Russell Berrie Nanotechnology Institute
TECHNION – ISRAEL INSTITUTE OF TECHNOLOGY

Modern Cryogenic-Temperature Electron Microscopy in the Nanostructural Study of Soft Matter

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&
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Birds of Agmon HaKhula Nature Reserve

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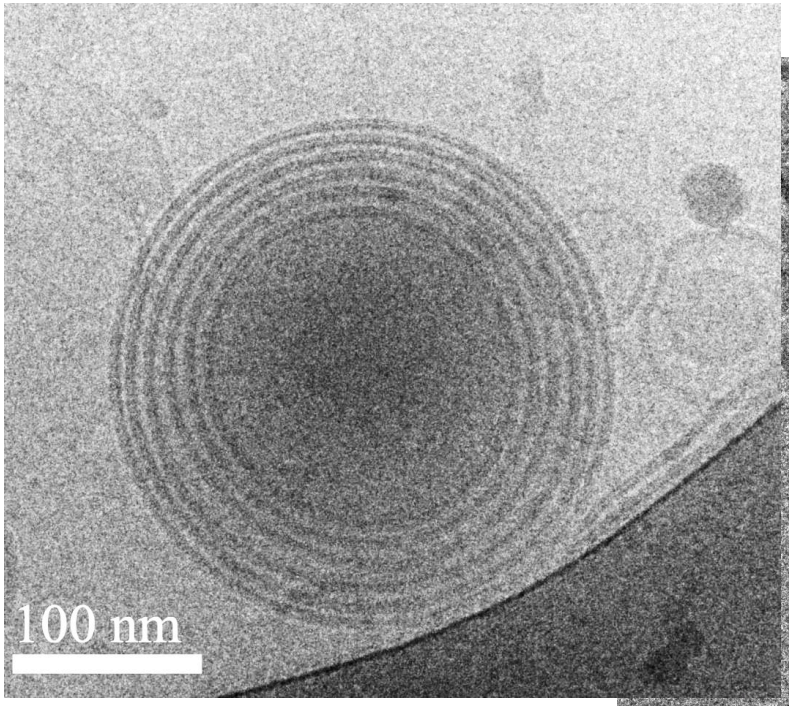
Judith Schmidt
Ellina Kesselman
Berta Shdemati

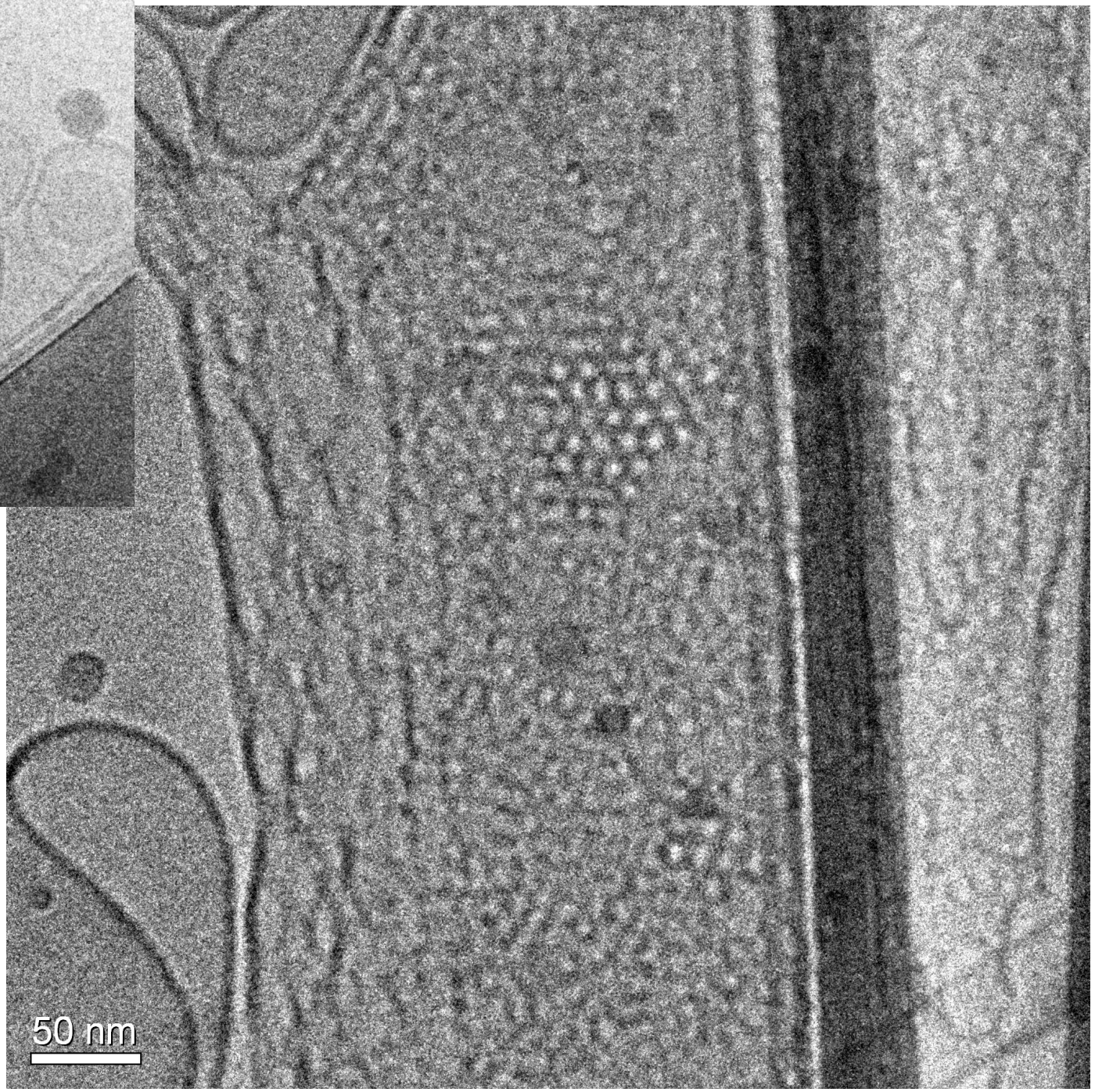


Shahrabani et al., J. Am. Chem. Soc. (2016)

Roy Beck, TAU

32.9% PE (29)
20.1% PC (25.9)
7.4% PS (7.0)
2.2% SM (6.2)
37.4% Chol (31.6)

10 mg/ml in 10mM MOPS buffer, 150mM NaCl, 2mM $CaCl_2$, pH=7.4



Myelin sheath complexes

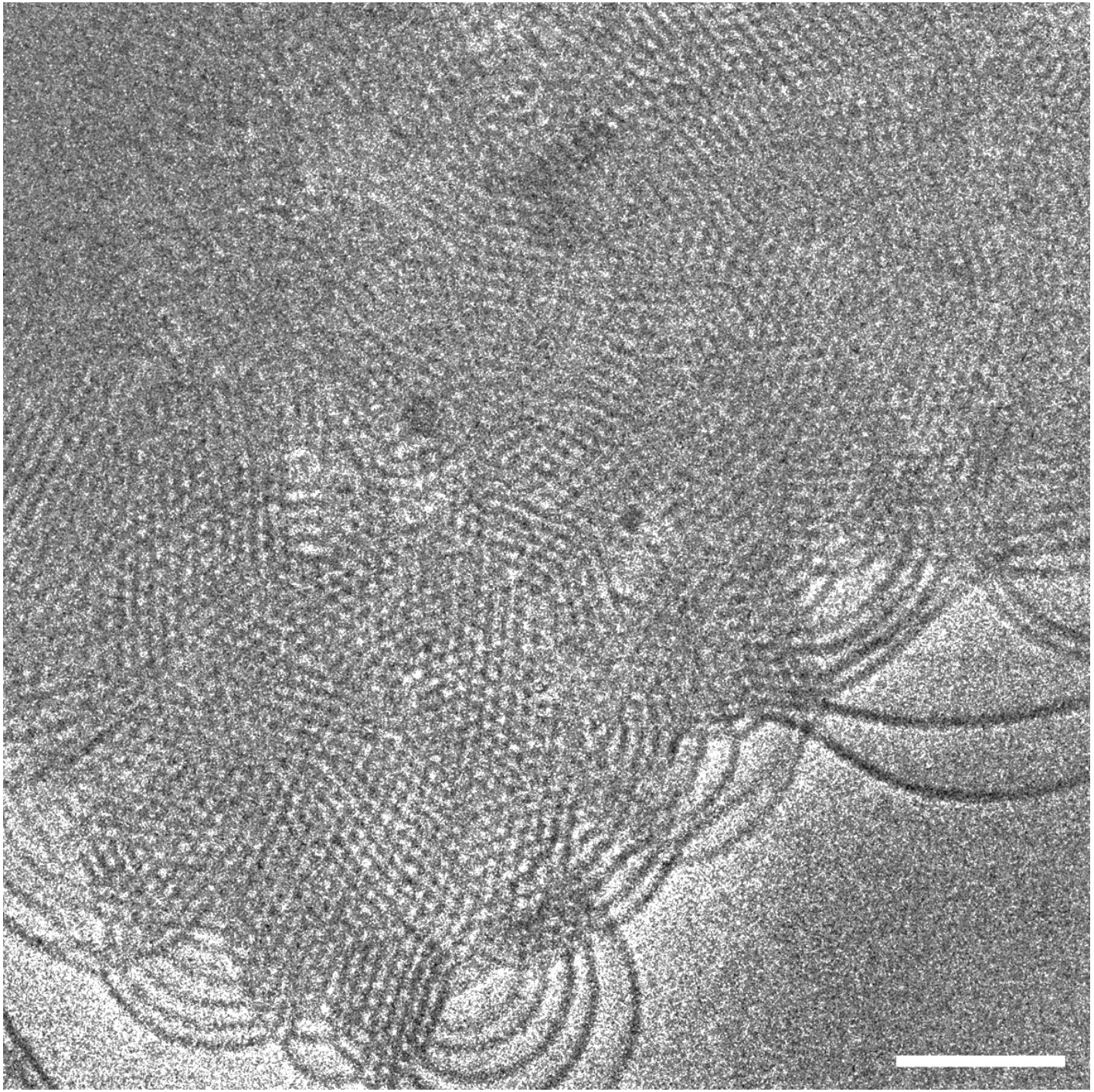
Maor Ram-On

Shahrabani et al.
PNAS (2018)

Roy Beck, TAU

Modified lipid composition
2 mM $MgCl_2$

Scale bar = 50 nm



Maor Ram-On

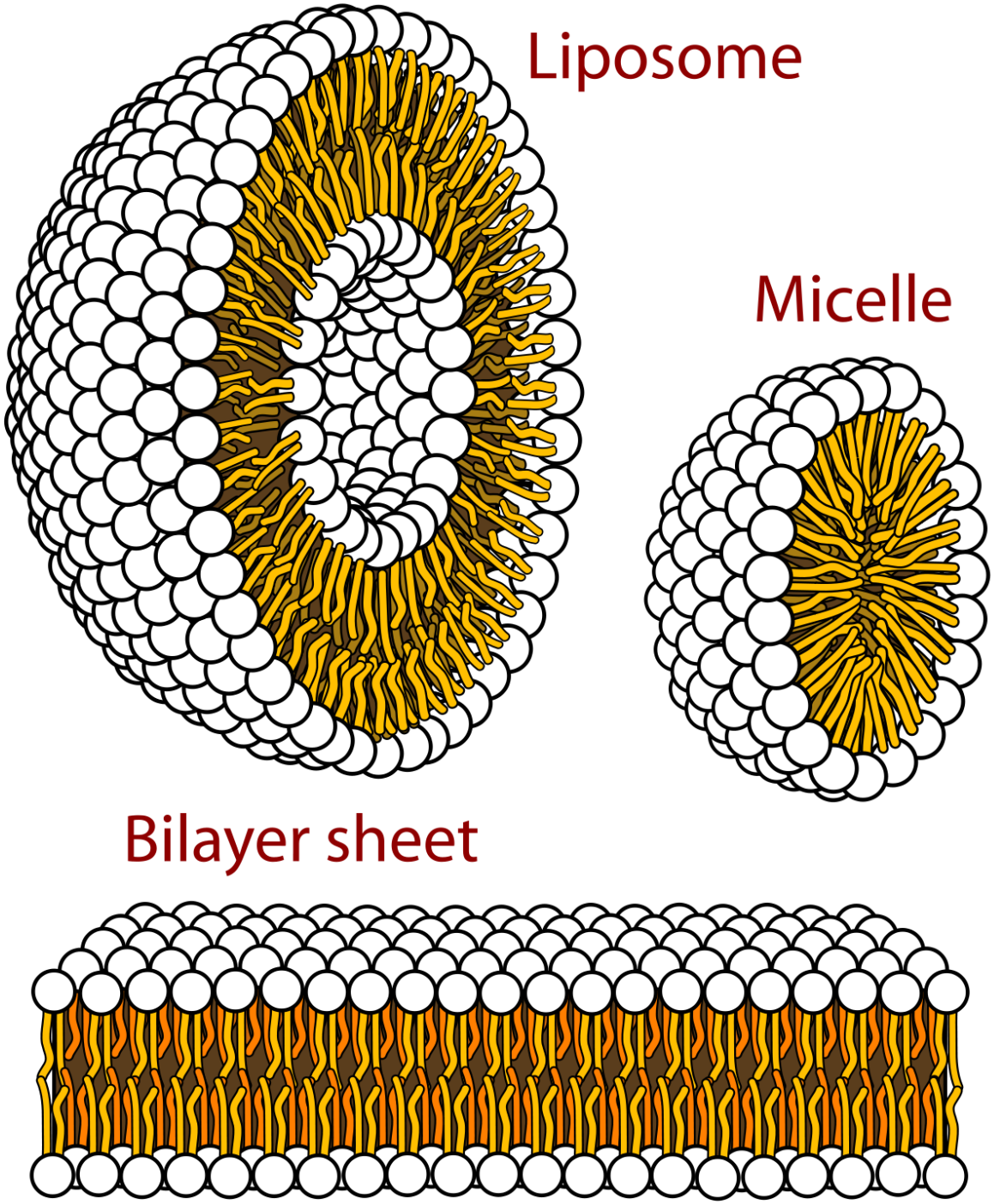
“Liquids are materials, too”

H. Ted Davis, ~1980

But they are not $SrTiO_3$….

Complex liquids or nanostructured liquids

Domain size on the order of 10^0 to 10^2 nm


Liposome
Micelle
Bilayer sheet

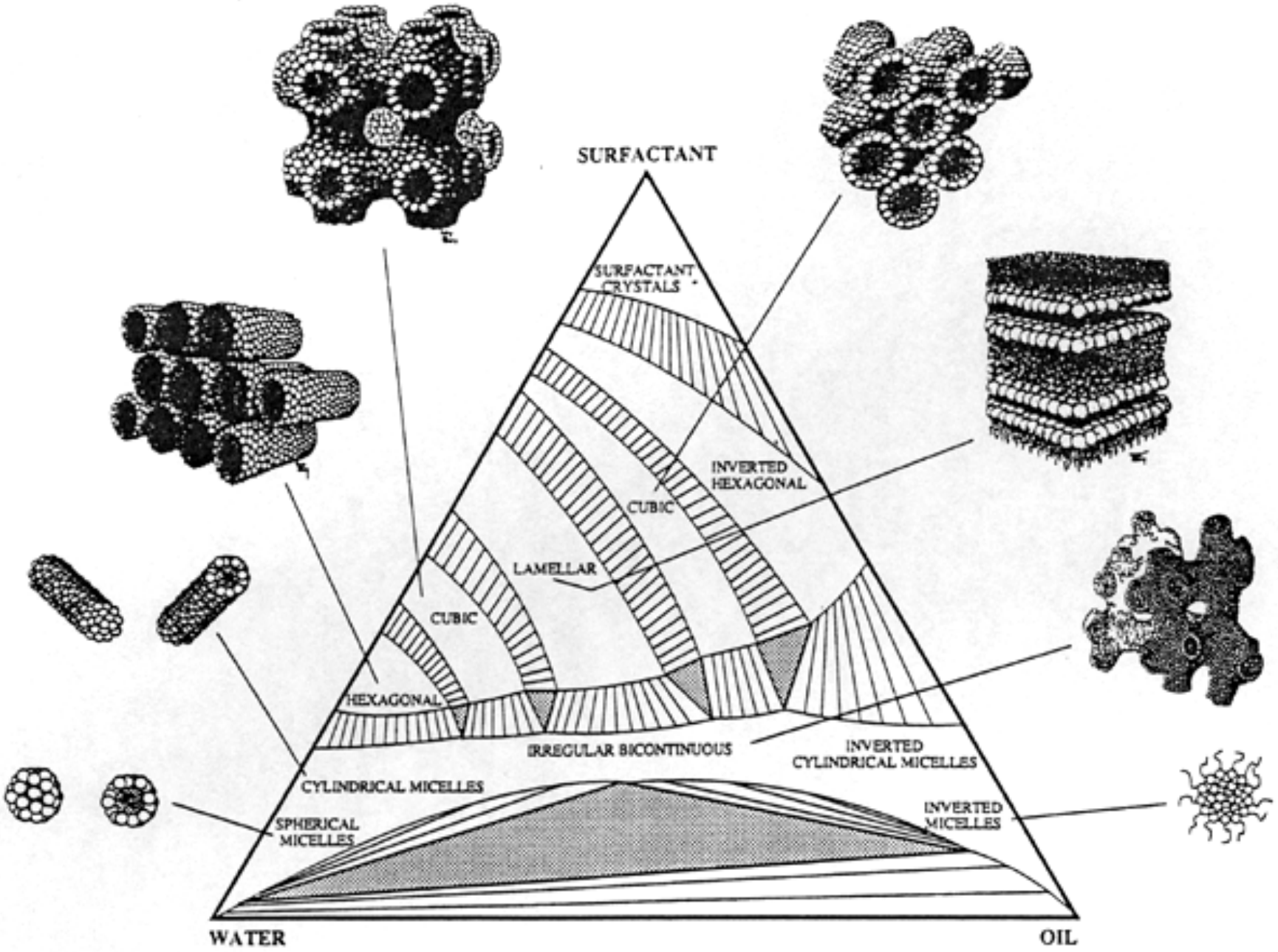


Figure 1.7 Idealized phase diagram for a surfactant-water-oil system (after Michels 1987)

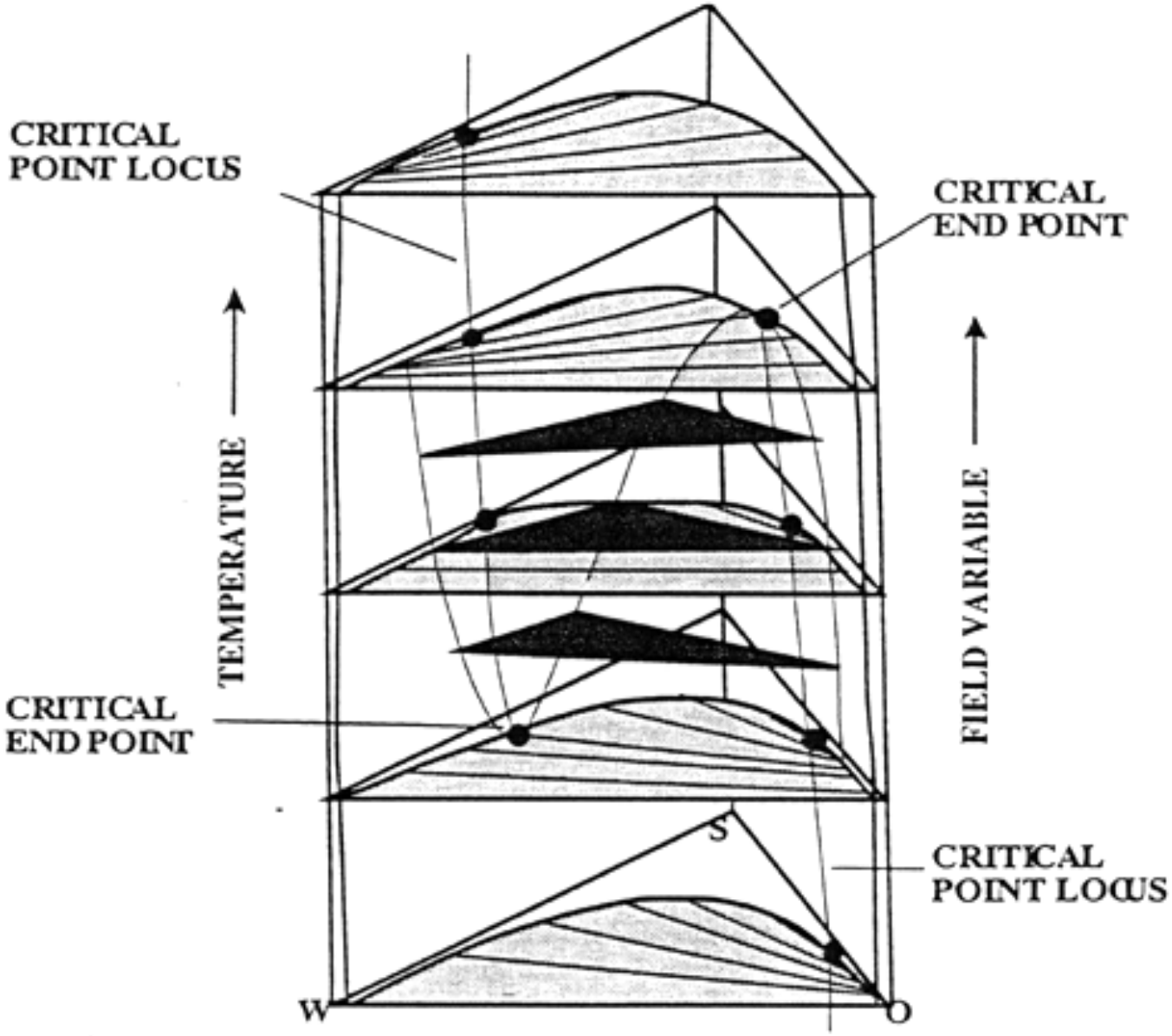


Figure 1.8 Generic temperature-composition prism of surfactant, oil and water ternary phase behavior.

The goals of EM of soft matter

- Direct images, at sufficiently high-resolution, of well-preserved specimens
- Determination of nanostructure development with change of system parameters
- Compare images to non-imaging experimental data
- Full nanostructural characterization of the system

The main challenges of EM of soft matter

- Nanostructure preservation during specimen preparation
- Minimize electron-beam radiation-damage
- Obtain sufficient image contrast
- Record micrographs at the required resolution

Cryo-EM (*EN*) of Complex (Nano-Structured) Liquids

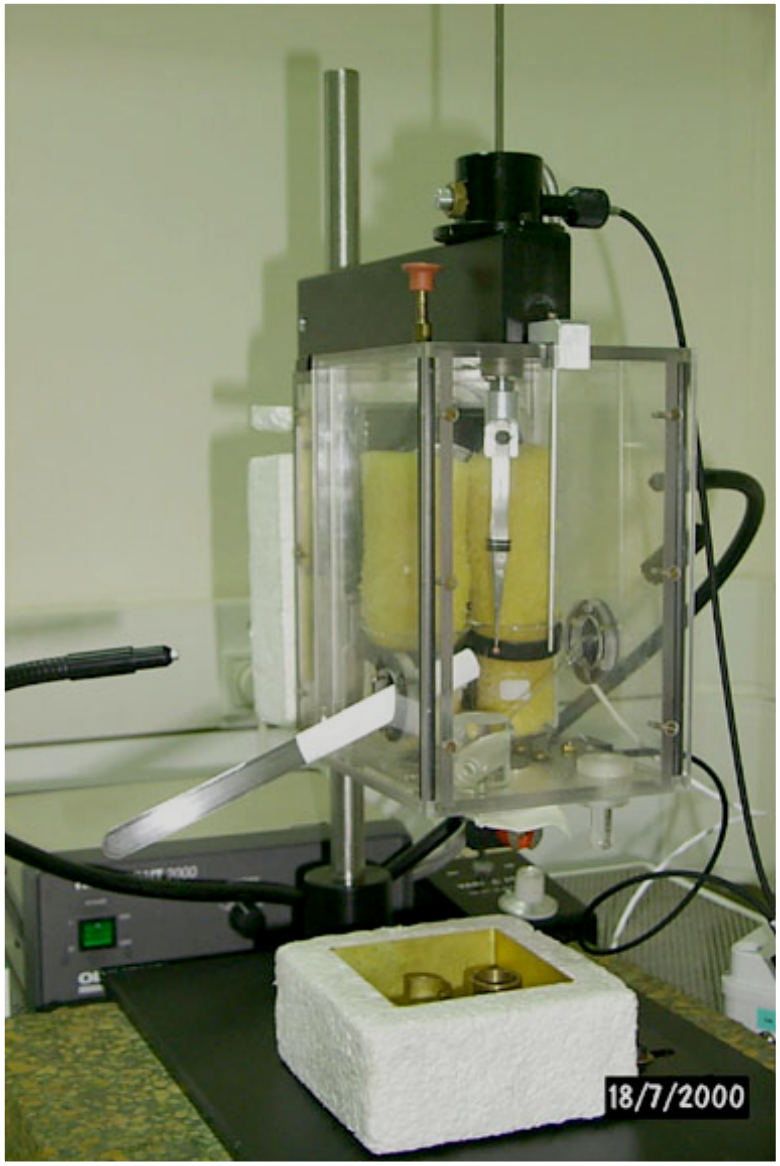
Cryo-TEM, Cryo-SEM (and Freeze-Fracture-Replication)

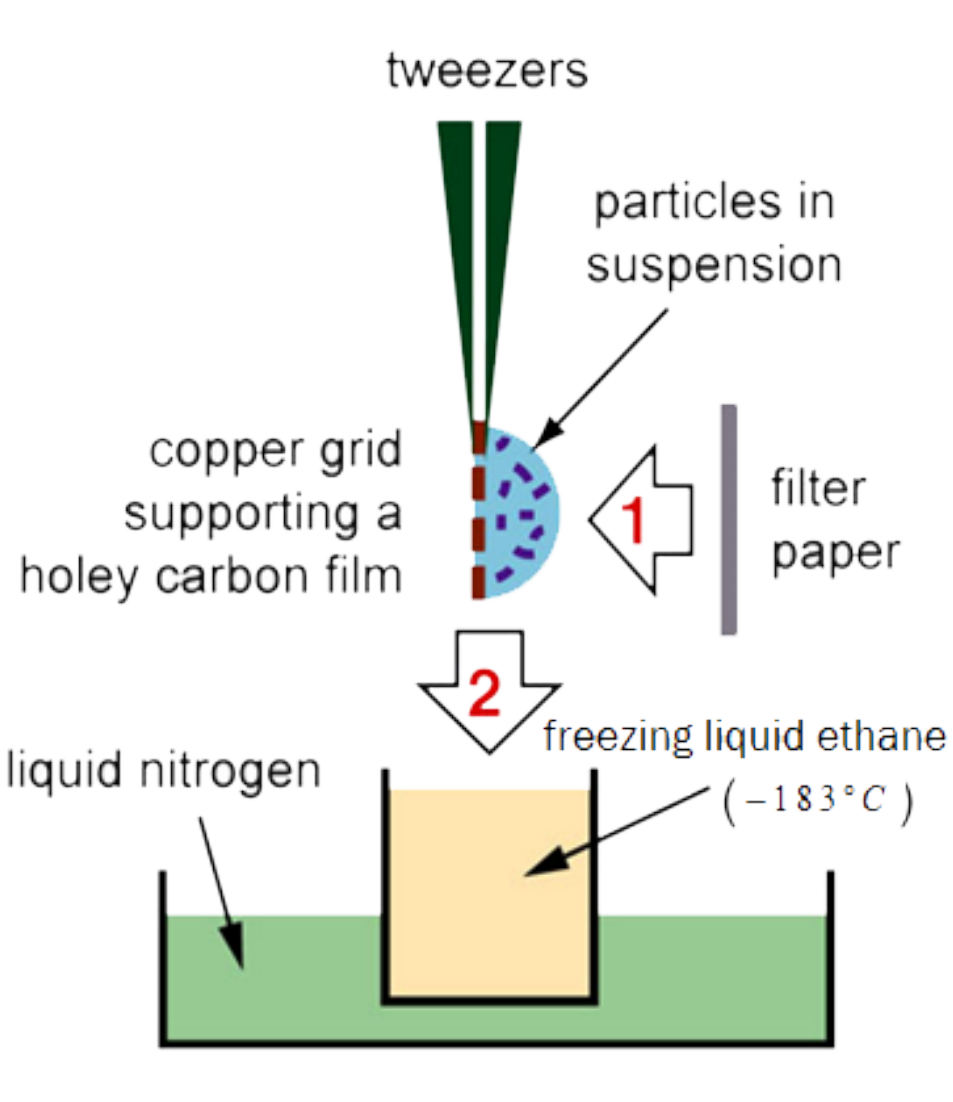
Sample Systems

- lyotropic liquid crystals
- nanotubes
- carbon nanotubes in strong acids
- metal and mineral nanoparticle dispersions
- micellar solutions, microemulsions
- biological liquids
- whole cells
- blood components


I_c
I_h
I_v
I_c
I_v

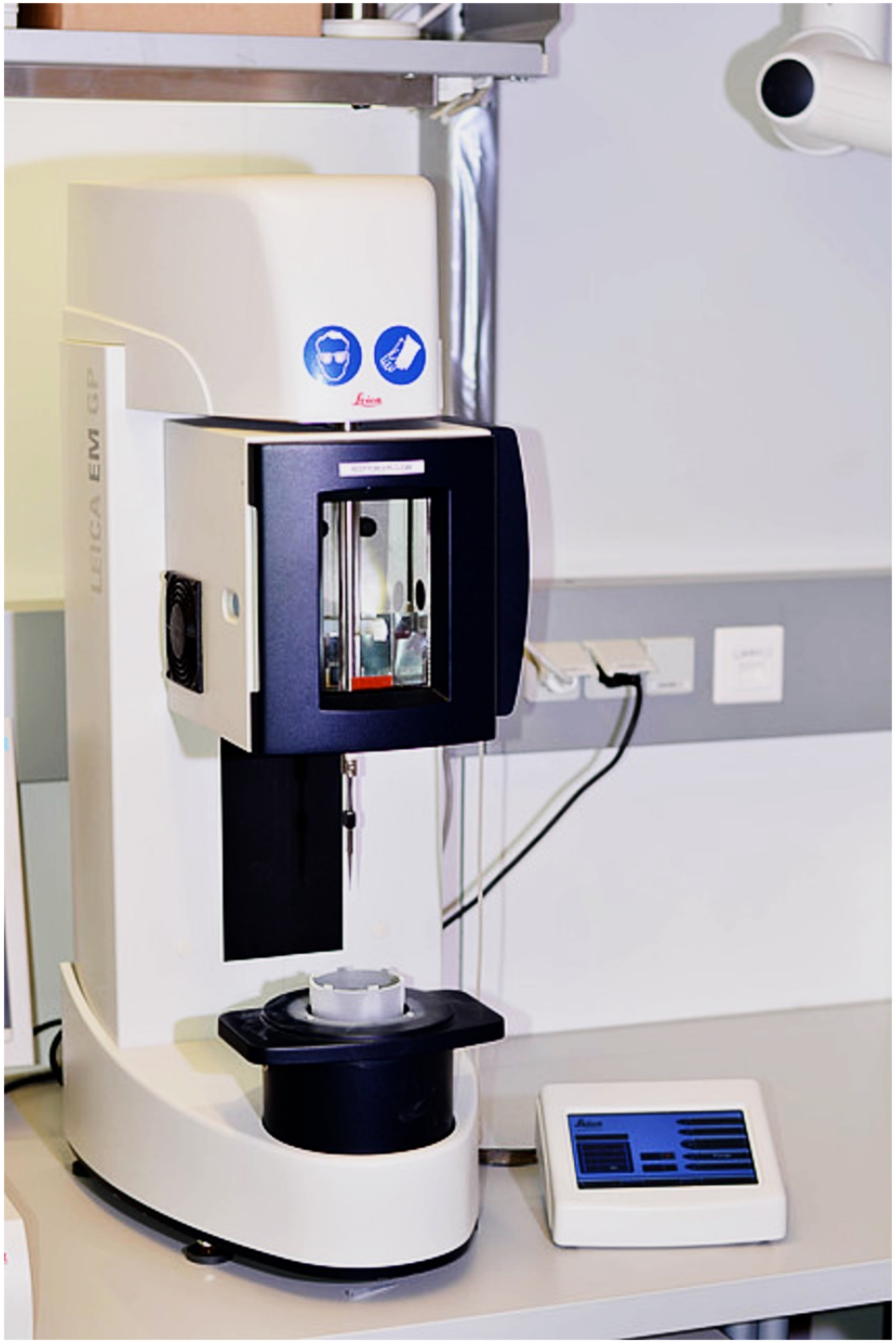
Cryo-TEM sample preparation
Controlled Environment Vitrification System (CEVS)



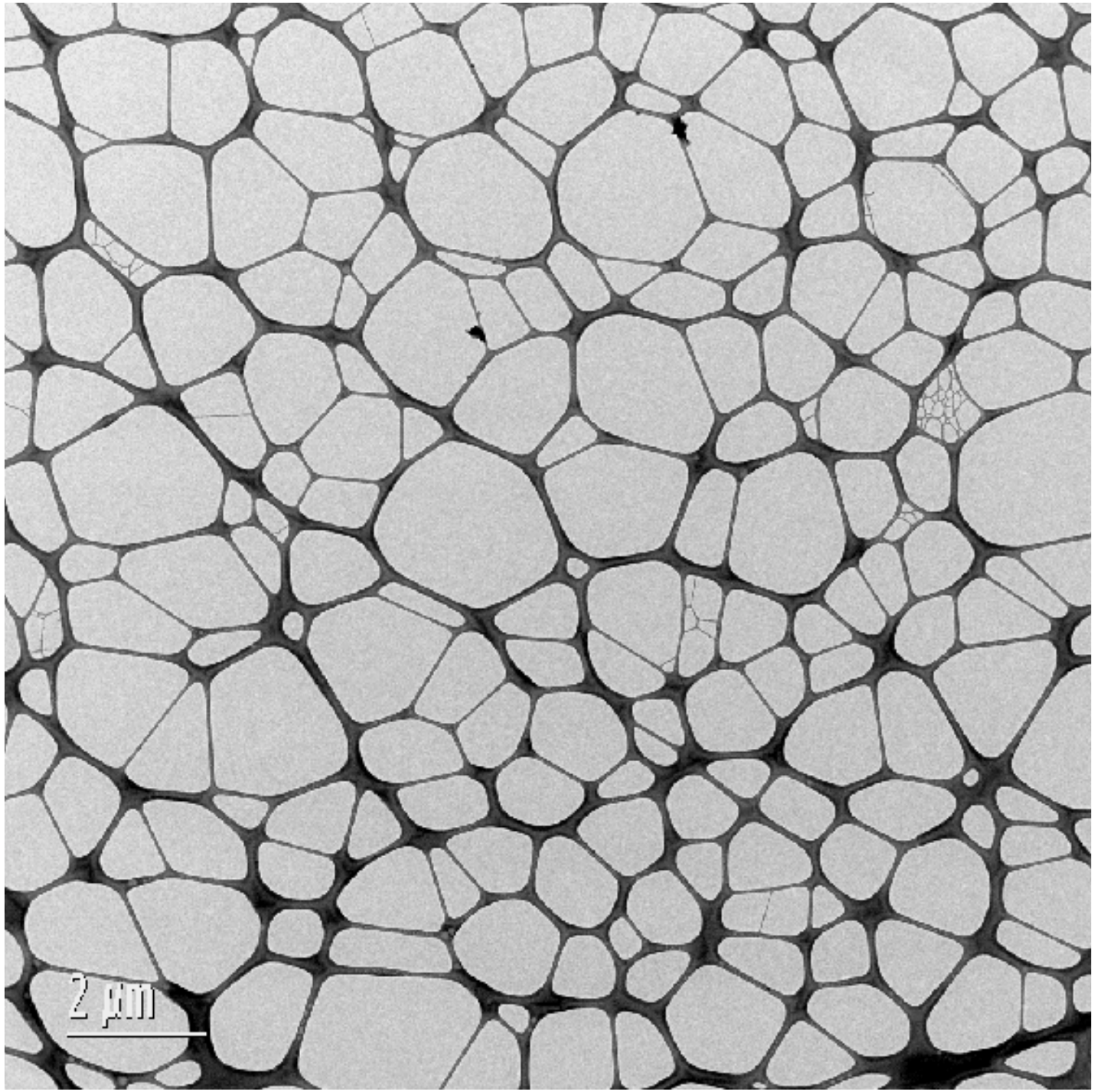


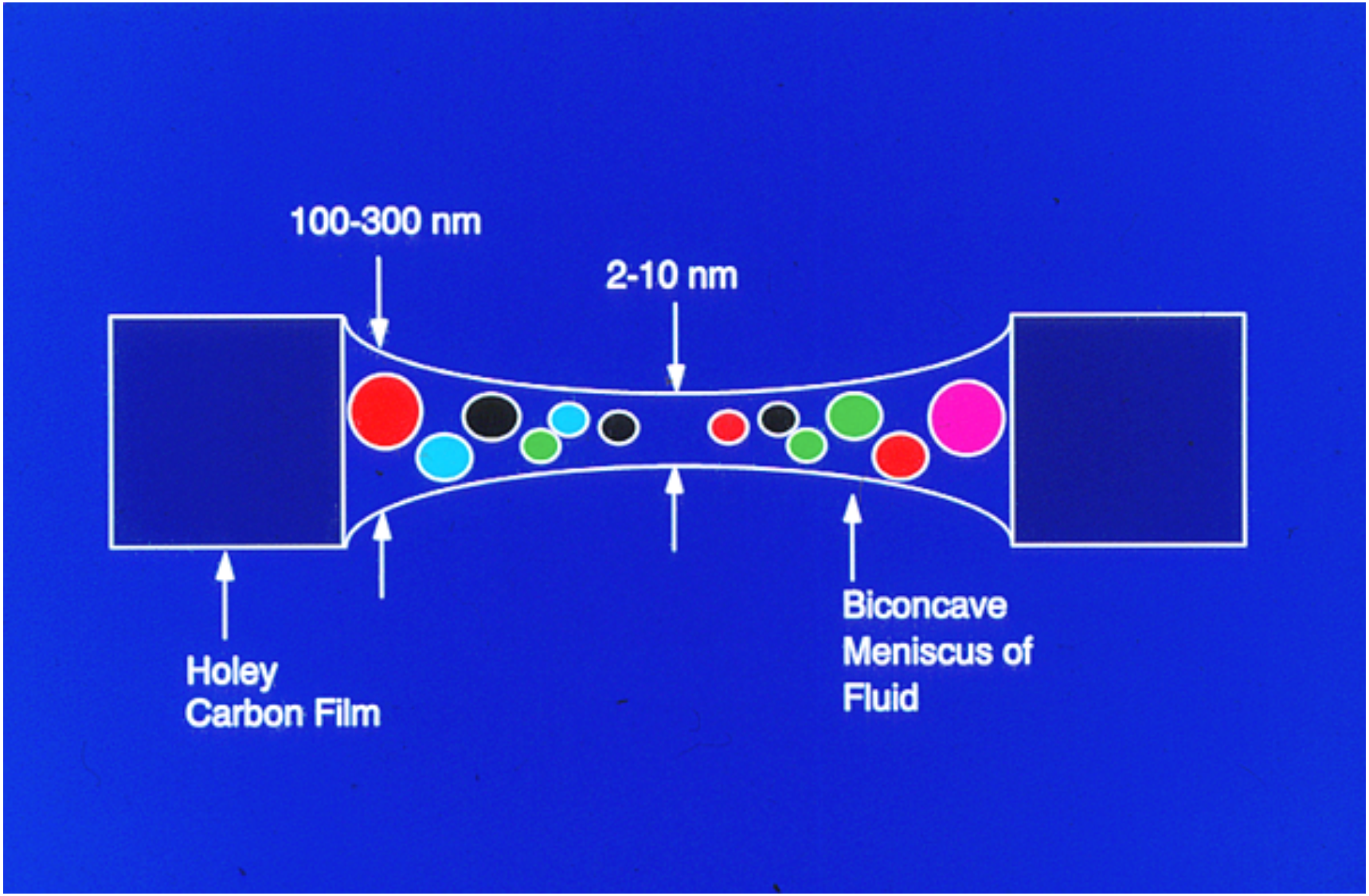
FEI
Vitrobot

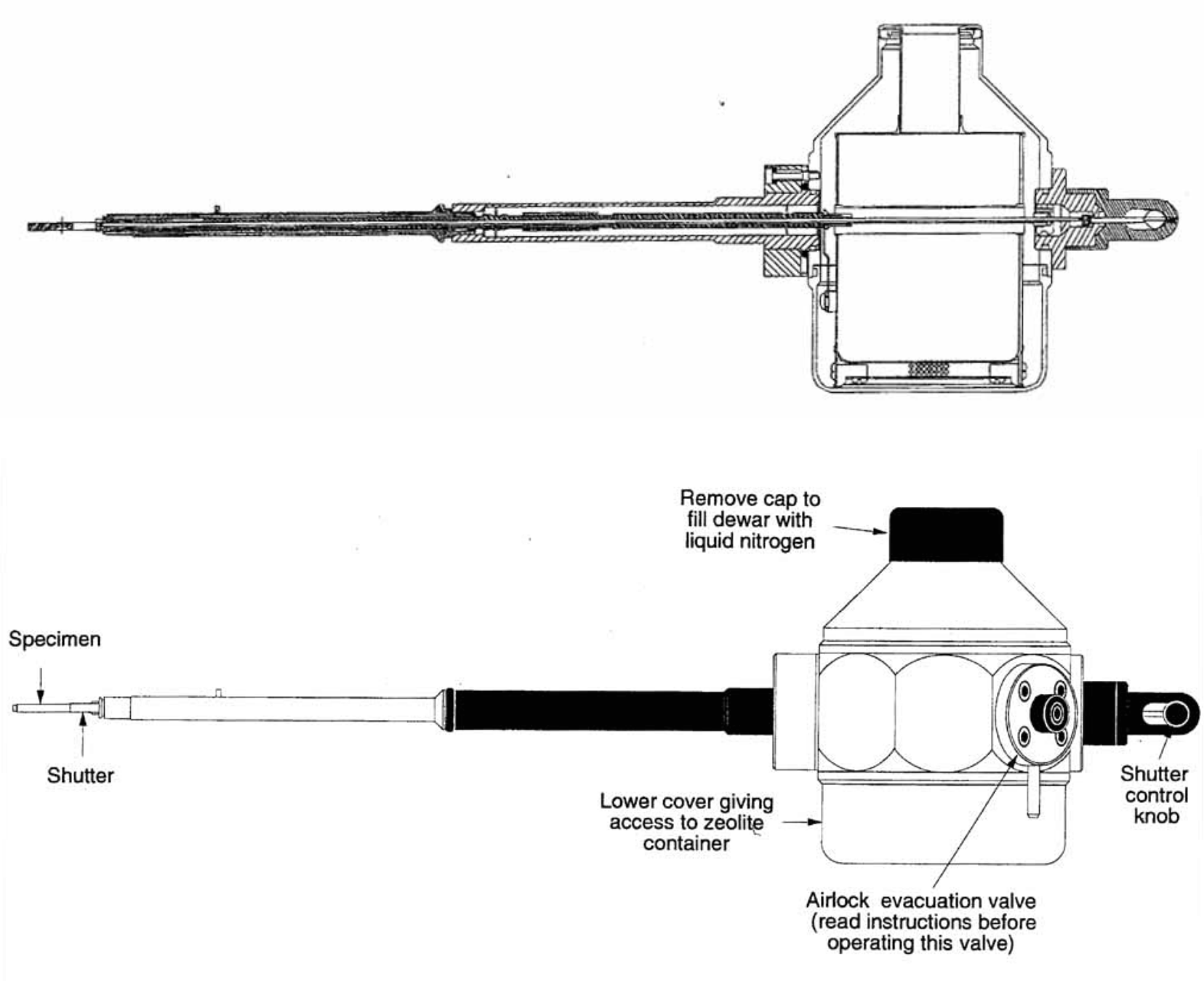
Leica EM GP



Perforated
("holey")
Carbon Film




100-300 nm
2-10 nm
Holey
Carbon Film
Biconcave
Meniscus of
Fluid



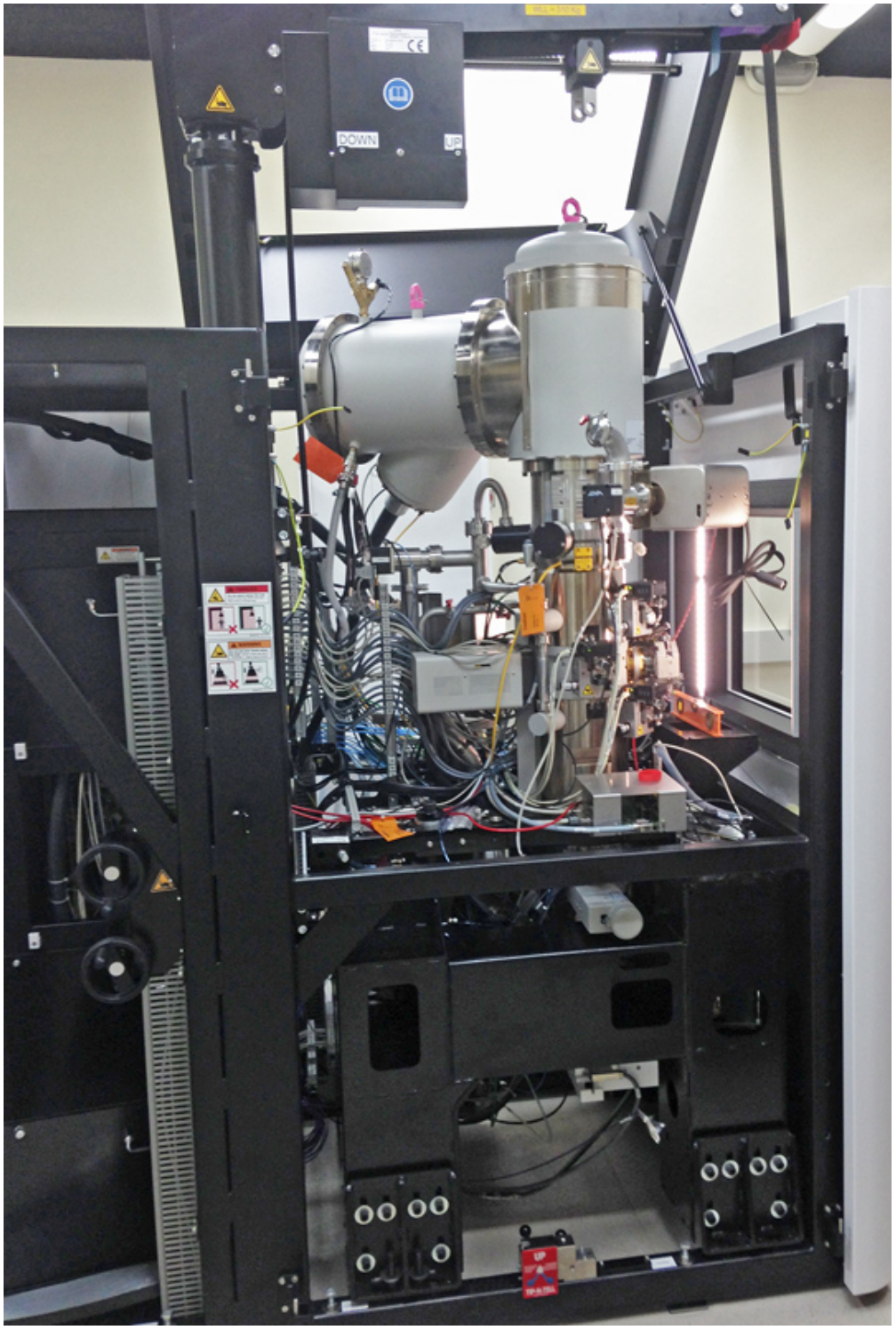
Gatan 626 Cryo-Holder

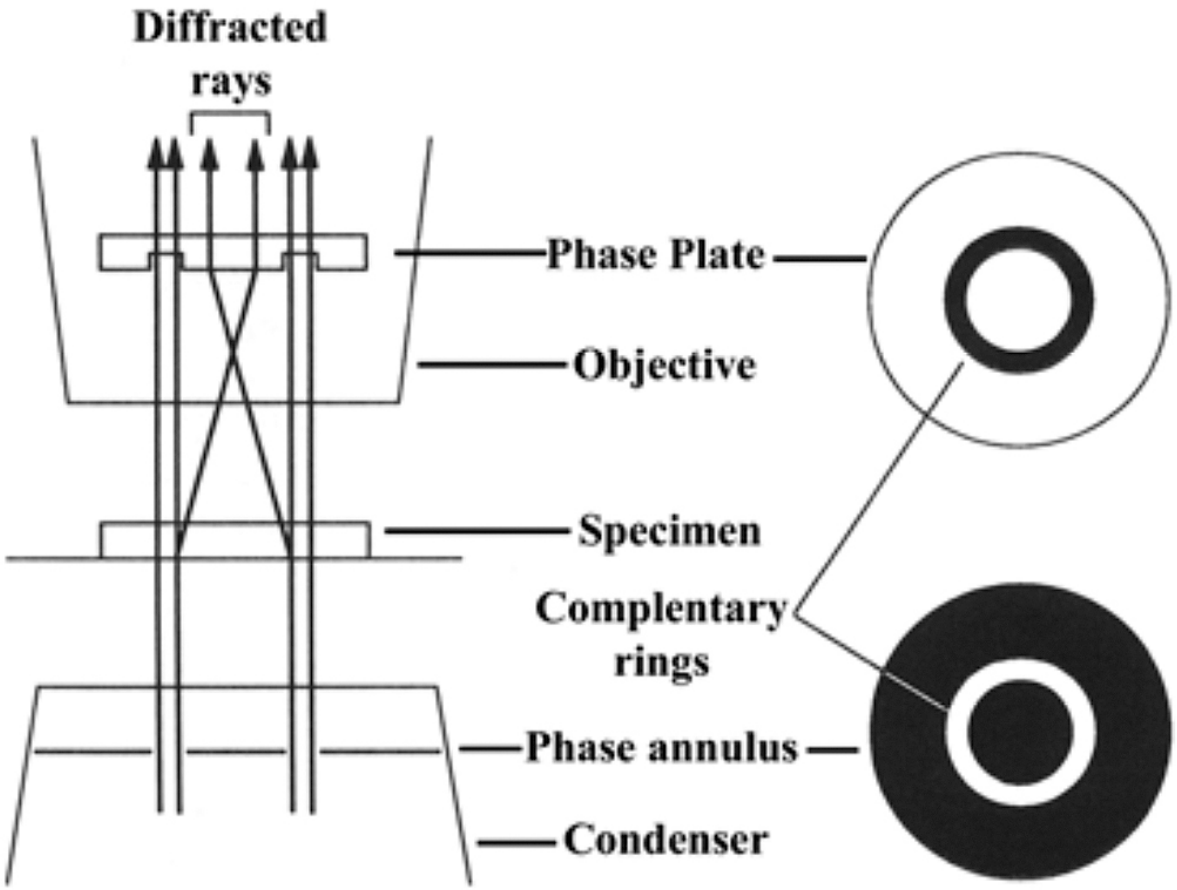
Since Sept. 2016

FEI Talos 200C

FEI Talos 200C

Technion, 4 Aug 2016



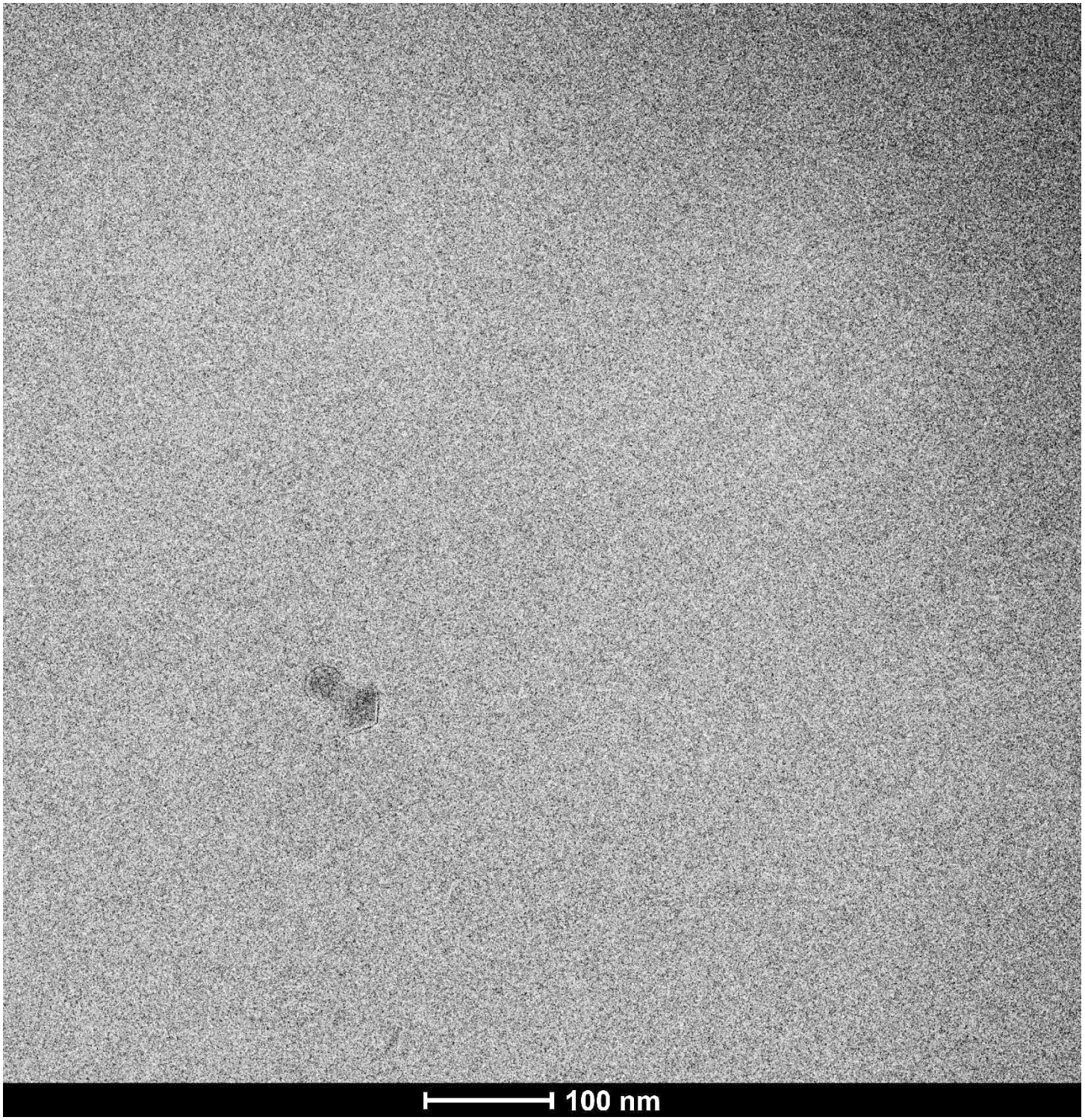


Phase Plates in LM

https://www.jic.ac.uk/microscopy/intro_EM.html

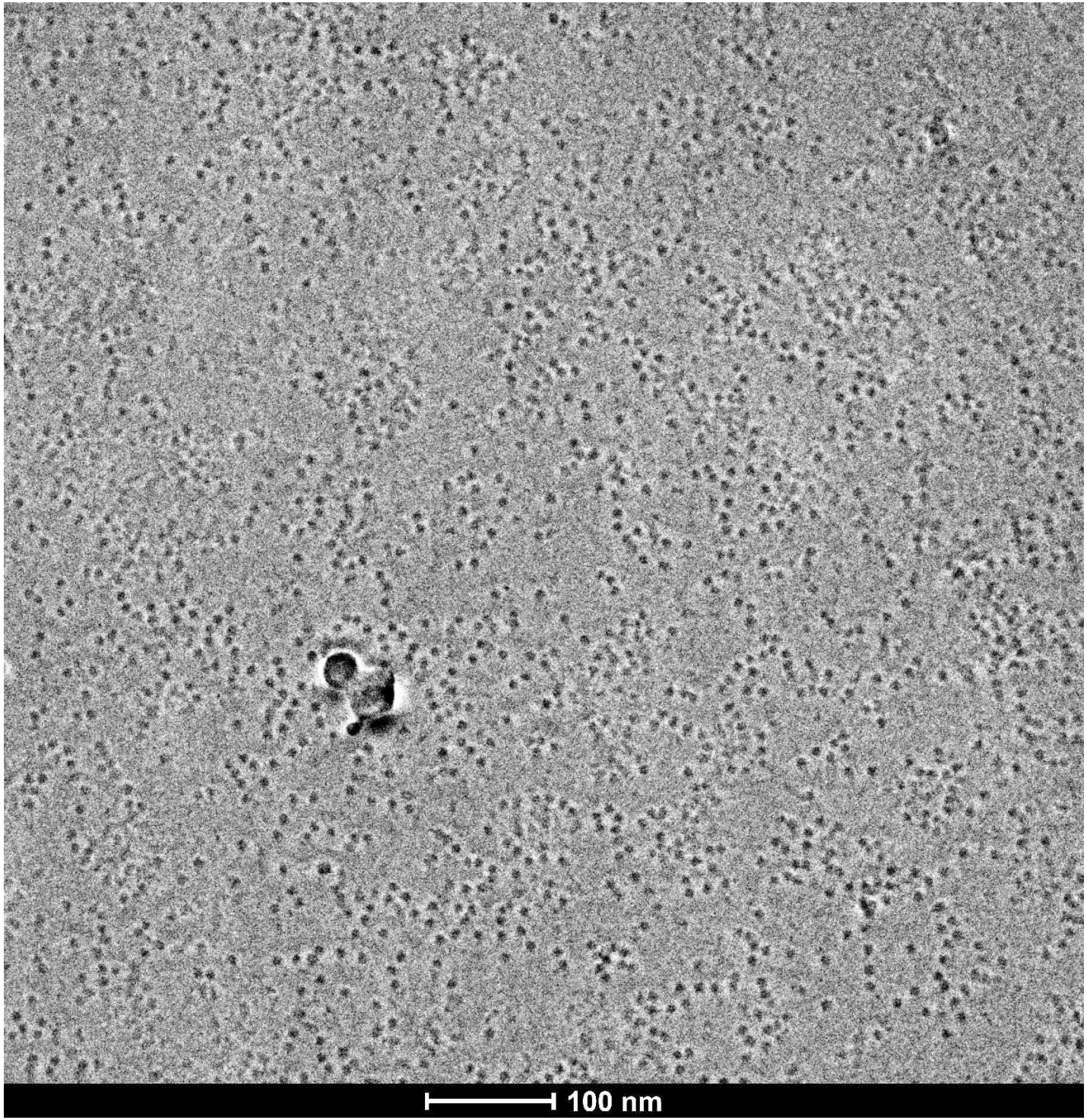
Fritz Zernike, 1933; Nobel Prize for Physics, 1953

EFF-1 A835
(protein trimer)
in-focus

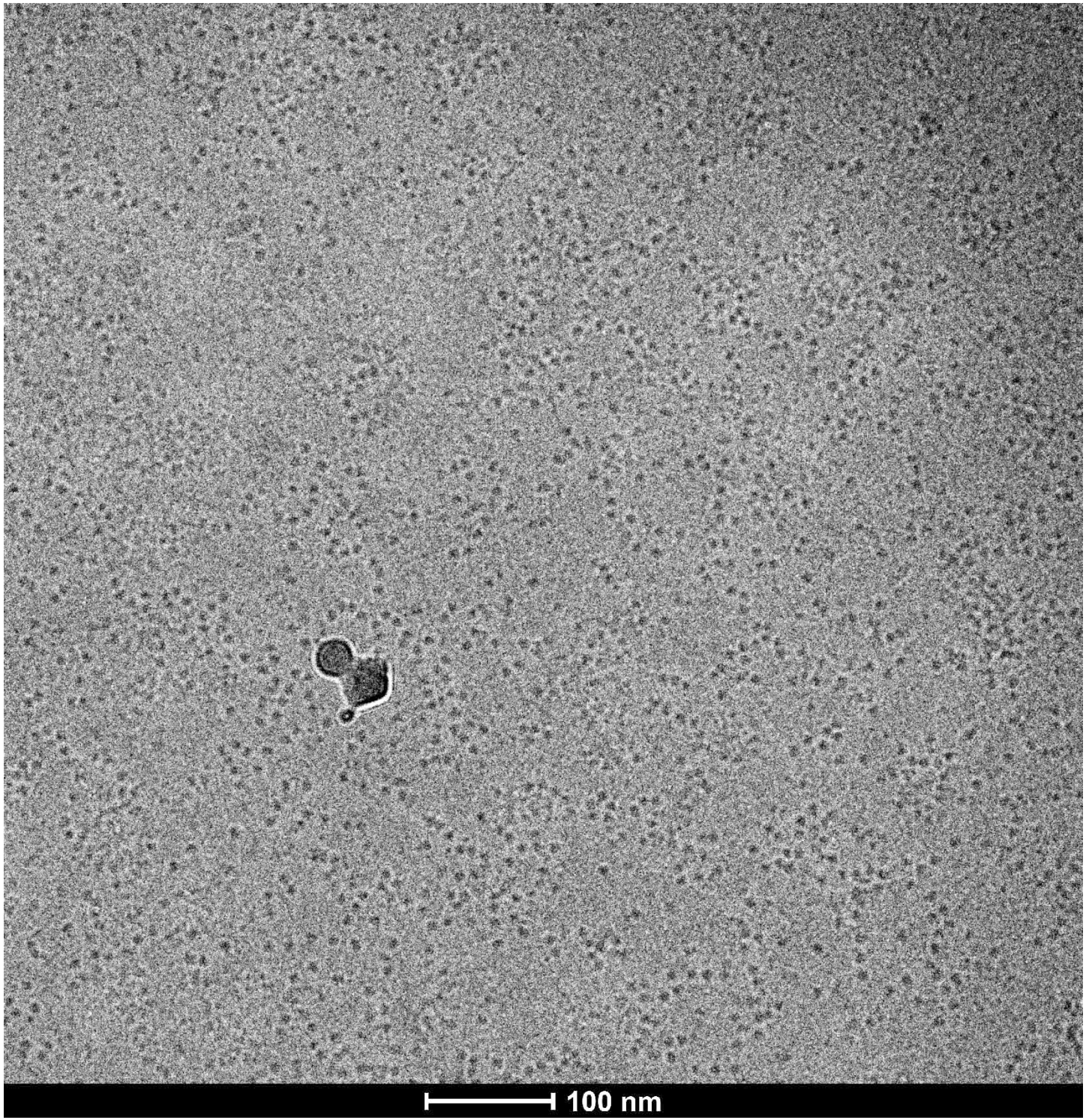


EFF-1 A835 aggregated
in-focus
with phase-plates

F. de Haas

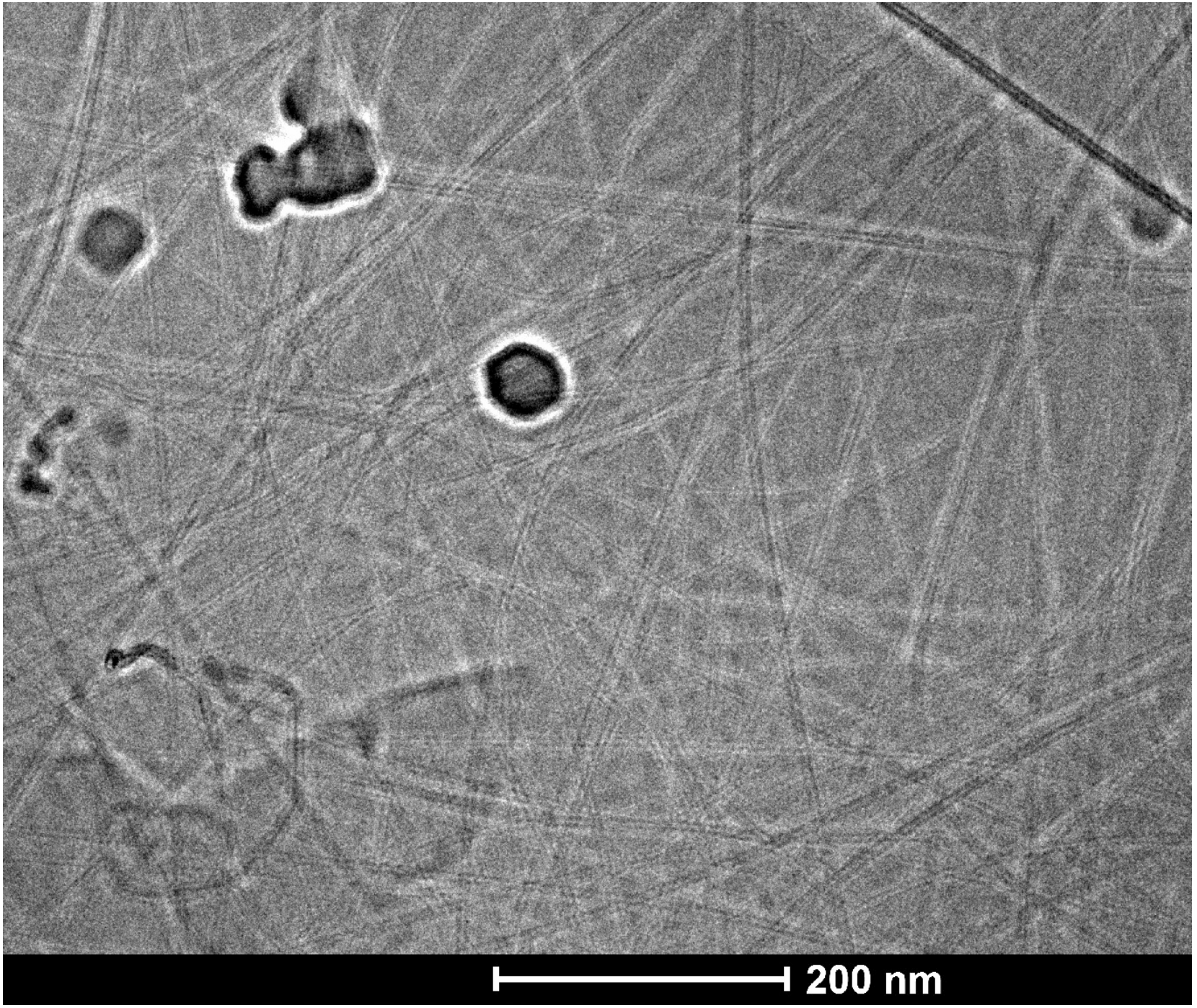


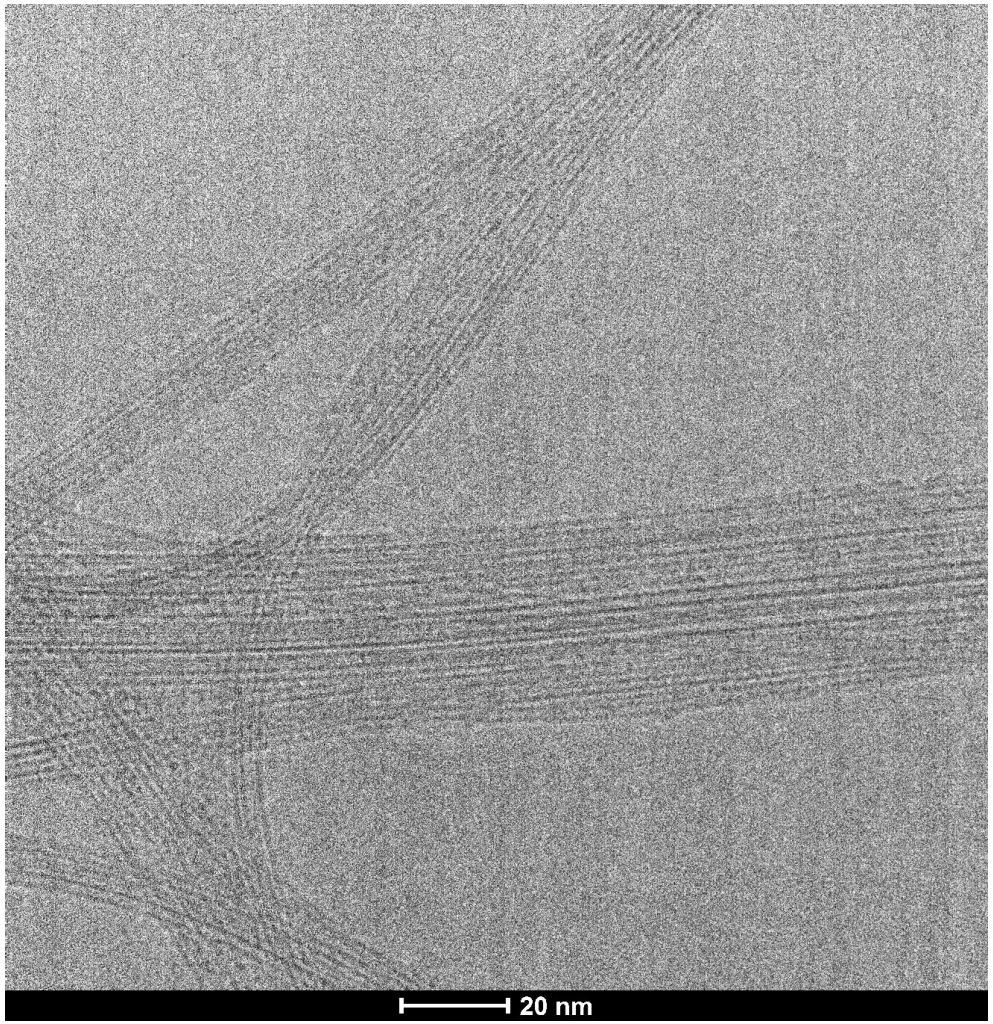
BP’s EFF-1 A835
7 µm underfocus
phase-plates out



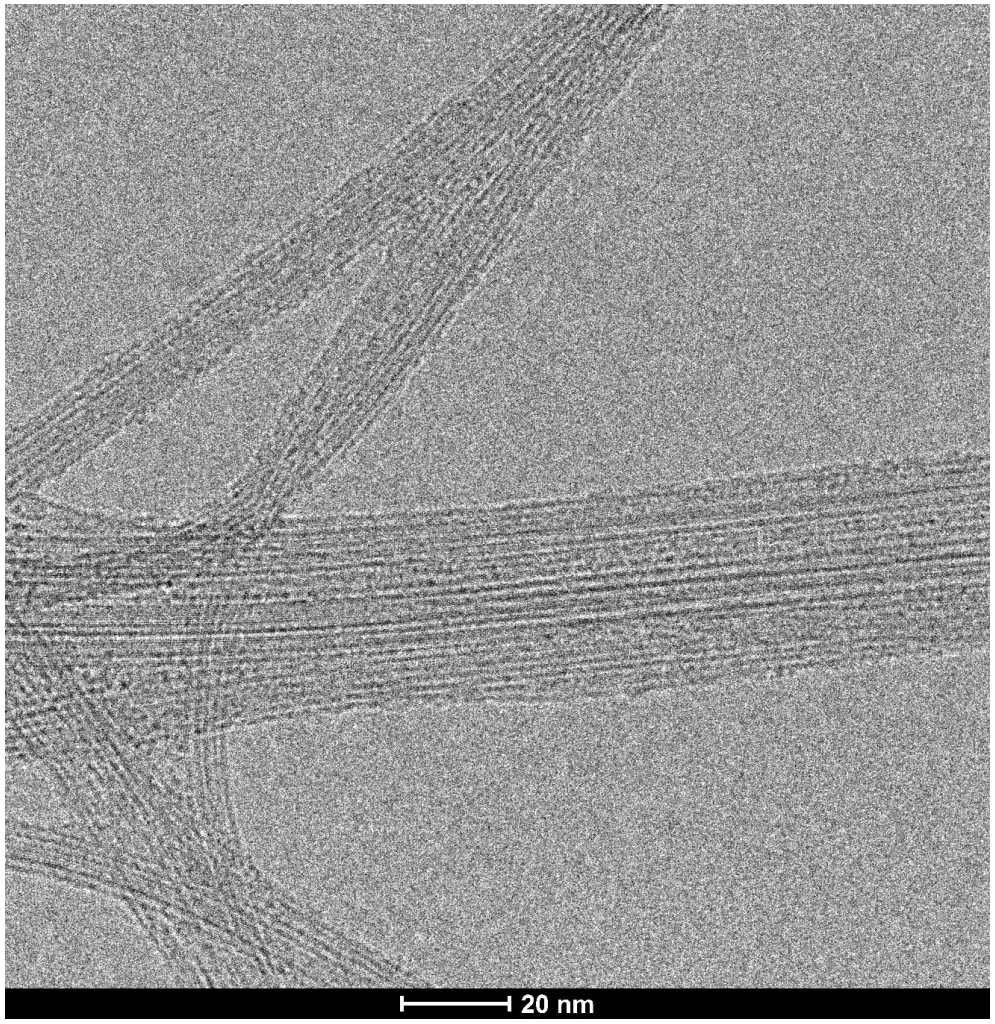
CCNI 1109 CNTs
30 ppm in CSA

in-focus
phase-plates
Lucy Liberman



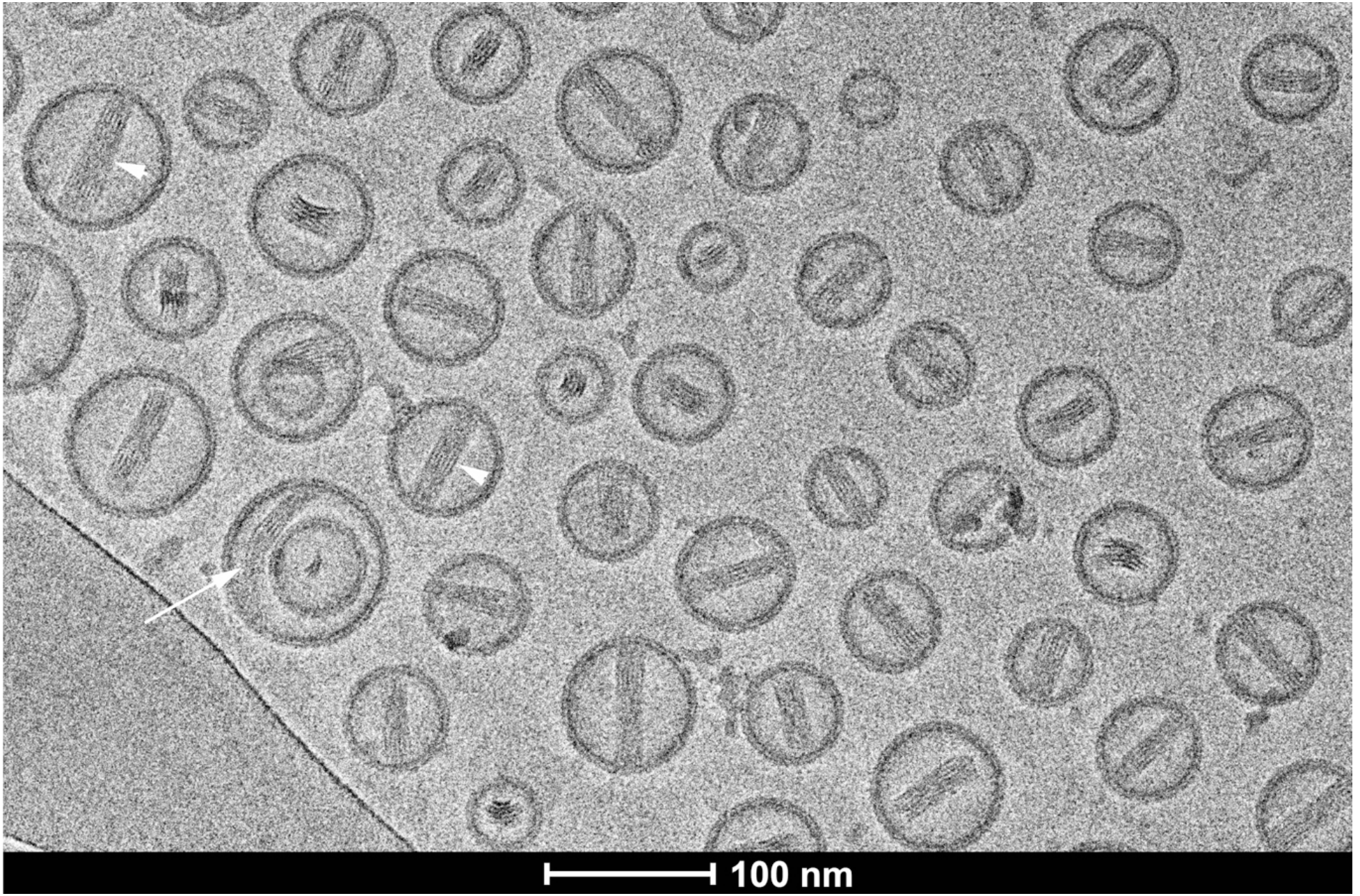


Ceta

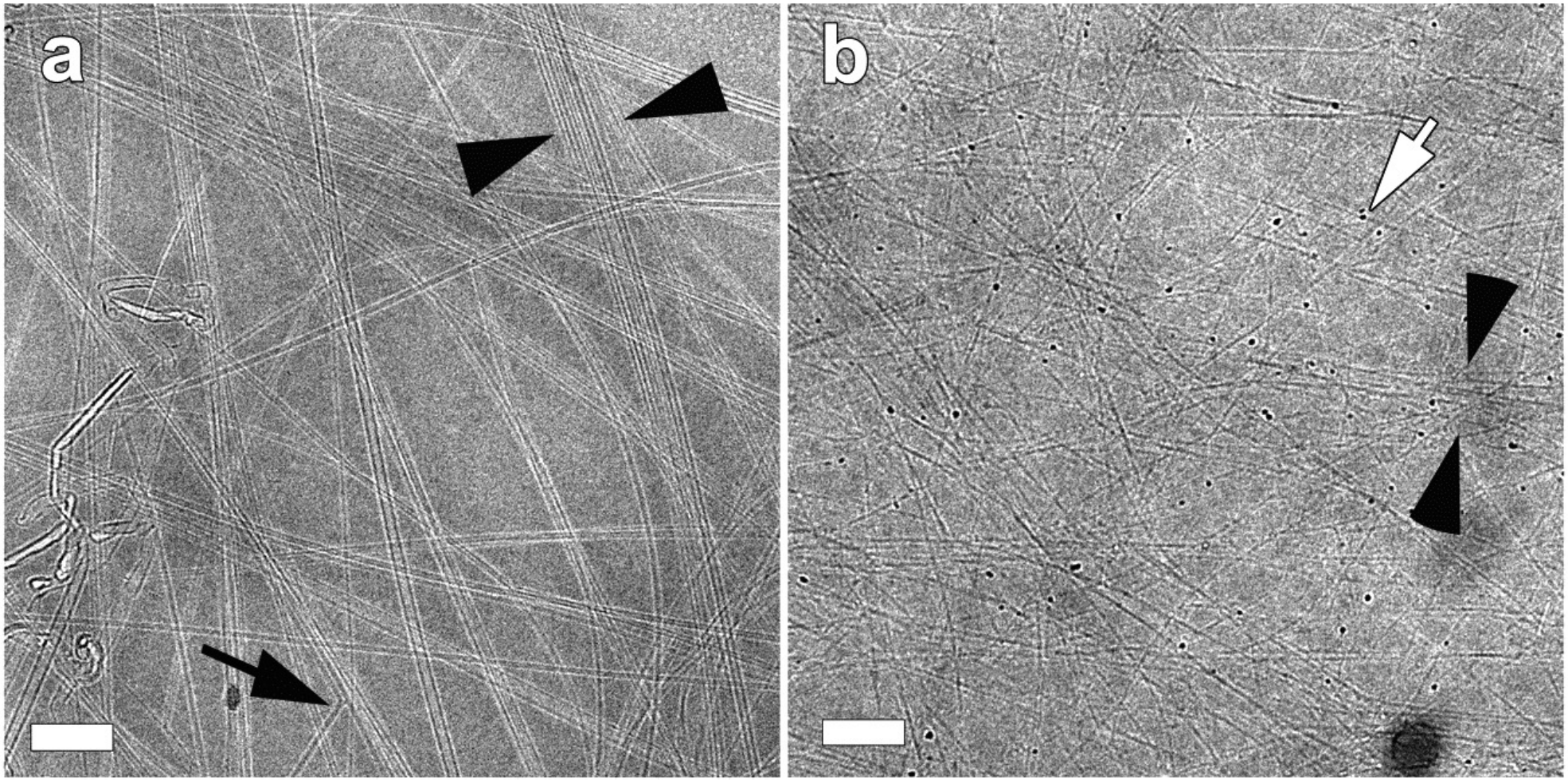


Falcon II

CNTs. As-recorded micrographs at the same conditions. ~ $2e^{-}/Å^{2}$

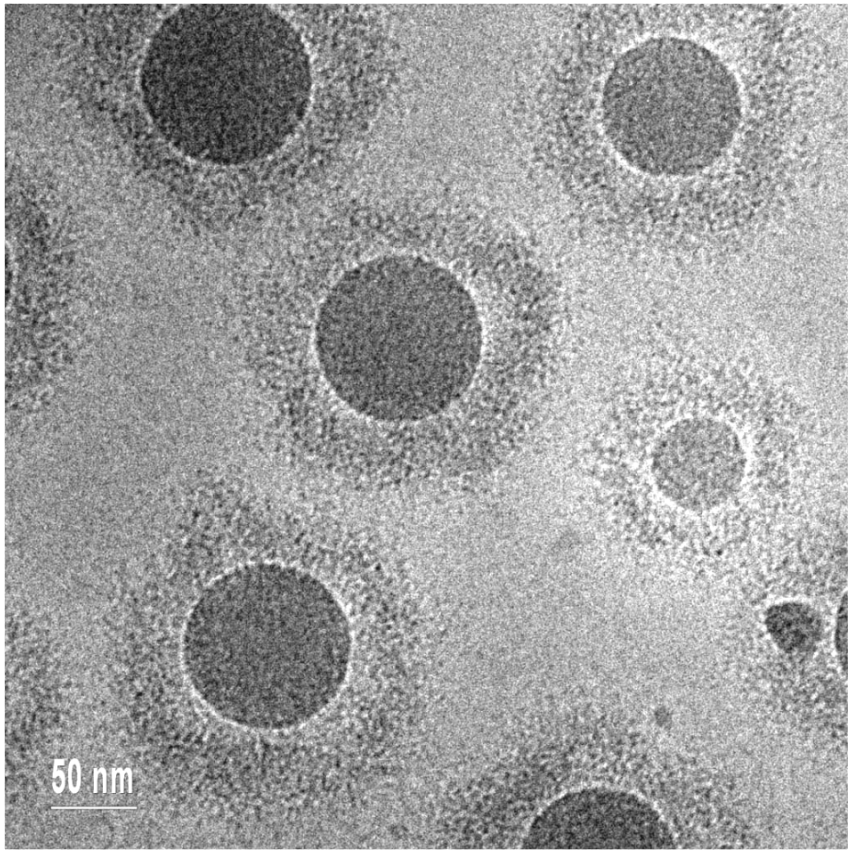


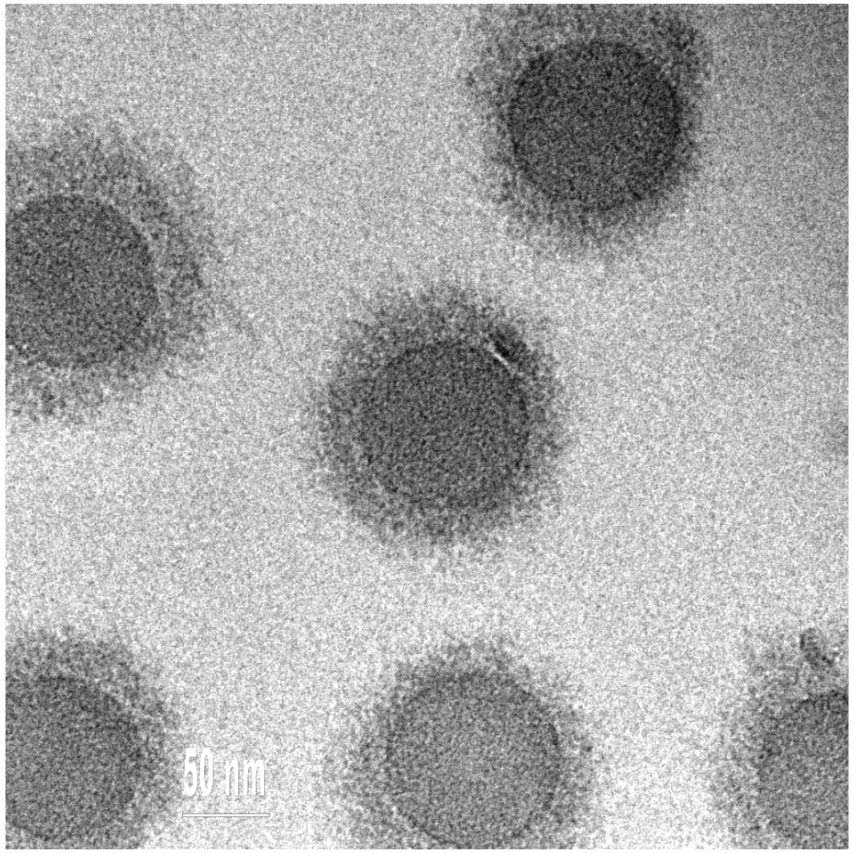
Doxil® particles (courtesy of Prof. Chezy Barenholz); Dr. N. Koifman



(a) CCNI 1002 DWNTs in CSA, 10 ppm by weight, and **(b)** HiPco 187.5 SWNTs, 250 ppm by weight.

Kleinerman, Liberman et al., Langmuir (2017)





5 °C

45 °C

Particles of a solid core of poly(styrene), with a corona of crosslinked poly(N-isopropylacrylamide) (PNIPAM)

Crassous et al., Langmuir (2006)

Matthias Ballauff, Bayreuth

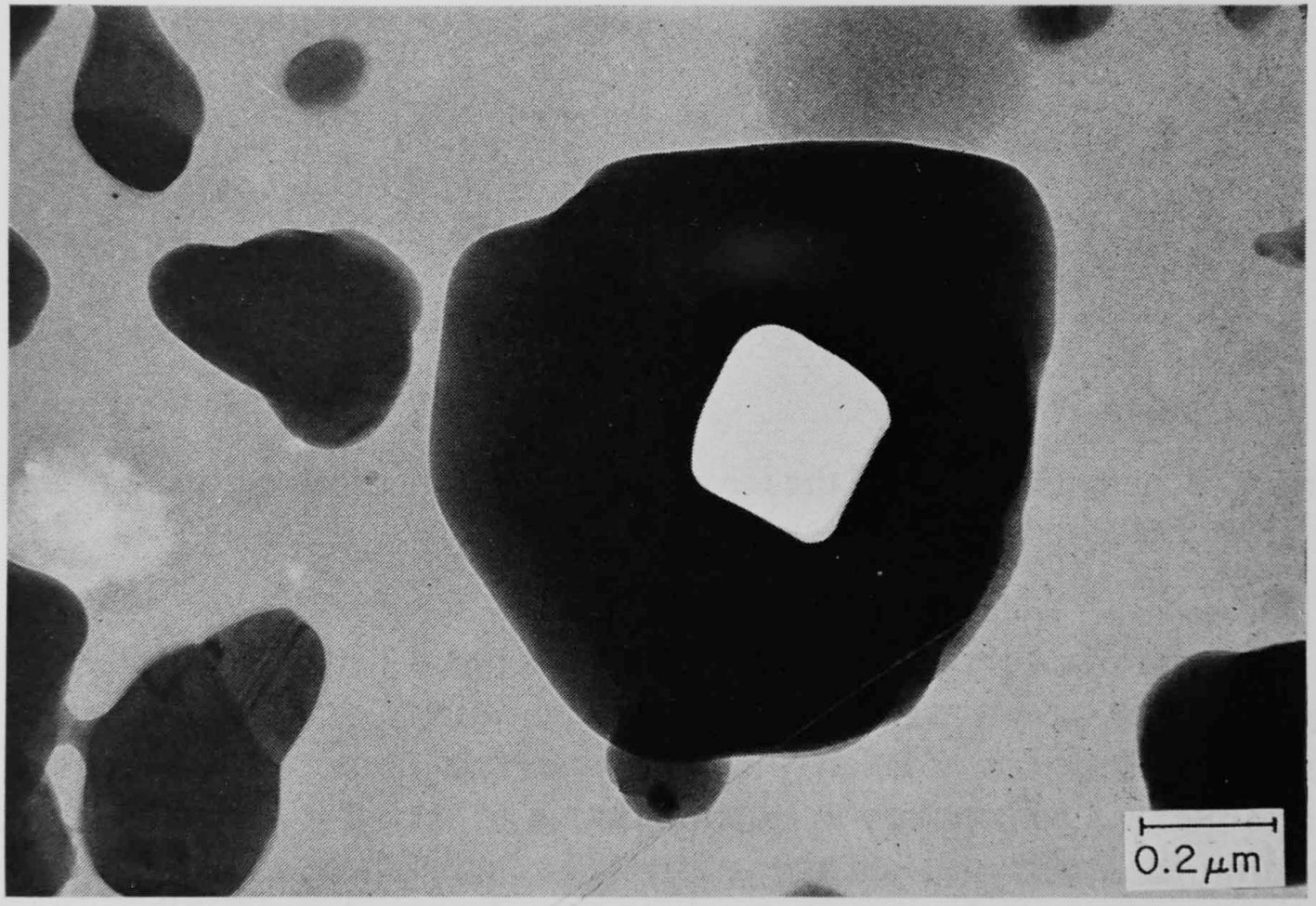
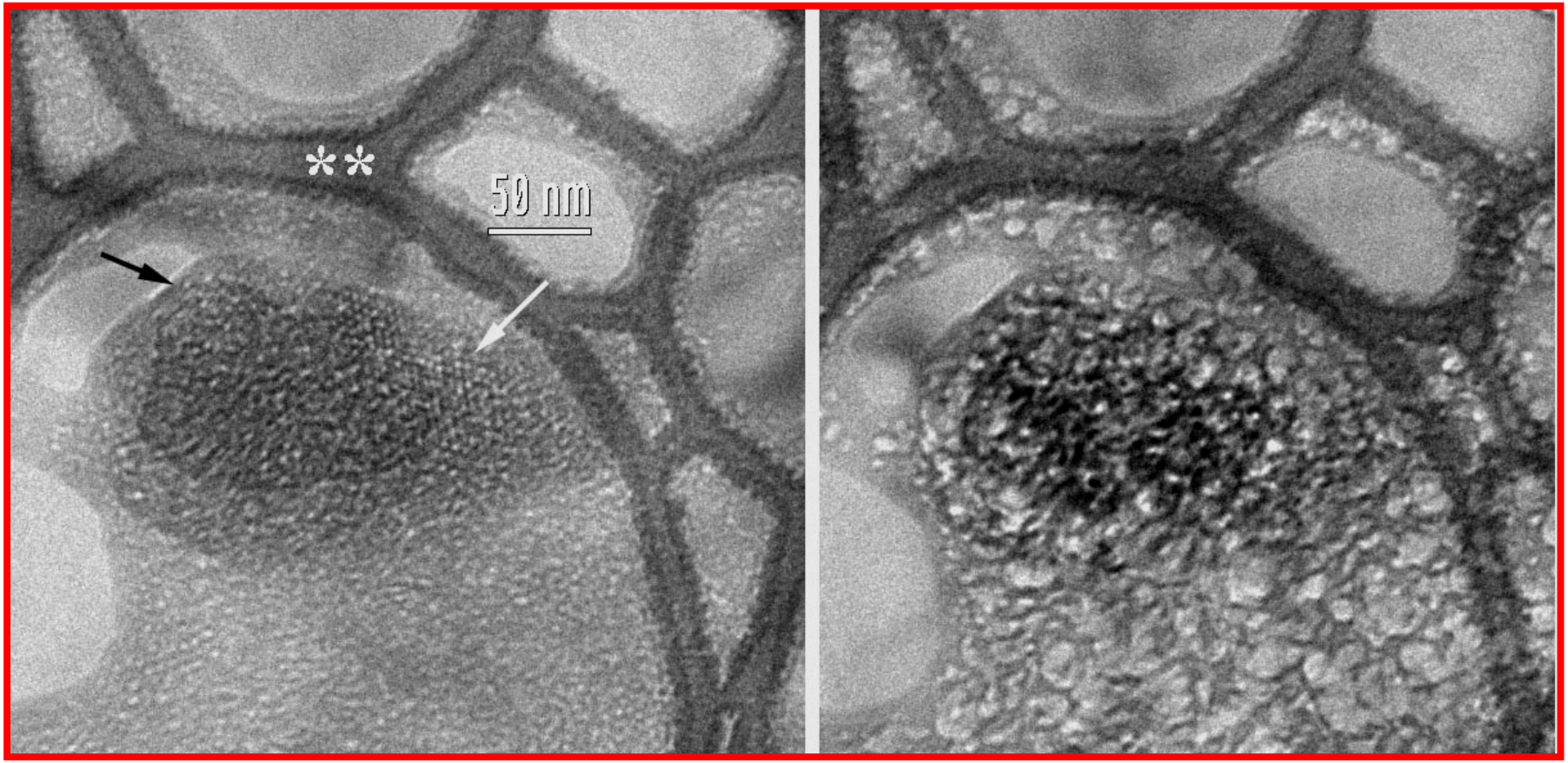
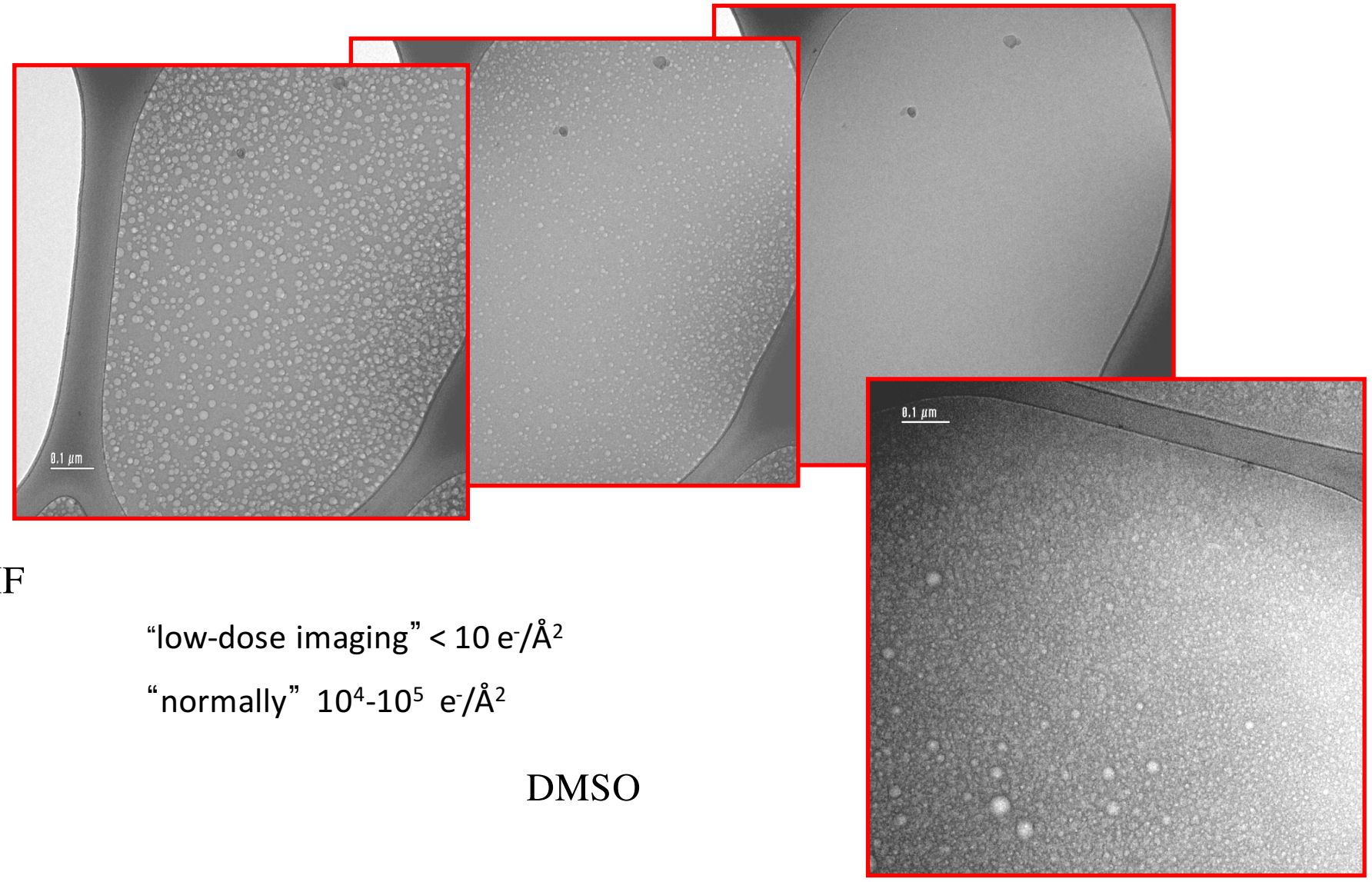


Fig. 6. A square hole etched in an ice crystal supported on a polyimide film (TEM micrograph). The etching was done by a $8{\cdot}8 \times 10^{-11}$ A electron beam in the STEM mode at CRT magnification of 40,000. The square area was scanned for 2 min.

Talmon et al., J Microscopy 1979



SDS/PDAC=0.738, 0.144% SDS, 0.1% PDAC



DMF

"low-dose imaging" < 10 $e^-/Å^2$

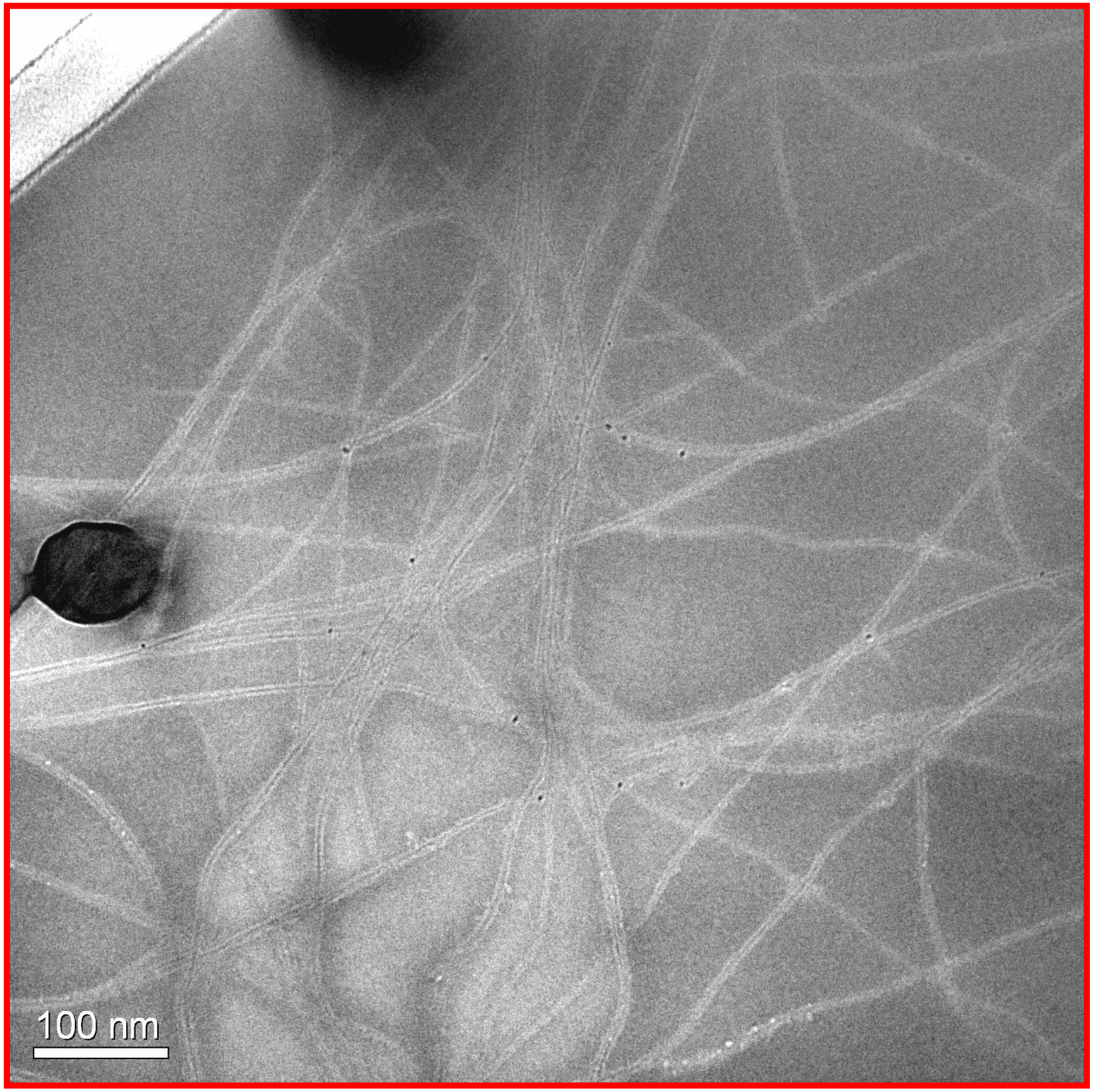
"normally" 10^4-10^5 $e^-/Å^2$

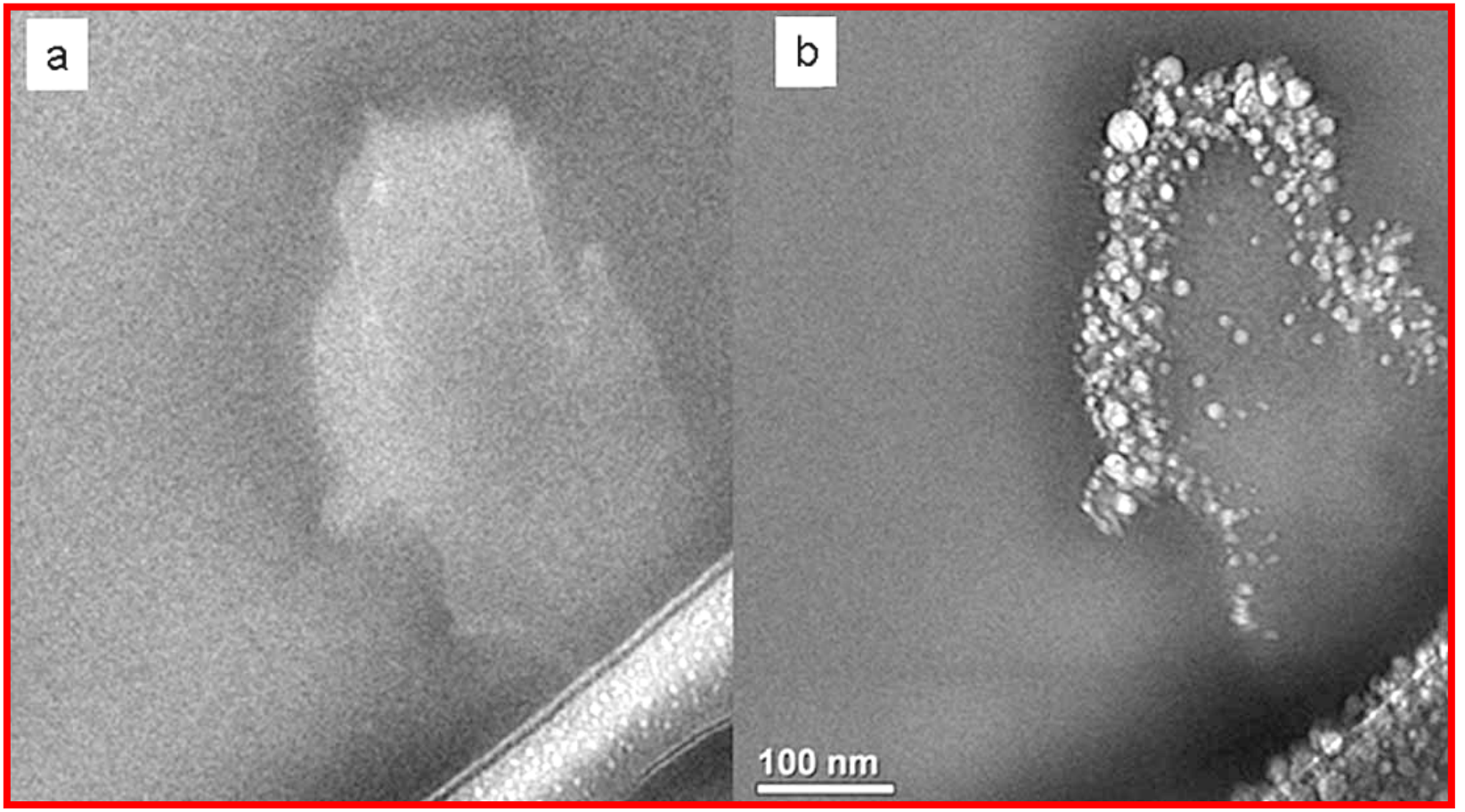
DMSO

1500 ppm CNTs
in 100% chlorosullfonic acid

M. Pasquali, Rice Univ.

V.A. Davis et al.,
Nature Nanotechnology (2009)





Graphene flakes dispersed in chlorosulfonic acid:
(a) Exposure < 10 electrons/Å^2. (b) After additional exposure of 70 electrons/Å^2

N. Behabtu et al., Nature Nanotechnology (2010)

Radiolysis of water

$H_2O \rightarrow H_2O^{\bullet +} + e^-$

$H_2O^{\bullet +} + e^- \rightarrow H_2O^* \rightarrow H^\bullet + {}^\bullet OH$

$H_2O^{\bullet +} + H_2O \rightarrow H_3O^+ + {}^\bullet OH$

$H_3O^+ + e^- \rightarrow H^\bullet + H_2O$

${}^\bullet OH + H_2O \rightarrow {}^\bullet O^- + H_3O^+$

$\bullet OH + e^- \rightarrow OH^-$

$H_2O_2 + e^- \rightarrow \bullet OH + OH^-$

Other radiolysis reactions at high electron flux

$H\bullet + \bullet OH \rightarrow H_2O$

Recombination reaction

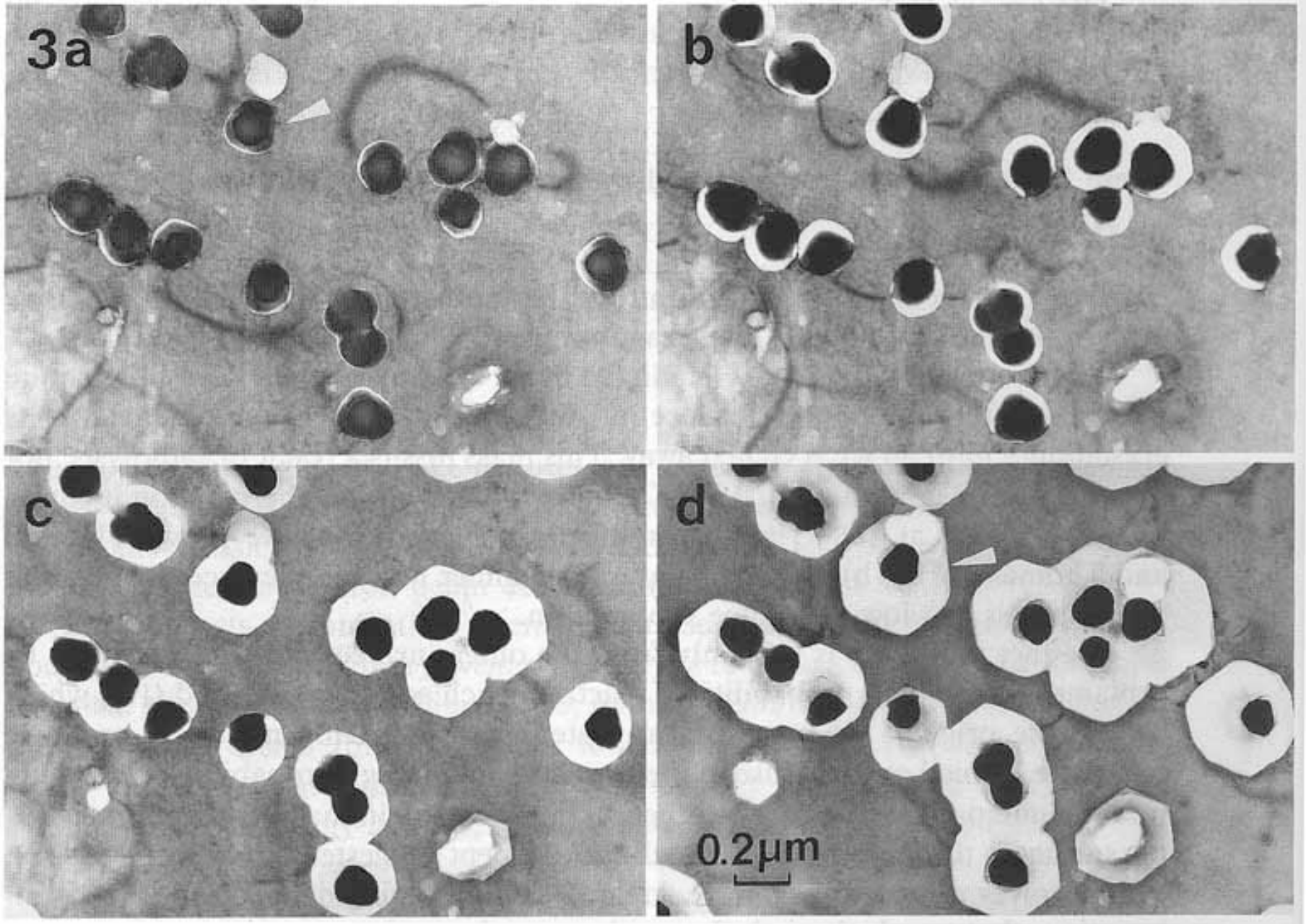
Effect of acceleration voltage

Electron mean-free-path increases with acceleration voltage,

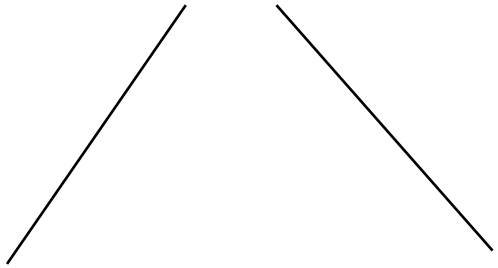
thus

E-beam radiation damage rate decreases with acceleration voltage

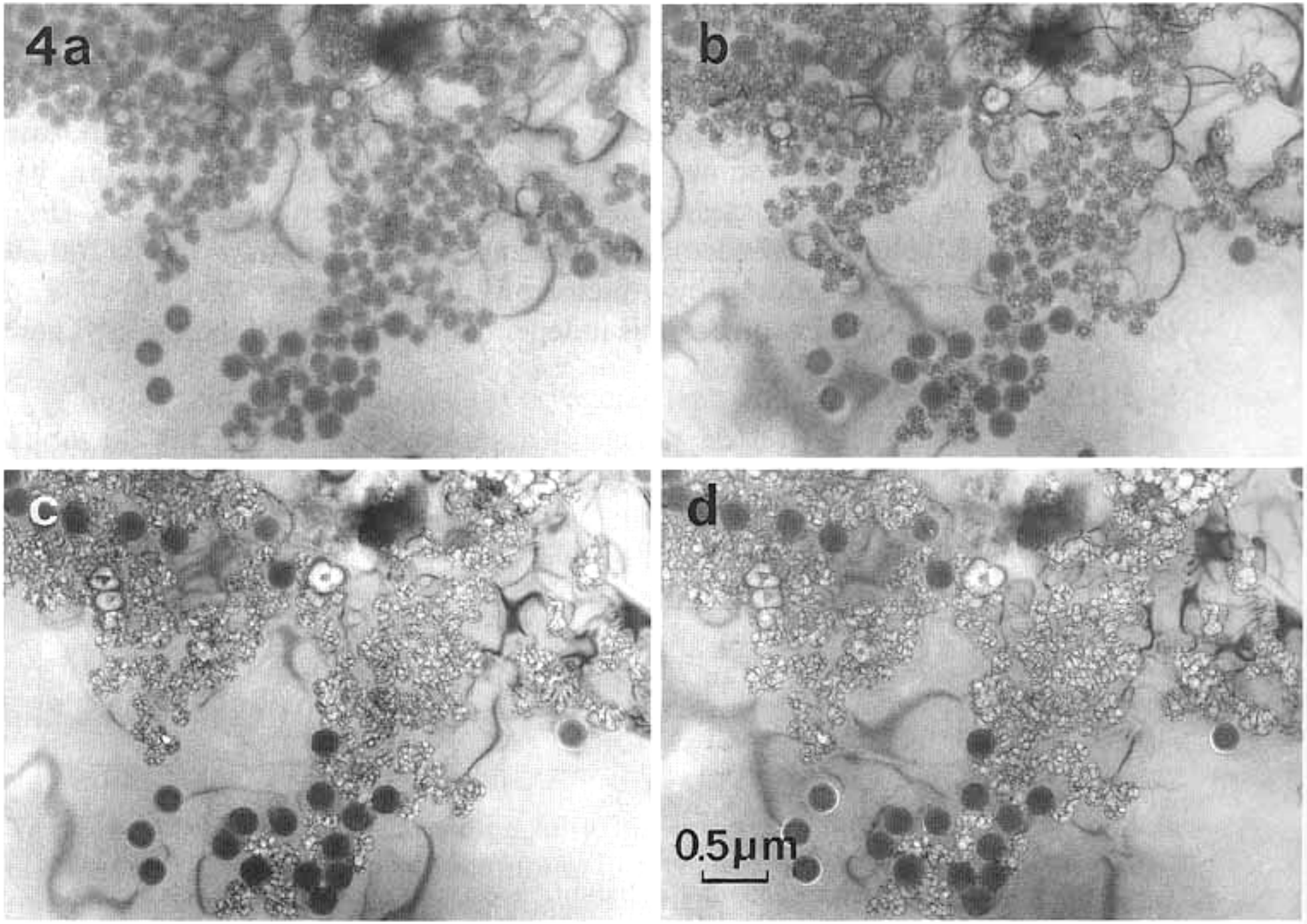
(but contrast decreases, too)

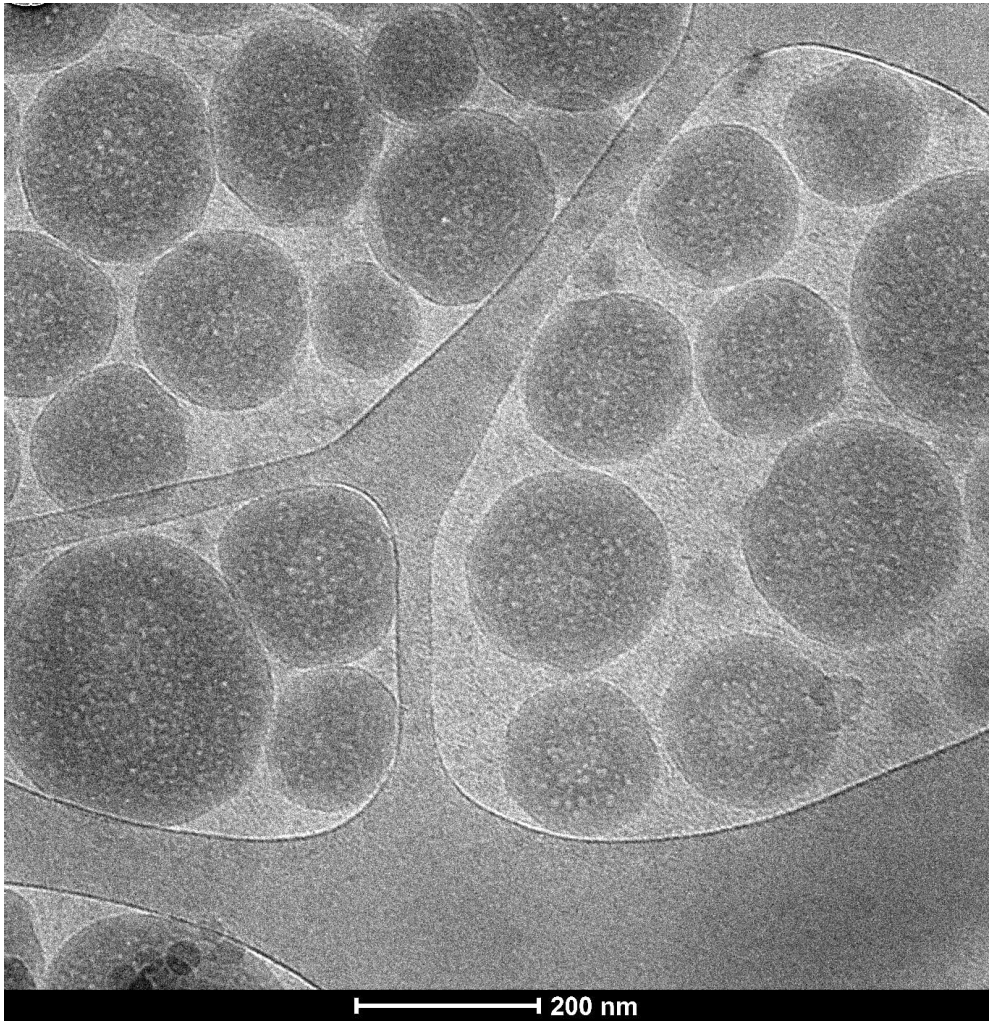

3a
b
c
d
0.2µm

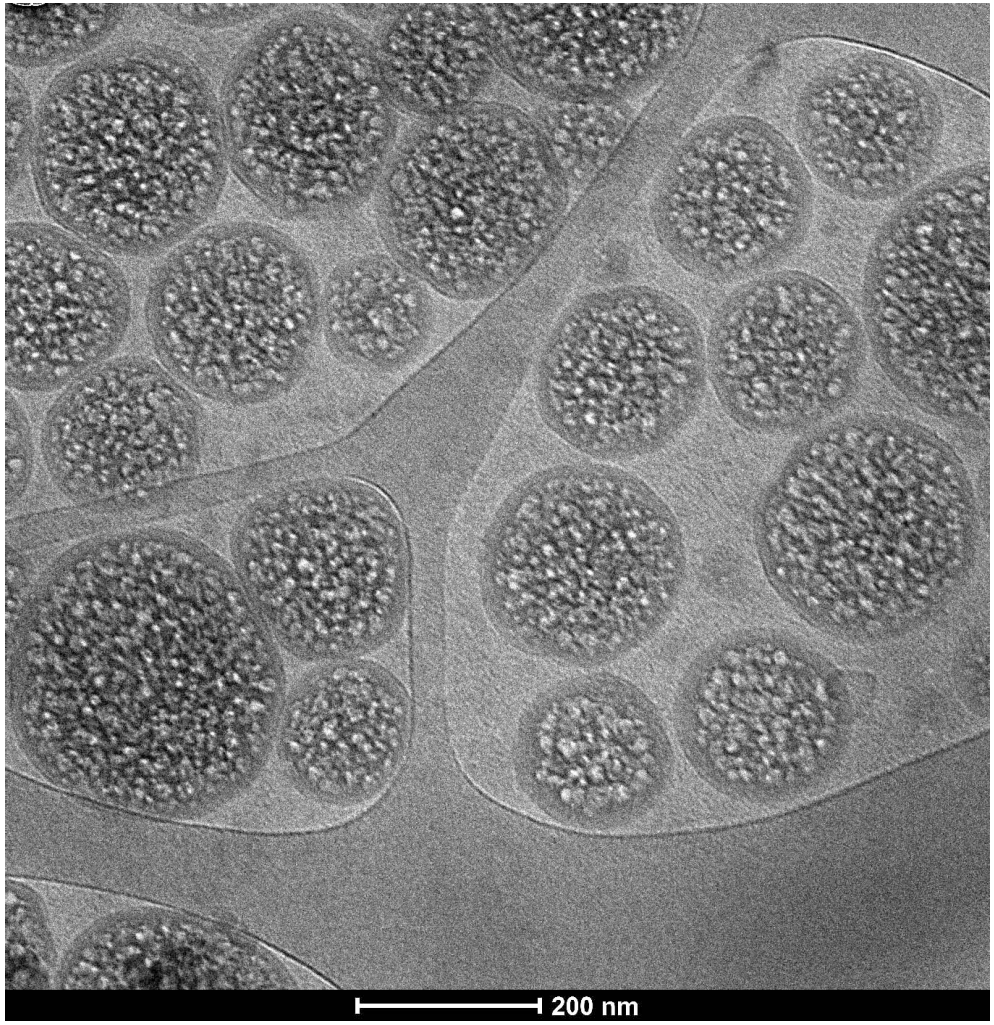
Soft materials under the electron beam



Scission-type	crosslinking-type
(ionization)	(polymerization)
e.g., PMMA	e.g., PS


4a
b
c
d
0.5µm





Cellulose-covered hexadecane drops in water: 10 $e^-/Å^2$ (left), 20 $e^-/Å^2$ (right)

Lucy Liberman