Supporting Information

Label-Free Visualization of Photosynthetic Microbial Biofilms using Mid-Infrared Photothermal and Autofluorescence Imaging

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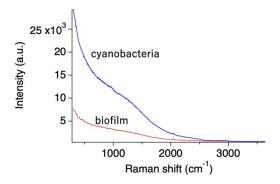


Figure S1. Raman spectra of cyanobacteria and their biofilms excited by incident light with a wavelength of 532 nm.

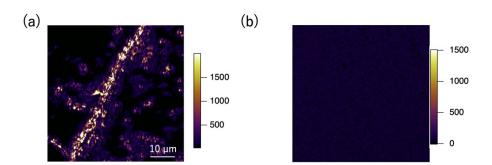


Figure S2. (a) MIP image of biofilms at 1550 cm⁻¹, mainly derived from amide II band of proteins. (b) MIP image of biofilms at 1180 cm⁻¹, which is the off resonant IR wavenumber.

Table S1. Comparison of performance of MIP and other vibrational imaging techniques.

Techniques	Imaging speed	Spatial resolution	Input power (visible)	Fluorescence interference
MIP (our work)	10 min (90000 pixels)	0.3~0.5 μm (Typical)	<1.0×10 ² kW/cm ⁻¹	No
Raman ^{1,2}	40 min (2500 pixels)	0.3~0.5 μm (Typical)	<1.0×10 ⁴ kW/cm ⁻¹	Yes
FTIR ^{3–5}	Tens of min – several hours	>10 μm	_	No

^{*} We would like to mention here that the comparison described above was made with the only references and the performance of each imaging technique depends on instruments, samples, and so on.

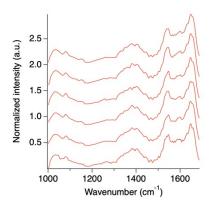


Figure S3. MIP spectra of cyanobacteria in biofilm matrix continuously recorded 6 times

References

- H. N. Noothalapati Venkata, N. Nomura and S. Shigeto, *J. Raman Spectrosc.*, 2011, **42**, 1913–1915.
- 2 R. Kato, T. Yano, T. Minamikawa and T. Tanaka, *Anal. Sci.*, 2022, **38**, 1497–1503.
- 3 J. L. Xu, K. V. Thomas, Z. Luo and A. A. Gowen, TrAC Trends Anal. Chem., 2019, 119, 115629.
- E. Levenson, P. Lerch and M. C. Martin, *Infrared Phys. Technol.*, 2006, **49**, 45–52.
- T. P. Wrobel, P. Mukherjee and R. Bhargava, *Analyst*, 2017, **142**, 75–79.