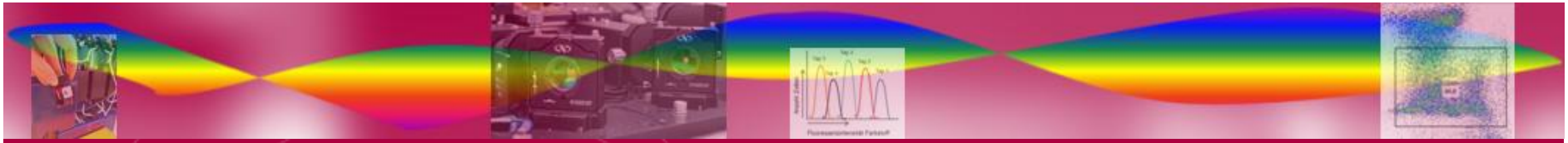
Universität Rostock Traditio et Innovatio

Universitätsmedizin Rostock



The perfect control - how and what to use?

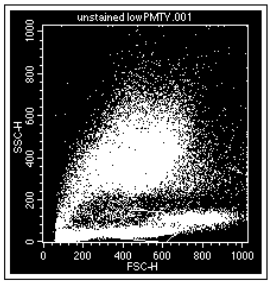
Die perfekte Kontrolle – aber wie und womit?

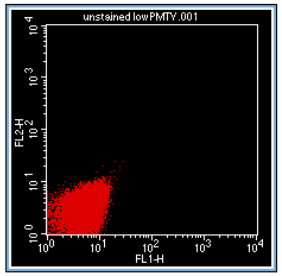
Core Facility for Cell sorting and Cell analysis

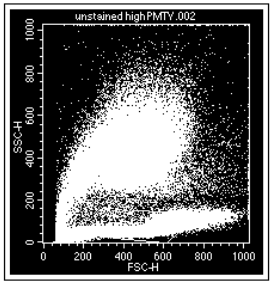
Core Facility für Zellsortierung und Zellanalyse
Universitätsmedizin Rostock

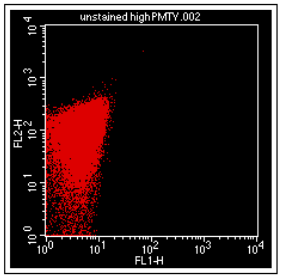
Controls – What‘s the problem?

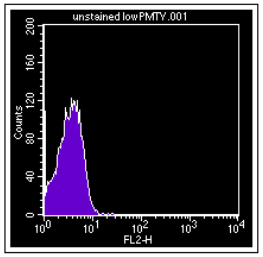
unstained → stained?



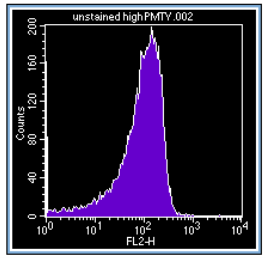


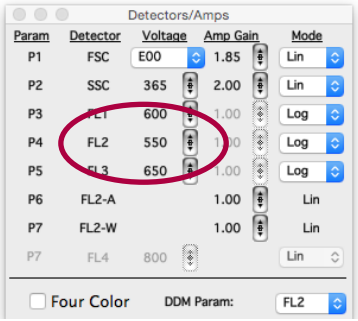


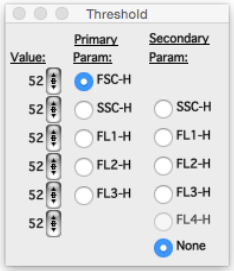


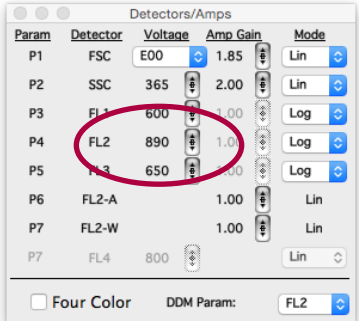


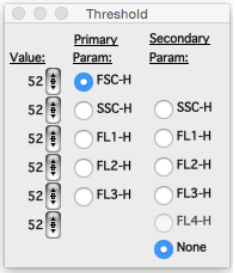
No! both unstained







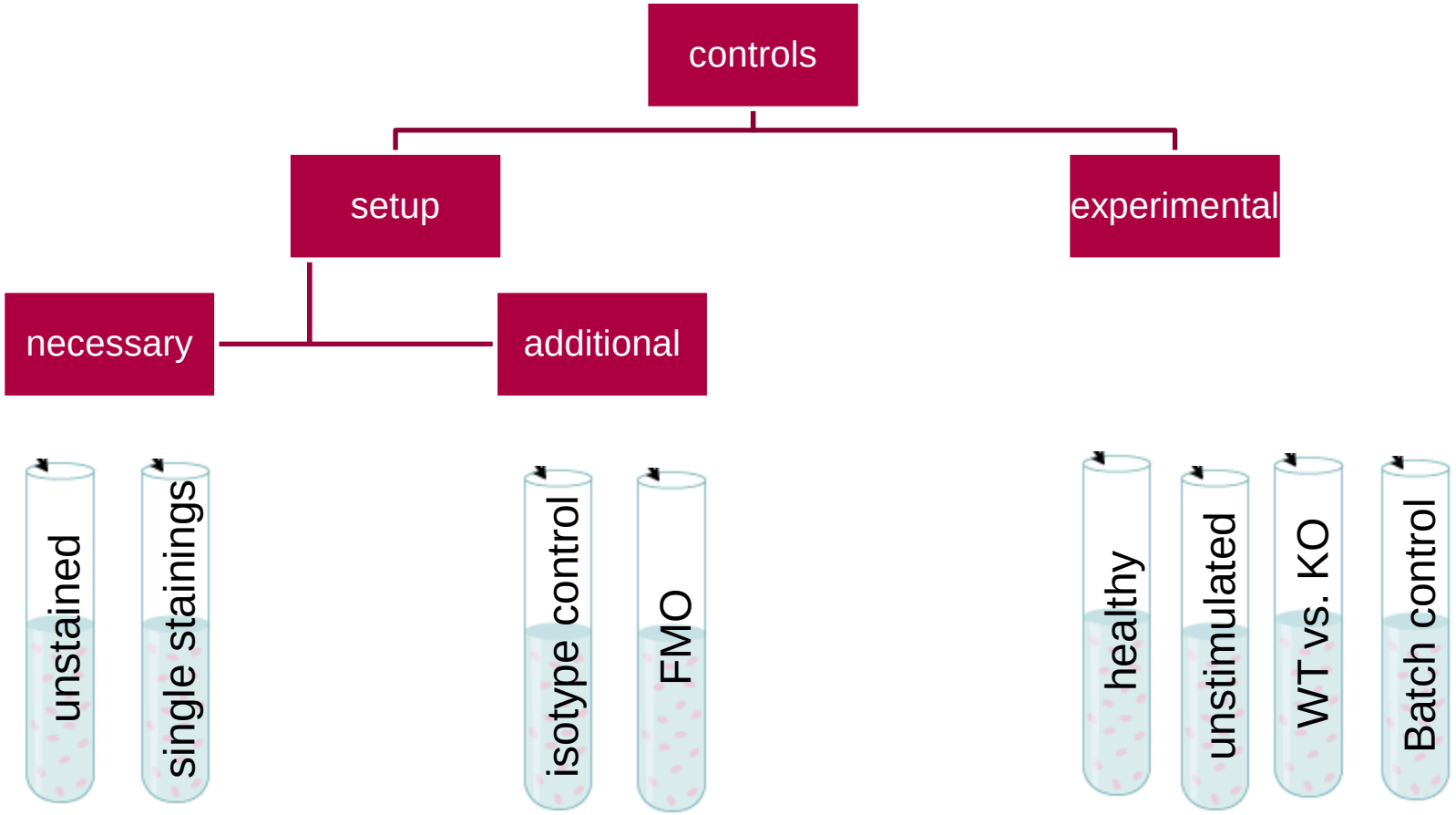




Controls – What‘s the problem?

- flow cytometry → multiparametric of single cell suspension
- But! Relative measurement → results depend on device configuration and settings (e.g. PMT voltages)
- Spectral spillover → compensation & unmixing single stains are needed
- wrong controls → wrong data and misinterpretation

different control types

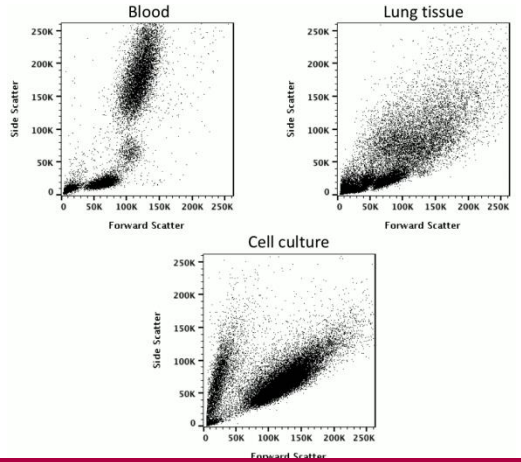


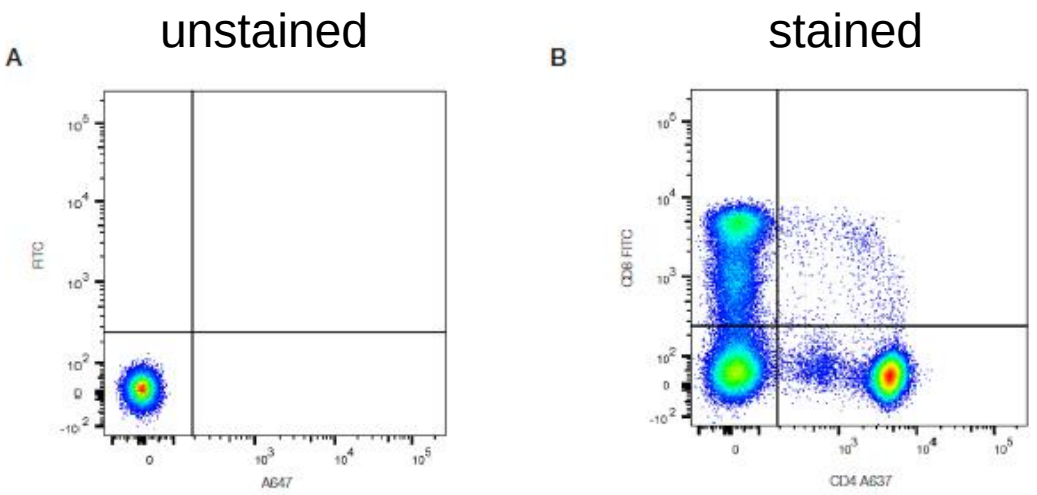
FMO = fluorescence minus one

https://resources.rndsystems.com/images/site/fmc002-protocol-step2andstep3.png

necessary controls

- **unstained:**
  - defines background and autofluorescence
  - should be within the first third of the plot/graph
  - can be used to determine where the staining starts
  - helpful to find your cells in the scatter plots

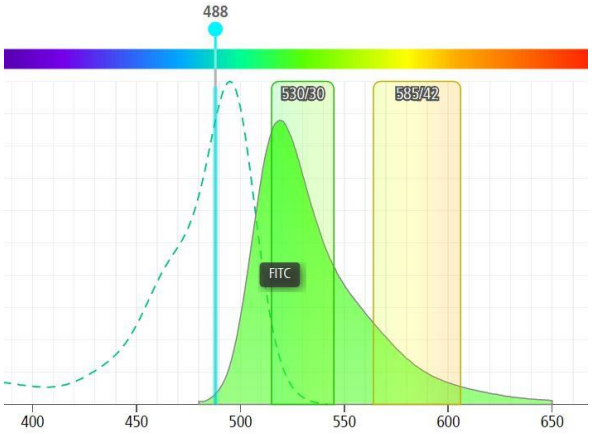


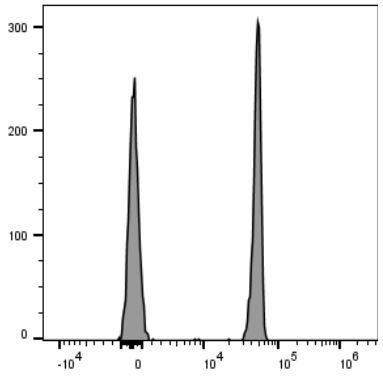


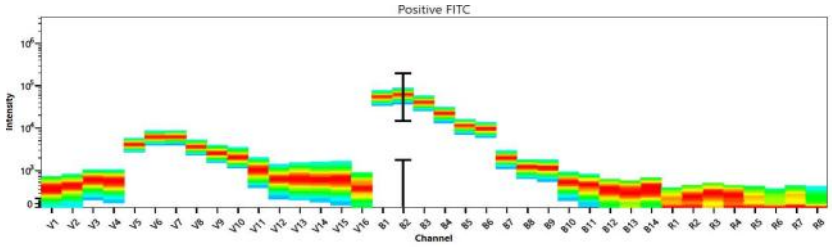
https://www.bio-rad-antibodies.com/flow-cytometry-unstained-controls.html

necessary controls

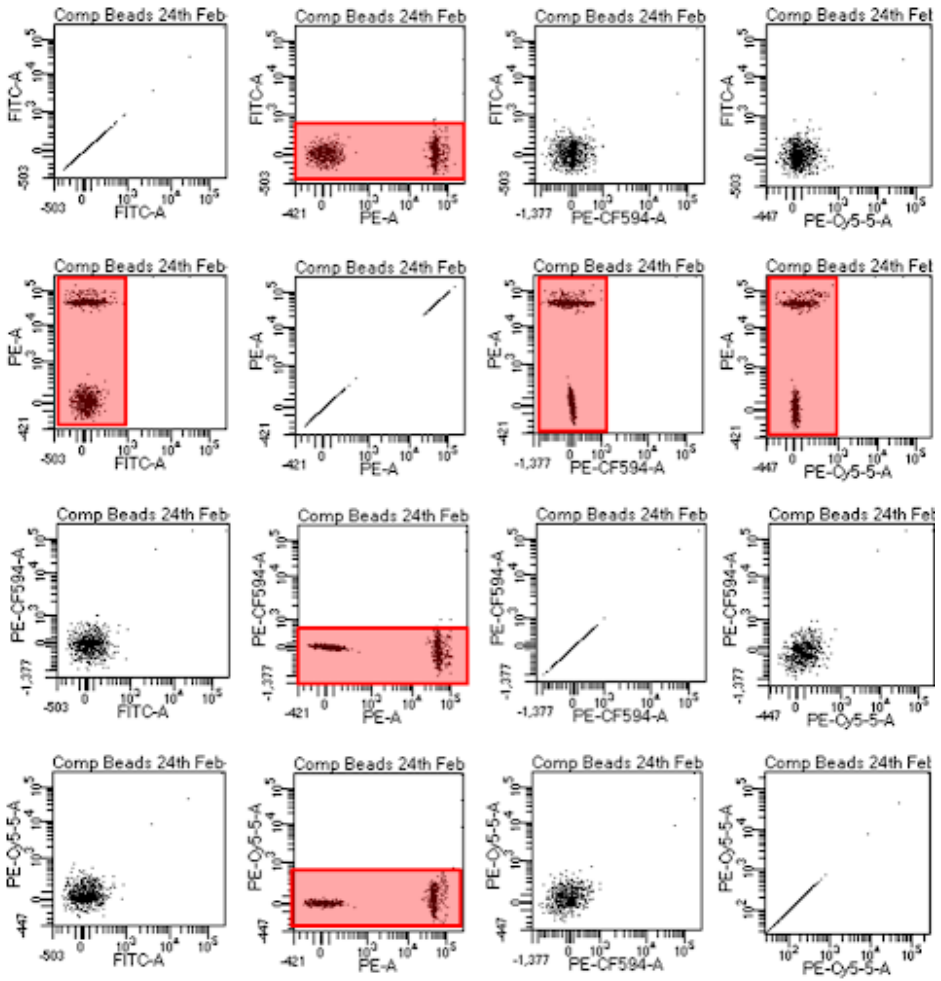
- **single stainings:**
  - positive control for each fluorescence separately
  - necessary for calculating compensation/unmixing
  - **minimum as bright as the real sample**
  - **background/autofluoresence of negative and positive control must be the same**
  - ! compare beads with beads, cells with cells !







NxN matrix to see compensation/unmixing errors

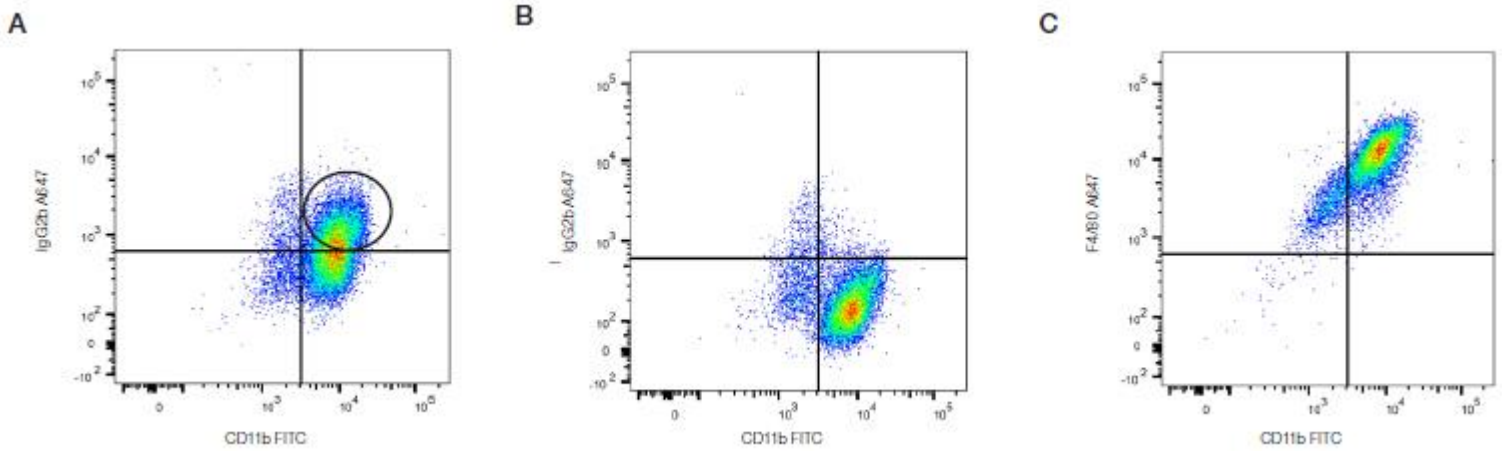


https://lh3.googleusercontent.com/proxy/pXrxzEOMnwVtjozy1lsTZqeO4T5phjMdZEEW6z5e9H3YLrUTD2Fzx4ZnLDxSUuKfvl5tE7u6x5yKdHMt7Uz8B_WGRROB9krjqGDeUnOClGB3xNuhCw7E7hHa6jzw90YXgMWyVV4MC7e4StbisOR_-0xR1YN92FVPM_k7G675sshB8iri

isotype control

w/o Fc-block

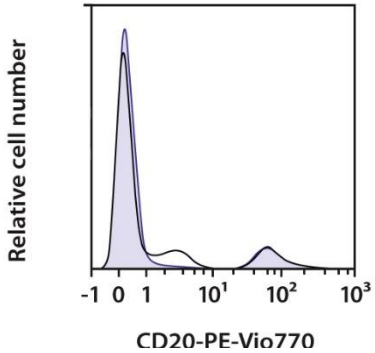
with Fc-block



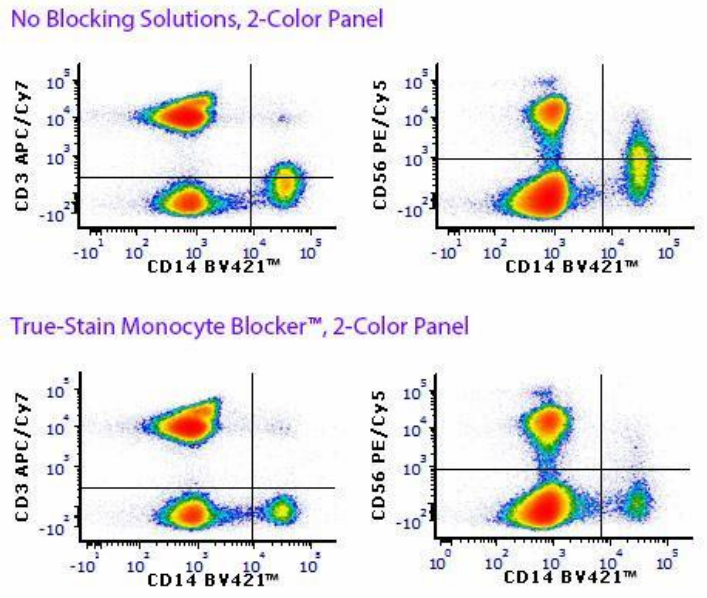
https://www.bio-rad-antibodies.com/flow-cytometry-guide-isotype-controls.html

different buffers prevent unspecific binding

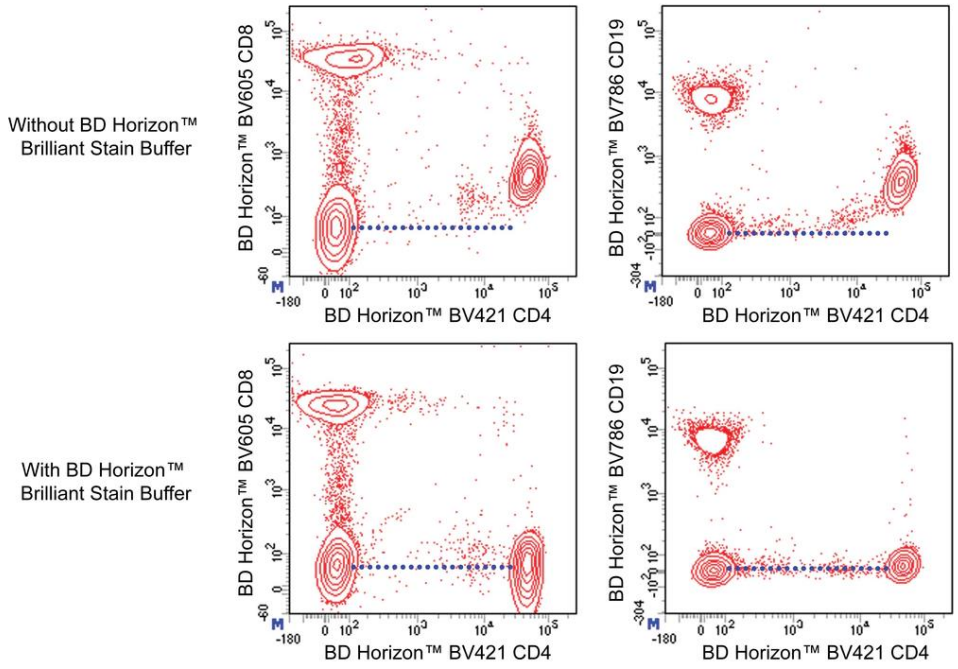
Miltenyi Tandem Signal Enhancer



BioLegend TruStain Monocyte Blocker

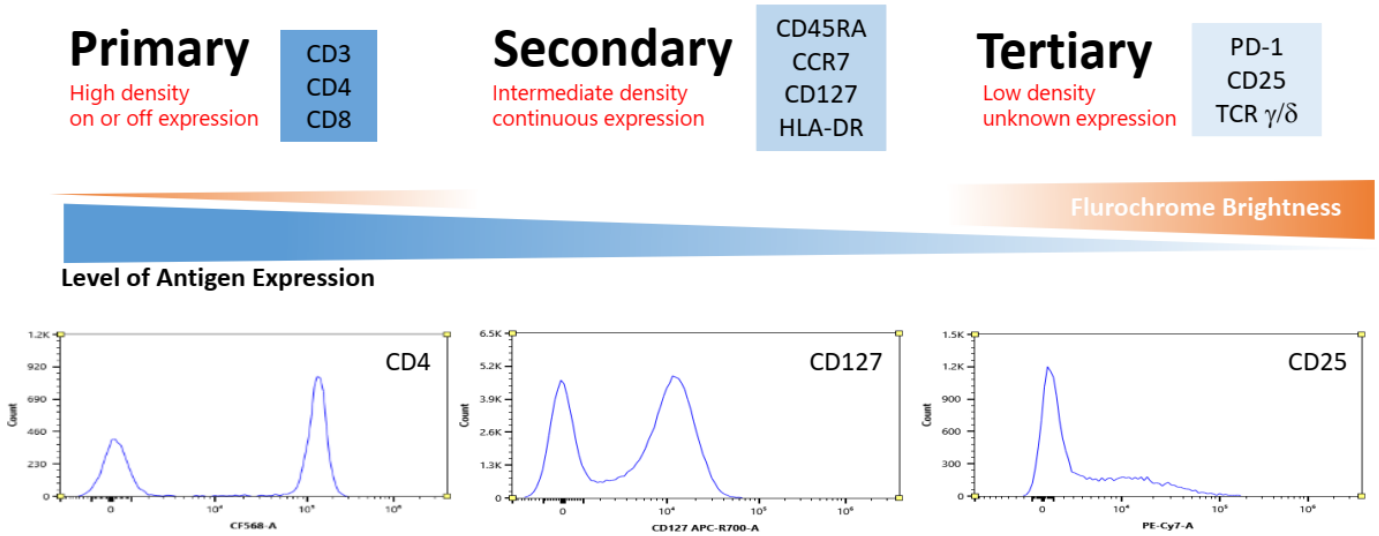


BD Brilliant Stain Buffer



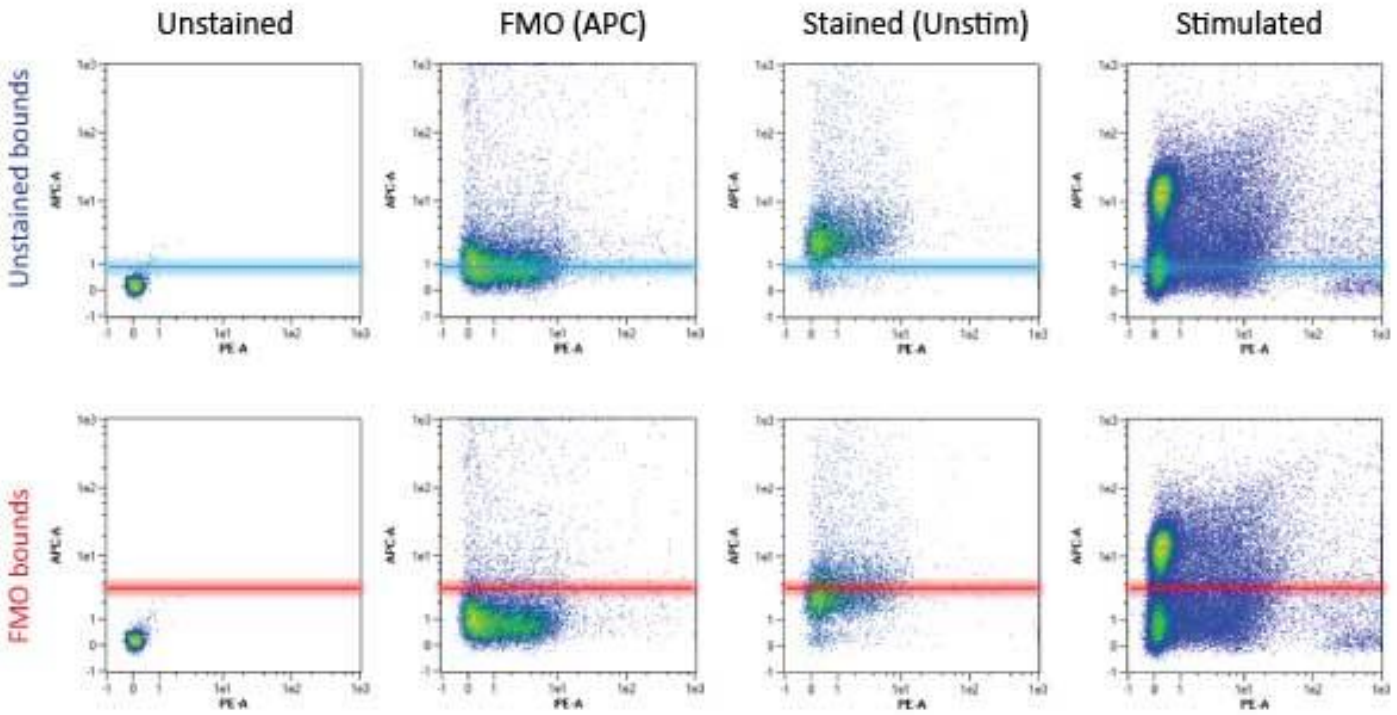
FMO = fluorescence minus one → gating control

- control staining without one antibody, all others stained
- defines gate for positive population
- can be combined with isotype control → most strict control
- often necessary for tertiary antigens



Mahnke, Y. and Roederer, M. Clin Lab Med. 2007 September ; 27(3): 469

FMO = fluorescence minus one



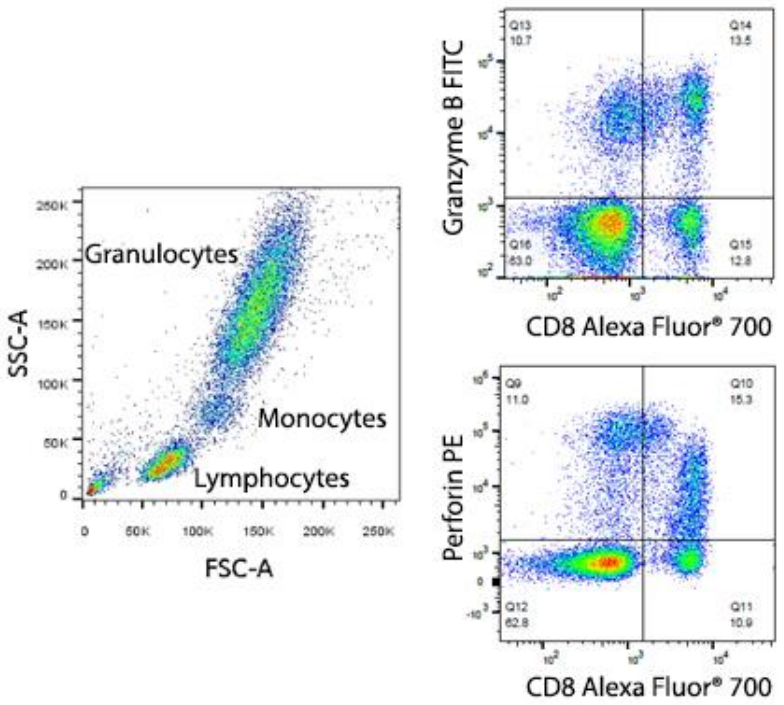
Fluorophore	VB FMO	FITC FMO	PE FMO	APC FMO
VioBlue	-	+	+	+
FITC	+	-	+	+
PE	+	+	-	+
APC	+	+	+	-

http://stmichaelshospitalresearch.ca/staff-services/research-facilities/facilities/flow-cytometry-core/immunophenotyping/

experimental controls

- depends on experiment
- WT vs KO
- healthy vs. disease
- control sample to rule out batch effects

BioLegend Veri Cells



controls - TAKE HOME MESSAGE

- unstained and single stainings absolut minimum
- **minimum as bright as the real sample**
- compare beads with beads, cells with cells

- always ask: what additional controls do I need?
- isotype → unspecific binding
- FMO → gating and analysis control
- experimental → healthy, unstimulated, batch control
- antibody producer often provides hints for QC/positive controls
- especially for spectral flow cytometry:
  - use the same treatments/buffers/fixative for controls and samples

- **unspecific binding → Block solution, different buffers**
- **no staining → search for positive cell line or stimulation**
- **unclear where to set gates → FMO controls**

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Thank you for your attention.

See you next month 06th April.

Next topic:
cell cycle analysis – simple but complicated?

Zellzyklusanalysen – einfach und doch kompliziert?