M5052

Characterization of Materials and Nanomaterials

Graduate Program in Nanotechnology

Electron Microscopy 2

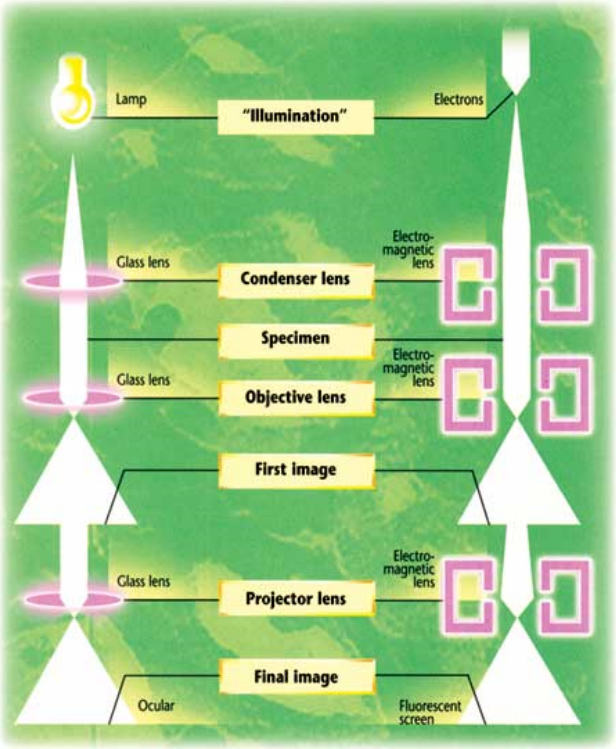
TEM: Transmission Electron Microscopy
EELS: Electron Energy Loss Spectroscopy

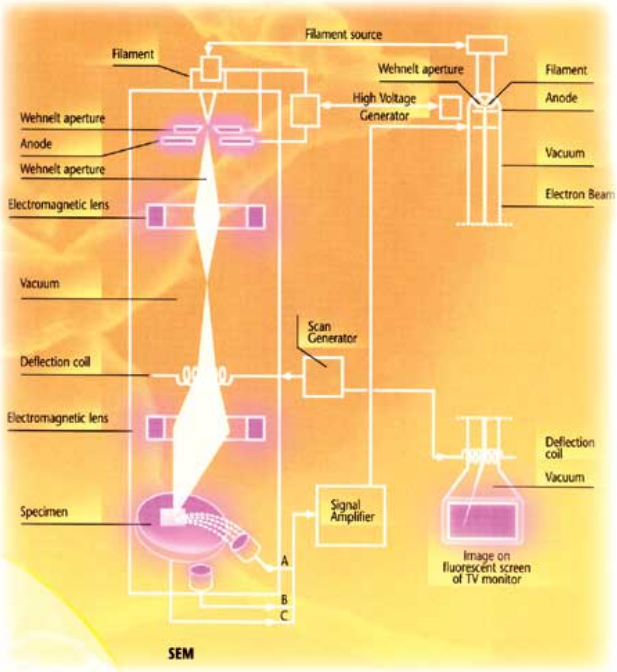
Dr. Fernando J. Rodríguez Macías
fernando.jrm@tec.mx
Profesor, Departamento de Ciencias – Química y Nanotecnología
Investigador, Grupo de Enfoque de Nanotecnología para Diseño de Dispositivos

Tecnológico de Monterrey
Escuela de Ingeniería y Ciencias

Comparison of Microscopy Techniques

- Optical
- TEM
- SEM

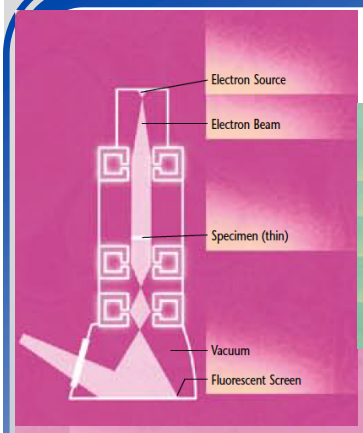


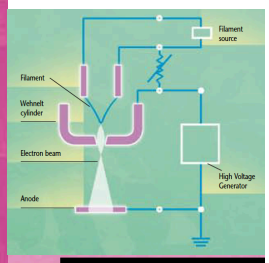


TEM

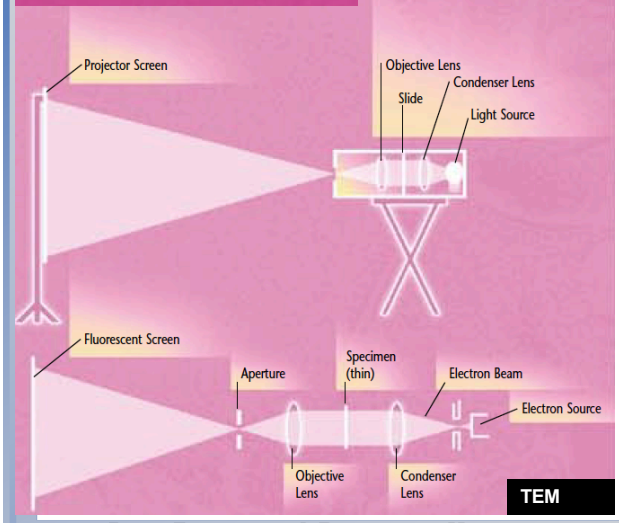
Transmission Electron Microscopy allows observations and characterizations that are impossible with SEM, but it is more difficult to use and instruments are usually more expensive

Transmission Electron Microscopy





Slide projector



TEM

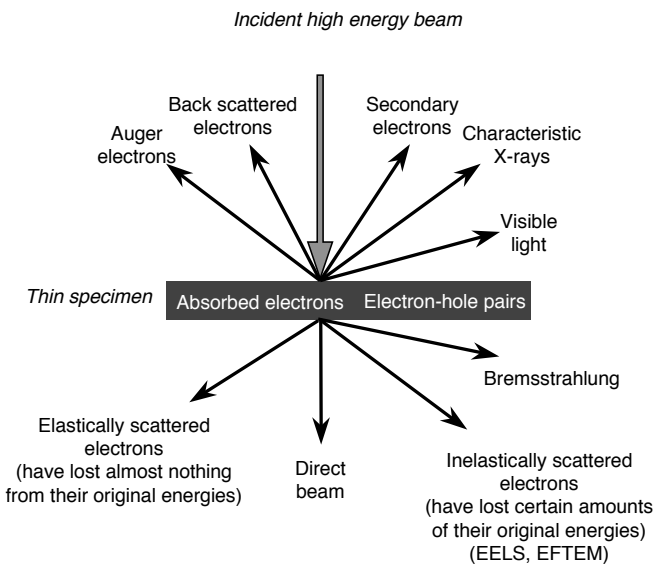
- Electron beam generated by field emission and adjusted with condenser lens
- Thin sample placed in the beam path
  - Beam diameter set with condenser lens defines observation area
- Objective lens used to amplify and project final image
  - Additional lenses for projection or selecting specific signals
- Magnification in TEM is changed with the electromagnetic lenses
  - Variable focal length, adjusted electronically
    - In SEM the magnification is adjusted by changing the scanning area
- TEM is more analogous to a slide projector or an optical microscope than it is to SEM
  - TEM is more versatile than optical microscopy: optical lenses have fixed focal length
    - Adjusting magnification requires changing the lens or moving the lens

Disadvantages and Limitations of TEM

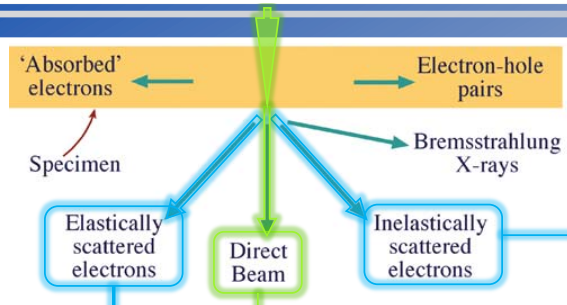
- Very limited sampling
  - Viewing area is extremely small
  - If a non-representative sample, or non-representative area of the sample is imaged results are not representative
    - Usually better to characterize materials with other techniques before use of TEM as well as in addition to it
- Two-dimensional images of 3D materials
  - TEM is analogous to a radiography
  - Image and other information acquired in the TEM is averaged through the thickness of the specimen
- Electron Beam Damage to some samples
  - High energy electrons give better resolution, but can also cause more damage
- Sample must be thin enough to be electron transparent
  - As a "rule of thumb" thickness should be < 100 nm
    - For HRTEM and EELS even thinner specimens are better
    - Actual maximum thickness depends on specimen, and on beam energy
  - Sample preparation to create thin samples can be complicated and can also damage some specimens introducing "artifacts"

Electron-Specimen Interactions in TEM

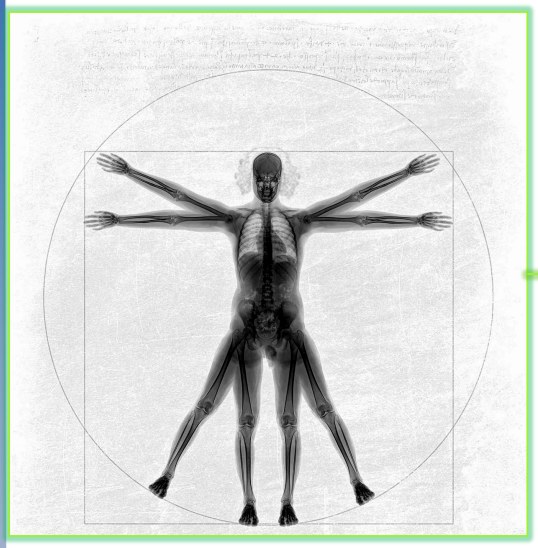
- Image formation depends on differences in absorption or scattering of electrons by different parts of the sample
- Some electrons are absorbed
  - "Amplitude contrast", depends on thickness and specimen composition
- Some electrons are scattered over small angles
  - "Phase contrast", depends on specimen composition
- In crystalline samples electrons are scattered in preferential directions
  - "Diffraction contrast", depends on structure of the specimen
- Larger atomic number (Z) elements scatter and absorb more electrons than elements with lower Z
  - In general terms higher-Z ("heavy") elements look darker in bright-field TEM images than lighter elements (and look brighter in dark-field TEM)

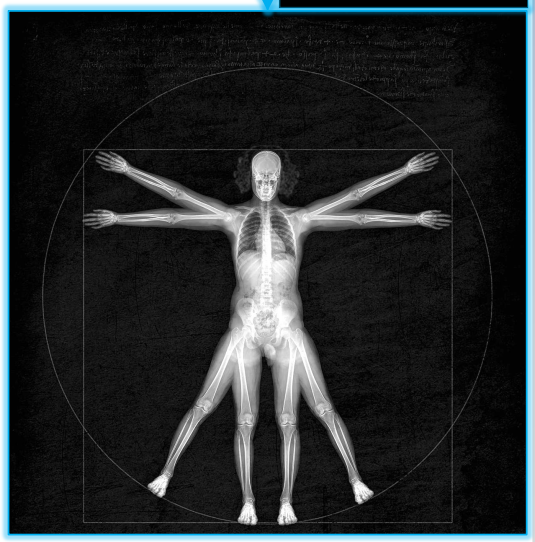


Bright Field TEM: image of direct beam (transmitted electrons)



Dark Field TEM: image of electrons scattered as they go through the sample





Bright Field TEM **Dark Field TEM**

Electron-Specimen Interactions in TEM (2)

- Other signals are generated (same as in SEM):
  - Characteristic X-rays (used for EDS/WDS analysis)
  - BSE and SE (not used in TEM)
  - Auger Electrons
  - Cathodoluminescence

- Some electrons lose a specific amount of energy due to interaction with the specimen
  - Electron Energy Loss Spectroscopy
  - Energy Filtered Imaging
  - *Note: These techniques are explained further below*

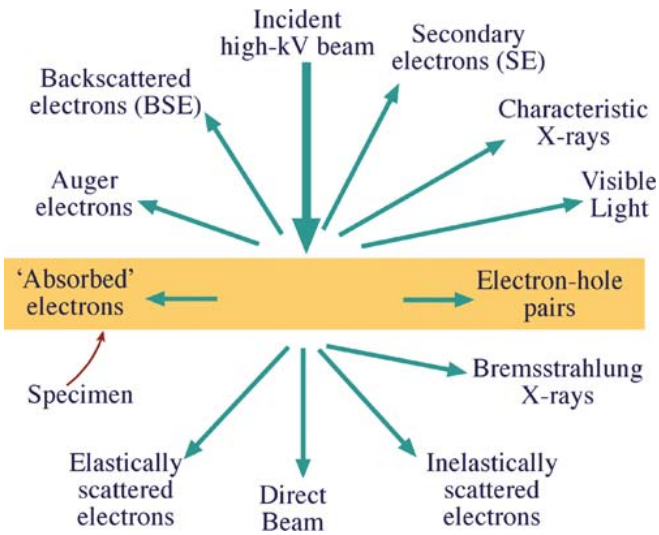
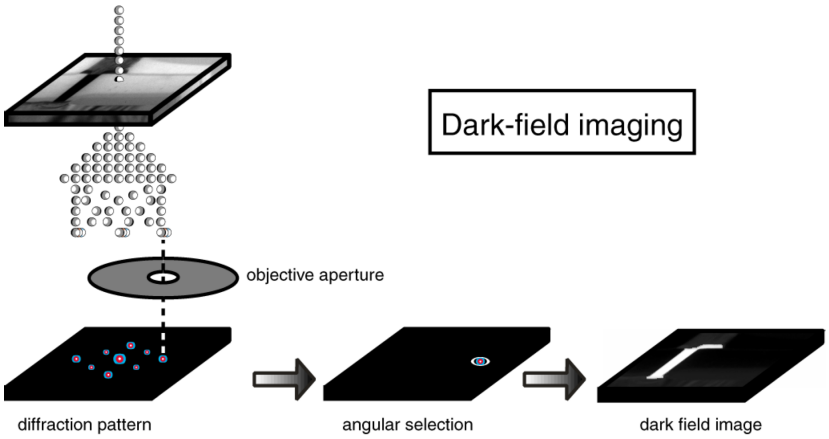


FIGURE 1.3. Signals generated when a high-energy beam of electrons interacts with a thin specimen. Most of these signals can be detected in different types of TEM. The directions shown for each signal do not always represent the physical direction of the signal, but indicate, in a relative manner, where the signal is strongest or where it is detected.

Figure from: David B. Williams, C. Barry Carter "Transmission Electron Microscopy: A Textbook for Materials Science" 2nd. ed. Springer, New York, USA, 2009

Imaging in TEM

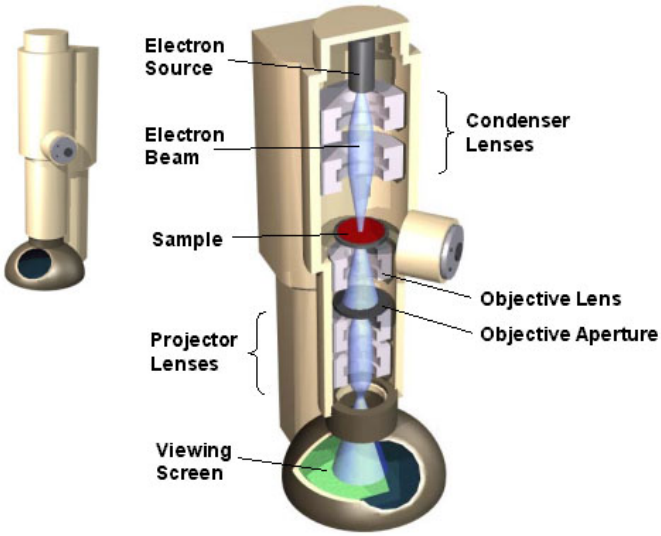
- For crystalline samples, phase and diffraction contrasts are most important for image formation
- For most non-crystalline specimens, amplitude and phase contrasts contribute most to the final image
- In addition to the lenses, several apertures can be used to remove electrons that will not be used in imaging
  - E.g. remove scattered electrons, or select only the scattered ones for dark field imaging
  - Different apertures can be placed in different lenses



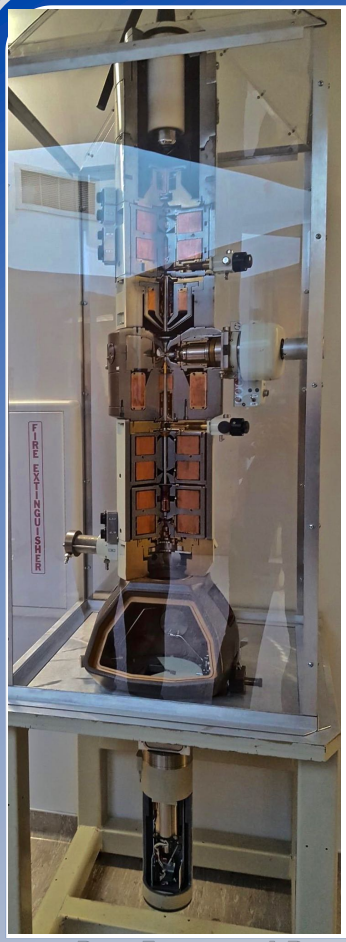
Imaging in TEM

- Bright Field Imaging uses the transmitted beam directly
- In all types of imaging the electrons are projected in a fluorescent screen forming the image
  - A smaller fluorescent screen together with a binocular microscope can be used for fine adjustment (focusing screen)
  - Screen is raised to take picture with a camera below

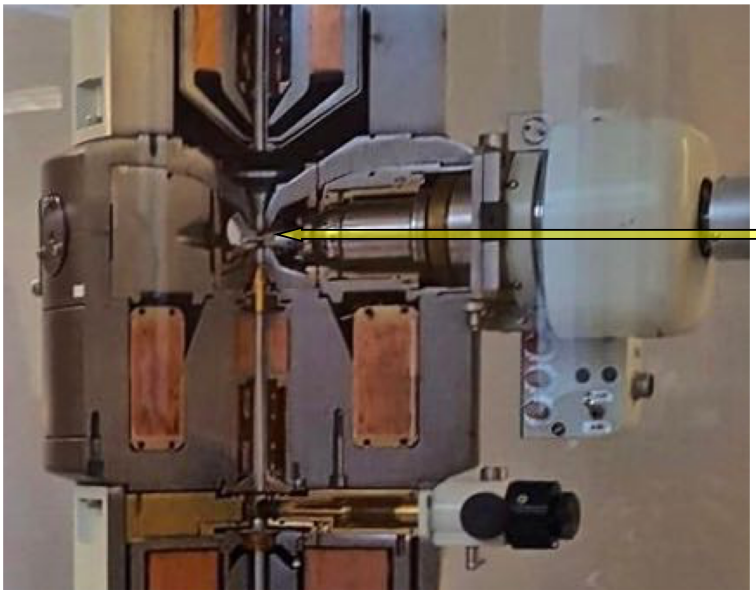
- Sample observation is mostly done on a digital camera in most modern microscopes
- Samples are inserted in a special chamber that is evacuated before opening the chamber to the column and inserting the sample in the beam path
  - Column pressure is $\sim\mathbf{10^{-5}}$ **Pa** ($\sim 10^{-7}$ torr)



TEM schematic from: http://barrett-group.mcgill.ca/tutorials/nanotechnology/nano02.htm



Cross section of an actual TEM



Sample grid would go here

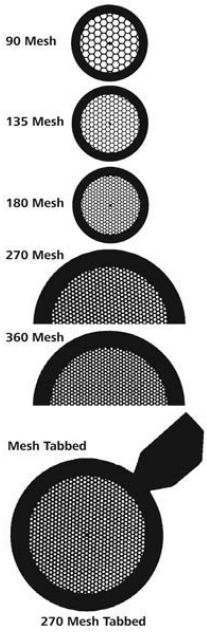
(TEM located at University of Minnesota)

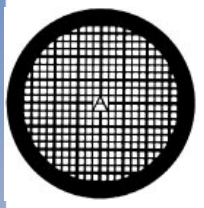
Photos courtesy of Prof. Karen Lozano,

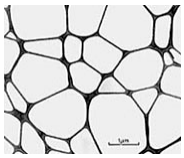
University of Texas, Rio Grande Valley `

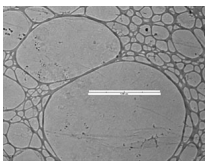
TEM support grids

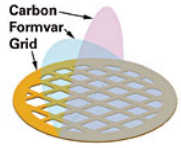
- Almost all samples are deposited over a 3.05 mm diameter grid made of copper (can be Ni or other metals or alloys)
  - Instead of square grids, different hole shapes are possible
- Support films can be used (formvar or carbon)
  - Formvar: vinyl formaldehyde polymer with PVA and PVAc
    - PVA= poly(vinyl alcohol); PVAc=poly(vinyl acetate)
    - Polymer films can be tens of nanometers thick, carbon films may be as thin as 3–4 nm
  - For nanomaterials, even if support film is very thin and amorphous, it can interfere with imaging
- Lacey-carbon or holey-carbon films are more suitable for nanostructure observations
- Graphene and few-layer graphene support films are now commercially available

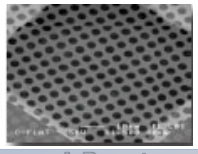


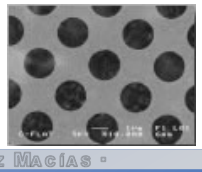


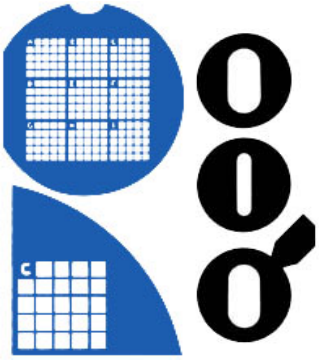


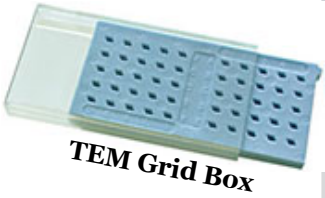










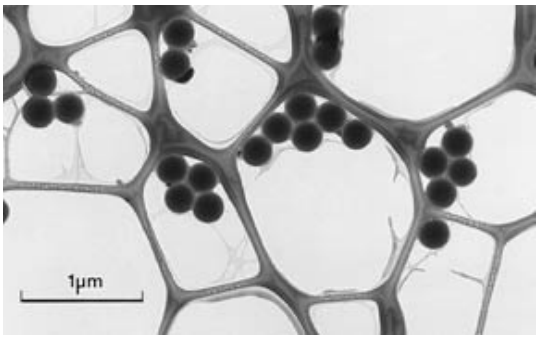


TEM Grid Box

Images from:
http://www.tedpella.com/

Sample Preparation for TEM

- Sample preparation is specially important in TEM to ensure that ”thin enough” samples can be observed
  - Thin enough is usually ≤100 nm, but for large atomic number elements a thinner sample may be preferable, and for lighter elements thicker samples may be used
  - The general recommendation is sample thickness between 20 and 150 nm
  - Consulting someone experienced may be advisable
- For nanomaterials in the form of particles (or aggregates even of micrometer size) preparation is simple: disperse the material in a volatile solvent, place a few drops over a TEM grid, dry, and should be ready
  - A proper support is needed, if atomic resolution is desired lacey or holey carbon is better to allow observation of samples without interference of the grid support material



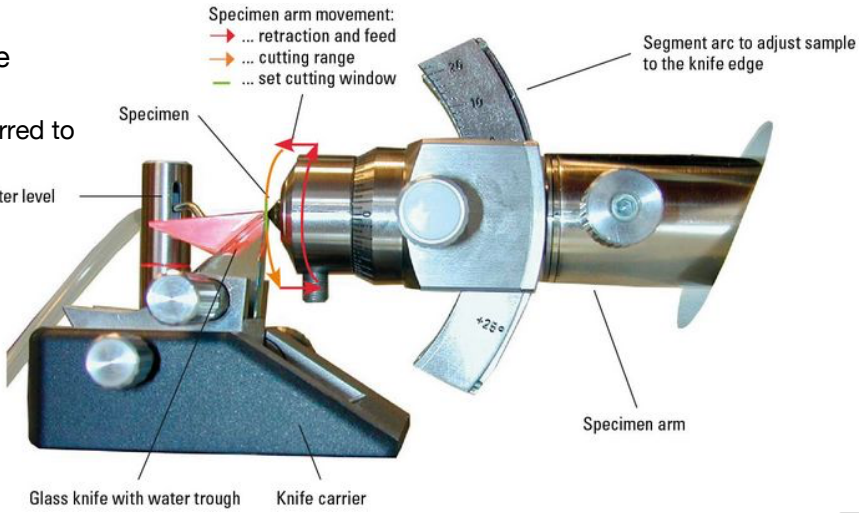
0.26μm dia. Latex on a
Lacey Carbon Support Film

Image from:
http://www.tedpella.com/Support_Films_html/Support_Films_and_Substrates_Overview.htm

TEM Samples: Ultramicrotomy

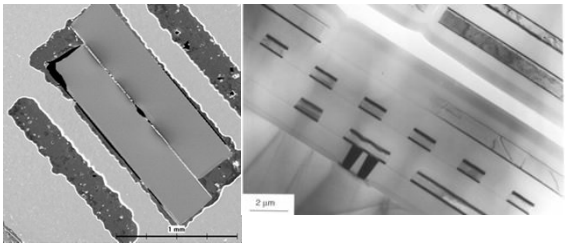
- For large samples thin cuts can be made with an ultramicrotome
  - A cryoultramicrotome is preferable for soft materials to avoid plastic distortion
  - Thin film can be mounted over copper grid without support film
  - Diamond or glass (freshly cleaved) blade can be used depending on sample hardness
  - Slices are collected on the surface of water
    - From there they are transferred to TEM grid
  - Some samples may need to be embedded in a support matrix (e.g. epoxy resin) before cutting

Video showing the use of an Ultramicrotome: https://youtu.be/es1sOxMPELo



Sample Preparation for TEM

- For harder, monolithic, materials mechanical grinding can be used, followed by ion beam milling (sputtering) to create thin enough sections
  - Different parameters may need to be adjusted (e.g. incidence angle of the ion beam, acceleration voltage)
    - Example image: cross-section of a semiconductor structure, sample was mechanically polished and then ion beam milled



Images from: https://www.leica-microsystems.com/science-lab/cross-sectional-preparation-of-structured-semiconductor-materials-for-tem/

- For soft materials that do not show natural contrast in the TEM "staining" may be necessary
  - Organic samples and polymers may be exposed to RuO_4 or OsO_4 vapors which react with several functional groups (e.g. double bonds, amines) providing atomic number contrast
  - Biological samples may be treated with uranyl acetate [$UO_2(CH_3COO)_2$], sodium phosphotungstate (or Phosphotungstic Acid, $H_3PW_{12}O_{40}$), lead citrate or other salts of heavy metals

- Other preparation techniques may be necessary depending on samples
  - In specific cases a FIB may be the best option to cut a thin film from a sample

Selected Area Electron Diffraction (SAED) in TEM

- Samples can diffract electrons from primary beam
- If the sample has a repeating structure constructive and destructive interference will form a diffraction pattern (DP)
  - In the image distance from center of the diffracted beam corresponds to the angle of scatter at the specimen
- Crystal structure can be determined from diffraction pattern
  - Amorphous materials do not show a defined pattern (A)
  - Monocrystals produce a diffraction pattern of spots (B)
  - Polycrystaline materials produce a pattern of circles with a spacing dependent on separation between crystal planes (C)
  - Convergent Beam Electron Diffraction (D) gives more information than SAED

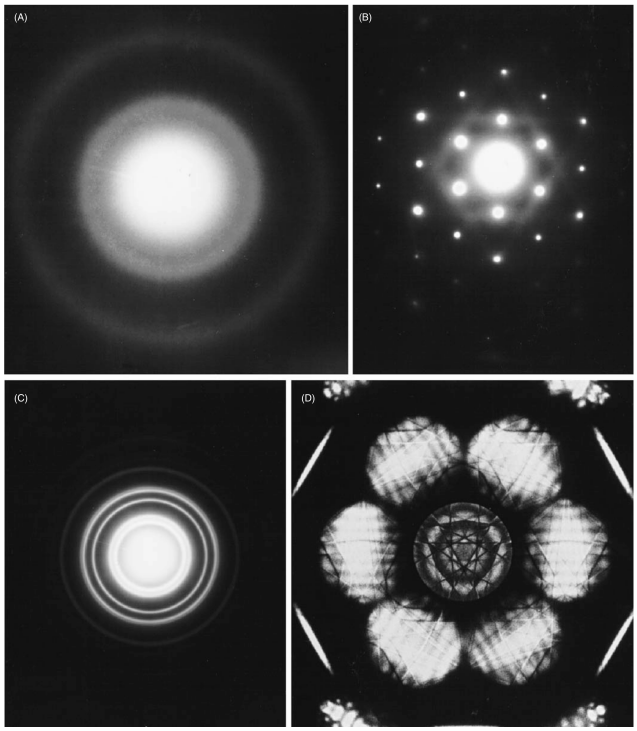
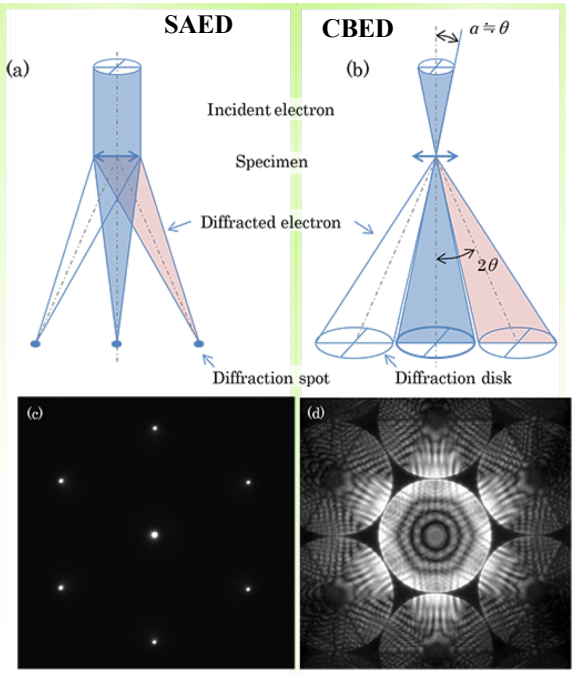


FIGURE 2.13. Several kinds of DPs obtained from a range of materials in a conventional 100-kV TEM: (A) amorphous carbon, (B) an Al single crystal, (C) polycrystalline Au, (D) Si illuminated with a convergent beam of electrons. In all cases the direct beam of electrons is responsible for the bright intensity at the center of the pattern and the scattered beams account for the spots or rings that appear around the direct beam.

Figure from: D.B. Williams, C.B. Carter "Transmission Electron Microscopy: A Textbook for Materials Science" 2nd. ed. Springer, New York, USA, 2009

Convergent Beam Electron Diffraction



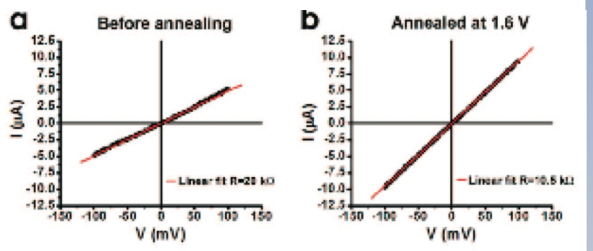
- Uses a cone-shaped electron beam focused on a small specimen area (≤10 nm)
- Disk diffraction pattern formed is magnified with imaging lenses and projected on the screen
  - In SAED spot diffraction pattern is magnified and projected
- CBED provides information on:
  - Specimen thickness
  - Lattice parameter
  - Crystal symmetry (point group and space group)
  - Characteristics of lattice defects
  - Crystal structure

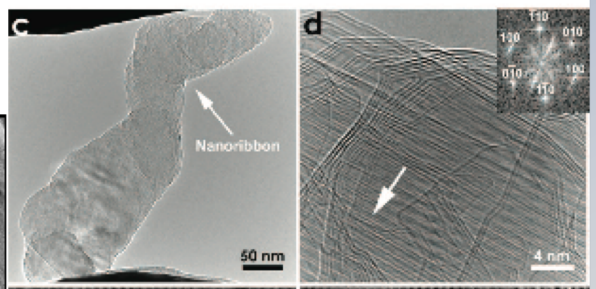
Comparison of SAED and CBED patterns for Si[111]. Both taken with an accelerating voltage of 200 kV. For the CBED pattern only elastically scattered electrons are used to form the image (zero-loss pattern) and inelastically scattered electrons are filtered out.

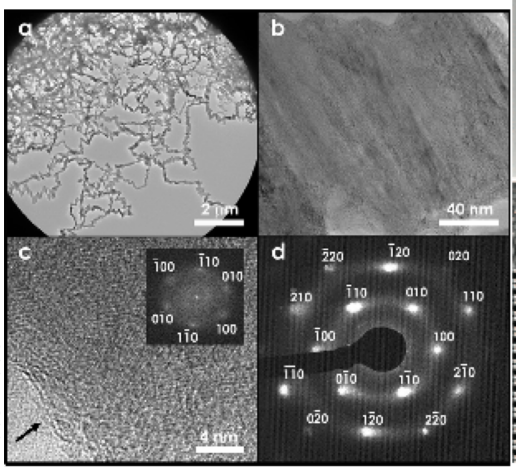
Images © JEOL Ltd. Downloaded April 2019 from the JEOL TEM Glossary pages
https://www.jeol.co.jp/en/words/emterms/search_result.html?keyword=convergent-beam%20electron%20diffraction

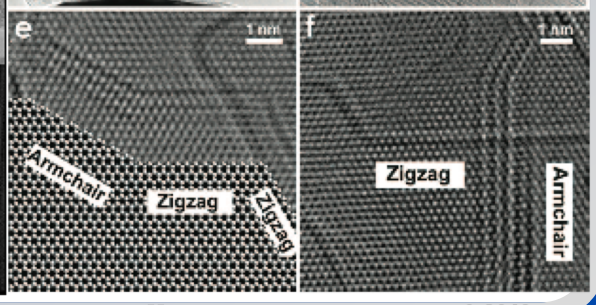
HRTEM+SAED Example: GNR by CVD

- Multi-layered Graphene Nanoribbons (GNR, thickness ≲ 10 nm) produced by chemical vapor deposition (CVD)
- Graphene Sheets show graphitic ordering
  - SAED shows graphitic diffraction pattern
- Heating increases order on edges
  - Joule heating while measuring resistance
  - High temperature treatments also result in fusion of edges
- HRTEM was essential for seeing these structures with atomic resolution





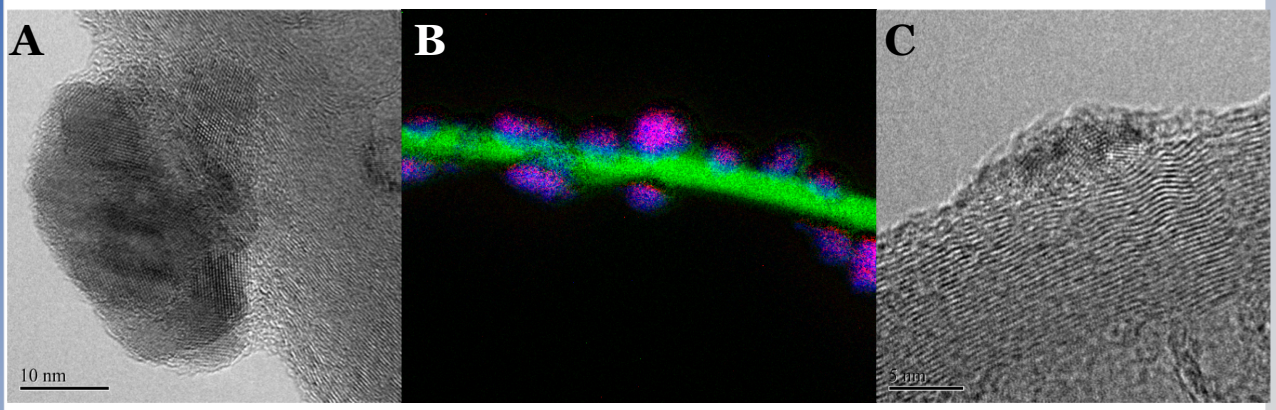




Figures taken from: J. Campos-Delgado, et al. "*Bulk Production of a New Form of sp² Carbon: Crystalline Graphene Nanoribbons*" Nano Letters **8** (2008) 2773–2778

HRTEM+EDS Example: CdSe-CN_x Composites

- HRTEM images of CdSe-CN_x showing CN_x graphitic planes and CdSe nanoparticle crystal planes. Good interface and adhesion appear to exist with both water (**A**) and methanol (**C**) as reaction solvents.
- Elemental mapping by EDX confirms that nanoparticles are composed of CdSe which deposits *in* situ over the CN_x (**B**, sample prepared with water as solvent) [green: C, red: Cd, blue: Se]

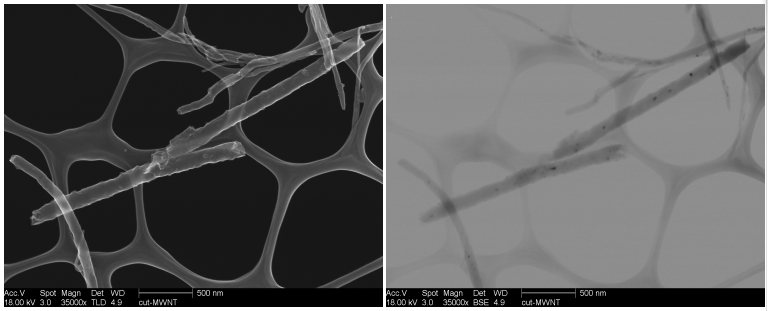


Y. Panécatl-Bernal, J.C. García-Gallegos, F.J. Rodríguez-Macías, *Efficient Anchoring of Nanostructured Cadmium Selenide on Nitrogen Doped Carbon Nanotubes*; IX Brazilian MRS Meeting (IX Encontro da SBPMat), Ouro Preto, MG, Brasil, 27–28 Octubre de 2010

Manuscript in Preparation: Y. Panecatl-Bernal, J. Alvarado, R. Lima-Juárez, M. Chávez-Portillo, M.A. Méndez-Rojas, J.C. García-Gallegos, F.J. Rodríguez-Macías, *"Efficient Anchoring of Nanostructured Cadmium Selenide with Different Carbon Nanotubes Types"*

SEM+TEM EXAMPLE: Chemical Modification of Carbon Nanotubes

- CNT functionalized by the "nanotube salts" method
  - Method can be used for *in situ* polymerization with several polymers
- SEM showed indications of partial opening as a "side effect"
  - Opening increased by exfoliation processes after intercalation
  - Under optimized conditions there is full opening ("unzipping") of carbon nanotubes to form graphitic nanoribbons
- STEM in SEM allowed to distinguish clearly between surface damage (observed by SEM) and nanotube "unzipping"
- HRTEM allowed to confirm unzipping and to characterize the graphitic nanoribbons
- First report that showed that carbon nanotubes could be "unrolled" into nanoribbons
  - Online publication Date: March 4, 2009



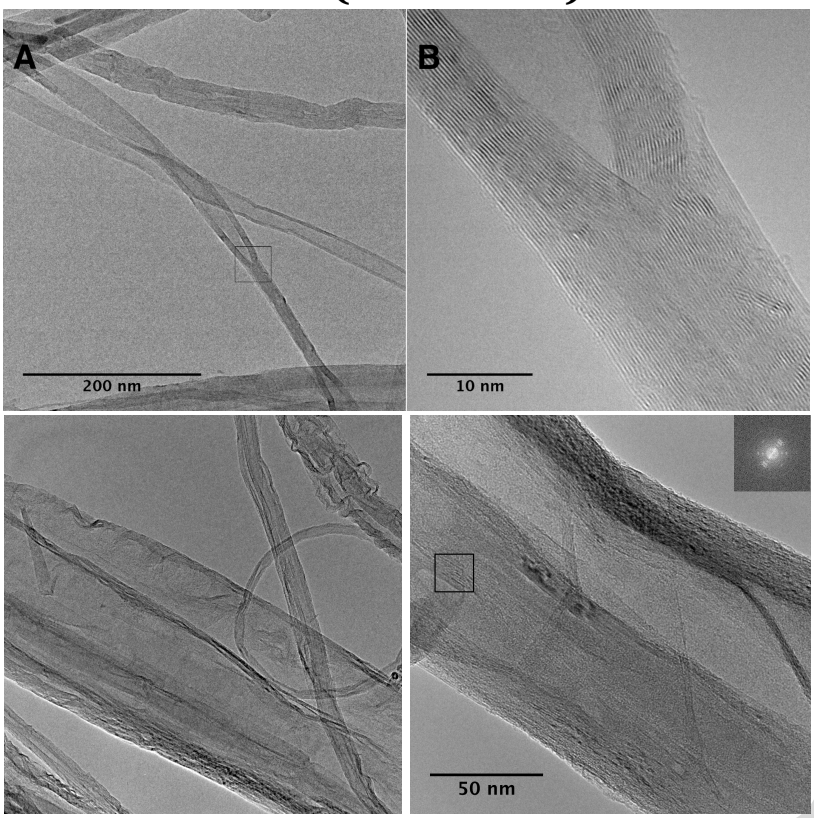
CNT with surface damage due to oxyacid cutting treatment

CNT opened longitudinally by an intercalation-exfoliation process ("exMWNT")

A.G. Cano-Márquez; F.J. Rodríguez-Macías; J. Campos-Delgado; C.G. Espinosa-González; F. Tristán-López; D. Ramírez-González; D.A. Cullen; D.J. Smith; Mauricio Terrones; Y.I. Vega-Cantú; "Ex-MWNTs: Graphene Sheets and Ribbons Produced by Lithium Intercalation and Exfoliation of Carbon Nanotubes", *Nano Letters,* 9(4), 1527-1533 (2009). DOI: 10.1021/nl803585s

TEM Example: Graphitic Nanoribbons by "unzipping" Multi-walled Carbon Nanotubes (exMWNT)

- TEM complements SEM
  - Observation of structures with higher resolution
  - Verification of nanotube opening and better understanding of intermediate and final structures
  - Observations on TEM suggested that the opening started due to intercalation of a lithium-ammonia complex
- TEM Images capture additional information
  - Fast Fourier transform (FFT) signal processing converts periodic variation of brightness due to periodic arrangement of atoms into a "diffraction" pattern



A.G. Cano-Márquez; F.J. Rodríguez-Macías; et al., *Nano Letters* **9** (2009) 1527-1533
Publication Date (Web): March 4, 2009,
DOI: 10.1021/nl803585s
Copyright © 2009 American Chemical Society

Example: Nanostructured Phase Separation of Block Copolymers

- J. T. Chen, E. L. Thomas, C. K. Ober, G.-p. Mao. "Self-Assembled Smectic Phases in Rod-Coil Block Copolymers". *Science*, 1996, **273**, :343-347
  - Poly(hexyl isocianate-*co*-polystyrene). P(HIC) forms "rods", Polystyrene (PS) forms "coils"
  - PS reacts with RuO_4 and those zones look darker

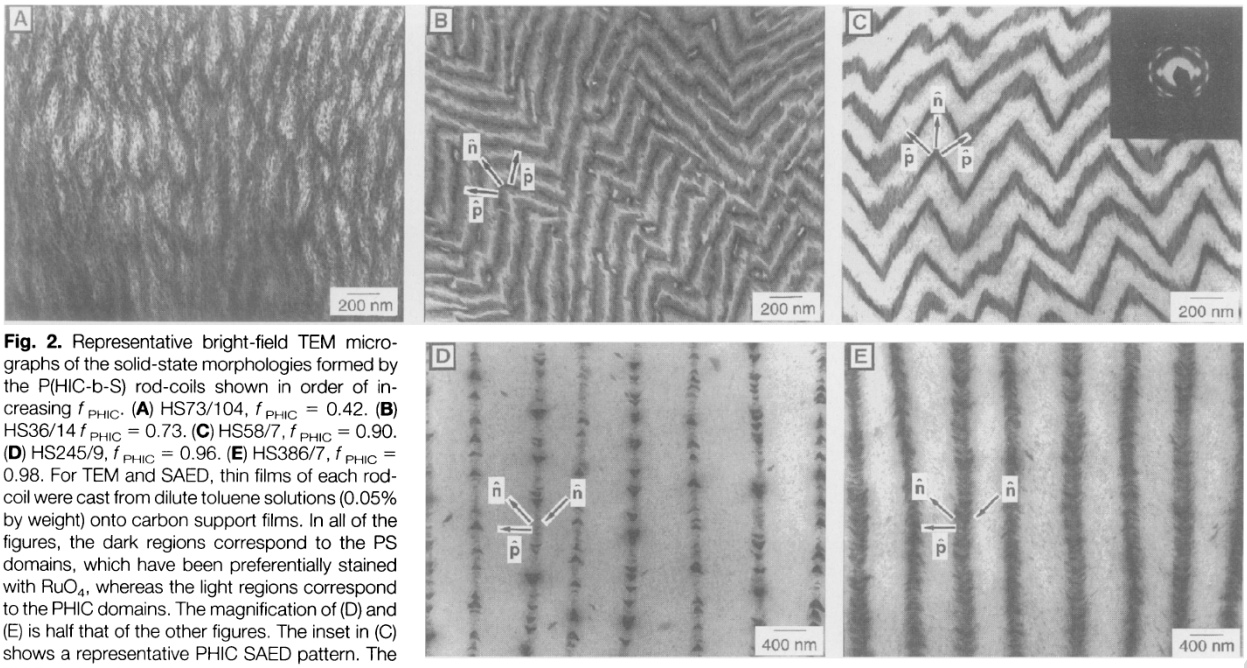
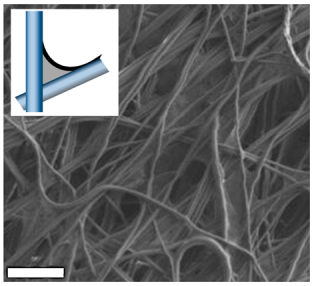
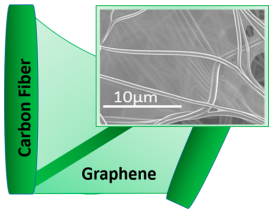


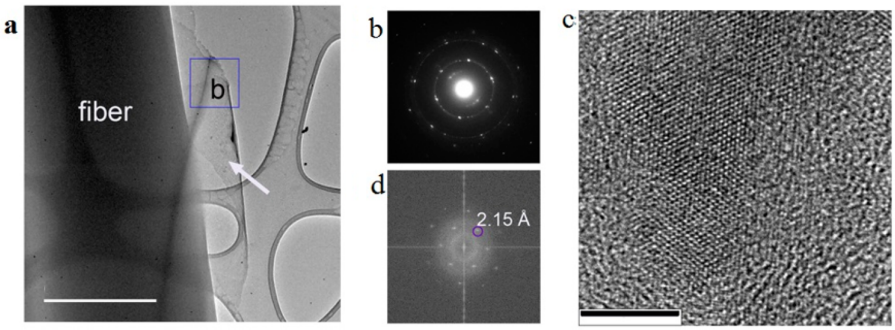
Fig. 2. Representative bright-field TEM micrographs of the solid-state morphologies formed by the P(HIC-b-S) rod-coils shown in order of increasing f_{PHIC}. (**A**) HS73/104, f_{PHIC} = 0.42. (**B**) HS36/14 f_{PHIC} = 0.73. (**C**) HS58/7, f_{PHIC} = 0.90. (**D**) HS245/9, f_{PHIC} = 0.96. (**E**) HS386/7, f_{PHIC} = 0.98. For TEM and SAED, thin films of each rod-coil were cast from dilute toluene solutions (0.05% by weight) onto carbon support films. In all of the figures, the dark regions correspond to the PS domains, which have been preferentially stained with RuO_4, whereas the light regions correspond to the PHIC domains. The magnification of (D) and (E) is half that of the other figures. The inset in (C) shows a representative PHIC SAED pattern. The PHIC chain axis and lamellar normals are denoted by $\hat{\mathbf{n}}$ and $\hat{\mathbf{p}}$ in (B) through (E), respectively.

Example: TEM of Graphene-Carbon Nanofiber Hybrids



- Polymer fibers by ForceSpinning produced with polymer membranes in between fibers
  - Use of salt in the spinning solution, and acid vapor dehydration treatment, with high relative humidity
- Pyrolysis treatment transforms those “veils” into graphene sheets
- TEM and SAED show that the membranes are graphene



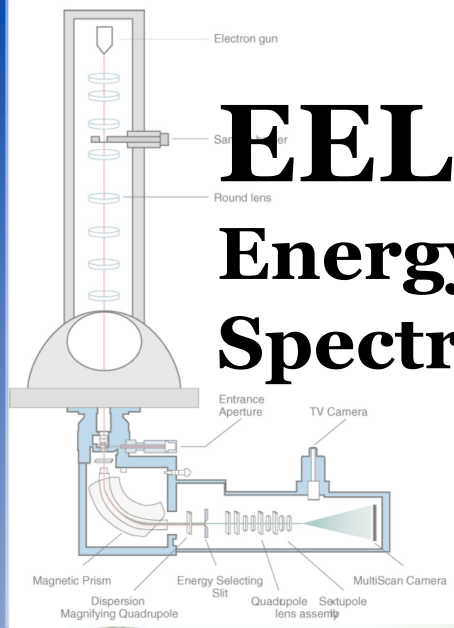


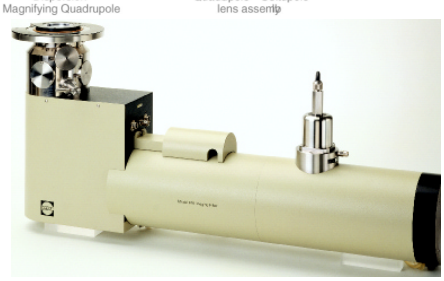
(a) TEM image of the hybrid structure. The white arrow shows a transparent graphene layer attached to a fiber. Scale bar, 500 nm. (b) SAED pattern from the square area marked in a. (c) A typical HRTEM image of the graphene layer, with a clear two-dimensional lattice showing the high crystallinity of the graphene sheets. Scale bar, 5 nm. (d) The corresponding FFT pattern of c.

Taken from M. Akia, L. Cremar, M. Chipara, E. Munoz, H. Cortez, H. De Santiago, F.J. Rodríguez-Macías, Y.I. Vega-Cantú, H. Arandiyan, H. Sun, T.P. Lodge, Y. Mao, K. Lozano, “In Situ Production of Graphene-Fiber Hybrid Structures.” ACS Applied Materials and Interfaces, 9(30), 25474-25480 (2017) DOI: 10.1021/acsami.7b07509 // © American Chemical Society

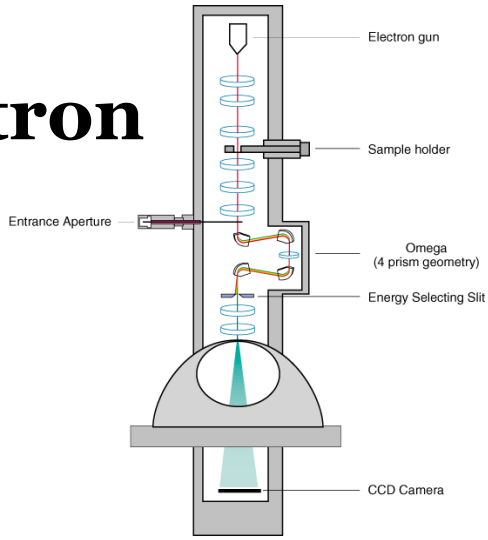
EELS: Electron Energy Loss Spectroscopy

Post-column filter (Gatan)





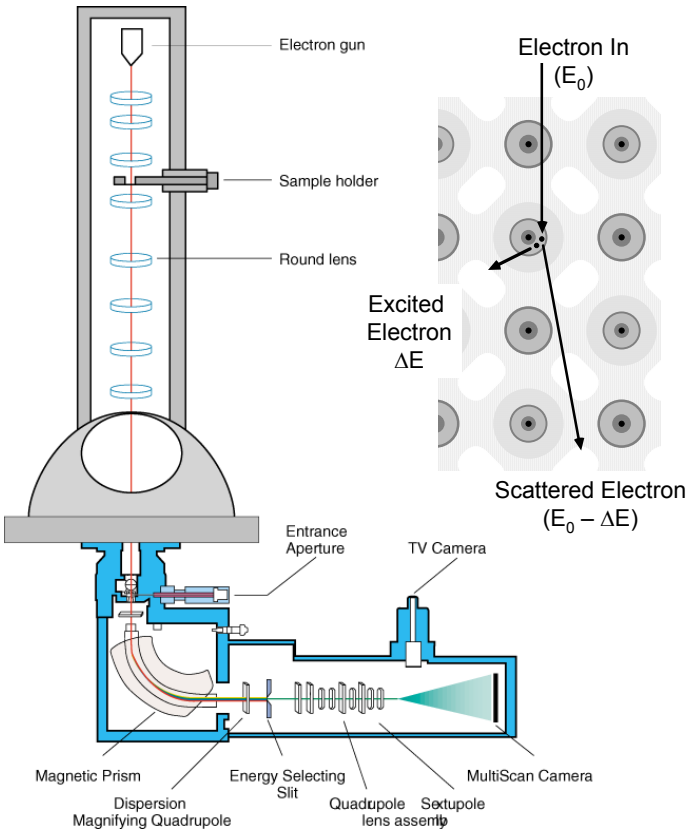
In-column filter (LEO, JEOL)



An optional technique for TEM that allows elemental and chemical information to be extracted from the electron beam interaction with the sample

The same detector can be used for Energy Filtered Imaging in TEM (EFTEM) which can provide some advantages in image clarity or in mapping composition

EELS: Basic Principles

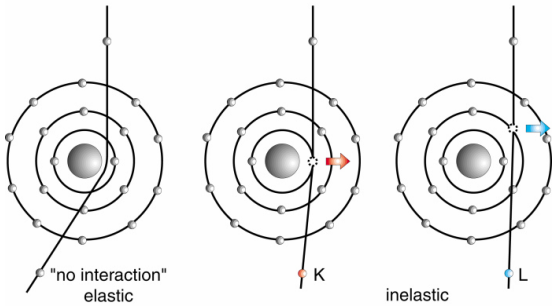


- Electrons can loose energy by inelastic scattering
- Loss of energy depends on the electron cloud of the atoms with which the beam electrons interact
  - Sensitive to the element and its oxidation or bonding state
  - Incident electron can lose a specific amount of energy by giving it to a K or L electron which then "jumps" out of the atom
  - Similarly, it can loose energy by exciting an electron to a higher energy level in the atom
- A series of magnetic and electric components separate electrons by energy and record number of electrons at different energies

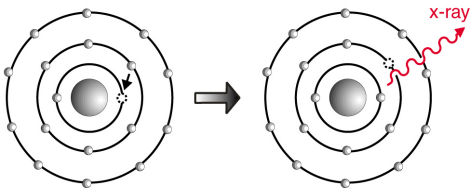
EELS vs. X-ray signal generation

- Signal acquisition for EELS is faster, has better energy resolution, and is more effective for low Z elements than EDX
- But the hardware for detection and the software for data analysis are much more complicated

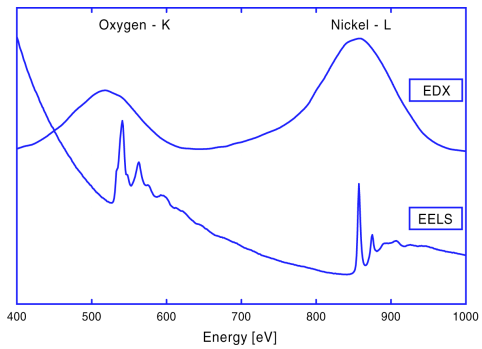
EELS signal generation



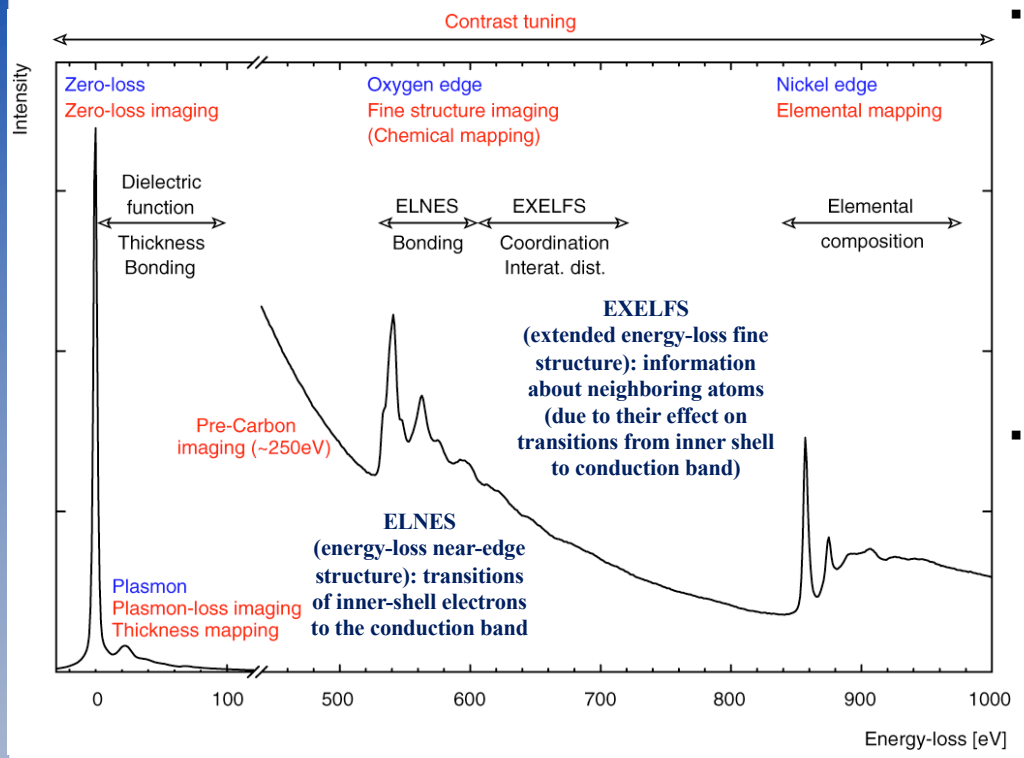
x-ray signal generation



x-ray and EELS spectra



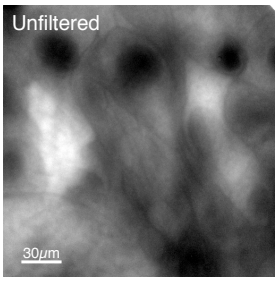
Different Energy-Loss Ranges are used for different purposes

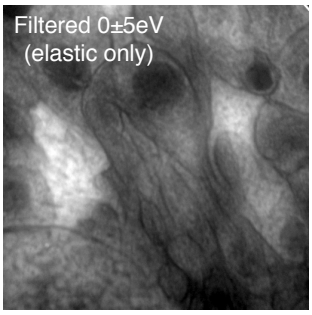


- EELS shows peaks at edges of ionization energy for elements
  - These appear above 100 eV
  - Peaks correspond to interaction with tightly bound (core) electrons
- Low-loss region corresponds to interactions with weakly bound electrons
  - Intensity of low-loss region bands is greater

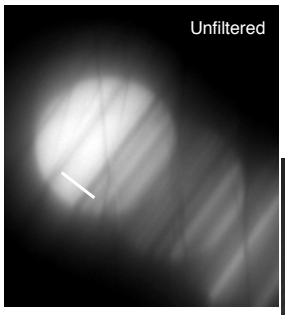
Enhanced Contrast using EFTEM/EELS

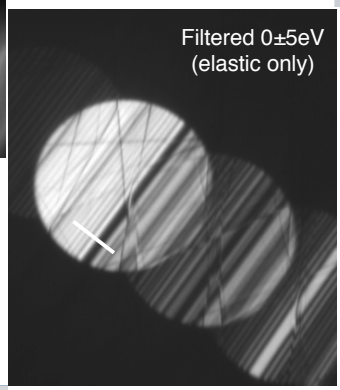
- Inelastically scattered electrons are not properly focused by the objective lens
  - By selecting zero-loss the electrons that are out of focus due to this chromatic aberration are removed from the image





Unstained/osmicated cerebellar cortex (thickness=0.4 μm, 100 keV)

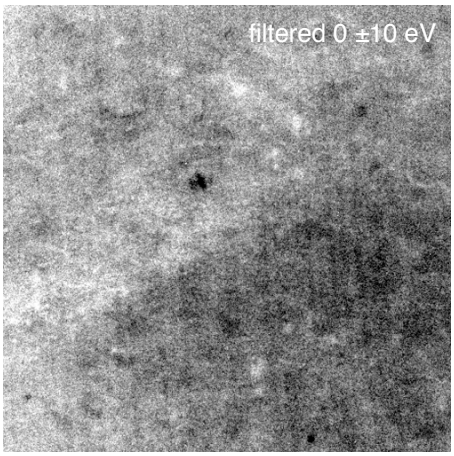


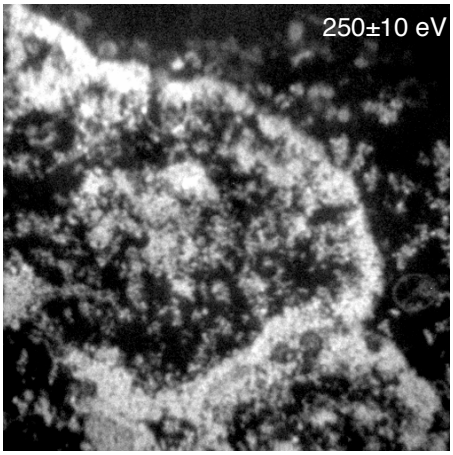


Si (100)

Enhanced Contrast using Pre-Carbon Filtering with EFTEM/EELS

- Removing energies right in front of the carbon K-edge allows signals of other elements to be seen more clearly by removing most of the carbon signal
  - Can be very useful in biological and organic samples

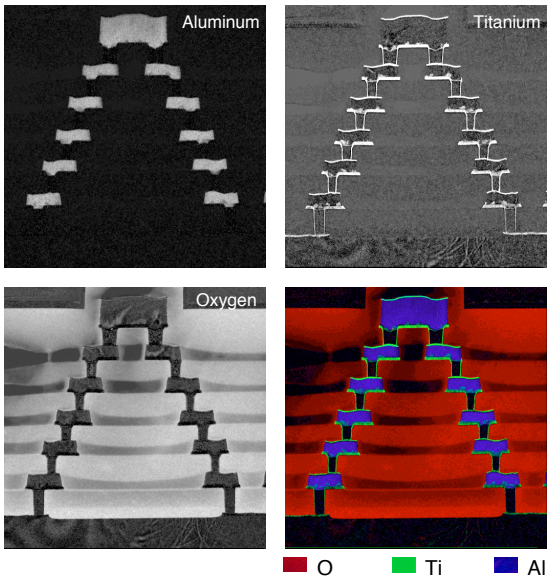




Pre-carbon K-edge energy-filtered image of a cell nucleus

- Energy Filtering in other energy ranges, depending on sample composition, can also be used to enhance contrast

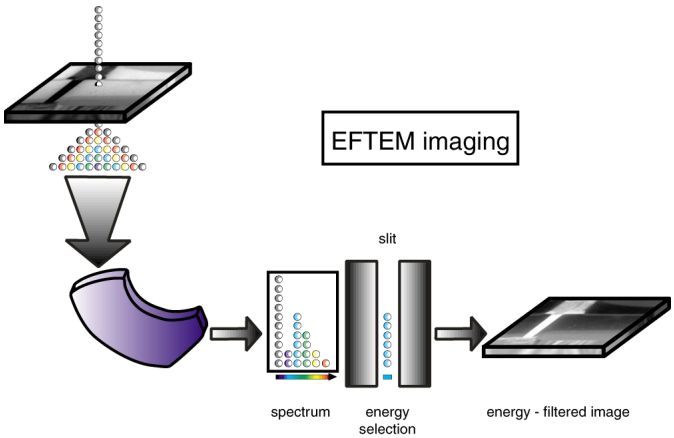
EFTEM: Energy Filtered TEM



O K edge: @ 532 eV
Ti L_{23} edge: @ 455 eV
Al K edge: @ 1560 eV

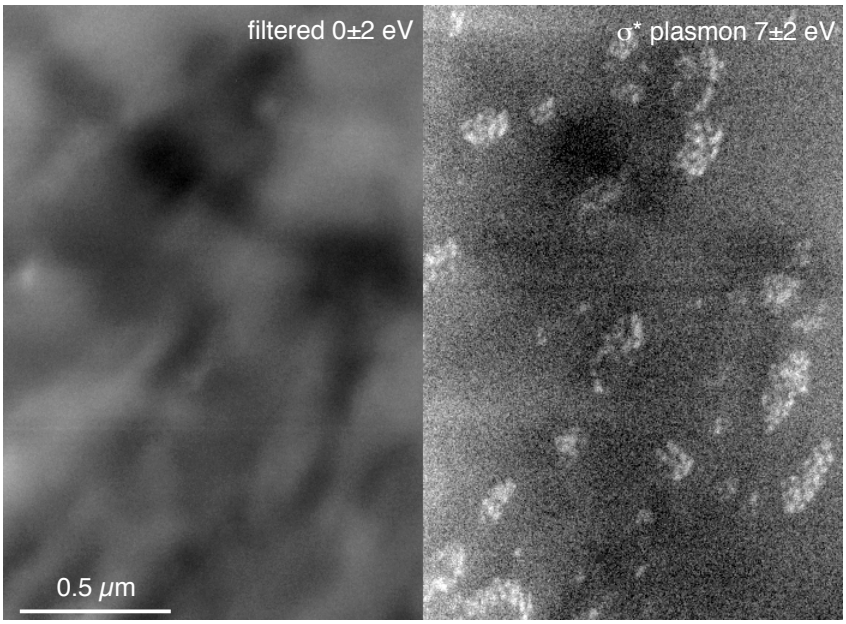
Images of a test structure for metallization in SiO_2/Si wafers

- Imaging using differences in energy of transmitted electrons by using an EELS detector
  - A color can be assigned to each energy loss and a false color image created
- **Elemental Mapping**



Chemical Mapping with EELS

- Some chemical species show spectral features that are specific to a type of chemical bond
  - E.g. EELS spectrum can be used to prove the presence of sp^2 vs. sp^3 carbon



- Example on the left: polystyrene (C-C double bonding) particles (unstained) in a polyethylene matrix
- Shows a small peak between zero-loss plasmon (ZLP) and a plasmon specific to polystyrene at 7 eV

Cryo-Electron Microscopy

- Imaging of frozen soft-matter, non-crystalline, samples in TEM
- Averaging of images of many copies of a biomacromolecule is used to reconstruct a 3D model of their shape
  - Has been widely used to find the 3D shape of proteins that cannot be crystalized for XRD analysis
- Nobel Prize in Chemistry 2017 to Jacques Dubochet, Joachim Frank and Richard Henderson "*for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution.*"

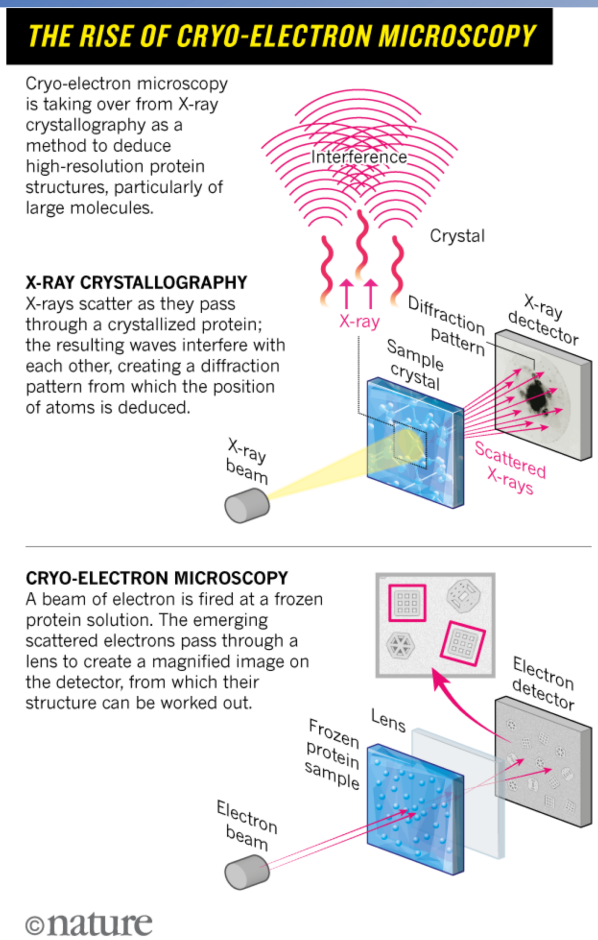
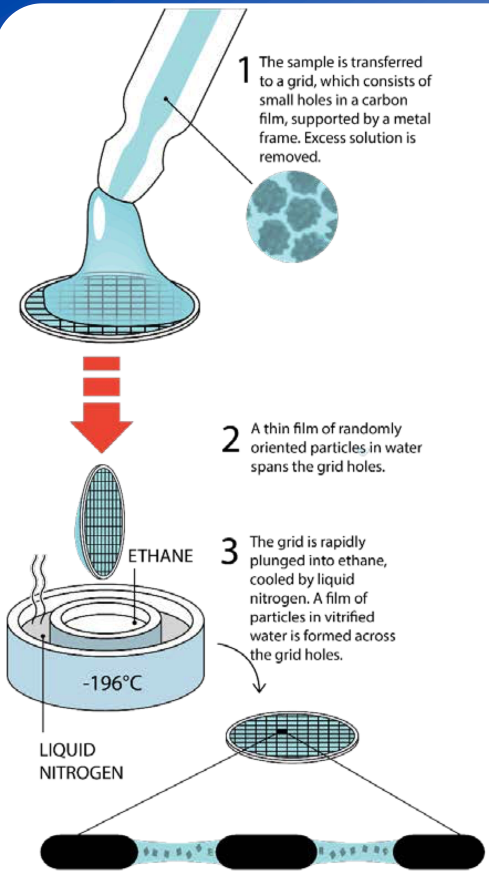


Figure downloaded April 2019 from: https://www.nature.com/news/cryo-electron-microscopy-wins-chemistry-nobel-1.22738

Sample Preparation for Cryo-EM



- Samples are in aqueous solution and frozen rapidly so that water does not form crystals
- Vitreous water provides a random background
  - Image of water should average out as noise during data processing
- Particles (e.g. proteins, viruses) frozen in place in random orientations

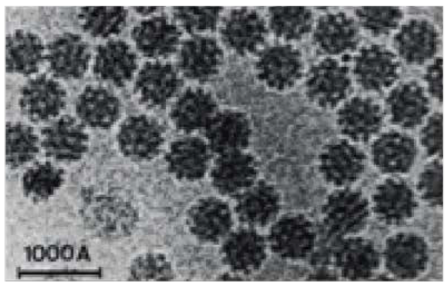


Fig.8. Sample-preparation procedure for cryo-EM. Illustration: © Johan Jarnestad/The Royal Swedish Academy of Sciences. The image of a Semliki Forest virus suspension at the bottom is from (57).

Figures from: The Royal Swedish Academy of Sciences "Scientific Background: The development of cryo-electron microscopy" NobelPrize.org. Nobel Media AB 2019. Downloaded Apr. 2019. <https://www.nobelprize.org/prizes/chemistry/2017/advanced-information/>.

Data Acquisition for Cryo-EM

- A series of images are acquired tilting the sample
  - Tomographic conical tilt series: 2D images correspond to the "shadow" of the shape of the molecule as projected in a cone
- Low energy electrons used to prevent damage to proteins
  - This makes the signal to noise ratio very low
- High sensitivity electron detectors for TEM were needed for faster acquisition of images with enough resolution and low noise
  - Direct electron detectors, with better signal to noise ration and resolution were introduced in 2012-2013
- Devices that improve phase contrast detection were also necessary to advance Cryo-EM
  - Energy filtering removes inelastically scattered electrons
    - Reconstruction of projected images is well-modelled only for elastically scattered electrons
  - Phase plate increases contrast by shifting the phase between scattered and un-scattered electrons
    - Analogous to phase contrast in optical microscopy

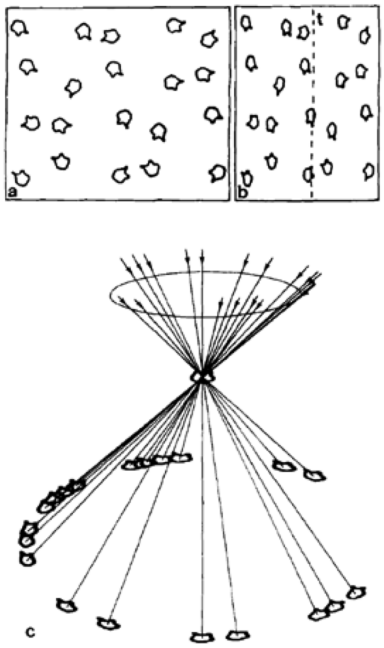


Fig. 6. Random conical reconstruction. (a) Randomly oriented particles; (b) projection of (a), tilted by 50°. The images in (b) form the conical tilt series, illustrated in (c) as a single particle that is randomly projected with all directions lying on the surface of a cone. Image from (47).

Image from: The Royal Swedish Academy of Sciences "Scientific Background: The development of cryo-electron microscopy" NobelPrize.org. Nobel Media AB 2019. Downloaded Apr. 2019. <https://www.nobelprize.org/prizes/chemistry/2017/advanced-information/>.

Data Processing for Cryo-EM of Proteins

- Thousands of images of 2D projections of the shapes of molecules are analyzed with specialized software
  - Data is naturally noisy due to background of water-ice
- Automated software has been very important for Cryo-EM
  - Reconstruction of the shape of a protein can require thousands of images of individual particles
  - Different particles are assumed to have the same shape for the reconstruction
- **Data interpretation is complicated**
  - *For examples of the problems of data interpretation read: Monya Baker* "Cryo-electron microscopy shapes up" *Nature* **561** (2018) 565-567 https://www.nature.com/articles/d41586-018-06791-6

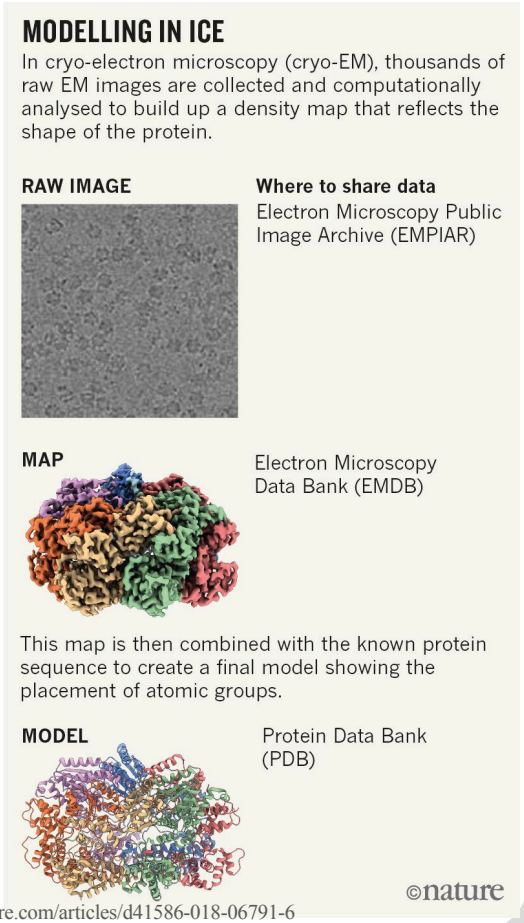
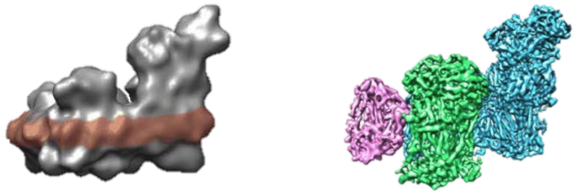


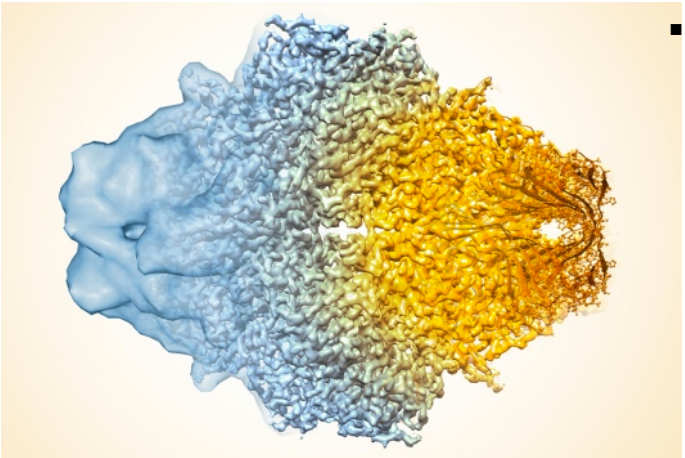
Image © Nature, downladed April 2019 from:https://www.nature.com/articles/d41586-018-06791-6

Resolution of Cryo-EM

- Resolution in 2011 was ca. 2 nm, by 2016 the resolution of Cryo-EM had improved to ca. 0.6 nm (6 Å)



Comparison of the resolution achiaveble in 2011 and 2016 for models of the electron-transport chain components in a mitochondrial supercomplex $I_1III_2IV_1$ (a supramolecular complex of proteins)



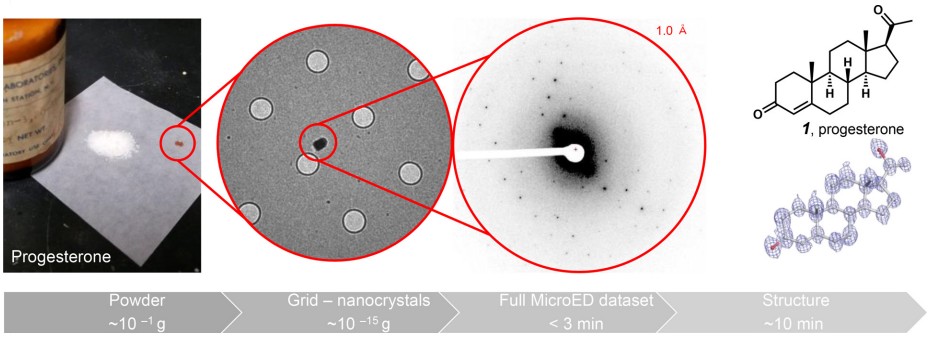
- The progression of resolution of Cryo-EM exemplified by a 3D reconstruction of β-galactosidase enzyme
  - Originally the resolution was just enough for the density map on the left (contour of the whole protein)
  - Advances in imaging and data processing can now allow to assign atomic coordinates as shown on the right hand side
  - Data on the protein sequence is also used for assignment of atom coordinates

Image top right from: The Royal Swedish Academy of Sciences "Scientific Background: The development of cryo-electron microscopy" NobelPrize.org. Nobel Media AB 2019. Downloaded Apr. 2019. <https://www.nobelprize.org/prizes/chemistry/2017/advanced-information/>. Image on bottom side © Nature, downloaded from https://www.nature.com/news/cryo-electron-microscopy-wins-chemistry-nobel-1.22738

Bonus: MicroED, using TEM for Chemistry.

Determination of Small Molecule Structures by Electron Diffraction in TEM

- Recent reports show that it is possible to find the 3D structures of organic molecules (including pharmaceuticals) by electron diffraction in the TEM
  - See for example: M. Warren, *"Why didn't we think to do this earlier?' Chemists thrilled by speedy atomic structures"*, Nature News, 29th October 2018
  - https://www.nature.com/articles/d41586-018-07213-3
- These studies rely on advances made in developing Cryo-EM techniques, but apply for molecules smaller than those analyzed by Cryo-EM

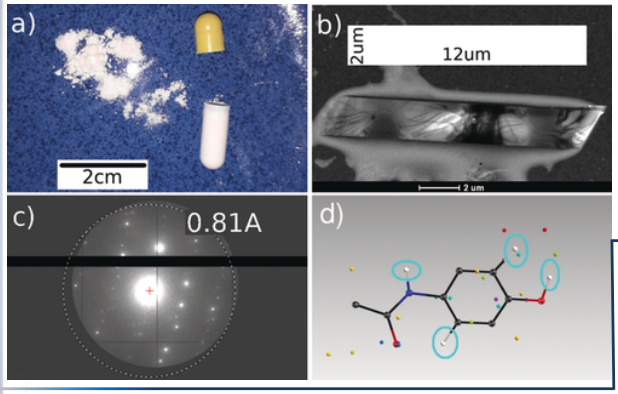


Process of applying MicroED to small molecule structural analysis. Here commercial progesterone (**1**) was analyzed, and an atomic resolution structure was determined at 1 Å resolution. Grid holes are 1 µm in diameter.

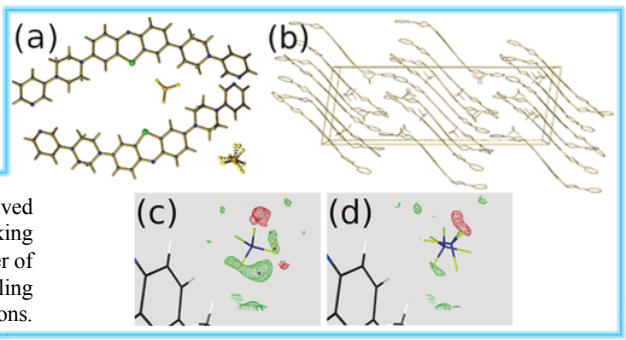
Figure taken from: Christopher G. Jones; Michael W. Martynowycz; Johan Hattne; Tyler J. Fulton; Brian M. Stoltz; Jose A. Rodriguez; Hosea M. Nelson; Tamir Gonen; "The CryoEM Method MicroED as a Powerful Tool for Small Molecule Structure Determination"ACS Central Science **4** (2018) 1587-1592.
DOI: 10.1021/acscentsci.8b00760 Copyright © 2018 American Chemical Society

Bonus: MicroED, using TEM for Chemistry.

- MicroED can be used to find the crystal structure of pharmaceuticals and other organic molecules with only a few microcrystals
  - Traditional X-ray Diffraction requires larger crystals, and typically gram amounts



Single-crystal structure from a powder blend. a) Drugs in powder form (Grippostad , STADA Arzneimittel AG) are usually a microcrystalline blend. b) The powder grains are too small for single-crystal X-ray analysis. The STEM micrograph shows a single crystal from the powder. c) The diffraction intensity is proportional to the crystal volume. Despite the minute volume, this crystal diffracts electrons to atomic resolution. d) High data quality localises some hydrogen atoms (encircled).



Crystal structure of MBBF4 [a methylene blue derivative] solved with electron crystallography. a) Structure of MBBF4. b) Stacking of MBBF4 in the crystal. c) The data quality shows the disorder of a counterion [BF_4^-]. d) The difference map reduces upon modelling of two conformations.

Figures taken from: T. Gruene, et al. "Rapid Structure Determination of Microcrystalline Molecular Compounds Using Electron Diffraction" Angew. Chem. Int. Ed. **57** (2018) 16313-16317
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