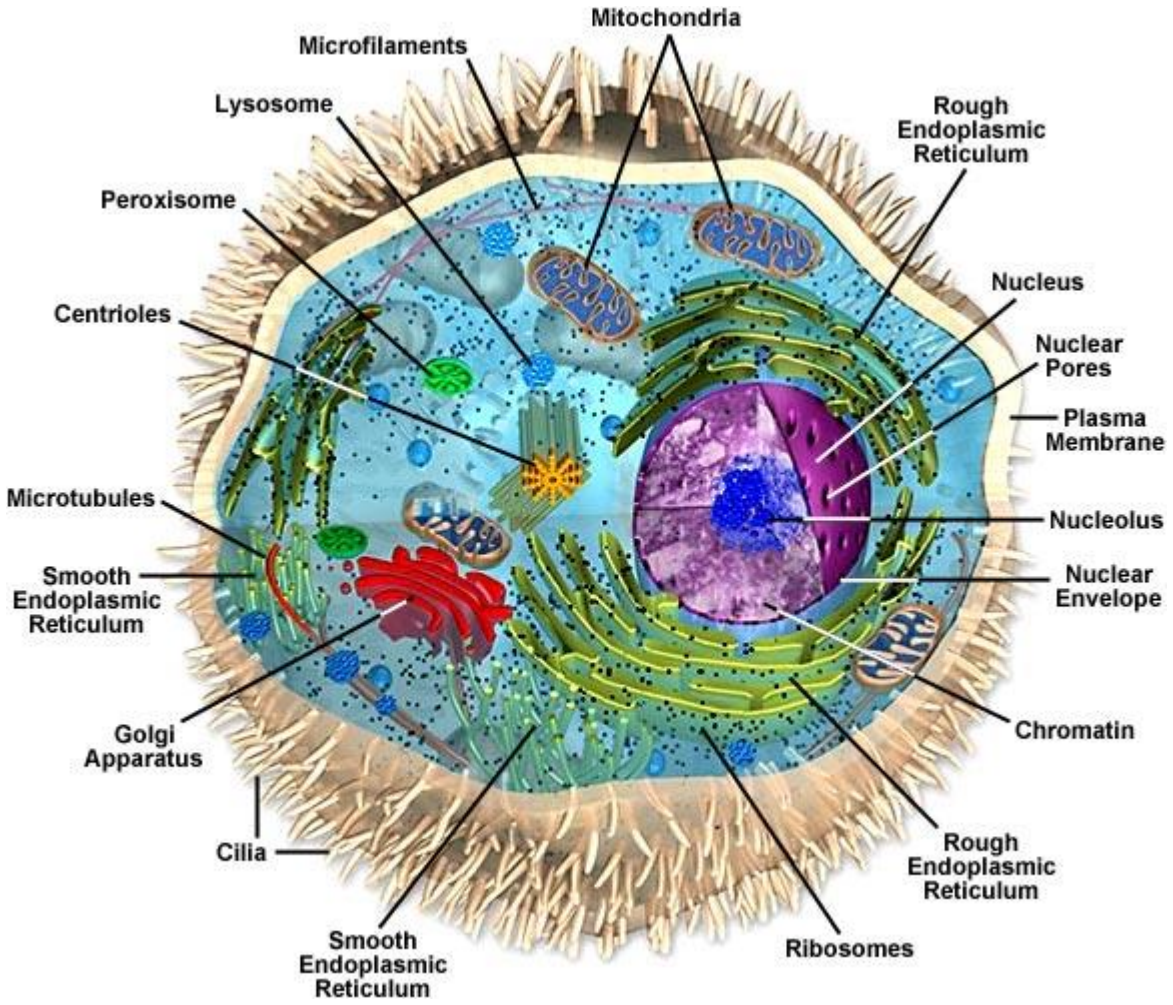
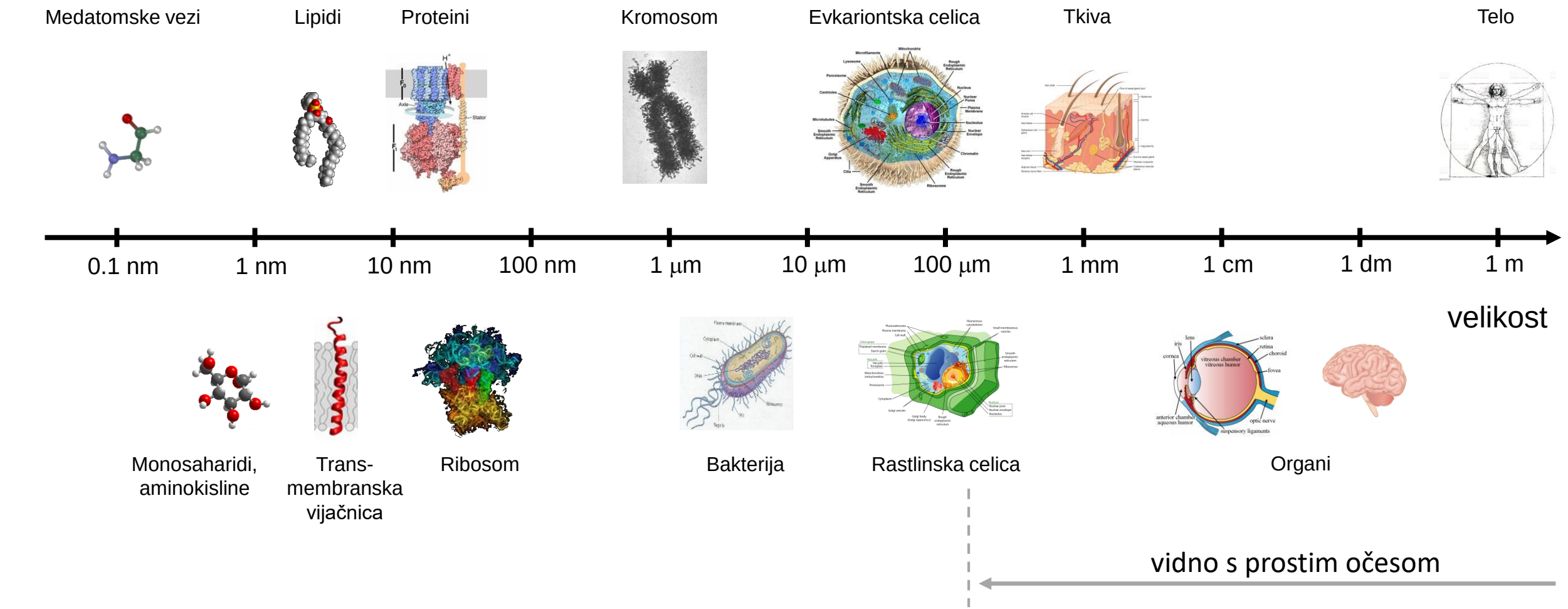
celica



Razdalje

https://www.youtube.com/watch?v=DWouwx3Hxmk

Velikostne skale življenja



Kaj je veliko in kaj majhno?

- Velikosti gradnikov primerjamo s tipično dimenzijo, npr. s premikom fronte molekul zaradi difuzije (**difuzijski premik**), ki je

  – odvisen od reologije (povezanosti prostora)
  – odvisen od velikosti in tipičnega časa sistema

$$\Lambda^2 \propto D\tau$$

  – tipični difuzijski premik v značilnem času spreminjanja konformacij (1 ns) je za majhne molekule:

    - v vodni raztopini 10 nm,
    - v membrani 1 nm,
    - v močno koncentrirani sladkorni raztopini pa manj kot 0.3 nm

Ravnila

- Če hočemo izmeriti velikost, moramo narediti “ravnilo” in definirati “enoto” (spodnjo mejo ločljivosti)

- “Enoto” definira orodje, s katerim preiskujemo snov

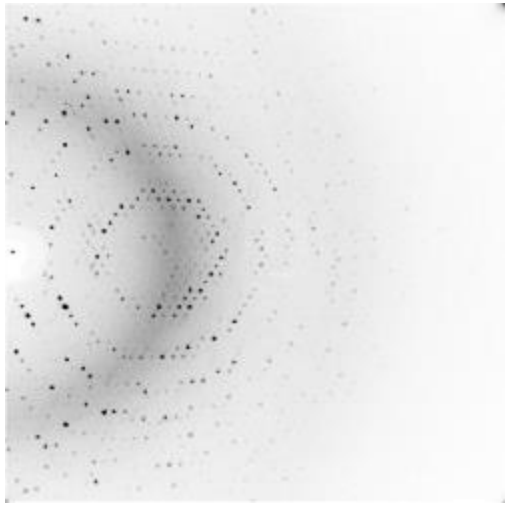
  – Če snov gledamo s svetlobo ali hitrimi delci, je to njihova **valovna dolžina**
    - vidna svetloba λ = 300 - 700 nm
    - rentgenska svetloba λ = 0.1 - 10 nm
    - elektroni λ = 0.02 - 0.1 nm

  – Če opazujemo sosledje difuzijskih dogodkov, je to **difuzijski premik**
    - fotonska korelacijska spektroskopija Λ = 10 nm

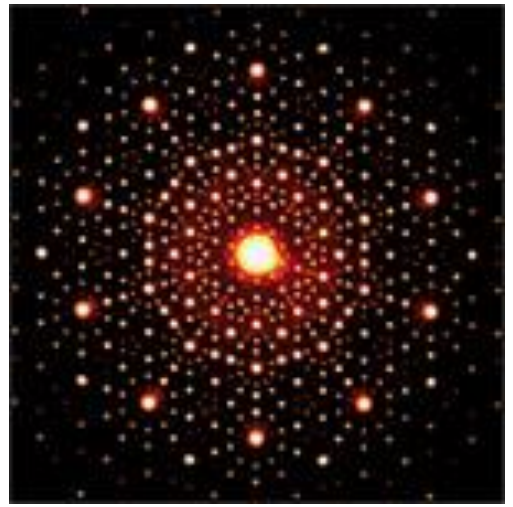
Sipanja kot ravnila

- Sipanja
  - ojačanje in slabljenje širjenja valovanja (interferenca) po uklonu na ovirah brez absorpcije
  - meja ločljivosti: valovna dolžina in urejenost vzorca
  - primeri:
    - sipanje rentgenskih žarkov
    - sipanje elektronov
    - sipanje nevtronov

SAXS



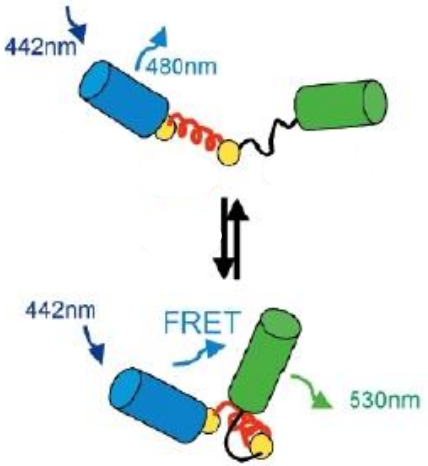
electron diffraction

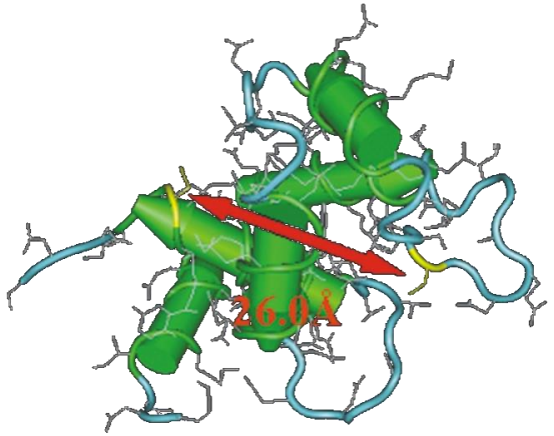


Spektroskopije kot ravnila

- Spektroskopije
  - odvisnost absorpcije svetlobe od njene energije
  - meja ločljivosti: najmanjša izmerljiva spektralna sprememba
  - doseg: najmanjša izmerljiva izmenjava energije

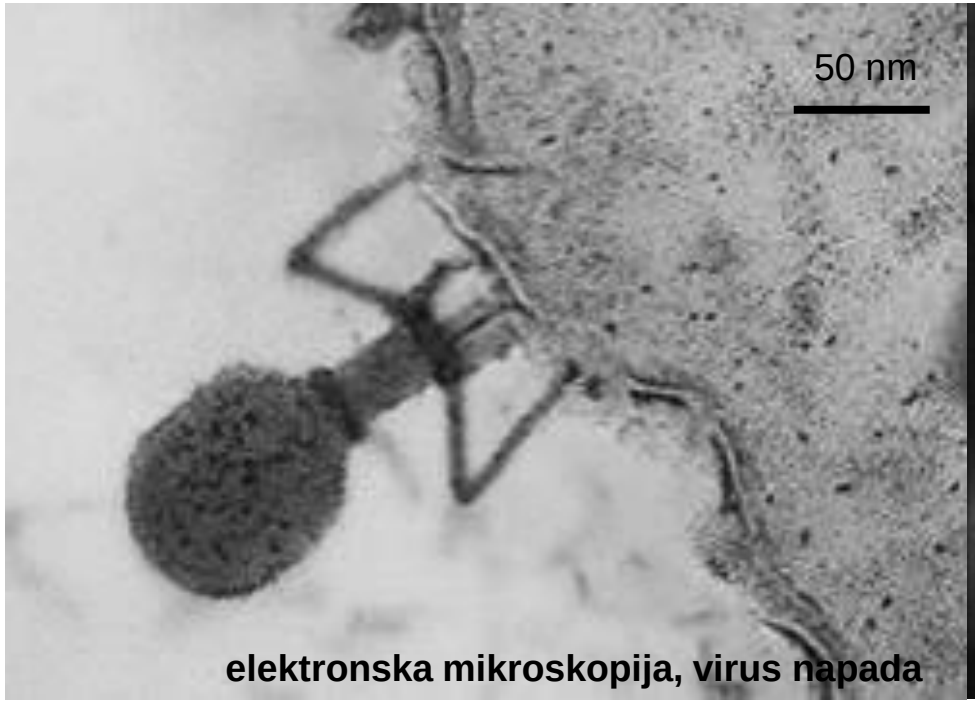
  - primeri:
    - FRET (fluorescence resonance energy transfer)
    - NOE (nuclear Overhauser effect)
    - ELDOR (electron-electron double resonance)



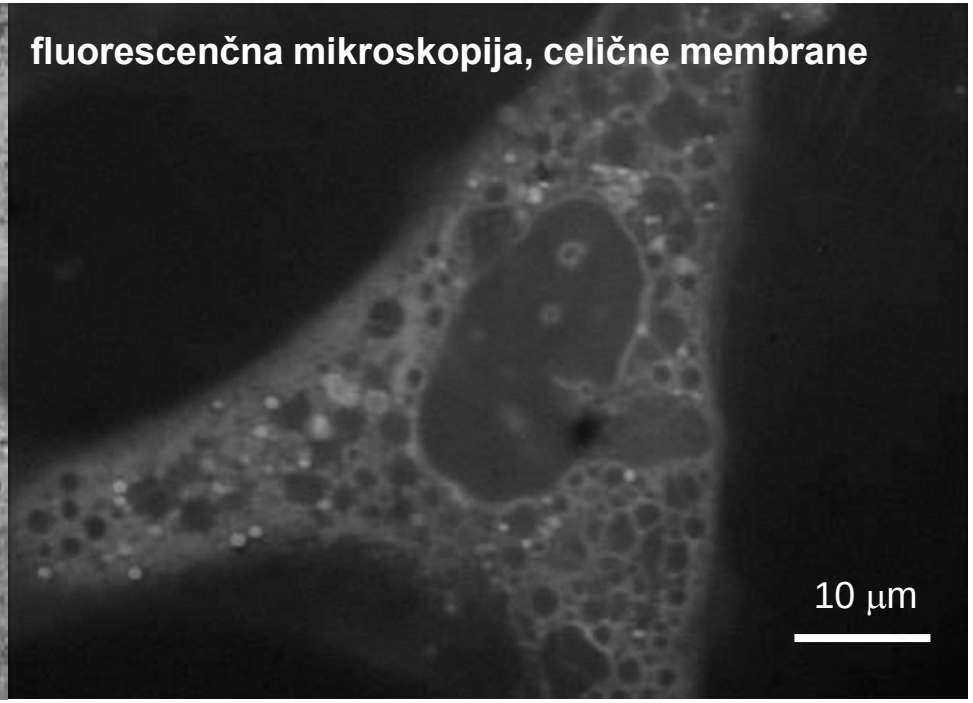


Mikroskopije kot ravnila

- Mikroskopije
  - krajevno odvisna absorbcija svetlobe ali sipanje delcev
  - meja ločljivosti: valovna dolžina



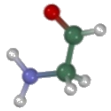
elektronska mikroskopija, virus napada



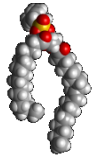
fluorescenčna mikroskopija, celične membrane

Fluorescenčna mikroskopija

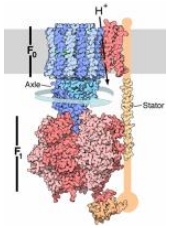
Velikostne skale življenja



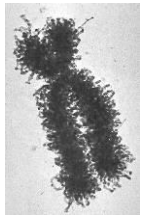
Medatomske vezi



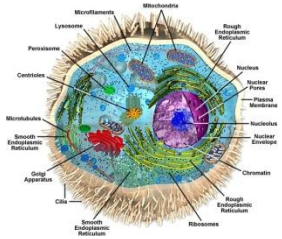
Lipidi



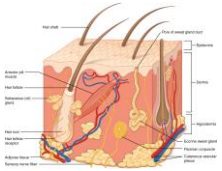
Proteini



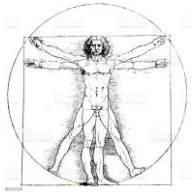
Kromosom



Evkariontska celica



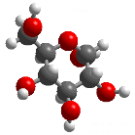
Tkiva



Telo

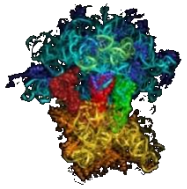
0.1 nm | 1 nm | 10 nm | 100 nm | 1 μm | 10 μm | 100 μm | 1 mm | 1 cm | 1 dm | 1 m

velikost

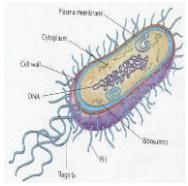


Monosaharidi, aminokisline

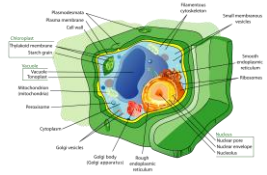
Trans-membranska vijačnica



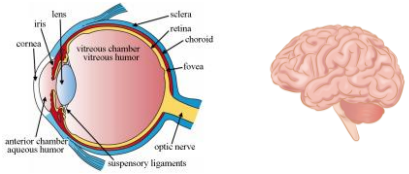
Ribosom



Bakterija



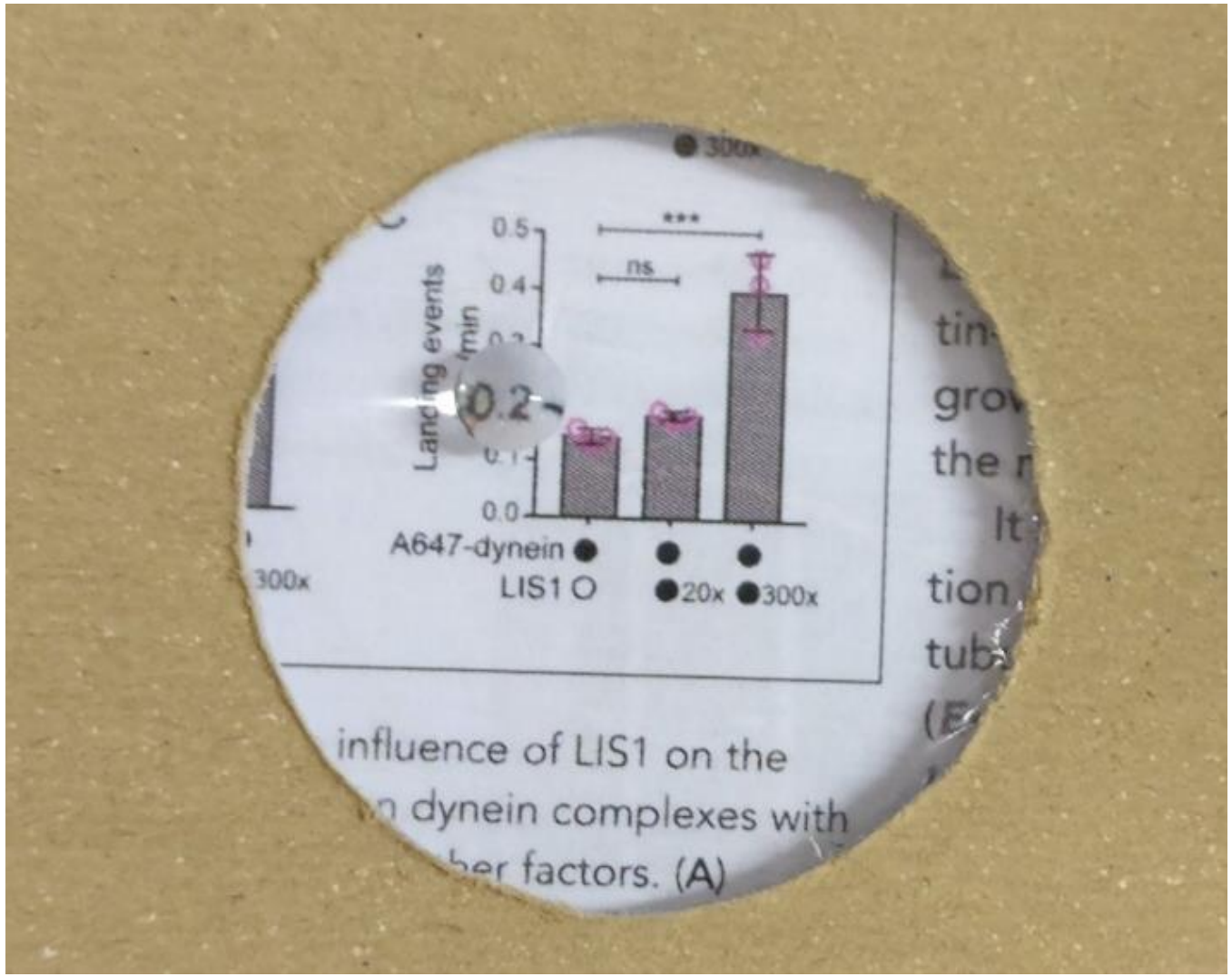
Rastlinska celica



Organi

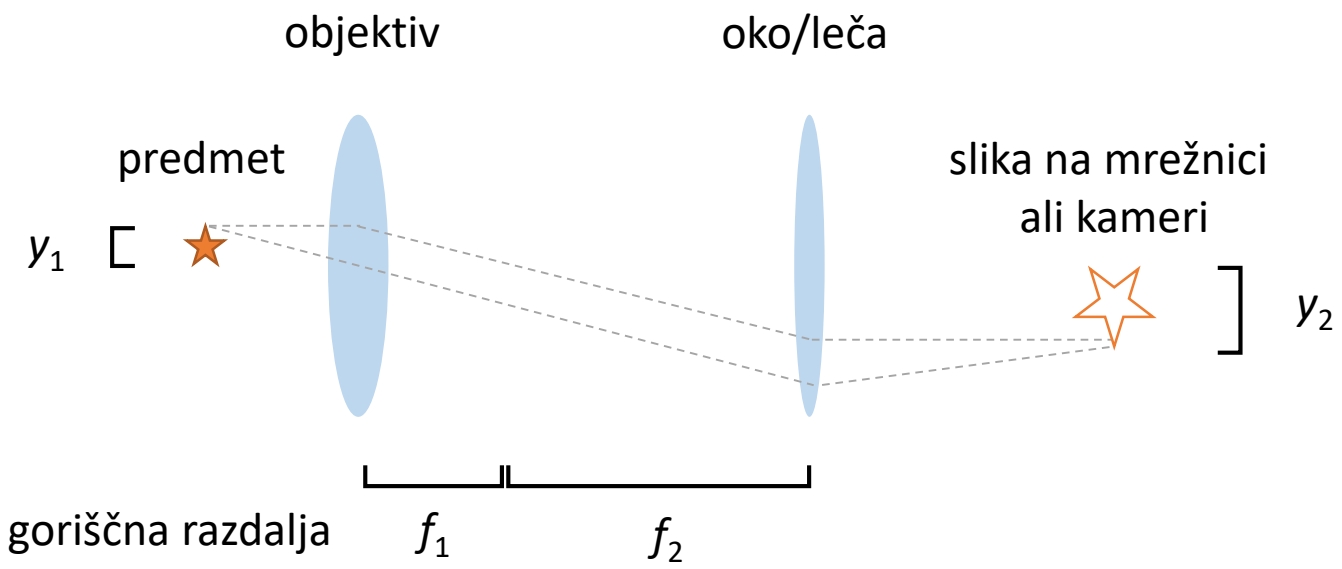
vidno s prostim očesom

Kako lahko vidimo majhne stvari?



Kako lahko vidimo majhne stvari?

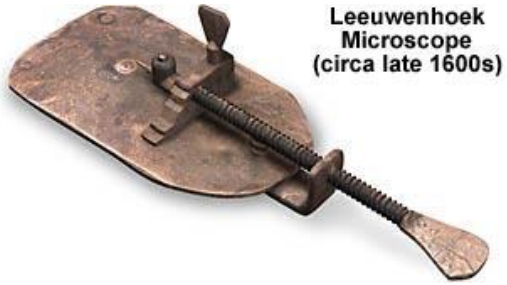
Povečava slike zaradi uklona svetlobe na ukrivljeni površini:



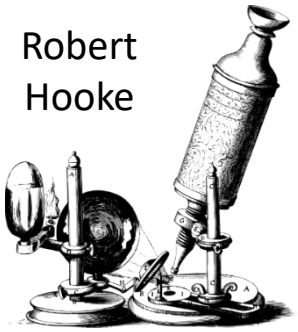
Optična povečava: $M = y_2 / y_1 = f_2 / f_1$

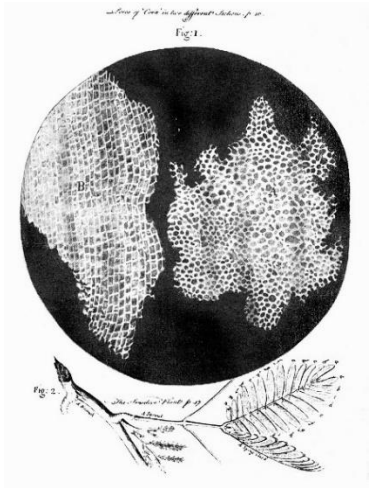
Kratka zgodovina svetlobne mikroskopije

17. stol.



Robert Hooke

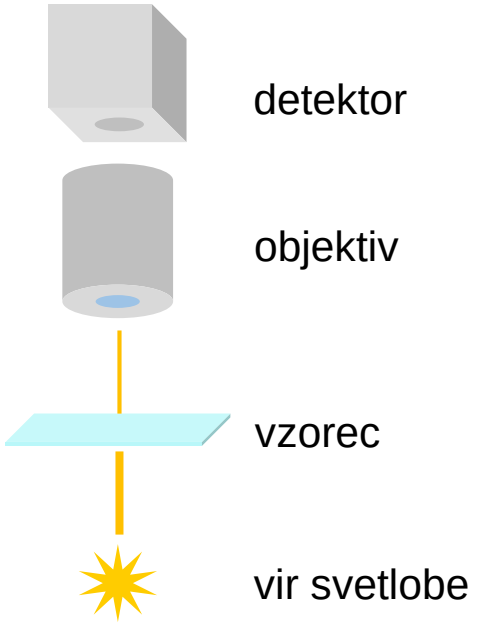




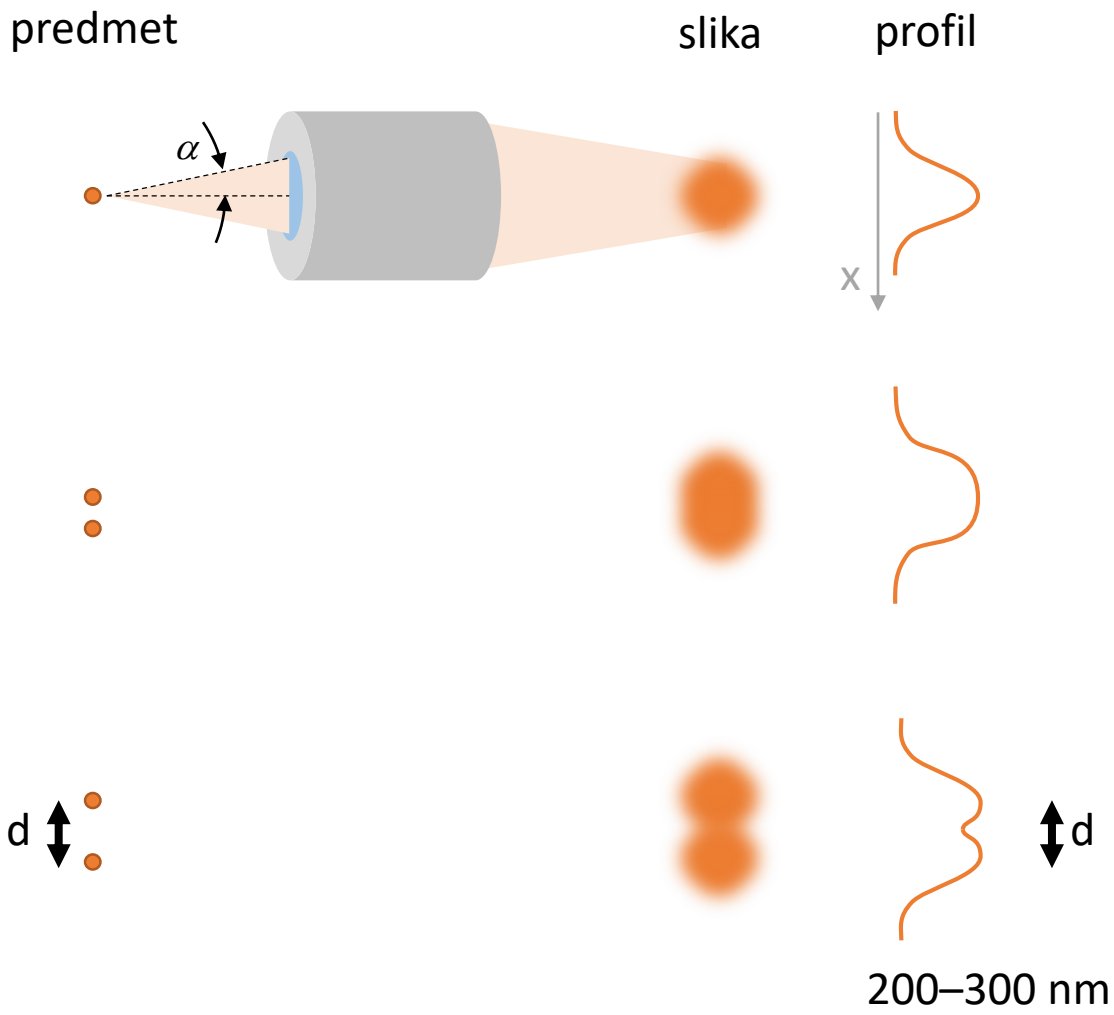
20. stol.

21. stol.

Zgradba presevnega mikroskopa



Kako podrobno vidimo majhne stvari?



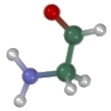
Ločljivost mikroskopa zaradi uklona svetlobe je odvisna od:

- valovne dolžine svetlobe - λ
- numerične odprtine objektiva - $NA = 2\ n \sin(\alpha)$
  n - lomni količnik medija
  α - polovični kot zajema svetlobe

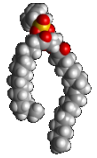
- ne od povečave!

Ernst Abbe

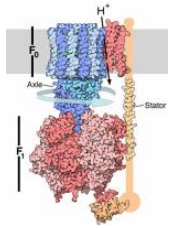
Velikostne skale življenja



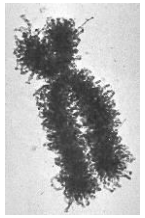
Medatomske vezi



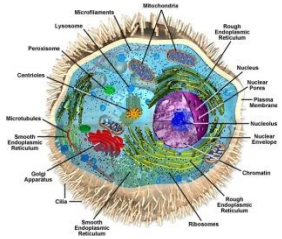
Lipidi



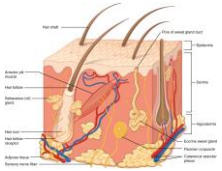
Proteini



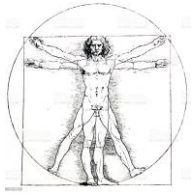
Kromosom



Evkariontska celica



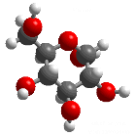
Tkiva



Telo

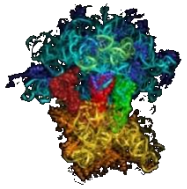
0.1 nm | 1 nm | 10 nm | 100 nm | 1 μm | 10 μm | 100 μm | 1 mm | 1 cm | 1 dm | 1 m

velikost

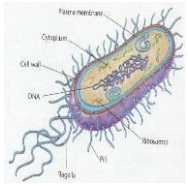


Monosaharidi, aminokisline

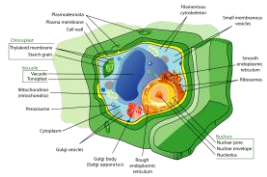
Trans-membranska vijačnica



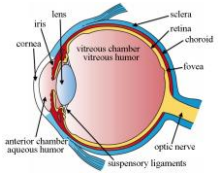
Ribosom



Bakterija



Rastlinska celica

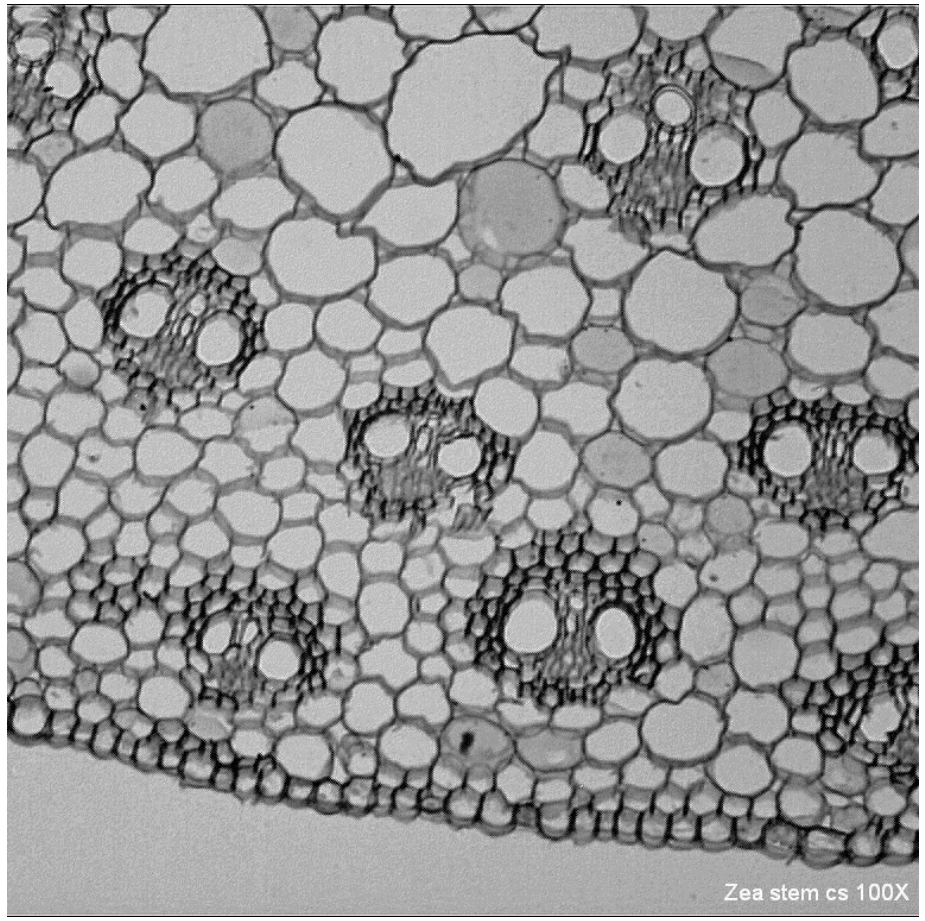


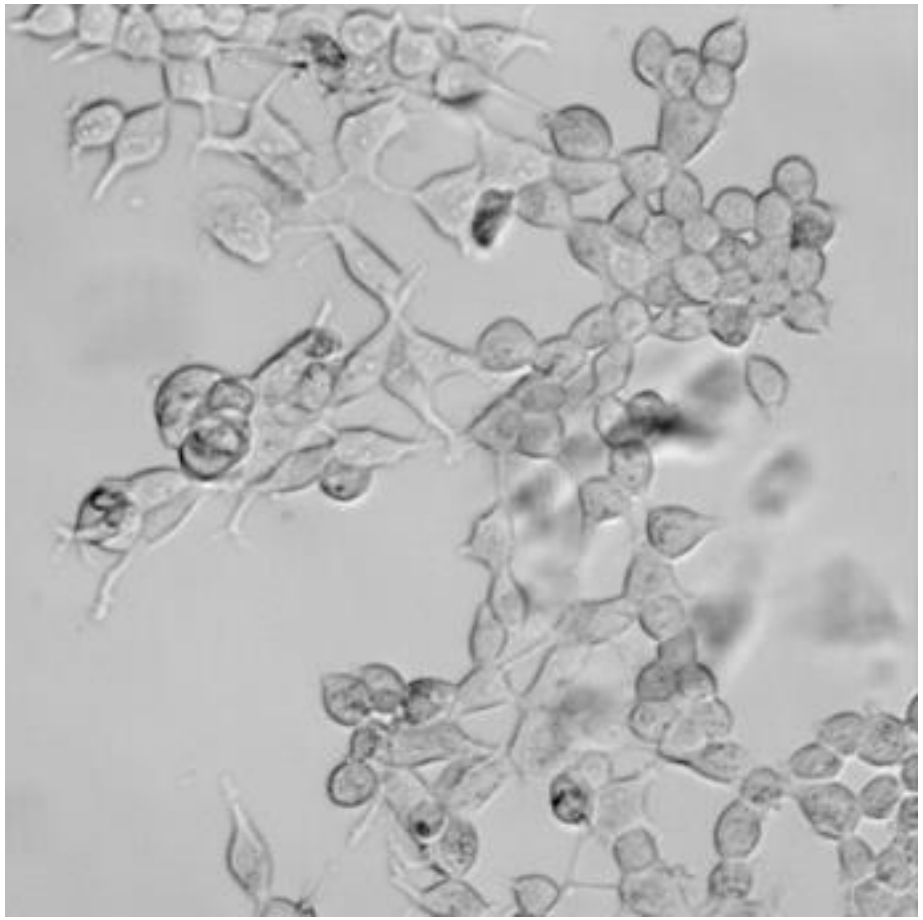
Organi

s svetlobnim mikroskopom

vidno s prostim očesom

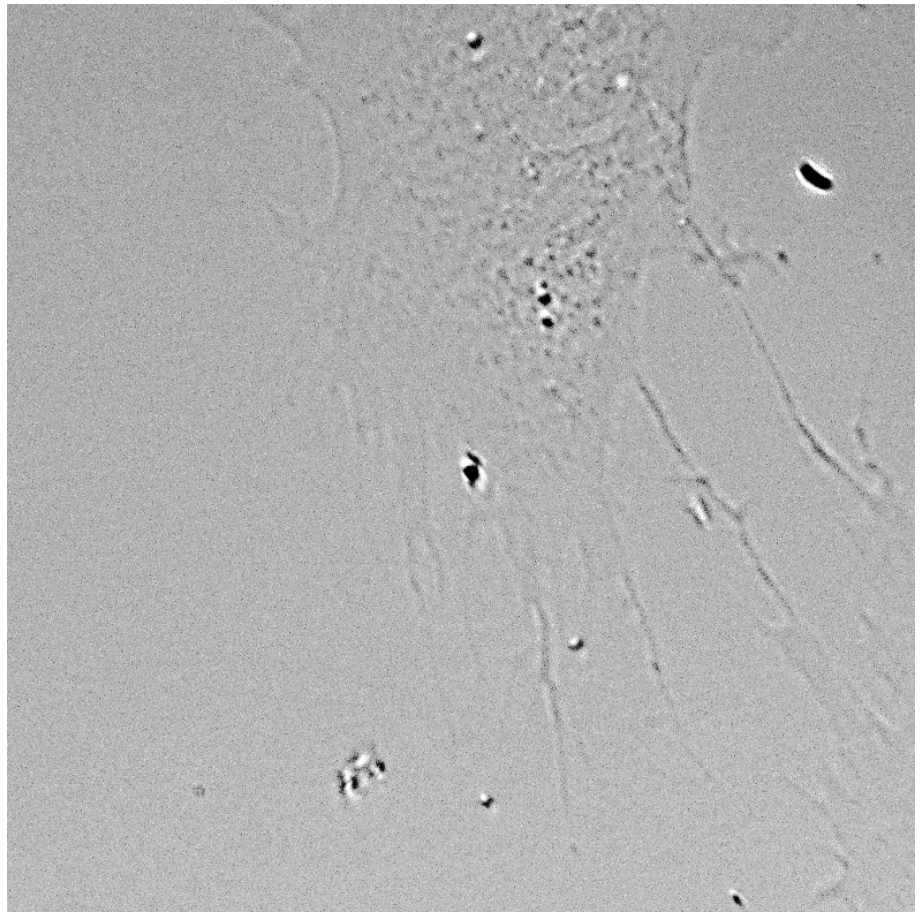
Kaj manjka tem slikam?



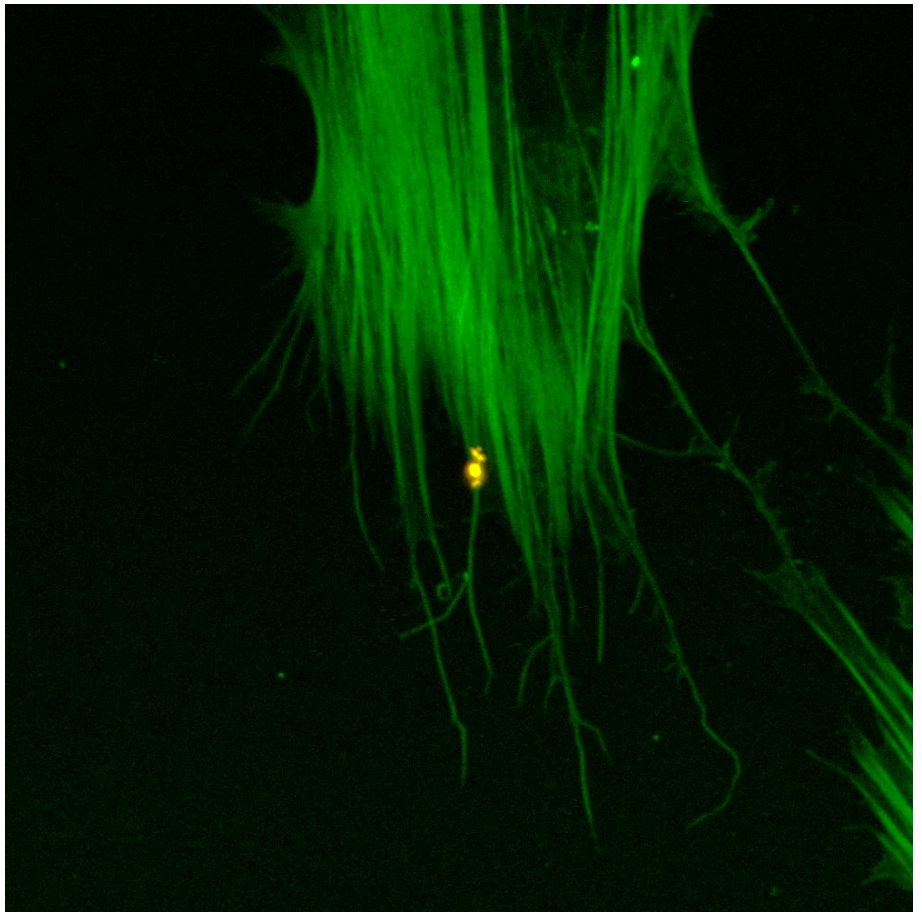


V čem se razlikujeta sliki iste celice?

Presevna mikroskopija



Fluorescenčna mikroskopija



citoskelet / nanomaterial / kolokalizacija

Fluorescenca: revolucija kontrasta

Osnove fluorescence

Energijski prehodi elektrona

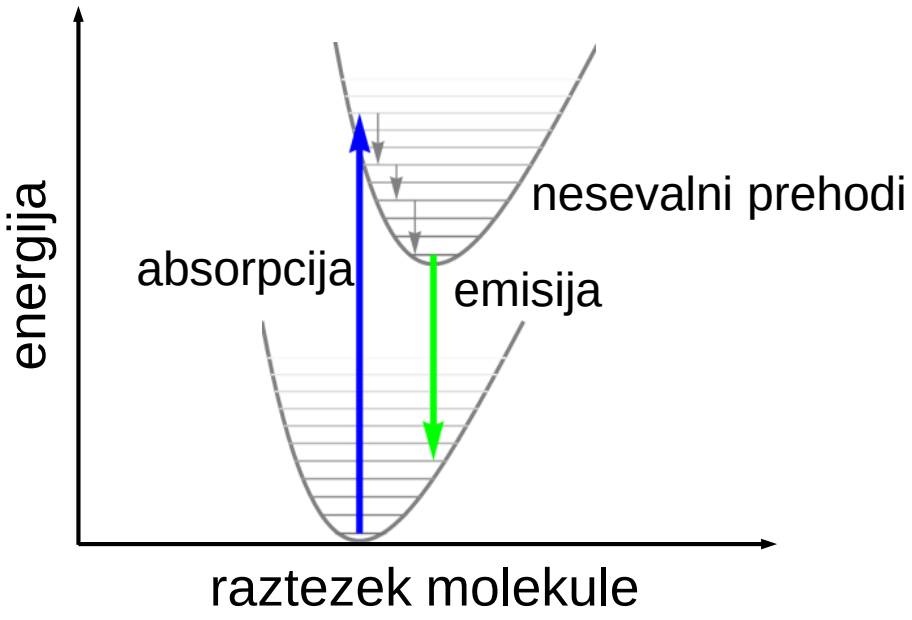
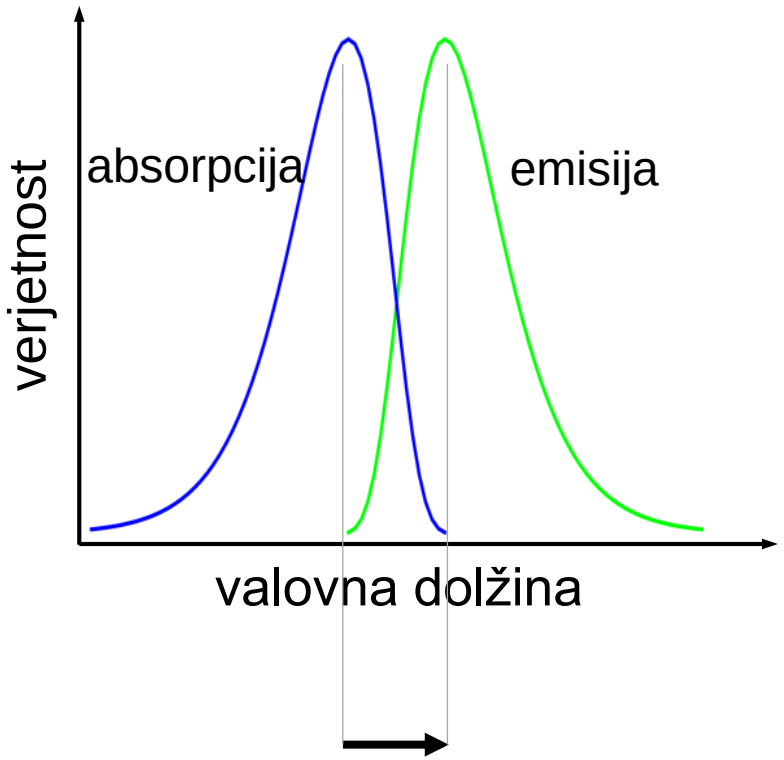


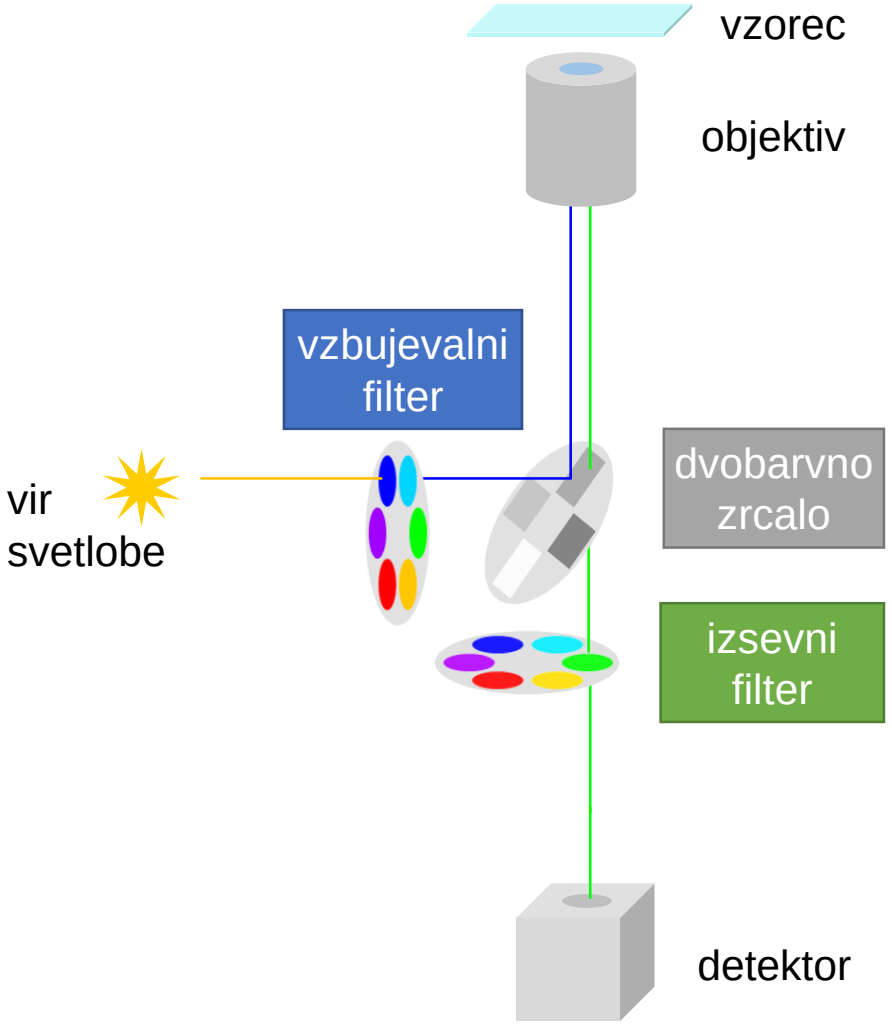
Diagram Jabłonskega

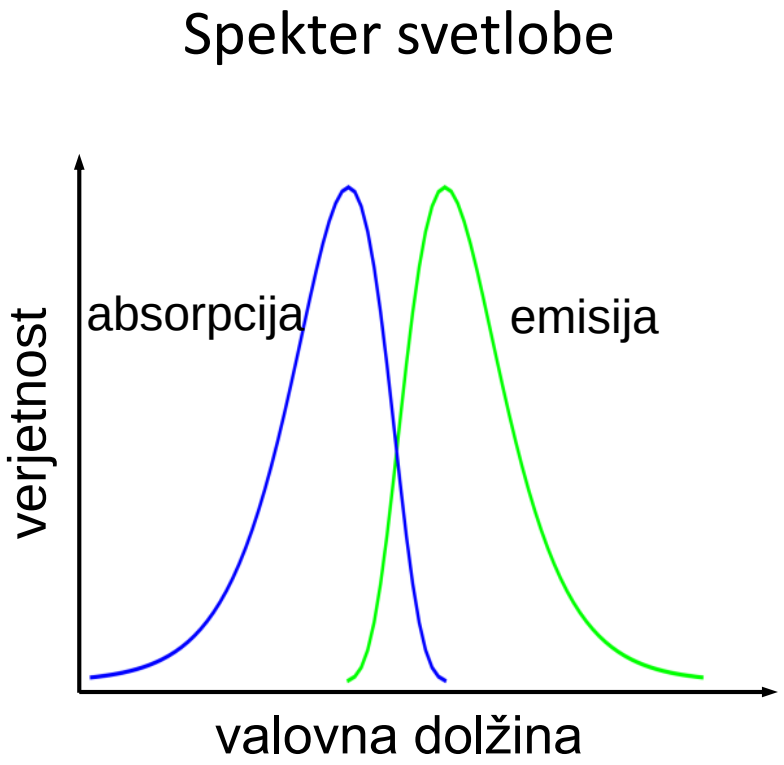
Spekter svetlobe



Stokesov premik

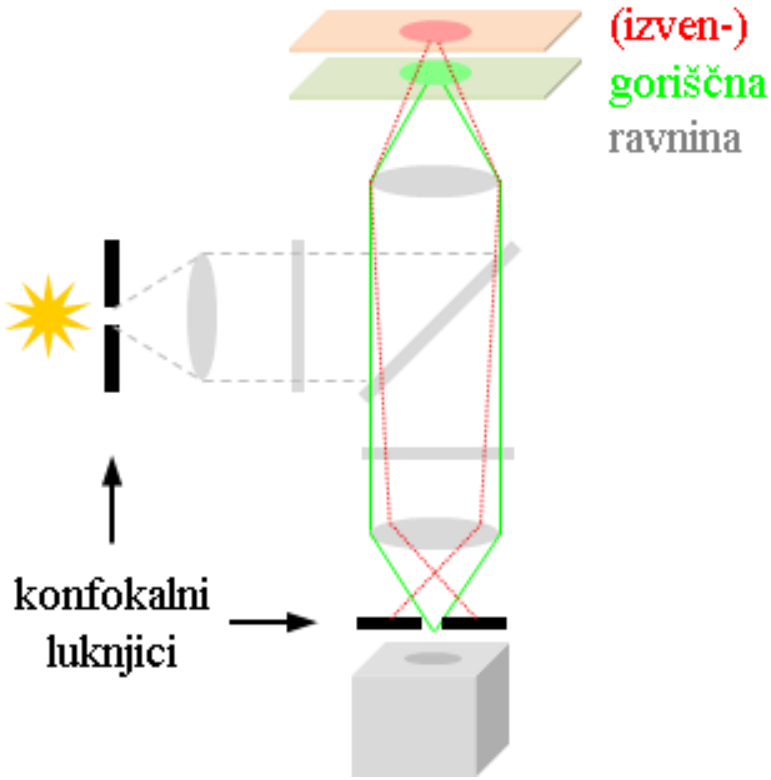
Fluorescenčni mikroskop



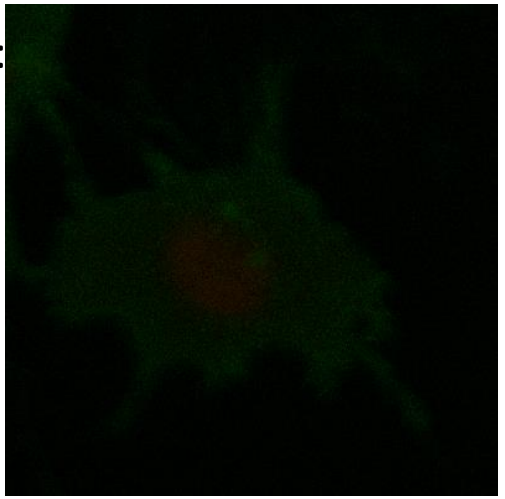


Konfokalni fluorescenčni mikroskop

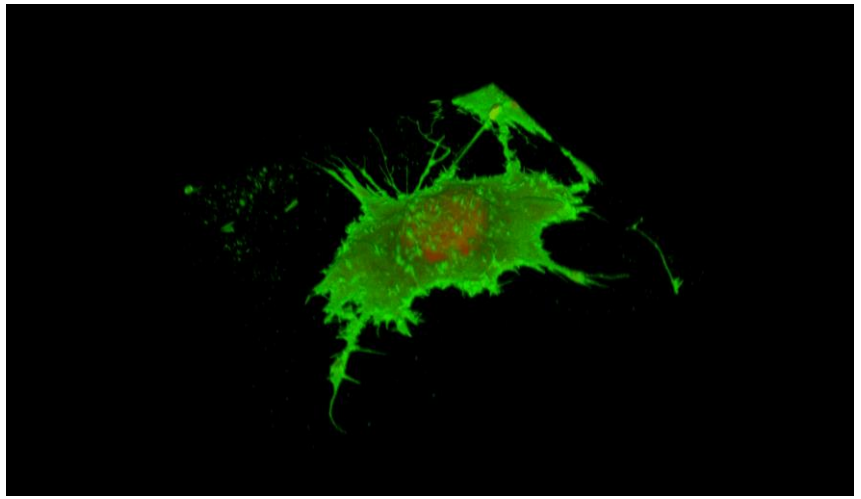
- Omogoča optično rezinjenje



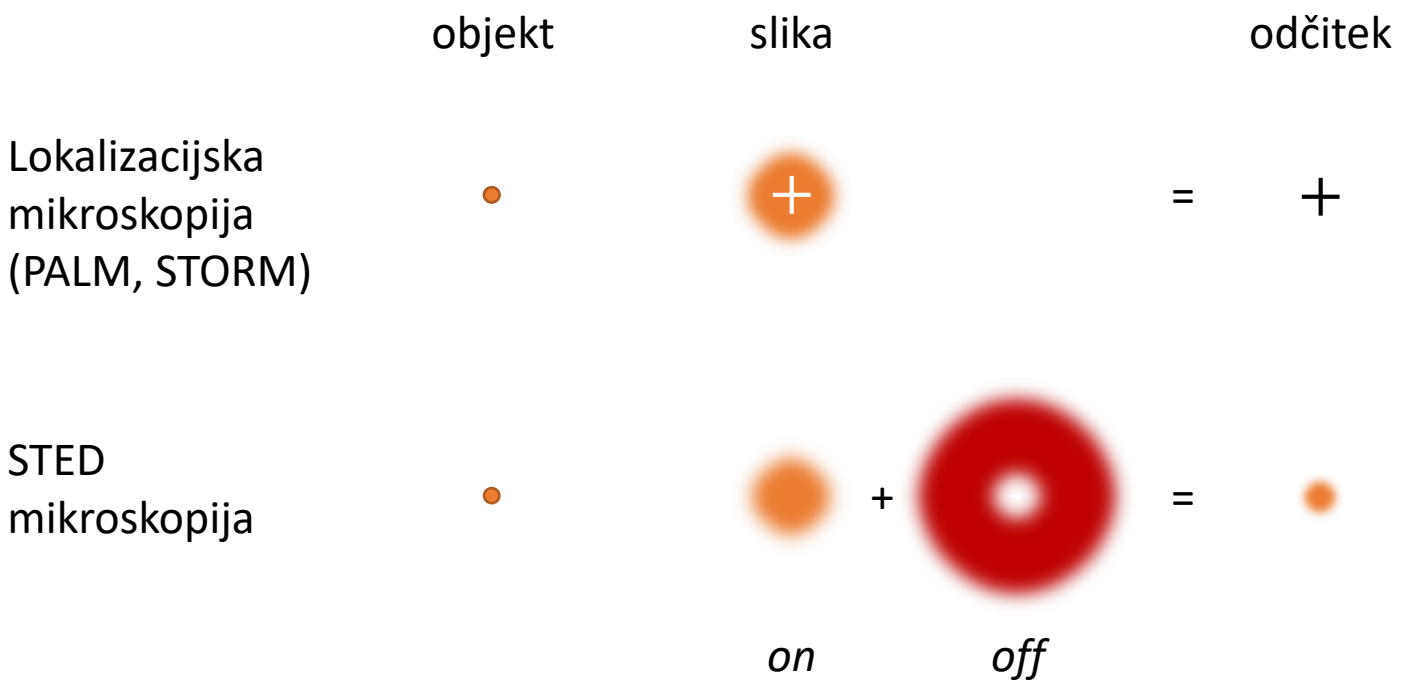
Niz slik po globini:



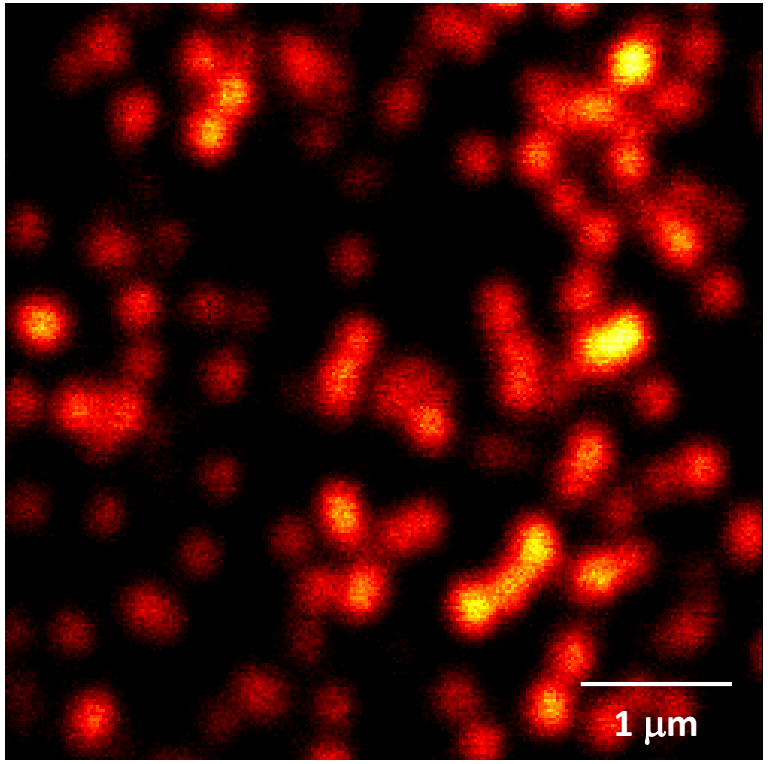
3D rekonstrukcija:



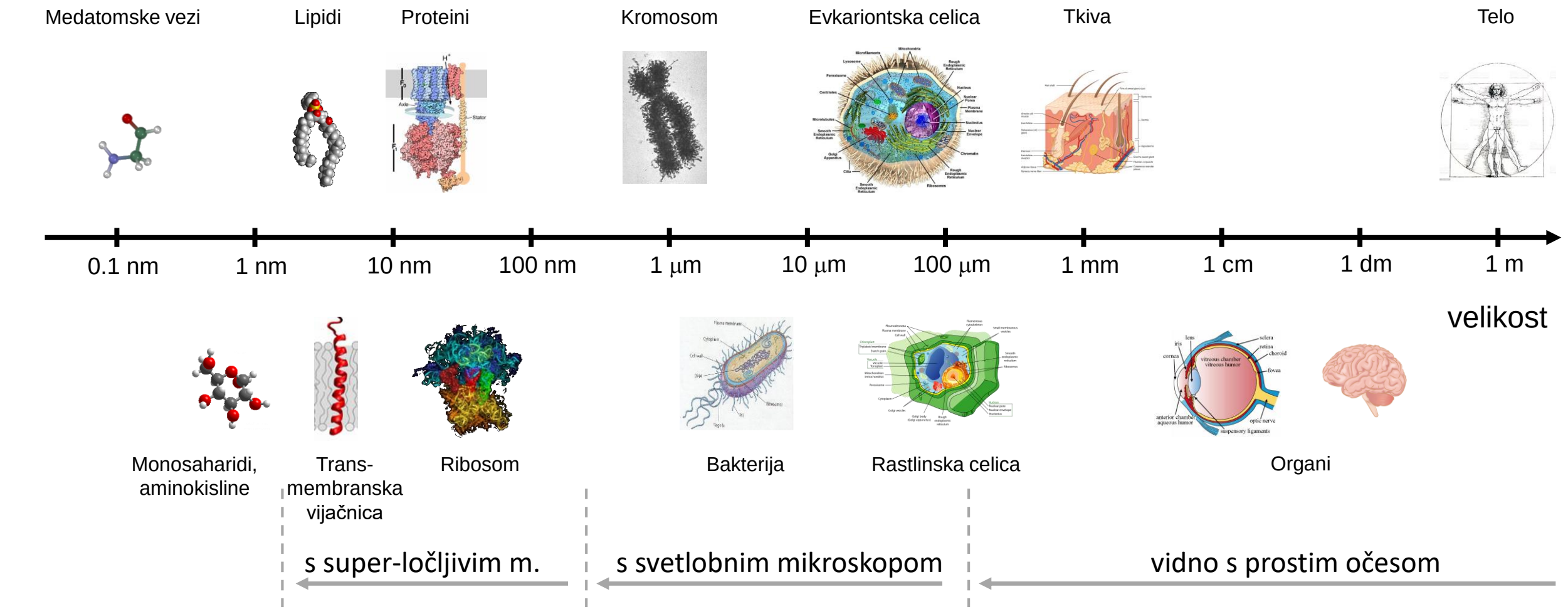
Superločljiv fluorescenčni mikroskop



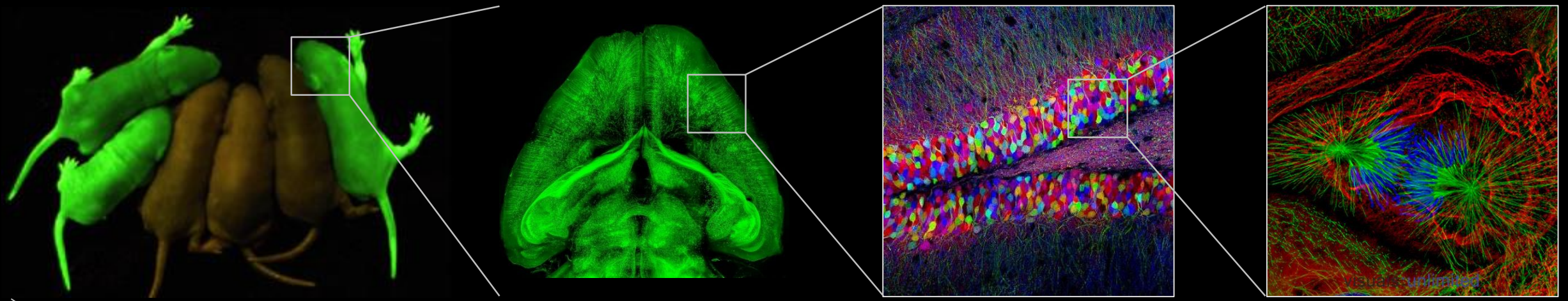
Fluorescenčne kroglice (40 nm)



Velikostne skale življenja



Fluorescenca: revolucija specifičnosti

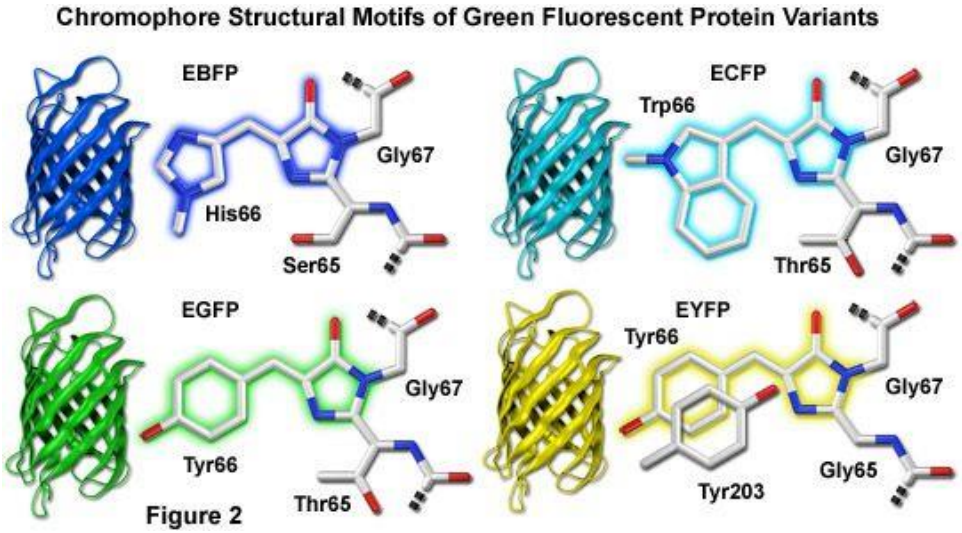


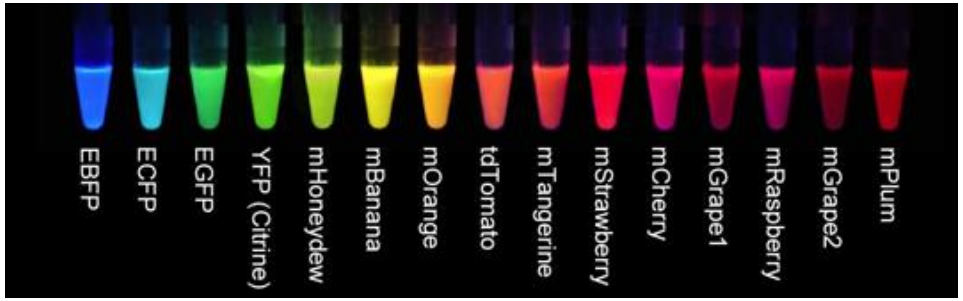
Organizem Organ Tkivo Celica Organel Molekula

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

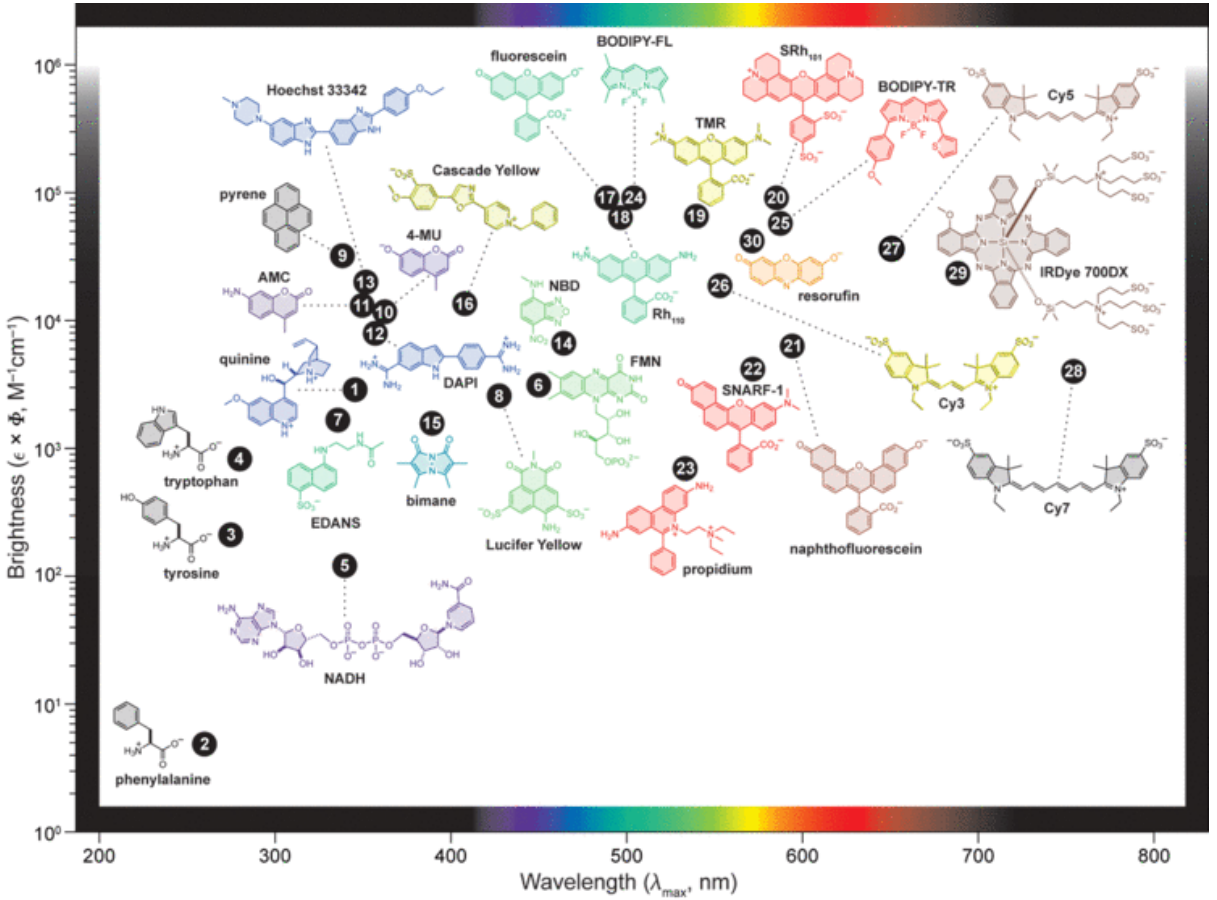
Fluorescenčna barvila

Fluorescenčni proteini





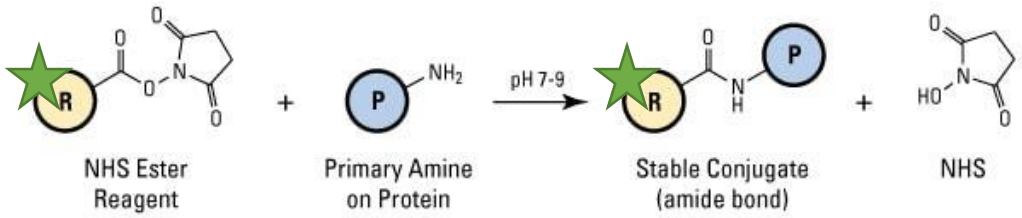
Organska barvila



Fluorescenčno označevanje proteinov

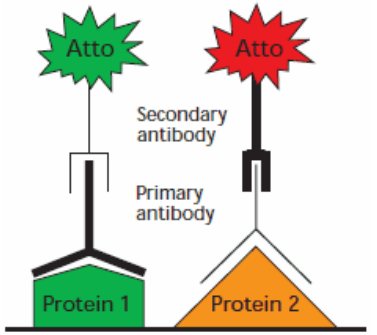
Nespecifično

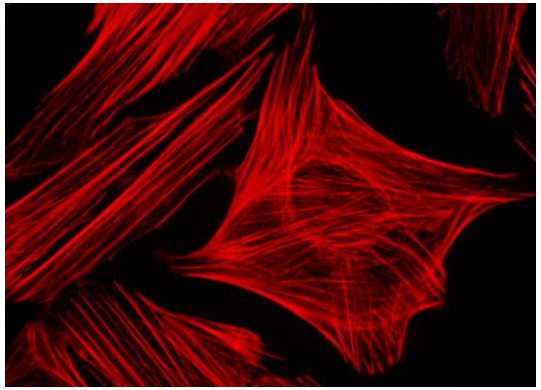
Označevanje izoliranih proteinov
(npr. protiteles)



Specifično

Fluorescenčno označena protitelesa



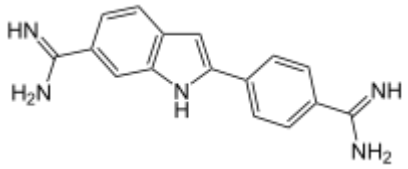


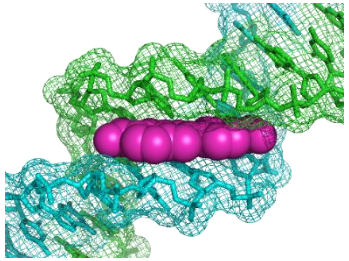
Ekspresija fluorescenčnih proteinov v celici

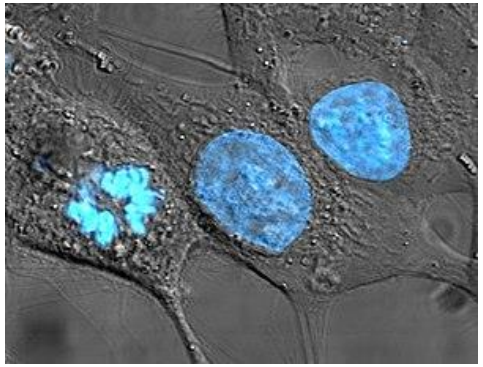
Fluorescenčno označevanje DNA/RNA

Nespecifično

DAPI, Hoechst, ...

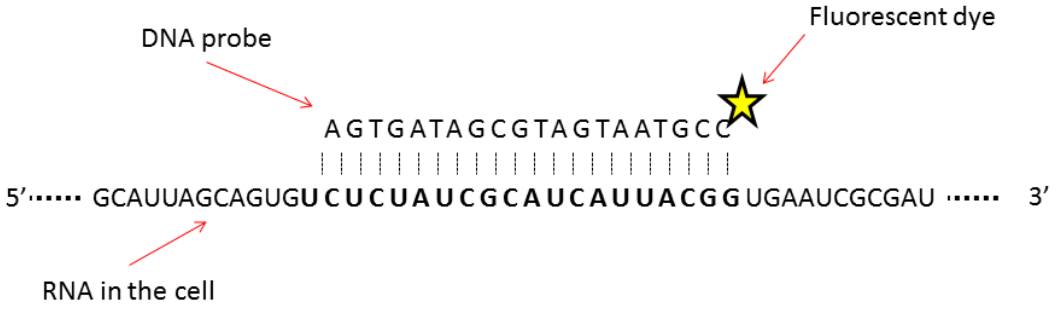


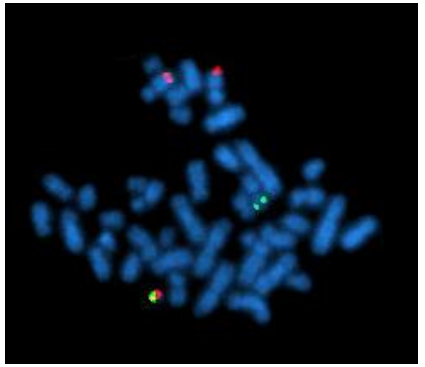




Specifično

Fluorescence in situ hybridization (FISH)





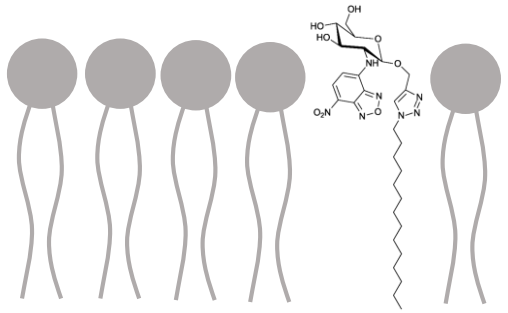
Fluorescenčno označevanje lipidov

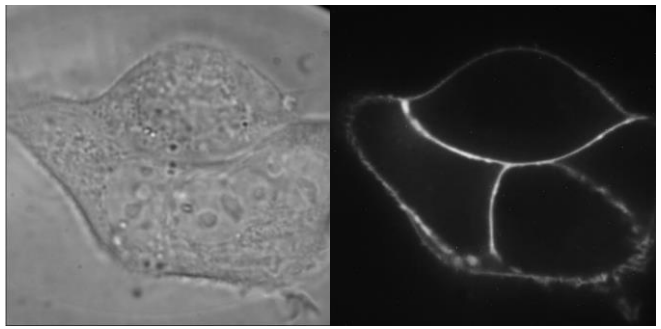
Nespecifično

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

hidrofilno

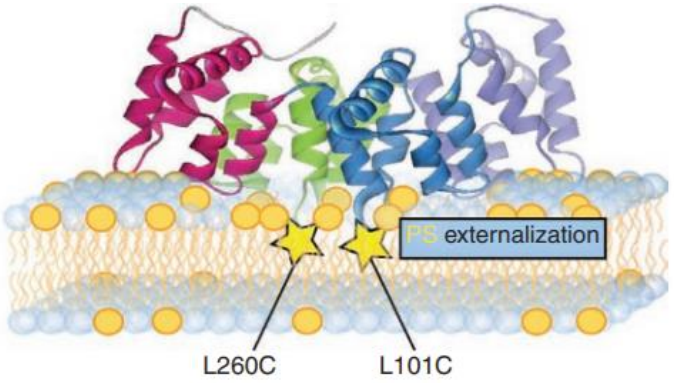
hidrofobno

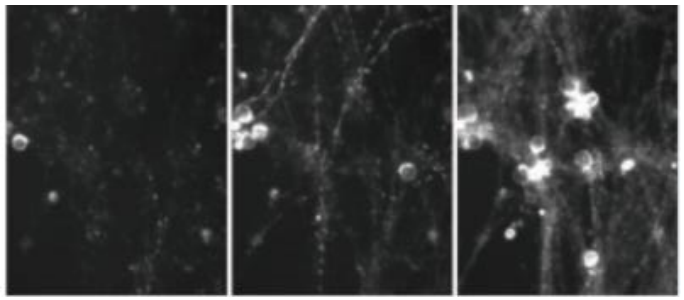




Specifično

Vezava na izbrano vrsto lipidov (fosfatidilserin)





čas

Kim *Nature Protocols* 2010

Fluorescenčna mikroskopija

Kontrast + specifičnost