Universität Rostock Traditio et Innovatio

Universitätsmedizin Rostock

Cell cycle analysis – simple but complicated?

Zellzyklusanalysen – einfach und doch kompliziert?

Core Facility for Cell sorting and Cell analysis

Cell cycle analysis – simple but complicated?

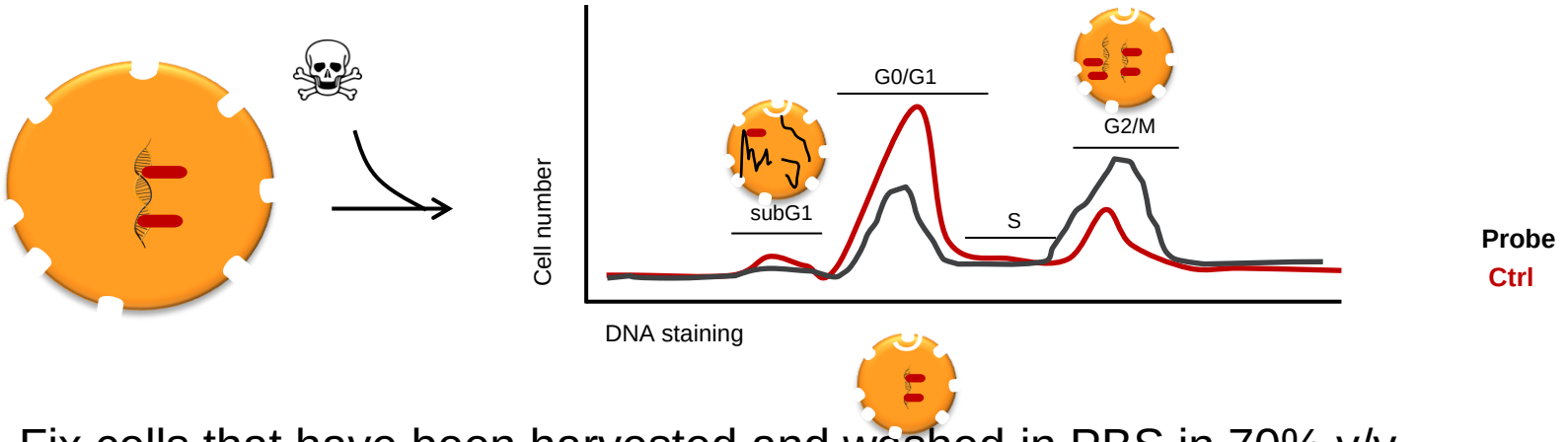
simple:

- use of intercalating DNA-Dyes
- only one colour needed → can be done on nearly any flow cytometer
- one of first applications of flow cytometry in history

complicated:

- many different protocols available
- small differences in treatment can have big effects
- RNA, doublets and clumps disturb cell cycle analysis

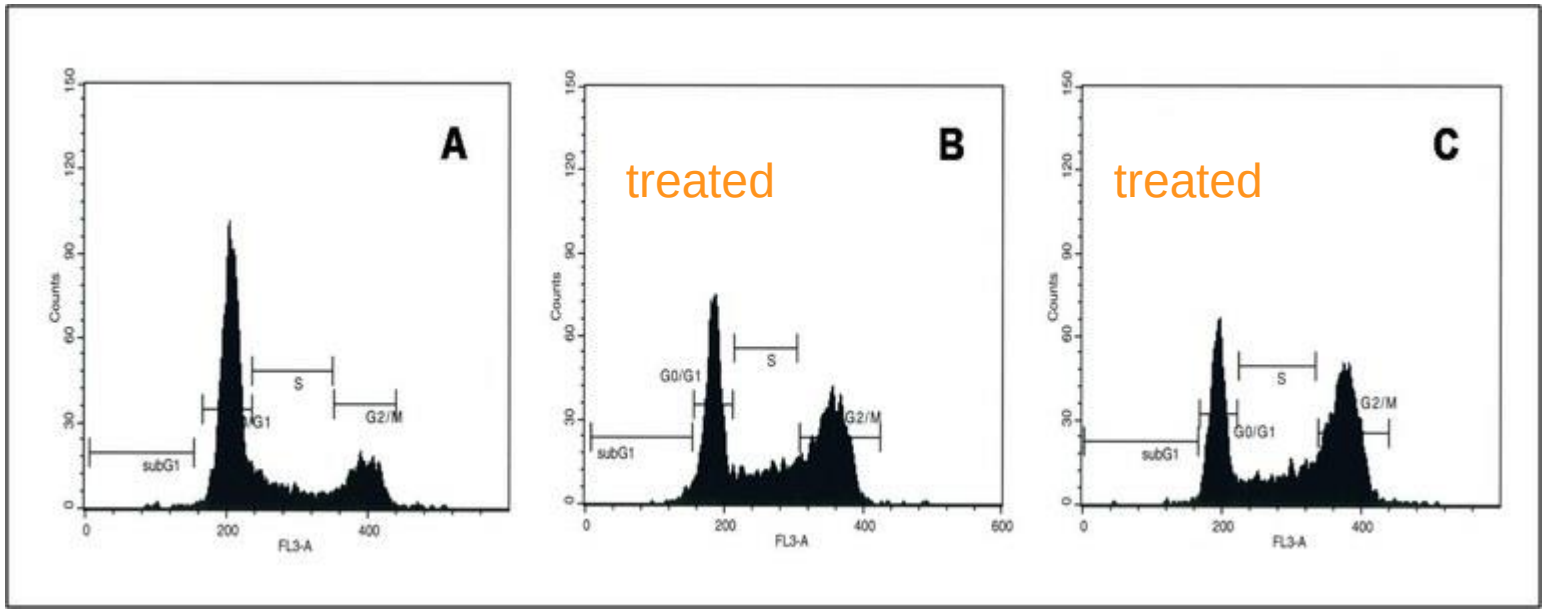
principle and general steps



1. Fix cells that have been harvested and washed in PBS in 70% v/v ethanol. Adding the ethanol dropwise to the cell pellet while vortexing will ensure that all cells are fixed and will minimize clumping.
2. Fix cells for 30 min at 4°C, after which wash cells twice in PBS (850 × *g*). Be careful to avoid cell loss when discarding the supernatants.
3. Treat cells with RNAse (50 µL, 100 µg/mL) in order to ensure that only DNA is stained.
4. Add PI (200 µL PI, 50 µg/mL stock solution) immediately before analyzing.

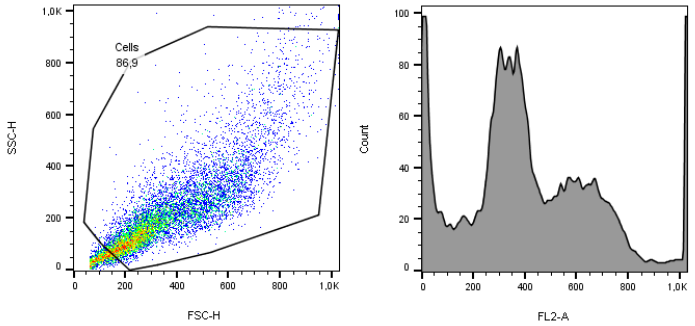
Cossarizza, A et al. “Guidelines for the use of flow cytometry and cell sorting in immunological studies” (second edition). (2019), Eur. J. Immunol., 49: 1457-1973. https://doi.org/10.1002/eji.201970107

Example of cell cycle arrest

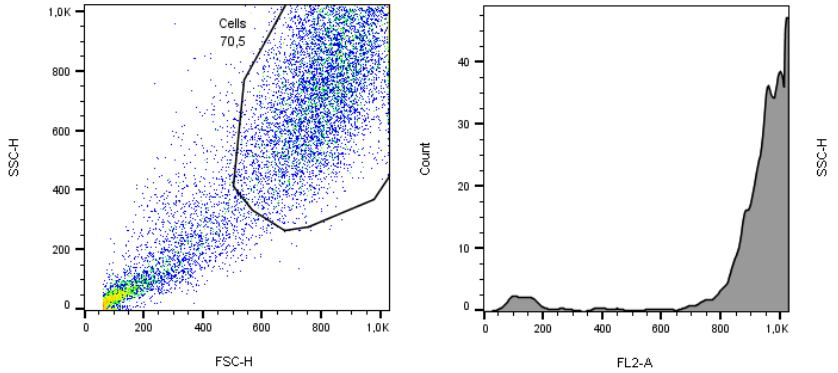


https://www.uni-giessen.de/fbz/fsp/meu/methodenplattform/analysen2/zellzyklusanalysen

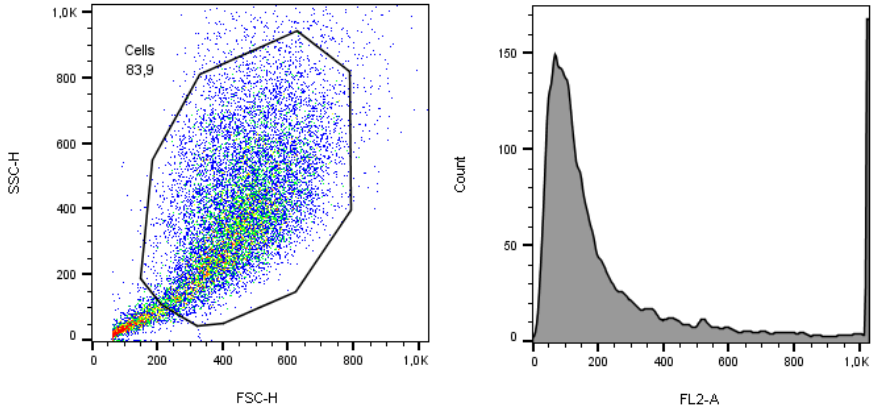
Cell cycle analysis – **What had happened here?**



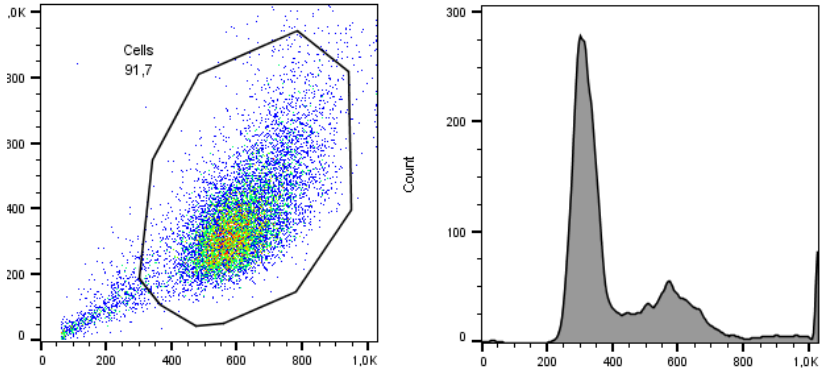
too many dead cells,
low number of living cell



PMTV for scatter and dye not set properly



too much PI added



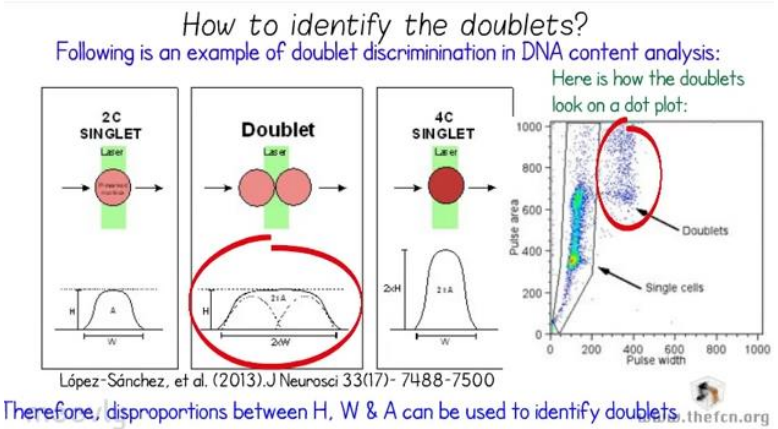
no doublet exclusion

What to consider?

- Many DNA Dyes available:
  - PI, Hoechst stains, TO-PRO-3, SYTOX, acridine orange, pyronin Y, 7-AAD, DAPI, DRAQ5™, and DRAQ7™
  - bind RNA (PI), others less (7-AAD, DAPI), staining of living cells (DRAQ Dyes)
- PFA-fixation reduces resolution, because of chromatin crosslinking
- Always count cells before DNA staining →

  DNA-dye – cell – ratio should be stable

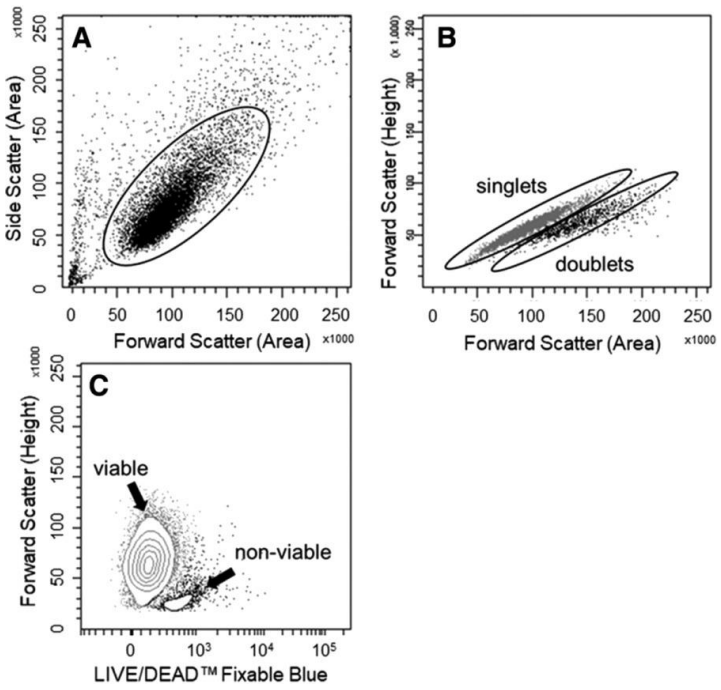
What to consider?



- exclude doublets
- if possible exclude dead cells

  (scatter or better fixable live-dead-stain)
- lower flow rate results in more accurate aquisition → sharp peaks
- measure & analyze with linear scaling
- record 10.000 – 20.000 cells
- adjust G1 peak to a definite value, e.g.~ 200, and check for the G2/M to appear at the doubled value , e.g. 400 (doubling of DNA content)

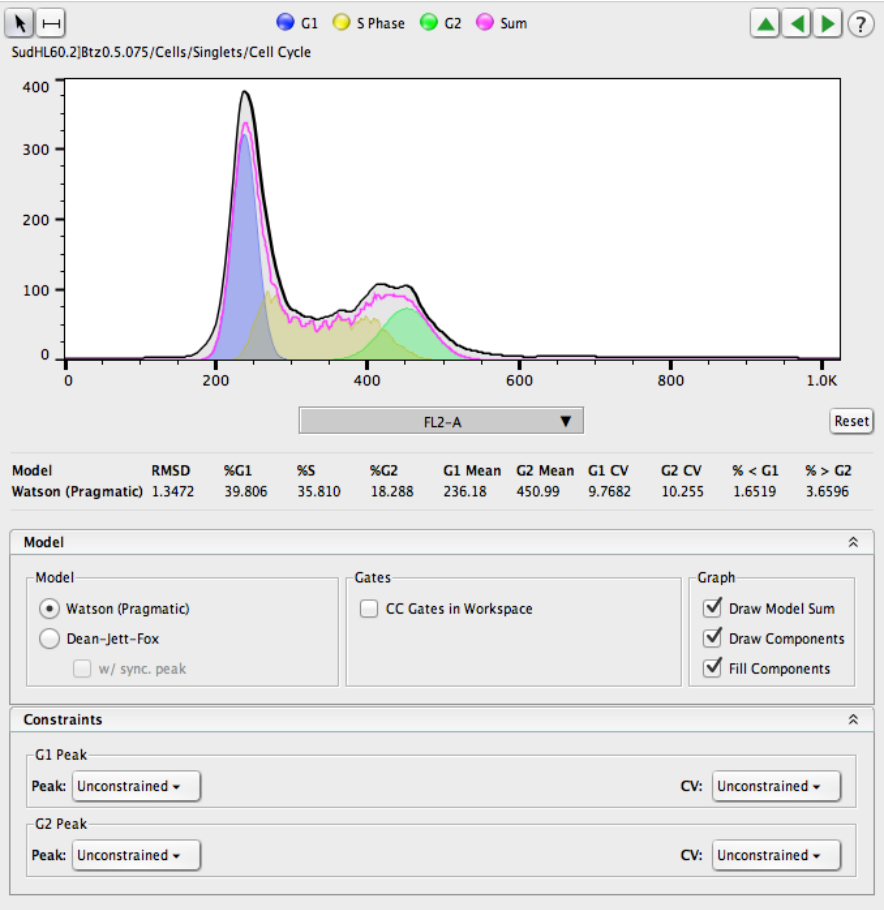
experimental improvements



- adjust concentration of treatment (too many apoptotic cells)
- include live-dead-staining
- include untreated control (always same confluency)
- maybe starve and synchronize cells before assays start

European Journal of Immunology, Volume: 49, Issue: 10, Pages: 1457-1973, First published: 21 October 2019, DOI: (10.1002/eji.201970107)

cell cycle analysis tools



- Specialized programs such as ModFit LT™ from Verity Software House (http://www.vsh.com/products/mflt/mfFeatures.asp) and Multicycle AV™ from Phoenix Flow Systems (http://www.phnxflow.com/MultiCycle.stand.alone.html) have been designed for this purpose.

European Journal of Immunology, Volume: 49, Issue: 10, Pages: 1457-1973, First published: 21 October 2019, DOI: (10.1002/eji.201970107)

https://docs.flowjo.com/flowjo/experiment-based-platforms/cell-cycle-univariate/

cell cycle analysis –TAKE HOME MESSAGE

- improve data quality:
  - dye selection and protocol optimization
  - doublet exclusion and low flow rate
  - because of linear scale small differences have big effect
  - stable cell number and dye concentration needed
- FlowJo provides easy analysis tool
  - needs clear G1-peak
  - good untreated control necessary

- ‚easy‘ cell cycle analysis can be more complicated than thought

Universität Rostock Traditio et Innovatio

Universitätsmedizin Rostock

Thank you for your attention.

See you next month 04th May.

Next topic:
Aggravating compensation –
discover errors and correct them

Die leidige Kompensation – Fehler erkennen und nachbessern