CEITEC

Central European Institute of Technology

BRNO | CZECH REPUBLIC

C9940 3-Dimensional Transmission Electron Microscopy
S1007 Doing structural biology with the electron microscope

Tanvir (Tapu) Shaikh

February 20, 2017

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

2007–13
OP Research and Development for Innovation

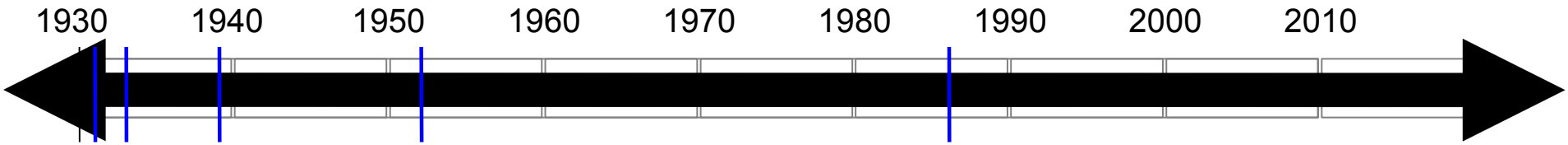
Syllabus

Week	Date	Instructor	Topic
1	20.2	T. Shaikh	Introduction/History/Optics
2	27.2	J. Novacek	Instrumentation/Tour (?)
3	6.3	J. Novacek	Specimen preparation
4	13.3	T. Shaikh	Image analysis I
5	20.3	T. Shaikh	Image analysis II
6	27.3	J. Novacek	Tomography I
7	3.4	J. Novacek	Tomography I
8	10.4	T. Shaikh	Image analysis III
	17.4		(Easter)
9	24.4	T. Shaikh	Image analysis IV
	1.5		(May Day)
	8.5		(Liberation Day)

History of electron microscopy

Munich: Ernst Ruska & Otto Scherzer

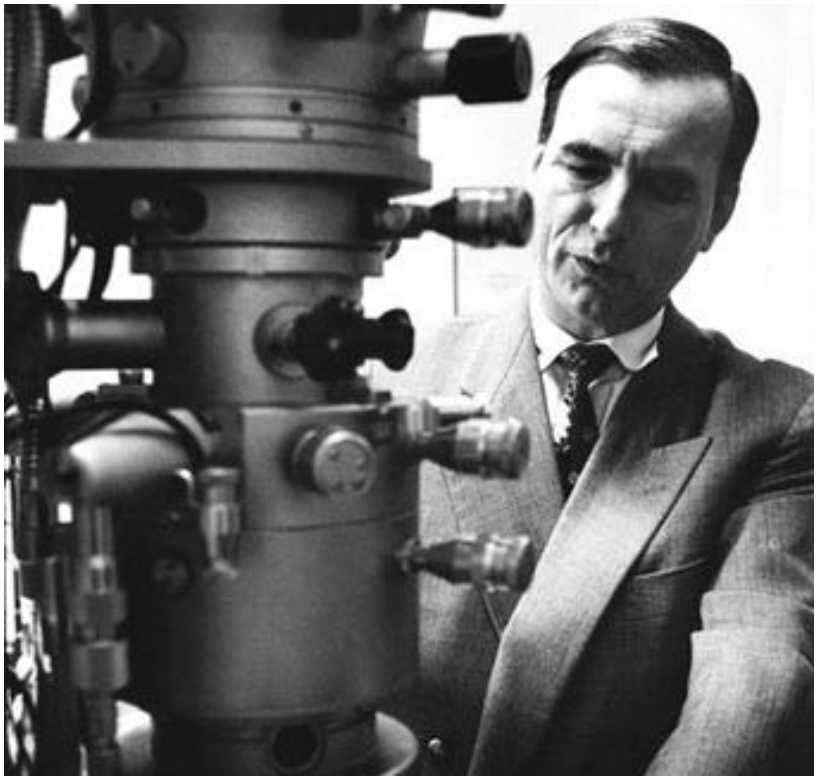
Ernst (and Helmut) Ruska: timeline



Milestones:

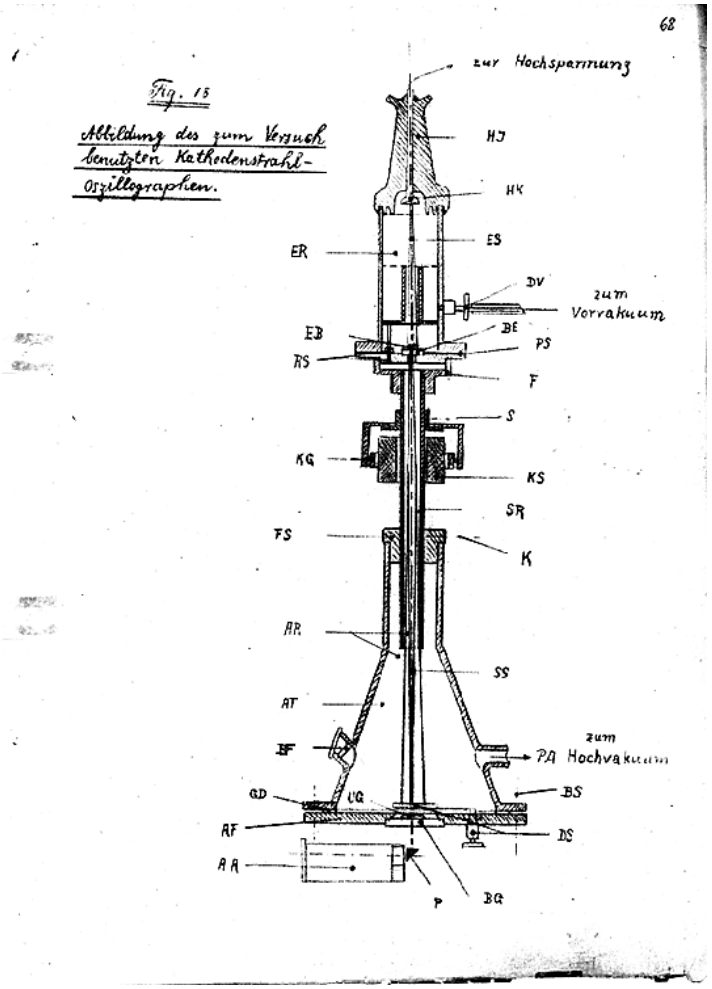
- 1931: Invention of the electrostatic lens
  - magnification: 400X
- 1933: First electron microscope
  - mag: 7000X (vs. LM: 2000X)
- 1933: Completed Ph.D. (!)
- 1939: First (viable) commercial EM
  - mag: 100,000X
- 1939: His brother Helmut Ruska images first virus (TMV)
- 1952: Helmut moves from Siemens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

Ernst Ruska



http://www.biografiasyvidas.com

Sketch from 1929



http://ernst.ruska.de

CEITEC

Replica of first electron microscope

http://www.bluesci.org

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

http://ernst.ruska.de

Helmut Ruska (standing)
next to Siemens-20
at Wadsworth Center in Albany

http://www.wadsworth.org

Clarification

First Siemens microscope, **1939**

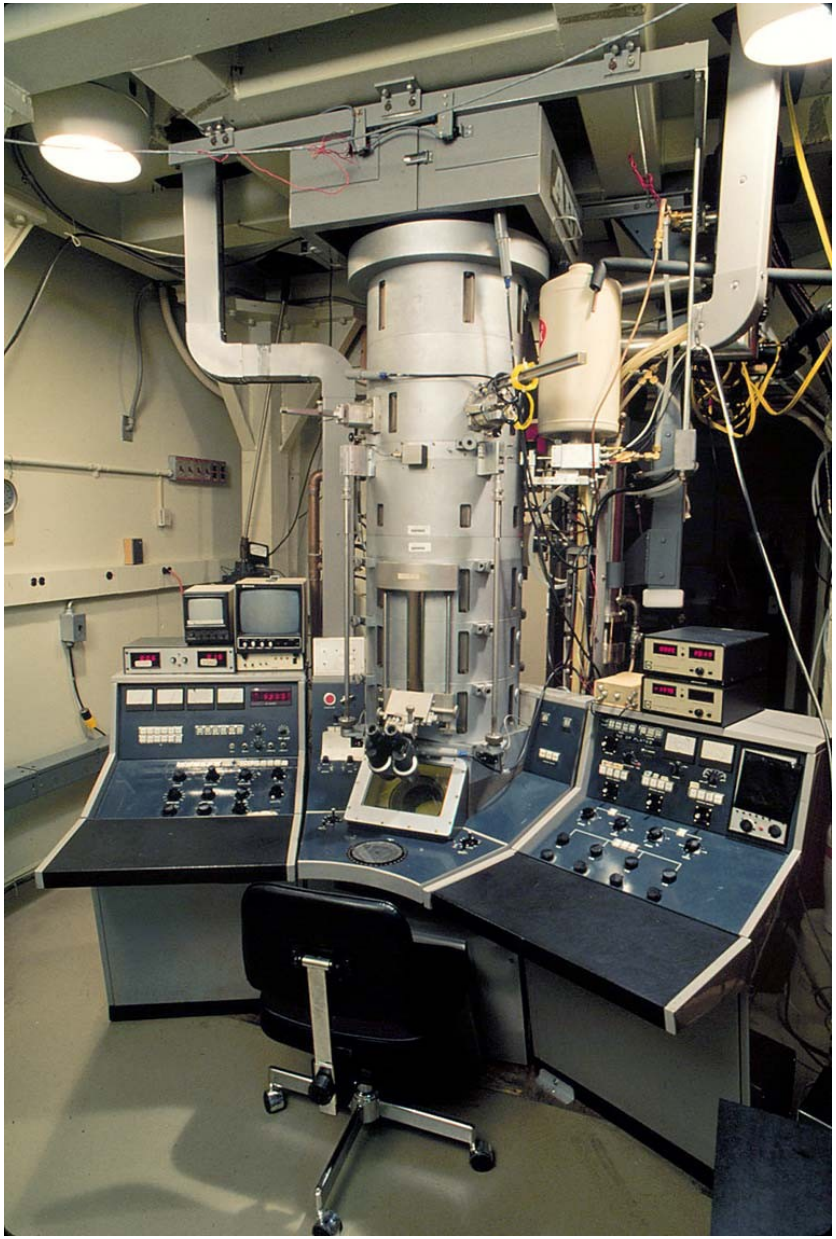
http://ernst.ruska.de

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

http://emu.msim.org.uk

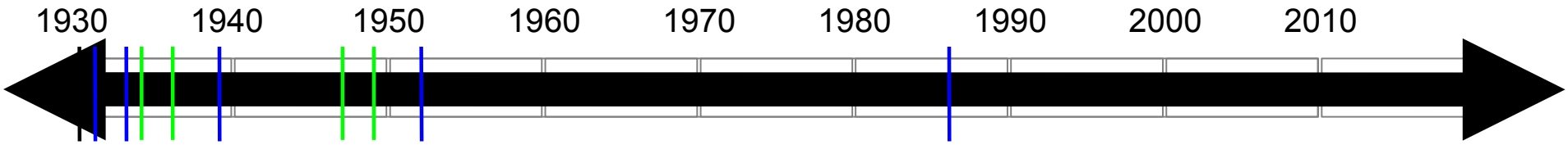
The first commercial electron microscope was actually by the British company Metropolitan-Vickers in 1937. However, the magnification was worse than for the light microscope, so the Siemens is considered “first.”

Metropolitan Vickers eventually became AEI, which built the 1.2 million volt EM-7.



http://www.wadsworth.org

Otto Scherzer: timeline



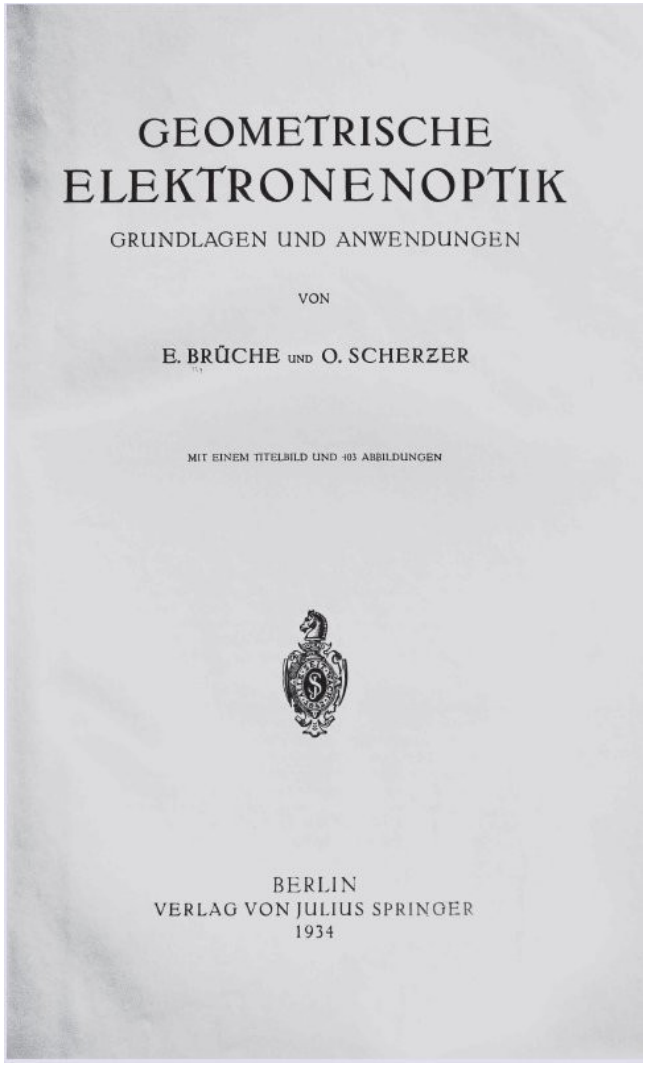
Milestones:

- 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"

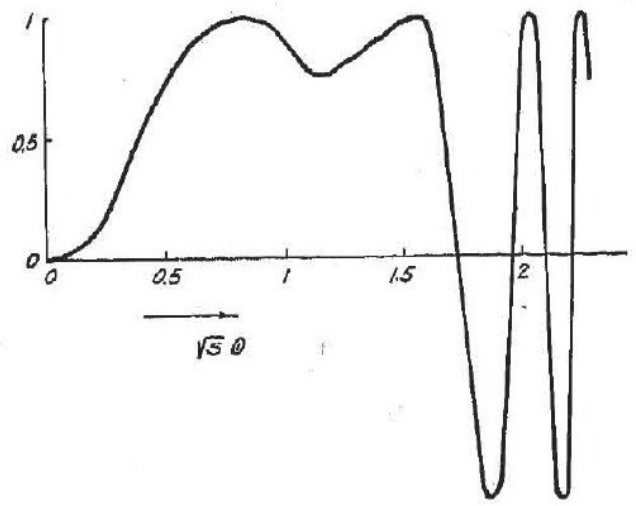
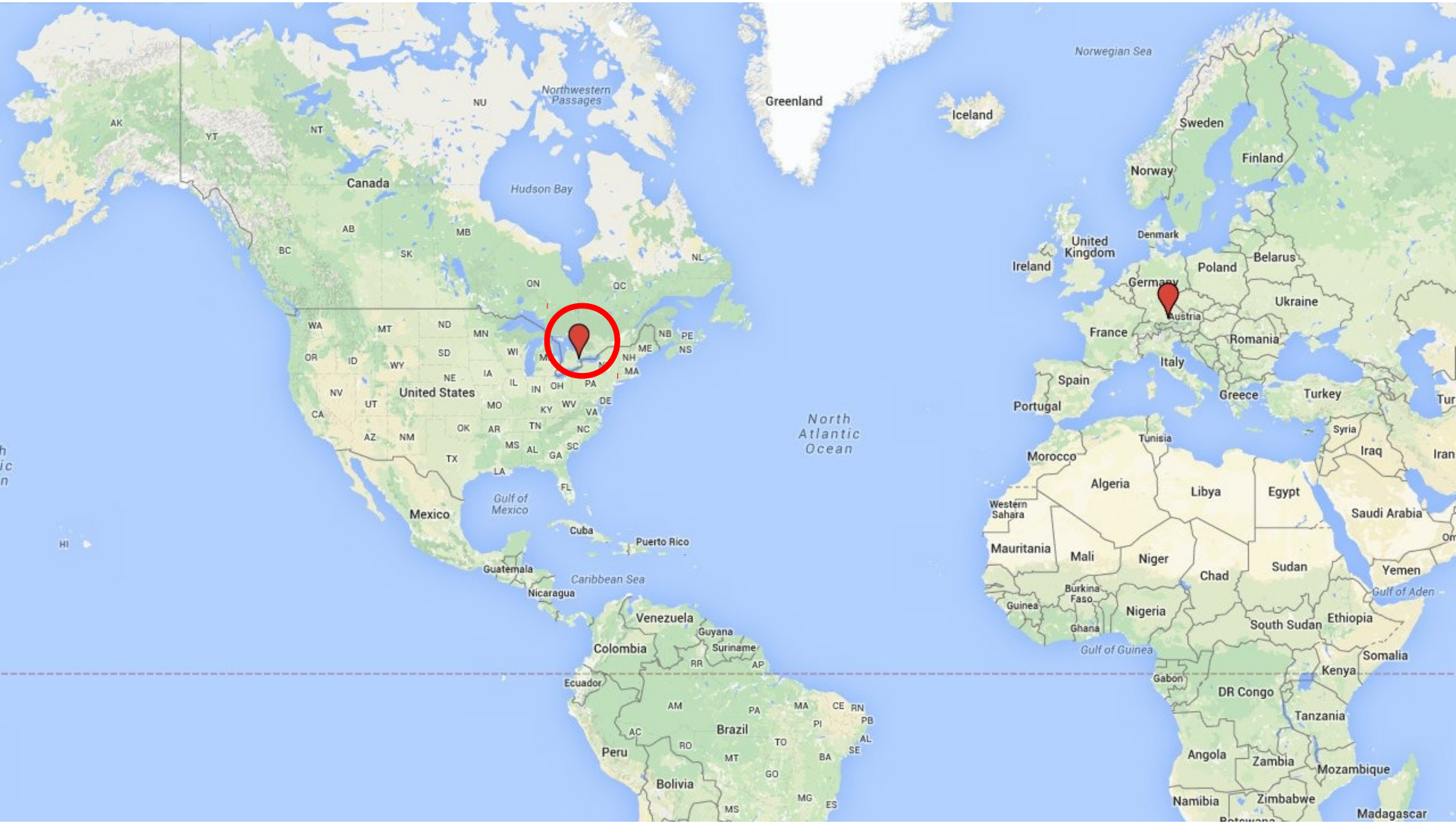


FIG. 4. The function $\sin(3s\Theta^2 - s^2\Theta^4)$, describing the phase shift in case of optimum contrast.

VOLUME 20, JANUARY, 1949

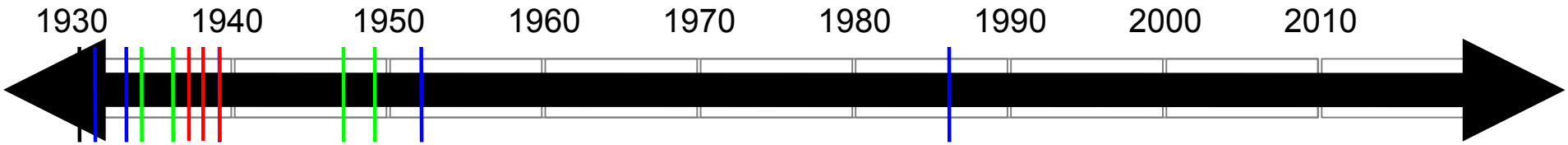
http://www.microscopy.org

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



CEITEC

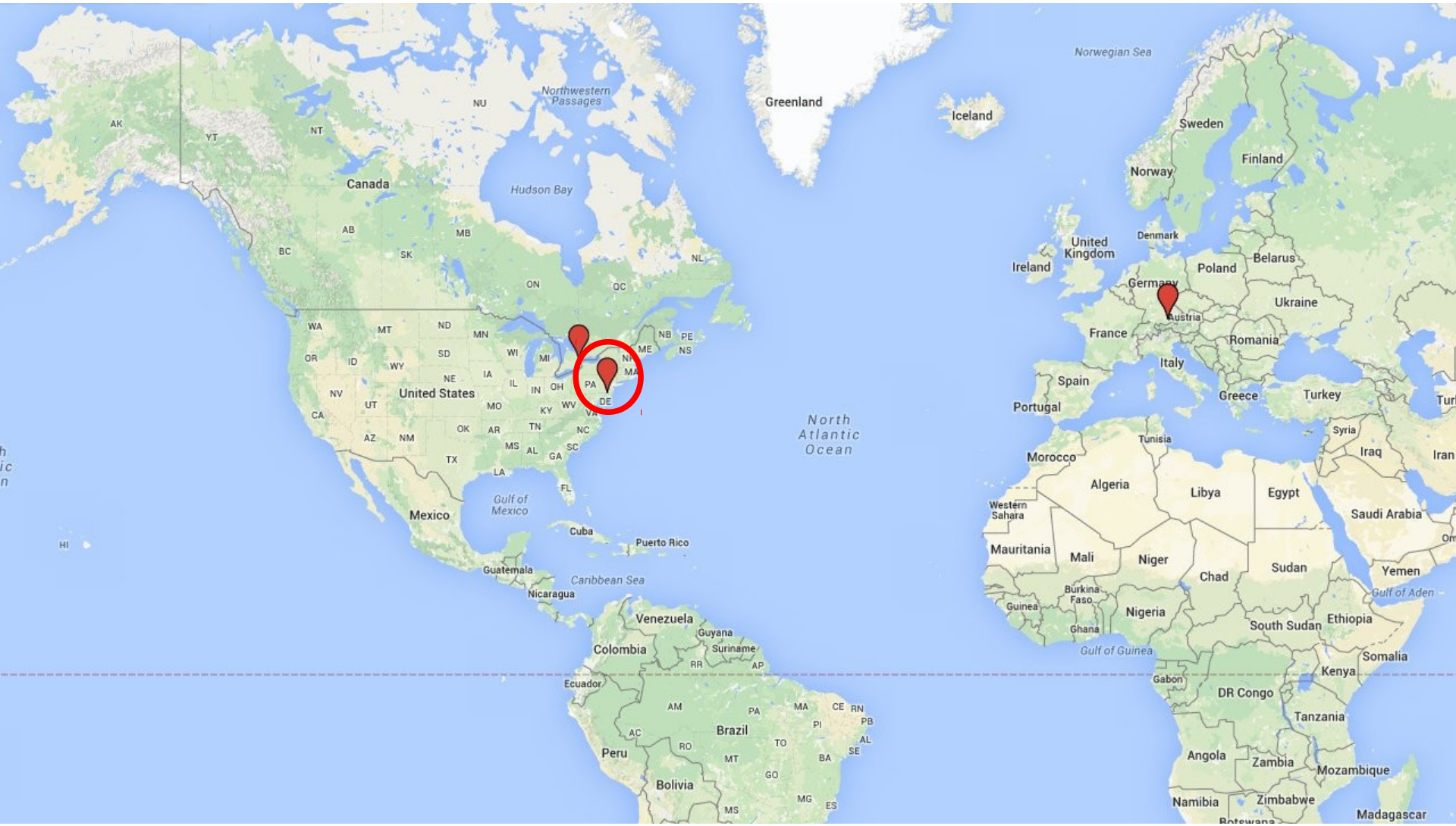
Toronto group: timeline



Milestones:

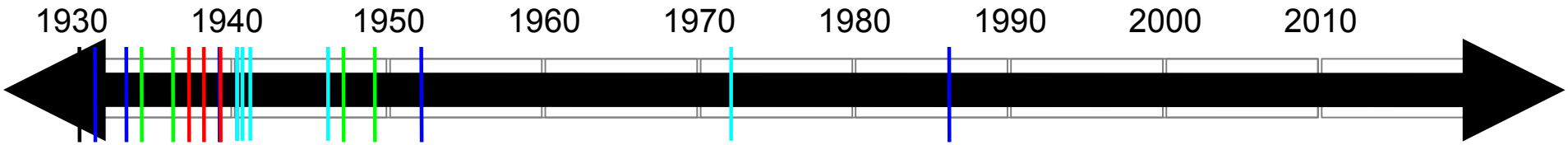
- 1937: Grad students James Hillier, Albert Prebus designed microscope over Christmas break
- 1938: First images
- 1939: E.F. Burton et al.
  - introduced airlock system
  - reduced specimen prep to 300nm thickness
  - resolution: 60Å, limited by specimen and not optics
  - maximum mag: 180,000X

Radio Corporation of America (RCA)



CEITEC

James Hillier: timeline



Milestones:

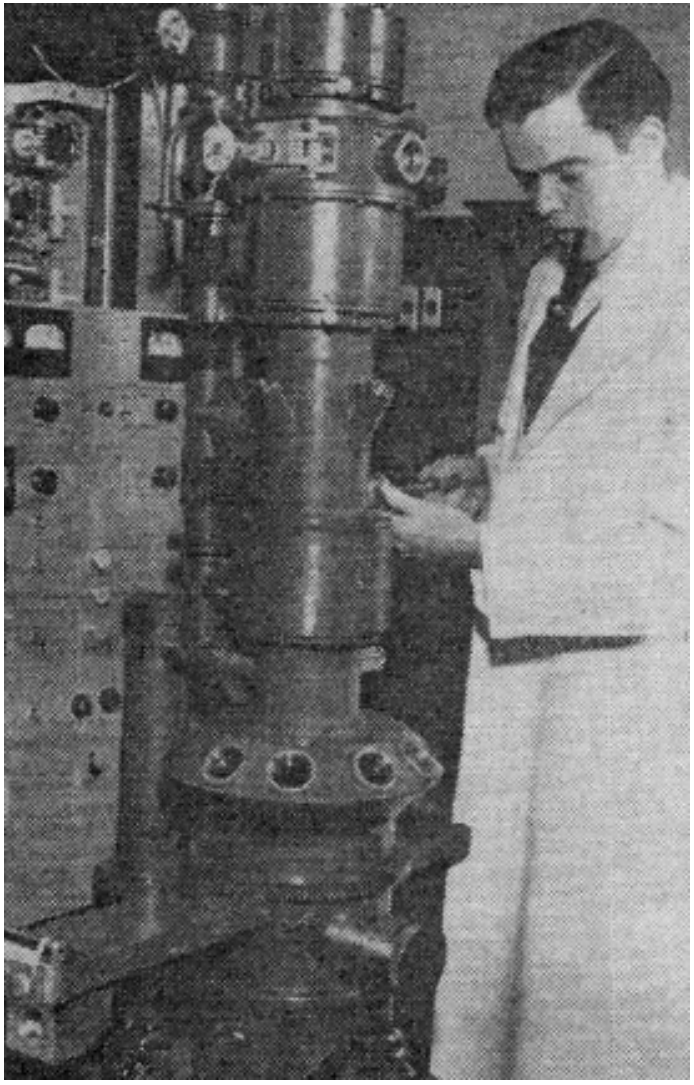
- 1940 February
  - Hillier started at RCA
  - enlisted by Vladimir Dworykin (cathode ray tube)
- 1940 Jul 4: Commercial EM, Model B (EMB)
- 1941: 300kV, for dealing with thick specimens
- 1947: first stigmator
  - stigmators were iron screws tapped into the pole piece
  - resolution → 1nm
- 1973 (as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

James Hillier

ed, with Albert Prebus standing

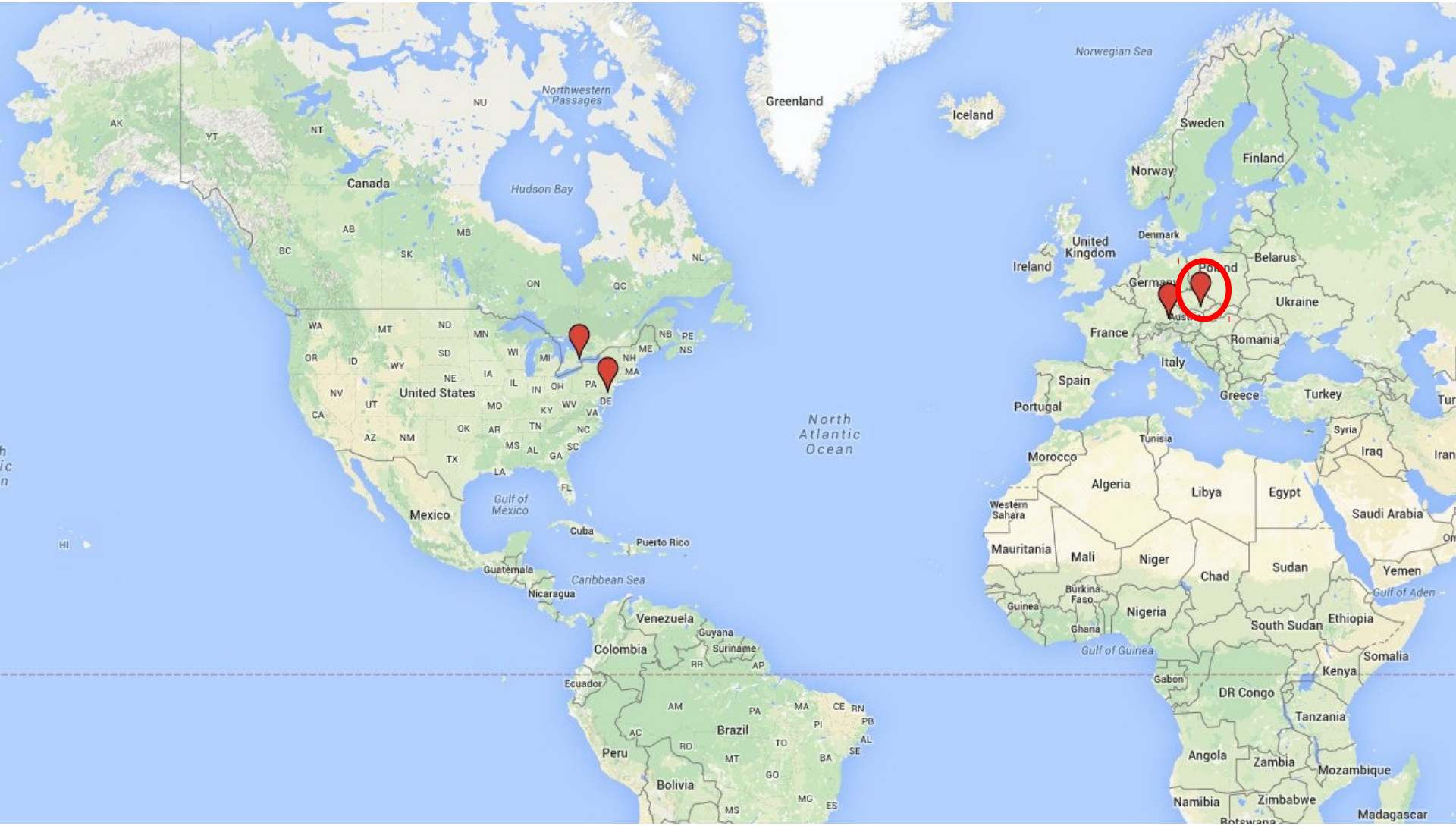
http://www.museevirtuel.ca

At RCA Model B, 1940



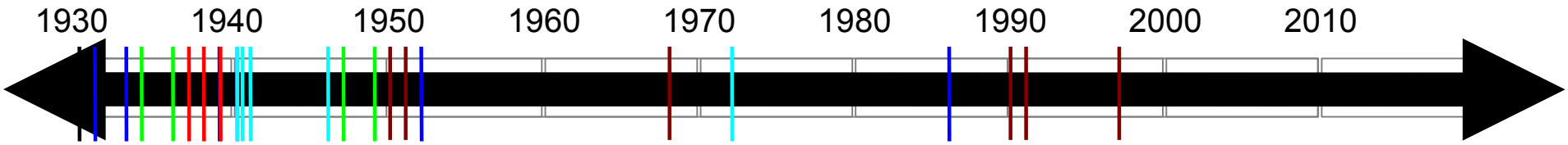
http://www.rfcafe.com

Electron microscopy in the Czech Republic



CEITEC

Electron microscopy in the Czech Republic

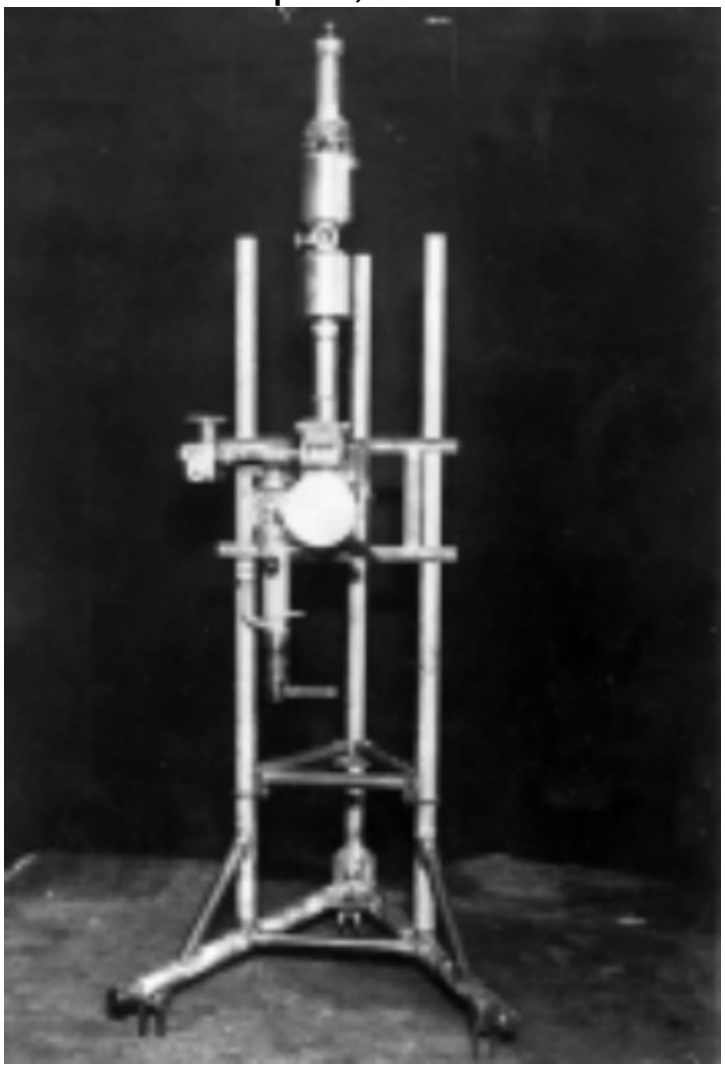


Milestones:

- 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

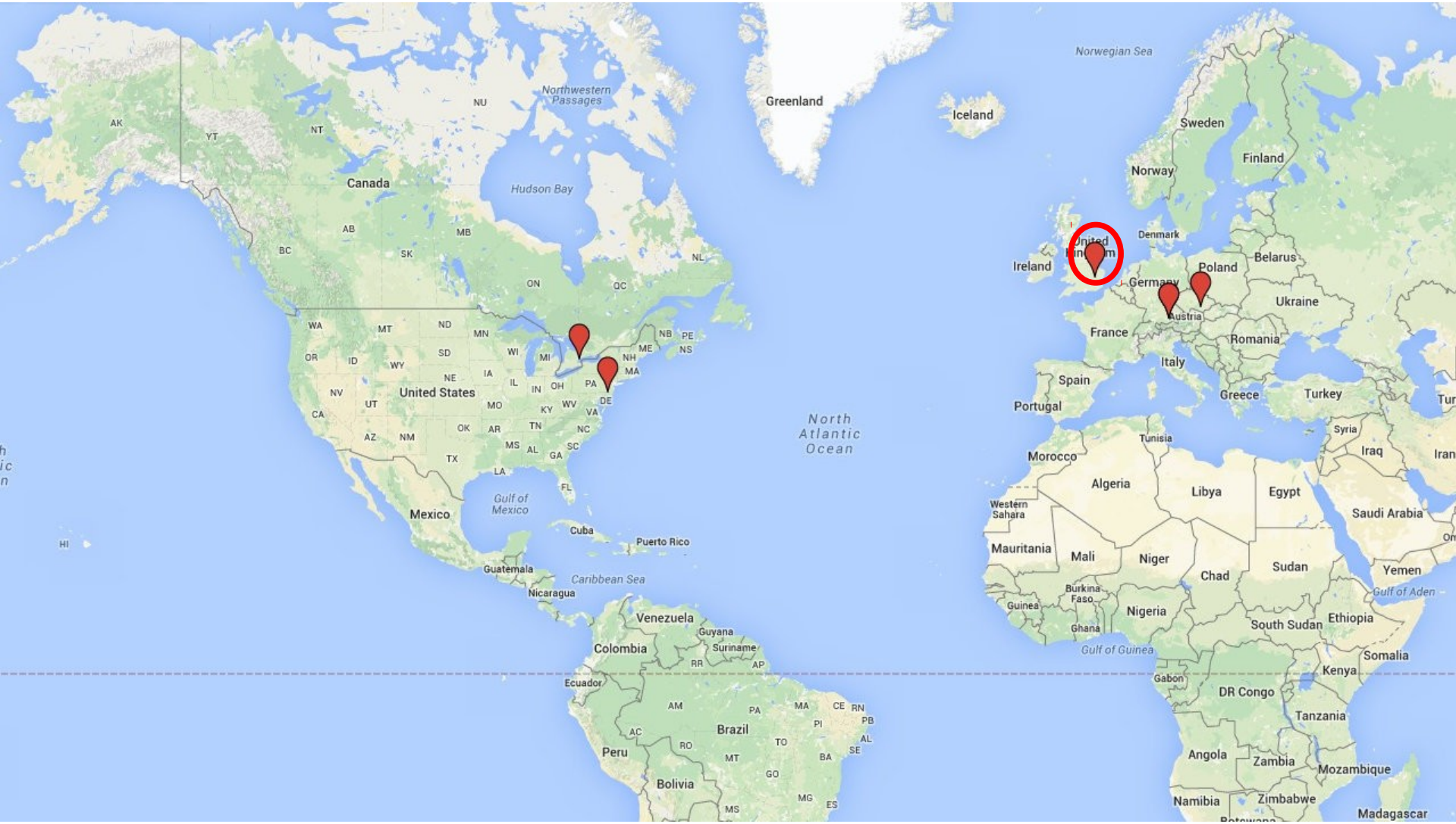
http://www.isibrno.cz

Electron microscopy in the Czech Republic

Tesla Factory in Brno

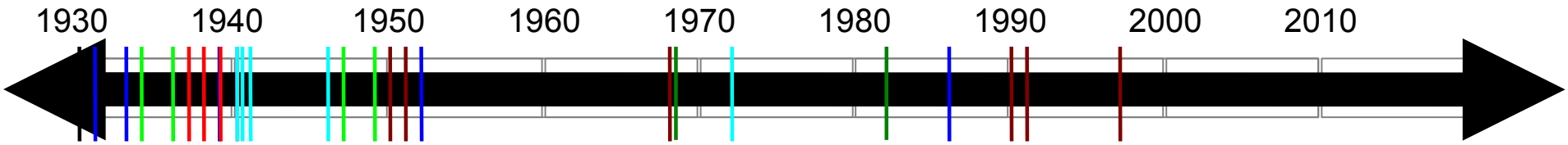
Technical Museum in Brno

Medical Research Council (MRC), Cambridge



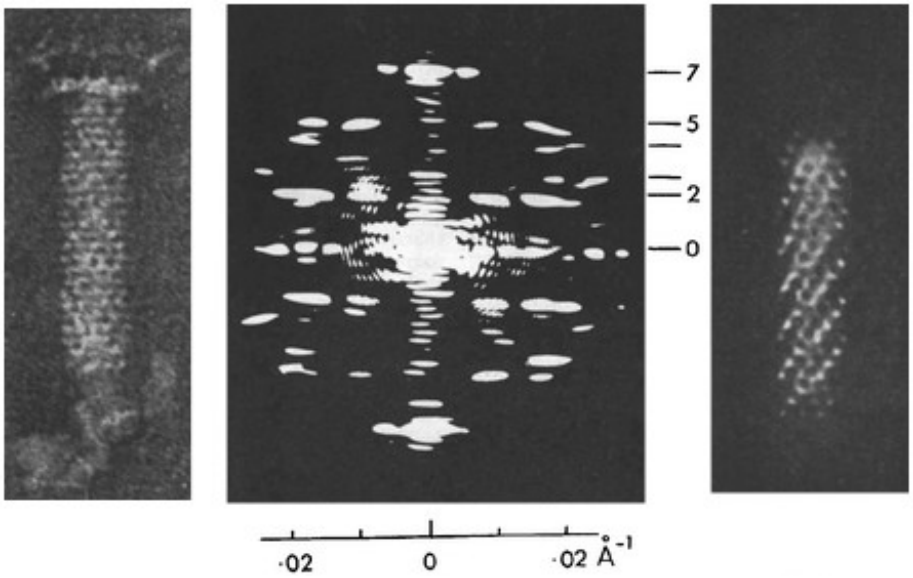
CEITEC

Aaron Klug: timeline

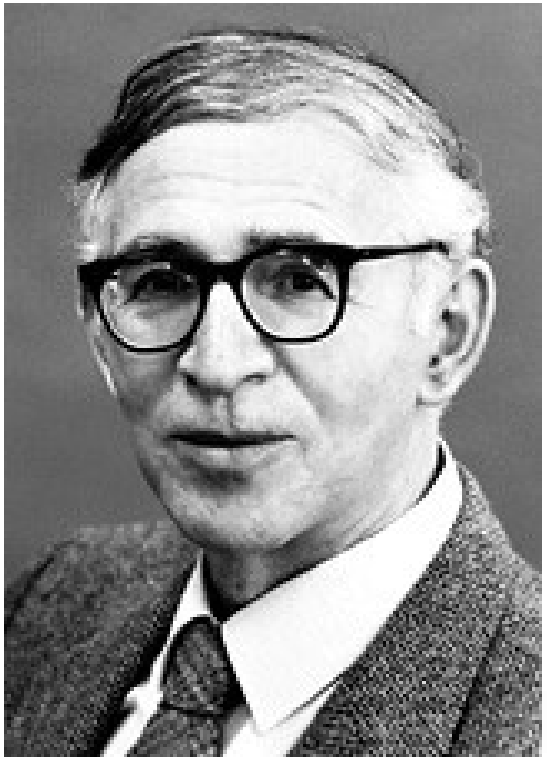


Milestones:

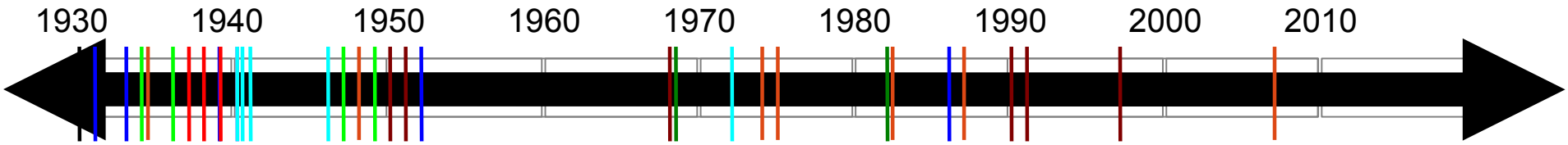
- 1968: DeRosier & Klug – first 3D EM reconstruction (phage T4)
- 1982: Nobel Prize in Chemistry



www.nobelprize.org



Other notable events



Milestones:

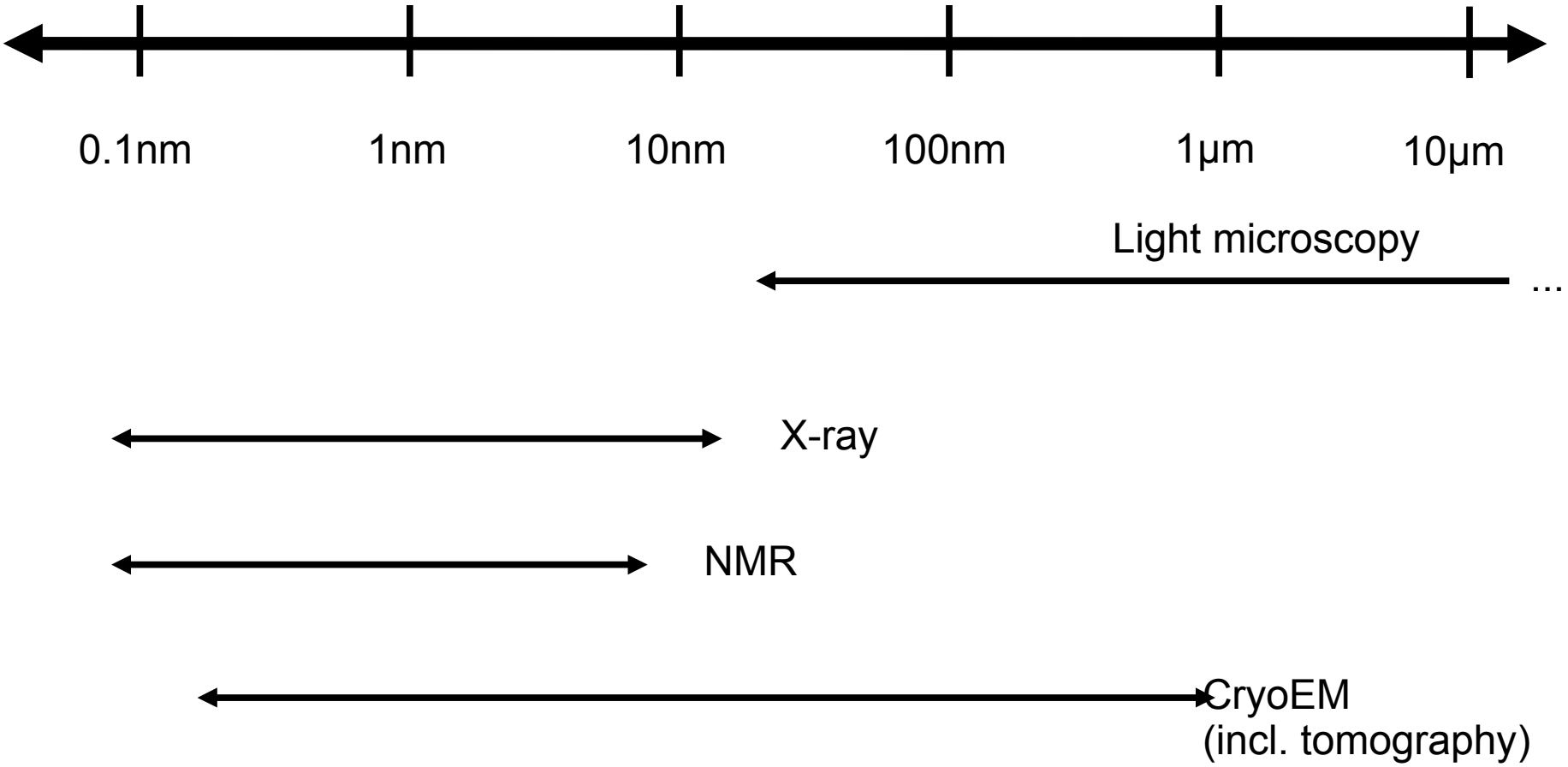
- 1934: Ladislaus Laszlo “Bill” Marton takes the first image of biological specimen: sections of a plant leaf
- 1937: Manfred von Ardenne (CRT) develops SEM
- 1948: Dennis Gabor develops electron holography (Nobel Prize in Physics, 1971)
- 1974: Ken Taylor & Bob Glaeser – electron crystallography of 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

“Oh no, not an electron microscope!
We have enough trouble trying to interpret the images we get with a light microscope!”
– Jules Bordet, 1934 (Nobel Prize, 1921)

The Beginnings of Electron Microscopy (1985), ed. Peter W. Hawkes

The basics

Size ranges for structural methods



Comparison of practical requirements

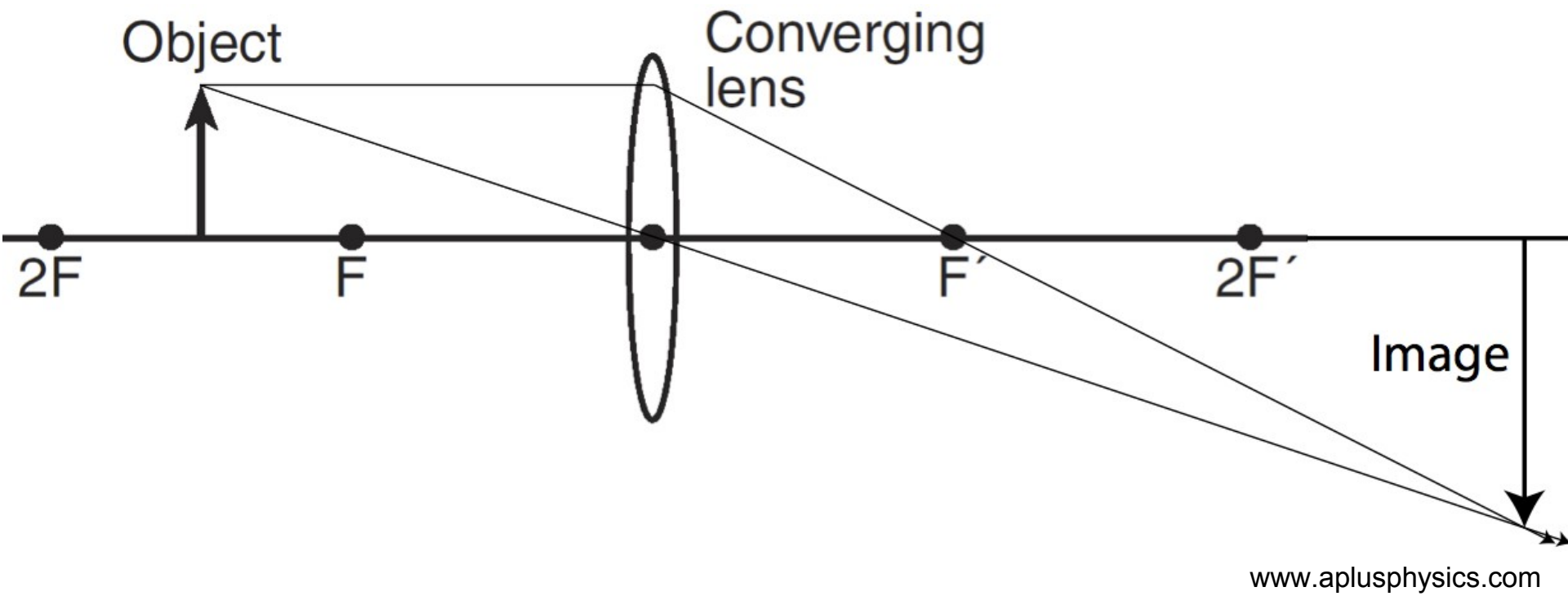
	CryoEM	X-ray	NMR
Sample volume	5µL[1]	10µl	400µl
Number of samples	5-10	1 crystal	1 (≤20kDa)[2] several (20-40kDa)
Concentration	50 nM	50 uM[3]	0.5-1.0 mM
Total amount of sample	0.25 pmol	500 pmol[4]	0.2-0.4 µmol
Time for data collection	1-7 days	2h (synchrotron) 48h (in-house)	1-2 weeks (≤20kDa) 6-8 weeks (20-40kDa)

[1]Droplet on grid, prior to blotting
[2]For stable proteins of this size a single U-13C, 15N sample is likely sufficient
[3]Based on 10 mg/ml of a 100 kDa protein
[4]Final amount required for the crystal. However, a multiple of this amount is required to try out different crystallization conditions

Adapted from Raj Agrawal

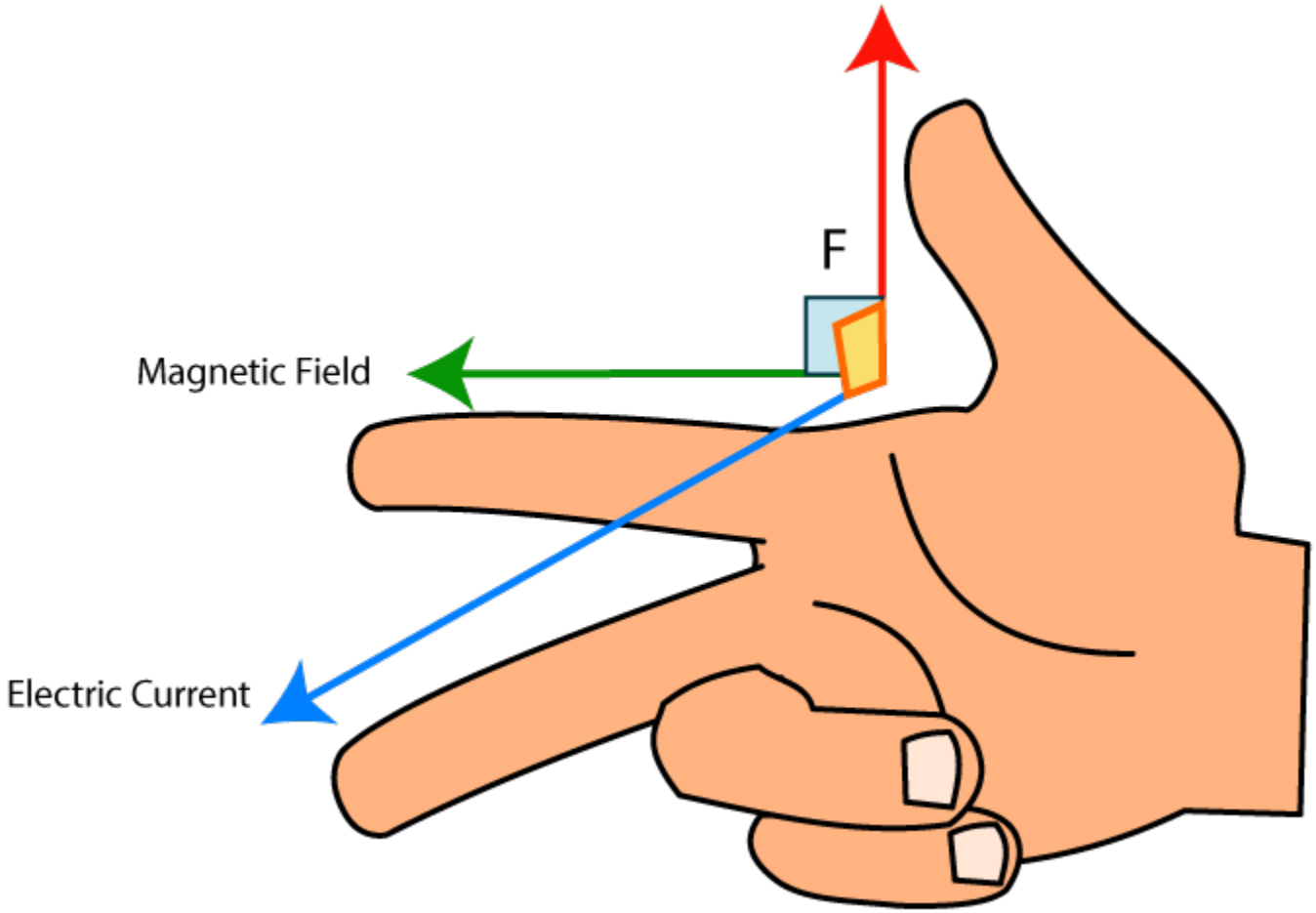
CEITEC

Quick review of light optics



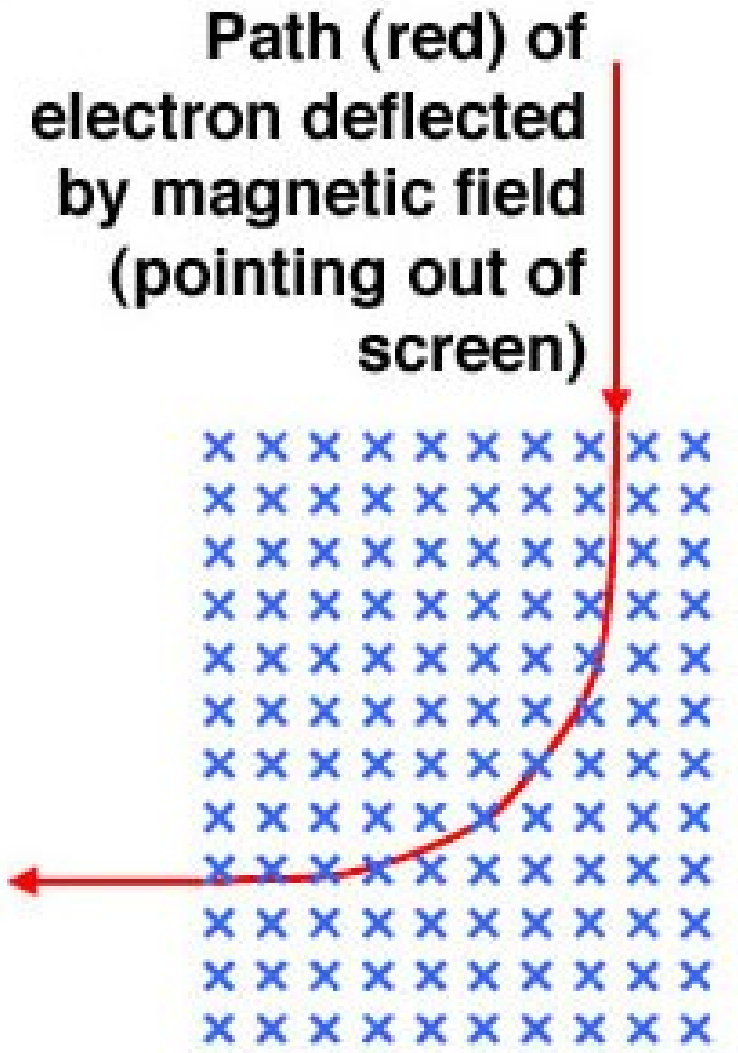
www.aplusphysics.com

Right-hand rule



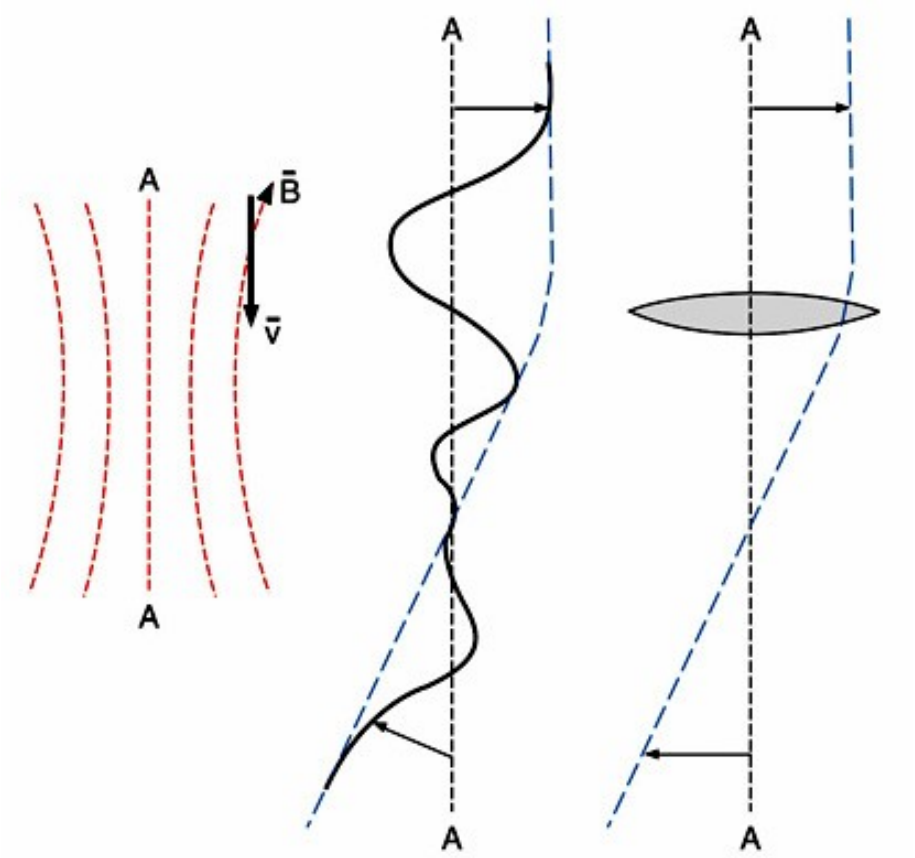
www.education.com

Right-hand rule

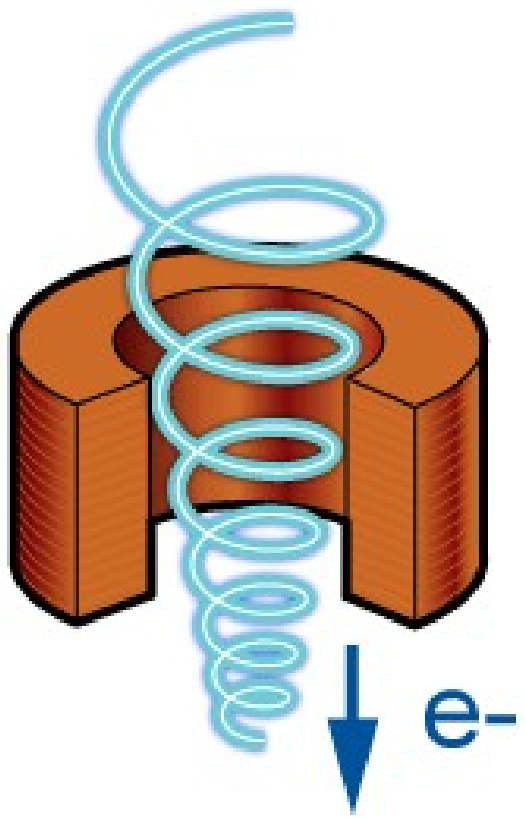


http://www.polywellnuclearfusion.com

Electron in a magnetic lens



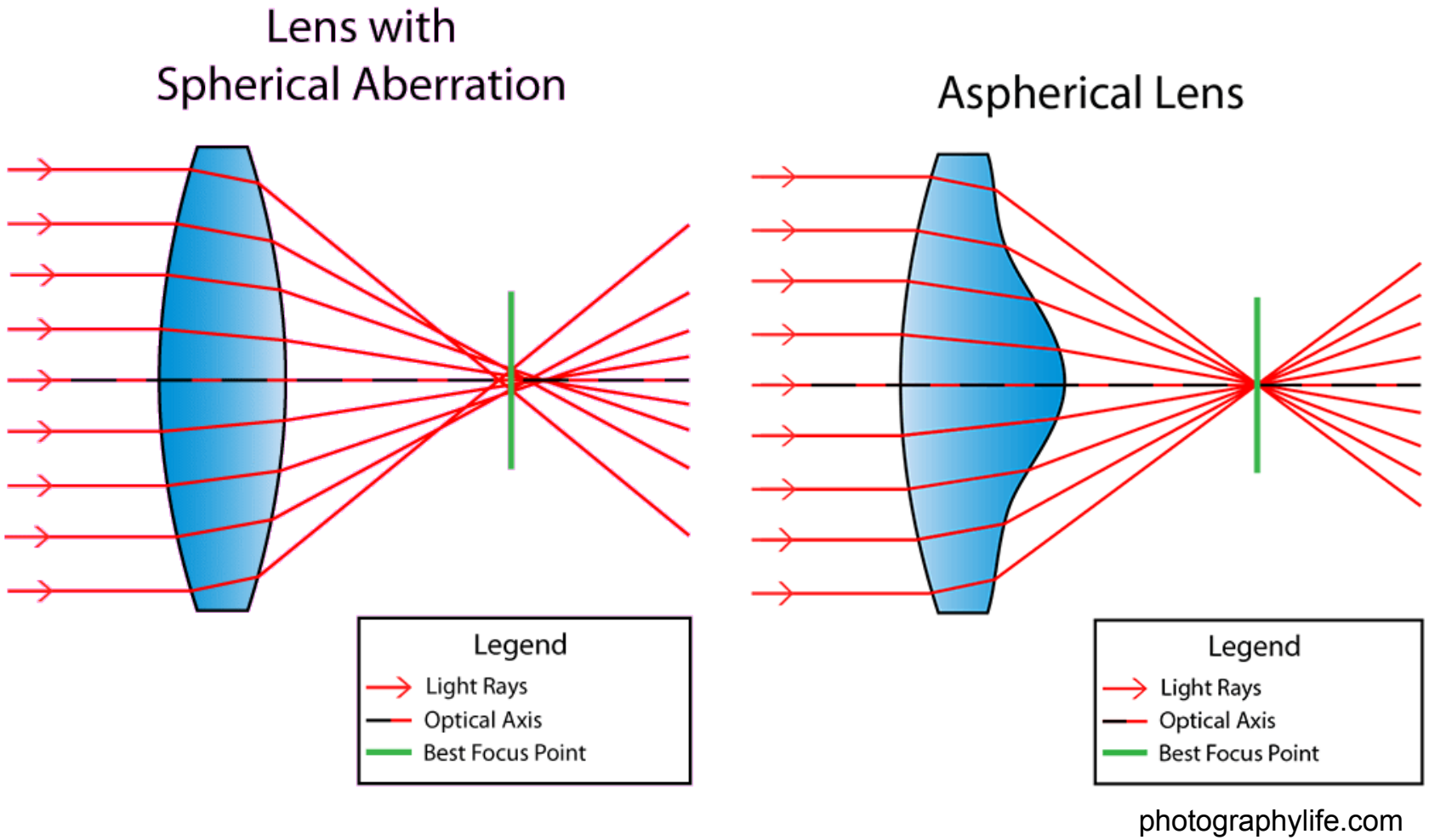
nau.edu



http://www.ammrf.org.au

One problem:
Magnetic lenses are terrible

Spherical aberration



photographylife.com

In electron optics, there is no lens setting* equivalent to an aspherical lens. Spherical aberration will thus be a problem (but also a benefit).

Syllabus

Week	Date	Instructor	Topic
1	20.2	T. Shaikh	Introduction/History/Optics
2	27.2	J. Novacek & T. Shaikh	Instrumentation/Tour (?)
3	6.3	J. Novacek	Specimen preparation
4	13.3	T. Shaikh	Image analysis I
5	20.3	T. Shaikh	Image analysis II
6	27.3	J. Novacek	Tomography I
7	3.4	J. Novacek	Tomography I
8	10.4	T. Shaikh	Image analysis III
	17.4		(Easter)
9	24.4	T. Shaikh	Image analysis IV
	1.5		(May Day)
	8.5		(Liberation Day)

Thank you for your attention

CEITEC

Central European Institute of Technology
Masaryk University
Kamenice 753/5
625 00 Brno, Czech Republic

www.ceitec.muni.cz | info@ceitec.muni.cz

UNIVERSITAS MASARYKIANA BRUNENSIS

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

2007–13
OP Research and Development for Innovation