## Supplementary information

## CAM/TMA-DPH as a promising alternative to SYTO9/PI for cell viability assessment in bacterial biofilms

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## Supplementary Table 1: Quantitative results obtained from CFU counting and microscopic analysis of biofilm surface area coverage and their correlation as

Spearman rank (r). Staining results are presented as mean values  $\pm$  standard deviation.

Bacterial strain	Treatment	Concentration in mg/L	CFU / mL	Calcein stained biofilm		SYTO9 – PI-stained biofilm	
				Area coverage	Spearman rank ( <i>r</i> )	Area coverage	Spearman rank ( <i>r</i> )
P. aeruginosa MIBI685	ciprofloxacin	0	$6.05 \times 10^{7}$	56.80% ± 8.33%	0.91 (****)	$0.56\% \pm 0.14\%$	- 0.65 (*)
		1.25	$7.90 \times 10^{5}$	48.48% ± 3.57%		$0.31\% \pm 0.08\%$	
		2.5	$5.31 \times 10^{5}$	45.25% ± 1.48%		$0.20\% \pm 0.07\%$	
		10	$8.45 \times 10^{4}$	$30.98\% \pm 6.99\%$		$0.24\% \pm 0.03\%$	
K. pneumoniae IIMK 217	ciprofloxacin	0	$2.35 \times 10^{8}$	25.23% ± 12.35%	0.59 (*)	1.0% ± 0.20%	0.04 (ns)
		2	$5.59 \times 10^{5}$	$2.30\% \pm 0.77\%$		$2.50\% \pm 1.70\%$	
		4	$2.48 \times 10^{5}$	1.0% ± 0.3 %		1.71% ±1.28%	
		8	$2.32 \times 10^{5}$	1.58% ±1.27%		7.71% ±5.69%	
S. aureus ATCC 43300	vancomycin	0	$7.24 \times 10^{8}$	85.85% ± 2.42%	0.88 (****)	$70.2\% \pm 6.99\%$	0.74 (**)
		512	$1.18 \times 10^{8}$	30.42% ± 3.97%		$8.31\% \pm 2.50\%$	
		2048	$6.22 \times 10^{7}$	$26.76\% \pm 7.63\%$		$36.6\% \pm 5.94\%$	
		10000	$5.50 \times 10^{6}$	$0.34\% \pm 0.45\%$		$3.18\% \pm 1.45\%$	
<i>E. faecium</i> MIBI701	gentamicin	0	$5.45 \times 10^{7}$	4.13% ± 1.26%	0.83 (****)	5.37% ± 1.59%	- 0.79 (***)
		64	$5.55 \times 10^{6}$	1.38% ± 0.38%		$0.75\% \pm 0.54\%$	
		256	$8.21 \times 10^{5}$	0.62% ± 0.11%		$0.57\% \pm 0.54\%$	
		1024	$4.78 \times 10^4$	$0.51\% \pm 0.11\%$		$0.17\% \pm 0.08\%$	

r = Spearman correlation rank. Significance was assumed at the *p* value < 0.05 and was indicated by asterisks: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, \*\*\*\* < 0.0001, ns = not significant.