Human Wound Healing Analysis using 2D-TOF-SIMS

Anthony Castellanos[1], Ivan Jozic[2], and
Francisco Fernández-Lima[1,3]

[1]Department of Chemistry and Biochemistry, Florida International University
[2]Department of Dermatology & Cutaneous Surgery, University of Miami Miller School of Medicine
[3]Biomolecular Sciences Institute, Florida International University

FIU
FLORIDA
INTERNATIONAL
UNIVERSITY

Background

- The skin is an important barrier to protect the body against microorganisms, abrasion, ultraviolet light, and water loss.[1]
- Chronic wounds fail to heal after ~4 weeks.
  - Venous leg ulcer, arterial ulcer, diabetic foot ulcer, pressure sores
- Chronic wounds of the skin affect an estimated 8 million people in the US at a cost of $28 billion.[2]
- Challenges in research:
  - Complexity of wound healing processes.[3]
  - Translational limitations of animal and skin equivalent models.[4,5]

1. K. R. Feingold, Journal of Lipid Research, 2007, 48, 2531-2546.
2. S. R. Nussbaum, M. J. Carter, C. E. Fife, J. DaVanzo, R. Haught, M. Nusgart and D. Cartwright, Value in Health, 2018, 21, 27-32.
3. S. A. Eming, P. Martin and M. Tomic-Canic, Science translational medicine, 2014, 6, 265sr266-265sr266.
4. S. Elliot, T. C. Wikramanayake, I. Jozic and M. Tomic-Canic, Journal of Investigative Dermatology, 2018, 138, 736-740.
5. C. M. A. Reijnders, A. van Lier, S. Roffel, D. Kramer, R. J. Scheper and S. Gibbs, Tissue engineering. Part A, 2015, 21, 2448-2459.

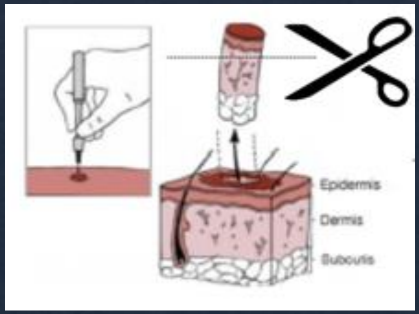
Goals

- Characterize acute skin wounds using healthy and newly-formed human epidermis using Mass Spectrometry Imaging.
  - Identify biomarkers for wound healing.
- Improve understanding of role of lipids in acute skin wounds for treatment of non-healing chronic wounds.
- Use a relevant *ex-vivo* model for skin repair.

Ex-vivo model and Experimental Design

Dr. Ivan Jozic

Tissue collection from abdominoplasty

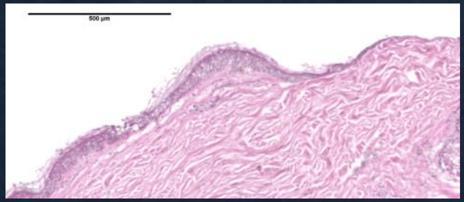


8mm biopsy punch followed by 3mm medial punch

Tissue grown on DMEM+FBS media

Cryosectioning

Freeze-drying



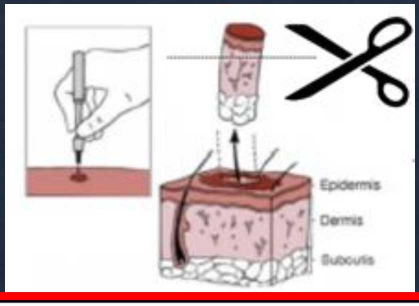
H&E staining of parallel tissue slice

TOF-SIMS analysis

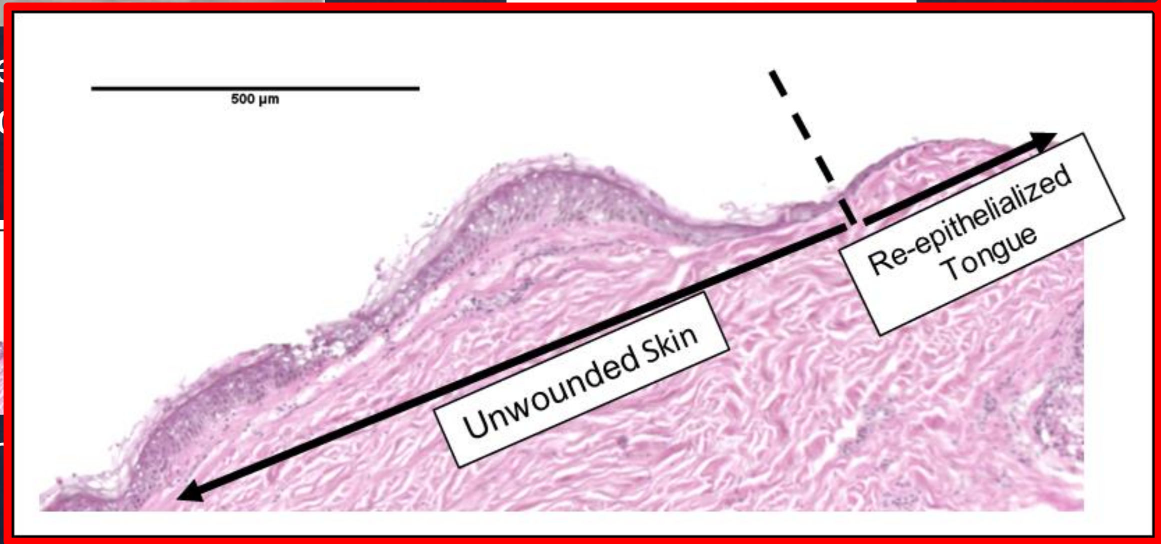
Ex-vivo model and Experimental Design

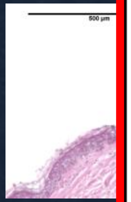
Dr. Ivan Jozic

Tissue
ab



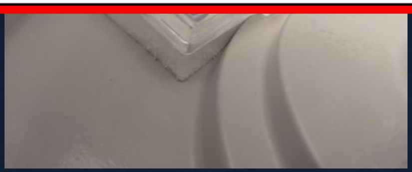
Tissue grown on DMEM+FBS media





H&E stai

Cryosectioning

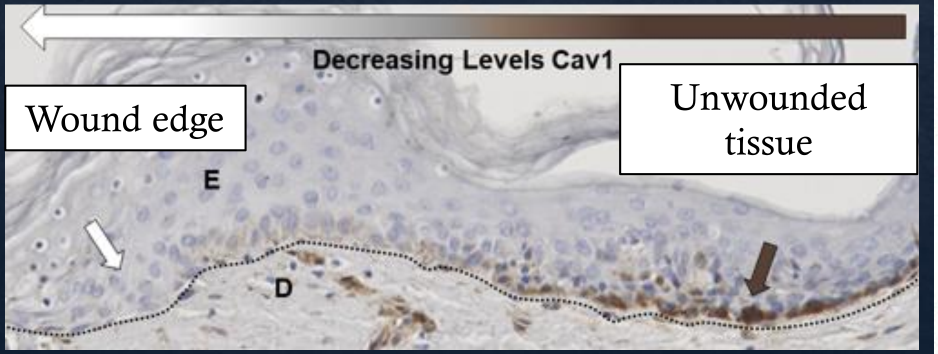


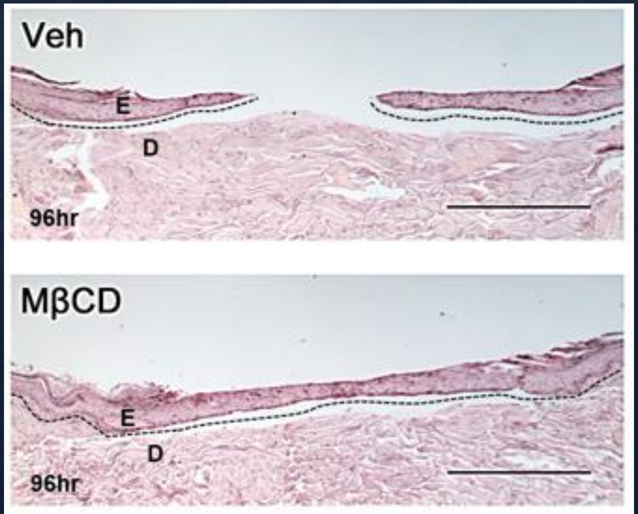
Freeze-drying

TOF-SIMS analysis

Targets for Wound Closure

- Caveolin-1, a scaffolding protein, slows wound closure when over-expressed.[1]
  - In healthy wounded skin, Cav-1 is downregulated at the epithelial tongue.
- Cav-1 is dependent on cholesterol for incorporation into membranes.[2,3]
  - Cholesterol removal limits Cav-1 expression and speeds wound closure.
- Cholesterol sulfate is a precursor to Cholesterol.[4]



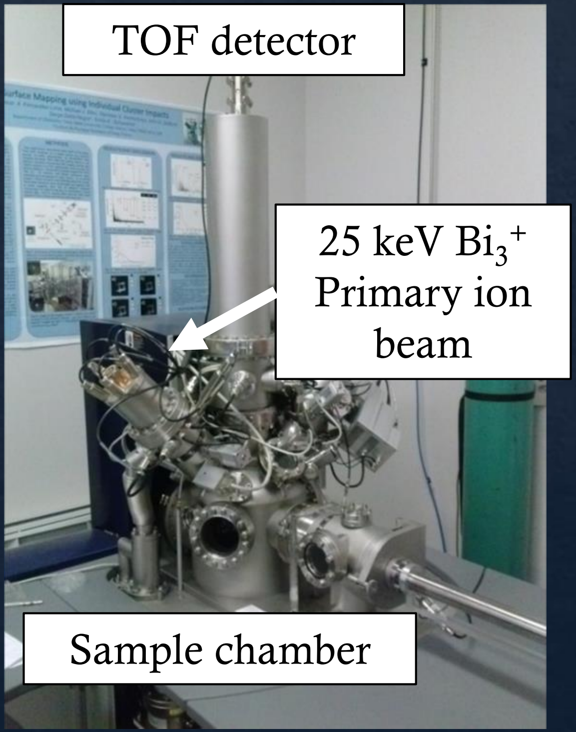


(Jozic et al., in revision)

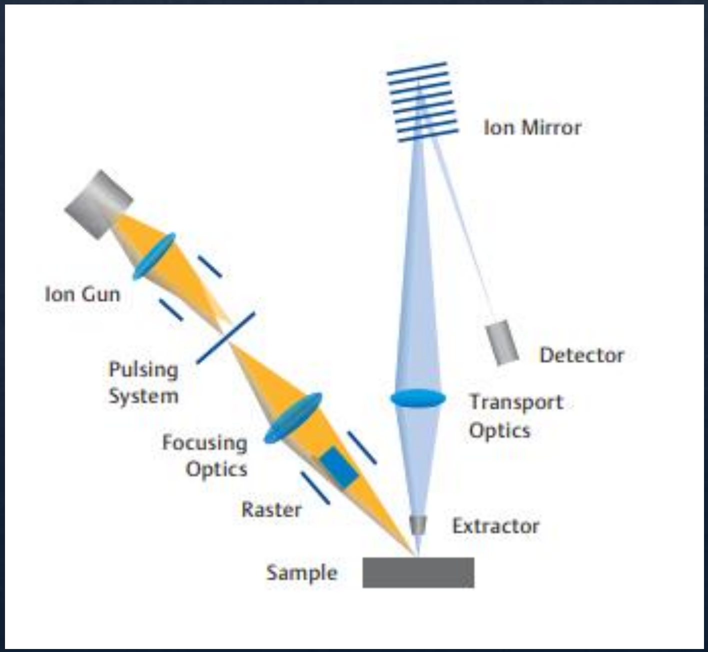
1. J. H. Rhim, J. H. Kim, E.-J. Yeo, J. C. Kim and S. C. Park, Molecular medicine (Cambridge, Mass.), 2010, 16, 527-534.
2. M. Murata, J. Peränen, R. Schreiner, F. Wieland, T. V. Kurzchalia and K. Simons, Proceedings of the National Academy of Sciences, 1995, 92, 10339.
3. S. Li, K. S. Song and M. P. Lisanti, J. Biol. Chem., 1996, 271, 568-573.
4. C. A. Strott and Y. Higashi, Journal of Lipid Research, 2003, 44, 1268-1278.

TOF-SIMS

- Time-of-Flight Secondary Ion Mass Spectrometry
  - Secondary Ions are extracted and analyzed in a TOF analyzer



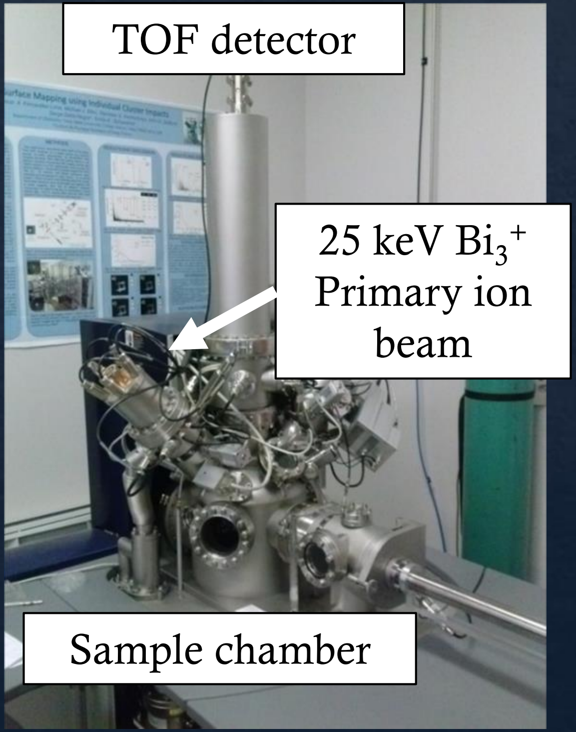
TOF-SIMS V
ION-TOF



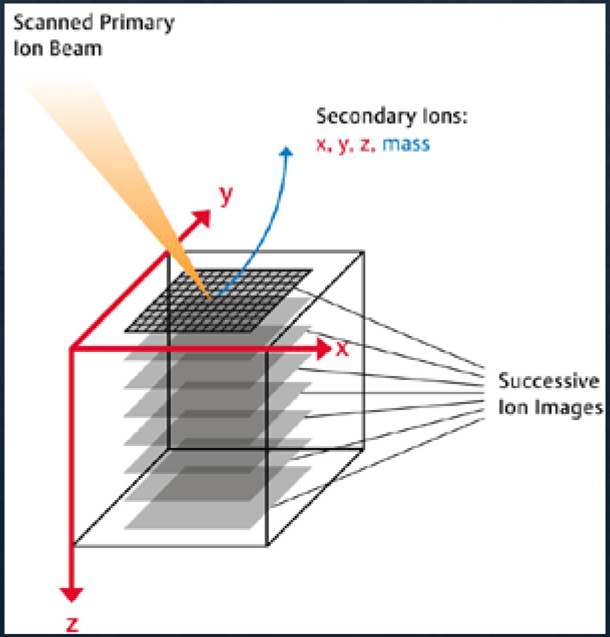
(ION-TOF)

TOF-SIMS

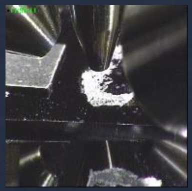
- Time-of-Flight Secondary Ion Mass Spectrometry
  - Secondary Ions are extracted and analyzed in a TOF analyzer
  - Mass Spectra are collected across a surface
  - Label-free technique and allows for retrospective analysis



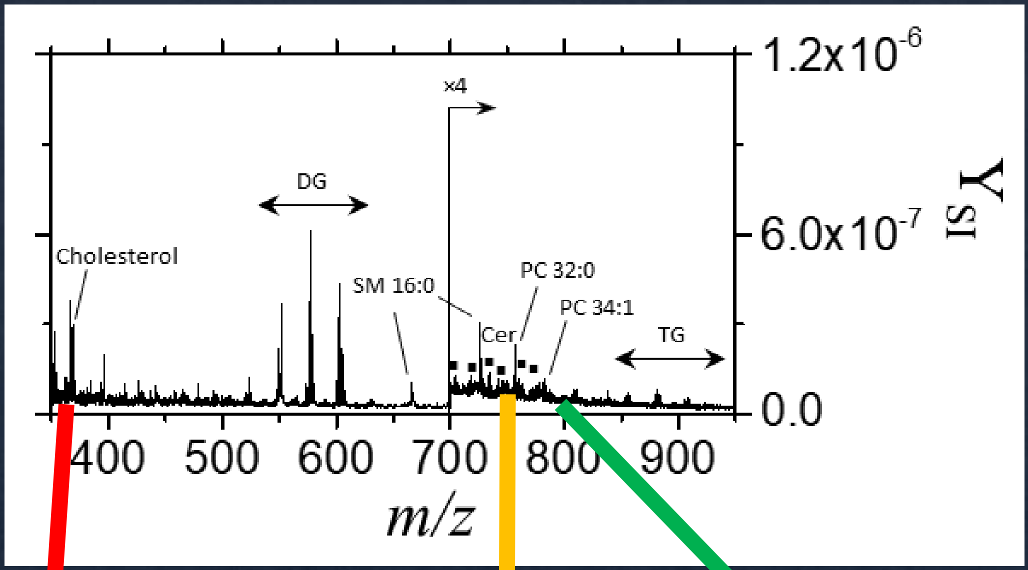
TOF-SIMS V
ION-TOF



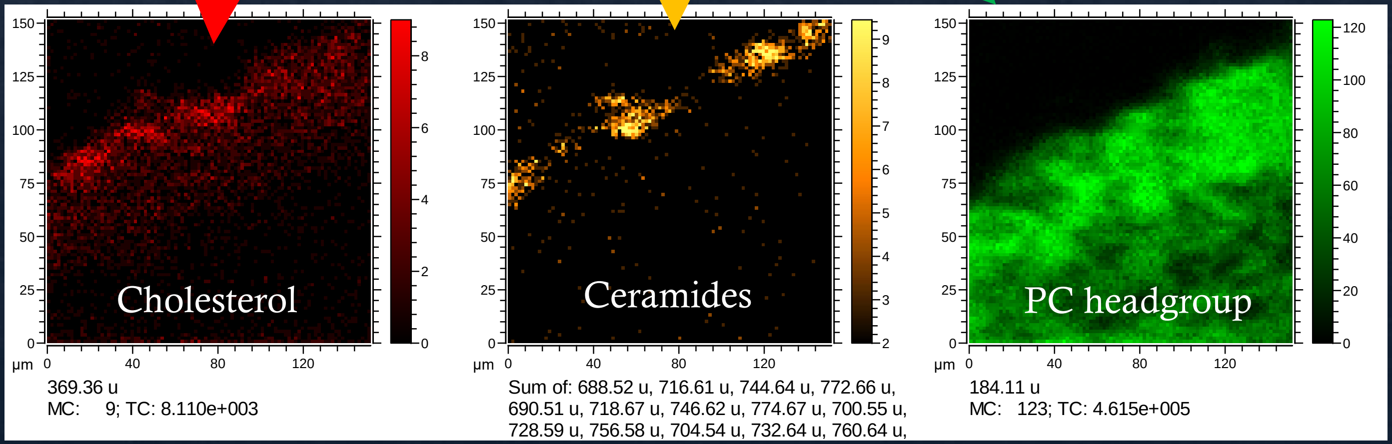
(ION-TOF)



Unwounded Human Skin Composition

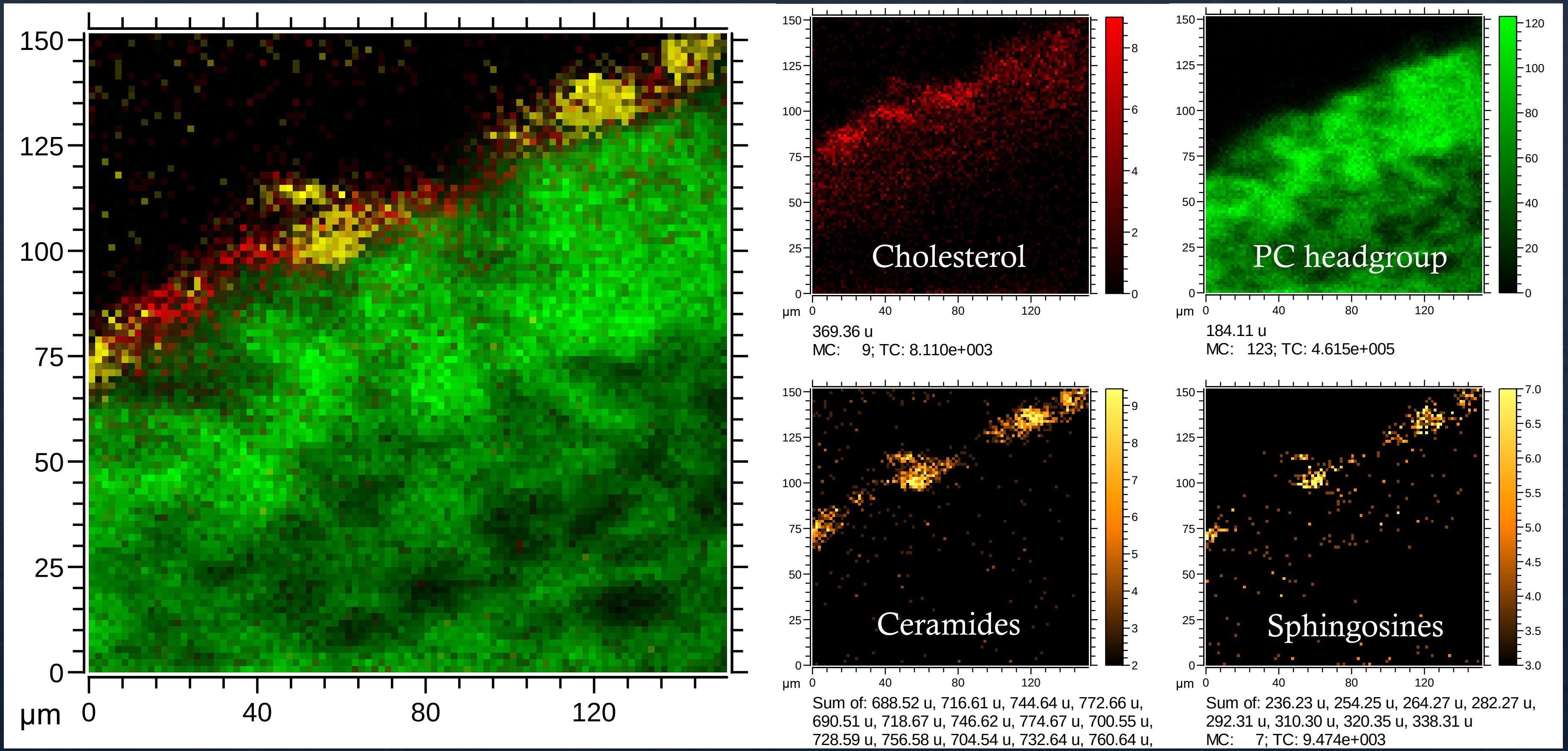


Positive polarity: Cholesterol, Diacyglycerides, Triacyglycerides, Ceramides, and Phosphatidylcholines



Imaging of Unwounded Human Skin

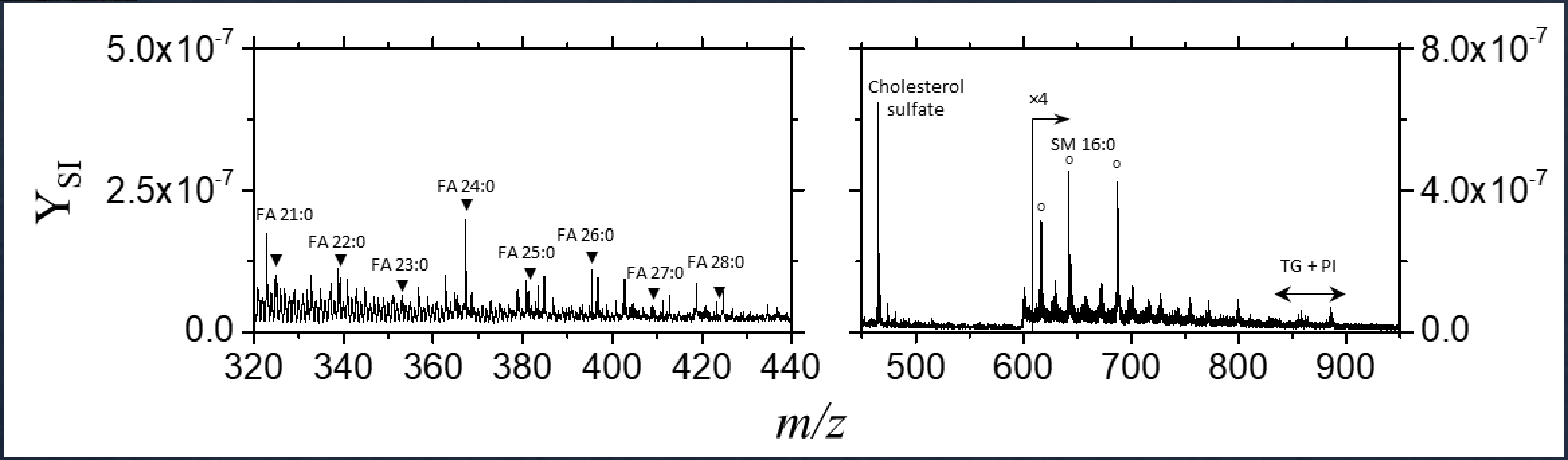
Positive polarity



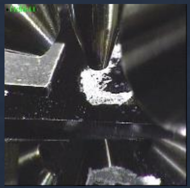
- Stratum Corneum is comprised of Cholesterol, Ceramides, and long chain fatty acids[1,2]

1. K. R. Feingold, *Journal of Lipid Research*, 2007, **48**, 2531-2546.
2. J. van Smeden et al., Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 2014, 1841, 70-79.

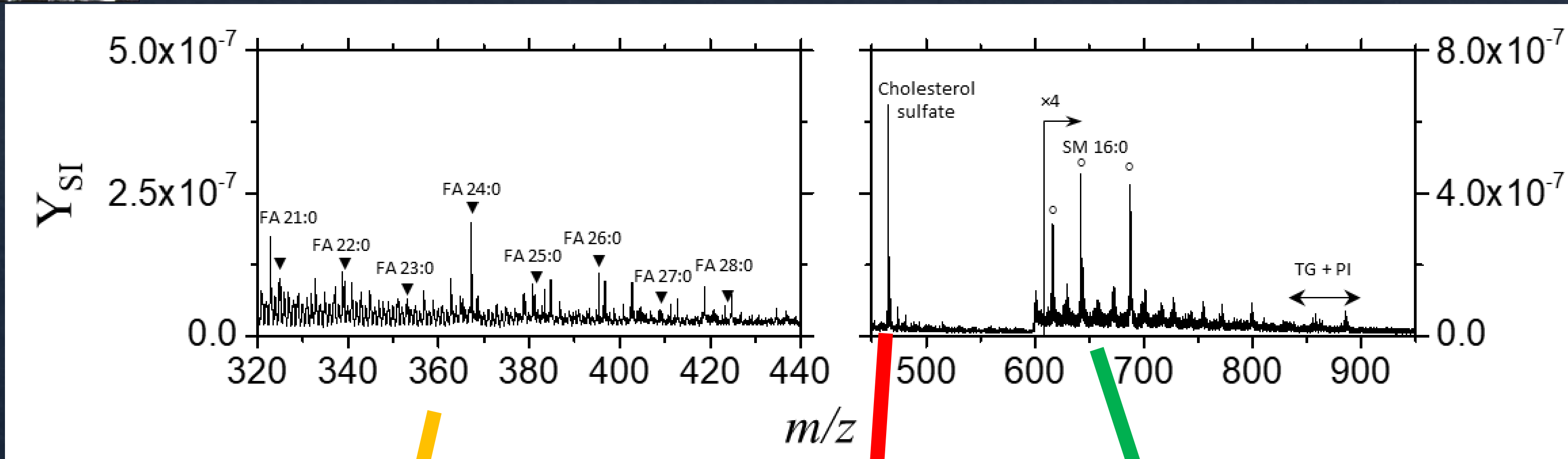
Unwounded Human Skin Composition



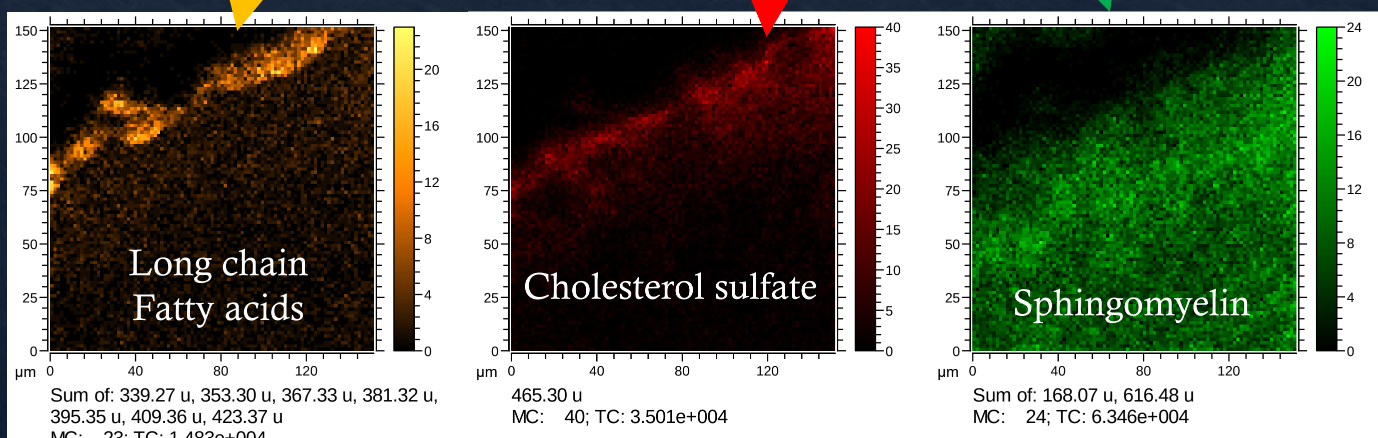
Negative polarity: Cholesterol sulfate, Sphingomyelin, Triacyglycerides, and Phosphoinositol



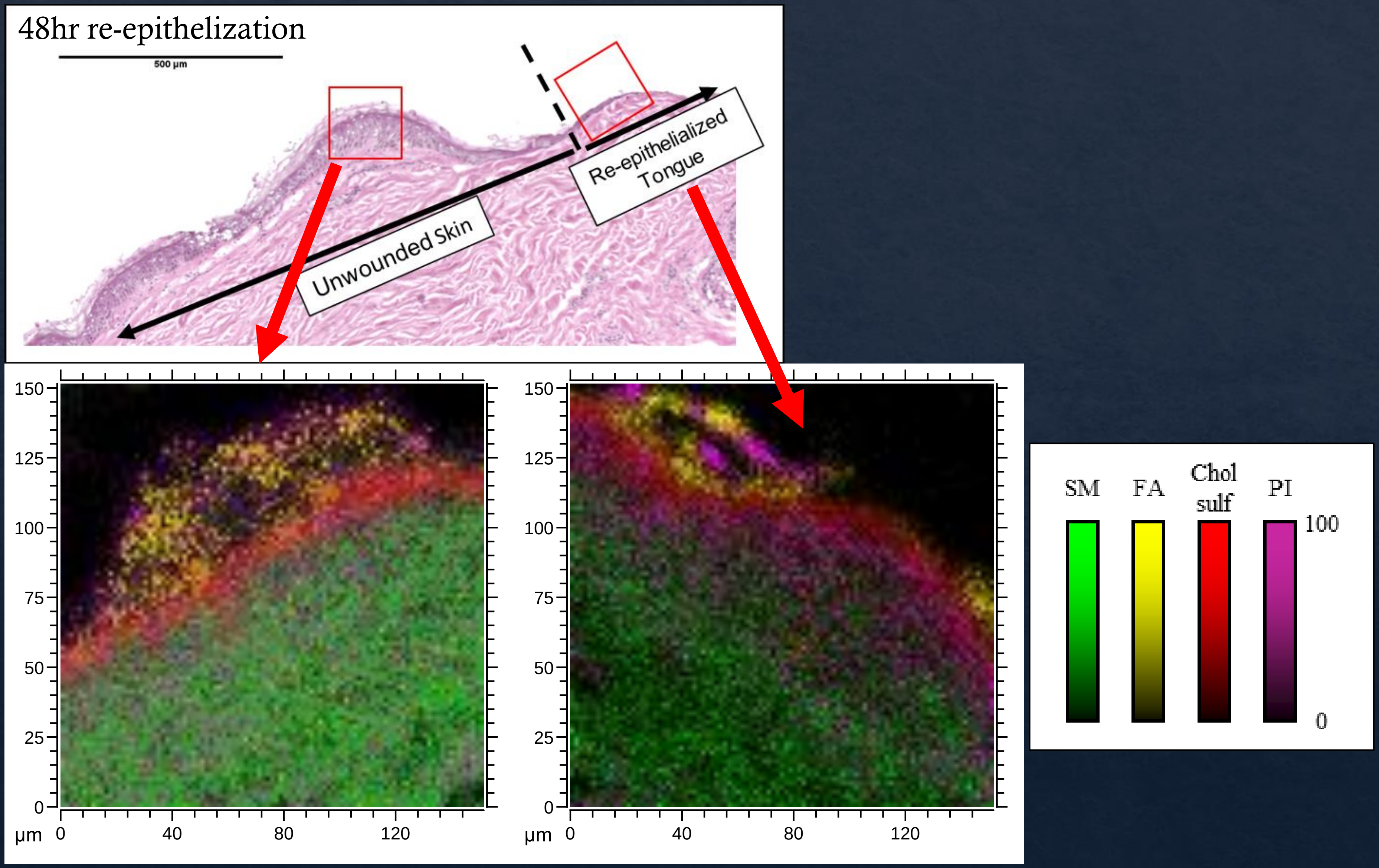
Unwounded Human Skin Composition



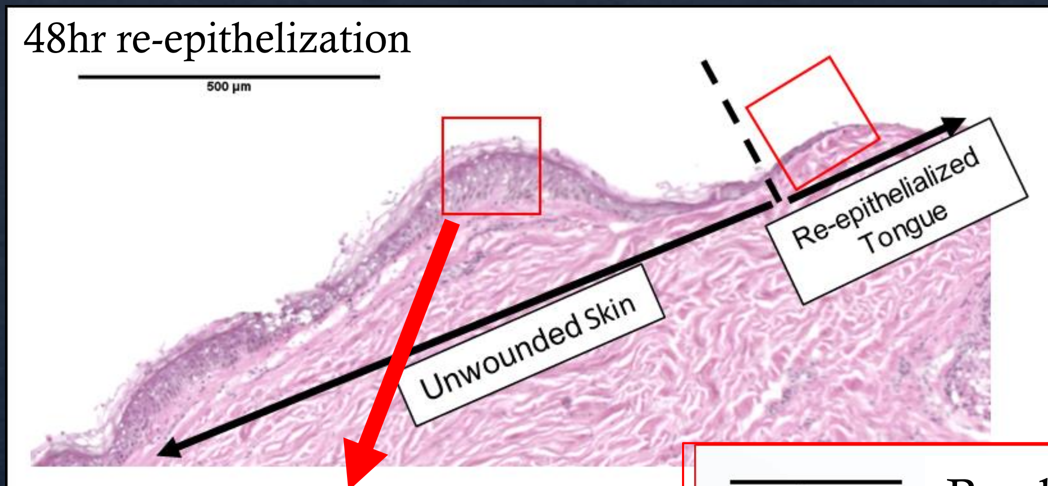
Negative polarity: Cholesterol sulfate, Sphingomyelin, Triacyglycerides, and Phosphoinositol



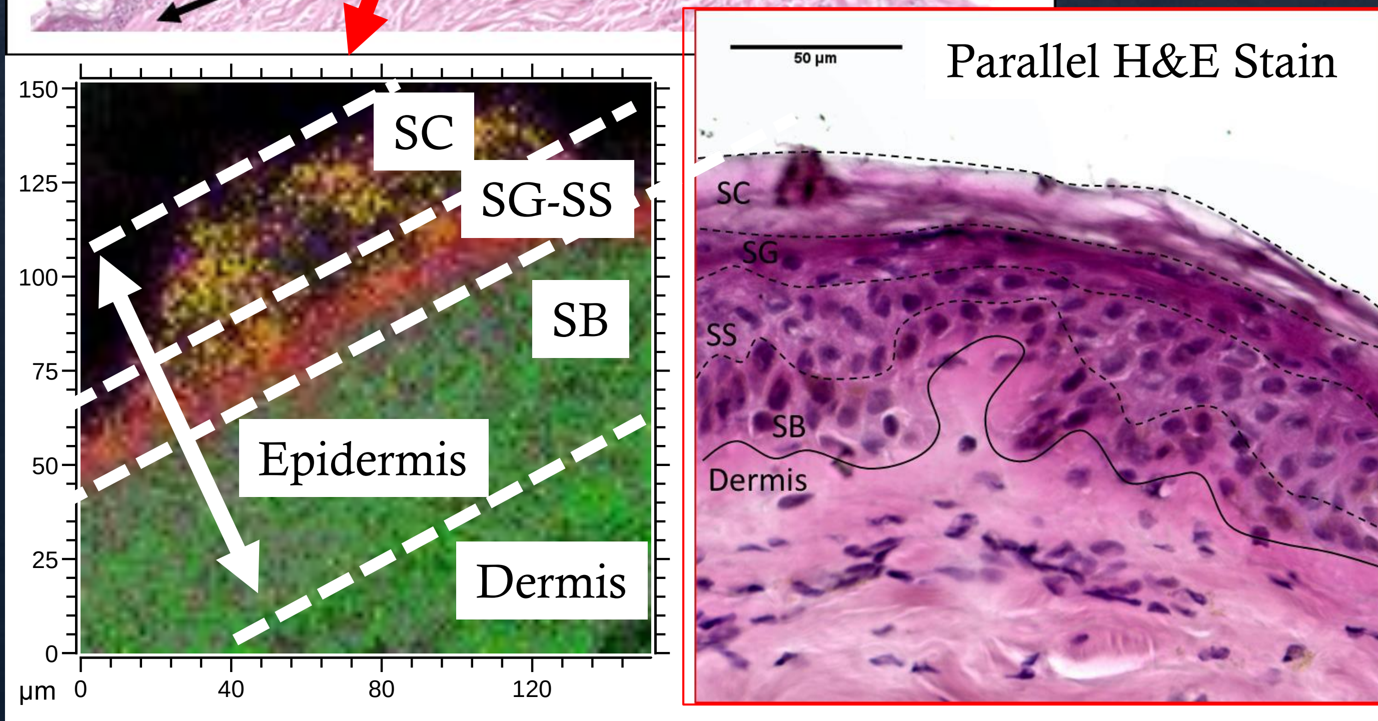
Imaging of Re-epitheliazed Human Skin

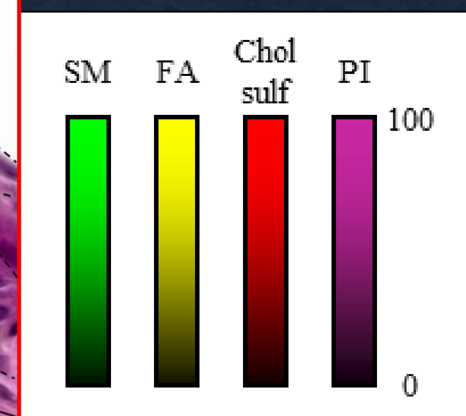


Imaging of Re-epitheliazed Human Skin

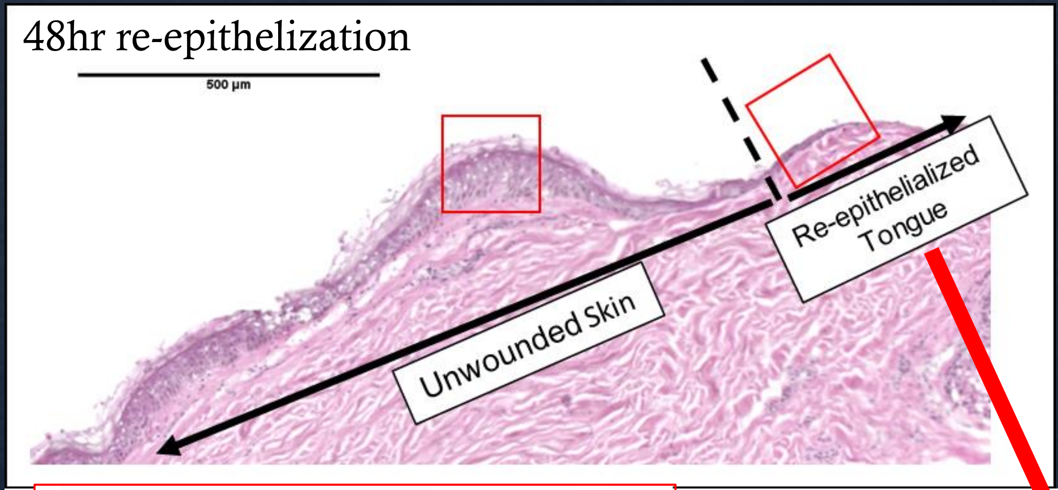


- Stratified epidermis
- Homogeneous lipid signal

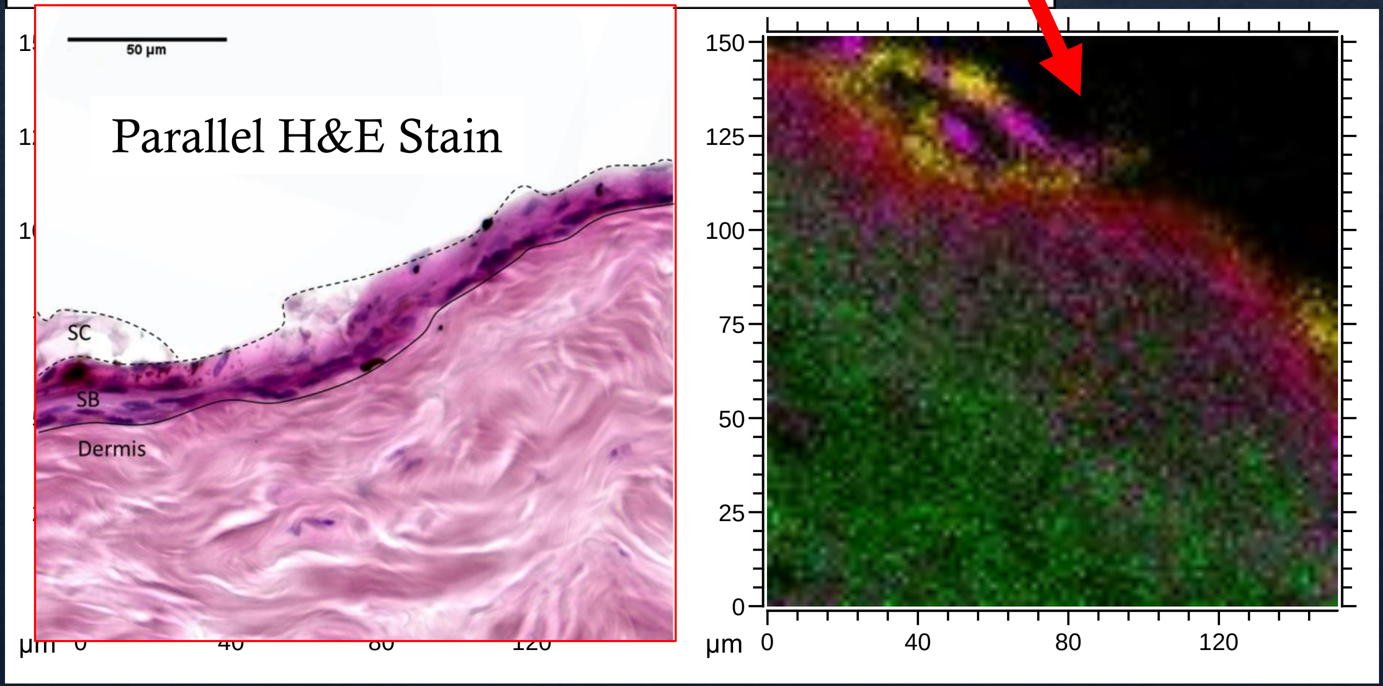


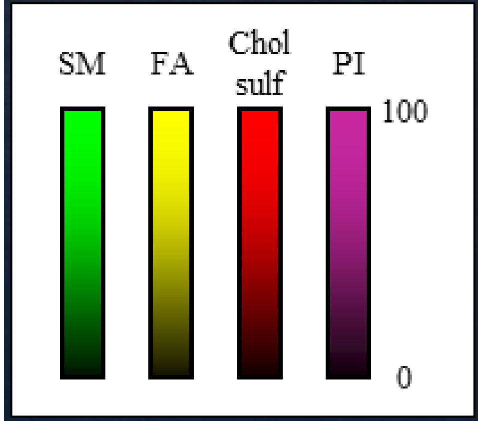


Imaging of Re-epitheliazed Human Skin



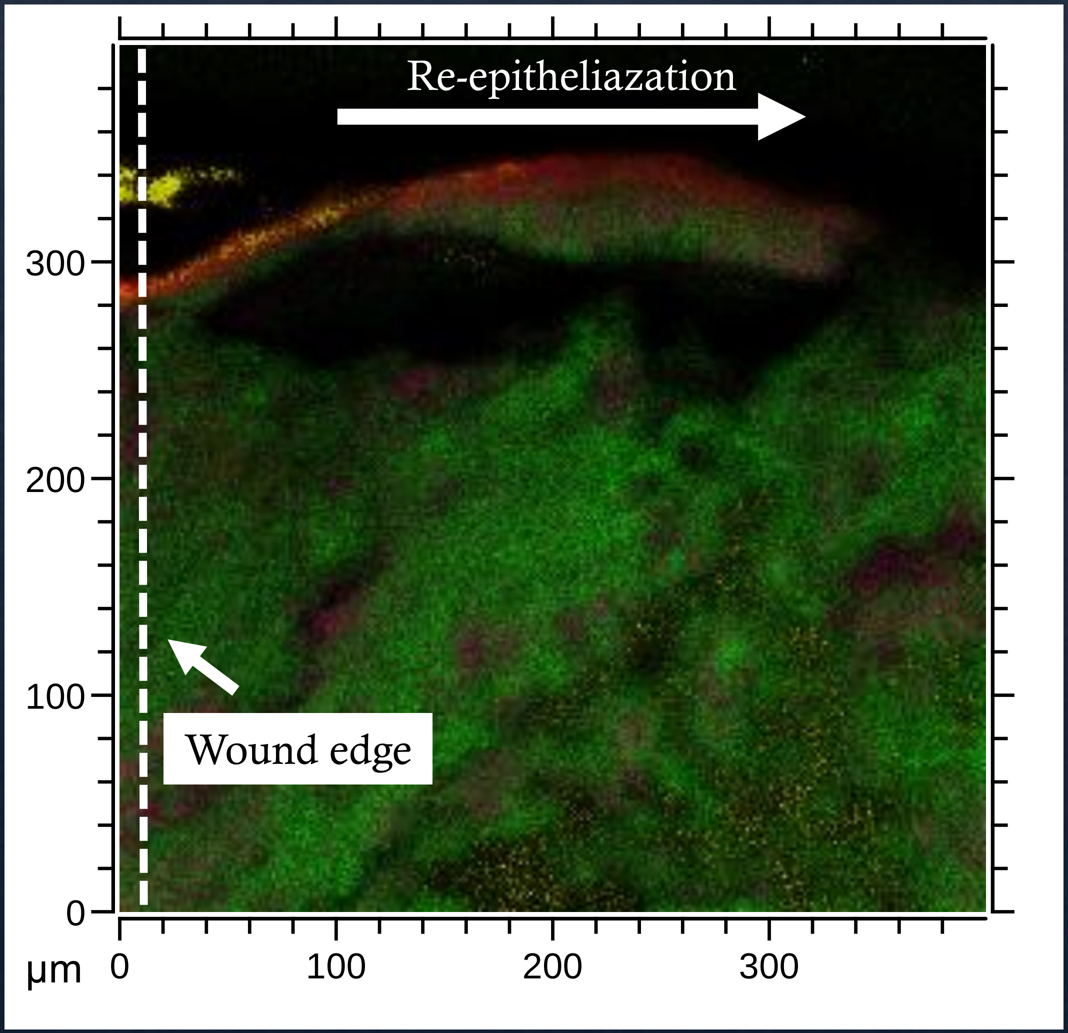
- Less stratified epidermis
- Diminished lipid signal



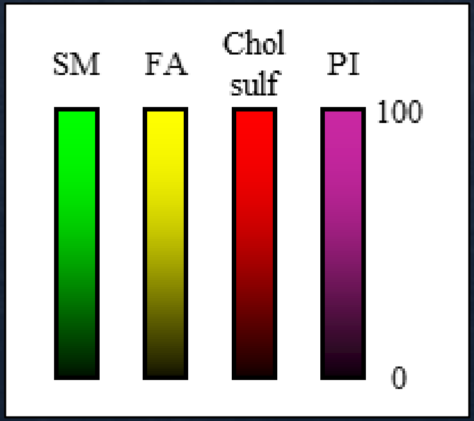


Imaging of Re-epitheliazed Human Skin

96hr re-epitheliazed tissue section

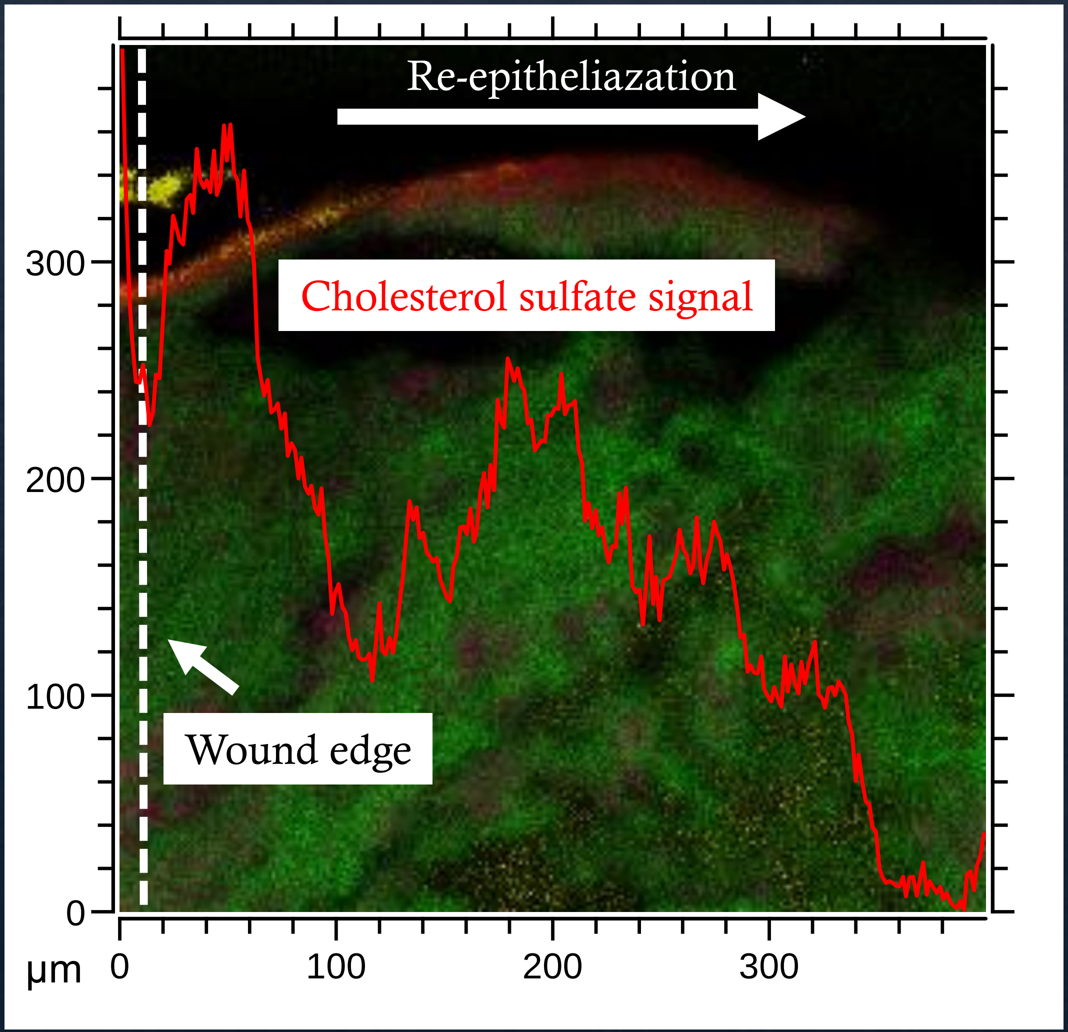


- Diminishing cholesterol sulfate across the epithelial tongue

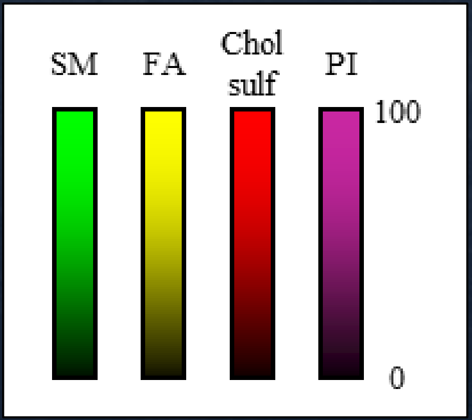


Imaging of Re-epitheliazed Human Skin

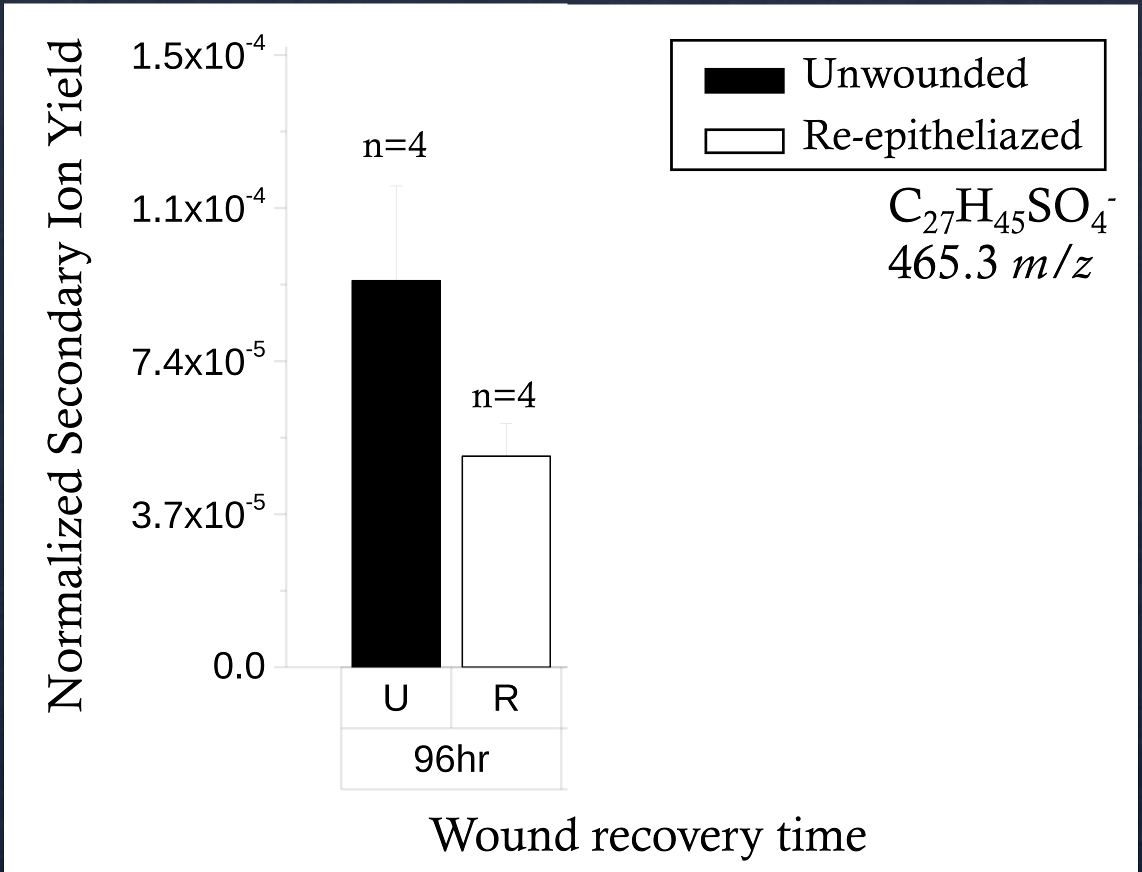
96hr re-epitheliazed tissue section



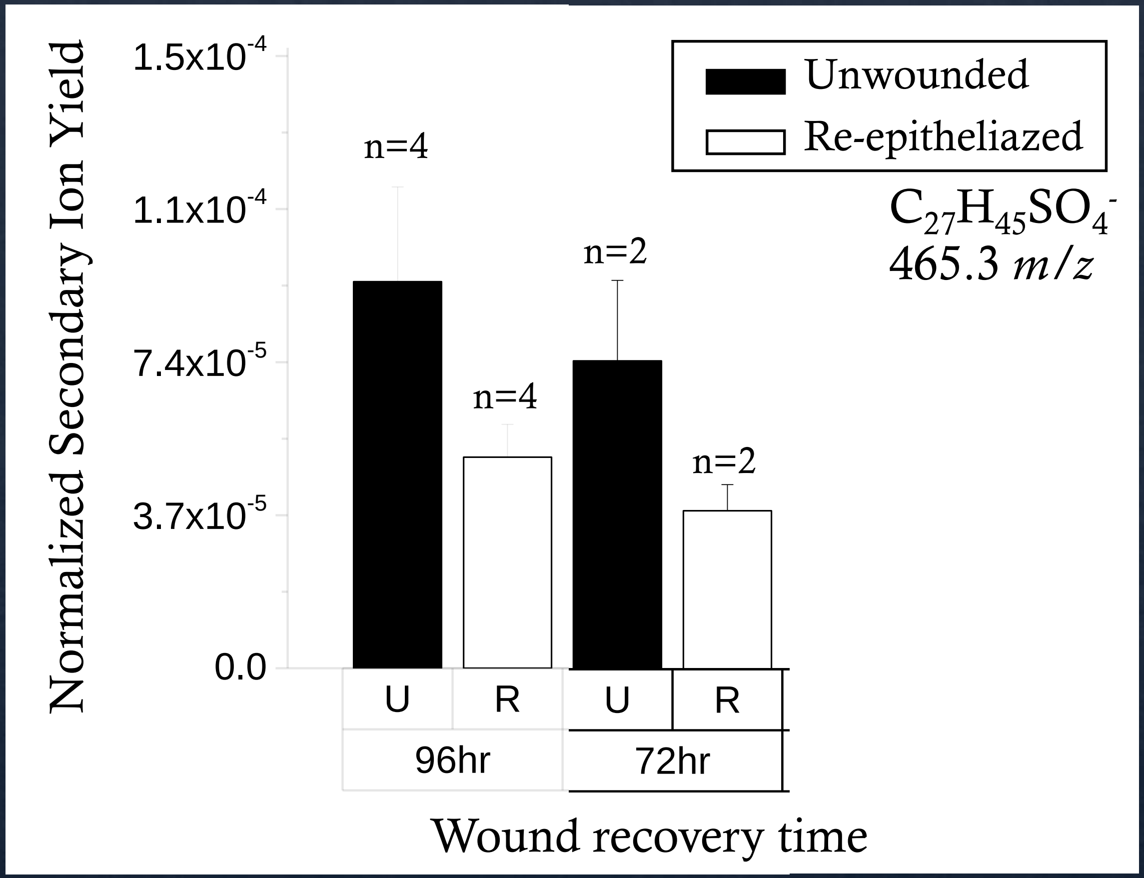
- Diminishing cholesterol sulfate across the epithelial tongue



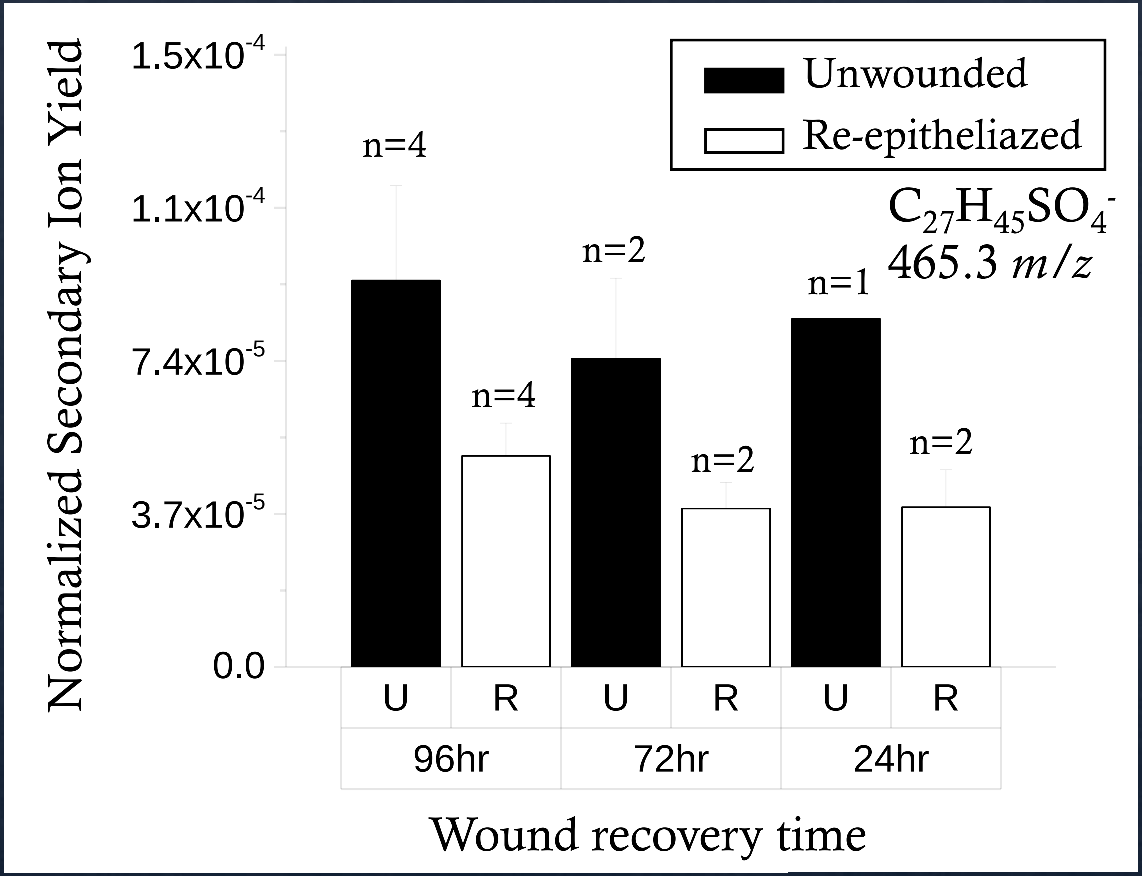
Semi-quantitation of Cholesterol sulfate



Semi-quantitation of Cholesterol sulfate

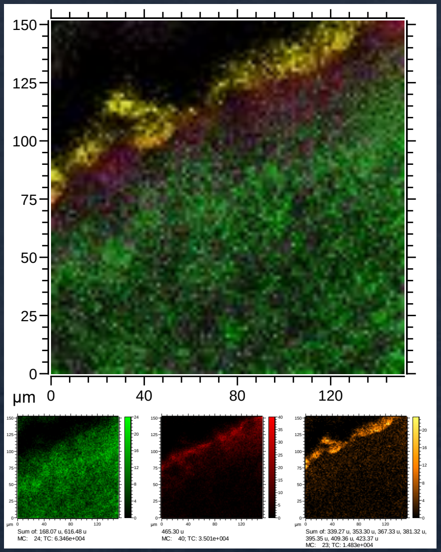


Semi-quantitation of Cholesterol sulfate



- Diminished cholesterol sulfate quantity is characteristic of the healing epithelial tongue

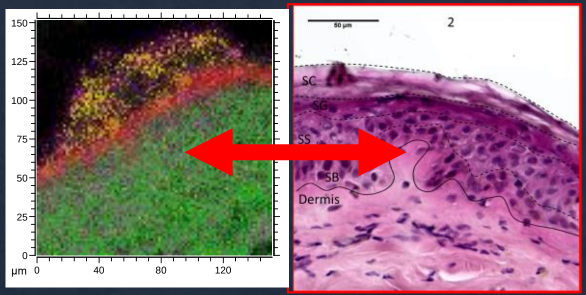
Conclusions



- TOF-SIMS imaging is an ideal method for studying lipid distribution and quantity
  - Cholesterol sulfate and other lipids may be related to the rate of wound closure and expression of proteins
  - *Ex-vivo* human tissue models will help understand wound healing and develop therapies for non-healing chronic wounds

Future Steps

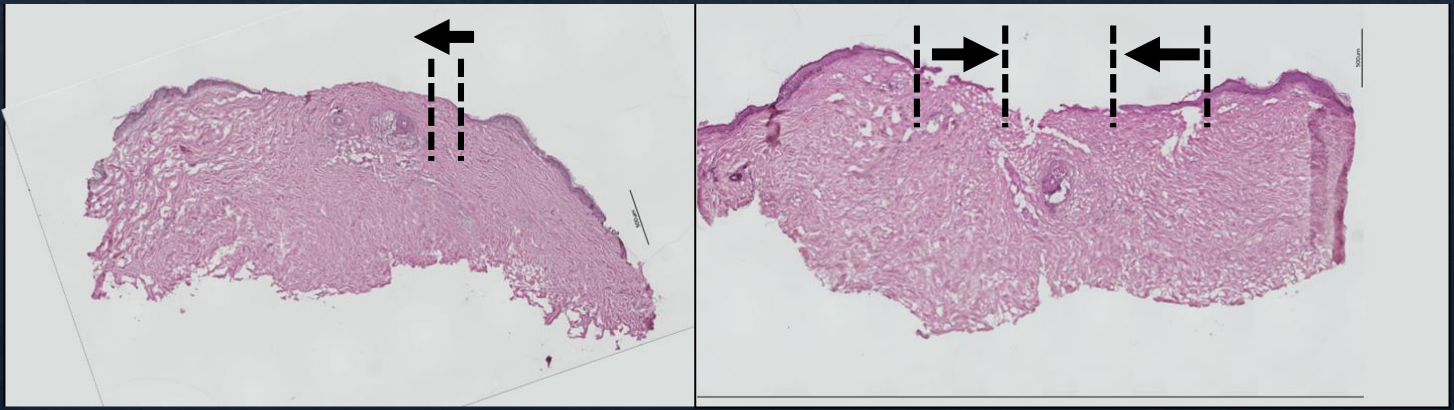
- Stain skin tissue post-TOF-SIMS analysis
  - Parallel slices are not exact
  - Use Cav-1 antibody stain to compare with cholesterol sulfate distribution



- Relate lipid composition to rate of re-epitheliazation

48hr tissue

96hr tissue



Thank you for your attention!

Acknowledgements

Group Members

Mentor

Dr. Francisco Fernandez-Lima

Post-docs

Kevin Jeanne Dit Fouque

Jean Haler

Collaborator

Dr. Ivan Jozic

UHealth UNIVERSITY OF MIAMI HEALTH SYSTEM | UNIVERSITY OF MIAMI MILLER SCHOOL of MEDICINE

Graduate students

Anthony Castellanos

Jacob Porter

Dennys Leyva

Yarixa Cintron

Kim Dang

Clement Olanrewaju

Elisa Shoff

Funding:

CREST NSF: HRD-1547798

FIU Center for Aquatic Chemistry and Environment

NSF Center of Research Excellence in Science and Technology

FIU FLORIDA INTERNATIONAL UNIVERSITY