# Imaging formulated product performance using optical spectroscopy

Natalie Belsey, UKSAF meeting, 5th January 2021

Formulated products

- The formulated products sector contributes ~ £150bn annually to the UK economy [£6.5 bn Aerospace, £17.1bn Automotive and £32.1bn for Construction, KTN, 2018].
- Not only critical to the economy, but also to our health and wellbeing.
- We need measurements to understand their structure, function and fate post-application in order to ensure efficacy & safety

Pharmaceuticals

Consumer health

Formulated products
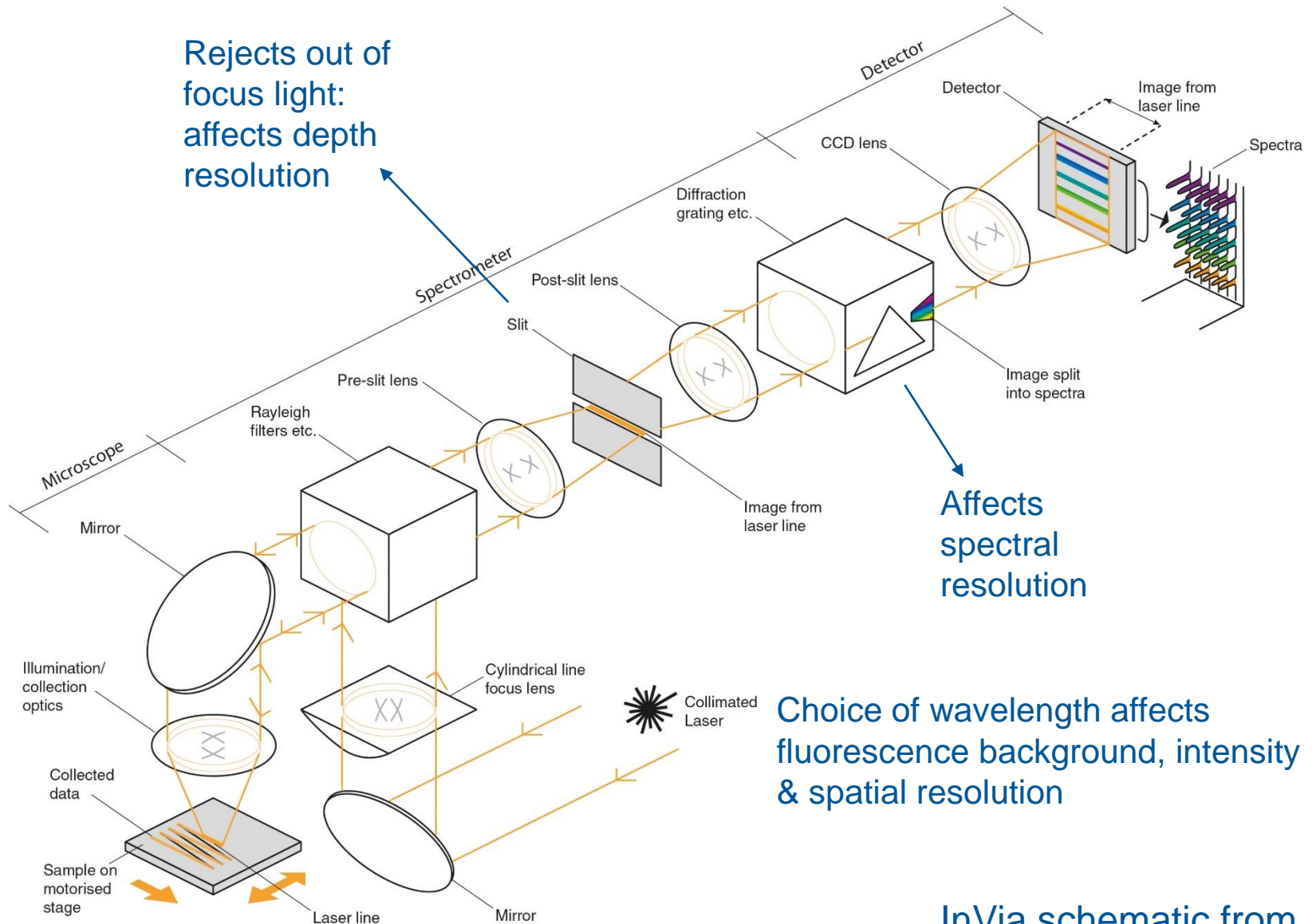
Food & Drink

Agrichemicals
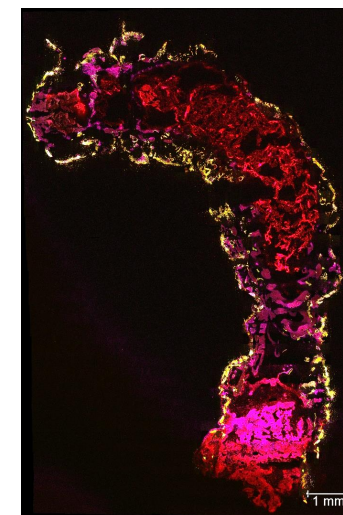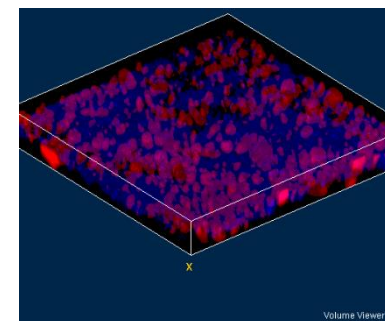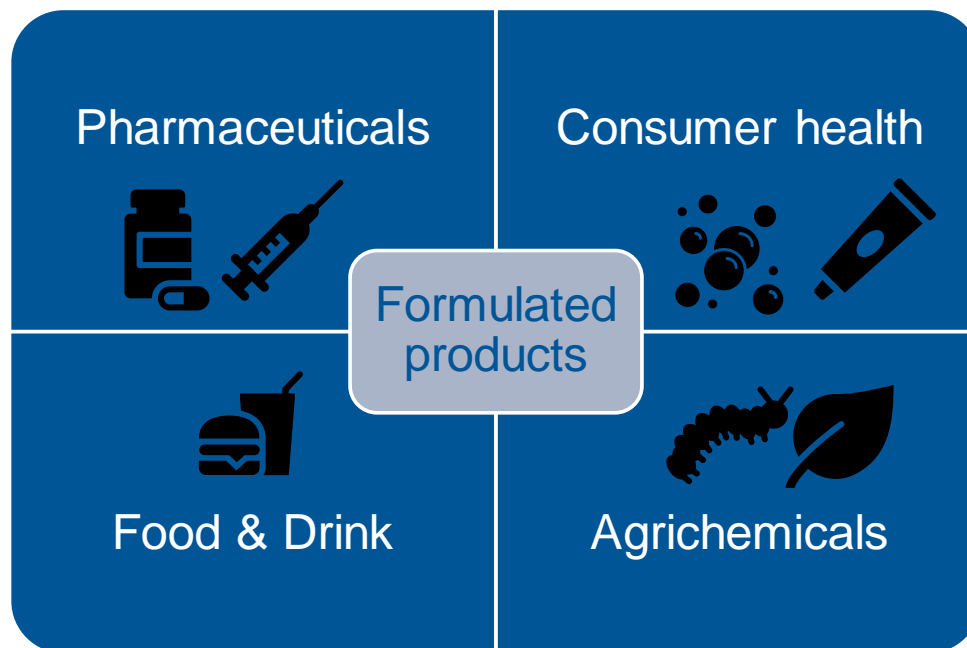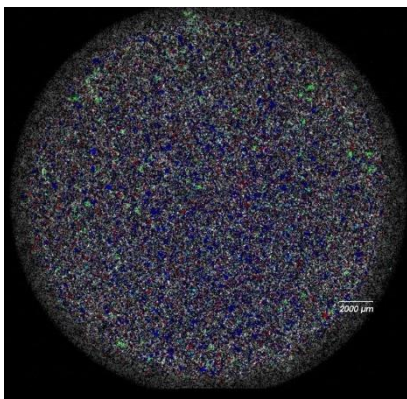
Raman spectroscopy

- Inelastic scattering of light by the sample: Energy needed to excite a molecular vibration depends on the masses of the atoms & type of bond(s) between them.
- 'Label-free', ambient analysis, no special sample preparation requirements.
- Non-invasive/destructive, possibility for in-line & *in vivo* measurement

Acetaminophen

- Aspirin
- Paracetamol
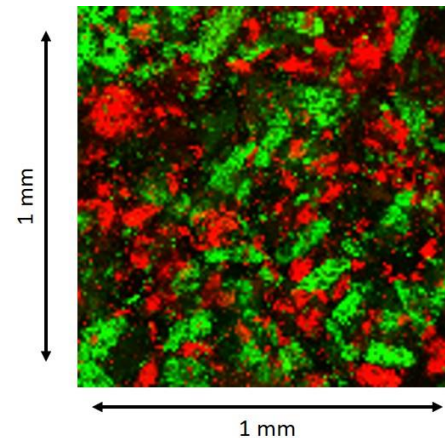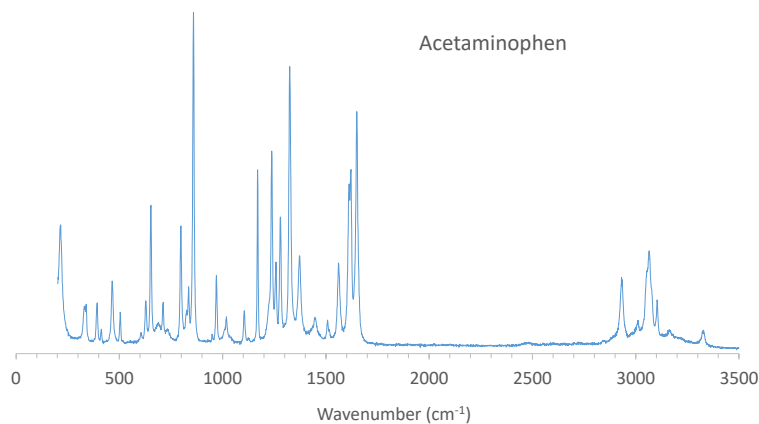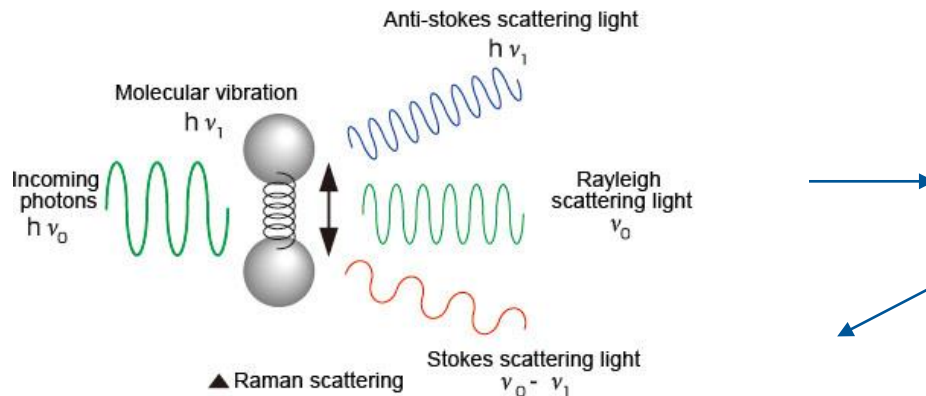
BUT confocal Raman mapping at high resolution is very slow, unsuitable for dynamic studies….

Confocal Raman spectrometer

Rejects out of focus light: affects depth resolution

Affects spectral resolution

Choice of wavelength affects fluorescence background, intensity & spatial resolution

InVia schematic from renishaw.com

Detector

Image from laser line

Spectra

CCD lens

Detector

Diffraction grating etc.

Image split into spectra

Post-slit lens

Slit

Spectrometer

Pre-slit lens

Rayleigh filters etc.

Image from laser line

Microscope

Mirror

Illumination/ collection optics

Cylindrical line focus lens

Collimated Laser

Collected data

Sample on motorised stage
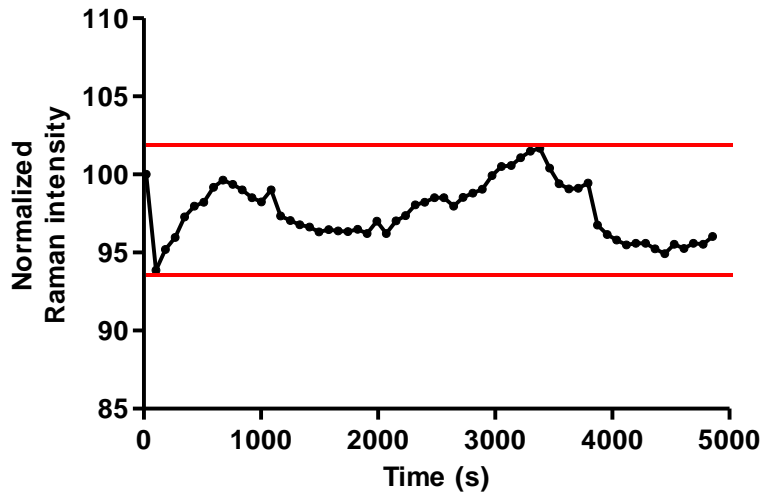
Laser line

Mirror

Measurement considerations

Intensity of Raman scattered radiation:

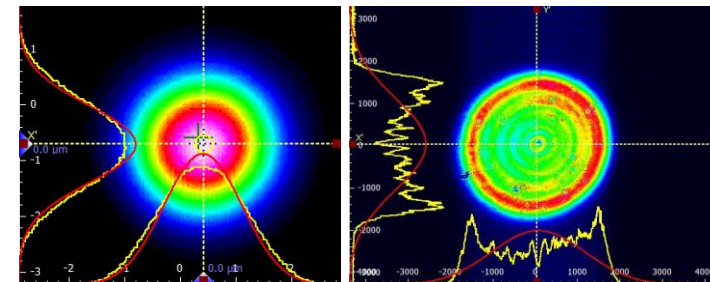$$I_R \propto \nu^4 I_0 N \left(\frac{\partial \alpha}{\partial Q}\right)^2$$

I_0 Incident laser intensity

N Number of scattering molecules in a given state

ν Frequency of excitation laser

α Polarizability of the molecule

Q vibrational amplitude

Calibration (wavelength)

Acetaminophen

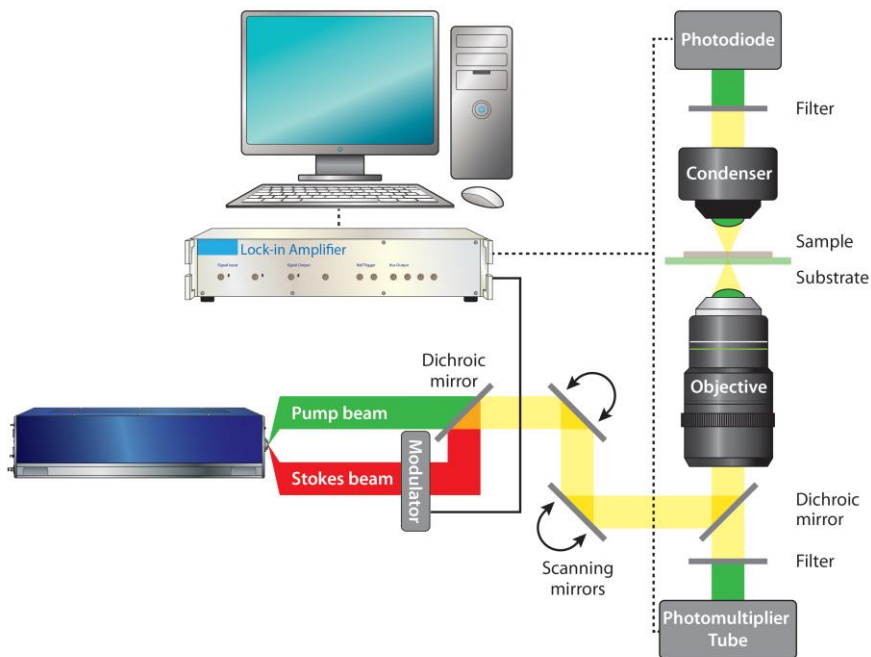Detector QE
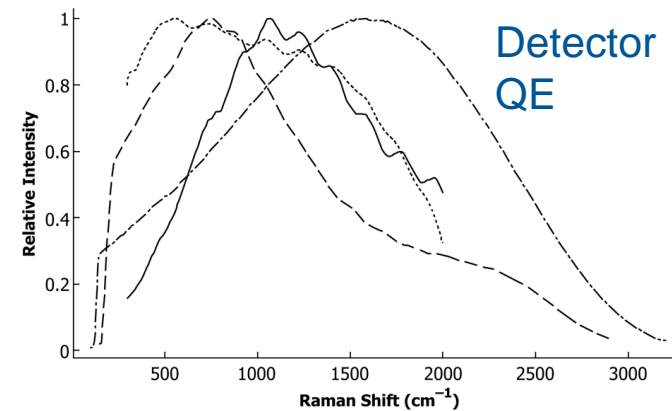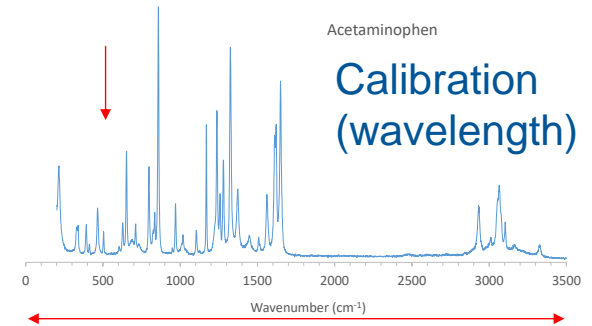
Laser intensity fluctuation

Beam power profile

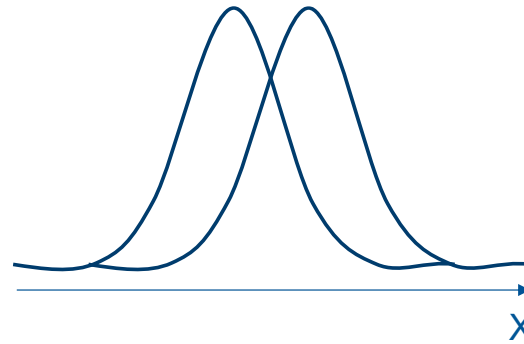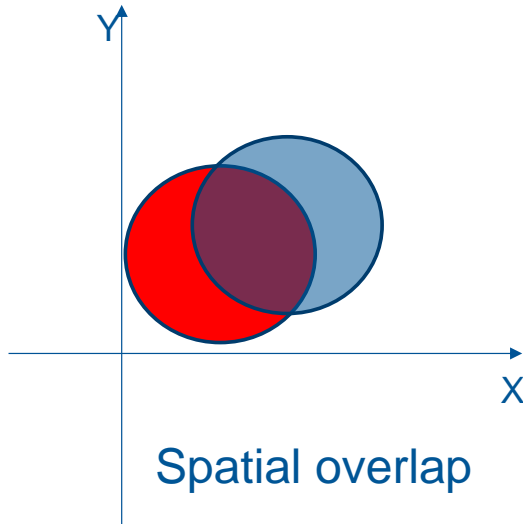# Stimulated Raman scattering (SRS) microscopy

- 2 lasers with a frequency difference tuned to match a vibrational mode of interest
- **'Real-time' label-free** chemical imaging at a single wavenumber at a time
- Other imaging modalities can also be collected, such as CARS, single & two photon fluorescence, and SHG.
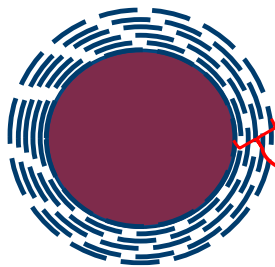- SRS signal is linear with concentration
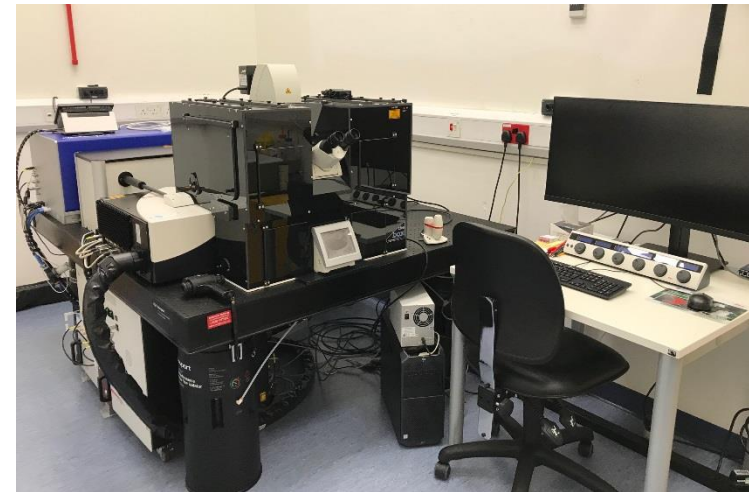
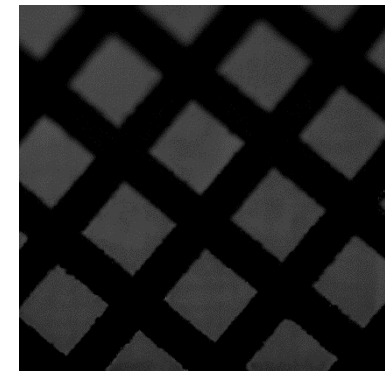Stimulated excitation when $\omega_{pump} - \omega_{Stokes} = \omega_{vib}$

Input pulse train   **Output pulse train**

ΔI SRL

Pump

Modulated Stokes
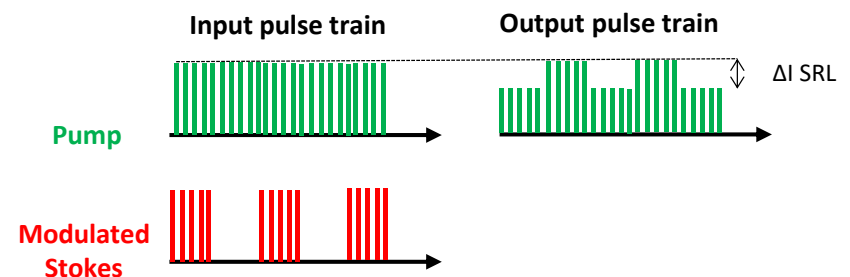
SRS: spatial & temporal alignment
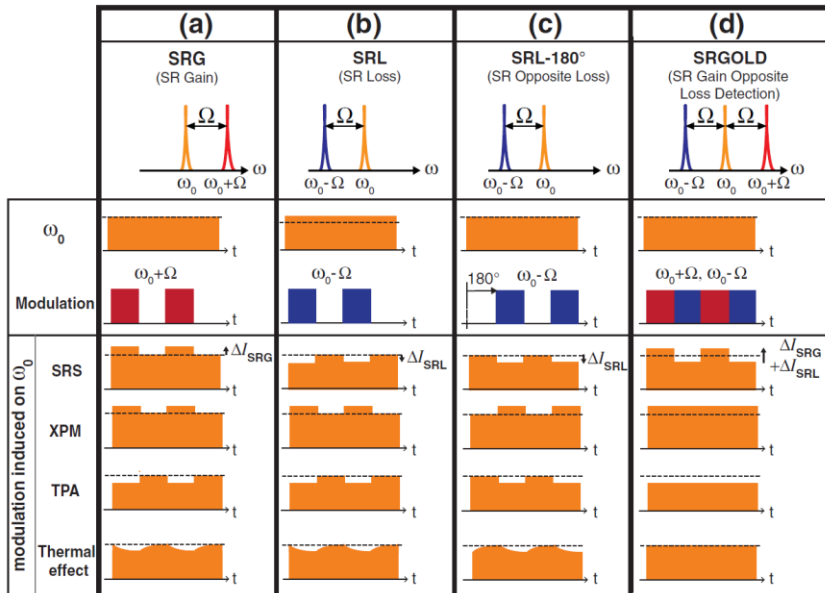

Spatial overlap

Temporal overlap

Variation of the pump beam diameter with wavelength
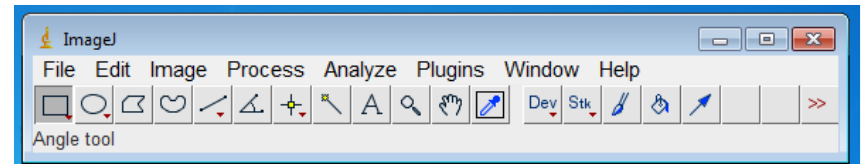
FOV & stage position

Non-Raman parasitic signals

Optical mechanisms for signal isolation

P. Berto, E. R. Andresen, H. Rigneault,
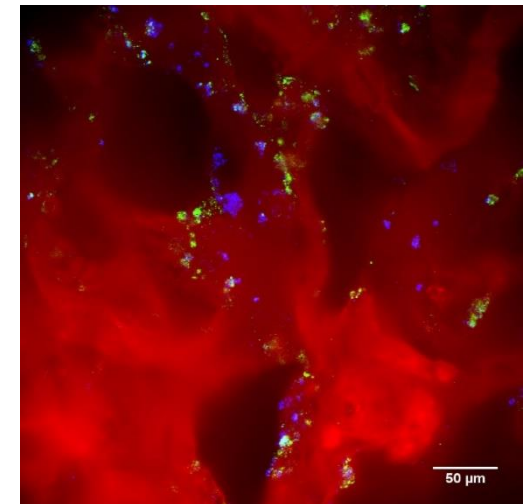*Physical Review Letters*, 2014, 112,
053905.

Empirical/data methods for signal isolation based around subtraction.
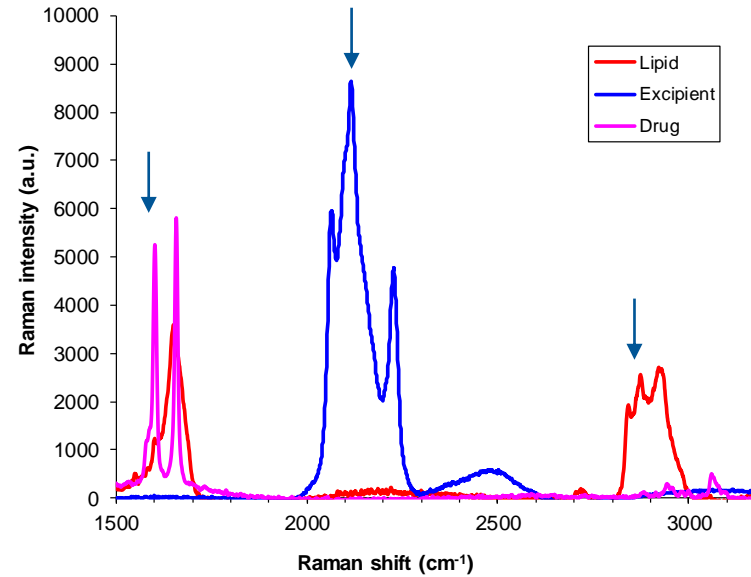

Measurement challenges:
- Detector sensitivity changes with wavelength
- Changes in parasitic signals with wavelength
- Sample movement requires image registration
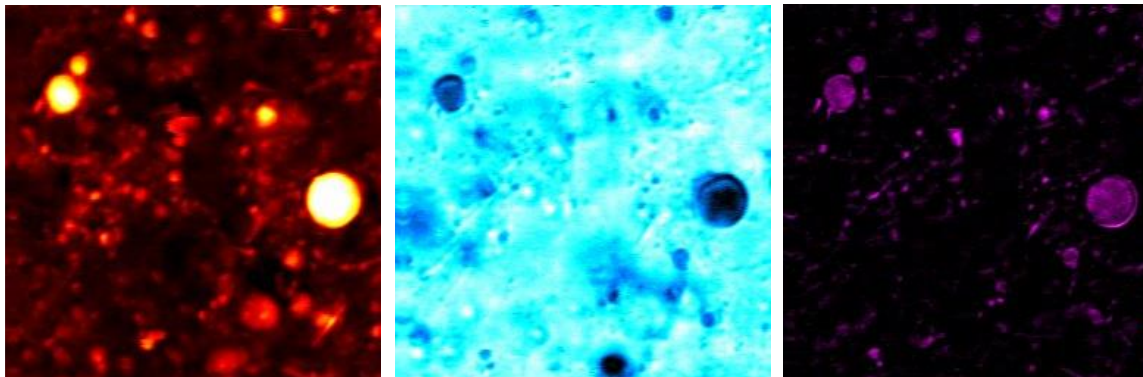

But its not all bad,
photothermal signals
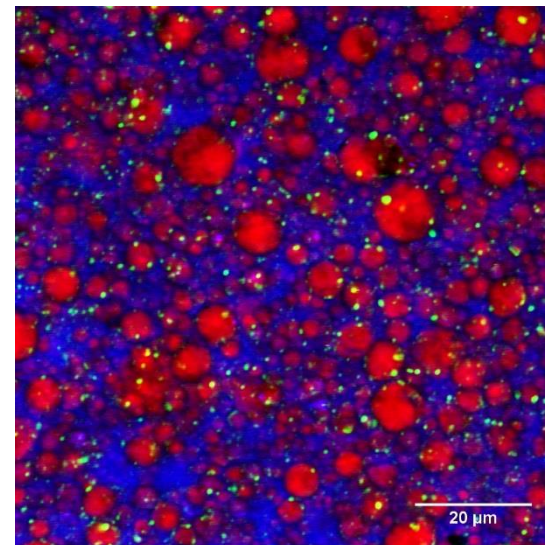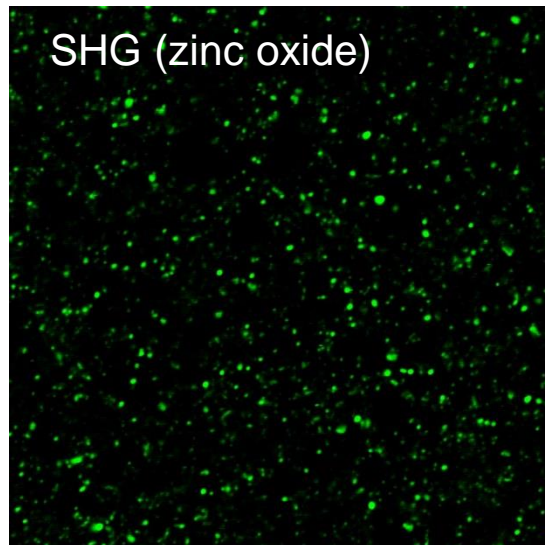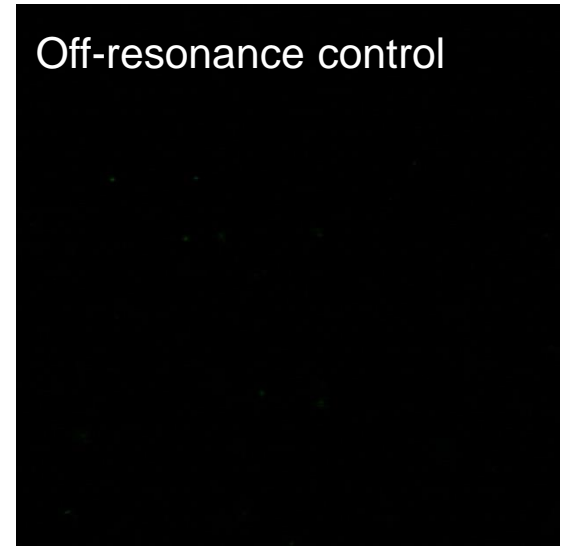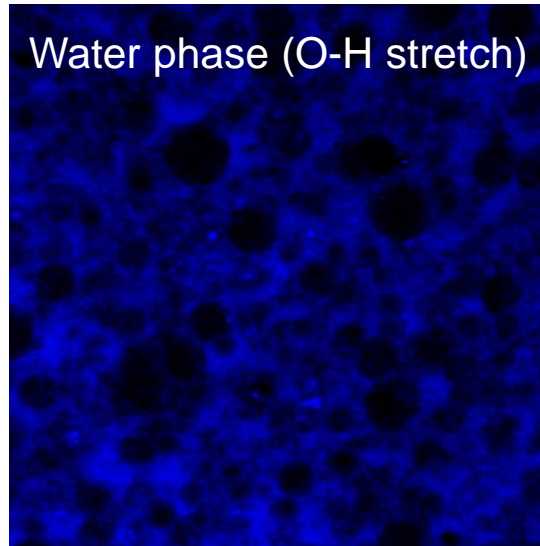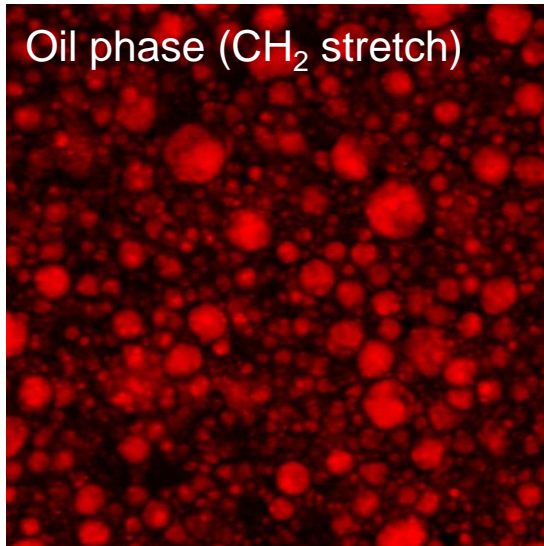can be very useful!

Confocal Raman to SRS workflow
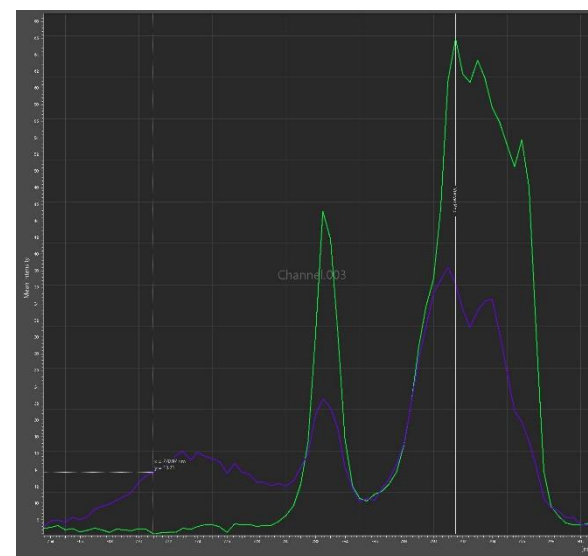
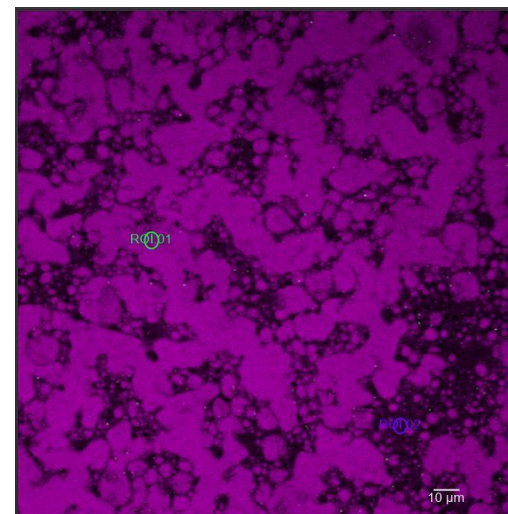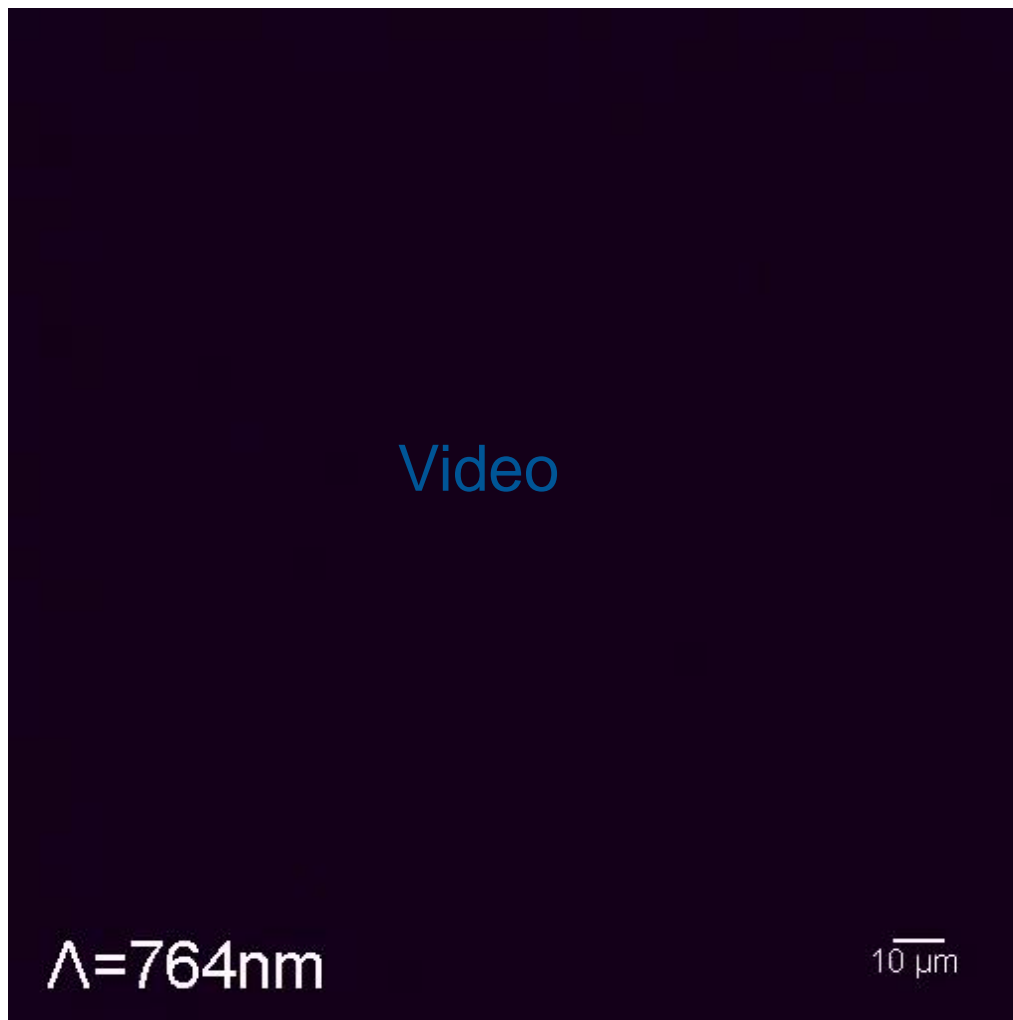1. Acquire Raman spectra of ingredients & matrix/tissue

2. Select peaks of interest (& controls) and tune SRS microscope to that frequency

Formulation characterisation: Sunscreen

Oil phase (CH$_2$ stretch)

Water phase (O-H stretch)

Off-resonance control

SHG (zinc oxide)

20 µm

SRS spectra: Lambda scanning

Further examples

Ibuprofen crystals

Aqueous cream

Wound dressing

50 µm
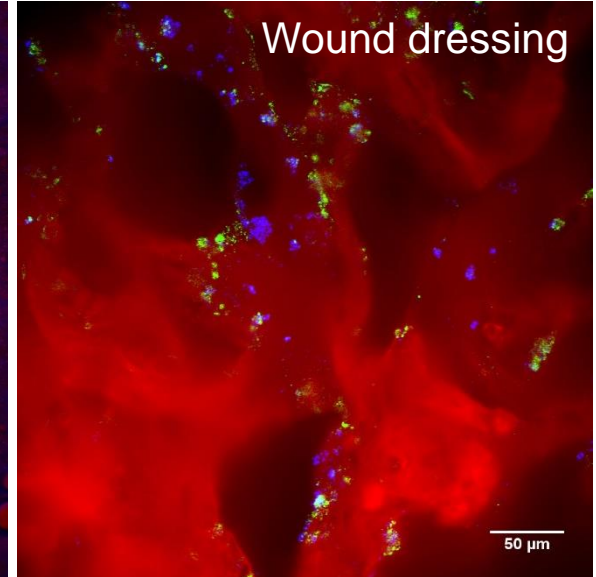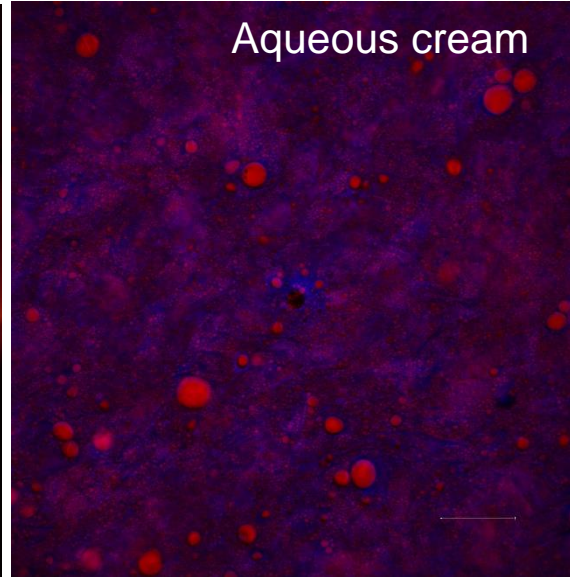
- Can be used to identify polymorphs, some isomers & co-crystals
- Particle distribution (metallic particles using SHG or photothermal lensing)
- Oxidation products
- & much more!

Product fate: Agrichemicals

■ uptake of agrichemicals into plants and insects e.g. lepidoptera:

SHG
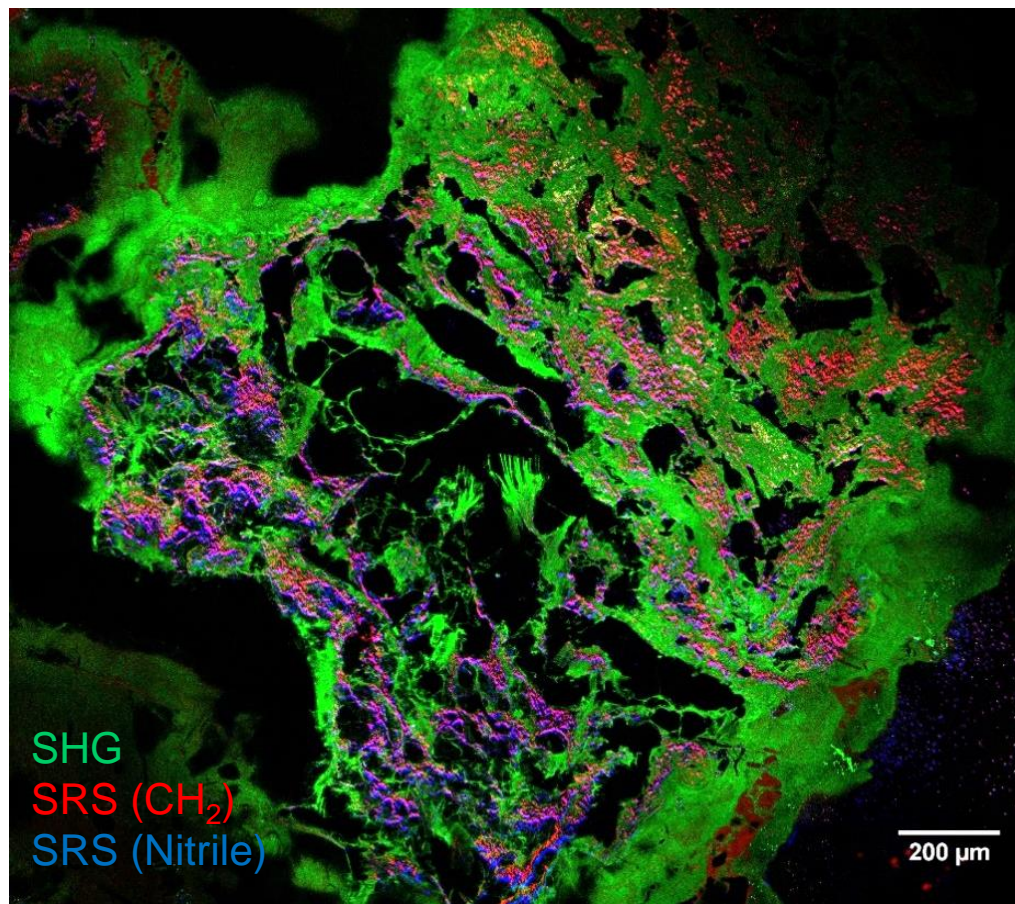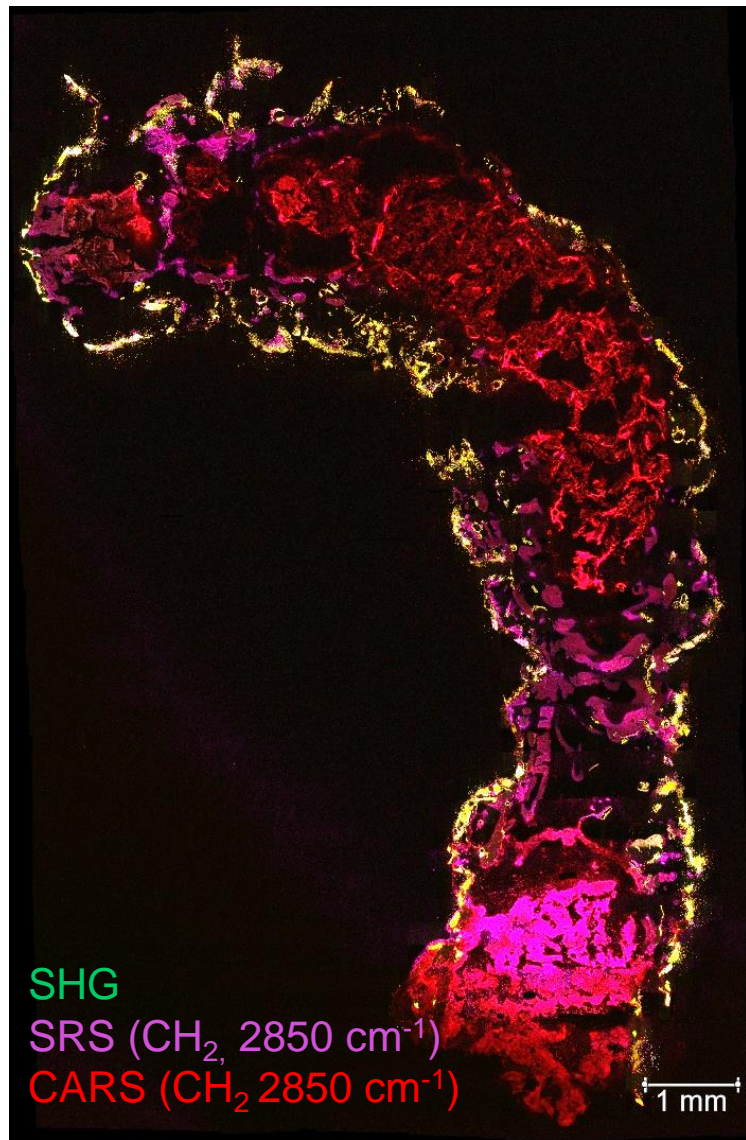SRS (CH$_2$, 2850 cm^{-1})
CARS (CH$_2$ 2850 cm^{-1})
1 mm

SHG
SRS (CH$_2$)
SRS (Nitrile)
200 µm

Product fate: Topical drugs

- Half of the UK population suffer from some form of skin disease which costs the NHS >£720M per year.
- Topical & transdermal: many other therapies are delivered across skin, e.g. nicotine patches

Its incredibly important to accurately measure chemical permeation through the skin:
- Intentional exposure: e.g. pharmaceutical drug products
- Unintentional exposure: e.g. risk assessment for industrial or agrichemical exposure

IVPT – Franz cell
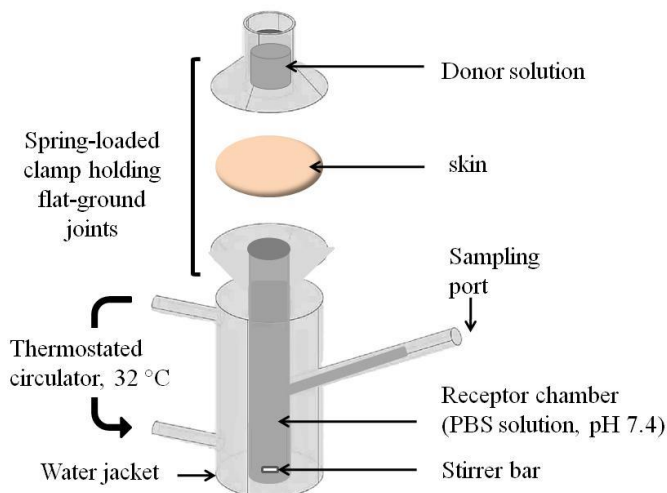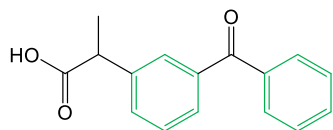

Adhesive tape stripping
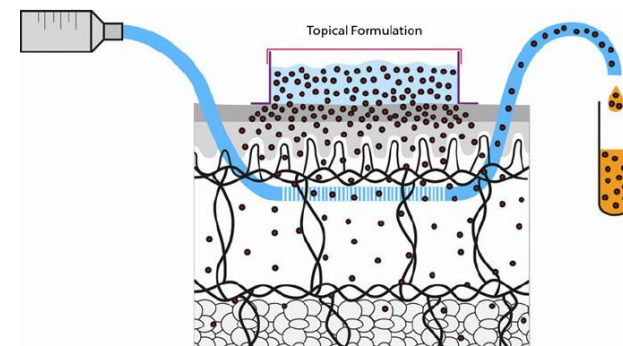
microdialysis

Many techniques are destructive, laborious & invasive. There is no lateral resolution or mechanistic insight into chemical penetration pathway or formulation metamorphosis.
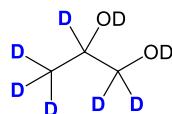**Note fluorescence imaging unhelpful since chemical permeation kinetics depend on LogP and MW…**
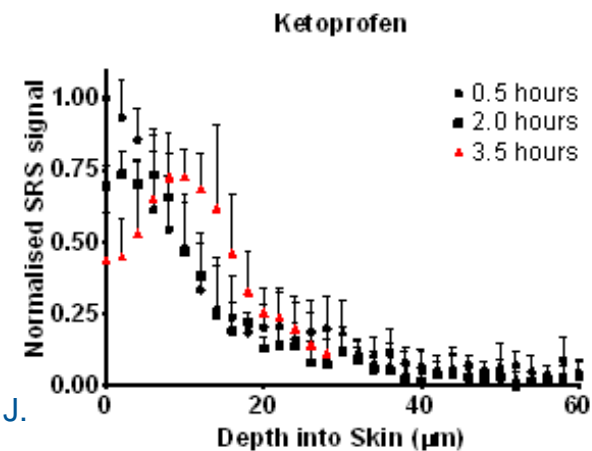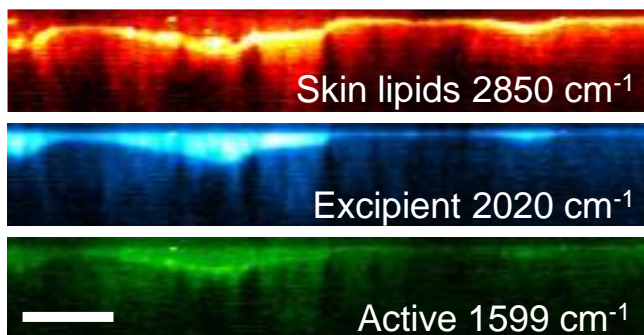
Visulaising topical drug delivery

Ketoprofen
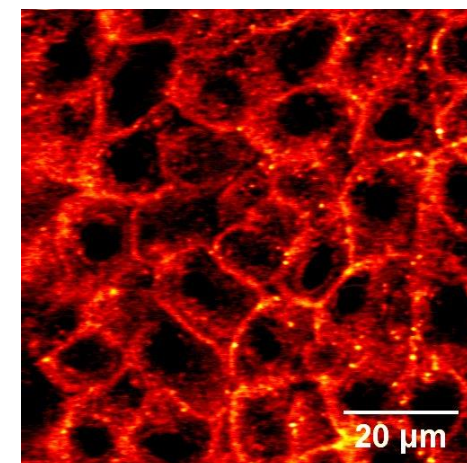1600 cm^{-1}

PG-d$_8$
2120 cm^{-1}

Skin: lipids (CH$_2$)
2850 cm^{-1}

Wavenumber (cm^{-1})

20 µm

Skin lipids 2850 cm^{-1}

Excipient 2020 cm^{-1}

Active 1599 cm^{-1}

Ketoprofen

- 0.5 hours
- 2.0 hours
- 3.5 hours

Normalised SRS signal

Depth into Skin (µm)

N. A. Belsey, L. R. Contreras-Rojas, N. L. Garrett, A. J. Pickup-Gerlaugh, G. J. Price, J. Moger, R. H. Guy. *Journal of Controlled Release*, 2014, 174, 37-42.
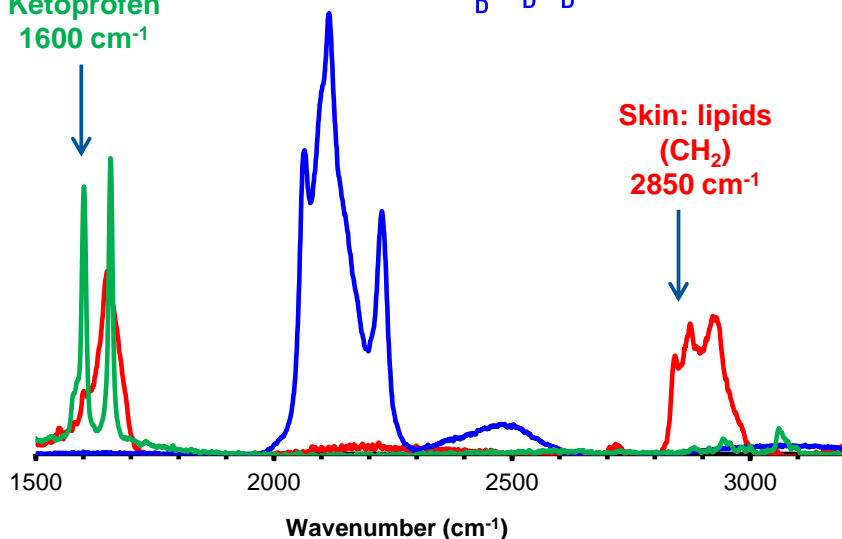
Visualisation of 'metamorphosis'

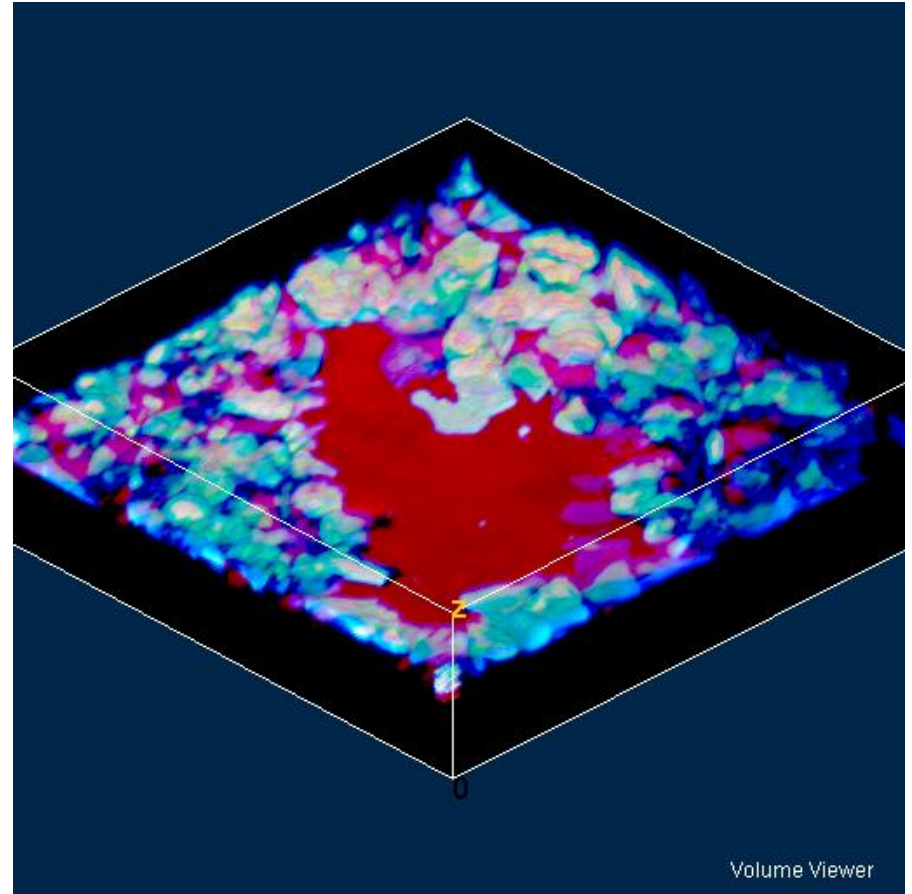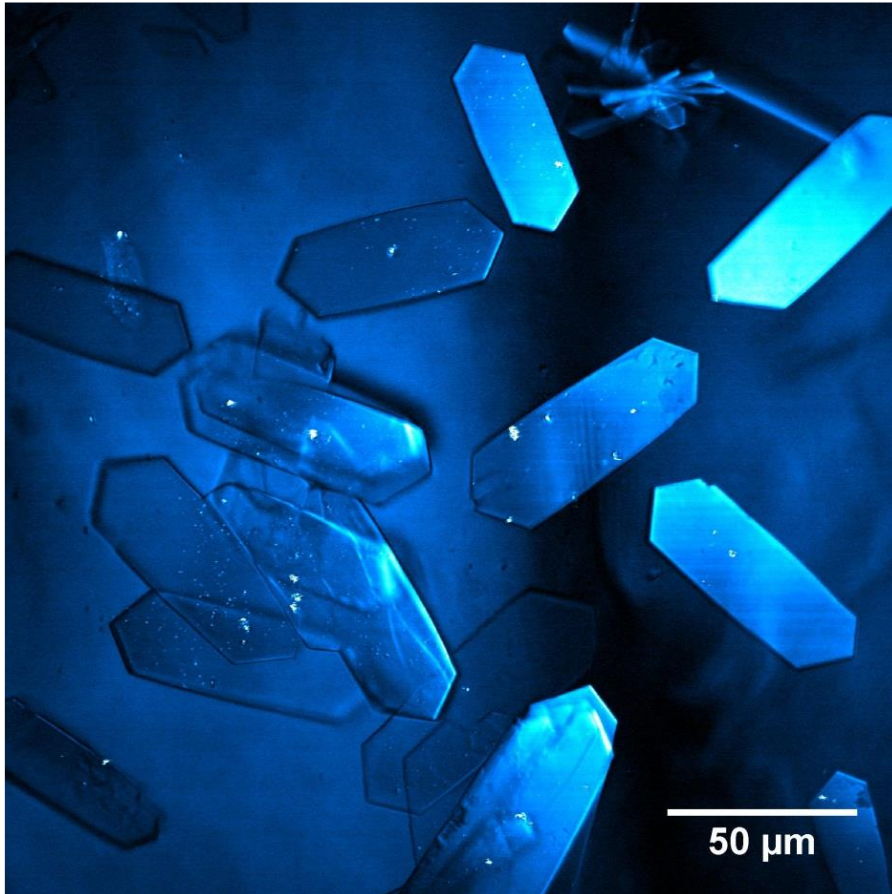Crystallisation of topically applied active often responsible for poor bioavailability.
SRS contrast at 2120 cm^{-1} Ibuprofen-d$_3$ in PG 30 min after topical application.

50 μm

Volume Viewer

N. A. Belsey, L. R. Contreras-Rojas, N. L. Garrett, A. J. Pickup-Gerlaugh, G. J. Price, J. Moger, R. H. Guy. *Journal of Controlled Release*, 2014, 174, 37-42.

Assessing skin pharmacokinetics & bioequivalence of topical drugs

50 µm

Aim: To characterise non-invasively the epidermal bioavailability of a topically applied drug and distinguish correctly between formulations that are bioequivalent and those that are not.

E.g. How can we measure if a generic formulation performs the same as the innovator?
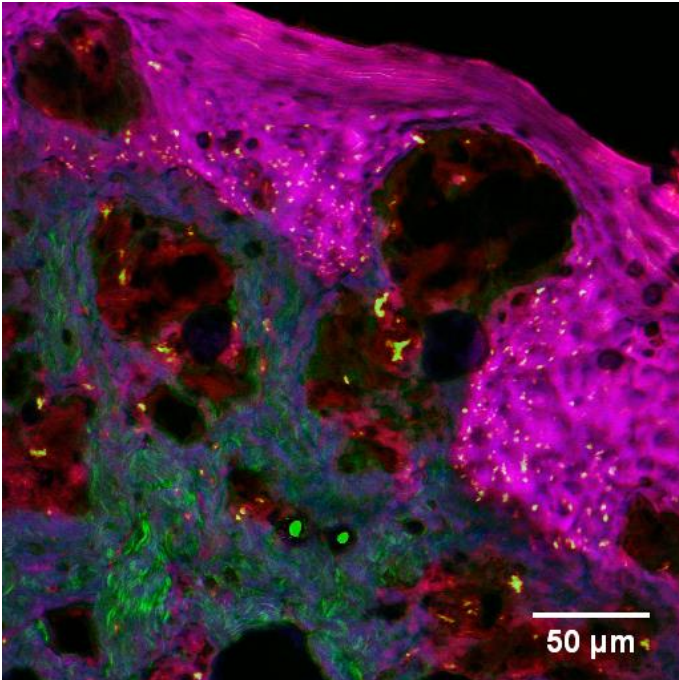
Approach:
Non-invasive Raman measurements, validated with MSI which offers greater chemical specificity to facilitate a deeper understanding.

Challenges

1. **Signal loss with depth**, due to increased scattering and absorption of the beam, also complicated by changes in the confocal volume. Compare data from endogenous skin components in 3D tissue (optical sectioning achieved with confocal microscopy). vs 2D physical cross section.
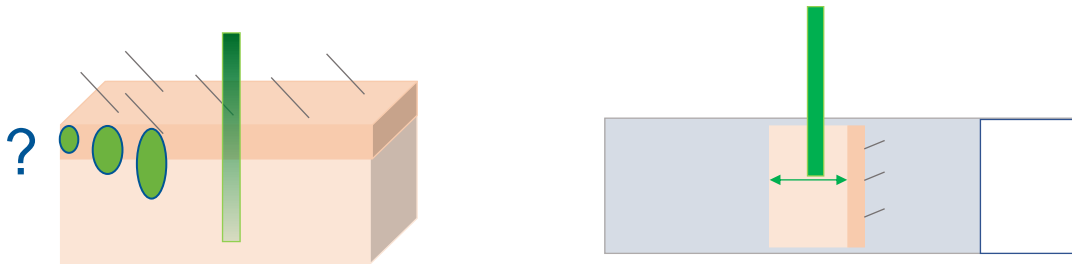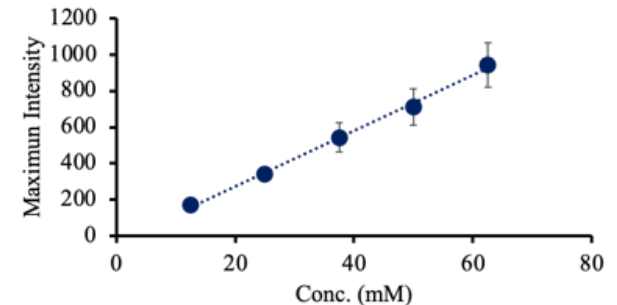

Endogenous signals e.g. amide I can be used as internal standards to correct the data for losses with depth.

2. **Separate drug signals from endogenous species**

A. start with unique chemistries

B. Use spectral unmixing techniques for chemistries shared with endogenous species
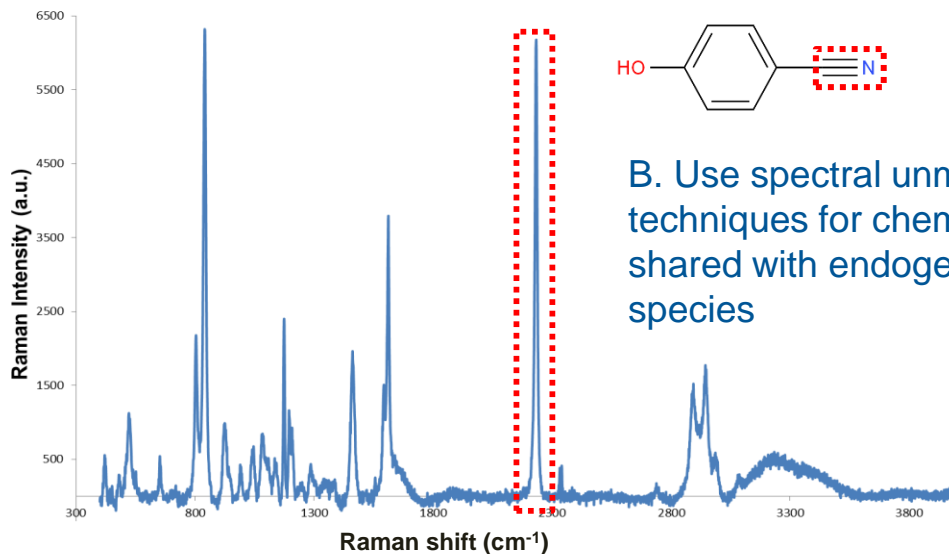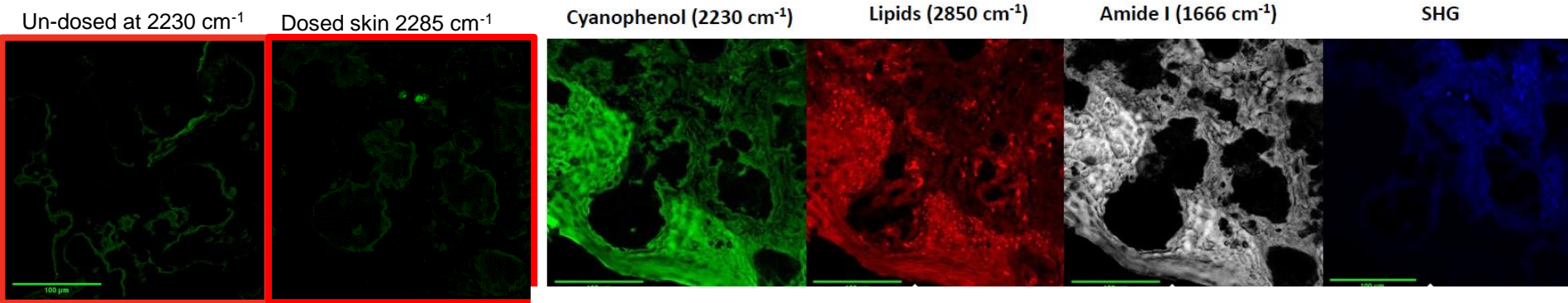
3. **Absolute concentration?**

ID & removal of spurious signals
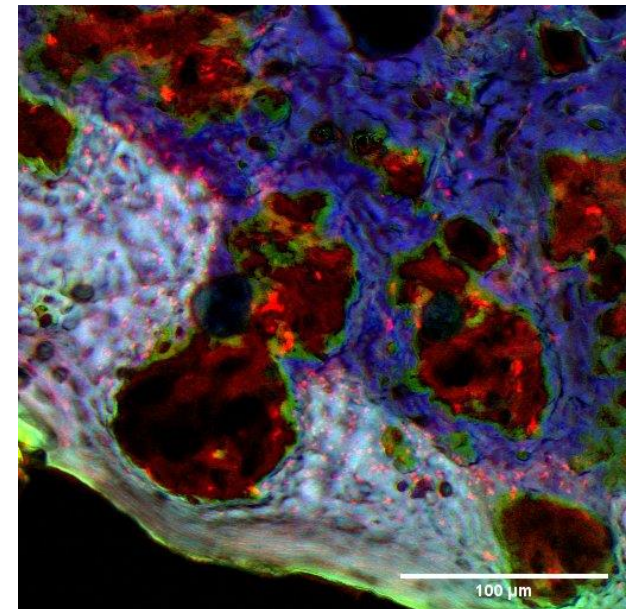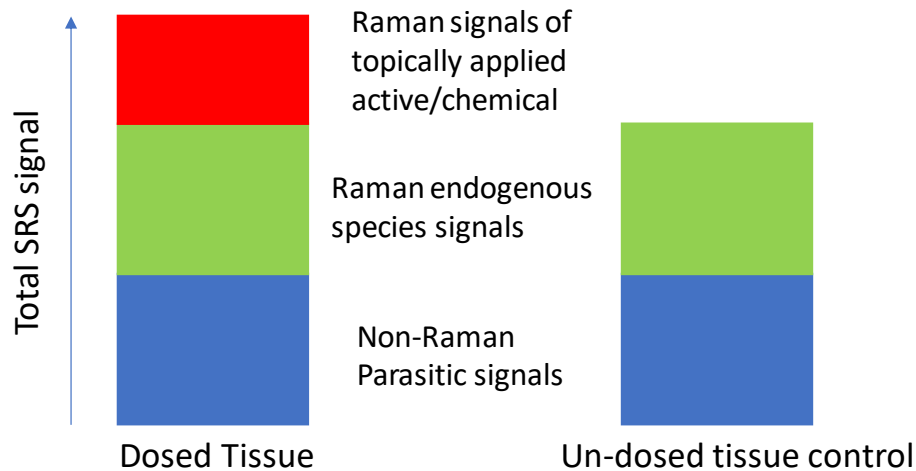
Multiple wavenumbers acquired sequentially; potential for artefacts relating to movement
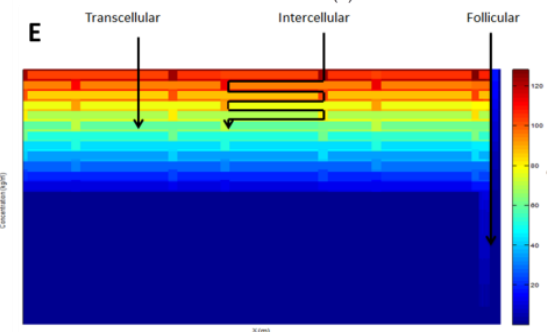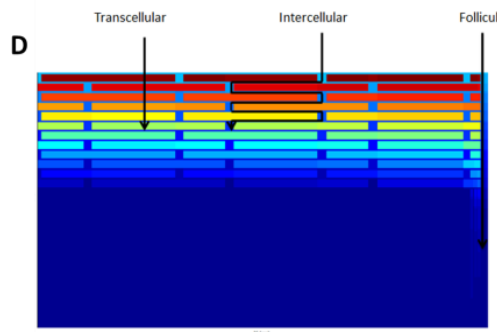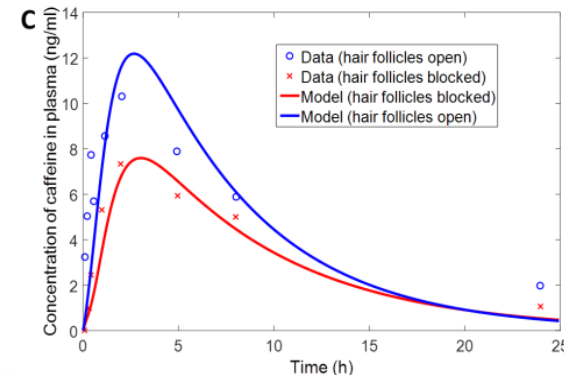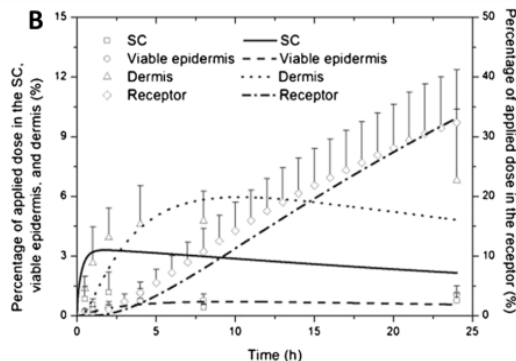
Un-dosed at 2230 cm^{-1}

Dosed skin 2285 cm^{-1}

Cyanophenol (2230 cm^{-1})

Lipids (2850 cm^{-1})

Amide I (1666 cm^{-1})

SHG

controls

Composite

Total signals in each pixel of your images:

Total SRS signal

Raman signals of topically applied active/chemical

Raman endogenous species signals

Non-Raman Parasitic signals

Dosed Tissue

Un-dosed tissue control

SRS-*In silico* modelling
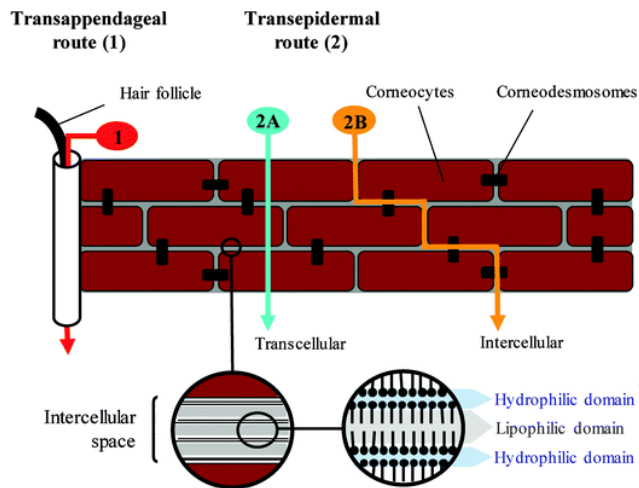
SRS: chemical pathway visualisation
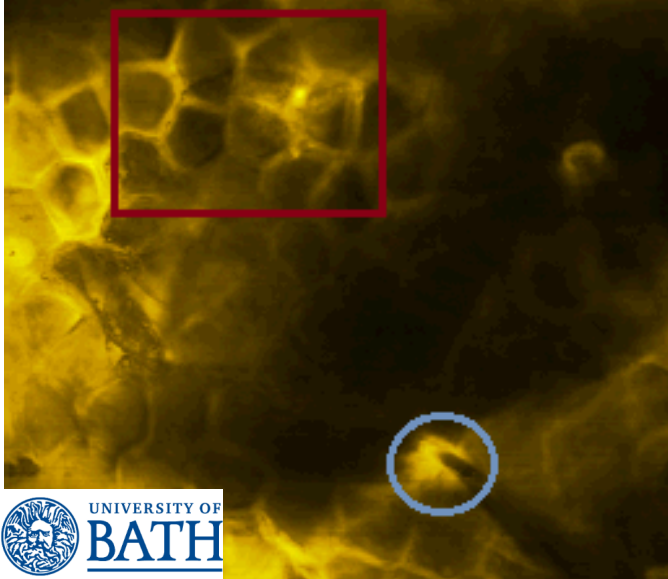
- SRS microscopy to provide new spatial information to inform & refine *in silico* models
- **Better prediction tools** for dermal uptake and iv/iv correlation (**enhanced drug delivery & chemical safety**)
- More confidence in models will translate to **reduced animal use**

Acknowledgements

- Dr Dimitrios Tsikritsis (NPL)
- Dr Jean-Luc Vorng (NPL)
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- Dr Alice Maciel Tabosa (Bath)
- Dr Pauline Vitry (Bath)
- Prof. Annette Bunge (Colorado)
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