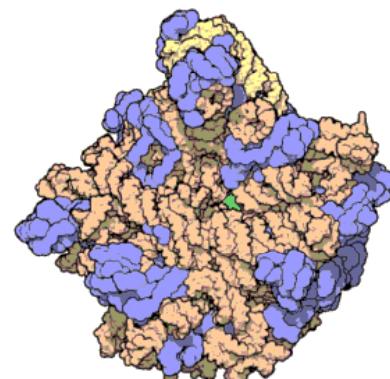




2018–2019学年秋季
“生物大分子电子显微三维重构”



Lecture 1: Overview of 3DEM



朱 平

zhup@ibp.ac.cn

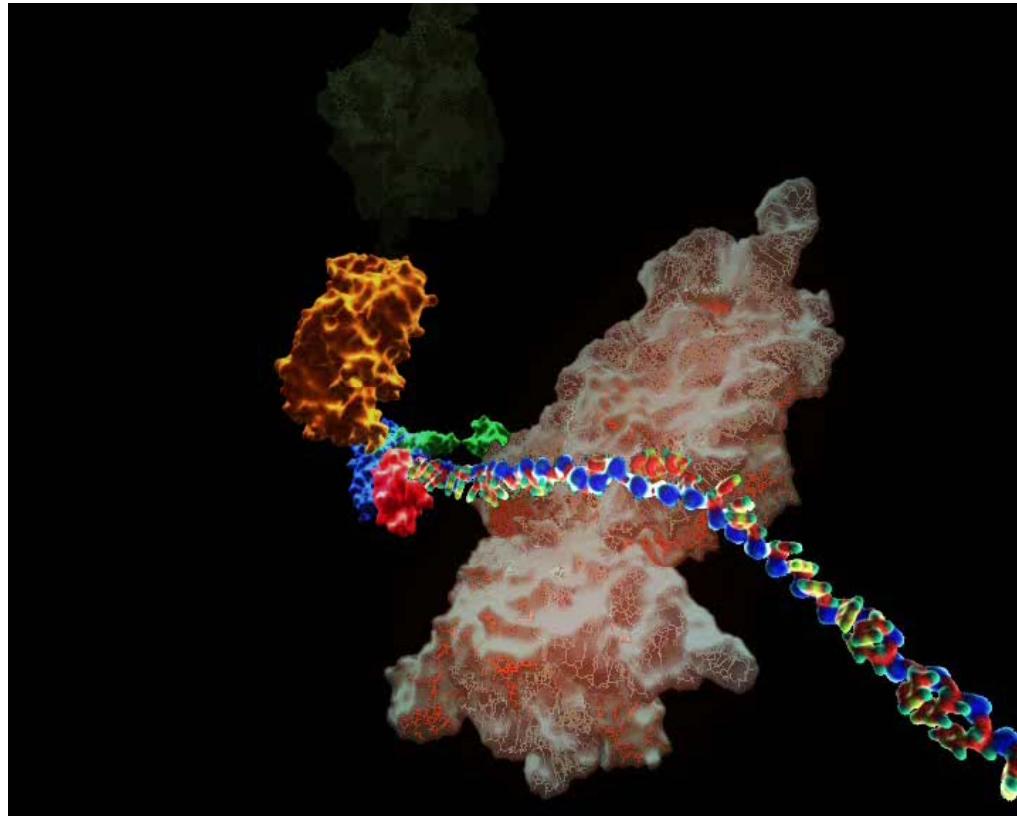
中国科学院生物物理研究所
生物大分子国家重点实验室



中国科学院生物物理研究所
INSTITUTE OF BIOPHYSICS CHINESE ACADEMY OF SCIENCES

2018年9月13日

超大分子复合体—生命活动的“执行者”



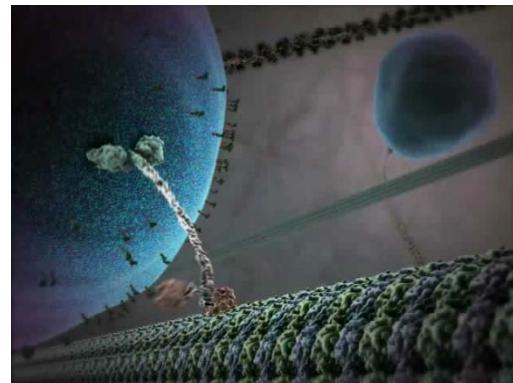
“生命机器”的运转依靠细胞内成千上万的超大分子复合体来执行

超大分子复合体——生命活动的“执行者”

生物体如何实现自身这一最为复杂、精密的“机器”的正常运转？



生命信息的
解码与复制
(核糖体)



物质的运输
(分子马达)



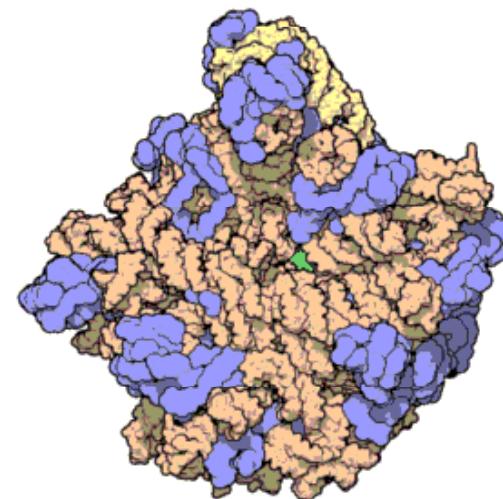
外界刺激的
感知与响应
(免疫感受复合体)

解析超大分子复合体——破解生命奥秘的关键

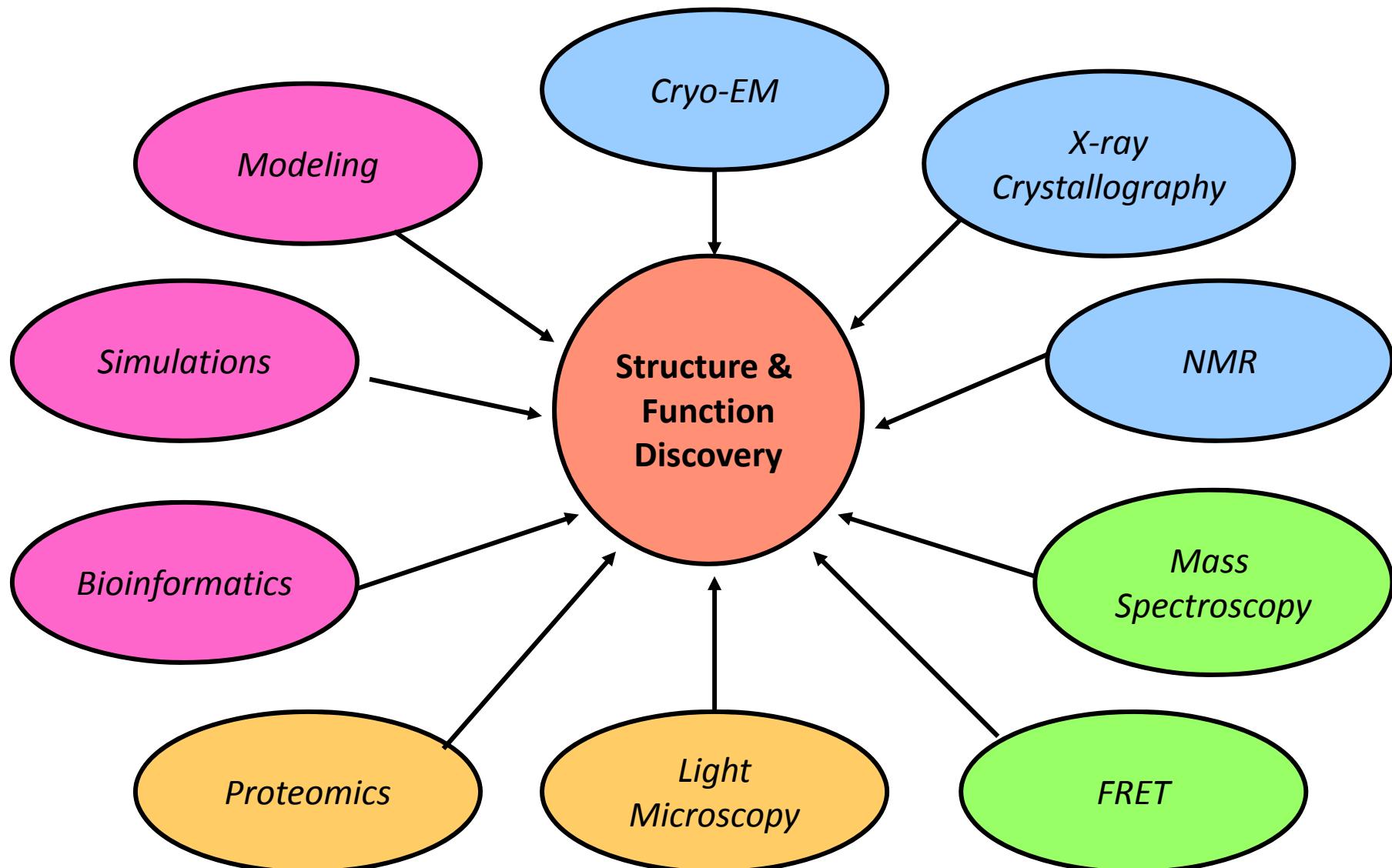
超大分子复合体—科学难题

两大基本特性：
——超大性和动态性

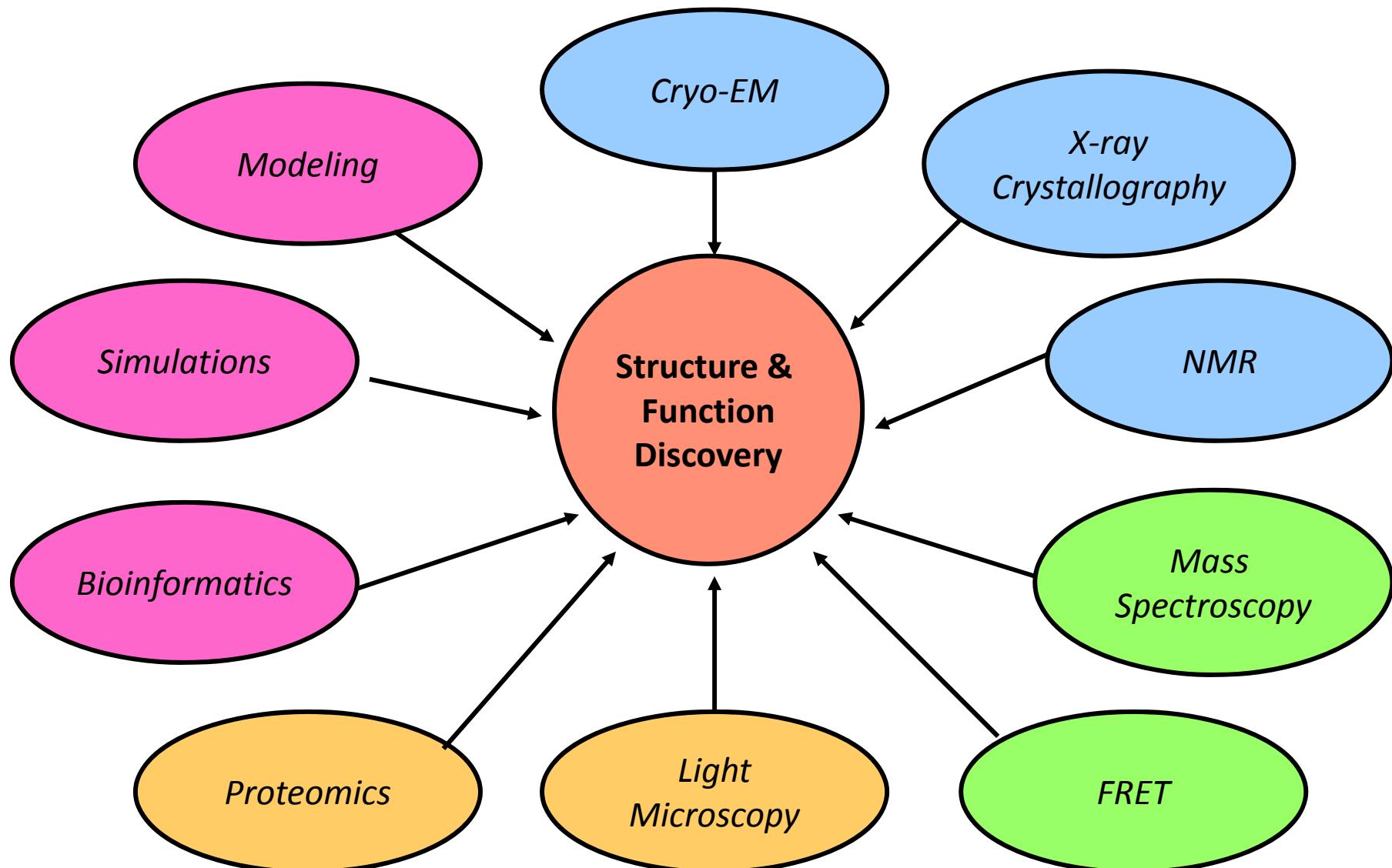
以核糖体为例，国际上有几十个研究组同期展开研究，历经三十多年，最终分别测定了原核来源核糖体的30S和50S亚基、原核完整70S核糖体及真核完整80S核糖体的三维结构。



结构生物学：主要方法和手段

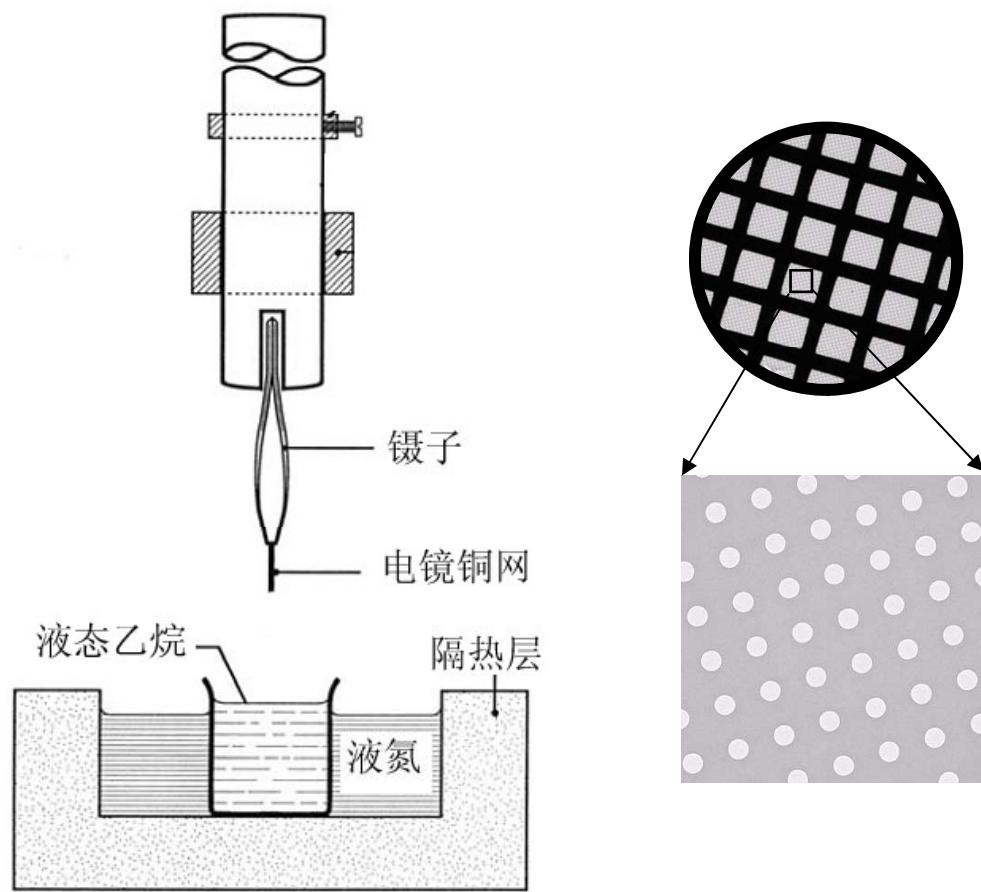


结构生物学：主要方法和手段

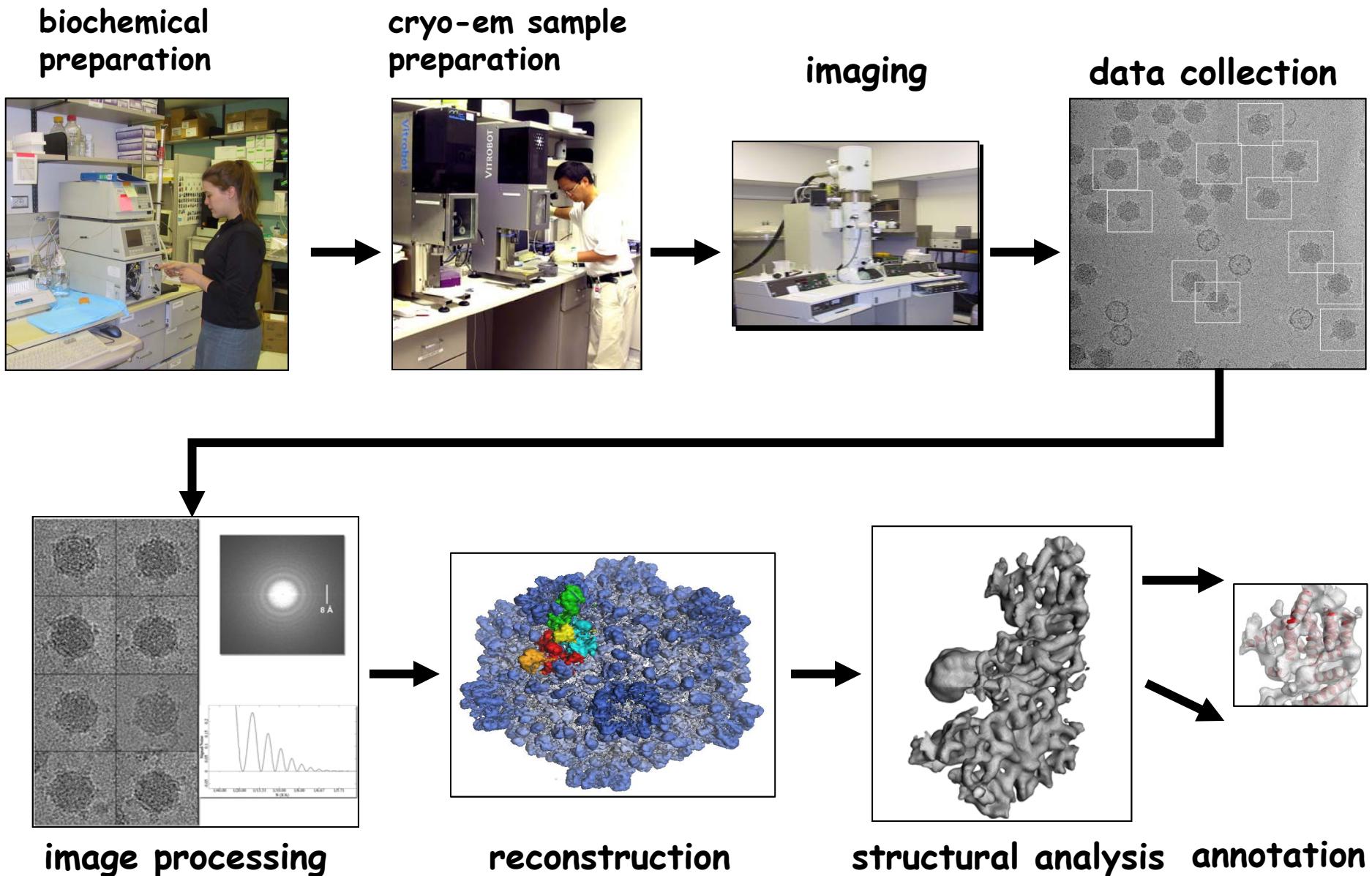


Cryo-EM

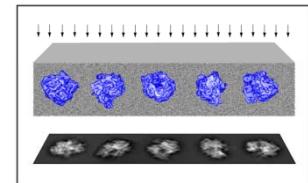
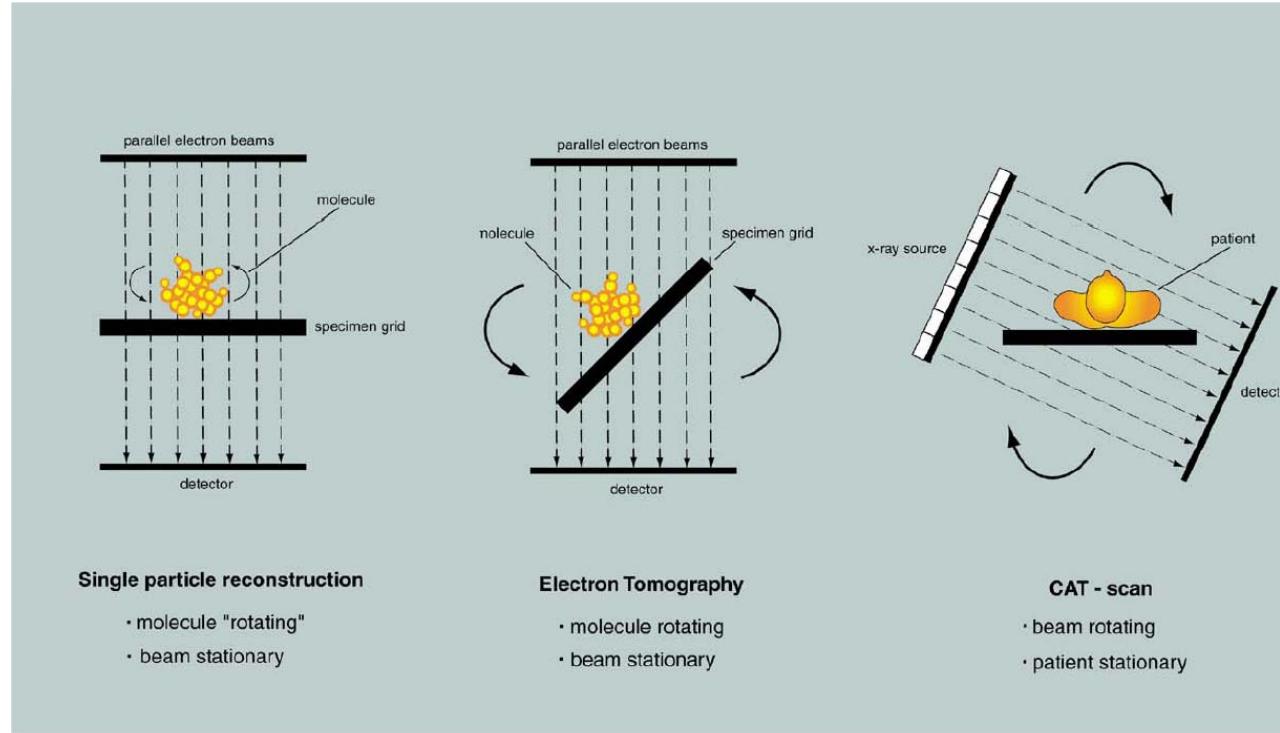
冷冻电镜成像技术是让生物大分子样品快速冷冻，使得水固化形成非晶态的冰，这样可以很好地保持生物样品在活性状态下的原有的结构不被破坏。保持样品温度在-160°C以下，用电镜对生物样品进行拍摄。



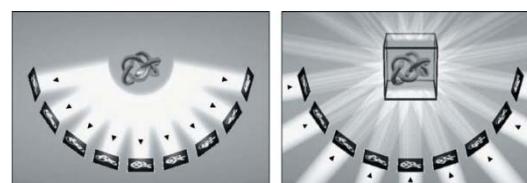
冷冻电镜结构解析



冷冻电镜三维重构方法



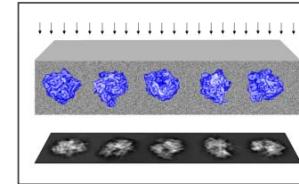
单颗粒分析方法
Single Particle Analysis



电子断层成像
Electron Tomography

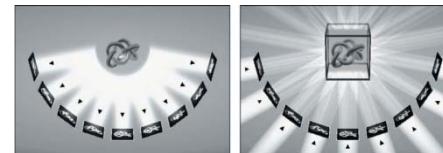
冷冻电镜三维重构方法

➤ **单颗粒分析方法**
(Single Particle Analysis)



对象：具有均一性的纯化样品，如二十面体对称病毒、核糖体、其它大分子复合物等

➤ **电子断层成像三维重构**
(Electron Tomography)



对象：不具有均一性的蛋白、病毒（如包膜病毒）、以及细胞器、细胞等

生物大分子的电镜三维结构

Three dimensional electron microscopic reconstruction of biological macromolecules

课程编号: 不填写

课程属性:不填写

学时/学分: 40/3

预备知识: 结构生物学导论、线性代数、数值分析、普通物理学

授课对象: 研究生和电镜结构生物学方面的初学者

教学目的和要求:

生物大分子电子显微三维重构技术作为结构生物学的三大研究手段之一,在近十年来得到了长足的发展,已经成为研究生物大分子特别是生物大分子复合体

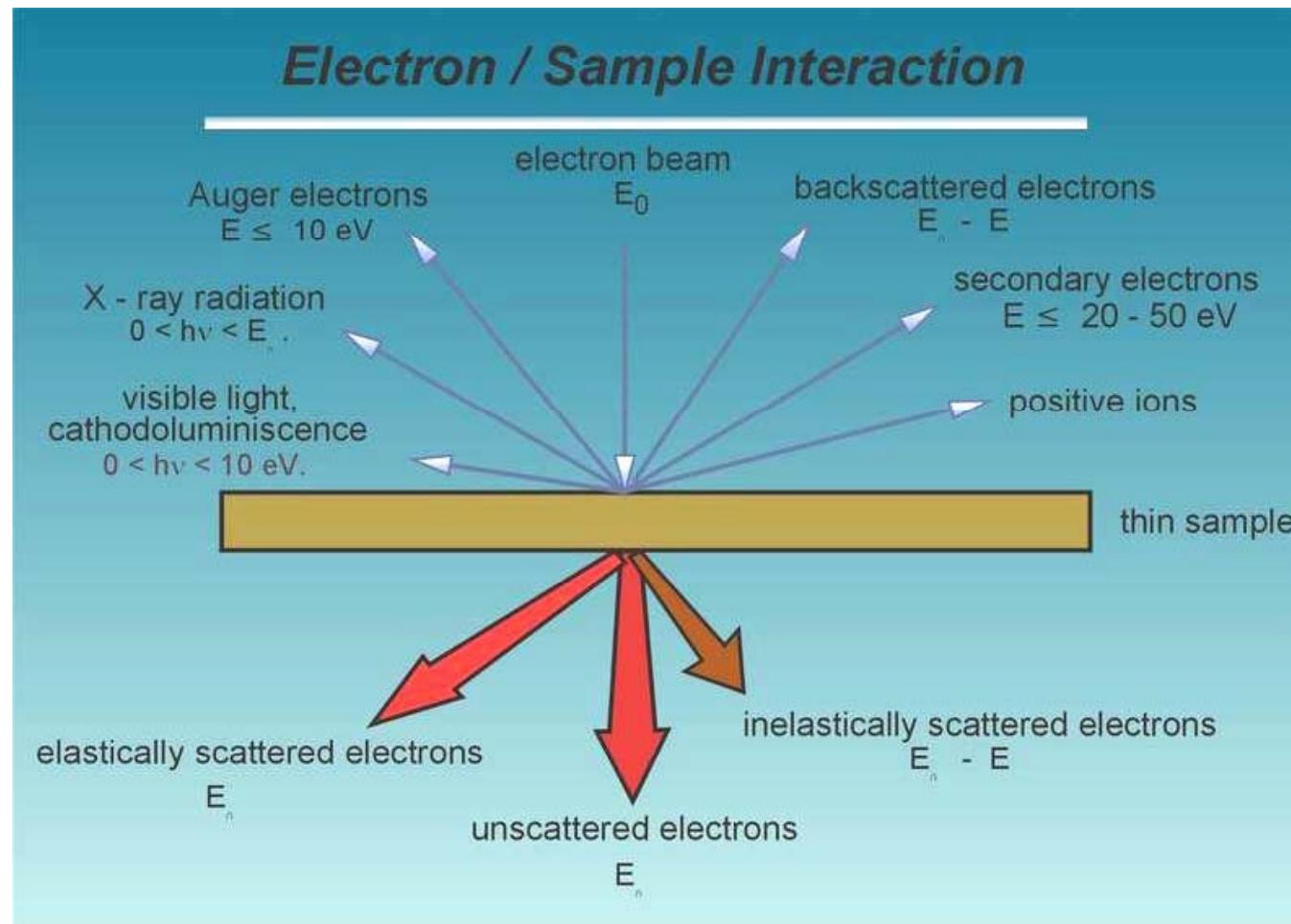
教学目的和要求：

生物大分子电子显微三维重构技术作为结构生物学的三大研究手段之一，在近十年来得到了长足的发展，已经成为研究生物大分子特别是生物大分子复合体三维结构的重要手段，是从事结构生物学研究的必要手段。作为一项前沿技术，我国尚没有系统地讲述该技术的课程和书籍，而我国在这个研究领域的投入巨大，已经有相当数量的研究人员在从事生物大分子电子显微三维重构研究，青年学生迫切需要系统学习和掌握这一技术方法。本课程的开设就是从这一背景下出发的，将从电子显微成像原理、生物样品成像本质、三维重构原理、电子显微三维重构方法和图像处理方法等方面对生物大分子的电子显微三维重构技术进行系统的讲述。本课程的教学重点是一、生物样品的电子显微成像本质；二、单颗粒三维重构图像处理和分析技术。通过对本课程的系统学习，学生可以深入理解电子显微镜在研究生物大分子复合体三维结构方面的优势、特点和前景，准确掌握生物大分子电子显微三维重构的技术方法，并利用该技术从事相关结构生物学研究。本课程要求学生事先掌握一些关于光学、傅里叶变换、线性代数和生物大分子结构方面的基本知识。

Cryo-EM study of molecules

Principle and procedures of Cryo-EM

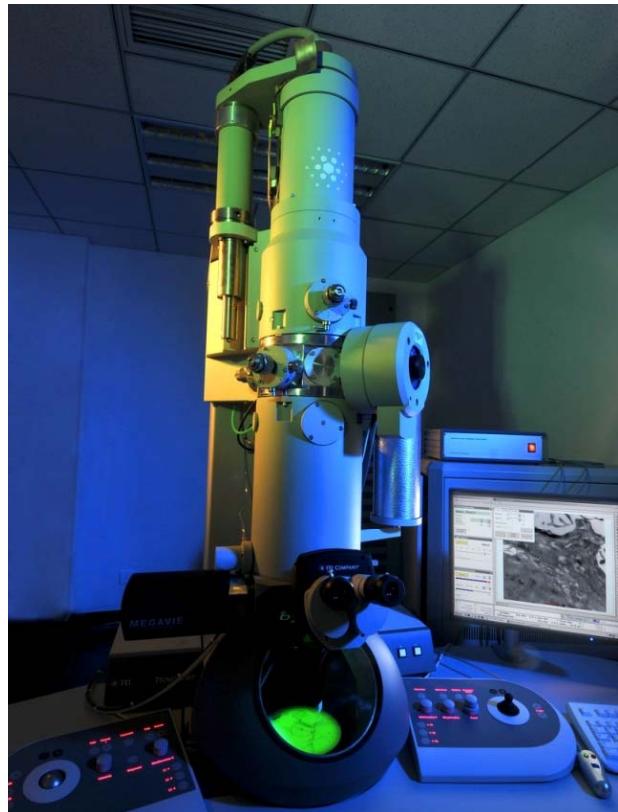
Electron-Sample Interaction



SEM

TEM

Principle: Transmission Electron Microscope (TEM)



TEM



Principle: Scanning Electron Microscope (SEM)

1.2.4 Scanning Electron Microscopy

The SEM is the most important electron-optical instrument for the investigation of bulk specimens [1.114–1.123]. An electron probe is produced by two- or three-stage demagnification of the smallest cross section of the electron beam after acceleration. This electron probe, 2–10 nm in diameter, is scanned in a raster over a region of the specimen (Fig. 1.3). The smallest diameter of the

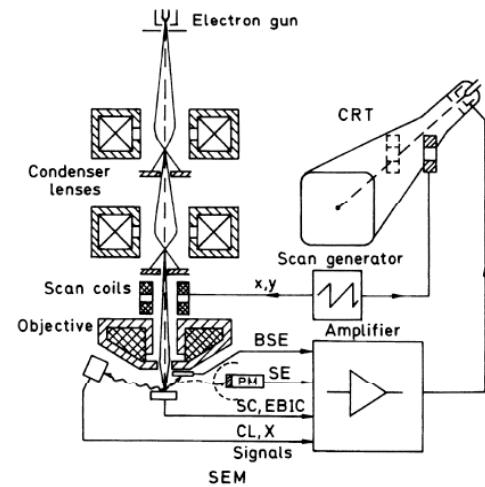
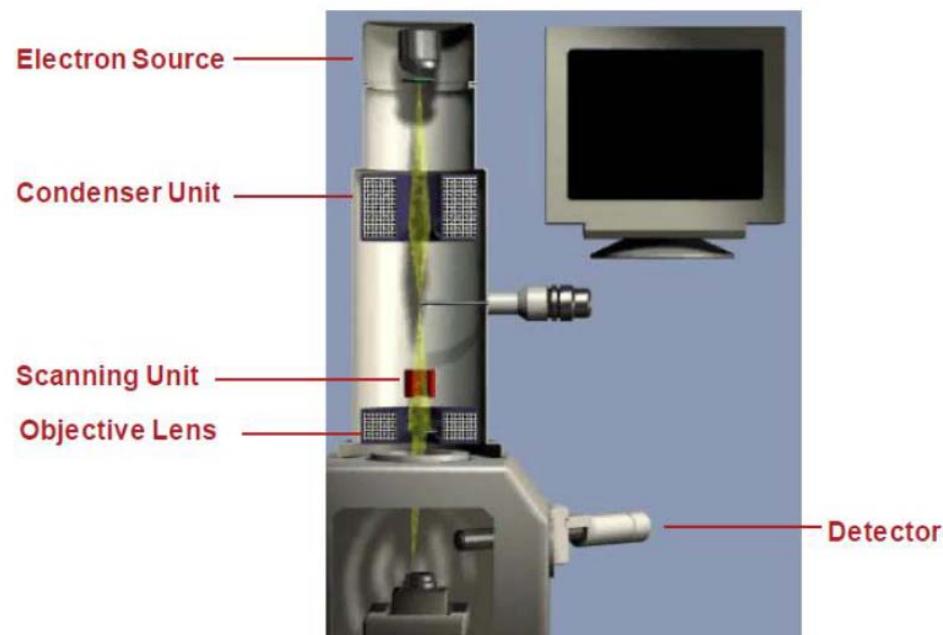


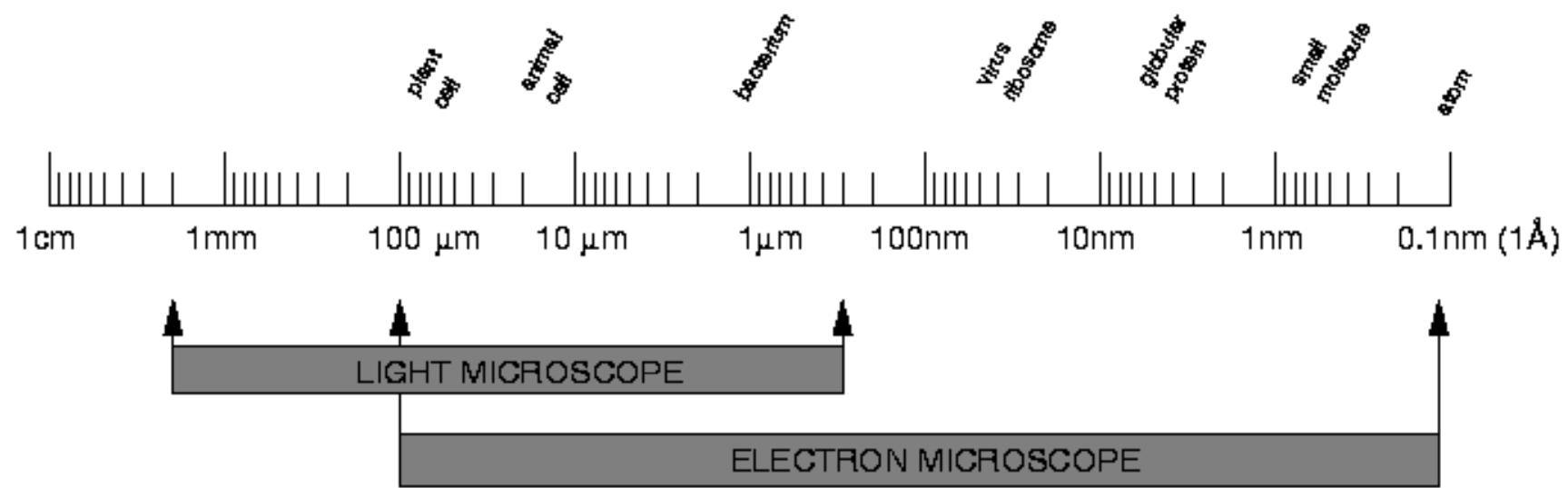
Fig. 1.3. Schematic ray path for a scanning electron microscope (SEM).

How a SEM works



SEM

Why Electron?



Visible Light:

$$\lambda = 400 - 600 \text{ nm}$$

Electrons:

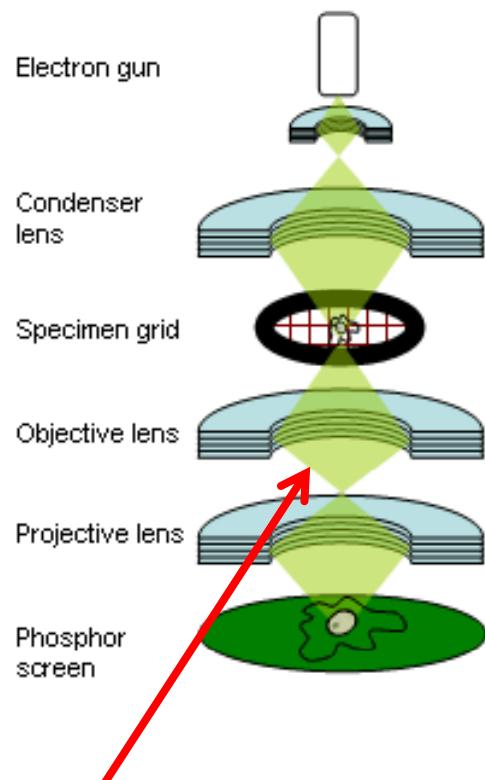
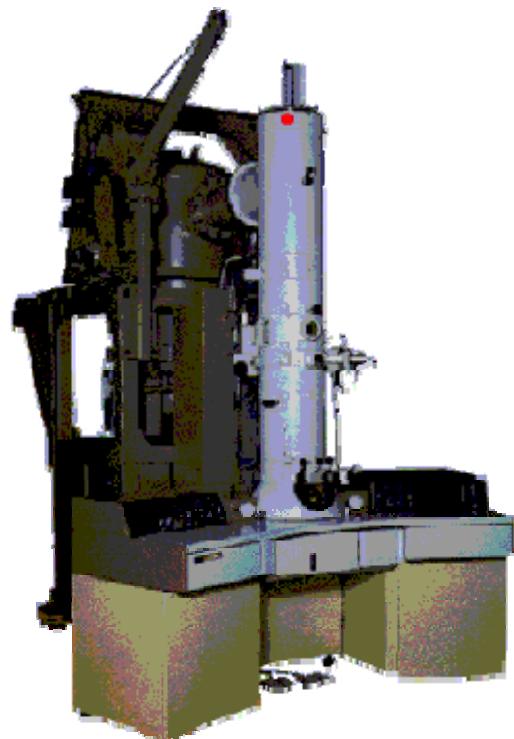
$$\lambda = 0.002 - 0.004 \text{ nm}$$

Remember: wave-particle dualism

$$\text{de Broglie: } \lambda = h / m * v$$

$$E = \frac{1}{2} * m * v^2$$

Principle: Transmission Electron Microscope (TEM)



Electron source:
Thermal emission from
heated cathode

Focussing:
Electro-magnetic Lenses

Detection:
Phosphor screen or CCD
camera (former times:
negative)

Vacuum!

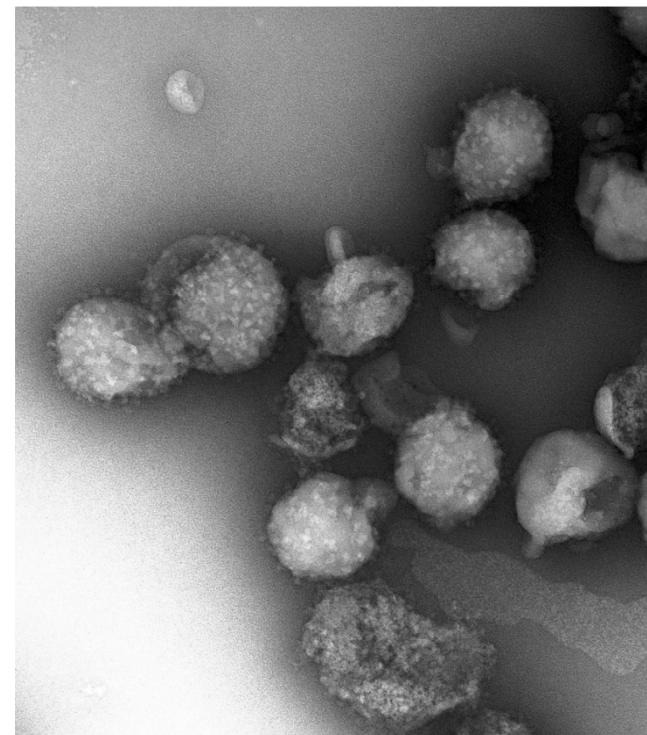
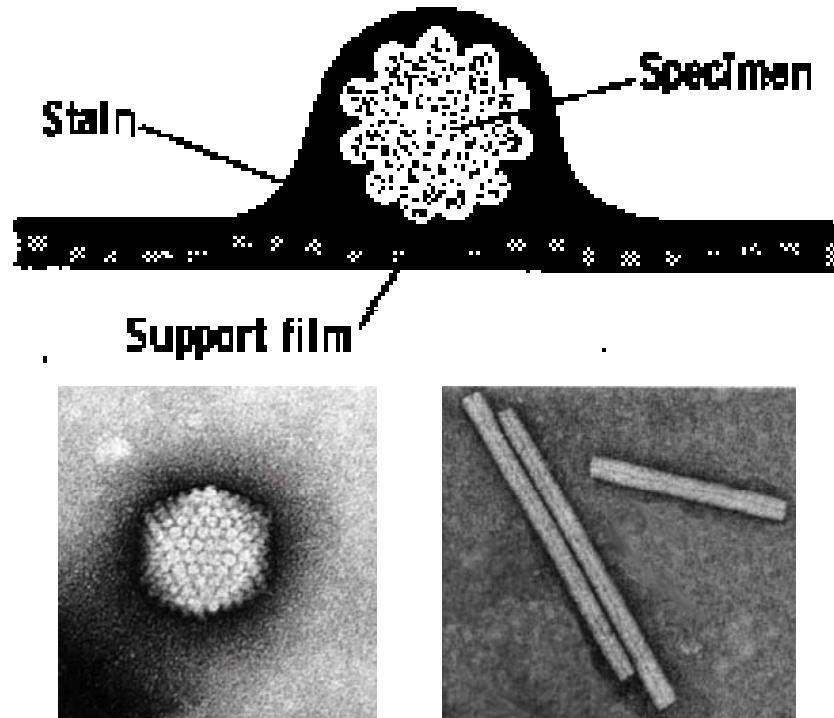
Electron Microscopy of Biological Samples

- Staining EM
- Cryo-EM

Staining EM

⇒ To increase contrast: heavy atoms interact stronger with e- than biomolecules (C, N, O, S, P)

Positive Staining, Negative Staining, Shadowing



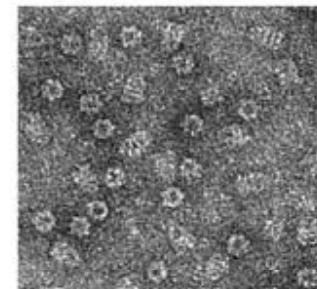
Disadvantage:
Size of stain reduces
resolution to about 20-30 Å

a

1. Add sample in buffer
2. Add heavy metal stain



3. Blot



4. Air dry

Carbon support film

Grid bars

b

1. Add small volume of sample

forceps

EM grid

2. Blot

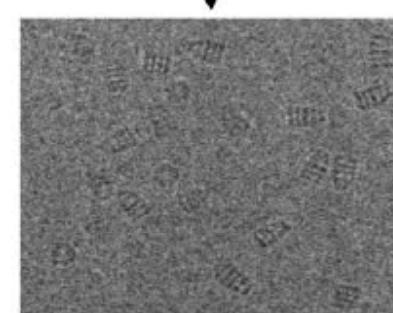
3. Plunge into liquid ethane

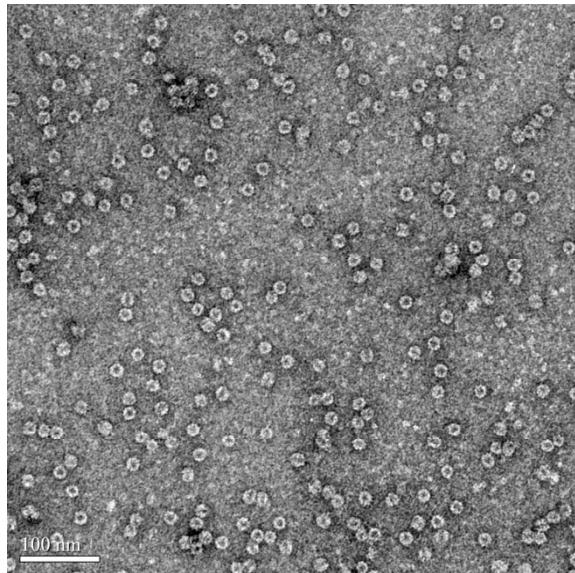
liquid ethane
(-160°C)

4. Keep the grid at liquid nitrogen temperature

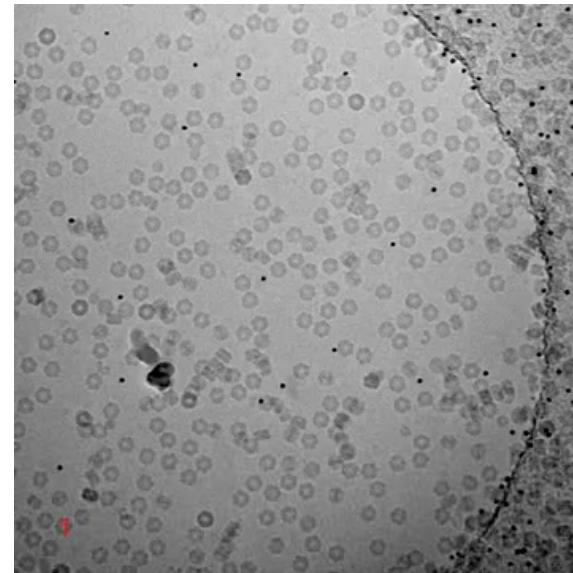


Edge-on view of an unsupported part of the water layer





TEM: Negative Staining



TEM: Cryo-EM

Characteristics of Secondary Electrons

- Product of inelastic scattering, not strongly affected by sample composition
- Stronger yield at edges, carry strong surface topography information
- Small energy, shallow escape depth, high resolution signal

sciences

SEM – Secondary Electrons

Characteristics of Backscattered Electrons

- Product of elastic scattering, strongly affected by Z
- Large energy, large escape depth, less high resolution signal

**中国科学院
物理所**

(b)

Atomic number (Z)	Theoretical Yield (Y _B)	Experimental Yield (Y _B)
10	0.20	0.20
20	0.25	0.25
30	0.35	0.38
40	0.40	0.42
50	0.45	0.45
60	0.48	0.48
70	0.50	0.48
80	0.52	0.48

SEM – Backscattered Electrons

Cryo-EM: Sample preparation history

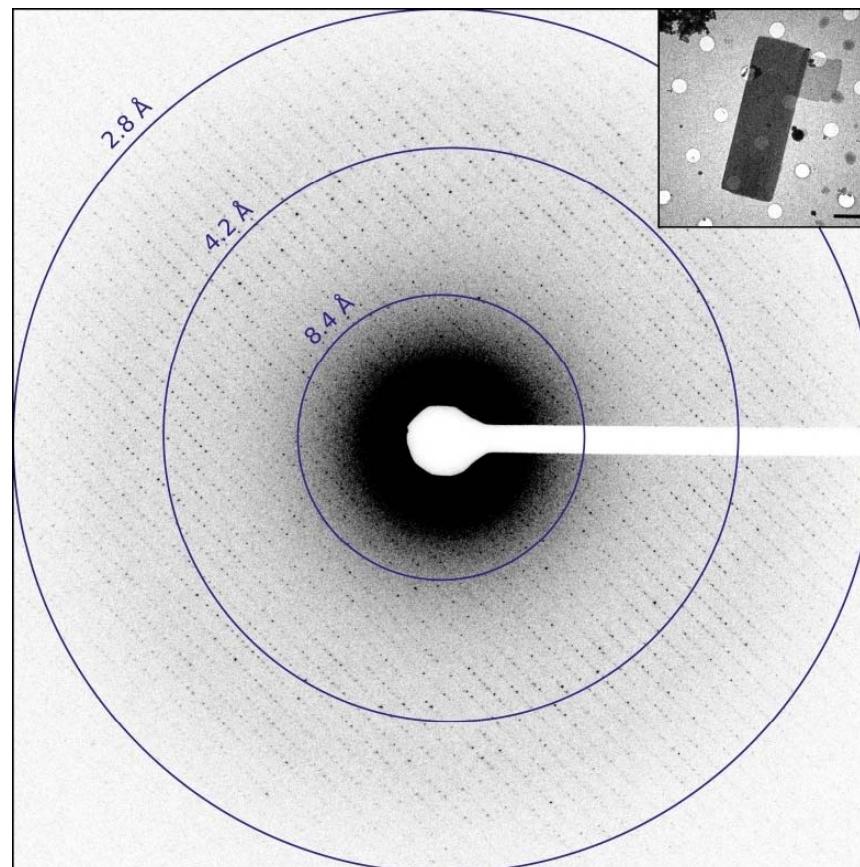
Cryo-EM : 2-D crystal



Glaeser RM



Taylor KA



Taylor KA, Glaeser RM. *Science*. 1974, 186:103

Cryo-EM : 2-D crystal

Henderson R



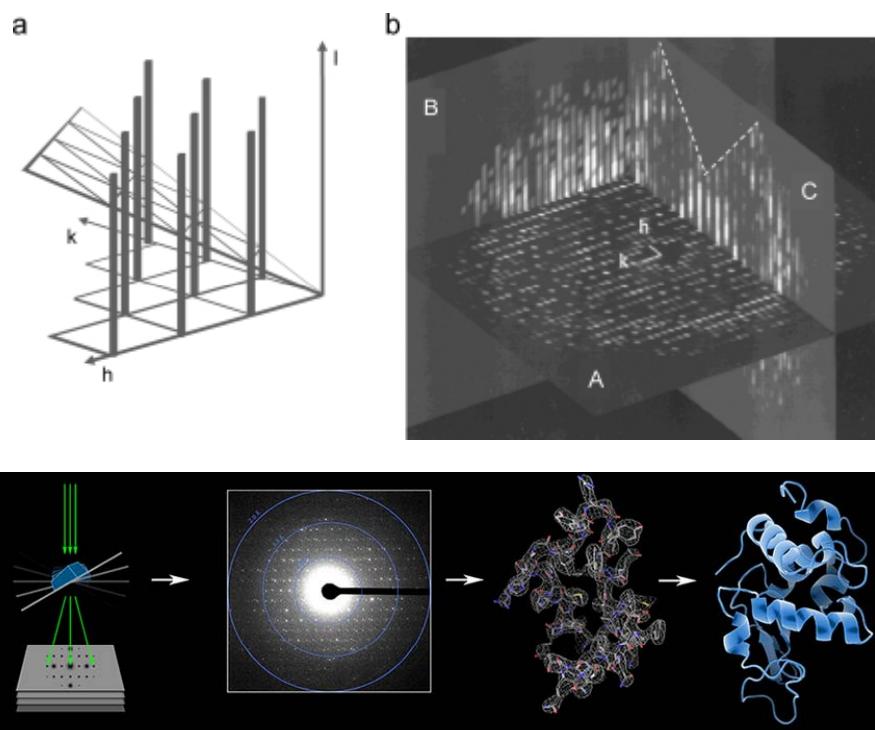
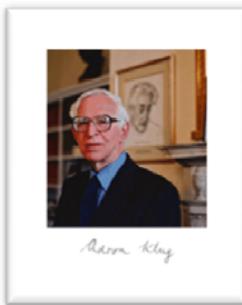
Unwin N



MRC Laboratory of Molecular Biology, Cambridge

Contributions in
Cryo-EM theory

Klug A

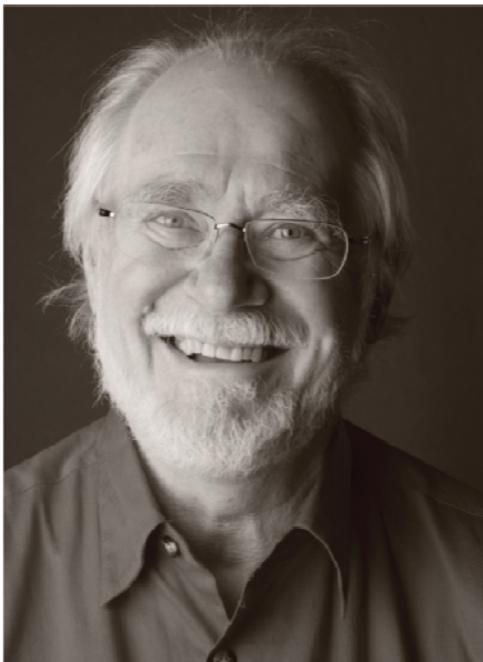


Cryo-Plunging Device

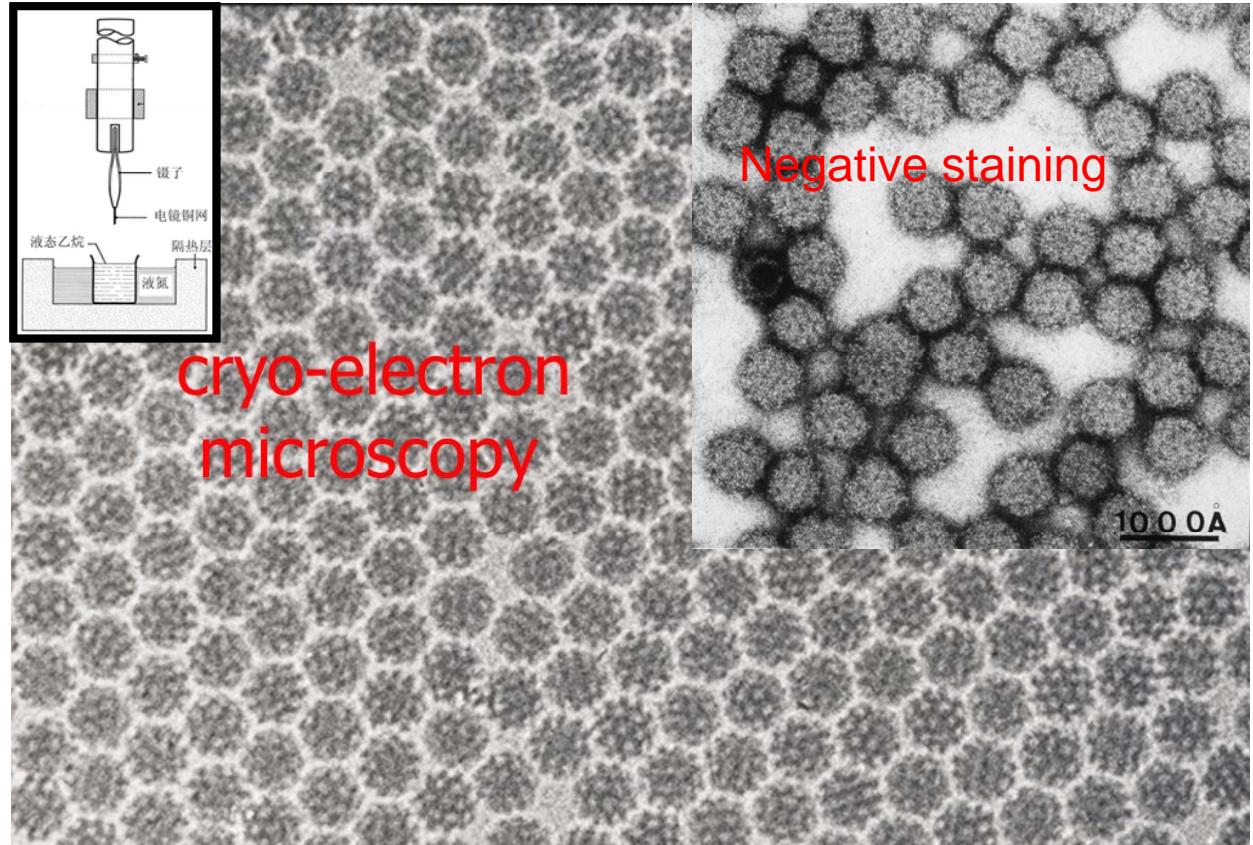
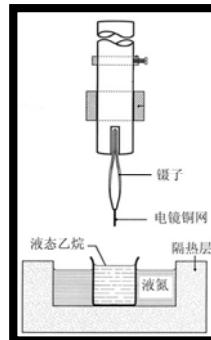


- The biological sample is applied to the grid, blotted to leave a thin film of water, and then plunge-frozen in ethane slush chilled by liquid nitrogen.
- Cryo sample preparation methods were developed in the mid 1980s

Cryo-EM : Single particle



Dubochet J

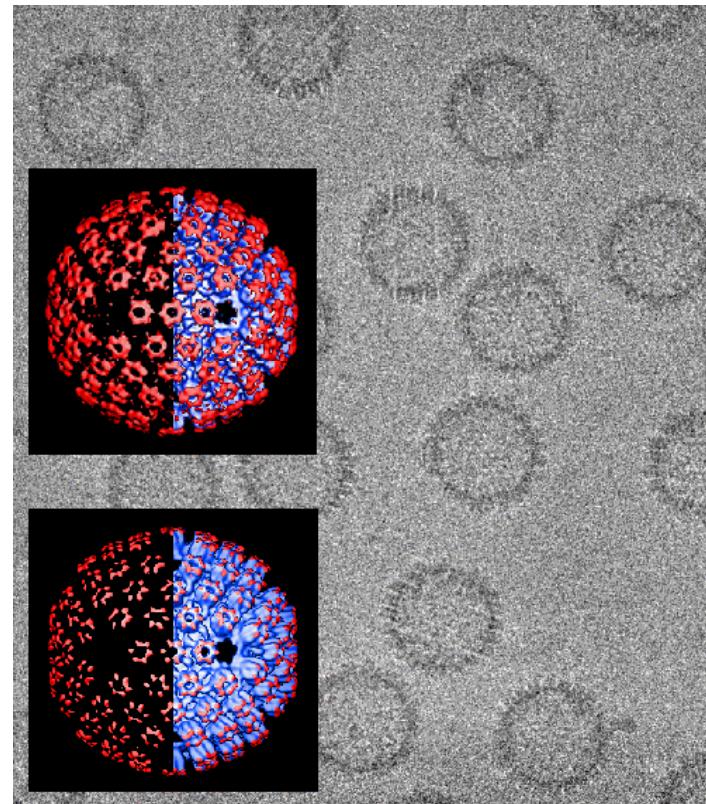
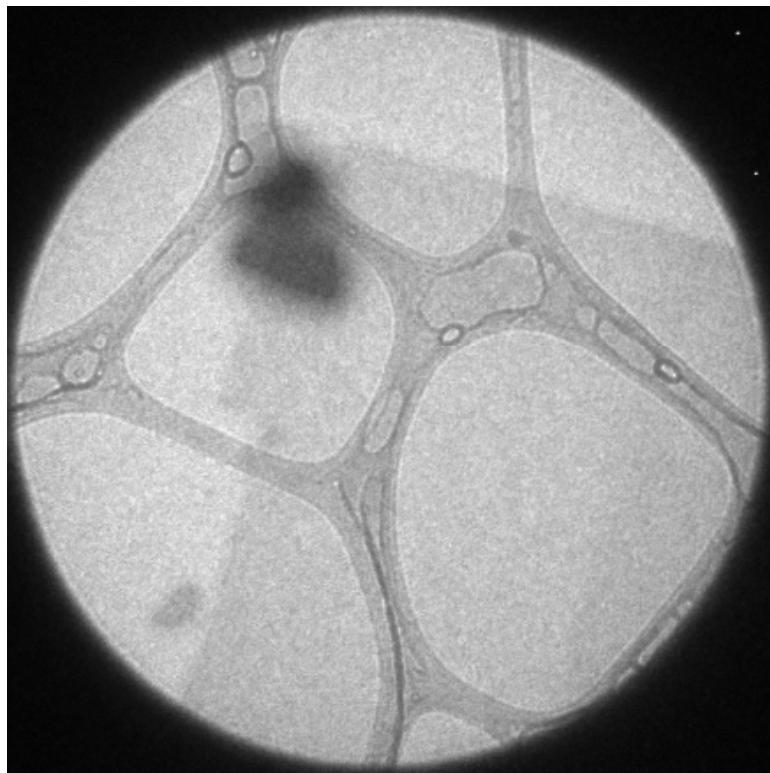


后期：冷冻切片

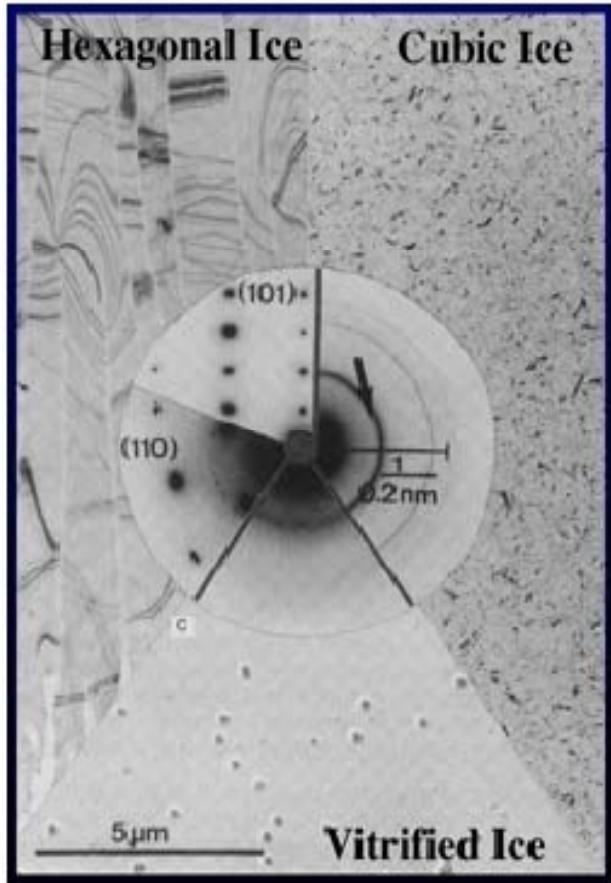
Adrian M, Dubochet J, et al. *Nature*. 1984, 308:32

Cryo-EM

H. Fernandez-moran
B. Glaeser
K. Taylor
J. Dubochet



Three Forms of Ice

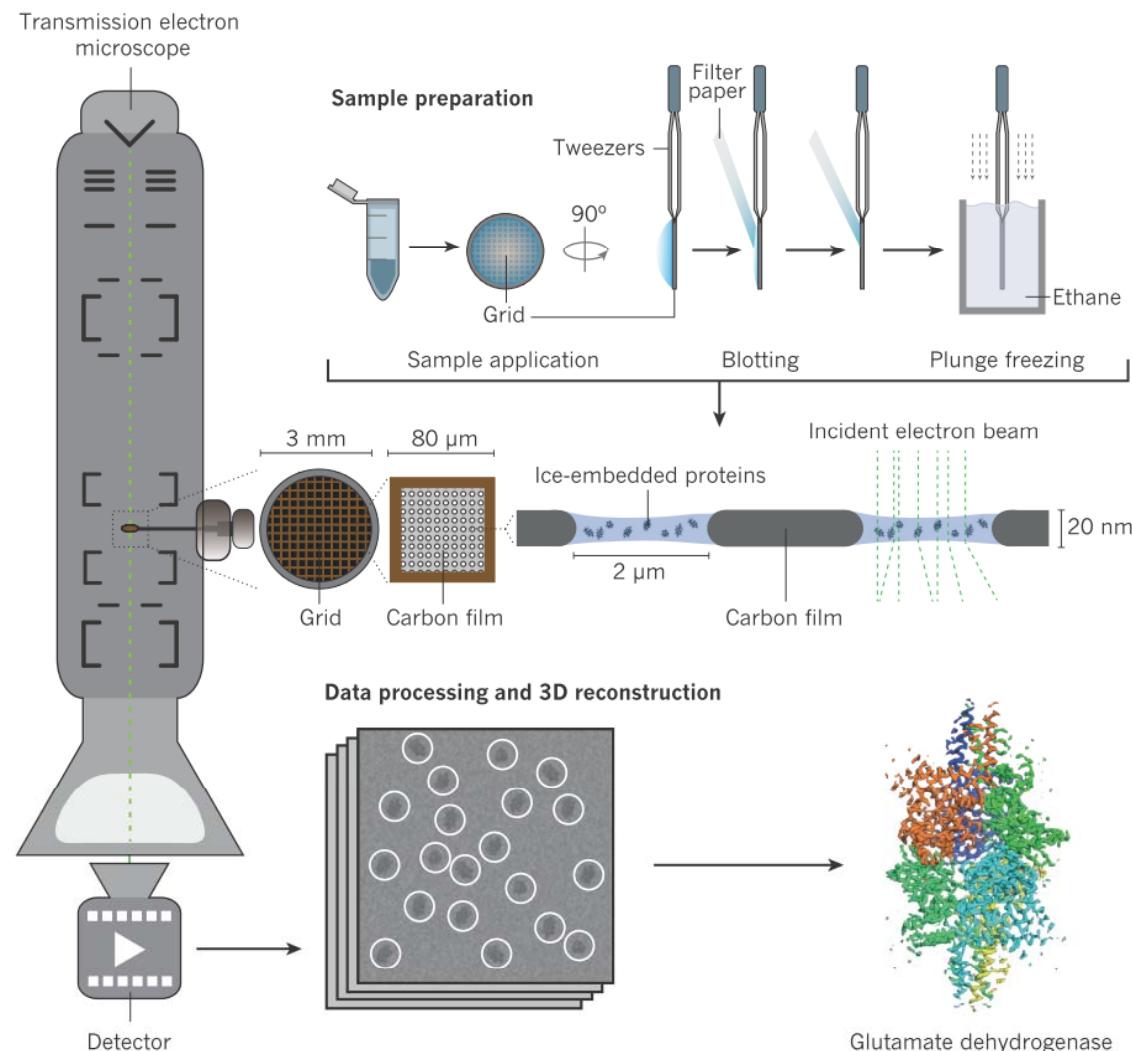


- Plunge freezing of a thin (~1,000Å) layer of water into a cryogen produces vitrified ice, or water in a glass-like state.
- The diffraction pattern of vitrified ice shows no regular diffraction spacing, indicating a non-crystalline structure.
- Hexagonal ice is the normal crystalline form of ice.
- Cubic ice is formed when vitrified ice warms up above approx. -130° C.
- Cubic and hexagonal ice both have a greater volume than liquid water - expansion occurs during non-cryogenic freezing.
- This expansion would distort the 3D structure of the biological sample.

Cryo-EM 3D Reconstruction

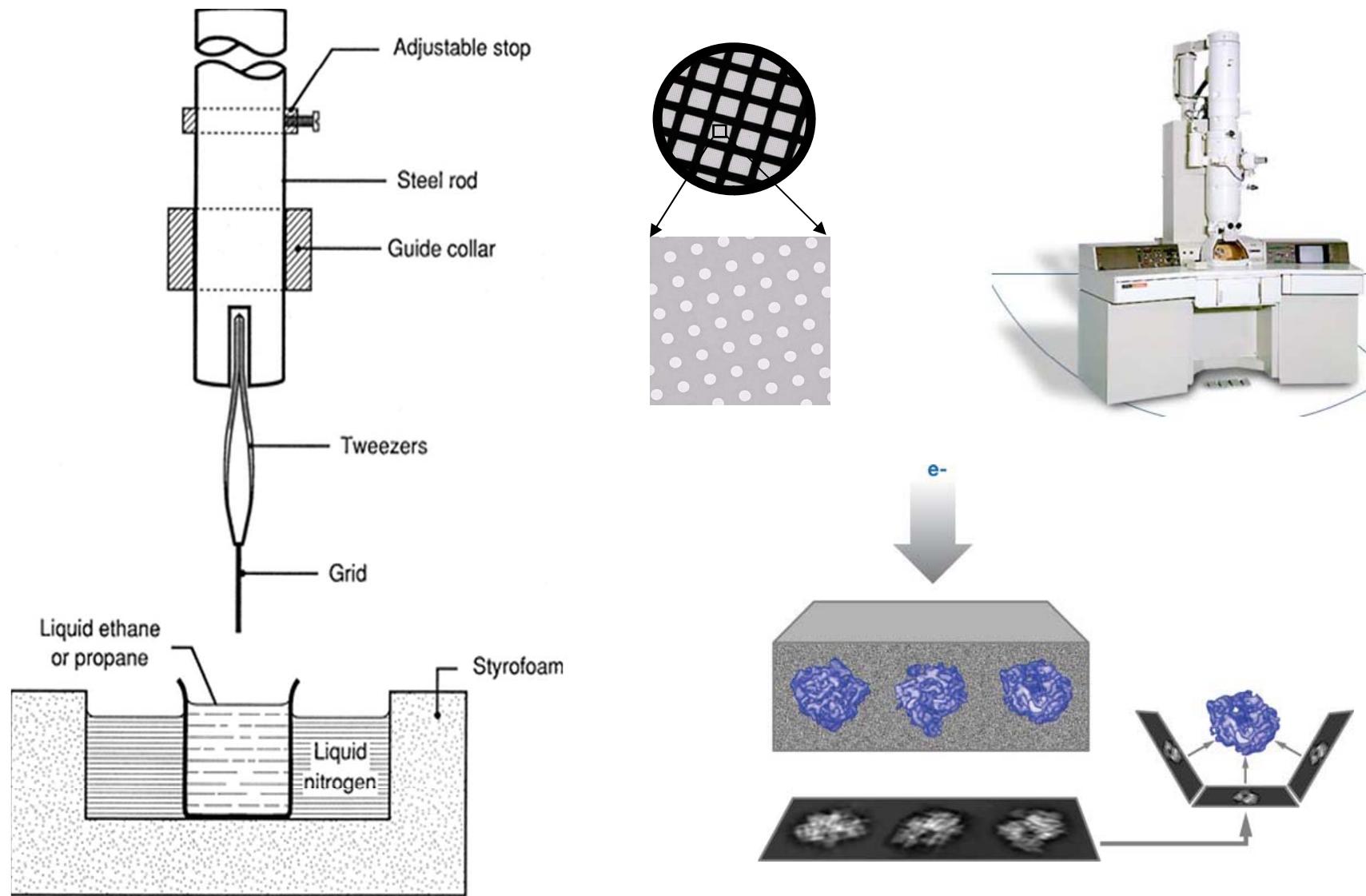
Single Particle Analysis

Cryo-SPA

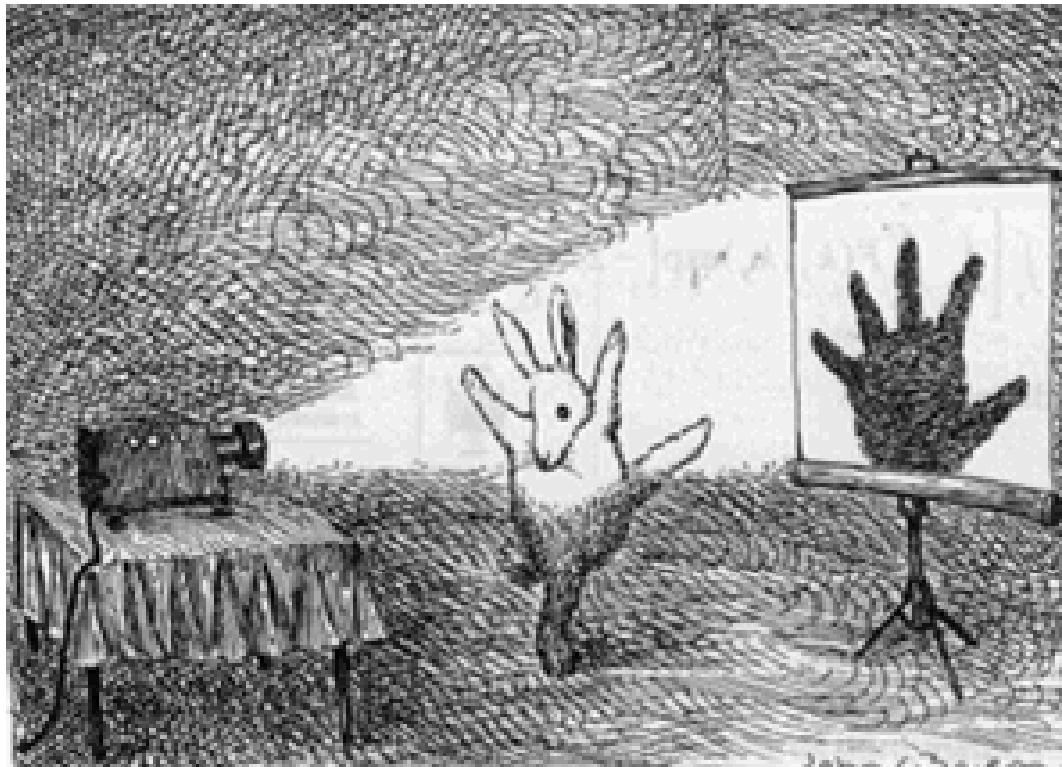


Nature.2016;537(7620):339-346.

Cryo-SPA



3-D Image Processing



- A single projection image is plainly insufficient to infer the structure of an object.
- (Originally from The New Yorker Magazine, 1991)

How many projection images are needed to generate a 3D reconstruction?

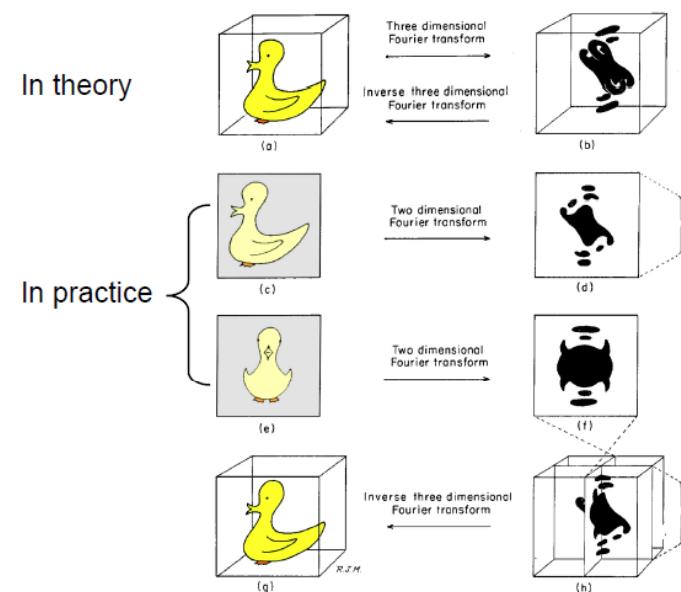
- Each particle image represents a 2D projection of the 3D object



3D object (a duck)



2D projections in different views

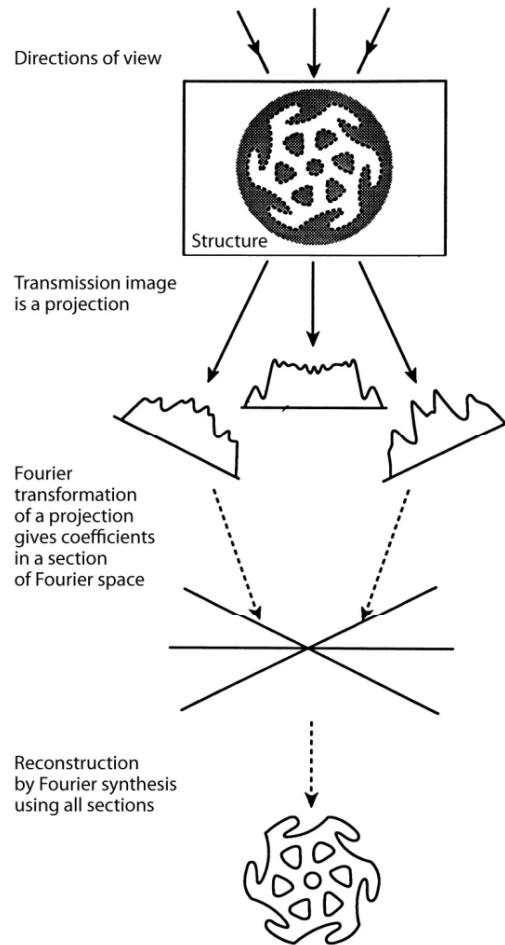


- The difficult step in 3D image processing is to determine the orientational angles (Euler angles) for each projection image

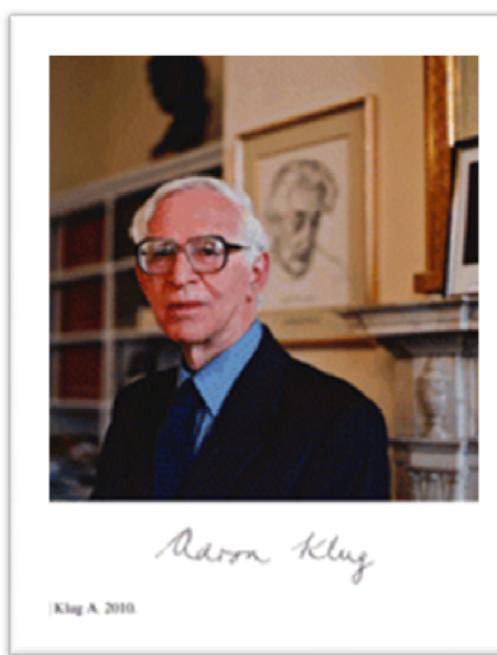
Principle of 3D reconstruction



David DeRosier



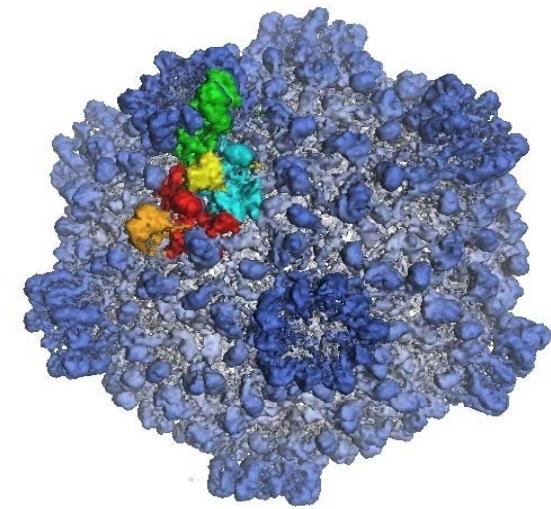
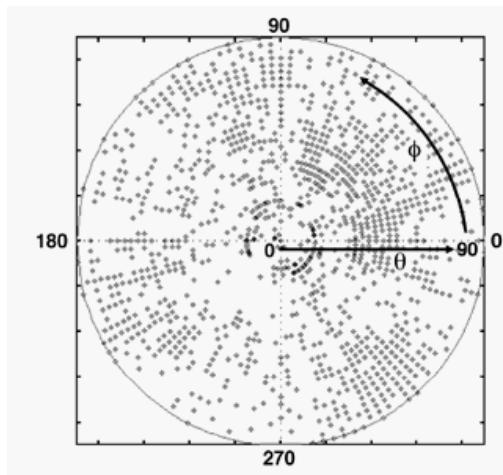
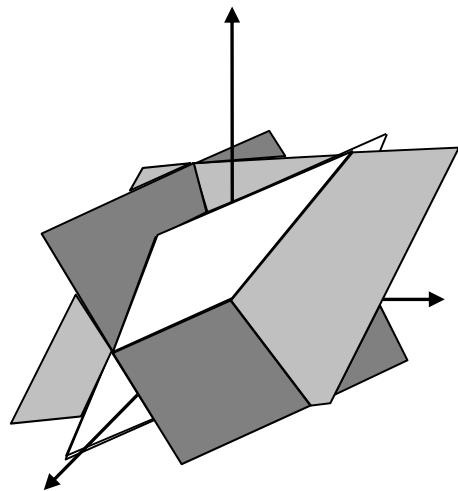
 Klug A. 2010.
Annu. Rev. Biochem. 79:1–35



Klug A

DeRosier DJ, Klug A. 1968. *Nature* 217:130–134

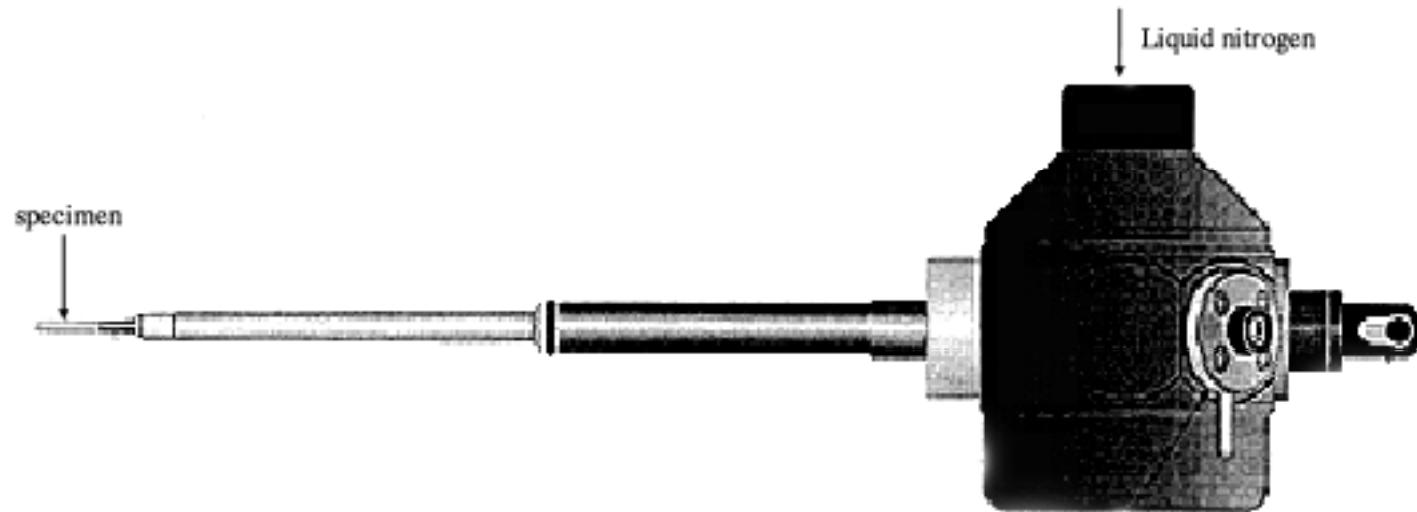
If the particle has no symmetry, projection images are needed for essentially all possible views



- This Euler sphere representation (which is like a flat map of the globe) shows each projection image in a cryoEM data set as a dot
- The entire Euler sphere should be spanned for an asymmetric particle.

Collect Cryo-Micrographs

Cryo Sample Holder



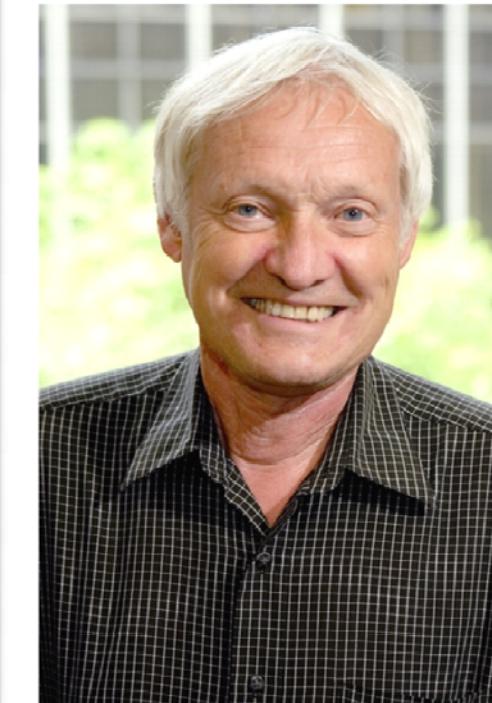
- The frozen sample grid is normally kept at liquid nitrogen temperature (approximately -185° C) while in the vacuum of the microscope by a cryo-holder.
- Liquid helium microscopes allow the sample grid to be kept even colder (~12 K, -261° C) in the microscope.

Collect Cryo-Micrographs

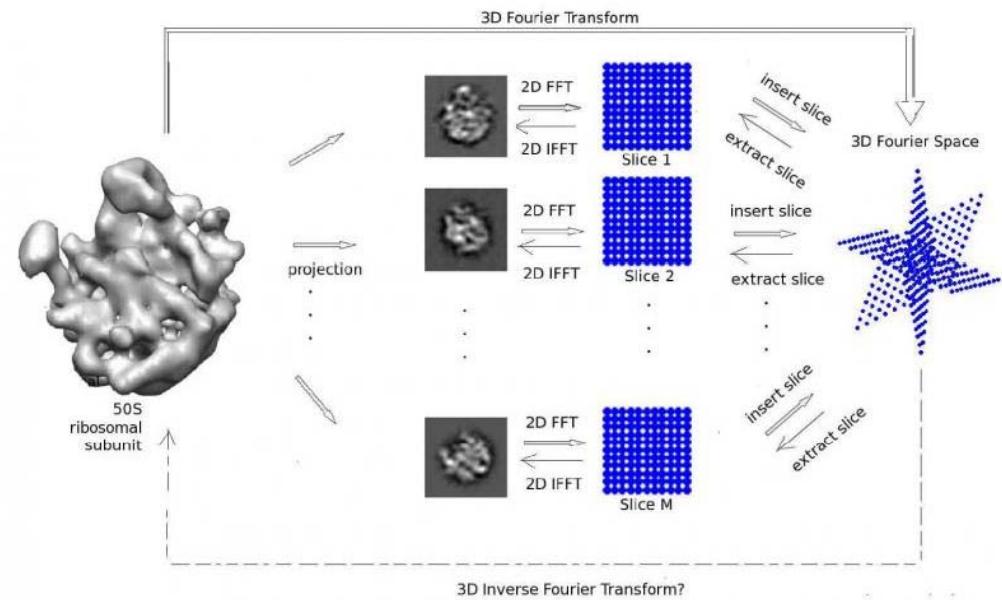


- Individual particle images must be selected from digital cryo-electron micrographs. This has been done interactively (non-automatically) in the past. Software is being developed for automatic particle selection.

Cryo-EM : Single particle 3-D reconstruction

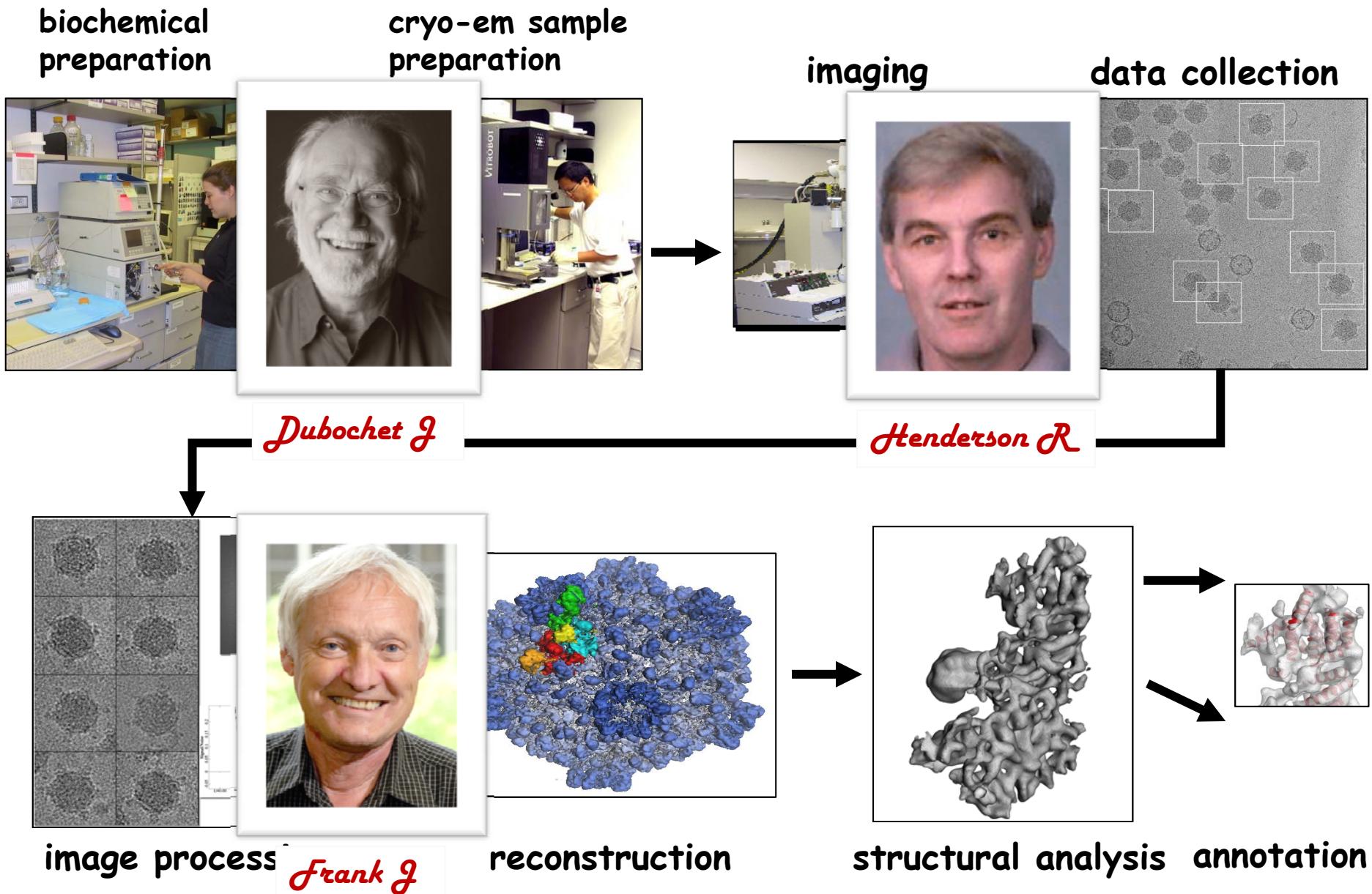


Frank J

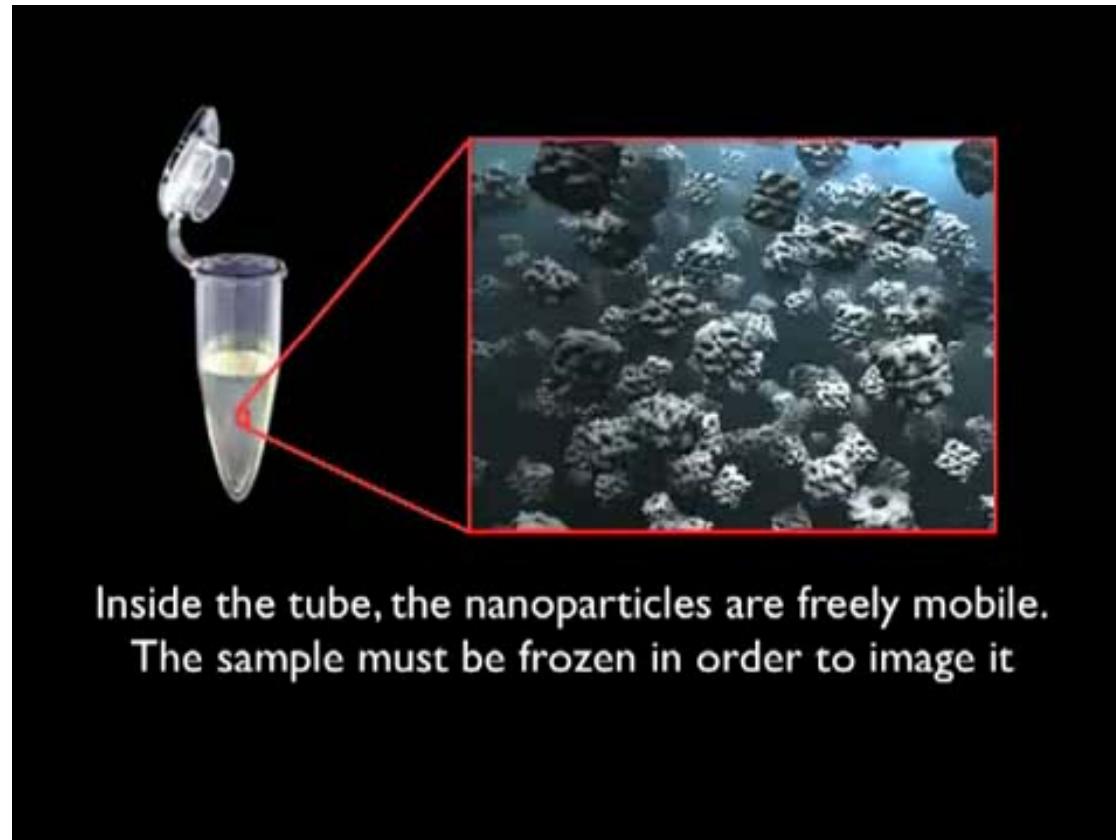


- Frank developed the method of SPA
- Frank and colleagues developed the 1st SPA software-SPIDER

Cryo-Electron Microscopy

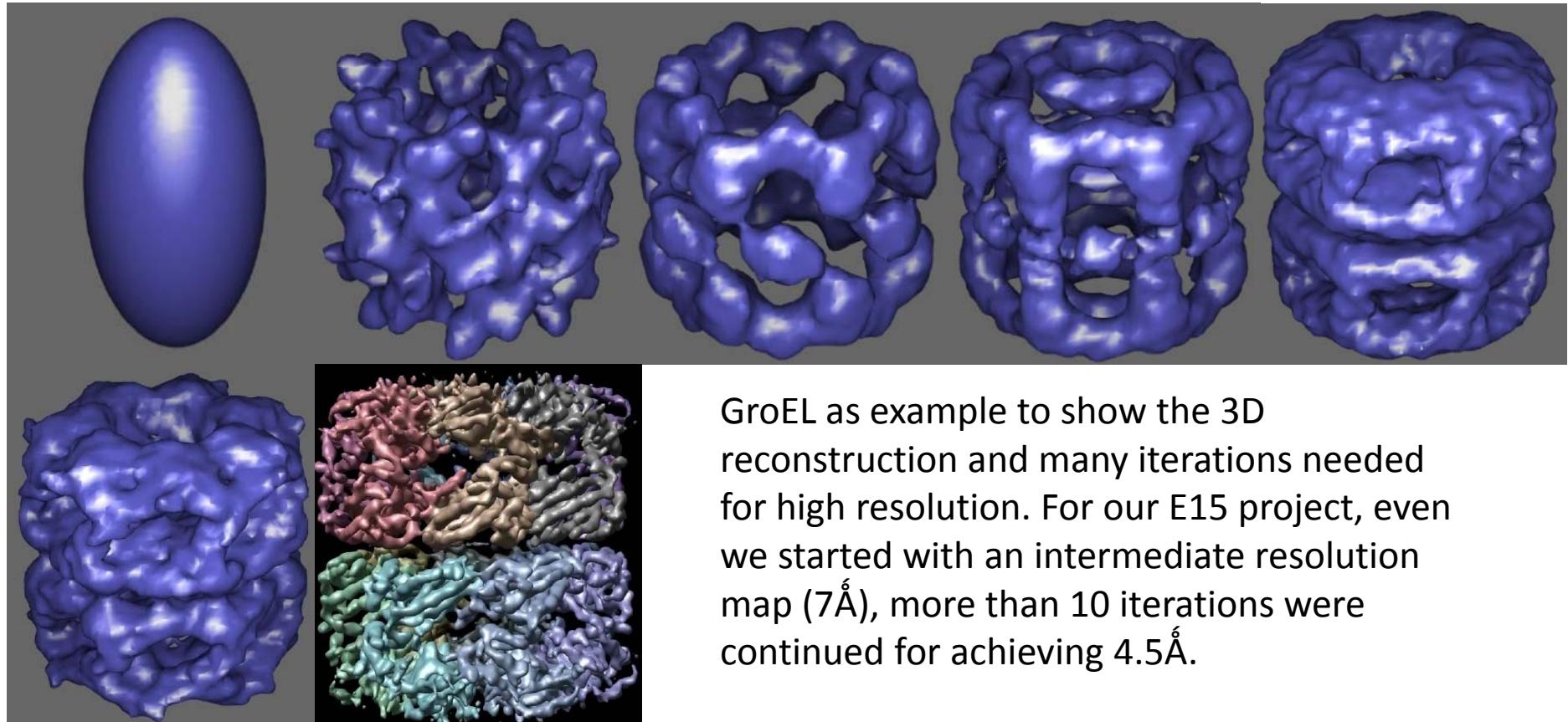


Cryo-EM single-particle 3-D reconstruction



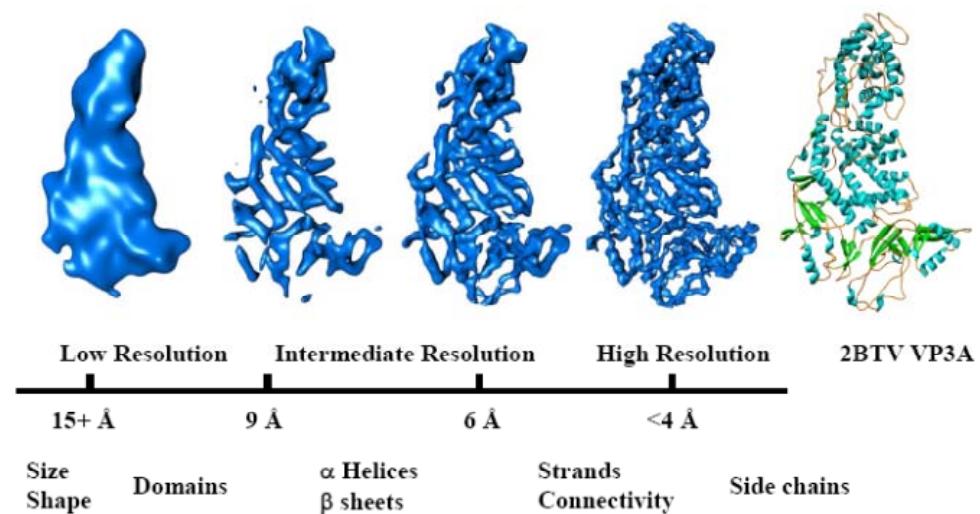
Cryo-EM 3D Reconstruction

Model building



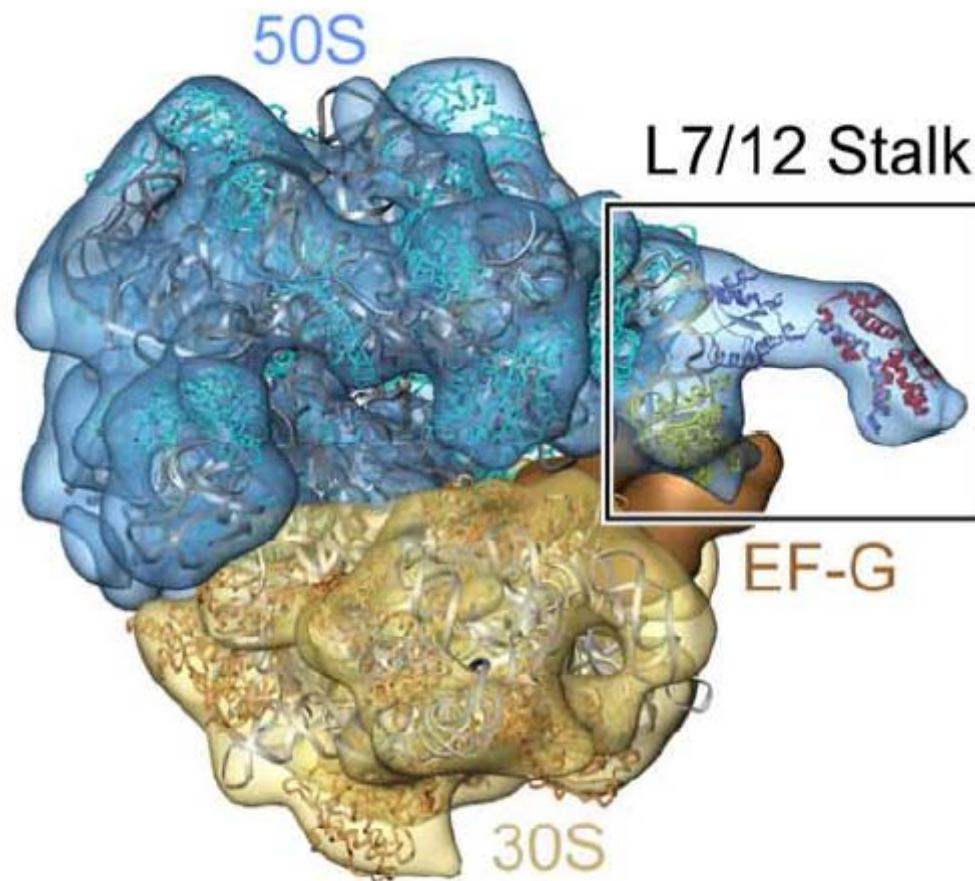
GroEL as example to show the 3D reconstruction and many iterations needed for high resolution. For our E15 project, even we started with an intermediate resolution map (7\AA), more than 10 iterations were continued for achieving 4.5\AA .

Features as a function of resolution to show how to evaluate the resolution qualitatively from density map



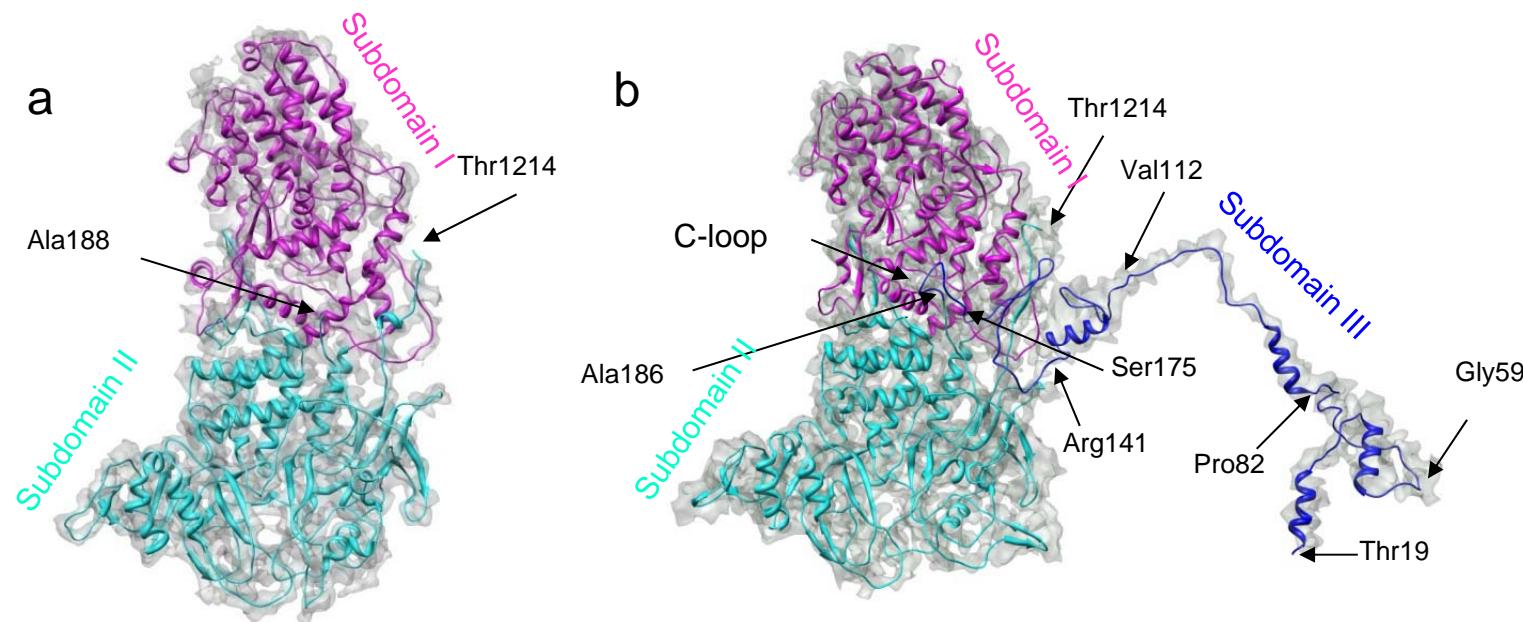
Structural Analysis & Modeling

- ◆ If 10~20 Å resolution is reached, we can try to interpret 3D map,
e.g. try to fit known crystal structures into electron density map



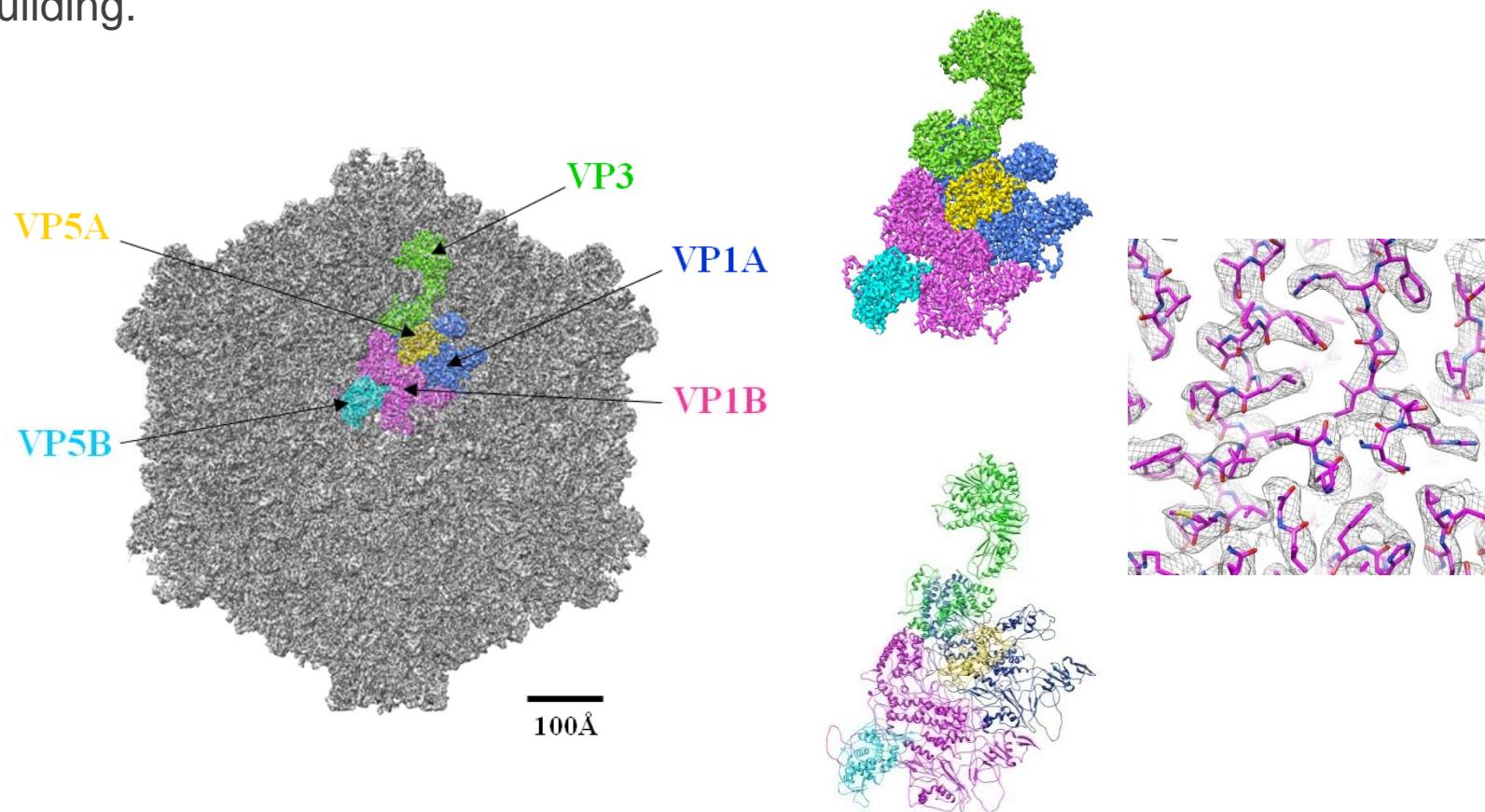
Structural Analysis & Modeling

- ◆ If ~5-6 Å resolution is reached, approaches fitting the cryoEM density with atomic structures of components help to interpret the cryoEM density and build the pseudomodel.



Structural Analysis & Modeling

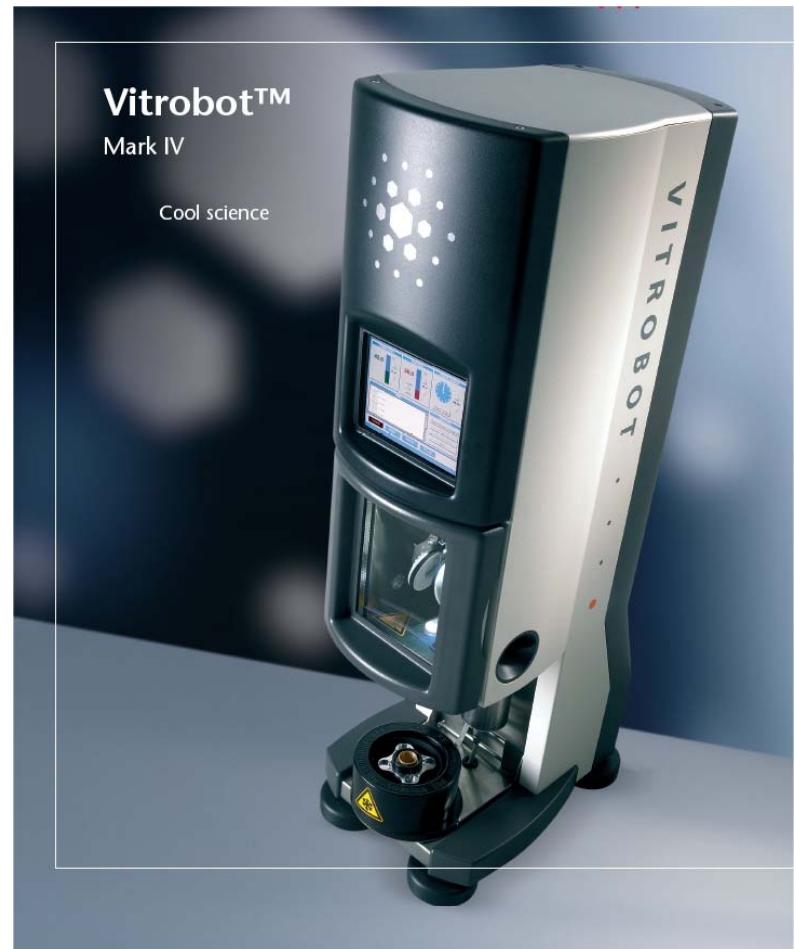
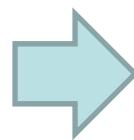
- ◆ If ~3-4 Å resolution is reached, we can do the de-novo atomic model building.



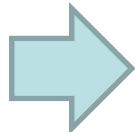
Advances in cryo-EM

single particle 3-D reconstruction

Cryo Plunging Device



Cryo Sample Transfer

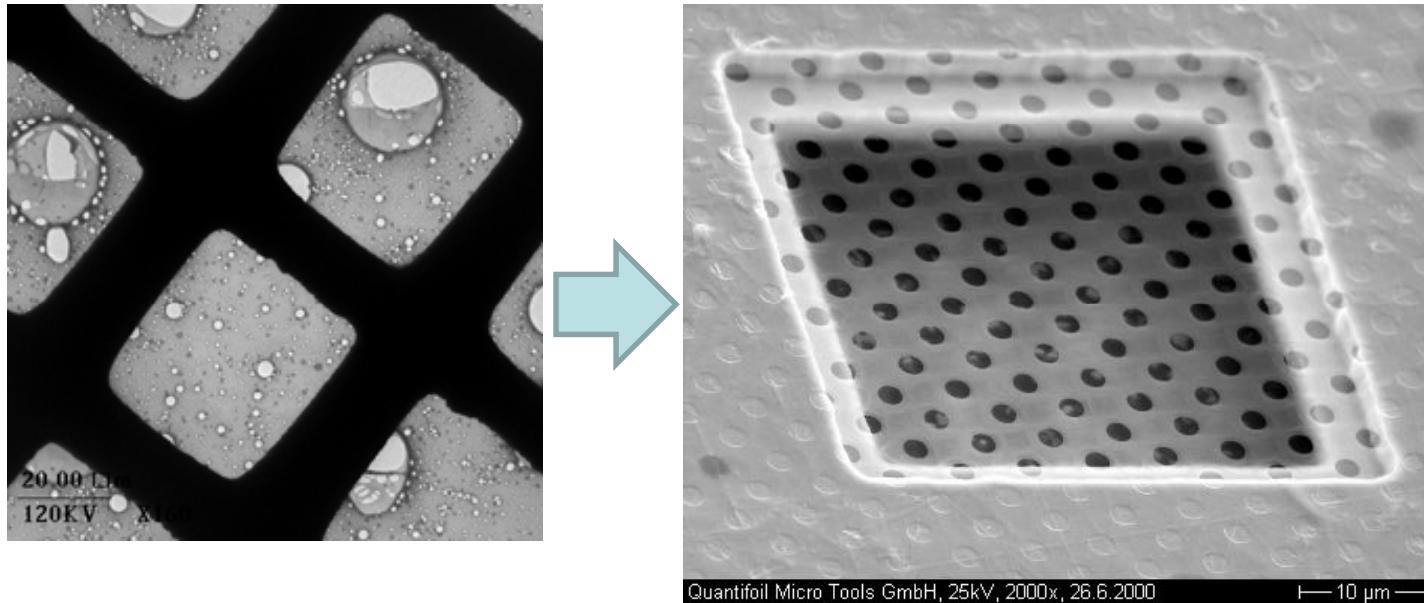


Titan Krios:

Fully Automatic EM grid Loading

12 Grids one time

EM Sample Grids



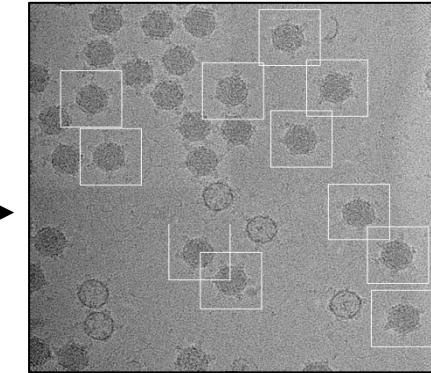
- Quantifoil Grid
- Automatic Point search and data collection

Advances in cryo-EM

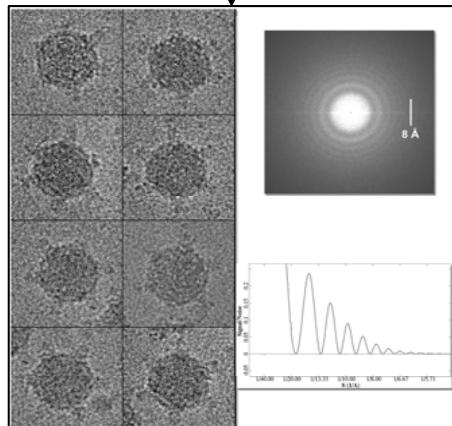
biochemical preparation



Automated acquisition and particle selection



Automated structure determination



Automated discovery

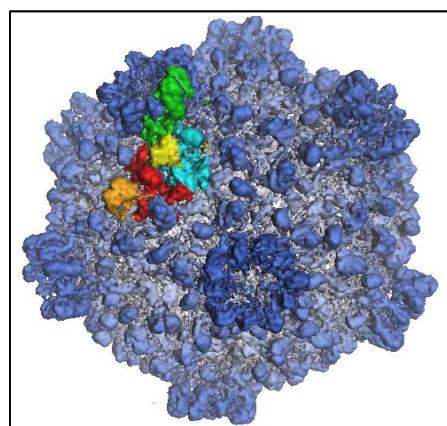
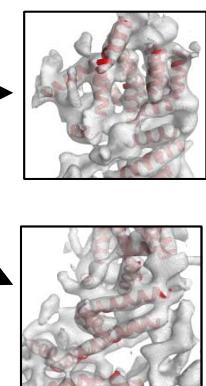
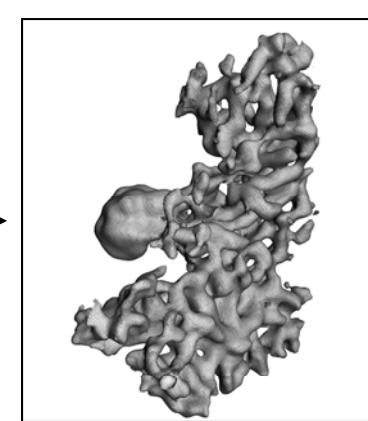


image processing

reconstruction

structural analysis annotation



Film -> CCD -> Direct Detector

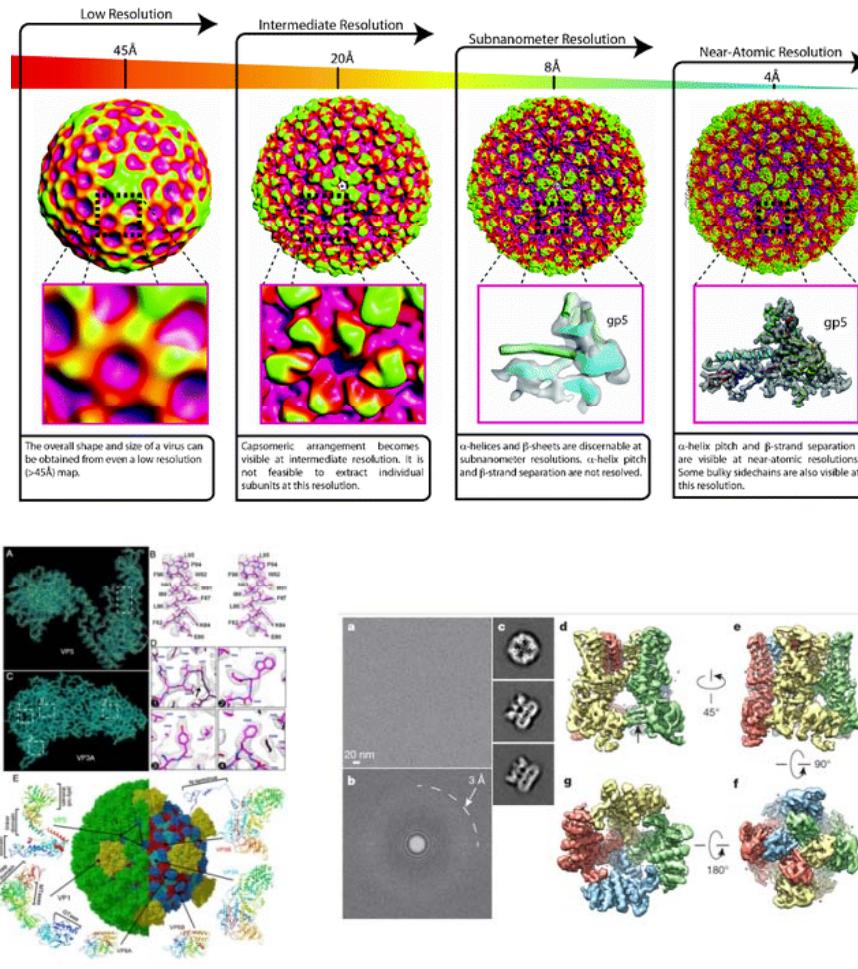
	Ceta (CMOS)	Eagle 2K (CCD)	Eagle 4K (CCD)	Falcon (CMOS, DDD)
CCD field of view:	60X60mm ² (~47%)	61.2 x 61.2 mm ² (47%)	61.2 x 61.2 mm ² (47%)	5.7 x 5.7 cm ² (~47%)
CCD size:	4,096 x4,096 pixels 14 x 14 μm^2	2048 x 2048 pixels 30 x 30 μm^2	4096 x 4096 pixels 15 x 15 μm^2	4096 x 4096 pixels 14 x 14 μm^2
Read-out speed:	1fps (4kx4k) 8fps (2kx2k) 18fps (1kx1k) 25fps (512x512)	0.4fps 2.5 Mpix/sec, 2.5 sec/full frame (4-port read-out)	0.2fps 4 Mpix/sec, 5 sec/full frame (4-port read-out)	0.4fps 2.5 sec/image

CCD



Direct
detector

Advances in cryo-SPA



近年来：

- 冷冻电镜向近原子分辨率方向取得重要突破，最高精度1.8 Å左右
- 病毒分子的氨基酸侧链清晰可见
- 基于冷冻电镜密度图构建病毒分子的三维空间结构模型成为可能

» Single-particle electron cryomicroscopy

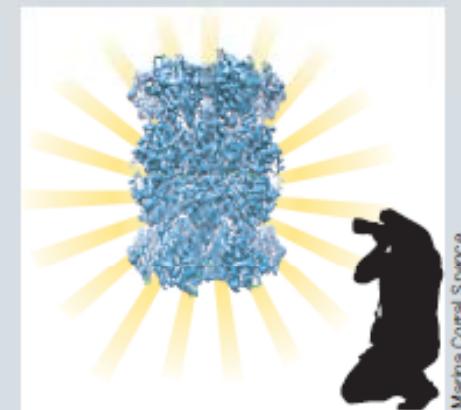
Single-particle electron cryomicroscopy reaches for atomic resolution.

Though first described several decades ago, single-particle electron cryomicroscopy (cryo-EM) has been a relatively specialized technique, straddling the shadowy area of structural biology between light microscopy and X-ray crystallography. Very recent technology advances that substantially improve the resolution of cryo-EM are currently reenergizing the field.

In a single-particle cryo-EM experiment, macromolecular assemblies are frozen in a thin layer of ice and imaged with an electron microscope. Thousands to millions of im-

ages have long been recognized that cryo-EM has the potential to reach atomic resolution, severe technical limitations have gotten in the way. These have included difficulties in producing sufficient amounts of sample, structural heterogeneity, radiation damage, electron beam-induced sample motion and poor camera efficiency.

Until very recently, cryo-EM users have had two options for capturing electron microscope images: an inefficient digital charge-coupled device (CCD) camera or inconvenient photographic film. New cameras that detect electrons directly allow much faster and more efficient image collection than either previous option, addressing several of the above limitations. These direct electron-sensing cameras allow a movie of a sample to be recorded over the



New cameras for cryo-EM promise to generate atomic-resolution macromolecular structures.

near-atomic resolution structures for relatively small, asymmetrical assemblies, the ribosome and proteasome. Notably, the resolution in these studies was so good that substantially fewer images than typical were needed for structure determination.

Combined with rapidly improving

冷冻电镜 《Nature Methods》
“2014年最受关注的技术”之一

Method of the Year 2015

The end of ‘blob-ology’: single-particle cryo-electron microscopy (cryo-EM) is now being used to solve macromolecular structures at high resolution.

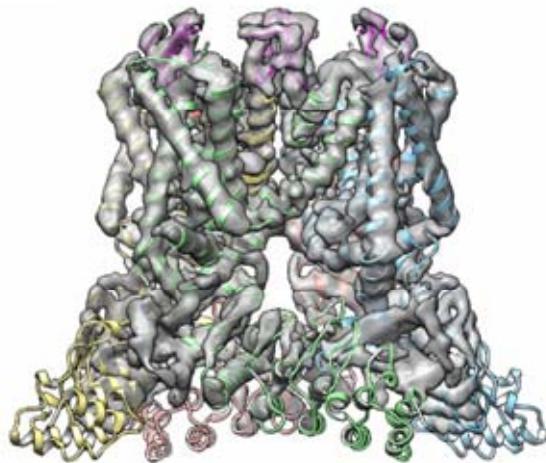
The three-dimensional structure of a protein or protein complex provides crucial insights into its biological function. As a structure-determination technique, cryo-EM has played second fiddle to the higher-resolution approaches of X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. This is rapidly

preparation as well as sophisticated image-processing software tools, as discussed in a Primer on page 23.

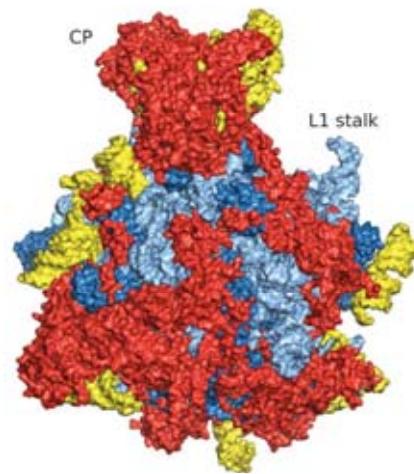
The cryo-EM resolution revolution is really just beginning, as discussed in a Commentary by Robert Glaeser on page 28. The sensitivity enhancements in detector technology have spurred opportunities for

冷冻电镜 《Nature Methods》
“2015年 – 最重要的技术”

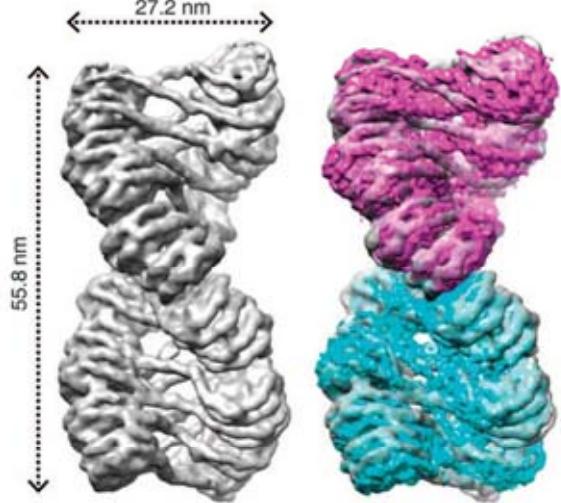
冷冻电镜：重要进展



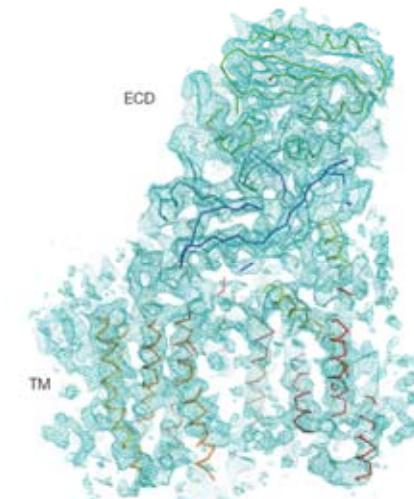
TRPV1, Nature 2013



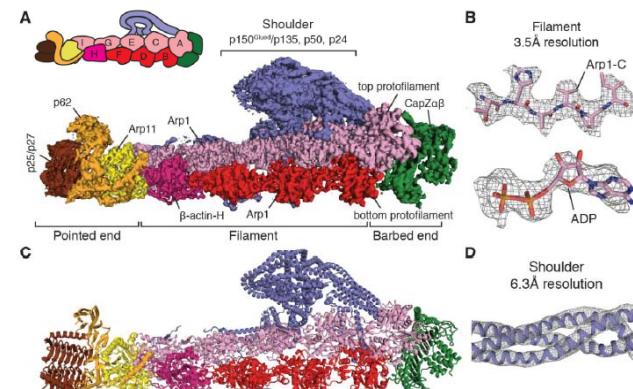
Ribosome, Science 2014



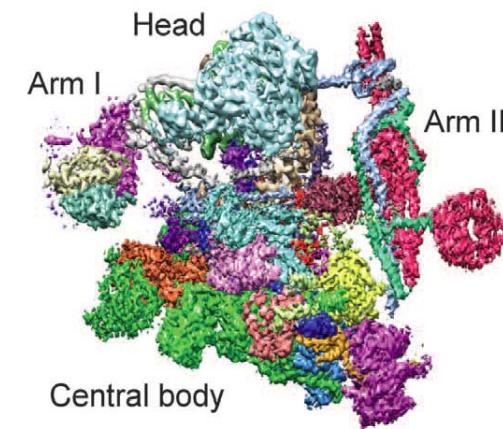
Chromatin, Science 2014



r-secretase, Nature 2014



Dynactin, Science 2015



Spliceosome, Science 2015

冷冻电镜：国内外重要进展

nature International weekly journal of science

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Nature 2015. 4. 23

NATURE | ARTICLE

日本語要約

Structure of the regulatory mech

Candice E. Paulsen, Jean-

Affiliations | Contributions

Nature 520, 511–517 (23 A
Received 26 November 2014

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Structure of the regulatory mech

Heena Khatter, Alexander G.

Affiliations | Contributions

Nature (2015) | doi:10.1038/nat Received 06 January 2015 | Accepted 26 February 2015 | Published online 23 March 2015

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Abstract • Introduction • Structure d interface • tRNA binding sites and m References • Acknowledgements •

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Current Issue > Letters > Article

NATURE | LETTER

日本語要約

Structure of the *E. coli* ribosome–EF-Tu complex at <3 Å resolution by C_s-corrected cryo-EM

Niels Fischer, Piotr Neumann, Andrey L. Konevega, Lars V. Bock, Ralf Ficner, Tatjana Rodnina & Holger Stark

Affiliations | Contributions | Corresponding authors

Nature 520, 567–570 (23 April 2015) | doi:10.1038/nature14275
Received 21 November 2014 | Accepted 30 January 2015 | Published online 23 Feb 2015

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Single particle electron cryomicroscopy (cryo-EM) has recently made significant progress in high-resolution structure determination of macromolecular complexes due to

arch

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deo | For Authors

Editor's summary

العربية

One of the cell's largest and most important macromolecular complexes, the ribosome has been the target of intensive structural study. Until now, crystallographic studies have provided the highest res...

▼

a nature conference

Nature Genetics and the Wellcome Trust present:

The Genomics of Common Diseases 2015

September 2-5, 2015
Hinxton, Cambridge, UK

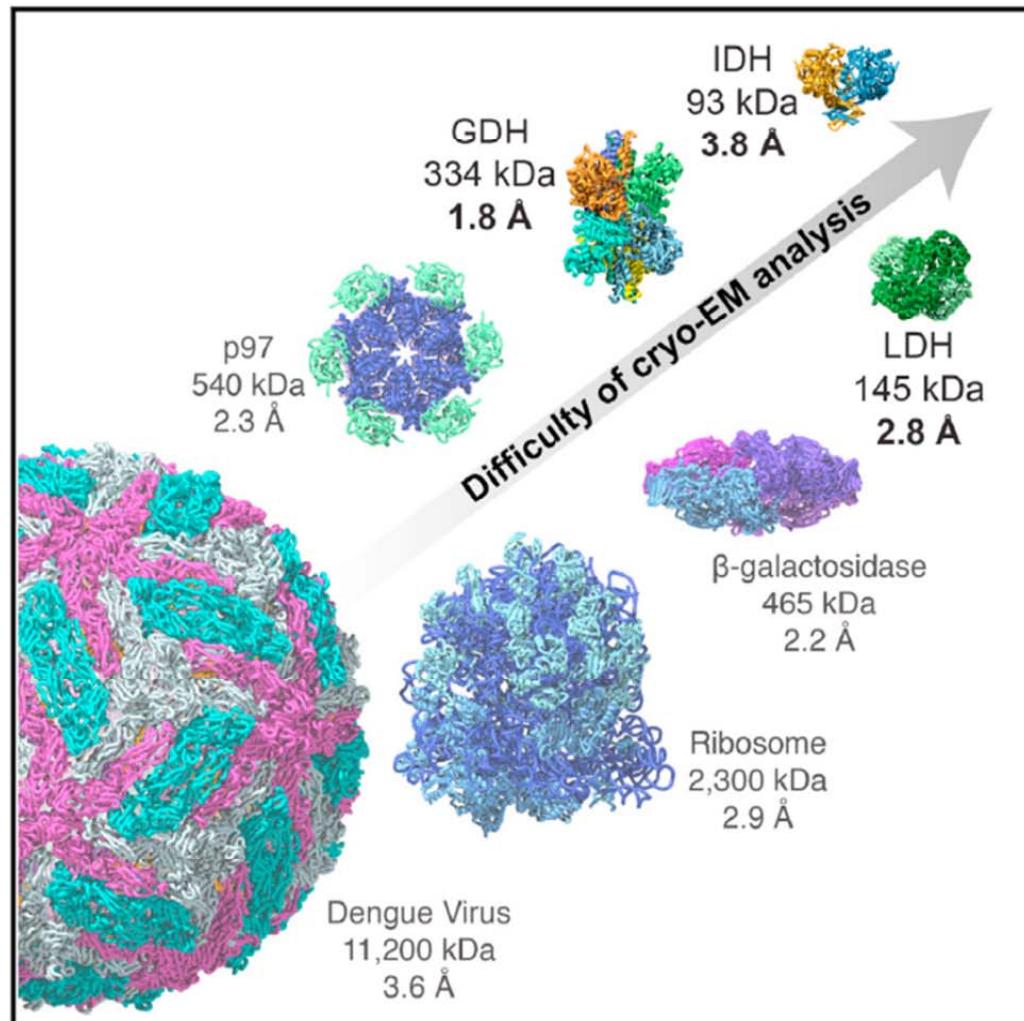
Register today!



Breaking Cryo-EM Resolution Barriers to Facilitate Drug Discovery

3D reconstruction of purified sample

Graphical Abstract



Authors

Alan Merk, Alberto Bartesaghi,
Soojay Banerjee, ..., Lesley A. Earl,
Jacqueline L.S. Milne,
Sriram Subramaniam

Correspondence

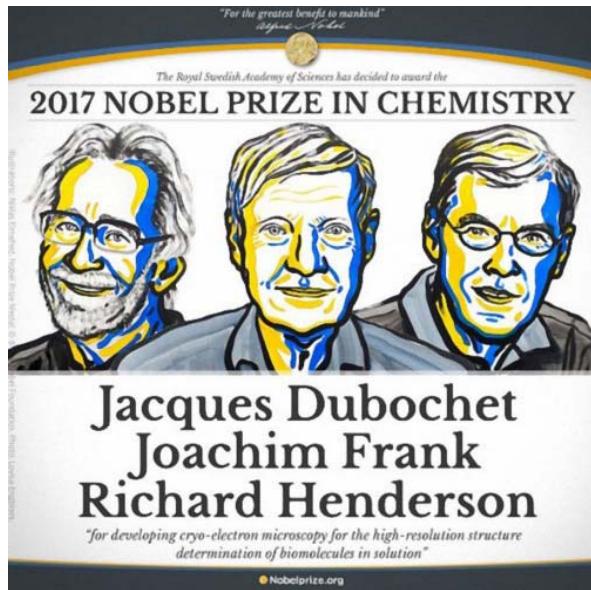
ss1@nih.gov

In Brief

By using cryo-EM methods, the structure of small metabolic enzymes as well as the localization of small-molecule inhibitors that bind to them can be determined at near-atomic resolution.

64 KD-3.2 Å
with phase plate
(2017)

Cryo-Electron Microscopy

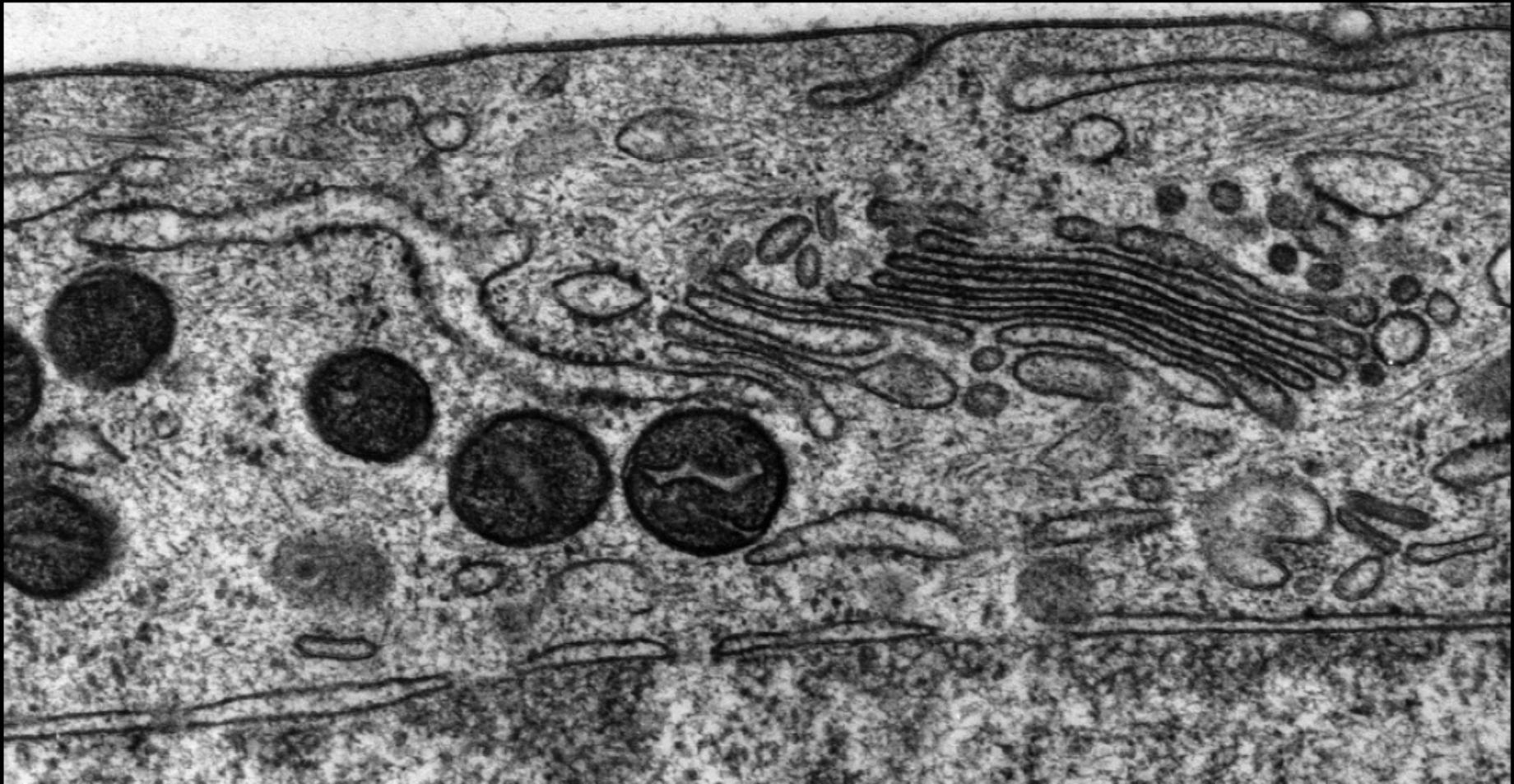


Cryo-EM : 2017 Nobel Prize in Chemistry

Cryo-EM 3D Reconstruction

Electron Tomography

Why Tomography?

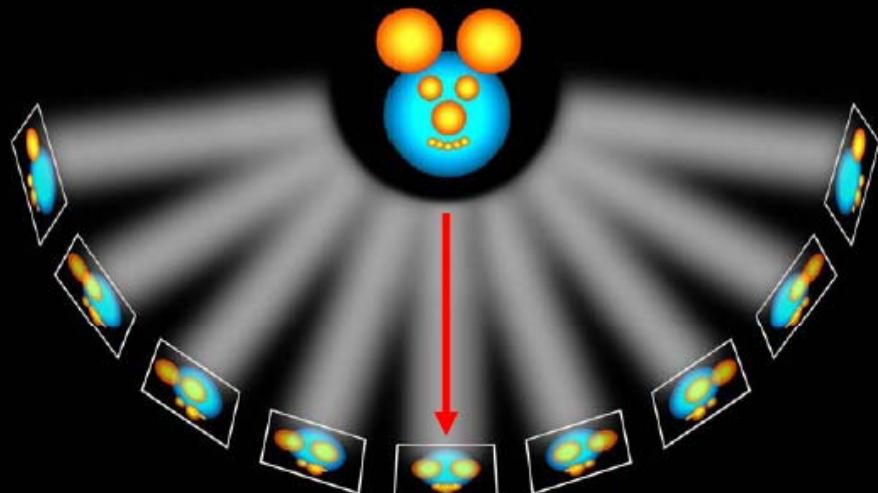


Tomography is needed to resolve features in the 1-20 nm size range

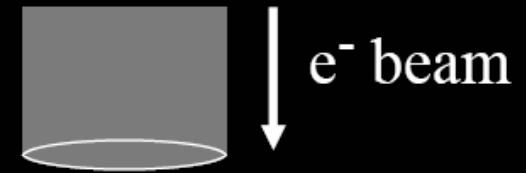
- To determine connectivity and relationships in 3-D
- To determine 3-D structure of organelles and associated macromolecules

Principle of electron tomography

- 3D object
- ⇒ set of 2D projections

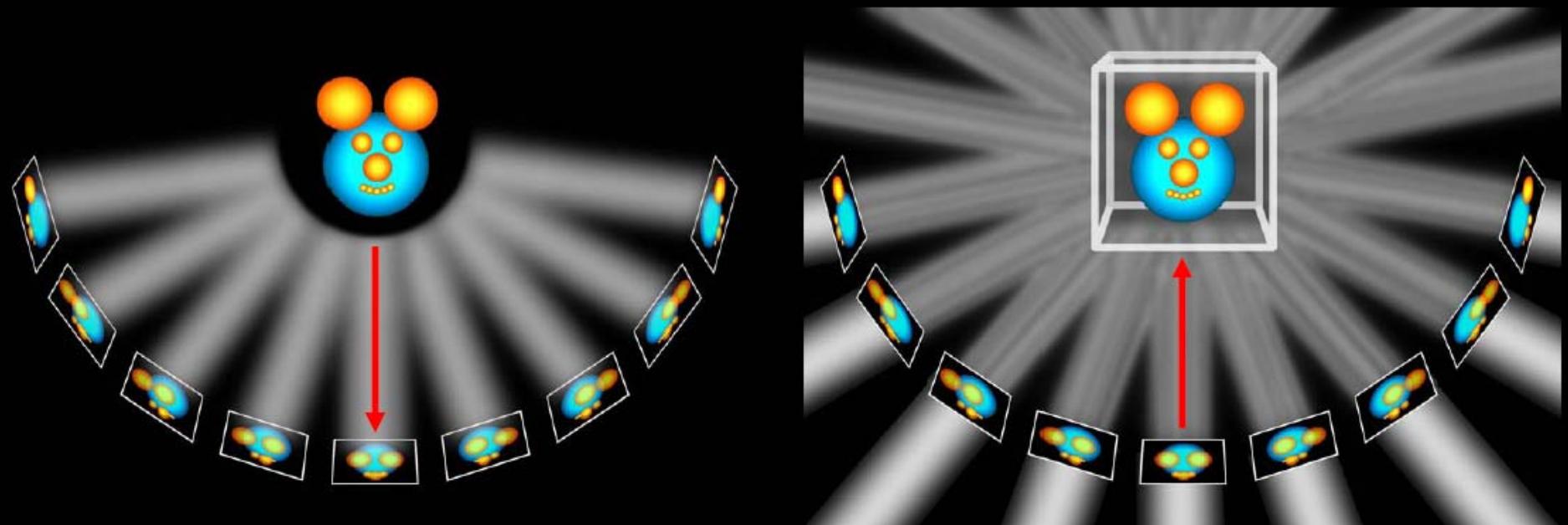


set of projections
 $(\pm 90^\circ, 2^\circ \text{ increment})$



Principle of electron tomography

3D object \Rightarrow set of 2D projections \Rightarrow 3D reconstruction



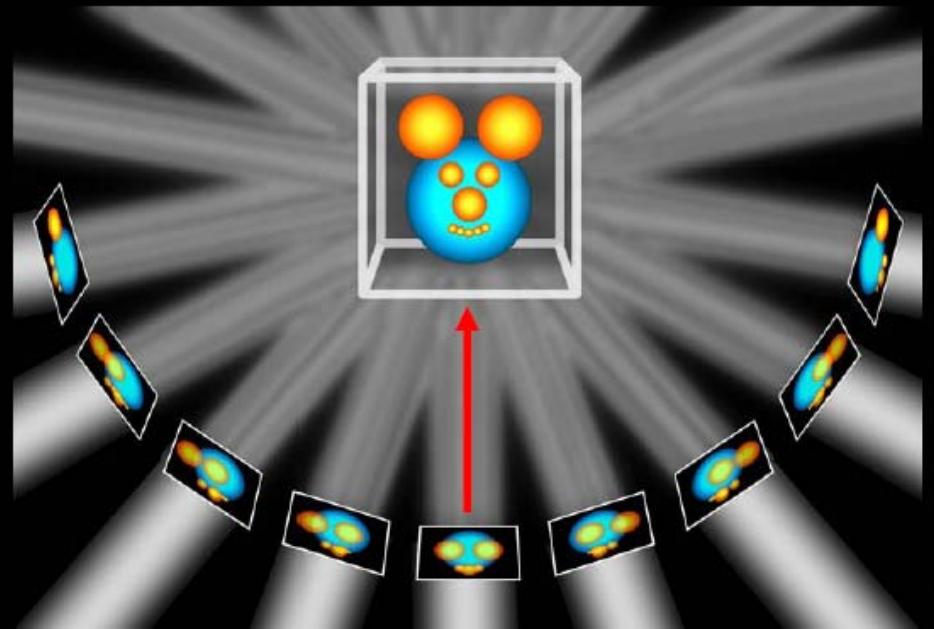
Principle of electron tomography

reconstruction



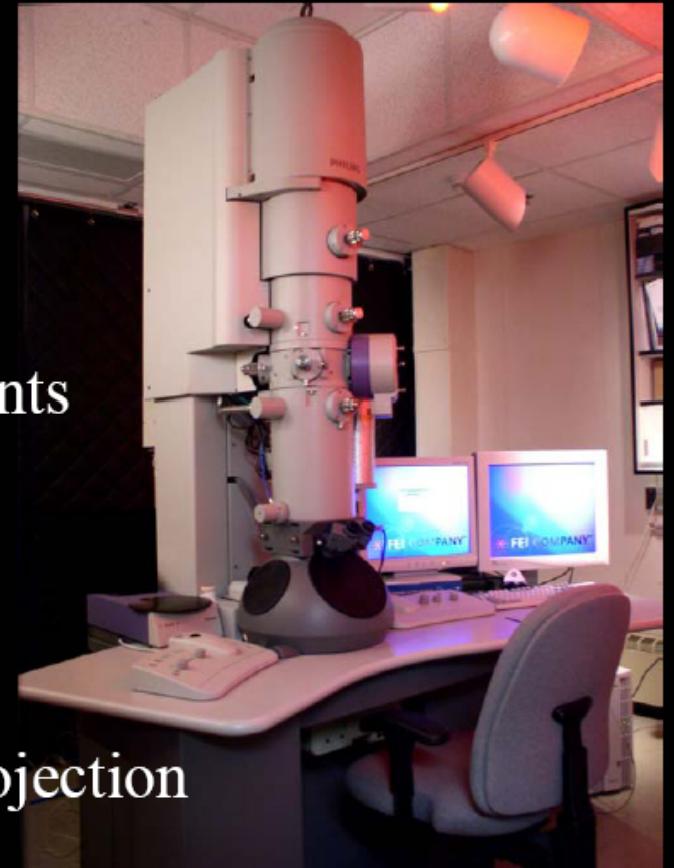
↑ by weighted
backprojection

set of 2D projections
⇒ 3D reconstruction

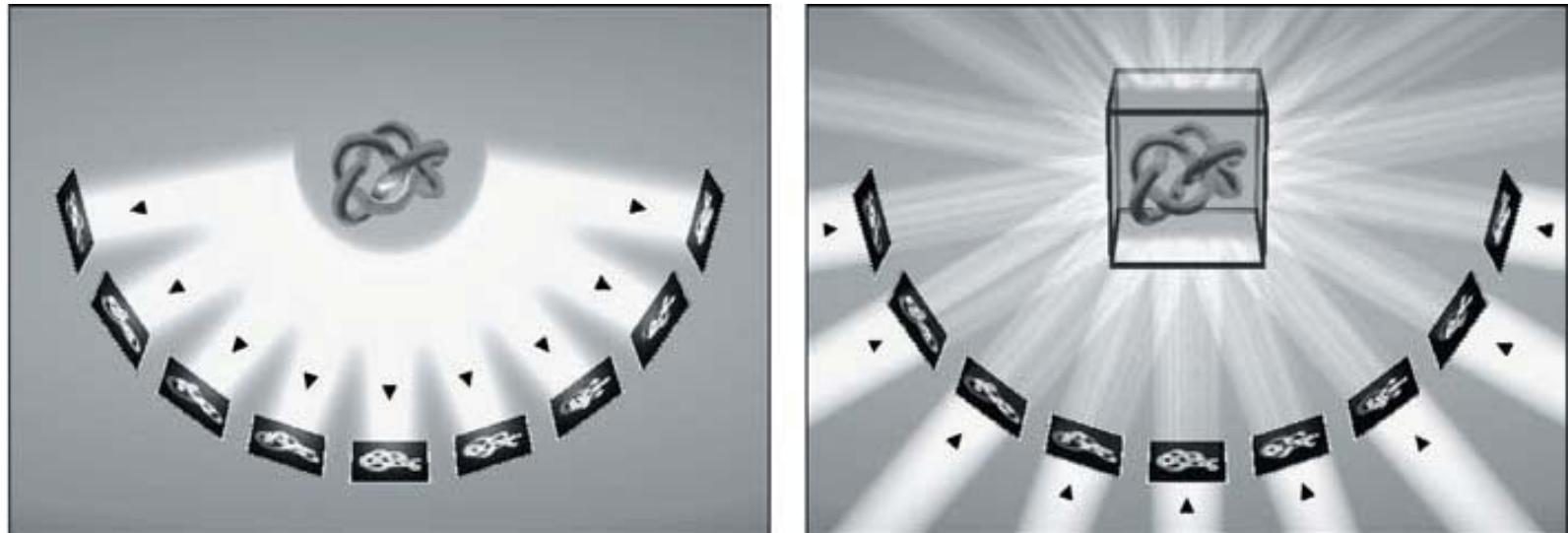


Tomography

- IVM: Tecnai F30 operated at 300KV
- Automated tilt-series acquisition software, SerialEM written by David Mastronarde
- $\pm 65^\circ$ single axis tilt-series at 1-1.5° increments
- Projection alignment using colloidal gold as fiducials (or by cross-correlation methods) and
Tomograms computed by weighted back-projection (using IMOD/Etomo software)

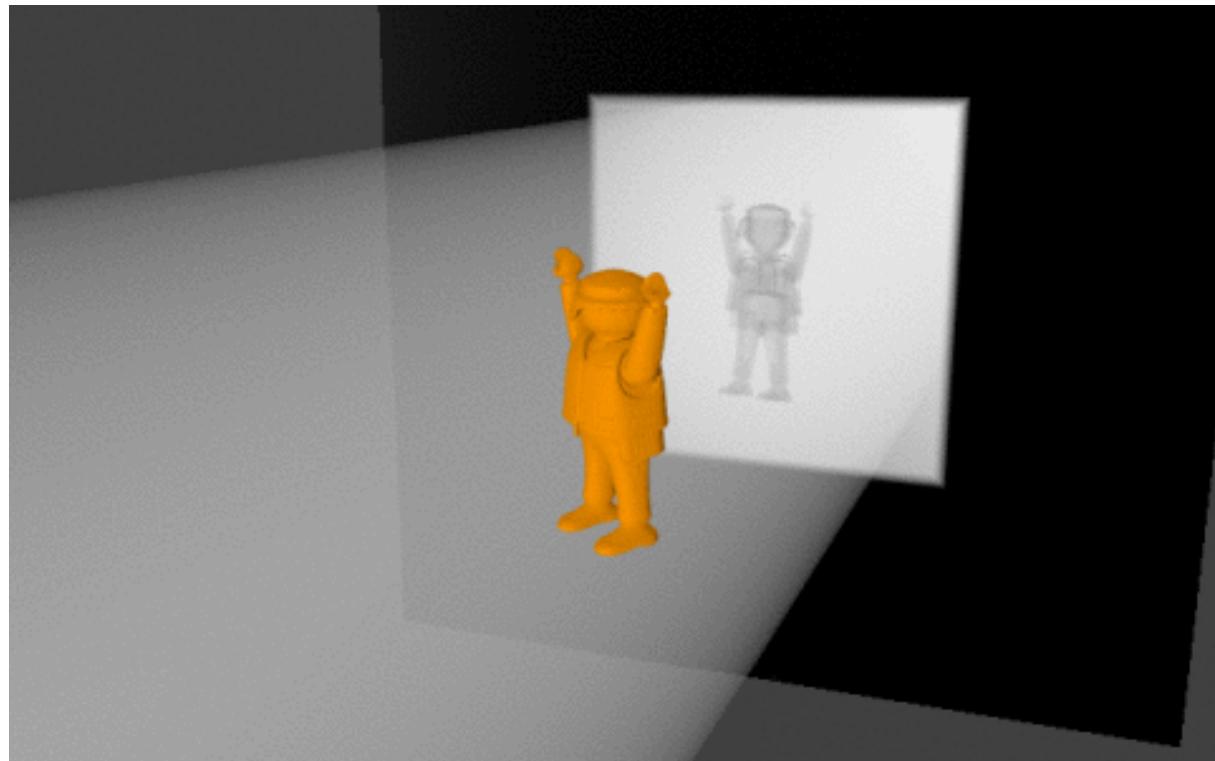


Electron Tomography



Limitation of Electron Tomography:

Limited tilt angle range → "Missing wedge"



ANIMATION. A visual simulation of
tomography

<https://www.cwi.nl/news/2016/vici-grant-joost-batenburg>

Tomography - imaging by sections

Greek - tomos means section/slice/cutting

Radiation source

X-rays

gamma rays

electron-positron annihilation

nuclear magnetic resonance

ultrasound

electrons

ions

Type of tomograph

CT (computed tomography)

SPECT (single photon emission tomography)

PET (positron emission tomog.)

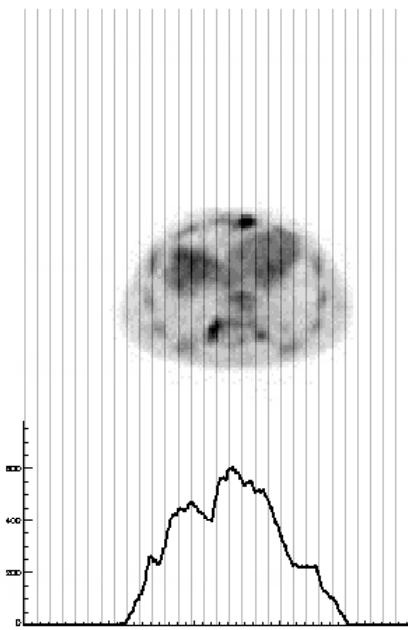
MRI (magnetic resonance imaging)

ultrasonography

3D TEM

atom probe

(True) Emission Volume



intensity profile:



Sinogram (stored data)

Forward
Projection

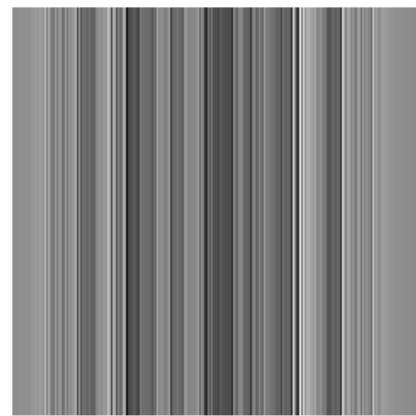
angle
0°

Theta (angle)

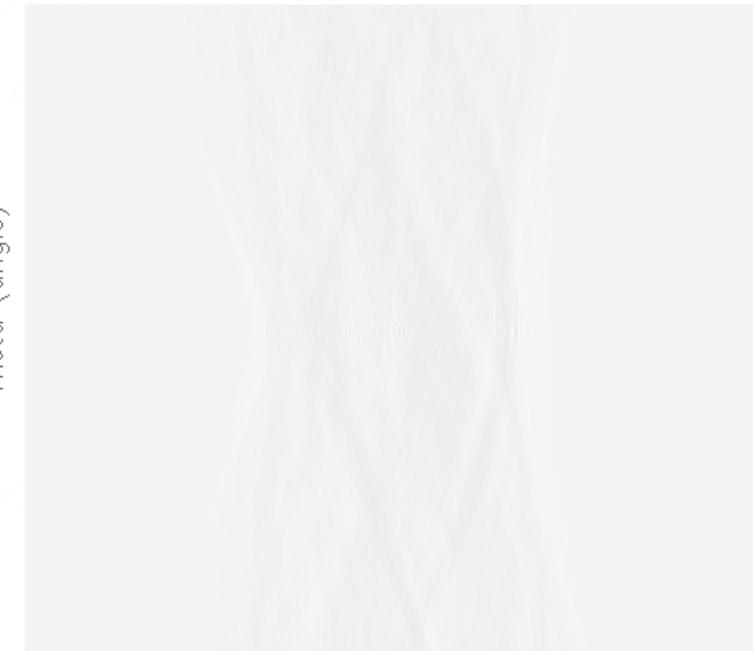
Rho (offset)

ANIMATION. Forward projection of a 2D slice

Reconstructed image



Filtered Sinogram



←
Filtered Back Projection Rho (offset)

ANIMATION. Back-projection with filtered data

Limitation of electron tomography

reconstruction of series with
 $\pm 90^\circ$ tilt angle range



reconstruction of series with
 $\pm 60^\circ$ tilt angle range



Dual-axis tilting and reconstruction

original image



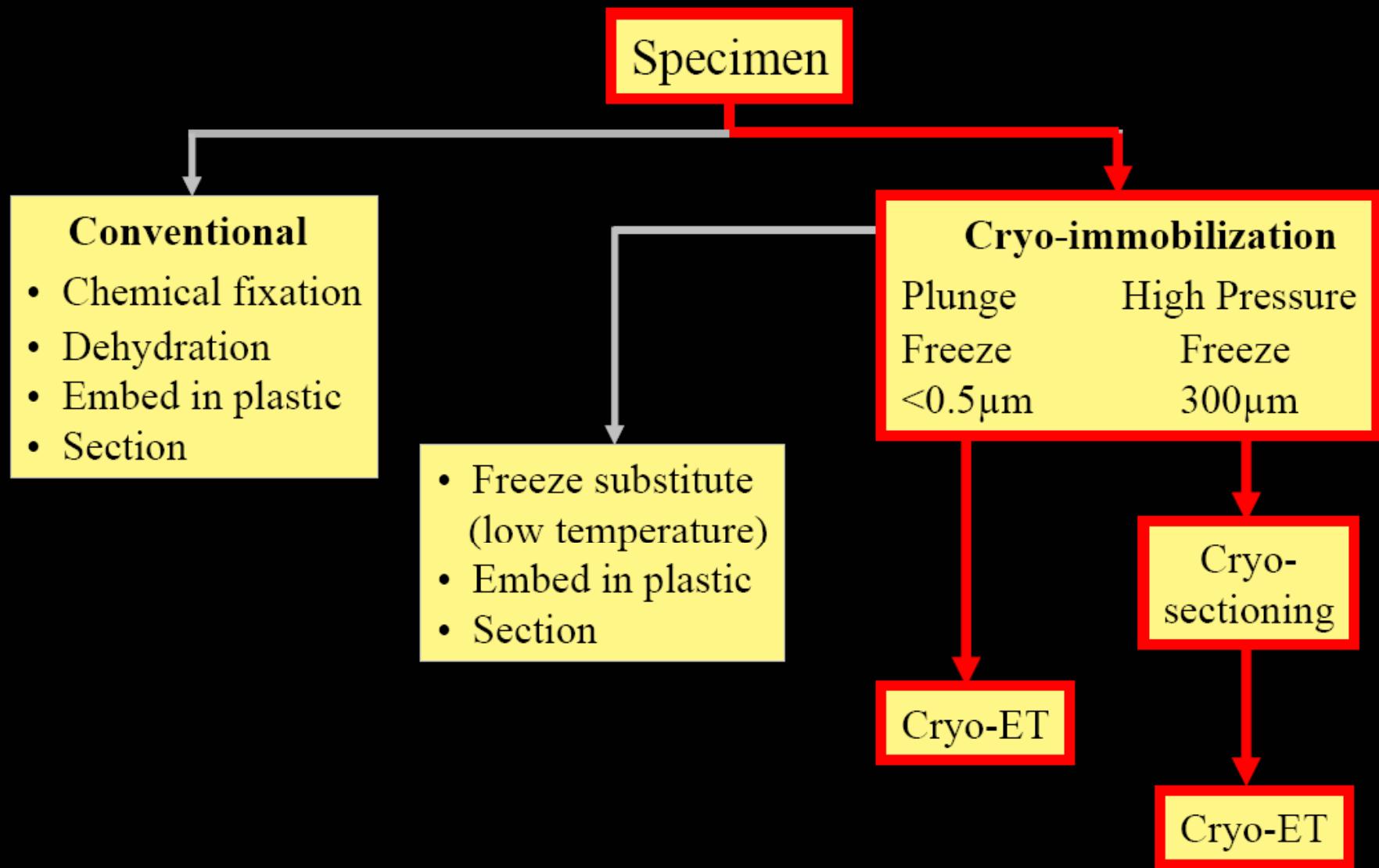
reconstr. of first axis

combined tomogram

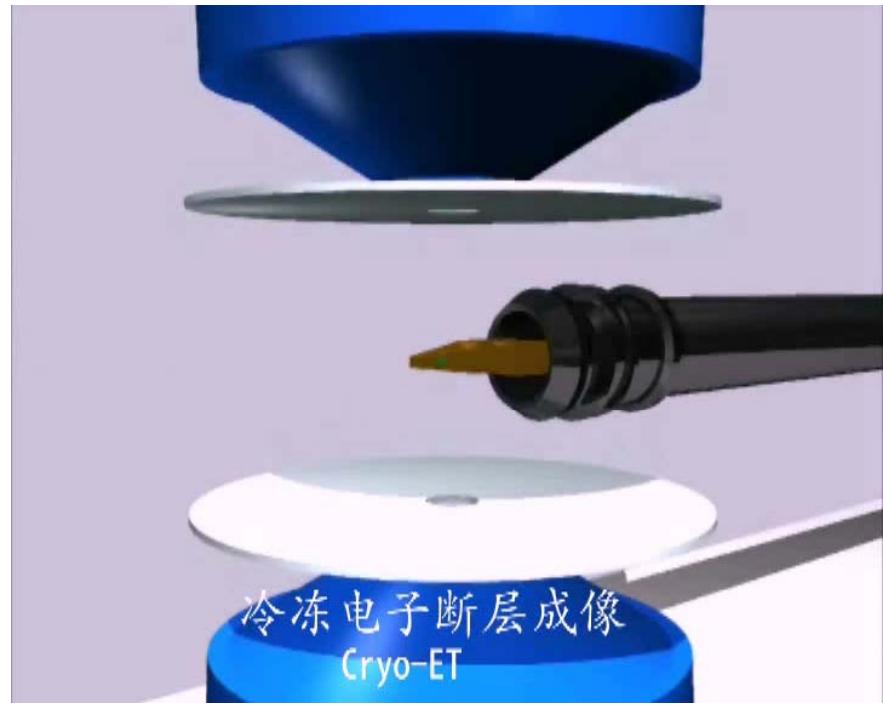
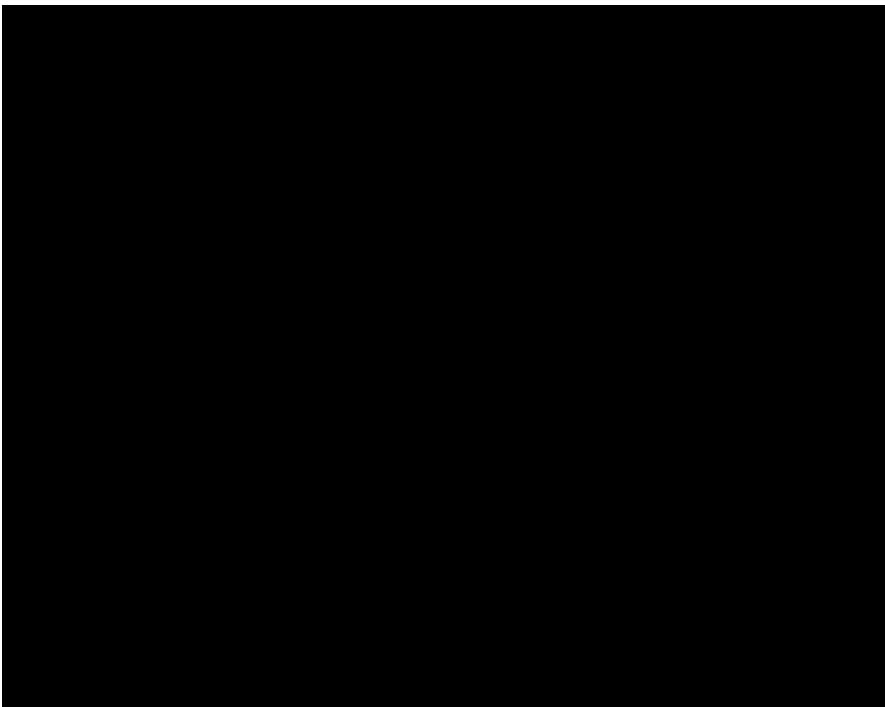


second axis (90° rotated)

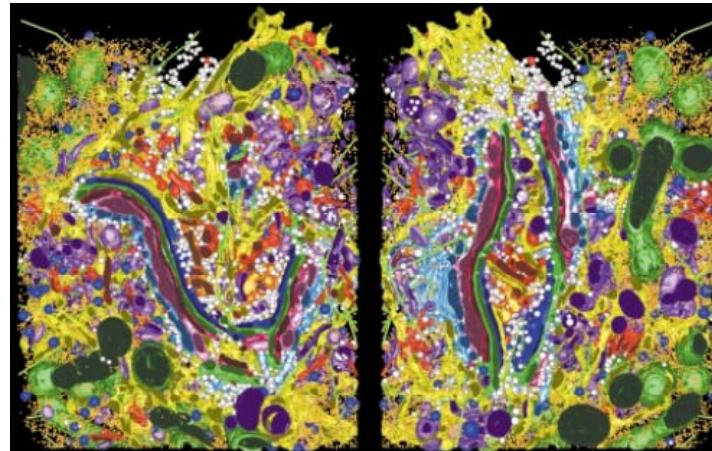
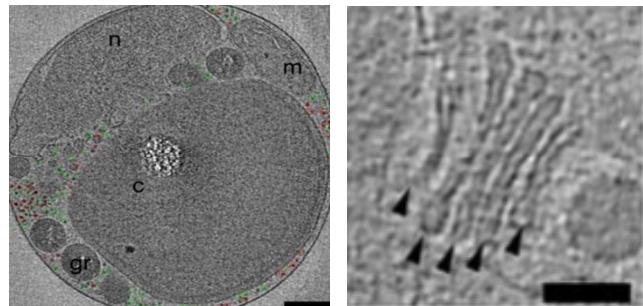
Cryo-Electron Tomography



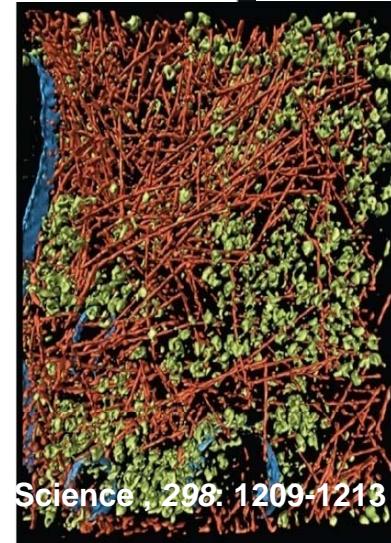
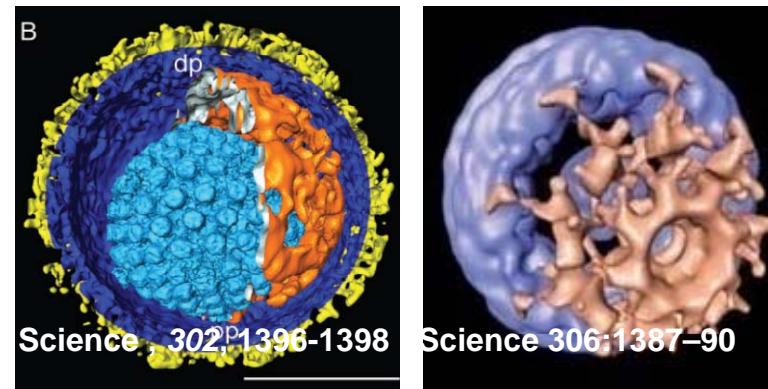
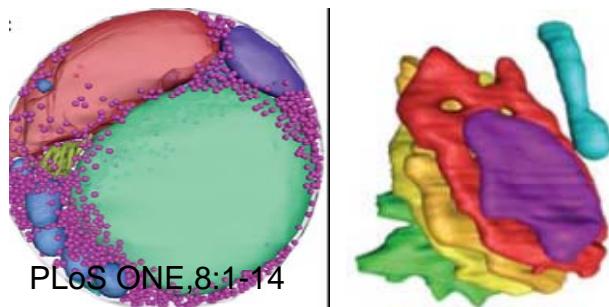
Electron Tomography



Electron Tomography: Examples



PNAS, 98:2399-2406.



Electron Tomography: Examples

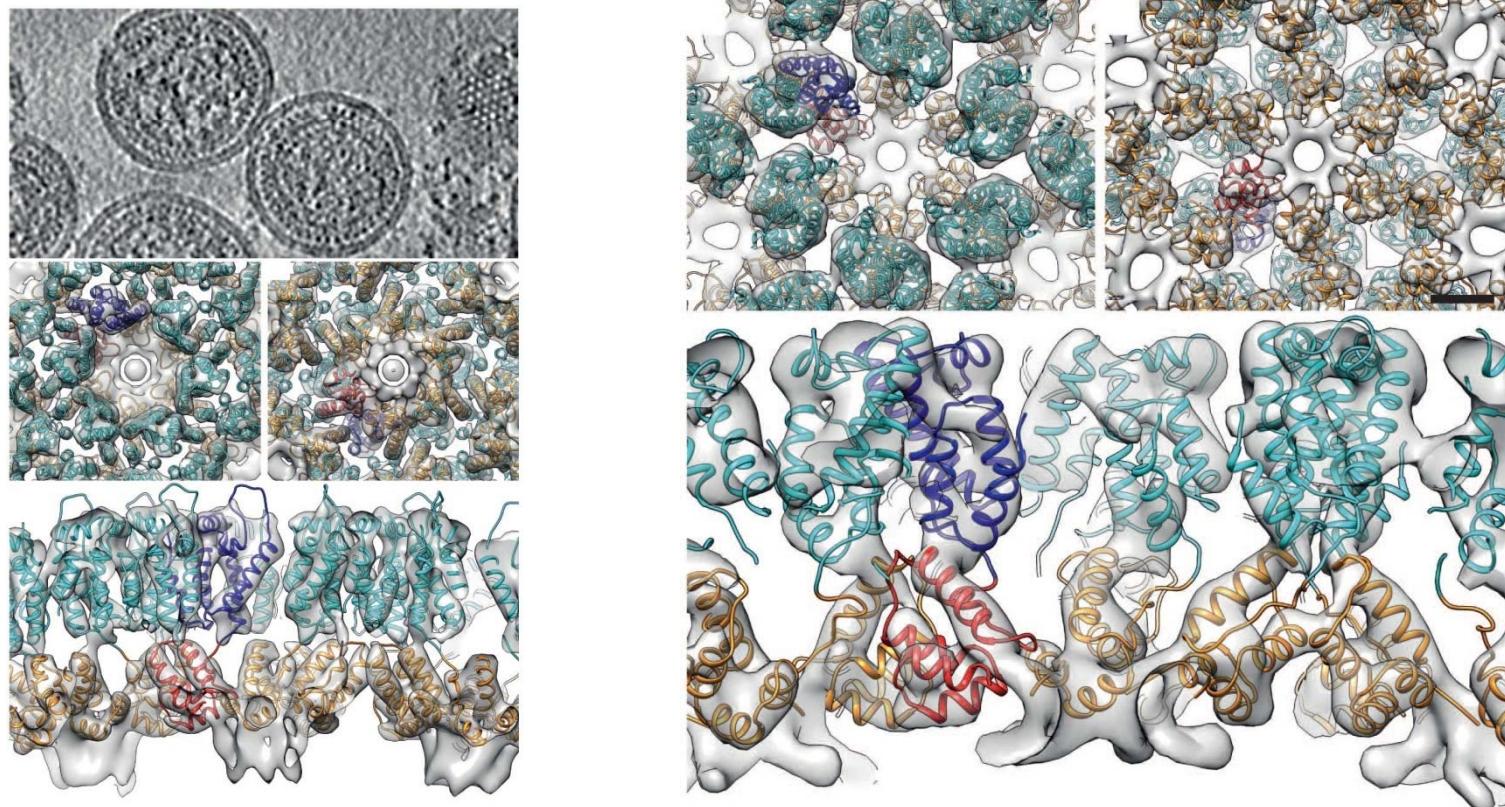
LETTER

doi:10.1038/nature13838

Structure of the immature HIV-1 capsid in intact virus particles at 8.8 Å resolution

Nature 2015. 1. 22

Florian K. M. Schur^{1,2}, Wim J. H. Hagen¹, Michaela Rumlová^{3,4}, Tomáš Ruml⁵, Barbara Müller^{2,6}, Hans-Georg Kräusslich^{2,6}
& John A. G. Briggs^{1,2}



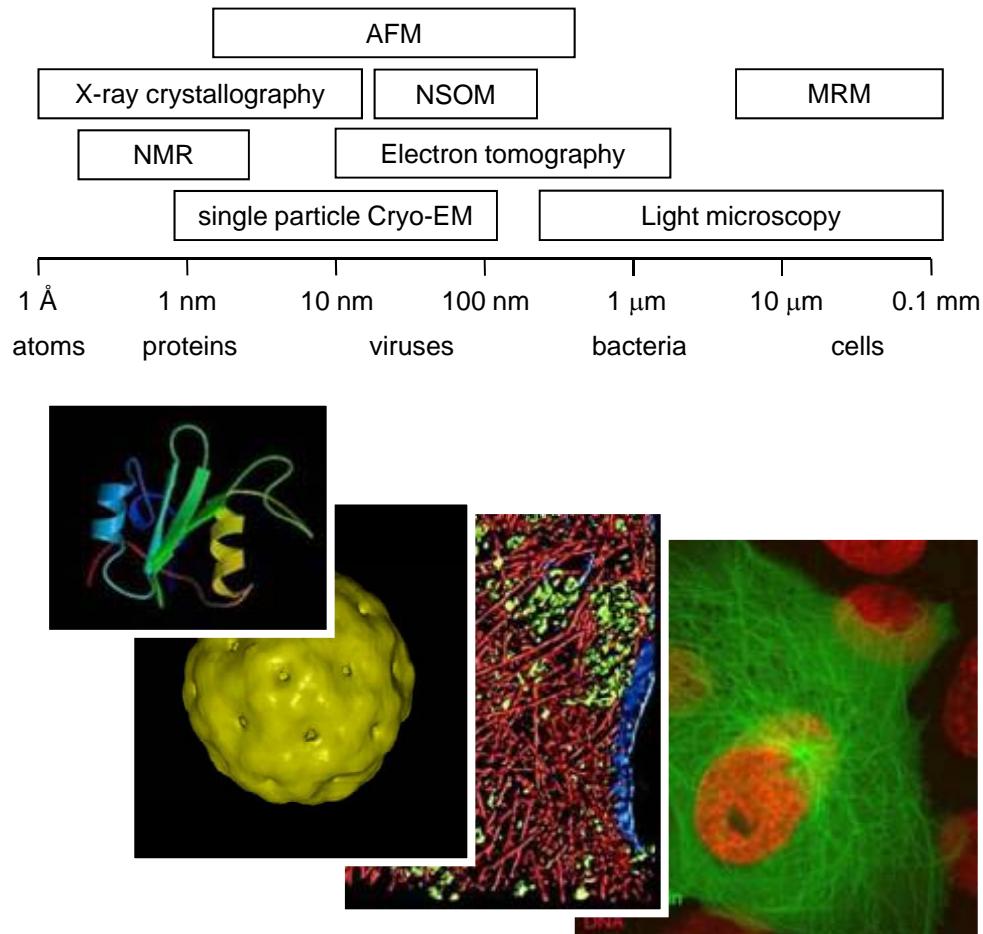
Electron Tomography



Courtesy of Jun Liu, Yale Univ.

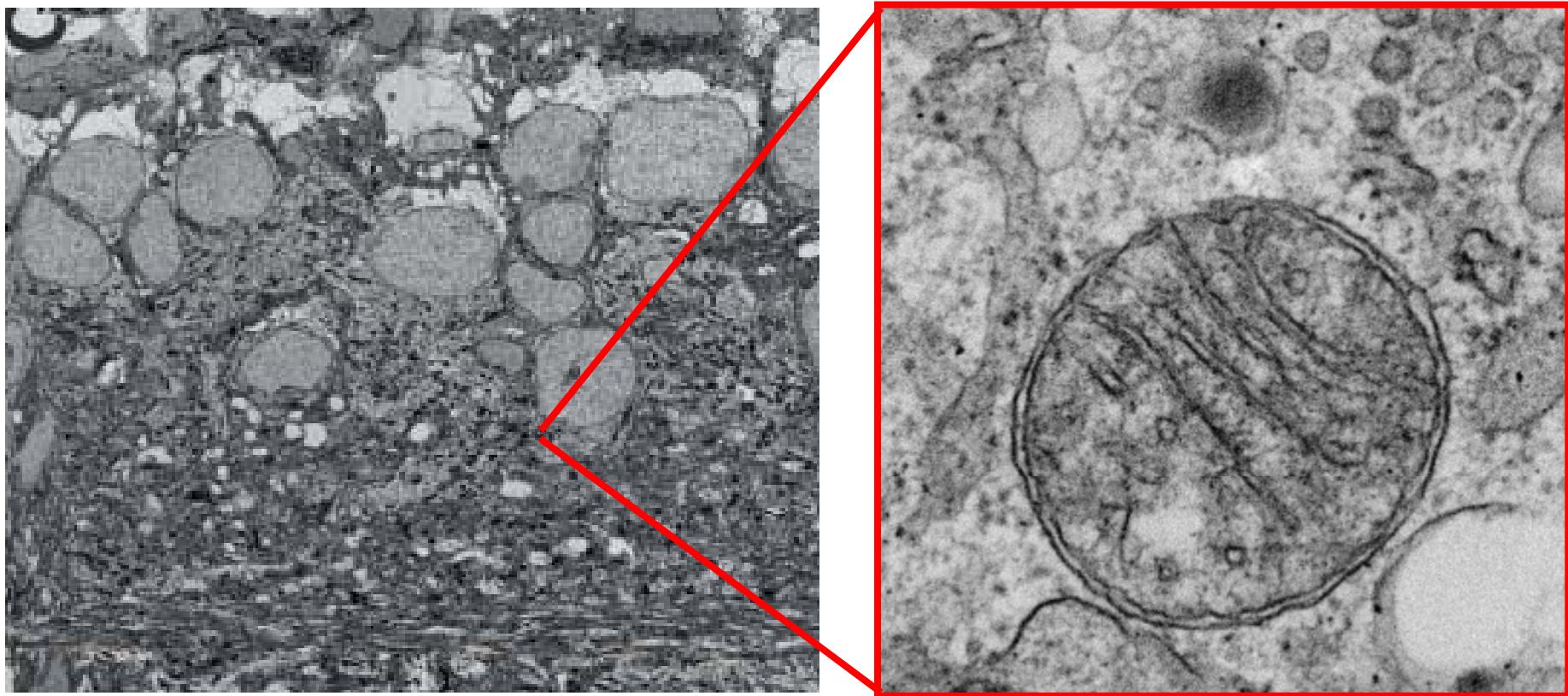
细胞环境下的蛋白是什么样的？

Complementary Bio-imaging

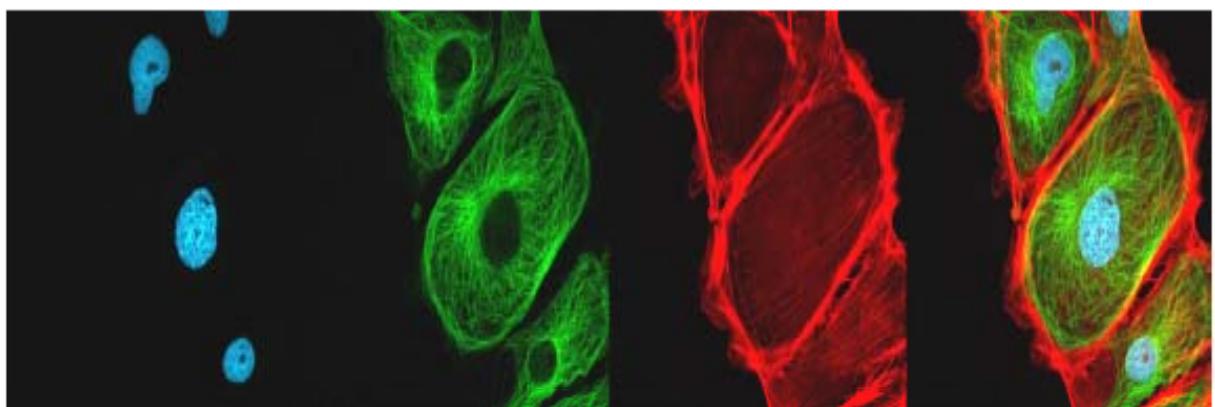
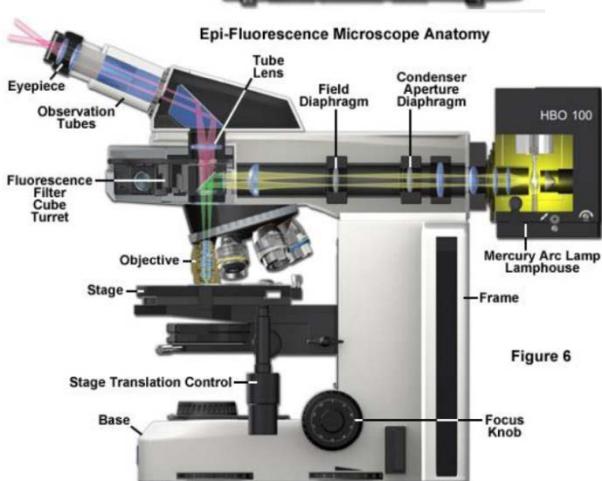
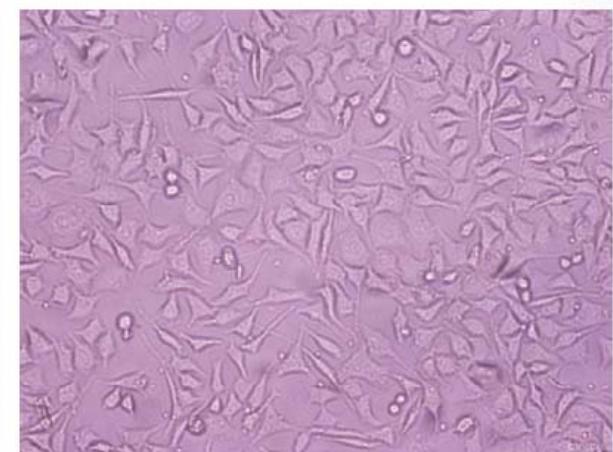
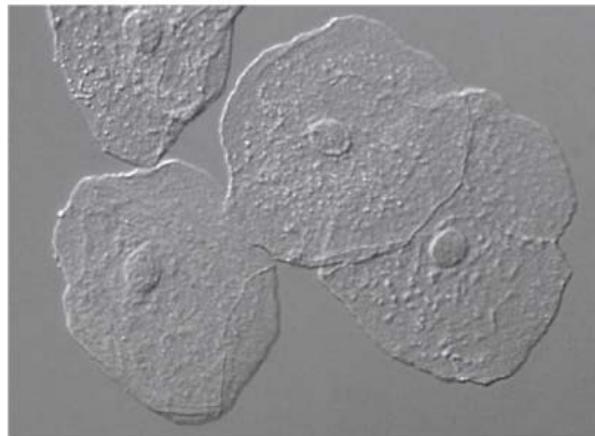
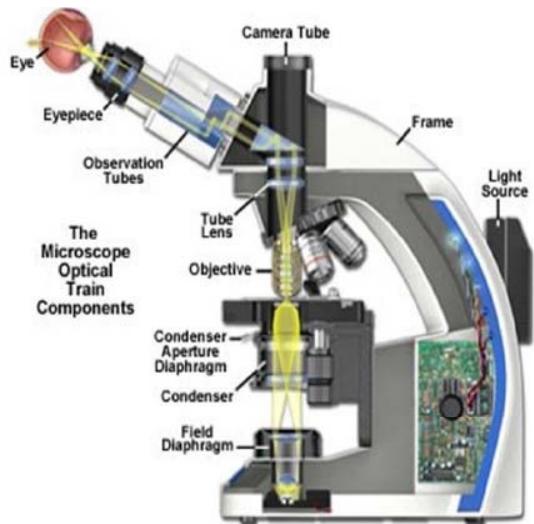


- **Structure Cell biology**
- **Visualization of biological process**
 - Virus-cell interaction
 - Binding, Fusion, Budding process?
 - Virus Life cycle
 - **Correlative microscopy: LM + EM**
 - **Electron tomography**

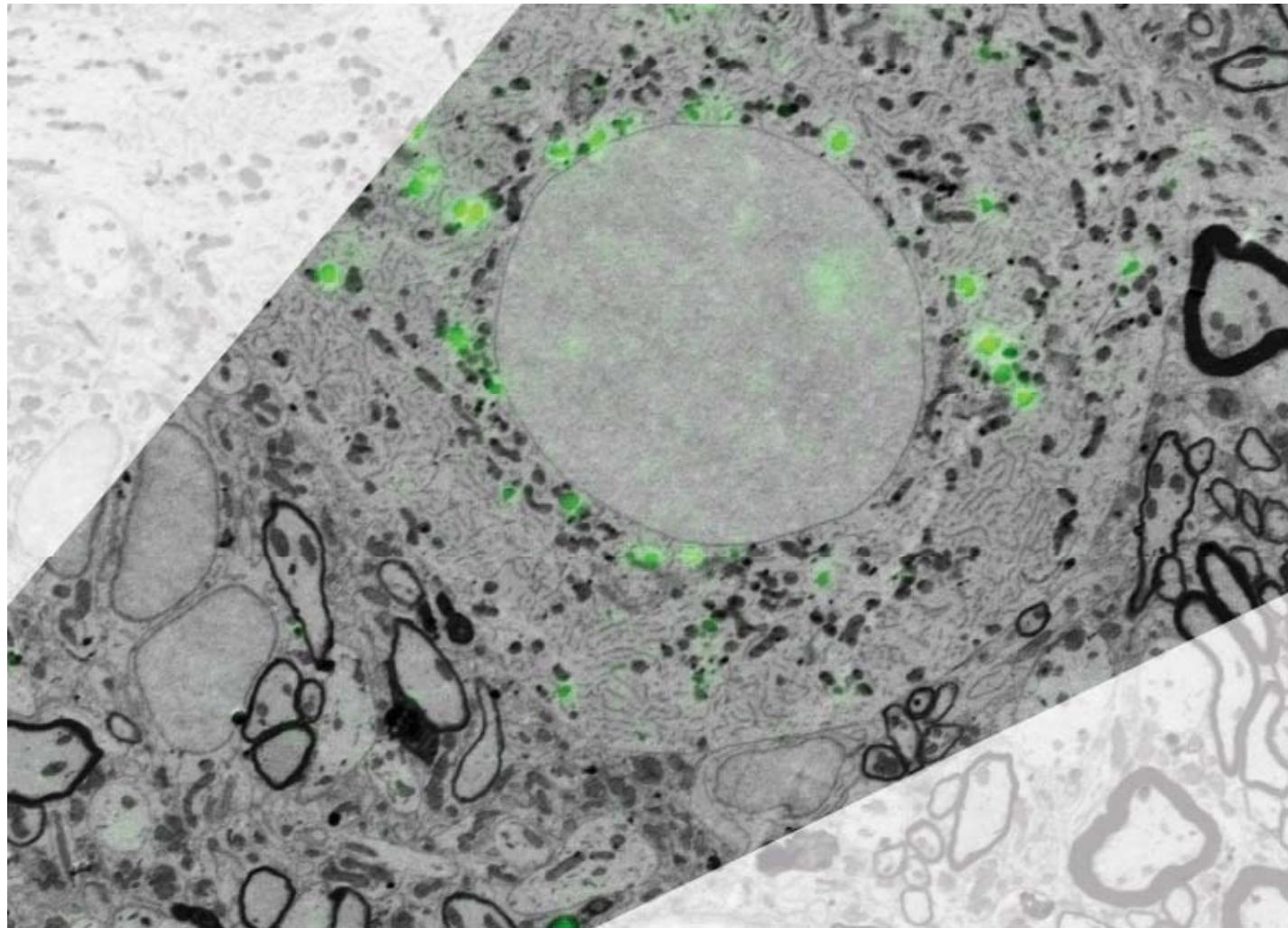
Correlative light-electron Microscopy (CLEM)



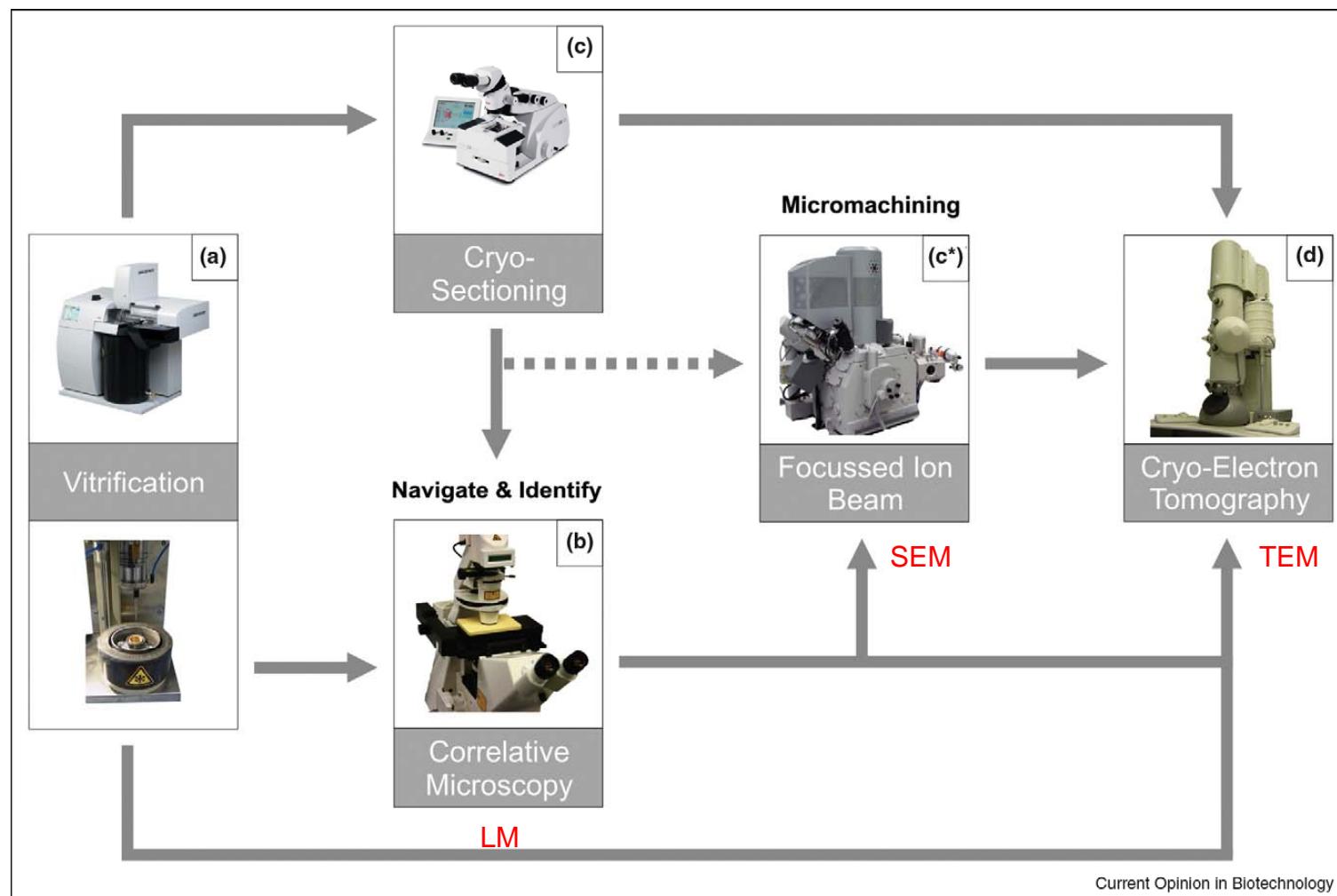
Correlative light-electron Microscopy (CLEM)



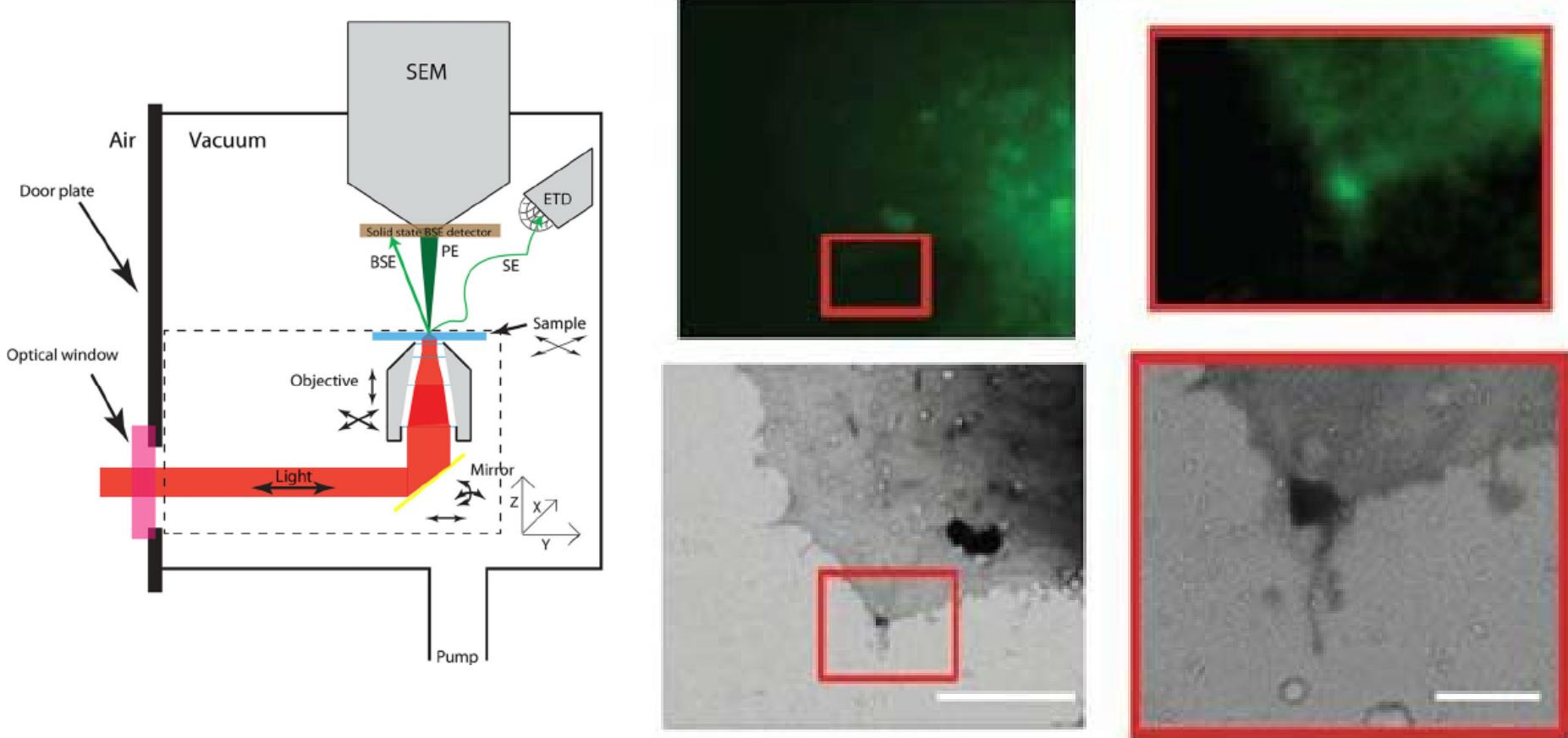
Correlative light-electron Microscopy (CLEM)



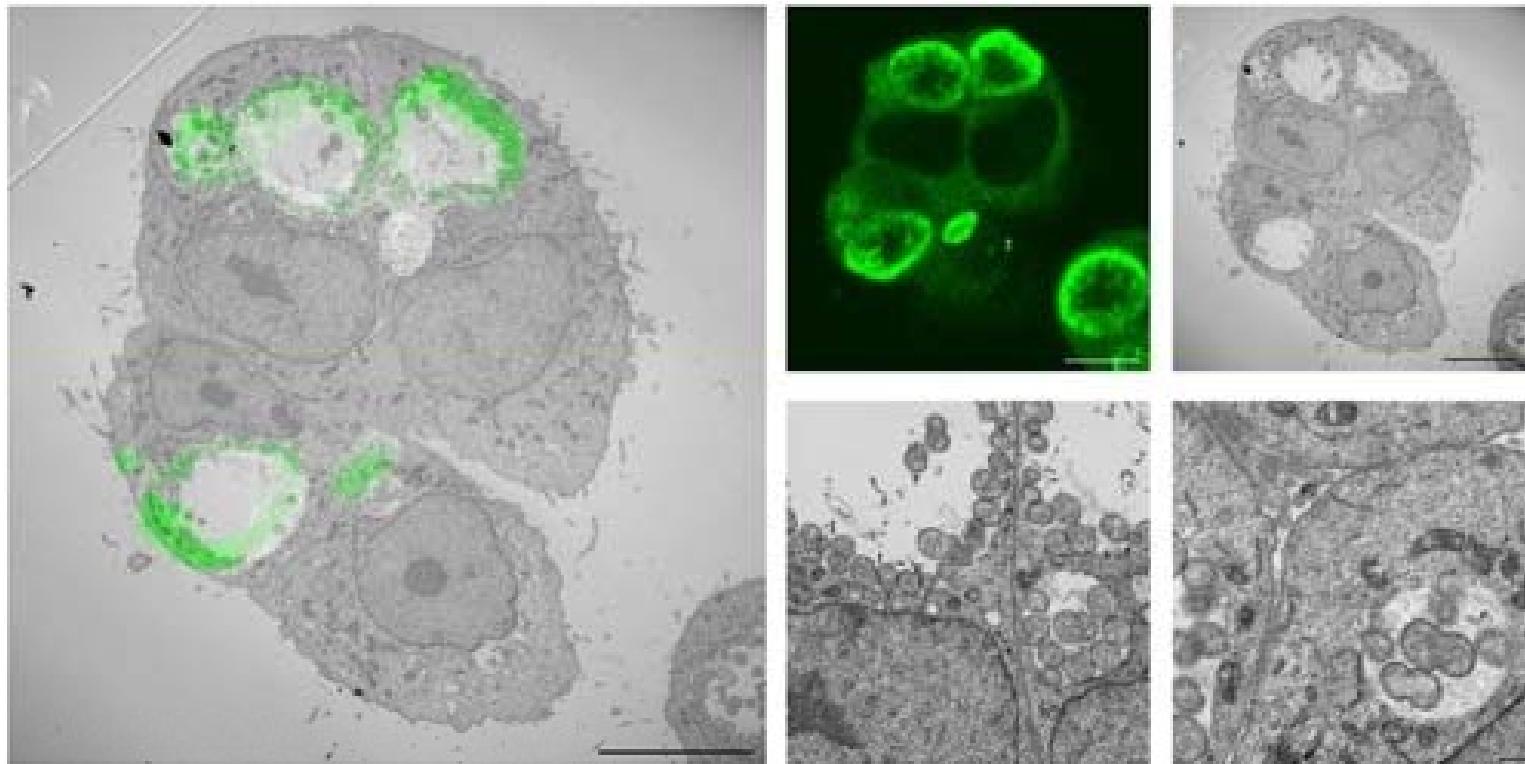
Correlative light-electron Microscopy (CLEM)



CLEM SEM

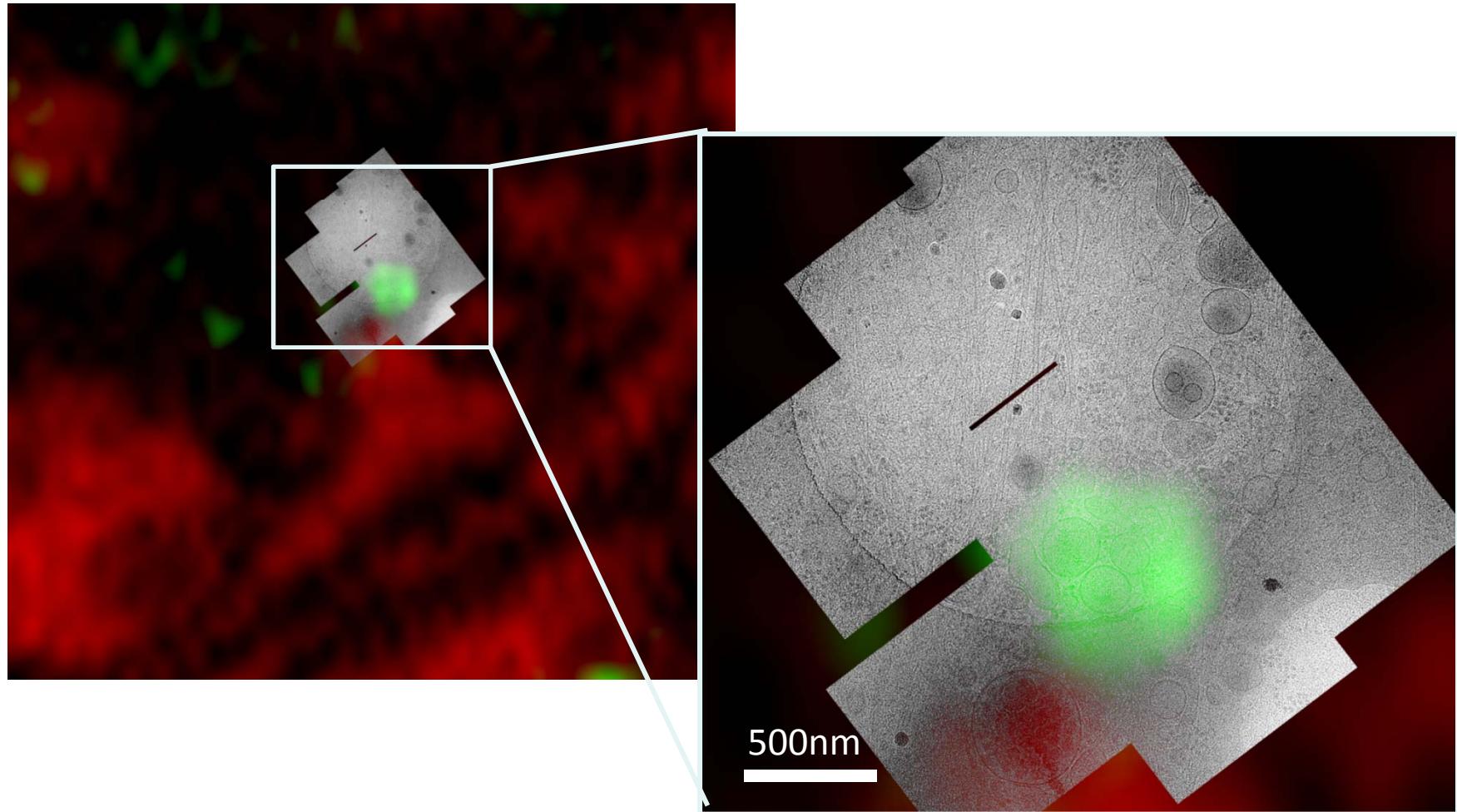


CLEM TEM



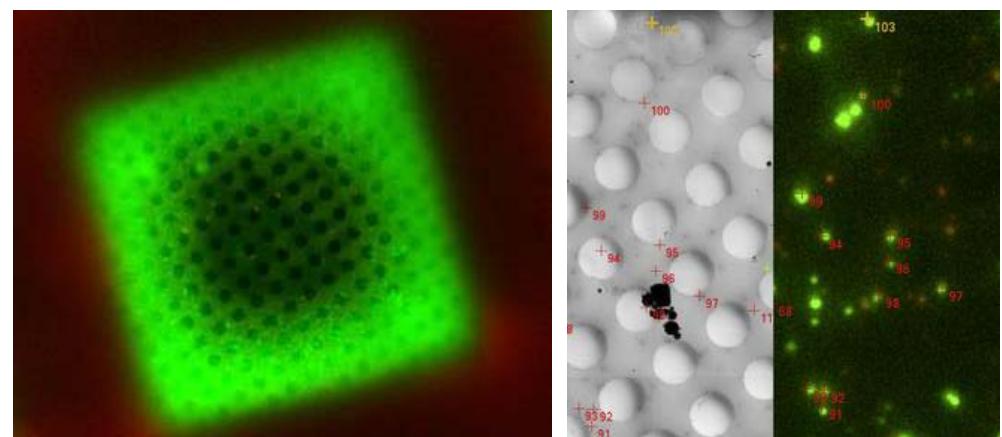
HeLa cells infected with *Chlamydia trachomatis* grown on photo-etched glass coverslips and labeled with fluorescent tagged anti-elementary body antibody. The TEM images reveal higher resolution information of the cells imaged by light microscopy.

Integrated Correlative Microscope: iCorr

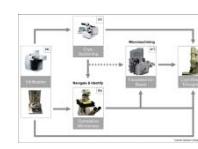
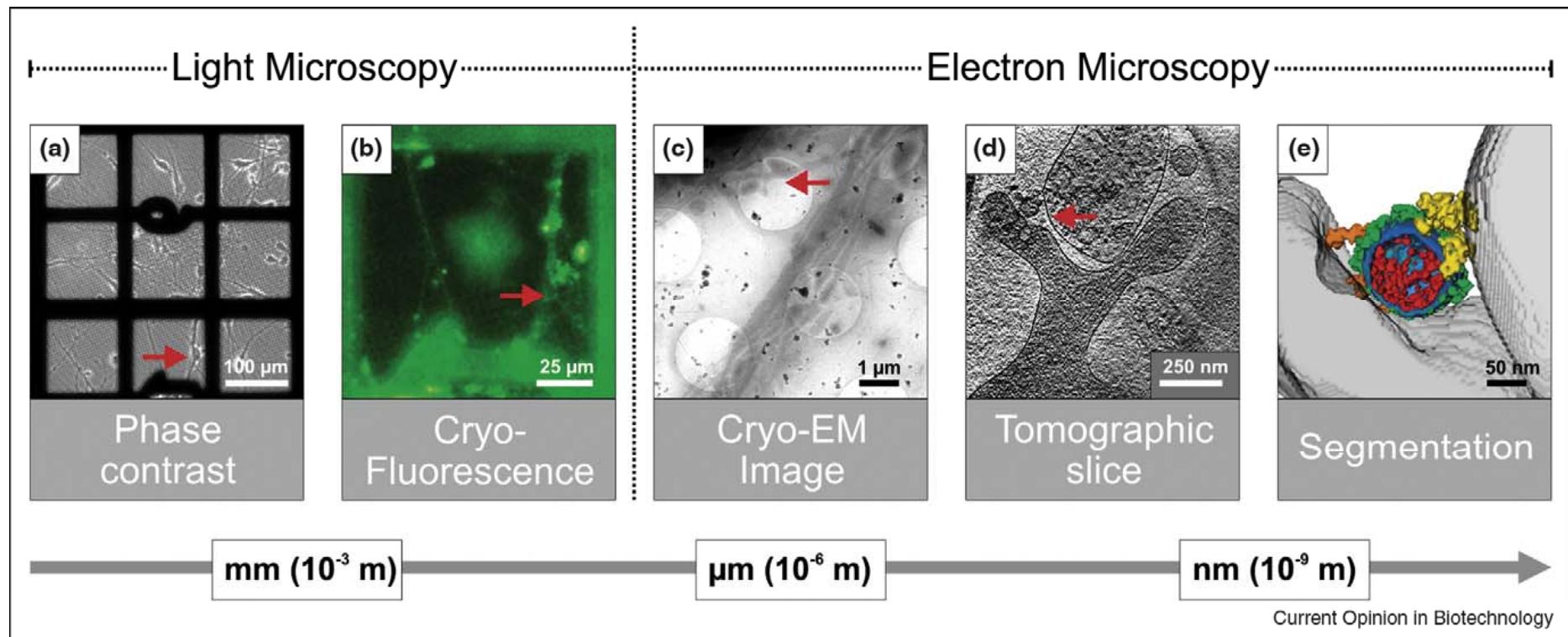


Non-Integrated CLEM: Cryo-stage

Leica
MICROSYSTEMS

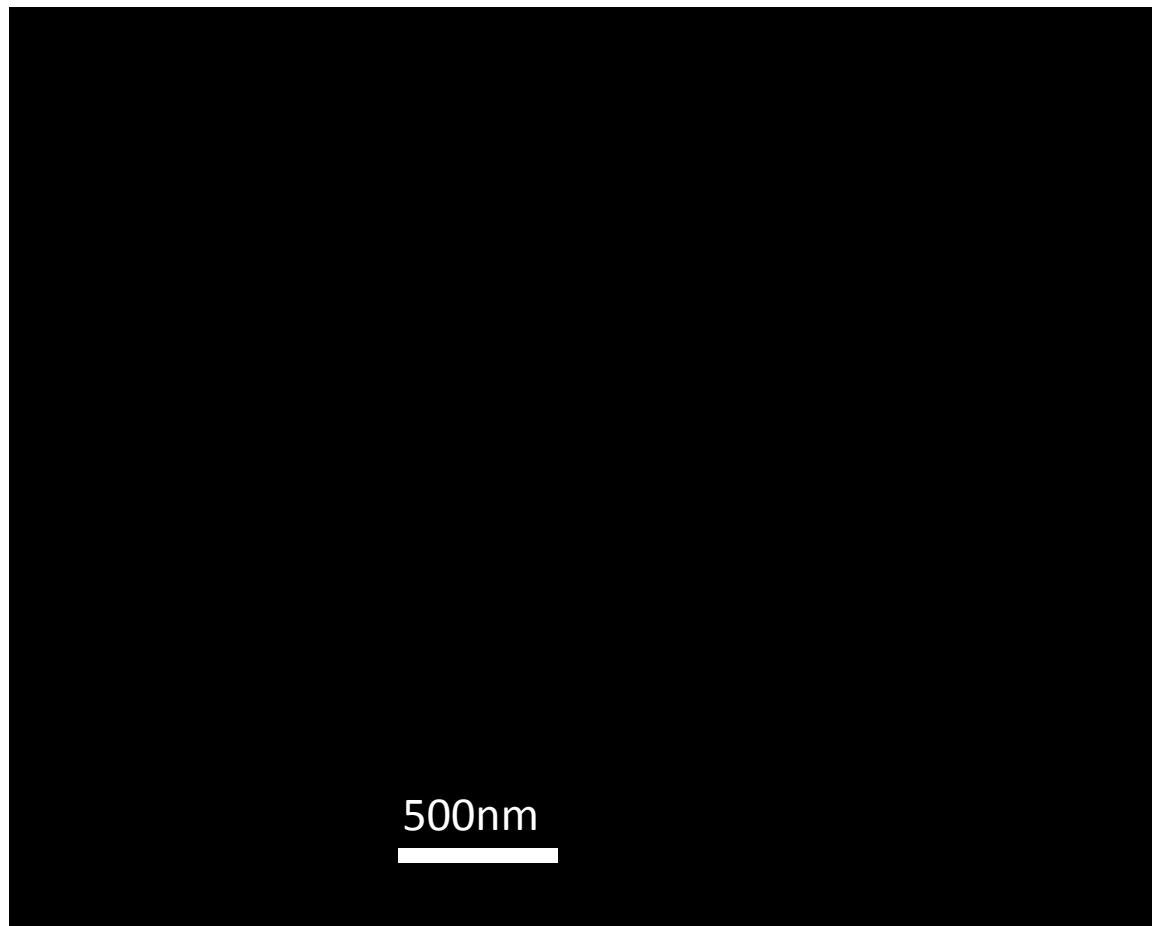
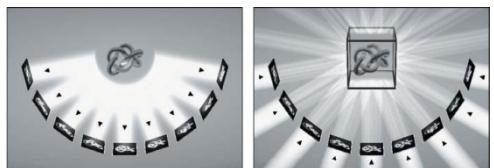
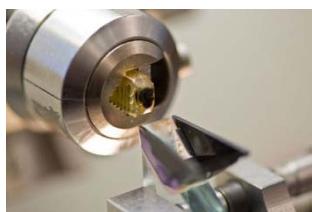


Correlative light-electron Microscopy (CLEM)



Correlative light-electron Microscopy

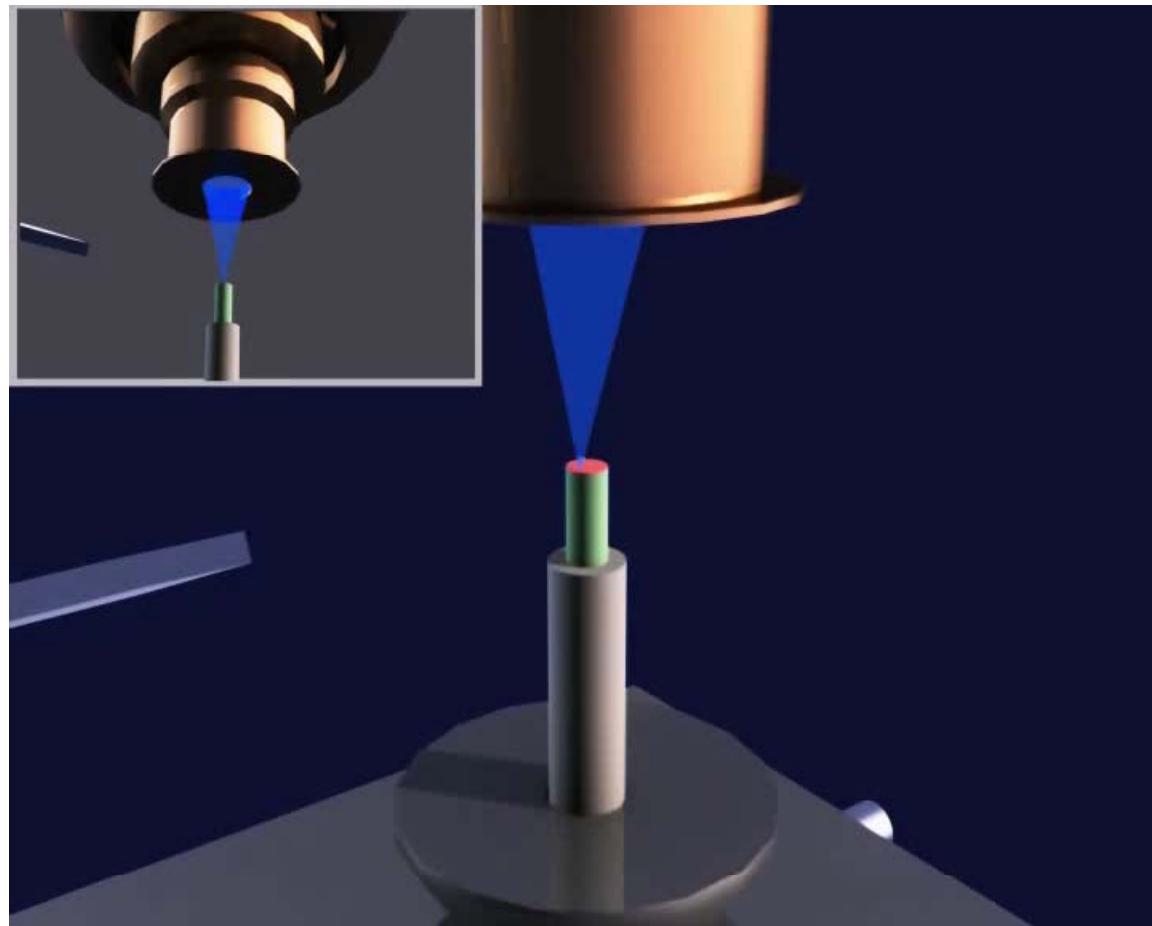
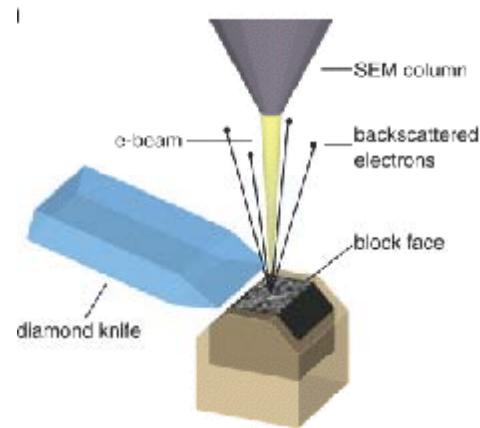
LM + Ultrathinsection + TEM + ET



Correlative light-electron Microscopy

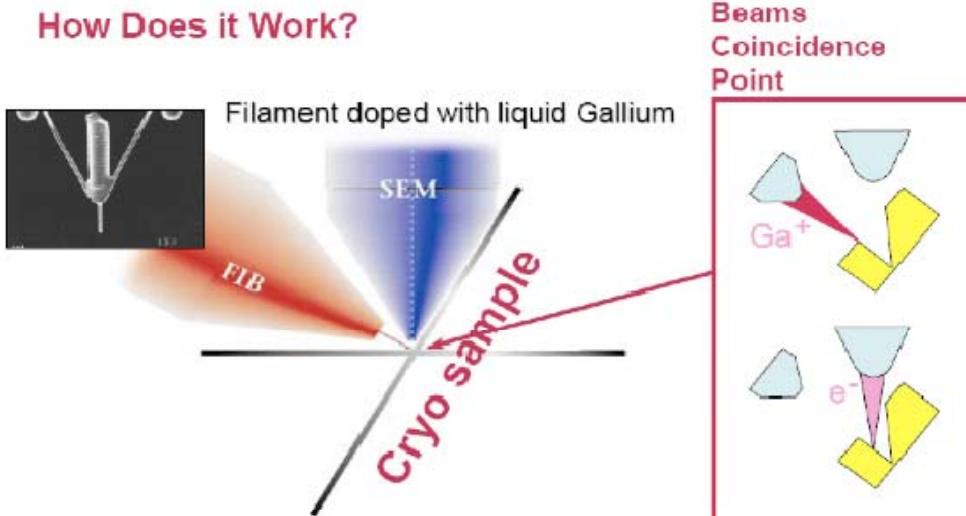
LM + 3View + SEM

Slicing Tomography

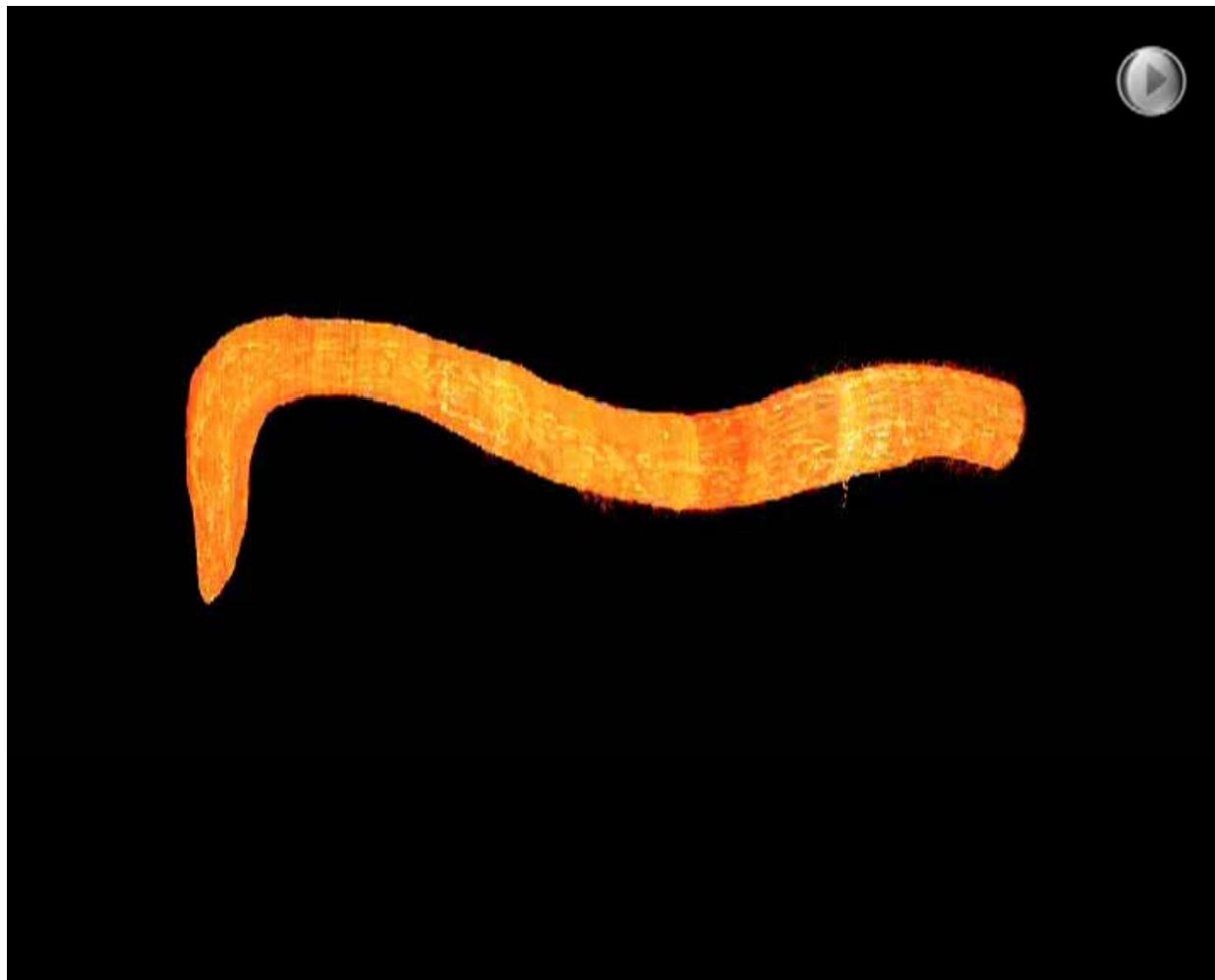


Correlative light-electron Microscopy

LM + dualBeam SEM



Visualization of Biological Sample



3D reconstruction with TEM

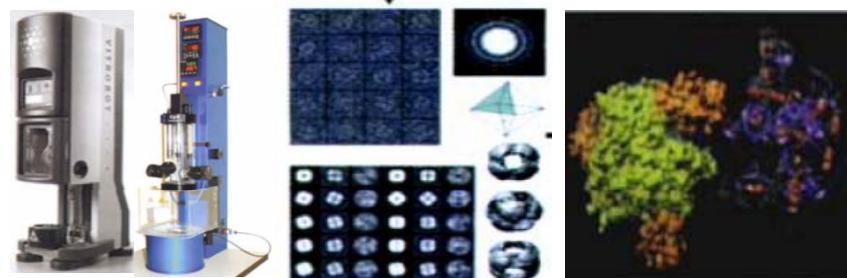
Resolution

0.3~2nm

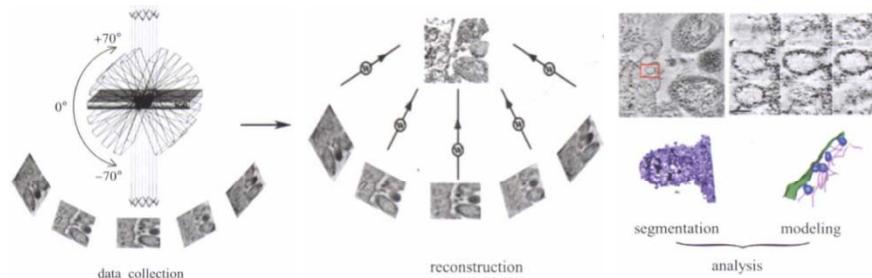
5 ~ 20 nm

**X ,Y: 1-2nm
Z:40-50nm**

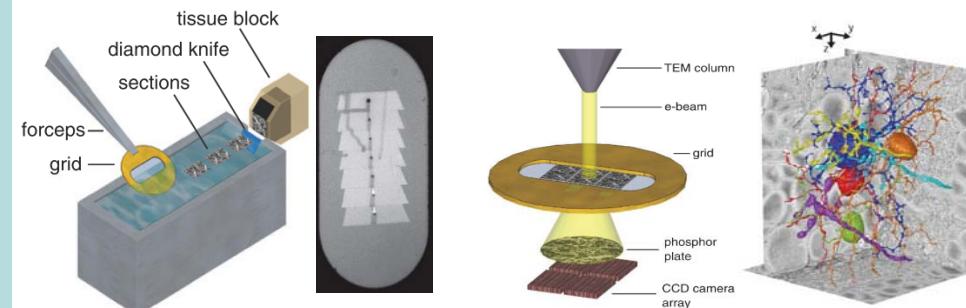
Single Partical Analysis



Electron Tomography



ssTEM



Dimension& Application

1~100nm

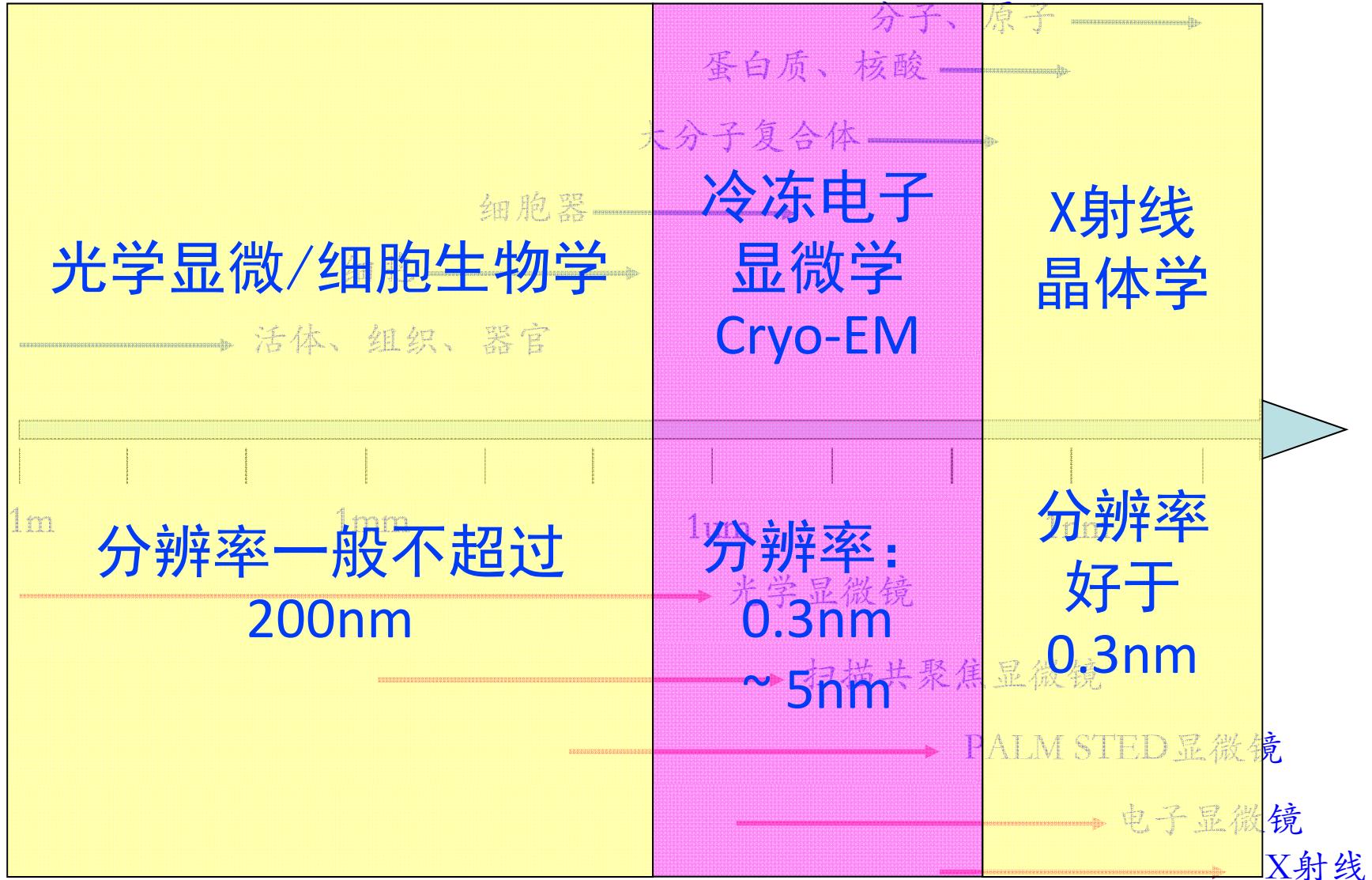
isotropic bio-macromolecular structure analysis

10~1000nm

Non-isotropic supermolecule, sub-cell,etc

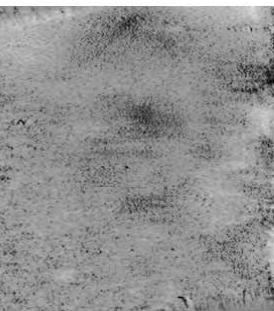
>1 μm
cell,tissue,etc

成像方法：研究尺度与精度



3DEM Technology

Meso Scale



ssSEM (500~700 Å)

SBF-SEM (~ 300 Å)

FIB-SEM (~ 50 Å)

STEM Electron
Tomography (~ 30 Å)

Electron Tomography
(~ 30 Å)

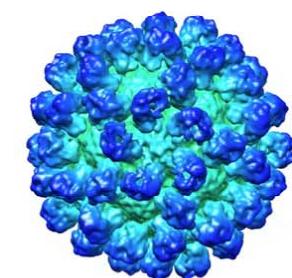
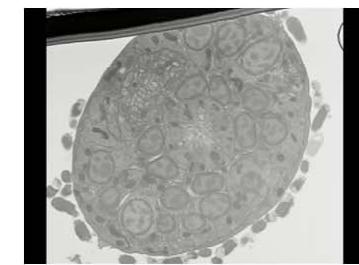
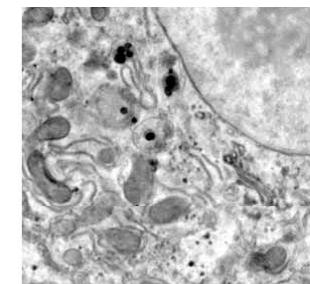
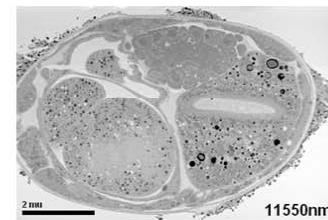
Single Particle Electron
Tomography (~ 8 Å)

Single Particle
Analysis (~ 2.2 Å)

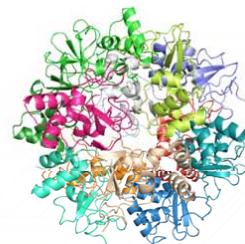
Micro Electron
Diffraction
(~ 2.5 Å)

Electron
Crystallography
(~ 1.9 Å)

Correlative Light and Electron Microscopy

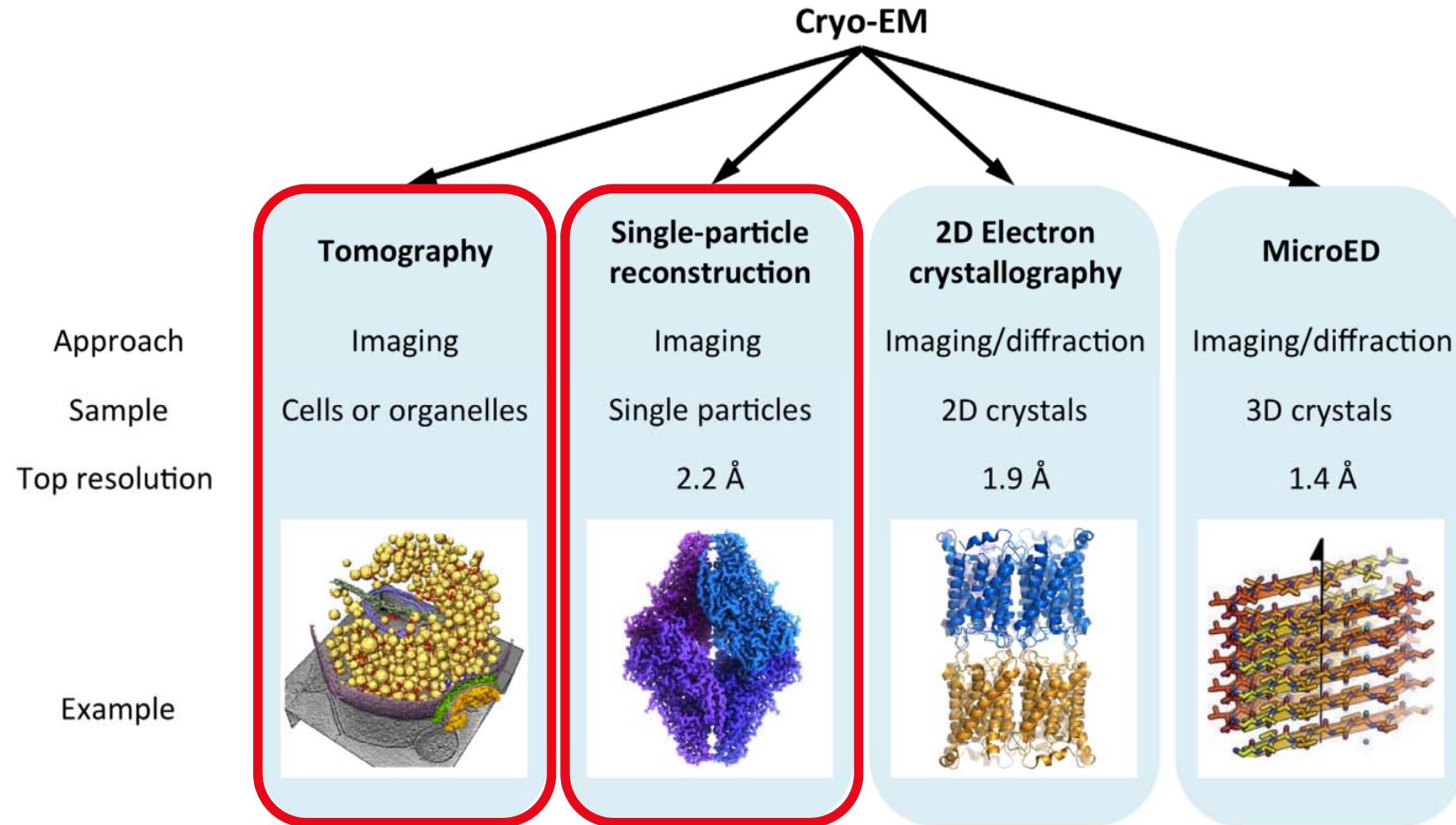


Nano Scale

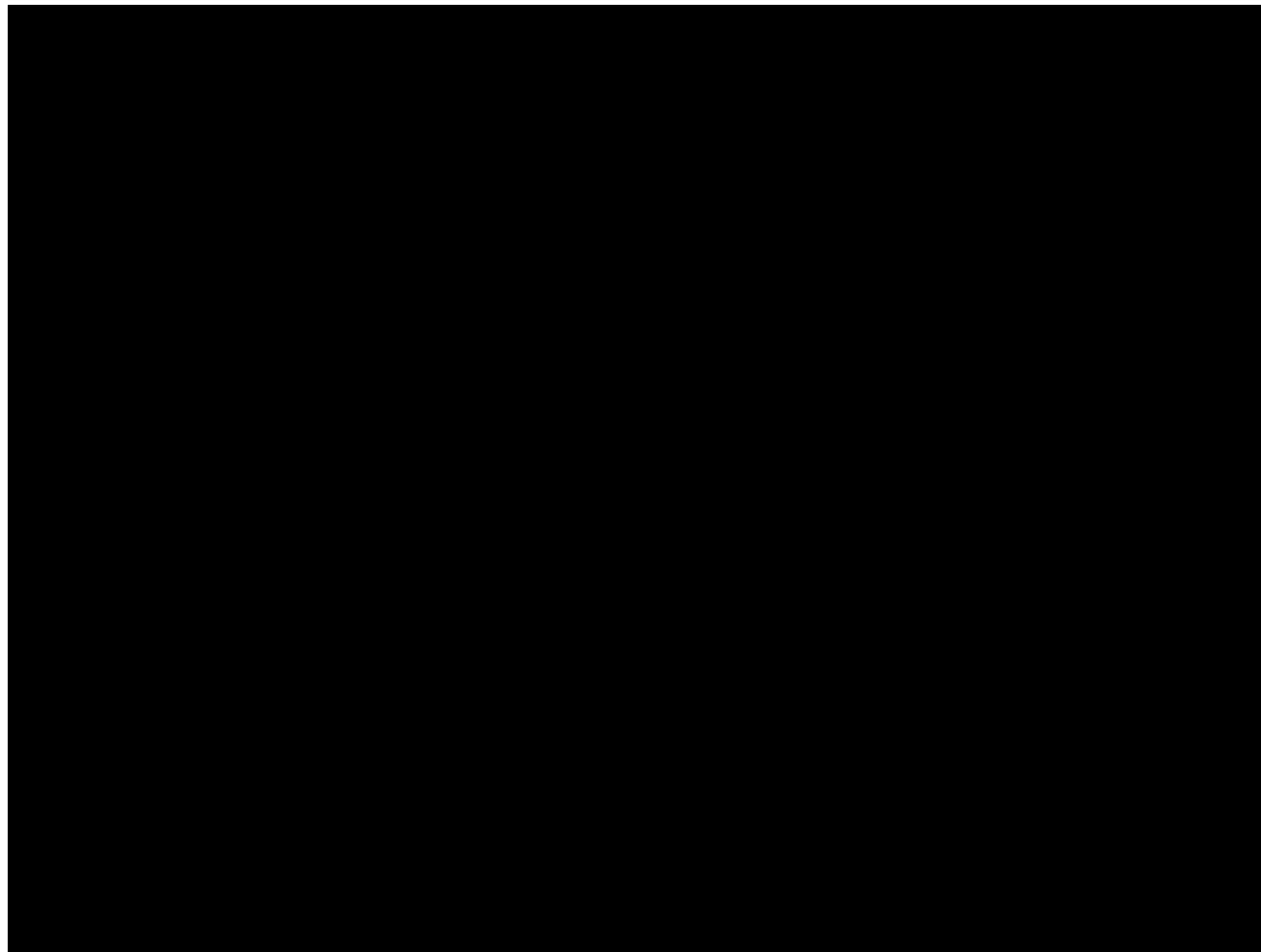


Resolution

冷冻电镜三维重构方法



Visualization of Biological Process



Institute of Biophysics, CAS

Olympic Science and Technology Park of CAS



IBP, CAS in Beijing



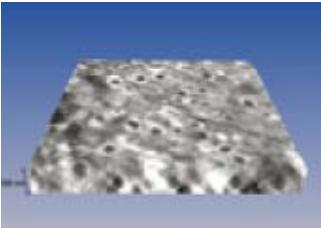
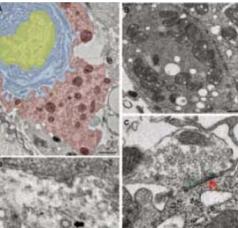
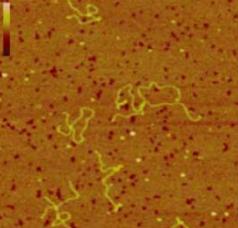
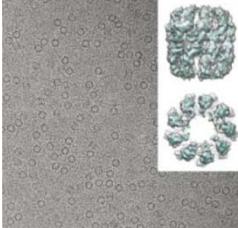
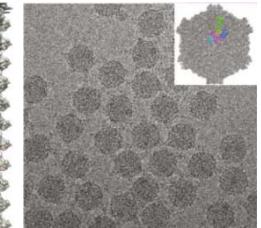
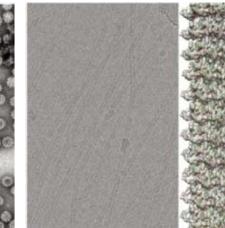
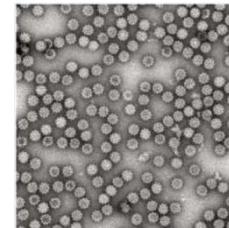
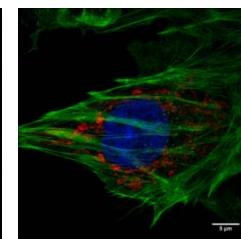
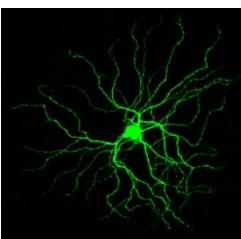
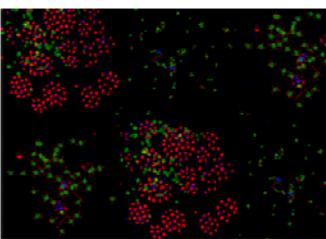
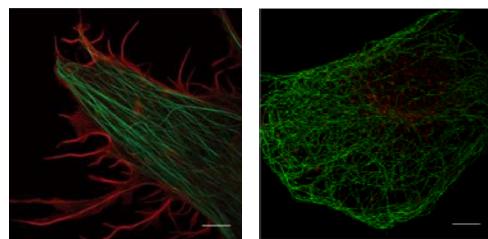
Center for Biological Imaging

Institute of Biophysics, Chinese Academy of Sciences

From Nano-scale to Meso-scale, 3D Nanometer Imaging

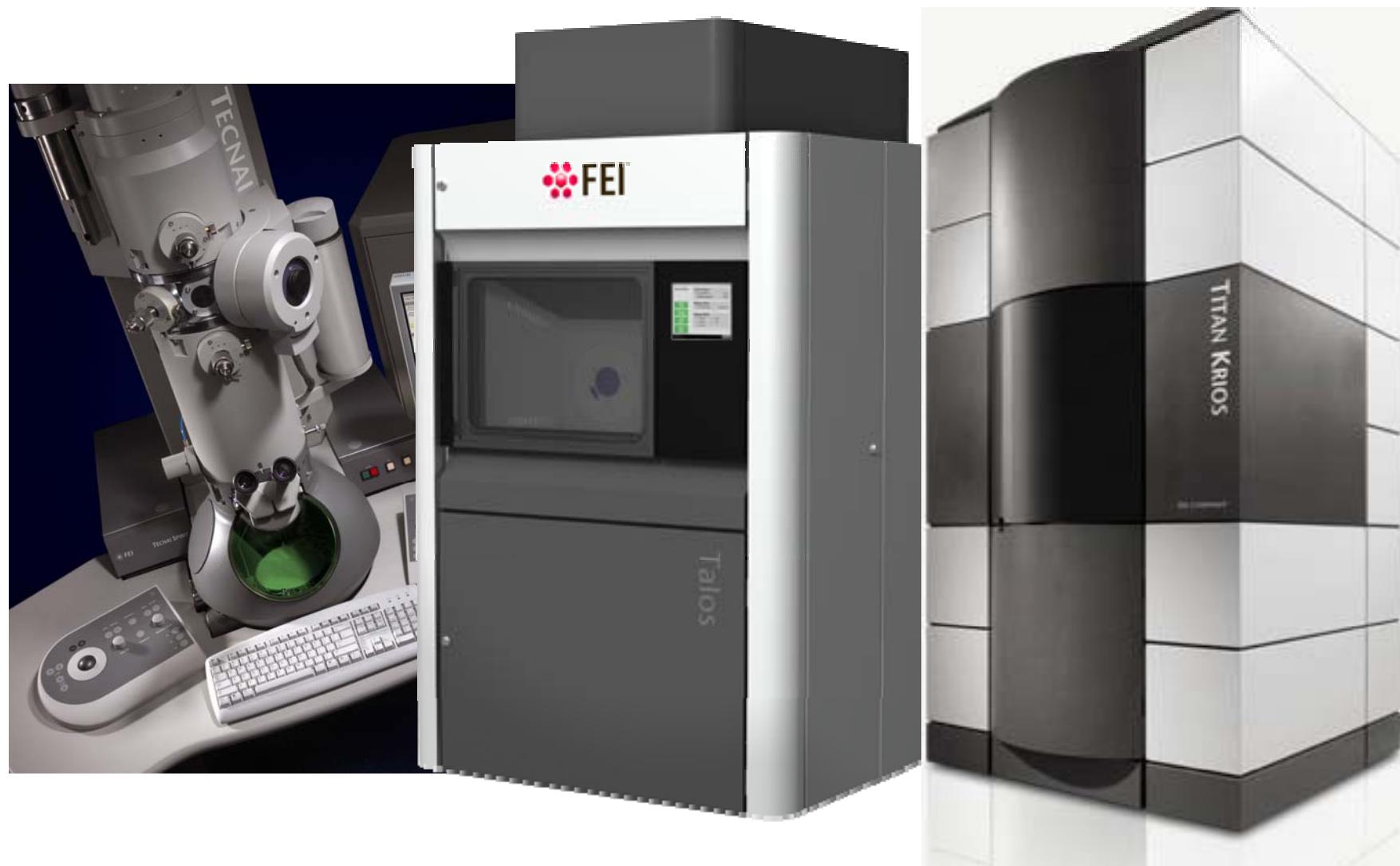
Ultrastructure of Cells, Supra Molecular Structures, Multi-scale Imaging

<http://cbi.ibp.ac.cn>



中科院生物物理所

— 冷冻电镜研究平台



2010年4月 建成并运行

超大分子复合体结构解析—硬件设施

中科院蛋白质科学平台

依托生物物理所，2004年启动建设，已投入运行



国家蛋白质科学上海设施

依托上海生科院，2013年底正式投入运行



合肥稳态强磁场实验装置
已投入运行





成像技术—推动生命科学发展的核心动力

“眼见为实” Seeing is believing

生命体结构与功能跨越了十个量级的时间和空间尺度，生物医学成像正在经历向多模态、跨尺度的革命性变化。

成像技术	获取信息	霍华德休斯医学研究所	本设施
结构连接图谱	电镜	形态	√
分子连接图谱	光电模态融合	形态+分子分布	√
功能连接图谱	多模态融合	形态+功能+动态	√

为什么需要多模态、跨尺度？
以脑连接图谱为例

10⁻⁹ 10⁻⁶ 10⁻⁵ 10⁻⁴ 10⁻² m
蛋白 突触 神经元 神经回路 全脑

X射线(1901年Nobel奖)
全息照相法(1971年Nobel奖)
CT 计算机断层成像(1979年Nobel奖)
电子显微镜(1986年Nobel奖)
MRI 磁共振成像(2003年Nobel奖)
超高分辨率显微成像(2014年Nobel奖)

PA-SIM
PET/CT
MRI (2003 Nobel Prize)
CT (1979 Nobel Prize)
X-ray (1901 Nobel Prize)

看得快
看得早
看得准
看得清
看得到

多模态、跨尺度生物医学成像

不同尺度下的生命活动是因果关联、不可割裂的；

空间尺度
时间尺度
原子 → 分子 → 复合大分子 → 细胞 → 组织 → 个体
分子事件 10⁻⁸s → 细胞信号转导 10⁻⁷s → 细胞运动 10⁻⁶s → 细胞分裂 10⁻⁵s → 个体寿命 >10⁻⁴s

不同成像模态获取不同的结构和功能信息。

成像模态	获取信息
电镜	微观结构
光镜	介观结构，离子活动、分子定位（高分辨）
MRI	介观-宏观结构、功能、代谢
CT/X-Ray	介观结构
PET/SPECT	分子定位（低分辨）
脑磁/脑电图	神经元电磁活动

如何整合多模态信息，如何打通尺度壁垒，从而精准描绘生命活动时空动态和内在联系。这是生命科学和医学面临的重大科学问题。

多模态融合成像的优势和不可替代性

核素成像 PET
X光成像 CT
光学成像 BLI FMI
磁共振成像 MRI

分辨率
灵敏度
特异性
对比度
信噪比

自主研发成像设备与多模态融合技术，建成三大装置

PAT: 光声成像
MRI: 核磁共振成像
PET: 正电子发射成像
CT: X射线计算机断层成像
SPECT: 单光子发射成像

设施包括三个跨尺度多模态成像装置，一个图像数据整合系统

设施工程指标

1. 成像空间尺度: 10-10 - 1 m;
2. 成像空间分辨率: 10-10 - 1 mm;
3. 成像时间尺度: 10-6 秒 - 1 年;
4. 成像时间分辨率: 10-6 秒 - 25 毫秒;
5. 实现 4 种以上模态融合;
6. 图像存储能力: 10 PB;

设施定位

由国家统筹布局，依托高水平创新主体，建设具有支撑科学和技术发展双重功能的国家生物医学成像基础设施。设施将成为：

- 在多模态、跨尺度上实现国际引领的生物医学成像平台
- 生物医学新技术新方法的创新动力源
- 生物医学成像人才培养和教育基地
- 生物医学成像国际合作基地

设施将面向全国基础科研、技术产业和临床应用提供开放共享服务，为国家重大科学计划和生物医学成像装备产业提供战略支撑。

科学目标

全景式揭示基因表达、生物大分子构象、细胞信号、组织代谢及功能网络的时空动态和内在联系，阐明大脑认知的基本原理、肿瘤和心脑血管疾病的发生和发展规律。|

工程目标

设施将融合光、声、电、磁、核素、电子等成像模态，提供从埃到米、从微秒到小时跨越十个空间与时间尺度的研究生命科学问题的能力，获取生命体的结构、化学组成、代谢、功能等相互关联的图像数据，实现多模态、跨尺度图像的整合与可视化。

科研需求

北京政府支持

习近平总书记指出，要坚持和强化首都全国科技创新中心的核心功能“加快向具有全球影响力的科技创新中心进军”。

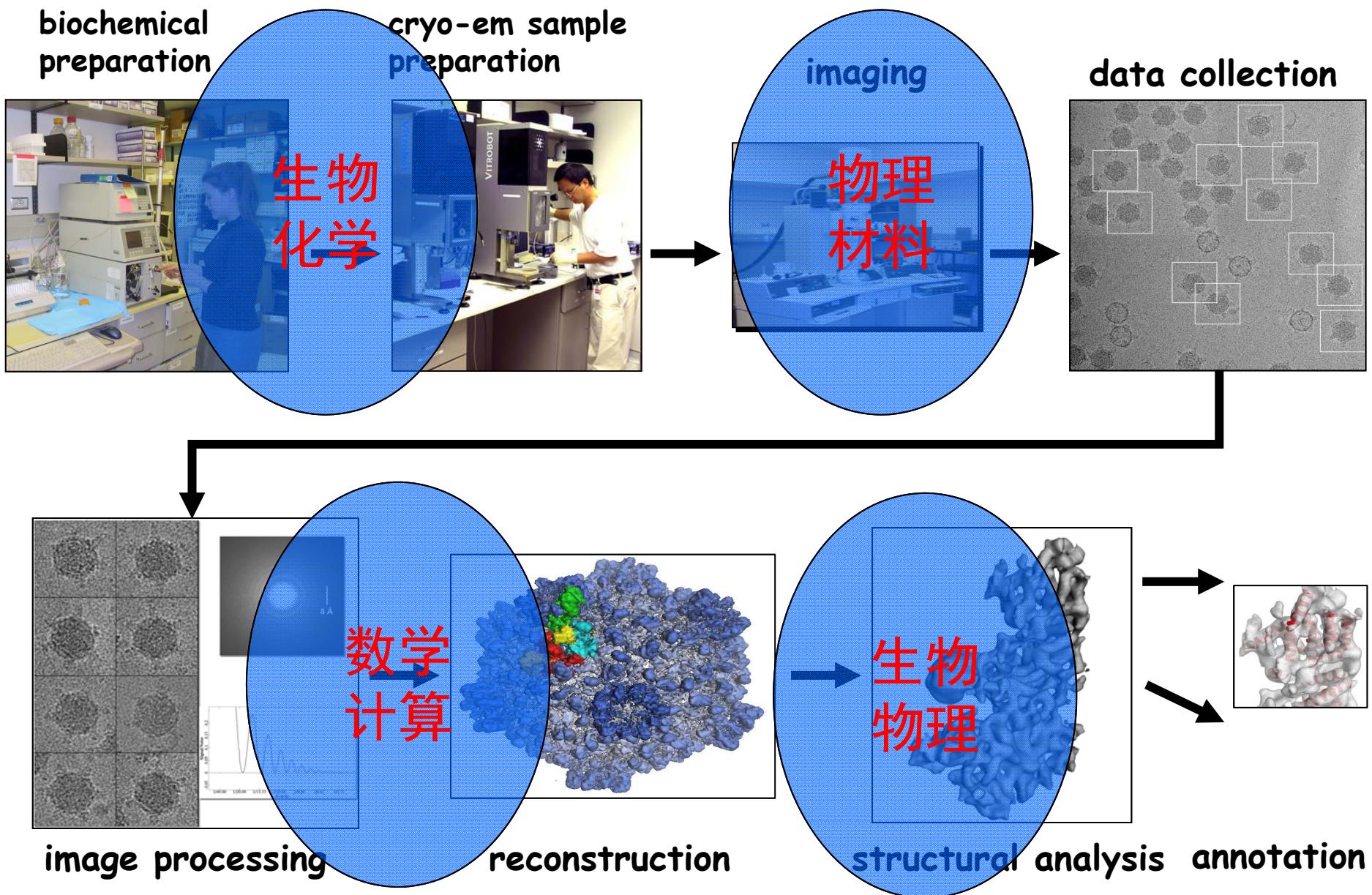
以北京综合性国家科创中心为核心，建设怀柔科学城，已正式纳入北京市“十三五”规划，多模态跨尺度生物医学成像设施已列为怀柔科学城重点工作工程。

北京综合研究中心已按照北京市规委要求，组织开展怀柔东北地块（怀柔雁栖经济开发区北部，中科院大学东南部，共计约 3500 亩）空间规划，其中 300 亩用于本设施建设。

联系我们

多模态跨尺度生物医学成像设施办公室
邮箱：sheshiban@ibp.ac.cn
联系人：王丹、张敏
联系方式：010-64888442、64884246

冷冻电镜涵盖的学科领域



Chiu et al (2006) JENOL News

Take home message:

- 结构生物学：
 - 冷冻电镜 vs 蛋白质晶体学
(优缺点，适用范围与对象，例子)
- 冷冻电镜三维重构：
 - 单颗粒分析 vs 电子断层成像
(优缺点，适用范围与对象，例子)
- 冷冻电镜研究的未来发展趋势：
 - 重构精度、硬件软件及方法、研究对象
(优缺点，适用范围与对象，例子)

生物大分子电子显微三维重构课程排课表			
上课时间：周四(5-7节)			
时间	授课教师	课程主题	课程内容
第1周(9月13号)	朱平	生物大分子电子显微三维重构概论(3 学时)	a. 结构生物学概述 b. 电子显微三维重构发展历史 c. 电子显微三维重构技术方法、应用与前沿
第2周(9月20号)	孙飞	傅立叶变换(3 学时)	a. 傅立叶级数与傅立叶变换 b. 离散傅立叶变换与快速傅立叶变换 c. 振幅与相位 d. 卷积与相关、卷积定理 e. Shannon-Nyquist 采样定理、Nyquist 频率 f. 傅立叶变换的插值 g. 滤波
第3周(9月27号)	孙飞	电子显微成像原理(3 学时)	a. 电子显微镜基本构造 b. 电子与生物样品的相互作用(弱相位近似)与成像衬度的产生(振幅衬度与相位衬度) c. 几何电子光学与像差(球差、像散、慧差、色差) d. 衬度传递函数、点分辨率、信息极限
第4周(10月11号)	朱平	透射电镜成像技术与要素(6 学时)	a. 透射电子显微镜的合轴 b. 聚焦技术(离焦、过焦、正焦、消像散;相位板技术) c. 低剂量成像技术(辐射损伤、剂量、自动化) d. 探测器(胶片、CCD、DED; DQE, MTF) e. 电子显微照片的质量评价
第5周(10月18号)	朱平	透射电镜成像技术与要素(6 学时)	a. 透射电子显微镜的合轴 b. 聚焦技术(离焦、过焦、正焦、消像散;相位板技术) c. 低剂量成像技术(辐射损伤、剂量、自动化) d. 探测器(胶片、CCD、DED; DQE, MTF) e. 电子显微照片的质量评价

月19号)		要素(6 学时)	d. 探测器(胶片、CCD、DED; DQE, MTF) e. 电子显微照片的质量评价
第6周(10月25号)	孙飞	生物电镜样品制备技术(3 学时)	a. 生物大分子高分辨率电镜成像的样品要求 b. 负染色技术 c. 冷冻玻璃化技术 d. 细胞和生物组织的制样技术
第7周(11月1号)	章新政	单颗粒三维重构技术方法(6 学时)	a. 单颗粒分析技术流程 b. 颗粒挑选、衬度传递函数矫正和滤波 c. 二维图像分析与分类(K-means 分类、多级递增分类、自组织图) d. 三维重构数学原理(中央截面定理、Radon 变换)与方法(WBP, SIRT/ART, FBP) e. 颗粒取向求解和三维结构精修(等价线方法、随机锥体重构、投影匹配方法) f. 三维结构后处理和评估(FSC 曲线、Gold Standard、分辨率、温度因子校正、Tilt Pair Validation) g. 统计学方法与三维结构分类 h. 重构软件介绍
第8周(11月8号)	章新政	单颗粒三维重构技术方法(6 学时)	a. 单颗粒分析技术流程 b. 颗粒挑选、衬度传递函数矫正和滤波 c. 二维图像分析与分类(K-means 分类、多级递增分类、自组织图) d. 三维重构数学原理(中央截面定理、Radon 变换)与方法(WBP, SIRT/ART, FBP) e. 颗粒取向求解和三维结构精修(等价线方法、随机锥体重构、投影匹配方法) f. 三维结构后处理和评估(FSC 曲线、Gold Standard、分辨率、温度因子校正、Tilt Pair Validation) g. 统计学方法与三维结构分类 h. 重构软件介绍
			a. 电子断层三维重构流程

第9周(11月15号)	朱平	电子断层三维重构技术与应用(3 学时)	a. 电子断层三维重构流程 b. 电子断层数据收集技术 c. 序列图像的对齐技术(mark-free and mark-based alignment) d. 倾转系统的几何参数求解 e. 三维重构与去噪 f. 三维体数据分析与平均技术 g. 三维体数据的可视化与分割
第10周(11月22号)	章新政	电镜三维重构应用(3学时)	a. 病毒的结构研究 b. 大分子复合体的高分辨率结构研究 c. 大分子复合体的动态构象研究 d. 细胞内部环境的三维超微结构研究
第11周(11月29号)	章新政	文献阅读与讨论 (3 学时)	
第12周(12月6号)	孙飞	文献阅读与讨论 (3 学时)	
第13周(12月13号)	孙飞、章新政	上机实习 (2+3 学时) 地点生物物理所 9 号楼 9212)	
注：考虑到学生周四上午可能有其他课程安排，上机实习课程时间可能需要调整，暂未给定正式确定时间			

教学方式： 90%课堂授课+ 10%上机练习

考核方式：课后作业 (20%) +考勤 (10%) +课堂笔记 (20%) +开卷考试
(50%)

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教材：

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Thank you!