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What is wrong with my flow cytometry data?

Hints, tricks and pitfalls

Core Facility for Cell sorting and Cell analysis

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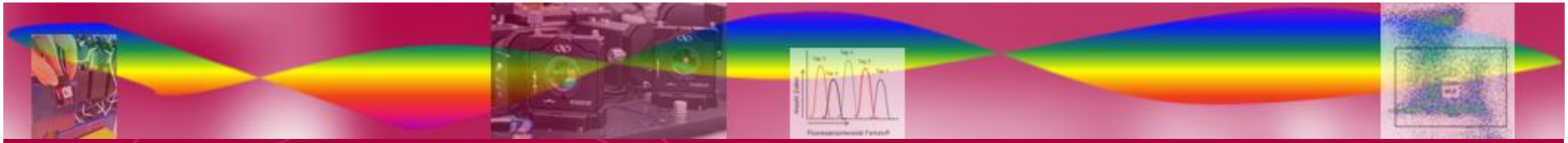

Dublets – misinterpretation preassigned

Dublettenausschluss - Fehlinterpretation vorprogrammiert.

Core Facility for Cell sorting and Cell analysis

Core Facility für Zellsortierung und Zellanalyse
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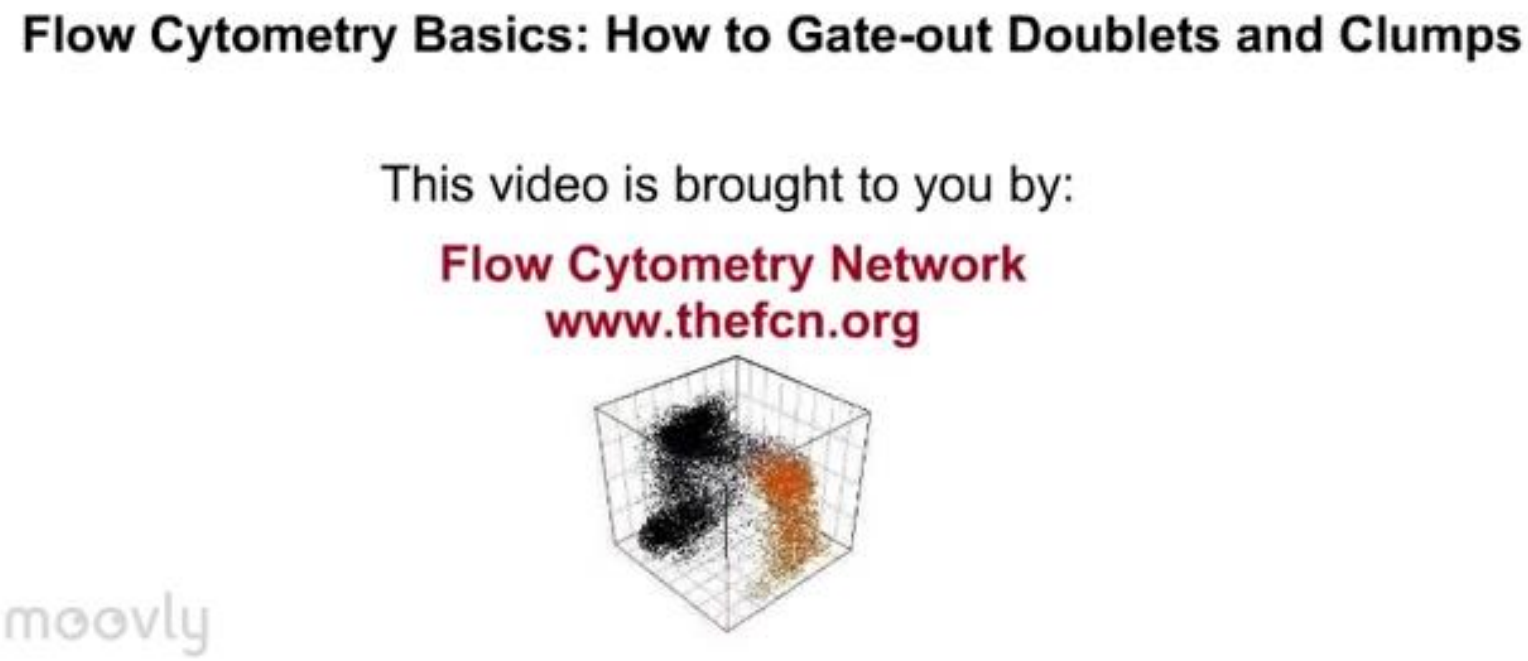
Doublets – What‘s the problem?

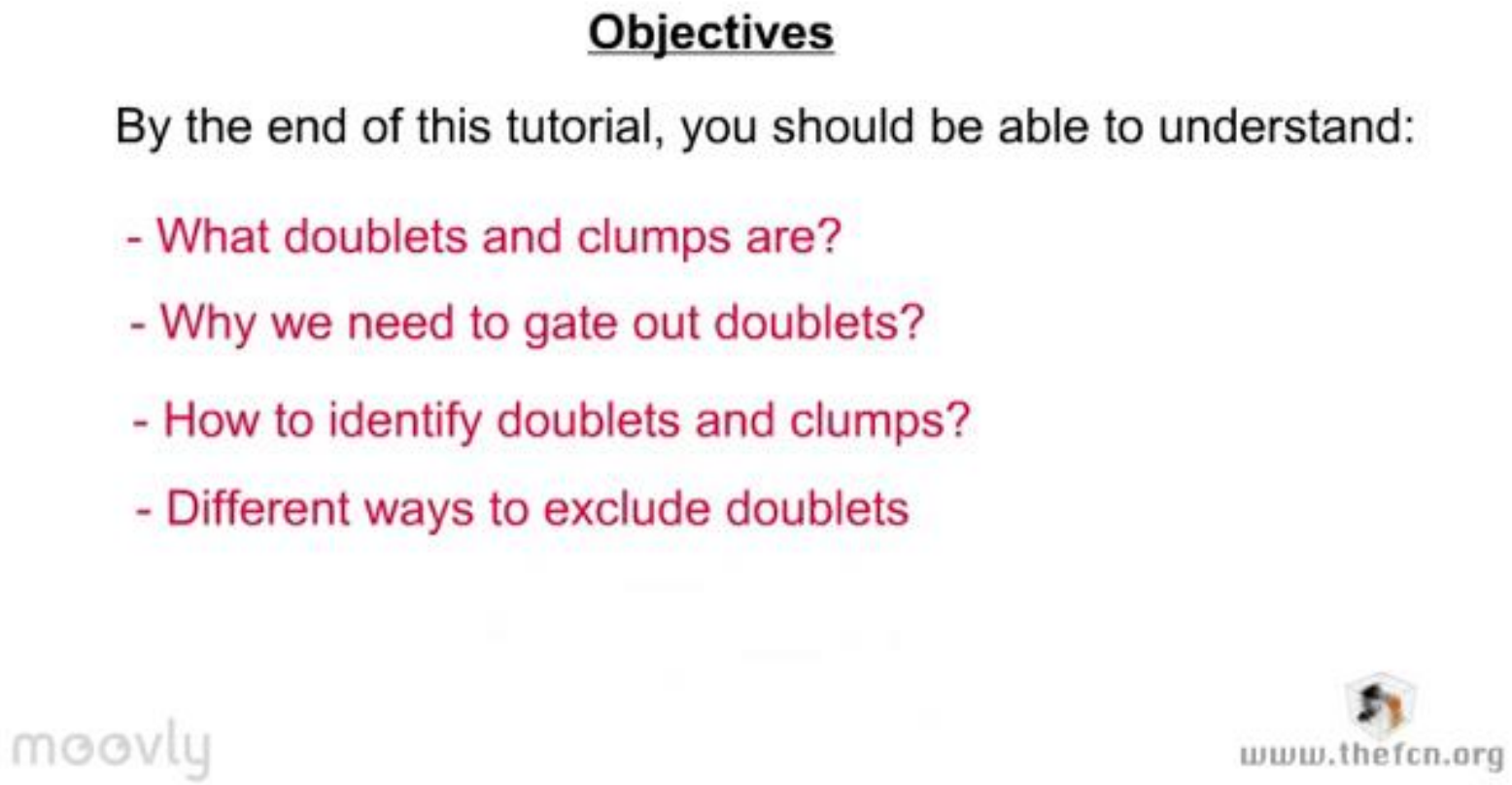
- flow cytometry → high-throughput analysis of single cell suspension
- dublets → 2 cells analysed at the same time
- signals can‘t be separated → loss of data accuracy
- false positive signal, higher MFI signals, more cells in G2/M phase in cell cycle

Doublet discrimination – Youtube tutorial



https://www.youtube.com/watch?v=MjMvwSUucKI

Doublet discrimination – Youtube tutorial



https://www.youtube.com/watch?v=MjMvwSUucKI

Doublet discrimination - Next NEWLETTER

NEWS + + + NEWS + + + NEWS + + + NEWS + + + NEWS + + + NEWS + + + NEWS + + + NEWS

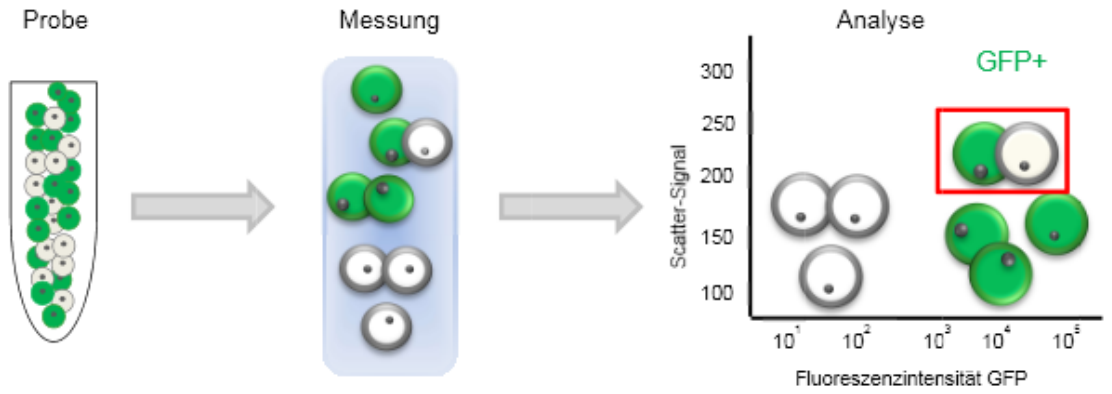
Aus der Core Facility für Zellsortierung und Zellanalyse

DOUBLE TROUBLE

- ÄRGER MIT DEN DUBLETTEN -

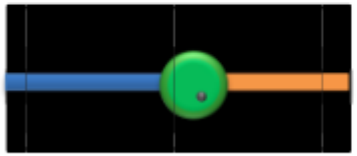
Kein Luxusproblem, sondern häufige Ursache von Fehlinterpretationen.

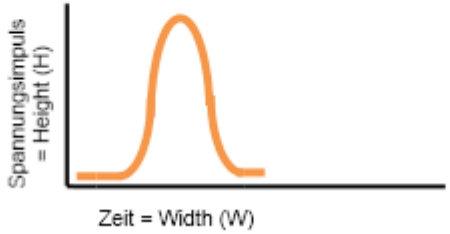
Trotz sorgfältiger Präparation kleben Zellen oft aneinander. Im Beispiel unten aggregiert eine GFP-negative mit einer GFP-positiven Zelle, so dass bei herkömmlichen Analysen beide als GFP-positiv wahrgenommen werden. Diese sogenannte Dublettenbildung führt bei 5- 20% aller Zellen – eher mehr bei Primärisolaten – zur Fehlinterpretation.

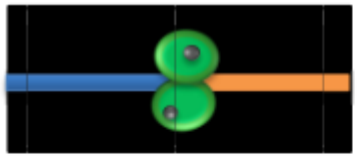


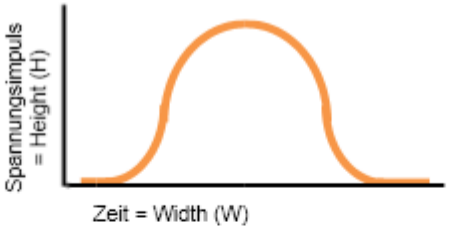
Die Lösung: **Dublettendiskriminierung!**

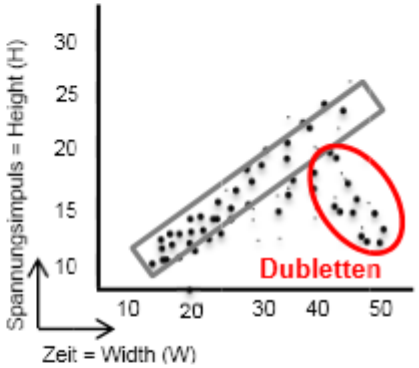
Dubletten benötigen mehr Zeit, den Laserstrahl zu passieren – damit wird das Weite-Signal größer. Über dieses vergrößerte Weite-Signal können Dubletten aus der weiteren Analyse ausgeschlossen werden.











Möchten Sie Ihre Analyse optimieren und haben Fragen dazu?

Dann einfach anrufen oder Email schreiben!

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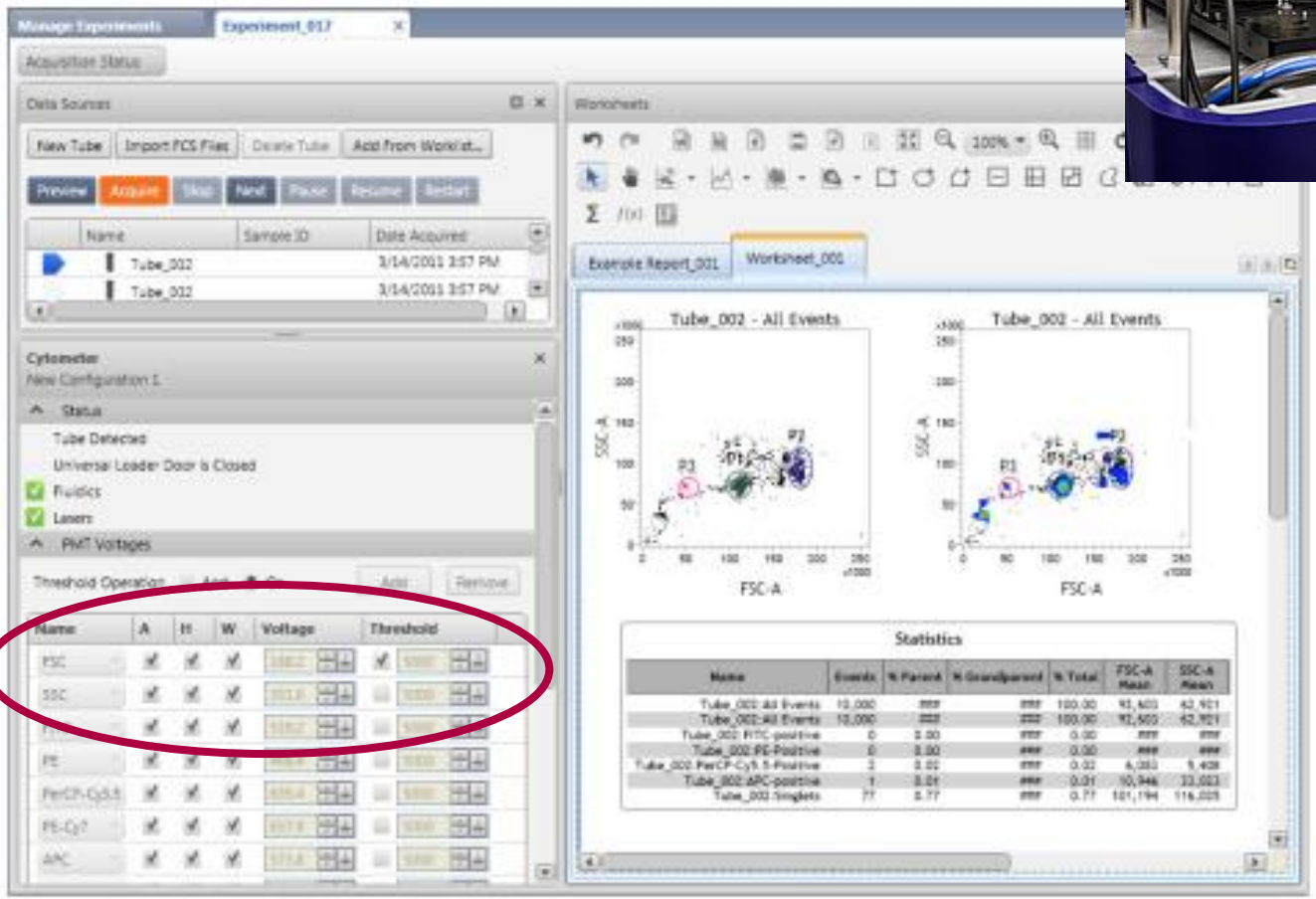
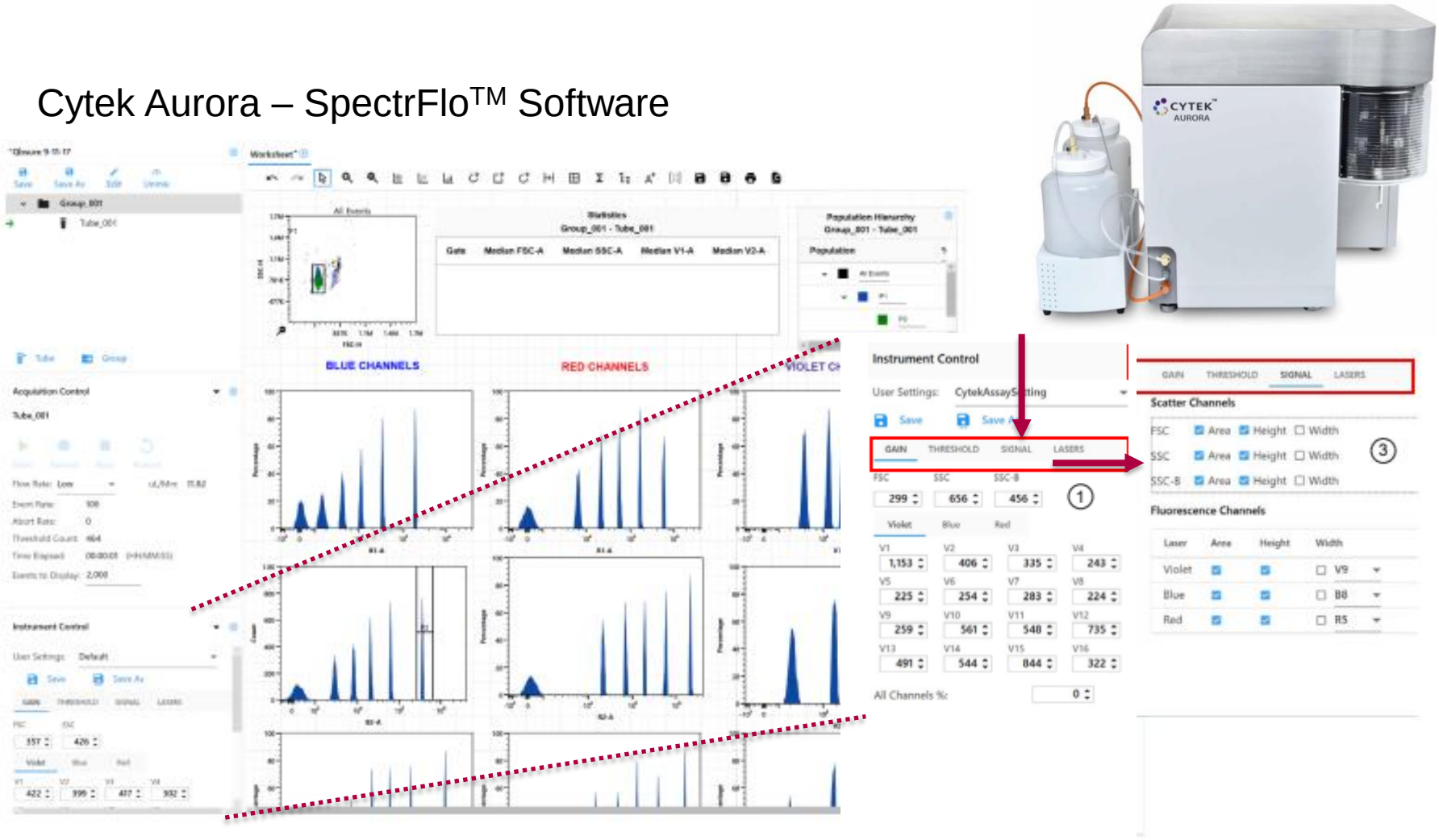
How to record FSC/SSC-Area Height Width

FACS Verse – FACS Suite Software



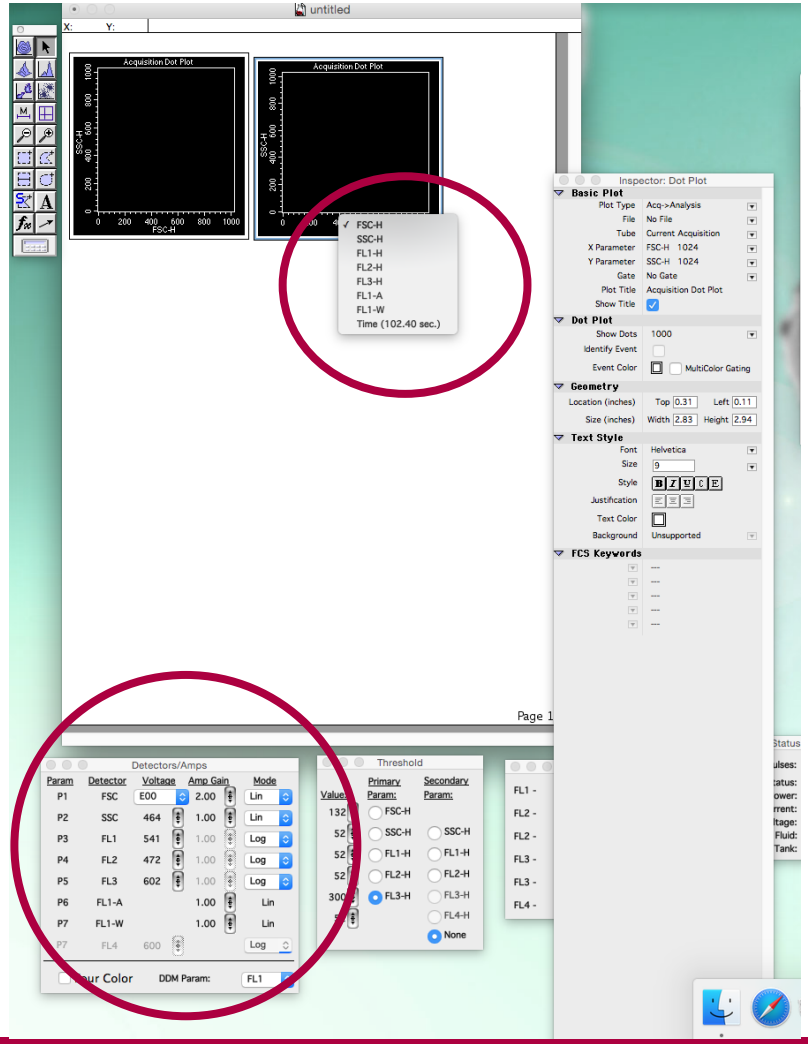
How to record FSC/SSC-Area Height Width

Cytek Aurora – SpectrFlo™ Software

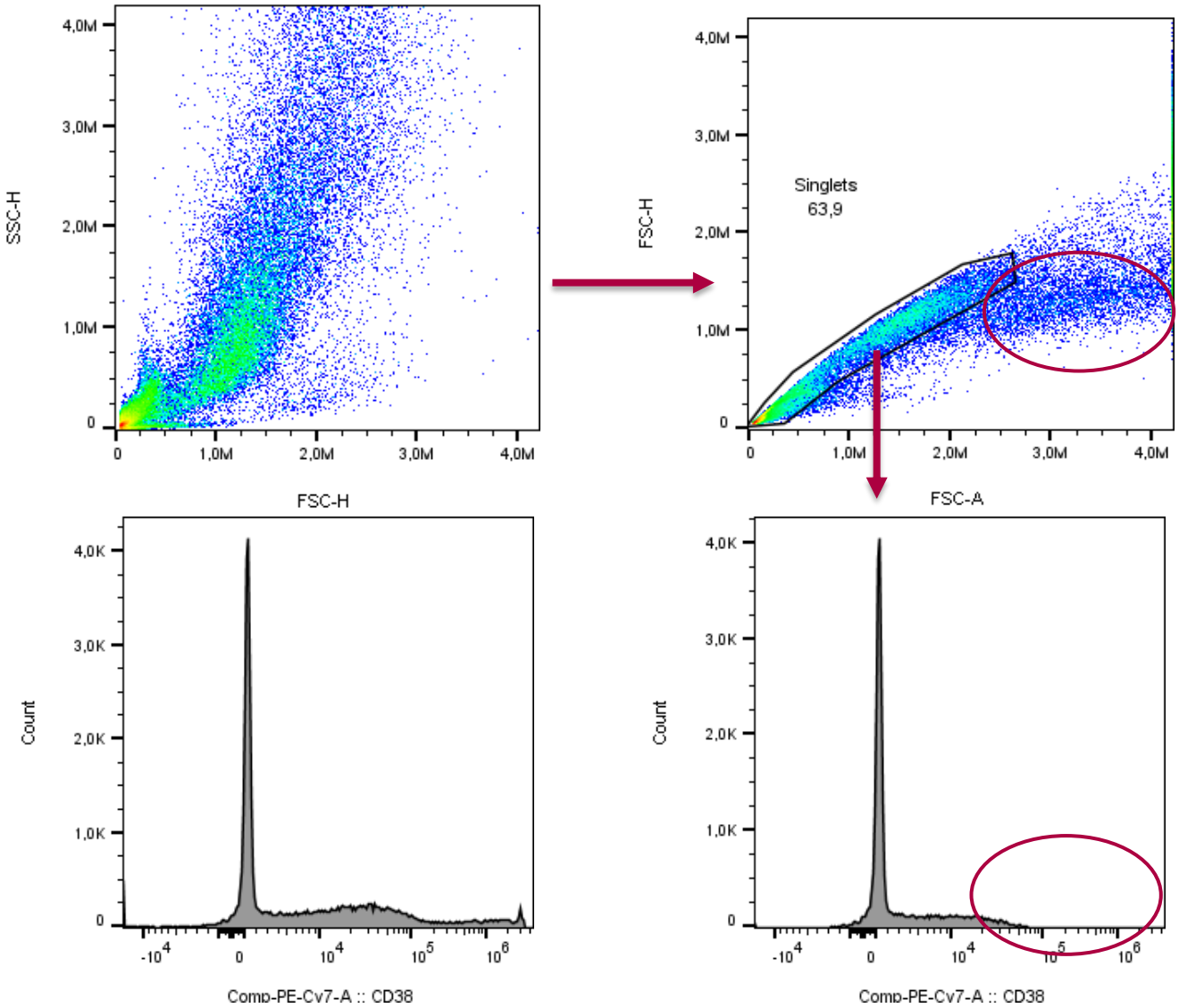


How to record FSC/SSC-Area Height Width

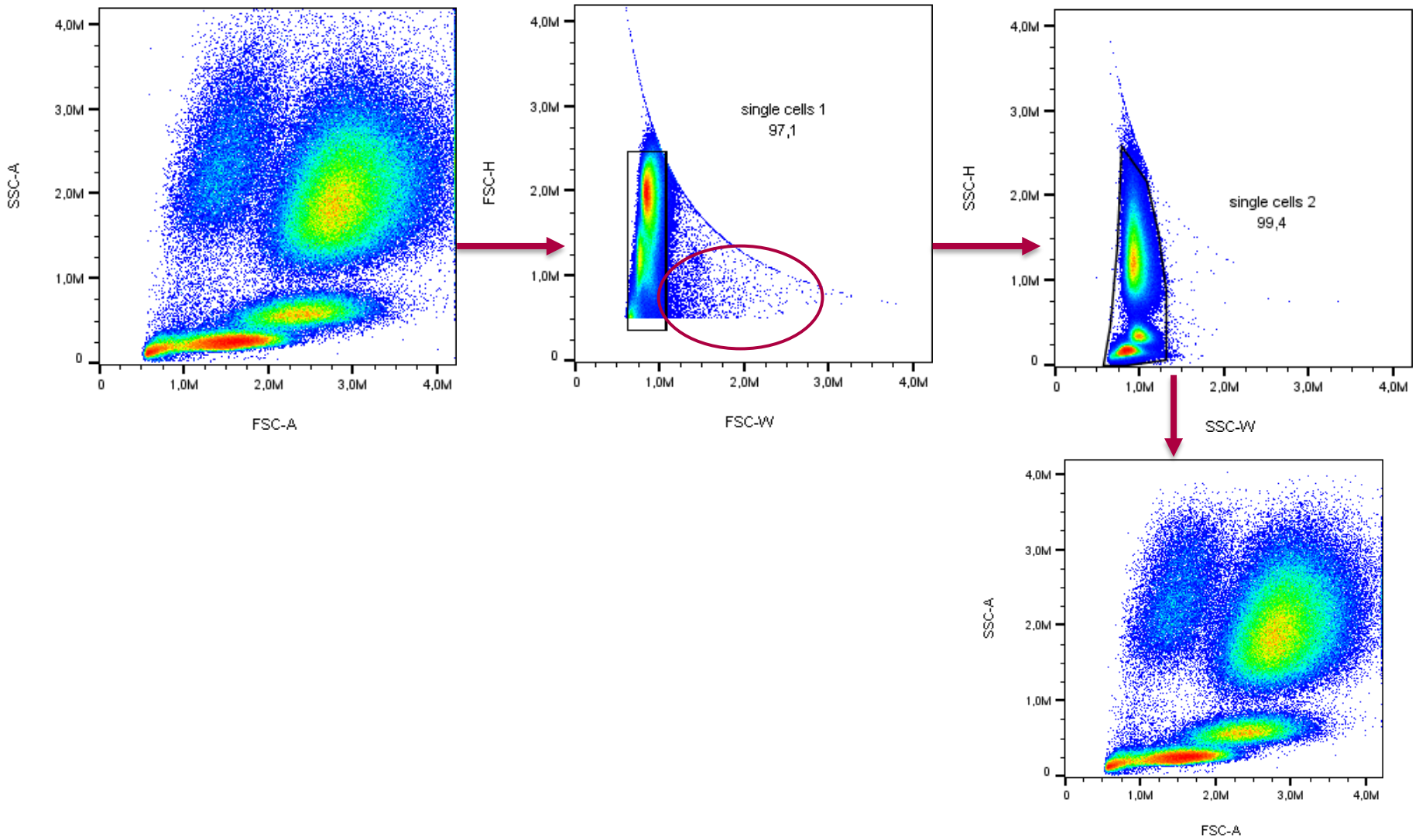
BD FACSCalibur™ – Cell Quest Pro



example data and gating



example data and gating



Doublet discrimination - TAKE HOME MESSAGE

- Prevent doublet formation:
  - Filter cells
  - Resuspend cells
  - 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 02nd March.

Next topic:
The perfect control – what to take?

Die perfekte Kontrolle – aber wie und womit?