Application of flow cytometry to assess microbial water quality

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Vannforsk Webinar - Drikkevann
7. oktober 2022

NTNU
Knowledge for a better world

Microbial contamination is the principal risk to public health associated with drinking water (WHO, 2017)

Ensuring Safe Drinking Water quality is challenging due to many factors such as population growth, global warming, and outbreaks of water-borne diseases.

Multibarrier approach focus on three layers:

1) Source of water protection
2) Water Treatment
3) Monitoring and Testing → Heterotrophic and selective plate count are legal standards

Presentation goal: Assessment of microbial water quality

There is a variety of online microbial monitoring techniques commercially available. The focus of the presentation is on flow cytometry

WHO, 2017, Guidelines for drinking water quality

Picture credit: crayon

Detection methods

State of the art

	Culture methods	Rapid Culture methods	qPCR
	 ISO 11731:2017		
Detection time	3 – 10 days	18hr – 7days	3 – 4 hrs
Sensitivity	moderate	moderate	very high
Skilled operator	yes	no	yes
Detection process	laborious	simple	laborious
Waste generation	high	high	high

Detection methods

Online microbial monitoring devices

	Enzymatic analysis	ATP	Online flow cytometry (BactoSense)
	 ColiMinder – VWMs GmbH	 EZ-ATP - Hach	 bNovate technologies
Detection time	15 min	7 – 10 minutes	20 minutes
Sensitivity	Very high		very high
Skilled operator	yes		no
Maintenance	laborious		simple
Waste generation	Replaceable reagent kit		None / reusable cartridges

Principle of Flow cytometry

1

Labeling the biological sample (cells) with a fluorescence material

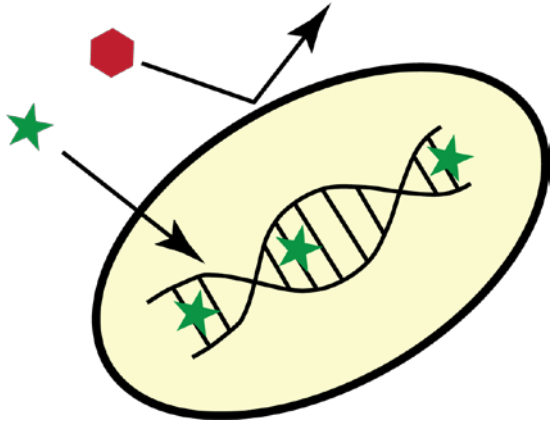
2

Measurement of fluorescence and scattering from each cells

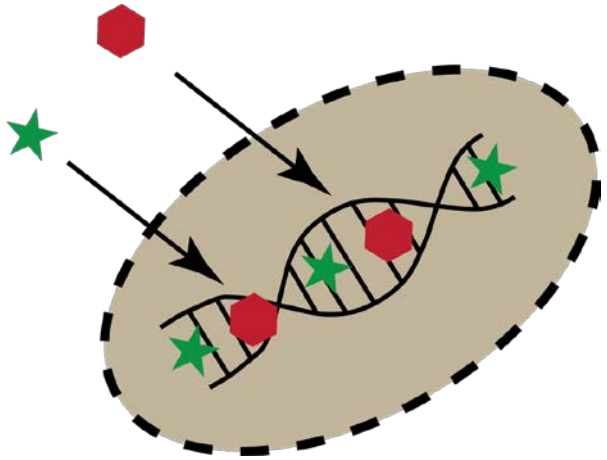
3

Grouping the different cell types and analyzing data

Labelling of bacterial cells



The total cell count (TCC) of any water sample can be determined using SYBR Green 1, which enters and stains all bacteria that contain nucleic acids independently of membrane integrity.

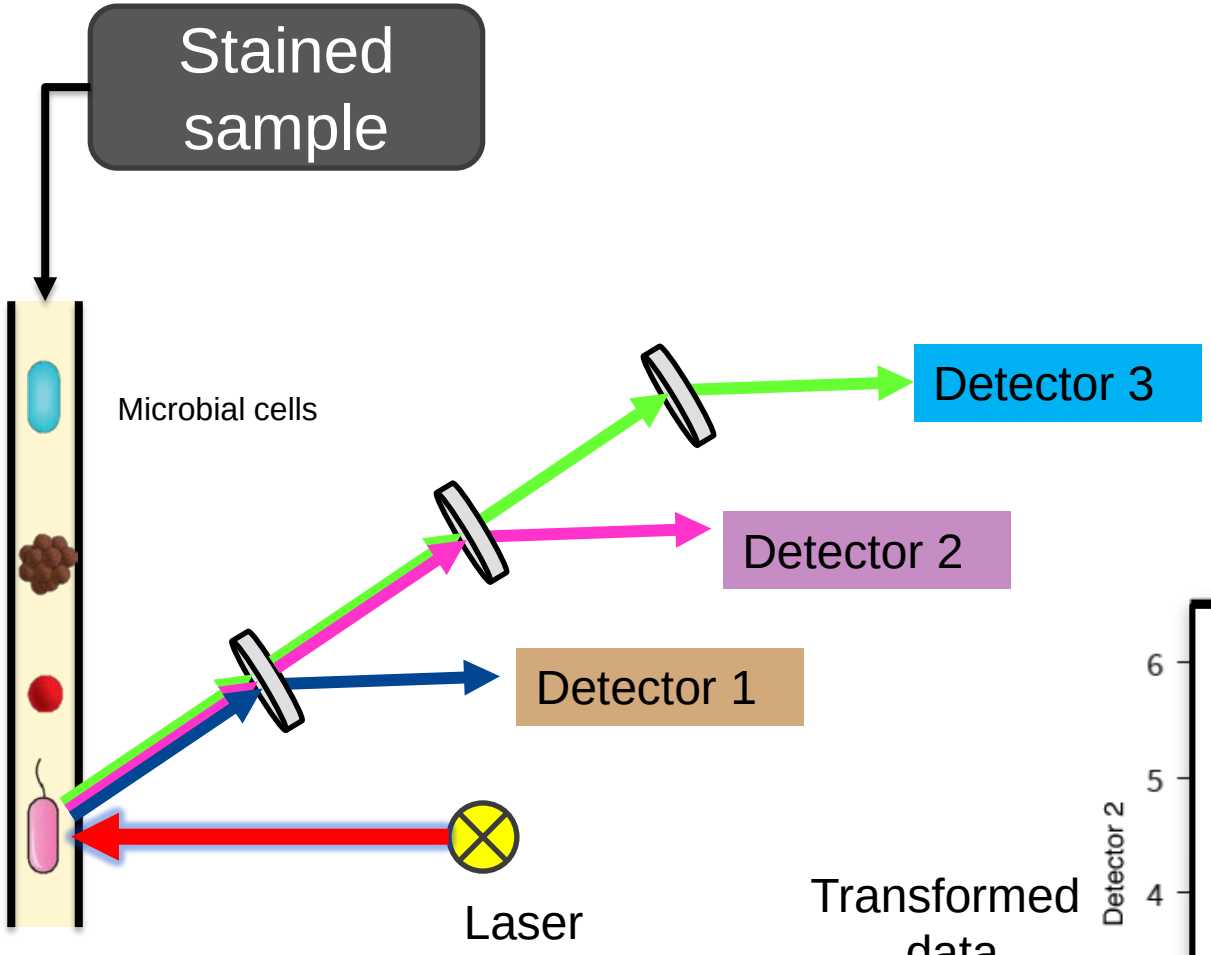


Intact cell count (ICC) can be distinguished from damaged bacteria and other inorganic particles using double staining SYBR Green 1 and Propidium iodine which can penetrate cells with damaged membranes.

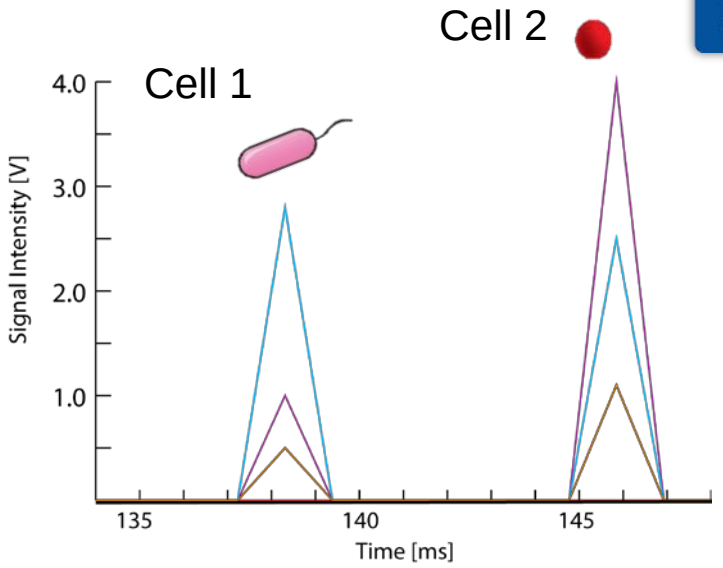
SYBER Green I Propidium iodide

Credit: bnovate

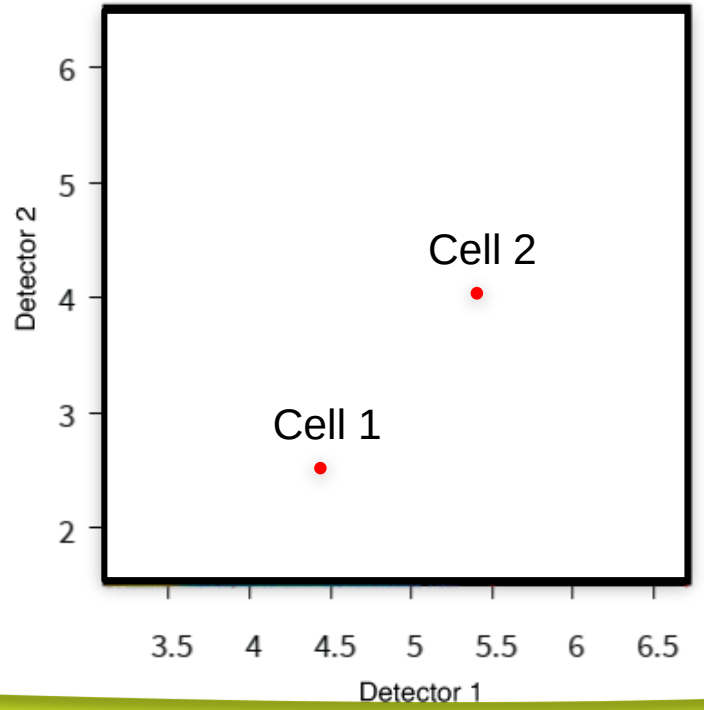
Detection of bacterial cells



Signals from the detectors

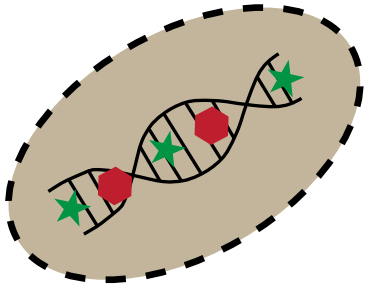


Transformed data

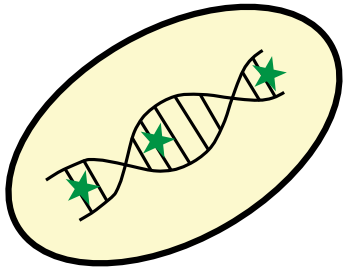


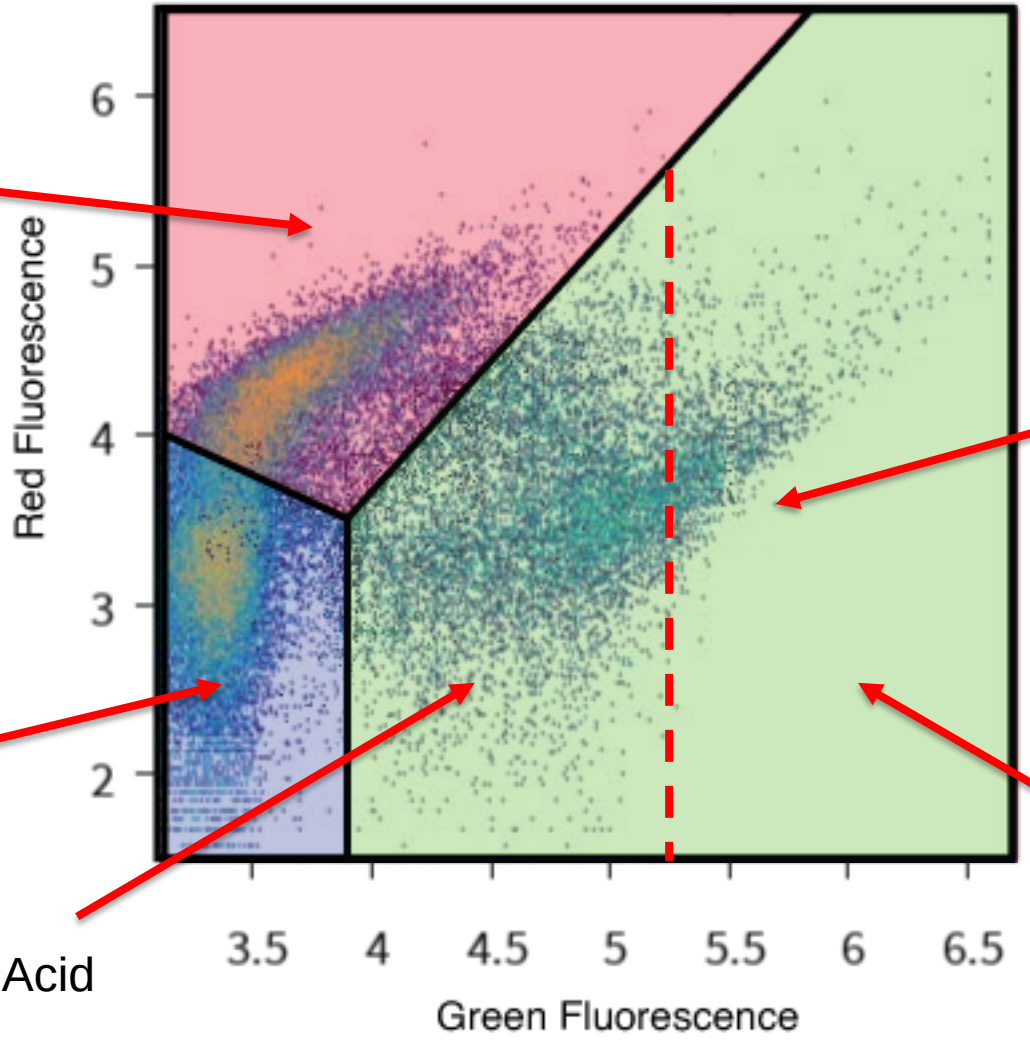
Interpreting FCM results

Damaged Cells



Intact cells



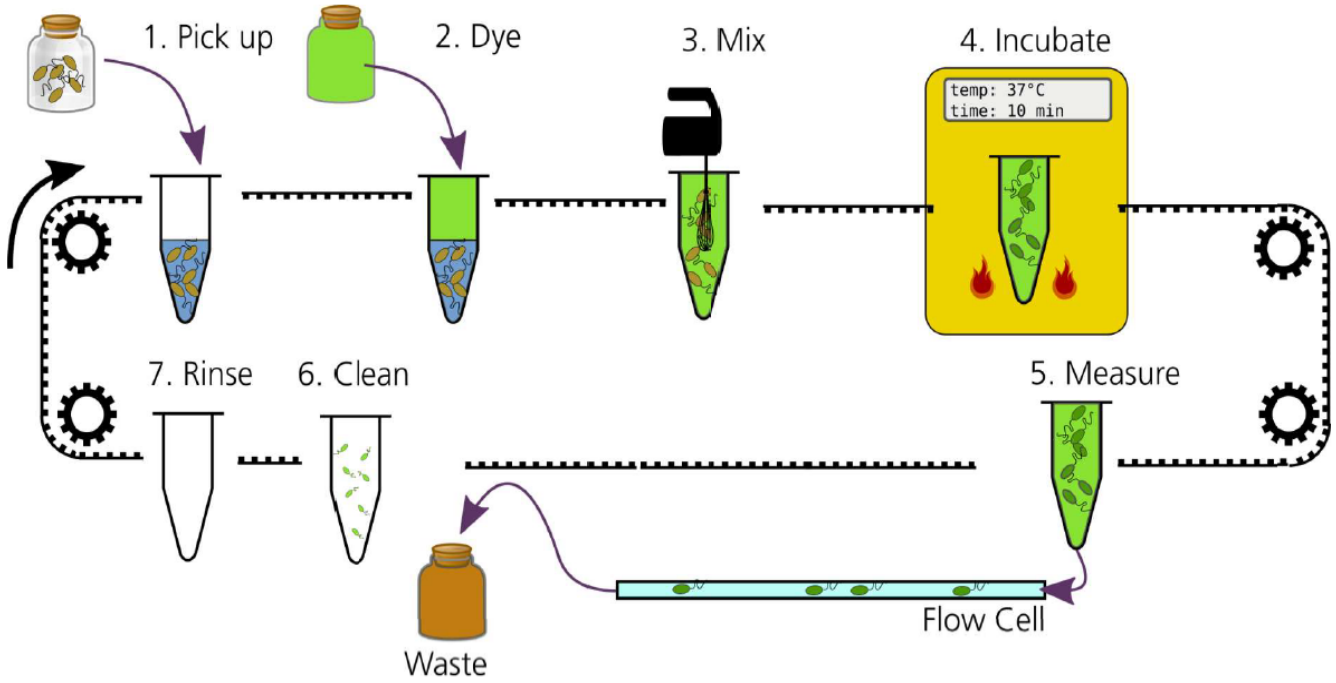


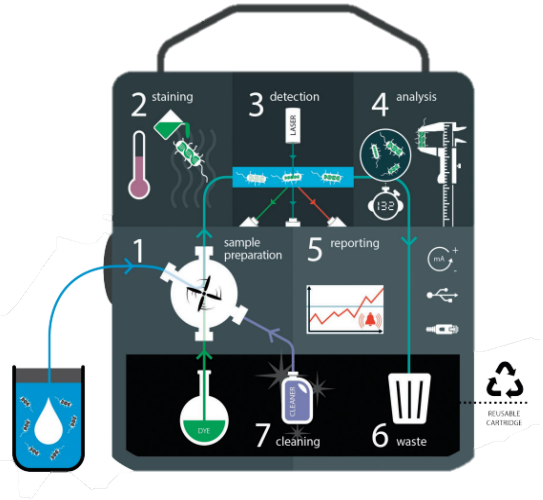
System noise

Low Nucleic Acid

High Nucleic Acid

Workflow of an online FCM
(BactoSense)





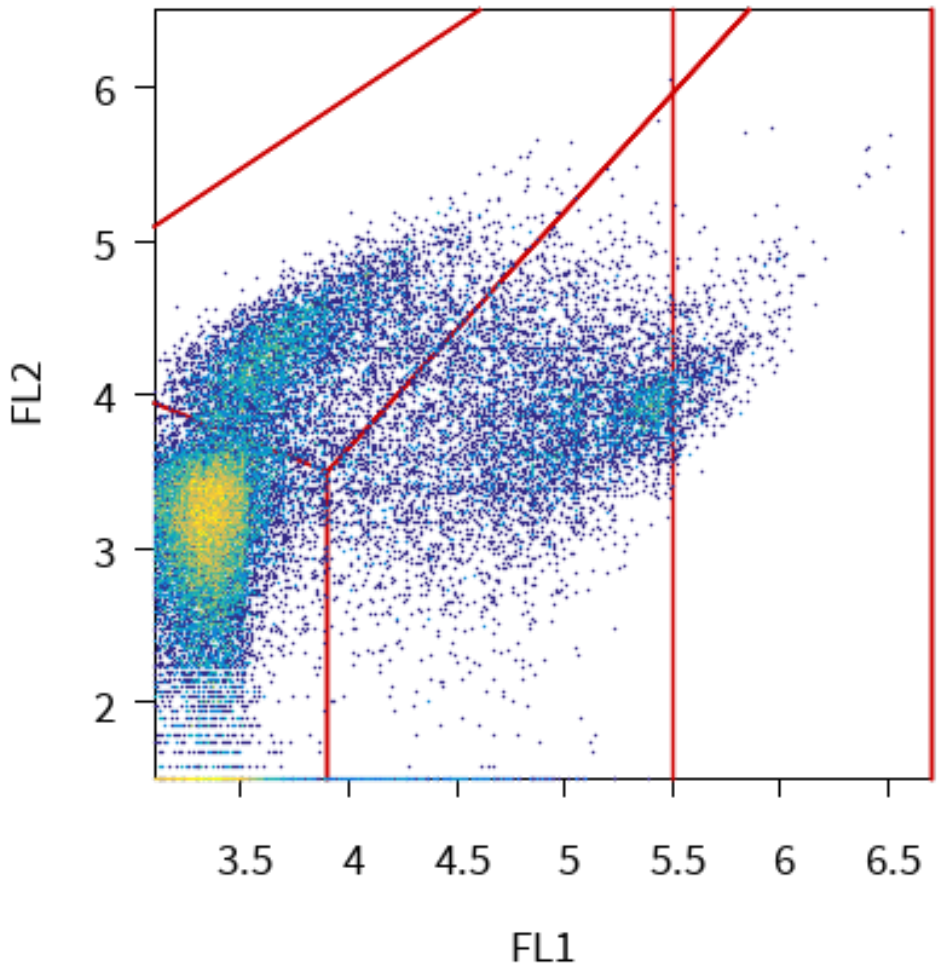
Developed by bNovate TECHNOLOGIES

Manufactured by SIGRIST PROCESS-PHOTOMETER

Fully automated and can be operated remotely
20 minutes process time
Compact and portable (possibility for online and offline measurements)
Maintenance is basic and estimated to 30 minutes every 3 months

Credit: bnovate

Typical results from an online Flow cytometer



Municipal tap water - Norway

Total cell count (TCC) : 158 288 cells/ml

Dead cell count (DCC): 74 488 cells/ml

Intact cell count (ICC) : 83 800 cells/ml

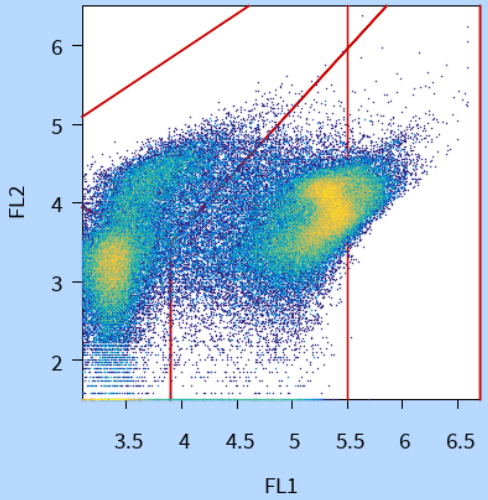
High nucleic acid percentage (HNAP): 9.74 %

Water Research Foundation (WRF 5033 project)

Demonstrating the Effectiveness of Flushing for Reducing the Levels of Legionella in Service Lines and Premise Plumbing

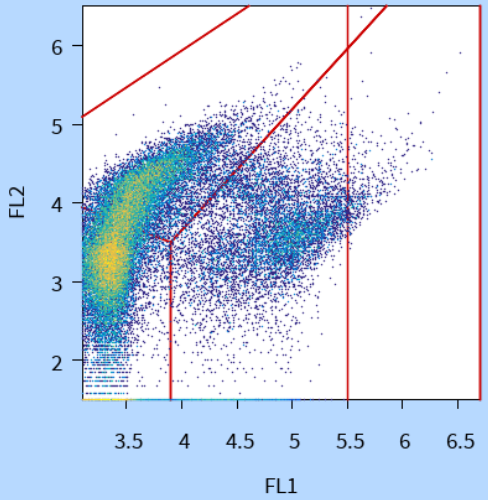
Cold water supply
Disinfection- 0 mg/L-Cl_2

Stagnant water



TCC: 903 481 cells/ml
ICC : 737 793 cells/ml
HNAP: 17.02 %

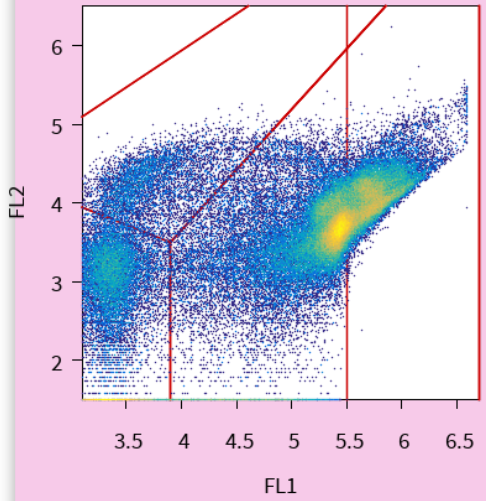
Immediately after a flush



TCC: 269 600 cells/ml
ICC : 107 711 cells/ml
HNAP: 4.66 %

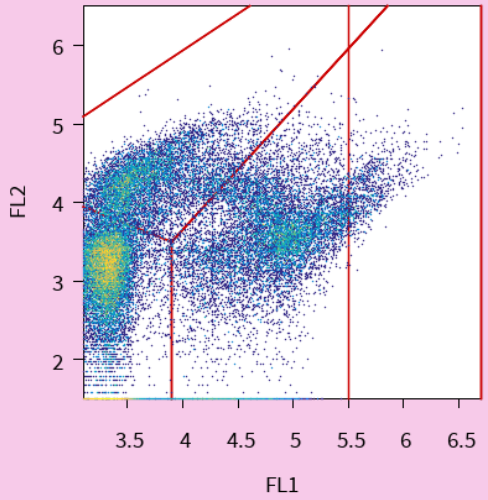
Cold water supply
Disinfection- 1 mg/L-Cl_2

Stagnant water



TCC: 1 104 444 cells/ml
ICC : 1 103 844 cells/ml
HNAP: 39.62 %

Immediately after a flush

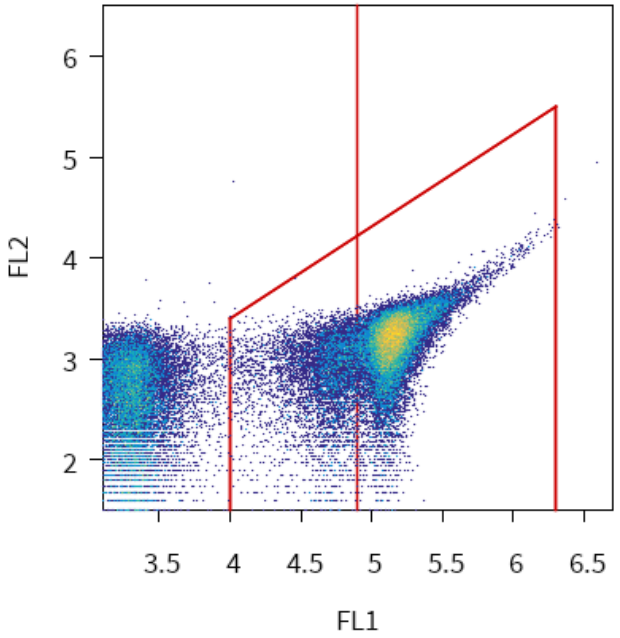


TCC: 230 458cells/ml
ICC : 140 811cells/ml
HNAP: 7.46 %

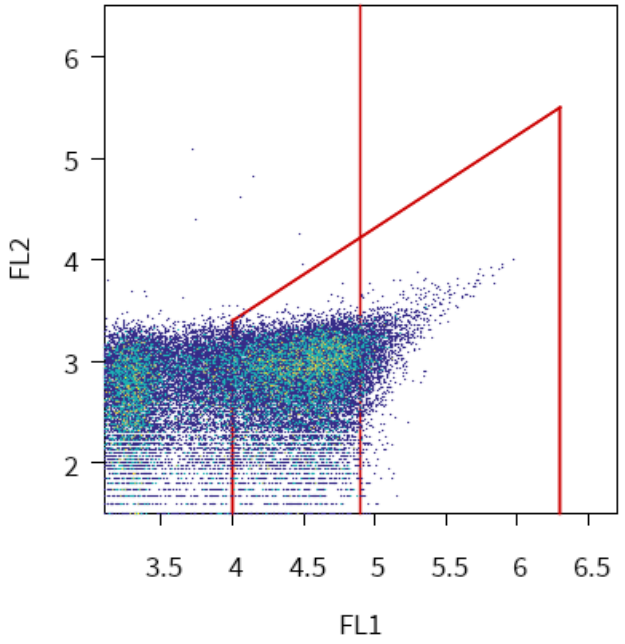
Effectiveness of disinfection

Add disinfectant
free chlorine (HOCl)

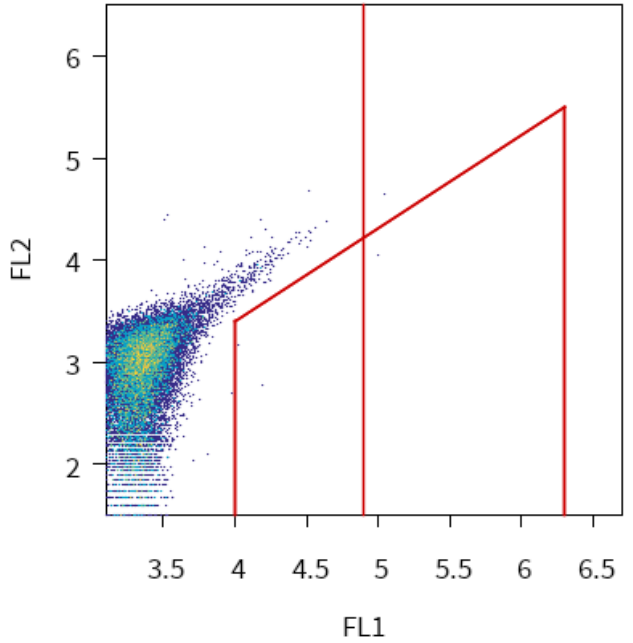
Old mineral water



1hr later



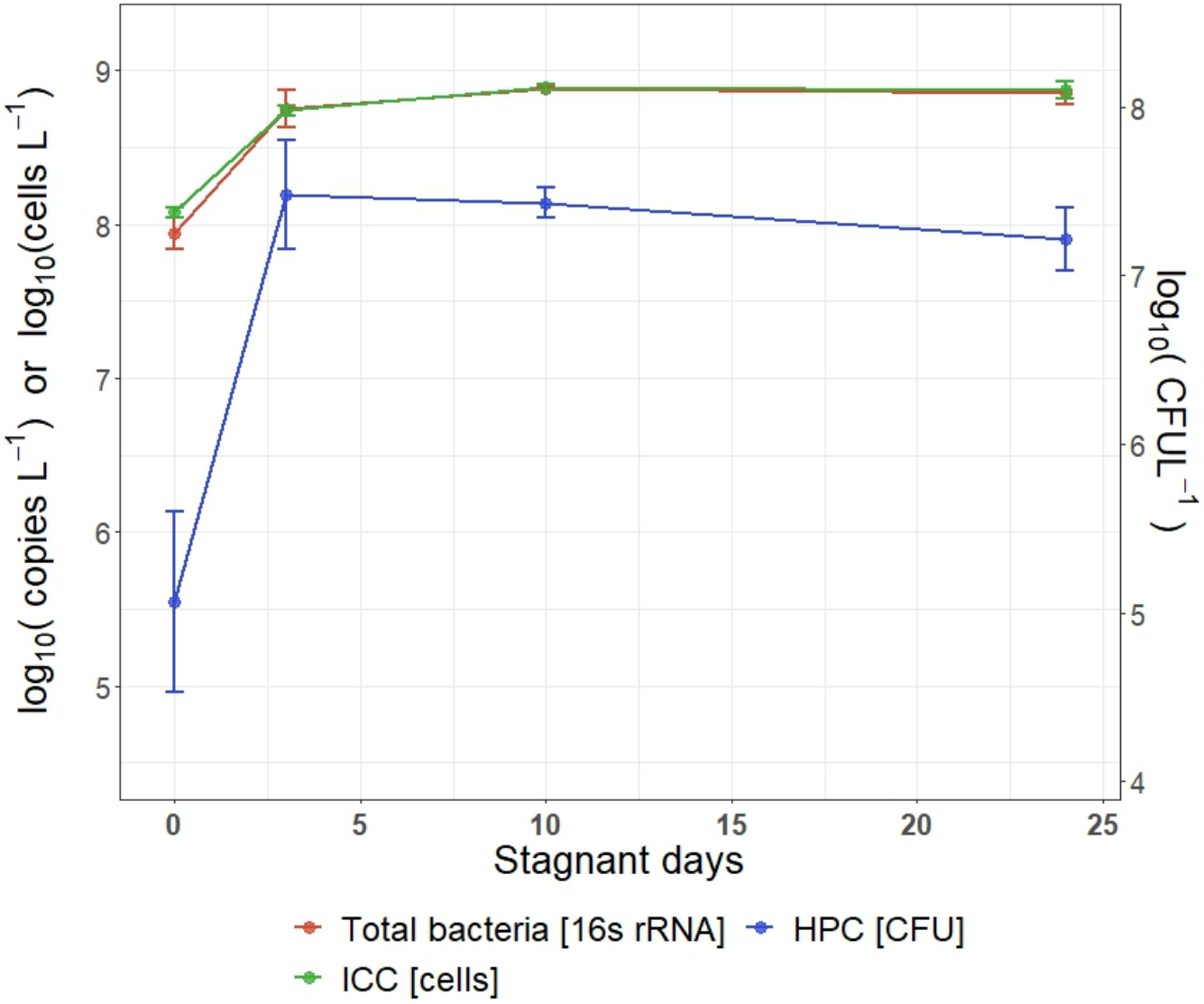
6hr later



Credit: SIGRIST

WRF 5033 project

Microbial growth in stagnant premise plumbing system



Applications of online FCM

- Follow the production process continuously.
- Analyze trends concerning operational conditions
- Can provide an answer about the change in water in the distribution network. For example, infiltration of rain- or groundwater due to damaged pipes. (Favere et al., 2021, Water Research)
- Can serve as an early warning system

Cost analysis

BactoSense

CAPEX: ca. 415 000 kr excl. mva

OPEX: 35 kr/samples + annual maintenance

Conventional plate count: ca. 500 to 650 kr/samples (include: shipping, skill worker, consumable, infrastructures)

Conclusions

Short time-to-results is an added value compared to conventional methods. fast (20 minutes)

Fully automated

Sensitive and accurate method for drinking water quality (consistent with qPCR results)

Research has shown that online microbial monitoring techniques can serve as a control to drinking water quality (I,.e. intrusion of rain- or groundwater in the to drinking water network)

Coupled with data analyses, online microbial monitoring can be used as an early-warning system

Challenges:
large amount of data
Data analysis
Still require approval from the regulators

For more information see :

www.ntnu.edu

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