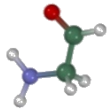
Opazovanje struktur

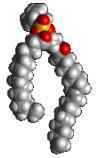
1. del - Optična mikroskopija

Janez Štrancar, Iztok Urbančič

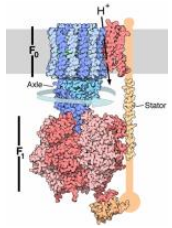
Velikostne skale življenja



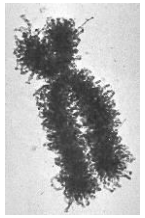
Medatomske vezi



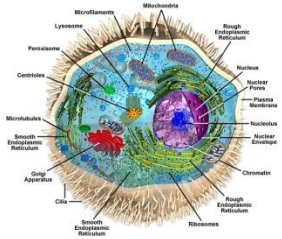
Lipidi



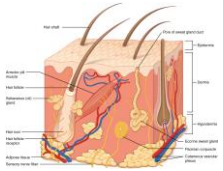
Proteini



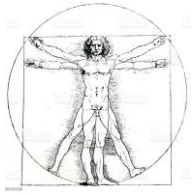
Kromosom



Evkariontska celica



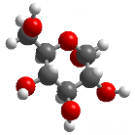
Tkiva



Telo

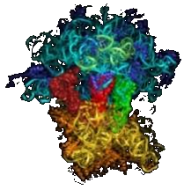
0.1 nm | 1 nm | 10 nm | 100 nm | 1 μm | 10 μm | 100 μm | 1 mm | 1 cm | 1 dm | 1 m

velikost

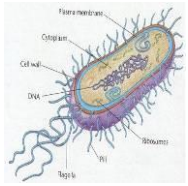


Monosaharidi, aminokisline

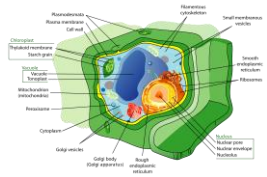
Trans-membranska vijačnica



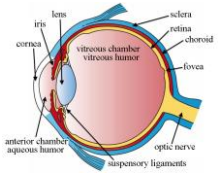
Ribosom



Bakterija



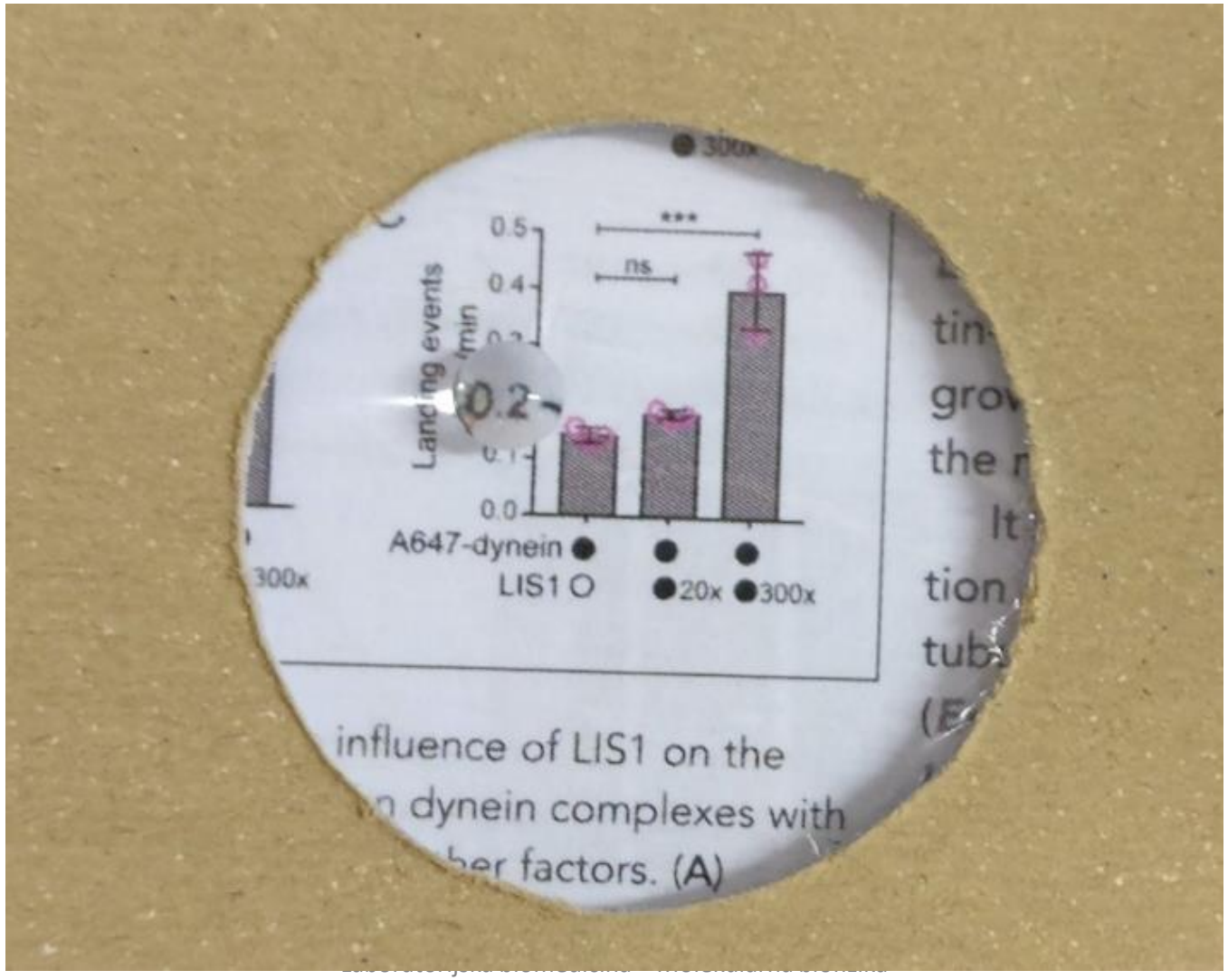
Rastlinska celica



Organi

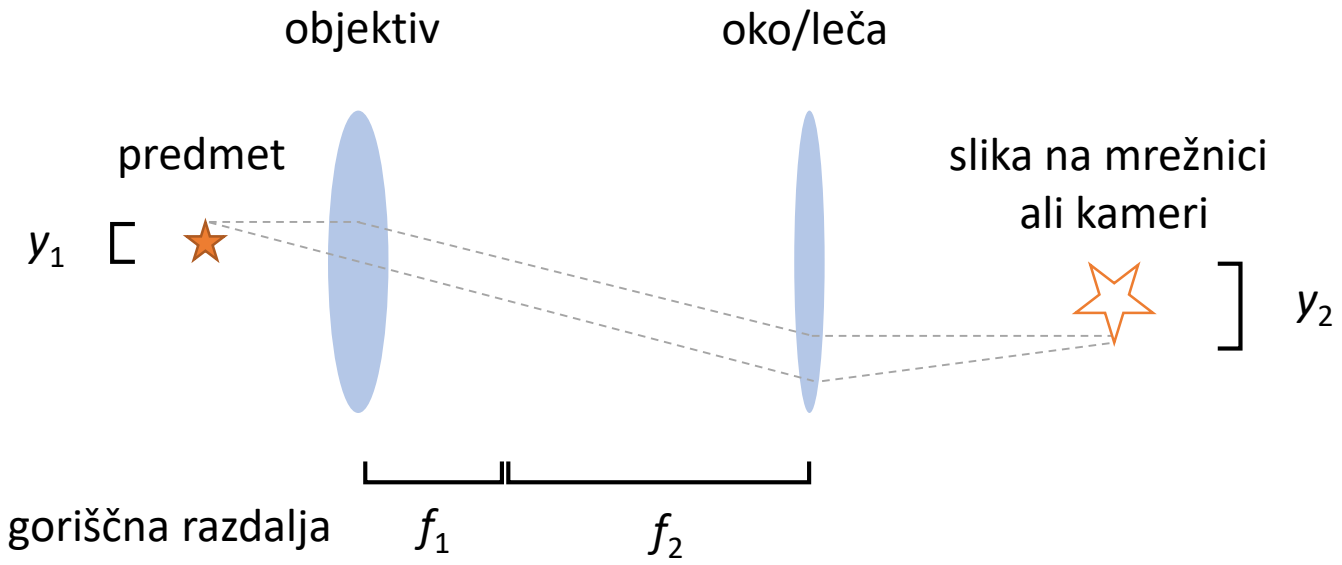
vidno s prostim očesom

Kako lahko vidimo majhne stvari?



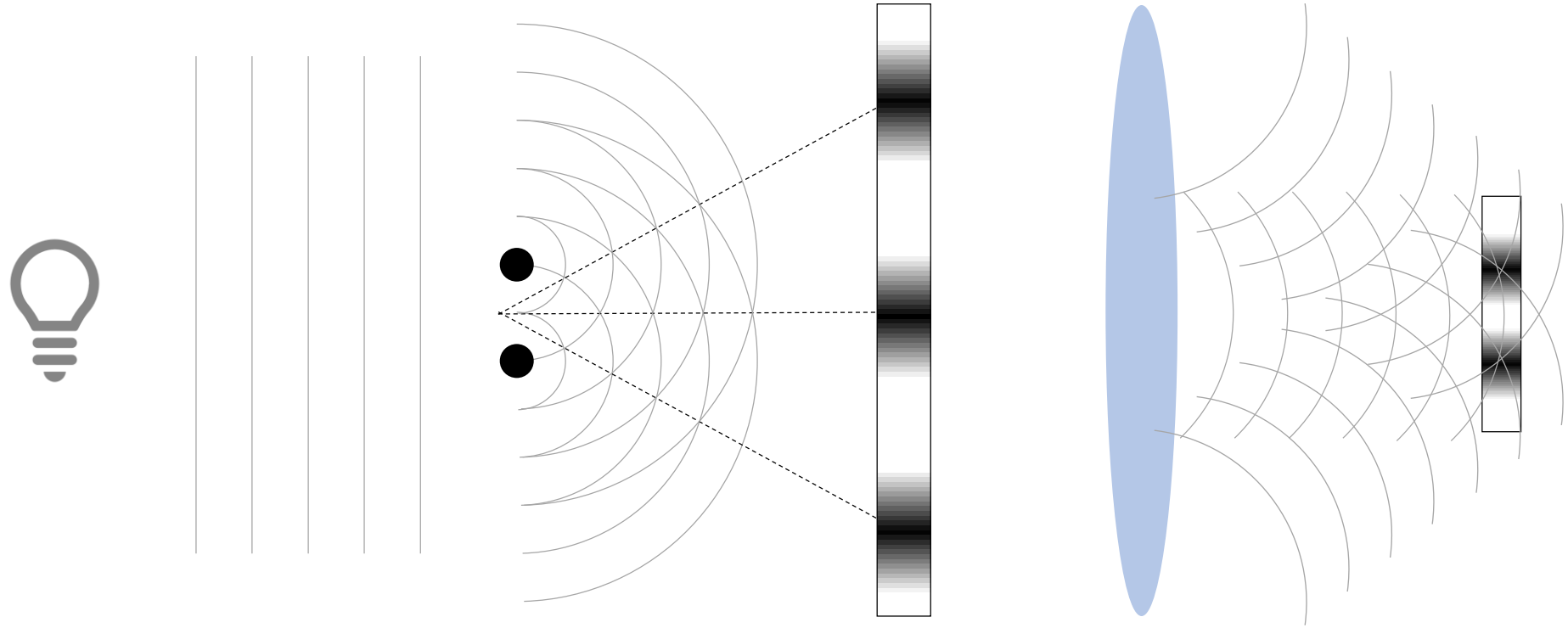
Kako povečamo majhne stvari?

Povečava slike zaradi uklona svetlobe na ukrivljeni površini:



Optična povečava: $M = y_2 / y_1 = f_2 / f_1$

Uklon svetlobe nam zamegli sliko



Vir svetlobe

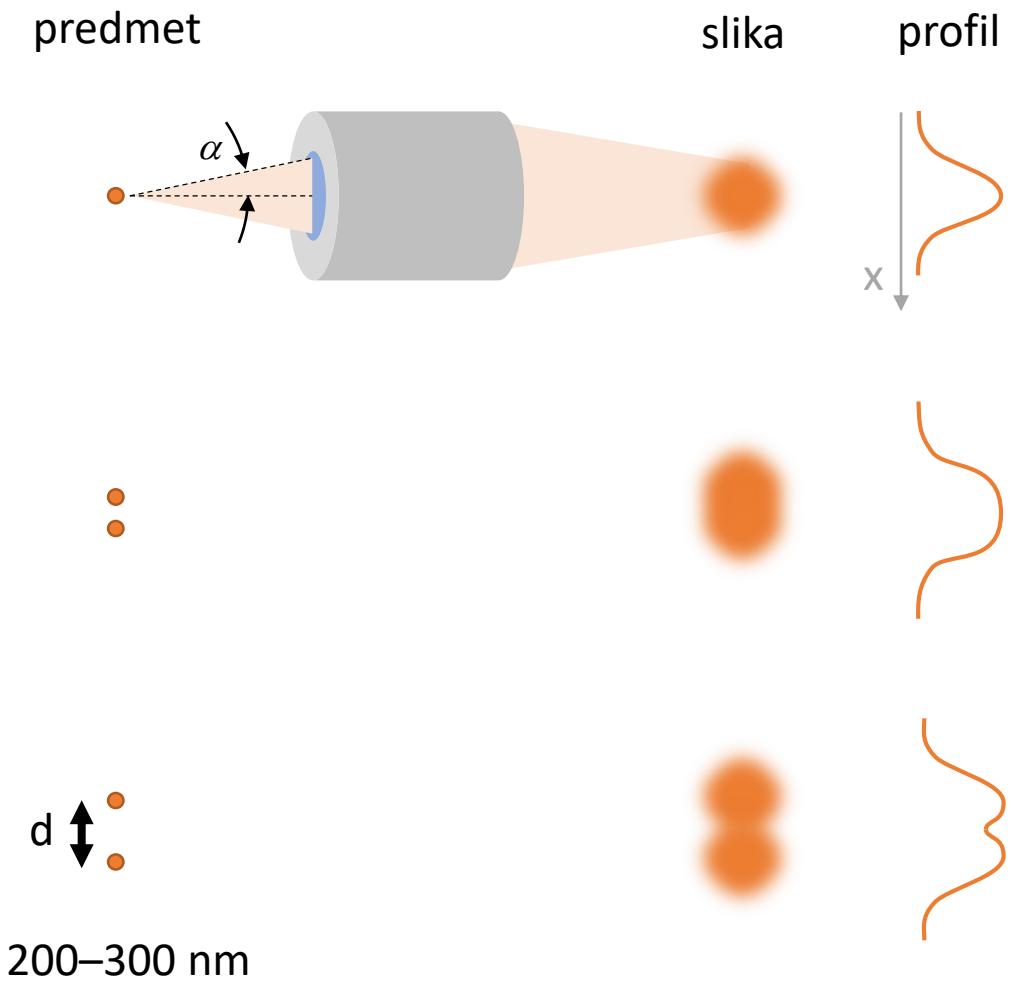
Objekt

Interferenčni vzorec

Lečje

Slika

Kako podrobno vidimo majhne stvari?



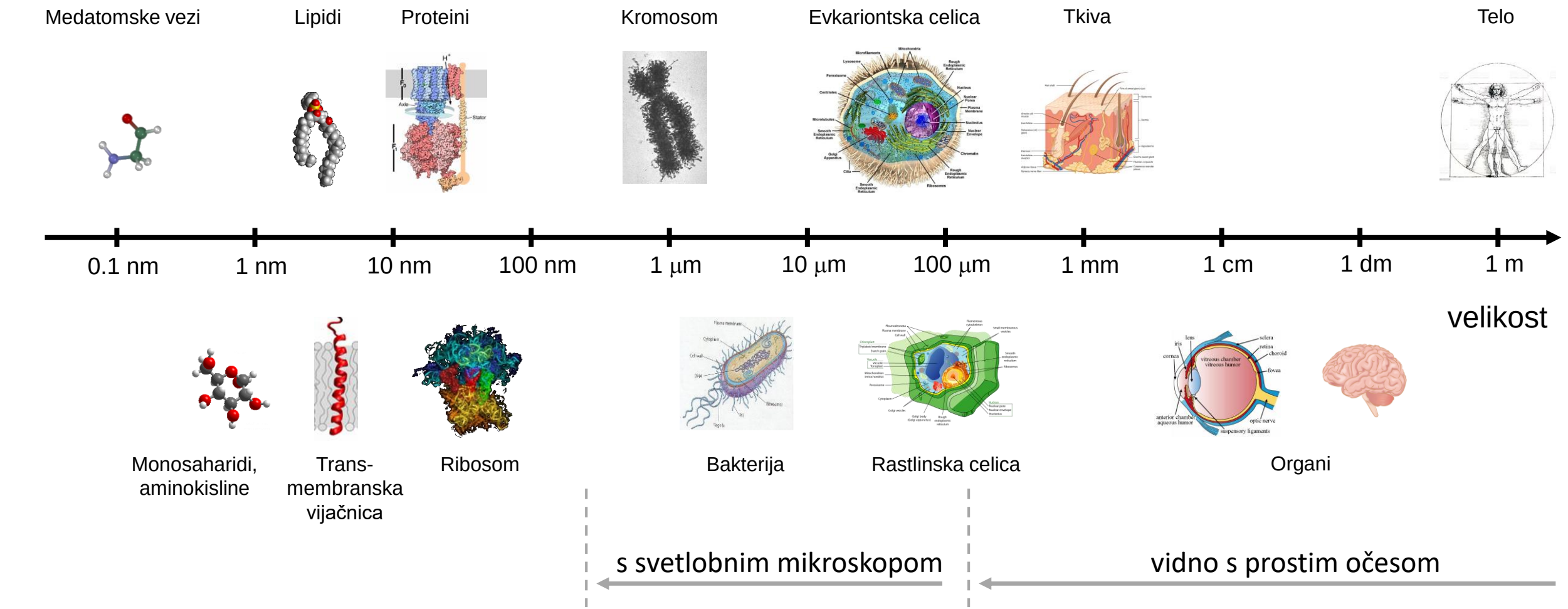
Ločljivost mikroskopa zaradi uklona svetlobe je odvisna od:

- valovne dolžine svetlobe - λ
- numerične odprtine objektiva - $NA = n \sin(\alpha)$
  n - lomni količnik medija
  α - polovični kot zajema svetlobe

- ne od povečave!

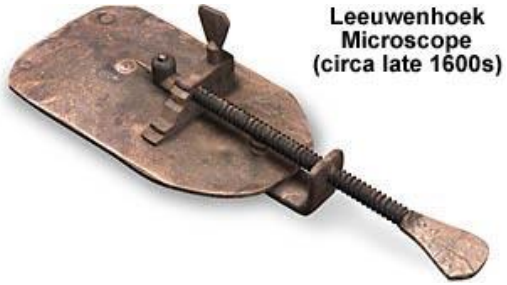
Ernst Abbe

Velikostne skale življenja

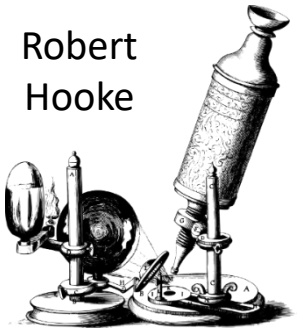


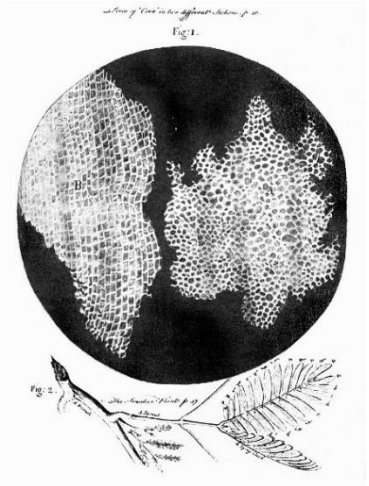
Kratka zgodovina svetlobne mikroskopije

17. stol.



Robert Hooke

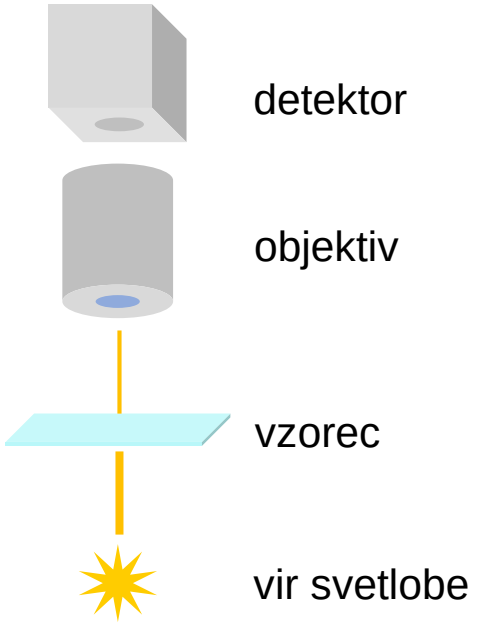




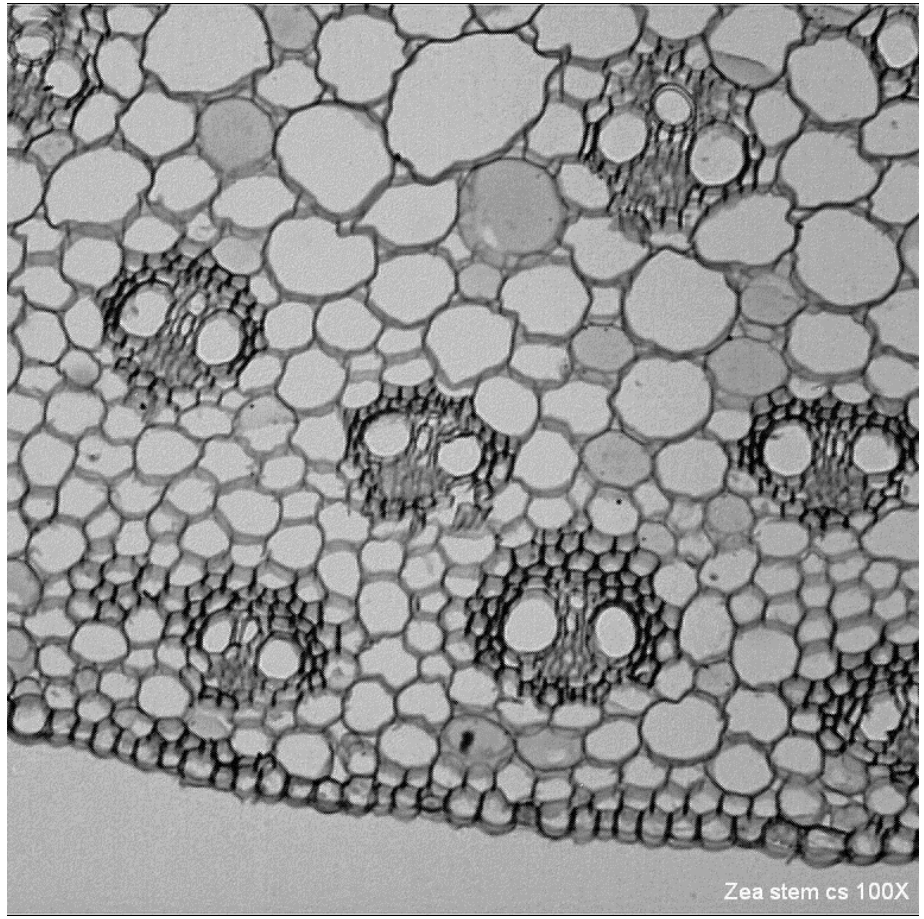
20. stol.

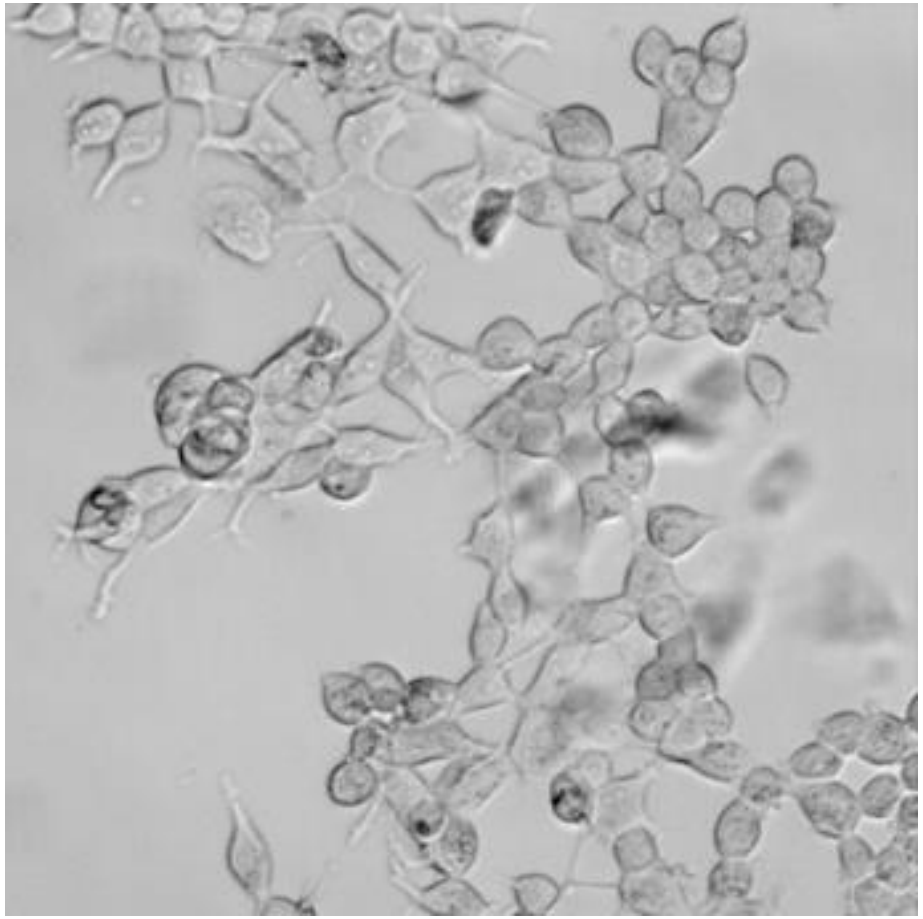
21. stol.

Zgradba presevnega mikroskopa



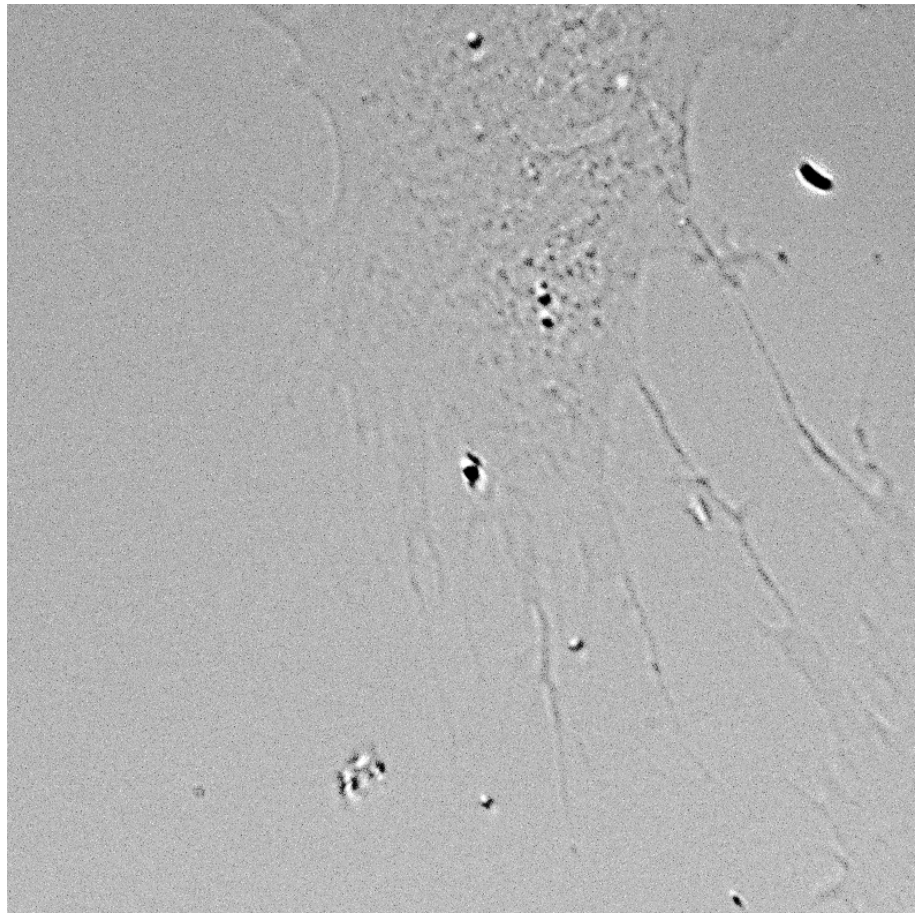
Kaj manjka tem slikam?



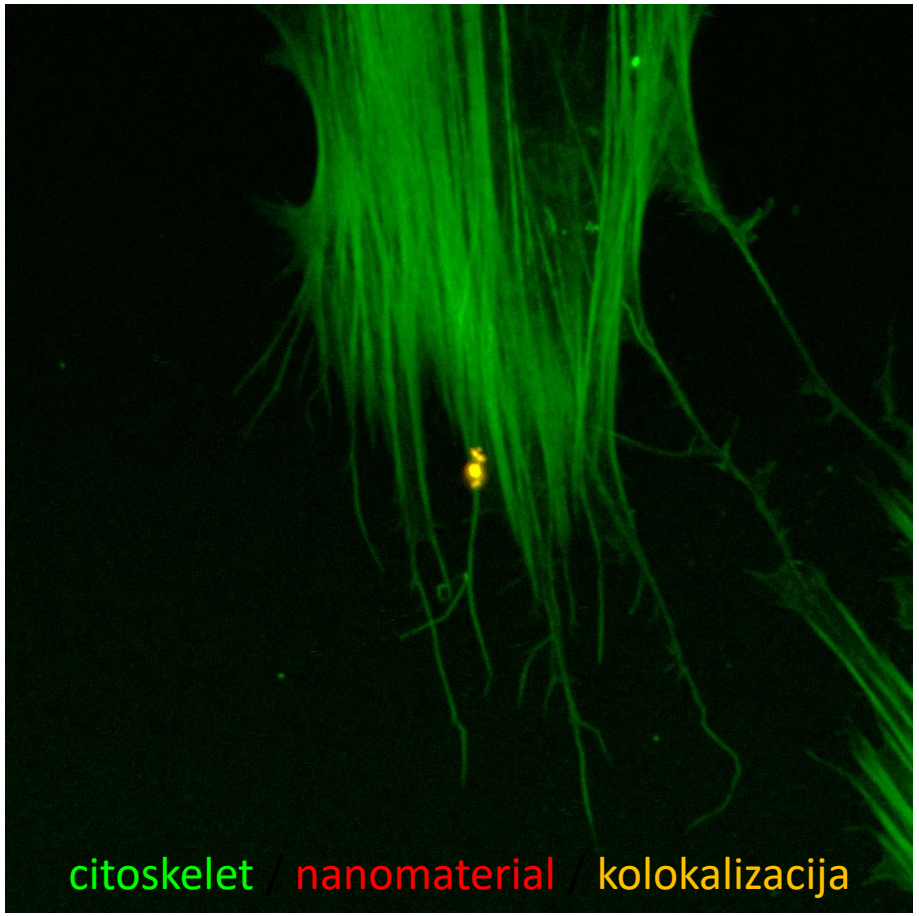


V čem se razlikujeta sliki iste celice?

Presevna mikroskopija



Fluorescenčna mikroskopija



Fluorescenca: revolucija kontrasta

Osnove fluorescence

Energijski prehodi elektrona

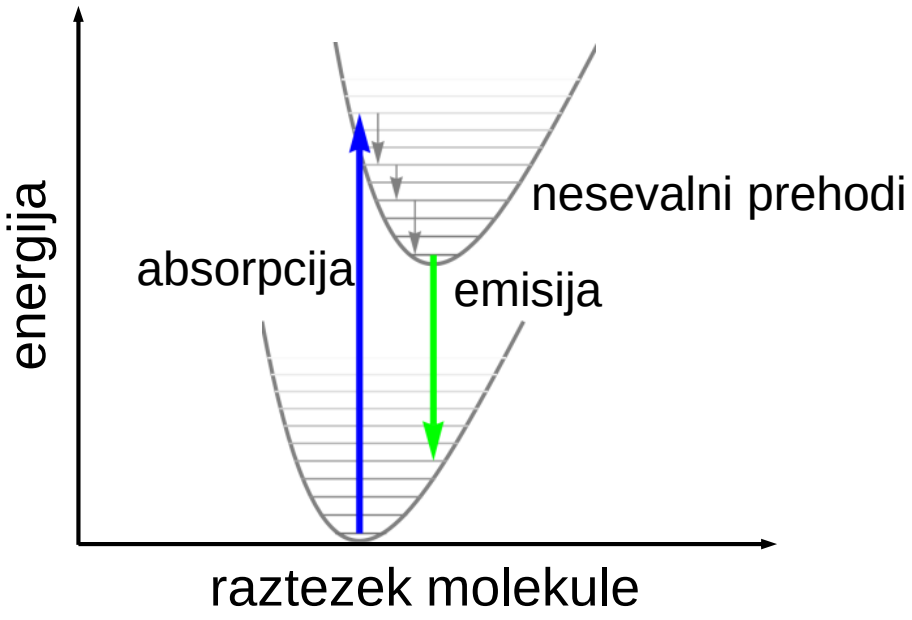
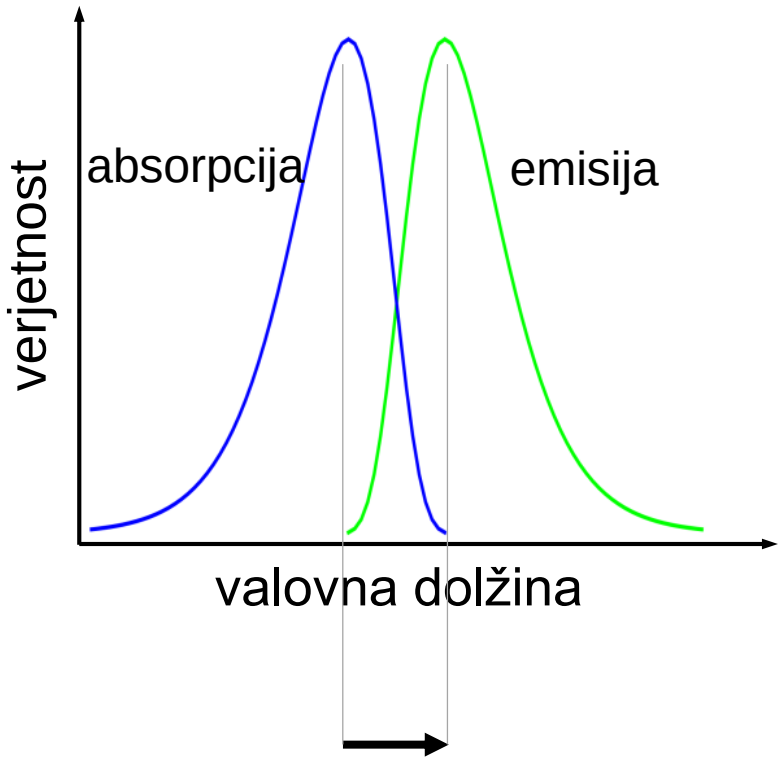


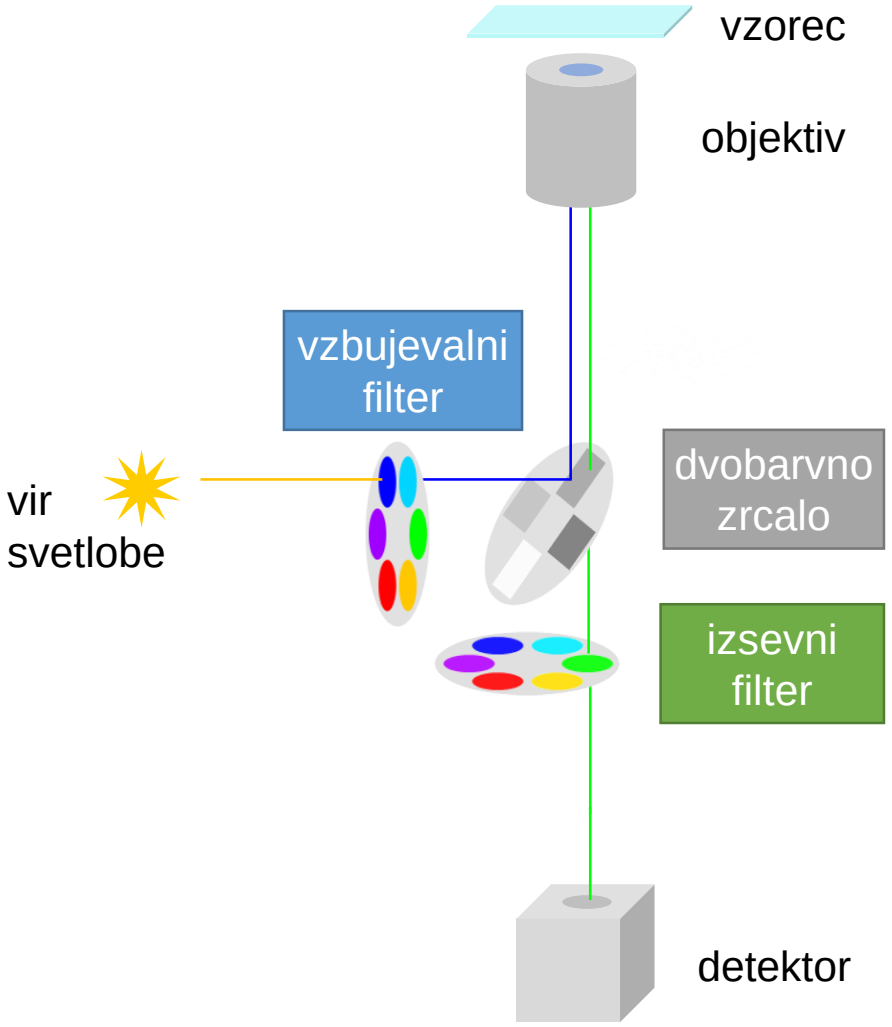
Diagram Jabłonskega

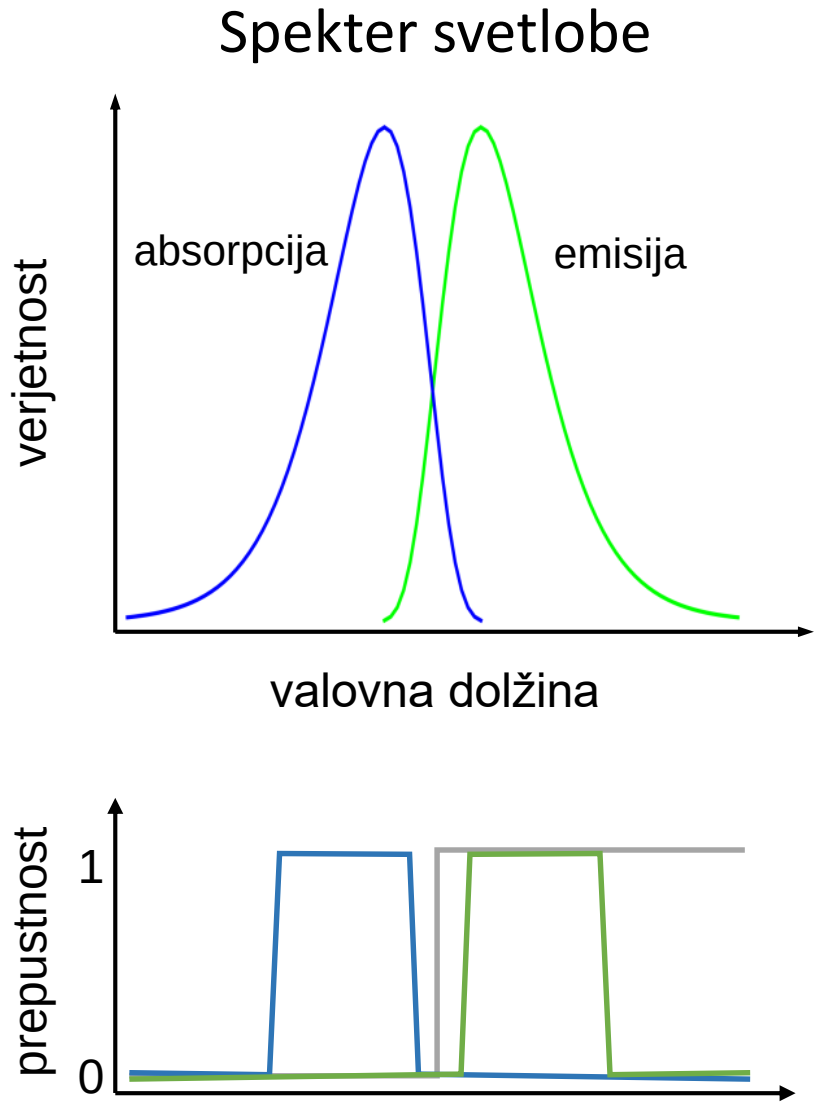
Spekter svetlobe



Stokesov premik

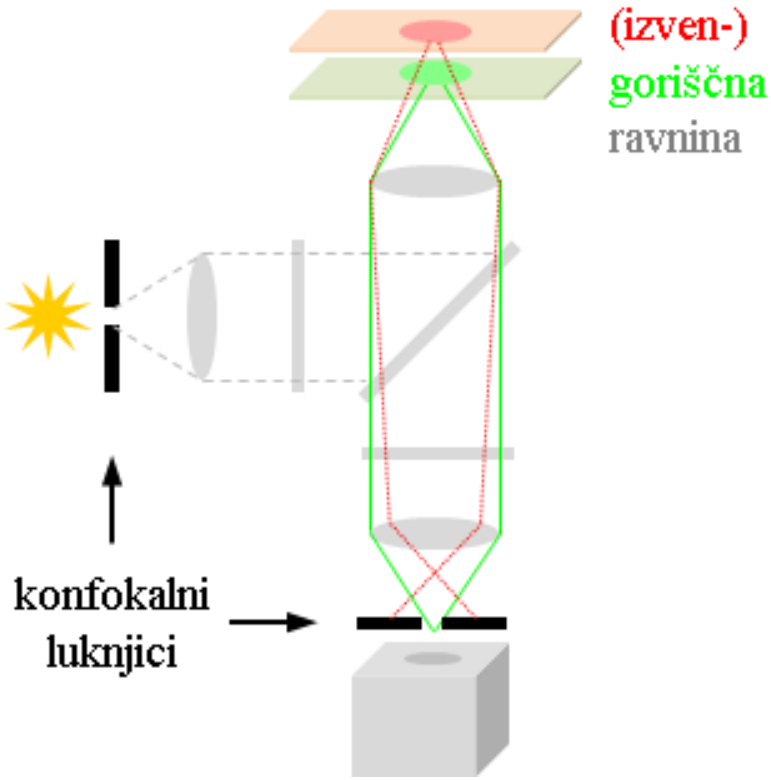
Fluorescenčni mikroskop



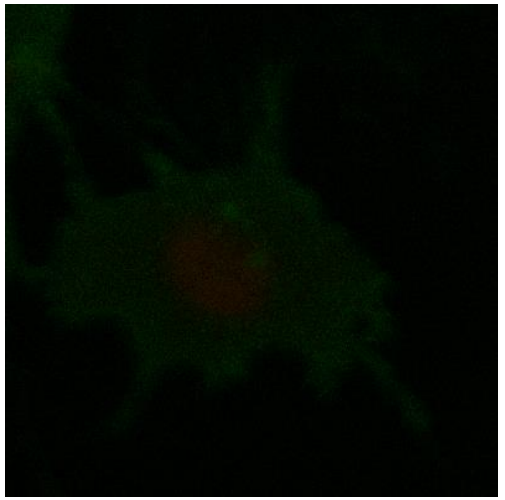


Konfokalni fluorescenčni mikroskop

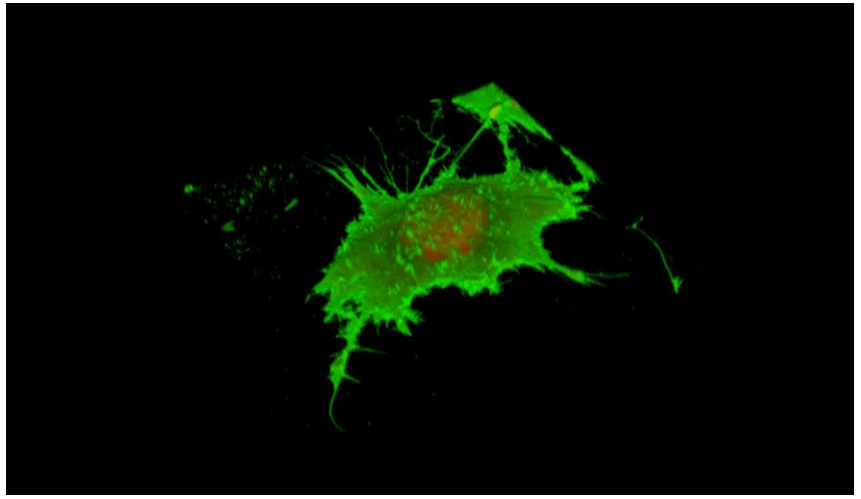
- Omogoča optično rezinjenje



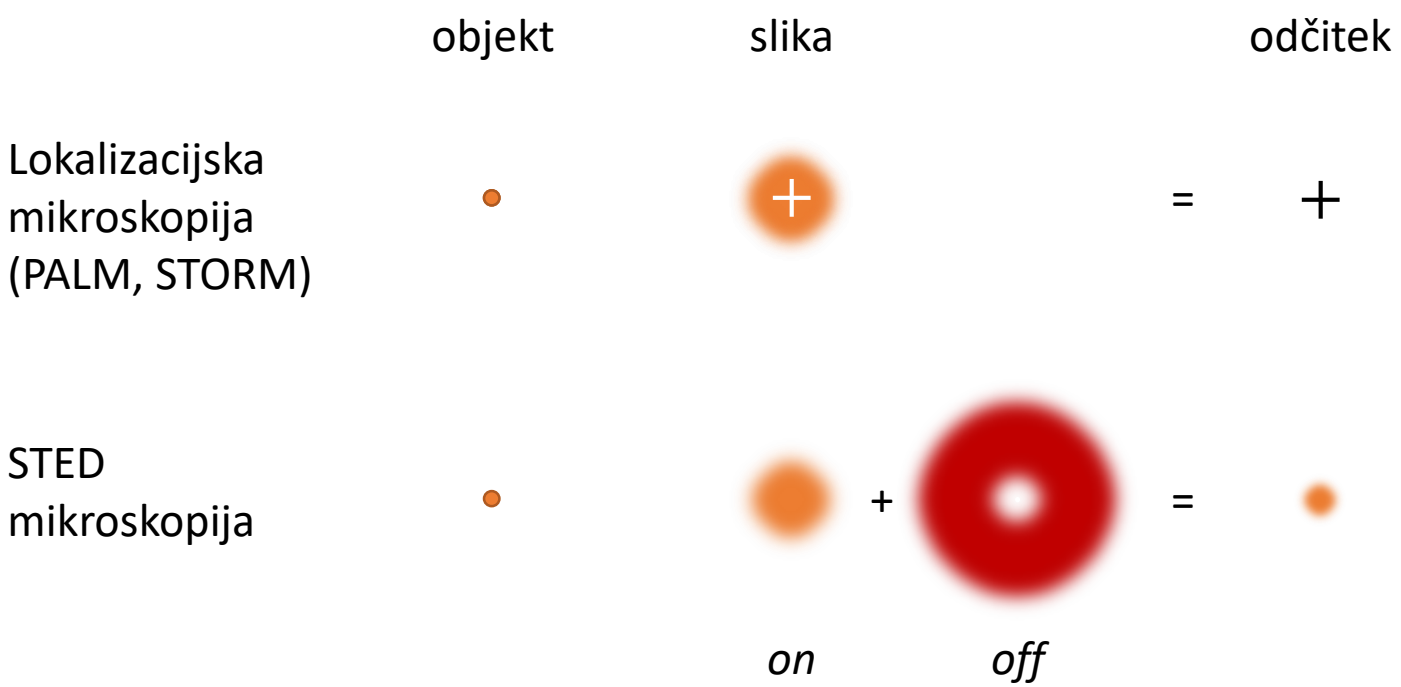
Niz slik po globini:



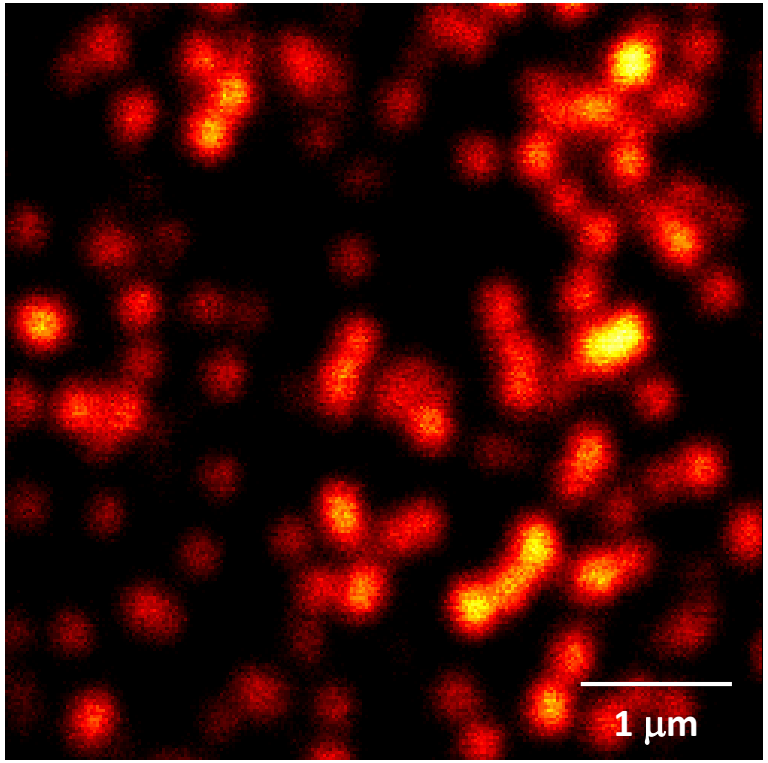
3D rekonstrukcija:



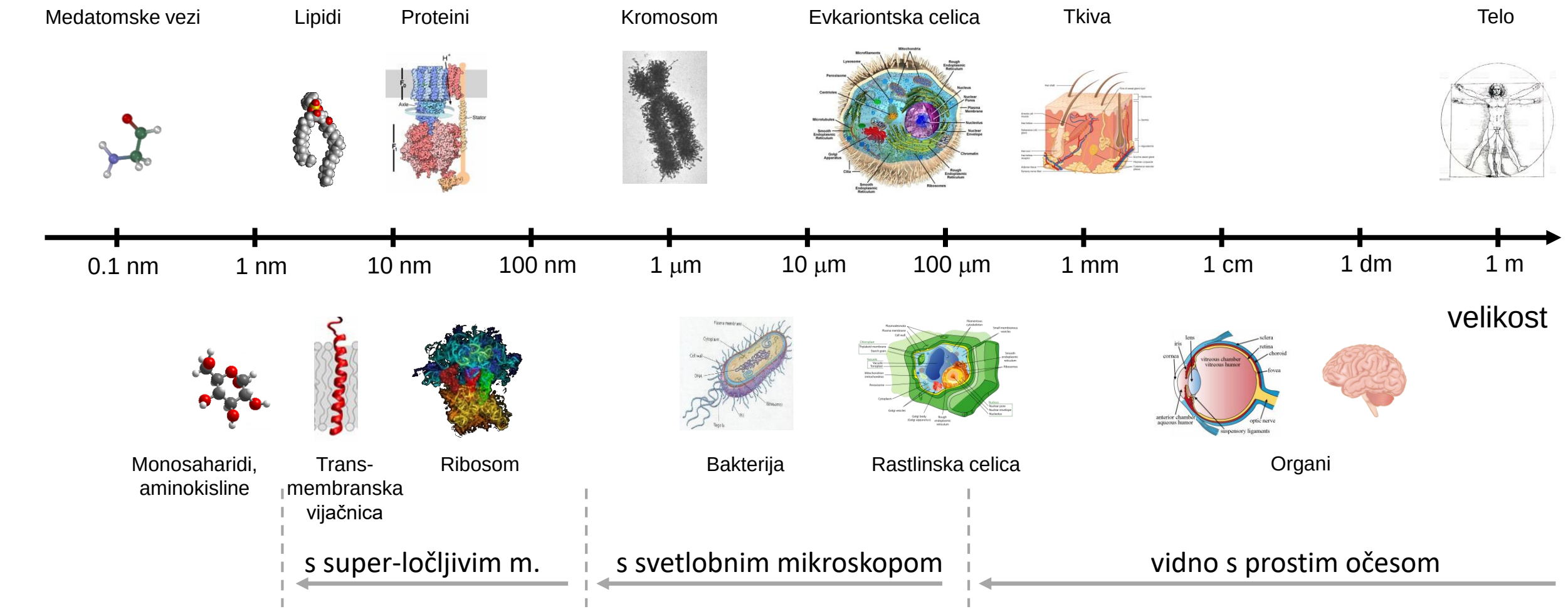
Superločljiv fluorescenčni mikroskop



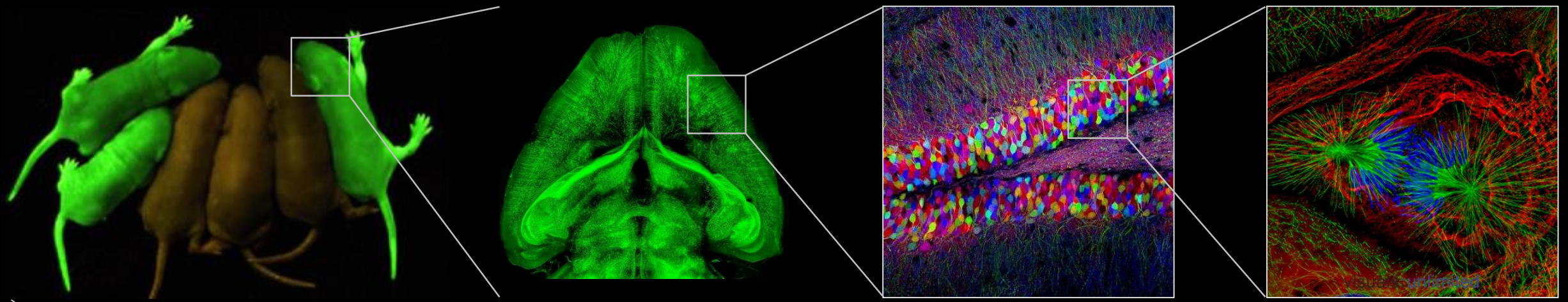
Fluorescenčne kroglice (40 nm)



Velikostne skale življenja



Fluorescenca: revolucija specifičnosti

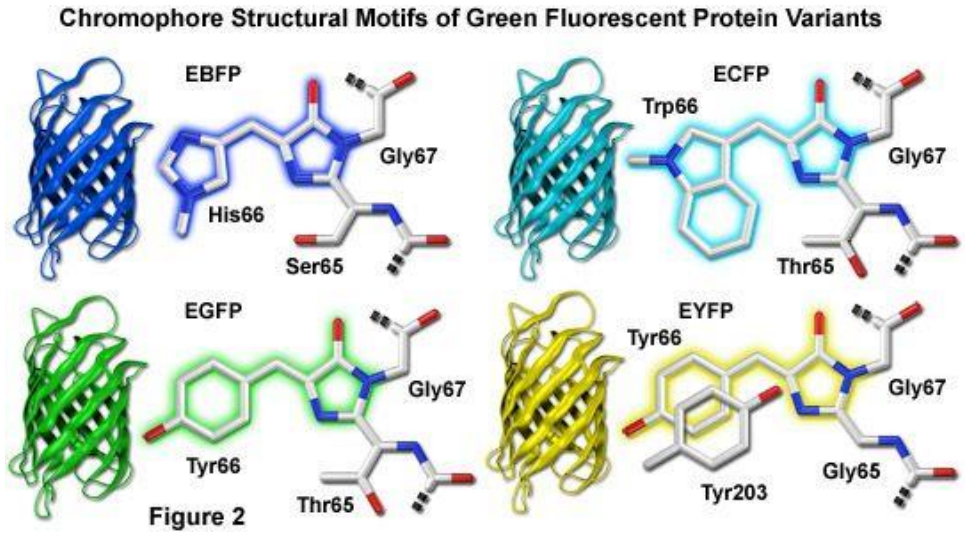


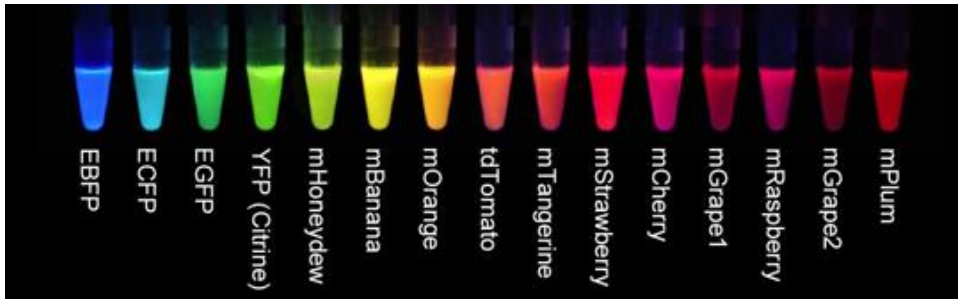
Organizem | Organ | Tkivo | Celica | Organel | Molekula

1 m | 10^{-1} m | 10^{-2} m | 10^{-3} m | 10^{-4} m | 10^{-5} m | 10^{-6} m | 10^{-7} m | 10^{-8} m | 10^{-9} m

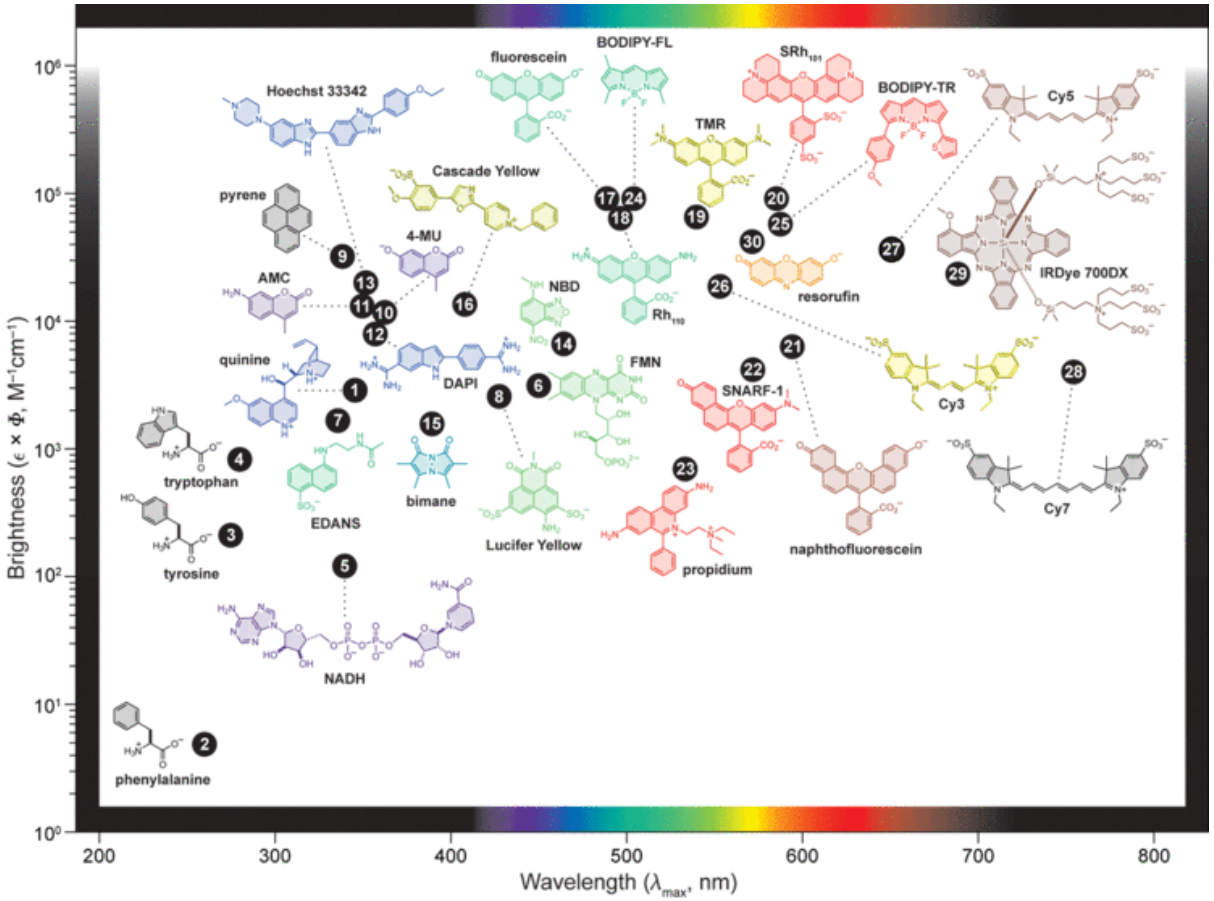
Fluorescenčna barvila

Fluorescenčni proteini





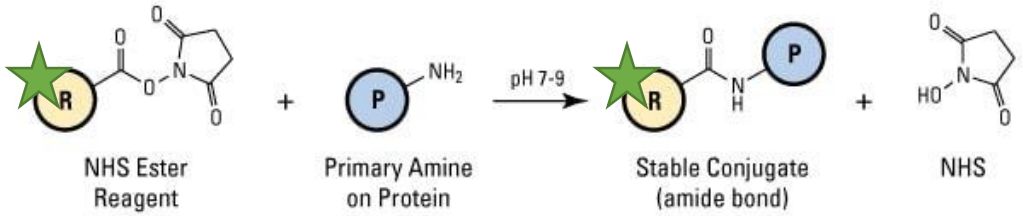
Organska barvila

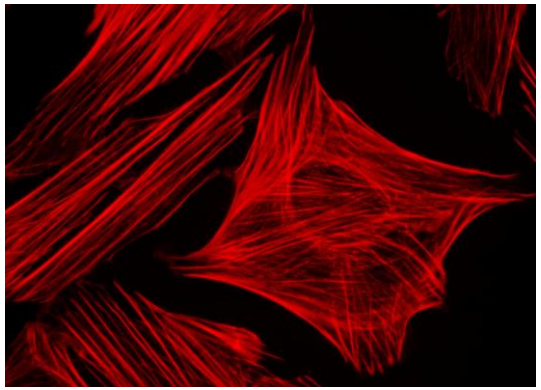


Fluorescenčno označevanje proteinov

Nespecifično

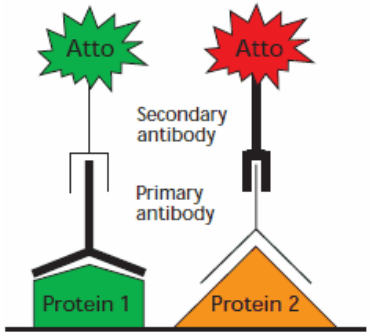
Označevanje izoliranih proteinov
(npr. protiteles)





Specifično

Fluorescenčno označena protitelesa

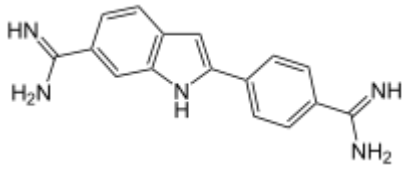


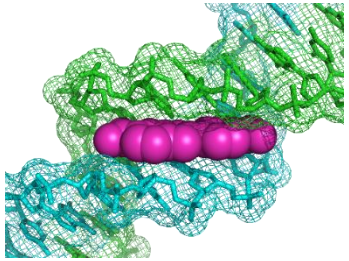
Ekspresija fluorescenčnih proteinov v celici

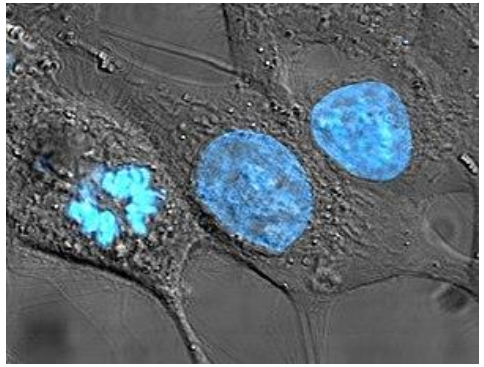
Fluorescenčno označevanje DNA/RNA

Nespecifično

DAPI, Hoechst, ...

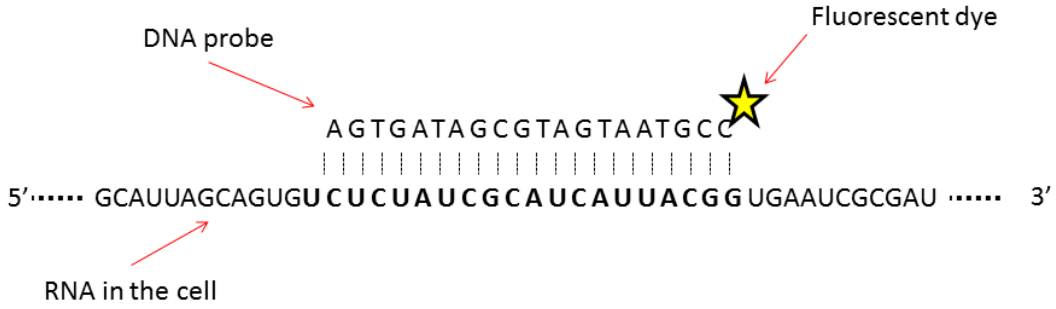


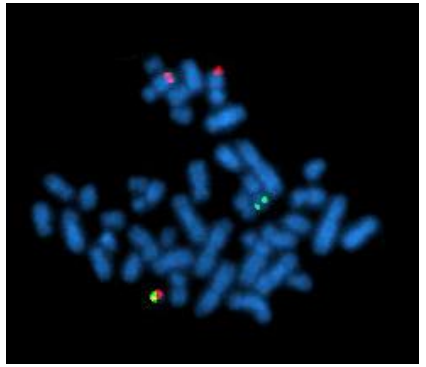




Specifično

Fluorescence in situ hybridization (FISH)





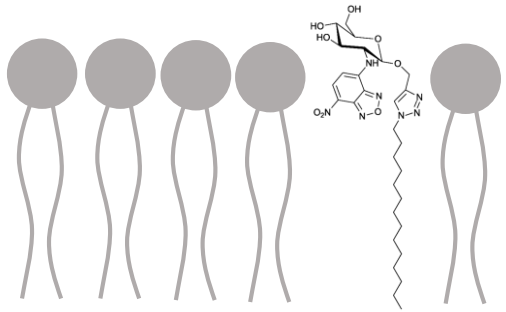
Fluorescenčno označevanje lipidov

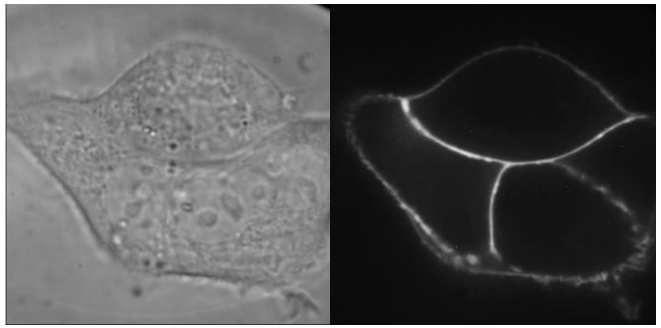
Nespecifično

Fluorescenčni analogi lipidov, maščobnih kislin, transmembranskih proteinov ipd. (amfifilne molekule)

hidrofilno

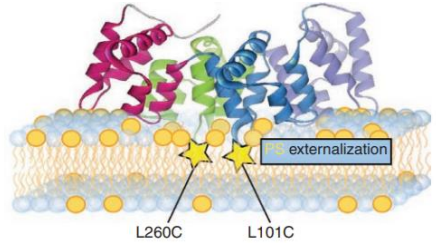
hidrofobno



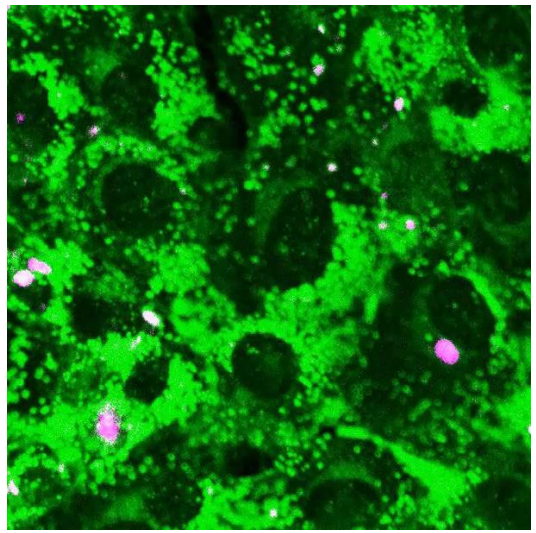


Specifično

Vezava na izbrano vrsto lipidov (fosfatidilserin)



Kim *Nature Protocols* 2010



Fluorescenčna mikroskopija

Kontrast + specifičnost