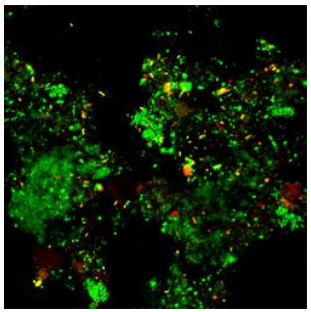
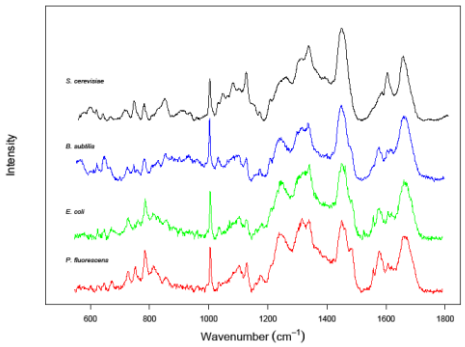
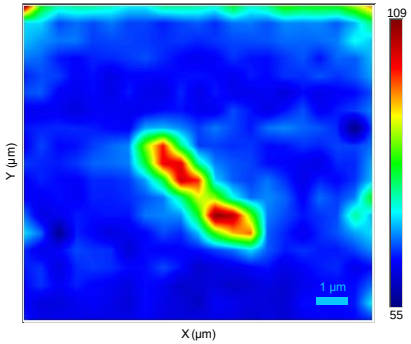
WHO, HOW AND WHERE? MICROBIAL ECOLOGY USING RAMAN SPECTROSCOPY

DR DANIEL READ
CENTRE FOR ECOLOGY & HYDROLOGY, WALLINGFORD







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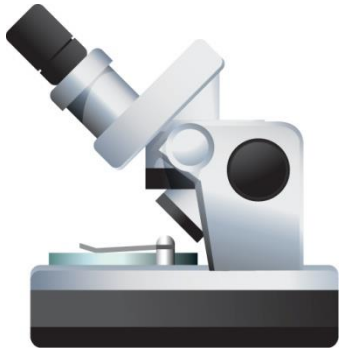
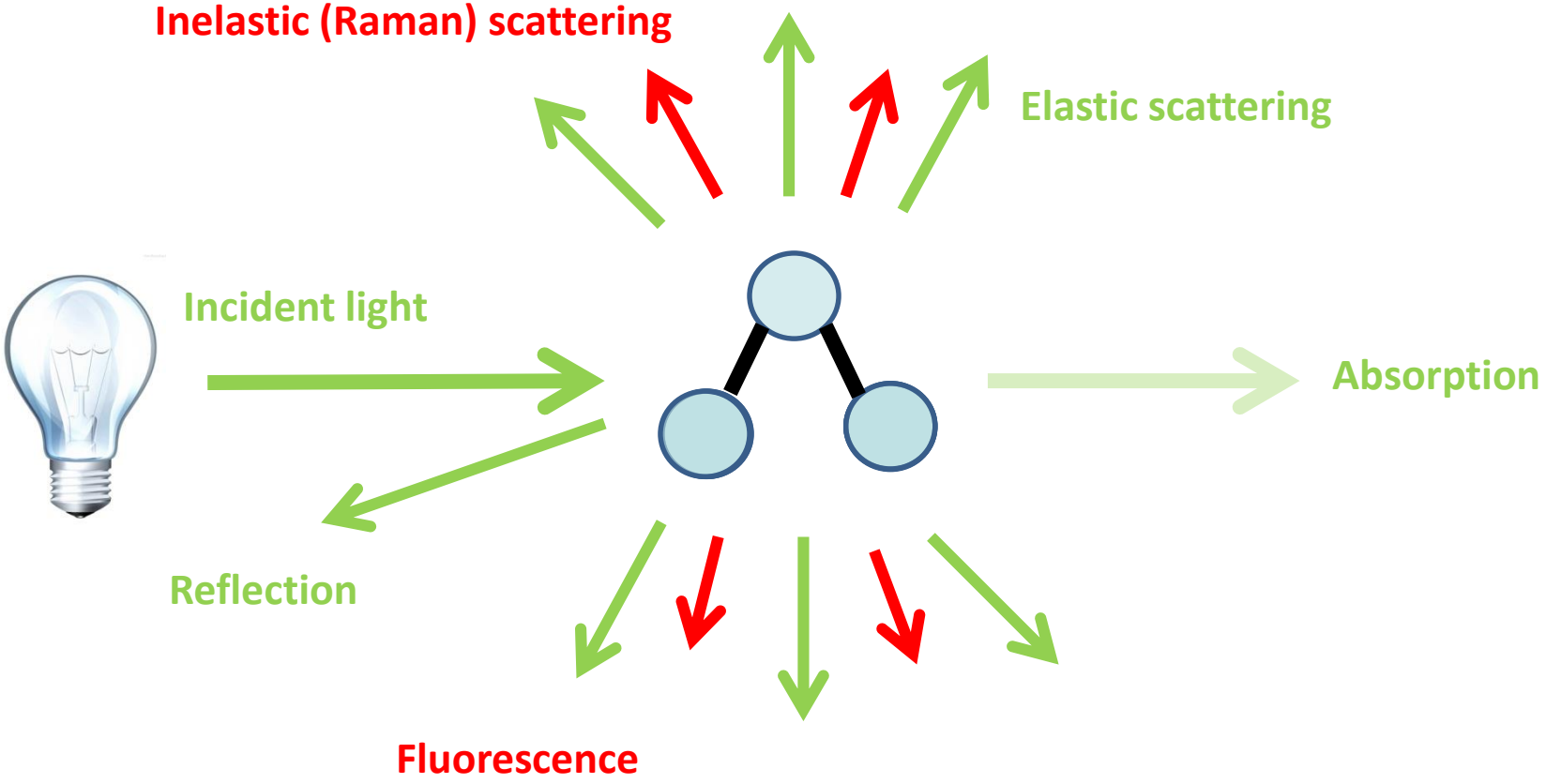
‘‘Progress in science depends on new techniques, new discoveries and new ideas, probably in that order’’. — Sydney Brenner



Outline

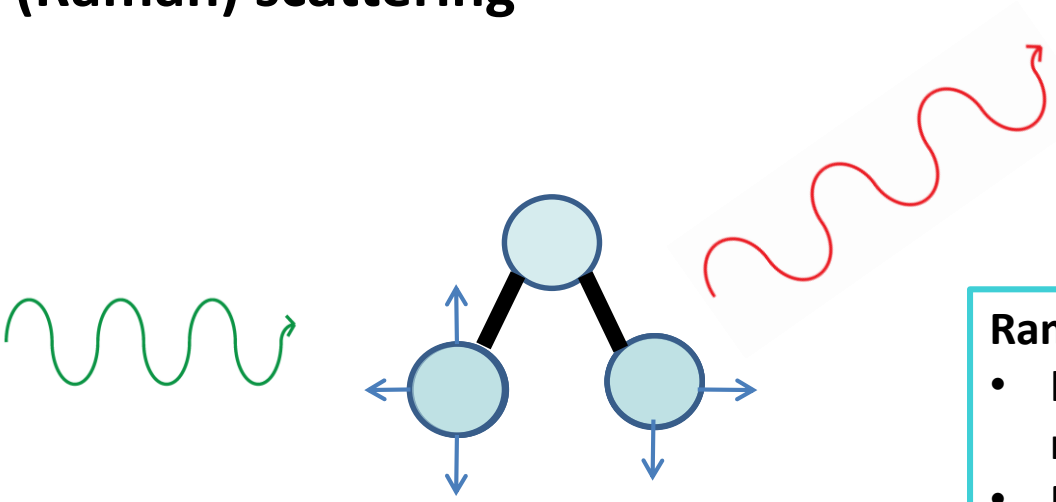
- A short intro to spectroscopy
- Raman spectroscopy
- Microbiological applications of Raman
  - Phenotyping **Who?**
  - Microbial function **How?**
  - Mapping with Raman **Where?**

Spectroscopy



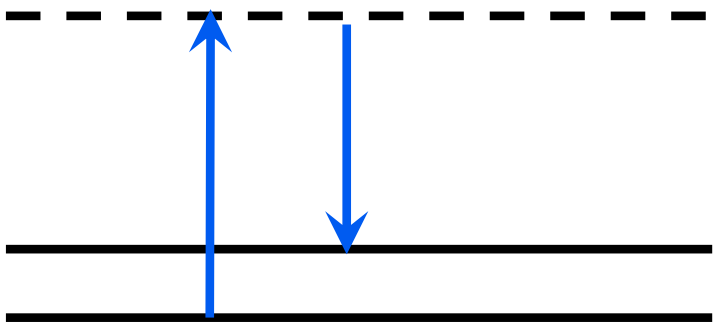
Scattering of light

Inelastic (Raman) scattering



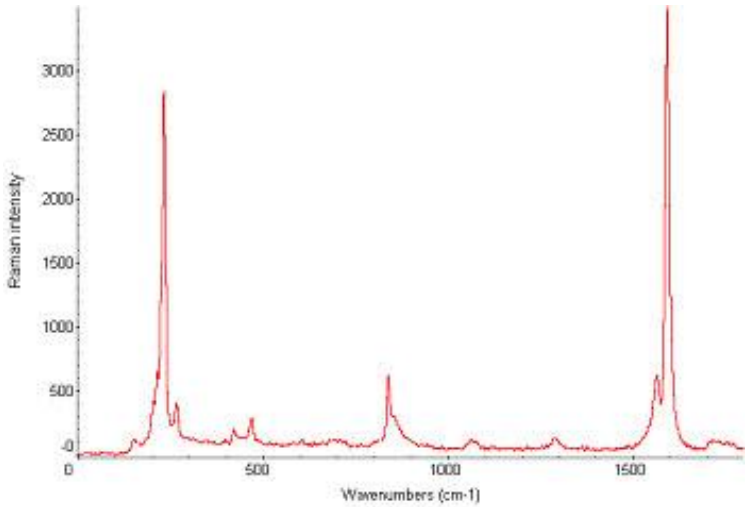
Raman spectroscopy:

- Identification of molecules
- Bonds
- Isotopic composition



Raman data

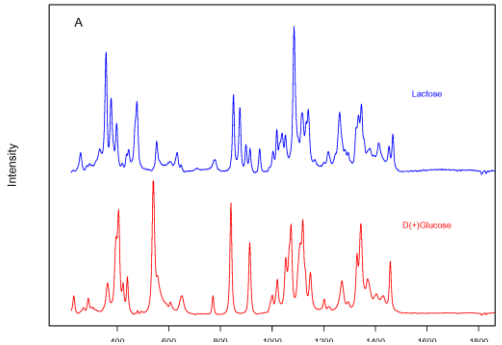
Data is presented relative to the excitation wavelength



Excitation wavelength

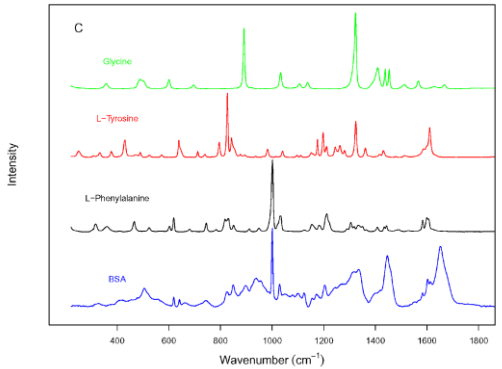
Shifted Raman scattered wavelengths

- Characteristic spectroscopic patterns results in a “fingerprint” for molecules
- Can be used to identify and characterise molecules of elements and compounds



Lactose

Glucose



Glycine

Tyrosine

Phenylalanine

BSA

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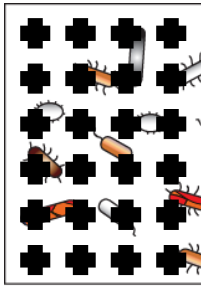
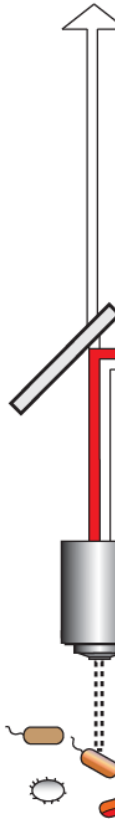
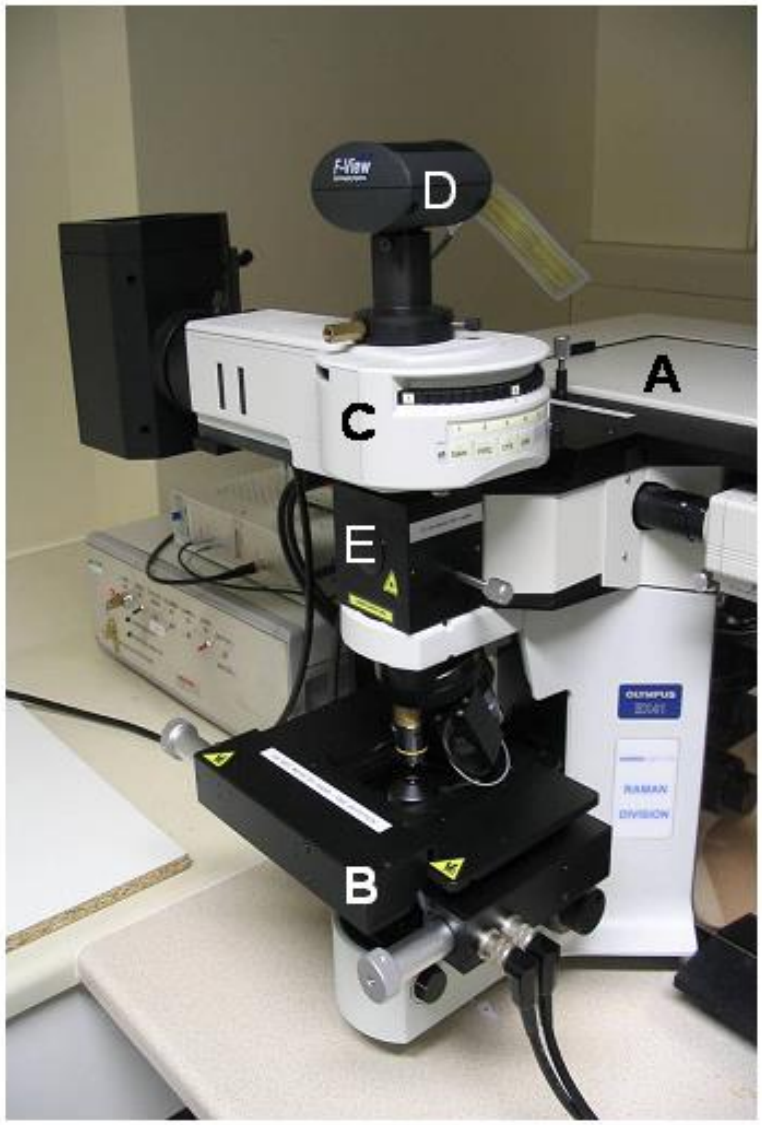
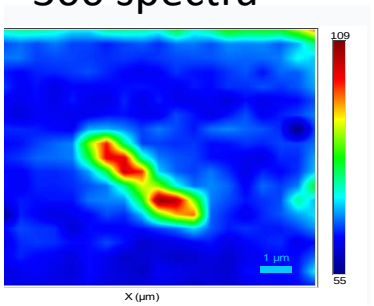
The Raman microspectrometer

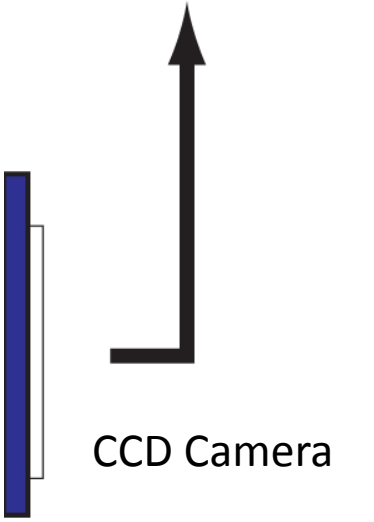






~300 spectra





CCD Camera

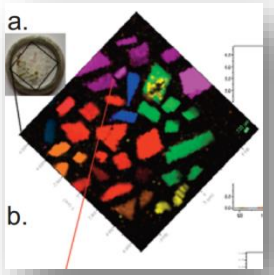
Raman applications:

Applications of Raman

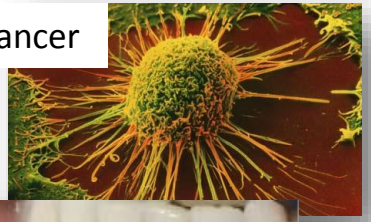
Art

Archaeology

Geology

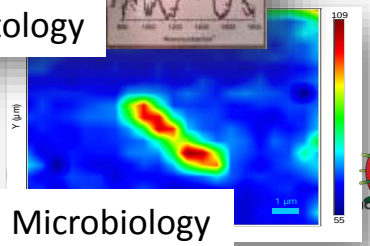


Cancer

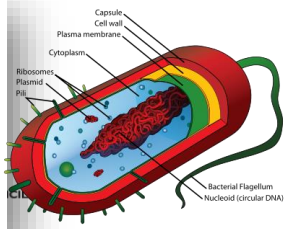


Histology

Biology

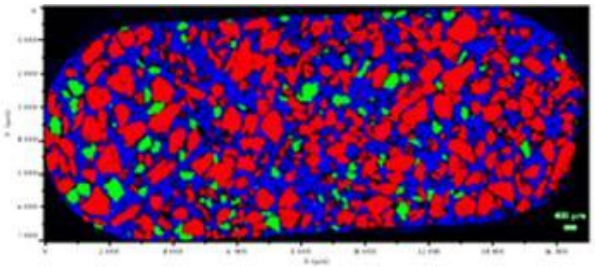


Microbiology

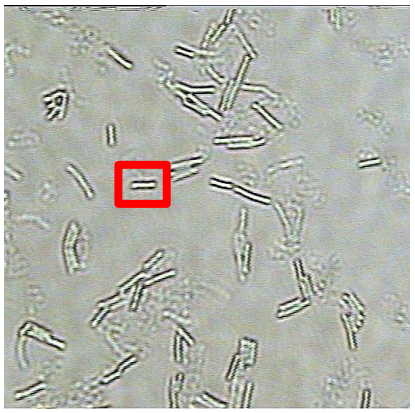


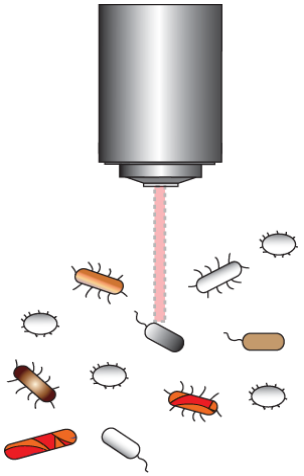
Forensics

Pharmaceuticals

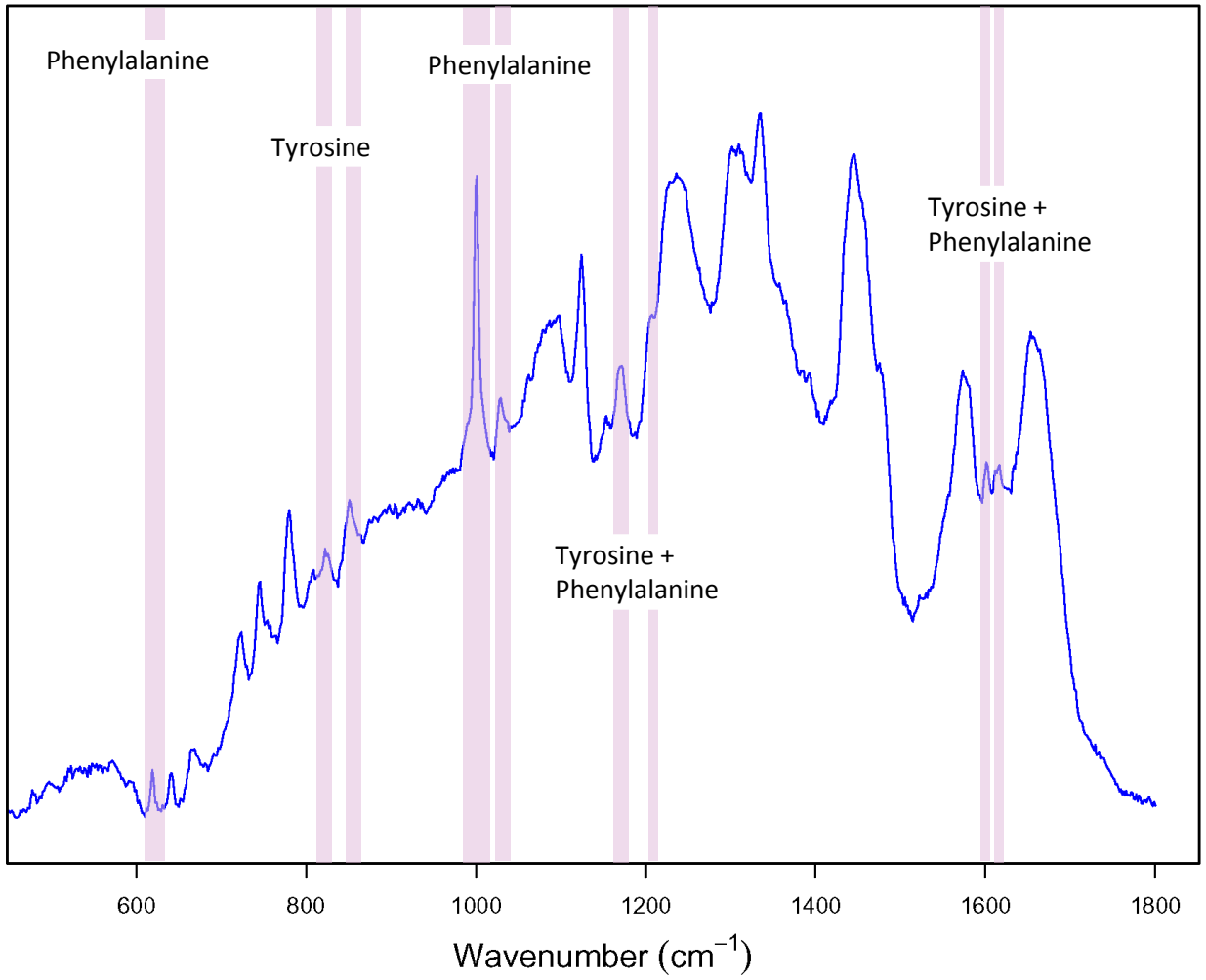


Raman applications: Biological sciences





Amino acids



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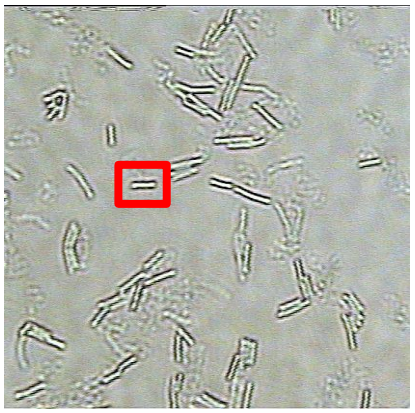
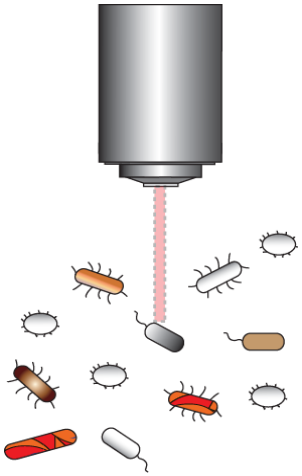
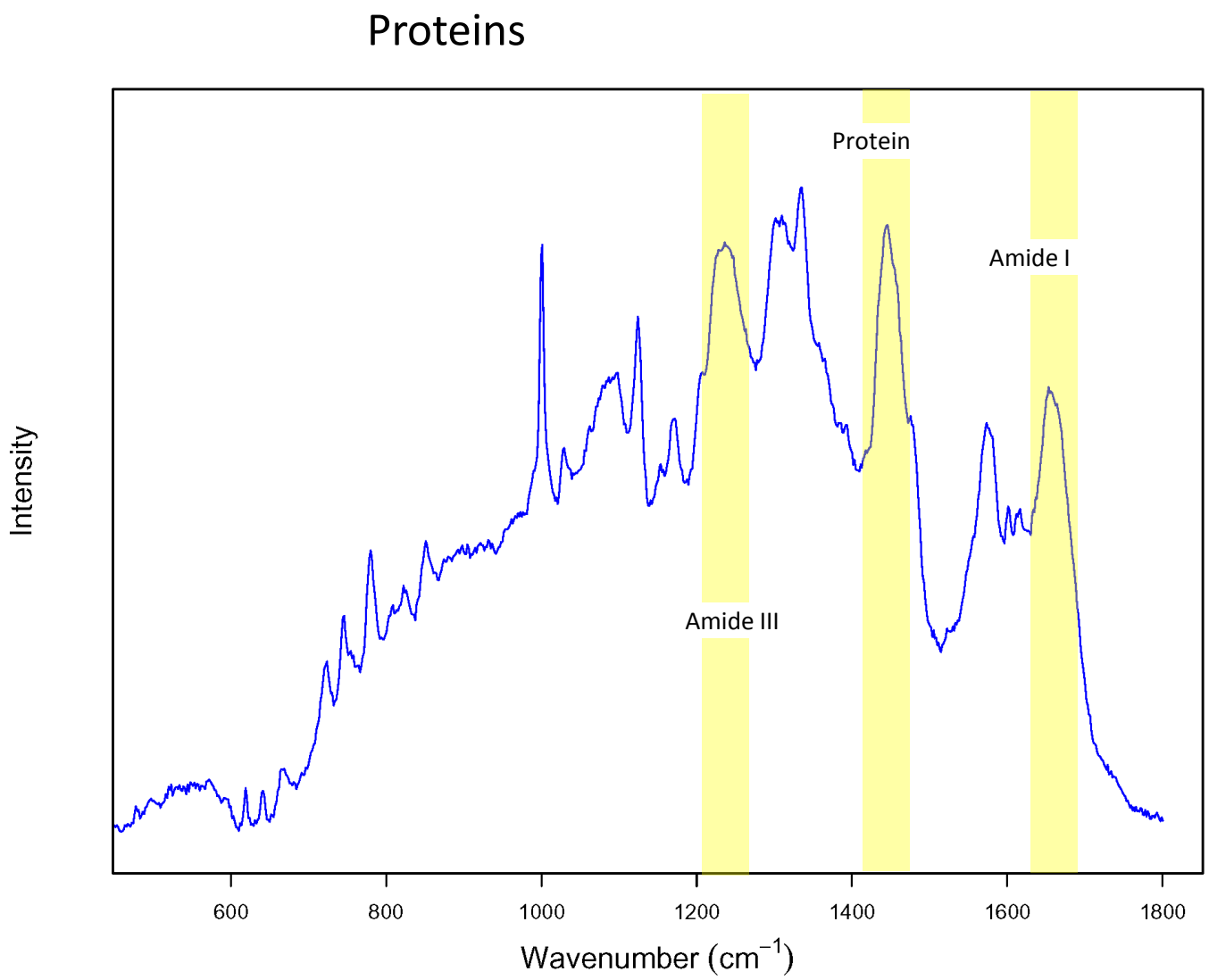
Raman applications: Biological sciences







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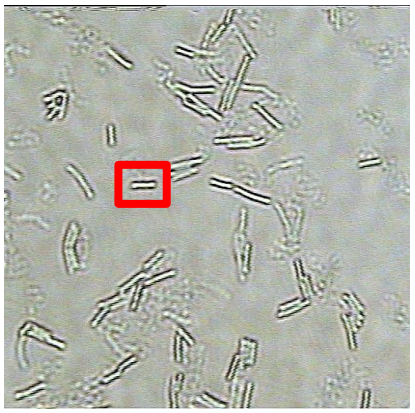
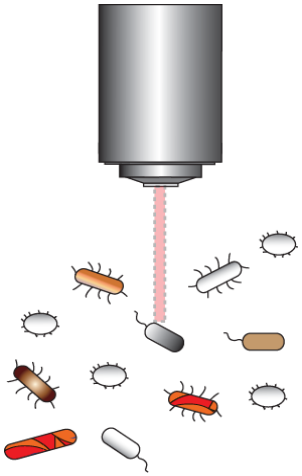
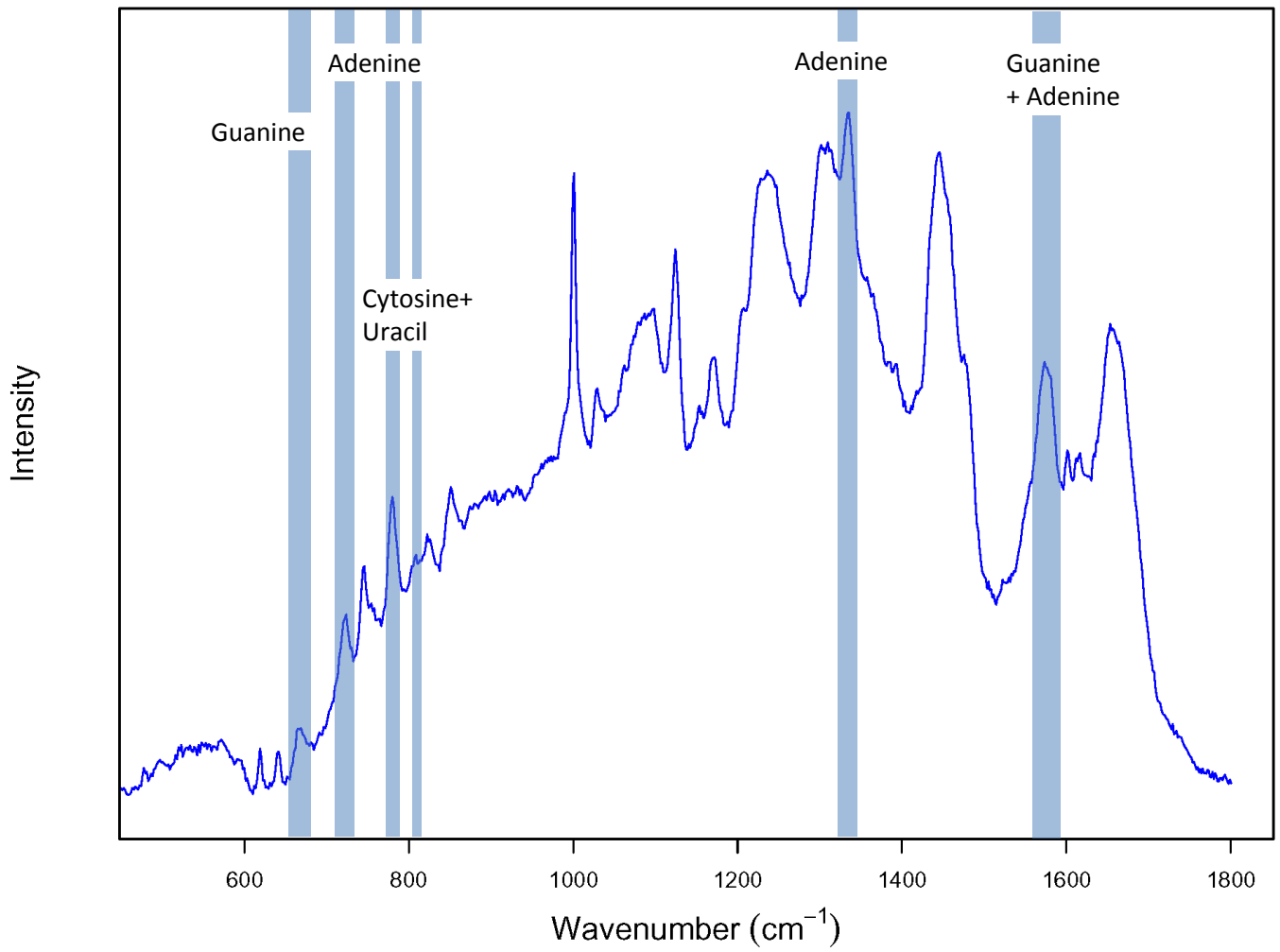
Raman applications: Biological sciences





Nucleic acids



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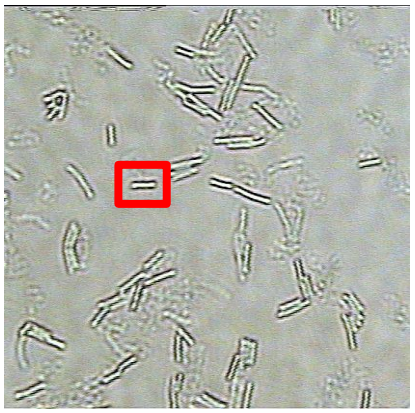
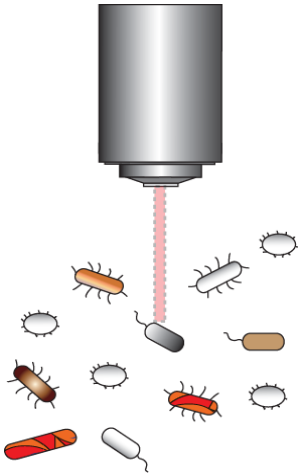
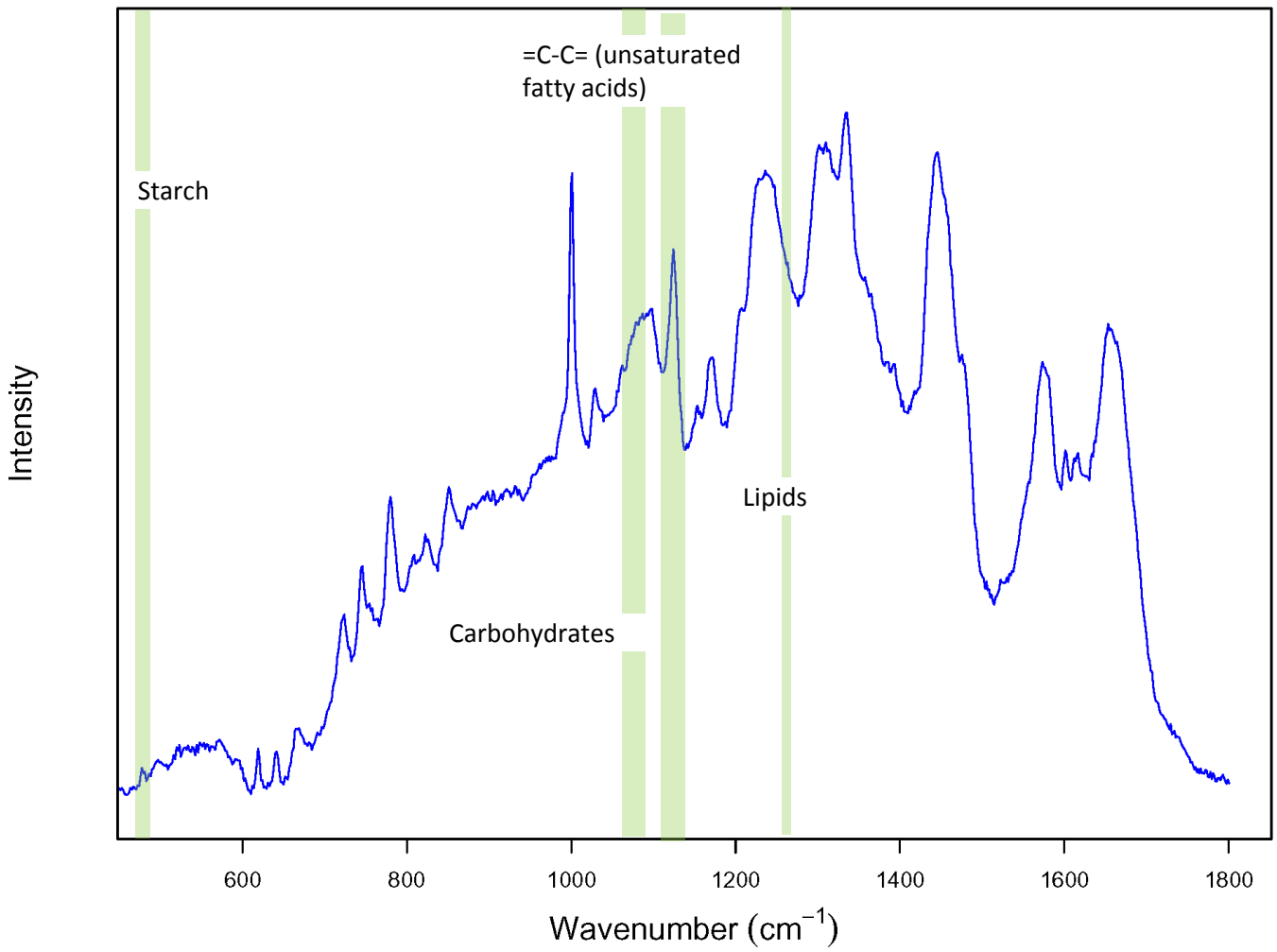
Raman applications: Biological sciences





Lipids and carbohydrates



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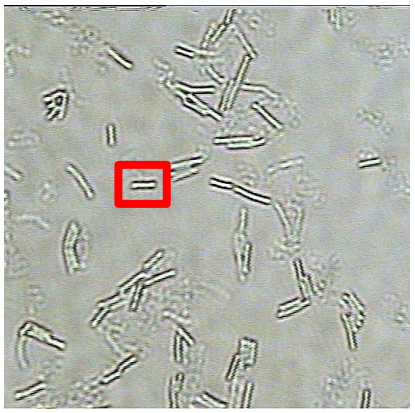
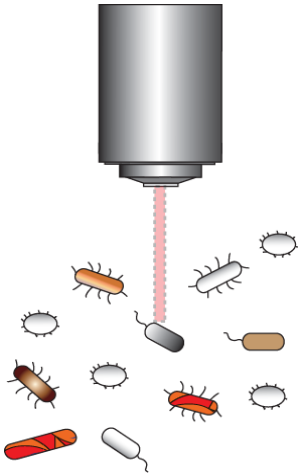
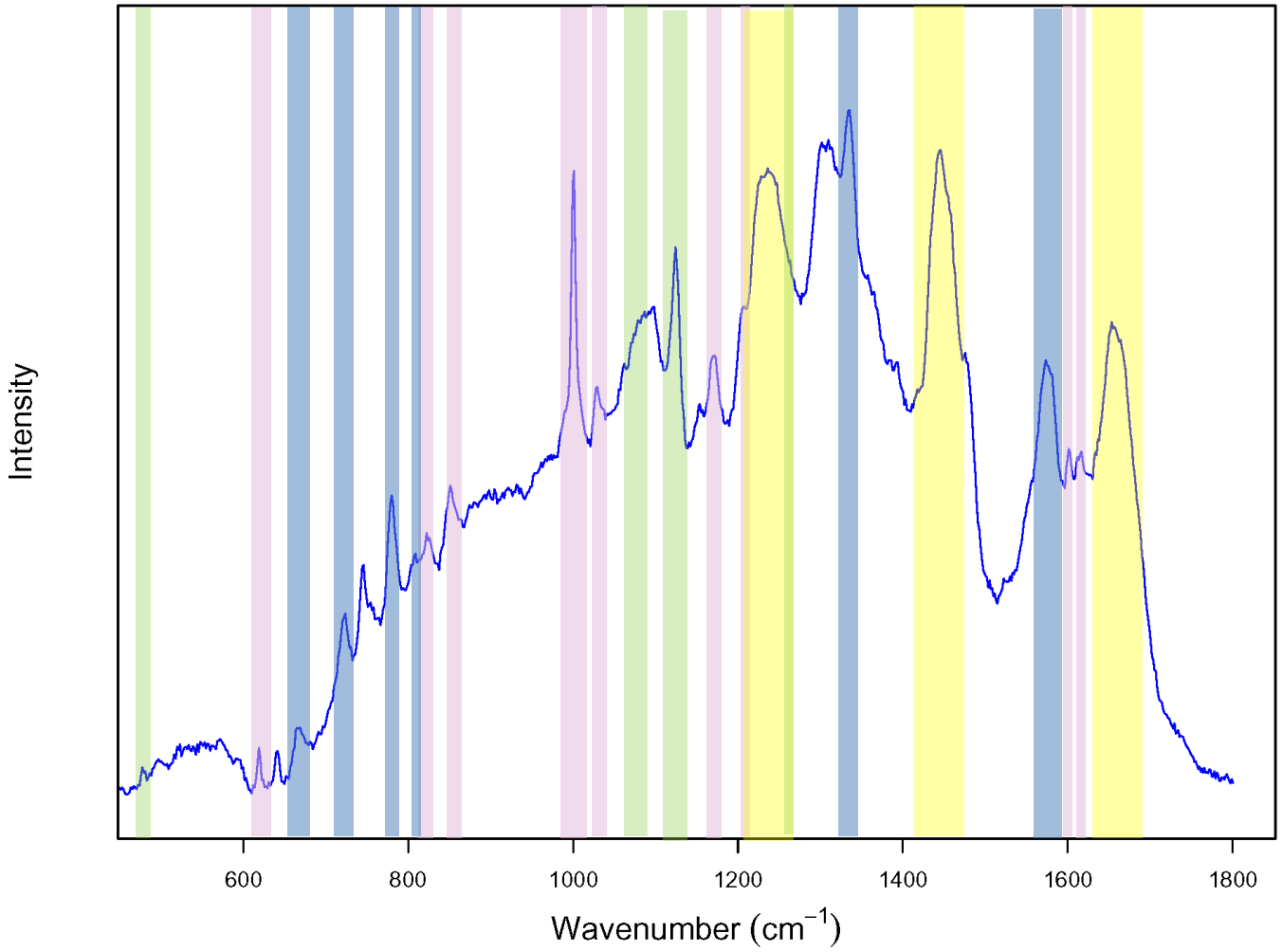
Raman applications: Biological sciences





Fingerprint or Phenotype



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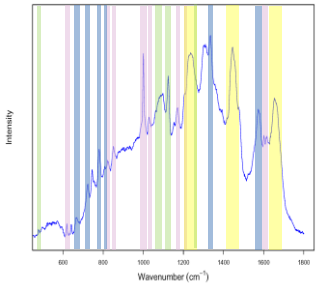
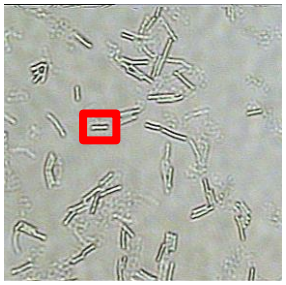
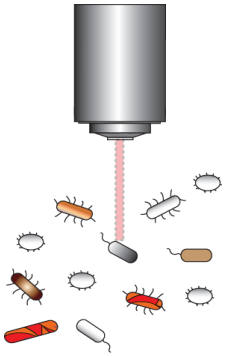
Raman applications: Biological sciences







Raman phenotyping or fingerprinting

- Fast (~30s per sample)
- No sample prep
- Cheap to run (after initial outlay)
- Quantitative (peak height)
- Diverse range of data in single assay
- Non-contact and non-destructive
- Single-cell analysis (sampling down to 1 µm dia)
    - microbiology at the level of the individual

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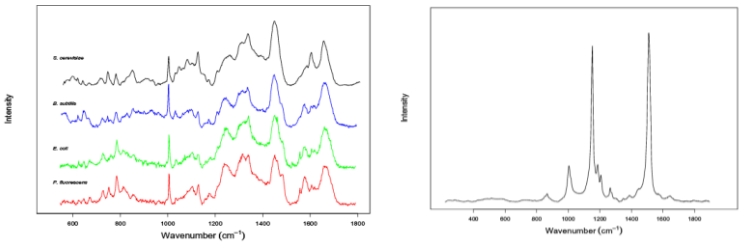
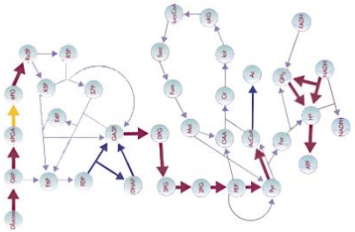
Raman applications: Microbial Ecology

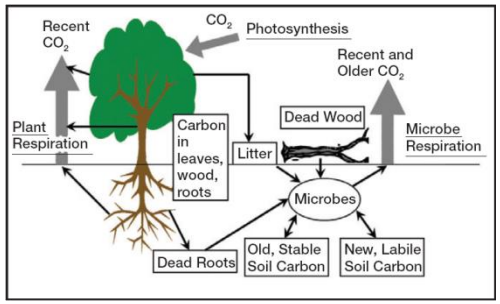
Phenotype **WHO?**

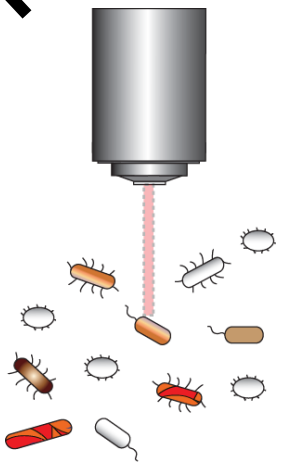


Metabolic history **HOW?**

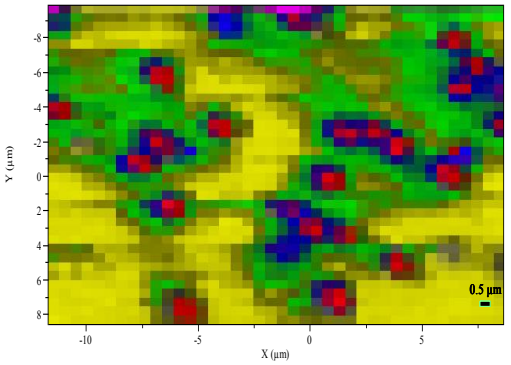


Function **HOW?**





Spatial distribution **WHERE?**



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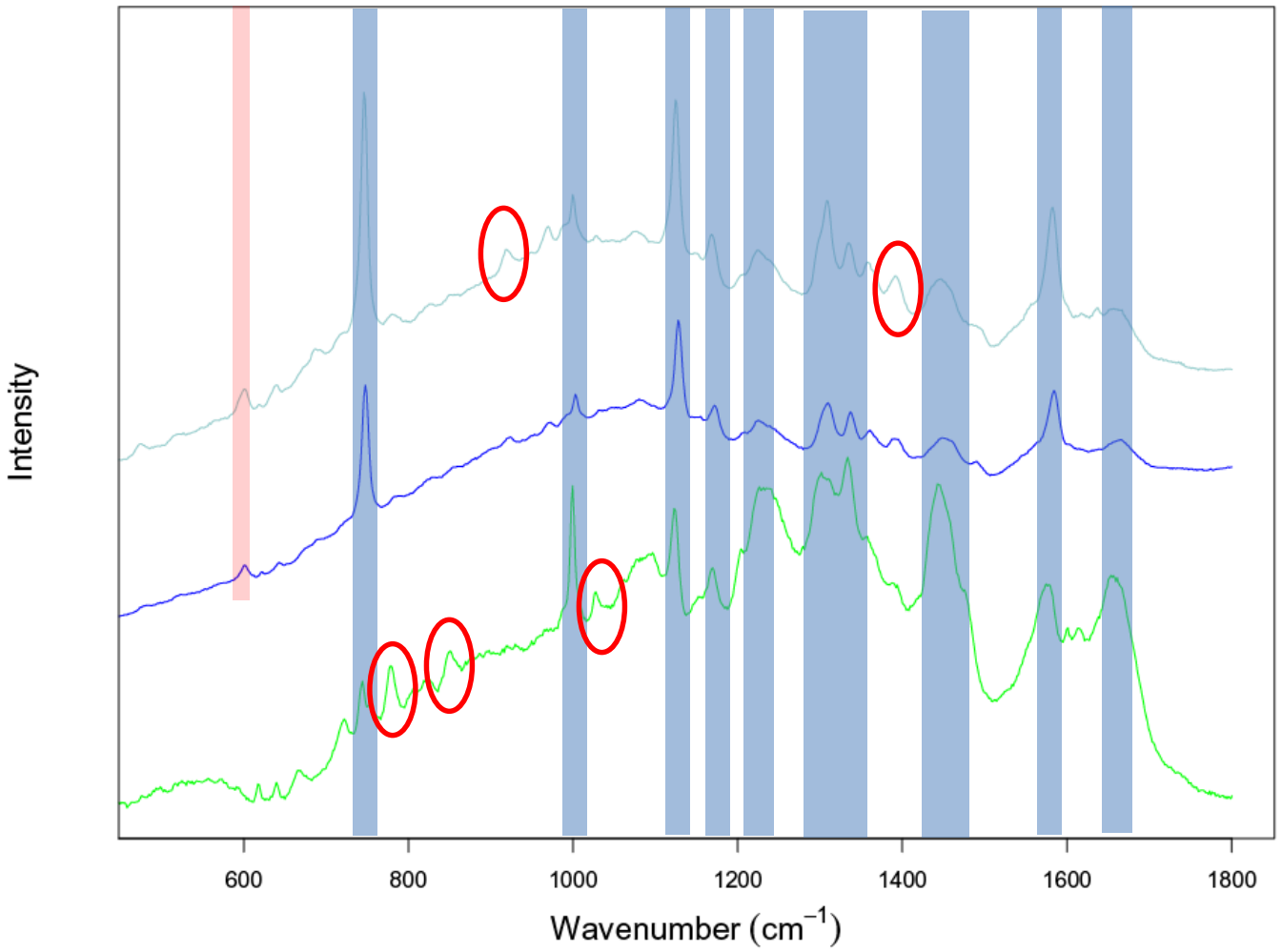
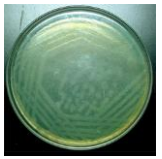
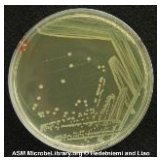
Who?: Raman Phenotyping



Pseudomonas fluorescens



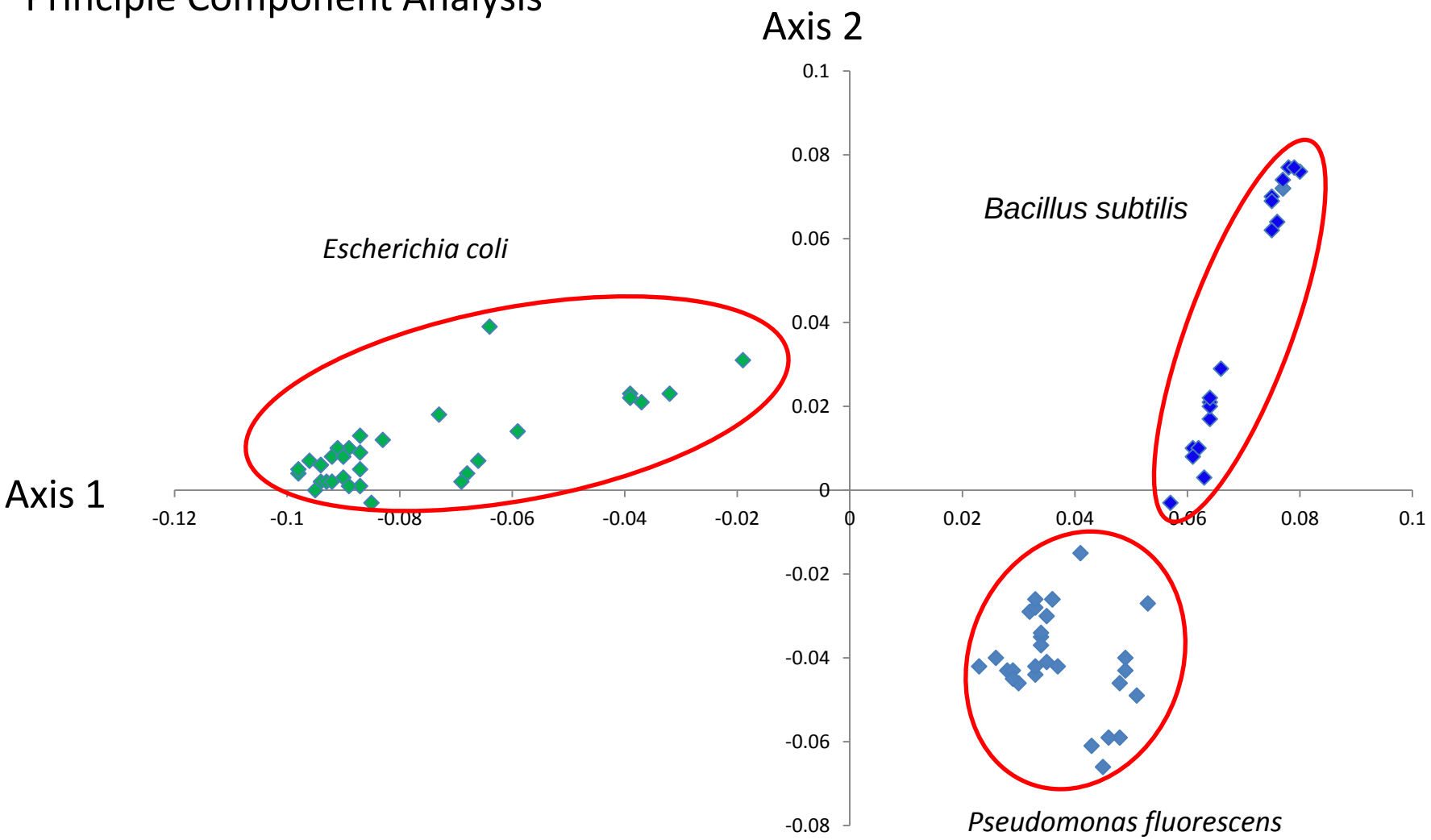
Bacillus subtilis



Escherichia coli

Who?: Raman Phenotyping

Principle Component Analysis



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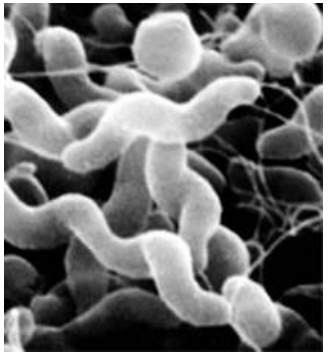
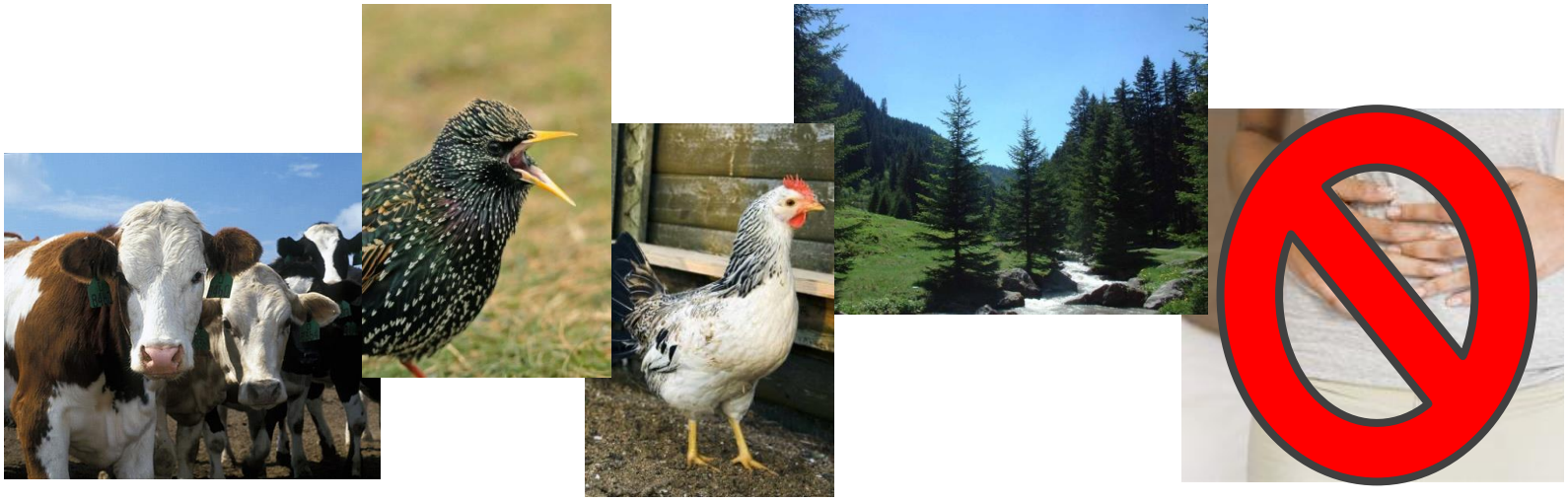
Who?: Pathogenic bacteria: linking genotype with phenotype

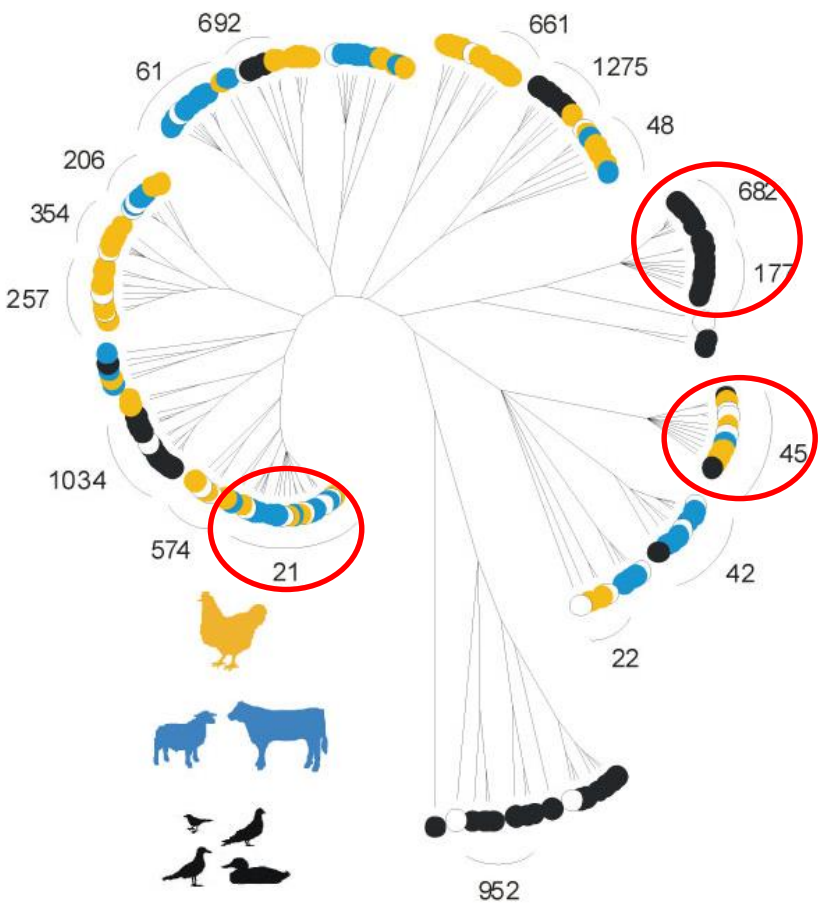


- *Campylobacter jejuni* and *C. coli* – campylobacteriosis
- Accounts for estimated **2.5 million cases** (US) and **1,340,000** (UK) each year
- x13 the number of cases caused by Salmonella, *E. coli*, and *Listeria* combined
- Estimated annual economic burden is £500 million in the UK
- Ability to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



Who?: Pathogenic bacteria: linking genotype with 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

<u>We wished to test whether:</u>

1. Campylobacter phenotype is related to genotype (Species, clonal complex and clade)

2. Phenotype is related to species of host organism it was isolated from, ***independent of MLST (genetic) classification***

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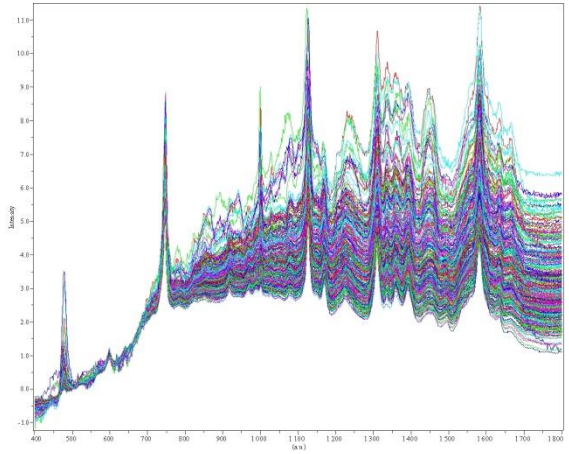
Sheppard, S.K., Maiden, M.C.J., Falush, D. (2009). Population Genetics of *Campylobacter*. In: Bacterial Population Genetics in Infectious Disease. Eds. Robinson, A.D., Falush, D., Feil, E.J. John Wiley & Sons, New Jersey. Pages 181-194.

Who?: Pathogenic bacteria: linking genotype with phenotype

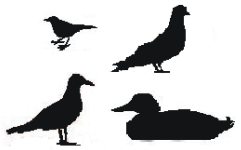
- Strains used in this study
  - 108 Strains of *Campylobacter* (cultured and analysed in triplicate)
  - 2 species - *C. jejuni* (85) and *C. coli* (23)
  - 66 Multilocus Sequence Types
  - 1,620 Raman spectra



27 Cattle faeces

5 Chicken gut
24 Chicken faeces
15 Chicken meat

23 Clinical



11 Wild bird

3 Pig

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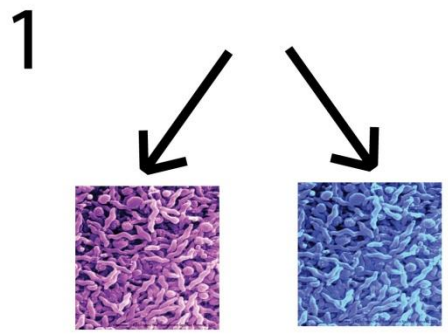
Who?: Pathogenic bacteria: linking genotype with phenotype

Neural network analysis – supervised statistical classification

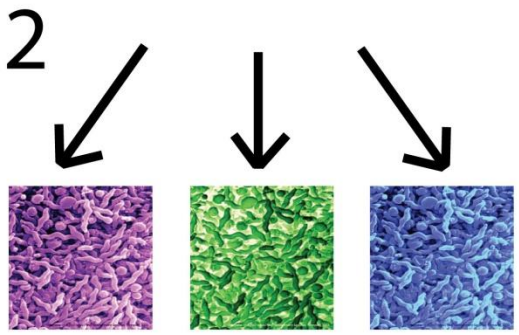
(Dr Dan Woodcock - Systems Biology Centre, University of Warwick)

- Identifies informative peaks and nonlinear relationships between the wavelengths
- Distinguish which inputs provide the most information in separating the classes
- Discards least informative peak until peak set with most discriminatory powers is left

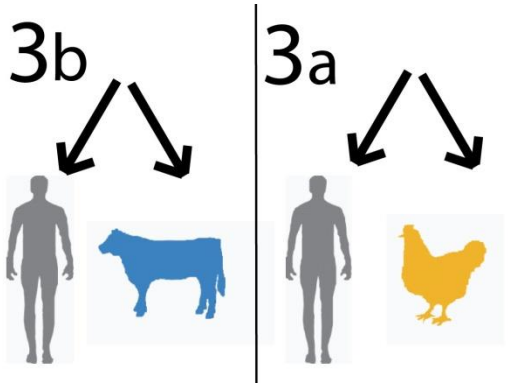
The two species, *C. jejuni* and *C. coli*



Clades 1, & 2 and 3 in *C. coli*



C. jejuni ST21 and ST45
a) human vs. cattle
b) human vs. chicken



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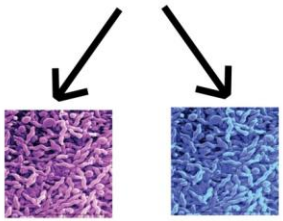
Read, DS., Woodcock, D., Whiteley, AS, Sheppard, SK (*In prep*) Classification of Campylobacter: linking phenotype, genotype and ecotype. PLOS Pathogens.

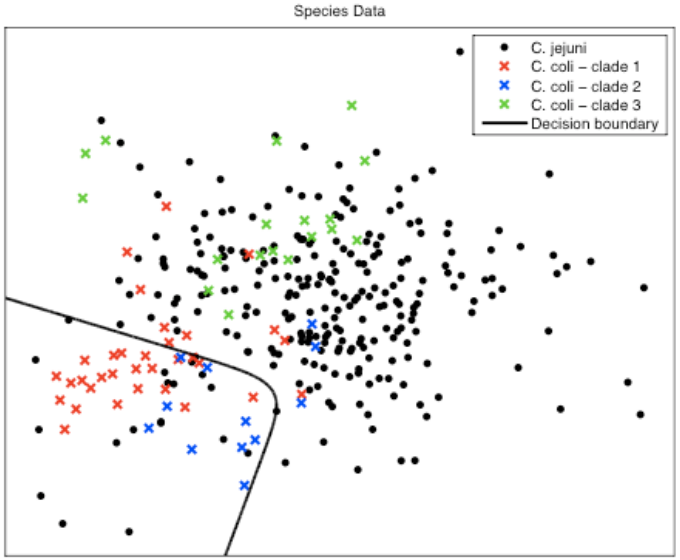
Who?: Pathogenic bacteria: linking genotype with phenotype

1

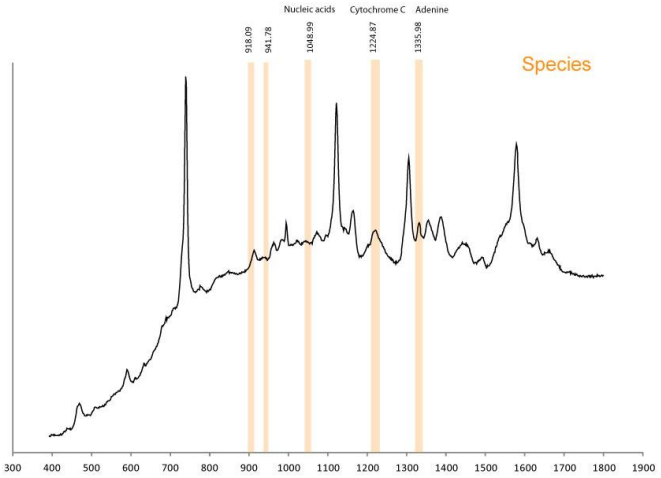


Comparison of *C. jejuni* and *C. coli*

89% classification success



Peaks used in model



Phenotypic signature associated with nucleic acids, cytochrome *c*, and adenine (a nucleobase)

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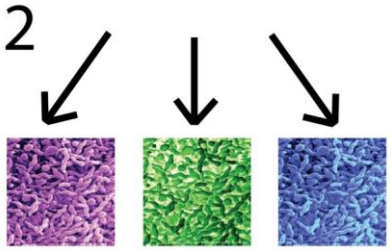
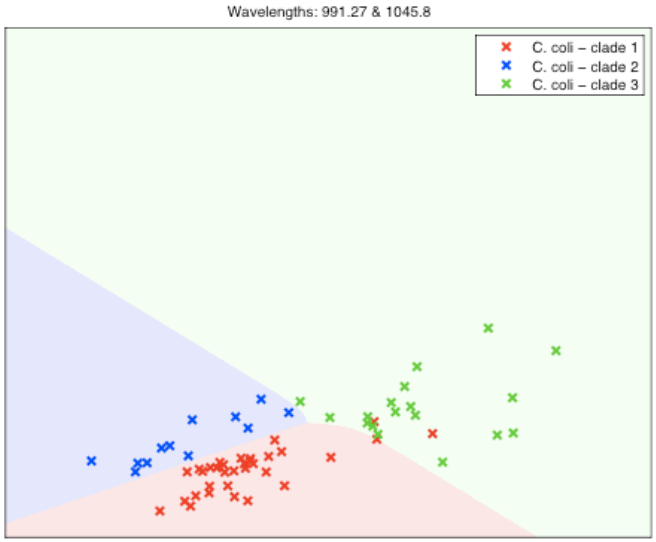
Read, DS., Woodcock, D., Whiteley, AS, Sheppard, SK (*In prep*) Classification of Campylobacter: linking phenotype, genotype and ecotype. PLOS Pathogens.

Who?: Pathogenic bacteria: linking genotype with phenotype

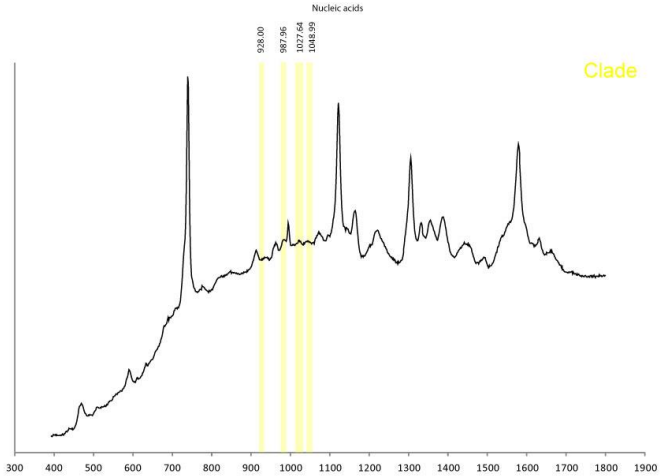


Campylobacter coli – Clade 1, 2, and 3 separation

79% classification success



Peaks used in model



Phenotypic signature associated with phenylalanine and nucleic acids

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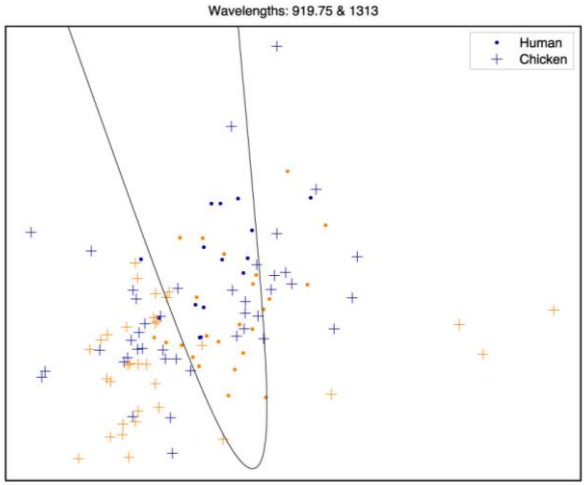
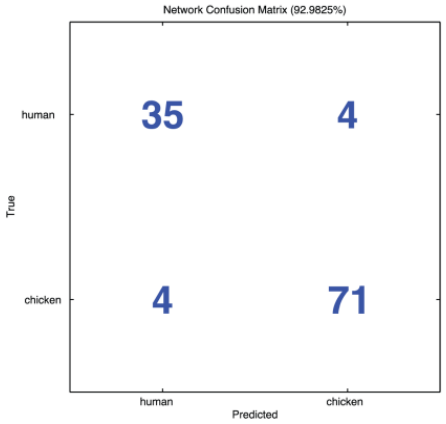
Read, DS., Woodcock, D., Whiteley, AS, Sheppard, SK (*In prep*) Classification of Campylobacter: linking phenotype, genotype and ecotype. PLOS Pathogens.

Who?: Pathogenic bacteria: linking genotype with phenotype

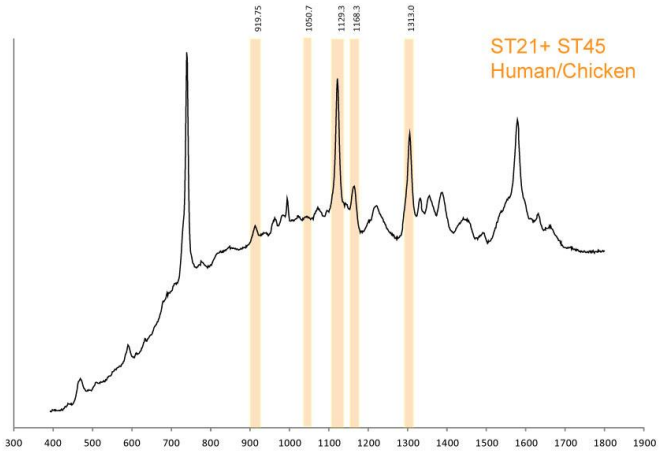
C. jejuni ST45 and ST21 human and chicken

93% classification success





3a



Phenotypic signature associated with cytochrome *c*, nucleic acids and one as yet unidentified peak

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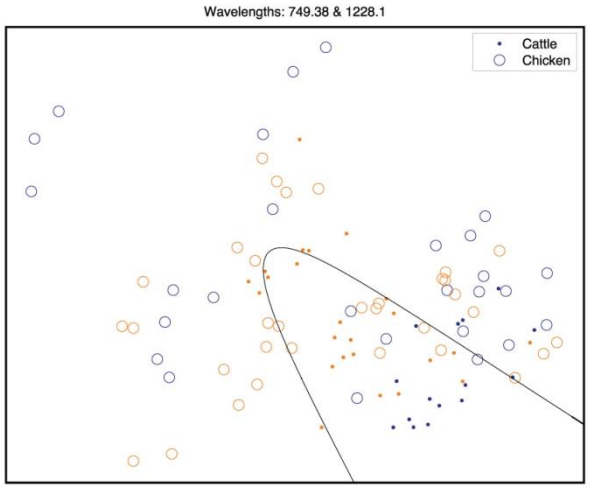
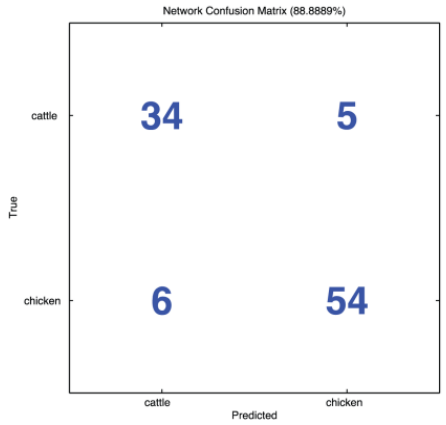
Read, DS., Woodcock, D., Whiteley, AS, Sheppard, SK (*In prep*) Classification of Campylobacter: linking phenotype, genotype and ecotype. PLOS Pathogens.

Who?: Pathogenic bacteria: linking genotype with phenotype

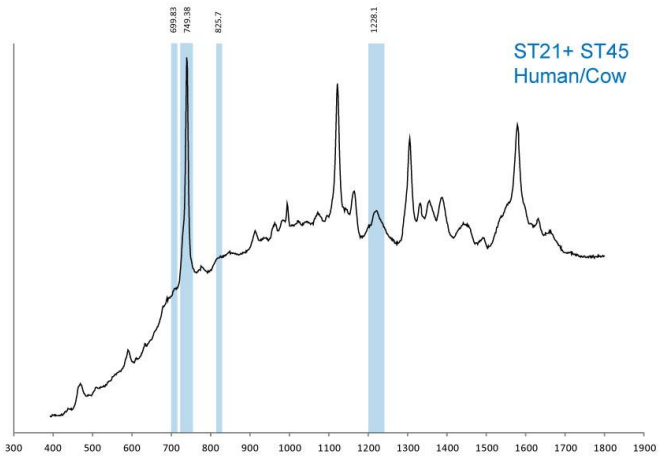
C. jejuni ST45 and ST21 human and cow

89% classification success





3b



Phenotypic signature associated with cytochrome *c*, and two as yet unidentified peaks

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Read, DS., Woodcock, D., Whiteley, AS, Sheppard, SK (*In prep*) Classification of Campylobacter: linking phenotype, genotype and ecotype. PLOS Pathogens.

Who?: Pathogenic bacteria: linking genotype with phenotype

- Links between phenotype and genotype comparatively weak in *Campylobacter* sp. (compared to other studies)
- Why Clade 3 *C. Coli* phenotypically more similar to *C. Jejuni*?

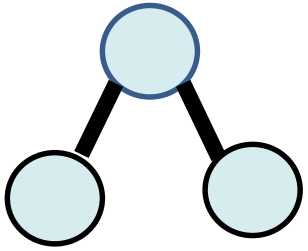
- Link between phenotype and host association proved to be stronger

- Possibly due to limitations of MLST technique (only 7 housekeeping genes)

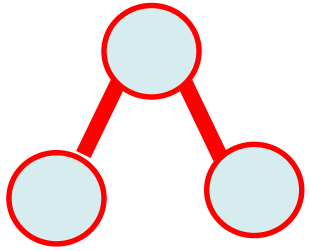
- Campylobacter possibly not a good study organism!

Read, DS., Woodcock, D., Whiteley, AS, Sheppard, SK (*In prep*) Classification of Campylobacter: linking phenotype, genotype and ecotype. PLOS Pathogens.

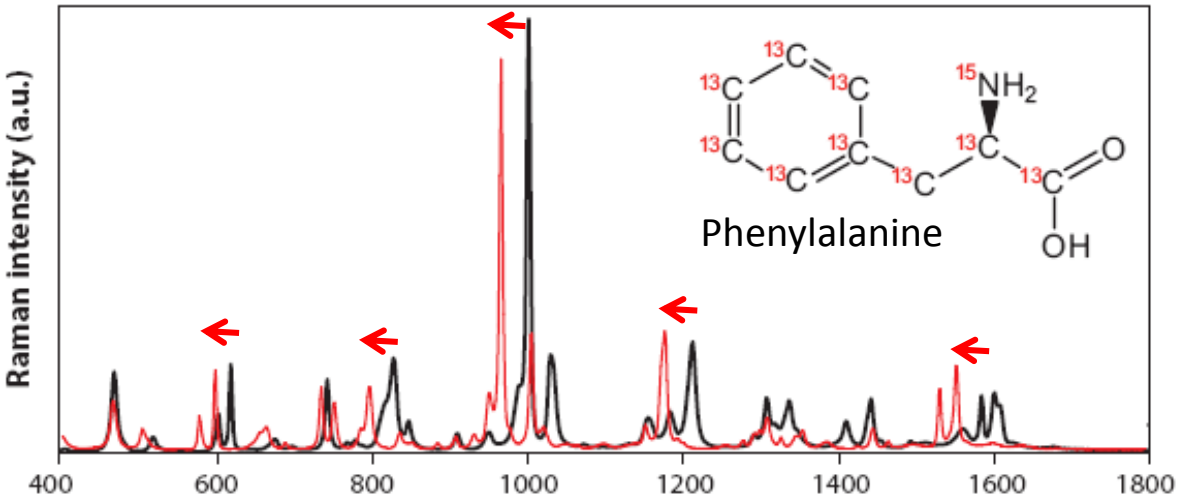
How?: Microbial function



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

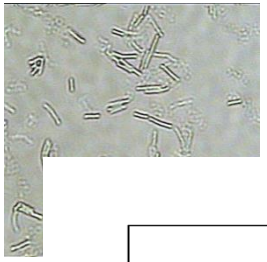


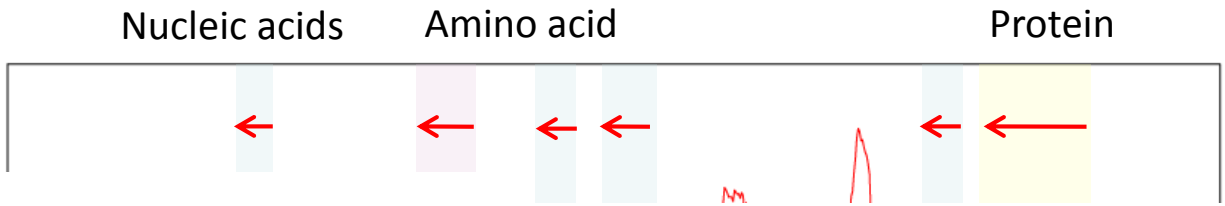
‘Heavy’ isotope
e.g. ^{13}C Carbon

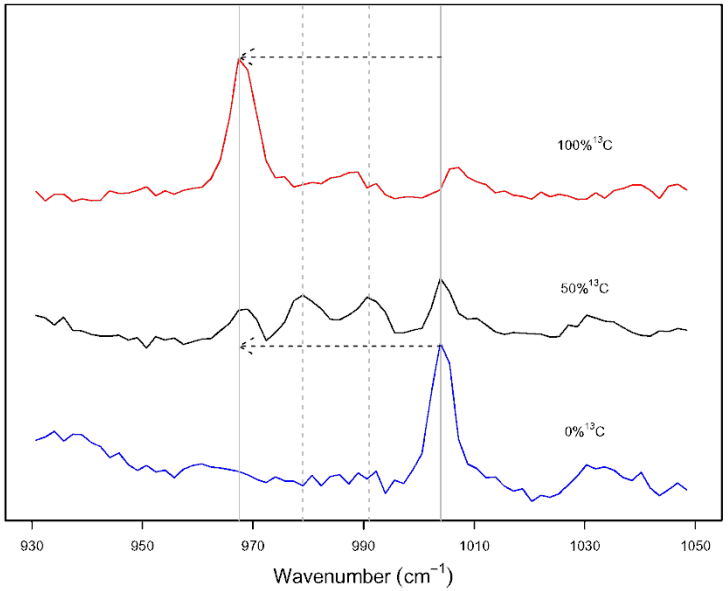


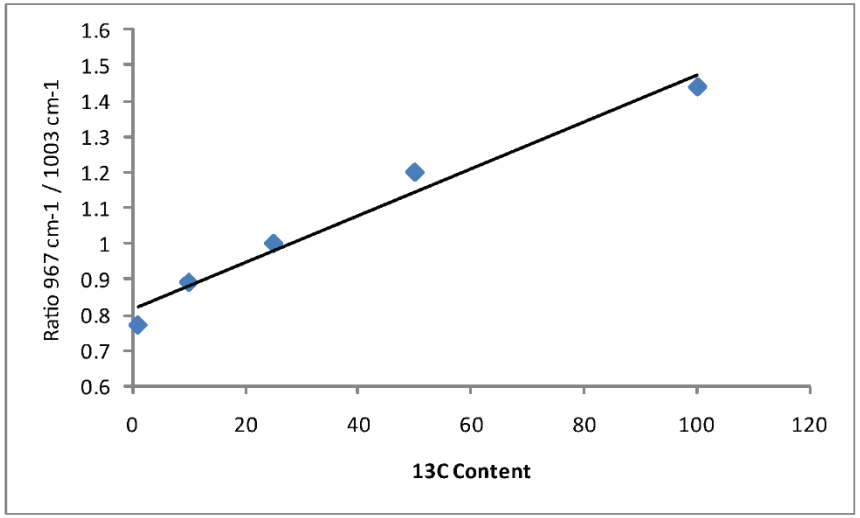
How?: Microbial function

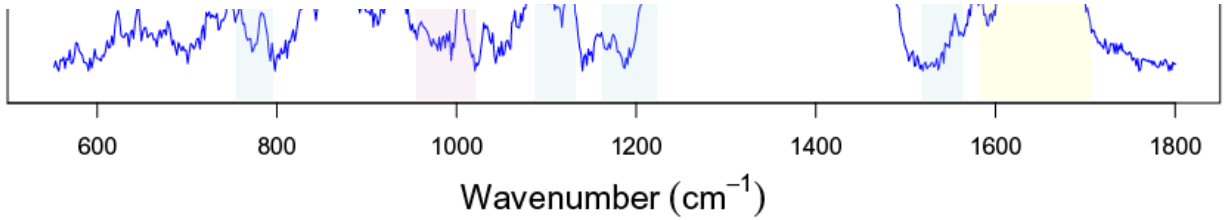
Escherichia coli











1% ^{13}C

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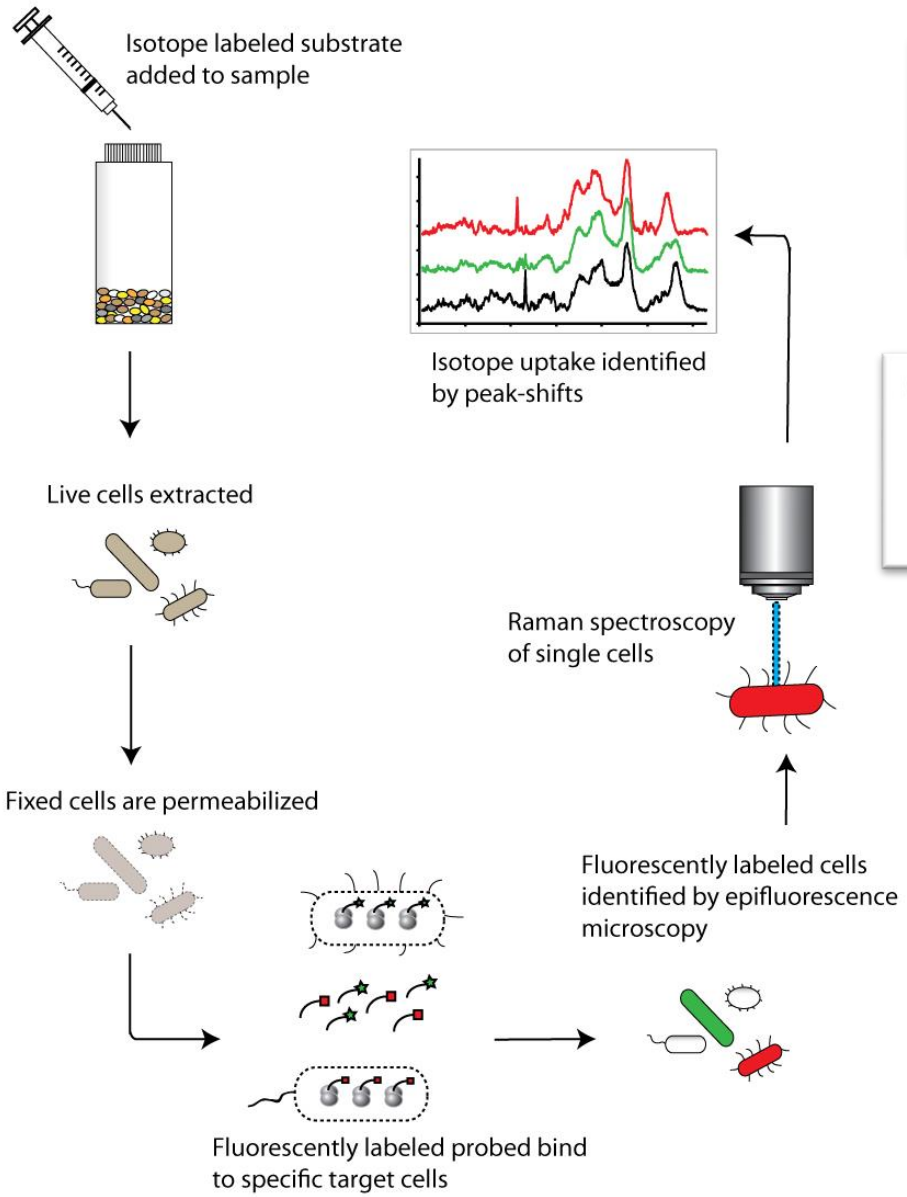
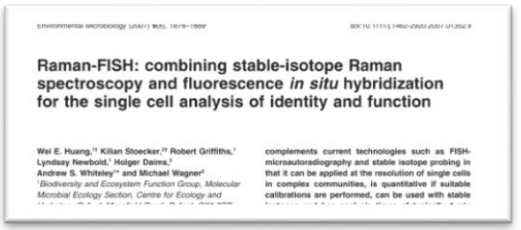
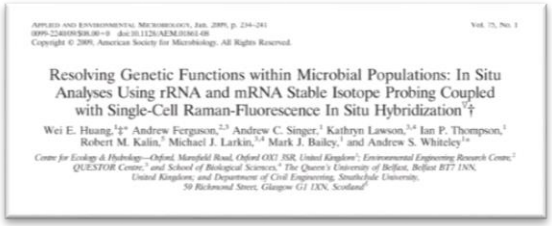
How?: Microbial function (Raman-FISH)




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


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

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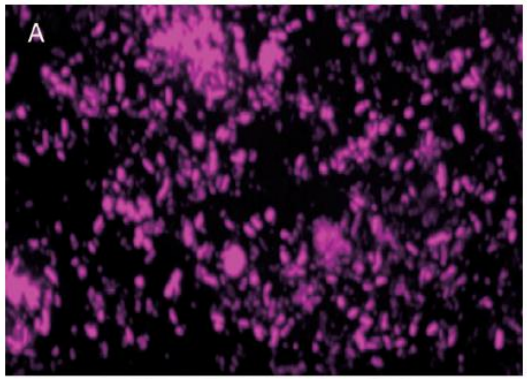
How?: Microbial function (Raman-FISH)

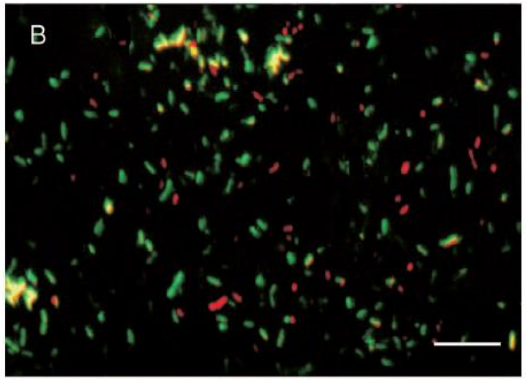
SEquenced REactive BARrier (SEREBAR)

- 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

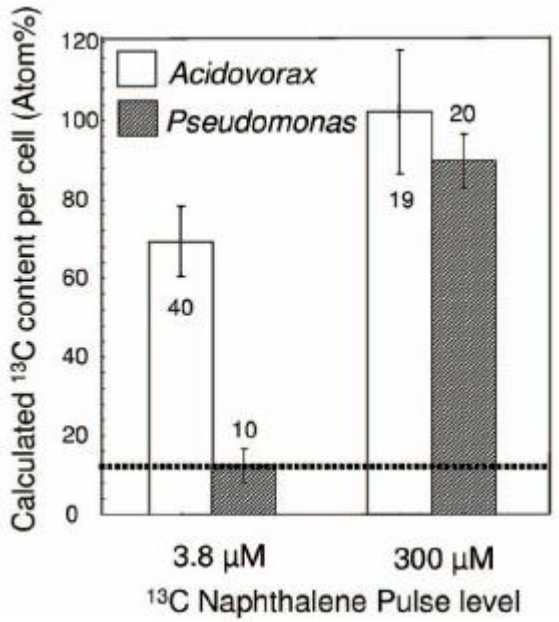


EUB338 (general bacteria)



Acidovorax sp. **Red**

Pseudomonas sp. **Green**



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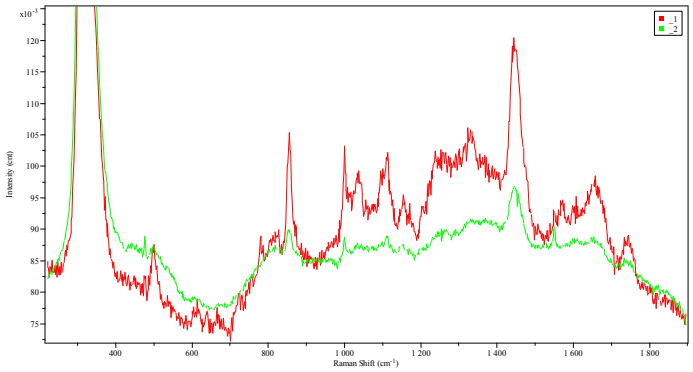
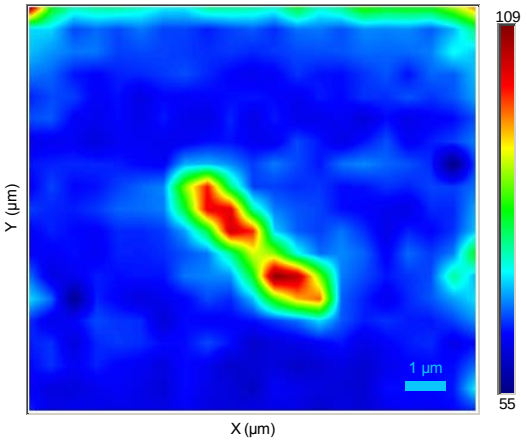
Huang, W. E., Stoecker, K., Griffiths, R., Newbold, L., Daims, H., Whiteley, A. S., & Wagner, M. (2007). Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence in situ hybridization for the single cell analysis of identity and function. *Environmental Microbiology*, *9*(8), 1878-1889

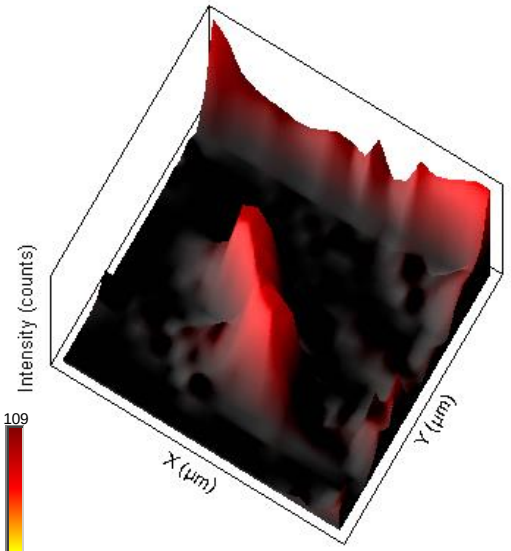
Where?: Raman mapping

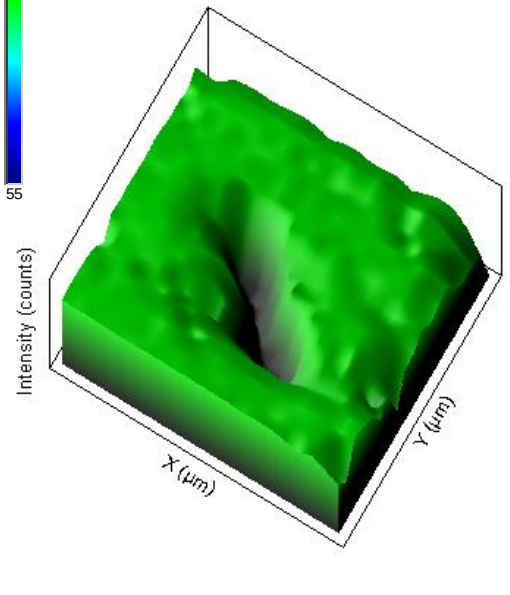
Single *Escherichia coli*

- Mapped over 20x20 grid
- 0.5µm between each point
- ~400 spectra









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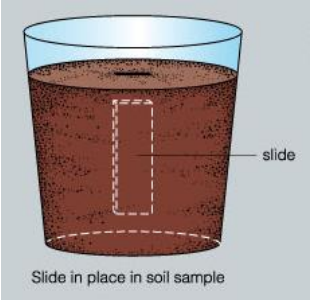
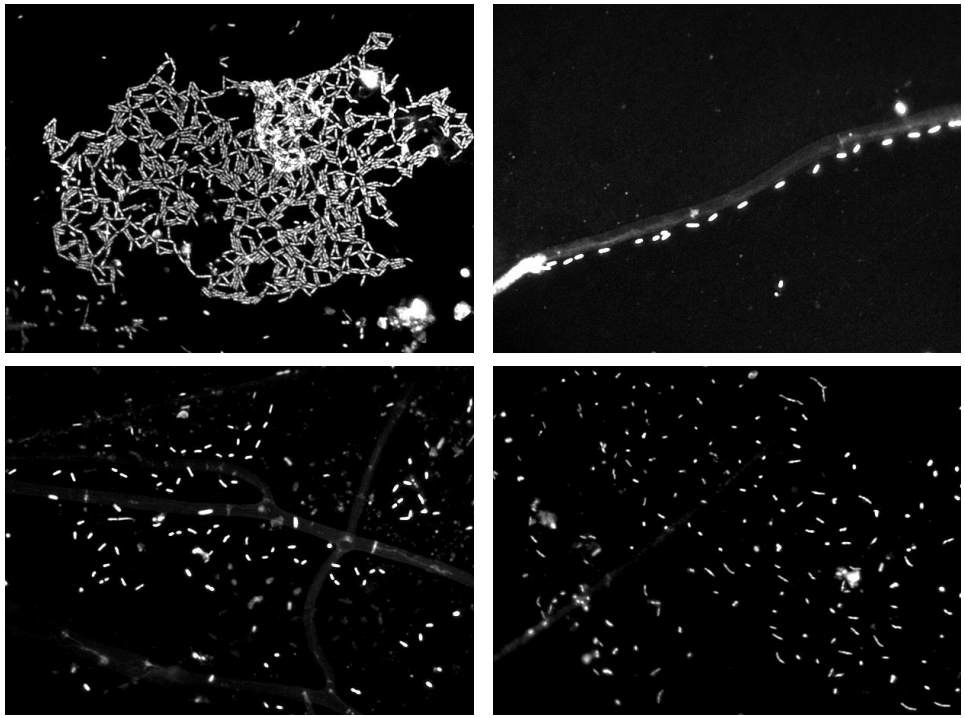
Where?: Raman mapping

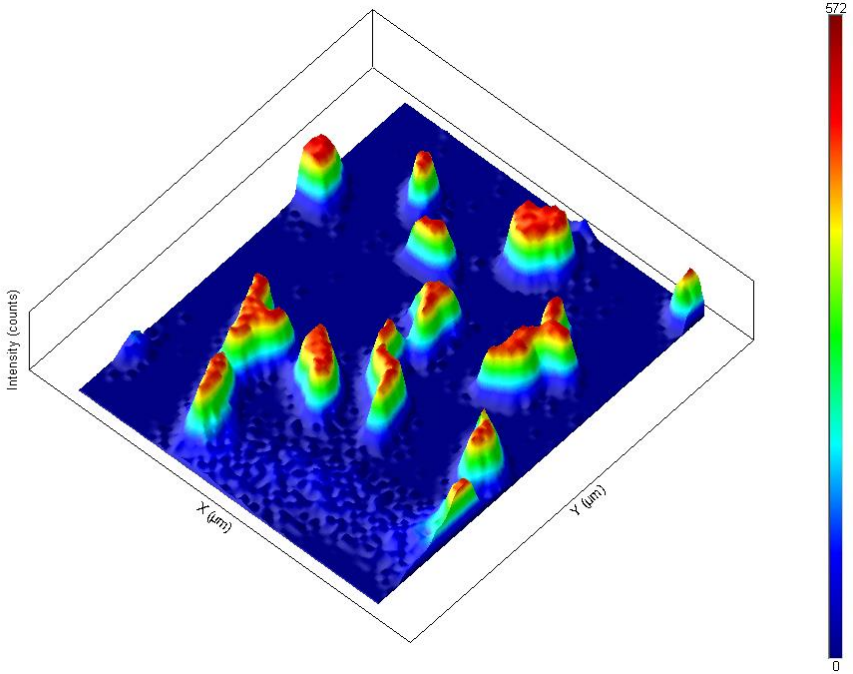
DIRECT MICROSCOPIC AND BACTERIOLOGICAL EXAMINATION OF THE SOIL

GIACOMO ROSSI
ASSISTED BY
S. RICCARDO, G. GESUÈ, M. STANGANELLI, AND TSU KAO WANG
Royal Higher Agricultural Institute, Portici, Italy

Received for publication May 29, 1935







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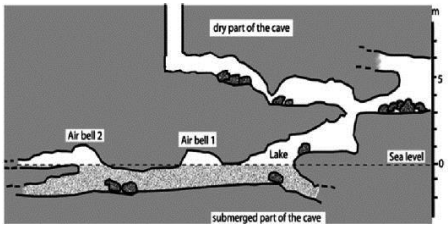
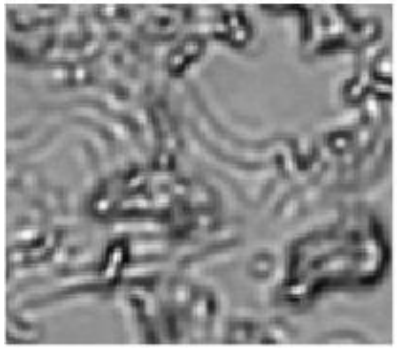
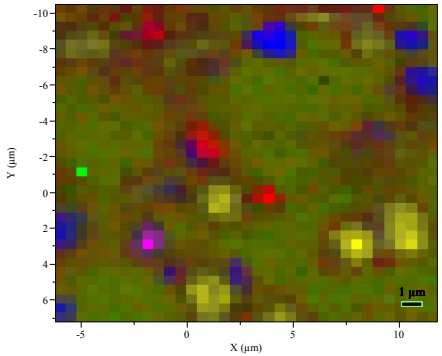
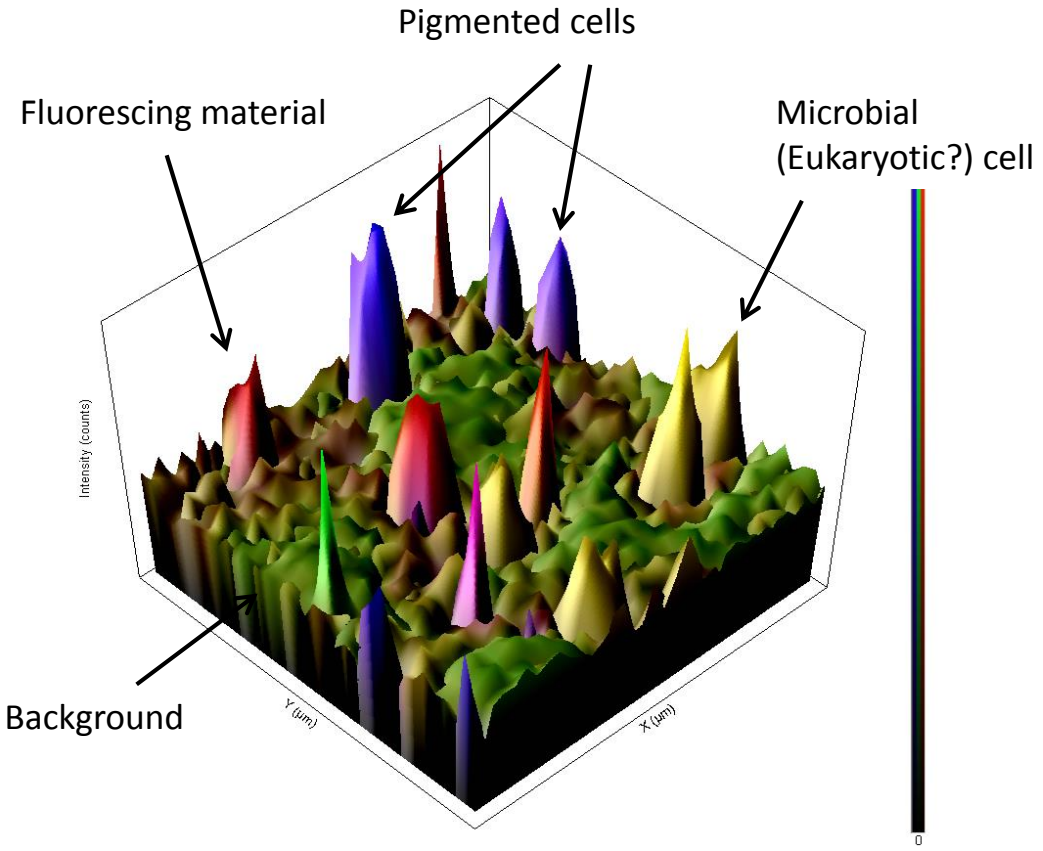
Where?: Raman mapping



Movile Cave







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Acknowledgements:

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Dr Sam Sheppard (University of Oxford)
Dr Dan Woodcock (University of Warwick)
Mr Simon Fitzgerald (Horiba Scientific)
Molecular Microbial Ecology Group (CEH Wallingford)

THANK YOU

Any Questions?...

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