MicrobioRaman: An open-access web repository for microbiological Raman spectroscopy data

Kang Soo Lee[1,*], Zachary Landry[1], Awais Athar[2], Uria Alcolombri[3], Pratchaya Pramoj Na Ayutthaya[1], David Berry[4,5], Philippe de Bettignies[6], Ji-Xin Cheng[7], Gabor Csucs[8], Li Cui[9], Volker Deckert[10,11], Thomas Dieing[12], Jennifer Dionne[13], Ondrej Doskocil[14], Glen D'Souza[15,16], Cristina García-Timermans[17], Notburga Gierlinger[18], Keisuke Goda[19,20,21], Roland Hatzenpichler[22], Richard J. Henshaw[1], Wei E. Huang[23], Ievgeniia Iermak[12], Natalia P. Ivleva[24], Janina Kneipp[25], Patrick Kubryk[26], Kirsten Küsel[27,28], Tae Kwon Lee[29], Sung Sik Lee[8,30], Bo Ma[31], Clara Martínez-Pérez[1], Pavel Matousek[32], Rainer U. Meckenstock[33], Wei Min[34,35], Peter Mojzeš[36], Oliver Müller[1], Naresh Kumar[37], Per Halkjær Nielsen[38], Ioan Notingher[39], Márton Palatinszky[4], Fátima C. Pereira[4,40], Giuseppe Pezzotti[41,42,43], Zdenek Pilat[14], Filip Plesinger[14], Jürgen Popp[10,11], Alexander J. Probst[44], Alessandra Riva[4,45], Amr. A. E. Saleh[46], Ota Samek[14], Haley M. Sapers[47], Olga T. Schubert[15,16], Astrid Stubbusch[15,16,48], Loza F. Tadesse[49,50,51], Gordon T. Taylor[52], Michael Wagner[4,38], Jing Wang[1,53], Huabing Yin[54], Yang Yue[1,53], Renato Zenobi[37], Jacopo Zini[55,56], Ugis Sarkans[2,*], and Roman Stocker[1,*]

[1] Institute for Environmental Engineering, Department of Civil, Environmental and Geomatic Engineering, ETH Zurich, Zurich, Switzerland
[2] European Molecular Biology Laboratory, European Bioinformatics Institute, EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridge, UK
[3] Department of Plant and Environmental Sciences, Institute of Life Science, The Hebrew University of Jerusalem, Jerusalem, Israel
[4] University of Vienna, Centre for Microbiology and Environmental Systems Science, Division of Microbial Ecology, Vienna, Austria
[5] Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna, Vienna, Austria
[6] HORIBA France SAS, France
[7] Department of Electrical and Computer Engineering & Department of Biomedical Engineering, Boston University, Boston, MA, USA
[8] Scientific Center for Optical and Electron Microscopy, ETH Zurich, Zurich, Switzerland
[9] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China
[10] Institute of Physical Chemistry (IPC) and Abbe Center of Photonics (ACP), Friedrich Schiller University Jena, member of the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany
[11] Leibniz Institute of Photonic Technology e.V. Jena, member of Leibniz Health Technology, member of the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany
[12] WITec GmbH, Ulm, Germany
[13] Stanford University, Department of Materials Science and Engineering, and Department of Radiology, USA
[14] Institute of Scientific Instruments of the Czech Academy of Sciences, v.v.i., Brno, Czech

Republic
[15] Department of Environmental Microbiology, Eawag, Dübendorf, Switzerland
[16] Institute of Biogeochemistry and Pollutant Dynamics, Department of Environmental System Sciences, ETH Zurich, Zurich, Switzerland
[17] CMET, Center for Microbial Technology and Ecology, Department of Biotechnology, Ghent University, Gent, Belgium
[18] Institute of Biophysics, Department of Bionanosciences, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
[19] Department of Chemistry, The University of Tokyo, Tokyo, Japan
[20] Institute of Technological Sciences, Wuhan University, Hubei, China
[21] Department of Bioengineering, University of California, Los Angeles, CA, USA
[22] Department of Chemistry and Biochemistry, Department of Microbiology and Cell Biology, Center for Biofilm Engineering, and Thermal Biology Institute, Montana State University, Bozeman, MT, USA
[23] Department of Engineering Science, University of Oxford, Oxford, UK
[24] Chair of Analytical Chemistry and Water Chemistry, Institute of Water Chemistry, TUM School of Natural Sciences (Dep. Chemistry), Technical University of Munich, Garching, Germany
[25] Department of Chemistry, Humboldt- Universität zu Berlin, Berlin, Germany
[26] Renishaw GmbH, Germany
[27] Institute of Biodiversity, Aquatic Geomicrobiology, Friedrich Schiller University Jena, Jena, Germany
[28] Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Jena, Germany
[29] Department of Environmental and Energy Engineering, Yonsei University, Wonju, Republic of Korea
[30] Institute of Biochemistry, Department of Biology, ETH Zurich, Zurich, Switzerland
[31] Single-Cell Center, CAS Key Laboratory of Biofuels and Shandong Key Laboratory of Energy Genetics, Qingdao Institute of Bioengineering and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, China
[32] Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, UKRI, Harwell Campus, OX11 0QX, UK
[33] Environmental Microbiology and Biotechnology (EMB), University of Duisburg-Essen, Essen, Germany
[34] Department of Chemistry, Columbia University, New York, New York, USA
[35] Kavli Institute for Brain Science, Columbia University, New York, New York, USA
[36] Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, CZ-1216 Prague, Czech Republic
[37] Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland
[38] Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark
[39] School of Physics and Astronomy, University of Nottingham, Nottingham, UK
[40] School of Biological Sciences, University of Southampton, Southampton, UK
[41] Ceramic Physics Laboratory, Kyoto Institute of Technology, Kyoto, Japan
[42] Department of Molecular Genetics, Institute of Biomedical Science, Kansai Medical University, Osaka, Japan
[43] Department of Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
[44] Environmental Metagenomics, Research Center One Health Ruhr of the University Alliance Ruhr, Faculty of Chemistry, University of Duisburg-Essen, Essen, Germany
[45] Chair of Nutrition and Immunology, School of Life Sciences, Technical University of Munich, Freising-Weihenstephan, Germany
[46] Department of Engineering Mathematics and Physics, Faculty of Engineering, Cairo University, Giza 12613, Egypt

[47] Centre for Research in Earth and Space Sciences, York University, Toronto, ON, Canada
[48] Geological Institute, Department of Earth Sciences, ETH Zurich, Switzerland
[49] Department of Mechanical Engineering, MIT, Cambridge, MA, USA
[50] Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA
[51] Jameel Clinic for AI & Healthcare, MIT, Cambridge, MA, USA
[52] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY, USA
[53] Advanced Analytical Technologies, Empa, Dübendorf, Switzerland
[54] Division of Biomedical Engineering, James Watt School of Engineering, University of Glasgow, Glasgow, UK
[55] Timegate Instruments Oy, Oulu, Finland
[56] Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

e-mail: romanstocker@ethz.ch; ugis@ebi.ac.uk; leeka@ethz.ch

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Here we present the establishment of an open-access web-based repository for microbiological Raman spectroscopy data. The data collection, called 'MicrobioRaman' (https://www.ebi.ac.uk/biostudies/MicrobioRaman/studies), was inspired by the great success and usefulness of research databases like GenBank and Uniprot. This centralized repository, residing within the BioStudies database[1] which is maintained by a public institution, the European Bioinformatics Institute (EMBL-EBI), minimizes the risk of data loss or eventual abandonment, offering a long-term common reference for analysis with advantages in accessibility and transparency over commercial data analysis tools. We feel that MicribioRaman will provide a foundation for this growing field by serving as an open-access repository for sharing of microbiological Raman data and through the codification of a set of reporting standards.

Raman spectroscopy is a type of vibrational spectroscopy that relies upon inelastic scattering, in which, after interaction with molecules in a sample, the wavelength of the scattered light differs from the wavelength of the incident light, which is typically provided by a laser. This shift in wavelength differs according to the type of molecules and their vibrational modes, allowing for the analysis of complex sample chemistry in a non-destructive manner (**Fig. 1a**). When Raman spectroscopy is applied for the measurement of microbiological samples, specific Raman peaks at different wavenumbers indicate the presence of macromolecules such as carbohydrates, proteins, lipids, nucleic acids, and pigments[2]. Recent advances in technology and data analysis now enable the investigation of molecular composition at the resolution of a single microorganism with high measurement sensitivity (**Fig. 1a**). By measuring the presence of peaks corresponding to specific macromolecules or differences in spectral shape, peak position, and relative intensity of peaks, and often in conjunction with complementary techniques, such as stable isotope probing (SIP)[3], fluorescence *in situ* hybridization (FISH)[4], or -omics[5], Raman spectroscopy enables investigation of cell identity and phenotypes. This analytical approach is increasingly being employed to address important questions in both fundamental and applied microbiology (**Fig. 1b**). Notable applications include the measurement of microbial diversity in terms of cell identity, metabolic phenotype, and functional role within complex microbial communities. Raman spectroscopy is also enabling researchers to untangle the complexity of microbial communities, by allowing for the tracking of molecular interactions between microorganisms or between a microorganism and its host, and the interactions between microorganisms and their environment. In comparison to other technologies offering similar capabilities (enabling analysis of molecular composition and structure of samples, for instance, Fourier-transform infrared (FTIR) spectroscopy, cryogenic electron microscopy (cryo–EM), nanoscale secondary ion mass spectroscopy (nanoSIMS), nuclear magnetic resonance (NMR) spectroscopy), the versatility in sample size and analysis

conditions (in liquid phase or dry form) and the ability to measure live microorganisms render Raman spectroscopy applicable to diverse sample types, ranging from large nematodes (and beyond) to minuscule viruses measuring a few tens of nanometres, collected from various environments spanning oceans, soils, and mammalian guts, and potentially even efforts to detect signals of life on other planets like Mars (see refs. [3,6–10] for comprehensive reviews about Raman technologies and applications in microbiology).

Despite the potential of Raman spectroscopy in microbiology, the reporting of analytical methods and data for microbiological systems has evolved in a haphazard manner and progress in the field is hindered by the lack of both a set of standards for data reporting and a common database to deposit microbiological Raman data. Raman data from microorganisms is relatively complex to analyse because proper interpretation is dependent upon (i) biological context, (ii) experimental conditions, and (iii) data processing.

Individual Raman spectra from microbiological samples, consisting of discretized wavenumbers (typically measured in cm^{-1}) and corresponding Raman scattering signals, typically encompass many (often overlapping) peaks that represent chemical bonds of diverse types of macromolecules. Identification of the source of each peak often depends on the biological context; for example, a peak at 1,570 cm^{-1} typically corresponds to C–C stretching of nucleic acids when analysing microorganisms more generally, but predominantly to calcium dipicolinic acids (CaDPA) when measuring endospore-forming bacteria[6]. Moreover, SIP or FISH, often coupled to Raman measurements to track metabolic exchange or identify microorganisms of interest, induce a red shift of Raman peaks (i.e., peak positions move to lower wavenumbers) or potentially a change in overall spectral shape (due to interference between some fluorescent dyes used for FISH and certain Raman lasers), respectively, adding further complexity to the interpretation of microbiological Raman data.

Experimental conditions further complicate the analysis of microbiological Raman data. Compared to Raman measurements in research fields in which samples are in the solid state (often the case in material science or electrical engineering) or target cells are relatively large (a few tens of micrometres, as in biomedical engineering), samples in microbiology often contain a diversity of molecules at relatively low concentrations (diverse cell components, with the majority in liquid phase) and target cells are rather small (e.g., bacteria or archaea ranging down to a few hundreds of nanometres). Microbiological measurements are thus substantially

influenced by sampling conditions and the biotic and abiotic environment of cells at the time of the analysis (**Fig. 1c**).

For both quantitative and qualitative analyses of large datasets, Raman data are often processed with computational algorithms[6] (**Fig. 1c**). Because interpretation can often depend on the presence of peak shoulders or small changes in peak locations on the order of a few tens of wavenumbers as is the case in isotope labelling, any computational treatment can potentially affect the interpretation of microbiological Raman data.

Considering these three aspects, microbiological Raman data share similarities with other types of microbiological data, namely those derived from -omics approaches. While fields that rely on the use of these data types have greatly benefitted from the availability of organized central and public repositories for published data with reporting standards, the lack of an actively maintained, open-access data repository for microbiological Raman data has been an obstacle to the wider adoption of Raman spectroscopy in microbiology. Currently, published data are scattered across various sources (e.g., deposited on a journal publication webpage or an author's personal or institutional repository) in the absence of rational and clear reporting standards, making it challenging for researchers to access and use the data. There are several databases commercially available, for example, KnowItAll (https://sciencesolutions.wiley.com/solutions/technique/raman/knowitall-raman-collection/) and one by S.T. Japan Inc. (https://www.stjapan.de/spectra-databases/raman-spectra-databases/). These databases aim to cover the broad range of organic and inorganic materials, and are not specific to microbiological Raman data. As such, considering the peculiarities of microbiological Raman data described above, a database tailored to microbiological Raman data would be highly beneficial to promote sharing and reuse of microbiological Raman data across diverse users within the community. Moreover, in light of how useful research databases like GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Uniprot (https://www.uniprot.org/) have proven to be, we are witnessing the unique power of 'collective intelligence', where each user plays an important role for data accumulation over time and the amassed data are used for further analyses from different perspectives by other users. In accordance with this, a bottom-up, open-access data repository would significantly reinforce the power and usefulness of Raman spectroscopy in microbiology.

The MicrobioRaman platform is now open for current and future Raman users — covering data from normal Raman spectroscopy to its advanced variant systems[6] such as, but not limited to,

resonance Raman spectroscopy, stimulated Raman spectroscopy (SRS), coherent anti-Stokes Raman spectroscopy (CARS), surface-enhanced Raman spectroscopy (SERS), tip-enhanced Raman spectroscopy (TERS), hyper Raman spectroscopy (HRS), spatially offset Raman spectroscopy (SORS), polarized Raman spectroscopy, and time-gated (TG) Raman spectroscopy. Step-by-step, recipe-style instructions for deposition of novel datasets are provided on the help page (https://www.ebi.ac.uk/biostudies/submissions/help).

MicrobioRaman aims to provide a comprehensive repository of Raman data acquired from fundamental and applied microbiology research (**Fig. 1b**). The platform was collaboratively developed among the authors of this Correspondence, and it establishes a set of standards for data reporting to ensure reproducible Raman measurements across different users.

The standards for data reporting consist of five sections (**Table 1**): (i) general information about the authors and project underlying the data submitted; (ii) biological context, including both general information and specific sample details; (iii) experimental conditions, encompassing the setup used for Raman measurements; (iv) data processing, particularly focusing on the treatment of the spectrum and classification of a dataset into subgroups; and (v) instrument metadata, such as the type of spike filter, detector specifications, details of the microscope objective or focusing lens, confocality, and spectral binning. Additionally, the platform allows data submitters to specify a public release date for newly deposited data, for example, to ensure compliance with publication embargos.

As MicrobioRaman grows, it will become a valuable resource with diverse applications. It will serve as a chemical catalogue, housing data on the distribution of compounds across taxa and ecosystems. Furthermore, it will function as a source of standardised experimental designs, inspiring novel approaches. The current wave of applications of machine learning is already beginning to impact Raman-based approaches in microbiology. The ability to collect Raman data and make them broadly accessible is timely in this regard, as the effectiveness of machine learning approaches often relies on collective intelligence — in particular, data in the repository may be reused as part of training datasets in supervised approaches[6].

In conclusion, we believe that, by establishing reporting standards and facilitating data sharing among Raman users, MicrobioRaman will play an important role in promoting the adoption of Raman spectroscopy in microbiology. This initiative represents a cornerstone for reproducible Raman measurements and will seed further developments in this field. We envision the

development of new functions for MicrobioRaman as it grows with active participation from Raman users in the community and the accumulation of novel microbiological Raman data. For example, creation of an open-access library of biological molecules for peak identification within spectra and its integration with MicrobioRaman could be considered. With this Correspondence, we pledge to deposit our future data into this newly constructed infrastructure and we encourage other Raman users to contribute, further reinforcing the power and potential of reproducible Raman measurements in microbiology.

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Additional information

Correspondence and requests for materials should be addressed to Roman Stocker, Ugis Sarkans, or Kang Soo Lee.

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Author contributions

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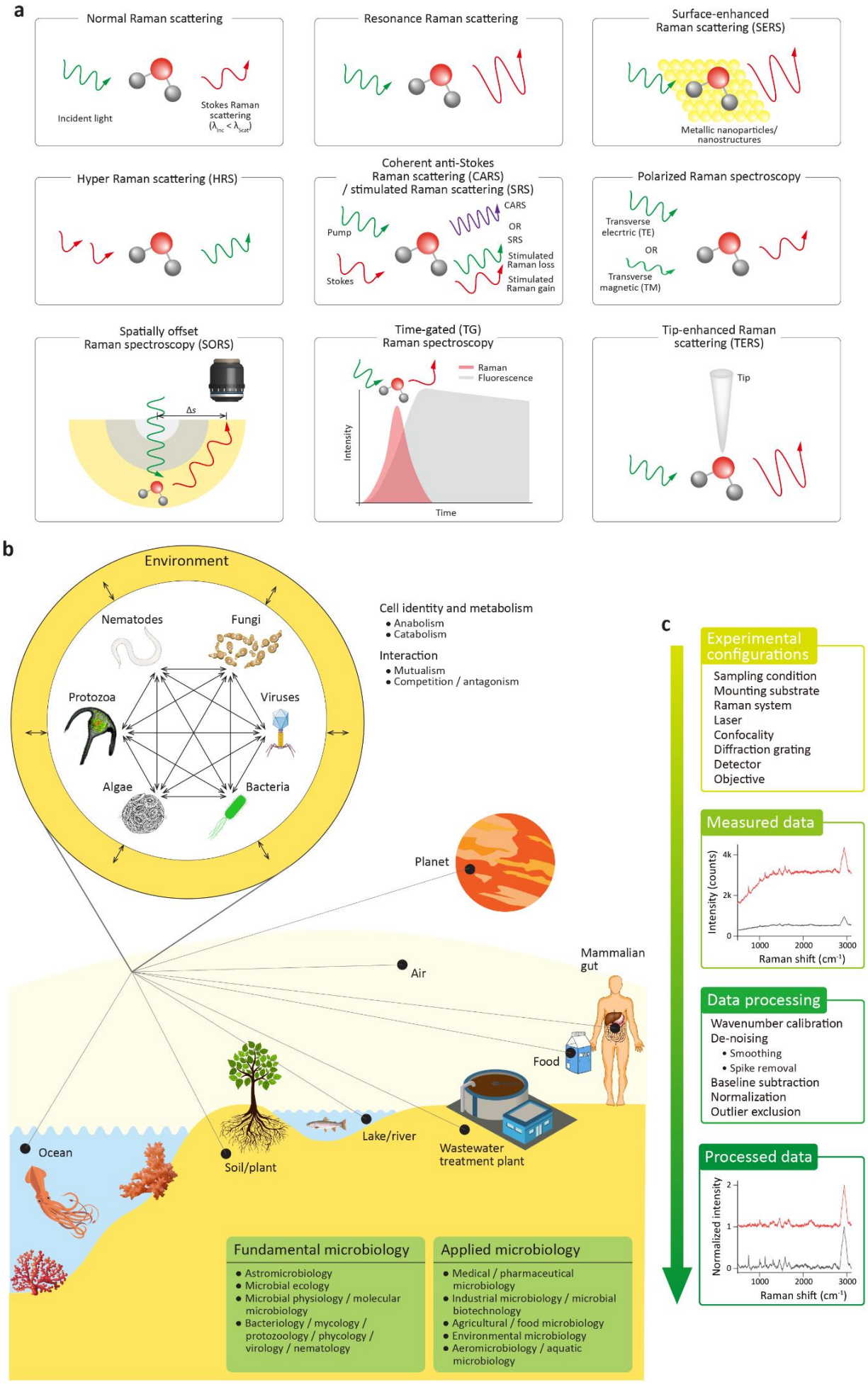
Competing interests

The authors declare no competing interests.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Normal Raman scattering
Incident light
Stokes Raman scattering
Resonance Raman scattering
Surface-enhanced Raman scattering (SERS)
Metallic nanoparticles/ nanostructures
Hyper Raman scattering (HRS)
Coherent anti-Stokes Raman scattering (CARS) / stimulated Raman scattering (SRS)
Pump
Stokes
CARS
OR
SRS
Stimulated Raman loss
Stimulated Raman gain
Polarized Raman spectroscopy
Transverse elecrtric (TE)
OR
Transverse magnetic (TM)
Spatially offset Raman spectroscopy (SORS)
Δs
Time-gated (TG) Raman spectroscopy
Raman
Fluorescence
Intensity
Time
Tip-enhanced Raman scattering (TERS)
Tip
b
Environment
Nematodes
Fungi
Protozoa
Viruses
Algae
Bacteria
Cell identity and metabolism
• Anabolism
• Catabolism
Interaction
• Mutualism
• Competition / antagonism
Planet
Air
Mammalian gut
Food
Ocean
Soil/plant
Lake/river
Wastewater treatment plant
Fundamental microbiology
• Astromicrobiology
• Microbial ecology
• Microbial physiology / molecular microbiology
• Bacteriology / mycology / protozoology / phycology / virology / nematology
Applied microbiology
• Medical / pharmaceutical microbiology
• Industrial microbiology / microbial biotechnology
• Agricultural / food microbiology
• Environmental microbiology
• Aeromicrobiology / aquatic microbiology
c
Experimental configurations
Sampling condition
Mounting substrate
Raman system
Laser
Confocality
Diffraction grating
Detector
Objective
Measured data
Intensity (counts)
4k
2k
0
1000
2000
3000
Raman shift (cm-1)
Data processing
Wavenumber calibration
De-noising
• Smoothing
• Spike removal
Baseline subtraction
Normalization
Outlier exclusion
Processed data
Normalized intensity
2
1
0
1000
2000
3000
Raman shift (cm-1)

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Fig. 1 | Overview of Raman technologies and their applications in microbiology. a The working principles underlying normal Raman spectroscopy and its advanced variant systems. For normal Raman spectroscopy, a laser beam interacts with molecules within a sample, resulting in Raman scattering after the interaction. Advanced variant systems, which rely upon modification of the system configuration, can be categorised into three groups depending on their specific advantages: techniques that provide a higher sensitivity for measurement (resonance Raman scattering, surface-enhanced Raman scattering, tip-enhanced Raman scattering), techniques that enable rapid measurement by virtue of the selection of specific wavenumbers (coherent anti-Stokes Raman scattering, stimulated Raman scattering), and techniques that provide other functions, such as the ability to measure peaks not detectable using normal Raman spectroscopy (hyper Raman scattering), among others including spatially offset Raman spectroscopy, polarized Raman spectroscopy, and time-gated Raman spectroscopy. **b** Applications of Raman spectroscopy in fundamental and applied microbiology. Raman spectroscopy is a versatile technique that enables the measurement of a broad size range of samples across diverse geographical regions and ecosystems — from large nematodes to minuscule viruses found in oceans, soils, mammalian guts, and industrial plant systems, and potentially even efforts to detect signals of life on other planets like Mars. **c** Pipeline for acquisition and analysis of microbiological Raman data and the parameters that influence the resulting data. Experimental configurations, in addition to the samples themselves, determine the resulting Raman data. When measured Raman spectra display a different level or shape of spectral background, they require computational data processing for quantitative or qualitative analyses and comparisons between samples. A section describing these factors is a part of the reporting standard in the ‘MicrobioRaman’ repository.

Table 1 | Reporting standards for microbiological Raman data. The 'general' section describes general information about the submission; the 'sample' section provides the biological context and treatment; the 'setup' section provides experimental conditions; the 'treated spectrum' section describes data processing; and the 'instrument metadata' section provides additional instrument information that could help users to reproduce the measurements. Parameters are colour-coded according to the level of recommended reporting: mandatory (in orange), if applicable (in grey), and recommended (in blue). See also the help page of BioStudies (https://www.ebi.ac.uk/biostudies/submissions/help) for general instructions for submission of novel data.

Section	Parameter	Description
General	Title	Project title
	Release date	Desired release date, for example, to ensure compliance with a publication embargo
	Description	Brief description of the project; details if isotopes (e.g., stable isotope probing (SIP)) or fluorescent probes (e.g., fluorescence *in situ* hybridization (FISH) probes) were used
	Contacts	Contact details for data authors
	Raw data files	Unprocessed raw Raman data composed of wavenumbers and corresponding Raman intensities
	Peak identification	Identification of peaks and methods used for identification (e.g., literature, public or commercial databases); peak shifts if isotopes (e.g., SIP) were used
	Software	If a commercial Raman system was used for measurement, the name and version of software
	Publications	Information about associated publications (authors, title, journal name, year)
Sample	Name of cell or compound	Sample names
	Source	Source of a sample, such as a strain collection, a chemical supplier, or the environment or tissue from which a sample was obtained
	Composition	Entities contained in the sample, including not just the cells of interest, but also the medium, as well as any extraneous materials such as tissue, debris, biofilm matrix, or soil
	Preparation	For example, preservation after sampling, culturing condition (e.g., sample volume, medium, light condition/diel cycle, pH, temperature, antibiotics, oxic/anoxic), sample age, fixed or unfixed, whether the cells were dry or wet for Raman measurement; whether isotopes (e.g., SIP) or fluorescent probes (e.g., FISH probes) were used
	Mounting substrate	E.g., glass coverslip, aluminum slide, CaF_2 slide, quartz slide

	Image files	Image files from Raman imaging; image files from light or electron microscopy (e.g., bright-field or SEM images of a sample, fluorescence images for FISH)
Setup	Raman system	Manufacturer and model of the scope
	Measurement type	E.g., normal Raman scattering; resonance Raman scattering; coherent anti-Stokes Raman scattering (CARS); stimulated Raman scattering (SRS); hyper Raman scattering (HRS); surface-enhanced Raman scattering (SERS); spatially offset Raman spectroscopy (SORS); polarized Raman spectroscopy; tip-enhanced Raman scattering (TERS); time-gated (TG) Raman spectroscopy
	Lasers and connected components/parameters	Wavelength and power of lasers; continuous wave (CW) or pulsed (if pulsed, pulse duration and repetition rate); if nonlinear Raman spectroscopy (e.g., CARS/SRS), excitation intensity; if polarized Raman spectroscopy, polarization state; laser spot shape (e.g., circular, elliptical, torus, square, rectangular); laser illumination spot size (e.g., diameter for a circular shape, lengths of major and minor axes for an elliptical shape, inner and outer diameters for torus, length and breadth for a square or rectangular shape); neutral density filter (100% if not used); grating (0 if not used); acquisition time and accumulation number (for averaging) for measurement; spectral window and resolution for measurement
	Lasers and connected components/parameters	Manufacturer and model of lasers
Treated spectrum	Processed data files	Processed Raman data and data analysis (e.g., principal component analysis (PCA), hierarchical cluster analysis (HCA), linear discriminant analysis (LDA))
	Data treatments	List of computational algorithms and their parameters and sources used for data processing and analysis
	Software	If manufacturer's software was used for data processing and analysis, the name and version of software
Instrument metadata	Annotations	For example, the type of spike filter, detector specifications, details of a microscope objective or focusing lens, confocality, spectral binning

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