CEITEC

Středoevropský technologický institut

BRNO | ČESKÁ REPUBLIKA

S1007 Doing structural biology with the electron microscope

C9940 3-Dimensional Transmission electron microscopy

Lecture 1: Introduction & history

EVROPSKÁ UNIE
EVROPSKÝ FOND PRO REGIONÁLNÍ ROZVOJ
INVESTICE DO VAŠÍ BUDOUCNOSTI

2007–13
OP Výzkum a vývoj pro inovace

Course organization

- Time and place: Monday 14:00 – 15:50, A35/211
- Language: English
- Finishing the course: exam (written form), 4 credits
- Jiri Novacek (jiri.novacek@ceitec.muni.cz)

Syllabus

12th March	History
19th March	No lecture
26th March	Anatomy of electron microscope
2nd April	Easter holiday (no lecture)
9th April	Specimen preparation
16th April	Principles of image formation
23rd April	Tomography
30th April	3D volume generation
7th May	Single particle analysis
14th May	Atomic modeling and validation
21st May	Recent advances

Resources

Literature:

- J. Frank, Three-dimensional electron microscopy of macromolecular assembliesvisualization of biological molecules in their native state.
- J. Frank, Electron Tomography: Methods for Three-Dimensional Visualization of Structures in the Cell
- Williams et al., Transmission electron microscopy

Video courses (youtube):

- Grant Jensen
- NRAMM SEMC
- Cryo-EM14 (LMB)
- Cryo-EM17 (LMB)

History of electron microscopy

Munich: Ernst Ruska & Otto Scherzer

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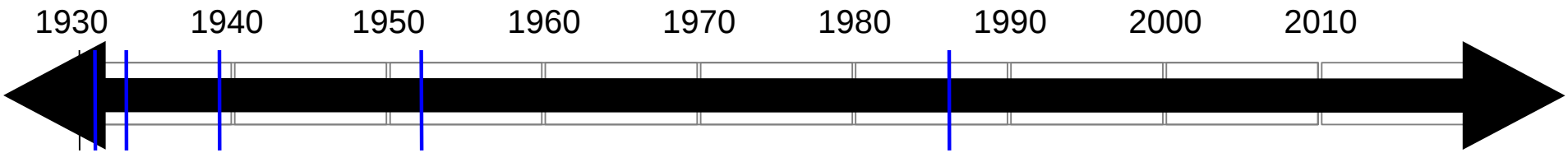
Ernst (and Helmut) Ruska: timeline



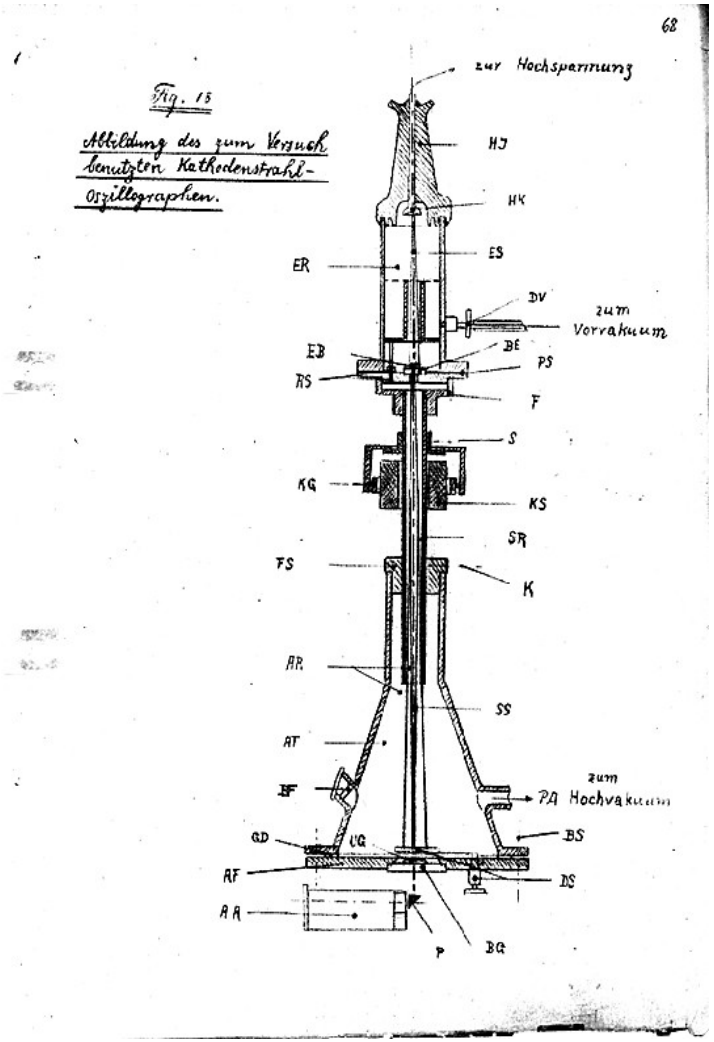
Milestones:

- 1931: Invention of the electrostatic lens
  - magnification: 400X
- 1933: First electron microscope
  - mag: 7000X (vs. LM: 2000X)
- 1933: Completed Ph.D. (!)
- 1939: First (viable) commercial EM
  - mag: 100,000X
- 1939: His brother Helmut Ruska images first virus (TMV)
- 1952: Helmut moves from Siemens to Albany
- 1986: Nobel Prize in Physics

Ernst Ruska

http://www.biografiasyvidas.com

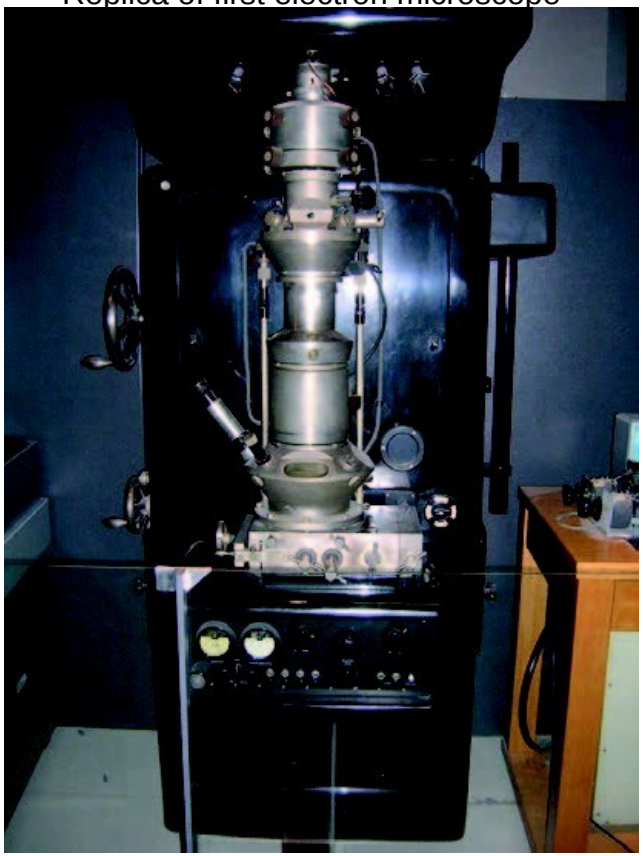
Sketch from 1929



http://ernst.ruska.de

Ernst Ruska

Replica of first electron microscope



http://www.bluesci.org

First (**viable**)
commercial microscope (Siemens)

http://ernst.ruska.de

Clarification

First Siemens microscope, **1939**

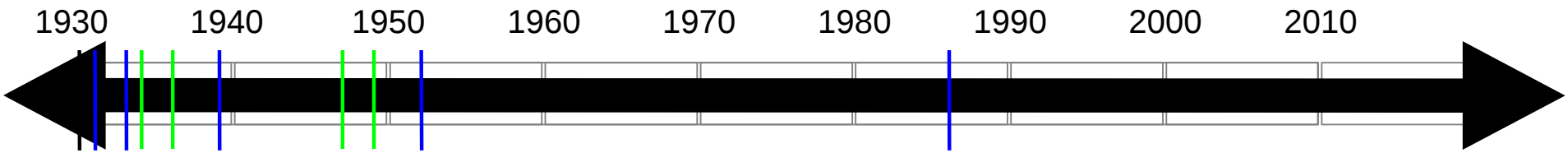
http://ernst.ruska.de

First **commercial** EM (**1937**) was Metropolitan-Vickers EM1 (EM2 shown)

http://emu.msim.org.uk

The first commercial electron microscope was actually by the British company Metropolitan-Vickers in 1937. However, the magnification was worse than for the light microscope, so the Siemens is considered “first.”

Otto Scherzer: timeline



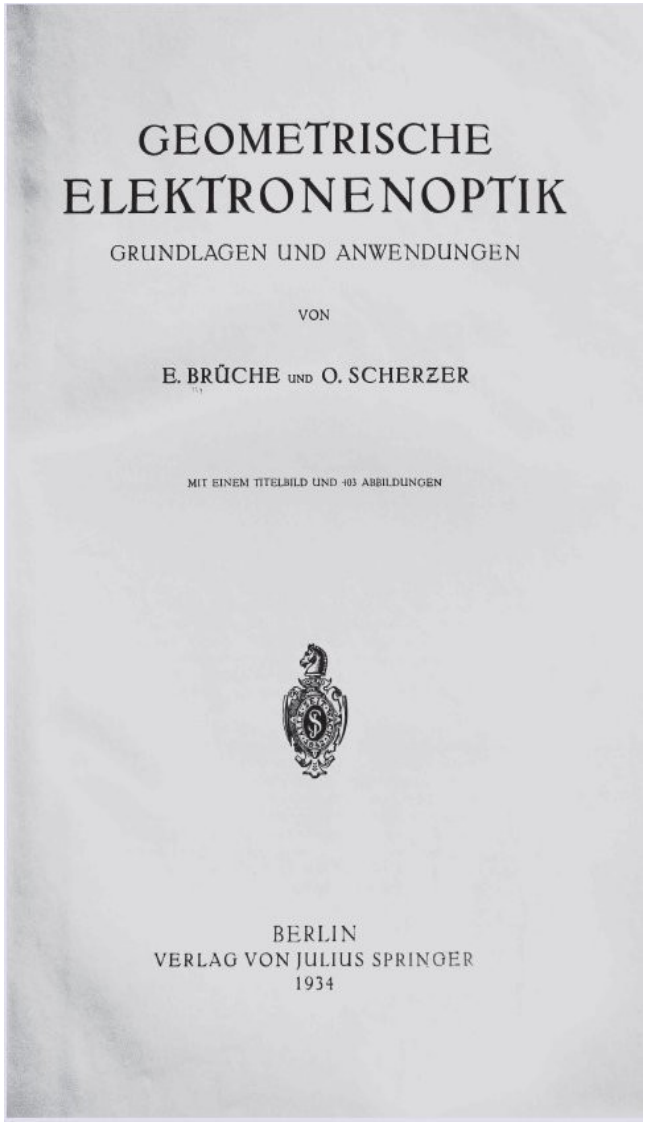
Milestones:

- 1934: First comprehensive book on electron optics
- 1936: Spherical aberration → resolution 50-100X the wavelength
- 1947-1951: Devised correction schemes for aberration correction
- 1949:
  - “Can atoms be visible in the electron microscope?”
  - “Scherzer focus”
- Scherzer → Harald Rose (Wadsworth, Darmstadt) → Max Haider

Otto Scherzer

http://www.microscopy.org

First book on electron optics



http://www.microscopy.org

Scherzer (1949) Physikalische Blätter & Scherzer (1949) Journal of Applied Physics

"Can atoms be visible in the electron microscope?"

PHYSIKALISCHE BLÄTTER
1949 Heft 10 11 Seite 460 – 463

Prof. O. Scherzer

Können Atome im Elektronen-Mikroskop sichtbar werden?

... des Auflösungsvermögens für möglich. Es ist also anzunehmen, daß die weitere Entwicklung des Elektronen-Mikroskops eines Tages nicht nur die schweren Jod-Atome des Moleküls, das wir unseren Betrachtungen zu Grunde gelegt haben, sichtbar machen wird, sondern auch die leichten Kohlenstoffatome und damit die Struktur von Molekülen, die weniger übersichtlich gebaut sind.

http://www.microscopy.org

"Scherzer focus"

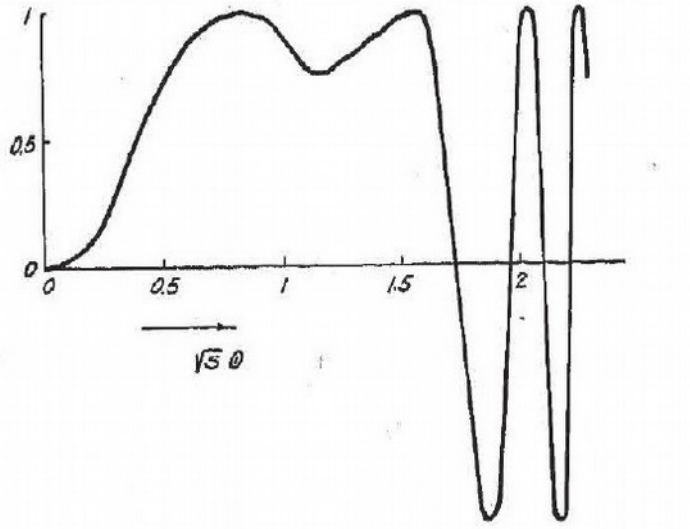
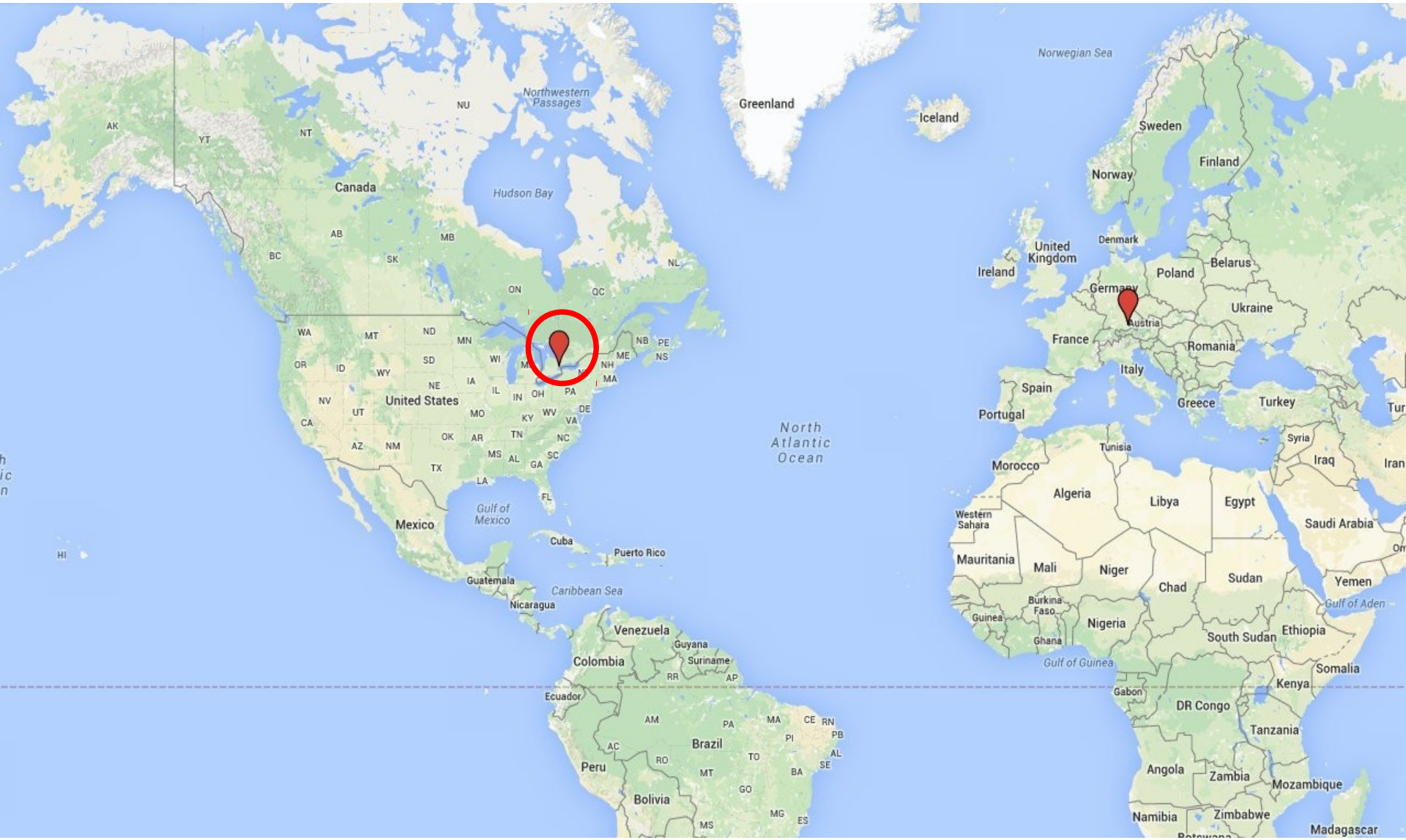


FIG. 4. The function $\sin(3s\Theta^2 - s^2\Theta^4)$, describing the phase shift in case of optimum contrast.

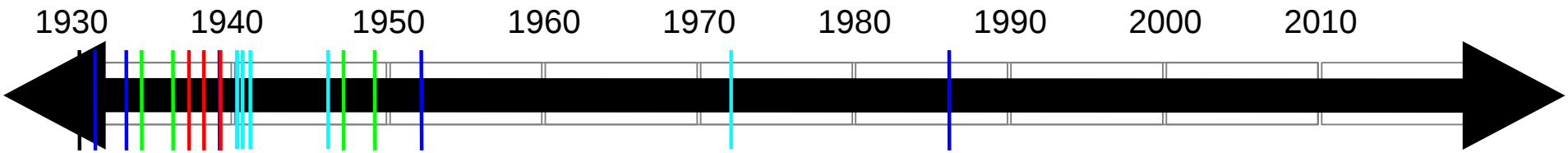
VOLUME 20, JANUARY, 1949

http://www.microscopy.org

Toronto group: E.F. Burton, James Hillier, etc.



James Hillier: timeline



Milestones:

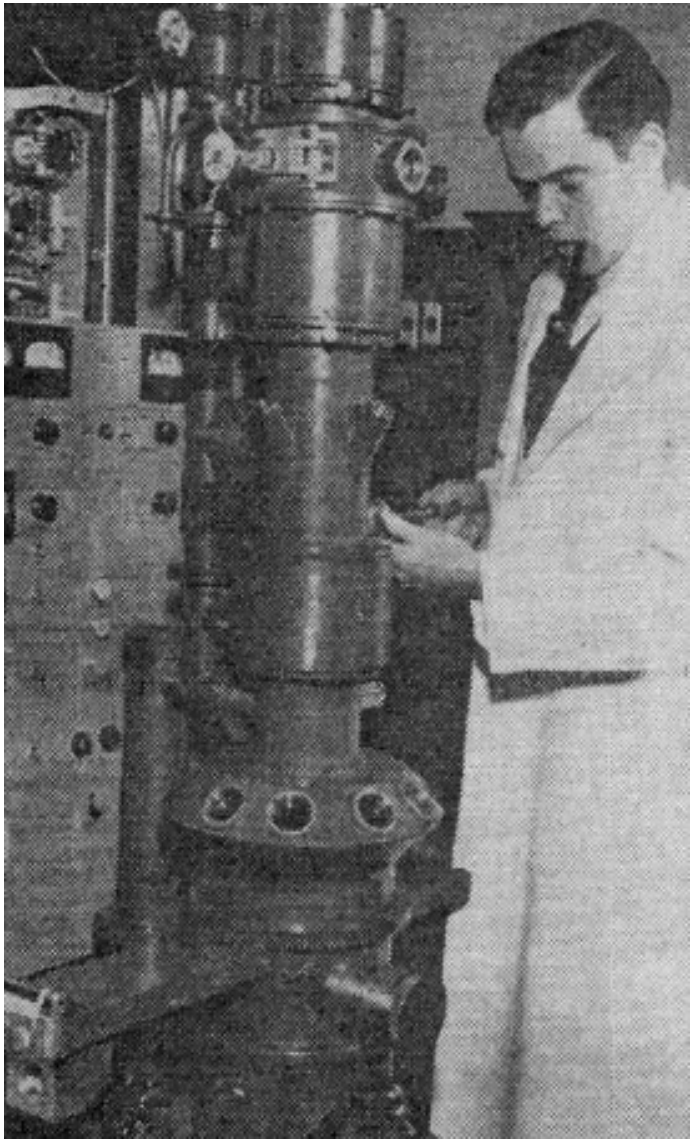
- 1940 February
  - Hillier started at RCA
  - enlisted by Vladimir Dworykin (cathode ray tube)
- 1940 Jul 4: Commercial EM, Model B (EMB)
- 1941: 300kV, for dealing with thick specimens
- 1947: first stigmator
  - stigmators were iron screws tapped into the pole piece
  - resolution → 1nm
- 1973 (as VP of RCA): first videodisc

James Hillier

Seated, with Albert Prebus standing

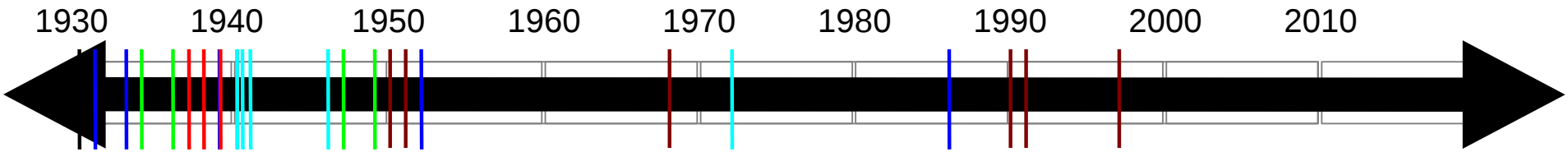
http://www.museevirtuel.ca

At RCA Model B, 1940



http://www.rfcafe.com

Electron microscopy in the Czech Republic

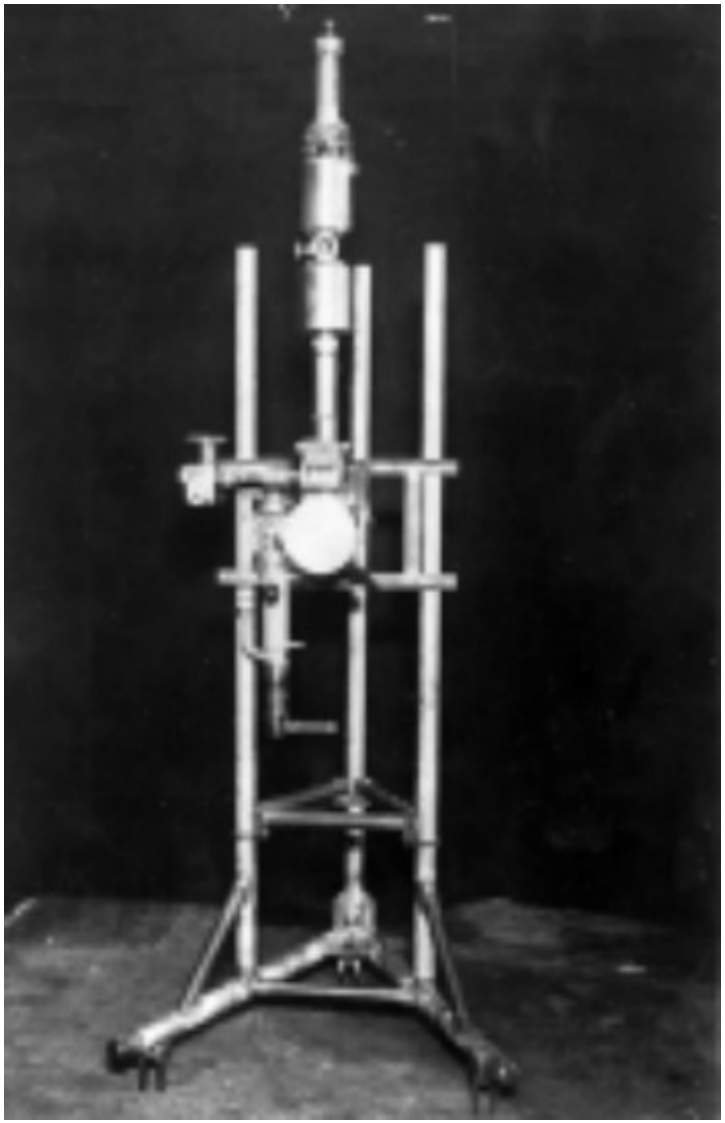


Milestones:

- 1950: Ales Blaha: “Tripod” at the Institute of Theoretical and Experimentation Electrotechnology of the Technical University
- 1951: Tesla BS241
  - first Czech commercial microscope
  - 50kV
  - resolution: 2nm
- 1968: First ultrahigh vacuum system (Institute of Scientific Instruments)
- 1990: Delong Instruments founded
- 1991: TESCAN founded by engineers from Tesla (TEsla SCANing)
- 1997: FEI builds factory in Brno

Electron microscopy in the Czech Republic

"Tripod," 1950



http://www.isibrno.cz

First high-vacuum system, 1961

http://www.isibrno.cz

Electron microscopy in the Czech Republic

Tescan Factory in Brno

Technical Museum in Brno

Electron microscopy of biological specimen

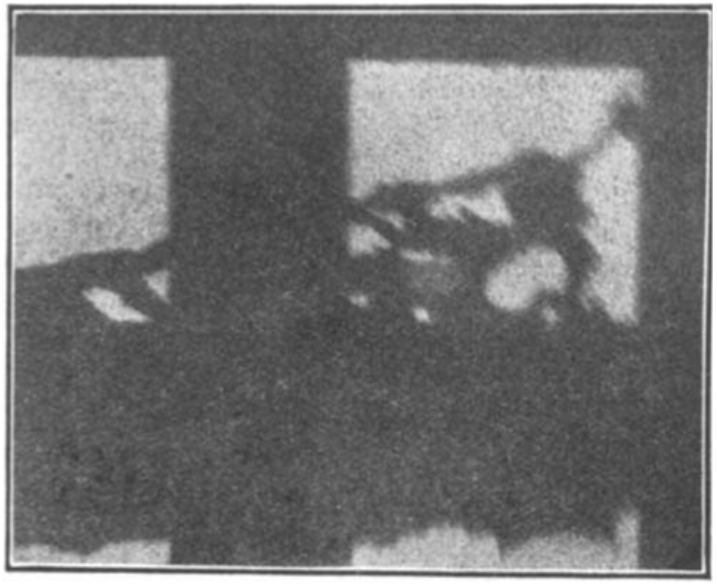
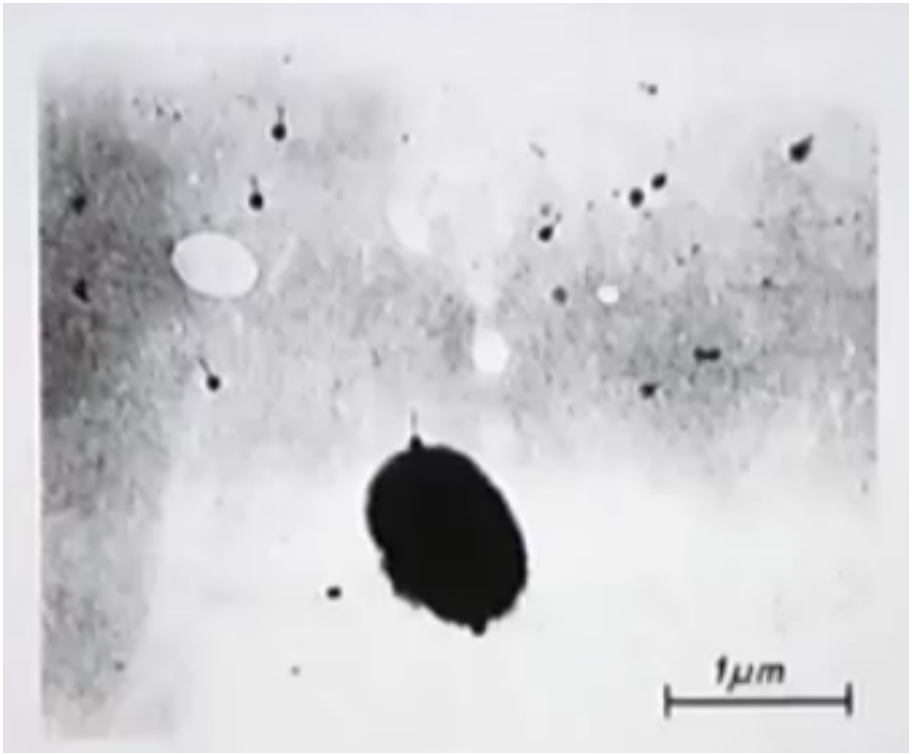


FIG. 2. × about 450.

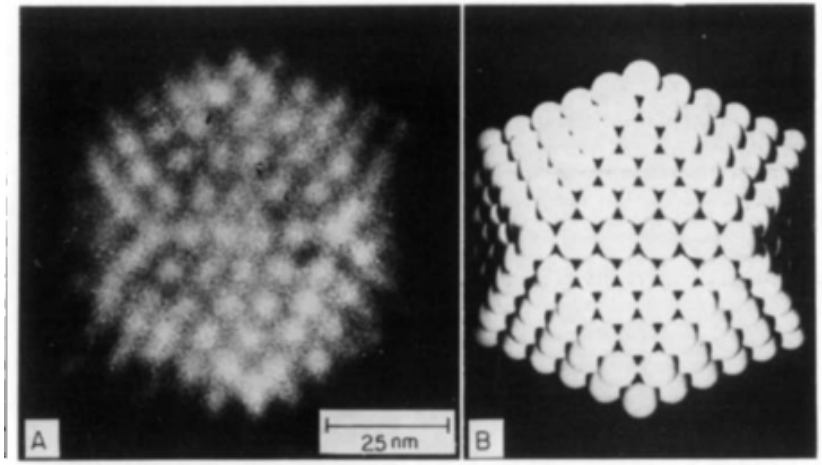
15um section of Drosera leaf, Marton, 1934

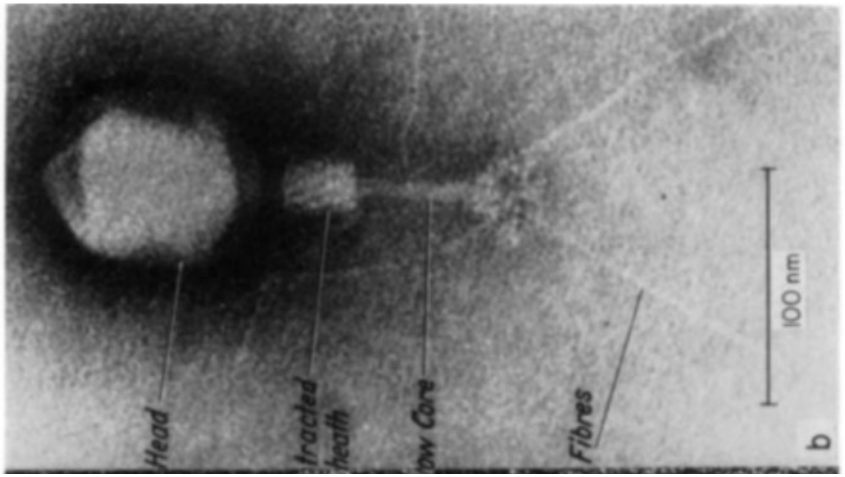


Bacteriophage, H. Ruska 1942

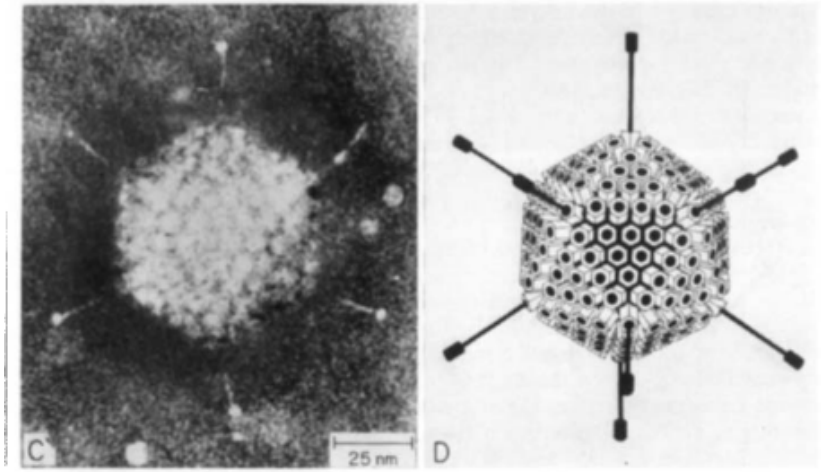
Electron microscopy of biological specimen

Negative staining (S. Brenner)

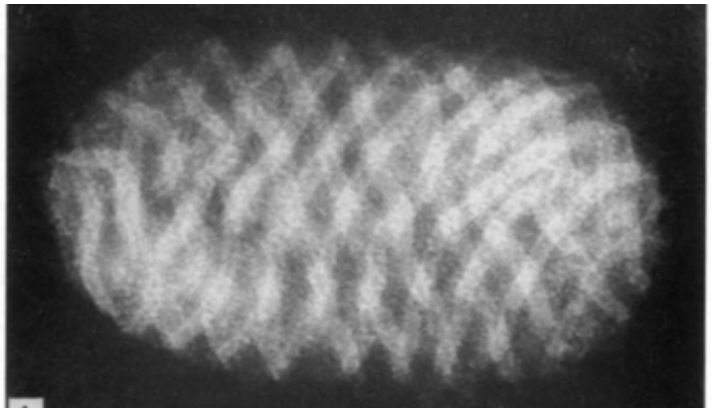




T4 bacteriophage



Adenovirus, W. Horn



Orf virus, W. Horn

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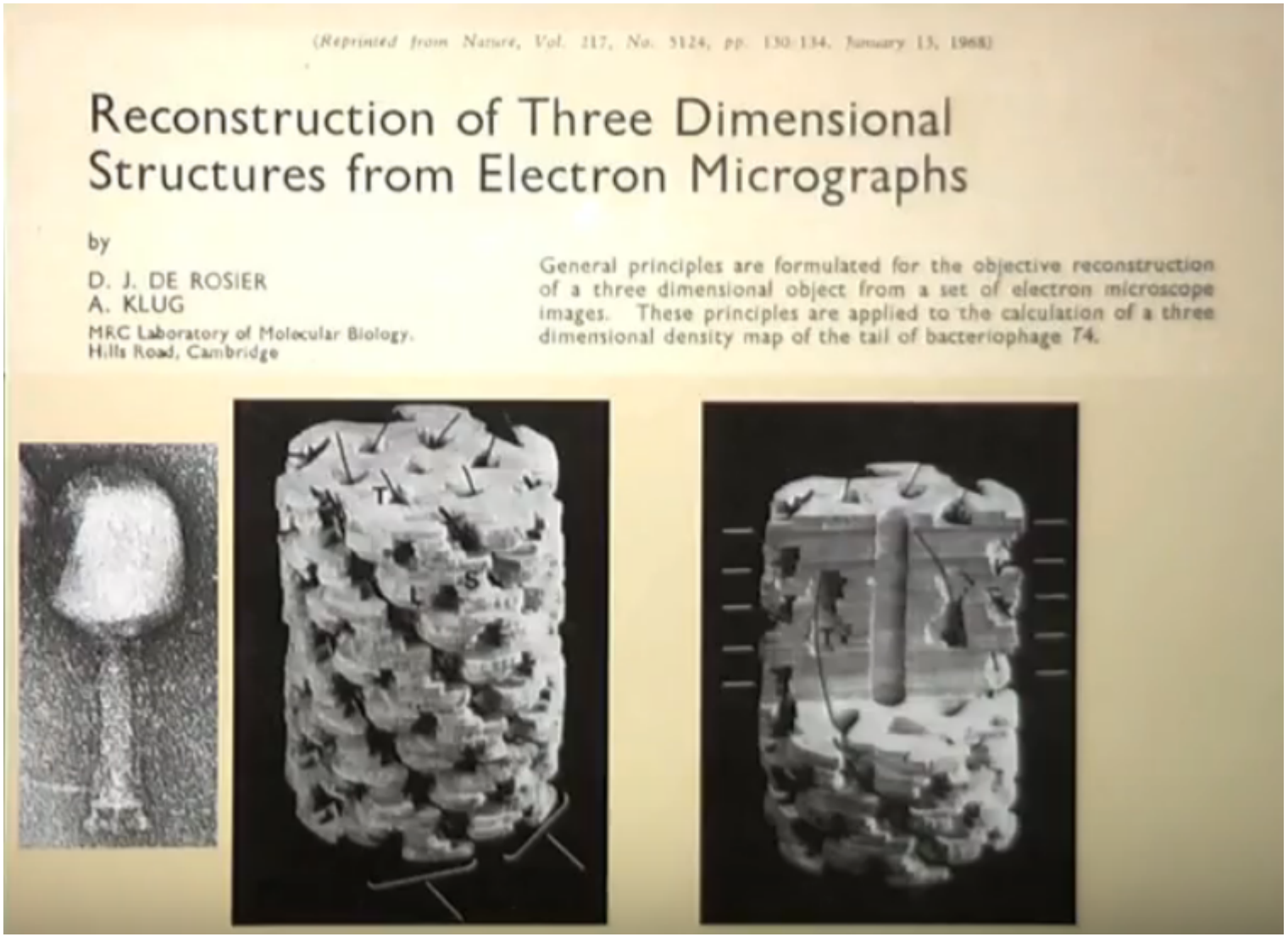
Electron microscopy of biological specimen



(Reprinted from Nature, Vol. 217, No. 5124, pp. 130-134, January 13, 1968)

Reconstruction of Three Dimensional Structures from Electron Micrographs

by

D. J. DE ROSIER
A. KLUG
MRC Laboratory of Molecular Biology, Hills Road, Cambridge

General principles are formulated for the objective reconstruction of a three dimensional object from a set of electron microscope images. These principles are applied to the calculation of a three dimensional density map of the tail of bacteriophage T4.

First qualitative image analysis

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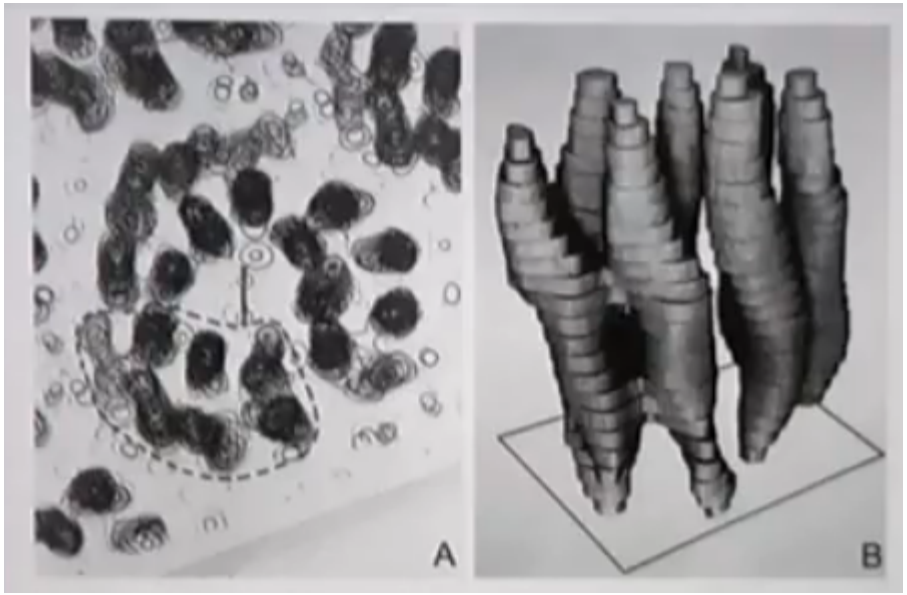
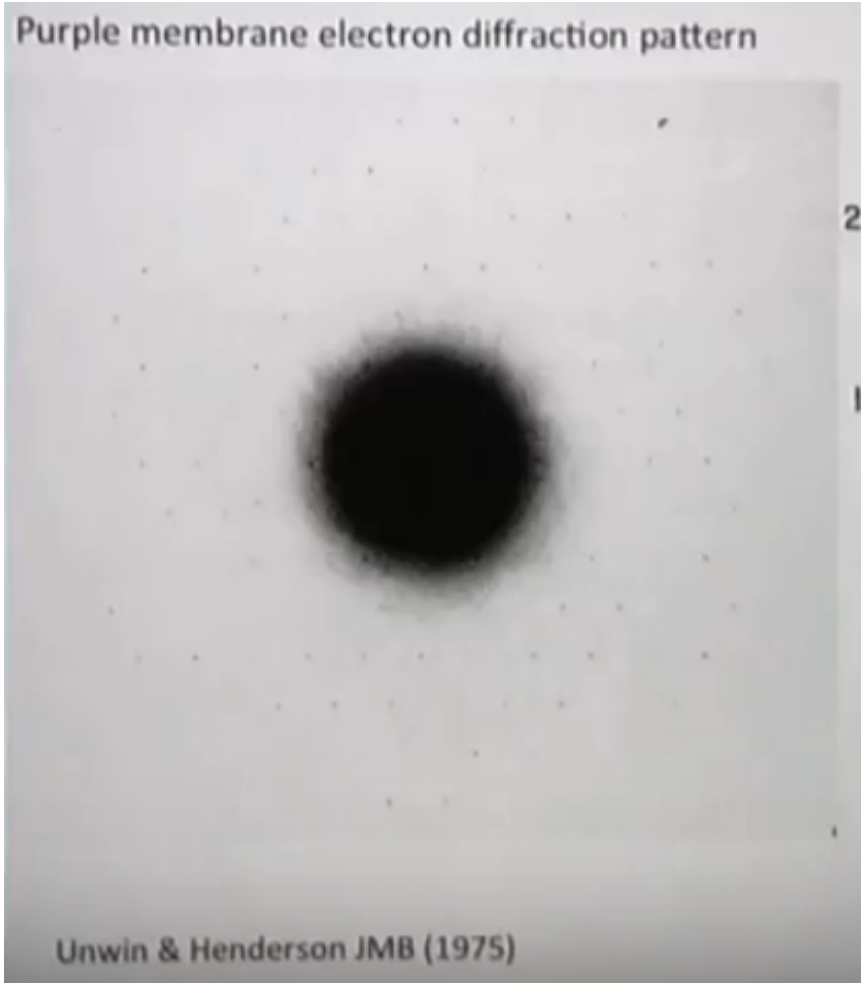
Electron microscopy of biological specimen

Electron cryostallography (R. Henderson, N. Unwin)

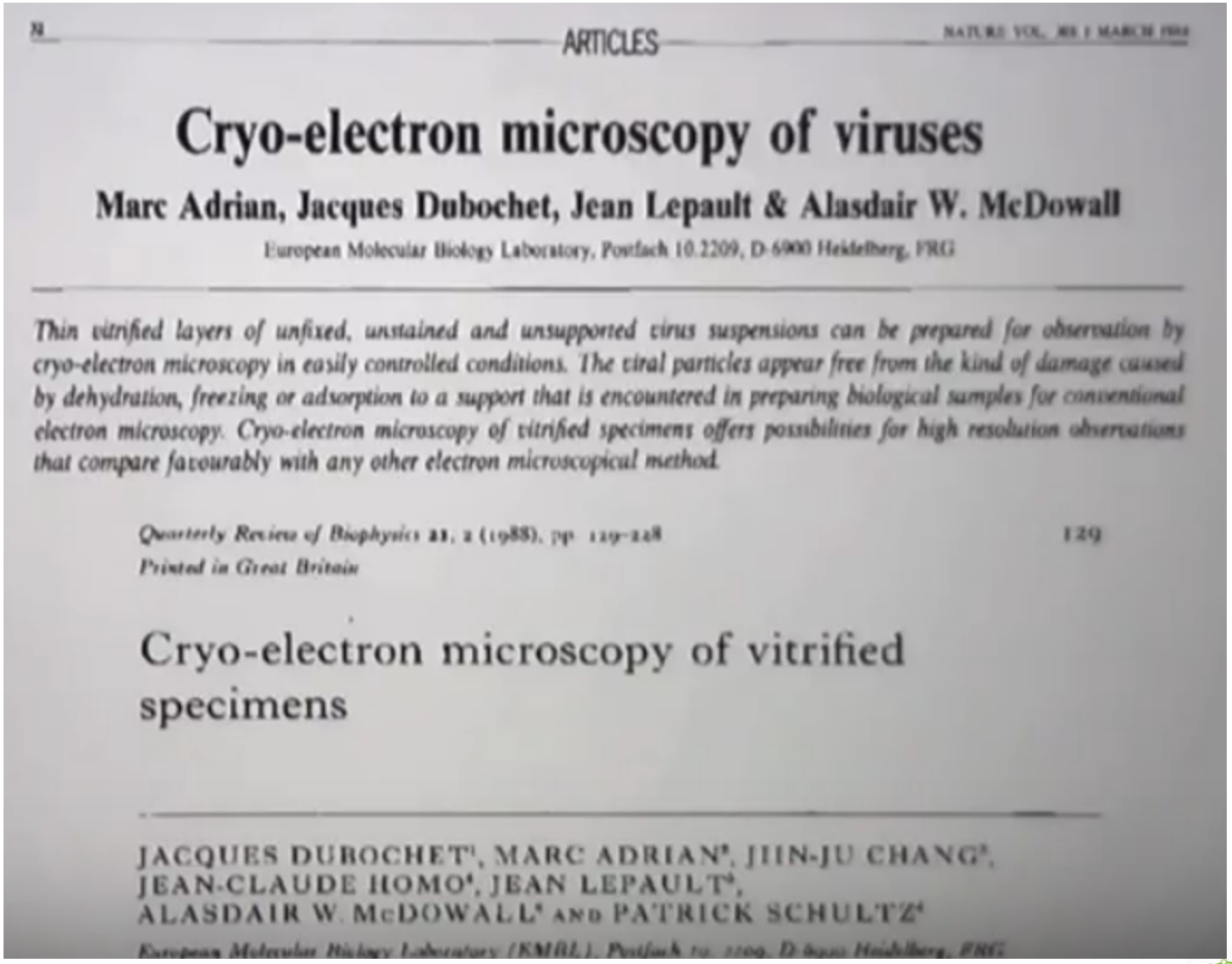




First sub-nanometer electron microscopy structure

Electron microscopy of biological specimen

Vitrification → Cryo-electron microscopy (J. Dubochet)



Cryo-electron microscopy of viruses

Marc Adrian, Jacques Dubochet, Jean Lepault & Alasdair W. McDowall

European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG

Thin vitrified layers of unfixed, unstained and unsupported virus suspensions can be prepared for observation by cryo-electron microscopy in easily controlled conditions. The viral particles appear free from the kind of damage caused by dehydration, freezing or adsorption to a support that is encountered in preparing biological samples for conventional electron microscopy. Cryo-electron microscopy of vitrified specimens offers possibilities for high resolution observations that compare favourably with any other electron microscopical method.

Quarterly Reviews of Biophysics 21, 2 (1988), pp. 129–228 129

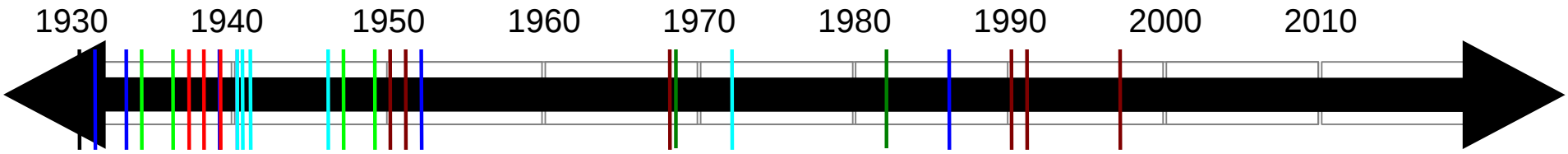
Printed in Great Britain

Cryo-electron microscopy of vitrified specimens

JACQUES DUBOCHET[1], MARC ADRIAN[2], JIIN-JU CHANG[3], JEAN-CLAUDE HOMO[4], JEAN LEPAULT[5], ALASDAIR W. McDOWALL[6] AND PATRICK SCHULTZ[4]

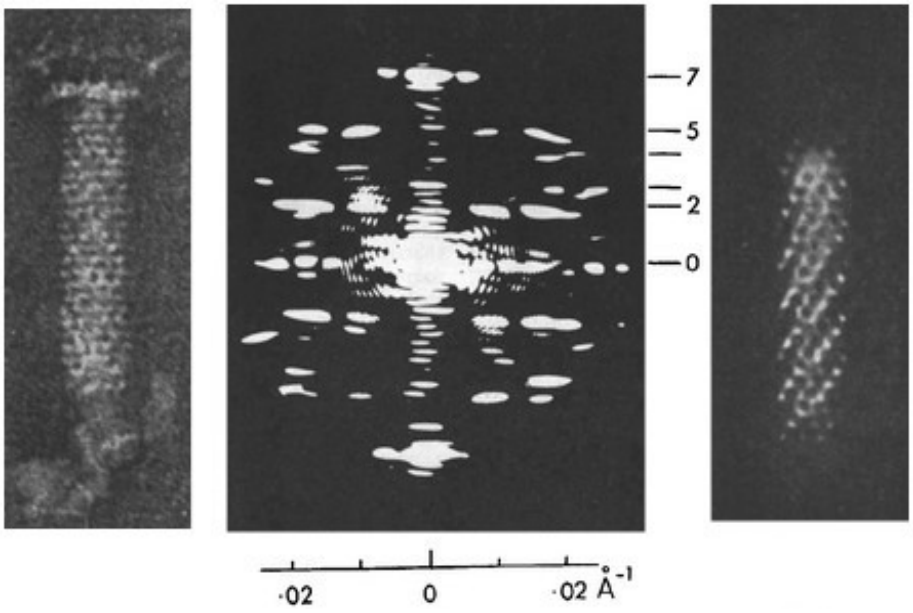
European Molecular Biology Laboratory (EMBL), Postfach 10.2209, D-6900 Heidelberg, FRG

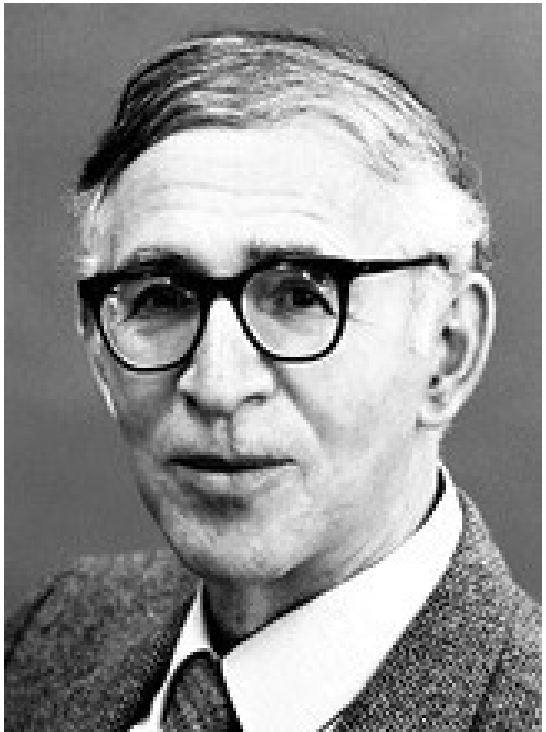
Aaron Klug: tim



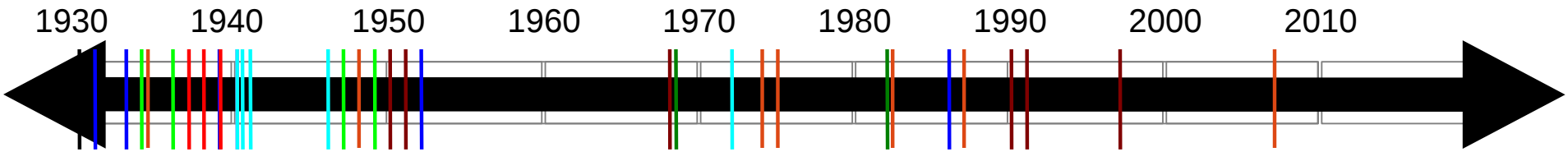
Milestones:

- 1968: DeRosier & Klug – first 3D EM reconstruction (phage T4)
- 1982: Nobel Prize in Chemistry





Other notable events



Milestones:

- 1934: Ladislaus Laszlo “Bill” Marton takes the first image of biological specimen: sections of a plant leaf
- 1937: Manfred von Ardenne (CRT) develops SEM
- 1948: Dennis Gabor develops electron holography (Nobel Prize in Physics, 1971)
- 1974: Ken Taylor & Bob Glaeser – electron crystallography of frozen hydrated catalase
- 1974: Walter Hoppe – 3D reconstruction of fatty acid synthase using tomography
- 1975: Richard Henderson – subnanometer electron crystallography
- 1982: Jacques Dubochet – modern cryo techniques
- 1987: Joachim Frank – “single particle” reconstruction of 50S ribosome
- 2007: Direct Electron develops first commercial direct electron detector

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