About me…

Conor Evans

- B.S. Chemical Physics, Brown University
- PhD Chemistry, Harvard
- Postdoc @ Wellman Center , MGH
- Faculty @ Wellman Center since 2009

- Wife, Rosa, and 2 sons, Atticus (8) and Wesley (3)

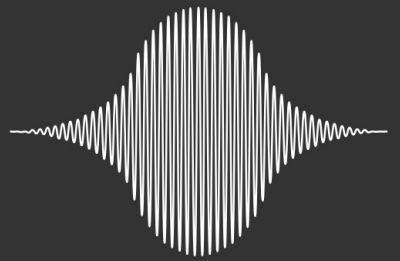
- **Favorites**
  - Swimming, cooking
- **Things I say "yes" to at GRCs**
  - Imaging & Microscopy
  - Translational science
  - Hiking & Kayaking

Barrier Function of Mammalian Skin

PK and PD Tomography:
Imaging and Quantifying Skin Pharmacology

Conor L. Evans

Wellman Center for Photomedicine
Massachusetts General Hospital
Harvard Medical School



Conflict of Interest

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

Acknowledgments

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Coherent Raman Scattering (CRS) Microscopy

Imaging based on intrinsic vibrational contrast

Two Colors: ω_p “Pump”

ω_s “Stokes”

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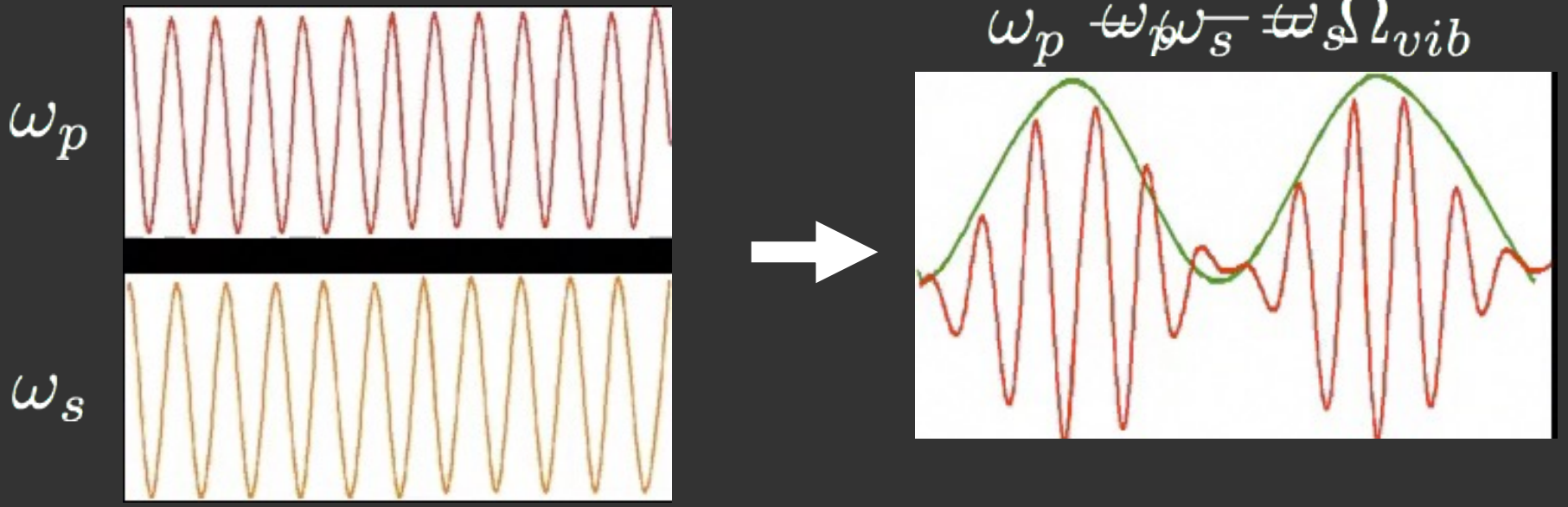
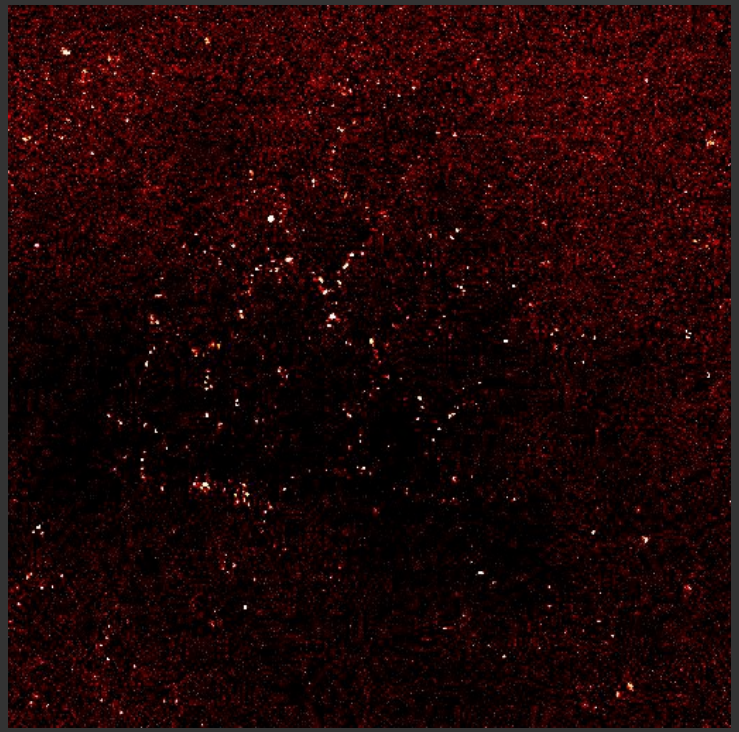
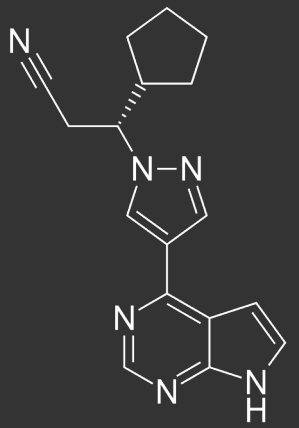


Direct visualization of APIs in skin

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





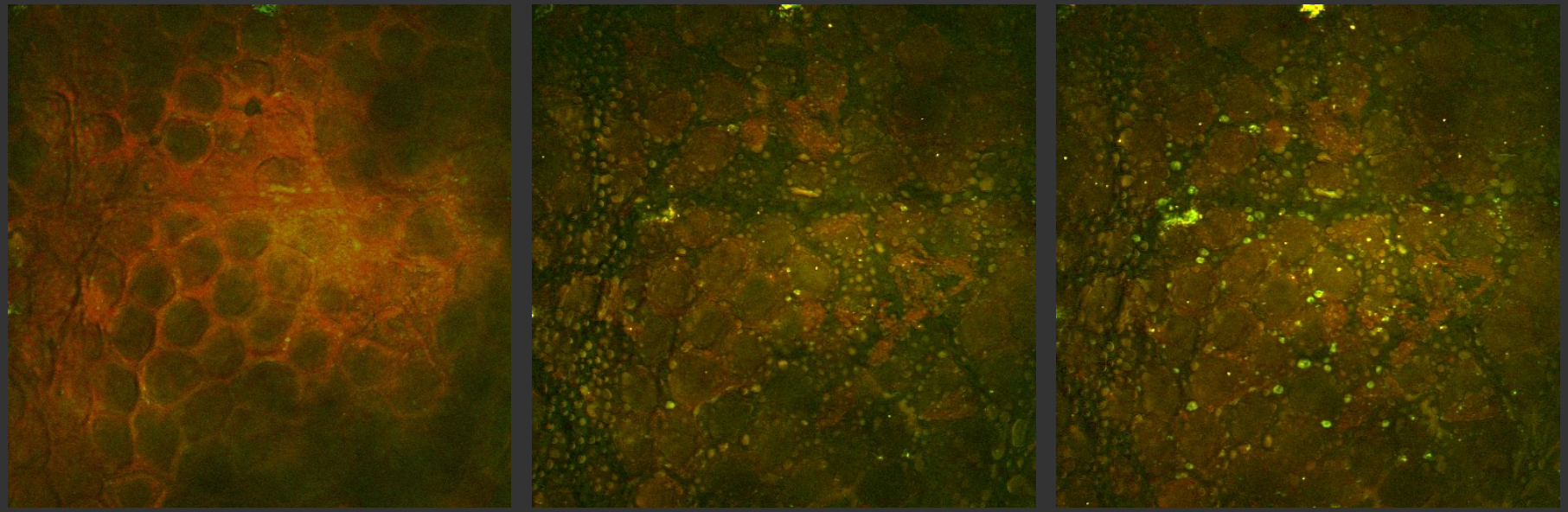
Ruxolitinib

Continuous, non-invasive and non-destructive visualization of APIs permeating through the skin

Simultaneous imaging of PK and skin structure

Red: Lipid 2845 cm^{-1}

Green: Nitrile 2250 cm^{-1}

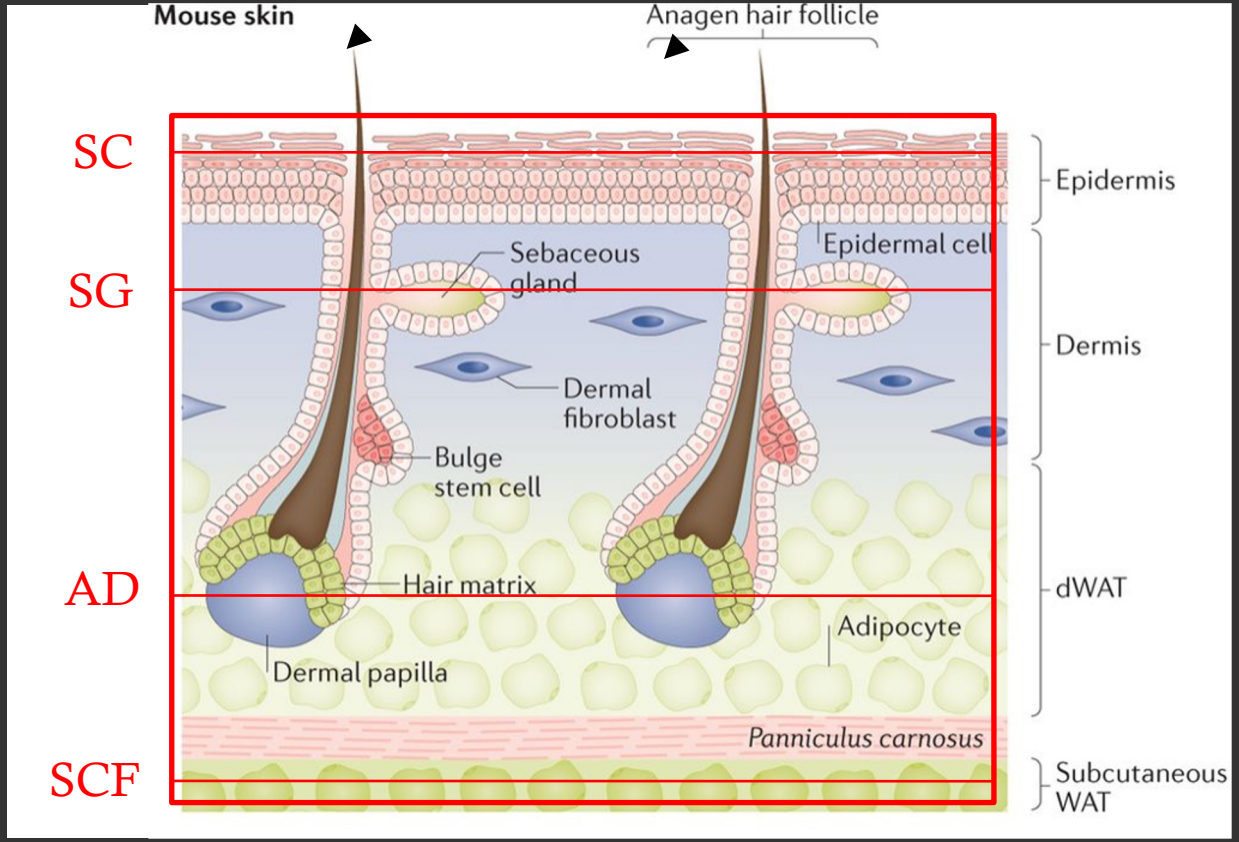


15 min **70 min** **120 min**

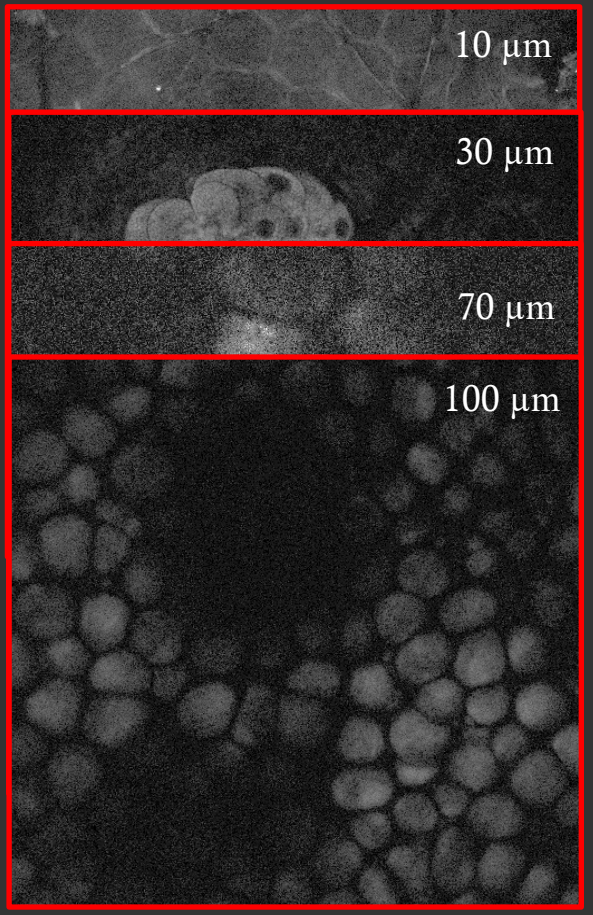
Observe the formation of “depots” at the lipid junctions between corneocytes

CRS can directly observe deposition/metamorphosis occurring within the stratum corneum surface

Mapping API permeation in depth



Nature Reviews Endocrinology **volume14**, pages163–173 (2018)

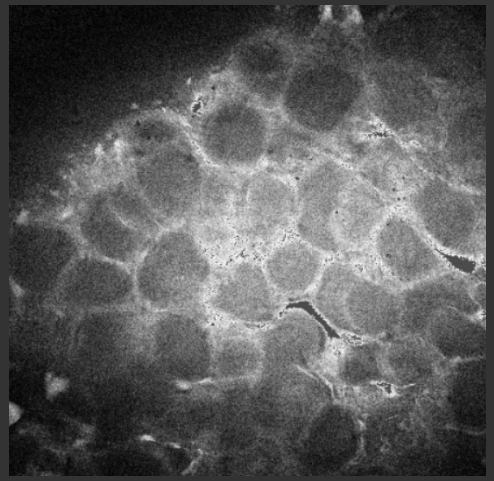


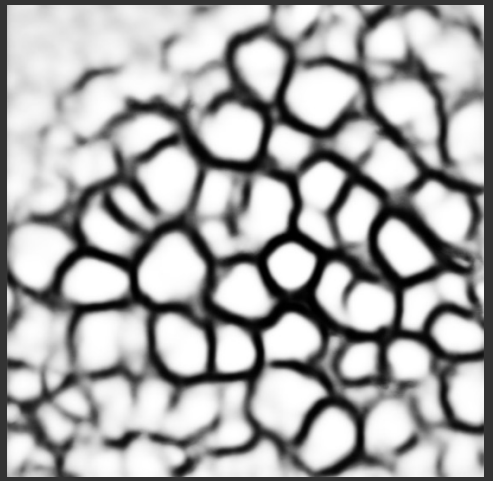
Quantifying PK with Deep Learning

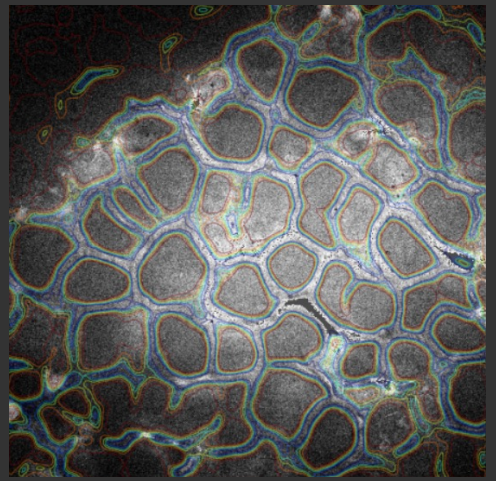
Detecting skin compartments can be challenging: irregular size, shape, connectivity, etc

Machine learning provides a robust method for consistent feature detection

We use a Unet Convolutional Neural Network (CNN) to
“segment” features within the skin

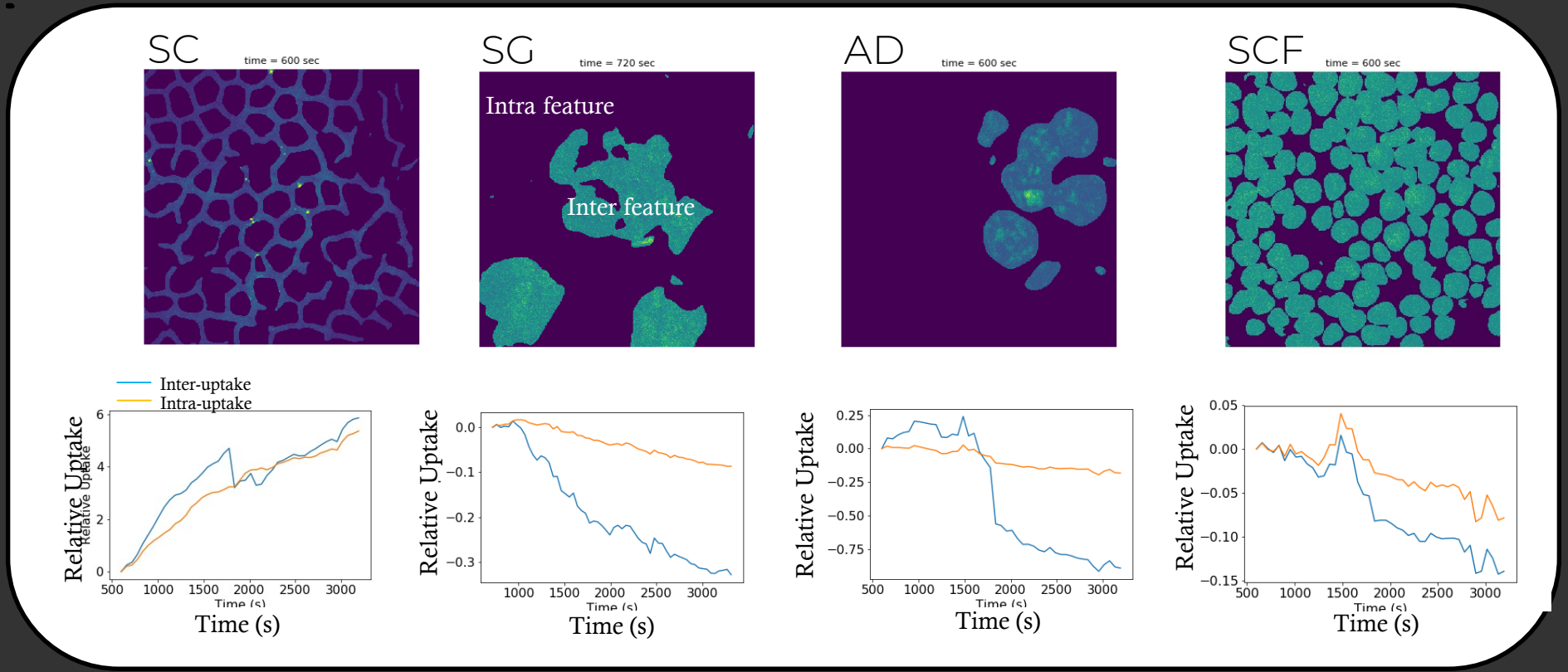






Following Drug Diffusion Through Skin Compartments

4 different layers of mouse skin. Drug: Ruxolitinib, Vehicle: Transcutol



Can observe the downward “wave” of the drug at the t_{max} peaks in the AD and SCF

Concentration-time profiles can be used to calculate PK parameters, including T_{max}, C_{max}, and AUC

Towards a General Method for SRS PK Tomography

Few drugs have a single unique peak in the silent region
The majority of drugs do not have any unique spectral bands

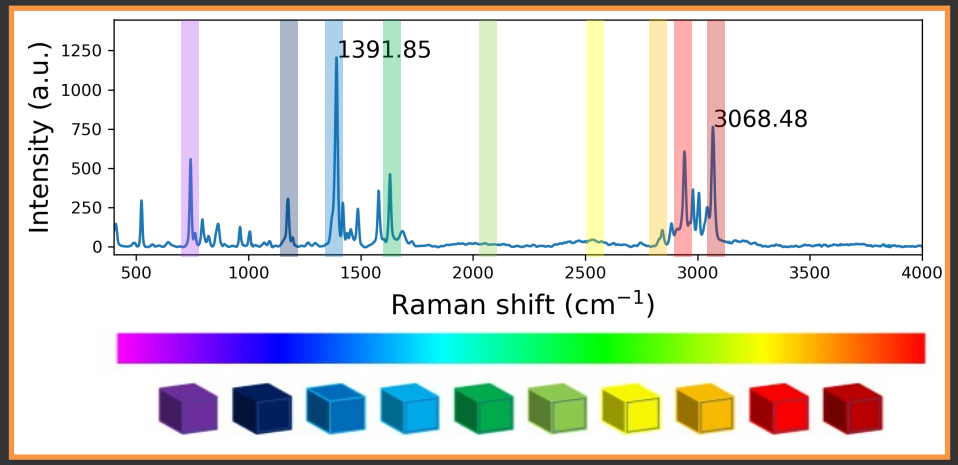
But almost every drug has a unique Raman spectrum

Identifying and quantifying drugs requires a means of acquiring complete molecular structure information -> spectra

BUT

- Spectral acquisition must be FAST (< 5s per spectral image)
- Spectral acquisition must be broad – across the ENTIRE Raman spectrum

Sparse Spectral Sampling SRS – S4RS



Raman spectrum of sample or combination of samples

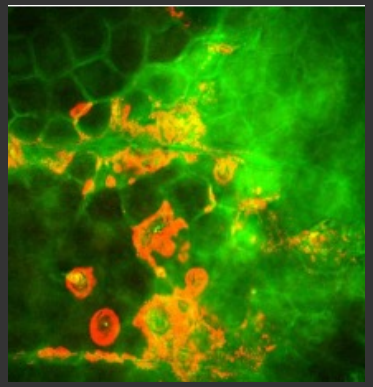
Conventional full spectrum sampling

Sparse Sampling

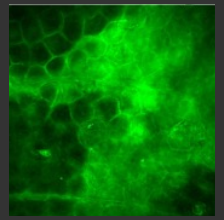
Spectral decomposition (OLS, NNLS, etc)

Spectral processing

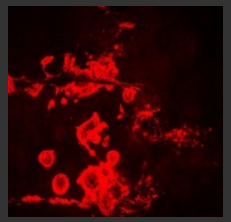
Composite



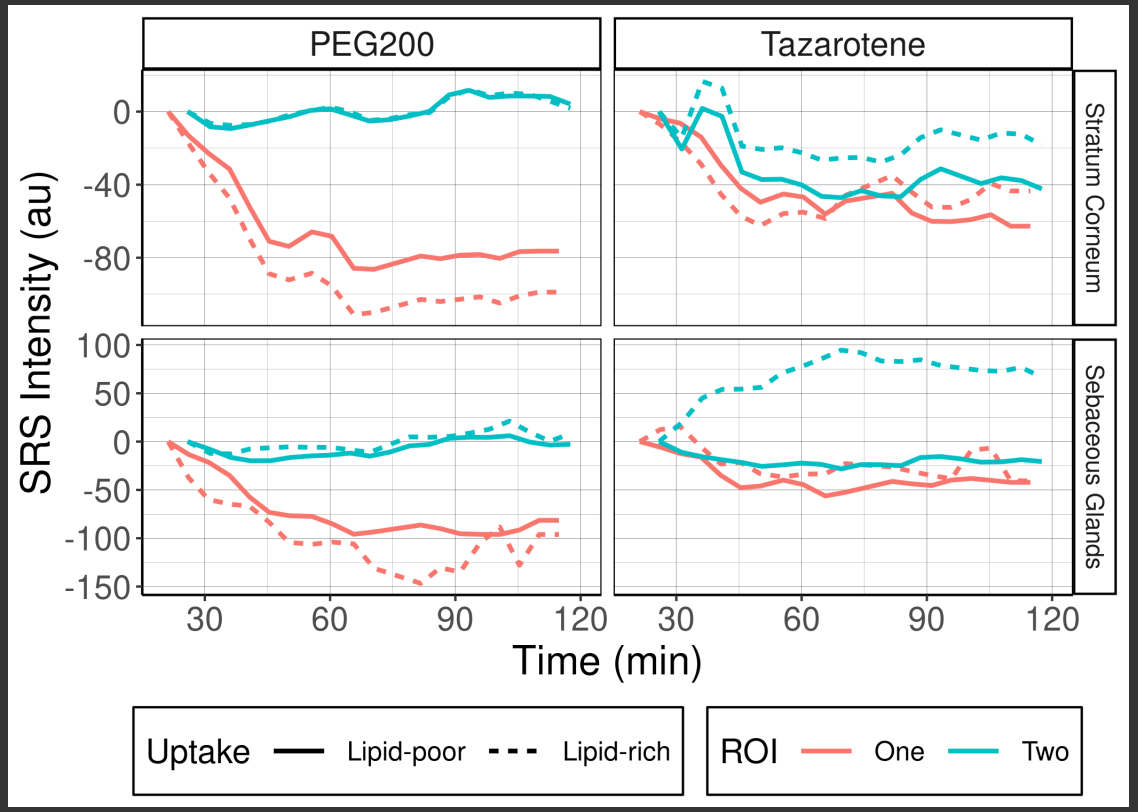
Skin structure



API conc.



Multicomponent PK Imaging with S4RS



Simultaneous imaging of the API, formulation excipients, and skin

Mapped and quantified permeation of *both* tazarotene and its PEG200 vehicle in skin compartments

CRS is not a pancea

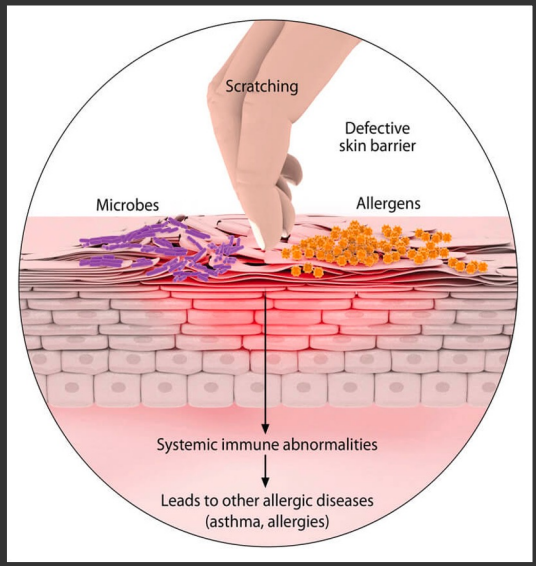
Limitations:

- Optical imaging has limited depth
  - ~200-250 μm with standard methods
  - ~500 μm with adaptive optics
- Lower detection sensitivity limit: 100 μM – 1 mM
- Not all APIs can be sensitively detected
  - S4RS is a more general method but is slightly more complex

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.



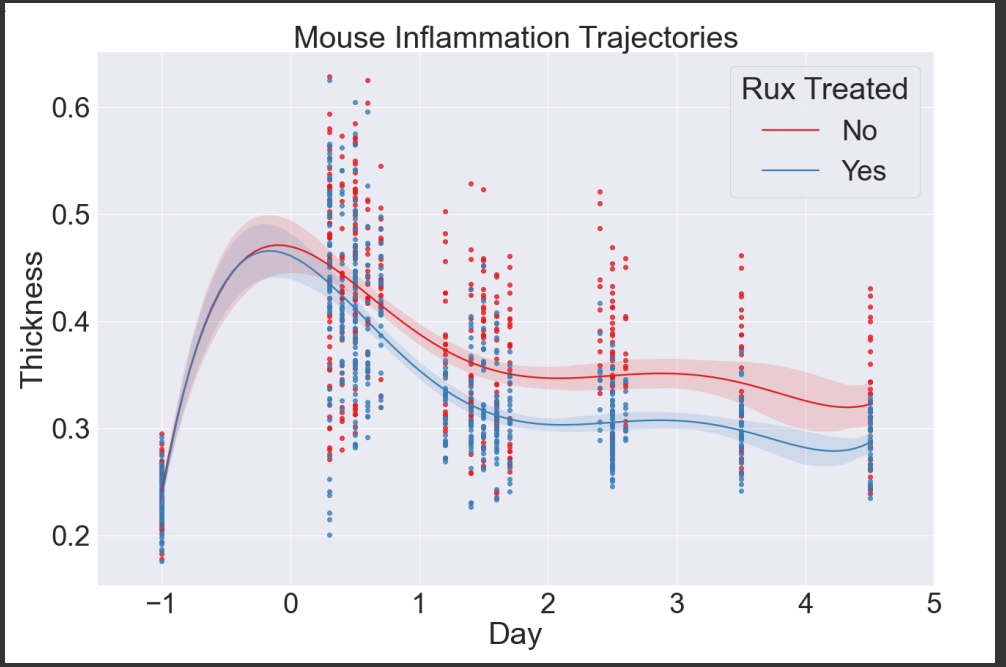
Quantifying PD with Imaging and Deep Learning

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 at peak inflammation



CRS Imaging reveals skin inflammatory state

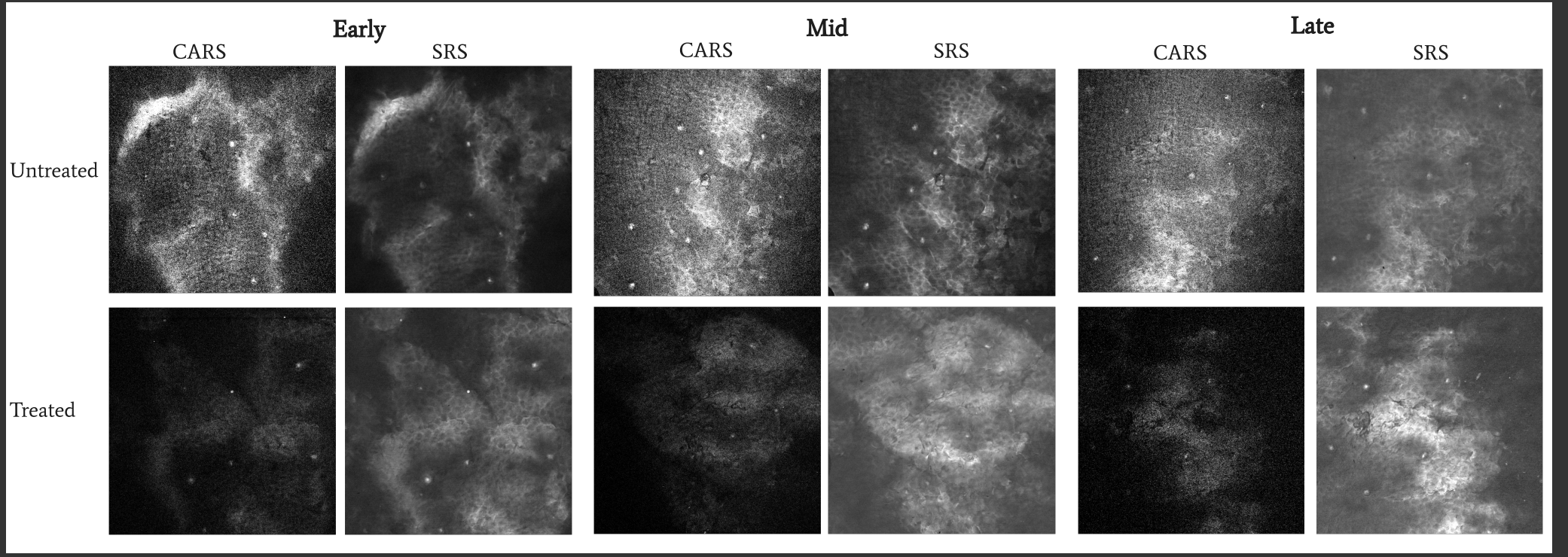
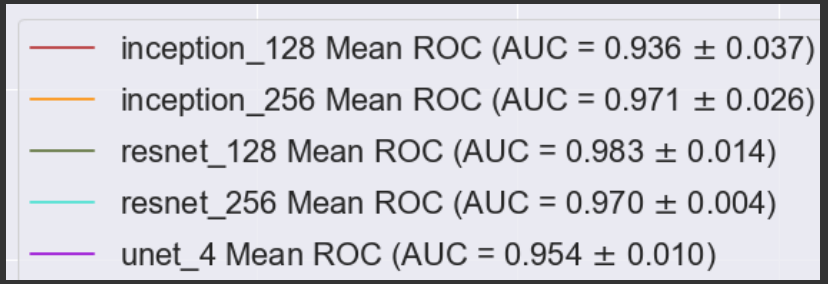


Image data used to train three neural networks to classify and score local inflammatory state

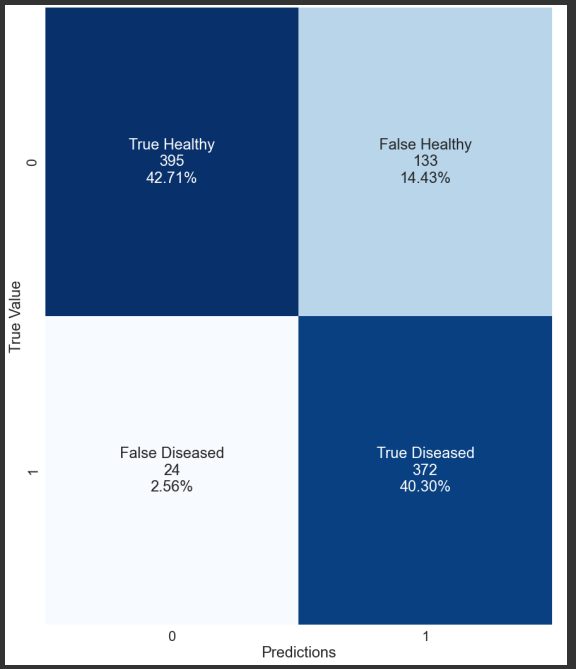
D

Binary Classification (inflamed/not inflamed)

False positive rate

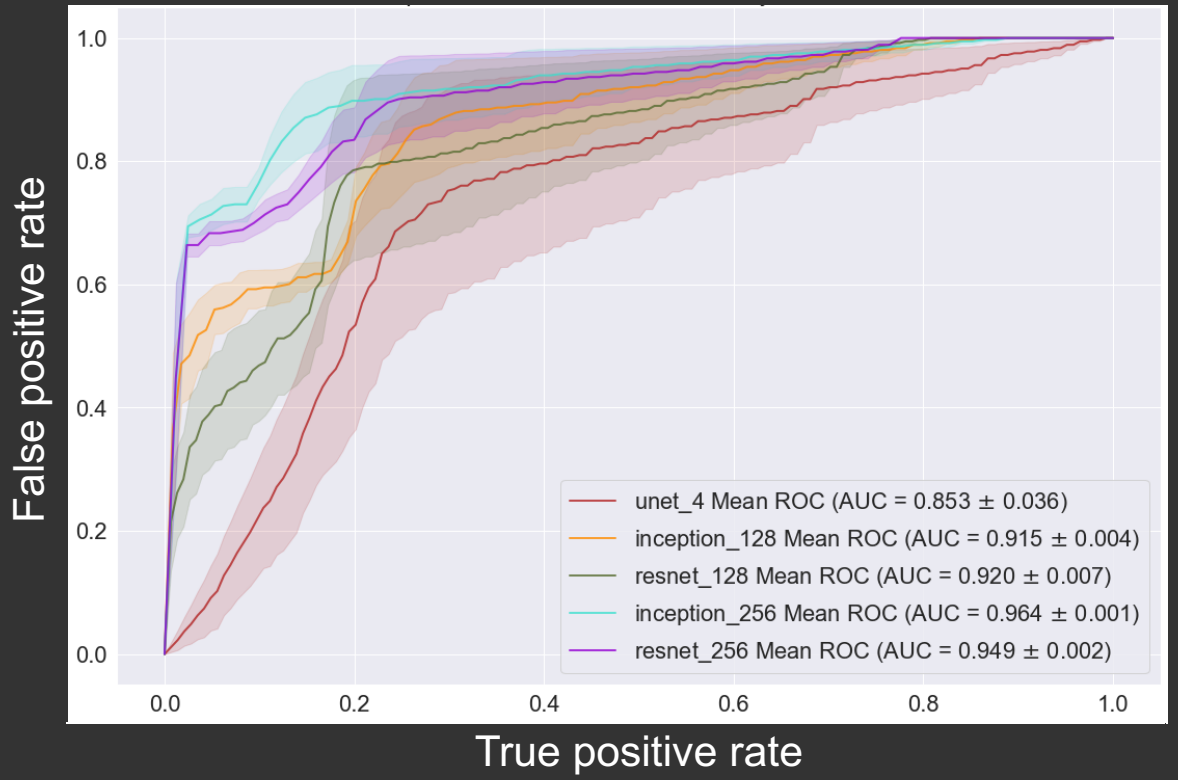


True positive rate

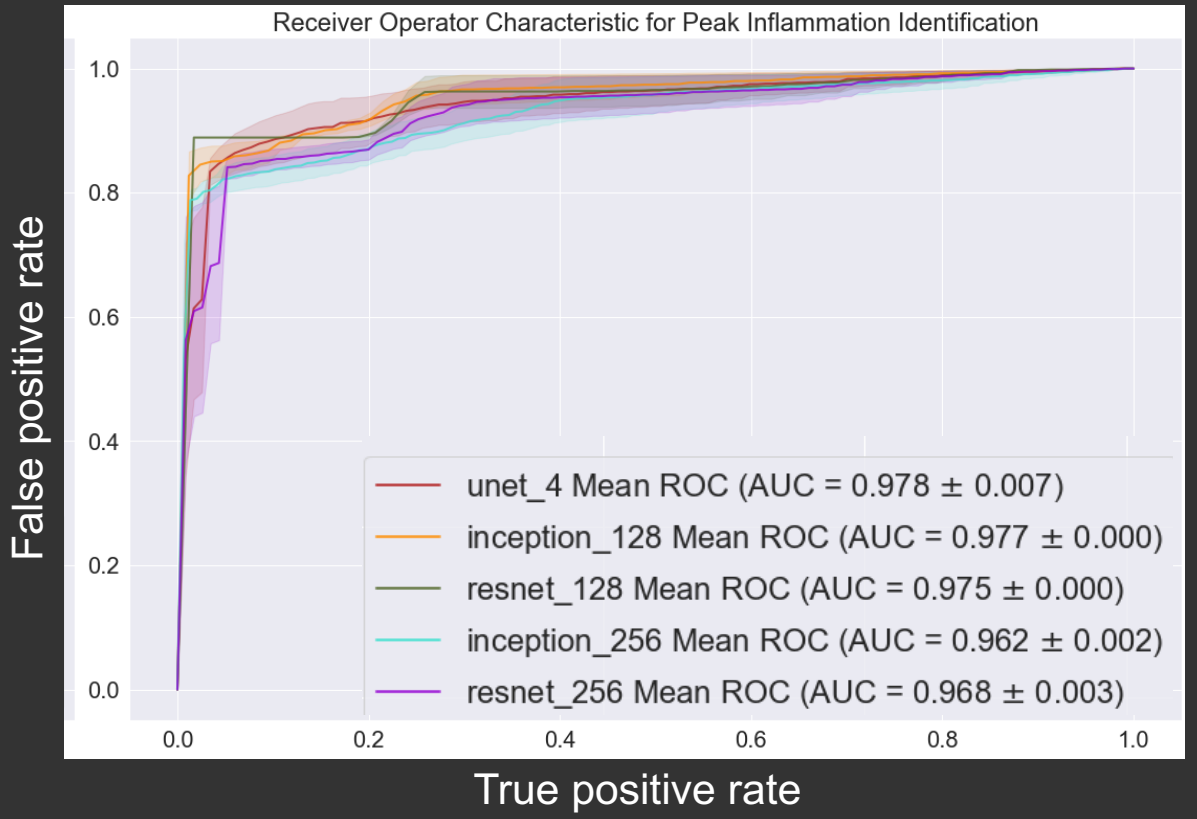


Binary classification, while successful, showed relatively high ”false” classification of diseased skin

Q earning

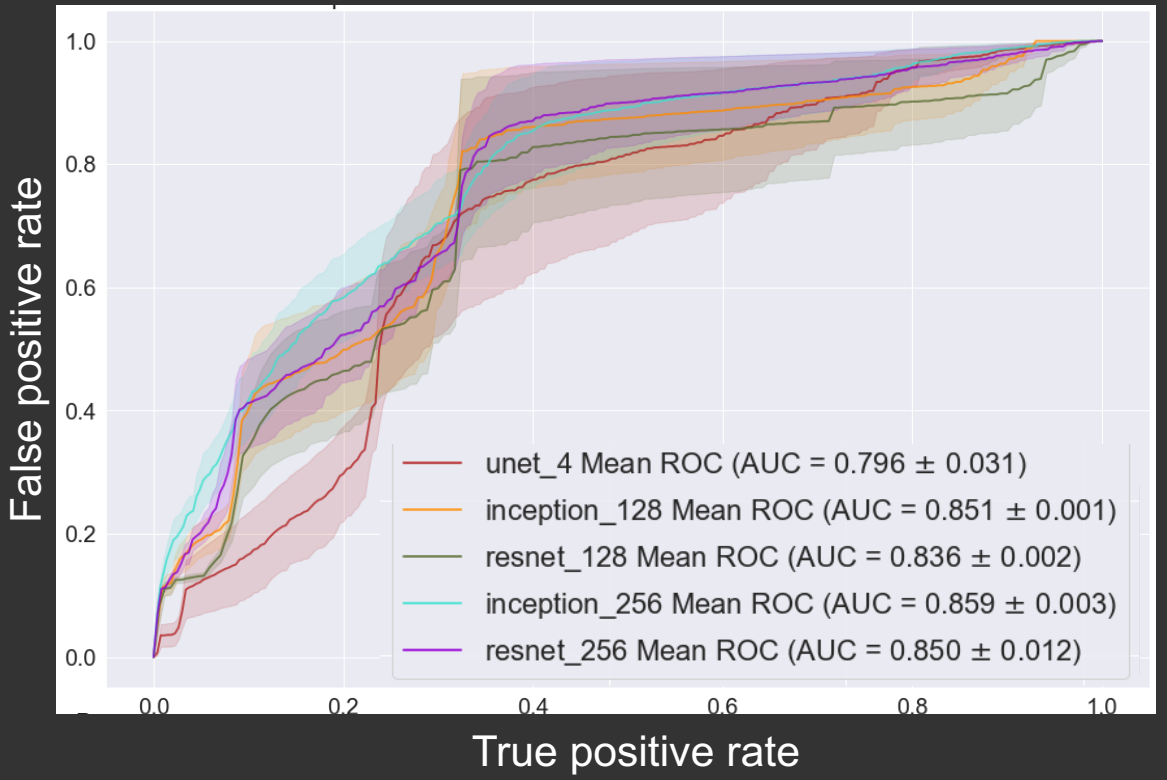

False positive rate
True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
unet_4 Mean ROC (AUC = 0.853 ± 0.036)
inception_128 Mean ROC (AUC = 0.915 ± 0.004)
resnet_128 Mean ROC (AUC = 0.920 ± 0.007)
inception_256 Mean ROC (AUC = 0.964 ± 0.001)
resnet_256 Mean ROC (AUC = 0.949 ± 0.002)

Q earning


Receiver Operator Characteristic for Peak Inflammation Identification
False positive rate
True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
unet_4 Mean ROC (AUC = 0.978 ± 0.007)
inception_128 Mean ROC (AUC = 0.977 ± 0.000)
resnet_128 Mean ROC (AUC = 0.975 ± 0.000)
inception_256 Mean ROC (AUC = 0.962 ± 0.002)
resnet_256 Mean ROC (AUC = 0.968 ± 0.003)

Quantifying PD with Deep Learning

Ternary Classification – Mild inflammation



Machine learning based scoring of *local* skin inflammation was considerably more accurate (>90% vs 53%) than expert annotation

Summary

CRS Imaging provides *local*, microscale PK and PD information

CRI PK imaging enables quantification of drug concentration and PK parameters within specific cells and skin compartments

Next steps: PK-PD imaging to study interactions of drugs, formulations, and **disease state**

Thank you!