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SRS microscopy and deep learning: Novel approach for evaluation of topical bioequivalence

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Fotis Iliopoulos, PhD

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Session Description and Objectives

- The bioequivalence (BE) of pharmaceutical formulations is typically demonstrated by assessing the pharmacokinetics (PK) of a generic product relative to a reference-listed drug (RLD) product.
- However, for topical products applied to the skin, the applicability of the PK-based approach has been limited.
- Here, we present a novel PK-based approach based on Stimulated Raman scattering (SRS) imaging and data processing via deep learning for image feature extraction and automated data processing.
- Discuss current challenges associated with the development of appropriate methods to assess topical bioequivalence
- Describe the use of pharmacokinetic measures for topical bioequivalence determinations
- Probe mechanisms of percutaneous absorption and permeation pathways across the *stratum corneum*

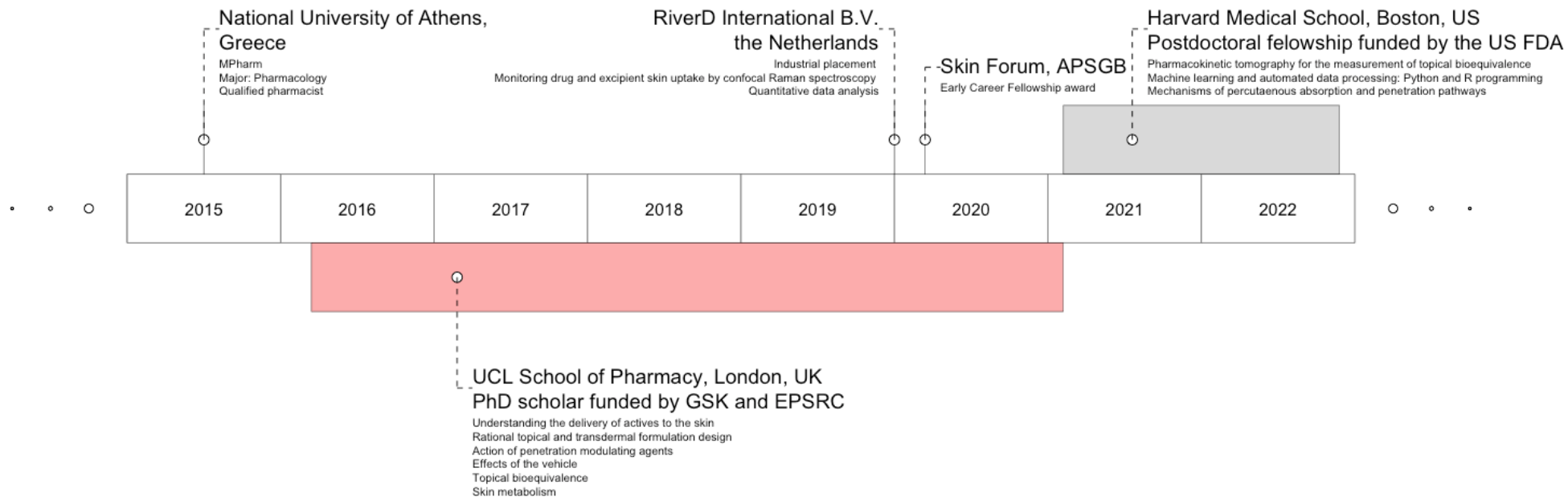
Biography and Contact Information

Fotis Iliopoulos, MPharm, PhD
Harvard Medical School
Massachusetts General Hospital
Wellman Center for Photomedicine

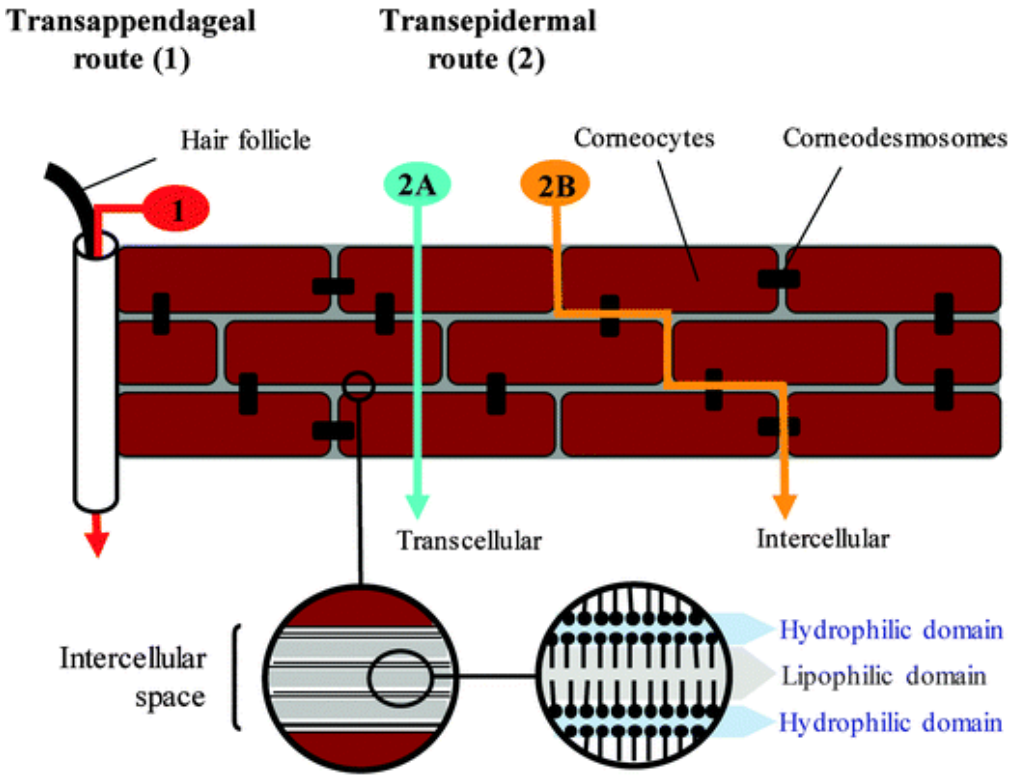
FILIOPOULOS@mgh.harvard.edu

+1 617-999-9082

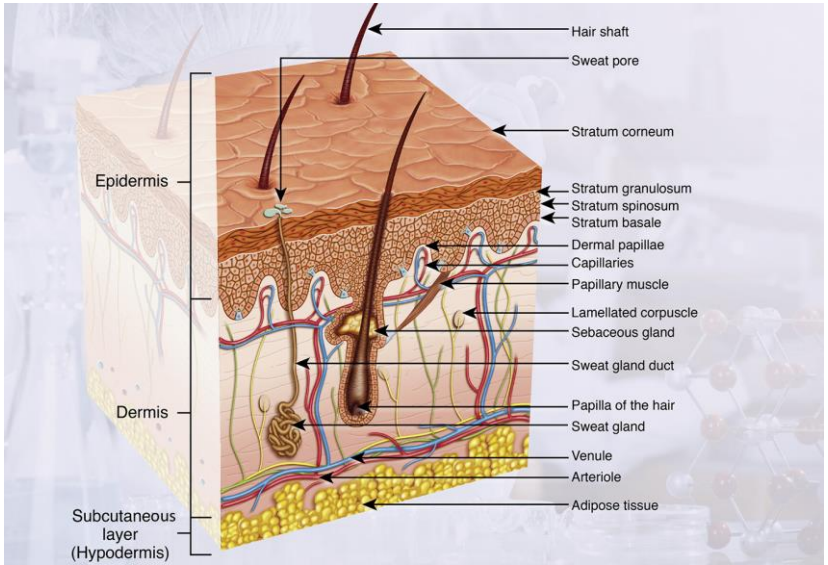
VisualResume (ver:0.1.1), RStudio (ver: 1.4.1717)



Dermal absorption is a complex process



- Physicochemical changes of product when applied to skin, may affect API bioavailability
- Excipients play significant role in drug delivery
- Solvent depletion due to evaporation or penetration to tissue -changes in thermodynamic activity of the drug and/or system on and inside the tissue
- API solubility in the residue
- Crystalline vs dissolved state



Hadgraft and Lane, *Phys. Chem. Chem. Phys*. **2011,** 13, 5215–22

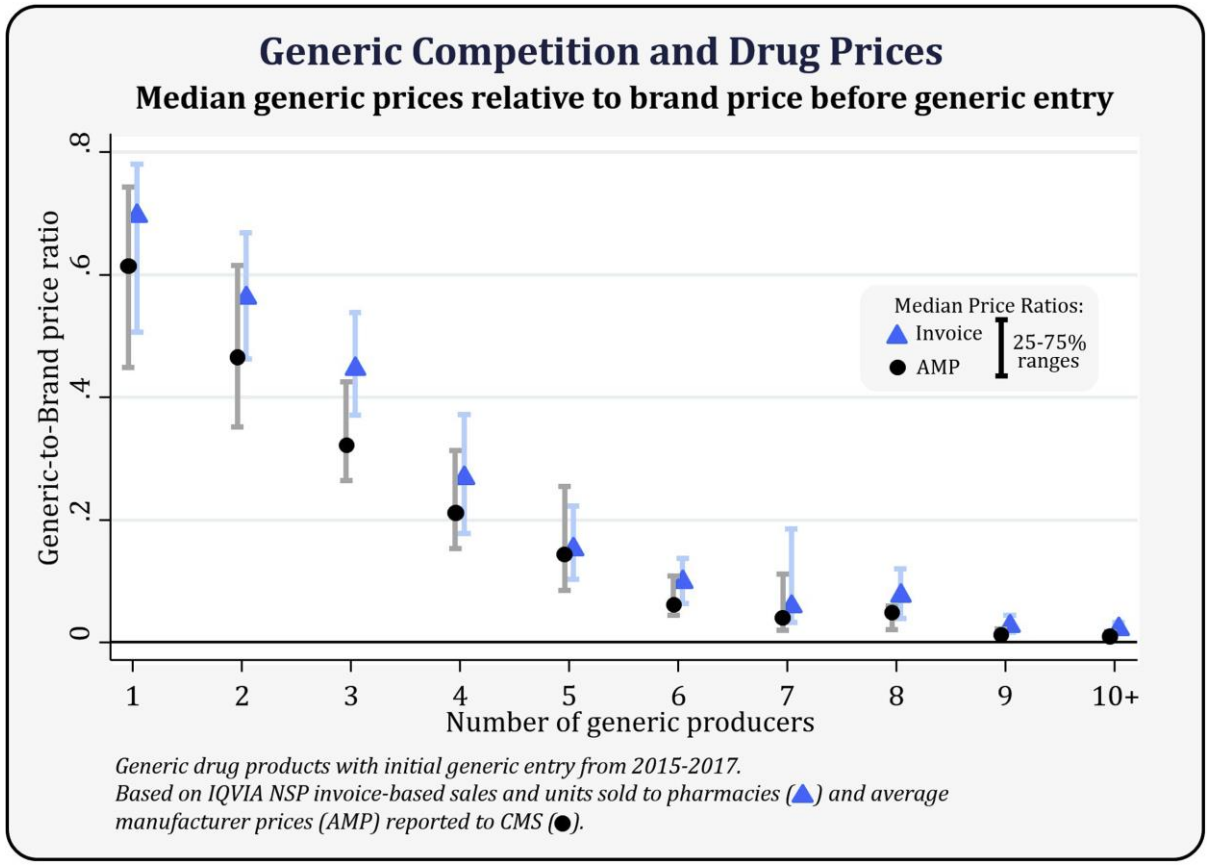
Mathes et al., *Adv Drug Deliv Rev.* **2014**, 69-70, 81-102

What is BE and why it is important

Bioequivalence (BE) of pharmaceutically equivalent formulations is defined as "*the absence of a significant difference in the rate and extent to which the active ingredient or active moiety become available* ***at the site of drug action*** *when administered at the same molar dose under similar conditions in an appropriately designed study*".

Effective BE assessment facilitates the development and availability of multisource generics

- Boosts market competition
- Reduces drug prices (estimated 20% price decline with each new market entrant)
- Widens patient access to treatment (availability & affordability)



US FDA analysis of prices and competition for all drug products with initial generic entry between 2015 and 2017, showing median generic-to-brand price ratios by the number of generic producers

CFR - Code of Federal Regulations; 21CFR320.23

Raney and Luke, *J Am Acad Dermatol*. **2020**; 82: 1570–1571

Challenges for assessing topical BE

Plasma concentrations may not be representative of local tissue concentration of the API

Most topical products have few or no approved generics

Lack of competition drives prices up

Uncertainty about formulation efficacy limits product development

There is **currently no established method** for measuring epidermal and superficial dermal drug concentrations, at or near the site of action

57% of topical drug products experienced a price increase of more than 100% between 2010 and 2015, with the average price of topical generic drugs 276% higher by 2015

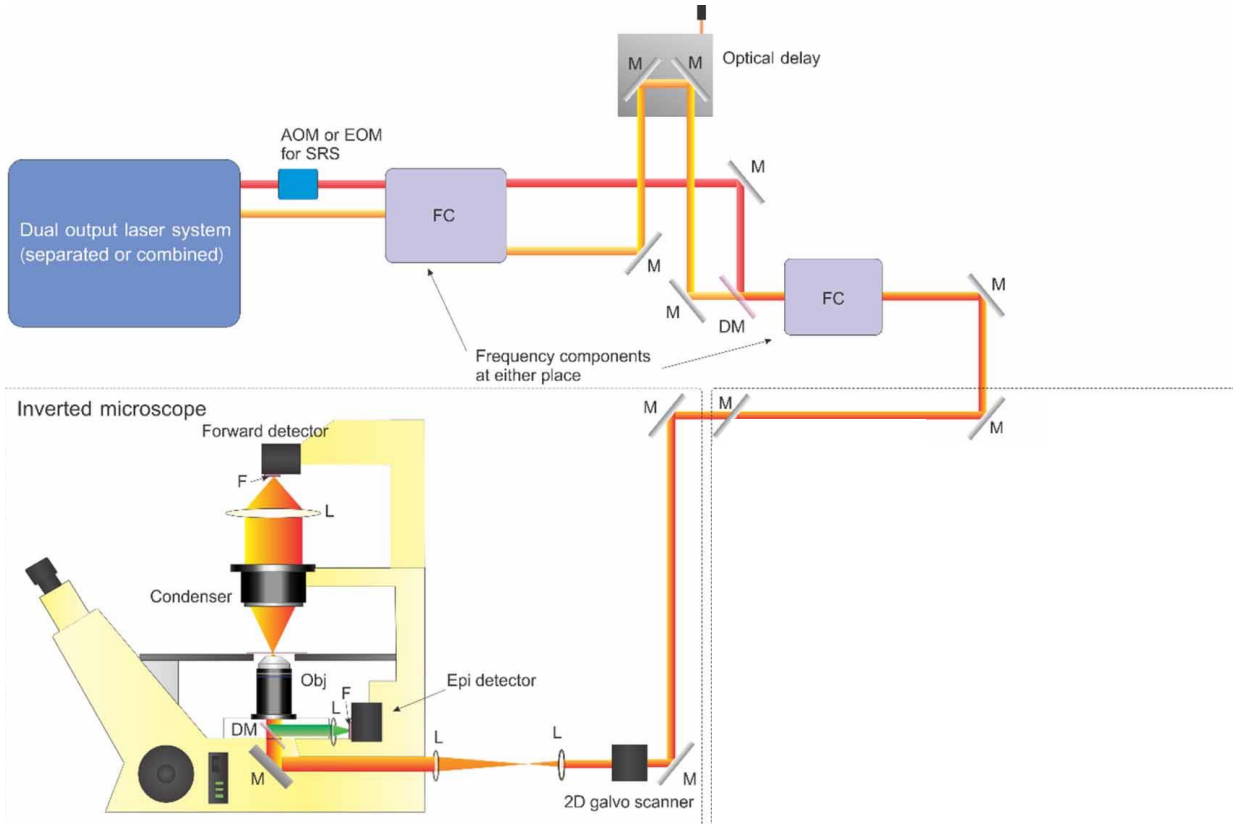
Table. Prices of Surveyed Prescription Drugs

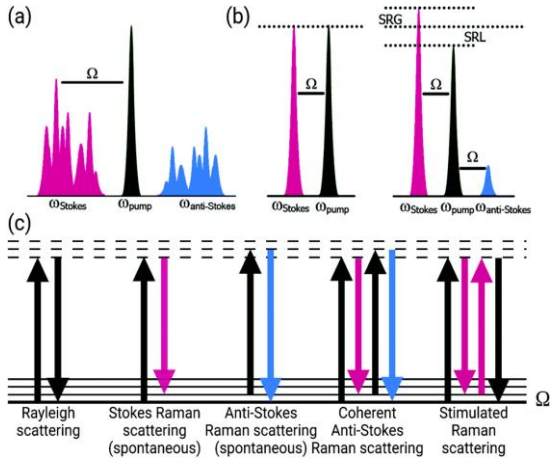
Drug	Type	Price, US $				Absolute Change, 2009-2015	% Change, 2009-2015
		2009	2011	2014	2015		
Altabax, 15 g	I	92.50	106.18	168.75	196.86	104.36	112.82
Benzaclin, 50 g	A	166.79	205.80	451.29	503.85	337.06	202.08
Carac cream, 30 g	N	159.40	227.16	2939.68	2864.70	2705.30	1697.18
Clobex spray, 4 oz	S	389.57	500.29	827.11	958.01	568.44	145.91
Cloderm cream, 30 g	S	96.47	132.92	220.75	360.02	263.55	273.19
Cutivate lotion 120 mL	S	305.00	493.92	918.63	1067.25	762.25	249.91
Derma-Smoothe FS oil, 4 oz	S	45.70	47.23	247.84	322.67	276.97	606.06
Finacea, 50 g	A	124.42	185.42	288.92	284.30	159.88	128.51
Olux-E foam, 100 g	S	307.58	382.79	750.79	841.76	534.18	173.67
Oracea, 40 mg (30 tablets)	A	439.01	416.09	632.80	702.46	263.45	60.01
Oxistat cream, 30 g	I	76.50	119.25	399.00	544.66	468.16	611.97
Oxsoralen-Ultra, 10 mg (50 capsules)	P	1227.32	2150.49	4568.54	5204.31	3976.99	324.04
Retin-A Micro, 0.1%, 50 g	A	178.05	335.73	791.47	914.52	736.47	413.64
Solaraze gel, 100 g	N	442.89	618.56	1738.91	1883.98	1441.09	325.38
Soriatane, 25 mg (30 capsules)	P	757.75	958.50	1452.50	1595.27	837.52	110.53
Taclonex, 60 g	P	465.99	522.58	848.21	962.90	496.91	106.64
Targretin gel, one 60-g tube	N	1686.78	1787.97	15 708.40	30 320.12	28 633.34	1697.51
Tazorac cream, 0.1%, 60 g	A	266.18	464.96	656.20	722.27	456.09	171.34
Xolegel, 30 g	I	212.50	278.00	389.25	641.96	429.46	202.10

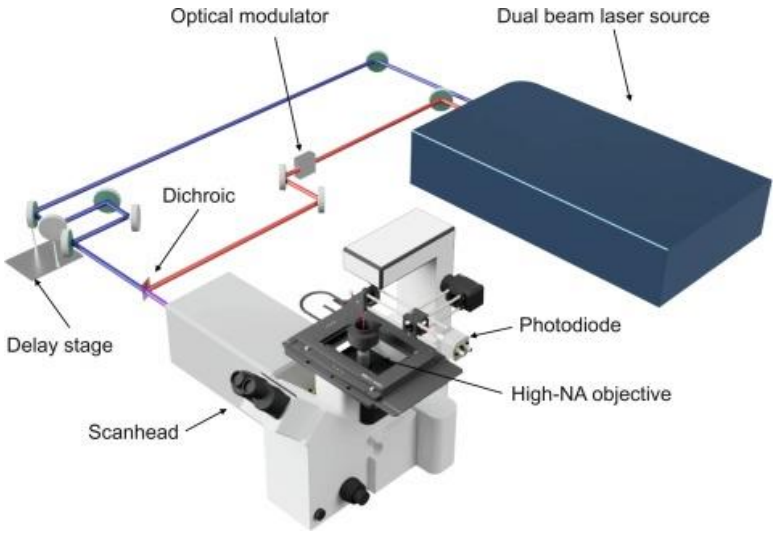
Abbreviations: A, acne and rosacea; I, antiinfective; N, antineoplastic; P, psoriasis; S, corticosteroid.

Raney and Luke, *J Am Acad Dermatol*. **2020**; 82: 1570– 1571
Rosenberg and Rosenberg, *JAMA Dermatol*, **2016**, 152, 158-163

Stimulated Raman Scattering Microscopy



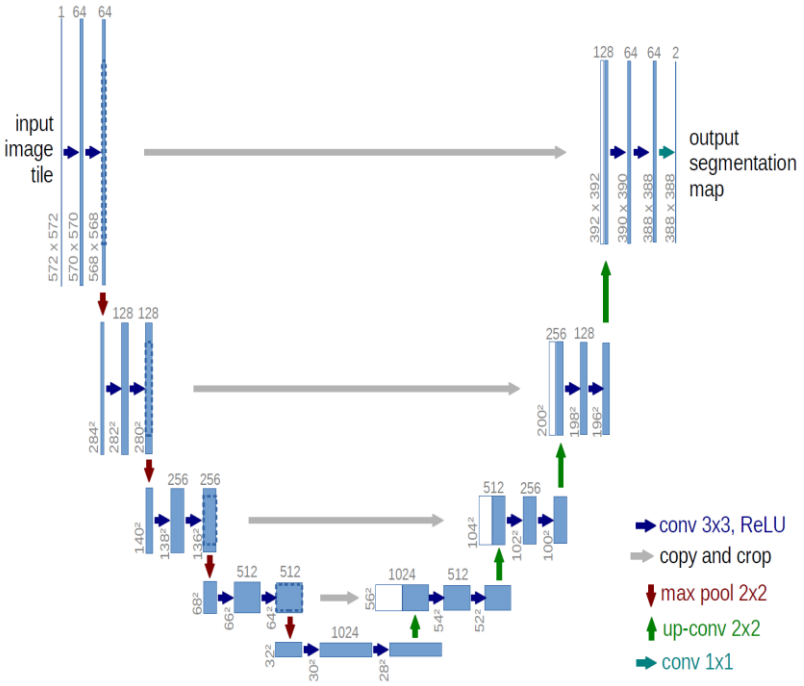


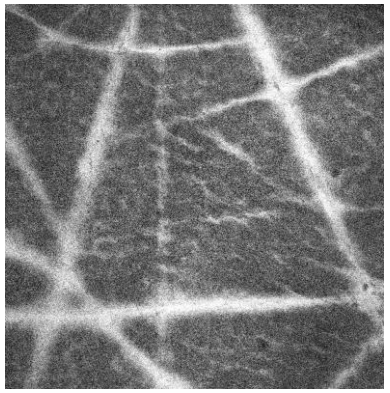


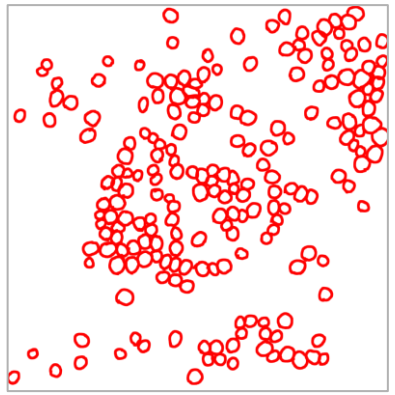
Prince and Potma, Stimulated Raman Scattering Microscopy Ch3, Elsevier, **2022**, 41-65,
Zhang and Aldana-Mendoza, *J. Phys. Photonics* **2021,** 3, 1–31
Pence and Evans *Analyst* **2021**, 146, 6379-6393

Determining cutaneous PK with a Deep Learning-based Pipeline

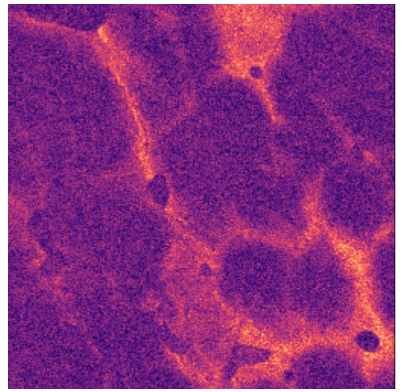
U-Net training for signal collection from selected skin regions

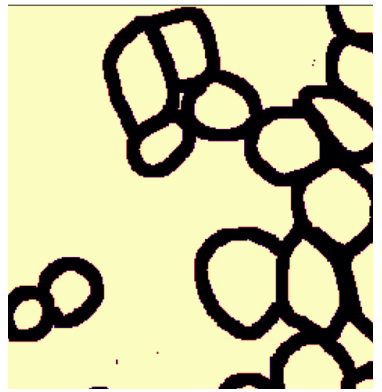






Sample image of human stratum corneum with corresponding hand-drawn annotations





U-Net training on Image/Annotations pairs

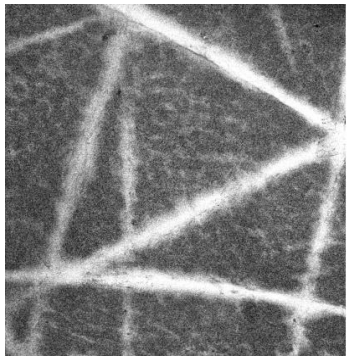
Libraries used:

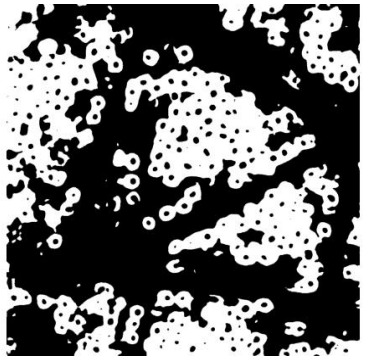
Python:

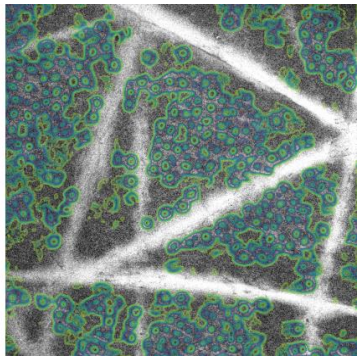
Tensorflow	Javabridge
Numpy	Python-Bioformats
Matplotlib	PyYaml

R:

Reticulate	Noncompart
Ggplot2	







Test image and machine generated output: probability image ranging from 0 to 1 and probability image as overlay over the original image.

Experimental design for Pivotal BE Study

1. Reference product (R1): Tazorac® cream Almirall, LLC

2. Generic product: Taro Pharmaceuticals U.S.A., Inc (cream)

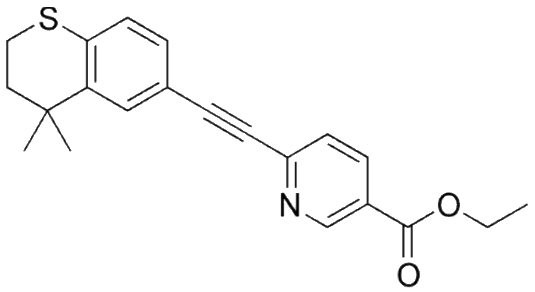
3. Reference product (R2): Same as reference product

4. Alternative formulation (gel): Tazorac® gel

5. Alternative formulation (PEG solution): Taz in PEG-200

Model drug & concentration in formulations	Tazarotene 0.1 % (*w/w*)
Number of donors	4
Skin preparation	Full-thickness, abdominal – Subcutaneous fat trimmed to allow SRS signal detection in the forward direction
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
Study duration	~6.5 hours of imaging (15 cycles)

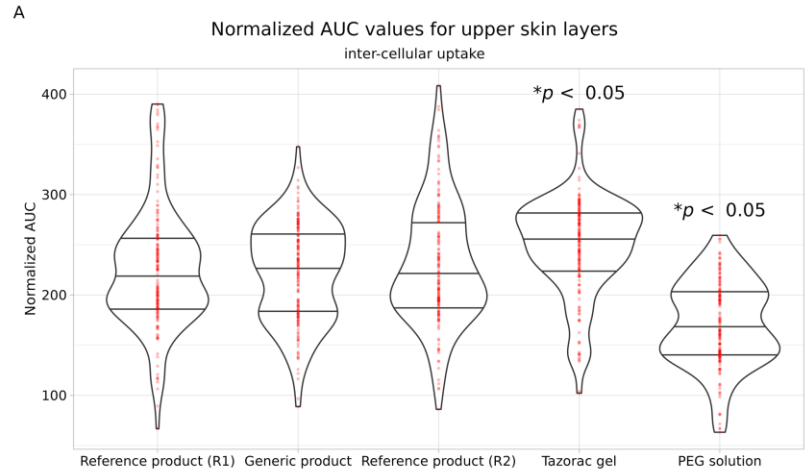
- SRS system tuned to 1590 cm^{-1} to target the delocalized C=C stretching vibration of the Taz backbone.
- The skin structure was imaged using the ~2870 cm^{-1} wavenumber to target the CH_2 methylene stretching vibration of lipids
- The tuning sequence was set to alternate between 1590 and 2870 to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

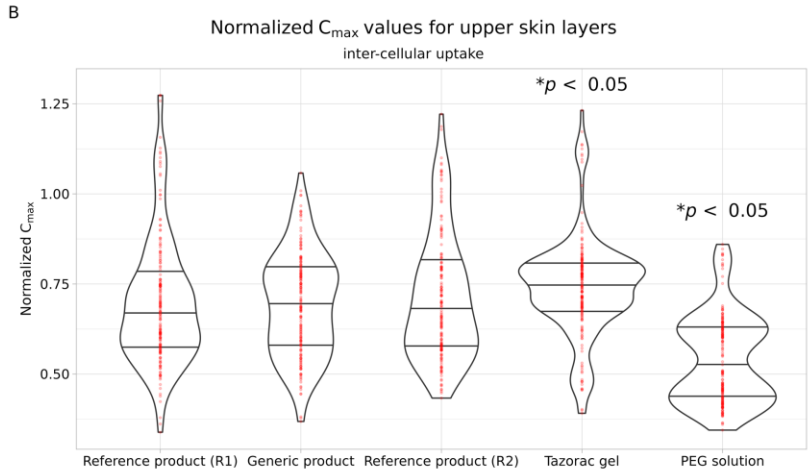


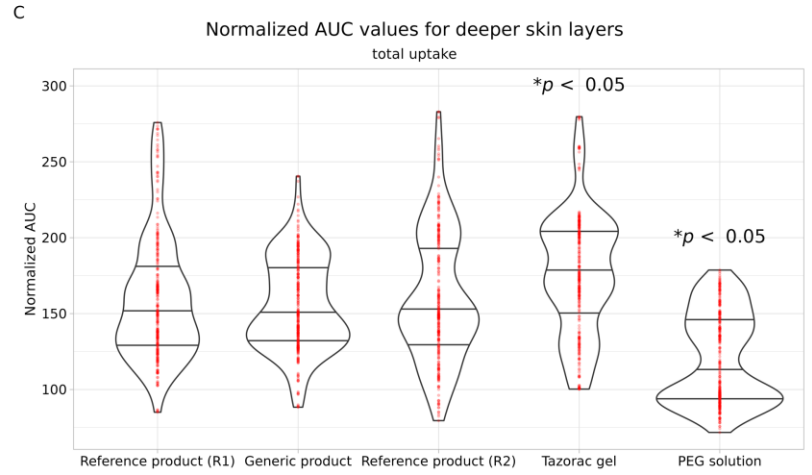
Molecular mass	351.5 g/mol
Melting point	97-106 °C
$logP_{(o/w)}$	5.6
Aqueous solubility	0.1 mg/mL

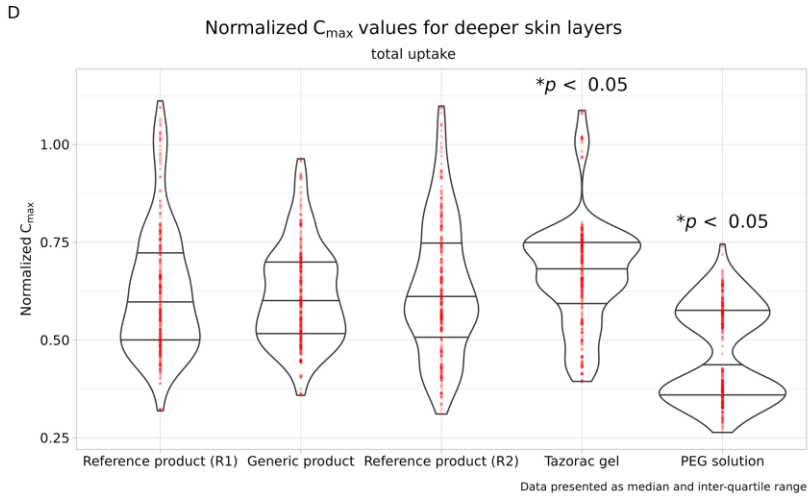
Tazarotene, DrugBank online , https://drugbank.com/drugs/DB00799 (accessed on Aug 5, 2022)

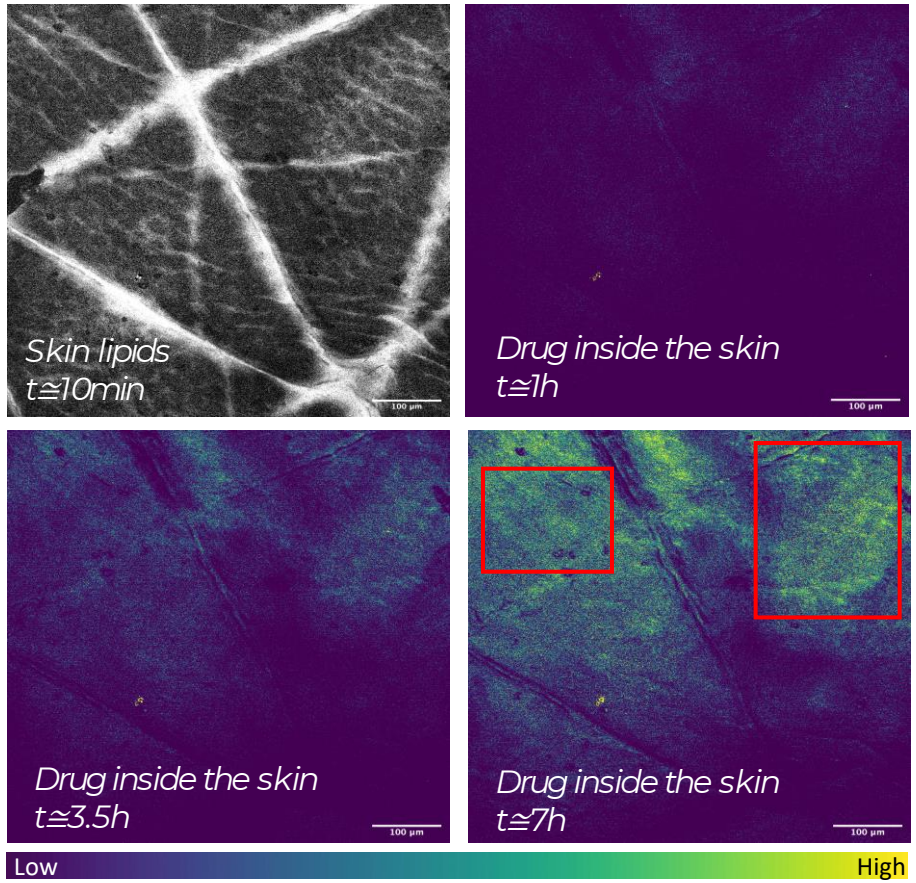
Visualization of Tazarotene disposition to human skin over time







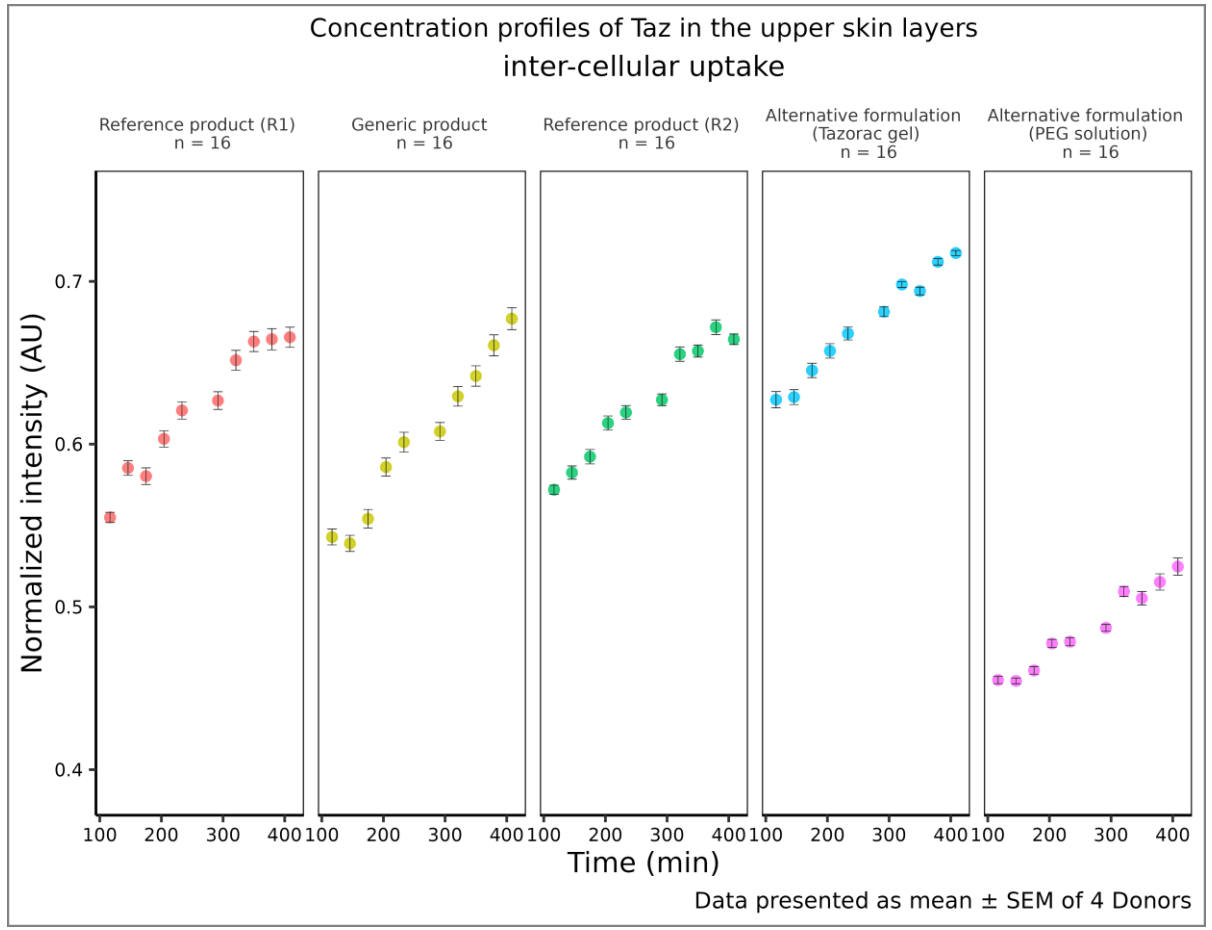


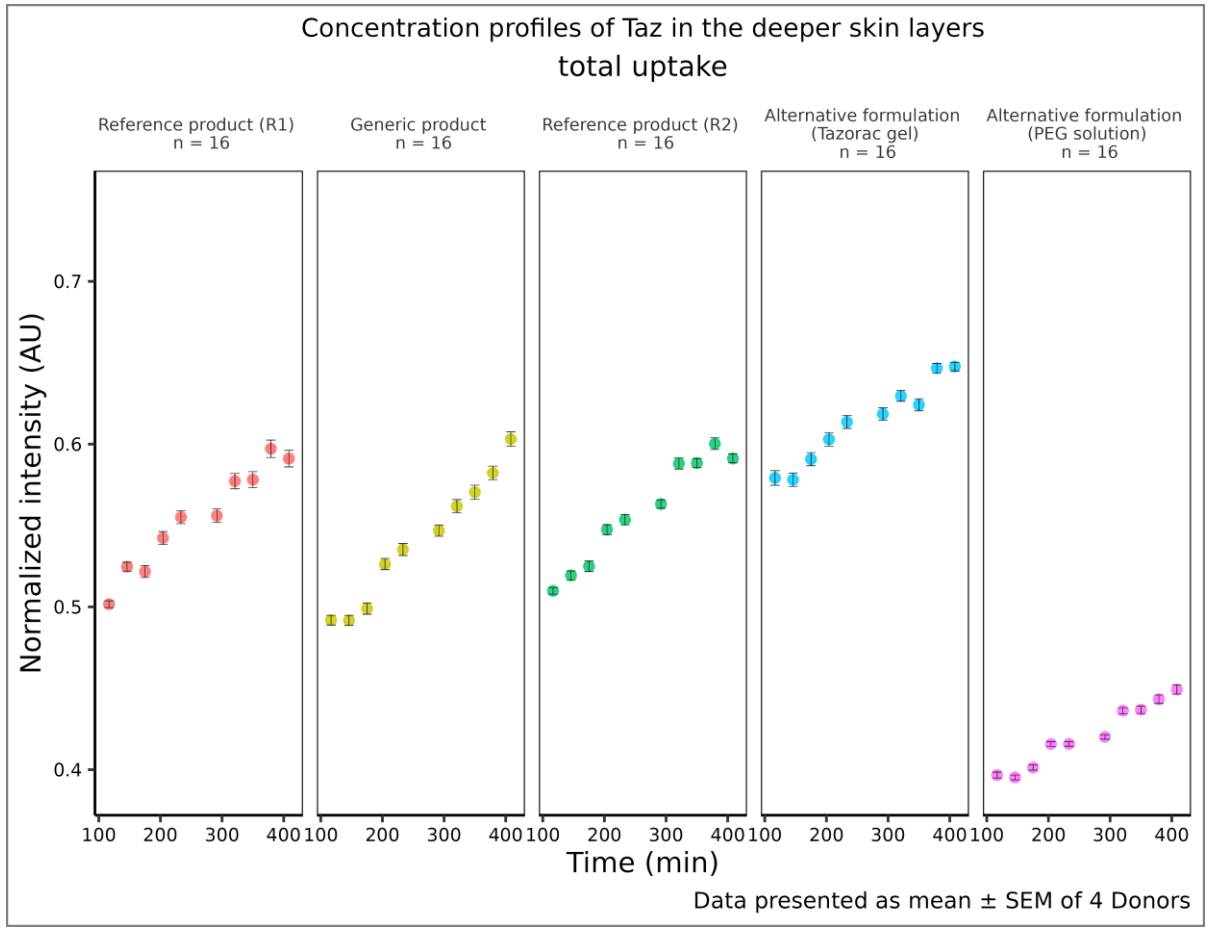


R1: Tazorac® cream; Generic product (cream): Taro Pharmaceuticals U.S.A., Inc; R2: Tazorac® cream; Alternative formulations: Tazorac® gel & Taz in PEG-200 solution (data from 4 donors; n=4 replicates per donor; 4 regions of interest (ROI) per replicate. (A-B) Peak drug concentration (C_{max}) and area under the drug penetration curve (AUC) values in the upper skin layers (0 – 16µm); (C-D) C_{max} and AUC values in the deeper skin layers (24 – 64µm).

Monitoring drug delivery into human skin (~8µm depth) by SRS microscopy ex vivo. SRS images showing Taz penetration over time. Images suggest a predominant route of drug permeation via the intercellular lipids. SRS contrast obtained at 1590 cm^{-1}.

Dermal distribution profiles of Tazarotene





Concentration vs time profiles of Tazarotene (AU) across the skin estimated by SRS microscopy for various formulations following finite dose application *ex vivo*. Reference product (R1): Tazorac® cream; Generic product: Taro Pharmaceuticals U.S.A (cream)., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & PEG-200 solution (mean ± SEM of 4 donors; n=4 replicates per donor; 4 regions of interest (ROI) per replicate). Upper skin layers: 0 – 16µm; Deeper skin layers: 24 – 64µm.

Conclusions & Future work

- ✓ Statistical analysis indicated that the RLD resulted in similar cutaneous PK parameter values of AUC and C_{max} compared to both itself (R1 vs R2; $p>0.05$) and the generic product (R1 vs Generic; R2 vs Generic; $p>0.05$). PEG-200 solution resulted in significantly lower amounts of Taz uptake by the tissue ($p<0.05$).
- ✓ Overall, the proposed method was found capable of detecting differences in the rate and extent of dermal drug absorption from different topical skin products.
- ✓ Real-time imaging of drug distribution across the tissue can additionally provide insights into drug permeation pathways and aid in pre-clinical evaluation of formulations

- Studies ongoing with additional drugs and dermatological products to examine the sensitivity and robustness of SRS and further explore this method as a novel cutaneous PK-based approach for evaluation of topical BE.
- Studies ongoing for assessing the utility of this method for determining skin uptake of chemicals with no unique vibrational bands.
- Future studies will focus on *in vivo* evaluation of topical BE by SRS

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Evans' team

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Michelle Wei
Tucker Raymond
Manolis Rousakis, PhD
Fei Peng, PhD
Juan Pedro Cascales, PhD
Maria Alice Tabosa, PhD
Badri Parshad, PhD
Saara Luna
Julia Slade
Dandan Tu, PhD
Anna Wiatrowski
Haley Marks, PhD
Wonsang Hwang, PhD

FDA team

Priyanka Ghosh, PhD
Markham C. Luke, PhD
Sam Raney, PhD
Tannaz Ramezanli, PhD
Anika Haq, PhD
Zane Arp, PhD

Refined Laser Systems

Sven Dobner, PhD
Niklas Lupken, PhD
Max Brinkman, PhD
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Questions

Fotis Iliopoulos, PhD

Wellman Center for Photomedicine
Massachusetts General Hospital
Harvard Medical School

FILIOPOULOS@mgh.harvard.edu

LinkedIn

Google Scholar