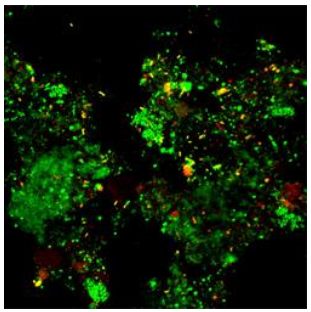
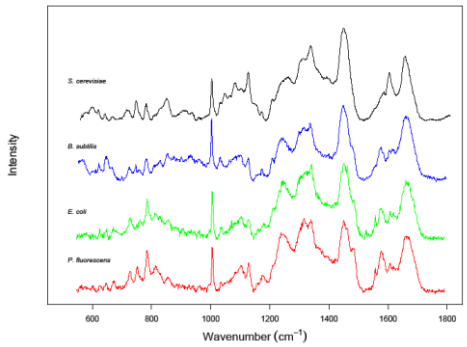
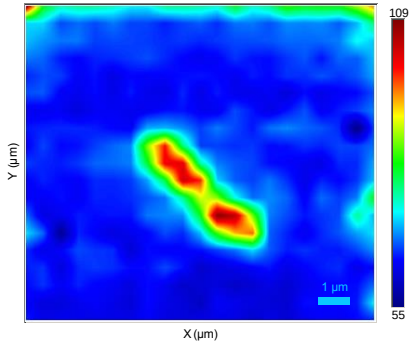
WHO, HOW AND WHERE? INSIGHTS INTO MICROBIAL ECOLOGY THROUGH RAMAN SPECTROSCOPY

DR DANIEL READ
CENTRE FOR ECOLOGY & HYDROLOGY, WALLINGFORD







Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

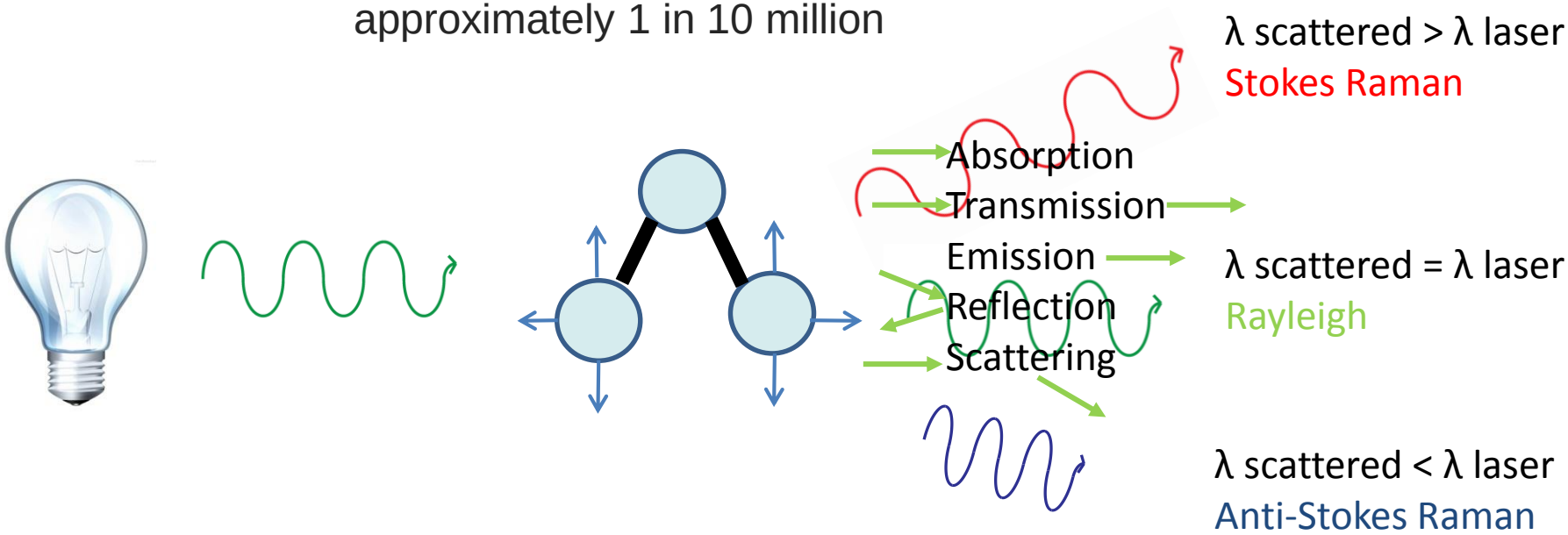
Outline

- A short introduction to Raman spectroscopy
- Microbiological applications of Raman
  - Phenotyping **Who?**
    - *Campylobacter* host association
    - *Escherichia coli* host association
  - Microbial function **How?**
    - Raman Stable Isotope Probing
  - Spatial Mapping with Raman **Where?**

Raman spectroscopy

Scattering of light

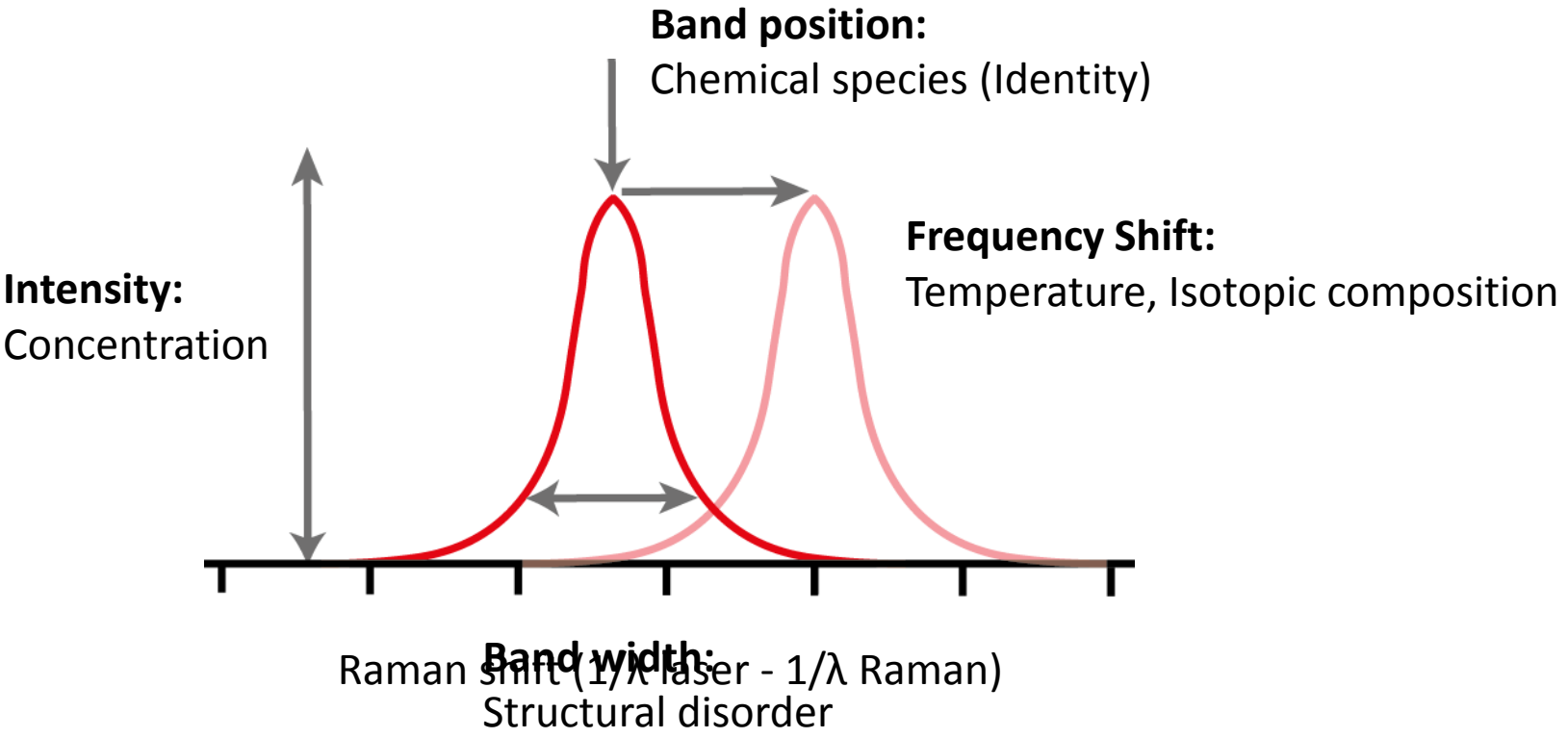
approximately 1 in 10 million



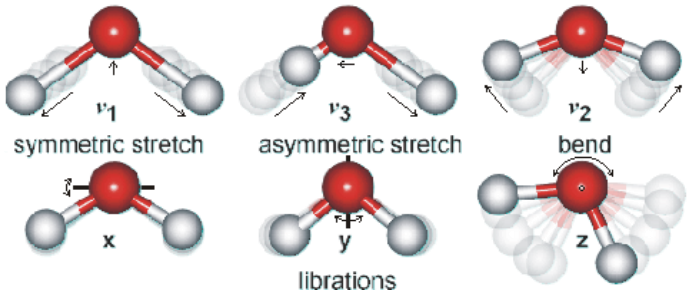
The frequency ($\nu = 1/\lambda$) difference between the incident and the scattered light characterises the molecule vibration.

ν scattered = ν laser ± ν vibration

Raman spectroscopy



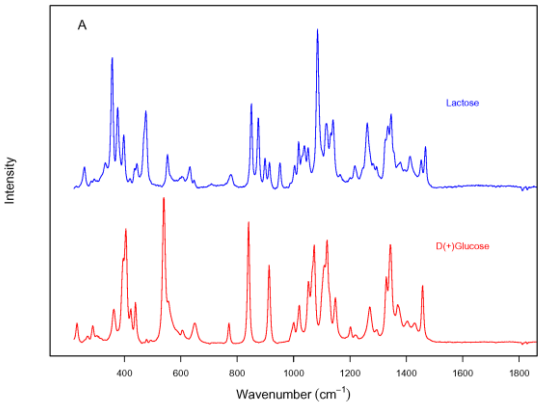
- A Raman spectrum provides a **molecular** or **chemical fingerprint**
- Vibrational frequencies are characteristic of **chemical bonds** or **groups of bonds**



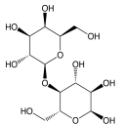
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Raman fingerprint

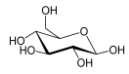
Carbohydrates



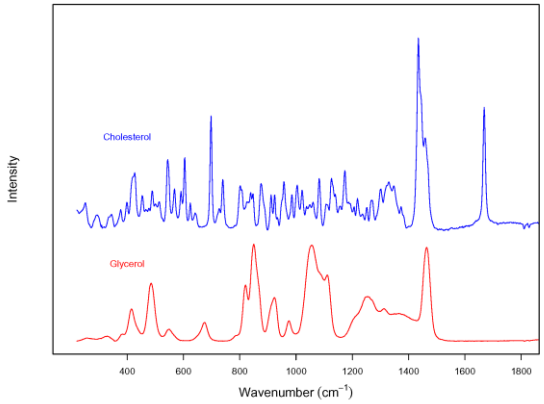
Lactose



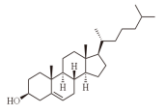
Glucose



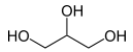
Lipids



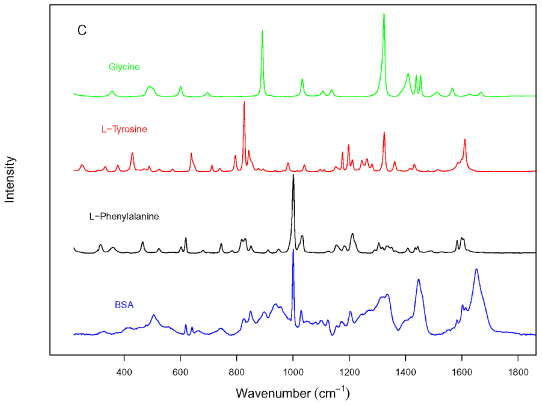
Cholesterol



Glycerol



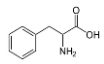
Amino acids and proteins



Glycine

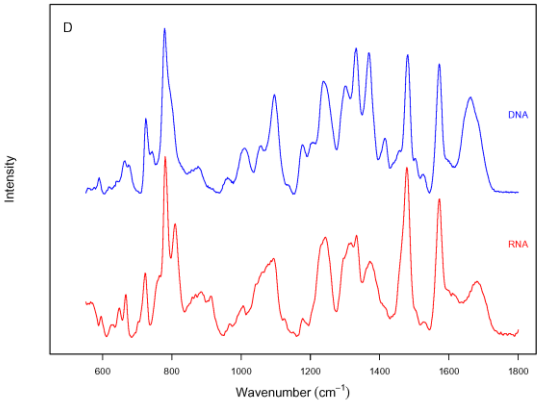
Tyrosine

Phenylalanine



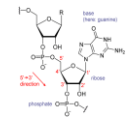
BSA

Nucleic acids



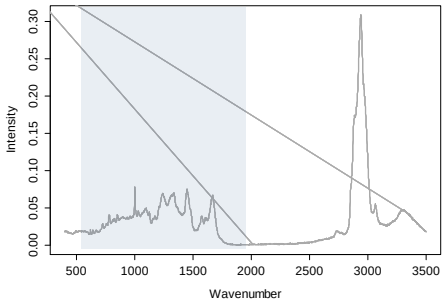
DNA

RNA

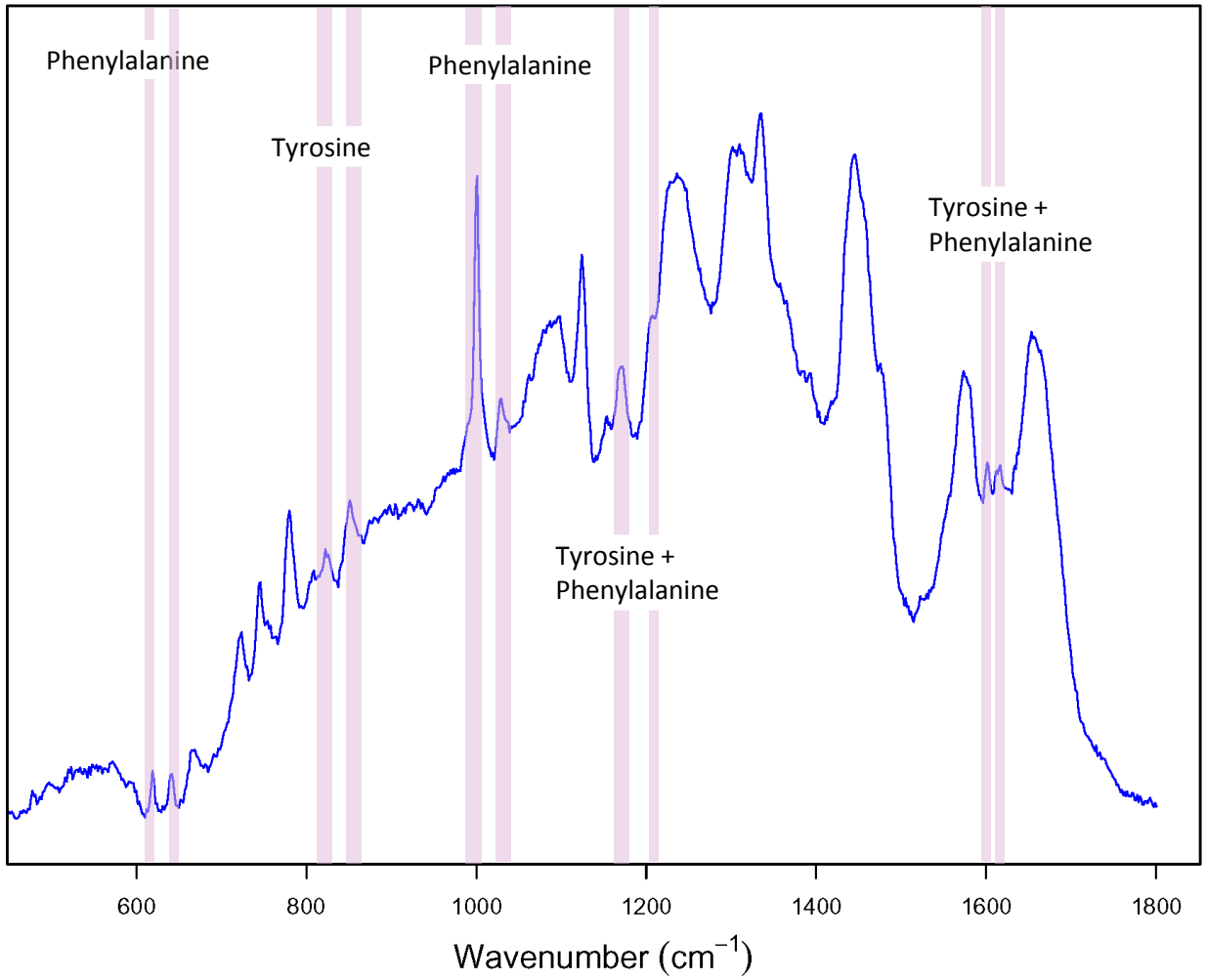


Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Raman and microbiology

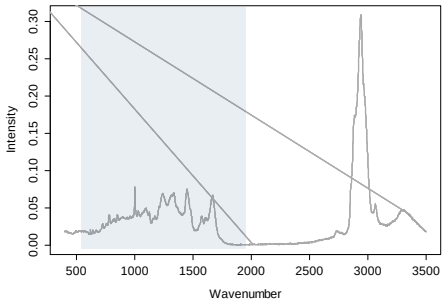


Amino acids

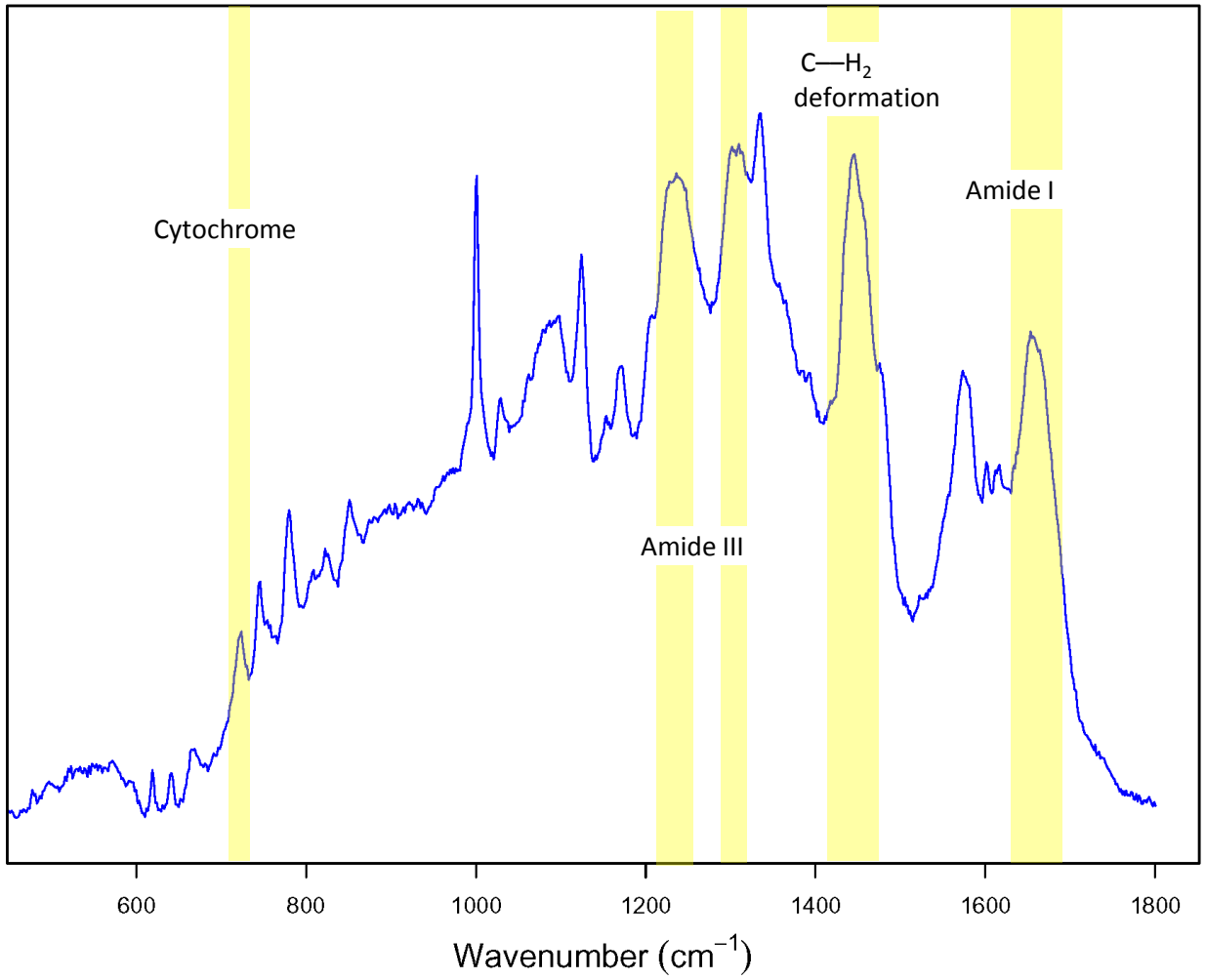


Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Raman and microbiology

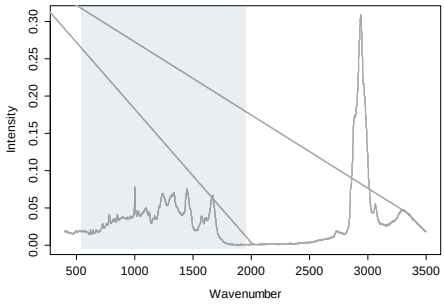


Proteins

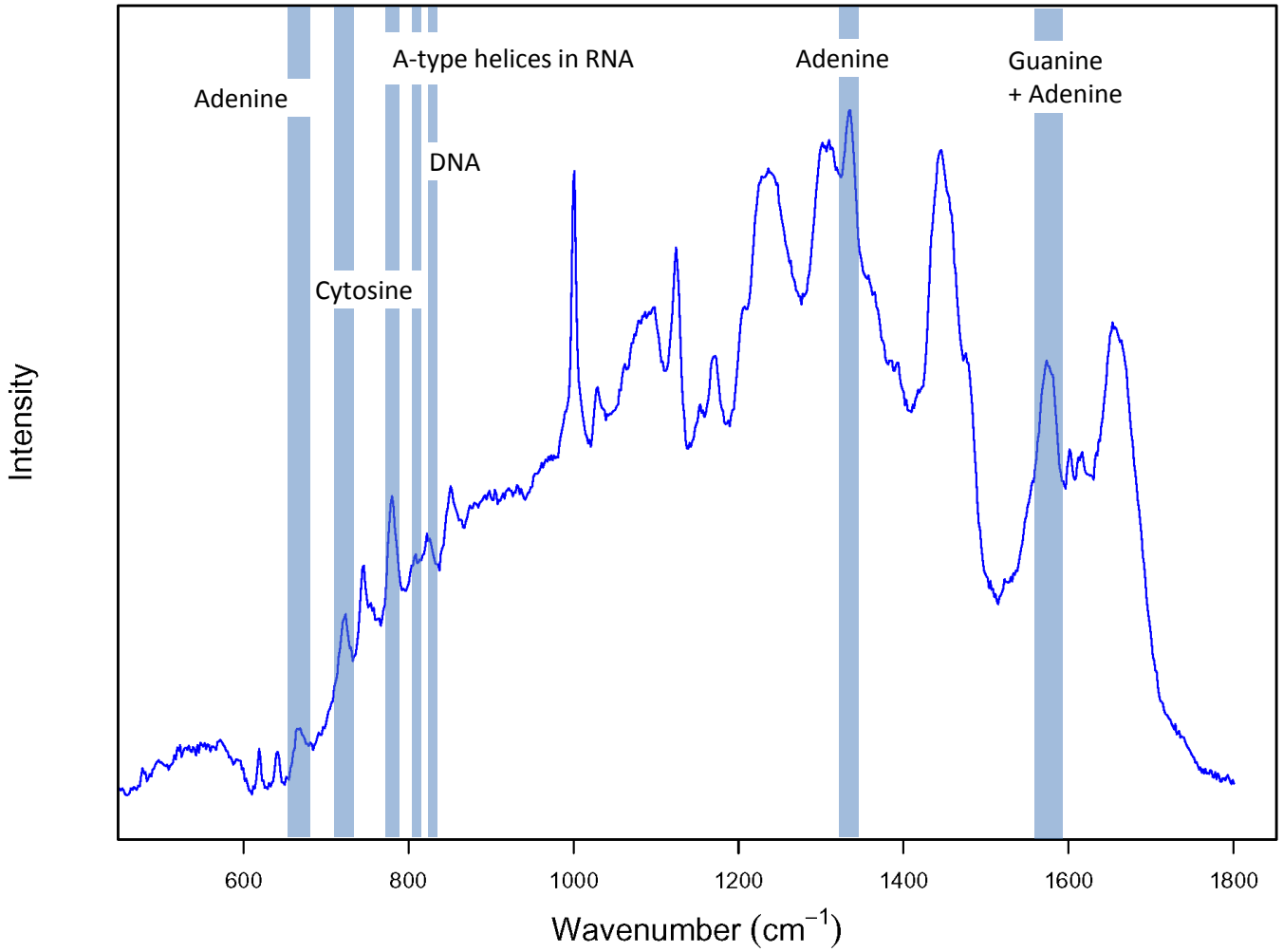


Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Raman and microbiology

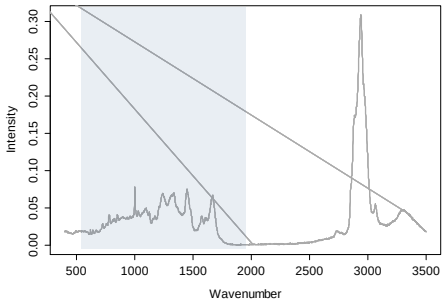


Nucleic acids

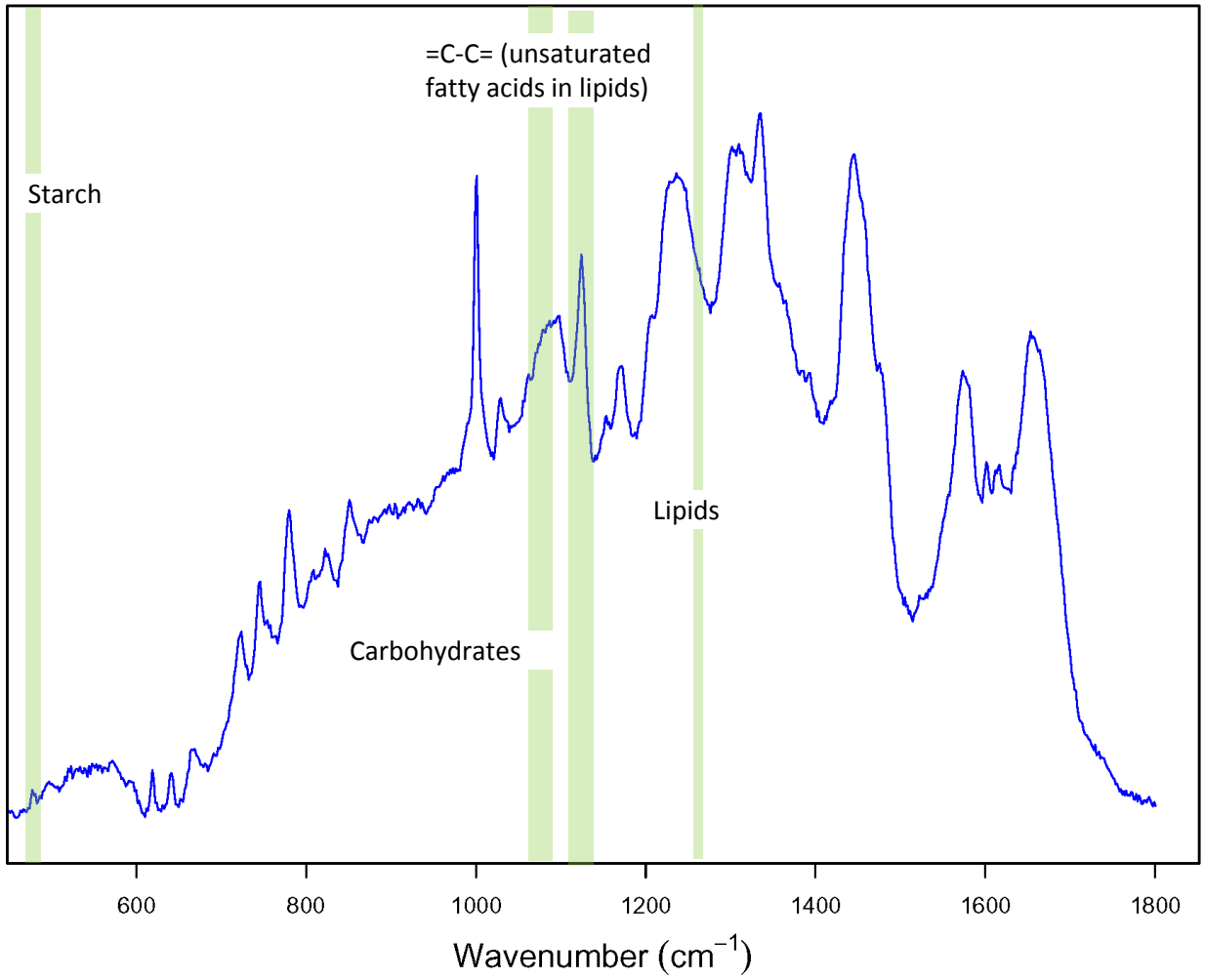


Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Raman and microbiology



Lipids and carbohydrates



Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Raman and microbiology

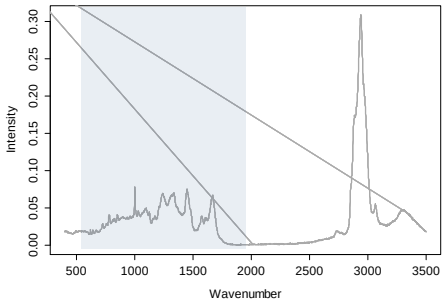
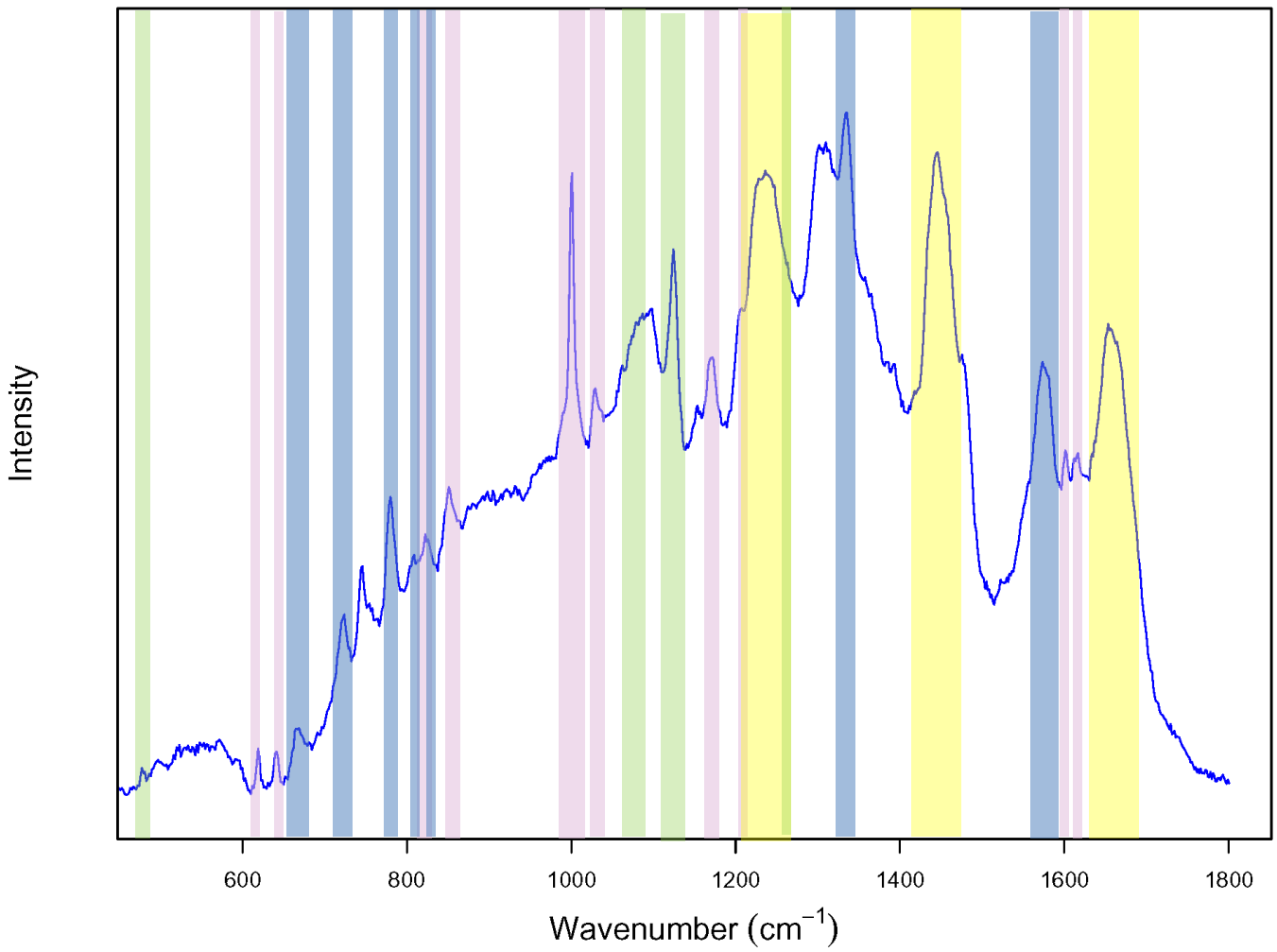


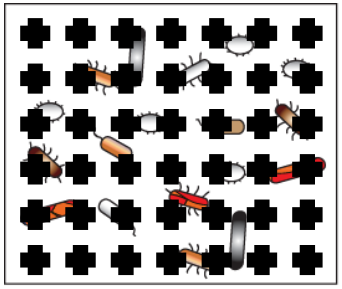
Table 1. Assignment of some bands frequently in Raman spectra respective of biological specimens.

Frequency (rel. cm^{-1})	Assignment	Reference
3059	(C—C—H) aromatic str	Maquelin *et al.* (2002)
2975	CH_3 str	Maquelin *et al.* (2002)
2935	CH_3 and CH_2 str	Maquelin *et al.* (2002)
2870–2890	CH_2 str	Maquelin *et al.* (2002)
1735	> C═O ester str	Maquelin *et al.* (2002)
1650–1680	Amido I	Maquelin *et al.* (2002)
1658	Unsaturated lipids	van Manen *et al.* (2005)
1614	Tyrosine	Maquelin *et al.* (2002)
1605–1606	Phenylalanine	Maquelin *et al.* (2002)
1582, 1593	Protein	Maquelin *et al.* (2002); Kneipp *et al.* (2006)
1575–1578	Guanine, adenine (ring str)	Maquelin *et al.* (2002)
1573	C═C, N—H def and C—N str (amide II)	Schuster *et al.* (2000b)
1510	Adenine	Uzunbajakava *et al.* (2003a)
1505, 1518, 1532, 1578	Adenine, Cytosine, Guanine	Uzunbajakava *et al.* (2003a)
1482–1487	Nucleic acids	Schuster *et al.* (2000a)
1441	Lipids	van Manen *et al.* (2005)
1440–1460	C—H_2 def	Maquelin *et al.* (2002)
1431–1481	Protein marker band 1451	Uzunbajakava *et al.* (2003b)
1421–1427	Adenine, Guanine	Uzunbajakava *et al.* (2003a); Kneipp *et al.* (2006)
1375	Thymine, Adenine, Guanine	Uzunbajakava *et al.* (2003a)
1336–1339	Adenine	Uzunbajakava *et al.* (2003a)
~1320	Amide III, C—H def	Schuster *et al.* (2000b)
1304	Adenine, amide III	Uzunbajakava *et al.* (2003a)
1295	CH_2 def	Maquelin *et al.* (2002)
1214, 1240, 1254	Thymine, Cytosine, Adenine, ring ν	Uzunbajakava *et al.* (2003a)
1254	Adenine, amide III	Uzunbajakava *et al.* (2003a)
1220–1290	Amide III random, lipids	Schuster *et al.* (2000b)
1267	Lipids	van Manen *et al.* (2005)
1209	Tyrosine, Phenylalanine, protein, amide III	Uzunbajakava *et al.* (2003a)
1175	Tyrosine, Phenylalanine	Uzunbajakava *et al.* (2003a)
1154	ν(CC, CN), β(CH3)	Maquelin *et al.* (2002)
~1130	═C—C═ (unsaturated fatty acids in lipids)	Maquelin *et al.* (2002)
1102	> PO_2 str (sym)	Maquelin *et al.* (2002)
1100	Glass background	Schuster *et al.* (2000b)
1098–1099	Phosphate, CC skeletal and COC str from glycosidic link	Maquelin *et al.* (2002)
1085	C—O str	Maquelin *et al.* (2002)
1061	C—N and C—C str	Maquelin *et al.* (2002)
1054	Nucleic acids, CO str; protein, C—N str	Uzunbajakava *et al.* (2003a)
1032	Phenylalanine; C—H str	Uzunbajakava *et al.* (2003a)
1030–1130	Carbohydrates, mainly —C—C— (skeletal), C—O, def (C—O—H)	Schuster *et al.* (2000b)
~1001	Phenylalanine	Maquelin *et al.* (2002)
897	COC str	Maquelin *et al.* (2002)
858	CC str, COC 1,4 glycosidic link	Maquelin *et al.* (2002)
~850	Buried tyrosine	Maquelin *et al.* (2002)
~830	Exposed tyrosine	Maquelin *et al.* (2002)
838	DNA	Deng *et al.* (1999)
813	A-type helices in RNA	Uzunbajakava *et al.* (2003b)
810–820	Nucleic acids (C—O—P—O—C in RNA backbone)	Schuster *et al.* (2000b)
778–785, 792	Cytosine, uracil (ring, str)	Maquelin *et al.* (2002); Uzunbajakava *et al.* (2003a)
748–751	O—P—O sym str	Takai *et al.* (1997)
752	T ring str	Uzunbajakava *et al.* (2003a)
730	A ring str	Uzunbajakava *et al.* (2003a)
720	Adenine	Maquelin *et al.* (2002)
665	Guanine	Maquelin *et al.* (2002)
640	Tyrosine (skeletal)	Maquelin *et al.* (2002)
620	Phenylalanine (skeletal)	Maquelin *et al.* (2002)
550 range	Glass background	Schuster *et al.* (2000b)
540	COC glycosidic ring def	Maquelin *et al.* (2002)
520–540	S—S str	Maquelin *et al.* (2002)
481	Skeletal modes of carbohydrates (starch)	Schuster *et al.* (2000b)
407	Skeletal modes of carbohydrates (glucose)	Schuster *et al.* (2000b)

Fingerprint or Phenotype

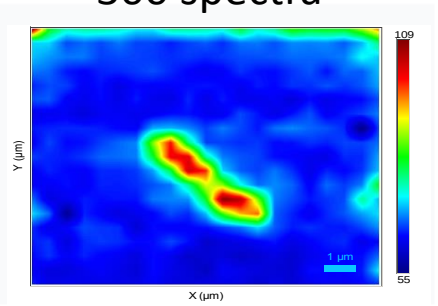


The Raman microspectrometer



Optical image

~300 spectra



Grating

Raman Microspectroscopy:

1. High spatial resolution (< 1 µm lateral)
2. Low interaction with water
3. Non-destructive*
4. Information rich

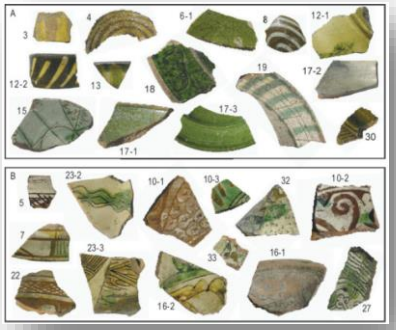
CCD Camera

Monochromatic laser

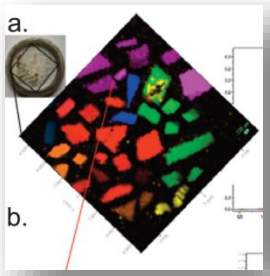
Applications of Raman Spectroscopy

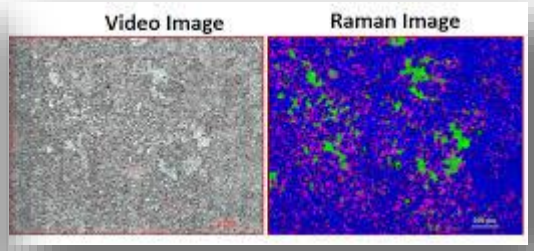
Art

Archaeology



Geology

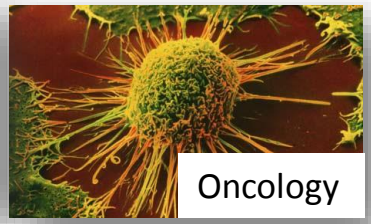




Forensics

Biology

Histology



Oncology

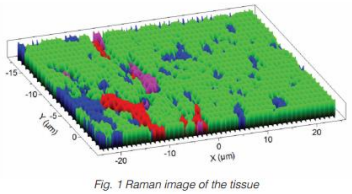
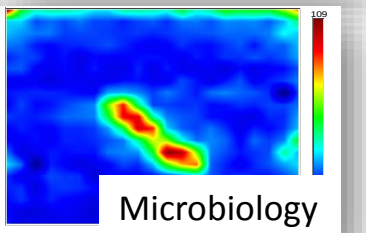
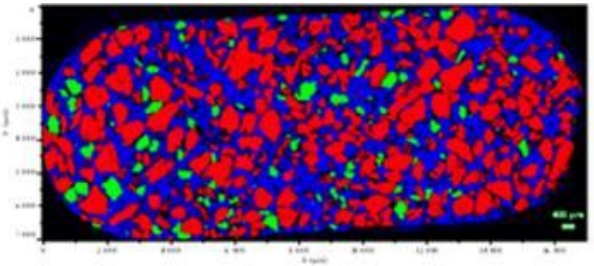


Fig. 1 Raman image of the tissue



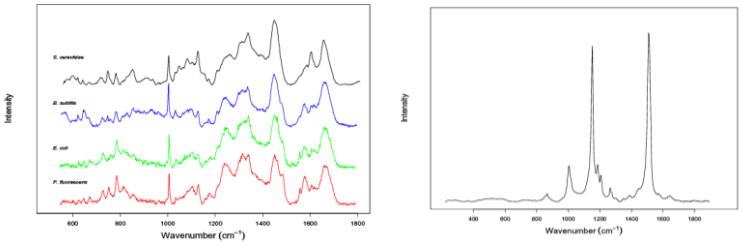
Microbiology

Pharmacy

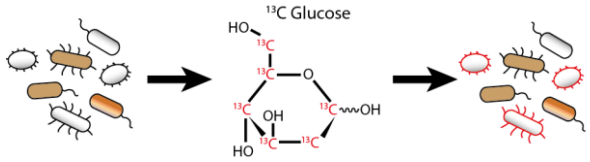


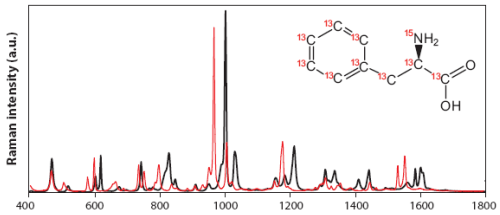
Raman and Microbial Ecology

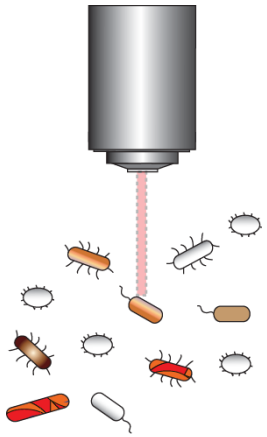
linking ecotype to phenotype **WHO?**



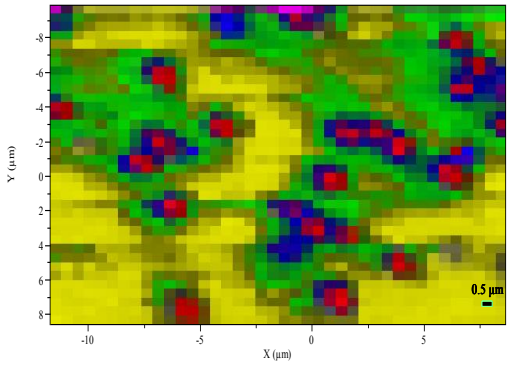
Raman Stable Isotope Probing **HOW?**





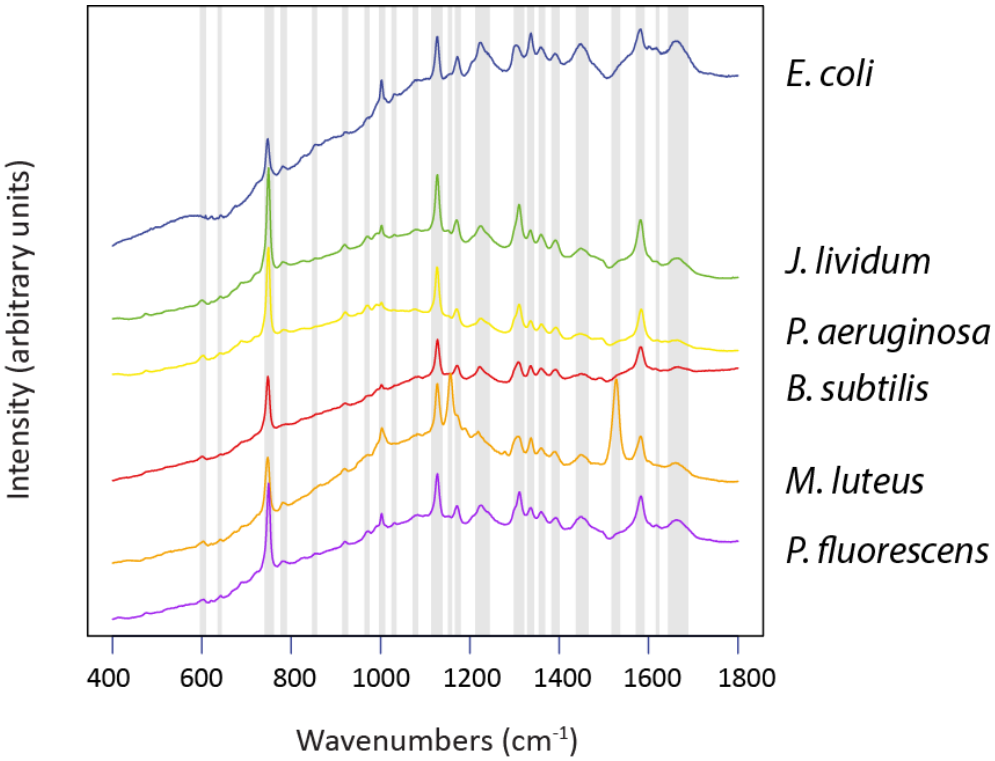


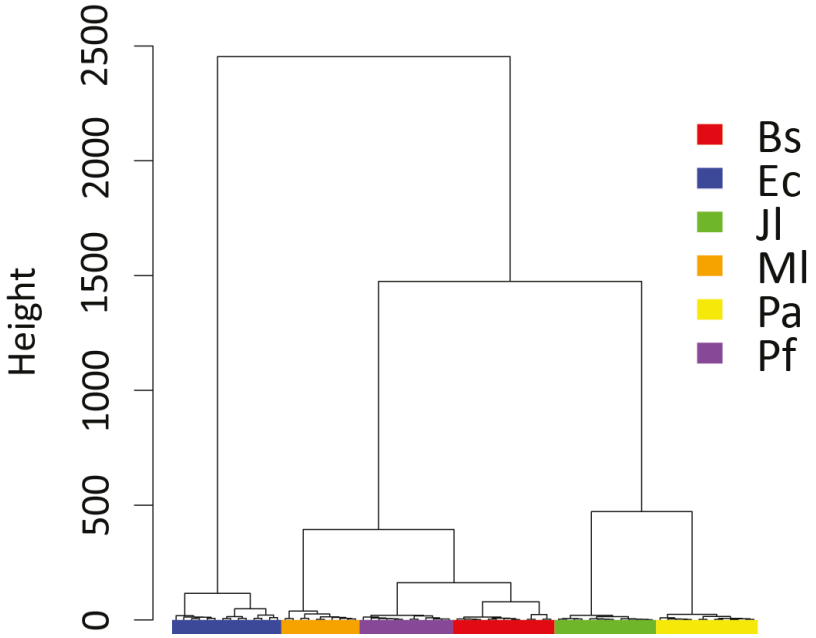
Raman Mapping **WHERE?**



Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Who? Raman Phenotyping





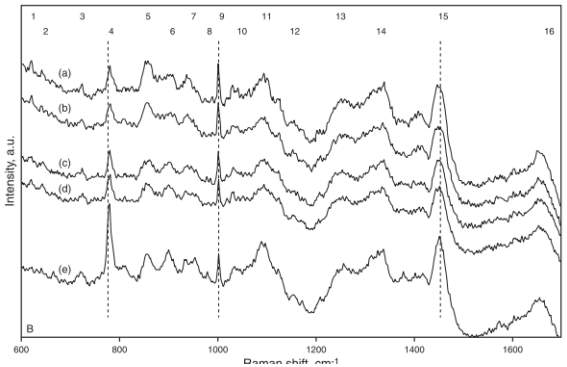
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Who? Raman Phenotyping

Raman spectroscopy for rapid discrimination of *Staphylococcus epidermidis* clones related to medical device-associated infections

O. Samek, [1,*] *H.H. Telle,* [1] *L.G. Harris,* [2] *M. Bloomfield,* [3] *and D. Mack* [2]

[1] Department of Physics, Swansea University, Singleton Park, Swansea, SA2 8PP, UK
[2] Medical Microbiology and Infectious Diseases, Institute of Life Science, School of Medicine, Swansea University, Singleton Park, Swansea, SA2 8PP, UK
[3] Renishaw plc, Spectroscopy Products Division, Wotton-under-Edge, Gloucestershire, GL12 7DW, UK



SpectraCell RA
Bacterial Strain Analyzer

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Identification of meat-associated pathogens via Raman microspectroscopy

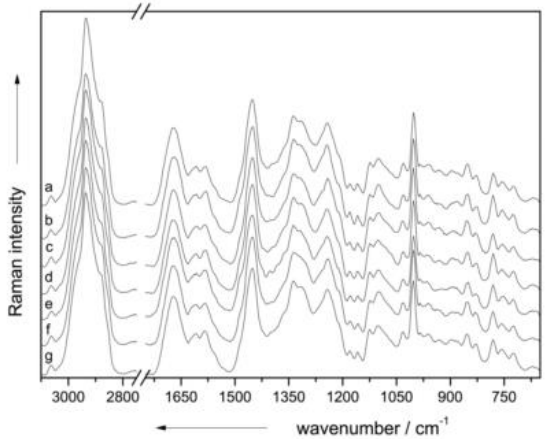
Susann Meisel [a], Stephan Stöckel [a], Petra Rösch [a,*], Jürgen Popp [a,b,*]

[a] Institute of Physical Chemistry and Abbe Center of Photonics, University of Jena, Helmholtzweg 4, D-07743 Jena, Germany
[b] Institute of Photonic Technology, Albert-Einstein-Straße 9, D-07745 Jena, Germany

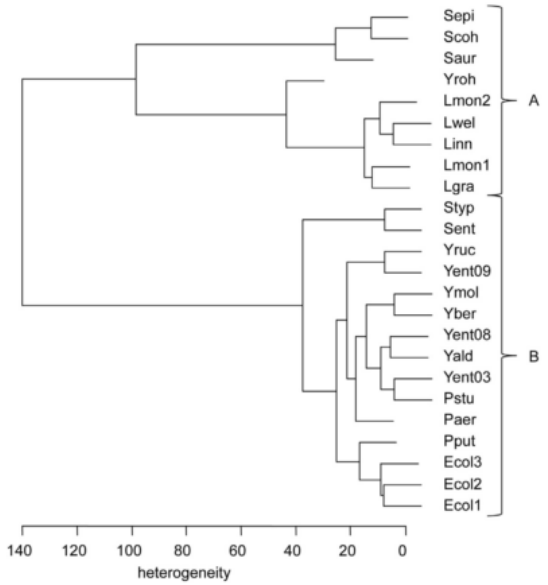
Raman Spectroscopy as a Potential Tool for Detection of *Brucella* spp. in Milk

Susann Meisel,[a] Stephan Stöckel,[a] Mandy Elschner,[b] Falk Melzer,[b] Petra Rösch,[a] and Jürgen Popp[a,c]

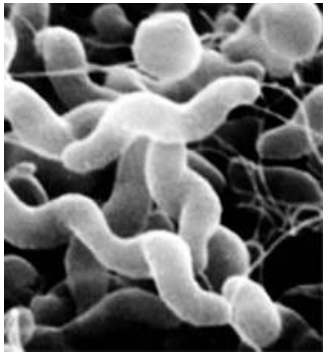
Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schi
Animal Health, Institute of Bacterial Infections and Zoonoses, Jena, Germany



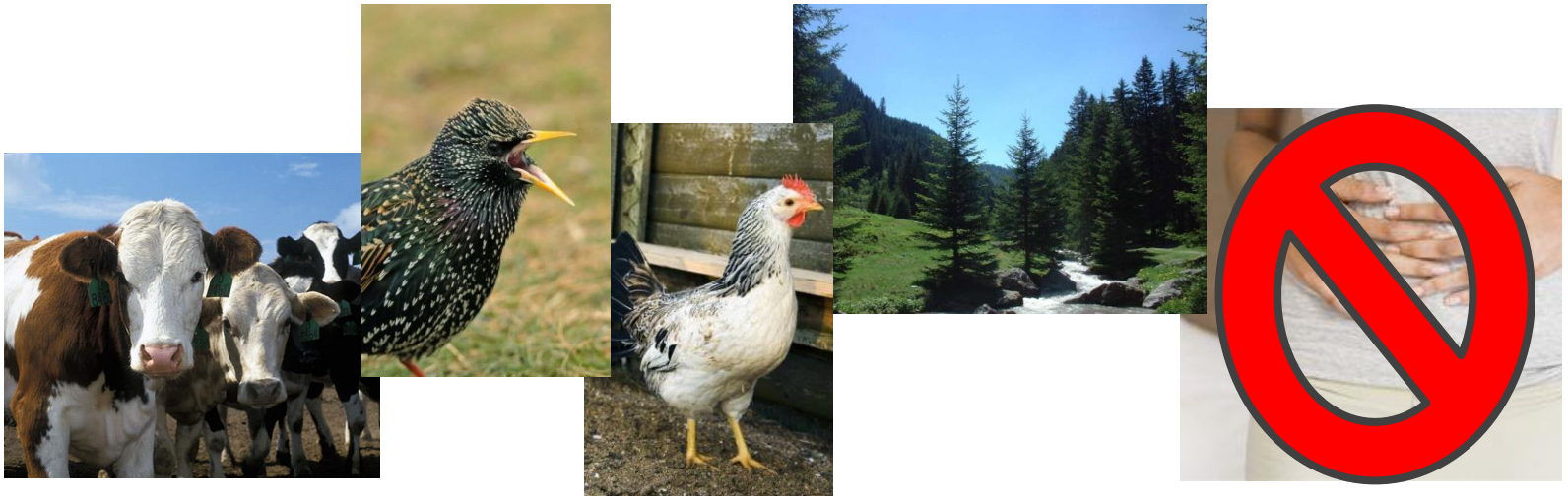
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL



Who? linking ecotype to phenotype

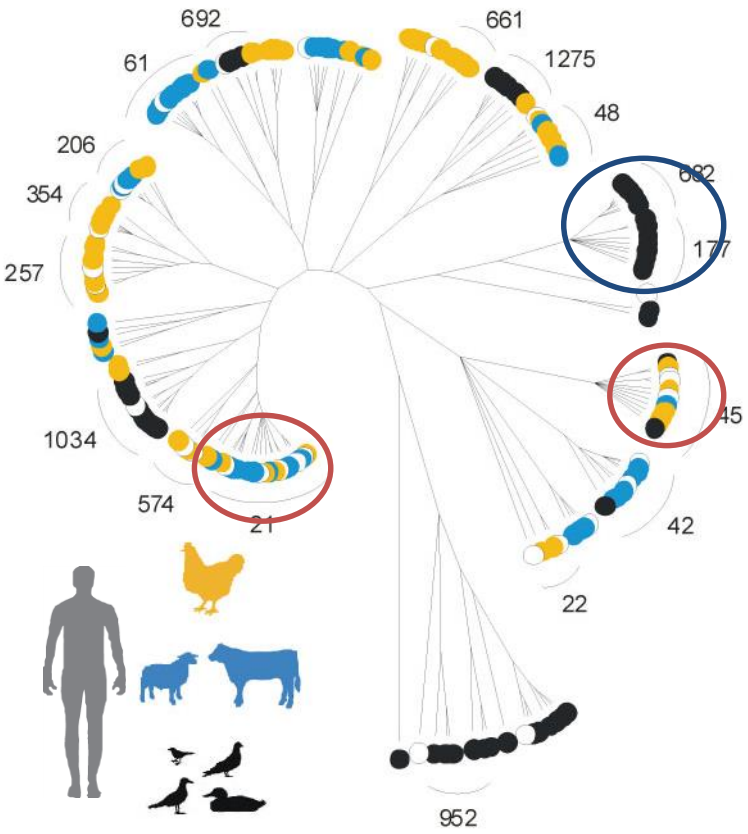


- *Campylobacter jejuni* and *C. coli* – campylobacteriosis
- Accounts for estimated **2.5 million cases** (US) and **1,340,000** (UK) each year
- x13 the number of cases caused by Salmonella, *E. coli*, and Listeria combined
- Estimated annual economic burden is £500 million in the UK
- Ability to colonize multiple hosts is a key feature of the ecology of Campylobacter



Who? linking ecotype to phenotype

Multilocus Sequence Typing (MLST)



Single host lineages

MLST 682 and 177 found only in wild birds

Multi-host lineages

MLST 45 and 21 found in chickens, cattle, wild birds and in clinical samples

Hypotheses:

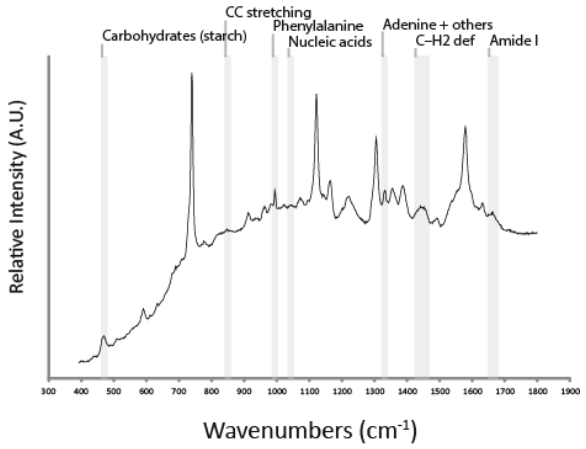
1. Phenotype is related to genotype
2. Phenotype is related to species of host organism/environment it was isolated from

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Sheppard, S.K., Maiden, M.C.J., Falush, D. (2009). Population Genetics of *Campylobacter*. In: Bacterial Population Genetics in Infectious Disease. Eds. Robinson, A.D., Falush, D., Feil, E.J. John Wiley & Sons, New Jersey. Pages 181-194.

Who? linking ecotype to phenotype

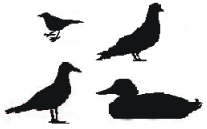
- **Strains used in this study:**
  - 108 Strains of *Campylobacter* (cultured and analysed in triplicate)
  - 2 species - *C. jejuni* (85) and *C. coli* (23)
  - 66 Multilocus Sequence Types
  - 1,620 Raman spectra



27 Cattle faeces

5 Chicken gut
24 Chicken faeces
15 Chicken meat

23 Clinical



11 Wild bird

3 Pig

Neural network analysis

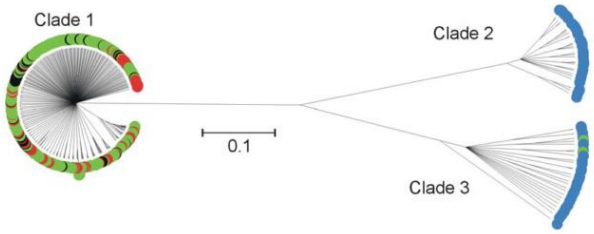
•Distinguish which peaks provide the most information in separating the classes

•Discards least informative peak until peak set with most discriminatory powers is left

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

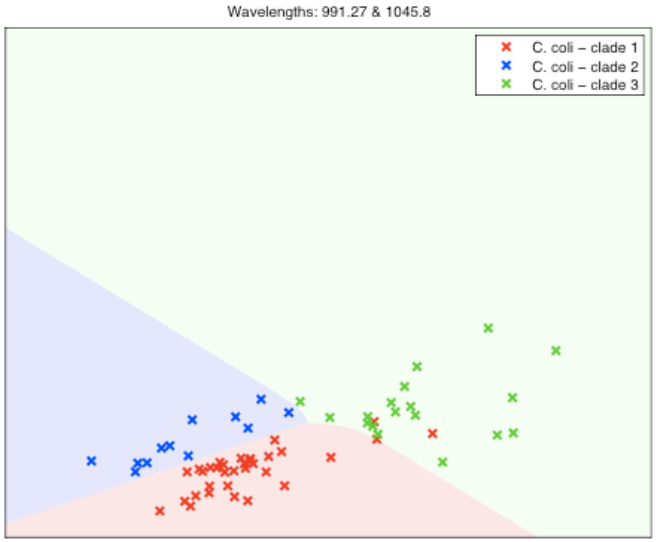
Who? linking ecotype to phenotype

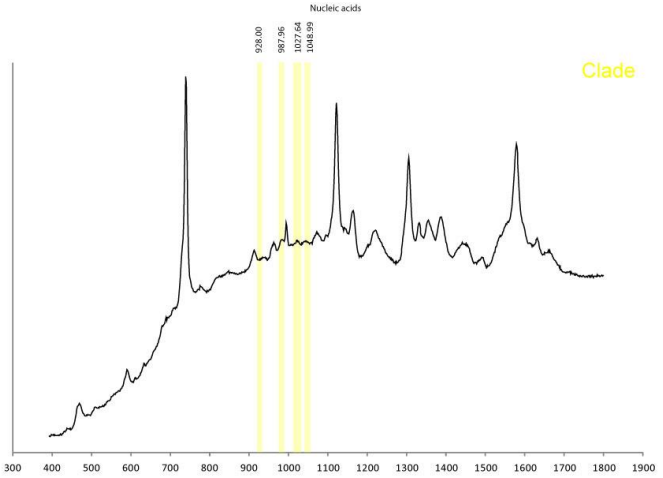
Campylobacter coli – Clade 1, 2, and 3



Farm
Riparian
Clinical
Farm and Clinical

79% classification success





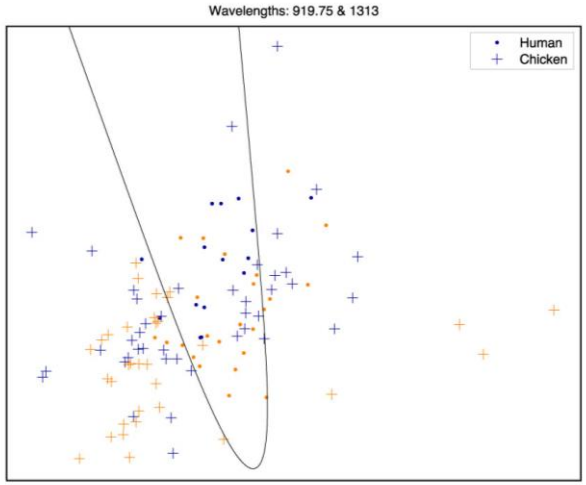
Optimal subset of four peaks belonging to phenylalanine and nucleic acids

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

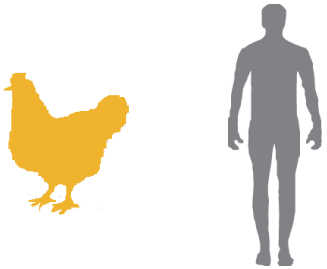
Read DS, Woodcock DJ, Strachan NJC, et al. (2013) Evidence for phenotypic plasticity among multihost *Campylobacter jejuni* and *C. coli* lineages, obtained using ribosomal multilocus sequence typing and Raman spectroscopy. Appl Environ Microbiol 79:965–73.

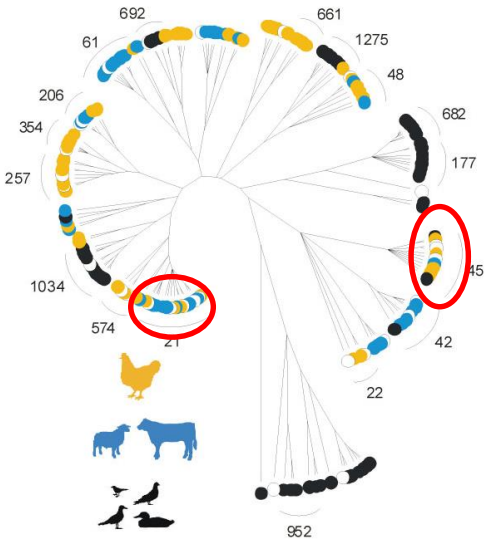
Who? linking ecotype to phenotype

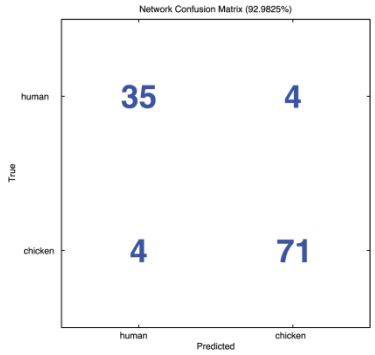
93% classification success



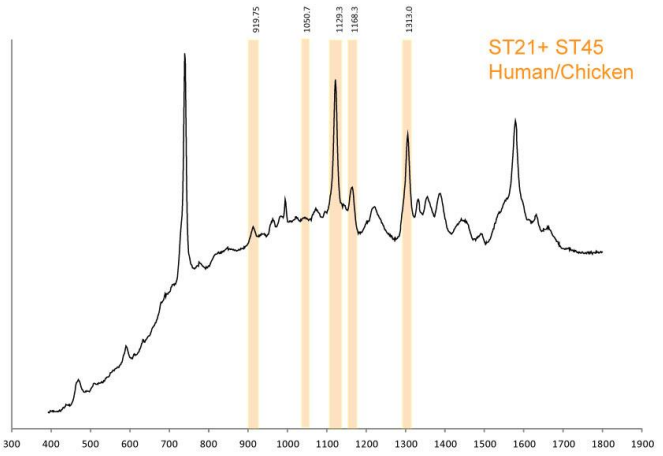
C. jejuni ST45 and ST21 human and chicken







Optimal subset of five peaks belonging to cytochrome, nucleic acids and one unidentified peak

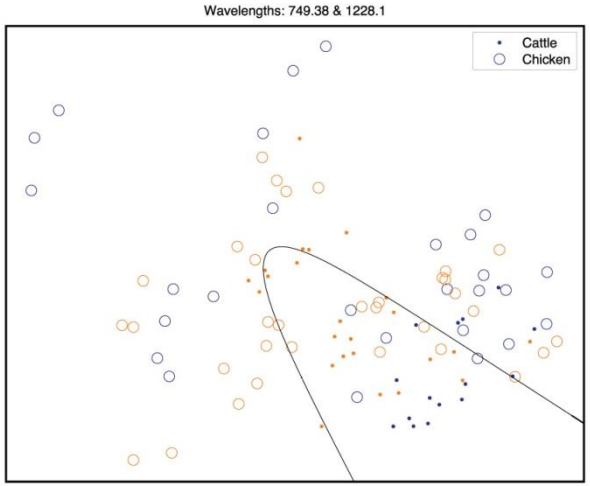


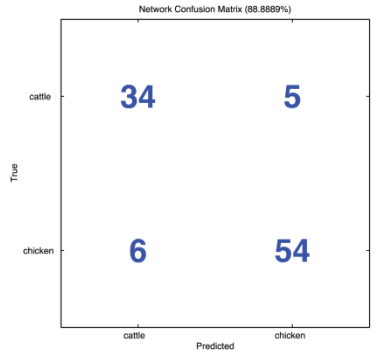
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Read DS, Woodcock DJ, Strachan NJC, et al. (2013) Evidence for phenotypic plasticity among multihost *Campylobacter jejuni* and *C. coli* lineages, obtained using ribosomal multilocus sequence typing and Raman spectroscopy. Appl Environ Microbiol 79:965–73.

Who? linking ecotype to phenotype

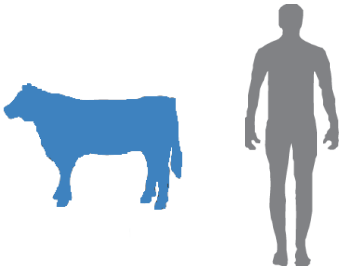
89% classification success

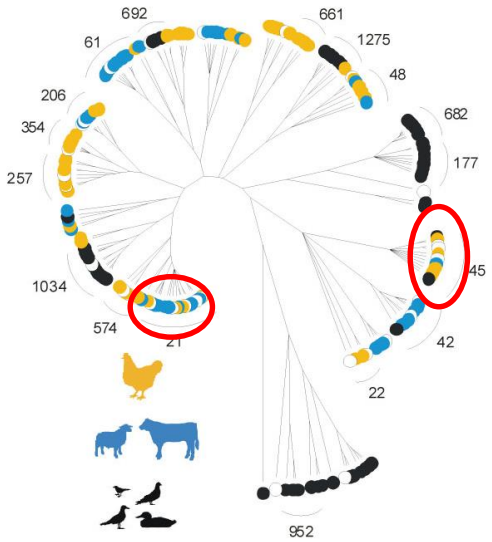


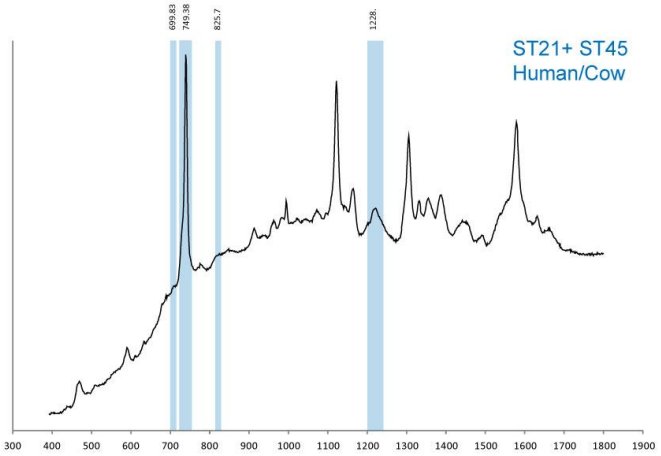


Optimal subset of four peaks belonging to cytochrome and two unidentified peaks

C. jejuni ST45 and ST21 human and cow







Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Read DS, Woodcock DJ, Strachan NJC, et al. (2013) Evidence for phenotypic plasticity among multihost *Campylobacter jejuni* and *C. coli* lineages, obtained using ribosomal multilocus sequence typing and Raman spectroscopy. Appl Environ Microbiol 79:965–73.

Who? linking ecotype to phenotype

Campylobacter phenotyping summary

- Phenotyping is a powerful method for typing bacteria
- Phenotype can be linked to the ecology of different strains
- Phenotype represents the sum of the genome interacting with the environment
- Limitations of MLST technique (only 7 housekeeping genes)
- Further studies on whole genomes needed

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Read DS, Woodcock DJ, Strachan NJC, et al. (2013) Evidence for phenotypic plasticity among multihost *Campylobacter jejuni* and *C. coli* lineages, obtained using ribosomal multilocus sequence typing and Raman spectroscopy. Appl Environ Microbiol 79:965–73.

Who? linking ecotype to phenotype

Two strain collections of *Escherichia coli* :

1. ‘ECOR’ collection (Ochman and Selander *et al*. 1984)
   - Host associated
   - Human and animal origin
   - 72 strains
2. ‘GMB’ collection (Méric *et al*. 2013)
   - Collected at IFR
   - Plant associated (salad crops)
   - 106 strains

Hypothesis:

1. Phenotype is related to environment of origin


environmental microbiology

Environmental Microbiology (2012) doi:10.1111/j.1462-2920.2012.02852.x

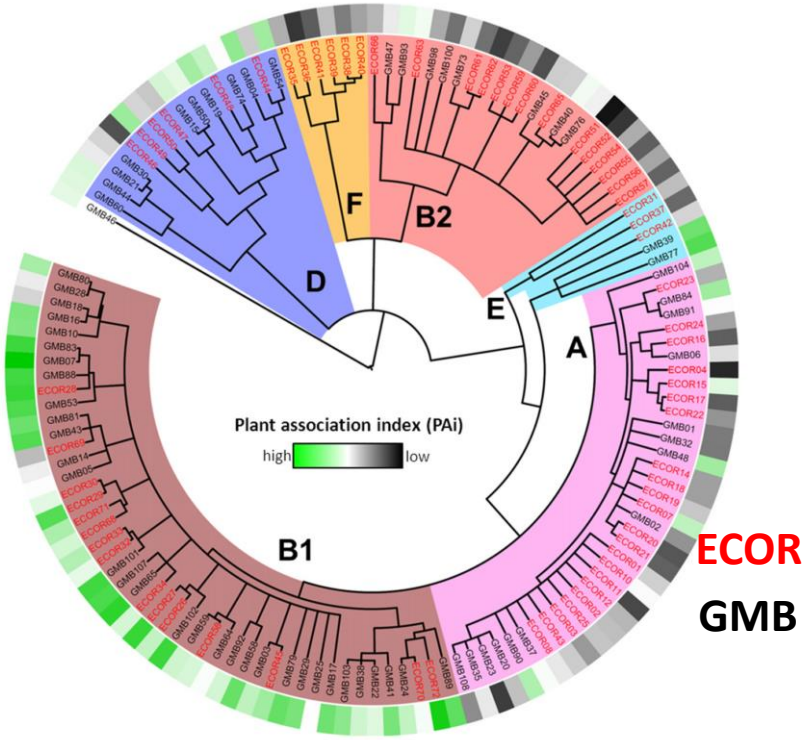
Phylogenetic distribution of traits associated with plant colonization in *Escherichia coli*

Guillaume Méric,[1†] E. Katherine Kemsley,[2] Daniel Falush,[4] Elizabeth J. Saggers[3] and Sacha Lucchini[1*]

[1]Gut Health and Food Safety, [2]Analytical Sciences Unit and [3]Microbial Ecology Exploitation Platform, Institute of Food Research, Norwich NR4 7UA, UK. [4]29 Glebe Road, London SW13 0DZ, UK.

Introduction

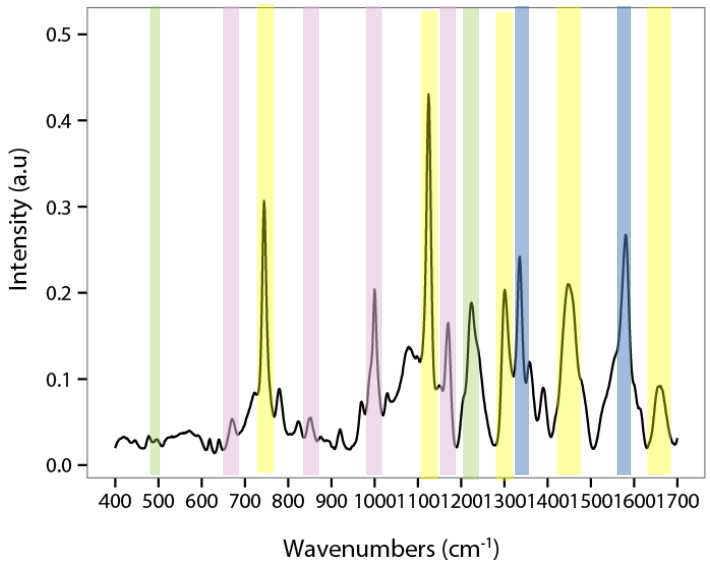
The primary habitat of *Escherichia coli* is the lower gastrointestinal tract of warm-blooded animals, and its presence in faecal (host) contents results in dispersal in secondary (non-host) environments (Savageau, 1983). *Escherichia coli* is believed to be able to persist in the environment with survival rates of several weeks, and it

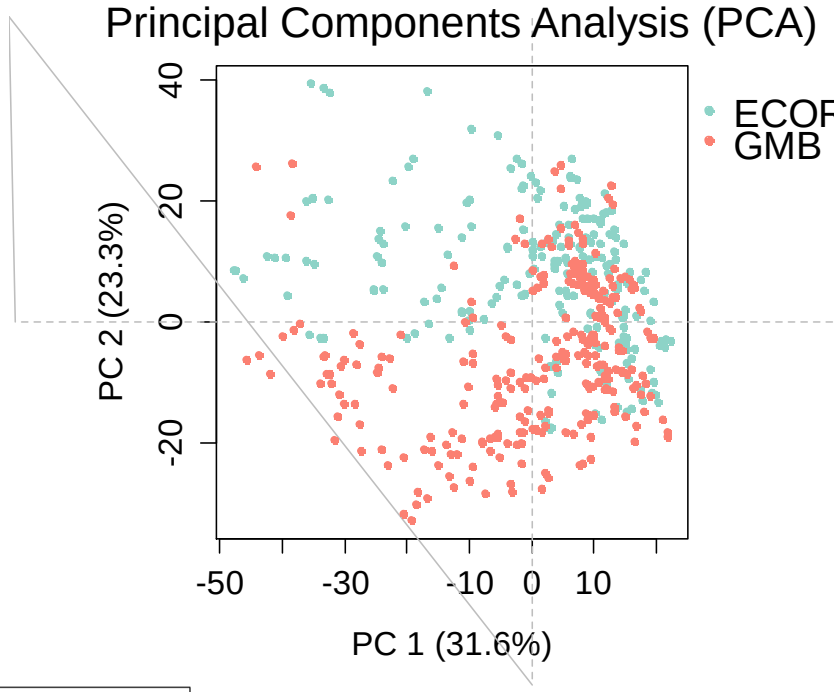


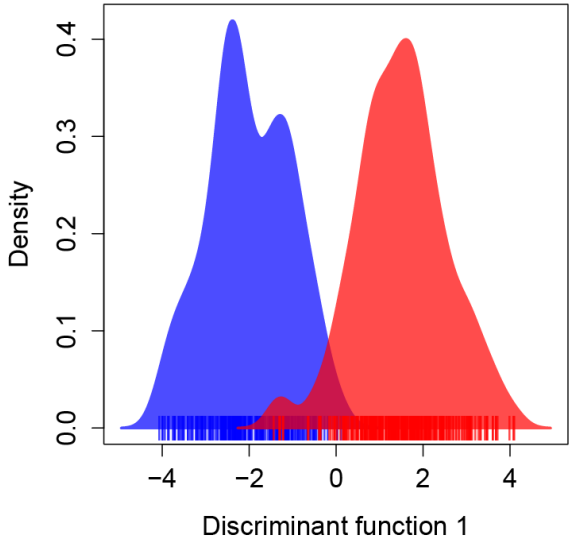
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Méric G, Kemsley EK, Falush D, et al. (2013) Phylogenetic distribution of traits associated with plant colonization in Escherichia coli. Environ Microbiol 15:487–501

Who? linking ecotype to phenotype







ECOR

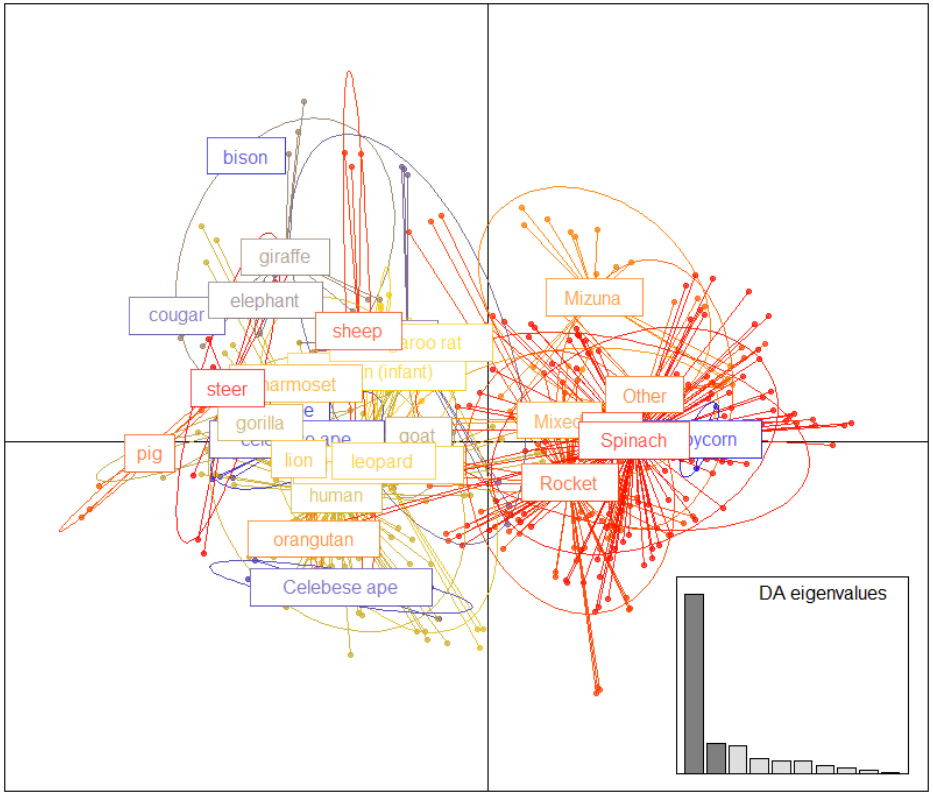
GMB

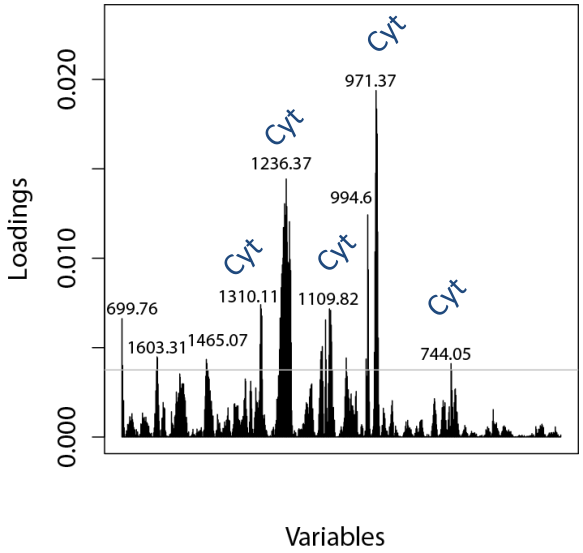
Discriminant Analysis of Principal Components (DAPC)

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Who? linking ecotype to phenotype

Discriminant Analysis of Principal Components (DAPC)





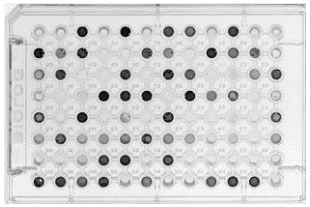
Cytochromes

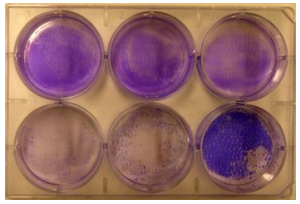
- Involved with electron transport, oxidative phosphorylation, assimilatory metabolism and detoxification
- Often located in the cytoplasmic membrane
- Some responsible for protection from UV exposure

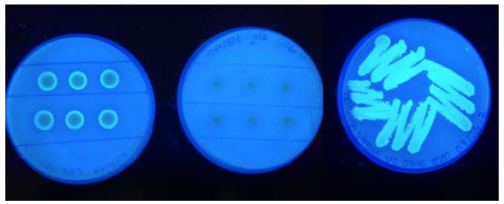
Who? linking ecotype to phenotype

Further *Escherichia* work:

1. Examination of the Raman phenotype with respect to other phenotypic traits:
   - C-source utilisation, biofilm formation, colony morphotypes (expression of curli fimbriae and cellulose)



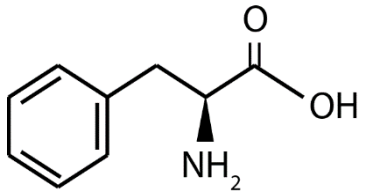




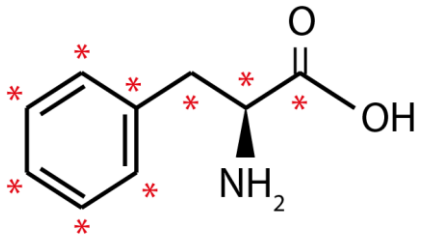
Phenotyping Summary:

1. Broad screening method
   - Measures multiple biomolecules simultaneously
2. Provides targets for further phenotypic/genomic investigation
3. Correlate phenotype to other aspects of microbe ecology? Pathogenicity, toxin production, persistence etc.

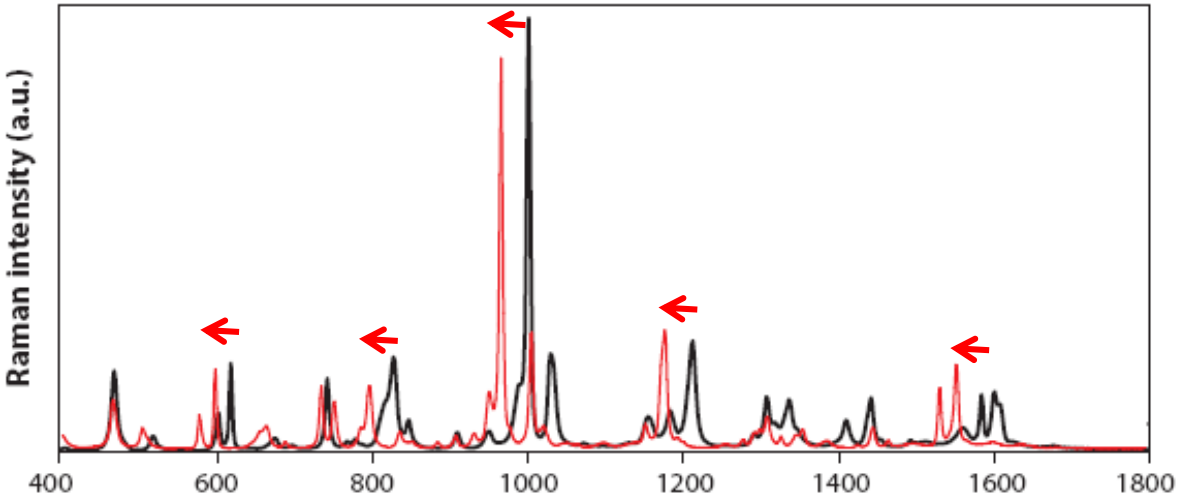
How? Raman Stable Isotope Probing



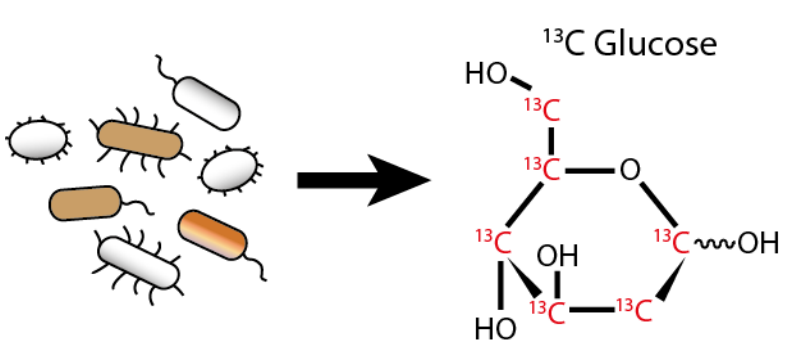
Common ‘light’ isotope
e.g. ^{12}C Carbon phenylalanine

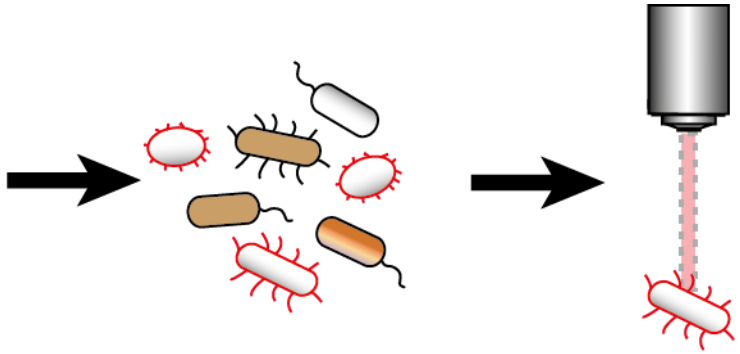


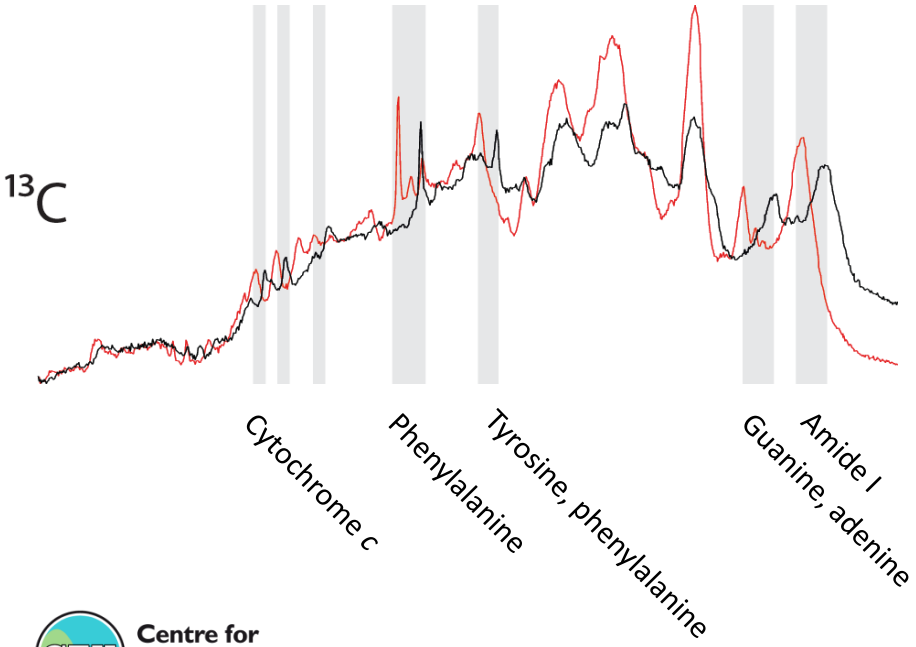
‘Heavy’ isotope
e.g. ^{13}C Carbon phenylalanine

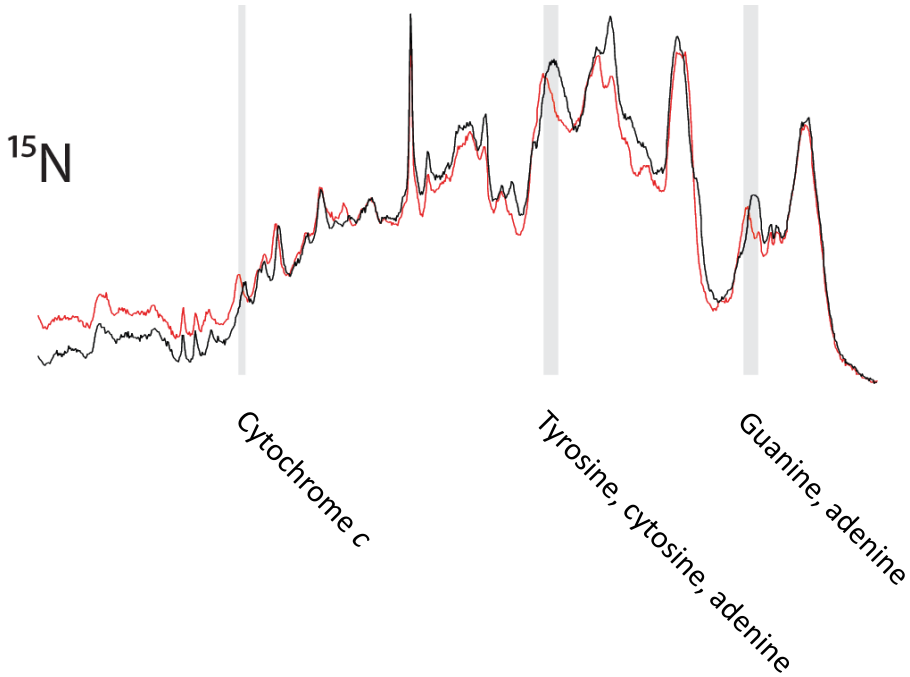


How? Raman Stable Isotope Probing



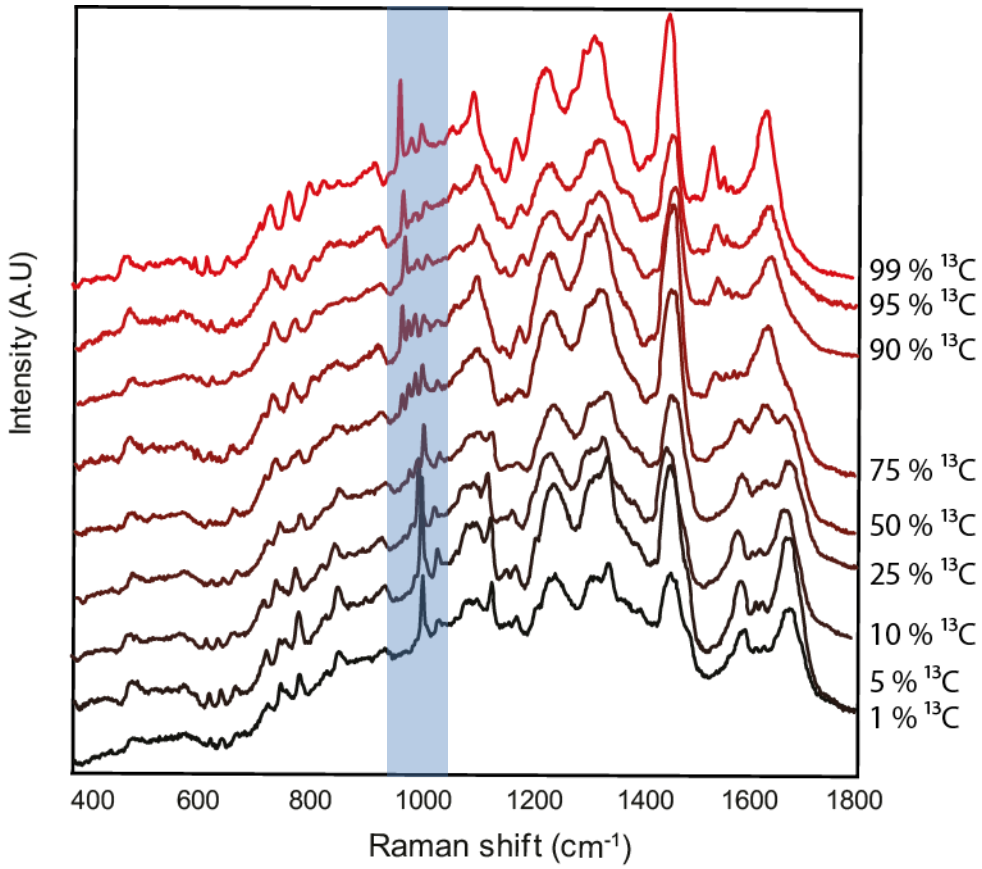


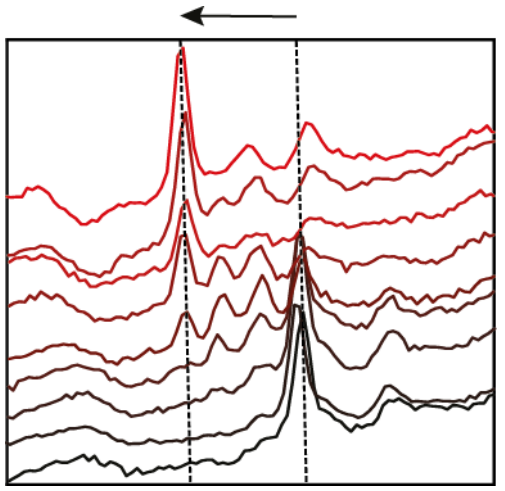


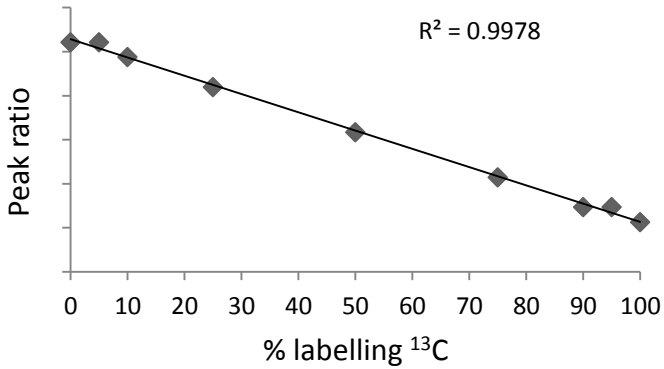


Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

How? Raman Stable Isotope Probing



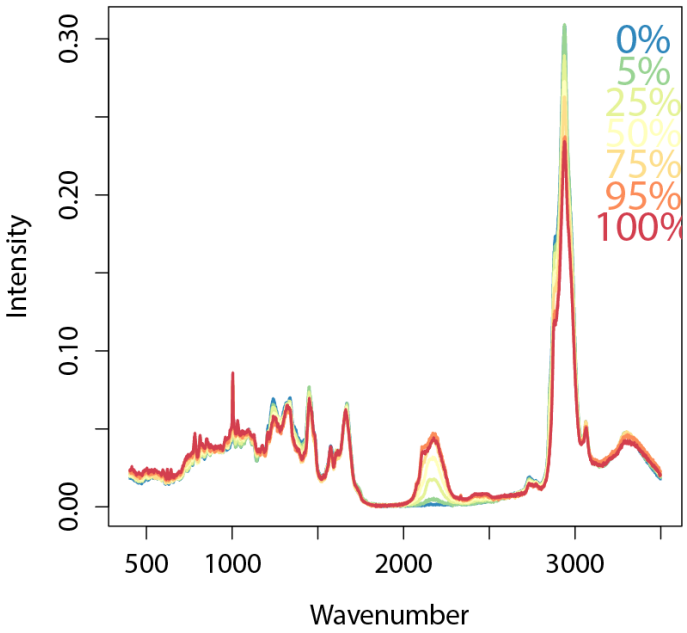




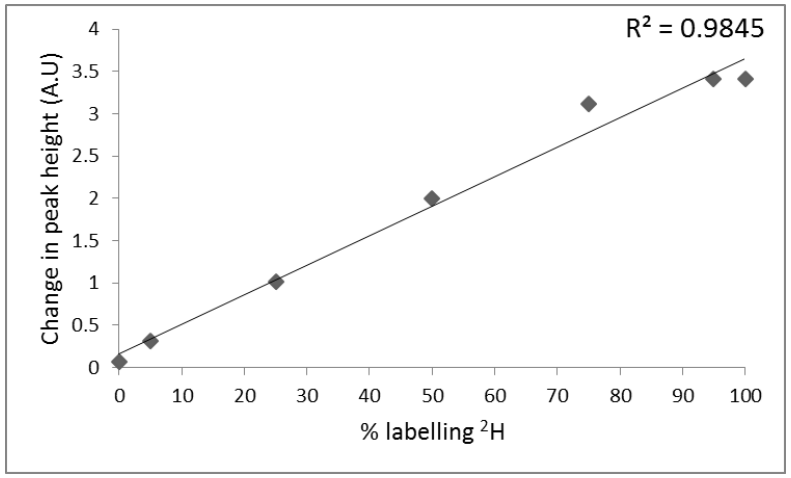
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

How? Raman Stable Isotope Probing

Deuterium oxide (^2H_2O) labelling



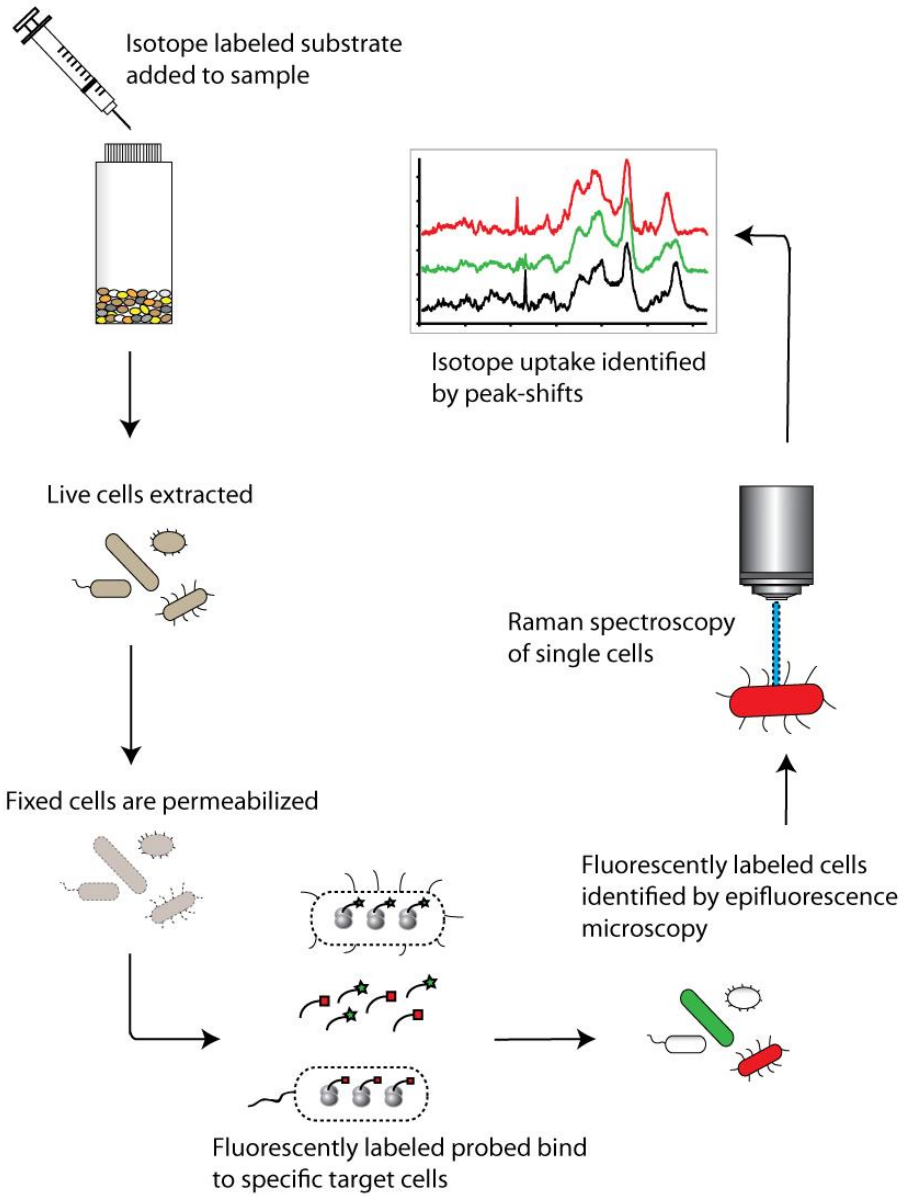
- C–D bond stretching in lipids
- Found in an empty region of the spectrum



- ^2H_2O can be used as a general marker of bacterial growth
- Cheaper than ^{13}C or ^{15}N labelled compounds

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

How? Raman Stable Isotope Probing



Environmental Microbiology (2007) 9(8), 1878–1889 doi:10.1111/j.1462-2920.2007.01352.x

Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence *in situ* hybridization for the single cell analysis of identity and function

Wei E. Huang,[1†] Kilian Stoecker,[2†] Robert Griffiths,[1] Lyndsay Newbold,[1] Holger Daims,[3] Andrew S. Whiteley[1*] and Michael Wagner[2]
[1]*Biodiversity and Ecosystem Function Group, Molecular Microbial Ecology Section, Centre for Ecology and Hydrology Oxford, Mansfield Road, Oxford, OX1 3SR, UK.*

complements current technologies such as FISH-microautoradiography and stable isotope probing in that it can be applied at the resolution of single cells in complex communities, is quantitative if suitable calibrations are performed, can be used with stable isotopes and has analysis times of typically 1 min per cell.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2009, p. 234–241 Vol. 75, No. 1
0099-2240/09/$08.00+0 doi:10.1128/AEM.01861-08

Resolving Genetic Functions within Microbial Populations: In Situ Analyses Using rRNA and mRNA Stable Isotope Probing Coupled with Single-Cell Raman-Fluorescence In Situ Hybridization[▽†]

Wei E. Huang,[1‡*] Andrew Ferguson,[2,3] Andrew C. Singer,[1] Kathryn Lawson,[3,4] Ian P. Thompson,[1] Robert M. Kalin,[5] Michael J. Larkin,[3,4] Mark J. Bailey,[1] and Andrew S. Whiteley[1*]
Centre for Ecology & Hydrology—Oxford, Mansfield Road, Oxford OX1 3SR, United Kingdom[1]; Environmental Engineering Research Centre,[2] QUESTOR Centre,[3] and School of Biological Sciences,[4] The Queen's University of Belfast, Belfast BT7 1NN, United Kingdom; and Department of Civil Engineering, Strathclyde University, 50 Richmond Street, Glasgow G1 1XN, Scotland[5]

Received 11 August 2008/Accepted 30 October 2008

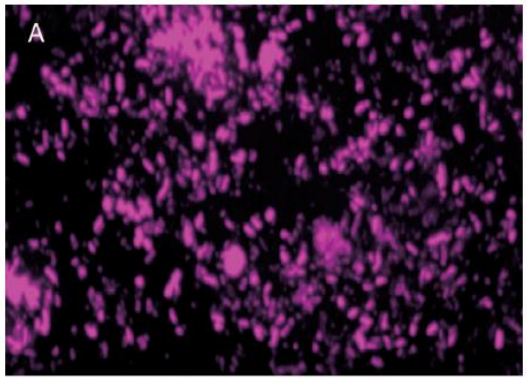
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

How? Raman Stable Isotope Probing

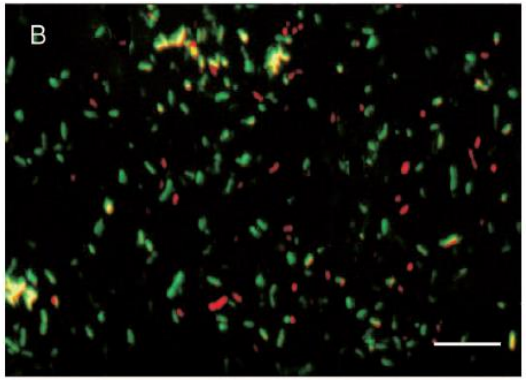
SEquenced REactive BARrier (SEREBAR)

- Former manufactured gas plant (FMGP) in the United Kingdom
- Groundwater polluted with Polycyclic Aromatic Hydrocarbons (PAHs) including Naphthalene

- RNA Stable Isotope Probing using ^{13}C labelled Naphthalene in microcosms
- Identified *Pseudomonas* sp. and *Acidovorax* sp. as main naphthalene degraders
- *Acidovorax* sp. unculturable
- Raman-FISH approach

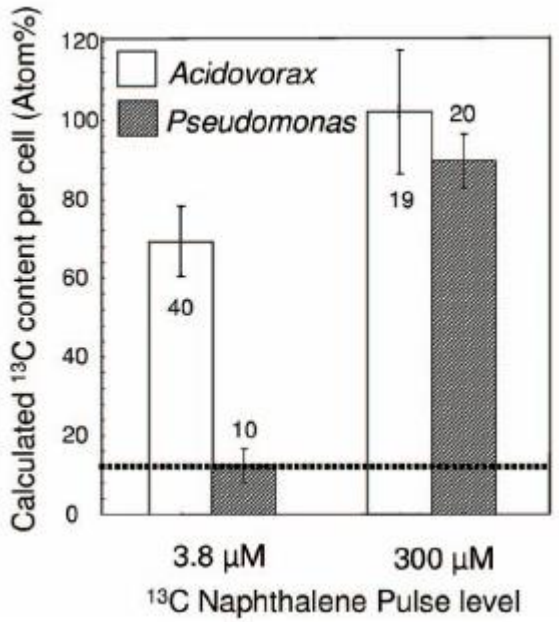


EUB338 (general bacteria)



Acidovorax sp. **Red**

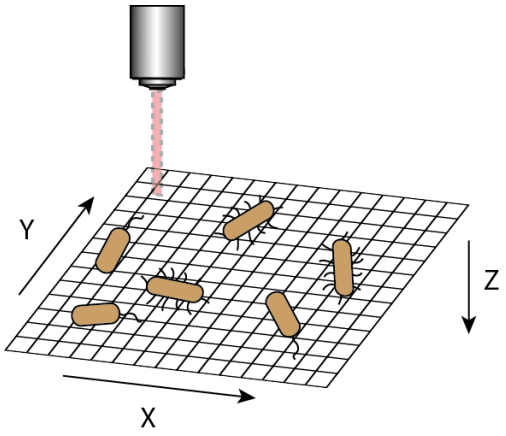
Pseudomonas sp. **Green**

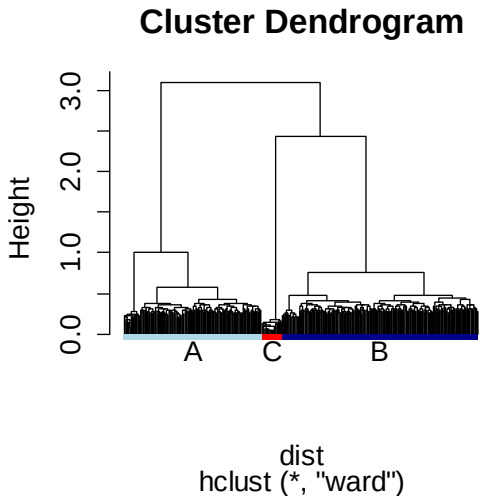


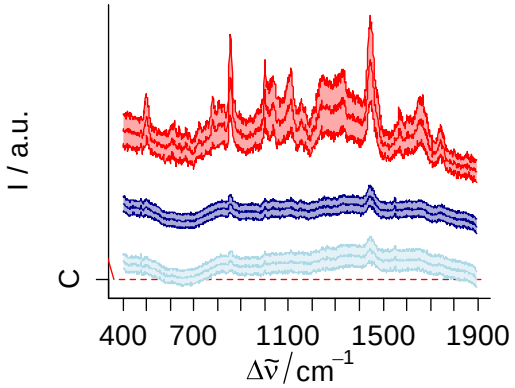
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Huang, W. E., Stoecker, K., Griffiths, R., Newbold, L., Daims, H., Whiteley, A. S., & Wagner, M. (2007). Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence in situ hybridization for the single cell analysis of identity and function. *Environmental Microbiology*, *9*(8), 1878-1889

Where? Raman mapping

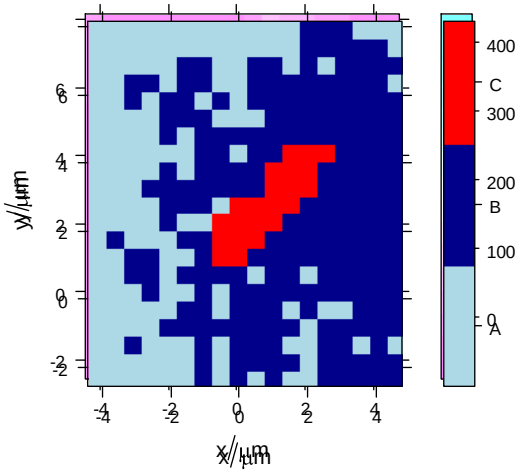






Single *Escherichia coli* cell

- Mapped over 18 x 21 spectra grid
- 0.5µm between each point
- 378 spectra



Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Where? Raman mapping

Bacillus thuringiensis

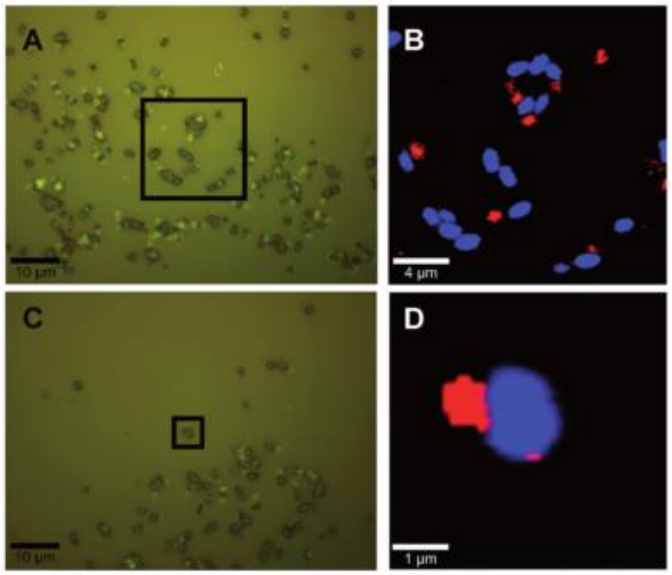
- Gram-positive, spore-forming
- spores containCa^{2+} dipicolinic acid (Ca-DPA) and Bt toxin
- **Red** Bt toxin, **Blue**, CA-DPA

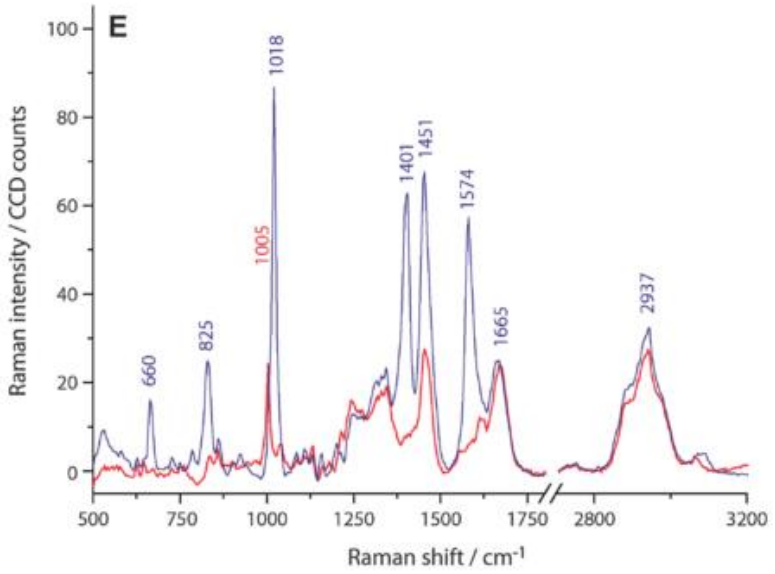
PAPER www.rsc.org/analyst | Analyst

Phenotypic heterogeneity within microbial populations at the single-cell level investigated by confocal Raman microspectroscopy†

Antje Hermelink, Angelika Brauer, Peter Lasch and Dieter Naumann*

Received 16th December 2008, Accepted 12th March 2009
First published as an Advance Article on the web 25th March 2009
DOI: 10.1039/b822574e





Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

1. Hermelink A, Brauer A, Lasch P, Naumann D (2009) Phenotypic heterogeneity within microbial populations at the single-cell level investigated by confocal Raman microspectroscopy. Analyst 134:1149–53. doi: 10.1039/b822574e

Where? Raman mapping

Legionella bozemanii

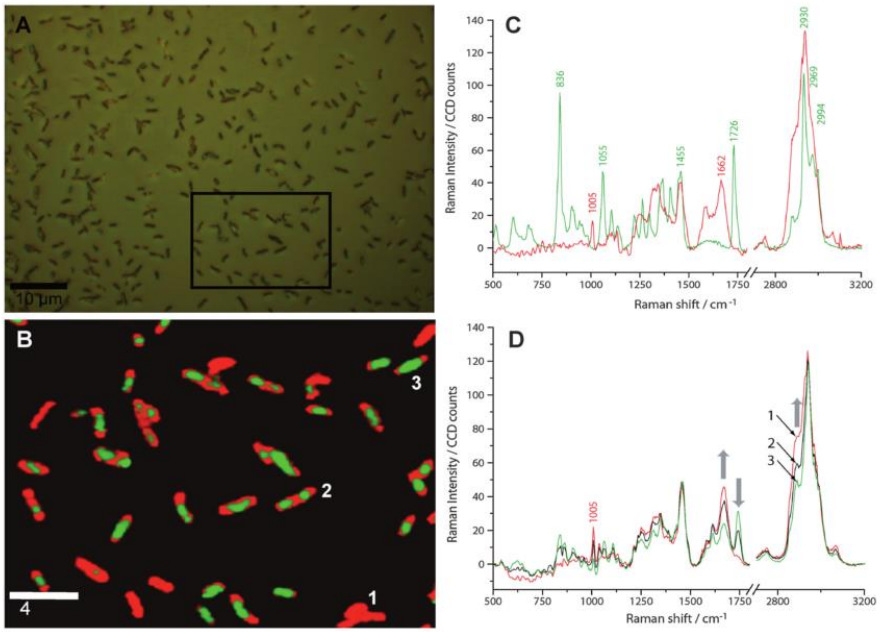
- Poly-b-hydroxybutyric acid (PHB) produced in response to response to physiological stress
- Energy storage molecule

PAPER www.rsc.org/analyst | Analyst

Phenotypic heterogeneity within microbial populations at the single-cell level investigated by confocal Raman microspectroscopy†

Antje Hermelink, Angelika Brauer, Peter Lasch and Dieter Naumann*

Received 16th December 2008, Accepted 12th March 2009
First published as an Advance Article on the web 25th March 2009
DOI: 10.1039/b822574e

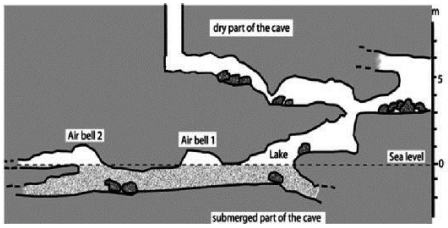


Mapped the intensity of the *v*(C=O) stretching vibration at 1726 cm^{-1} (**green** – PHB) and peptide linkage at 1662 cm^{-1} (**red** – cell)

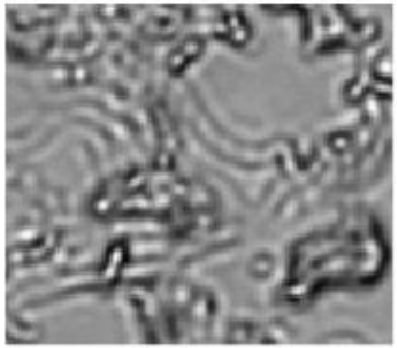
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

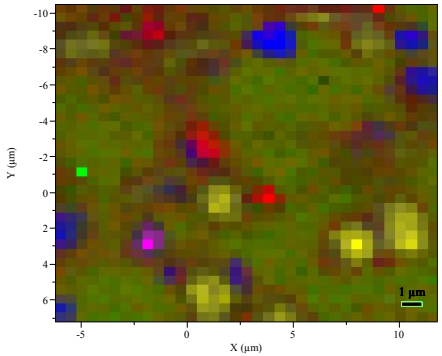
1. Hermelink A, Brauer A, Lasch P, Naumann D (2009) Phenotypic heterogeneity within microbial populations at the single-cell level investigated by confocal Raman microspectroscopy. Analyst 134:1149–53. doi: 10.1039/b822574e

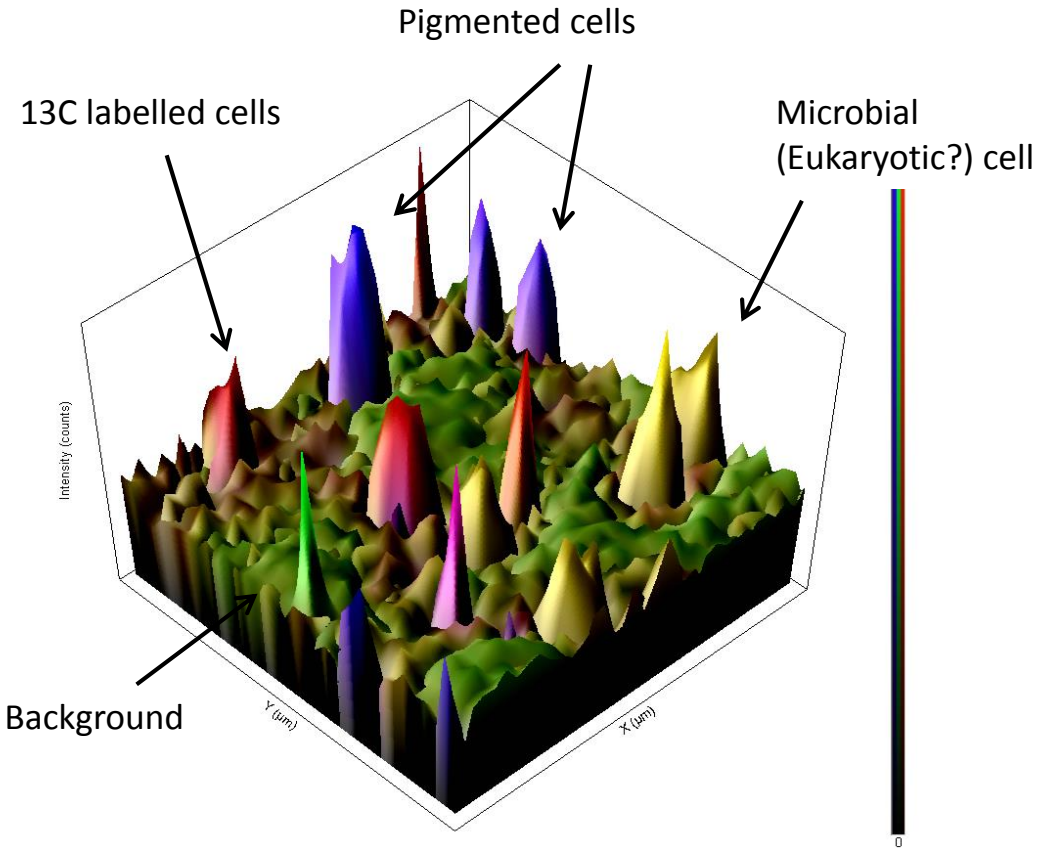
Where? Raman mapping



Movile Cave



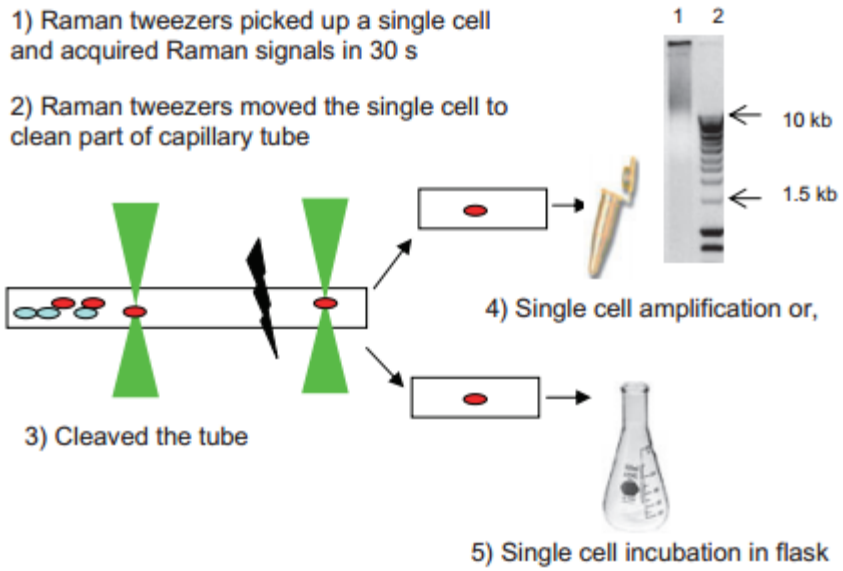




Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Future prospects

Raman Activated Cell Sorting (RACS)



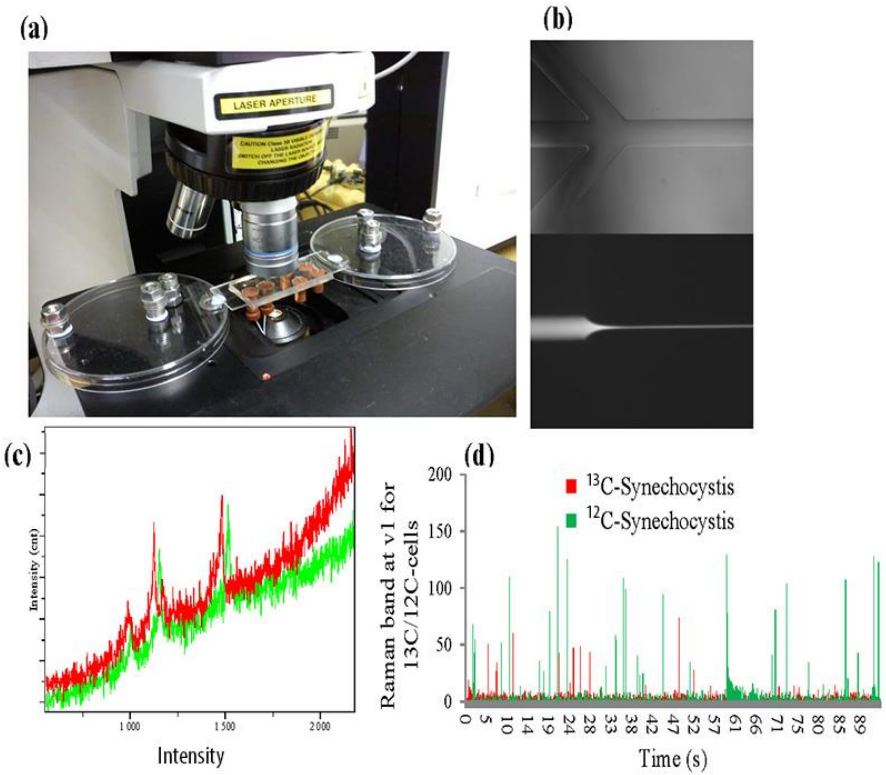
Environmental Microbiology Reports (2009) 1(1), 44–49 doi:10.1111/j.1758-2229.2008.00002.x

Raman tweezers sorting of single microbial cells

Wei E. Huang,[1*†] Andrew D. Ward[2] and Andrew S. Whiteley[1]
[1]*Molecular Microbial Ecology, CEH-Oxford, Mansfield Road, Oxford OX1 3SR, UK.*
[2]*Science and Technology Facilities Council, Rutherford*

Introduction

Cell sorting techniques have revolutionized cell biology research and one of the most powerful tools to separate cells populations is flow cytometry (Davey and Kell,



Probing and sorting single cells—the application of a Raman-activated cell sorter

07 Oct 2013

Yun Wang,[a,c] Yizhi Song,[b] Di Zhu,[a] Yuetong Ji,[c] Tingting Wang,[c] David McIlvenna,[d] Huabing Yin,[d] Jian Xu[c] and Wei E. Huang[a,*]
[a]Kroto Research Institute, The University of Sheffield, Broad Lane, Sheffield, S3 7HQ, UK. E-mail: w.huang@sheffield.ac.uk

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Acknowledgements:

Dr Wei Huang (University of Sheffield)
Prof Andrew Whiteley (CEH Wallingford)
Dr Sam Sheppard (Swansea University)
Dr Dan Woodcock (University of Warwick)
Mr Simon Fitzgerald (Horiba Scientific)
Molecular Microbial Ecology Group (CEH Wallingford)

THANK YOU

Any Questions?...

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL