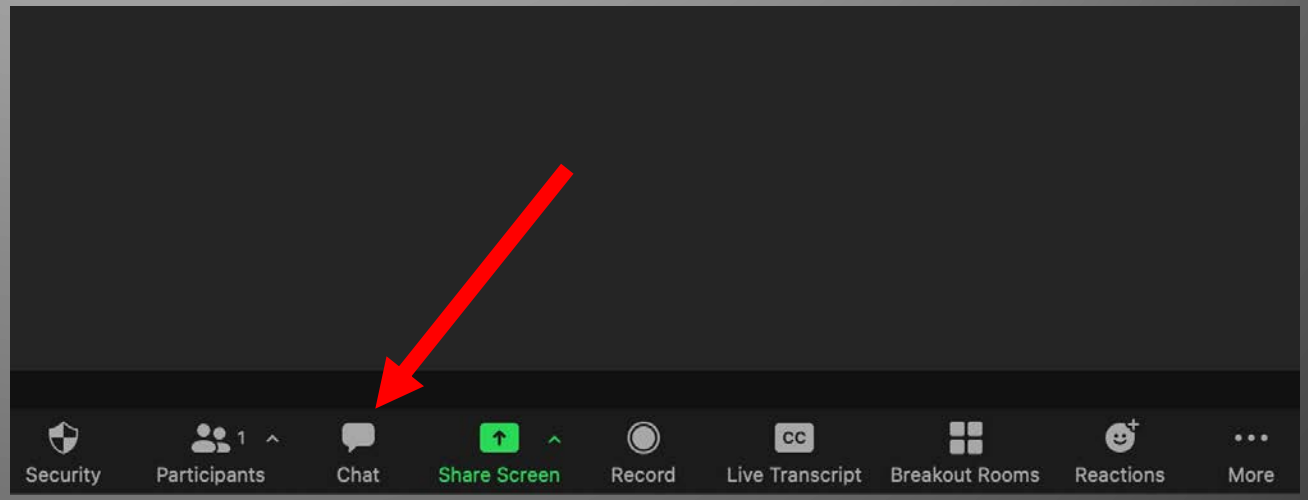






TOPICS COVERED

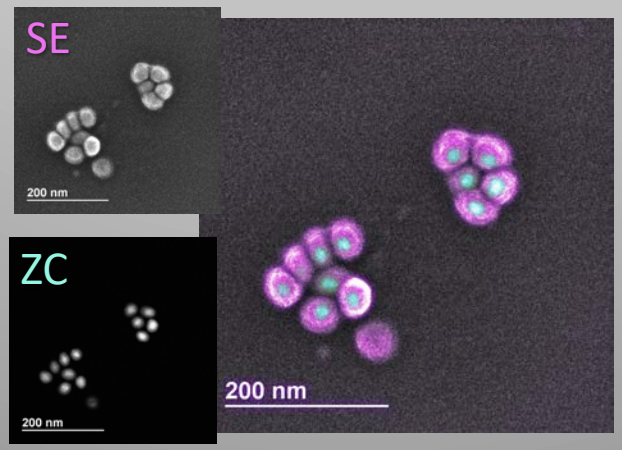
- Basic cell ultrastructure/organelles as seen by TEM
- Cell Death Morphology
- Sample processing artefacts

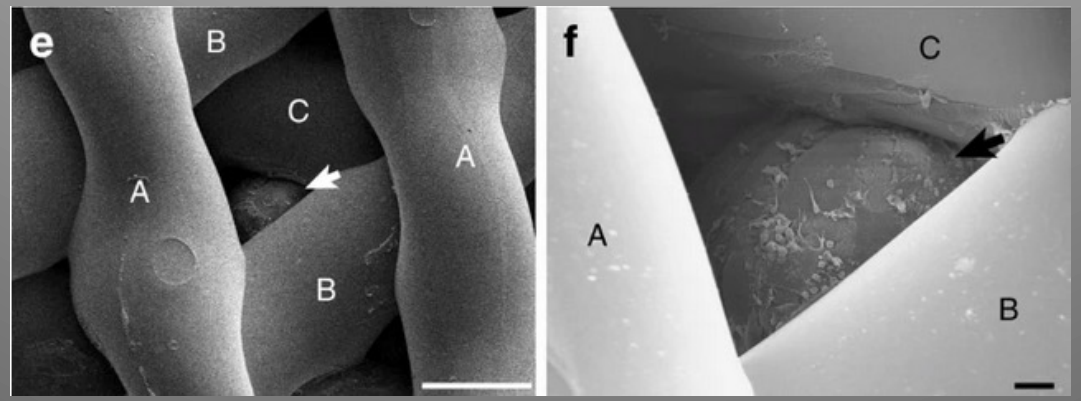


Material-Bio interfaces

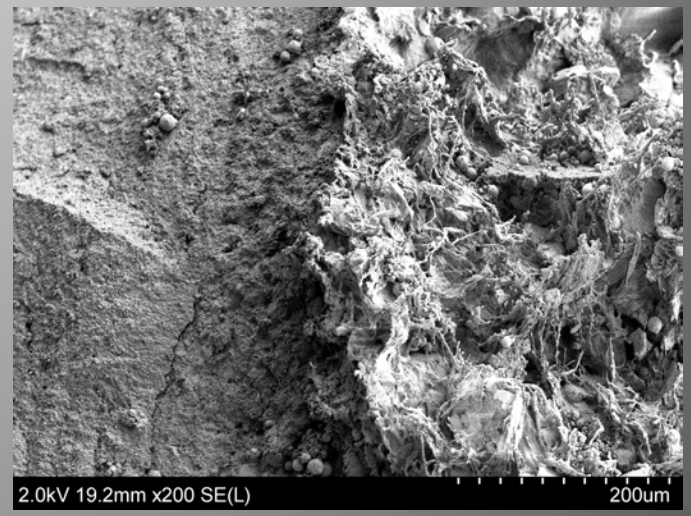
- Food
- Cancer drugs
- Sensors and Monitors
- Battery Tech
- Vaccines
  - COVID mRNA vaccine impossible without Liposomes
- Tissue Engineering
  - 3D scaffolding with primary cultures

40 nm Fe w/ polymer shell

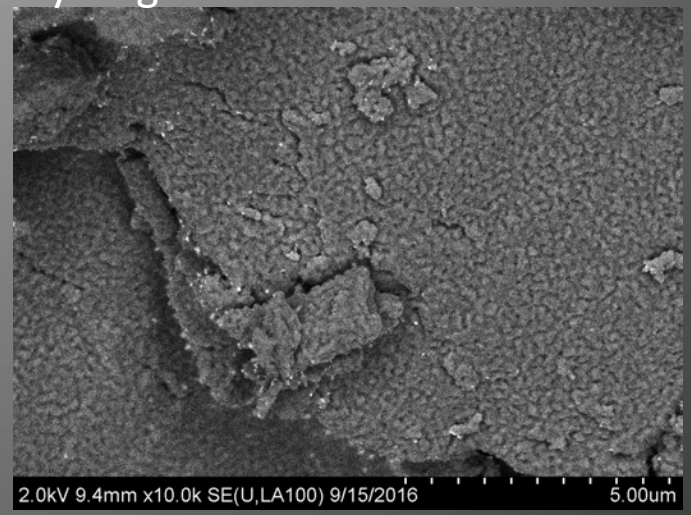




Candy shell / gum



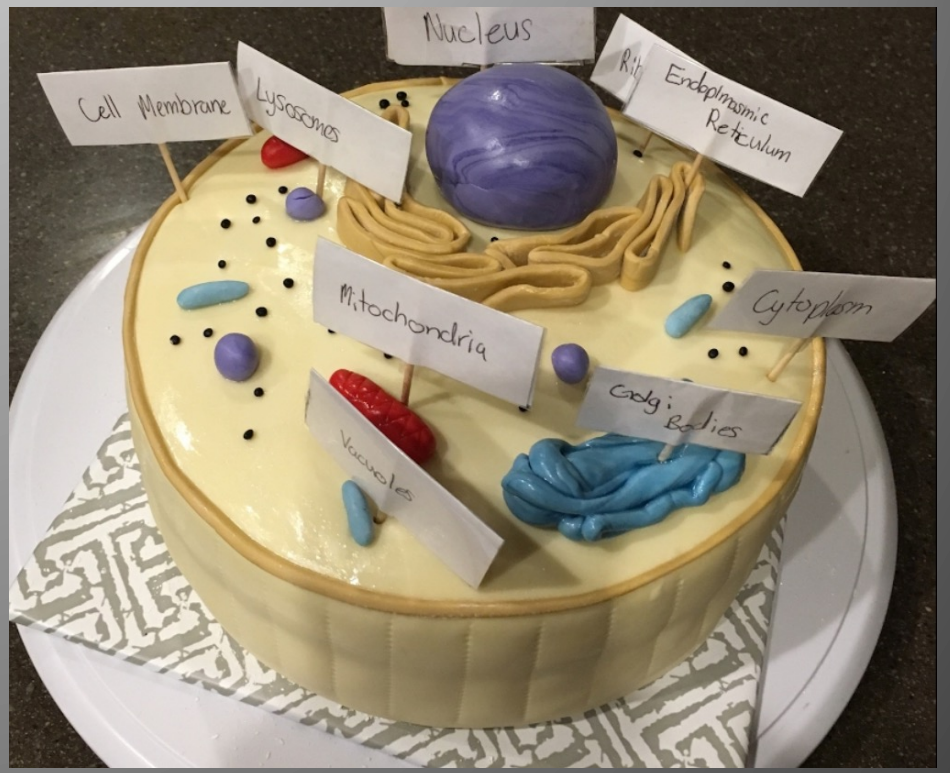
Hydrogel with NP's



Basic Cell Ultrastructure in TEM

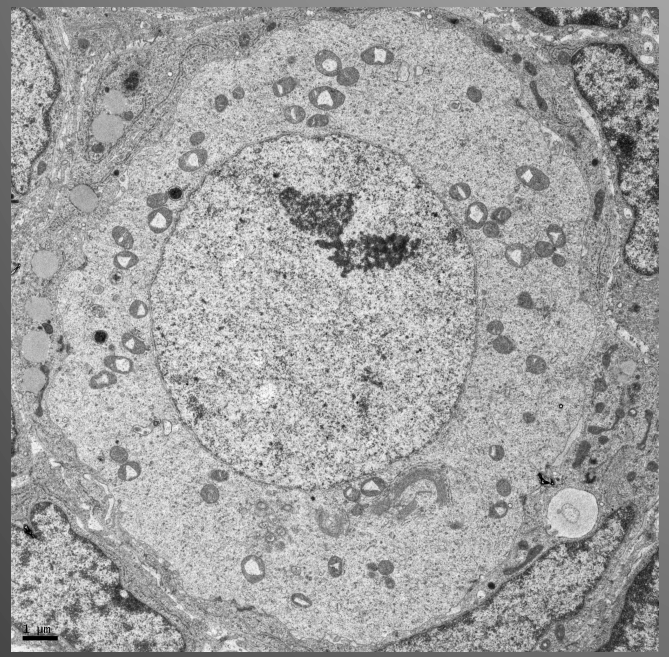
"I know what I'm looking for, but not what I'm looking at." "This doesn't look like the cell parts I learned." -Researcher

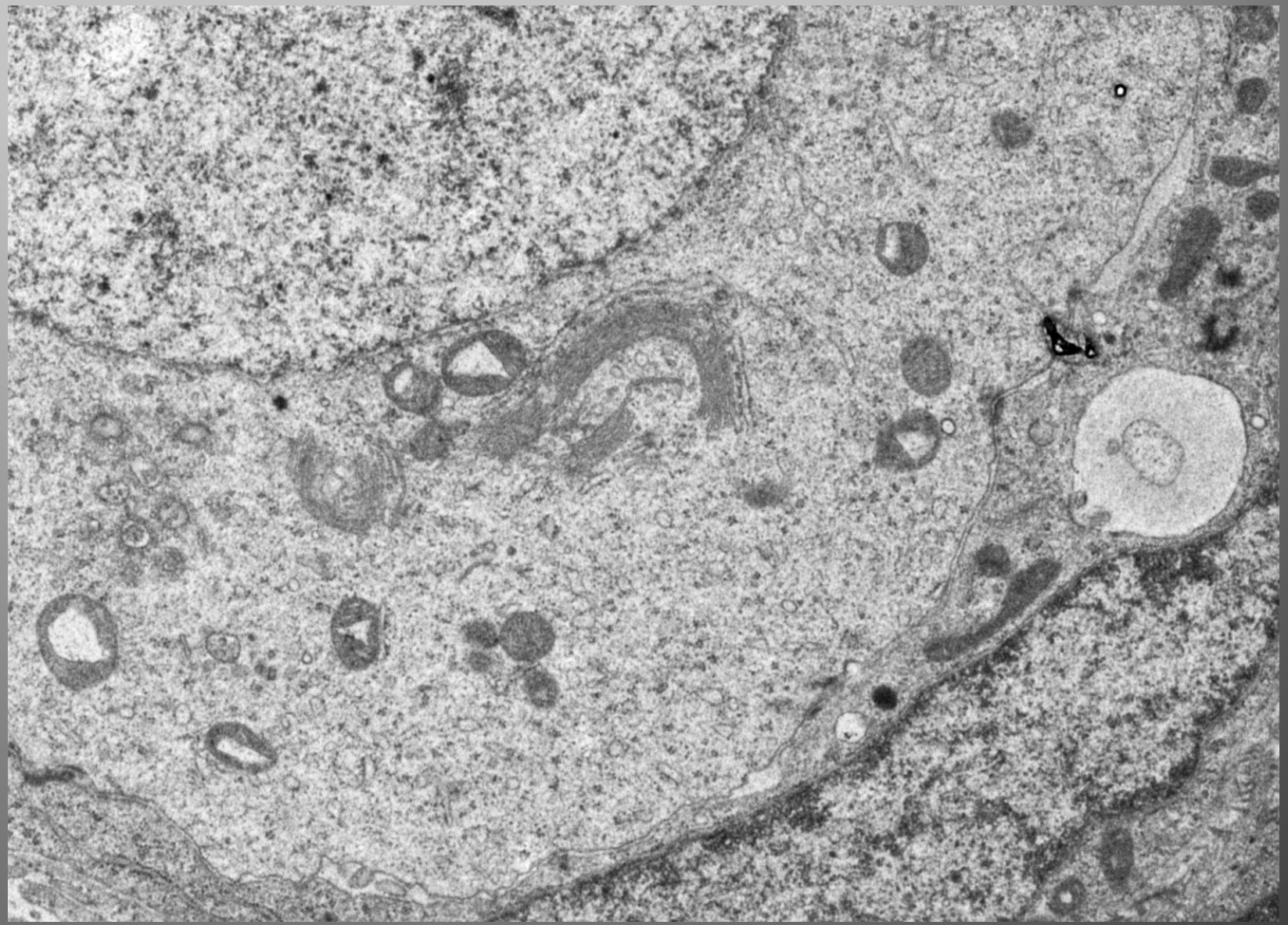
- Nuclear membrane, Nucleus, and Nucleolus
  - Chromatin (Heterochromatin and Euchromatin), RNA/Ribosomes
- ER (RER Vs. SER) (presence of ribosomes)
- Golgi
- Digestive bodies (lysosomes, etc.)
- Mitochondria
- Cell membrane and junctional complexes
- Centrioles



Cytoplasm

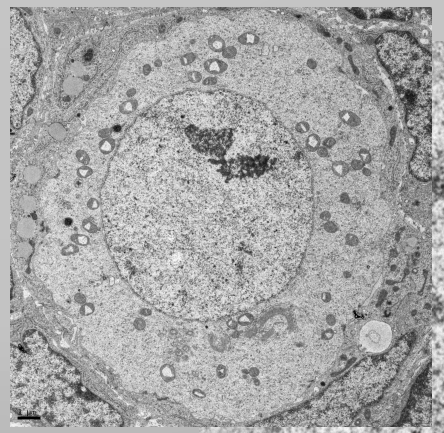
Space in cell that contains all organelles (nucleus, mitochondria, ER, etc.)

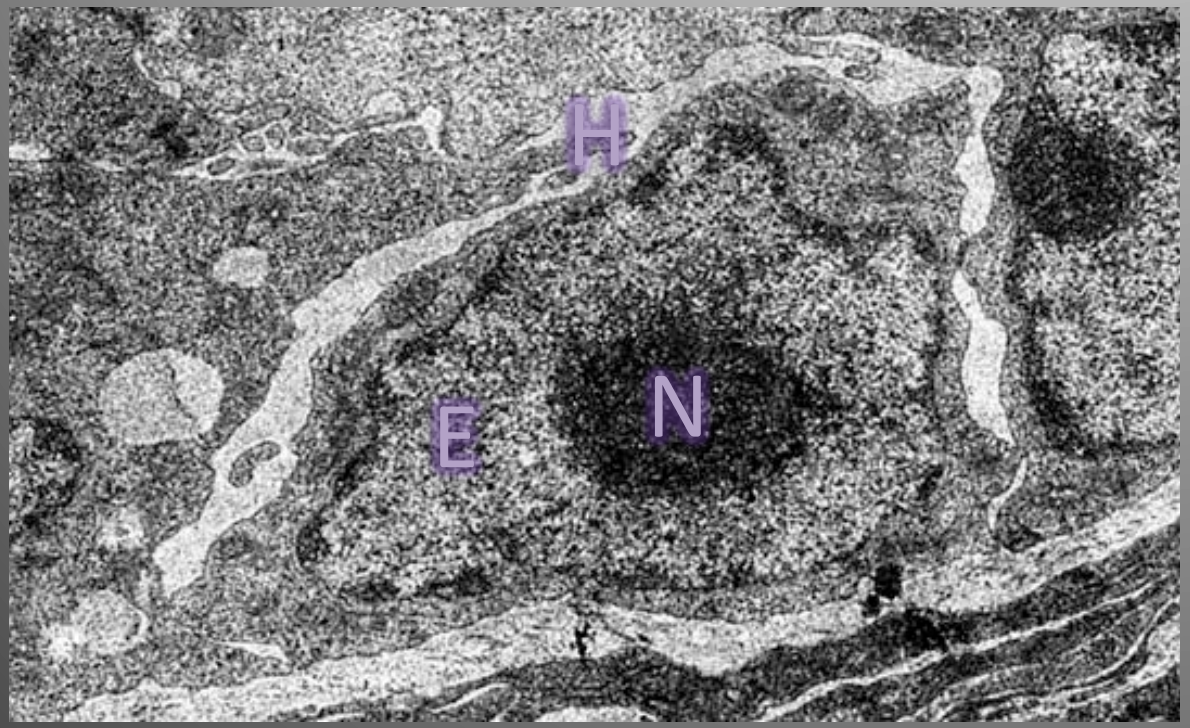


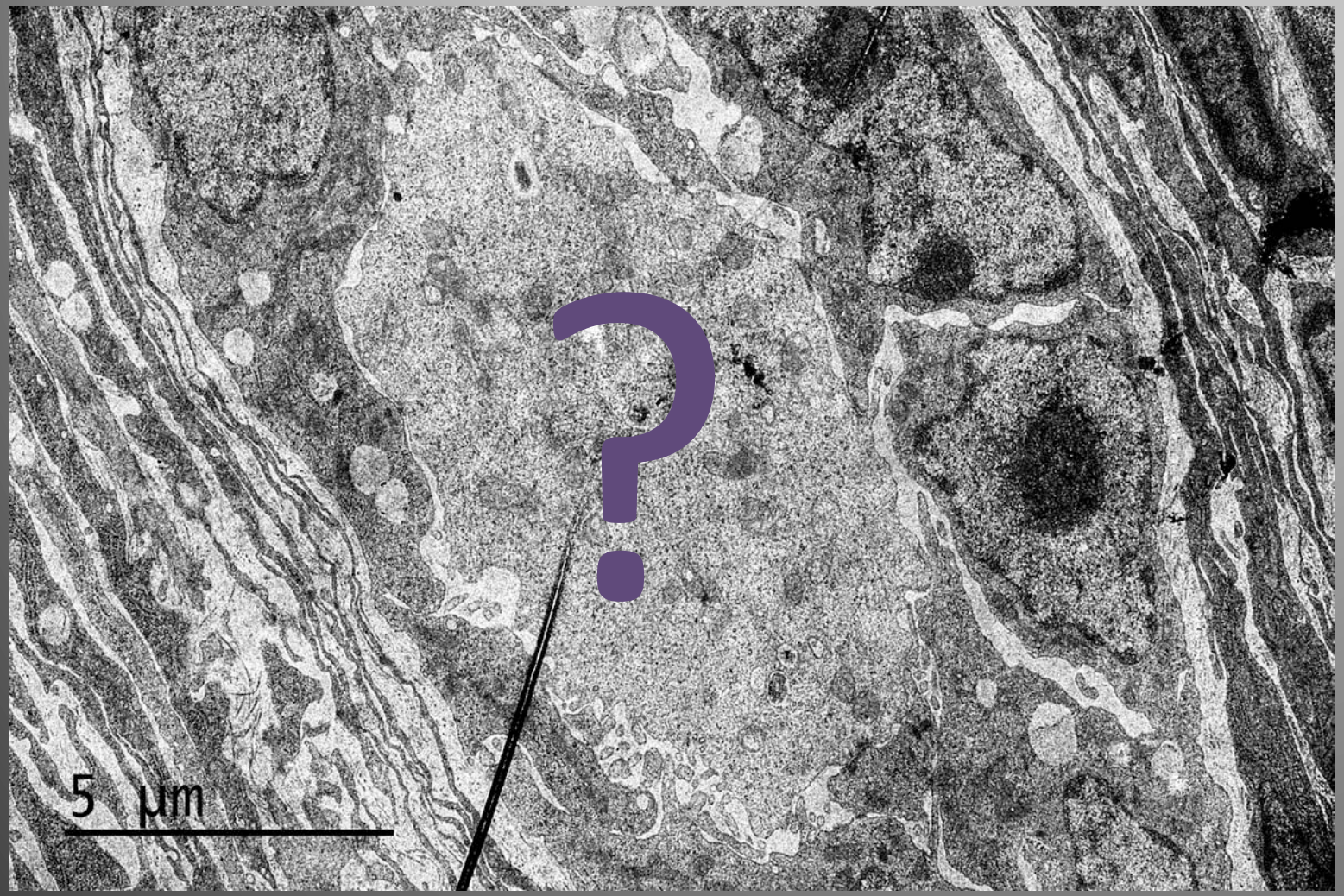


Nucleus

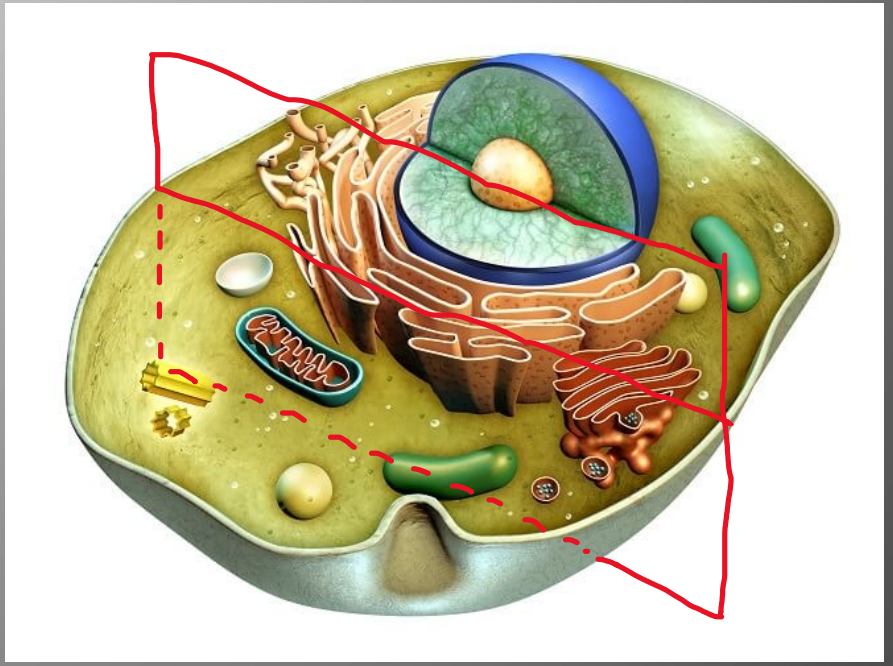
Bound by Nuclear Envelope containing pores
Contains: Nucleolus (RNA), Chromatin (DNA) made up of Heterochromatin (inactive) and Euchromatin (active)





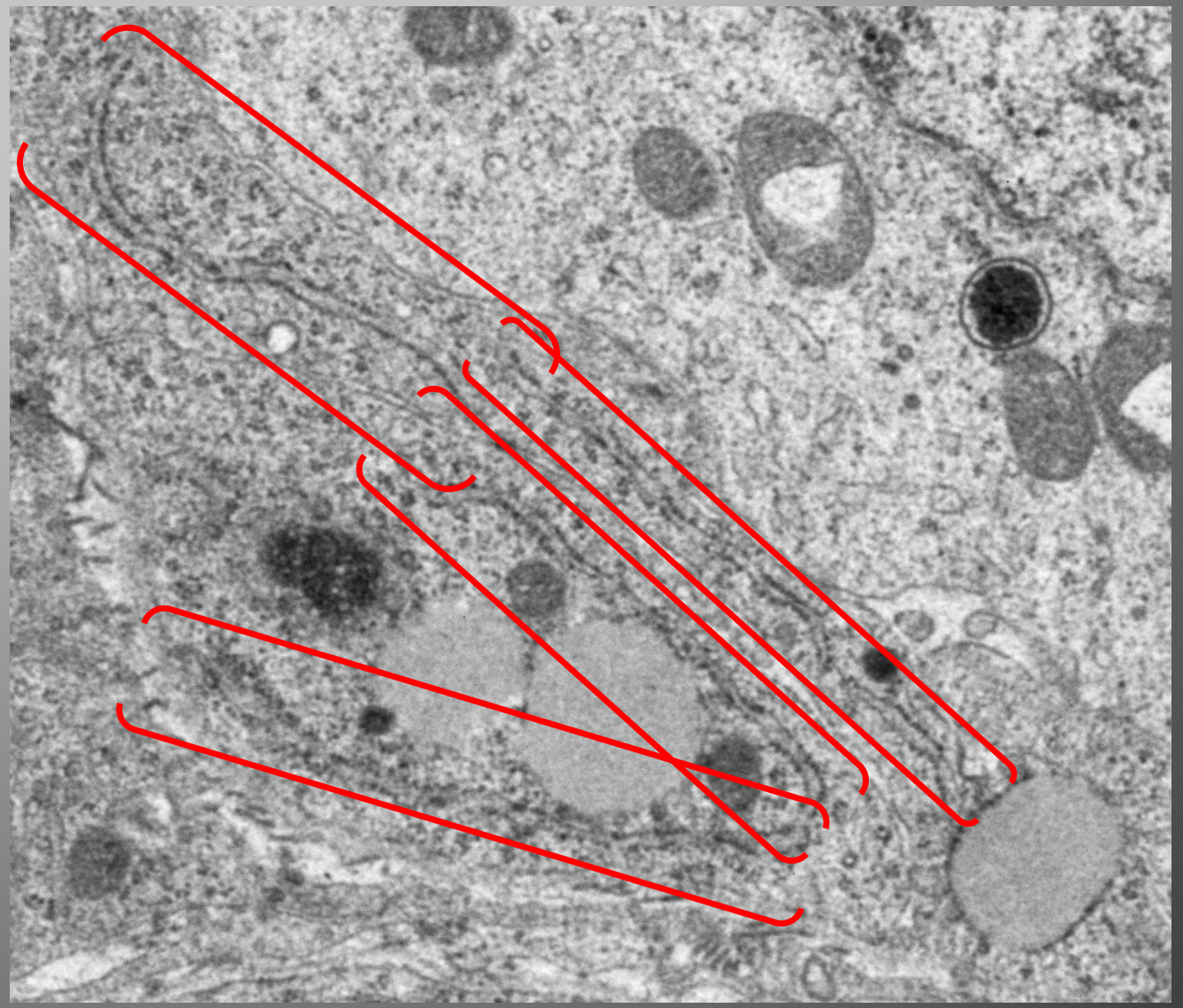


Where's the nucleus in this cell?

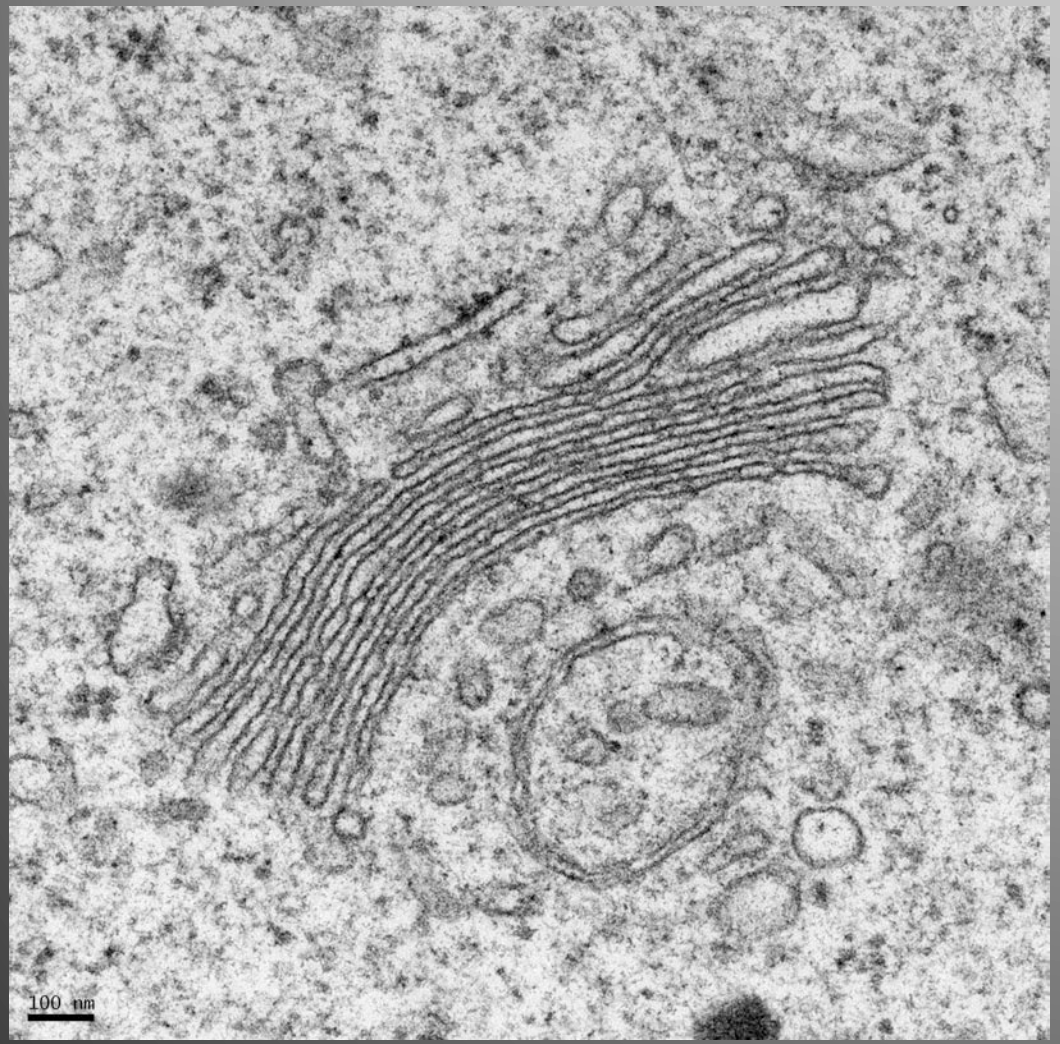


Endoplasmic Reticulum and Ribosomes

- Ribosomes composed of RNA produce proteins
  - Typically trimers
  - Produce proteins
  - Found floating freely in cytoplasm and RER
- Rough Endoplasmic Reticulum
  - Shares membrane with nucleus
  - Plays role in protein synthesis with ribosomes
- Smooth Endoplasmic Reticulum
  - Produces Lipids (fats)



Golgi



"The cell's take out restaurant"

Modifies, packages, and transports proteins

Ingredients

- Input from ER proteins created by ribosomes

Cooking

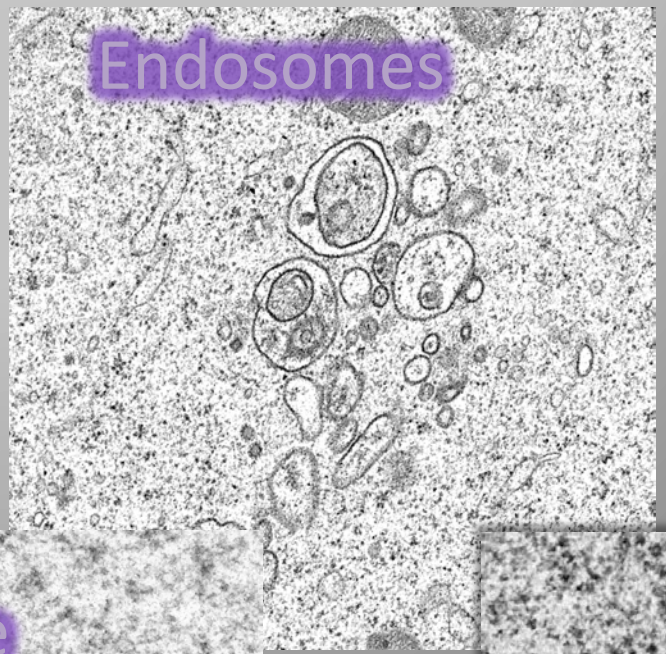
- Modifies protein amino acid code

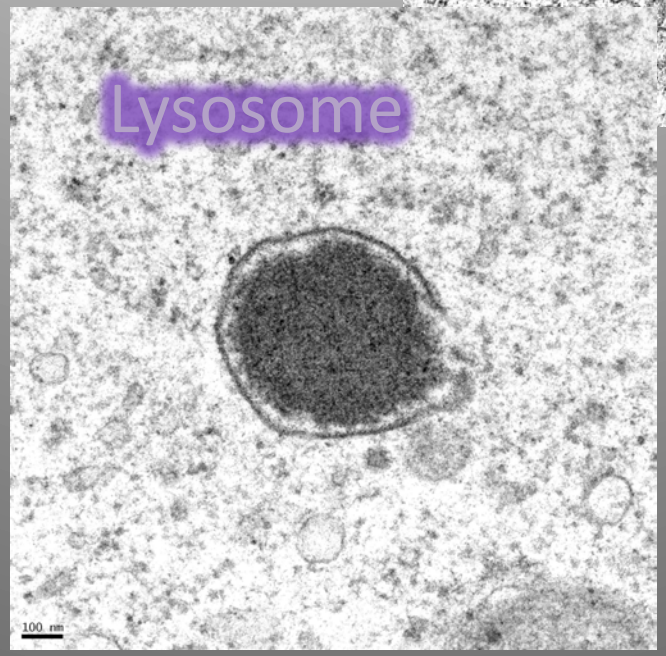
Delivery

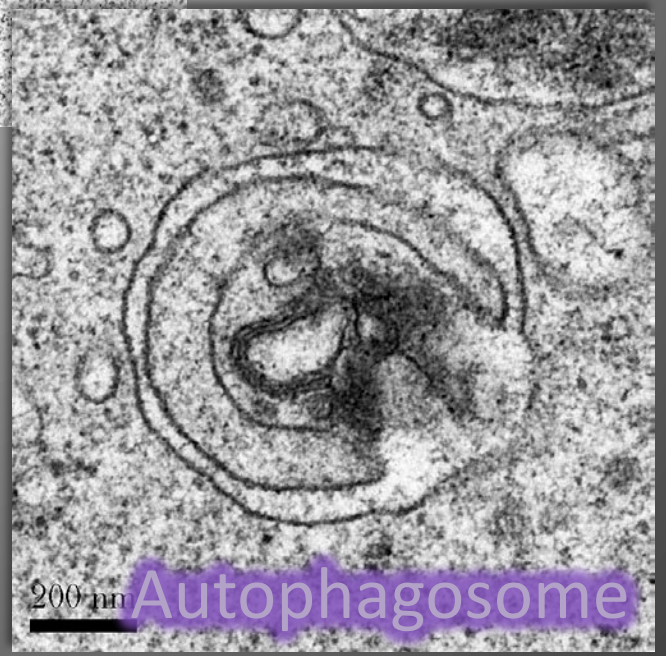
- Proteins contained in vesicles and transported to other parts of cell

Digestive bodies

Nutrients enter cell through clathrin-mediated endocytosis (endosomes). Digestion and removal of proteins, membranes, fats, organelles, and other molecules from cell through multi-vesicular bodies, lysosomes, autophagosomes/residual bodies/mylinic figures, to exosomes exiting cell

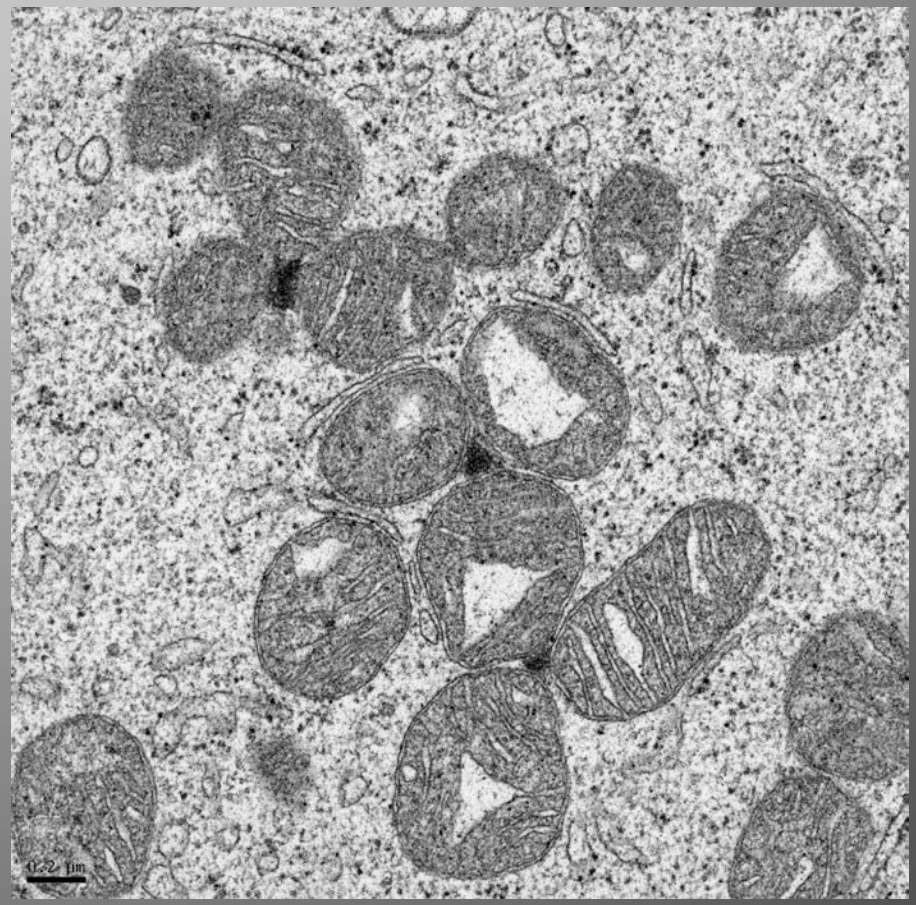






Mitochondria

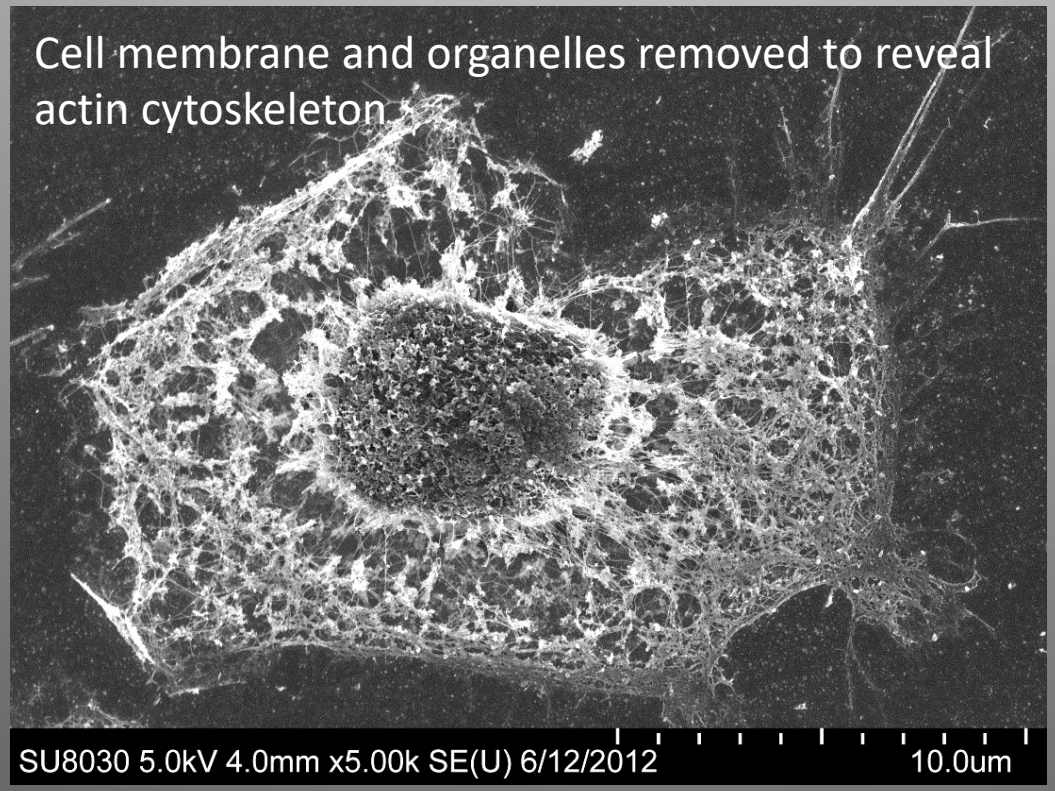
Transforms glucose into ATP to give cells energy
Contains cristae and Ca+ Ions
Abundant in cells requiring a large amount of energy (example: cardiac muscle)

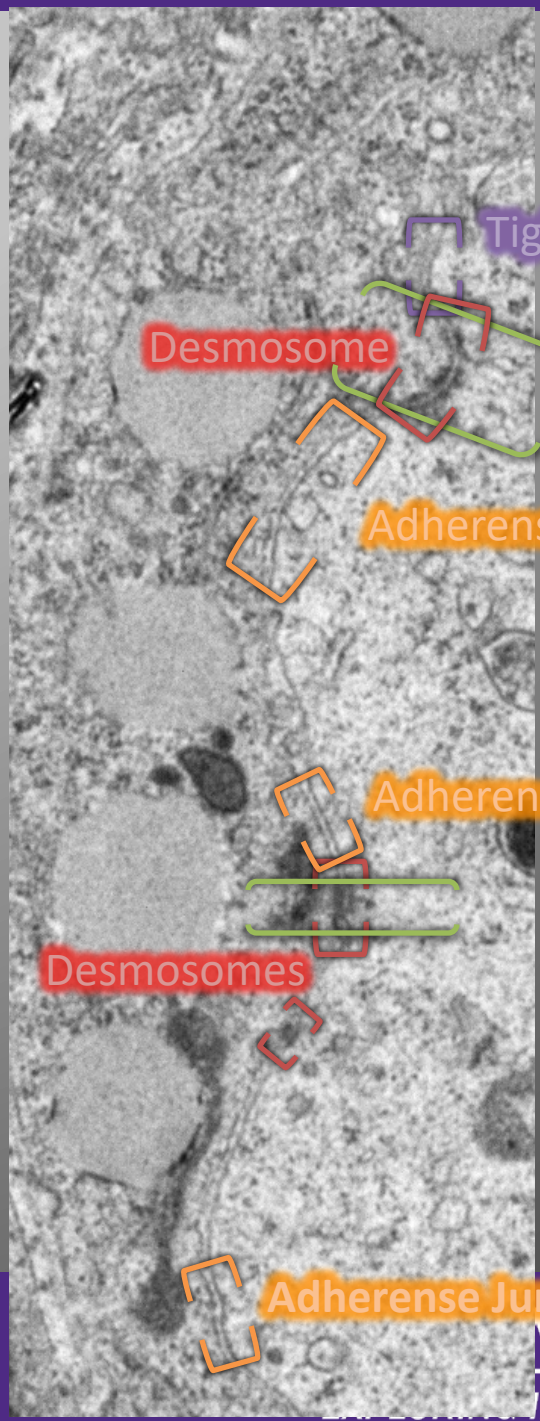


Cell Membrane, Junctional Complexes, and Cytoskeleton

Joins cells together
Transport ions, chemical communication, and vesicles
Actin, intermediate filaments, and microtubules offer skeletal-like support to cells

Cell with Au substrate





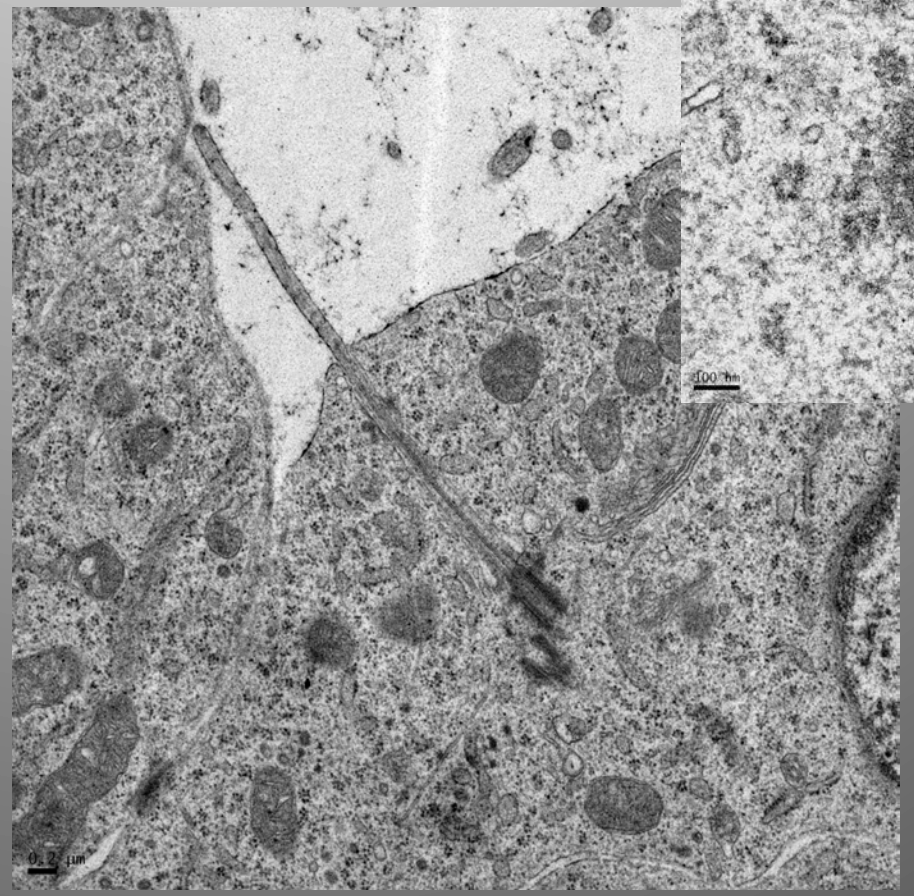
NUANCE
Northwestern University Atomic and Nanoscale Characterization Experimental Center

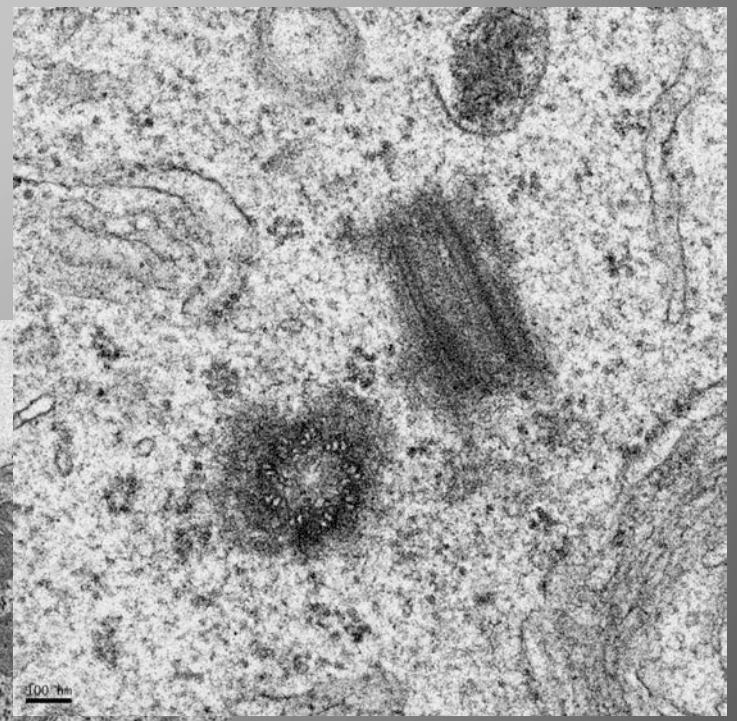
BioCryo Facility

Western
INNER SPACE

Centrioles/Centrosome

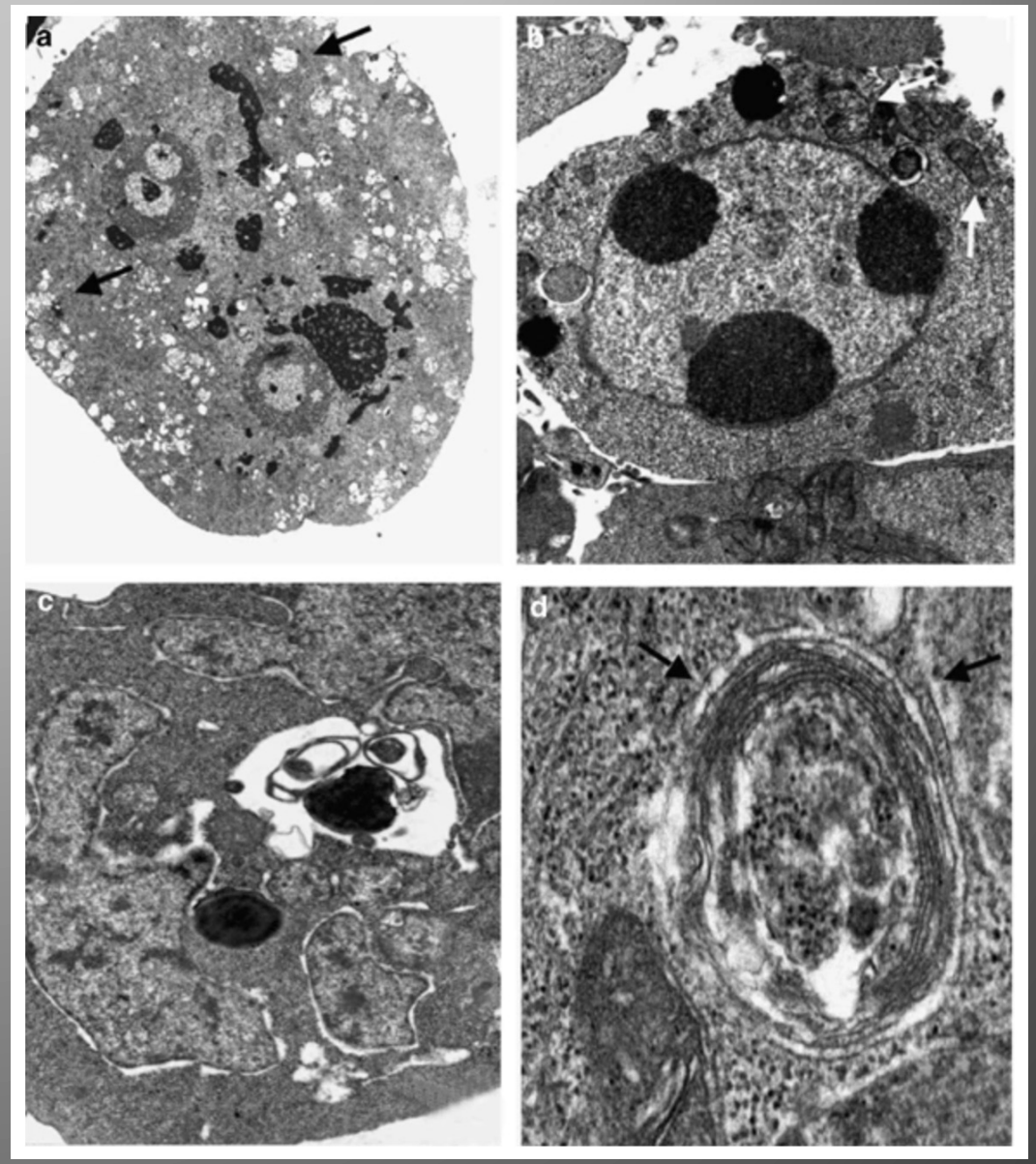
- Cylinder-like organelles critical to cellular division/mitosis
- Only one pair in entire cell
- Very rare to see in EM ultrathin section





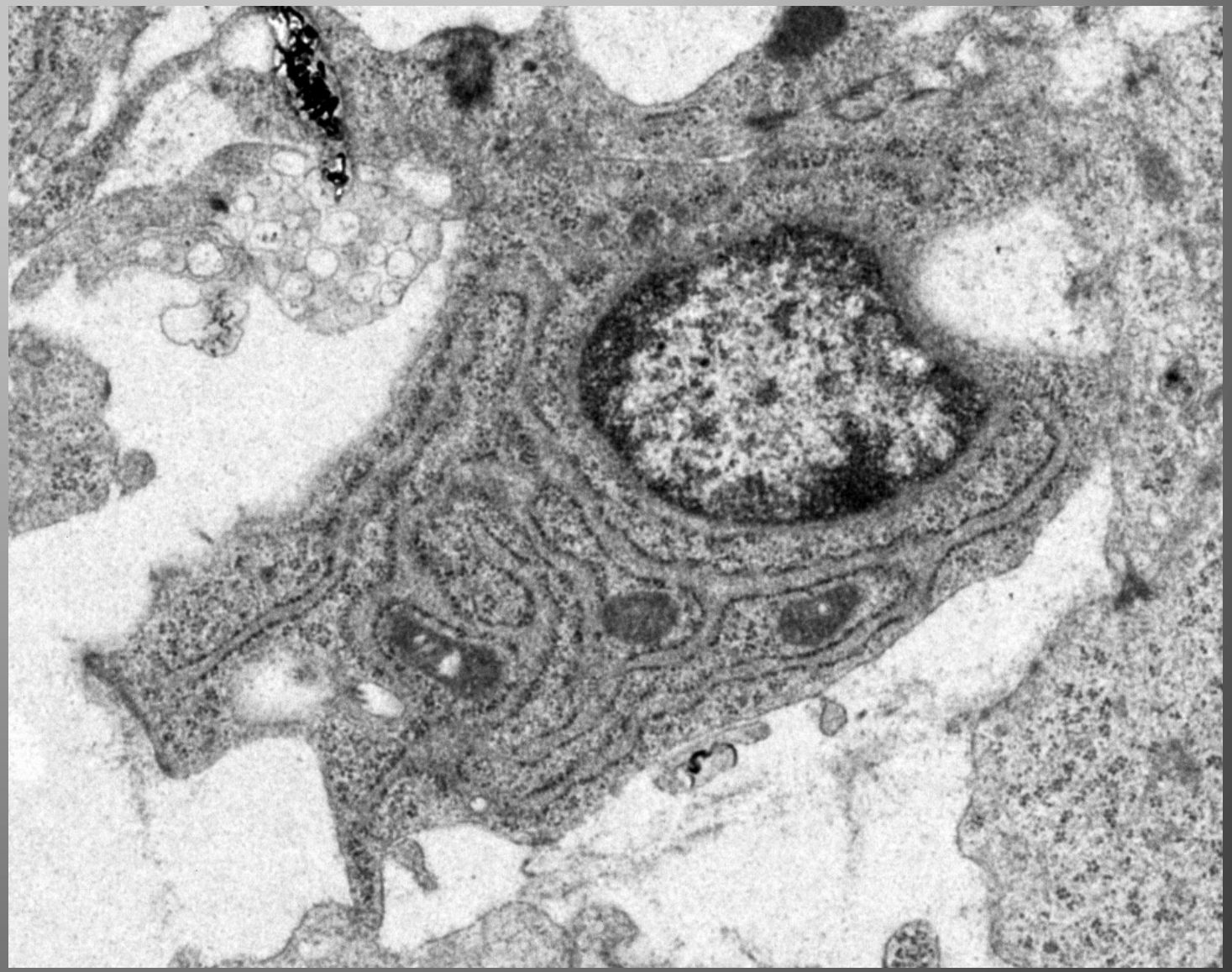
Cell Death

- Programmed
  - ***Apoptosis***
    - Paraptosis
    - Parthanatos
  - ***Autophagocytosis***
- Entosis (One cell eats another)
- Hyperpahgia (Cell eats apoptotic cell and dies of indigestion?)
- Catastrophic Mitosis Failure
- ***Necrosis***



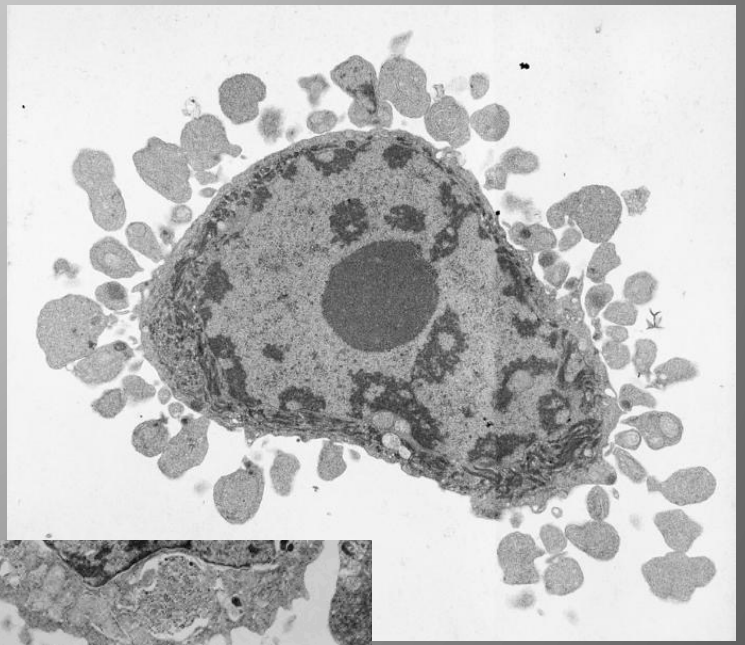
Paraptosis

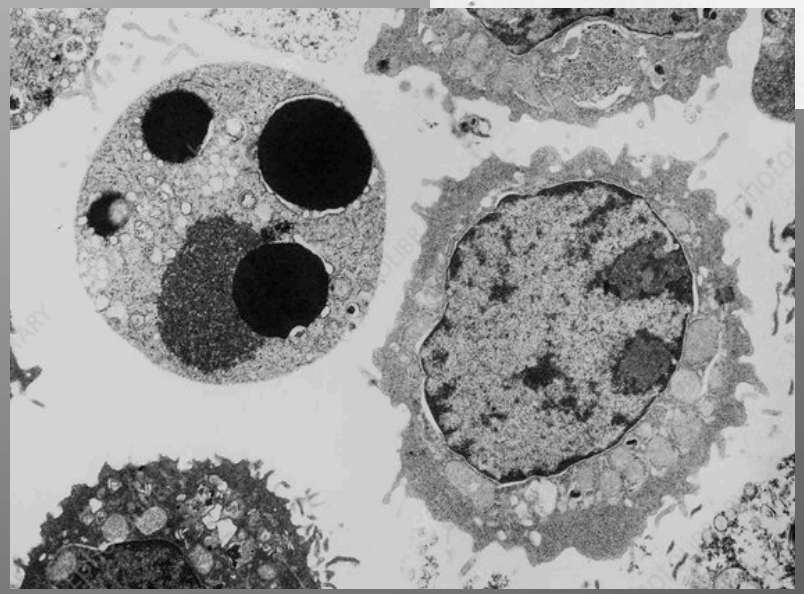
- Programmed Cell Death
  - Cells lyse in final stage releasing signals to nearby tissue resulting in inflammation
- Morphology
  - Vacuoles form in cytoplasm and get worse over time until lysis
  - Appearance of swollen mitochondria and ER
  - No dense bodies or condensed chromatin (as seen in Apoptosis)



Apoptosis

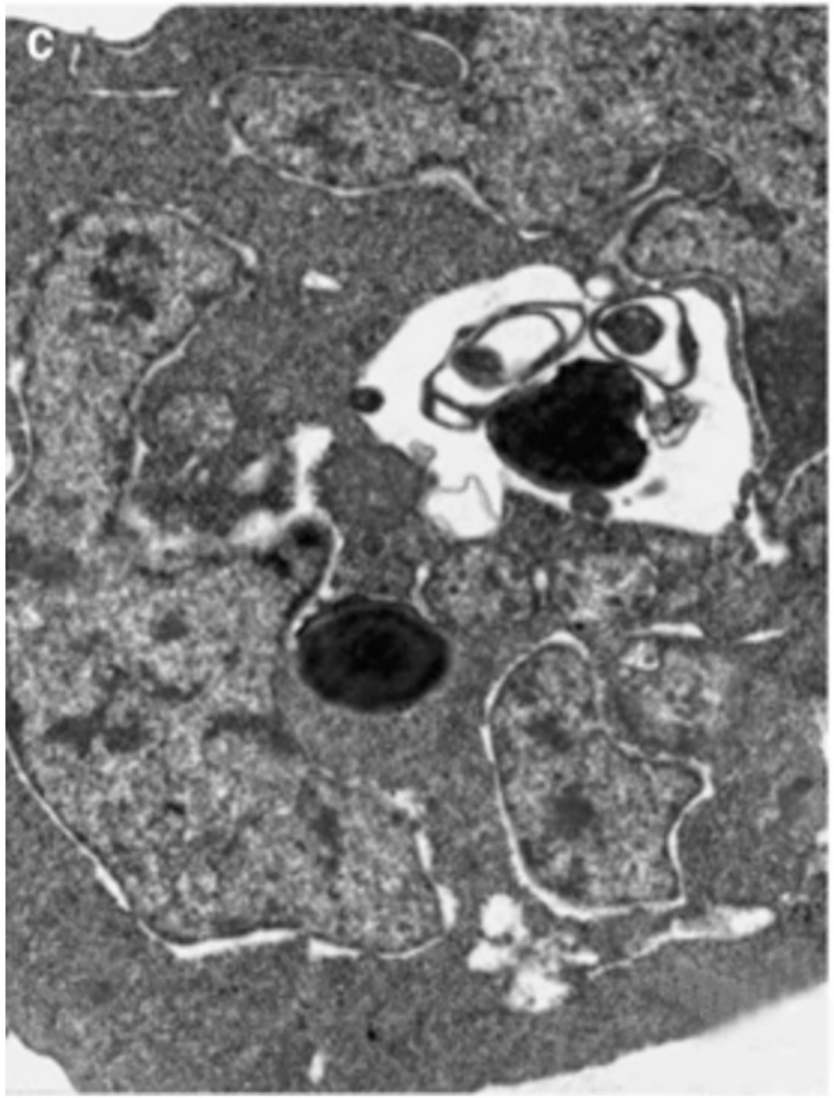
- Programmed Cell Death
  - Sparked biochemical events from nearby cell stress or cell signaling
  - Natural cell death vital to development and eliminating mutations
- Morphology
  - Condensed chromatin in nucleus, nuclear fragmentation, swollen mitochondria, blebbing of membranes
  - Cell eventually fragments resulting in apoptotic bodies (membrane-bound vesicles)





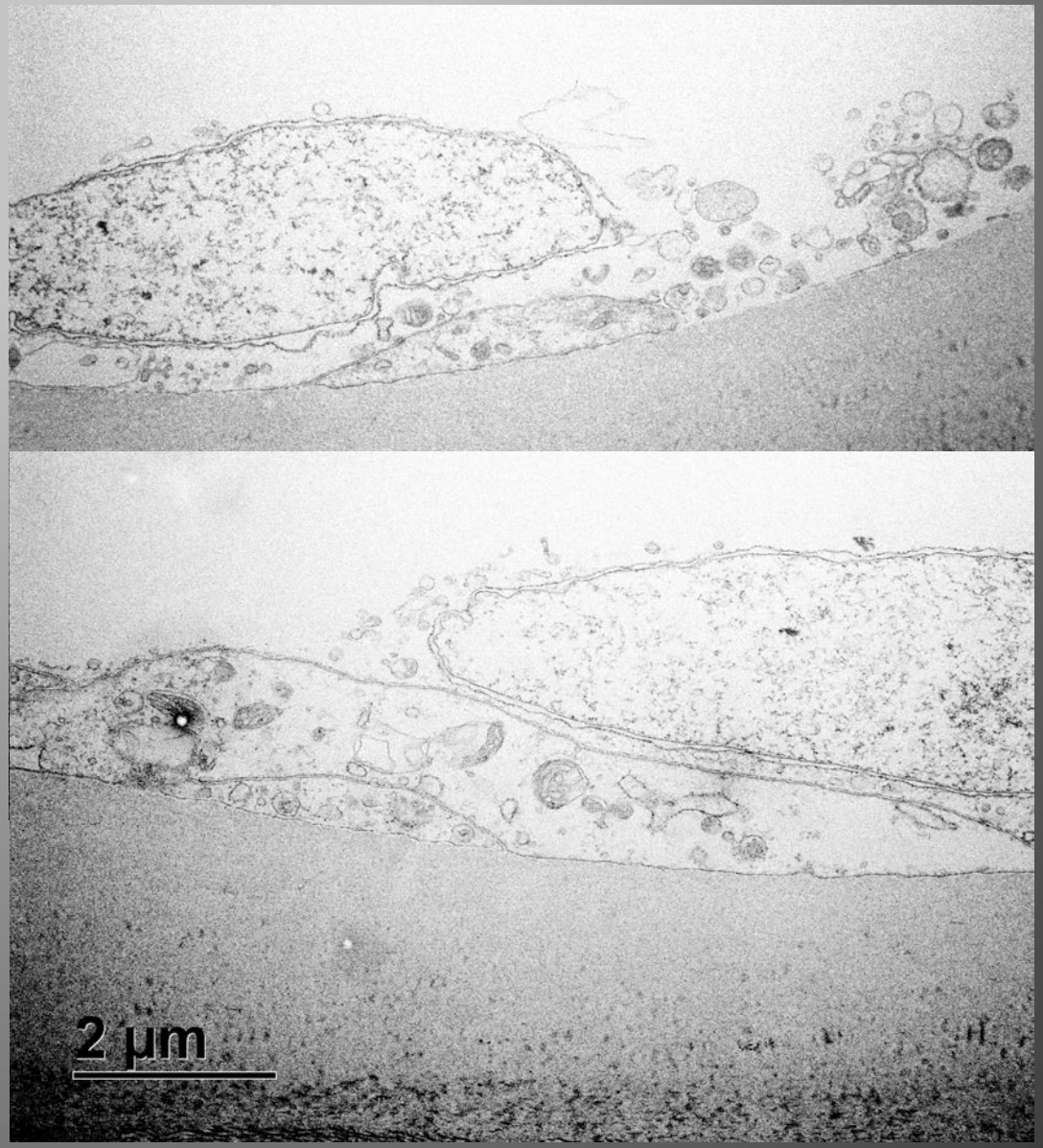
Autophogy/Autophagocytosis

- Programmed Cell Death
  - Cell digests itself (yum! (insert homer as donut?)
  - Response to stress, disease, infection, etc.
- Morphology
  - Organelles in cytoplasm (defuct mitochondria, etc.) or excess proteins are sequestered in a double-membrane bound vesicle (autophagosome) and sent through the digestion process
- Significance
  - Prevents mutations, maintains cellular homeostasis
  - Dying cells often demonstrate autophogy, but it is unclear if the process is a cause or symptom (in an attempt to save the cell)



Necrosis

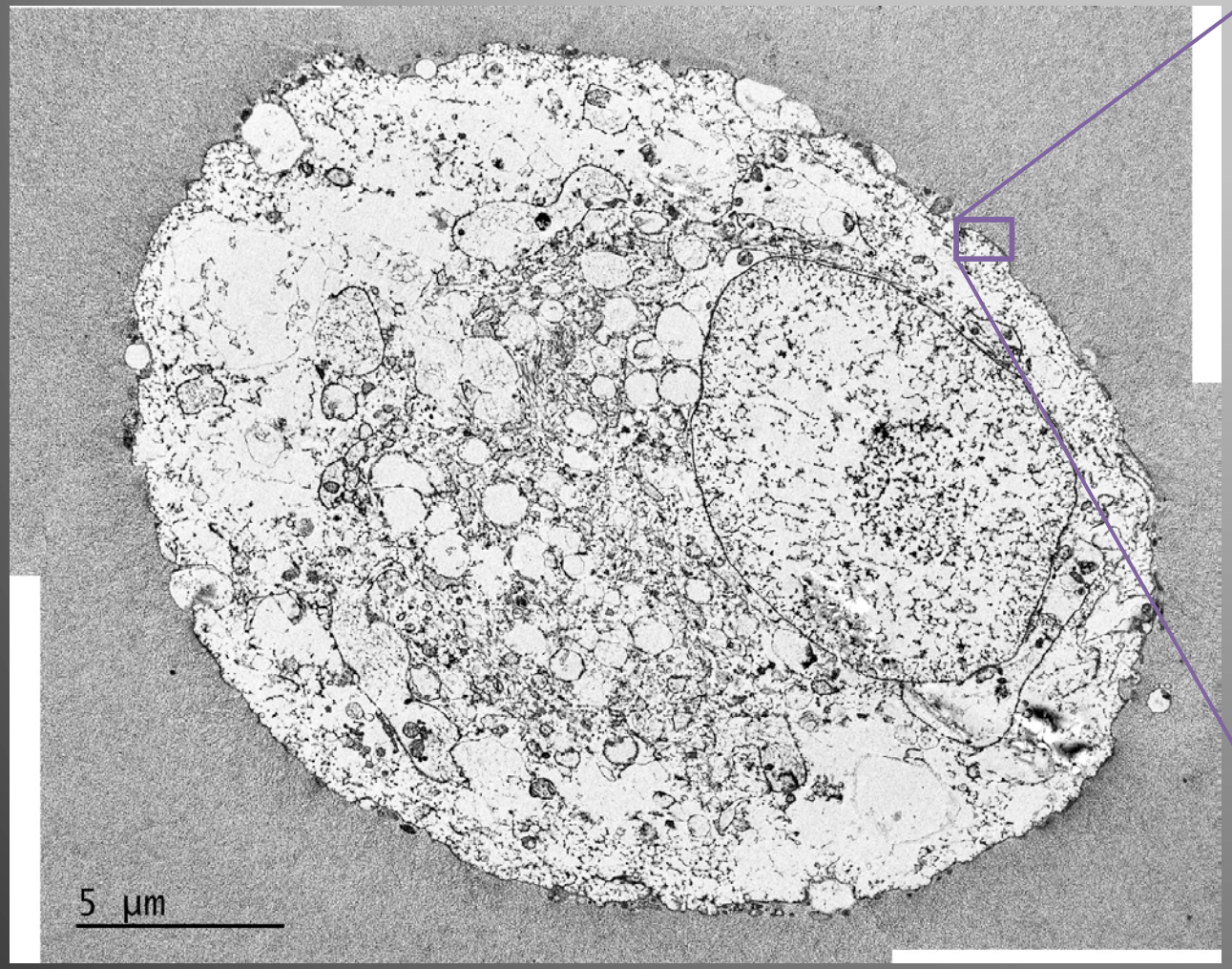
- Non-Programmed Cell Death
  - Caused by physical tissue/cell damage
- Morphology
  - Vacuoles, swollen mitochondria, membrane separation/blebbing, extraction of cytoplasm, nucleus often appears normal

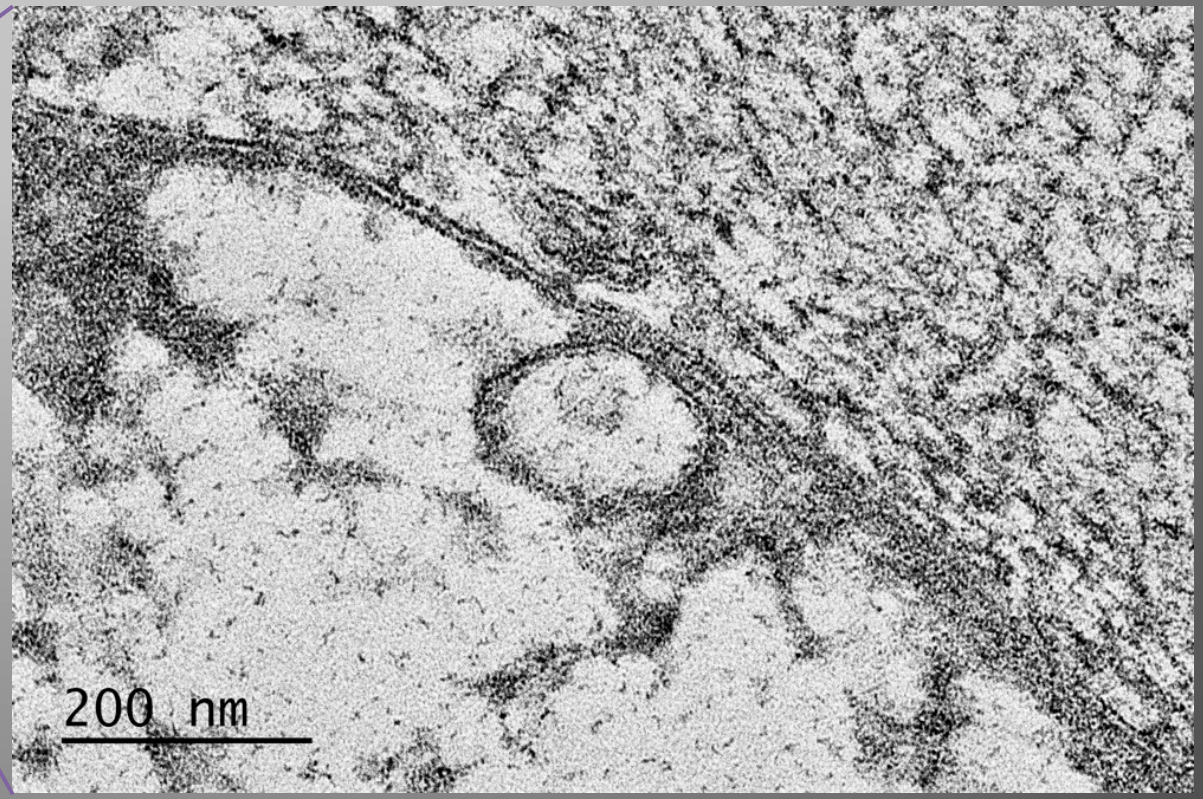


Preservation/Processing artefacts

- Ice crystal damage
  - Freezing to slowly, accidental thawing
- Mishandling tissue during processing, especially after critical point drying and during stub-mounting for SEM
- Improper fixation, dehydration, or infiltration (can be cascading problem)
  - Improper buffer used for cell or tissue
  - Not long enough time, not enough gradation in series, tissue too large to penetrate by aldehydes and other reagents
  - Precipitation of heavy metals during chemical processing
    - Improper flushing out of buffer salts that react with osmium, uranium, lead, etc. during postfixation

Freezing artefacts / Ice crystal damage

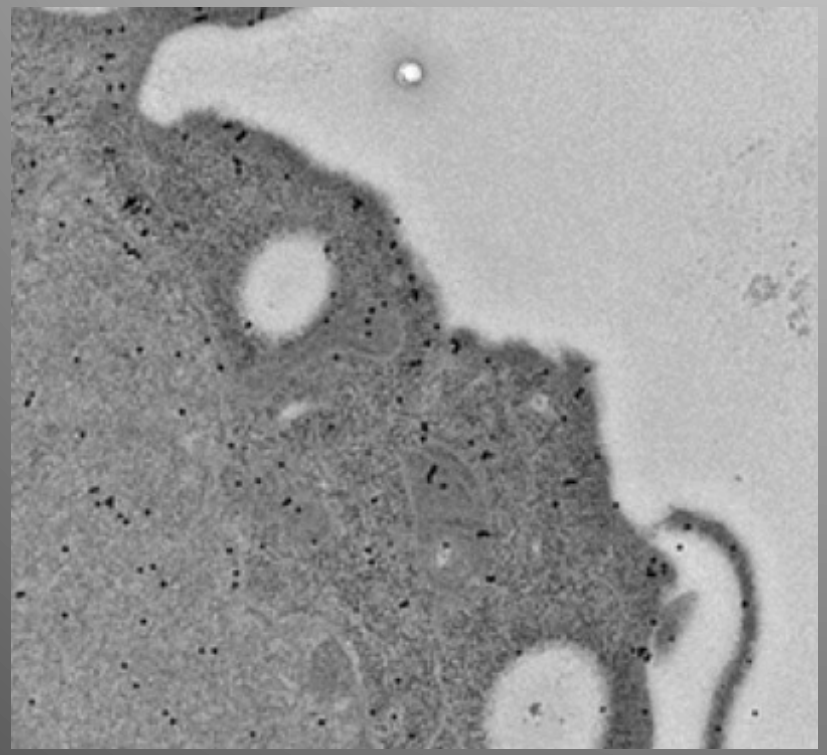




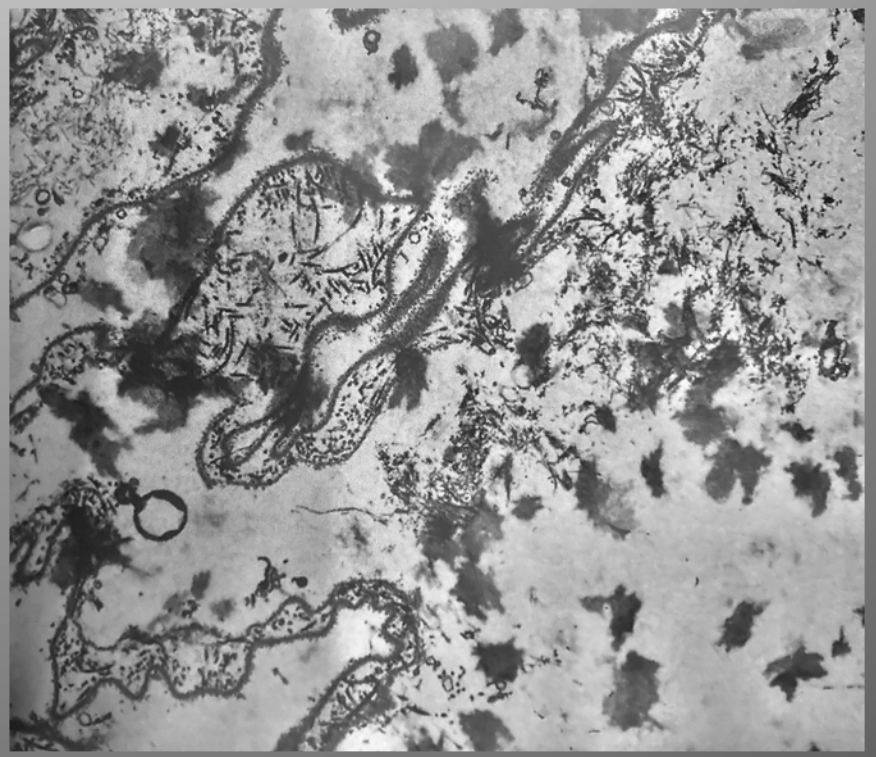
Cell poorly frozen within a hydrogel matrix
Cytoplasm and organelles appear damaged and extracted
Some membranes and vesicles preserved…that's about it.

Chemical Processing Artefacts

Cells/tissue fixed without glutaraldehyde lacks membrane definition

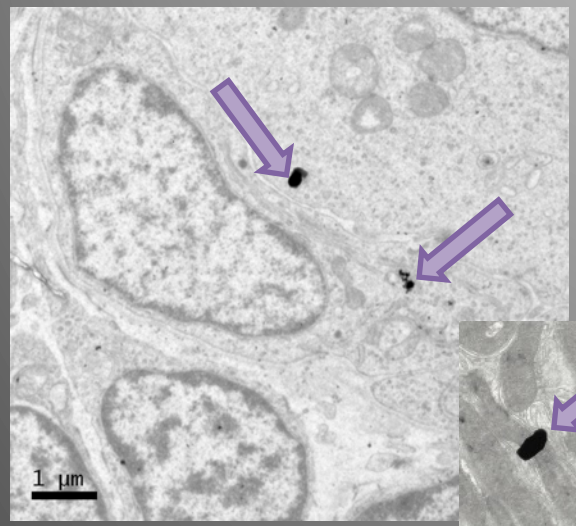


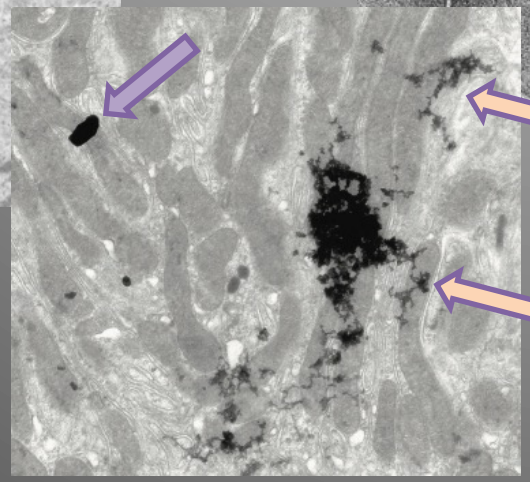
Poor fixation and extracted cytoplasm from excessive dehydration



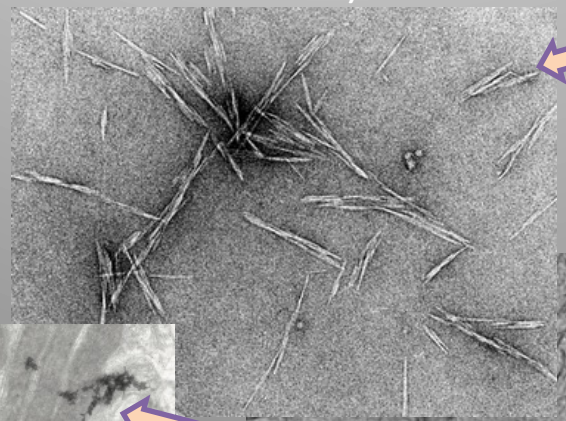
Heavy Metal Precipitates

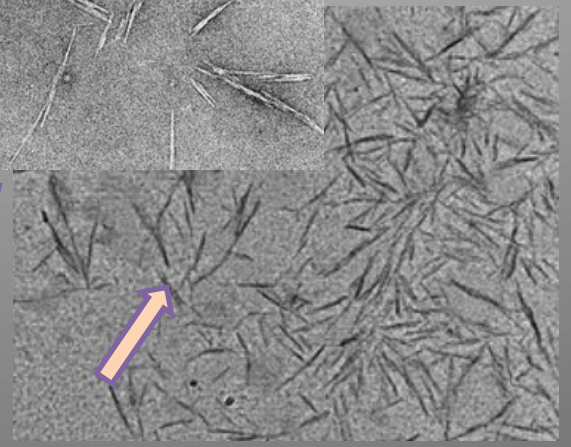
Contamination from Lead citrate stain



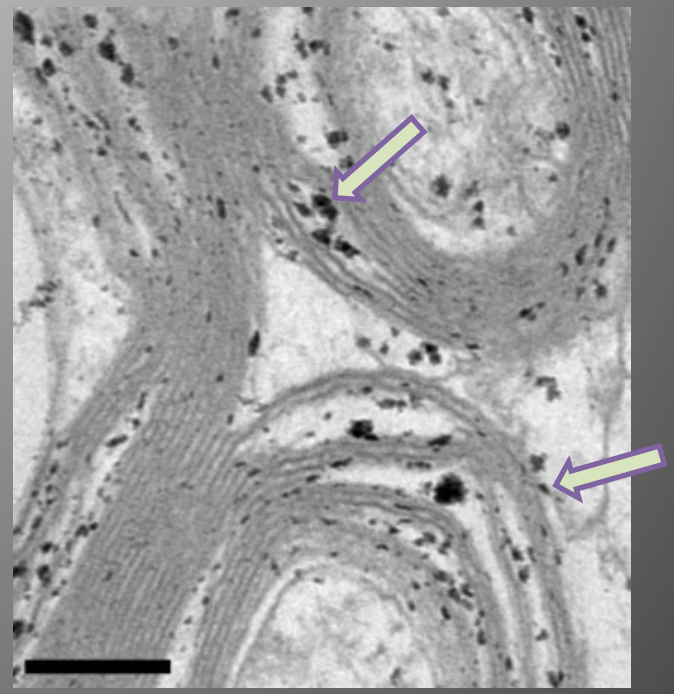


Uranium precipitate (negative stain and on tissue section)





Osmium precipitates with phosphate in buffer



Mitigating Preservation Arterfacts

Lecia VT1200 S Vibrating-blade Microtome ("Vibratome")

- The VT1200 S is a fully automatic microtome with vibrating blade.
- It can be operated in automatic as well as in semiautomatic sectioning mode.
- The specimen size can be up to 30 x 40 mm (in automatic mode).
- The section thickness can be set in 1 μm increments to maximally 1000 μm (=1mm).

Lecia High Pressure Freezer HPM100 (Leica)

- High-pressure freezing is the method of choice for cryofixation of pristine samples with thickness of up to 400 μm without chemical fixation and drying steps.
- The formation and growth of ice crystals is suppressed by applying 2100 bar high pressure to the sample and cryo immobilization in less than 10 msec.
- Once the sample is high pressure frozen, it can be processed for cryoSEM, cryoTEM, freeze substitution, and other applications.
- Available is the flat carrier system and the tube system.
- Location: Hogan, 5-150.

Leica EM AFS2 Automatic Freeze Substitution and Low Temperature Embedding System

- Freeze substitution and progressive lowering of temperature (PLT) techniques along with allowing low temperature embedding and polymerization of resins.
- LED illumination for easy viewing and working within the chamber.
- The automatic reagent handling system EM FSP dispenses and automatically dilutes reagents from 100% stock containers.

ASP-1000 mPrep Automated Specimen Processor

- The ASP-1000 automates the full range of electron microscopy sample preparation; fully programmable uses both mPrep/s and mPrep/g capsules to perform an unlimited range of lab preparation tasks, including:
- TEM specimen preparation (resin embedment)
- TEM grid staining
- Immuno-labeling of TEM grids
- Immuno-labeling of en bloc tissue

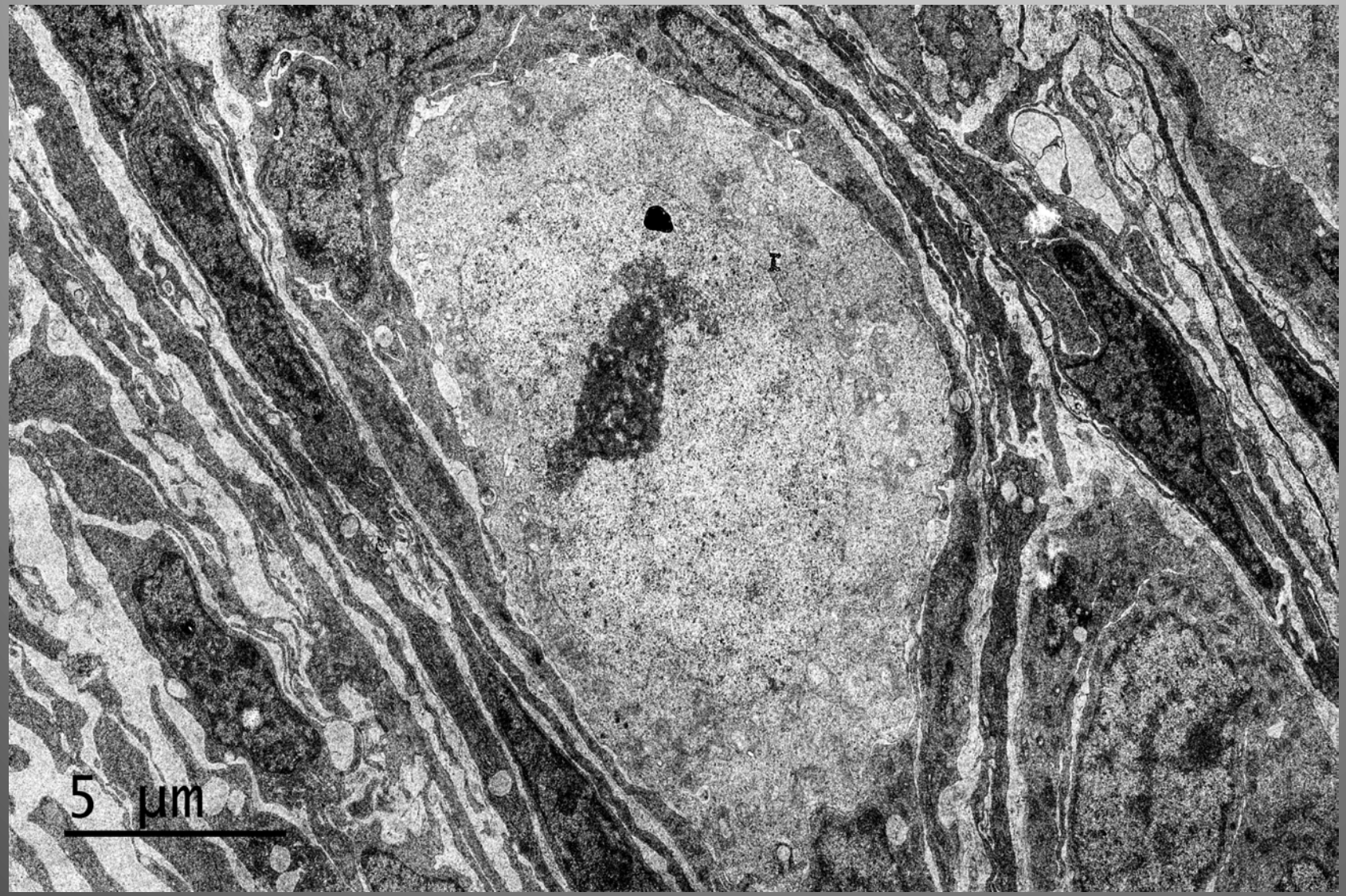
FEI Vitrobot Mark IV and Mark III- Plunge Freezer

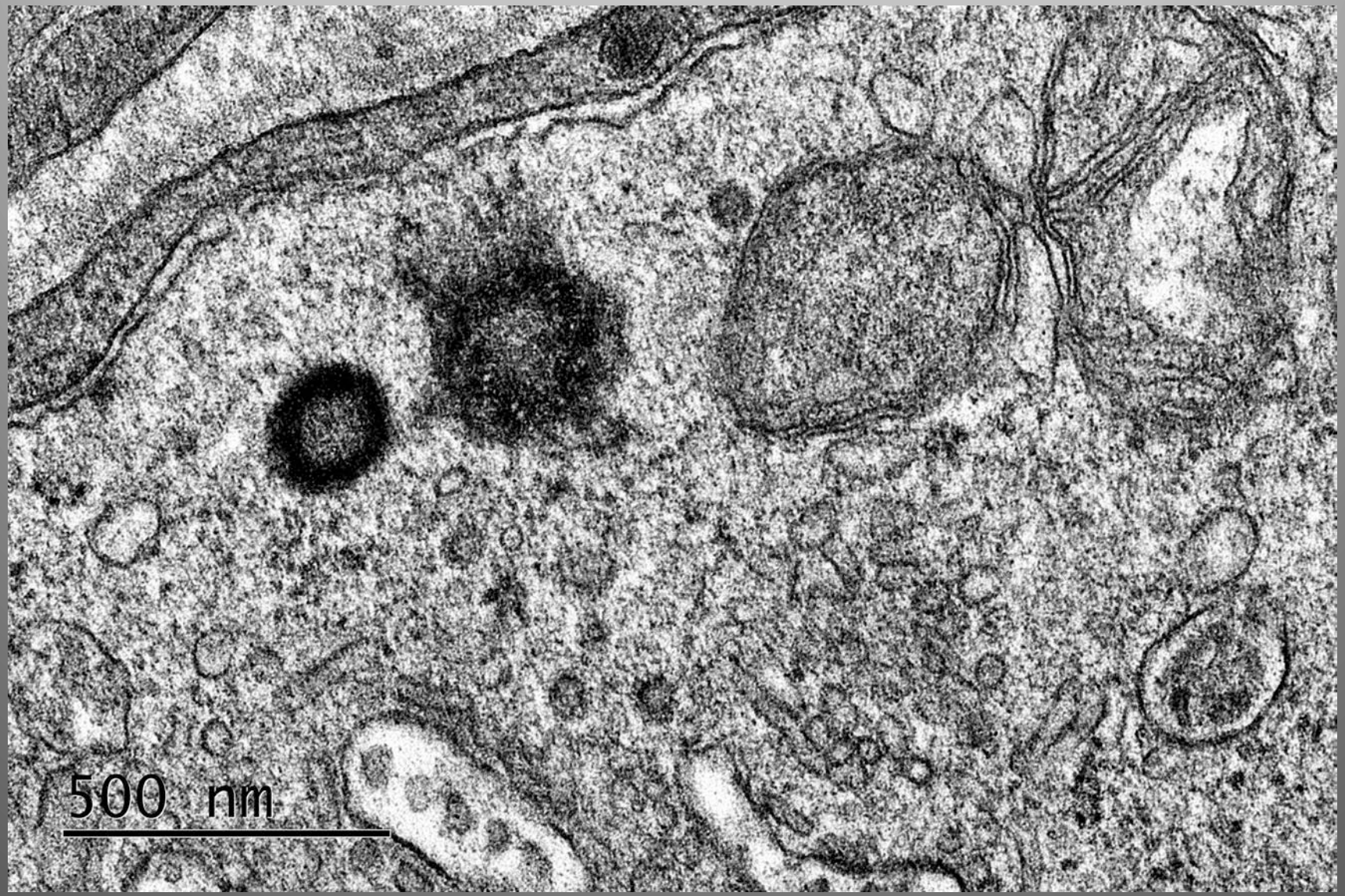
- Fully automated vitrification robot for plunge-freezing of aqueous sample suspensions.
- All essential vitrification parameters, such as temperature, relative humidity, the pressure and quantity of blottings, and drain time can be programmed for each individual application and set for automatic retrieval.
- Operating parameters: Working temperature 4 – 60°C (at an ambient temperature range between 18 – 25°C) Peltier controlled heating/cooling Relative humidity ambient humidity – 100% (no condensation at an RH < 85%) Ultrasonic controlled humidification.
- Location: the Mark IV is in Hogan 5-150, the Mark III is in Technology Institute, AG 76
- Vitrobot Manual is avaiable in NUCore in Docs tab

Q&A

Parthanatos

- Programmed Cell Death
  - Sparked by DNA damage
- Morphology
  - Similar to apoptosis with nuclear fragmentation and condensed chromatin, but lacking apoptotic bodies and swollen mitochondia
- Significance
  - Heart disease, Parkinson's, diabetes


5 µm


500 nm