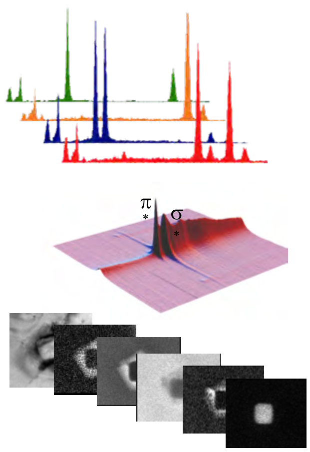
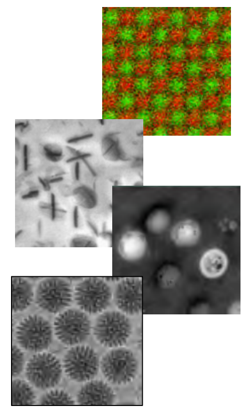
Hyperspectral Imaging of Hard & Soft Matter
In the Analytical Electron Microscope

Current and Futures Prospects





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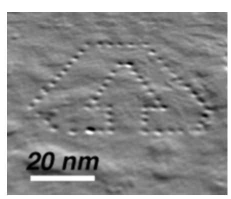
Acknowledgements



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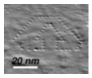
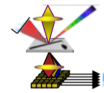


Soft / Hard Matter Characterization at ANL



Soft Matter (SM) and its Interface with Hard Matter (HM) involves a broad research community (Materials , Chemistry, Physics, Life Science) and numerous fields of research including:

- Monolayers/2D Materials/Interfacial Species/ Defects
- Molecular, Colloid and Membrane Frameworks
- Complex Fluids, Emulsions, Gels, "amorphous" materials.
- Organic/Inorganic Heterostructures - Smart Materials for Advanced Functional systems
- Polymeric Systems: self-assembly, templated growth,
- Templates for Semi-conductors
- Hybrid/Smart nanostructures for therapeutic medicine, intelligent coatings for encapsulation and separation, nano-optics, sensors, liquid crystals, photo-activated/enhanced processes
- Single particles, macromolecular complexes, inter/intra cellular processes, biosystems
- Structural Biology/Life Sciences/Medicine

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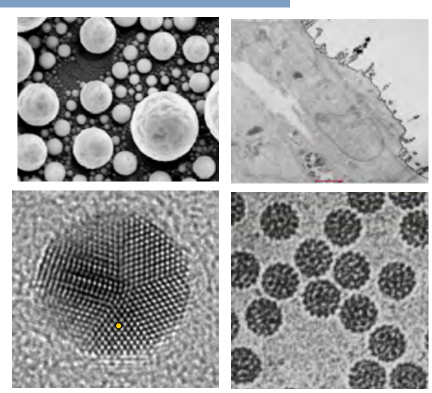
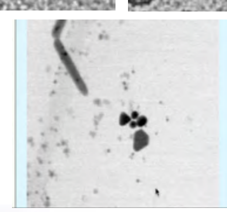
What are the basic questions we all ask ?

- **State**
  - Morphology
  - Crystallography
  - Elemental/Chemical Constituents
  - Bonding/Electronic Structure
- **Static vs Dynamic**
  - Temporal
  - Temperature
  - Stress/Strain/Mechanical Deformation
  - Vacuum/Gaseous/Liquid Environment
  - EM Fields
  - Irradiation Environment
    - Charged Particles
    - Photons….
- **Key Challenges:**
  - In-situ observation of real time processes
  - In-situ high-spatial resolution elemental analysis
  - Simultaneous imaging of hard/soft components
  - Dynamics - Fast detection schemes, detectors, and sources
  - Radiation Damage





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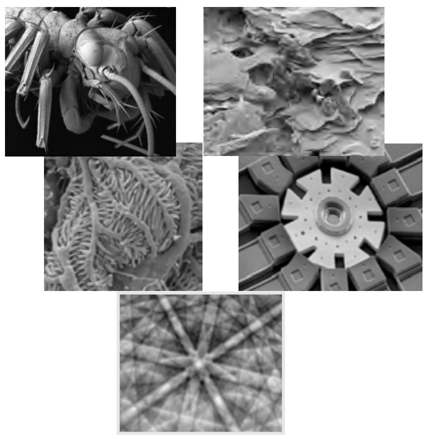
Role of Traditional Electron Microscopy

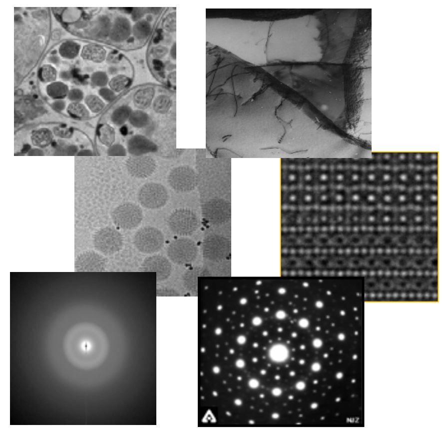
SEM

Scanning Electron Microscopy



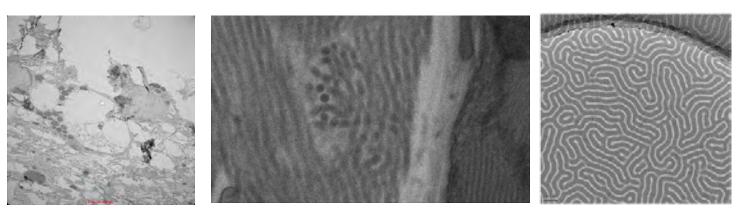
TEM / STEM / HREM

Transmission - Scanning Transmission - High Resolution Electron Microscopy

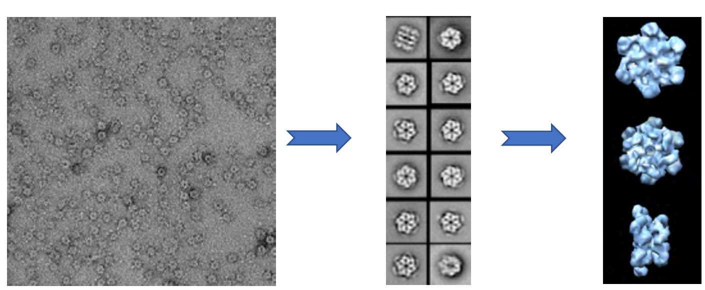


Conventional Transmission Electron Microscopy of non-Crystalline (Soft) Matter

Structural Imaging typically uses thin sections to visualize localized structures



Single Particle Imaging uses Statistical Averaging of Thousands of Images

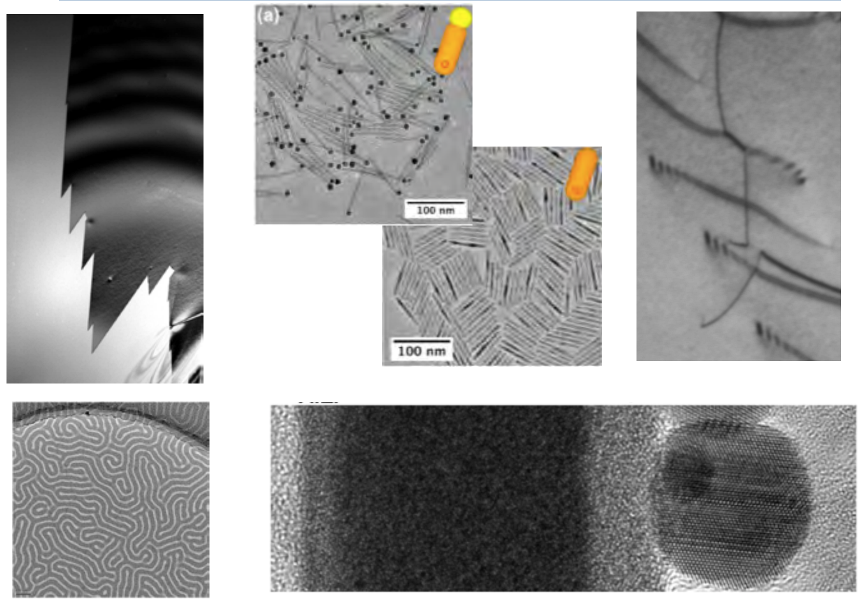


Hundreds of Thousands of Particles

Class Average Images

Model Fits

Conventional Transmission Electron Microscopy of Crystalline (Hard) Matter



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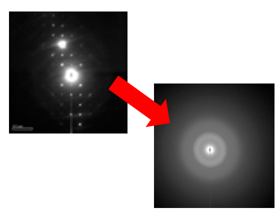
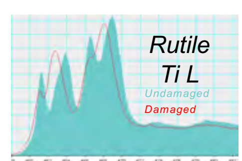
The Microanalyst's Bane :
Electron Radiation Damage

- ***Knock-on / Displacement Damage***
  - Mainly due to Elastic Scattering with Nucleus
  - Metals / Semiconductors / Ceramics /
    - Crystalline
    - Amorphous
  - Threshold Energy exists and varies with Materials
  - Increases with Beam Energy
  - Temperature dependence exists but is variable with Material
  - Sputtering / Melting are associated variants
  - Dose Dependent



- ***Radiolysis / Ionization Damage***
  - Mainly due to Inelastic Events with electron shells
  - Most prominent in Organics, i.e. bond breakage
  - Decreases with Increasing Beam Energy
  - Very low Threshold for the onset (< 1 keV)
  - Some Evidence of decrease with specimen cooling
  - Dose Dependent

- ***Secondary Electron Damage***
  - Emission of secondary's from inelastic scattering events
  - Increases radiolysis by additional low energy events
  - Also causes charging
    - Dielectric breakdowns
    - Coulomb explosion
    - Charging issues (drift, beam deflection ……)



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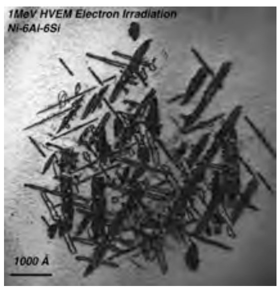
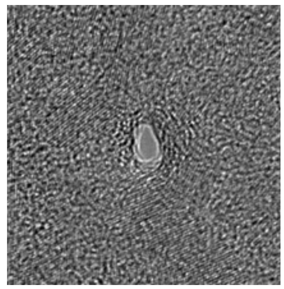
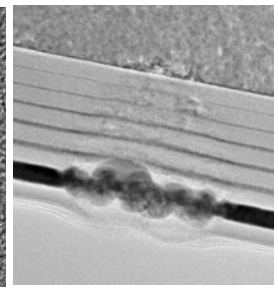
The Microanalyst's Bane : Hard Materials



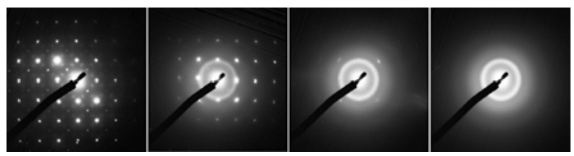
Atomic Displacement



Sputtering

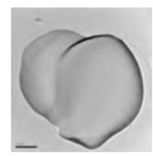


Melting



0.0 D_c 0.4 D_c 0.8 D_c 1.0 D_c

Amorphization



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Knock-On - Displacement Damage

Energy Transfered by an Incident Electron to an Atomic Nucleus

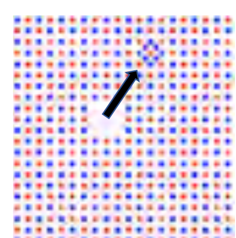
$$T_T = \frac{2*T_0*(T_0+2*m_0c^2)*\sin^2(\frac{\phi}{2})}{Mc^2}$$

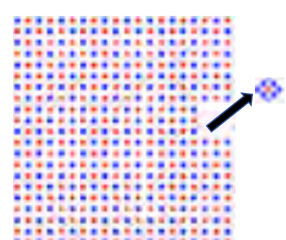
To = eVo, M= nuclear mass, mo = electron mass, f = scattering angle

Element	<----------- --	-----------	-T_T--------- -	------------- >	T_d[1]	T_s[2]
	100 kV	200 kV	300 kV	400 kV		
Al	8.93	19.5	31.6	45.3	16	3.5-7.0
Ti	5.00	11.0	17.8	25.5	15	4.9-9.8
V	4.73	10.3	16.72	24.0	29	5.3-10.6
Cr	4.63	10.1	16.38	23.5	21	4.1-8.2
Fe	4.31	9.40	15.25	21.8	16	4.3-8.6
Co	4.08	8.91	14.45	20.7	23	4.4-8.8
Ni	4.10	8.94	14.5	20.8	21	4.5-9.0
Cu	3.79	8.26	13.4	19.2	18	3.5-7.0
Zn	3.69	8.03	13.03	18.7	16	1.4-2.8
Nb	2.59	5.65	9.17	13.2	24	7.5-15.0
Mo	2.51	5.47	8.88	12.7	27	6.8-13.6
Ag	2.23	4.87	7.90	11.3	28	3.0-6.0
Cd	2.14	4.67	7.58	10.9	20	1.2-2.4
Ta	1.33	2.90	4.71	6.75	33	8.1-16.2
Pt	1.23	2.69	4.37	6.26	33	5.9-11.8
Au	1.22	2.67	4.32	6.2	36	3.8-7.6

T_d ~ 3-5 * $E_{sublimination}$ T_s ~ 1.5-2 * $E_{sublimination}$

$T_{Displacement} > T_{Sputtering}$





$T_x = f(\text{structure})$

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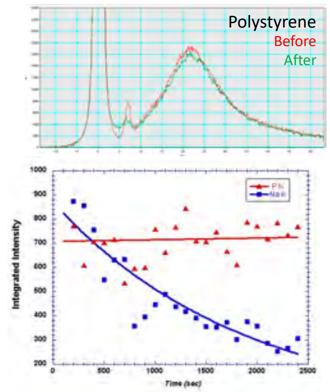
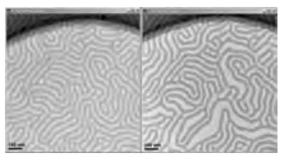
The Microanalyst's Bane : Soft Materials
Radiolysis - Ionization Damage
Inelastic Interations with Electron Shells

Physical
- Inelastic scattering -> Secondary Electron Creation
- Secondary Electrons -> Efficient Low Energy Ionization
- Ionization -> Bond Breakage
- Bond Breakage-> Structural Changes

Chemical
- Bond Breakage -> Reactivity Changes
- Chemical Reactions with Neighboring Environment
- Mass Transport - > Mass Loss





PS/PMMA Block CoPolymer

Radiation Damage to Organic and Inorganic Specimen
Ray Egerton ; Micron V119 73-87 (2019)

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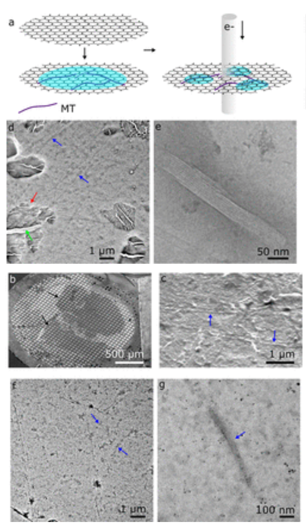
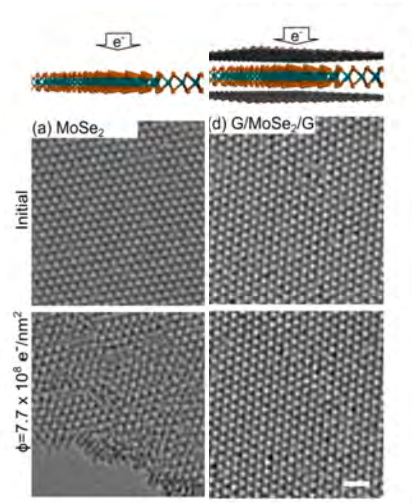
Mitigating e⁻ damage using Graphene Encapsulation





Reduced Radiation Damage in Transmission Electron Microscopy of Proteins in Graphene Liquid Cells
Sercan Keskin† and Niels de Jonge
Nano Lett. 2018, 18, 7435−744

Electron radiation damage mechanisms in 2D MoSe2
T. Lehnert,a) O. Lehtinen, G. Algara–Siller,b) and U. Kaise
APPLIED PHYSICS LETTERS 110, 033106 (2017)

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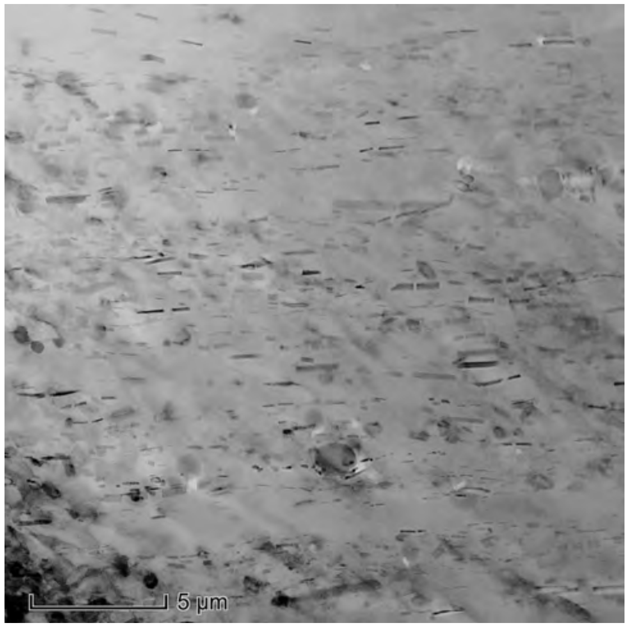
5E61 aluminum alloy: Al-6Mg-0.9Mn-0.07Zr-0.2Er (wt.%)



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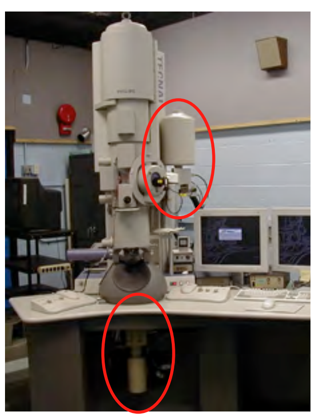
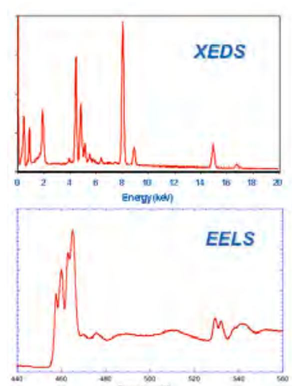
Analytical Electron Microscopy
Information from Inelastic Scattering Events





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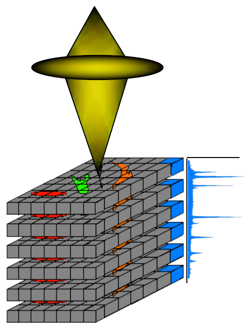
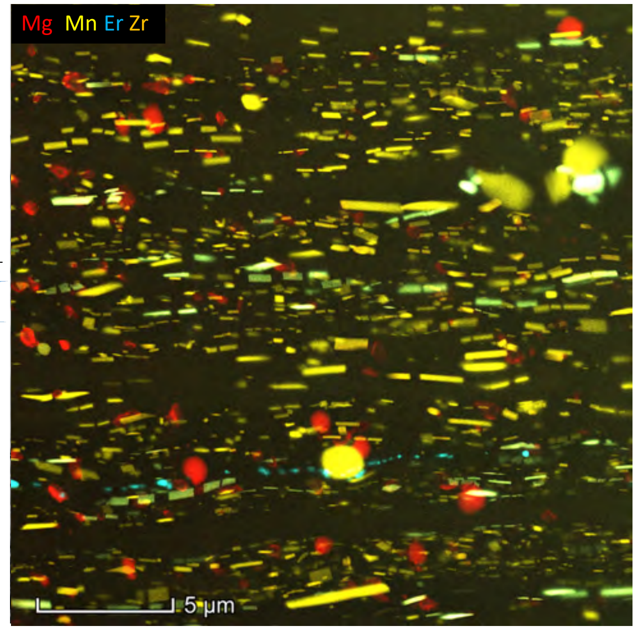
14

5E61 aluminum alloy: Al-6Mg-0.9Mn-0.07Zr-0.2Er (wt.%)





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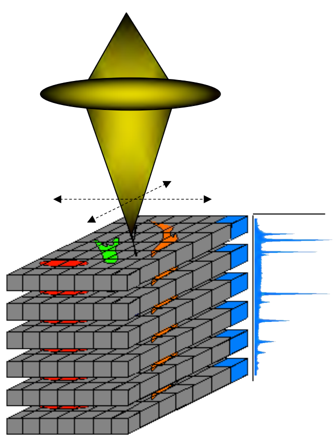
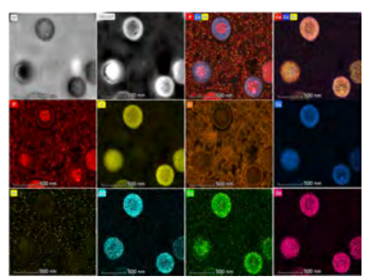
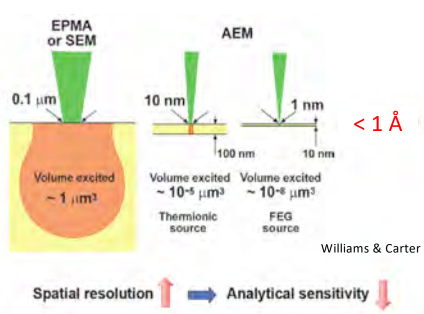
Hyperspectral Imaging in Today's Analytical Electron Microscope

Correlated/Simultaneous ***Localized & Temporally Resolved*** *Spatially Varying Signals*







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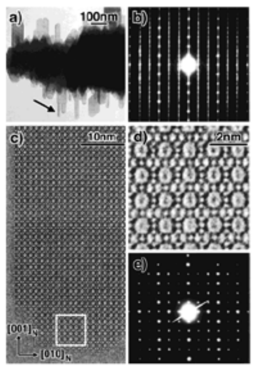
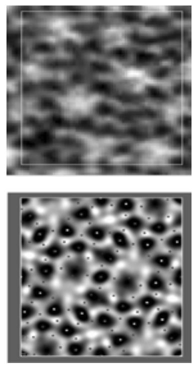
Small Particles: nanomaterials and catalysis

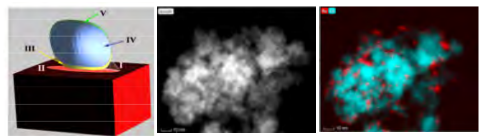
Nanomaterials and processes challenge our ability to understand them:



Zeolite structure solved by imaging and diffraction (Terasaki)



$Mo_1V_{0.32}Nb_{0.1}Te_{0.25}O_x$
HREM image (top) simulation (bottom)
(Zajac - Inovene)



Multimetallic Electrocatalyst Nanoparticles

Key Challenges:

- Quantification - nanocrystallography at atomic resolution (automated w/ real time modeling and simulation)
- atomic scale 3D imaging and diffraction
- In situ: controlled environments for simultaneous imaging and spectroscopy during reactions
- Dynamics: real-time observation

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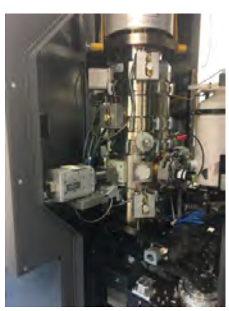
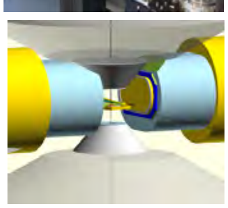
Applications of Soft Matter Visualization and Characterization by Electron Optical Beam Lines

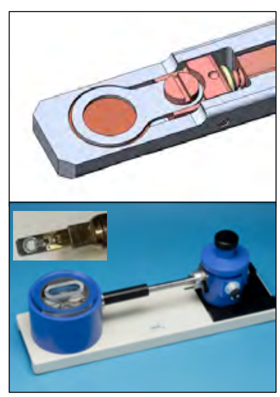
Analytical Electron Optical Beam Lines
ThermoFisher Tecnai/Talos/Titan



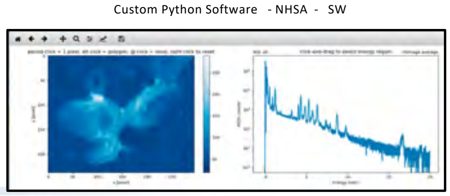


Next Generation Detectors

Custom Cryo-Tomo-XEDS



Custom Python Software - NHSA - SW



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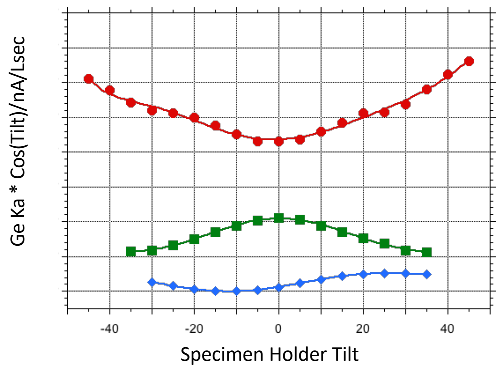
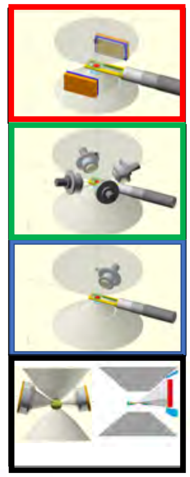
Optimized Detectors for XEDS

Dual / Quad / Single





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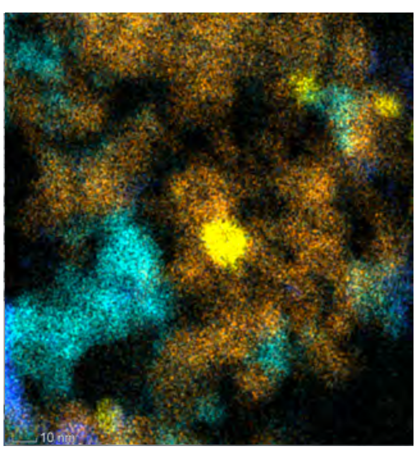
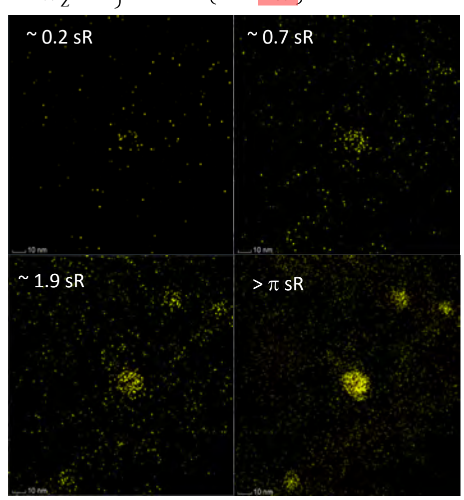
$$I_\alpha = \{\sigma_\alpha(E_o,Z)\cdot\Gamma_\alpha\cdot\omega_\alpha\}\cdot\left\{\frac{C_Z\cdot N_o\cdot\rho}{W_Z}\right\}\cdot\xi_o\cdot t\cdot\left\{\varepsilon_\alpha\cdot\frac{\Omega}{4\pi}\right\}$$



Automotive PGM Catalysts

PdCaTiCeZr



10usec/pixel = 2.6 sec full frame

Quad Actual Data

All others Scaled to Detector Collection Solid Angles

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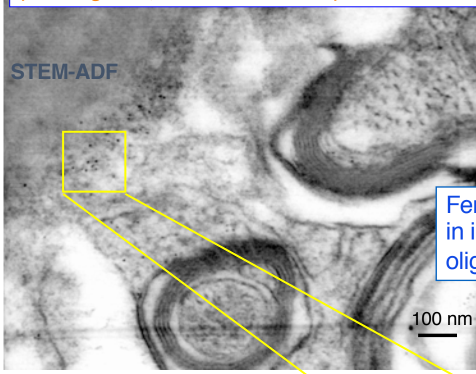
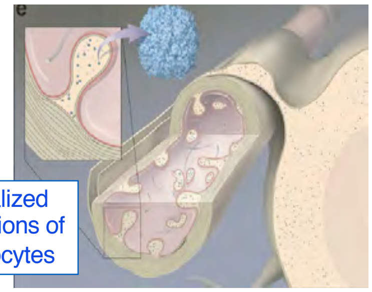
Mapping Ferritin in Brain: Misregulation of Iron Metabolism in IRP Knockout Mice

(P. Zhang et al., J. Struct. Biol. 2005)



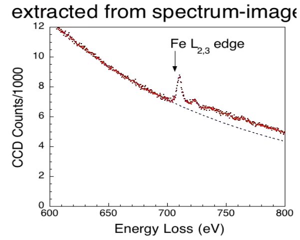
Model of axon and oligodendrocyte showing anatomy of degeneration



Ferritin localized in invaginations of oligodendrocytes

STEM-EELS

EELS of ferritin molecule extracted from spectrum-image



Courtesy of Richard Leapman – NIH

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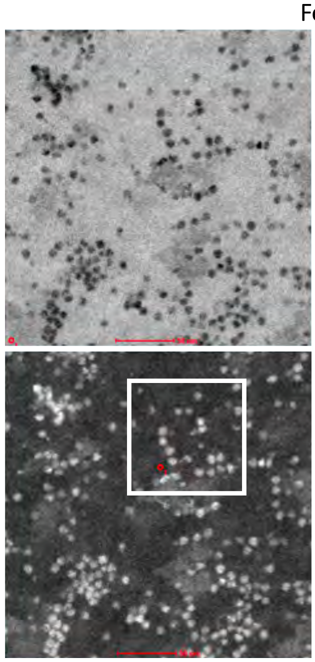
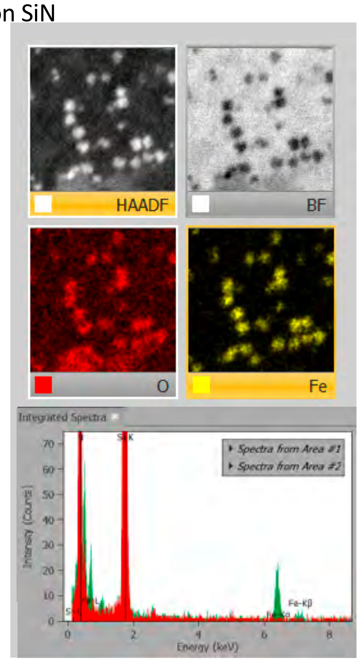
Ferritin on SiN





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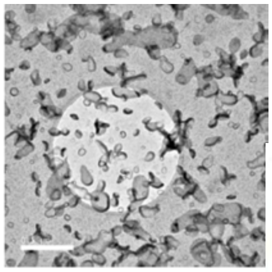
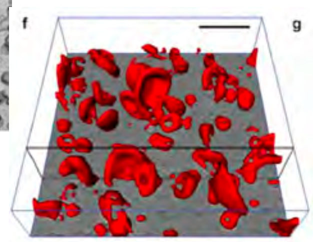
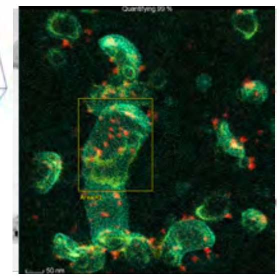
Folded Thin Film Composite Polyamide Filtration Nanomembranes for Waste Water Reclaimation



Complex nanoscale morphologies emerge from nano-membrane synthesis, these kinds of morphologies have proven difficult to characterize, and therefore manipulate, because they are three-dimensional (3D), nanoscopic, and often highly irregular.



Electron tomographic reconstruction of membrane structure



Is there a correlation of metal ion adsorption with morphology?

ACS Appl. Mater. Interfaces 2019, 11, 8517−8526

Xiaohui Song, John W. Smith, Juyeong Kim,.Nestor J. Zaluzec ,Wenxiang Chen
Hyosung An, Jordan M. Dennison David G. Cahill, Matthew A. Kulzick and Qian Chen*.

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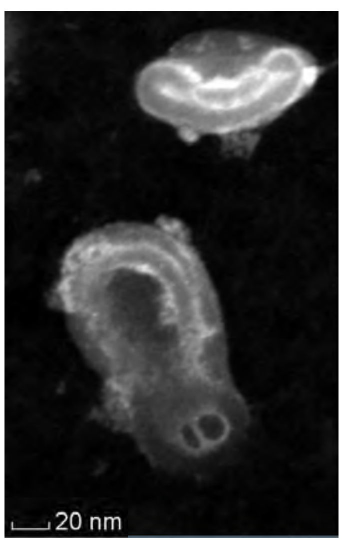
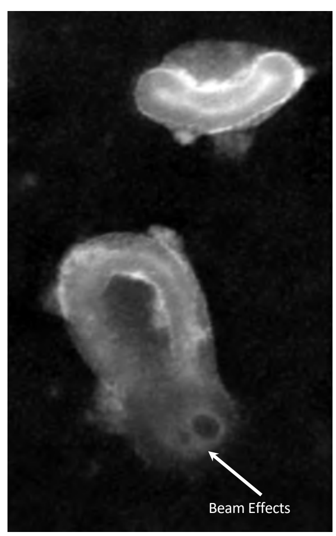
Polyamide Filtration Nanomembranes





t = 0 @ 100 usec/pixel
~300 e$^-$/Å^2

Δt = 33 msec/pixel
~1.05x10^{5}e$^-$/Å^2

defocused probe!

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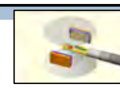
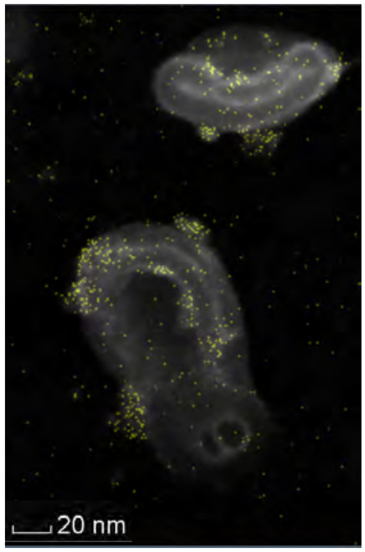
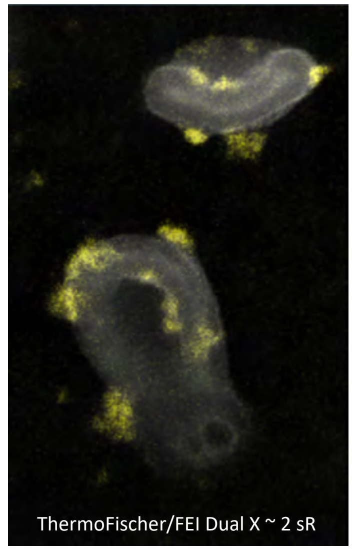
Polyamide Filtration Nanomembranes

Hyperspectral Imaging *+ Spatial Temporal Event Spectroscopy (X,Y,E,t)*







$\triangle$t = 1 msec/pixel

$\triangle$t = 33 msec/pixel

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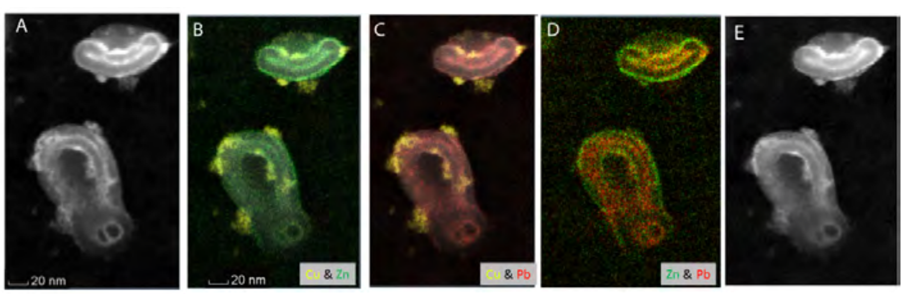
Polyamide (PA) Filtration Nanomembranes

Localization of Heavy Metals to
Site specific Membrane features



$\triangle$t = 0 ← $\triangle$t = 33 msec/pixel →

Distribution of heavy metal adsorbates on the PA film demonstrates an interplay between nanoscale film structure, and film- solute interactions, which may serve as a connection between the physicochemical properties of solutes and their filtration behavior.

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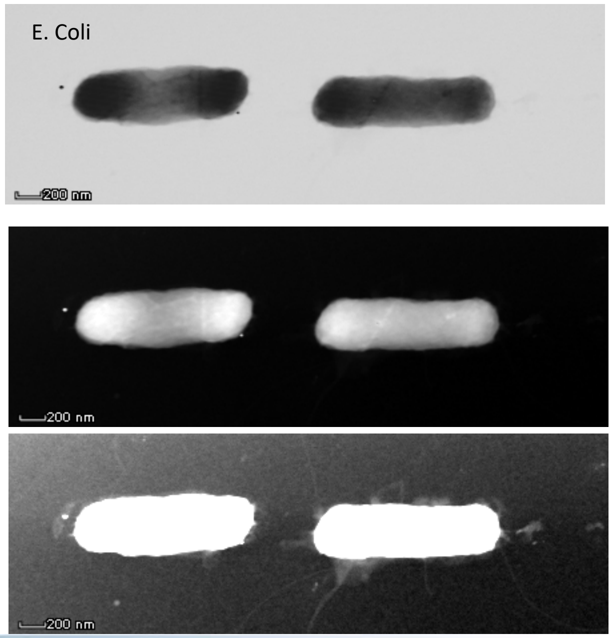
Beam Damage is not always easily visible

2018052752



Frame 1@
250µsec/pixels
@ 100 pA

~ 1.6×10^6 e^-

~ 1.6×10^2 $e^-/Å^2$

Flagellum
Intact on
1st Frame
Expanded
Intensity
Display

Whole E. Coli mounted on SiN TEM support film

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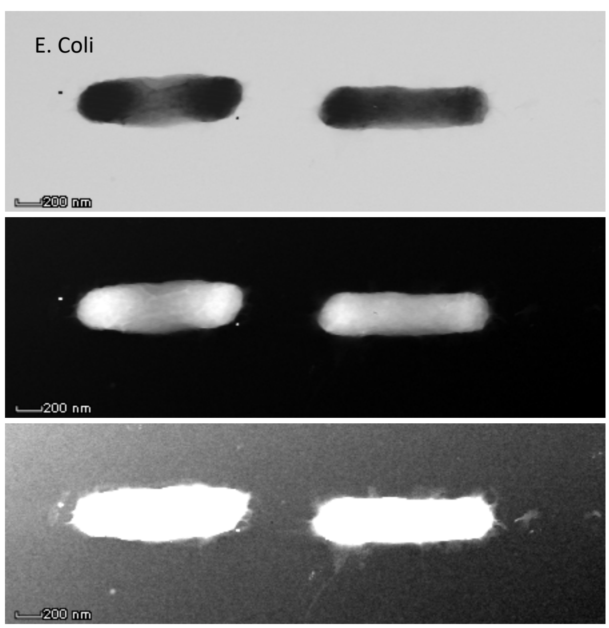
2018052752



Frame 1600
@250µsec/pixels
400 msec Total

~ 2.5×10^9 e^-
2.5×10^5 $e^-/Å^2$

Diameter
Reduced
Flagellum
Damaged

Expanded
Intensity
Display

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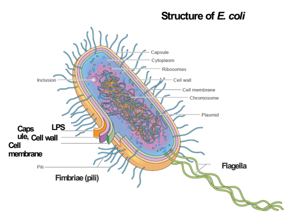
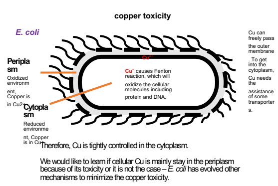
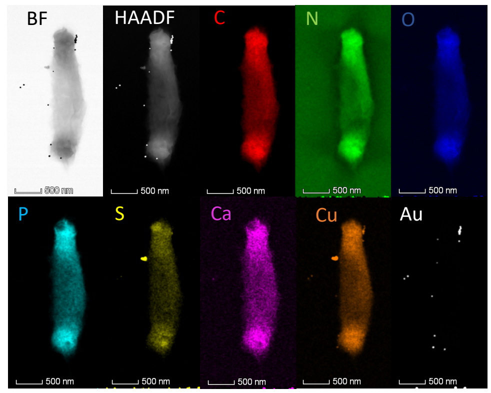
Biological Systems

Dramatically challenge our ability to understand them and characterize them







Key Challenges:

- ♦ Quantification : Imaging & Spectroscopy vs Radiation Effects
- ♦In situ: controlled environments for simultaneous imaging an spectroscopy – "wet environments"

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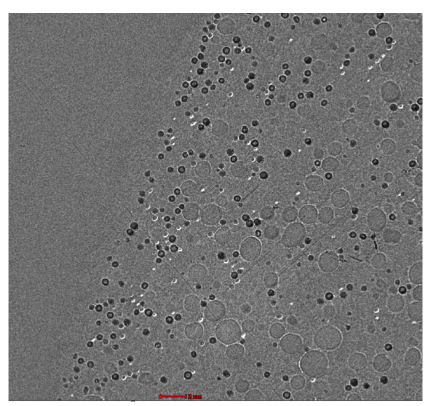
Xenopus (African Frog) eggs and embryos are a tool in biomedical research.

- embryos tolerate extensive manipulation (e.g. single cell , germ layer dissections, tissue transplantations
- easy to inject of a range of materials (e.g. nucleic acids, proteins, whole nuclei) into whole embryo or specific cells
- cell fate of each early embryonic cell is known, allowing targeted gene knock-out, knockdown and overexpression studies
- eggs and embryos provide abundant source for high-throughput biochemical studies
- cell-free extracts made from Xenopus oocytes are a premier in vitro system for studies of fundamental aspects of cell and molecular biology
- Xenopus oocytes are a leading system for studies of ion transport and channel physiology
- Xenopus oocytes widely used assay environmental toxicology
- large-scale genetic screens has identified genes involved in diverse developmental and physiological processes



Elemental changes in egg vesicles correlate with biological processes

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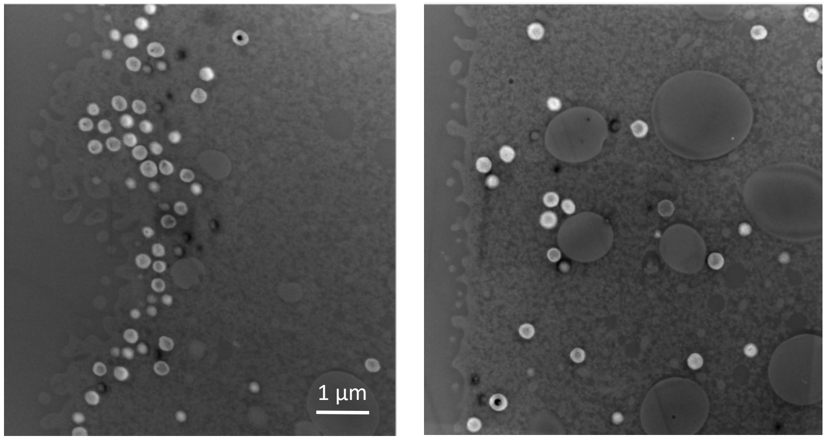
Xenopus - Animal Pole

Egg < – > Embryo



Identification of elemental changes in egg vesicles which correlate with biological processes

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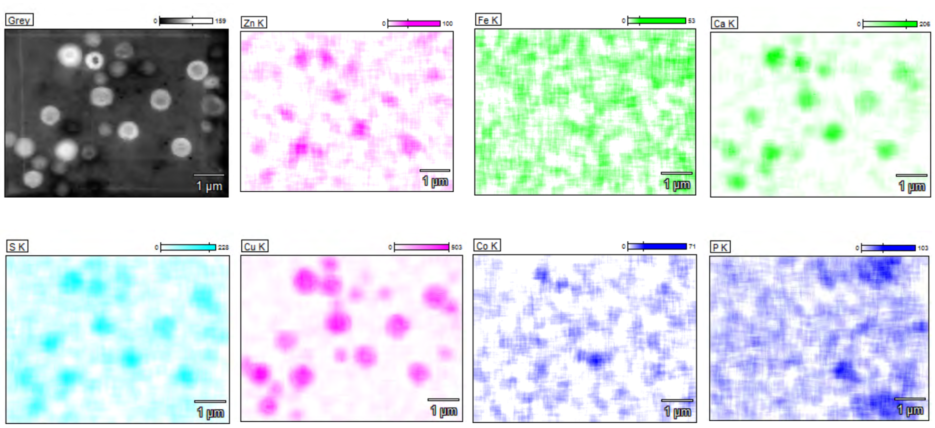
Xenopus Vesicles

µTome section ~ 150nm on Al Slot Grid

Fixed/Embedded



Detectable Signal but Not very Useable

Data from 7/3/18. 43.75 min scan. 200 kV. 24,000X. EDX mode. 13 x 13 kernel.

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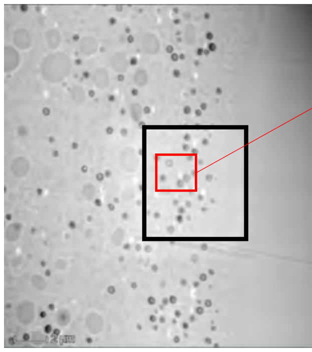
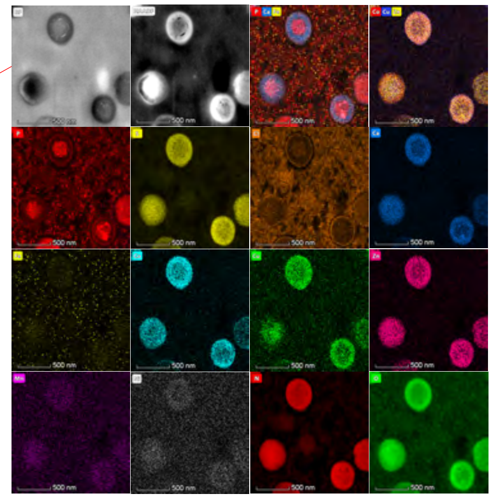
Elemental Redistribution in Xenopus Cortical Compartments

John F. Seeler, Ajay Sharma, Nestor J. Zaluzec, Reiner Bleher, Barry Lai, Emma G. Schultz, Brian M. Hoffman, Carole LaBonne, Teresa K. Woodruff, Thomas V. O'Halloran



200 kV, Hyperspectral Imaging
RT – Cryo-Tomo-Be- XEDS Holder
~100 pA/MultiFrame/Drift Corrected/
Temporally resolved / 719 frames
~ 20 nm probe ~ 400 µsec/pixel low dose
~ total DAQ/pixel ~ 300 msec/pixel

µTome section ~ 150nm on Al Slot Grid
Fixed/Embedded NU protocol



Elemental changes in egg vesicles correlate with biological processes

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Environments: in-situ, growth processes and hard/soft materials

Materials behave differently in growth environments - understanding growth processes, especially self-assembly via organic or bio, presents a challenge

Nanowires grown in situ, characterized post-growth (F. Ross, IBM)

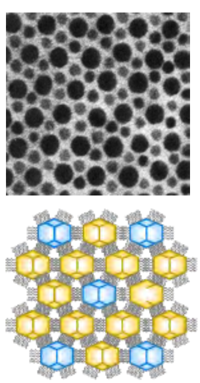
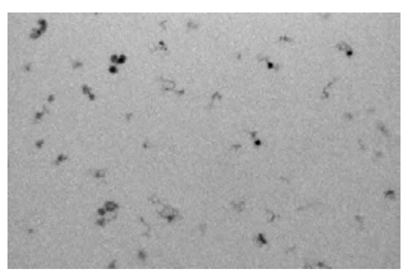


Image (top) and model (bottom) of self-assembled metamaterial (C. Kiely, Lehigh)



Key Challenges:

- In-situ observation of growth processes - at atomic resolution in growth environment
- In-situ high-spatial resolution chemical analysis
- Simultaneous imaging of hard/soft components
- Dynamics - Fast detection schemes, detectors, and sources

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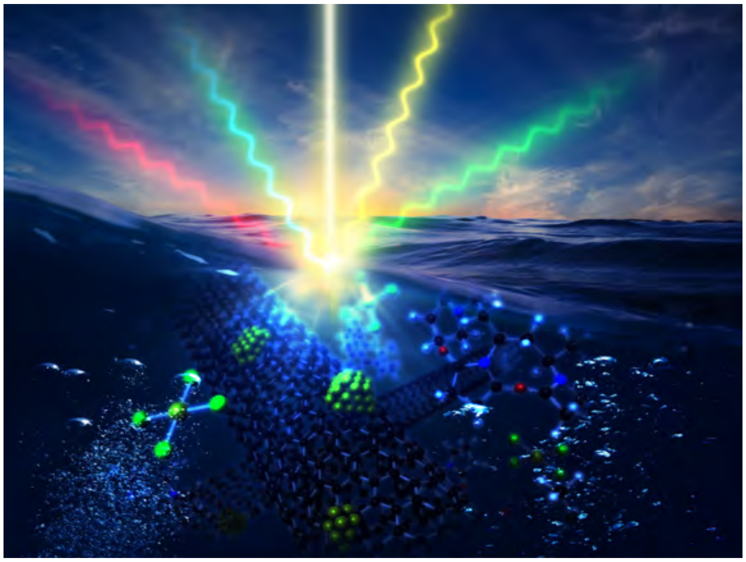
Most of Today's Technology is not Operated/Functionalized in Vacuum



In-Situ Operando Microscopy is of Great Interest

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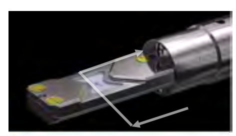
In-situ Characterization: The game has changed

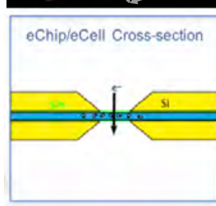
- *Improved Electron Optics*
  - *Improved Performance*
  - *Improved Experimental Space*
  - *In-Situ Holders/Environments*
- *Improved Detector/Geometry*
  - *Higher Resolution*
  - *Higher Speed DAQ*
  - *Higher Efficiency*
- *Computationally Mediated Experiments*
  - *Exploit Electron Solid Interactions for State-of-the-Art Materials Characterization*

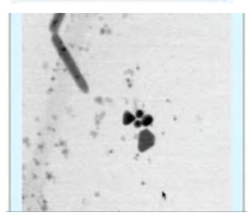
Challenges:

- *In-situ observation of processes - at resolution in growth environment*
- *Simultaneous imaging of hard/soft components*
- *Dynamics - Fast detection schemes, detectors, and sources*
- *In-situ / high-spatial resolution elemental spectroscopy*

eCell Holders







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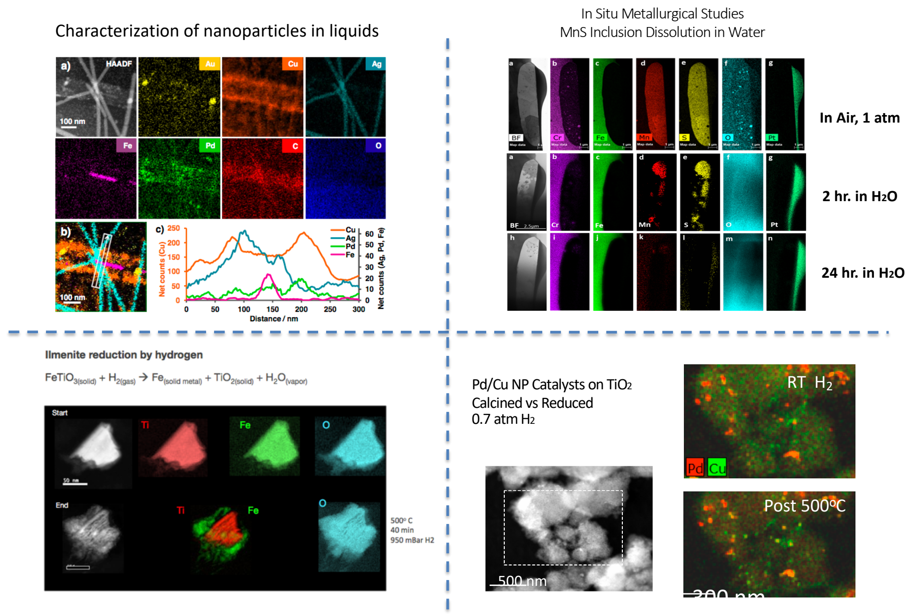
Hyperspectral Imaging in Liquids & Gases @ 1 ATM @ Temperature



Collaborative work with Manchester & BP

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The Characterization Wish list

- Imaging resource with mm to sub-Å resolution ✓
- Imaging resource with spectrographic (elemental/ chemical/electronic) contrast ✓
- Capable of dynamic processes (time scales) ✓
- Capable of environmental conditions (in-operando) ✓
- Quantitative in all modes ✓
- Non intrusive ➛ Non destructive ✓
- Applicable to Hard & Soft materials ✓
- In multi-dimensions (x,y,z,t,…..) ✓
- Multi-modal platform which integrates other complementary resources (probes/signals) ✓

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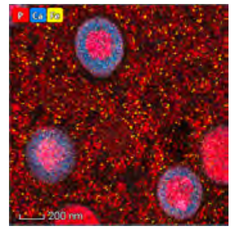
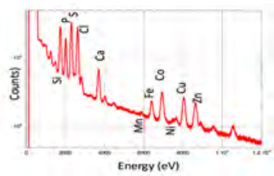
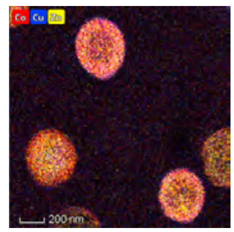
Capabilities for Hard Matter XEDS has been significantly improved
Soft Matter XEDS is now practical in many (but not all systems)

- New Detector Geometries
- High Collection Solid Angles ~ 2 sR
- Temporally Resolved Hyperspectral Imaging
- Low/Controlled Dose DAQ
- New and optimized holders (including cryo-modes)







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Thanks !

Questions ?

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Zaluzec @microscopy.com

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