FLUORESCENCE ASSAY FOR SPORE GERMINATION DETECTION

FAST DECONTAMINATION ASSAY

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RESUME OF PRESENTER

Dr. Andreas Tortschanoff, (* 1973) has studied Chemistry at the University of Vienna, Austria. He received his Ph.D. in the field of laser spectroscopy in 1999. After 5 years in Switzerland at the EPFL he returned to Austria to work at the Carinthian Tech Research (CTR) in the field of photonic micro-systems with focus on the development and integration of MOEMS-based sensor devices. In 2019 he joined Silicon Austria Labs.

Currently, he is working as principal scientist at Silicon Austria Labs in the Photonic Systems Department. He is author of more than 50 publications in the field of spectroscopy and MOEMS based devices.

PHOS RESEARCH UNIT @SAL

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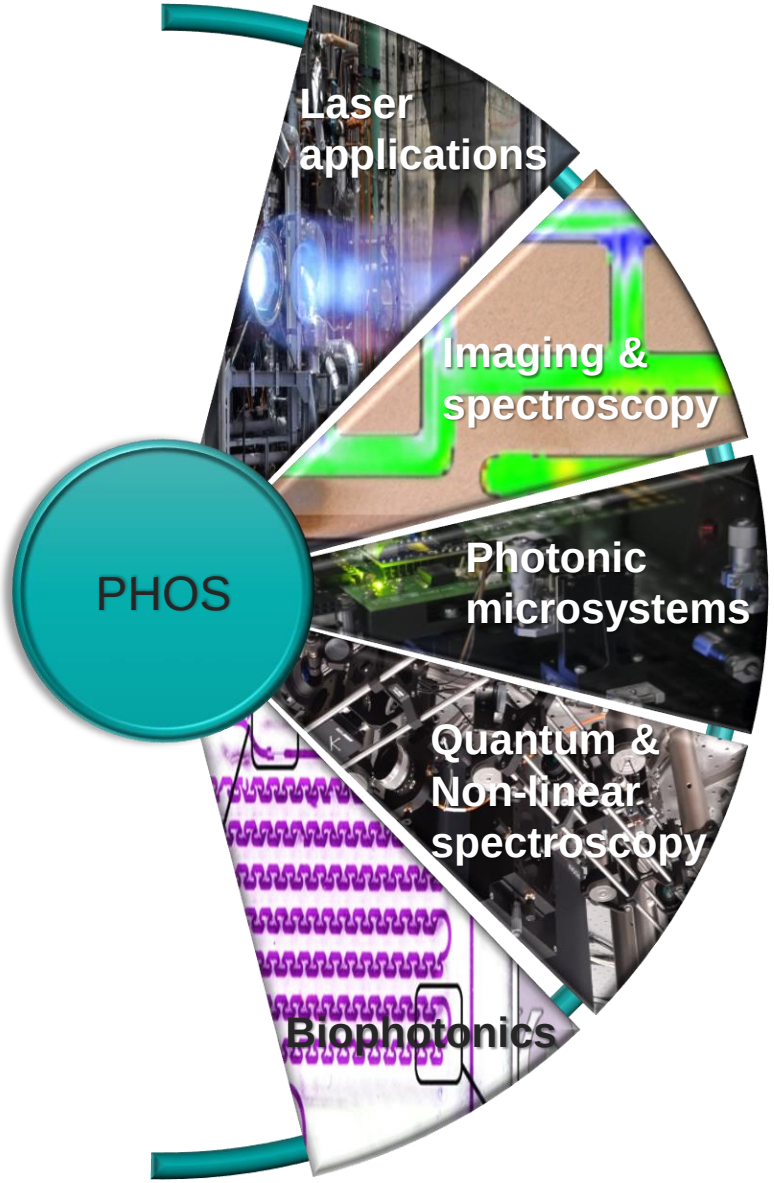
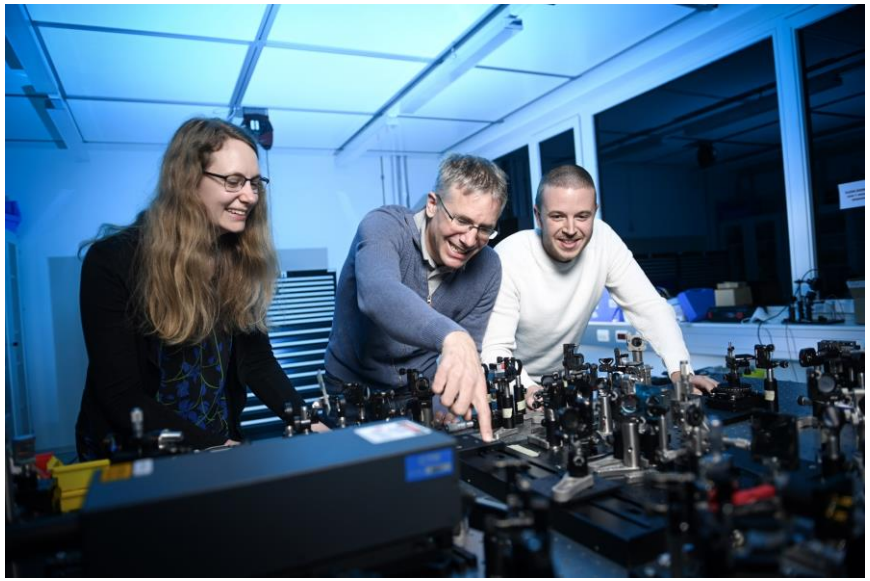


- Research infrastructure (laser class 4):
  - General-purpose Optics Lab
  - Laser development Lab
  - Raman (Micro)Spectroscopy Lab
  - Ultra-fast / Quantum Sensing Lab
  - Photonics 7 lab
- Spectroscopy Lab
- Chemistry Lab
- Application Lab for dedicated test stands and out-of-lab functional demonstrators & prototypes
- Comprehensive competence in component & system design & simulation software



FT-IR microscope
Bruker, Lumos II

Raman microscope
Renishaw inVia™ Qontor

Laser Vibrometer
Polytec MSA-500

Glass processor
Fiber splice/taper/combine

TERS

Profilometer

3D photonic assembly

Photonic probe station

MOTIVATION

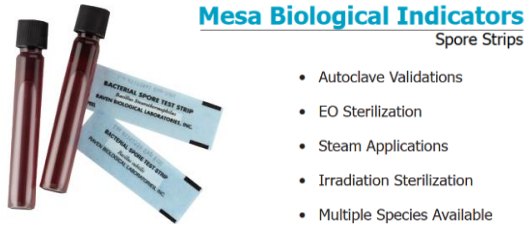
- Assessing disinfection and sterilization quality is of uttermost importance in medical and other clean environments.
- Biological indicators, or spore tests, are the most accepted means, and assess the sterilization process directly by killing known highly resistant microorganisms.
- Classical tests for spore contamination rely on bacterial growth, which is slow!
- Slow also translates to expensive in the case of test for disinfection success of rooms. And slow translates to increased risk, in the context of diseases (e.g. anthrax)
- Assays are needed which are easy to implement and provide RAPID results without sacrificing sensitivity and accuracy.

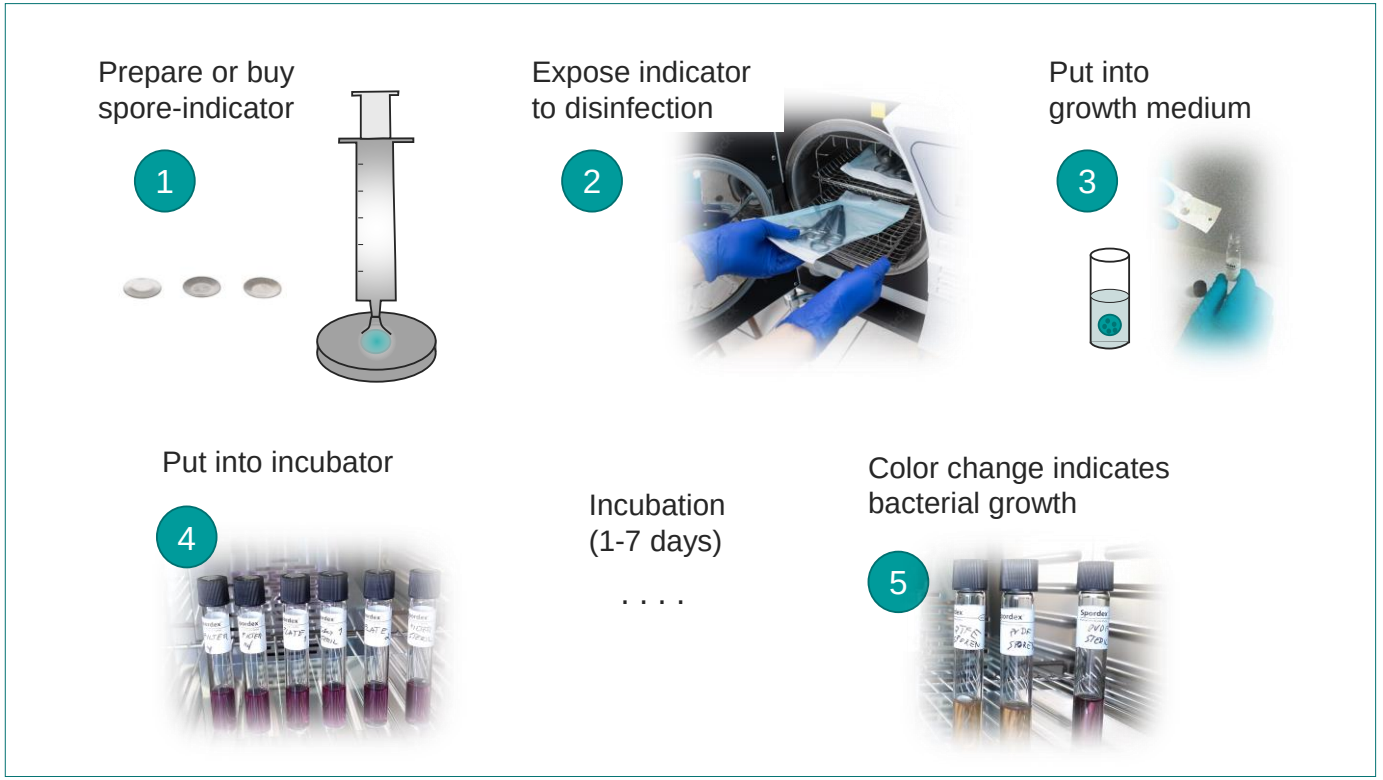
DISINFECTION VALIDATION STATE-OF-THE-ART

Check for surviving spores using bacterial growth:

1. Spore indicators
2. Apply disinfection procedure
3. Put into growth medium
4. Incubate to check for remaining viable spores.
5. After incubation residual bacterial growth is reflected in color change and turbidity.

For reliable verification, incubation must last for several days!





GERMINATION DETECTION:

Decontamination assessment based on *Geobacillus stearothermophilus* spores

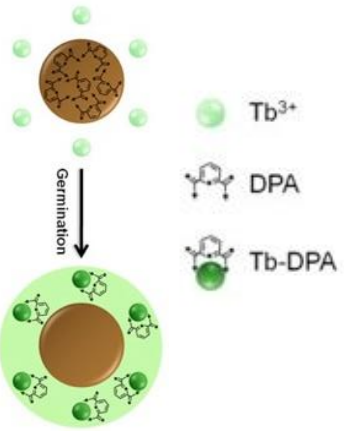
Focus on GERMINATION rather than growth.

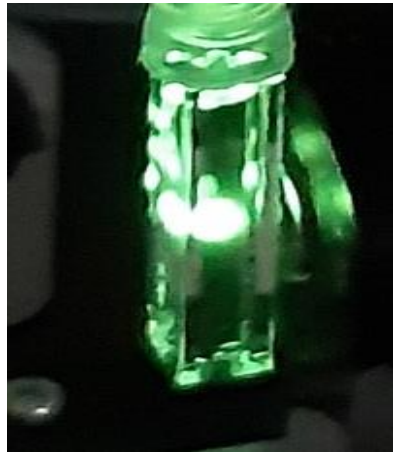
Can be detected with highest sensitivity via fluorescence-based measurements.

Assays can be prepared based on an (micro-)fluidic automated scheme.

Reliable results after 30 minutes.

>20x reduced measurement times!!





EXPERIMENT SCHEME

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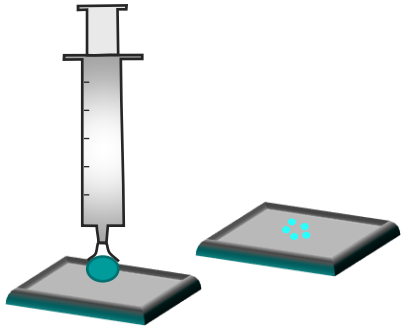
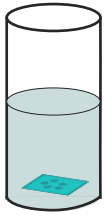
1 Centrifuge and dilute spore solution

2 Deposit on substrate

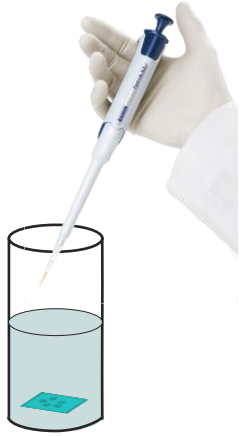


3 Sterilization

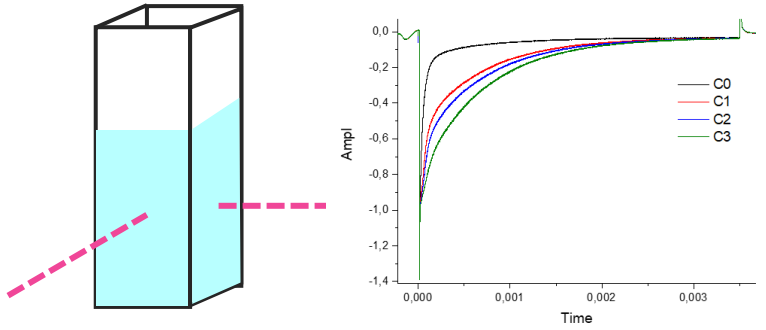
4 Incubation with L-Valin (Production of DPA)



5 Sampling + $TeCl_3$



6 Measurement by Fluorescence



TIME RESOLVED FLUORESCENCE

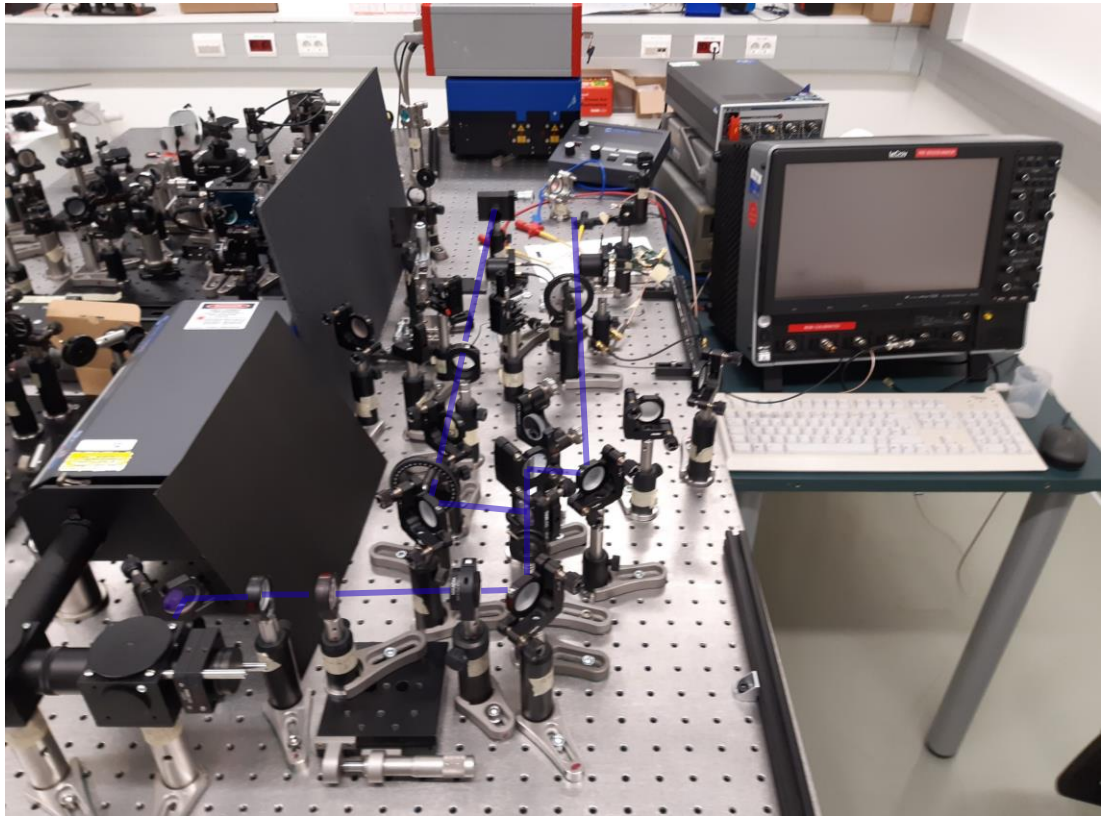
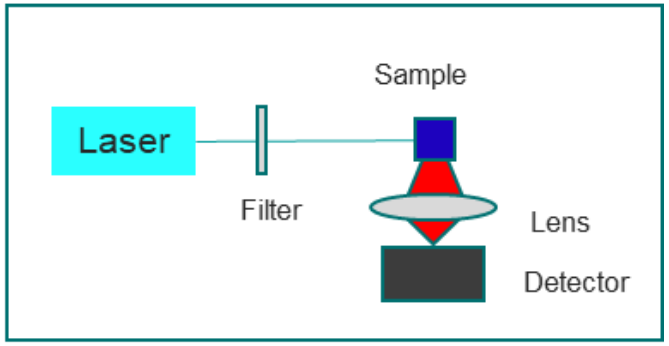
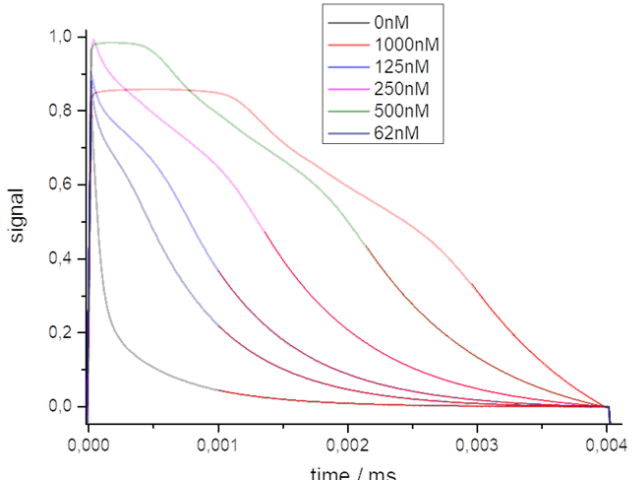


Photo of the fluorescence set-up



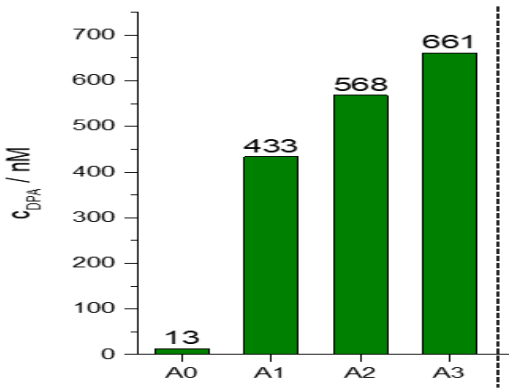
Scheme of the set-up



Fluorescence intensity of TbDPA in 10mM Trizma buffer for the DPA concentrations marked in the legend; Curves at high concentrations show detector saturation.

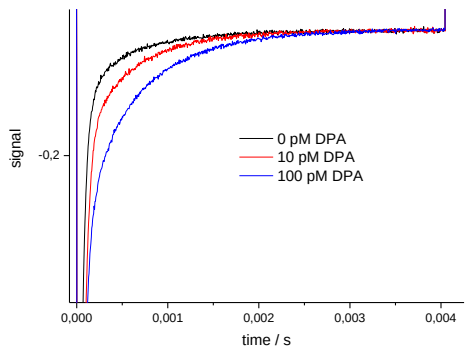
FEASIBILTY: “MIKOSENSE PROJECT”

Feasibility was proven at the ASSIC K1-center within the „Mikosense“ project.

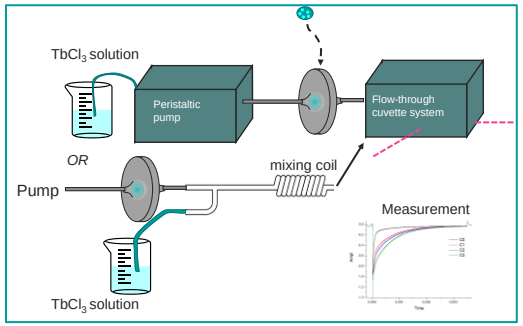


10 min till germination

Versatile solution for chemical desinfection validation.



Highest sensitivity

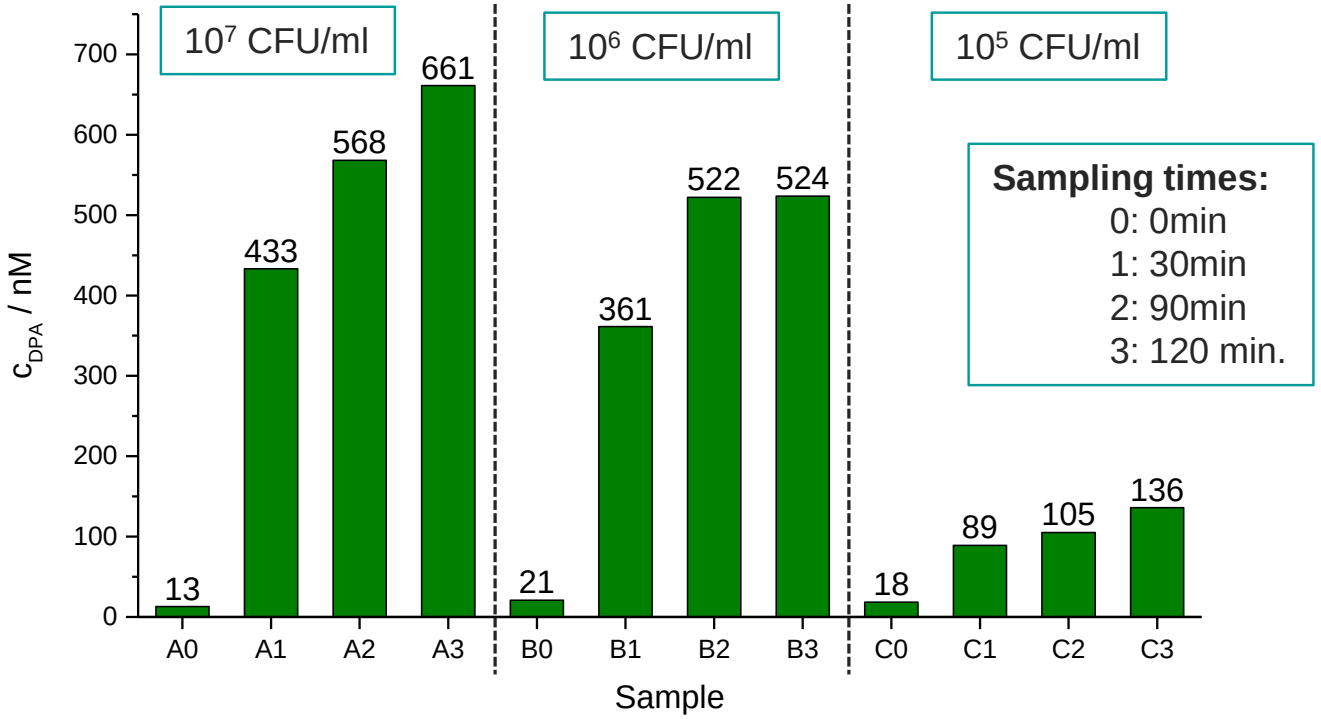


Automated measurement scheme; (proposed)

Patent pending!

GERMINATION KINETICS

After 30 minutes more than 60% of the spores have germinated !



DPA-concentration measured on the germination samples. Sampling times are indicated in the inset. Concentrations of the original spore solutions were 10^7, 10^6, 10^5 CFT/ml for sample-series A, B, C, respectively.

ESTIMATIONS

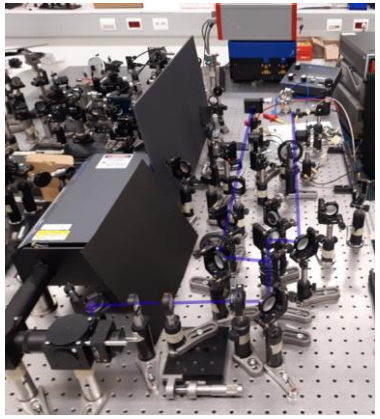
Spore Solution:	Value	comment
DPA/spore	2.3 10^8 molecules /spore	** zhou et al, PLOS one 8, e74987 (2013)
Spore concentration	1 10^7 CFU/ml	Suspension 1:10 diluted
DPA amount	3.8 nmol/ml = 3.8µM	Amount contained in the spores. (Would be released if cell desintegrate (380 attomol/spore))
DPA released at germination	114 nM	Assuming fraction of **3%** of the DPA released by the spores.

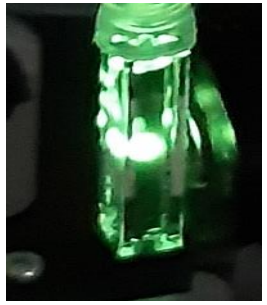
Single Spore/nl	Value	comment
DPA/spore	2.3 10^8 molecules /spore	** zhou et al, PLOS one 8, e74987 (2013)
Spore concentration	1 CFU/nl = 1 10^6 CFU/ml	Assuming single spore in 100x100x100 μm^3
DPA amount	380 attomol/nl = 0.38 µM	Amount contained in the spores. (Would be released if cell desintegrate (380 attomol/spore))
DPA released at germination	11.4 nM	Assuming fraction of **3%** of the DPA released by the spores.

→ Single Spore Detection feasible but microfluidics or microscopy necessary!

CONCLUSION AND OUTLOOK

- Rapid assay for spore germination detection, which can be used for sterilization validation.
- By optimized combination of germination conditions and a highly sensitive time-resolved fluorescence detection set-up based on pulsed laser excitation, we could determine the DPA, which is released during germination with nM sensitivity under realistic conditions.
- Single spore detection could be realistic, however, in order to reach sensitivities approaching the single spore regime, a microfluidic environment would be necessary.



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THANK YOU