Supplement to

Spectral Intensity Bioaerosol Sensor (SIBS): A new Instrument for Spectrally Resolved Fluorescence Detection of Single Particles in Real-Time

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Supplementary Figures S1 to S14

Table S1. Summary of physical properties of Polystyrene latex spheres (PSLs) and polystyrene-divenylbenzene particles (PS-DVB) used in this study. Stated properties are taken from manufacturer information. SD: Standard deviation, RI: Refraction index at 589 nm and 25˚C.

Diameter (µm)	SD (µm)	Confidence	RI	Material	Color / Dye	$\lambda_{ex}/\lambda_{em}$ (nm)	Provider	Catalog code
0.3	0.0148	CV= 5.1%	1.59	PSL	Non-fluorescent	Non-fluorescent	Polysciences Inc.	64015
0.356	0.014	CV= 3.9%	1.59	PSL	Non-fluorescent	Non-fluorescent	Polysciences Inc.	64016
0.4	0.0073	CV= 1.8%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	3400A
0.5	0.0079	CV= 1.6%	1.59	PSL	Non-fluorescent	Non-fluorescent	Duke Scientific Corporation	3500A
0.53	N/A	N/A	1.59	PSL	Plum Purple / Proprietary	360 / 420	Bangs Laboratories Inc.	FS03F
0.6	0.010	CV= 1.7%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	3600A
0.7	0.0083	CV= 1.2%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	3700A
0.8	0.0083	CV= 1%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	3800A
0.9	0.0041	CV= 0.5%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	3900A
1	0.010	CV = 1.0%	1.59	PSL	Non-fluorescent	Non-fluorescent	Duke Scientific Corporation	4009A
1.6	0.020	CV= 1.3	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	4016A
2	0.021	CV= 1.0%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	4202A
2	N/A	CV= < 5%	1.59	PSL	Red / Firefli™ Fluorescent Red	542 / 612	Thermo-Fisher	R0200
2	N/A	CV= < 5%	1.59	PSL	Green / Firefli™ Fluorescent Green	468 / 508	Thermo-Fisher	G0200
2	N/A	CV= < 5%	1.59	PSL	Blue / Firefli™ Fluorescent Blue	368,388,412 / 445, 445, 473	Thermo-Fisher	B0200B
2.07	0.15	N/A	1.59	PSL	Plum Purple / Proprietary	360 / 420	Bangs Laboratories Inc.	FS05F
3	0.032	CV=1.1%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	4203A
4	0.04	CV = 1.0%	1.59	PSL	Non-fluorescent	Non-fluorescent	Thermo-Fisher	4204A
4.52	0.15	CV = 3.0%	1.59	PSL	Non-fluorescent	Non-fluorescent	Polysciences Inc.	17135
5	0.6	CV=11%	1.59	PS-DVB	Non-fluorescent	Non-fluorescent	Thermo-Fisher	DC-05
7	0.7	CV= 10%	1.59	PS-DVB	Non-fluorescent	Non-fluorescent	Thermo-Fisher	DC-07
8	0.8	CV= 10%	1.59	PS-DVB	Non-fluorescent	Non-fluorescent	Thermo-Fisher	DC-08
10	0.9	CV=9.2%	1.59	PS-DVB	Non-fluorescent	Non-fluorescent	Thermo-Fisher	DC-10
15	1.8	CV=11%	1.59	PS-DVB	Non-fluorescent	Non-fluorescent	Thermo-Fisher	DC-15
20	1.7	CV=8.9%	1.59	PS-DVB	Non-fluorescent	Non-fluorescent	Thermo-Fisher	DC-20

Table S2. Summary of reference particles used within this study. All biofluorophores, iron oxide (Fe_3O_4), and carbon nanotubes were purchased from Sigma-Aldrich, St. Louis, MO, USA. Ammonium sulfate was purchased from Fisher Scientific, Hampton, NH, USA.

Reference particles	**CAS Nr.**
Bacteriochlorophyll	17499-98-8
Chlorophyll *a*	479-61-8
Chlorophyll *b*	519-62-0
NAD	606-68-8
Riboflavin	83-88-5
Tryptophan	73-22-3
Tyrosine	556-02-5
Fe_3O_4	1317-61-9
Carbon nanotubes	308068-56-6
Ammonium sulfate	7783-20-2

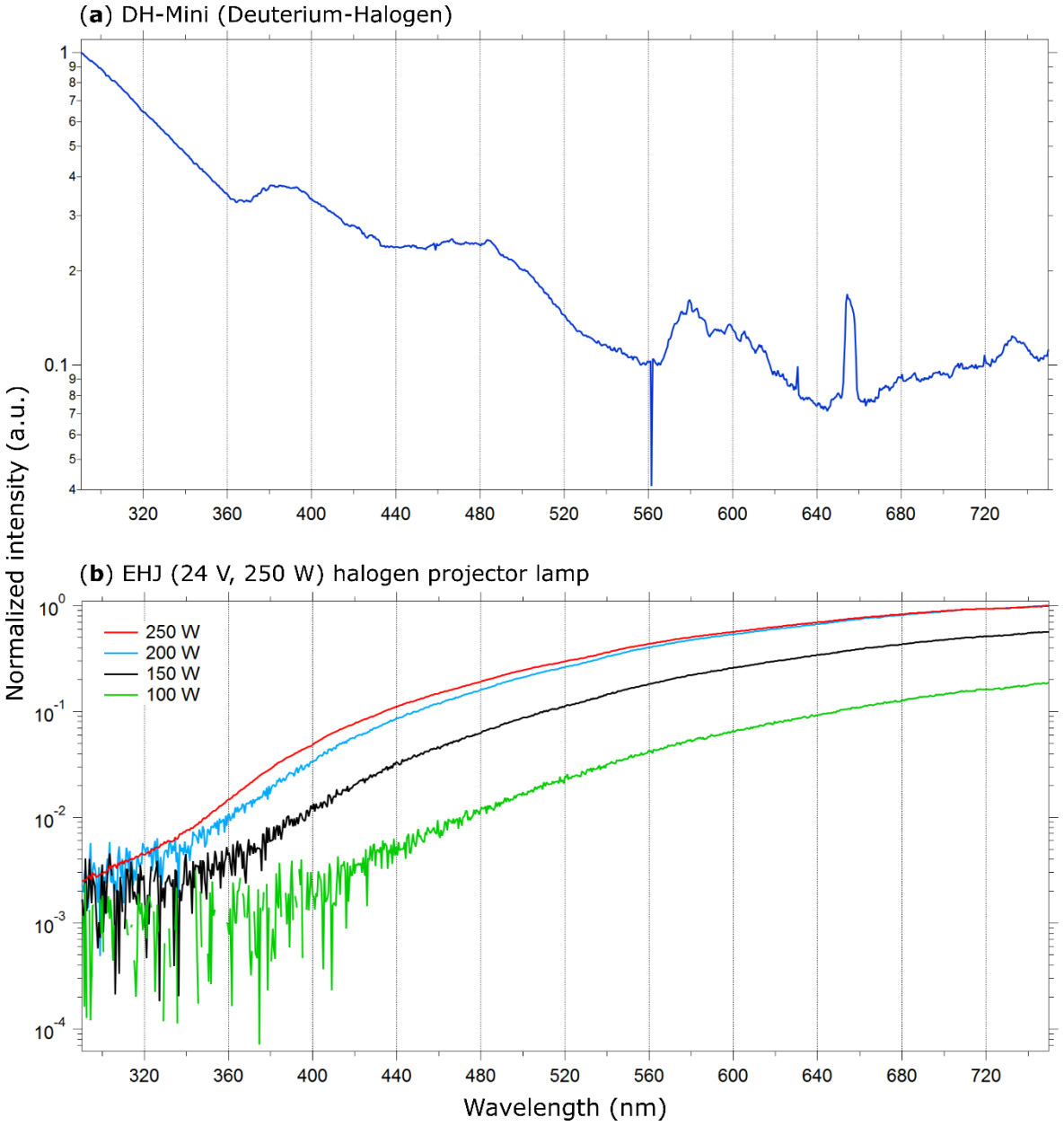


Figure S1. Normalized and averaged calibration lamp spectra. In (**a**), the spectrum of a deuterium-halogen lamp (DH-Mini, Ocean Optics) is shown, in (**b**) the spectra of a halogen projector lamp (EHJ 24V250W, Ushio), both measured with the Dual-FL spectrometer (Horiba).

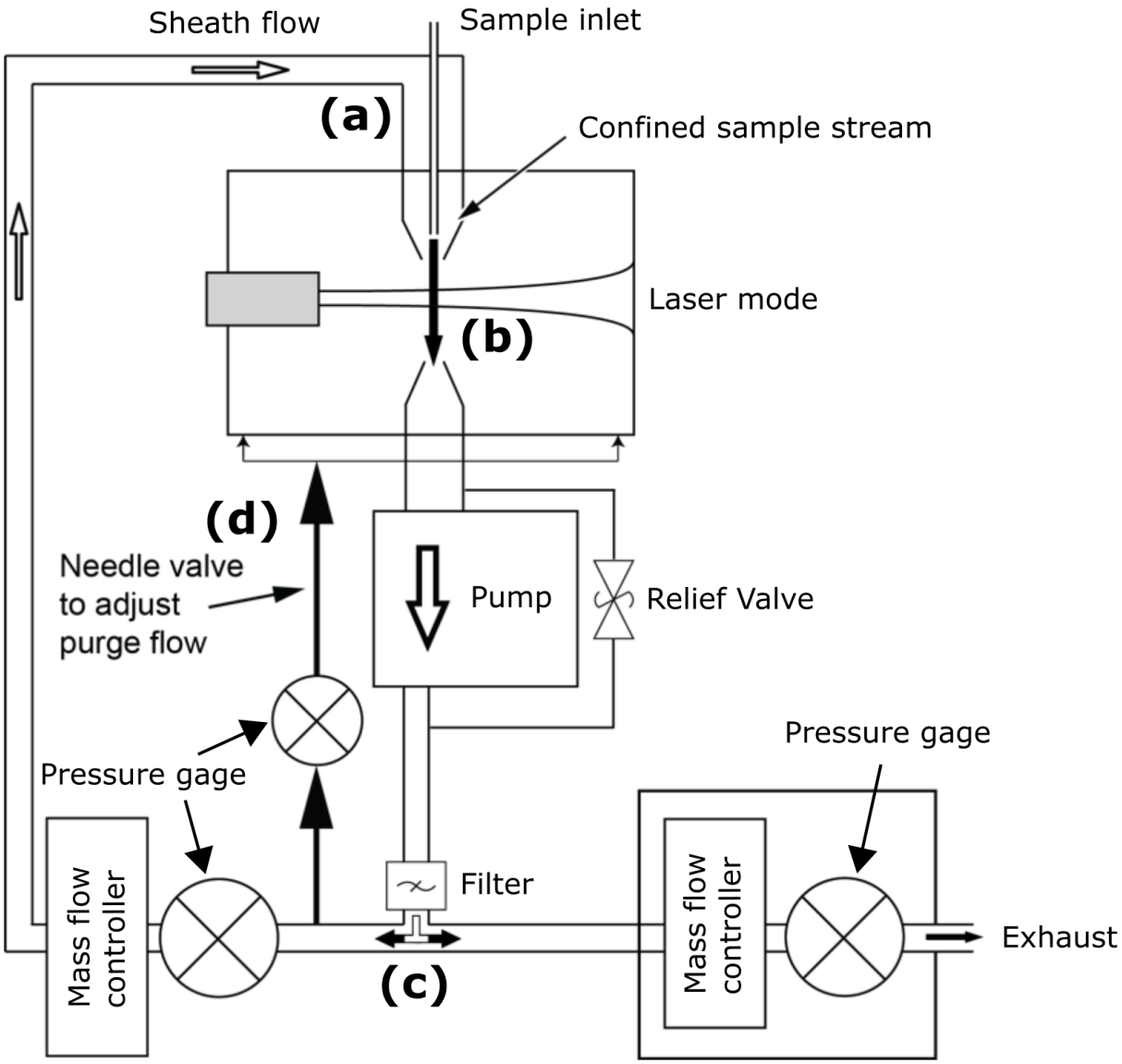


Figure S2. Flow diagram for the SIBS. Aerosol is drawn via a tapered delivery nozzle (**a**) into the optical cavity. The intersection of sample flow and laser beam defines a sampling volume with approximately 0.7 mm in diameter and 130 µm depth (**b**). The sheath flow is filtered through a HEPA filter and recirculates in the system (**c**). A small purge flow, which is adjusted by a needle valve (**d**), constantly purges the optical cavity (Modified, image courtesy: DMT).

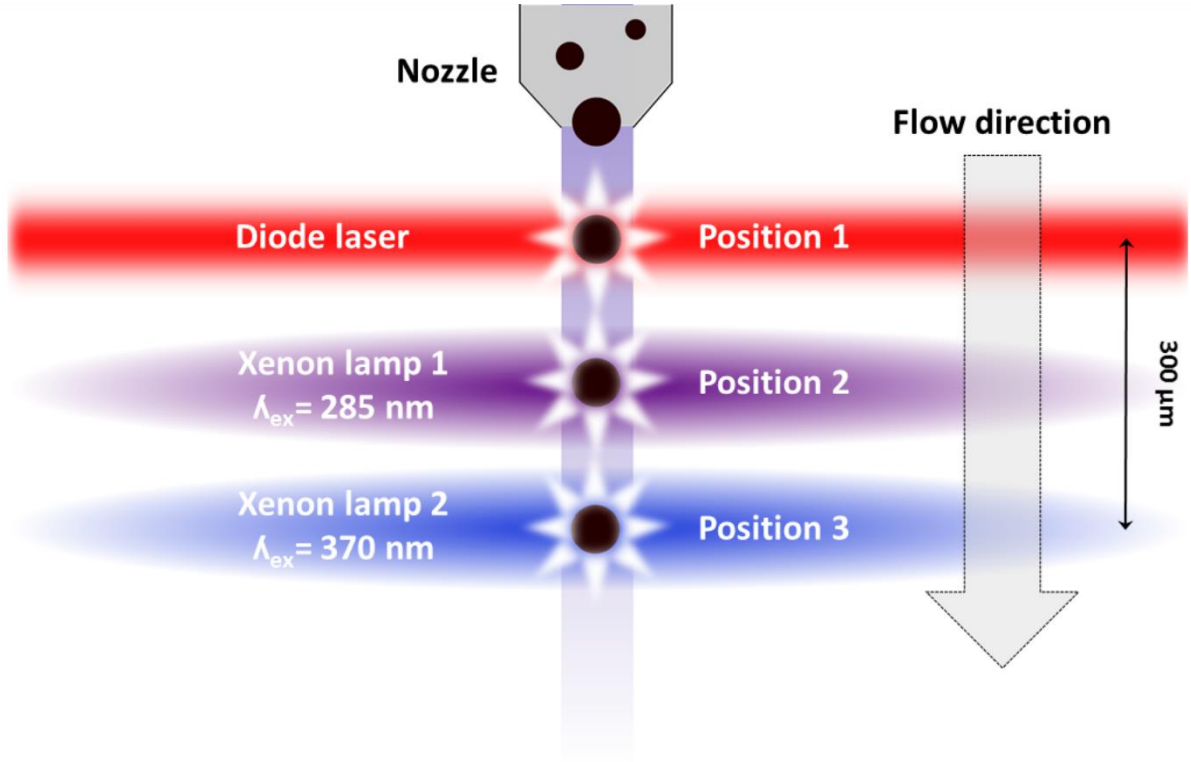


Figure S3. Schematic diagram of particle size and fluorescence detection. Position 1: Particles scatter light in all directions after being illuminated by a diode laser (λ = 785 nm). Position 2: Xenon lamp 1 is firing at λ_{ex} = 285 nm. Position 3: Xenon lamp 2 is firing at λ_{ex} = 370 nm. The measurement cycle from position 1 to position 3 takes ~25 µs over a distance of ~300 µm. (Modified, adapted from WIBS-4A service manual (DOC-0345 Rev A), DMT; 2012).

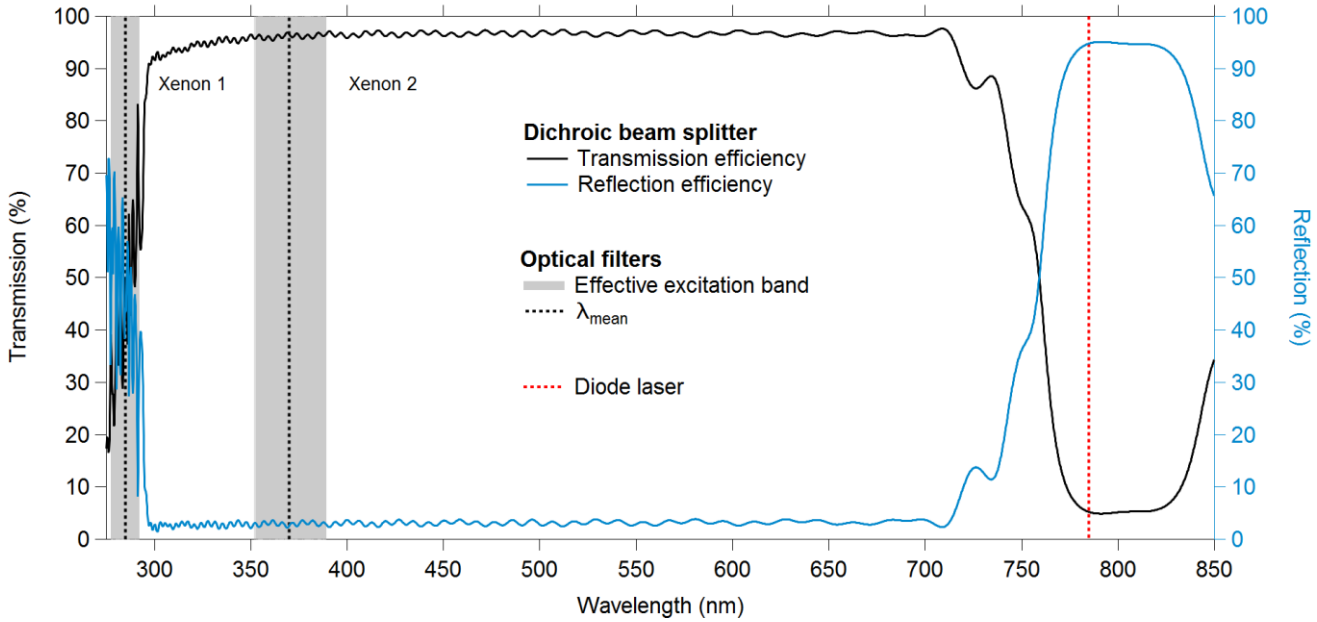


Figure S4. Transmission and reflection efficiency of the dichroic beam splitter. The beam splitter transmits fluorescence emission (black line) to the grating polychromator and reflects scattering light (blue line) to the particle sizing- and detection PMT. (Data courtesy: Semrock).

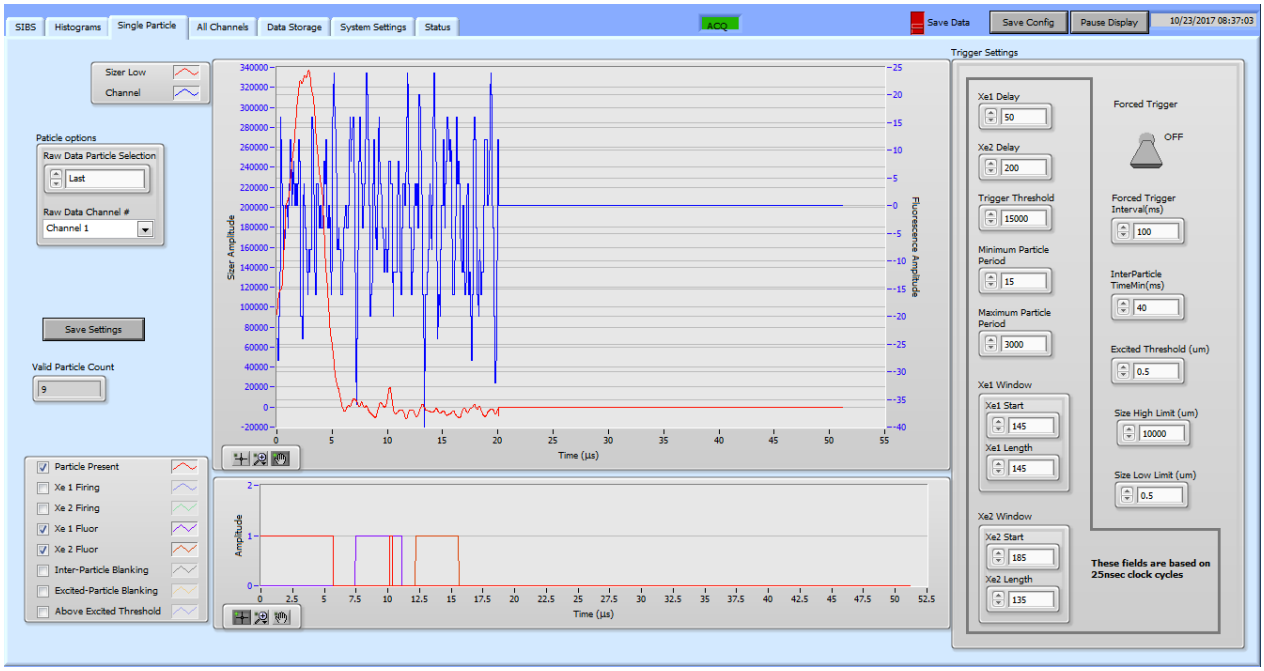


Figure S5. "Single Particle" tab of the SIBS user interface.

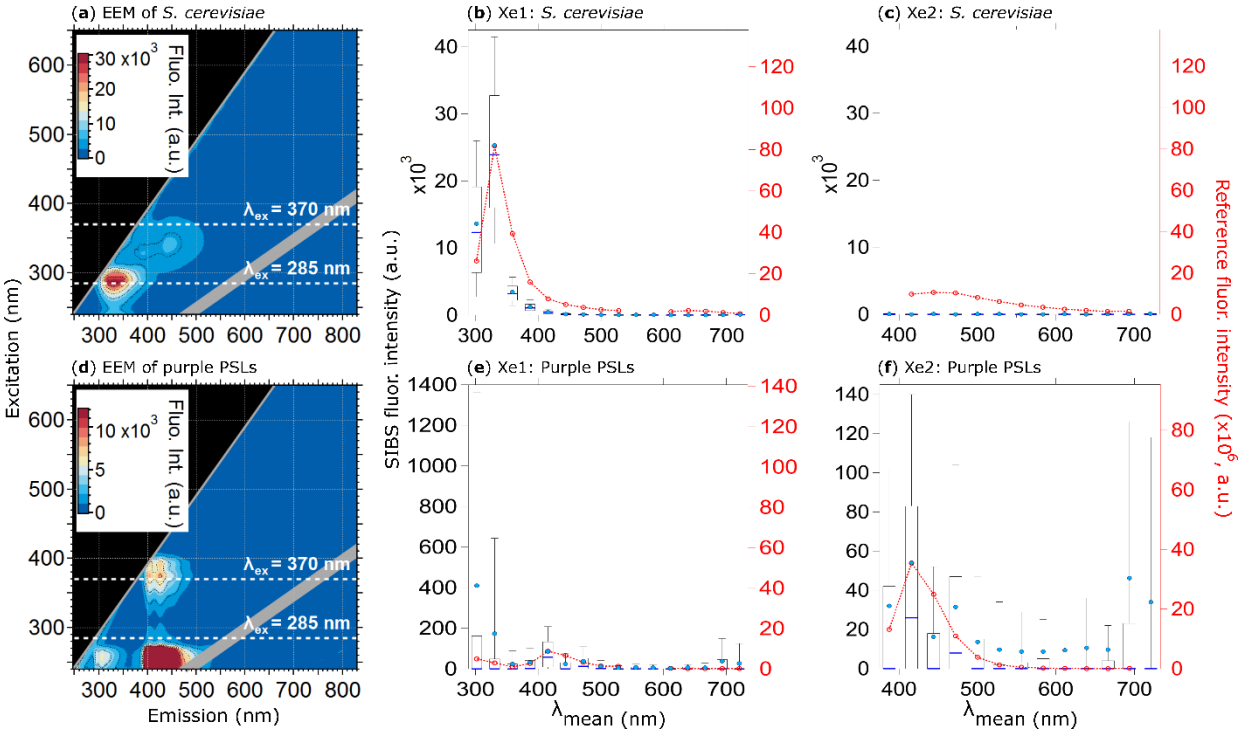


Figure S6. Corrected fluorescence emission of *S. cerevisiae* and 0.53 µm purple PSLs. Steady-state fluorescence signatures displayed as EEMs (left column) and spectra at Xe1 and Xe2 (middle, right columns) for: *S. cerevisiae* (**a, b** and **c**, size range between 4 - 10 µm, 1057 particles), and 0.53 µm purple PSLs (**d**, **e,** and **f**, 5260 particles). Within EEMs: white dashed lines show SIBS excitation wavelengths (λ_{ex} = 285 and 370 nm), grey diagonal lines indicate 1st and 2nd order elastic scattering bands (both bands were subtracted automatically by the Aqualog V3.6 software).

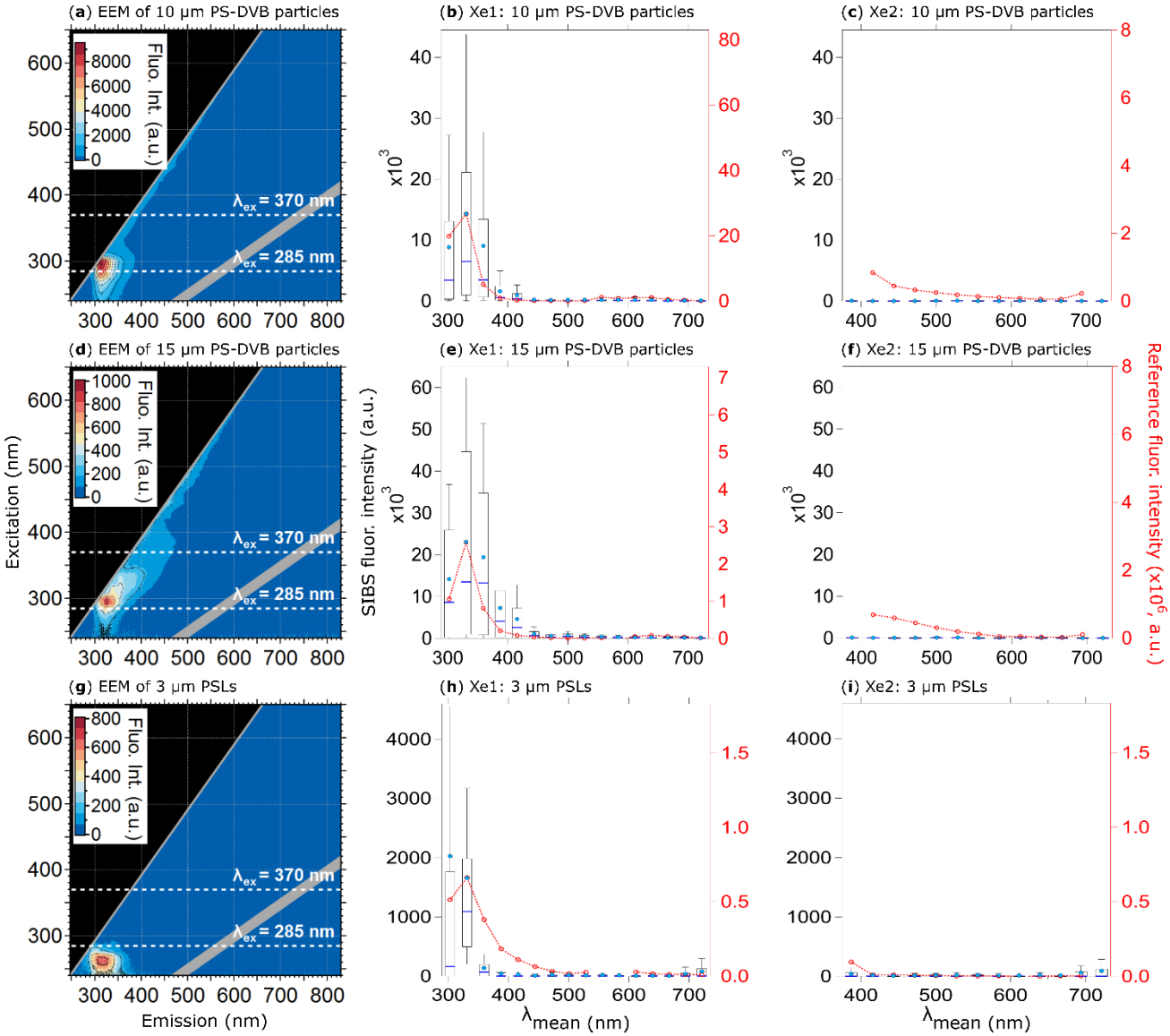


Figure S7. Fluorescence signatures of non-fluorescent particles. Highlighted are EEMs (left column) and spectra at Xe1 and Xe2 (middle, right columns) for: 10 µm (**a**, **b,** and **c**, uncorrected, 367 particles) and 15 µm (**d**, **e,** and **f**, uncorrected, 400 particles) PS-DVB particles, and 3 µm PSLs (**g**, **h,** and **i**, corrected, 2396 particles).

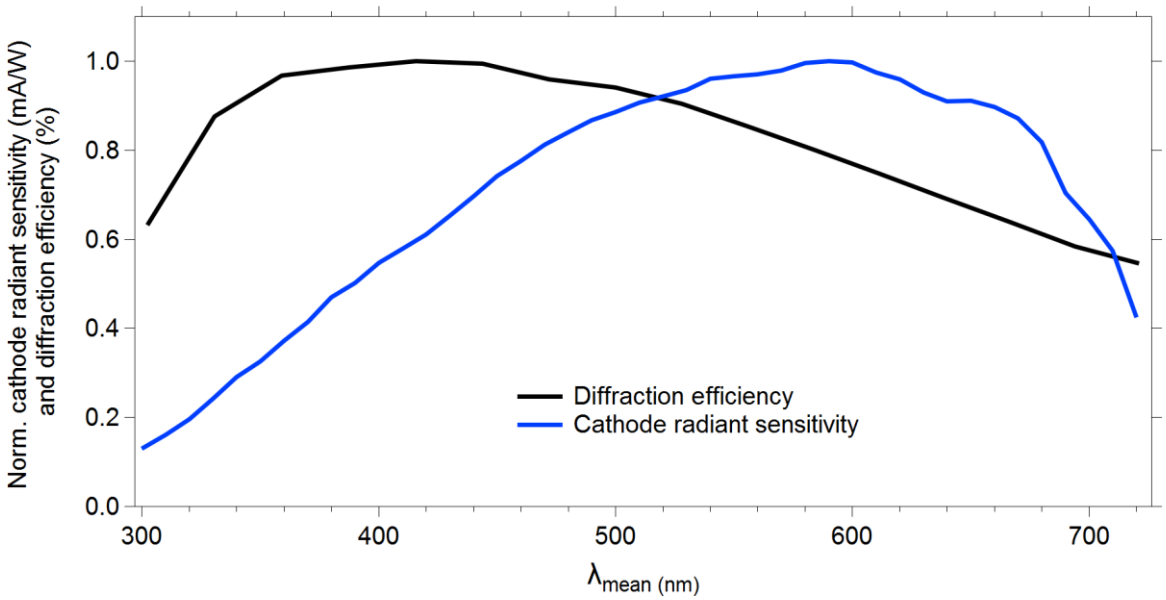


Figure S8. Normalized cathode radiant sensitivity of the PMT and diffraction efficiency of the grating. The cathode radiant sensitivity multiplied by the diffraction efficiency results in the theoretical detector responsivity shown in Figure 6. (Data courtesy: Hamamatsu).

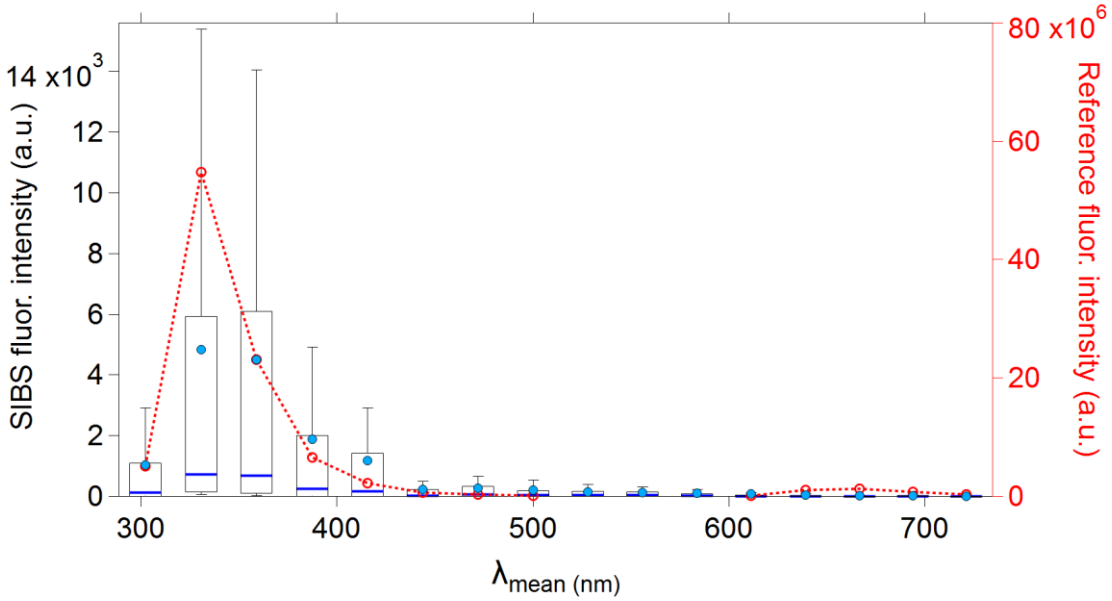


Figure S9. Uncorrected fluorescence spectra of tryptophan at Xe1 in a size range between 1 – 2 µm.

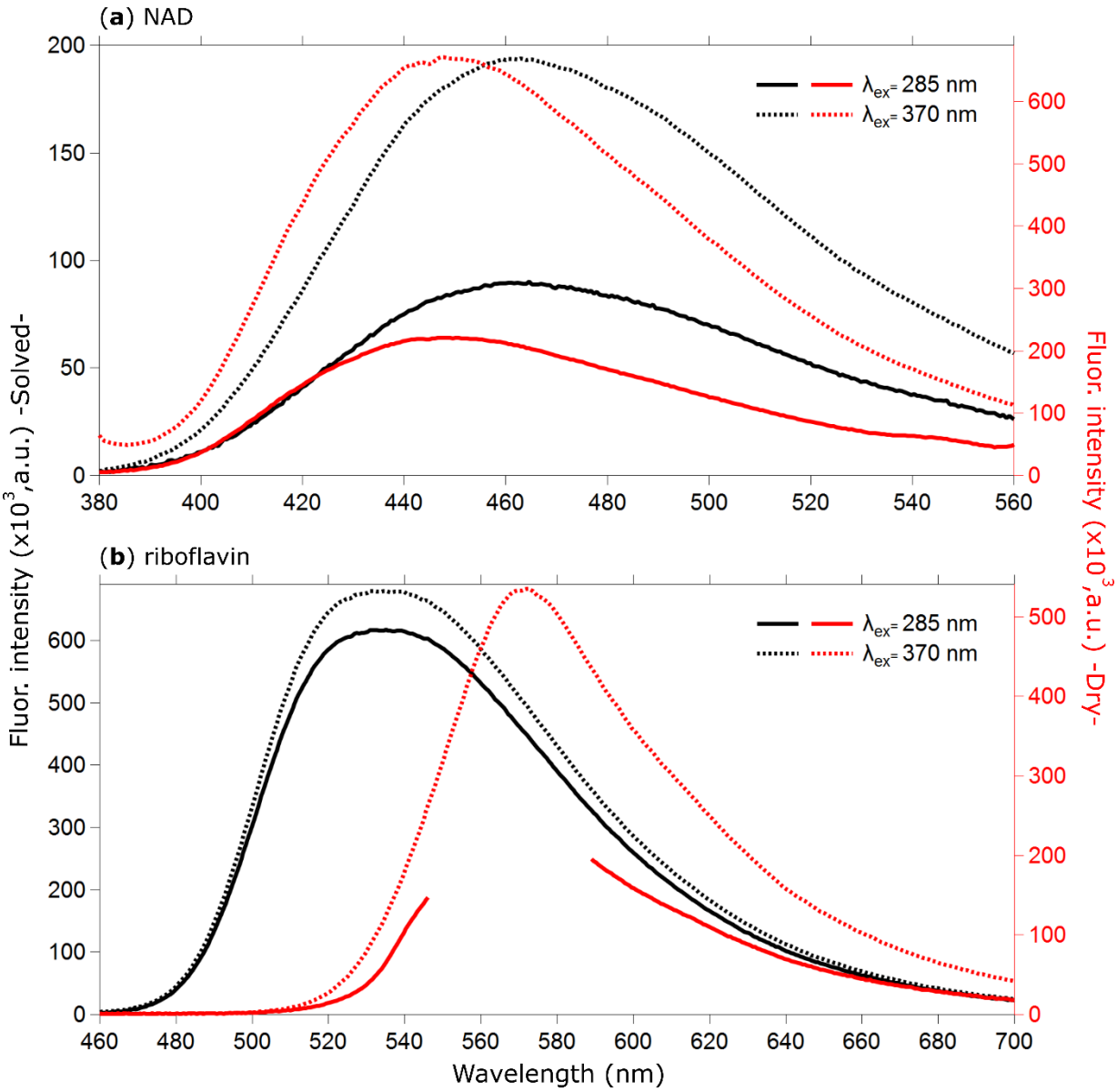


Figure S10. Dry vs. solved. Shown are reference spectra for NAD (**a**) and riboflavin (**b**) in dry and solved state. Data coinciding with 2nd order elastic scattering were removed (**b**, red solid line). Peak maxima: NAD (dry): ~448 nm, NAD (solved): ~463 nm, riboflavin (dry): ~572 nm, riboflavin (solved): ~535 nm.

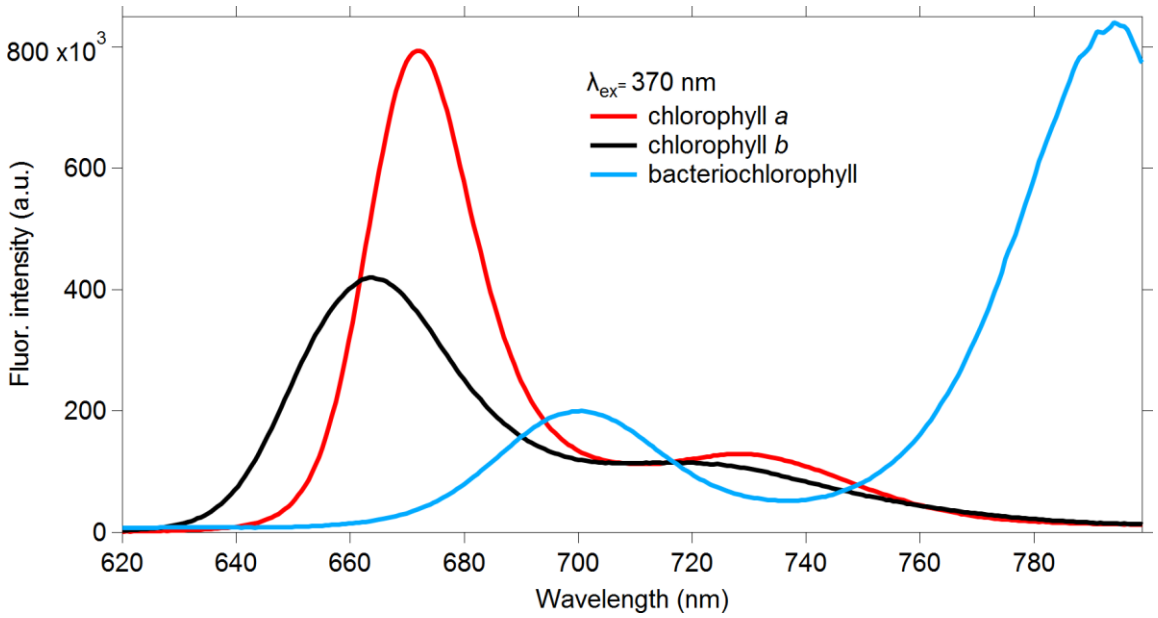


Figure S11. Fluorescence spectra of different chlorophyll types. Shown are reference spectra for chlorophyll *a*, *b*, and bacteriochlorophyll at λ_{ex} = 370 nm.

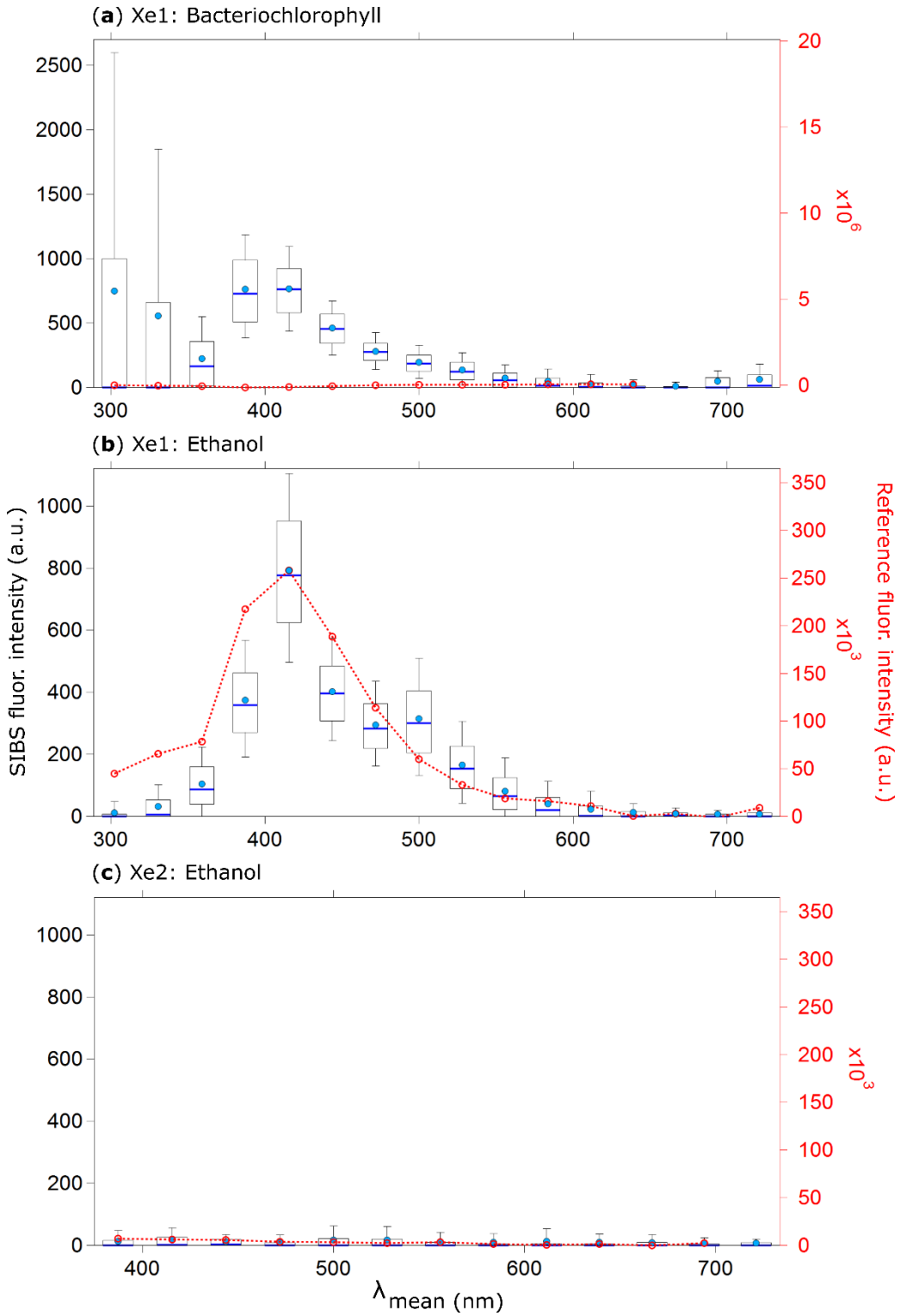
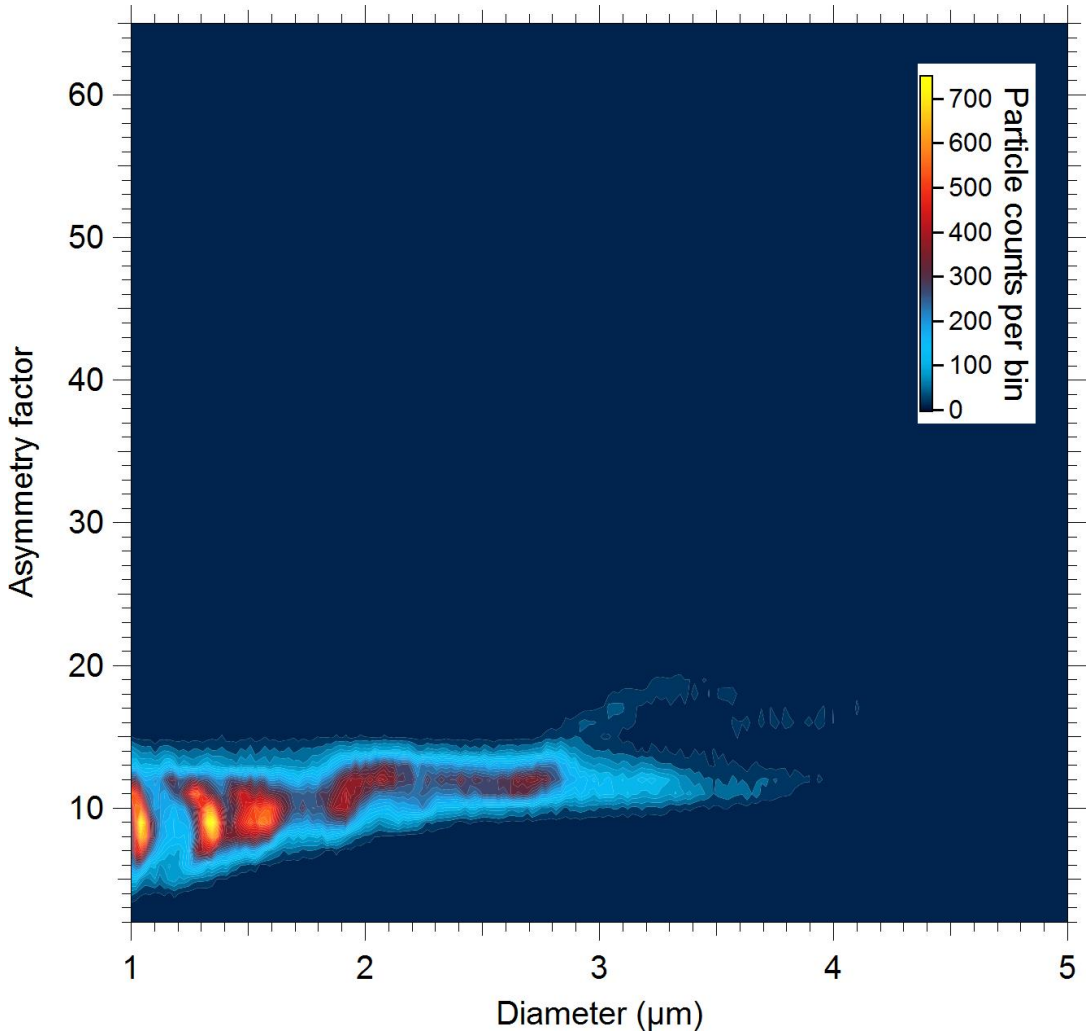


Figure S12. Fluorescence spectra of ethanol artefact. Highlighted are fluorescence spectra of bacteriochlorophyll at Xe1 (**a**) and uncorrected spectra of ethanol, after being vortexed for 15 min in nebulizer plastic bottles, at Xe1 (**b**) and Xe2 (**c**). Since no distinct fluorescence signal is detectable at Xe2 (**c**), the fluorescence emission of chlorophyll *a*, *b* and bacteriochlorophyll is considered to be unaffected.



74 **Figure S13.** Particle asymmetry of ultrapure water droplets (163178 particles) displayed as
75 particle density histogram.

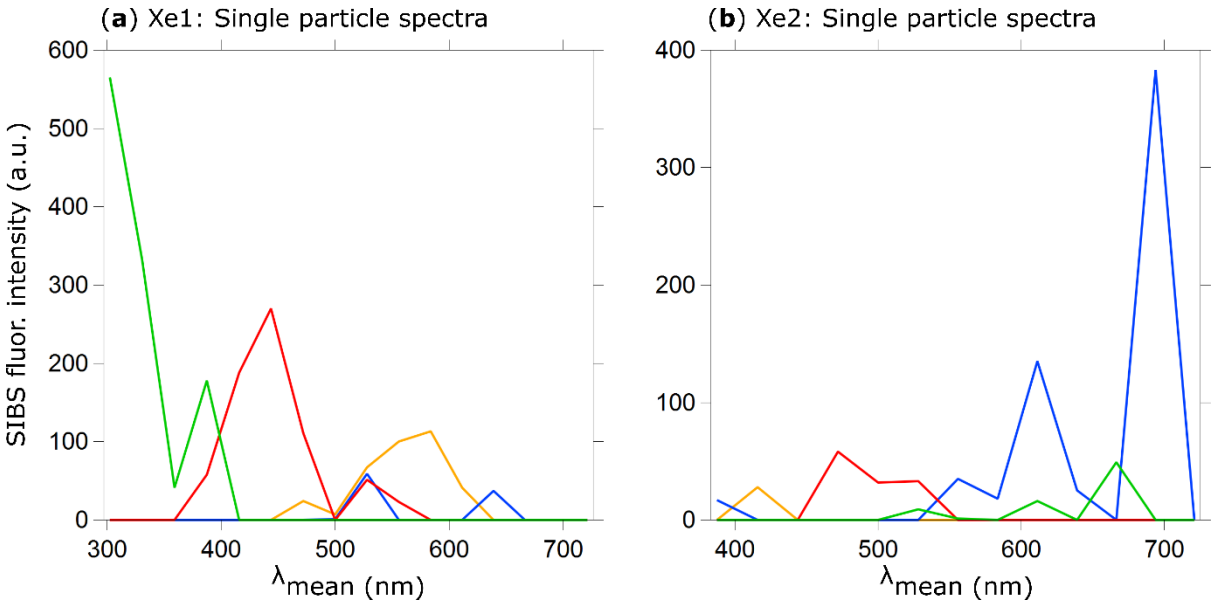


Figure S14. Exemplary fluorescence spectra of single ambient particles at Xe1 (**a**) and Xe2 (**b**).