3D Electron Microscopy: 
a collection of methods...

History Electron Microscopy....
1933...das “Übermikroskop”....

1936-40: μm.....nm!!!

Modern Electron Microscope....

Tools for Studying the Nano-Cosmos:
Scanning-force & Scanning-tunneling Microscope (SPM)
Field-Ion Microscope (1955 E.W. Müller - first image of an atom!)
X-ray diffraction &-microscope
Ion (He-) Microscope
Scanning Electron Microscopy: SEM

- Surface morphology (length, surface, width, depth, height)
- Element/Chemistry (X-ray, Auger, EBSD)

A virtual image built pixel by pixel is formed!

Transmission electron microscope (TEM)

- Internal morphology (length, surface, width, depth, relation)
- Element/Chemistry (X-ray, EELS, Auger)

Imaging Modes - LM vs. EM: (Light vs. Electron Optics)

- Ernst Abbe: Resolution Power
  \[ d = \frac{\lambda}{2n \sin \alpha} \]

  Angular aperture of the lens - The aperture thus controls the ability of the lens to gather information about the object e.g. the eye at 25 cm corresponding to an angle of about 0.9° for a 4 mm exit pupil diameter of the eye lens; a typical LM with an oil immersion objective lens has 2\(\alpha\) of -175°. For EM typically 8-10mrad (0.5-0.9°)

Obtainable resolution: (Electron vs. Light Optics)

- Angular aperture for EM typically 8-10mrad (0.5-0.9°)
- Magnetic fields not homogeneous!

\[ \lambda_{EM}/\lambda_{LM} = 100'000x \rightarrow \text{Resolution only 1000x better} \]
3D - Beam Transparent: Confocal Imaging -> optical sectioning in Light Microscopy....for EM?

- EM:
  - you need a high convergent beam -> Cs Corr.
  - a “beam transparent” specimen (<50-100nm)
  - high contrast sample....

- z-slice imaging possible for solid state material


=> for all other samples we need other approaches

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EM in Life-Science: Cellular & Molecular....

**Cellular EM**

**Molecular TEM**

Objects “thick” not e-transparent

Objects “thin” e-transparent

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3D Electron Microscopy

**“Thick”**

- not e-transparent
  -> serial section
  real section or “en-bloc”

**“Thin”**

- e-transparent
  -> “Tomography”
  (various angle views...)

=> TEM Tomo

section projections or bloc-face view
  => Image Stack

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3D - Beam Transparent EM

- macromolecular complexes (helices...)
- 2D crystals (protein crystals)
- symmetrical objects (icosahedral viral particle)
- single particle (isolated > 100k Da)
- tomographic reconstruction - tilt series

collect as many view angle as possible - use fourier space maths or tomographic procedure to reconstruct 3D volume
3D - Beam Transparent EM

History of Electron Microscopy and 3D Reconstruction Methods

- 1950s: membrane topology of cellular structures, e.g. mitochondria
- 1950s: Crick, Klug et al. FT of helical structures, selection rules
- 1964: (Parsons and Martius) high resolution electron diffraction on fibers
- 1968: DeRosier and Klug) first 3D structure determination of T4 Bacteriophage tail based on helical reconstruction
- 1970: Crowther et al) first icosahedral viruses
- 1972 (Matthews et al.), 1974 (Taylor and Glaeser), 1975 (Unwin and Henderson): 2D crystals
- 1983 (Kauzer et al): ribosome 3D reconstruction (asymmetric single particle)
- 1990 (Henderson et al): atomic resolution of bacteriophytopin (2D crystal)

W. Wriggers....

References-Helical Reconstruction

- Cochran, Crick, & Vand, 1952 (FT of helix)
- Klug, Crick, & Wyckoff, 1958 (selection rule, n-l plot)
- DeRosier & Klug, 1968 (first ever 3D reconstruction from EM)
- Stewart, 1988 (great review of helical reconstruction technique)
- Moody, 1990 (of course)
Can 3-D reconstructions of F-actin filaments be interpreted in atomic terms?

- 3D - Beam Transparent EM
- EM: - 2D crystals (protein crystals)
  - -> e-diffraction (amplitude) or FT of real images (amplitude & phase)...  
  - -> periodic structure (real and reciprocal space)
  - -> collect different view angle - tilt series
  - -> add in fourier space the layers to a 3D frequency representation

Real space / Fourier space

- Real space
  - 1D-lattice
  - 2D-
  - Images

- Fourier (reciprocal) space
  - Diffractogram (power spectrum)

Image enhancement: signal, noise and averaging
improving the signal-to-noise ratio

- Image: sum of signal and noise
- \( S/N = 0.25 \)

Literature

- Electron diffraction processing
  - Baddeley & Henderson, (1994) Ultramicroscopy, 14, 319
- Image processing
  - Henderson et al. (1989) Ultramicroscopy, 19, 147
  - Processing of tilt images and data merging

- Retinement
3D - Beam Transparent EM

- EM: single particle (isolated > 100k Da) & tomographic reconstruction - “tilt series”
  - -> collect as many images and projection of your sample (real)
  - -> > 100'000 images of single particle (statistics)
  - -> Multivariate statistics selects “classes” of different projection views
  - -> average n particles per class -> merge 2D transfers in 3D in Fourier space -> back-transformation (rFFT)
3D-volume reconstruction: single particles
overview of the various iterative refinements

- Sample
- Cryo-electron Microscopy
- Digitisation
- Alignment
- Classification
- Angle Assignment
- Realignments
- Reconstruction
- Structure Interpretation

Electron Tomography - macromolecular complexes..

2D-projections of single particles
a random series of of 2D-images
aligned by man/computer selection
-> selection of different
projection classes of images
-> Tomographic reconstruction

2D-projection tilt series
by tilting the specimen stage...
-> TEM Tomography

Electron tomography
“weighted back projection (real space)
-> generate direct tilt series...(S/N!!!)

2D-projections
a 3D-object is projected at various tilt angles into a series of of 2D-images

3D-reconstruction
to reconstruct the 3D-object all the backprojections bodies are summed

W. Baumeister, MPI Martinsried

EM Tomo: resolution and weighting

Crowther criterion
Elongation factor

\[ d_r = \pi \frac{D}{N} \]
\[ \epsilon_r = \frac{\alpha + \text{SIZE OF WEDGE}}{\alpha - \text{SIZE OF WEDGE}} \]
\[ d_r = d_r \cdot \epsilon_r \]

Missing wedge

d = lateral resolution
D = thickness of sample
N = number of projections
\( \alpha \) = missing wedge angle

Lit.: see also S.Nickel et al..., Nature Reviews Molecular Cell Biology....
Electron Tomography - macromolecular complexes.

**Single Particle Analysis**

<table>
<thead>
<tr>
<th>Principle to obtain multiple views</th>
<th>Electron tomography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merged many particles with various views in solution</td>
<td>Take micrographs of one particle tilted at various angles in the microscope</td>
</tr>
</tbody>
</table>

**Crystallization**

<table>
<thead>
<tr>
<th>Structural heterogeneity</th>
<th>Electron tomography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Averaged out</td>
<td>Visualized individually</td>
</tr>
</tbody>
</table>

**Current resolution**

<table>
<thead>
<tr>
<th>Resolution</th>
<th>Electron tomography</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (up to 8 A)</td>
<td>Low (30 A)</td>
</tr>
</tbody>
</table>

**Missing information**

<table>
<thead>
<tr>
<th>Missing information</th>
<th>Electron tomography</th>
</tr>
</thead>
<tbody>
<tr>
<td>None / Missing cone</td>
<td>Missing wedge/pyramid</td>
</tr>
</tbody>
</table>

Flow diagram 3D (cryo-) TEM from sample preparation to 3D-map interpretation

**Tomography**

- Cellular TEM
- Tilt series (same specimen area)
- Averaging not possible
- Resolution: 100-50Å

**Single particles**

- MW >250kD
- Tilt not necessary
- Averaging (after classification)
- Resolution: 20-10Å

**1D-crystals (helices)**

- Tilt not necessary
- Averaging
- Resolution: 30-10Å

**2D-crystals**

- Tilt series (different crystals)
- Averaging
- Resolution: 20-3Å

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Surface relief reconstruction - TEM

- “optimal granularity”
- Statistical nucleation
- Averaging out granularity

**Polyhead freeze-dried and rotary shadowed (30°)**

- Elevation angle 45°
- TaW (5Å)

**Surface relief reconstruction - SEM**

- Freeze-dried and shadowed with 1nm W or from negative staining...

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H. Gross...

J.D. Woodward...2009
- 3D - Data from sections....

- classical serial sectioning... -> TEM
- serial sectioning (arrays) for SEM
- serial sectioning in the SEM... ->

- serial sectioning in the FIB/SEM... ->

- tomographic view of section volume... -> TEM Tomo

- serial section TEM-tomography...

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**From serial sections to 3-D model:**

[Diagram showing serial sections and 3D reconstruction]

**Paramecia 3-D reconstruction:**

[Images of Paramecia 3D reconstruction with context embedded models]

**Serial section array**

SEM imaging:

K. D. Micheva, S.J. Smith
Neuron 55, 2007

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**3D - Data from sections....**

[Diagram showing steps from embedding to reconstruction]
A Compromise: a modified „Freeze Substitution“ (or embedding) for correlative LM/EM

1. transfer frozen sample into substitution media at -110°C or -90°C
   Media (organic solvent saturated with fluorochrome and Uranyl Acetate)
2. substitution according to tissue experience (-90/-70/-50°C)
3. wash excess fluorochrome and fixative until no colour bleeding (-50°C)
4. start infiltrating with resin (-50°C HM20, 0°C Epon...)
5. polymerization under UV or with heat
6. trimming for CLSM or SEM, sectioning for LM, TEM....

+ Fluorescent dyes
e.g. DiIC18 or Safranin O

3D - Data from sections....

- classical serial sectioning...-> TEM
- serial sectioning (arrays) for SEM
- serial sectioning in the SEM...-> W. Denk
- serial sectioning in the FIB/SEM...-> a new way to section embedded sample (resin and cryo...)

Acquisition of 3D image stacks with FIB-SEM

1. Deposition of protecting C-layer
2. Milling of a trench, milling current 6.5 - 13nA
3. Polish the cross section, milling current 1.5nA
4. Imaging with SEM (ESB)
5. cut again a slice away with ion beam
6. repeat 4.-5. for acquisition of a 3D serial section stack (fully automatized)
3D - Data from sections....

- tomographic view of section volume...-&gt;TEM Tomo
- serial section TEM-tomography...

Electron tomography “weighted back projection (real space)-&gt; generate direct tilt series...(S/N!!)

2D-projections
a 3D-object is projected at various tilt angles into a series of 2D-images

3D-reconstruction
to reconstruct the 3D-object all the backprojections bodies are summed
courtesy from W. Baumeister, MPI Martinsried

Electron Tomography - Four Steps

1. Acquisition
2. Reconstruction
3. Visualization
4. 3D-reconstruction

TEM Tomography:
multilamellar bodies in the Stratum Granulosum..

80nm HM20 section from a sample freeze-substituted 1999
Resin embedded samples are a “Storage” device for “morphomic data”
-&gt; “Data block” & “Data slices”
Reinvestigated 2002 by TEM Tomography...
-&gt; membrane visibility!

- Stratum Corneum lipids are synthesised in the TGN (GluCer) and exported in Multivesicular lamellar bodies into the intercellular space (Cer)...

Stratum Corneum lipids are synthesised in the TGN (GluCer) and exported in Multivesicular lamellar bodies into the intercellular space (Cer)....
3-D reconstruction of Golgi-TGN from TEM Tomography:

http://bio3d.colorado.edu/pubs/Golgi/GolgiAnalysis.html

3-D reconstruction of a whole cell.....(B. Marsh group):

Correlating FM and cryo-ET: Full Correlation Cycle

LM
Cryo-ET

NG108 neuroblasto/ glioma hybrid cell line


A cell has roughly about something like 1x10^8-1x10^10 individual bio-molecular entities not counting water, ions, metabolites....
3D - Data from sections....

Serial section array
SEM imaging:
K. D. Micheva, S.J. Smith
Neuron 55, 2007

A good biological EM Lab needs:
- a Fluorescence LM
- a (Cryo)-HR-SEM
- a Ultramicrotome

New solutions for correlative microscopy....

Correlative Microscopy for Materials Analysis

How to Read 3D EM data...

see. Lit: Saibil, HR (2007) How to read papers on three-dimensional structure determination by electron microscopy. in Evaluating techniques in biomedical research, Cell Press
Some further reading on 3D EM data...

Reviews

Cellular tomography

Helical reconstruction

Electron cryotomography

Comprehensible “Nano-cosmos” in its natural context
Thank you for your attention...