

# Use of flow cytometry and DNA sequencing as a proxy for characterising microbial communities during managed aquifer recharge

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**SMART CONTROL, Water JPI Project**  
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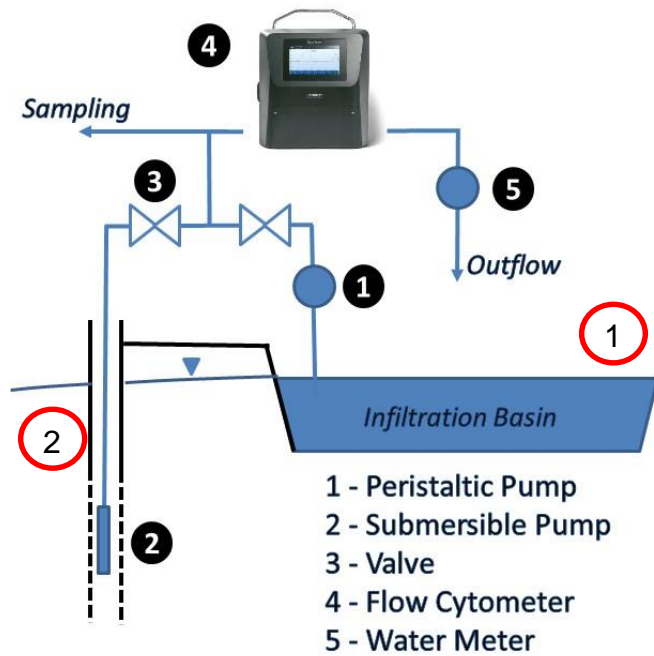


# Motivation and Problem Statement

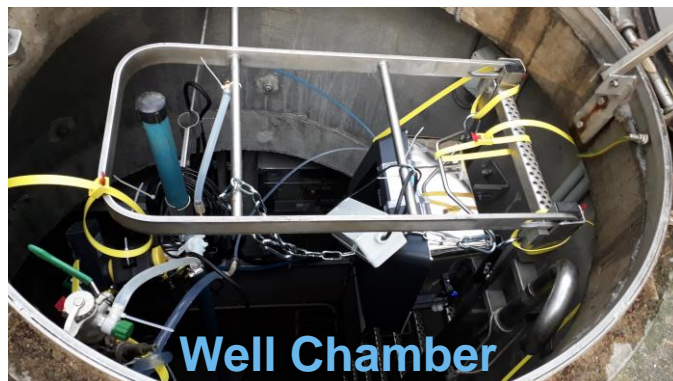
- Measure the microbial dynamics influenced by operational or natural events in ground- und surface water at a Managed Aquifer Recharge site
- Employ Flow cytometry (FCM) to measure temporal bacterial dynamics at high frequency
- Characterize microbial communities by metagenomic DNA sequencing

Can we derive any hygienic implications from FCM counts?

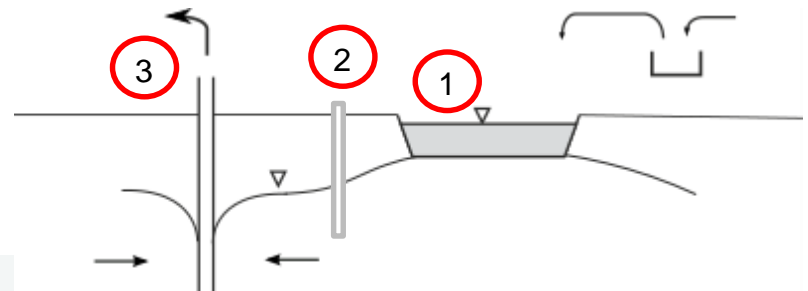
# Monitoring set-up for Flow Cytometer (FCM) at WaterWorks Berlin-Spandau



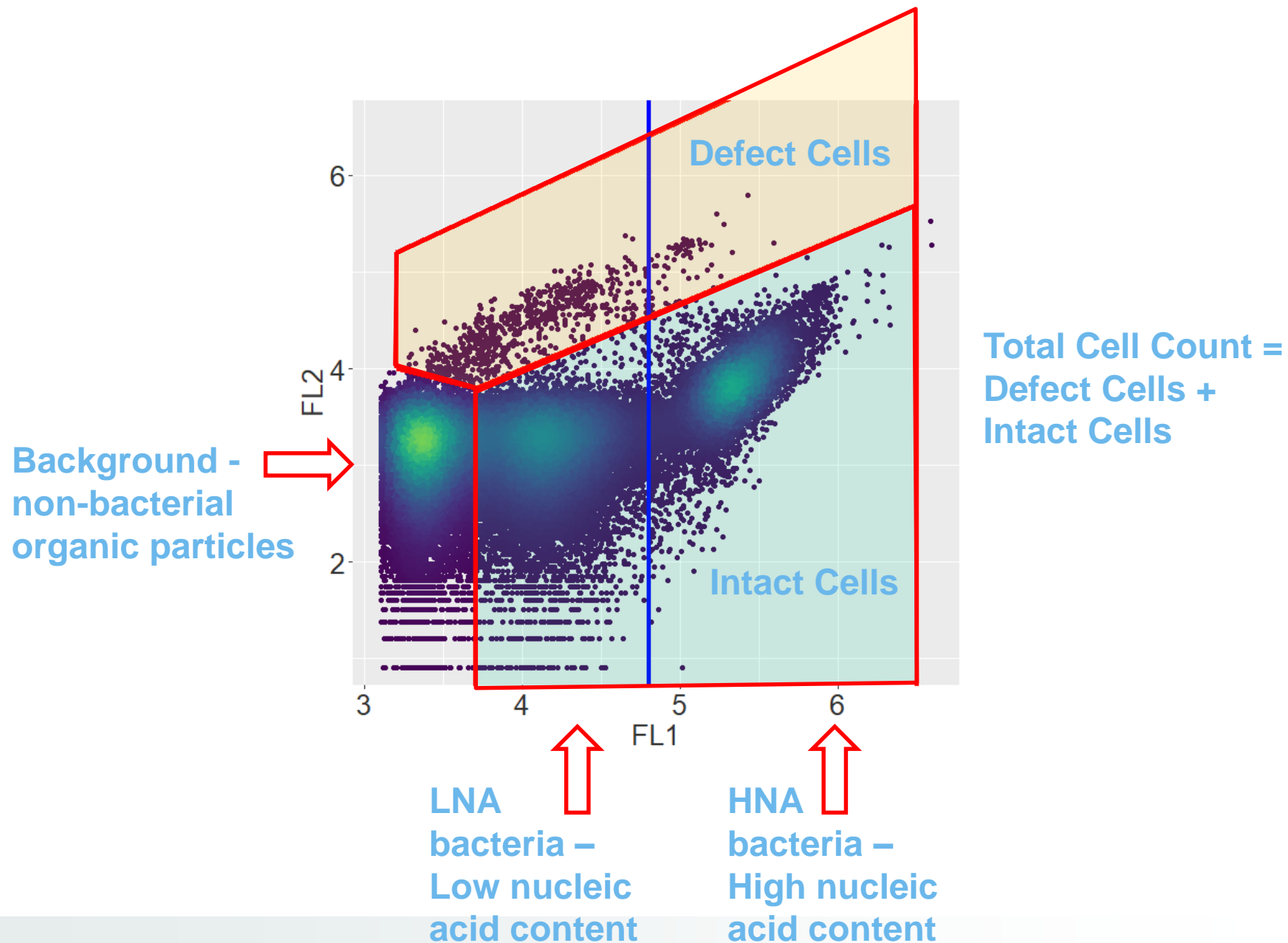
- Fully automatic sampling and analysis FCM device (Sigrist BactoSense©)
- FCM measurement interval 6h
- FCM installation 1 month at Basin and observation well and 1 month at Abstraction well
- Sampling of *E.Coli*, Somat. coliphages, *Clostridium perfringens* and *Enterococci*
- Sampling for DNA sequencing



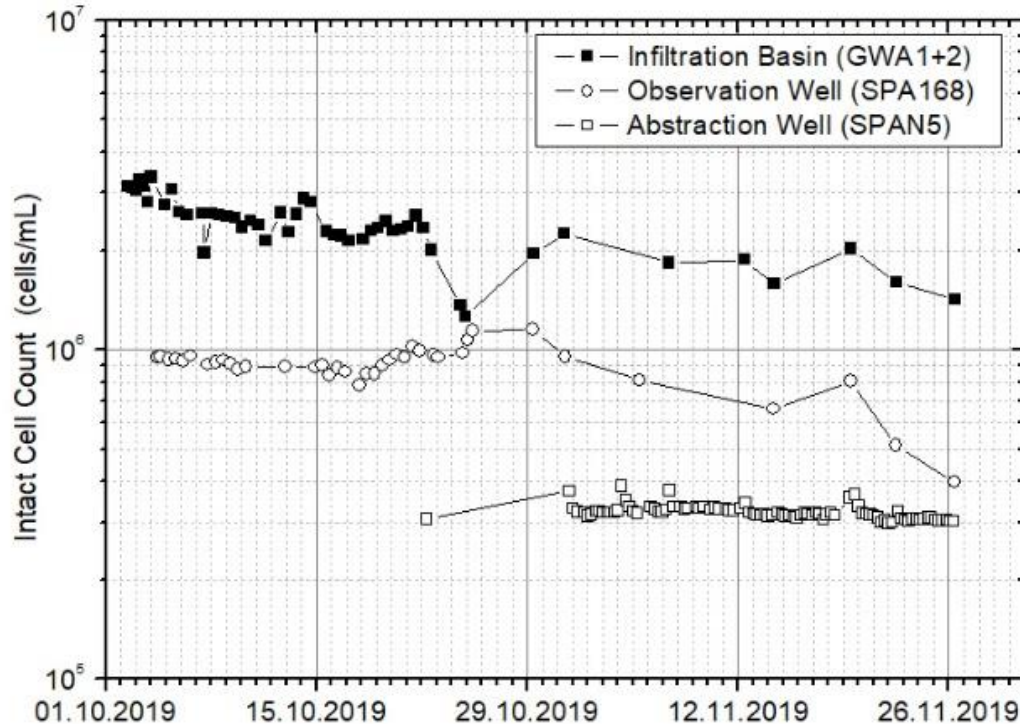
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# Flow Cytometry - Methods



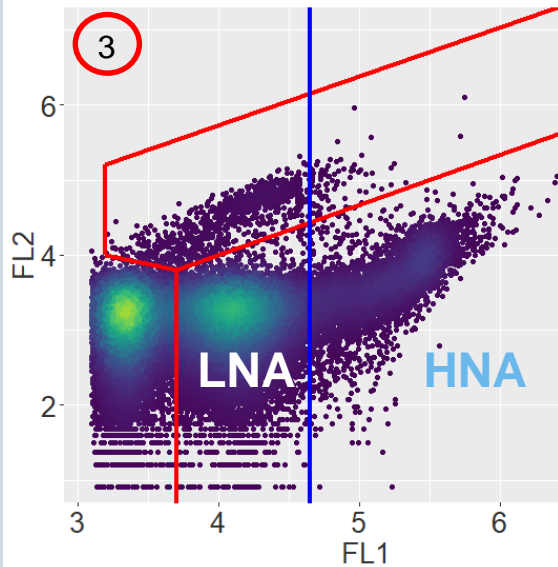
# Flow Cytometry (FCM) – Time series Intact Cell Count (ICC)



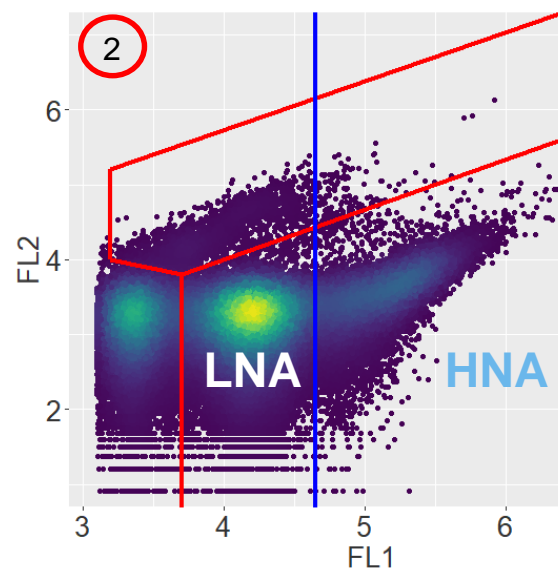
- Fairly constant ICC in abstraction well
- No detections of *E.Coli*, *somat. coliphages*, *Clostridium perfringens* and *Enterococci* in abstraction well (not shown)
- Intact cell counts from FCM were multiplied by the relative abundances of taxa (DNA sequencing) to reveal absolute abundance (adapted from Props et al. 2017)

# Flow Cytometry – Density plots

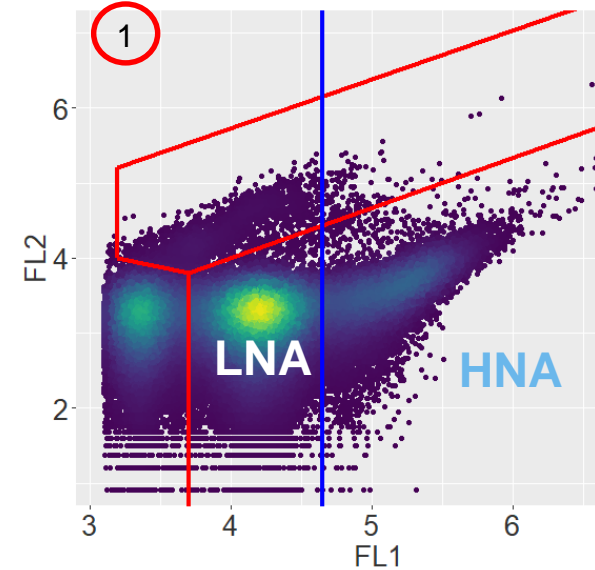
Abstraction well  
(40-50 d HRT)



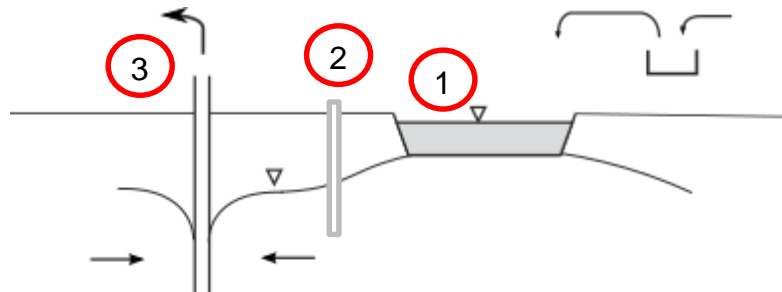
Observation Well  
(2-4 d HRT)



Infiltration Basin



- HNA counts in basin water are in absolute abundance and percentage higher than in the measured groundwater samples

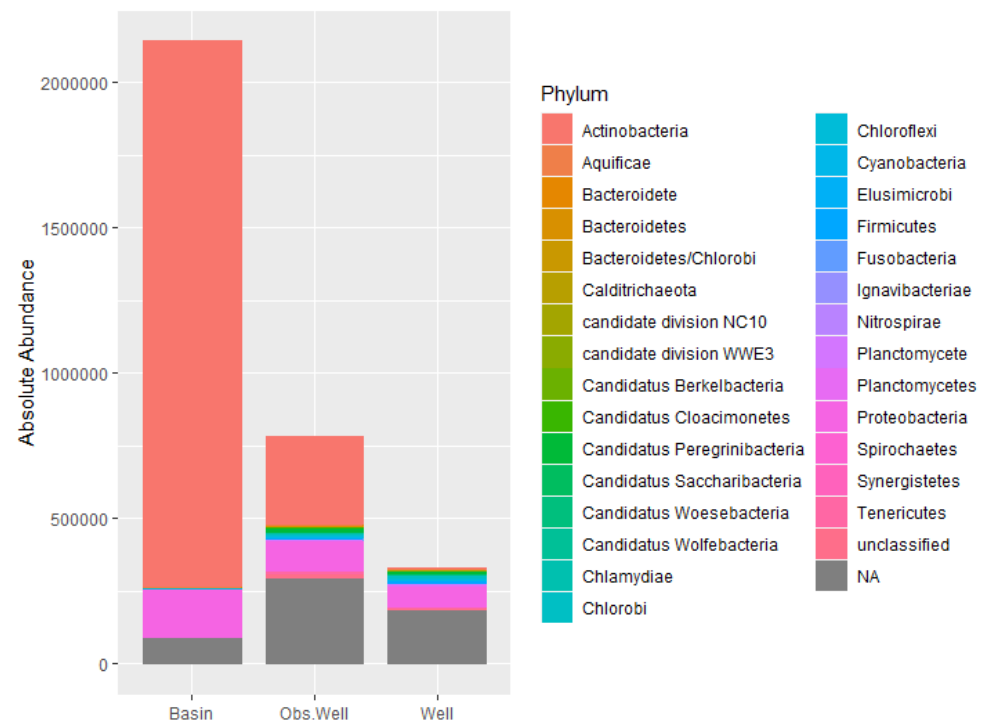
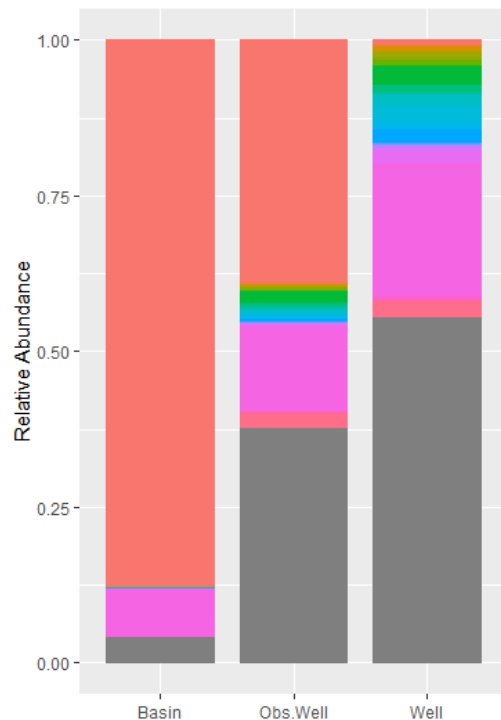


# Results DNA sequencing

Relative and absolute abundance of phyla (NA = cut-off value 0.1%)

## Overview

Sample	Bacteria	Viruses	Eukaryota	Archaea
Basin	81 %	5 %	12 %	1 %
Obs.Well	87 %	0.5 %	9 %	3 %
Well	86 %	0.1 %	8 %	4 %



## Bacteria only



# Preliminary conclusions and practical implications ....open for discussion

- Increase in TCC/ICC from FCM has no direct hygienic implication

But: Fairly constant cell counts in the abstraction well may can be used to optimize the hygienic monitoring?

- DNA metagenomic sequencing can be a supplementary tool for water quality assessments, due to high costs and complex interpretation requirements, it is still not viable for broad scale routine measurements though

But: based on known metabolic properties (database available?) it is valuable tool for evaluating well ageing processes and identify pathogenic species?



# Literature

Props, R., Kerckhof, F.-M., Rubbens, P., De Vrieze, J., Hernandez Sanabria, E., Waegeman, W., Monsieurs, P., Hammes, F. and Boon, N. (2017) Absolute quantification of microbial taxon abundances. The ISME Journal 11, 584-587.