

FLOW CYTOMETRIC MONITORING IN THE WATER INDUSTRY

Applied research within industry-academia partnerships

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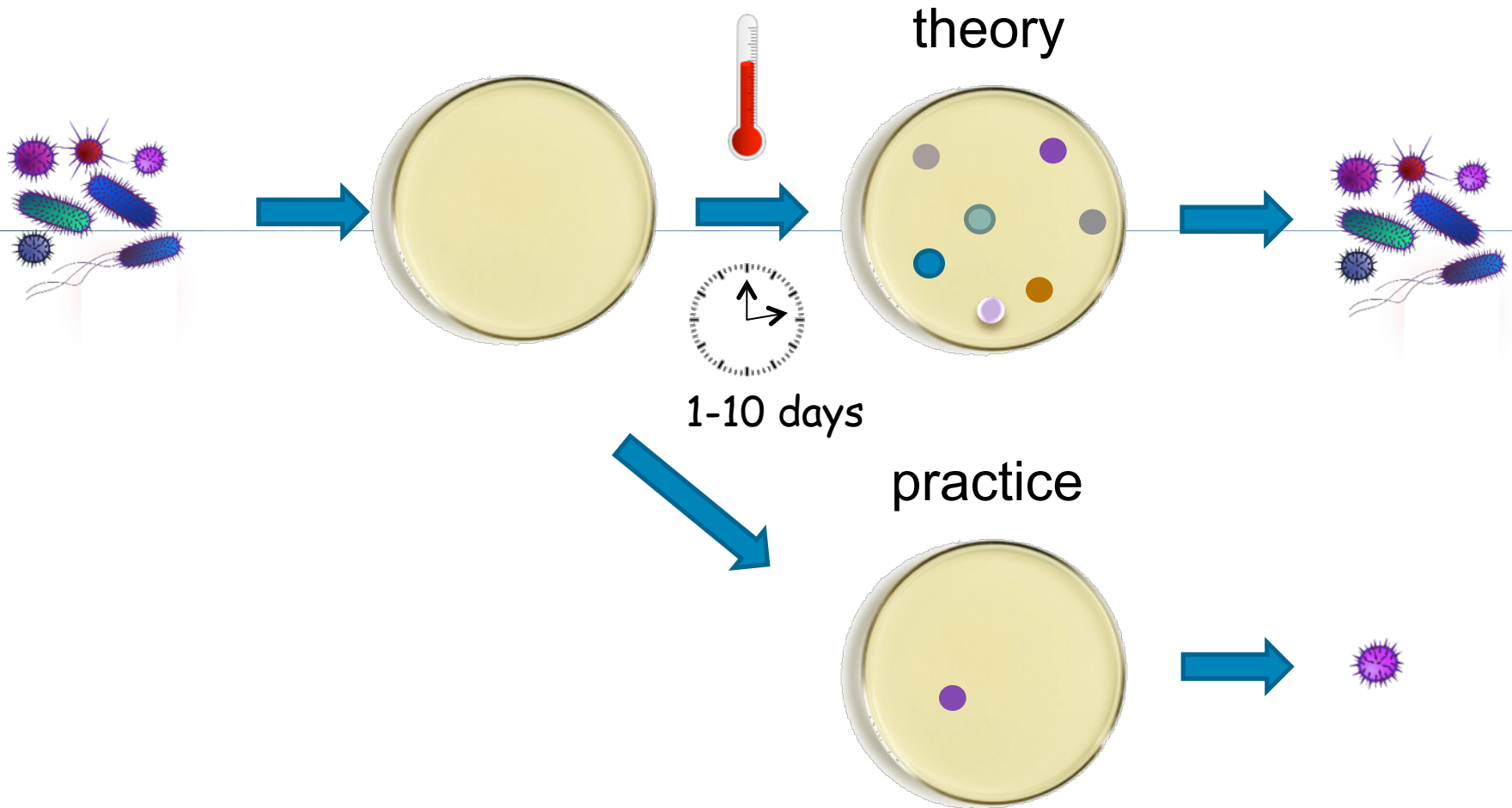
IMPROVED Workshop Gent

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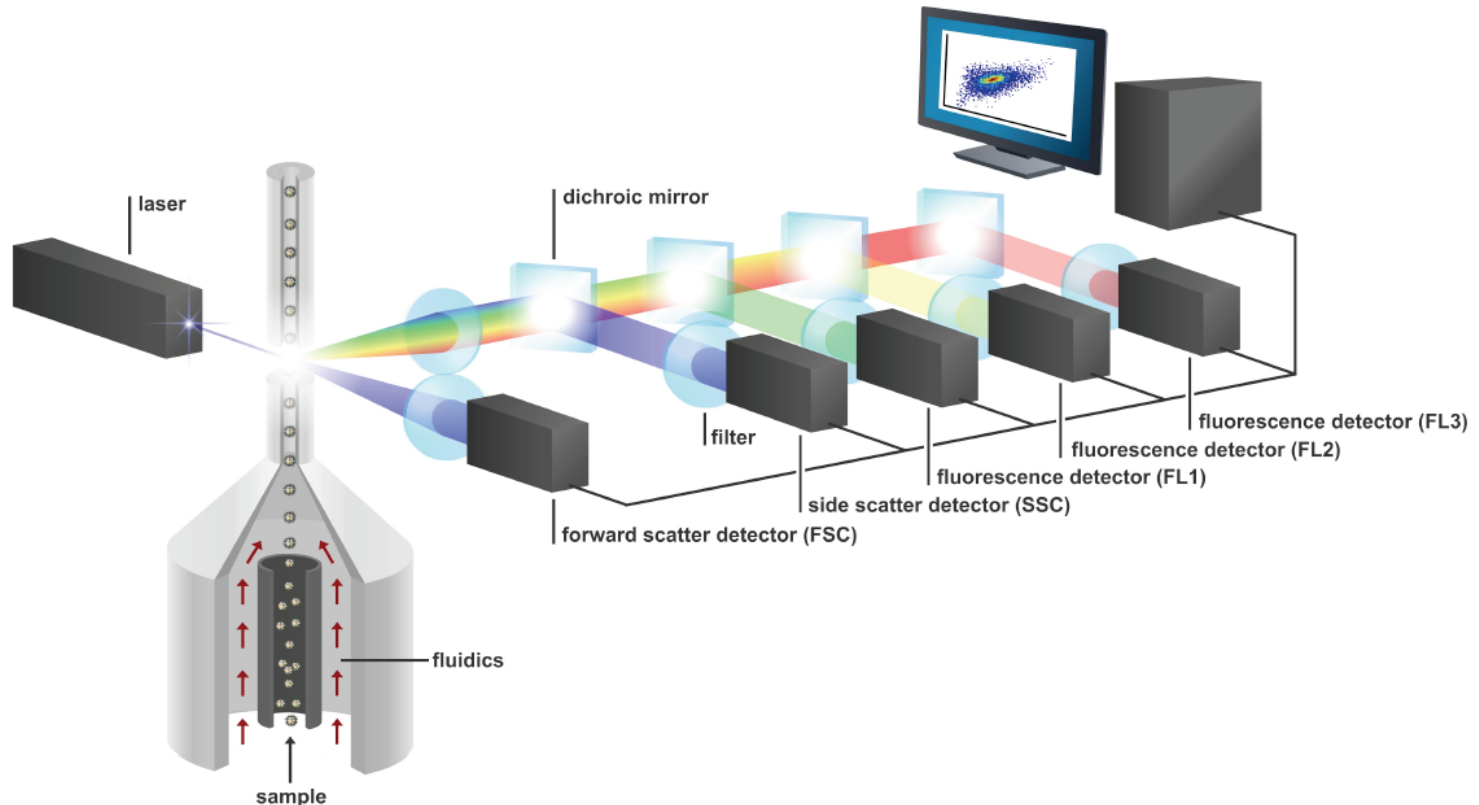
Monitoring water quality today

- ❖ Plate counting to detect bacteria in drinking water (legislation)

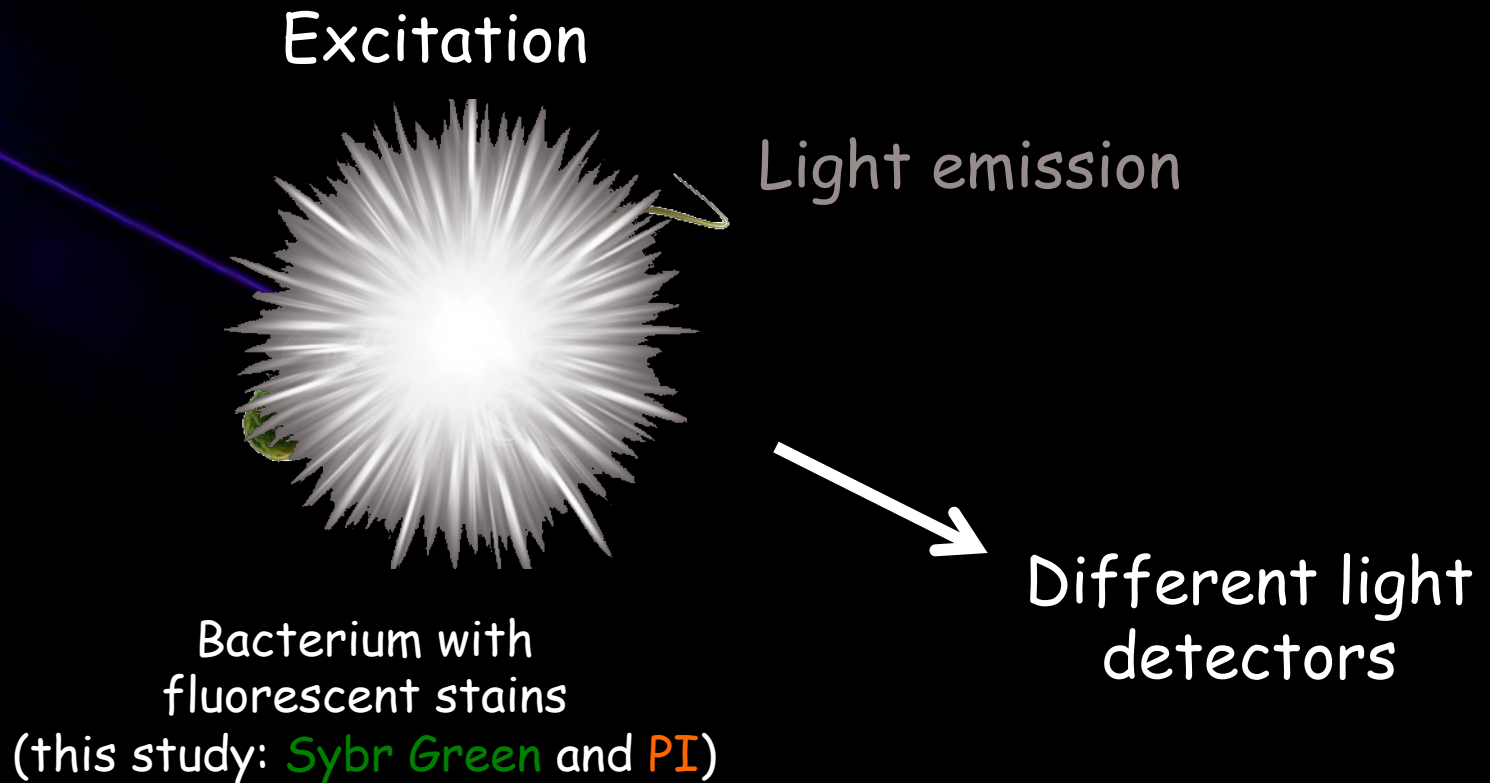


Flow Cytometric Monitoring (FCM)

- ❖ FCM: faster, more complete and accurate than cultivation-based methods
- ❖ Useful to assist plate-counting, not to replace it



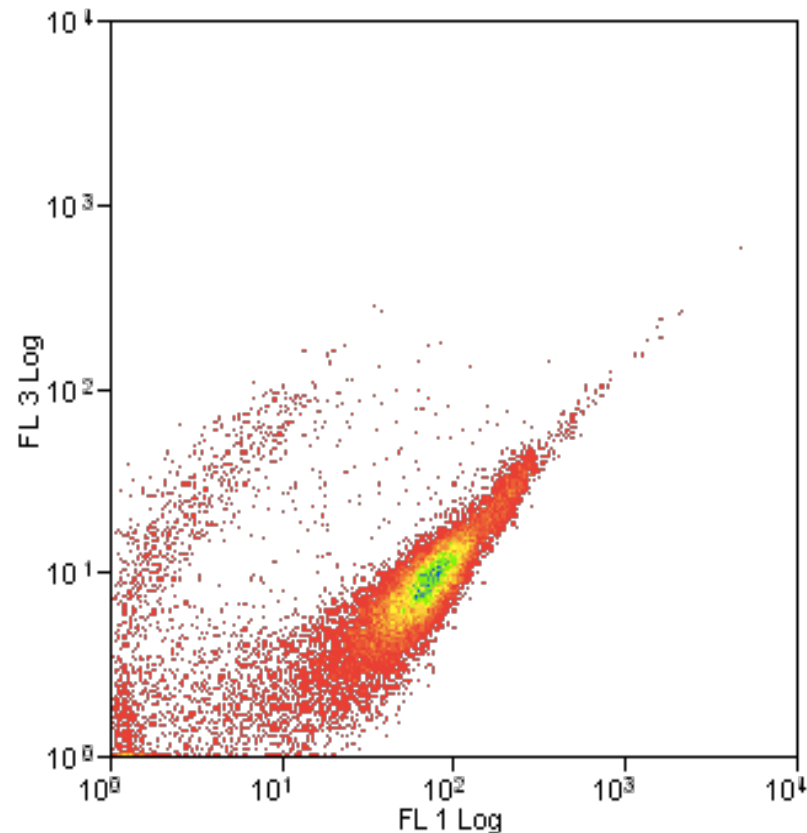
Flow cytometry: basic principle



Flow Cytometric Monitoring (FCM)

❖ Scatter plots

- ▣ 1 dot = 1 particle (bacterium) -> more complete and accurate
- ▣ Results ready withing minutes ⇔ plating

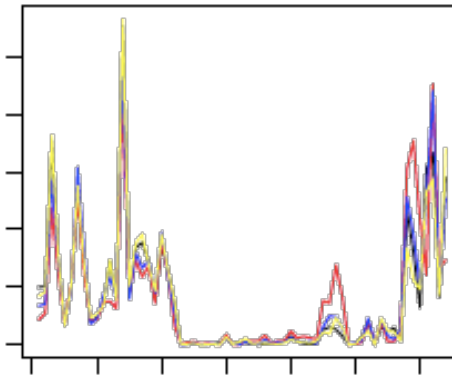


FCM: Fingerprinting (1)

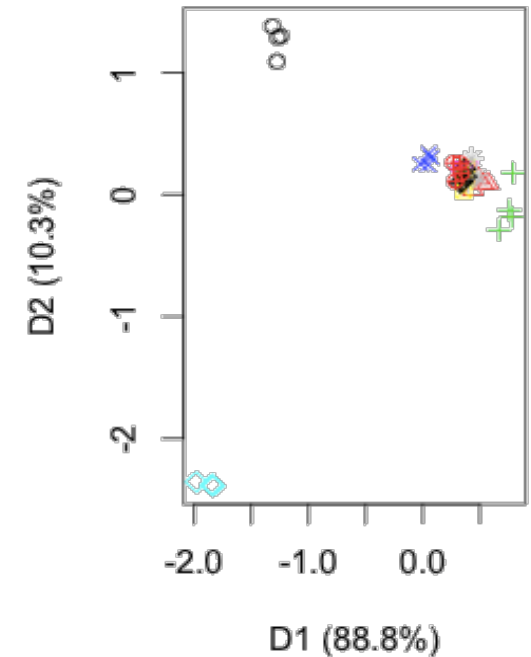
Raw data

Cell #	FS Log	SS Log	FL 1 Log	FL 2 Log	FL 3 Log	FL 4 Log	FL 5 Log
1	6590	0	1410	0	7073	5096	0
2	0	0	543	4961	11209	0	0
3	1028	0	471	4214	9788	1891	0
4	15768	31078	39700	49590	49959	42585	36900
5	1320	0	635	3694	3091	8637	0
6	2939	0	657	2405	1428	804	0
7	0	0	1692	1086	3339	17	0
8	16356	30593	53553	62467	62184	53112	43913
9	14447	26617	39063	47761	46191	36766	25530
10	0	0	1755	3329	6712	7648	0
11	19275	29566	53147	62234	61717	53024	43955
12	0	0	2463	12371	9518	10154	0
13	6848	0	26550	35007	31985	23072	14045
14	14048	28571	4321	13587	14727	4161	0
15	0	6396	5669	13604	20223	6598	0
16	0	0	3692	5100	16109	4914	0
17	4654	0	32784	41094	38916	30031	23044
18	0	0	1727	645	8346	0	0
19	0	0	2931	18918	17284	11783	0
20	0	0	3788	13196	12835	12890	0

Processing



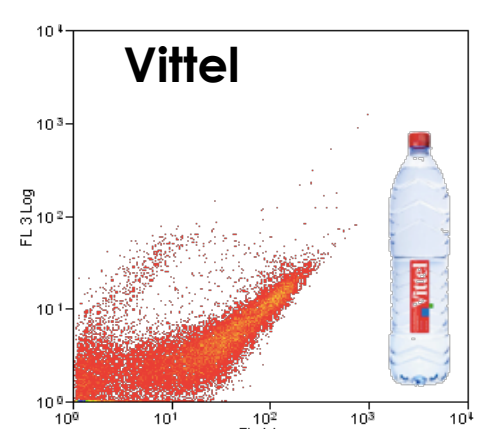
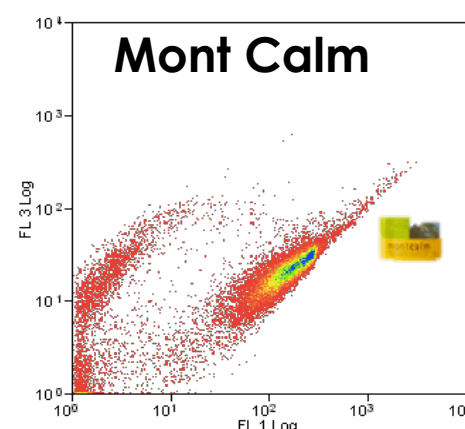
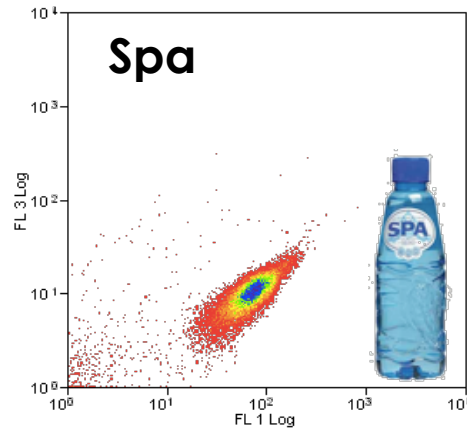
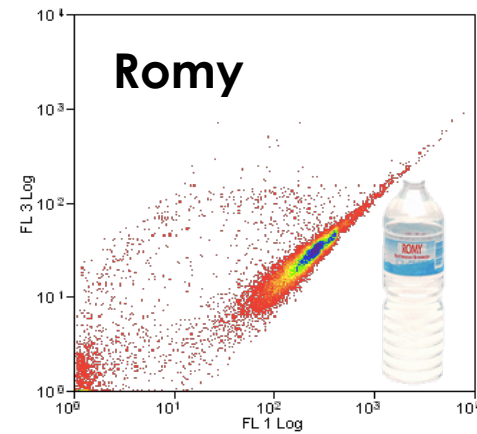
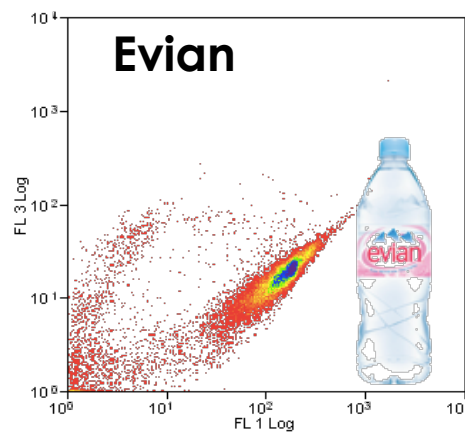
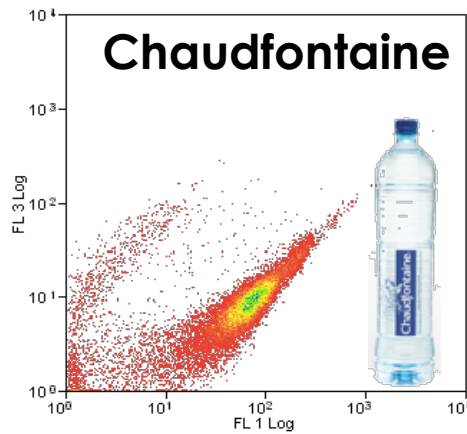
Statistical analysis:
similarities/differences





FCM: Fingerprinting (2)

- ❖ 6 different brands of bottled water = 6 different communities = 6 different fingerprints



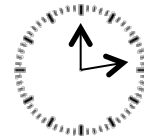
To practice: drinking water

- ❖ **IDEA:** can we use FCM fingerprinting for fast control of drinking water?
 - ▣ Determine the “reference” fingerprint of “good water”
 - ▣ Rapid analysis (minutes) and comparison of fingerprints allows fast screening of water samples (pipelines/networks)



To practice: drinking water

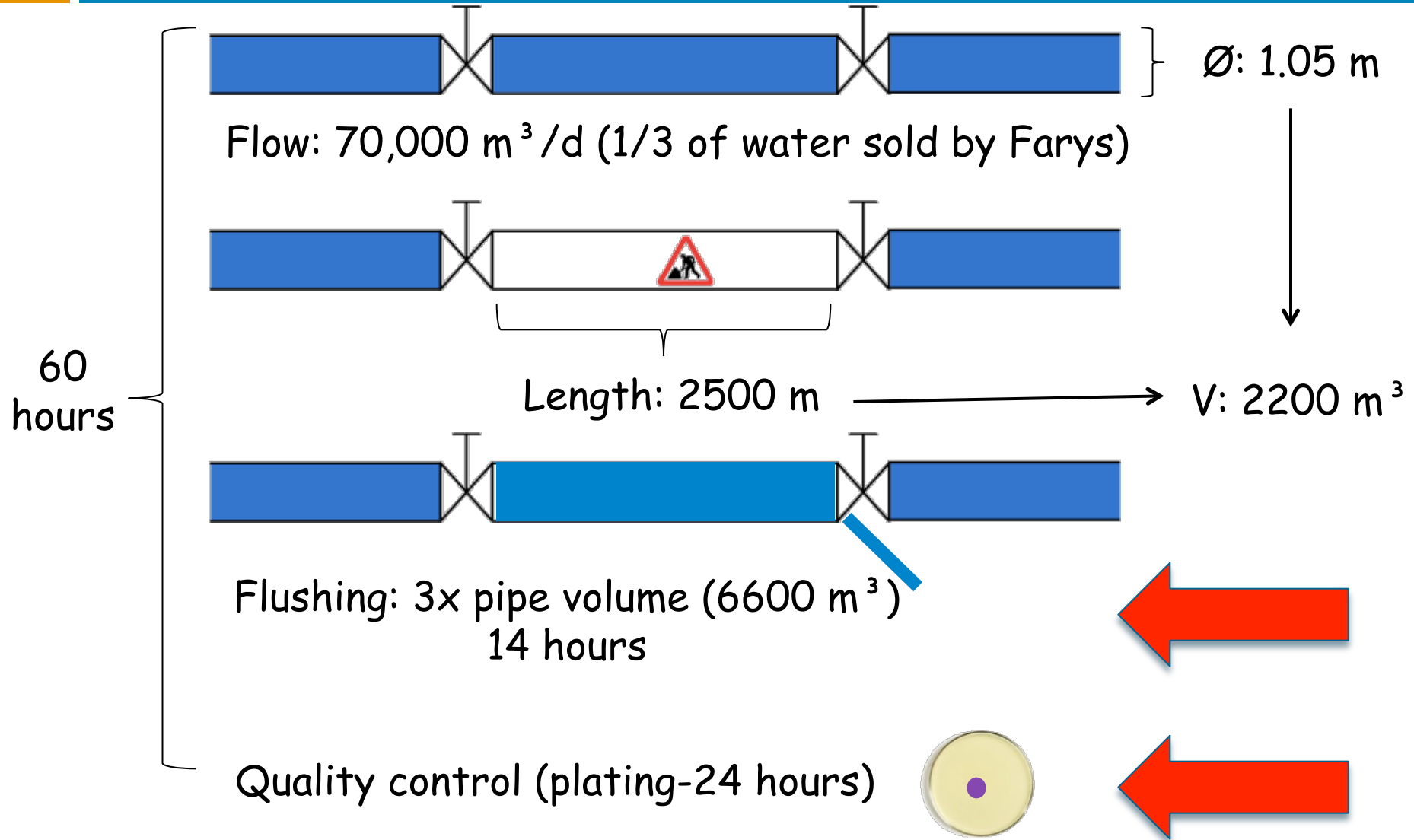
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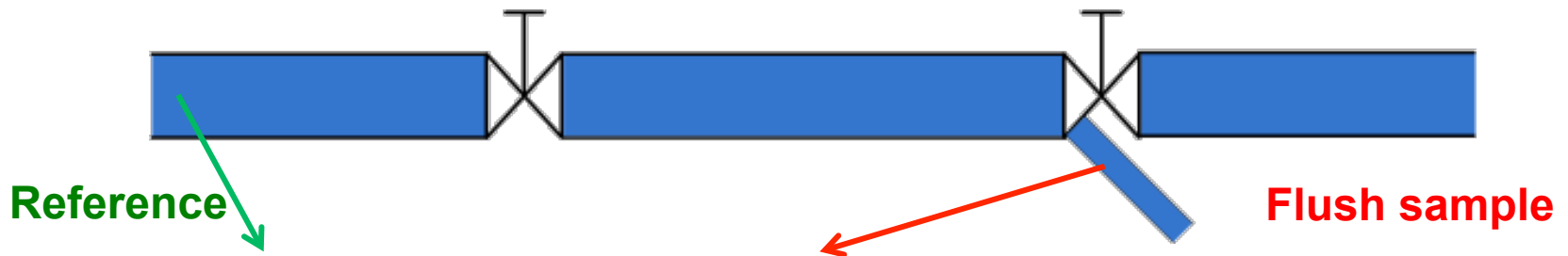
Time is crucial !



To practice: drinking water feeder

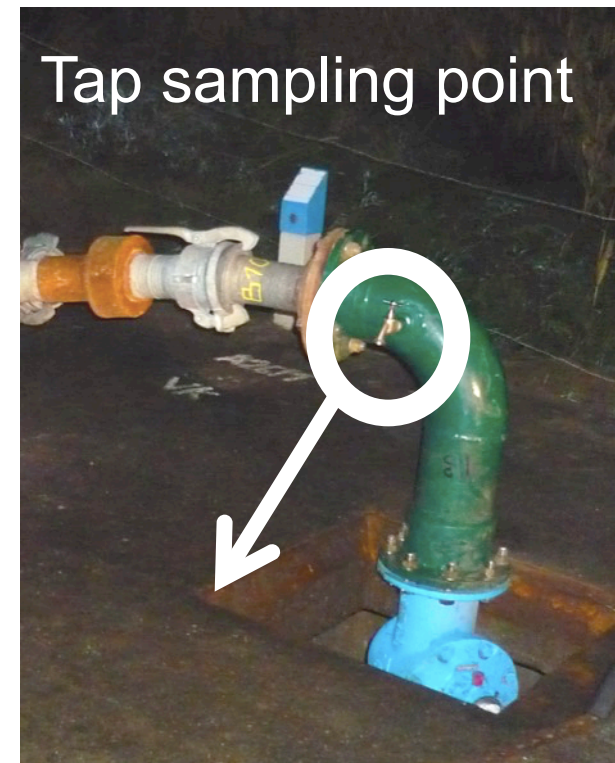


To practice: drinking water feeder



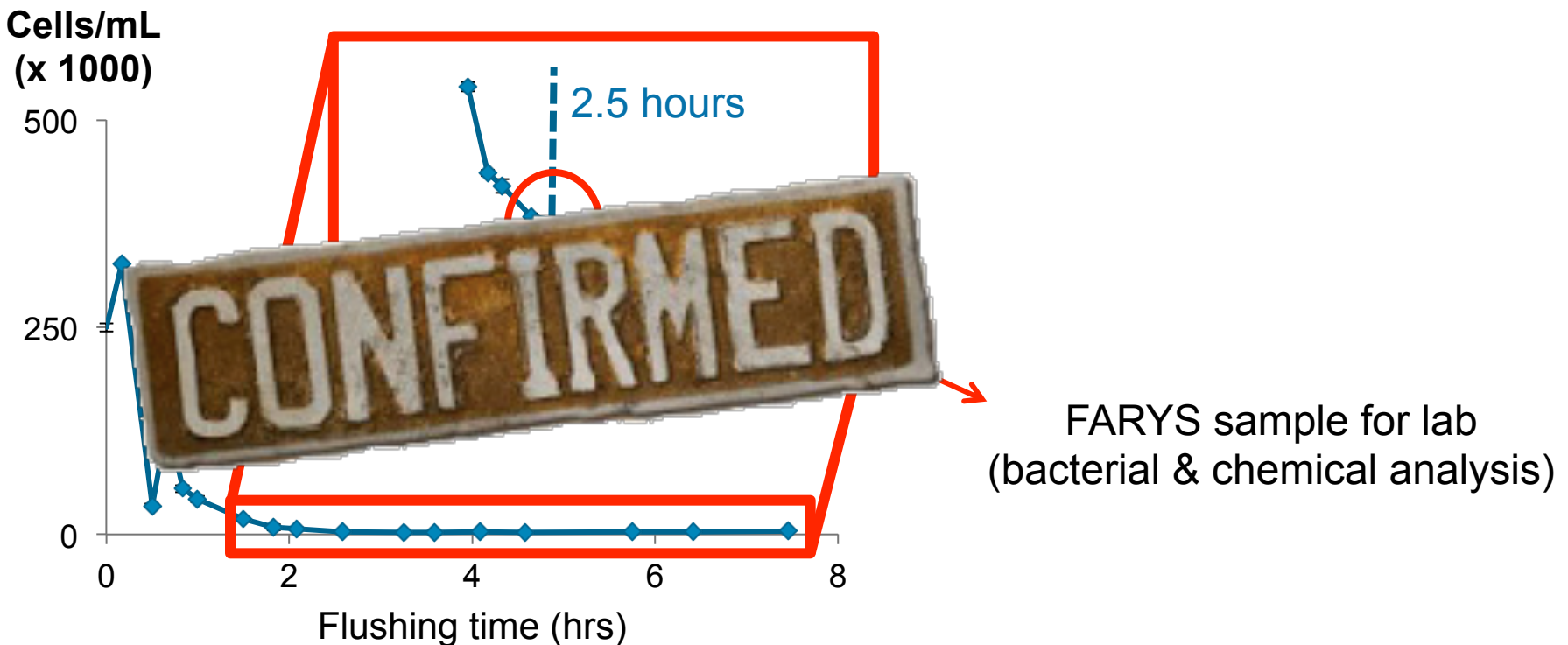
Same quality?

Cell count & flow cytometric fingerprinting



Downstream users: drinking water

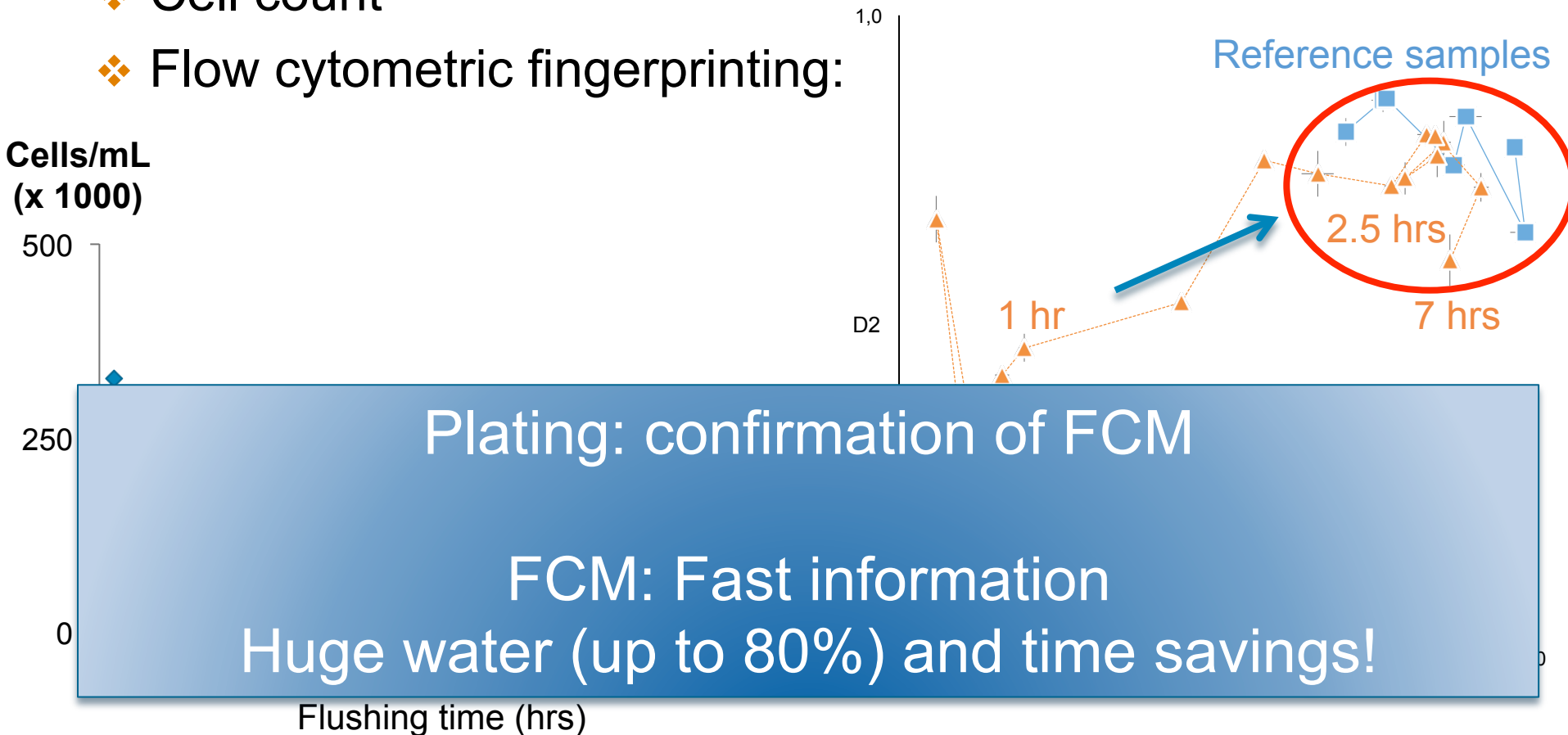
❖ Cell count:



Van Nevel *et al.* (2015). Flow cytometry for immediate follow-up of drinking water networks after maintenance. *Water Research submitted.*

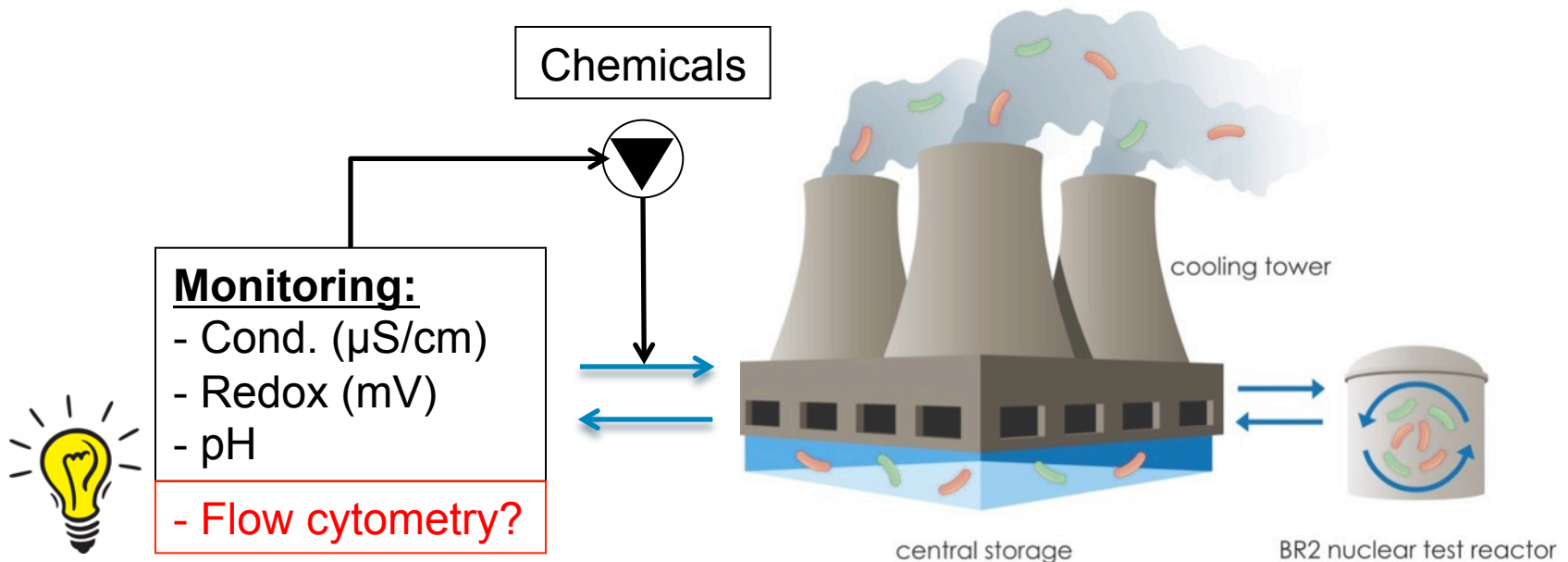
Downstream users: drinking water

- ❖ Cell count
- ❖ Flow cytometric fingerprinting:



Process water

- ❖ Monitoring of community dynamics in closed circuits
 - ▣ Example: cooling water
 - ▣ Open cooling towers susceptible to contamination
 - ▣ Biological growth: MIC, Legionella, ...
 - ▣ Dosing of biocides (NaOCl), pH control and corrosion inhibitors



Process water

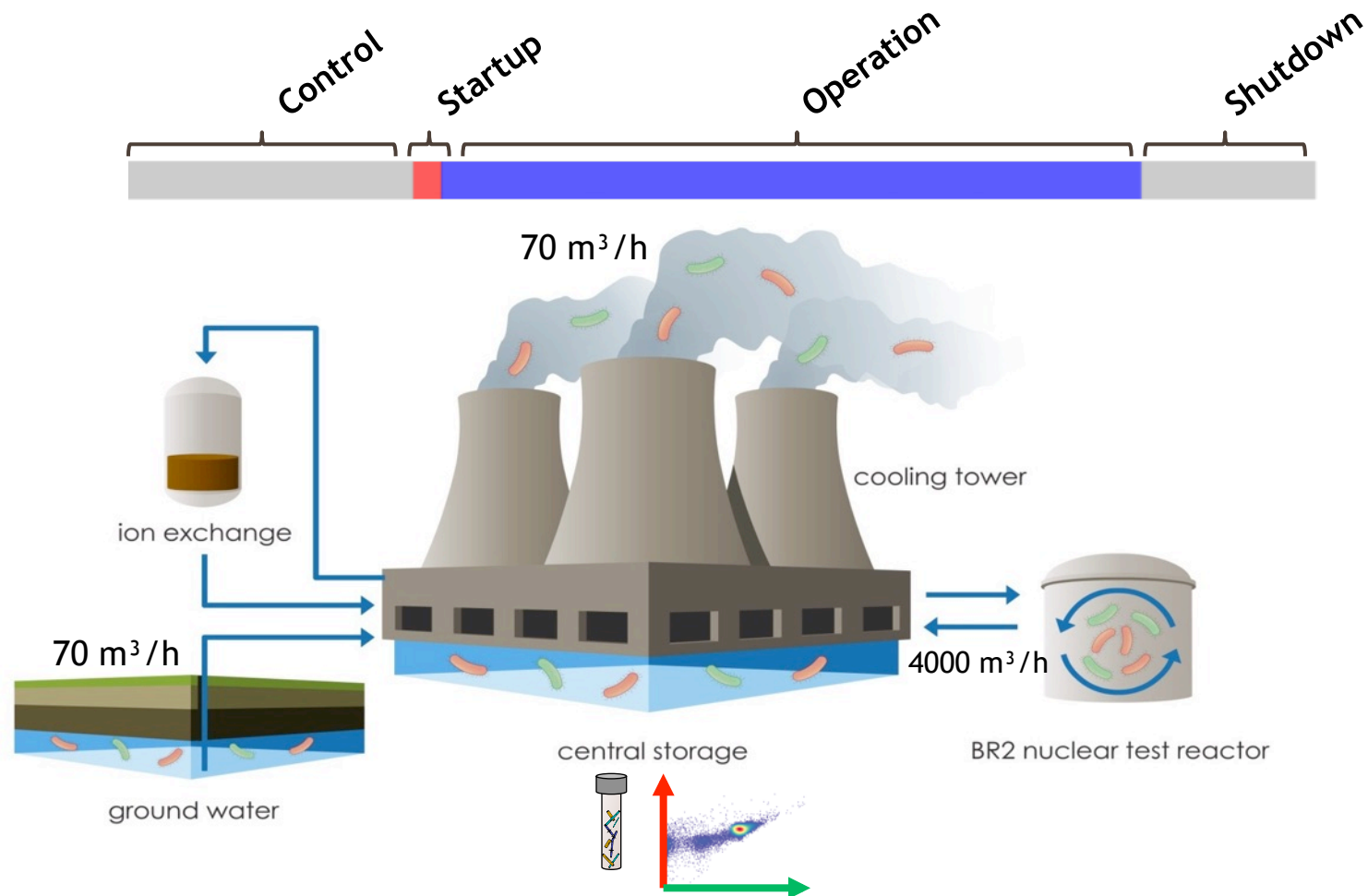
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 - ▣ Example: cooling water
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- ❖ **IDEA:** can we use FCM for on-line monitoring of closed circuits?
 - ▣ Link community dynamics to changing water quality
 - ▣ “Early-warning system” for contamination
 - ▣ On-line tool to control/steer dosing of biocides



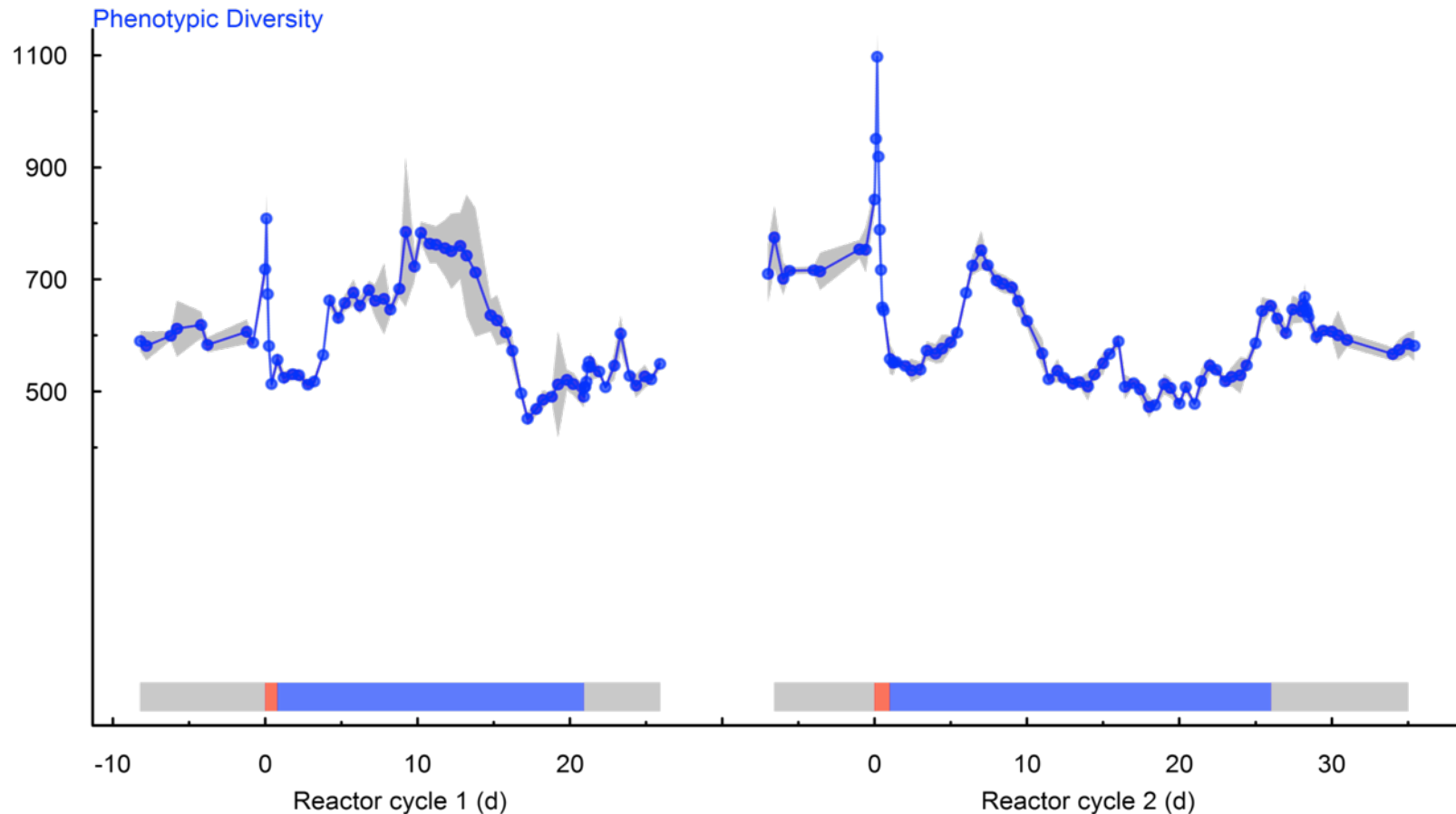
Process water: nuclear test reactor

- ❖ Exploring community dynamics in cooling towers with FCM



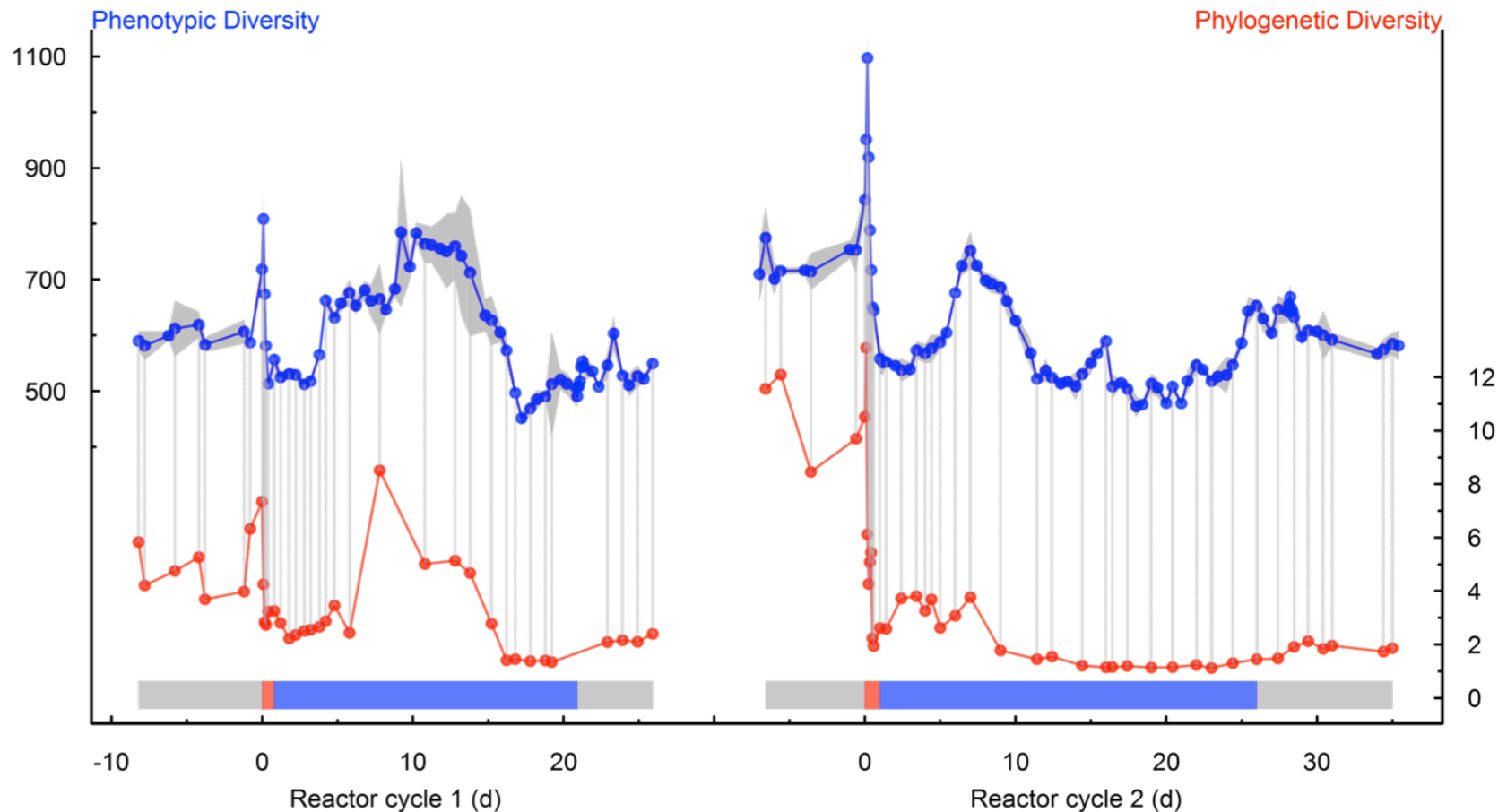
Process water: nuclear test reactor

- ✓ Phenotypic diversity (derived from FCM) indicates dynamic changes



Process water: nuclear test reactor

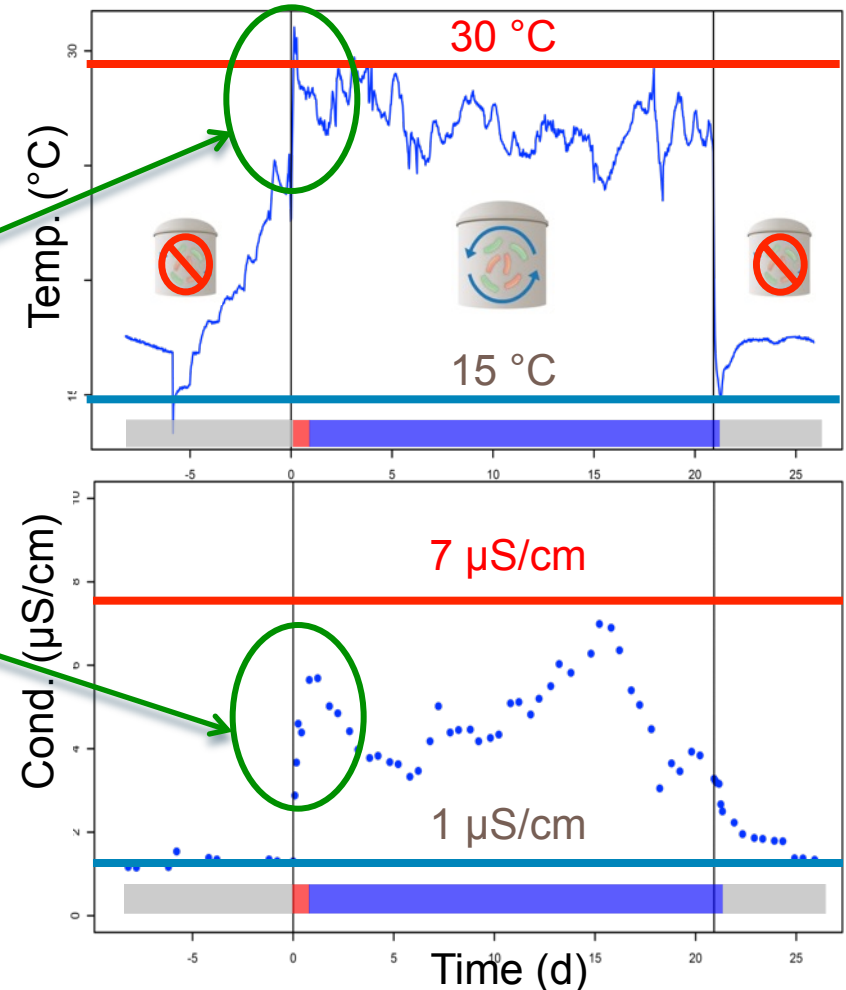
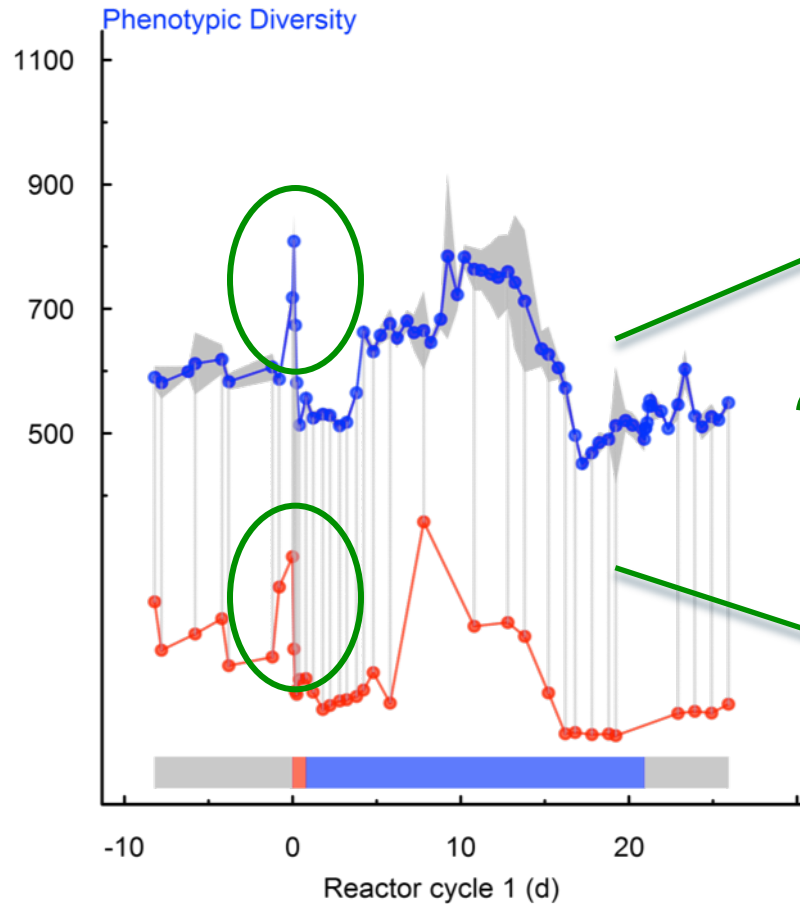
- ✓ Phenotypic diversity link with phylogenetic diversity (illumina seq.)



Props et al. (2015). Measuring the biodiversity of microbial communities by single-cell analysis. *submitted.*

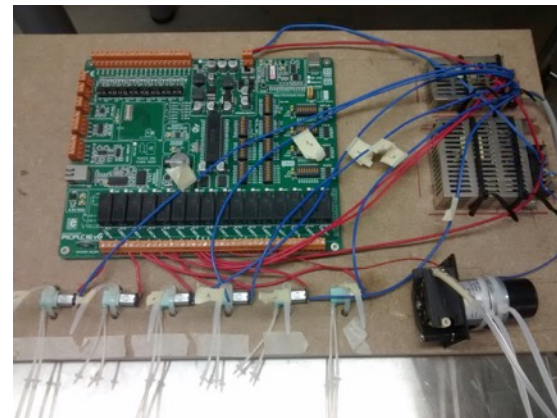
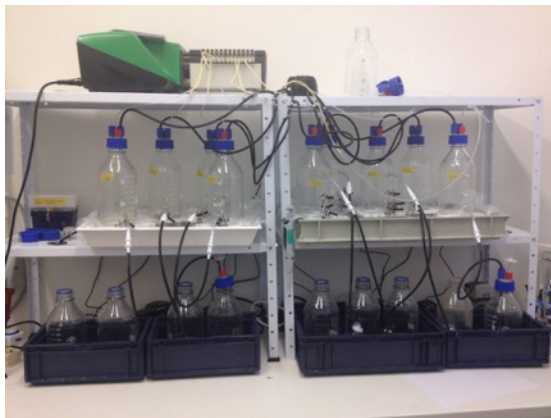
Process water: nuclear test reactor

Can we link the community dynamics to changing water quality?



Process water: on-line monitoring

- ❖ Link the community dynamics to changing water quality?
 - ▣ Determine “detection limit” of community respons
 - ▣ Different pulses in batch and continuous chemostats:
 - ▣ Yeast extract
 - ▣ TOC, N, P, salt, ...
 - ▣ Development of autosampler and staining robot for on-line application



Valerie Baetens: master thesis 2015-2016 (CMET)

IMPROVED: on-line fingerprinting

- ❖ (Process) water production and reuse
 - ▣ Control water resources: surface water, condensate, ...
 - ▣ Monitoring treatment e.g. RO permeate quality, MBR membrane integrity, ... (*Lotte Vermijs, master thesis*)
 - ▣ Development of early-warning system (*Agathi Naka*)
- ❖ Distribution
 - ▣ Monitoring microbial water quality and regrowth in network (*Pidpa*)
 - ▣ Linking planktonic community to biofilm and MIC (*Marjolein Minne*)
- ❖ End-users
 - ▣ On-line control of cooling towers & circuits
 - ▣ Automated steering of biocide dosing



Thanks for your attention

Benjamin Buysschaert
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Agathi Naka
Sam Van Nevel
Lieven Clement
Olivier Thas
and Frederik Hammes (Eawag)



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