

Apoptoos, rakutsükkel ja läbivoolutsütomeetria

Dmitri Lubenets

Tartu 11.2015

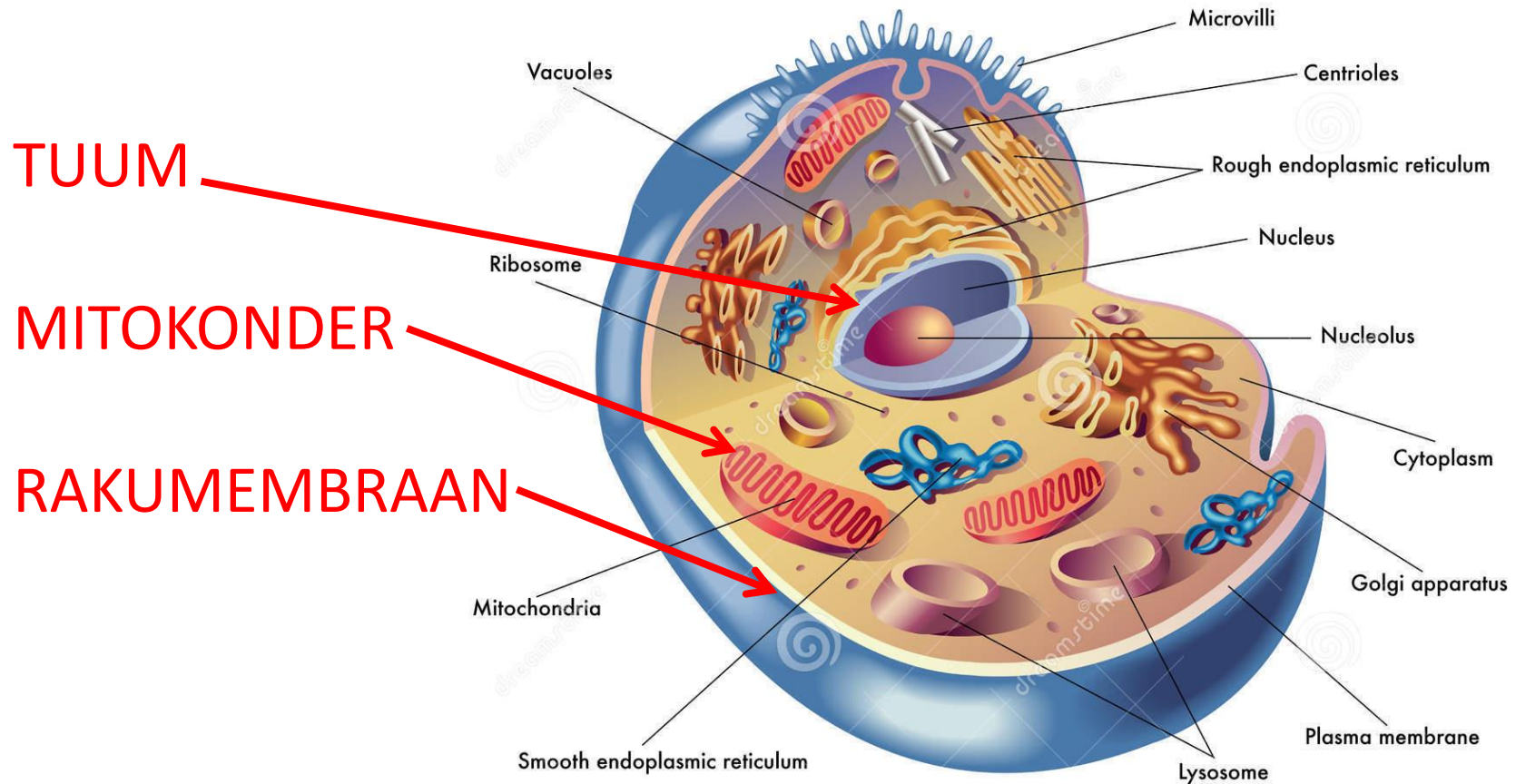
Loeng koosneb 3. osast

- Apoptoos – programmeeritud rakusurm
- Rakutsükkel – raku eluperiood
- Läbivoolutsütomeetria – meetod apoptoosi ja rakutsükli tuvastamiseks

1. osa – Apoptoos

- Kreeka keelest: ***apo*** – ära hoidmine või -st, ***ptosis*** - kukkumine
- Programmeeritud rakusurm

Rakud on omavahel tihedalt seotud ning iga rakk koosneb paljudest osadest



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Apoptoos on loomulik protsess



15.5 päeva vana hiire käpp

Rakusurma võimalused

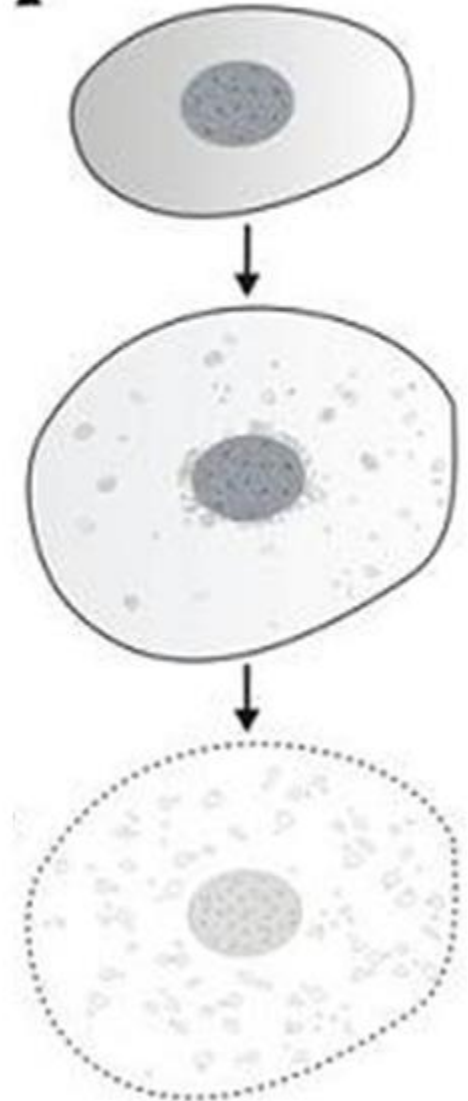


APOPTOOS

- Kindla sise- või välissignaali tagajärjel
- Kromatiini ja organelle lõigatakse tükkideks
- Rakumembraani omadused muutuvad
- Rakusiseseid komponente pakitakse membraaniga kaetud apoptootiliste kehade sisse
- Raku ohutu kõrvaldamine – põletiku EI tekki

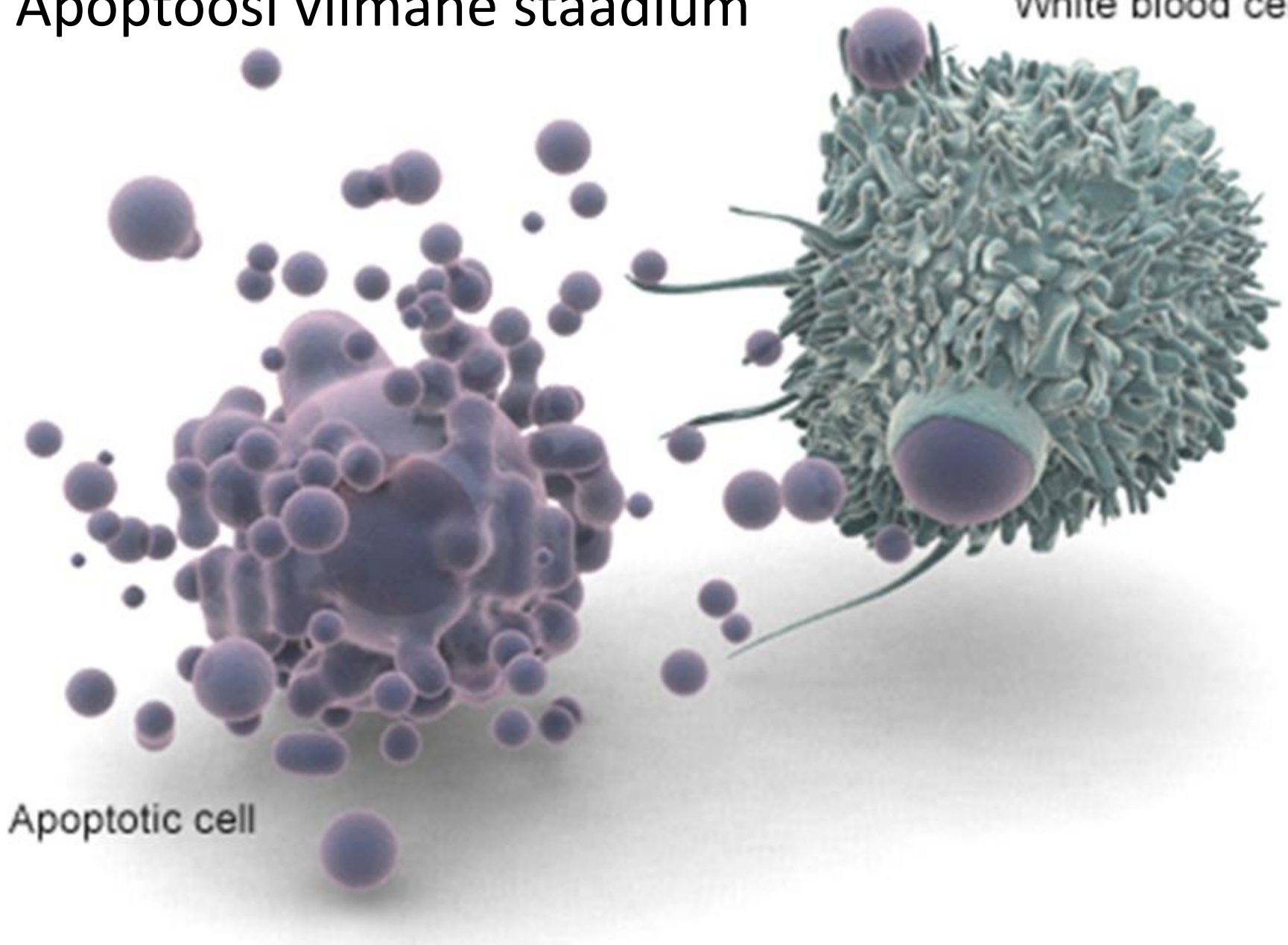


NEKROOS



Apoptoosi viimane staadium

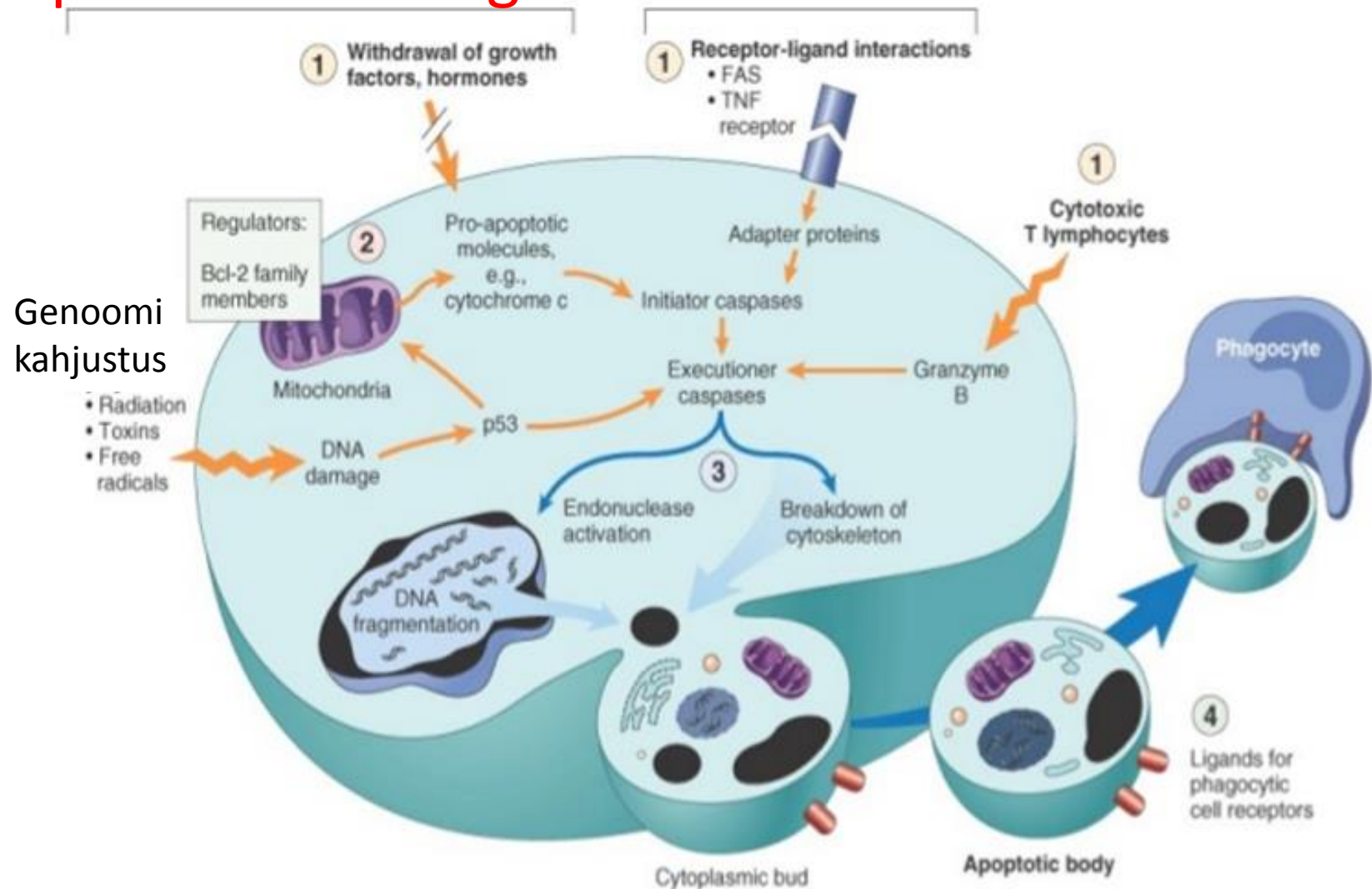
White blood cell



Apoptotic cell

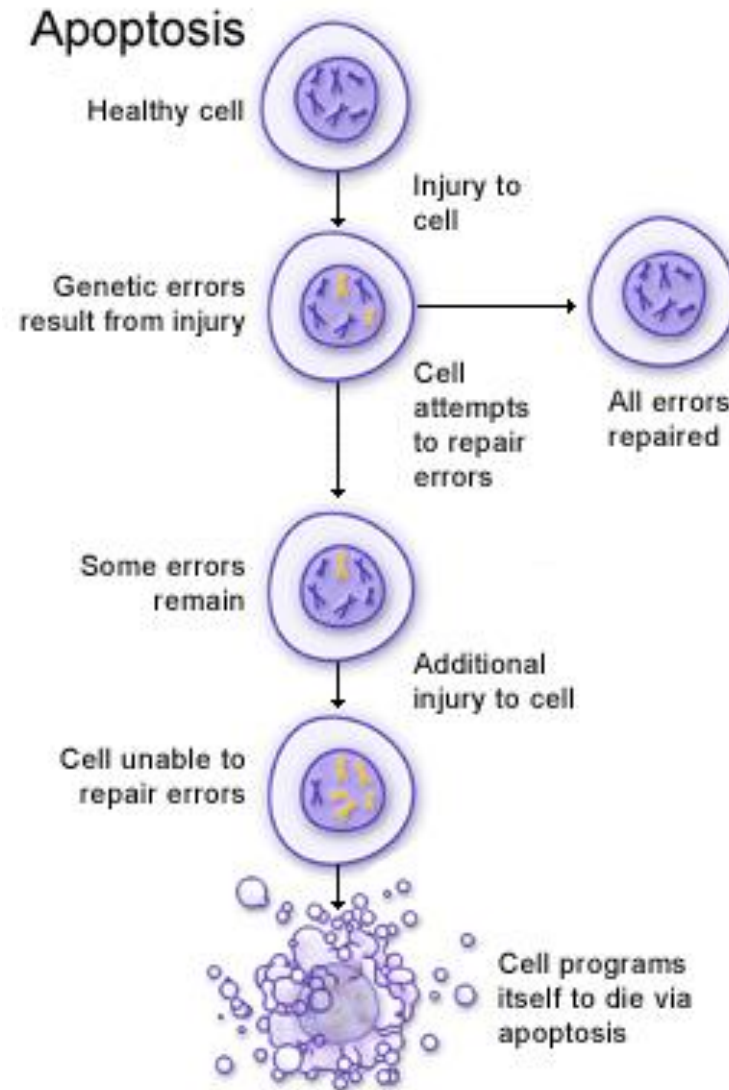
Apoptoosi sisene signaal

Apoptoosi väline signaal



Genoomi
kahjustus

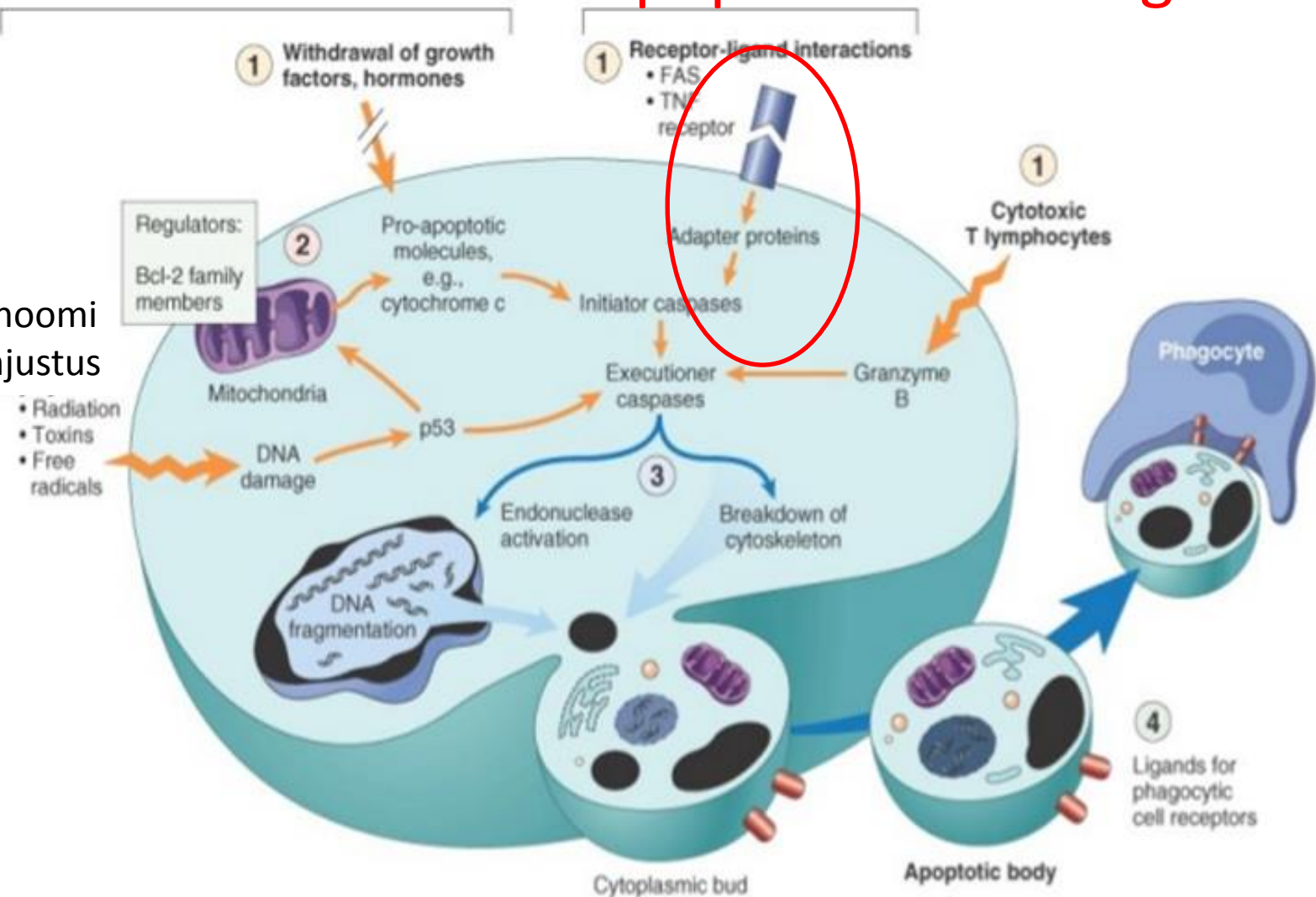
Apoptoosi rakusisesed signaalid



Apoptoosi sisene signaal

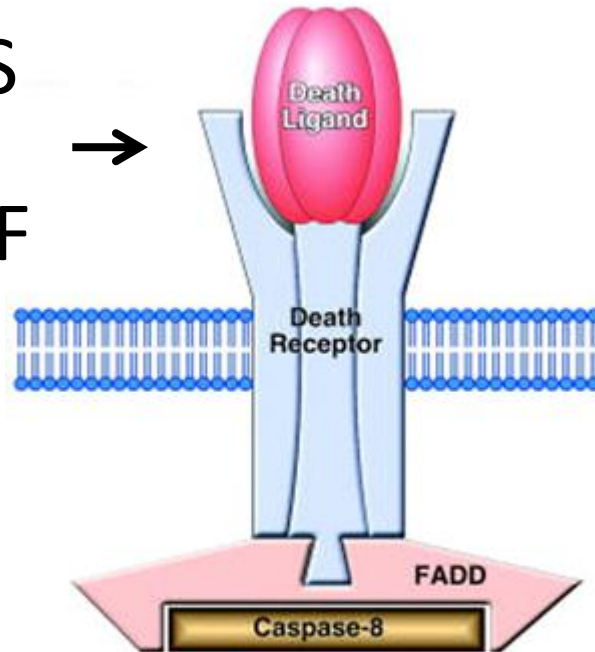
Apoptoosi väline signaal

Genoomi
kahjustus



Apoptoosi väline signaal

FAS
või
TNF



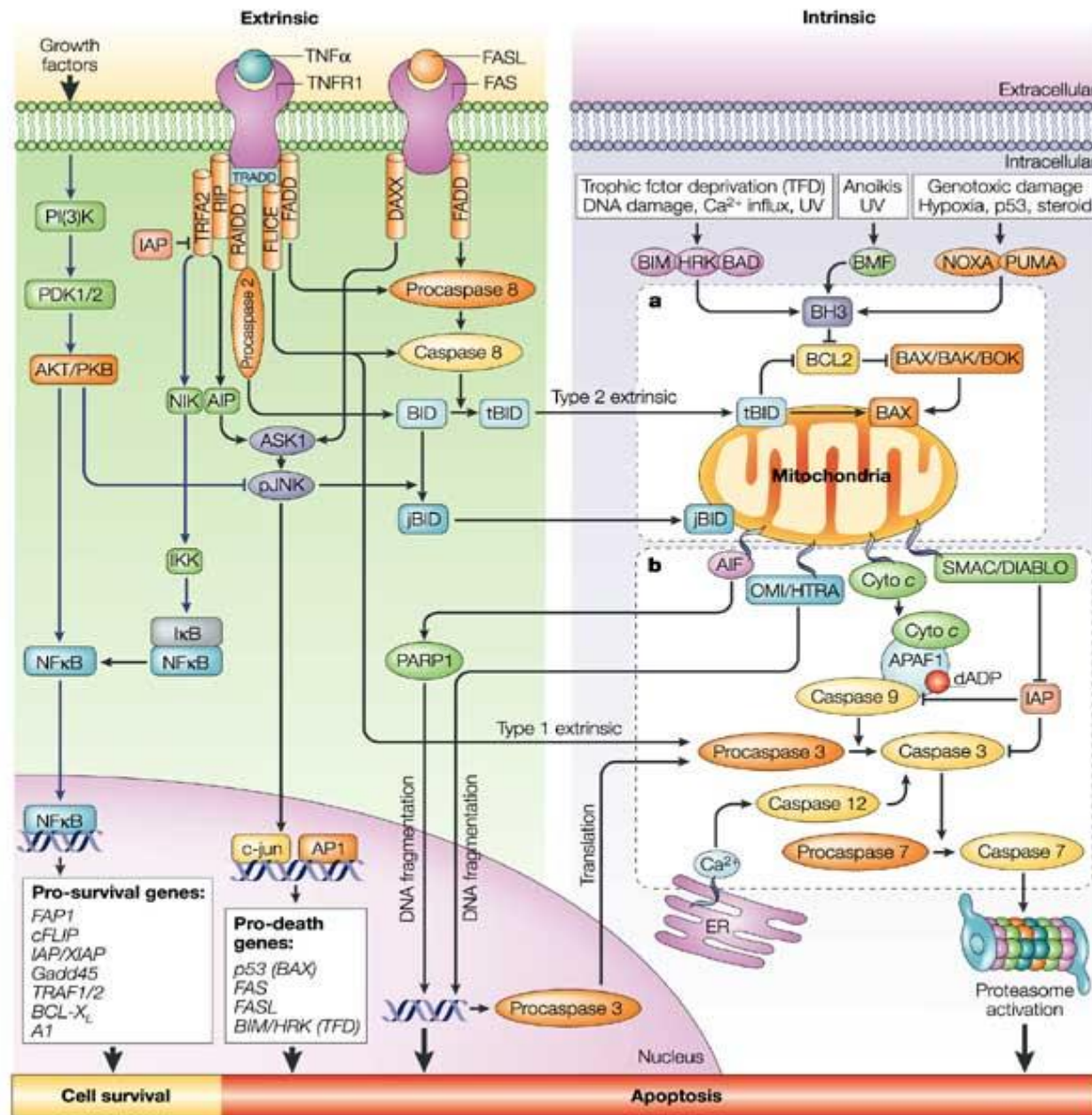
cFLIP



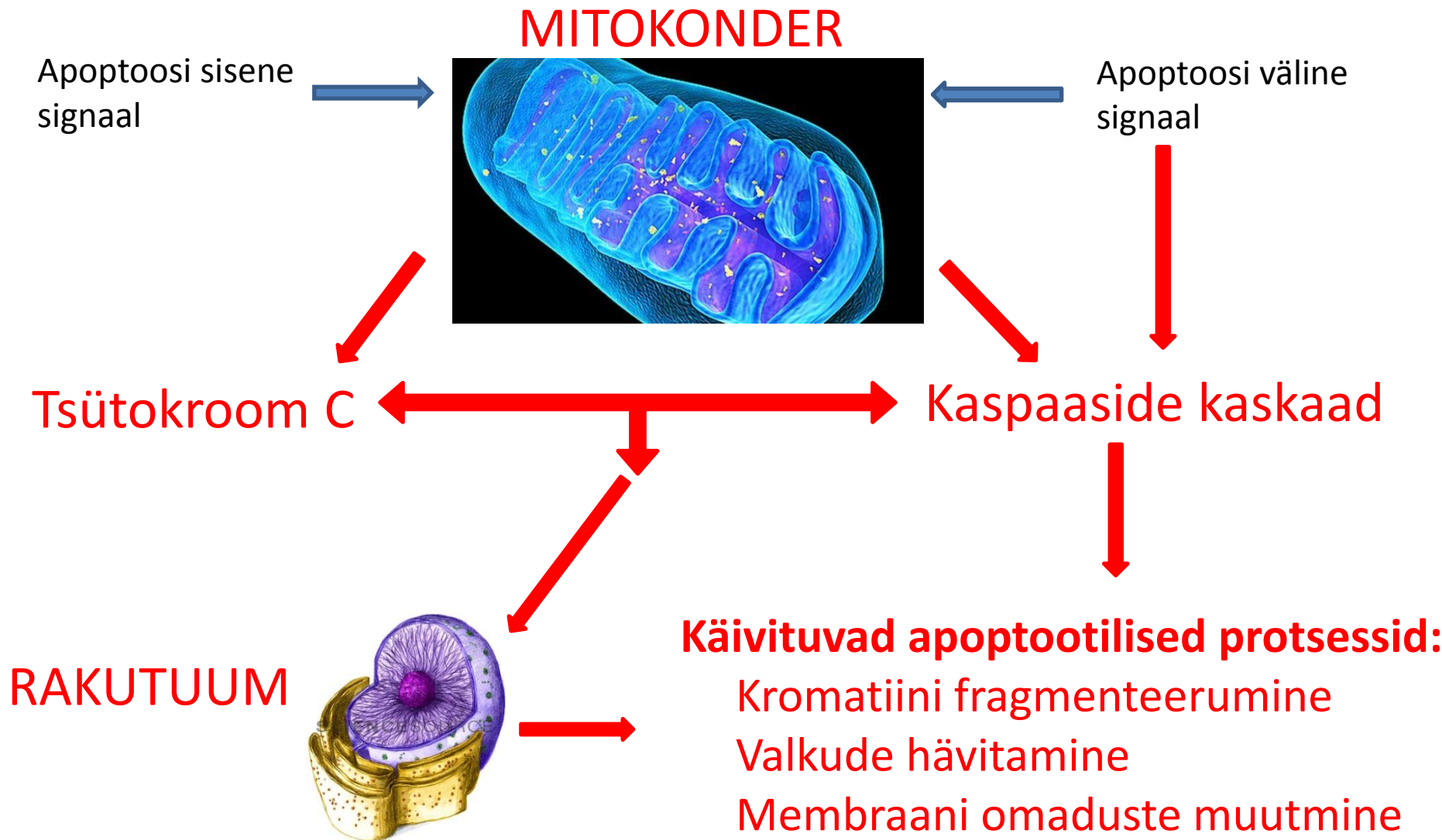
Caspase-3/7



APOPTOOS



Apoptoosi rada



Apoptoosi rada võtmesõnad

1. Raku sisene- või välisesignaali
2. Mitokondrid
3. Tsütokroom C
4. Kaspasid ja kaspaside kaskaad
5. Rakutuum
6. Apoptootiliste protsesside käivitamine

KÜSIMUSED??

Apoptoosi tuvastamine

Apoptoosi
indutseerijad

Mitokonder

Kaspaaside
aktiveerumine

1 Kromatiini
fragmenteerumine

Happelisus

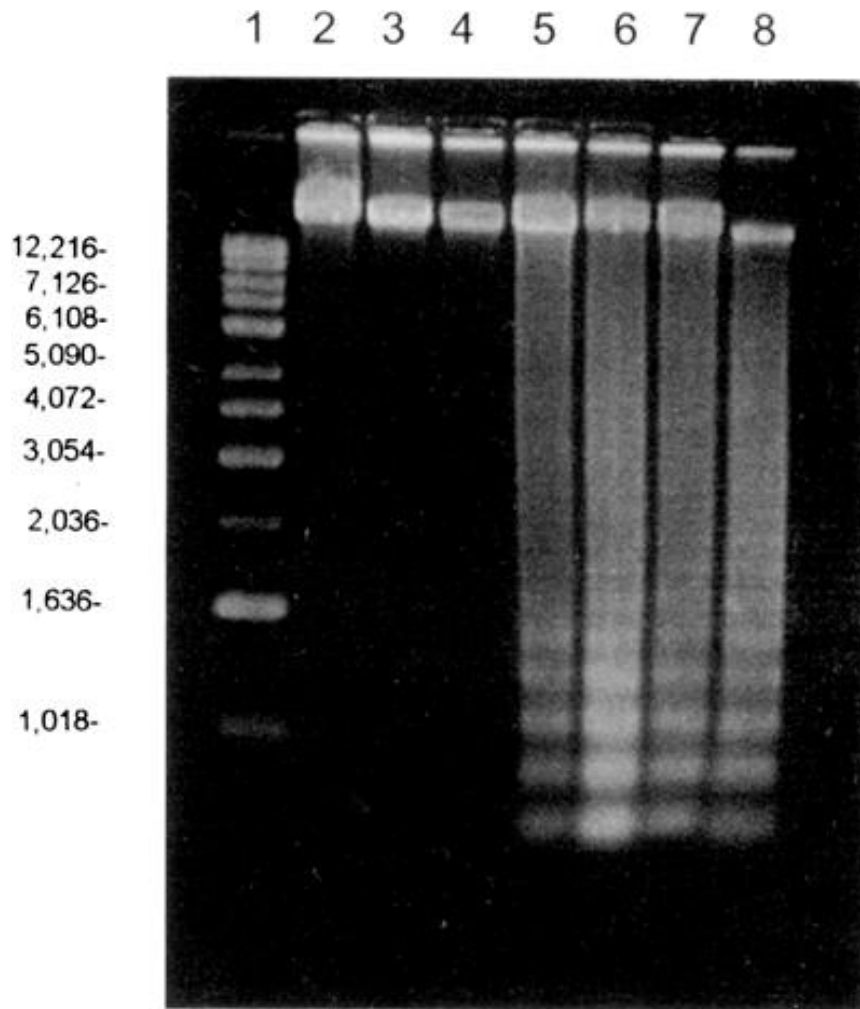
Apoptootiliste
kehade teke

2
Tuneli test

3 Fosfatidüülseriini
paljastamine

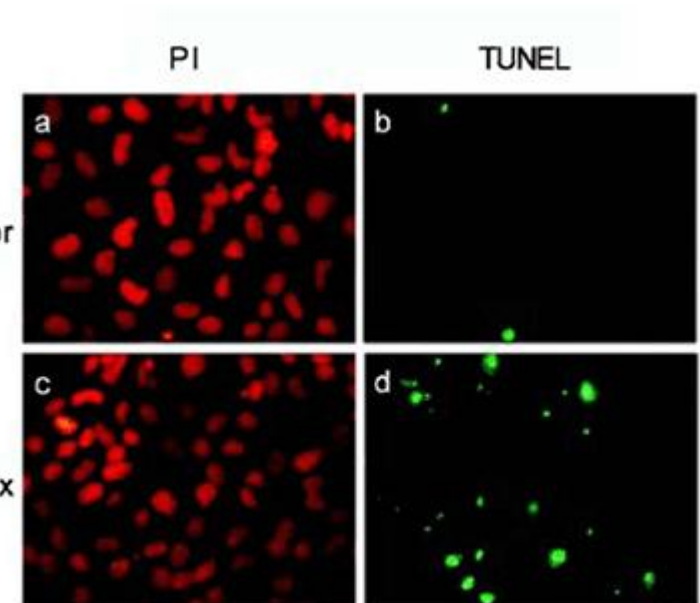
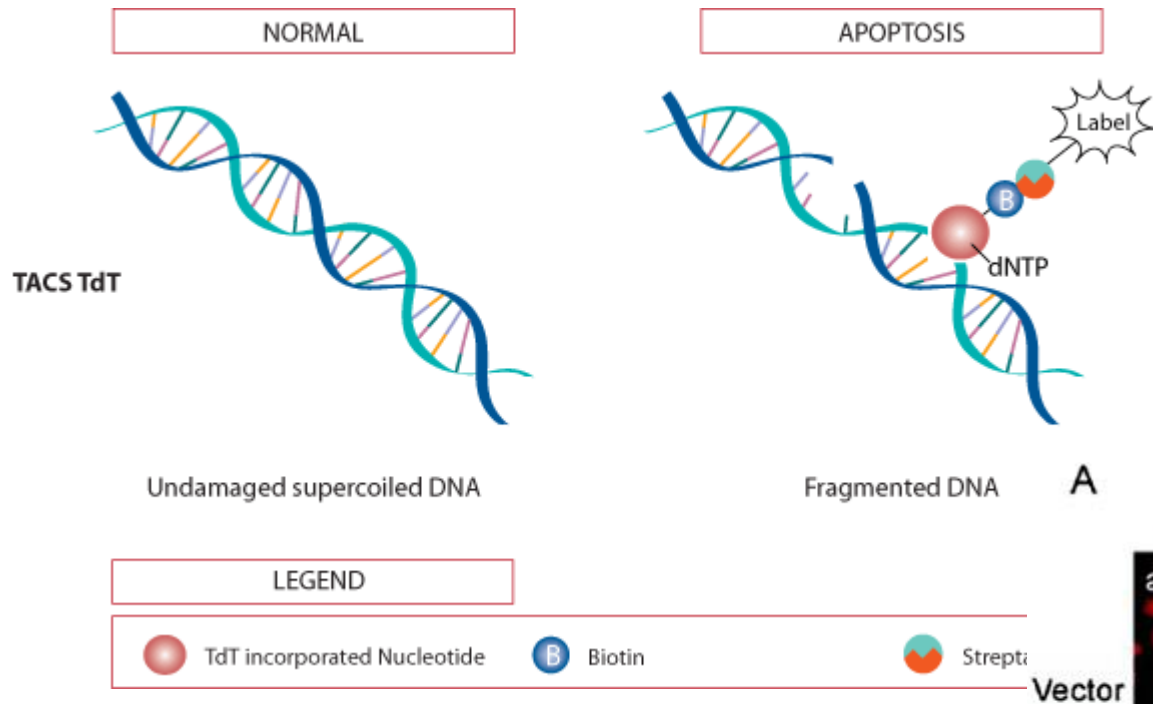
4 Membraani
läbilaskvus

Kromatiini fragmenteerumine



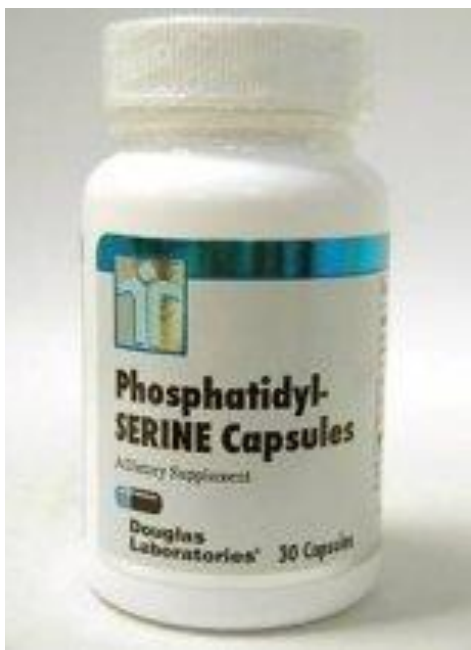
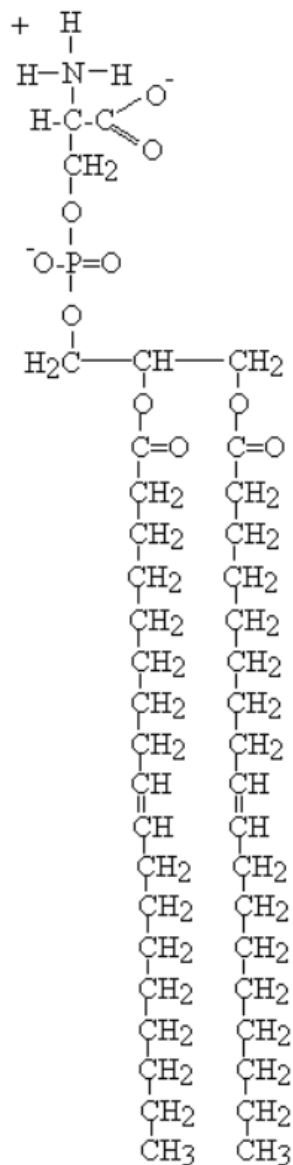
Agaroos
geelektroforees

Tuneli test



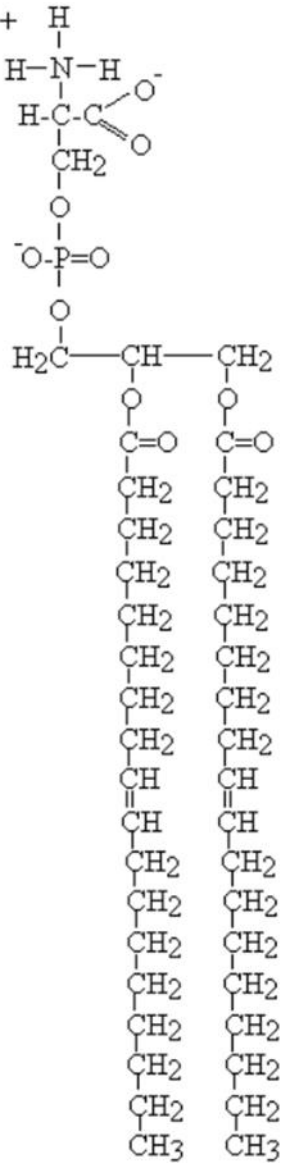
https://www.rndsystems.com/products/tdt-in-situ-apoptosis-detection-kit--dab_4810-30-k

Fosfatidüülseriini paljastamine

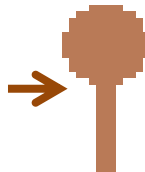


- Populaarne toidulisand
- Soodustab peaaju tööd
parandades kontsentratsiooni
ja mälu
- Hoiab ära enneaegset
vananemist
- Hoab ära või vähendab
kaalu juurde võtmist

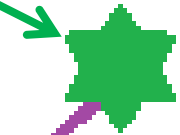
Fosfatidüülseriini paljastamine



Fosfatidüülseriin



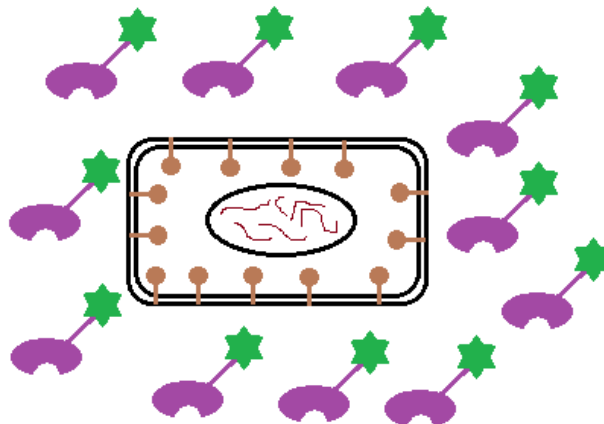
Fluorokroom, **FITC**



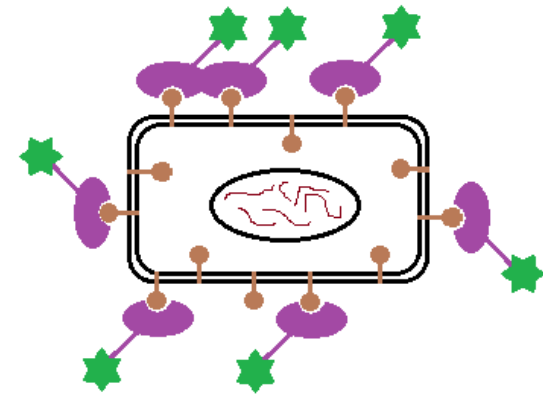
Annexiin V



Terve rakk

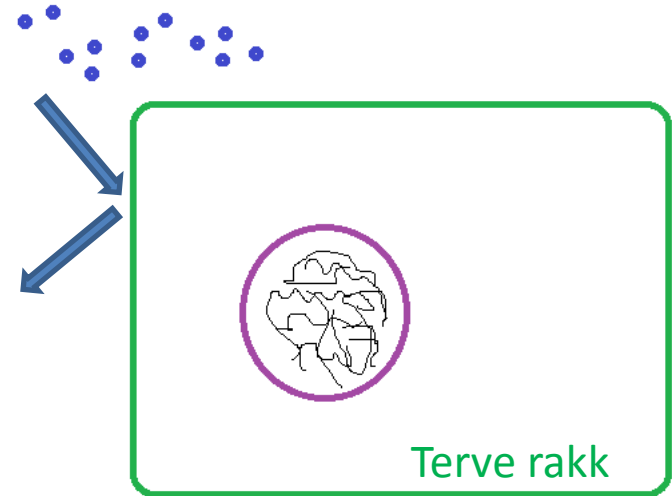
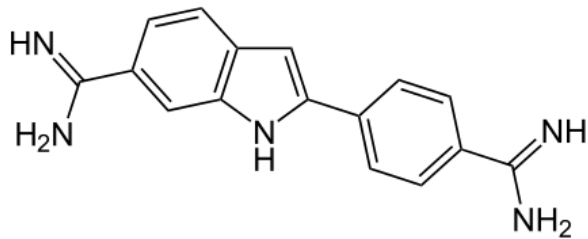


Apoptootiline rakk

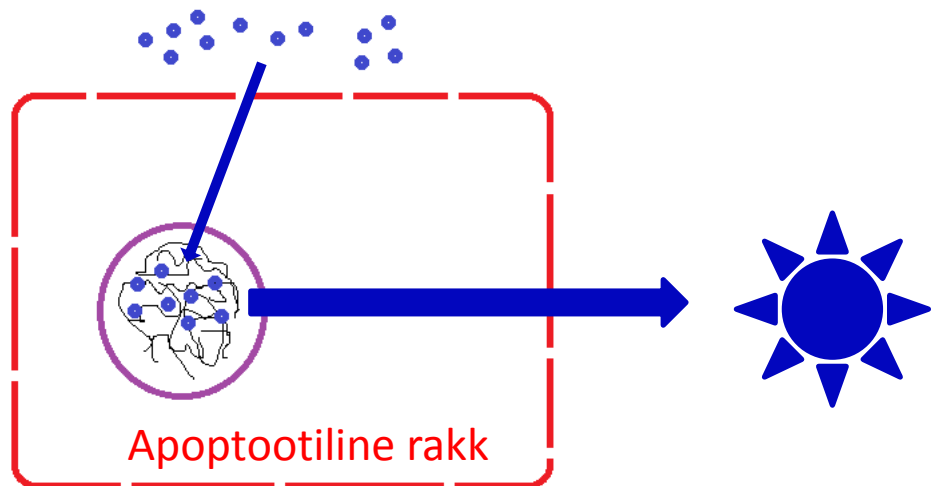
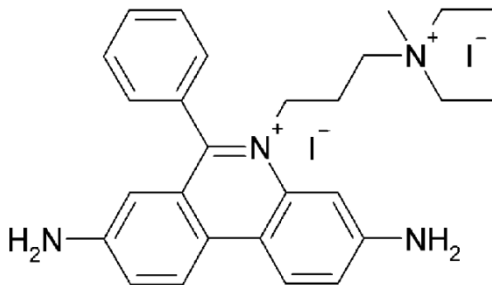


Membraani läbilaskvus

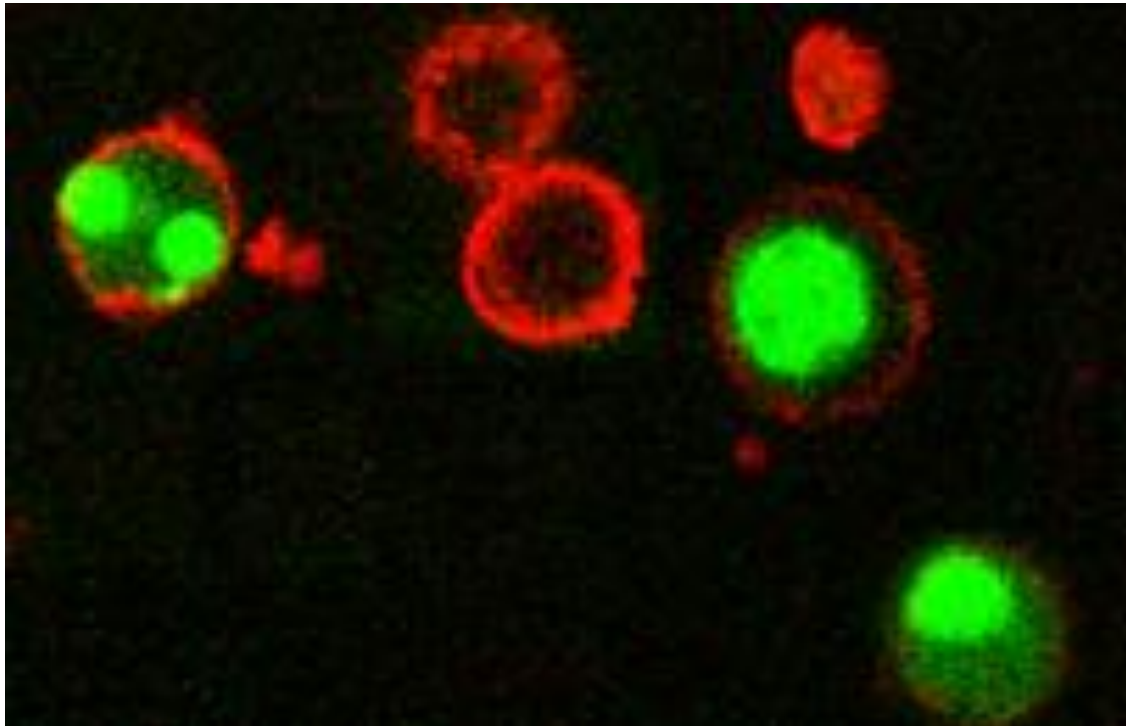
DAPI, 4',6-diamidino-2-phenylindole



PI, Propidium iodiid

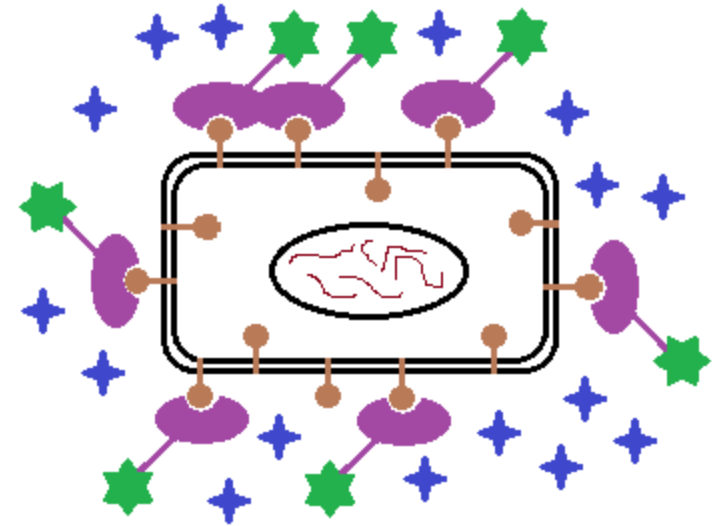
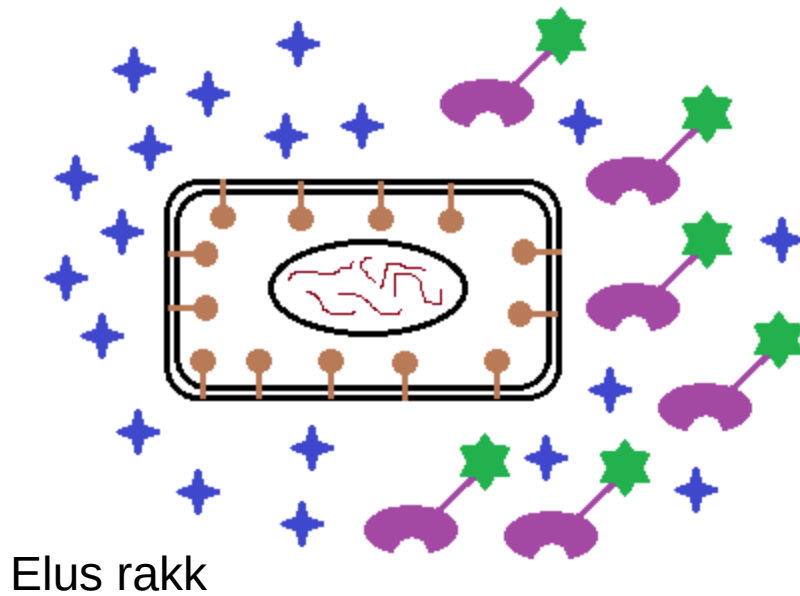


Fosfatidüülseriini paljastamine ja membraani läbilaskvus. 2 meetodit koos

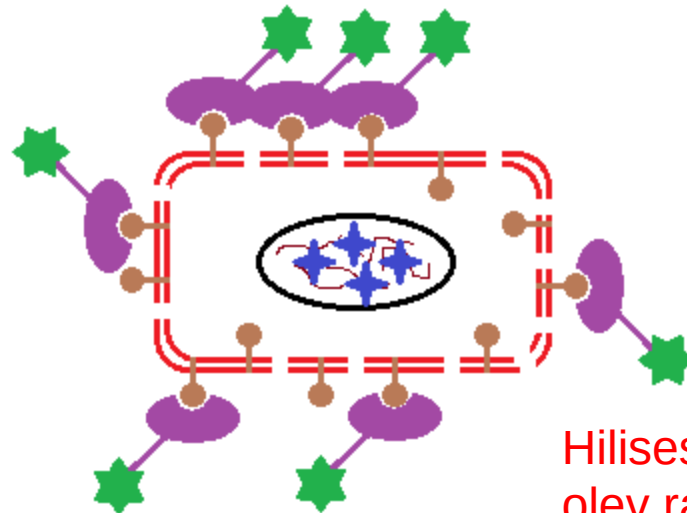


DAPI

AnneksiinV-FITC



Varajases apoptoosis
olev rakk

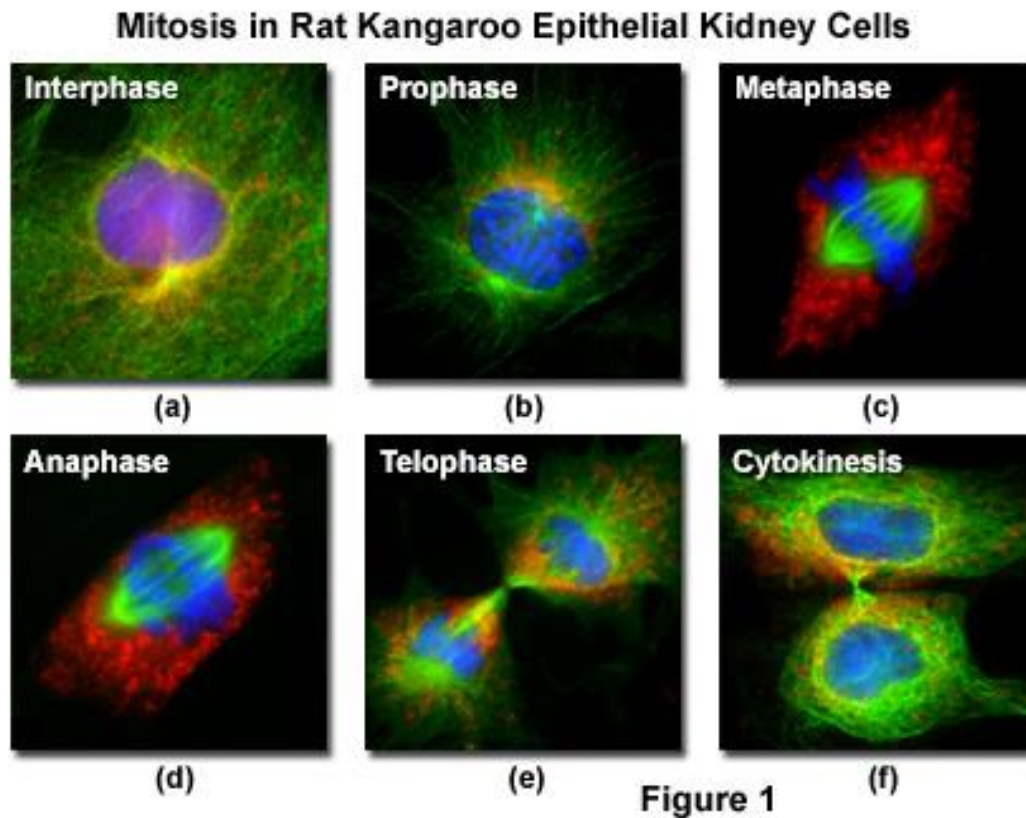


Hilises apoptoosis
olev rakk

Küsimused?

2. osa – Rakutsükkel

- Raku elutsükkel – raku eluperiood ühest jagunemisest teiseni



Rakutsükkel



Mitoos = M-faas

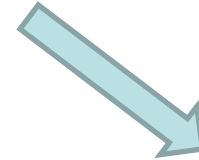


1. Profaas

2. Metafaas

3. Anafaas

4. Telofaas



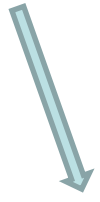
Interfaas



G1



S



G2

Interfaas

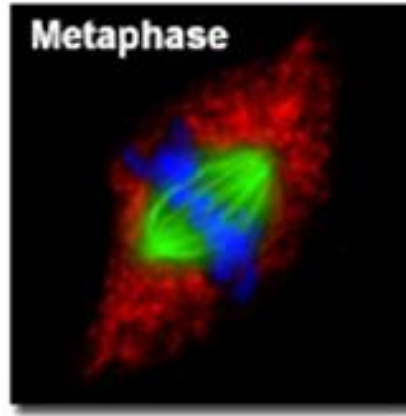
- G1 = kasvufaas 1 – rakk kasvab varub energiat
- S = DNA sünteesi faas – rakk kahekordistab oma genoomi, ehk sünteesib DNA-t
- G2 = kasvufaas 2 – rakk kontrollib sünteesitud DNA õiglust ja varub energiat järgunemiseks

Mitoos = M-faas

1. Profaaas =
prometafaas



2. Metafaas

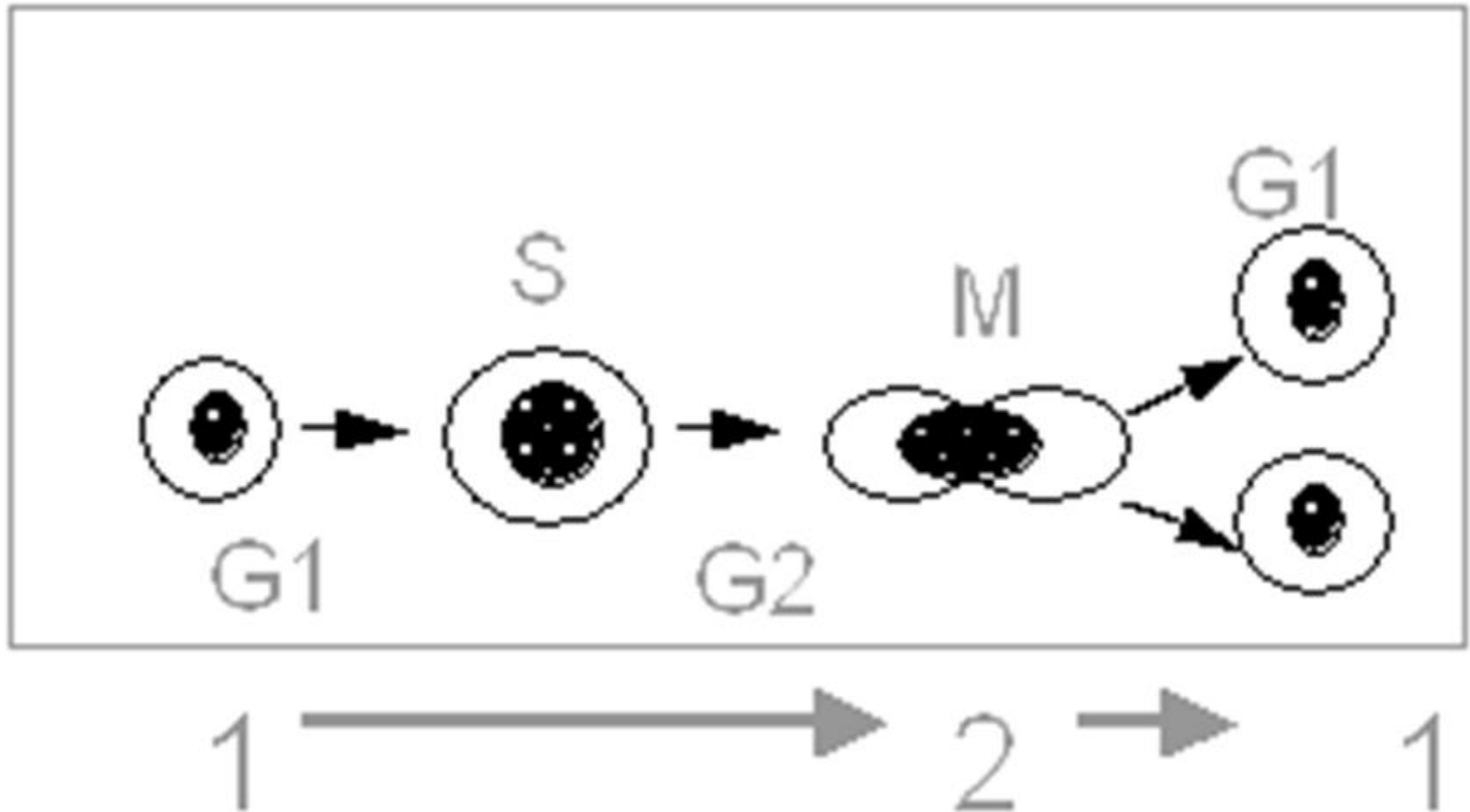


3. Anafaas



4. Telofaas

Rakutsükli jooksul muutub DNA
hulk rakus ja ühest rakust saab 2
rakku!!!



Miks rakutsükliit uurida?

Erinevate kemikaalide, valkude, keskkonnatingimuste mõju rakkude kasvule

Erinevad rakud kasvavad erineva kiirusega – normaalne rakk vs vähirakk

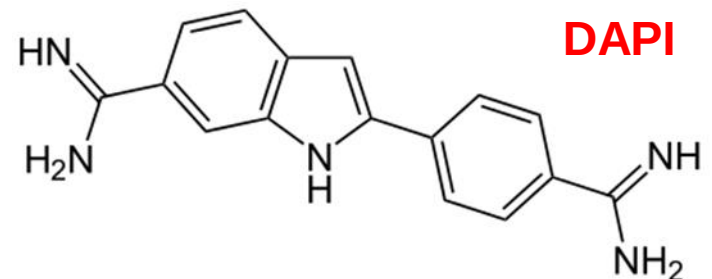
Erinevate valkude ekspressiooni uurimine sõltuvalt rakutsüklist

Kuidas tuvastada rakutsükliit?

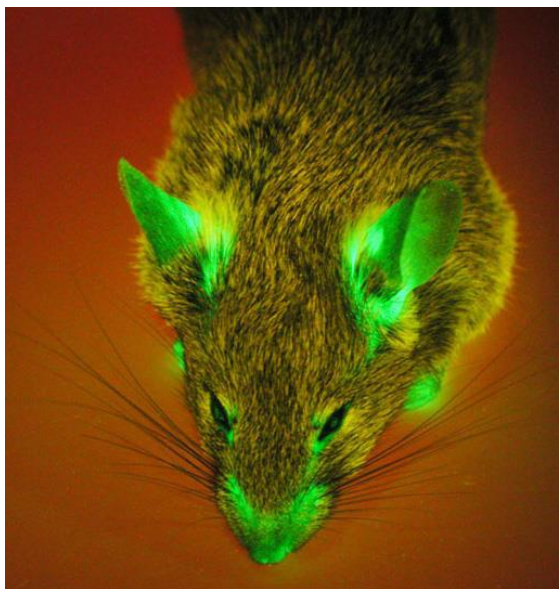


DNA/RNA fluorestsentsvärvid

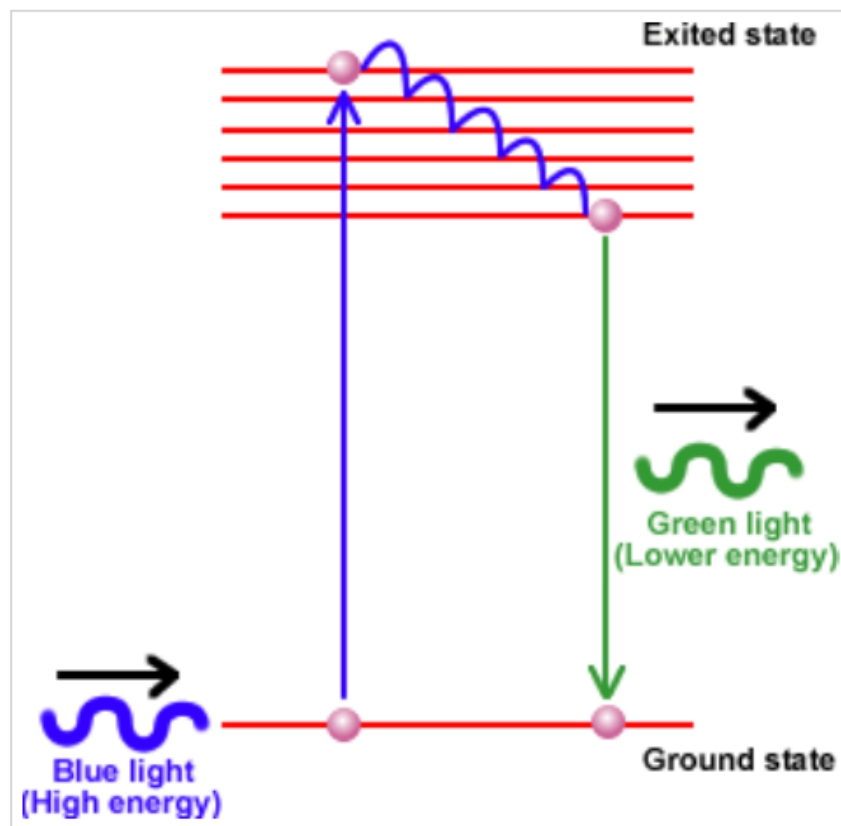
- Propidium Iodide
- Ethidium Bromide
- Hoechst dyes
- Acridine Orange
- Mithramycin, Chromomycin
- 7 Aminoactinomycin D (7AAD)
- **Diamino-2-phenylindole (DAPI)**
- Syto 9
- SYBR Green
- DRAQ5 ja DRAQ7



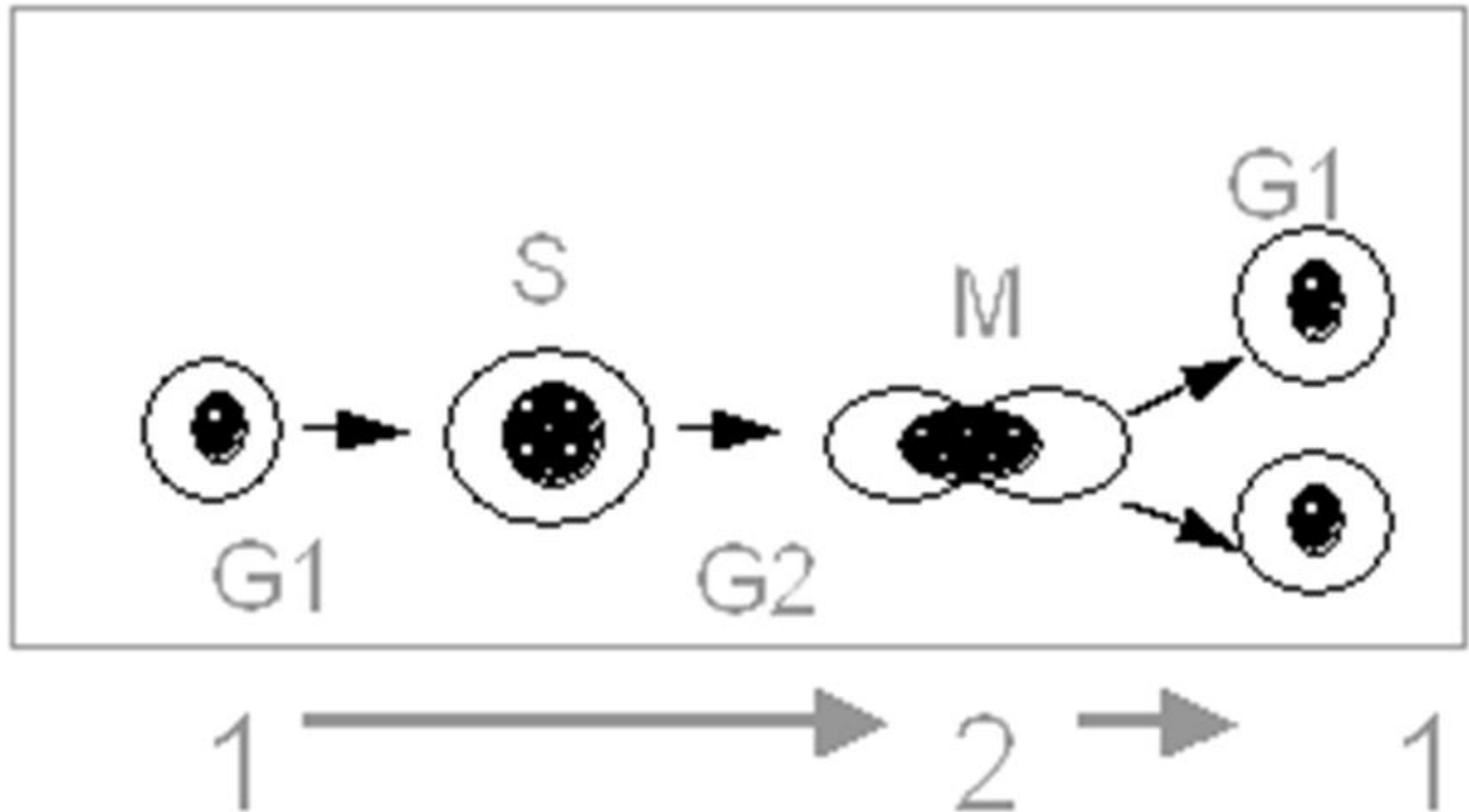
Fluorestsents ja fluorestseeruvad molekulid



Fluorestsents on nano sekundite küsimus



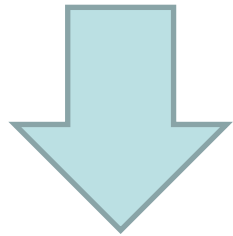
Rakutsükli jooksul muutub DNA hulk rakus!!!



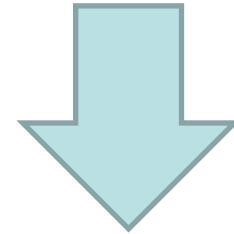
Kuidas tuvastada märgistatud DNA hulka rakus?



Fluorestsentsmikroskoopia
+ tarkvara



Võimalik, kuid aeglane



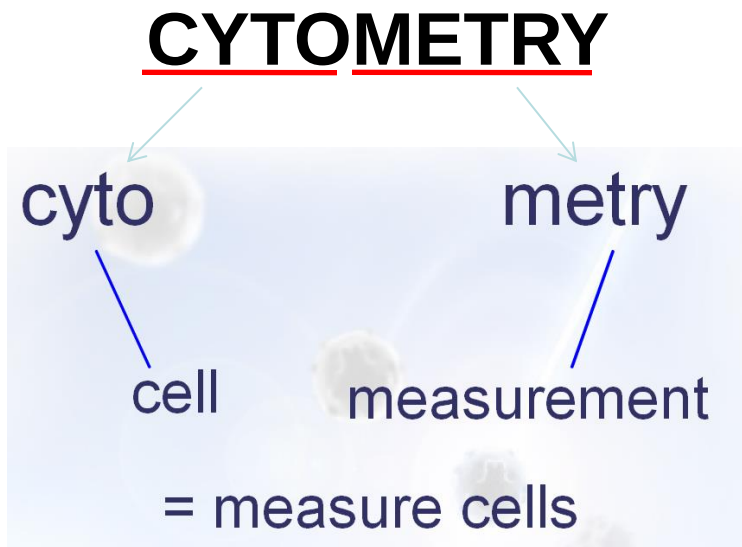
olutsütomeetria



alike, kiire ja mugav

Küsimused?

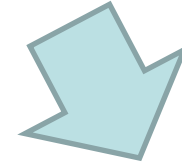
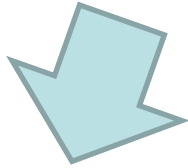
Mis on läbivoolutsütomeetria, ehk flow cytometry?



Läbivoolutsütomeetria on tehnoloogia, mida kasutatakse osakeste analüüsimiseks ja nende füüsiliste omaduste kirjeldamiseks.

Läbivoolutsütomeetria TÜMRIs

Becton Dickinson



FacsCalibur



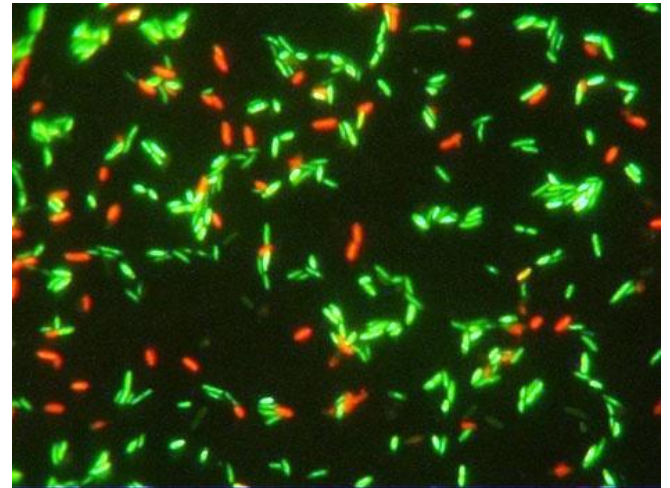
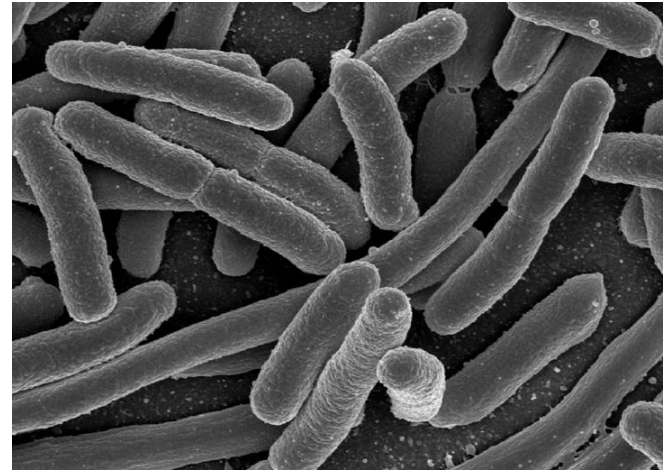
FacsAria I



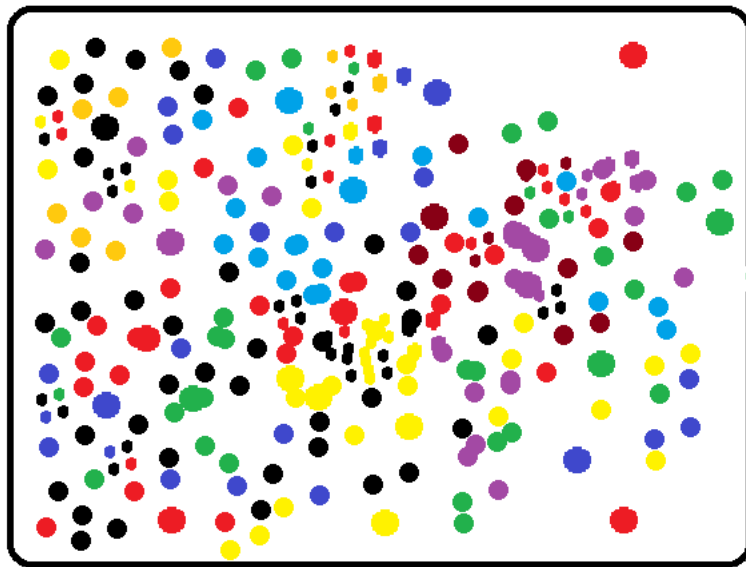
Miks see masin hea on?



“Hello Kitty”™ mikroskoop



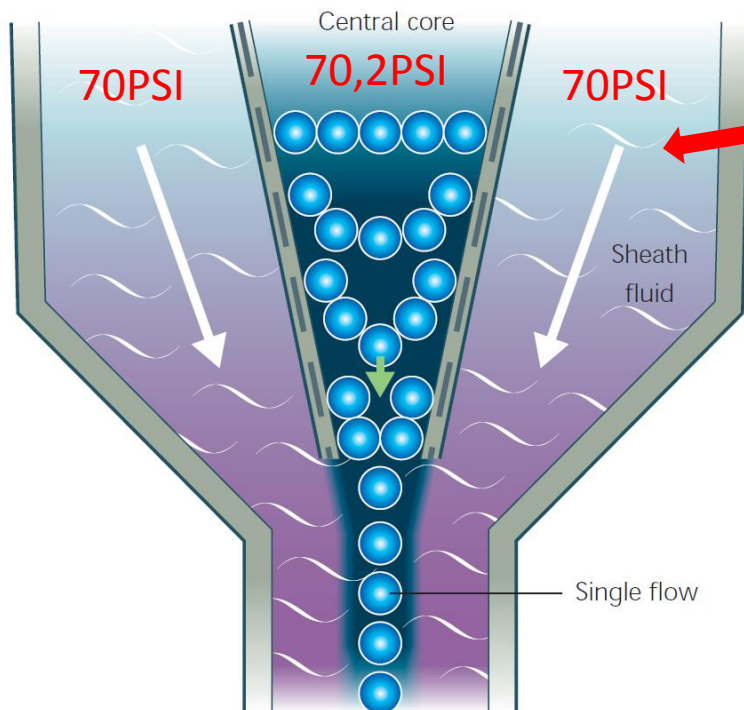
Läbivoolutsütomeetri põhimõte



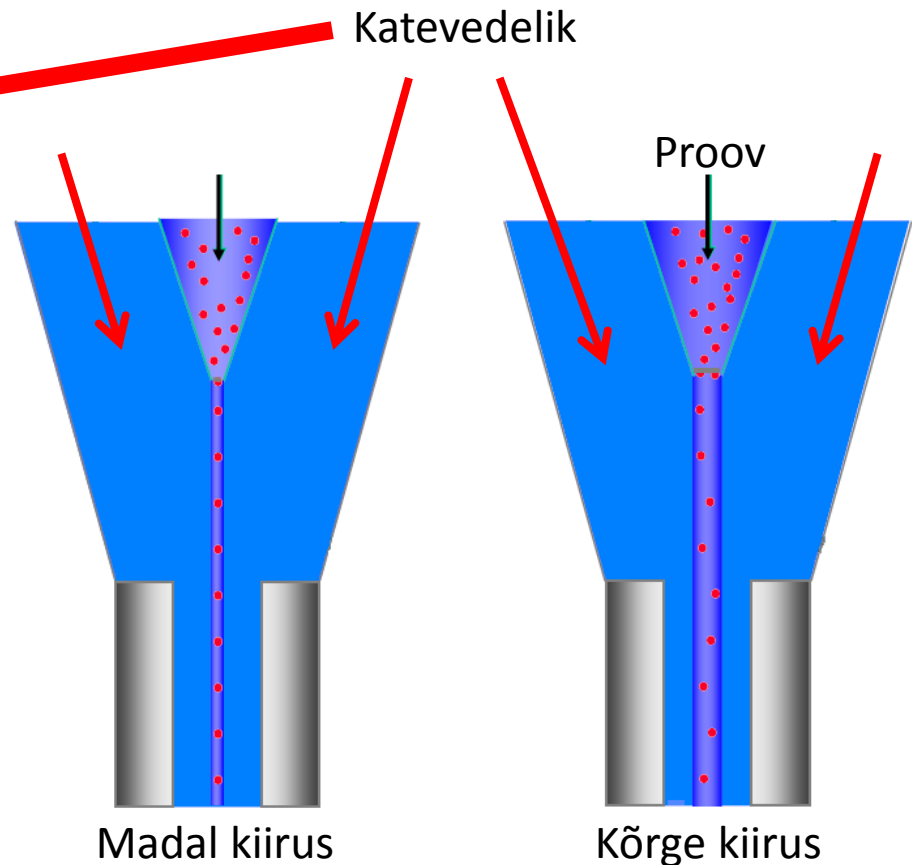
Loendamise koht

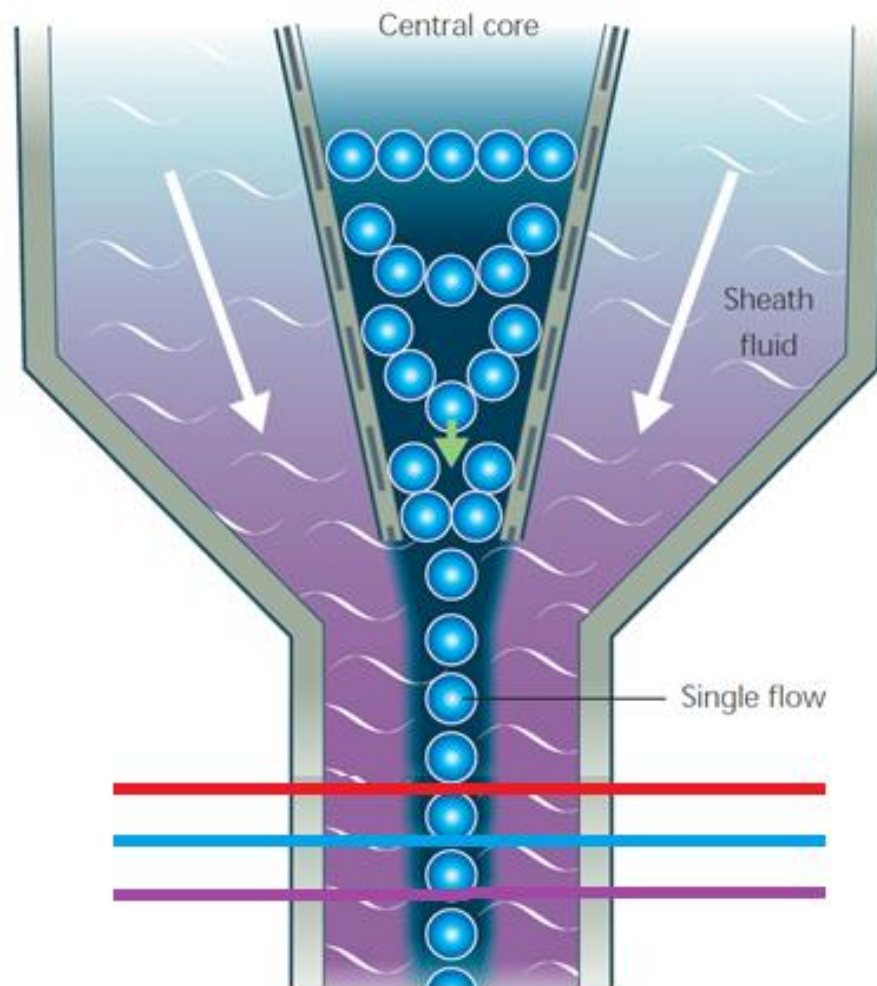
Hüdrodünaamiline fokusserimine

Küvetti tulevad erineva rõhuga vedelikud!

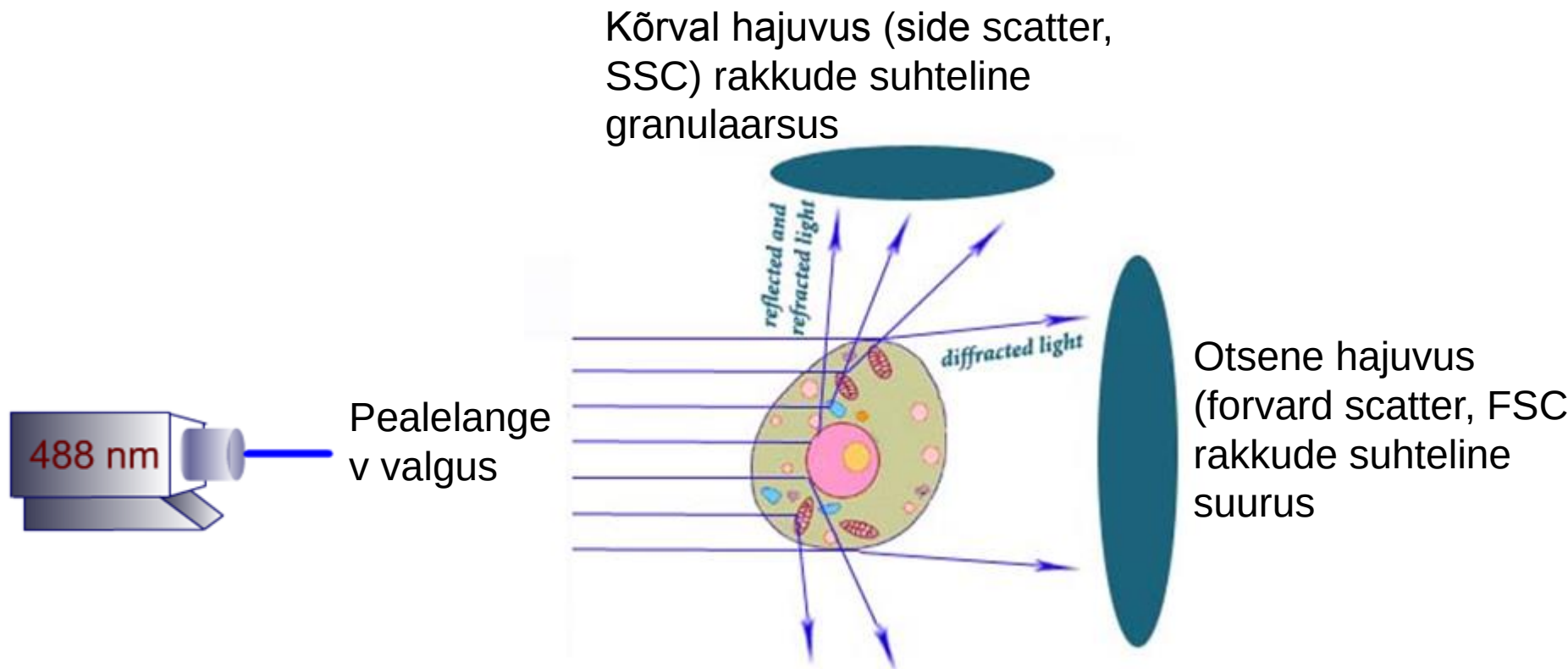


BD

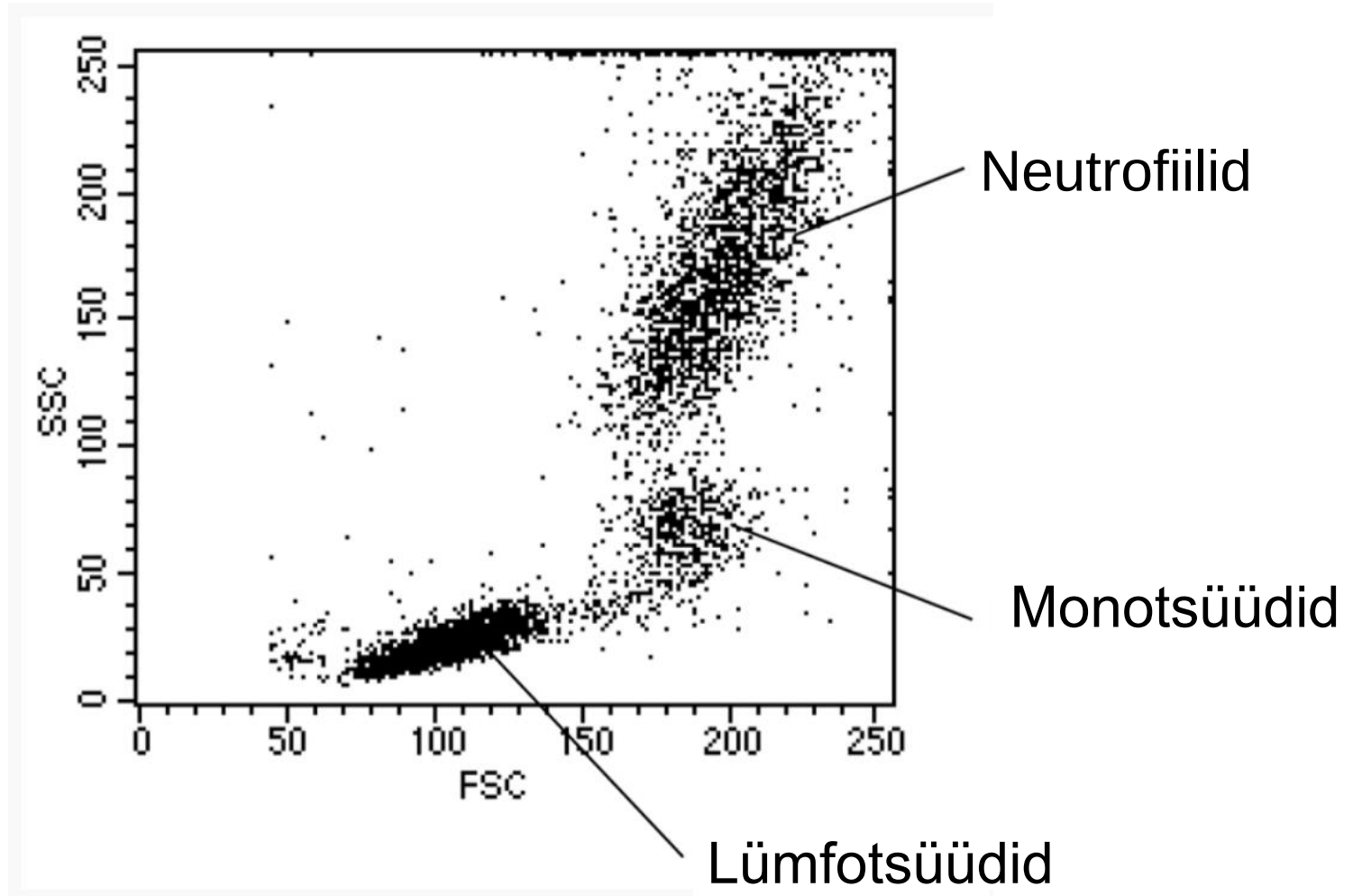




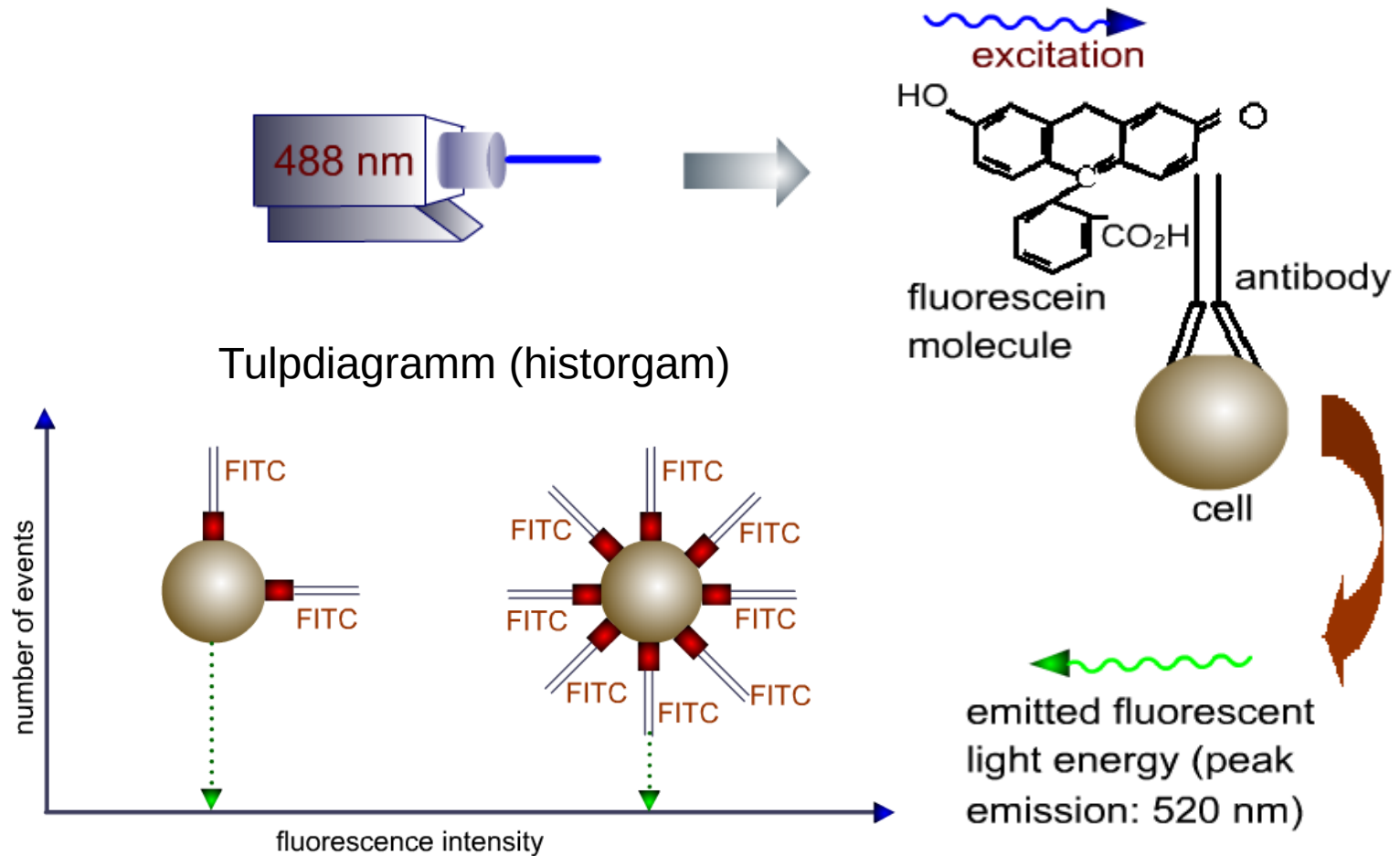
Rakke leitakse ülesse suhtelise suuruse ja suhtelise granulaarsuse järgi



Rakke leitakse ülesse suhtelise suuruse ja suhtelise granulaarsuse järgi



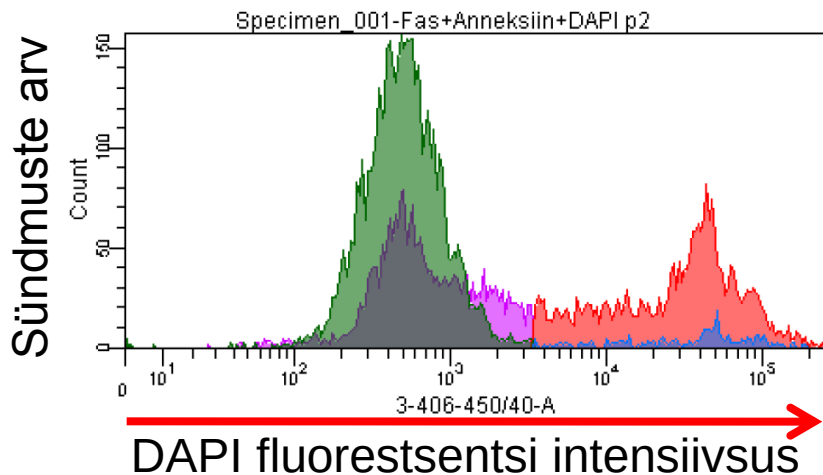
FACSi andmete esitamine



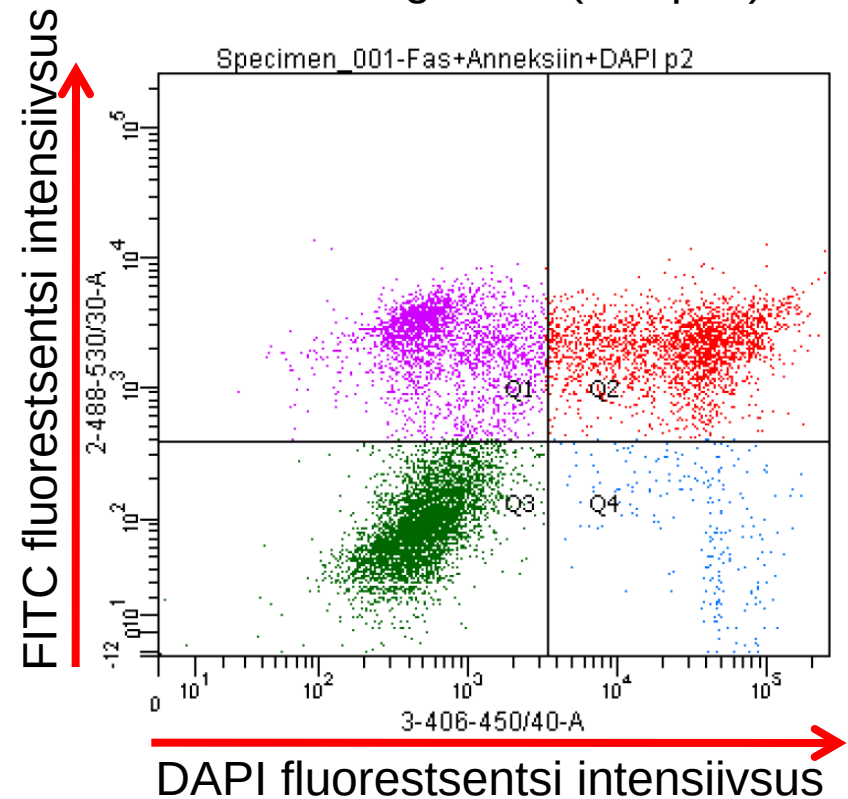
FITCi asemel võib ka DAPI olla!

FACSi andmete esitamine

Tulpdiagramm (Histogram)

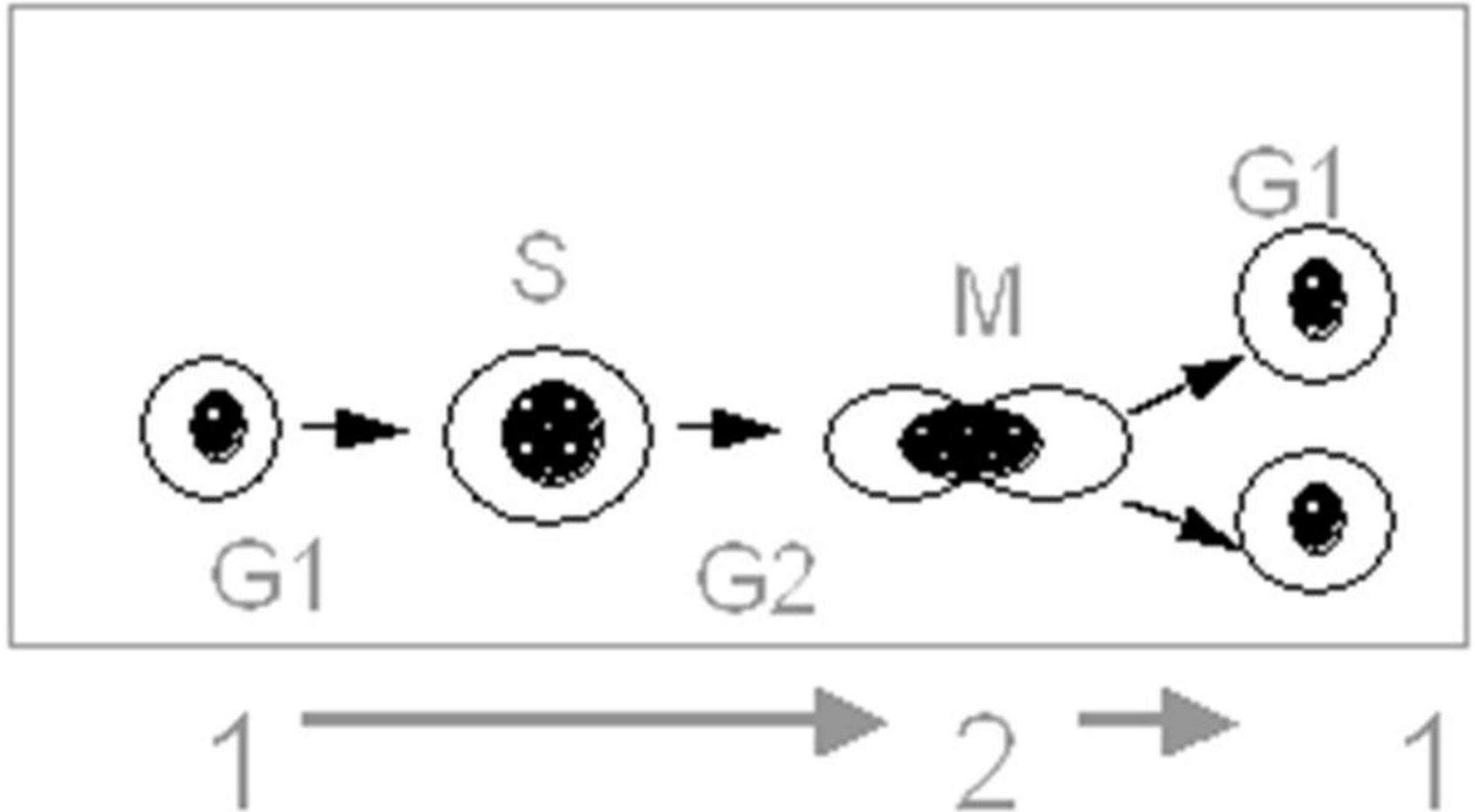


Punktdiagramm (Dot plot)

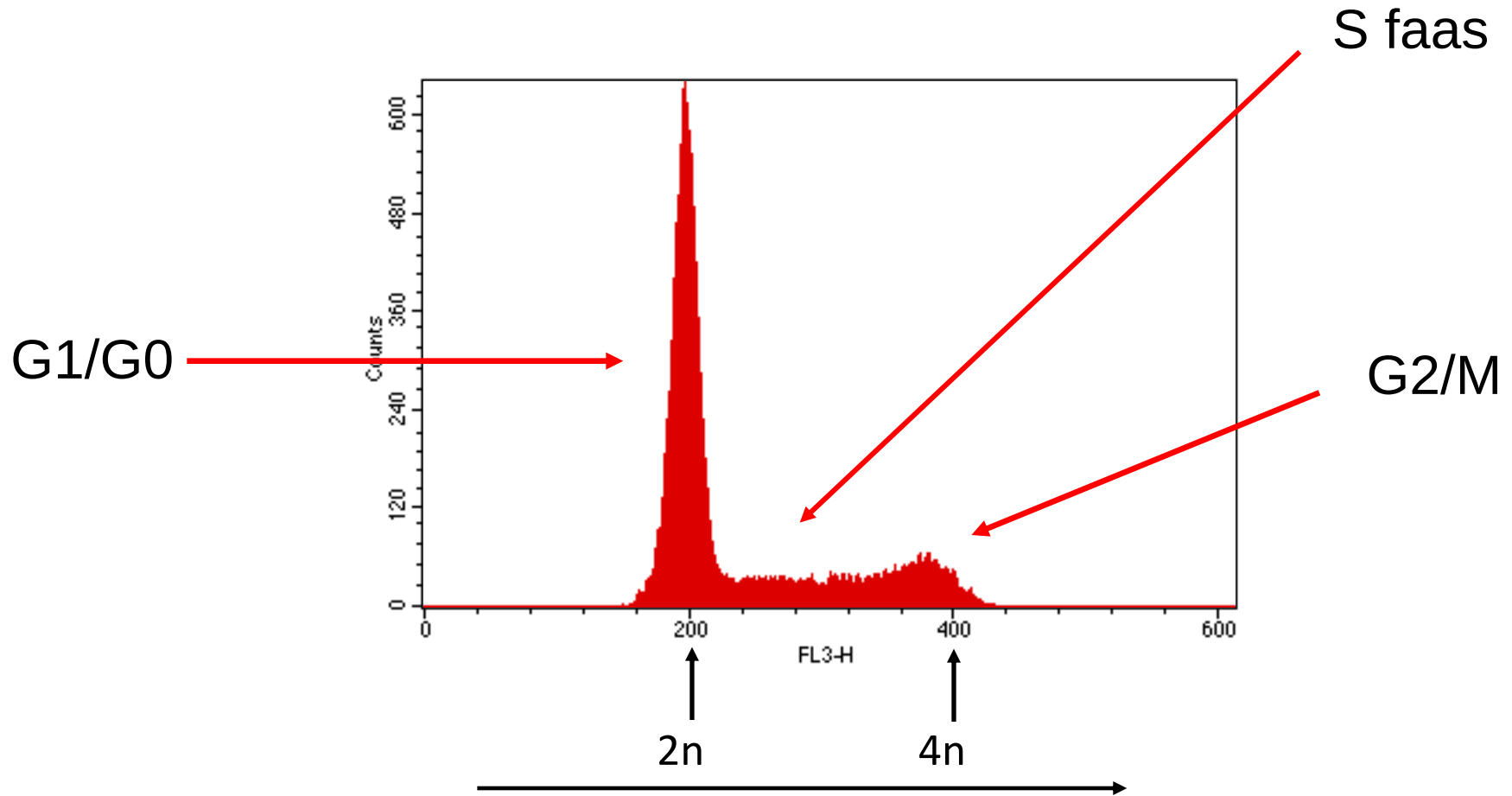


Küsimused?

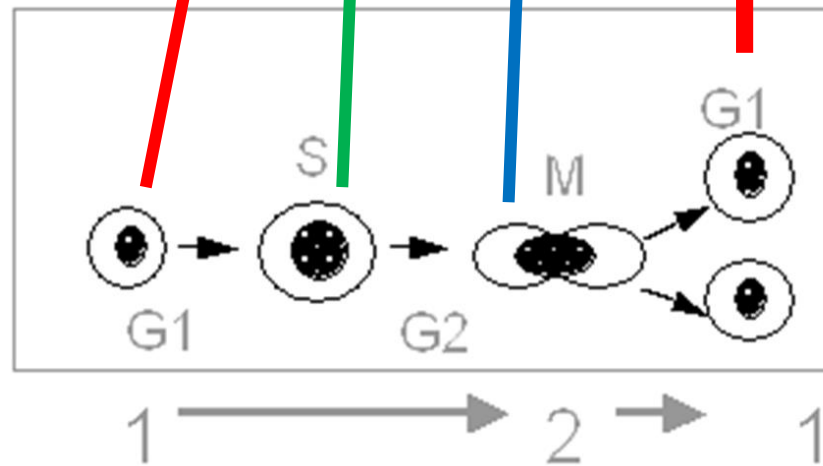
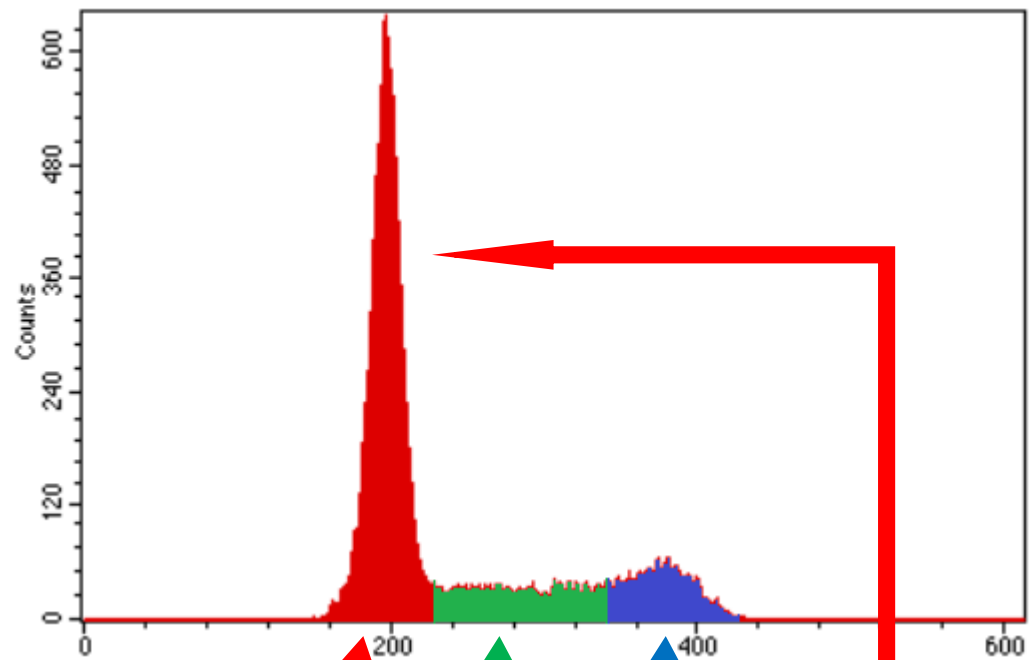
Tagasi rakutsükli juurde!



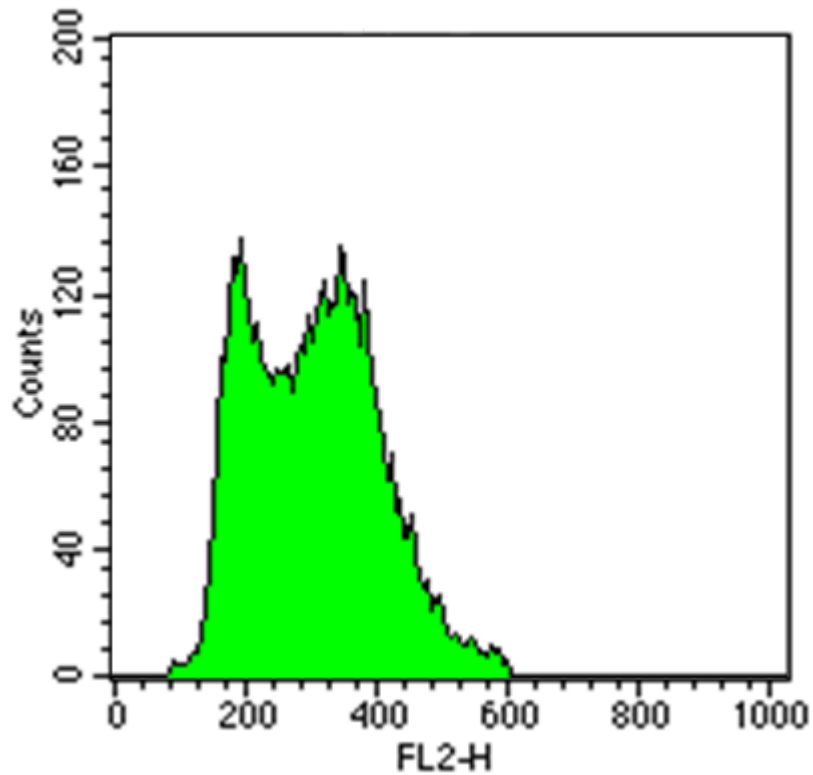
Kuidas näeb välja rakutsükli pilt?



DNA hulk rakus, DAPI fluorestsentsi intensiivsus

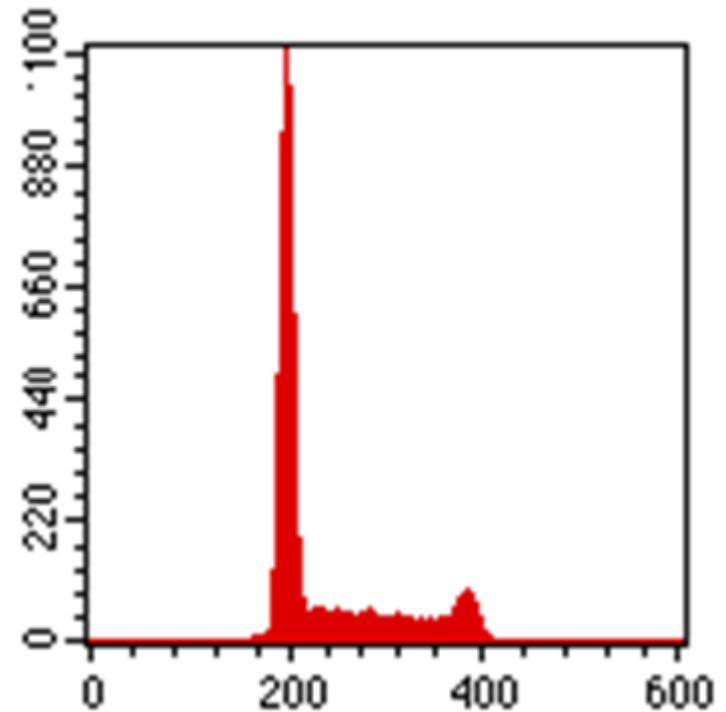


Pärmirakkude rakutsükkel:



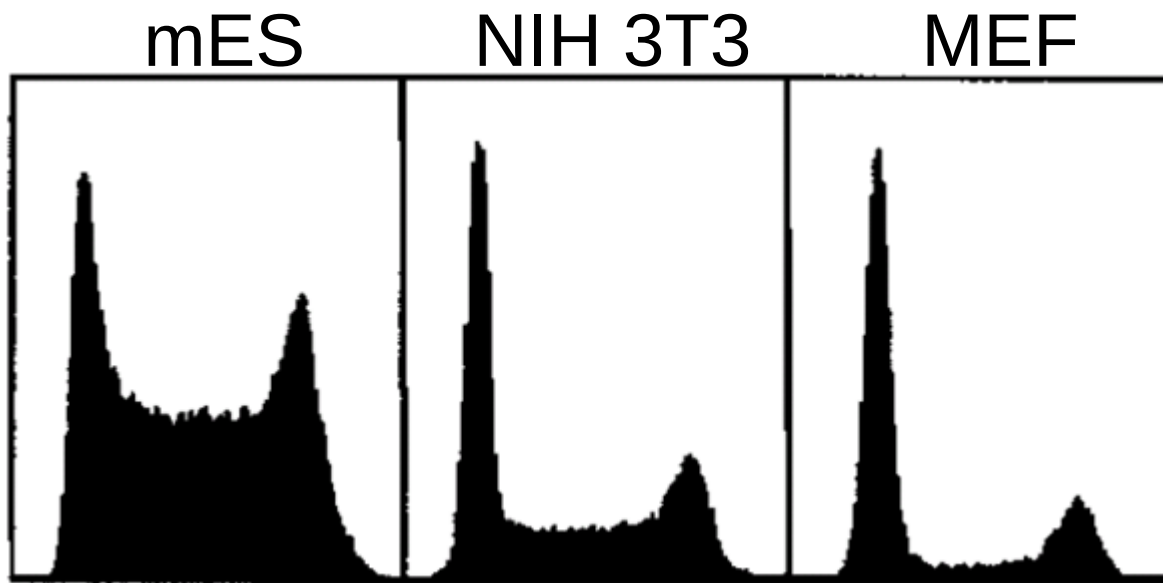
propidium iodide
(DNA hulk)

Imetajarakkude rakutsükkel:



propidium iodide
(DNA hulk)

(sündmuste ehk rakkude arv)



DAPI
(DNA hulk)

Rakkude sünkroniseerimine – et saada ühes rakutsükli faasis olev rakupopulatsioon

DNA sünteesi inhibeerimine erinevate kemikaalidega

aminopterin
thymidine
hüdoksüurea
cytosine arabinoside

rakutsükkel peatub **enamasti** S-faasis
ning rakud jäävad ellu

DNA-e kahjustuse tekitamine:

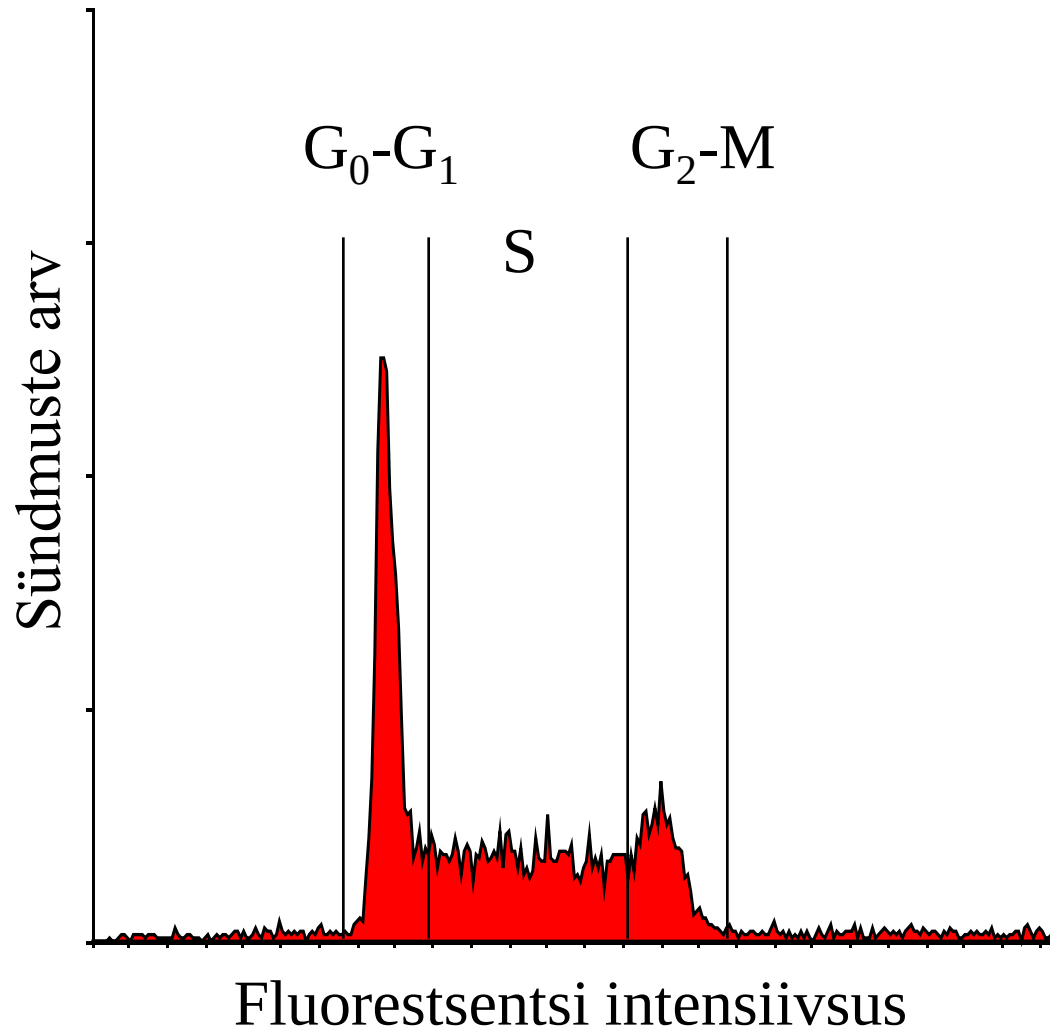
UV-kiirgus
reaktiivsed hapnikuühendid
kamptotetsiiniga (cpt)- rakud peatuvad G2 faasis

rakkude näljutamine

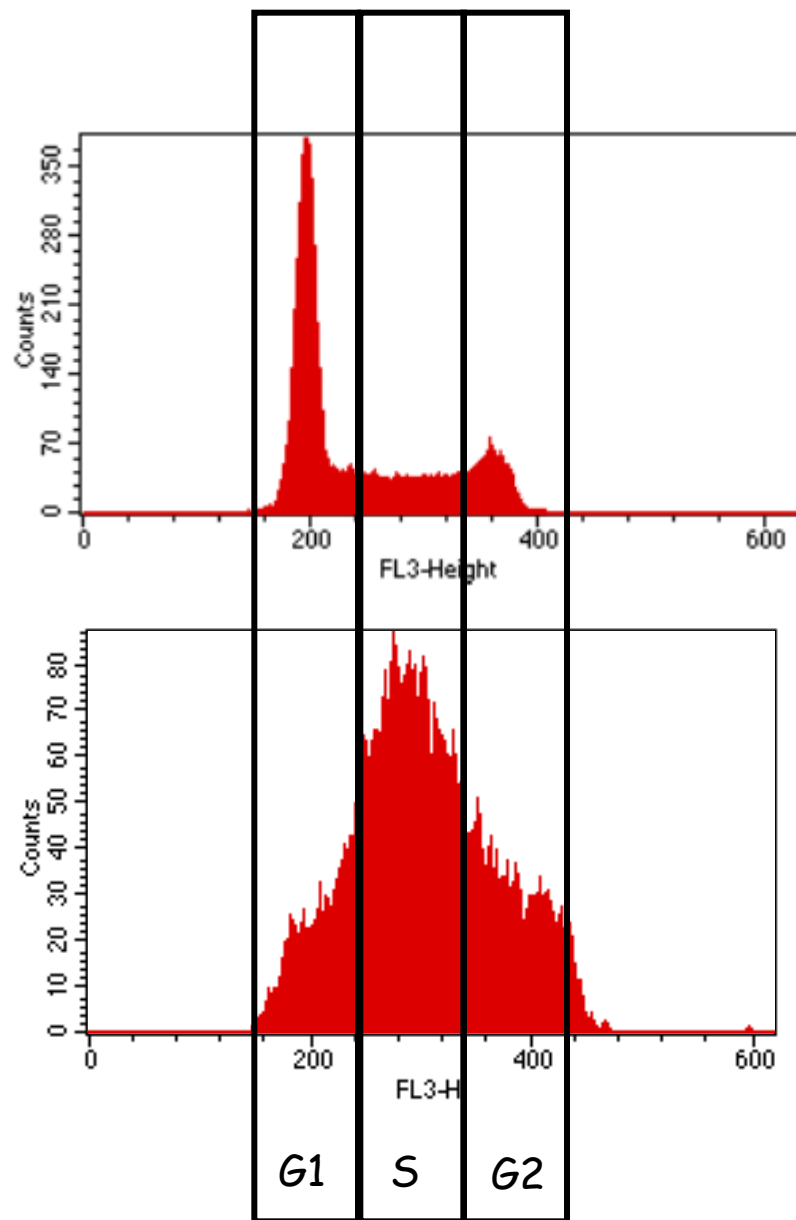
rakkude söötmele ei lisata seerumit

24 h ilma seerumita olnud rakud kogunevad G1 faasi

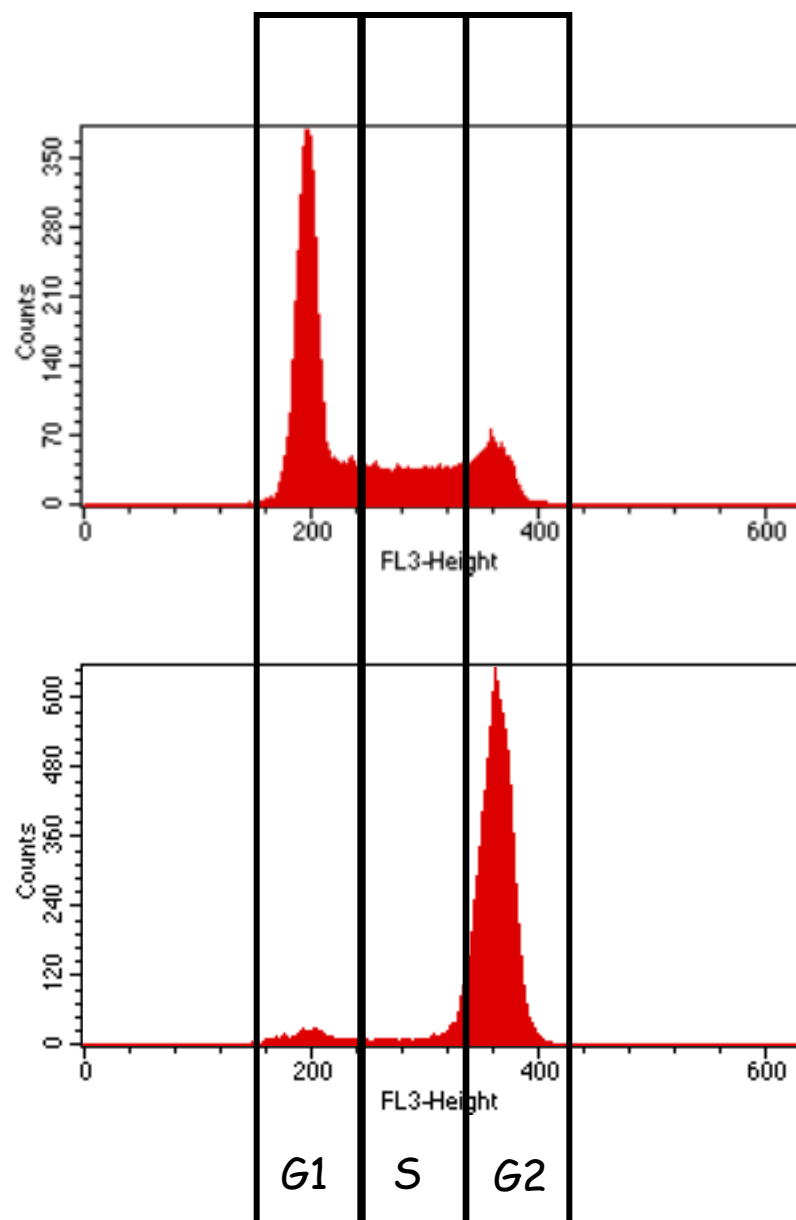
Tüüpiline DNA tulpdiagramm (histogram)



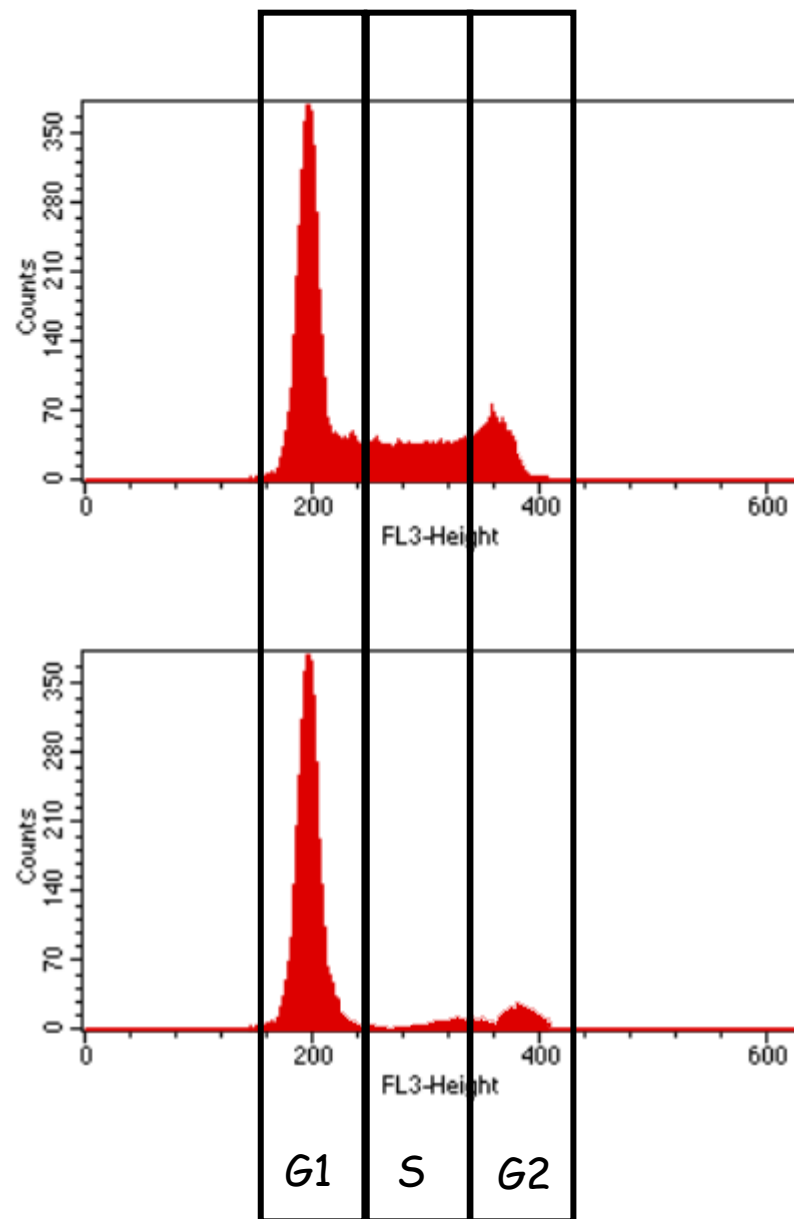
S faasi plokk



G2/M faasi plokk



G1 faasi plokk



Küsimused?