Airborne bacteria: names and numbers, please…

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My background

- Microbial ecology
- Marine microbiology group- Bergen, Norway
- Focus: algae, bacteria, protozooa, virus

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- Methods
  - Nutrient uptake/turnover
  - Production rates
  - Genetic diversity/biodiversity
  - Viral activity
  - Modelling (vs experiments)

Why airborne bacteria?

- Curiosity…new field
- Biodiversity and transport rates
- Microbial production
- Improved/new methods…possibilities?

Bacteria in the air …from where?

	Bacteria	Virus
Soil (terrestrial)	~10^9 g^{-1}	??
Sea /fresh water	~10^6 mL^{-1}	~10^7 mL^{-1}

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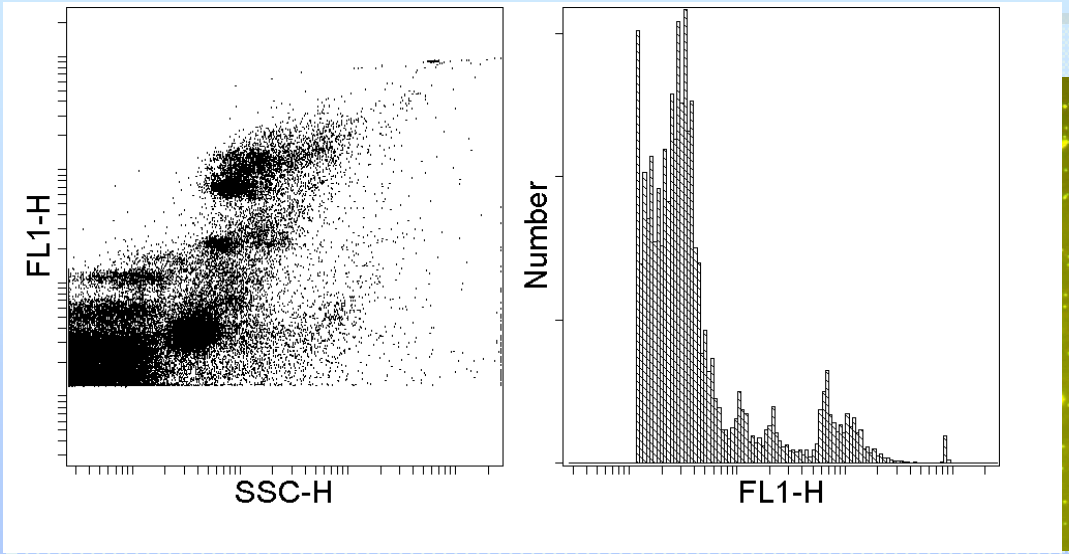
- **We know:**
- Most marine bacteria are assumed to be alive
- Nutrient limited N , P or C
- <1% growing on agar plates (sea water and soil samples!)

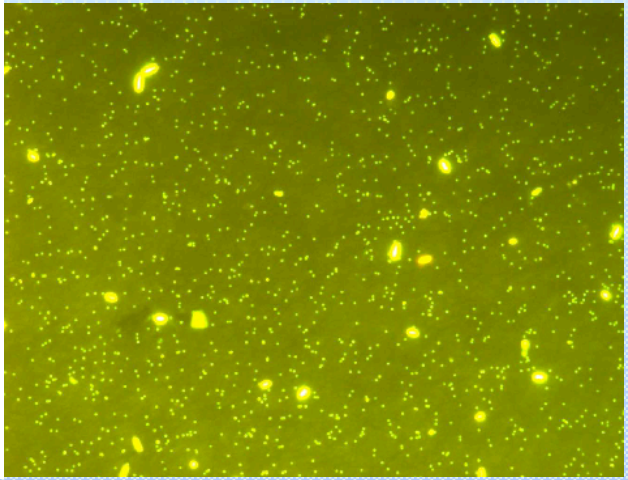
Counting airborne bacteria

- Liquid samples!
  - Rain or air sampled in liquids
- Methods:
  - Flow cytometry (FCM)
    - DNA/RNA staining - fluor /scatter signals from single particles
  - Quantitative PCR (Q-PCR)
    - DNA staining – amount of bacterial DNA in a bulk sample
  - Colony forming units (CFU)
    - Bacteria willing to grow on agar plates
  - Epifluorescense microscopy (EFM)
    - DNA/RNA staining - fluor signals from single particles

FCM plot / EFM picture

sea water examples:





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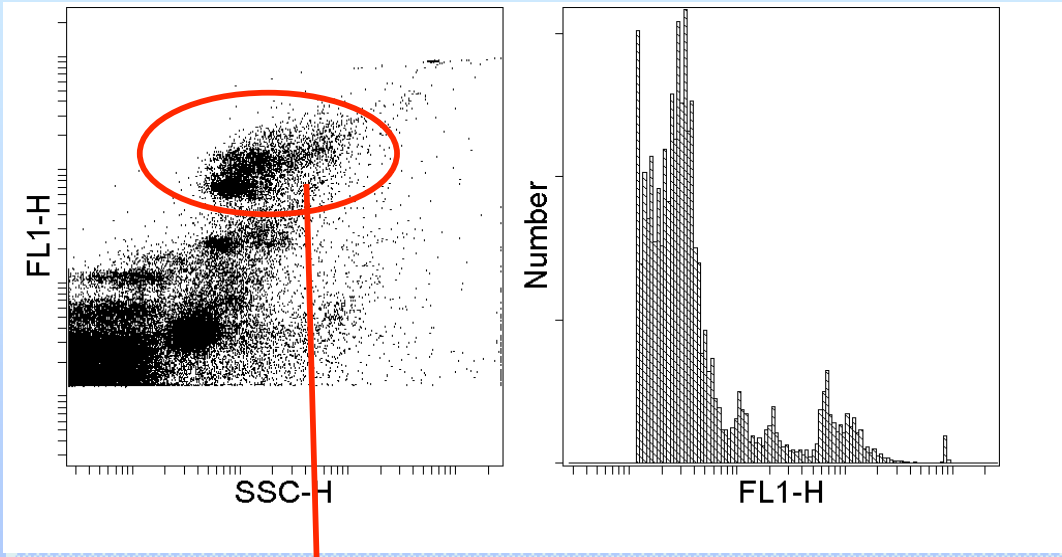

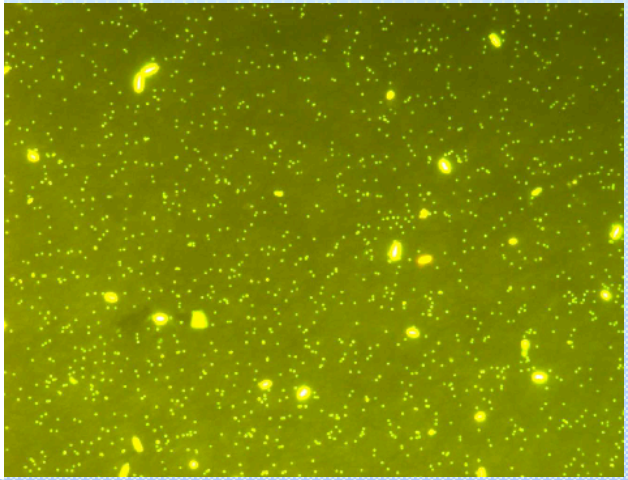

Marine bacteria

FCM plot / EFM picture

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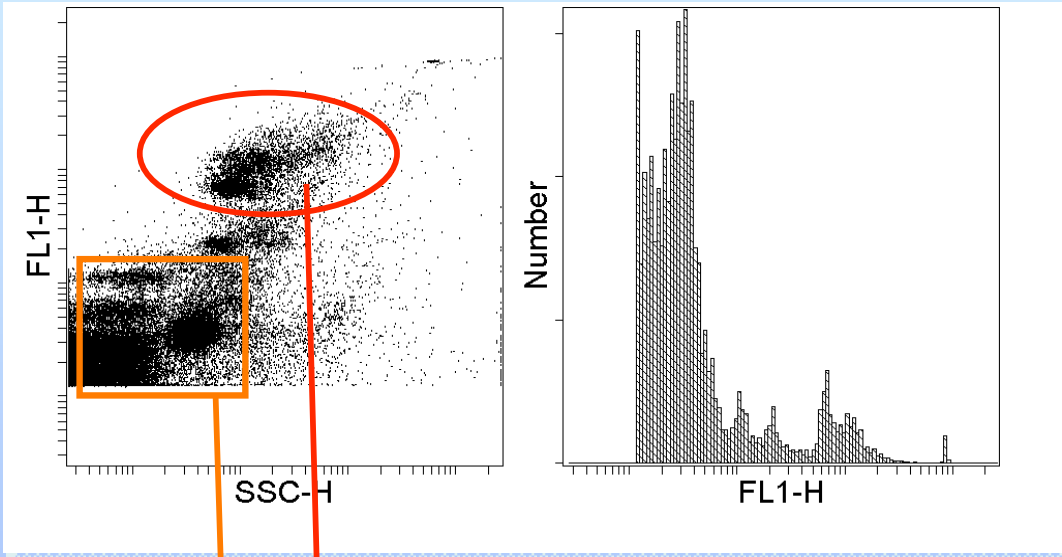

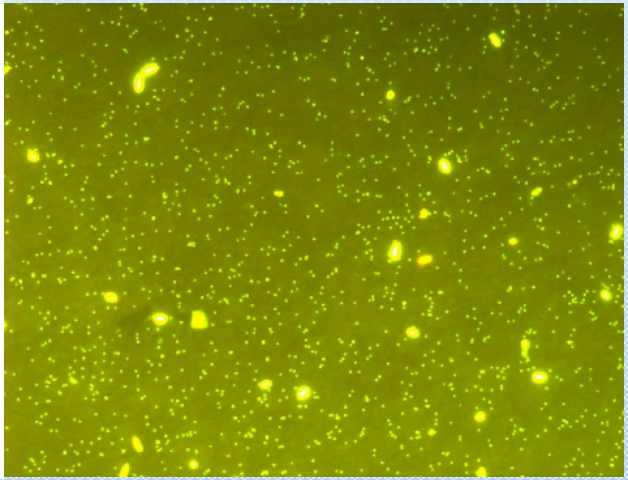

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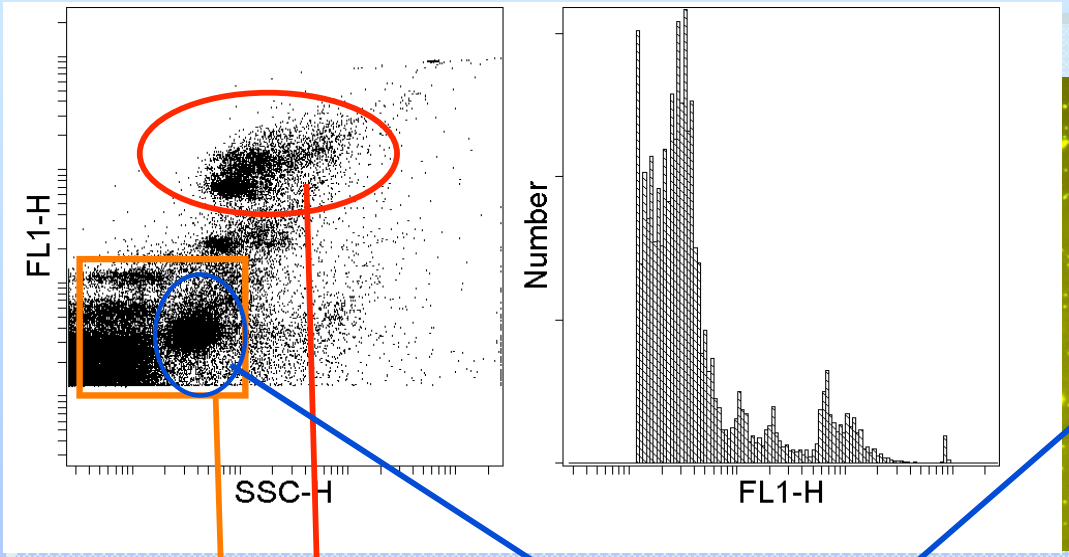
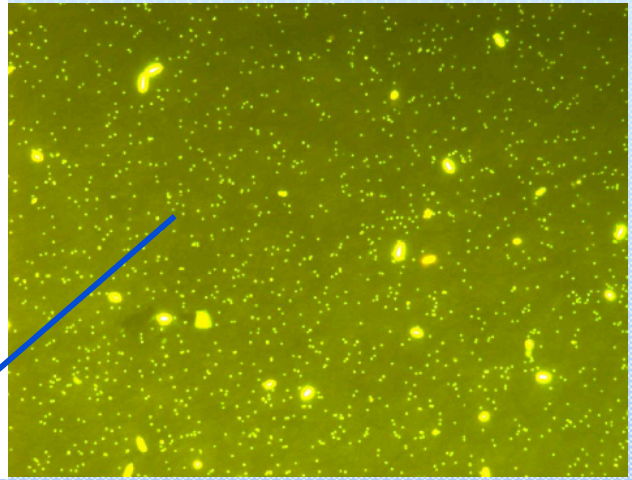




Emiliania huxleyi virus isolate (EhV)

Marine bacteria

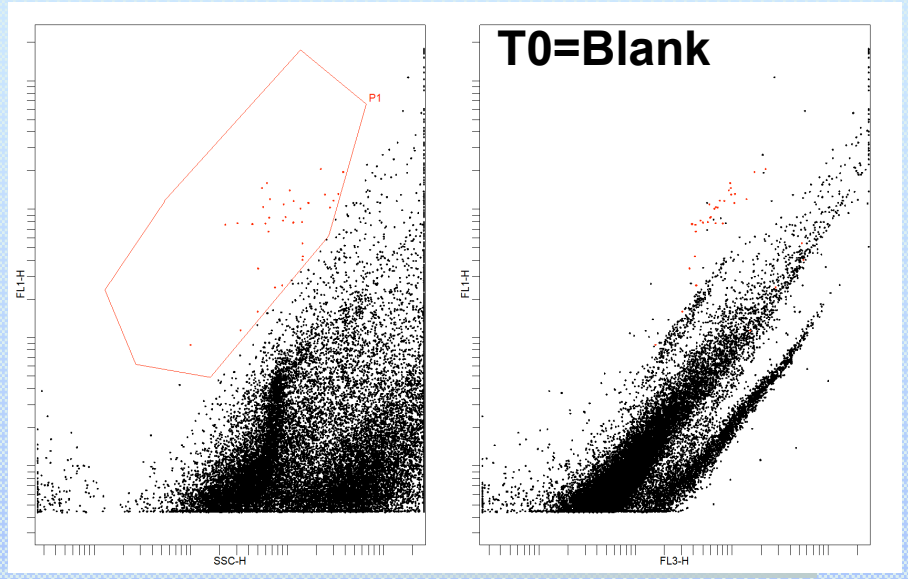
EhV

Marine viruses

Sampling airborne bacteria

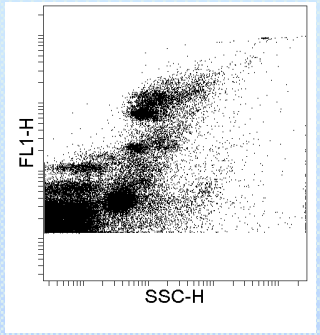
- Xmx-cv (Dycor)
  - 0,7 m^3 min^{-1}
  - 5 mL PBS
  - Suggested max sampling time: 5 min
  - Our max: 10 min

Flow cytometry of air samples



Fluorescence

Scatter signal



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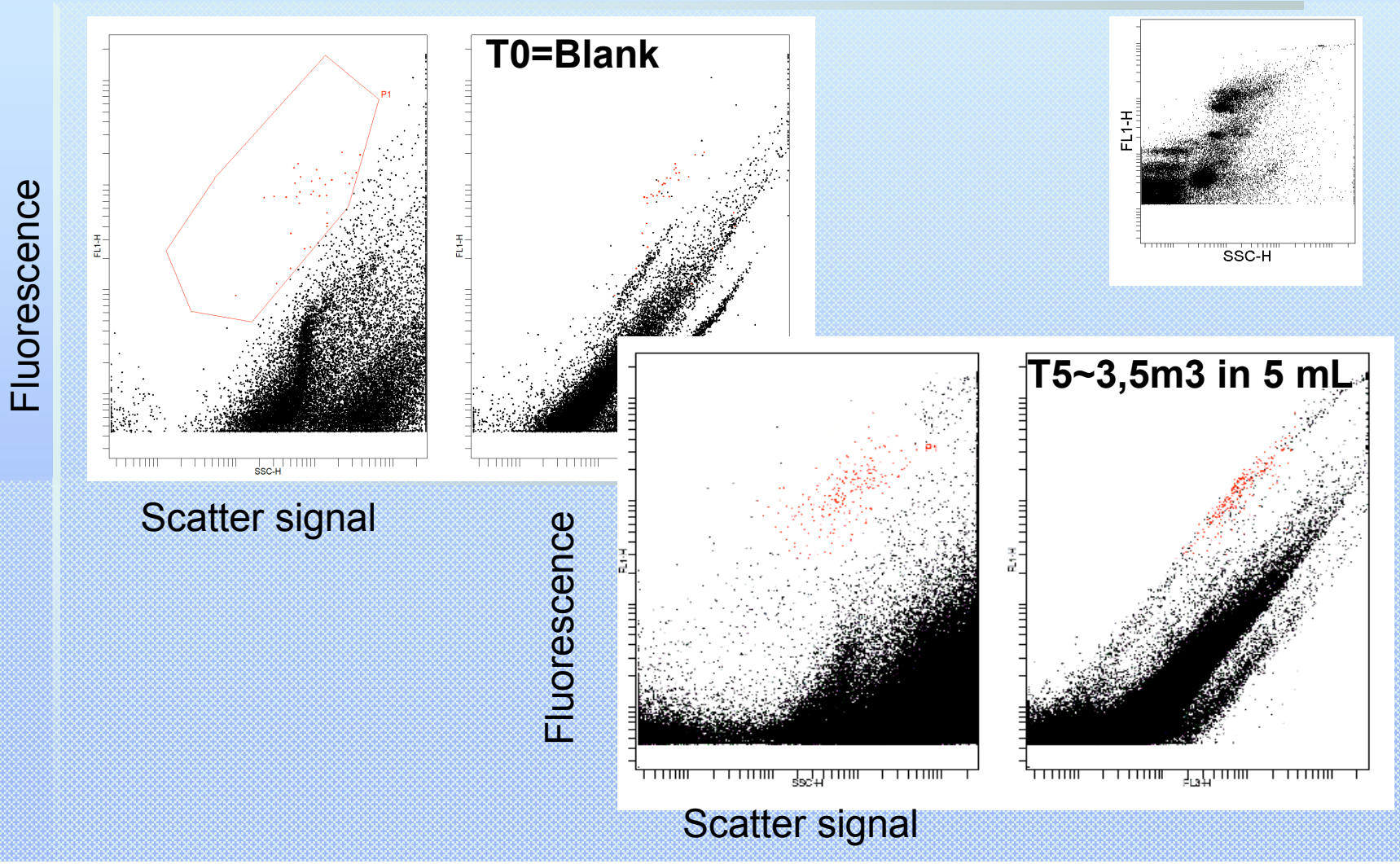

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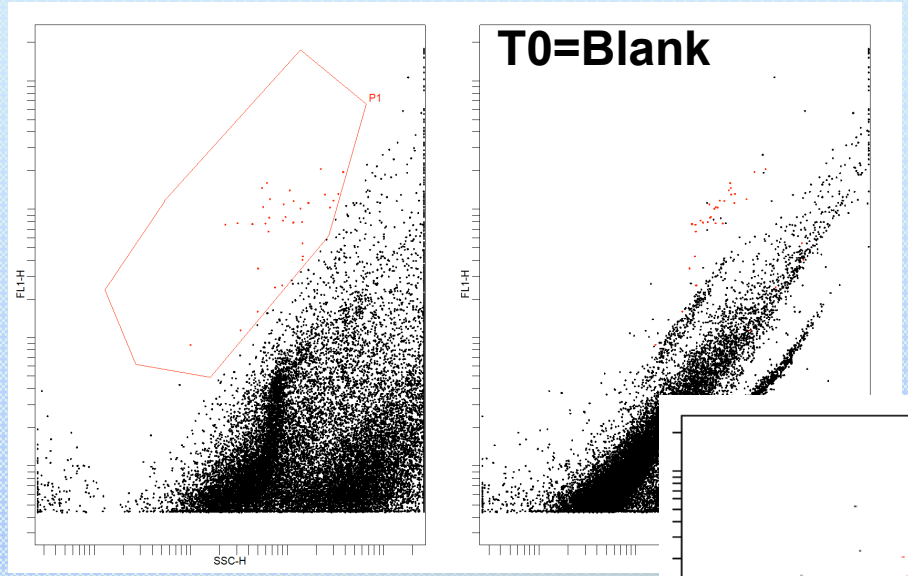
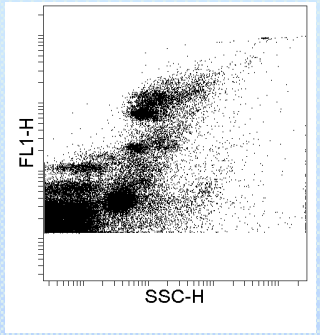
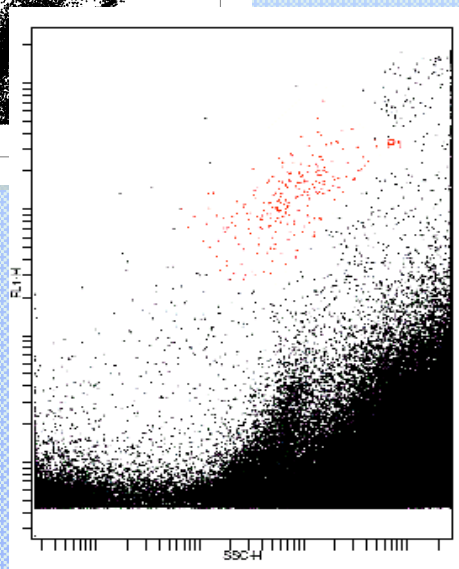


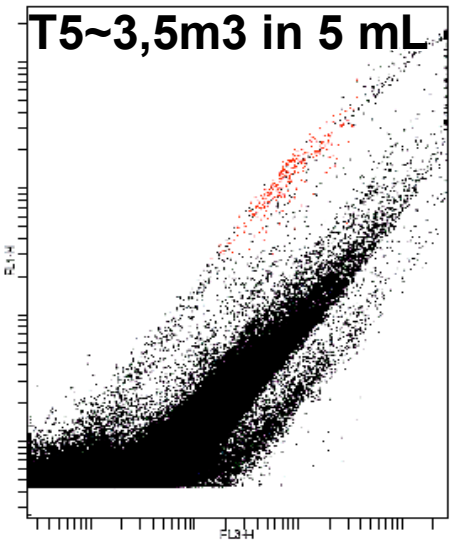


Fluorescence

Scatter signal

~10^3 pr m^3



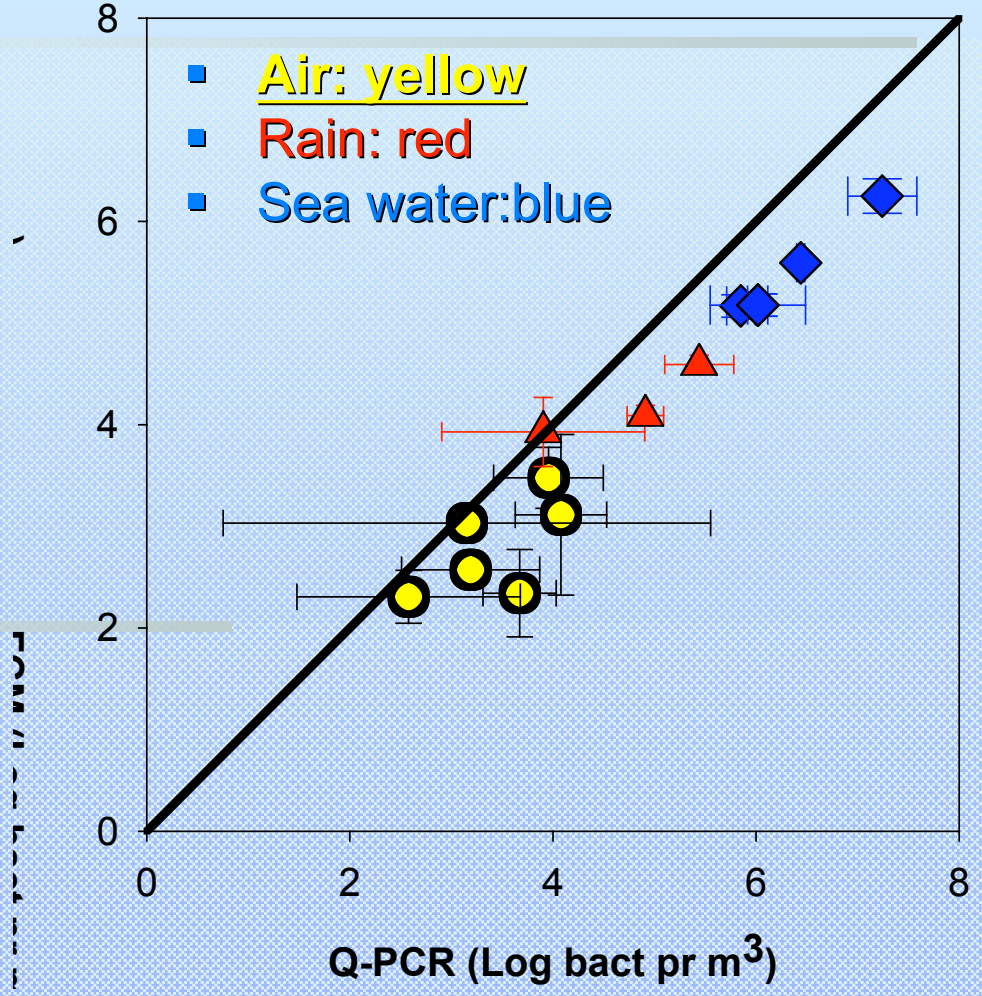


Fluorescence

Scatter signal

Flow cytometry vs Q-PCR

- Q-PCR:FCM ~10
- Calibration going on!

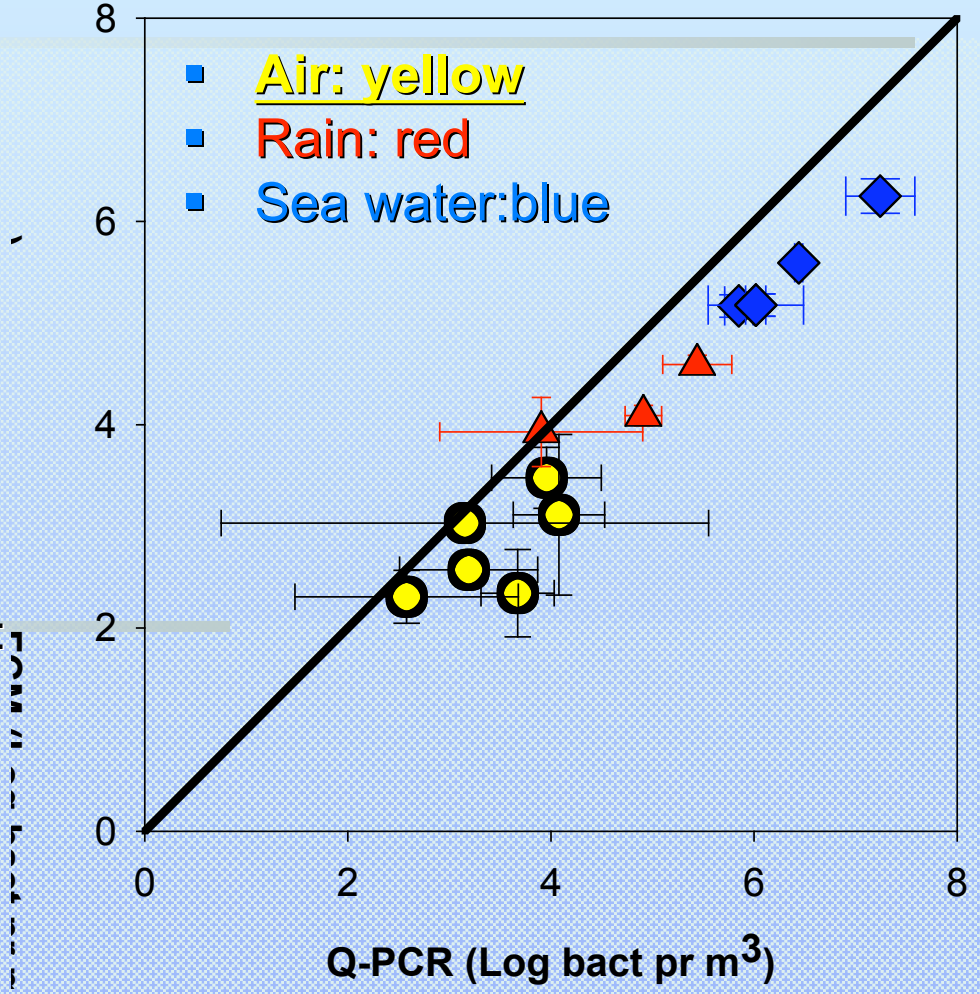


Flow cytometry vs Q-PCR

- Q-PCR:FCM ~10
- Calibration going on!

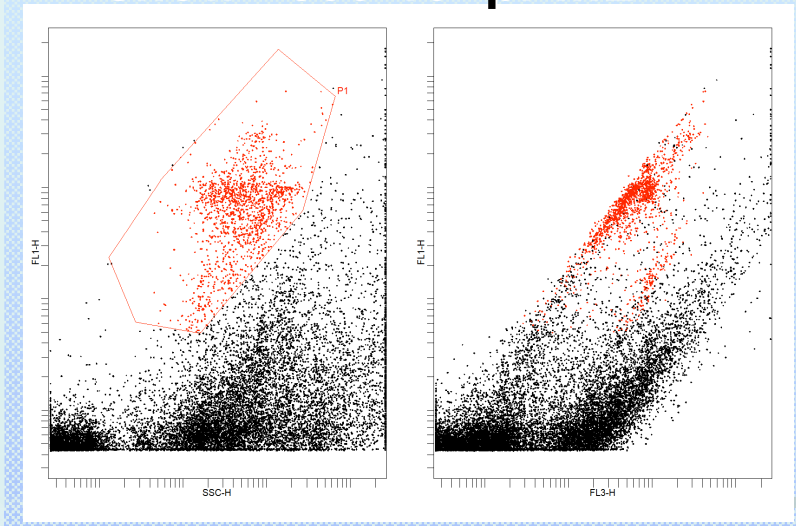
CFU: around 1-2% compared to FCM

Epifluorescence microscopy: difficult to discriminate signal/noise



Snow and rain samples

snow ~500-10^4 pr mL



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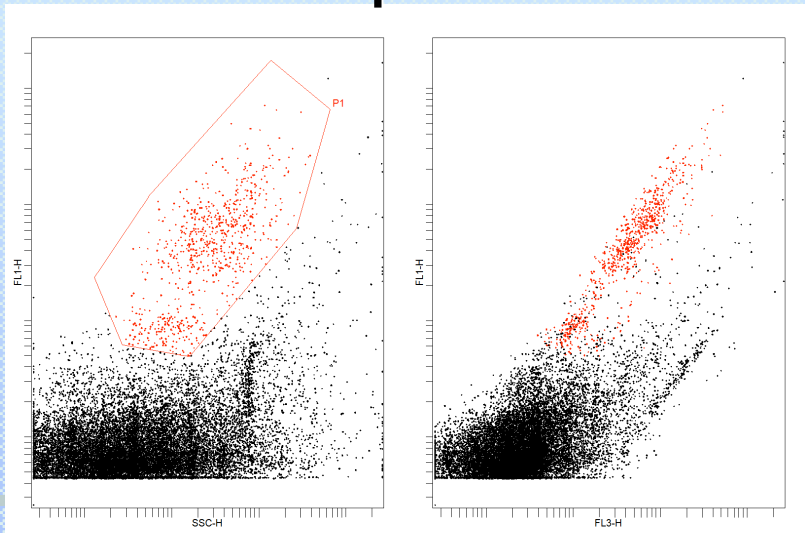

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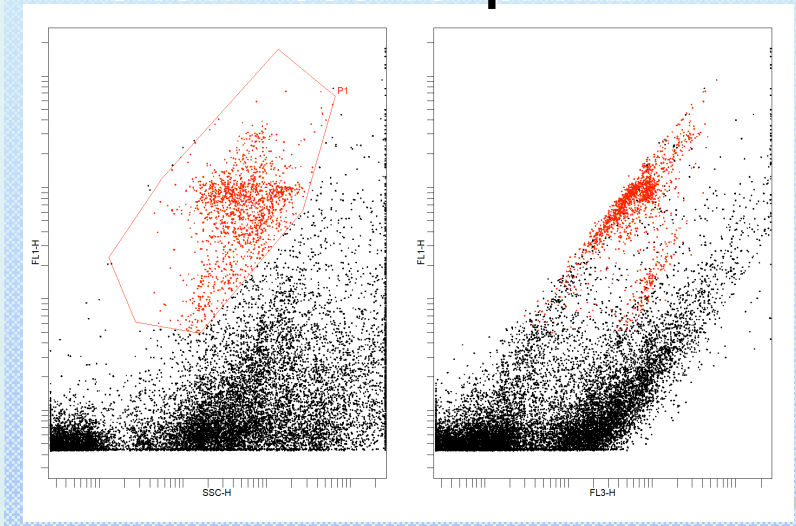

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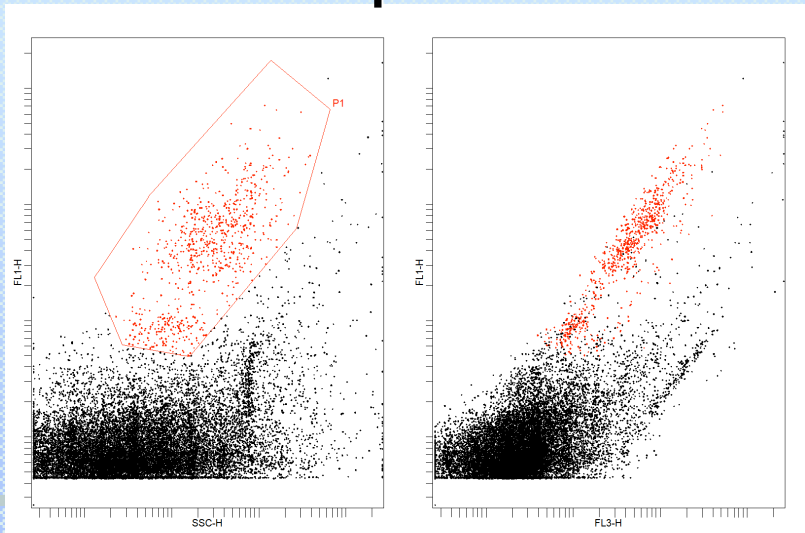

Bacterial production:

Addition of H^3 leucine, 24 h incubation

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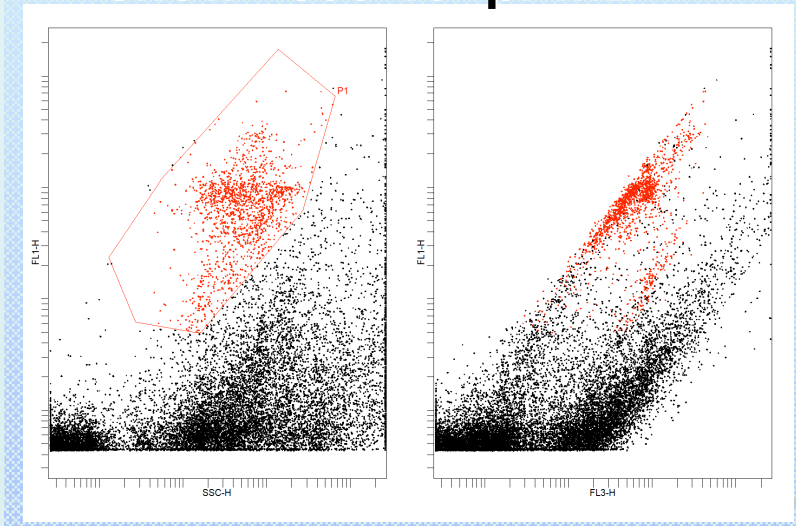

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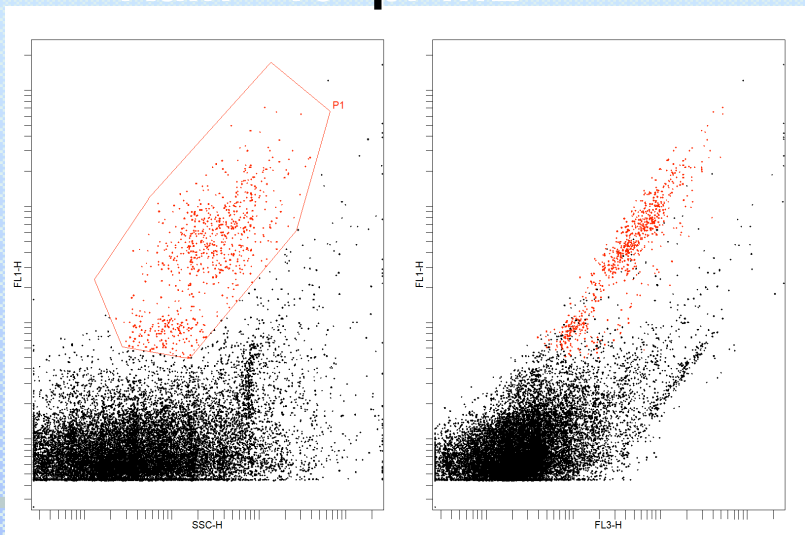


Bacterial production:

Addition of H^3 leucine, 24 h incubation

Result: activity pr bacteria ~ as in sea water

Estimated generation time~ 1day

Comparison of 3 bioaerosol samplers

- Time series
- FCM and CFU counts
- Liquid loss rates

Sampler 1

- Xmx-cv (Dycor)
  - 0,7 m^3 min^{-1}
  - 5 mL
  - Suggested max

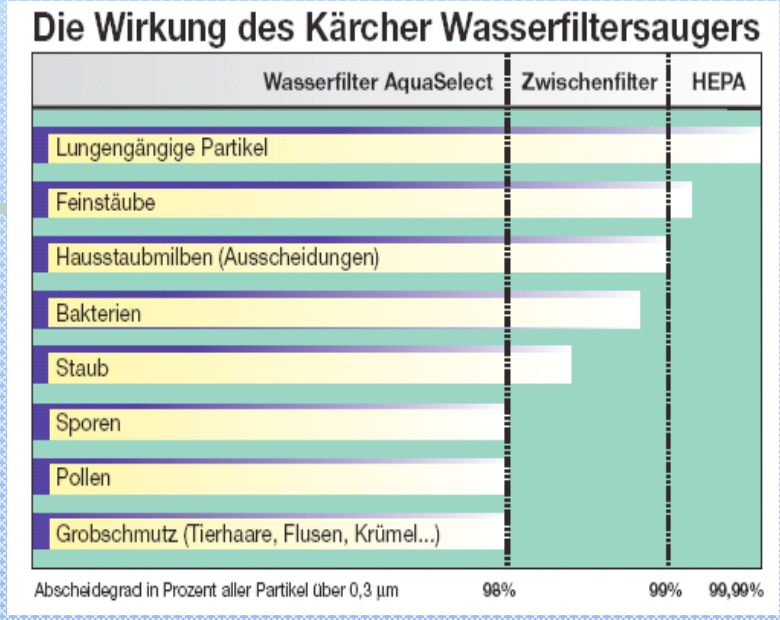
  sampling time: 5 min
  - Our sampling time 0-10 min
  - Liquid: PBS
  - ~10% PBS loss after 10 min

Sampler 2

- **SKC 225-9595 (SKC)**
  - 0,0125 m^3 min^{-1}
  - Sampling time 0-60 min
  - Liquid: MilliQ
  - Refill~25% milliQ at 10 min

Sampler 3

- Kärcher DS 5550



Sampler 3

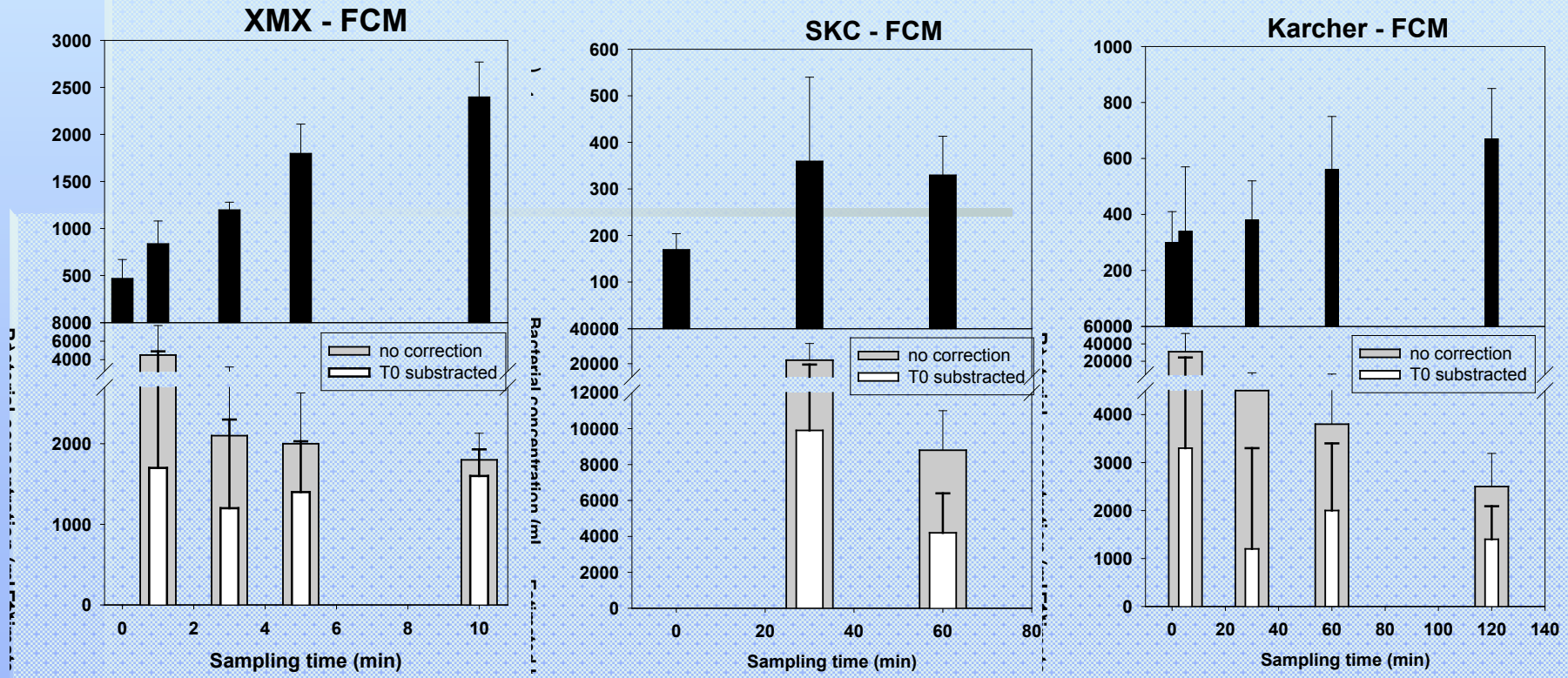
- **Kärcher DS 5550**
  - 3,3 m^3 min^{-1}
  - 1500 mL
  - Max sampling time: 120 min
  - Liquid: PBS
  - ~10% pbs loss after 120 min

FCM counts - time series

Black: direct counts FCM *"bacteria pr mL"*

Grey: estimated bacterial concentration pr m^3 air

White: estimated bacterial conc pr m^3 air after substracting blank (T0 sample)



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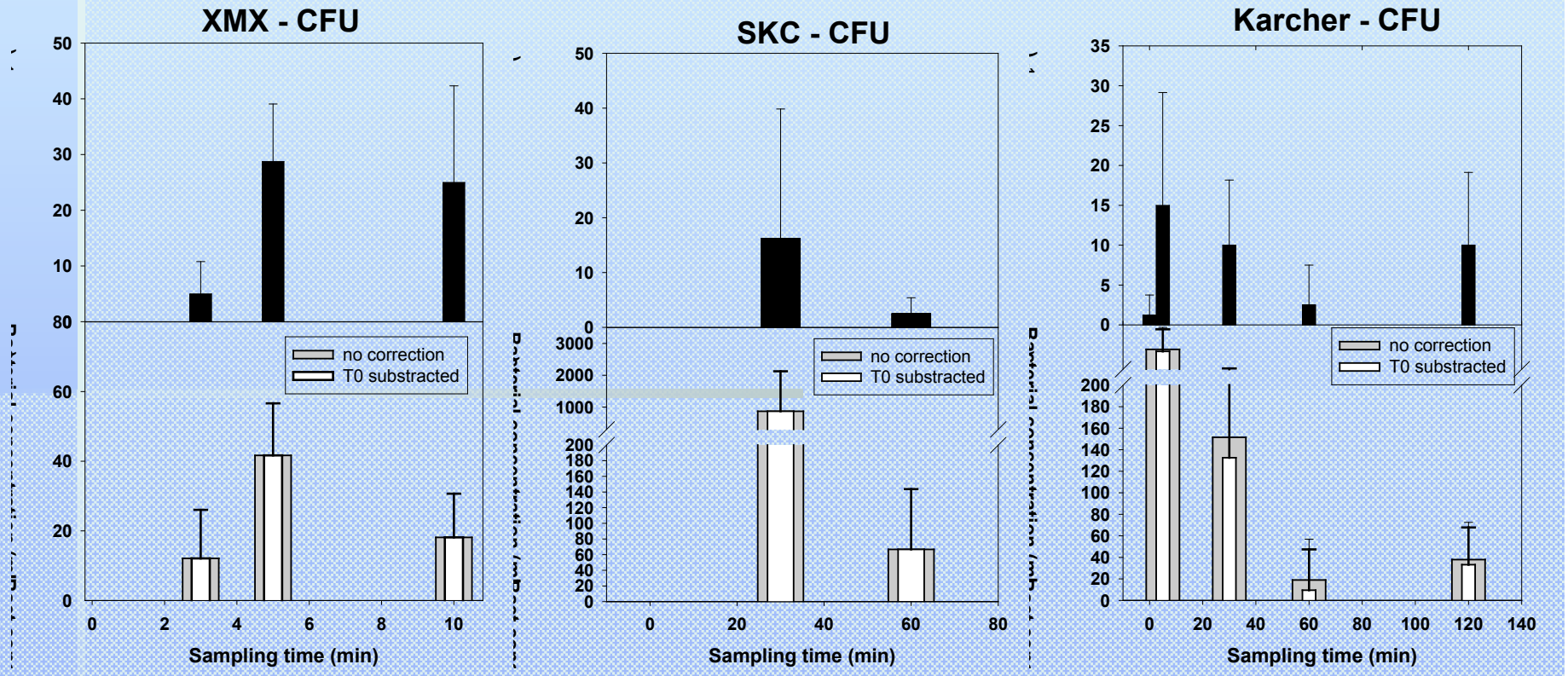

CFU as % of FCM

Sampler	sampling data		CFU pr m^3		FCM m^3		CFU as % of FCM	
	time (min)	volume (m^3)	CFU±err[3]	n	FCM±std. dev[4] raw data	FCM±std.dev[4] T0 substr	FCM raw data	FCM data T0 subst.
XMX-CV	10	1.4[1]	18±13	4	1800±330	1600±330	1.0	1.1
Karcher	120	0.26[1]	33±35	4	2500±690	1400±690	1.3	2.4
SKC	60	0.038[1]	67±77	4	8800±2200	4200±2200	0.8	1.6
slit sampler	60	1.8[2]	37	1				
Mas-100	20	2.0[2]	40±14	2				

Table xx. cfu and fcm bacterial counts in samples with the highest theoretical concentration factor in this experiment.
1. estimated air volume sampled pr ml sampling liquid.
2. Air volume sampled.
3. std dev except slitsamplers,
4. std dev of 3 dilutions

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The dice is thrown…

- Evaluation
  - Background noise
  - Liquid volume
  - Sampling volume
  - Sampling time
  - Sampling efficience – concentration factors
  - Reliability

The dice is thrown…

+ Robust

+ Low backgroud

+ Fast sampling

- Low liquid volume

The dice is thrown…

XMX-CV

+ Robust

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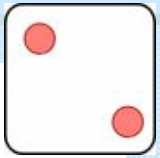
- Low liquid volume



SKC

+ ok background

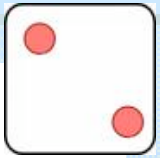
-Very low efficiency

-Highest liquid loss rate

The dice is thrown…

XMX-CV

+ Robust
+ Low backgroud
+ Fast sampling
- Low liquid volume



SKC

+ ok background
-Very low efficiency
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Kärcher

+ High volume rates
+ High volumes
- High volumes
- Highest backgroud

In the end

- **Bacteria in air pr m^3:**
  - **CFU 10-50**
  - **FCM ~10^3**

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- **Bacteria in snow/rain pr mL:**
  - CFU 10-200
  - FCM ~10^4
  - Bacterial activity in snow/rain ~ SW bacteria

In the end

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Sampler test:

1. XMX-CV
2. Kärcher
3. SKC

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Thanks to:

- University of Bergen, Dep Biology
  - Jørn Einen
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  - Mikal Heldal

- Norwegian Defence Research Establishment
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  - Else-Marie Fykse
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