Imaging Drug Delivery in the Skin using Coherent Raman Scattering Microscopy

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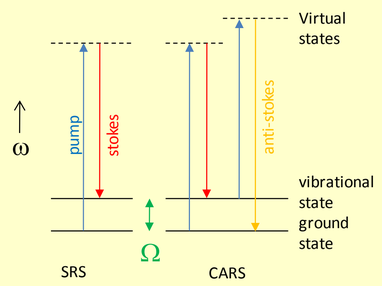
Objective

Efficient drug delivery to the skin is central to the effective treatment of major dermatologic diseases . However, treatment of most skin diseases is currently inefficient; typically less than a few % of the topically applied dose is absorbed to the target in the tissue.[1]

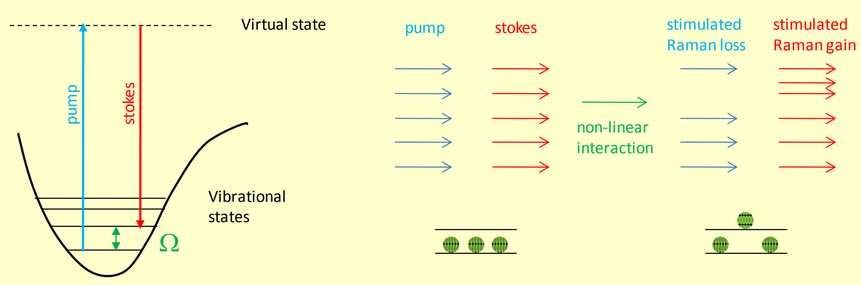
Our objective is to use coherent Raman scattering microscopy to image drug disposition and pharmacokinetics in the skin following application with excipients typically used in dermatological formulations.

This recently-developed, label-free imaging tool enables the acquisition of high resolution 3D images of multiple chemical components of a formulation as they penetrate the skin. Preliminary *in vitro* studies have been conducted on mouse skin,[2] however pig skin is a considerably better model for the human barrier, so has been utilised in this study.

Principles of Coherent Raman Scattering Microscopy



Stimulated Raman Scattering (SRS)



Advantages of stimulated Raman scattering (SRS) over coherent anti-stokes Raman scattering (CARS)

- No non-resonant background
- Linear sample concentration dependence
- No phase-matching effects: straightforward quantitative analysis
- High sensitivity due to high frequency modulation

Advantages of Coherent Raman Scattering Microscopy

Current method to quantify drug penetration is adhesive tape stripping:

- stratum corneum is progressively removed for chemical analysis e.g. By HPLC.
- laborious & invasive: can only be performed once on a given region of skin.
- no lateral resolution: no mechanistic insight into penetration pathways.

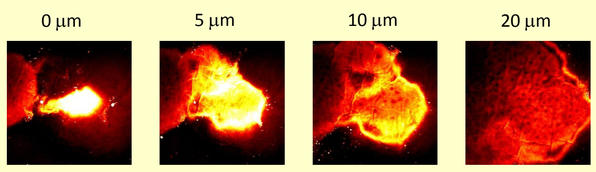
Coherent Raman Scattering Microscopy offers the following advantages:

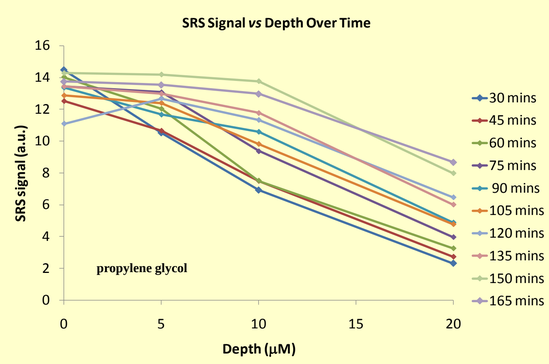
- Non-destructive analysis
- Label-free imaging:
- High resolution 3D images of multiple chemical components of a formulation as they penetrate the skin.

Imaging Drug Disposition : Propylene Glycol-d_8 and Ketoprofen

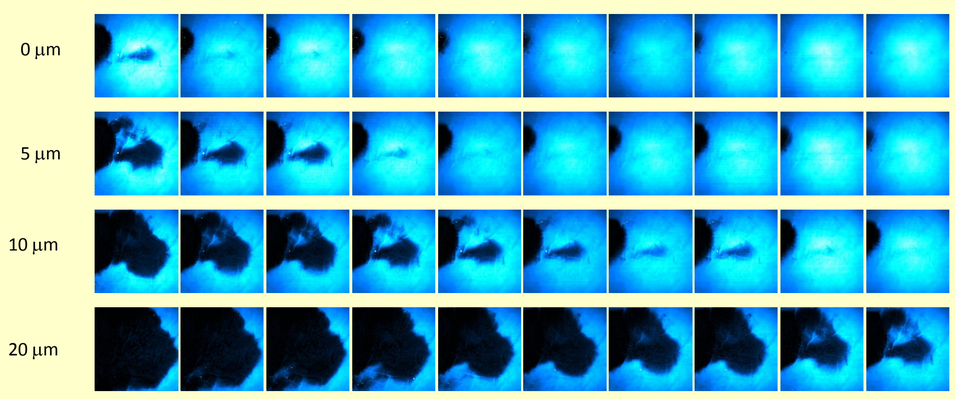
- Ketoprofen in Propylene glycol-d_8 applied to the skin
- SRS tuned to CH_2-stretch to visualise skin lipids & surface
- Laser tuned to C-D stretch to visualise Propylene glycol-d_8, or to C-H wavelength to visualise ketoprofen.
- Image intensity can subsequently be analysed to extract quantitative information.

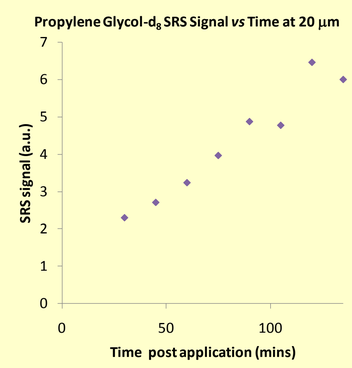
Lipid CH_2-stretch (2845 cm^{-1}, 816 nm):



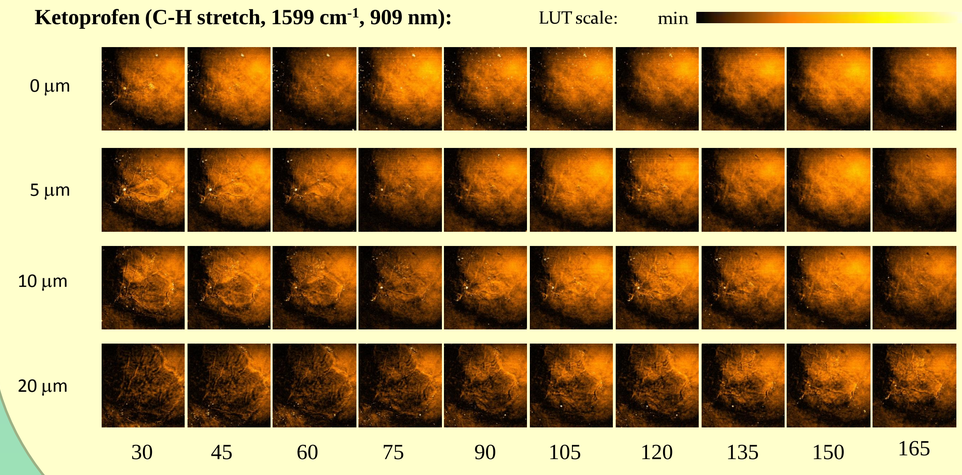


Propylene glycol-d_8 (C-D stretch, 2120 cm^{-1}, 868 nm): LUT scale: min max

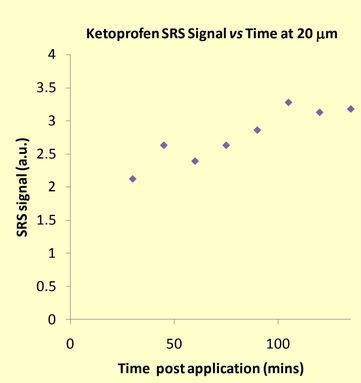




Ketoprofen (C-H stretch, 1599 cm^{-1}, 909 nm): LUT scale: min max

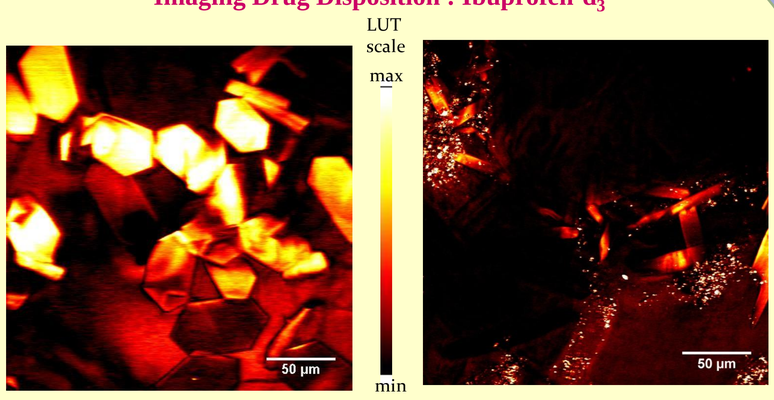


Time (min) post application



Scale: all images 250 x 250 µm.

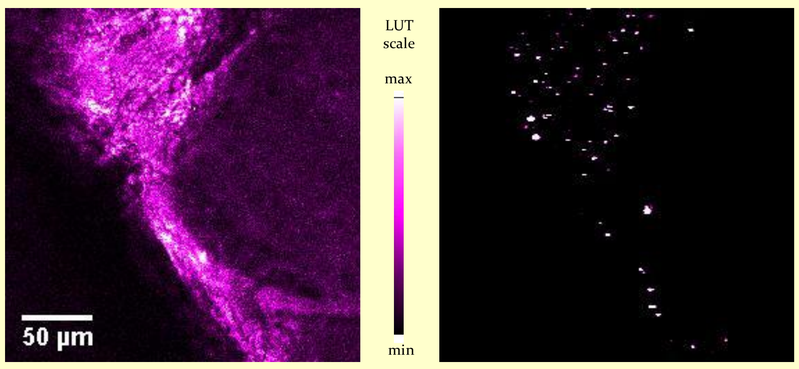
Imaging Drug Disposition : Ibuprofen-d_3



SRS Images showing crystallization of Ibuprofen in the skin 30 minutes after *in vitro* application. Mouse (left) and pig (right).

Imaging Deuterated Nanoparticles

- Deuterated nanoparticles (~60 nm) with and without fluorescein.
- SRS imaging performed in conjunction with multiphoton microscopy.
- SRS microscopy can be used to detect the C-D stretch of deuterated nanoparticles while the multiphoton detects skin auto-fluorescence.
- Alternatively, multiphoton microscopy can be employed to detect fluorescent particles while SRS is simultaneously tuned to the lipid CH_2 stretch wavelength to image the skin.



Multiphoton image showing skin auto-fluorescence

SRS tuned to C-D wavelength to image deuterated nanoparticles

Challenges

- Currently, 'defining' the surface of the skin is achieved by choosing the 'depth' the greatest intensity of CH_2 stretch. A reproducible method to determine '0 µM' must be developed.
- Formulation: absorption often leads to swelling of the tissue which over the timeframe of the experiment can lead to a change in the surface height of the tissue.

Conclusions and Future Directions

- Drugs and excipients have been successfully tracked through pig skin using SRS microscopy.
- Evidence has been obtained for crystallisation of drugs on the skin as the excipient permeates more rapidly.
- Validation of SRS *vs* tape stripping technique.
- Develop improved formulations based on knowledge gained from tracking their constituents.

References

1. S. Wiedersberg, C.S. Leopold, R.H. Guy. Bioavailability and Bioequivalence of Topical Glucocorticoids. *Eur. J. Pharm. Biopharm.*, 68: 453-466 (2008).
2. B.G. Saar, L.R. Contreras-Rojas, X.S. Xie, R.H. Guy. Label-free Imaging of Pharmacokinetics in Skin with Stimulated Raman Scattering Microscopy. *Manuscript submitted for publication*.

Funding:

This project is supported by Leo Pharma (Ballerup, Denmark).