BIOLOGIE CELULARA SI MOLECULARA

LP01. Glosar. Notiuni de microscopie.

Biologie Celulara si Moleculara

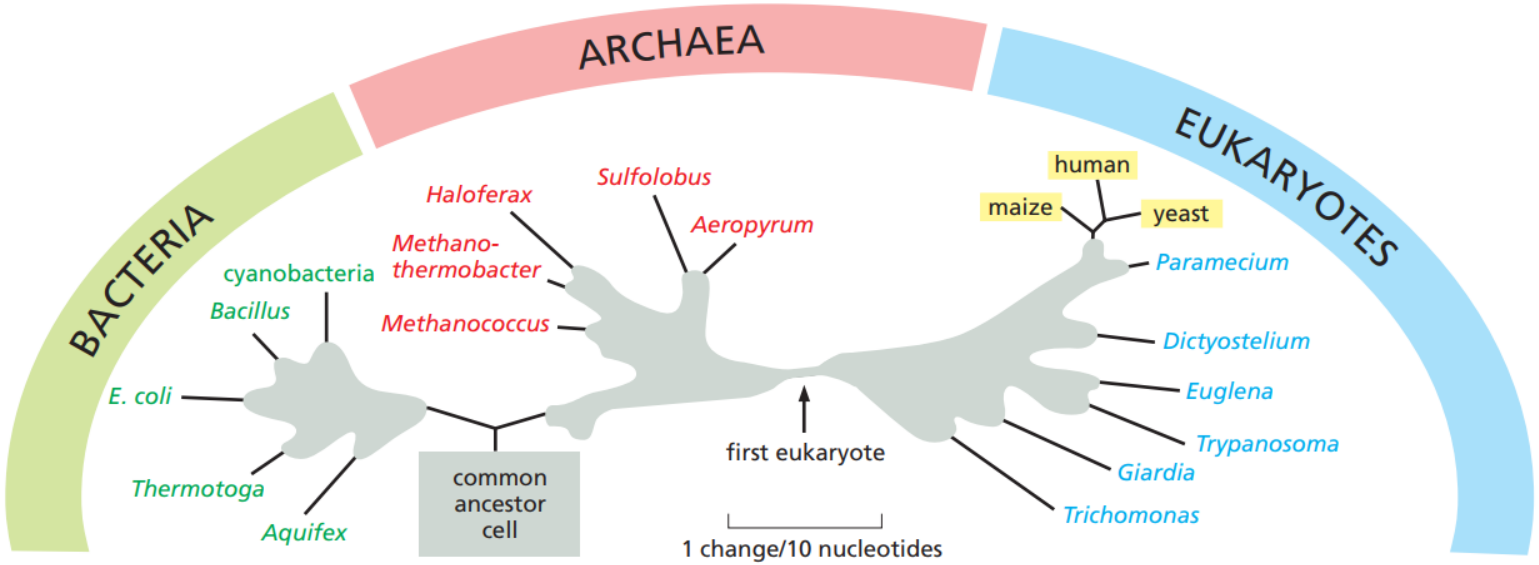
Modul Biologie celulara:
Notiuni microscopie
Evaluare organite celulare;
Culturi de celule;

Modul Biologie moleculara:
Izolarea ADN şi ARN;
Amplificarea ADN/ARN
Electroforeza
Tehnici de detectare a mutaţiilor:
- ASO.
- RFLP.
- DGGE.
- Real-Time PCR.
- Secvenţiere.

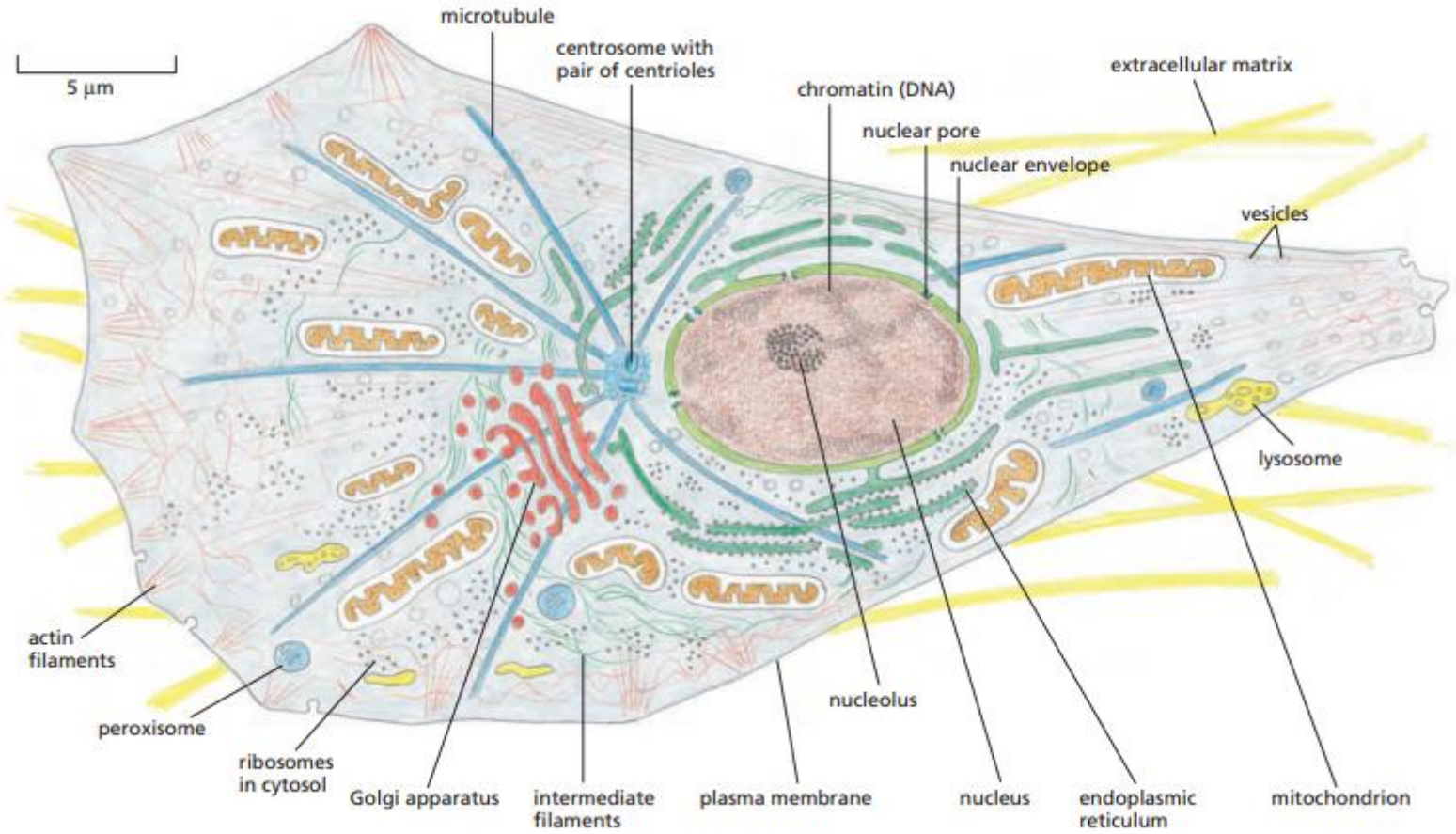
ADN şi ARN non-self.

ABC in biologie celulara si moleculara

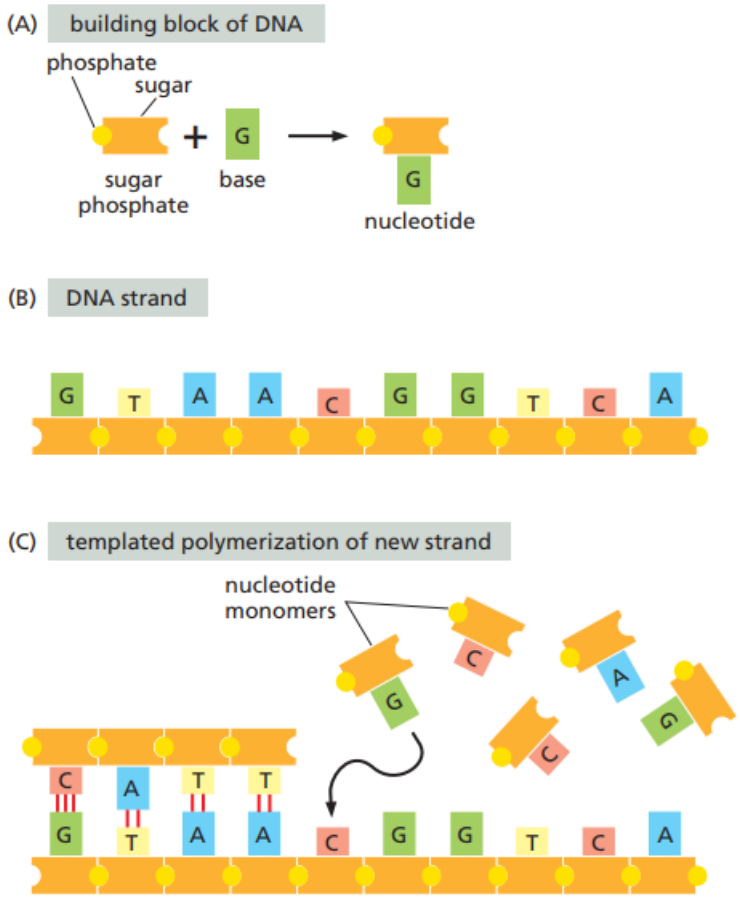
eucariot
procariot



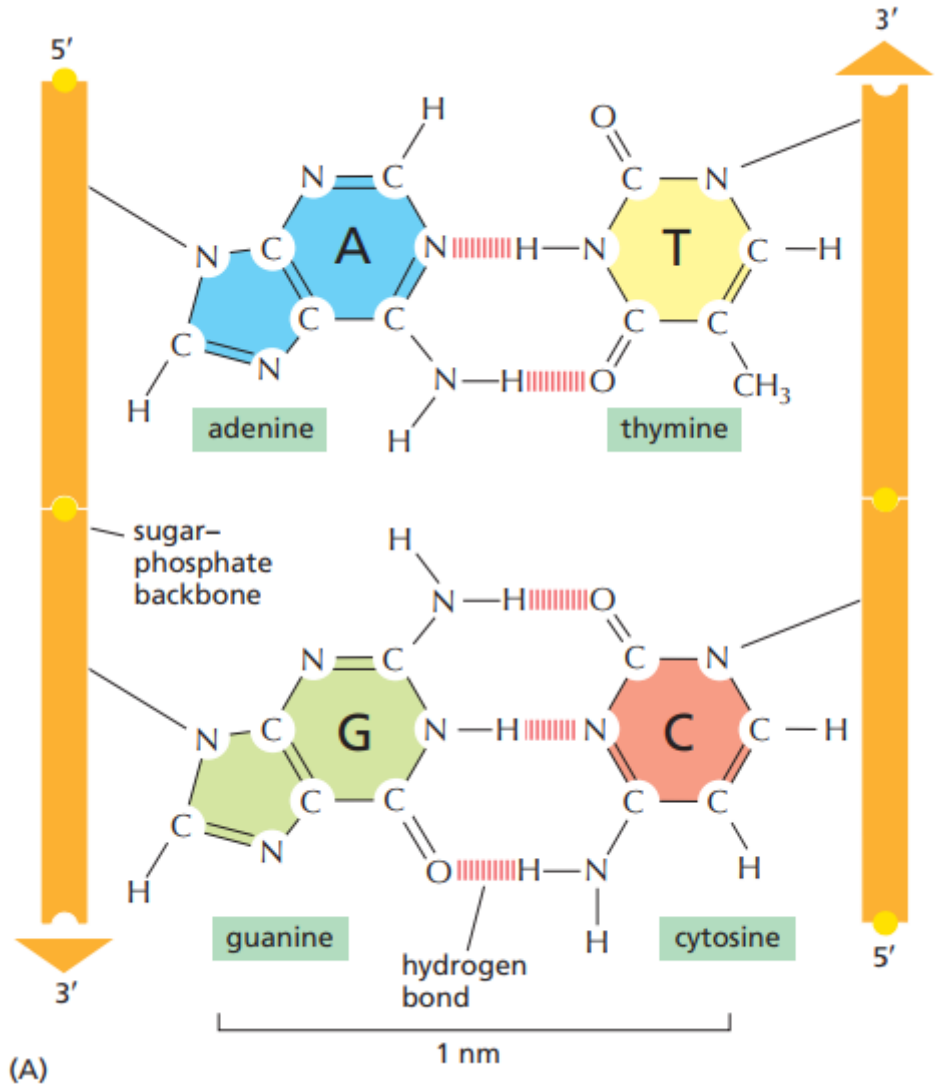
eucariot
procariot



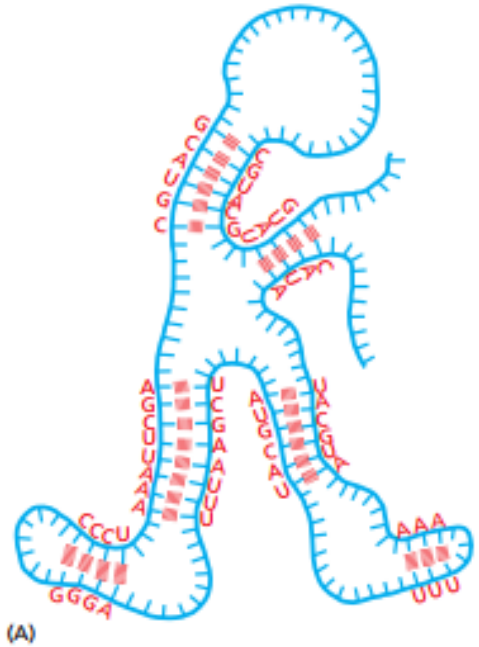
ADN

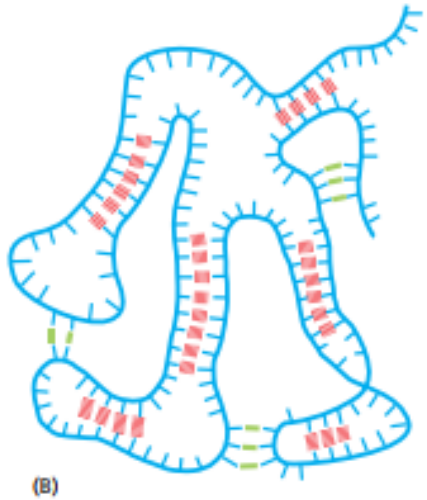

(A) building block of DNA
phosphate
sugar
sugar phosphate
base
nucleotide
(B) DNA strand
(C) templated polymerization of new strand
nucleotide monomers
(D) double-stranded DNA
sugar-phosphate backbone
hydrogen-bonded base pairs
(E) DNA double helix

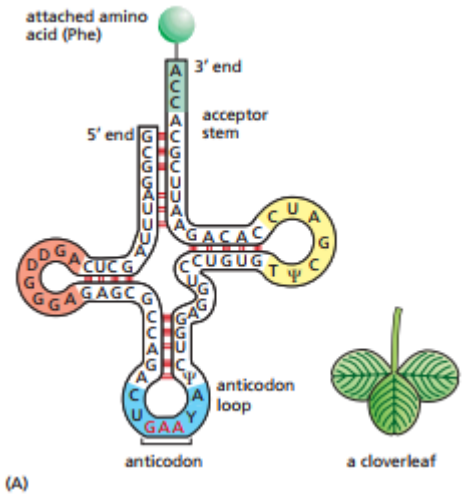
nucleotid


5′
3′
A
T
adenine
thymine
sugar–
phosphate
backbone
G
C
guanine
cytosine
hydrogen
bond
1 nm
3′
5′
(A)

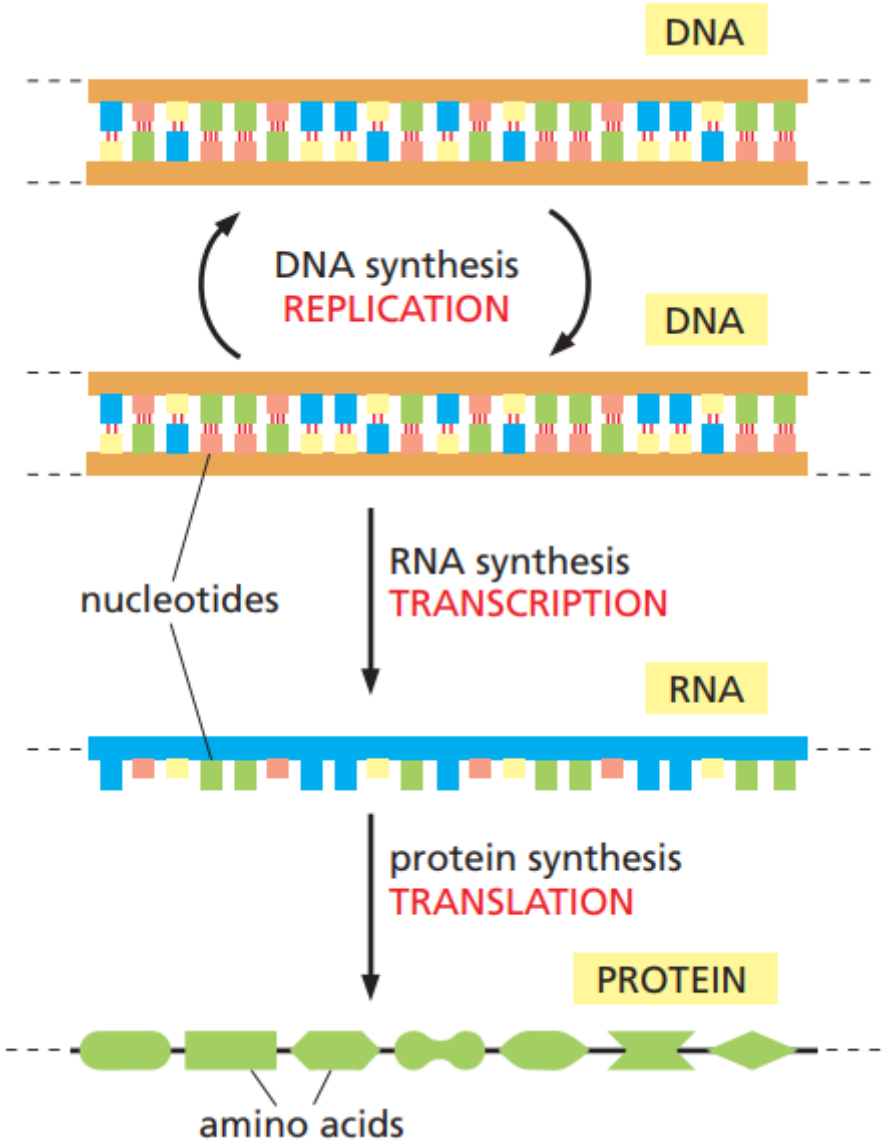
ARNm,t


(A)


(B)

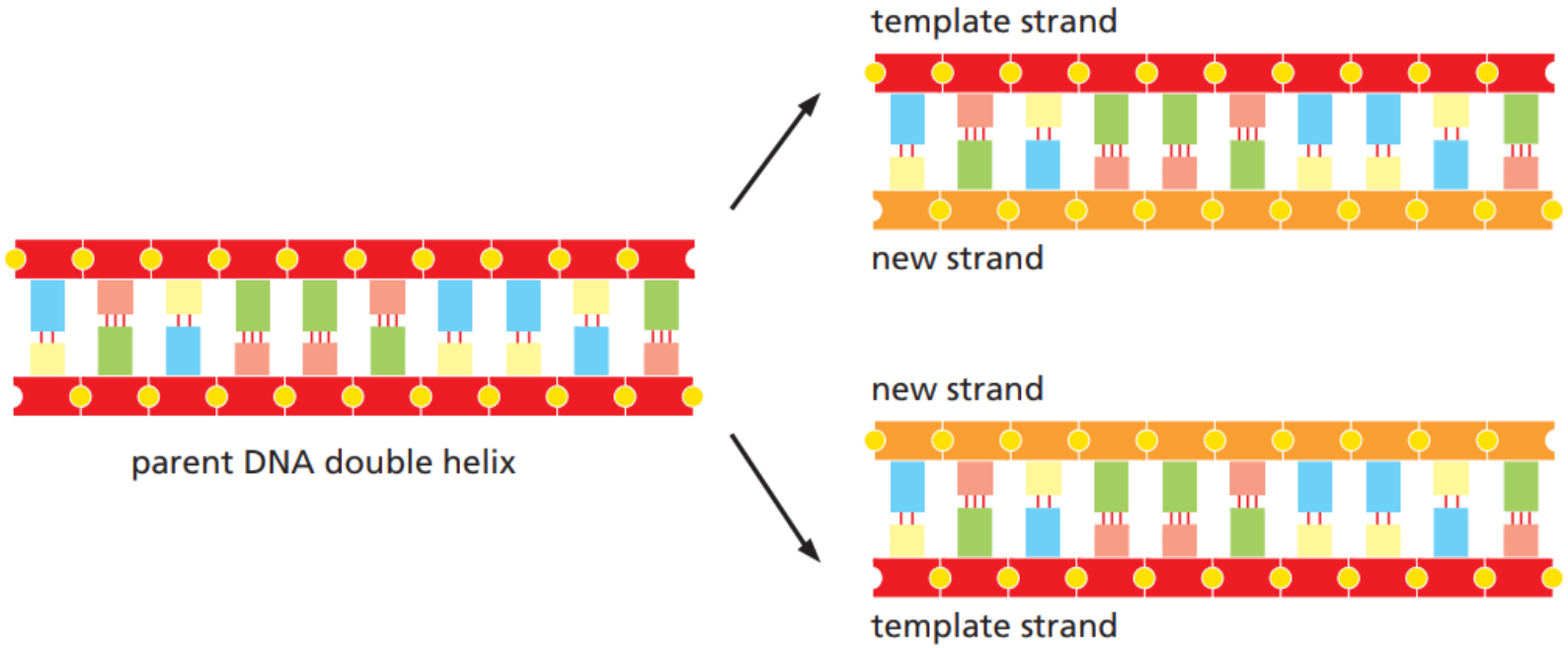

attached amino acid (Phe)
3′ end
acceptor stem
5′ end
anticodon loop
anticodon
a cloverleaf
(A)

Dogma centrala a biologiei



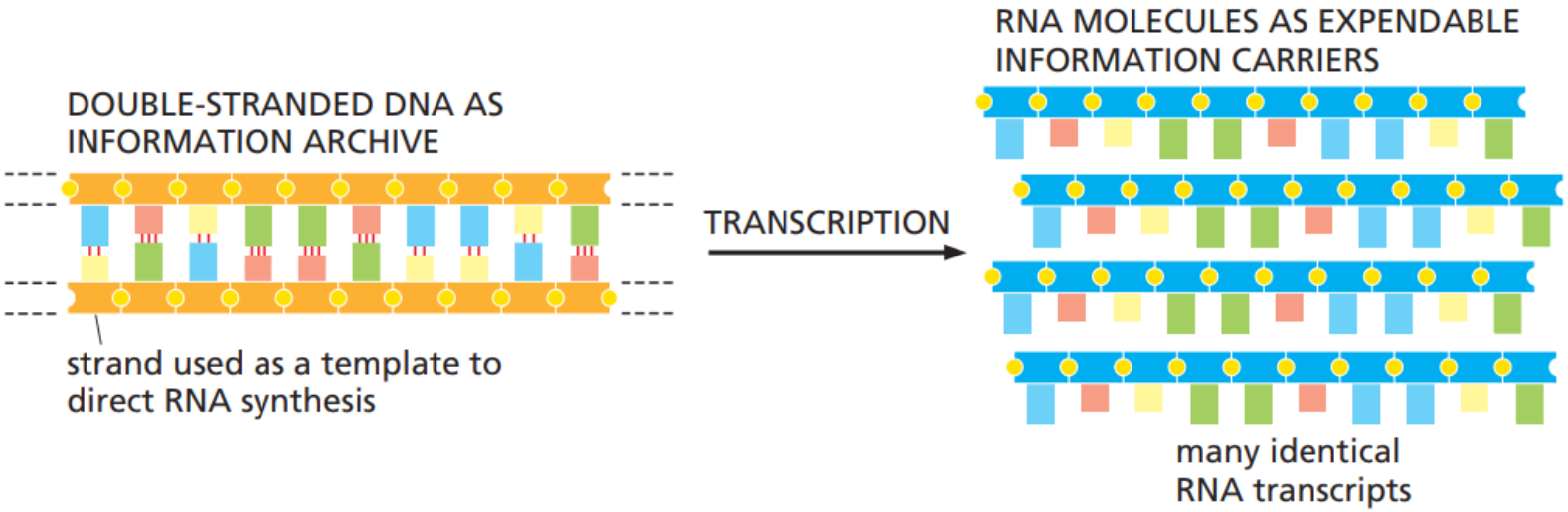
Dogma centrala a biologiei

replicare

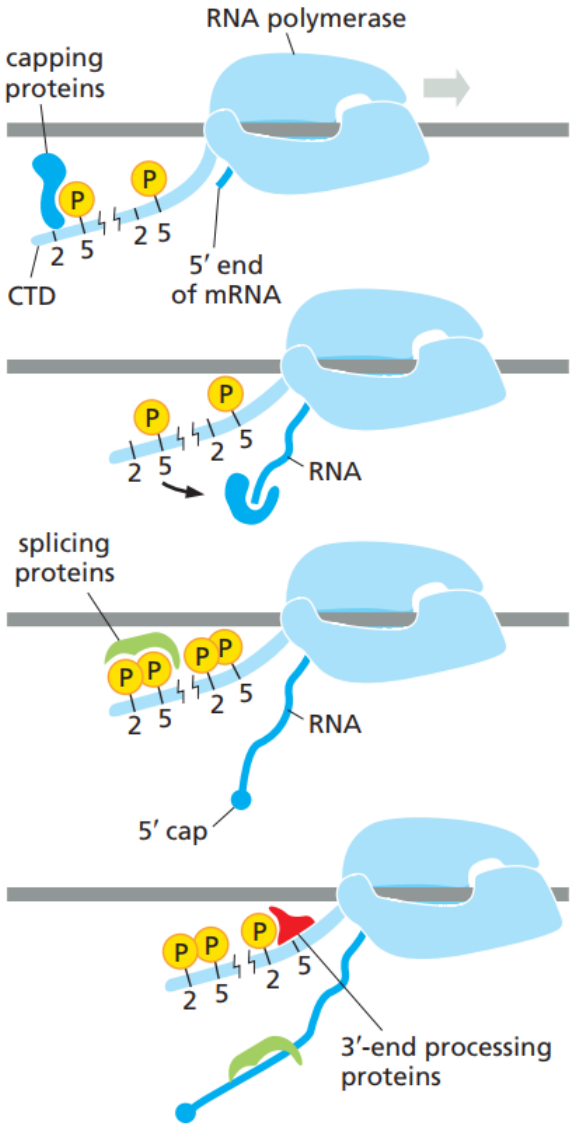


Dogma centrala a biologiei

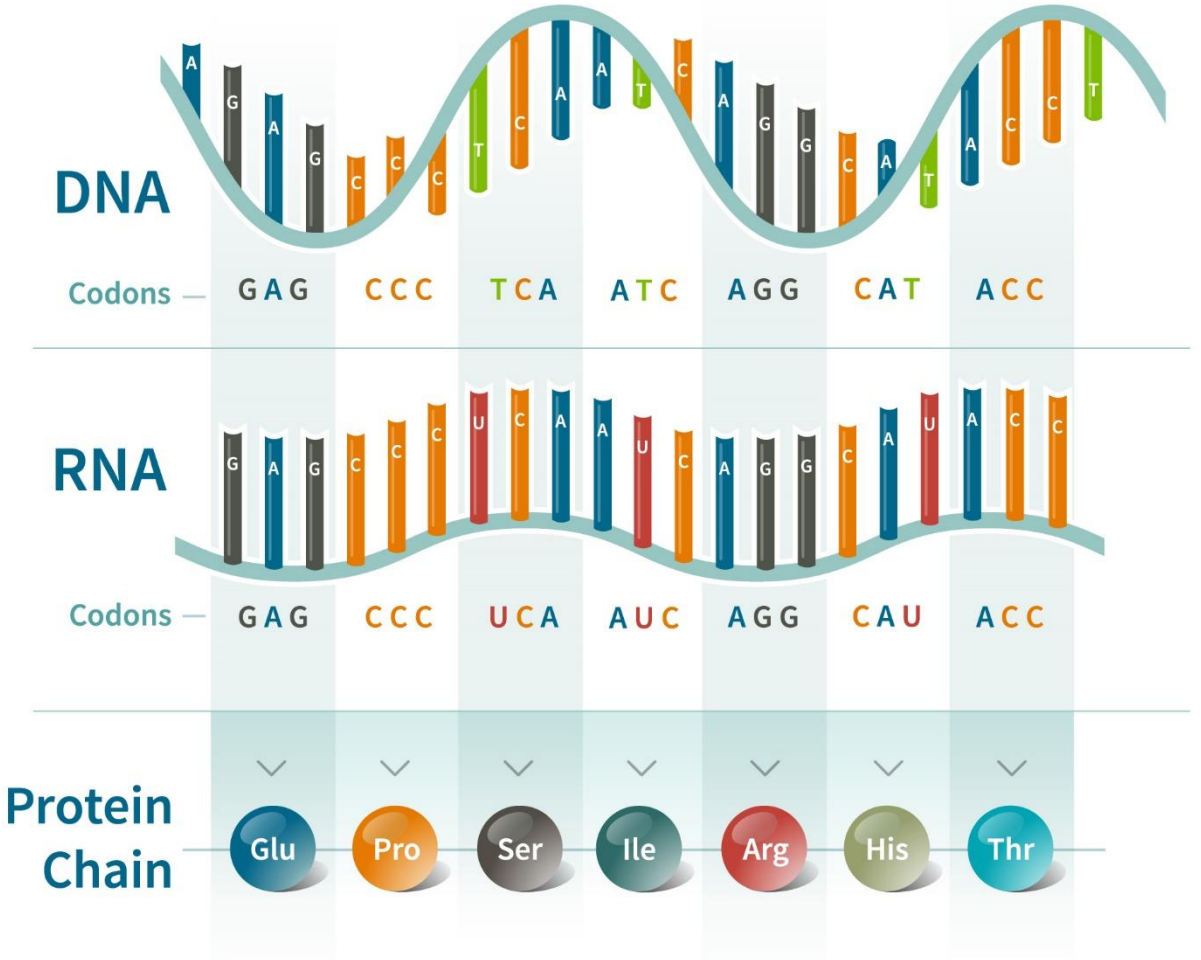
transcriptie



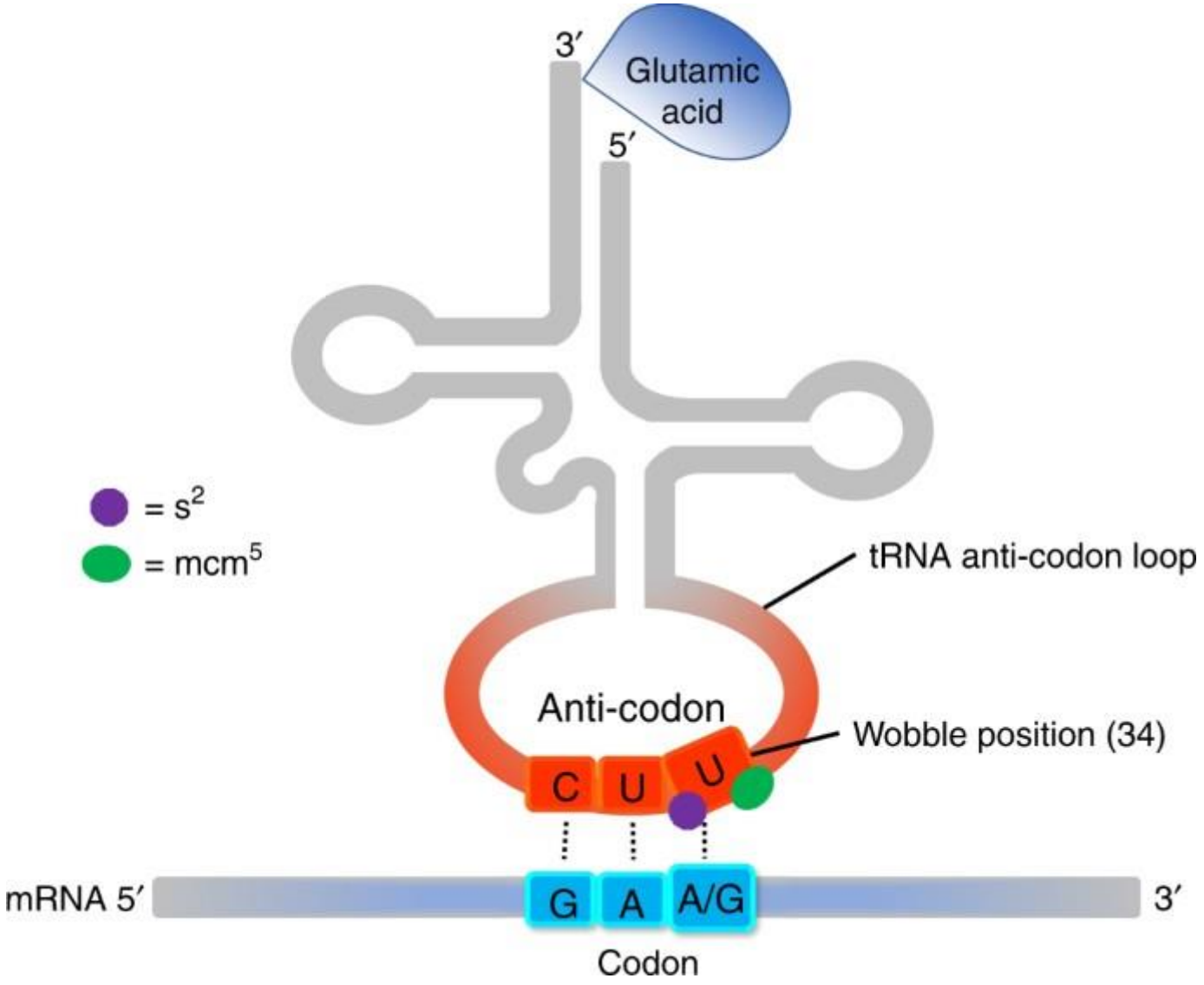
translatie


RNA polymerase
capping proteins
P
P
2 5
2 5
CTD
5′ end of mRNA
P
P
2 5
2 5
RNA
splicing proteins
P
P
P
P
2 5
2 5
RNA
5′ cap
P
P
P
2 5
2 5
3′-end processing proteins

codon


DNA
Codons
GAG
CCC
TCA
ATC
AGG
CAT
ACC
RNA
Codons
GAG
CCC
UCA
AUC
AGG
CAU
ACC
Protein Chain
Glu
Pro
Ser
Ile
Arg
His
Thr

codon


3′
Glutamic acid
5′
= s2
= mcm5
tRNA anti-codon loop
Anti-codon
Wobble position (34)
C
U
U
mRNA 5′
G
A
A/G
3′
Codon

codon

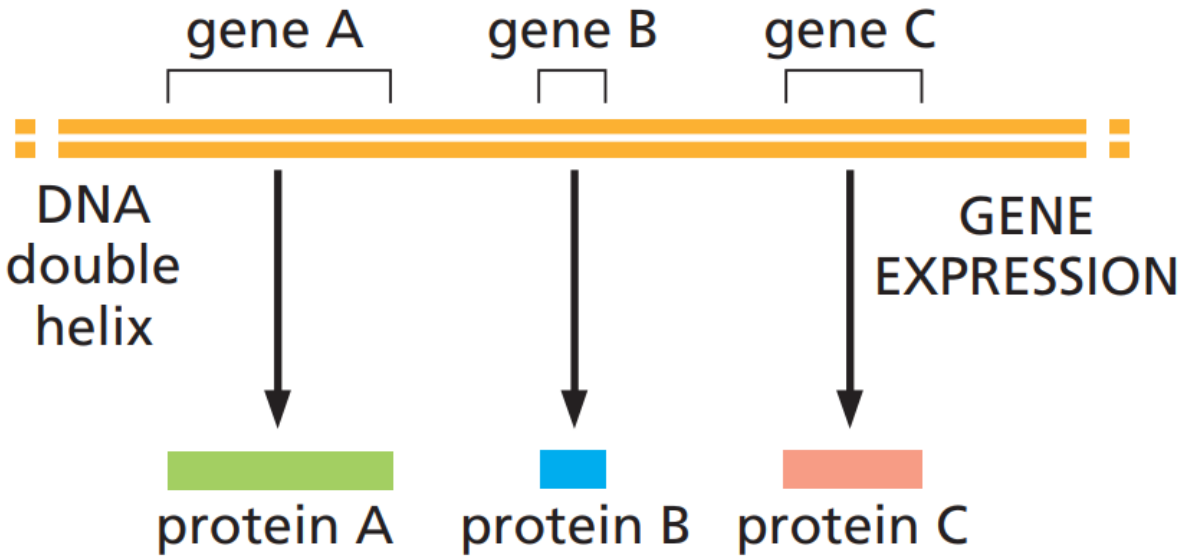
First Base	Second Base: U		Second Base: C		Second Base: A		Second Base: G		Third Base
U	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	U
	UUC		UCC		UAC		UGC		C
	UUA	leu	UCA		UAA	STOP CODON	UGA	STOP CODON	A
	UUG		UCG		UAG		UGG	trp	G
C	CUU	leu	CCU	pro	CAU	his	CGU	arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	ile	ACU	thr	AAU	asn	AGU	ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	lys	AGA	arg	A
	AUG	met (START CODON)	ACG		AAG		AGG		G
G	GUU	val	GCU	ala	GAU	asp	GGU	gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	glu	GGA		A
	GUG		GCG		GAG		GGG		G

TABLE 14–3 Some Differences Between the "Universal" Code and Mitochondrial Genetic Codes*

Codon	"Universal" code	Mitochondrial codes: Mammals	Invertebrates	Yeasts	Plants
UGA	STOP	*Trp*	*Trp*	*Trp*	STOP
AUA	Ile	*Met*	*Met*	*Met*	Ile
CUA	Leu	Leu	Leu	*Thr*	Leu
AGA AGG	Arg	*STOP*	*Ser*	Arg	Arg

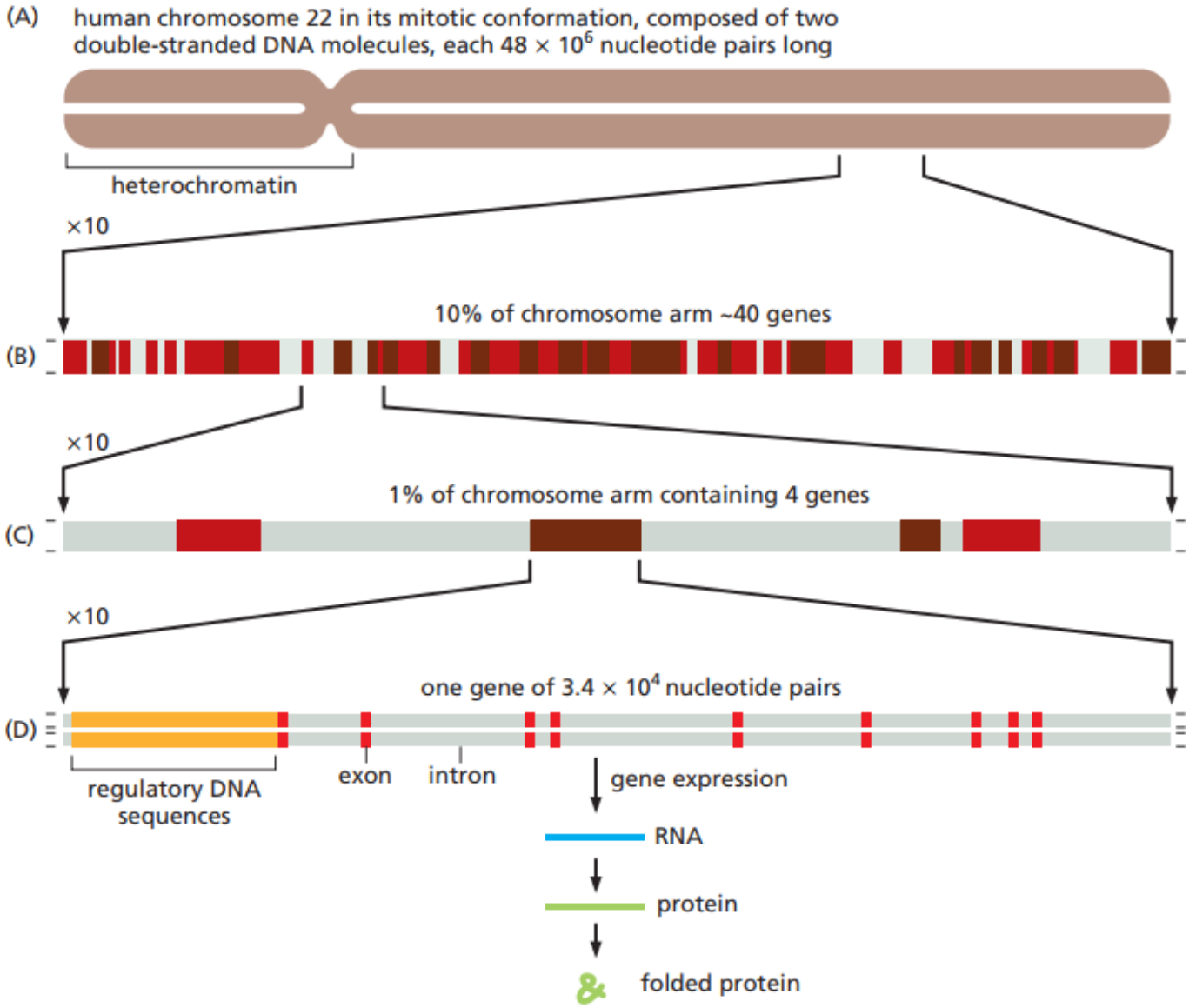
*Red italics indicate that the code differs from the "Universal" code.

gena




```
GTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAACCTCACCC  human
GCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTTAAAGGAATTGGCGGGGGAGCACTACAACGGGTGGAGCCTGCGGTTTAATTGGATTCAACGCCGGGCATCTTACCA  Methanococcus
ACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC.ACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT  E. coli
GTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAACCTCACCC  human
```

intron
exon


(A) human chromosome 22 in its mitotic conformation, composed of two double-stranded DNA molecules, each 48 × 10^6 nucleotide pairs long
heterochromatin
×10
10% of chromosome arm ~40 genes
(B)
×10
1% of chromosome arm containing 4 genes
(C)
×10
one gene of 3.4 × 10^4 nucleotide pairs
(D)
regulatory DNA sequences
exon
intron
gene expression
RNA
protein
folded protein

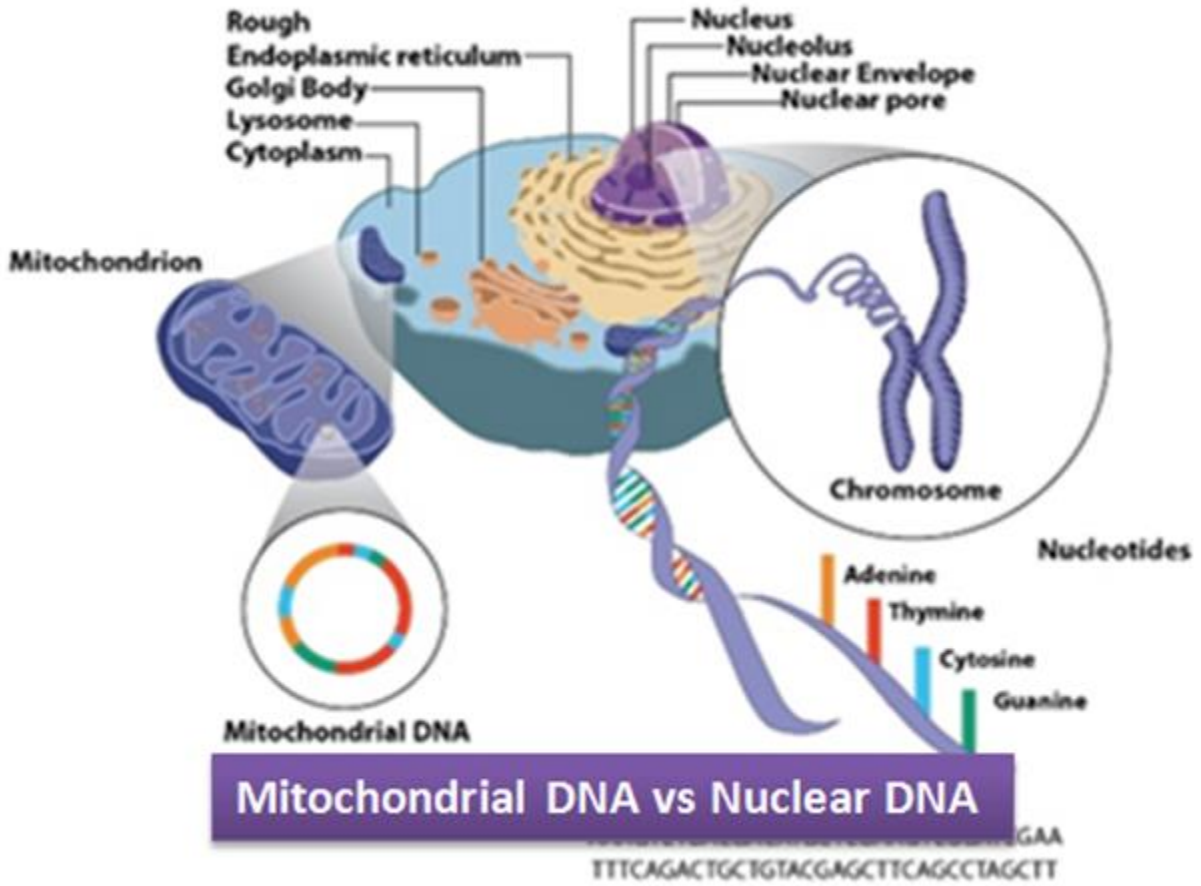
genom

TABLE 1–2 Some Model Organisms and Their Genomes

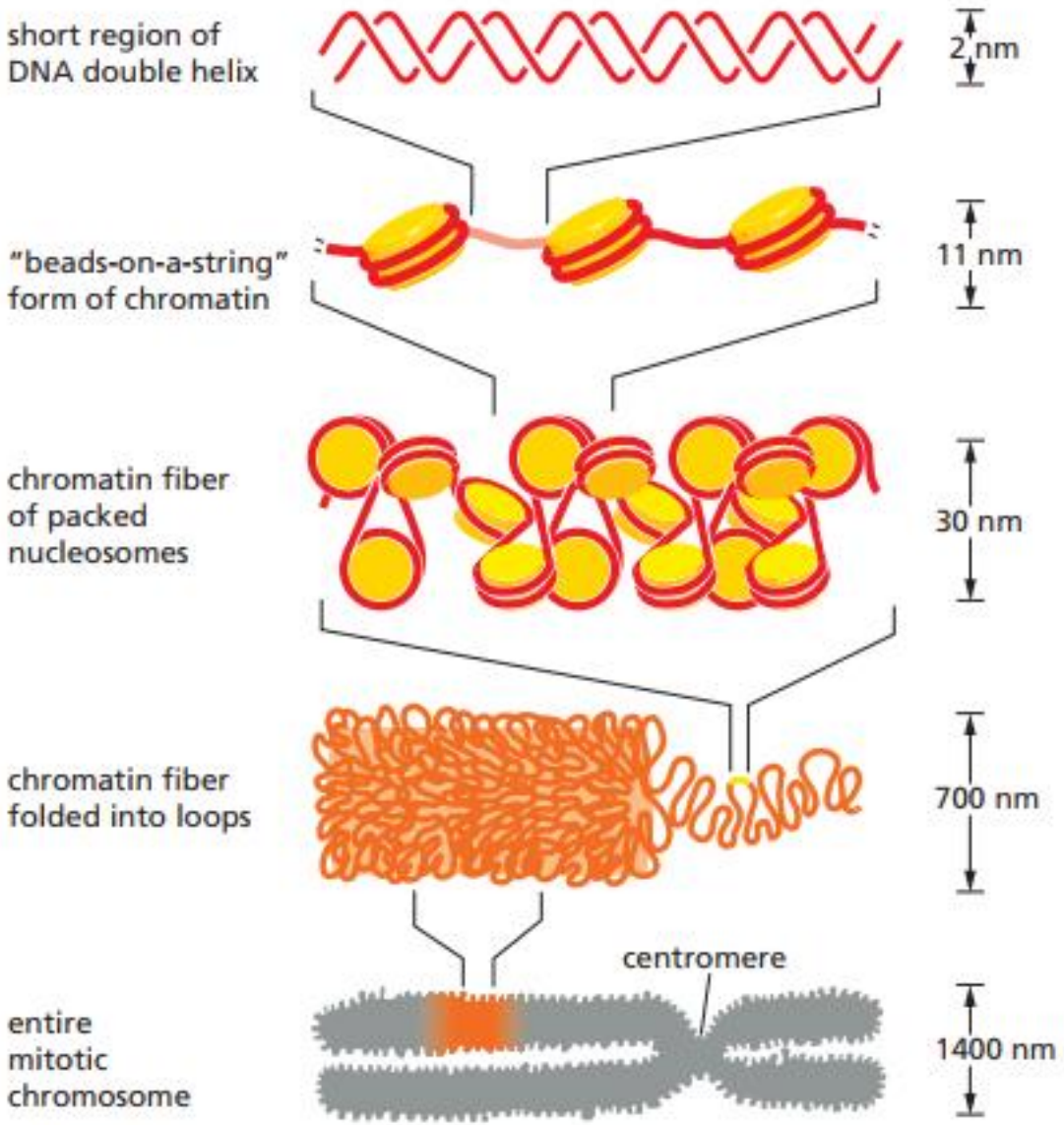
Organism	Genome size* (nucleotide pairs)	Approximate number of genes
Escherichia coli (bacterium)	4.6×10^6	4300
Saccharomyces cerevisiae (yeast)	13×10^6	6600
Caenorhabditis elegans (roundworm)	130×10^6	21,000
Arabidopsis thaliana (plant)	220×10^6	29,000
Drosophila melanogaster (fruit fly)	200×10^6	15,000
Danio rerio (zebrafish)	1400×10^6	32,000
Mus musculus (mouse)	2800×10^6	30,000
Homo sapiens (human)	3200×10^6	30,000

*Genome size includes an estimate for the amount of highly repeated DNA sequence not in genome databases.

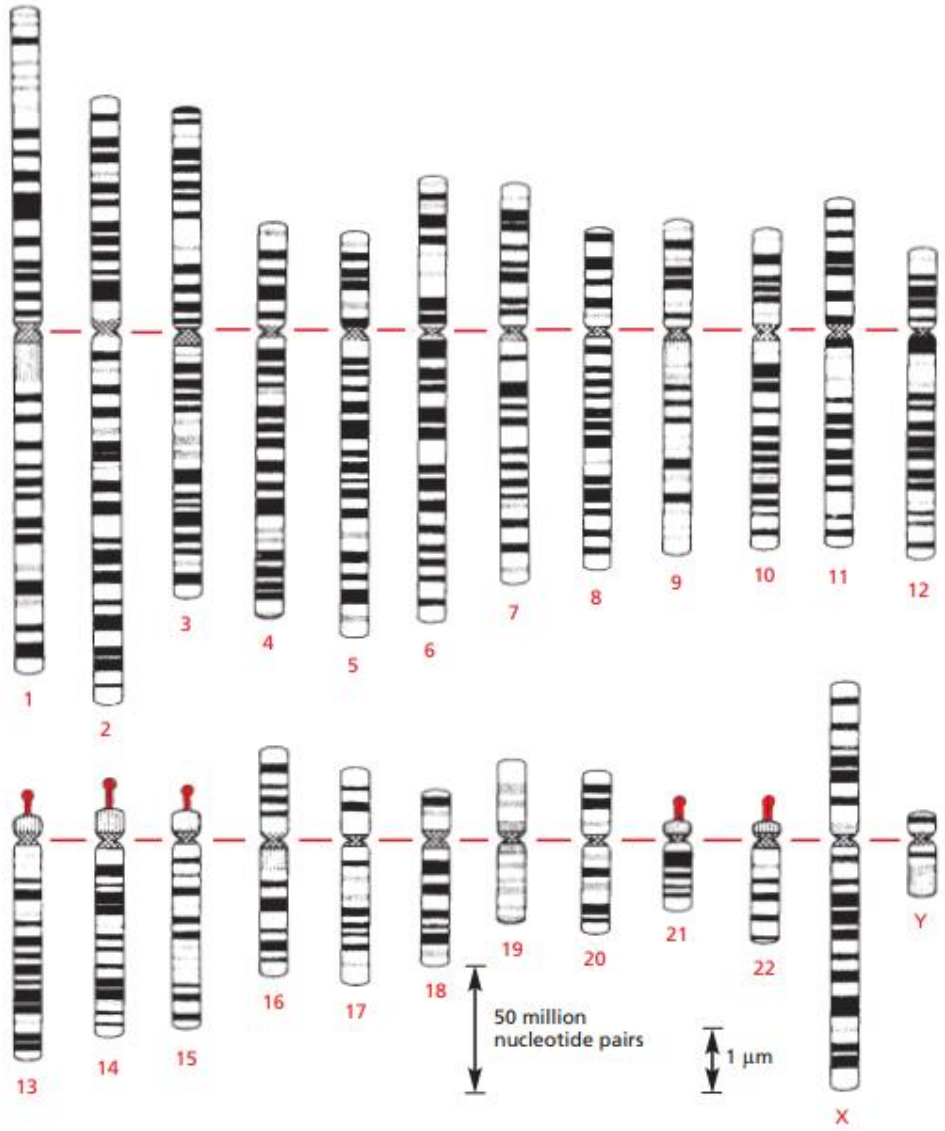
genom nuclear, mitocondrial



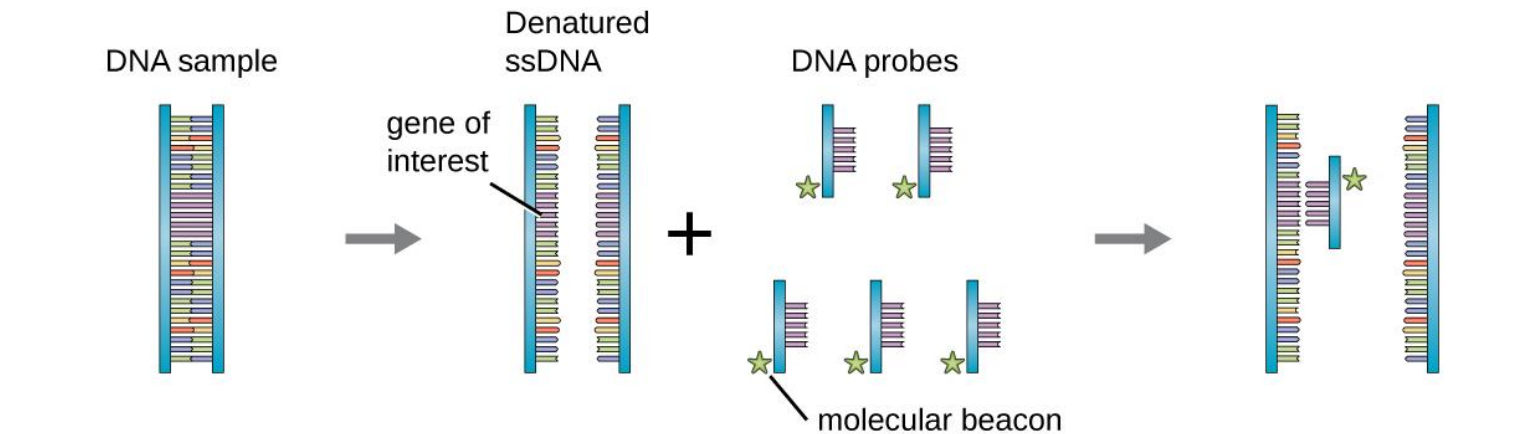
cromatina


short region of
DNA double helix
2 nm
"beads-on-a-string"
form of chromatin
11 nm
chromatin fiber
of packed
nucleosomes
30 nm
chromatin fiber
folded into loops
700 nm
centromere
entire
mitotic
chromosome
1400 nm

cromozom
cariotip



sonda


DNA sample
Denatured ssDNA
gene of interest
DNA probes
molecular beacon
1 Isolate DNA from body fluid sample.
2 Denature DNA sample and combine with DNA probes. Probes are complementary to the gene of interest and labeled with a molecular beacon.
3 DNA probes will bind to the gene of interest if it is present in the DNA sample.

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Electroforeza

Tehnici de detectare a mutaţiilor:

- ASO.
- RFLP.
- DGGE.
- Real-Time PCR.
- Secvenţiere.

ADN şi ARN non-self.

Principiile microscopiei optice. Tipuri speciale de microscopie optică. Microscopul electronic. Principii. Interpretarea imaginilor de ME.

- Scopul microscopiei:
  - Diagnostic
  - Cercetare
- Microscopia optica:
  - Componentele microscopului optic
  - Principiul de functionare
  - Preparate pentru microscopie optica
- Vizualizare la microscopul optic. Imagini.
- Microscopia cu fluorescenta
- Microscopia electronica

Principii microscopie

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- Diagnostic
- Cercetare

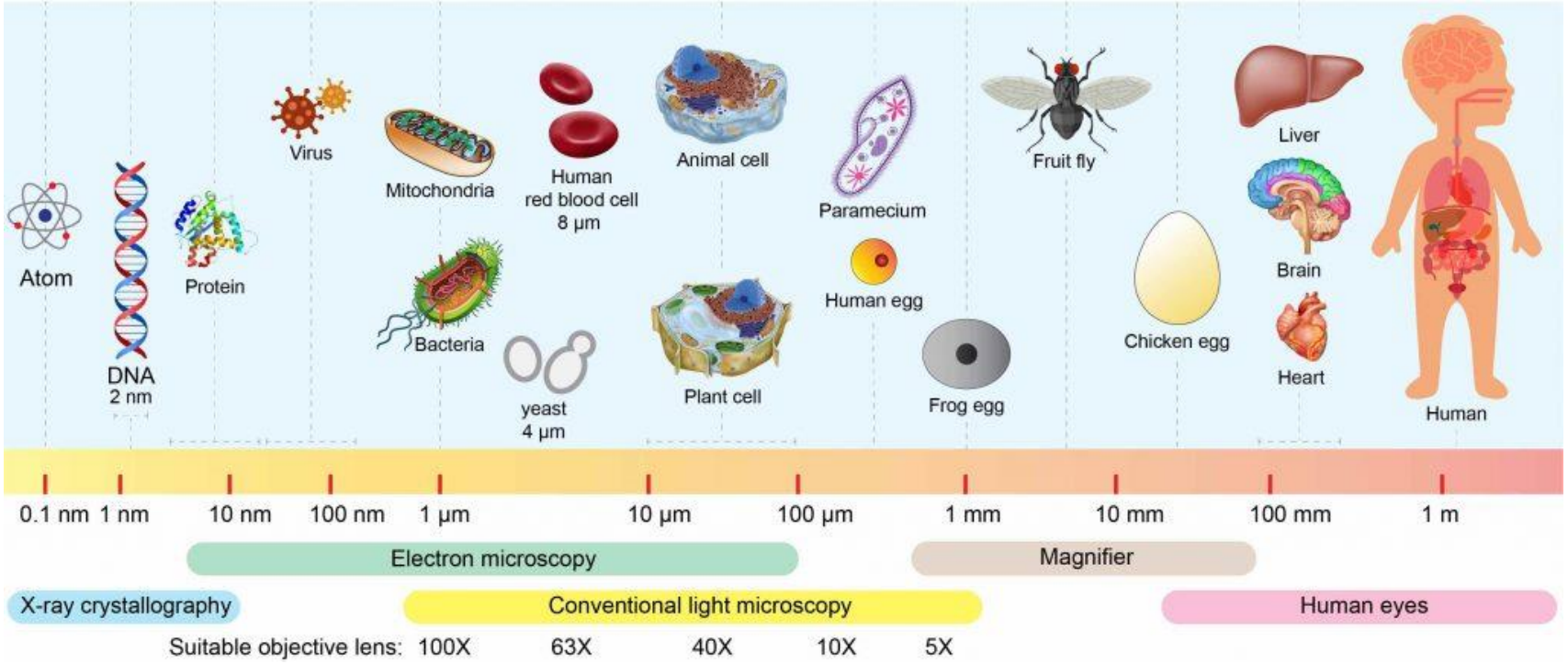
Microscopia optica:

- Componentele microscopului optic
- Principiul de functionare
- Preparate pentru microscopie optica

Vizualizare la microscopul optic. Imagini.

Microscopia cu fluorescenta

Microscopia electronica



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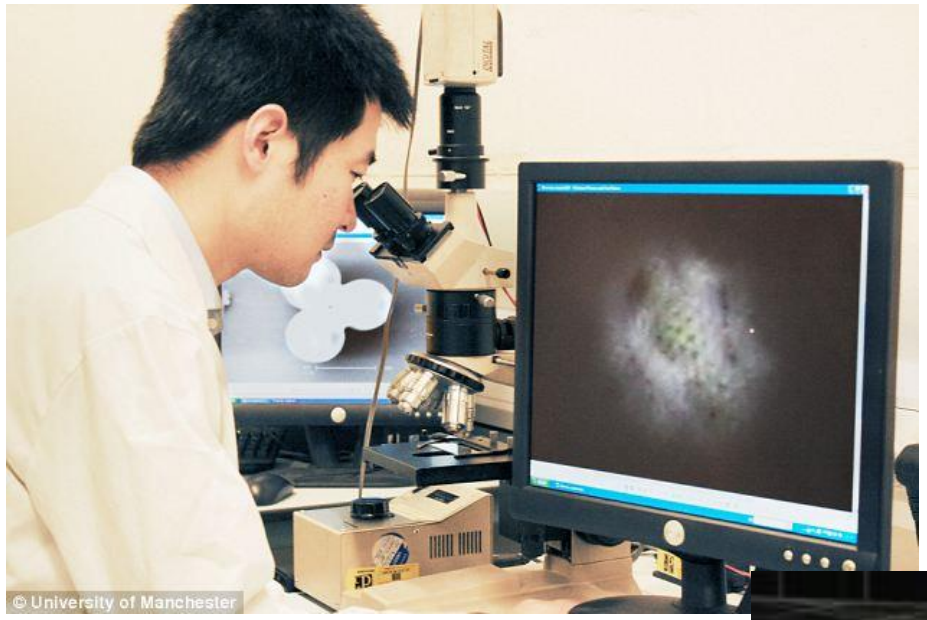
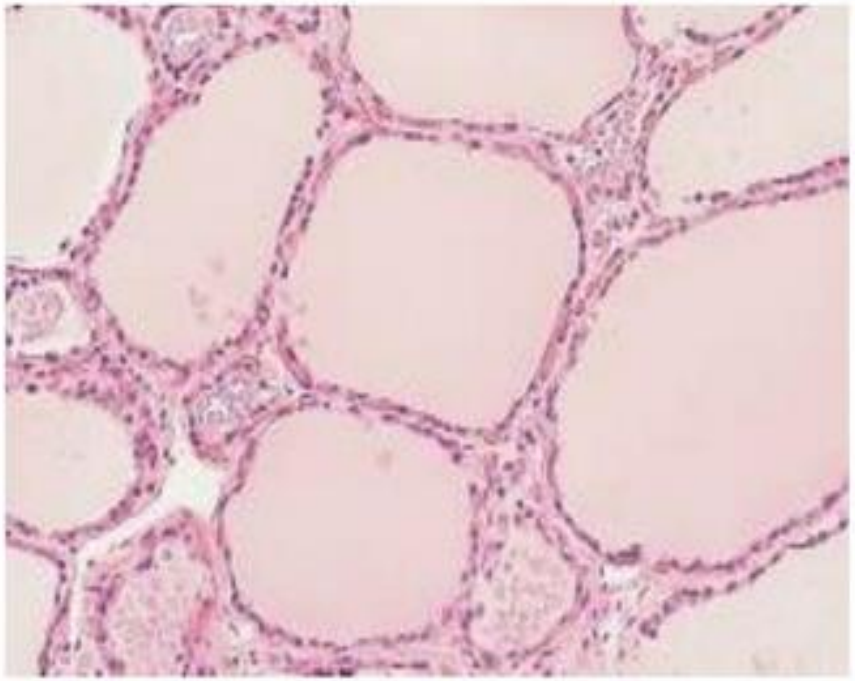
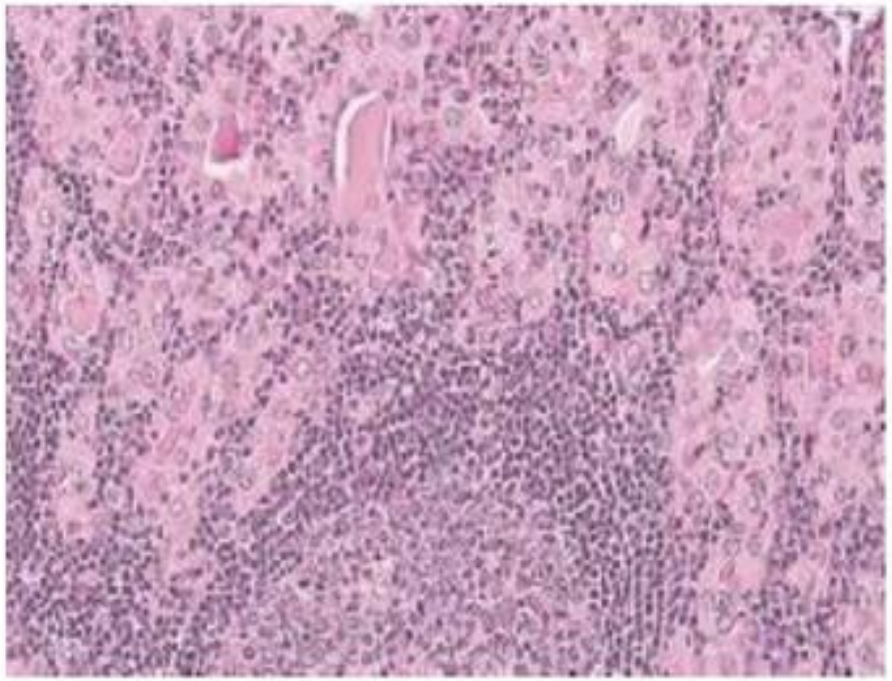
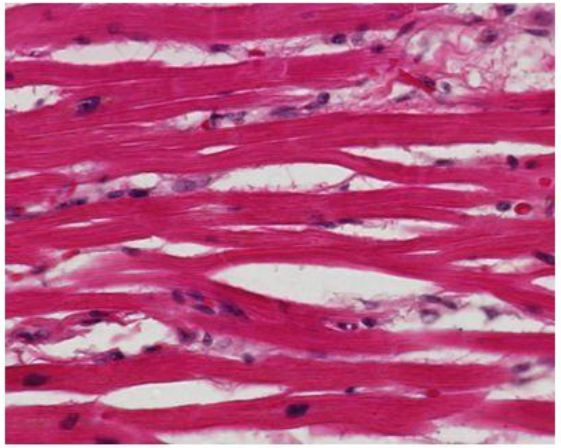




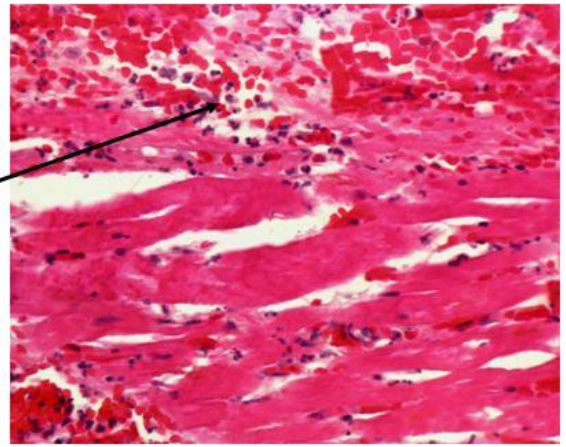


Histology Report

Myocardial Reference Slides



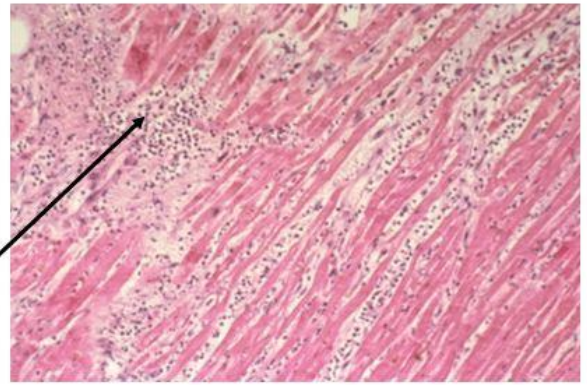
Normal Heart Tissue



Increased number of black dots indicates inflammation.

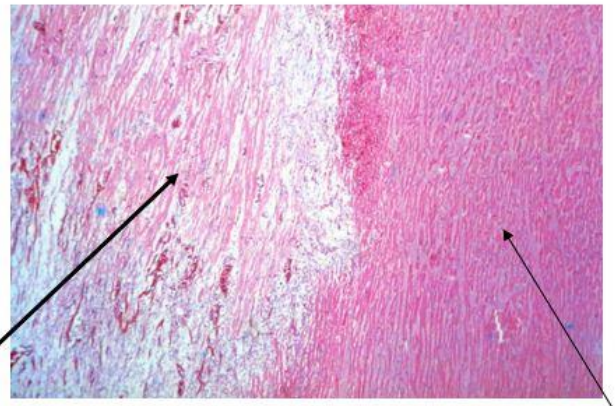
8 – 12 Hours After Myocardial Infarction (MI)

Myocardial Reference Slides



Increased number of black dots indicates inflammation.

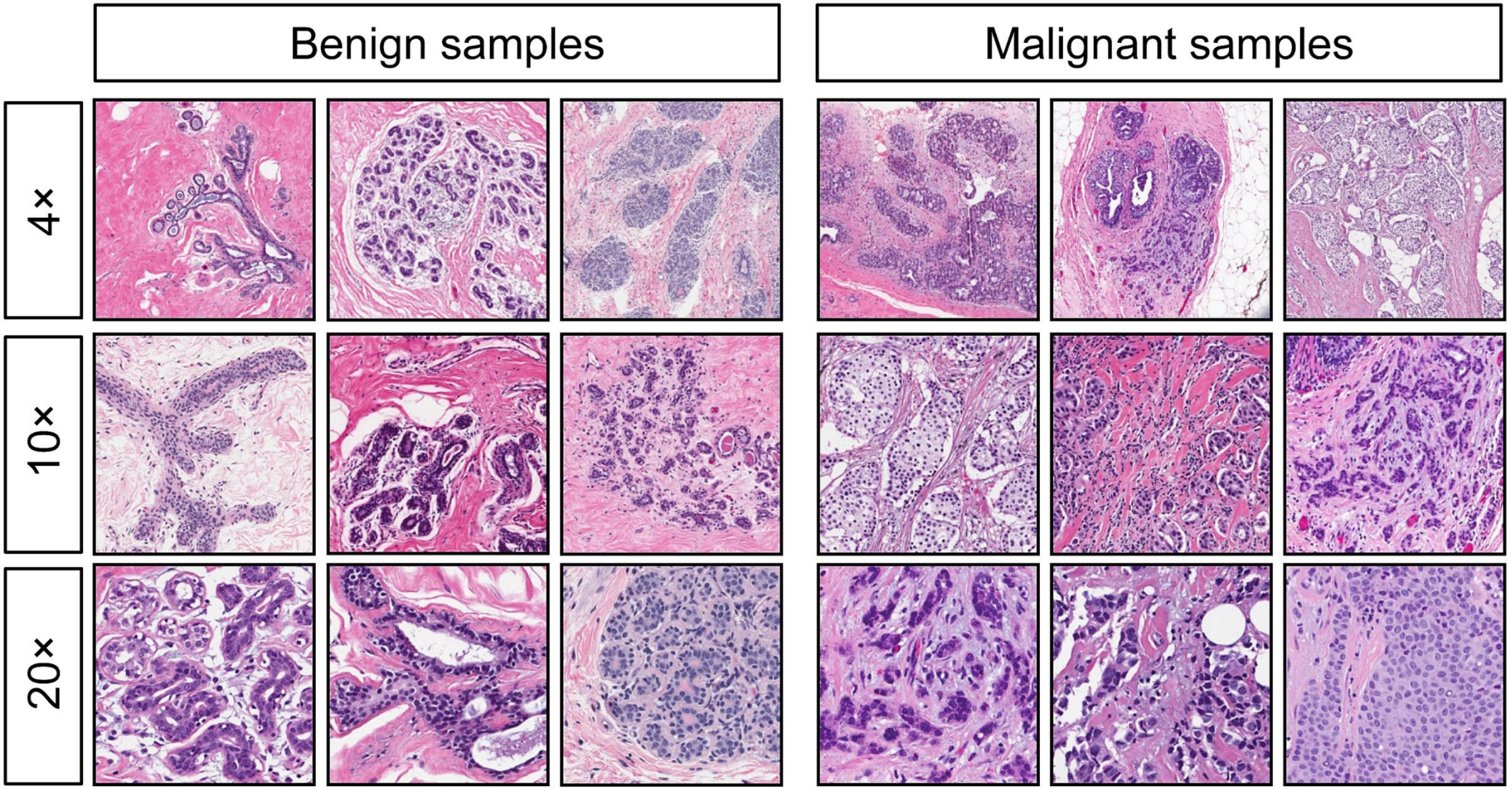
2 – 4 Days After MI Shows Loss of Muscle Tissue



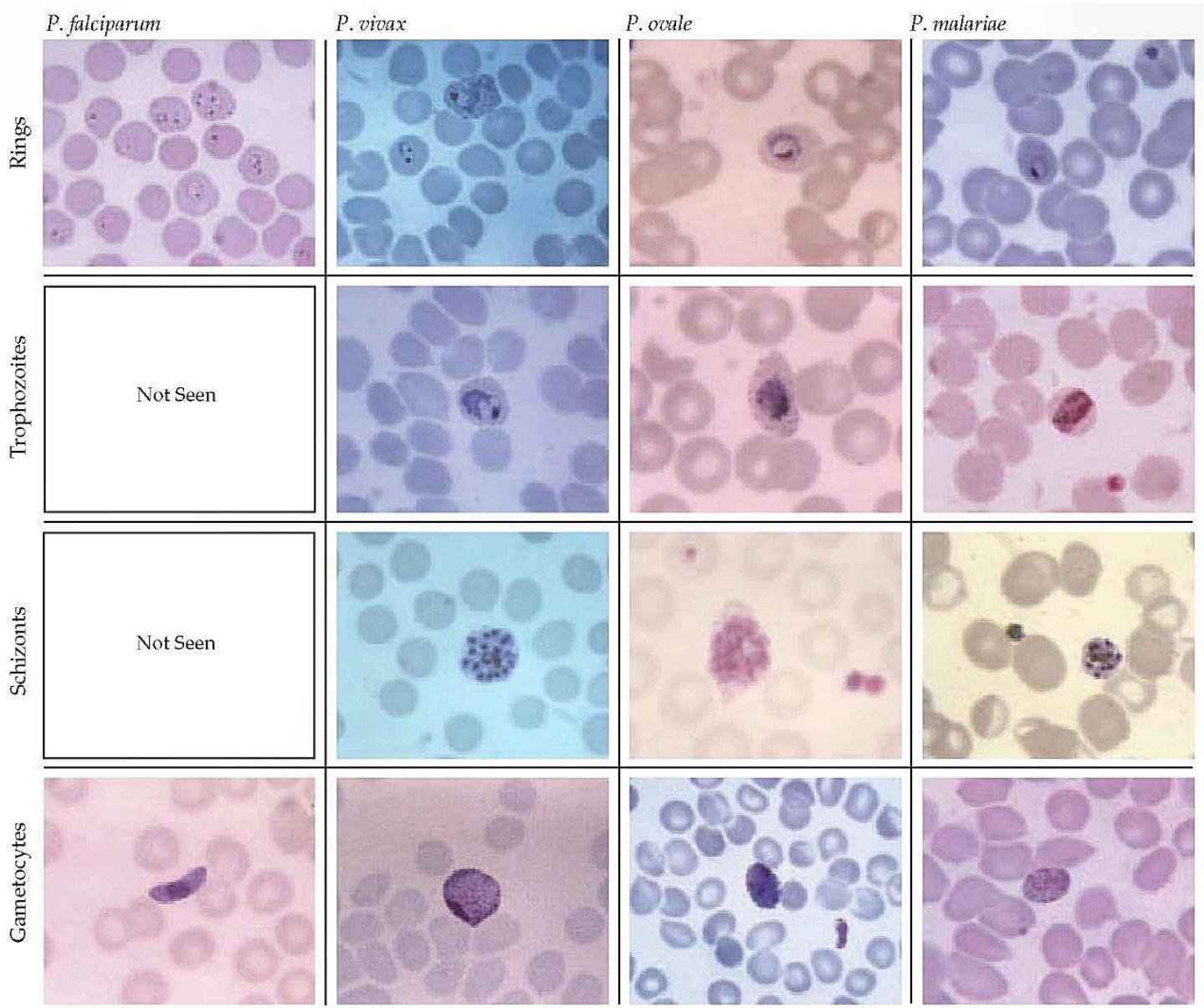
White tissue indicates healing - granulation of tissue.

Healing MI

Healthy tissue with good blood supply



https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0141357.g002



http://clinical-laboratory.blogspot.com/2016/08/laboratory-identification-of-malaria.html?m=1

Principii microscopie

Scopul microscopiei:

- Diagnostic
- Cercetare

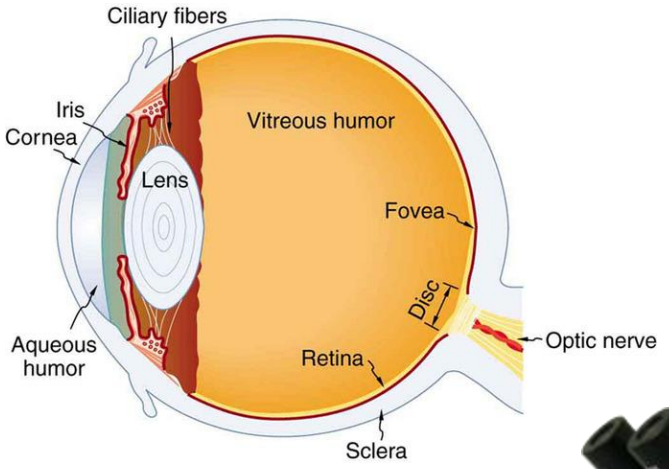
Microscopia optica:

- **Sisteme optice**
  - Componentele microscopului optic
  - Principiul de functionare

Vizualizare la microscopul optic. Imagini.

Microscopia cu fluorescenta

Microscopia electronica



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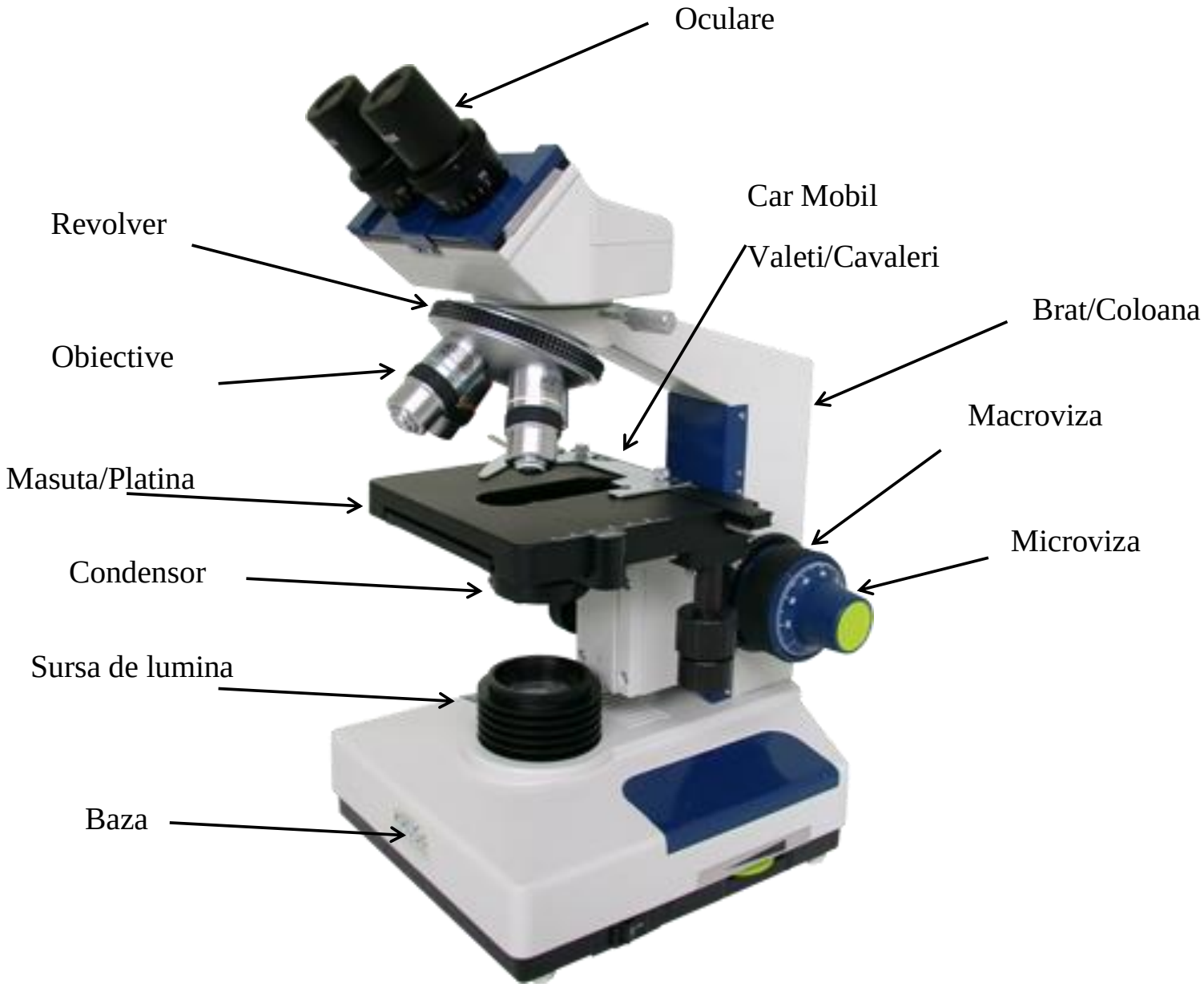
Microscopia electronica


Oculare
Car Mobil
Revolver
Valeti/Cavaleri
Brat/Coloana
Obiective
Macroviza
Masuta/Platina
Microviza
Condensor
Sursa de lumina
Baza

Principii microscopie

Scopul microscopiei:

- Diagnostic
- Cercetare

Microscopia optica:

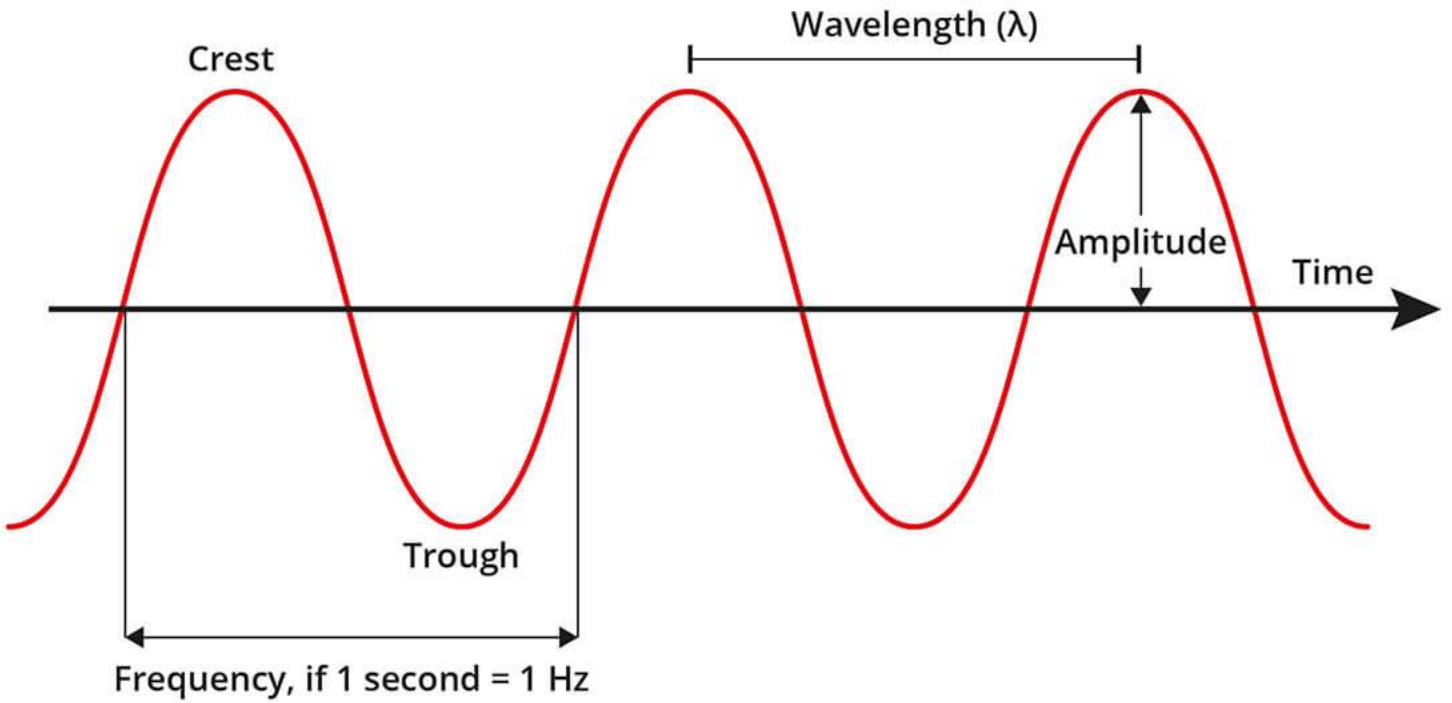
- Sisteme optice
- Componentele microscopului optic
- **Principiul de functionare**

Vizualizare la microscopul optic. Imagini.

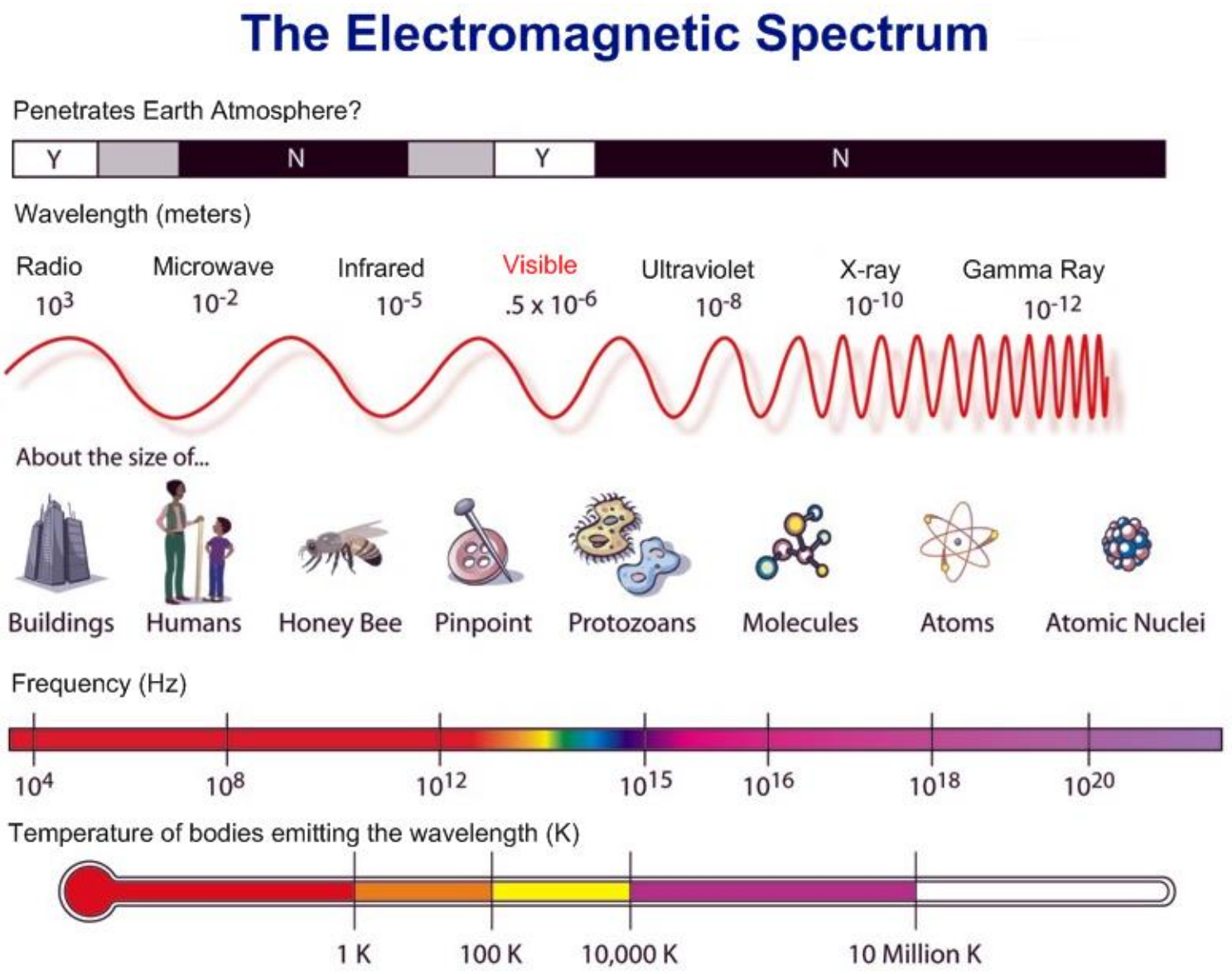
Microscopia cu fluorescenta

Microscopia electronica

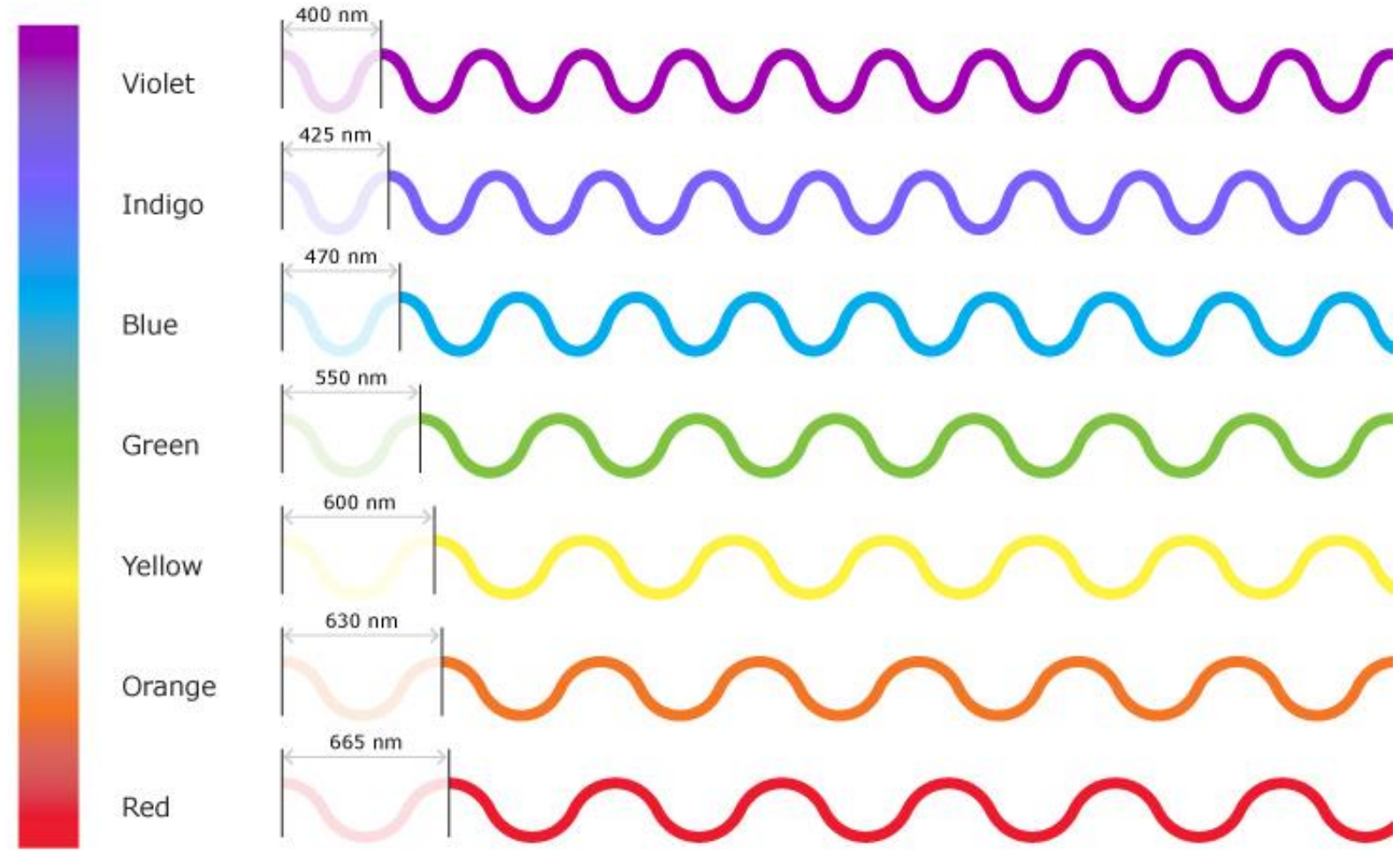
Caracteristicile unei unde



Spectrul electromagnetic



Spectrul vizibil



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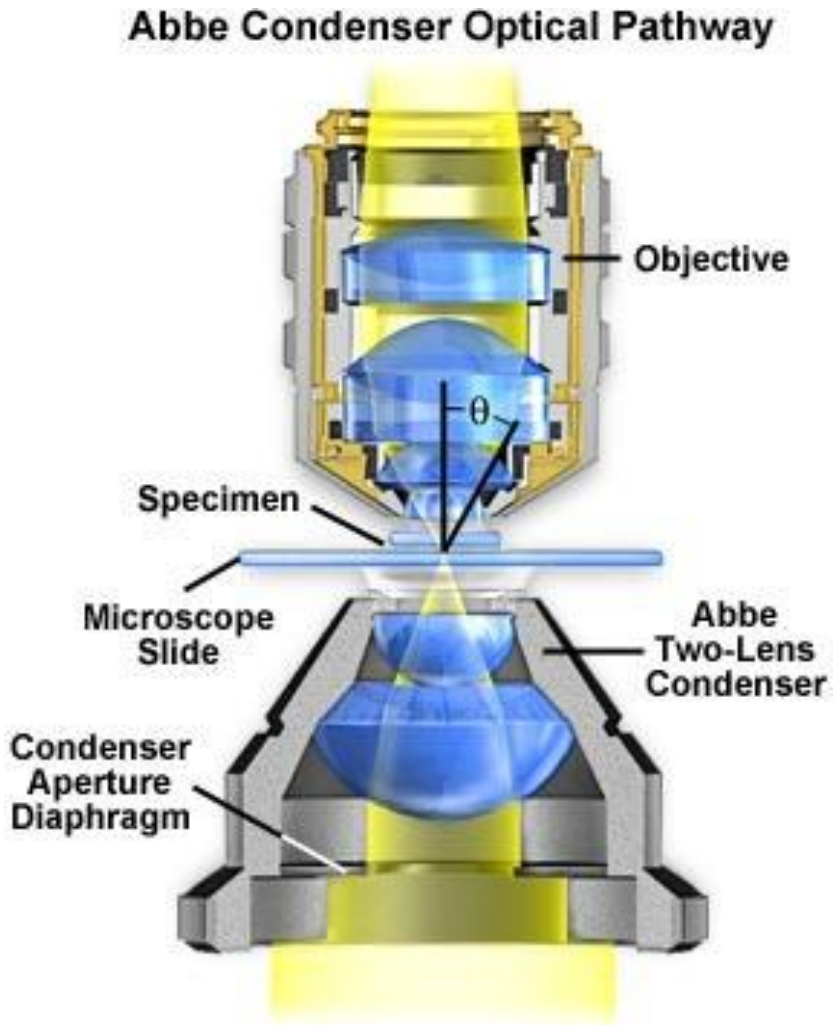
Traiectul optic

Traiectul optic



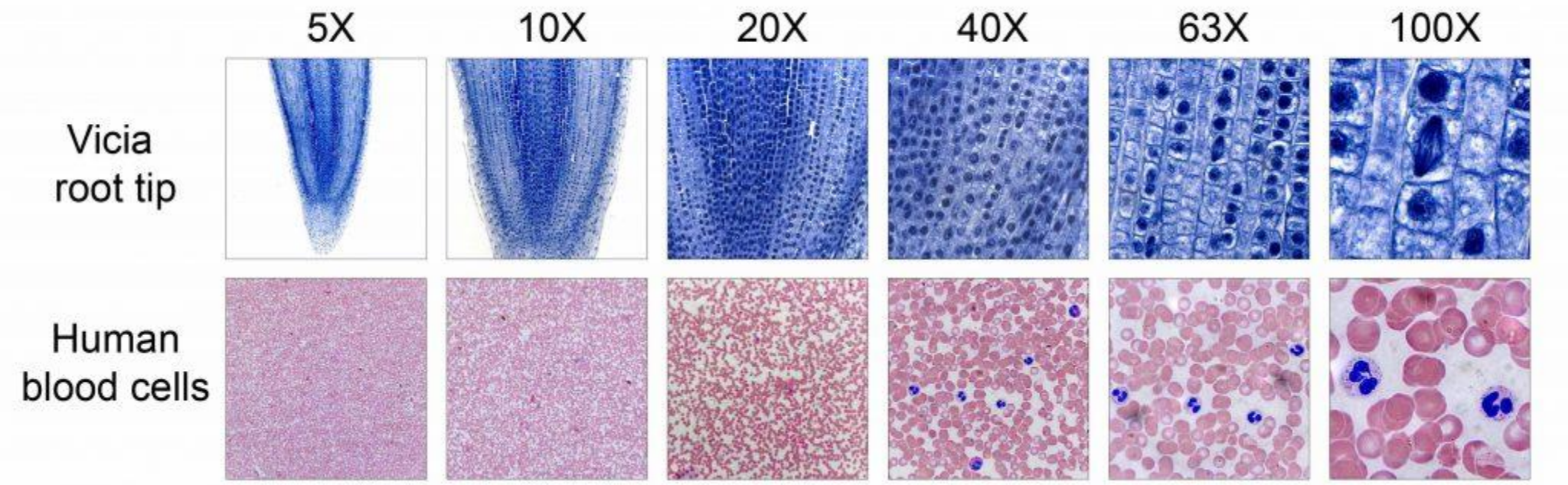
PUTEREA DE MARIRE

Puterea de mărire este produsul puterilor de mărire ale componentelor optice componente:

$\mathbf{M}_{\text{Microscop}} = \mathbf{M}_{\text{Obiectiv}} * \mathbf{M}_{\text{Ocular}}$, unde

M = puterea de mărire

A 4X/0.10
∞/–
A 10X/0.25
∞/–
A 40X/0.65
∞/0.17
A 100X/ 1.25OIL
∞ / 0.17


5X
10X
20X
40X
63X
100X
Vicia root tip
Human blood cells

PUTEREA DE REZOLUȚIE

LEGEA LUI ABBÈ

Rezoluția maximă

$$R0=(0.61*\lambda)/(n*\sin\alpha\ ),\ \text{unde}$$

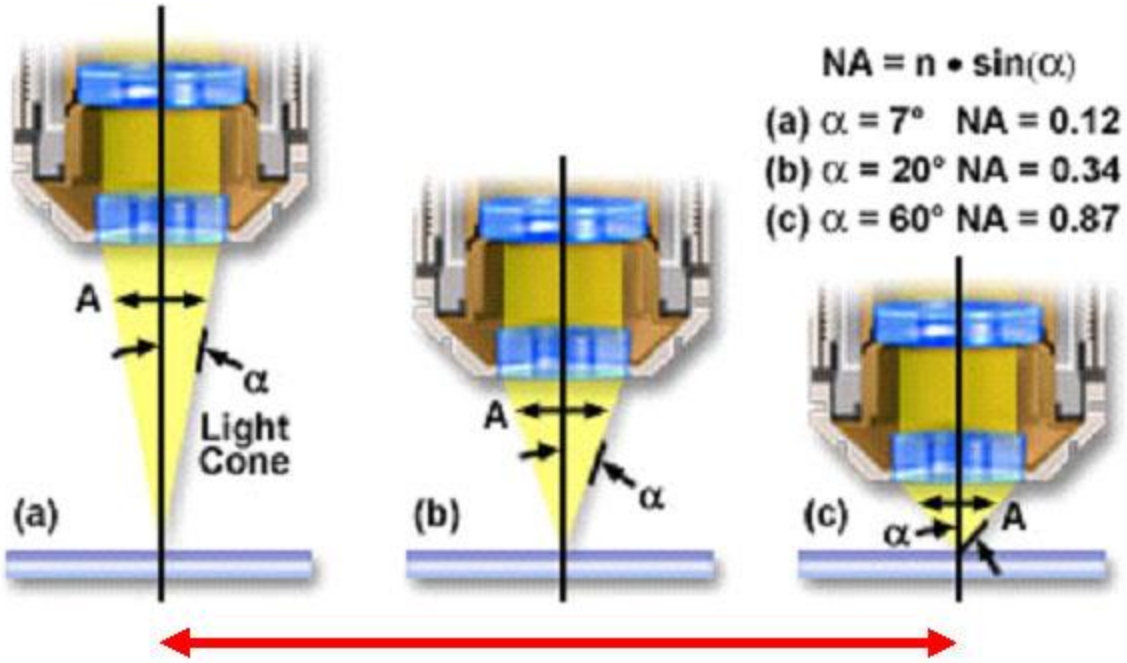
0.61 este o constantă geometrică general valabilă sistemelor optice

$\boldsymbol{\lambda}$ = lungimea de undă a luminii

$n*\sin\alpha$ = apertura numerica

n = index de refracție a mediului,

α = unghiul de apertură



Small Angle
Low NA
Large Details

Large Angle
High NA
Small Details

Oil Immersion and Numerical Aperture

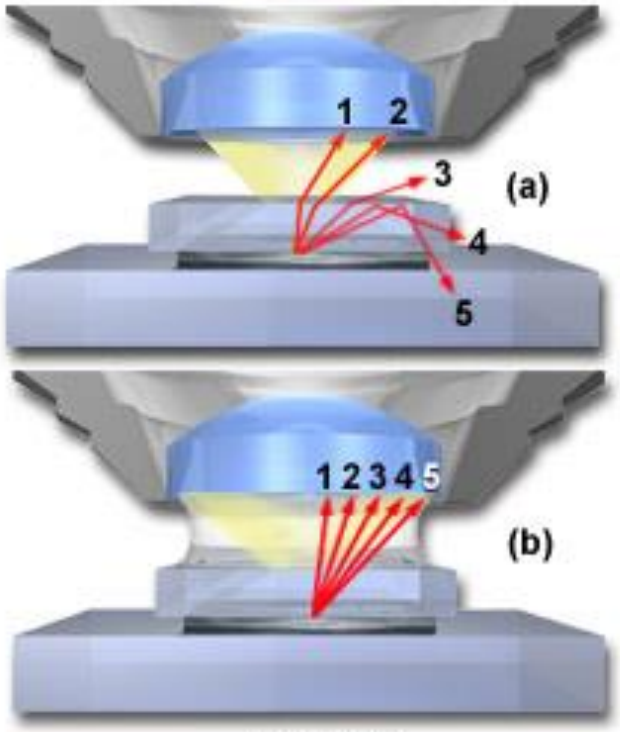


Figure 1

Oil-Immersion Microscope Objective

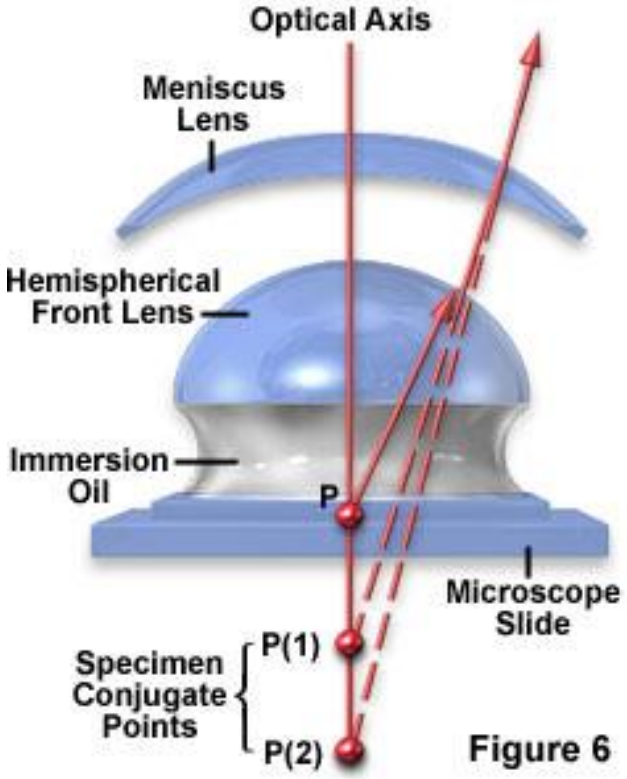


Figure 6

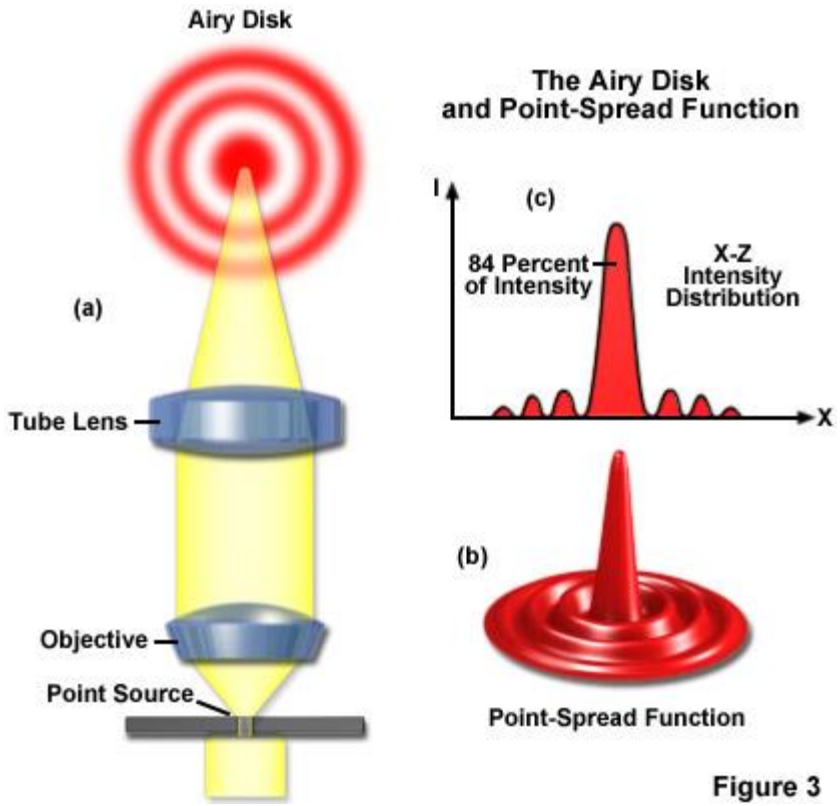
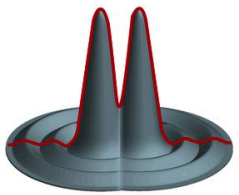
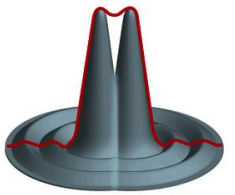


Figure 3



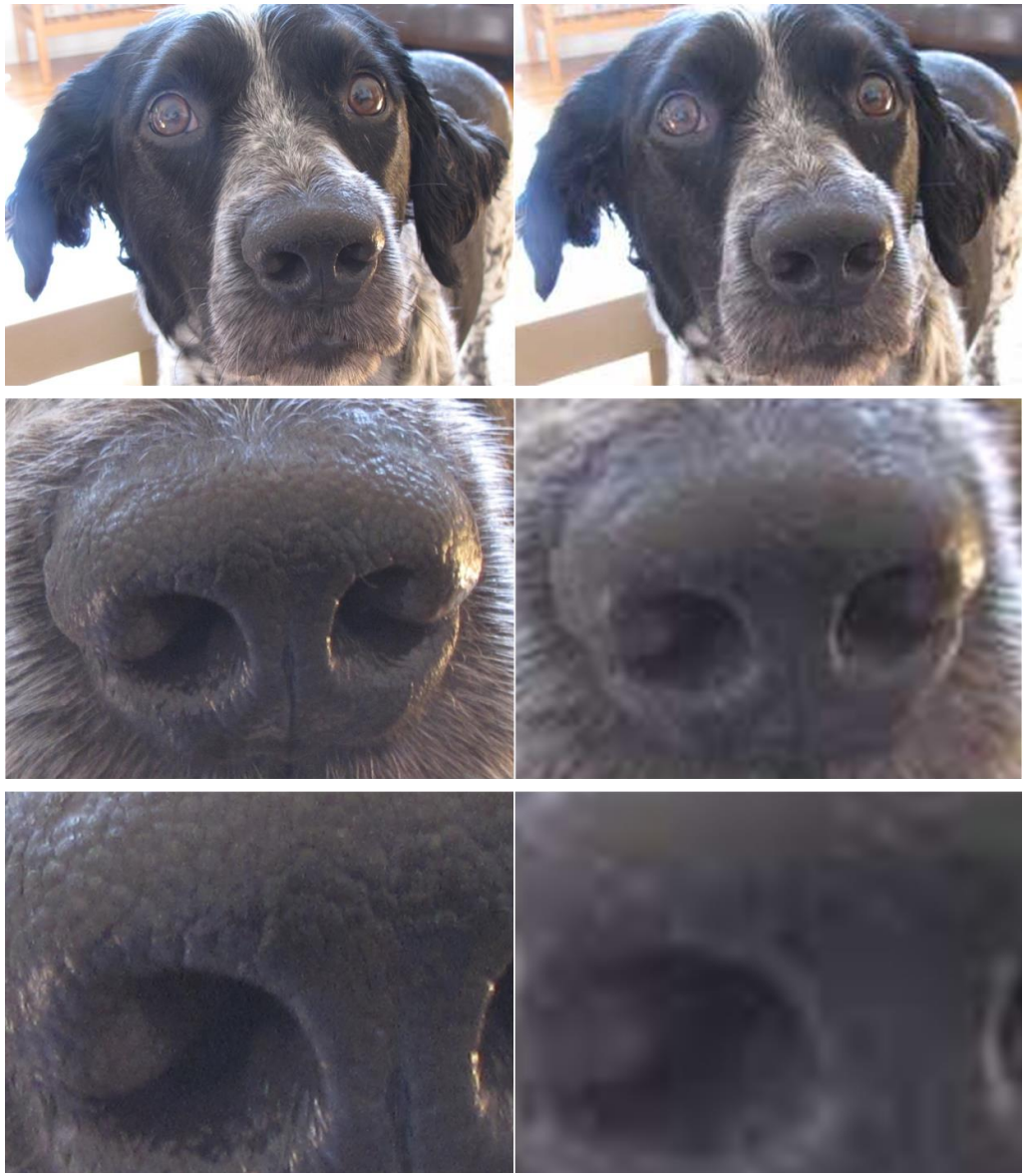
Resolved

Rayleigh Limit



Not Resolved

Rezolutie vs Marire



Principii microscopie

Scopul microscopiei:

- Diagnostic
- Cercetare

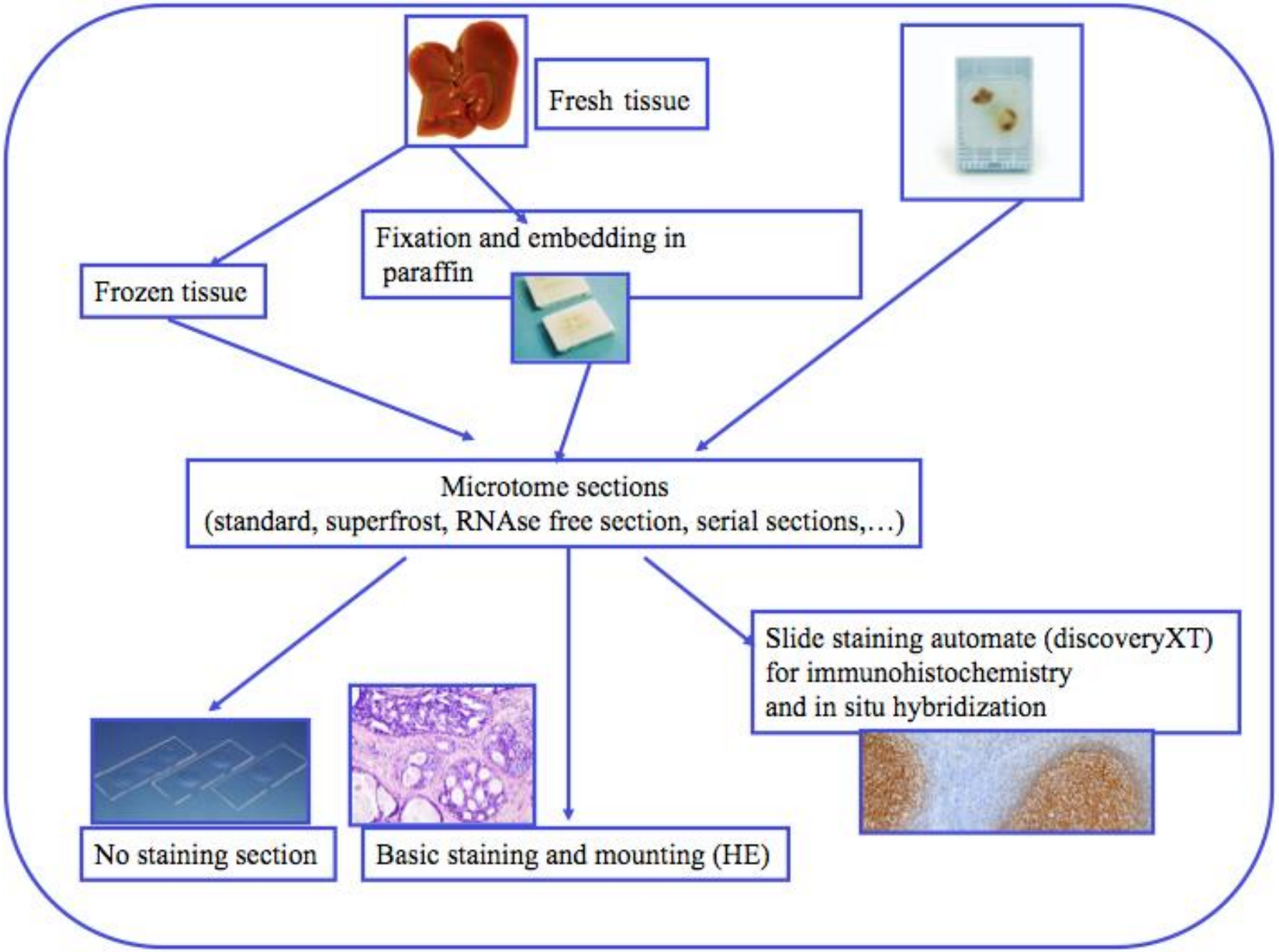
Microscopia optica:

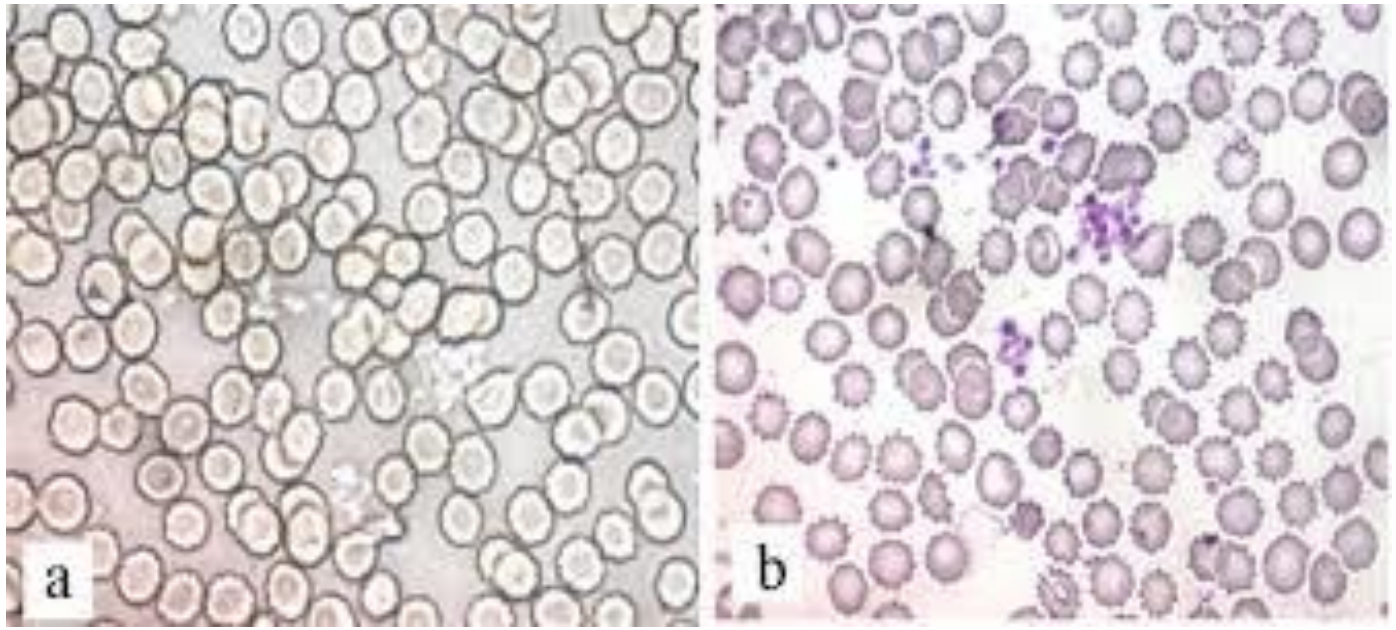
- Sisteme optice
- Componentele microscopului optic
- Principiul de functionare
- **Preparate pentru microscopie optica**

Vizualizare la microscopul optic. Imagini.

Microscopia cu fluorescenta

Microscopia electronica

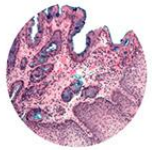

Fresh tissue
Fixation and embedding in paraffin
Frozen tissue
Microtome sections
(standard, superfrost, RNAse free section, serial sections,…)
Slide staining automate (discoveryXT)
for immunohistochemistry
and in situ hybridization
No staining section
Basic staining and mounting (HE)


a
b

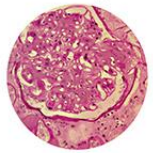
Amyloid

Congo Red
Cat # 24614

Carbohydrates



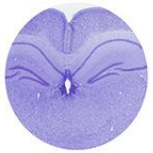
Alcian Blue/ PAS
Cat # 25086



PAS
Cat # 24200

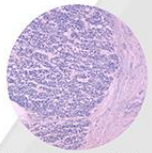
Rapid Mucin
Cat # 24208

Neuronal Tissue



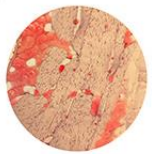
Cresyl Violet
Cat # 21063

Bielschowsky
Cat # 25994



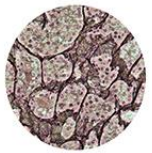
Luxol® Fast Blue
Cat # 24611

Triglycerides & Lipids

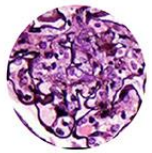


Oil Red O
Cat # 25962

Reticulin Fibers

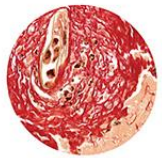


Reticulin
Cat # 25094

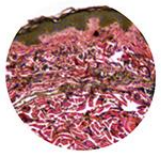


Jones PAS-M
Cat # 25091

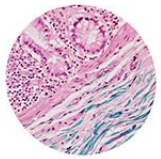
Connective Muscle Tissue



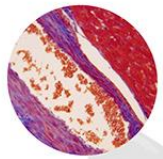
Picrosirius Red
Cat # 24901



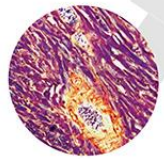
Verhoeff Van Gieson
Cat # 25089



Gomori's Trichrome
Cat # 24205

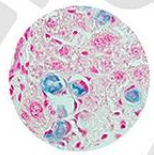


Masson's Trichrome
Cat # 25088

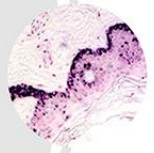


Rapid PTAH
Cat # 25715

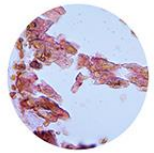
Pigment, Minerals & Granules



Prussian Blue Iron
Cat # 24199



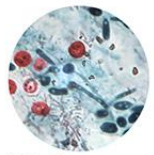
Fontana Masson
Cat # 25104



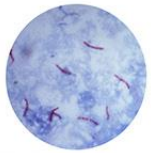
Von Kossa Method
Cat # 24633

Villanueva Osteochrome Bone
Cat # 16280

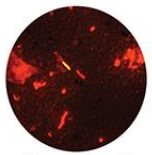
Microorganisms



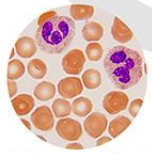
AFB Kinyuon
Cat # 25765



AFB Ziehl-Neelson
Cat # 24669

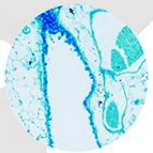


Auramine O
Cat # 24665

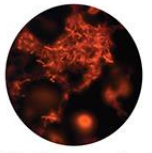


Differential Quik
Cat # 24606

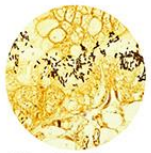
Fungi-Fluor®
Cat # 17442



Grocott Methenamine Silver
Cat # 25462



TB Fluorostain
Cat # 22422



Warthin-Starry
Cat # 25093

Gram's Stain
Cat # 24668

Special Stains

Principii microscopie

Scopul microscopiei:
- Diagnostic
- Cercetare

Microscopia optica:
- Sisteme optice
- Componentele microscopului optic
- Principiul de functionare
- Preparate pentru microscopie optica

Vizualizare la microscopul optic. Imagini.

Microscopia cu fluorescenta

Microscopia electronica

PAȘII FIXĂRII UNEI IMAGINI LA MICROSCOPUL OPTIC

Se aduce obiectivul 4x in axul optic;
Se ridica platina la punctul maxim cu ajutorul macrovizei;
Se fixeaza preparatul microscopic pe platina cu ajutorul valetilor;
Se ridica condensatorul la punctul maxim;
Se aduce in axul optic o portiune de tesut cu ajutorul carului mobil;
Se coboara condensatorul;
Se regleaza distanta pupilara privind in microscop;
Se coboara platina pana la punctul minim cu ajutorul macrovizei;

Se ridica platina pana in momentul prinderii imaginii;

Se clarifica imaginea cu ajutorul microvizei;

Se aduce in centrul campului microscopic portiunea de tesut care prezinta interes, dupa care, prin rotirea revolverului de deplaseaza, pe rand, obiectivele cu putere de marire mai mare in axul optic;

Examinarea cu imersie presupune punerea unei picaturi de ulei pe suprafata preparatului microscopic si aducerea in axul optic a obiectivului cu imersie, dupa care se clarifica imaginea;

Dupa terminarea examinarii, obiectivul de 4X se aduce in axul microscopului, se inlatura preparatul microscopic, se coboara condensatorul.

Principii microscopie

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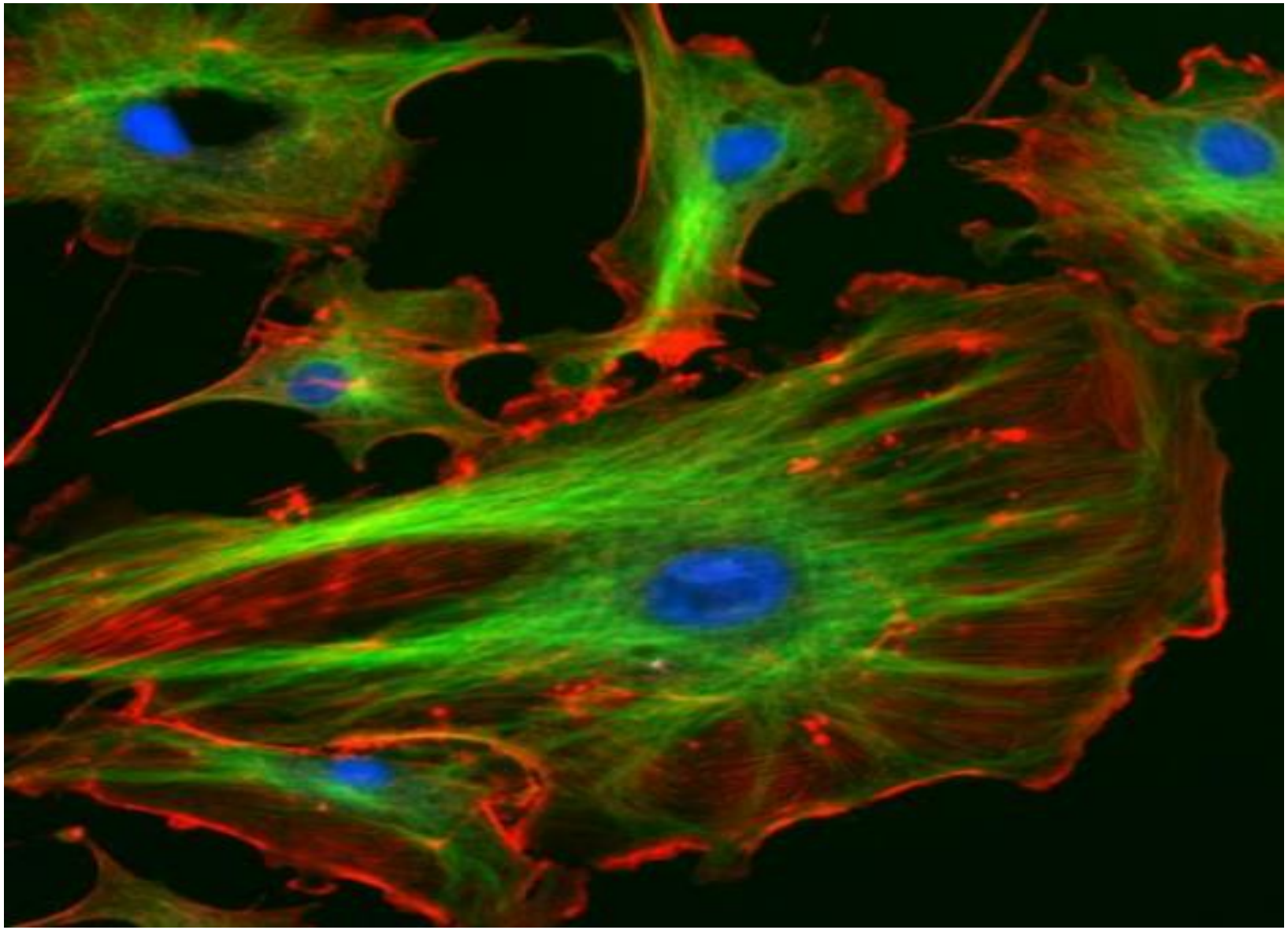


Anatomy of the Fluorescence Microscope

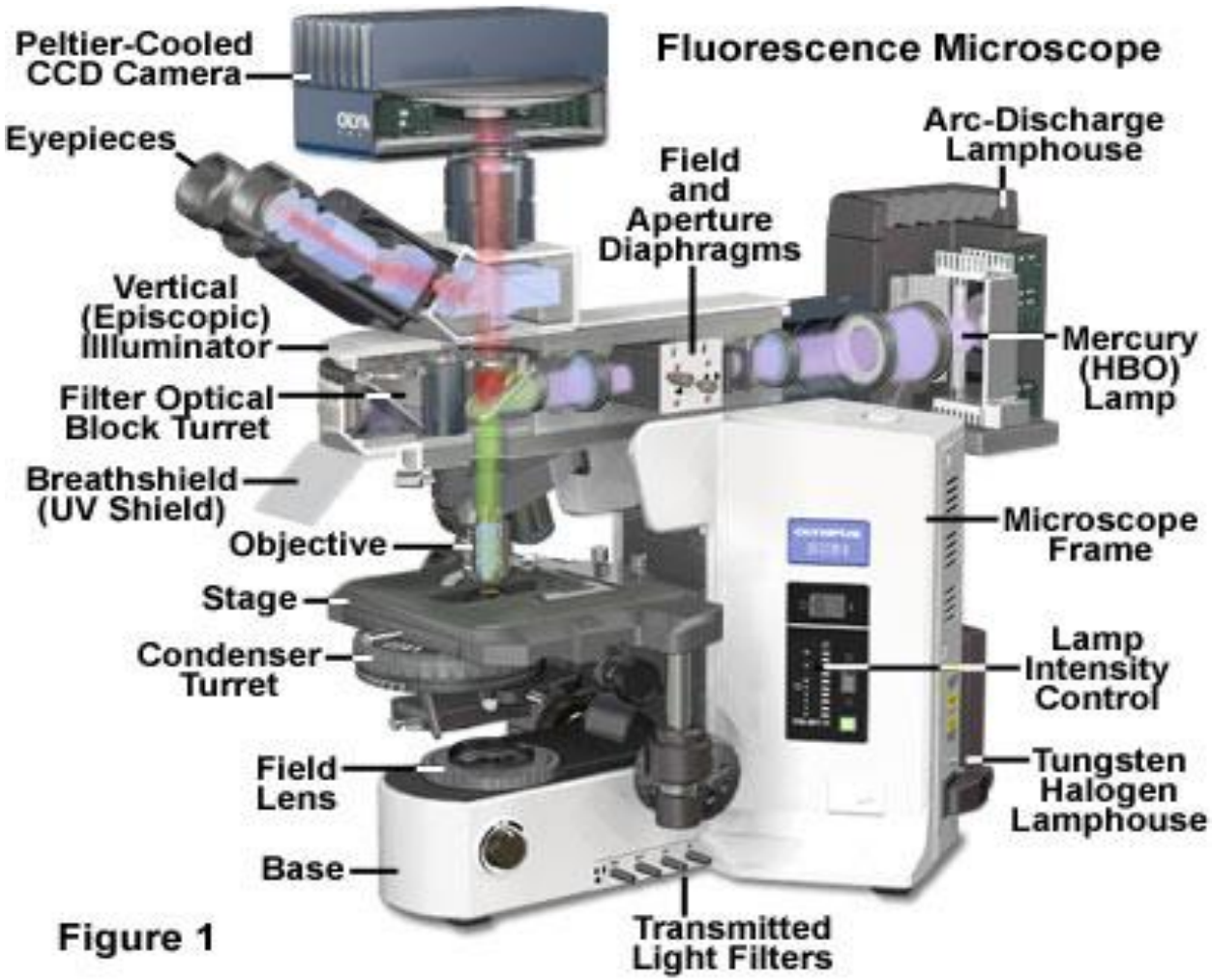


Figure 1

Principle of Excitation and Emission

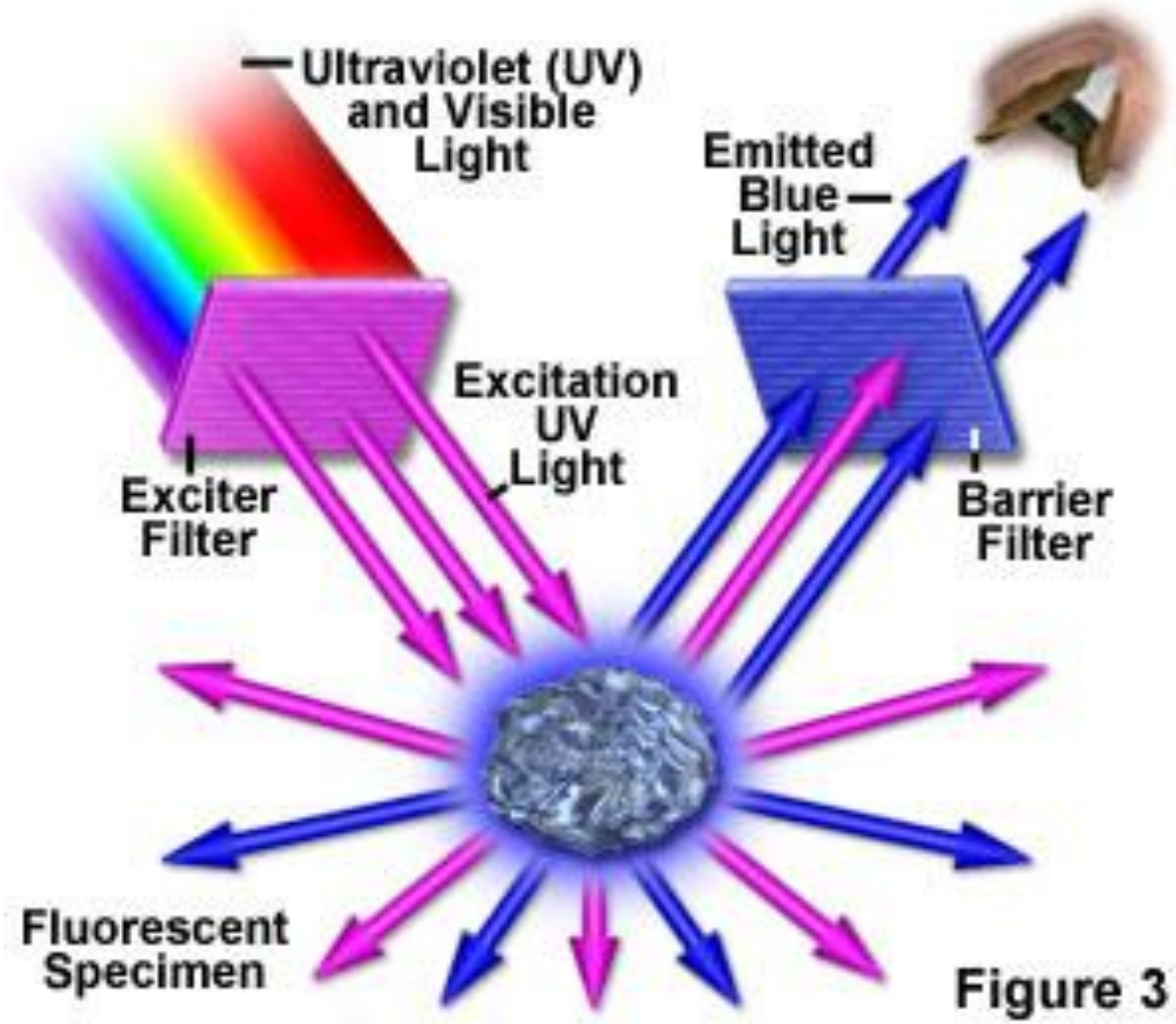
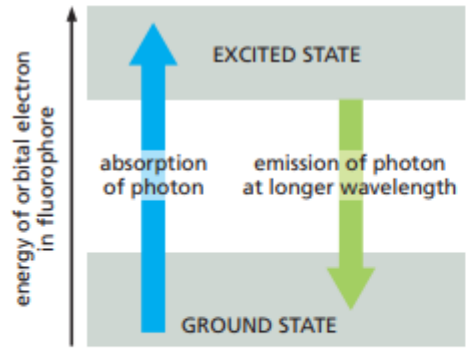
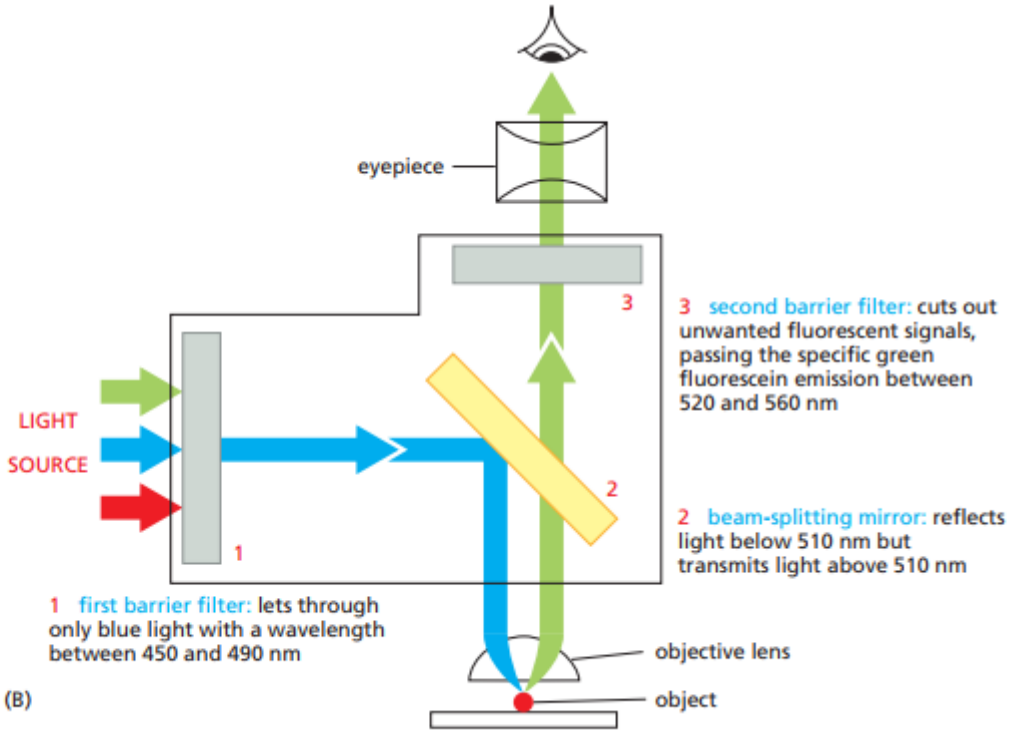


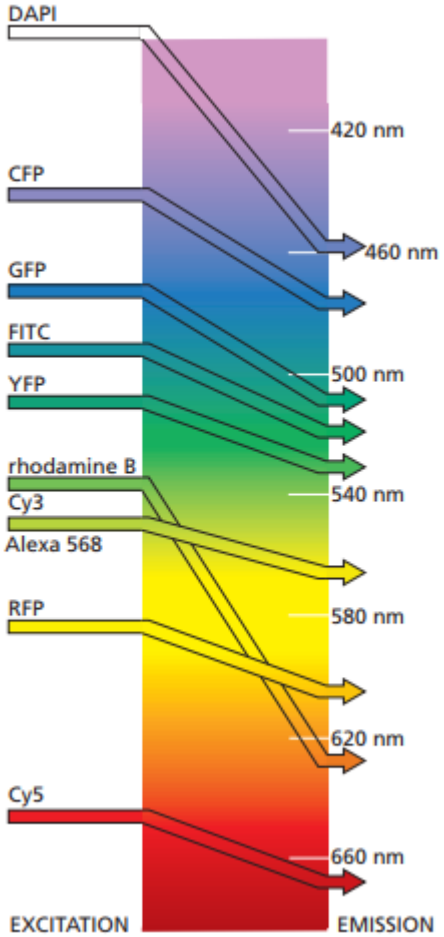
Figure 3

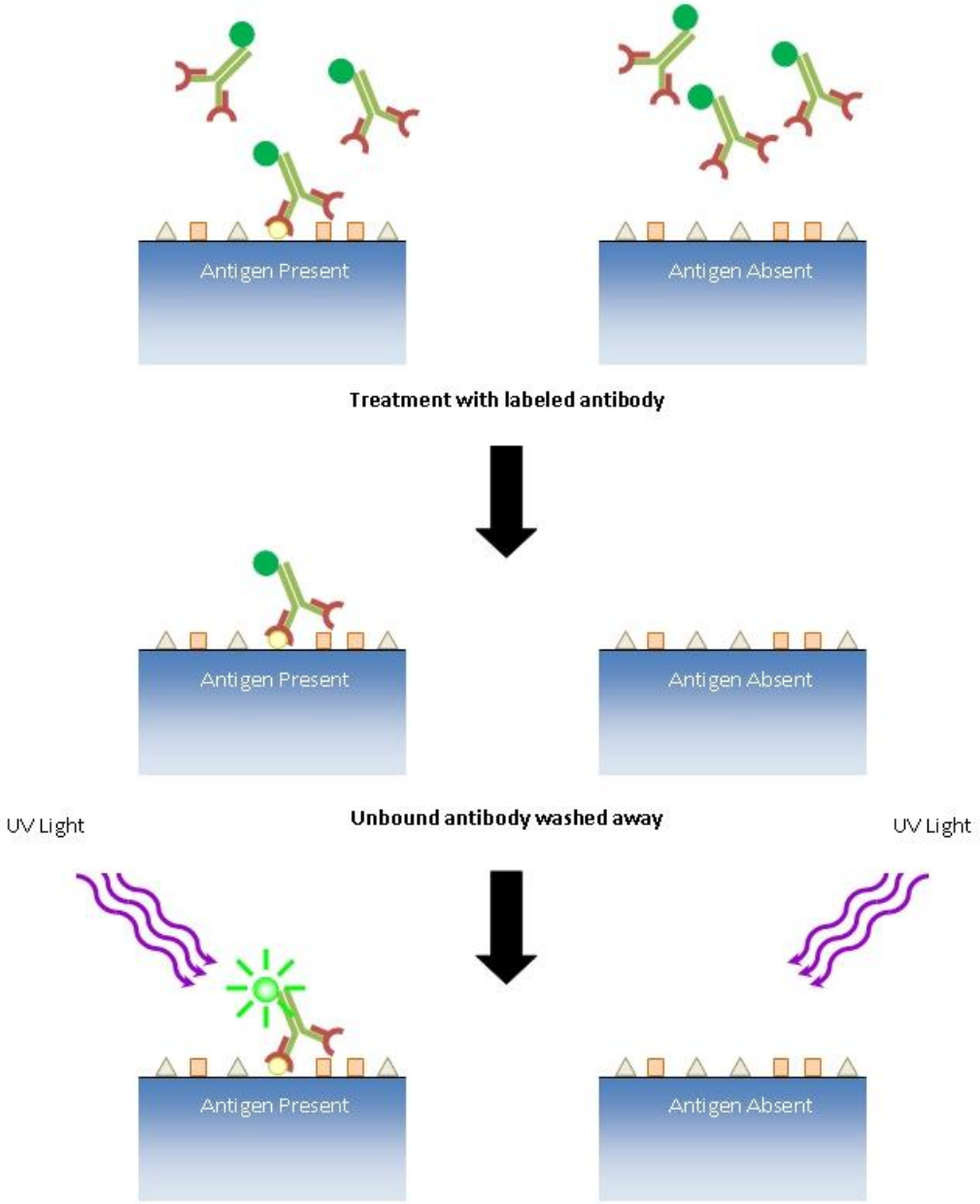

energy of orbital electron in fluorophore
EXCITED STATE
absorption of photon
emission of photon at longer wavelength
GROUND STATE

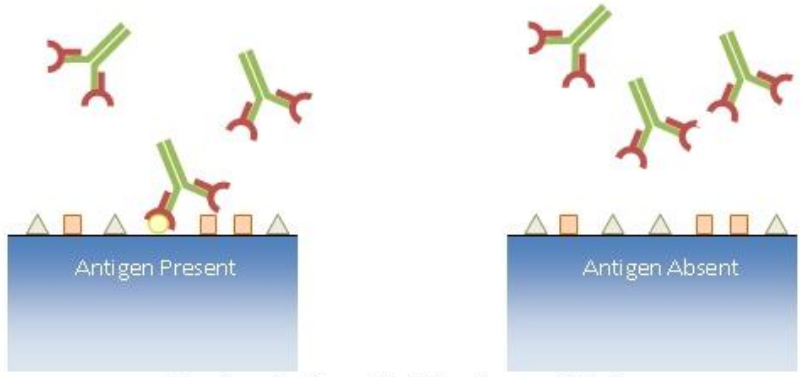
(A)


eyepiece
LIGHT SOURCE
3
3 second barrier filter: cuts out unwanted fluorescent signals, passing the specific green fluorescein emission between 520 and 560 nm
2
2 beam-splitting mirror: reflects light below 510 nm but transmits light above 510 nm
1
1 first barrier filter: lets through only blue light with a wavelength between 450 and 490 nm
objective lens
object

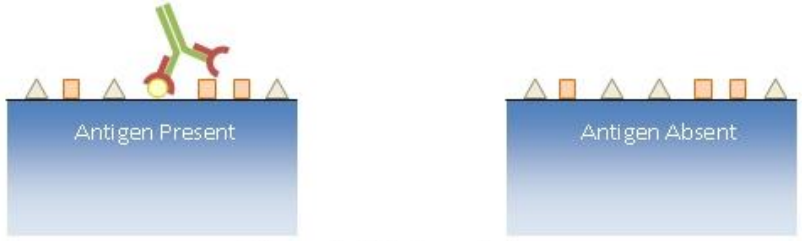
(B)


DAPI
420 nm
CFP
460 nm
GFP
FITC
500 nm
YFP
rhodamine B
Cy3
540 nm
Alexa 568
RFP
580 nm
620 nm
Cy5
660 nm
EXCITATION
EMISSION

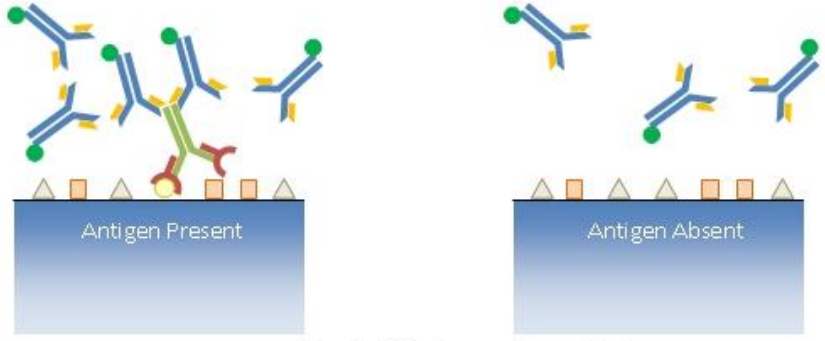

Antigen Present
Antigen Absent
Treatment with labeled antibody
Antigen Present
Antigen Absent
UV Light
Unbound antibody washed away
UV Light
Antigen Present
Antigen Absent
Fluorescence observed where antigen is located



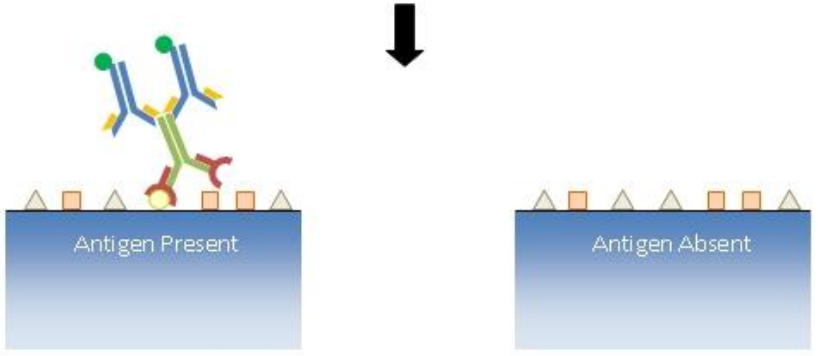
Treatment with unlabelled primary antibody



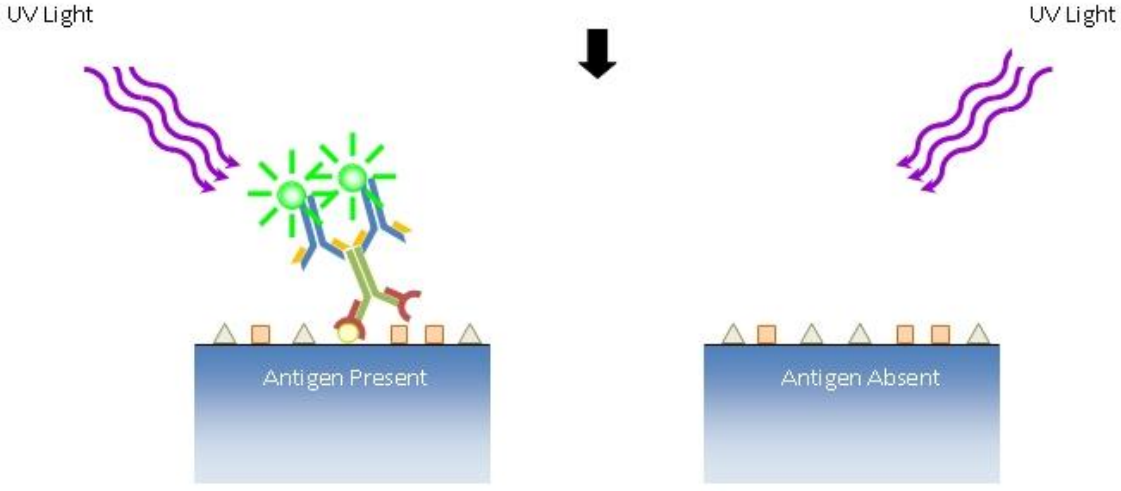
Unbound antibody washed away



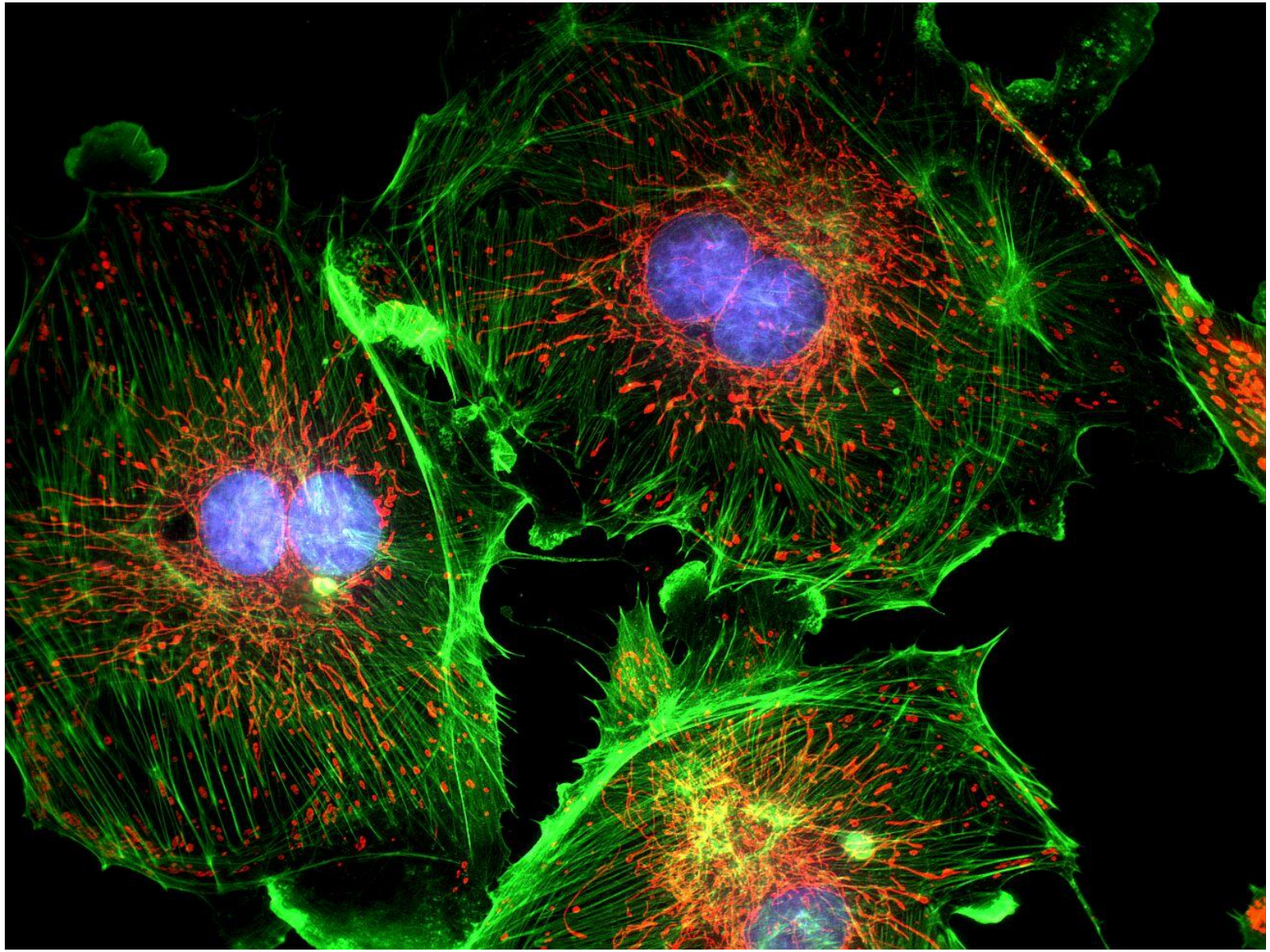
Treatment with unlabelled secondary antibody



Unbound secondary antibody washed away



Fluorescence observed wherever antigen is located



Principii microscopie

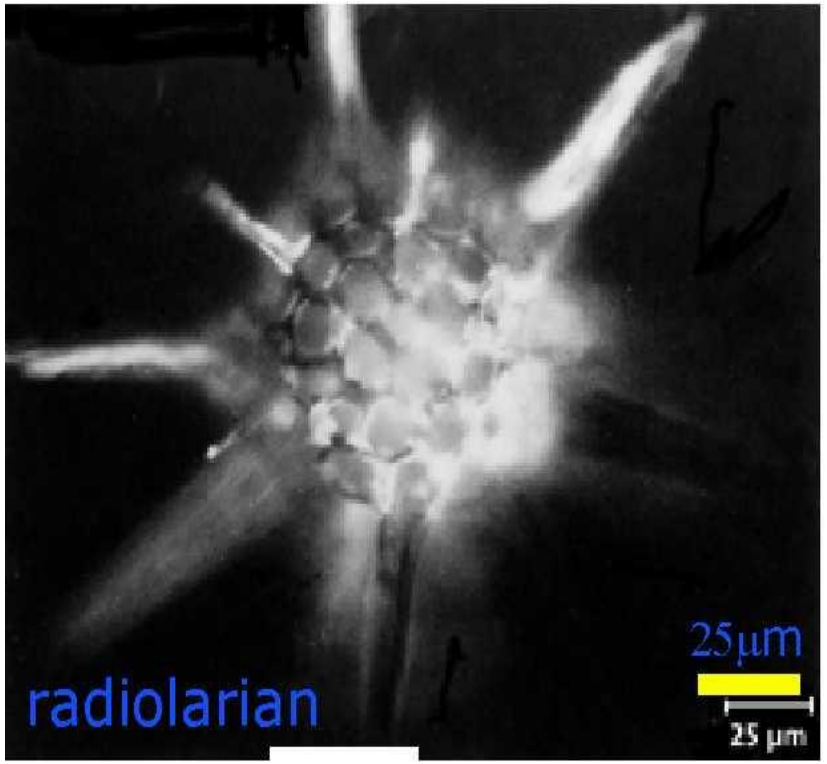
Scopul microscopiei:
- Diagnostic
- Cercetare

Microscopia optica:
- Sisteme optice
  - Componentele microscopului optic
  - Principiul de functionare
  - Preparate pentru microscopie optica

Vizualizare la microscopul optic. Imagini.

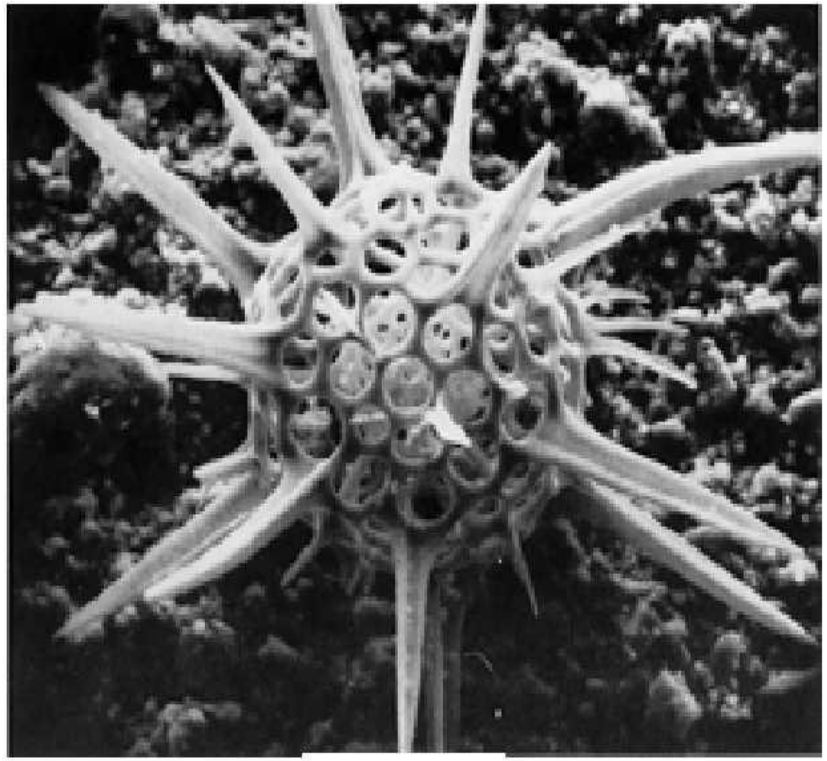
Microscopia cu fluorescenta

Microscopia electronica



M.O.

Rezoluție scăzută



M.E.

Rezoluție înaltă

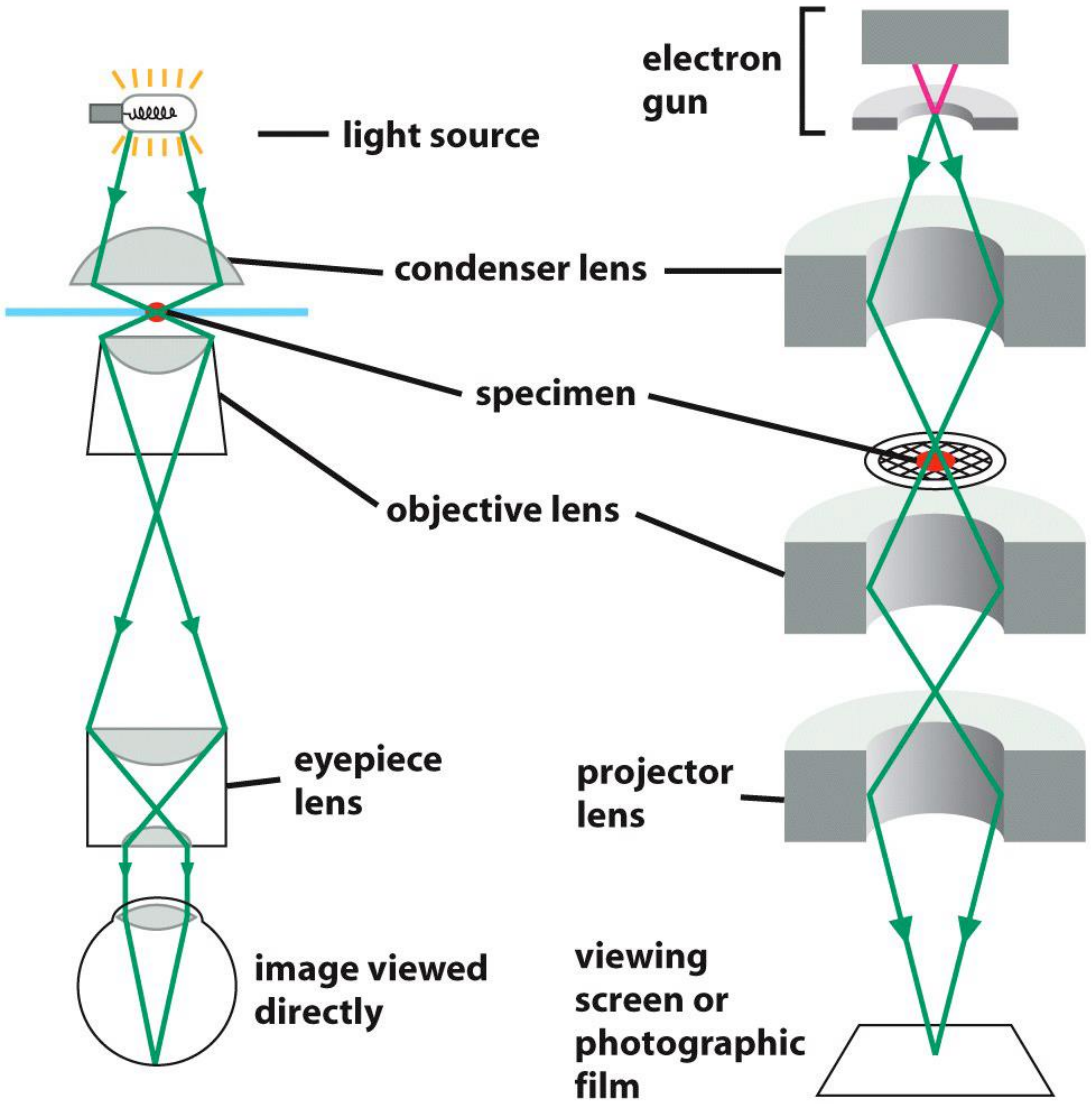
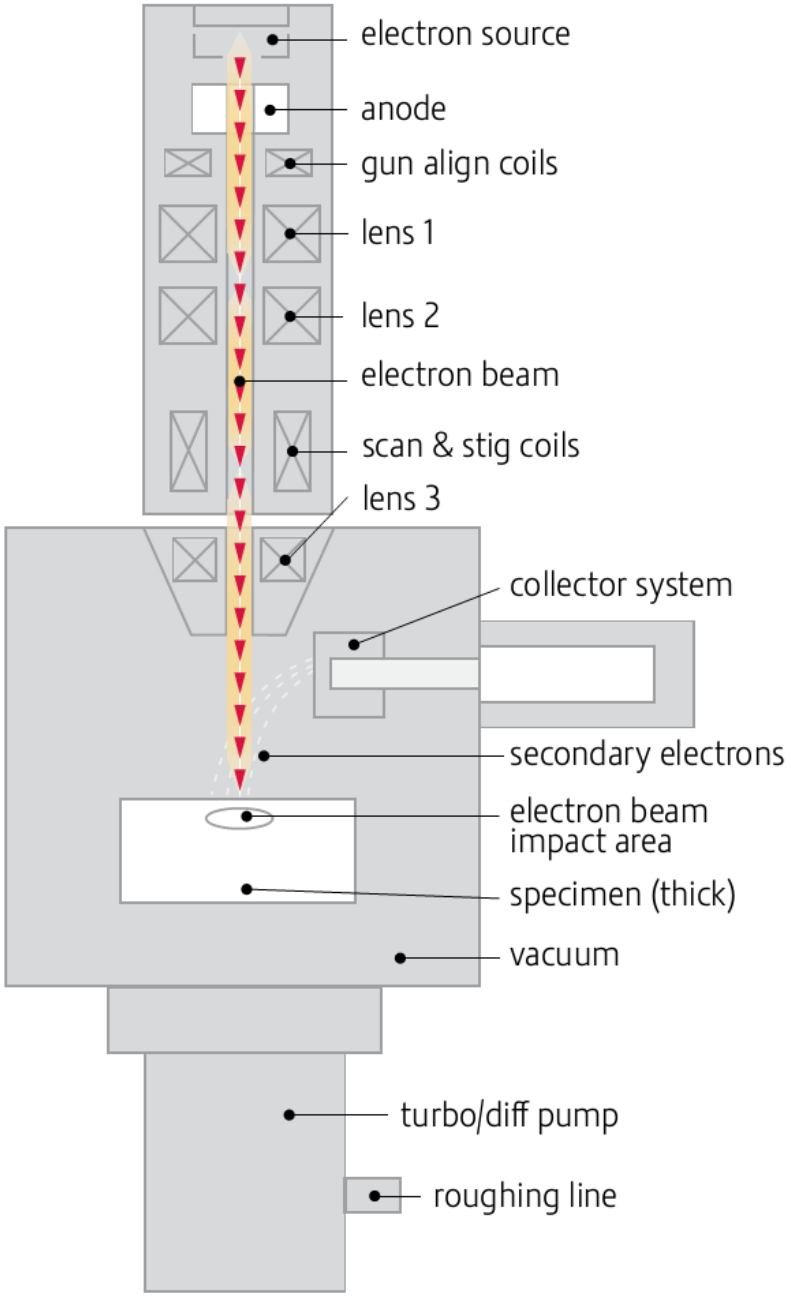
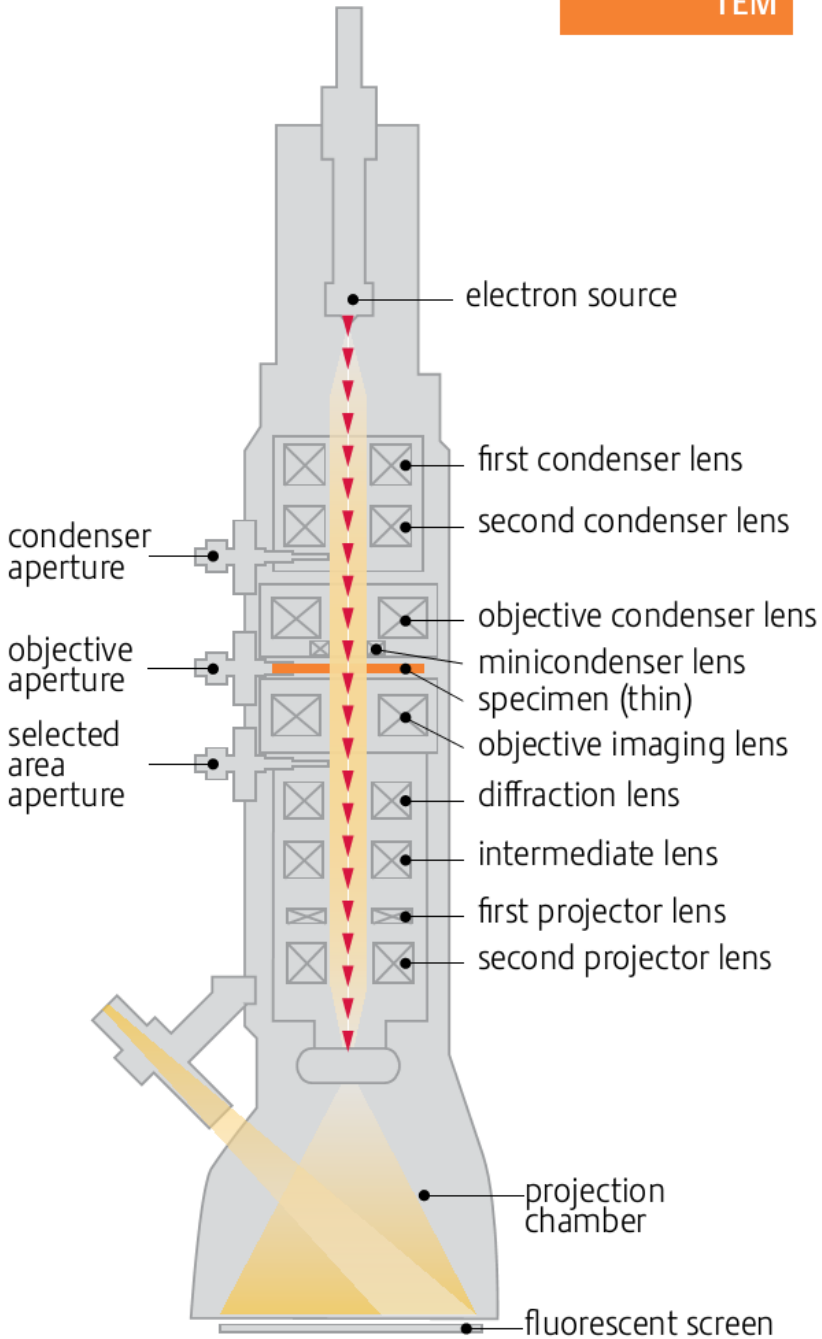


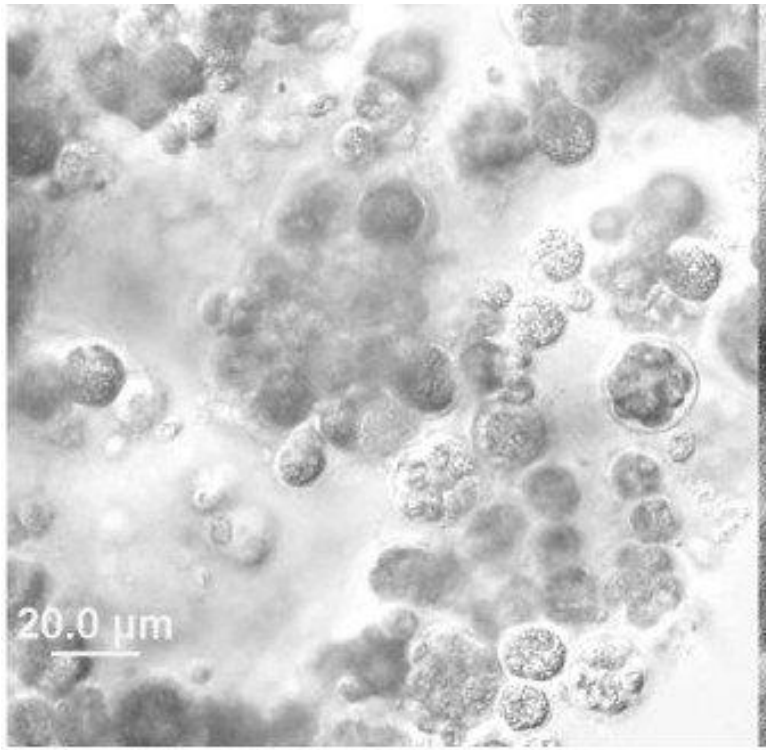
Figure 9-42 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

SEM

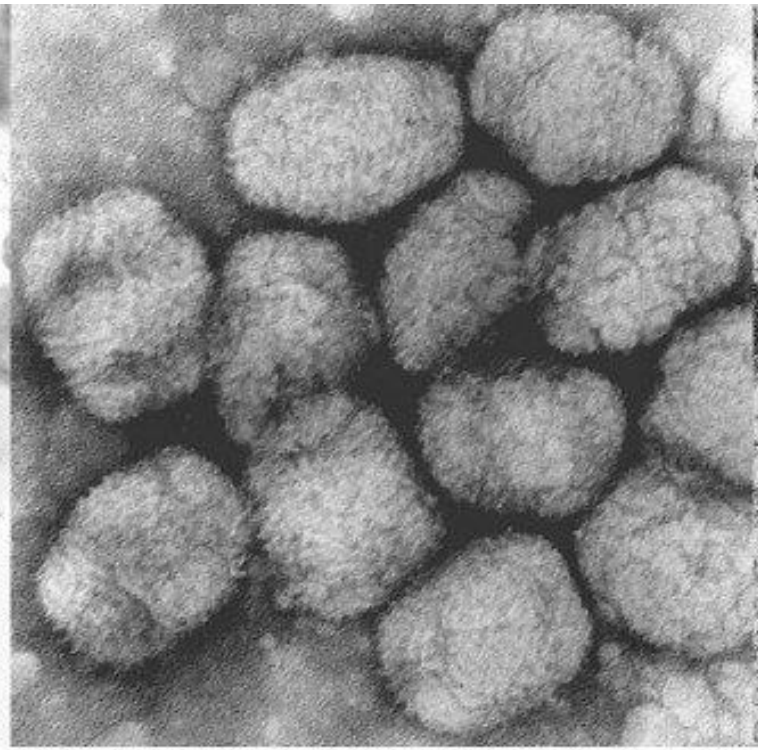

electron source
anode
gun align coils
lens 1
lens 2
electron beam
scan & stig coils
lens 3
collector system
secondary electrons
electron beam impact area
specimen (thick)
vacuum
turbo/diff pump
roughing line

TEM

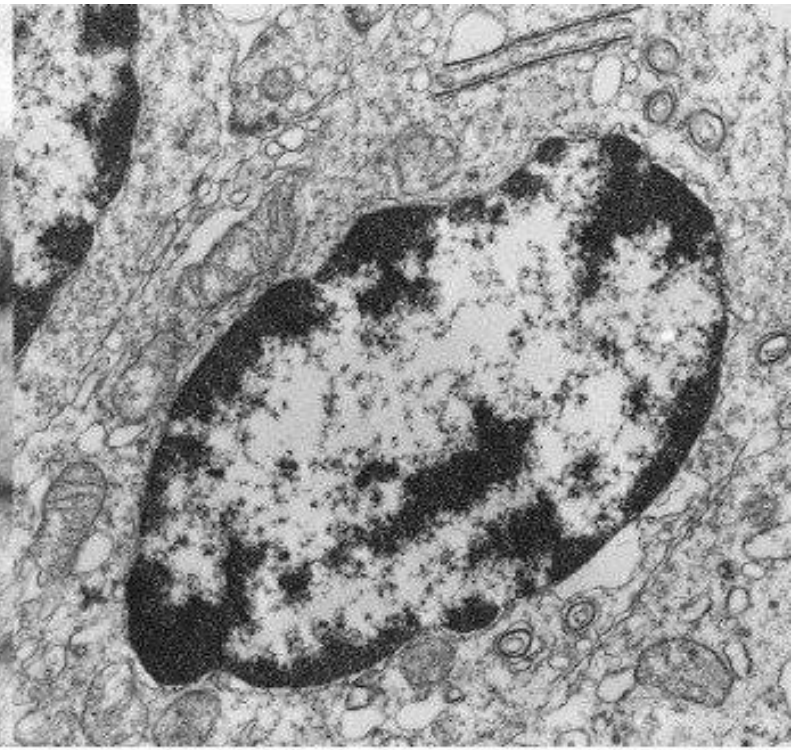

electron source
first condenser lens
second condenser lens
condenser aperture
objective condenser lens
objective aperture
minicondenser lens
specimen (thin)
selected area aperture
objective imaging lens
diffraction lens
intermediate lens
first projector lens
second projector lens
projection chamber
fluorescent screen



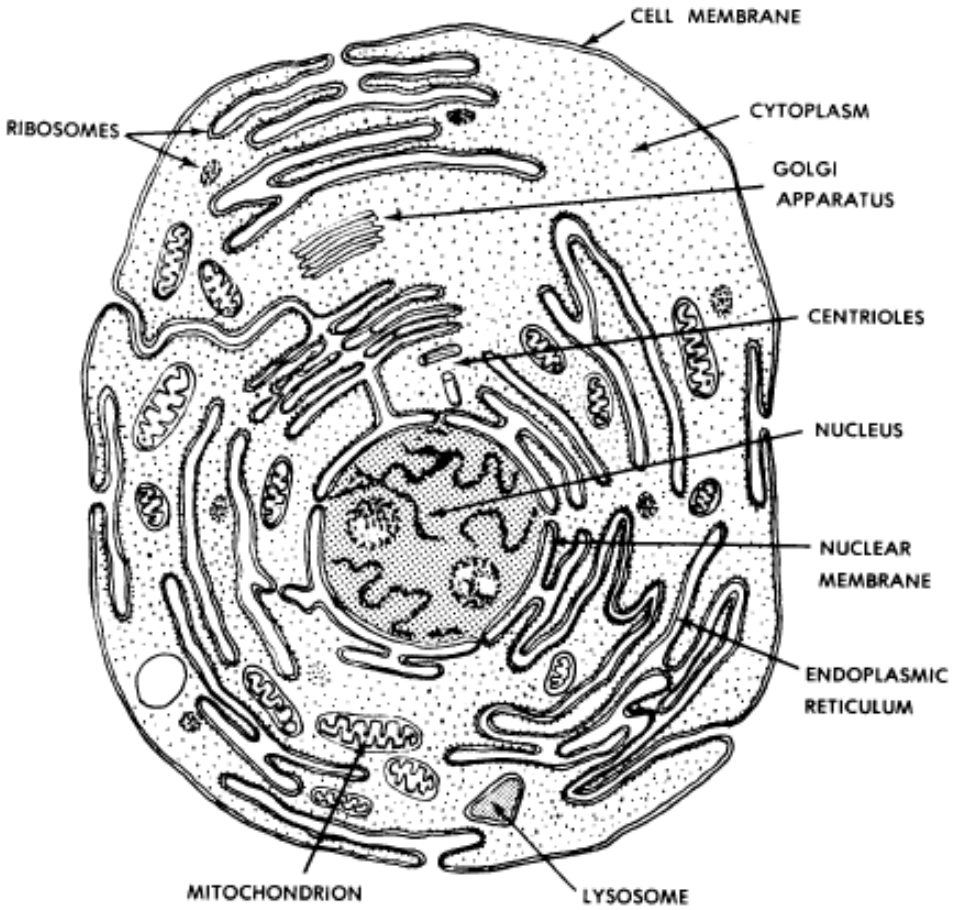
Light Microscope

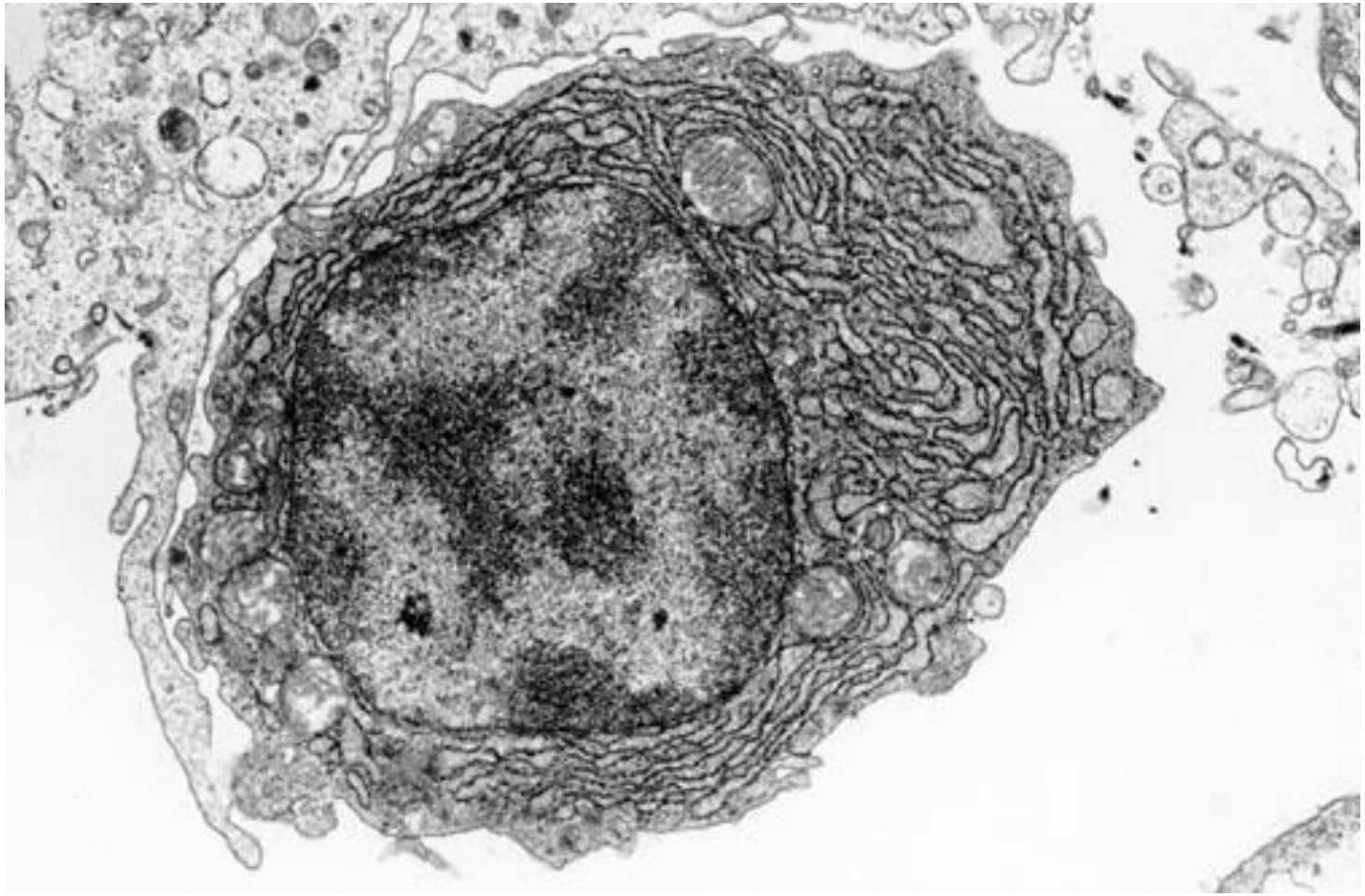


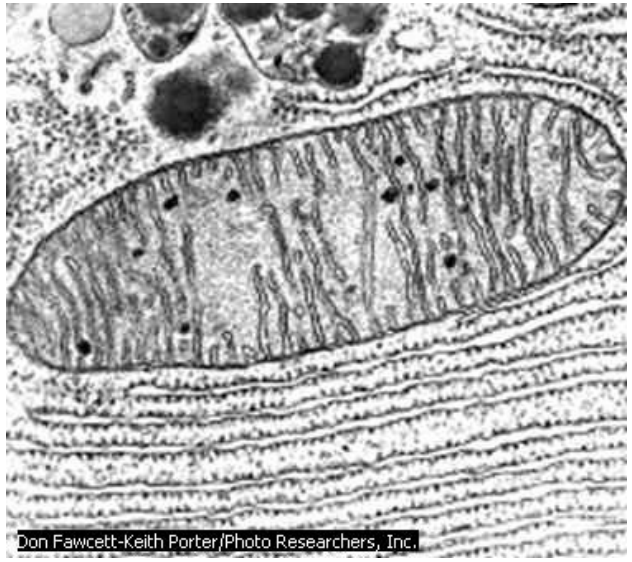
Scanning Electron Microscope (SEM)

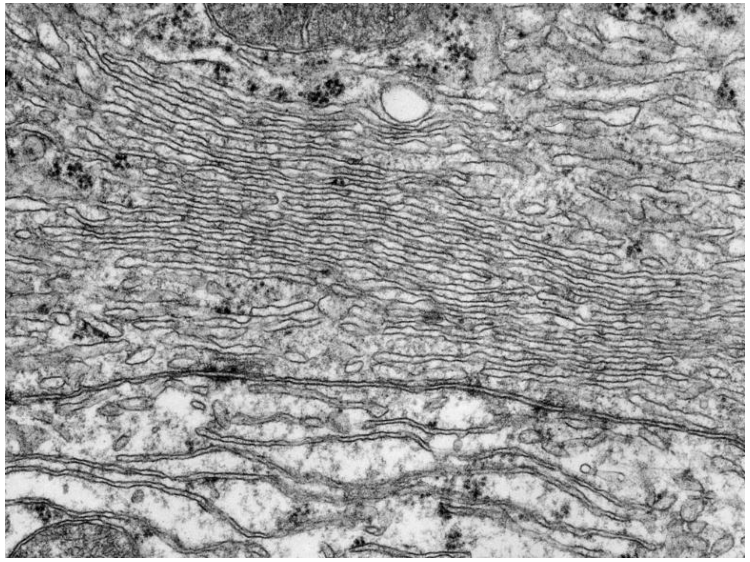


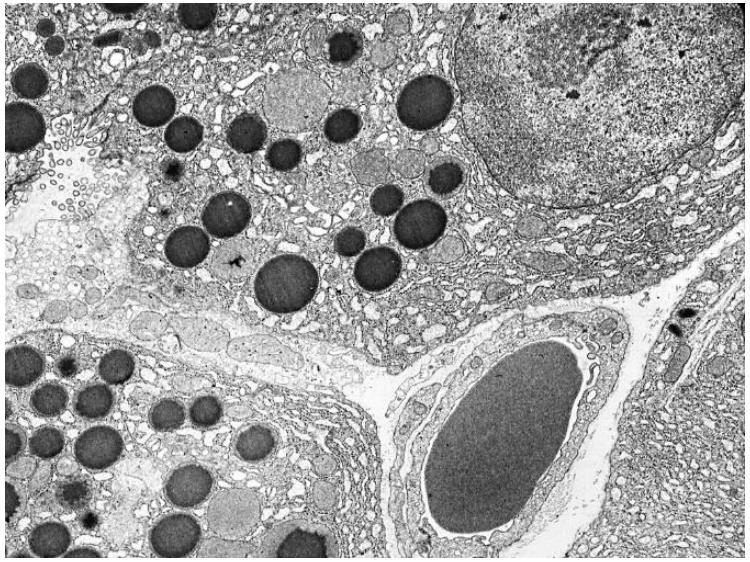
Transmission Electron Microscope (TEM)

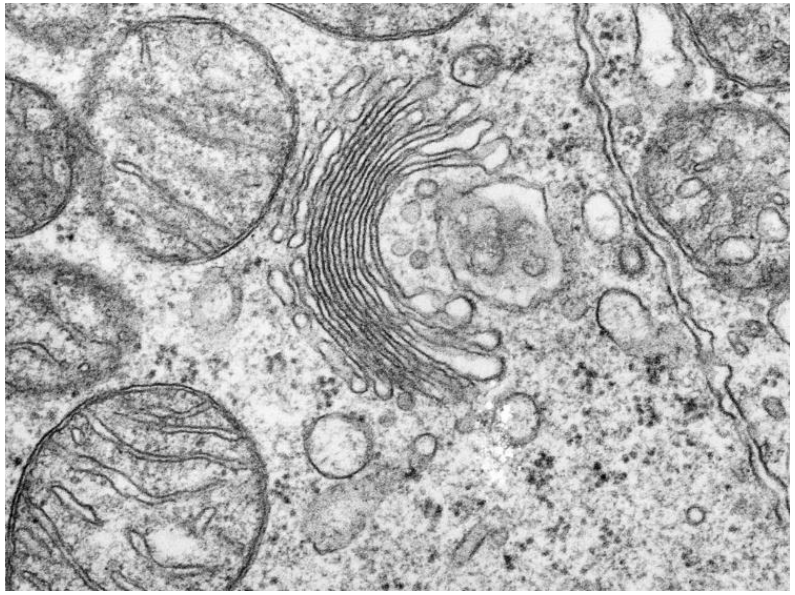

CELL MEMBRANE
CYTOPLASM
RIBOSOMES
GOLGI APPARATUS
CENTRIOLES
NUCLEUS
NUCLEAR MEMBRANE
ENDOPLASMIC RETICULUM
MITOCHONDRION
LYSOSOME

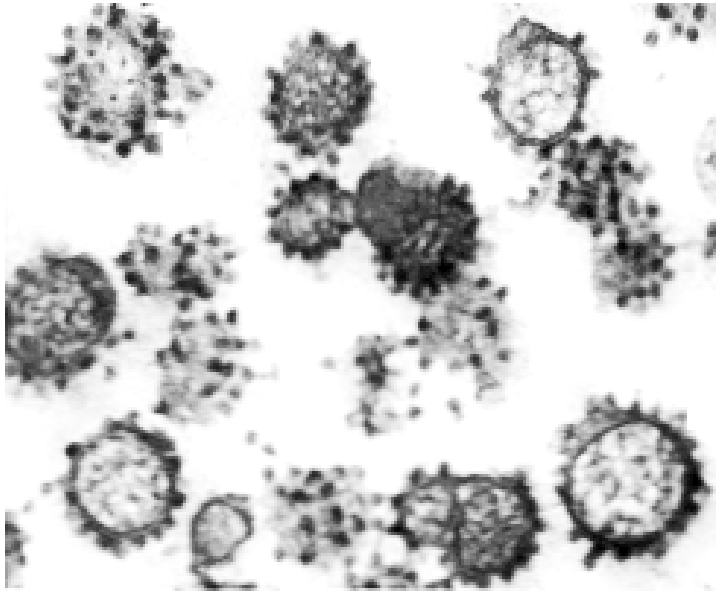



Don Fawcett-Keith Porter/Photo Researchers, Inc.









DISCUȚII