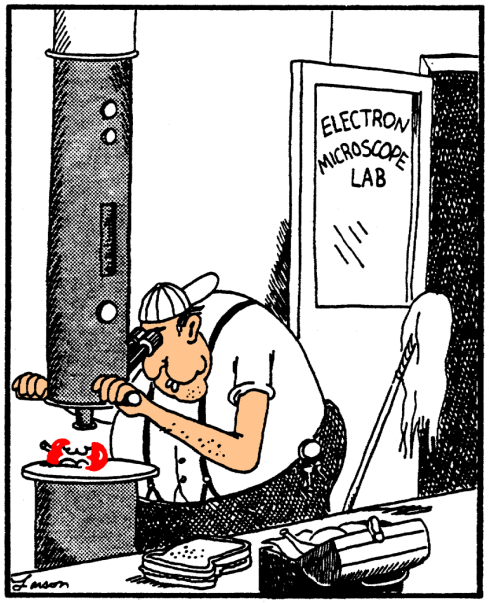
CHEM 165,265 / BIMM 162 / BGGN 262 Winter 2013

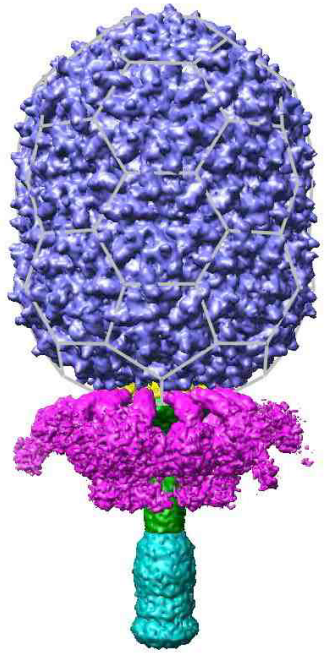
3D Electron Microscopy of Macromolecules



When: TuTh 8:00-9:20 AM
Jan 8 - Mar 14

Where: Peterson Hall 103

Who: Tim Baker
Hector Viadiu



EVERYONE (students and auditors)

Please provide requested info on **CLASS ROSTER** handout and return to T. Baker asap (today or Thursday)

CHEM 165,265 / BIMM 162 / BGGN 262 Winter 2013

3D Electron Microscopy of Macromolecules

PRIMARY GOAL

- Basic, theoretical understanding of the principles and practice of TEM and 3D image reconstruction

- Emphasis is on BIOLOGICAL specimens

CHEM 165,265 / BIMM 162 / BGGN 262 Winter 2013

3D Electron Microscopy of Macromolecules

Rule #1

CHEM 165 = CHEM 165,265 / BIMM 162 / BGGN 262

3D Electron Microscopy of Macromolecules

COURSE MATERIALS / HANDOUTS

- Class Roster (please check, fill in, and return to T. Baker asap)
- Basic Info and Syllabus (also posted on Web)

BASIC INFO AND SYLLABUS (2013)

LECTURERS: Timothy S. Baker, Natural Sciences NSB 4-105, 4-5845, tsb@ucsd.edu
Hector Viadiu, Natural Sciences NSB 4-316, 2-4486, viadiu@ucsd.edu

ADMIN. ASSIST.: Irene Acosta: NSB 4-103, 2-2514, riacosta@ucsd.edu

WHERE & WHEN: Peterson Hall 103; Tue/Thu 8:00-9:20 a.m.

COURSE GOALS

Gain a basic understanding of the principles of transmission electron microscopy (TEM) and three-dimensional (3D) image reconstruction as applied primarily to the study of biological macromolecules, macromolecular complexes, and small organelles and cells. Knowledge of these principles form a foundation for those who may be interested in obtaining practical experience and training in TEM and 3D reconstruction. Even if you never record a TEM image or compute a 3D reconstruction, the course should help broaden your knowledge about powerful, rapidly evolving tools of structural biology and enable you to critically evaluate research results reported in the scientific literature that rely on the use of these popular technologies.

COURSE FORMAT

1. Lectures on Tuesdays and Thursdays (8:00-9:20 am) from Jan 8 through Mar 14.
2. One or two optional lab "demos" *might* be offered outside of the normal class period (*e.g.* to tour a modern TEM facility) and will provide a means to earn some extra credit.
3. Optional, 1-hour recitation/help sessions will be held on some Fridays (time/place yet to be determined) where students can ask burning questions about lectures or optional homework assignments and to help prepare for exams. This time might also be used to cover certain topics in more depth than possible in class, or that couldn't be covered in class but which are covered in required reading materials.

GRADING

Undergraduate students (those taking CHEM 165 or BIMM 162): There will be one midterm exam (100 pts), a comprehensive final exam (200 pts), and three, two-page written critiques (50 pts each) chosen from six different image processing topics. You may submit up to six critiques, but the final grade will be based on an un-weighted sum of the midterm and final exam plus the three highest scored critiques (for grand total of 450 possible points). There are **NO MAKE-UP EXAMS**.

Graduate students (those taking CHEM 265 or BGGN 262): The rules are essentially the same as for undergraduates except that graduate students must submit four and only four critiques. Hence, the final grade will be based on an un-weighted sum of the midterm and final exam plus the four critiques (for grand total of 500 possible points). In addition, the comprehensive final exam will include questions that differ from those given to undergraduate students and will also include more challenging versions of questions given to the undergraduates. There are **NO MAKE-UP EXAMS**.

CLASS HANDOUTS AND OTHER MATERIALS

Course reading material is posted on the class website at http://cryoem.ucsd.edu. Access to this and additional material requires a username and password provided during the first class meeting. Lecture notes include most of the illustrations that will be shown as Powerpoint presentations. Having access to this should minimize the need for frantic note taking and allow you to listen carefully and concentrate more on understanding the basic principles being presented.

Lecture notes, PowerPoint slides, optional homework, and other supplementary materials (e.g. reference lists) are available as PDF documents on the class website to help solidify your understanding of the topics being discussed. The lecture notes and select literature articles are **required** reading because it is impossible to cover materials in adequate depth during lectures alone.

3D Electron Microscopy of Macromolecules

COURSE FORMAT

19 lectures (80 min each)

Midterm exam: Feb 5 (100 pts.)

Final exam: Mar 21 (200 pts.)

3-4 two-page critiques: after Feb 15 (50 pts. each)

1-2 optional demos (extra credit pts.)

Several optional recitation help sessions (to review optional homework assignments and lectures)

3D Electron Microscopy of Macromolecules

SYLLABUS (Tentative)

Date(s)	Lec #	Topic(s)
Jan 8	1	Course introduction; Analogy between light microscopy and transmission electron microscopy
Jan 10	2	Electrons/waves/interference/resolution
Jan 15	3	Optics and electromagnetic lenses
Jan 17	4	Design of the TEM and lens aberrations (Top to bottom description of instrument)
Jan 18		**Optional recitation session (laser demo; review of homework and lectures, etc.)**
Jan 22	5	Design of the TEM and lens aberrations (Continued)
Jan 24	6	Contrast and image formation (electron scattering)
Jan 25		**Optional recitation session (review of homework and lectures, etc.)**
Jan 29	7	Basics of TEM alignment, performance, operation, and image recording
		Other modes of TEM operation – self taught from notes §I.F
Jan 31	8	Overview of specimen preparation (emphasis on cryoTEM); Radiation effects
		Other biological specimen preparation methods – self taught from notes §II.A.2-II.A.5
Feb 1		**Optional recitation session (Help session to prep for midterm exam)**
Feb 5		**Midterm Exam (100pts) – Covers material through Jan 31 lecture**
Feb 7	9	Introduction to image analysis; Sources of noise
Feb 12	10	Crystals, symmetry and diffraction
Feb 14	11	Crystals, symmetry and diffraction (Continued)
Feb 19	12	Fourier processing techniques
Feb 21	13	Fourier processing techniques (Continued)
Feb 22		**Optional recitation session (review of homework and lectures, etc.)**
Feb 26	14	Principles of 3D image reconstruction
Feb 28	15	3D reconstruction of thin 2D crystals
Mar 5	16	3D reconstruction of helical assemblies
Mar 7	17	3D reconstruction of macromolecular complexes with icosahedral symmetry
Mar 8		**Optional recitation session (review of homework and lectures, etc.)**
Mar 12	18	3D reconstruction of macromolecular complexes with no symmetry
Mar 14	19	Electron cryo-tomography of unique specimens, organelles, and cells
Mar 15		**Optional recitation session (Help session to prep for final exam)**
Mar 21		**FINAL EXAM (200pts) – All inclusive, but focuses on 2nd half of course**

3D Electron Microscopy of Macromolecules

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3D Electron Microscopy of Macromolecules

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Accessing course materials on the Internet

Handout presented in class

Class Web Page: Jan 7, 2013

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Dr. Timothy S. Baker

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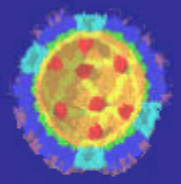
Cryoelectron Microscopy at UCSD

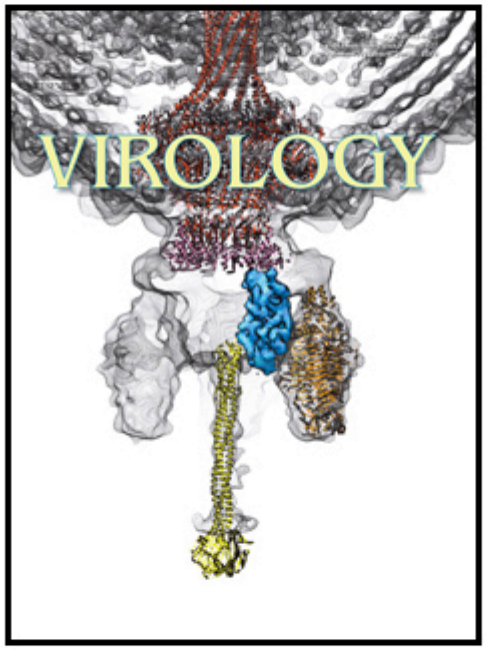
Contact | Site Map | Search | Position Openings



Depts. Chemistry, Biochemistry and Molecular Biology
MC-0378
La Jolla, CA 92093-0378

Structural Evolution of P22-like Phages

Parent, K. N., E. B. Gilcrease, S. R. Casjens, and T. S. Baker (2012). Structural evolution of the P22-like phages: Comparison of Sf6 and P22 procapsid and virion architectures. *Virology* **427:177-188.**
(PDF)



Produced by the Timothy S. Baker Cryoelectron Microscopy Laboratory
Main Address: Department of Chemistry and Biochemistry MC-0378,
University of California, San Diego,
9500 Gilman Dr., La Jolla, CA 92093-0378

Webmaster
Date Modified: October 12, 2012

Class Web Page: Jan 7, 2013

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CHEM 165,265 / BIMM 162 / BGGN 262 - 3D Electron Microscopy of Macromolecules
Winter Quarter 2013

→ Syllabus (PDF)

→ Book list (PDF)

→ Reference list (PDF)

Lecture Notes

→ • Sec. IA. Principles of the transmission electron microscope (9.2 MB)

Home | Contact | Site Map | Search | Calendar

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9500 Gilman Dr., La Jolla, CA 92093-0378

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Date Modified: January 7, 2013

3D Electron Microscopy of Macromolecules

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Most of the references listed here are in the **Science & Engineering** (S&E), the **Biomedical Library** (BML), at **Geisel East** (GE), or at **Scripps Institution of Oceanography** (SIO). Those highlighted in **red boldface** are good places to start. The Library call numbers are given at the end of each reference. "TSB" signifies that Professor T. S. Baker has a copy or copies that you may borrow temporarily if you can't find a copy at UCSD or SIO.

ELECTRON MICROSCOPY AND OPTICS: GENERAL TEXTBOOKS

Electron Microscopy of Cells and Tissues. Vol. 1. Instrumentation and Techniques. F. S. Sjostrand. **1967.** Classic text on electron microscopy with emphasis on thin sectioning techniques. Is now outdated with regard to thin sectioning methods, but still provides a useful treatment of basic principles of transmission EM. (TSB)

Electron Microscopy: Principles and Techniques for Biologists. J. J. Bozzola and L. D. Russel. **1992, 1999**. Comprehensive, well-illustrated text of biological TEM and SEM and specimen preparation (mostly histological samples). (1992: SIO QH212.E4 B69 & TSB; 1999: BML QH 212.E4 B793 & TSB)

Introduction to Electron Microscopy. C. E. Hall. 1st ed. **1953**, 2nd ed. **1966, 1983**. Classic reference on electron optical theory and design. (1966: BML QH 205 H174i & TSB)

Introduction to Electron Microscopy. S. Wischnitzer. 1st ed. **1962**, 2nd ed. **1970**, 3rd ed. **1981**. Excellent survey of the principles and practice of electron microscopy. (1962: BML QH 205 W811i; 1970: BML QH 205 W811i & TSB; 1981: BML QH 212 E4 W811i & TSB)

Light and Electron Microscopy. E. M. Slayter and H. S. Slayter. **1992**. Basic text covering a wide range of topics including LM, TEM, STEM, SEM, STM, AFM, etc.. (BML QH 502.2 S631L & TSB)

Practical Electron Microscopy for Biologists. G. A. Meek. 1st ed. **1970**, 2nd ed. **1976**. Excellent general reference, more comprehensive than Wischnitzer. (1970: TSB; 1976: BML QH 212 E4 M494p & TSB)

Principles and Practice of Electron Microscope Operation. A. W. Agar, R. H. Alderson and D. Chescoe. In Practical Methods in Electron Microscopy, Vol. 2, **1976**, A. M. Glauert (ed.). Excellent coverage of most aspects of the operation of a TEM. (GE QH212.E4 P7 & TSB)

The Principles and Practice of Electron Microscopy. I. M. Watt. 1st ed. **1985, 1989,** 2nd ed. **1997**. Basic text covering TEM and SEM. (1985: TSB; 1997: BML QH 212 E4 W344p & TSB)

OTHER TEM BOOKS & ANNUAL REVIEWS (Alphabetical list)

Biomedical Electron Microscopy: Illustrated Methods and Interpretations. A. B. Maunsbach and B. A. Afzelius. **1999.** Academic Press, San Diego. Lots of beautiful illustrations, mostly of histological samples, and includes practical tips. (GE QH212.E4 M38 & TSB)

Electron Microscopy of Proteins. Vols. 1-4, J. R. Harris (ed.); Vols. 5-6, J. R. Harris & R. W. Horne (eds.). Academic Press, London. (BML QU 55 E387 & TSB)

Vol	Date	Contents
1	1981	Haemocyanins; Nuclear envelope and nuclear pore complex; Intermediate filaments; Protein synthesis in prokaryotes and eukaryotes; Glycoproteins; Coated vesicles; Cilia and flagella
2	1982	Multienzyme complexes; Nonenzymic proteins; Bacterial appendages; Plasma lipoproteins; Fibrous proteins of connective tissue; HREM of unstained, hydrated protein crystals; Specialized membranes
3	1982	Algal cell walls; Bacterial cell walls and membranes; Chromatin and chromosomal proteins; Extracellular haemoglobins/chlorocruorins of annelids; Amyloid; Tubulin & tubulin associated proteins
4	1983	Actin and thin filaments; Myosin molecules, thick filaments and the actin-myosin complex; Erythrocyte membrane proteins; Plasma membrane intercellular junctions
5	1986	Bacteriophage T7; Bacteriophage morphogenesis; Crystalline arrays of adenovirus and their components; Influenza virus; Filamentous plant viruses; Human hepatitis viruses; Reoviruses; Immunoelectron microscopy of extracts of virus-infected plants; Structure and assembly of herpesviruses
6	1987	Freeze fracture of integral membrane proteins; Plasma membrane and cell wall of yeast; Microvillar membrane hydrolases of small intestine; Bacterial surface layers; Photosynthetic membranes and membrane proteins; Phycobilisomes and thylakoids; Sarcoplasmic recticulum

+ 2 more pages

3D Electron Microscopy of Macromolecules

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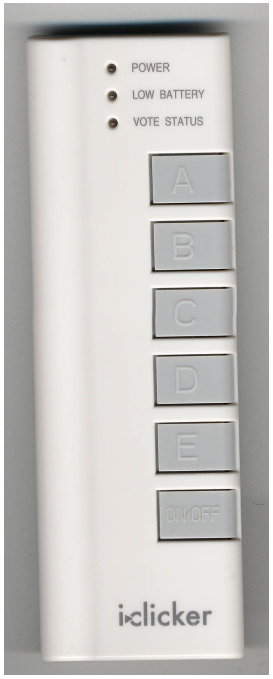
- Textbook List (also posted on Web)
- Reading References List (posted on Web)
- Lecture Notes (16 pages ***today***; all will be posted on Web)
- Powerpoint lectures (*will be* posted on Web)
- "Virtual Homework" questions (*will be* posted on Web)
- "Bottom Line" (*will be* posted on Web)
- "*p-Flasher*" (substitute for *i-Clickers*: please bring to every lecture)

i>clicker®


POWER
LOW BATTERY
VOTE STATUS
A
B
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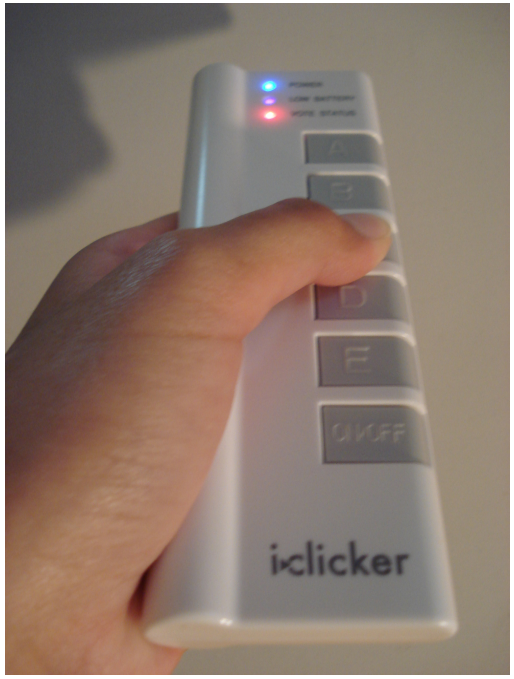
ON/OFF
i>clicker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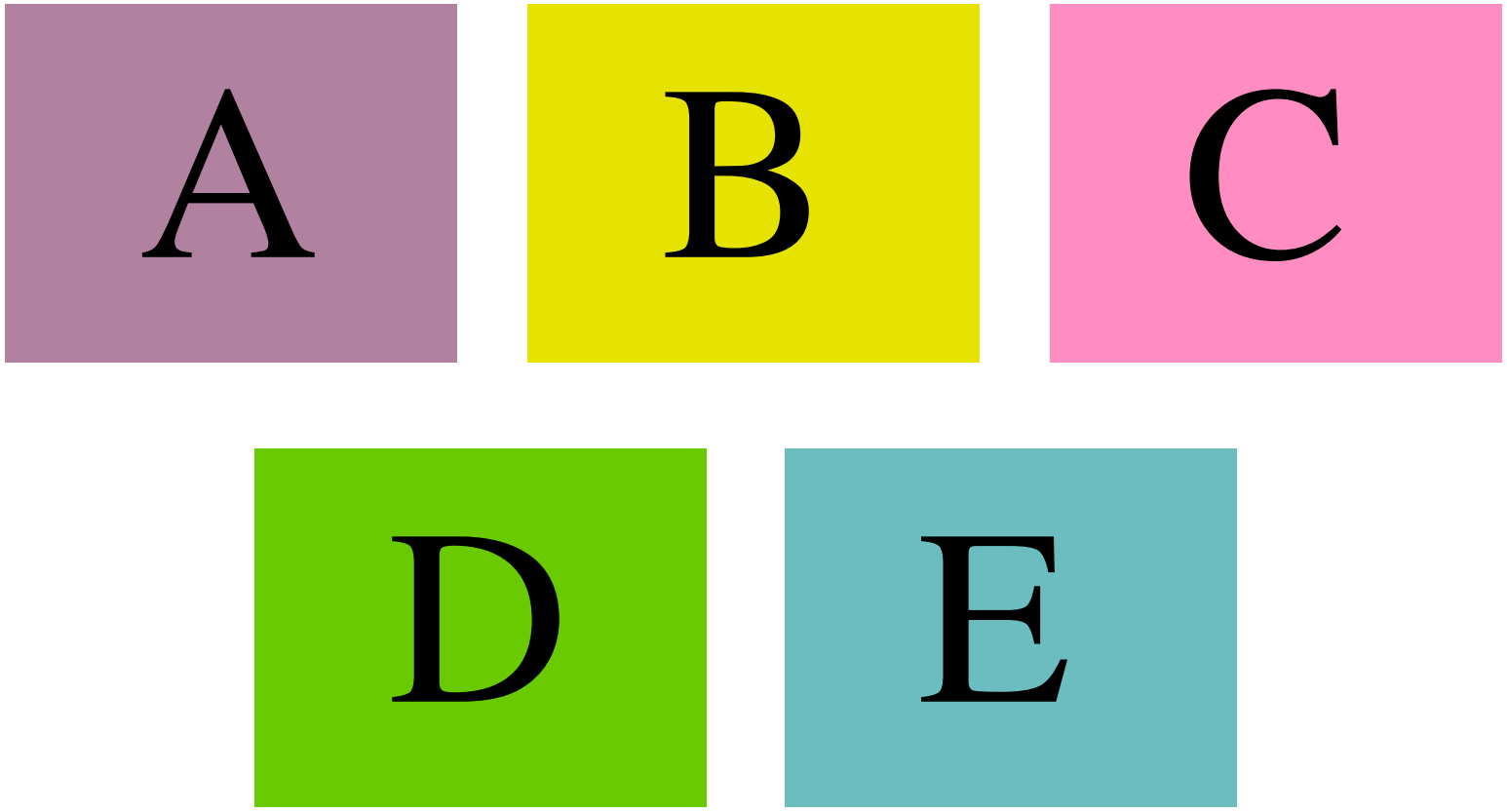
p-Flasher

(AKA the ‘poor man’s’ low-tech substitute)



3D Electron Microscopy of Macromolecules

LECTURES

- Concentrate on key concepts

- Get details from notes and supplementary reading

- Images, images, images!!!

3D Electron Microscopy of Macromolecules

This course **IS NOT** about:

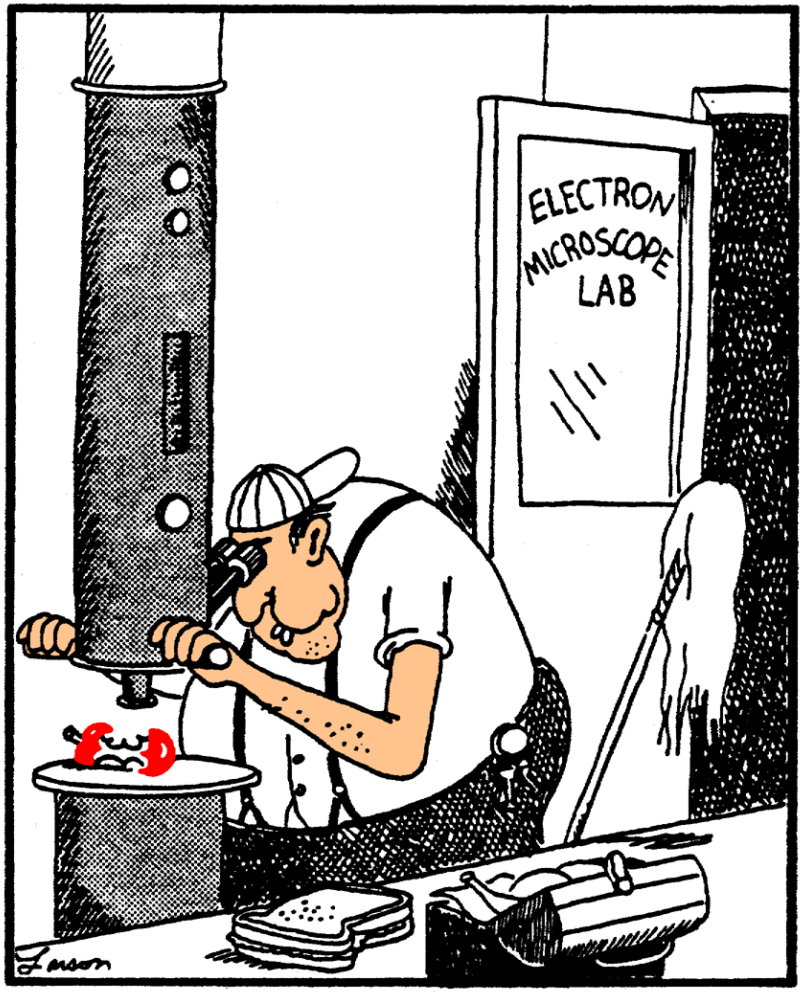
- SEM (Scanning Electron Microscopy)
- STEM (Scanning Transmission Electron Microscopy)
- AFM (Atomic Force Microscopy)
- STM (Scanning Tunneling Microscopy)
- SPM (Scanning Probe Microscopy)
- LM (Light Microscopy - or any related version)

3D Electron Microscopy of Macromolecules

This course **IS** about:

- TEM (Transmission Electron Microscopy)

Sounds simple, right?



BASIC DESIRES
(of any microscopist)

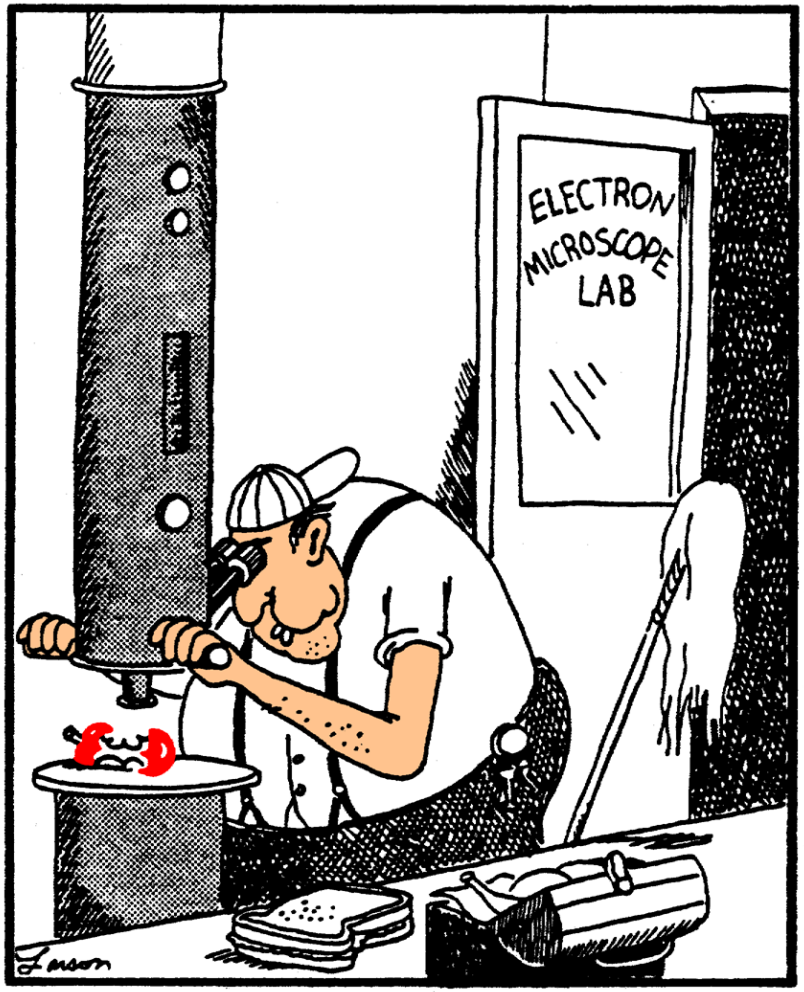
- See **magnified view** of object details

- Need **optical instrument** to get REALLY REALLY 'close' to object

- Need to **form AND record images**

- **TEM** specimens need **special preparation** for observation in 'harsh' environment

- Need **theoretical** and **practical** training to become a proficient 'expert'



TOPICS

- Principles of TEM
  - Electrons, lenses, and optics
- Design of TEM
  - Components top to bottom
- Contrast and image formation
  - Electron scattering from object
- Optimizing TEM performance
  - Alignment assures ‘best’ images
- Operation of TEM
  - “What do all these buttons do?”
- Other modes of TEM
  - Many ways to ‘observe’ specimens
- Specimen preparation for TEM
  - Getting the specimen ready
- Radiation damage
  - Less is better
- 3D reconstruction
  - Specimen 3D structure from 2D images

A few loose ends

- Lectures start promptly at 8:00 AM

- Equations: not necessary to memorize!
  You ***do*** need to understand them

- Practice questions often posted before 8 AM
  (some may appear on exams)

And then there is always…

…and other devices

TRIVIAL BUT IMPORTANT

$1\ nm = 1 \times 10^{-9}$ meter

1 nanometer = 1 billionth of a meter

TRIVIAL BUT IMPORTANT

1 nm = 1 x 10^{-9} meter

1 nm = 10 Å (Å = Angstrom)

1 Å = 0.1 nm

1 μm = 1 x 10^{-6} meter

1 micron = 1 millionth of a meter

TRIVIAL BUT IMPORTANT

1 nm = 1 x 10^{-9} meter

1 nm = 10 Å (Å = Angstrom)

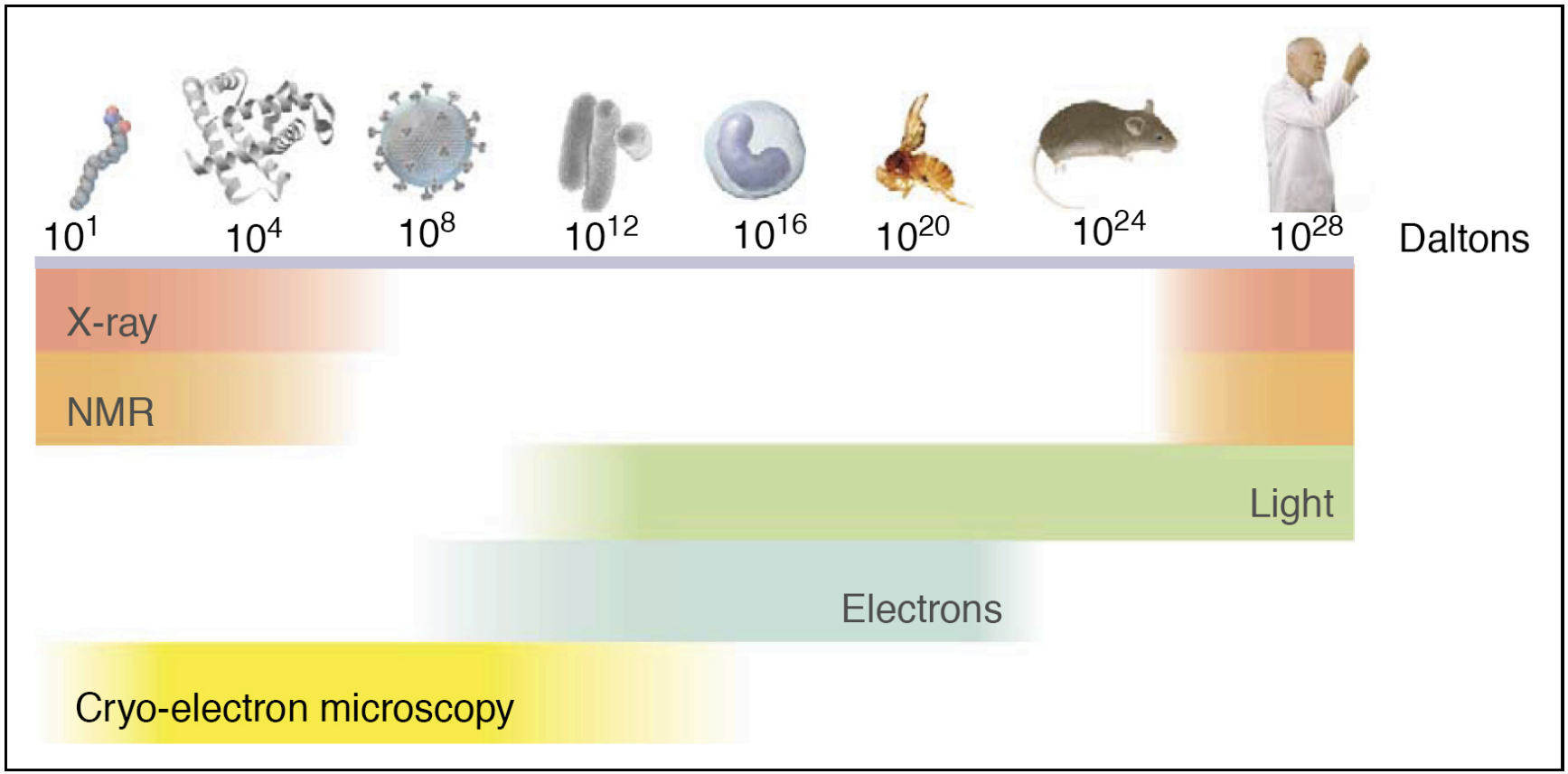
1 Å = 0.1 nm

1 μm = 1 x 10^{-6} meter

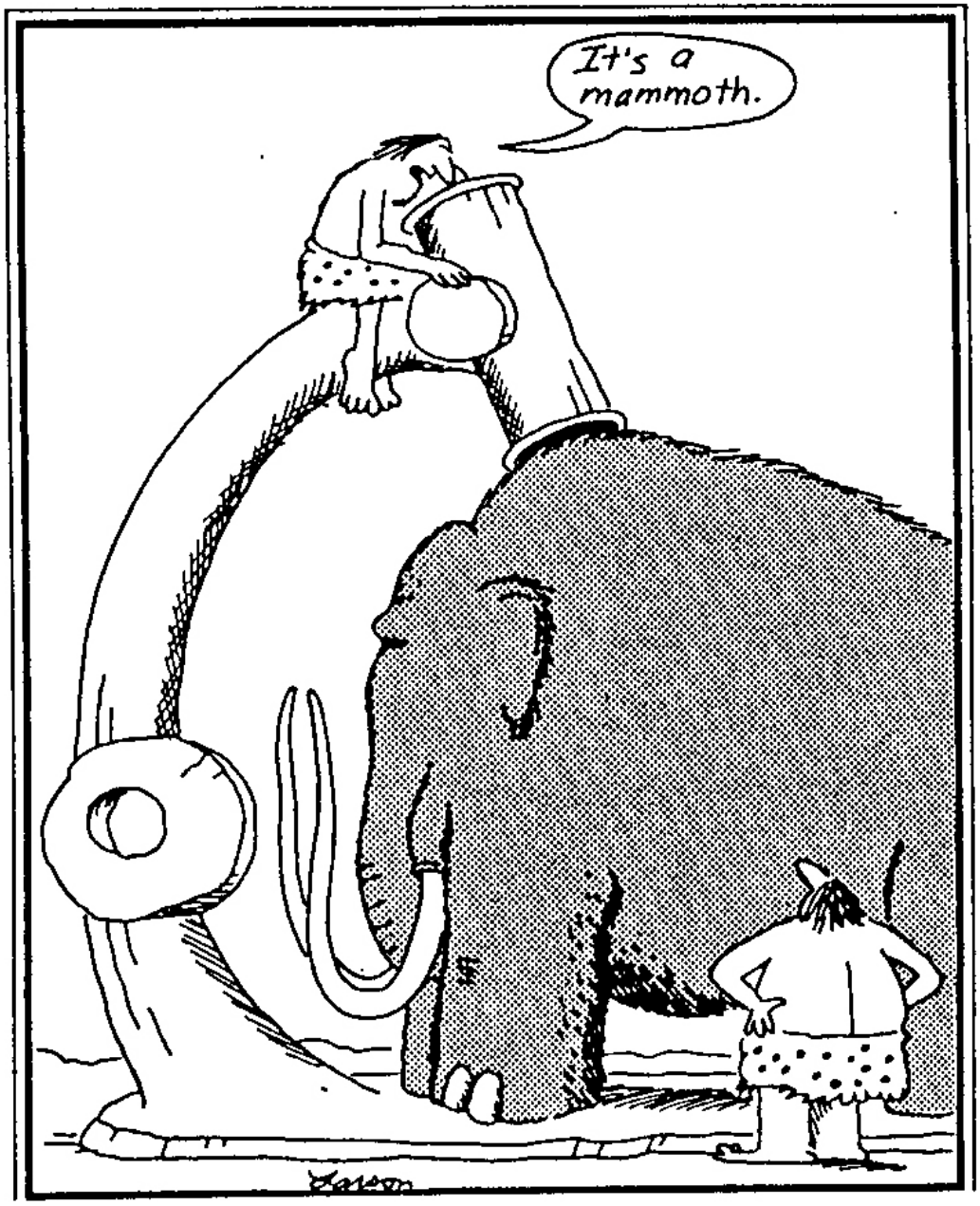
1 μm = 1000 nm

1 μm = 10,000 Å

Scale of Biological Structures



From Subramaniam (2005) Fig. 1, p.2



Early microscope

How small are macromolecules?

Scale: 2^0
1X

FEI Tecnai 30 Polara

Light microscope

Four happy men

1 meter

How small are macromolecules?

Scale: 2^1
2X

FEI Tecnai 30 Polara

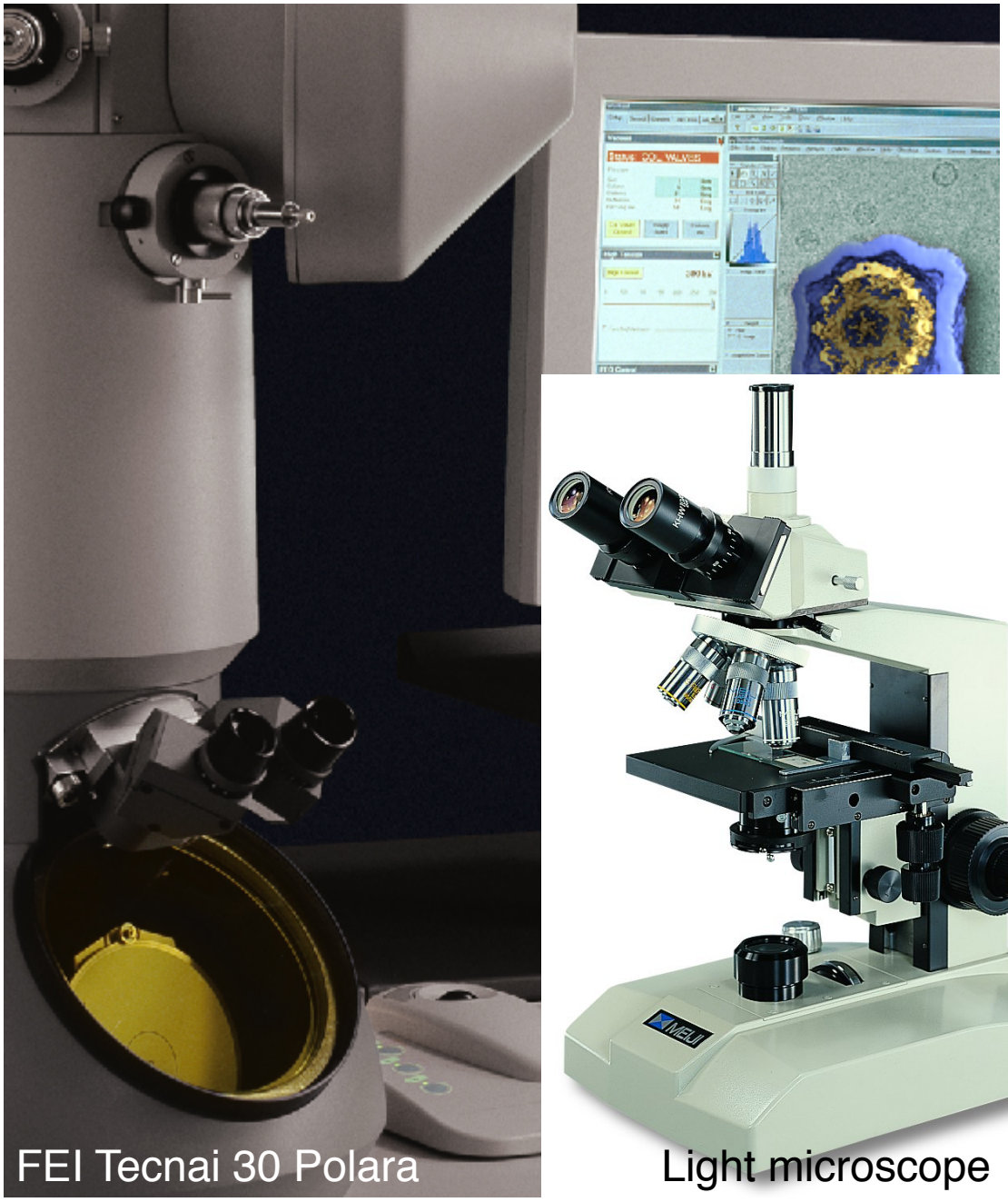
Light microscope

One man

500 mm

How small are macromolecules?

Scale: 2^2
4X



FEI Tecnai 30 Polara

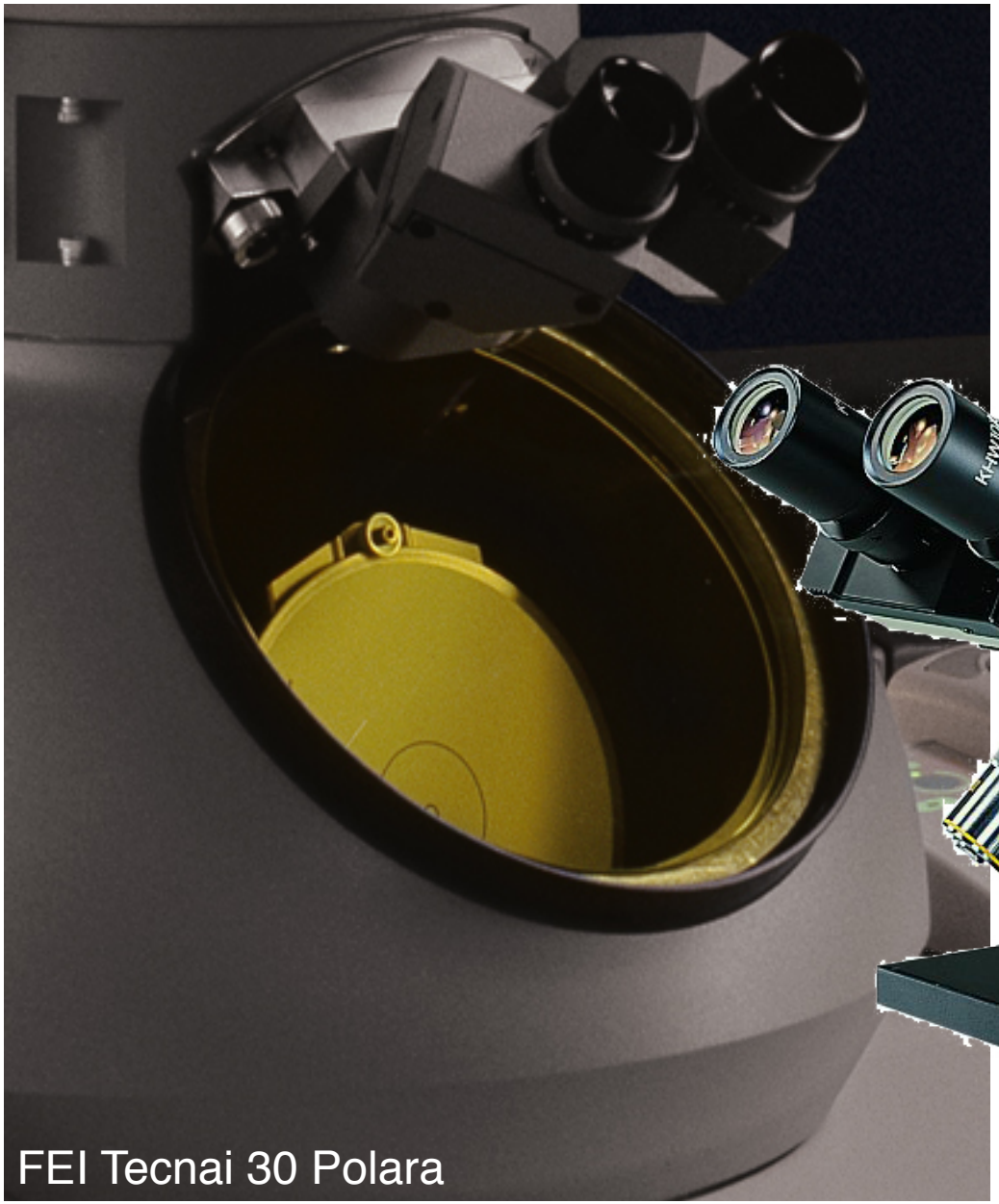
Light microscope

One man

200 mm

How small are macromolecules?

Scale: 2^3
8X



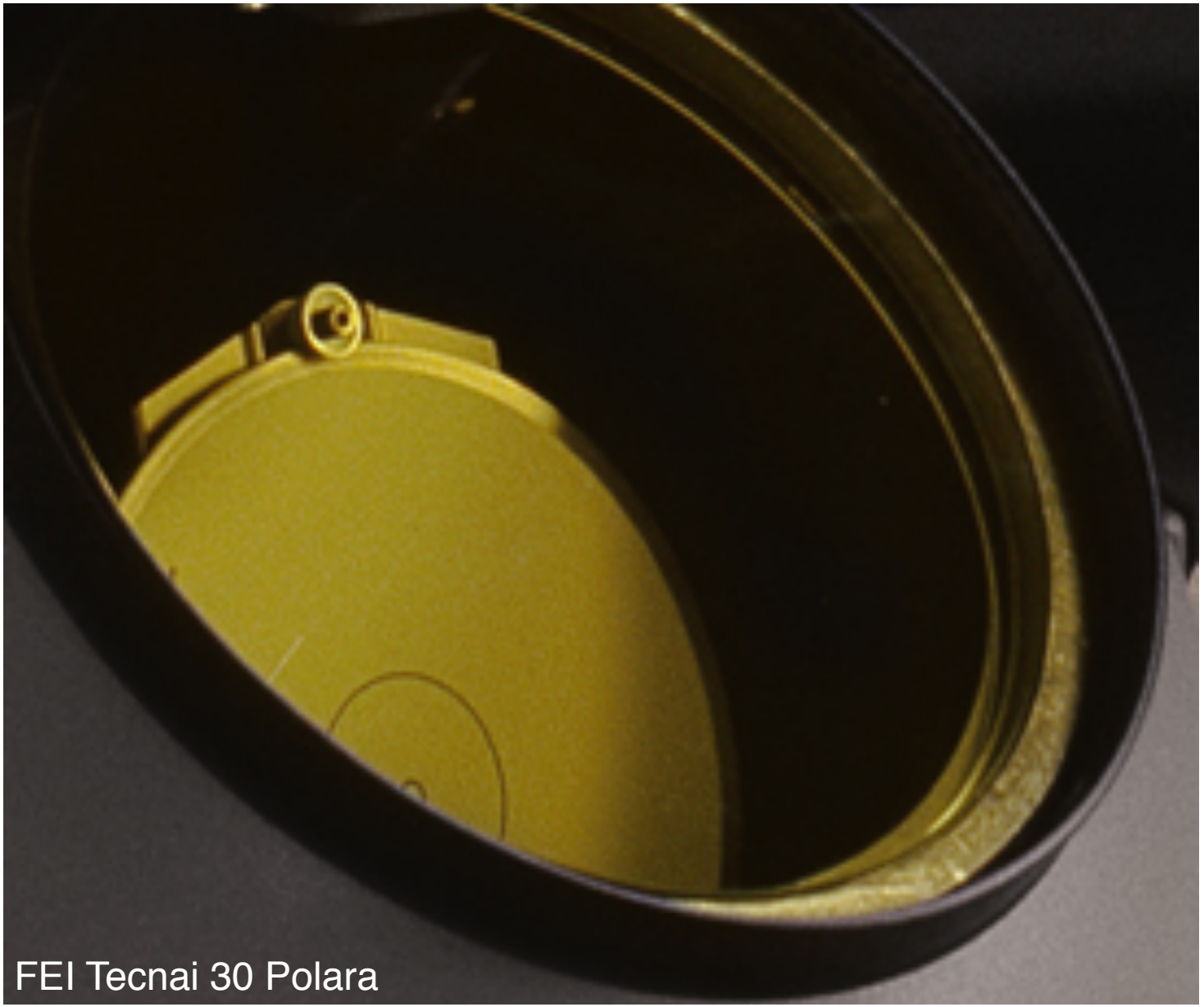
One man

Quarter

100 mm

How small are macromolecules?

Scale: 2^4
16X



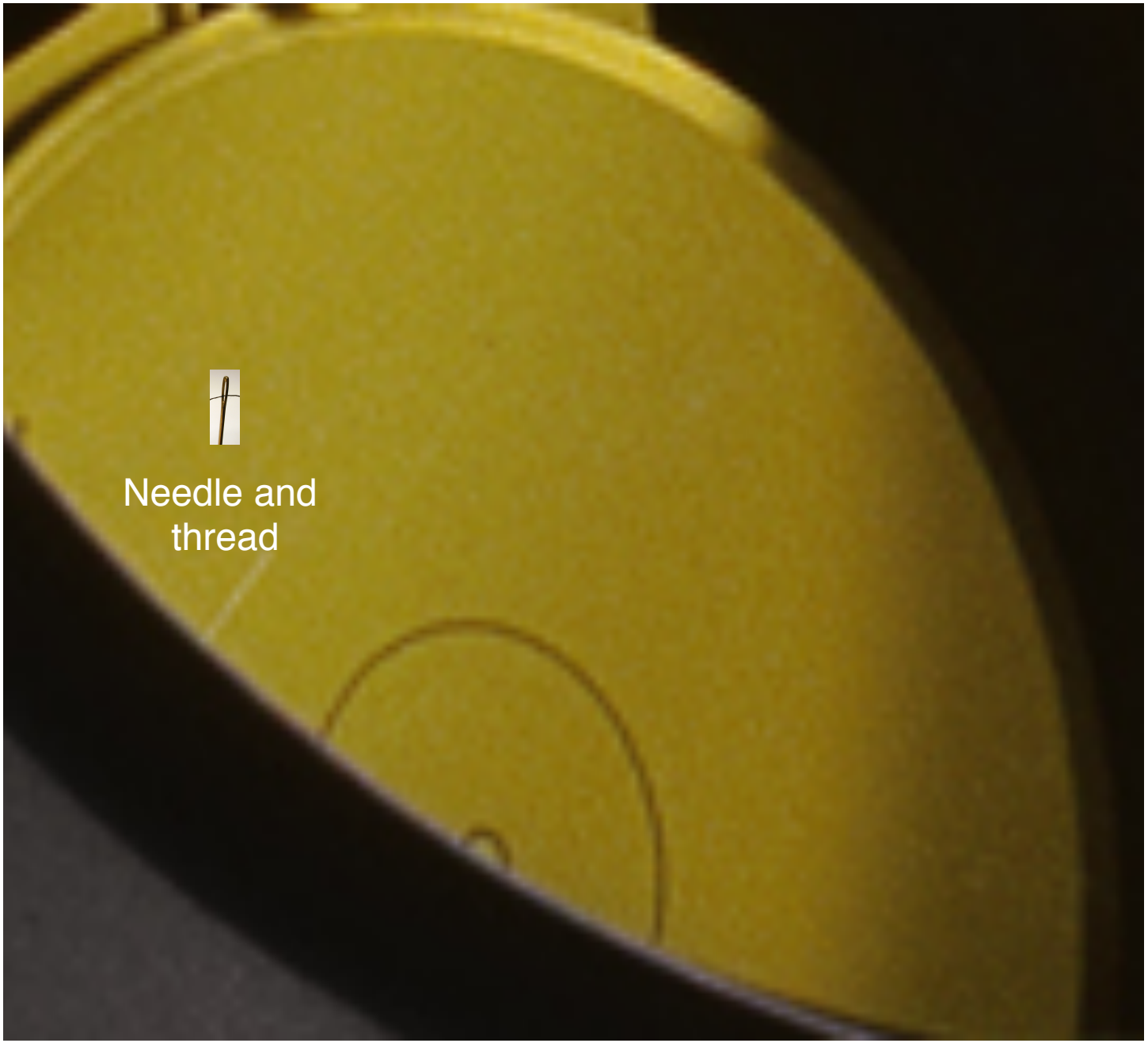
Quarter

House fly

50 mm

How small are macromolecules?

Scale: 2^5
32X



Quarter

House fly

20 mm

How small are macromolecules?

Scale: 2^6
64X

Needle and thread

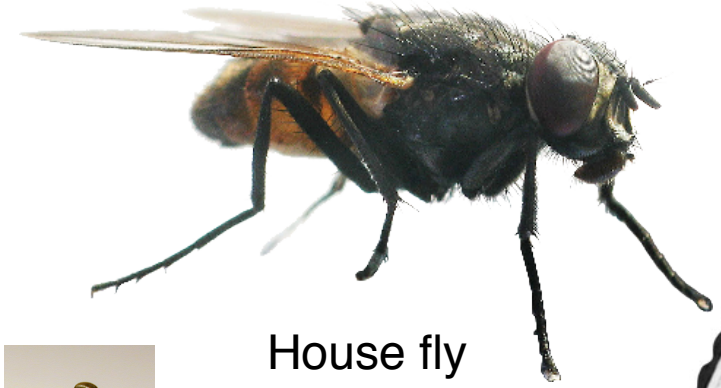
House fly

Quarter

10 mm

How small are macromolecules?

Scale: 2^7
128X



House fly

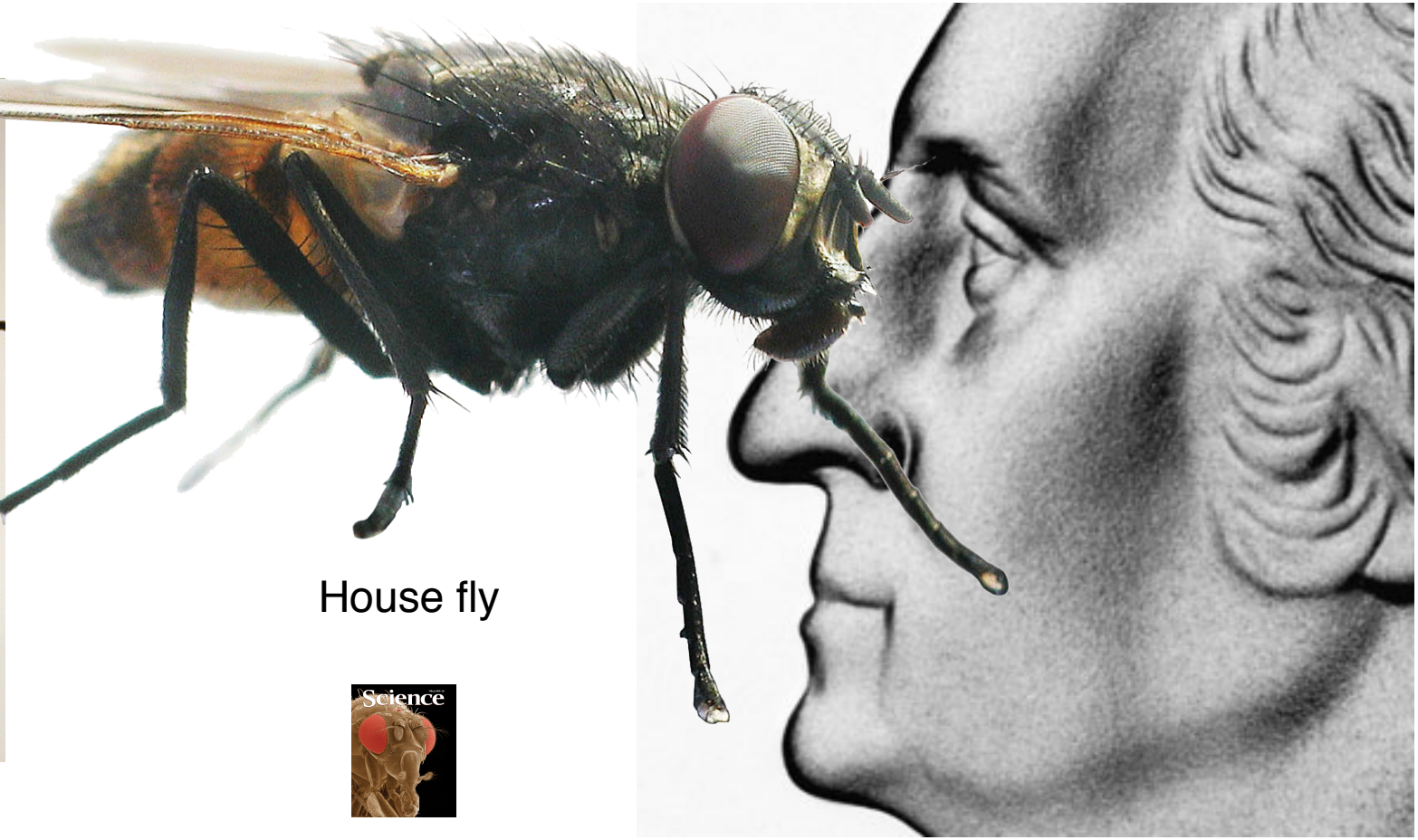
Needle and thread

5.0 mm

How small are macromolecules?

Scale: 2^8
256X

Needle and thread



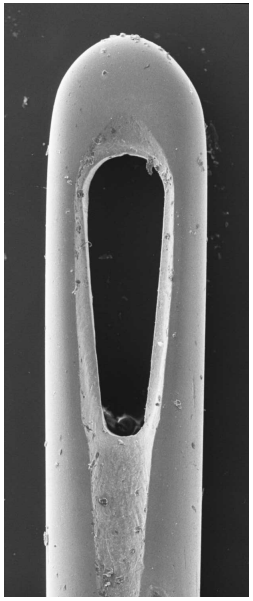
House fly

Drosophila

2.0 mm

How small are macromolecules?

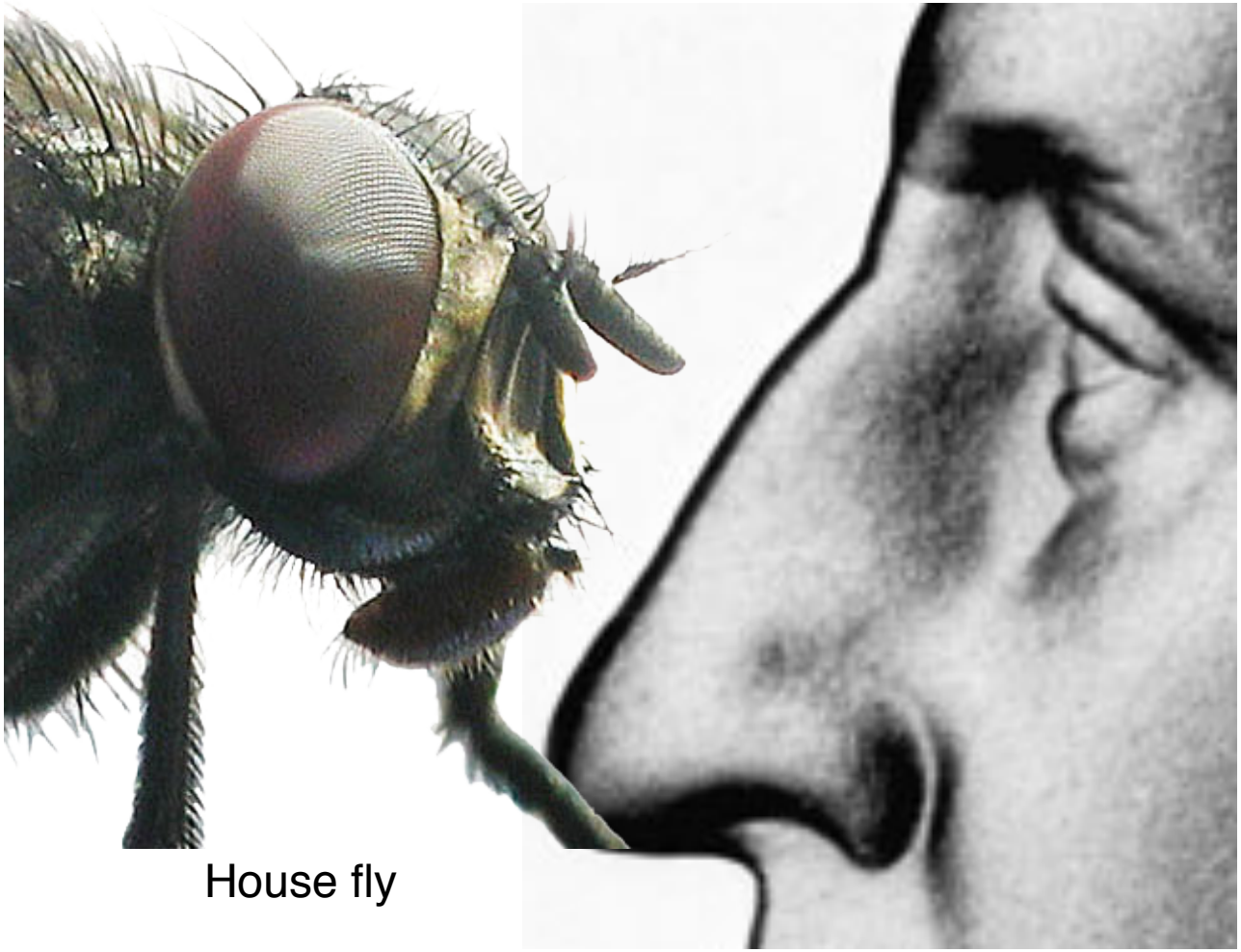
Scale: 2^9
512X



Eye of needle

Bed bug

Drosophila

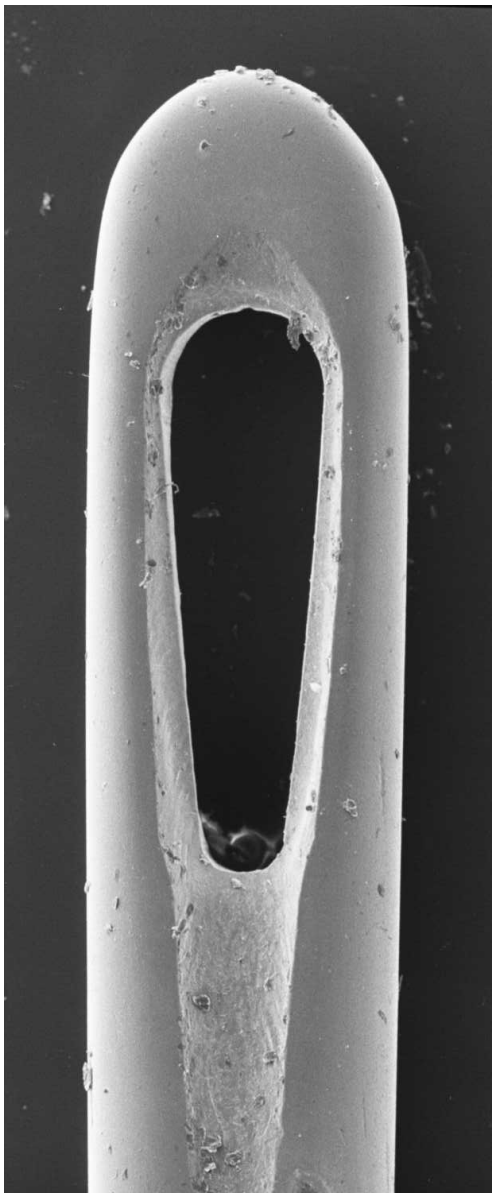


House fly

1.0 mm

How small are macromolecules?

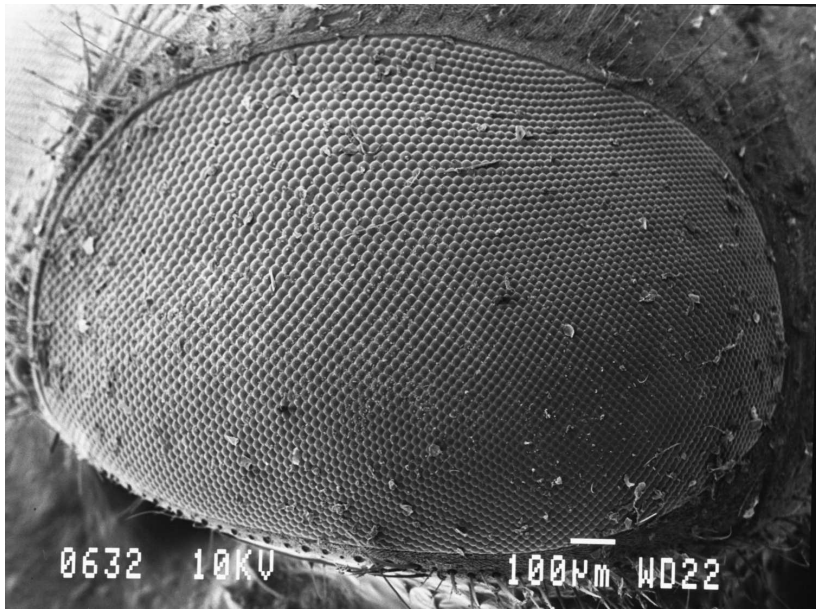
Scale: 2^{10}
1024X



Eye of needle

Bed bug

Drosophila

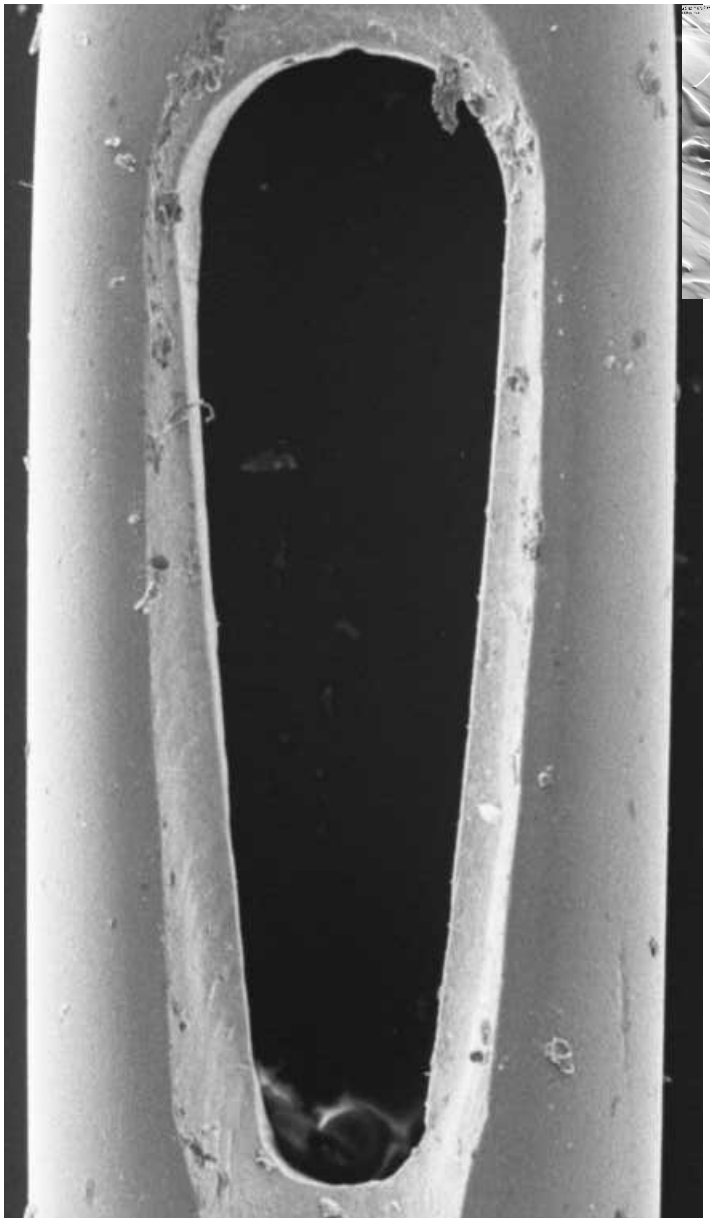


House fly eye

1.0 mm

How small are macromolecules?

Scale: 2^{11}
2048X



Eye of needle

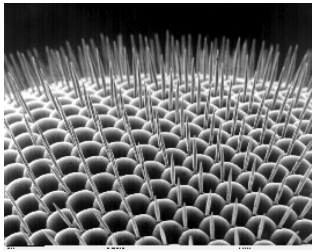
Bed bug

0.5 mm

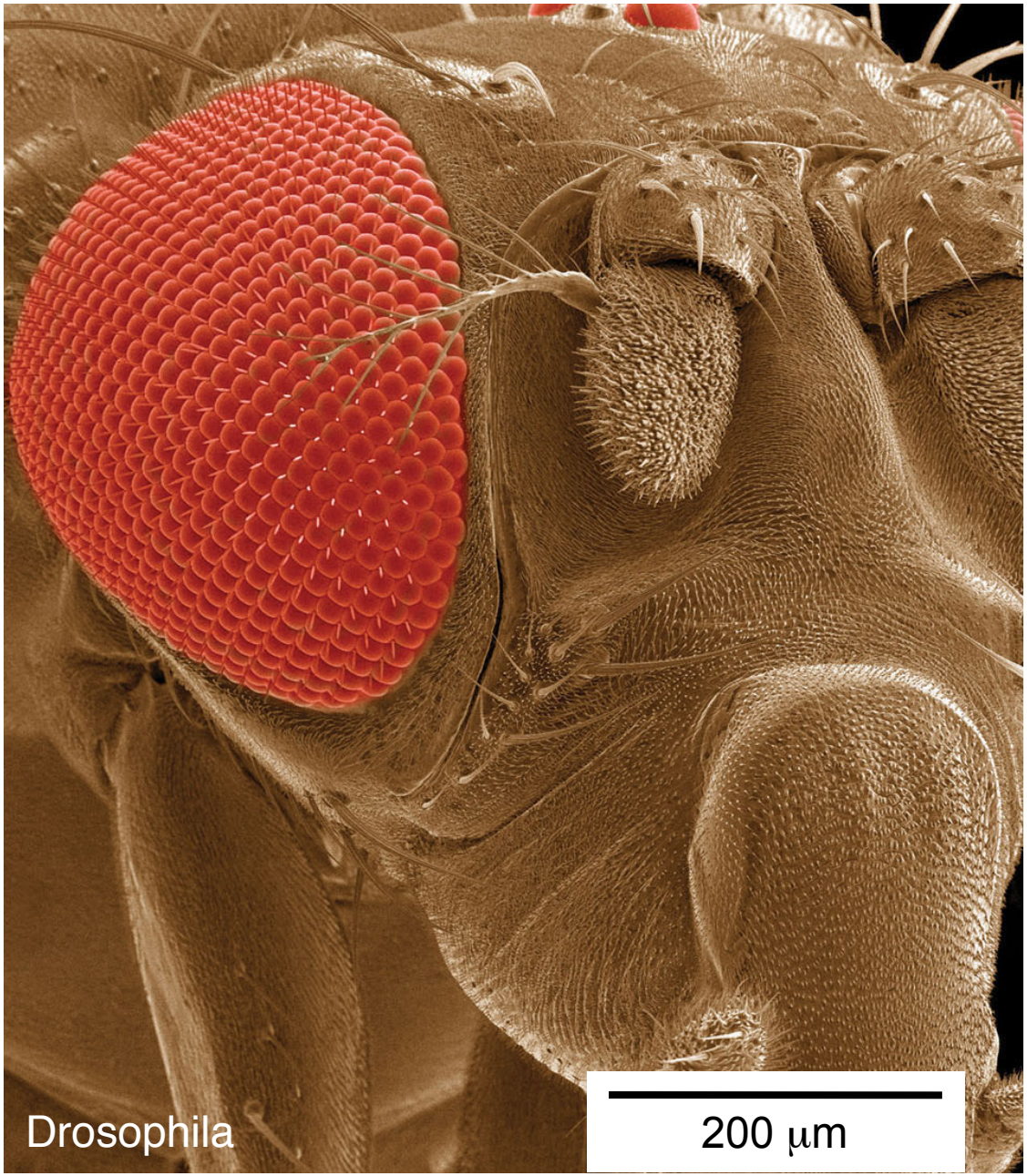
How small are macromolecules?

Scale: 2^{12}
4096X

Bed bug

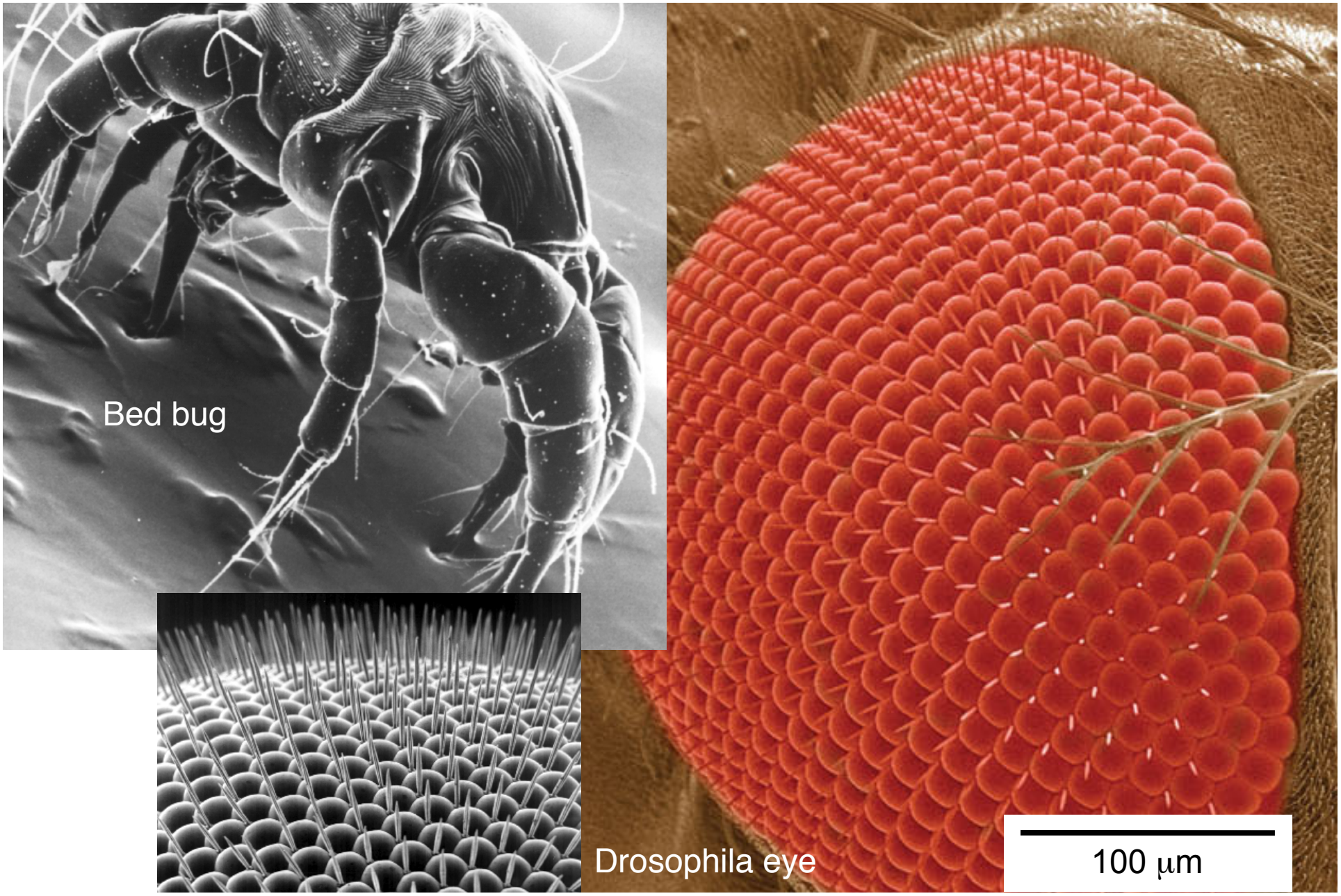


Drosophila eye



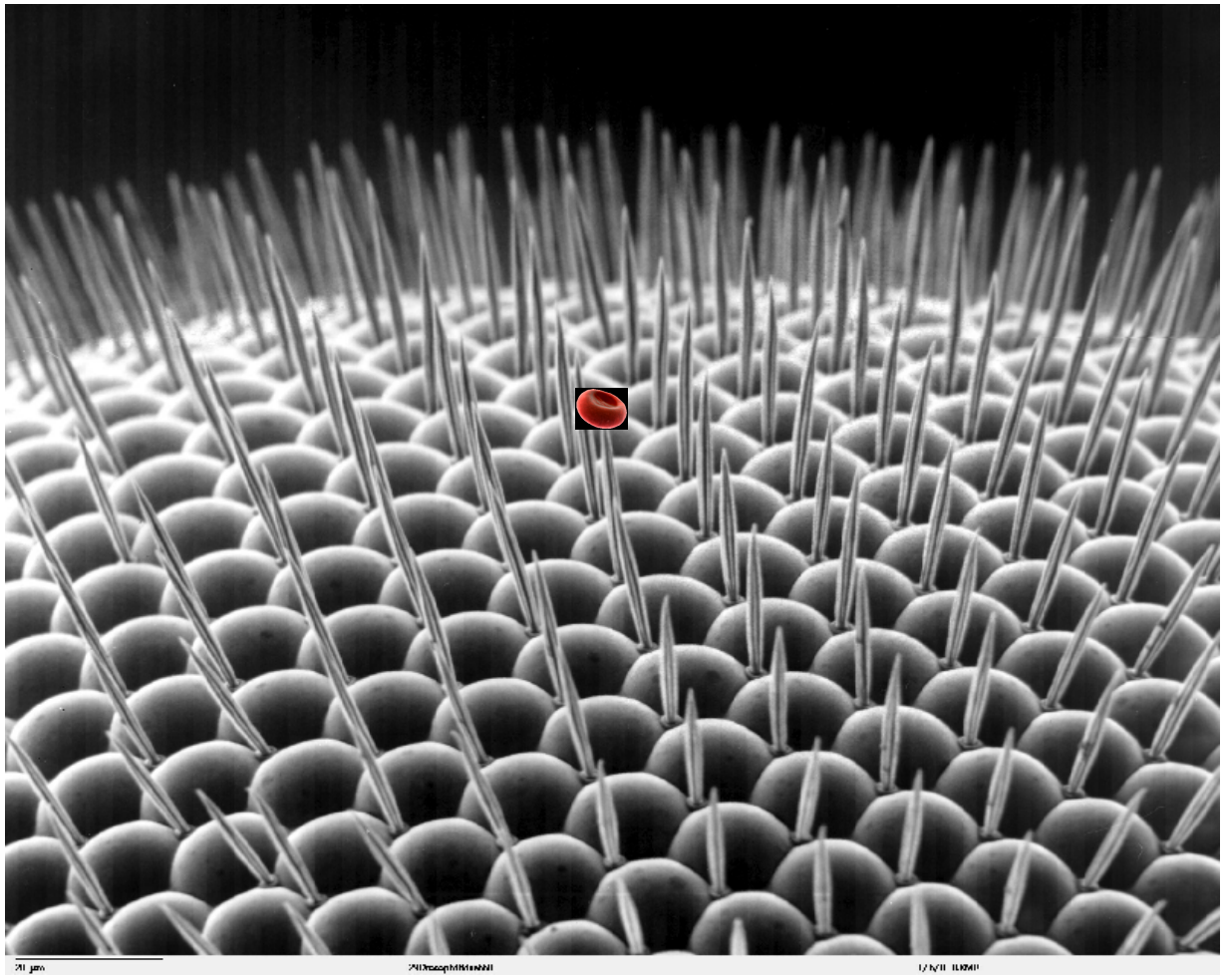
How small are macromolecules?

Scale: 2^{13}
8192X

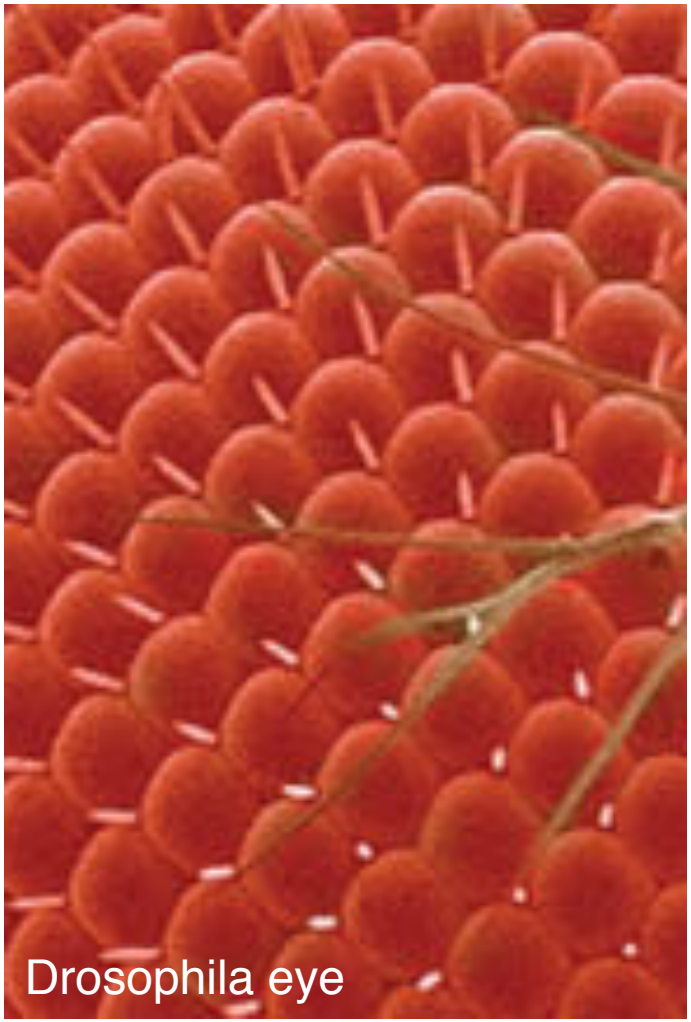


How small are macromolecules?

Scale: 2^{14}
16,384X



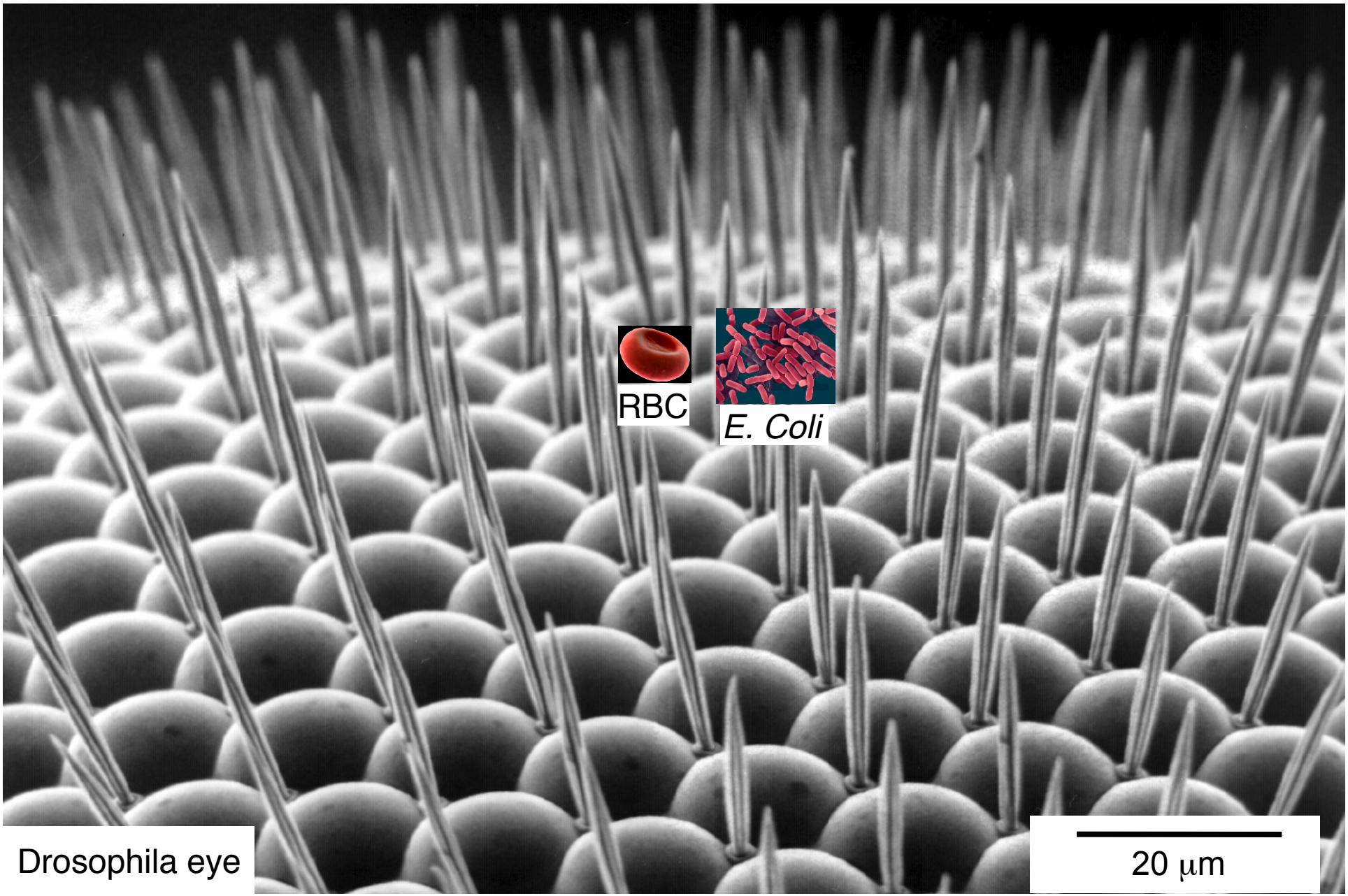
Drosophila eye



50 μm

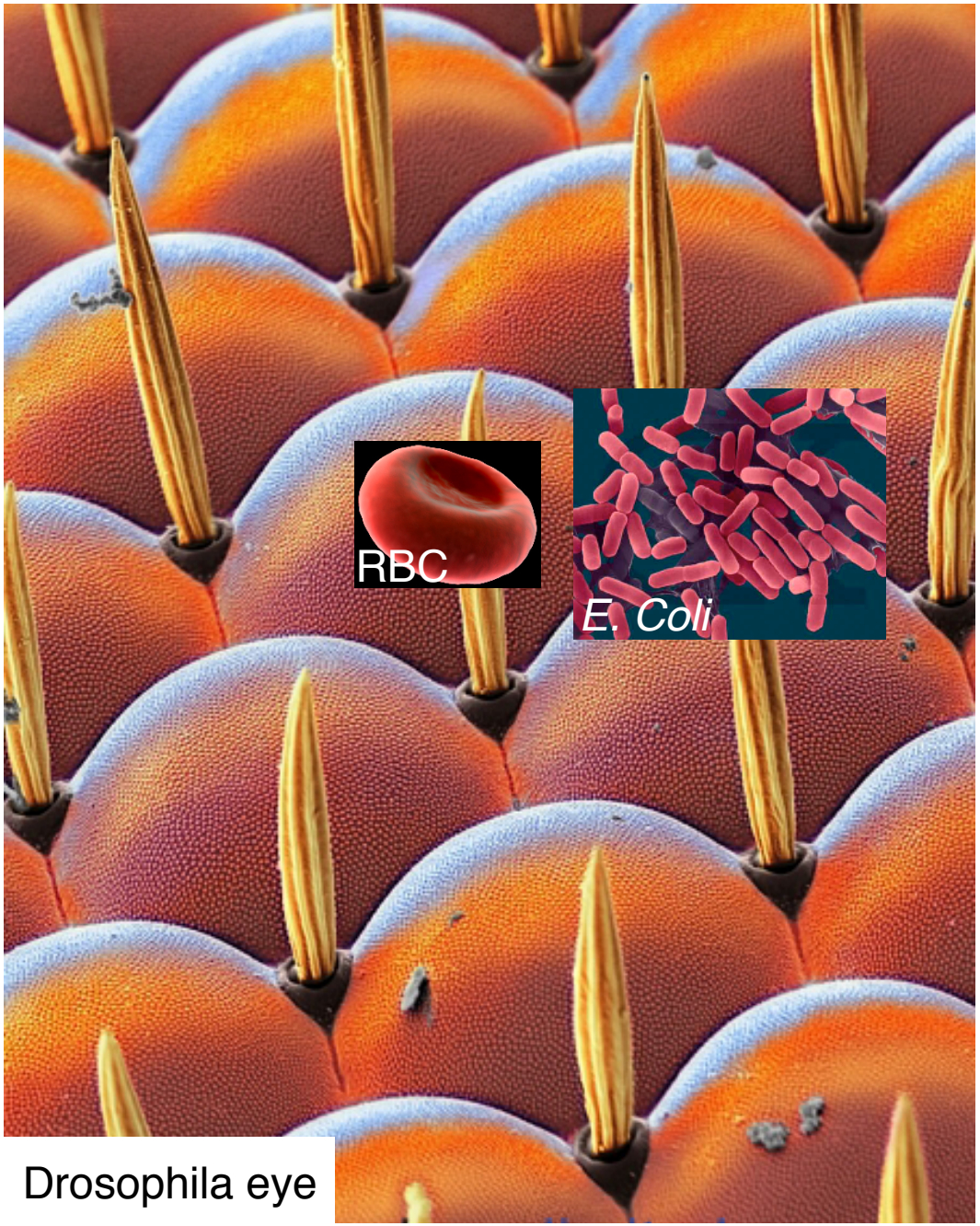
How small are macromolecules?

Scale: 2^{15}
32,768X



How small are macromolecules?

Scale: 2^{16}
65,536X

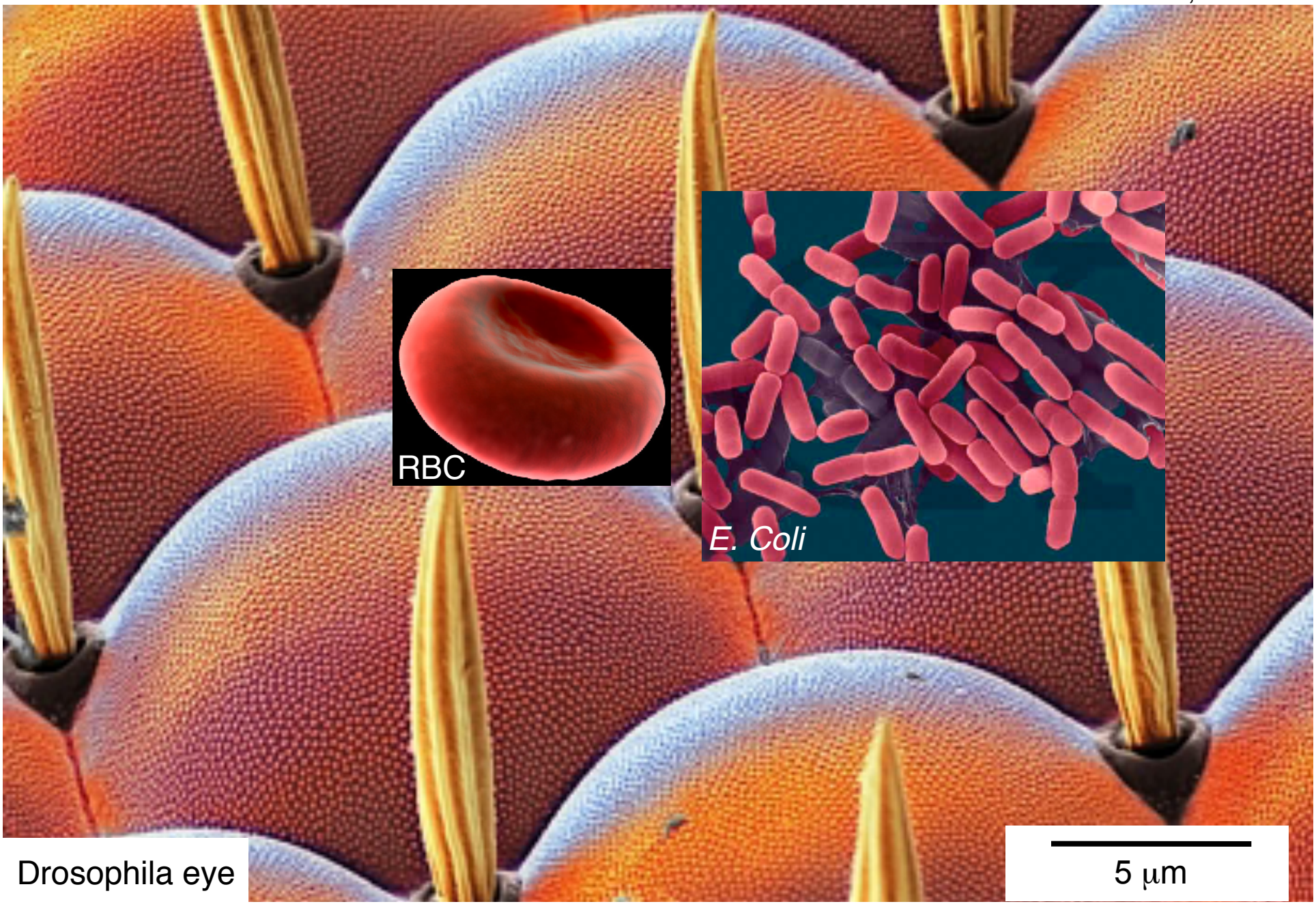


Drosophila eye

10 μm

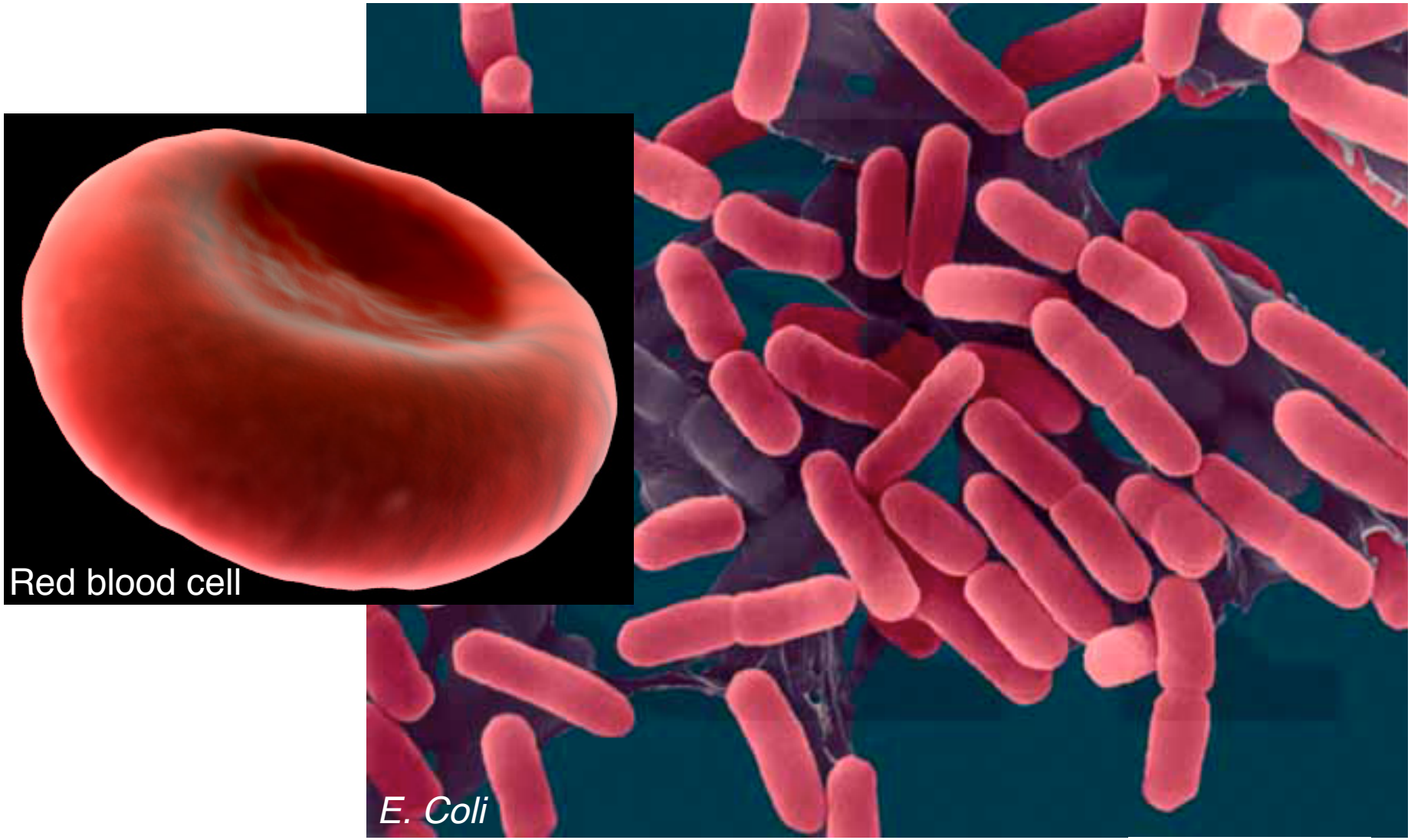
How small are macromolecules?

Scale: 2^{17}
131,072X



How small are macromolecules?

Scale: 2^{18}
262,144X

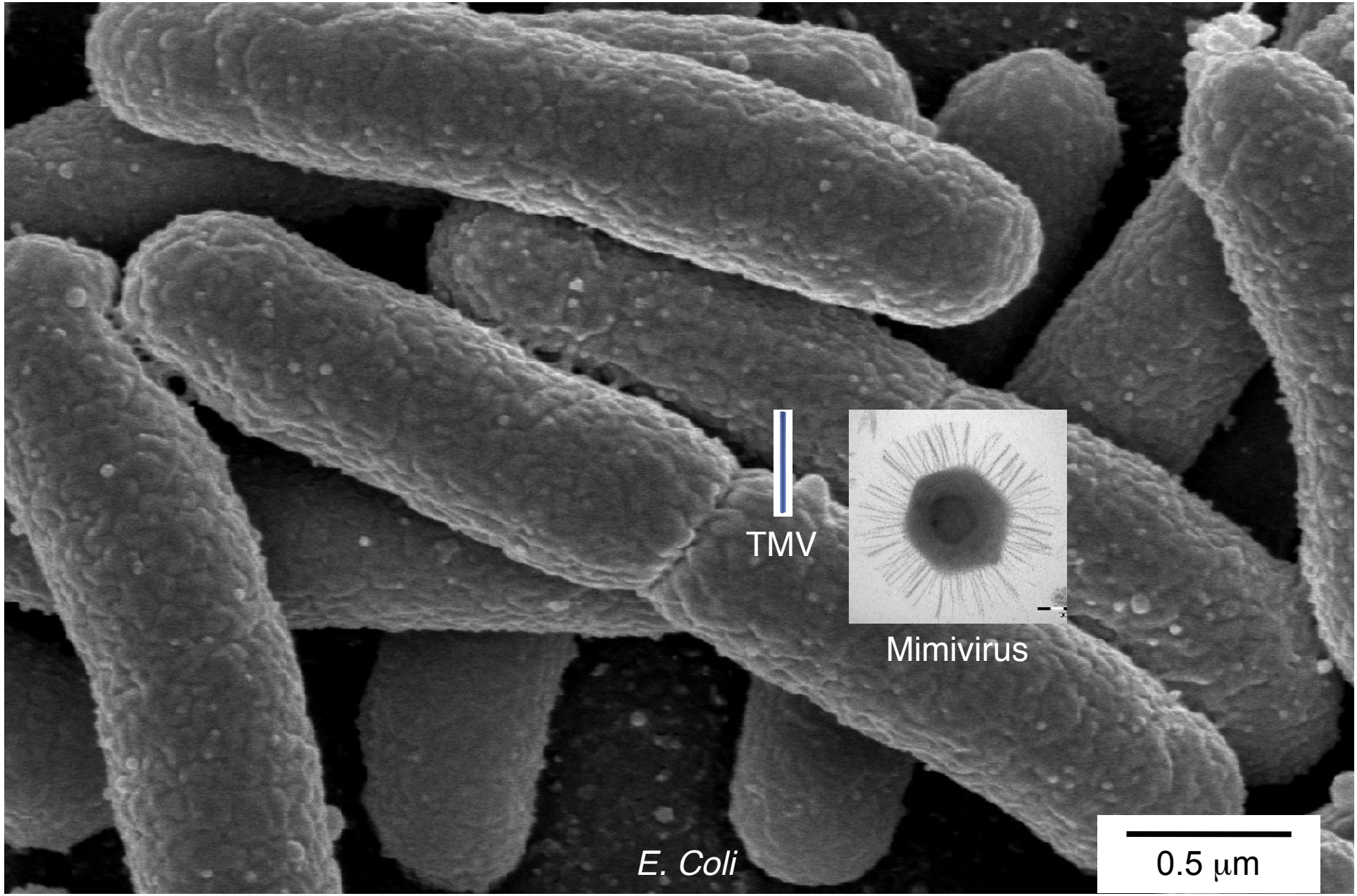


How small are macromolecules?

Scale: 2^{19}
524,288X

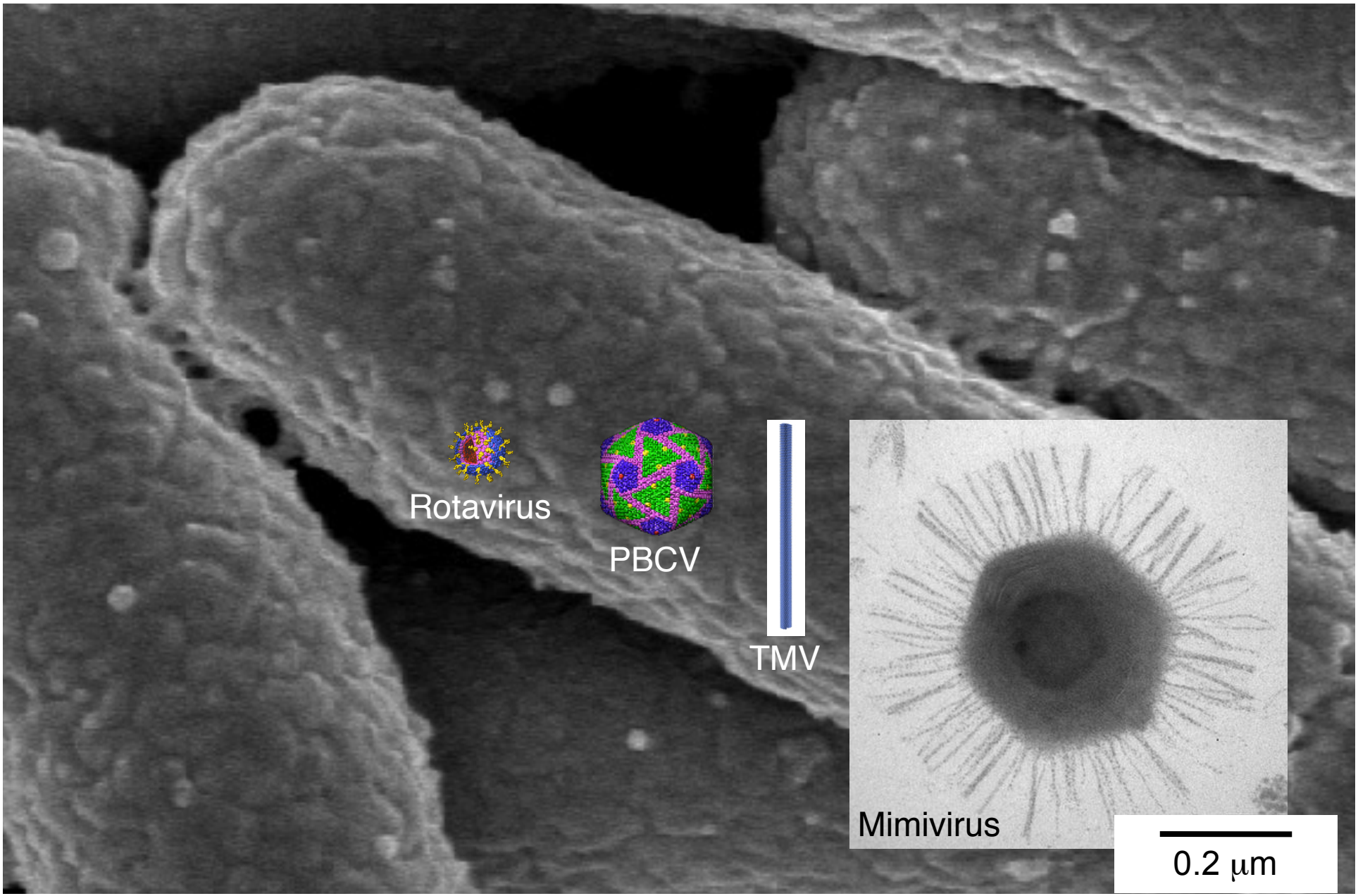
How small are macromolecules?

Scale: 2^{20}
1,048,576X



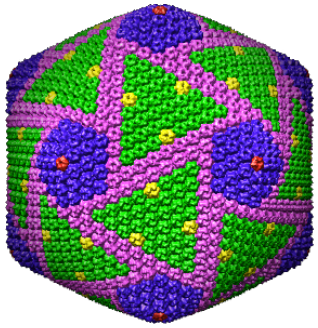
How small are macromolecules?

Scale: 2^{21}
2,097,152X



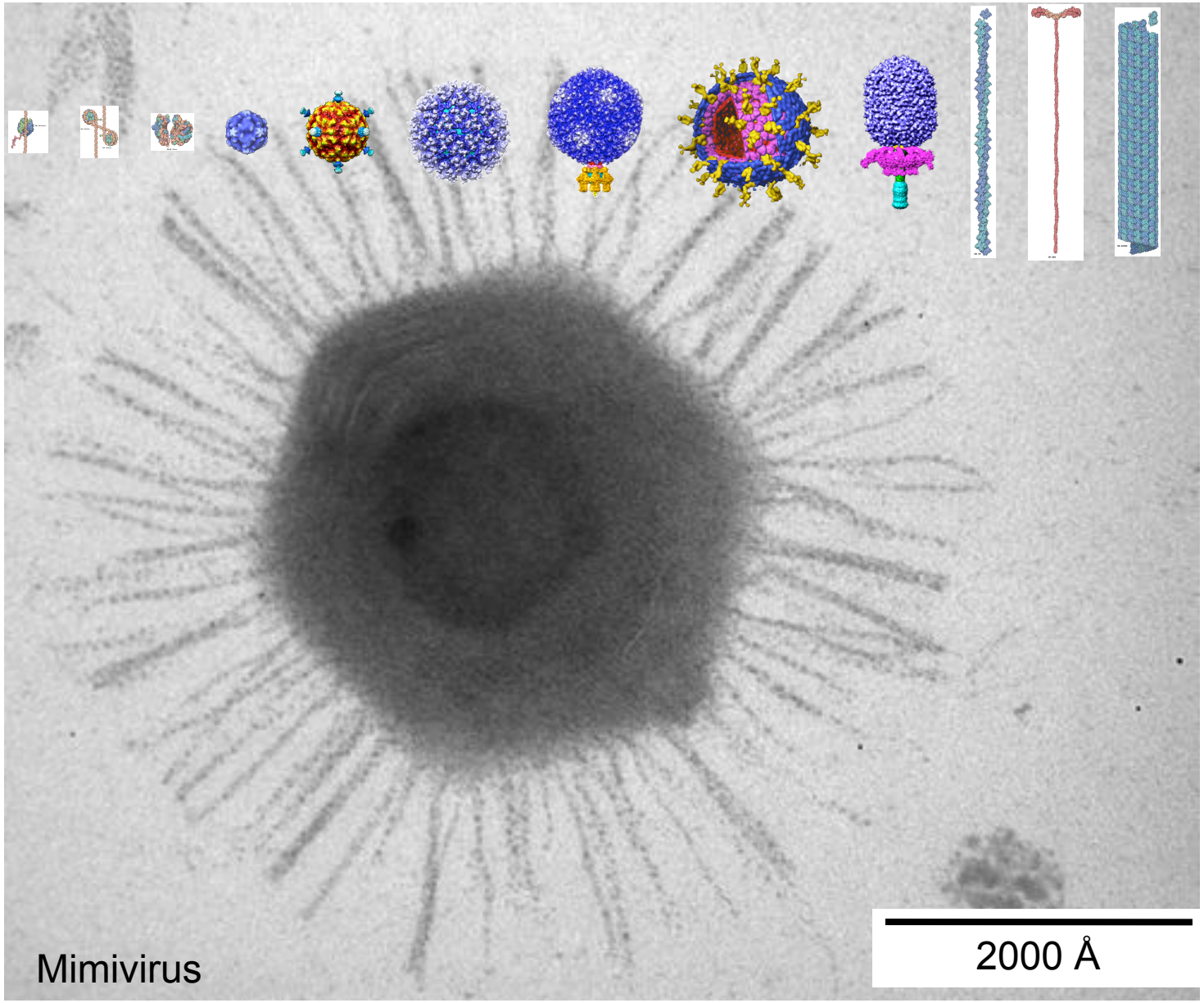
How small are macromolecules?

Scale: 2^{22}
4,194,304X



PBCV-1

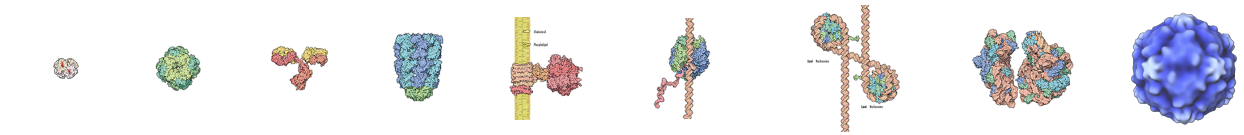
TMV



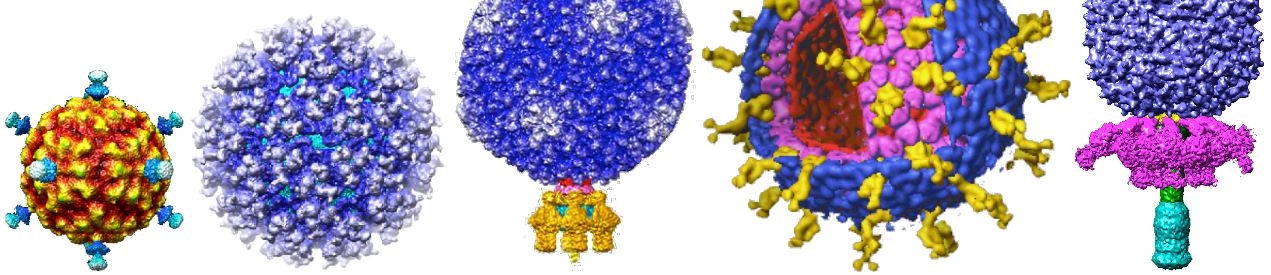
www.stanford.edu/group/virus/mimi/2005/index.htm

How small are macromolecules?

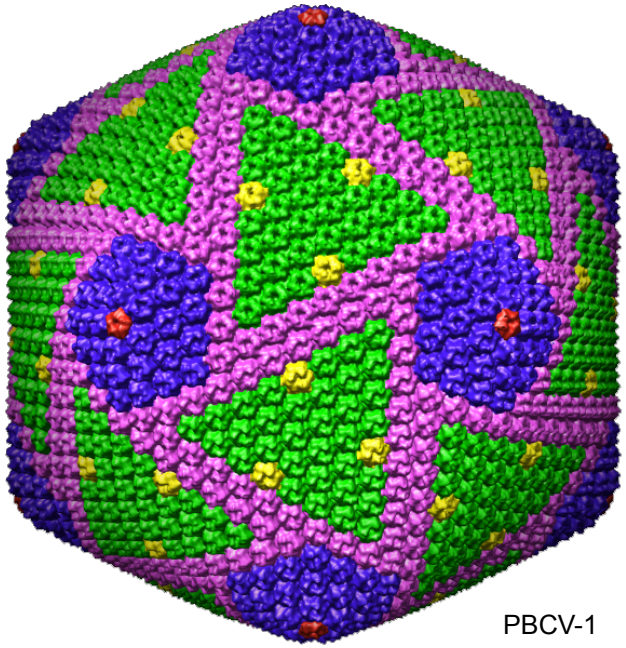
Scale: 2^{23}
8,388,608X



1000 Å

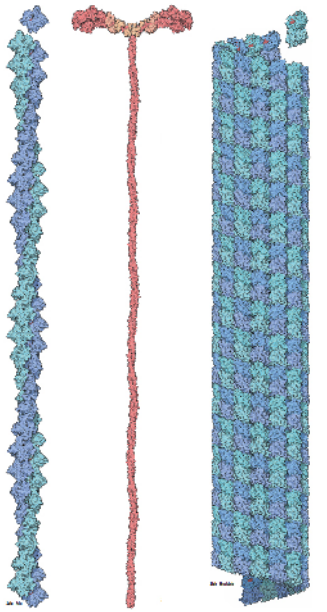


IMNV Sindbis P22 Rotavirus ϕ29



PBCV-1

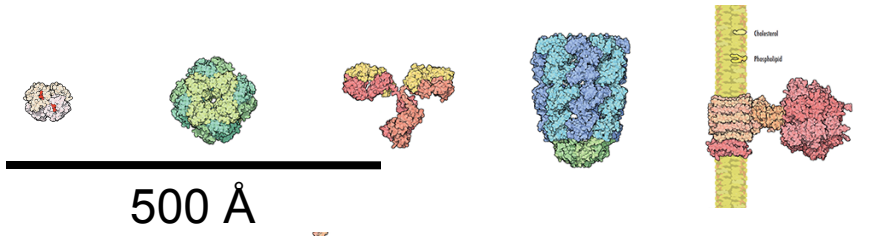
TMV

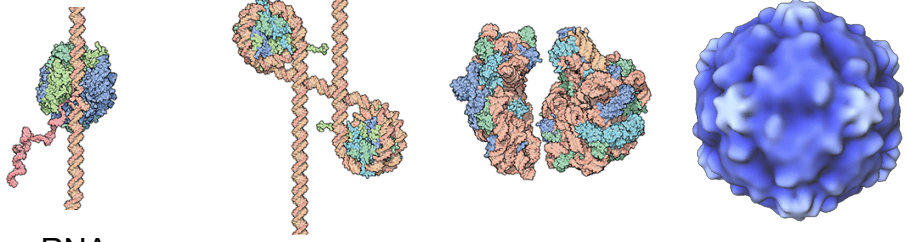


Actin Myosin Microtubule

How small are macromolecules?

Scale: 2^{24}
16,777,216X



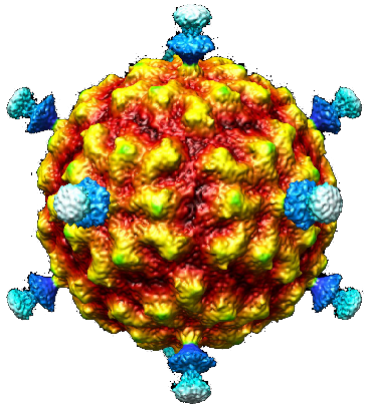


RNA Polymerase

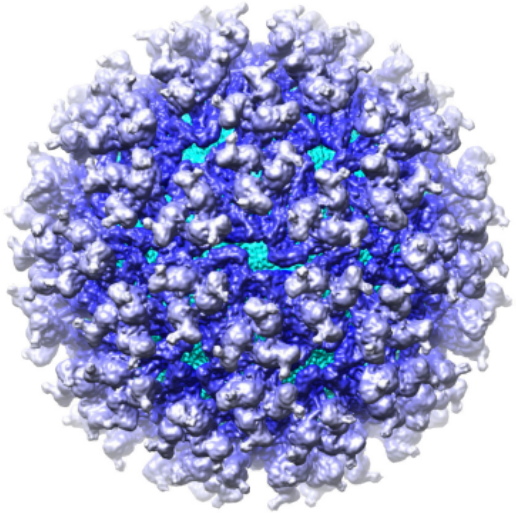
Nucleosome

Ribosome

HRV

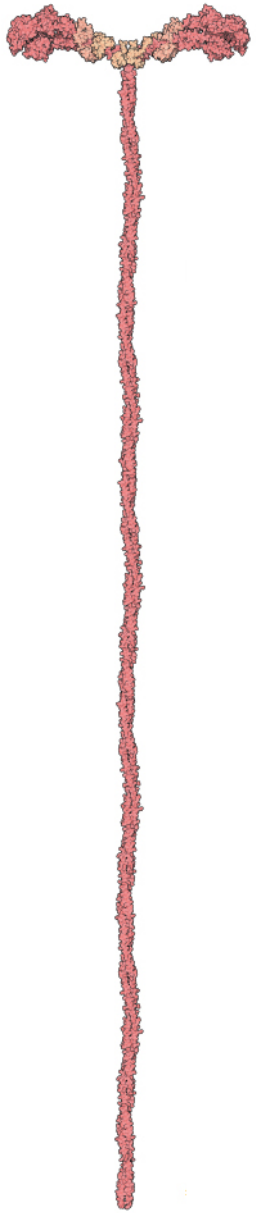


IMNV

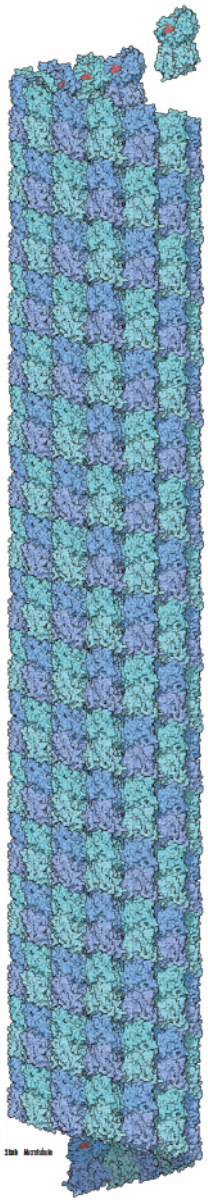


Sindbis

Actin



Myosin



Microtubule

3D Electron Microscopy of Macromolecules

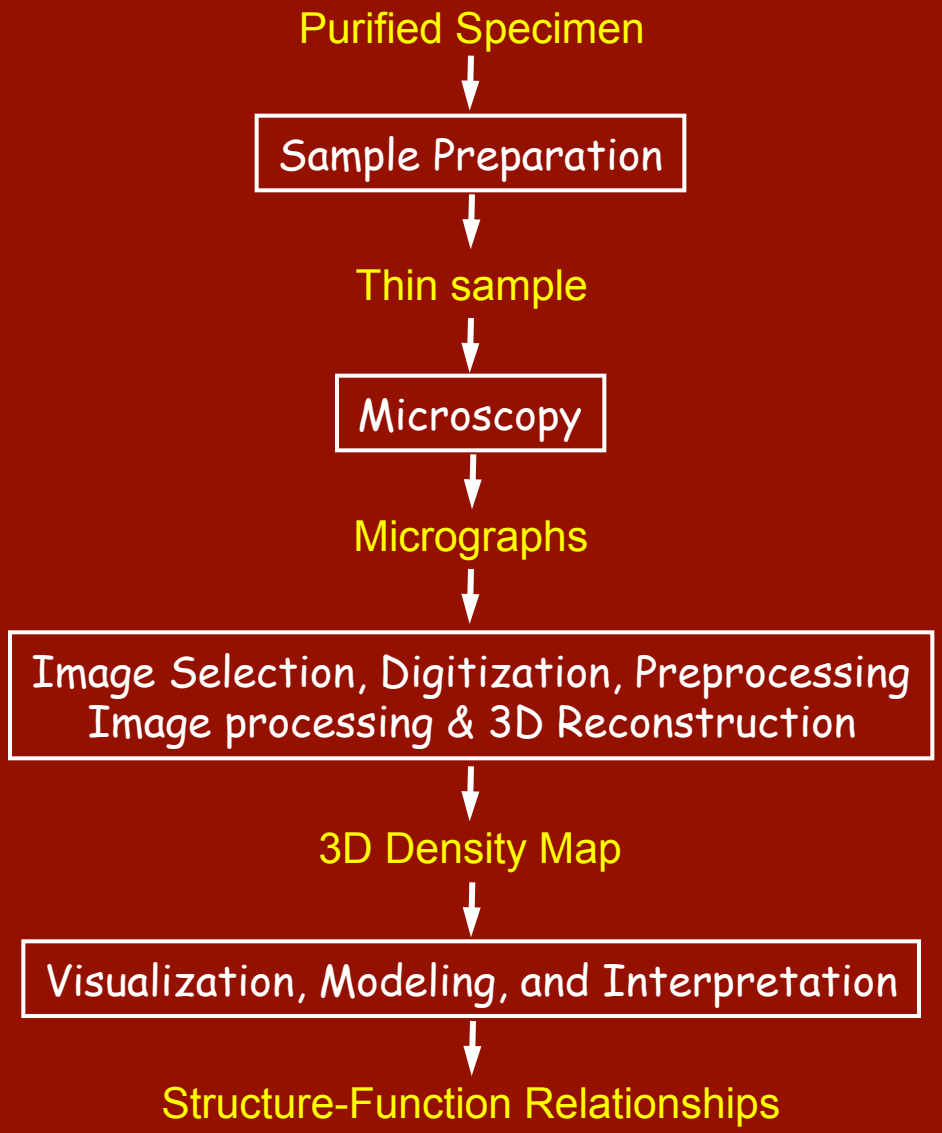
The "Story" in Three Parts

- The Microscope

- The Specimen

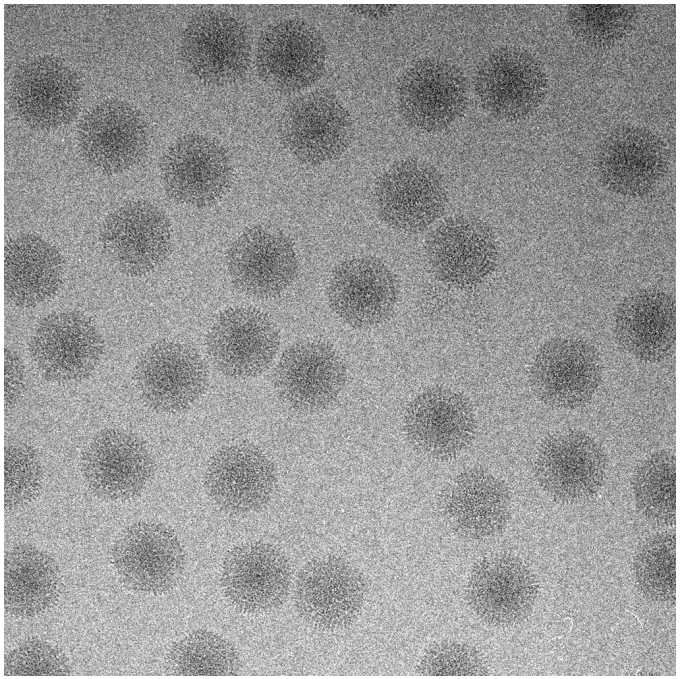
- The Structure

Electron Microscopy and 3D Image Reconstruction



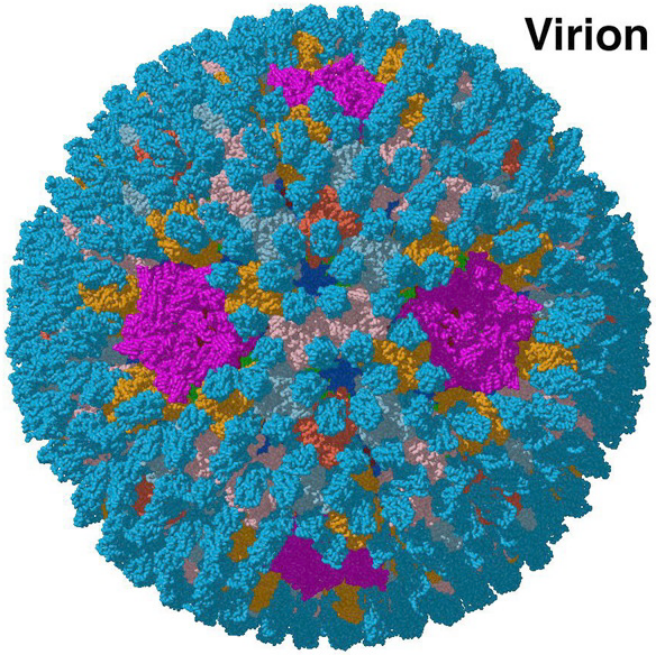
3D Electron Cryo-Microscopy of Macromolecules

How do we get this…



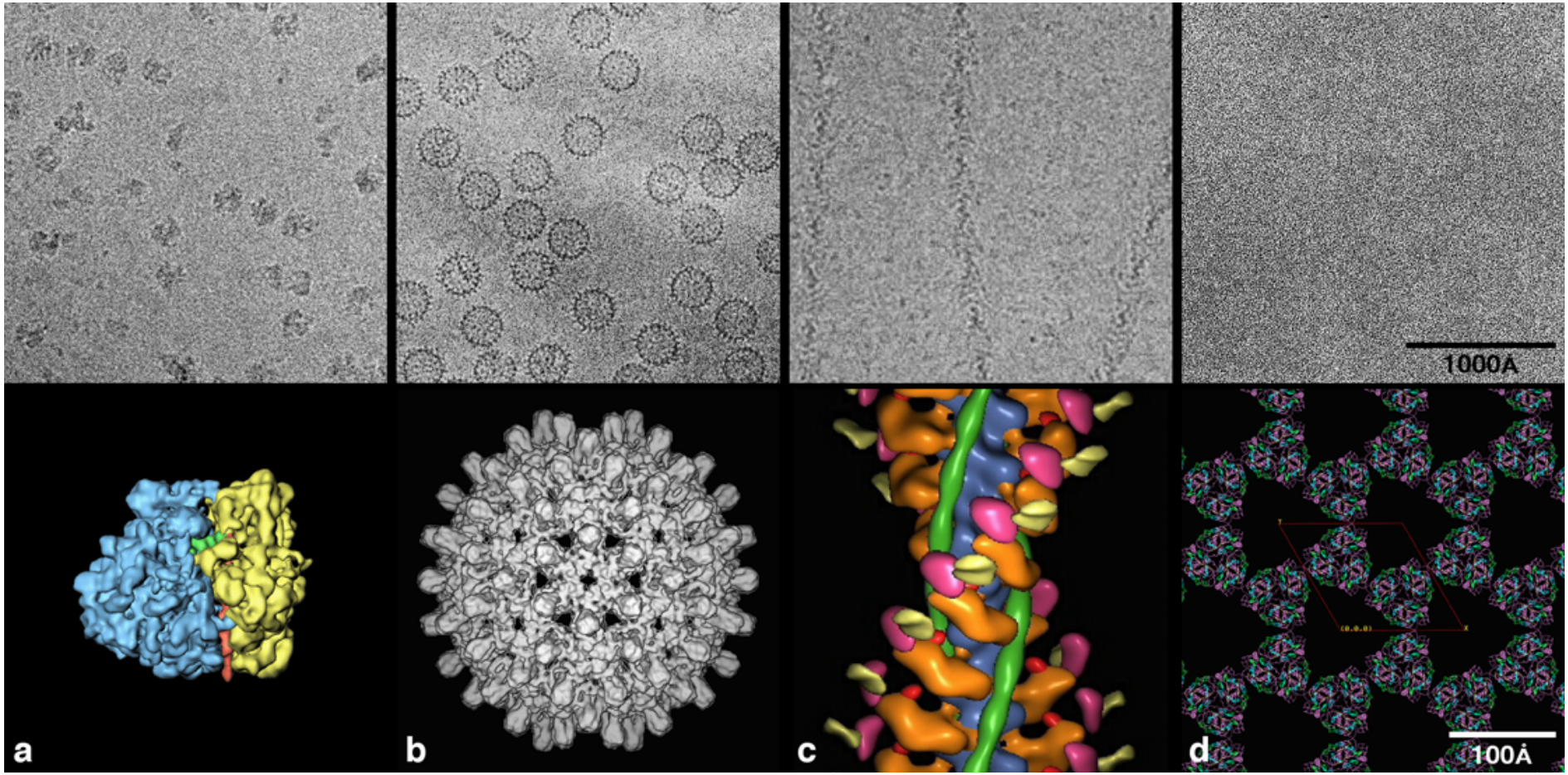
Cryo-TEM image of human reovirus

…and eventually this?



3D pseudo-atomic model
of human reovirus

3D Electron Cryo-Microscopy of Macromolecules



70S *E. coli* ribosome

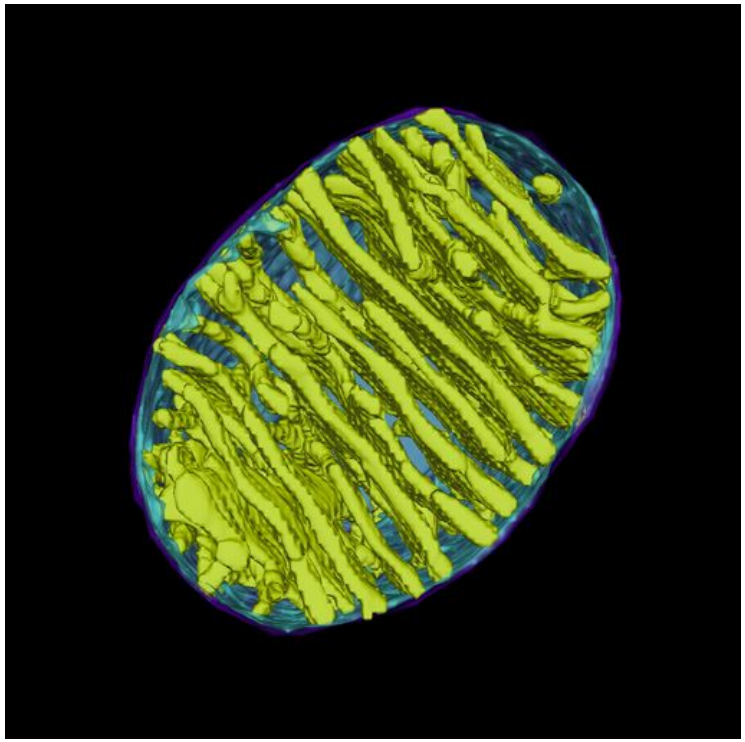
Hepatitis B virus core

Actin-myosin filament

Light-harvesting 2D crystal

3D Electron Tomography of Organelles /Cells

Tomographic series of a stained, thick section of mitochondrion



Animation of a segmented version of the mitochondrion on left

Courtesy of Guy Perkins (Biomedical Sciences, UCSD)

Allright, so much for the introductory stuff…

Sample Question

Rearrange the following list of dimensions according to increasing size:

0.15 μm
0.3 mm
0.5 nm
1 cm
1 μm
1 nm
1 mm
1 Å
7.5 Å
10 m
25 Å
30 μm
65 μm
400 nm
500 mm

Note: m = meter; cm = centimeter; mm = millimeter; μm = micron; nm = nanometer; Å = Ångstrom

Sample Question

Which is larger, 150 Å or 0.15 μm?

Which is larger, 151 nm or 0.149 μm?

Which is larger, 999 μm or 9.9 mm?

Questions like this may be displayed **RIGHT BEFORE** or **DURING** class, but only those shown during class will be posted on the Class Web site along with the Powerpoint lecture notes