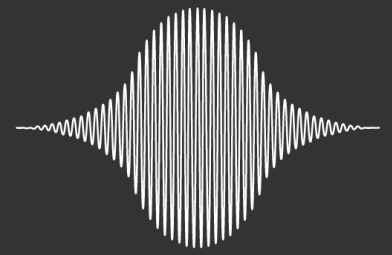


Advanced Techniques for Measuring Cutaneous Pharmacokinetics Using Pharmacokinetic Tomography

Conor L. Evans
Wellman Center for Photomedicine
Massachusetts General Hospital
Harvard Medical School

Disclosures

Patents in coherent Raman imaging licensed to both Leica and Zeiss

Consultant for Compass Skincare

Acknowledgments

Current Team

Alice Chao
Dan Greenfield
Helen Keshishian
Rachel Keller
Xiaolei Li
Tucker Raymond
Michele Wei
Anna Wiatrowski
Dr. Juan Cascales
Dr. Fotis Ilioupolous
Dr. Ben Kuzma
Dr. Matthias Muller

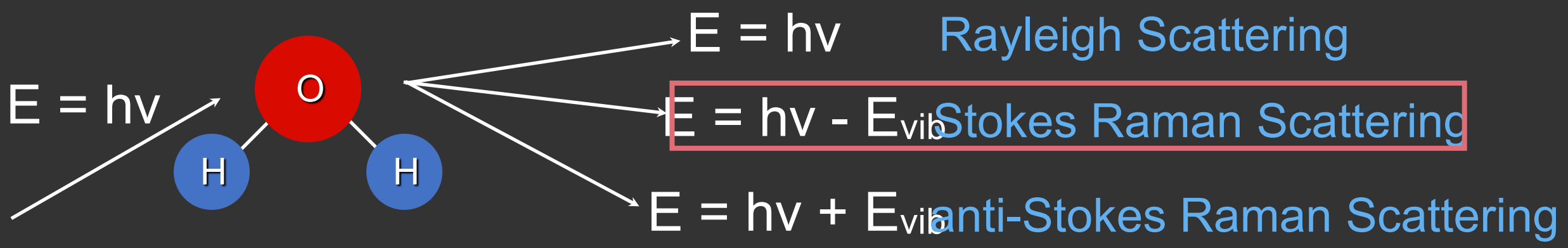
Dr. Isaac Pence
Dr. Fei Peng
Dr. Manolis Rousakis
Dr. Maria Alice Tabosa

Dr. Ben Kuzma

FDA

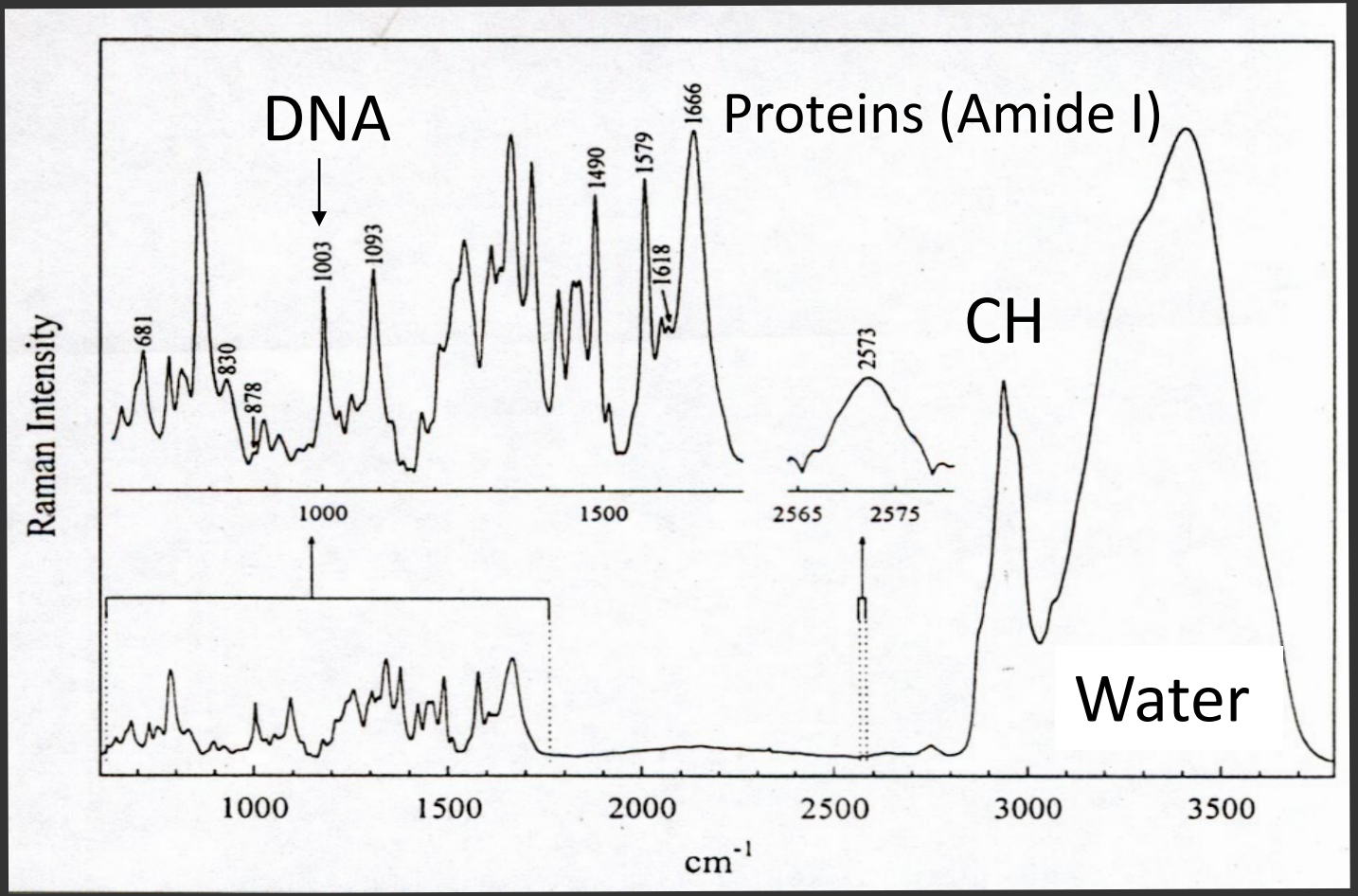
Dr. Priyanka Ghosh
Dr. Sam Raney
Dr. Markham Luke

Spontaneous Raman Scattering



Light can undergo Raman scattering from any molecular vibration in a sample, leading to a spectrum of scattered light energy

Spontaneous Raman Scattering



Raman spectrum of a HeLa Cell

The Raman spectrum of a molecule can be used as a unique "fingerprint"

Problem: Raman scattering is *very* weak

Coherent Raman Scattering (CRS) Microscopy

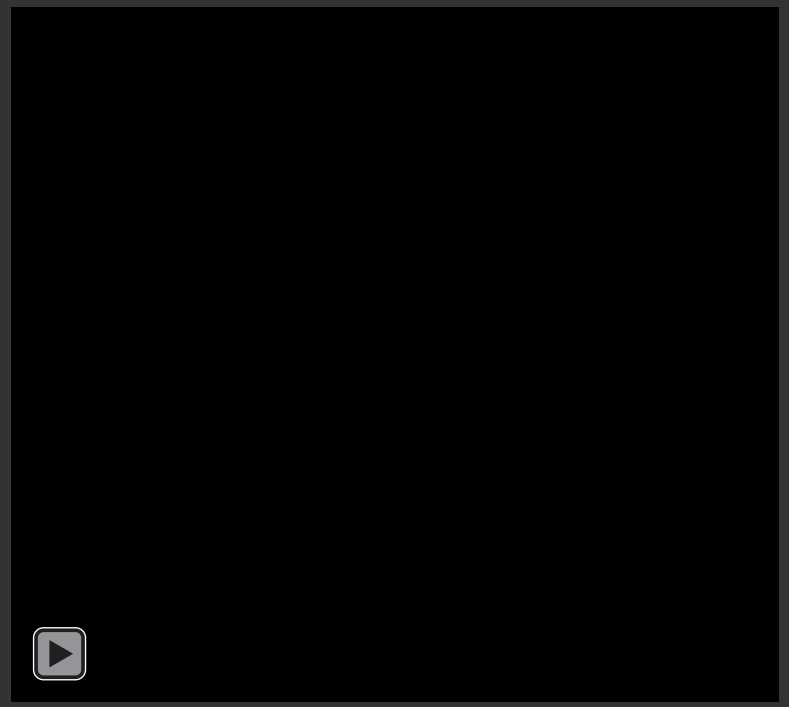
Imaging based on intrinsic vibrational contrast

Two Colors: ω_p "Pump"

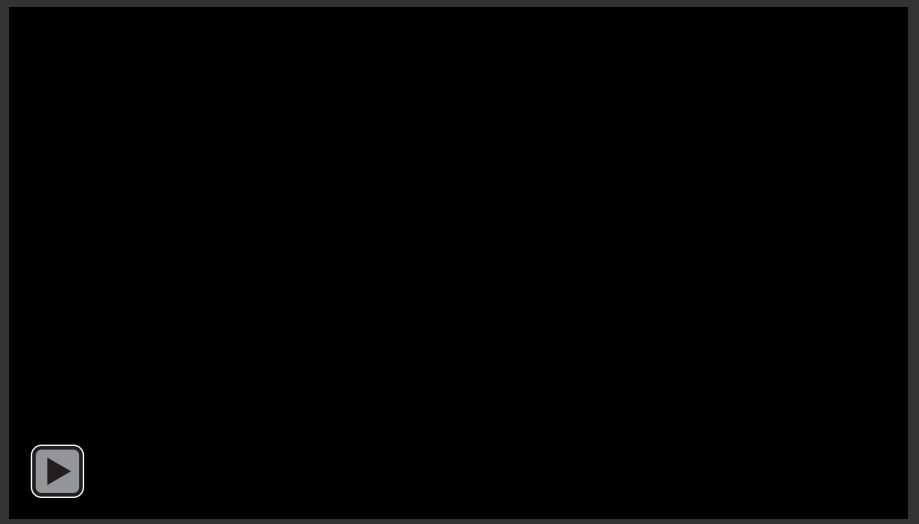
ω_s "Stokes"

ω_p

ω_s



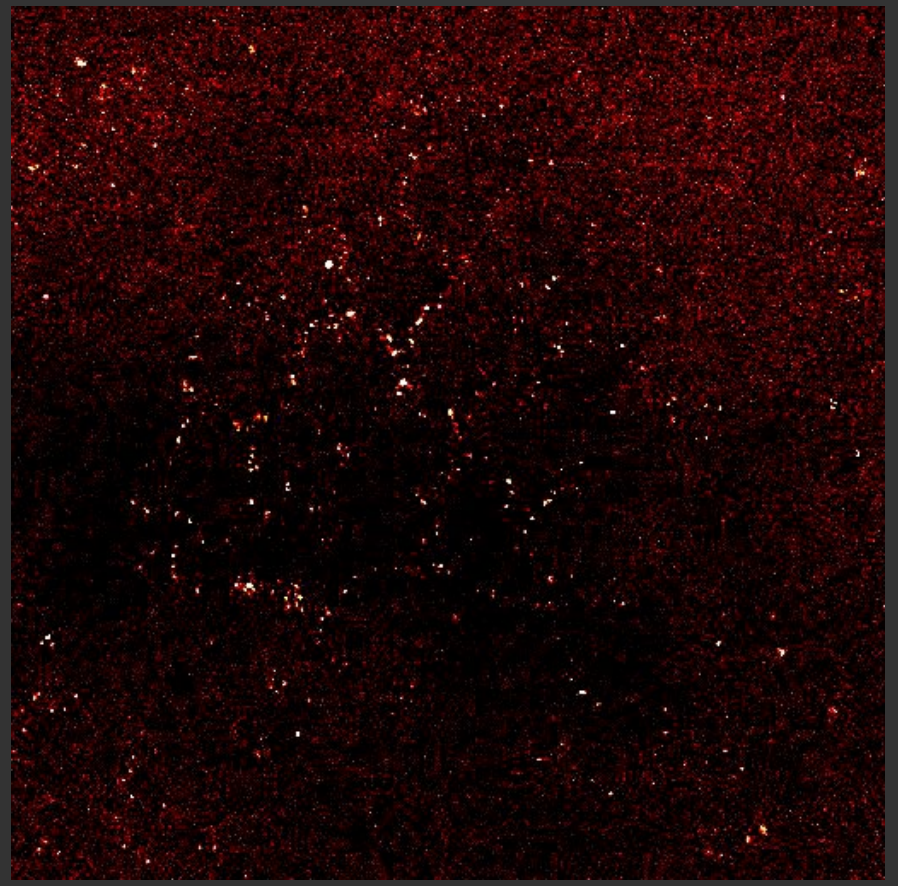
$\omega_p - \omega_s = \Omega_{vib}$



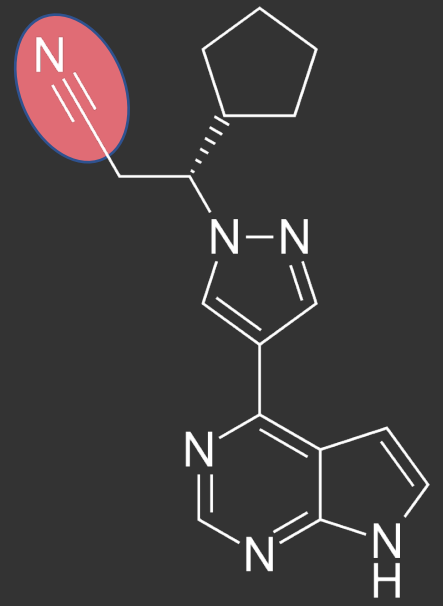
Direct Imaging of Active Pharmaceutical Compounds (APIs)

Stimulated Raman Scattering Microscopy

Nitrile Stretch: 2250 cm-1
100% resonant signal
120 min



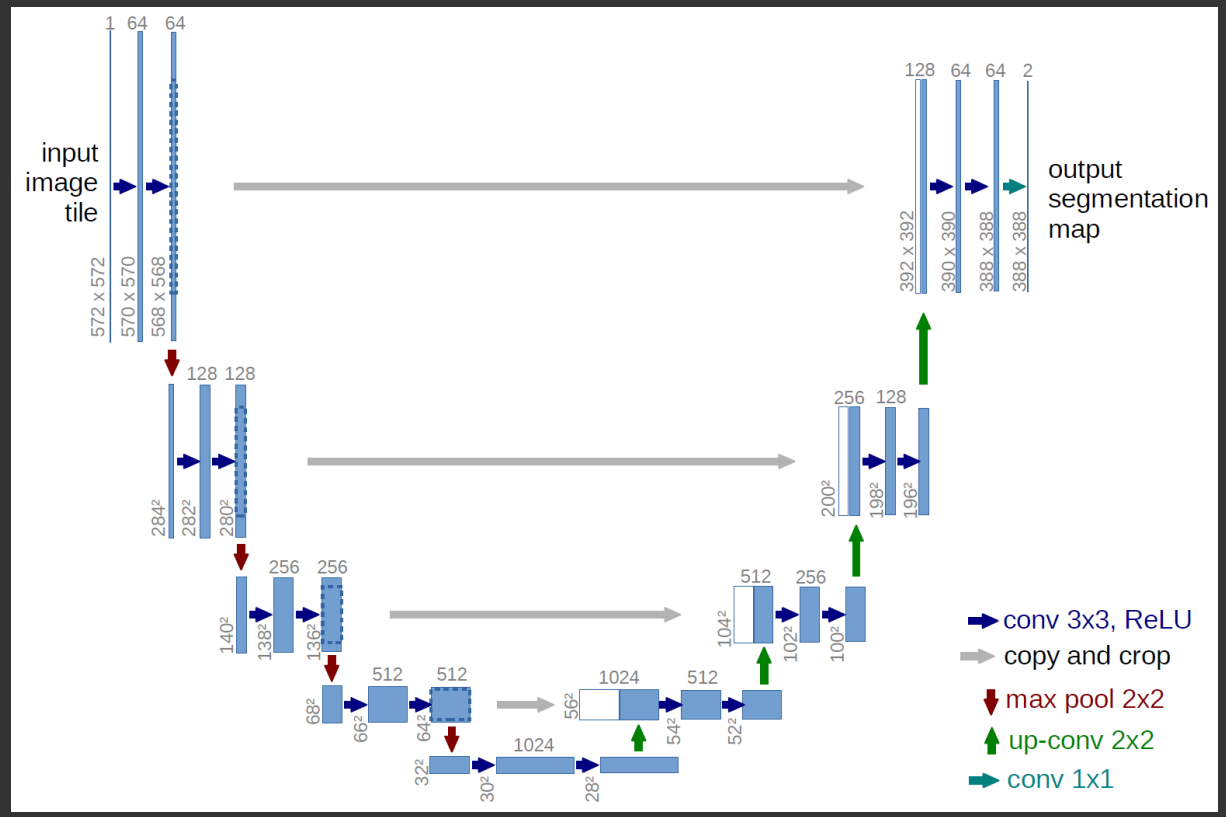
Direct visualization of flux in the epidermis of mouse skin without background signal



Ruxolitnib

Quantifying PK with a Deep Learning-based Pipeline

We use a Unet Convolutional Neural Network (CNN) along with a server-based python pipeline and R-based automated non-compartmental analysis



Libraries:

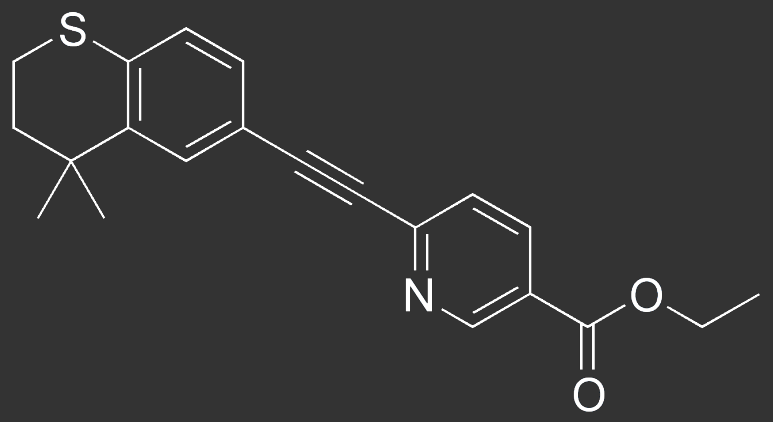
Python:

- Tensorflow
- Numpy
- Matplotlib
- Javabridge
- Python-Bioformats
- PyYaml

R:

- Reticulate
- Ggplot2
- Noncompart

Tazarotene (Taz) Pivotal Study



3rd Generation Retinoid for the treatment of numerous skin conditions including acne, rosacea, and psoriasis

Goal: Quantify the Bioequivalence of multiple Tazarotene topical products

- **Experimental parameters** :
  - SRS system tuned to 1590 cm^{-1} to target the delocalized CC stretching vibration of the Taz backbone.
  - The skin structure was imaged using the 2870 cm^{-1} wavenumber to target the CH_2 methylene stretching vibration of lipids
  - The tuning sequence was set to alternate between 1590 and 2870 to monitor & confirm the focal depth during imaging
  - A polymeric concentration standard loaded with Taz was used in all experiments

Treatment groups for pivotal study

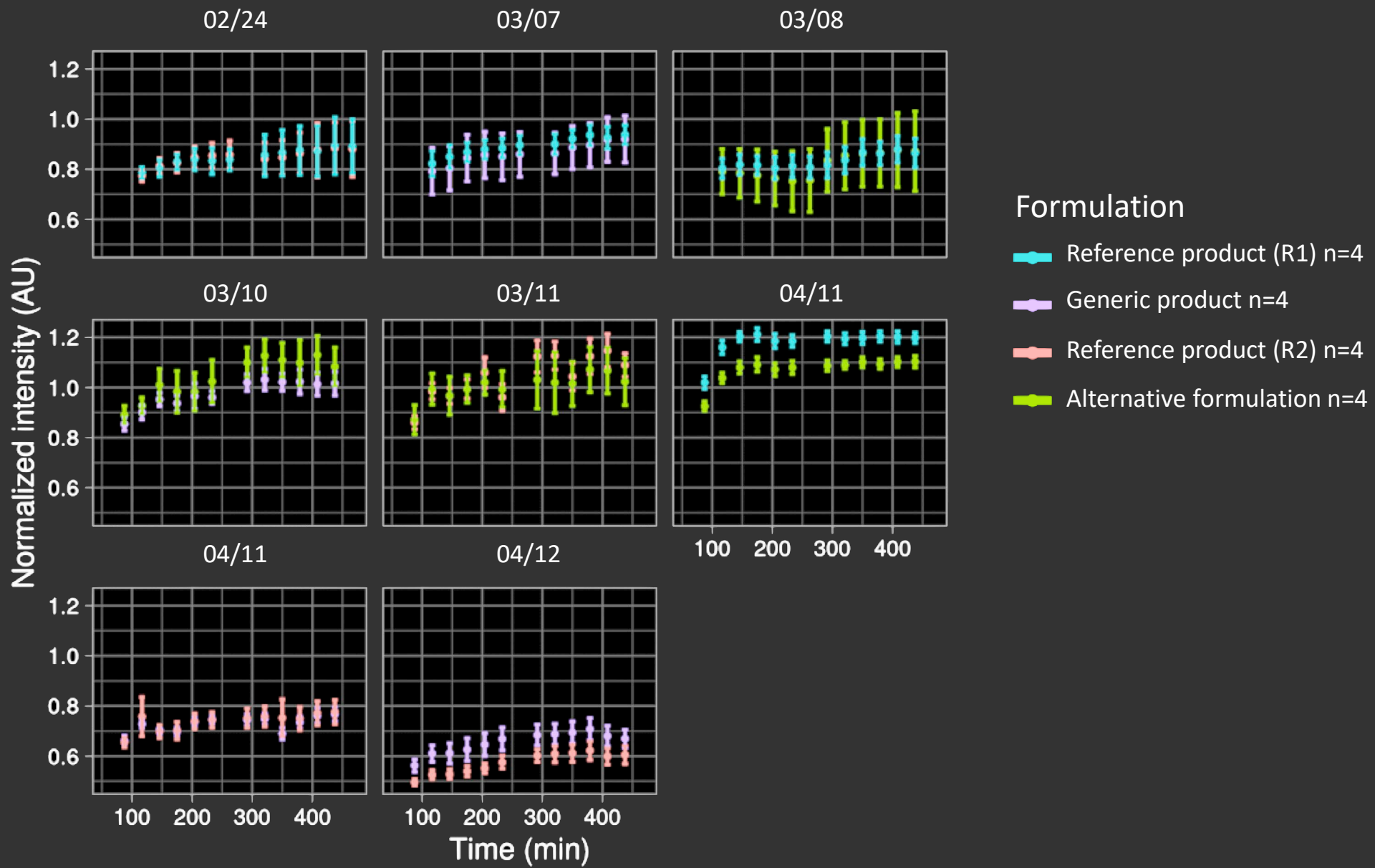
Treatment groups	**1. Reference product (R1)**: Tazorac® Almirall, LLC; Dosage form: cream; NDC package code: 16110-0916-30; Appl. No: NDA021184 **2. Generic product**: Taro Pharmaceuticals U.S.A., Inc; Dosage form: cream; NDC package code: 51672-1373-02; Appl. No: ANDA208258 **3. Reference product (R2)**: Same as reference product (Provides a measure of inter-experimental variability) **4. Alternative Formulation**: Tazorac®; Dosage form: gel; NDC package code: 16110-0042-30; Appl. No: NDA020600

Experimental details for pivotal study

API & drug concentration in formulations	Tazarotene 0.1% (*w/w*)
Skin donors	**Donor 1**: 39 years old; Female; Abdomen **Donor 2**: 48 years old; Female; Abdomen **Donor 3**: 42 years old; Female; Abdomen **Donor 4**: 54 years old; Male; Abdomen
Skin preparation	Full-thickness – Subcutaneous fat trimmed to allow SRS signal detection in the forward direction
Source of skin procured	Massachusetts General Hospital; Cooperative Human Tissue Network
Number of skin samples & regions of interest (ROIs)	4 samples per formulation; 4 ROIs per skin sample (1024 x 1024 pixel)
Depth stack	Step size: 8 μm; number of slices: 9; final depth at 64 μm
Time per cycle (8 ROIs – pair of formulations)	~25 min
Study duration	~6.5 hours of imaging (15 cycles)
Skin uptake conditions	Finite dose (5 μL); Occlusive; 32°C

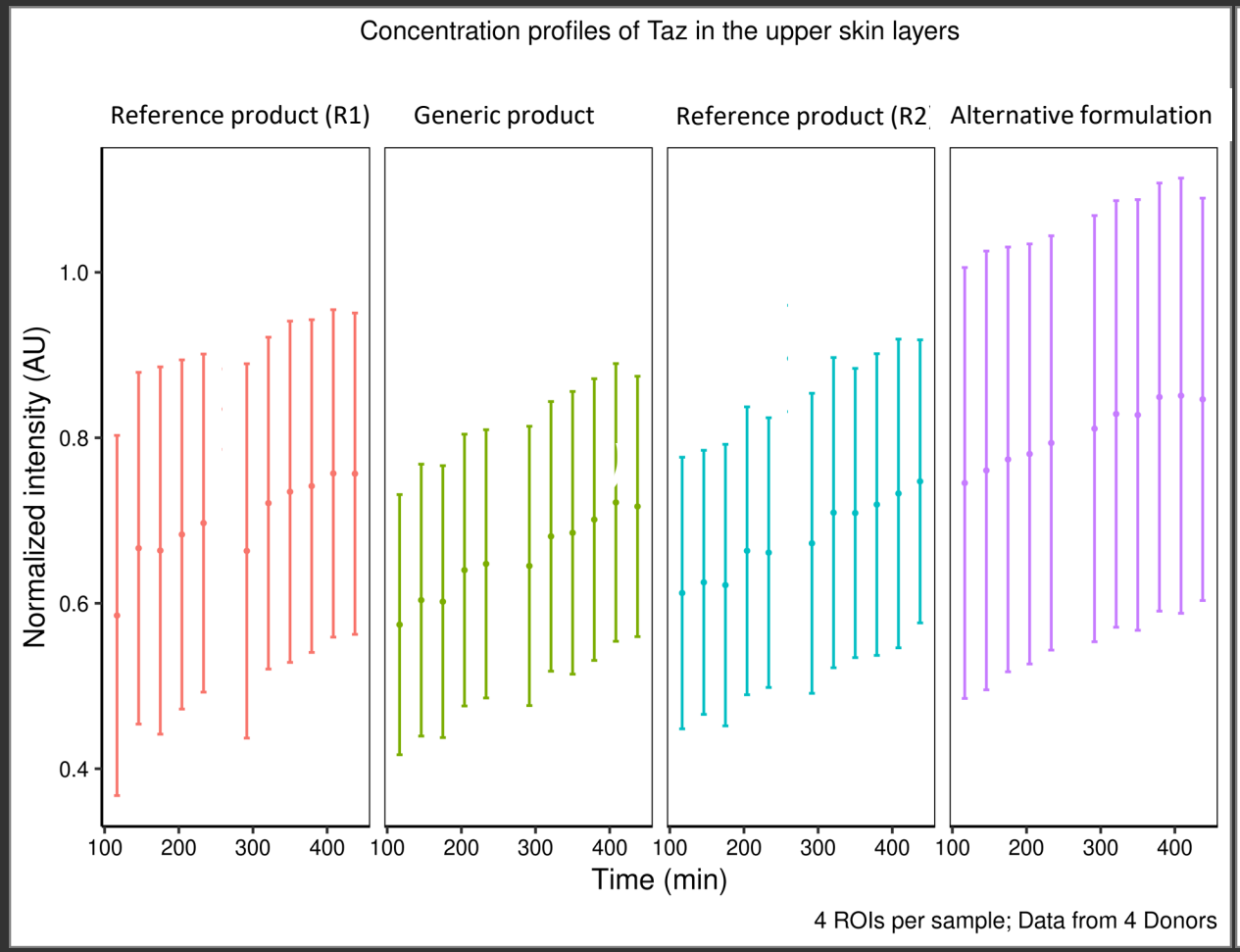
Extracting Concentration Time Profiles

Example data from a single skin donor

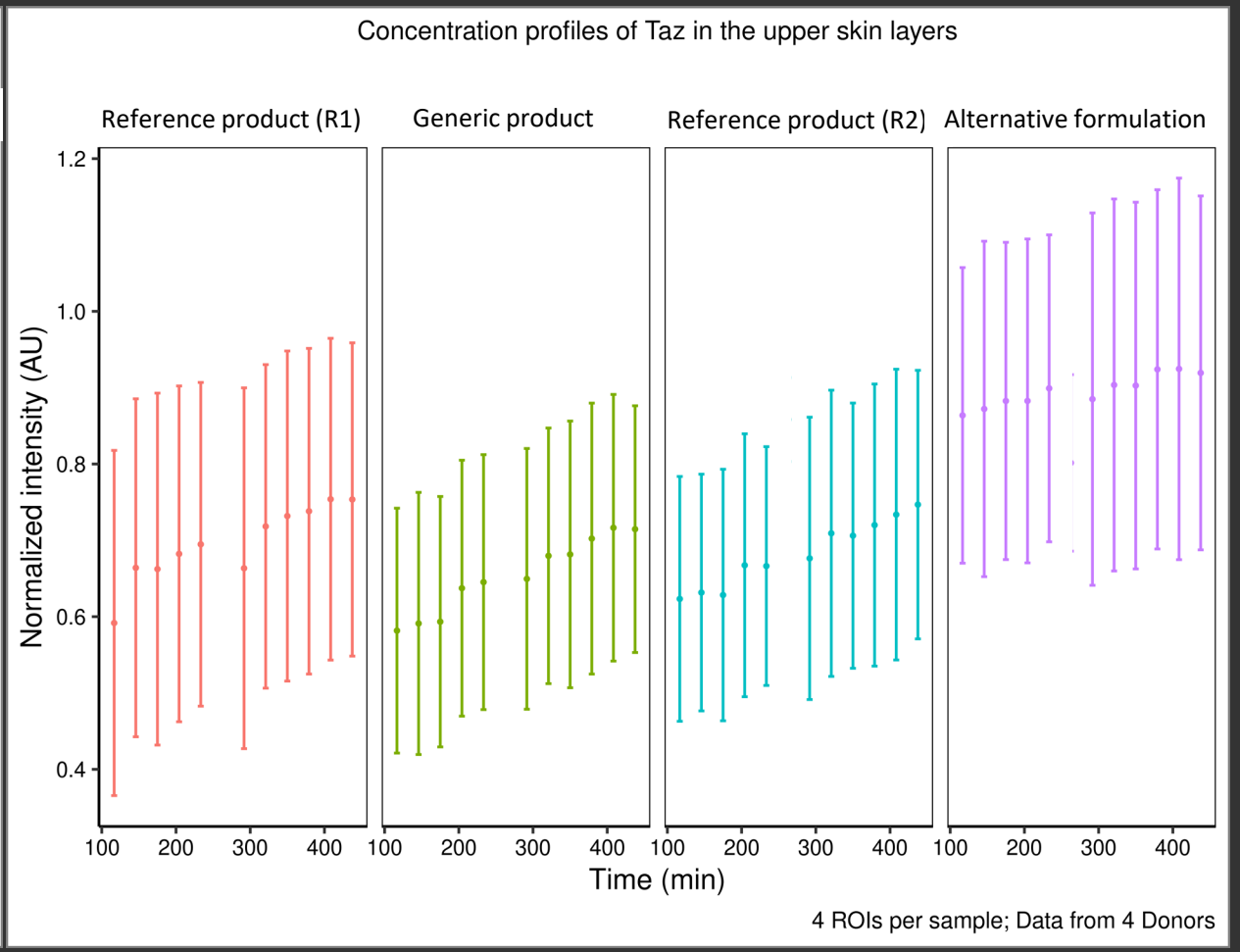


Average Concentration Time Profiles

Within Lipid-Rich Skin Regions



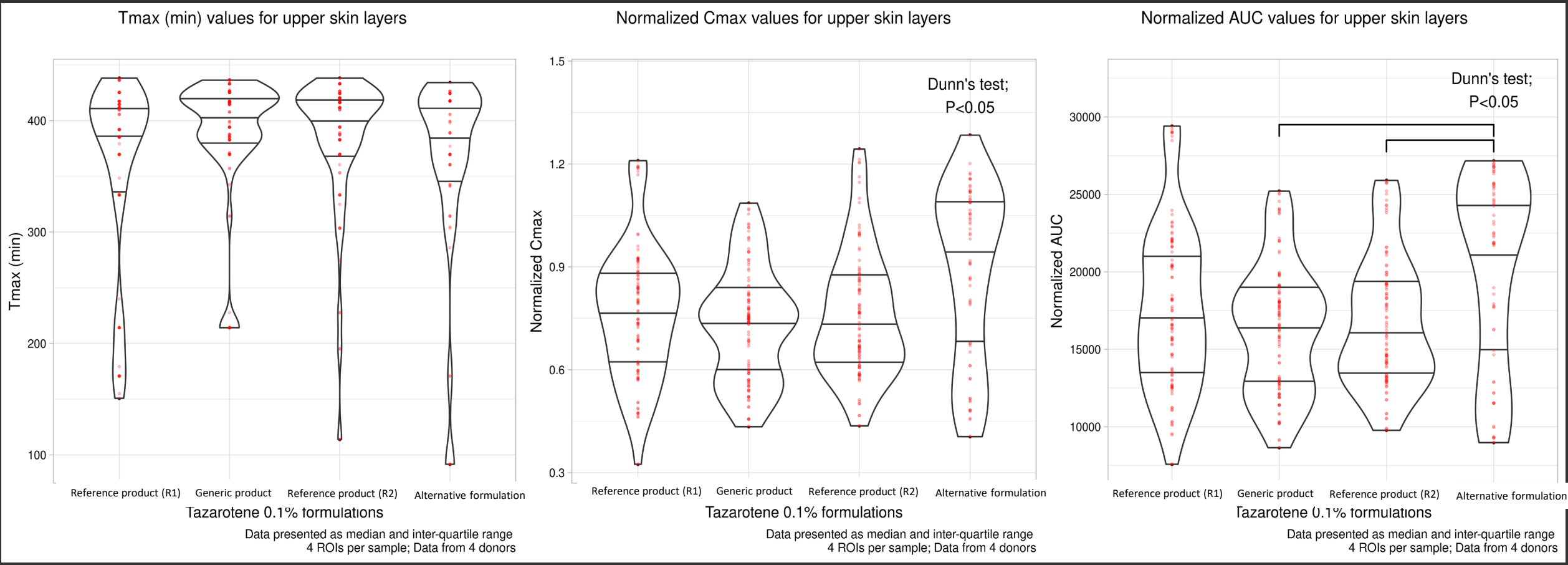
Within Lipid-Poor Skin Regions



Concentration profiles of Taz for the first two depths (upper 16µm) for various 0.1% cream formulations following finite dose application. Reference product (R1): Tazorac®; Generic product: Taro Pharmaceuticals U.S.A., Inc; Reference product (R2): Tazorac®; Alternative formulation: Tazorac® gel.

Donor 1 (Group 1: n=2; Group2: n=3; Group3: n=3), Donor 2 (Group 1: n=3; Group2: n=2; Group3: n=3), Donor 3 (n =4 for all groups), Donor 4 (n =4 for all groups)

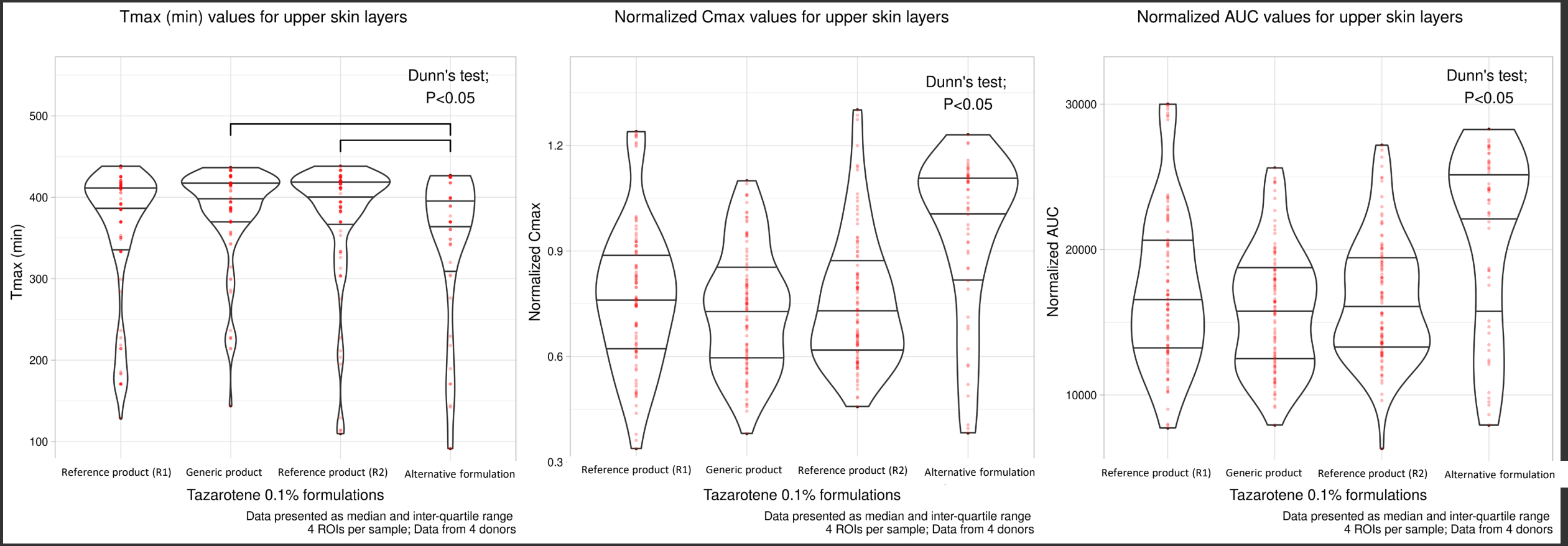
Pharmacokinetic Parameters: Lipid-Rich



Concentration profiles of Taz for the first two depths (upper 16µm) for various 0.1% cream formulations following finite dose application. Reference product (R1): Tazorac®; Generic product: Taro Pharmaceuticals U.S.A., Inc; Reference product (R2): Tazorac®; Alternative formulation: Tazorac® gel.

Donor 1 (Group 1: n=2; Group2: n=3; Group3: n=3), Donor 2 (Group 1: n=3; Group2: n=2; Group3: n=3), Donor 3 (n =4 for all groups), Donor 4 (n =4 for all groups)

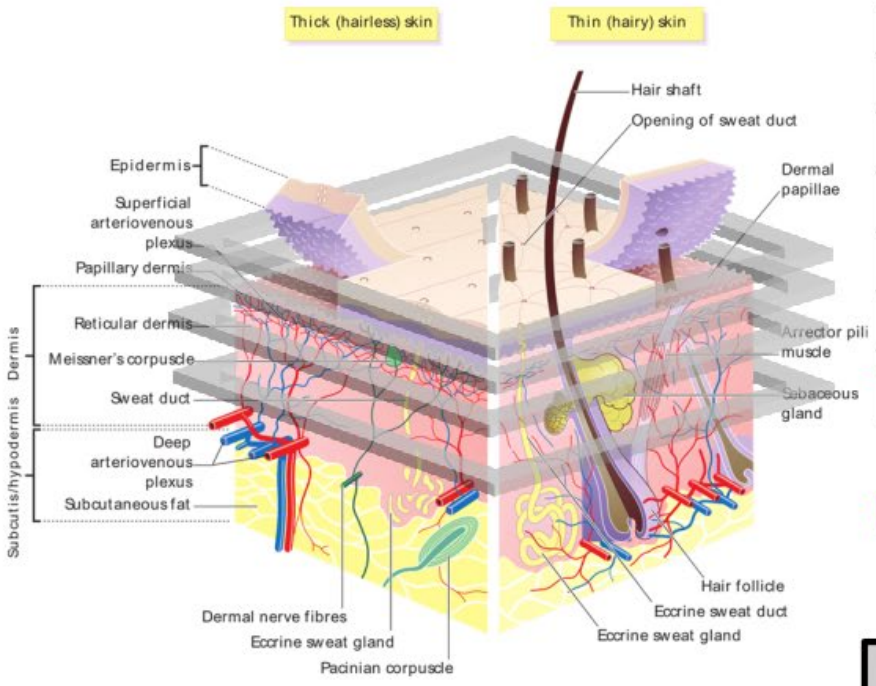
Pharmacokinetic Parameters: Lipid-Poor

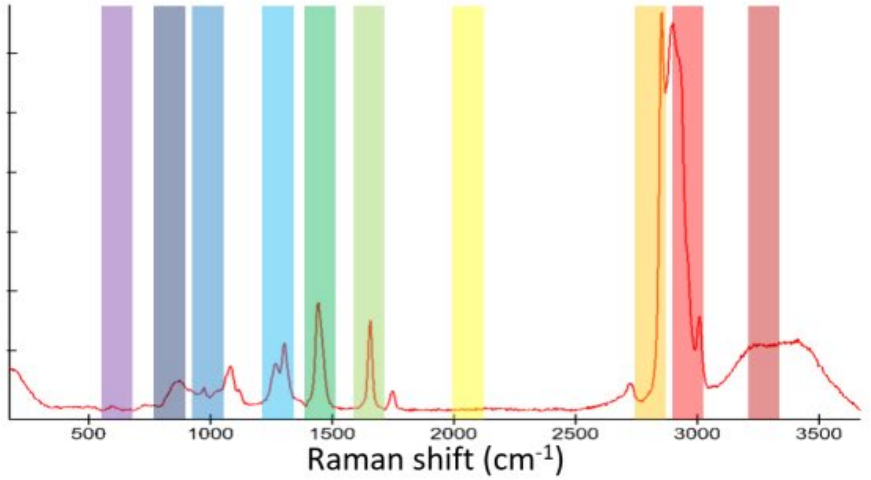


Concentration profiles of Taz for the first two depths (upper 16µm) for various 0.1% cream formulations following finite dose application. Reference product (R1): Tazorac®; Generic product: Taro Pharmaceuticals U.S.A., Inc; Reference product (R2): Tazorac®; Alternative formulation: Tazorac® gel.

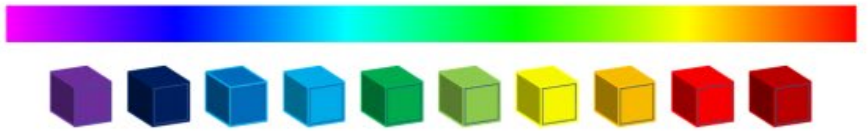
Donor 1 (Group 1: n=2; Group2: n=3; Group3: n=3), Donor 2 (Group 1: n=3; Group2: n=2; Group3: n=3), Donor 3 (n =4 for all groups), Donor 4 (n =4 for all groups)

Developing a more General Method: S⁴RS





Raman spectrum of tissue +/- drug



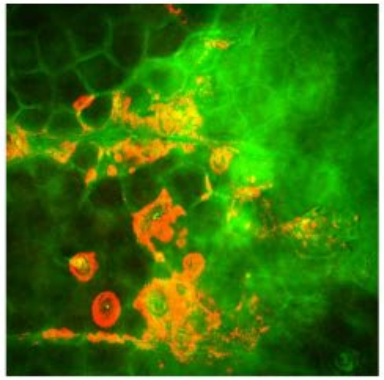
Conventional Sampling

Sparse Sampling

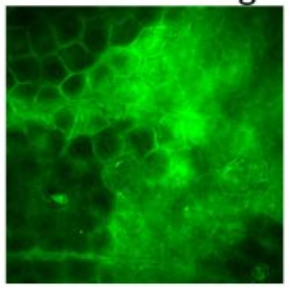
Principal Component Analysis (PCA)	Generalized Linear Models (GLM)	Deep Learning (Autoencoder, Unet)

Spectral processing

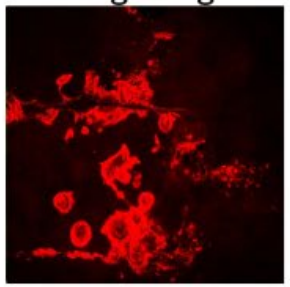
Composite: structure & drug



Structural image



Drug image



Green: CH_2 (2845 cm^{-1}) in the tissue, vehicle (a lipid-rich gel) and ruxolitinib
Red: Nitrile (2250 cm^{-1}) only in the ruxolitinib

Sparse Spectral Sampling Stimulated Raman Scattering – S⁴RS, a generalized method for Topical Product Quantification

Conclusions and Next Steps

- Coherent Raman Imaging (CRI) is capable of quantifying the permeation of APIs within the epidermis
- CRI datasets can be processed to extract concentration-time profiles and the PK parameters T_{max}, C_{max}, and AUC
- Preliminary analysis suggests that CRI can assess bioavailability and bioequivalence of APIs in different topical formulations
- Upcoming Sparse Spectral Sampling SRS (S^4RS) methods will enable CRI bioequivalence experiments in a wide range of topical products