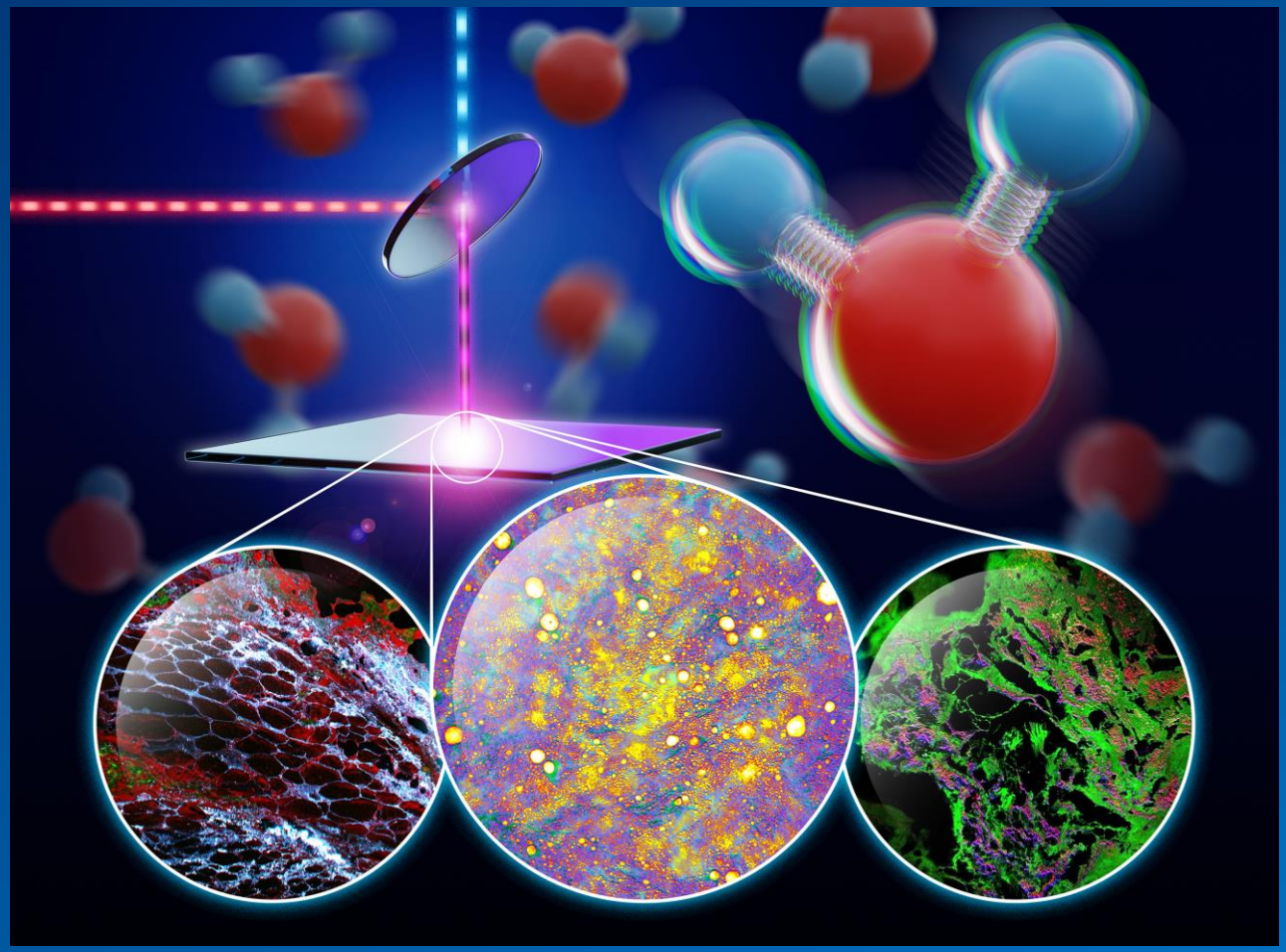
NPL
National Physical Laboratory

UNIVERSITY OF SURREY

Visualization of Topical Drug Delivery with Label-Free Chemical Imaging



Natalie Belsey

National Measurement Institute of the UK

NPL

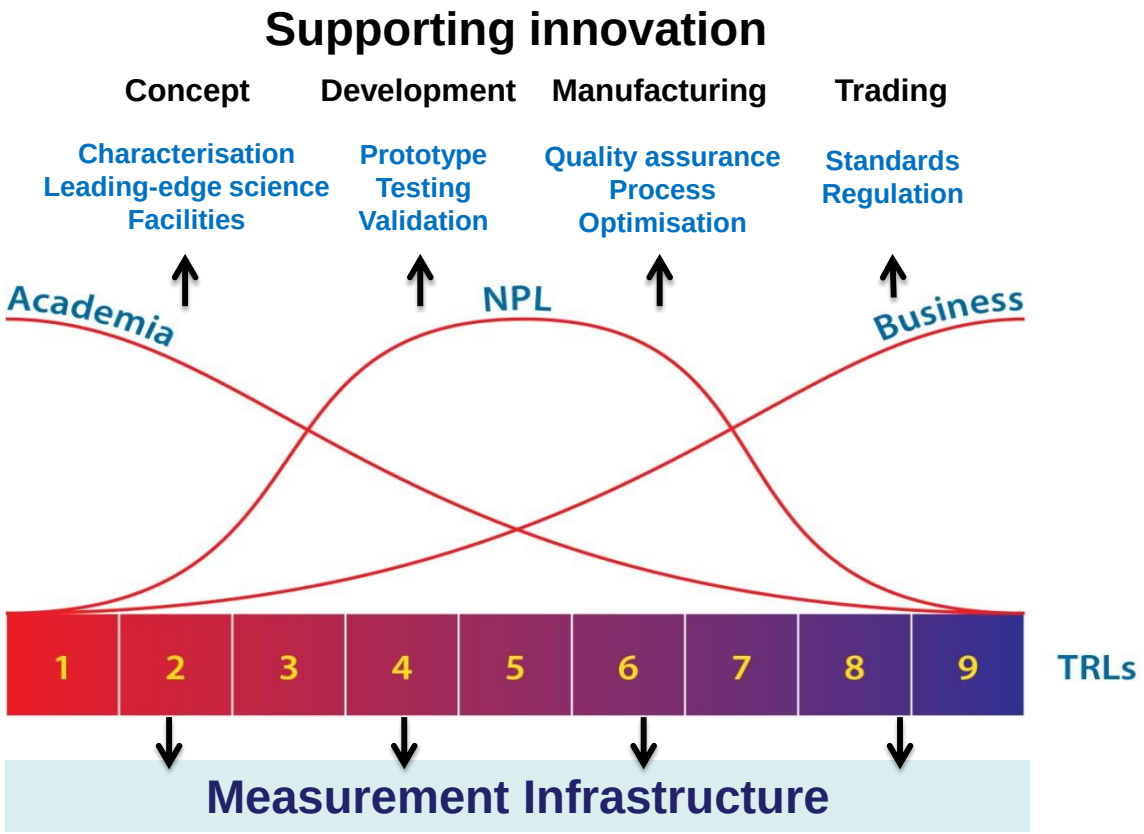
- Develop & disseminate UK's measurement standards
- Multidisciplinary R&D and technical services for public and private sector
- Knowledge transfer between industry, government and academia

Department for Business, Energy & Industrial Strategy

FUNDED BY BEIS

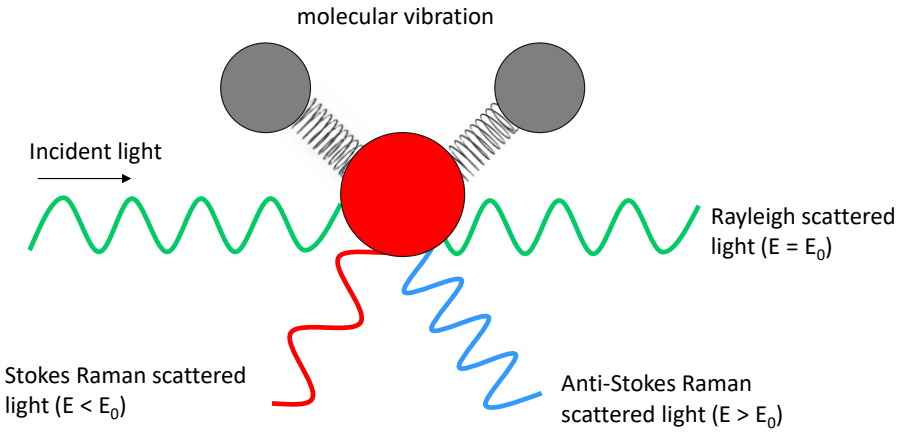
Innovate UK

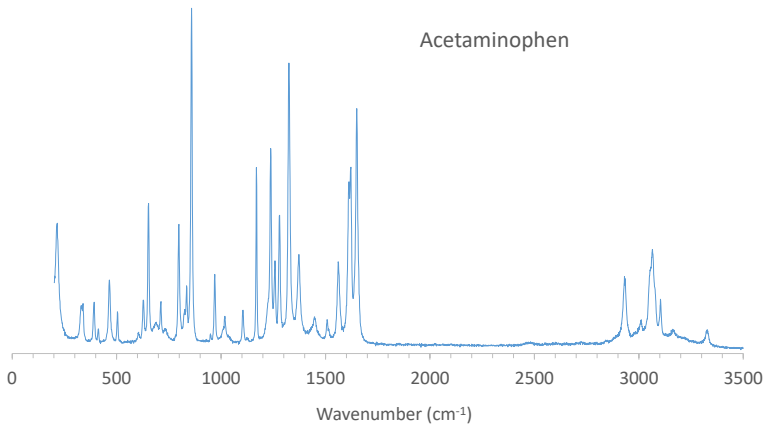
A4I Analysis for Innovators

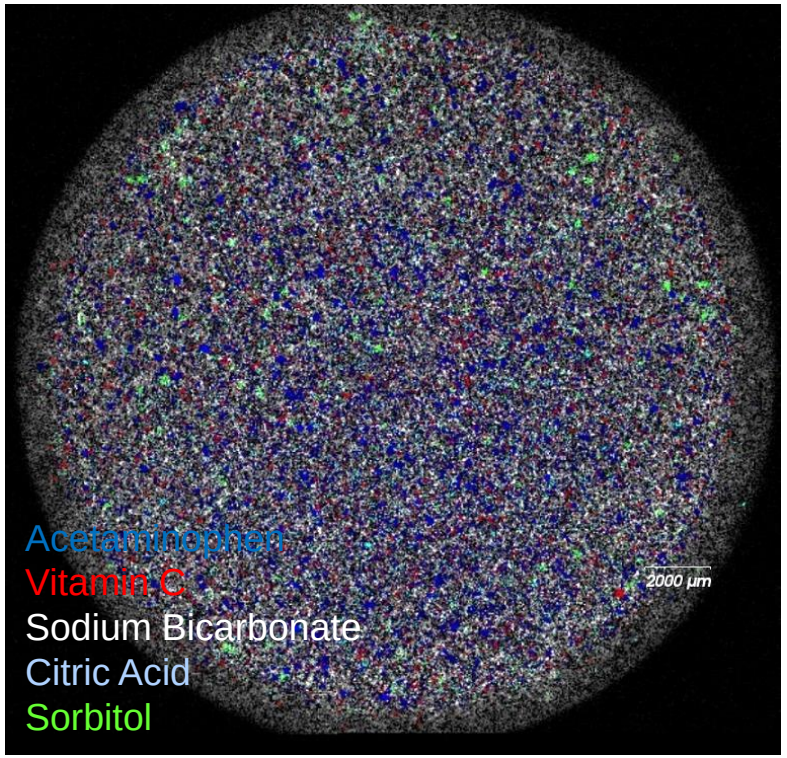


Measurement tool: Raman scattering

- 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, non-invasive/destructive, possibility for in-line & *in vivo* measurements.



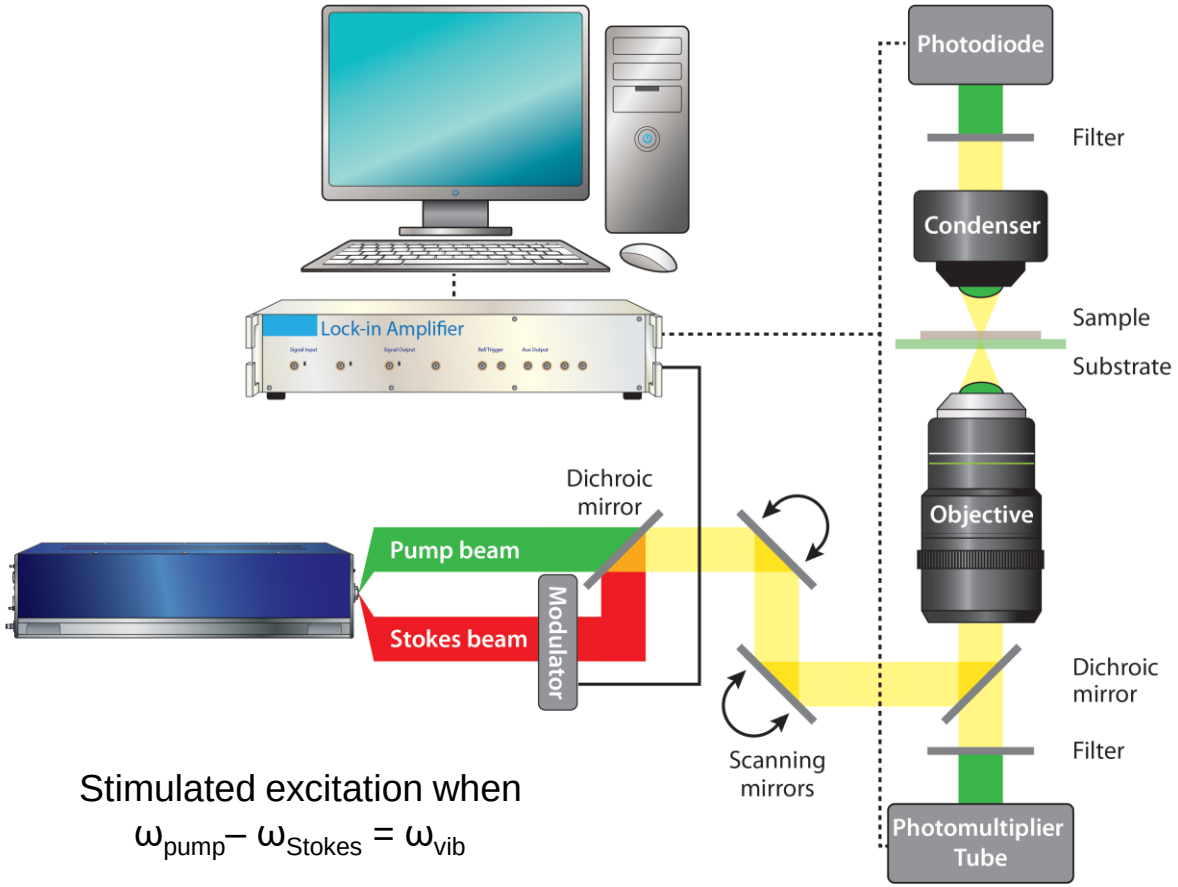




Well-established method for chemical measurements and mapping
….but too slow for high-resolution imaging studies of dynamic systems

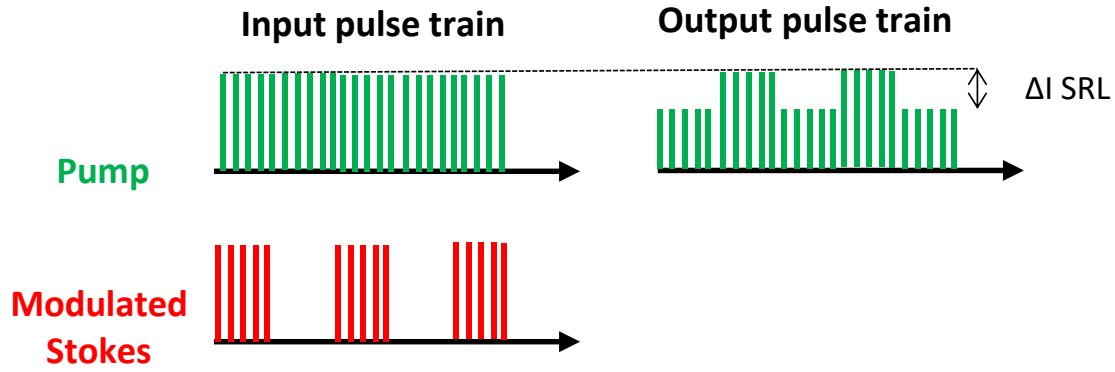
Stimulated Raman scattering (SRS) microscopy

- Label free, ambient, non-destructive video rate chemical imaging.
- SRS signal is linear with chemical concentration: prospects for non-invasive quantitative chemical analysis.



Stimulated excitation when

$\omega_{pump} - \omega_{Stokes} = \omega_{vib}$



Analyst

ROYAL SOCIETY OF CHEMISTRY

TUTORIAL REVIEW

Cite this: DOI: 10.1039/d2an00817c

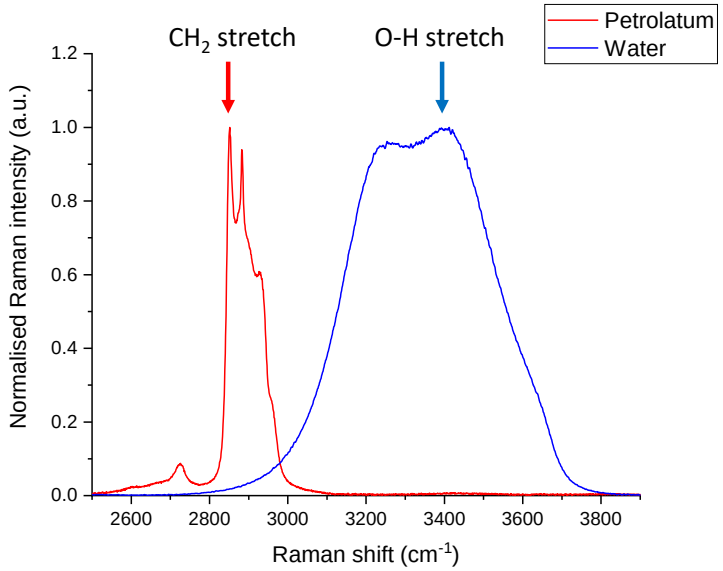
Practical considerations for quantitative and reproducible measurements with stimulated Raman scattering microscopy†

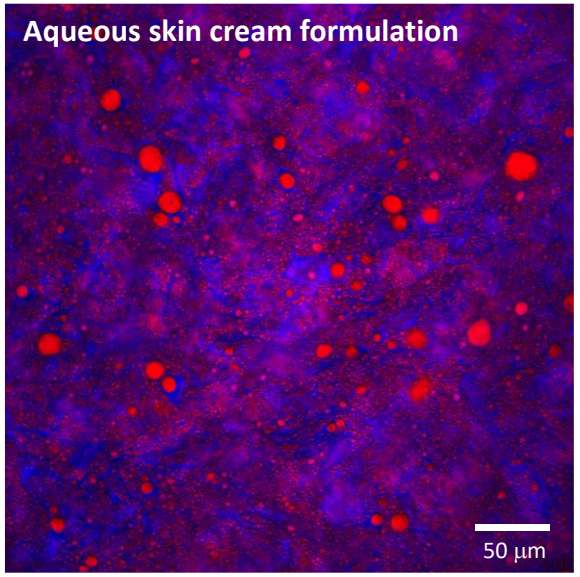
Dimitrios Tsikritsis, [a] Elizabeth J. Legge [a] and Natalie A. Belsey *[a,b]

Stimulated Raman scattering (SRS) microscopy provides rapid label-free 3D chemical imaging with wide-ranging applications including histology, pharmacokinetic studies, and materials characterisation. SRS microscopy has seen a steady increase in utilisation since the early 2000s and has become more accessible due to the increase in availability of facilities, and the development of user-friendly instrumentation. Although some complete SRS systems are now commercially available, many instruments are home-built with highly varied laser sources, optics, and detection mechanisms. Signal intensity is also dependent on the effective spatiotemporal overlap of two (or more) laser beams, thus any drift in alignment can result in

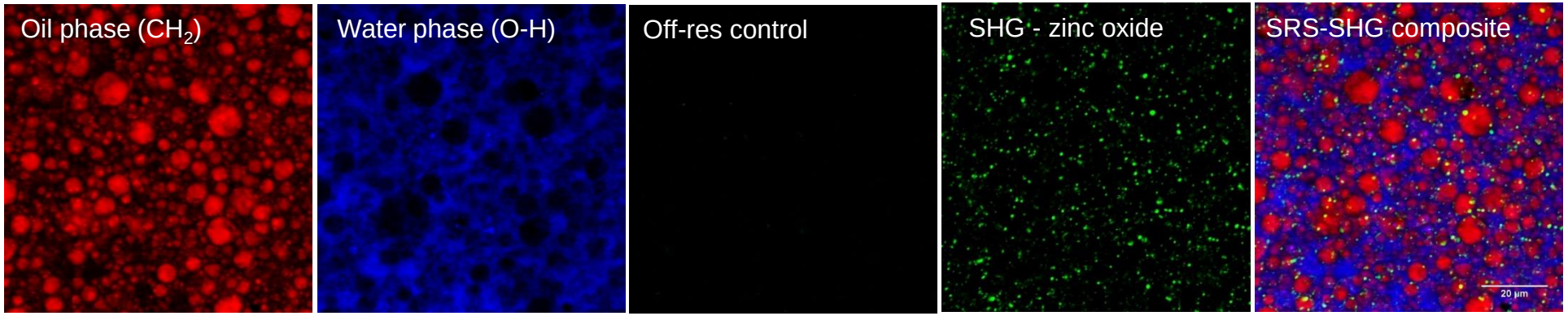
Confocal Raman to SRS workflow

1. Acquire Raman spectra of ingredients
2. Select peaks of interest (& controls) and tune SRS microscope to that wavenumber:

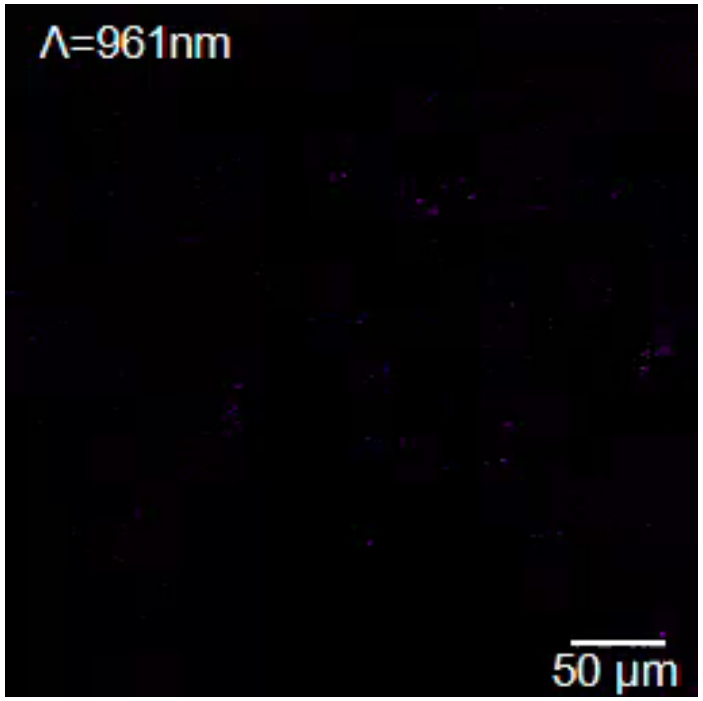


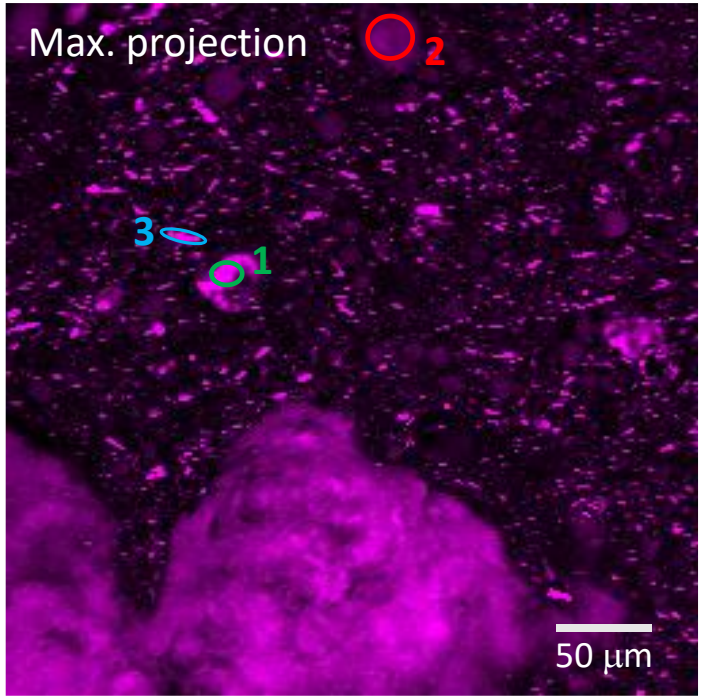


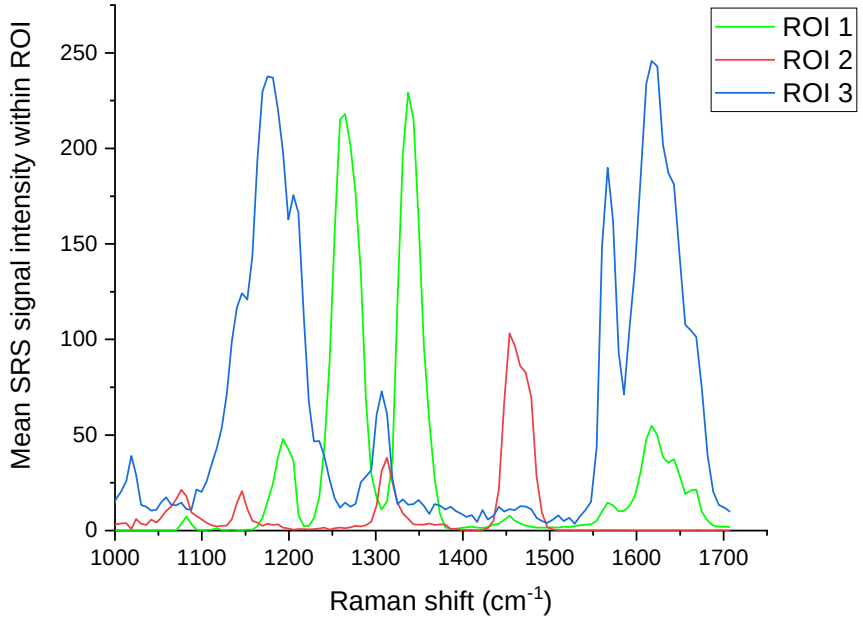
Sunscreen:



SRS spectra: Lambda scanning





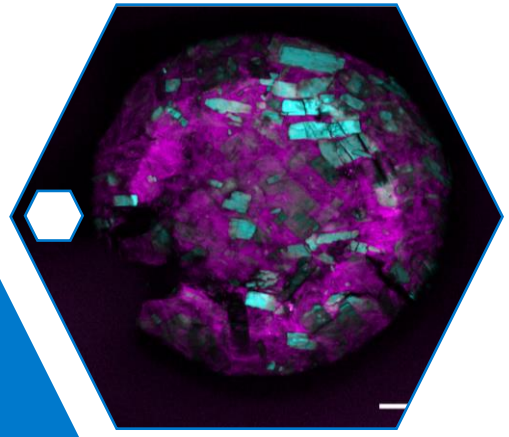


Generation of spectra by acquiring a library of images across a range of wavenumbers, ideal for:

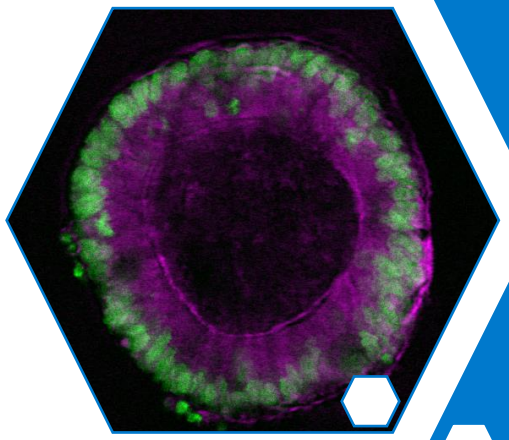
- Identification of ingredient co-localisation/structures formed
- crystalline phase including co-crystals
- Impurities & degradation products etc

Environmental sample chamber available to allow us to monitor temperature-dependent processes

Understanding Formulated Products & Therapies

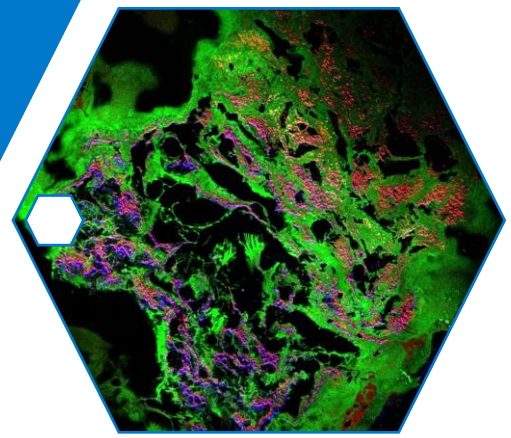


Drug form & distribution, stability of formulations



Formulation fate in biological systems

Effects of formulations on cells or tissues



Accelerate innovation and access to drugs & therapies

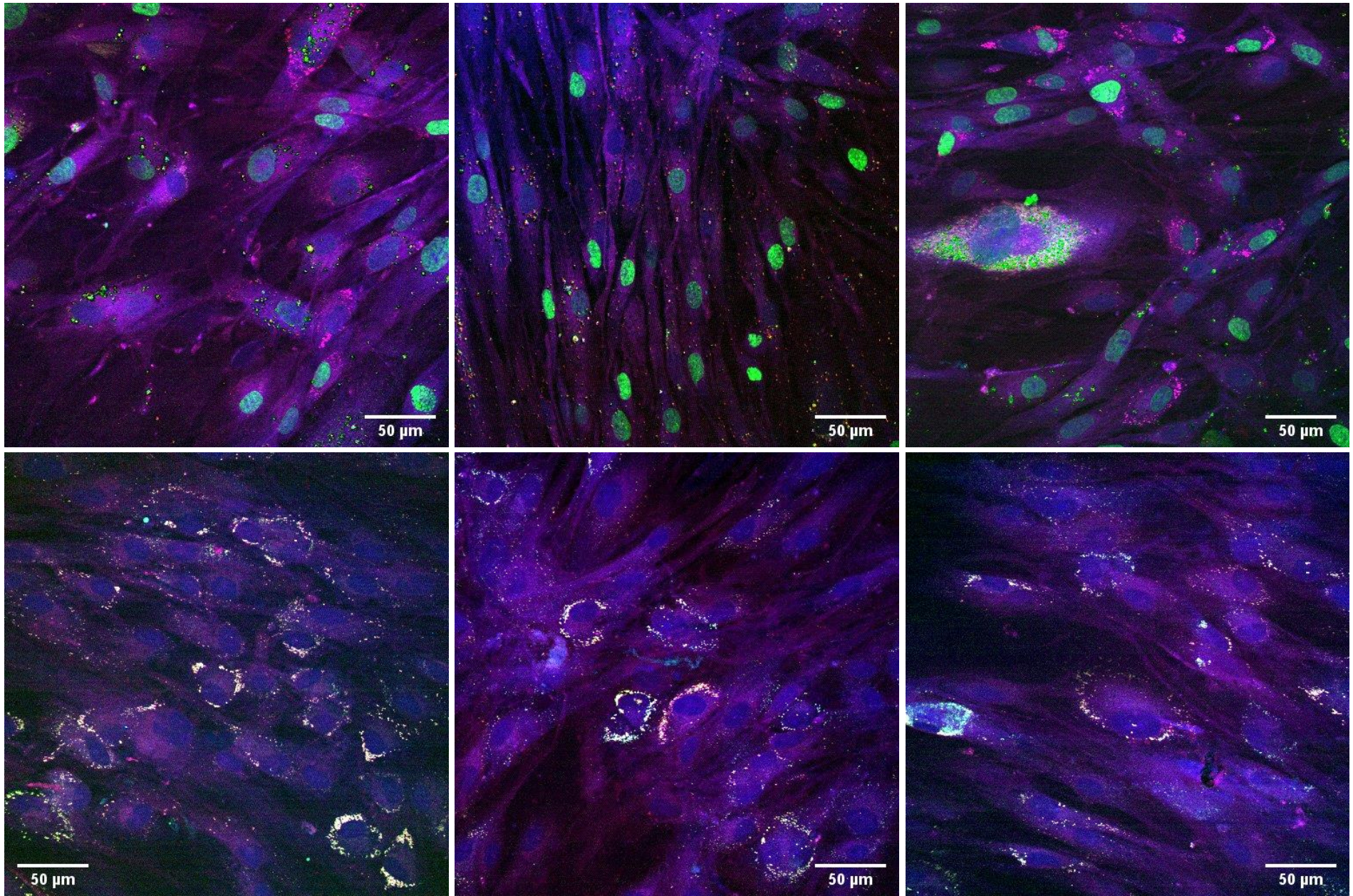
Accelerate innovation and access to medical devices & technologies

Visualising Cellular Response to Active Ingredients

No Active control (at 7 days)

SRS CH_3; SRS CH_2, SHG/TPEF

Active ingredient treatment (at 7 days)



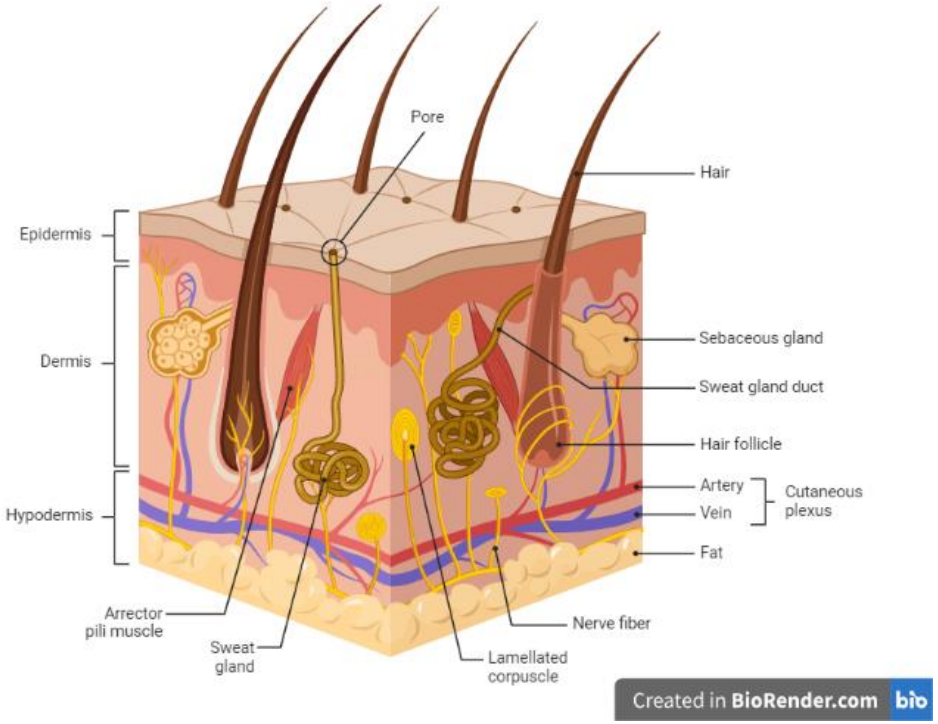
Formulation Fate: Topical drug delivery

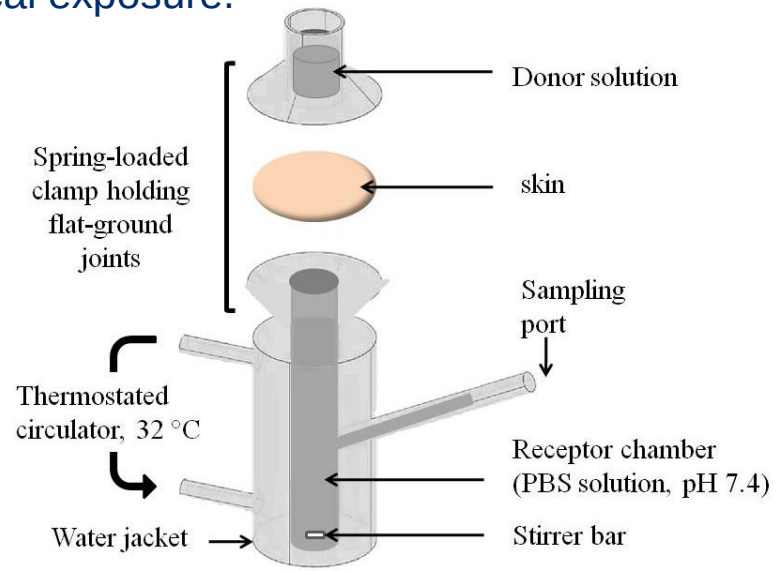
Topical and transdermal delivery is growing because of several advantages:

- Reduced side effects, bypassing the GI tract.
- Reduced drug-drug interactions compared to systemic medication.
- Continuous drug release from reservoirs in the skin.

Half of the population suffer from some form of skin disease, but many other therapies are delivered across skin.
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 chemical exposure.

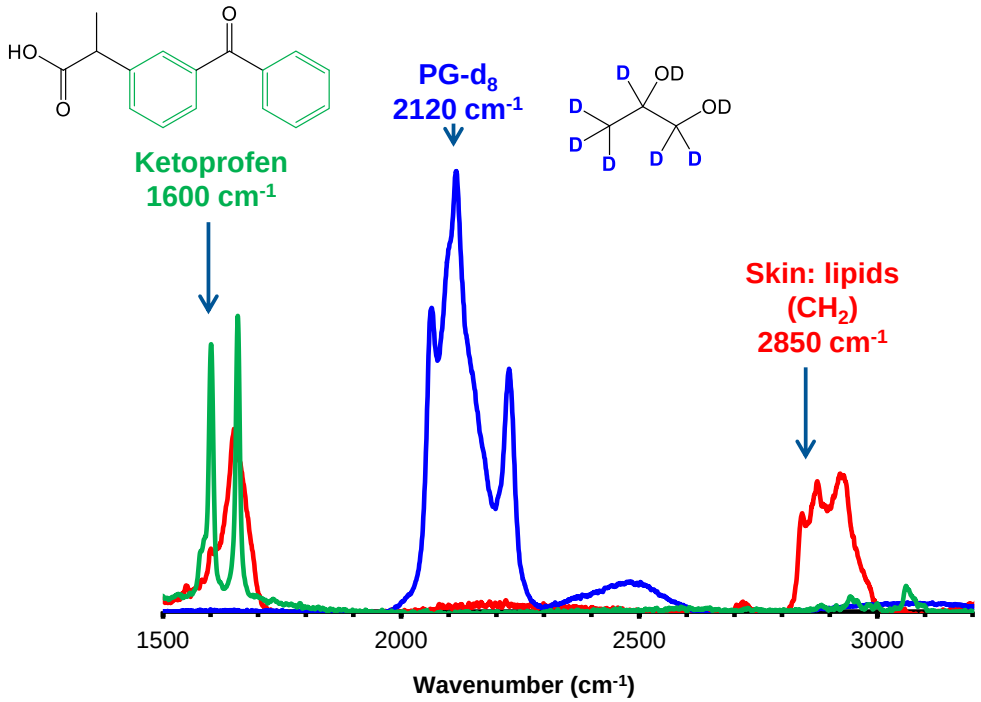


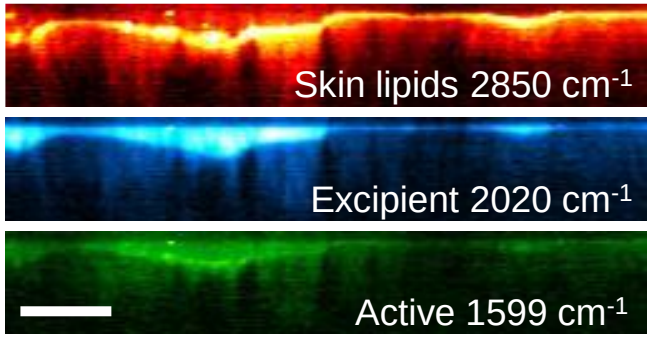


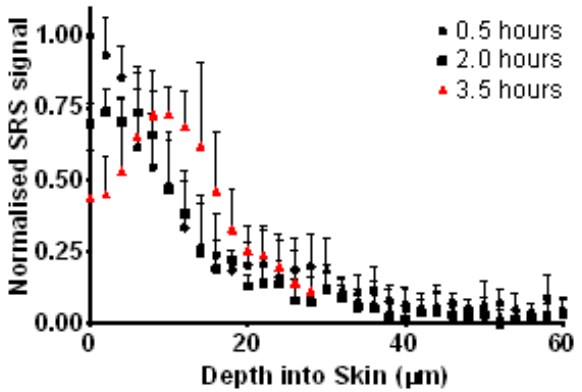
Destructive, laborious & invasive. No lateral resolution or mechanistic insight
Note fluorescence imaging unhelpful since chemical permeation kinetics depend on LogP and MW…

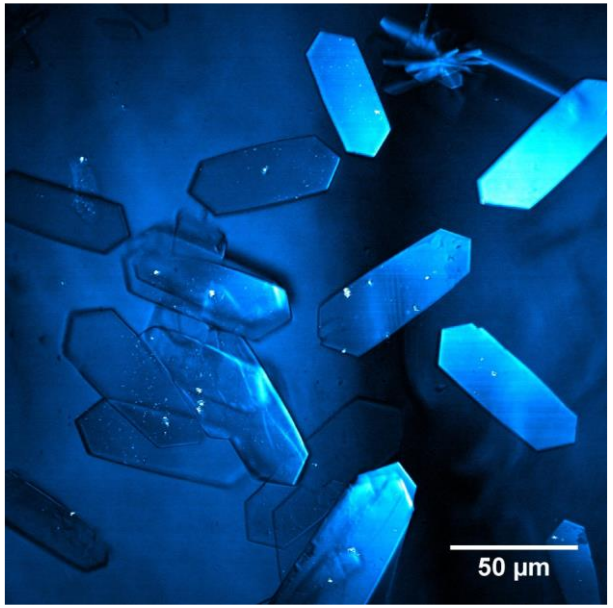
Measuring transdermal drug delivery with SRS

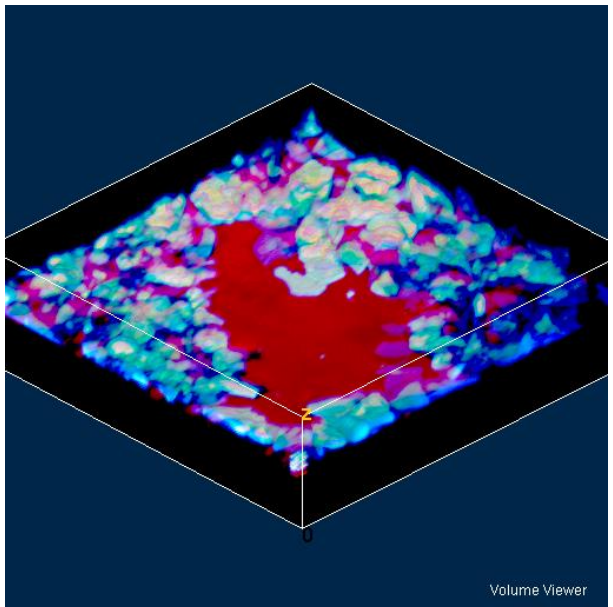
Proof of concept experiments tracking ketoprofen permeation through excised pig skin:











N. A. Belsey *et al*. Journal of Controlled Release, 2014, 174, 37-42.

Assessing the skin pharmacokinetics & the bio(in)equivalence of topical drugs

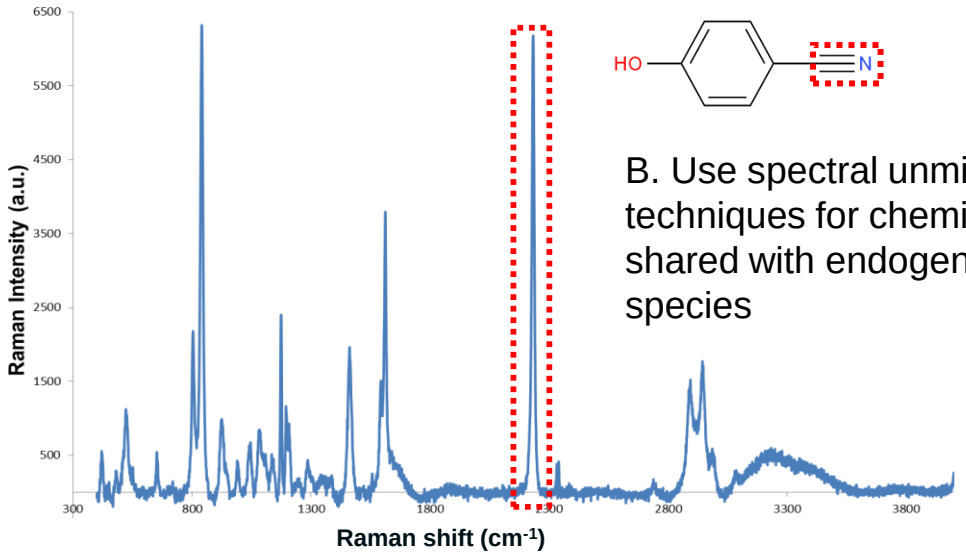
Aim: To characterise non-invasively the epidermal bioavailability of a topically applied drug and distinguish correctly between formulations that are bioequivalent and those that are not.

Confocal Raman sampling measurements supported by SRS microscopy & MSI for mechanistic insight.

Lots of measurement challenges!

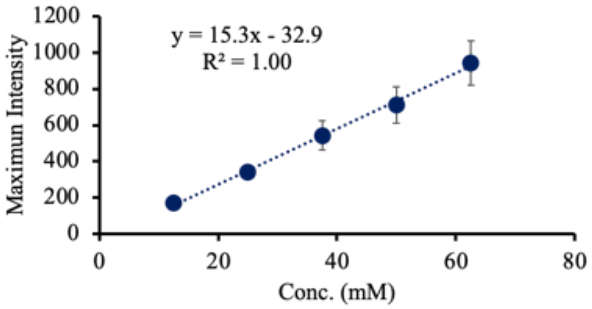
Separate drug signals from endogenous species?

A. start with unique chemistries



B. Use spectral unmixing techniques for chemistries shared with endogenous species

Absolute concentration?

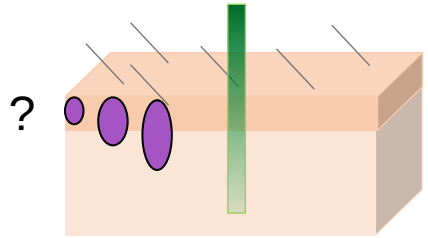


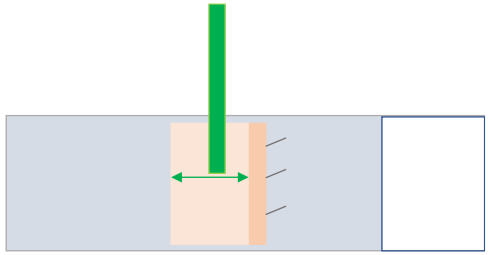
Dr Panagiota Zarmpi

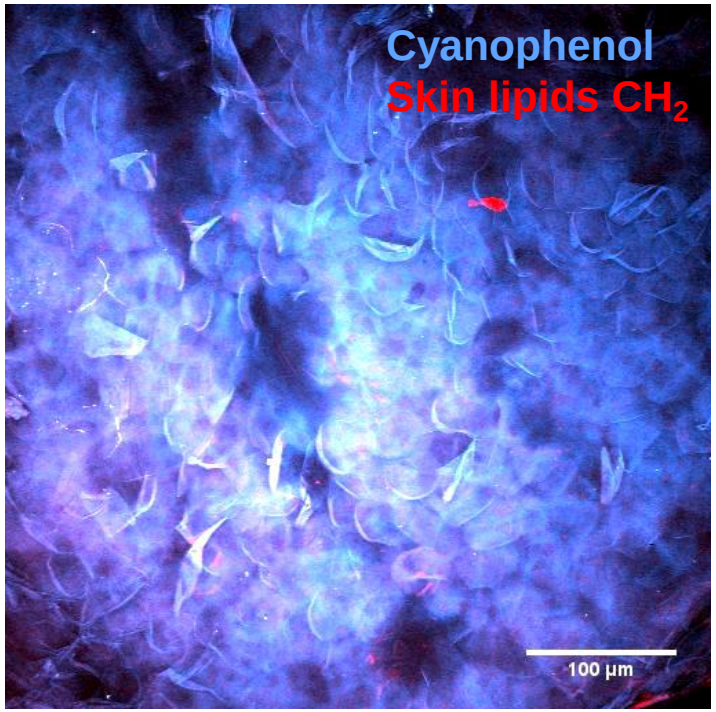
Losses with depth

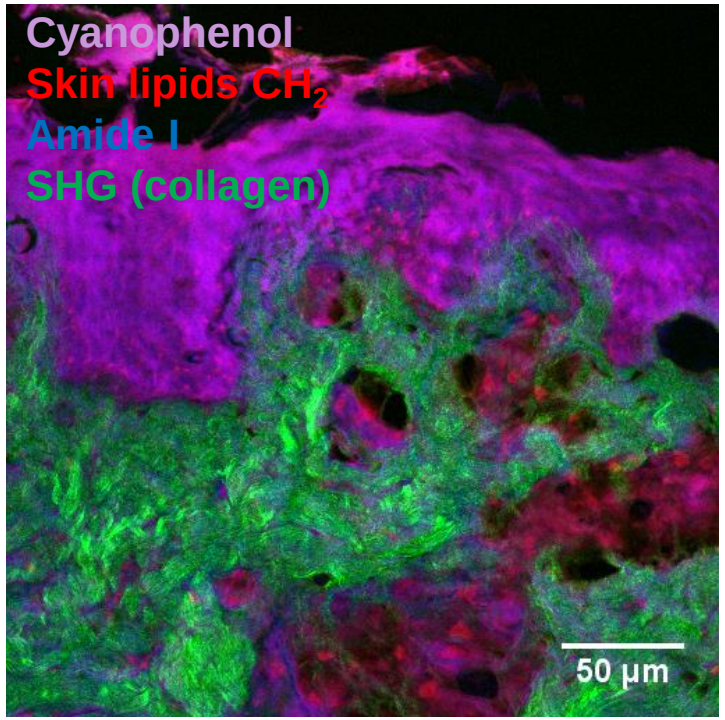
Signal loss with depth, due to increased scattering and absorption of the beam:

Compare data from endogenous skin components in 3D tissue (optical sectioning achieved with confocal microscopy). vs 2D physical cross section.



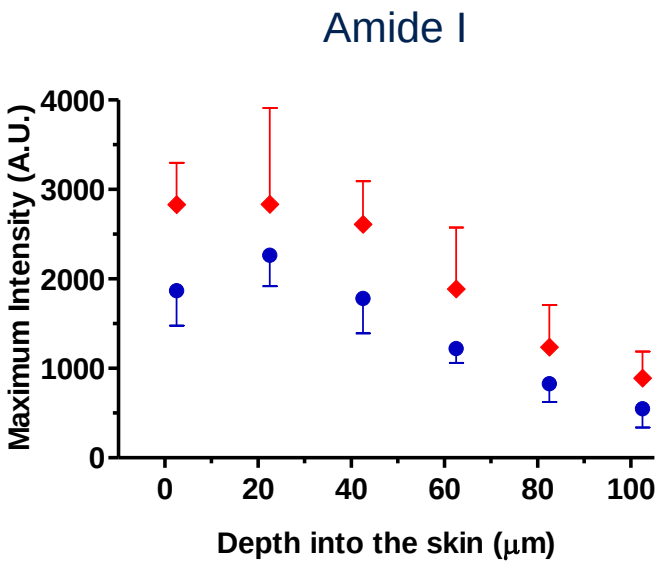


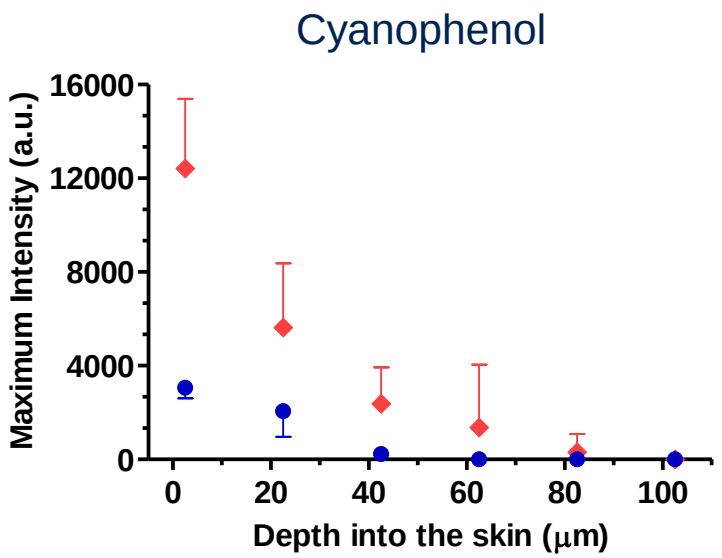


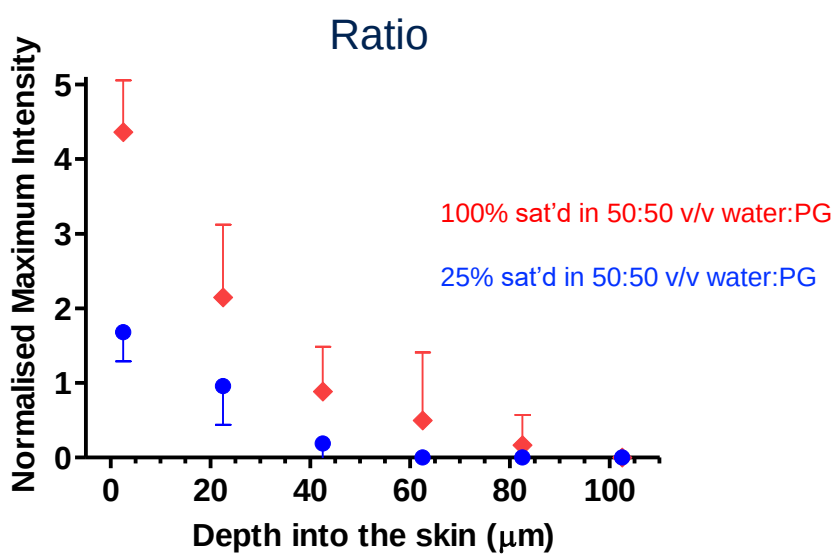


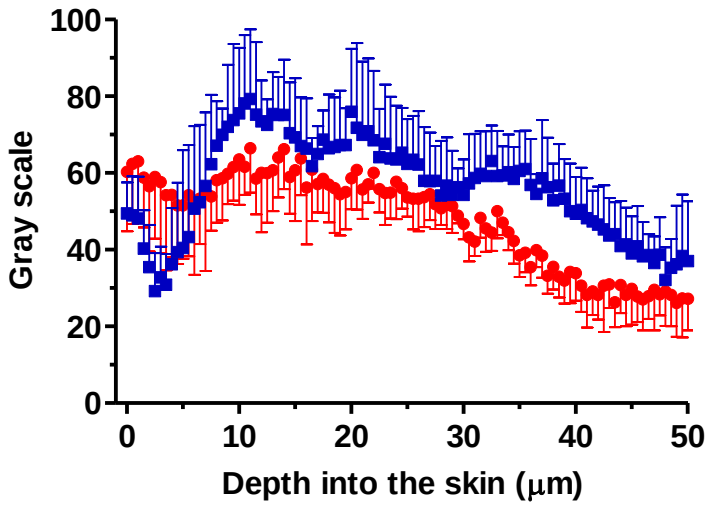
Endogenous signals can be used as internal standards to correct the data for losses with depth.

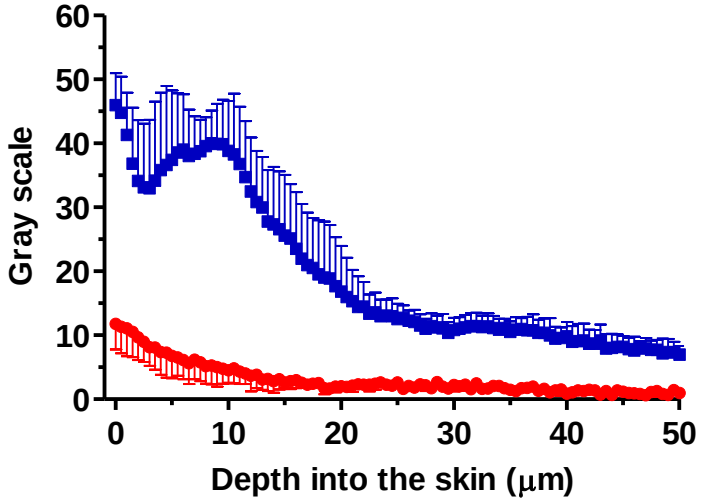
Cyanophenol: confocal Raman vs SRS

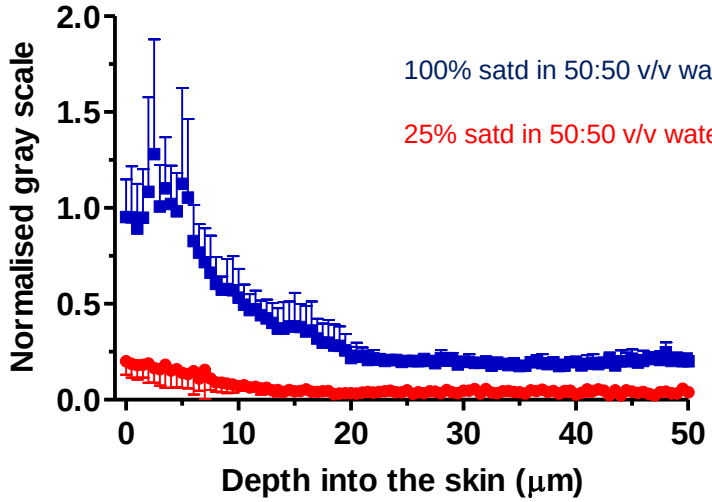






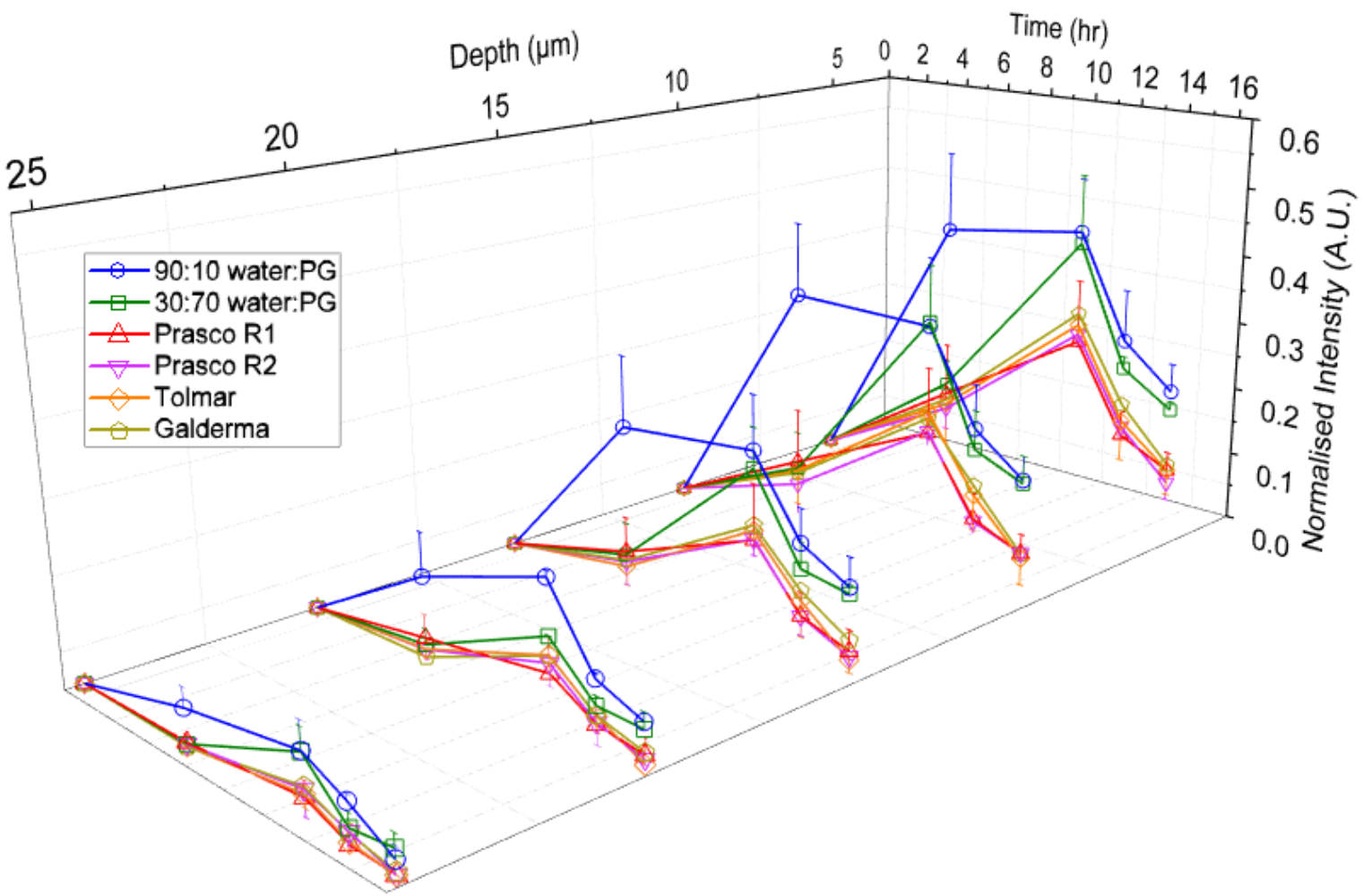




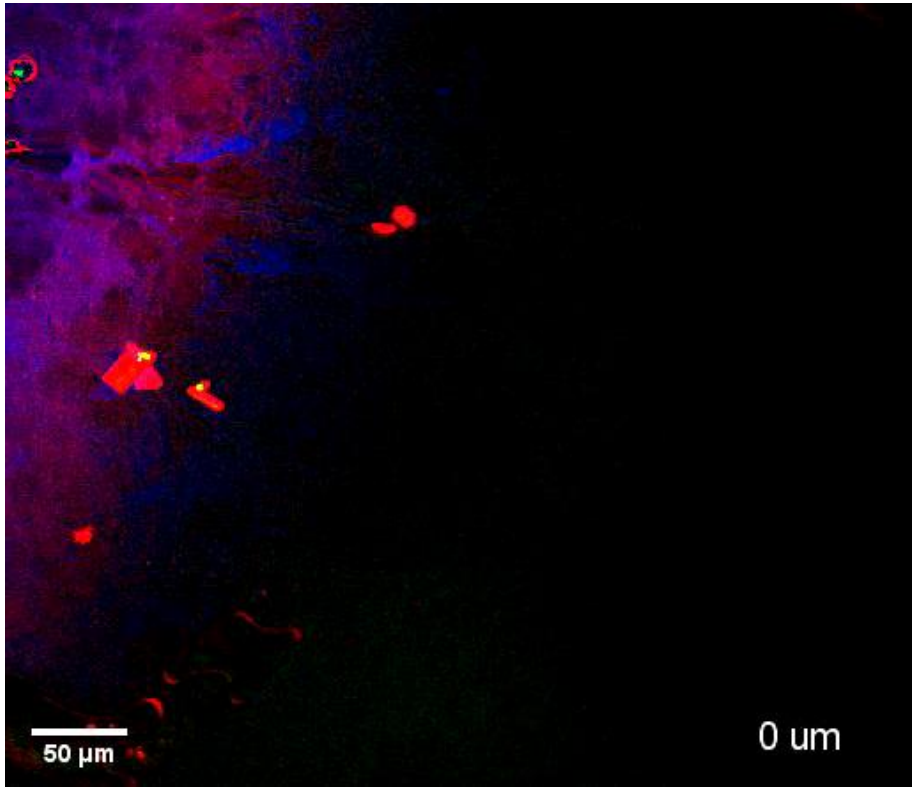


Metronidazole bioequivalence study results

Measured by confocal Raman (spectra)



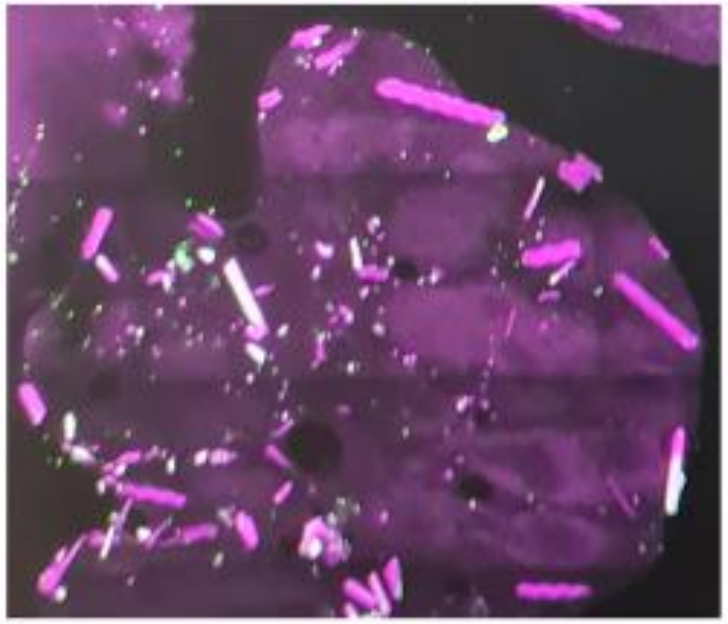
Measured by SRS microscopy (imaging)



MTZ
Skin proteins
Skin Collagen

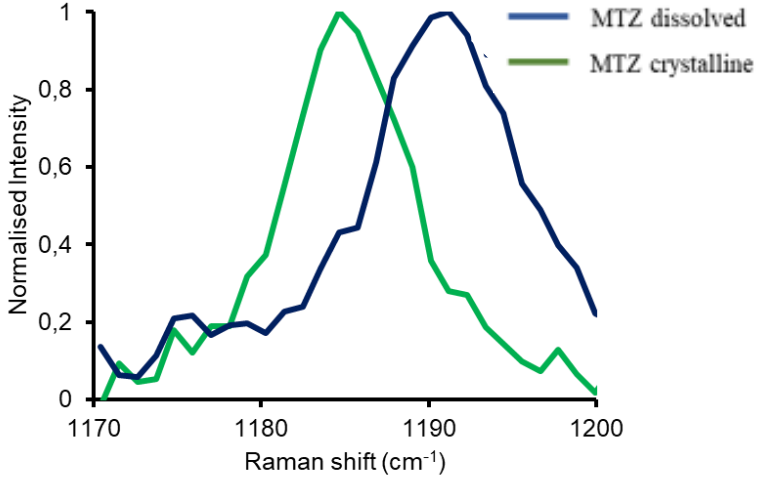
Measurement of Metamorphosis: Poster #30

Metronidazole in 90:10 v/v water:propylene glycol (PG)

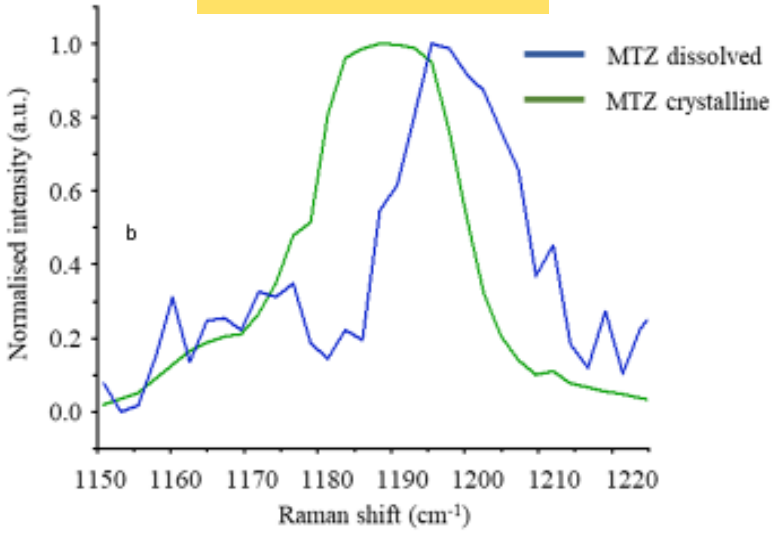


Tile view of the skin surface after treatment with the 90:10 v/v water/PG formulation for 6 hours

Confocal Raman



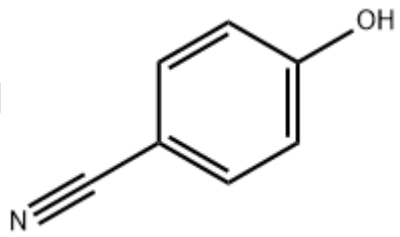
SRS

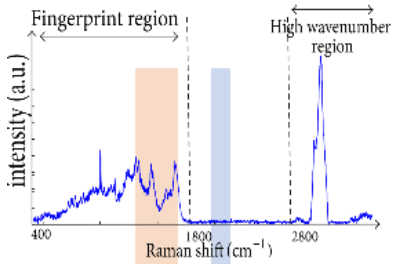


Can we trust multivariate data methods?

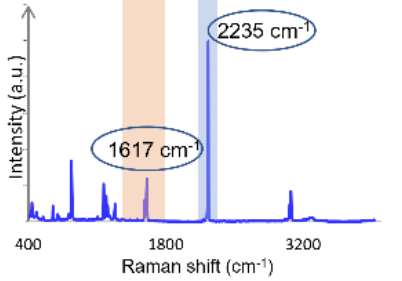
Testing spectral unmixing methods for skin analysis using molecules with unique chemistries

Cyanophenol

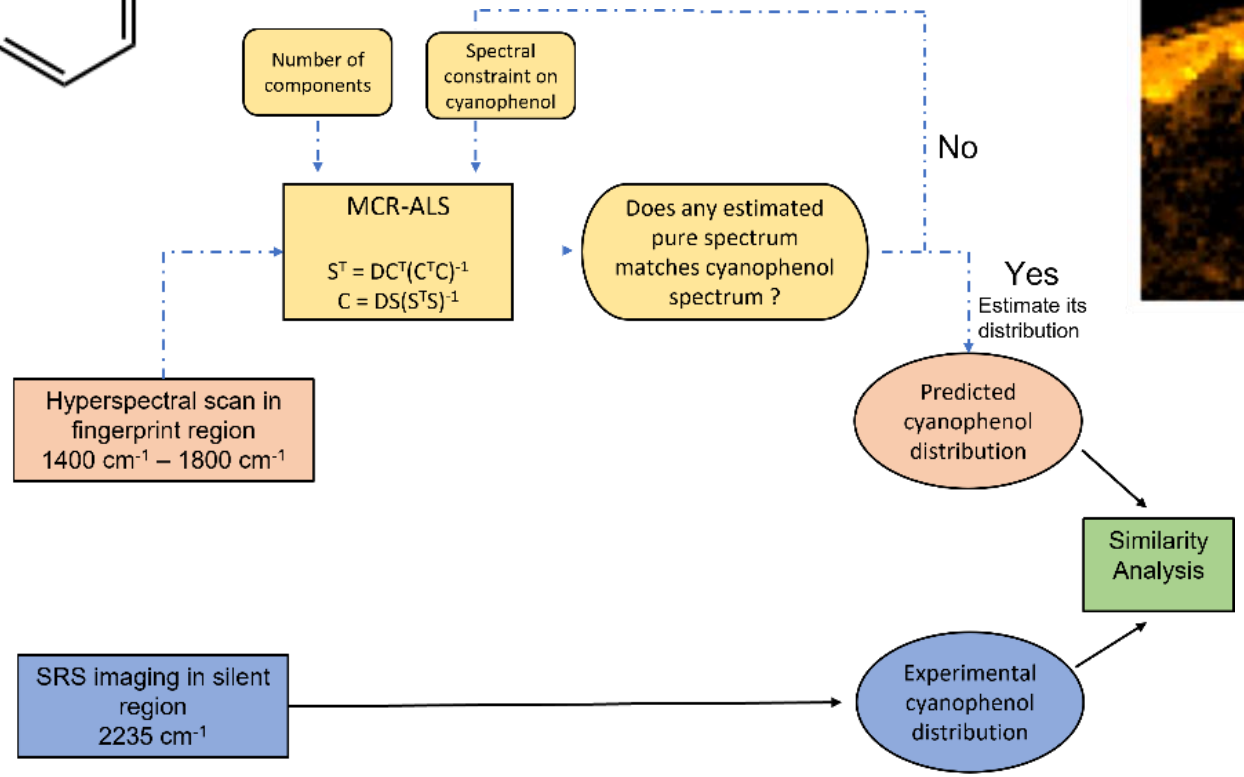


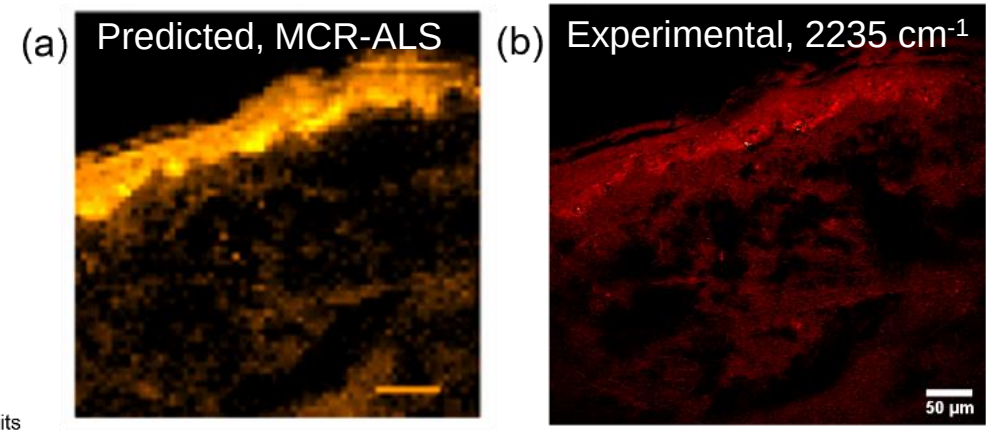


Raman spectra of skin



Raman spectra of 4-cyanophenol





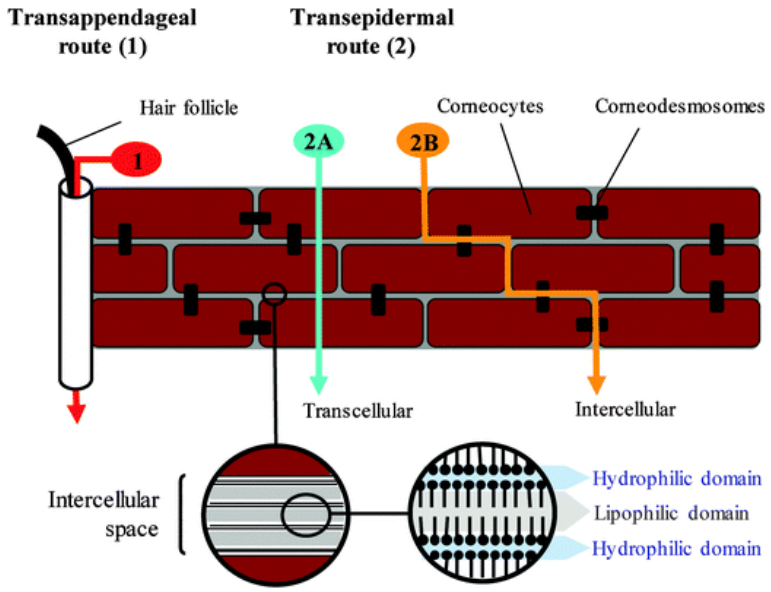
MCR-ALS reconstructed images agreed well with the experimentally-determined distribution. Poorer correlations with depth due to S/N. (using Pearson's correlation)

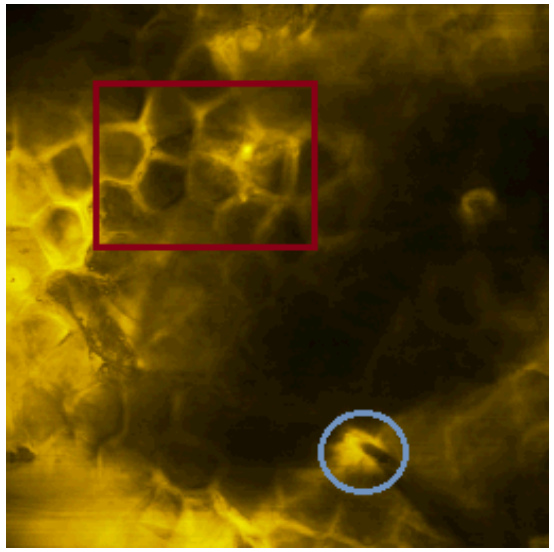
Measurement of Chemical Penetration in Skin using Stimulated Raman Scattering Microscopy and Multivariate Curve Resolution - Alternating Least Squares
A. Goel *et al*, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2023.

Prof. Tao Chen

Anukrati Goel

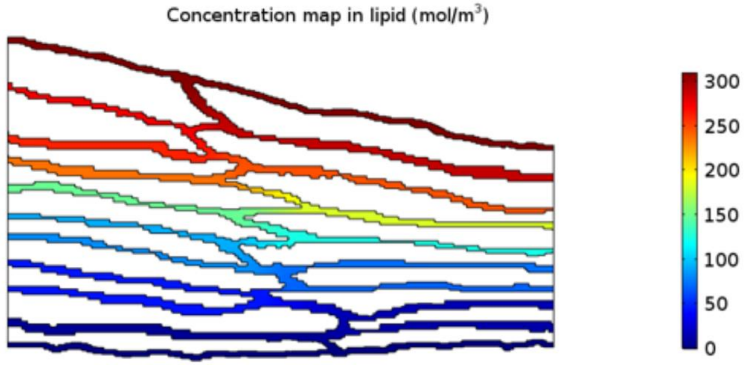
Integrating SRS data with *In silico* modelling: Poster #14

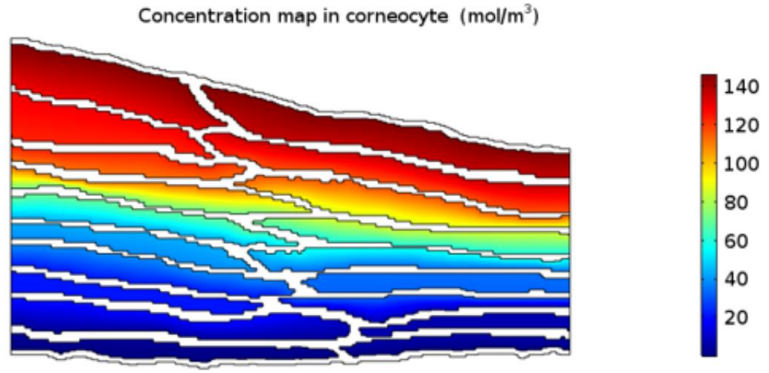




Saar et al, Mol. Pharmaceutics

- 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.**





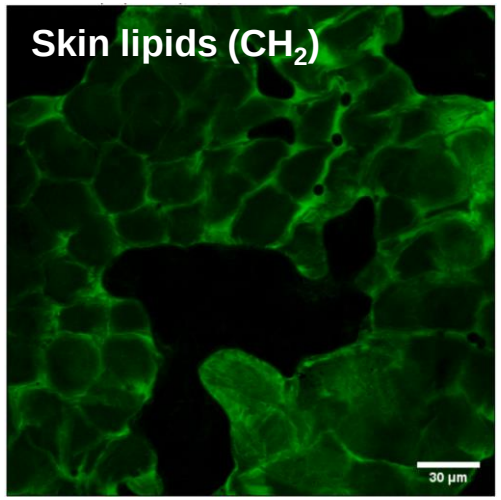
Unilever

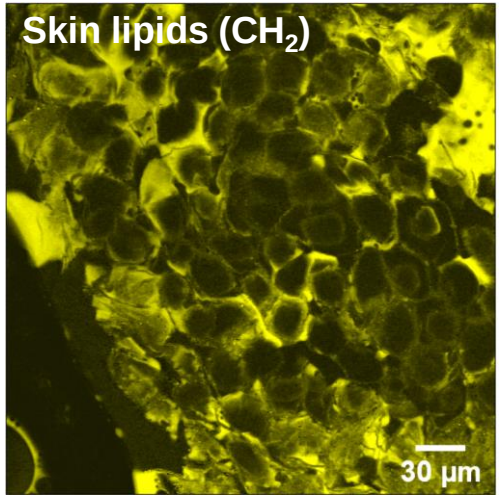
NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

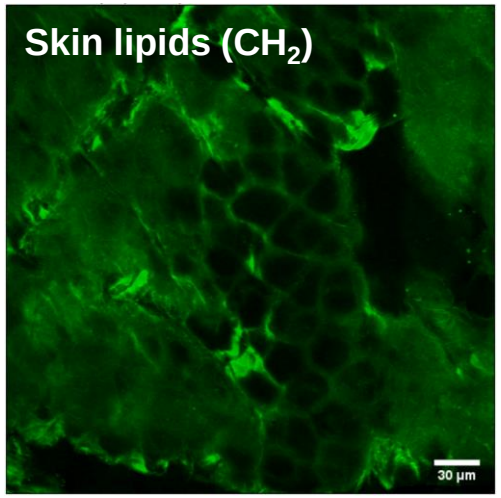
UNIVERSITY OF SURREY

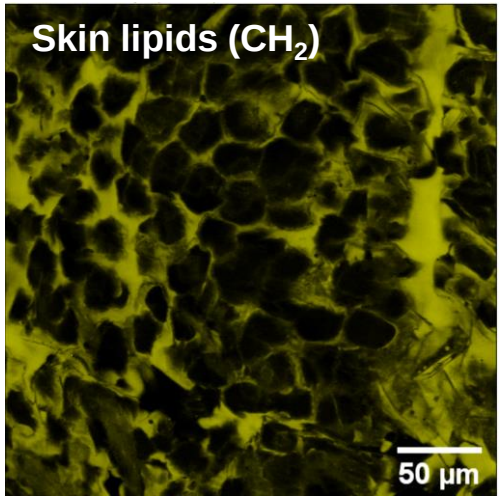
Permeation pathways in human skin

Test compounds selected to cover a range of MW and LogP:

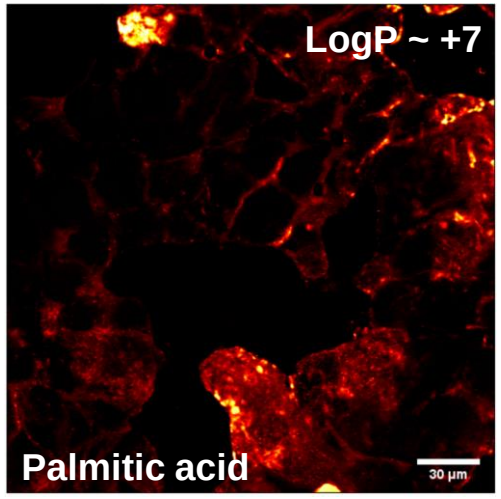


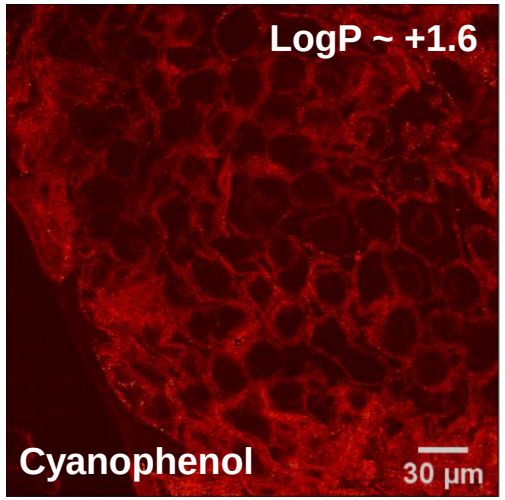


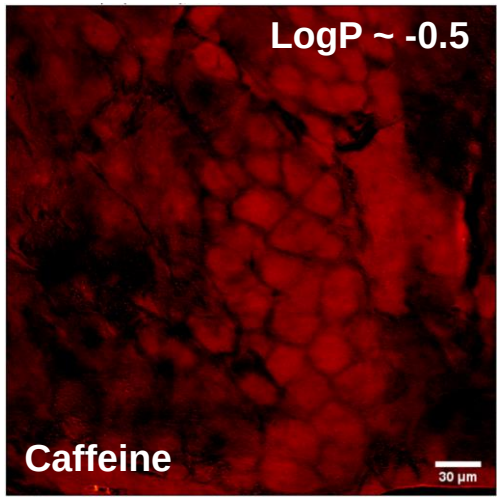


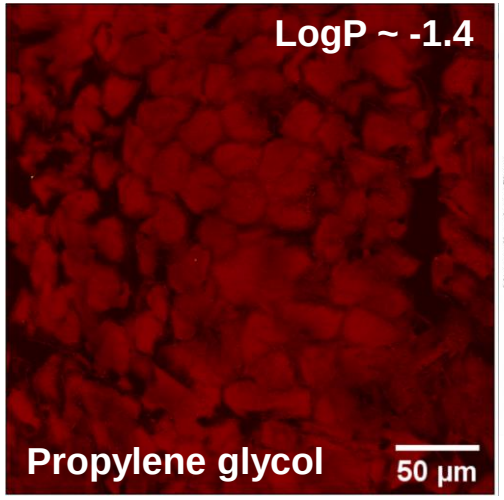


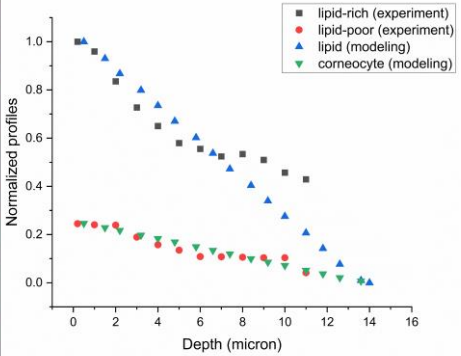
Partition factor $= \frac{AUC_{Lipid-rich}}{AUC_{lipid-poor}}$



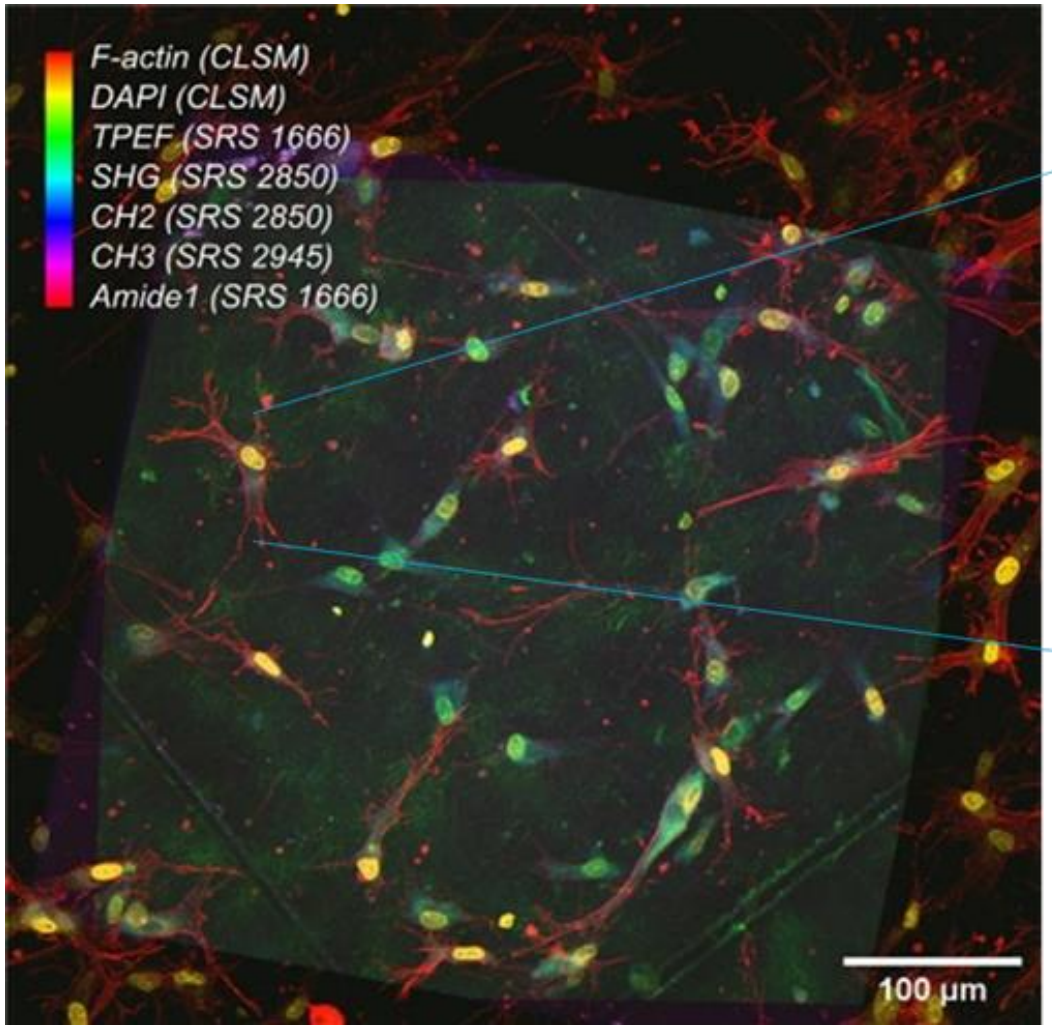




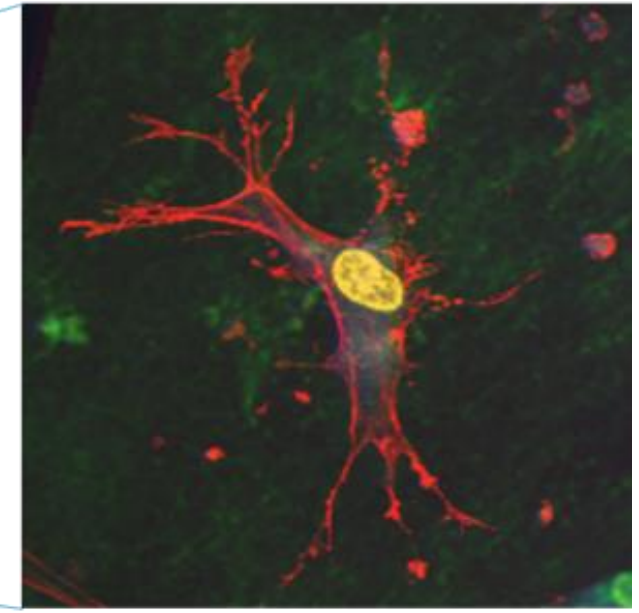




Correlative Imaging – why it's important?



NPL Correlative Imaging project



Dimitrios Tsikritsis (Camilla Dondi & Mike Shaw)

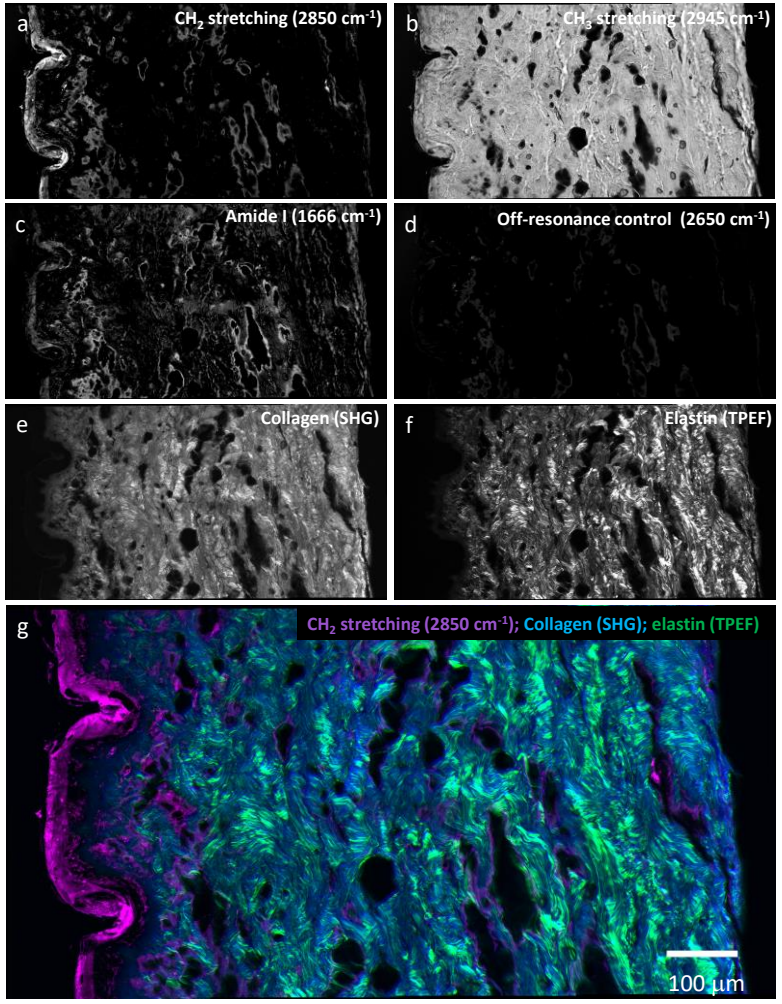
3D fibroblast culture model (collagen-based scaffold)

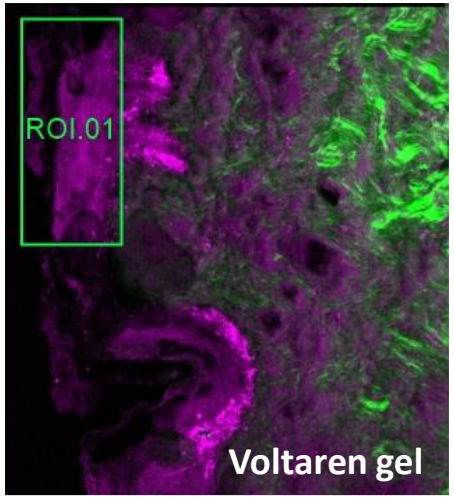
- **Learning more about our sample**: Maximise information by combining techniques that offer complementary information: e.g. spatial resolution vs. chemical specificity vs. chemical sensitivity.
- **Learning more about our instruments**: why don't the results match? E.g. artefacts from matrix effects, issues with tags, different LOD, different sample probe volumes, instrument conditions, e.g. ambient vs high vacuum, sample damage e.g. by the analysis beam.

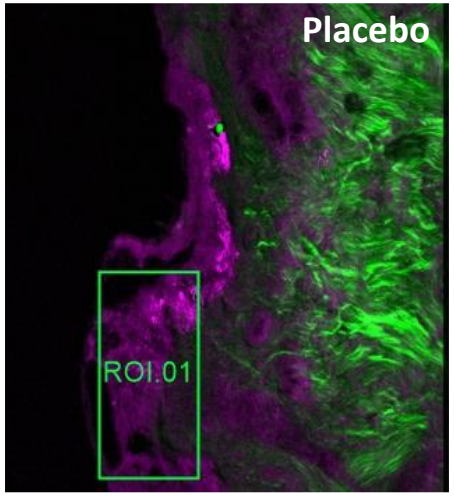
Correlative imaging example

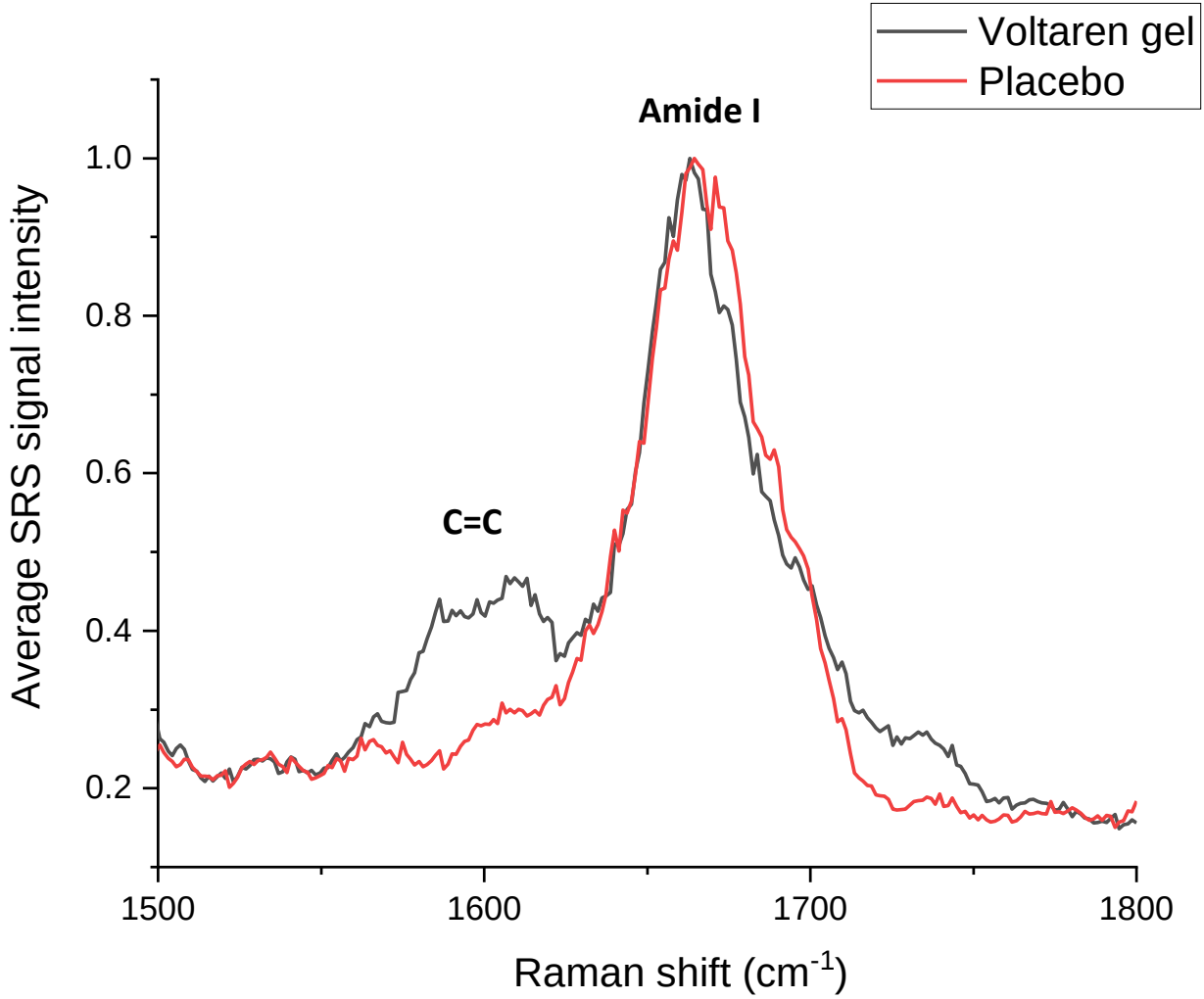
HALEON NPL

Visualization of diclofenac in skin requires sub micron spatial resolution coupled with high chemical sensitivity & specificity.
Need to combine this information in a correlative manner from several spectroscopic imaging techniques.

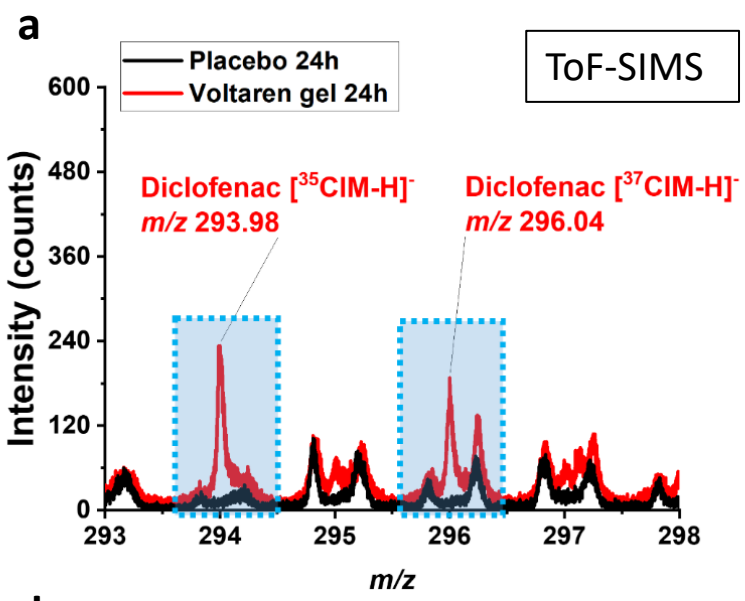


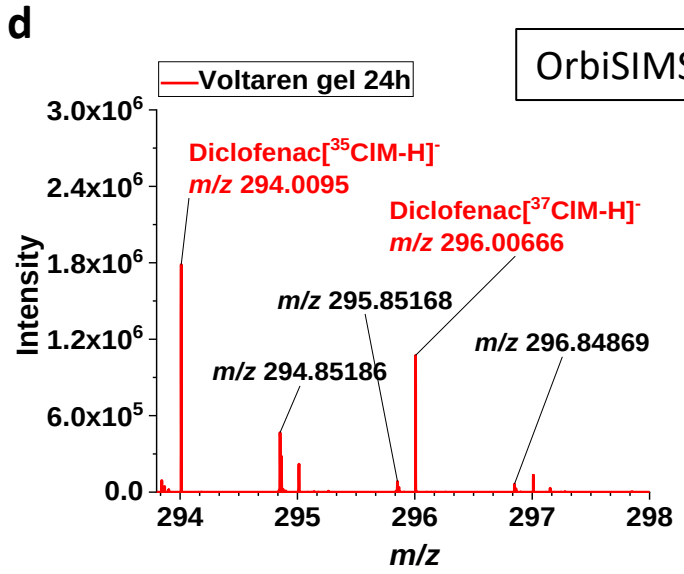


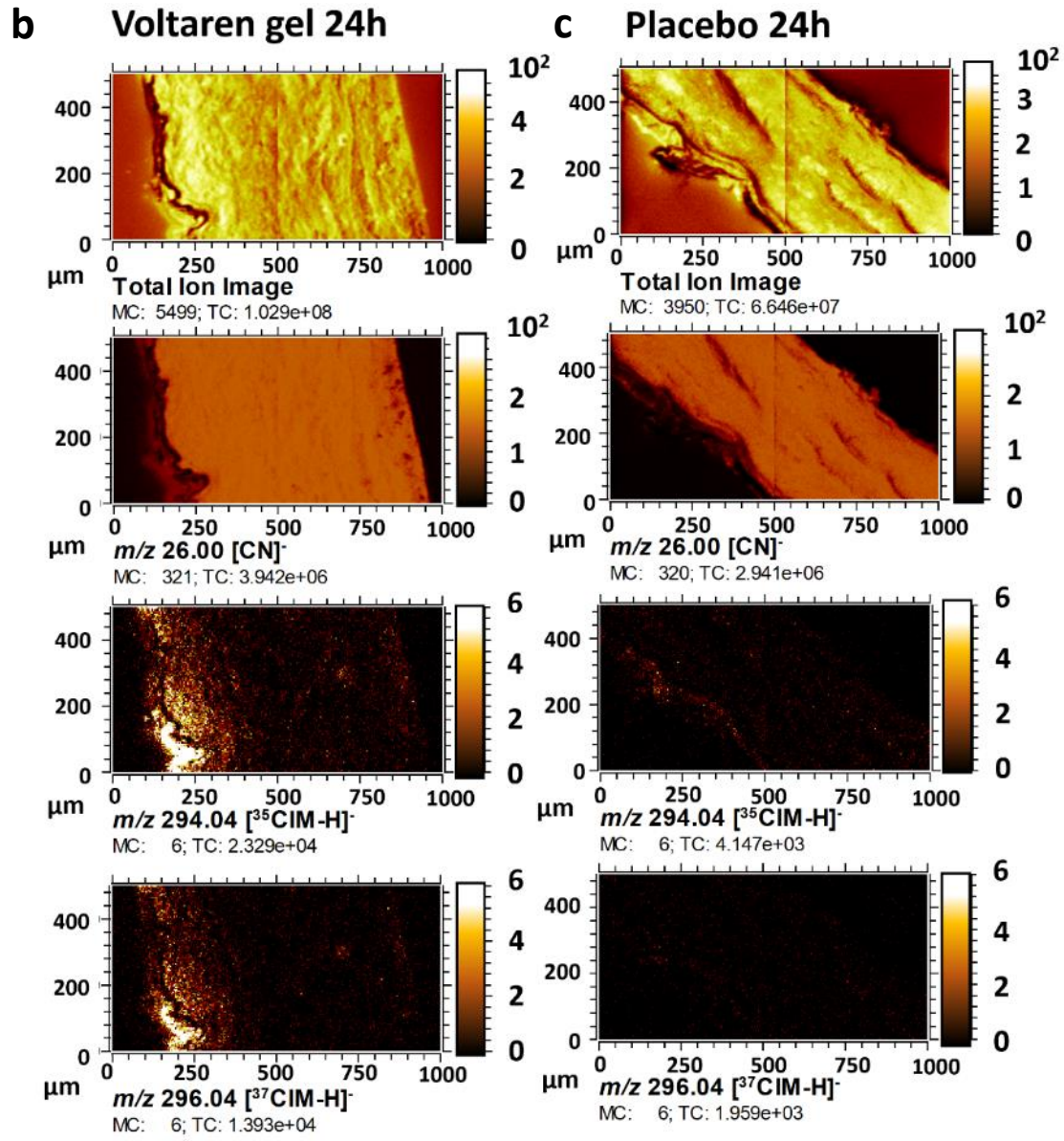




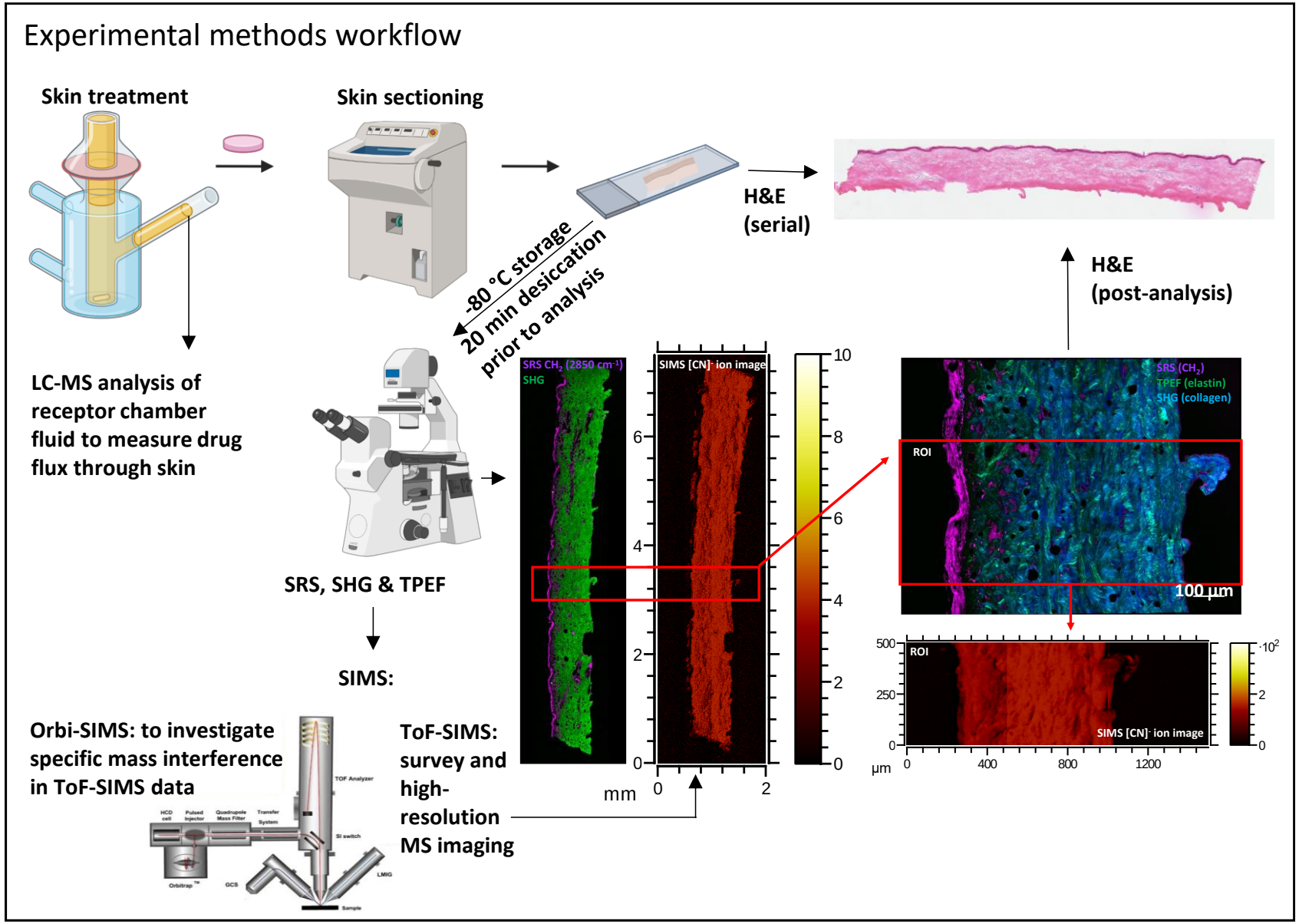
Secondary Ion Mass Spectrometry (SIMS)







Experimental Approach



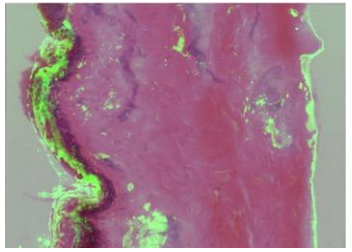
Data Approach

Data methods workflow

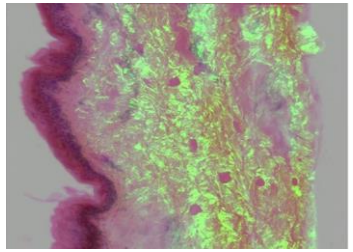
Image registration

H&E – optical spectroscopy validation

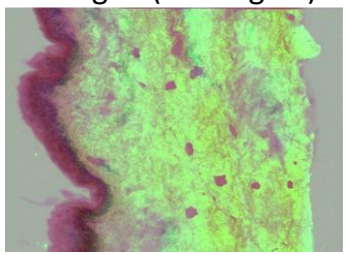
Overlay H&E to lipids (SRS CH_2 signal)



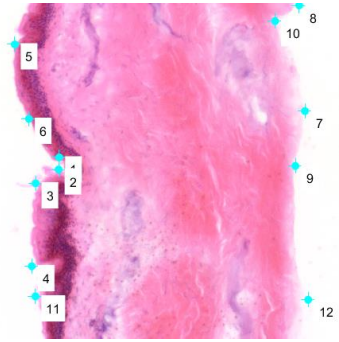
Overlay H&E to elastin (TPEF signal)



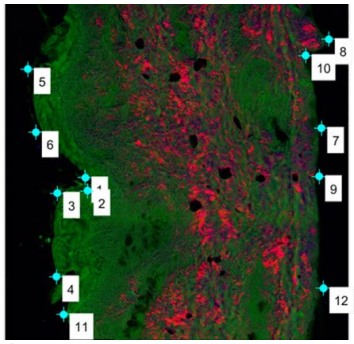
Overlay H&E to collagen (SHG signal)



H&E

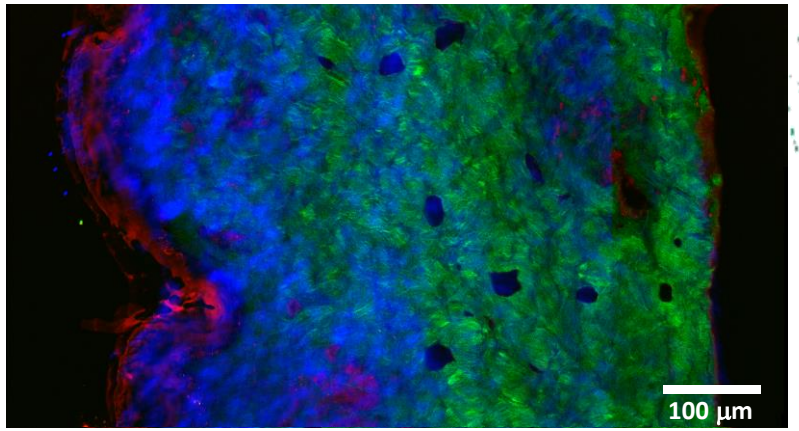


Optical spectroscopy (SRS-SHG-TPEF)

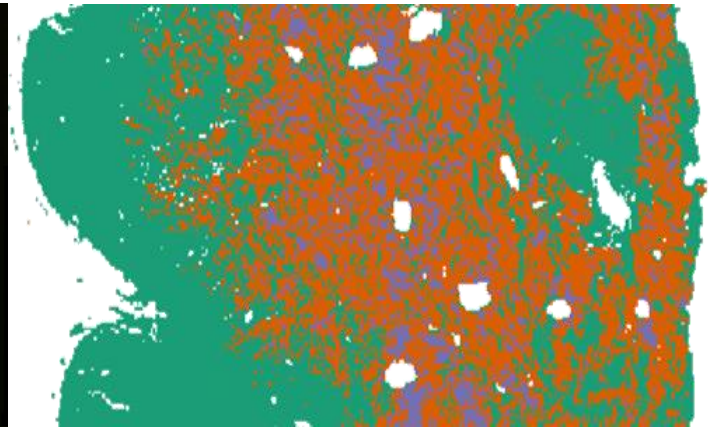


Overlay: optical spectroscopy with SIMS

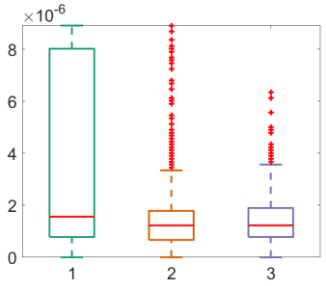
SRS contrast for lipids in red; SHG contrast for collagen in green; SIMS drug signals in blue:



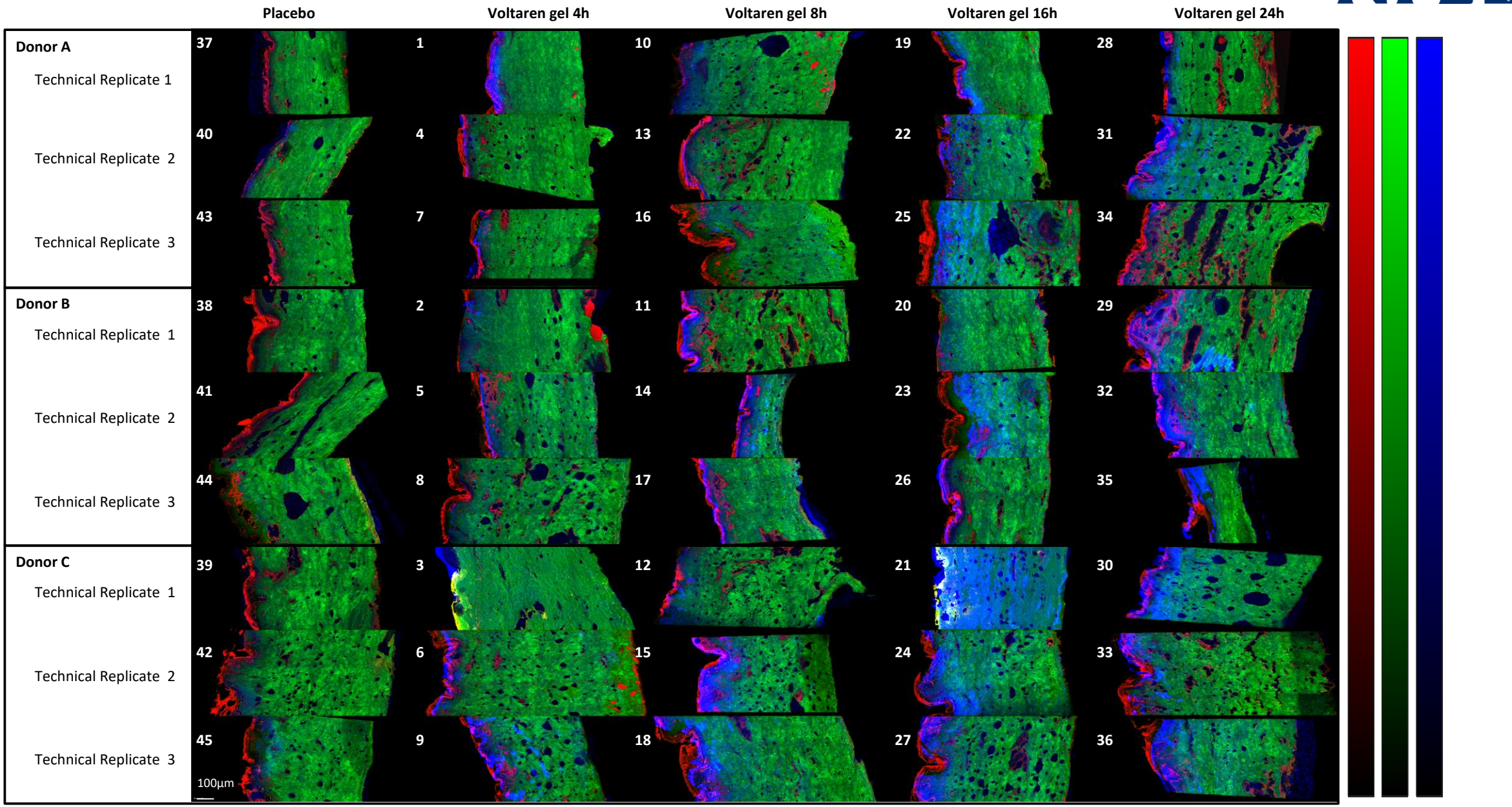
Data analysis, e.g. clustering



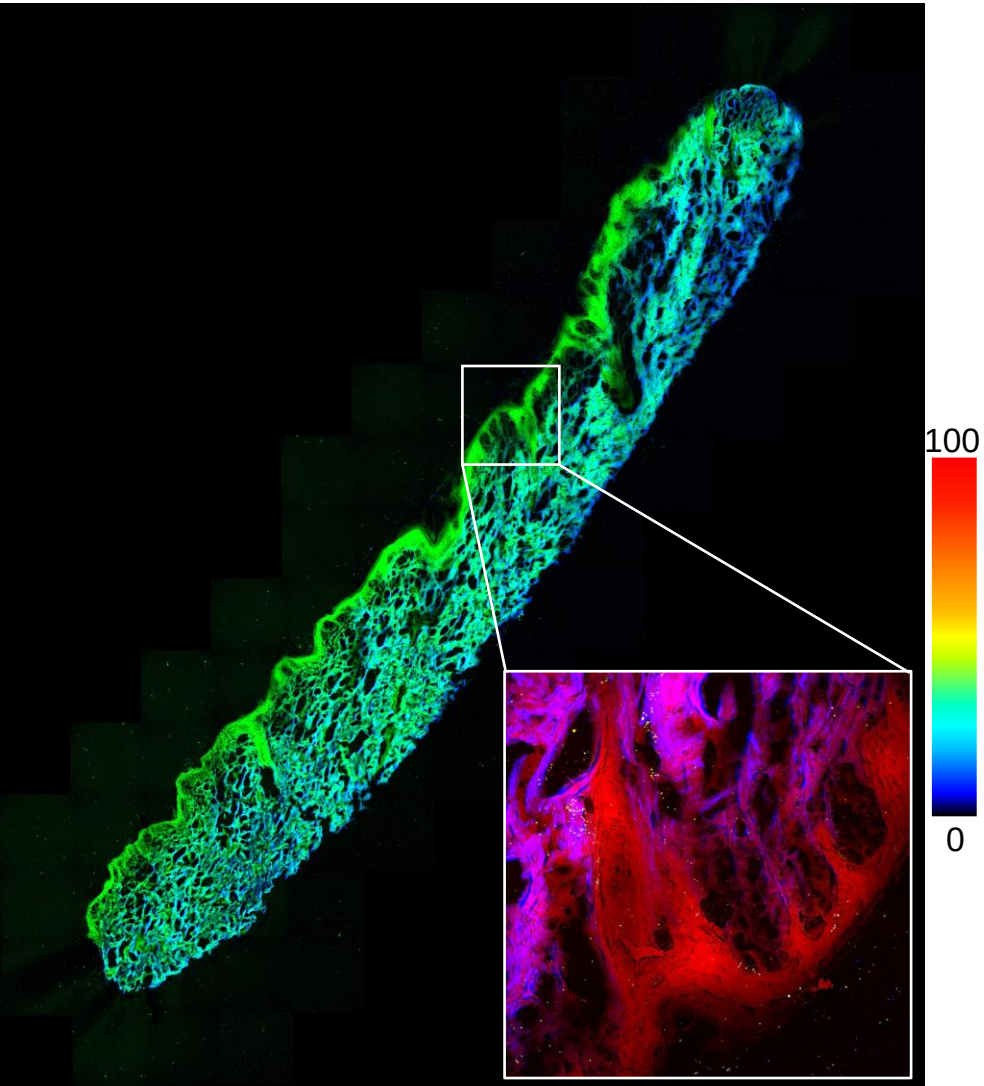
Analysis of average drug concentration within each cluster.



For more info please see Poster #8



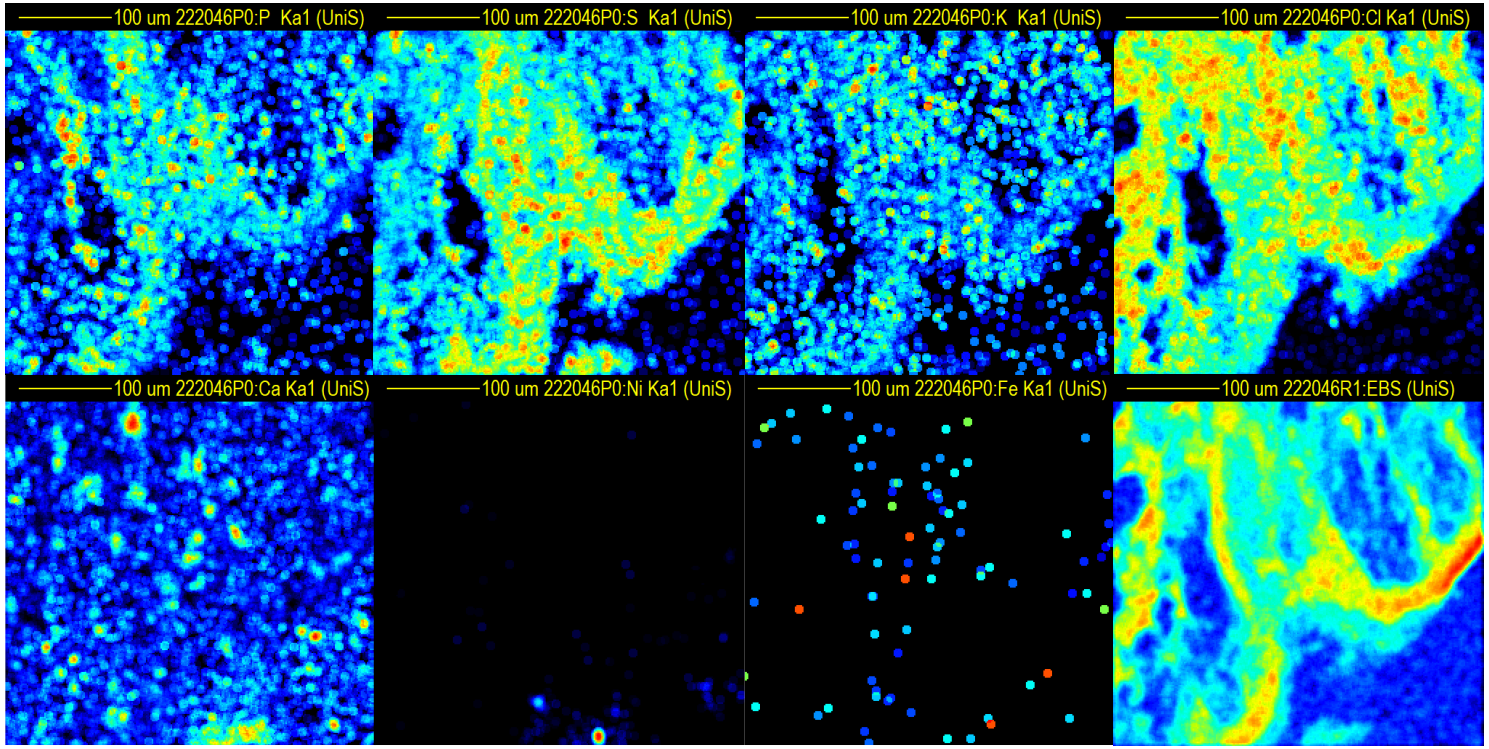
Integrated framework to risk assessment of dermal absorption of metal compounds



Work in progress!

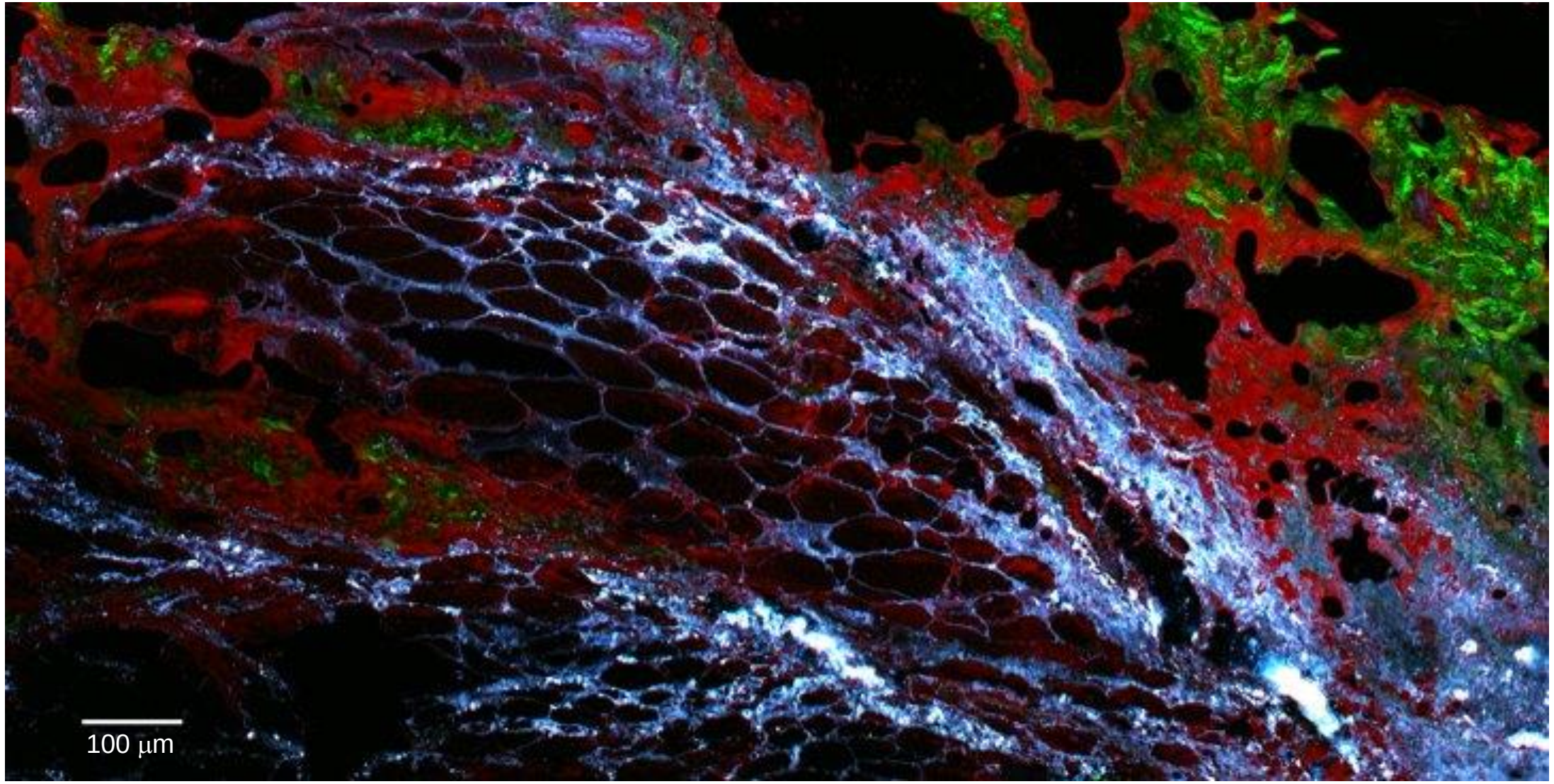
Correlative SRS-PTL with PIXE (Particle induced X-ray emission) @ Surrey Ion Beam Centre.

Normalised by EBS



Photothermal Lensing: Metallic Particles

Wounded rat skin model



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