

We've Got The Flow

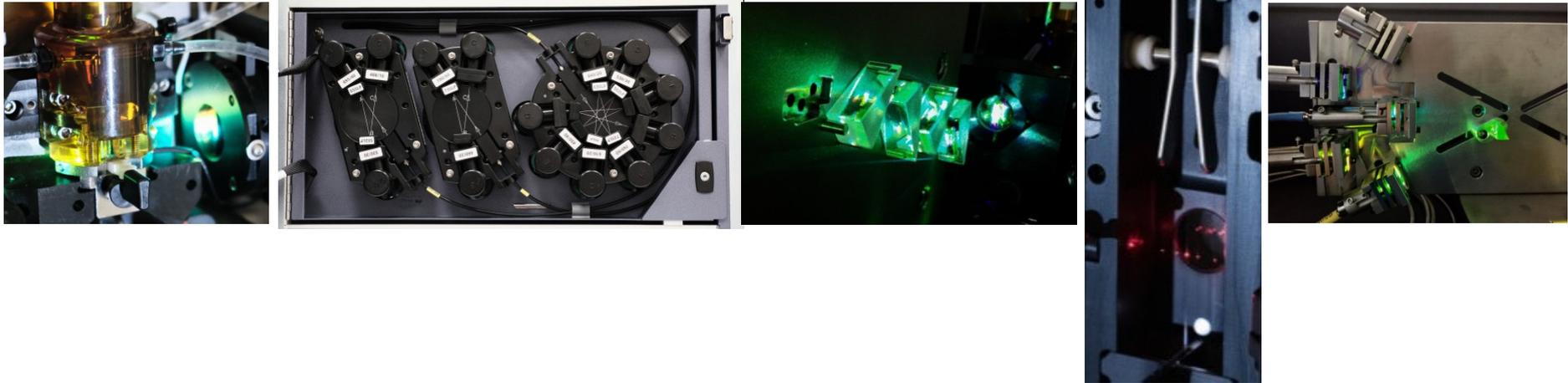
Brigitte Müller-Hilke

Core Facility für Zellsortierung und Zellanalyse

zsa@med.uni-rostock.de



Introducing our flow cytometers.....



FACSCalibur™
6 parameters
4 colors
+ FSC
+ SSC

FACSVerse™
8 parameters
6 colors
+ FSC
+ SSC

FACSAria™
16 parameters
14 colors
+ FSC
+ SSC



EUROPÄISCHE UNION
Europäischer Fonds für regionale Entwicklung



Mecklenburg
Vorpommern



Europäische Fonds EFRE, ESF und ELER
in Mecklenburg-Vorpommern 2014-2020

When (and why) to use flow cytometry.....

cellular status

- Immune-phenotyping
- Cell cycle analysis / Apoptosis
- Viability / vitality
- Quantifying DNA/RNA/Proteins (single cells)
- Redox status
- Mikrovesikel / Exosomes

cellular functions

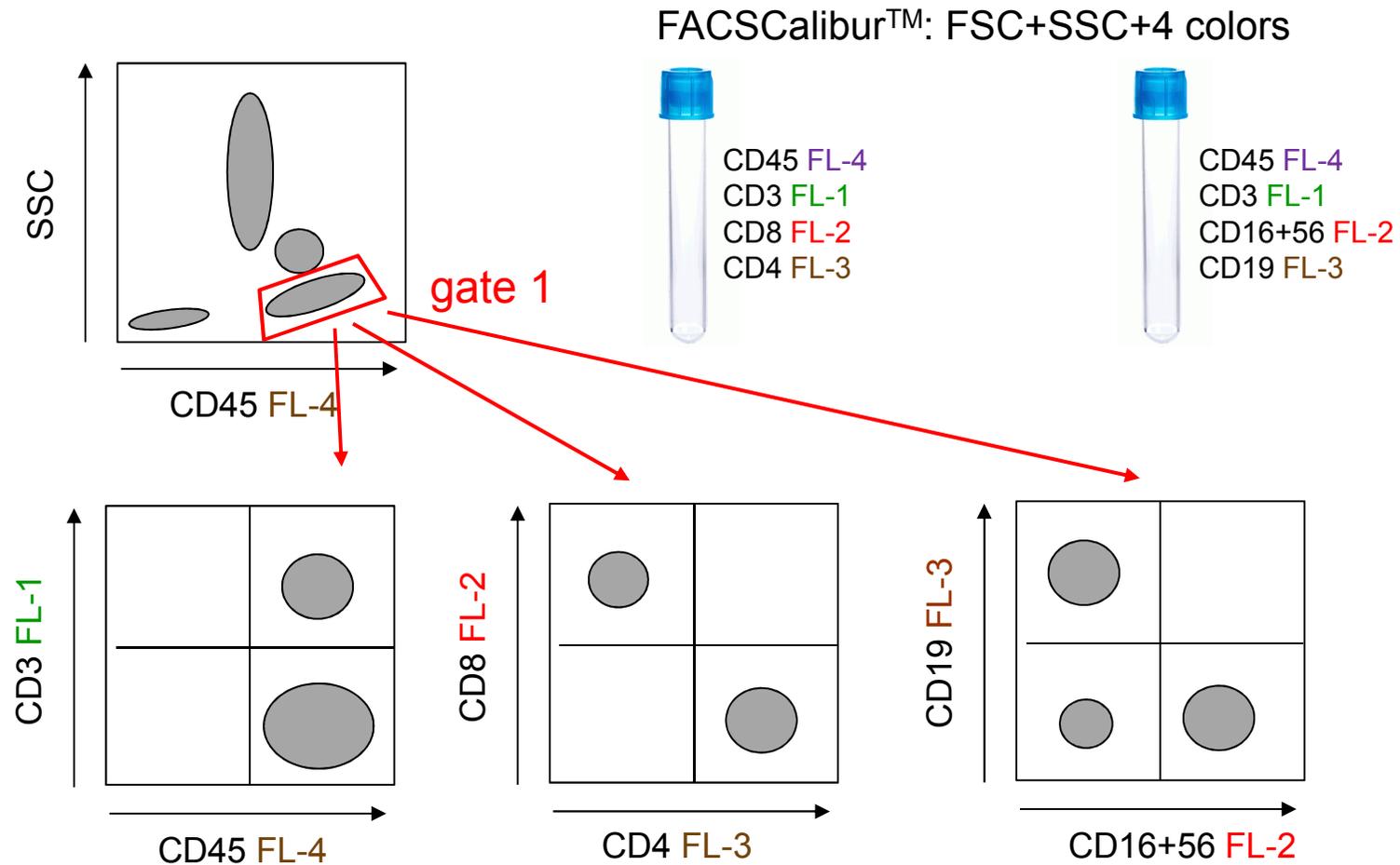
- Activation
- Ca⁺⁺-Flux
- cytokine secretion
- Phosphorylation / signalling cascades
- Enzyme activity
- Intrazellular pH
- Phagozytosis

miscellaneous

- Identification of transgene expressing cells
- Quantifying absolute cell numbers
- Live / dead exclusion
- Oxidative stress
- Sorting
- Protein-protein Interaction (FRET)
- Hypoxia
- Flow Fish: telomer length determination

Cellular status: immune phenotyping

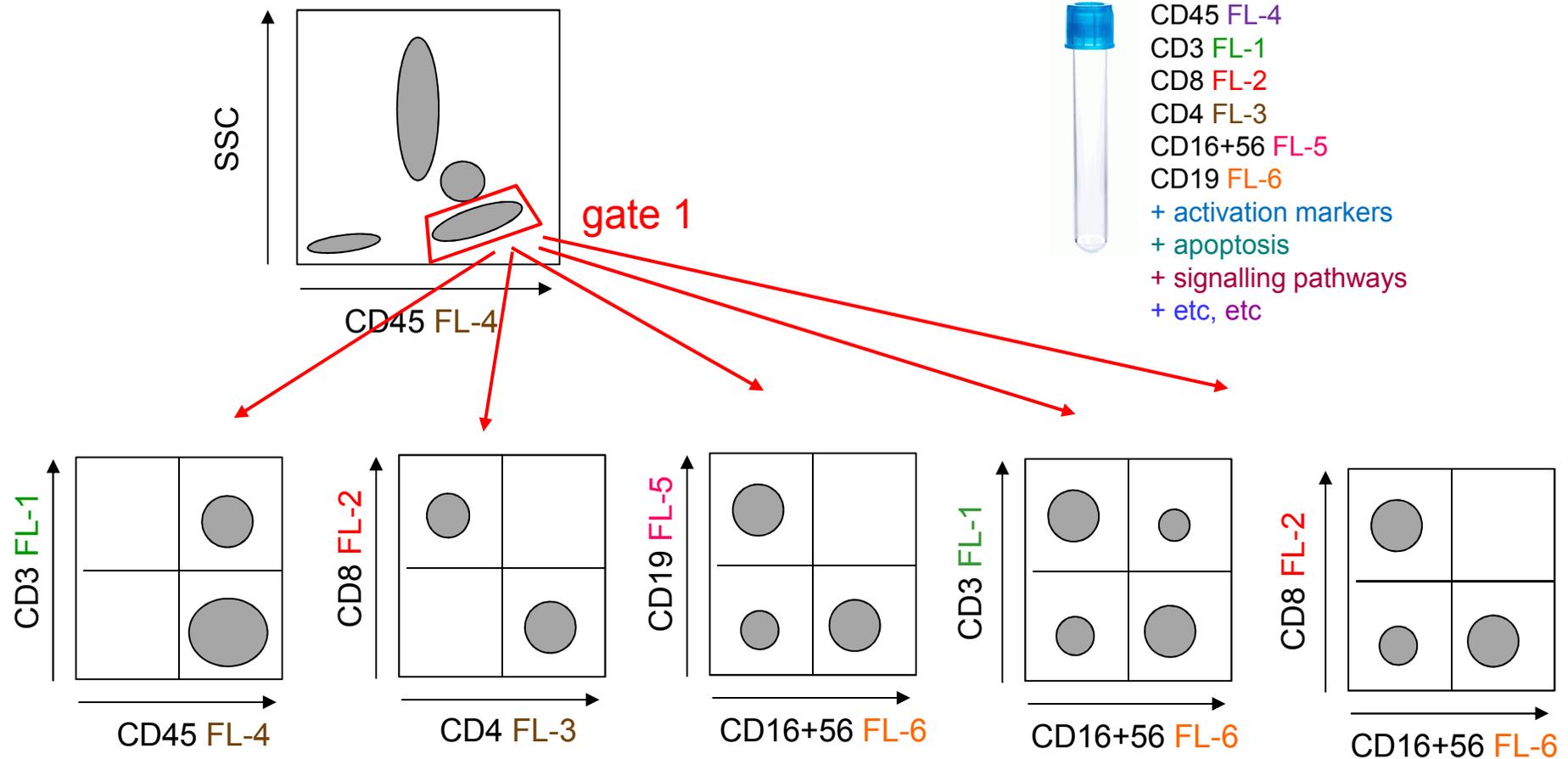
.... and why 16 parameters are better than 6



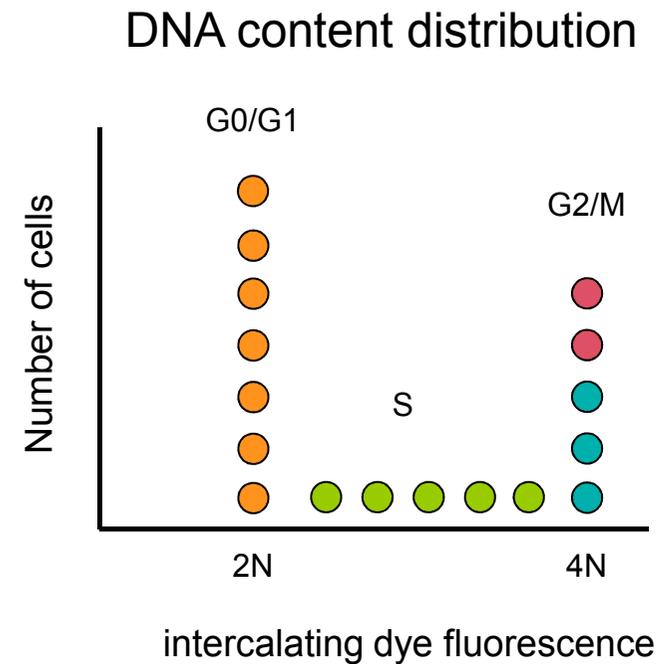
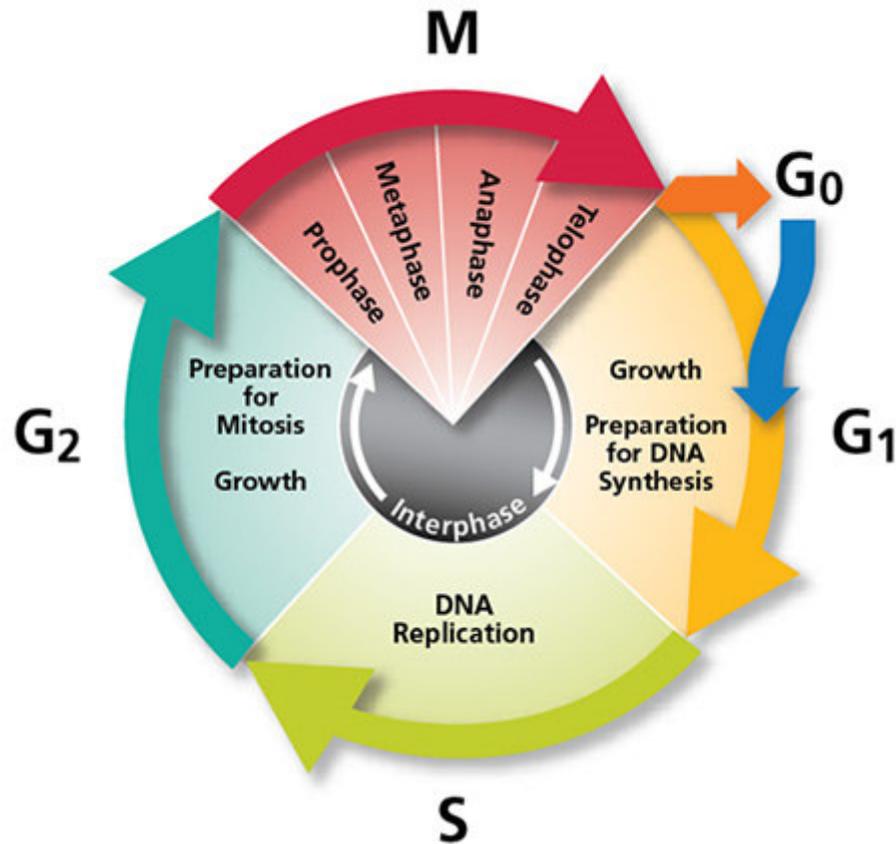
Cellular status: immune phenotyping

.... and why 16 parameters are better than 6

FACSAria™: FSC+SSC+14 colors

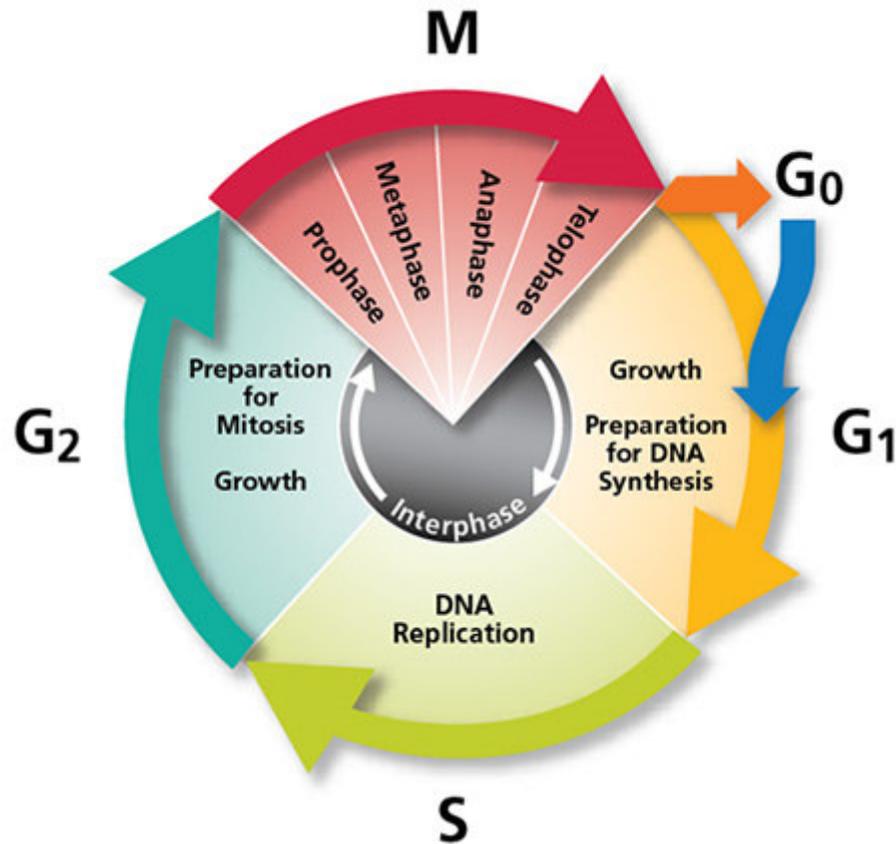


Cellular status: cell cycle analysis by DNA content

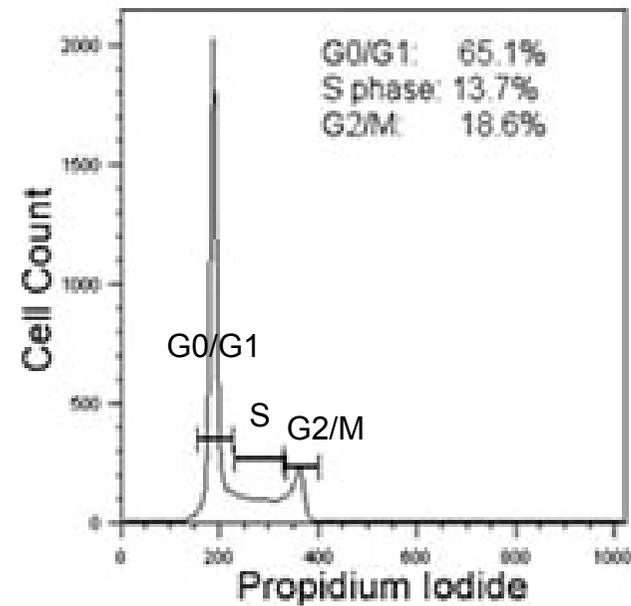


<https://m.bdbiosciences.com/sg/research/apoptosis/analysis/index.jsp>

Cellular status: cell cycle analysis by DNA content



DNA content distribution



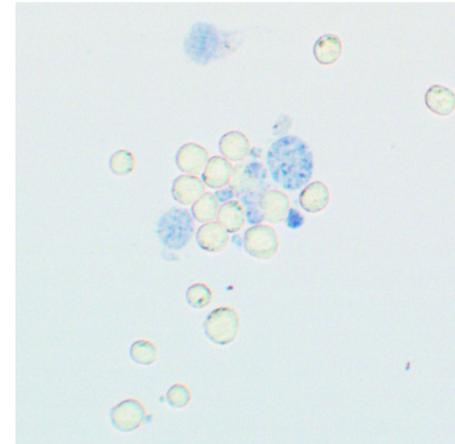
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Cellular status: viability and vitality

checking plasmamembrane integrity

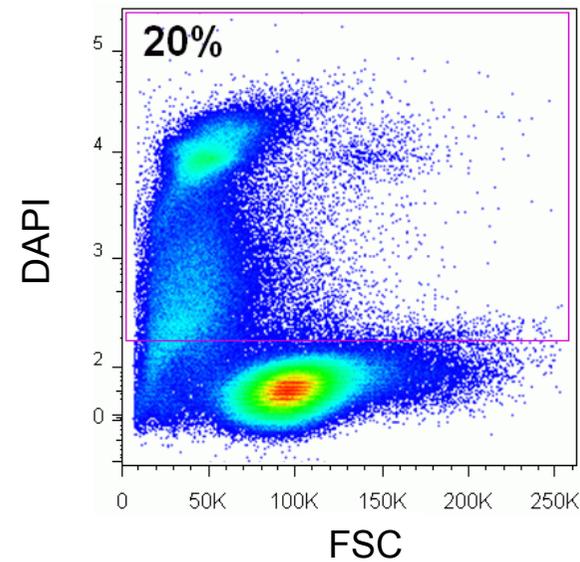
Microscope

- ◆ Trypan blue

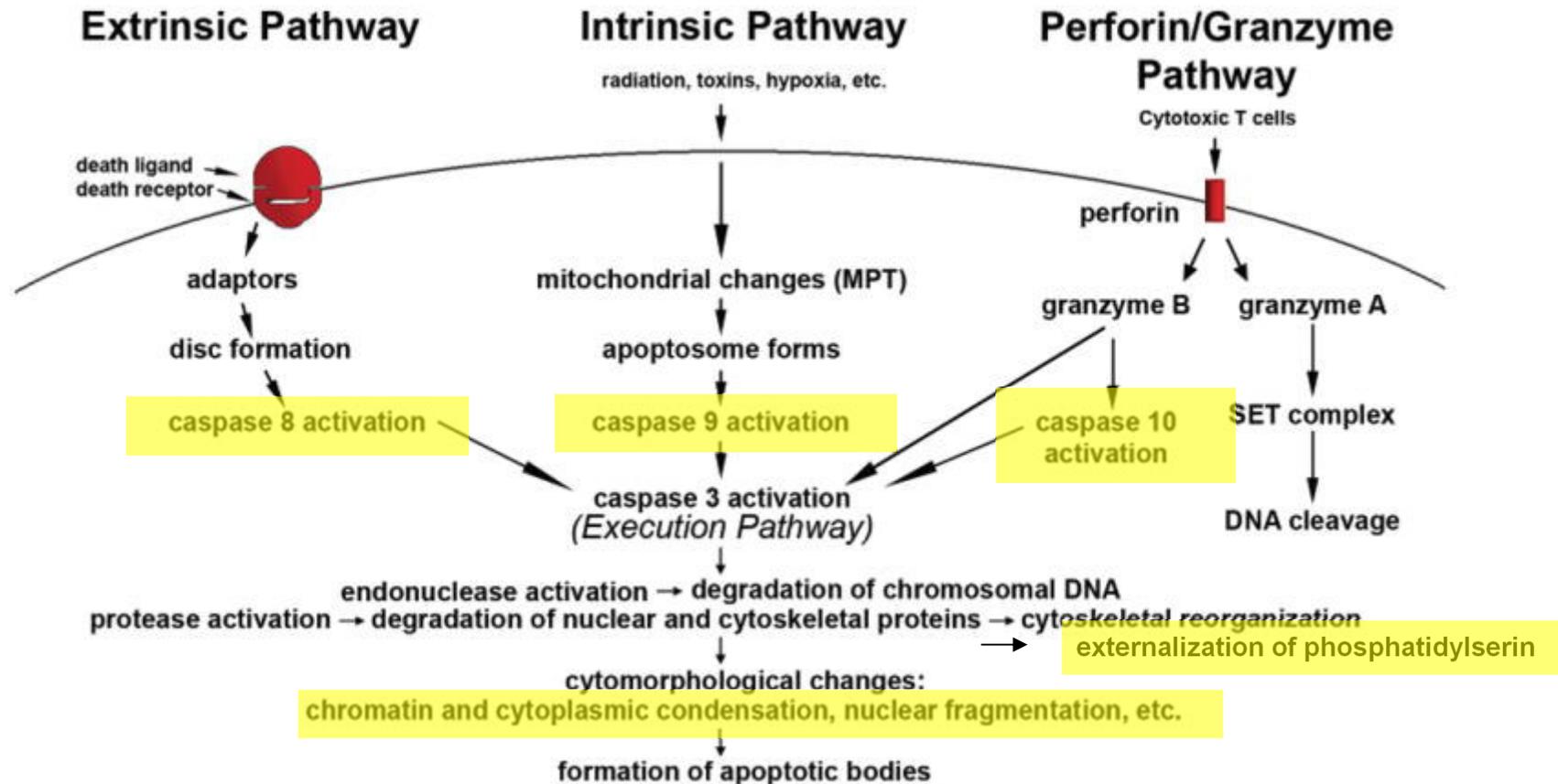


Flow Cytometer

- ◆ Propidium iodide
- ◆ DAPI

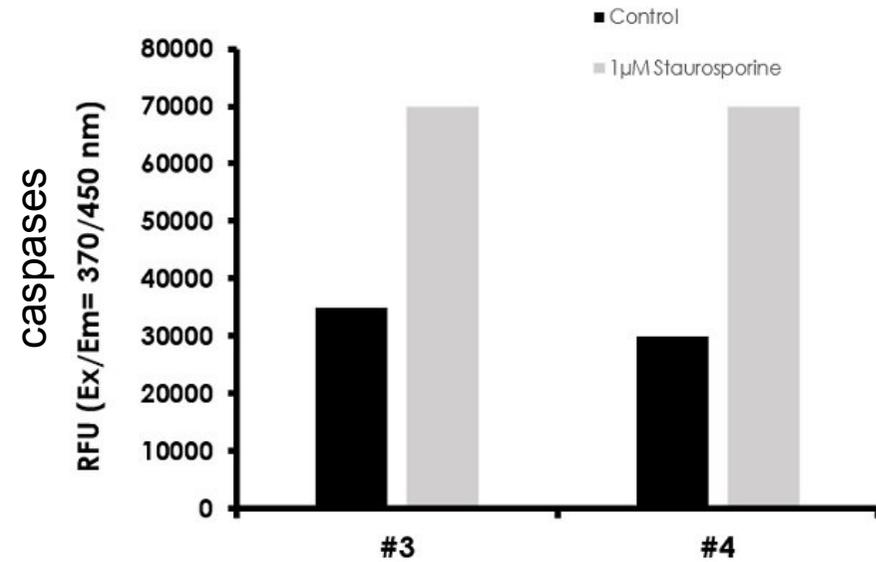
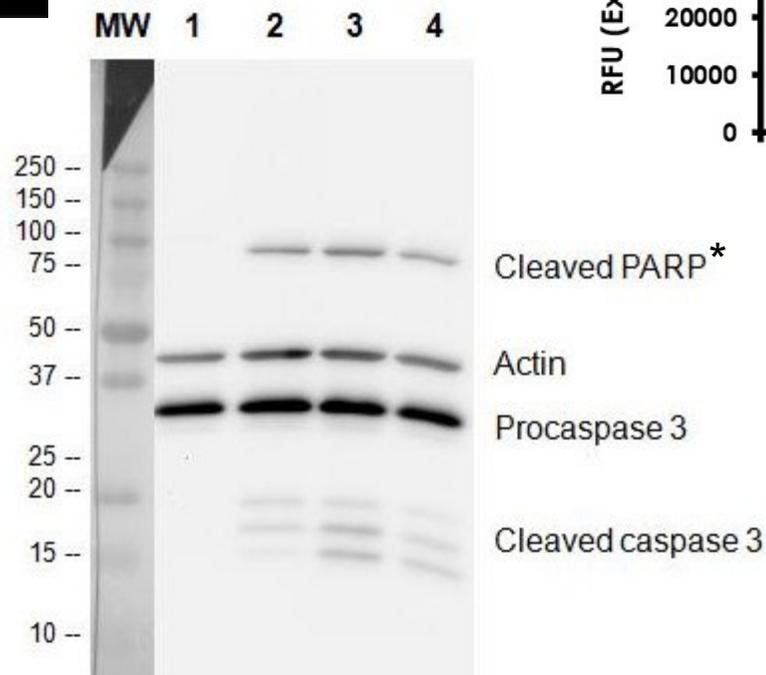
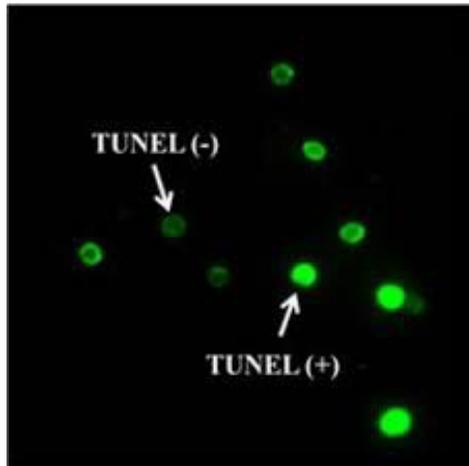


Cellular status: viability and vitality - apoptosis



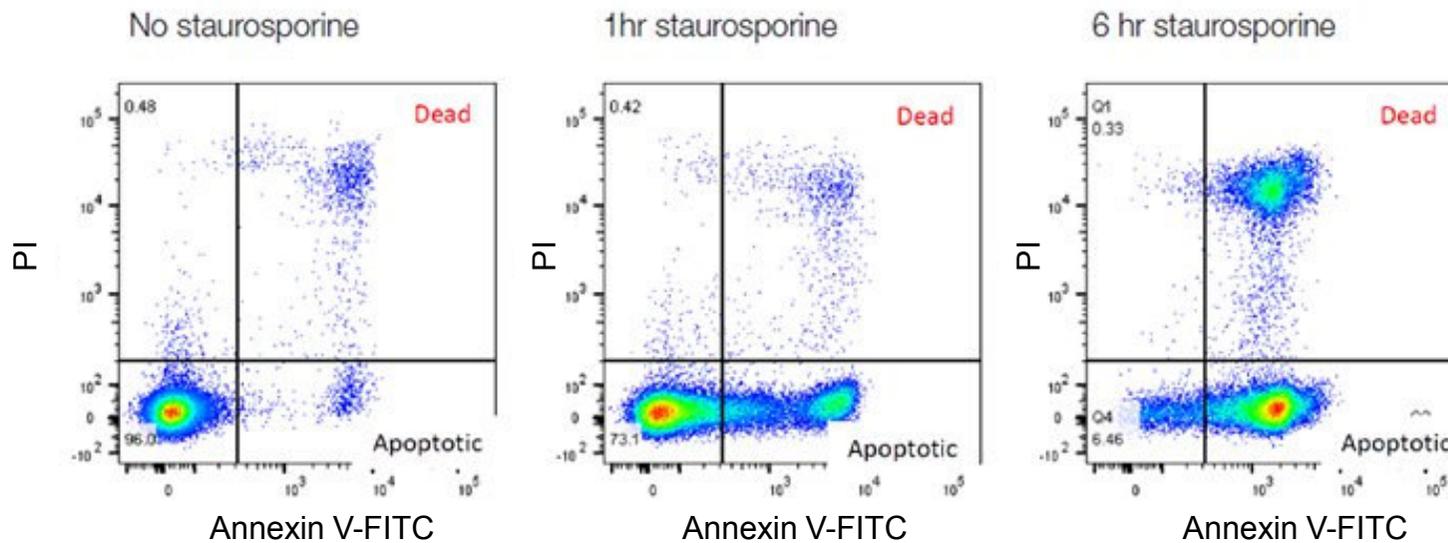
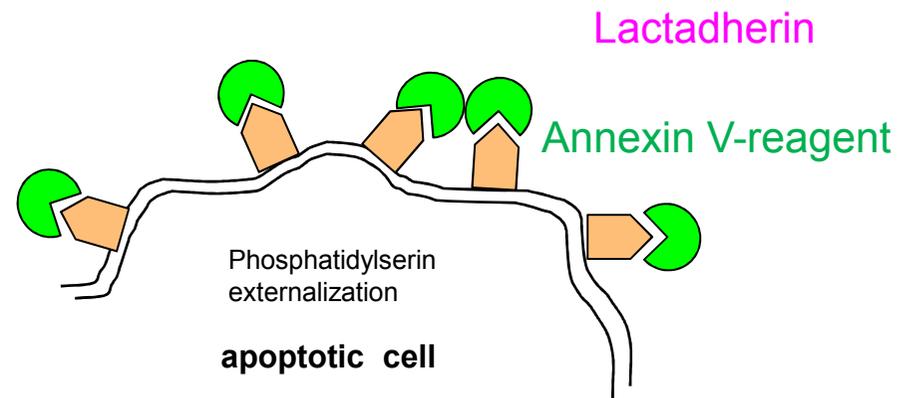
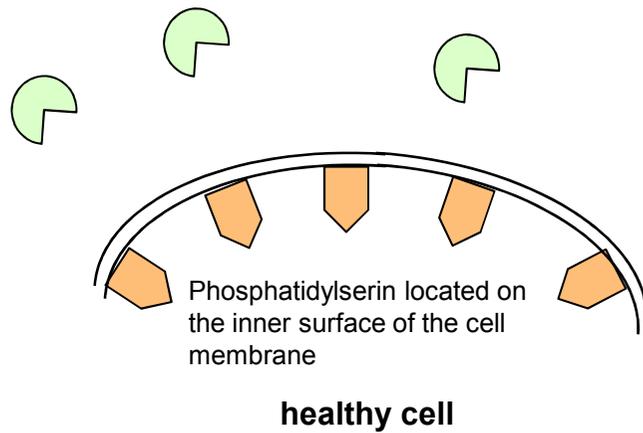
[Susan Elmore](#); Apoptosis: A Review of Programmed Cell Death; [Toxicol Pathol.](#) 2007; 35(4): 495–516.

Cellular status: viability and vitality - apoptosis



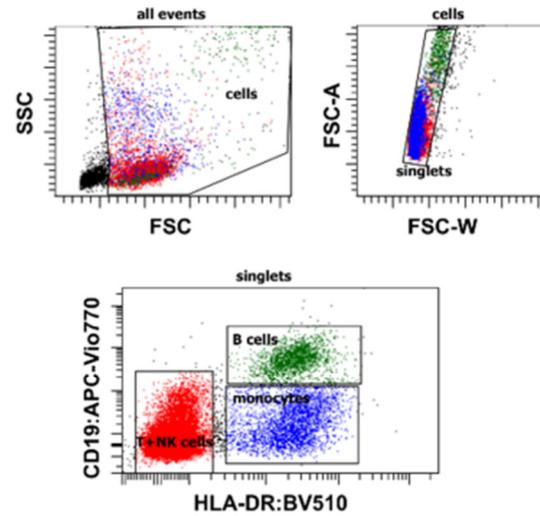
*Poly (ADP-ribose) Polymerase

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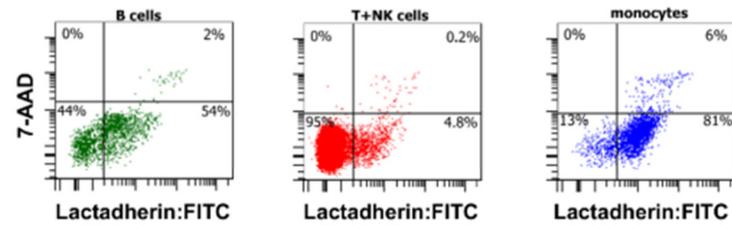


<https://www.bio-rad-antibodies.com/flow-cytometry-apoptosis.html>

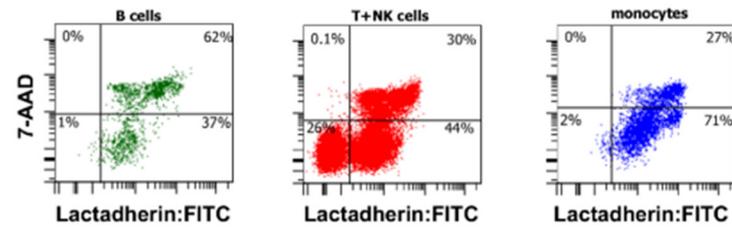
Cellular status: viability and vitality - apoptosis



staurosporine - 5h



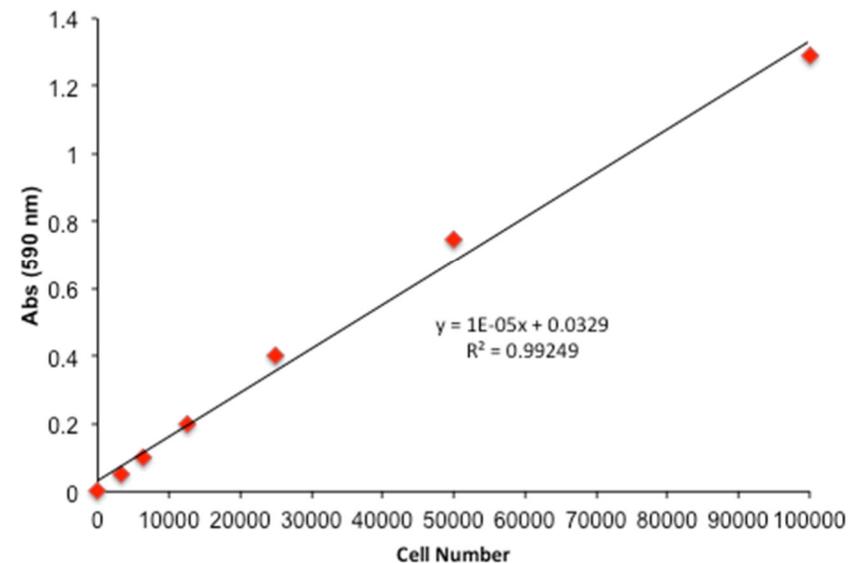
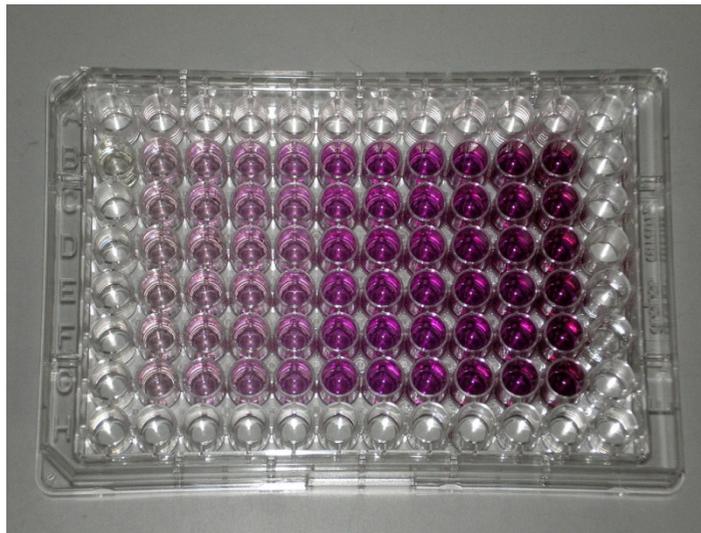
staurosporine - 24h



Cellular status: viability and vitality – cell proliferation

WST-8 or MTT assays (metabolic activity)

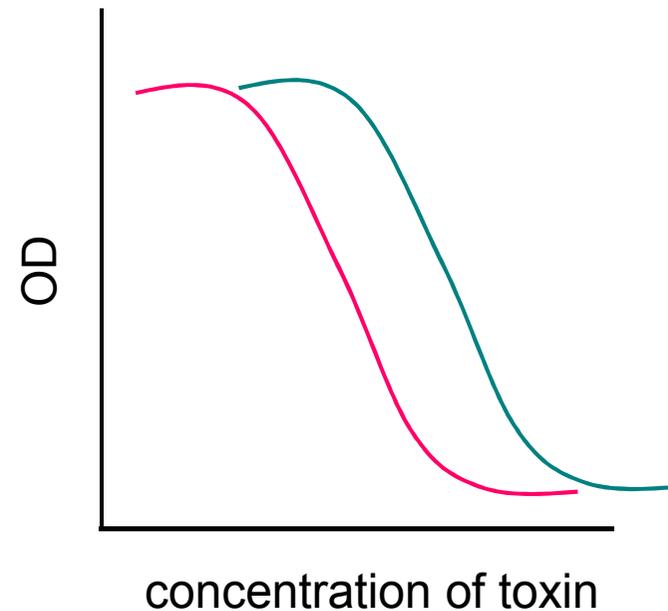
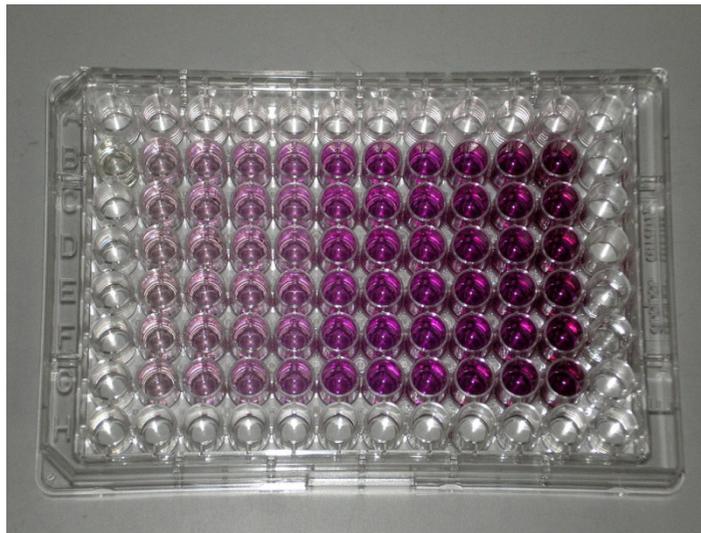
Metabolically active cells cleave tetrazolium salts to form water-insoluble (MTT) or water-soluble (WST) formazan salts



Cellular status: viability and vitality – cell proliferation

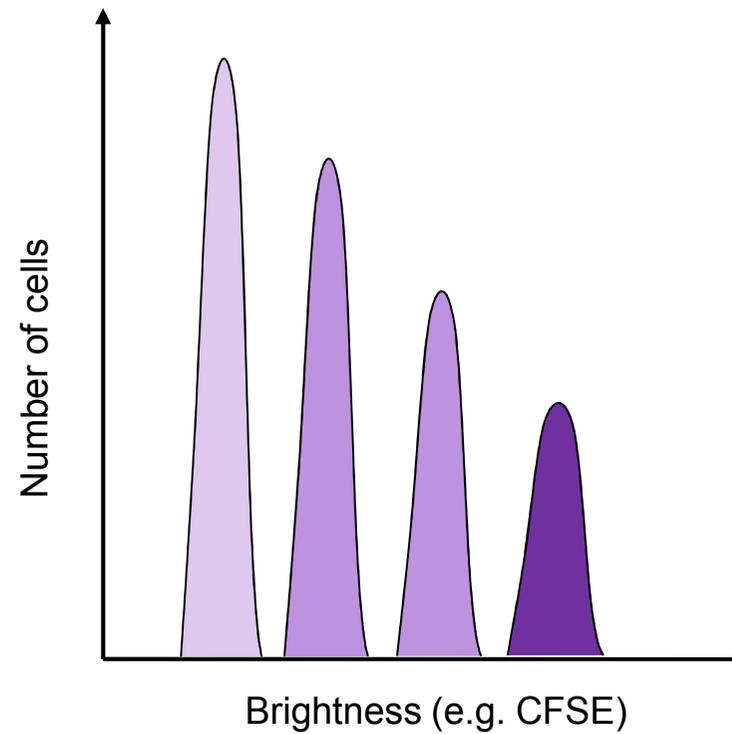
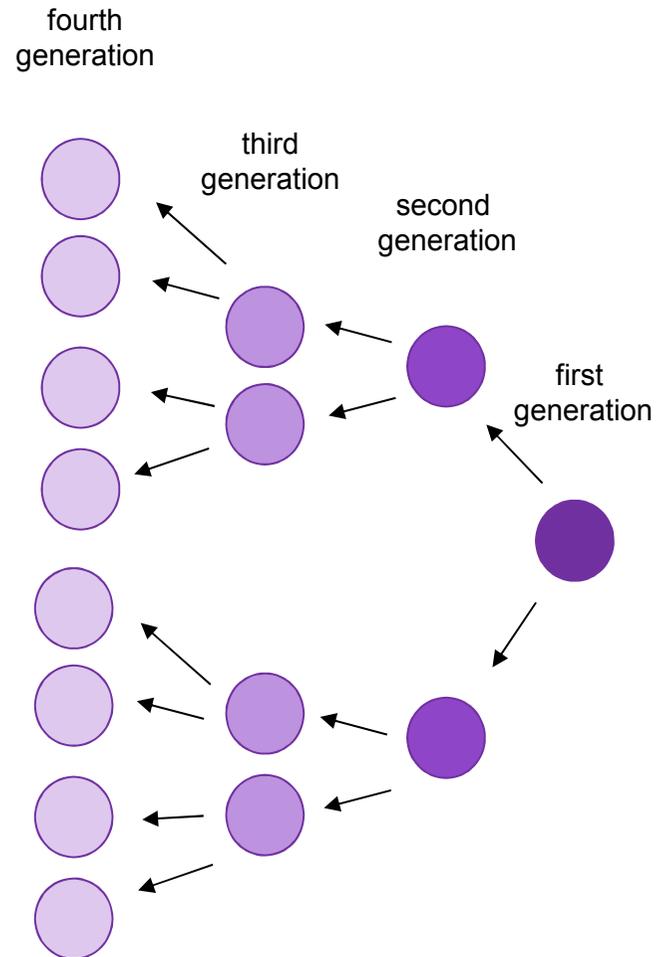
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Cellular status: viability and vitality – cell proliferation

via dye dilution and FACS



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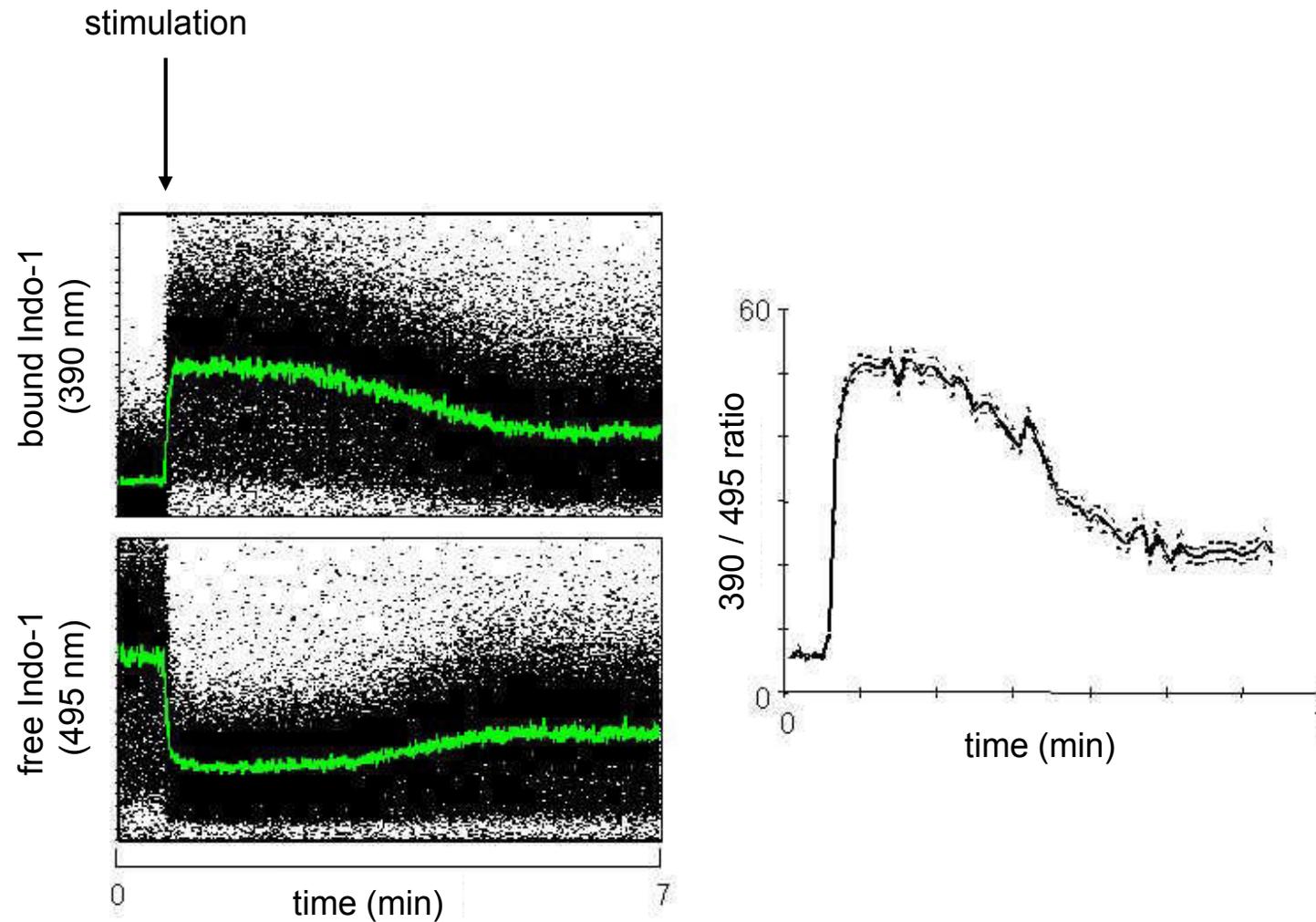
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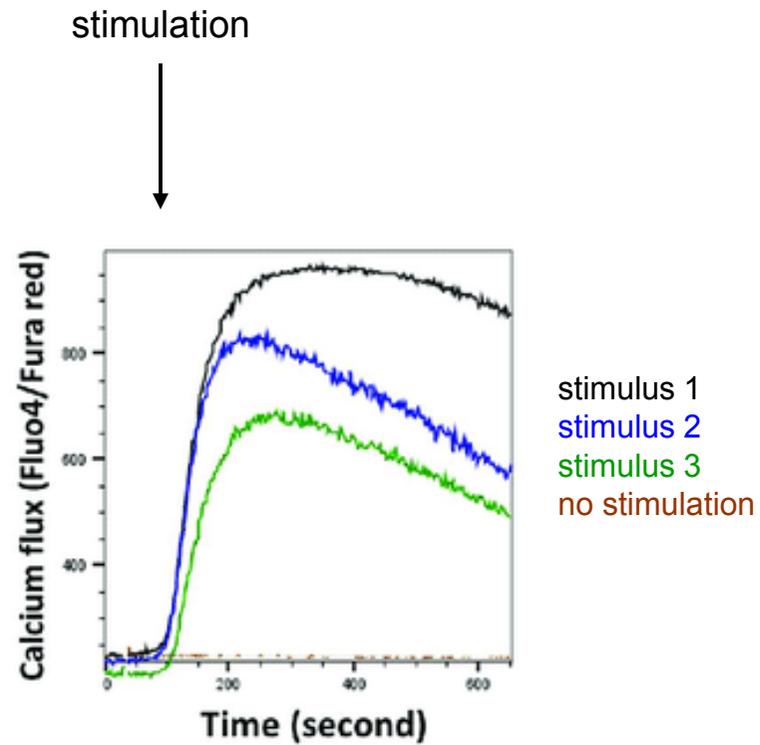
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Cellular function: activation and Ca⁺⁺-flux

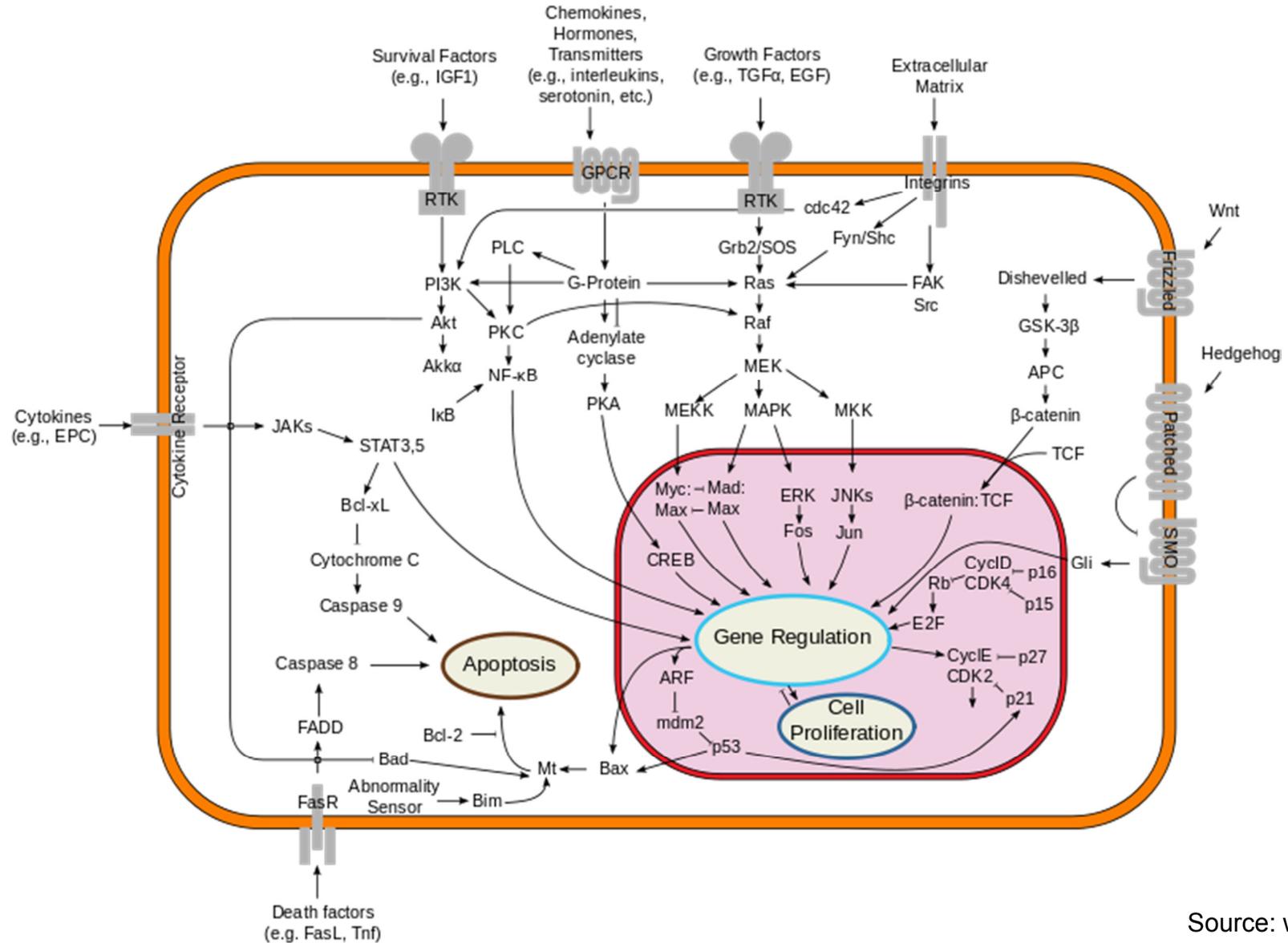


<https://bcf.technion.ac.il/portfolio/calcium-flux/>

Cellular function: activation and Ca⁺⁺-flux



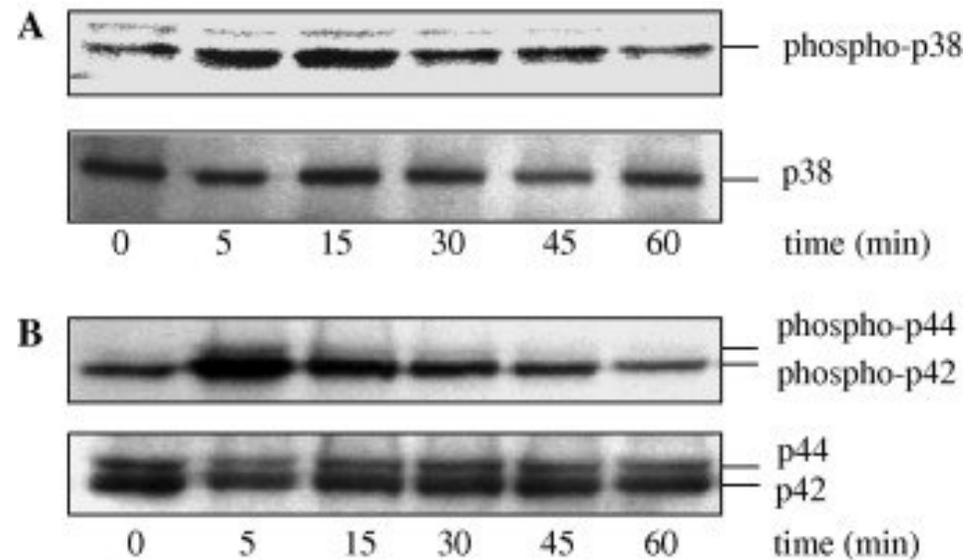
Cellular function: phosphorylation and signalling cascades



Source: wikipedia

Cellular function: phosphorylation and signalling cascades

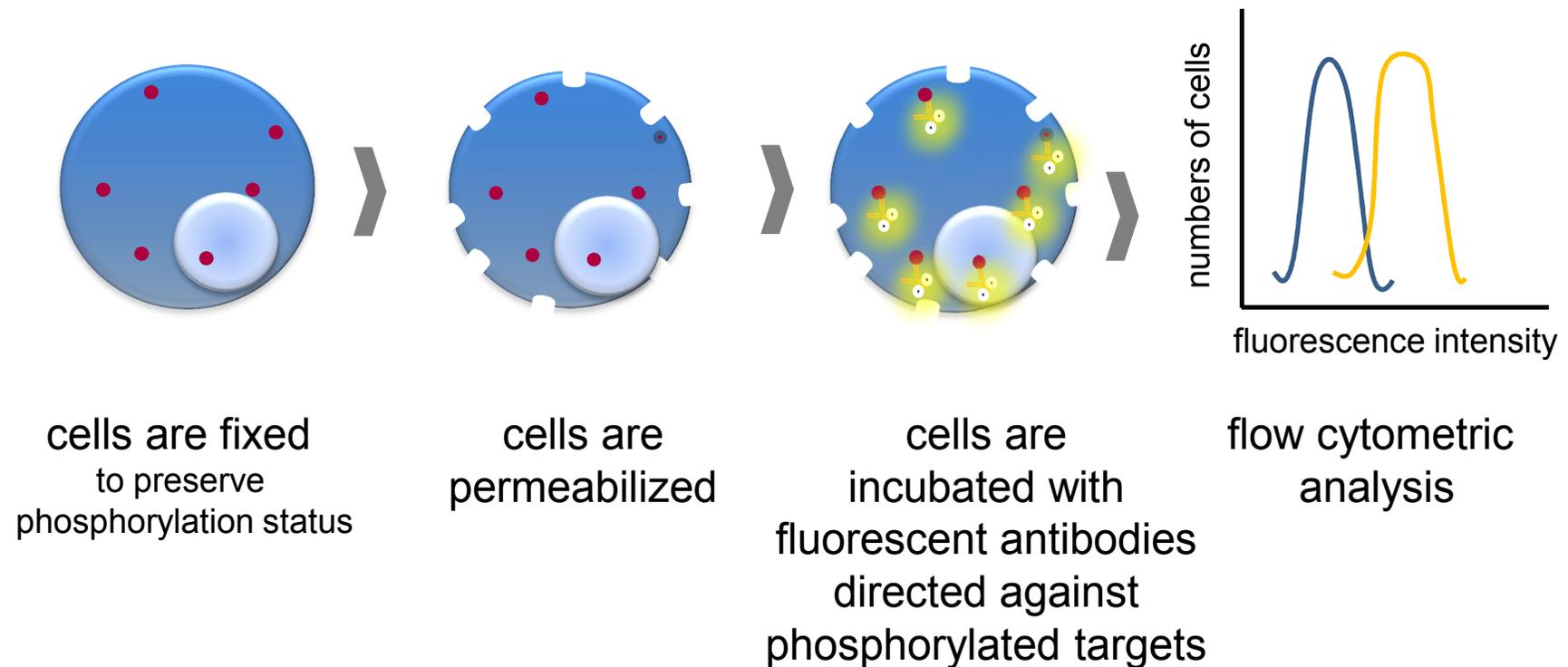
..... the classic way



Rösch et al. 2005; Bioch. Biophys. Res. Comm. 338(2): 1171-1178

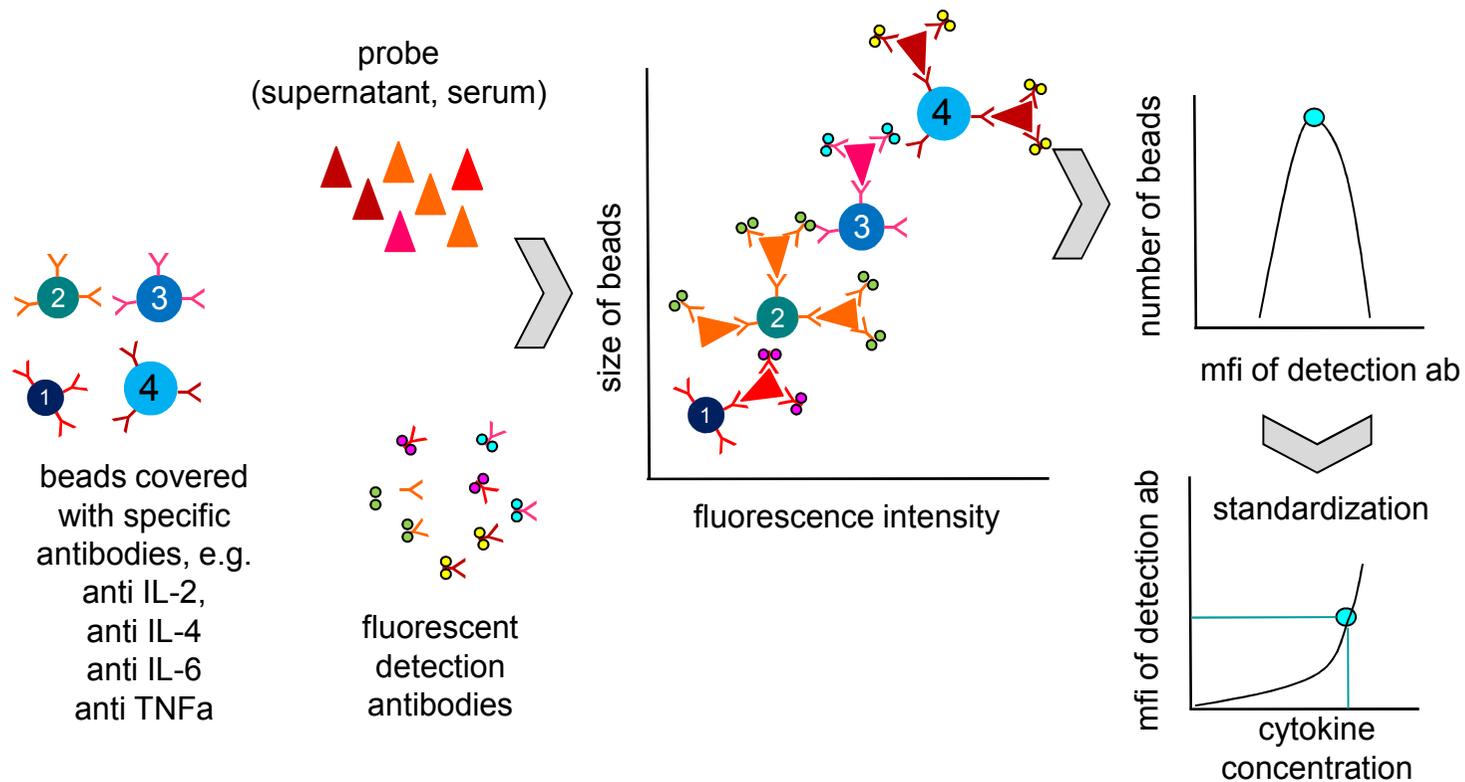
Cellular function: phosphorylation and signalling cascades

..... the phos flow way



Cellular function: measuring cytokines

and why ELISA are so „yesterday“



	ELISA (Kit)	Multiplex
numbers of cytokines (analytes)	1	≤ 30
min. volume of probe	100 μ L	12,5 μ L
Time spent (e.g. 6 cytokines)	6 x 4,5 h = 27 h	6 h
costs (e.g. 6 cytokines)	6 x ~ 400 € = 2400 €	~ 1000 €
sensitivity	< 1 pg/mL	< 1 pg/mL

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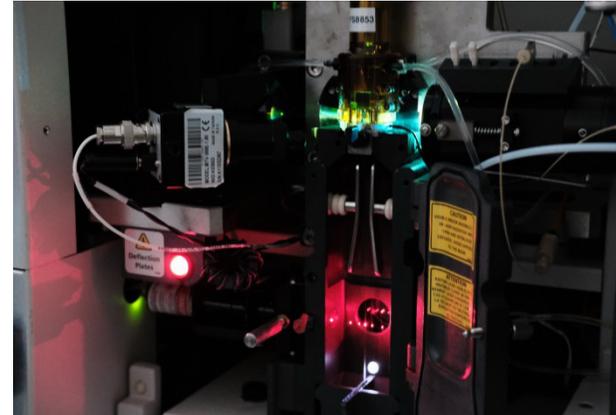
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miscellaneous: cell sorting

autoMACS[®] Pro



FACS Aria[™] IIIu
16 parameters
14 colors
+ FSC
+ SSC

Magnetic cell sorting:

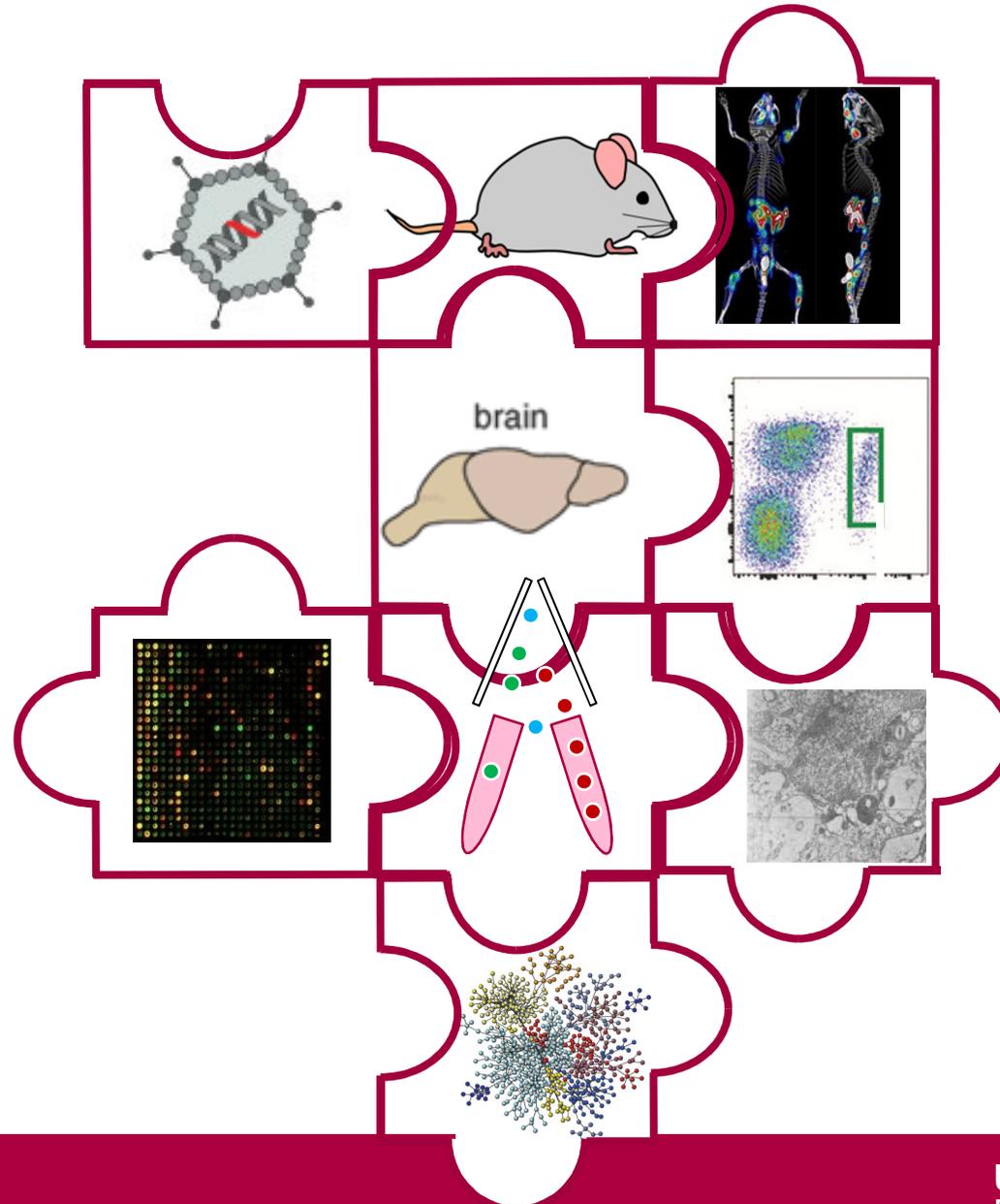
- ◆ positive selection of target cells
- ◆ isolation of untouched target cells via depletion of non-target cells
- ◆ isolation of target cells from whole blood
- ◆ concentration of rare target cells for subsequent flow cytometric isolation via FACS Aria[™] IIIu.

Flow cytometric cell sorting:

Sorting (single) cells for subsequent

- ◆ (co)culture (direct sorting into culture plate)
- ◆ transfusion and transplantation into animals
- ◆ characterization of transfected / transduced cells
- ◆ cloning, sequencing, proteomics, transkriptomics

When (and why) to use flow cytometry.....



Thanks for your attention.....



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