



Imaging and Quantifying Topical Drug Uptake in Human Tissue

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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

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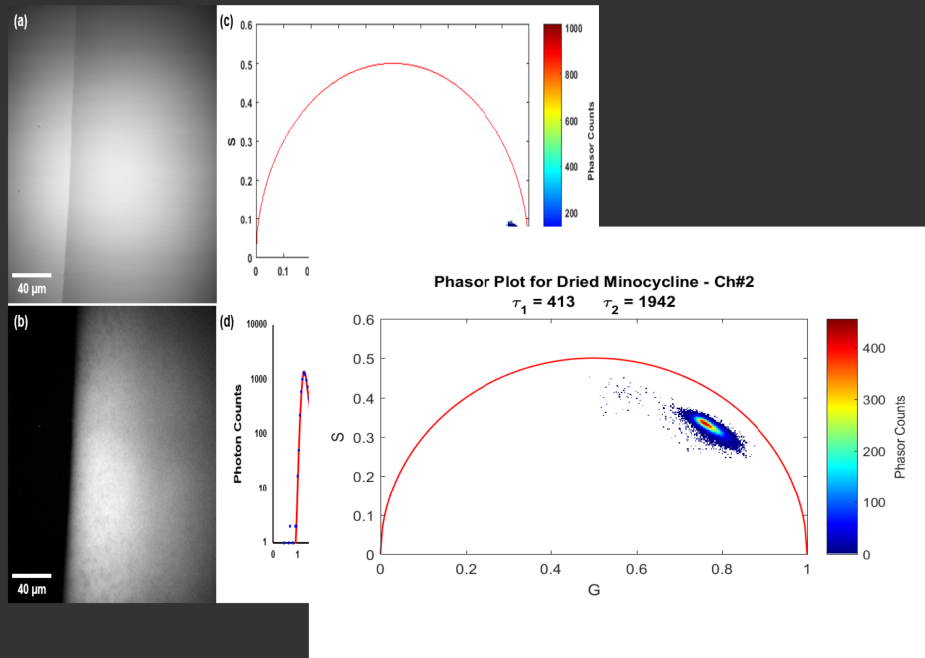
Pharmacokinetics

- ◆ A central concern in drug development is that a drug reaches its intended target
- ◆ Challenging for topical and transdermal administration
- ◆ Radiographic methods (e.g. MARG) give uptake, but not dynamics
- ◆ Modifications to drugs for tracking (e.g. fluorescence) often fundamentally alter pharmacokinetics
- ◆ Ideally, would aim to follow drug uptake in subjects, not model systems

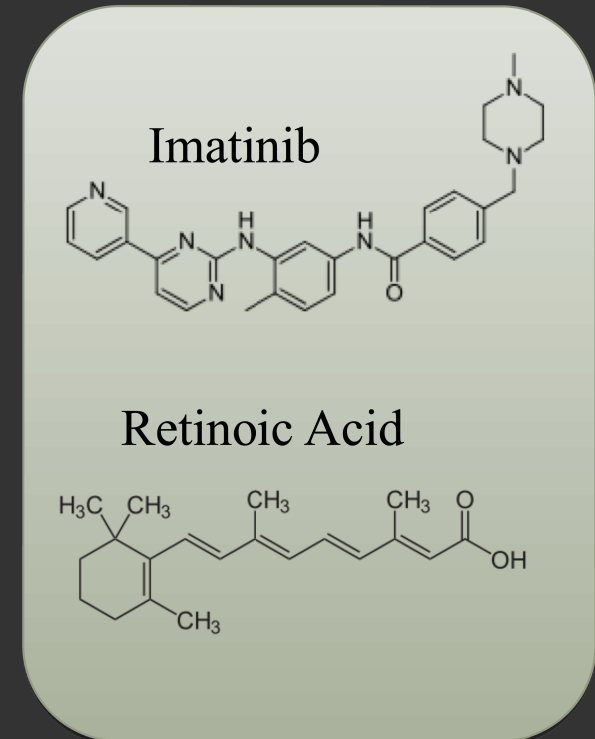
Our goal is to overcome this limitation and create quantitative
optical imaging methods

Pharmacokinetic Tomography

A paradigm for the microscopic imaging and quantification of drugs based on *intrinsic* sources of contrast:

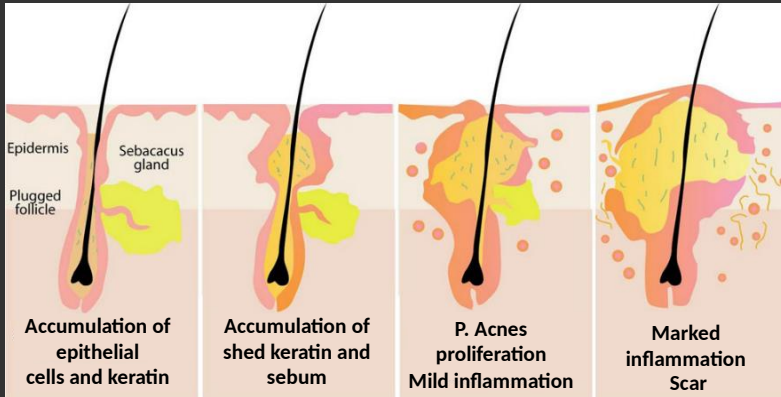
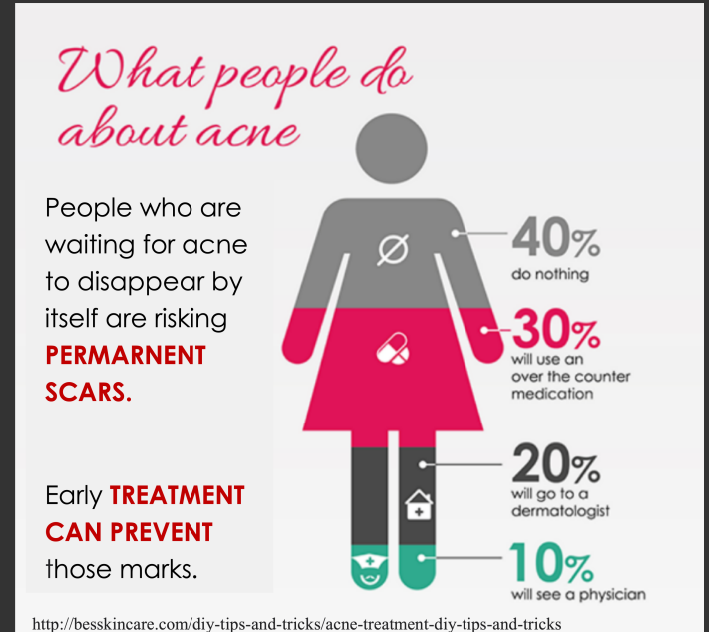
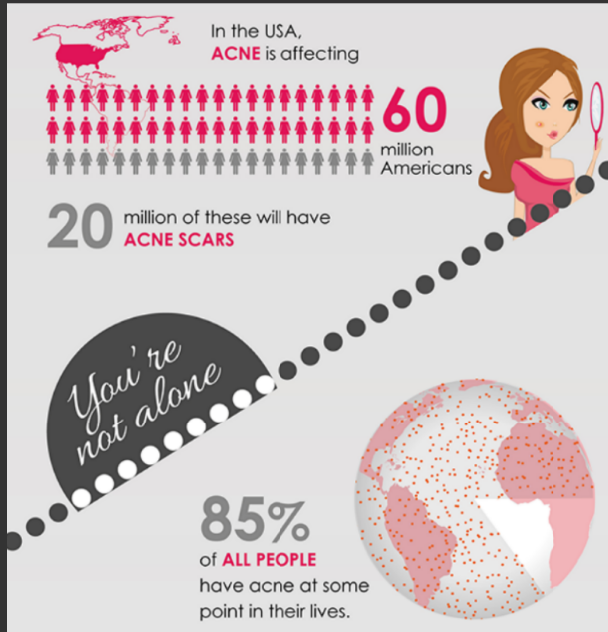


Fluorescence
Intensity/Lifetime



Molecular Vibrations

Acne Vulgaris



Acne vulgaris

1. Abnormal keratinization
2. Sebum Production
3. P. Acnes
4. Inflammation

← **Minocycline and Retinoids!**

Minocycline and Retinoids

» Minocycline



Recommended minocycline dose for acne
= 45-135 mg/day

Duration of therapy
= 12 weeks

Solodyn™ is a trademark of Valeant Pharmaceuticals for oral minocycline

Required high dose of minocycline for effective treatments

- Dizziness
- Nausea
- Joint and muscle pain
- Skin discoloration for long-term usage



Br. J. Dermatol. 129(4), 403-407 (1993).

» Isotretinoin



Severe acne

Duration of therapy
= 15-20 weeks
(3.5-4.5 months)

Isotretinoin

- Extreme dry skin and eyes
- Vomiting
- Diarrhea
- Dangerous for unborn babies

Topical Minocycline and Retinoid Formulations

Topical minocycline and retinoid

- *Topical* versus oral (current standard of care)
 - » *Topical* dosage form of minocycline not commercially available
 - » Minimal systemic side effects

Are they successfully delivered to their intended target as we expected?

Can we visualize a topical drug uptake within the skin at its *native* daily dose?

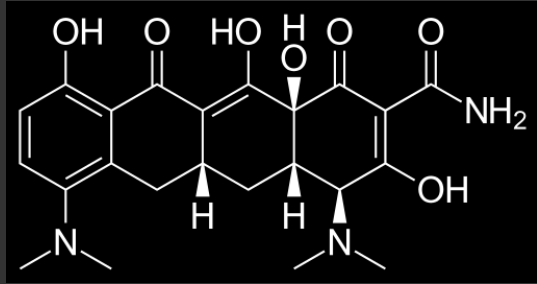
Current Evaluation of a topical drug delivery

- Franz-type diffusion cells
- Tape stripping + HPLC
- MALDI-MS imaging technique

Can we do better through imaging?

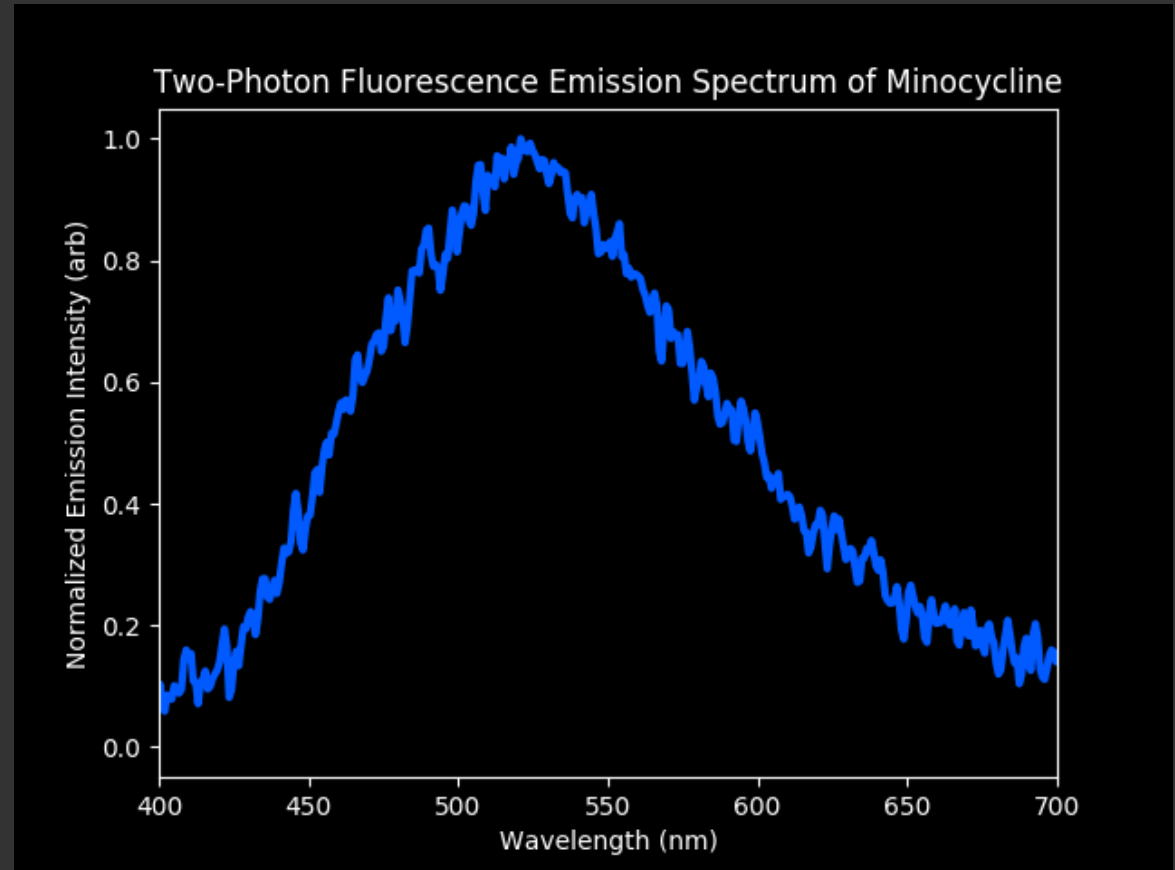


A New Topical Formulation of Minocycline for Tx of Acne

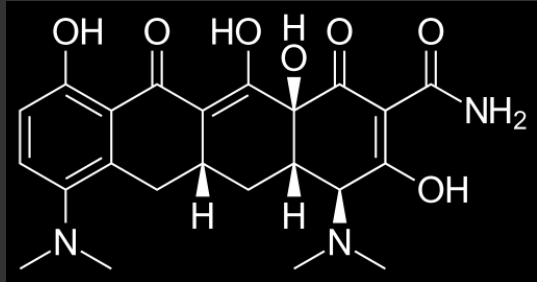


BPX-01

Use Two-Photon
Microscopy for deep
tissue imaging

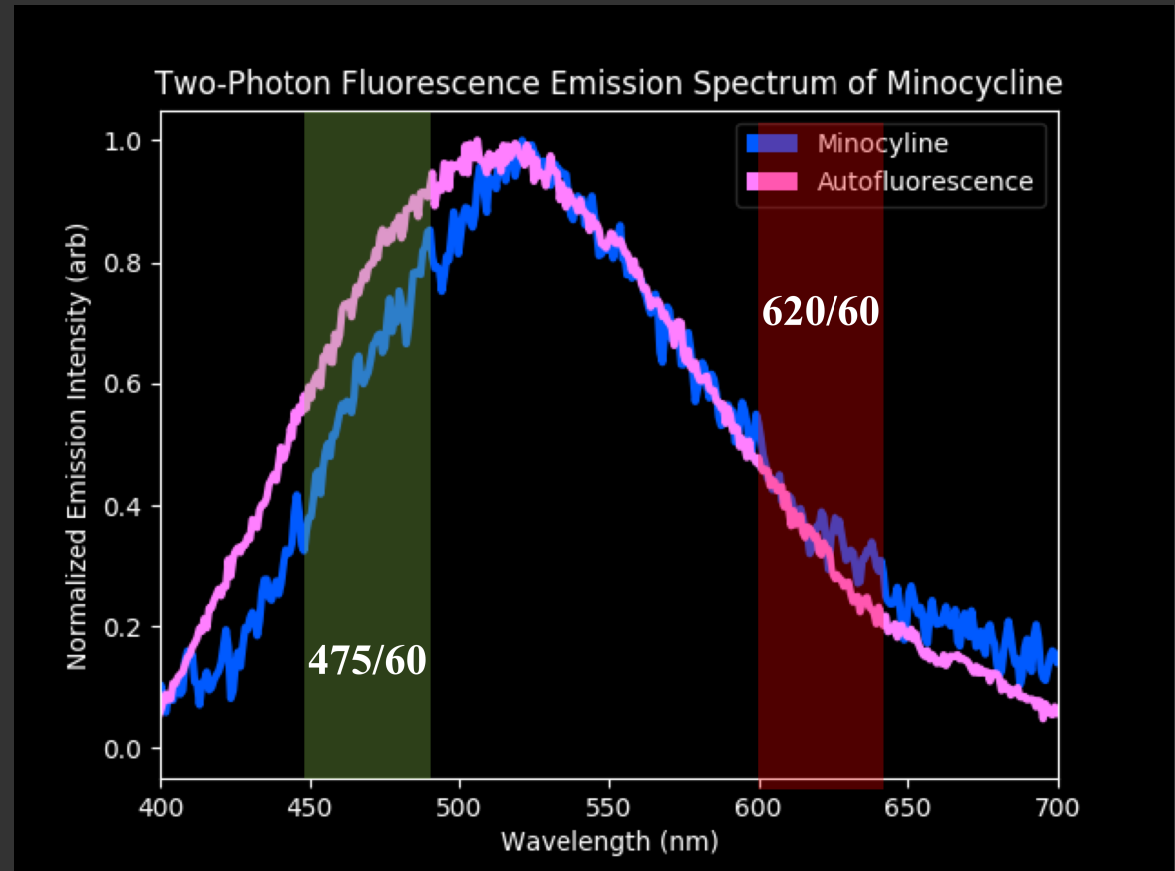


A New Topical Formulation of Minocycline for Tx of Acne

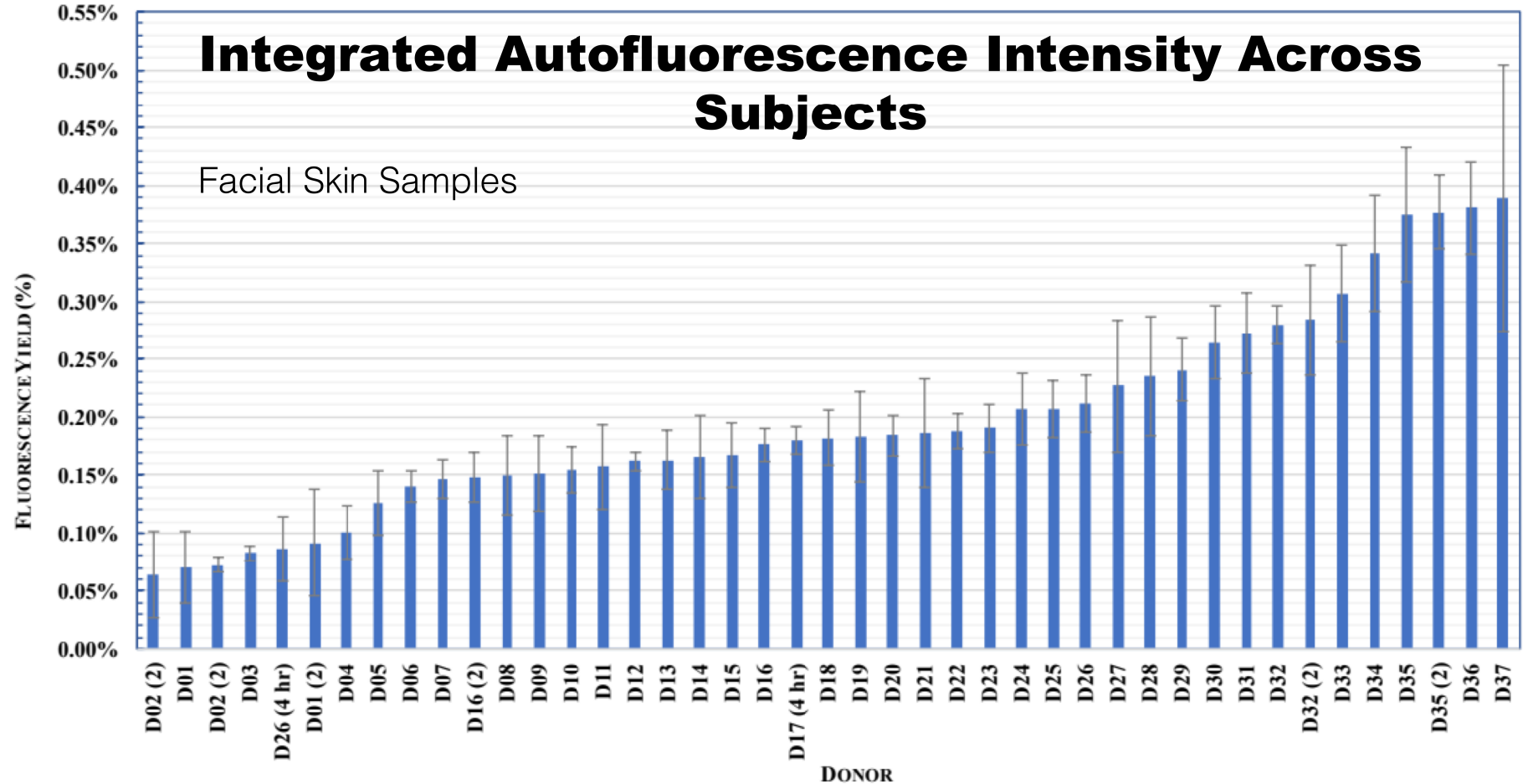


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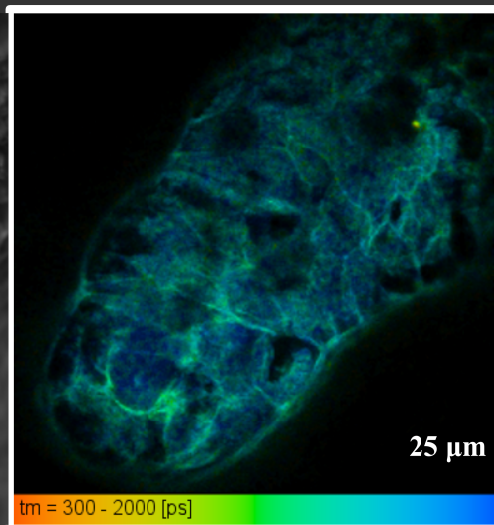
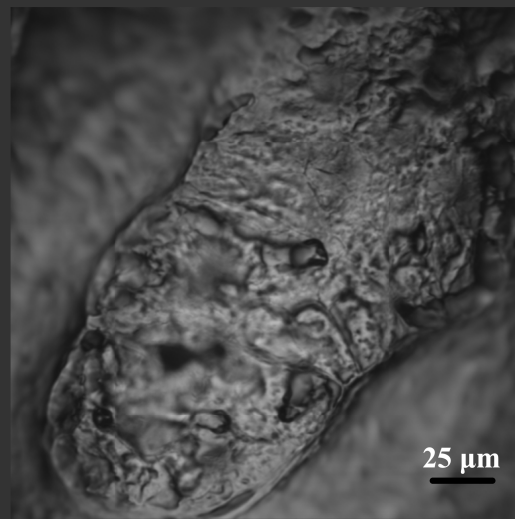


Tissue Autofluorescence is Heterogenous Across Subjects

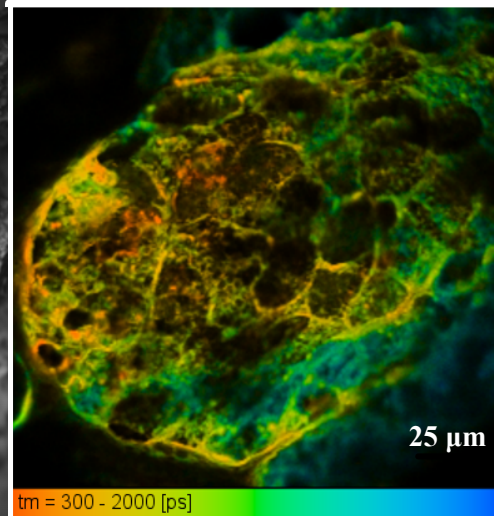
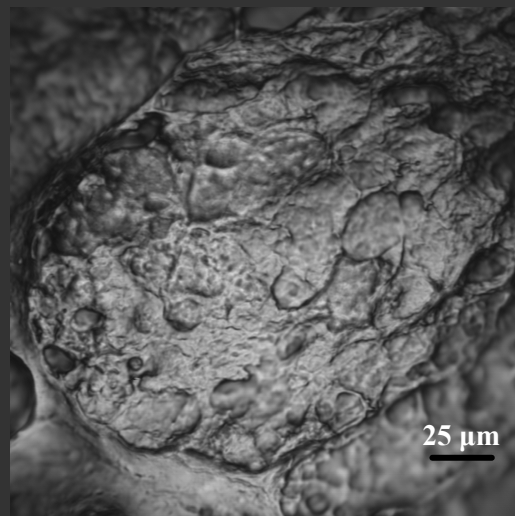


Teasing out Minocycline via FLIM

SG No Drug

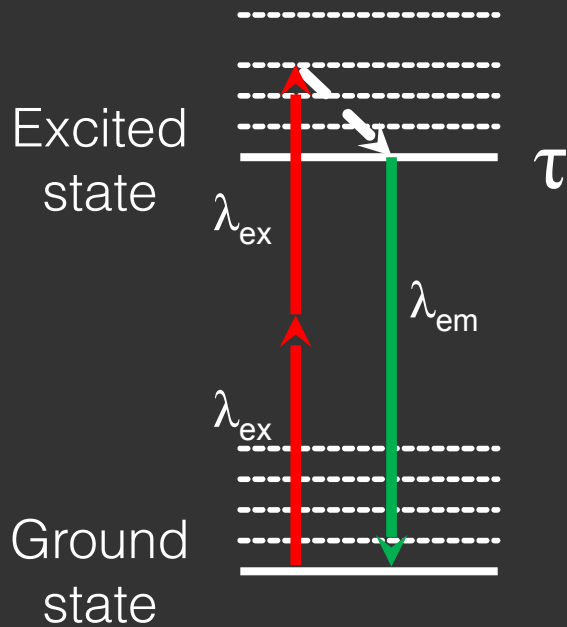


SG +Drug

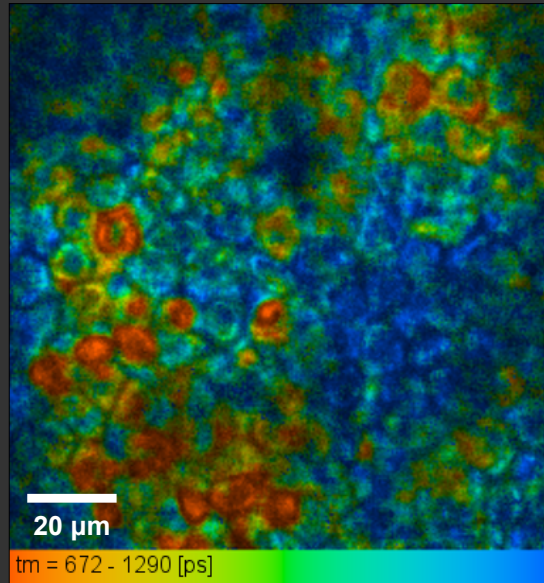


Fluorescence Lifetime Imaging Microscopy

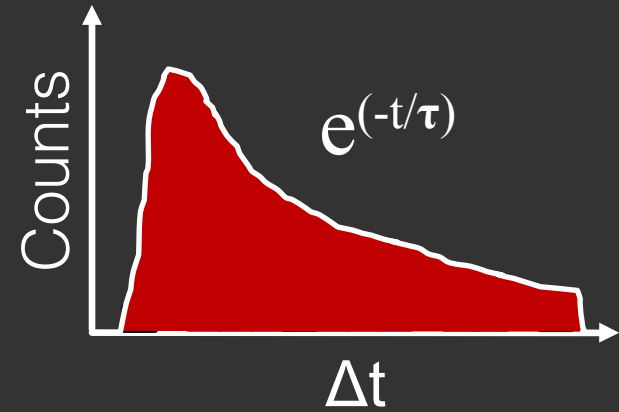
2PEF



FLIM



TCSPC

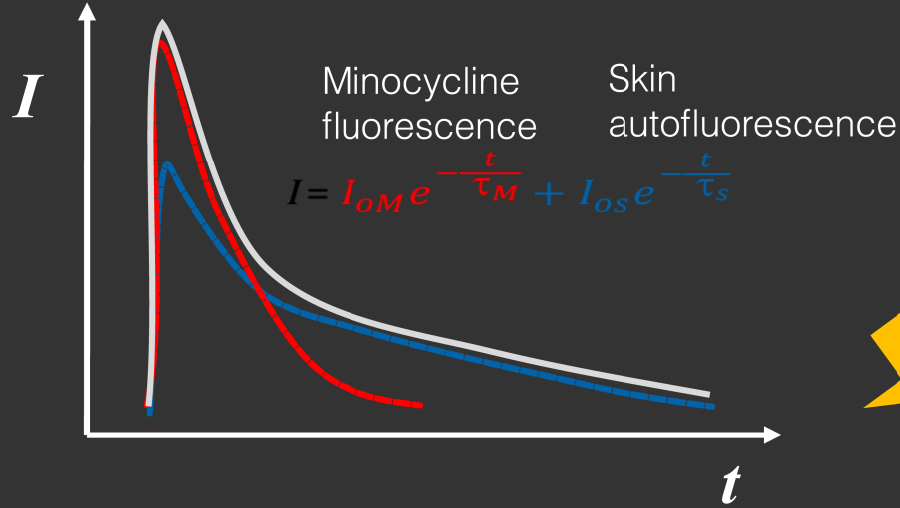


Single: $e^{(-t/\tau)}$

Double: $a_1 e^{(-t/\tau_1)} + a_2 e^{(-t/\tau_2)}$

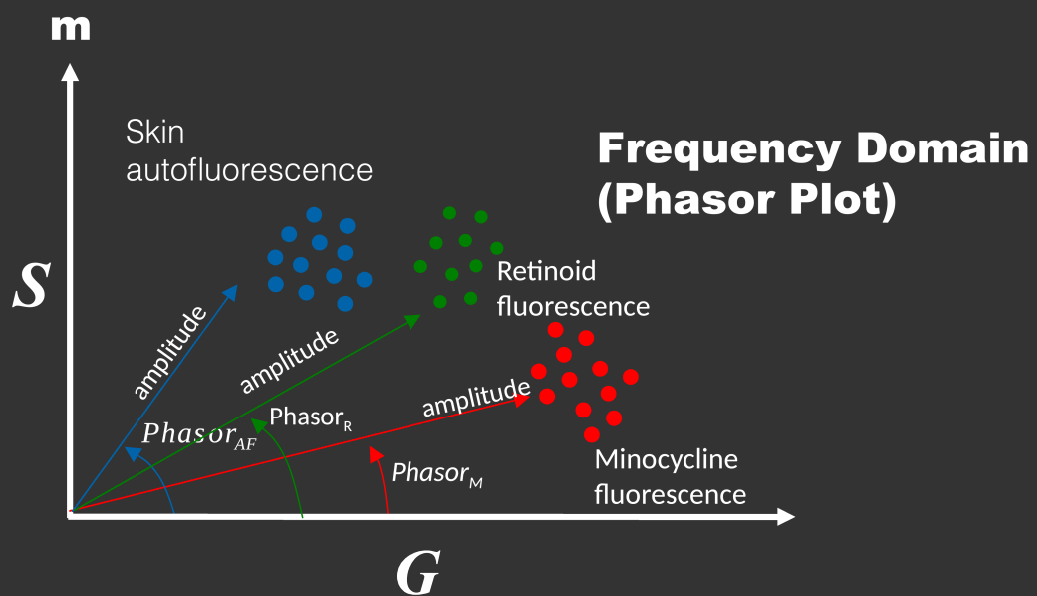
Phasor Analysis

Time Domain (Exponential decay)



Fourier Transform

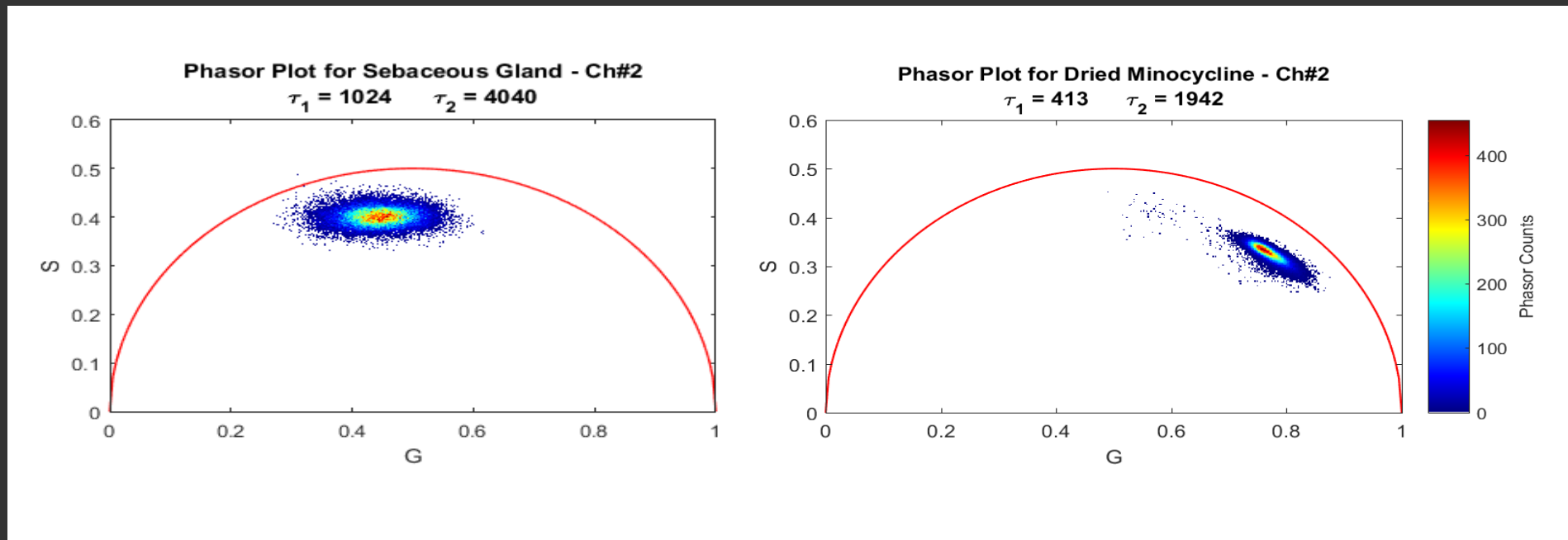
$$G_{i,j}(\omega) = \frac{\int_0^{\infty} I_{i,j}(t) \cos(\omega t) dt}{\int_0^{\infty} I_{i,j}(t) dt}$$
$$S_{i,j}(\omega) = \frac{\int_0^{\infty} I_{i,j}(t) \sin(\omega t) dt}{\int_0^{\infty} I_{i,j}(t) dt}$$



Autofluorescence and Minocycline have Unique “Phasor” Signatures

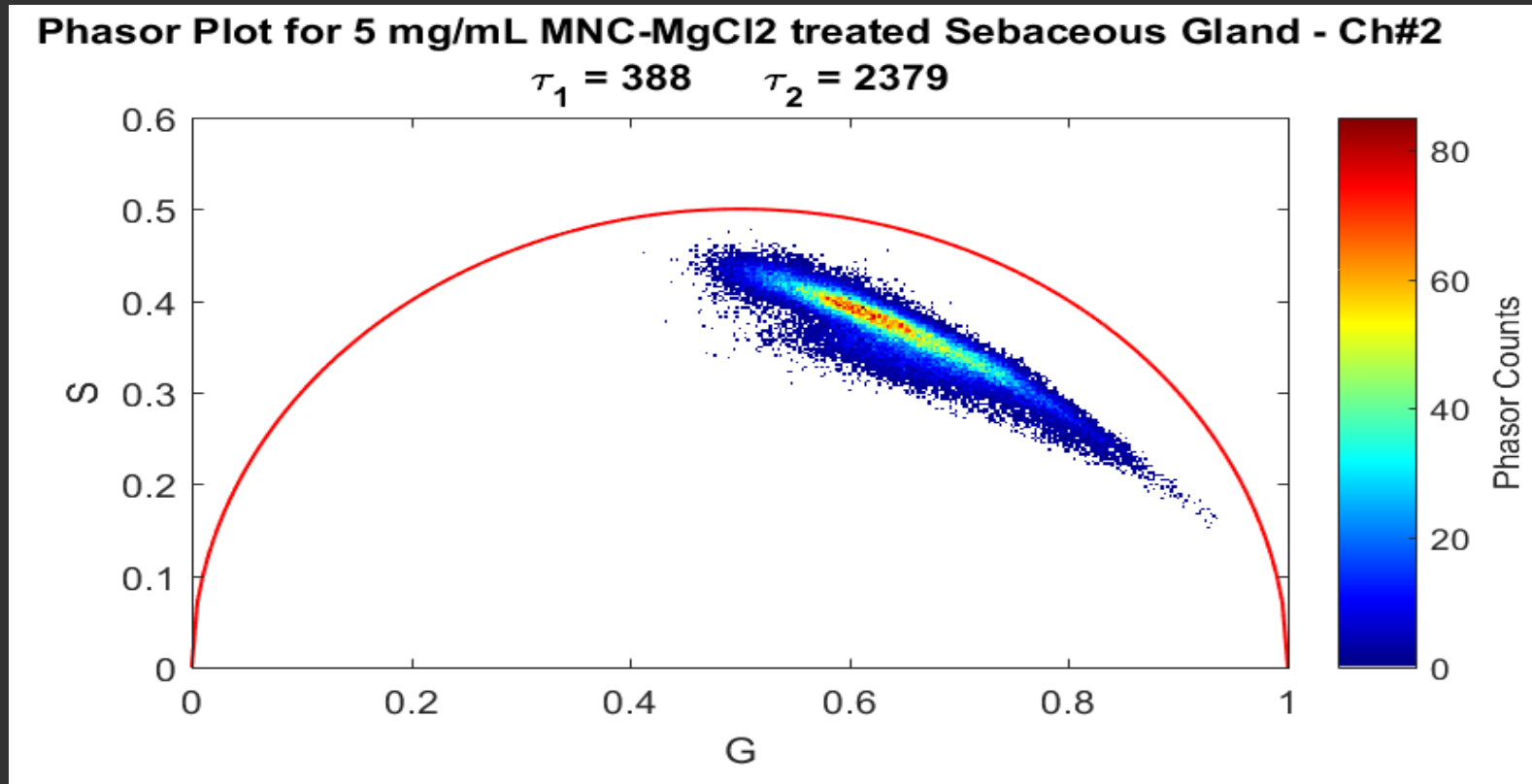
Not at all simple; requires pixel-by-pixel separation of lifetime components

Problem akin to “big” data issues in Flow Cytometry and CyTOF



Autofluorescence and Minocycline have Unique “Phasor” Signatures

Not at all simple; requires pixel-by-pixel separation of lifetime components

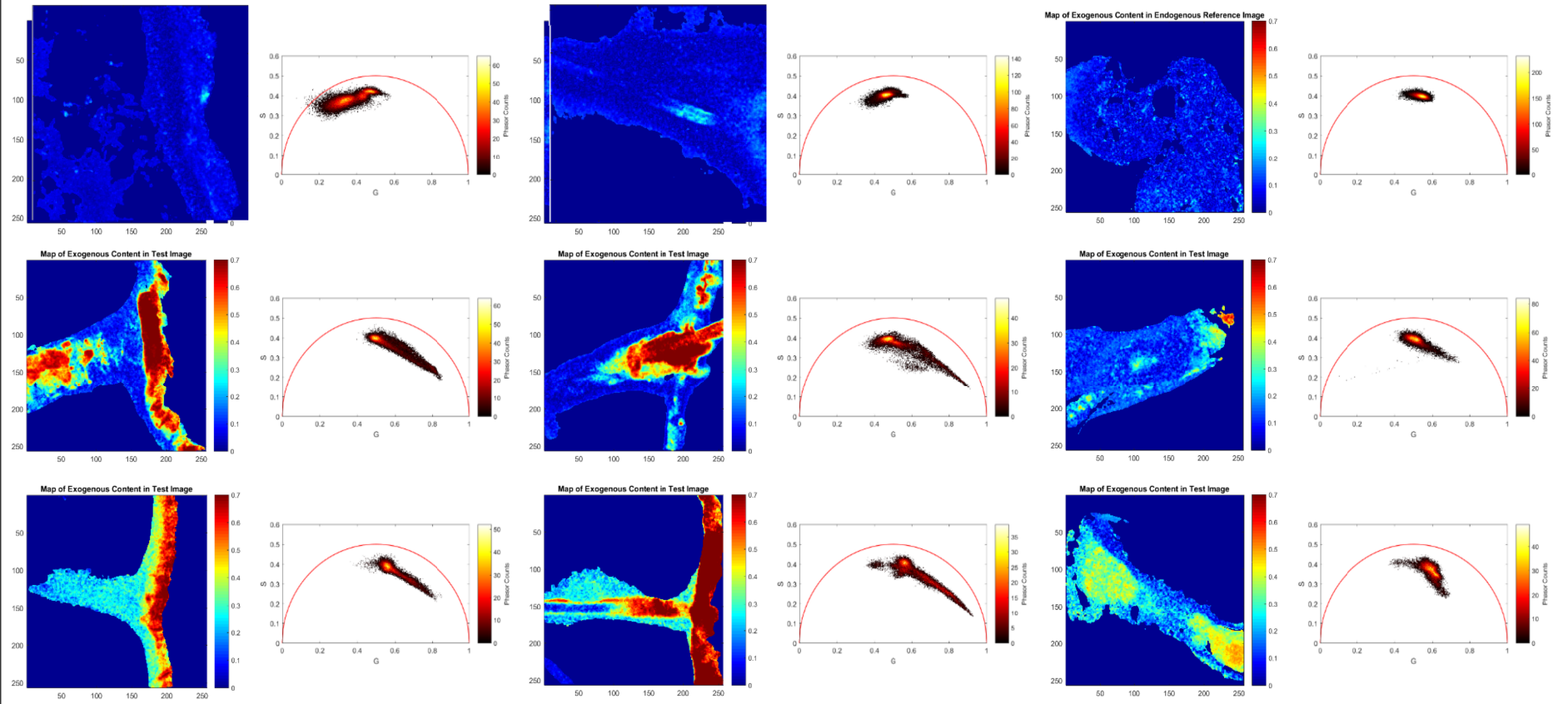


Quantitative Assessment of Minocycline Uptake

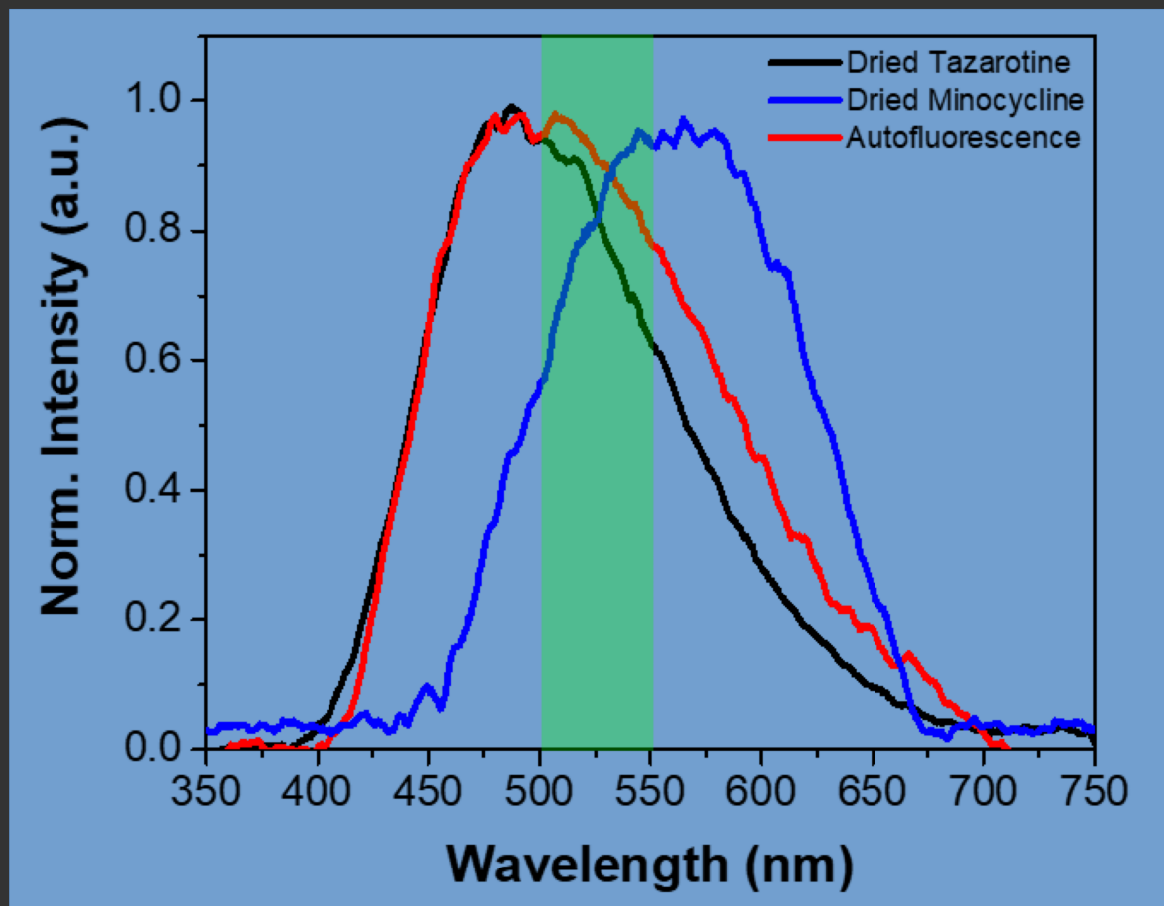
Epidermis

Hair Follicles

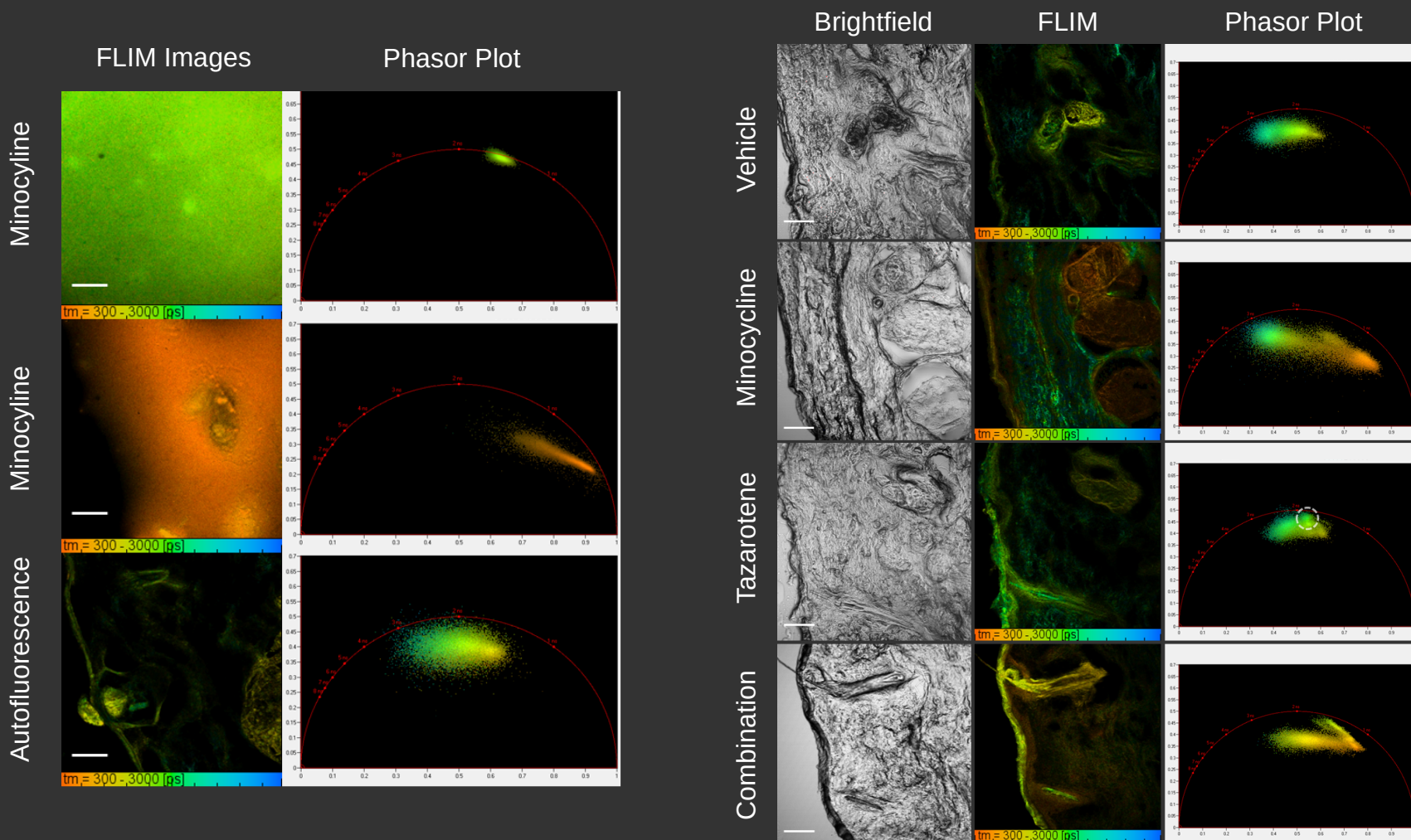
Sebaceous glands



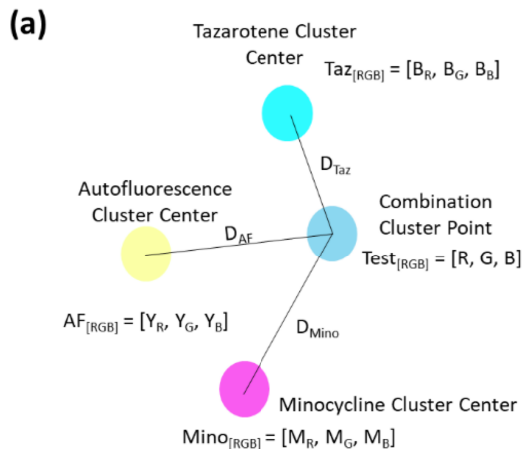
Challenge: Substantial Spectral Overlap



Quantifying Individual Drugs in a Multicomponent API



Separating and Quantifying Clusters



(b) Calculations

$$UC_{AF} = 1/D_{AF}$$

$$UC_{Taz} = 1/D_{Taz}$$

$$UC_{Mino} = 1/D_{Mino}$$

$$C_{AF} = UC_{AF} / C_{Tot}$$

$$C_{Taz} = UC_{Taz} / C_{Tot}$$

$$C_{Mino} = UC_{Mino} / C_{Tot}$$

$$C_{Tot} = UC_{AF} + UC_{Taz} + UC_{Mino}$$

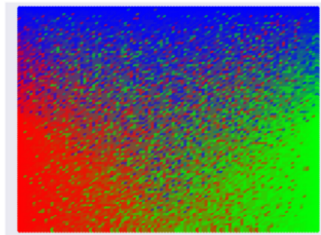
$$Test_{[RGB]} = [R = Y_R * C_{AF} + B_R * C_{Taz} + M_R * C_{Mino},$$

$$G = Y_G * C_{AF} + B_G * C_{Taz} + M_G * C_{Mino},$$

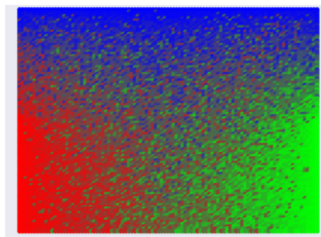
$$B = Y_B * C_{AF} + B_B * C_{Taz} + M_B * C_{Mino}]$$

Variables
UC: Unscaled Component Contribution
C_{Tot} : Total Contribution
$C_{Component}$: Scaled Component Contribution
$Test_{[RGB]}$: Phasor plot pixel RGB value

(c) Simulated Ground Truth Drug Distribution Image

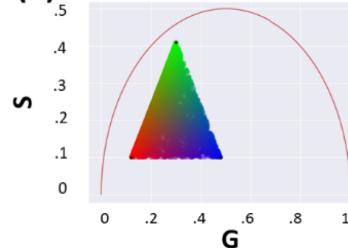


(e) Reconstructed Ground Truth Drug Distribution

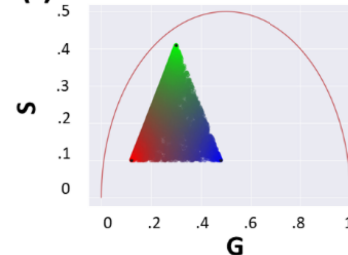


■ Component A (Red) ■ Component B (Green) ■ Component C (Blue)

(d) Ground Truth Phasor Plot



(f) Reconstructed Phasor Plot

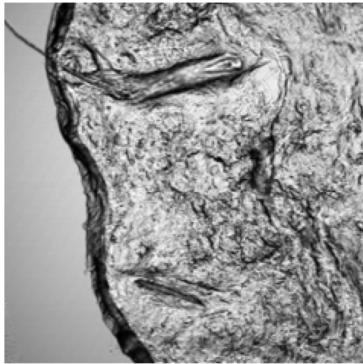


(g)

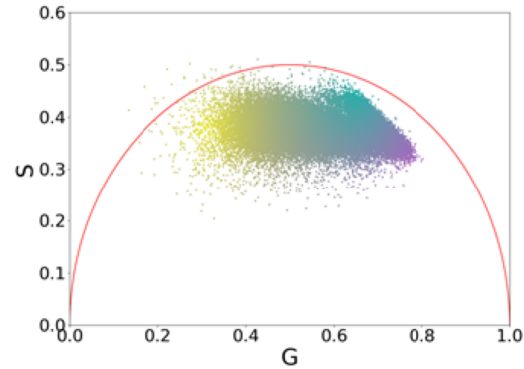
	Mean Error (%)	Standard Deviation (%)
Component A Contribution (Red)	1.67	± 2.77
Component B Contribution (Green)	1.71	± 2.74
Component C Contribution (Blue)	4.29	± 5.65

Separation and Quantification

Bright Field



Phasor plot



Local Distribution Map

