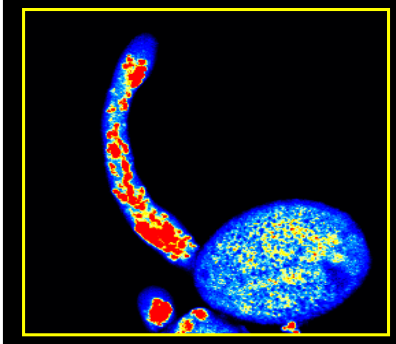
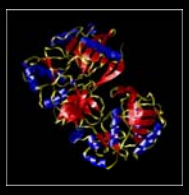
Microscopia

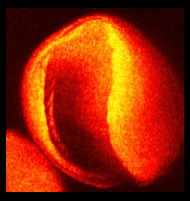
- A Ciência *"rente ao olhar"* -

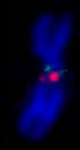


Rui Malhó

Dept. Biologia Vegetal
Univ de Lisboa



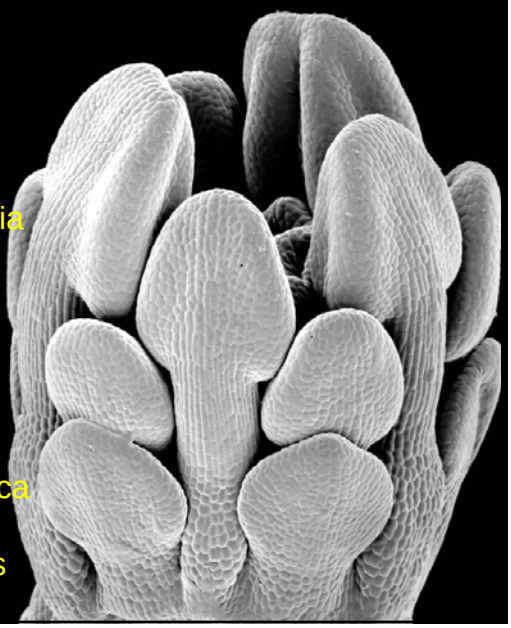


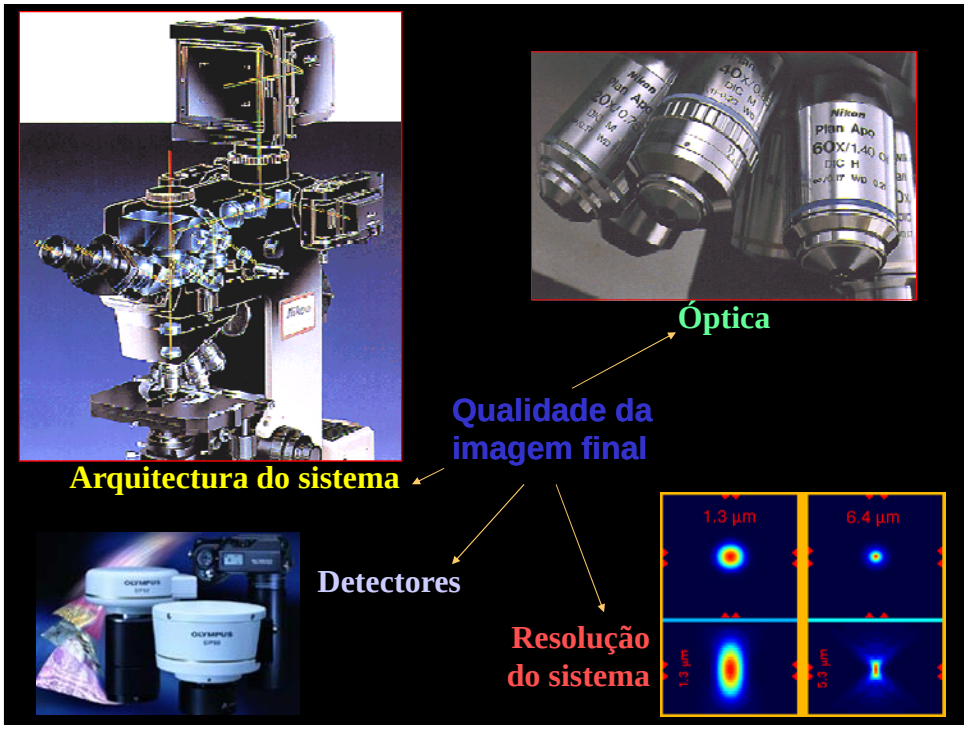


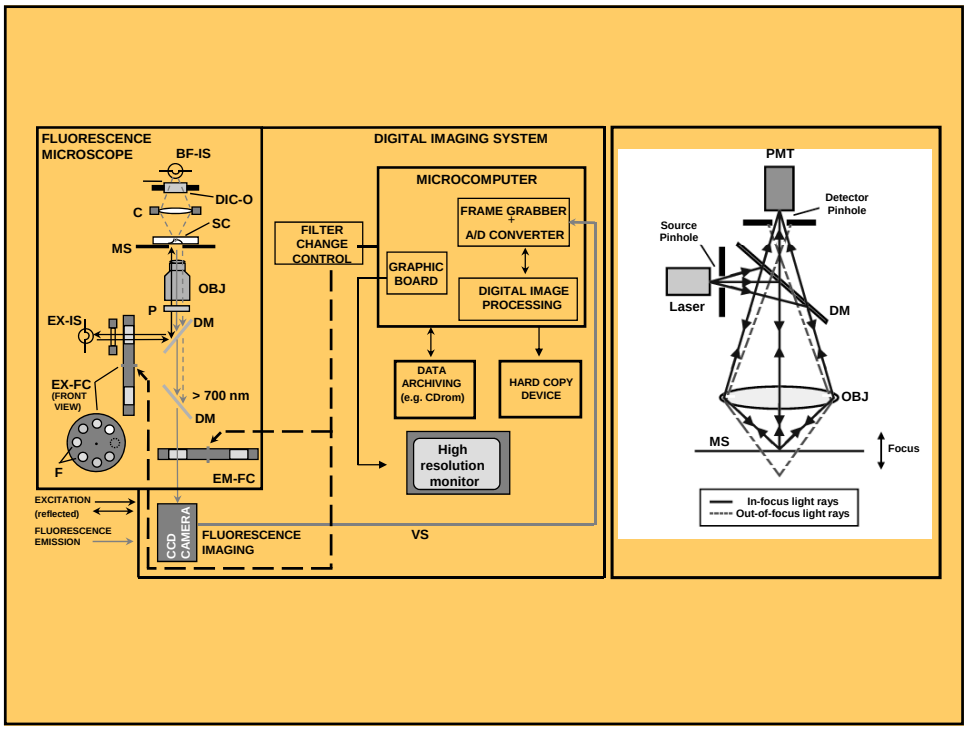
UNIVERSIDADE·DE·LISBOA FACULDADE·DE·CIÊNCIAS

Técnicas de Microscopia:

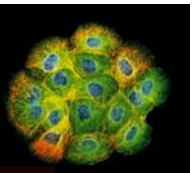
- Microscopia óptica
  - campo claro
  - campo escuro
  - contraste de fase
  - DIC
- Microscopia de Fluorescência
  - Vídeo Digital
  - Confocal
  - Convencional
- Microscopia Electrónica
  - Transmissão
  - Varrimento
- Microscopia de Força Atómica
- Microsondas
  - Microsondas vibráteis
  - Patch-clamp



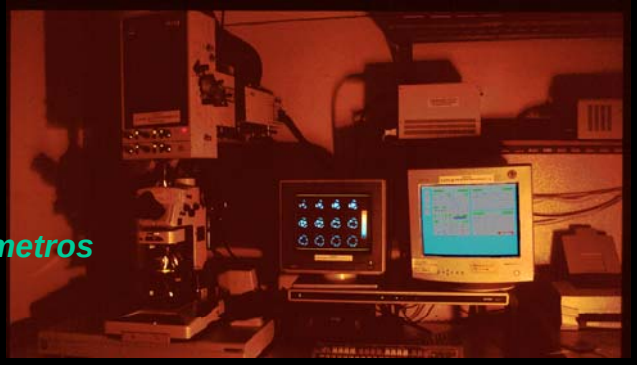

Óptica
Qualidade da imagem final
Arquitectura do sistema
Detectores
Resolução do sistema
1.3 µm
6.4 µm


FLUORESCENCE MICROSCOPE
BF-IS
DIC-O
C
SC
MS
OBJ
P
DM
EX-IS
EX-FC
(FRONT VIEW)
> 700 nm
DM
F
EM-FC
EXCITATION (reflected)
FLUORESCENCE EMISSION
CCD CAMERA
FLUORESCENCE IMAGING
DIGITAL IMAGING SYSTEM
MICROCOMPUTER
FRAME GRABBER + A/D CONVERTER
FILTER CHANGE CONTROL
GRAPHIC BOARD
DIGITAL IMAGE PROCESSING
DATA ARCHIVING (e.g. CDrom)
HARD COPY DEVICE
High resolution monitor
VS
PMT
Detector Pinhole
Source Pinhole
Laser
DM
OBJ
MS
Focus
In-focus light rays
Out-of-focus light rays

Câmaras Digitais 8-16 bit

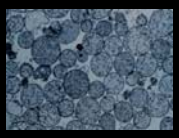


Câmaras 35 mm

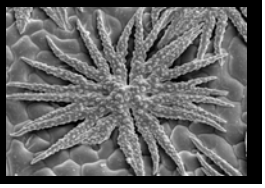


CCDs (cool)

Luminómetros

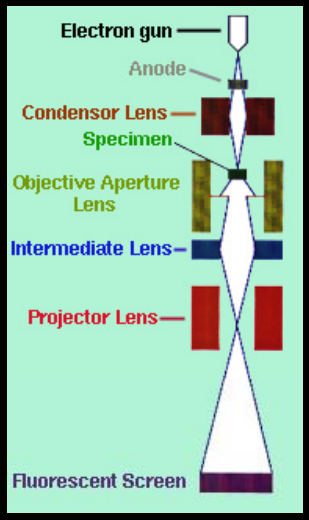


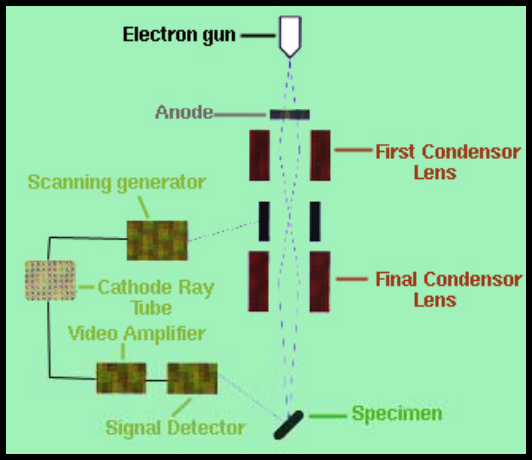
Sistema confocal

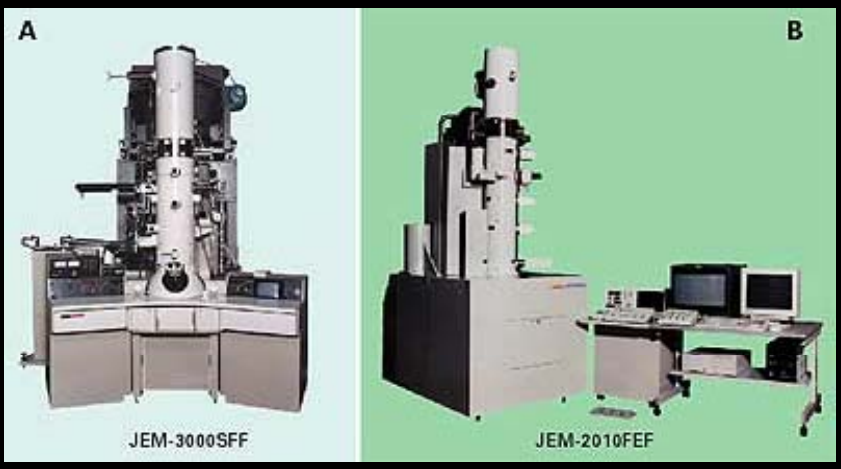


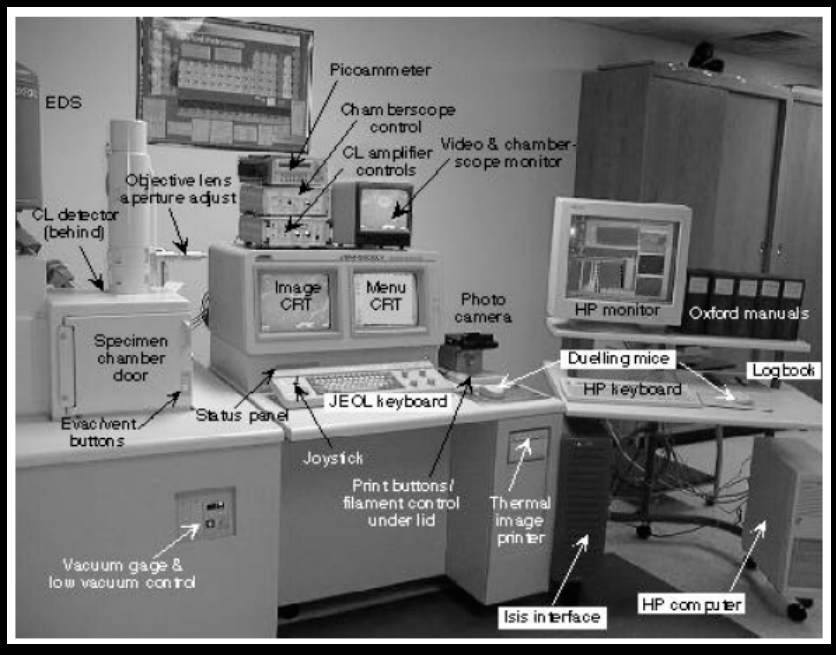
Fotomultiplicadores

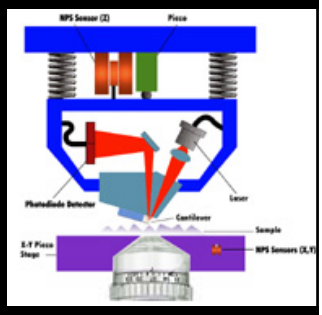
MET vs MEV






A
B
JEM-3000SFF
JEM-2010FEF


Picoammeter
EDS
Chamberscope control
CL amplifier controls
Video & chamber-scope monitor
Objective lens aperture adjust
CL detector (behind)
Image CRT
Menu CRT
Photo camera
HP monitor
Oxford manuals
Specimen chamber door
Dueling mice
Logbook
HP keyboard
JEOL keyboard
Status panel
Evac/vent buttons
Joystick
Print buttons/ filament control under lid
Thermal image printer
Vacuum gage & low vacuum control
Isis interface
HP computer



GFP 101

Aequoria victoria uses GFP to emit green light.

Noticed in 1962 by Osamu Shimomura

GFP cloned in 1992

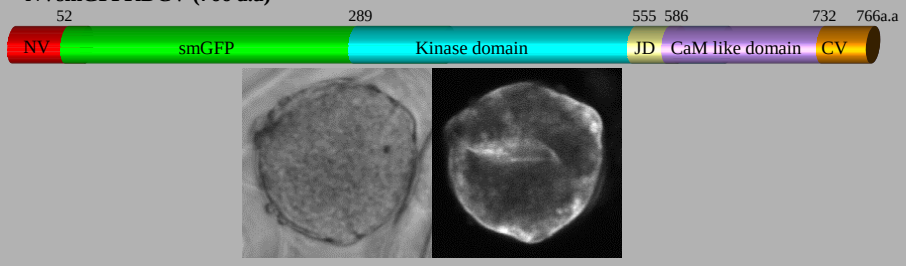
Crystal structure solved in 1996

Many mutations have made GFP brighter, added different colors, enhanced stability at 37°C and at physiological pH.

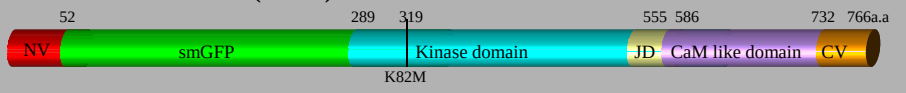
GFP is now one of the most widely used reporter molecules.

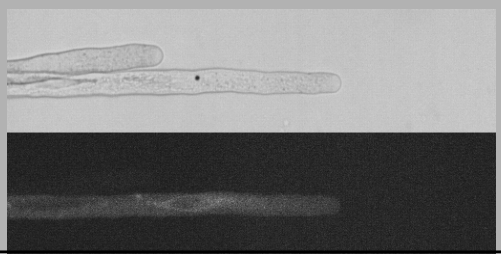
NpCDPK7 localises to the generative cell membrane

NVsmGFPKDCV (766 a.a)

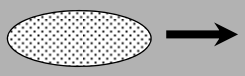


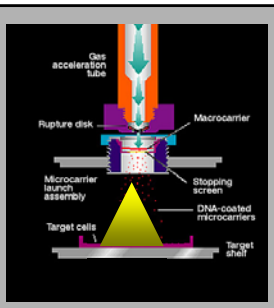
NVsmGFPKDK82MCV (766 a.a)





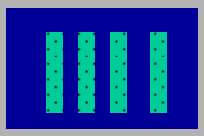
Capture the tissue / cell suspension on the membrane filter

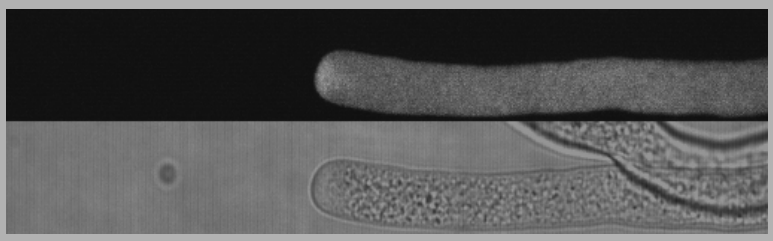




Transform by biolistic gun

Transfer the cells to solid culture media on the slide glass

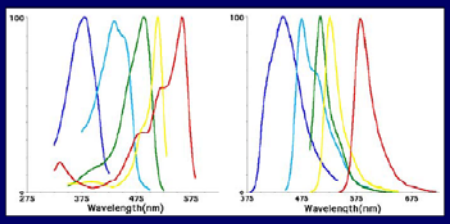


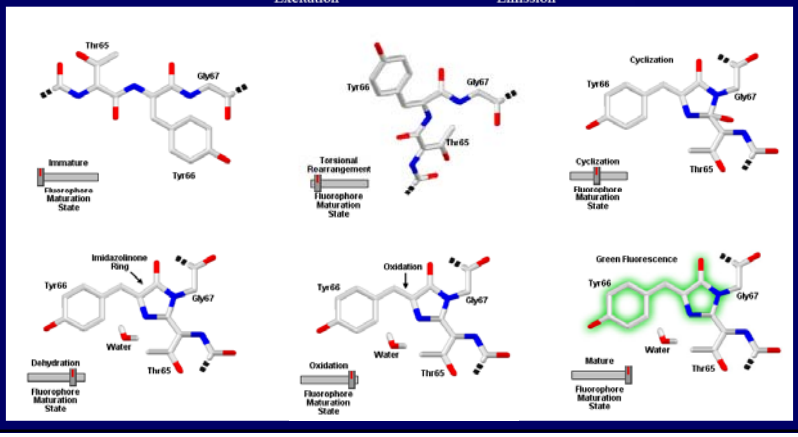


GFP fluorescence image

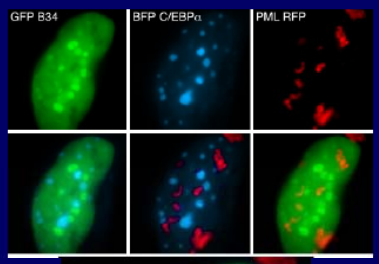
Bright field image

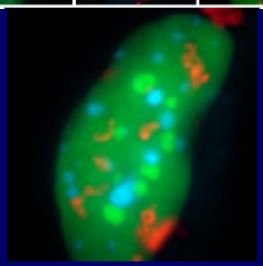
Palette of GFP Mutants



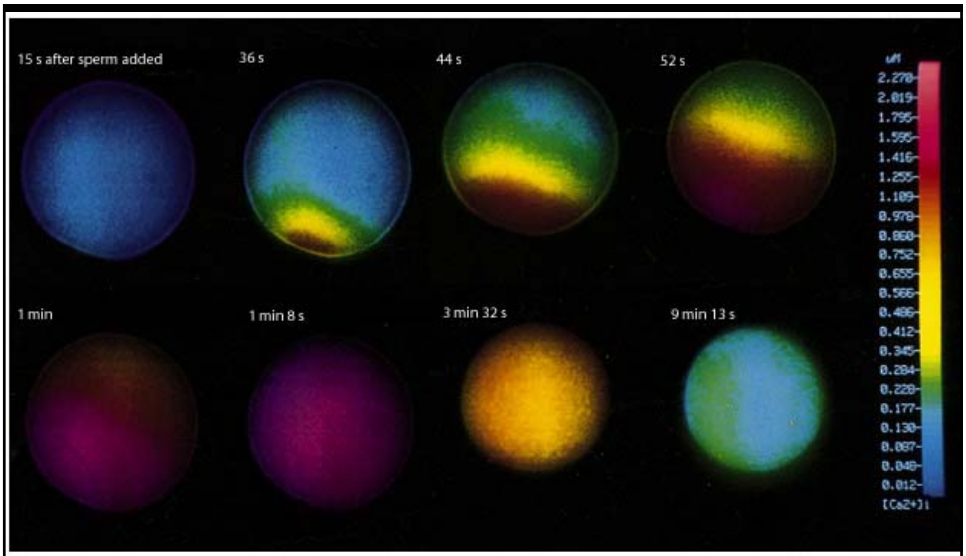


Three Color GFP Imaging





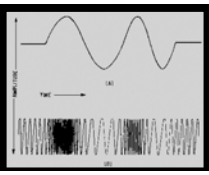
BFP
GFP
RFP

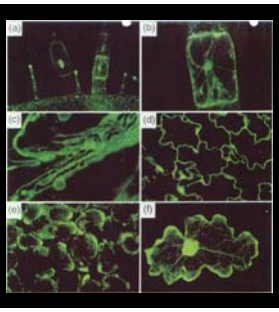


Equação Difusão (onda de cálcio):

$\delta c / \delta t = D [\delta^2 C / \delta x^2]$

em que: D (coeficiente de difusão); t (tempo - amplificação); c (concentração do mensageiro); x (distância)





Circularly permuted green fluorescent proteins engineered to sense Ca^{2+}

Takeharu Nagai, Asako Sawano, Eun Sun Park, and Atsushi Miyawaki*

Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

Communicated by Roger Y. Tsien, University of California, San Diego, CA, December 30, 2000 (received for review December 11, 2000)

PNAS | March 13, 2001 | vol. 98 | no. 6 | 3197–3202

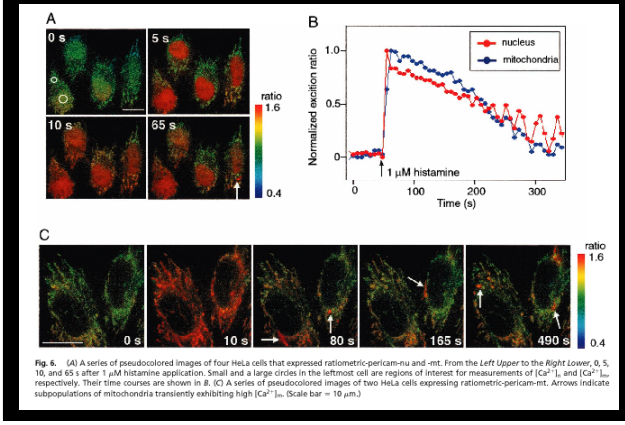
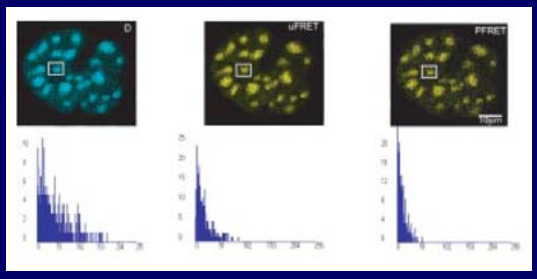
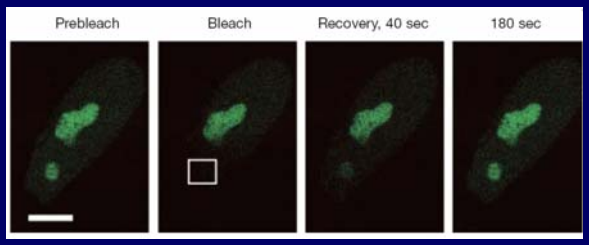


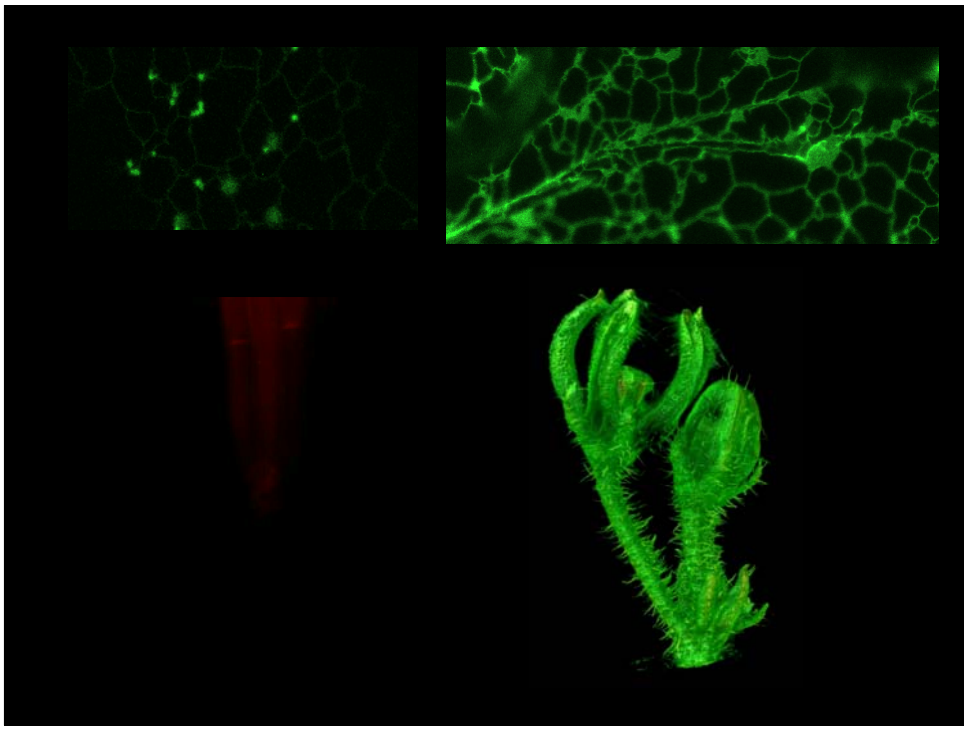
Fig. 6. (*A*) A series of pseudocolored images of four HeLa cells that expressed ratiometric-pericam-nu and -mt. From the *Left Upper* to the *Right Lower*, 0, 5, 10, and 65 s after 1 μM histamine application. Small and a large circles in the leftmost cell are regions of interest for measurements of $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$, respectively. Their time courses are shown in *B*. (*C*) A series of pseudocolored images of two HeLa cells expressing ratiometric-pericam-mt. Arrows indicate subpopulations of mitochondria transiently exhibiting high $[Ca^{2+}]_m$. (Scale bar = 10 μm.)

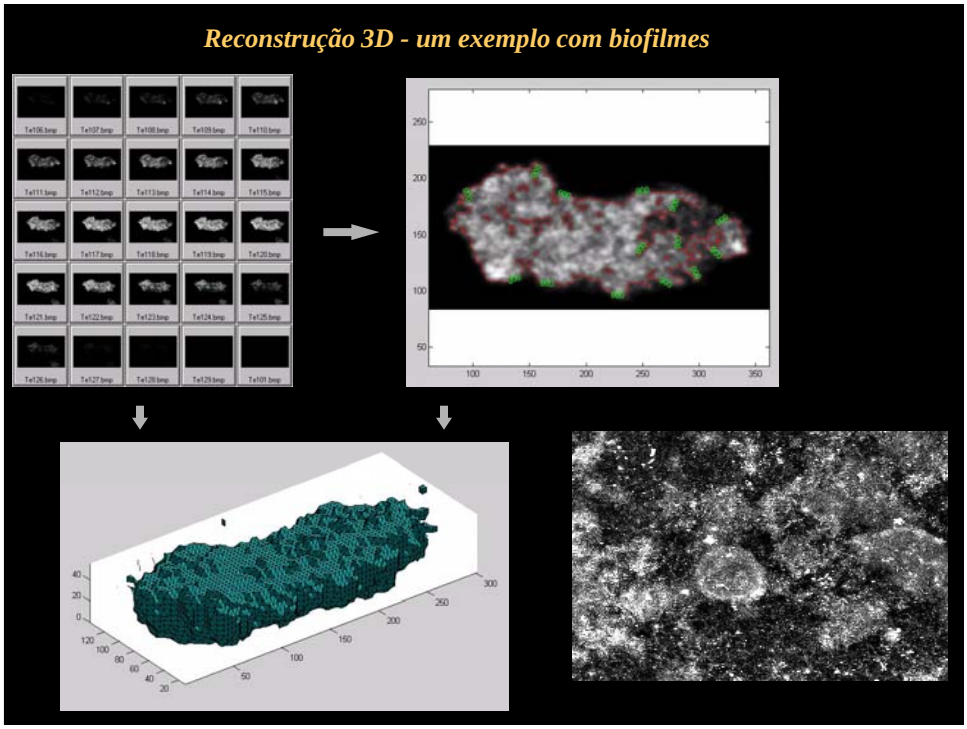
GloFish

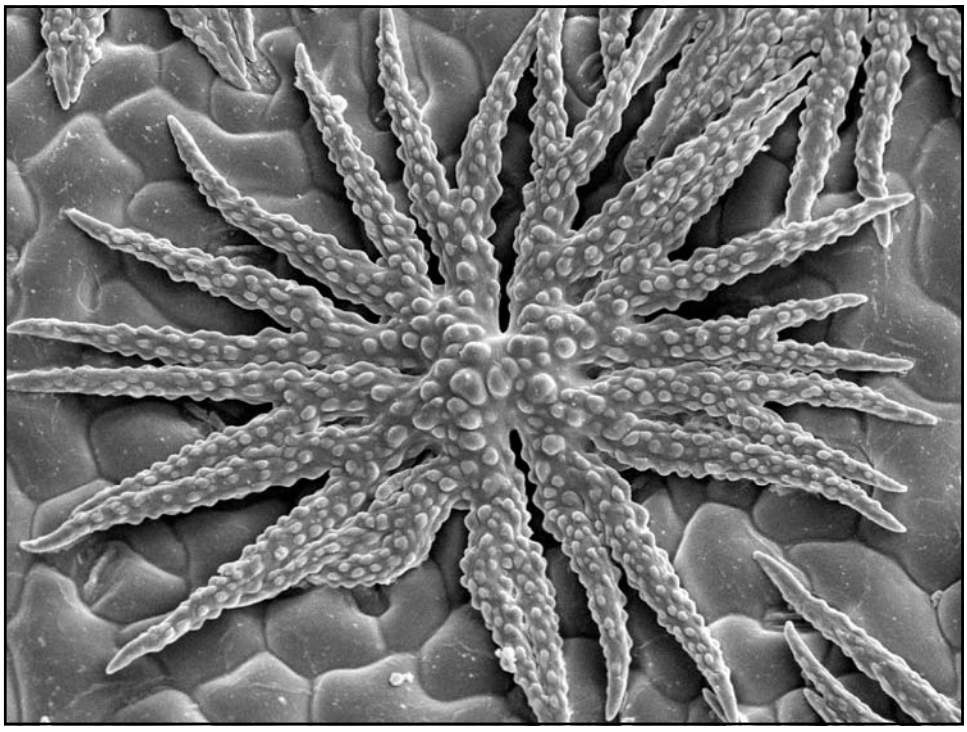
FRET & FRAP
bRET

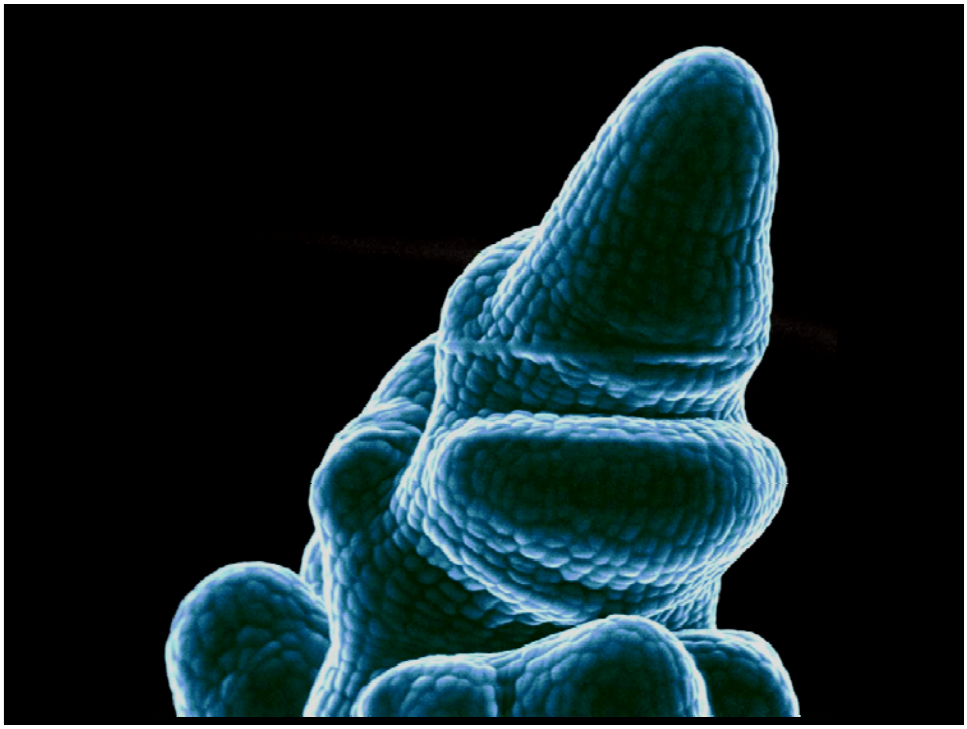


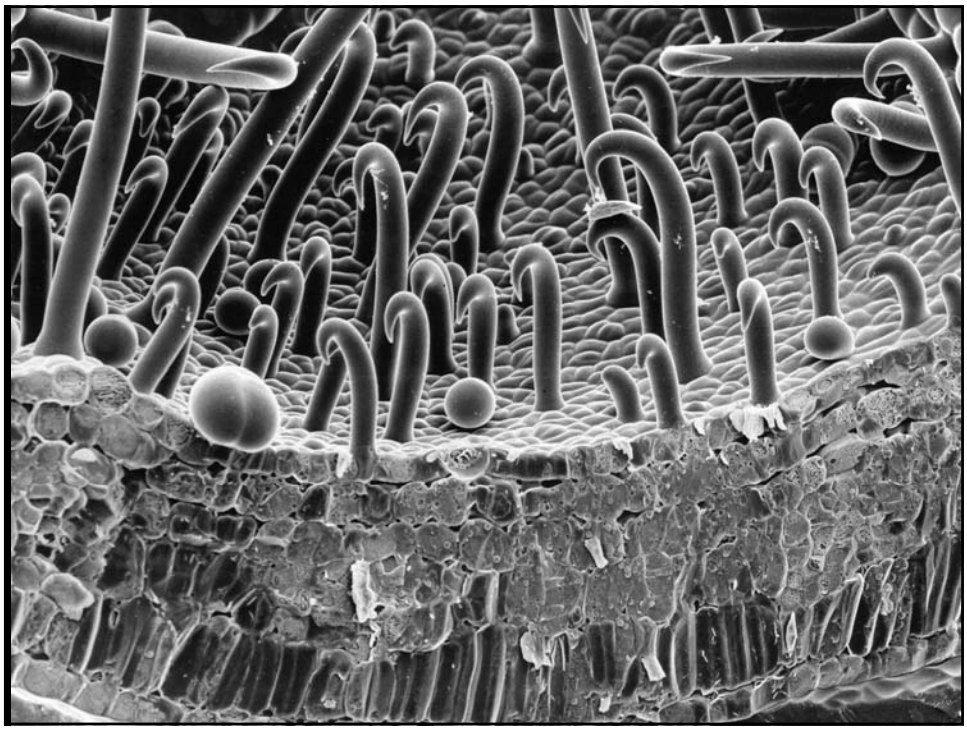


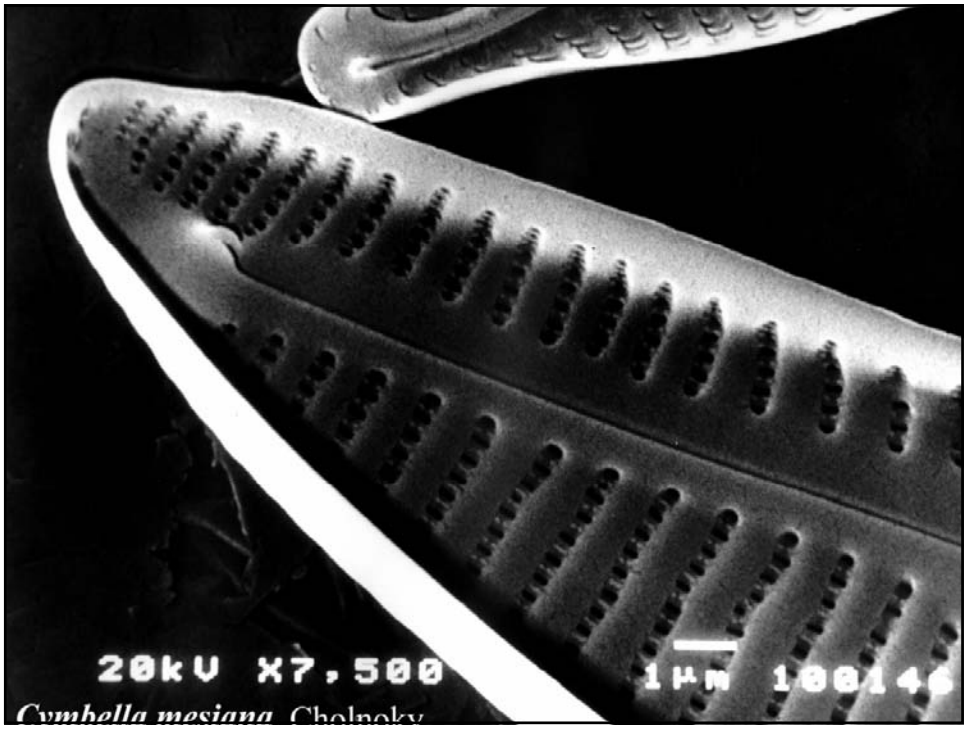


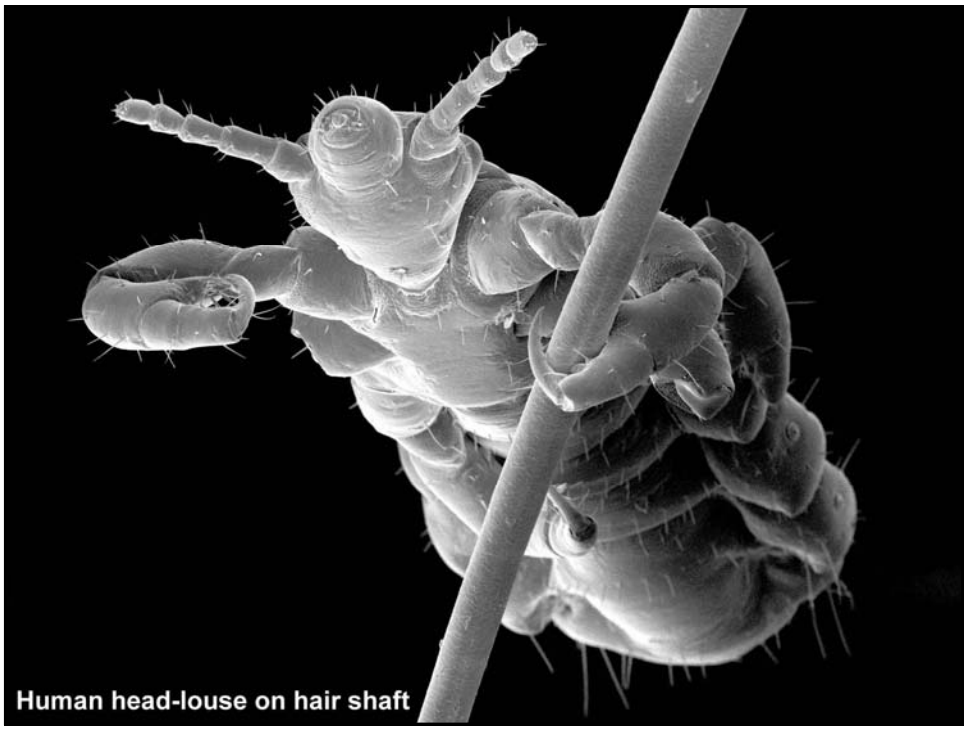

Reconstrução 3D - um exemplo com biofilmes

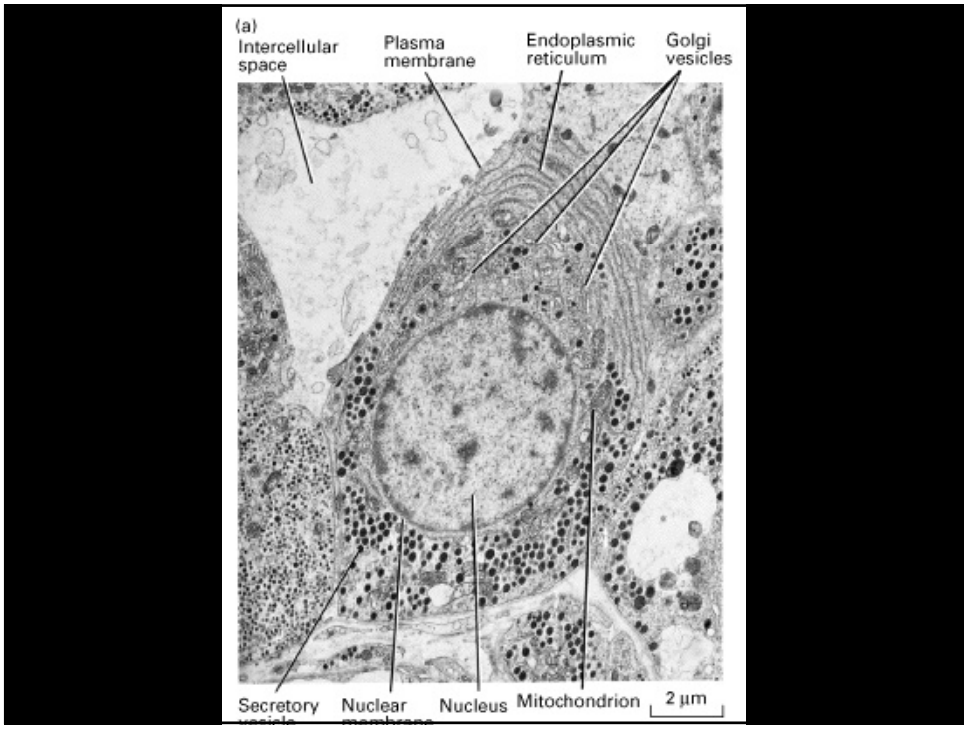


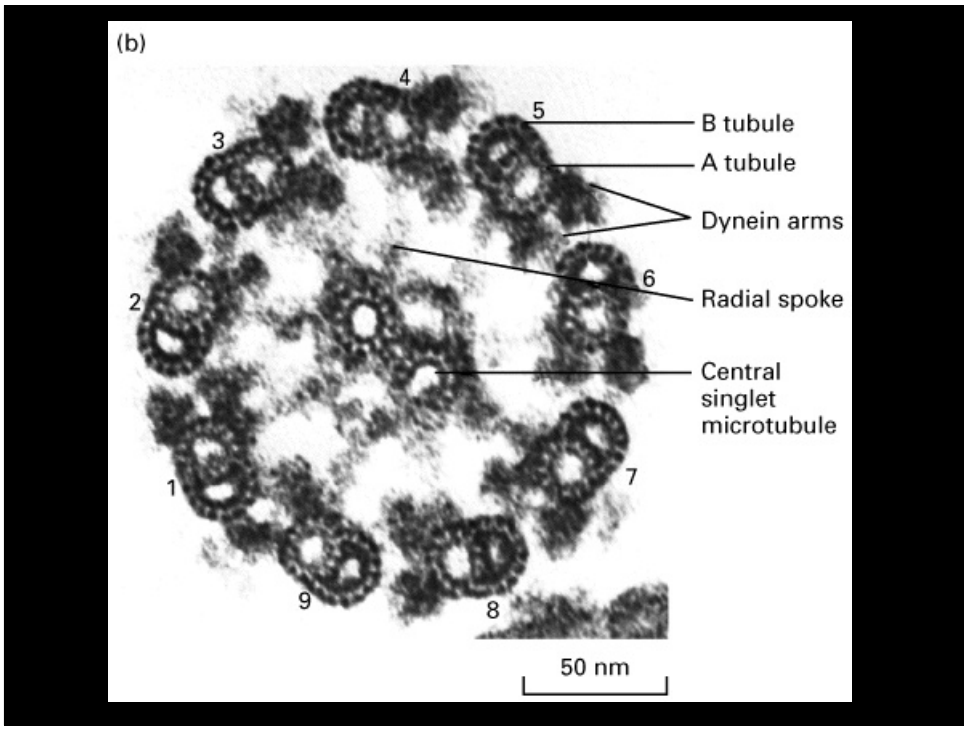


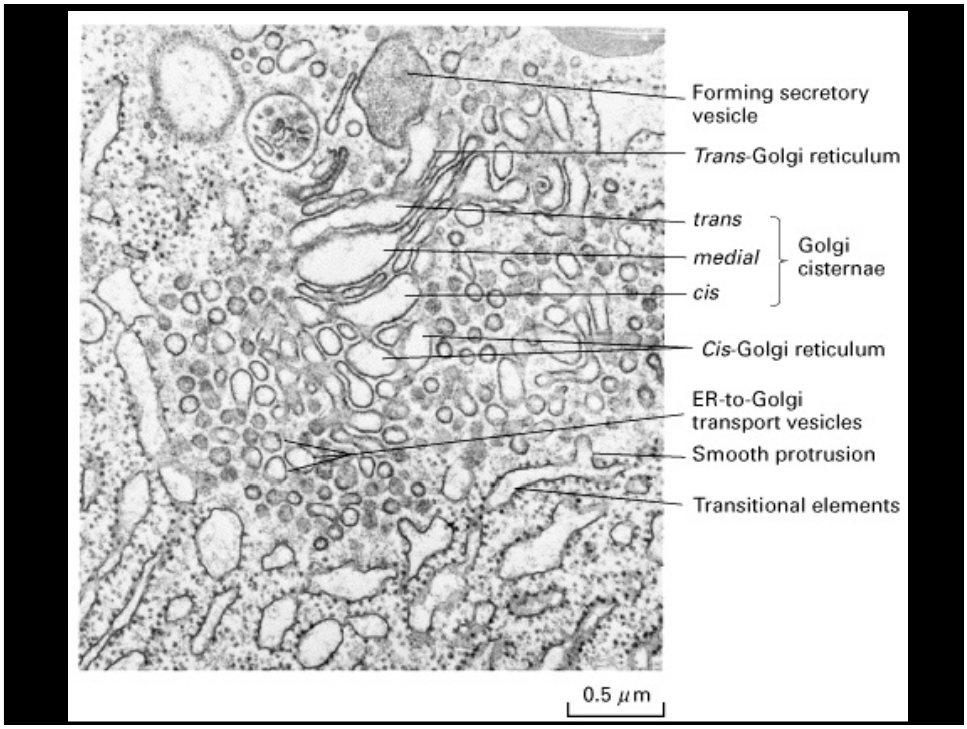


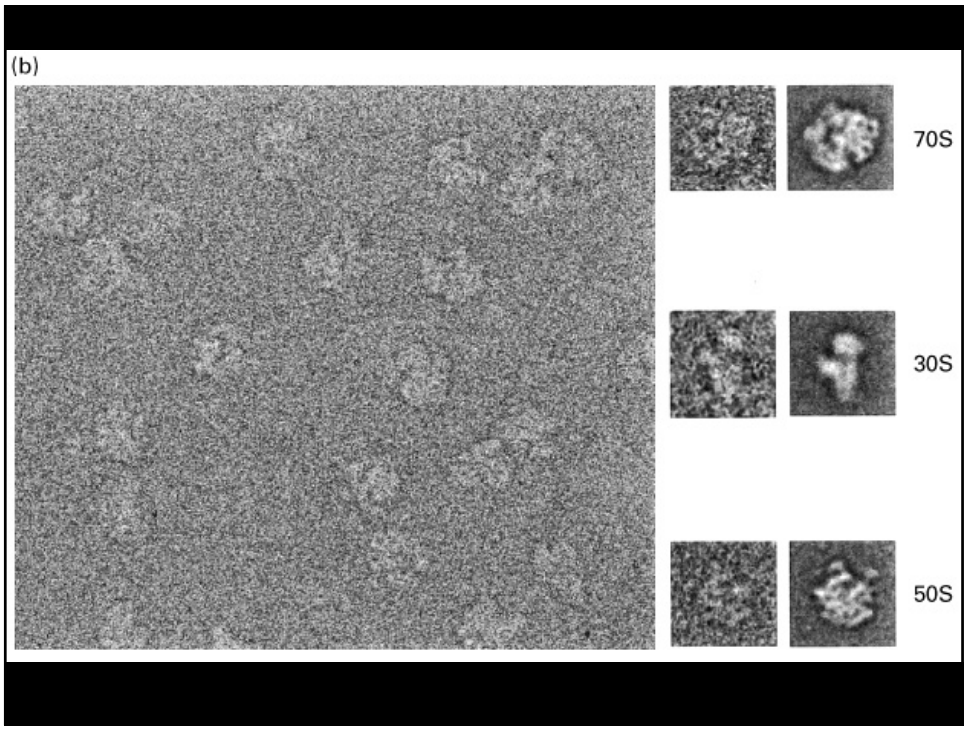

20kV X7,500
1µm 100146
Cymbella mesiana Cholnoky

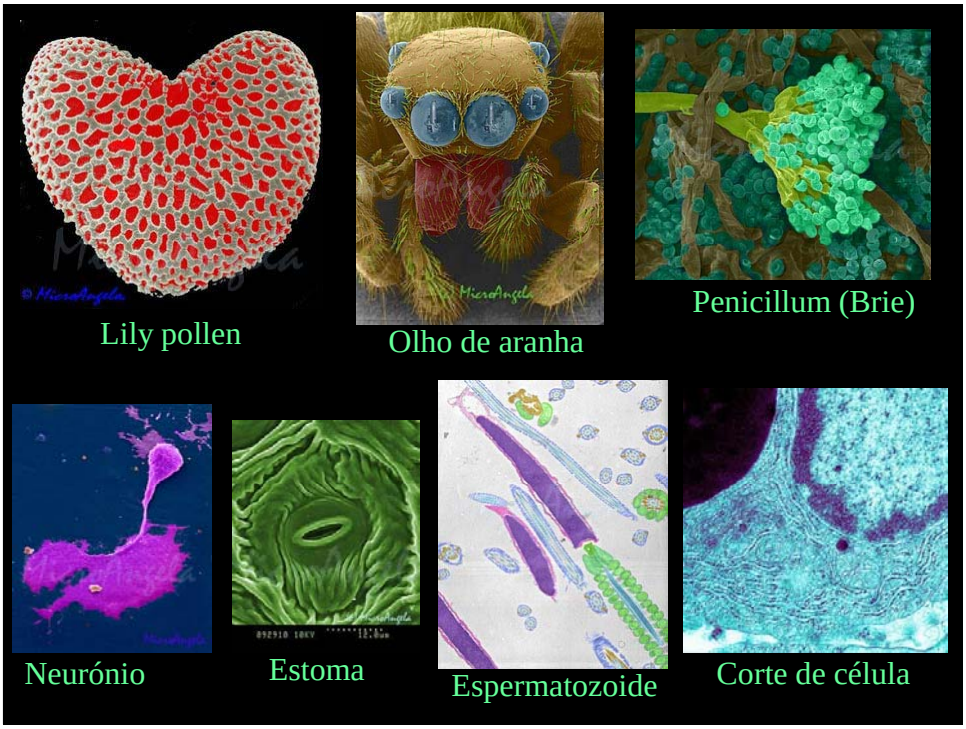

Human head-louse on hair shaft


(a)
Intercellular space
Plasma membrane
Endoplasmic reticulum
Golgi vesicles
Secretory
Nuclear
Nucleus
Mitochondrion
2 µm


(b)
1
2
3
4
5
6
7
8
9
B tubule
A tubule
Dynein arms
Radial spoke
Central singlet microtubule
50 nm


Forming secretory vesicle
Trans-Golgi reticulum
trans
medial
cis
Golgi cisternae
Cis-Golgi reticulum
ER-to-Golgi transport vesicles
Smooth protrusion
Transitional elements
0.5 μm


(b)
70S
30S
50S


Lily pollen
Olho de aranha
Penicillum (Brie)
Neurónio
Estoma
Espermatozoide
Corte de célula