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What is wrong with my flow cytometry data?

Hints, tricks and pitfalls

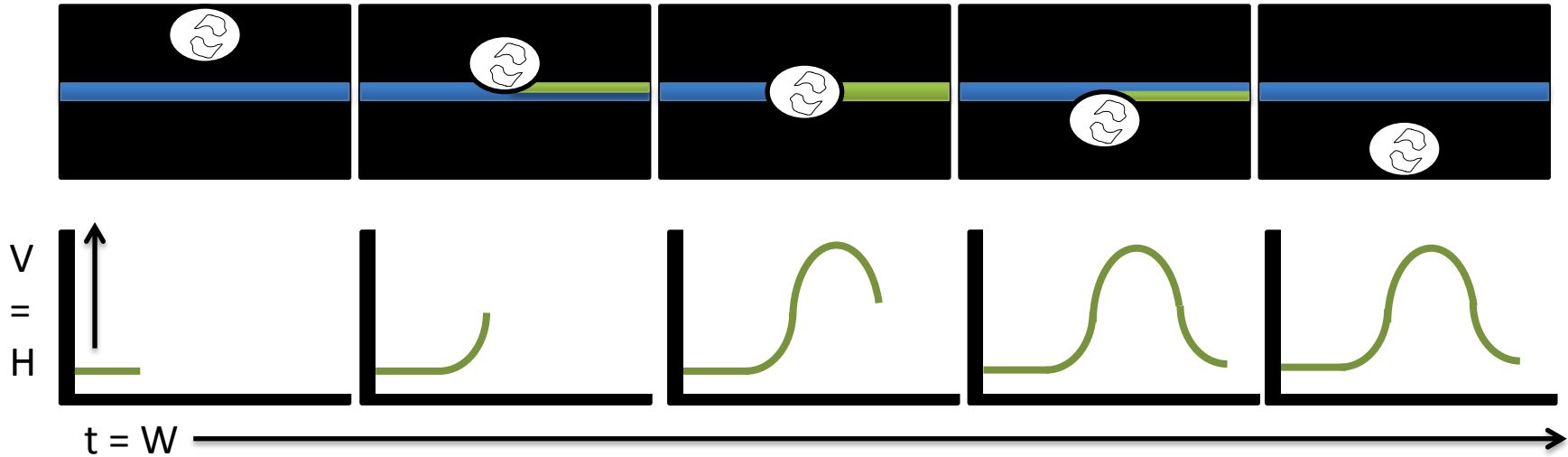
Webinar 1
07.02.2023



Doublets: Misinterpretation preassigned.

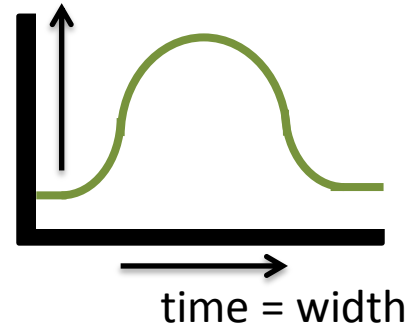
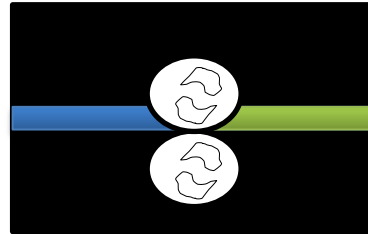
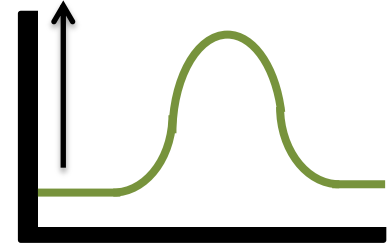
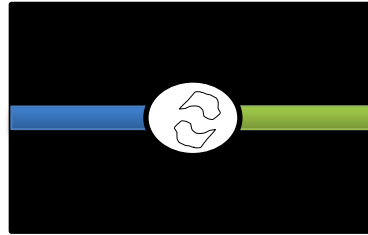
Doublet? Never heard of this...

- flow cytometry → high-throughput analysis of **single cell** suspension



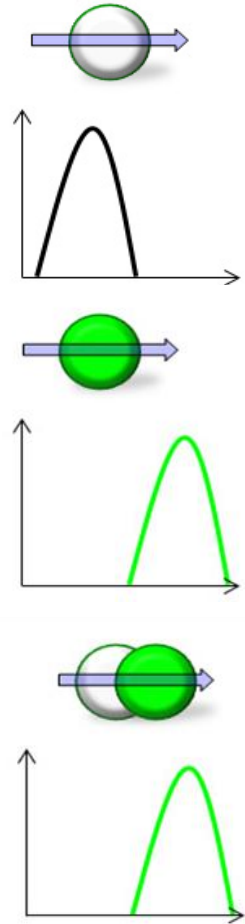
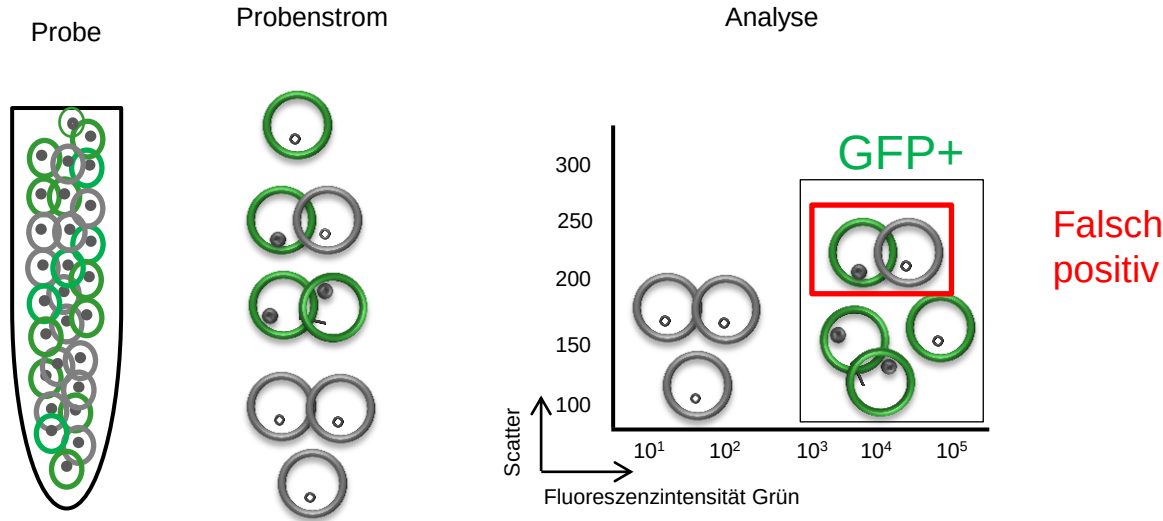
Doublet? Never heard of this...

- Singlets vs. doublets
- Doublets → 2 events analysed at the same time
- Higher width parameter

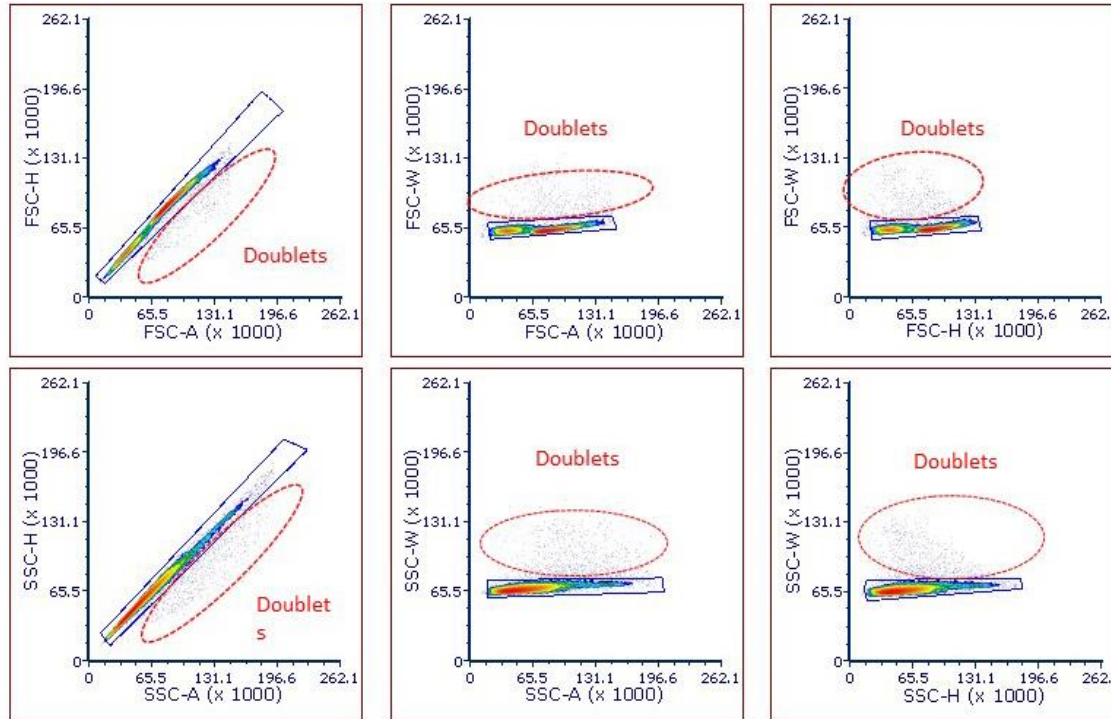


Doublets? What is the problem about...

- signals can't be separated → loss of data accuracy
- false positive signal, higher MFI signals, more cells in G2/M phase in cell cycle



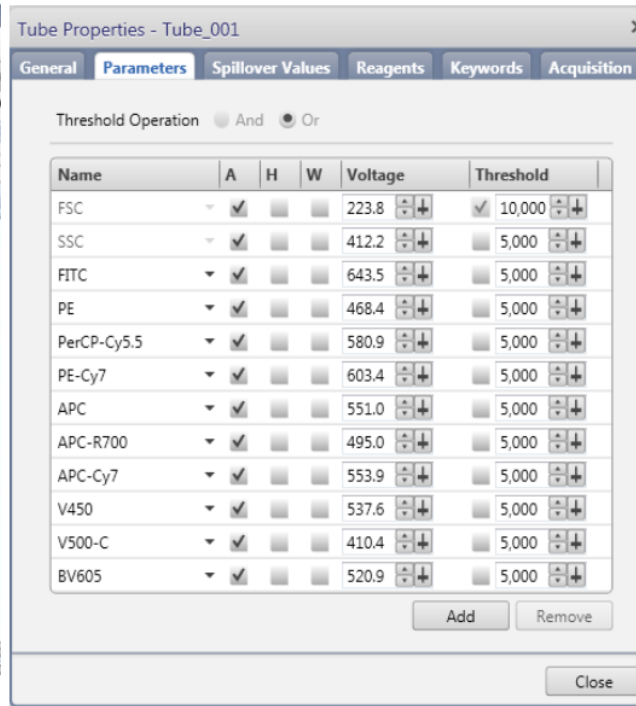
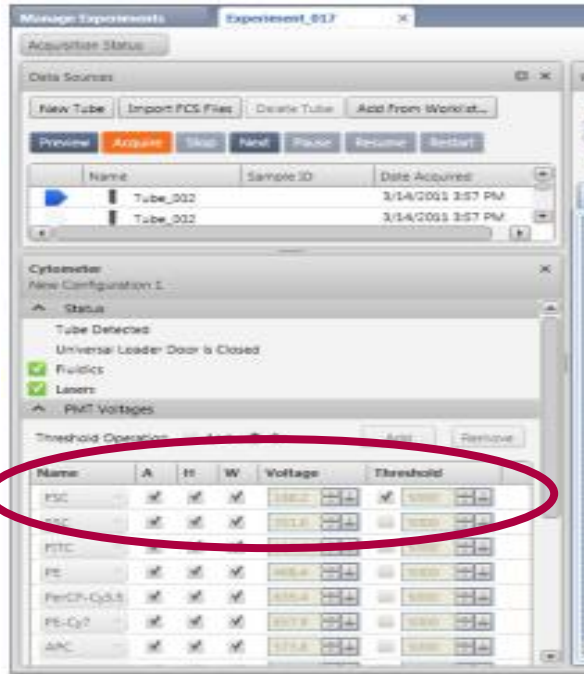
Doublets? How to exclude dublets



FSC or/and SSC

W vs. H
H vs. A

Doublets? How to record A, W and H...





Doublets? How to record A, W and H...



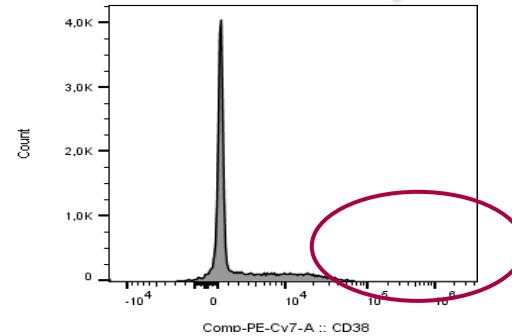
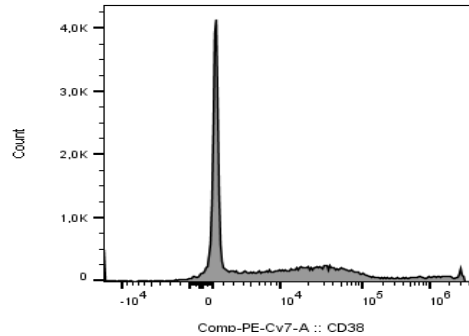
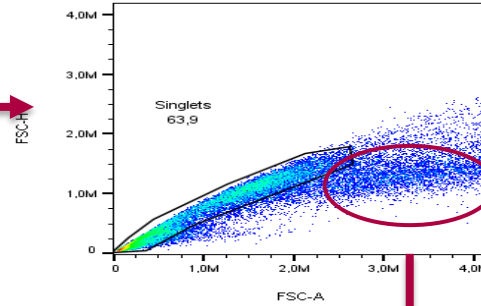
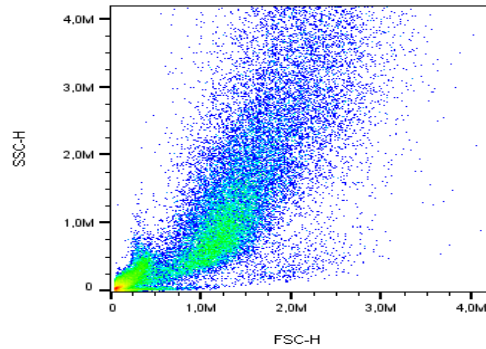
The screenshot shows the 'Detectors/Amps' panel with the following data:

Param	Detector	Voltage	Amp Gain	Mode
P1	FSC	E00	2.14	Lin
P2	SSC	288	1.00	Lin
P3	FL1	551	1.00	Log
P4	FL2	628	1.00	Log
P5	FL3	597	1.00	Log
P6	FL2-A		1.00	Lin
P7	FL2-W		1.00	Lin
P7	FL4	672		Log

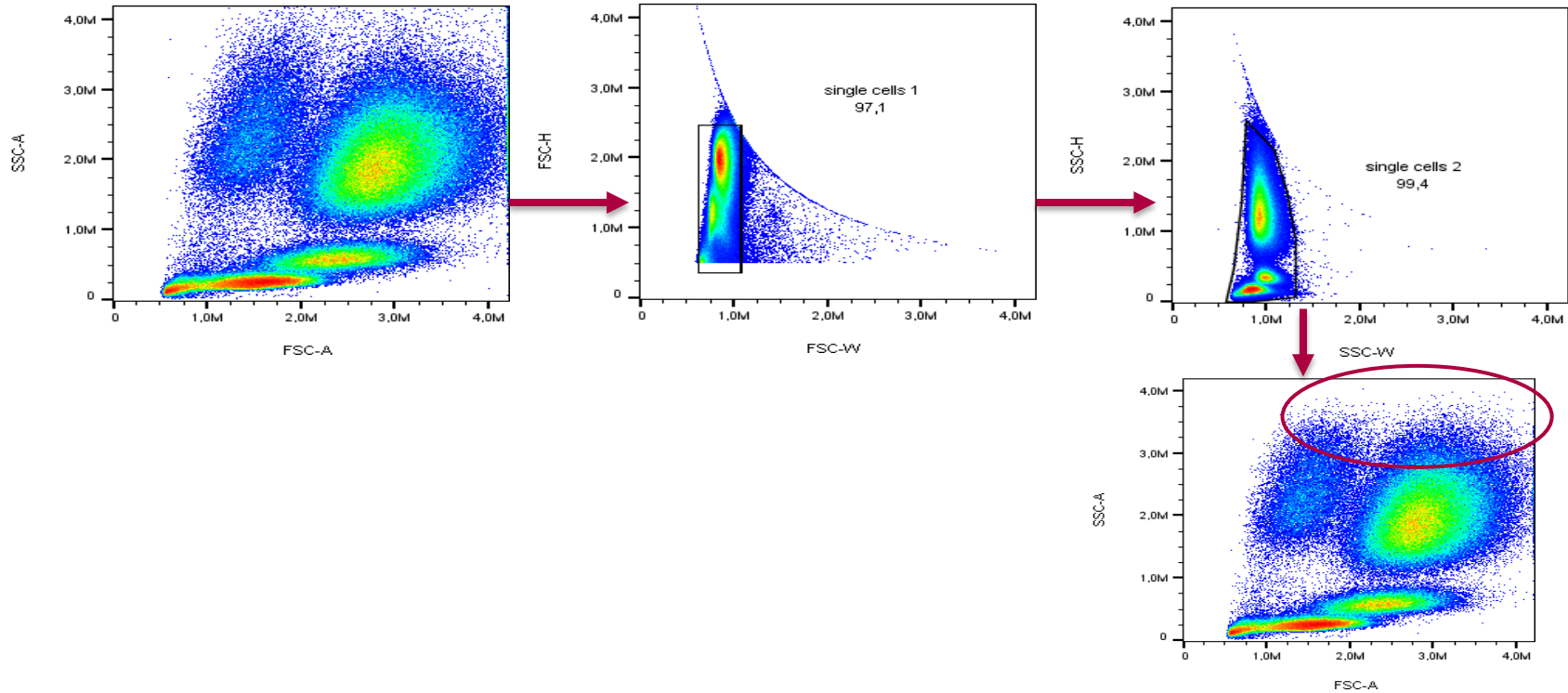
Additional settings shown in the interface include:

- Four Color** checkbox: checked.
- DDM Param:** FL2.
- Slider control** for Amp Gain: indicated by an arrow.
- Threshold** panel: showing various parameters like FSC-H, SSC-H, FL1-H, etc.

Doublets? How do they look like...



Doublets? How do they look like...

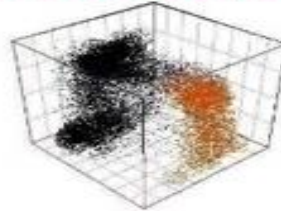


Doublets? I want more...

Flow Cytometry Basics: How to Gate-out Doublets and Clumps

This video is brought to you by:

Flow Cytometry Network
www.thefcn.org



moovly

<https://www.youtube.com/watch?v=MjMvwSUuckI>

Doublets? Take home message, not doublets...

- Prevent doublet formation:
 - Filter cells or/and
 - Resuspend cells or/and
 - Vortex sample
 - Add EDTA
 - Reduce BSA/FCS concentration
 - Change to PP tubes instead of using PS tubes
- Select A, W and H parameter to be recorded
- Exclude doublet from analysis via A vs.W or H vs. W
- Reduce acquisition speed / flow rate or dilute cells more

Thank you for your attention.

See you next month **7th March**.

Next topic:

The perfect control – what to take?