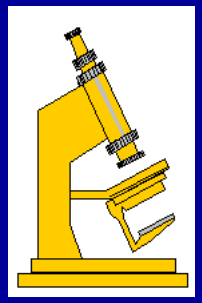
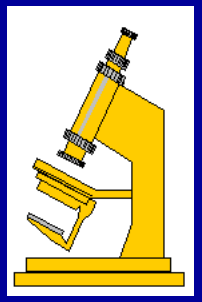




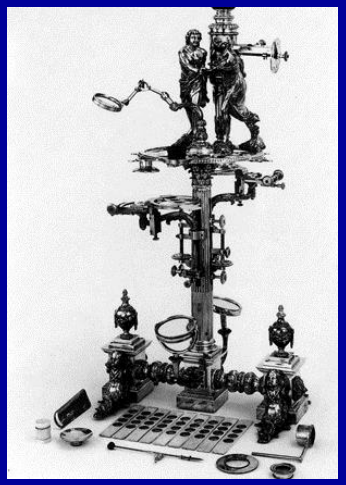
# Microscopy

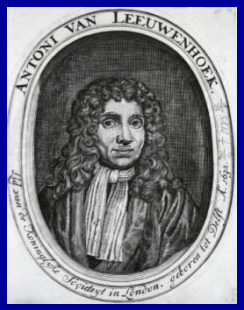
## Past & Future?

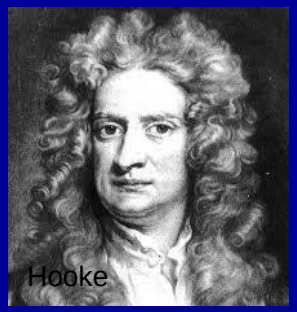

140th Year !

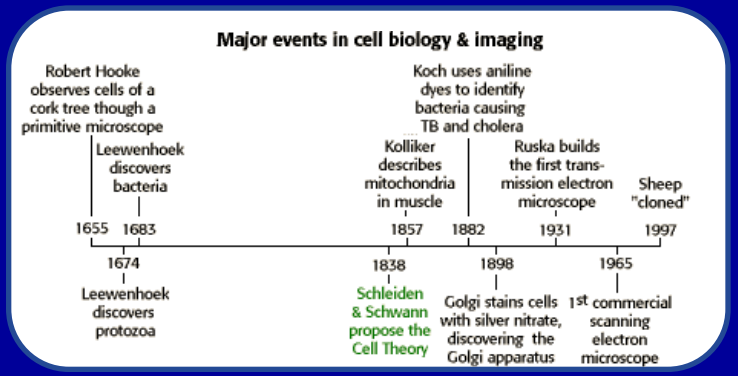
**Mike Mahon**
Manchester Microscopical & Natural History Society
July 2019

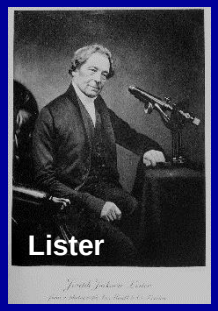
# Historical




ANTONI VAN LEEUWENHOEK


Hooke


Major events in cell biology & imaging
Robert Hooke observes cells of a cork tree though a primitive microscope
Leewenhoek discovers bacteria
Koch uses aniline dyes to identify bacteria causing TB and cholera
Kolliker describes mitochondria in muscle
Ruska builds the first trans-mission electron microscope
Sheep "cloned"
1655
1683
1857
1882
1931
1997
1674
1838
1898
1965
Leewenhoek discovers protozoa
Schleiden & Schwann propose the Cell Theory
Golgi stains cells with silver nitrate, discovering the Golgi apparatus
1st commercial scanning electron microscope


Lister


Dancer


Figure 2
Ernst Abbe (1840-1905)
Carl Zeiss (1816-1888)


Rayleigh


Frits Zernike
(1888-1966)


Ernst Ruska
(1906-1988)

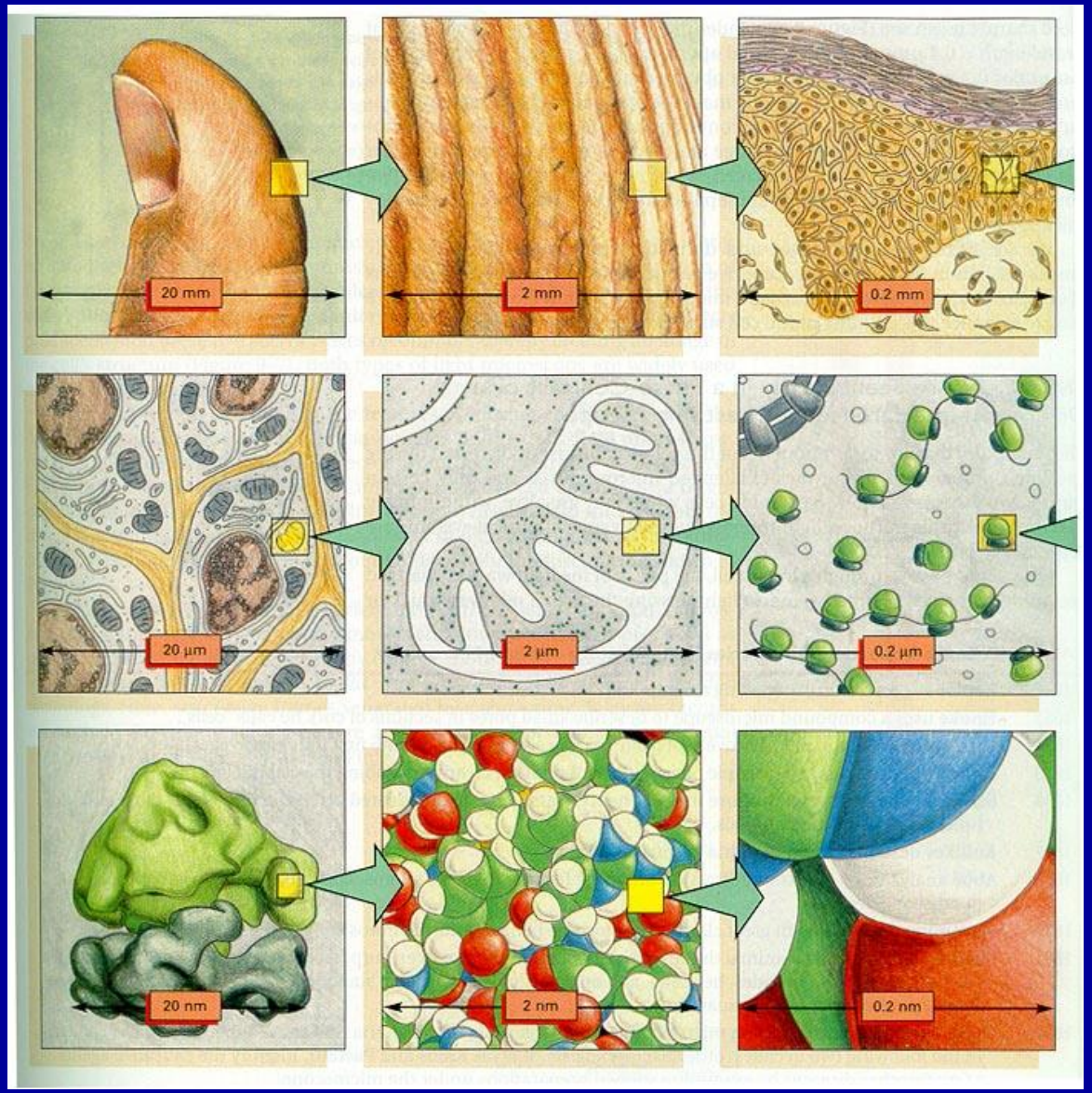

Photo: IBM
Binnig & Rohrer

# Microscopy Landmarks

- <1600s magnifying lens
- 1600s simple microscopes
- 1650s compound microscope
- 1830s expansion of light microscopy + pol
- 1900-60 UV & fluorescence microscopy
- 1930s phase and interference microscopes
- 1930-60 electron microscopes developed
- 1980s confocal microscopy
- 1990s-2010s scanning probe microscopies
- 2010s µCT, super-resolution, X-ray, .... ???

# Remember Scale

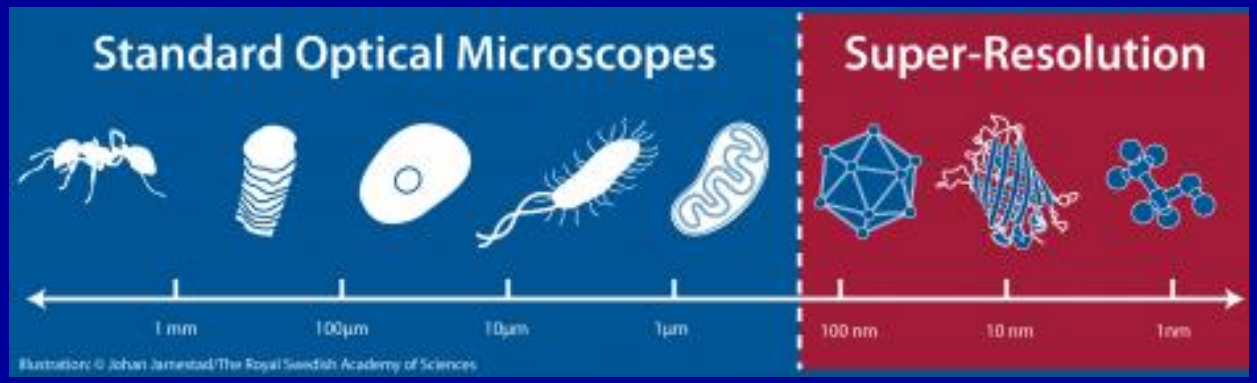
| | | |
|---|---|---|
| m | | |
| mm | $10^{-3}$ | |
| µm | $10^{-6}$ | 0.001 mm |
| nm | $10^{-9}$ | 0.000001 mm |
| pm | $10^{-12}$ | 0.000000001 mm |
| fm | $10^{-15}$ | 0.000000000001 mm |



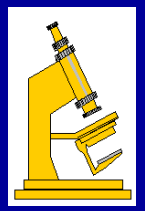

- keep in mind what it is possible to see

# -scopy

- Macroscopy - endoscopes, stereomicroscopes
- Microscopy - optical microscopes
- Nanoscopy - electron microscopes
- Picoscopy - scanning probe microscopies
- Femtoscopy - gas ion microscopes



# Uses of the Microscope



*… the scientific instrument (RMS)*

*MMC Exhibition 2019 !*

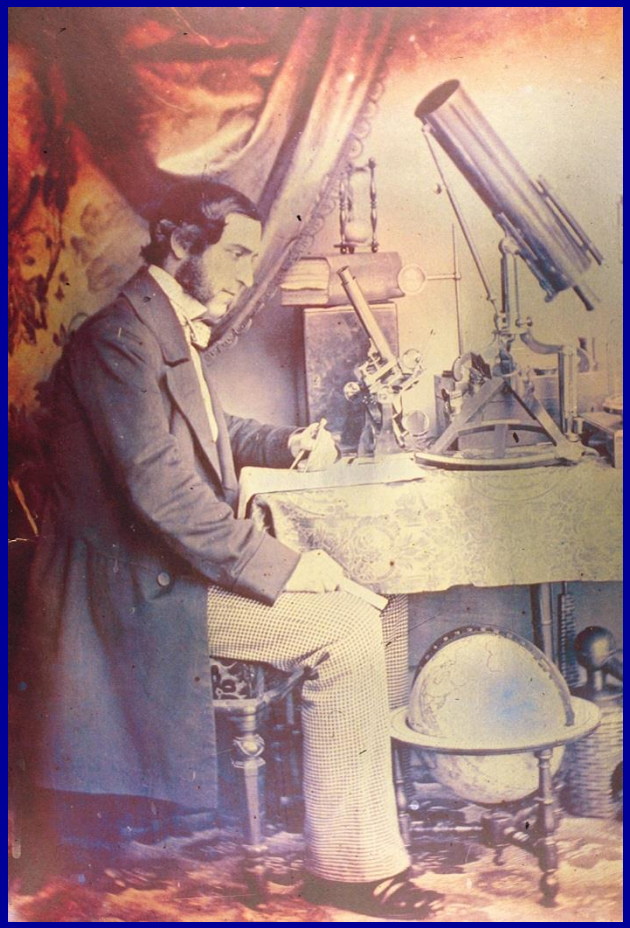
- Biology
- Medicine
- Forensic
- Geology
- Metallurgy
- Computer Science
- Astrophysics

Qualitative / Quantitative / Analytical

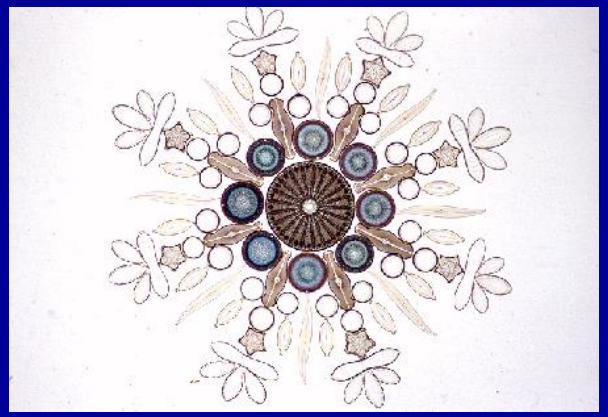
# Social History of the Microscope

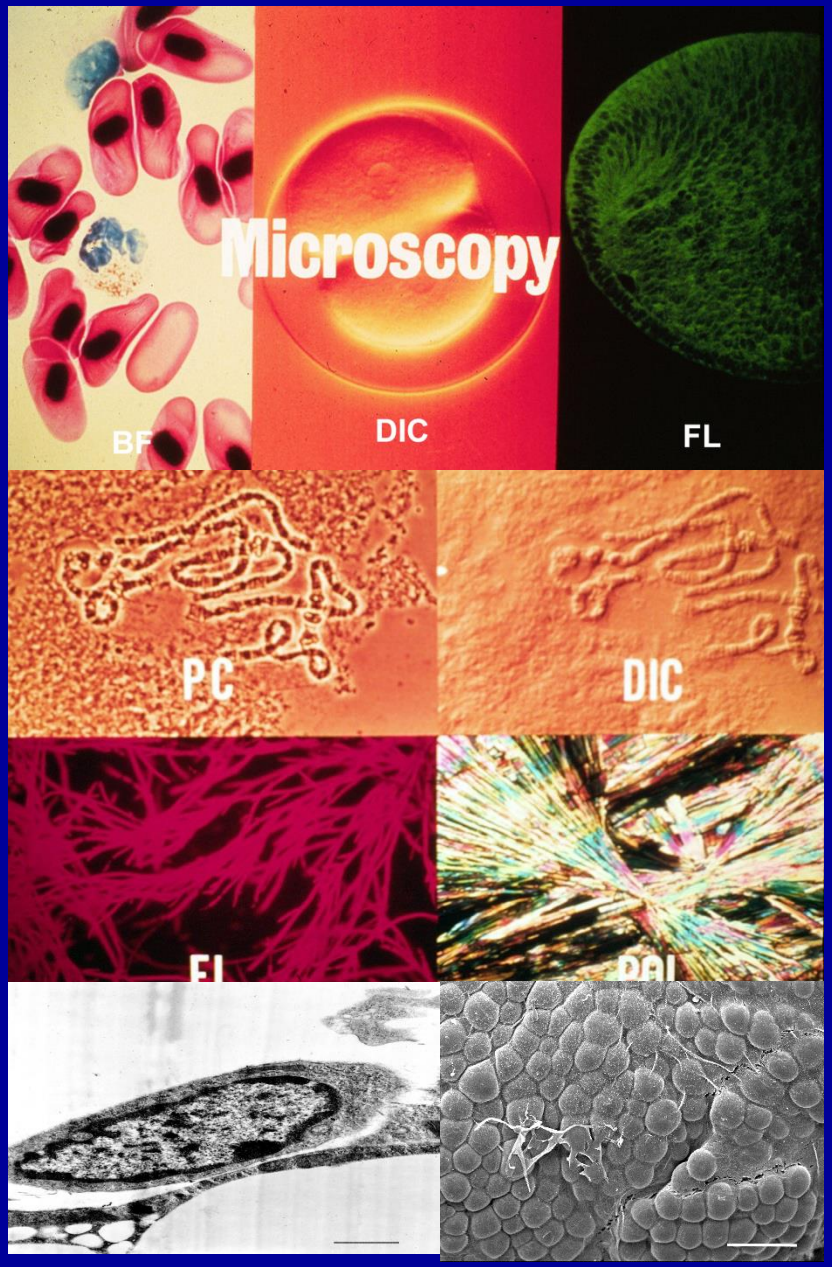
- Science
- Medicine
- Public Health

- Soires
- Exhibitions
- Photomicrography
- Microphotography



- Societies
  RMS, Queckett
  Manchester MS
  Leeds MS, ….



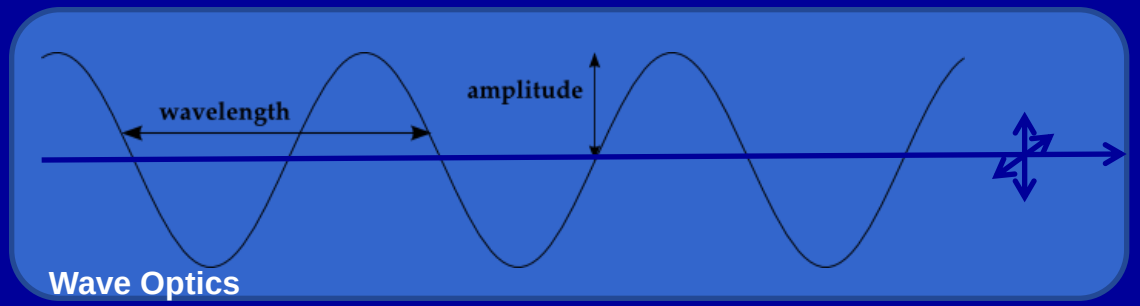



# Which microscope to use ?

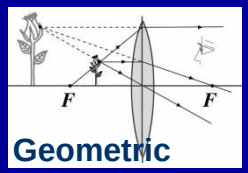
- Light Microscopy
  - bright field
  - dark field / reflectance
  - phase contrast
  - polarising
  - interference
  - fluorescence
  - computerised / confocal
- Electron Microscopy
  - transmission
  - scanning
- Scanning Probe/Stylus

# Light Microscope Theory

| Properties of Light | Microscopies |
|---|---|
| • Wave Optics | |
| – direction | – reflection,refraction,diffraction / BF / DF |
| – amplitude | – absorption / BF |
| – wavelength | – colour |
| – phase | – phase contrast, interference |
| – vibrational plane | – polarisation |
| – velocity | – refraction / oil immersion |
| • Geometric Optics | |
| – image location | |
| – Magnification / Res | |
| • Quantum Optics | |
| – formation & destruction | |
| – fluorescence | |



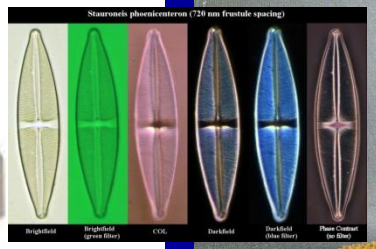
Wave Optics

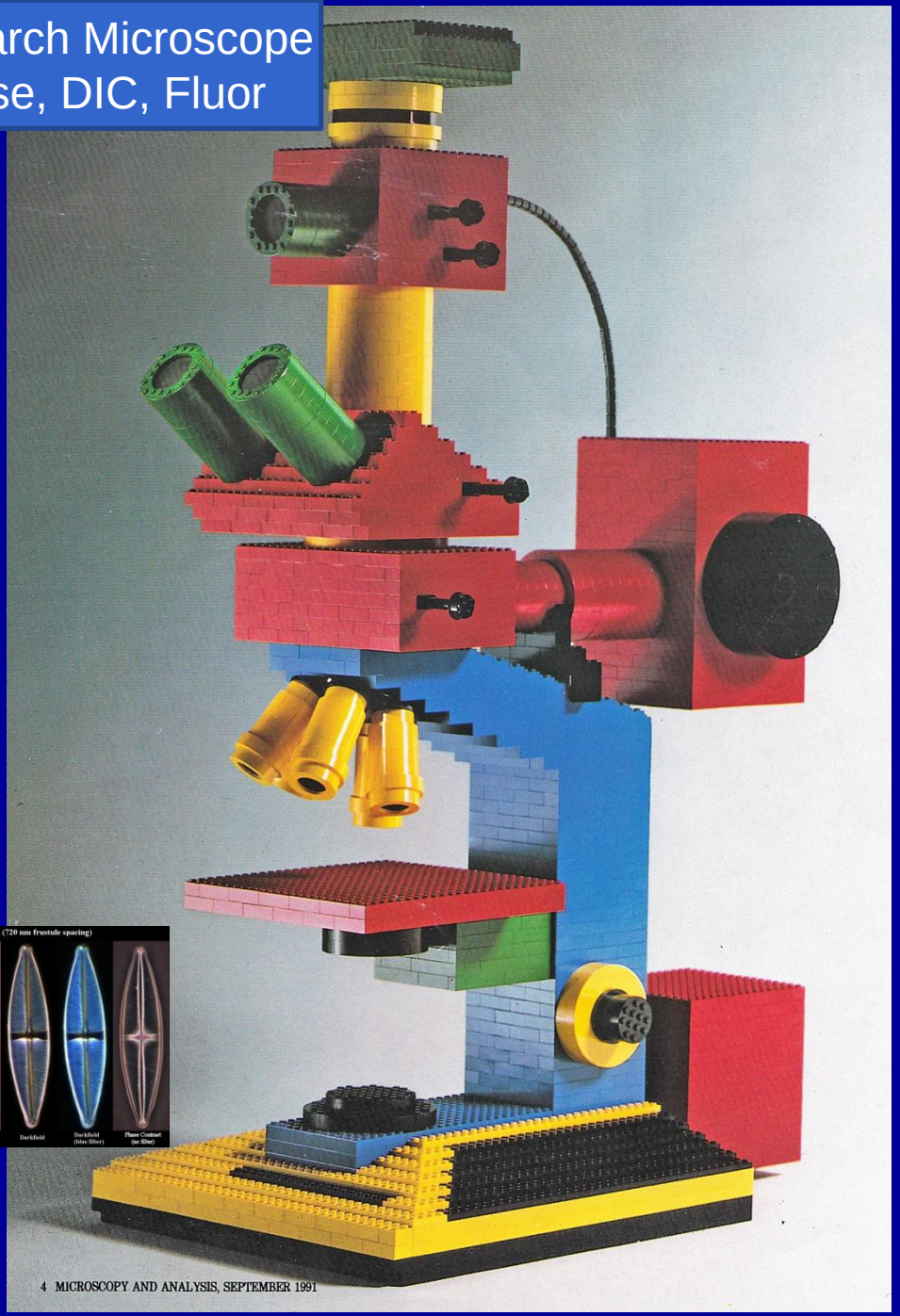


Geometric


Quantum

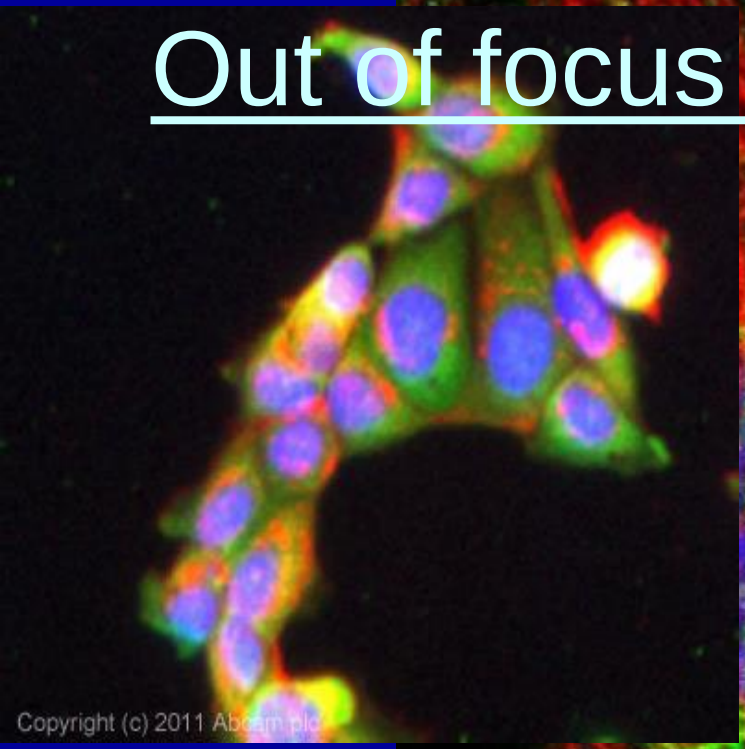
# Modular Research Microscope
# BF, DF, Phase, DIC, Fluor

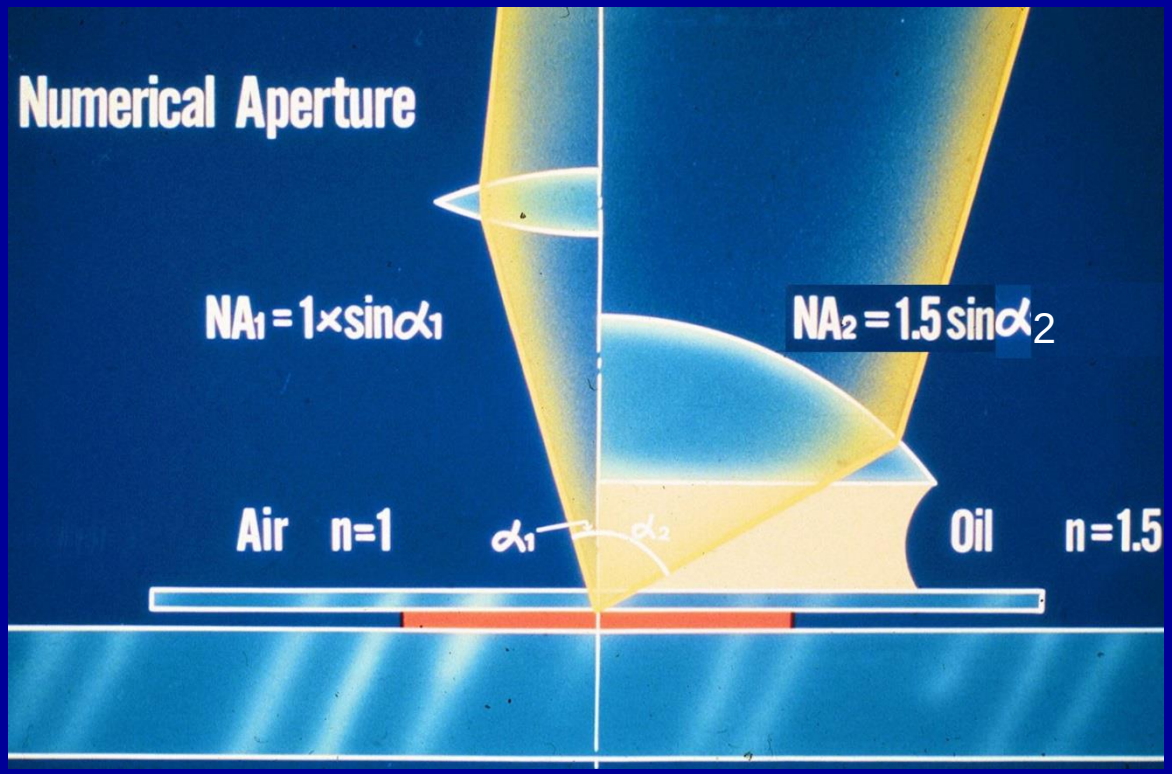
# Confocal Microscopy

## Out of focus fluorescence rejected



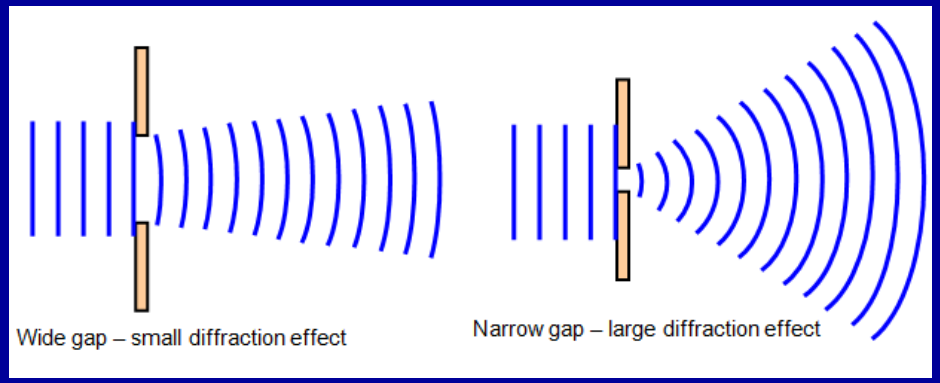
But how can we see even more detail …… get greater resolution ?

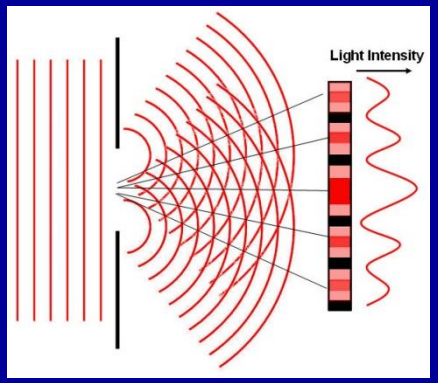
# Numerical aperture

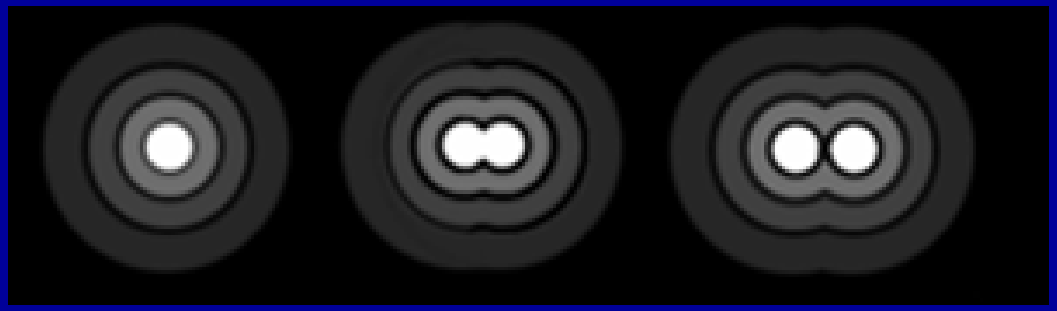



- NA numerical aperture of objective = n sinα
  - α = half intake angle of lens
  - n = refractive index
    - oil helps both
- high magnification objectives have high NA (1.3)

# Numerical aperture and resolution



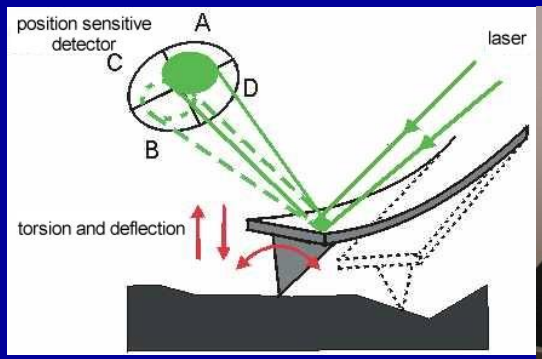



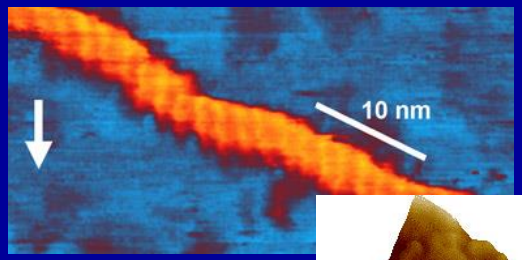
- Airy disc - diffraction pattern arising from pinhole
- If objects are close their diffraction pattern overlaps, they cannot be resolved as two objects
- **r = 0.61λ / NA** Rayleigh, Abbe, Zeiss, Helmholtz
- Resolution in green light (λ500nm) = 250 nm or 0.25µm

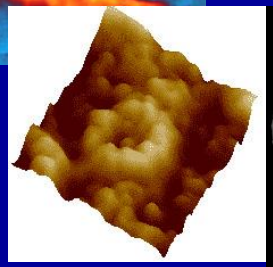
**???**
UV
Electrons
X-rays

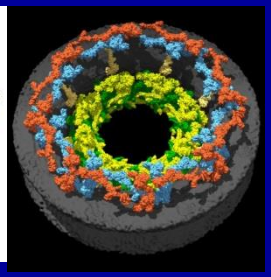
# Scanning Probe Microscopies (Picoscopy)

Scanning Tunnelling Microscope
Invented 1981
Nobel Prize 1986





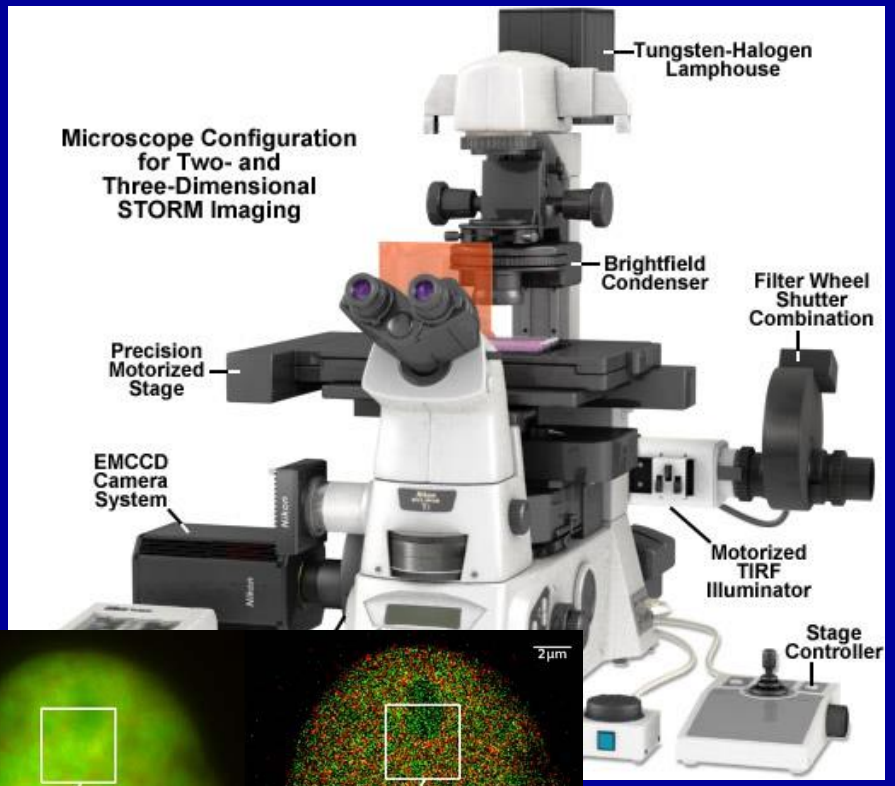
- AFM, atomic force microscopy [2]
  - Contact AFM
  - Non-contact AFM
  - Dynamic contact AFM
  - Tapping AFM
  - AFM-IR
- BEEM, ballistic electron emission microscopy[3]
- CFM, chemical force microscopy
- C-AFM, conductive atomic force microscopy[4]
- ECSTM electrochemical scanning tunneling microscope[5]
- EFM, electrostatic force microscopy[6]
- FluidFM, Fluidic force microscopy[7]
- FMM, force modulation microscopy[8]
- FOSPM, feature-oriented scanning probe microscopy[9]
- KPFM, kelvin probe force microscopy[10]
- MFM, magnetic force microscopy[11]
- MRFM, magnetic resonance force microscopy[12]
- NSOM, near-field scanning optical microscopy (or SNOM, scanning near-field o
- PFM, Piezoresponse Force Microscopy[14]
- PSTM, photon scanning tunneling microscopy[15]
- PTMS, photothermal microspectroscopy/microscopy
- SCM, scanning capacitance microscopy[16]
- SECM, scanning electrochemical microscopy
- SGM, scanning gate microscopy[17]
- SHPM, scanning Hall probe microscopy[18]
- SICM, scanning ion-conductance microscopy[19]
- SPSM spin polarized scanning tunneling microscopy[20]
- SSM, scanning SQUID microscopy
- SSRM, scanning spreading resistance microscopy[21]
- SThM, scanning thermal microscopy[22]
- STM, scanning tunneling microscopy[23]
- STP, scanning tunneling potentiometry[24]
- SVM, scanning voltage microscopy[25]
- SXSTM, synchrotron x-ray scanning tunneling microscopy[26]
- SSET Scanning Single-Electron Transistor Microscopy [27]

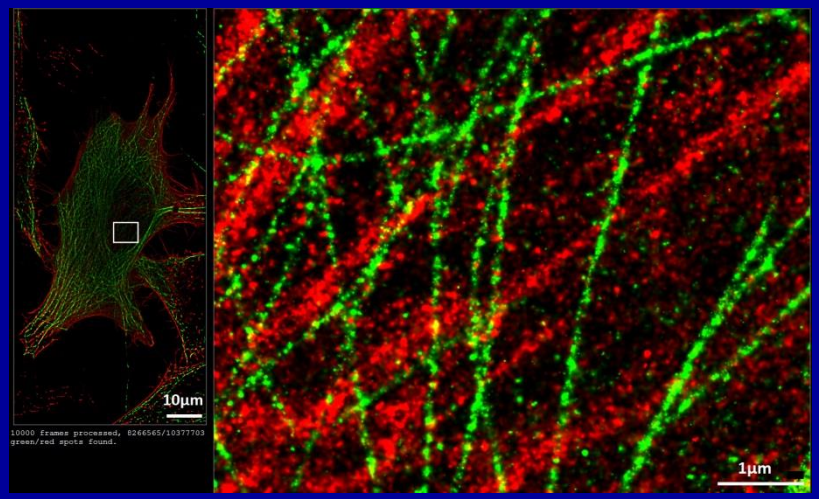
# Super-Resolution Microscopy ? ↓25nm

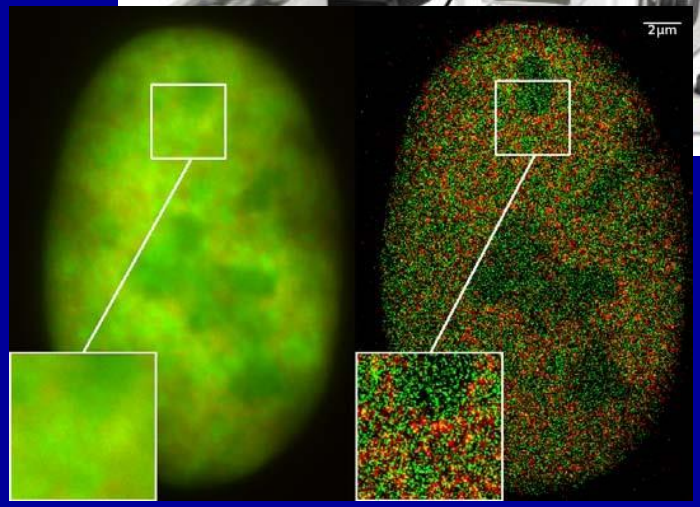
'Fluorescence microscopy with clever chemicals'

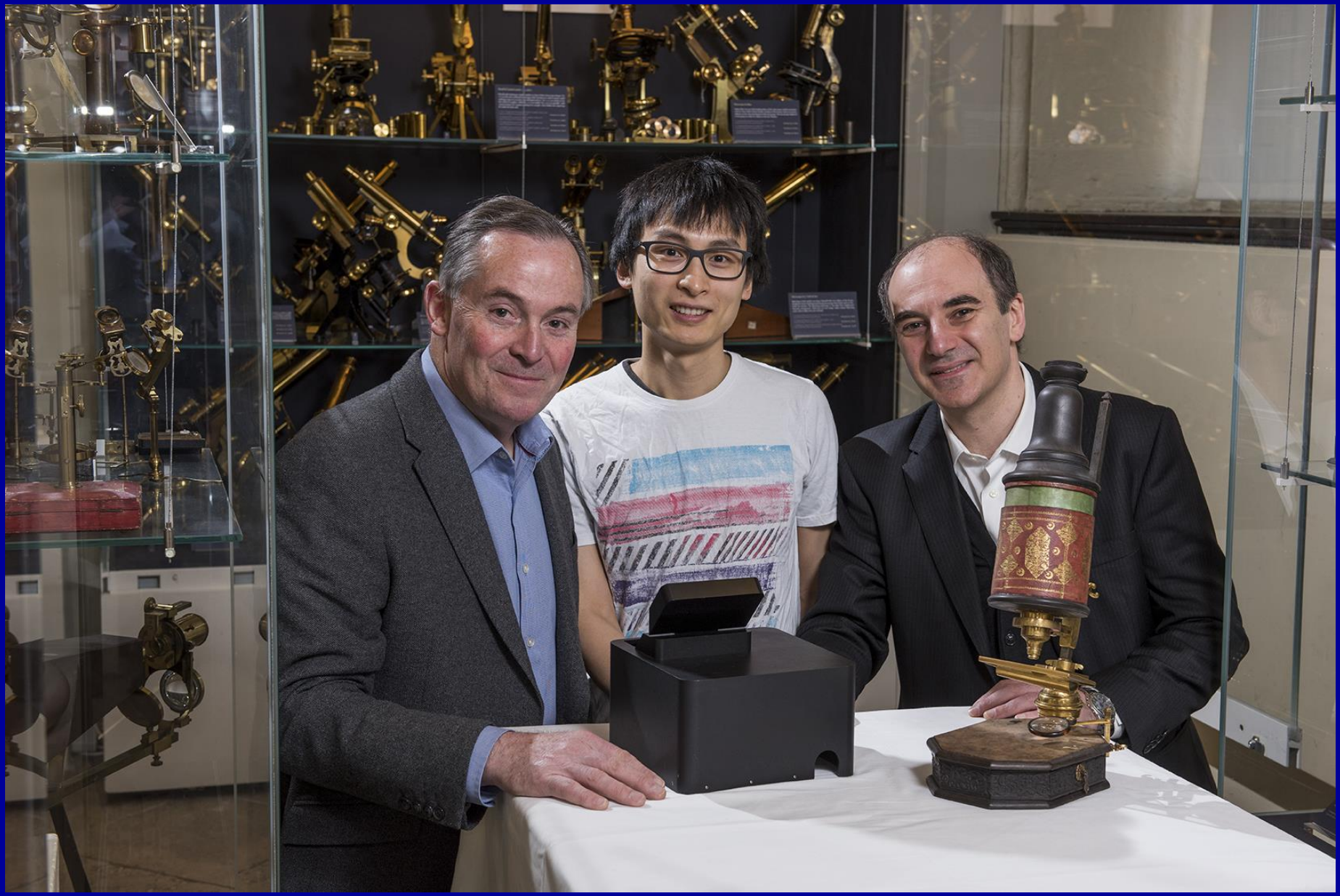
(2014 Nobel Prize)

- **TIRF**
- **FRET**
- **FIONA**
- **STORM**
- **PALM**



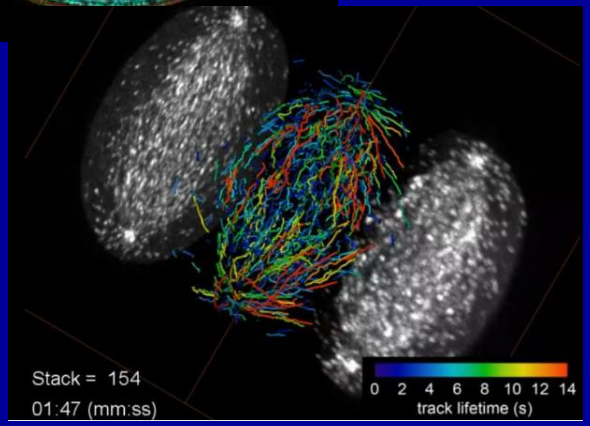


# Future ?

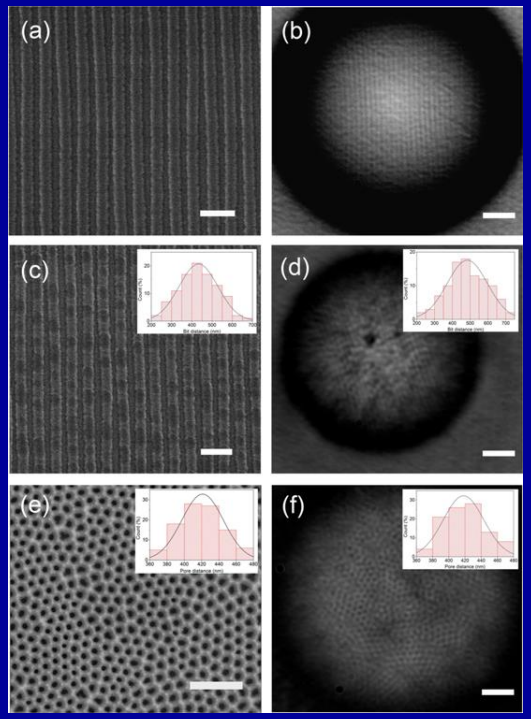
- **Last MMC 2017**
- Stereo/3D – moving objective
- Super res staining
- Light sheet
- Globular / silk / metal lenses

- **This MMC 2019 (Nanoscopy !)**
- * Multiple tilted light sheet microscopy, fast, CTLS
- * Microsphere lens mics (SMAL) ri 5.4, x400, r=50-100nm
- * Big Image Data microscopy – fast 3D/4D VR, holotomography
- EM lithography to 1.7um, FIB, cryo, multimodal/correlative
- Xray & Gas ion microscopes
- Acoustic microscopy
- Miniature endoscopic microscope
- * **AI Microscopy / Machine Learning !!!**

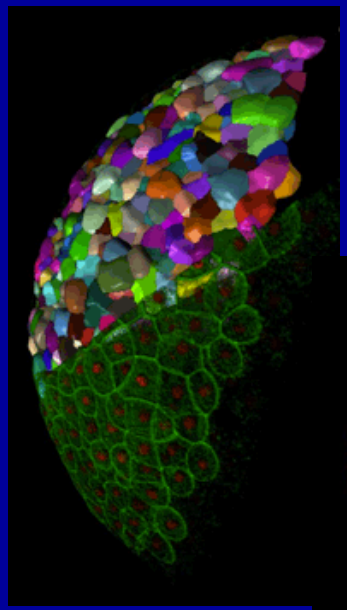
## Light Sheet Microscopy

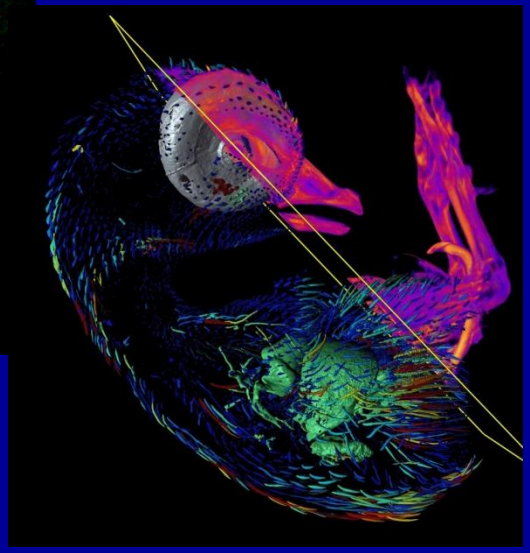


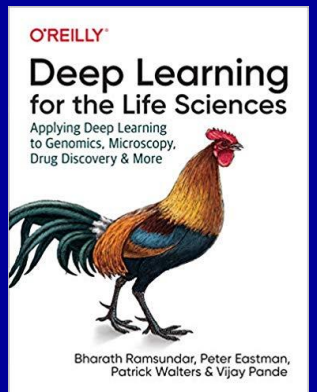

## Microsphere Nanoscope




## Big Image Data








## AI Microscopy

# The Ultimate Microscope ???

## Diamond Light Beamline Facility

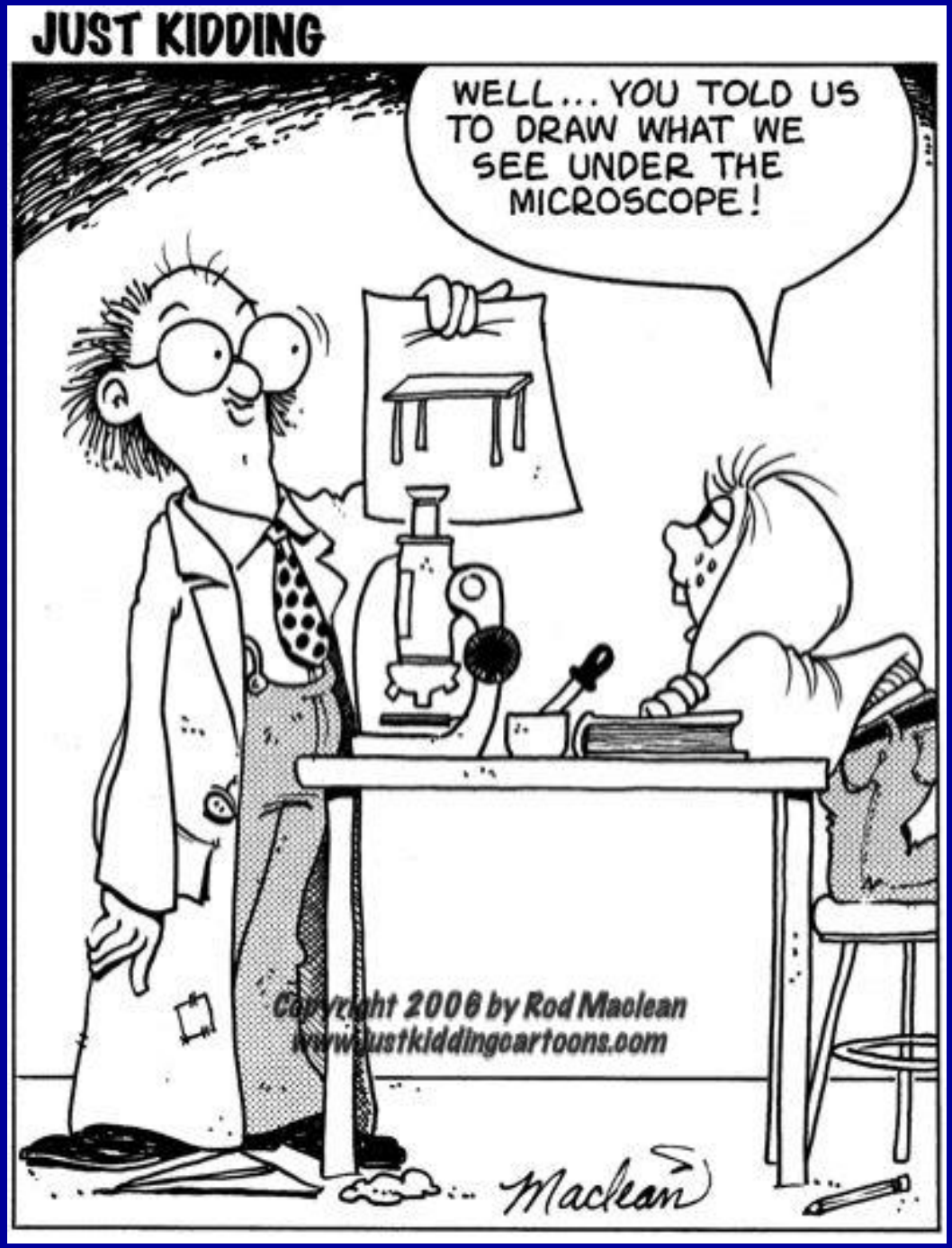
2007-2018 - ….

https://www.diamond.ac.uk/Instruments.html

Diamond Light Source is the UK's national synchrotron. It works like a giant microscope, harnessing the power of electrons to produce bright light that scientists can use to study anything from fossils to jet engines to viruses and vaccines.

The machine accelerates electrons to near light speeds so that they give off light 10 billion times brighter than the sun. These bright beams are then directed off into laboratories known as 'beamlines'. Here, scientists use the light to study a vast range of subject matter, from new medicines and treatments for disease to innovative engineering and cutting-edge technology.

…. a machine that is 10,000 times more powerful than a traditional microscope.


JUST KIDDING
WELL... YOU TOLD US TO DRAW WHAT WE SEE UNDER THE MICROSCOPE!
Copyright 2006 by Rod Maclean
www.justkiddingcartoons.com
Maclean

# How a microscope specimen sees you !!

# References for Microscopy

- An Introduction to the Optical Microscopy Saville Bradbury (1989) OUP/RMS
- Light Microscopy by D.J.Rawlins (BIOS Scientific) 1992
- Phase Contrast and Interference Microscopy for Cell Biologists by K.F.A. Ross (Edward Arnold) 1967
- Pioneers & Leaders in Microscopy by R Pool (Wiley/M&A) 2017
- Polarising Microscopy
- New: Understanding Light Microscopy by J Sanderson (2018) RMS/Wiley