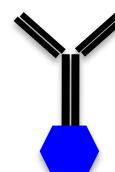
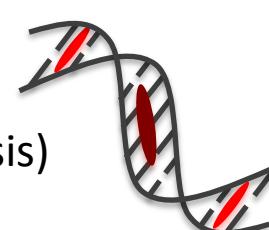
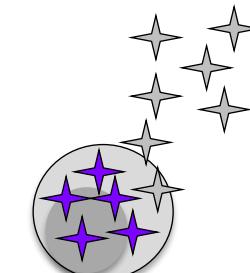
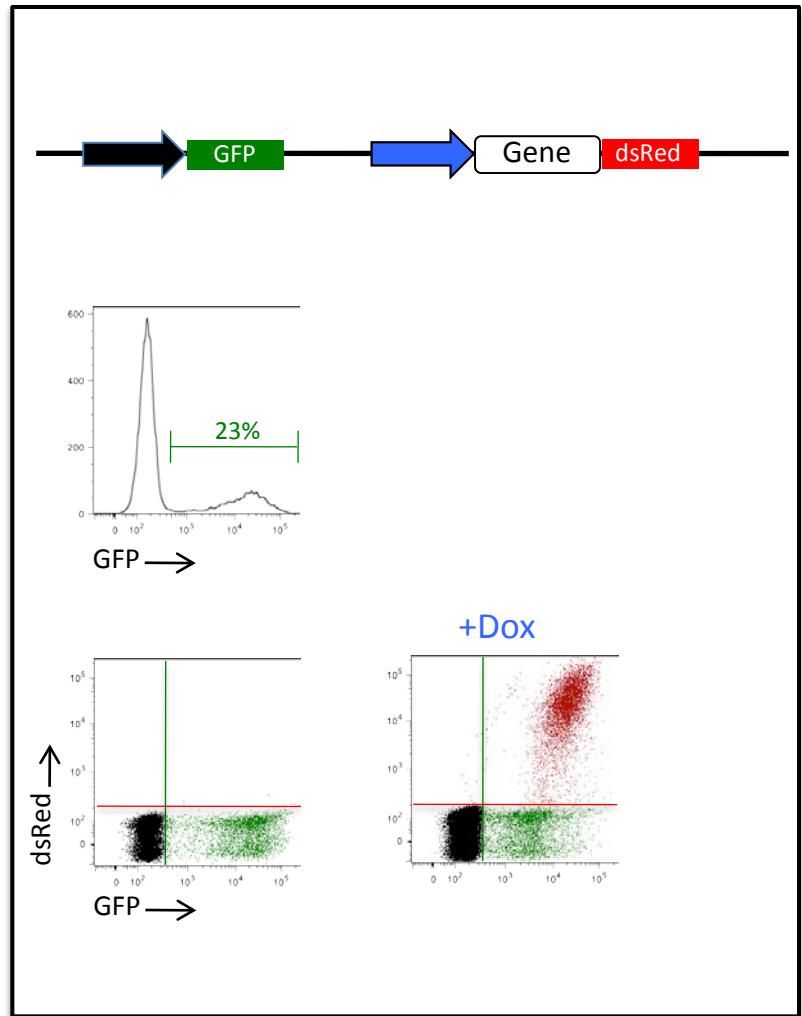


Applications Flow Cytometry

- Genetically encoded fluorescent reporter proteins
(transfection-, infection-, induction- rates, expression, deletion...) 
- Immunostainings of cell-specific antigens
(extra- and intracellular) 
- DNA stainings
(cell cycle, ploidy, apoptosis/necrosis) 
- Functional assays and Kinetics
(apoptosis, cell proliferation, cell signaling: Ca^{2+} -flux) 

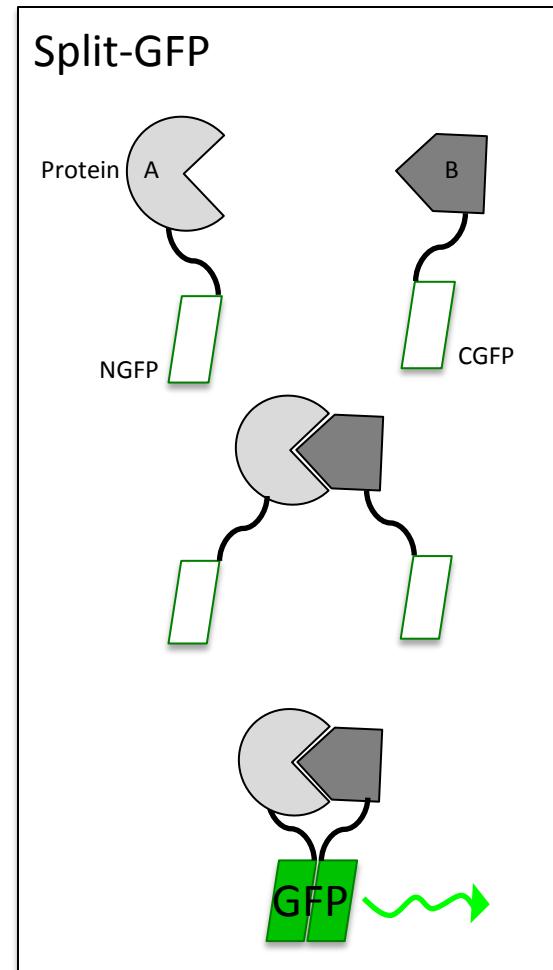
Genetically encoded fluorescent proteins report for ...

- Transfection
Infection ...
- Expression
Induction
Deletion ...
- Protein interaction (FRET, split-GFP)
- Location (Photoactivatable GFP)



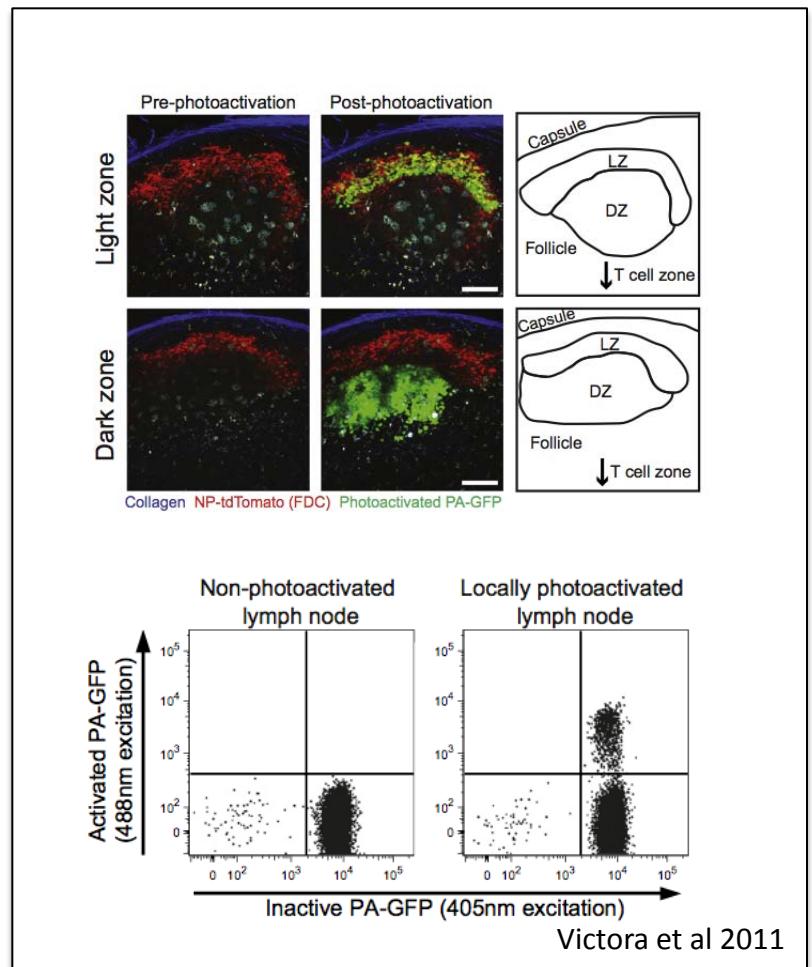
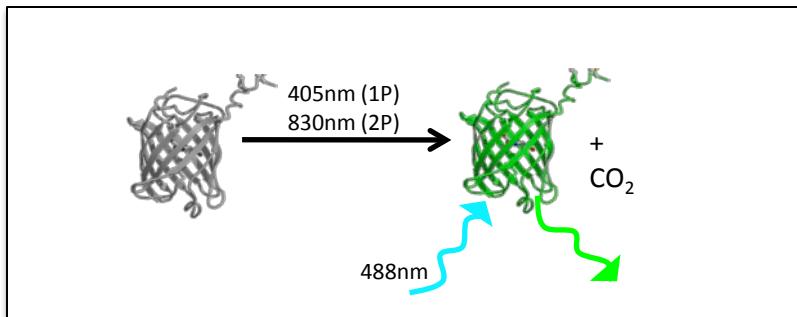
Genetically encoded fluorescent proteins report for ...

- Transfection
Infection ...
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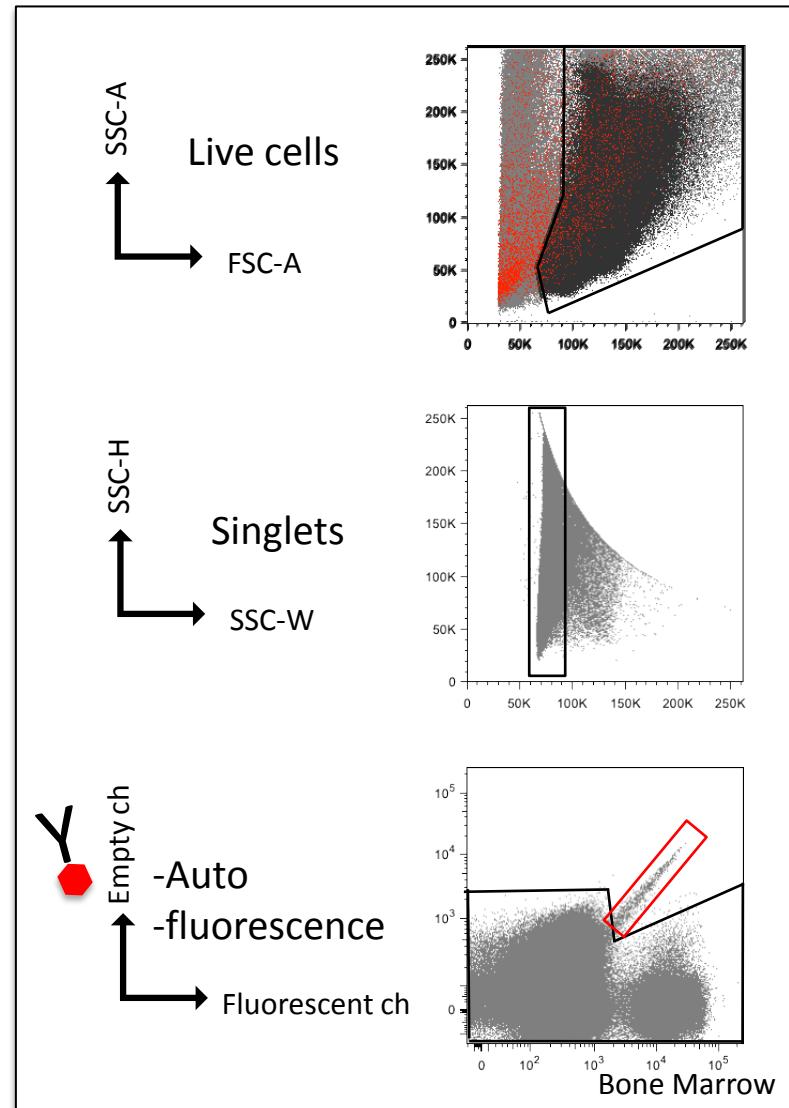
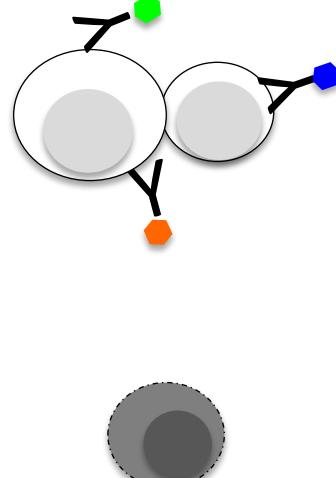
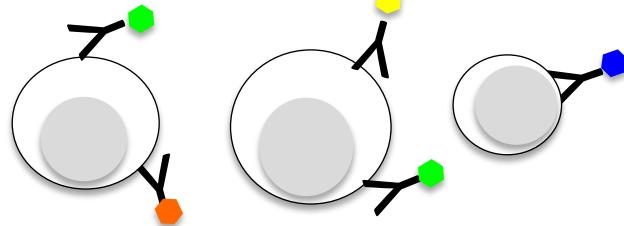


Genetically encoded fluorescent proteins report for ...

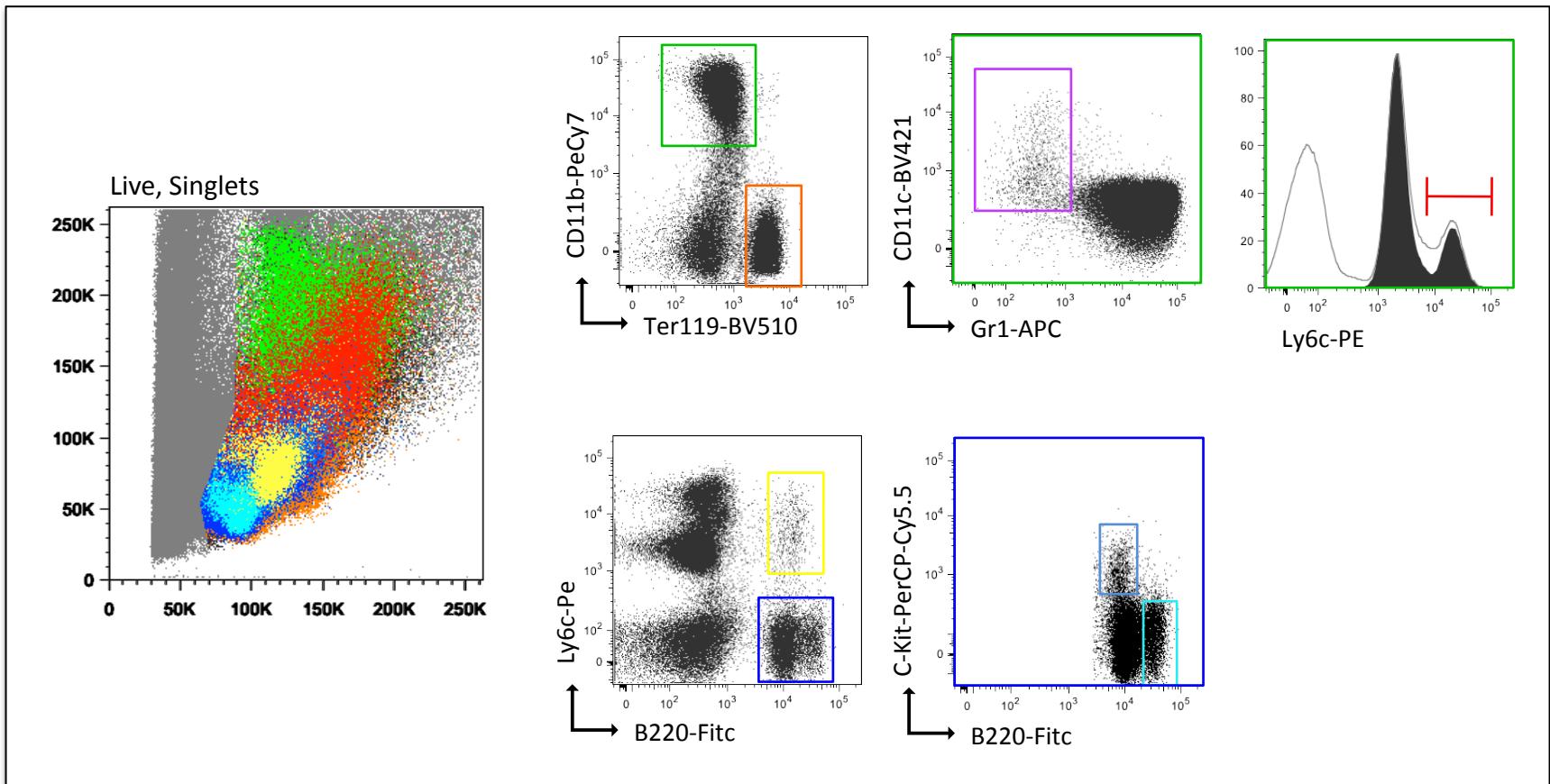
- Transfection
Infection ...
- Expression
Induction
Deletion ...
- Protein interaction (FRET, split-GFP)
- Location (Photoactivatable GFP)



Immunophenotyping by cell-specific surface staining



Immunophenotyping by cell-specific surface staining



Used by Biooptics with permission of Tanja Schwickert

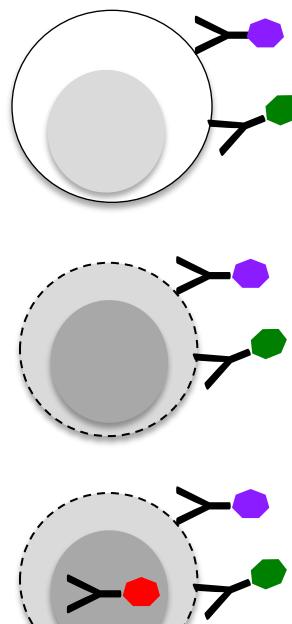
Immunophenotyping: Multi-color FACS

	Laser 1: 405nm				Laser2: 488nm		Laser3: 561nm			Laser4: 640nm	
X-color	450/50 PacBlue BV421	525/50 BV510	585/40 BV605	780/60 BV785	530/30 Fitc AF488 GFP	695/40 PerCP- Cy5.5	582/15 PE	610/20 PE- CF594	780/60 PE-Cy7	670/30 APC A647	780/60 APC-Cy7
4	X				X		X				X
5	X				X		X		X	X	
6	X			X	X		X		X	X	
7	X		X	X	X		X		X	X	
8	X		X	X	X	X	X		X	X	
9	X		X	X	X	X	X		X	X	X
10	X	X	X	X	X	X	X		X	X	X
11	X	X	X	X	X	X	X	X	X	X	X

Laser & filter settings: Fortessa

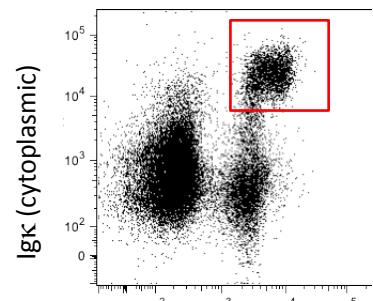
Phenotyping and functionality by cytoplasmic-, nuclear staining

- Staining of surface antigen
- Fixation
- Permeabilization
- Staining of cytoplasmic & nuclear antigens

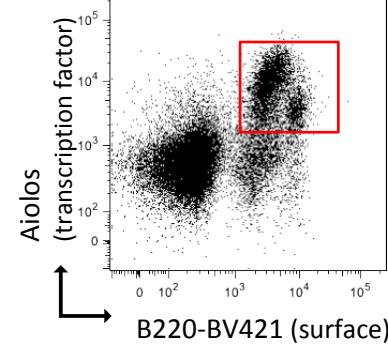


Intracellular stainings

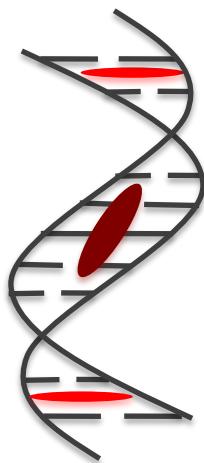
Cytoplasmic



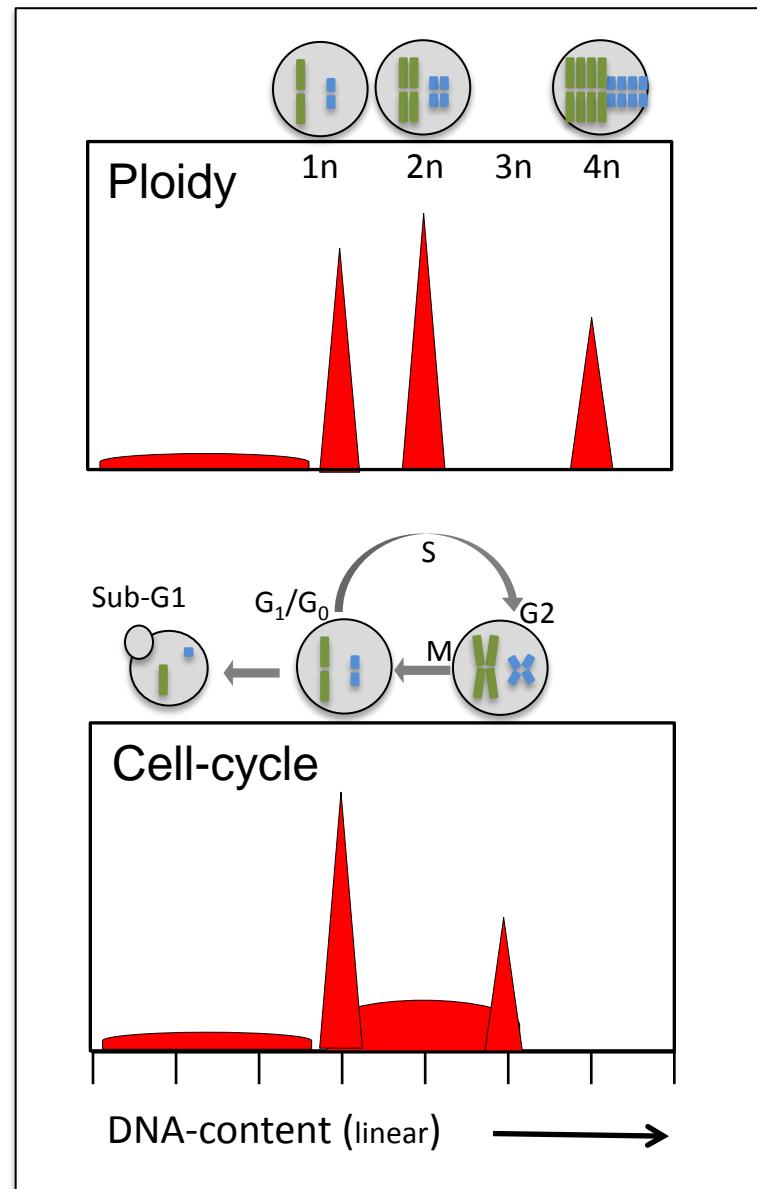
Nuclear



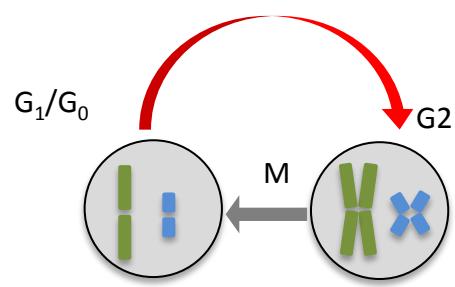
DNA-content



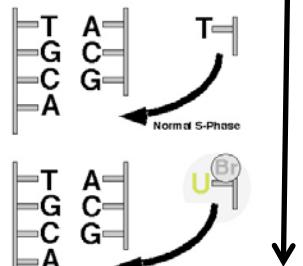
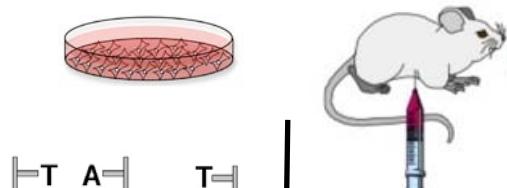
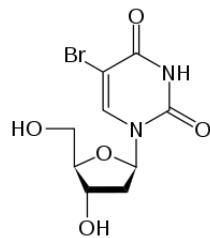
dsDNA dye	Excitation Laser [nm]	Emission [nm]	Notes
PI Propidium iodide	488/561	620	dsDNA/RNA Fix/Perm
7-AAD 7-Aminoactinomycin D	488/561	645	dsDNA Fix/Perm
DAPI	350/375	460	dsDNA Fix/Perm
Hoechst 33342	350/375	460	dsDNA Cell- membrane permeable



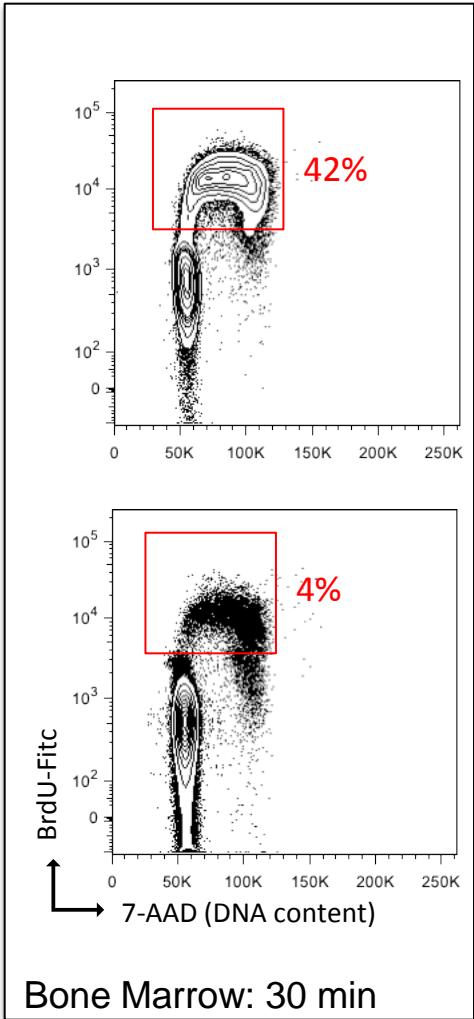
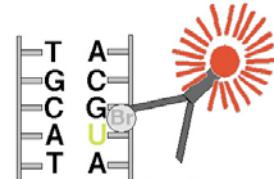
S-phase analysis by BrdU uptake



- In vitro/ in vivo Bromodeoxyuridine (BrdU) incorporation



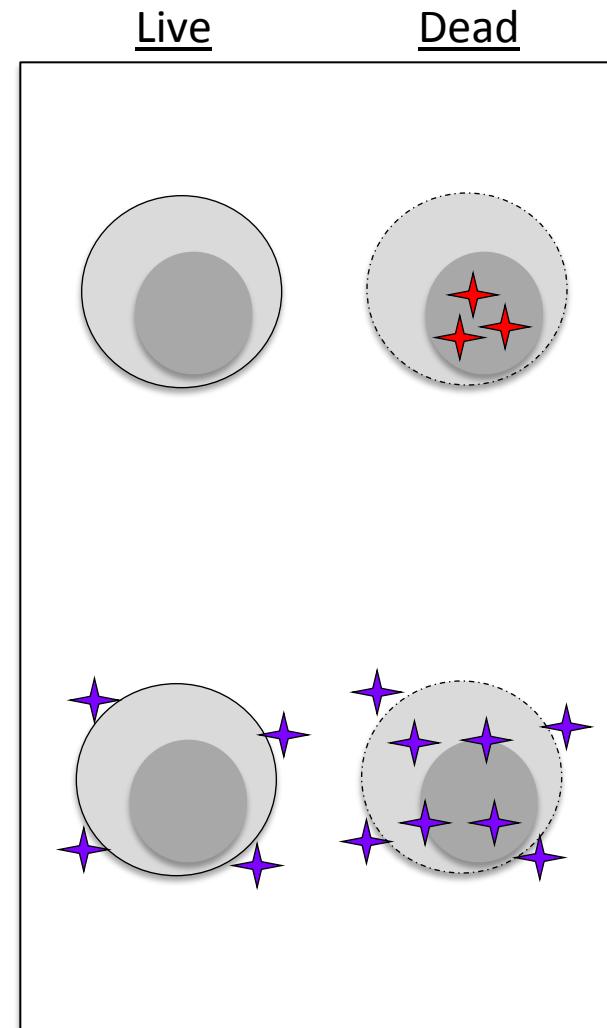
- Harvest cells/organ
- Staining of surface antigen
- Fixation/Permeabilization
- DNase-treatment
- Staining of BrdU and total DNA



(alternative: Click-it EdU assay)

Live/dead discrimination with impermeable dyes

DNA dye	Excitation [nm]	Notes
PI Propidium iodide	488/561	
7-AAD 7-Aminoactinomycin D	488/561	Non-fixable
DAPI	350	

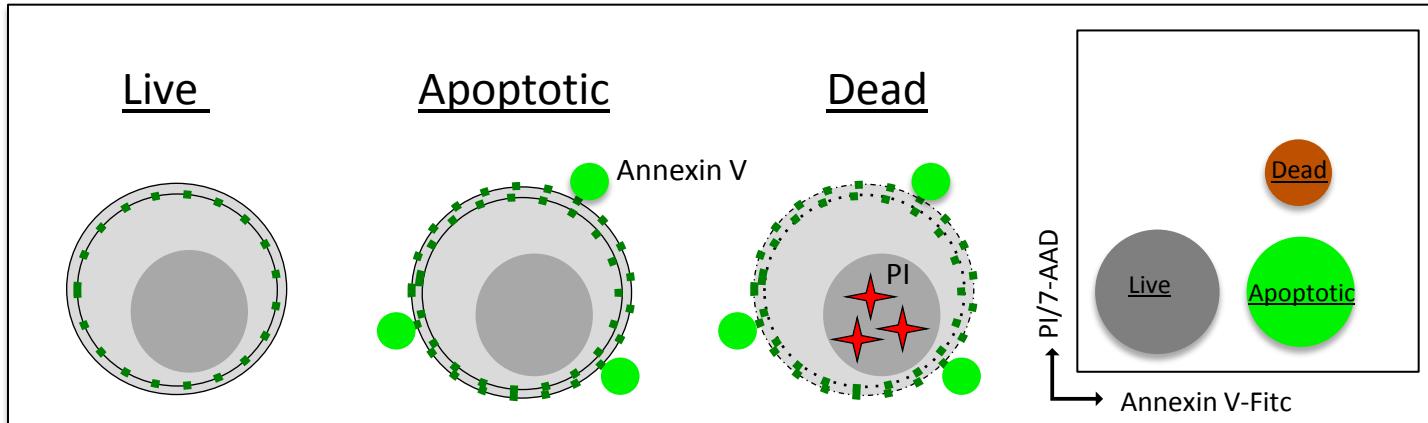


Protein dye	Excitation [nm]	Notes
LIVE/DEAD Fixable (Molecular Probes)	350/405/488/561/640	
Fixable viability dye eFluor (eBioscience)	350/405/488/640	Fixable
Zombie (BioLegend)	350/405/640	

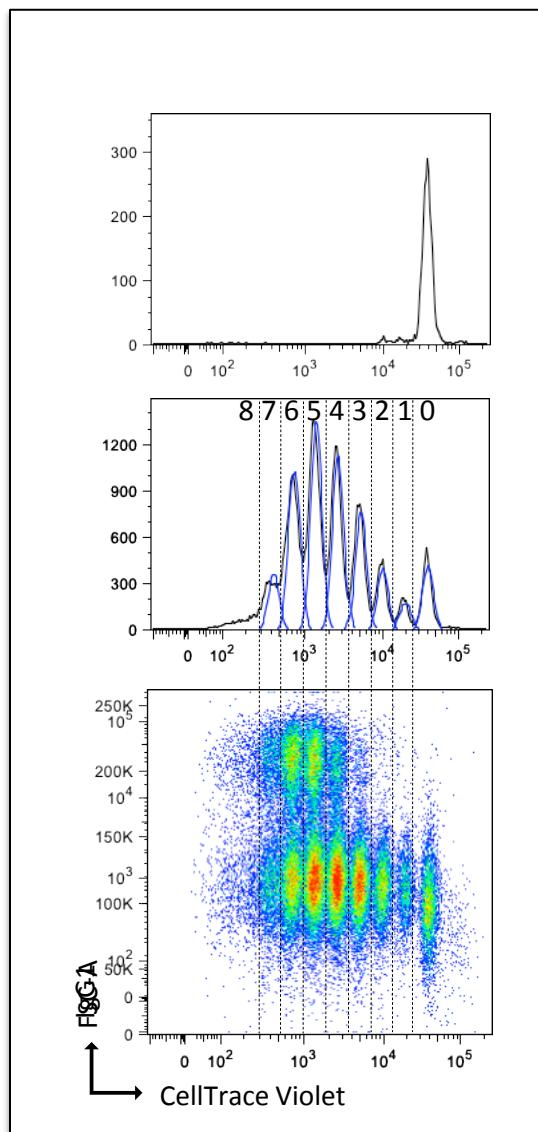
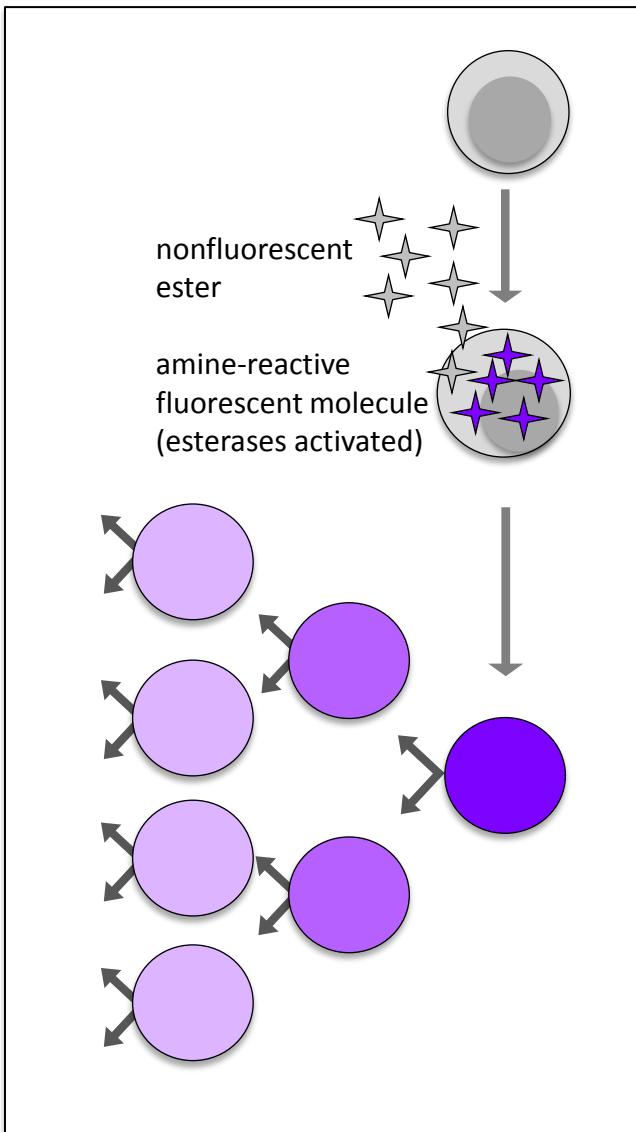
...and many more

Apoptosis

Staining/dye	method	note
Annexin 5	Phosphatidylserine (PS) translocation to outer membrane	No Fix/Perm Ca ²⁺ buffer
dsDNA dye: PI, 7-AAD, DAPI	Membrane impermeable DNA-fragmentation (Sub-G1)	No Fix/Perm Fix/Perm
Anti-pro-apoptotic molecules	Active form of caspase-3 Cleaved PARP	Fix/Perm
JC-1	Mitochondrial Membrane Potential, Emission: 530nm (monomer in cytoplasm, apoptotic) 590nm (aggregates in mitochondria, live)	No Fix/Perm ratio metric



Proliferation



Cell proliferation dyes	Excitation [nm]
CFSE	488
CellTrace Violet (Molecular Probes)	405
eFluor 450/670 (eBioscience)	405/640
VPD450 (BD)	405

...and many more

Intracellular signaling: Ca^{2+} -flux

Calcium sensor dye	Excitation	Emission
eFluor 514	490nm	514nm
Indo-1	346nm	410 nm (Ca^{2+}) 485nm
Flura-2	300nm (Ca^{2+}) 400nm	510nm

- Loading of cells with calcium sensor dye
- Incubation at 37°C
- Acquisition & Stimulation at 37°C
Fortessa I (water bath)

