3° SCIENTIFIC INTERNATIONAL CONFERENCE ON CBRNE

Determination of spectral fluorescence cross sections of bacteria aerosols: Achievements, challenges, and limitations.

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Motivation and challenges

Spectral fluorescence cross sections at selected excitation wavelengths are material properties, solely depending on bacteria (species, growth status, life form)

Basic information on

- Strength of fluorescence response of bacteria
- Distinguishability of bacteria by fluorescence

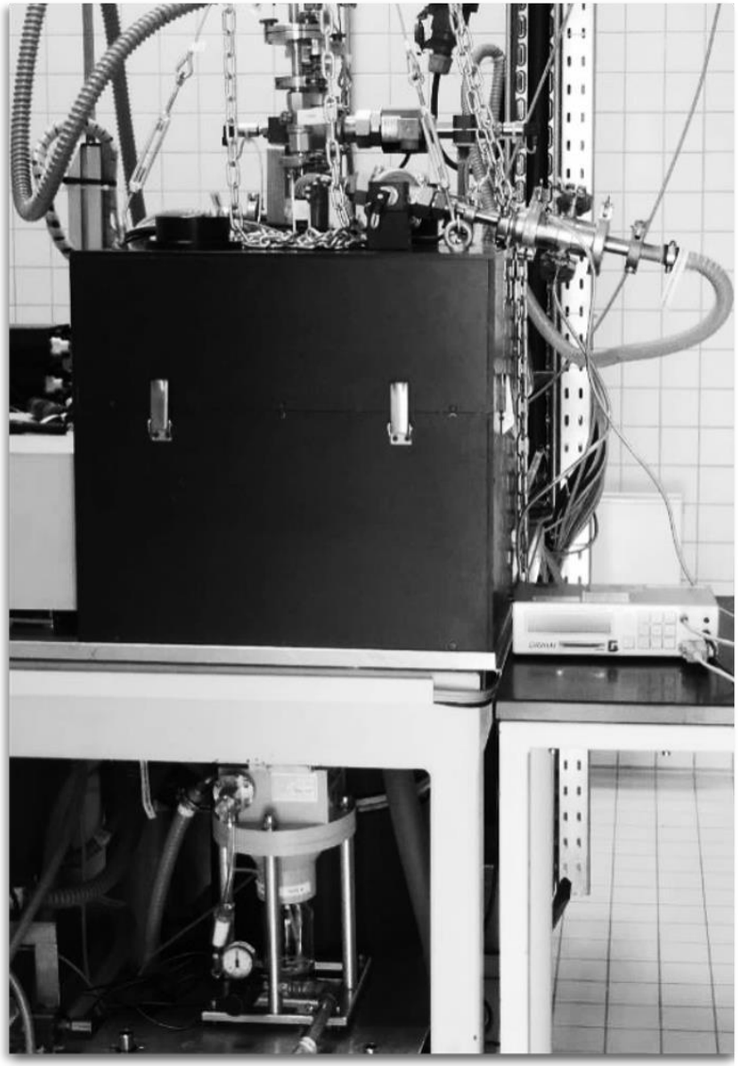
to support LIF stand-off detection

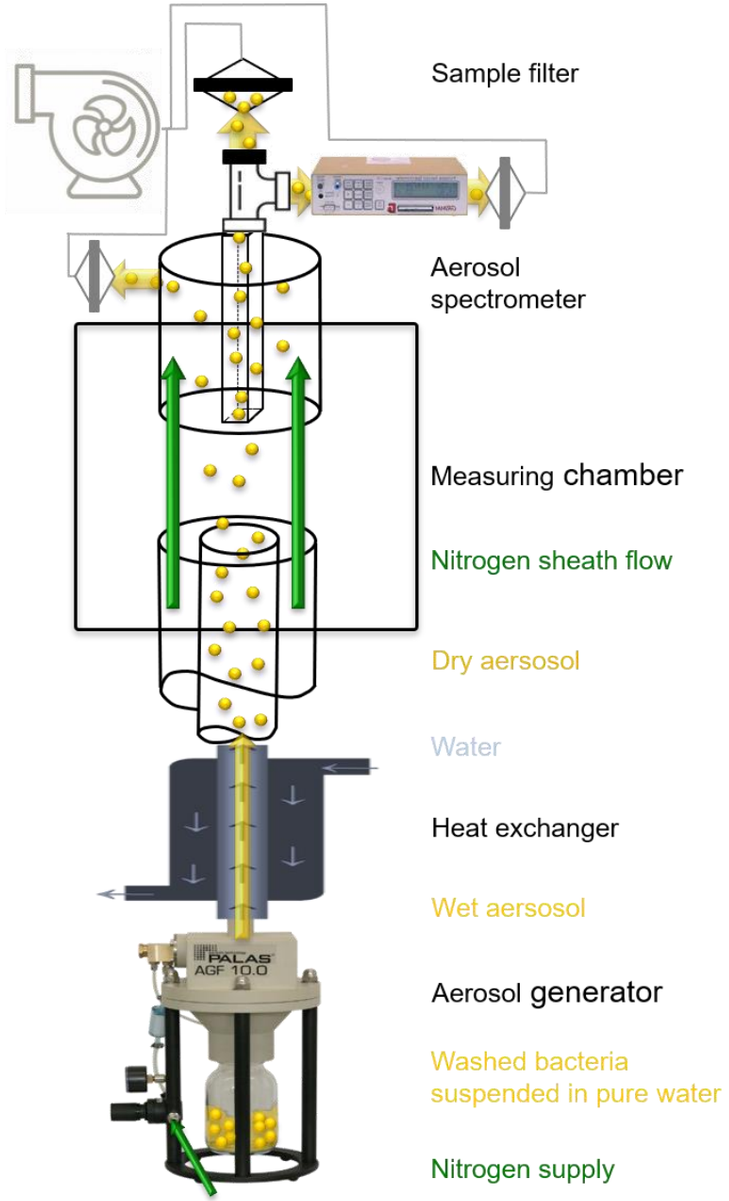
- Detector conception and application tailored design
- Careful choice of optical surrogates for test environments
- Classification
- Modeling

Measurement challenges:

- Precise absolute calibration of the measuring system
- Excact characterization of the aerosol flow

Aerosol provision





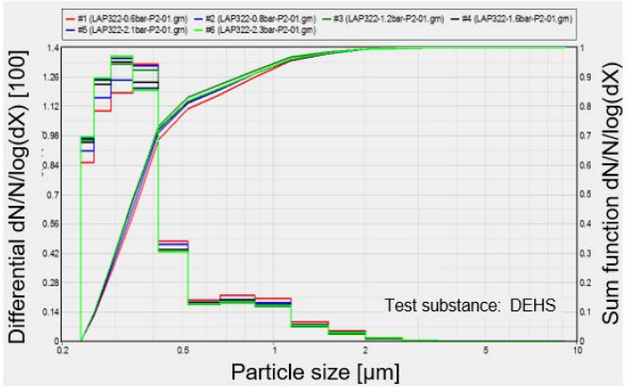
Measuring system

- BSL2 ready
- Remote operation
- Safety-related process control

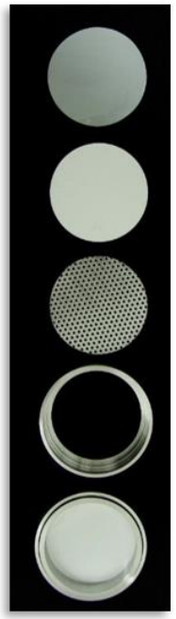
Aerosol

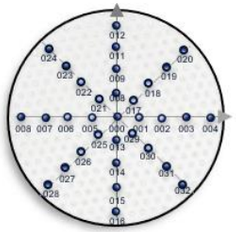
- Vegetative Bacteria, washed
- Bacteria spores, washed

Aerosol generation



Aerosol characterization

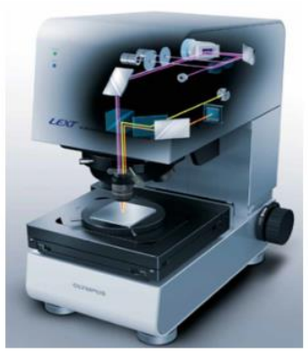


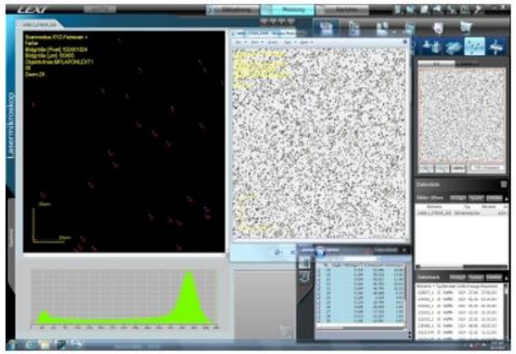


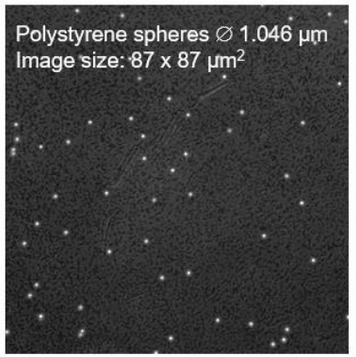
Sample filter with 13…33 analyzing positions

Bacteria density range: $10^8 \ldots n \cdot 10^{10}$ #/m^3

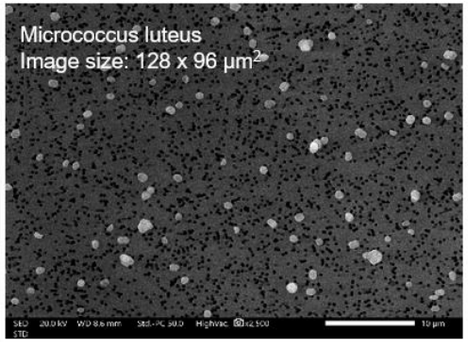
Particle / Bacteria counting





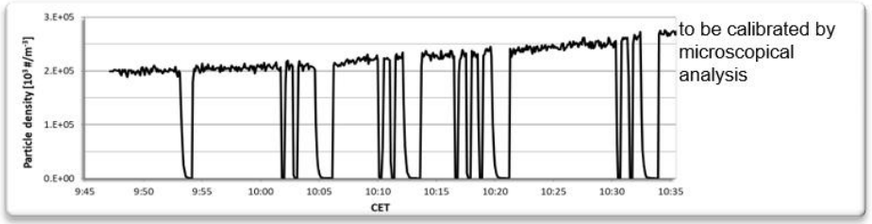


Laser scanning microscope



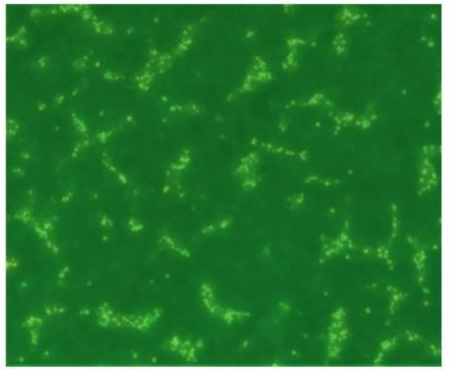
Scanning electron microscope

Aerosol spectrometer

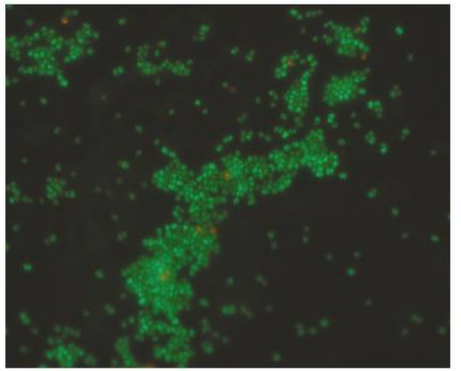


to be calibrated by microscopical analysis

Life-dead analyses of vegetative cells on a random basis



Sample filter,
green = alive / red = dead

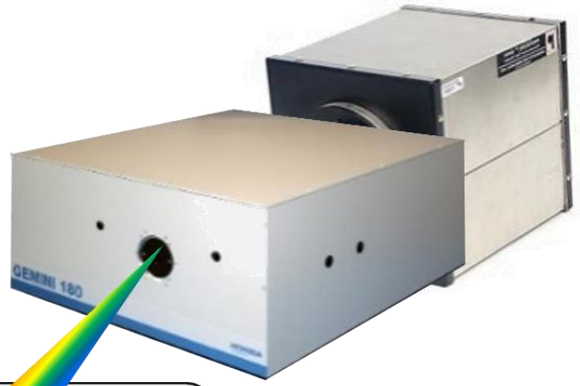


Residual bacteria suspension after test series,
green = alive / red = dead

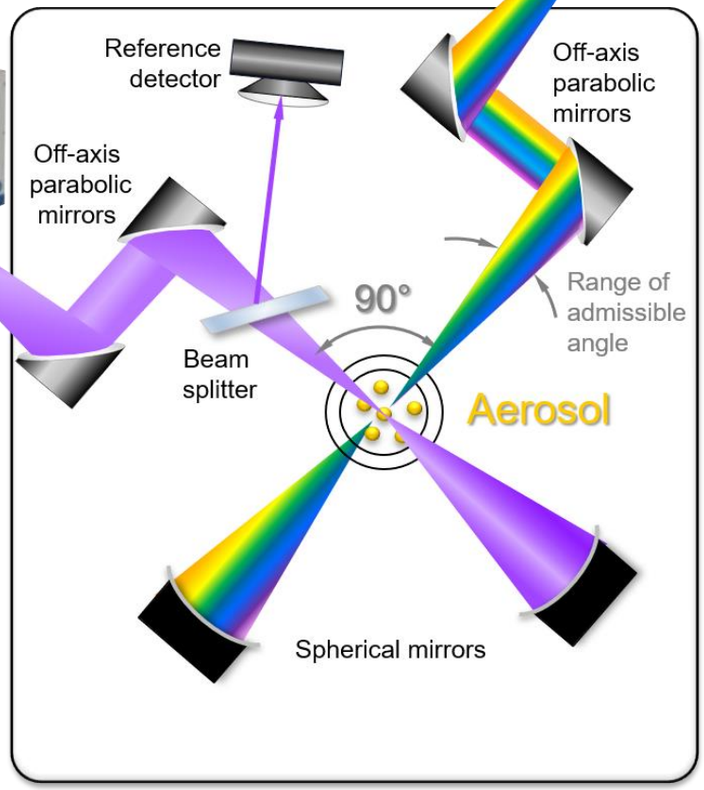
Optical set-up

Double monochromator

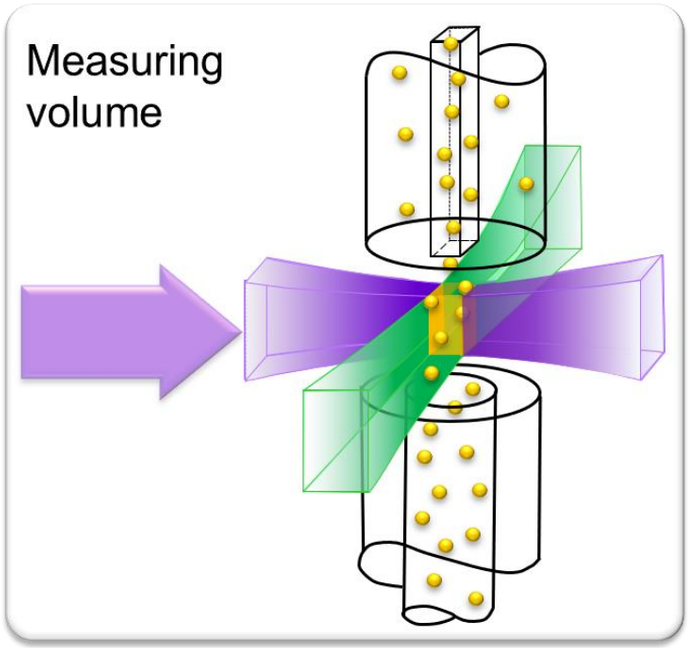
Double spectrometer



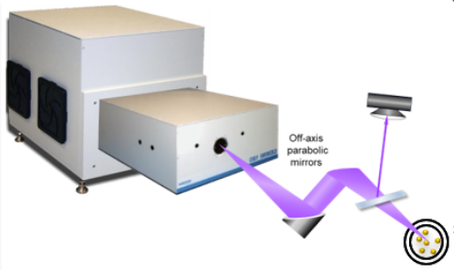
Photon counter
Peltier cooled

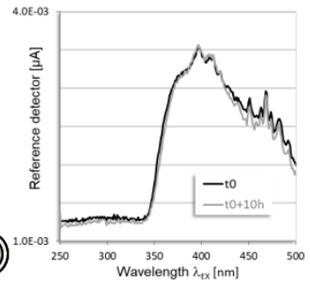


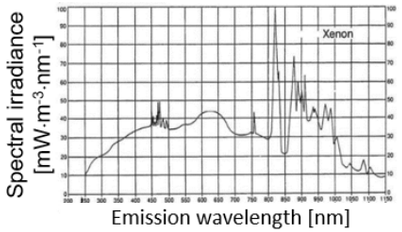
Measuring chamber

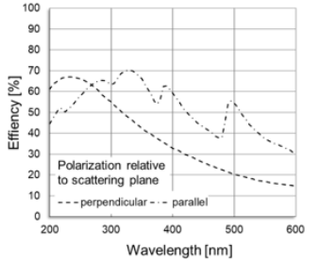


Calibration - Overview

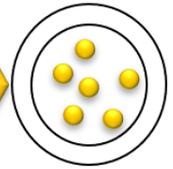








Absolute calibration of excitation path



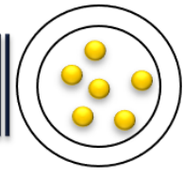
Emission measurement
- Substance: DCM ($C_{19}H_{17}N_3O$) dissolved in ethanol
- Reference: HeNe laser 0.3 mW @ 543 nm

Substances with quantitatively specified/specifiable absorption/emission properties

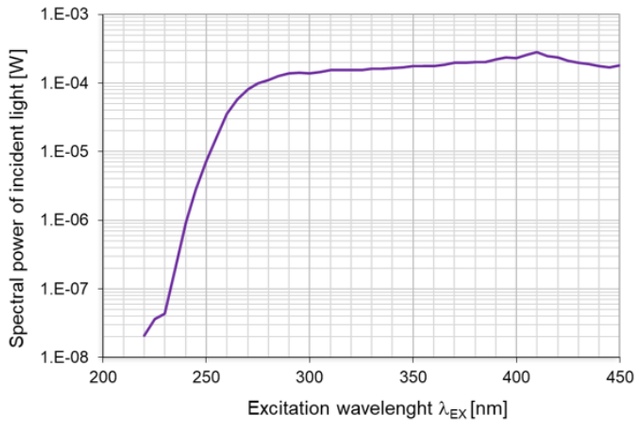
Mie Scattering
- Substance: Monodisperse polystyrene spheres
- Reference: Mie theory for single particles

Raman scattering
- Substance: Pure water, Starna reference
- Reference: Calculated Raman intensity

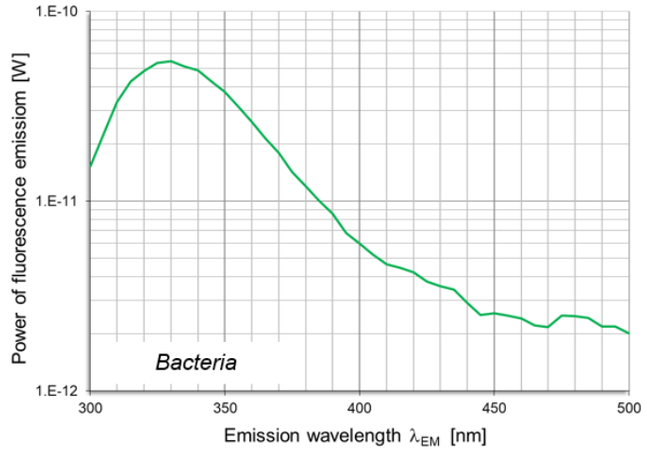
Absolute calibration of emission path

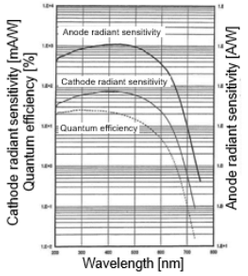


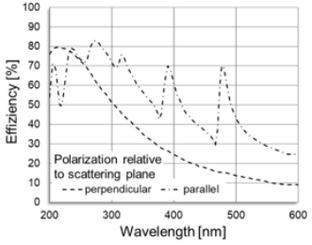
Power of incident light at the position of the aerosol

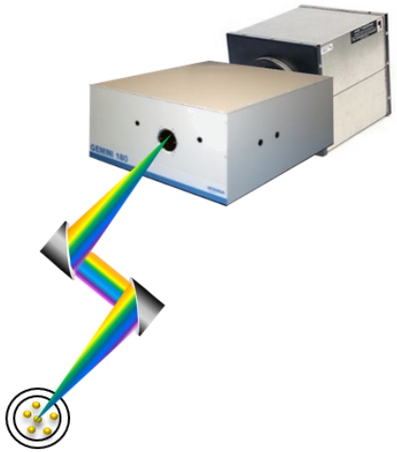


Power of fluorescence emission

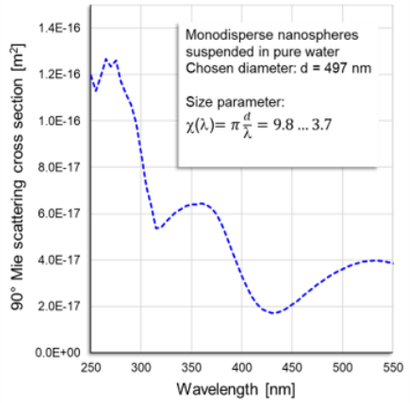
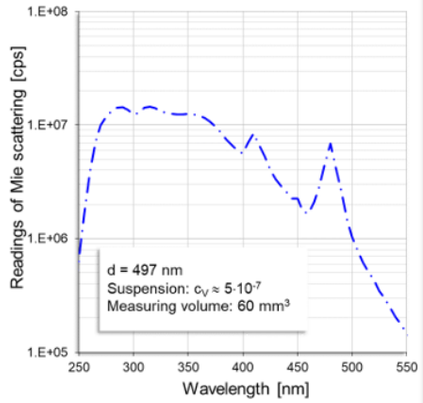
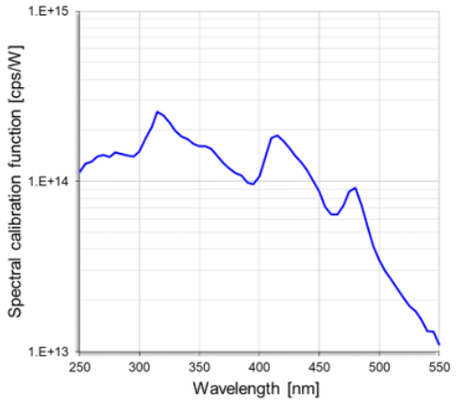
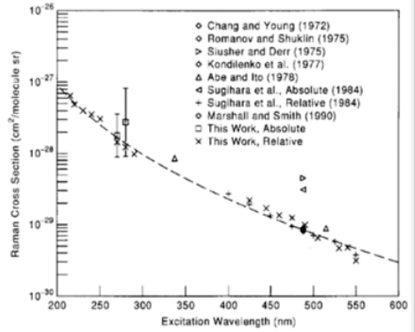
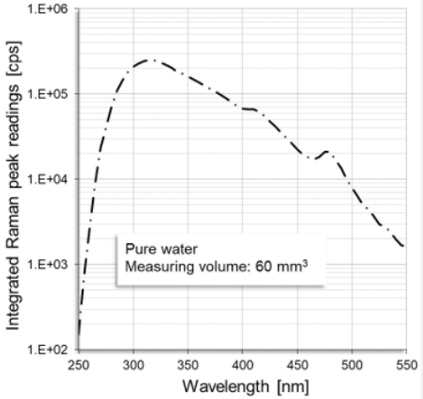
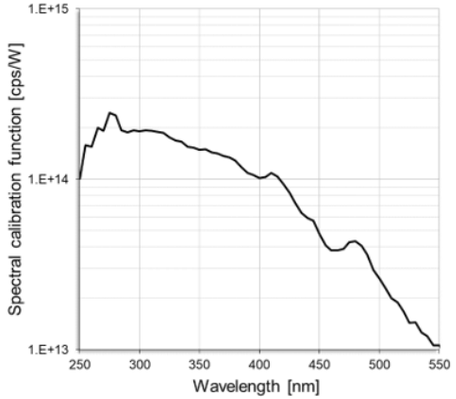


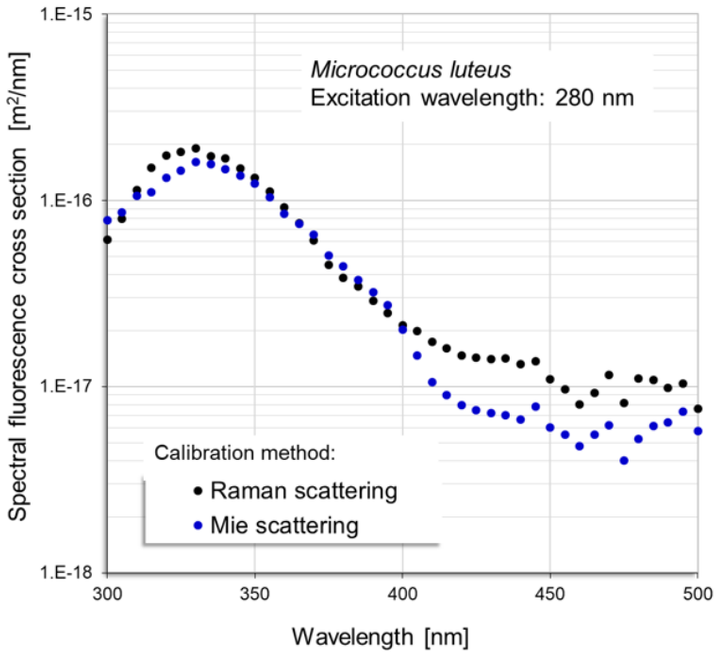




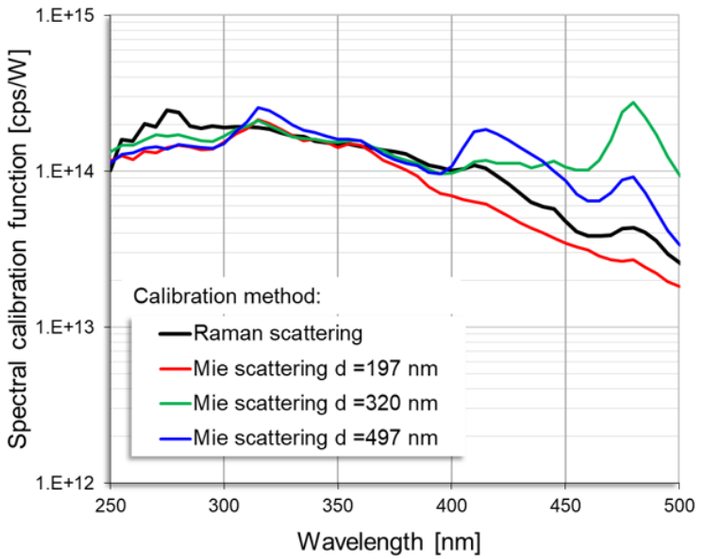


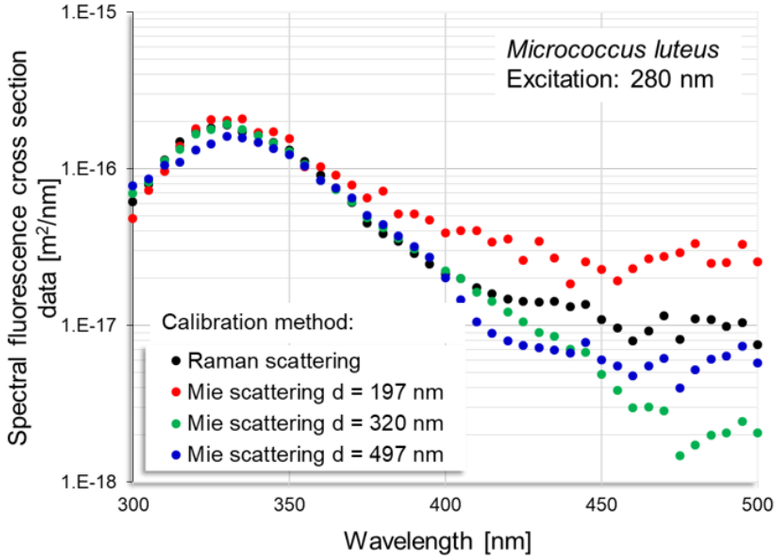
Calibration – Comparison of methods

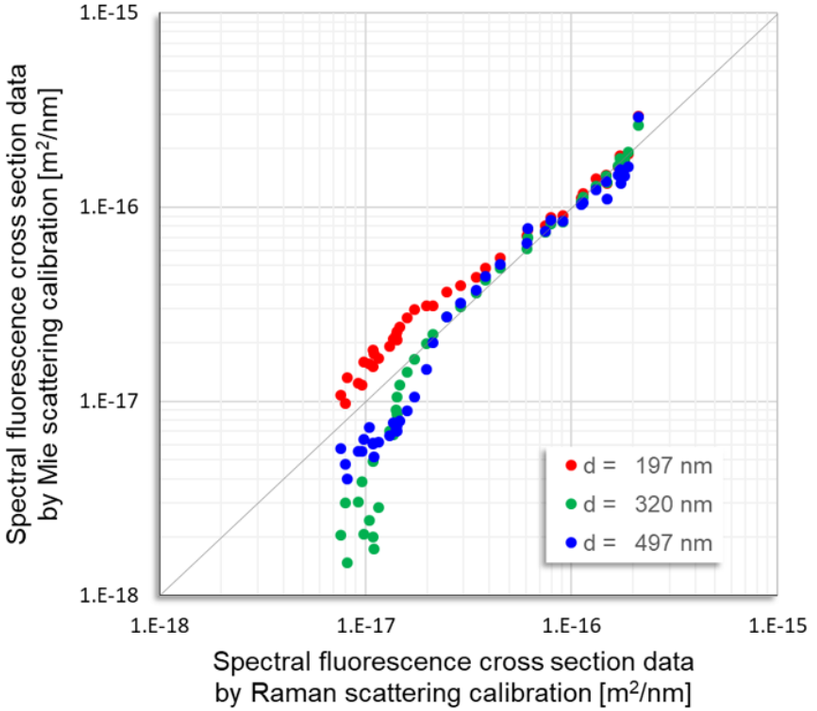
	Theory	Measurement	Calibration function
Mie scattering			
	$C_s(\lambda) = \left(\frac{\lambda}{2\pi n_i r}\right)^2 \int j\left(\frac{\pi d_{PST}}{\lambda_i}, m_{PST}, \Theta\right) dF$, i : Medium PST: Polystyrene sphere		$CF^{Mie}(\lambda) = \frac{Readings}{Theoretical\ power\ value}\ \frac{[cps]}{[W]}$
Raman scattering	 Faris, G. W., Copeland, R. A. "Wavelength dependence of the Raman cross section for liquid water", Appl. Opt., Vol. 36, No. 12, 1997		
	C_R: Referenced data		$CF^{Raman}(\lambda_{EM}) = \frac{Integrated\ differential\ readings}{Theoretical\ power\ value}\ \frac{[cps]}{[W]}$

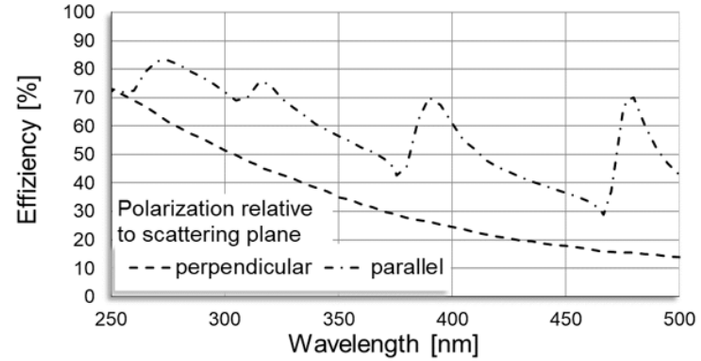


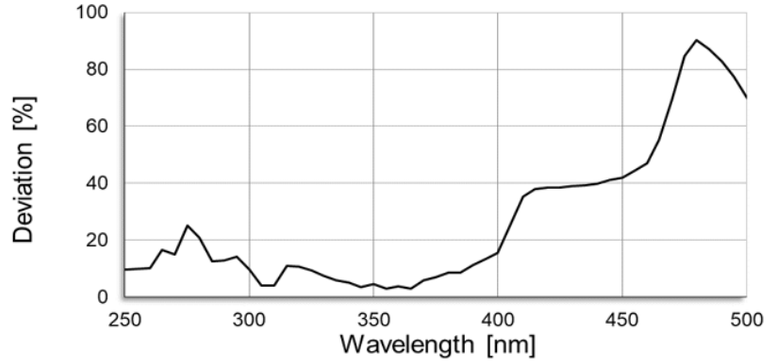
Calibration – Adverse Impacts







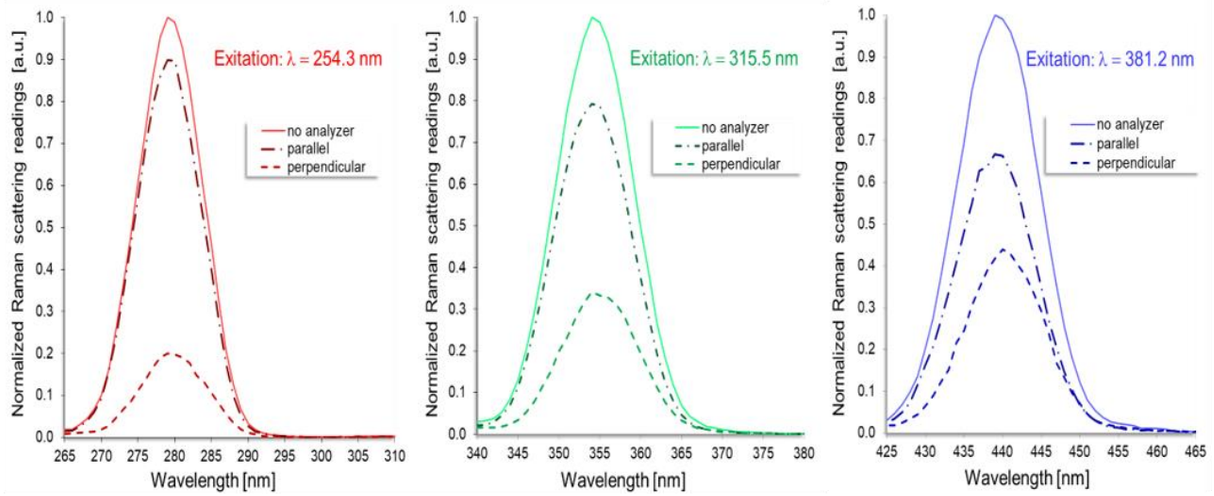


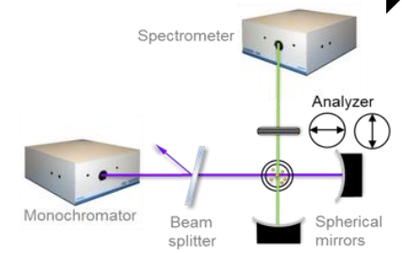


Mie scattering: Polarization dependencies on sphere size, scattering angle, and wavelength
Polarization sensitive optics: Gratings, detectors, cameras, windows, filters, beam splitters, …

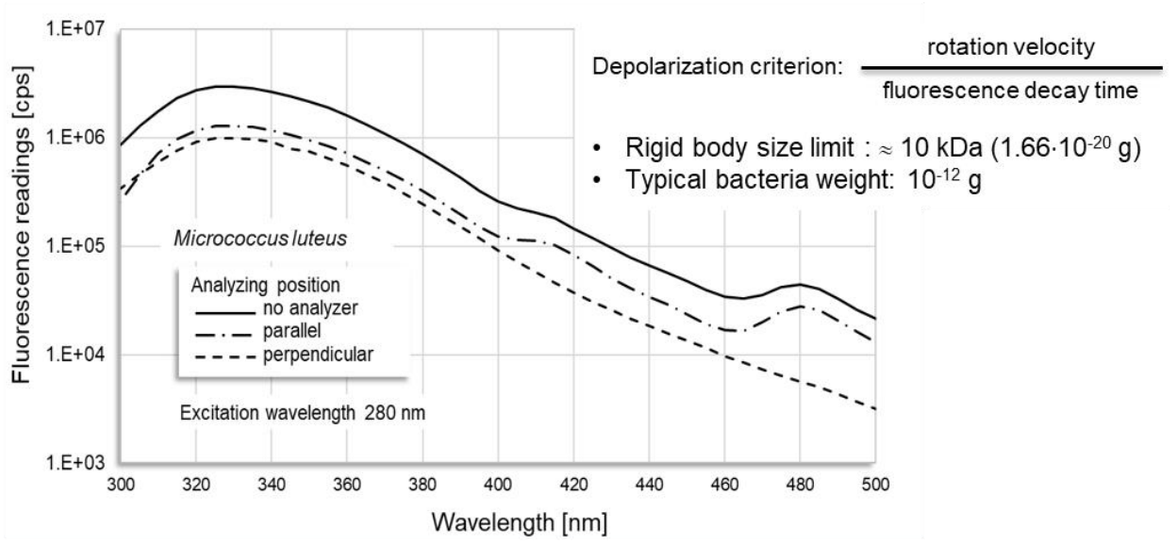
Calibration – Polarization cross check

Raman scattering



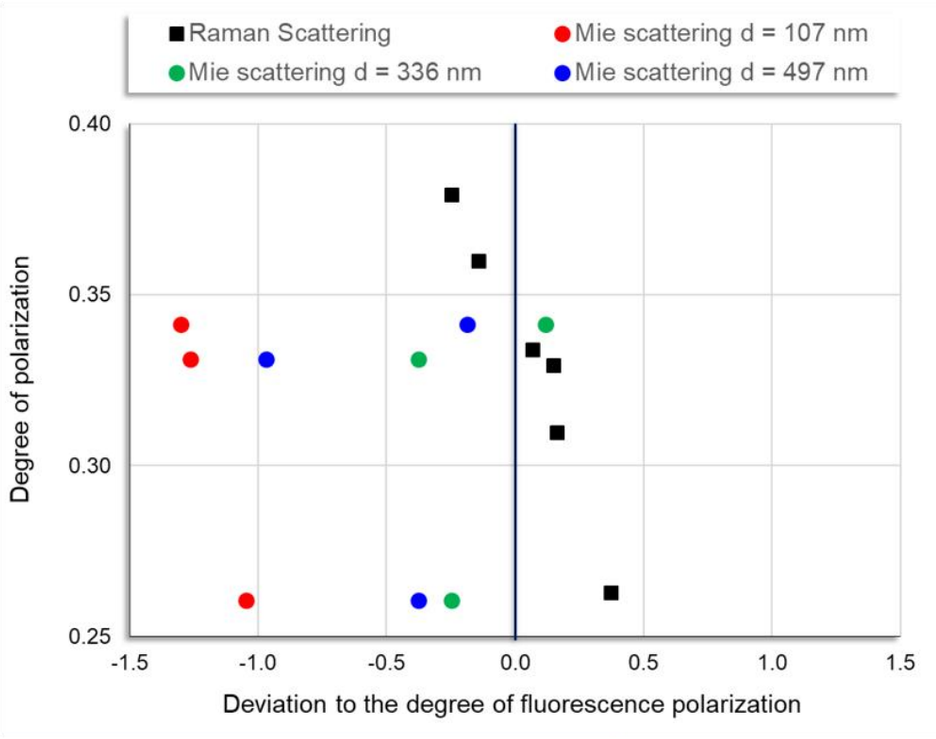


Fluorescence



Depolarization criterion: $\frac{\text{rotation velocity}}{\text{fluorescence decay time}}$

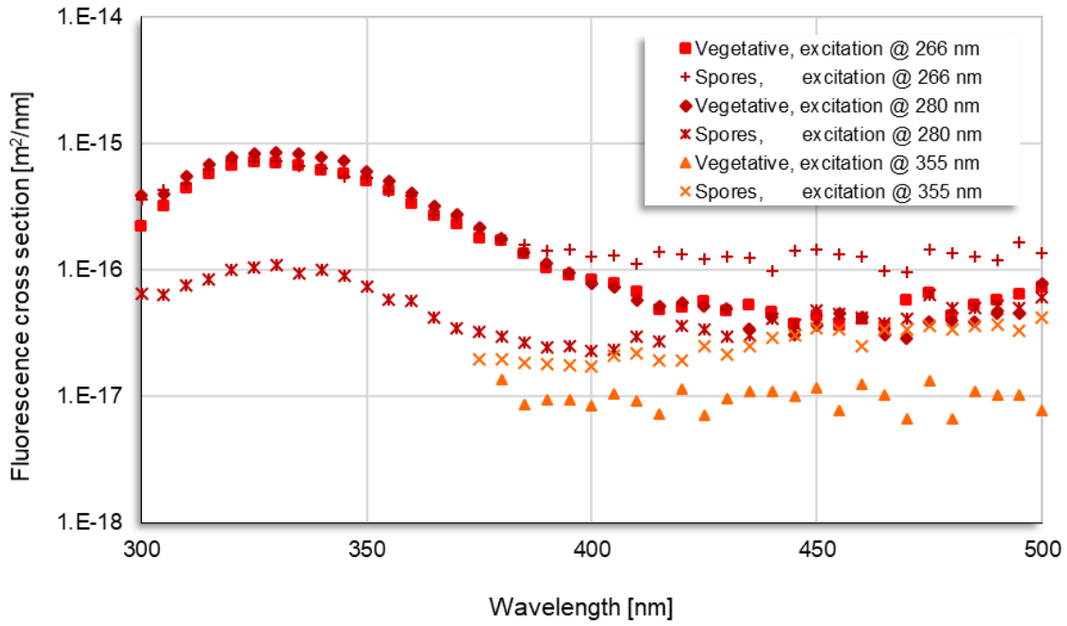
- Rigid body size limit : ≈ 10 kDa ($1.66 \cdot 10^{-20}$ g)
- Typical bacteria weight: 10^{-12} g

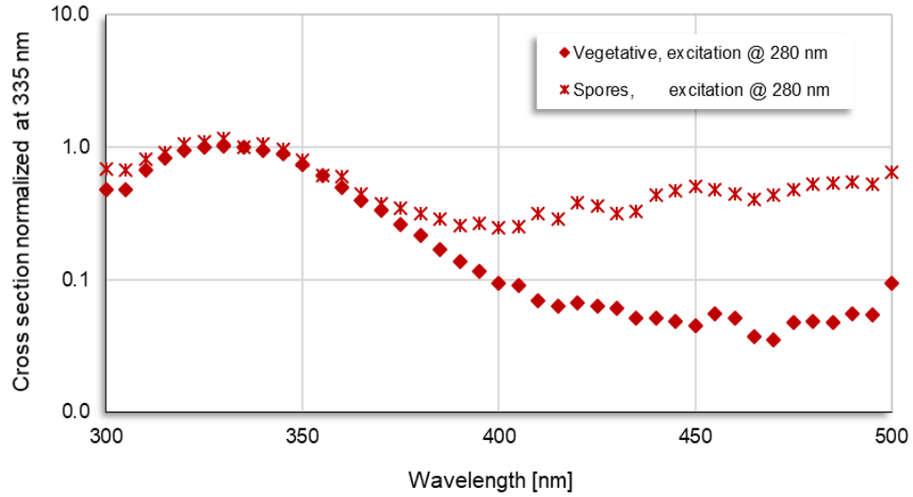


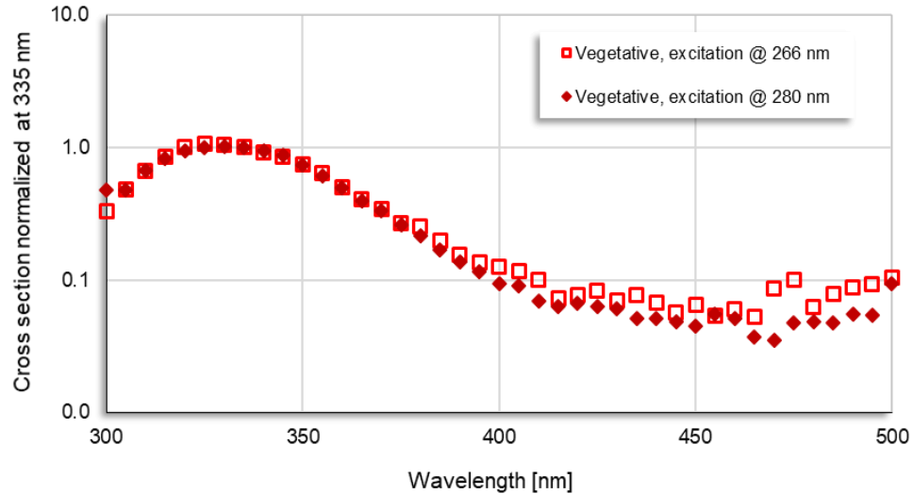
Raman scattering: Polarization depending on frequency
Fluorescence depolarization

Fluorescence cross sections

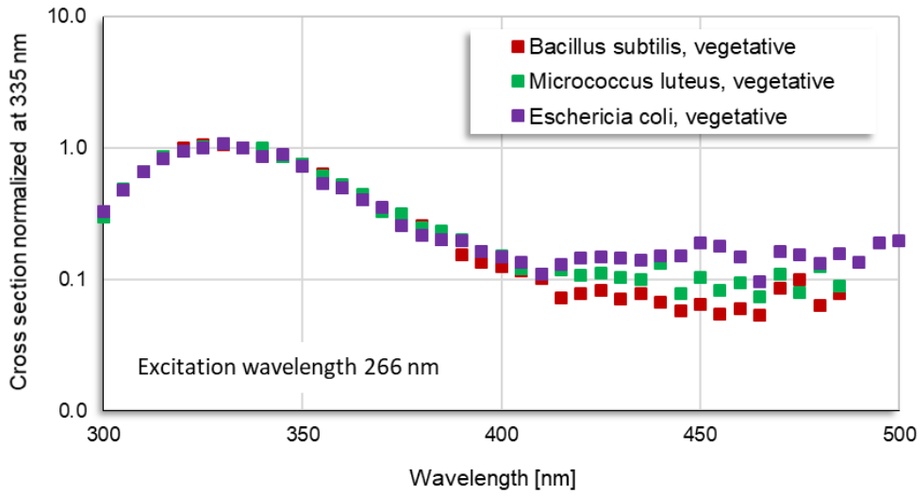
Bacillus subtilis

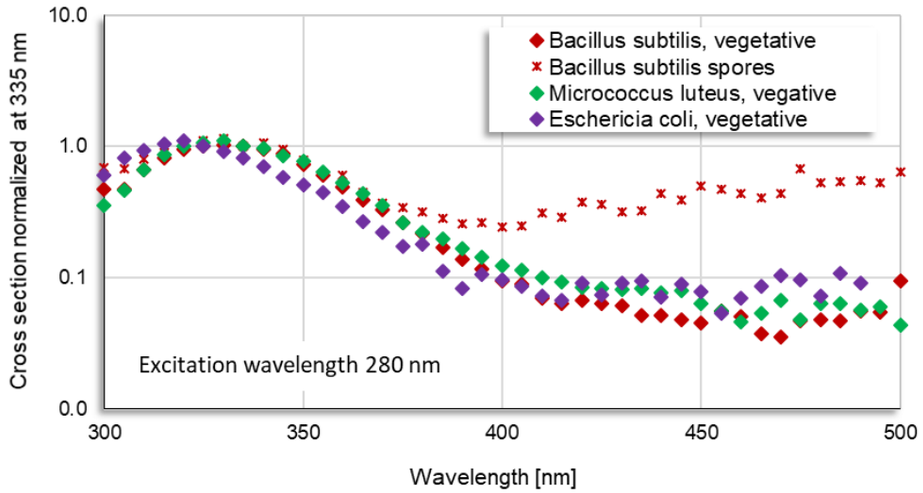






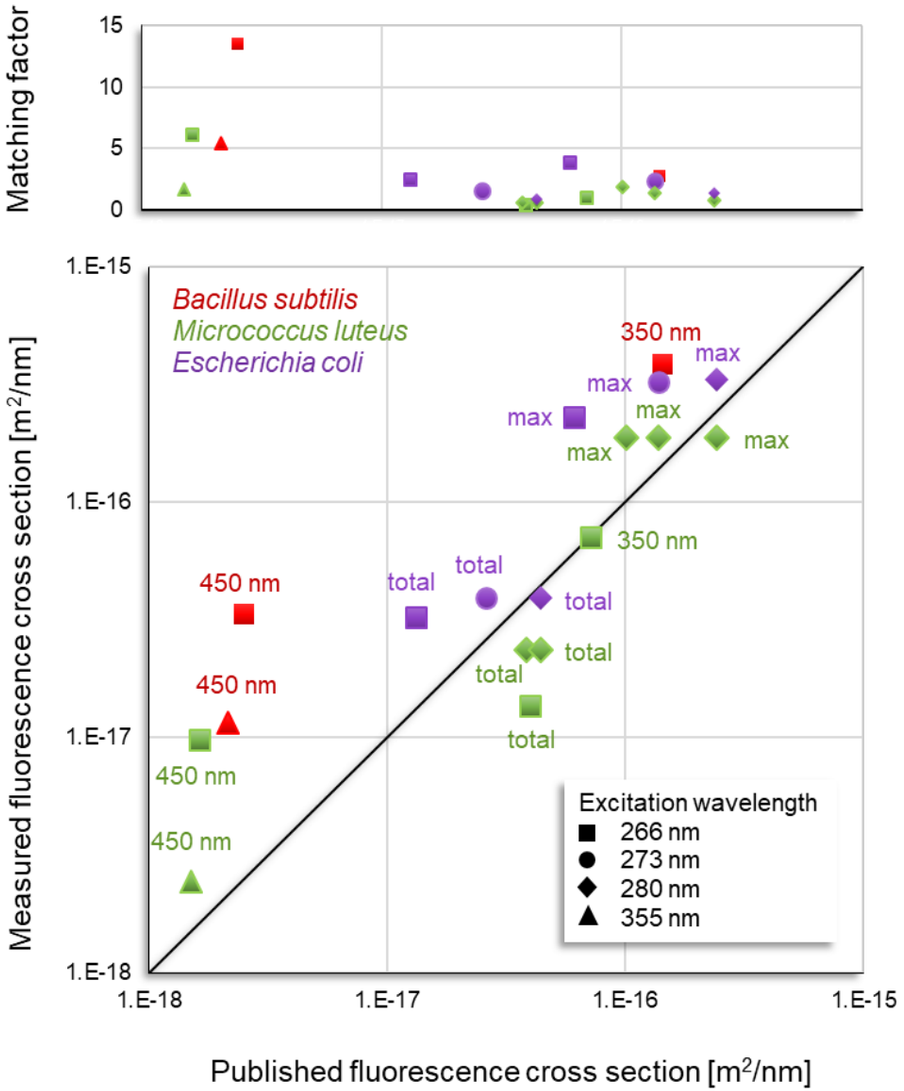
Fluorescence cross sections





	B. subt. veg. @ 266 nm	B. subt. veg. @ 280 nm	B. subt. spores @ 280 nm	E.coli. veg. @ 266 nm	E. coli veg @ 280 nm	M. lut. veg. @ 266 nm	M. lut. veg. @ 280
B. subt. veg. @ 266 nm	1	0.996	0.880	0.993	0.937	0.997	0.998
B. subt. veg. @ 280 nm	0.996	1	0.881	0.989	0.959	0.993	0.996
B. subt. spores @ 280 nm	0.880	0.881	1	0.907	0.858	0.872	0.868
E. coli veg. @ 266 nm	0.993	0.989	0.907	1	0.929	0.992	0.990
E. coli veg. @ 280 nm	0.937	0.959	0.858	0.929	1	0.930	0.941
M. lut. veg. @ 266 nm	0.997	0.993	0.872	0.992	0.930	1	0.998
M. lut. veg. @ 280 nm	0.998	0.996	0.868	0.990	0.941	0.998	1

Comparison of data



Comparison compromises due to partially differing conditions

- Single particle / aerosol flow systems
- Bacteria preparation
- Aerosol dryness
- Excitation wavelengths in a range of ± 2 nm

Adaptation of literature values for comparability

- Width of total spectral range
- Spectral resolution

Data references

M. Seaver, D. C. Roselle, J. F. Pinto, J. D. Eversole, "Absolute emission spectra from Bacillus subtilis and Escherichia coli vegetative cells in solution," Applied Optics, vol. 37, no. 22, 1998.

V. Sivaprakasam, A. L. Houston, C. Scotto, J. D. Eversole, "Multiple UV wavelength excitation and fluorescence of bioaerosols," Optics Express, vol. 12, no. 19, pp. 4457-4466, 2004.

Y. L. Pan, S. C. Hill, J. L. Santarpia, K. Brinkley, T. Sickler, M. Coleman, C. Williamson, K. Gurton, M. Felton, R. G. Pinnick, N. Baker, J. Eshbaugh, J. Hahn, E. Smith, B. P. A. Alvarez, W. Gardner, "Spectrally-resolved Fluorescence Cross Sections of Aerosolized Biological Live Agents and Simulants Using Five Excitiation Wavelengths in a BSL-3 Laboratory," Optics Express, vol. 22, no. 7, pp. 8165-8189, 2014.

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S. C. Hill, Y. L. Pan, C. Williamson, J. l. Santarpia, H. H. Hill, "Fluorescence of bioaerosols: mathematical model including primary fluorescing and absorbing molecules in bacteria", Optics Express, 22285-22313, 2013.

max : Maximum value of cross section within the spectrum at any wavelength
total: Total value of cross section for the chosen excitation wavelength
… nm: Emission wavelength

Outlook

Achievements

- Raman scattering - preferred calibration method
- Fluorescence response of single bacteria
- Distinguishability of spectral fluorescence cross sections of bacteria
- Comparison with literature in good agreement with some overestimation

Challenges

- Eliminaton of all optical system effects
- Polarization insensitive optical components
- Identical optical process for calibration and measurement
- Improvement of bacteria countability

Limitations

- Cross section data approaching true bacteria properties
- Necessity to compare data within the same system for reliable results
- Availability of alternative calibration substances
- Present restriction to bacteria of BSL1

Thank you for your attention

also in the name of my colleagues, who contributed to this work

Interdisciplinary science
Frank Duschek, Lea Fellner, Thomas Hall, Marian Kraus, Arne Walter

Engineering
Kirsten Klaffki

Chemical / Microbiological assistance
Bjoern Prietzel, Lara-Cathrin Wahlprecht

Mechanics / Electrics /Electronics
Thomas Schlagenhaufer, Andreas Walther, Ralf Zimmermann