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What is wrong with my flow cytometry data?

Hints, tricks and pitfalls

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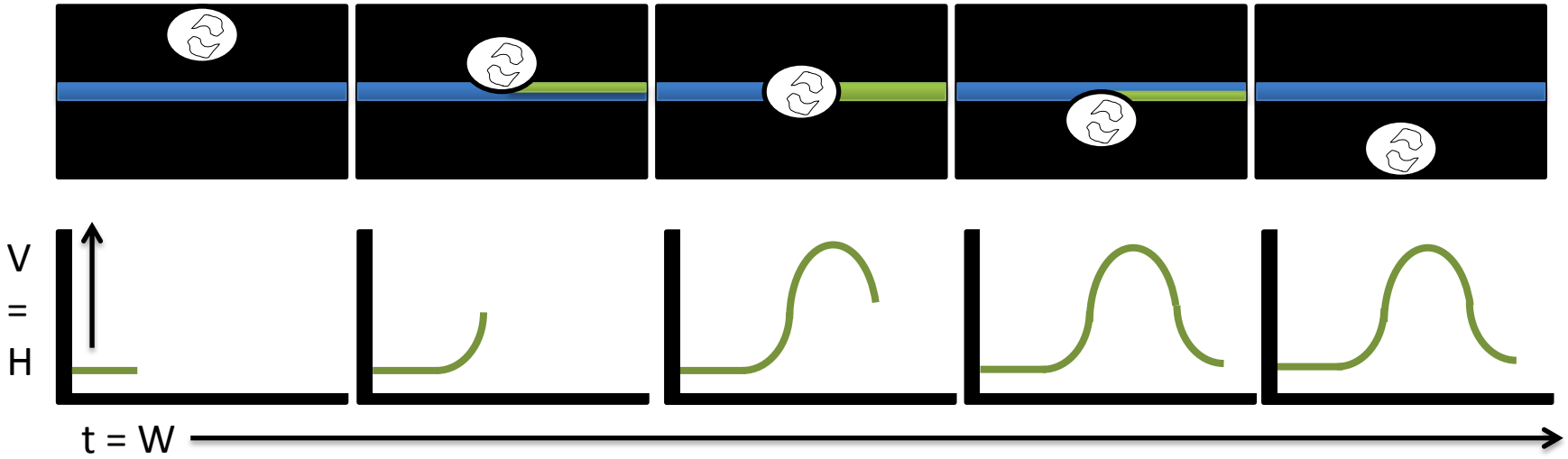
Core Facility für Zellsortierung und Zellanalyse Universitätsmedizin Rostock

Webinar 1
07.02.2023

Doublets: Misinterpretation preassigned.

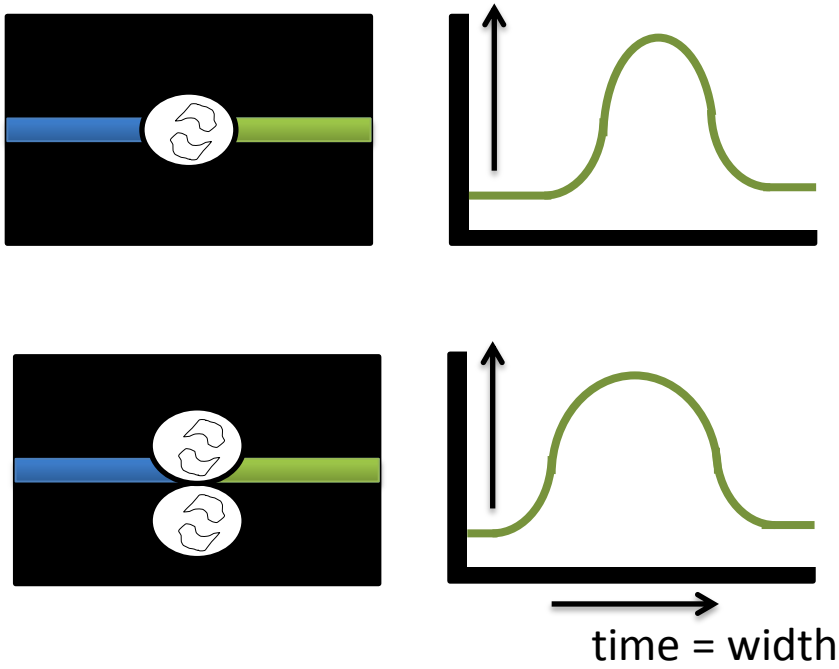
Doublet? Never heard of this…

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



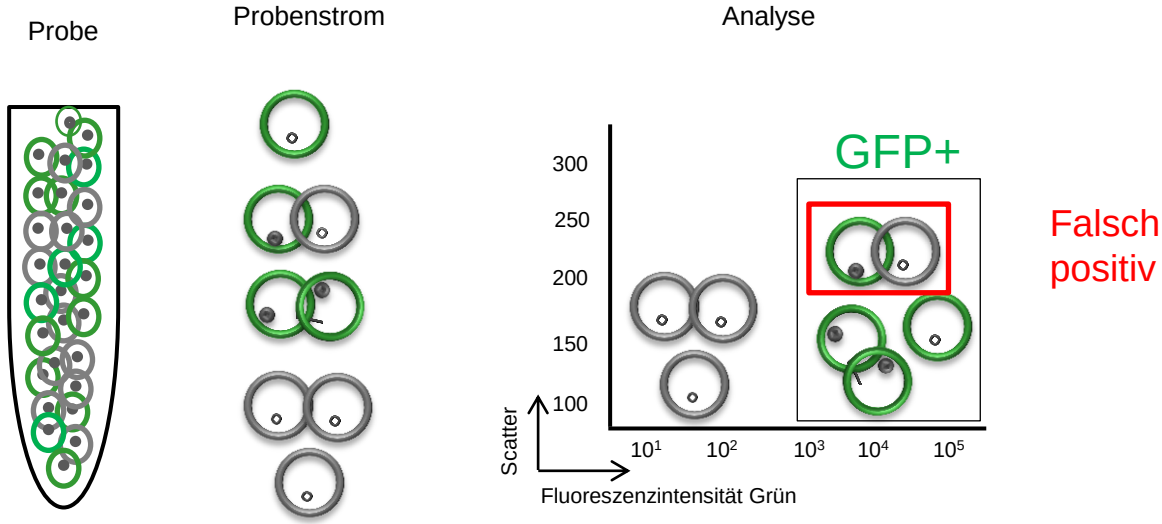
Doublet? Never heard of this…

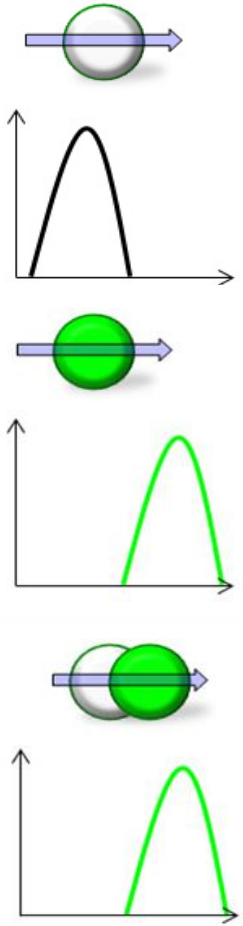
- Singlets vs. dublets
- ➢ Doublets → 2 events analysed at the same time
- ➢ Higher width parameter



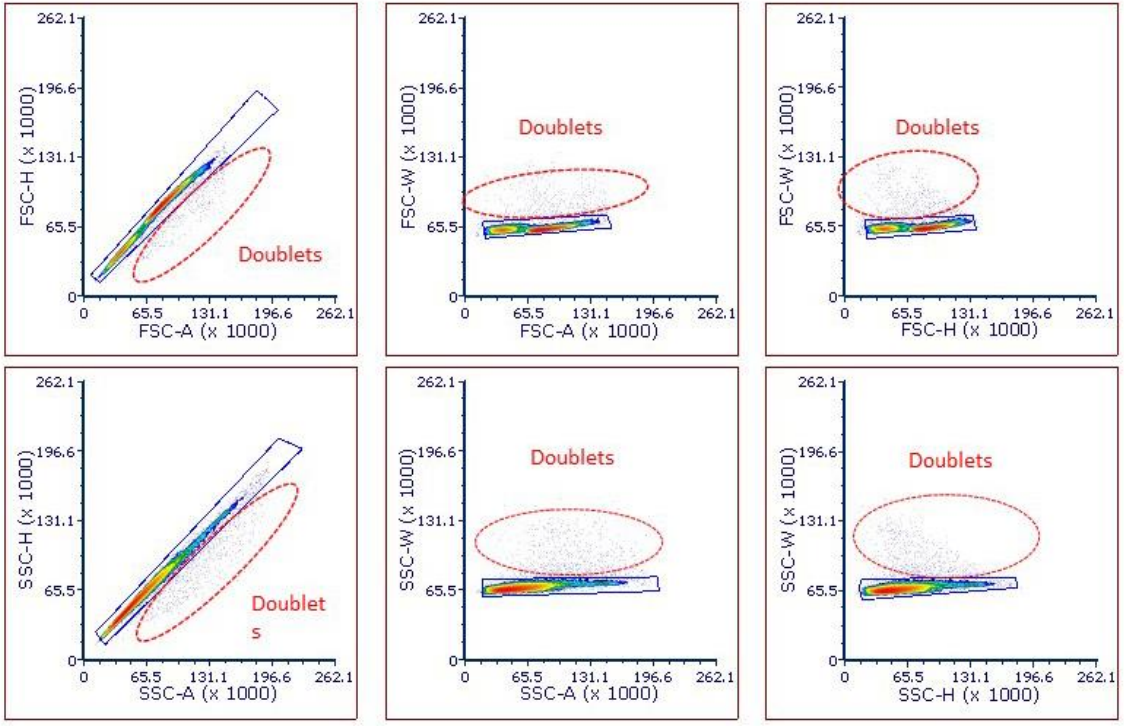
Doublets? What is the problem about…

- signals can‘t be separated → loss of data accuracy
- ➢ false positive signal, higher MFI signals, more cells in G2/M phase in cell cycle





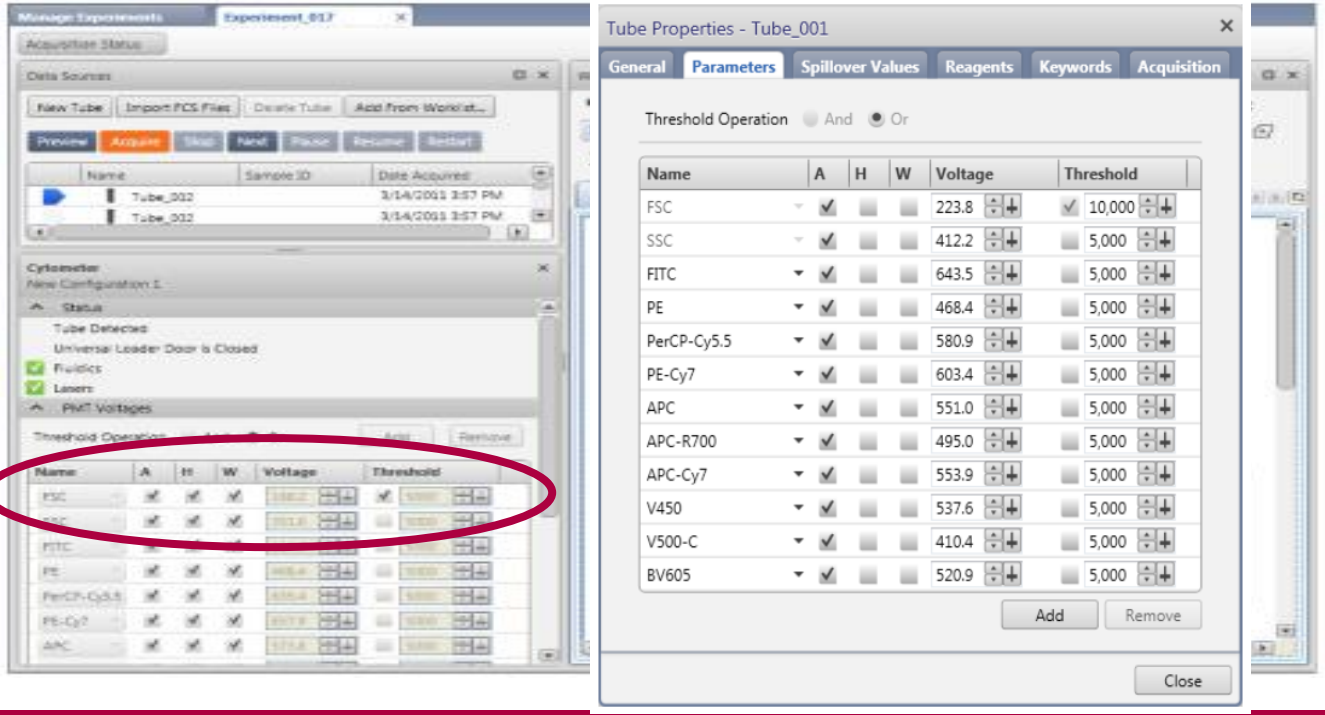
Doublets? How to exclude dublets



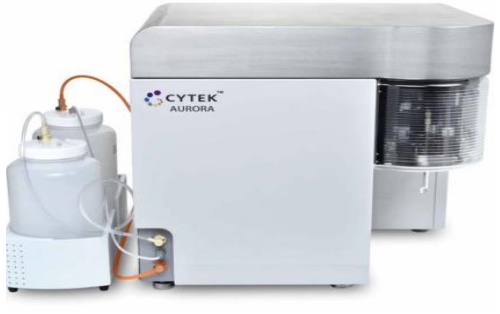
FSC or/and SSC

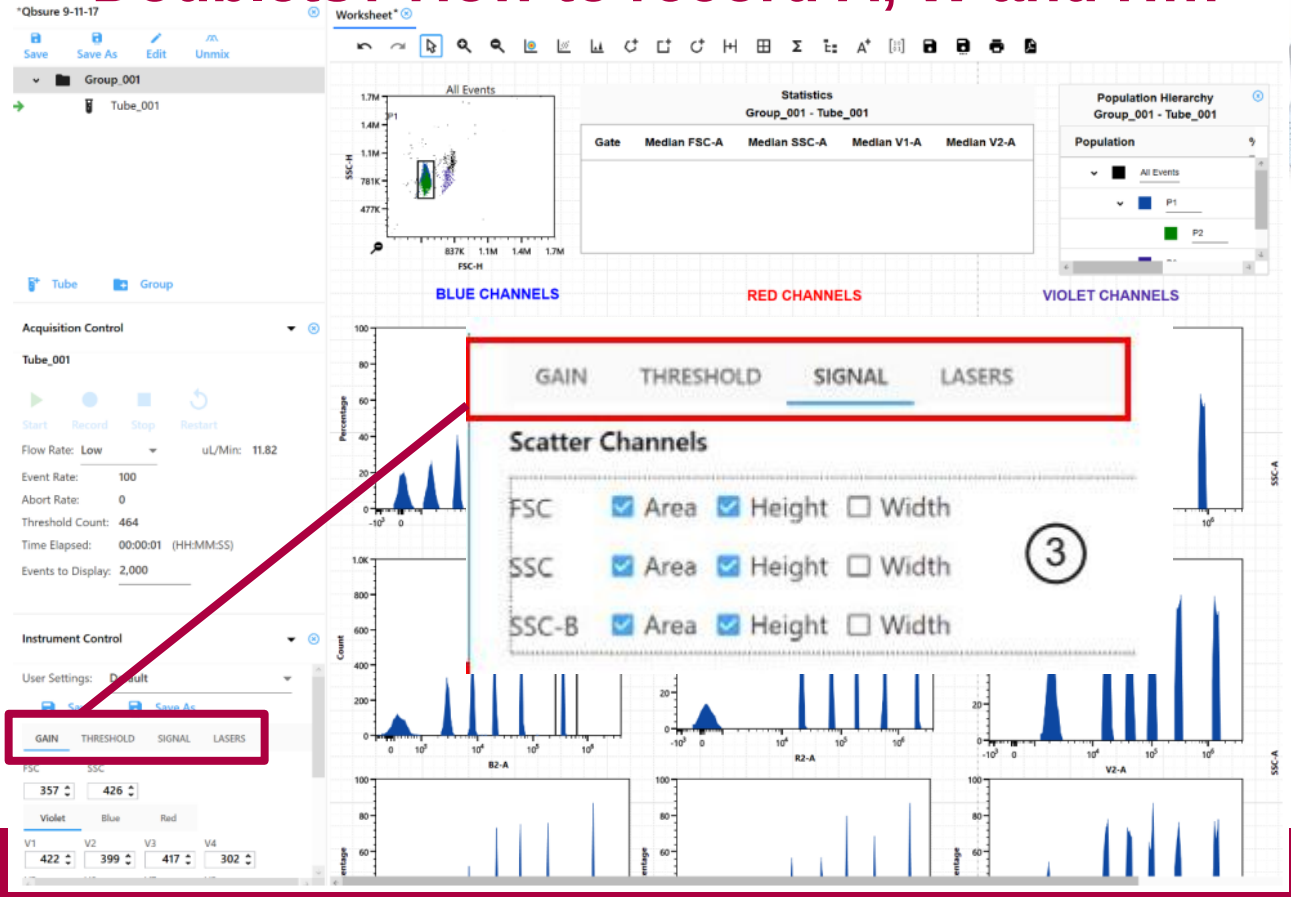
W vs. H
H vs. A

Doublets? How to record A, W and H…

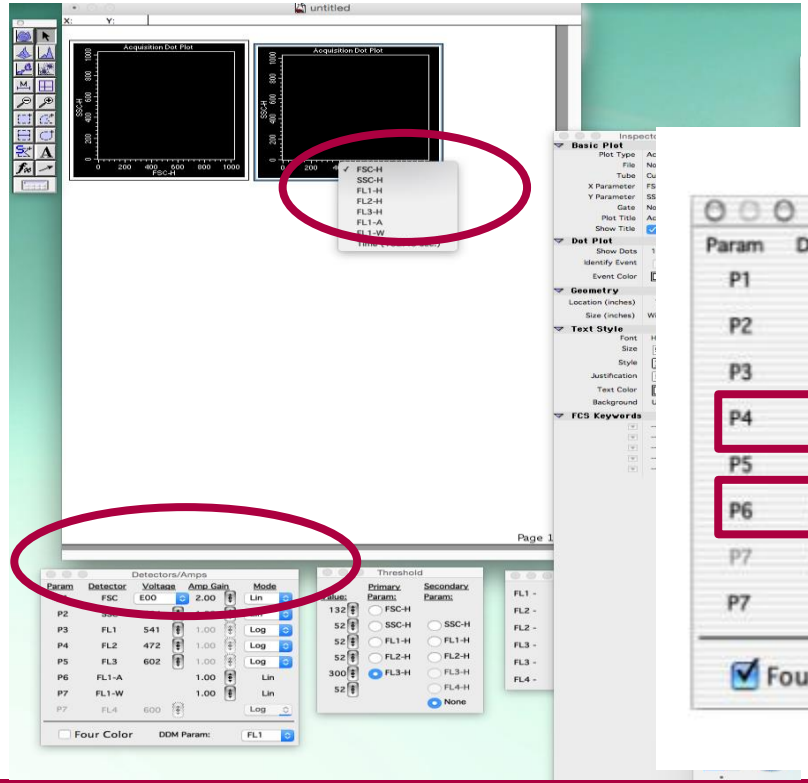


Doublets? How to record A, W and H…

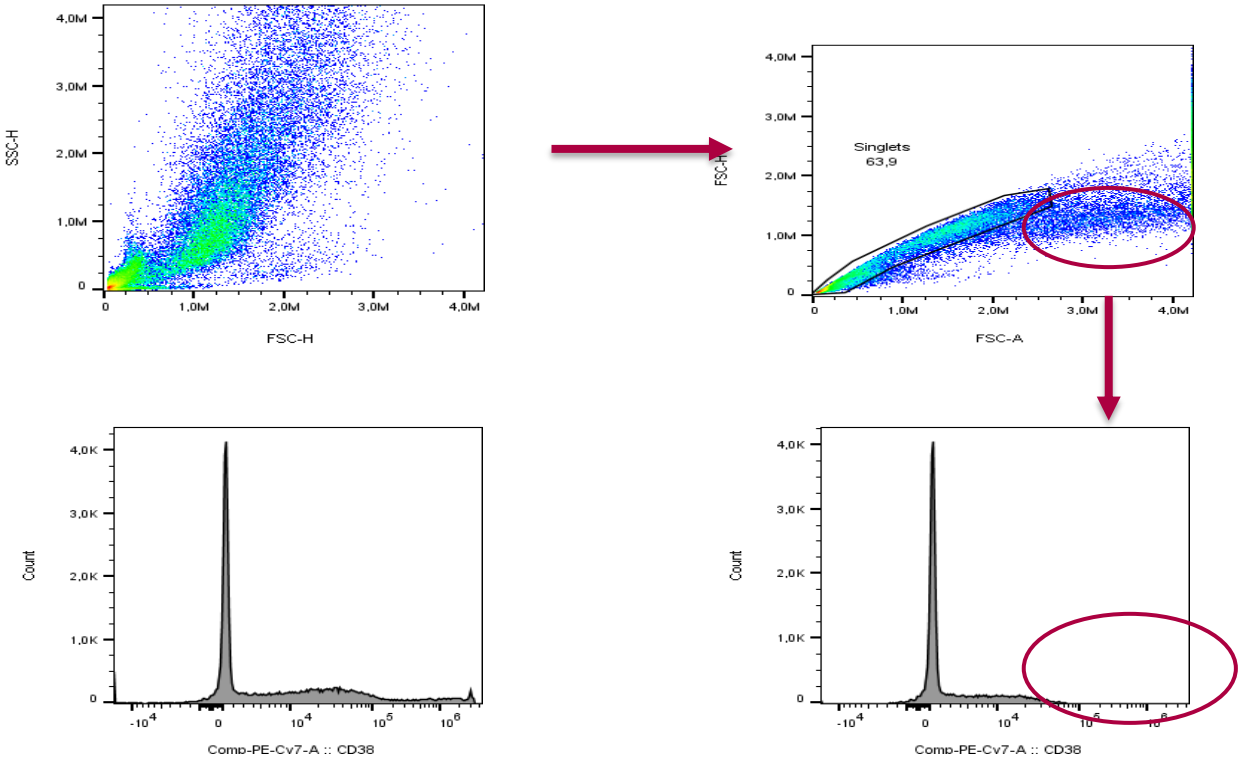




Doublets? How to record A, W and H…



Doublets? How do the ylook like…



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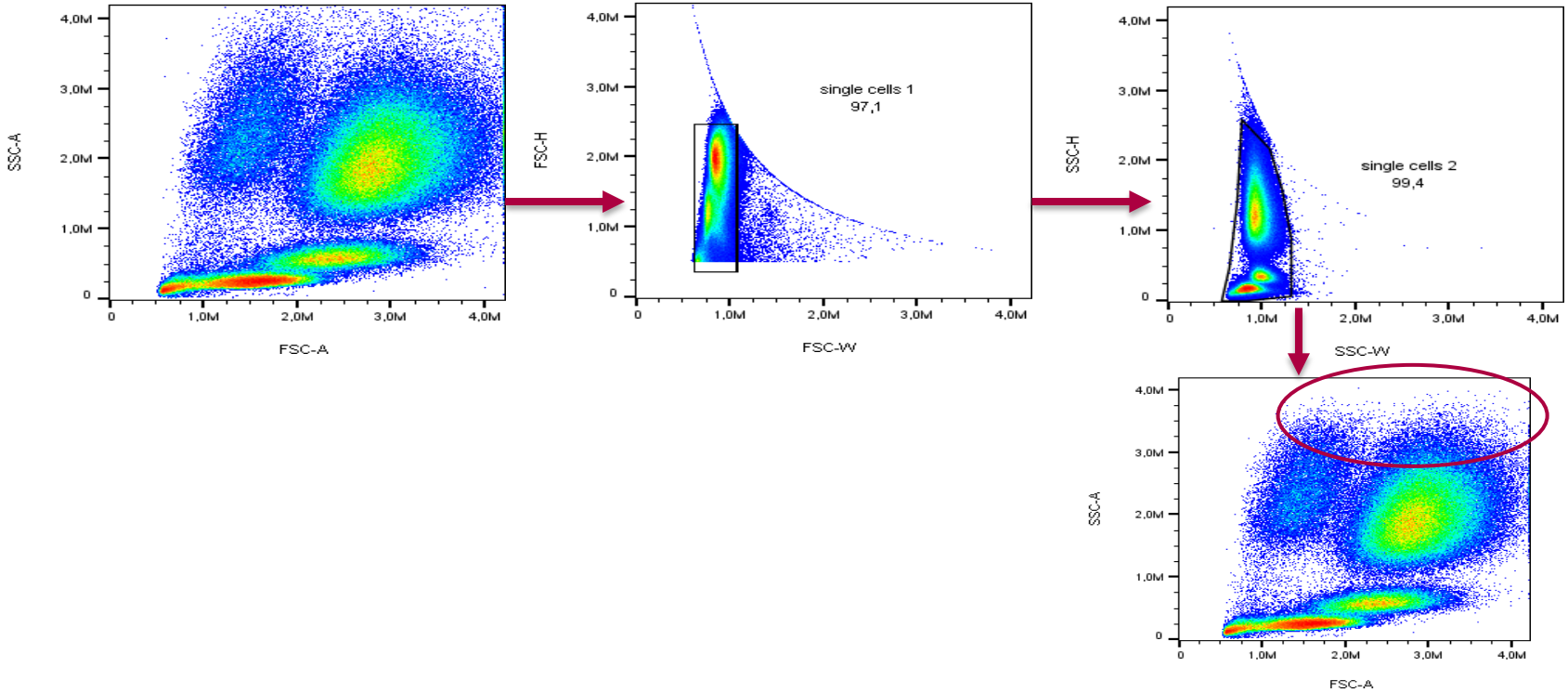
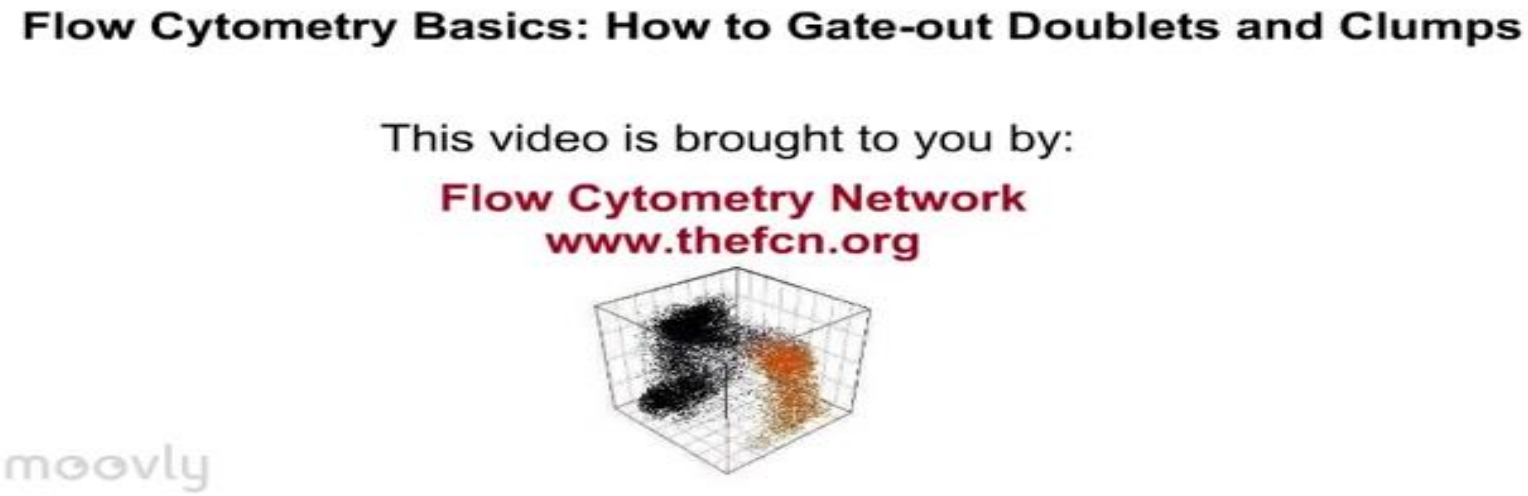


Doublets? I want more…



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 aquisition speed / flow rate or dilute cells more

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Thank you for your attention.

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

Next topic:
The perfect control – what to take?