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

*Tanvir (Tapu) Shaikh*

**February 22, 2016**

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# Syllabus

| Week | Date | Instructor | Topic |
|---|---|---|---|
| 1 | 02/22 | T. Shaikh | Introduction/History/Optics |
| 2 | 02/29 | J. Novacek & T. Shaikh | Instrumentation/Tour (?) |
| 3 | 03/07 | J. Novacek | Specimen preparation |
| 4 | 03/14 | T. Shaikh | Image analysis I |
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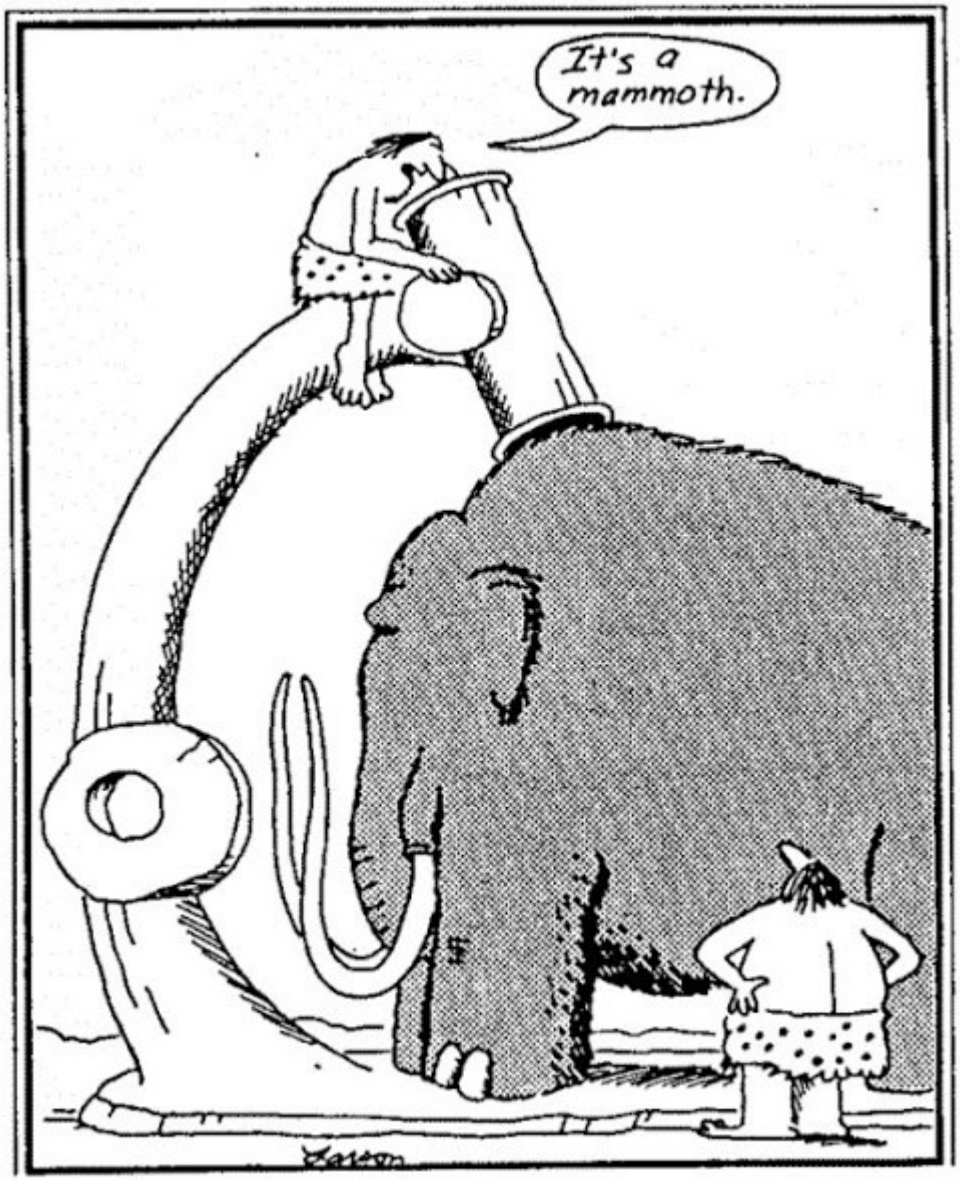
# *History of electron microscopy*




Early microscope

http://www.thefarside.com/

# Munich: Ernst Ruska & Otto Scherzer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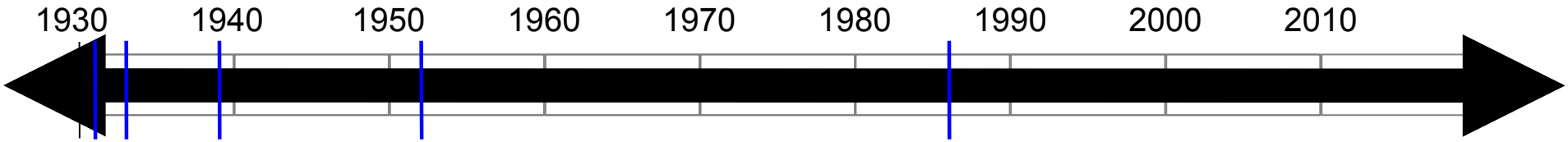
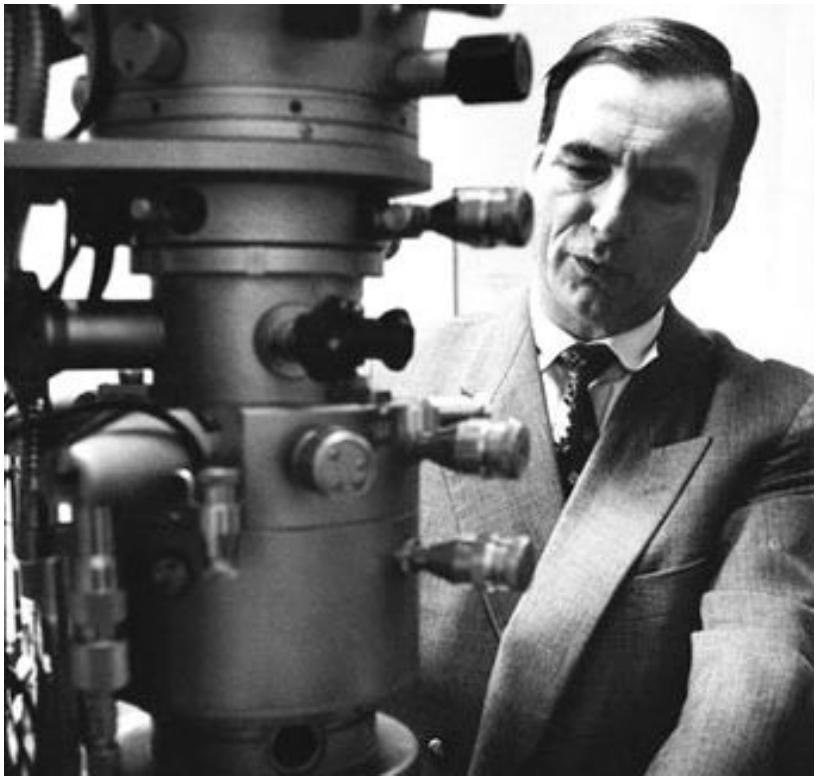
# Ernst Ruska: timeline




## Milestones:

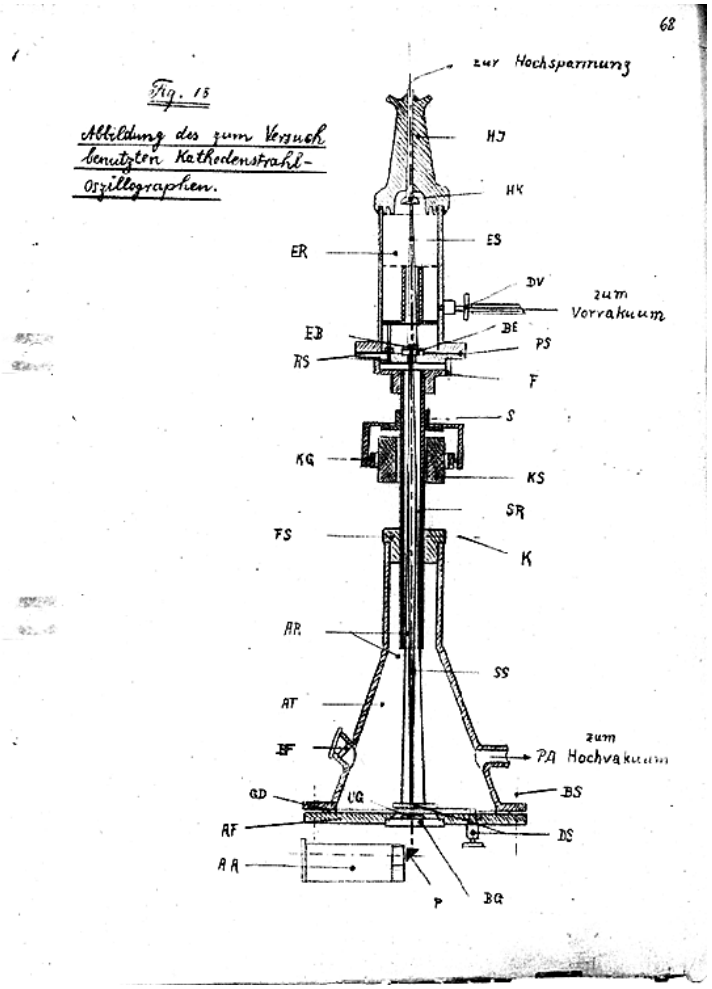
- 1931: Invention of the electrostatic lens
  - magnification: 400X
- 1933: First electron microscope
  - mag: 7000X (vs. LM: 2000X)
- 1933: Completed Ph.D. (!)
- 1939: First (viable) commercial EM
  - mag: 100,000X
- 1952: His brother Helmut Ruska moves from Siemens to Albany
- 1986: Nobel Prize in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

# Ernst Ruska



http://www.biografiasyvidas.com

Sketch from 1929




http://ernst.ruska.de

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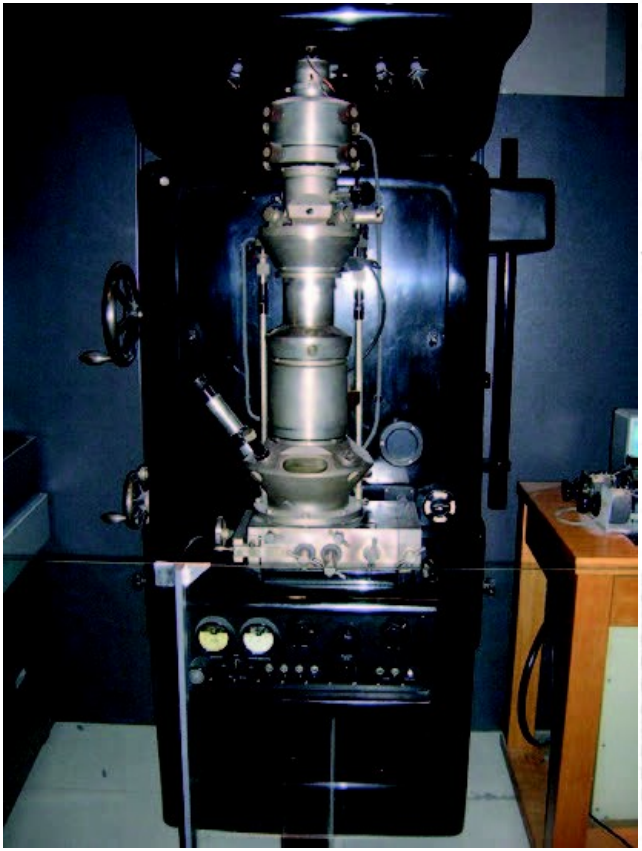
Replica of first electron microscope



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

# Correction/clarification

First Siemens microscope, **1939**

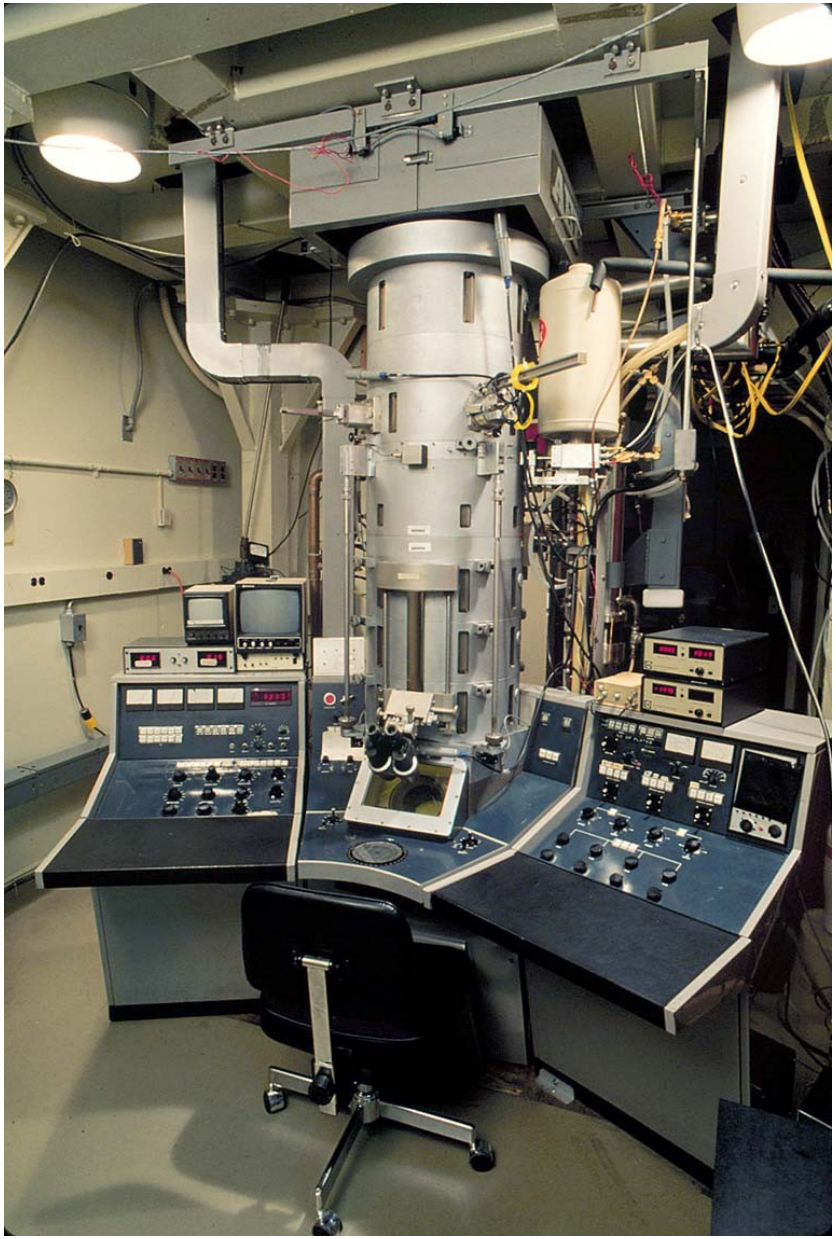
http://ernst.ruska.de

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

http://emu.msim.org.uk

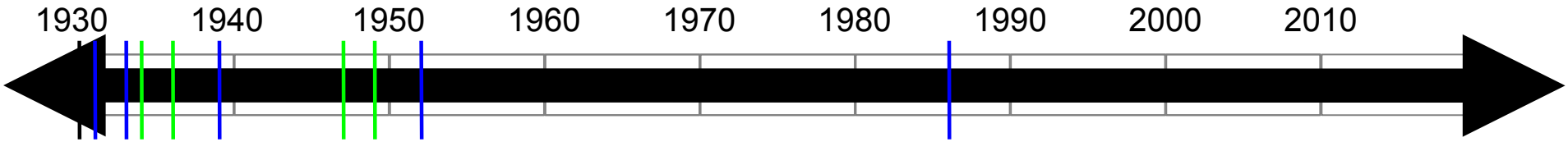
The first commercial electron microscope was actually by the British company Metropolitan-Vickers in 1937. However, the magnification was worse than for the light microscope, so the Siemens is considered "first."

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



http://www.wadsworth.org

# Otto Scherzer: timeline



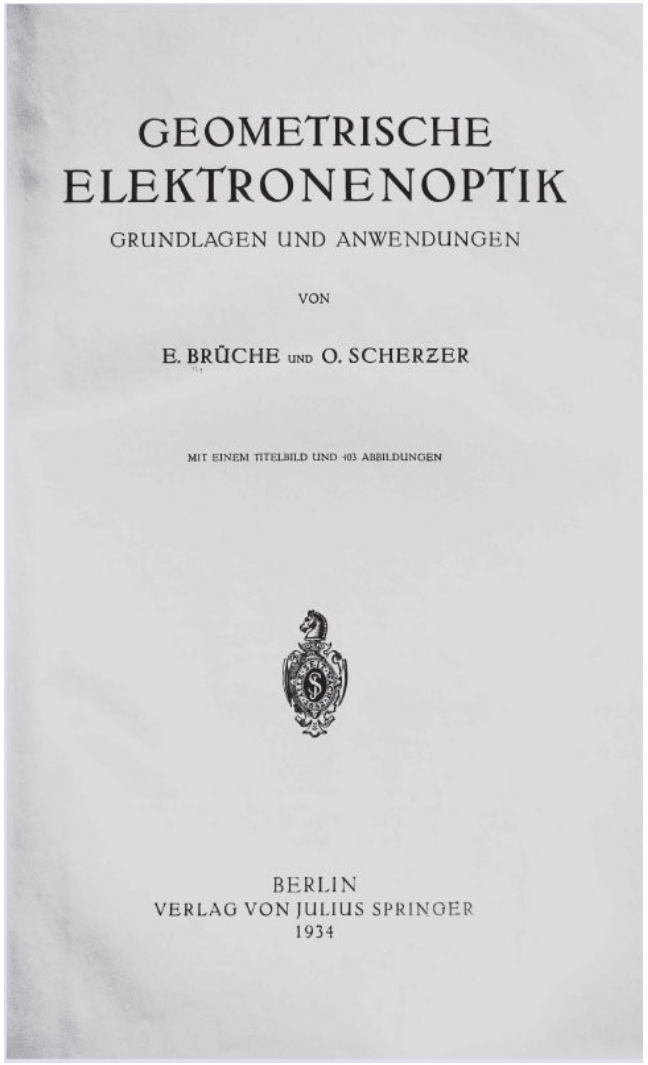

## Milestones:

- 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"

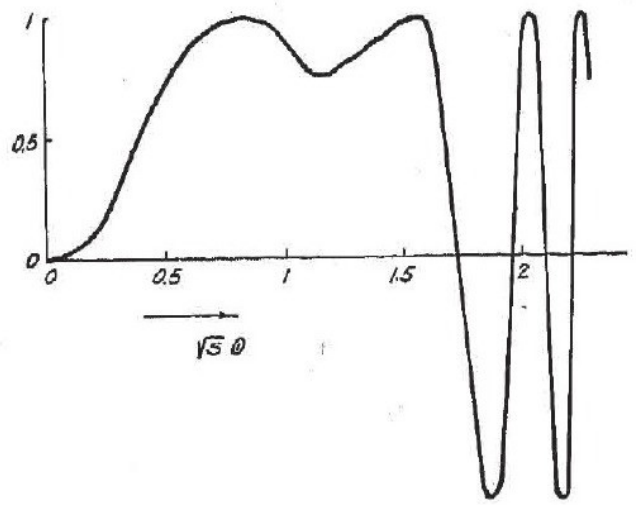
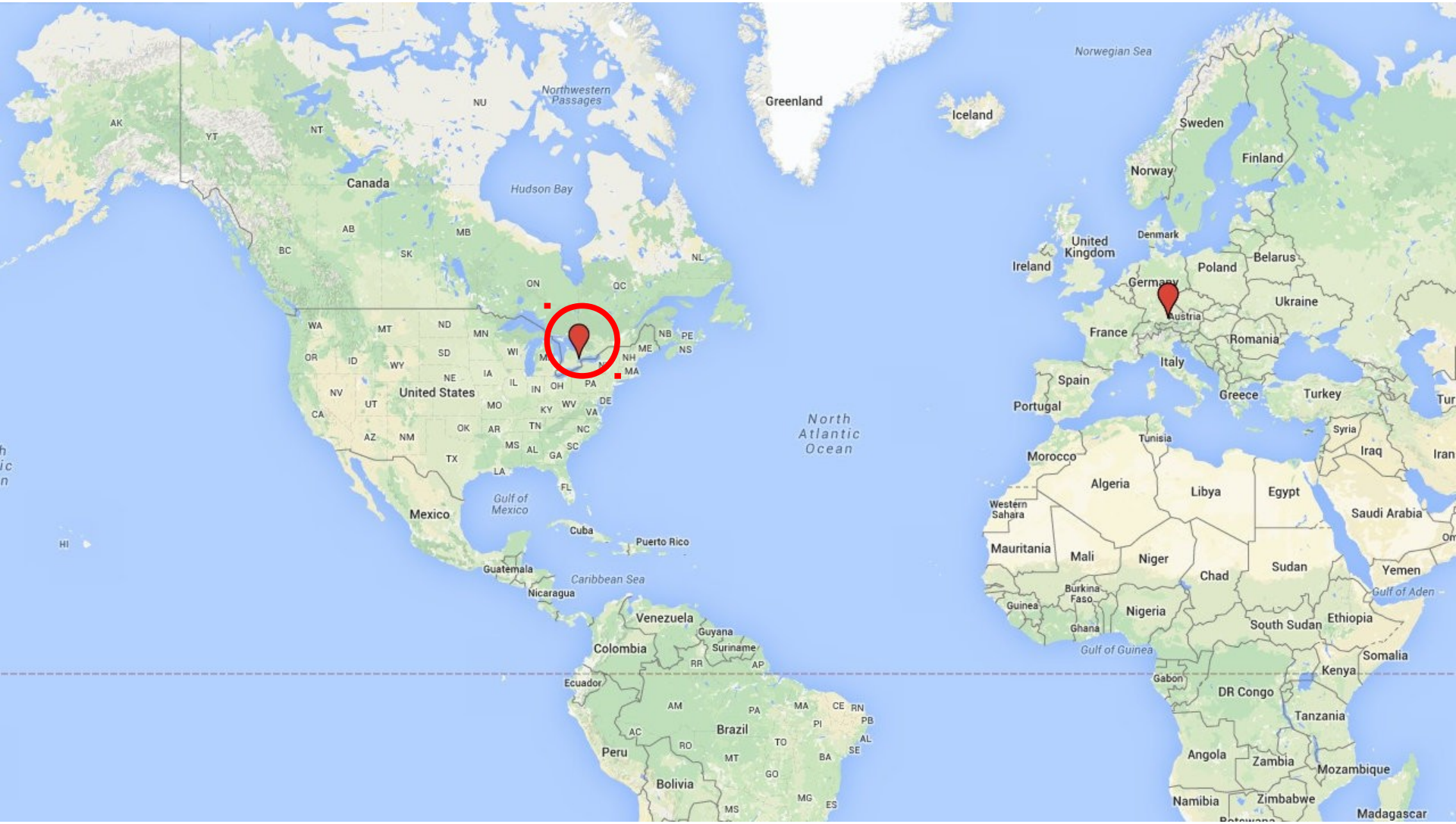



FIG. 4. The function $\sin(3s\Theta^2 - s^2\Theta^4)$, describing the phase shift in case of optimum contrast.

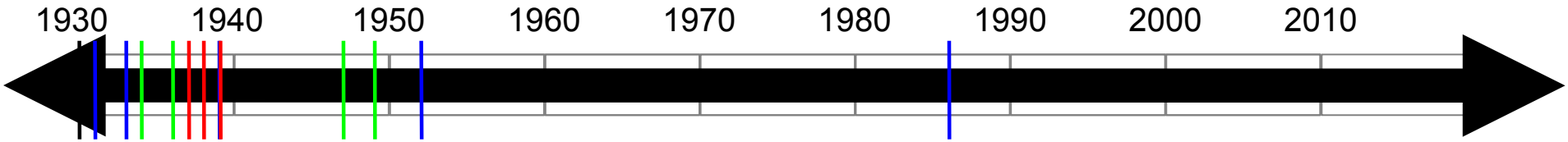
VOLUME 20, JANUARY, 1949

http://www.microscopy.org

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



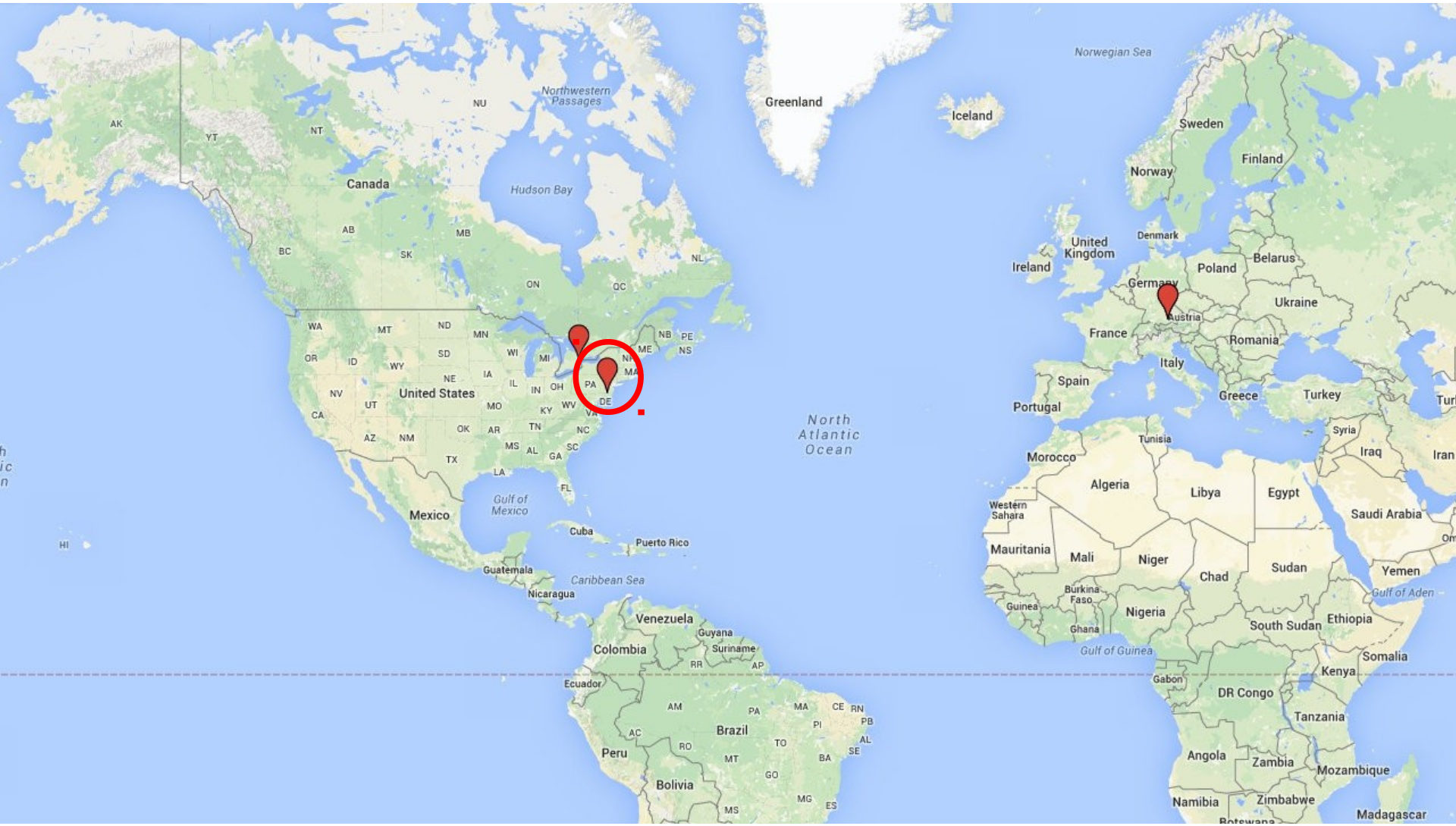
# Toronto group: timeline




## 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)

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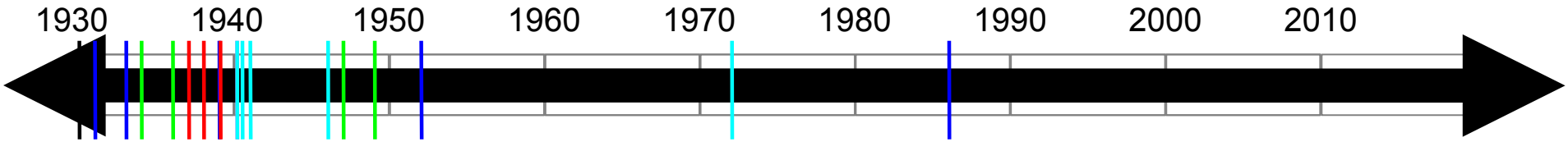
# James Hillier: timeline




## Milestones:

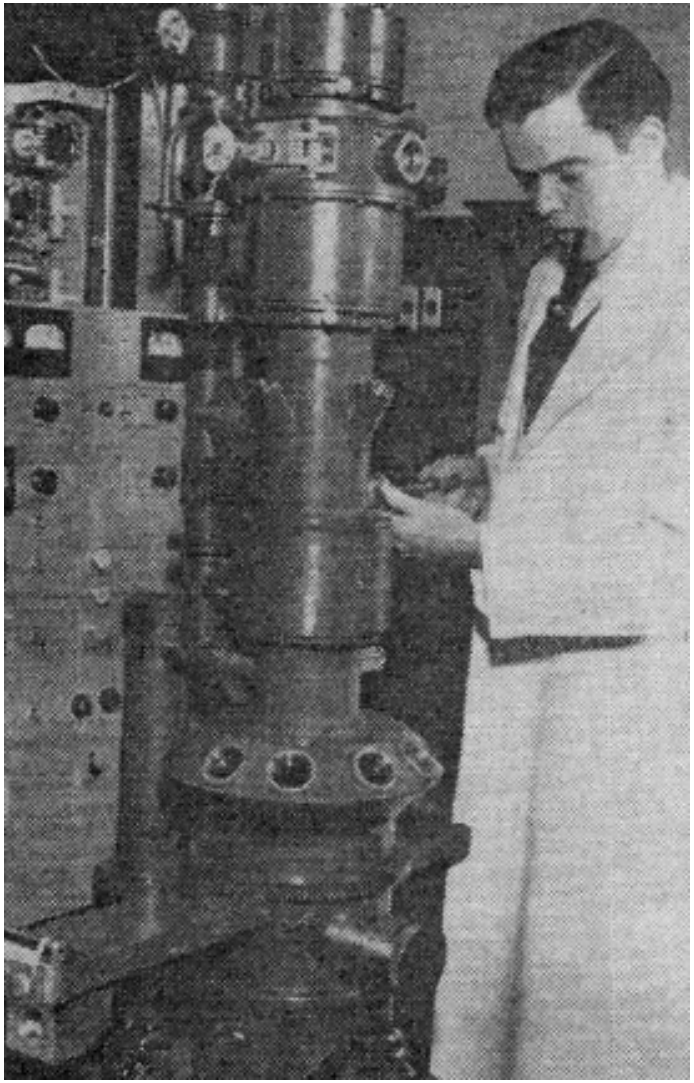
- 1940 February
  - Hillier started at RCA
  - enlisted by Vladimir Dworykin (cathode ray tube)
- 1940 Jul 4: Commercial EM, Model B (EMB)
- 1941: 300kV, for dealing with thick specimens
- 1947: first stigmator
  - stigmators were iron screws tapped into the pole piece
  - resolution → 1nm
- 1973 (as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

# James Hillier

Seated, with Albert Prebus standing

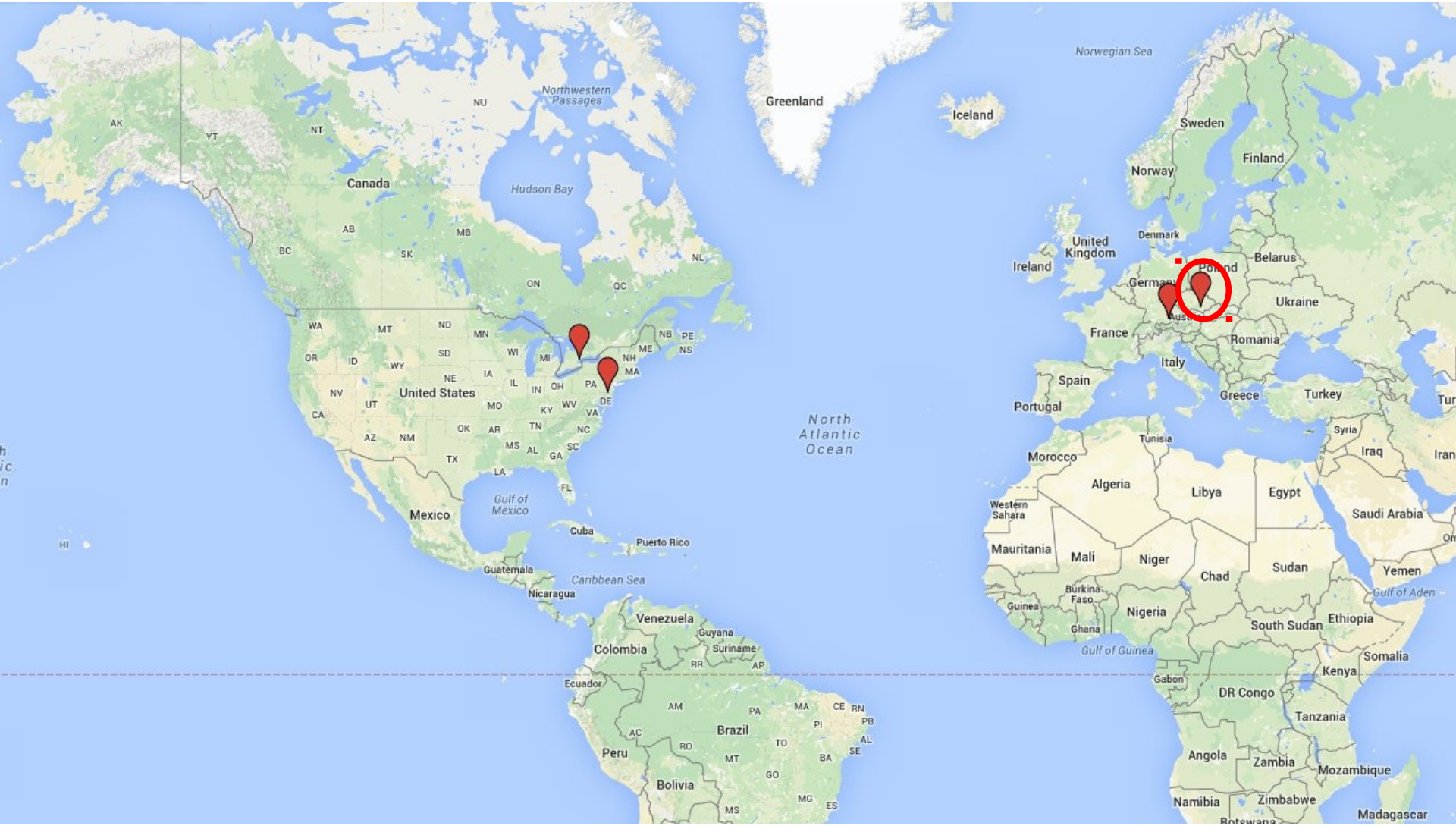
http://www.museevirtuel.ca

At RCA Model B, 1940



http://www.rfcafe.com

# Electron microscopy in the Czech Republic

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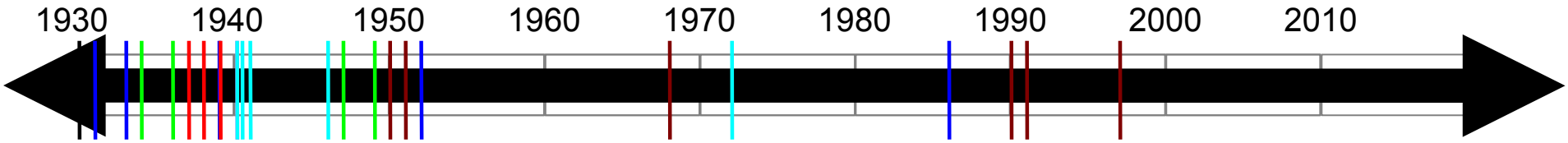
# Electron microscopy in the Czech Republic

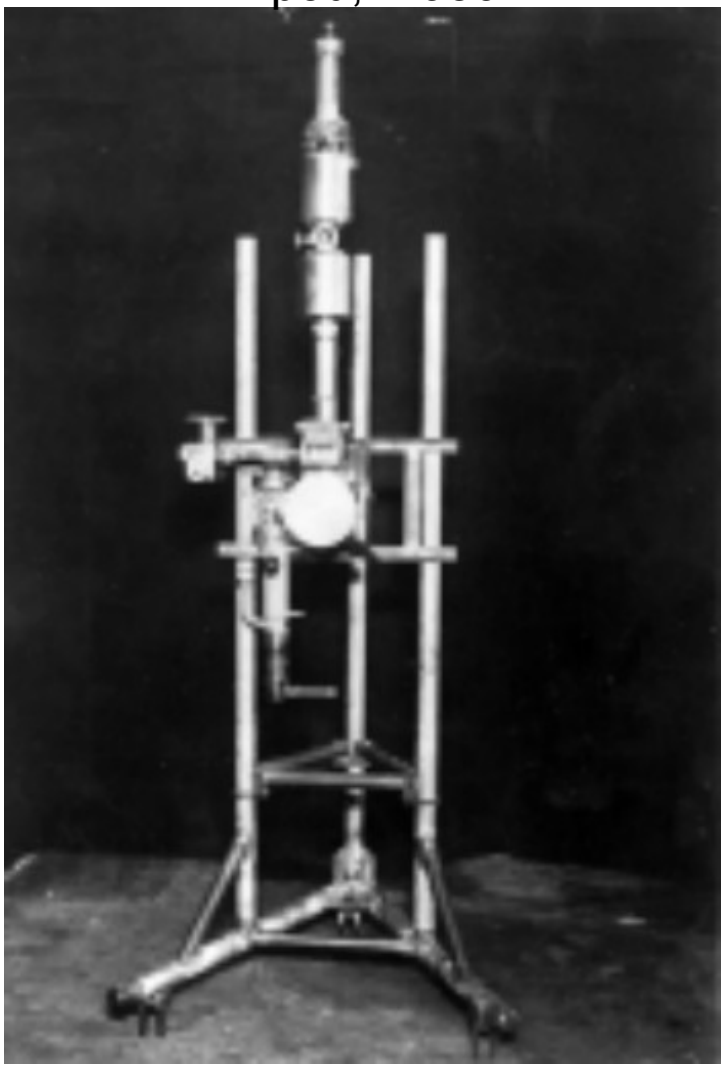



Milestones:

- 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

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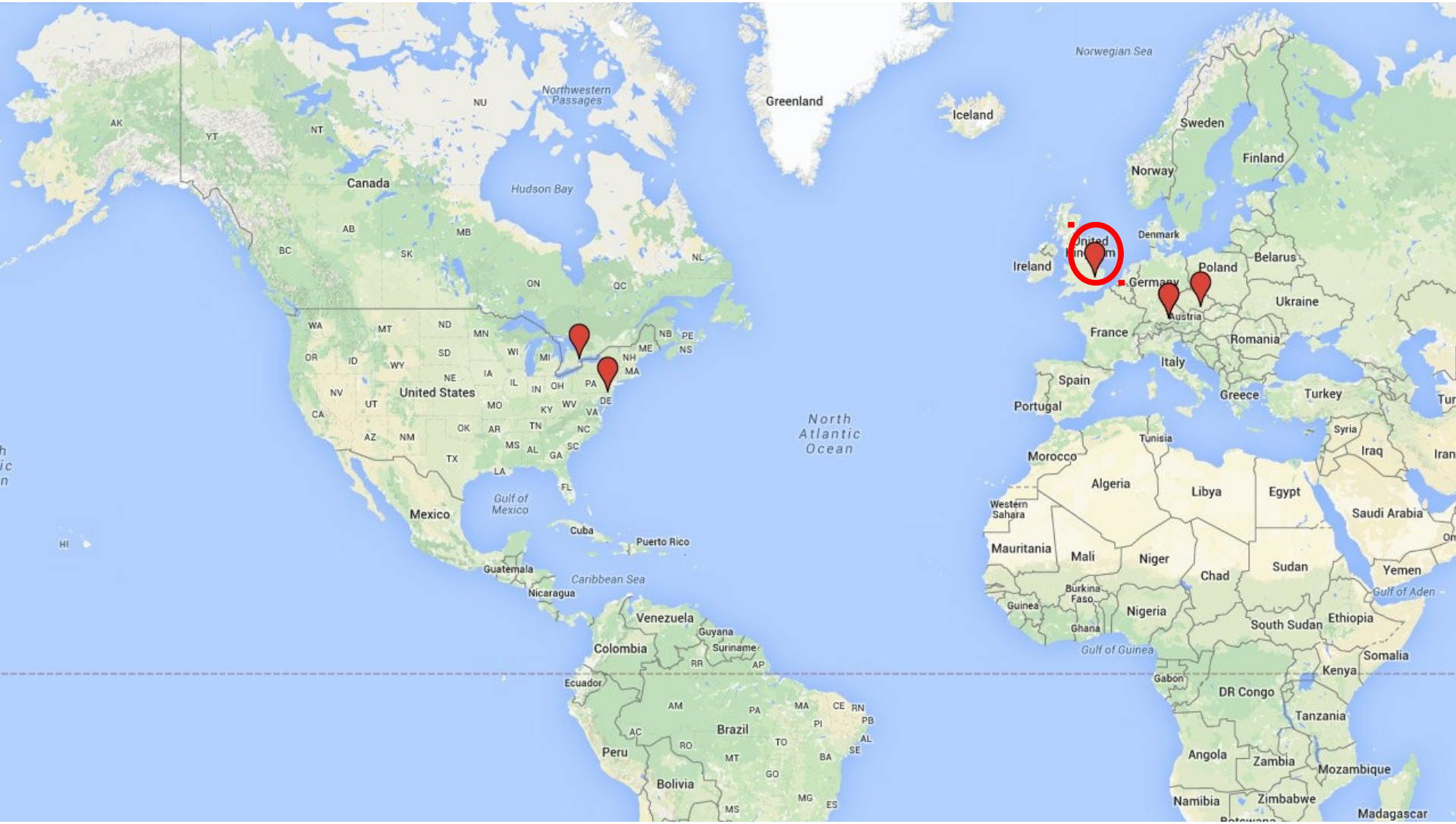
# Electron microscopy in the Czech Republic


Technical Museum in Brno
(electron microscopy exhibit undergoing
renovations until autumn 2016)

# Medical Research Council (MRC), Cambridge

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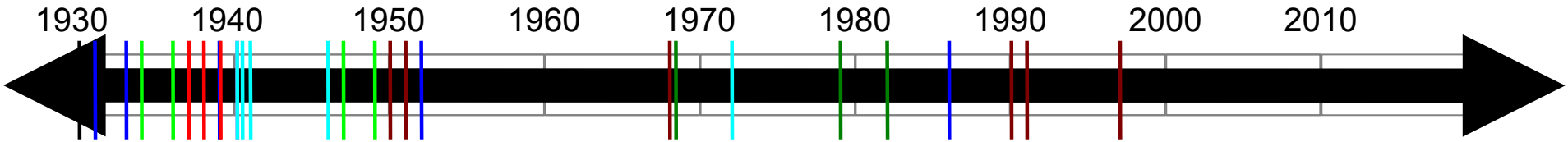
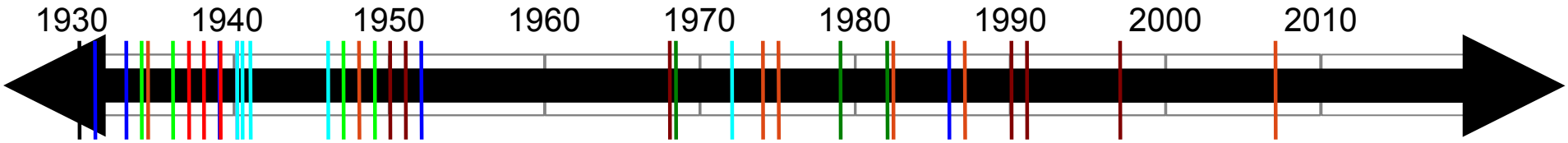
# Aaron Klug: timeline




## Milestones:

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

# Other notable events

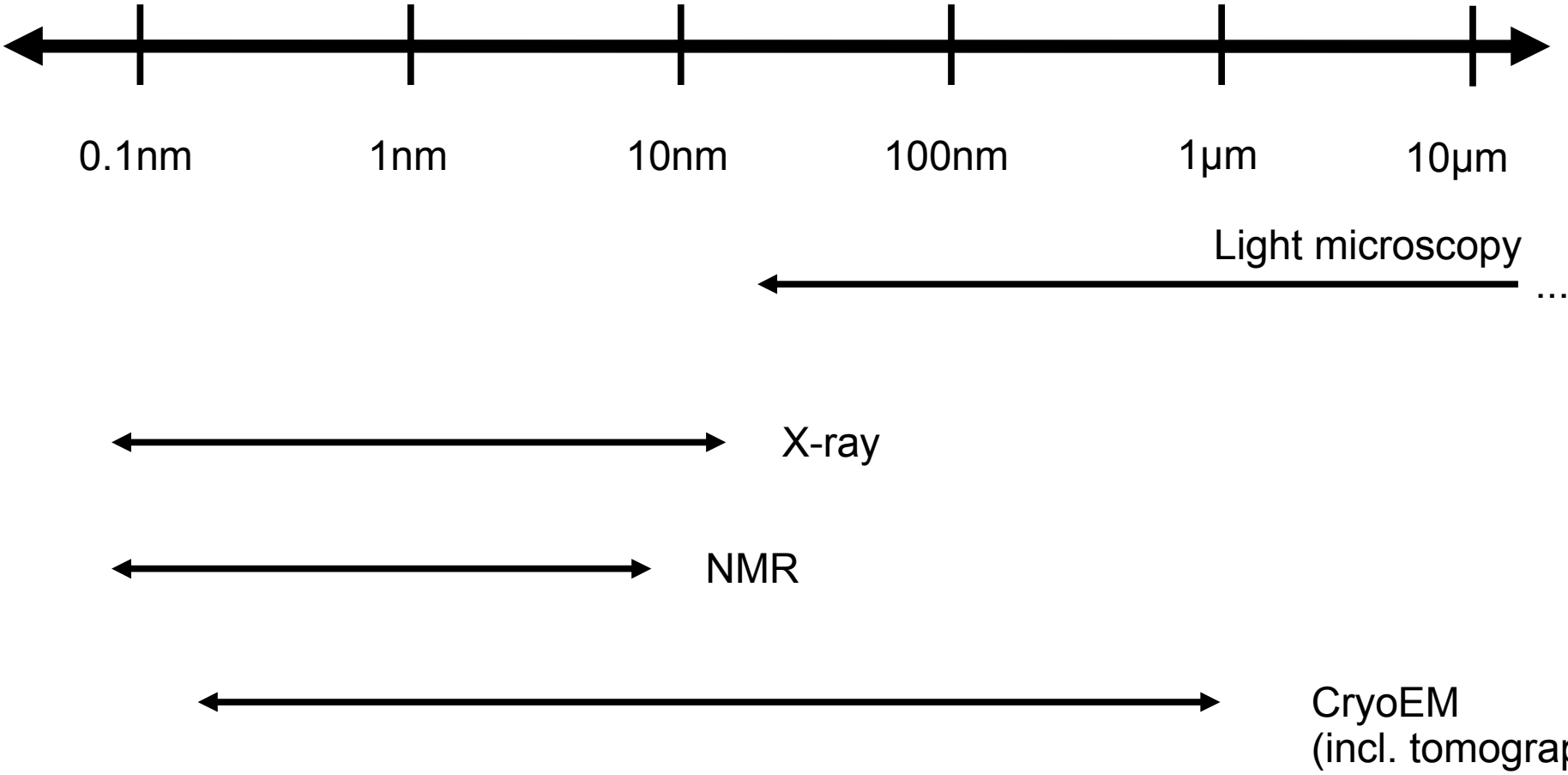



Milestones:

- 1934: Ladislav L. Marton takes the first image of biological specimen
- 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 specimens
- 1975: Richard Henderson – subnanometer electron crystallography
- 1982: Jacques Dubochet – modern cryo techniques
- 1987: Joachim Frank determines 3D reconstruction of an asymmetric specimen (50S ribosome)
- 2007: Direct Electron develops first commercial direct electron detector

# *The basics*

# Size ranges for structural methods



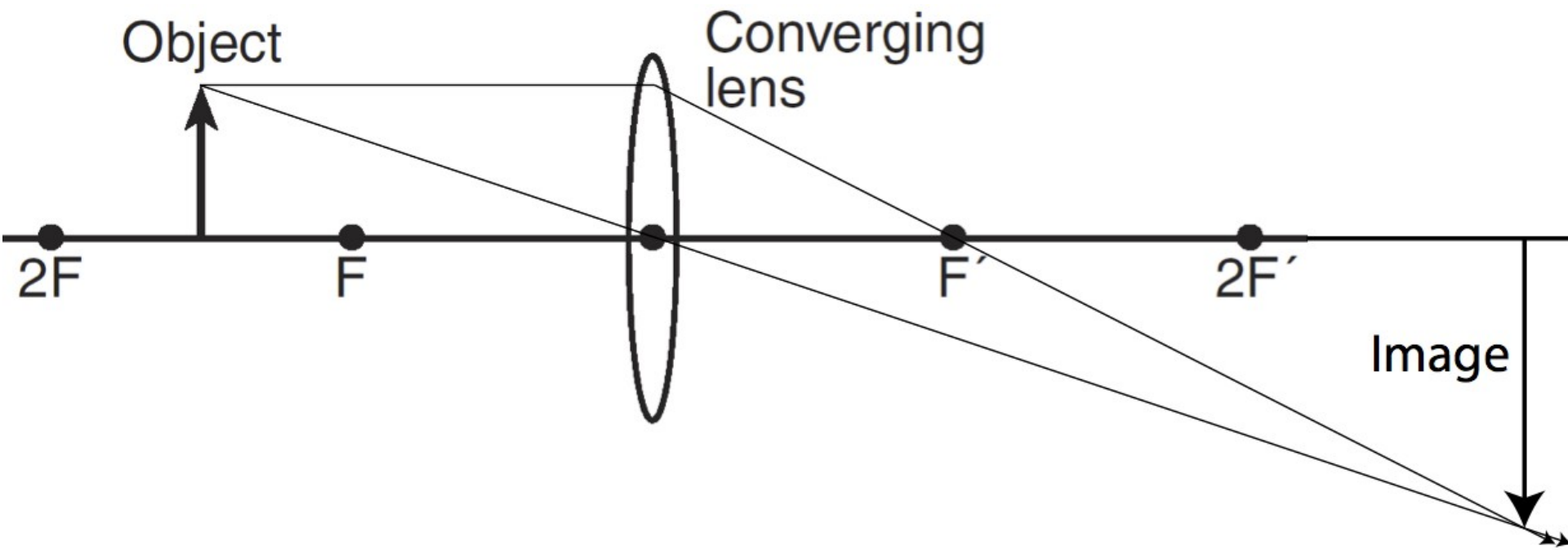
# Comparison of practical requirements

| | CryoEM | X-ray | NMR |
|---|---|---|---|
| Sample volume | 5µL[1] | 10µL | 400µL |
| Number of samples | 5-10 | 1 crystal | 1 (<20kDa)[2]<br>several (20-40kDa) |
| Concentration | 50 nM | 50 µM[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 (synchotron)<br>48h (in-house) | 1-2 weeks (<20kDa)<br>6-8 weeks (20-40kDa) |

Adapted from Raj Agrawal

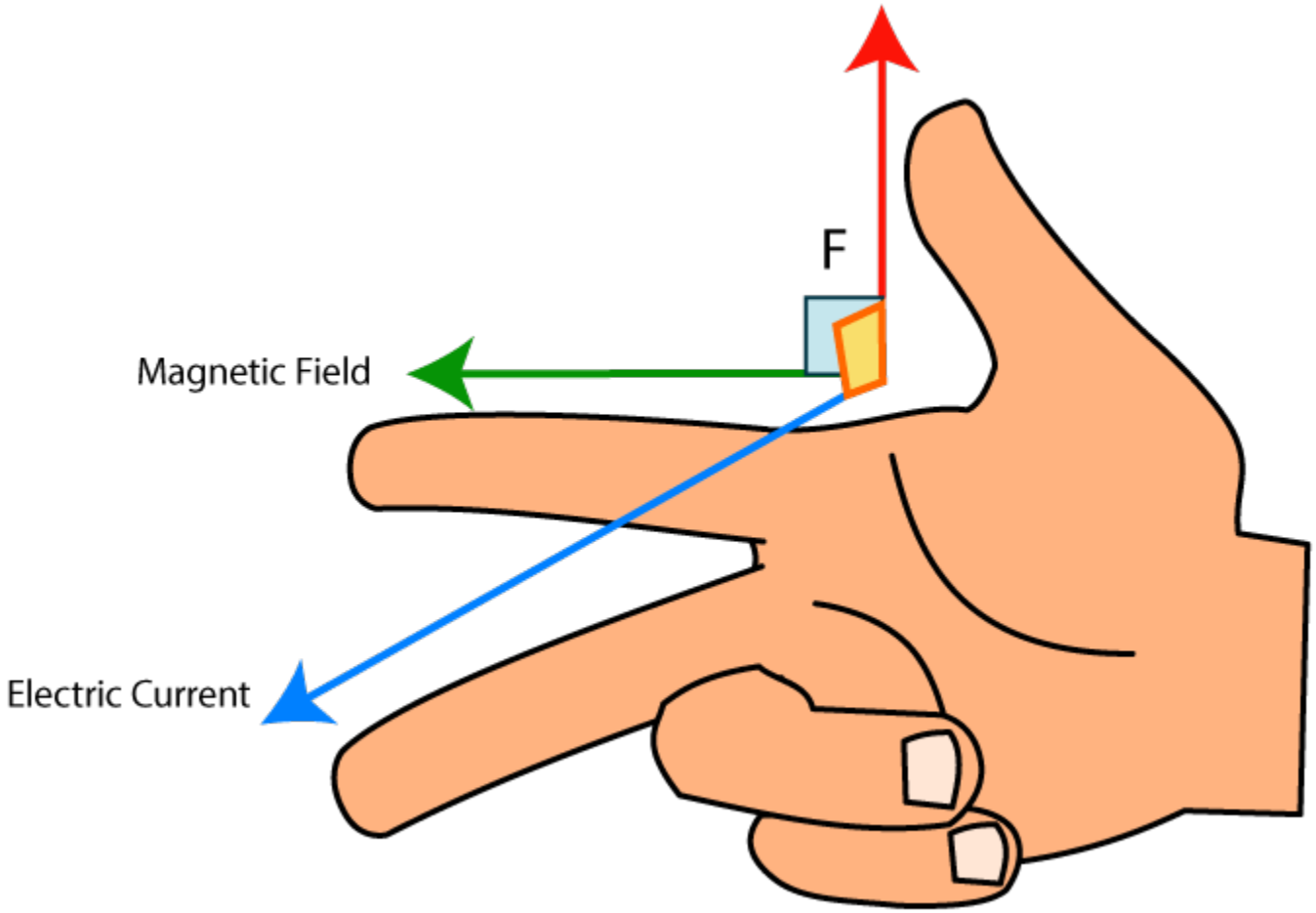
[1]Droplet on grid, prior to blotting
[2]For stable proteins of this size, a single U-13C, 15N sample is likely sufficient
[3]Based on 10 mg/ml of a 100 kDa protein
[4]Final amount required for the crystal. However, a multiple of this amount is required to try out different crystallization conditions.

# Quick review of light optics




www.aplusphysics.com

# Right-hand rule




www.education.com

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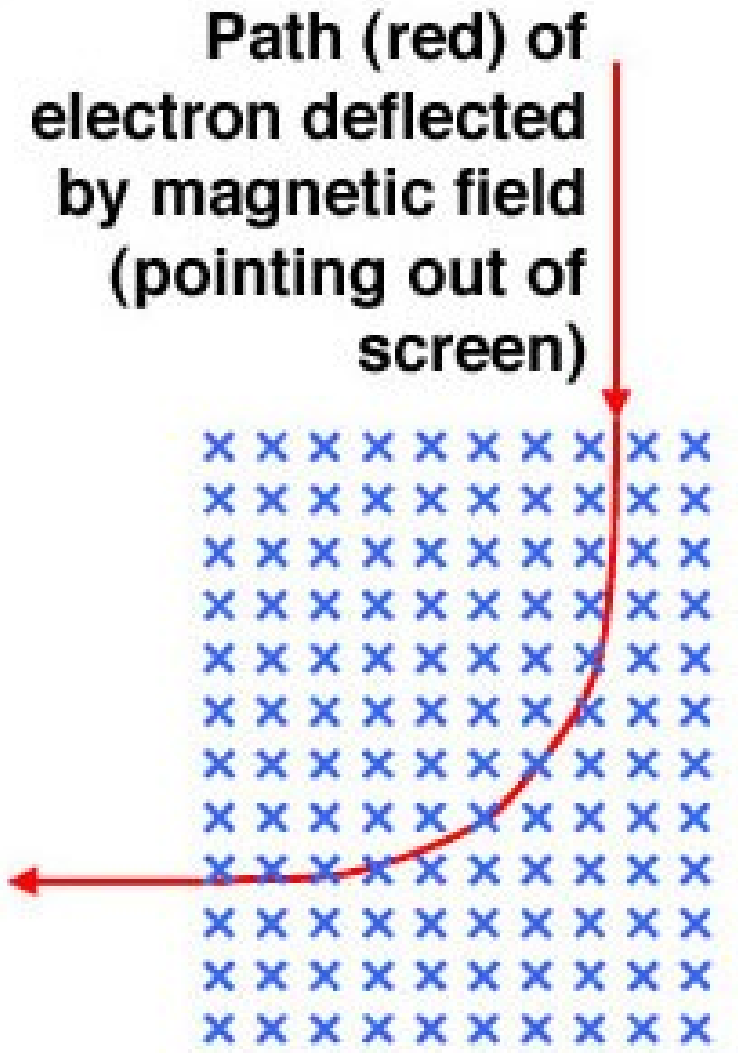
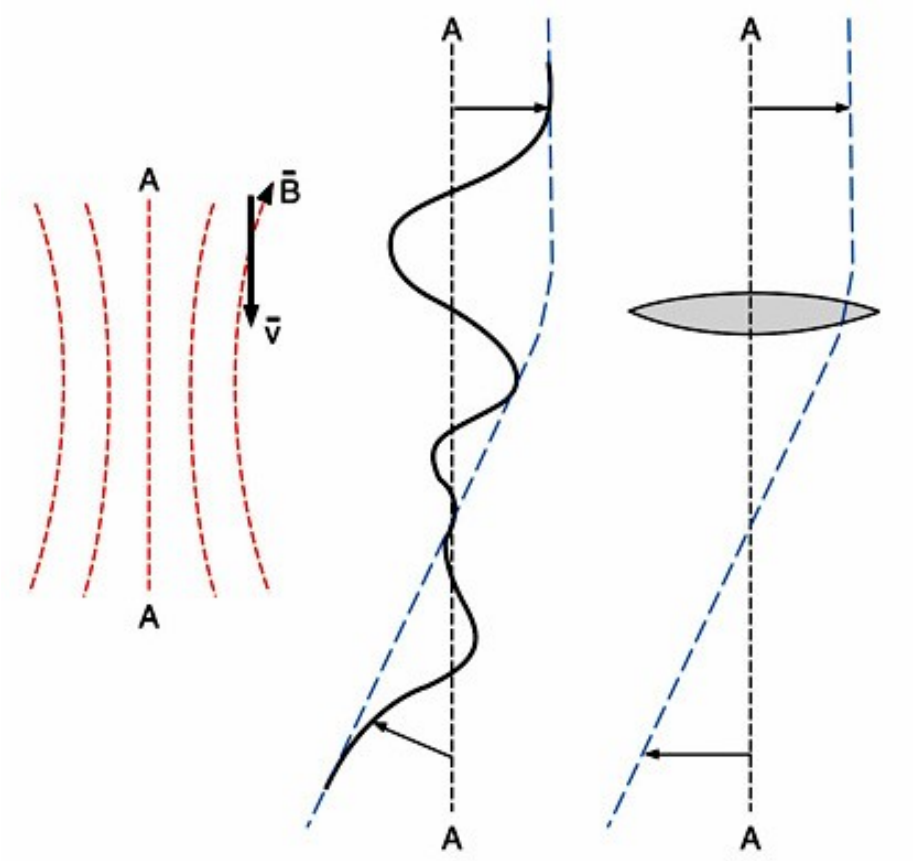
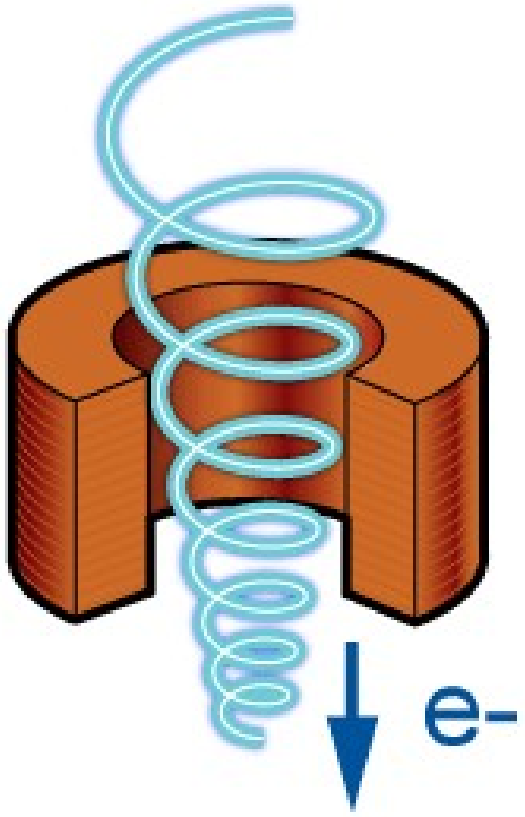



http://www.polywellnuclearfusion.com

# Electron in a magnetic lens



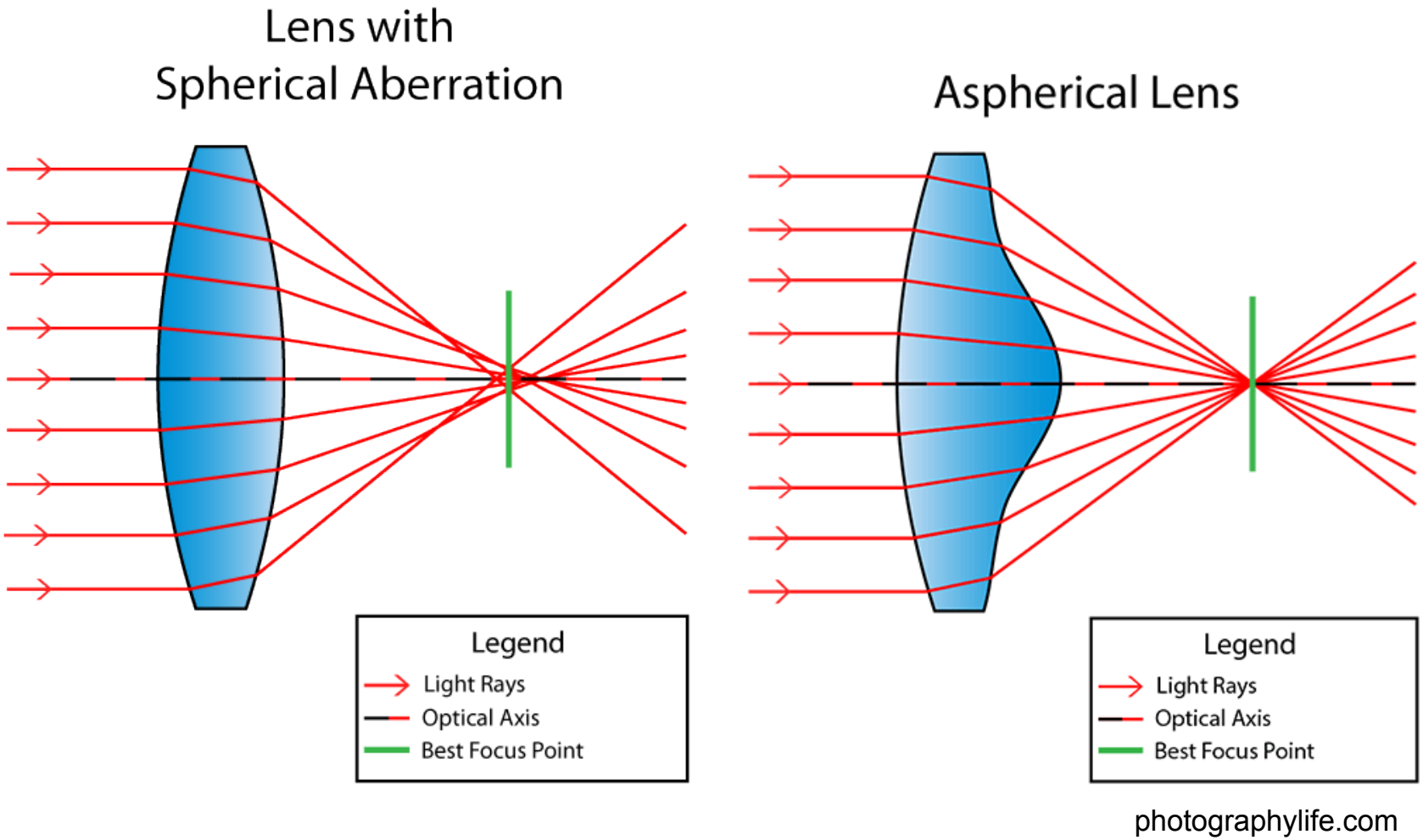

nau.edu




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).

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# Thank you for your attention

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