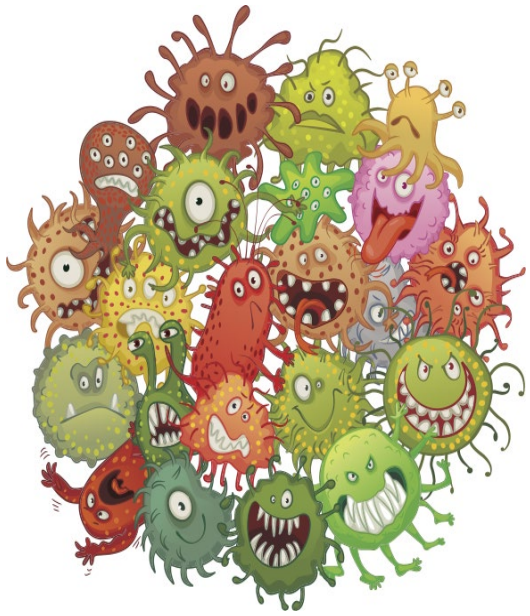
Flowcytometry applications

Gene expression/
Fluorescent
proteins

Immune
Phenotyping



Activity / metabolism dyes:

- Cell proliferation: CFSE
- Cell Cycle: PI
- Apoptosis: Annexin
- Mitoprobes
- ROS-assay (oxidative stress)
- Ca-flux: FuraRed
- Caspase-activity: FLICA

Phosphorylation

Cytokine expression

Cell isolation (sorting)

Flowcytometry applications

Cell viability
(PI, 7-AAD,
Amine dyes)

Proliferation
(CFSE, CytoTRACK dyes,
BrdU, EdU, Ki-67)

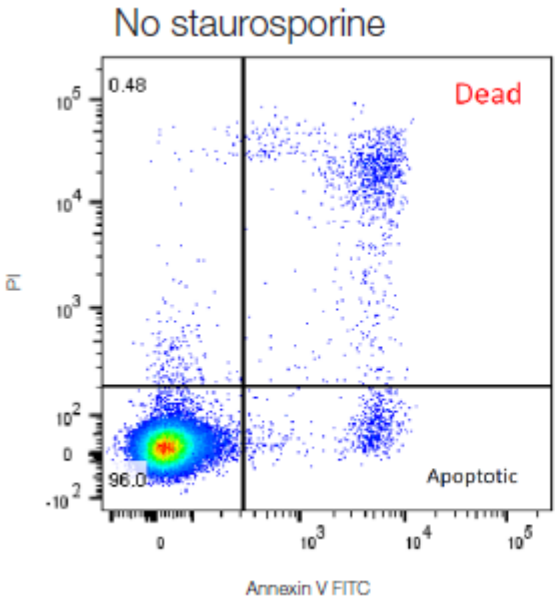
Apoptose
(AnxV,
Caspase FLICA kits)

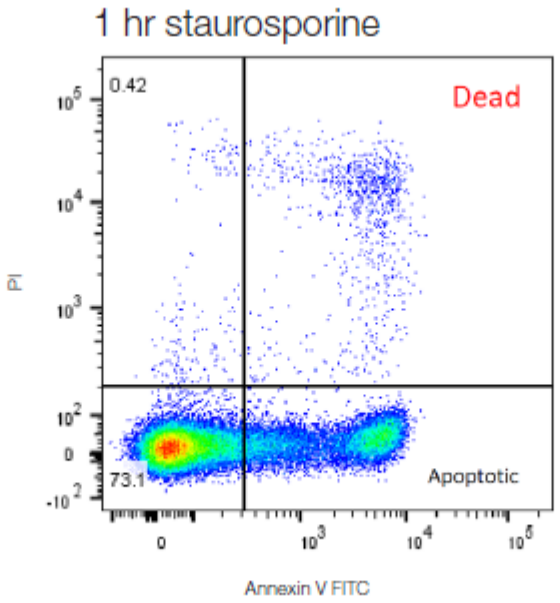
Autophagy
(Cathepsins, autophagy probe
Beclin-1, Lamp-1/2)

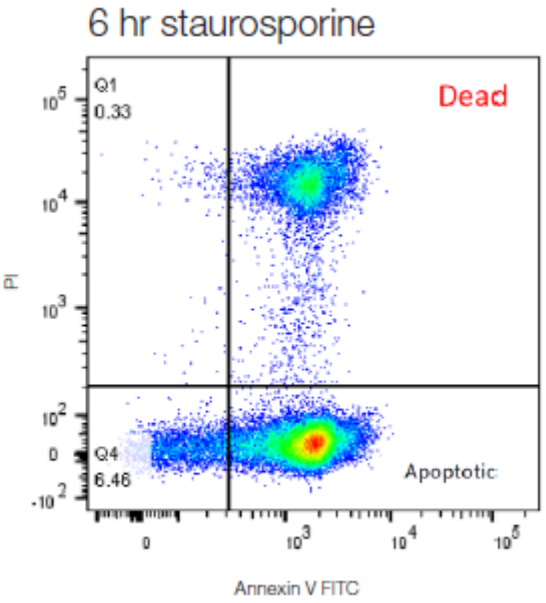
Cell Cycle
(PI)

Flowcytometry applications

Apoptosis

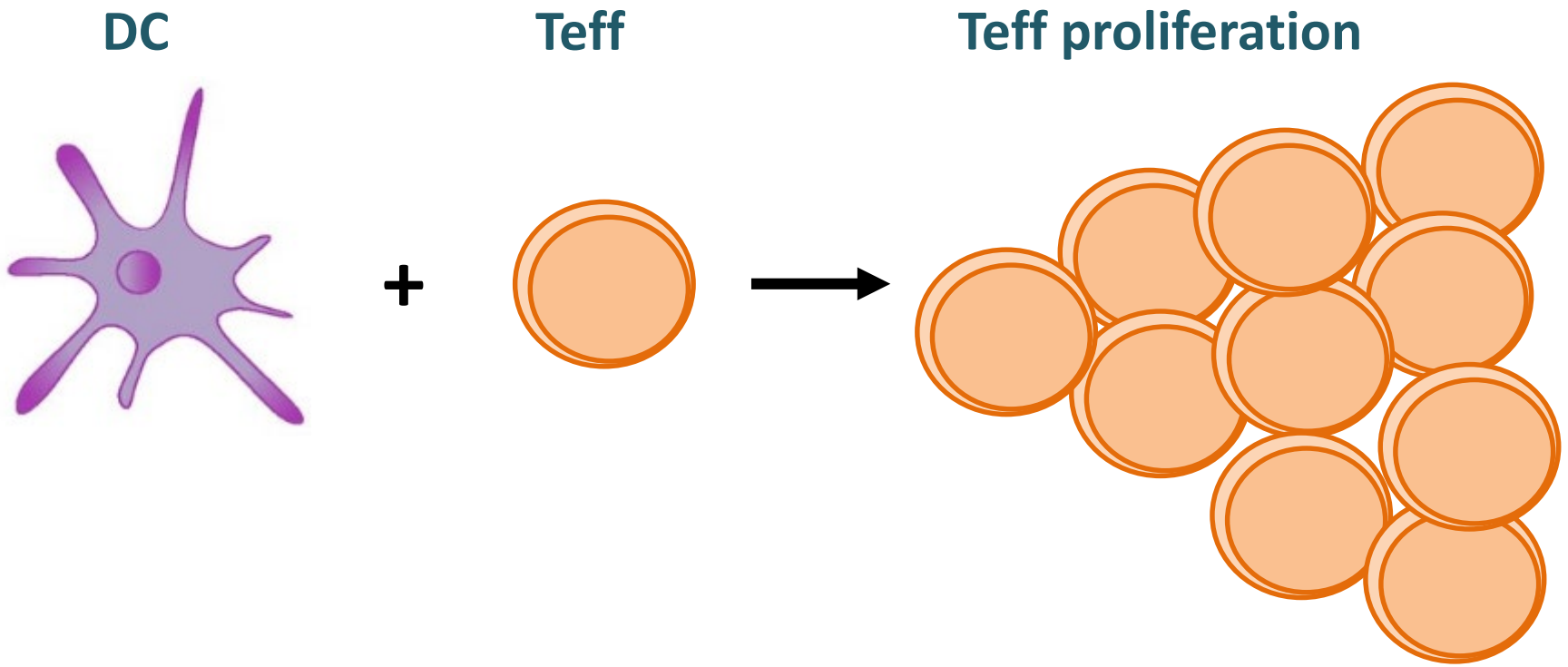




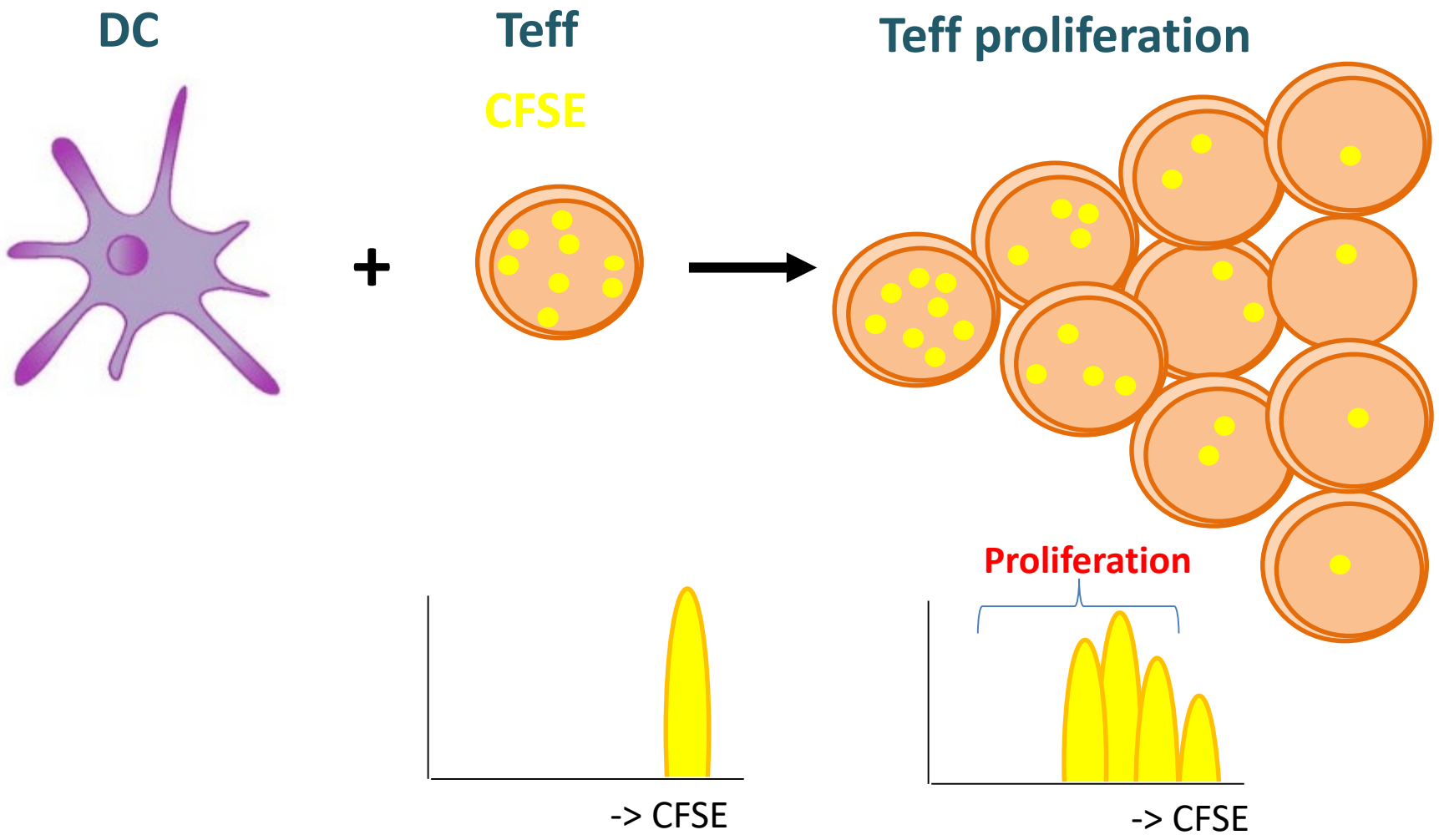


BioRad

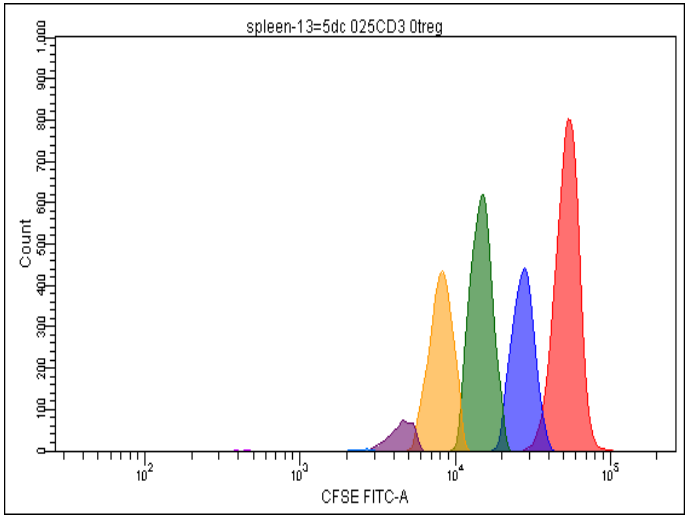
Teff-proliferation and Treg supression

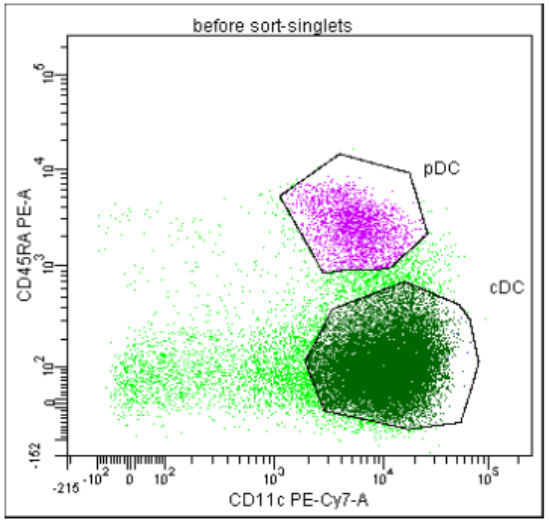


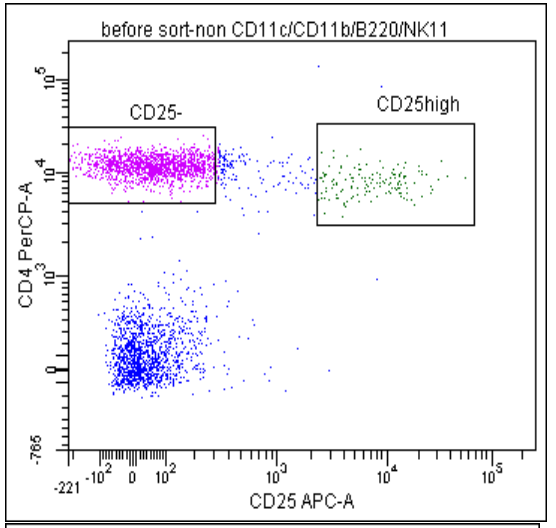
Teff-proliferation and Treg supression

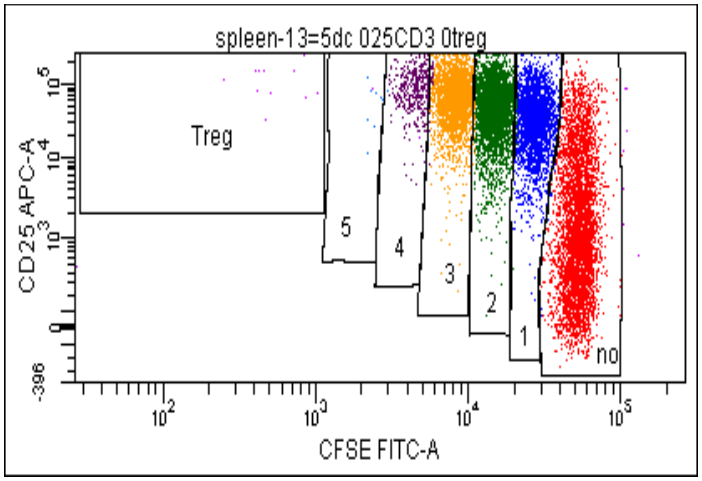


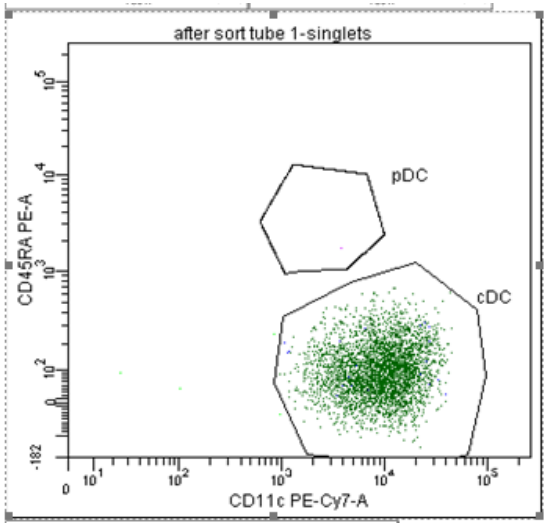
Teff-proliferation and Treg supression

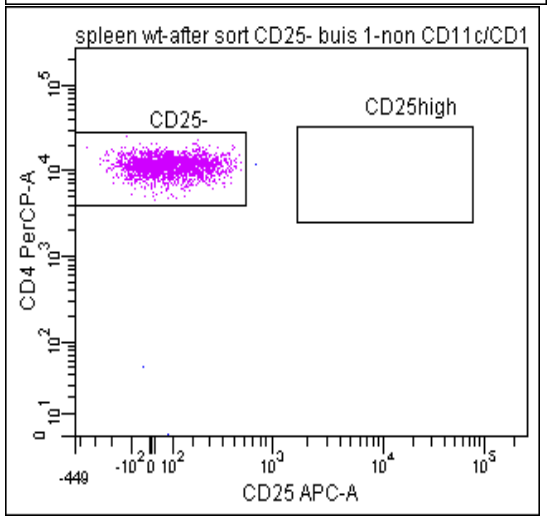




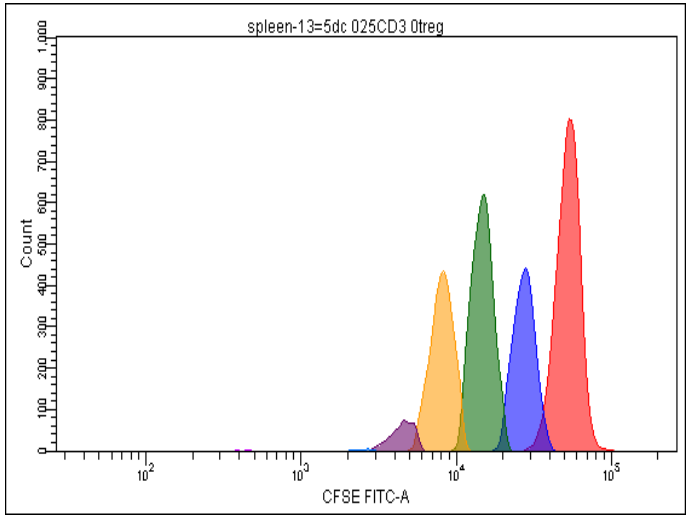


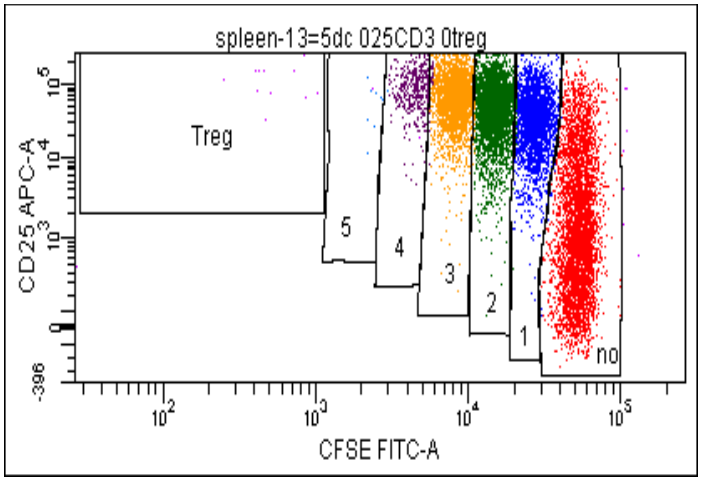


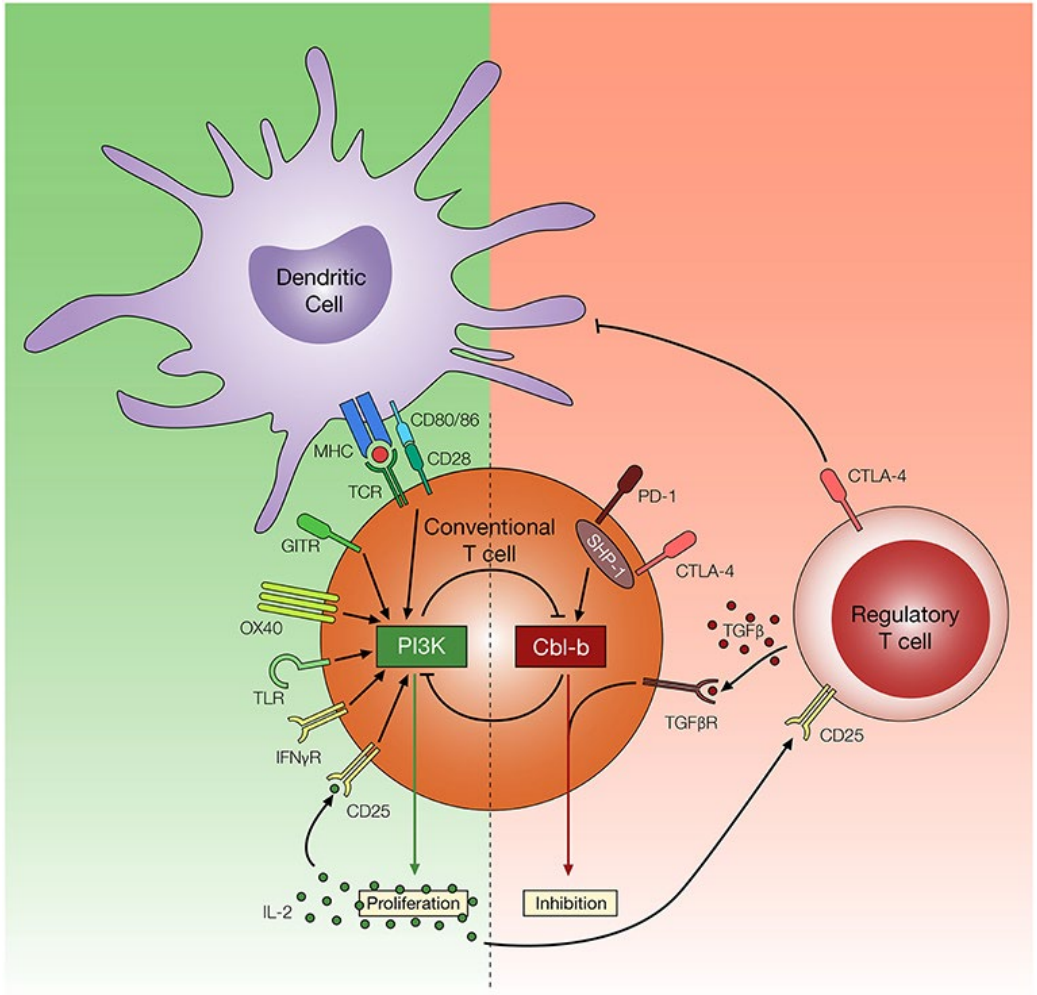




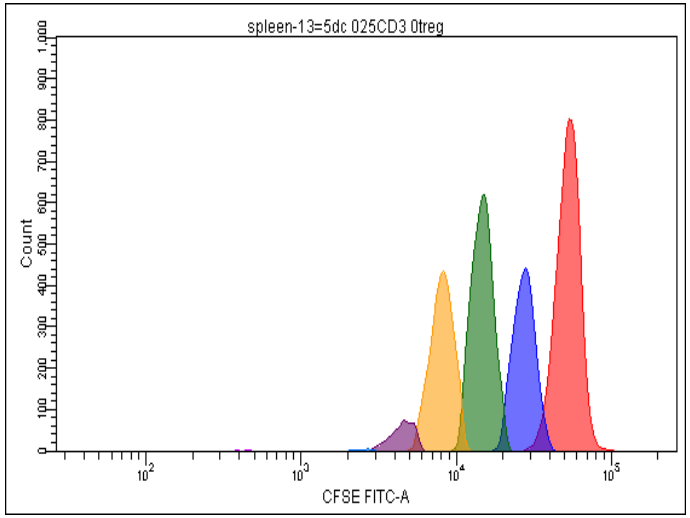
Teff-proliferation and Treg supression

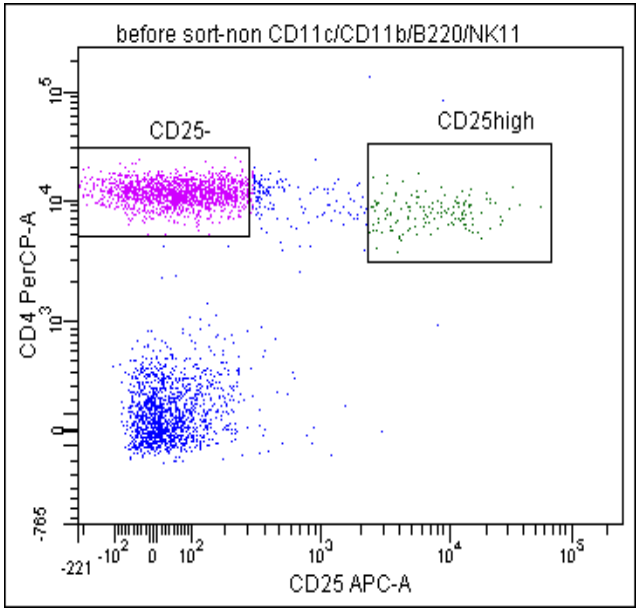


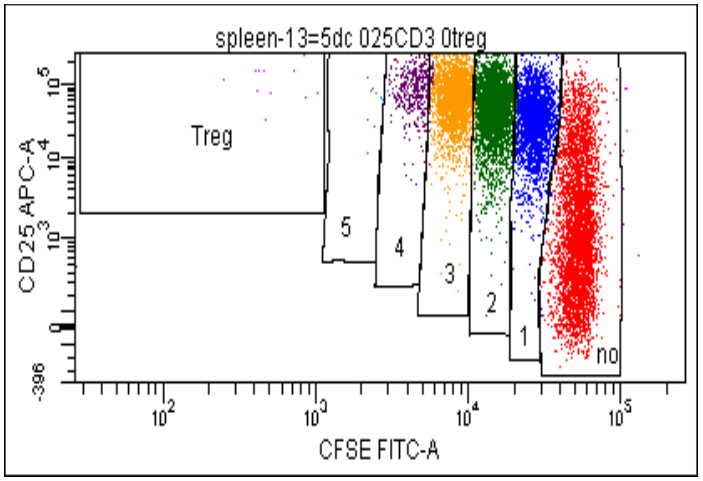


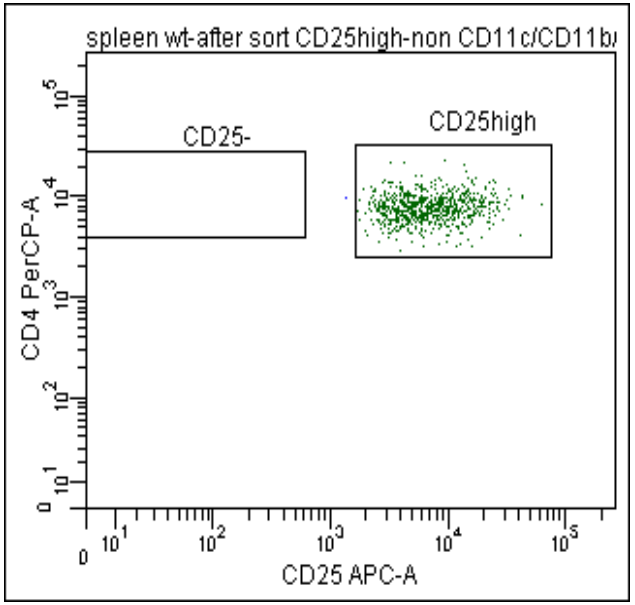


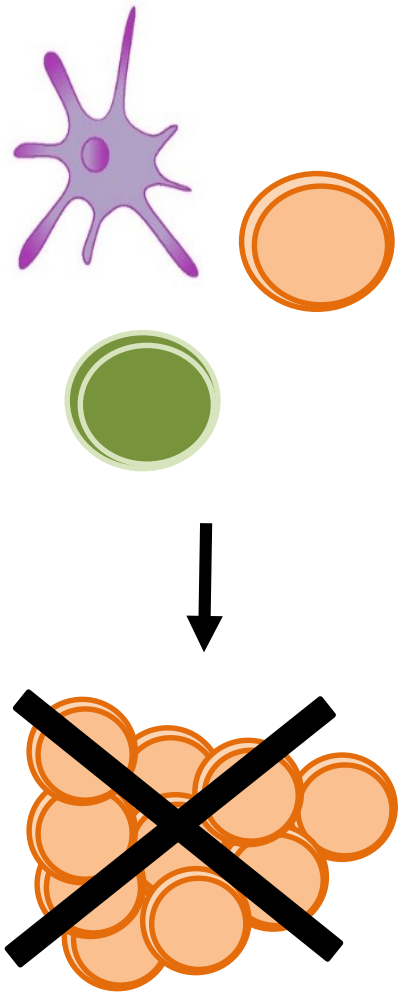
Teff-proliferation and Treg supression





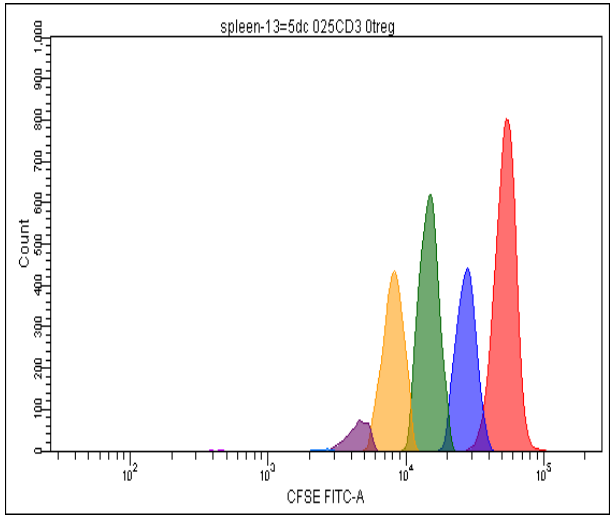


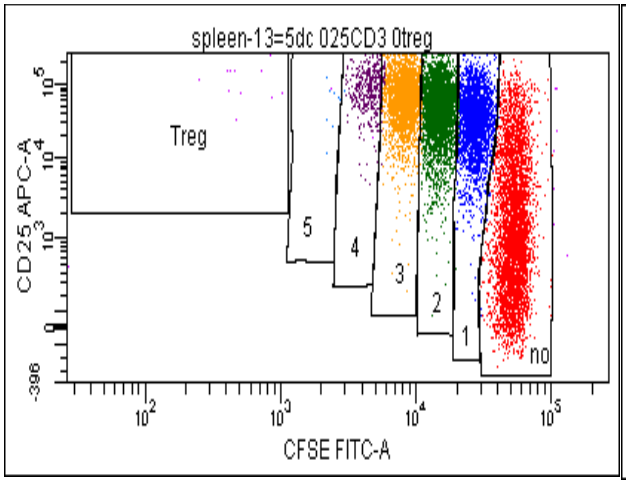




Teff-proliferation and Treg supression

Teff only





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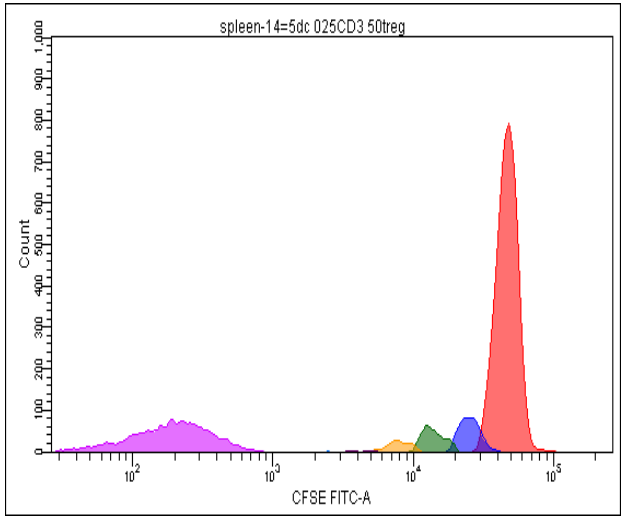

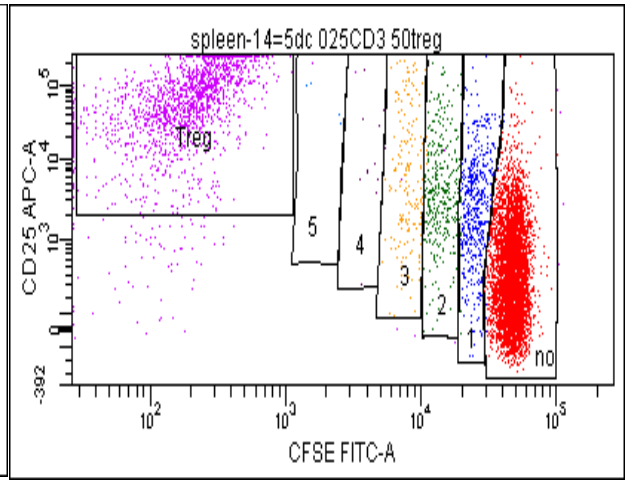

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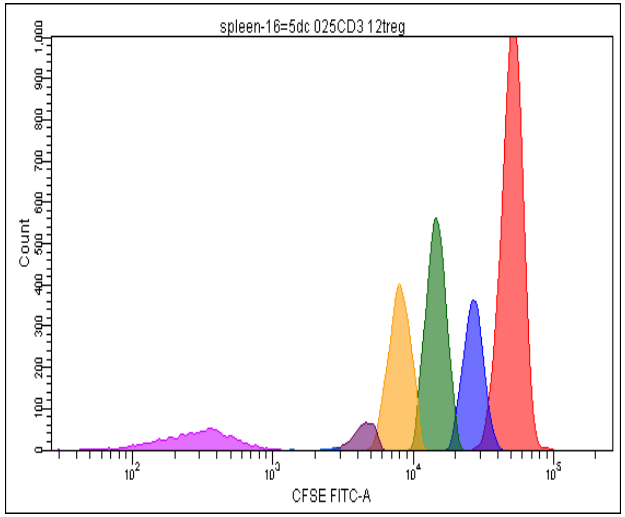
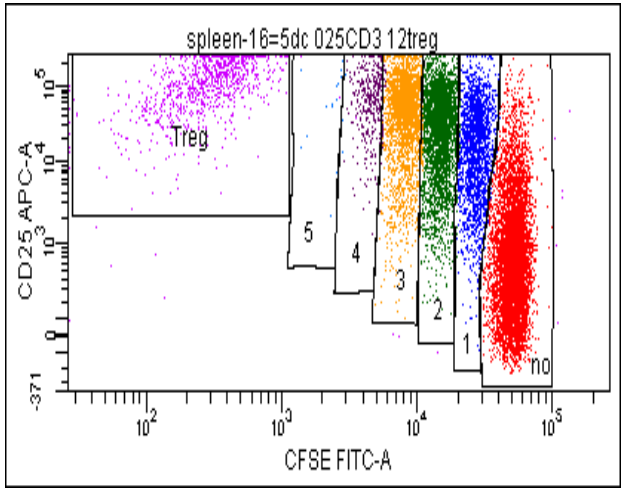
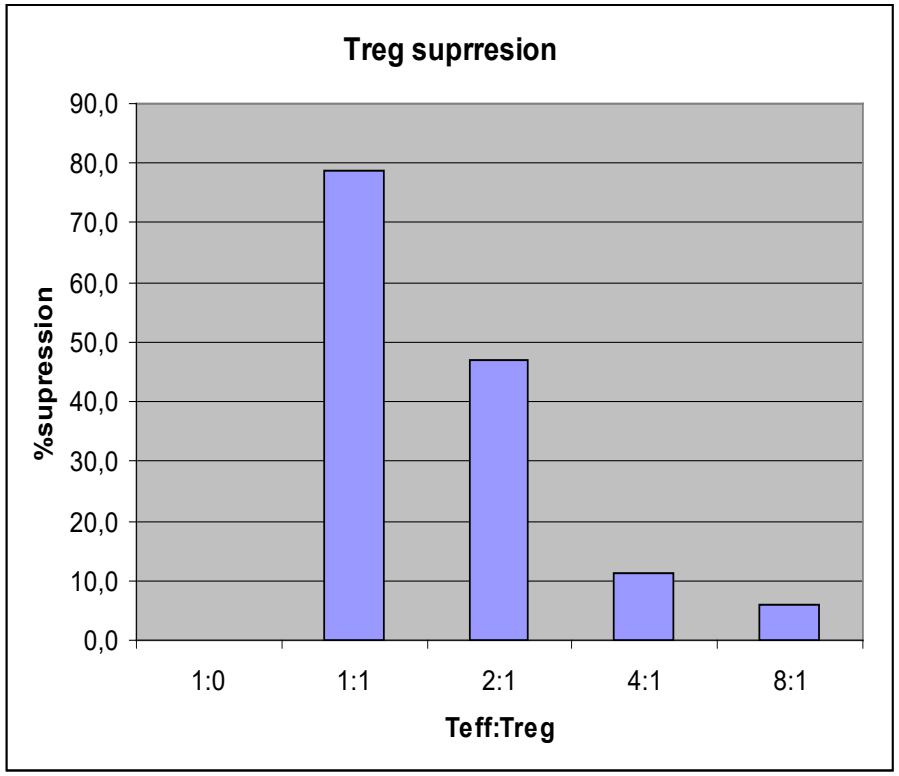
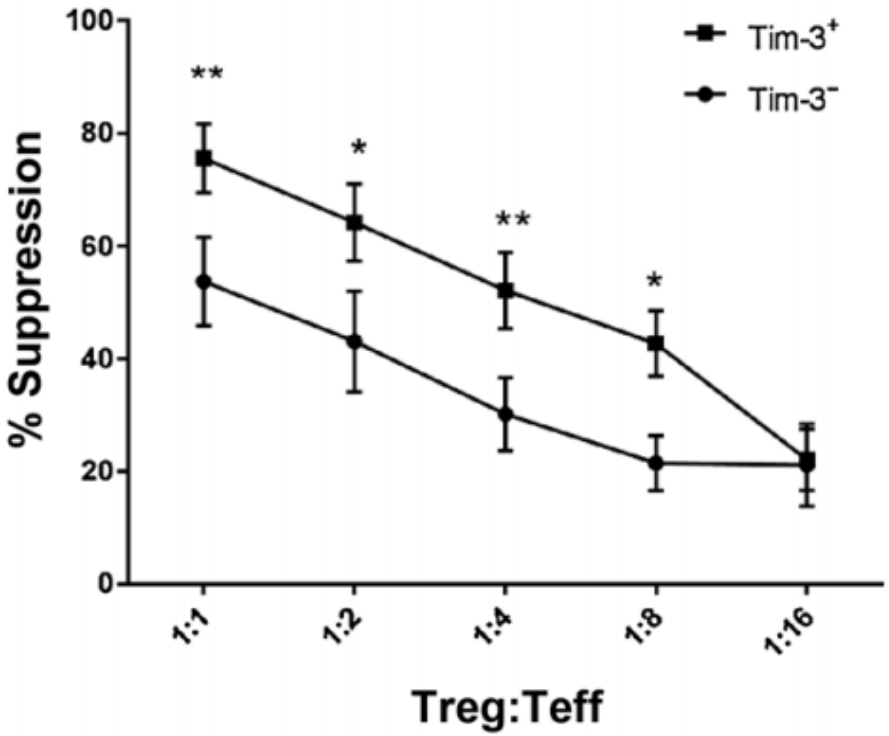




Teff-proliferation and Treg supression



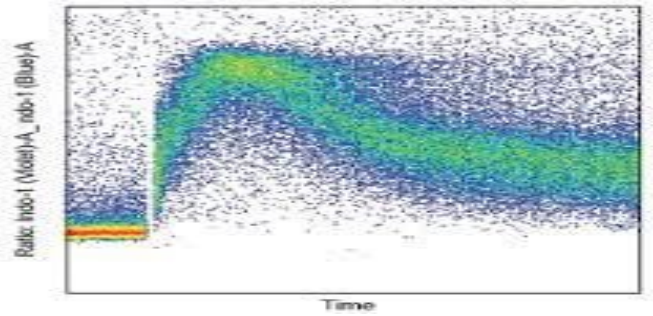


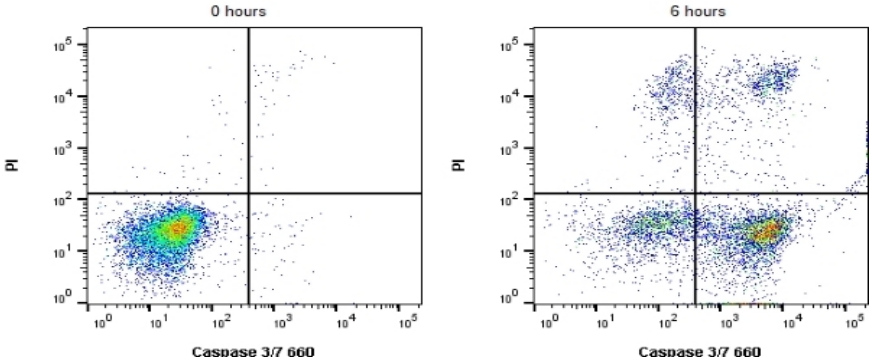
Liu et al, Clin Canc Res, 2018

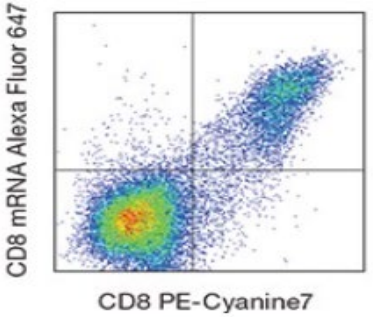
Flowcytometry applications

Activity / metabolic dyes

- Ca-flux: FuraRed / Indo-1
- Mitoprobes (Mitochondrial activity)
- Caspase-activity: FLICA
- ROS-assay (oxidative stress)
- mRNA detection

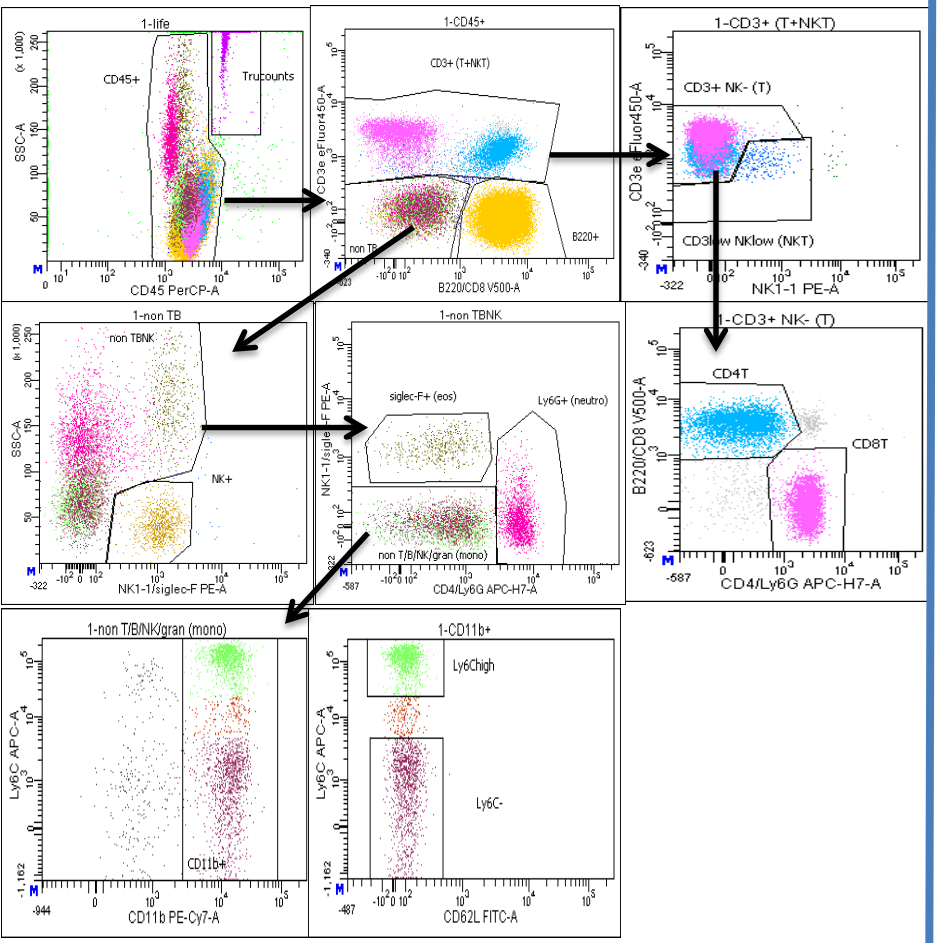




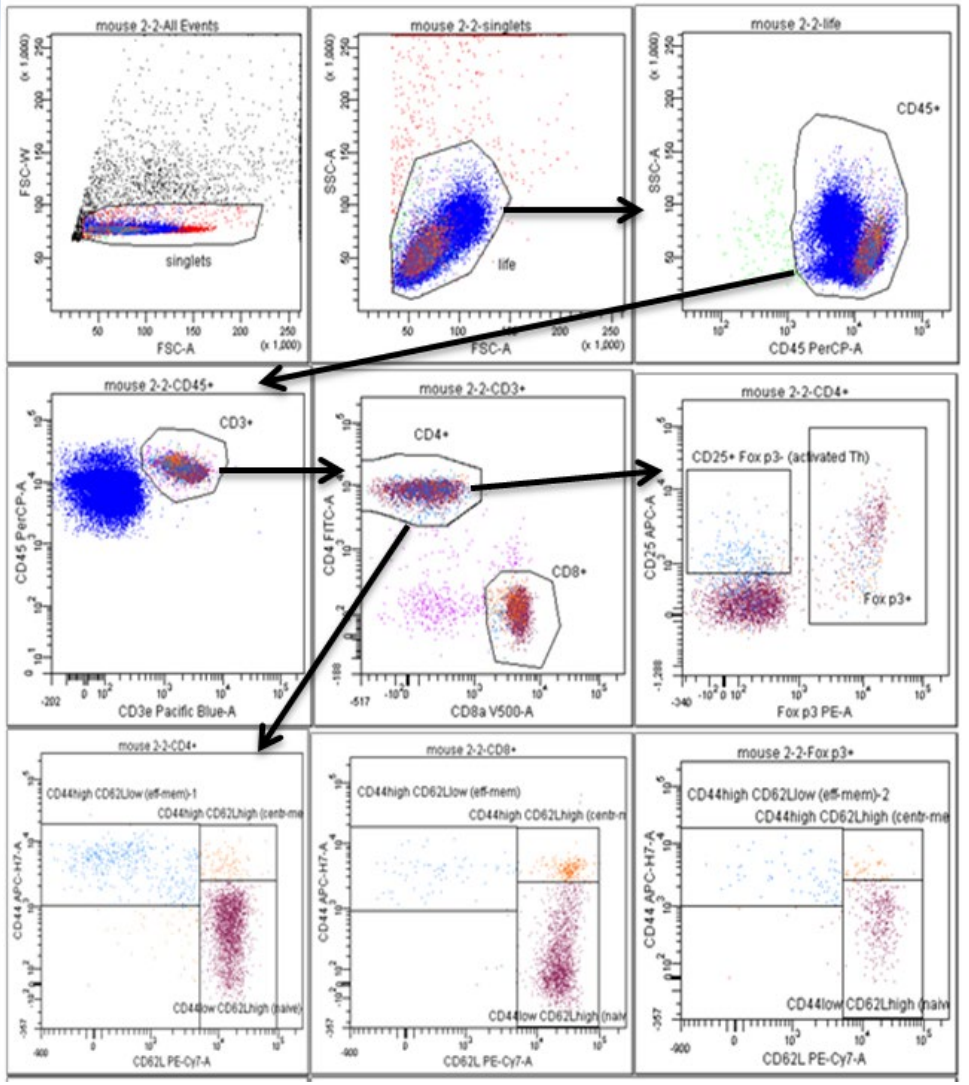


Immune phenotyping

Blood leucocytes

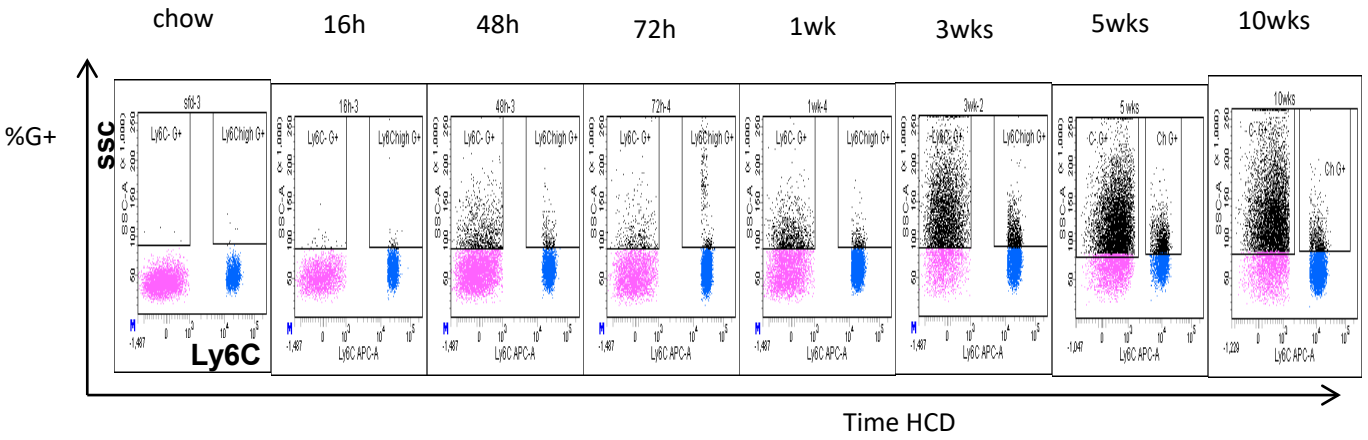


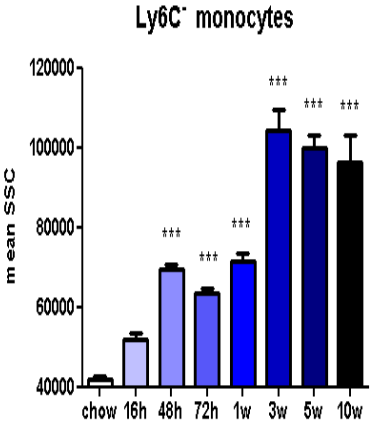
T-cells

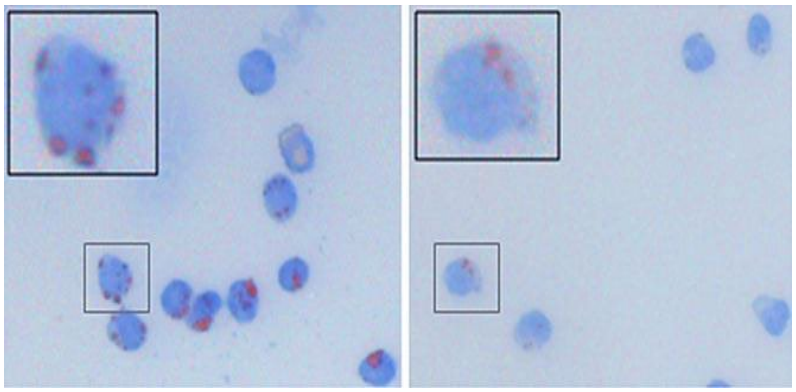


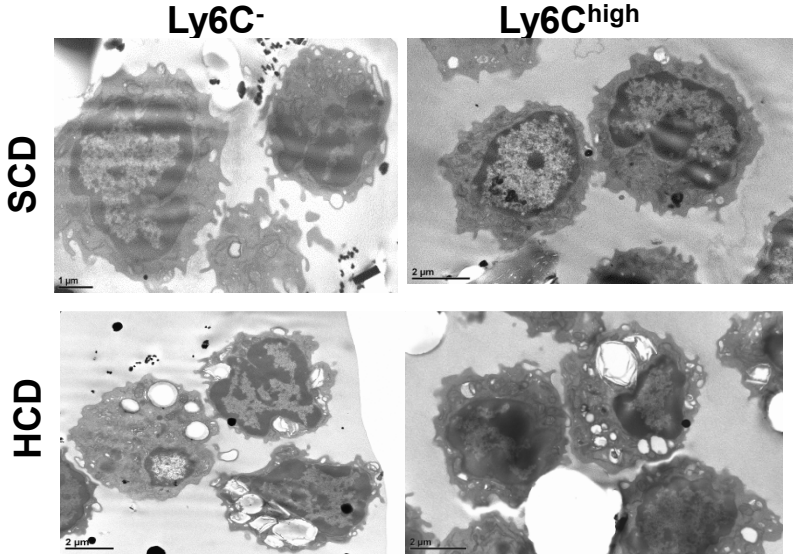
Immune phentyping

Monocytes: SSC is also a parameter

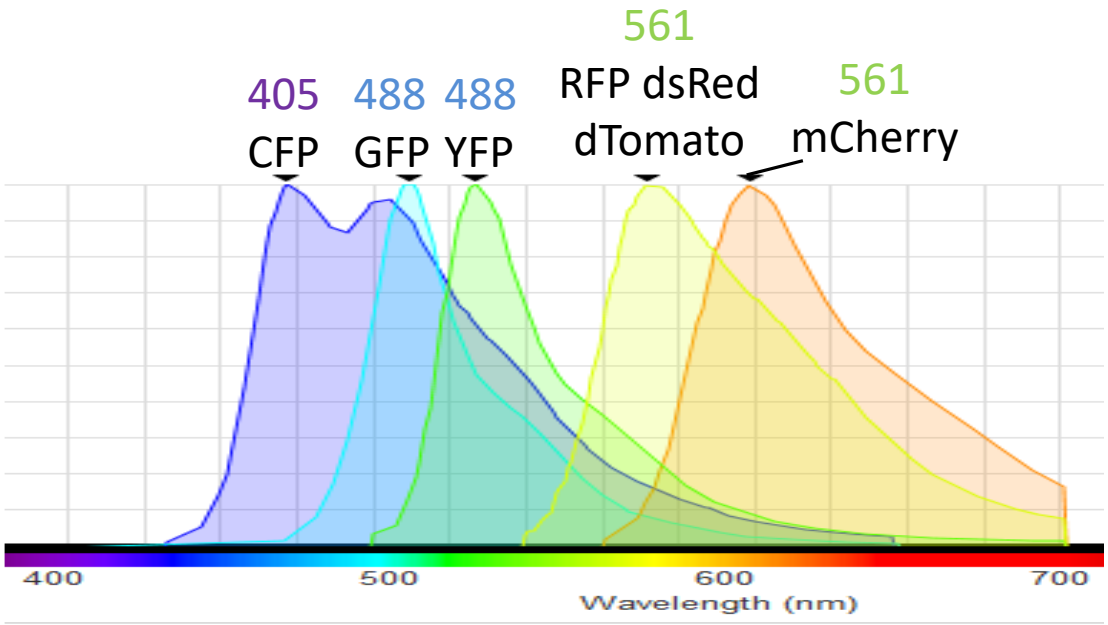




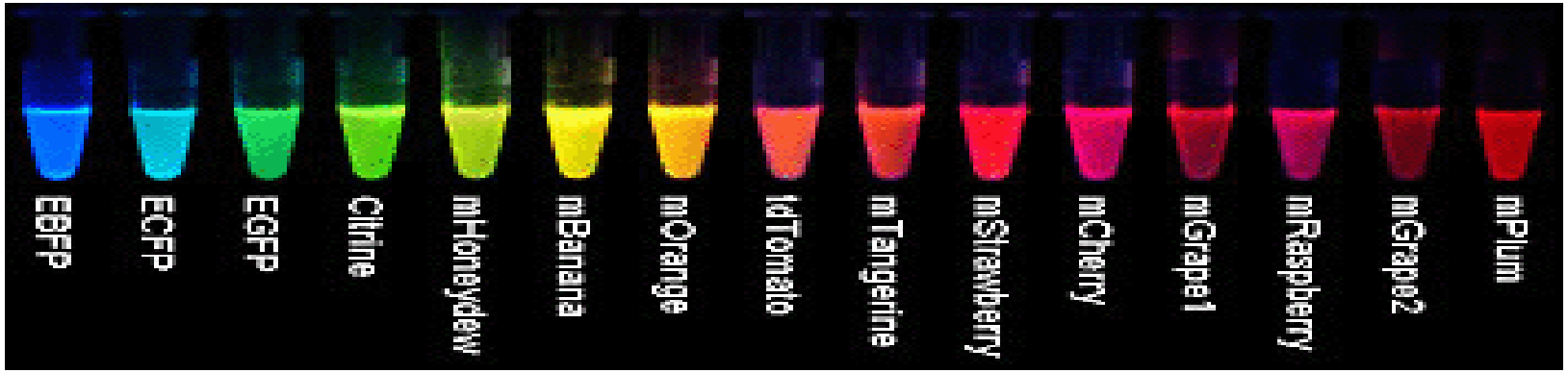




Fluorescent proteins



Extra filters-sets for CFP, GFP and YFP



Fluorescent proteins

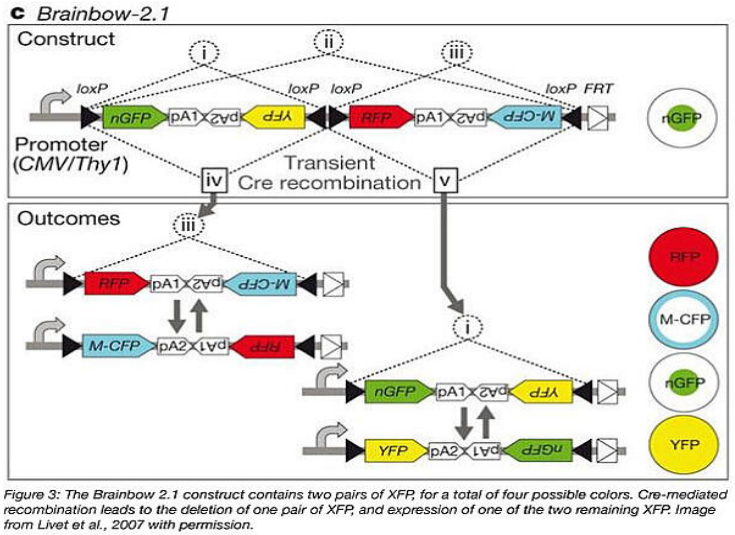
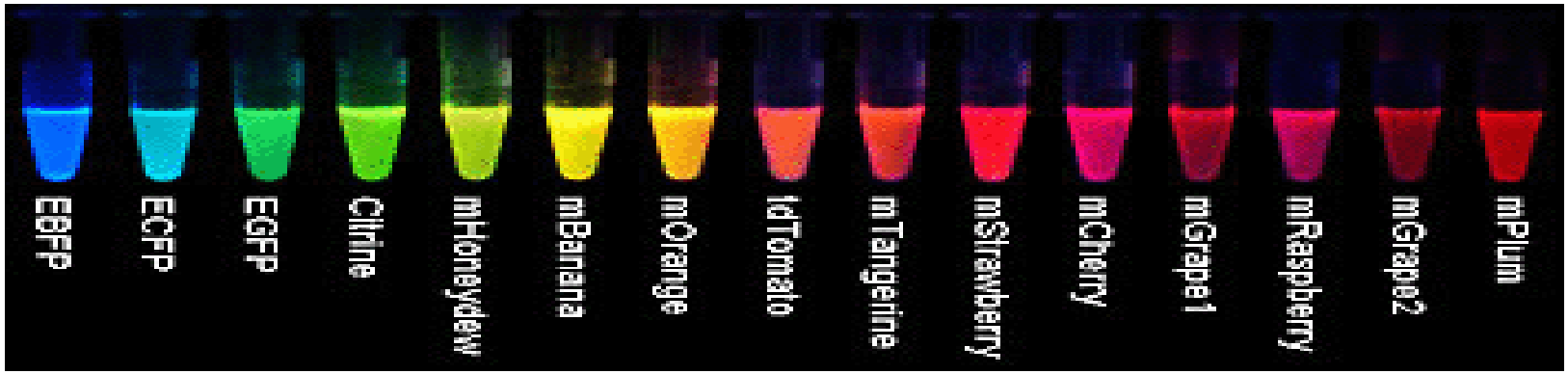


Figure 3: The Brainbow 2.1 construct contains two pairs of XFP, for a total of four possible colors. Cre-mediated recombination leads to the deletion of one pair of XFP, and expression of one of the two remaining XFP. Image from Livet et al., 2007 with permission.

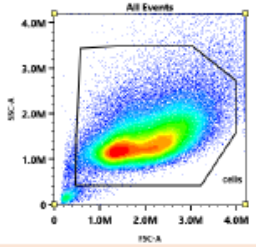
Extra filters-sets for CFP, GFP and YFP



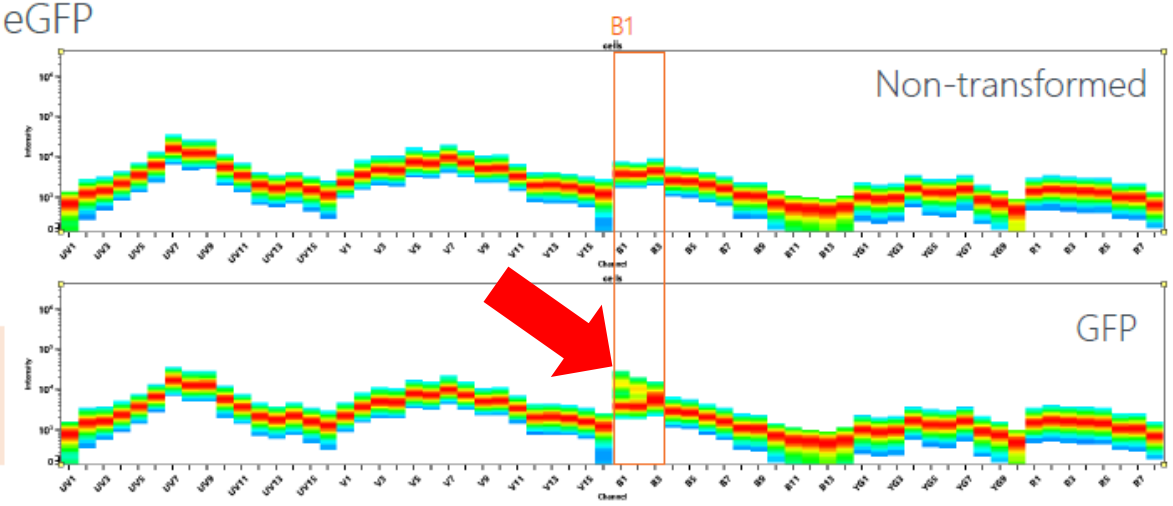
Autofluorescence extraction

Full spectrum flowcytometry: Aurora

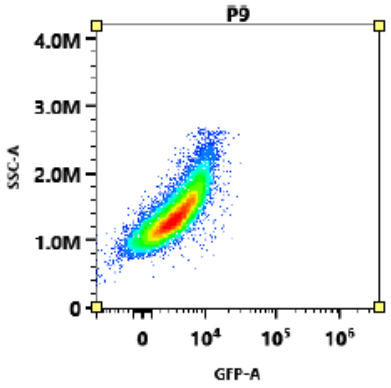
iPS cells transformed to express eGFP



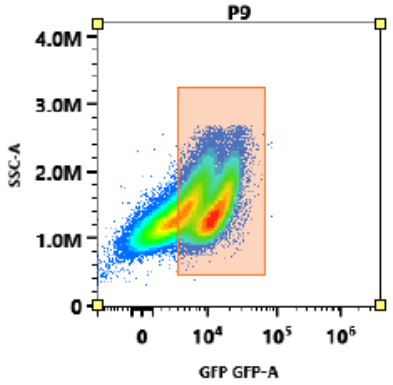
The cells have homogenous signal which indicates we are dealing with 1 AF signature



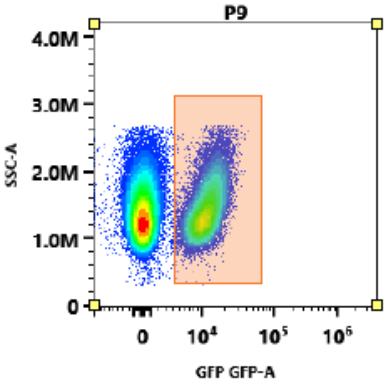
Non-transformed



Unmixing

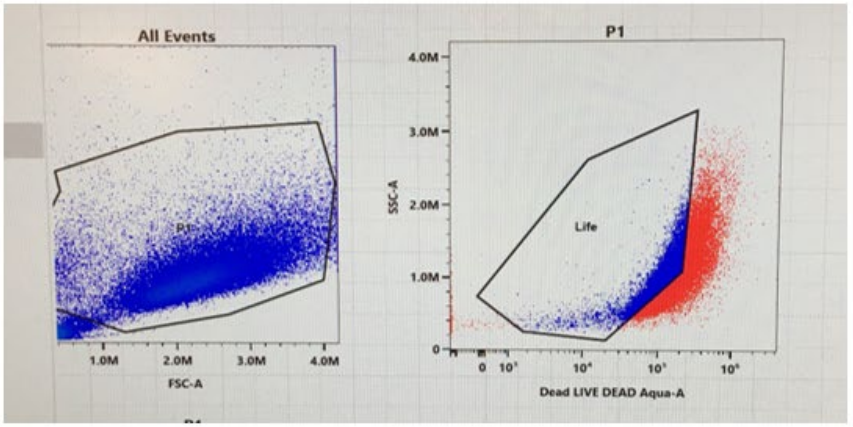


Unmixing with AF Extraction



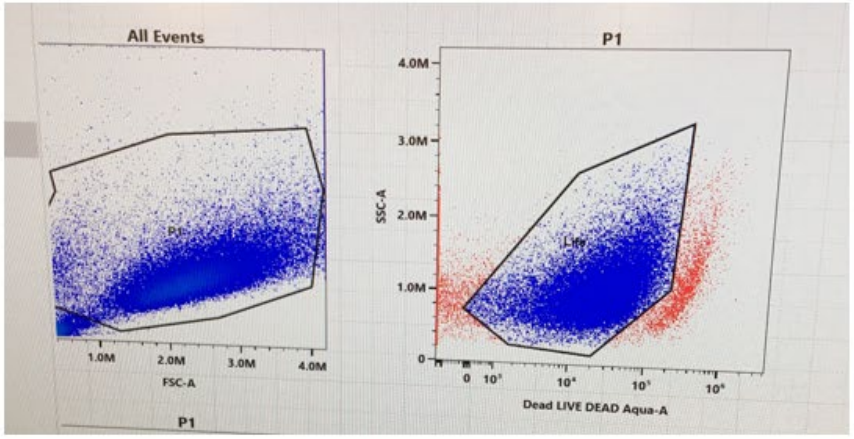
Autofluorescence extraction

Full spectrum flowcytometry: Aurora



Life-dead resolution not possible

Autofluorescence extraction

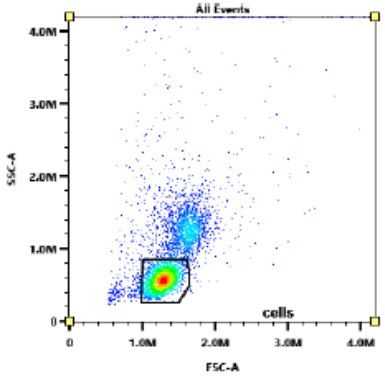


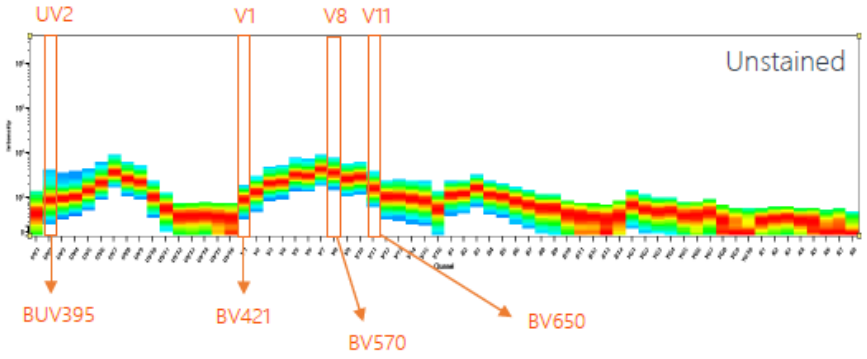
Life-dead resolution clear!

Autofluorescence extraction

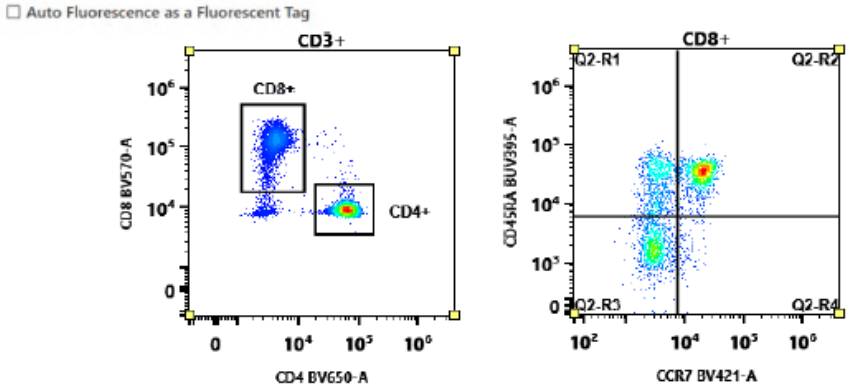
Full spectrum flowcytometry: Aurora

Human PBMCs – 18C Panel

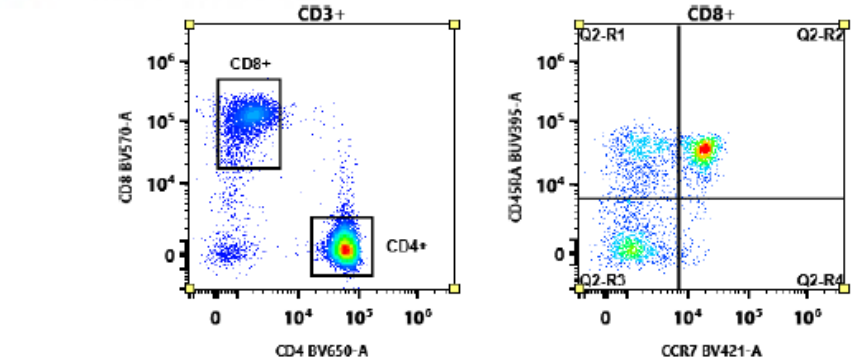




Unmixing



Unmixing with AF Extraction



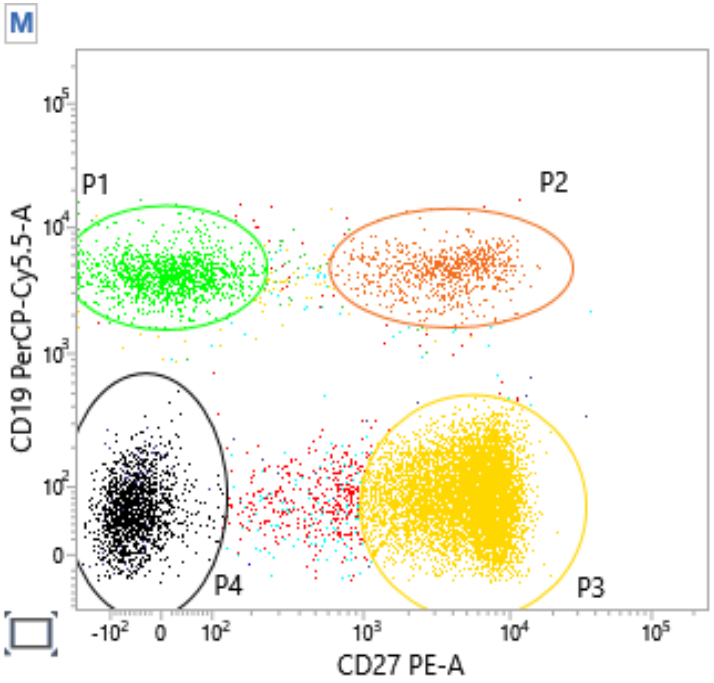
CELL SORTING

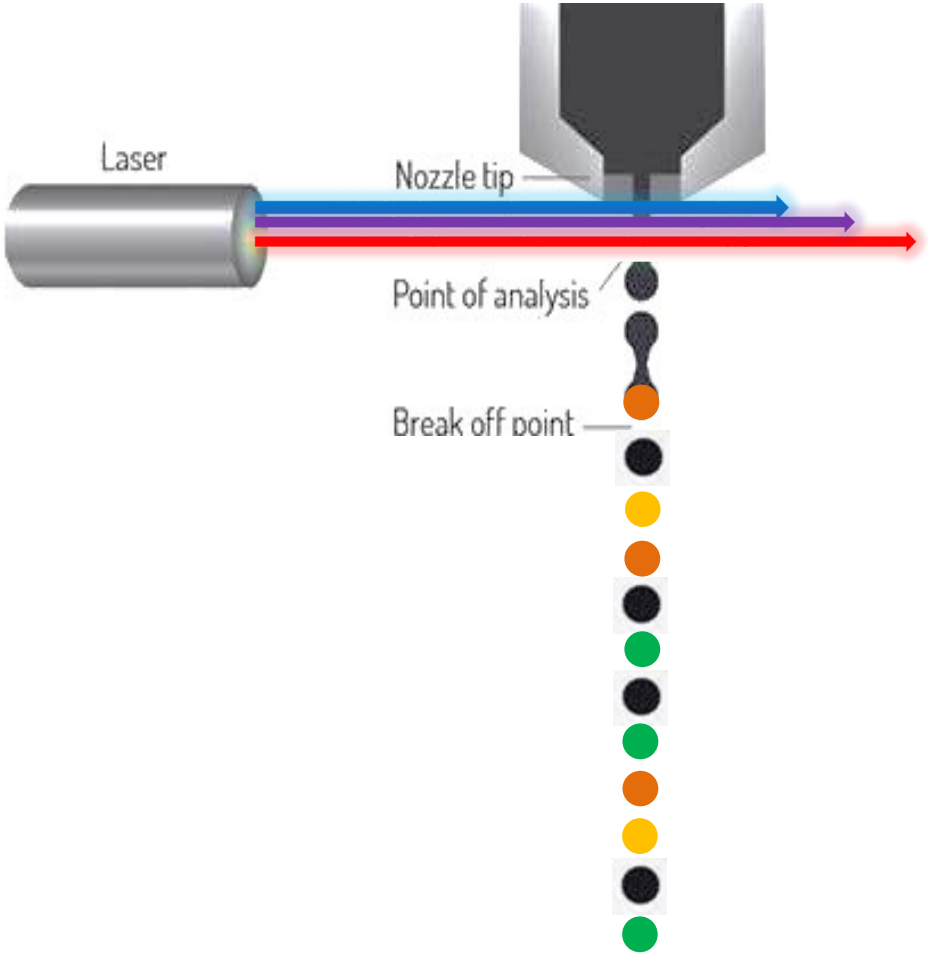
Maastricht University Medical Center

Flow Cytometry Core Facility

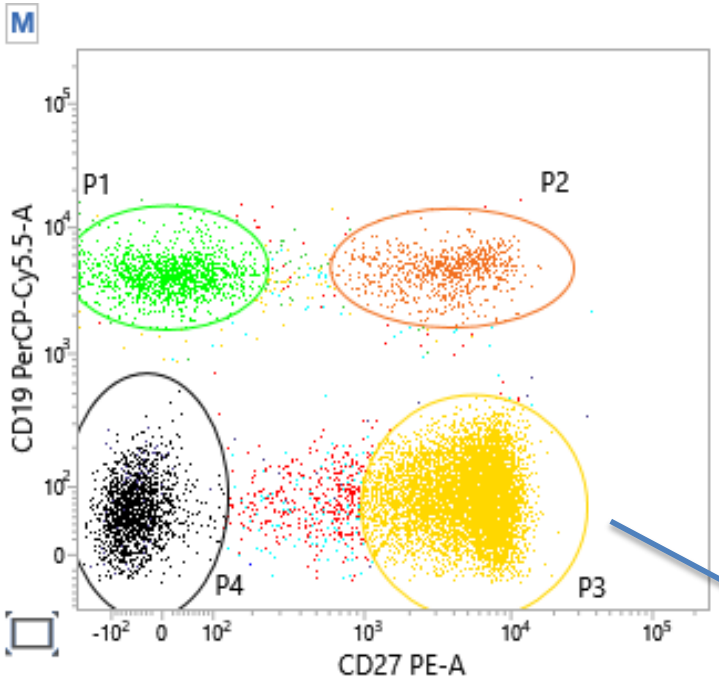
Erwin Wijnands

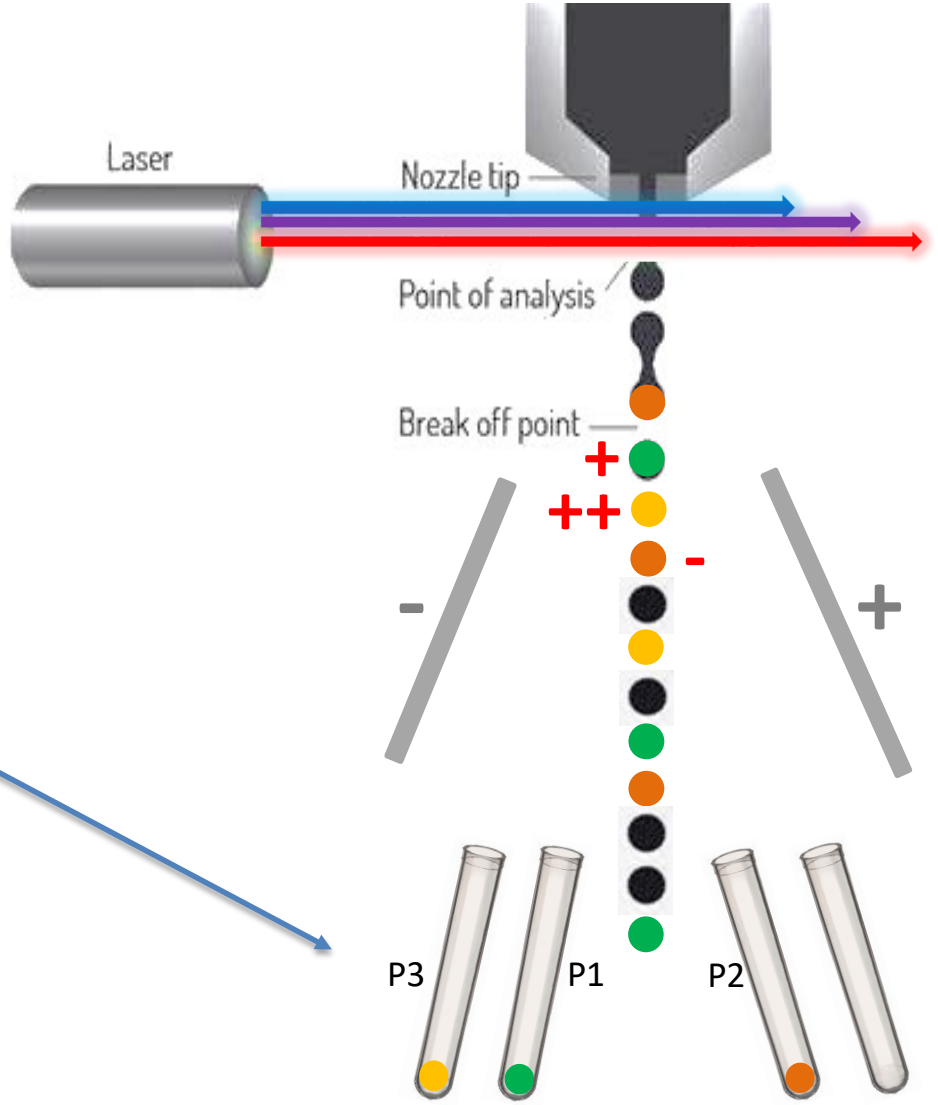
Principle of cell sorting



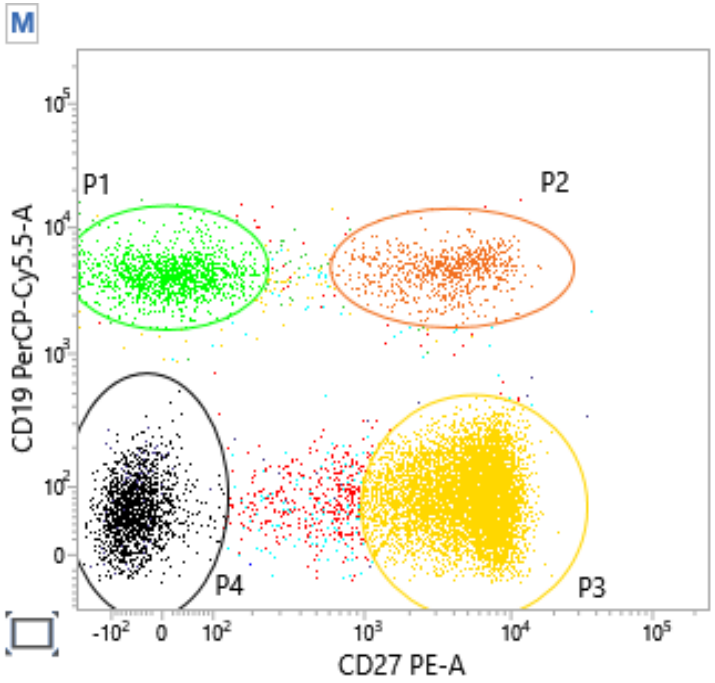


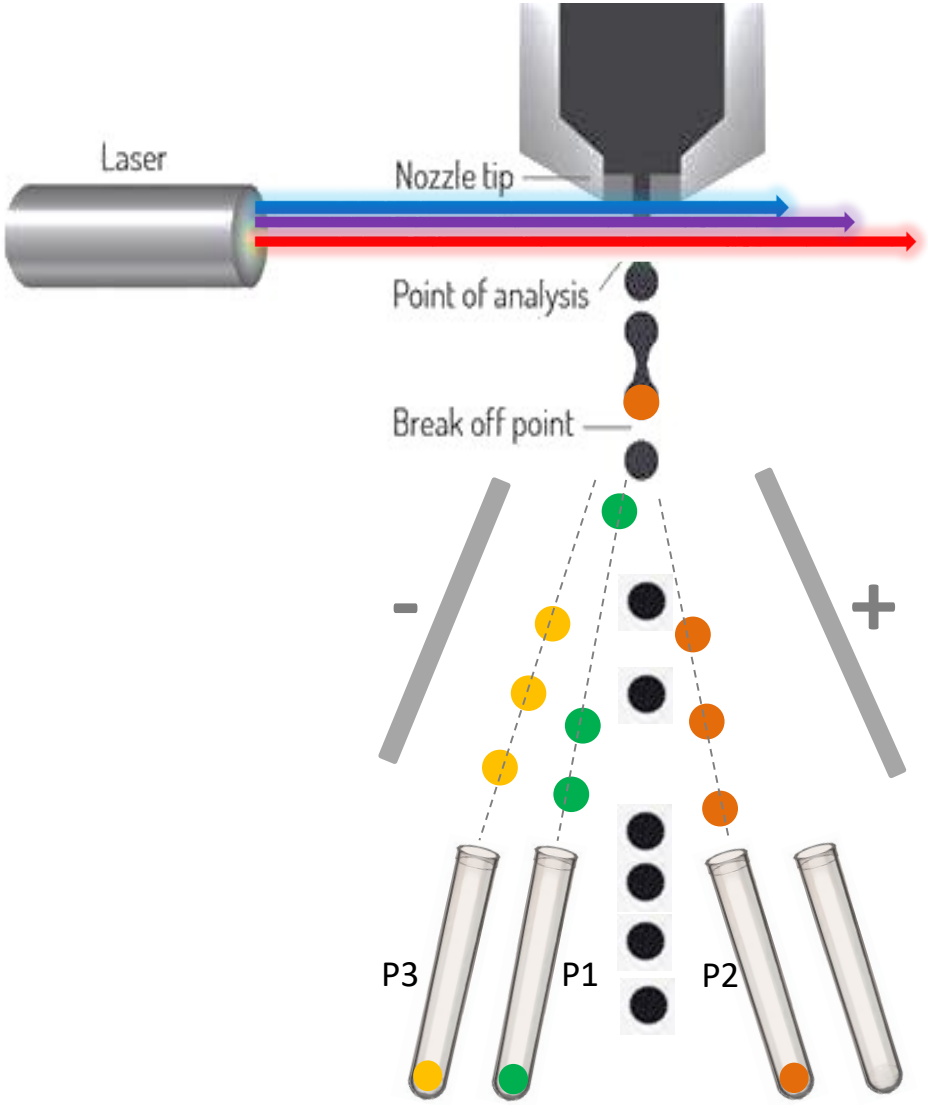
Principle of cell sorting





Principle of cell sorting





BD Fusion cell sorter

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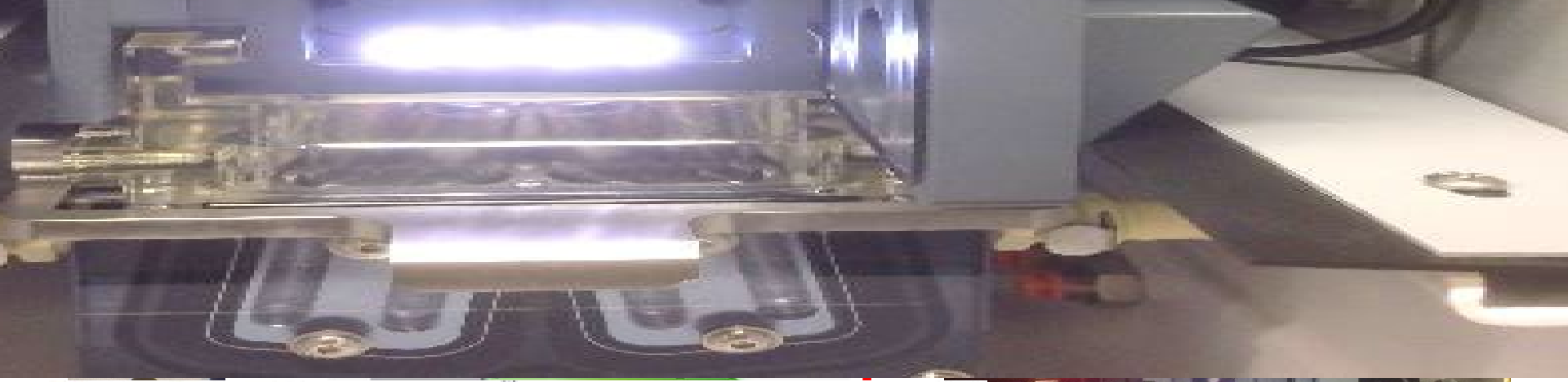
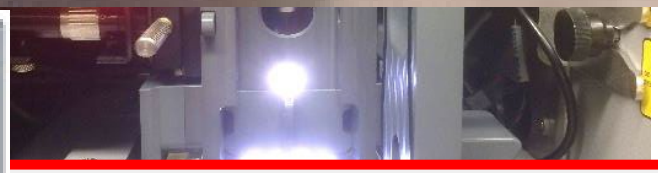
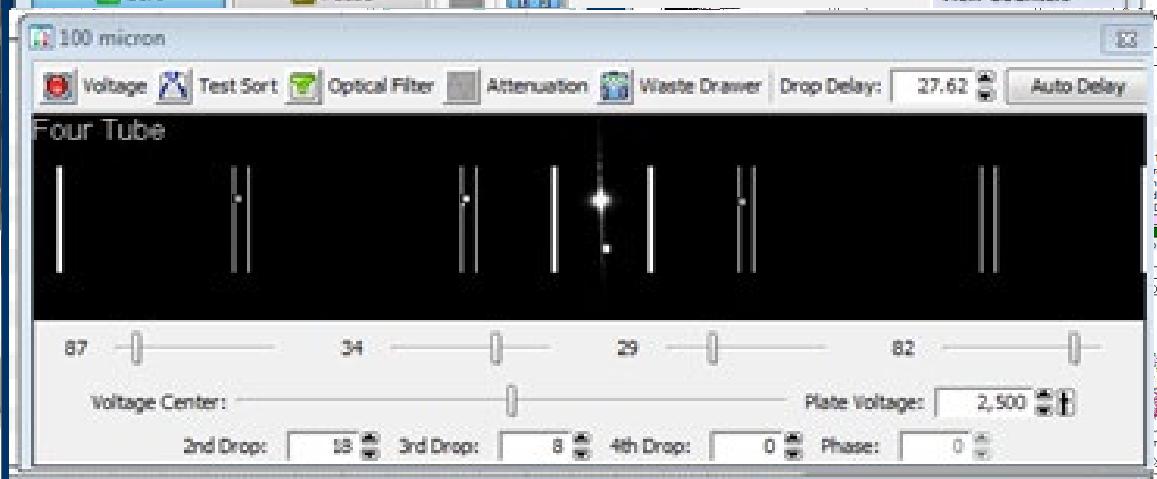
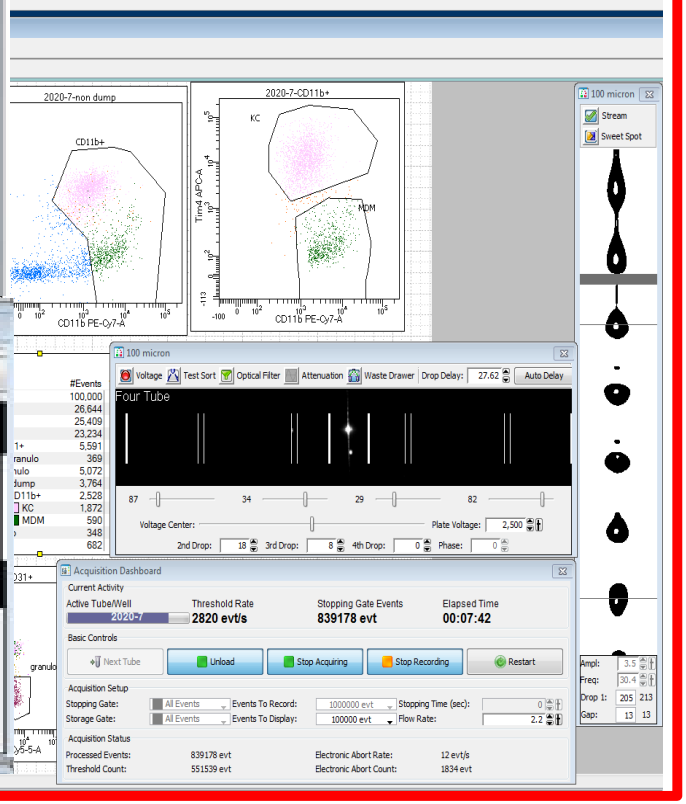




after sort: Sort Layout_001
Device:
Precision:
Target Events:
Save Sort Reports:
Save Conflicts
Index Sorting
4 Tube
4-Way Purity
Continuous
Ask User
Far Left
Left
Right
Far Right
MDM : 2844
KC : 8980
granuloo : 1556
Sort Rate:
4 evt/s
32 evt/s
8 evt/s
NA
Confl. Cnt:
460 evt
1414 evt
274 evt
NA
Confl. Rate:
0 evt/s
8 evt/s
0 evt/s
NA
Efficiency:
88%
93%
87%
NA
Sort
Pause
View Counters

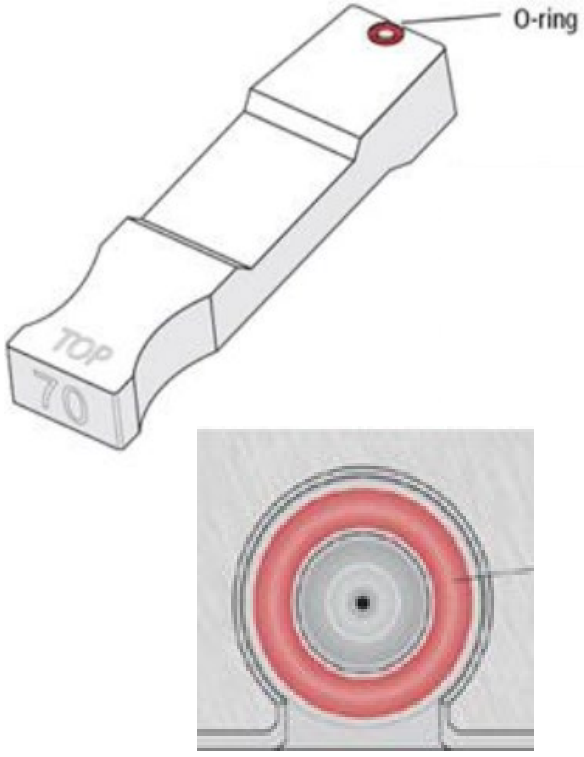

100 micron
Voltage
Test Sort
Optical Filter
Attenuation
Waste Drawer
Drop Delay:
27.62
Auto Delay
Four Tube
87
34
29
82
Voltage Center:
Plate Voltage:
2,500
2nd Drop:
18
3rd Drop:
8
4th Drop:
0
Phase:
0


2020-7-non dump
CD11b+
CD11b PE-Cy7-A
2020-7-CD11b+
KC
MDM
Trim4 APC-A
CD11b PE-Cy7-A
100 micron
Stream
Sweet Spot
Four Tube
Acquisition Dashboard
Current Activity
Active Tube/Well
2020-7
Threshold Rate
2820 evt/s
Stopping Gate Events
839178 evt
Elapsed Time
00:07:42
Basic Controls
Next Tube
Unload
Stop Acquiring
Stop Recording
Restart
Acquisition Setup
Acquisition Status
Processed Events:
839178 evt
Threshold Count:
551539 evt
Electronic Abort Rate:
12 evt/s
Electronic Abort Count:
1834 evt

Options of the BD Fusion cell sorter

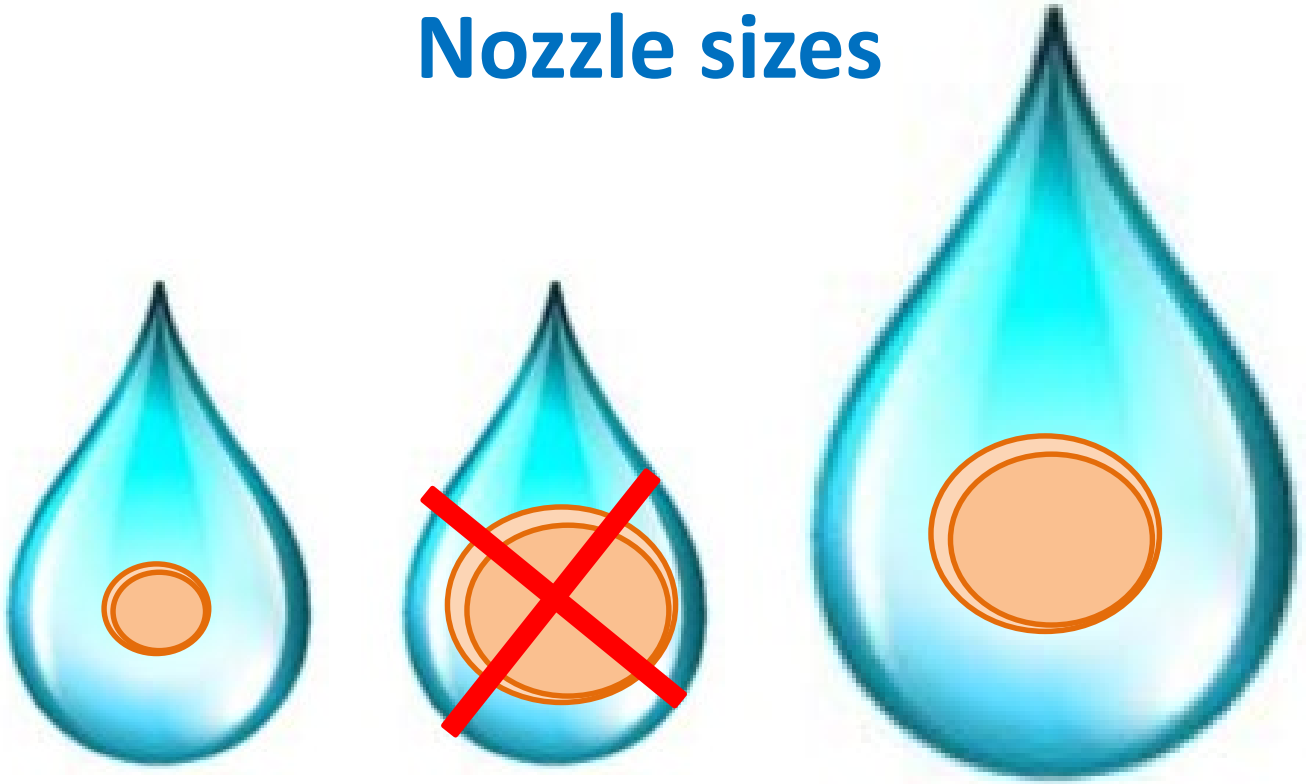
Nozzle sizes

Cell types	Nozzle size
Lymphocytes	70
Activated lymphocytes, small cell-lines	85
Monocytes, large adherent cells, iPSC's, solid tissue preparations	100
Cardiomyocytes, fibroblasts, very large cells	130



Options of the BD Fusion cell sorter

Nozzle sizes



70uM

70uM

100uM

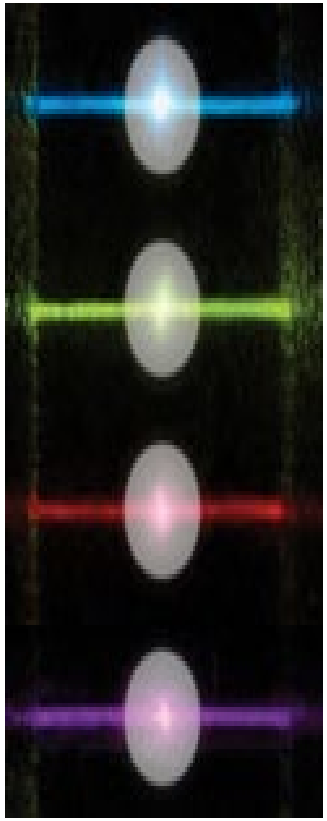
Options of the BD Fusion cell sorter

Nozzle sizes

Nozzle size	Max event/rate	Max # Cells/hour
70	20K/s	40*10e6
85	10K/s	20*10e6
100	8K/s	15*10e6
130	2K/s	7*10e6

Options of the BD Fusion cell sorter

Lasers and detectors



Laser	LP	BP	Examples
488	655	695/40	PerCp-Cy5.5, PerCp, YFP
	502	530/30	FITC, CFSE, GFP
	-	488/10	SSC
561	735	780/60	PE-Cy7
	685	710/50	PE-Cy5.5
	630	670/14	PE-Cy5
	600	610/20	PE-Cy594, PI, mCherry
	-	582/15	PE, DsRed
640	755	780/60	APC-Cy7, APC-H7
	-	670/30	APC, Alexa647
	690	730/45	Alexa700
405	750	780/60	BV786
	690	710/50	BV711
	630	660/20	BV650
	595	610/20	BV605
	505	525/50	BV480, BV510, V500, CFP
	-	450/40	BV421, V450, PB, DAPI

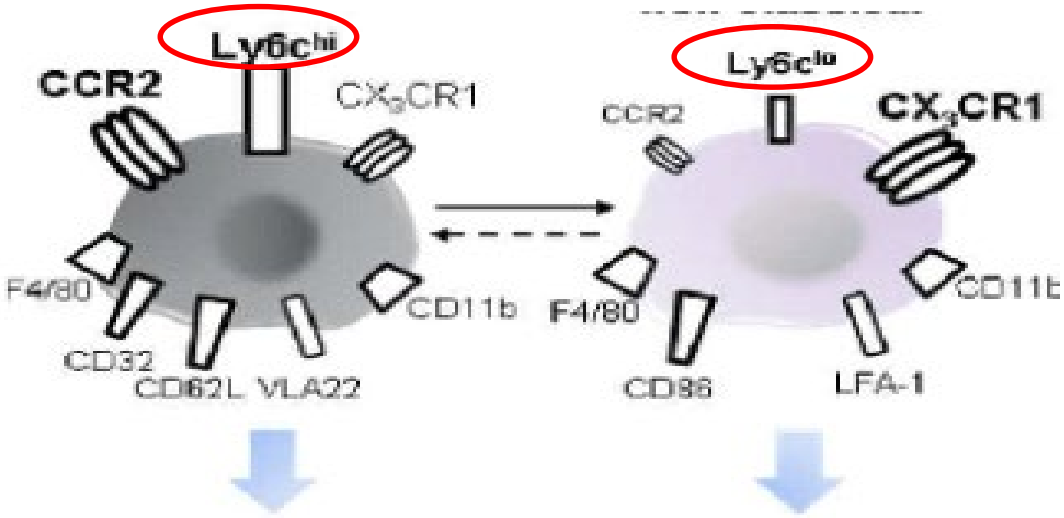
Fluorescent Activated Cell sorting

Applications

Monocyte-subsets in high fat diet

Isolation of monocyte subsets by FACS-sorting:

Classical **Non-classical**



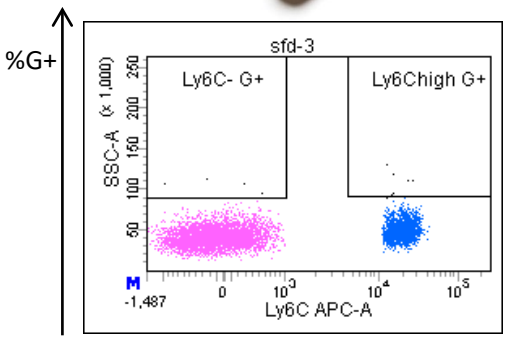
Inflammatory cytokines

Patrolling vessels

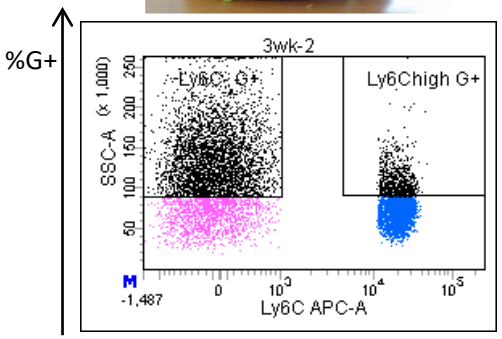
Monocyte-subsets in high fat diet

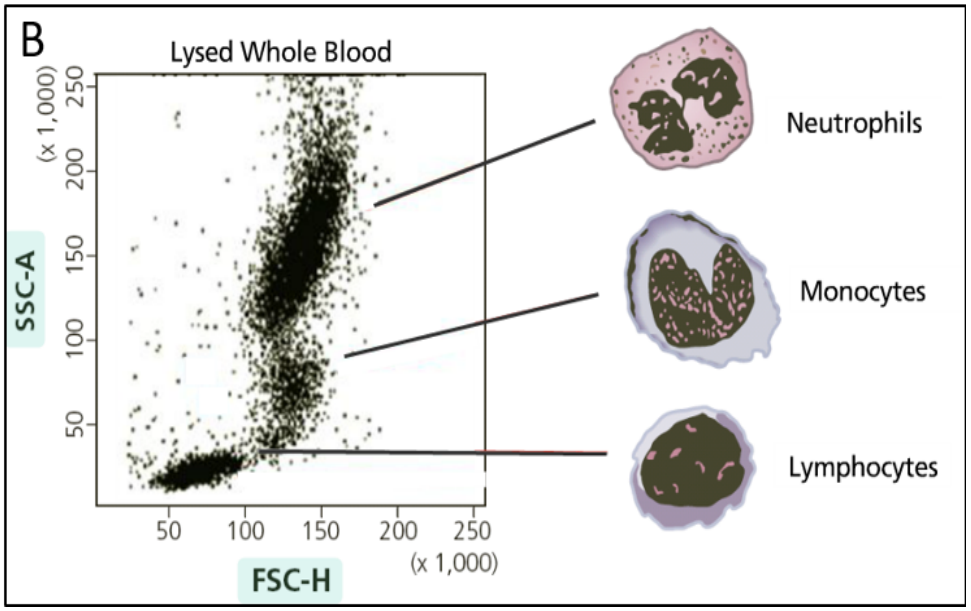
Isolation of monocyte subsets by FACS-sorting:

chow



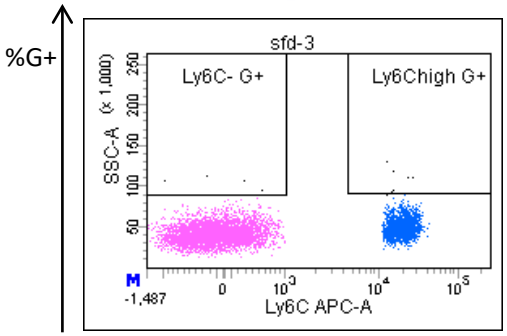
3wks HFD

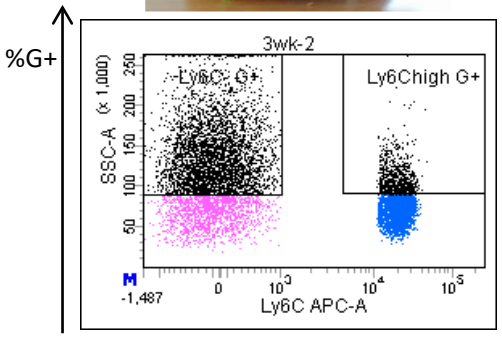


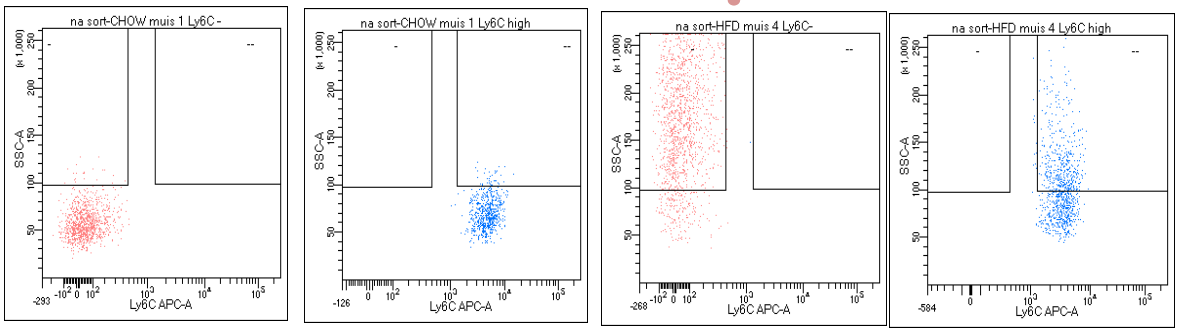


Monocyte-subsets in high fat diet

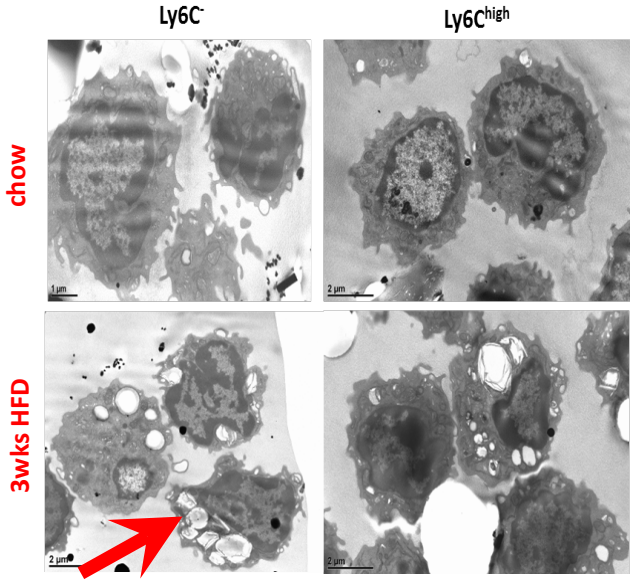
Isolation of monocyte subsets by FACS-sorting:



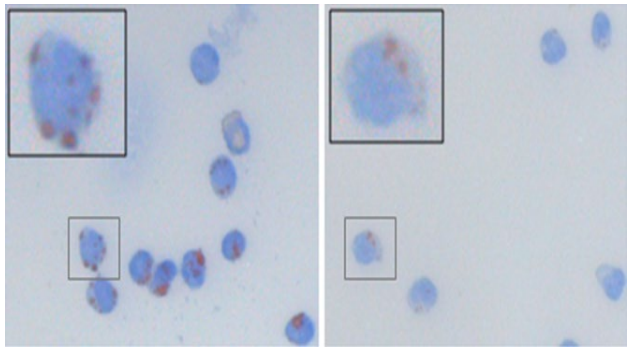




EM:



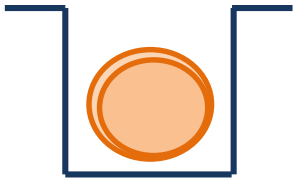
OilRedO fat stain:



Single Cell Sorting

Single cell RNAseq (uencen)

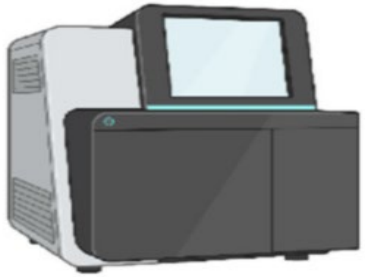
Single-cell



RNA extractie

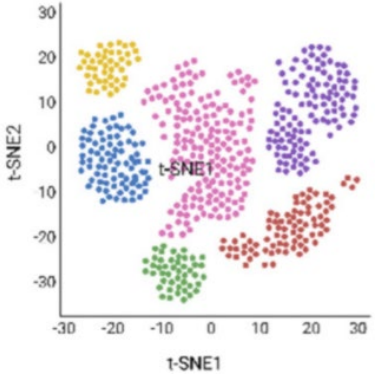
cDNA synthese

Sequencen



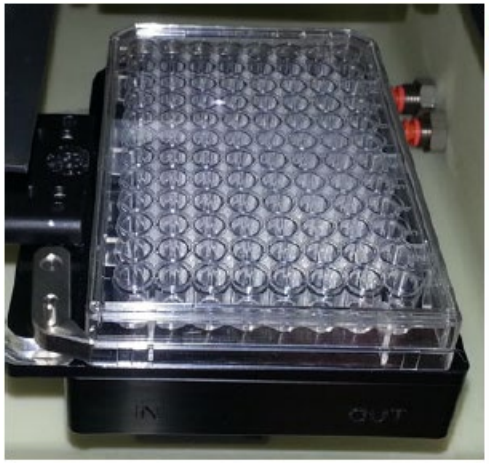
Expressie profielen

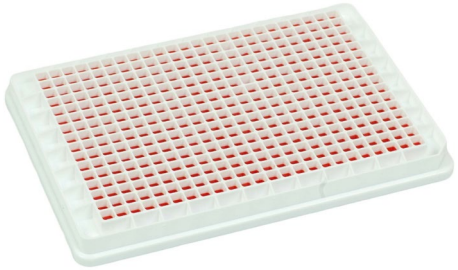
Cel type identificatie



Single Cell Sorting

Single cell RNAseq (uencen)





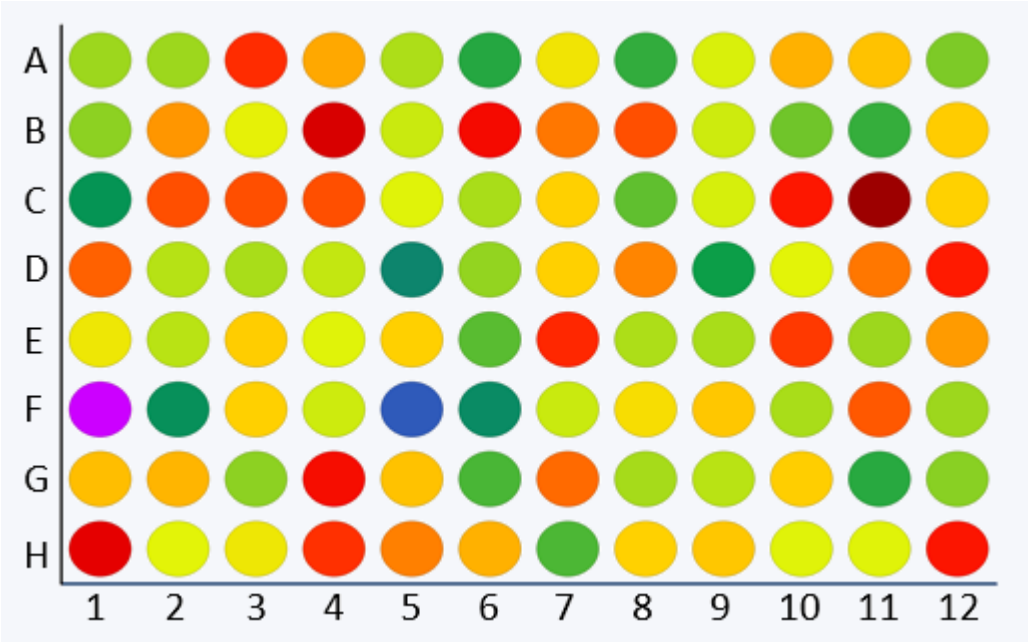
Global Sheet1: Sort Layout_002

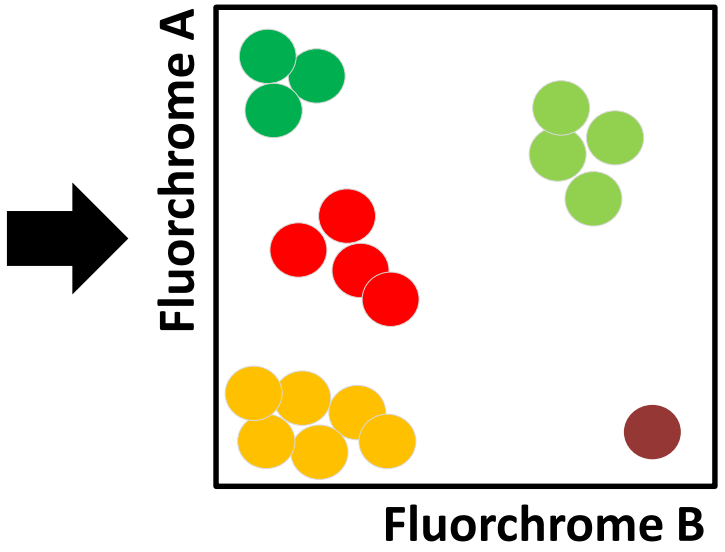
Device:	Precision:	Target Events:	Save Sort Reports:	Save Conflicts	Index Sorting
96 Well - Falcon	Single Cell	1	Ask User	☐	☑

	1	2	3	4	5	6
A	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1
B	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1
C	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1
D	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1
E	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1
F	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1
G	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1
H	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1	GFP : 1

Single Cell Sorting

Index sorting



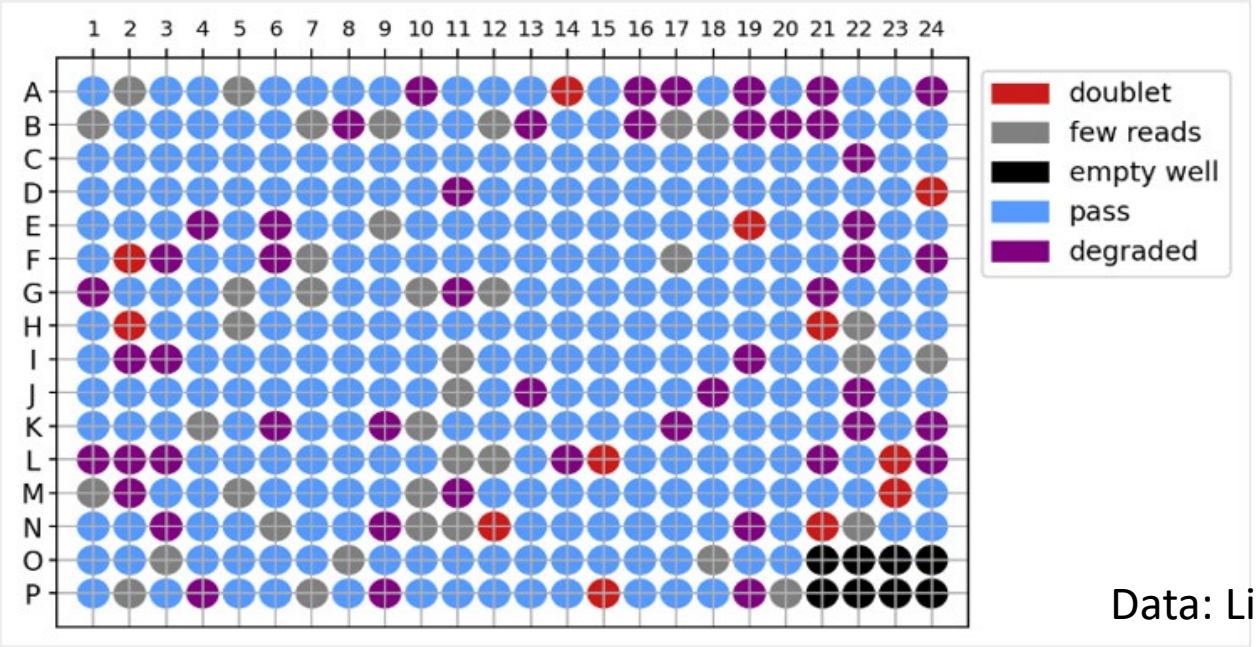


Met terugwerkende kracht toekenen van populaties aan het moleculiare profiel van oppervlakte marker expressie

Single Cell Sorting

Single cell RNAseq (uencen)

Sample Name	Enzyme	Condition	Library type	Sample	Mapped reads %
SCC-scKaryo-MMC-VOO-007	NlaIII	Organoid	sc	PGO-007	89.8%
SCC-scKaryo-MMC-VOO-008	NlaIII	Organoid	sc	PGO-027	87.2%
SCC-scKaryo-MMC-VOO-009	NlaIII	Organoid	sc	PGO-027	86.4%
SCC-scKaryo-MMC-VOO-010	NlaIII	Organoid	sc	PGO-030	87.1%



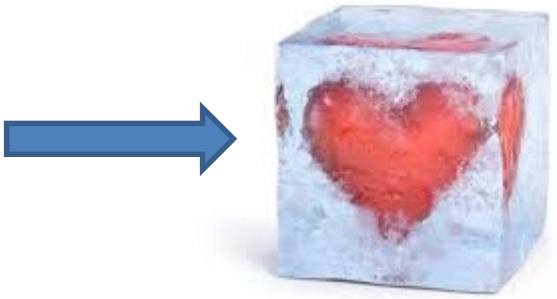
Data: Linde Hoosemans
MAASTRO-lab

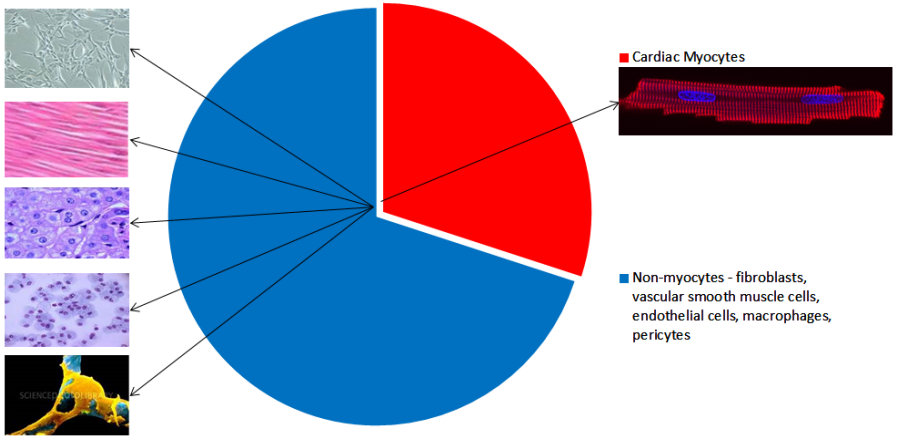
Nuclear sorting

Cell-type specific transcriptomics and epigenomics

Cardiomyocytes:

- Too big
- Changes in genoom during long isolation





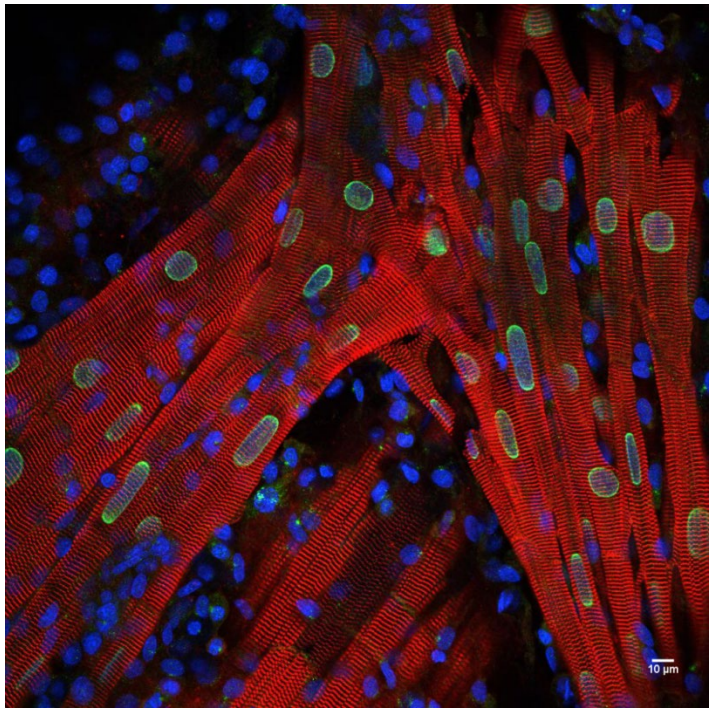
Data: Emma Robinson
JCI, Vol 127, Nr1, Jan 2017

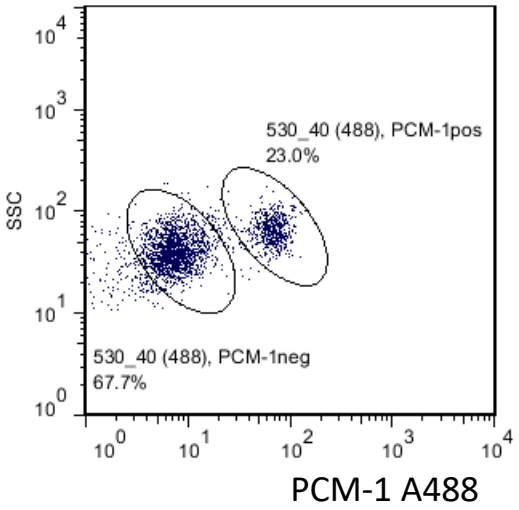
Nuclear sorting

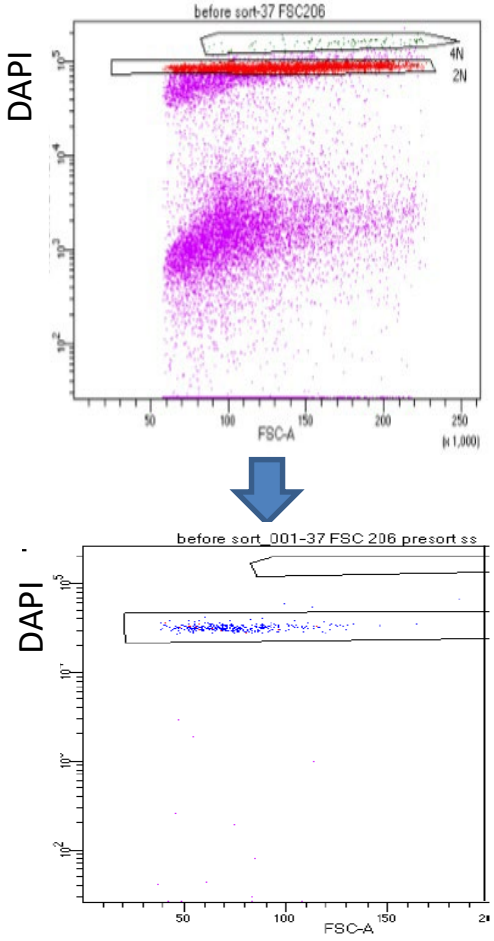
Cell-type specific transcriptomics and epigenomics

PCM-1 is cardiomyocyte specific nuclear marker

PCM-1, alpha actinin, DAPI



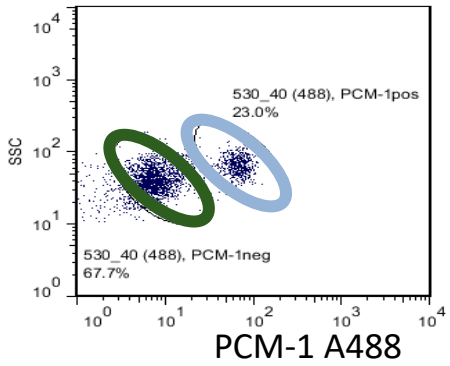


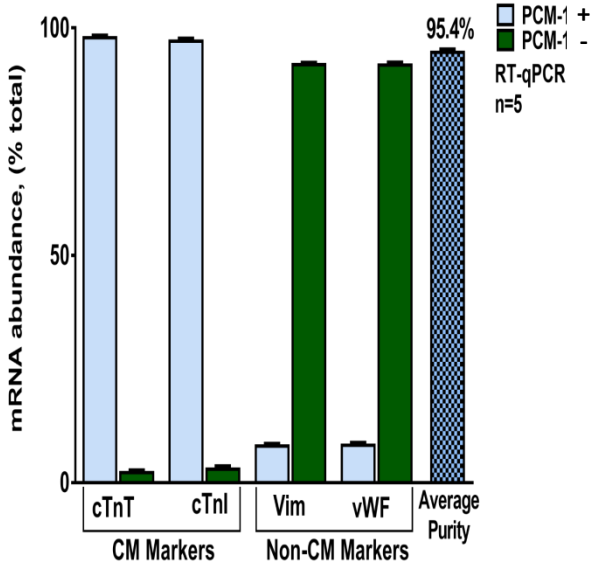


Data: Emma Robinson
JCI, vol 127, Nr1, Jan 2017

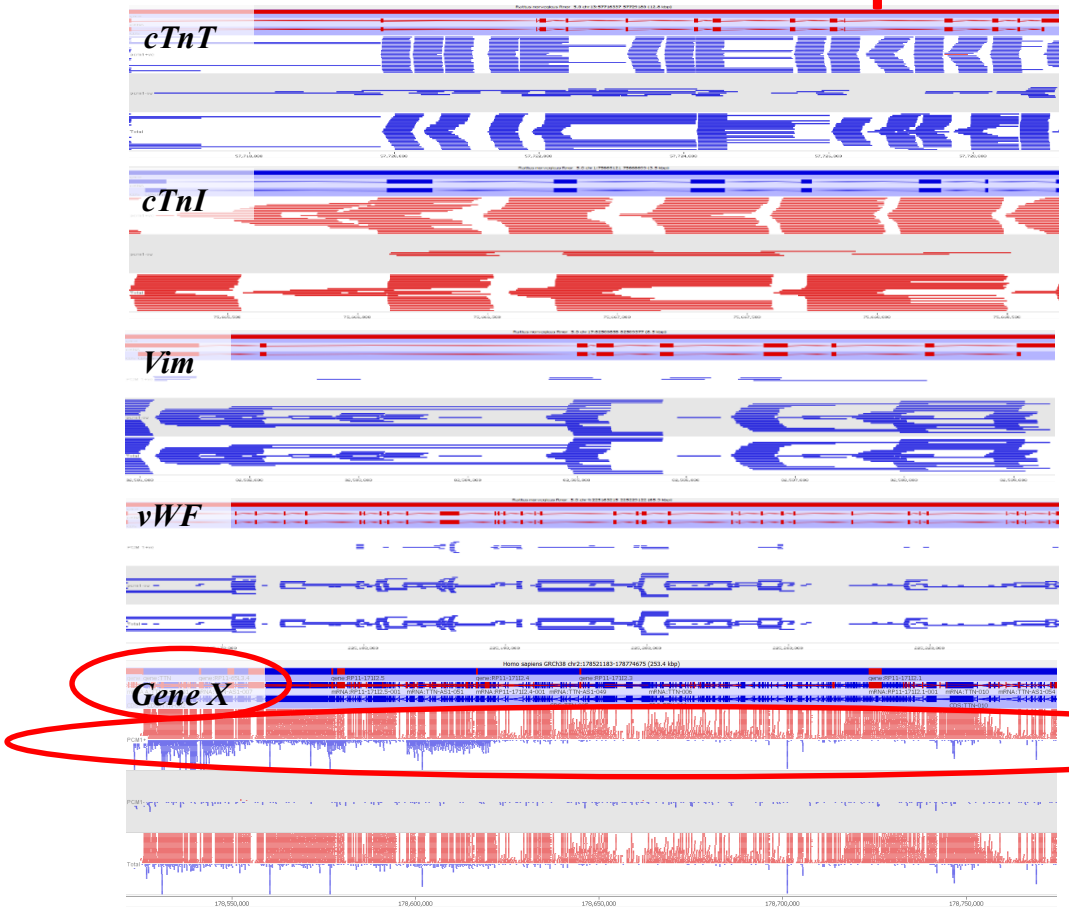
Nuclear sorting

Cell-type specific transcriptomics and epigenomics



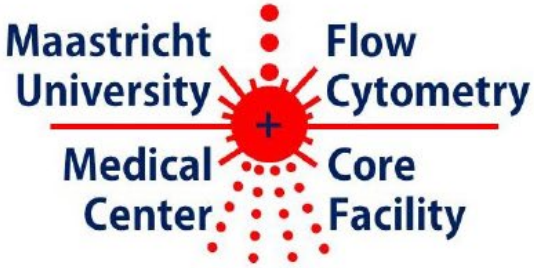


RNAseq:



Data: Emma Robinson JCI, vol 127, Nr1, Jan 2017

Guidelines for Cell Sorting



Sample preparation is a key to a successful sort. These guidelines state how to prepare and collect your cells in the best way.

Questions?

fccf.cdl@mumc.nl

Maastricht University Medical Center
Flow Cytometry Core Facility