Cutaneous pharmacokinetic and pharmacodynamic imaging with coherent Raman scattering

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Conflict of Interest

I hold patents on technologies related to Coherent Raman Imaging that have been licensed to both Leica and Zeiss

Disclaimer

This project is supported by the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services (HHS) as part of a financial assistance award [FAIN] totaling $1.25 Million with 100 percent funded by FDA/HHS. The contents are those of the author(s) and do not necessarily represent the official views of, nor an endorsement, by FDA/HHS, or the U.S. Government.

Acknowledgments

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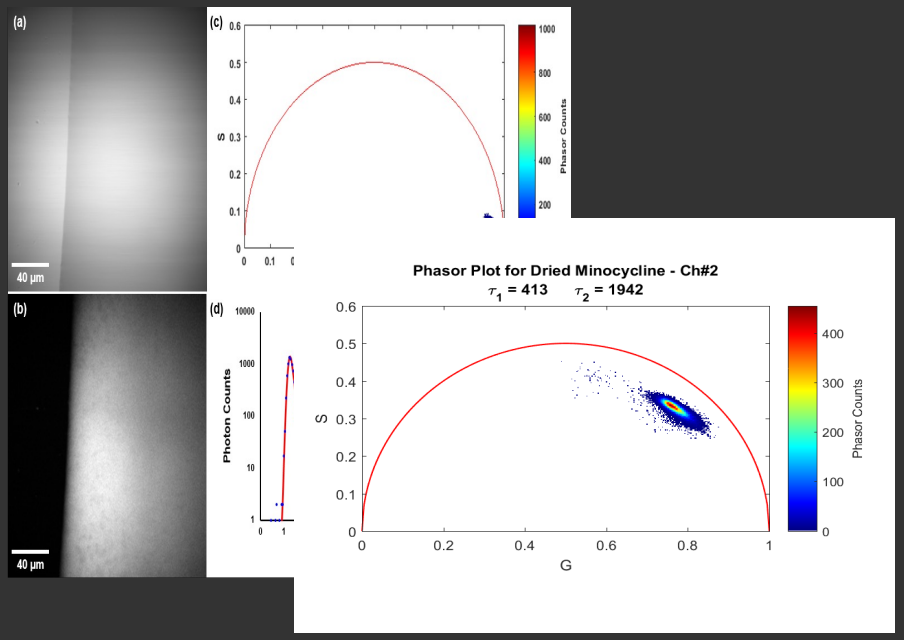
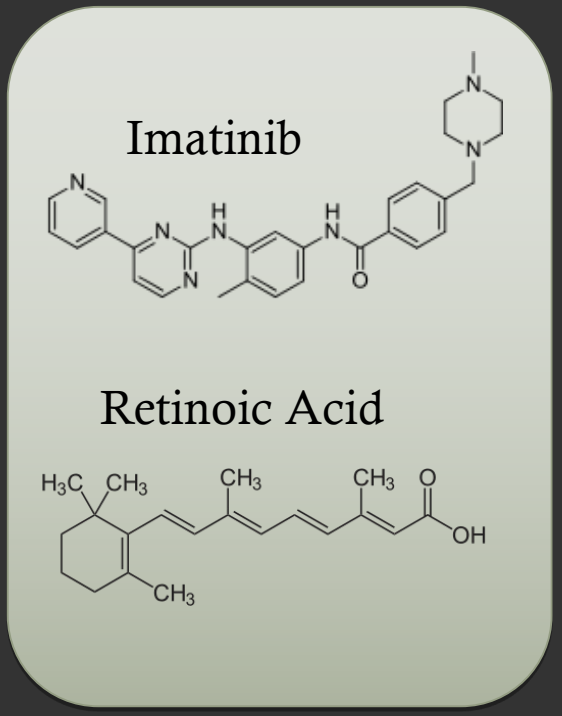
Pharmacokinetic and Pharmacodynamic Tomography

A paradigm for the microscopic imaging and quantification of drugs and their effects



Fluorescence Intensity/Lifetime



Molecular Vibrations

Coherent Raman Scattering (CRS) Microscopy

Imaging based on intrinsic vibrational contrast

Two Colors: ω_p “Pump”

ω_s “Stokes”

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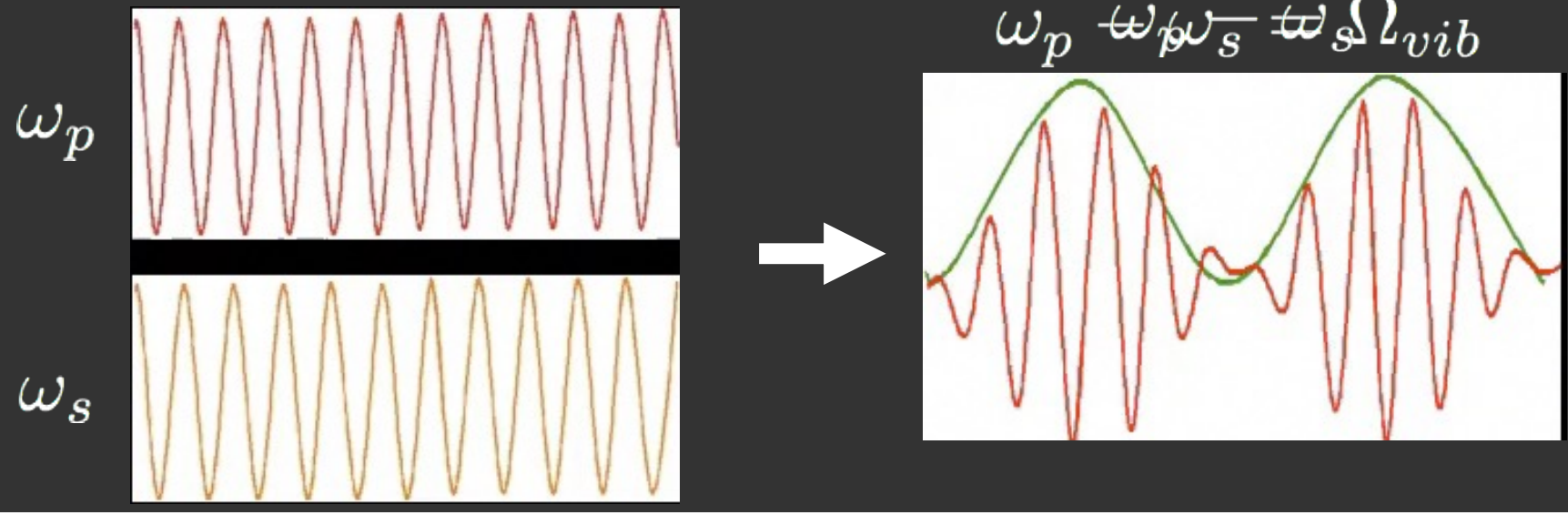
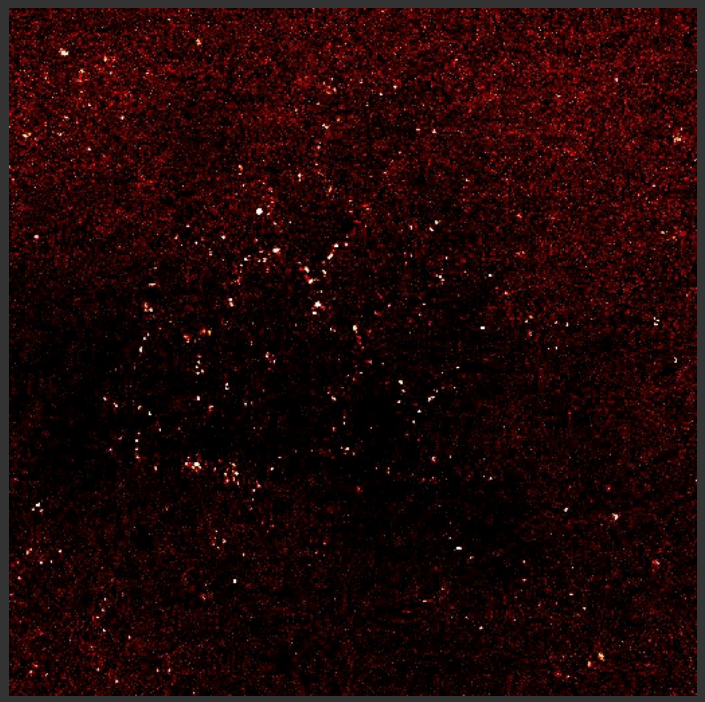
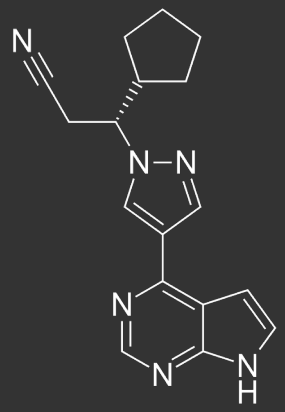


Drug Uptake Dynamics in the Stratum Corneum

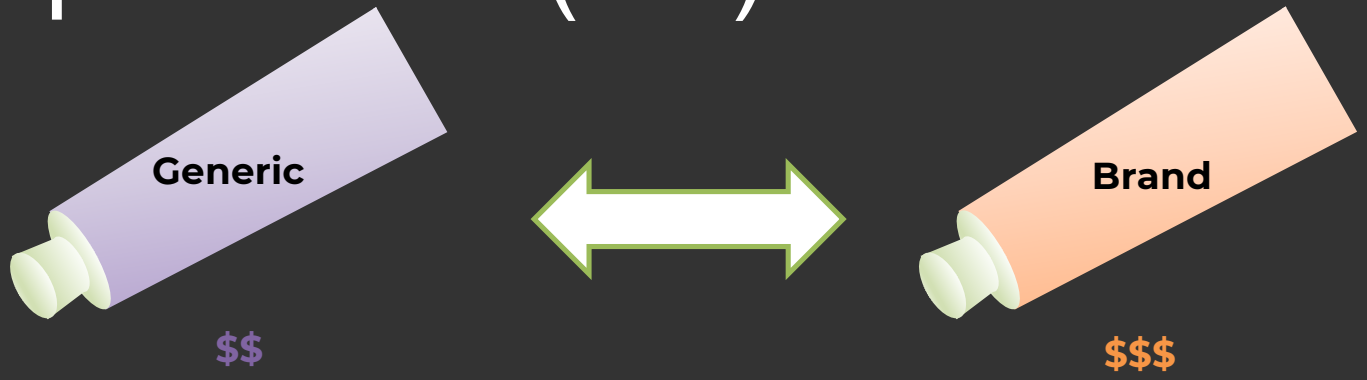
SRS Microscopy
Nitrile Stretch: 2250 cm-1
100% resonant signal
120 min





Direct visualization of Rux depositing on the surface of skin without background signal

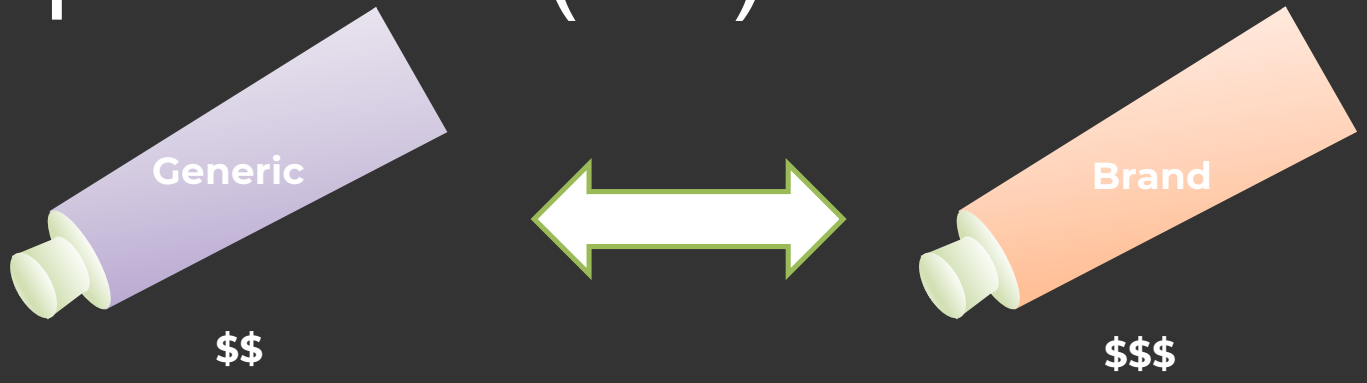
Bioequivalence(BE)



A generic drug is a medication created to be the same as an already marketed brand-name drug in dosage form, safety, strength, route of administration, quality, performance characteristics, and intended use[1].

[1] https://www.fda.gov/drugs/generic-drugs/what-approval-process-generic-drugs

Bioequivalence(BE)

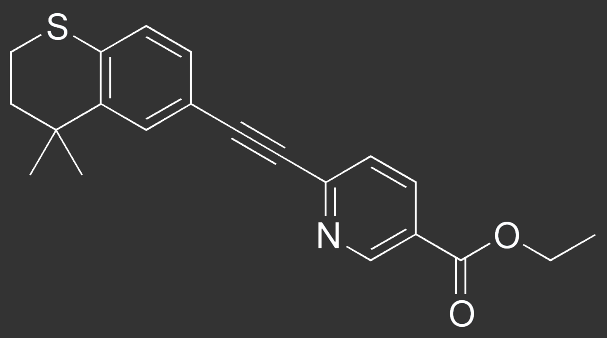


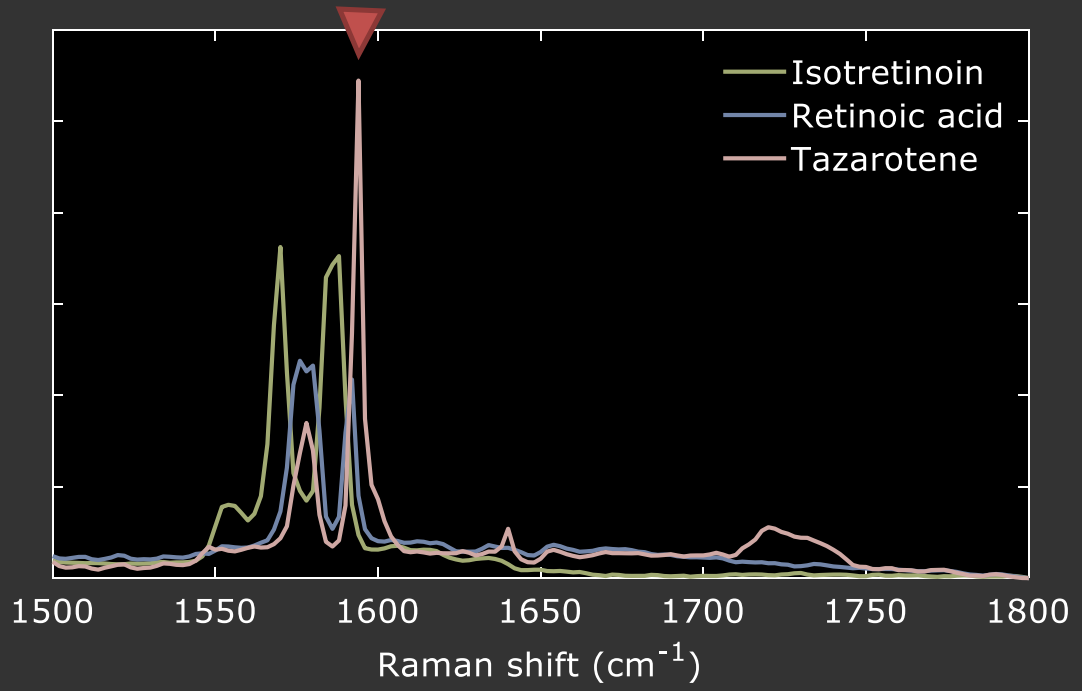
- The generic medicine is bioequivalent to the brand-name medicine.
- Two products are considered to be bioequivalent when they are equal in the rate and extent to which the active pharmaceutical ingredient (API) becomes available at the site(s) of drug action[2].

[2] https://www.fda.gov/animal-veterinary/abbreviated-new-animal-drug-applications/bioequivalence

SRS for Evaluation of Topical BE

- Tazarotene
- 3rd Generation Retinoid for the treatment of numerous skin conditions including **acne vulgaris and psoriasis**
- SRS tune to wavenumber ~1594 cm^{-1} , attributed to delocalized vinyl stretch



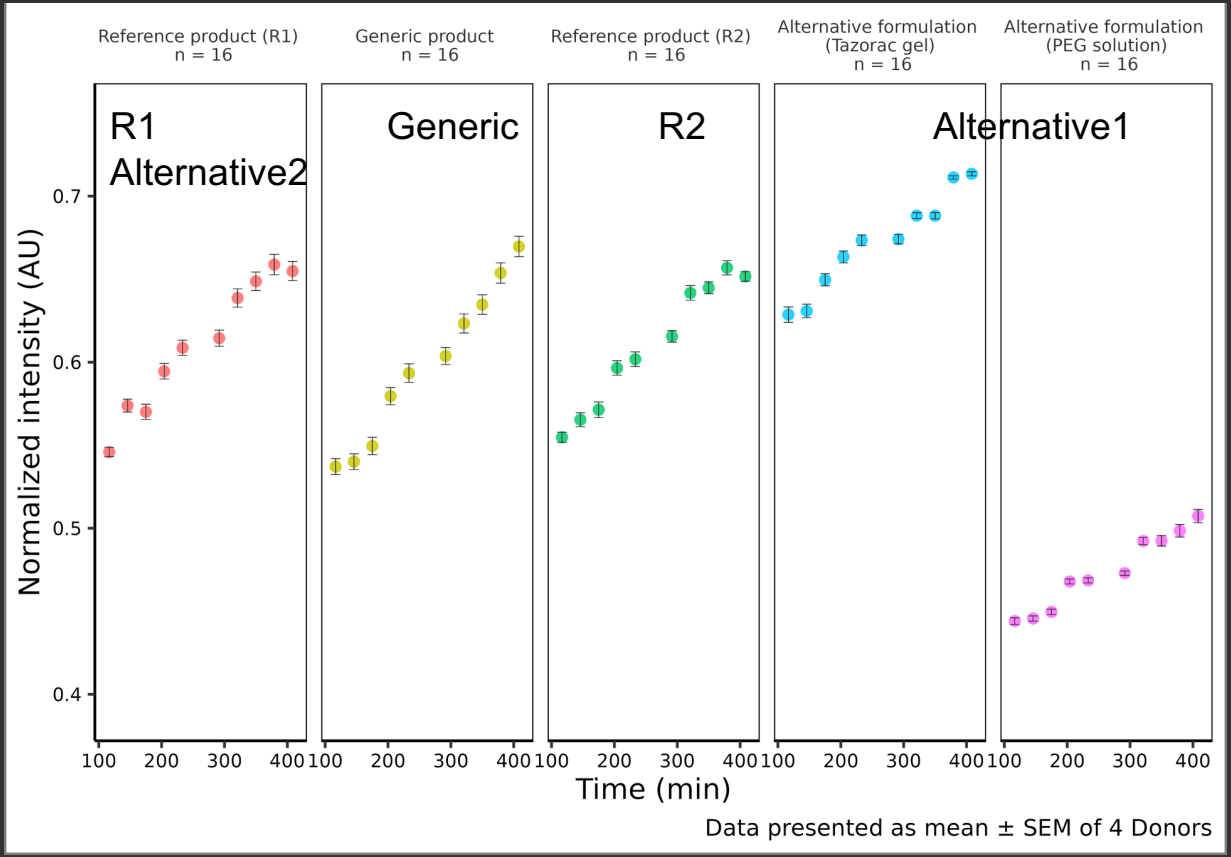


Experiment Methods

Treatment groups:

- **Reference product (R1)**: Tazorac® Almirall, LLC;
  Dosage form: cream;
- **Generic product**: Taro Pharmaceuticals U.S.A., Inc;
  Dosage form: cream;
- **Reference product (R2)**: Same as reference product
  (Provides a measure of inter-experimental variability)
- **Alternative Formulation**: Tazorac®;
  Dosage form: gel;
- **Alternative formulation (PEG solution):** Taz in PEG-200 (0.1 % w/w)

Mean concentration profiles for all donors

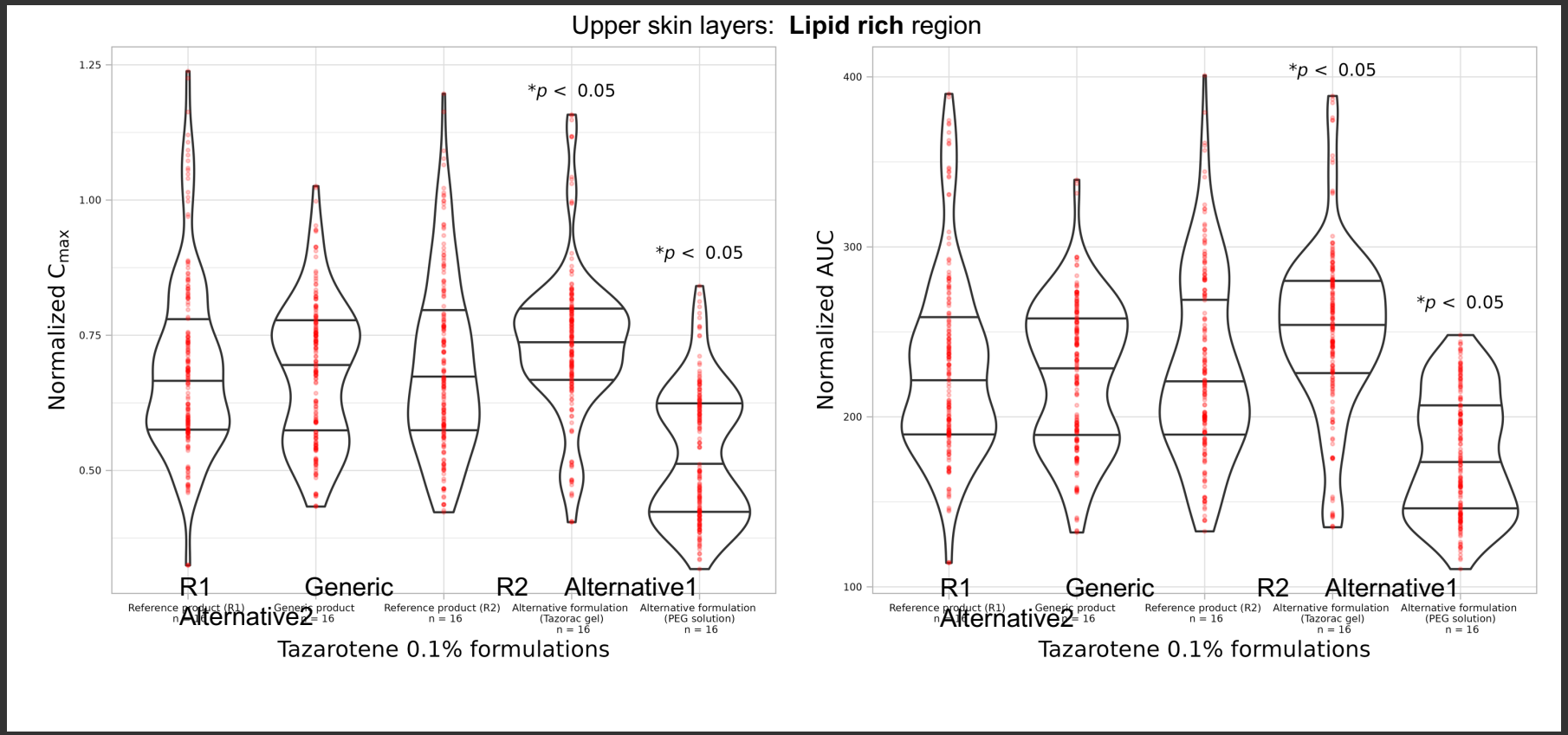


- Upper skin layers
  Lipid rich region

Concentration 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. Upper skin layers: 0-16µm.

(Mean±SEM of 4 donors; n=4 replicates per donor; 4 regions of interest (ROI) per replicate)

Pharmacokinetic Parameters: Lipid-Rich



Estimated cutaneous pharmacokinetic parameters following finite dose application of Tazarotene-containing formulations to human skin *ex vivo*. Reference product (R1): Tazorac® cream; Generic: Taro Pharmaceuticals U.S.A (cream)., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & PEG-200 solution. Statistical significance determined by Kruskal-Wallis Test and Dunn test for multiple pairwise comparisons. The family-wise error rate was controlled using Dunn's Bonferroni adjustment. Upper skin layers: 0-16µm.

Positive Control Shows Bioequivalence

Donor number	Difference of the means of the log-transformed C_{max} values (Reference product (R1) – Generic product)	Difference of the means of the log-transformed AUC values (Reference product (R1) – Generic product)
Abd 39y F	0.12	0.12
Abd 42y F	0.10	0.10
Abd 48y F	-0.07	-0.06
Abd 54y M	-0.14	-0.14
Mean(x)	0.03	0.01
SEM	0.06	0.06
90% CI [LL, UL]	[-0.15, 0.15]	[-0.14, 0.15]

Mean values of both metrics are found between the ln(0.8) and ln(1.25) limits (i.e., -0.22, 0.22), suggesting bioequivalence

Reference product (R1): Tazorac® cream; Generic product: Taro Pharmaceuticals U.S.A (cream)., Inc

Data from SC intercellular uptake values

Alternative formulation is NOT Bioequivalent

Donor number	Difference of the means of log-transformed C_{max} values (Alternative formulation (PEG-200))	Difference of the means of the log-transformed AUC values (Reference product (R1) – Alternative formulation (PEG-200))
Abd 39y F	0.26	0.42
Abd 42y F	0.17	0.14
Abd 48y F	0.36	0.29
Abd 54y M	0.22	0.27
Mean(x)	0.25	0.28
SEM	0.04	0.06
90% CI [LL, UL]	[0.16, 0.35]	[0.15, 0.42]

Reference product (R1): Tazorac® cream; Alternative formulation (PEG-200)

Mean values of both metrics are found outside the ln(0.8) and ln(1.25) limits (i.e., -0.22, 0.22), suggesting the two products are not bioequivalent

Data from SC intercellular uptake values

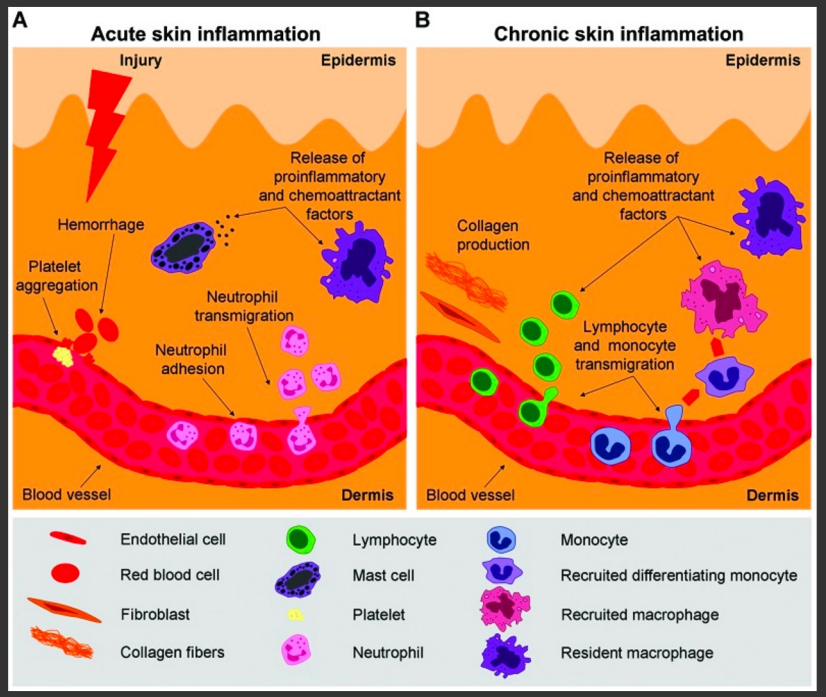
Skin Inflammation: Dermatitis

Collection of skin diseases that cause itch, redness and rash, also known as **eczema**.

Affects >245 Million people worldwide, **atopic dermatitis** being the most common.
Atopic dermatitis affects up to 30% of people in the US.

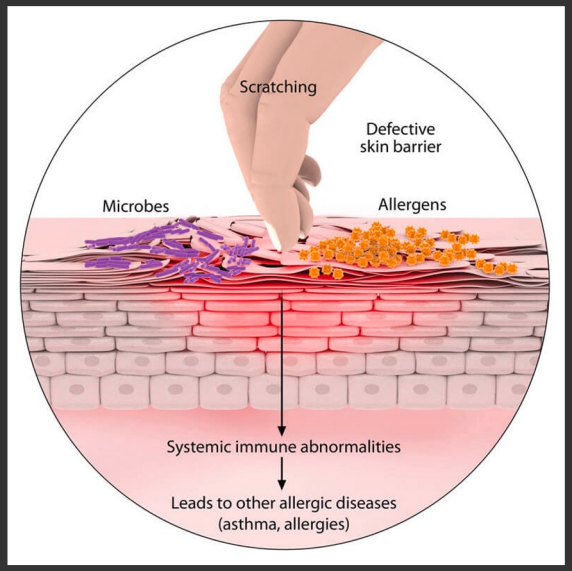
Quantifying PD with Imaging and Deep Learning

Inflammation is a dynamic, variable, complex process



Neagu, Monica, et al. *Oncology Reports* 35.5 (2016): 2516-2528.

Atopic dermatitis is one of the most common inflammatory skin conditions



Day	Procedure
-7	50µL oxazolone & ear thickness measurement
-1	10µL oxazolone & ear thickness measurement
0	4x Mouse imaging & ear thickness measurement
1	3x Mouse imaging & ear thickness measurement
2	1x Mouse imaging & ear thickness measurement
3	1x Mouse imaging & ear thickness measurement
4	1x Mouse imaging & ear thickness measurement

Oxa sensitization of mouse skin creates ”atopic dermatitis”

CARS and SRS imaging carried out at timepoints

“Gold Standard” of imaging was ear thickness

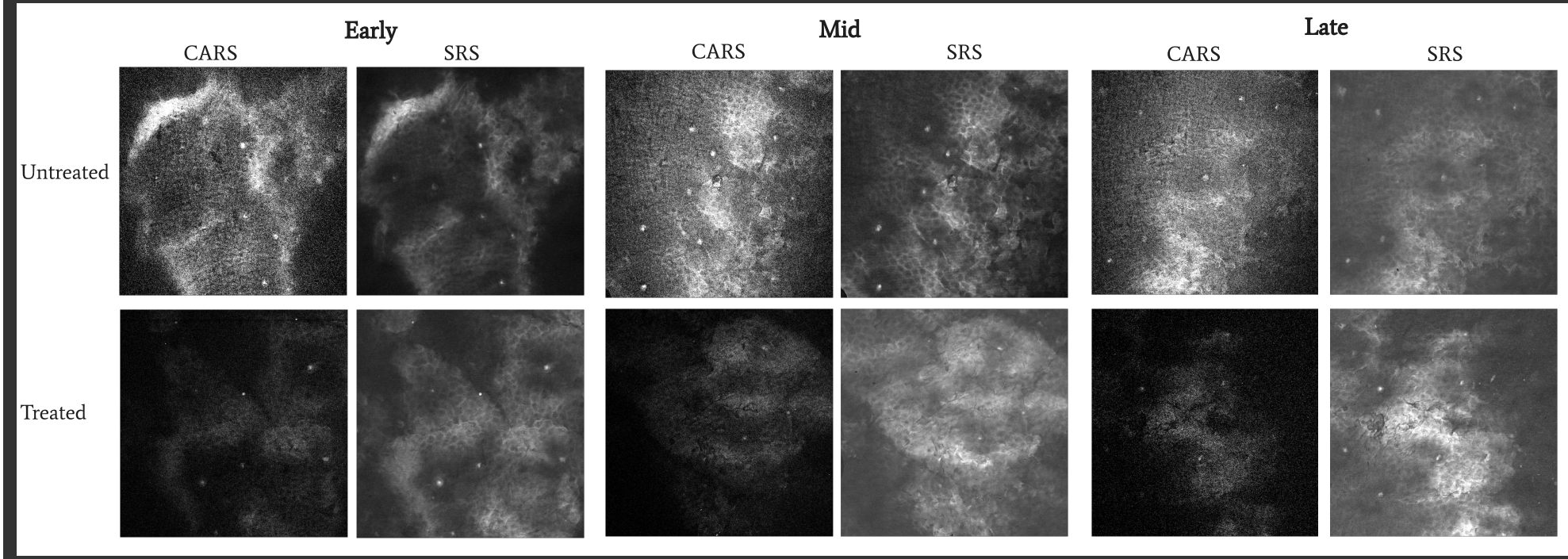
Single Tx of Ruxolitinib on Day 0

Rux Tx Significantly Reduces Inflammation

Linear mixed effects model fit with exponential regression:
log(Thickness) ~ Day + Treated + Day:Treated + (1|MouseID)

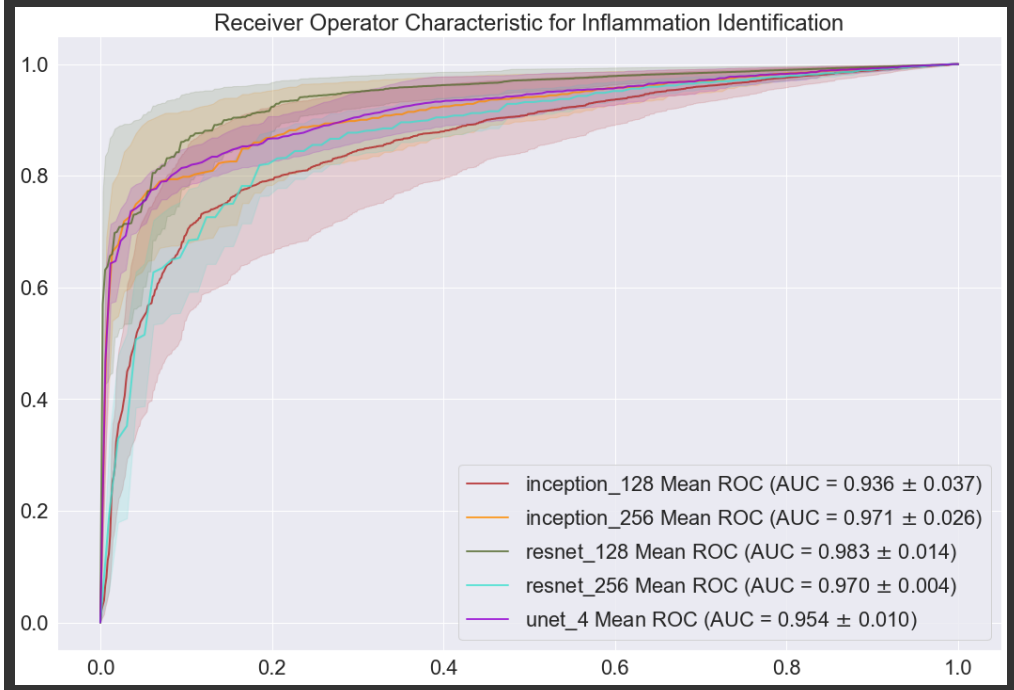
Fixed effects	Estimate	Std. Error	df	t-value	Pr(> |t|)
Intercept	-.885	0.039	10.418	-22.97	$2.91e^{-10}$ ***
Day	-.07	0.006	811.30	-12.49	$< 2e^{-16}$ ***
Treated	-.068	0.016	810.05	-4.234	$2.56e^{-05}$ ***
Day:Treated	-.012	0.008	810.05	-2.512	0.0122 *

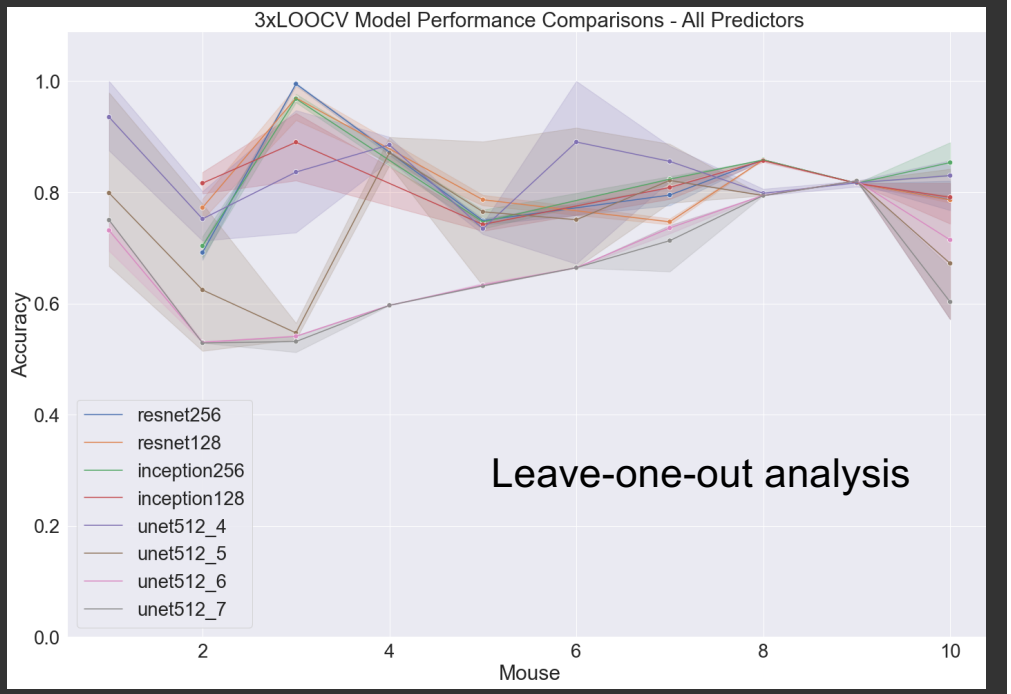
Quantifying PD with Deep Learning



Quantifying Skin with Deep Learning

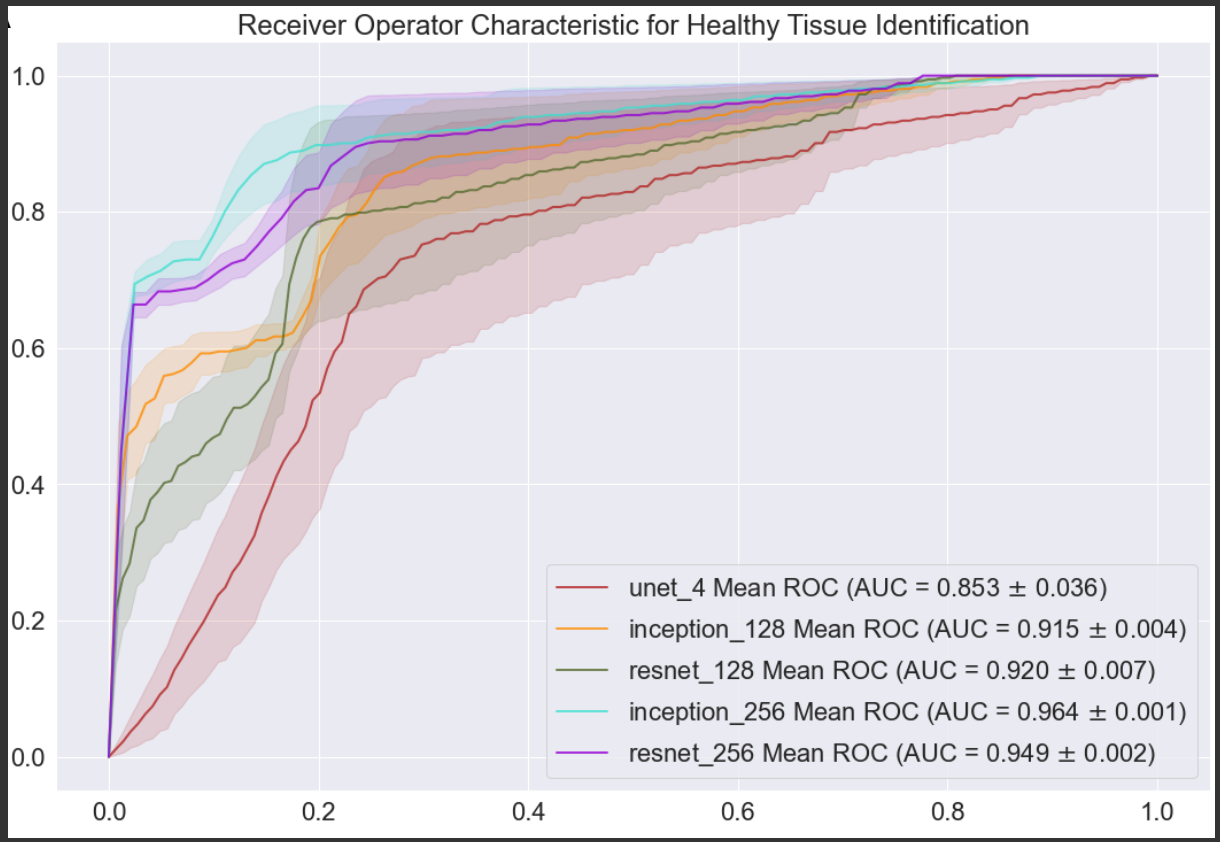
Binary Classification





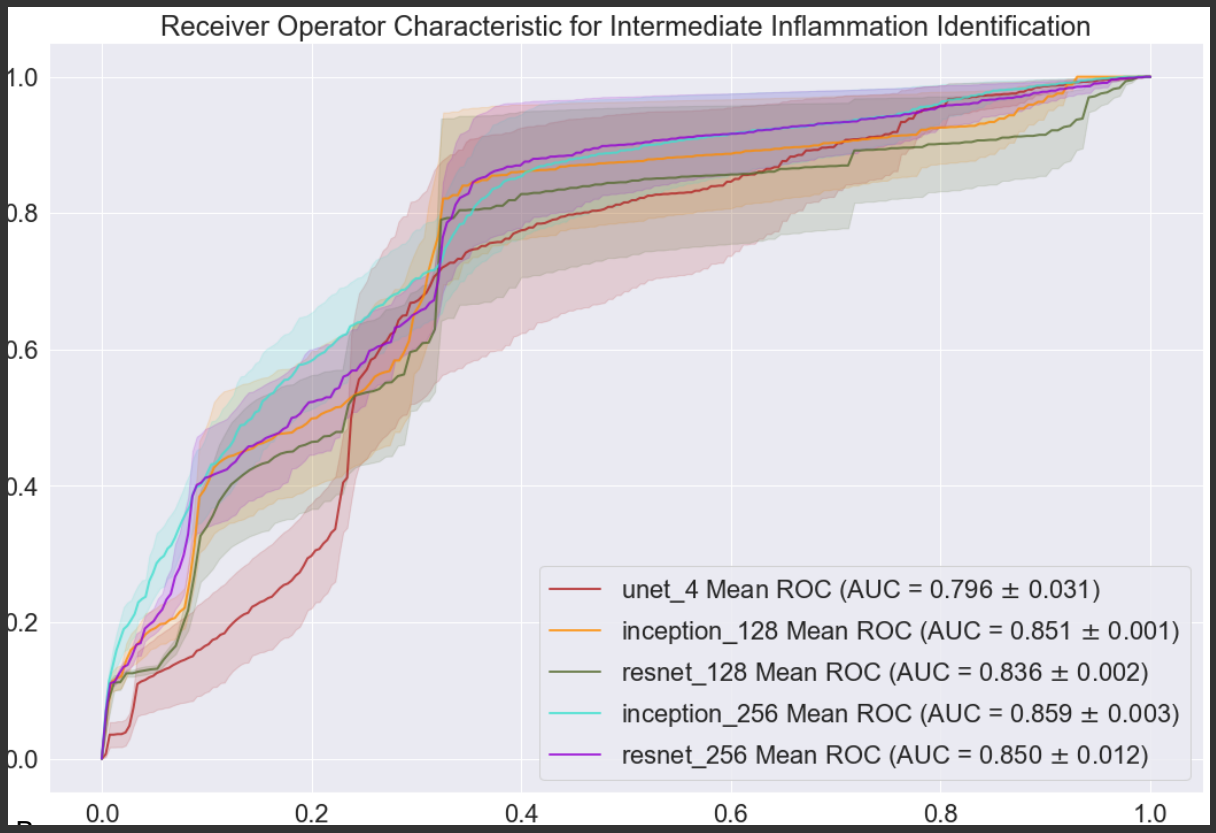
Quantifying PD with Deep Learning

Ternary Classification



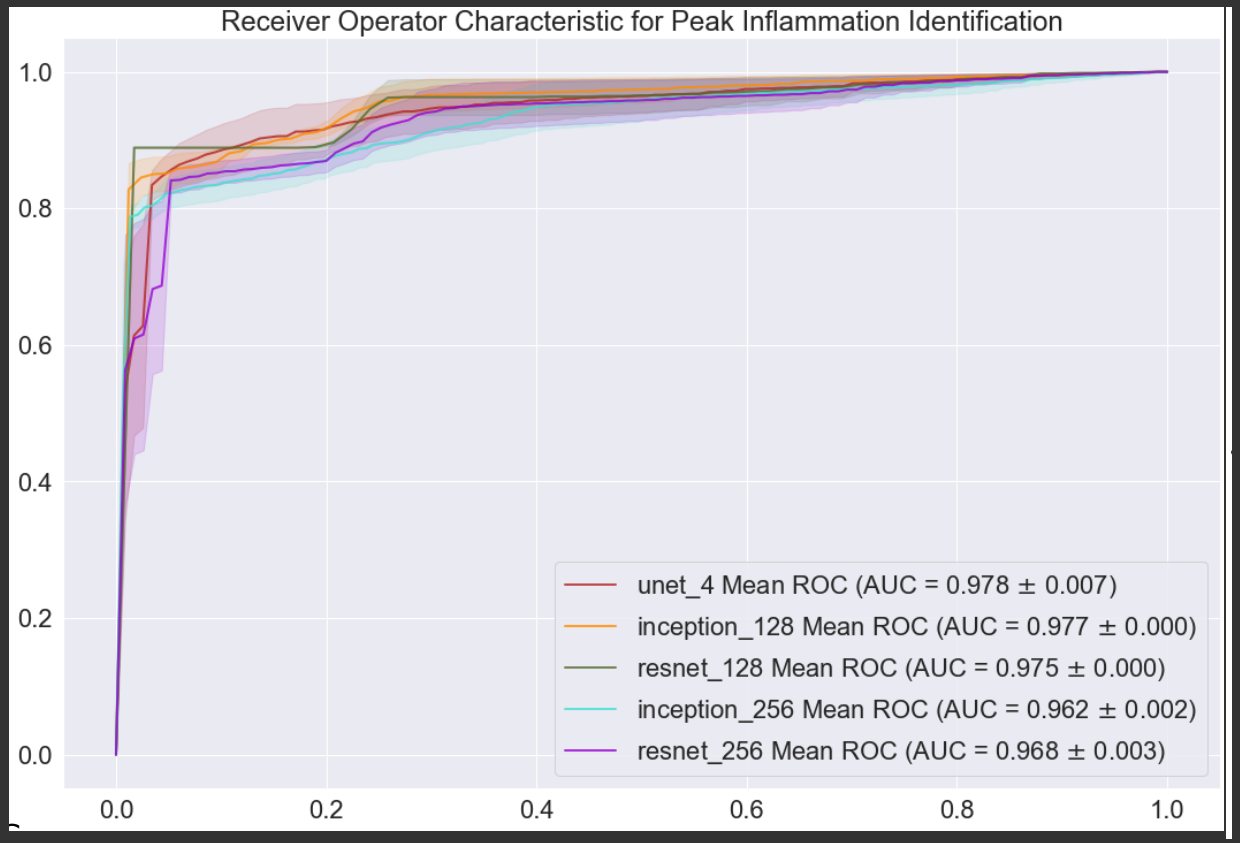
Quantifying PD with Deep Learning

Ternary Classification



Quantifying PD with Deep Learning

Ternary Classification



Next Steps

- Two current clinical studies:
  - PK Study in Healthy and Atopic Dermatitis patients
  - Disease profiling study in Atopic Dermatitis, Psoriasis, and Vitiligo patients
- Laboratory Development of dual PK-PD imaging and quantification
- Clinical studies exploring PK-PD in diseased subjects

Thank you!